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Publication Date

2025-09-09

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Los Angeles

Molecular Mechanisms of Coenzyme Q Deficiency: Biochemical and Computational Insights
into COQ5, A *C*-Methyltransferase in Coenzyme Q Biosynthesis

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Biochemistry, Molecular and Structural Biology

by

Sining Wang

2025

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ABSTRACT OF THE DISSERTATION

Molecular Mechanisms of Coenzyme Q Deficiency: Biochemical and Computational Insights
into COQ5, A C-Methyltransferase in Coenzyme Q Biosynthesis

by

Sining Wang

Doctor of Philosophy in Biochemistry, Molecular and Structural Biology

University of California, Los Angeles, 2025

Professor Catherine F. Clarke, Co-Chair

Professor Steven G. Clarke, Co-Chair

Coenzyme Q (CoQ) is a redox-active lipid functioning at the intersection of diverse metabolic processes. Its unique chemical and physical properties enable it to function both as a mitochondrial electron carrier and as a chain-breaking antioxidant, supporting cellular respiration and inhibiting lipid peroxidation within the lipid bilayer. In eukaryotes, CoQ is synthesized by nuclear-encoded polypeptides that assemble into a lipid-rich complex, termed the CoQ synthome, peripherally associated with the matrix face of the inner mitochondrial membrane. Defects in CoQ biosynthesis cause primary CoQ deficiency, a condition with diverse clinical manifestations. Supplementation with CoQ₁₀, the native human form, has shown inconsistent efficacy due to poor

bioavailability, underscoring the need for a comprehensive understanding of CoQ biosynthesis. Chapter 1 of this work provides an overview of CoQ's essential roles in metabolism, as well as current functional and structural evidence supporting our understanding of the CoQ biosynthetic polypeptides. Chapter 2 examines the genetic underpinnings of primary CoQ deficiency by classifying missense single nucleotide variants (SNVs) affecting functional regions of core synthome subunits. Building on this analysis, Chapters 3 and 4 focus on biochemical validation of functional regions in the *C*-methyltransferase COQ5/Coq5 in *Saccharomyces cerevisiae*. Chapter 3 combines liquid chromatography–tandem mass spectrometry (LC-MS/MS) with molecular dynamics (MD) simulations and other computational approaches to identify catalytic residues and proposes a mechanism for aromatic carbon nucleophile activation and methyl-transfer. Chapter 4 builds on this work by linking Coq5 dimerization to catalytic activity through an SNV in the dimer interface that disrupts both oligomerization and CoQ biosynthesis. Appendices I–III extend beyond CoQ biosynthesis, addressing *C*-methyltransferase substrate selectivity, methylation as a post-translational modification, and mechanistic organic chemistry. Additional supporting data are provided as supplementary files: Files 1–5 relate to Chapter 3 and include an AlphaFold-predicted COQ5 structure, a table summarizing analyses of COQ5 SNVs, and representative MD structures; Files 6–7, associated with Appendix II, contain interaction energies and structural analyses of methylarginine-containing complexes. Together, this work provides insights into how genetic variation, active-site chemistry, and protein–protein interactions shape CoQ biosynthesis and highlights a broader interest in the functionally diverse family of methyltransferases.

The dissertation of Sining Wang is approved.

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*To Mimi, Dada, Adongdong, and Abadou
who did everything they could to support my education
and to help me see the world with curious eyes.*

*To my penguinmate, my role model, greatest thinker and speaker
who gave me strength when things felt impossible.*

*Berry, we made it!
Keep on swimming, Science Penguin.*

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ACKNOWLEDGEMENTS

I would like to begin by expressing my deepest gratitude to my advisors, Drs. Catherine F. Clarke and Steven G. Clarke, whose guidance, patience, enthusiasm, and unwavering support have been invaluable to my growth as a researcher. As an undergraduate student in Steve's biochemistry class, I was inspired by his passion for teaching and was immediately intrigued by his lectures on the molecular mechanisms underlying the aging process, which prompted me to stay and pursue a PhD in the Biochemistry, Molecular and Structural Biology program, where I then met Cathy, who shares this passion. Steve and Cathy recognized my interests and strengths, supporting me in pursuing both multidisciplinary research and undergraduate teaching. I am incredibly fortunate to have them as my advisors.

I am profoundly grateful to my thesis committee, Dr. Kendall N. Houk and Dr. Aldons J. Lusis, for the unique perspectives they brought to my work. Their guidance and professional support have been indispensable to my development as a scientist. I would like to thank Jake for his advice on the genetics aspects of my work, which broadened the scope of my research and suggested future directions I hope to explore in my career. I am especially thankful to Ken, as my research journey began in his lab as an undergraduate. He, along with the Houkies, provided mentorship early in my career, helping me gain confidence and challenging me to think critically and independently. The experience I gained in computational organic chemistry not only strengthened my skills but also enabled me to participate in multidisciplinary research and expand my horizons.

Chapter 2 is a reprint of *Antioxidants* (2022) 11(12), 2308 (doi: 10.3390/antiox11122308), reproduced with permission from the Multidisciplinary Digital Publishing Institute. I would like to thank my co-authors Akash Jain, Dr. Noelle Alexa Novales, Audrey Nashner, Fiona Tran, and

my advisor Dr. Catherine Clarke, who oversaw this work, for their collaboration and permission to include it in my dissertation. This project was among my very first in graduate school, and it has been especially rewarding to see a COVID-19 pandemic remote project come to fruition. I am particularly thankful to my co-primary author Akash, who was only a freshman when he joined this project and has been incredibly supportive throughout the write-up process.

Chapter 3 is a manuscript in preparation, made possible through the contributions of several exceptional collaborators. My mentee Celeste Medina-Seymour performed computational analysis of missense variants and assisted with site-directed mutagenesis and biochemical characterization. Qingyang Zhou provided mentorship on docking calculations and molecular dynamics simulations, while Dr. Kendall N. Houk advised on *ab initio* calculations and mechanistic hypotheses. Chapter 4 describes a project I worked on with Celeste. This project arose from an incidental finding in Chapter 2 and would not have been feasible without her support. Mentoring Celeste and watching her develop into an independent researcher has been a true privilege. I also thank Dr. Eric Pang and Cameron Flowers for their assistance with protein purification and biophysical characterization. Eric performed the size-exclusion chromatography experiments; Cameron performed preliminary native top-down mass spectrometry analysis, which, though not included in this work, helped inform future directions. Finally, for the liquid chromatography–tandem mass spectrometry experiments outlined in Chapters 3 and 4, I am grateful to Dr. Yu Chen from the UCLA Molecular Instrumentation Center for providing essential assistance with instrument usage and maintenance.

Appendix I describes a side project involving bacteria that I worked on during the first few years of graduate school. I thank Dr. Jennifer Shepherd and Dr. Gilles Basset for the insightful discussions on C-methyltransferase homologs in prokaryotes. Dr. Shepherd kindly provided the

farnesylated substrate analogs I used for early characterizations of enzymatic activity in bacteria. Appendix II is a manuscript in preparation under the guidance of Dr. Steven Clarke and Dr. Kendall N. Houk. My mentee Jenai Blazina performed extensive density functional theory calculations that built on early work I performed during the pandemic and made this project possible. Appendix III is reproduced from *Organic & Biomolecular Chemistry* (2021) 19, 8025-8029 (doi: 10.1039/D1OB01343B) with permission from the Royal Society of Chemistry. I am grateful to my co-authors, Drs. Atena Solea, Xiao-Song Xue, Aurelien Crochet, and Katharina Fromm, as well as faculty advisors, Drs. Kendall N. Houk, Olimpia Mamula, and Christophe Allemann, for their collaboration and permission to use this material.

The use of all reproduced material has been approved by the thesis committee. All computational work described herein utilized computational and storage resources provided by the Hoffman2 Cluster, operated by the UCLA Office of Advanced Research Computing's Research Technology Group. In addition, I would like to acknowledge support from the UCLA Chemistry-Biology Interface Predoctoral Training Program, funded by NIH NIGMS award T32GM136614. I am grateful for additional support from the Edwin W. Pauley Fellowship (College of Letters & Science, UCLA), the Audree Fowler Fellowship in Protein Science (Molecular Biology Institute, UCLA), and the Dissertation Year Fellowship (Division of Graduate Education, UCLA). This work was also supported by National Science Foundation (NSF) grant MCB-2343997 (PI: Dr. Catherine Clarke). Chapters 3 and 4, as well as Appendices I and II, were further supported by NSF grant MCB-1714569 (PI: Dr. Steven Clarke).

During my time here, I got to work alongside the talented scientists in the Steve Clarke and Cathy Clarke Labs, whose camaraderie and support have made this journey unforgettable. To former members, Dr. Troy Lowe, Dr. Eric Pang, Dr. Alexa Novales, Miranda Kelly, thank you for

helping me get started with wet bench work and providing both guidance and friendship throughout my time in the lab. To current members Michael Guile and Kelsey Feustel, thank you for your insightful advice on science and on life in general, as well as for being a good friend. To the undergraduates I have had the pleasure of working with—Akash, Celeste, Jenai, Kyle, and others—thank you for making my time here more enjoyable and for inspiring me with your curiosity and dedication. I have learned as much from this experience as I hope you have from your time here. I am also grateful to my future postdoctoral advisor, Dr. Danielle Schmitt, for her guidance, career advice, and support during my final year of graduate school. Her dedication to fostering my growth as a scientist and educator has been invaluable.

Aside from research, I got to serve as a teaching assistant in numerous courses in the UCLA Department of Chemistry and Biochemistry. It was a rewarding experience to work alongside my advisors Steve and Cathy, who entrusted me with creative freedom in designing my discussion sections and course materials. Serving as a teaching assistant was incredibly fulfilling, and I truly enjoyed supporting my students both in mastering course material and navigating opportunities in undergraduate research. I would also like to thank Dr. Ryan Lannan and the Undergraduate Learning Assistant program, with whom I worked on improving the introductory biochemistry curriculum. Through this experience, I got to work on fun projects such as developing tactile models and learning tools to enhance student engagement and understanding, which was an exciting change of pace from wet bench work. I also got to meet some amazing fellow teaching assistants and learning assistants, who made the implementation of these tools possible.

To my parents and grandparents, thank you for your unwavering patience and for all that you've done to support my education. I know that I can call at any time—and you'll drop everything, no matter what you're in the middle of—to listen, encourage, or just to keep me

company. To my friends, Yilan Qin, Yi Feng, Yining Hu, Cynthia Zhao, and Jeongmin Son, you have always been there to cheer me on when things got tough. Even though we barely see each other these days, know that you are always in my thoughts.

To Jukka, my favorite person in the world—where do I even begin? You have been by my side every step of the way, helping me focus on the positives, look ahead, and recognize and build on my strengths. You inspire me every day with your kindness and resilience. I am so glad I found you, and I look forward to the next chapter in our lives together.

And finally, to Berry, I think your moral support worked.

VITA

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PUBLICATIONS

Wang, S., Medina-Seymour, C., Zhou, Q., Houk, K. N., Clarke, S. G., Clarke, C. F. Mutations in the Coq5 substrate-binding pocket reveal insights into C-methyltransferase catalytic mechanism. **Manuscript in preparation.**

Wang, S.[‡], Blazina, J.[‡], Clarke, S. G., Houk, K. N. Computational characterization of hydrogen bonding interactions in methylated arginine residues. **Manuscript in preparation.**

Wang, S.[‡], Jain, A.[‡], Novales, N. A., Nashner, A. N., Tran, F., and Clarke, C. F. (2022) Predicting and Understanding the Pathology of Single Nucleotide Variants in Human *COQ* Genes. *Antioxidants* 11, 2308.

Solea, A. B., **Wang, S.**, Xue, X.-S., Crochet, A., Fromm, K. M., Houk, K. N., Mamula, O., and Allemann, C. (2021) Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids. *Org. Biomol. Chem.* 19, 8025–8029.

Chen, S., **Wang, S.**, and Houk, K. N. (2019) Origins of Contrasteric π -Facial Selectivity in Epoxidations of Encumbered Tetrahydropyridines by a Bifunctional Peracid. *Synlett* 30, 459–463.

[‡] These authors contributed equally to this work.

Chapter 1

Understanding coenzyme Q: function, biosynthesis, and deficiencies in humans

INTRODUCTION TO COENZYME Q

Coenzyme Q (CoQ), also known as ubiquinone, is a redox-active, lipophilic molecule found in the cellular membranes of eukaryotes and many prokaryotes (1,2). Its chemical structure consists of a tetrasubstituted benzoquinone head group attached to a polyisoprenyl tail (Figure 1A) of variable unit-length—ten isoprene units in humans (CoQ₁₀), six in the yeast *Saccharomyces cerevisiae* (CoQ₆), and eight in *Escherichia coli* (CoQ₈). The quinone head group allows CoQ to participate in redox reactions (Figure 1B), while its hydrophobic tail allows for its integration into the midplane of the lipid bilayer (3).

FUNCTIONS OF COENZYME Q

Mitochondrial CoQ serves as a central electron carrier, accepting electrons from respiratory complexes I and II as well as from various dehydrogenases involved in key metabolic pathways, including fatty acid oxidation, branched-chain amino acid catabolism (4), uridine biosynthesis (5), and one-carbon metabolism (6,7) (Figure 2). Beyond its role in the mitochondria, the reduced form of CoQ, ubiquinol (CoQH₂), acts as a lipophilic antioxidant that protects cellular membranes against lipid peroxidation (8–13) and contributes to the regeneration of vitamin E (14). The broad and essential nature of these functions underscores the importance of maintaining sufficient cellular CoQ levels. Achieving this requires a dedicated multi-step biosynthetic pathway, supported by a large set of conserved enzymes and polypeptide factors that coordinate the production and distribution of CoQ within mitochondria.

POLYPEPTIDES INVOLVED IN COENZYME Q BIOSYNTHESIS

Many of the genes required for CoQ biosynthesis were first elucidated in the yeast *S. cerevisiae* (15), establishing it as a valuable model for studying this pathway. In yeast, CoQ biosynthesis can begin with either 4-hydroxybenzoic acid (4-HB), produced from tyrosine or the

shikimate pathway (16), or with *para*-aminobenzoic acid (*p*ABA) derived from chorismate as an alternative ring precursor (17,18). By contrast, in humans, 4-HB derived from phenylalanine and tyrosine serves as the predominant precursor (19) (Figure 3). Once 4-HB is synthesized and imported into mitochondria, the remaining steps of CoQ biosynthesis take place within the mitochondrial matrix.

In yeast, CoQ biosynthesis requires at least nine nuclear-encoded polypeptides (Coq1–Coq9), which correspond to human homologs PDSS1, PDSS2, COQ2–7, COQ8A, COQ8B, and COQ9. With the exception of Coq2, an integral membrane protein, these polypeptides are peripherally associated with the inner mitochondrial membrane (15). The yeast ferredoxin Yah1 (human homolog FDX1 and FDX2) and ferredoxin reductase Arh1 (human homolog FDXR) are also essential for Coq6-dependent monooxygenation (18), although the involvement of the human homologs in this step remains to be established. Two additional yeast polypeptides, Coq10 (human homologs COQ10A and COQ10B) and Coq11, enhance CoQ biosynthetic efficiency (20,21), although their human homologs are poorly characterized. Notably, Coq11 shares only a distant homology with the human short-chain dehydrogenase/reductase NDUFA9, a subunit of the mitochondrial respiratory complex I (22).

In yeast, Coq3–Coq9 and Coq11 form a high-molecular-weight assembly known as the CoQ synthome (Figure 4), or complex Q (3,15). Evidence indicates that humans also possess a homologous complex (23,24). The members of this complex are mutually dependent for stability, and its assembly into spatially defined domains is required for efficient CoQ biosynthesis (25–31). However, the exact stoichiometry and spatial organization of the complex's constituent polypeptides is not yet known. Interestingly, recent computational models suggest that the precise arrangement of the assembly may not be strictly required for catalytic efficiency (32). Functionally,

extensive work in yeast and human cells has now identified the catalytic polypeptides responsible for each step of the CoQ biosynthetic pathway (Figure 3), including the most recently assigned reaction, an oxidative decarboxylation catalyzed by Coq4 (33). Nevertheless, the precise order of several enzymatic steps remains equivocal, as knockout and mutagenesis studies have yielded conflicting results (18,33–36).

The genetic tractability of yeast, coupled with the viability of CoQ-deficient strains on fermentable carbon sources, has made it an indispensable system for dissecting this pathway. This high degree of conservation between yeast and human CoQ biosynthesis provides a strong foundation for analyzing the individual PDSS and COQ polypeptides, which will be discussed in detail in the following sections.

Human PDSS1/DPS1 and PDSS2/DLP1 (Yeast Coq1)

In both yeast and humans, the polyprenyl tail of CoQ is derived from the non-sterol branch of the mevalonate pathway (3). Condensation of an allylic pyrophosphate with isopentenyl diphosphate (IPP) units is catalyzed by members of the class III *trans*-prenyltransferase family, with the number of polymerized isoprene units being species-dependent (37,38). In yeast, this reaction is catalyzed by the single-subunit hexaprenyl pyrophosphate synthetase Coq1 (39), whereas in humans it is catalyzed by a heterotetrameric complex composed of decaprenyl diphosphate synthase subunits 1 and 2 (PDSS1 and PDSS2) (40,41). *In vitro* studies indicated that yeast Coq1 can use dimethylallyl pyrophosphate, geranyl pyrophosphate, or farnesyl pyrophosphate as the initial substrate for the condensation, although the preferred substrate *in vivo* has not been definitively established (3,42). Both Coq1 and PDSS1/2 are thought to be peripherally associated with the matrix-facing side of the inner mitochondrial membrane and are distinct from the CoQ synthome (Figure 4) (3,43). However, the mechanism by which IPP units are imported

into the mitochondrial matrix remains unknown. The product of this condensation step is essential for the formation of polyprenylated CoQ intermediates, which in turn are required for maintaining the polypeptide levels of other synthome members and, consequently, the stability of the CoQ synthome (30,43,44).

Human COQ2 (Yeast Coq2)

COQ2, also known as Coq2 in yeast, is an integral membrane enzyme embedded in the inner mitochondrial membrane (30), responsible for the attachment of the polyprenyl tail to the aromatic ring precursor in the CoQ biosynthetic pathway (45). In yeast, Coq2 catalyzes the formation of 3-hexaprenyl-4-hydroxybenzoic acid (HHB) from 4-HB and 3-hexaprenyl-4-aminobenzoic acid (HAB) from *p*ABA (15,17), producing the first polyprenylated CoQ intermediate. Human COQ2 has also been shown to restore CoQ₆ content in *coq2Δ* yeast (46), highlighting their functional conservation and the ability of COQ2 to attach polyprenyl tails of varying lengths.

The yeast Coq2 polypeptide is imported into mitochondria via the Tim23 pathway (47). Its presence is required for the stabilization of the synthome, as the loss of polyprenylated CoQ intermediates in *coq2Δ* yeast mutants results in markedly decreased levels of other Coq polypeptides, which cannot be rescued by either supplementation of CoQ₆ or overexpression of the atypical kinase Coq8 (29,30). While the requirement for COQ2 in synthome assembly is likely conserved in humans, evidence for its role in maintaining the expression of other COQ polypeptides is less conclusive, as one study demonstrated that the knockdown of *COQ2* expression in human osteosarcoma 143B cells did not result in significant changes in the levels of PDSS and COQ polypeptides (44).

Based on homology with prokaryotic homologs of COQ2 in the UbiA family of aromatic prenyltransferases, as well as structural models, human COQ2 is predicted to contain nine transmembrane helices, with an active site facing the matrix (48,49). A conserved aspartate-rich motif shared between yeast and humans serves as the putative polyprenyl pyrophosphate binding site (45,46). Similar to its prokaryotic homologs, the eukaryotic COQ2/Coq2 enzyme is also believed to be Mg^{2+} -dependent and can utilize a broad spectrum of polyprenyl diphosphates as substrates (38,49,50). Interestingly, a recent model of human COQ2 identified a channel that may serve to transport 4-HB across the inner mitochondrial membrane (51), although this function has not been experimentally validated.

Functional studies in yeast (29,35,52,53), mice (54,55), and human cells (36,55–58) have demonstrated that supplementation with alternative ring precursors, such as 3,4-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, and vanillic acid, may help bypass COQ6 or COQ7 deficiency, suggesting that COQ2 is able to bind and act on head-group-modified substrates as well. The substrate promiscuity of COQ2 points to a promising new mode of therapy for primary CoQ₁₀ deficiency.

Human COQ3 (Yeast Coq3)

Human COQ3 and yeast Coq3 are *S*-adenosyl-L-methionine (AdoMet)-dependent methyltransferases that catalyze both *O*-methylation steps in the CoQ biosynthetic pathway in the presence of a divalent metal cation, converting 4,5-dihydroxy-3-polyprenylbenzoic acid (DHPB) to 4-hydroxy-5-methoxy-3-polyprenylbenzoic acid (HMPB) as well as 3-methyl-6-methoxy-2-polyprenyl-1,4,5-benzenetriol (demethyl-QH₂ or DMeQH₂) to CoQH₂ (59–61). Given its participation in both early- and late-stage CoQ biosynthesis, one question that arises is the

molecular basis for its substrate specificity and regioselectivity. However, no such study has been performed to date.

Both human and yeast homologs of COQ3 contain several conserved motifs found in the seven- β -strand (class I) methyltransferase family, which provide the structural basis for the conserved Rossmann fold core (62,63). While no structures of human or yeast COQ3 are available, a model of yeast Coq3 was generated and used for the identification and validation of coevolved residues of functional importance (63). An AlphaFold model of human COQ3 was also analyzed, revealing eight α -helices and eight β -strands flanked by long disordered regions on both termini. Further insights into functional regions have been gained from apo and *S*-adenosyl-L-homocysteine (AdoHcy)-bound structures of the *O*-methyltransferase homolog UbiG from *Escherichia coli* (PDB: 4KDC and 5DPM) (64,65), which shares 34% sequence identity with human COQ3 and is part of a soluble ubiquinone biosynthetic complex (2,62). Members of the UbiG/COQ3 family contain a conserved hydrophobic stretch of ten residues that is thought to interact with the membrane. A model has been proposed in which a helix connected to the hydrophobic region controls cofactor entry to the active site upon membrane association (65).

Both human and yeast homologs associate with the CoQ synthome in their respective species and are thought to be required for maintaining synthome stability (22,23). Given their involvement in two steps in CoQ biosynthesis, the loss of COQ3 is extremely detrimental. The *coq3* Δ yeast mutant exhibits significantly reduced levels of Coq4, Coq6, Coq7, and Coq9 (28), as well as the loss of the Coq4- and Coq9-containing megadalton-sized polypeptide assembly representing the CoQ synthome (30). Partial knockdown of *COQ3* in the human 143B cell line has also been shown to result in a significant decrease in the levels of the homologous polypeptides (44). The *coq3* Δ yeast mutant accumulates the early polyprenylated intermediate HHB and HAB,

even under the overexpression of the atypical kinase Coq8, which enhances the stability of the Coq polypeptides in the absence of a complete synthome (29,30). Notably, subsequent studies showed that polypeptide levels of Coq3 were unaffected by *coq4–coq9* null mutants in the presence of phosphatase and protease inhibitors (29), suggesting that the dependence for stability is not mutual.

Coq3 is hypothesized to be a phosphorylation target of Coq8, as the detection of a phosphorylated form of Coq3 is dependent on expression of Coq8 (66). However, the significance of this phosphorylation event is not clear. While the phosphorylation of Coq3 by Coq8 has never been shown directly *in vitro* (67), recent studies using ancestral COQ polypeptides derived from ancestral sequence reconstruction demonstrated that COQ8B, a paralog of Coq8, phosphorylates COQ3 to enhance the catalytic efficiency of the COQ6:COQ3 subcomplex (68).

Interestingly, *COQ3* gene expression was found to be upregulated in esophageal squamous cell carcinoma and was associated with poor prognosis (69,70), suggesting that COQ3 may have a unique role in humans.

Human COQ4 (Yeast Coq4)

Human COQ4 and yeast Coq4 are integral members of the CoQ synthome and were previously believed to play a scaffolding role in the assembly of the synthome, helping to maintain the stability of other Coq polypeptides (26,28,29,71). Yeast lacking Coq4 accumulate HHB and HAB and do not form any detectable subcomplexes of the synthome (29,30). However, recent studies have identified COQ4 as the oxidative decarboxylase that converts the C1 carboxylate of HMPB to a hydroxyl group in 2-methoxy-6-polyprenyl-1,4-benzohydroquinone, also known as demethyl-demethoxy-QH₂ (DDMQH₂). This conversion is carried out in a single oxygen-dependent step, filling the longstanding gap in the CoQ biosynthetic pathway (33,72). Interestingly, *in vitro* studies on the ancestral COQ4 polypeptide demonstrated that it exhibits C1-

decarboxylation activity but not C1-hydroxylation, which requires ancestral COQ6 (68). The lack of sequence conservation between COQ4 and the *E. coli* functional equivalent, the UbiD/UbiX complex, further suggests that the catalytic function of these polypeptides and the exact sequence of steps may have undergone several rounds of evolution (2,33).

Prior to the elucidation of COQ4's catalytic function, it was known that the COQ4 homolog Alr8543 from *Nostoc sp.* (PDB: 6E12) contained long hydrophobic α -helices that may serve as a lipid-binding site, as well as a conserved Zn^{2+} -binding motif, HDXXH-(X)₁₀₋₁₃-E, which co-crystallized with two Mg^{2+} ions (71). However, the exact mechanism of oxidative decarboxylation and the identity of the liganded metal ion *in vivo* remain unknown. Nicoll *et al.* proposed that the O4' hydroxyl group of HMPB and the Lewis acidic properties of Zn^{2+} may facilitate this decarboxylation without the need of other cofactors (68). However, the way in which this mechanism fits in with the requirement of oxygen is unclear. Attempts to detect *in vitro* activity in the presence of various metal cations, cofactors, and soluble substrate analogs were unsuccessful (33), potentially due to the requirement of a lipid environment.

Unlike the other *COQ* genes, mutations in which are generally autosomal recessive, mutations in the *COQ4* gene can result in haploinsufficiency, as seen in yeast models and patients with *COQ4* mutations (73). Whether this phenotype is due to COQ4's catalytic activity or its scaffolding role remains unknown.

Human COQ5 (Yeast Coq5)

COQ5/Coq5 is an AdoMet-dependent C-methyltransferase responsible for the C5-methylation of DDMQH₂, forming 2-methoxy-6-polyprenyl-1,4-benzohydroquinone, or demethoxy-QH₂ (DMQH₂) (74–76). In isolated yeast mitochondria, this reaction requires the addition of NADH, which is hypothesized to keep the headgroup in a reduced state (25,75). The

catalytic mechanism of COQ5 has not been fully elucidated, although several residues implicated in substrate binding and catalysis have been reported (77). No *in vitro* activity assay has yet been reported for COQ5. A detailed discussion of the catalytic mechanism can be found in Chapter 3.

The structures of apo and AdoMet-bound yeast Coq5 have been solved (PDB: 4OBX and 4OBW), revealing its seven- β -strand Rossmann fold structure, conserved in class I methyltransferases. Several functional regions have been identified, including the AdoMet-binding and substrate-binding pockets, as well as the hydrophobic dimer interface (77). An AlphaFold model of human COQ5 further revealed an N-terminal α -helix not visible in the yeast crystal structure, which may act as a gate to the active site (62). Helix α 3 of human COQ5 is extended compared to that of the yeast polypeptide and reflects an insert found exclusively in vertebrate species (62). However, the residue conservation within this insert is low, and its functional significance remains unknown.

As with other integral members of the synthome, yeast Coq5 is required for the stability of other Coq polypeptides (25,28–30), while a partial knockdown of *COQ5* in human cells was shown to have the strongest effect on COQ7 (44). The *coq5* Δ yeast mutant is unable to form the Coq4- and Coq9-containing synthome and accumulates the early polyprenylated intermediates HHB and HAB. However, upon overexpression of the atypical kinase Coq8, DDMQ₆ can be formed, suggesting that the synthome may assemble in the absence of Coq5 under the cryptic activity of Coq8 (29,30).

Similar to the *O*-methyltransferase Coq3, the Coq5 polypeptide remains stable in the absence of other Coq polypeptides (28,29), suggesting that it may play a role in regulating the assembly of the synthome. Indeed, two mechanisms of post-transcriptional regulation have been identified for *COQ5* in yeast. The RNA binding protein Puf3 binds to the 3' untranslated region

of the *COQ5* mRNA to prevent the overaccumulation of Coq5, which is detrimental to yeast growth and leads to the accumulation of HHB and HAB (78). Furthermore, Coq5 polypeptide stability is enhanced by N-terminal cleavage via the mitochondrial processing protease (MPP) and the mitochondrial intermediate peptidase Oct1, which occurs after its import into the mitochondria (79). It has also been suggested that Coq5 is a phosphorylation target of Coq8, although this has not been confirmed *in vitro* and the effect of phosphorylation remains unclear (66).

Interestingly, the *E. coli* homolog of COQ5, UbiE, is also involved in the biosynthesis of menaquinone, a bulkier naphthoquinone that functions in anaerobic respiration (80). Both human and yeast COQ5 were shown to convert demethylmenaquin(ol)-8 (DMK₈(H₂)) to menaquin(ol) (MK₈(H₂)) when expressed in an *E. coli ubiEΔ* mutant, despite neither human nor yeast naturally synthesizing MK (81). Several distant homologs of COQ5 have evolved to catalyze the methylation of a variety of quinones involved in anaerobic respiration and photosynthesis, and the targeted expression of a homolog with incorrect substrate specificity may be toxic (81). However, the residues conferring substrate specificity in these homologs have not been elucidated.

Human COQ6 (Yeast Coq6)

COQ6/Coq6 is a flavin-dependent monooxygenase responsible for the first head-group modification step after attachment of the polyprenyl tail by COQ2, catalyzing the conversion of 3-polyprenyl-4-hydroxybenzoic acid (HPB) to DHPB (34,35). In yeast, Coq6 also performs C4-deamination when *p*ABA is used as a precursor (72). The yeast enzyme co-purifies with a tightly bound FAD cofactor (82), which receives electrons indirectly from NADPH via ferredoxin (Yah1) and ferredoxin reductase (Arh1) (18,83). However, the human ferredoxin homologs FDX1 and FDX2, as well as the ferredoxin reductase homolog FDXR, have not been implicated in coenzyme Q biosynthesis. Several functional regions in COQ6 have been characterized. The FAD-binding

motifs 1 and 2, also referred to as the ADP-binding fingerprint (GxGxxG) and the ribityl-binding motif (GDAXH), respectively, are well conserved between COQ6 and the *E. coli* CoQ biosynthetic flavin-dependent monooxygenases UbiF, UbiH, and UbiI (34,62,82,84). A third region containing a conserved aspartate residue was initially hypothesized to recognize NAD(P)H but was later shown, in a yeast Coq6 homology model, to interact with the adenine and pyrophosphate moiety of FAD (34,82).

COQ6 is peripherally associated with the inner mitochondrial membrane and is an integral member of the CoQ synthome (22,23,34). Human COQ6 can rescue the yeast *coq6Δ* mutant, underscoring their functional conservation. Like other yeast null mutants, *coq6Δ* mutant yeast accumulates HHB and HAB. However, its effects on the levels of other Coq polypeptides and on synthome assembly appear to be comparatively limited (28–30), with partial synthome assembly observed even in its complete absence. Overexpression of the atypical kinase Coq8 or expression of a stable but inactive Coq6 polypeptide in yeast leads to the accumulation of 3-hexaprenyl-4-hydroxyphenol (4-HP₆, Figure 3) or 3-hexaprenyl-4-aminophenol (4-AP₆), suggesting that when synthome assembly is efficient, oxidative decarboxylation by Coq4 can precede hydroxylation (29,35). The equivalent intermediate, 4-HP₁₀, has also been observed in human cells harboring a deletion affecting the FAD-binding site (36), further contributing to the uncertainty regarding the preferred sequence of reactions *in vivo*.

Both yeast and human homologs interact with the atypical kinase in their respective species—Coq8 in yeast and COQ8B in humans (22,85). This interaction is further supported by studies on ancestral COQ polypeptides, in which COQ8B enhances the catalytic activity of COQ6 within the COQ6:COQ3 subcomplex (68). However, since COQ6 was not previously proposed to

be a phosphorylation target of COQ8, the functional significance of this interaction remains unclear and continues to warrant further investigation.

Human COQ7/CAT5/CLK-1 (Yeast Coq7/Cat5)

Human COQ7 and yeast Coq7 are part of the di-iron carboxylate hydroxylase family, catalyzing the penultimate step of CoQ biosynthesis by converting DMQ(H₂) to DMeQ(H₂) (86–88). COQ7 is unique to eukaryotes, as the prokaryotic functional homolog UbiF uses flavin-dependent chemistry similar to COQ6 (84). The hydroxylation activity of COQ7 relies on a carboxylate-bridge di-iron binding motif, which accepts electrons from NADH via the substrate DMQ, subsequently activating O₂ (89,90). A consequence of this mechanism is the requirement that the substrate be in the oxidized benzoquinone form, DMQ, which was observed *in vitro* for ancestral COQ7 (68). Purified recombinant human COQ7 can utilize substrate analogs DMQ₀ and DMQ₂ as substrates (90), indicating that the polyprenyl tail is not required for substrate recognition. Experiments in murine cell cultures further demonstrated that, in addition to Fe²⁺, the enzyme can utilize Co²⁺ to carry out its hydroxylation activity, whereas Mn²⁺ inhibits it (91).

COQ7 functions closely and forms physical interactions with the lipid-binding protein COQ9 (92–94). Cryo-EM structures of hetero-octamers consisting of 1:1 ratios of the apo or NADH-bound human COQ7 with its binding partner COQ9 have been determined (PDB: 7SSP and 7SSS) (24). Consistent with a prior structural model of *Pseudomonas aeruginosa* Coq7 (88), COQ7 consists of a four-helical bundle core. Two additional helices, α 3 and the C-terminal helix α 6, help form a hydrophobic tunnel with parts of the helical bundle, allowing entry of the quinone substrate. A nearby water-filled channel binds NADH, which may be shared between several heterodimers of COQ7:COQ9 (24). Structural evidence further supports the hypothesis that COQ9 facilitates the delivery of the quinone substrate to COQ7. A molecular dynamics simulation of the

subcomplex suggests that the heterotetrameric arrangement of (COQ7)₂:(COQ9)₂ may facilitate the local deformation of the lipid bilayer to extract CoQ intermediates (24).

Yeast Coq7 is a member of the CoQ synthome and is peripherally associated with the matrix face of the IMM (30). Loss of Coq7 results in accumulation of HHB and HAB, as well as the total absence of the CoQ synthome (29,30). This is partially rescued by the overexpression of Coq8, which restores the levels of other Coq polypeptides and the presence of a high-molecular-weight complex containing Coq4, and results in the formation of the diagnostic intermediate and Coq7 substrate, DMQ₆ (29,30,95). However, comigration of Coq9 with the complex is not observed, suggesting that Coq7 is required to mediate the interaction between Coq9 and the synthome (30).

The Coq8-dependent phosphorylation of Coq7 in yeast has been shown to decrease CoQ₆ content (22,66,96), indicating that Coq7 is a control point in CoQ biosynthesis. Coq7 is dephosphorylated by Ptc7, a mitochondrial serine/threonine protein phosphatase (97). The human homolog PPTC7, however, has not been linked to CoQ biosynthesis. Furthermore, the kinase directly responsible for the downregulation of Coq7 has not been identified.

Human COQ8A/ADCK3 and COQ8B/ADCK4 (Yeast Coq8)

Yeast Coq8 and its human homologs COQ8A and COQ8B are members of the UbiB protein kinase-like (PKL) family (98). To date, the function of this family of proteins remains elusive. A low-level ATPase activity essential for CoQ biosynthesis (99) and a dispensable autophosphorylation activity (67,98) have been observed for human COQ8A. However, while the expression of yeast Coq8 or human COQ8A restores the phosphorylation state of Coq3, Coq5, and Coq7 in yeast (66), no phosphopeptides from COQ3, COQ5, or COQ7 were detected in wild-type or *Coq8a*^{-/-} mice (67). Furthermore, protein kinase activity in *trans* has never been observed for

any UbiB family member. Thus, the mechanism by which COQ8's ATPase activity promotes CoQ biosynthesis remains unknown.

Compared to canonical kinases, the UbiB family kinases possess an additional N-terminal extension and an insert within the N-lobe harboring conserved KxGQ and ExD motifs, respectively (98). Structural studies on mature human COQ8A (PDB: 4PED and 5I35) revealed that the KxGQ domain occludes the substrate-binding site found in canonical protein kinases (98). Upon ATP binding, two hydrophobic pockets are formed in the KxGQ domain (67), which may bind putative small-molecule substrates or allosteric effector molecules such as isoprenoids or lipids. An alanine-rich loop replaces the canonical glycine-rich loop for nucleotide binding, resulting in a preference for ADP over ATP and the inhibition of autophosphorylation activity (98). The ExD motif forms a salt bridge with the KxGQ motif, potentially assembling a catalytic triad (98). However, this motif clashes with the putative phosphoryl acceptor binding site present in canonical kinases, which may explain the lack of kinase activity in *trans*.

Both yeast and human homologs of COQ8 associate with the synthome in their respective species (22,23). However, this interaction is thought to be dynamic (67). COQ8 is required for CoQ biosynthesis and the maintenance of COQ polypeptide levels in yeast and mice (28,29,66,67). Overexpression of yeast Coq8 in several *coq* mutants also promotes the assembly of the CoQ synthome (66,67). In addition to its interactions within the synthome, COQ8 is thought to make extensive contacts with the lipid bilayer. Both human and yeast homologs contain a putative transmembrane helix (98), and human COQ8A and yeast Coq8 have been shown to bind liposomes (67). Recent studies further revealed that human COQ8A and yeast Coq8 are activated by cardiolipin, which is proposed to interact with the conserved KxGQ domain and a nearby membrane-interacting helix (99). Interestingly, phenolic compounds have also been found to

activate COQ8A and yeast Coq8 (99). Therefore, one model of COQ8 function is that it interacts with cardiolipin-rich membranes and extracts CoQ precursors from the membrane through its ATPase activity, thereby promoting the assembly of the CoQ synthome (99).

Interestingly, two other UbiB family proteins in yeast, Cqd1 and Cqd2/Mcp2, reside on the opposite face of the inner mitochondrial membrane, facing the intermembrane space (100,101). These proteins have been shown to reciprocally regulate the mitochondrial export of CoQ in an ATPase domain-dependent manner (100). Cqd1 has been further implicated in the formation of a contact site between the outer and inner mitochondrial membranes, mediating the formation of endoplasmic reticulum (ER)–mitochondria contacts and phospholipid homeostasis (102). However, the function of the human homologs of Cqd1 (ADCK2) and Cqd2 (ADCK1/5) has not been investigated. Finally, another Coq8 paralog in yeast, named Coq21, was recently found to co-purify with the CoQ synthome (103). A deeper understanding of these paralogs may provide clues to the mechanism of COQ8 function.

Human COQ9 (Yeast Coq9)

COQ9/Coq9 is a lipid-binding protein that is believed to lack any enzymatic function. However, its presence is required for CoQ biosynthesis, maintenance of the levels of other Coq polypeptides, and assembly of the CoQ synthome (28,29,94,104). It functions in tandem with COQ7 and is hypothesized to present CoQ intermediates to COQ7 (24,92–94). Yeast *coq9Δ* mutants overexpressing Coq8 accumulate DMQ₆, suggesting that Coq7 is inactive in the absence of Coq9 (29). A functional relationship between Coq9 and Coq6 also exists, as these mutants additionally accumulate 4-HP₆. When yeast *coq9Δ* mutants are fed *p*ABA, the aminated intermediates 4-AP₆ and imino-demethoxy-Q₆ (IDMQ₆) accumulate, suggesting that yeast Coq9 modulates the C4-deamination performed by Coq6 (105). Meanwhile, human COQ9 appears to

lack this function, as the expression of human COQ9 in the temperature-sensitive yeast mutant *coq9-ts19* enhances CoQ₆ biosynthesis from 4-HB, but not from *p*ABA (106).

Crystallographic studies (PDB: 4RHP, 6AWL, and 6DEW) and computational simulations of human COQ9 revealed a lipid-binding pocket and an exposed tryptophan that mediates interaction with the aromatic head group of quinone substrates, as well as with COQ7 (92,94). A C-terminal amphipathic helix facilitates the binding to and deformation of cardiolipin-rich membranes and may control access to the lipid-binding pocket (94).

Human COQ10A and COQ10B (Yeast Coq10)

Yeast Coq10 (human homologs COQ10A and COQ10B) is present on the matrix face of the inner mitochondrial membrane and does not associate with the CoQ synthome (107,108). While Coq10 is not considered essential for CoQ biosynthesis, the deletion of *COQ10* in yeast results in defects in respiration, impairments in *de novo* CoQ₆ biosynthesis, destabilization of the CoQ synthome, and a reduction in the number of mitochondrial CoQ biosynthetic domains (31,107–110). Coq10 homologs contain a steroidogenic acute regulatory protein-related lipid transfer (START) domain that binds CoQ and CoQ intermediates (20,108–111). Combined with the respiratory defects seen in *coq10Δ* yeast, current evidence suggests that Coq10 may act as a CoQ chaperone delivering CoQ to respiratory complexes. Curiously, the expression of human COQ10A or COQ10B rescues the respiratory growth of *coq10Δ* yeast but fails to restore *de novo* CoQ biosynthesis and synthome assembly, suggesting that more than one mechanism is at play (108). Indeed, a recent study using nonfunctional yeast *coq10* mutants showed that replacing the *COQ10* gene with select auxotrophic markers may interfere with the expression of *MDM12* (112). *MDM12* encodes a cytosolic component of a yeast-specific ER-mitochondria contact site, the ER-mitochondria encounter structure (ERMES) (113), and is co-expressed with *COQ10* via a shared

promoter (112). Since ERMES was previously shown to colocalize with CoQ biosynthetic polypeptides and spatially coordinate the formation of CoQ domains (31,114), current evidence suggests that only the respiratory defects can be attributed to the loss of Coq10, consistent with the phenotypes observed in the rescue of *coq10Δ* yeast by human COQ10A and COQ10B.

Yeast Coq11

Coq11 is a recently identified component of the yeast CoQ synthome (22). Deletion of *COQ11* in yeast impairs *de novo* CoQ₆ biosynthesis but does not affect the respiratory growth of the *coq11Δ* mutant (21,22). Interestingly, Coq11 appears to act as a negative modulator of CoQ synthome assembly, as the *coq11Δ* mutant exhibits elevated Coq polypeptide content and an enlarged CoQ synthome (21,31). Furthermore, subsequent deletion of *COQ11* in the *coq10Δ* mutant and select ERMES mutants rescues their respiratory growth defect (21,112,115). However, this effect does not appear to be mediated by restoration of *de novo* CoQ₆ biosynthesis, as the subsequent deletion of *COQ11* in the *coq10Δ* mutant, the nonfunctional *coq10-R147** mutant, or the ERMES *mmm1Δ* mutant result in either similar or less efficient CoQ₆ biosynthesis compared to the respective single mutants (112,115). Instead, these results may point to alternative mechanisms such as altered CoQ₆ spatial distribution or oxidation state.

Coq11 is a member of the short-chain dehydrogenase/reductase (SDR) superfamily (22), which contains a nucleotide-binding Rossmann fold structural motif (116). However, the catalytic function of Coq11 has yet to be characterized. The closest human homolog of Coq11 is NDUFA9 (22), a structural component of the mitochondrial respiratory complex I that is not known to have any catalytic activity (117,118). Given NDUFA9's essential role in complex I assembly (119), its involvement in CoQ biosynthesis in humans is difficult to evaluate. Coq11 also interacts with the mitochondrial organization of gene expression (MIOREX) complex, which consists of

mitoribosomes and factors involved in mitochondrial post-transcriptional gene expression (120). Since many components of the respiratory complexes are encoded in the mitochondrial genome, this dual function may allow Coq11 to coordinate CoQ biosynthesis with respiration.

Other polypeptides implicated in coenzyme Q biosynthesis

One recently identified regulator of CoQ biosynthesis is the human reticulon 4-interacting protein 1 (RTN4IP1), a mitochondrial matrix protein with NAD(P)H-dependent quinone oxidoreductase activity (121). TurboID interactome profiling revealed COQ3 and COQ5 as RTN4IP1 interactors, implicating it in CoQ biosynthesis. Knockout of RTN4IP1 reduced CoQ levels in both mammalian cell culture and *Drosophila*, an effect at least partly explained by its ability to enhance the *O*-methyltransferase activity of COQ3 in the presence of NAD(P)H. However, its effect on COQ5 activity has not been examined. Notably, both methyltransferases are thought to act on reduced hydroquinone substrates (25,75,122). It is therefore tempting to think that RTN4IP1 may function as the *in vivo* reductase of DDMQ and DMeQ. Furthermore, both RTN4IP1 and its yeast homolog Yim1 have been shown to mediate resistance to oxidative stress (121,123), though a direct role for Yim1 in CoQ biosynthesis has yet to be established.

PRIMARY COENZYME Q DEFICIENCY

Given the multifaceted roles of CoQ, deficiencies in this molecule—particularly those arising from defects in its biosynthesis—can have profound consequences on human health. Mutations in the genes directly involved in CoQ biosynthesis cause primary CoQ₁₀ deficiency, a rare autosomal recessive disorder. By 2023, more than 300 patients had been described in the literature (124), with an estimated global prevalence of ~120,000 affected individuals (125). Human patients with primary CoQ₁₀ deficiency exhibit striking variability in both clinical presentation and age of onset (126), likely reflecting differences in genetic background, tissue-

specific expression, and the extent of functional disruption. Commonly affected systems include the central nervous system (CNS), heart, peripheral nervous system (PNS), kidneys, and skeletal muscle, although less frequent manifestations can involve the lungs, liver, thyroid, and broader metabolic or cardiovascular pathways (127).

Therapeutic supplementation with CoQ₁₀, the native human form, has shown inconsistent efficacy (124). Treatment must begin early, as organ damage is irreversible, yet the strong hydrophobicity of CoQ hampers uptake, resulting in low bioavailability and uneven tissue distribution (3,124,126). Moreover, receptor-mediated uptake mechanisms remain poorly understood (128). These challenges highlight the importance of understanding the enzymatic and structural components of the CoQ biosynthetic pathway, which could inform the development of more effective therapeutic strategies.

Among the polypeptides implicated in CoQ biosynthesis, clinical data remain especially sparse for two methyltransferases, COQ3 and COQ5, as well as the CoQ chaperones COQ10A and COQ10B. To date, no patients with primary CoQ₁₀ deficiency associated with COQ3 and COQ10A/B have been identified, and only seven cases associated with COQ5 have been reported in the literature (129–132). Nevertheless, genetic variants in *COQ3* and *COQ5* predicted to be pathogenic have been detected in humans (62,125), and polymorphisms in *COQ10A* and *COQ10B* have been associated with increased susceptibility to statin-associated myopathy (133). These gaps highlight the need for further investigation into the function and clinical relevance of these understudied CoQ biosynthetic polypeptides.

OTHER QUINONES IN BIOLOGY

Other prenylquinones are found in electron transport systems of photosynthetic or anaerobic organisms (Figure 5). While CoQ, with a reduction potential $E^{\circ'}$ of +100 mV, is well

suited as a midpoint carrier for electron transport to oxygen, alternative terminal acceptors like fumarate require carriers with much lower reduction potentials (134). Menaquinone (MK; $E^{\circ'} = -74$ mV) is found in archaea and many bacterial species and is believed to have evolved in lower oxygen environments before the emergence of CoQ (134). *E. coli* synthesizes both CoQ and MK, providing redundancy and enabling adaptation to fluctuating oxygen availability (135,136). Notably, *E. coli* uses the same C-methyltransferase, the COQ5 homolog MenG/UbiE, for both MK and CoQ biosynthesis, providing a key point of regulation (80). Similarly, many organisms living in low-oxygen or anaerobic environments possess rhodoquinone (RQ; $E^{\circ'} = -63$ mV), including certain nematodes, mollusks, protists, and bacteria (137). In contrast, plastoquinone and phyloquinone serve as electron carriers in the photosynthetic electron transport chains of plants, algae, and cyanobacteria (134,138,139).

Several of these prenylquinones are also relevant to human health. Both menaquinone and the structurally related phyloquinone are forms of vitamin K, an essential nutrient for humans required for blood coagulation and bone metabolism (140,141). Vitamin K deficiency, although rare, can lead to bleeding and increased risk of bone fractures, and may arise secondary to other conditions such as malabsorption, liver disease, and cholestasis, or the use of vitamin K antagonist oral anticoagulants (140,141). In humans, dietary phyloquinone can be converted to menaquinone via the UbiA prenyltransferase containing 1 (UBIAD1) (142), resulting in menaquinone enrichment in the brain, kidney, and pancreas (143). Disorders associated with mutations in UBIAD1 are further complicated by an additional role of this enzyme in the regulating cholesterol metabolism (144–146).

Taken together, these examples illustrate the diversity of quinones in nature, ranging from bacterial cofactors in energy production to essential human nutrients. This broader context underscores the unique position of CoQ as the central quinone in human bioenergetics and health.

CONCLUSION

Together, these perspectives on CoQ biology—from its essential redox functions to the diverse enzymatic steps of its biosynthetic pathway and the pathologies arising from its deficiency—underscore both the complexity and the biomedical importance of this molecule. Having established this foundation, the remainder of this work turns to a more focused analysis of CoQ biosynthesis at the molecular level. Chapter 2 surveys human missense single nucleotide variants in genes encoding the core members of the CoQ synthome, with an emphasis on those of potential clinical significance. Chapter 3 narrows in on the *C*-methyltransferase Coq5, probing how mutations in its substrate-binding pocket inform our understanding of its catalytic mechanism. Chapter 4 extends this inquiry by examining the role of the Coq5 dimer interface in enzymatic activity. Together, these studies provide new insights into how individual residues, structural features, and genetic variation shape CoQ biosynthesis, with particular relevance for understanding primary CoQ deficiency linked to mutations in the *COQ5* gene.

FIGURES

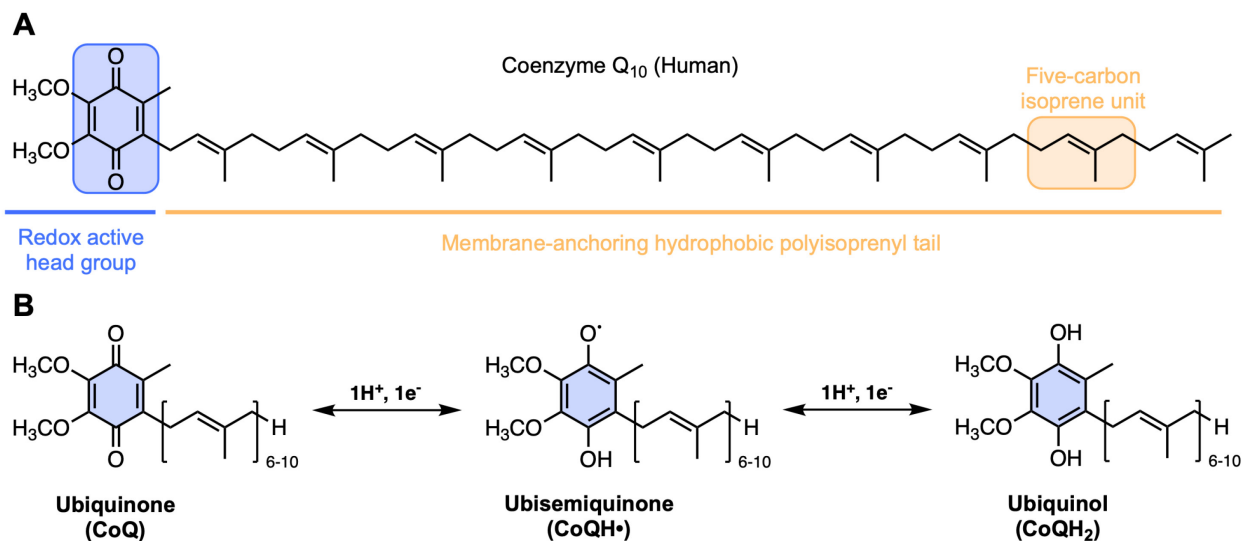


Figure 1. Coenzyme Q structure and reactivity. (A) Coenzyme Q (CoQ), or ubiquinone, contains a redox-active head group and a membrane-anchoring polyisoprenyl tail ranging from 6–10 isoprene units. The hydrophobicity of its tail positions it within the midplane of the lipid bilayer. The ten-isoprene unit human isoform is pictured. (B) CoQ can undergo two sequential single-electron reductions to form ubiquinol (CoQH₂) via a ubisemiquinone radical.

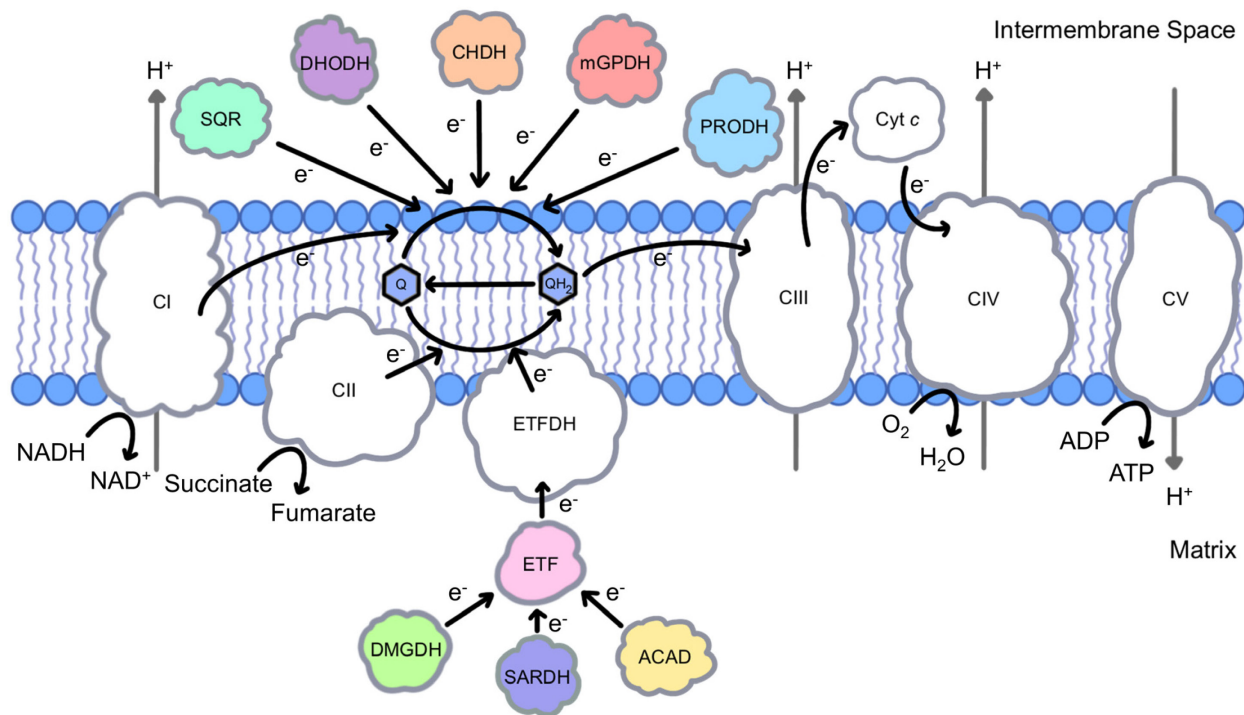


Figure 2. CoQ in the electron transport chain. Mitochondrial CoQ accepts electrons from respiratory complexes I and II, as well as dehydrogenases involved in a variety of metabolic pathways, and passes them to complex III. Black arrows accompanied by (e⁻) indicate the direction of electron flow. Grey arrows denote the flow of protons. Diagram is not drawn to scale or to indicate stoichiometry. Figure adapted from Guile and Jain *et al.* (128). Abbreviations are listed in alphabetical order: ACAD, acyl-CoA dehydrogenase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CHDH, choline dehydrogenase; CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; CV, complex V; Cyt *c*, cytochrome *c*; DHODH, dihydroorotate dehydrogenase; DMGDH, dimethylglycine dehydrogenase; ETF, electron transfer flavoprotein; ETFDH, electron transfer flavoprotein dehydrogenase; SQR, sulfide:quinone oxidoreductase; mGPDH, mitochondrial glycerol-3-phosphate dehydrogenase; NAD⁺/NADH, nicotinamide adenine dinucleotide; PRODH, proline dehydrogenase; SARDH, sarcosine dehydrogenase.

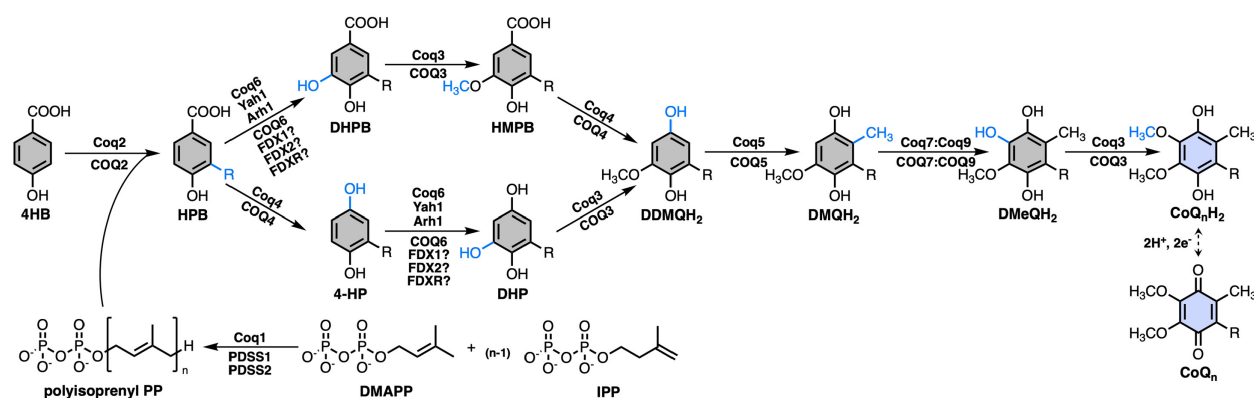


Figure 3. The CoQ biosynthetic pathway in yeast and humans. The CoQ biosynthetic pathways in the yeast *S. cerevisiae* (polypeptides labeled above arrows) and humans (below arrows) are highly conserved. 4-hydroxybenzoic acid (4-HB) is the common aromatic CoQ precursor in yeast and humans. The order of enzymatic steps remains an open debate due to conflicting evidence from knockout and mutagenesis studies. Intermediates pictured in the pathway include: DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; HPB, 3-polyprenyl-4-hydroxybenzoic acid; 4-HP, 4-hydroxy-2-polyprenylphenol; DHPB, 4,5-dihydroxy-3-polyprenylbenzoic acid; HMPB, 4-hydroxy-5-methoxy-3-polyprenylbenzoic acid; DHP, 4,5-dihydroxy-3-polyprenylphenol; DDMQH₂, 2-methoxy-6-polyprenyl-1,4-benzohydroquinone; DMQH₂, 2-methoxy-5-methyl-6-polyprenyl-1,4-benzohydroquinone; DMeQH₂, 3-methyl-6-methoxy-2-polyprenyl-1,4,5-benzenetriol. R represents the polyprenyl tail (n = 6 in yeast, n = 10 in humans).

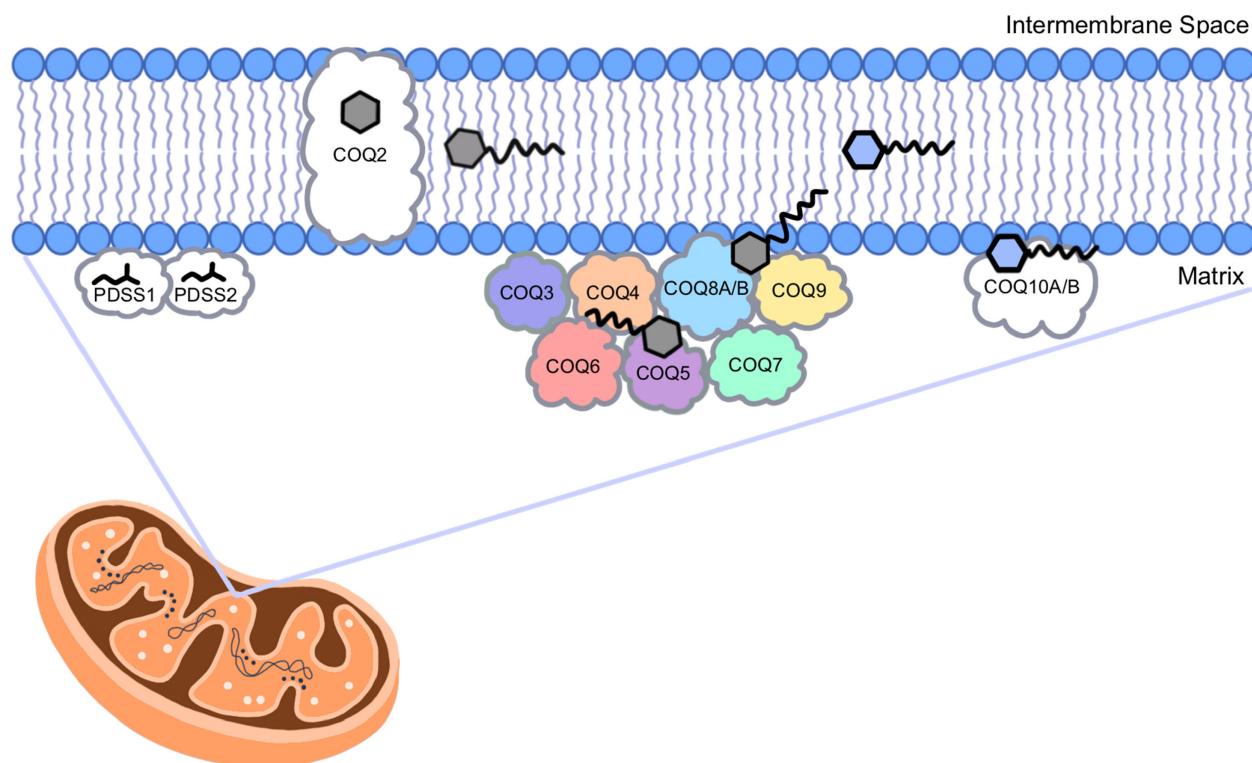


Figure 4. The CoQ biosynthetic metabolon in humans. Human polypeptides involved in CoQ biosynthesis are shown. Colored proteins indicate components of the multi-subunit biosynthetic complex referred to as the CoQ synthome (or Complex Q in humans). The PDSS proteins (homologous to yeast Coq1), COQ2 (homologous to yeast Coq2), and the CoQ chaperone COQ10A and COQ10B (homologous to yeast Coq10) function outside of the synthome. Both human COQ8A/COQ8B, along with their yeast homolog Coq8, are dynamically associated members of the synthome with undefined catalytic roles. In yeast, the synthome also contains Coq11, which has no close human homolog. Grey hexagons denote CoQ biosynthetic intermediates, and the final CoQ product is shaded in blue. Diagram is not drawn to scale or stoichiometry.

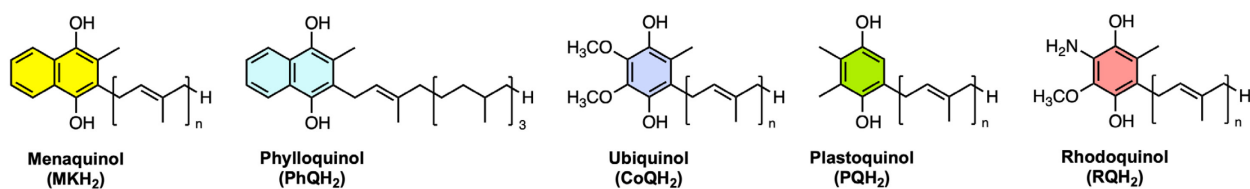


Figure 5. Other quinones found in biology. The reduced forms of biologically relevant quinones involved in electron transfer are shown. Phylloquinone and plastoquinone are found in photosynthetic organisms such as cyanobacteria, algae, and plants. Menaquinone serves as the primary electron carrier in many bacteria living in anaerobic conditions. Rhodoquinone independently evolved in diverse lineages spanning animals, protists, and prokaryotes living in anaerobic conditions.

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Chapter 2

Predicting and understanding the pathology of single nucleotide variants in human *COQ* genes

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Wang, S., Jain, A., Novales, N. A., Nashner, A. N., Tran, F., and Clarke, C. F. (2022) Predicting and Understanding the Pathology of Single Nucleotide Variants in Human *COQ* Genes. *Antioxidants* 11, 2308. doi: 10.3390/antiox11122308.

Article

Predicting and Understanding the Pathology of Single Nucleotide Variants in Human *COQ* Genes

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Abstract: Coenzyme Q (CoQ) is a vital lipid that functions as an electron carrier in the mitochondrial electron transport chain and as a membrane-soluble antioxidant. Deficiencies in CoQ lead to metabolic diseases with a wide range of clinical manifestations. There are currently few treatments that can slow or stop disease progression. Primary CoQ₁₀ deficiency can arise from mutations in any of the *COQ* genes responsible for CoQ biosynthesis. While many mutations in these genes have been identified, the clinical significance of most of them remains unclear. Here we analyzed the structural and functional impact of 429 human missense single nucleotide variants (SNVs) that give rise to amino acid substitutions in the conserved and functional regions of human genes encoding a high molecular weight complex known as the CoQ synthome (or Complex Q), consisting of the *COQ3*–*COQ7* and *COQ9* gene products. Using structures of CoQ polypeptides, close homologs, and AlphaFold models, we identified 115 SNVs that are potentially pathogenic. Further biochemical characterizations in model organisms such as *Saccharomyces cerevisiae* are required to validate the pathogenicity of the identified SNVs. Collectively, our results will provide a resource for clinicians during patient diagnosis and guide therapeutic efforts toward combating primary CoQ₁₀ deficiency.

Keywords: coenzyme Q; ubiquinone; primary CoQ deficiency; mitochondrial disease; single nucleotide variants; Missense3D; *COQ* genes



Citation: Wang, S.; Jain, A.; Novales, N.A.; Nashner, A.N.; Tran, F.; Clarke, C.F. Predicting and Understanding the Pathology of Single Nucleotide Variants in Human *COQ* Genes. *Antioxidants* **2022**, *11*, 2308. <https://doi.org/10.3390/antiox11122308>

Academic Editor: Rafael Artuch

Received: 25 October 2022

Accepted: 14 November 2022

Published: 22 November 2022

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1. Introduction

Coenzyme Q (CoQ), also known as ubiquinone, is a redox-active lipophilic molecule required for cellular respiration. Its structure consists of a tetra-substituted benzoquinone ring and includes a lipid-anchoring polyisoprenyl tail of variable unit-length (CoQ_n, *n* = ten isoprene units in humans, CoQ₁₀) [1]. In the mitochondria, CoQ functions as an electron carrier in metabolic processes, such as oxidative phosphorylation, fatty acid β -oxidation, and choline metabolism [1,2]. In its oxidized form, CoQ accepts electrons from Complex I, Complex II, and many other mitochondrial dehydrogenases, producing its reduced form, ubiquinol (CoQH₂), which donates electrons and protons to Complex III [1–3]. In addition, CoQH₂ serves as a general lipophilic antioxidant [4–6] that has been implicated in protection from lipid autooxidation [7,8], and regeneration of vitamin E [9], another small molecule antioxidant. More recently, CoQH₂ has also been identified as having a role in ferroptosis suppression [10,11].

In humans, the biosynthesis of CoQ begins with 4-hydroxybenzoic acid (4-HB), a tyrosine derivative [12] (Figure 1A). The bulk of CoQ biosynthesis occurs in the mitochondrial matrix, where at least thirteen nuclear-encoded CoQ polypeptides (PDSS1, PDSS2, COQ2–COQ7, COQ8A (ADCK3), COQ8B (ADCK4), COQ9, COQ10A, and COQ10B) reside in or are peripherally associated with the inner mitochondrial membrane (IMM) [13] (Figure 1B). In addition, ferredoxin Yah1 (human homolog FDX1 and FDX2) and ferredoxin reductase Arh1 (human homolog FDXR) from *Saccharomyces cerevisiae* have been shown

to be important for CoQ biosynthesis through the monooxygenation step performed by Coq6, although the involvement of their human homologs in CoQ biosynthesis has not been demonstrated [14]. The PDSS1 and PDSS2 polypeptides catalyze the formation of the decaprenyl diphosphate tail characteristic of CoQ₁₀, and COQ2 attaches the tail to 4-HB [15]. The PDSS1/PDSS2 complex and the COQ2 polypeptides appear to work independently. Polypeptides COQ3–COQ7 and COQ9 assemble into a high molecular weight complex, termed the CoQ synthome (or complex Q), which has been confirmed by mass spectrometry [16] and co-purification methods [17–20]. COQ8A and COQ8B are reported to have dynamic interactions with the remaining protein components [16]. Similarly in yeast, while the Coq8 polypeptide is necessary for CoQ synthome assembly [21,22] and co-purified with the Coq6 polypeptide [23], fluorescence microscopy of yEGFP-tagged Coq8 reveals the protein is dispersed throughout the mitochondria, forming few domain-like patterns, unlike the remaining polypeptides in the core complex that specifically reside in discrete puncta [24]. In addition to protein components, this CoQ synthome in yeast and complex Q in human cells also contains lipid intermediates and small molecule ligands [23–27].

Mutations in the genes encoding COQ polypeptides lead to primary CoQ₁₀ deficiency, as they directly affect the biosynthesis of CoQ. This rare condition is typically caused by autosomal recessive mutations and often results in highly variable clinical manifestations, ranging from isolated pathologies, such as in the kidneys or central nervous system (CNS), to a fatal multi-system disorder [28–30]. The high variability may be attributed to discrepancies in genetic backgrounds, variable tissue-specific expression levels, and a varying degree of pathogenicity for each mutation. Systems and tissues generally impacted by primary CoQ₁₀ deficiencies include the CNS, heart, peripheral nervous system (PNS), kidneys, and muscles. Some rarer manifestations can involve the lungs, liver, thyroid, and more general metabolic or cardiovascular disorders [28,29]. To date, there are approximately 200 patients with primary CoQ₁₀ deficiency described in the literature, spanning mutations in ten of fourteen genes encoding COQ polypeptides [28,29]. Additionally, secondary CoQ₁₀ deficiencies can result from aging or treatment with statins and have also been linked to mutations in genes such as *APTX* (aprataxin), *BRAF* (B-Raf), *ETFDH* (mitochondrial electron transfer flavoprotein-ubiquinone oxidoreductase), and *MUT* (methylmalonyl-CoA mutase), that are not directly linked to the biosynthesis of CoQ₁₀ [1]. Currently, the only treatment for CoQ₁₀ deficiency is exogenous CoQ₁₀ supplementation. However, therapeutic benefits of exogenous supplementation are limited due to the extreme hydrophobicity and low bioavailability of CoQ₁₀ [31]. Supplementation with CoQ₁₀ may slow or stop the progression of disease but cannot reverse the damage already incurred in the patient [30]. In cases of early intervention in patients diagnosed with CoQ₁₀ deficiency-associated nephropathy, CoQ₁₀ supplementation has been shown to be remarkably effective in resolving the nephrotic syndrome, underscoring the significance of prompt intervention [32].

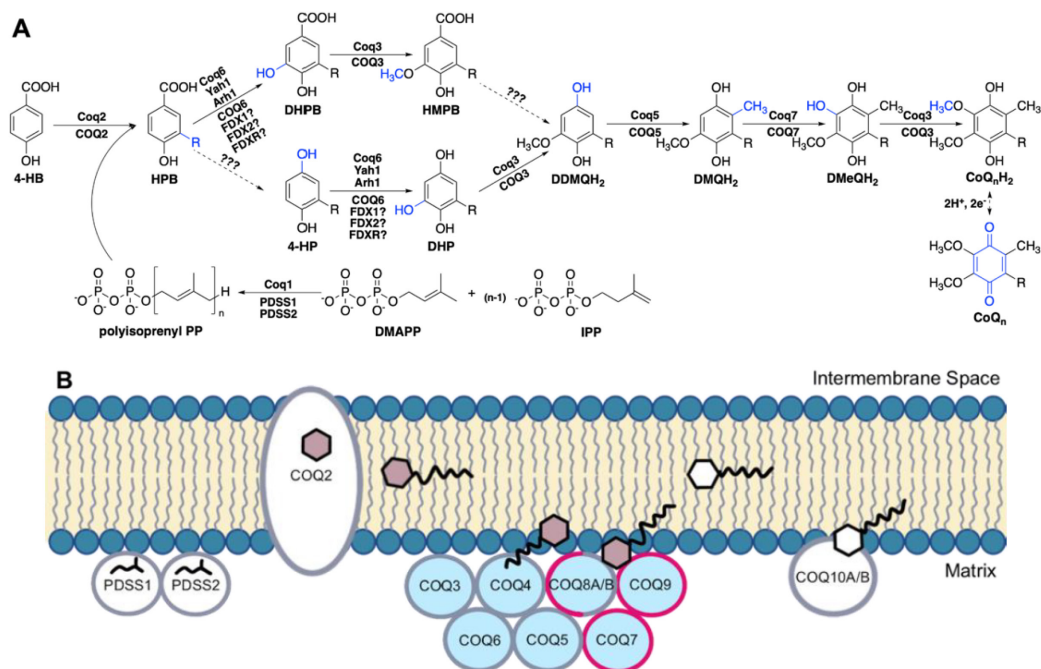


Figure 1. Biosynthesis of coenzyme Q. **(A)** The CoQ biosynthetic pathway is largely homologous between *S. cerevisiae* (polypeptide names above arrows) and humans (polypeptide names below arrows). In humans, at least seven nuclear-encoded catalytic proteins are directly responsible for the biosynthesis of CoQ from 4-hydroxybenzoic acid (4-HB), a tyrosine derivative. The enzyme(s) responsible for the decarboxylation and hydroxylation step(s) (dashed arrows) at ring position 1 has not been found. Hence, there is uncertainty about the order of steps. The decarboxylation step may precede the Coq6/COQ6 hydroxylation step based on the accumulation of 4-hydroxy-3-polyprenylphenol (4-HP) in yeast and human cells harboring mutations in Coq6/COQ6 data from [14,33,34]. In addition to the yeast Coq and human COQ polypeptides, other polypeptides involved in CoQ biosynthesis include PDSS1 and PDSS2 (decaprenyl diphosphate synthase subunits 1 and 2), Yah1 (yeast ferredoxin), Arh1 (yeast ferredoxin reductase), FDX1 and FDX2 (human ferredoxins 1 and 2), and FDXR, human ferredoxin reductase. Intermediates in the pathway include: DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; HPB, 3-polyprenyl-4-hydroxybenzoic acid; DHPB, 4,5-dihydroxy-3-polyprenylbenzoic acid; HMPB, 4-hydroxy-5-methoxy-3-polyprenylbenzoic acid; DHP, 4,5-dihydroxy-3-polyprenylphenol; DDMQH₂, 2-methoxy-6-polyprenyl-1,4-benzohydroquinone; DMQH₂, 2-methoxy-5-methyl-6-polyprenyl-1,4-benzohydroquinone; DMeQH₂, 3-methyl-6-methoxy-2-polyprenyl-1,4,5-benzenetriol. Note that the intermediates found in *S. cerevisiae* contain a hexaprenyl tail, while humans make decaprenylated CoQ₁₀ intermediates. **(B)** The CoQ synthome (Complex Q in humans) is a high-molecular mass protein and lipid complex data from [1], consisting of polypeptides COQ3-COQ9. The PDSS proteins (homologous to *S. cerevisiae* Coq1), COQ2, and the lipid-binding proteins COQ10A and COQ10B do not associate with the complex. Colored hexagons indicate CoQ intermediates; white hexagons indicate the final CoQ product. Polypeptides for which the human protein structures have been solved are highlighted in pink. The COQ8A/COQ8B polypeptides are thought to have a dynamic association with complex Q. Polypeptides are not drawn to scale and their stoichiometry has not been determined.

In this study, we utilized exome and genome sequencing data of large populations, as well as clinical variants reported in the literature and the NCBI ClinVar database [35] to generate a comprehensive list of missense single nucleotide variants (SNVs) found in genes encoding the core CoQ synthome or complex Q (COQ3–COQ7 and COQ9). The COQ8A and COQ8B genes were excluded due to their aforementioned dynamic interactions with the core complex. We structurally and functionally characterized variants that occurred in conserved and functional regions of the protein, using multiple sequence alignment and structural analyses with available models and crystal structures. In addition, clinically reported variants and those with high allele frequency were also included in our analyses. Our final list consisted of 429 variants compiled from gnomAD [36], ClinVar, Missense3D-DB [37], as well as the published research literature. We predicted the pathogenicity of each variant using the Missense3D mutation classifier, which relies purely on structural information and local structural changes [38]. When an experimentally determined structure was not available, as is often the case for the COQ polypeptides, Missense3D presented the advantage of being able to make predictions based on computational models such as those generated by AlphaFold [39]. This is in contrast to other classifier methods, including SIFT [40] and Polyphen-2 [41], that rely on evolutionary conservation and a mixture of sequence- and structure-based predictive features, respectively. In total, Missense3D identified 115 variants that are structurally damaging and potentially pathogenic. Confirmation of these predictions will require biochemical characterization in model systems, such as *Saccharomyces cerevisiae*. The results presented will provide a resource for clinicians with the aim of guiding efforts toward the treatment of primary CoQ₁₀ deficiency.

2. Materials and Methods

2.1. Multiple Sequence Alignment

All human COQ polypeptide and orthologous sequences were obtained from NCBI GenBank Release 246.0 [42]. Multiple sequence alignments were generated using the ClustalW package of Clustal Omega [43] and visualized with Jalview2 [44]. Residues were classified as highly conserved if the percent agreement at that position was higher than 80%, as calculated by the built-in feature of Jalview and indicated by a dark blue shading in the sequence alignment. Prior biochemical characterization and crystal structure of *Escherichia coli* ubiquinone biosynthesis O-methyltransferase UbiG (the COQ3 ortholog) was used to determine the functional regions in human COQ3 (PDB: 4KDC and 5DPM) [45,46]. Molecular genetic analyses of *S. cerevisiae* Coq4 were used to determine the potential zinc-liganding residues in human COQ4 [47]. Apoenzyme (apo; PDB: 4OBX) and AdoMet-bound (PDB: 4OBW) form of *S. cerevisiae* Coq5 crystal structures were used to determine homologous residue functionality in human COQ5 [48]. Functional residues in human COQ6 were determined using an *S. cerevisiae* Coq6 model [49]. Prior biochemical characterization, a structural model, and a recent structure of human COQ7 were used to identify the functional regions of COQ7 [50–52]. Prior biochemical characterization, crystal structures, and molecular dynamics simulations were used to outline the functional residues in COQ9 (PDB: 6AWL and 6DEW) [50,52].

2.2. Analysis of Single Nucleotide Variants

Human SNVs occurring in the canonical transcripts, as reported in NCBI RefSeq [53] for COQ3–COQ7 and COQ9, were gathered from gnomAD v2.1.1 [36], NCBI ClinVar [35], Missense3D-DB v1.5.1 [37], and available literature. Only SNVs that resulted in a missense variant were analyzed. In addition, a filter was applied to retain variants that occur in highly conserved (see Section 2.1) or functional amino acid positions. Variants documented in the ClinVar database or literature, as well as variants with an allele frequency larger than 1.00×10^{-4} as reported in gnomAD v2.1.1 (labeled as “Frequent Polymorphisms”), were also included in our analysis. Finally, exonic variants that are within two nucleotides of a splice site were included as variants of potential interest. Since the Missense3D-DB had already published variant classification using the crystal structure of COQ9, any variant that

was reported as structurally damaging in the database was also included in our analysis, regardless of amino acid position or conservation.

The selected variants were classified with three mutation classifiers, each using a different algorithm. Missense3D (<http://missense3d.bc.ic.ac.uk/missense3d/>, accessed on 30 March 2022) reports deleteriousness based on local structural feature changes that may be destabilizing [38]. SIFT (<https://sift.bii.a-star.edu.sg/>, accessed on 30 March 2022) scores variants as “Tolerated” or “Deleterious” based on evolutionary conservation [40]. Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>, accessed on 30 March 2022) uses a Naïve Bayes classifier to report the likelihood of a mutation being damaging based on a combination of sequence- and structure-based criteria, whenever structures are available. Variants are scored as “Benign”, “Possibly Damaging”, and “Probably Damaging” [41]. Default settings were used whenever applicable. Results and interpretations from the three mutation classifiers can be found in Supplemental Table S1. The pipeline for SNV selection and classification is illustrated in Figure 2.

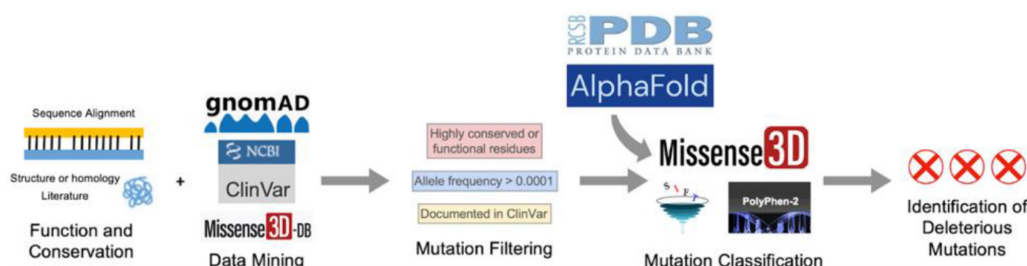


Figure 2. Schematic diagram of the overall approach used in this study.

2.3. Missense3D Analysis

Selected variants were submitted to the Missense3D online server for analysis of structural impact. For COQ3–COQ7, all variants were analyzed using the structural predictions of the human protein obtained from the AlphaFold Protein Structure Database [39,54]. For COQ9, variants occurring in regions modeled by its crystal structure (PDB 6AWL) were assessed using the crystal structure, while the remaining variants were assessed using their AlphaFold models. AlphaFold models used in this study include Q9NZJ6 (COQ3), Q9Y3A0 (COQ4), Q5HYK3 (COQ5), Q9Y2Z9 (COQ6), Q99807 (COQ7), and O75208 (COQ9). Structures were visualized using PyMOL (PyMOL Molecular Graphics System, Version 2.4.2, Schrödinger, LLC, New York, NY, USA).

3. Results

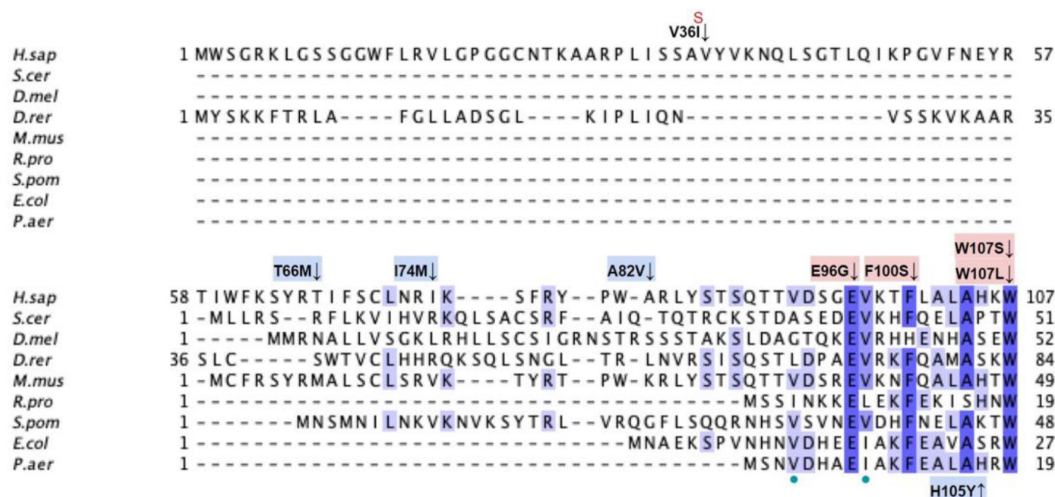
3.1. COQ3

COQ3 is an *S*-adenosyl-L-methionine (AdoMet)-dependent methyltransferase that catalyzes both *O*-methylation steps in CoQ biosynthesis in both *S. cerevisiae* and humans [55–57]. Namely, it is responsible for the conversion of DHPB to HMPB as well as the last step in CoQ biosynthesis, DMeQH₂ to CoQH₂ (Figure 1A). Overexpression of human COQ3 has been shown to rescue the growth CoQ biosynthesis of respiratory deficient *coq3Δ* yeast [56]. COQ3 and its yeast homolog are required for synthome stabilization, as partial knockdown of COQ3 in human cells results in a significant decrease in the levels of COQ4–COQ9, with the exception of COQ5 [58]. This result is corroborated by studies in yeast harboring a deletion in COQ3 [59]. Interestingly, Coq3 is stable in *coq4-coq9* null mutant yeast strains studied in the presence of phosphatase and protease inhibitors, and its level is dependent on Coq8 [21,59]. There are currently no crystal structures available for eukaryotic homologs of COQ3. Structures are available for the orthologous *O*-methyltransferase UbiG from *E. coli*, which shares 34% sequence identity with human COQ3 and is part of a soluble ubiquinone/menaquinone biosynthetic complex [60]. Crystal structures of

the apo and S-adenosyl-L-homocysteine (AdoHcy)-bound UbiG monomer have guided subsequent biochemical characterizations and confirmed its seven β -strand Rossmann-fold structure [45,46].

To date, there have not been reported cases of primary CoQ₁₀ deficiency associated with mutations in the COQ3 gene [28–30], and pathogenic SNVs have not been identified. The lack of clinical data is echoed in yeast studies, which have primarily focused on COQ3 gene deletions. As a result, no point mutations conferring pathogenic effects have been characterized in yeast. However, COQ3 gene expression was found to be upregulated in esophageal squamous cell carcinoma and was associated with poor prognosis, hinting at a potentially unique role of COQ3 in humans [61,62].

From gnomAD, ClinVar, and Missense3D-DB, we identified 44 missense SNVs of interest (Figure 3; Supplemental Table S1; see Section 2.2 for the selection criteria). Of these variants, 29 coincided with a highly conserved region or functional residue determined from structural and biochemical studies, as well as analyses of sequence conservation [45,46,63]. Sixteen SNVs occurring in highly conserved or functional residues were classified as structurally damaging by Missense3D. Twelve additional variants from gnomAD that were neither highly conserved nor functional residues were included due to their notable allele frequency. Interestingly, three of these frequent variants were also classified as structurally damaging. V202M was the only variant listed in ClinVar, although its clinical significance was “Likely Benign”. Finally, a total of four variants were marked as potential splice site variants in addition to their apparent amino acid sequence change. Since the effect on splicing may be confounding, this group of variants was not subjected to extensive study.

Figure 3. *Cont.*

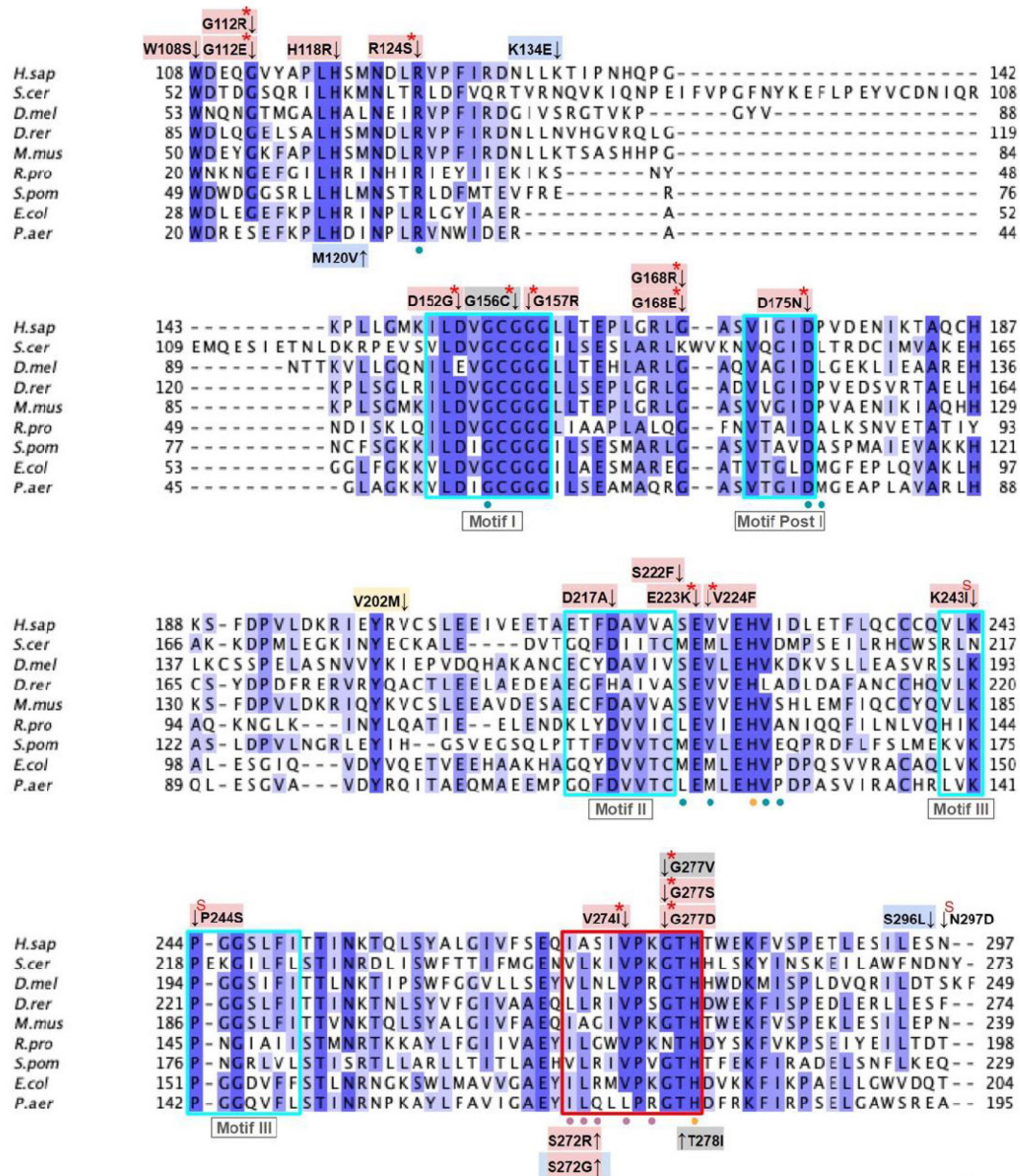
Figure 3. *Cont.*



Figure 3. Labeled and annotated multiple sequence alignment of COQ3. Amino acid sequences of COQ3 were analyzed as described in Materials and Methods and include *Homo sapiens* (NCBI accession number NP_059117.3) and homologs in *Saccharomyces cerevisiae* (NP_014545), *Drosophila melanogaster* (NP_610092.2), *Danio rerio* (NP_001002620.1), *Mus musculus* (NP_766275.1), *Rickettsia prowazekii* (WP_004596275.1), *Schizosaccharomyces pombe* (NP_588239.2), *Escherichia coli* (NP_416735.1), and *Pseudomonas aeruginosa* (WP_003122245.1). See KEY for descriptions of the figure annotations. Putative AdoMet binding residues are denoted by a blue dot, and residues thought to interact with the cell membrane in *E. coli* UbiG are denoted by a purple dot, data from [45,46]. A pair of co-evolving, highly conserved, and structurally nearby histidines may be involved in metal ion coordination (orange dot), data from [63]. Methyltransferase motifs I, post-I, II, and III are boxed in cyan data from [56,64]. The putative membrane-interacting hydrophobic region is boxed in red, data from [45]. For all multiple sequence alignments, conservation is indicated via shaded residues, which represent a percent identity great than 80%, 60%, and 40%, from darkest to lightest. Residues with less than 40% identity are unshaded.

The human COQ3 model was obtained from the AlphaFold Protein Structure Database (Figure 4) [39,54]. The core structure of the model consists of eight α -helices (as rendered by the AlphaFold 3D viewer) and eight β -sheets, flanked by long disordered regions on the N- and C-termini. The overall structure of the model aligned well with the crystal structure of AdoHcy-bound (PDB: 5DPM; Figure 5A) and apo *E. coli* UbiG (PDB: 4KDC; Figure A1). Four sequence motifs shared among class I methyltransferases, motifs I–III and post-I, each form a β -strand that aligns the residues in the AdoMet binding pocket (Figure 5B) [64,65].

In addition to the conserved Rossmann fold structure, the UbiG/COQ3 family contains a hydrophobic stretch of ten residues (residues 270–279 in the human COQ3 sequence) that is thought to interact with the membrane in UbiG [46]. An isolated helix linked to the hydrophobic region is visible in the COQ3 model, while this region was truncated in the crystal structure of UbiG (Figure 5A) [46]. This region is thought to act as a gate to the AdoMet binding pocket, which is consistent with its position in the model (Figure 5B) [46]. However, it is not clear how this putative membrane interaction helix is compatible with the recent structure of the soluble Ubi metabolon [66].

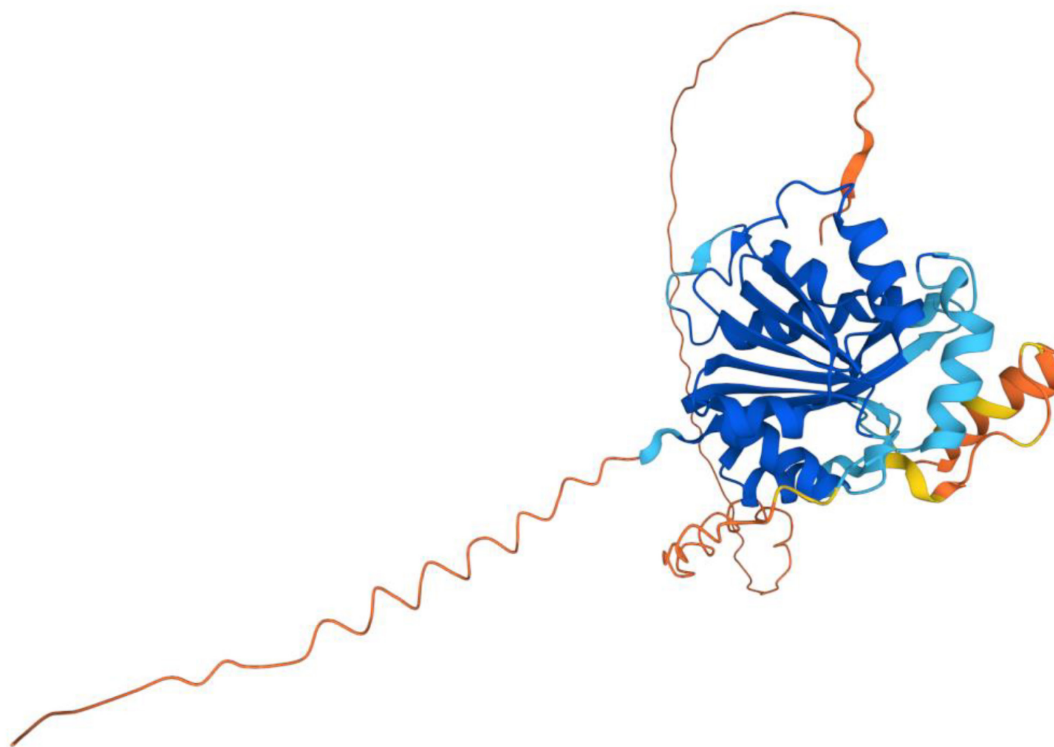


Figure 4. AlphaFold model of human COQ3. Model color corresponds to model confidence in each region. Very high confidence in dark blue (pLDDT > 90); confident in light blue (90 > pLDDT > 70); low confidence in yellow (70 > pLDDT > 50); very low confidence in orange (pLDDT < 50). pLDDT is a measure of per-residue confidence. pLDDT < 50 is a reasonably strong predictor of disorder for the corresponding residue. Figure was generated by the AlphaFold Protein Structure Database; adapted with permission from [39,54].

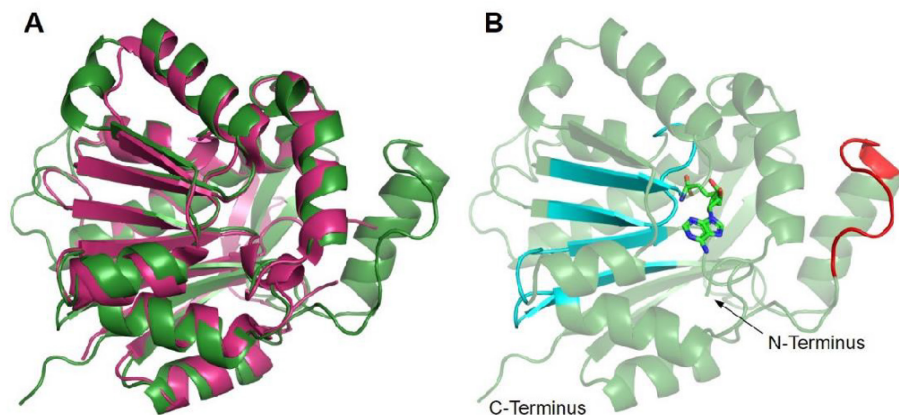


Figure 5. Alignment of AlphaFold-generated model of human COQ3 with existing *E. coli* UbiG crystal structure. (A) Superimposition of the human COQ3 model (residues 91–336, shown in dark green), adapted with permission from [39,54], and the crystal structure of AdoHcy-bound *E. coli* UbiG (shown in magenta, PDB: 5DPM). The N- and C-termini of the model were omitted due to low confidence. Note that this and subsequent figures about COQ3 were generated using PyMOL, which classifies a short-coil region as a helix (top left of figure). This gives a total of nine helices as opposed to eight rendered in Figure 4. (B) Human COQ3 model with conserved methyltransferase motifs I–III and post-I highlighted in cyan and the hydrophobic region in red. AdoMet (shown in light green) was modeled via structural alignment with PDB 5DPM using PyMOL.

3.1.1. SNVs in Methyltransferase Motifs I and Post I

Motif I (residues I150 to G1158) is found in the first β -strand followed by a Gly-rich loop that extends into the putative AdoMet binding site of COQ3 (Figure 6A) [65]. It closely resembles the nine-residue consensus sequence, with two aliphatic residues followed by a (D/E) (V/I) GXGXG motif [64]. The backbone carbonyl of the G66 in *E. coli* in this motif (G156 in humans) has been shown to form a hydrogen bond with the amino group of AdoHcy in UbiG (PDB: 5DPM) [46].

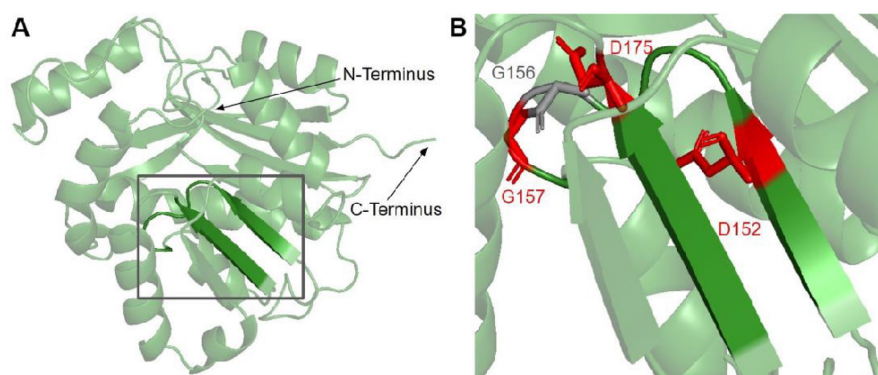


Figure 6. SNVs found in motifs I and post I of COQ3. (A) Motifs I (right β -strand and loop, residues I150 to G1158) and post I (left β -strand, residues V171 to D175) highlighted on residues 91–336 of the COQ3 model. N- and C-termini were truncated for simplicity. (B) Locations of SNVs in motifs I and post-I are depicted. Residues are colored according to their corresponding SNVs in Figure 3.

There are three SNVs of interest in this region, all of which occur on highly conserved residues (Figures 3 and 6B). D152G from gnomAD maps to the β -strand region, which is critical for the alignment of AdoMet binding residues. Missense3D classifies this variant as structurally damaging due to the replacement of a buried charge and the loss of a buried hydrogen bond. These disruptions may lead to destabilization of the core structure and more profound changes to the CoQ synthome. G156C and G157R were reported in Missense3D-DB and gnomAD, respectively. These two SNVs map to the flexible Gly-rich loop. Missense3D classifies both variants as structurally damaging due to the replacement of buried Gly residues. In addition, G156C was flagged for altering the buried/exposed status of this residue position, which likely refers to the mutant Cys residue protruding into the AdoMet binding site. G157R, on the other hand, introduces a buried charge in proximity with the putative docking region of the amino and carboxyl groups of AdoMet. Indeed, mutating the homologous residue in yeast, G133, to an Ala resulted in partial suppression of respiratory growth and significantly reduced CoQ levels, despite stable expression of the polypeptide [63]. Taken together, these two variants likely affect cofactor binding and enzyme activity.

Motif post-I (V171 to D175) is part of the second β -strand that contains a short stretch of hydrophobic residues followed by a highly conserved acidic residue (Figure 6A) [65]. The acidic residue in *E. coli* UbiG, D85 (D175 in humans) was shown to form hydrogen bonds with the O2' and O3' hydroxyl groups on the ribose ring of AdoHcy in the crystal structure of UbiG (PDB: 5DPM), while residue M86 (P176 in humans) immediately following motif post-I interacts with the adenine ring [46]. D175N is the only SNV of interest in this region (Figures 3 and 6B). This variant was classified as structurally damaging by Missense3D due to the replacement of a buried charge, which may disrupt the aforementioned interactions with the ribose ring.

3.1.2. SNVs in Methyltransferase Motifs II and III

Motif II, roughly E214 to A221, maps to residues immediately preceding and including the fourth β -strand (Figure 7A). The sequence of motif II is not as well conserved among COQ3 homologs, although one highly conserved Asp is thought to make contact with motif I (Figure 3) [65]. Aliphatic residues following this Asp mark the start of β 4, which leads to a loop containing residues forming a hydrophobic environment for the adenine ring of AdoHcy in UbiG [46]. In addition, this region following motif II is thought to interact with the methyl group in AdoMet as well as the substrate, giving it a possible role in determining substrate specificity [65,67]. This hypothesis is supported by coevolution studies of UbiG/COQ3, in which residue position 196 in yeast Coq3 (222 in humans) was shown to coevolve in other methyltransferases but was distinct in Coq3 and *E. coli* UbiG. One additional residue of interest downstream is H227 in human COQ3. Its homologous residue in UbiG, H134, was shown to be required for growth in *E. coli*, and in the *Arabidopsis thaliana* small RNA 2'-O-methyltransferase HEN1 the homologous residue was described as magnesium-binding [45,68]. Mutating this residue to an Ala in yeast (H201A) completely destabilized the polypeptide [63]. In motif II and the loop following it, there are four SNVs of interest in highly conserved or functional residues, all of which were reported in gnomAD (Figures 3 and 7B). D217A immediately preceding β 4 was, while highly conserved, not classified as damaging by Missense3D due to the residue being solvent-exposed in the model. S222F, E223K, and V224F are located in the loop following β 4. S222 and V224 are thought to bind the amino group and the adenine ring of AdoMet, respectively, by homology with *E. coli* UbiG (M129 and M131) [46]. However, only V224F was classified as structurally damaging due to a change in cavity size, likely referring to the binding pocket for the adenine ring of AdoMet. S222F and E223K were also solvent-exposed in the model, which resulted in their classification as neutral despite their disruption of hydrogen bonds or salt bridges. In yeast, an Ala mutation in the homologous position of E223 completely destabilized the Coq3 polypeptide and disrupted respiratory growth. Coq4

was also destabilized as a consequence [63]. This strongly suggests that all three variants in the loop are likely disruptive.

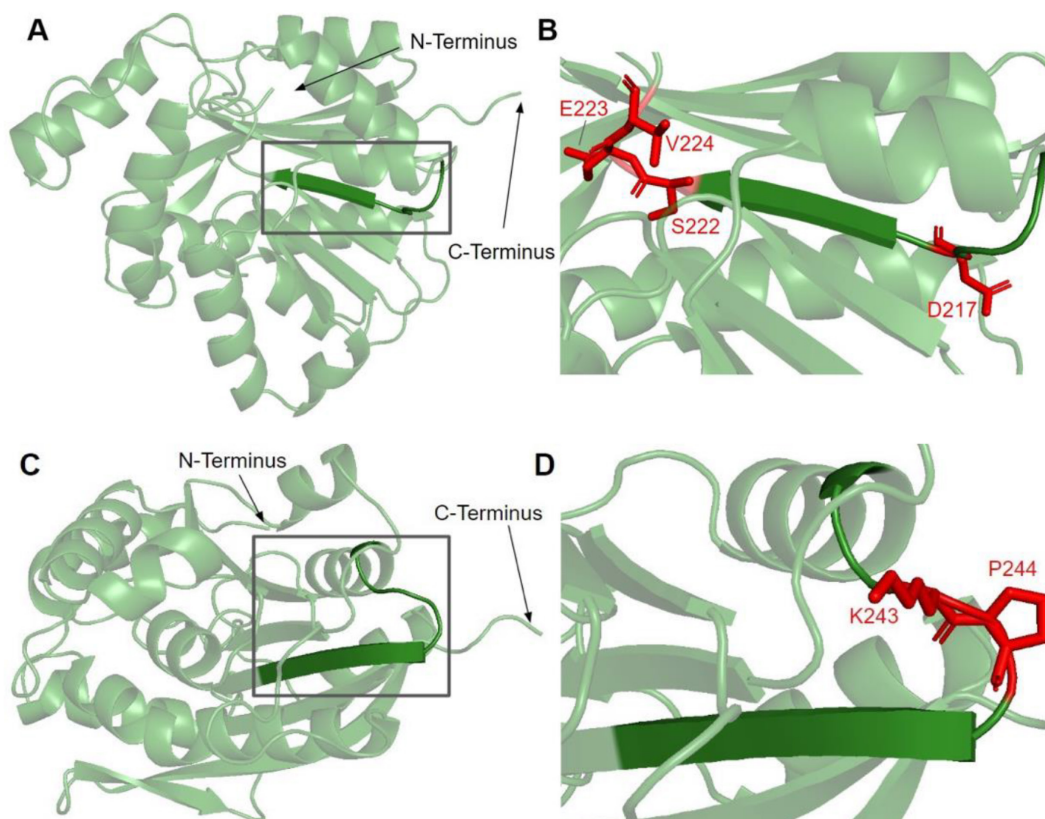


Figure 7. SNVs found in motifs II and III of COQ3. (A,C) Residues 91–336 of the COQ3 model with E214 to A221 as motif II (A) and V241 to I250 as motif III (C) are highlighted. (B,D) Locations of SNVs in motif II and nearby residues (B) and motif III (D) are depicted. N- and C-termini were truncated for simplicity. Residues are colored according to their corresponding SNVs in Figure 3.

Motif III (V241 to I250) lies on the fifth β -strand as well as the loop and parts of the helix preceding it (Figure 7C). This loop is essential for the alignment of the methyltransferase motifs and consists of Pro and Gly residues to facilitate the turn [65]. GnomAD reported two SNVs, K243I and P244S, in highly conserved residues of this motif (Figure 7D). Neither SNV was classified as structurally damaging by Missense3D. However, it is worth noting that these SNVs occur near a splice junction and may therefore result in more profound changes to protein expression in addition to changes in protein sequence.

3.1.3. SNVs in the Membrane Interacting Hydrophobic Region

Previous studies demonstrated that a region consisting of several hydrophobic and basic residues in *E. coli* UbiG and human COQ3 is required for their interaction with liposomes containing cardiolipin [45]. Furthermore, mutating multiple residues in this region to Ala resulted in reduced growth in *E. coli* [45]. In the human COQ3 model, this region can be seen mapping to a linker connecting an isolated α -helix to the core structure

(Figure 8A). This is consistent with a model in UbiG in which this region acts as a gate to the binding site of the AdoMet cofactor [46]. Upon binding to the membrane, this helix is thought to dissociate from the core structure, allowing the cofactor to diffuse into the active site. Mutating residues in and preceding this region to disrupt its interaction with the core structure indeed resulted in enhanced binding of AdoHcy [46]. Nevertheless, it is worth noting that there are key differences in CoQ biosynthesis in *E. coli* and humans. UbiG is part of a soluble complex in the cytosol, while COQ3 is part of a membrane-associated complex in the mitochondrial matrix [60]. Implications of this membrane interaction in human COQ3 have not been investigated. Its preferential binding to liposomes containing cardiolipin, a unique component of the inner mitochondrial membrane, suggests a similar cofactor gating role in human COQ3. Meanwhile, a more recent coevolution study of the UbiG/COQ3 family identified the His residue at the end of this region (H279 in humans, H254 in yeast) as coevolving with the putative metal binding H201 in yeast (H227 in humans) [63]. The close proximity of the two His residues in our model supports an alternative role of metal binding. However, the functional significance of this region in human COQ3 remains unelucidated.

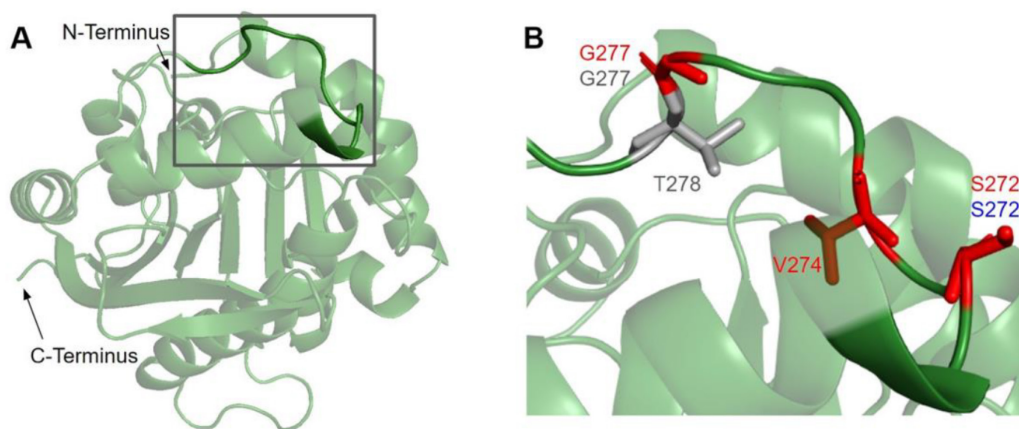


Figure 8. SNVs found in the membrane interacting hydrophobic region of COQ3. **(A)** Residues I270 to H279 are highlighted on residues 91–336 of the COQ3 model. N- and C-termini were truncated for simplicity. **(B)** Locations of SNVs in the hydrophobic region are depicted. Residues are colored according to their corresponding SNVs in Figure 3.

In this region, we have identified seven variants spanning five residue positions from gnomAD and Missense3D-DB (Figure 8B). S272G, S272R, and V274I all coincide with residues known to affect liposome binding in UbiG when mutated [45]. Interestingly, S272G is a frequent polymorphism with an allele frequency of 8.45×10^{-1} , suggesting that this variant may be benign. V274I was classified as structurally damaging by Missense3D due to changes in cavity size, which likely refers to the active site of COQ3. This classification suggests a possible change in either substrate or cofactor binding and therefore activity. G227S, G227V, G227D, and T278I were not tested in UbiG, although the high conservation suggests functional importance. All three variants occurring on G227 were classified as damaging by Missense3D due to disallowed phi/psi angle.

3.1.4. Frequent Polymorphisms in COQ3

In this study, we have defined “frequent polymorphism” as a variant having an allele frequency larger than 1.00×10^{-4} . There are 12 such variants found in COQ3 that do not fall into the previously described functional regions. Most of them occur in regions

of poor conservation such as the N- and C-termini. Two variants, Y329H and K134E, stand out with their exceptionally high allele frequency of 9.84×10^{-1} and 3.03×10^{-1} , respectively. Interestingly, Y329H, located on $\beta 7$, was classified as structurally damaging by Missense3D due to the introduction of a buried charge. However, given the large number of homozygotes that result from its allele frequency, this variant is unlikely to be detrimental. Two additional variants, G348E (allele frequency 8.14×10^{-4}) and E365D (allele frequency 6.07×10^{-4}), were classified as structurally damaging. The former was flagged for replacement of a Gly residue in a bend, which may affect the flexibility of the region. The latter was flagged for steric clash, which may be an artifact of the Missense3D program since the region is disordered.

3.2. COQ4

COQ4 has not been characterized as having an enzymatic function, though it appears to play an important structural role in organizing the CoQ synthome [13]. Human transcripts encode two *COQ4* isoforms; isoform 2 lacks both the first exon and the mitochondrial leader sequence and fails to rescue yeast *coq4* null mutants [69]. The polypeptide encoded by *COQ4* isoform 1 complements *coq4* null mutant yeast [69]. This demonstrates conserved functionality across species as well as the power of *S. cerevisiae* as a model organism to study human *COQ4* mutations [69]. Human COQ4 is located within mitochondria [69], as is the yeast Coq4 polypeptide that is peripherally associated with the matrix side of the IMM [70].

The first structure determined for a COQ4 homolog, the Alr8543 polypeptide from *Nostoc* sp. (PDB: 3KB4), revealed a geranylgeranyl monophosphate bound between hydrophobic helices. However, PDB: 3KB4 is now obsolete and has been replaced with PDB: 6E12. The Alr8543 protein in the more recent 6E12 structure is in complex with two magnesium ions. Both Mg^{2+} ions are associated with a conserved HDXXH-(X)₁₀₋₁₃-E signature motif required for COQ4 functionality and is similarly considered to chelate a zinc ion [47]. Mutations in the human *COQ4* gene are associated with primary CoQ₁₀ deficiency and with several disease states including a range of neurological afflictions, cardiomyopathy, and respiratory distress [29].

From gnomAD, ClinVar, Missense3D-DB, and literature, we identified 97 missense SNVs of interest (Figure 9; Supplemental Table S1). Of these variants, 60 coincided with a highly conserved region or functional residues, as determined from the multiple sequence alignment as well as structural studies with Alr8543. Of 22 variants classified as structurally damaging by Missense3D, 18 were contained within a highly conserved or functional region. From the ClinVar database and published research literature, 15 missense SNVs were determined to be either pathogenic or likely pathogenic. Four of these pathogenic or likely pathogenic variants were identified by Missense3D to be structurally damaging. Fifteen additional variants from gnomAD were included as frequent polymorphisms. Intriguingly, three of these frequent polymorphisms were identified in the research literature as deleterious. G124S (allele frequency of 1.13×10^{-4}) has been identified as a founder mutation in the southern Chinese population [71]. Another, E161D (allele frequency of 6.03×10^{-3}) was found in a patient who suffered from primary CoQ₁₀ deficiency and later died from rhabdomyolysis [72,73]. A third, R240C (allele frequency of 1.79×10^{-4}), occurred in a patient with progressive spasticity, motor impairment, and ataxia [74]. This patient harbored biallelic variants of COQ4 (P193S and R240C), and individual tests of these alleles of human COQ4 in yeast showed that neither variant could rescue a *coq4* yeast mutant [74].

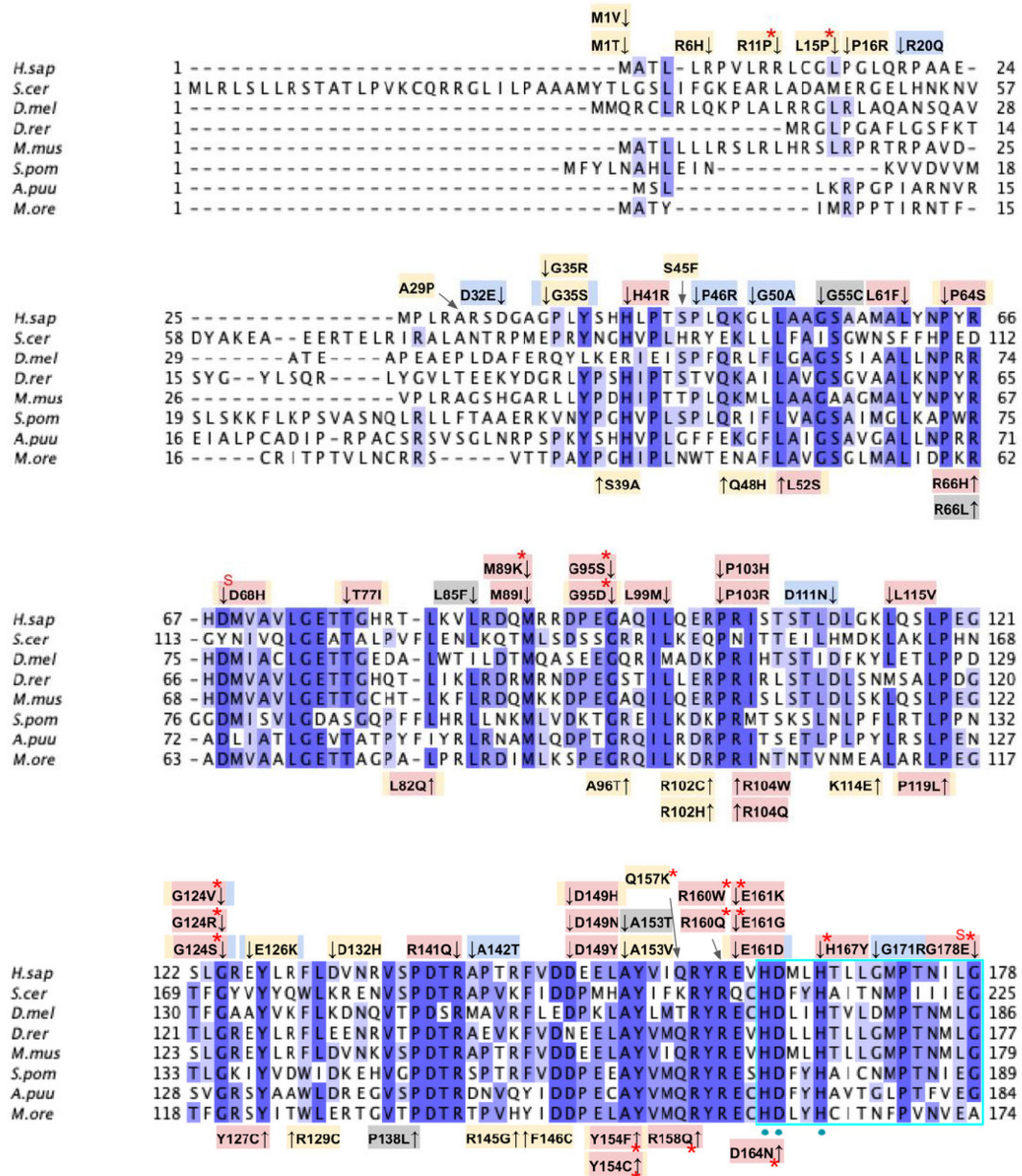


Figure 9. Cont.

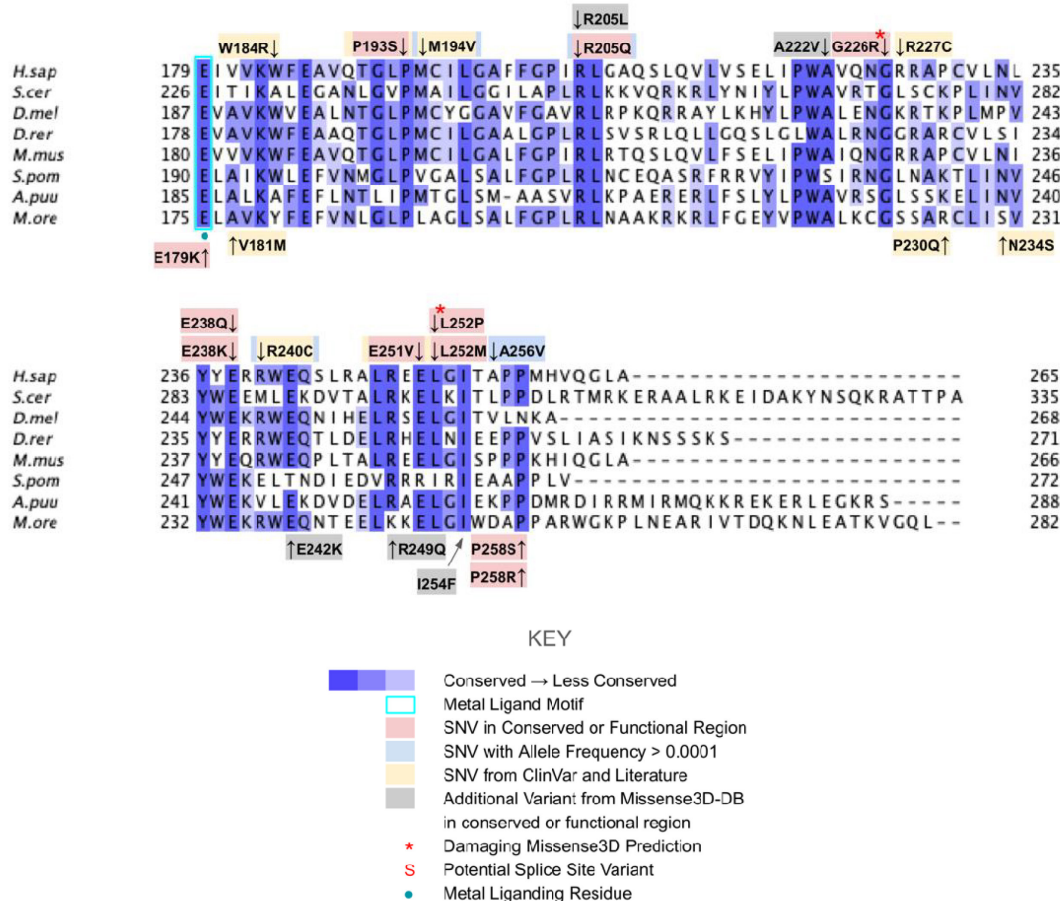


Figure 9. Labeled and annotated multiple sequence alignment of COQ4. Amino acid sequences of COQ4 were analyzed as described in Materials and Methods and include *Homo sapiens* (NCBI accession number NP_057119.3) and homologs in *Saccharomyces cerevisiae* (NP_010490.1), *Drosophila melanogaster* (NP_730270.1), *Danio rerio* (NP_001108192.1), *Mus musculus* (NP_848808.1), *Schizosaccharomyces pombe* (NP_593130.1), *Aspergillus puulaauensis* (XP_041555109.1), and *Marasmius oreades* (XP_043005754.1). See KEY for descriptions of the figure annotations. The metal ligand motif is boxed in cyan, and putative residues that ligand the metal ions are denoted by a blue dot, data from [47].

The human COQ4 model was obtained from the AlphaFold Protein Structure Database (Figure 10A) [39,54]. The per-residue model confidence is shown in Figure 10A. A structural alignment of the human COQ4 AlphaFold model with the PDB 6E12 structure of the Alr8543 polypeptide, a COQ4 homolog from *Nostoc* sp., is shown in Figure 10B.

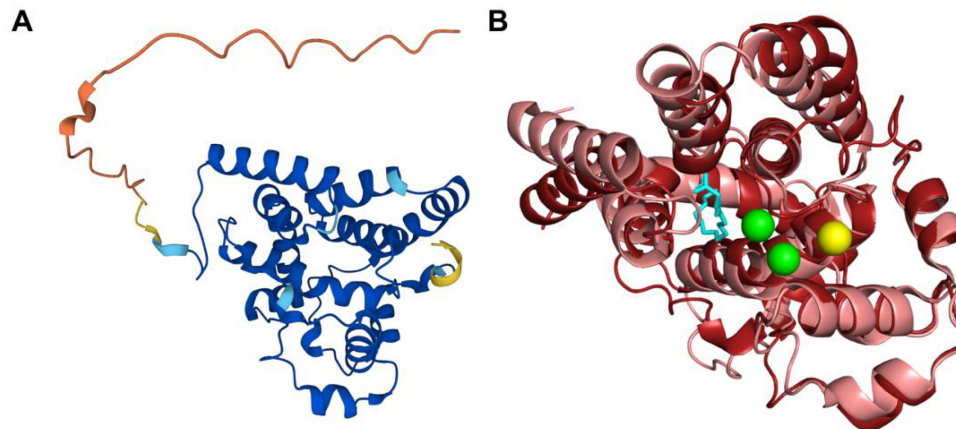


Figure 10. Comparison of the COQ4 AlphaFold model and the structure of Alr8543. (A) AlphaFold model of single chain of COQ4. Model color corresponds to model confidence as described in Figure 4. Figure was generated by the AlphaFold Protein Structure Database; adapted with permission from [39,54]. (B) Structural alignment of the human COQ4 AlphaFold model (shown in dark red) and Chain B of the crystal structure of Alr8543 protein (shown in salmon, PDB: 6E12) in complex with oleic acid (shown in cyan), Mg²⁺ (shown in green), and Cl⁻ (shown in yellow). Residues 1–44 of the AlphaFold model were omitted due to disordered structure.

3.2.1. SNVs in the Metal Liganding Motif

The amino acid sequence of COQ4 contains the highly conserved HDXXH-(X)₁₁-E motif indicative of a metal ligand [47]. While the function and identity of the metal ligand remain unknown, it has been hypothesized that the ligand is sensitive to the redox state of mitochondria. Mutations in this region may destabilize coordination of the metal and CoQ synthome formation [47]. The motif spans residues H163 to E179 (Figure 11A). In the Alr8543 structure, the analogous E136 in the conserved HDXXH-(X)₁₁-E motif ligands one Mg²⁺ ion (Figure A2). H124, the second H residue of the motif, forms an interaction with E. The D121 of this motif in Alr8543 ligands the second Mg²⁺ ion.

There are four SNVs reported in Missense3D-DB that occur at highly conserved residues within the metal-liganding motif: D164N, H167Y, G178E, and E179K (Figure 11B). Of these, D164, H167, and E179 correspond to residues predicted to ligand the metal ion. D164 is proposed to be analogous to the Mg²⁺ ion-liganding D121 in Alr8543 (Figure A2). H167 is proposed to be analogous to H120 in Alr8543, which stabilizes interactions with E136 that ligands the Mg²⁺ ion. E179 is proposed to be analogous to E136, which ligands the second Mg²⁺ ion in Alr8543 (Figure A2). A homologous point mutation of E179K in Coq4 of *S. cerevisiae* was unable to form the high molecular mass CoQ synthome [47]. Three SNVs in this region, D164N, H167Y, and G178E, were identified by Missense3D as being structurally damaging. None of these mutations have a reported clinical association on ClinVar.

The most common flags generated by Missense3D relate to the change or introduction of a buried charge, such as at D164 and H167. Such changes may destabilize the tertiary structure of COQ4 and may also affect the ability of COQ4 to interact with metal ligands.

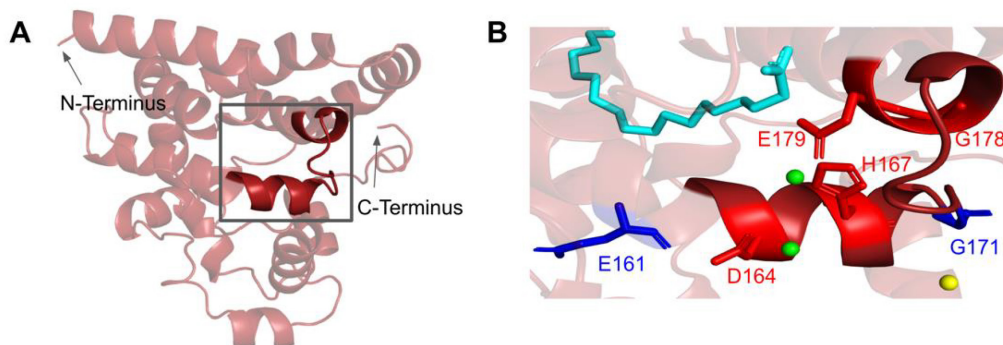


Figure 11. SNVs found in the putative COQ4 metal-liganding motif. (A) The metal-liganding motif (encompassing residues H163 to E179) is highlighted in red on the COQ4 model. (B) Locations of SNVs are depicted. Structural alignment of Alr8543 and COQ4 identifies the metal-liganding motifs in COQ4. Metal ions and oleic acid were obtained from this structural alignment. Residues are colored according to their corresponding SNVs in Figure 9. Mg^{2+} ions are designated as green spheres and Cl^{-} in yellow. Oleic acid is represented by the cyan structure.

3.2.2. COQ4 Haploinsufficiency

Salviati et al. first identified the *COQ4* gene as being haploinsufficient [75]. This property is unusual because heterozygous carriers harboring a mutation in one of the other *COQ* genes are generally described as asymptomatic and produce normal levels of CoQ. *COQ4* haploinsufficiency was confirmed in both fibroblast and yeast cell models [75]. Intriguingly, one of the SNVs identified as a frequent polymorphism (E161D, Figure 11B) was detected as a heterozygous mutation in a patient with CoQ₁₀ deficiency who had minor mental retardation and died of rhabdomyolysis [72,73]. Correction of the sequence variant in a cell culture model with CRISPR-mediated editing rescued the CoQ-deficient phenotype [73]. It is tempting to speculate that the haploinsufficiency of COQ4 may be related to its putative role in “scaffolding” the CoQ synthome, as proteins that serve analogous functions appear to be sensitive to changes in gene dosage [76].

3.3. COQ5

COQ5 is an AdoMet-dependent C-methyltransferase and a component of the CoQ synthome [18,77]. It is required for the conversion of DDMQH₂ to DMQH₂ in both *S. cerevisiae* and humans (Figure 1A) [18,78,79]. The human protein has been shown to rescue Coq5-deficient yeast that harbor stable but inactive Coq5 polypeptides, and it can also rescue *coq5* null mutants that overexpress Coq8 [18,80]. COQ5 is thought to be crucial for the stability of the synthome, as yeast lacking Coq5 are CoQ-less, respiratory-deficient, and do not form an intact synthome [59,80,81]. Partial knockdown of *COQ5* in human cells corroborates the findings observed in yeast *coq5* mutants and supports the crucial role of COQ5 in CoQ synthome formation and CoQ biosynthesis [20]. Crystal structures of the apo and AdoMet-bound yeast Coq5 dimers have been determined [48], revealing its seven β -strand Rossmann fold structure typical of the most common class I methyltransferases [64,82]. These crystal structures, in addition to known methyltransferase motifs [64], have helped elucidate the AdoMet and substrate-binding pockets as well as key residues involved in protein dimerization.

To date, only three patients with a mutation in *COQ5* have been documented. These related patients carried a 9590-bp duplication of the last four exons and part of the 3' UTR of *COQ5*, resulting in an elongated 3' UTR. They were deficient in CoQ₁₀ and exhibited symptoms exclusive to the central nervous system, including encephalopathy, intellectual disability, ataxia, and cerebellar atrophy. The clinical outcome of long-term CoQ₁₀

supplementation on these patients was equivocal [83]. Meanwhile, pathogenic SNVs in the *COQ5* gene have not been identified, although yeast studies have identified point mutations affecting the activity and stability of the Coq5 polypeptide, all of which resulted in respiratory deficiency [80].

From gnomAD, ClinVar, and Missense3D-DB, we identified 88 missense SNVs of interest (Figure 12; Supplemental Table S1). Of these variants, 76 coincided with a highly conserved region or functional residue, which were determined from the multiple sequence alignment as well as structural studies on yeast Coq5 [48]. All 24 variants classified as structurally damaging by Missense3D were contained in this group. In addition, ten variants from gnomAD that did not occur in highly conserved or functional residues were included as frequent polymorphisms. None of these variants were predicted to be structurally damaging.

The human COQ5 model was obtained from the AlphaFold Protein Structure Database (Figure 13) [39,54]. Compared to the crystal structure of AdoMet-bound yeast Coq5 (PDB: 4OBW) and apo yeast Coq5 (PDB: 4OBX), this model consisted of an additional α -helix N-terminal to the core structure, which was not visible in the yeast Coq5 crystal structure (Figures 14A and A3) [48]. The α 3 of the human COQ5 model is extended compared to that of the yeast Coq5 structure and is followed by a loop region containing a shorter helix. This region reflects an insert that spans residues 134–164, found exclusively in vertebrate species (Figure 12). The core seven β -strand structure, as well as the AdoMet and substrate-binding pockets, remain largely similar. In addition to their conserved three-dimensional structure, each of the four methyltransferase motifs (motifs I–III and post-I) in COQ5 approximately correspond to one β -strand connected to loops that make up the AdoMet binding site (Figure 14B) [64,65].

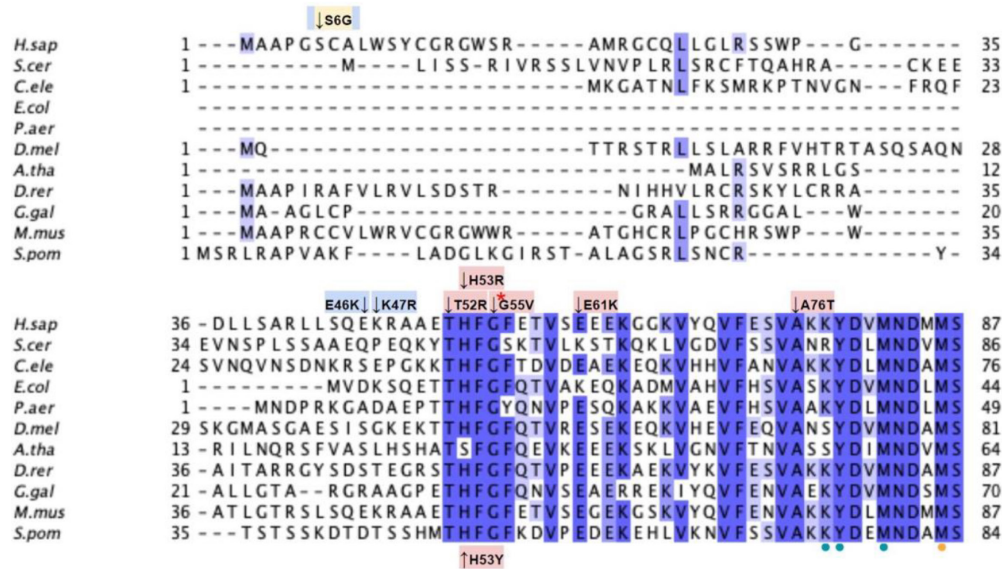


Figure 12. Cont.

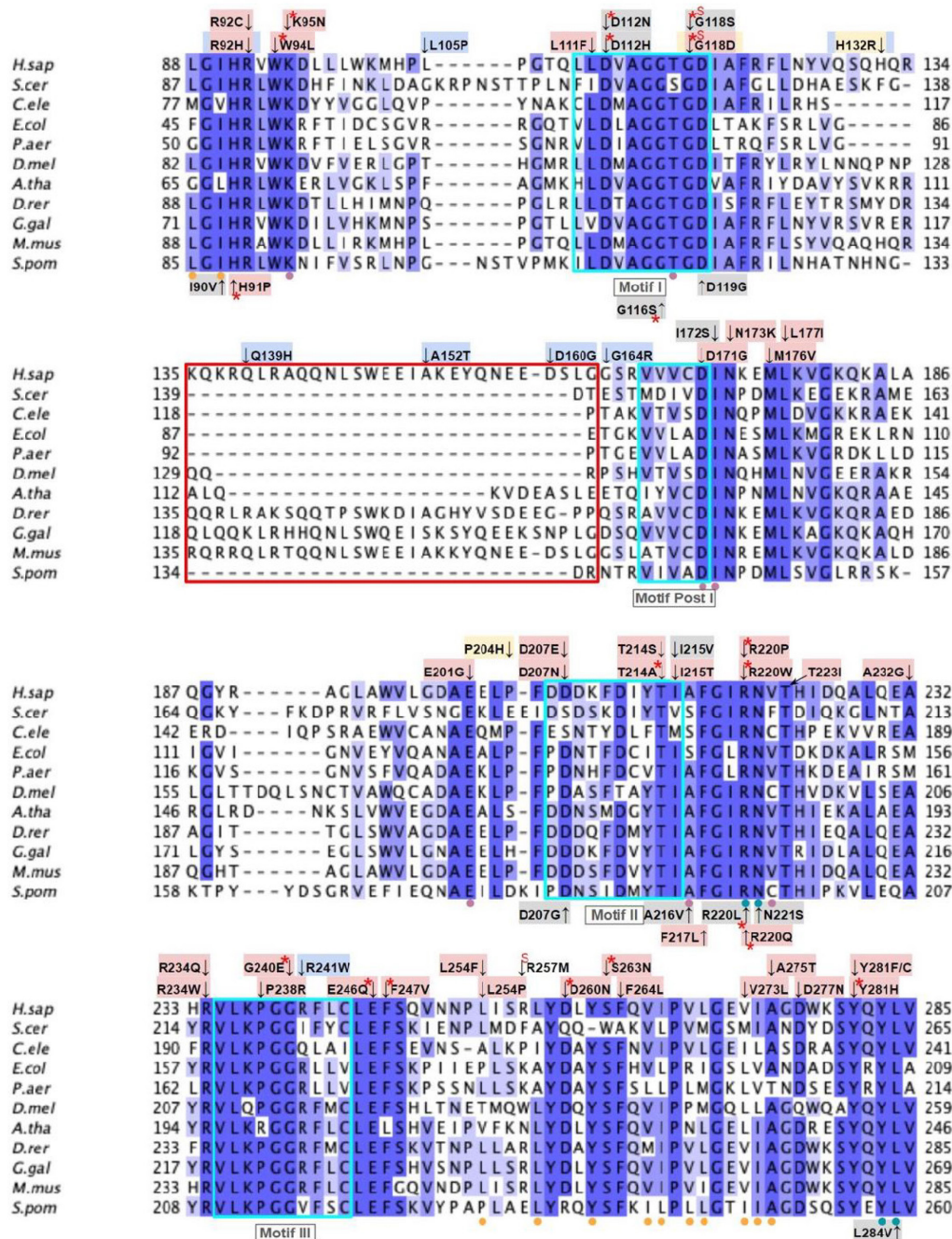


Figure 12. Cont.

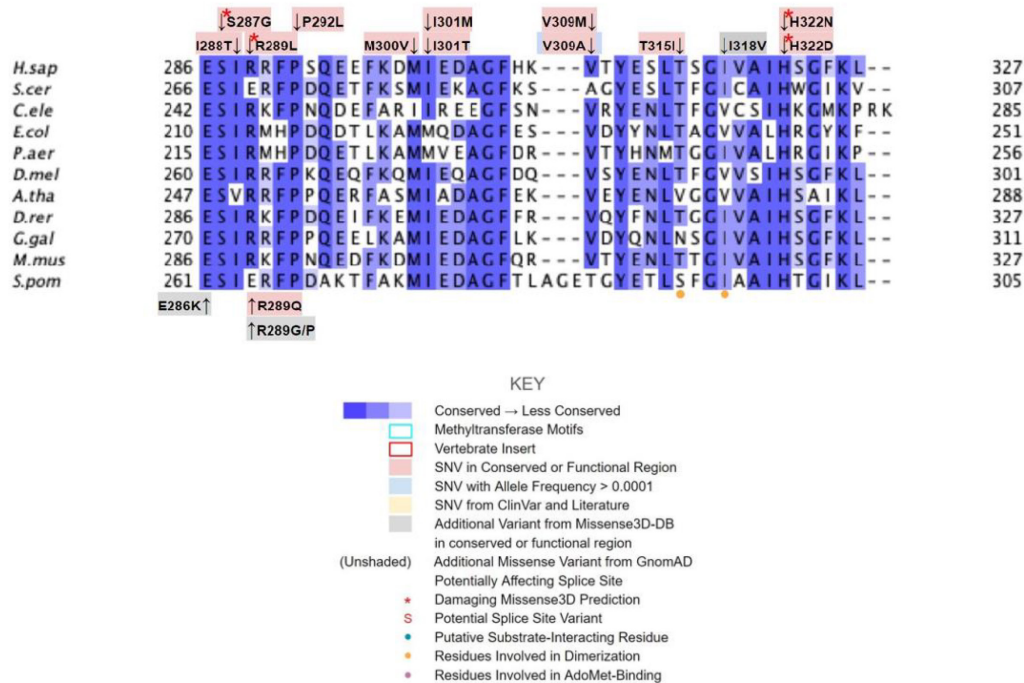


Figure 12. Labeled and annotated multiple sequence alignment of COQ5. Amino acid sequences of COQ5 were analyzed as described in Materials and Methods and include *Homo sapiens* (NCBI accession number NP_115690.3) and homologs in *Saccharomyces cerevisiae* (NP_013597.1), *Caenorhabditis elegans* (NP_498704.1), *Escherichia coli* UbiE (YP_026260.1), *Pseudomonas aeruginosa* UbiE (NP_253750.1), *Drosophila melanogaster* (NP_572865.1), *Arabidopsis thaliana* (NP_200540.1), *Danio rerio* (NP_001004541.1), *Gallus gallus* (NP_001006194.1), *Mus musculus* (NP_080780.1), and *Schizosaccharomyces pombe* (NP_587834.1). See KEY for descriptions of the figure annotations. Functional residues (dotted) were determined from the yeast Coq5 crystal structure (data from [48]). Methyltransferase motifs I, post-I, II, and III are boxed in cyan (data from [18]). An insert exclusively found in vertebrate species is boxed in red. Note that W243 in *S. cerevisiae* (aligned to S263 in the human sequence) is the residue involved in dimerization based on the crystal structure. However, since all other species in the multiple sequence alignment have a Tyr insertion at that position, Y262 was chosen as the functional residue instead due to its similar aromatic nature.

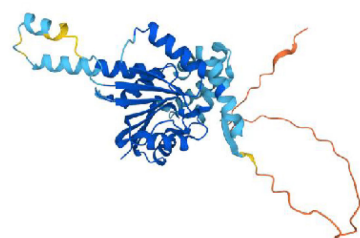


Figure 13. AlphaFold model of the COQ5 monomer. Model color corresponds to model confidence as described in Figure 4. Figure was generated by the AlphaFold Protein Structure Database; adapted with permission from [39,54].

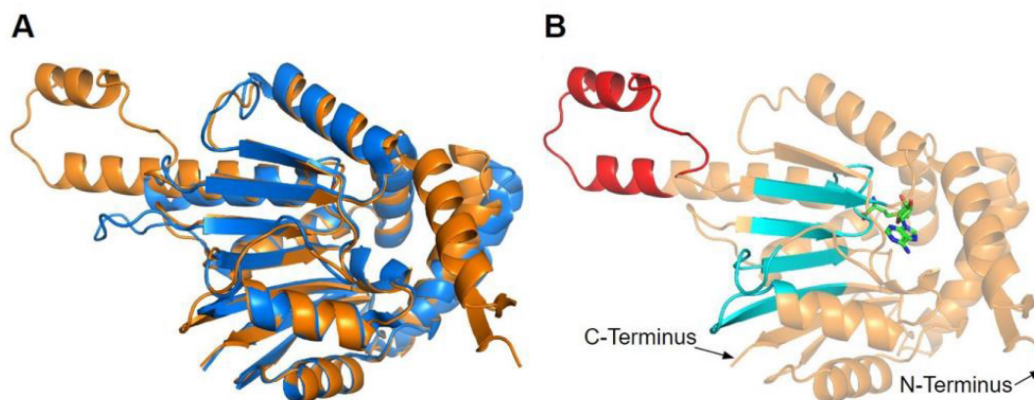


Figure 14. Alignment of AlphaFold-generated model of human COQ5 with existing yeast Coq5 crystal structures. (A) Superimposition of the human COQ5 model (shown in orange), adapted with permission from [39,54], and the crystal structure of AdoMet-bound yeast Coq5 (shown in blue, PDB: 4OBW). The first 47 residues of the AlphaFold model, which contains a disordered region with low confidence, were omitted for clarity. (B) Human COQ5 model with conserved methyltransferase motifs I-III and post-I highlighted in cyan and vertebrate insert in red. AdoMet (shown in green) was modeled via structural alignment with the crystal structure of AdoMet-bound yeast Coq5 using PyMOL.

3.3.1. SNVs in Methyltransferase Motifs I and Post-I

The first of the four motifs, motif I, is a Gly-rich motif that makes up the first β -strand followed by an extended turn (Figure 15A) [65]. The homologous residue of T117 within this motif (S122 in yeast) has been shown to interact with the amino group of AdoMet in yeast Coq5, while the remaining residues line the AdoMet binding pocket (PDB: 4OBW) [48]. Mutations occurring in this region, which roughly corresponds to residues L110 to D119, may affect the binding of the AdoMet cofactor and therefore enzyme activity. In total, we identified seven SNVs of interest spanning five distinct amino acid positions in this motif, all of which occur on highly conserved residues (Figures 12 and 15B). D112N, D112H, G116S, G118S, and D119G were reported in Missense3D-DB, and all but D119G were classified as structurally damaging by Missense3D. These point mutations may result in the loss of stabilizing interactions within the protein or with the cofactor, as well as the loss of buried Gly residues, which may destabilize the extended turn. L111F and G118D were reported in gnomAD, with G118D having an unknown clinical significance in ClinVar. G118D was classified as structurally damaging due to similar reasons as the previous group of variants, while L111F was neutral. In yeast, a G120R point mutant, homologous to G115 in human COQ5, was found to be structurally stable but catalytically inactive [80], further supporting the Missense3D classifications. However, it is worth noting that the two SNVs at position 118, G118S and G118D, flank the splice sites between exons 2 and 3 on the 5' and 3' ends, and may affect splicing in addition to protein structure.

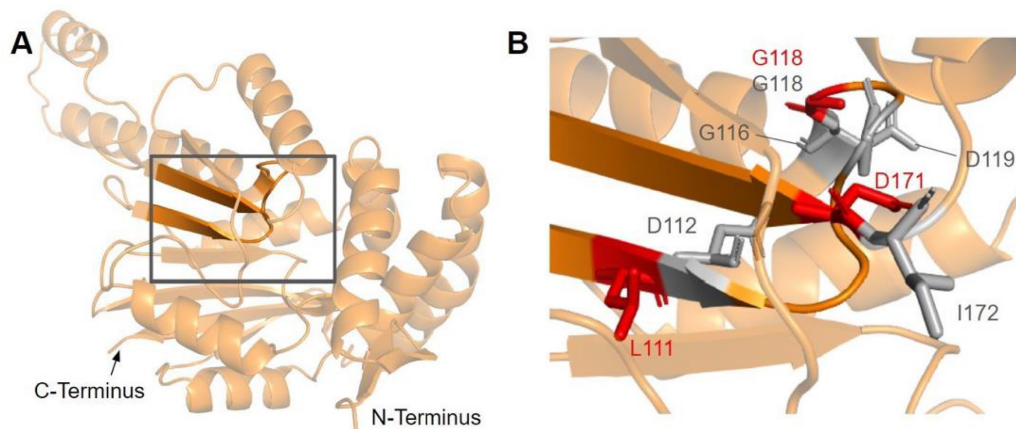


Figure 15. SNVs found in motif I and post-I of COQ5. (A) Motif I (lower β -strand, residues L110 to D119) and post-I (upper β -strand, residues V167 to D171) highlighted on the COQ5 model. The first 47 residues were truncated for simplicity. (B) Locations of SNVs in motifs I and post-I are depicted. Residues are colored according to their corresponding SNVs Figure 12.

After motif I, the second β -strand is partially composed of motif post-I, which contains a highly conserved acidic residue responsible for interacting with the hydroxyl groups on the ribose ring of AdoMet (Figure 15A) [48,65]. We have defined motif post-I as residues V167 to D171, although flanking residues I172 and N173 are also highly conserved. There are two particular SNVs of interest in the motif post-I region found in gnomAD, namely D171G at the conserved acidic residue and I172S adjacent to it, both of which extend into the AdoMet binding pocket and are annotated as AdoMet-binding (Figures 12 and 15B). These mutations likely abolish interaction with the ribose ring of AdoMet, but were not classified as damaging by Missense3D due to the residues being solvent-exposed in the binding pocket of the model.

3.3.2. SNVs in Methyltransferase Motifs II and III

Motif II (D206 to I215) is located in the fourth β -strand (Figure 16A), where residues K209 to D211 are thought to make extensive contacts with motifs I and III to help align the methyltransferase domain [65]. Residues immediately after motif II form a loop that extends into the AdoMet and substrate-binding pockets. This loop region includes A216 and V222, which are homologous to yeast Coq5 residues interacting with AdoMet, as well as R220 and N221, which are thought to interact with the substrate, DDMQH₂ [48]. In motif II and the loop that follows it, there are 15 SNVs of interest spanning eight distinct amino acid positions, all of which are highly conserved (Figures 12 and 16B). D207G, A216V, A215V, R220L, and N221S were reported in Missense3D-DB, although all of them were classified as neutral by Missense3D except for R220L. From gnomAD, we identified variants D207E, D207N, T214S, T214A, I215T, F217L, R220P, R220W, R220Q, and T223I. Of these, T214A, R220P, R220W, and R220Q were classified as deleterious by Missense3D. The most common flags pertain to the loss of buried charges, residue-residue interactions, as well as a change in cavity size (R220). Cavity size likely refers to a change in the substrate binding pocket and is likely to result in a loss of activity. In yeast Coq5, the introduction of G199D (the residue homologous to G218 in human COQ5) resulted in a structurally stable but catalytically inactive mutant that was respiratory-deficient [80], suggesting that mutations in this region are likely pathogenic.

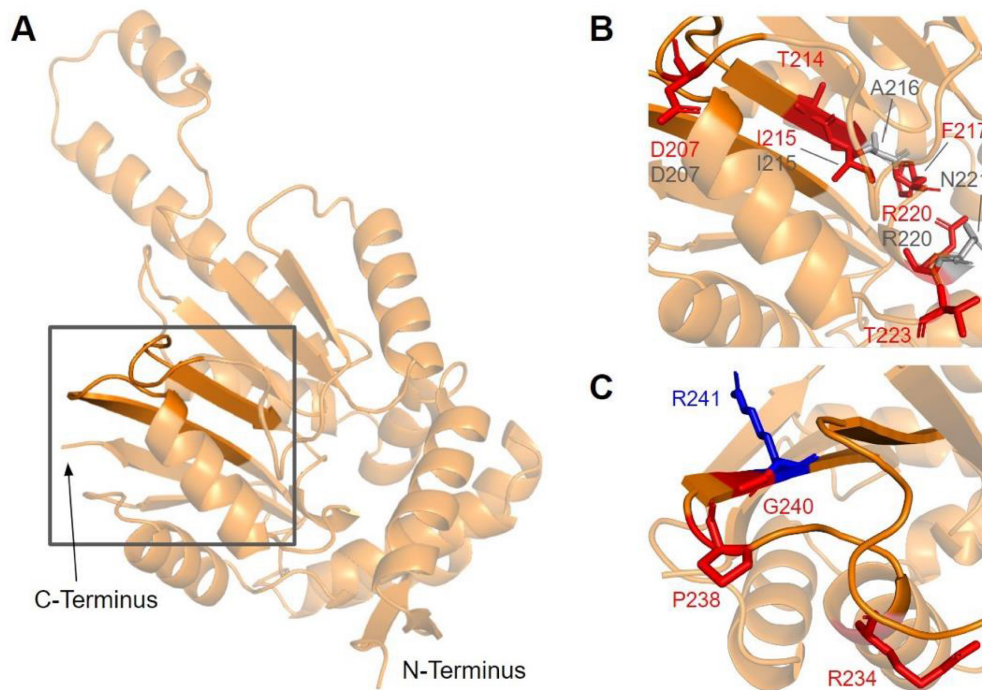


Figure 16. SNVs found in motifs II and III of COQ5. (A) Motif II (upper β -strand, residues D206 to I215) and motif III (lower β -strand, residues V235 to F242) highlighted on the COQ5 model. The first 47 residues were truncated for simplicity. (B,C) Locations of SNVs in motif II (B) and motif III (C) are depicted. Residues are colored according to their corresponding SNVs in Figure 12.

Motif III (V235 to C244) and the residues preceding it are located in a coil that becomes β -strand five (Figure 16A). The residues in this coil are responsible for aligning the residues in β 5 and the active site [65]. From gnomAD, we identified five SNVs of interest spanning four distinct amino acid positions in this region (Figures 12 and 16C). They are R234Q, R234W, P238R, G240E, and R241W, all of which are highly conserved, with the exception of R241W, which is a frequent polymorphism (allele frequency of 4.21×10^{-4}). Only G240E was classified as damaging by Missense3D.

3.3.3. SNVs on the Dimerization Interface

The dimerization interface, as determined from the crystal structures of the yeast Coq5 dimer (PDB: 4OBX and 4OBW), consists of hydrophobic residues homologous to M86, L88, I90, L254, L258, Y262, V266, I267, V269, L270, V273, I274, A275, T315, and I318 [48]. We identified a total of seven SNVs spanning six unique amino acid positions (Figure 17A). Among them, L254F, L254P, V273L, A275T, and T315I were found in gnomAD, while I90V and I318V were found in Missense3D-DB (Figure 17B). None of these variants were flagged as damaging by Missense3D, as the mutant residues were often hydrophobic. It is worth noting that all seven SNVs would appear as surface residues in the COQ5 model, which does not include an oligomeric structure. The effectiveness of Missense3D is limited in this region; thus, we have also provided the SIFT and Polyphen-2 classifications (Supplemental Table S1).

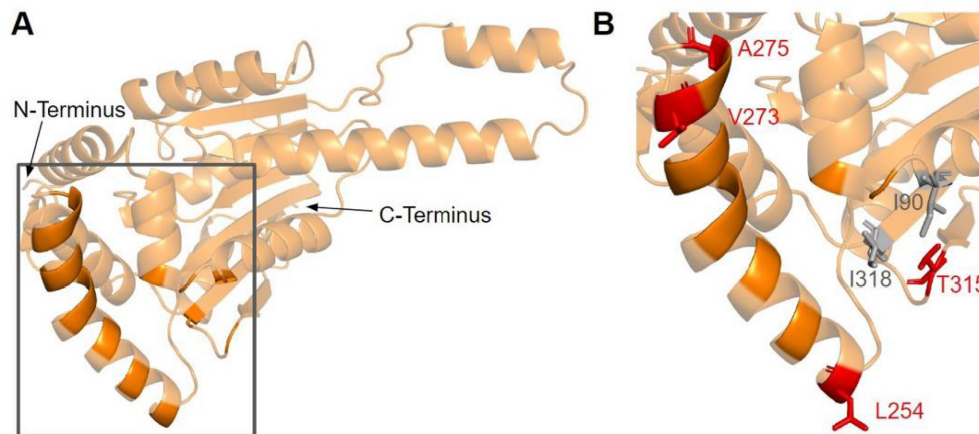


Figure 17. SNVs found in the COQ5 dimerization interface. (A) The dimerization interface of COQ5 largely consists of hydrophobic residues located on the $\alpha 6$ helix (left highlight). The first 47 residues were truncated for simplicity. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 12.

3.3.4. Additional SNVs in Potentially Significant Regions

Two additional regions on COQ5 are worth noting. The first is an insert roughly located at residues K135 to G163, which is found exclusively in vertebrate species. A BLAST search comprising the amino acid sequence of this region gave no significant hits [84]. Three SNVs with allele frequency larger than 1.00×10^{-4} are found in this region: Q139H, A152T, and D160G (Figure 18A). A152T was estimated to have an allele frequency of 1.18×10^{-1} by gnomAD, the most common among all missense SNVs found in the COQ5 coding region. While none of these variants were classified as structurally damaging by Missense3D, this region may have evolved new functions, such as protein-protein interactions, that may be affected by these mutations. The second region of interest is the C-terminus of COQ5. Previous studies in yeast have identified a Coq5 point mutant located four residues away from the C-terminus. This mutant resulted in a partially active enzyme that resulted in a decrease in the steady-state levels of Coq3, Coq4, and Coq5, as well as respiratory deficiency [80]. The underlying cause of this phenotype is unclear, although it does suggest that the C-terminus of COQ5 may play a key role in stabilizing Coq5, whether it be through residue-residue interactions or some other means. Two SNVs, H322N and H322D, are found in this region, located just six amino acids away from the C-terminus (Figure 18B). Residue H322 was predicted to interact with Q294 by Missense3D. Both variants were classified as damaging, for reasons related to the loss of buried charges and hydrogen-bonding interactions.

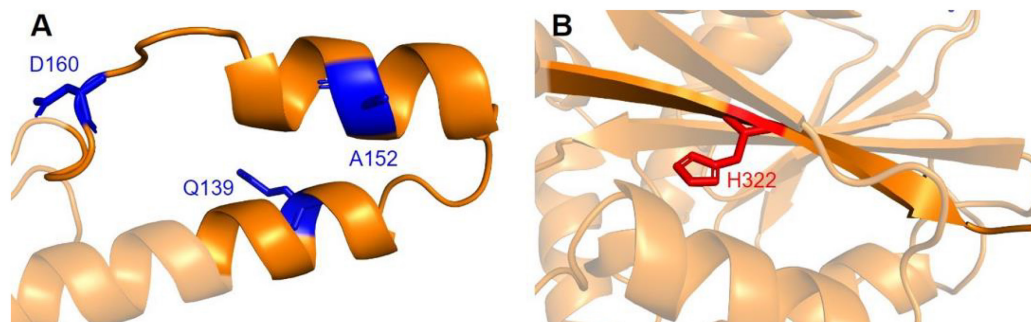


Figure 18. SNVs found in other potentially significant regions of COQ5. Locations of SNVs are depicted in (A) the vertebrate insert (left, residues K135 to G163), and (B) in the C-terminus (right, residues V319 to L327). Residues are colored according to their corresponding SNVs in Figure 12.

3.4. COQ6

COQ6 is characterized as a class A flavoprotein monooxygenase with a tightly bound FAD cofactor [33,85,86]. However, unlike typical flavin-dependent monooxygenases which receive electrons directly via an NAD(P)H coenzyme, NAD(P)H instead delivers electrons to yeast Coq6 via ferredoxin Yah1 (FDX1 and FDX2 are human homologs) and ferredoxin reductase Arh1 (FDXR is a human homolog) [13]. It is not yet known if human homologs FDX1, FDX2, and FDXR perform the same electron transfer roles for COQ6. Coq6 is peripherally associated with the IMM and is responsible for the addition of a hydroxyl group to the C5 position on the CoQ ring precursor, converting HBPB to DHPB (Figure 1A) [33]. While both humans and yeast use 4-HB as a ring precursor for CoQ biosynthesis, yeast cells also utilize para-aminobenzoic acid (pABA) as a ring precursor to CoQ [87]. Ozeir et al. showed that yeast Coq6, in addition to hydroxylating C5, is also able to deaminate the ring C4 position on the intermediate derived from pABA [88]. Yeast *coq6* null-mutants accumulate 3-hexaprenyl-4-hydroxybenzoic acid (HHB) and 3-hexaprenyl-4-aminobenzoic acid (HAB) [13,89]. Yeast *coq6* point mutants that express a stable but inactive Coq6 polypeptide accumulate 3-hexaprenyl-4-hydroxyphenol (4-HP₆) [33]. The equivalent intermediate, 4-HP₁₀, has also been observed to accumulate in human cells harboring a COQ6 disruption [34]. This finding indicates that the C1-decarboxylation and C1-hydroxylation steps can occur prior to the C5-hydroxylation step (Figure 1A). Resolving the order of steps will require an in vitro assay for Coq6 and would also benefit from identification of the enzyme(s) that mediate the decarboxylation and hydroxylation at C1. Expression of human COQ6 partially complements yeast lacking Coq6, demonstrating the usefulness of *S. cerevisiae* as a model organism to study human COQ6 mutations [90].

Mutations in the human COQ6 gene are associated with primary CoQ₁₀ deficiency and are further associated with several disease states, predominantly steroid-resistant nephrotic syndrome (SRNS) and sensorineural hearing loss (SNHL) [29,91–93]. Importantly, supplementation with either 3,4-dihydroxybenzoic acid (3,4-diHB) or vanillic acid (VA) partially restores CoQ biosynthesis in yeast *coq6* null mutants expressing human COQ6 genes harboring pathogenic mutations; such a bypass of the C5-hydroxylation step with VA or 3,4-diHB also occurs when yeast *coq6* null mutants overexpress Coq8 [33,90]. Supplementation with VA may provide a potential avenue of treatment for patients with CoQ₁₀ deficiency due to mutations in the COQ6 gene. However, clinical trials are required to determine both the safety and efficacy of this “bypass” therapy.

Drovandi et al. list 48 patients who likely have pathogenic mutations in COQ6 [91]. While mutations in COQ6 are rare, they result in severe disease phenotypes across multiple physiological systems. A report by Perrin et al. detailed a case study of a Turkish patient born to consanguineous parents with a missense mutation in COQ6 [94]. The patient

presented with end-stage renal failure at five years old and subsequently received an organ transplant. At 17 years old, the patient presented with sudden loss of vision. Genetic analysis of the *COQ6* gene revealed the presence of the SNV A353D. The younger brother of this patient harbored the same homozygous mutation and presented with primary CoQ₁₀ deficiency with SRNS and SNHL. Replacement therapy with idebenone (a hydrophilic short-chain CoQ₁₀ analog) began at an earlier age for the second patient. Doimo et al. expressed the human A353D mutation, amongst others, in *S. cerevisiae coq6* at the corresponding residue A361 [90]. Results indicated that the mutation was hypomorphic, as yeast were viable but showed decreased growth on medium containing a nonfermentable carbon source. A recent study indicates that clusters of the A353D occur in Kazak, Turkish, and Iranian populations, and show a comparatively later disease onset [91]. In contrast, patients with a variant that is predominant in the Middle East (G255R), present with early disease onset, severe phenotype, and higher odds of mortality [91]. Further mutagenesis studies using yeast can help to elucidate the effects of mutations found in patients. In addition to the 13 known pathogenic mutations in *COQ6*, there have also been several reported heterozygous SNVs that may confer deleterious consequences.

From gnomAD, ClinVar, Missense3D-DB, and literature we identified 82 missense SNVs of interest (Figure 19; Supplemental Table S1). Of these variants, 55 SNVs coincided with a highly conserved or functional residue, determined from the multiple sequence alignment and from structural studies on yeast, respectively [49]. Identification of functional residues was performed by in silico prediction of substrate-access tunnels and protein-cofactor interactions [49]. Residues predicted to be functional were subsequently corroborated by site-directed mutagenesis and in vivo functional assays in the yeast model [49]. Of the 27 variants classified as structurally damaging by Missense3D, 21 overlapped with the group of highly conserved or functional residues (Figure 19). From the ClinVar database, seven missense variants were determined to be pathogenic or likely pathogenic. Of those seven variants, three were identified by Missense3D to be structurally damaging. There were 18 SNVs in gnomAD identified as frequent polymorphisms. Intriguingly, one of the frequent polymorphisms T446M (allele frequency of 1.70×10^{-4}) was identified as being structurally damaging by Missense3D.

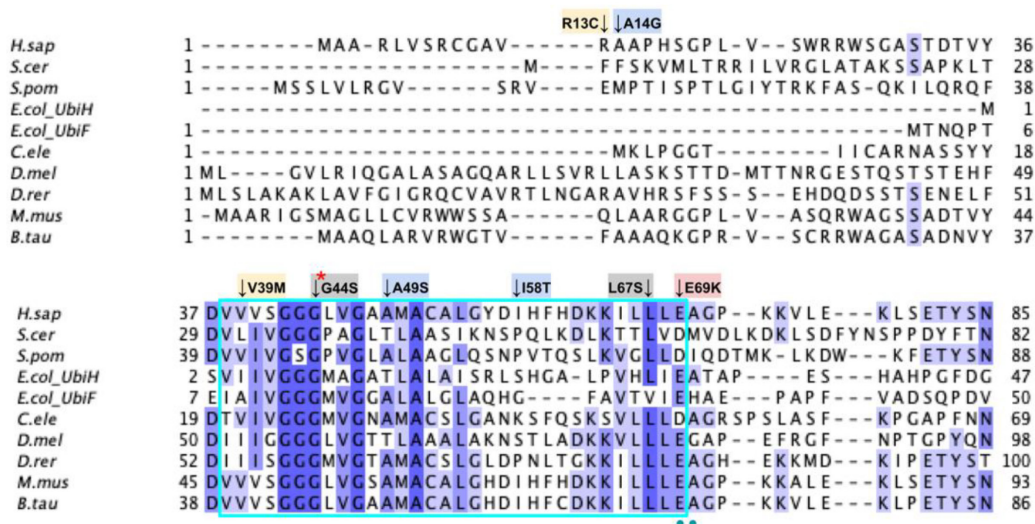


Figure 19. Cont.

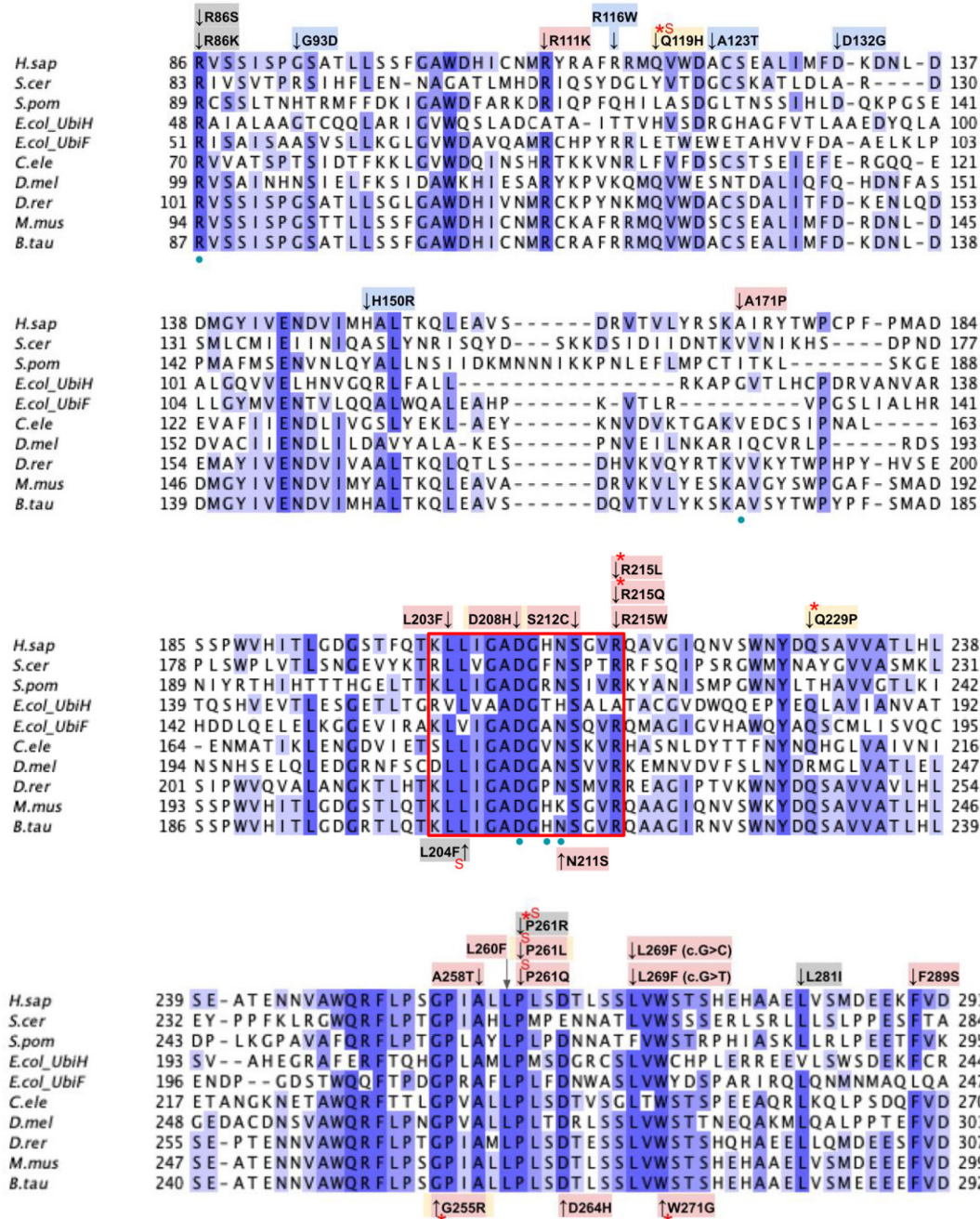


Figure 19. Cont.

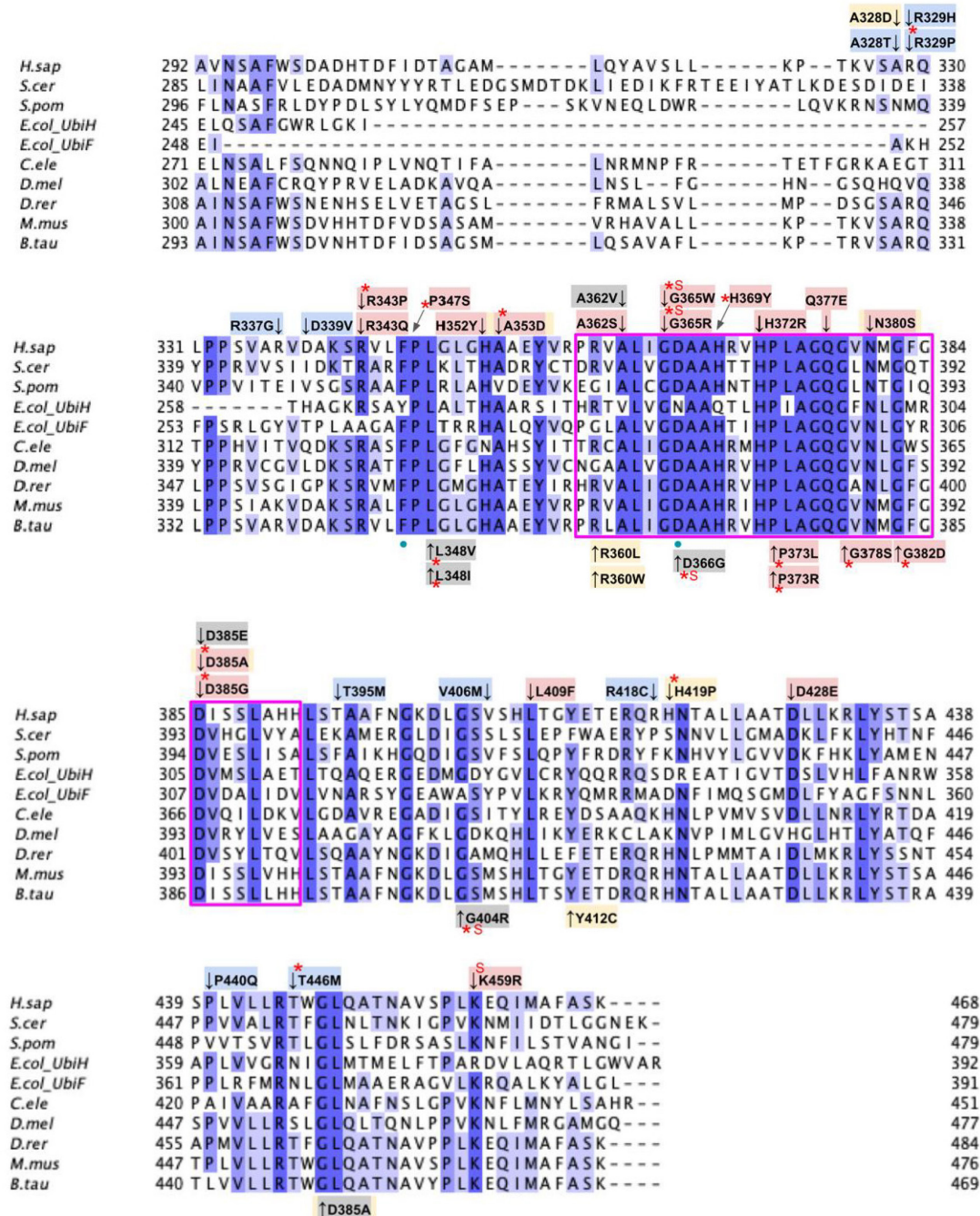


Figure 19. Cont.

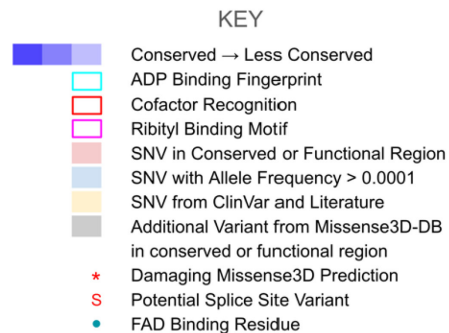


Figure 19. Labeled and annotated multiple sequence alignment of COQ6. Amino acid sequences of COQ6 were analyzed as described in Materials and Methods and include *Homo sapiens* (NCBI accession number NP_872282.1) and homologs in *Saccharomyces cerevisiae* (NP_011771.1), *Schizosaccharomyces pombe* (NP_595401.2), *Escherichia coli* UbiH (NP_417383.1), *Escherichia coli* UbiF (NP_415195.1), *Caenorhabditis elegans* (NP_505415.2), *Drosophila melanogaster* (NP_608934.1), *Danio rerio* (NP_001038869.1), *Mus musculus* (NP_766170.2), and *Bos taurus* (NP_001039558.1). See KEY for descriptions of the figure annotations. The ADP Binding Fingerprint is boxed in cyan, the Cofactor Recognition motif is boxed in red, and the Ribityl Binding motif is boxed in magenta, data from [89]. FAD-binding residues are marked with blue dots, data from [49].

The human COQ6 model was obtained from the AlphaFold Protein Structure Database (Figure 20) [39,54].

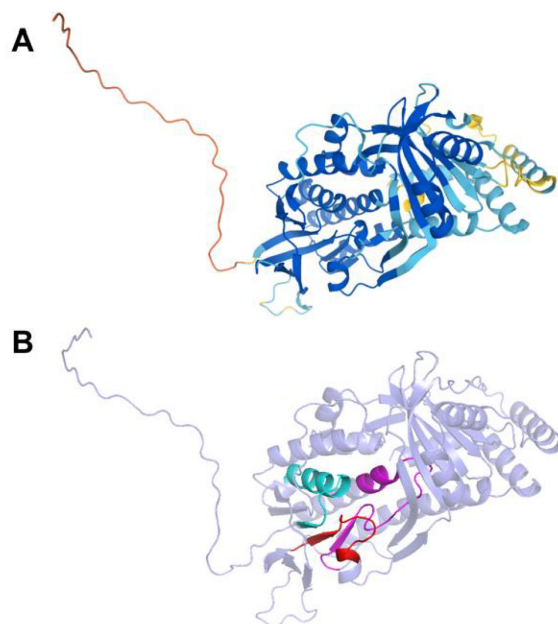


Figure 20. AlphaFold model of single chain of COQ6. (A) Model color corresponds to model confidence as described in Figure 4. Figure was generated by the AlphaFold Protein Structure Database [39,54]. (B) Structural motifs shown in Figure 19 are highlighted.

3.4.1. SNVs in the ADP-Binding Fingerprint

The COQ6 amino acid sequence contains the characteristic GXGXXG fingerprint necessary for binding the ADP-moiety of the FAD cofactor, along with other conserved residues involved in FAD/NAD⁺ recognition [89,95]. The segment forms a characteristic $\beta\alpha\beta$ -fold that binds FAD, which is reflected in the COQ6 multiple sequence alignment and model (Figures 19 and 21A). The $\beta\alpha\beta$ -fold occurs near the N-terminus of COQ6 from residues D37 to E69 (Figure 21A). In this domain, we encounter six SNVs with relevance to this analysis. Mutations occurring in this region may affect COQ6 cofactor-binding capabilities. Two SNVs reported in Missense3D-DB occur at highly conserved residues within the ADP-binding fingerprint: G44S and L67S (Figure 21B). Of these two, G44S was identified by Missense3D as being structurally damaging, due to the replacement of Gly at a bend. Neither mutation has a clinical association listed on ClinVar. In this same motif gnomAD reports a SNV that occurs at a residue identified as FAD-binding: E69K (Figure 21B). This variant is of particular interest because of its potential to disrupt cofactor binding. However, E69K does not have any clinical association with primary CoQ₁₀ deficiency, nor is it predicted to be structurally damaging by Missense3D. Two SNVs reported in gnomAD are identified as frequent polymorphisms: A49S and I58T (Figure 21B). Neither of these variants occurs at highly conserved residues. According to the ClinVar database, A49S has been listed as a benign mutation. There is no ClinVar annotation for I58T. Both of these variants are characterized as neutral by Missense3D. One additional SNV is reported in ClinVar in this region: V39M (Figure 21B). This variant has an uncertain significance label on ClinVar and is characterized as structurally neutral by Missense3D.

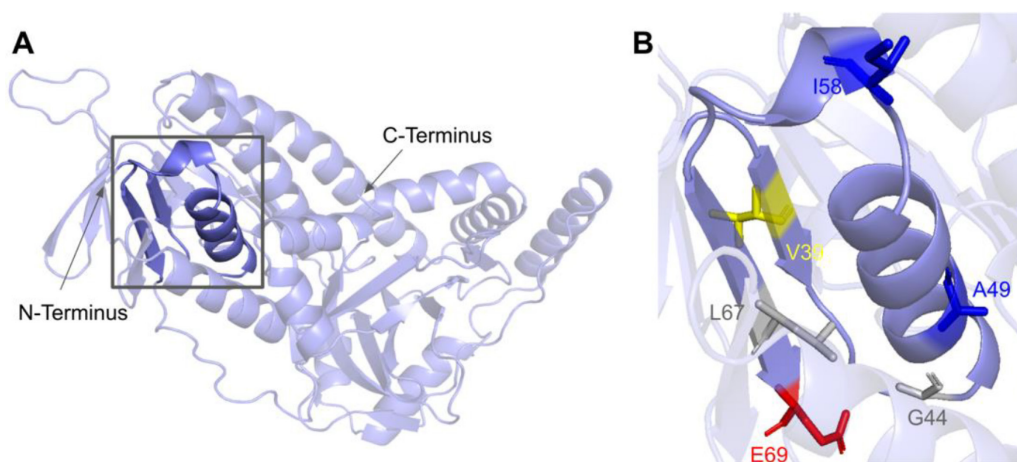


Figure 21. SNVs in the COQ6 ADP-binding fingerprint. (A) The ADP-binding $\beta\alpha\beta$ -fold is highlighted on the COQ6 model and includes residues D37 to E69. Residues 1–35 have been truncated. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 19.

3.4.2. SNVs in the Cofactor Recognition Structure

This second region on the COQ6 polypeptide is suggested to recognize the pyrophosphate moiety of the FAD cofactor [89]. The cofactor recognition structure occurs from residues K202 to R215 (Figure 22A). In this domain, we encounter eight SNVs with relevance to this analysis. Mutations in functional residues contributing to FAD-binding are predicted to diminish COQ6 function, resulting in a deficiency in CoQ₁₀. There are six SNVs reported in gnomAD that affect highly conserved residues within this region:

L203F, N211S, S212C, R215L, R215Q, and R215W (Figure 22B). Of these six variants, R215L and R215Q were reported as structurally damaging by Missense3D. N211S may disrupt catalytic function, as it occurs at a residue identified as FAD-binding and is flagged by Missense3D as a buried charge replaced. However, this mutation does not have any clinical association. There is one additional SNV reported in Missense3D-DB occurring at a highly conserved residue: L204F (Figure 22B). This mutation is predicted to be structurally neutral by Missense3D. L204F does not have a clinical association with CoQ10 deficiency. ClinVar reports one SNV in this region: D208H (Figure 22B). This variant occurs at a highly conserved residue identified as FAD-binding and has a clinical association of unknown significance. This mutation is reported to lead to haploinsufficiency of COQ6, predisposing individuals harboring the mutation to schwannomatosis, which is a rare genetic disorder resulting in multiple benign schwannomas growing on peripheral nerves [96]. However, further studies must be conducted to confirm this link [97].

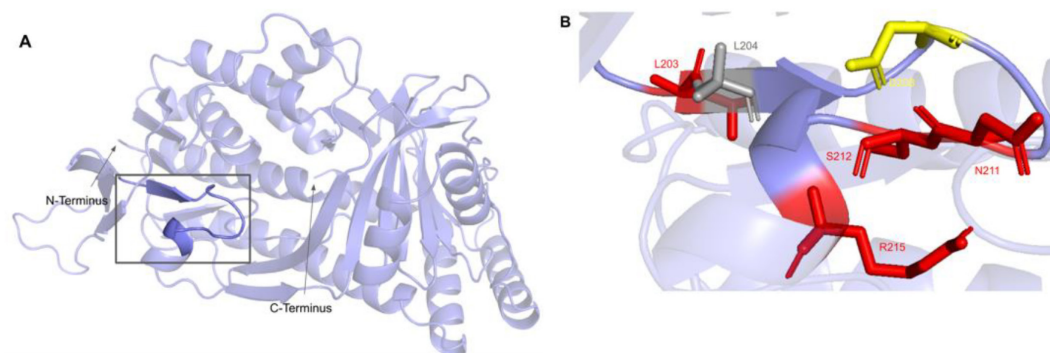


Figure 22. SNVs found in the COQ6 NAD(P)H/FAD recognition sequence. (A) NAD(P)H/FAD recognition structure highlighted on COQ6 model, residues K203 to R215. Residues 1–35 have been truncated. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 19.

3.4.3. SNVs in the Ribityl Binding Motif

The third region of known importance on the COQ6 polypeptide has been implicated in binding to the ribityl moiety on FAD. It contains the consensus fingerprint for ribityl binding, including the conserved Asp necessary to hydrogen bond with O-3 of the ribityl moiety [89,98]. This region is predicted to reside near the putative catalytic region containing the other amino acid fingerprints necessary for FAD-binding. Mutations in functional residues contributing to coenzyme binding are predicted to diminish COQ6 function, resulting in a deficiency in CoQ10.

This region spans residues P359 to H392 (Figures 19 and 23A). In this domain, we encounter 18 SNVs relevant to this analysis. There are 11 SNVs reported in gnomAD that occur in highly conserved residues within this region. Eight of these 11 variants, G365W, G365R, H369Y, P373L, P373R, G378S, G382D, and D385G are reported to be structurally damaging by Missense3D. The high number of variants predicted to be structurally damaging in this region may be indicative of its structural importance to COQ6 integrity. Three additional SNVs are reported in Missense3D-DB to occur at highly conserved residues: A362V, D366G, and D385E (Figure 23B). Of the three, only D366G is predicted to be structurally damaging by Missense3D. This variant is also located at the conserved Asp predicted to bind FAD and may therefore disrupt COQ6 function. None of these mutations have clinical associations.

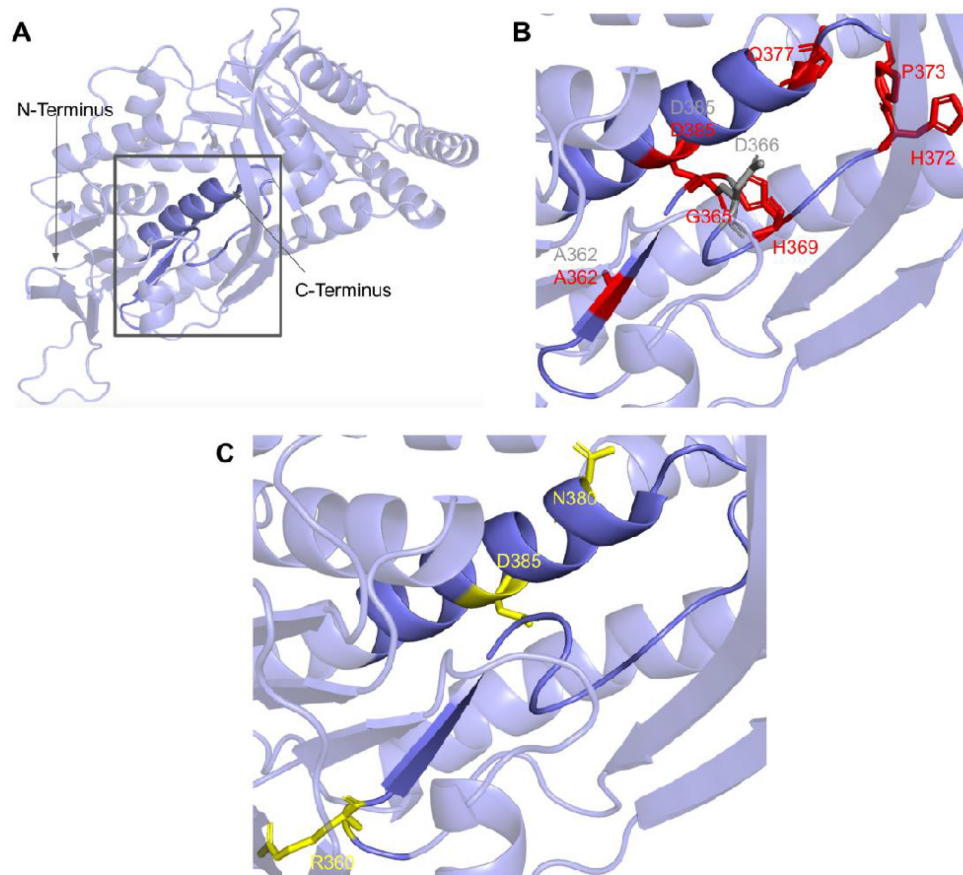


Figure 23. SNVs found in the COQ6 ribityl binding motif. (A) Ribityl binding motif is highlighted on the COQ6 model, from residues P359 to H392. Residues 1–35 have been truncated. (B) Locations of SNVs in conserved or functional regions are depicted. (C) Locations of ClinVar and literature variants are depicted. Residues are colored according to their corresponding SNVs in Figure 19.

In this region, four SNVs are reported in ClinVar and the research literature: R360L, R360W, N380S, and D385A (Figure 23C). R360L is associated with primary CoQ₁₀ deficiency and is labeled as likely pathogenic. R360W was found in a compound-heterozygous patient with the frameshift c.804delC mutation and was determined to be pathogenic [99]. Patients homozygous for R360W have been identified in China and Central/Eastern Europe and have been associated with a higher risk of cardiomyopathy, growth retardation, and neurologic involvement [91]. The N380S variant does not have a condition associated with it but is labeled as likely pathogenic by ClinVar. The D385A variant is reported and has been predicted in silico to be deleterious but has not been associated with any clinical manifestations [100]. This D385A variant is the only one of these four SNVs that is predicted to be structurally damaging by Missense3D.

The most common flags generated by Missense3D for this region concern the change of buried residues. The most consequential changes pertain to the replacement of buried charges and Gly residues. Such variants have the potential to disrupt FAD-binding and protein folding.

3.5. COQ7

COQ7 is characterized as a di-iron carboxylate hydroxylase [13]. It is peripherally associated with the matrix side of the IMM and is responsible for the penultimate step of CoQ biosynthesis, hydroxylating DMQH₂ to DMeQH₂ [101,102]. Addition of 2,4-diHB was shown to restore synthesis of CoQ₆ in the yeast *coq7* null mutant when Coq8 was overexpressed [59]. The overexpression of Coq8 stabilizes the yeast Coq polypeptides that are otherwise degraded in the *coq* null mutants. The use of an alternate ring precursor to “bypass” the blocked Coq7 hydroxylase step in CoQ biosynthesis is similar to the previously described VA bypass of certain yeast *coq6* point mutants, or the *coq6* null mutant overexpressing Coq8 [33,90]. Tests of 2,4-diHB bypass therapy in patient fibroblasts harboring COQ7 mutations indicated that such a bypass was not uniformly successful and seemed to have the most benefit in cells with a more profound deficiency in COQ7 [103]. Hence, the outcome of such bypass therapies is likely to be quite dependent on the nature of the mutations.

Human COQ7 is able to complement yeast lacking Coq7, demonstrating conserved functionality across species as well as the viability of *S. cerevisiae* as a model organism to study human COQ7 mutations [104]. Mutations in the human COQ7 gene are associated with primary CoQ₁₀ deficiency and are further associated with several disease states, predominantly hypertonia and sensorineural hearing loss (SNHL) [29].

From gnomAD, ClinVar, Missense3D-DB, and literature we identified 60 missense SNVs of interest (Figure 24; Supplemental Table S1). Of these variants, 47 coincided with a highly conserved region or functional residue, which were determined from the multiple sequence alignment as well as structural studies on human COQ7 [50,52]. Eleven of the twelve variants classified as structurally damaging by Missense3D were contained in this group (Figure 24). From the ClinVar Database and literature, four missense variants were determined to be pathogenic or likely pathogenic. None of these four variants were identified by Missense3D to be structurally damaging. There were 13 SNVs in gnomAD identified as frequent polymorphisms. One of these, R65C (allele frequency of 2.76×10^{-4}) was identified as being structurally damaging by Missense3D.

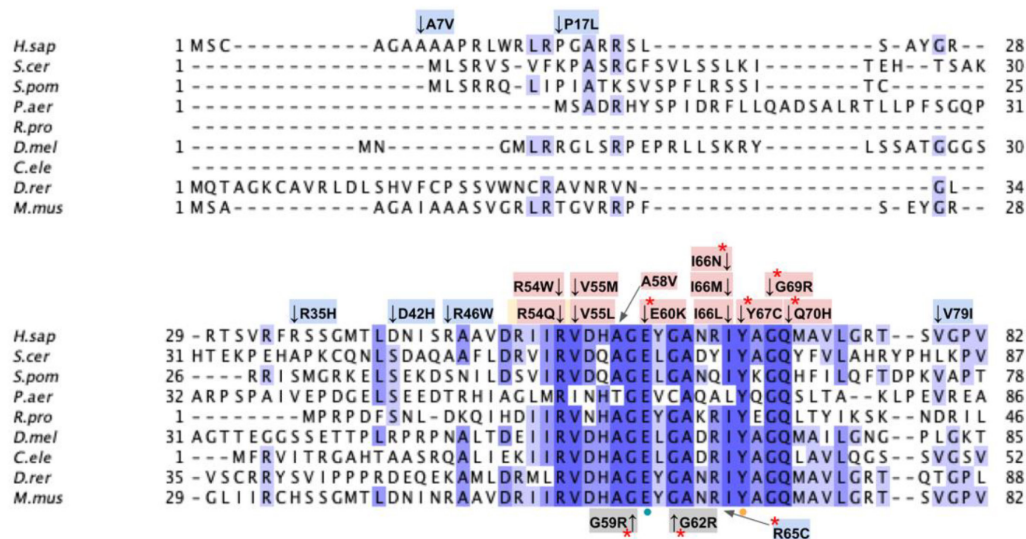


Figure 24. Cont.

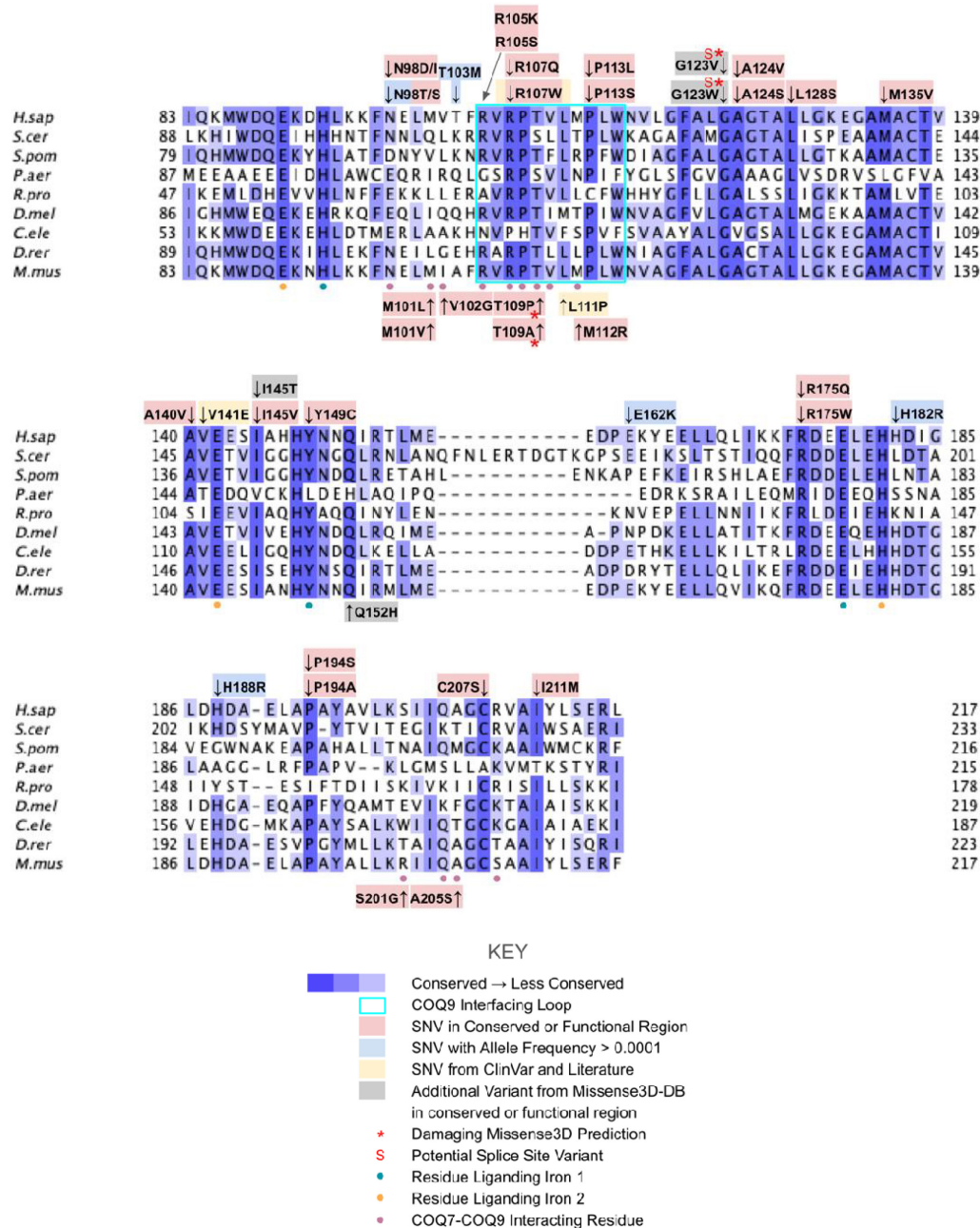


Figure 24. Labeled and annotated multiple sequence alignment of COQ7. Amino acid sequences of COQ7 were analyzed as described in Materials and Methods and include *Homo sapiens* (NCBI accession number NP_057222.2) and homologs in *Saccharomyces cerevisiae* (NP_014768.2), *Schizosaccharomyces*

pombe (NP_595416), *Pseudomonas aeruginosa* (QJ91605.1), *Rickettsia prowazekii* (ADE29699), *Drosophila melanogaster* (NP_651967.2), *Caenorhabditis elegans* (NP_498128.1), *Danio rerio* (NP_001076480.1), and *Mus musculus* (NP_034070.1). See KEY for descriptions of the figure annotations. The COQ9 interfacing loop is boxed in cyan, data from [50]. Residues that ligand iron 1 are marked with blue dots, and those that ligand iron 2 are marked with orange dots, data from [52]. COQ7-COQ9 interacting residues are marked with purple dots, data from [50,52].

The human COQ7 model was obtained from the AlphaFold Protein Structure Database (Figure 25) [39,54]. The structure of a human COQ7:COQ9 complex was recently determined (PDB: 7SSS and 7SSP) [52].

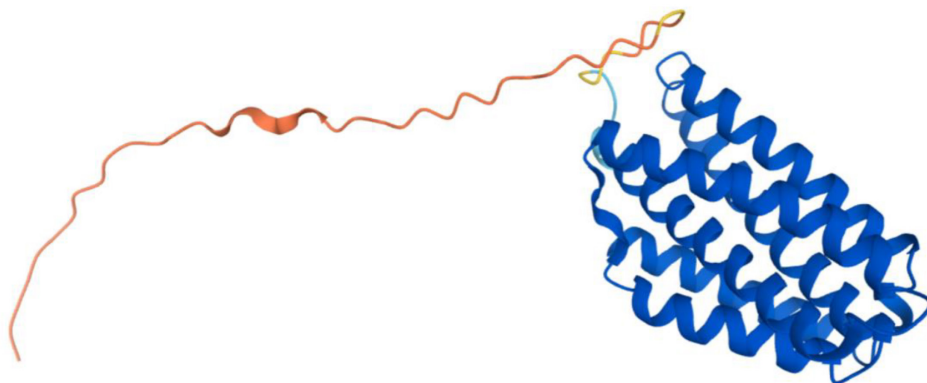


Figure 25. AlphaFold model of single chain of COQ7. Model color corresponds to model confidence as described in Figure 4. Figure was generated by the AlphaFold Protein Structure Database [39,54].

3.5.1. SNVs in a Loop of COQ7 That Interfaces with COQ9

The COQ7 polypeptide contains a loop (residues R105 to W115) that is situated between α -helix 1 and α -helix 2 and is composed predominantly of residues with hydrophobic side-chains. This loop was predicted to interact with COQ9 [50] and was recently observed at the interface between COQ7 and COQ9 in the structure determined for an octameric COQ7:COQ9 complex [52]. The residues in this loop are situated on the outer face of COQ7 (Figure 26A). In this domain, we encounter six SNVs with relevance to this analysis. Mutations in this region have the potential to decrease COQ7-COQ9 hydrophobic, hydrogen-bonding, and salt bridge interactions noted by [52]. There are nine SNVs reported in gnomAD occurring at highly conserved or functional residues in this region (Figure 26B). Of these nine SNVs from gnomAD, only variants at T109 were flagged by Missense3D as structurally damaging (buried H-bond breakage). None of these variants are present as homozygotes in gnomAD, nor are they associated with a deficiency in CoQ10. The variant R107W was identified in ClinVar as having uncertain significance and occurs at a highly conserved residue (Figure 26B). This variant is adjacent to P108, a key residue predicted to interact with COQ9 [50]. Missense3D characterizes R107W as neutral. L111P was identified as a homozygous mutation in a six-year-old girl presenting with SNHL and spasticity by Wang et al. (Figure 26B) [103]. However, the severity of this mutation was determined to be dependent on the presence of another variant, T103M, which is identified here as a frequent polymorphism (allele frequency of 6.24×10^{-1}). Missense3D characterizes T103M as neutral.

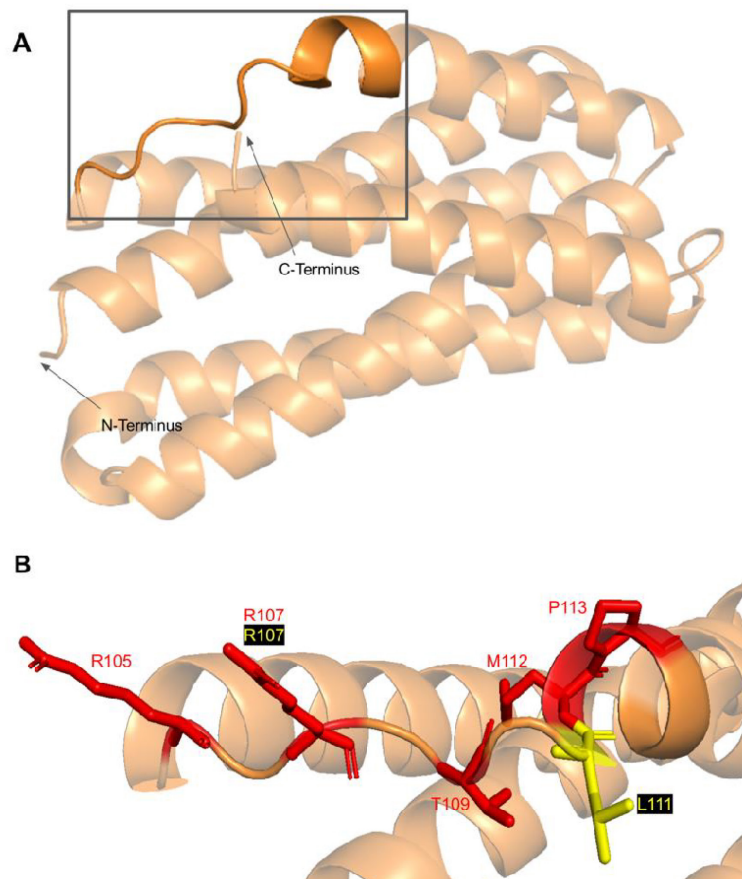


Figure 26. SNVs found in the COQ7 loop that interfaces with COQ9. (A) The loop (residues R105 to W115) that interfaces with COQ9 are highlighted on the COQ7 model. Residues 1–43 have been truncated. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 24.

3.5.2. Residues near the C-terminus of COQ7 That Form Contacts with COQ9

The COQ7:COQ9 complex co-crystallized with a core of phospholipids, suggesting that membrane association is crucial for complex formation [52]. Four residues located in the C-terminal α -helix 6 make additional contacts with COQ9: S201, Q204, A205, and R208. It seems possible that the variant C207S, nestled among these residues and occurring at a highly conserved Cys, might impact the association of COQ7 with COQ9. There are also two variants that occur at reported interacting residues: S201G and A205S. Both variants are characterized by Missense 3D as neutral but may have the potential to disrupt COQ7:COQ9 interaction.

3.5.3. SNVs in the Predicted Iron-Liganding Motif

COQ7 was first identified as a di-iron protein with the motif $E X_{n1} EXXH X_{n2} E X_{n3} EXXH$, where n represents a variable number of connecting residues [51]. These residues form carboxylate ligands that are predicted to bind to two Fe (II) atoms located within the four-helix bundle of COQ7 [101]. Using three-dimensional mapping of their COQ7 cryo-EM

structure, Manicki et al. suggested that the motif E X₆ Y X₂₂ E X₂ H X₄₈ E X₆ Y X₂₈ E X₂ H is functionally important in forming these di-iron carboxylate ligands [52]. However, it is important to note that the structure determined for COQ7 did not possess metals, hence the residues that function to ligand the di-iron are surmised based on structural similarity to other di-iron carboxylate proteins [52]. SNVs at or near residues in the motif are predicted to disrupt the ability of COQ7 to ligand iron atoms. We encounter three SNVs at or near residues in the predicted di-iron-liganding motif. G59R and E60K occur at or adjacent to E60, which ligands iron 1 (Figure 27B). G59R was reported in Missense3D-DB and was characterized as altering a cavity by Missense3D. This is likely to reduce substrate access to the active site. E60K was reported in gnomAD and was characterized as switching a buried charge by Missense3D. It is important to note that Missense3D does not take into account the roles played by residues that act as metal ligands when making predictions about functionality.

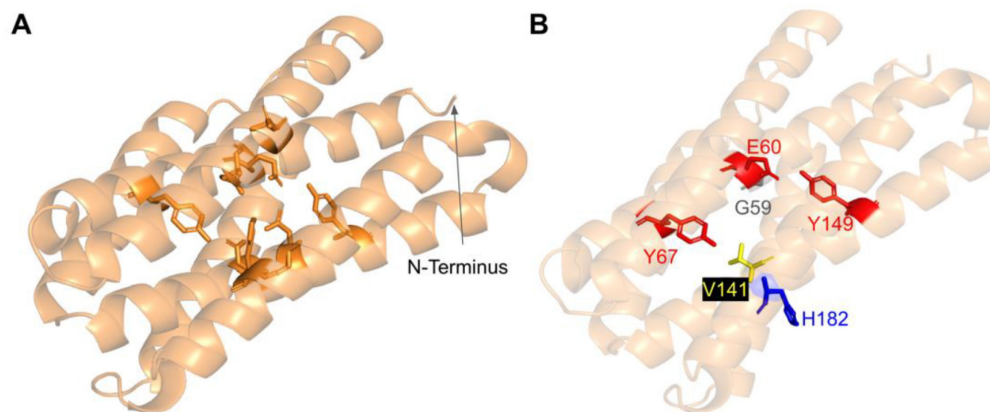


Figure 27. SNVs found in COQ7 residues that are adjacent to or that ligand the iron atoms. (A) The residues predicted to ligand the Fe (II) atoms are depicted in orange on the COQ7 model; E60, Y67, E90, H93, E142, Y149, E178, and H181. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 24. Residues 1–43 have been truncated in both panels.

The V141E variant occurs adjacent to E142, which is predicted to ligand iron 2 (Figure 27B). This variant was reported in the ClinVar database and is labeled as deleterious. Missense3D characterizes this variant as neutral. It is likely that V141E disrupts ligand formation with the iron.

3.5.4. SNVs Predicted to Impact Redox Chemistry of COQ7

COQ7 utilizes NADH to reduce the iron atoms, which then activate O₂ to perform the hydroxylation step of the DMQ substrate [101]. Several of the residues predicted to act as ligands for the two iron atoms (E60, H148, Y149) are thought to mediate a sequential electron-proton-electron relay [101], and a DMQ quinone may act as a conduit between NADH and the di-iron site [105]. Intriguingly, Manicki et al. observed that the NADH cofactor is localized adjacent to a water-filled channel formed by the triad [52]. The variants E60K and Y149C discussed previously (Figure 27) likely disrupt this redox chemistry.

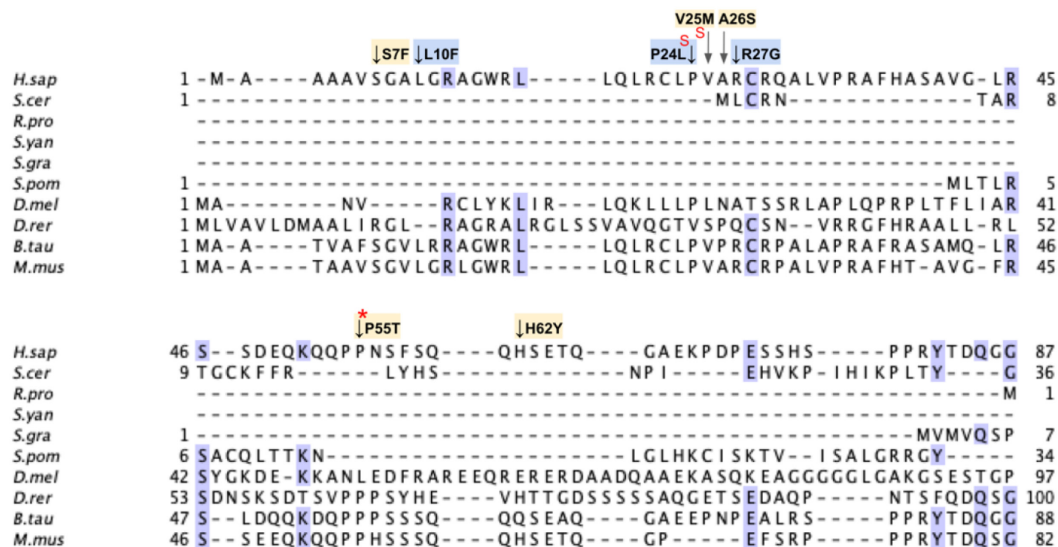
3.6. COQ9

COQ9 is peripherally associated with the IMM and is shown to be essential for CoQ synthome formation and stabilization in both human and yeast cells [50,106]. COQ9 homologs play slightly different roles in different organisms. Coq9, the yeast COQ9 homolog,

plays a supportive role in the hydroxylation steps mediated by Coq6 and Coq7 [13]. Certain *coq9* yeast mutants accumulate demethoxy-Q (DMQ), the penultimate intermediate of the CoQ biosynthetic pathway [107]. In humans, COQ9 plays a supportive role in the COQ7 hydroxylation of DMQ in addition to the stabilization of the CoQ synthome [25]. Patients with deficiencies in COQ9 have been shown to accumulate DMQ₁₀, the same intermediate that accumulates in COQ7-deficient cells [108].

To date, pathogenic cases of genetic variants in the *COQ9* gene have been identified in seven patients across four families [29]. While the determination of pathogenic variants in *COQ9* is rare, mutations in the *COQ9* gene are associated with primary CoQ₁₀ deficiency and are further associated with several disease states, predominantly encephalomyopathy and an autosomal-recessive neonatal-onset CoQ₁₀ deficiency [29]. Low levels of CoQ₁₀ in human cells resulting from *COQ9* deficiency can be partially restored by treatment with 2,4-diHB or VA [109]. For 2,4-diHB, the addition of the hydroxyl group at the 2-carbon position allows it to bypass the COQ7 hydroxylation step in COQ7-deficient cells. VA was shown to increase the production of CoQ₁₀ in COQ9-deficient cells [109]. Neither 2,4-diHB nor VA have been used as treatments in patients.

From gnomAD, ClinVar, and Missense3D-DB, we identified 52 missense SNVs of interest (Figure 28; Supplemental Table S1). Of these variants, 17 coincided with a highly conserved region or functional residue, which were determined from the multiple sequence alignment as well as structural studies on human COQ9 [50,52]. Two of the twelve variants classified as structurally damaging by Missense3D were contained in this group (Figure 28). From the ClinVar Database and literature, 11 missense variants were determined to be pathogenic or likely pathogenic. Two of these variants (P55T and H148P) were identified by Missense3D to be structurally damaging. There were 17 SNVs in gnomAD identified as frequent polymorphisms. Two of these, N252K and M281V (allele frequencies of 1.41×10^{-4} and 1.70×10^{-4} , respectively), were identified as being structurally damaging by Missense3D.

Figure 28. *Cont.*

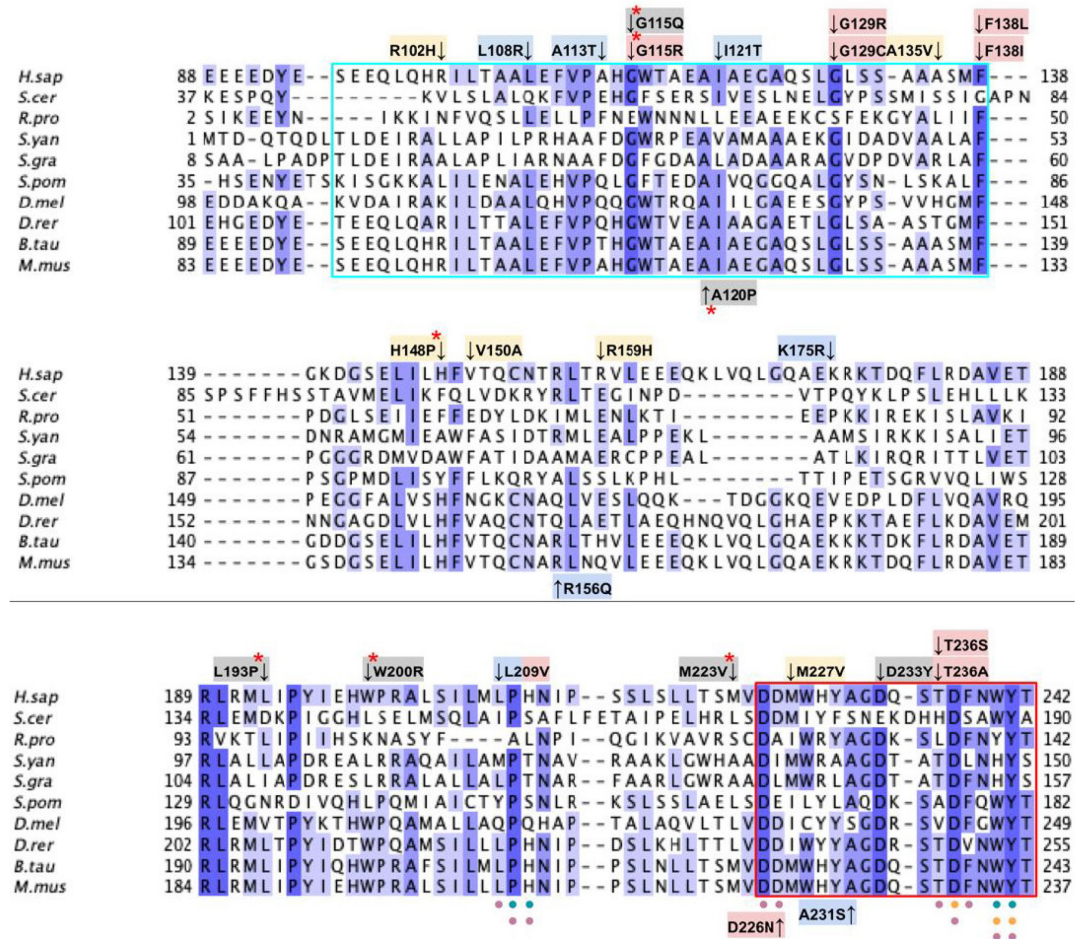


Figure 28. Cont.

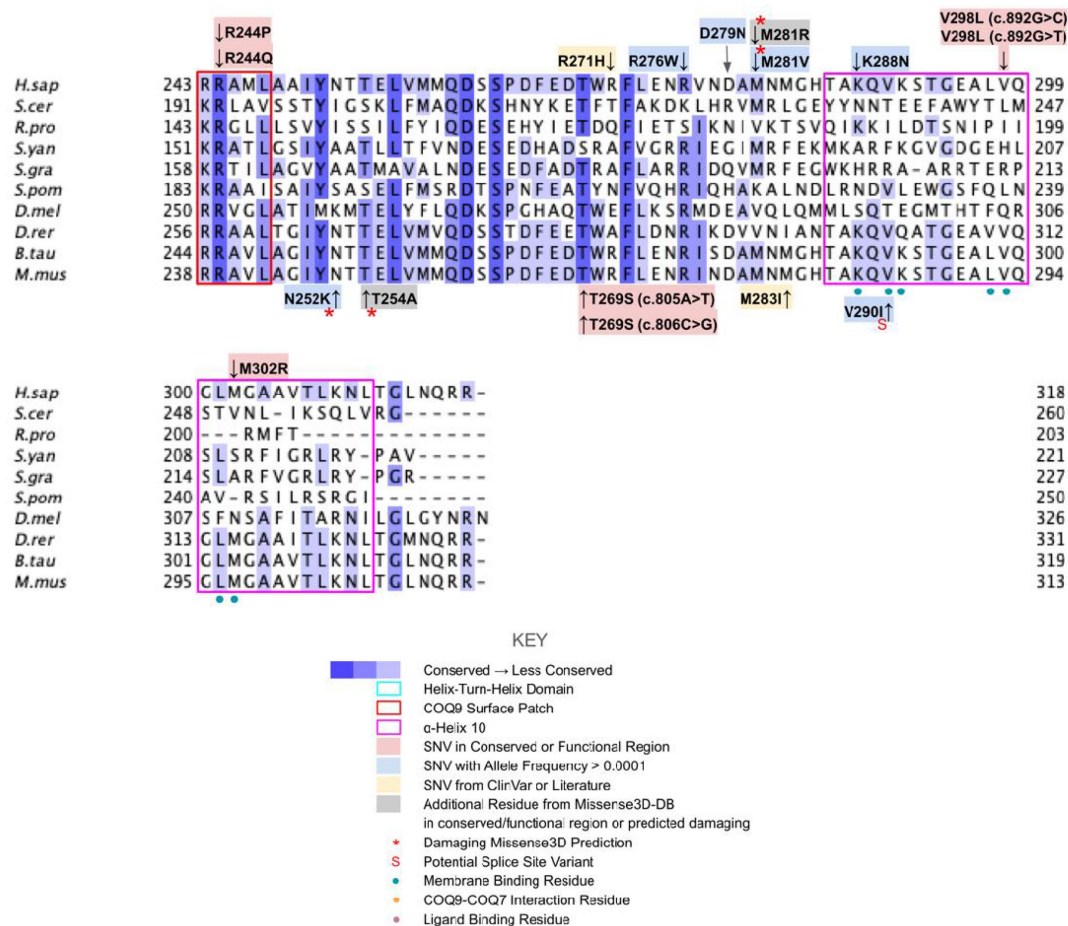


Figure 28. Labeled and annotated multiple sequence alignment of COQ9. Amino acid sequences of COQ7 were analyzed as described in Materials and Methods and include *Homo sapiens* (NCBI accession number NP_064708.1) and homologs in *Saccharomyces cerevisiae* (QHB10353.1), *Rickettsia prowazekii* (WP_004596292.1), *Sphingomonas yanoikuyae* (EKU74250.1), *Sphingopyxis granuli* (WP_082737038.1), *Schizosaccharomyces pombe* (NP_594426.1), *Drosophila melanogaster* (NP_724594.1), *Danio rerio* (NP_001092216.1), *Bos taurus* (NP_001039767.1), and *Mus musculus* (NP_080728.1). See KEY for descriptions of the figure annotations. Residues in the HTH domain are boxed in cyan and COQ9 surface patch in red, data from [25]. COQ7 interaction residues are marked with orange dots, data from [52]. Ligand-binding residues are marked with purple dots, data from [50].

The human COQ9 structure determined by X-ray diffraction at 2.00 Å resolution by Lohman et al. (PDB: 6AWL) was used as a model for visualizing the protein in PyMOL [50]. The COQ9 protein exists as a dimer with a hydrophobic cavity. Two co-crystallized molecules are visible in this structure: phosphatidylethanolamine and bis-tris (Figure 29A). A second COQ9 structure, determined by X-ray diffraction at 2.00 Å resolution by Lohman et al., PDB: 6DEW, co-crystallized with various isoprenes (Figure 29B) [50]. Additionally, Lohman et al. expressed COQ9^{ΔN79} (lacking the amino-terminal 79 residues)

in *E. coli* and found six co-purified enriched lipids including CoQ₈, menaquinone-8, and 2-octaprenyl phenol, which had the highest intensity signal of any lipid.

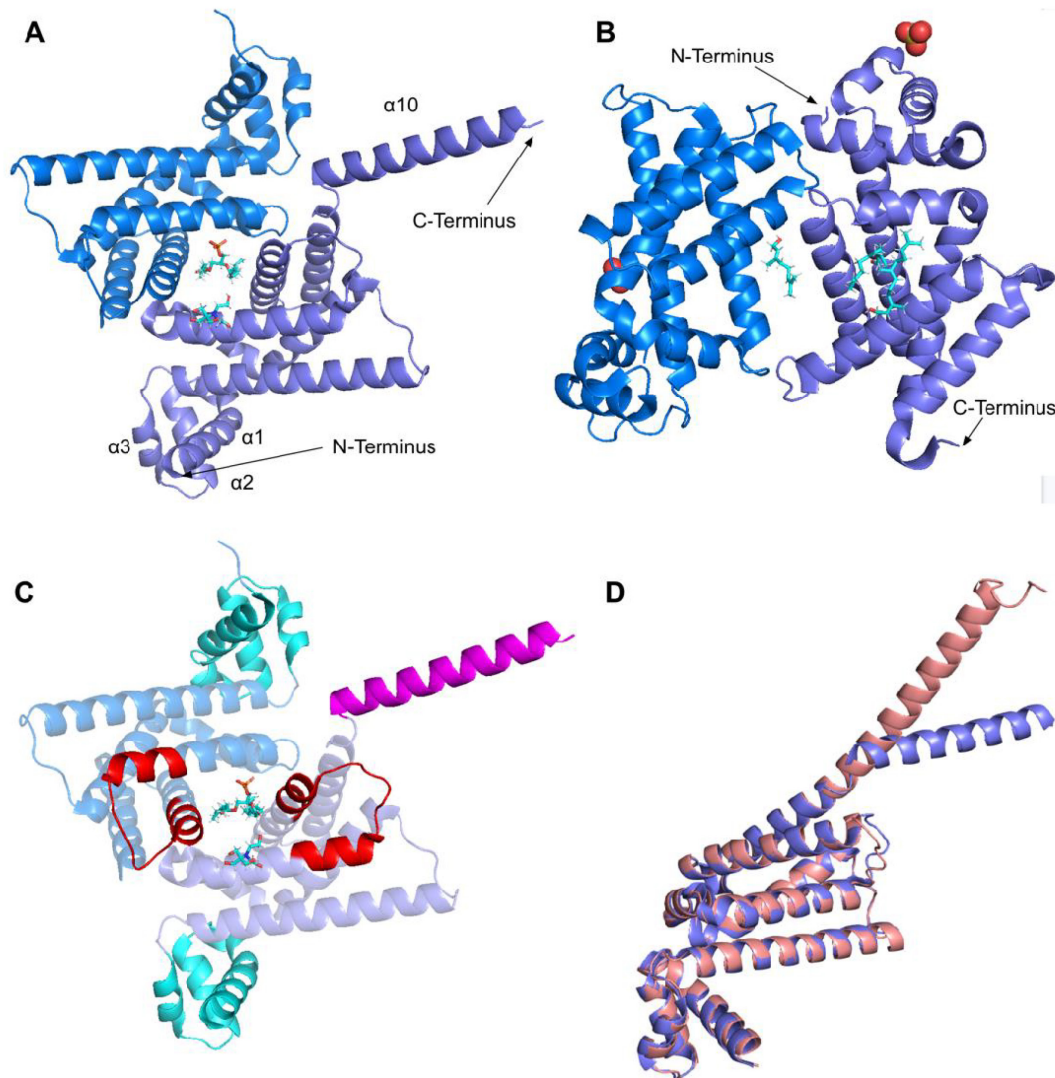


Figure 29. Comparison of existing structures of COQ9 and the AlphaFold model of COQ9 (A) PDB: 6AWL structure of COQ9 Δ N79 with co-crystallized phosphatidylethanolamine and bis-tris shown in cyan, data from [50]. Chain A colored in purple, chain B colored in blue. (B) PDB: 6DEW structure of COQ9 Δ N79 Δ C36 with co-crystallized geraniol, cis-trans-farnesol, trans-trans-farnesol, and cis-cis-farnesol shown in cyan and sulfate molecules shown as ball structures; Chain A colored in purple, chain B colored in blue, data from [50]. (C) Structural motifs shown on 6AWL structure. Colors match Figure 28. (D) Structural alignment of 6AWL chain A shown in purple with the AlphaFold model obtained from the AlphaFold Protein Structure Database [39,54], shown in pink.

This indicates COQ9 as lipid-binding and its hydrophobic pocket as a potential binding site. Structural motifs noted in Figure 28 are designated on the 6AWL structure (Figure 29C). A model for human COQ9 was obtained using the AlphaFold Protein Structure Database (Figure A4) [39,54]. This model shows 79 residues at the N-terminus that the crystal structure for COQ9 (PDB: 6AWL) lacked (Figure 29D).

3.6.1. SNVs in the Helix-Turn-Helix Domain

COQ9 has been determined to have structural homology to proteins in the TetR family of regulators (TFRs) [25]. TFR proteins are typically found in prokaryotes with small-molecule binding capability, allowing many in the family to regulate transcription [110]. All TFR proteins consist of an N-terminal helix-turn-helix (HTH)-domain DNA binding motif consisting of α -helices 1–3, or residues E91–F138 (Figure 30A) and a larger C-terminal ligand-binding motif consisting of α -helices 4–9 (Figure 29A). However, COQ9 is not predicted to bind DNA, making it an atypical TFR protein [25].

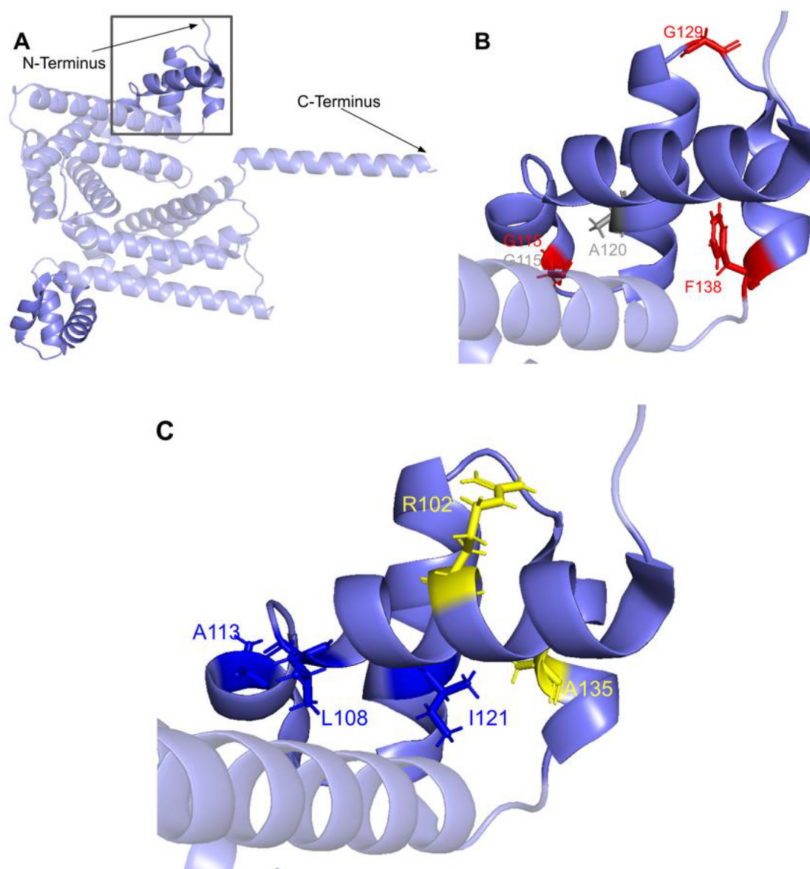


Figure 30. SNVs in the HTH-domain of COQ9. (A) N-terminal HTH-domains highlighted on the COQ9 dimer structure (PDB:6AWL), corresponding to residues S95 to F138 and D92 to F138, on chains A and B, respectively. (B) Locations of SNVs in conserved or functional regions are depicted. (C) Locations of ClinVar variants and frequent polymorphisms are depicted. Residues are colored according to their corresponding SNVs in Figure 28.

In the COQ9 HTH domain, we encounter twelve SNVs with relevance to this analysis. There are five SNVs reported in gnomAD occurring at highly conserved residues in this segment of amino acids: G115R, G129R, G129C, F138L, and F138I (Figure 30B). Of these five SNVs from gnomAD, only G115R was flagged by Missense3D as damaging. None of these variants are present as homozygotes in gnomAD, nor are they associated with a deficiency in CoQ₁₀. Additionally, there are two SNVs reported in Missense3D-DB that are predicted to be structurally damaging by Missense3D: G115Q and A120P.

Three SNVs reported in gnomAD are identified as frequent polymorphisms: L108R, A113T, and I121T (Figure 30C). Only one variant, L108R, occurs at a highly conserved residue. According to the ClinVar database, one report states that L108R is likely benign whereas four others list an uncertain significance. Two of the four uncertain significance interpretations list primary CoQ₁₀ deficiency as the associated condition. The L108R variant is of particular interest because of its frequency and possible pathogenicity; however, no homozygotes have been reported to date. A113T has been determined to be a likely benign SNV. I121T occurs at a semi-conserved residue, and there are conflicting reports ranging from being likely benign to having uncertain significance. All of these variants are characterized as neutral by Missense3D. Two additional SNVs are reported in ClinVar in the HTH-domain: R102H and A135V (Figure 30C). Both of these variants potentially lead to primary CoQ₁₀ deficiency; however, the association must be investigated further in order to elucidate a certain link. Both SNVs are characterized as neutral by Missense3D.

3.6.2. SNVs in the COQ9 Surface Patch

The second motif present in typical TFR proteins is a C-terminal ligand-binding domain [110]. The COQ9 surface patch is comprised of several highly conserved residues necessary for interaction with COQ7, ranging from residues D225 to L247 [25]. The surface patch resides near the lipid-binding pocket (Figure 31A). Mutagenesis of conserved residues in this region of COQ9 and co-purification with COQ7 has been shown to disrupt COQ9-COQ7 interaction, resulting in reduced production of CoQ and accumulation of the COQ7 substrate DMQ. Thus, we predict that mutations in highly conserved regions along the surface patch may result in either disruption of protein-protein interaction within the CoQ synthome or destabilization of the COQ9 protein itself. Variants in this region are not likely to disrupt protein stability, as these are solvent-exposed and will thus appear neutral to Missense3D.

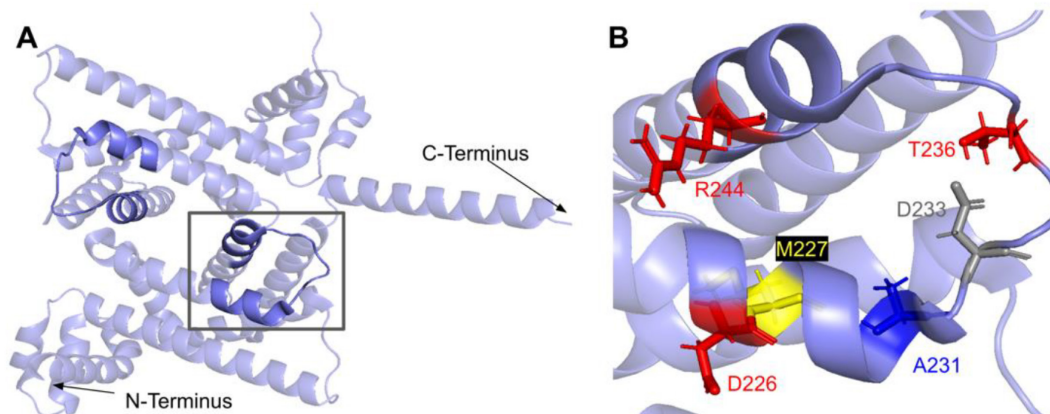


Figure 31. SNVs found in the COQ9 surface patch. (A) Surface patch highlighted on the COQ9 dimer (PDB:6AWL), corresponding to residues D225 to L247. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 28.

In COQ9, the surface patch stretches from the end of α -helix 7 and the beginning of α -helix 8. In this domain, we encounter eight SNVs with relevance to this analysis. There are five SNVs reported in gnomAD occurring at highly conserved or functional residues in this segment of amino acids (Figure 31B). None of these mutations are predicted to be structurally damaging by Missense3D. None of these mutations has a clinical association with CoQ₁₀ deficiency.

There is one additional SNV reported in Missense3D-DB occurring at a highly conserved residue: D233Y. This mutation is predicted to be structurally neutral by Missense3D. D233Y does not have a clinical association with CoQ₁₀ deficiency. Another SNV reported in gnomAD is identified as a frequent polymorphism, A231S. This variant does not occur at a highly conserved residue. It is predicted to be neutral by Missense3D and has been labeled as likely benign by ClinVar. There is one SNV reported in ClinVar in the surface patch region, M227V (Figure 31B). This variant is associated with primary CoQ₁₀ deficiency; however, it has been labeled as having unknown significance in ClinVar. M227V is characterized as neutral by Missense3D.

3.6.3. SNVs in α -Helix 10

α -helix 10 (α 10), a C-terminal amphipathic helix not present in typical TFR proteins, has been found to have importance in membrane binding [50]. Lohman et al. found that COQ9 binds the matrix side of the IMM in a series of five steps. In the second step, the hydrophobic residues along α 10 associate with the membrane phospholipids. Mutating these hydrophobic residues to Ser significantly reduced liposome association. We predict that, despite low evolutionary conservation of residues along α 10, mutations occurring at residues implicated in membrane binding may result in reduced membrane association and lower levels of synthome formation, thus resulting in lowered CoQ production.

α 10 occurs at the C-terminus of COQ9 from T286 to R311 (Figure 32A). In this domain, we encounter five SNVs with relevance to this analysis. Three SNVs reported in gnomAD occur at residues implicated in membrane binding: V298L (c.892G>C and c.892G>T) and M302R (Figure 32B). Both variants are characterized as neutral by Missense3D and neither are clinically associated with primary CoQ₁₀ deficiency. For V298L, the SNV mutates a hydrophobic residue to another hydrophobic residue, minimizing the impact of the change. For M302R, the SNV mutates a hydrophobic residue to a positively charged residue, which may potentially have a large impact on membrane binding ability. There are two SNVs reported in gnomAD that were identified as frequent polymorphisms: K288N and V290I. Both variants are characterized as neutral by Missense3D and K288N is labeled as benign by ClinVar. V290I did not appear in ClinVar.

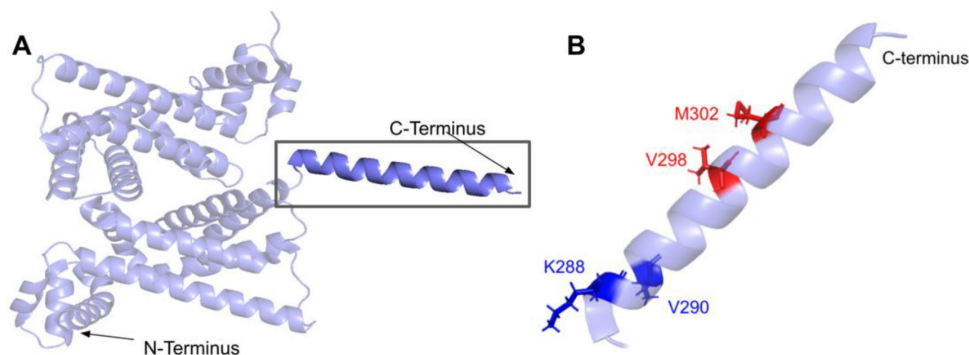


Figure 32. SNVs found in α 10 of COQ9. (A) α 10 highlighted on COQ9 dimer structure (PDB: 6AWL), corresponding to residues T286 to L311. (B) Locations of SNVs are depicted. Residues are colored according to their corresponding SNVs in Figure 28.

4. Discussion

Herein, we analyzed the functional and structural impacts of missense SNVs in *COQ3–COQ7* and *COQ9*. From a large pool of genome and exome sequencing data (gnomAD and Missense3D-DB), as well as clinically documented variants (NCBI ClinVar and literature), 429 variants were selected; these consisted of SNVs in conserved and functional regions, with potential clinical significance or with high allele frequency. Of these, 115 SNVs were classified as potentially structurally damaging by the Missense3D server. AlphaFold models were used for classification wherever crystal structures were not available. As expected, we found frequent polymorphisms to be less deleterious than those found in highly conserved or functional residues. The mutation classifiers used, Missense3D, SIFT, and PolyPhen-2, often had disagreements with their respective assessments. Some of this disagreement can be attributed to the assessment criteria used by each classifier. Missense3D is more stringent in its structural consequence criteria whereas the SIFT and PolyPhen-2 algorithms are less conservative when classifying a mutation as deleterious. Nevertheless, when the data obtained across classifiers are compared in the context of function, one can make an educated prediction based on the considerations described here.

It is imperative to note that predicted models may not accurately reflect the true physiological structures. The dynamic residue-residue interactions between polypeptides may result in different local conformations that are not represented in the individual structural models, including disordered regions. A further consequence of this limitation is that mutation classifiers such as the ones used in this study cannot predict the extent to which the activity of an enzyme is affected. Additionally, mutation classifiers may underestimate the deleteriousness of certain mutations, such as point mutations within binding pockets deemed “benign” that may impair essential cofactor or substrate binding, polymorphisms within the mitochondrial targeting sequence that may affect localization, or surface mutations that may alter protein-protein interaction interfaces. In the case of Missense3D, it is currently unable to model mutations at a protein-protein or protein-ligand interaction interface, as additional polypeptide chains or ligands in the provided structure are removed during analysis. One example of a mutation that was overlooked by Missense3D is the D208H mutation in *COQ6*, which occurs at a putative FAD-binding residue. This mutation has been associated with schwannomatosis in a family of patients [96]. While this linkage has been contested, it has been shown that human *COQ6* harboring this mutation is unable to rescue *coq6* null yeast, suggesting a loss of function. However, Missense 3D had classified the mutation as neutral.

Differential gene expression in distinct tissues may also account for pathogenic effects that cannot be characterized solely by predicted structures. For example, mutations associated with the atypical kinase *COQ8B* present a perplexing case where the pathogenic phenotypes appear to be kidney-specific [29]. While the methods described in this study can identify potentially pathogenic SNVs, they cannot predict the extent to which a structurally destabilizing mutation decreases protein levels in a given tissue, nor whether a resulting decrease is sufficient to affect tissue function. It is also worth noting that distinct cell types may also be differentially impacted by CoQ deficiency due to particular energy demands or exposure to oxidative damage.

Despite these limitations, multiple sequence alignments and structural models have revealed highly conserved and functionally relevant residues, which allowed us to narrow our search in identifying variants with potential clinical significance. The pipeline used in this work may also serve as a scaffold for future SNV studies. Multiple sequence alignments can help identify highly conserved regions of unknown function such as post-translational modification sites. Meanwhile, structural models can be used in computational studies to predict protein-protein interaction interfaces as well as the effect of SNVs on ligand binding and catalysis. All of these studies are invaluable in elucidating the regulatory mechanisms of CoQ biosynthesis. Furthermore, our pipeline provides a structural basis for analyzing existing pathogenic mutations. For example, Ling et al. identified a patient with a homozygous mutation G124S in *COQ4* inherited from heterozygous parents. Skin

fibroblasts isolated from the patient exhibited low CoQ₁₀ content [111]. A separate study found another patient homozygous for the G124S allele with poor cardiac contractility and developmental delays [71]. In silico analyses from Missense3D, SIFT, and Polyphen-2 all correctly predicted the deleterious nature of this mutation. In particular, Missense3D classified the mutation as damaging due to the replacement of a buried Gly, suggesting deleterious structural consequence. Additionally, this pipeline can be combined with other publicly available high-throughput data, such as gene expression profiles of different human tissues, to yield further insight into the phenotype variability in primary CoQ deficiencies.

Primary CoQ₁₀ deficiency is often caused by autosomal recessive mutations. Compounded with the limited accessibility to genome sequencing, it is likely that a significant portion of pathogenic variants has not been identified. Furthermore, early intervention of primary CoQ₁₀ deficiency has shown to be effective [32], illuminating the importance of detecting such mutations early. As the cost of genetic sequencing declines over time, we expect more variants to be identified in the future.

To aid the prompt diagnosis of pathogenic variants causing primary CoQ₁₀ deficiency, further in vitro and in vivo biochemical characterizations in model organisms such as *S. cerevisiae* are required to validate the SNV classifications made in this study. Regarding COQ3 and COQ5, there has been little to no clinical information on COQ3- and COQ5-associated primary CoQ₁₀ deficiency. This may appear to suggest that COQ3 and COQ5 mutations are not pathogenic. However, we speculate that many structurally damaging variants may be embryonic lethal, resulting in an apparent lack of known pathogenic variants. Conversely, mutations in the COQ8A and COQ8B polypeptides are reported to have several pathogenic effects [29], likely due to the aforementioned dynamic interactions and/or redundancy of function. For this reason, the clinical effects of mutations in the COQ8A and COQ8B polypeptides would require a more elaborate structural analysis and stringent biochemical characterization to fully understand the consequences of such SNVs.

Current treatment for CoQ deficiency relies on oral supplementation with CoQ₁₀. However, this method is only partially successful due to poor uptake of this extraordinarily hydrophobic lipid molecule. It will be important to identify the gene products responsible for the cellular trafficking of both exogenous and endogenous CoQ and its delivery to the mitochondria [112,113]. A potential alternative is a “bypass” treatment, in which a CoQ precursor can be administered to circumvent any faulty enzymatic steps in the biosynthetic pathway, as demonstrated previously in yeast [90], mice [114], and human fibroblasts [115]. Unfortunately, the success of this potential avenue is contingent upon the nature of the mutation. The COQ polypeptide harboring the SNV must be able to retain a structurally stable CoQ complex. Given the extensive interactions between the COQ polypeptides, membrane association, and binding of CoQ and CoQ-biosynthetic intermediates in the high molecular mass CoQ synthome, it seems likely that many surface residue SNVs may impact these interactions.

Similar to the bypass approach, another option for treating CoQ deficiency is supplementation with MitoQ. MitoQ is composed of the lipid molecule conjugated to a triphenylphosphonium cation, which enables the antioxidant to be targeted to the mitochondria [116]. Recently, clinical studies involving the supplementation of this CoQ derivative have been shown to attenuate mitochondrial DNA damage in skeletal muscle [117,118], although the direct role of MitoQ in this protective effect remains unclear. Given that MitoQ does not support the respiratory electron transport function of CoQ, it is unlikely on its own to rescue primary CoQ₁₀ deficiency [119].

A recent innovative method to serve as a potential therapy for mitochondria-related diseases is mitochondria transplantation. The fusion and fission of mitochondria within the cell are essential for the maintenance of healthy mitochondrial functions [120], and such dynamics are an attractive process to exploit for therapeutic applications. Recent studies explored the potential for mitochondrial transplants as a targeted therapy for repairing defunct mitochondria in different cells and, in a larger application, tissue revital-

ization [121,122]. Similarly, given the association of mitochondrial dysfunction with disease, another avenue for therapeutics involves the design of drugs that induce mitochondrial biogenesis, thereby promoting the turnover of defunct mitochondria and subsequently the emergence of healthy mitochondria. Several naturally occurring polyphenols have been identified as mitochondrial biogenesis inducers, such as resveratrol and phytoestrogens [123]. However, similar to oral CoQ₁₀ supplements, the therapeutic advantage of these inducers is limited by poor absorption. To target mitochondrial biogenesis from a transcriptional approach, a number of nuclear transcription factors have also been implicated in promoting mitochondrial biogenesis, which would help coordinate the expression of both mitochondrial-encoded genes as well as those that are nuclear-encoded, such as our COQ genes [123,124]. Unfortunately, the response resulting from this approach would be nonspecific, as targeting the induction of transcription factors would activate several other pathways, potentially leading to more severe side effects. Currently, targeting mitochondrial disease through the induction of mitochondrial biogenesis remains unsuccessful. While these approaches may have promise for treating mitochondrial dysfunctions, primary CoQ₁₀ deficiencies result from mutations in the nuclear-encoded COQ genes, making this method suitable for only certain types of secondary CoQ₁₀ deficiencies.

In summary, this work provides a thorough structural and functional analysis of clinically relevant SNVs in several of the COQ genes. We have identified 115 SNVs that are likely pathogenic due to structural perturbations, using multiple sequence alignments, mutation classifiers, and predicted or solved protein structures. This analysis highlights the intricate interactions in local regions of a given protein, as well as the large-scale dynamic interactions between the individual constituents of protein complexes. Together, this serves as a scaffold for future studies that seek to characterize the biochemical consequences of SNVs that result in pathogenic effects caused by primary CoQ₁₀ deficiencies. Finally, the approach employed takes advantage of recent advances in protein tertiary structure prediction and should be applicable to any human gene with identified homologs and reported SNVs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antiox11122308/s1>, Table S1: Comprehensive list of analyzed variants. References [71,72,74,90–93,96,99,100,103,111,115,125–148] were cited in supplementary.

Author Contributions: Conceptualization, F.T., S.W. and C.F.C.; methodology, S.W., A.J., F.T. and C.F.C.; validation, A.J. and S.W.; formal analysis, A.J. and S.W.; investigation, A.J. and S.W.; data curation, A.J., S.W. and A.N.N.; writing—original draft preparation, A.J., S.W. and A.N.N.; writing—review and editing, C.F.C., A.J., S.W., N.A.N., F.T. and A.N.N.; visualization, S.W. and A.J.; supervision, C.F.C.; project administration, C.F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the UCLA Graduate Division and the UCLA Edwin W. Pauley Fellowship (S.W.) and by the UCLA Department of Chemistry & Biochemistry Summer Undergraduate Research Fellowships to A.J., A.N. and F.T. We also acknowledge funding from the National Science Foundation grant MCB-1714569.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All models and crystal structures used in Missense3D analysis were obtained from the AlphaFold Protein Structure database (<https://alphafold.ebi.ac.uk/> accessed on 14 October 2022) and the Protein Data Bank (<https://www.rcsb.org/> accessed on 14 October 2022), respectively. Accession numbers of these structures can be found in the Materials & Methods. Accession numbers of the protein sequences analyzed in this study can be found in the captions of Figures showing the respective multiple sequence alignments. All SNVs analyzed in this study were obtained from gnomAD (<https://gnomad.broadinstitute.org/> accessed on 30 March 2022), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/> accessed on 30 March 2022), and Missense3D-DB (<http://missense3d.bc.ic.ac.uk:8080/> accessed on 30 March 2022). A full list can be found in Table S1.

Acknowledgments: We thank the UCLA Division of Physical Sciences for supporting this project. We also thank Michael Guile for helpful advice during the initial phase of this project, as well as Robert Clubb and Steven G. Clarke for advice on structures and methyltransferases. S.W. participates in the UCLA Chemistry-Biology Interface Predoctoral Training Program funded by NIH NIGMS award T32GM36614. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

AdoHcy, S-adenosyl-L-homocysteine; AdoMet, S-adenosyl-L-methionine; apo, apoenzyme; *APT*X, gene encoding aprataxin; Arh1, yeast ferredoxin reductase; *BRAF*, gene encoding B-Raf; CoQ, coenzyme Q or ubiquinone; CoQH₂, reduced CoQH₂ hydroquinone or ubiquinol; CoQ_n, *n* = number of isoprene units in polyprenyl tail; DDMQH₂, 2-methoxy-6-polyprenyl-1,4-benzohydroquinone; DHP, 4,5-dihydroxy-3-polyprenylphenol; DHPB, 4,5-dihydroxy-3-polyprenylbenzoic acid; 2,4-diHB, 2,4-dihydroxybenzoic acid; 3,4-diHB, 3,4-dihydroxybenzoic acid; DMAPP, dimethylallyl pyrophosphate; DMeQH₂, 3-methyl-6-methoxy-2-polyprenyl-1,4,5-benzenetriol; DMQH₂, 2-methoxy-5-methyl-6-polyprenyl-1,4-benzohydroquinone; *ETFDH*, gene encoding mitochondrial electron transfer flavoprotein-ubiquinone oxidoreductase; FDX1, human ferredoxin 1; FDX2, human ferredoxin 2; FDXR, human ferredoxin reductase; HAB, 3-hexaprenyl-4-aminobenzoic acid; 4-HB, 4-hydroxybenzoic acid; HHB, 3-hexaprenyl-4-hydroxybenzoic acid; HMPB, 4-hydroxy-5-methoxy-3-polyprenylbenzoic acid; 4-HP, 4-hydroxy-3-polyprenylphenol; 4-HP₆, 3-hexaprenyl-4-hydroxyphenol; HPB, 3-polyprenyl-4-hydroxybenzoic acid; IMM, inner mitochondrial membrane; IPP, isopentenyl pyrophosphate; *MUT*, gene encoding methylmalonyl-CoA mutase; pABA, para-hydroxybenzoic acid; PDB, protein data bank; PDSS1, decaprenyl diphosphate synthase subunit 1; PDSS2, decaprenyl diphosphate subunit 2; SNV, single nucleotide variant; SNHL, sensorineural hearing loss; SRNS, steroid-resistant nephrotic syndrome; TFRs, TetR family of regulators; UbiG, *E. coli* ubiquinone biosynthesis O-methyltransferase; VA, vanillic acid; Yah1, yeast ferredoxin.

Appendix A

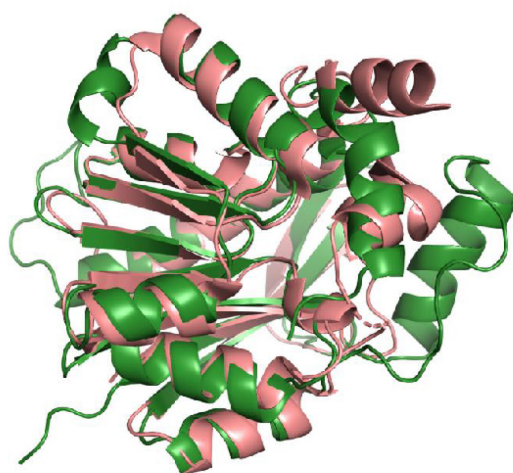


Figure A1. Structural alignment of the human COQ3 model (shown in dark green) and the crystal structure of *E. coli* UbiG, apo form (shown in pink, PDB: 4KDC). Residues 1–90 and 337–369 of the AlphaFold model were omitted due to disordered structure. Alignment was performed using PyMOL.

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<i>H.sap</i>	69	-MVAV	L	G	E	T	T	G	H	-R	T	L	K	V	L	R	D	Q	M	R	R	D	P	E	C	A	Q	I	L	Q	E	R	P	R	I	S	T	S	T	L	D	L	G	K	L	Q	S	L	P	E	G	S	L	123					
<i>S.cer</i>	115	-N	I	V	Q	L	G	E	A	T	A	L	P	V	F	L	E	N	L	K	Q	T	M	L	S	D	S	S	G	R	R	I	L	K	E	Q	P	N	I	T	T	E	I	L	H	M	D	K	L	A	K	L	P	H	N	T	F	170	
<i>Nostoc</i>	29	A	S	I	G	K	L	S	H	V	L	N	D	P	D	T	L	Q	K	V	V	A	V	L	S	L	T	P	Q	G	K	Q	A	F	E	D	R	P	M	L	G	-K	I	D	L	E	Q	L	H	Q	L	P	N	Y	T	L	83		
<i>H.sap</i>	124	C	R	E	Y	L	R	F	L	D	V	N	R	V	S	P	D	T	R	A	P	T	R	F	V	D	E	E	L	A	Y	V	I	Q	R	Y	R	E	V	H	D	M	L	H	T	L	L	G	M	P	T	N	I	L	G	E	I	180	
<i>S.cer</i>	171	C	Y	V	Y	Q	W	L	K	R	E	N	V	S	P	D	T	R	A	P	V	K	F	I	D	D	P	M	H	A	I	F	K	R	Y	R	Q	C	H	D	F	Y	H	A	I	T	N	M	P	I	I	E	G	E	I	227			
<i>Nostoc</i>	84	C	Y	M	Y	A	D	H	M	I	R	N	Q	L	T	P	P	V	---	N	E	N	V	N	H	P	F	M	F	L	A	A	H	L	G	E	T	H	D	I	W	H	V	V	T	G	C	D	T	D	K	P	G	E	V	137			
<i>H.sap</i>	181	V	V	K	W	F	E	A	V	Q	T	G	L	P	M	C	I	L	G	A	F	F	G	P	I	R	L	G	A	Q	-----	S	L	Q	V	L	V	S	E	L	I	P	W	A	V	Q	N	G	R	R	A	P	C	231					
<i>S.cer</i>	228	T	I	K	A	L	E	G	A	N	L	G	V	P	M	A	L	G	G	I	L	A	P	L	R	L	K	K	V	-----	Q	R	K	R	L	Y	N	I	Y	L	P	W	A	V	R	T	G	L	S	C	K	P	278						
<i>Nostoc</i>	138	K	L	E	A	F	Y	T	A	Q	L	I	P	D	R	L	F	L	-A	L	A	K	N	L	K	T	A	M	Y	E	V	E	L	C	E	Q	I	L	D	G	L	T	Q	G	W	--M	M	G	K	R	A	K	P	191					
<i>H.sap</i>	232	V	L	N	L	Y	Y	E	R	R	W	E	Q	S	L	R	A	L	R	E	E	L	G	I	T	A	P	P	M	H	V	Q	G	L	A	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	265											
<i>S.cer</i>	279	L	I	N	V	Y	W	E	E	M	L	E	K	D	V	T	A	L	R	K	E	L	K	I	T	L	P	P	D	L	R	T	M	R	K	E	R	A	A	L	R	K	E	I	D	A	K	Y	N	S	Q	K	R	A	T	T	P	A	335
<i>Nostoc</i>	192	L	F	G	I	E	W	N	K	L	W	E	T	F	L	E	E	L	Q	T	S	L	N	I	V	P	I	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	217					

Figure A2. Multiple sequence alignment of *H. sapiens* COQ4, *S. cerevisiae* COQ4, and *Nostoc* sp. homolog Alr8543 (NCBI accession number: WP_010999537.1). Metal-liganding motif is boxed in cyan, and residues in Alr8543 (H120, D121, H124, and E136) that act to ligand the Mg^{2+} ions are marked with blue dots.

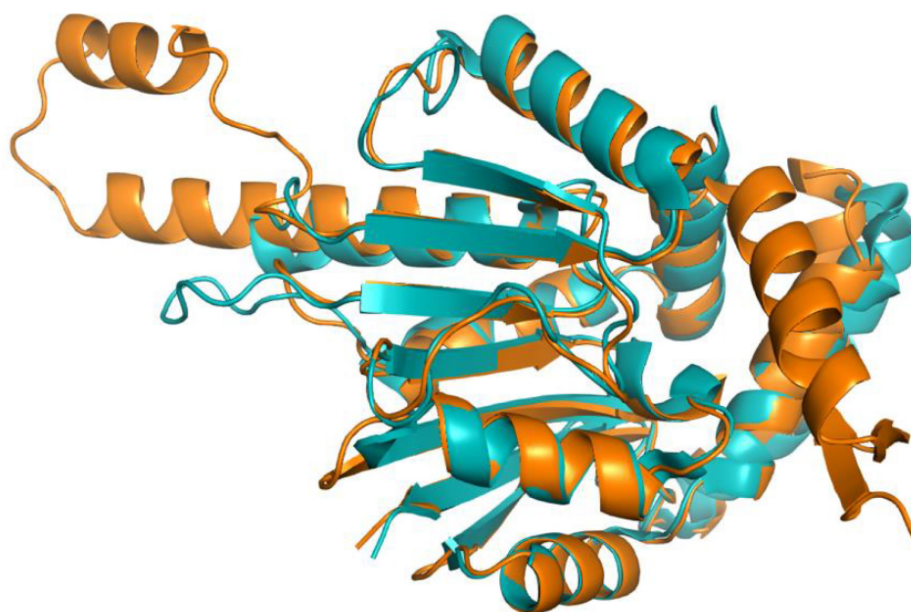


Figure A3. Structural alignment of the human COQ5 model (shown in orange) and the crystal structure of *S. cerevisiae* Coq5, apo form (shown in teal, PDB: 4OBX). Residues 1–47 of the AlphaFold model were omitted due to disordered structure. Alignment was performed using PyMOL.

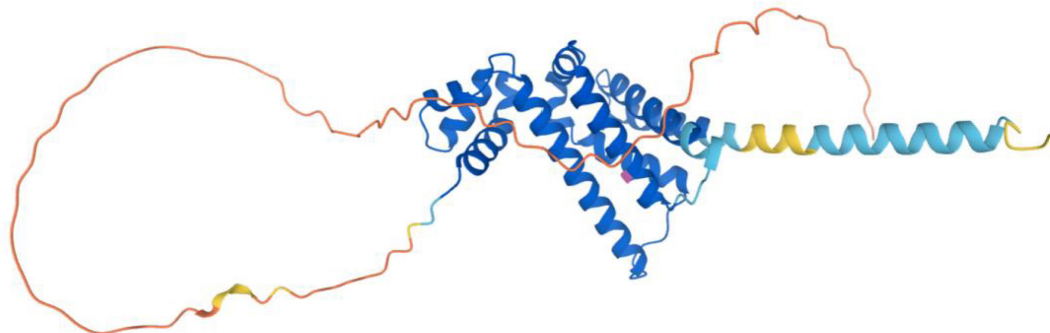


Figure A4. AlphaFold model of single chain of COQ9. Model color corresponds to the model confidence described in Figure 4. Figure was generated by the AlphaFold Protein Structure Database [39,54].

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Chapter 3

**Mutations in the Coq5 substrate-binding pocket reveal insights into *C*-methyltransferase
catalytic mechanism**

ABSTRACT

Coenzyme Q (CoQ) is an essential lipid-soluble quinone that functions in mitochondrial electron transport, redox homeostasis, and antioxidant defense. In eukaryotes, its biosynthesis requires the multi-subunit CoQ synthome, which coordinates a series of membrane-associated reactions. *COQ5* encodes a C-methyltransferase responsible for C5-methylation of demethyl-demethoxy-QH₂, a key step in CoQ biosynthesis. Although conserved from bacteria to humans, the catalytic mechanism of COQ5 and the functional consequences of human *COQ5* variants remain poorly understood. Here, we combined structural modeling, mutagenesis, and biochemical assays to investigate COQ5 substrate recognition and catalysis. An AlphaFold 3 multimeric model of human COQ5, together with the AlphaMissense pathogenicity predictor, identified potentially deleterious single-nucleotide variants (SNVs), many within the putative substrate-binding pocket. Using an adapted LC-MS/MS assay in the yeast *Saccharomyces cerevisiae*, we assessed the markers of catalytic activity and synthome stability in selected mutants, revealing an alternative substrate-binding mode supported by flexible docking and molecular dynamics simulations. Site-directed mutagenesis identified residues essential for function and pointed to a catalytic mechanism in which an active-site glutamate (E227 in yeast, E246 in humans) facilitates aromatic carbon nucleophile activation, methyl transfer, and rearomatization. These findings refine our understanding of COQ5 catalysis, highlight structural determinants of substrate recognition, and provide a framework for interpreting *COQ5* variants of uncertain significance in the context of mitochondrial disease.

INTRODUCTION

Coenzyme Q (CoQ), also known as ubiquinone, is a redox-active, lipophilic molecule found in the cellular membranes of eukaryotes and many prokaryotes (1,2). In eukaryotes, CoQ serves as an essential mitochondrial electron carrier involved in key processes such as oxidative phosphorylation, fatty acid oxidation, and uridine biosynthesis (3,4). Outside of the mitochondria, CoQ is reduced by the plasma membrane redox system to form ubiquinol (CoQH₂), which acts as a lipophilic, radical scavenging antioxidant for cell membranes, capable of preventing lipid autoxidation and regenerating vitamin E (5–9). More recently, CoQ has been implicated in ferroptosis—a form of cell death caused by lipid peroxidation and a potential target in cancer therapies—as well as redox signaling and metabolic state sensing (8–10). Given the complex and vital roles of CoQ, human patients with genetic defects in CoQ biosynthesis, a condition known as primary CoQ₁₀ deficiency, display a wide array of illnesses, including cardiovascular, kidney, and neurodegenerative disorders. Supplementation with CoQ₁₀, the native form in humans, has had limited success due to its low bioavailability and disproportionate organ distribution (11,12). Altogether, understanding the machinery responsible for CoQ biosynthesis is crucial to developing therapeutics targeting cancer, age-related CoQ₁₀ loss, and primary CoQ₁₀ deficiency.

The CoQ biosynthetic pathway is largely conserved among eukaryotes, with minor species-specific differences in the length of the polyisoprene tail. Both humans and the yeast *Saccharomyces cerevisiae* can utilize 4-hydroxybenzoic acid (4-HB) as the precursor for CoQ (Figure 1A), with humans deriving it from phenylalanine and tyrosine, and yeast from tyrosine and the shikimate pathway (13,14). CoQ biosynthesis occurs in the mitochondrial matrix, where several nuclear-encoded polypeptides residing in or peripherally associated with the inner mitochondrial membrane (Coq1–Coq9 in yeast, PDSS1/2, COQ2–7, COQ8A/B, and COQ9 in

humans) are essential for this process (15). Two additional yeast polypeptides, Coq10 (human homolog COQ10A/B) and Coq11, promote efficient biosynthesis of CoQ, although the closely related human homologs have not been well characterized or, in the case of Coq11, are absent (16,17). In yeast, it has been shown that Coq3–Coq9 and Coq11 form a large multi-subunit complex called the CoQ synthome, whose assembly is required for efficient CoQ biosynthesis (12,15,18–21). Current evidence strongly suggests the existence of a homologous complex in humans as well (Figure 1B) (22,23).

The *COQ5* gene, conserved between humans and yeast, encodes a matrix-localized, *S*-adenosyl-L-methionine (AdoMet)-dependent methyltransferase responsible for the *C*5-methylation of the hydroquinone ring of demethyl-demethoxy-QH₂ (DDMQH₂) during CoQ biosynthesis, producing demethoxy-QH₂ (DMQH₂) (Figure 1A). Human COQ5 shares 45% sequence identity with yeast Coq5, with an additional vertebrate insert found in humans that is not present in yeast (24). A homolog is also found in *Escherichia coli*, named UbiE. Together, the UbiE/COQ5 family of proteins belongs to the class I AdoMet-dependent methyltransferase superfamily. All members of this class contain a seven- β -strand Rossmann fold core structure, as well as conserved AdoMet-binding sequence motifs (25–27). The class I methyltransferase S_N2-like catalytic mechanism has been studied extensively in *O*- and *N*-methyltransferases (28–33). However, the catalytic mechanism of methyl transfer to a less nucleophilic atom, as in *C*-methyltransferases like COQ5, is less well understood and may involve diverse mechanisms for carbon nucleophile activation. To gain a deeper understanding of the catalytic mechanism, structures of apo and AdoMet-bound yeast Coq5 dimers have been solved (PDB: 4OBX and 4OBW) (34). These structures have been crucial in elucidating the AdoMet- and substrate-binding pockets, as well as enabling the identification of putative substrate-binding residues. However, the

UbiE/COQ5 family catalytic mechanism and these hypothesized protein-substrate interactions have not been experimentally validated.

Much of our current understanding of the CoQ synthome originates from early studies in yeast. Yeast harboring a *COQ5* deletion (*coq5Δ*) are unable to grow on non-fermentable carbon sources like glycerol and are hypersensitive to polyunsaturated fatty acid treatment as a result of CoQ(H₂) deficiency (6,18,27,35–37). Furthermore, the absence of the Coq5 polypeptide destabilizes the CoQ synthome, resulting in the loss of the megadalton complex and reduced levels of other Coq polypeptides—effects can be partially restored by Coq8 overexpression or CoQ supplementation (21,38,39). Partial knockdown of *COQ5* in human cell lines corroborates observations in yeast *coq5* mutants and underscores the crucial role of Coq5 catalysis and in maintaining CoQ synthome integrity (40). The human protein has also been shown to rescue yeast expressing stable but catalytically inactive Coq5 polypeptides, as well as *coq5Δ* mutants overexpressing Coq8 (18,27), highlighting their functional conservation.

To date, only seven patients with mutations in *COQ5* have been reported in the literature (41–44). Three individuals from the same family carry alleles with a 9590-bp duplication that produces an elongated 3' untranslated region (42). These patients present with developmental delays and central nervous system (CNS) phenotypes—including encephalopathy, cerebellar atrophy, ataxia, and intellectual disability—that improve with CoQ₁₀ supplementation. Two additional patients with CNS or cognitive disorders harbor single-nucleotide variants (SNVs) at or near splice sites (41,44). The remaining two cases involve distinct compound heterozygous *COQ5* variants predicted to cause early termination or missense changes, with primary symptoms of retinitis pigmentosa (43). The functional impact of most human *COQ5* missense SNVs detected in population databases remains largely unknown. Because developmental delays and organ

damage from CoQ₁₀ deficiency are often irreversible, early genetic diagnosis and treatment are most critical.

In this study, we report the identification of potentially pathogenic SNVs using an AlphaFold 3 multimeric model of human COQ5 and the machine learning-based mutation classifier AlphaMissense, many of which are located in the conserved putative substrate-binding pocket. Using an adapted liquid chromatography–tandem mass spectrometry (LC-MS/MS) method, we examined several substrate-binding pocket mutations and SNVs for markers of catalytic defect and synthome destabilization in a yeast model. Molecular dynamics and mutagenesis studies on yeast Coq5 supported a novel substrate-binding mode distinct from one that was previously identified (34), enabling the identification of residues likely involved in substrate recognition and catalysis. Based on these findings, we propose a general catalytic mechanism for human COQ5 and yeast Coq5 in which acid-base catalysis, mediated by an active-site glutamate residue (E227 in yeast, E246 in humans), facilitates the activation of the aromatic carbon nucleophile and drives proton transfer and rearomatization following methylation.

MATERIALS AND METHODS

Multiple Sequence Alignment

Multiple sequence alignment was previously performed in (24). Briefly, protein sequences of human COQ5 and orthologs from *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Drosophila melanogaster*, *Arabidopsis thaliana*, *Danio rerio*, *Gallus gallus*, *Mus musculus*, and *Schizosaccharomyces pombe* were aligned with Clustal Omega using default parameters (45,46) and visualized with Jalview2 (47). Accession numbers for all polypeptide sequences used in the alignment are listed in Table 1. Highly conserved residues were defined as percent agreement $\geq 80\%$, while functional residues were

determined from crystal structures of the *S. cerevisiae* Coq5 apoenzyme (PDB: 4OBX) and AdoMet-bound enzyme (PDB: 4OBW) (34), as well as subsequent biochemical validation.

Analysis of Single-Nucleotide Variants

Human *COQ5* missense single-nucleotide variants (SNVs) were gathered from gnomAD v.2.1.1 (gnomad.broadinstitute.org/, accessed on 1 July 2025) (48), NCBI ClinVar (www.ncbi.nlm.nih.gov/clinvar/, accessed on 1 July 2025) (49), Missense3D-DB v1.5.1 (missense3d.bc.ic.ac.uk:8080/, accessed on 1 July 2025) (50), and literature (41,43,44,51). The selection criteria for SNVs is described in (24). Briefly, we analyzed all variants that occur in highly conserved or functional residues, appear in ClinVar, have an allele frequency larger than 0.0001 as reported in gnomAD v2.1.1, or appear within two nucleotides of a splice site. Variants were classified using Missense3D-PPI (missense3d.bc.ic.ac.uk/~missense3d/indexppi.html, accessed on 1 July 2025) (52), SIFT (sift.bii.a-star.edu.sg, accessed on 1 July 2025) (53), PolyPhen-2 (genetics.bwh.harvard.edu/pph2/, accessed on 1 July 2025) (54), and AlphaMissense (alphamissense.hegelab.org, accessed on 1 July 2025) (55) using default settings. For Missense3D-PPI analyses, a model of the apo human COQ5 dimer was obtained from the AlphaFold Server (alphafoldserver.com, accessed on 1 July 2025) (56) and provided in Supplementary File 1. The resulting model is subject to the AlphaFold Server Output Terms of Use found at alphafoldserver.com/output-terms. All structures were aligned and visualized using PyMOL version 2.5.1 (Schrödinger, LLC, New York, NY, USA) unless otherwise stated. Results from the mutation classification can be found in Supplementary File 2.

Yeast strains and growth medium

Wild-type and *coq5Δ* *S. cerevisiae* strains used in this study were derived from W303 (57) (Table 2). Growth media were prepared as previously described (58) and included: YPD (1% yeast

extract, 2% peptone, 2% glucose), YPG (1% yeast extract, 2% peptone, 3% glycerol), and synthetic dextrose media (SD-Complete, SD-Ura, and SD-Trp). SD medium contained 2% dextrose, 0.18% yeast nitrogen base without amino acids, 0.14% NaH₂PO₄, 0.5% (NH₄)₂SO₄, and amino acids at final concentrations described in (36), minus uracil or tryptophan for selective media. All synthetic dextrose media were adjusted to approximately pH 6. Plate medium contained 2% bacto-agar.

Construction of *COQ5* mutant plasmids and complementation in yeast

All plasmids used in this study are described in Table 3. The *coq5-2* and *coq5-3* alleles were previously described in (18). All other mutations were introduced into the pGBmCOQ5 and pGBsCOQ5 plasmids using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Cat. #210518), according to the manufacturer's instructions (Revision F1). Primers used during the mutagenesis of each plasmid are described in Table 4. Plasmids were purified using standard miniprep procedures and mutagenesis was confirmed by whole-plasmid sequencing (Plasmidsaurus). Yeast transformation procedures were adapted from standard protocols (58). Briefly, 0.5 mL aliquots of yeast cultured in YPD overnight were pelleted. To each pellet, the following reagents were added in order followed by brief vortexing: 100 µg of heat-treated single stranded carrier DNA, 1 µg of plasmid DNA, 0.5 mL of sterile PLATE solution (40% PEG 3350, 0.1 M lithium acetate, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA), and 57 µL of DMSO. The resuspension is incubated at room temperature for 15 min followed by incubation at 42 °C for 15 min. Cells are pelleted and resuspended in 200 µL of sterile phosphate-buffered saline (PBS), then plated onto the appropriate selective plate medium. Plates were incubated at 30 °C for up to three days and single colonies were maintained on a selective plate for subsequent biochemical assays.

Drop dilution plate assays

Yeast were grown overnight in 5 mL of SD medium with the appropriate amino acid selection for transformed yeast strains and SD-Complete medium for W303 wild type and *coq5Δ*. The following day, cultures were diluted to OD₆₀₀ of 0.2 in sterile PBS. 200 μL aliquots of the cell suspensions were transferred to a 96-well plate, where four five-fold serial dilutions were performed. 2 μL of each dilution were spotted onto YPD, YPG, and selective plate medium, corresponding to a final OD₆₀₀ = 0.2, 0.04, 0.008, 0.0016, and 0.00032. All plates were incubated at 30 °C for two or three days.

Stable isotope labeling and analysis of CoQ and CoQ intermediates

Transformed yeast cells were cultured overnight in 5 mL of selective media at 30 °C with shaking. The following day, pre-cultures were sub-inoculated to OD₆₀₀ = 0.6 in 15 mL of fresh selective media. For analysis of *de novo* CoQ biosynthesis, cultures were treated with either 8 μg/mL (56 μM) ¹³C₆-*p*ABA (Sigma-Aldrich) or ethanol as a vehicle control, with a final ethanol concentration of 0.2% (v/v). Cultures were grown at 30 °C with shaking for 5 h to a final OD₆₀₀ of ~2.7. For each culture, 9 OD₆₀₀ units of cells (as determined by OD₆₀₀ × volume in mL) were harvested by centrifugation at 4000 × *g* for 5 min. For cells treated with vehicle control, another identical aliquot was harvested for immunoblot analysis (see *SDS-PAGE and immunoblot analysis of steady-state protein expression*). Cell pellets were stored in –20 °C until use.

To prepare lipid extracts, cell pellets were washed in 500 μL of PBS and resuspended in 100 μL of fresh PBS. 2 mL of methanol and glass beads were added to each resuspension, and cells were lysed by vortexing for 30 s. Equal amounts of the internal standard CoQ₄ (Sigma-Aldrich) were added, typically ranging from 100–150 pmol. Lipids were then extracted twice, each time with 2 mL of petroleum ether and 30 s vortexing. A standard curve containing ~0.4 pmol to

~1.3 nmol of CoQ₆ (Avanti Polar Lipids) and the same amount of CoQ₄ per sample was resuspended and extracted alongside the experimental samples. Extracted lipids in petroleum ether were dried under N₂ gas and stored at -20 °C.

On the day of analysis, lipids were reconstituted in 200 µL of ethanol containing 1 mg/mL benzoquinone. LC-MS/MS analyses were performed as previously described (27,59). Briefly, 20 µL aliquots of each sample were injected into a Luna phenyl-hexyl column (100 × 4.6 mm, 5 µm; Phenomenex) connected to either an API4000 linear MS/MS spectrometer (Applied Biosystems) or a 4000 QTRAP linear MS/MS spectrometer (Applied Biosystems). Precursor-to-product ion transitions are listed in Table 5. Data acquisition and processing was performed using Analyst version 1.4.2 (AB Sciex, Framingham, MA, USA). For each analyte, the relative amount was calculated by summing the peak area of the protonated species and that of its corresponding ammonium adduct, where applicable, corrected for extraction efficiency by the amount of internal standard CoQ₄ recovered, then normalized by OD₆₀₀ unit. Relative levels of CoQ intermediates calculated by this method were reported as Arbitrary Units/OD₆₀₀. For CoQ₆, absolute amounts were determined by an additional normalization to the standard curve. One-way ANOVA with Tukey's HSD test was used to compare each strain with every other strain within a treatment group. Where only two strains were present within a treatment group, an unpaired *t*-test was used. Strains that produced no signal above background in any biological replicate or had values below the quantification limit were labeled as 'n.d.' (not detected) and 'n.q.' (not quantifiable), respectively, and were excluded from statistical analyses. In cases where only one or two biological replicates yielded no detectable signal, values were conservatively assigned as zero to allow comparison within the group. All statistical analyses and data presentation were performed using GraphPad Prism version 10.4.1 (GraphPad Software, Boston, MA, USA).

SDS-PAGE and immunoblot analysis of steady-state protein expression

Yeast whole-cell extracts were prepared as described (60). Briefly, 9 OD₆₀₀ pellets of vehicle control-treated cultures from the stable isotope labeling experiments (see *Stable isotope labeling and analysis of CoQ and CoQ intermediates*) were resuspended in 300 µL of 2 M lithium acetate, incubated on ice for 5 min, and pelleted. Pellets were then resuspended in 300 µL of 0.4 M NaOH, incubated on ice for 5 min, and pelleted again. The final cell pellet was reconstituted in 200 µL of SDS sample buffer (50 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 0.1% bromophenol blue, 5% v/v β-mercaptoethanol), boiled for 5 min, and stored immediately at −20 °C. Immediately before SDS-PAGE analysis, samples were boiled for 5 min and briefly centrifuged. 10 µL (corresponding to 0.45 OD₆₀₀ units) of each sample were loaded onto 10% Tris-glycine polyacrylamide gels for electrophoretic separation. Proteins were transferred to Immobilon®-FL 0.45 µm PVDF membranes (Millipore) and blocked overnight at 4 °C with blocking buffer (5% milk in PBS-T, consisting of PBS with 0.1% Tween-20). Coq5 and the mitochondrial protein loading control Atp2 were detected using rabbit polyclonal antibodies, diluted as specified in Table 6, in either 0.5% bovine serum albumin (BSA) or 5% milk in PBS-T containing 0.05% (v/v) ProClin 300. Primary antibody incubation was performed overnight at 4 °C. IRDye 680LT Goat Anti-Rabbit IgG secondary antibodies (LI-COR) were diluted 1:20,000 in PBS-T containing 0.02% SDS, 0.5% BSA, and 0.05% (v/v) ProClin 300 (Sigma-Aldrich), and incubated for 1 h at room temperature. Membranes were imaged using the LI-COR Odyssey Infrared Scanner (LI-COR) and immunoblots were manually quantified using the ImageJ version 1.5.3 (National Institutes of Health, Bethesda, MD, USA) (61). One-way ANOVA with Dunnett's multiple comparison test was used to compare the mutant Coq5 band intensities to those of *coq5Δ* yeast transformed with plasmids expressing wild-type *COQ5*.

Density functional theory calculations

Density functional theory (DFT) calculations were performed using Gaussian 16, Revision A.03 (Gaussian Inc., Wallingford, CT, USA) (62). Initial structures were manually built in GaussView 6.0.16 (Semichem Inc., Shawnee Mission, KS, USA) (63) to approximate the active-site geometry of the plausible pre-reactive conformation derived from molecular docking (see *Substrate docking*). To reduce computational complexity, glutamate and AdoMet were modeled as acetate and trimethylsulfonium (TMS), respectively, and the polyprenyl tail of the substrate (DDMQH₂) was truncated to a methyl group. Proton donors and acceptors involved in proposed mechanism were modeled in separate calculations, independent of the TMS-substrate complex.

All geometry optimizations, frequency calculations, and single-point energy evaluations were performed using the B3LYP functional (64–67), augmented with Grimme's D3 empirical dispersion correction (68), and the 6-311+G(d,p) basis set. Solvation effects were included in both optimization and single-point calculations using the CPCM solvation model (69,70) with diethyl ether or water as the solvent. All atoms were allowed to relax freely during geometry optimizations. Normal vibrational mode analysis was used to confirm the identity of optimized structures as either minima (zero imaginary frequencies) or transition states (one imaginary frequency). Intrinsic reaction coordinate (IRC) calculations (71) were performed to verify the connectivity of each transition state to its associated pre- and post-transition state complexes. All Gibbs free energies are calculated at 298 K. Final geometries were rendered with CYLview20 (Université de Sherbrooke, Sherbrooke, QC, Canada) (72). Mechanisms and free energy diagrams were illustrated in ChemDraw version 23.1.2 (Revvity Signals Software Inc., Waltham, MA, USA).

The average pK_a of residues in the substrate-binding pocket across all four subunits of the AdoMet-bound yeast Coq5 crystal structure (PDB: 4OBW) was calculated using PROPKA version 2.0 (73) to verify the catalytic base, E227, in the proposed mechanism.

Substrate docking

All docking calculations were performed using AutoDock Vina version 1.1.2 (The Scripps Research Institute, La Jolla, CA, USA) (74). Chain A of the crystal structure of yeast Coq5 in complex with AdoMet (PDB: 4OBW) was used as the target of docking. All waters and other co-crystallized ligands were removed. Polar hydrogens were added using AutoDockTools version 1.5.7 (The Scripps Research Institute, La Jolla, CA, USA) (75) with default settings. The grid box of size $20 \text{ \AA} \times 20 \text{ \AA} \times 20 \text{ \AA}$ contained the entire active-site pocket. The structure of the geranylated substrate analog, DDMQ₂H₂, was optimized using DFT in diethyl ether (see *Density functional theory calculations*) and docked to the active site. All rotatable single bonds in DDMQ₂H₂, as well as the side chains of the following residues lining the active site, were treated as flexible and allowed to rotate freely during docking: R77, Y78, M81, N82, F198, R201, N202, F228, Y263, L264. The calculation was repeated where E227 was added to the flexible region, but its inclusion did not alter the docking results. This process was repeated four times, and 60 binding poses were generated. A cluster containing four members was the lowest in energy as calculated by AutoDock Vina (−9.3 kcal/mol) and was selected for further studies. The coordinates of the top-ranked member of this cluster are given in Supplementary File 3. A four-membered cluster representing a binding intermediate along the substrate-entrance tunnel (−7.1 kcal/mol) and a four-membered cluster in which the hydroquinone head group is rotated 180° (−8.5 kcal/mol) were also observed. All other poses placed the reactive atoms >5 Å apart and were discarded. Rigid docking was also

performed using the same parameters, but failed to produce any catalytically relevant conformations in which the reactive head group was positioned near AdoMet.

Molecular dynamics simulations

Molecular dynamics (MD) simulations were carried out using the Amber 16 software package (76) (University of California, San Francisco, San Francisco, CA, USA), with AmberTools 24 (77) (University of California, San Francisco, San Francisco, CA, USA) and AmberTools 16 used for system setup and trajectory post-processing, respectively. The initial coordinates for the simulations were derived from the top-ranked docking pose. Hydrogens on the AdoMet and DDMQ₂H₂ were optimized using DFT in diethyl ether (see *Density functional theory calculations*). Hydrogens were added onto the protein using *reduce* and *pdb4amber* from AmberTools 24. Simulations were conducted in explicit water solvent using the GPU accelerated code (*pmemd*). The *ff14SB* force field parameters were applied to the protein scaffold (78), and TIP3P parameters were used for water molecules, and GAFF parameters (79) for small molecules. The partial charges of AdoMet and DDMQ₂H₂ were derived by fitting the electrostatic potential computed at the HF/6-31 G(d) level of theory using the restrained electrostatic potential (RESP) model. These charges were calculated using the Merz-Singh-Kollman scheme implemented in the Gaussian 16 software package. The protein complexed with ligands was placed in a cubic box of TIP3P water molecules with a 10 Å buffer using the *tleap* module in AmberTools 24. Explicit counterions (Na⁺) were added to neutralize the system. Molecular dynamics simulations were performed according to the following steps: (i) The system was minimized in two stages, each with a maximum cycle of 5000, the first 2500 of which are steepest descent cycles. Non-periodic constant volume conditions were applied. The first stage applied strong positional restraints (500 kcal mol⁻¹ Å⁻²) to all atoms, while the second applied lighter restraints (2 kcal mol⁻¹ Å⁻²) to

backbone atoms. (ii) The system was heated under constant volume (NVT) without periodic boundary conditions. Temperature increased from 0 K to 300 K over 0.3 ns, using Langevin dynamics with a collision frequency of 5 ps⁻¹. The SHAKE algorithm was activated during heating and all subsequent steps constrain bonds involving hydrogen atoms. Backbone atoms were restrained with a force constant of 30 kcal mol⁻¹ Å⁻². (iii) Equilibration was performed in two 2 ns stages under constant pressure (NPT) at 300 K at 1 atm, using periodic boundary conditions and the particle mesh Ewald (PME) method for long-range electrostatics. A Langevin thermostat (collision frequency 5 ps⁻¹) and Monte-Carlo barostat were applied. Backbone atoms were restrained with a force constant of 30 kcal mol⁻¹ Å⁻² during the first equilibration stage and 0.5 kcal mol⁻¹ Å⁻² during the second. (iv) A 100-ns or 1000-ns production was performed with NPT conditions as specified during equilibration. For all steps of the simulation, an 8 Å cutoff was applied to Lennard-Jones and electrostatic interactions. Analysis of interatomic distances, root mean square deviation (RMSD), and clustering of trajectory frames were performed in Chimera (University of San Francisco, San Francisco, CA, USA) (80). Representative frames from most populated clusters were selected for further analysis. For the representative 1000-ns production, coordinates of the frames of the early-state and late-state conformations can be found in Supplementary Files 4 and 5, respectively.

RESULTS

Classification of *COQ5* variants reveals potential disruption of functional regions

Single-nucleotide variants (SNVs) of potential clinical significance in the human *COQ5* gene were previously identified based on residue conservation and function, ClinVar annotation, allele frequency, as well as Missense3D prediction of structural impact on the AlphaFold-predicted structure of human COQ5 (24,81). However, the structural impact of these resulting point

mutations was not examined in the context of a dimeric state, which is thought to be the native state of yeast Coq5 as reflected by its crystal structure (PDB: 4OBX and 4OBW) (34). To model the homology with yeast Coq5, we generated the structure of the full length human COQ5 dimer using AlphaFold 3 (Figure 2A). Consistent with the conservation between class I methyltransferases, the seven- β -strand methyltransferase core structure of human COQ5 aligns well with that of yeast Coq5 (Figures 2B, S1). However, the dimer interface, which includes the hydrophobic region along helices $\alpha 6$ and $\alpha 7$ homologous to the yeast Coq5 dimer interface, as well as parts of helices $\alpha 1$ –4, was predicted with lower confidence (34), likely due to lower degree of conservation. Other low confidence regions include the N-terminus including portions of helix $\alpha 1$, whose homologous region is not visible in the yeast crystal structure, as well as a vertebrate-specific insert spanning roughly K135 to G163 that is absent in yeast (24). A total of 118 SNVs obtained from gnomAD, ClinVar, and Missense3D-DB databases were then classified using Missense3D-PPI, an extension of the Missense3D classifier that supports mutation classification on multimeric structures (Figure S2, Supplementary File 2) (52). 27 variants were classified as structurally damaging, including 24 variants in highly conserved or functional residues and one variant associated with retinitis pigmentosa (R123W) (43). Furthermore, four of the structurally damaging variants in highly conserved residues disrupted the AlphaFold-predicted human COQ5 dimer interface.

In addition to traditional rule-based mutation classifiers that rely solely on sequence conservation or structure- and function-based features, we also incorporated the results from the machine learning-based mutation classifier AlphaMissense (55). A total of 43 variants were classified as likely pathogenic by AlphaMissense. Notably, nearly all of the variants occurring in AdoMet binding residues and all of those occurring in putative substrate-interacting residues were

classified as likely pathogenic (Figure 2B). These functional residues were identified by Dai *et al.* based on the AdoMet-bound yeast Coq5 crystal structure (PDB: 4OBW) and subsequent molecular docking of a substrate analog (34). However, their importance in COQ5's methyltransferase activity has not been experimentally validated. Given that the active-site residues involved in AdoMet-binding are well characterized in other class I methyltransferases (25,26,82), we chose to focus on the residues responsible for COQ5 substrate recognition and catalysis in the context of these variants.

Development of yeast *in vivo* assay to assess Coq5 catalytic function and synthome integrity

Although the catalytic role of COQ5 and its involvement in maintaining CoQ synthome stability have been established in yeast and human cells (36,18,27,35,21,38,39,83), no direct functional assay for COQ5 activity has been described in the literature. We chose to perform mutational analysis in yeast, as yeast Coq5 shares 45% sequence identity with human COQ5 and its substrate-binding pocket is highly conserved (Figure 2). As a peripheral membrane protein embedded within the interdependent CoQ synthome, Coq5's activity may not be recapitulated in a soluble, aqueous environment. Indeed, our attempts to measure activity of purified yeast Coq5 in solution were unsuccessful (data not shown), likely due to the absence of essential binding partners or suboptimal assay conditions.

Instead of a direct activity assay, we adapted a previously described LC-MS/MS-based analysis of *de novo* and steady-state levels of CoQ₆ and CoQ₆ intermediates (27,59) as an indirect measure of Coq5 activity *in vivo*. In addition to the canonical ring precursor 4-HB, yeast can utilize an additional ring precursor, *para*-aminobenzoic acid (*p*ABA) to synthesize CoQ₆ (84,85). To trace the flux through the CoQ₆ biosynthetic pathway, we chose to label cells with ¹³C₆-*p*ABA. The first prenylated intermediate resulting from ¹³C₆-*p*ABA, ¹³C ring-labeled 4-amino-3-

hexaprenylbenzoic acid ($^{13}\text{C}_6\text{-HAB}$), can be readily separated and detected by LC-MS/MS. Subsequent deamination performed by Coq6 and modulated by Coq9 (86,87) allows for the formation of hydroxylated late-stage intermediates such as $^{13}\text{C}_6\text{-DDMQ}_6\text{H}_2$, which is the substrate of Coq5. In strains expressing catalytically impaired Coq5, we expect to observe the accumulation of the diagnostic intermediates $^{12}\text{C-DDMQ}_6$ and $^{13}\text{C}_6\text{-DDMQ}_6$, corresponding to the oxidized quinone form of the substrate. Furthermore, the accumulation of such intermediates offers insight into the integrity of the CoQ synthome. Previous studies have shown that when the synthome is destabilized, as in *coq* deletion strains (38,39), the early intermediate HAB accumulates and no diagnostic intermediate is formed. In contrast, when synthome integrity is preserved, as in strains stably expressing a defective *coq* point mutant (86,88,89), Coq8 overexpression (38), or CoQ₆ supplementation (39), the accumulation of the diagnostic intermediate observed.

To validate this assay, we labeled previously reported CoQ-less *coq5* point mutants with $^{13}\text{C}_6\text{-pABA}$. The *coq5-2* and *coq5-3* alleles encode G199D and G304D substitutions, respectively (18). Both mutants are respiratory-deficient and fail to complement the growth of *coq5* Δ yeast on glycerol, a non-fermentable carbon source (Figure S3A, B). The *coq5-2* allele encodes a stably expressing but catalytically inactive polypeptide, due to a disruption of the AdoMet-binding site (Figure S3C). In contrast, the *coq5-3* mutation disrupts the core seven- β -strand Rossmann fold (Figure S3C) and its encoded polypeptide exhibits reduced stability and protein expression levels, despite retaining partial activity *in vitro* (18). As expected, both *coq5-2* and *coq5-3* mutants show defects in *de novo* CoQ biosynthesis when overexpressed, as seen in the near absence of $^{13}\text{C}_6\text{-CoQ}_6$ (Figure S4B). Steady-state CoQ₆ levels were also reduced, as seen in the levels of $^{12}\text{C-CoQ}_6$ and total CoQ₆ (Figure S4A, C). The *coq5-2* mutant, but not the *coq5-3* mutant, accumulates $^{13}\text{C}_6\text{-DDMQ}_6$ (Figure S4E), consistent with its stable expression and suggesting the preservation of

synthome integrity in the *coq5-2* mutant. Meanwhile, the *coq5-3* mutant accumulates $^{13}\text{C}_6\text{-HAB}$ at levels similar to *coq5Δ* (Figure S4F), indicating that its reduced expression is insufficient to support synthome assembly. Taken together, these results support the use of $^{13}\text{C}_6\text{-pABA}$ labeling and LC-MS/MS as a robust indirect assay of Coq5 activity and synthome integrity.

Functional impact of substrate binding mutants suggests alternative substrate binding mode

Prior structural studies of yeast Coq5 by Dai *et al.* identified a putative substrate-binding pocket and highlighted residues R77, Y78, R201, and N202 as potential contributors to substrate binding and catalysis (34). Docking calculations in the model by Dai *et al.* suggest that R77 and N202 (corresponding to human COQ5 residues K78 and N221) anchor the hydroquinone head group of the substrate by forming polar contacts with the hydroxyl groups at O1' and O4', respectively. Dai *et al.* also hypothesized that Y78 and R201 (corresponding to human Y79 and R220) may perform base catalysis on the methylation site, carbon C5. Given that the protonation state of arginine at physiological pH would not allow it to act as a base catalyst, we focused on Y78 as the putative catalytic residue. To evaluate the functional significance of these interactions, we generated Coq5-R77S and N202S mutants, hypothesizing that both mutations would disrupt hydrogen bonding with the substrate while altering the steric environment of the active site, as well as Coq5-Y78F, which would remove the hydroxyl group required for base catalysis. Notably, the N202S mutation corresponds to the human SNV N221S (rs1048333814). Missense3D-PPI predicted that mutations R77S and Y78F would alter the size of the active-site cavity in the yeast Coq5 dimer structure (PDB: 4OBW) (52). While N202S is structurally neutral, its homologous SNV was predicted to be pathogenic by AlphaMissense (55).

We expressed either wildtype or mutant forms of Coq5 in the yeast *coq5Δ* mutant in low-copy (lc; Figure S5A, B) or high-copy (hc; Figure S5C, D) plasmids. All mutants expressed at

levels comparable to the corresponding wild-type Coq5 expression, with low-copy levels similar to endogenous expression in wild-type W303 yeast (Figure S5A, B). We then assessed their ability to support respiratory growth on glycerol-containing plate medium. Surprisingly, all three mutants complemented the growth defect of the *coq5Δ* mutant, under both low- or high-copy expression (Figure 3), suggesting that these mutants retained CoQ₆ biosynthetic activity.

Given that only low amounts of CoQ₆ are necessary to support growth on plate medium, we examined the *de novo* synthesis and steady-state levels of CoQ₆ and CoQ₆ intermediates in low-copy mutants using our ¹³C₆-*p*ABA labeling assay (Figure 4A). Under ¹³C₆-*p*ABA labeling conditions, we observed that complementation of the *coq5Δ* mutant with wild-type Coq5 (lc *COQ5*) led to a statistically significant reduction in *de novo* CoQ biosynthesis (Figure 4C) and total CoQ₆ levels (Figure 4D) compared to the W303 wild-type strain (WT lc EV). This indicates that plasmid-based complementation is not fully efficient, despite comparable levels of protein expression (Figure S5B). We next examined ¹²C-CoQ₆ and ¹³C₆-CoQ₆ levels in strains complemented with mutant Coq5. Coq5-R77S exhibited no defect in CoQ biosynthesis (Figure 4B, C) compared to those complemented with wild-type Coq5. In contrast, Coq5-Y78F supported only moderate CoQ₆ biosynthesis, displaying reduced *de novo* and steady-state CoQ₆ levels (Figure 4A–C) compared to strains complemented with wild-type Coq5. Mutants expressing Coq5-N202S exhibited the most severe phenotype, with a marked reduction in ¹²C-CoQ₆, ¹³C₆-CoQ₆, and total CoQ₆ under both ¹³C₆-*p*ABA labeling and unlabeled (EtOH) conditions (Figure 4A–C). Consistent with a catalytic impairment in the Coq5 step, mutants expressing Coq5-Y78F and Coq5-N202S, but not those expressing Coq5-R77S, accumulated the Coq5 diagnostic intermediate ¹²C-DDMQ₆ (Figure 4E) and ¹³C₆-DDMQ₆ (Figure 4F). However, none of the

mutants accumulated the early intermediate $^{13}\text{C}_6\text{-HAB}$ (Figure 4G), suggesting that these mutations preserve synthome integrity.

To verify that these defects were due to impaired catalysis in the Coq5 step, we repeated the $^{13}\text{C}_6\text{-pABA}$ labeling assay under high-copy expression. We observed similar trends in the levels of CoQ₆ and CoQ₆ intermediates in our mutants (Figure S6). Notably, overexpression partially rescued the CoQ₆ biosynthetic defects in mutants expressing Coq5-Y78F and Coq5-N202S (Figure S6A–C), consistent with a catalytic deficiency that renders the Coq5 step rate limiting.

Overall, these results demonstrate that R77 is dispensable for substrate binding and Coq5 function. The partial retention of CoQ₆ biosynthetic activity in Coq5-Y78F and N202S strongly suggests that Y78 and N202 contribute to substrate binding, but neither residue is essential for catalysis. In particular, N202 appears to play a more critical role in substrate binding than Y78, although the N202S mutant may retain partial hydrogen-bonding capacity through the serine side chain, albeit with altered geometry. These findings underscore the need for a revised substrate binding model and catalytic mechanism.

Flexible docking and molecular dynamics identify novel substrate-interacting residues

Based on the mechanism proposed by Dai *et al.*, the conversion of the polyprenylated substrate DDMQH₂ to the Coq5 product DMQH₂ requires at least one deprotonation step, which occurs at the methylation site, C5 (34). To identify candidate residues capable of acting as catalytic bases, we re-examined the putative substrate-binding pocket using molecular docking. A geranylated substrate analog, DDMQ₂H₂, was docked into the substrate-binding pocket of the AdoMet-bound yeast Coq5 crystal structure (PDB: 4OBW) using AutoDock Vina (Figure 5A). Side chains of residues lining the pocket were allowed to rotate freely during the calculation. The

top-ranked had an estimated binding affinity of -9.3 kcal/mol (Figure 5B, C). In agreement with our mutagenesis results, R77 and Y78 were not positioned near the hydroquinone head group of DDMQ₂H₂. Although Y78 is unlikely to participate in catalysis, it may engage in hydrophobic interactions with the polyprenyl tail, along with M81, Y263, and L264, potentially explaining the partial activity retained in the Coq5-Y78F mutant. Two phenylalanine residues, F198 and F228, line the deep end of the substrate-binding pocket near the methoxy group of DDMQ₂H₂. The O4' of DDMQ₂H₂ is positioned next to a row of polar residues along the substrate-entrance tunnel—E227, R201, and N202—where it forms polar contacts with the E227-R201 salt-bridge pair (Figure 5C). The pK_a's of E227 and R201 in the absence of the substrate were estimated to be 4 and 12, respectively, making E227 a plausible catalytic base. The opposite end of the head group, O1', is anchored by a hydrogen bond with N82. Importantly, the AdoMet methyl carbon and C5 carbon of DDMQ₂H₂ are positioned within 4.0 Å of each other in this pose (Figure 5C). The short distance between the reactive atoms, coupled with the proximity of a candidate catalytic base, suggests that this docking pose may approximate the pre-reactive geometry and was defined as the productive pose.

Two lower-ranking alternative binding poses were also identified (Figure S7A, B). In the first pose, the hydroquinone head group is rotated relative to the top-ranked pose, positioning O1' adjacent to R201 and N202 (Figure S7A). The second pose resembles a binding intermediate along the substrate-entrance tunnel, in which O4' is positioned near residues R201 and N202 instead of E227 (Figure S7B). While the reactive carbon distances are within 4.0 Å for both poses, the candidate catalytic base E227 is not positioned to interact with DDMQ₂H₂ in either pose, and thus they were not further evaluated.

To assess the stability of the top-ranked pose, we performed five independent molecular dynamics (MD) simulations, each initiated from the same geometry but with randomized initial velocities. In a representative 1000-ns simulation, the substrate DDMQ₂H₂ remained stably bound throughout. Clustering of trajectory frames revealed two major conformational sub-states. In the early-state conformation (~0–200 ns), E227 remains positioned near the O4' hydroxyl group (Figure 6A), consistent with the initial geometry capable of undergoing base catalysis. The late-state conformation (~450–1000 ns) resembles an alternative docking pose (Figure S7B), in which N202 forms hydrogen bonds with O4' (Figure 6B). The conformational transition between these two residues is seen in their respective distances to O4' throughout the simulation (Figure 6D). Additionally, R201 consistently forms a salt bridge with E227 across both states (Figure 6A, B) and forms transient interactions with O4' throughout the simulation. Meanwhile across the active site, although the initial geometry positioned O1' within hydrogen bonding distance of N82, O1' does not form persistent polar contacts with any residue over the course of the simulation. The reactive methyl–C5 distance remains near 5 Å throughout, briefly dropping below 4 Å during the transition between the two substates (Figure 6C), indicating a geometry compatible with methyl transfer. To further evaluate the structural integrity of the active site, we calculated root-mean-square-deviation (RMSD) values across simulation time. AdoMet and active site residues remained stable for the entire duration of the simulation, supporting a largely rigid catalytic pocket (Figure S8B, C). The substrate exhibited more movement, likely due to the dynamics of its polyprenyl tail (Figure S8B).

In an independent 100-ns simulation with the same starting geometry, the substrate undergoes head group rotation and samples an alternative conformation resembling one observed in docking calculations (Figures S7A, S9A). In this simulation, O4' rotates away from E227, R201,

and N202, and instead forms transient polar contacts with N82. This unproductive conformation appears to be interconvertible with the initial geometry on the ns-to- μ s timescale, suggesting that they are metastable.

Overall, these data support the physiological relevance of the top-ranked pose and highlight the dynamic and synergistic roles for E227, R201, N202, and N82 in substrate stabilization. The interconvertible nature of these conformations suggests that multiple metastable conformations with similar energies may exist in equilibrium to guide the substrate toward a reactive conformation.

Novel substrate-interacting residues are essential for Coq5 function

To validate the importance of novel substrate interactions identified in MD simulations, we performed mutational analysis on these residues. Coq5-R201Q and E227Q are homologous to human SNVs R220Q (rs528148814) and E246Q (rs749890516), respectively. Either mutation would abolish the salt bridge between these residues, while E227Q would additionally disrupt the hypothesized base catalysis mechanism. Given that the N202S mutation led to a marked reduction—but not complete loss—of CoQ₆ production, we considered the possibility that substrate binding is not completely disrupted by the N202S mutation. To further assess the role of N202, we generated the Coq5-N202L mutant, replacing the polar side chain with a nonpolar, isosteric residue. A similar rationale was used to generate Coq5-N82L, to evaluate the contribution of the only polar residue on the opposite side of the substrate-binding pocket. As a control, we also introduced a nonpolar leucine at R77, a residue previously shown to be nonessential for substrate binding and catalysis in the R77S mutant.

All five mutants stably expressed in *coq5* Δ yeast (Figure S10) but failed to support respiratory growth on glycerol-containing plate medium, with the exception of Coq5-R77L (Figure

7A, B), which fully complemented the growth defect (Figure 7B). Overexpression of protein in respiratory-deficient mutants did not rescue growth (Figure 7, top row), suggesting a severe catalytic defect. Indeed, five-hour $^{13}\text{C}_6$ -*p*ABA labeling of mutants under both low-copy (Figure 8B) and high-copy (Figure S11B) expression failed to produce detectable amounts of *de novo* synthesized $^{13}\text{C}_6$ -CoQ₆ in those expressing Coq5-R201Q, E227Q, and N82L, and only trace amounts of $^{13}\text{C}_6$ -CoQ₆ in those expressing Coq5-N202L. Similar trends were observed for ^{12}C -CoQ₆ levels in labeled and unlabeled cells (Figures 8A, S11A). In contrast, Coq5-R77L restored wild-type levels of ^{12}C -CoQ₆, $^{13}\text{C}_6$ -CoQ₆, and total CoQ₆ (Figures 8A–C, S11A–C), reinforcing the dispensability of R77 in substrate binding and catalysis. All four CoQ₆-deficient mutants accumulated ^{12}C -DDMQ₆ and $^{13}\text{C}_6$ -DDMQ₆ (Figure 8D, E) but not $^{13}\text{C}_6$ -HAB (Figure 8F) under low-copy expression, consistent with a catalytic defect at the Coq5 step. Curiously, under high-copy expression, the intermediate profiles were largely similar except in cells overexpressing Coq5-R201Q, which accumulated $^{13}\text{C}_6$ -HAB but not ^{12}C -DDMQ₆ nor $^{13}\text{C}_6$ -DDMQ₆ (Figure S11D–F). Despite its stable expression (Figure S10C, D), overexpression of Coq5-R201Q may interfere with synthome assembly or flux through the CoQ₆ biosynthetic pathway via alternative mechanisms.

Taken together, these results highlight the importance of the R201-E227 salt bridge and support the role of E227 as a catalytic base. The hydrogen-bonding capacity of N202 also appears to be essential for substrate binding, as the N202L mutation nearly abolished catalytic activity, while N202S retained partial function. Interestingly, the N82L mutation was also inactivating, suggesting that the transient polar contacts formed by N82 with O1' or O4' play a more substantial role in substrate binding than previously thought.

E227-assisted Coq5 catalytic mechanism supported by DFT

Given the MD simulations and subsequent mutagenesis data, we considered a preliminary mechanism in which E227 deprotonates C5 of DDMQH₂ and the resulting carbanion nucleophile attacks the methyl carbon of AdoMet (Figure S12). However, given the relative pK_a's of E227 and an aromatic C–H, the resulting unstabilized carbanion is unlikely to form. Furthermore, we were unable to find a transition state for the formation of the C–C bond in this mechanism. Instead, we propose an alternative mechanism in which E227 abstracts the phenolic proton from O4', increasing the electron density across the aromatic ring and enhances the nucleophilicity of the *ortho* carbon C5, enabling its nucleophilic attack on the methyl group of AdoMet (Figure 9A). Indeed, in the early-state conformation of the 1000-ns MD simulation, the average distances from the oxygen atom of the E227 carboxylate to O4' and H4' were 2.99 Å and 2.15 Å, respectively (Figures 6D, S8A), compatible with deprotonation of the O4' hydroxyl. The methylated intermediate can then undergo solvent- or E227-assisted rearomatization to form the product, DMQH₂. Standard Gibbs free energy of the mechanism on simplified substrates was calculated using density functional theory (DFT) at 298 K in diethyl ether or water, simulating nonpolar or polar active site, respectively. O4' deprotonation by E227 and subsequent methylation in diethyl ether were calculated to have a ΔG of –13.7 kcal/mol and –26.5 kcal/mol, respectively, with an activation barrier $\Delta G^\ddagger$ of +18.4 kcal/mol for the methylation step (Figure 9B). Meanwhile, deprotonation is slightly uphill in water (+5.2 kcal/mol) and energetics of methylation is less favorable (ΔG_{net} : –17.9 kcal/mol; $\Delta G^\ddagger$: +20.3 kcal/mol) (Figure S13). The methylation transition states in diethyl ether (TS1a) and water (TS1b) features an approximately linear geometry, characteristic of an S_N2-like displacement (Figures 9B, S13). E227-assisted rearomatization is generally favorable in both solvents, although active-site water molecules may also mediate the

proton transfer. Collectively, our computational and experimental findings are consistent with this proposed mechanism.

Like CoQ, the hydroquinone in DDMQH₂ can also undergo spontaneous autoxidation. While the Coq5 step is generally thought to require the reduced form of the substrate, we assessed whether a mechanism involving the oxidized quinone, DDMQ, is feasible (Figure S14A). A preliminary DFT calculation was performed on simplified substrates. The activation barrier was calculated to be +42.7 kcal/mol and +41.1 kcal/mol in diethyl ether and water, respectively (Figure S14B, C), significantly higher than that of the reduced substrate. Therefore, our DFT calculations strongly favor DDMQH₂ as the substrate of Coq5.

Based on both computational and experimental evidence, we propose a model of Coq5 catalysis in which the substrate, DDMQH₂, samples multiple local minima before adopting a catalytically competent conformation. Residue R201 forms a salt bridge with E227, positioning E227 to deprotonate the O4' hydroxyl and stabilizing the resulting negative charge. N202 contributes to substrate binding via hydrogen bonding with O4'. N82 appears to support catalysis indirectly by stabilizing a transient, nonproductive binding mode that may help prevent substrate dissociation as it samples different poses within the active site. Together, these findings provide a coherent mechanistic framework that explains the catalytic role of key active-site residues and the observed defects in our mutants.

DISCUSSION

The yeast Coq5 substrate-binding pocket was previously identified by Dai *et al.* through structural studies (34). However, the catalytic mechanism and the specific roles of active-site residues had not been verified biochemically. In this study, we combined an LC-MS/MS-based assay of Coq5-dependent biosynthetic flux with computational modeling to reassess Coq5–

substrate interactions and propose a revised catalytic mechanism for both yeast and human COQ5. While yeast residues R77 and Y78 (human K78 and Y79) were previously implicated in substrate binding or catalysis, our data indicate that R77 is completely dispensable and Y78 plays only a limited role. Instead, our model highlights critical contributions from R201, N202, and N82 (human R220, N221, and N83) in substrate binding, and supports a catalytic role of E227 (human E246). Together, these findings provide a more complete picture of substrate binding and catalysis in Coq5 and its conserved human homolog, and offer mechanistic insight into how certain human SNVs affecting this region may lead to pathogenic outcomes.

Implications of *COQ5* SNVs for disease

Most SNVs identified in the *COQ5* gene remain of uncertain clinical significance. In this study, we modeled three rare human SNVs—N221S (rs1048333814; yeast N202S), R220Q (rs528148814; yeast R201Q), and E246Q (rs749890516; yeast E227Q)—in the yeast *S. cerevisiae* to assess their functional impact. While R201Q and E227Q mutations led to a complete loss of CoQ₆ production and varying levels of DDMQ₆ accumulation (Figures 8A–E, S11A–E), the N202S variant led to a partial retention in total CoQ₆ levels (28% in labeled and 27% in unlabeled cells). It should be emphasized that in the clinical context, even moderate decreases in CoQ levels may contribute to disease. For example, patients with CNS symptoms associated with aberrant *COQ5* mRNA processing exhibited reduced leukocyte CoQ₁₀ levels ranging from 54–90% of normal levels (42,44). Notably, while a more severe CoQ₁₀ deficiency is generally associated with severe bioenergetic defect, the association between CoQ₁₀ levels and oxidative stress may not be linear, with maximal reactive oxygen species (ROS) production observed in patient-derived fibroblasts with 25–45% residual CoQ₁₀ levels (90). Therefore, the connection between CoQ₁₀ deficiency and pathogenesis is likely multi-dimensional.

While the stability of Coq5 is not strongly dependent on the presence of other Coq polypeptides in yeast, its absence reduces the levels of other Coq polypeptides, making it a critical stabilizing component of the yeast CoQ synthome (21,38,39). None of the mutations examined here led to a significant decrease in Coq5 protein expression in yeast. However, it remains possible that other *COQ5* variants may contribute to pathogenicity by impairing protein stability or promoting degradation, as seen in the *coq5-3* mutant (18). Importantly, as protein abundance alone may not reflect functional competency, caution should be taken when using expression data as the sole proxy for pathogenicity during patient diagnosis.

Unlike many other CoQ biosynthetic genes, relatively few pathogenic *COQ5* variants have been reported in patients, most of which are associated with CNS-related phenotypes. This scarcity may reflect an underrepresentation of functional studies that directly test the impact of SNVs on *COQ5* function. Alternatively, it is possible that many deleterious mutations are embryonic lethal, or that some SNVs exert only subtle effects of CoQ biosynthesis and are insufficient to cause disease. Further *in vivo* and patient-derived cell studies will be critical to evaluate these possibilities and improve clinical interpretation of rare *COQ5* variants.

Structural considerations in understanding COQ5 function

The recent availability of high-quality *in silico* prediction tools, such as multimeric structural prediction in AlphaFold 3 and the variant classification tool AlphaMissense, has expanded opportunities to study the structure-function relationships of human *COQ5* and homologs. Both human *COQ5* and yeast Coq5 share a highly conserved catalytic site (Figure 2), reinforcing the utility of *S. cerevisiae* as a good system for functional investigation of human SNVs. However, structural predictions of the hydrophobic dimer interface in human *COQ5* were less robust, possibly due to sequence divergence from yeast in this region. Four human SNVs were

identified by Missense3D-PPI as potentially disruptive to the predicted dimer interface, all of which were located in highly conserved residues (Supplementary File 2). This suggests that while the AlphaFold-predicted dimer model may be uncertain, the functional importance of the interface is nevertheless supported by evolutionary conservation of key residues and variant classification. Further experimental work is required to investigate the role of the dimer interface in overall protein structural integrity, catalysis, and synthome assembly.

In addition to limitations in predicting quaternary structure, discrepancies between AlphaFold and crystallographic models also highlight uncertainty in COQ5's flexible regions. For example, AlphaFold predicts an N-terminal helix near the active site that is not visualized in the available yeast Coq5 crystal structure, raising the possibility that this region is dynamic and may act as a "gate" regulating access to the active site. Unfortunately, both AlphaFold models and crystal structures ultimately offer only static representations with respective limitations. For example, crystal structures often miss disordered regions, while AlphaFold cannot distinguish between different conformational states, such as ligand-bound versus unbound states. Thus, despite the potential of these models to offer valuable insight into structure and function of human COQ5 and yeast Coq5, they must be interpreted cautiously and complemented by experimental validation.

Catalytic insights into COQ5 mechanism

Using yeast as model organism, we have characterized the effects of mutations in the substrate-binding pocket on catalytic activity, an insight applicable to both human COQ5 and yeast Coq5 due to their high degree of conservation within the substrate-binding pocket. Our yeast Coq5 substrate-binding mutants, which exhibited CoQ₆ biosynthetic defects, accumulated the diagnostic intermediate DDMQ₆ but not the earlier intermediate HAB when stably expressing near-

endogenous levels of Coq5 polypeptide (Figures 4E–G, 8D–F). This accumulation pattern is consistent with a catalytic defect at the Coq5 step. However, the absolute LC-MS/MS signal intensities for intermediates were significantly lower than those for CoQ₆, potentially reflecting the reduced stability or intrinsic toxicity of these intermediates. The variability in intermediate abundance across experiments—particularly in wild-type or *coq5*Δ control strains—necessitated that our interpretation of intermediate accumulation remain qualitative (binary “yes/no”) rather than quantitative. Moreover, since Coq5 operates within the multi-protein CoQ synthome, our *in vivo* measurements cannot fully isolate its catalytic contribution from possible flux compensation by other biosynthetic enzymes. Future work to optimize an *in vitro* activity assay for purified recombinant yeast Coq5, or to perform labeling experiments in yeast strains carrying catalytically inactive point mutants of specific CoQ synthome components, could help quantify intermediate flux more accurately and directly assess the catalytic capacity of individual variants.

To complement these *in vivo* observations and probe underlying molecular mechanisms, we performed *in silico* docking and MD simulation studies. The docking pose reported by Dai *et al.* could not be reproduced under our conditions, and the mutations Y78F and R77S/L did not significantly affect CoQ₆ biosynthesis in yeast, indicating that these residues are not essential for substrate binding nor catalysis. The inability of rigid docking to yield catalytically competent poses suggests that Coq5 substrate binding may involve induced-fit rearrangements not captured by the AdoMet-bound Coq5 crystal structure. MD simulations further support the view of substrate binding as a dynamic process—in our experiments, substrate binding was not especially stable in any of the conformations observed, suggesting that the substrate actively samples multiple conformations to avoid dissociation before reaching the catalytically competent state. Interestingly, all-atom RMSD also exhibited small fluctuations over the course of the simulation

(Figure S8D). These fluctuations may reflect larger-scale changes in overall protein conformation, potentially stemming from the absence of a membrane environment. Although Coq5 activity has been demonstrated in *E. coli* in the absence of known binding partners (91), association with the membrane or native lipids may still enhance substrate retention. In addition, the longer polyprenyl tails of the native substrates may influence binding dynamics relative to the shorter analogs typically used in modeling. Alternatively, the fluctuations could reflect the necessity of the second subunit for efficient catalysis. Dimerization may contribute to active-site architecture by positioning substrate-binding residues, such as those located on helix $\alpha 1$. These possibilities further underscore the need for more comprehensive modeling of the protein environment, as well as experimental validation of the dimer interface.

Further mechanistic insights can be gained from several distant homologs of Coq5. The first example is the structurally related *C*-methyltransferase CouO from *Streptomyces rishiriensis* (PDB: 5M58), which participates in the biosynthesis of the antibiotic coumermycin A1. Both CouO and Coq5 methylate an aromatic *ortho* to a hydroxyl group. Structural alignment of CouO and Coq5 reveals that residues H120, R121, and S146 in CouO correspond to R201, N202, and E227 in Coq5, respectively (92). Despite positional variation in the residues implicated in negative charge stabilization and base catalysis, a similar mechanism has been proposed, in which a general base H120 deprotonates the hydroxyl group while R121 stabilizes the negatively charged intermediate. Secondly, a plastid-localized homolog in plants, MENG, participates in the biosynthesis of phyloquinone, which features a larger naphthoquinone head group compared to the benzoquinone head group in CoQ. In *Arabidopsis thaliana* MENG (UniProtKB: Q3ED65), the residues corresponding to R201 and N202 are conserved (R156 and N157), while E227 is replaced by D182, a residue with similar properties capable of base catalysis. The conservation of putative

catalytic residues in these catalytically similar homologs reinforces their functional importance. Several other structurally related enzymes bind benzoquinone-derived substrates, including the CoQ biosynthetic *O*-methyltransferase Coq3/UbiG (2,93–96) and the distant homolog RquA from *Rhodospirillum rubrum*, the latter of which employs AdoMet to donate an amino group to the opposing *ortho* position of CoQ (C3) to synthesize rhodoquinone (97,98). Although these enzymes diverge functionally, their shared Rossmann-fold scaffold underscores the catalytic versatility of this structural framework, illustrating how Coq5 and its distant *C*-methyltransferase homologs employ a unique mechanism adapted to their specific biological role.

While our yeast Coq5 studies provide insight into substrate-binding dynamics, several mechanistic questions surrounding both the human and yeast protein remain unanswered. First, the origin of COQ5's regioselectivity is unclear. COQ5 must reliably distinguish between two opposing *ortho* positions on the aromatic ring, C3 and C5, with C5 being more sterically hindered due to the adjacent polyprenyl tail at C6. Whether catalytically competent conformations strongly favor methylation at C5 requires further investigation. Off-pathway products, if formed in our studies, may evade detection due to their indistinguishable mass spectra. Second, a recent study found that both human COQ5 and yeast Coq5 exhibit methylation activity toward demethylmenaquinol (DMKH₂), a bulkier naphthoquinol-derived substrate found in *E. coli* and other anaerobic prokaryotes (91). This unexpected substrate promiscuity may represent an evolutionary remnant from ancestral anaerobic organisms that use the product, menaquinol, as an alternative electron carrier (91). However, the residues mediating substrate selectivity versus promiscuity has not been elucidated. Based on our docking calculations, large aromatic residues lining the substrate-binding pocket, such as yeast F198/F228 (human F217/F247), may facilitate π -stacking interactions with DMKH₂. Further computational modeling with DMK(H₂) may clarify

the molecular determinants of substrate selectivity versus promiscuity. Finally, although this study focused on substrate-binding residues, future work could examine how cofactor binding and release modulate COQ5's conformation and substrate or product affinity. Such insights would deepen our understanding of its catalytic mechanism within the context of substrate channeling in the larger CoQ synthome.

Outstanding questions in CoQ biosynthesis

Our studies provide insight into the activity of COQ5/Coq5 in the broader context of its integration into the CoQ synthome. In our experiments, varying yeast Coq5 expression levels alone did not substantially alter CoQ production under the tested conditions. In *coq5Δ* yeast, rescuing with either high- or low-copy expression of Coq5 produced similar CoQ levels, both remaining below those of the true wild-type strain, suggesting that Coq5 is not a rate-limiting step in CoQ biosynthesis. In addition, we did not observe any mutations that led to significant accumulation of $^{13}\text{C}_6\text{-HAB}$, a marker for synthome destabilization. These findings suggest that the presence of the late-stage intermediate DDMQ₆ may be sufficient to stabilize the synthome, and that stoichiometric imbalances caused by excess inactive Coq5 do not substantially impair complex formation. An intriguing exception was the overexpression of the Coq5-R201Q mutant, which led to the unexpected accumulation of $^{13}\text{C}_6\text{-HAB}$ (Figure S11F). The origin of this product remains unclear and may point to alternative regulatory mechanisms that influence metabolic flux in the CoQ pathway.

The activity of Coq5 is generally believed to require the substrate in its reduced hydroquinone form (DDMQH₂), as supported by prior *in vitro* studies showing increased methylation activity in fresh or freeze-thawed isolated mitochondria following NADH addition (36). This is further corroborated by our DFT calculations, which predict a significantly lower

activation barrier for the methylation of the reduced substrate compared to its oxidized form (Figures 9B, S14B). A similar dependence on reducing agents such as NADH and dithionite has also been observed for the final *O*-methylation step in CoQ biosynthesis catalyzed by Coq3 in rat liver mitochondria (99), where the requirement for reducing agents was bypassed by pre-reducing the demethyl-Q (DMeQ) substrate. However, the mechanistic basis for this reduced-substrate requirement in Coq3 remains less well understood. Furthermore, the identity of the oxidoreductase that maintains DDMQH₂ in its reduced state *in vivo* remains unknown. Recent evidence implicates the mitochondrial NAD(P)H oxidoreductase RTN4IP1/OPA10 and its yeast homolog Yim1 as plausible candidates (100). RTN4IP1 has been shown to interact with both COQ3 and COQ5 in human HEK293T cells. Purified RTN4IP1 enhances the *O*-methylation activity of COQ3 in the presence of NADH or NADPH (100), suggesting that it may perform a similar role in *C*-methylation by COQ5. In yeast, Coq11—an atypical short-chain dehydrogenase/reductase and component of the yeast CoQ synthome—represents another possibility (101). Coq11 has been implicated in regulating respiration, CoQ biosynthesis, cellular CoQ redox state, CoQ synthome assembly, and CoQ domain formation (17,102), although its exact catalytic role has not been defined. Intriguingly, the step catalyzed by COQ7, which bridges the COQ5 and COQ3 steps, is thought to require an oxidized substrate that undergoes NADH-mediated reduction and electron transfer to a di-iron center within the protein (103,104). Thus, how the CoQ synthome coordinates substrate channeling between enzymes with differing substrate oxidation state requirements remains a key open question, highlighting the intricate orchestration of CoQ biosynthesis.

Beyond substrate recognition, COQ5's function may also be influenced by post-translational modification and its spatial organization within the CoQ synthome. One potential regulatory mechanism involves phosphorylation. The detection of a phosphorylated form of yeast

Coq5 has been shown to depend on the presence of Coq8, the atypical kinase component of the CoQ synthome (105). Reconstruction of the synthome using ancestral COQ polypeptides demonstrated that phosphorylation of COQ3 by COQ8B, the higher eukaryotic homolog of Coq8, enhanced its *O*-methylation activity (104), raising the possibility that a similar regulatory mechanism could apply to COQ5 and its homologs. Although homologs of COQ5 physically associate with those of COQ8, direct phosphorylation of COQ5 has not been demonstrated *in vitro* (22,106), leaving open the possibility of indirect effects mediated by an alternative protein kinase. A second layer of potential regulation arises from the spatial organization required for substrate channeling in CoQ biosynthesis. Substrate channeling helps improve catalytic efficiency in a biosynthetic pathway while limiting side reactions and preventing the escape of toxic intermediates. Mechanisms of substrate channeling in the CoQ synthome remain poorly defined due to the lack of a spatially resolved model of the entire synthome. Recent work by Launay *et al.* used AlphaFold2 to construct a structural model of the minimal functional *E. coli* Ubi metabolon assembly (107). However, such predictions are inherently speculative and the current model is incompatible with the incorporation of additional subunits or possible homodimers such as the COQ5 homolog UbiE. Moreover, the stoichiometry of the synthome *in vivo* is not yet known. Recently, Wang *et al.* performed coarse-grained molecular dynamics simulations that offered a different perspective, suggesting that close proximity, rather than the precise spatial organization of the synthome, is necessary for efficient substrate channeling and metabolon yield (108). Nevertheless, existing protein–protein interaction data point to at least some level of defined organization; for instance Coq4, now identified as the enzyme acting immediately upstream of Coq5 (109), directly associates with Coq5 (27). Interestingly, both human and yeast homologs of Coq5 can function in *E. coli* in the absence of native partner proteins (91), a finding that may

challenge the necessity of post-translational modification and strict spatial organization, or alternatively imply that these homologs can physically associate with the corresponding *E. coli* CoQ biosynthetic complex, the Ubi metabolon. Taken together, these findings imply that both phosphorylation state and spatial arrangement could fine-tune Coq5's activity within the synthome, but the precise interplay between these factors, and whether they are universally required across all species, remains to be determined. Future work to identify and characterize sites of post-translational modification and protein–protein interaction interfaces may yield further catalytic insights.

Broader significance and applications

In this work, we identified and characterized the substrate-binding pocket of COQ5 through combined computational and biochemical approaches in the yeast system. The mechanistic insights gained from this work extend beyond COQ5 and its homologs to the broader question of how multi-enzyme complexes coordinate redox chemistry and substrate channeling in living cells. Given CoQ's essential role in mitochondrial respiration, redox homeostasis, and antioxidant defense, elucidating its biosynthetic machinery carries clear biomedical significance, particularly for conditions associated with CoQ deficiency and mitochondrial dysfunction. Further exploration of COQ5's interplay with other synthome components, along with the regulatory mechanisms governing CoQ biosynthesis, may ultimately inform improved diagnostic tools and targeted therapeutic interventions for affected patients.

Table 1. Protein sequences used in the multiple sequence alignment.

<i>Species</i>	Protein Name	NCBI Accession Number
<i>Homo sapiens</i>	COQ5	NP_115690.3
<i>Saccharomyces cerevisiae</i>	Coq5	NP_013597.1
<i>Caenorhabditis elegans</i>	COQ-5	NP_498704.1
<i>Escherichia coli</i>	UbiE	YP_026269.1
<i>Pseudomonas aeruginosa</i>	UbiE	NP_253750.1
<i>Drosophila melanogaster</i>	Coq5	NP_572865.1
<i>Arabidopsis thaliana</i>	Coq5	NP_200540.1
<i>Danio rerio</i>	Coq5	NP_001004541.1
<i>Gallus gallus</i>	COQ5	NP_001006194.1
<i>Mus musculus</i>	Coq5	NP_080780.1
<i>Schizosaccharomyces pombe</i>	Coq5	NP_587834.1

Table 2. Genotype and source of yeast strains.

<i>Strain</i>	<i>Genotype</i>	<i>Source</i>
W303-1B	<i>MATα leu2-3,-112; his3-11,-15; trp1-1; ura3-1; ade2-1; can1-100</i>	R. Rothstein ^a
W303-1B <i>coq5Δ</i>	<i>MATα leu2-3,-112; his3-11,-15; trp1-1; ura3-1; ade2-1; can1-100 coq5:HIS3</i>	(36)
JM6	<i>MATα his-4 ρ⁰</i>	J. E. McEwen ^b
JM8	<i>MATα ade-1 ρ⁰</i>	J. E. McEwen ^b

^a Dr. Rodney Rothstein, Department of Human Genetics, Columbia University

^b Dr. Joan E. McEwen

Table 3. Plasmid constructs used in this study.

<i>Plasmid</i>	Construction Description	Source
pRS314	Yeast shuttle vector carrying the <i>TRP1</i> gene; low-copy	(110)
pRS316	Yeast shuttle vector carrying the <i>URA3</i> gene; low-copy	(110)
pRS426	Yeast shuttle vector carrying the <i>URA3</i> gene; high-copy	(111)
pGBsCOQ5	Wildtype yeast <i>COQ5</i> open reading frame with 400 bp upstream and 100 bp downstream regions (~1500 bp) inserted into the <i>SmaI</i> site of pRS316	(18)
pRS314/coq5-2	Yeast allele <i>coq5-2</i> (G199D) along with 400 bp upstream and 100 bp downstream regions inserted into the <i>SmaI</i> site of pRS314; low-copy	(18)
pRS316/coq5-3	Yeast allele <i>coq5-3</i> (G304D) along with 400 bp upstream and 100 bp downstream regions inserted into the <i>SmaI</i> site of pRS316; low-copy	(18)
pGBsCOQ5-R77S	pGBsCOQ5 carrying the cDNA change c.229_230delinsAC, expressing Coq5-R77S	This work
pGBsCOQ5-R77L	pGBsCOQ5 carrying the cDNA change c.230G>T, expressing Coq5-R77L	This work
pGBsCOQ5-Y78F	pGBsCOQ5 carrying the cDNA change c.233A>T, expressing Coq5-Y78F	This work

Table 3. Plasmid constructs used in this study. (Cont.)

<i>Plasmid</i>	Construction Description	Source
pGBsCOQ5-N82L	pGBsCOQ5 carrying the cDNA change c.244_246delinsCTA, expressing Coq5-N82L	This work
pGBsCOQ5-R201Q	pGBsCOQ5 carrying the cDNA change c.601_603delinsCAG, expressing Coq5-R201Q	This work
pGBsCOQ5-N202S	pGBsCOQ5 carrying the cDNA change c.603A>G, expressing Coq5-N202S	This work
pGBsCOQ5-N202L	pGBsCOQ5 carrying the cDNA change c.604_606delinsCTA, expressing Coq5-N202L	This work
pGBsCOQ5-E227Q	pGBsCOQ5 carrying the cDNA change c.679_681delinsCAG, expressing Coq5-E227Q	This work
pGBmCOQ5	Wildtype yeast <i>COQ5</i> open reading frame with 400 bp upstream and 100 bp downstream regions (~1500 bp) inserted into the <i>SmaI</i> site of pRS426; high-copy	(18)
pRS426/coq5-2	Yeast allele <i>coq5-2</i> (G199D) along with 400 bp upstream and 100 bp downstream regions inserted into the <i>SmaI</i> site of pRS426; high-copy	(18)
pRS426/coq5-3	Yeast allele <i>coq5-3</i> (G304D) along with 400 bp upstream and 100 bp downstream regions inserted into the <i>SmaI</i> site of pRS426; high-copy	(18)

Table 3. Plasmid constructs used in this study. (Cont.)

<i>Plasmid</i>	Construction Description	Source
pGBmCOQ5-R77S	pGBmCOQ5 carrying the cDNA change c.229_230delinsAC, expressing Coq5-R77S	This work
pGBmCOQ5-R77L	pGBmCOQ5 carrying the cDNA change c.230G>T, expressing Coq5-R77L	This work
pGBmCOQ5-Y78F	pGBmCOQ5 carrying the cDNA change c.233A>T, expressing Coq5-Y78F	This work
pGBmCOQ5-N82L	pGBmCOQ5 carrying the cDNA change c.244_246delinsCTA, expressing Coq5-N82L	This work
pGBmCOQ5-R201Q	pGBmCOQ5 carrying the cDNA change c.601_603delinsCAG, expressing Coq5-R201Q	This work
pGBmCOQ5-N202S	pGBmCOQ5 carrying the cDNA change c.603A>G, expressing Coq5-N202S	This work
pGBmCOQ5-N202L	pGBmCOQ5 carrying the cDNA change c.604_606delinsCTA, expressing Coq5-N202L	This work
pGBsCOQ5-E227Q	pGBmCOQ5 carrying the cDNA change c.679_681delinsCAG, expressing Coq5-E227Q	This work

Table 4. List of primers used in this study.

<i>Resulting Mutation</i>	<i>Primers</i>
Coq5-R77S	F 5'-gtcttttctccgtggccaatagctatgacttgatgaatgatgt-3' R 5'-acatcattcatcaagtcatactattggccacggaagaaaagac-3'
Coq5-R77L	F 5'-cttttctccgtggccaatctgtatgacttgatgaatgatg-3' R 5'-catcattcatcaagtcatactattggccacggaagaaaag-3'
Coq5-Y78F	F 5'-cttcgtggccaatcggttgacttgatgaatgat-3' R 5'-atcattcatcaagtcacaccgattggccacggaag-3'
Coq5-N82L	F 5'-cgtggccaatcggtatgacttgatgctagatgttatgtcattaggaattcata-3' R 5'-tatgaattcctaataacataacatctagcatcaagtcataccgattggccacg-3'
Coq5-R201Q	F 5'-catctacacagtctccttcggtatccagaatttcaccgatattcaaaagggtt-3' R 5'-aacccttttgaatatcggtgaaattctggataccgaaggagactgtgtagatg-3'
Coq5-N202S	F 5'-agtctccttcggtatcagaagtttcaccgatattcaaaagg-3' R 5'-ccttttgaatatcggtgaaacttctgataccgaaggagact-3'
Coq5-N202L	F 5'-ctacacagtctccttcggtatcagactattcaccgatattcaaaagggtttaa-3' R 5'-ttaaaccttttgaatatcggtgaatagtctgataccgaaggagactgtgtag-3'
Coq5-E227Q	F 5'-cgggcggtatttttattgtctacagtttccaaaattgagaatcccct-3' R 5'-aggggattctcaattttggaaaactgtagacaataaaaaataccgcccg-3'

Table 5. Precursor-to-product ion transitions used in this study.

<i>Analyte</i>	<i>m/z</i> [M+H] ⁺	<i>m/z</i> [M+NH ₄] ⁺
HAB	546.4/150.0	563.0/150.0
¹³ C ₆ -HAB	552.4/156.0	569.0/156.0
DDMQ ₆	547.4/153.1	Not used
¹³ C ₆ -DDMQ ₆	553.4/159.1	Not used
CoQ ₆	591.4/197.0	608.0/197.0
¹³ C ₆ -CoQ ₆	597.4/203.0	614.0/203.0
CoQ ₄	455.4/197.0	472.0/197.0

Table 6. Description and source of antibodies.

<i>Antibody</i>	Working dilution	Source
Atp2	1:4,000	C. M. Koehler ^c
Coq5	1:5,000	(18)

^c Dr. Carla M. Koehler, Department of Chemistry and Biochemistry, UCLA

FIGURES

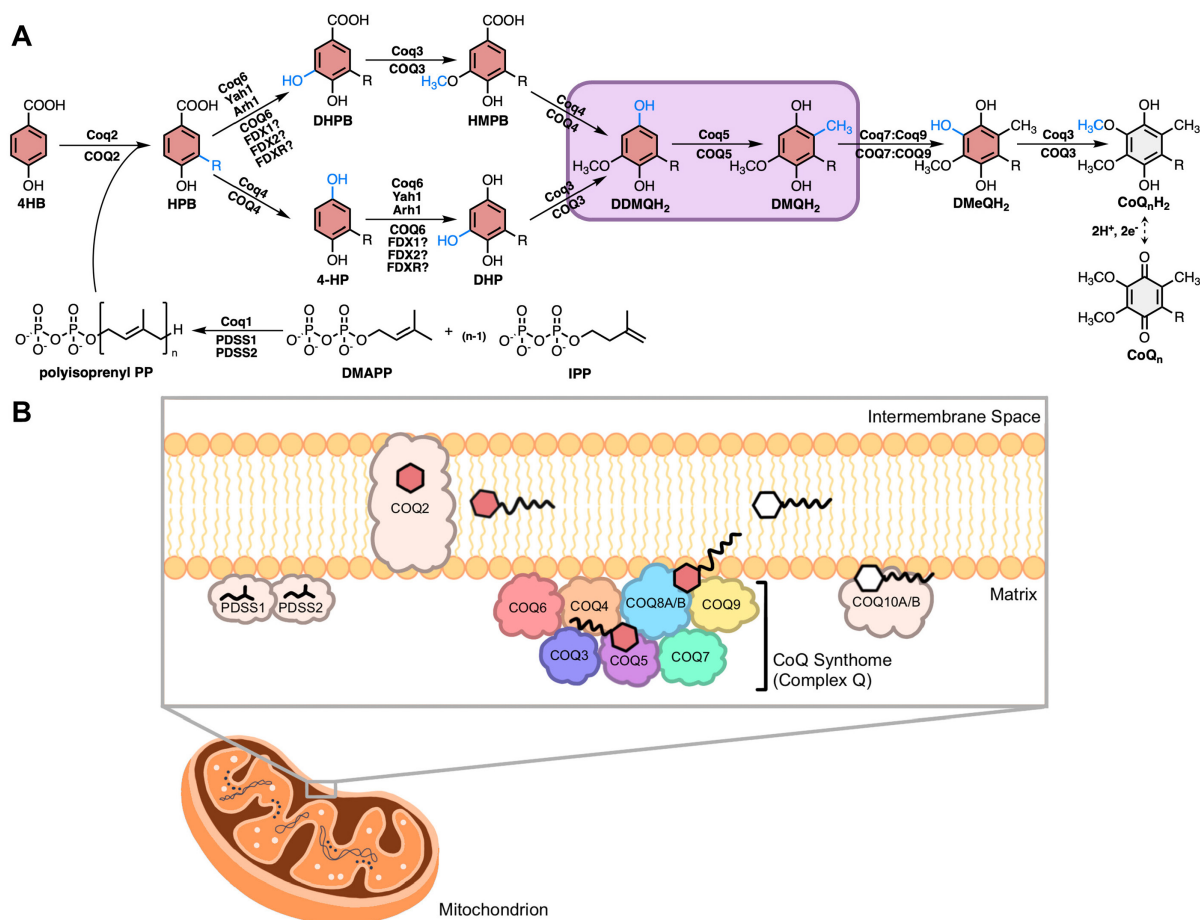


Figure 1. Overview of the CoQ biosynthetic pathway and associated mitochondrial biosynthetic proteins. (A) The CoQ biosynthetic pathways in the yeast *S. cerevisiae* (polypeptides labeled above arrows) and humans (below arrows) are highly conserved. 4-hydroxybenzoic acid (4-HB) is the common aromatic CoQ precursor in yeast and humans, while yeast can utilize an additional precursor, *para*-aminobenzoic acid (*p*ABA) (84,85). Recent evidence suggests that both human COQ4 and its yeast homolog possess oxidative decarboxylation activity, filling the longstanding gap in the pathway (109). However, the order of enzymatic steps remains an open debate due to conflicting evidence from knockout and mutagenesis studies (85,88,89,109,112). Intermediates pictured in the pathway include: DMAPP, dimethylallyl pyrophosphate; IPP,

isopentenyl pyrophosphate; HPB, 3-polyprenyl-4-hydroxybenzoic acid; 4-HP, 4-hydroxy-2-polyprenylphenol; DHPB, 4,5-dihydroxy-3-polyprenylbenzoic acid; HMPB, 4-hydroxy-5-methoxy-3-polyprenylbenzoic acid; DHP, 4,5-dihydroxy-3-polyprenylphenol; DDMQH₂, 2-methoxy-6-polyprenyl-1,4-benzohydroquinone; DMQH₂, 2-methoxy-5-methyl-6-polyprenyl-1,4-benzohydroquinone; DMeQH₂, 3-methyl-6-methoxy-2-polyprenyl-1,4,5-benzenetriol. R represents the polyprenyl tail (n = 6 in yeast, n = 10 in humans). The step catalyzed by human COQ5 and its yeast homolog are highlighted in purple. **(B)** Human polypeptides involved in CoQ biosynthesis are shown. Colored proteins represent the multi-subunit biosynthetic complex known as the CoQ synthome (also known as Complex Q) (12). The PDSS proteins (homologous to yeast Coq1), COQ2, and the CoQ chaperone proteins COQ10A and COQ10B are not part of the complex. Both human COQ8A/COQ8B and its yeast homolog, Coq8, are dynamically associated members of the synthome. The yeast CoQ synthome also contains Coq11, a polypeptide that lacks a close human homolog (17,101,102). Shaded and unshaded hexagons represent CoQ intermediates and the final CoQ product, respectively. Polypeptides are not drawn to scale. The exact spatial arrangement and stoichiometry of the synthome remain undetermined. Figure adapted from Wang *et al.* (24).

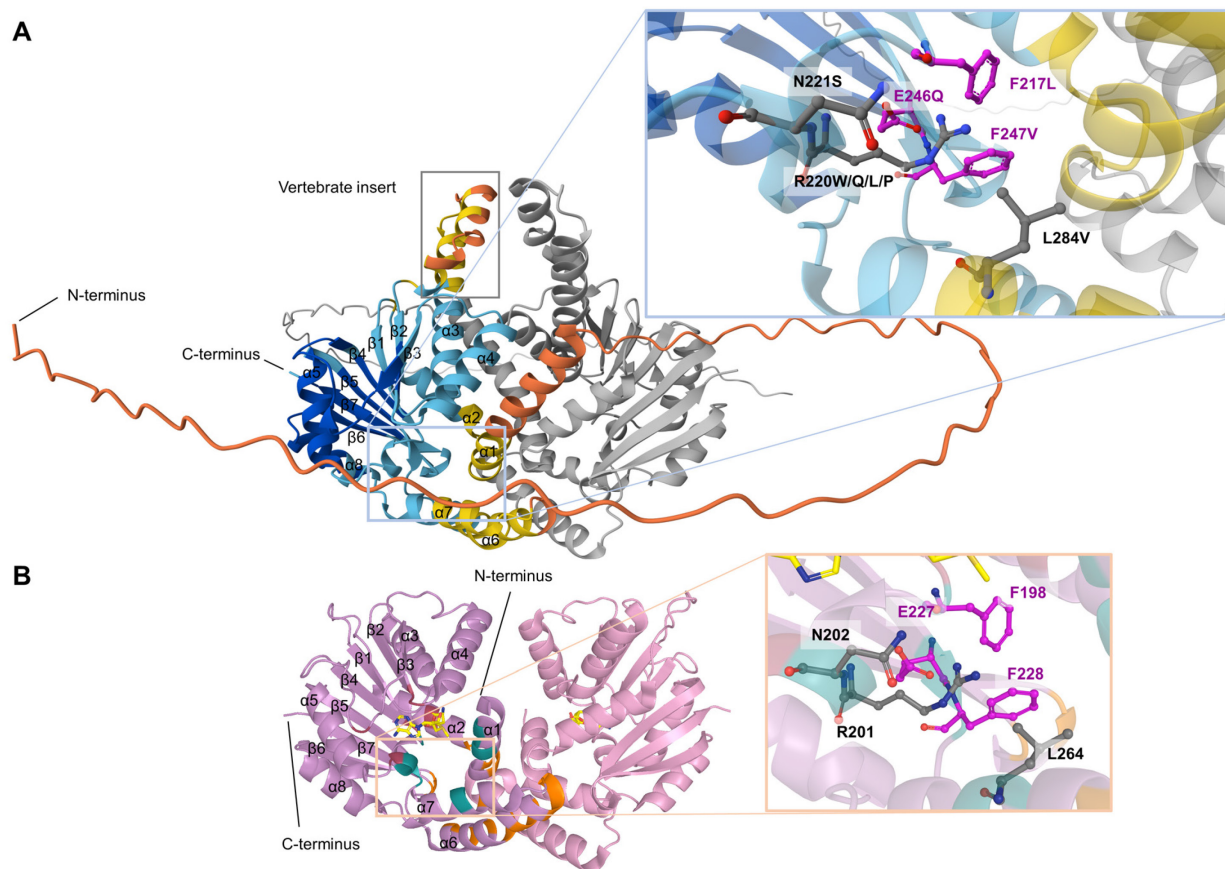


Figure 2. Comparison of the AlphaFold-predicted human COQ5 dimer with the AdoMet-bound yeast Coq5 dimer crystal structure. (A) The AlphaFold-predicted human COQ5 dimer is shown. Colors reflect local confidence according to the predicted local distance difference test (pLDDT). Regions with very high confidence are shown in dark blue (pLDDT > 90), confident in light blue (90 > pLDDT > 70), low confidence in yellow (70 > pLDDT > 50), and very low confidence in orange (pLDDT < 50). Regions with very low confidence may correspond to flexible or dynamic regions. Secondary structure labels are adapted by homology to yeast Coq5 (34). Locations of SNVs affecting the substrate binding pocket are depicted in the top right panel. Three newly identified substrate-interacting residues from this study for which an SNV was analyzed are highlighted in purple (F217, E246, F247, corresponding to yeast residues F198, E227, F228). Note that other substrate-interacting residues that do not have an SNV mapped to them (Y79, M82, N83,

and Y283, corresponding to yeast residues Y78, M81, N82, and Y263) are not shown. Structure was generated by the AlphaFold Server (alphafoldserver.com) (56) and visualized using the Mol* 3D viewer (113). The coordinates are provided in Supplementary File 1. This information is subject to AlphaFold Server Output Terms of Use found at alphafoldserver.com/output-terms. **(B)** The AdoMet-bound yeast Coq5 dimer crystal structure (PDB: 4OBW) is shown in lavender and pink. Segments of α -helices $\alpha 1$, $\alpha 6$, $\alpha 7$, and loop regions involved in dimerization in the monomer on the left are highlighted in orange. Regions interacting with AdoMet are colored in dark red. Regions corresponding to putative substrate-interacting residues identified in this study are colored in teal. AdoMet is shown in yellow. Yeast residues homologous to those highlighted in **(A)** are shown in the right panel.

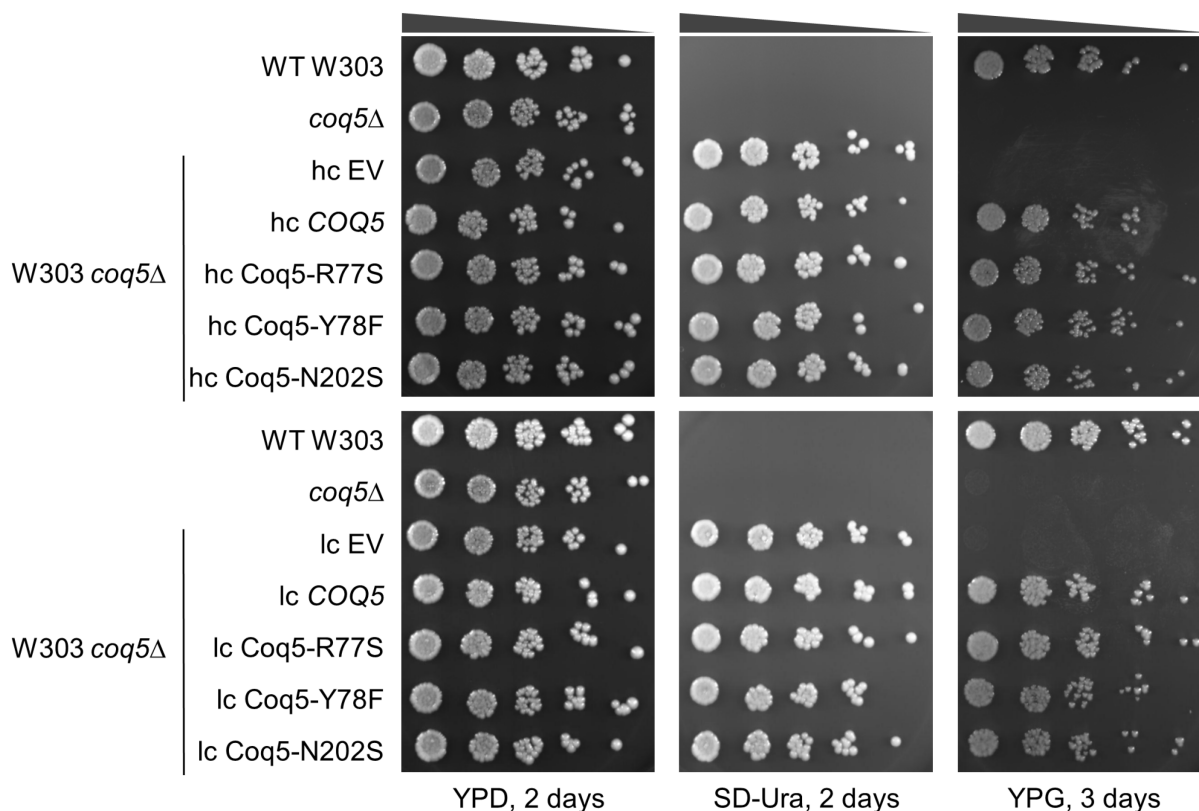


Figure 3. Yeast Coq5-R77S, Y78F, and N202S mutants support growth on respiratory medium. W303 *coq5Δ* yeast were transformed with designated high-copy (top row) or low-copy (bottom row) plasmids. Cultures were grown overnight in selective media (or non-selective synthetic media for untransformed strains), then diluted to $OD_{600} = 0.2$. 2 μ L of 5-fold serial dilutions were spotted onto fermentable YPDextrose (YPD), respiratory YPGlycerol (YPG), or synthetic selective media containing dextrose (SD-Ura). Plates were incubated at 30 °C for two days for YPD and SD-Ura plates or three days for YPG plates before imaging. Data are representative of three biological replicates.

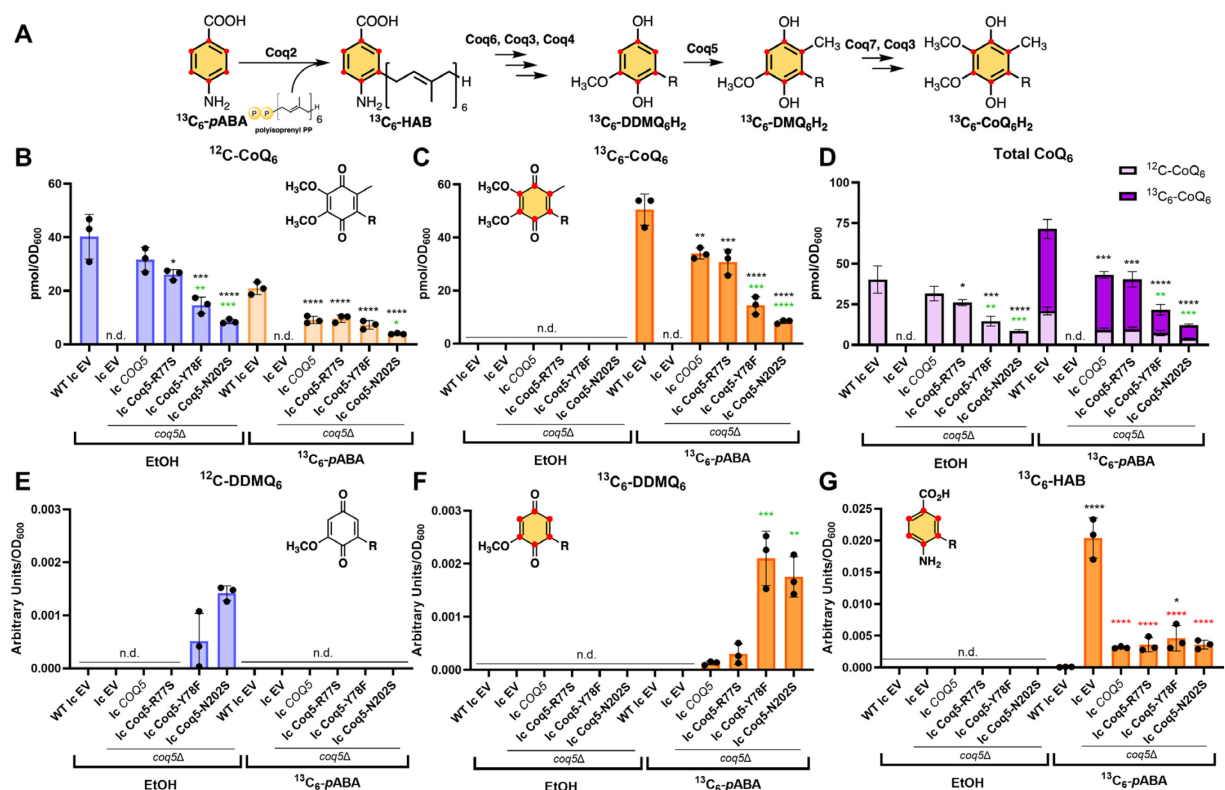


Figure 4. Mutants expressing Coq5-Y78F and N202S retain partial CoQ biosynthetic activity. Wild-type yeast transformed with empty vector and *coq5Δ* yeast transformed with the indicated low-copy plasmids were labeled at OD₆₀₀ = 0.6 with 8 μg/mL $^{13}\text{C}_6$ -para-aminobenzoic acid ($^{13}\text{C}_6$ -pABA) or ethanol as a vehicle control. Lipids were analyzed via LC-MS/MS after 5 h of labeling. (A) The yeast CoQ₆ biosynthetic pathway and $^{13}\text{C}_6$ -pABA labeling strategy are shown. Incorporation of $^{13}\text{C}_6$ -pABA into CoQ₆ ($^{13}\text{C}_6$ -CoQ₆) or CoQ intermediates results in a +6 Da mass shift (indicated by red dots). (B–G) Quantification of ^{12}C -CoQ₆ (B), $^{13}\text{C}_6$ -CoQ₆ (C), total CoQ₆ (D), ^{12}C -DDMQ₆ (E), $^{13}\text{C}_6$ -DDMQ₆ (F), and $^{13}\text{C}_6$ -HAB (G) in the transformants are shown. Total CoQ₆ was calculated as the sum of ^{12}C -CoQ₆ and $^{13}\text{C}_6$ -CoQ₆. ^{12}C -HAB was not quantified due to a co-eluting peak. All data depict mean ± SD of three biological replicates. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test. Black, red, and green asterisks denote comparisons to wild-type yeast

expressing the low-copy empty vector (WT lc EV), *coq5Δ* yeast expressing the low-copy empty vector (*coq5Δ* lc EV), and *coq5Δ* expressing the low-copy *COQ5* plasmid (lc *COQ5*), respectively. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$, and ns = not significant. n.d. = peak not detected; n.d. results were excluded from statistical analyses. See Materials and Methods for details. DDMQ₆, 2-methoxy-6-hexaprenyl-1,4-benzoquinone; HAB, 3-hexaprenyl-4-aminobenzoic acid.

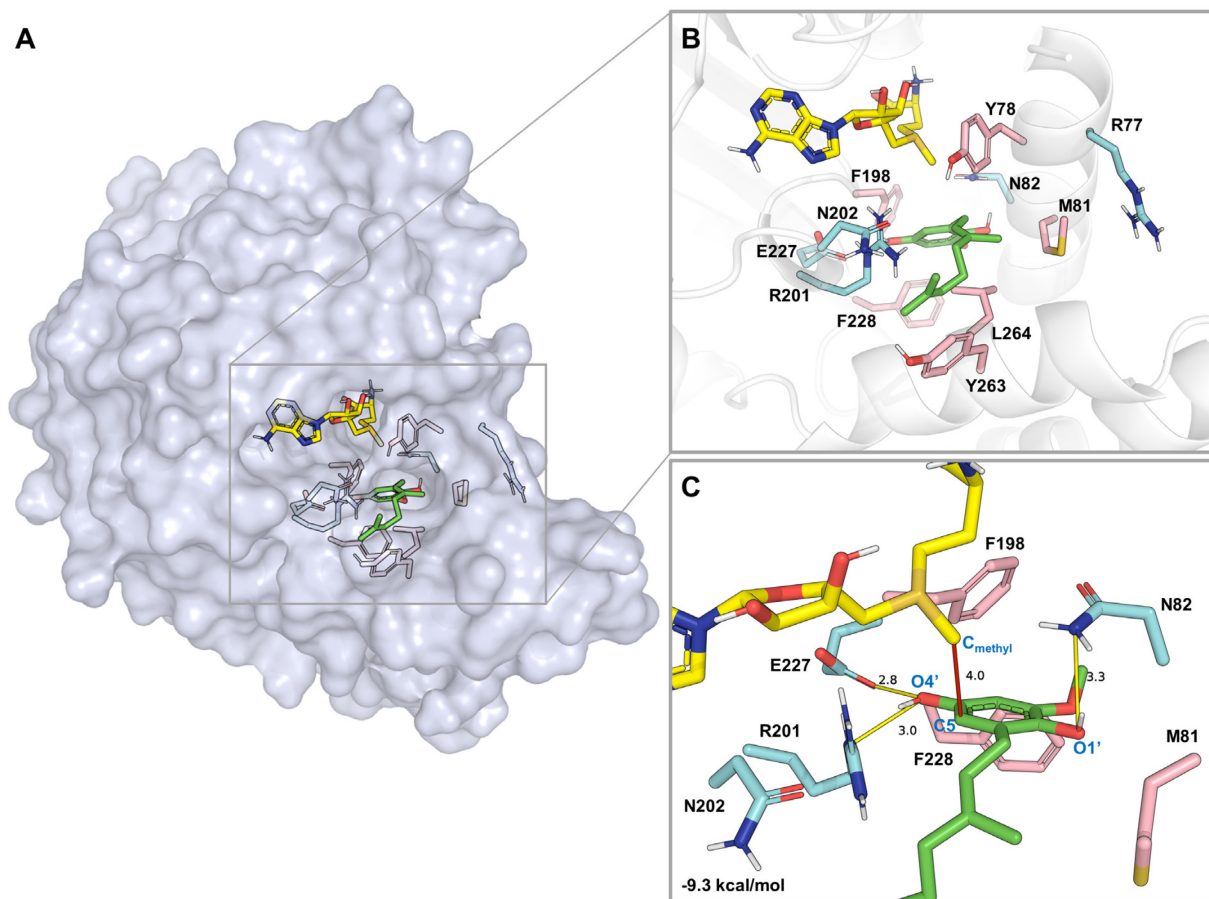


Figure 5. Docking reveals additional substrate-interacting residues. (A) The geranylated substrate analog, DDMQ₂H₂, was docked into chain A of the AdoMet-bound crystal structure of yeast Coq5 (PDB: 4OBW) using AutoDock Vina. The top-ranked docking pose has an estimated binding affinity of -9.3 kcal/mol. AdoMet (yellow) and DDMQ₂H₂ (green) occupy their respective binding pockets. (B) A close-up view of the binding site with surrounding residue side chains is shown. Nonessential residues R77 and Y78, previously highlighted by Dai *et al.* as important for hydrogen bonding and catalysis, respectively, are included for comparison (34). Nonpolar hydrogens are omitted for clarity. (C) The distance between the reactive atoms (the AdoMet methyl carbon and C5 of DDMQ₂H₂) is shown in red. Polar contacts within 3.5 Å between side chains and the substrate are shown in yellow. All distances (in Ångströms) are measured between heavy atoms. Atom labels are shown in blue. Polar and nonpolar residues are colored in light blue

and pink, respectively. The coordinates of this docking pose are provided in Supplementary File 3.

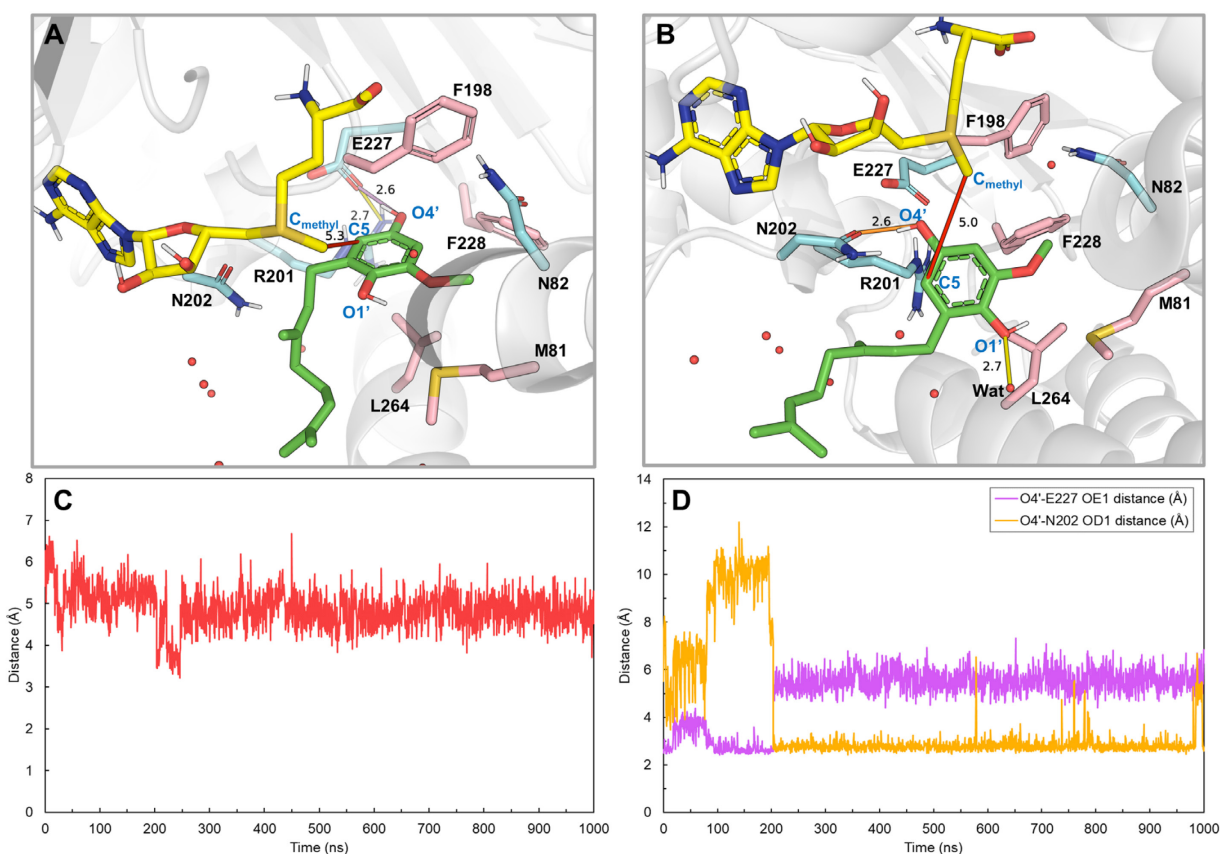


Figure 6. MD simulations reveal stable binding of DDMQ₂H₂ and involvement of R201, N202, and E227. A 1000-ns MD simulation was performed on the top-ranked docking pose shown in Figure 5. **(A)** A representative frame from the second most populated cluster spanning the first ~200 ns (15% of all frames analyzed) is shown. AdoMet (yellow) and DDMQ₂H₂ (green) are pictured next to active-site residues. The distance from O4' to E227 OE1 is shown in lilac. **(B)** A representative frame from the most populated cluster spanning ~450–1000 ns (22.6% of frames) is shown. The distance from O4' to N202 OD1 is shown in orange. The distance between the reactive atoms (the AdoMet methyl carbon and C5 of DDMQ₂H₂) is shown in red. Other polar contacts within 3.5 Å are shown in yellow. Distances (in Ångströms) are measured between heavy atoms. The ten nearest water molecules (Wat) in each frame are depicted as red spheres. Atoms and residues are labeled and colored as in Figure 5. **(C)** The distance between the reactive atoms

throughout the 1000-ns simulation is shown in red, with average distance 4.88 Å. **(D)** Distances between O4' and E227 OE1 (lilac) and N202 OD1 (orange) over 1000 ns is shown. Average distance to E227 OE1: 2.99 Å (0–203 ns), 5.54 Å (204–1000 ns). Average distance to N202 OD1: 8.32 Å (0–203 ns), 2.84 Å (204–1000 ns). Representative coordinates of the early-state and late-state conformations are provided in Supplementary Files 4 and 5, respectively.

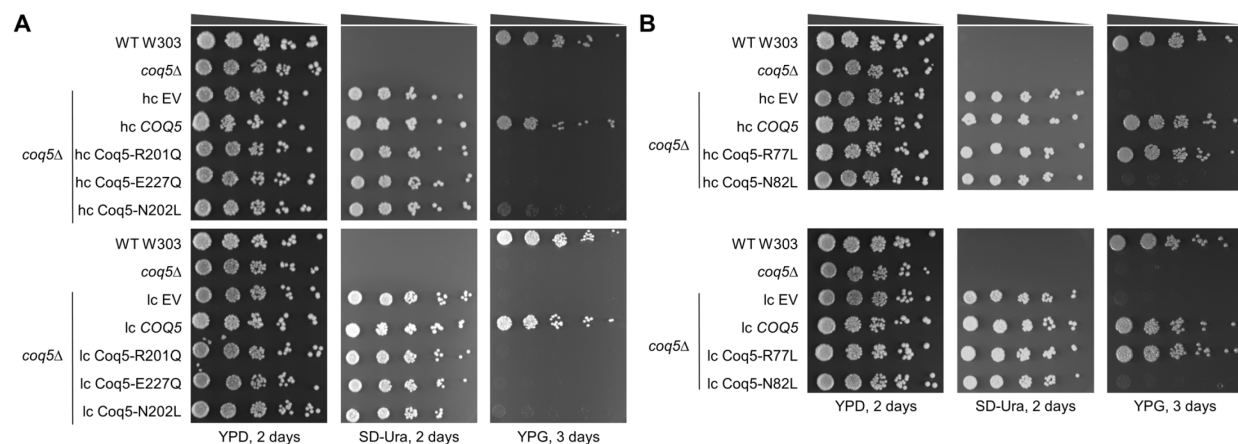


Figure 7. Mutations at substrate-interacting residues predicted by docking and MD result in respiratory deficiency. W303 *coq5Δ* yeast were transformed with either high-copy (top row) or low-copy (bottom row) plasmids. Mutants targeting candidate residues for O4' interaction (**A**) or O1' interaction (**B**) were grown and spotted as described in Figure 3. Data are representative of three biological replicates.

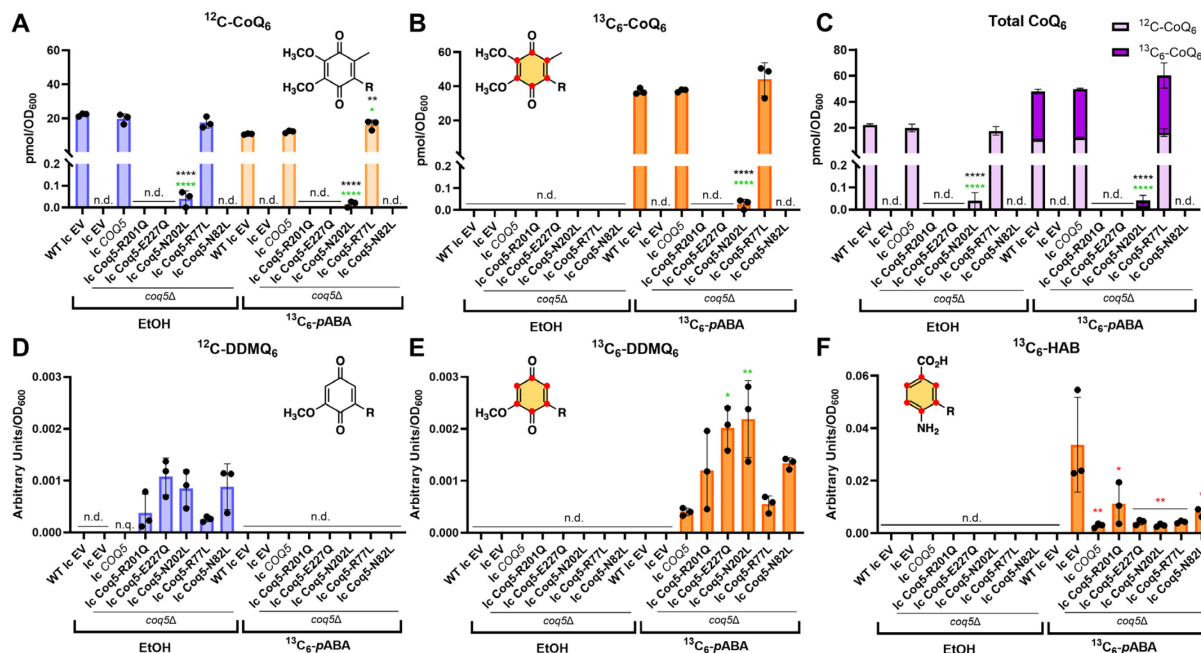


Figure 8. Coq5-R201Q, E227Q, N202L, and N82L mutants severely impair CoQ biosynthesis and lead to DDMQ₆ accumulation. Wild-type yeast transformed with empty vector and *coq5Δ* yeast transformed with the indicated low-copy plasmids were labeled with ¹³C₆-*pABA* as described in Figure 4. Lipids were analyzed by LC-MS/MS after 5 h of labeling. (A–F) Quantification of ¹²C- and ¹³C₆-CoQ₆ (A, B), total CoQ₆ (C), ¹²C- and ¹³C₆-DDMQ₆ (D, E) and ¹³C₆-HAB (F) in the transformants are shown. All data depict mean ± SD of three biological replicates. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey’s HSD test. Black, red, and green asterisks denote comparisons to wild-type yeast expressing the low-copy empty vector (WT lc EV), *coq5Δ* yeast expressing the low-copy empty vector (*coq5Δ* lc EV), and *coq5Δ* expressing the low-copy COQ5 plasmid (lc COQ5), respectively. **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001, *****p* ≤ 0.0001, and ns = not significant. n.d. = peak not detected; n.d. results were excluded from statistical analyses. See Materials and Methods for details.

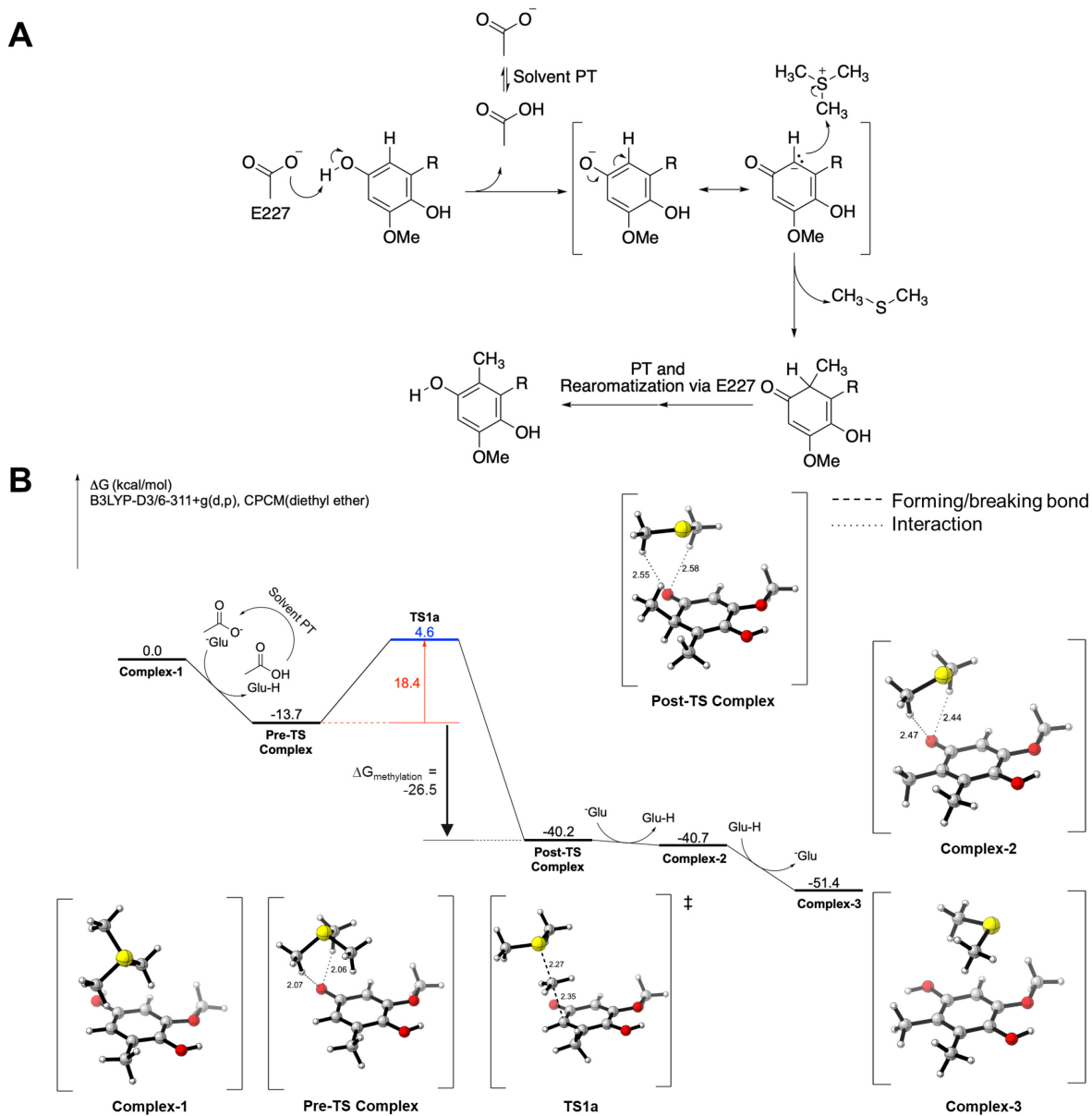


Figure 9. DFT-computed standard Gibbs free energy of the proposed mechanism. (A) Proposed mechanism demonstrating the deprotonation of O4' by E227 and the subsequent activation and attack by the carbon nucleophile is depicted. The methylated product then undergoes a E227-assisted isomerization to rearomatize. The polyprenyl tail of the substrate, DDMQH₂, is denoted as R. Reactants are simplified as follows: acetate as E227, trimethylsulfonium as AdoMet, and R = CH₃ in DDMQH₂. **(B)** Favorable standard Gibbs free

energy diagram of the simplified reaction in (A) in diethyl ether solvent, calculated by density functional theory using the methods stated. Optimized structures of the reactant and product complexes, as well as transition states for the reaction are pictured. Energies are reported in kcal mol⁻¹. Interatomic distances are denoted in Ångströms.

SUPPLEMENTAL INFORMATION

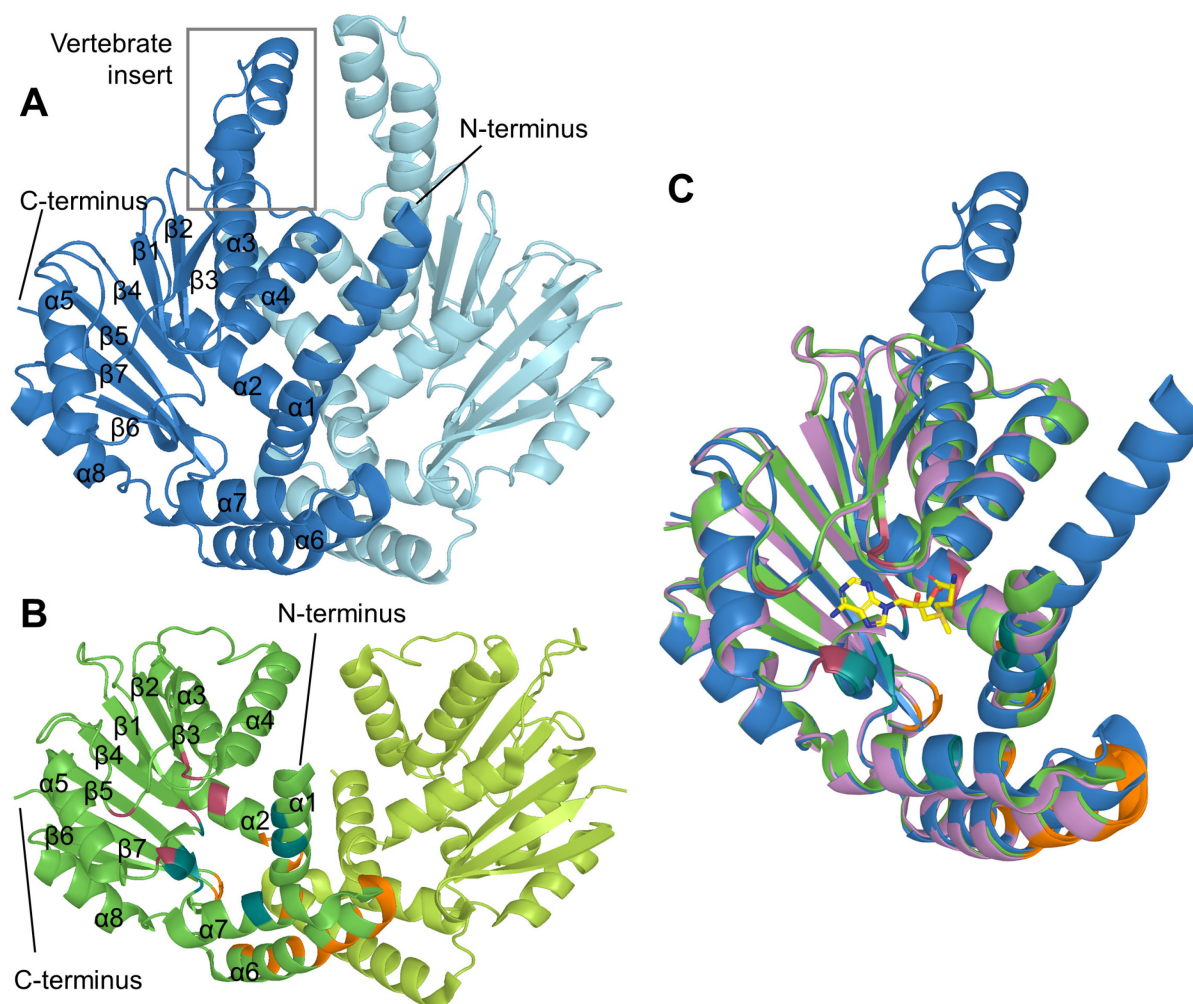


Figure S1. Comparison of the AlphaFold-predicted human COQ5 dimer with apo- and AdoMet-bound yeast Coq5 dimer crystal structures. (A) The AlphaFold-predicted human COQ5 dimer is shown in blue, with the vertebrate insert boxed in gray. The insert is predicted to form an extended α -helix and loop region and may be necessary for interactions with other polypeptides, possibly the other Coq5 monomer. The first 60 residues of the human COQ5 model were truncated for simplicity. Secondary structure labels and residue function are adapted from previous structural studies on yeast Coq5 (34). (B) The yeast apo-Coq5 dimer crystal structure (PDB: 4OBX) is shown in green. Regions are colored according to Figure 2B. (C) Yeast Coq5 and

human COQ5 monomers demonstrate good alignment. Blue, AlphaFold-predicted human COQ5 dimer; lavender, AdoMet-bound yeast Coq5 (PDB: 4OBW); green, yeast apo-Coq5 (PDB: 4OBX). Dimeric structures aligned poorly (not shown), likely due to the limitations in AlphaFold in predicting protein-protein interaction interfaces.

Figure S2. Location of SNVs analyzed in this study. Amino acid sequences of human COQ5 and its homologs were analyzed as described in **Materials and Methods**. See KEY for descriptions of the figure annotations. Functional residues (dotted) were determined from the yeast Coq5 crystal structure (34) and this study. K78 (yeast residue R77) has been removed from the list of substrate-interacting residues, while N83, F217, E246, and F247 (yeast N82, F198, E227, and F228) have been added as substrate-interacting residues. Methyltransferase motifs I, post-I, II, and III are boxed in cyan (27). Homologs from vertebrate species contain a ~30 residue insert between motifs I and post-I and are boxed in red. Conservation at each residue is indicated by blue shading, which represent percent agreement >80%, >60%, and >40%, from darkest to lightest. Residues with less than 40% agreement are unshaded.

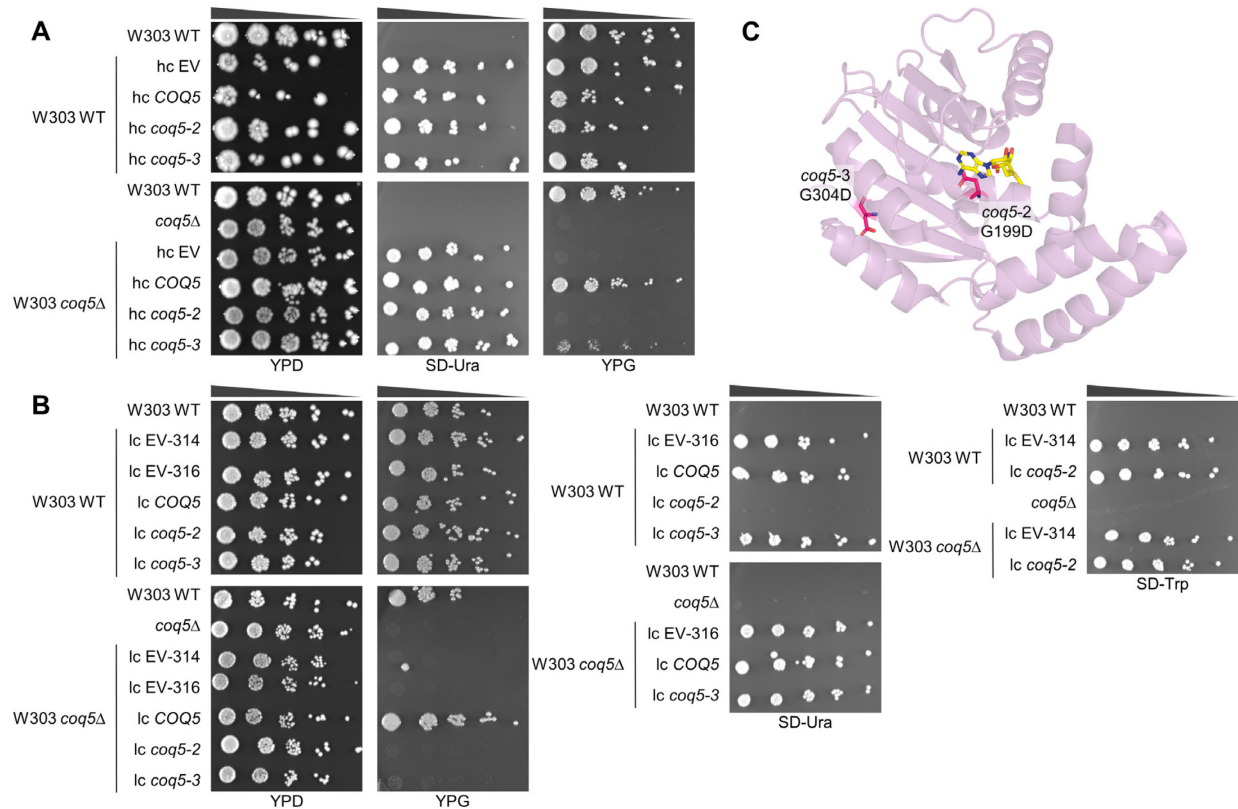


Figure S3. Yeast expressing the *coq5-2* or *coq5-3* mutant allele are respiratory deficient.

W303 WT or *coq5*Δ yeast were transformed with designated high-copy (A) or low-copy (B) plasmids. Cultures were grown overnight in the appropriate selective media (or non-selective synthetic media for untransformed strains), then diluted to OD₆₀₀ = 0.2. 2 μL of 5-fold serial dilutions were spotted onto fermentable YPDextrose (YPD), respiratory YPGlycerol (YPG), or synthetic selective media containing dextrose (SD-Ura or SD-Trp). Note that the low-copy plasmid carrying the *coq5-2* allele uses a different selection marker; therefore, both the empty vector with the *TRP1* marker (EV-314) and the one with the *URA3* marker (EV-316) were used. Plates were incubated at 30 °C prior to imaging for the following number of days (listed as YPD/synthetic/YPG): W303 WT high-copy (3/4/4); W303 *coq5*Δ high-copy (2/3/3); all low-copy transformants (2/2/4). Data are representative of three biological replicates. (C) Mutations encoded

by the *coq5-2* (G199D) and *coq5-3* (G304D) alleles are shown in magenta on the yeast Coq5 structure (PDB: 4OBW).

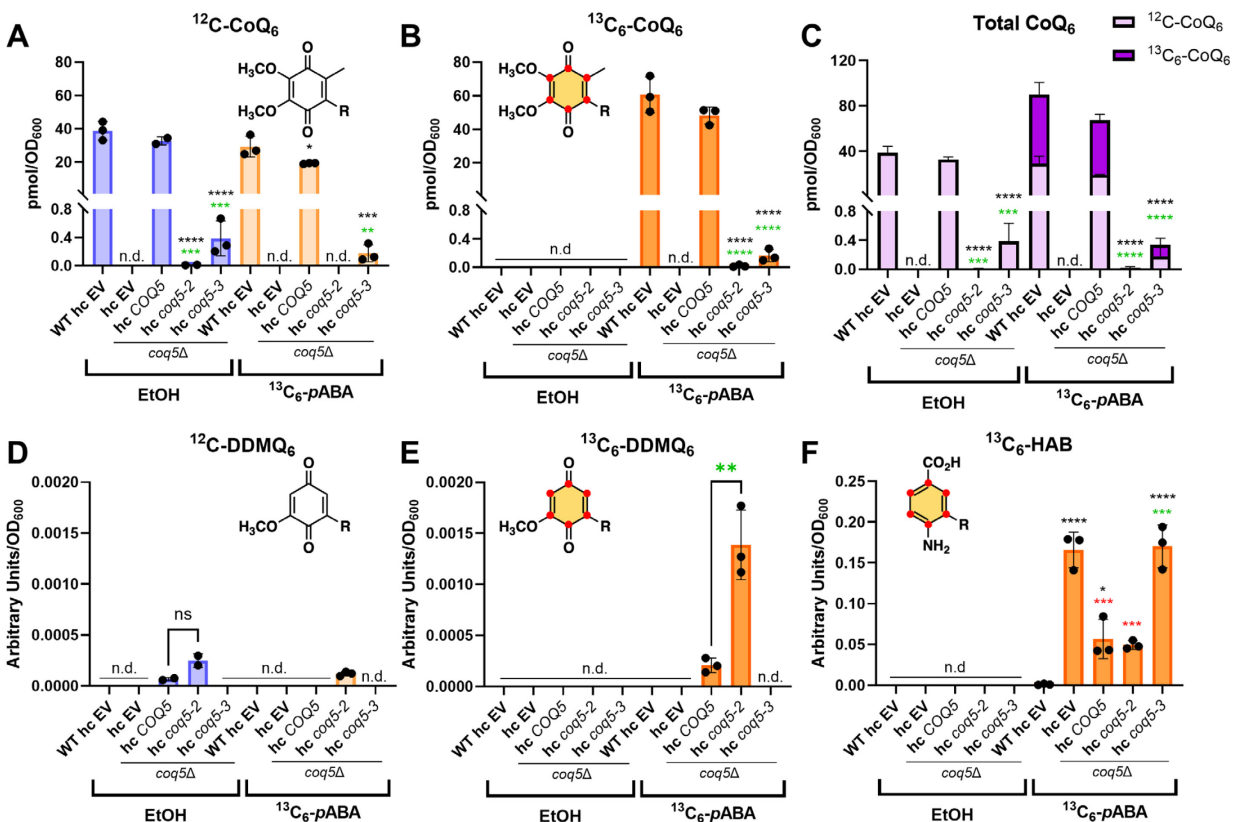


Figure S4. *coq5-2* and *coq5-3* mutants accumulate distinct intermediates. Wild-type yeast transformed with empty vector and *coq5Δ* yeast expressing the indicated high-copy plasmids were labeled at OD₆₀₀ = 0.6 with 8 μg/mL $^{13}\text{C}_6$ -para-aminobenzoic acid ($^{13}\text{C}_6$ -pABA) or ethanol as a vehicle control. Lipids were analyzed via LC-MS/MS after 5 h of labeling. (A–F) Quantification of ^{12}C - and $^{13}\text{C}_6$ -CoQ₆ (A, B), total CoQ₆ (C), ^{12}C - and $^{13}\text{C}_6$ -DDMQ₆ (D, E) and $^{13}\text{C}_6$ -HAB (F) in the transformants are shown. Total CoQ₆ was calculated as the sum of ^{12}C -CoQ₆ and $^{13}\text{C}_6$ -CoQ₆. Data depict mean ± SD of three biological replicates except in the vehicle control of *COQ5* and *coq5-2*, where n = 2. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test for comparison between multiple strains and unpaired *t*-test for two strains. Black, red, and green asterisks denote comparisons to wild-type yeast expressing the high-copy empty vector (WT hc EV), *coq5Δ* yeast

expressing the high-copy empty vector (*coq5*Δ hc EV), and *coq5*Δ expressing the high-copy COQ5 plasmid (hc *COQ5*), respectively. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns = not significant. n.d. = peak not detected; n.d. values were excluded from statistical analyses. DDMQ₆, 2-methoxy-6-hexaprenyl-1,4-benzoquinone; HAB, 3-hexaprenyl-4-aminobenzoic acid.

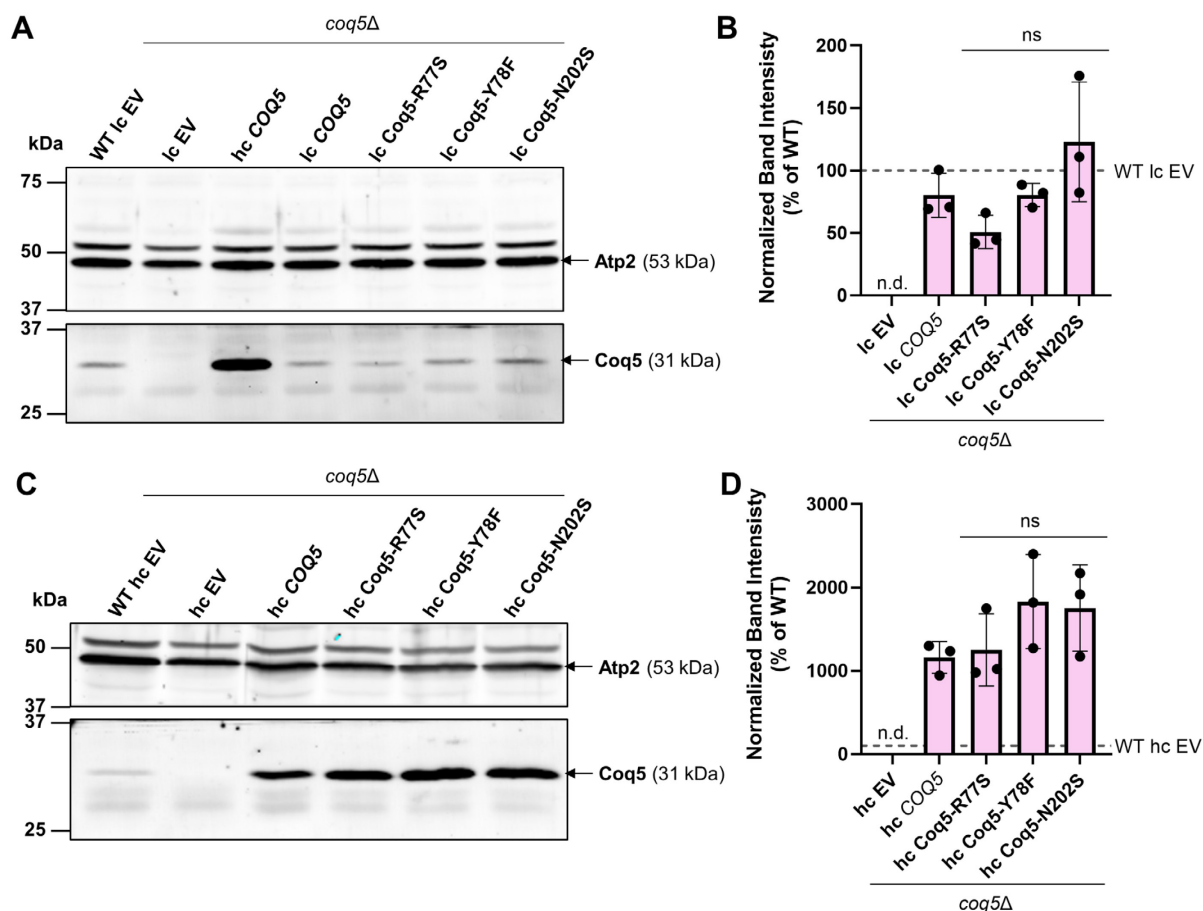


Figure S5. Coq5-R77S, Y78F, and N202S mutants stably express. Aliquots of whole cell protein extracts (equivalent to 0.45 OD₆₀₀ units) were resolved on 10% Tris-glycine SDS-PAGE. (A) Representative Western blot analysis of the steady state levels of wild-type and mutant Coq5 polypeptides in the indicated low-copy transformants is pictured. A high-copy plasmid carrying the yeast *COQ5* gene was included as a control but not quantified. The β -subunit of the mitochondrial ATP synthase (Atp2) was included as a loading control; the size of the mature Atp2 polypeptide was previously determined (114). (B) Quantification of low-copy Coq5 band intensities is shown. Atp2-normalized band intensities were plotted as a percentage of the corresponding WT EV band on the same blot, which represent endogenous levels of expression in W303 wild type. (C, D) The corresponding representative Western blot analysis (C) and

quantification (**D**) for high-copy transformants overexpressing wild-type or mutant Coq5 are shown. Western blot statistical significance was performed with respect to band intensity in *coq5Δ* yeast expressing the wild-type *COQ5* plasmids (hc *COQ5* or lc *COQ5*), using one-way ANOVA with Dunnett's multiple comparison test. Data in (**B**) and (**D**) depict mean $\pm$ SD of three biological replicates. ns = not significant.

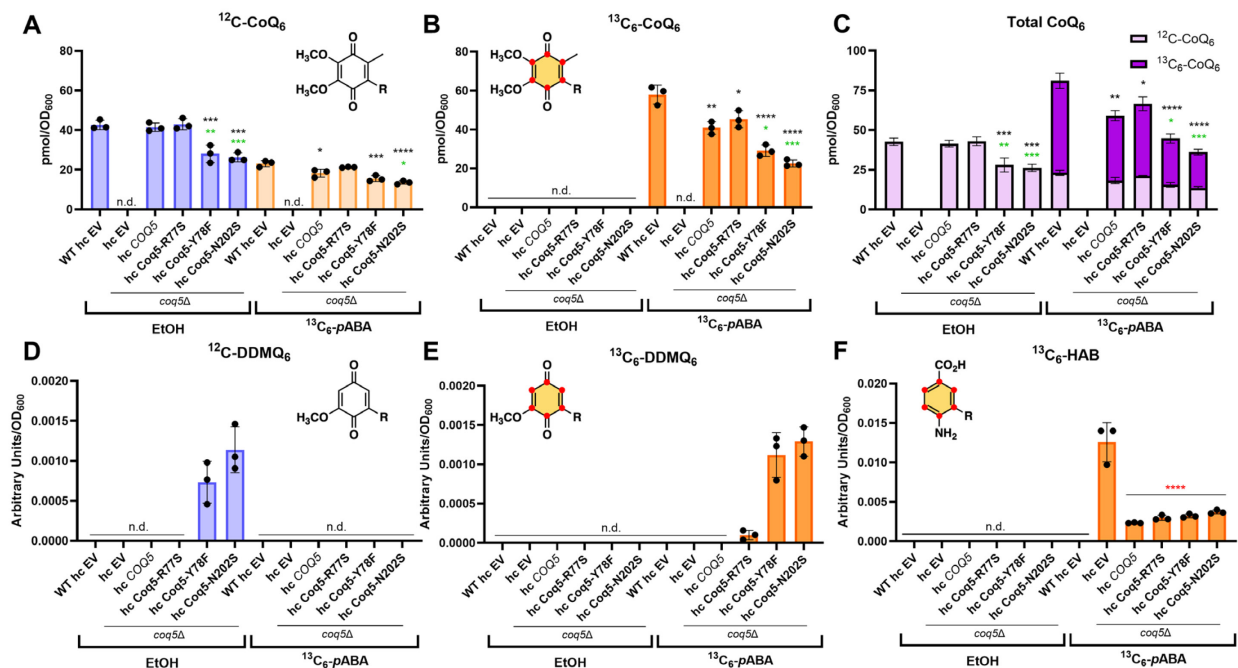


Figure S6. Overexpression of Coq5-R77S, Y78F, or N202S mutants results in partial retention of CoQ biosynthetic activity comparable to or higher than that of mutants expressed on low-copy plasmids. Wild-type yeast transformed with empty vector and *coq5Δ* yeast transformed with the indicated high-copy plasmids were labeled with $^{13}\text{C}_6\text{-pABA}$ as described in Figure 4. (A–F) Quantification of ^{12}C - and $^{13}\text{C}_6$ -CoQ₆ (A, B), total CoQ₆ (C), ^{12}C - and $^{13}\text{C}_6$ -DDMQ₆ (D, E) and $^{13}\text{C}_6$ -HAB (F) in the transformants are shown. All data depict mean $\pm$ SD of three biological replicates. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test. Black, red, and green asterisks denote comparisons to wild-type yeast expressing the high-copy empty vector (WT hc EV), *coq5Δ* yeast expressing the high-copy empty vector (*coq5Δ* hc EV), and *coq5Δ* expressing the high-copy COQ5 plasmid (hc COQ5), respectively. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns = not significant. n.d. = peak not detected; n.d. results were excluded from statistical analyses. See Materials and Methods for details.

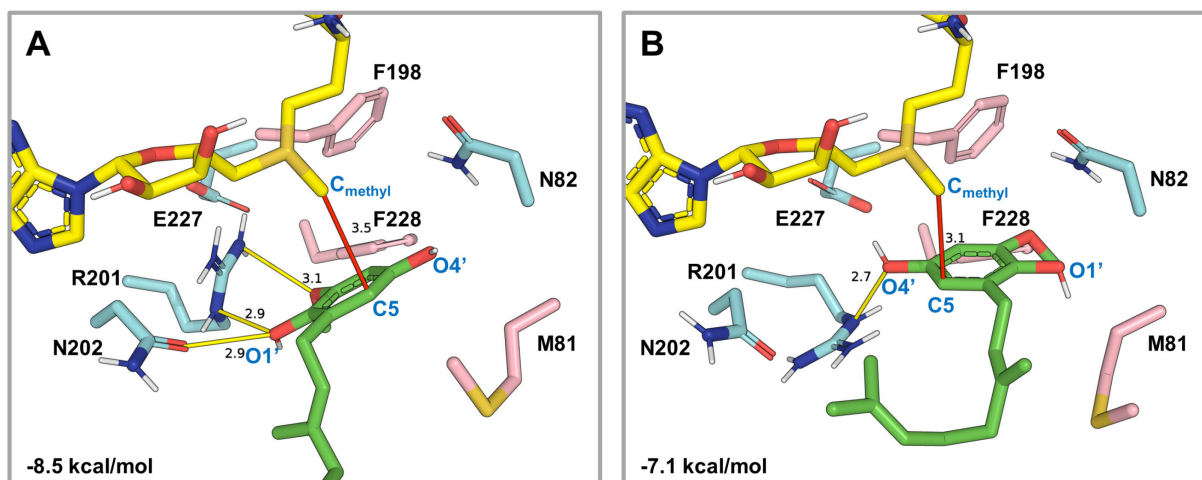


Figure S7. Lowest-energy poses from additional docking clusters. Representative lowest-energy poses from two clusters in which the hydroquinone head group of DDMQ₂H₂ (green) is in close proximity to the methyl group of AdoMet (yellow) are shown. **(A)** Lowest-energy pose from a cluster in which the hydroquinone head group is rotated relative to the top-ranked pose shown in Figure 5. The O4' atom faces away from polar residues E227, R201, and N202. **(B)** Lowest-energy pose from a cluster likely representing an intermediate along the substrate entry pathway. The head group is less buried in the active site and does not interact with E227. The distance between the reactive atoms is shown in red. Polar contacts within 3.5 Å between side chains and the substrate are shown in yellow. All distances (in Ångströms) are measured between heavy atoms. Atom labels are shown in blue. Polar and nonpolar residues are colored in light blue and pink, respectively. Estimated binding affinities, as calculate by AutoDock Vina, are shown in the bottom left corner of each panel.

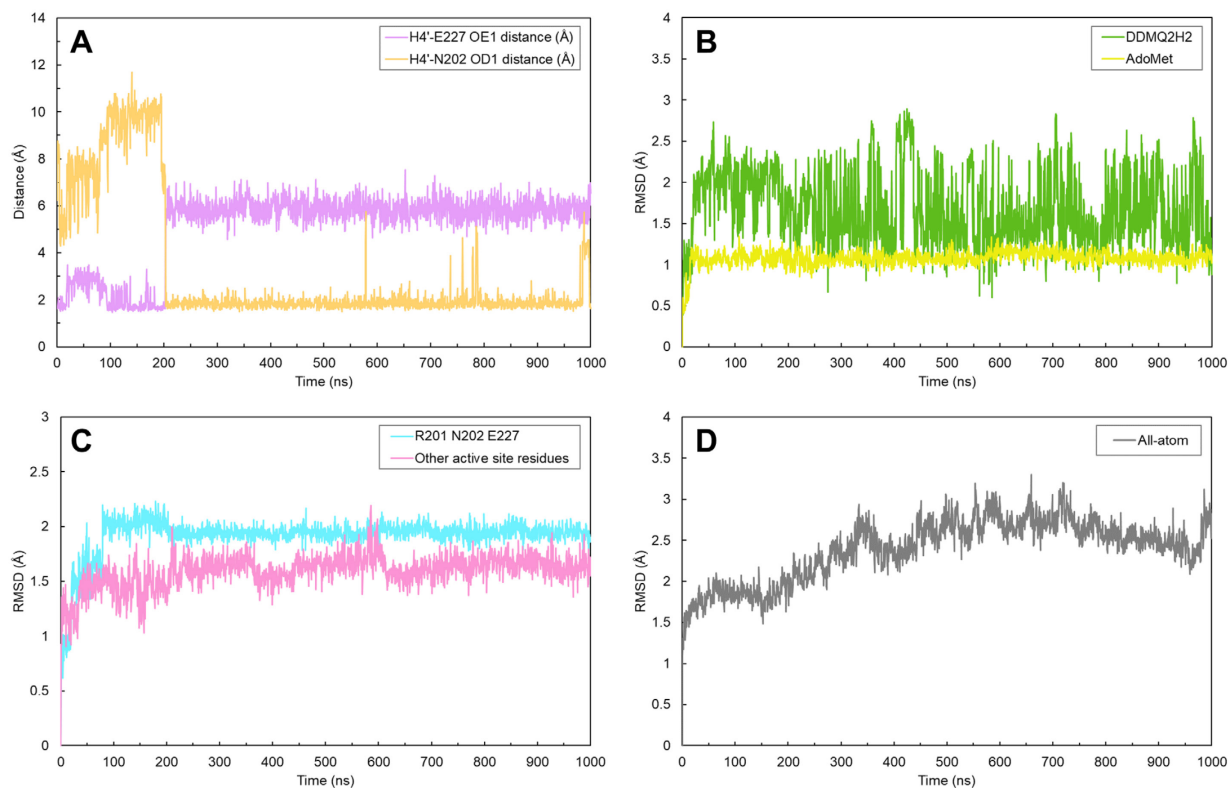


Figure S8. Additional analysis of the 1000-ns molecular dynamics simulation of the Coq5-AdoMet-substrate complex. (A) Distances between the hydroxyl hydrogen (H4') of DDMQ₂H₂ and polar side chain atoms of E227 (OE1, pale purple) and N202 (OD1, pale orange) over the course of the simulation are plotted. (B–D) Root-mean-square-deviation (RMSD) traces of DDMQ₂H₂ (green) and AdoMet (yellow) (B), O4'-interacting residues (blue) and other active-site residues (pink) (C), and all protein and ligand atoms (D) throughout the simulation is plotted. O4'-interacting residues include R201, N202, E227. Other active-site residues correspond to those shown in Figure 6A, with the addition of Y78. Residue RMSD calculations are performed with the inclusion of backbone atoms.

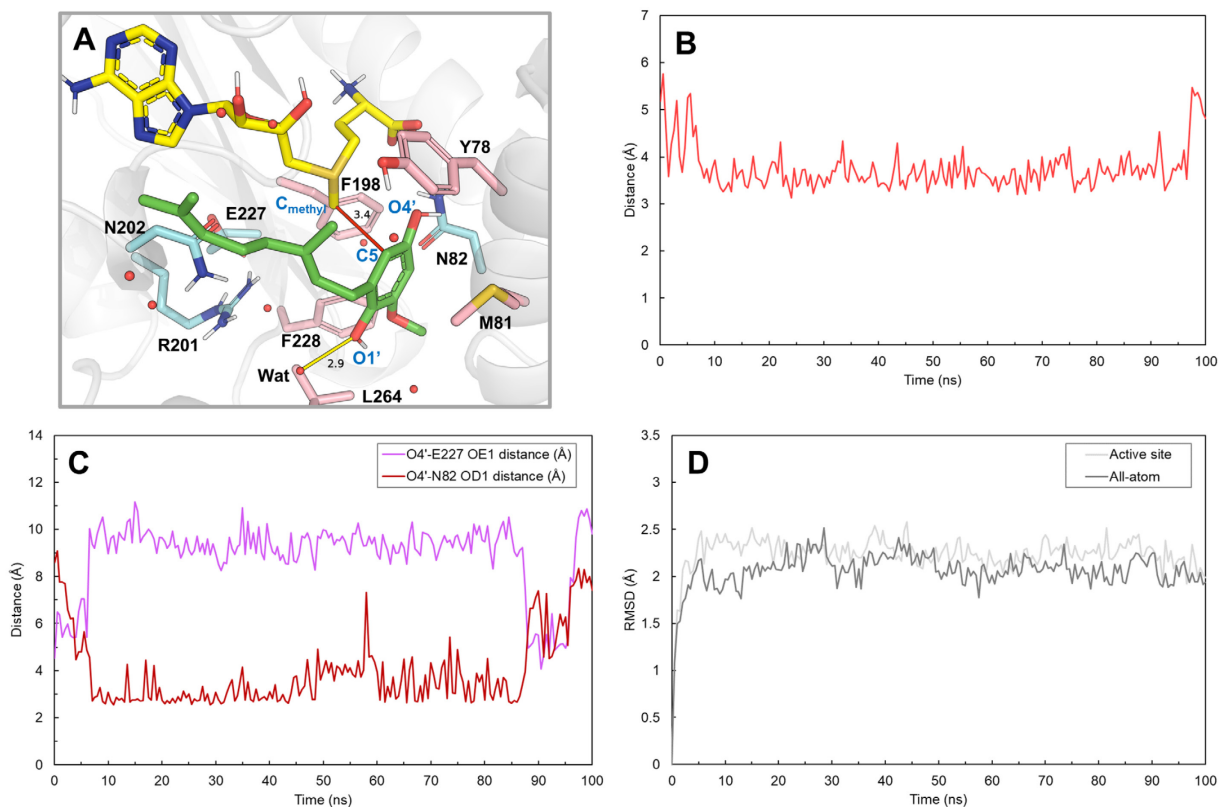


Figure S9. An alternative binding pose suggests role of N82 in substrate binding. An independent 100-ns MD simulation was performed on the top-ranked docking pose shown in Figure 5. (A) A representative frame from most populated cluster spanning the entire 100-ns simulation is shown. AdoMet (yellow) and DDMQ₂H₂ (green) are pictured next to active-site residues. O4' is positioned in close proximity to N82 and away from R201, N202, and E227. (B) The distance between the reactive atoms throughout the 100-ns simulation is shown in red, with average distance 3.75 Å. (C) Distances between O4' and E227 OE1 (lilac) and N82 OD1 (maroon) over 100 ns is shown. The average distances to E227 and N82 throughout the 100-ns simulation are 8.87 Å and 3.92 Å, respectively. (D) Active-site (light grey) and all-atom (dark grey) RMSD traces are plotted. Active site includes backbone and side chain of all residues highlighted in (A), as well as AdoMet and DDMQ₂H₂.

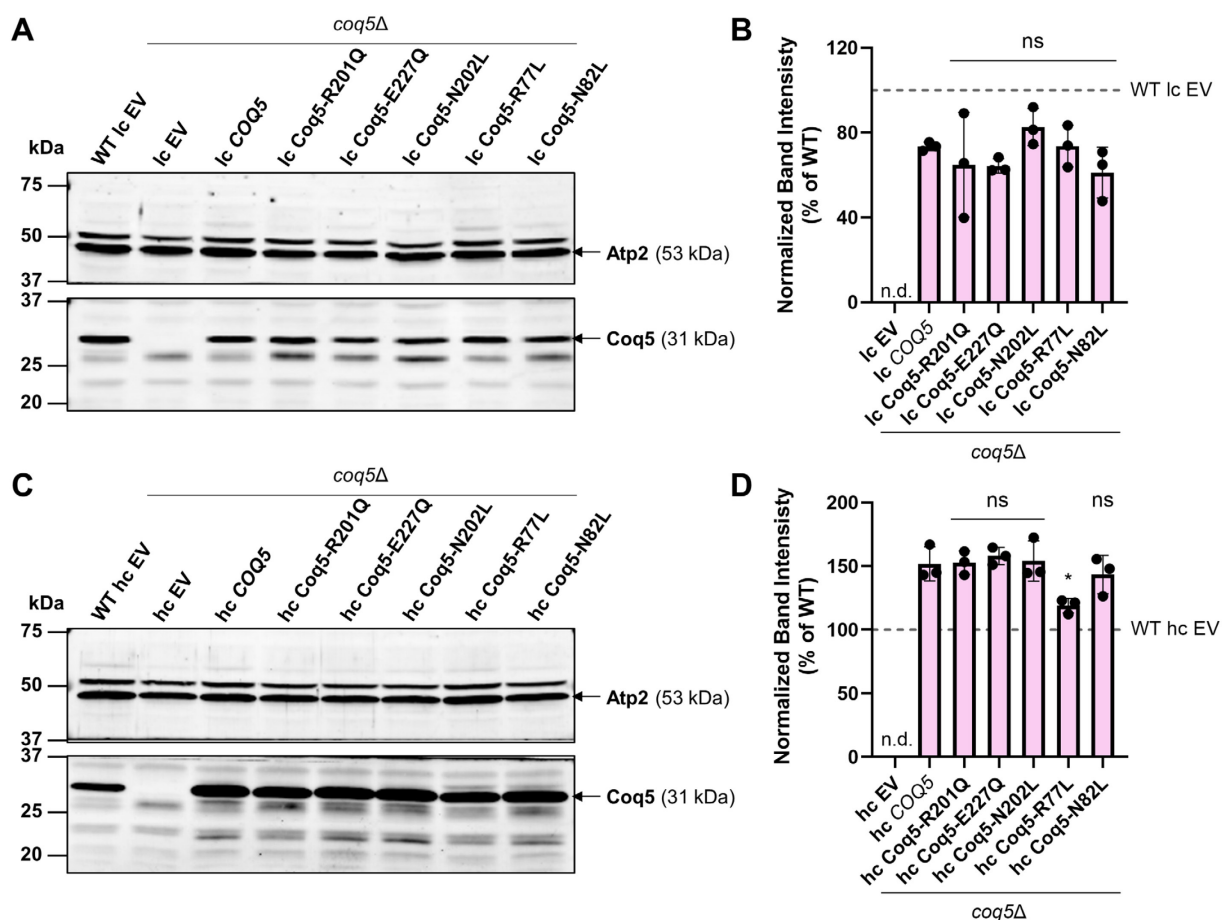


Figure S10. Coq5-R201Q, E227Q, N202L, R77L, and N82L mutants stably express. (A, B) Representative Western blot analysis (A) and quantification (B) of the steady state levels of wild-type and mutant Coq5 polypeptides expressed in a low-copy plasmid was performed as described in Figure S5. (C, D) The corresponding representative Western blot analysis (C) and quantification (D) for high-copy transformants overexpressing wild-type or mutant Coq5 are shown. Western blot statistical significance was performed with respect to band intensity in *coq5Δ* yeast expressing the wild-type *COQ5* plasmids (hc *COQ5* or lc *COQ5*), using one-way ANOVA with Dunnett's multiple comparison test. Data in (B) and (D) depict mean $\pm$ SD of three biological replicates. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns = not significant.

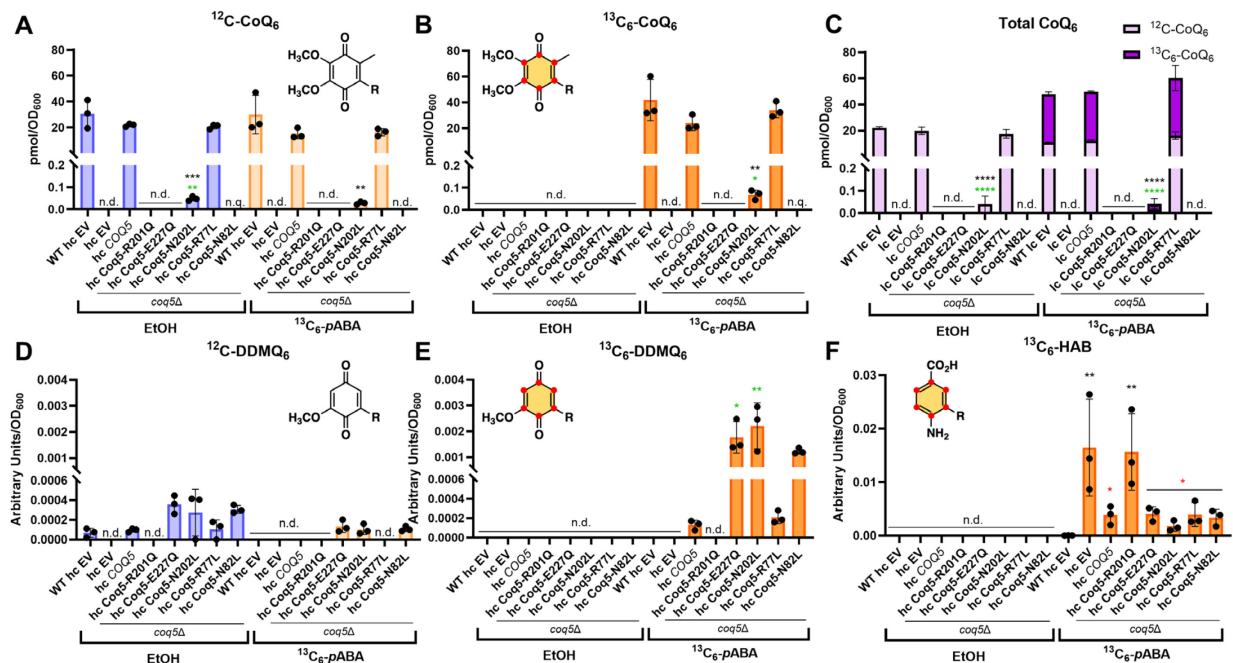


Figure S11. CoQ biosynthetic defects in transformants expressing Coq5-R201Q, E227Q, N202L, or N82L are not rescued by overexpression. Wild-type yeast transformed with empty vector and *coq5Δ* yeast transformed with the indicated high-copy plasmids were labeled with $^{13}\text{C}_6$ -*pABA* as described in Figure 4. (A-F) Quantification of ^{12}C - and $^{13}\text{C}_6$ -CoQ₆ (A, B), total CoQ₆ (C), ^{12}C - and $^{13}\text{C}_6$ -DDMQ₆ (D, E) and $^{13}\text{C}_6$ -HAB (F) in the transformants are shown. All data depict mean $\pm$ SD of three biological replicates. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test. Black, red, and green asterisks denote comparisons to wild-type yeast expressing the high-copy empty vector (WT hc EV), *coq5Δ* yeast expressing the high-copy empty vector (*coq5Δ* hc EV), and *coq5Δ* expressing the high-copy COQ5 plasmid (hc COQ5), respectively. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns = not significant. n.d. = peak not detected; n.q., not quantifiable; n.d. and n.q. results were excluded from statistical analyses. See Materials and Methods for details.

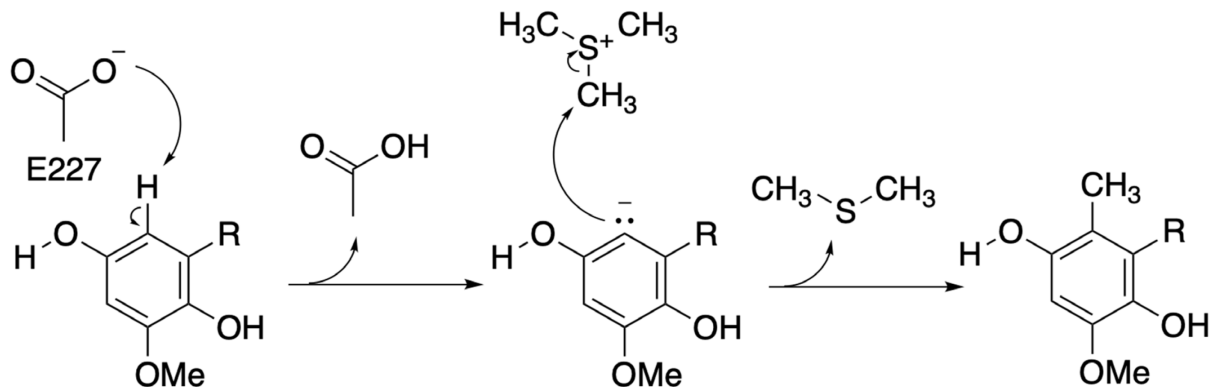


Figure S12. An unlikely alternative mechanism involving the formation of a high-energy carbanion. A preliminary mechanism demonstrating the deprotonation of C5' by E227 and the subsequent attack by the carbanion nucleophile is depicted. The polyprenyl tail of the substrate, DDMQH₂, is denoted as R. Reactants are simplified as in Figure 9.

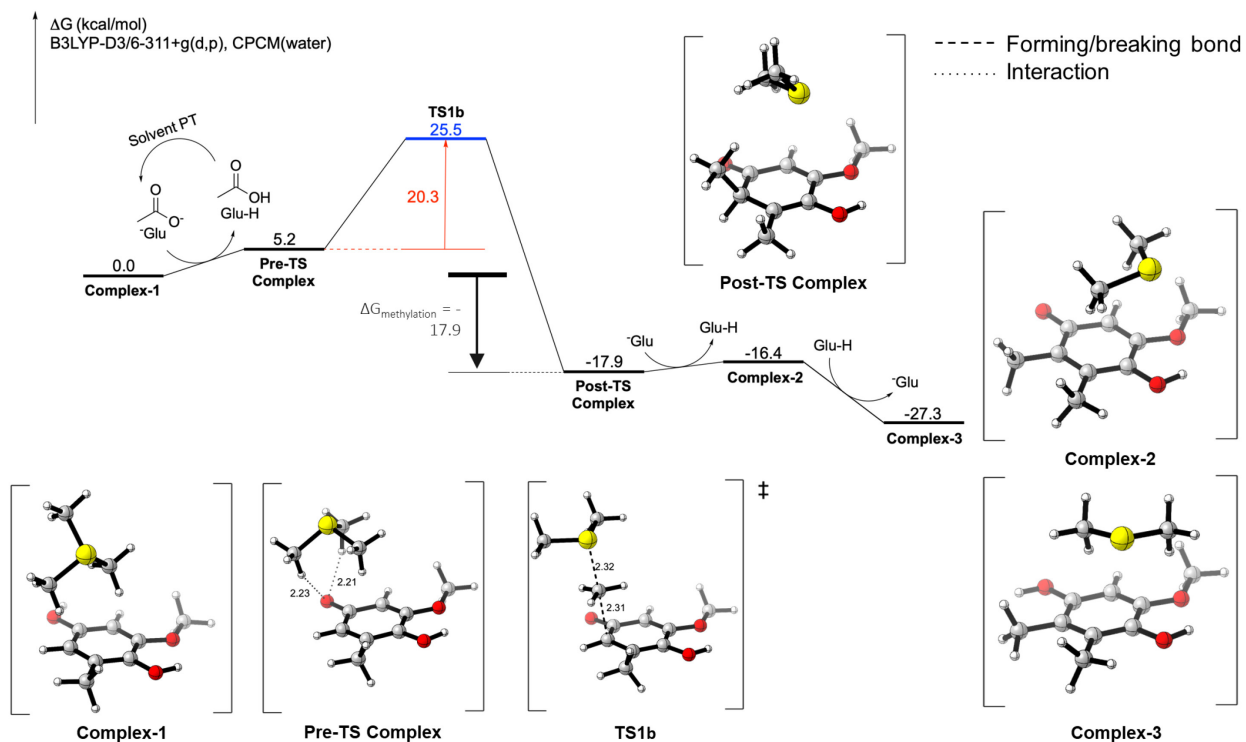


Figure S13. DFT-computed standard Gibbs free energy of the proposed mechanism in water.

Standard Gibbs free energy diagram of the simplified reaction in Figure 9 in water solvent, calculated by density functional theory using the methods stated. Optimized structures of the reactant and product complexes, as well as transition states for the reaction are pictured. Energies are reported in kcal mol⁻¹. Interatomic distances are denoted in Ångströms.

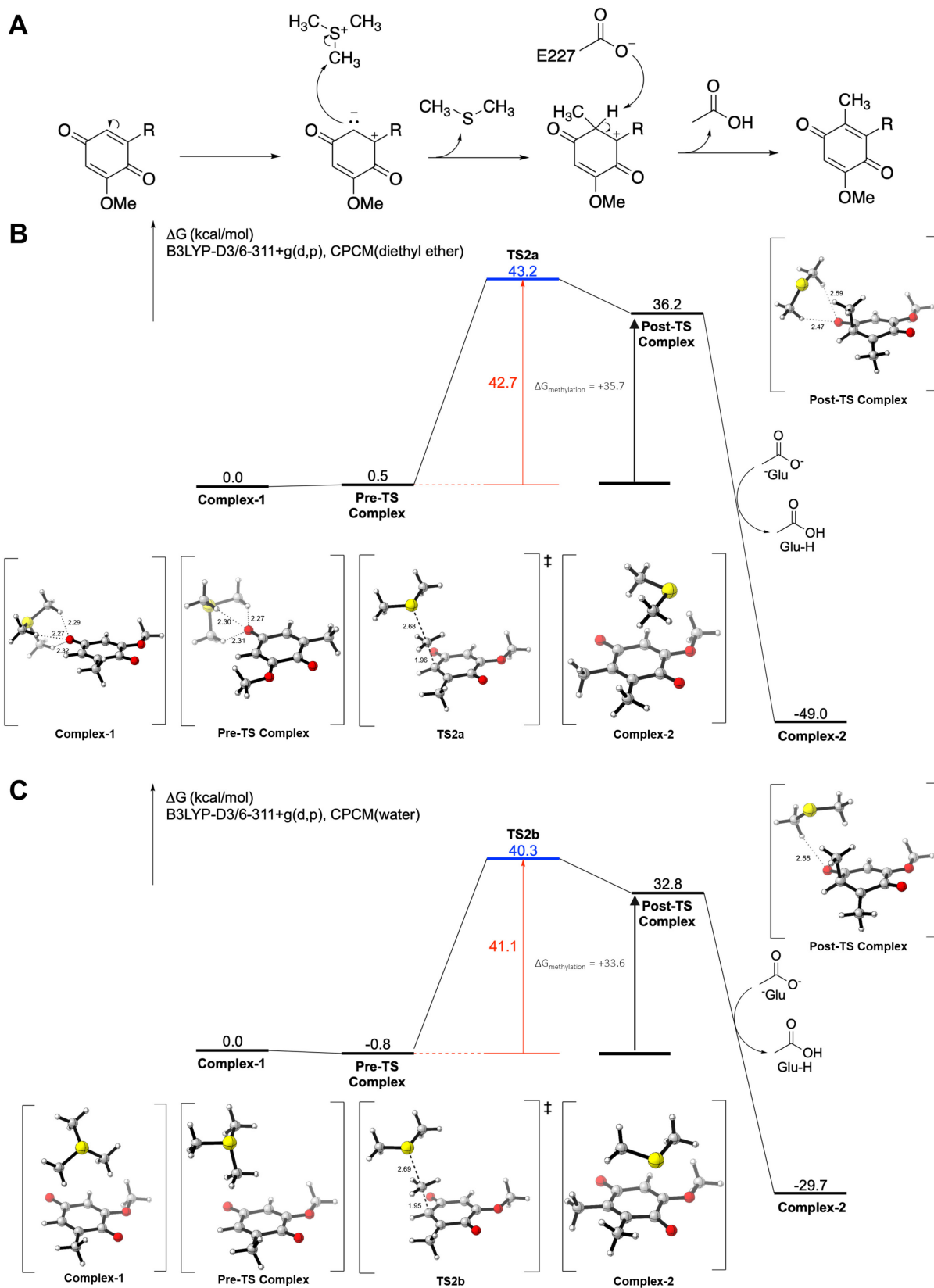


Figure S14. DFT-computed standard Gibbs free energy of an alternative mechanism involving the oxidized benzoquinone substrate in diethyl ether or water. (A) An alternative mechanism in which the oxidized benzoquinone form of the substrate performs the nucleophilic attack is depicted. E227 deprotonates C5 instead of O4' to generate the nucleophile carbanion is depicted. The polyprenyl tail of the substrate, DDMQ, is denoted as R. Reactants are simplified as in Figure 9. (B, C) Standard Gibbs free energy diagram of the reaction in (A) in diethyl ether solvent (B) or water solvent (C), calculated by density functional theory using the methods stated. Optimized structures of the reactant and product complexes, as well as transition states for the reaction are pictured. Energies are reported in kcal mol⁻¹. Interatomic distances are denoted in Ångströms.

Cartesian Coordinates

The following coordinates are listed vertically and columns continue on the next page.

Proposed Mechanism, Diethyl Ether (Figure 9B)

Complex-1

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O -1.22747400 -0.30818900 -2.22340600
C 1.61038000 -2.80311200 0.94880200
H 1.94150500 -3.58423100 0.25505700
H 0.75174500 -3.21151400 1.49258600
H 2.41238100 -2.62591200 1.66491400
O 3.00260800 -0.34434500 1.37032500
H 3.31071900 0.56991000 1.41192900
O 2.38097100 1.90931000 0.10634800
S -2.62426400 0.83647800 1.43366700
C -0.58781800 -2.82311400 -0.97817400
H 0.07045400 -3.63726800 -1.30704100
H -1.11462700 -3.18856900 -0.08664500
H -1.33019000 -2.64577800 -1.75686900
C -2.74285600 1.91905800 -0.03657300
H -2.30383300 2.88122100 0.23127600
H -3.78642500 2.06707900 -0.32376300
H -2.18450200 1.46959000 -0.85939900
C -3.14968300 -0.73947200 0.66751300
H -4.21413200 -0.71448000 0.42339300
H -2.96475200 -1.53323000 1.39242700
H -2.56002300 -0.91547000 -0.23228100
C 2.02376100 3.17382600 -0.43575800
H 2.72516600 3.89426600 -0.01550300
H 1.00151000 3.45060000 -0.15582200
H 2.10919300 3.17828200 -1.52784100

H 0.78416900 2.08438300 0.87887100
H 1.30360000 2.75529100 -0.67757500
C 2.96924700 -1.65714000 -0.00220800
H 2.41468900 -2.53101300 0.34105200
H 4.02346100 -1.92023100 -0.10781700
H 2.56793700 -1.32019100 -0.95896400
C 3.65290400 0.99689100 0.38808600
H 4.70605300 0.73716700 0.26566300
H 3.57281800 1.89370600 1.00304500
H 3.19149000 1.18041100 -0.58307900
H -0.88794600 2.19197300 -1.67896400
C -1.38202900 -3.42513800 -0.24094000
H -1.99292600 -4.20978500 0.19809900
H -0.35601400 -3.49779600 0.12623400
H -1.39928700 -3.50044600 -1.33044500

Complex-3

C 0.11943600 0.06722500 1.38441900
C -0.11522600 1.24261700 0.68910800
C -1.19574900 1.33174000 -0.20393000
C -2.04441100 0.24568500 -0.40833100
C -1.81475000 -0.95677800 0.29883200
C -0.72809800 -1.02138500 1.17544400
H 0.95555300 -0.04222700 2.05967000
O -0.40649000 -2.16485400 1.87811200
C -3.18085400 0.36903500 -1.39647700
H -4.15148200 0.27973600 -0.89830600
H -3.13086700 -0.41646600 -2.15587300
H -3.15458700 1.33118600 -1.90440200
O -1.39613300 2.50475300 -0.89639700
H -0.68897000 3.11454900 -0.64441500
O 0.64463000 2.37837300 0.77031300
S 2.88049000 -0.45739300 -1.07712200
C -2.69048900 -2.17485000 0.11212200
H -2.97138900 -2.62139800 1.07303600
H -3.62141900 -1.93601200 -0.39831400
H -2.18788700 -2.95029600 -0.47884400
C 2.73796300 -2.02405800 -0.14785000
H 3.61371000 -2.09977300 0.49792800
H 2.72440700 -2.87588800 -0.83067300
H 1.83617800 -2.02434500 0.46564900
C 1.33316000 -0.55029600 -2.04289600
H 1.36160400 -1.39522800 -2.73367300
H 1.24788300 0.37623200 -2.61153200
H 0.47577600 -0.63857000 -1.37617700
C 1.82867100 2.34897000 1.57313100
H 2.28965400 3.32813300 1.45856900
H 2.51051600 1.57176600 1.21752400
H 1.58063700 2.18207900 2.62586100
H -1.01113000 -2.87879900 1.65026600

Proposed Mechanism, Water (Figure S13)

Complex-1

C -1.62022100 -0.86598000 -1.06647400
C -2.04864000 -0.36101100 0.16046600
C -1.65449400 0.91968200 0.58212600
C -0.83090900 1.70593000 -0.22259800
C -0.40413800 1.19370600 -1.45406700
C -0.79232900 -0.07576800 -1.86820500
H -1.91128700 -1.85505100 -1.39440300

Pre-TS Complex

C -0.70800500 -0.89637200 -1.08599300
C -1.75218400 -0.71183100 -0.18584300
C -2.10674600 0.57001500 0.26064700
C -1.41090100 1.68267500 -0.20896900
C -0.35937600 1.49786600 -1.11498100
C 0.04884000 0.21367100 -1.57658600
H -0.42155100 -1.88791800 -1.41152600

O -0.31841700 -0.51682700 -3.08150400
 C -0.41453000 3.07403100 0.25085000
 H 0.23553200 3.56072100 -0.47769700
 H 0.11467800 3.01498000 1.20693900
 H -1.28639400 3.71388700 0.41509300
 O -2.07396700 1.40872000 1.79558400
 H -2.61834300 0.73079900 2.21997600
 O -2.84784300 -1.02165300 1.04894100
 S 2.97150300 -0.31501200 0.97983800
 C 2.93649900 1.02326400 -0.24770100
 H 3.91134900 1.50648200 -0.21519800
 H 2.16145200 1.71976000 0.06308800
 H 2.72639200 0.61618500 -1.23470700
 C 1.40488100 -1.17353000 0.65163200
 H 0.60287900 -0.48758700 0.91668000
 H 1.38011400 -2.04974500 1.29691100
 H 1.35003000 -1.45050600 -0.39881900
 C 4.18665300 -1.45606600 0.25470300
 H 4.21015100 -2.34228600 0.88642500
 H 5.15167000 -0.95299400 0.27925900
 H 3.89474900 -1.70423500 0.76435900
 H 0.23968600 1.78373300 -2.09504900
 C -3.26463700 -2.35747700 0.74495400
 H -3.86653600 -2.67988200 1.59165400
 H -2.40026900 -3.01832000 0.63403900
 H -3.86973200 -2.37909700 -0.16567200
 H -0.63716400 -1.40927000 -3.25984700

TS1b

C -1.48999200 -1.05685300 -0.97301200
 C -2.21556700 -0.50771300 0.06383400
 C -2.07991000 0.86132100 0.43088500
 C -1.20454600 1.67197200 -0.25311200
 C -0.39752700 1.10290100 -1.27882300
 C -0.57014500 -0.25555600 -1.73556100
 H -1.61118400 -2.09367500 -1.25691600
 O 0.09355600 -0.73357000 -2.71453600
 C -1.03734500 3.11634300 0.14058900
 H -0.50006800 3.67299500 -0.62937400
 H -0.47138200 3.20401500 1.07549600
 H -2.00382100 3.59730500 0.30993100
 O -2.83086400 1.36584800 1.47792500
 H -3.39840300 0.65264800 1.80049100
 O -3.12130400 -1.17886400 0.83722700
 S 3.39669400 -0.13610400 1.06834600
 C 1.47372800 0.53167100 -0.04485400
 H 1.63404800 1.58083400 0.14052400
 H 0.82131100 -0.01975200 0.61235000
 H 1.80910200 0.08865000 -0.96942800
 C 3.27192000 -1.91458400 0.69834600
 H 2.51822900 -2.33112300 1.36550400
 H 4.23400400 -2.38959500 0.88711600
 H 2.97270000 -2.05909000 -0.34065300
 C 4.62239200 0.37577800 -0.17741300
 H 5.57692200 -0.10219700 0.04017500
 H 4.72594800 1.45757700 -0.10327400
 H 4.27838600 0.10049400 -1.17511800
 H 0.16921000 1.76917800 -1.92073200
 C -3.36154100 -2.56516000 0.57700400
 H -4.10264100 -2.88096900 1.30828200
 H -2.44514300 -3.14850300 0.70320500
 H -3.75421700 -2.70915400 -0.43334700

O 1.08047400 0.04373200 -2.35167000
 C -1.79480000 3.05765300 0.28089000
 H -1.15975000 3.82346600 -0.16821100
 H -1.70299300 3.13048100 1.36940300
 H -2.83671000 3.29008400 0.03844600
 O -3.14873200 0.73716000 1.16621900
 H -3.48301600 -0.14337300 1.38281100
 O -2.51766500 -1.72242900 0.35763800
 S 3.04185600 -0.21901600 1.01753400
 C 1.38871700 0.06653600 1.71935900
 H 1.46695600 0.95379500 2.34575100
 H 1.15464800 -0.80284500 2.33133000
 H 0.66306900 0.20355100 0.92196400
 C 2.76121500 -1.61625400 -0.11547000
 H 2.35419200 -2.43226000 0.47944800
 H 3.73934300 -1.89264600 -0.50719700
 H 2.08772800 -1.29791800 -0.91481000
 C 3.21751100 1.16700300 -0.14966800
 H 4.22288700 1.09610700 -0.56271300
 H 3.11705900 2.08347400 0.42922800
 H 2.45870900 1.07412000 -0.93015300
 H 0.19740700 2.36276400 -1.46328400
 C -2.19446400 -3.07353800 0.03070400
 H -2.89961000 -3.69315200 0.58211700
 H -1.17255800 -3.31801400 0.33694400
 H -2.30697000 -3.25771100 -1.04213100

Post-TS Complex

C -0.67190500 -0.76543300 -1.40917800
 C -1.07760900 -1.14775500 -0.16505000
 C -1.58563800 -0.20134500 0.82919900
 C -1.63772500 1.11675400 0.57209200
 C -1.10421700 1.65551800 -0.73010500
 C -0.70207200 0.62291200 -1.78394200
 H -0.32810000 -1.47798700 -2.14544100
 O -0.38911100 1.00652800 -2.91804800
 C -2.16869000 2.09867200 1.57683200
 H -2.69527900 2.91656500 1.07714200
 H -1.35493900 2.54681600 2.15865100
 H -2.84993300 1.61869400 2.27992900
 O -2.00663100 -0.71381500 2.03115000
 H -1.91297900 -1.67628500 2.00150100
 O -1.08405400 -2.40591000 0.29977100
 S 2.38344100 -0.47026200 0.75847100
 C 0.10611100 2.59214200 -0.47363500
 H -0.18977500 3.44646800 0.13669400
 H 0.89447100 2.04759800 0.05051700
 H 0.49371200 2.95877200 -1.42496500
 C 2.81406600 -0.17119800 -0.99107500
 H 2.21400900 -0.85286700 -1.59266200
 H 3.87306700 -0.36932600 -1.16458000
 H 2.57666500 0.85515700 -1.27621200
 C 3.49301400 0.74903200 1.54746000
 H 4.53712400 0.52748400 1.31982700
 H 3.33768900 0.68079800 2.62461200
 H 3.24921000 1.75987300 1.21456700
 H -1.89061200 2.26189900 -1.19767100
 C -0.59850500 -3.47727200 -0.53054400
 H -0.69283000 -4.37686100 0.07177000
 H 0.44770900 -3.30622400 -0.79151000
 H -1.20688900 -3.56297300 -1.43301600

Complex-2

C 0.49043400 1.66078700 -0.70572200
 C -0.12939900 1.37926100 0.50368400
 C 0.34961400 0.35979700 1.33430700
 C 1.45762900 -0.39003800 0.93829900
 C 2.09278700 -0.12624800 -0.29107500
 C 1.62879800 0.92036100 -1.15822000
 H 0.11866200 2.44513100 -1.35284700
 O 2.18870900 1.19609500 -2.29870100
 C 1.95369100 -1.51004800 1.82485600
 H 3.01644500 -1.39093700 2.05842200
 H 1.84867300 -2.48031900 1.32633200
 H 1.40231400 -1.55433500 2.76333500
 O -0.29819700 0.08931600 2.54040000
 H -1.06792800 0.67219400 2.57794300
 O -1.24783000 2.03008900 0.99708700
 S -2.58291100 -1.77222600 -0.35175100
 C 3.27235200 -0.96463600 -0.72144700
 H 4.12364000 -0.87282800 -0.03447600
 H 3.02511200 -2.03281200 -0.76603000
 H 3.60401100 -0.65044600 -1.71139700
 C -2.39472200 -0.54807700 -1.69577400
 H -3.28562200 0.08113500 -1.69096700
 H -2.31315100 -1.04791400 -2.66285500
 H -1.51333800 0.06922200 -1.51594800
 C -0.96855700 -2.61628300 -0.49784700
 H -0.88230200 -3.11394400 -1.46565200
 H -0.91910900 -3.36305400 0.29540100
 H -0.15914300 -1.89709300 -0.36751800
 C -1.86808200 3.02319600 0.18456500
 H -2.73562000 3.37181100 0.74294900
 H -2.19339600 2.60362900 -0.77256100
 H -1.19140600 3.86376000 -0.00014200

Complex-3

C -0.92237400 1.60744000 0.67126800
 C 0.01754900 1.51813500 -0.34462600
 C -0.03129100 0.45705300 -1.26344500
 C -1.01378400 -0.52553600 -1.16446700
 C -1.98602000 -0.43594800 -0.14260900
 C -1.91346600 0.62768400 0.76167700
 H -0.90342400 2.40358100 1.40203100
 O -2.79934600 0.77628200 1.80837900
 C -1.00980600 -1.68044600 -2.13764600
 H -1.90020300 -1.66814000 -2.77436700
 H -1.00051100 -2.63708700 -1.60832300
 H -0.13535400 -1.64463000 -2.78443800
 O 0.91651000 0.38977300 -2.26049700
 H 1.52490000 1.13157600 -2.13714100
 O 1.05219800 2.39299600 -0.55028200
 S 2.22076000 -2.11301200 0.57553300
 C -3.06261300 -1.48393700 0.01983200
 H -3.21478300 -2.05764300 -0.89265500
 H -2.81479600 -2.19710400 0.81515400
 H -4.03174300 -1.03679000 0.26621500
 C 3.06743200 -0.49495400 0.63280000
 H 3.92760500 -0.55381200 -0.03486300
 H 3.41290800 -0.27811000 1.64518100
 H 2.40172700 0.29770600 0.29117800
 C 0.85335100 -1.77249700 1.73795600
 H 1.24057800 -1.57265400 2.73875200
 H 0.22192900 -2.66116500 1.76660400
 H 0.26255100 -0.92742500 1.38689400
 C 1.25900700 3.44424400 0.39783300
 H 2.14484300 3.97869400 0.06139100
 H 1.43136000 3.03622000 1.39801800
 H 0.40401900 4.12599500 0.41799600
 H -3.40658300 0.02915300 1.84160900

Alternative Mechanism, Diethyl Ether (Figure S14B)*Complex-1*

C -1.15441800 -0.97389600 -0.05189000
 C -2.50389900 -0.86381000 -0.00594900
 C -3.17319500 0.49548800 0.01877400
 C -2.29804900 1.70788400 -0.00805600
 C -0.96515400 1.54915100 -0.05266900
 C -0.32116700 0.21832200 -0.07677000
 H -0.64166800 -1.92553500 -0.07172300
 O 0.91258400 0.13676900 -0.11600700
 C -2.99020000 3.03380600 0.01723500
 H -2.27085200 3.85233900 -0.00400300
 H -3.60926700 3.12329500 0.91407000
 H -3.66520500 3.12716400 -0.83789700
 O -4.38275400 0.58798500 0.05921000
 O -3.38482700 -1.85497500 0.02172000
 S 4.52115300 -0.09022500 0.06195800
 C 3.78943900 1.55440900 0.33004700
 H 4.22234300 2.21768100 -0.41729500
 H 4.08791900 1.87455700 1.32721300
 H 2.70725000 1.48197600 0.23181800
 C 3.58796300 -1.11697500 1.23927200
 H 3.87387300 -0.79303700 2.23883800
 H 3.90217300 -2.14710800 1.07848200
 H 2.52261100 -0.98590900 1.05829200

Pre-TS Complex

C -1.16718800 -0.98323400 0.00128400
 C -2.51623900 -0.85899700 0.00033800
 C -3.17167800 0.50725400 0.00013600
 C -2.28338500 1.71038800 0.00030600
 C -0.95152100 1.53763000 0.00065400
 C -0.32108000 0.20008800 0.00154000
 H -0.66409300 -1.94021700 0.00180600
 O 0.91239300 0.10564100 0.00227300
 C -2.96190400 3.04358100 -0.00024800
 H -2.23356200 3.85440600 -0.00006600
 H -3.60864600 3.14208100 0.87587700
 H -3.60786600 3.14175500 -0.87698700
 O -4.38085400 0.61268300 -0.00017400
 O -3.40787500 -1.84090700 -0.00019300
 S 4.52779300 -0.08617600 -0.00385300
 C 3.79506900 1.57449400 -0.13856300
 H 4.15267600 2.00282400 -1.07365000
 H 4.17117700 2.15558300 0.70228700
 H 2.70981200 1.48596500 -0.11915200
 C 3.69937900 -0.74374500 1.47724600
 H 4.06059900 -0.16589600 2.32662300
 H 4.00752700 -1.78328000 1.57810800
 H 2.62218300 -0.65184300 1.35225300

C 3.77882200 -0.57670100 -1.52688000
H 4.08881100 -1.60237400 -1.72125500
H 4.19130300 0.08607000 -2.28623500
H 2.69651400 -0.48780800 -1.45610500
H -0.29051900 2.39808100 -0.07408300
C -2.90170700 -3.21020700 0.00252800
H -3.78979100 -3.83556300 0.03126400
H -2.27699400 -3.40351100 0.87808100
H -2.33758600 -3.39977800 -0.91401600

TS2a

C -1.73597800 -1.04353100 -0.92486400
C -2.37300500 -0.49218800 0.14102400
C -2.06526500 0.90811000 0.59170500
C -1.08208300 1.70408400 -0.22521600
C -0.30684900 1.05274600 -1.16718900
C -0.71959700 -0.31413300 -1.66650600
H -1.94813800 -2.04526700 -1.27226300
O -0.16952100 -0.76627900 -2.65761200
C -0.96748400 3.14375100 0.07981100
H -0.18364100 3.62630700 -0.50094000
H -0.80262100 3.28962400 1.15237000
H -1.92760900 3.63014800 -0.13395600
O -2.59591200 1.40275300 1.56244800
O -3.29890700 -1.05931100 0.89550900
S 3.57157700 -0.18766300 1.00755300
C 1.25628400 0.60889500 -0.07846000
H 1.58061000 1.59121200 0.22841500
H 0.83156000 -0.04193800 0.67019700
H 1.74639800 0.15419600 -0.92720300
C 3.40732800 -1.98085100 0.72033300
H 2.70590400 -2.36449200 1.46051200
H 4.37782400 -2.45884300 0.85722600
H 3.03170500 -2.17702800 -0.28454200
C 4.70971100 0.26031900 -0.34524200
H 5.67335500 -0.22287300 -0.18143000
H 4.83524500 1.34215600 -0.31747700
H 4.30013800 -0.04154700 -1.30988300
H 0.21787400 1.65492600 -1.90400800
C -3.71856700 -2.40705000 0.59550600
H -4.46940100 -2.64642000 1.34295600
H -2.87256300 -3.09318900 0.67609400
H -4.15412800 -2.45258600 -0.40508100

Complex-2

C 0.09167200 0.11831900 1.50782100
C 0.15955200 1.19673200 0.69808000
C -0.89026200 1.42795300 -0.36154500
C -1.99771300 0.44117900 -0.47936500
C -2.04322800 -0.64235900 0.32901400
C -0.98523200 -0.85538200 1.37306600
H 0.82945500 -0.09354400 2.26874800
O -1.01925700 -1.84832800 2.09790800
C -3.02694000 0.69204500 -1.54546900
H -4.03777600 0.61417400 -1.13729700
H -2.94241300 -0.05517900 -2.34187200
H -2.89890000 1.67752100 -1.98916700
O -0.80764400 2.39530400 -1.09795000
O 1.09211600 2.14489900 0.70435600
S 2.69781700 -0.71473900 -1.07826400
C -3.12876100 -1.67262200 0.22089000
H -2.93740600 -2.51067400 0.88803600

C 3.67470700 -0.98468800 -1.33703700
H 3.99491100 -2.02366100 -1.27451000
H 4.00903200 -0.54980200 -2.27790300
H 2.59934800 -0.88333000 -1.20251900
H -0.26782100 2.37953800 0.00075600
C -2.93852600 -3.20111300 -0.00005600
H -3.83354700 -3.81716400 -0.00060000
H -2.34626100 -3.39881500 0.89680100
H -2.34528700 -3.39861000 -0.89631500

Post-TS Complex

C -1.18001800 -1.18675700 -0.62207800
C -2.30521500 -0.70039500 -0.02598300
C -2.44735500 0.75448500 0.29775200
C -1.35625000 1.66644100 -0.20182100
C -0.05323500 1.11702300 -0.45360600
C -0.03412800 -0.34556800 -0.92152200
H -1.07514300 -2.23294400 -0.87575400
O 0.96178900 -0.75735100 -1.48494000
C -1.68022800 3.07493100 -0.37983500
H -0.79799600 3.70920700 -0.44963000
H -2.39989200 3.42042800 0.36587800
H -2.21287000 3.13522900 -1.34859300
O -3.40337600 1.20932000 0.88610300
O -3.37844200 -1.38270400 0.31756800
S 3.90306100 0.01700100 0.79387800
C 0.60566600 1.17680500 1.00077000
H 0.65909700 2.20523000 1.35512200
H 0.06589100 0.55849700 1.71735000
H 1.63025600 0.78290900 0.86192300
C 3.54435900 -1.77017200 0.68971300
H 3.30120700 -2.11071300 1.69605600
H 4.42479400 -2.30475000 0.32970800
H 2.69985400 -1.94576500 0.02415100
C 4.16033500 0.39190600 -0.97260500
H 5.00902600 -0.17879700 -1.35306100
H 4.37873900 1.45712400 -1.04779900
H 3.25989700 0.15374800 -1.53968900
H 0.55821700 1.73135200 -1.11523600
C -3.42505300 -2.80657200 0.06853200
H -4.39408700 -3.12771100 0.43863700
H -2.62458000 -3.30869000 0.61541100
H -3.34442000 -3.00326000 -1.00226400

H -4.09945000 -1.23695800 0.48047500
 H -3.21024900 -2.04358600 -0.80487700
 C 2.49301800 -2.19717700 -0.03186000
 H 3.33736200 -2.22678100 0.65773800
 H 2.49582100 -3.10371200 -0.63964500
 H 1.56640100 -2.13733200 0.54174100
 C 1.21551100 -0.89118900 -2.13010200
 H 1.28837400 -1.78138600 -2.75723000
 H 1.15782600 -0.00648100 -2.76470900
 H 0.31525700 -0.94939100 -1.51672500
 C 2.18882800 2.01115900 1.62260400
 H 2.82601800 2.87350600 1.44337800
 H 2.73621700 1.08862000 1.41570600
 H 1.82800200 2.02009100 2.65461100

Alternative Mechanism, Water (Figure S14C)

Complex-1

C -1.14511800 -0.76067600 -1.30416300
 C -1.74290900 -0.90342200 -0.09875400
 C -2.05967500 0.30079100 0.75937800
 C -1.68859100 1.65051800 0.23980500
 C -1.09745700 1.74758800 -0.96204000
 C -0.77515400 0.56434600 -1.79308000
 H -0.89862400 -1.59774700 -1.94268000
 O -0.18416200 0.71010100 -2.86408400
 C -2.00398300 2.82020400 1.11846600
 H -1.69187800 3.75389600 0.65108000
 H -1.50012900 2.71780500 2.08361000
 H -3.07656800 2.86461800 1.32620300
 O -2.59717400 0.16417500 1.84238000
 O -2.11363900 -2.04246300 0.47998700
 S 3.15129800 0.09632600 0.88486800
 C 2.35486600 1.35948600 -0.15055300
 H 3.10227000 2.12854700 -0.33741900
 H 1.53043300 1.77345400 0.42679100
 H 2.00526600 0.91719700 -1.08144100
 C 1.87134300 -1.18808200 1.00164600
 H 1.07588500 -0.79146600 1.62928300
 H 2.33267000 -2.04732700 1.48543800
 H 1.50695300 -1.44202500 0.00839500
 C 4.33479900 -0.65454100 -0.27284200
 H 4.81246900 -1.47969700 0.25260700
 H 5.07025300 0.11062400 -0.51503100
 H 3.81528000 -1.00151100 -1.16434200
 H -0.80717000 2.70621600 -1.37810900
 C -1.88560000 -3.27721000 -0.22476300
 H -2.27512800 -4.05782700 0.42309600
 H -0.81680400 -3.42830600 -0.39327700
 H -2.42126600 -3.27617700 -1.17695800

TS2b

C -1.72073700 -1.02208100 -0.95157200
 C -2.34947700 -0.50588500 0.13575100
 C -2.07026300 0.89346700 0.60406000
 C -1.08603200 1.71305800 -0.18682200
 C -0.31297400 1.09149300 -1.15128200
 C -0.73405600 -0.25454400 -1.69445100
 H -1.92171400 -2.01910700 -1.31811200
 O -0.21787600 -0.66143300 -2.72417000
 C -0.96448800 3.14071500 0.16557700

Pre-TS Complex

C -1.73231200 -0.97878800 -1.02755400
 C -2.34586900 -0.60300900 0.11820600
 C -2.21622700 0.80874500 0.64259100
 C -1.39294300 1.77658200 -0.14276300
 C -0.80607900 1.36407900 -1.27791600
 C -0.92329200 -0.02515300 -1.78061800
 H -1.79669100 -1.98060600 -1.42896400
 O -0.33036000 -0.35624100 -2.80828900
 C -1.27436200 3.16234700 0.40973000
 H -0.66262800 3.78959700 -0.23835900
 H -0.82648800 3.13617000 1.40713700
 H -2.26245400 3.61721500 0.51991700
 O -2.76624000 1.13874100 1.67665400
 O -3.10019800 -1.36412500 0.90764300
 S 3.60420900 -0.24226900 0.90999100
 C 1.96807900 0.36933500 0.40677700
 H 1.94664900 1.43762600 0.61268300
 H 1.23536500 -0.14598800 1.02503900
 H 1.80898500 0.16653800 -0.65059100
 C 3.56081900 -1.95825000 0.31071200
 H 2.82715500 -2.48800100 0.91562700
 H 4.55177300 -2.37901000 0.47188200
 H 3.28974800 -1.97413900 -0.74347600
 C 4.70111700 0.55784700 -0.29895000
 H 5.69992500 0.15581300 -0.13794600
 H 4.68862100 1.62437300 -0.08200300
 H 4.35151400 0.35489000 -1.30957100
 H -0.19795900 2.03108300 -1.87932300
 C -3.31062700 -2.73849200 0.53521400
 H -3.94568900 -3.15682900 1.31148700
 H -2.35835600 -3.27318600 0.49960800
 H -3.81225900 -2.79770600 -0.43364700

Post-TS Complex

C 0.70291100 0.95185300 -0.87877800
 C 1.82931800 0.96456700 -0.11040200
 C 2.41603300 -0.30385400 0.42068500
 C 1.80392400 -1.58744700 -0.08873100
 C 0.41770000 -1.57704400 -0.47125100
 C -0.02451400 -0.27165500 -1.16562900
 H 0.28356900 1.86004700 -1.28937600
 O -1.00007800 -0.31460500 -1.88781900
 C 2.64883100 -2.76284200 -0.15223100

H -0.19230600 3.64186100 -0.41438300
 H -0.77156200 3.24655000 1.23827400
 H -1.92891700 3.63334800 -0.00869800
 O -2.62646700 1.36947800 1.57132500
 O -3.25109000 -1.10923100 0.89420100
 S 3.55783400 -0.20456600 1.00396300
 C 1.23738700 0.62057500 -0.07135200
 H 1.55996100 1.59237300 0.26937600
 H 0.81584000 -0.05709700 0.65499900
 H 1.74005400 0.19497600 -0.92804400
 C 3.38868700 -1.99293000 0.69241100
 H 2.68253400 -2.38272400 1.42467200
 H 4.35724600 -2.47631800 0.82408900
 H 3.01400000 -2.17220400 -0.31576600
 C 4.69250200 0.26034000 -0.34565400
 H 5.65713300 -0.22381800 -0.19090000
 H 4.81416800 1.34213900 -0.30587700
 H 4.27888500 -0.03074400 -1.31170200
 H 0.20940200 1.71778200 -1.86895500
 C -3.62796200 -2.46772100 0.58397400
 H -4.35652100 -2.74326400 1.34100200
 H -2.75651600 -3.12321200 0.64119700
 H -4.07908400 -2.51692500 -0.40927000

Complex-2

C -0.41324300 -1.44430200 0.93461800
 C -0.92192500 -1.13630500 -0.27807400
 C -0.02624900 -0.61154000 -1.37116100
 C 1.37011900 -0.25104800 -1.00945100
 C 1.87437400 -0.58315000 0.20076600
 C 0.99855000 -1.22463200 1.23250800
 H -1.00856400 -1.86353600 1.73396200
 O 1.45685000 -1.54370300 2.33138400
 C 2.18833600 0.42520900 -2.07266500
 H 2.86127700 -0.29025600 -2.55814000
 H 2.80753000 1.21505000 -1.64331000
 H 1.54804900 0.85375200 -2.84205900
 O -0.43429200 -0.54348100 -2.51927700
 O -2.18534100 -1.28311600 -0.67649800
 S -0.81673200 2.29887700 -0.12885700
 C 3.30051500 -0.30351900 0.58017100
 H 3.98578000 -0.59711000 -0.21803100
 H 3.44676600 0.76827000 0.75534800
 H 3.56812600 -0.83232800 1.49314800
 C -2.24771300 1.88194700 0.92461800
 H -3.09507600 1.69305600 0.26562600
 H -2.48611300 2.70776400 1.59639900
 H -2.03198800 0.98344100 1.50522100
 C 0.46508400 2.43775700 1.15837000
 H 0.22166200 3.23647000 1.86057300
 H 1.40658100 2.66684800 0.65976200
 H 0.56378300 1.49260600 1.69573200
 C -3.13410200 -1.84317000 0.24812100
 H -4.08127700 -1.86737400 -0.28437500
 H -3.22275900 -1.21455400 1.13711400
 H -2.83627900 -2.85522100 0.53293400

H 2.08625500 -3.69458900 -0.18749800
 H 3.42930200 -2.74765400 0.61079200
 H 3.18035500 -2.66802200 -1.12223500
 O 3.35377400 -0.33548800 1.18539200
 O 2.53714300 2.01754200 0.24034900
 S -3.66635000 -0.18024000 0.86112400
 C -0.38730000 -1.68491000 0.90060000
 H -0.20669900 -2.65502500 1.36006000
 H -0.11364200 -0.88819300 1.59099100
 H -1.44450300 -1.57845700 0.63037500
 C -2.70725500 1.36397600 1.01158200
 H -2.21755900 1.35133900 1.98518000
 H -3.36860400 2.22946000 0.94861700
 H -1.95145600 1.42559100 0.22721500
 C -4.31532000 0.01870200 -0.83469200
 H -4.93873400 0.91212400 -0.89632400
 H -4.92183300 -0.86068500 -1.05161400
 H -3.49262700 0.07948500 -1.54649400
 H 0.14045000 -2.43272100 -1.08743200
 C 2.11341900 3.33177400 -0.19542000
 H 2.84343000 4.01804900 0.22274300
 H 1.11772700 3.54966500 0.19463500
 H 2.12296500 3.38513000 -1.28539400

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Chapter 4

Understanding the role of dimerization on Coq5 catalytic activity and synthome assembly

ABSTRACT

Coenzyme Q (CoQ or Q) is an essential redox-active lipid that acts as an electron carrier in the mitochondrial electron transport chain and a chain-terminating antioxidant. The biosynthesis of CoQ relies on a multi-subunit complex within the mitochondrial matrix termed the CoQ synthome. Pathogenic mutations in the components of the CoQ synthome lead to primary CoQ deficiency, a condition with diverse clinical presentations including cardiomyopathy, kidney disease, and neurodegeneration. One particular component of the synthome is an *S*-adenosyl-L-methionine (AdoMet)-dependent *C*-methyltransferase encoded by the *COQ5* gene that is believed to form a homodimer *in vivo*. Using the yeast *Saccharomyces cerevisiae* as a model organism, we examined two human missense single-nucleotide variants (SNVs) with unknown clinical significance that occur on the dimer interface, A275T and Y281H (A255T and Y261H in yeast). Although both variants supported respiratory growth in yeast, liquid chromatography–tandem mass spectrometry experiments revealed an apparent reduction of *in vivo* steady-state and *de novo* CoQ levels in the Y261H mutant. Biophysical analyses of purified Coq5 wild-type and Coq5-Y261H proteins showed that the Y261H mutation does indeed disrupt Coq5 dimerization. This data suggests that dimerization supports optimal Coq5 catalytic activity. These findings demonstrate that dimerization is structurally conserved and functionally essential for Coq5, and provide mechanistic insight into how interface variants may compromise CoQ production.

INTRODUCTION

Coenzyme Q (CoQ or ubiquinone) is an essential lipid that participates in redox reactions across cellular membranes. It consists of a redox-active quinone headgroup attached to a hydrophobic polyisoprenoid tail. The best-characterized function of CoQ is its role in the mitochondrial electron transport chain, where it transfers electrons from complexes I and II to complex III, thereby supporting oxidative phosphorylation and cellular energy production (1,2). In addition, the reduced form, ubiquinol (CoQH₂), protects cells against oxidative stress by scavenging free radicals and inhibiting lipid peroxidation in conjunction with the plasma membrane redox system and vitamin E (3–5), and serves as a line of defense against ferroptosis (6,7).

In the yeast *Saccharomyces cerevisiae*, CoQ is synthesized *de novo* within the mitochondrial matrix by a multi-protein assembly known as the CoQ synthome (Figure 1A, B). This complex is composed of at least eight peripheral membrane proteins bound to the matrix side of the inner mitochondrial membrane (Coq3–Coq9 and Coq11), together with three additional polypeptides (Coq1, Coq2, and Coq10) that do not associate with the complex (Figure 1B). These proteins catalyze the conversion of 4-hydroxybenzoic acid (4-HB) or *para*-aminobenzoic acid (pABA) and activated isoprene units into CoQ (Figure 1A) (8–10). Different organisms produce isoforms of CoQ with tails of varying lengths, designated CoQ_n, where “n” indicates the number of isoprene units. For instance, yeast synthesizes CoQ₆, whereas *Escherichia coli* and humans produce CoQ₈ and CoQ₁₀, respectively.

The *COQ5* gene, conserved between humans and yeast, encodes an *S*-adenosyl-L-methionine (AdoMet)-dependent *C*-methyltransferase member of the CoQ synthome (11–14). Coq5 catalyzes the C5-methylation of the hydroquinone ring of 2-methoxy-6-polyprenyl-1,4-

benzohydroquinone (DDMQH₂), producing 2-methoxy-5-methyl-6-polyprenyl-1,4-benzohydroquinone (DMQH₂). Structural studies have shown that yeast Coq5 adopts a Rossmann-fold methyltransferase core and forms a homodimer (Figure 2A) (15). The dimer interface is primarily located on three α -helices, originally designated as α 1, α 6, and α 7, and largely consists of hydrophobic residues (Figure 2B, C). However, the effect of Coq5 dimerization on its catalytic activity and CoQ synthome assembly has not been investigated.

To date, only seven clinical cases of *COQ5* mutations have been reported, almost all of which are associated with neurological phenotypes such as cerebellar ataxia, encephalopathy, generalized tonic-clonic seizures, or varying degrees of cognitive impairment (16–19). Despite the limited number of clinical cases, single-nucleotide variants (SNVs) in *COQ5* have been identified in the general population through exome and genome sequencing databases such as gnomAD (20) and NCBI ClinVar (21). Deleterious SNVs are most often predicted to occur within functional regions of the protein, such as the methyltransferase motifs and substrate-binding site, which are highly conserved (22). However, due to low residue-level conservation of the hydrophobic dimer interface, traditional mutation classifiers may overlook the effects of mutations in this region.

Previous work established *S. cerevisiae* as a suitable model system to evaluate the functional consequences of human *COQ5* variants (8,13). In this study, we focused on two variants located at the dimer interface: A255T (corresponding to A275T in humans) and Y261H (Y281H in humans). Drop dilution assays revealed that neither mutation caused significant respiratory defects. However, liquid chromatography–tandem mass spectrometry (LC-MS/MS) analyses showed that the Y261H variant led to a markedly reduced *de novo* CoQ biosynthesis and accumulation of the Coq5 substrate, consistent with a catalytic defect without major disruptions to the CoQ synthome. Size-exclusion chromatography analysis of purified Coq5-Y261H further

revealed an altered oligomeric state in solution, supporting the idea that dimerization plays an important role in Coq5 catalytic function.

MATERIALS AND METHODS

Multiple Sequence Alignment

Multiple sequence alignment was previously conducted and adapted from (22), which was carried out using Clustal Omega with default parameters (23,24), incorporating protein sequences from human COQ5 and orthologs from *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Drosophila melanogaster*, *Arabidopsis thaliana*, *Danio rerio*, *Gallus gallus*, *Mus musculus*, and *Schizosaccharomyces pombe*. Sequence accession numbers are listed in Table 1. The alignment was subsequently visualized using Jalview2 (25). Residues showing $\geq 80\%$ sequence identity across species were classified as highly conserved. Functionally relevant residues were identified based on structural data from *S. cerevisiae* Coq5 apoenzyme (PDB: 4OBX) and its AdoMet-bound form (PDB: 4OBW) (15).

Yeast strains and growth medium

Wild-type and *coq5* Δ *S. cerevisiae* strains in this study were derived from the W303 genetic background (26) (see Table 2). Growth media were prepared as previously described (27): YPD (1% yeast extract, 2% peptone, 2% glucose), YPG (1% yeast extract, 2% peptone, 3% glycerol), and synthetic dextrose (SD) media with (SD-Complete) and without uracil SD-Ura. All SD media were composed of 2% dextrose, 0.18% yeast nitrogen base without amino acids, 0.14% sodium phosphate monobasic (NaH_2PO_4), 0.5% ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), and a defined amino acid mix at concentrations specified in (11). All SD media were adjusted to approximately pH 6. Solid plate media were prepared with 2% bacto-agar.

Construction of *COQ5* mutant plasmids and complementation in yeast

All plasmids used in this study are listed in Table 3. Mutations were introduced into the pGBmCOQ5 and pGBsCOQ5 backbones using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Cat. #210518), following the manufacturer's protocol (Revision F1). Primer sequences used for mutagenesis are detailed in Table 4. The resulting plasmid sequences were verified using whole-plasmid sequencing (Plasmidsaurus). Yeast were transformed using a modified lithium acetate (LiOAc)-based protocol (27). Briefly, 0.5 mL aliquots of overnight YPD cultures were collected by centrifugation and each pellet was sequentially mixed with 100 µg of denatured single-stranded carrier DNA, 1 µg of plasmid DNA, 0.5 mL of PLATE solution (containing 40% PEG 3350, 0.1 M LiOAc, 10 mM Tris-HCl pH 7.5, and 1 mM EDTA), and 57 µL DMSO, vortexing gently after each addition. The transformation mixtures were incubated at room temperature for 15 minutes, followed by a 15-minute heat shock at 42 °C. Cells were then pelleted, resuspended in 200 µL of sterile PBS, and spread onto SD-Ura plate medium. Plates were incubated at 30 °C for 2–3 days, after which single colonies were isolated and maintained on selective plates for subsequent biochemical experiments.

Drop dilution plate assays

Yeast were grown overnight in 5 mL of SD-Ura for transformed strains or SD-Complete medium for the W303 wild type and *coq5*Δ strains. The next day, cultures were diluted in sterile PBS to an OD₆₀₀ of 0.2. From these suspensions, 200 µL aliquots were dispensed into a 96-well plate, and four successive five-fold serial dilutions were prepared. A 2 µL volume from each dilution was then spotted onto YPD, YPG, and SD-Ura plate medium, corresponding to final OD₆₀₀ values of 0.2, 0.04, 0.008, 0.0016, and 0.00032. Plates were incubated at 30 °C for 2–3 days before analysis, with incubation time depending on cell growth rates on each plate medium.

Stable isotope labeling and analysis of CoQ and CoQ intermediates

Cultures of transformed cells were grown in 5 mL of selective media overnight at 30 °C with agitation. Cells were then sub-inoculated into 15 mL of fresh selective media to an OD₆₀₀ of 0.6 the next day. To assess *de novo* CoQ biosynthesis, the 15 mL cultures were supplemented with either 8 µg/mL (56 µM) ¹³C₆-pABA (Sigma-Aldrich) or ethanol as a vehicle control, resulting in a final concentration of 0.2% (v/v) ethanol. Cultures were incubated for 5 hours at 30°C with agitation, reaching a final OD₆₀₀ of 2–3. From each culture, 9 OD₆₀₀ units of cells (calculated as OD₆₀₀ × culture volume in mL) were collected and centrifuged at 4000 × g for 5 minutes. For vehicle control cells, another identical aliquot of 9 OD₆₀₀ units was harvested for immunoblot analysis. The supernatant was aspirated, and the remaining cell pellets were stored at –20°C until use.

For lipid extraction, cell pellets were washed with 500 µL of PBS and resuspended in 100 µL of fresh PBS. To each suspension, 2 mL of methanol and glass beads were added, followed by vortexing for 30 seconds. An equal amount (typically 100–150 pmol) of internal standard, CoQ₄ (Sigma-Aldrich) was added to each sample. A CoQ₆ standard curve (Avanti Polar Lipids) ranging from approximately 0.4 pmol to 1.3 nmol, containing the same amounts of CoQ₄, was made in parallel with the experimental samples. Lipids were extracted twice using 2 mL of petroleum ether per extraction, with 30-second vortexing for each extraction. Petroleum ether fractions containing the extracted lipids were dried under nitrogen gas and stored at –20 °C until further analysis.

On the day of analysis, lipid extracts were reconstituted in 200 µL of 1 mg/mL benzoquinone and LC-MS/MS analyses were conducted as previously described (14,28). 20 µL of each sample was injected onto a Luna phenyl-hexyl column (100 × 4.6 mm, 5 µm; Phenomenex), coupled to the 4000 QTRAP linear ion trap mass spectrometer (Applied Biosystems). Precursor-

to-product ion transitions for each analyte are detailed in Table 5. Data acquisition and analysis were performed using Analyst software version 1.4.2 (AB Sciex, Framingham, MA, USA). For each analyte, relative abundance was calculated by summing the peak areas of the protonated molecular ion and, where applicable, its corresponding ammonium adduct. These values were corrected for extraction efficiency using the recovery of the internal standard CoQ₄ and normalized to the OD₆₀₀ of the harvested sample. For CoQ₆, absolute quantification was achieved via normalization to the standard curve and are expressed in pmol/OD₆₀₀. Relative levels of CoQ intermediates were expressed as Arbitrary Units/OD₆₀₀. Statistical comparisons between strains within each treatment group were performed using one-way ANOVA followed by Tukey's HSD post hoc test. Strains that yielded no detectable signal in any biological replicate were designated 'n.d.' (not detected) and excluded in statistical analysis. All statistical analyses and data visualizations were conducted using GraphPad Prism version 10.4.1 (GraphPad Software, Boston, MA, USA).

SDS-PAGE and immunoblot analysis of steady-state protein expression

Yeast whole-cell extracts were prepared as previously described (29). The 9 OD₆₀₀ units of cells from vehicle control-treated cultures used in the stable isotope labeling experiments (see *Stable isotope labeling and analysis of CoQ and CoQ intermediates*) were resuspended in 300 μ L of 2 M LiOAc, incubated on ice for 5 minutes, and centrifuged. Pellets were then resuspended in 300 μ L of 0.4 M NaOH, incubated on ice for an additional 5 minutes, and pelleted. The resulting pellets were solubilized in 200 μ L of SDS sample buffer (50 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 0.1% bromophenol blue, 5% v/v β -mercaptoethanol), boiled for 5 minutes, and immediately stored at -20°C . Immediately before running SDS-PAGE, samples were boiled for 5 minutes and centrifuged briefly to remove insoluble material and debris.

For electrophoresis, 0.45 OD₆₀₀ equivalents (10 µL) of each extract was loaded onto 10% Tris-glycine polyacrylamide gels. Following separation, proteins were transferred to Immobilon®-FL 0.45 µm PVDF membranes (Millipore) and blocked overnight at 4 °C in blocking buffer (5% non-fat dry milk in PBS-T; PBS with 0.1% Tween-20). Detection of Coq5 and the mitochondrial loading control Atp2 was carried out using rabbit polyclonal primary antibodies (see Table 6), diluted in either 0.5% BSA or 5% milk in PBS-T supplemented with 0.05% (v/v) ProClin 300. Primary antibody incubations were performed overnight at 4 °C or 6 hours at room temperature. IRDye 680LT goat anti-rabbit IgG secondary antibodies (LI-COR) were diluted 1:20,000 in PBS-T containing 0.02% SDS, 0.5% BSA, and 0.05% (v/v) ProClin 300, and incubated with the membranes for 1 hour at room temperature. Membranes were imaged using the LI-COR Odyssey Infrared Imaging System, and immunoblot signals were quantified manually using the ImageJ version 1.5.3 (National Institutes of Health, Bethesda, MD, USA) (30). Statistical comparisons of Coq5 band intensities of mutant strains against *coq5Δ* yeast expressing wild-type Coq5 were performed using one-way ANOVA with Dunnett's multiple comparisons test on GraphPad Prism version 10.4.1.

Recombinant protein expression, purification, and oligomeric state determination

Plasmids used for the recombinant expression of yeast Coq5-WT (pTHQ5-1) were previously described (13). The Y261H mutation was introduced into pTHQ5-1 using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Cat. #210518), generating pTHQ5-Y261H. *E. coli* BL21(DE3) cells (New England Biolabs) were transformed with pTHQ5-1 or pTHQ5-Y261H under ampicillin selection. Cultures were grown in LB media and induced at OD₆₀₀ ~ 0.6 with 1 mM IPTG for 6 hours at 37 °C. Cells were collected by centrifugation at 1900 × g at 4 °C for 40 minutes and resuspended in pre-chilled lysis buffer (50

mM HEPES, 500 mM NaCl, 20 mM imidazole, 1 mM DTT, pH 7.5) containing one tablet of Pierce protease inhibitor (Thermo Fisher Scientific) per 50 mL and lysed by sonication. The resuspension was subjected to centrifugation at $17,000 \times g$ at 4 °C for 50 minutes. The resulting supernatant was loaded onto a 5 mL HisTrap HP column (Cytiva) maintained at 4 °C for a total of three times and washed with buffer A (50 mM HEPES, 500 mM NaCl, 50 mM imidazole, 1 mM DTT, pH 8.0) for 15 column volumes. Proteins were eluted with a step-wise gradient of increasing concentrations of buffer B (50 mM HEPES, 500 mM NaCl, 300 mM imidazole, 1 mM DTT, pH 8.0). The step-wise gradient began at 0% Buffer B and increased by 20% every column volume. After 5 column volume elutions, 100% buffer B was run for an additional column volume. 1-mL fractions were collected, and fractions containing Coq5, assessed by SDS-PAGE and A_{280} , were pooled and concentrated using Amicon® Ultra Centrifugal Filter, 10 kDa MWCO (Millipore Sigma). Oligomeric states of purified Coq5-WT and Coq5-Y261H were determined by size-exclusion chromatography (SEC) at 22 °C using a Superdex 200 10/300 GL column (Cytiva) with void volume of 9.01 mL in SEC buffer (50 mM HEPES, 150 mM NaCl, 1 mM DTT, pH 7.5). Elution volumes were determined based on the A_{280} trace and normalized according to the void volume. Apparent molecular weight estimates were determined by comparison to a set of SEC standards (Gel Filtration Markers Kit for Protein Molecular Weights 29,000–700,000 Da, Sigma-Aldrich, Cat. #MWGF1000).

Protein expression and sample purity were assessed by SDS-PAGE. Briefly, samples were diluted two-fold in SDS sample buffer with a final concentration of 50 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 0.1% bromophenol blue, and 5% v/v β -mercaptoethanol, boiled for 5 min, and stored immediately at –20 °C. Immediately before SDS-PAGE analysis, the samples were boiled for 5 minutes and then briefly centrifuged. 10-30 μ L of purified protein samples and 20 μ L of the

flow-through and unpurified lysates were loaded onto 4–16% Tris-MOPS polyacrylamide gels. The identity of the Coq5 band was confirmed by immunoblotting using rabbit polyclonal primary antibodies.

RESULTS

Structural modeling identifies conserved dimer interface in Coq5

To investigate the role of Coq5 dimerization in CoQ biosynthesis and the functional impact of human COQ5 SNVs in this region, we employed *Saccharomyces cerevisiae* as a model system, whose dimer interface has been structurally characterized. A cluster of hydrophobic residues—including V84, L87, I89, M236, A239, V246, L247, V249, M250, M253, I254, A255, Y261, T295, and I298—form a tightly packed interface predicted to stabilize Coq5 dimer formation (Figure 2A–B). To determine the potential evolutionary significance of this interface, we performed sequence alignments with homologs from eleven organisms, including *Homo sapiens*, *S. cerevisiae*, *Caenorhabditis elegans*, *E. coli*, and *Danio rerio*. A representative segment encompassing $\alpha 6$ and $\alpha 7$ is shown in Figure 2C. While the hydrophobicity of these residues is maintained across species, residue agreement was in general much lower than that of other functional regions, such as the AdoMet- and substrate-binding pockets (22). Given the lower residue conservation, we considered the possibility that mutation classifiers may overlook the deleteriousness of mutations in this region. We selected human SNVs A275T (rs201170387) and Y281H (rs761451242), corresponding to yeast mutations A255T and Y261H, for further biochemical characterization as both residues are highly conserved across the analyzed species. Residues A255 and Y261 are located on $\alpha 6$ and $\alpha 7$, respectively. While the SNV Y281H was previously predicted to be deleterious with consensus from Missense3D, SIFT, and PolyPhen-2 (22), the interpretation of A275T was conflicting. Since both mutations disrupt the hydrophobicity

of the interface, we hypothesized that both A255T and Y261H mutations in yeast would disrupt dimerization and result in catalytic defects.

Coq5 dimerization variants support respiratory growth

To evaluate the functional impact of interface variants, we introduced Coq5-A255T and Coq5-Y261H using either high-copy (Figure 3A, B) or low-copy (Figure 3C, D) expression plasmids into *coq5Δ* yeast. In strains harboring high-copy plasmids, wild-type Coq5 was readily detected at the expected ~31 kDa, while Atp2 at ~53 kDa served as a loading control. The polypeptide content of Coq5-A255T and Coq5-Y261H was comparable to the overexpression of wild-type Coq5 (HC COQ5; Figure 3A, B). Similar trends were observed in the low-copy plasmid background, with polypeptide expression comparable to that of endogenous levels of expression in a wild-type W303 strain (Figure 3C, D). We then assessed their respiratory competence by drop dilution assays. On rich fermentable (YPD) and selective fermentable medium (SD-Ura), all strains grew comparably, as expected given that respiration is not required. Interestingly, when plated on the non-fermentable carbon source glycerol (YPG), the Coq5-A255T and Coq5-Y261H mutants maintained respiratory competency regardless of copy number (Figure 4). These results suggest that CoQ₆ is present in both mutants at levels sufficient for respiratory growth on glycerol.

Coq5-Y261H but not Coq5-A255T impairs CoQ₆ biosynthesis

We next quantified levels of CoQ₆ and CoQ₆ intermediates in lipid extracts prepared from these strains using LC-MS/MS. Under overexpression, steady-state levels of ¹²C-CoQ₆ in the Coq5-Y261H mutant were significantly lower than that of strains overexpressing wild-type COQ5 (HC COQ5) and the wild-type W303 strain (WT HC EV) (Figure 5A). *De novo* synthesis of ¹³C₆-CoQ₆ exhibited similar trends, with the mutant strain expressing Coq5-Y261H having diminished amounts compared to HC COQ5 and WT HC EV (Figure 5B, C). We also examined the content

of DDMQ₆ and DMQ₆, the oxidized forms of the substrate and product of the Coq5 step, respectively. Consistent with impaired methyltransferase activity, the steady-state and *de novo* content of DMQ₆ were similarly reduced in the Coq5-Y261H mutant compared to HC *COQ5* and WT HC EV (Figure 5D–F), while steady-state levels and *de novo* content of DDMQ₆ were elevated (Figure 5G, H). In contrast, the CoQ₆, DDMQ₆, and DMQ₆ content in the Coq5-A255T mutant was indistinguishable from wild type. In fact, when overexpressed, Coq5-A255T exhibited slightly elevated content of *de novo* and total DMQ₆ (Figure 5E, F), indicating catalytic activity comparable to or exceeding wild-type levels. Lastly, *de novo* content of the early intermediate 3-hexaprenyl-4-aminobenzoic acid (¹³C₆-HAB) was analyzed, but neither dimerization mutant accumulated this intermediate (Figure 5I), suggesting that the CoQ synthome is stably forming. Similar trends were observed in strains containing low-copy plasmids, with Coq5-Y261H exhibiting a slightly exacerbated CoQ₆ biosynthetic defect (Figure 6). These quantitative measurements provide direct evidence that the Coq5-Y261H dimerization variant alters enzymatic function and impairs CoQ₆ biosynthesis. Given its lack of effect *in vivo*, the Coq5-A255T was not further analyzed.

The Y261H mutation disrupts dimerization *in vitro*

To probe directly the oligomeric state of the Coq5-Y261H variant, we expressed recombinant mature Coq5-WT and Coq5-Y261H harboring N-terminal His₆-tags in *E. coli* and purified using Ni-NTA affinity chromatography (Figure 7A–B). Fractions containing Coq5-WT (Figure 7A) or Coq5-Y261H (Figure 7B) were pooled, concentrated, and subjected to size-exclusion chromatography (SEC) in succession. Coq5-WT eluted at 13.4 mL, consistent with a higher-order oligomeric state, whereas Coq5-Y261H eluted later at 16.3 mL, corresponding to a smaller apparent size (Figure 7C). These data suggest that the Y261H mutation alters the

oligomeric state of Coq5, providing a mechanistic explanation for its inability to support CoQ₆ biosynthesis in yeast.

Together, these findings suggest that Coq5 dimerization is structurally conserved and functionally essential. While the A255T variant behaves indistinguishably from wild type, our data suggest that the Y261H variant disrupts dimerization and impairs catalytic activity, leading to CoQ₆ deficiency while maintaining the stability of the CoQ synthome. These findings underscore the importance of the dimer interface in maintaining Coq5 activity.

DISCUSSION

Our findings provide evidence that Coq5 dimerization is critical in CoQ biosynthesis. Biochemical analyses provide a mechanistic basis for the reduction in CoQ₆ content observed in strains expressing Coq5-Y261H. Specifically, the accumulation of DDMQ₆ in this mutant points to a defect at the Coq5-catalyzed step, while the lack of upstream HAB accumulation indicates that the CoQ synthome remains intact. Western blotting confirmed that mutant Coq5 protein levels were comparable to wild type, ruling out decreased protein abundance as the basis for the phenotype. SEC analysis further reveals that the Y261H variant adopts a smaller oligomeric state than wild type. Together, these results indicate that the reduction in CoQ content stems from impaired catalytic function due to disrupted dimerization, rather than secondary effects on protein abundance or synthome integrity. In the case of Coq5, dimer formation is not merely a structural feature but directly contributes to enzymatic efficiency and biosynthetic output, highlighting the importance of protein–protein interactions in regulating both individual CoQ biosynthetic enzymes and overall pathway output.

Our findings are consistent with previous work showing that dimerization is critical for the catalytic activity of many other methyltransferases. For example, the representative member of the

protein arginine methyltransferase family, PRMT1, requires dimerization for AdoMet binding and methyltransferase activity (31,32), despite the fact that its dimerization arm is distal to the active site. Furthermore, PRMT1's substrate preference and processivity can be modulated by its oligomerization state (33,34). Zhou *et al.* identified an allosteric pathway in which conformational changes can be propagated to the active site (32), suggesting that a similar mechanism may occur for Coq5. Moreover, similar dimer-dependent behavior has been reported in bacterial small-molecule C-methyltransferases such as CouO from *Streptomyces rishiriensis*, confirmed by size-exclusion chromatography and crystallographic analyses (35).

However, in contrast to the more extensively characterized PRMT1 and other cytosolic methyltransferases, there is very little information available regarding dimerization of the mitochondrial methyltransferases involved in CoQ biosynthesis. It remains unclear whether more distant Coq5 homologs, such as *Pseudomonas aeruginosa* and *E. coli* UbiE, would also require dimerization due to lower conservation in this region. Notably, the structure of the Coq3 O-methyltransferase homolog UbiG from *E. coli* was solved as a monomer (36,37). To our knowledge, this study is among the first to directly link the oligomeric state of a CoQ biosynthetic enzyme to its enzymatic output, thereby expanding the current understanding of how protein–protein interactions regulate this essential pathway.

Extending these insights to human relevance, the yeast Coq5-Y261H mutation models a region homologous to residues implicated in human *COQ5* variants. Our results suggest that naturally occurring SNVs at the dimer interface can impair CoQ biosynthesis, providing a potential mechanistic explanation for certain cases of primary CoQ₁₀ deficiency. These findings emphasize the translational importance of studying Coq5 oligomerization and protein–protein interactions

within large mitochondrial biosynthetic machineries and could inform future therapeutic strategies for mitochondrial diseases aimed at stabilizing or compensating for disrupted interfaces.

The mechanistic basis for the Coq5-Y261H defect remains incompletely defined, and our data do not permit a definitive structural conclusion about how the Y261H mutation disrupts Coq5 function. One possible mechanistic explanation is that dimerization stabilizes the active-site conformation of Coq5, thereby maintaining proper geometry for AdoMet binding and catalysis. Changes to Y261 on α -helix $\alpha 7$ may propagate conformational changes through the nearby helix $\alpha 1$, which lines the face of the substrate-binding pocket distal to AdoMet, in a manner similar to the allosteric pathway observed in PRMT1 (32). However, it must be noted that the Coq5-A255T variant behaved indistinguishably from wild type, suggesting that not all interface residues are equally critical. Alternatively, the dimer interface may facilitate cooperative substrate channeling by mediating contacts with other polypeptide partners of the CoQ synthome, or allosteric communication between monomers, enhancing catalytic turnover independently of synthome organization. These observations suggest that monomer–monomer interactions may represent a critical regulatory checkpoint in CoQ production, with potential implications for understanding how oligomerization contributes to the control of mitochondrial biosynthetic enzymes more broadly.

The interplay between Coq5 expression level, dimerization, and CoQ synthome assembly remains an interesting question. In yeast, the RNA-binding protein Puf3 has been shown to regulate Coq5 polypeptide levels by binding to the 3' untranslated region of the Coq5 mRNA (38). Puf3 binding prevents the overaccumulation of Coq5, which was shown to be detrimental to yeast growth, resulting in the accumulation of early intermediates such as 3-hexaprenyl-4-hydroxybenzoic acid (HHB) and HAB and reduction of late-stage intermediates (38). The

excessive abundance of one CoQ synthome member may dysregulate synthome assembly, possibly due to decreased availability of protein–protein interaction interfaces. Whether Coq5 dimerization plays a role in this observed toxicity is not known. In our growth conditions, however, we did not observe significant differences in growth or CoQ content between Coq5 expression on low-copy and high-copy plasmids, regardless of oligomeric state.

Several limitations of this study should be noted. First, the functional residues obtained from the Coq5 crystal structure capture only a static view of the dimer interface and do not reflect protein dynamics, hindering mechanistic interpretation. Second, the inherent instability of purified Coq5 limited our ability to perform direct biochemical and biophysical characterizations (e.g., activity assays, native PAGE, and native top-down mass spectrometry). In yeast, Coq5 stability is enhanced by N-terminal processing via the mitochondrial processing protease and Oct1 (39), suggesting that the N-terminal tag present on the recombinant mature Coq5 may contribute to its instability. Additional stabilization may also require chaperones or binding partners absent during purification. Future work may benefit from optimizing recombinant Coq5 constructs for direct mechanistic interrogation, as well as employing molecular dynamics simulations to capture the dynamic interplay of dimerization and catalysis.

In summary, our study demonstrates that Coq5 dimerization is essential for its catalytic role in CoQ biosynthesis. The Y261H mutation disrupts this interaction, blocking the Coq5-catalyzed methylation step and reducing CoQ production despite preserved synthome integrity. By connecting these results to prior work on other methyltransferases, we establish that dimerization is a conserved strategy for stabilizing active-site geometry and promoting efficient catalysis. Importantly, our findings provide a foundation for investigating human *COQ5* variants and their potential contribution to mitochondrial disease. More broadly, examining whether dimerization is

a general regulatory principle for other enzymes in the CoQ biosynthetic pathway may reveal new layers of control in mitochondrial metabolism. Taken together, these findings highlight the importance of oligomeric assembly in enzyme function and open new opportunities for both mechanistic insight and translational application.

Table 1. Protein sequences used in the multiple sequence alignment.

<i>Species</i>	Protein Name	NCBI Accession Number
<i>Homo sapiens</i>	COQ5	NP_115690.3
<i>Saccharomyces cerevisiae</i>	Coq5	NP_013597.1
<i>Caenorhabditis elegans</i>	COQ-5	NP_498704.1
<i>Escherichia coli</i>	UbiE	YP_026269.1
<i>Pseudomonas aeruginosa</i>	UbiE	NP_253750.1
<i>Drosophila melanogaster</i>	Coq5	NP_572865.1
<i>Arabidopsis thaliana</i>	Coq5	NP_200540.1
<i>Danio rerio</i>	Coq5	NP_001004541.1
<i>Gallus gallus</i>	COQ5	NP_001006194.1
<i>Mus musculus</i>	Coq5	NP_080780.1
<i>Schizosaccharomyces pombe</i>	Coq5	NP_587834.1

Table 2. Genotype and source of yeast strains.

<i>Strain</i>	<i>Genotype</i>	<i>Source</i>
W303-1B	<i>MATα leu2-3,-112; his3-11,-15; trp1-1; ura3-1; ade2-1; can1-100</i>	R. Rothstein ^a
W303-1B <i>coq5Δ</i>	<i>MATα leu2-3,-112; his3-11,-15; trp1-1; ura3-1; ade2-1; can1-100 coq5:HIS3</i>	(11)
JM6	<i>MATα his-4 ρ⁰</i>	J. E. McEwen ^b
JM8	<i>MATα ade-1 ρ⁰</i>	J. E. McEwen ^b

^a Dr. Rodney Rothstein, Department of Human Genetics, Columbia University

^b Dr. Joan E. McEwen

Table 3. Plasmid constructs used in this study.

<i>Plasmid</i>	Construction Description	Source
pRS316	Yeast shuttle vector carrying the <i>URA3</i> gene; low-copy	(40)
pRS426	Yeast shuttle vector carrying the <i>URA3</i> gene; high-copy	(41)
pGBsCOQ5	Wildtype yeast <i>COQ5</i> open reading frame (ORF) with 400 bp upstream and 100 bp downstream regions (~1500 bp) inserted into the <i>SmaI</i> site of pRS316	(13)
pGBsCOQ5-A255T	pGBsCOQ5 carrying the cDNA change c.763G>A, expressing Coq5-A255T	This work
pGBsCOQ5-Y261H	pGBsCOQ5 carrying the cDNA change c.781T>C, expressing Coq5-Y261H	This work
pGBmCOQ5	Wildtype yeast <i>COQ5</i> ORF with 400 bp upstream and 100 bp downstream regions (~1500 bp) inserted into the <i>SmaI</i> site of pRS426; high-copy	(13)
pGBmCOQ5-A255T	pGBmCOQ5 carrying the cDNA change c.763G>A, expressing Coq5-A255T	This work
pGBmCOQ5-Y261H	pGBmCOQ5 carrying the cDNA change c.781T>C, expressing Coq5-Y261H	This work

Table 3. Plasmid constructs used in this study. (Cont.)

<i>Plasmid</i>	Construction Description	Source
pTHQ5-1	Wildtype yeast <i>COQ5</i> ORF segment (+91 to +924) containing the mature Coq5 polypeptide inserted into the BglII site of the pTrcHisB backbone (Invitrogen) for expression in <i>E. coli</i>	(13)
pTHQ5-Y261H	pTHQ5-1 carrying the cDNA change c.781T>C, expressing mature Coq5-Y261H	This work

Table 4. List of primers used in this study.

<i>Resulting Mutation</i>	Primers
Coq5-A255T	F 5'-ctgtaatgggctcgatgattactaatgactacgactcttac-3' R 5'-gtaagagtcgtagtcattagtaatcatcgagcccattacag-3'
Coq5-Y261H	F 5'-tcgatgattgctaatactgactacgactctcaccagtatttggtg-3' R 5'-caccaaatactggtgagagtcgtagtcattagcaatcatcga-3'

Table 5. Precursor-to-product ion transitions used in this study.

<i>Analyte</i>	<i>m/z</i> [M+H] ⁺	<i>m/z</i> [M+NH ₄] ⁺
HAB	546.4/150.0	563.0/150.0
¹³ C ₆ -HAB	552.4/156.0	569.0/156.0
DDMQ ₆	547.4/153.1	Not used
¹³ C ₆ -DDMQ ₆	553.4/159.1	Not used
DMQ ₆	561.4/167.0	578.0/167.0
¹³ C ₆ -DMQ ₆	567.4/173.0	584.0/173.0
CoQ ₆	591.4/197.0	608.0/197.0
¹³ C ₆ -CoQ ₆	597.4/203.0	614.0/203.0
CoQ ₄	455.4/197.0	472.0/197.0

Table 6. Description and source of antibodies.

<i>Antibody</i>	Working dilution	Source
Atp2	1:4,000	C. M. Koehler ^c
Coq5	1:5,000	(13)

^c Dr. Carla M. Koehler, Department of Chemistry and Biochemistry, UCLA

FIGURES

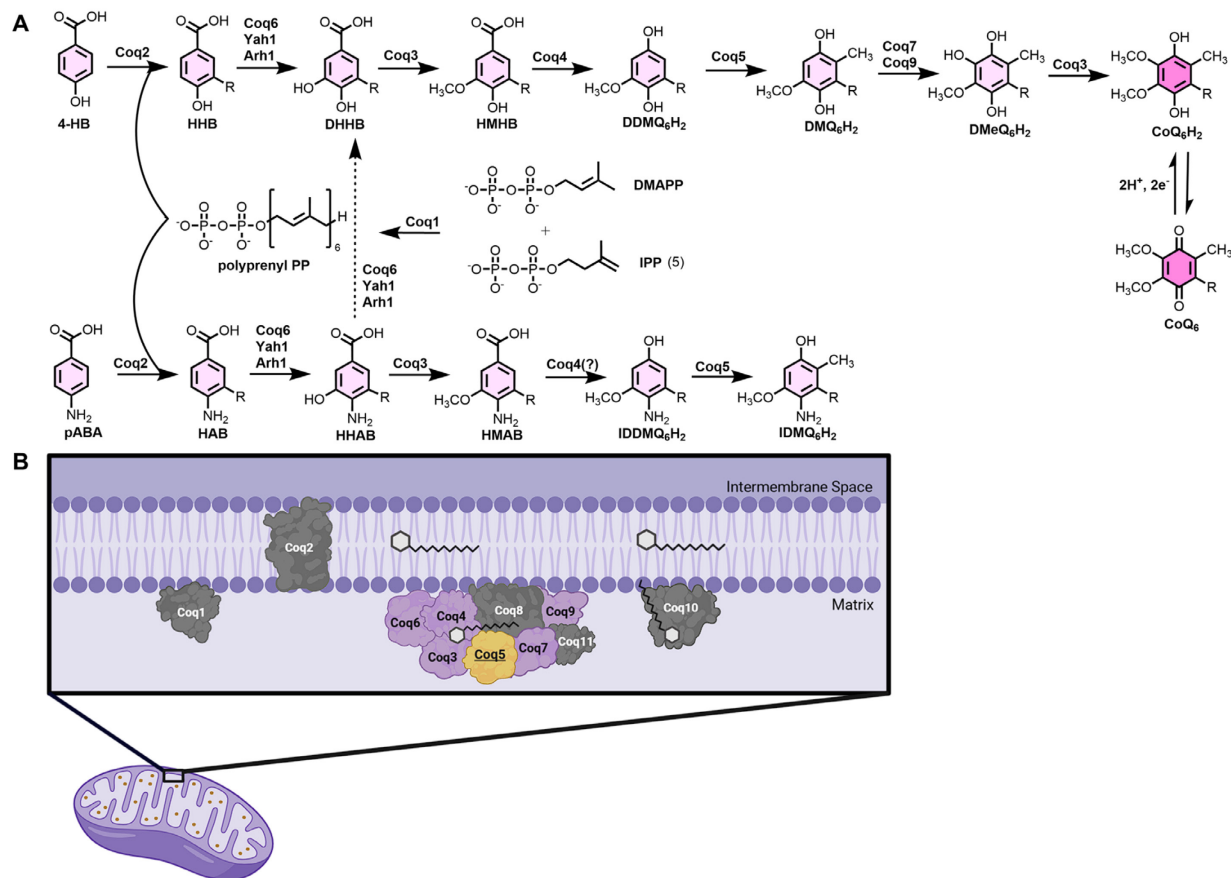


Figure 1. CoQ₆ biosynthetic pathway in *S. cerevisiae*. (A) The CoQ₆ biosynthetic pathway in yeast begins with precursors 4-hydroxybenzoic acid (4-HB) and *para*-aminobenzoic acid (pABA) (9,10), which are prenylated by Coq2 and subsequently modified by other enzymes in the pathway. Recent evidence suggests that yeast Coq4 possesses oxidative decarboxylation activity, although the ability of Coq4 to act on the aminated intermediate HMAB is unclear (42). (B) Schematic representation of the Coq polypeptides responsible for efficient synthesis of CoQ₆. Peripheral membrane proteins Coq3–Coq9 and Coq11, along with additional non-synthome members Coq1, Coq2, and Coq10, work synergistically to ensure the efficient conversion of ring precursors and activated isoprene units into CoQ₆. Non-synthome members and synthome members with unknown functions are colored in grey. Coq5 is highlighted in yellow. DMAPP, dimethylallyl

pyrophosphate; IPP, isopentenyl pyrophosphate; HHB, 3-hexaprenyl-4-hydroxybenzoic acid; DHHB, 4,5-dihydroxy-3-hexaprenylbenzoic acid; HMHB, 4-hydroxy-5-methoxy-3-hexaprenylbenzoic acid; DDMQ₆H₂, 2-methoxy-6-hexaprenyl-1,4-benzohydroquinone; DMQ₆H₂, 2-methoxy-5-methyl-6-hexaprenyl-1,4-benzohydroquinone; DMeQH₂, 3-methyl-6-methoxy-2-hexaprenyl-1,4,5-benzenetriol; HAB, 3-hexaprenyl-4-aminobenzoic acid; HHAB, 3-hexaprenyl-5-hydroxy-4-aminobenzoic acid; HMAB, 3-hexaprenyl-5-methoxy-4-aminobenzoic acid; IDDMQ₆H₂, 4-amino-2-methoxy-6-hexaprenylphenol; IDMQ₆H₂, 4-amino-2-methoxy-5-methyl-6-hexaprenylphenol. R represents the polyprenyl tail (n = 6 in yeast).

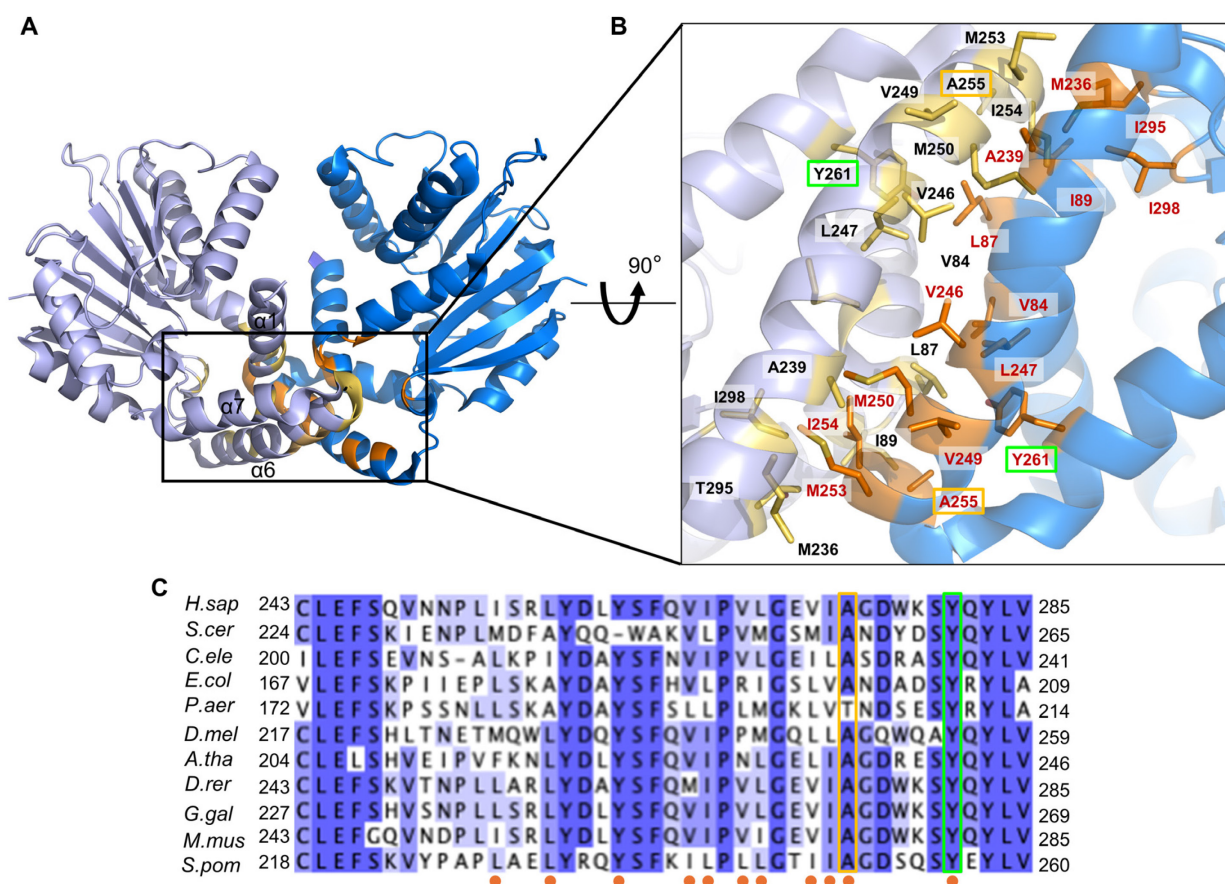


Figure 2. Structure and dimer interface of yeast Coq5. (A) The yeast Coq5 apoenzyme dimeric crystal structure (PDB: 4OBX) is pictured in pale (chain A) and deep blue (chain D). Key hydrophobic residues (V84, L87, I89, M236, A239, V246, L247, V249, M250, M253, I254, A255, Y261, T295, I298) are highlighted in yellow for chain A and orange for chain D. The α -helices are numbered according to Dai *et al.* (15). (B) A rotated view of the dimer interface is shown, with chain A residues labeled in black and chain D residues labeled in red. Adapted from Dai *et al.* (15). (C) Sequence alignment of Coq5 homologs from representative species demonstrates conservation of residues at the dimer interface. Alignment of residues located in $\alpha 6$ and $\alpha 7$ is adapted from Wang and Jain *et al.* (22) and pictured here. Interface residues marked by an orange dot. Yeast Coq5 residues A255 (human A275) and Y261 (human Y281) are boxed in yellow and green, respectively. Conservation at each residue is indicated by blue shading, which represent percent

agreement >80%, >60%, and >40%, from darkest to lightest. Residues with less than 40% agreement are unshaded.

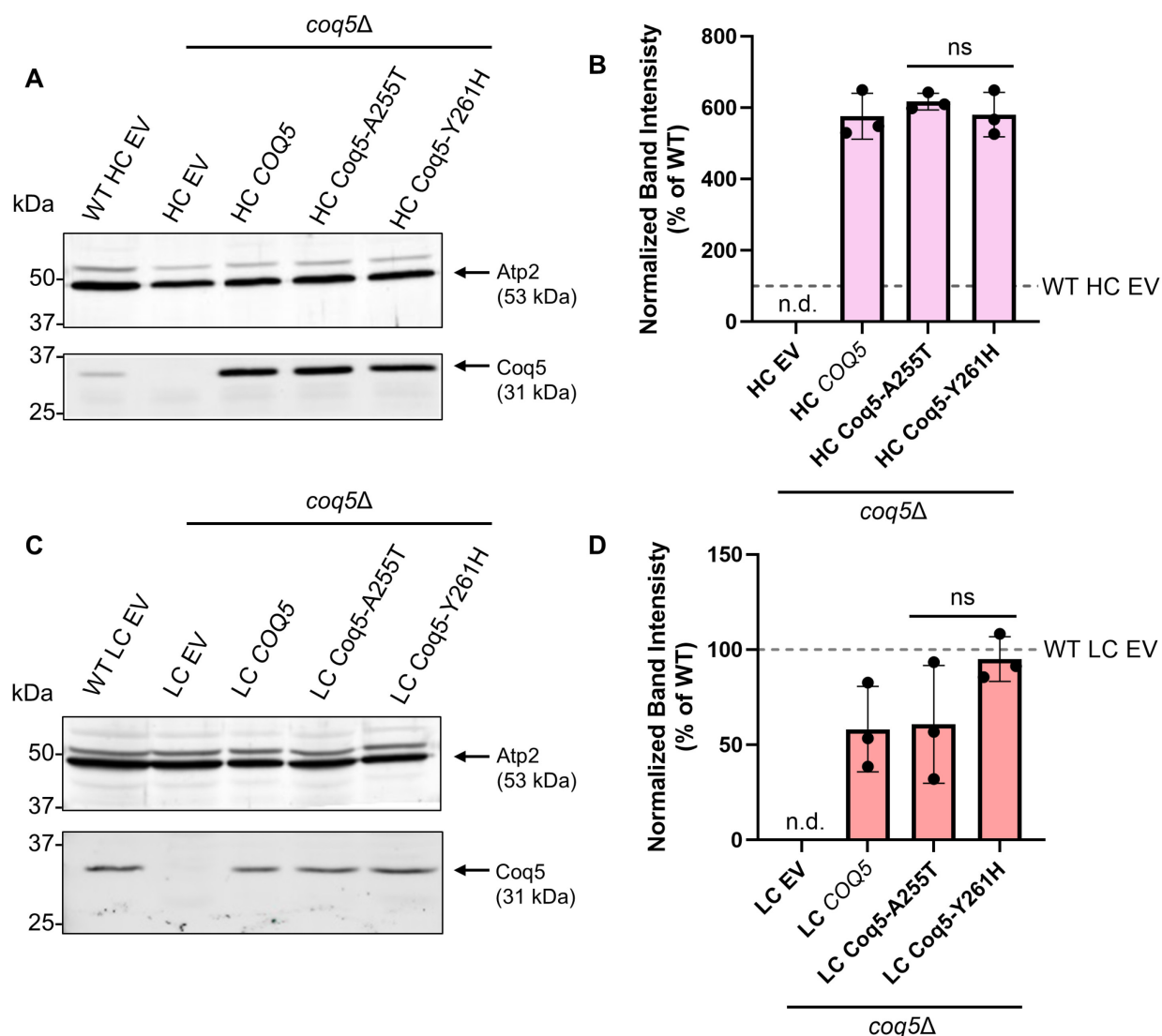


Figure 3. Expression of Coq5 mutants in yeast. Aliquots of whole cell protein extracts (equivalent to 0.45 OD₆₀₀ units) were resolved on 10% Tris-glycine SDS-PAGE. (A, C) Representative Western blot analysis of Coq5 expression in the indicated high-copy (A) and low-copy (C) transformants is pictured. The β -subunit of the mitochondrial ATP synthase (Atp2) was included as a loading control; the size of the mature Atp2 polypeptide was previously determined (43). (B, D) Quantification high-copy (B) and low-copy (D) Coq5 band intensities is shown. Western blot statistical significance was performed with respect to band intensity in *coq5*Δ yeast expressing the wild-type *COQ5* plasmids (HC *COQ5* or LC *COQ5*), using one-way ANOVA with

Dunnett's multiple comparison test. Data in **(B)** and **(D)** depict mean $\pm$ SD of three biological replicates. ns = not significant.

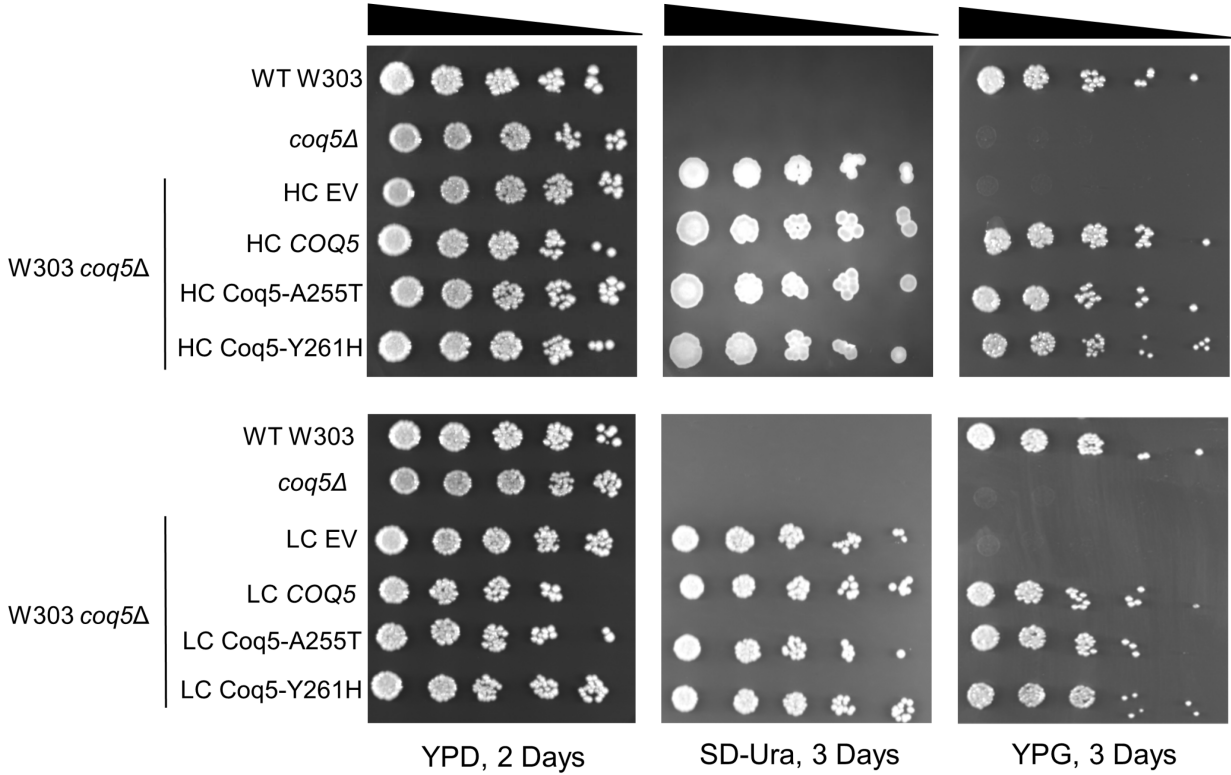


Figure 4. Coq5 dimerization mutants are respiratory-competent. W303 *coq5Δ* yeast were transformed with designated high-copy (HC, top row) and low-copy (LC, bottom row) plasmids. Cultures were grown overnight in selective media (or non-selective synthetic media for untransformed strains), then diluted to $OD_{600} = 0.2$. 2 μ L of 5-fold serial dilutions were spotted and growth was assessed on fermentable (YPD, SD-Ura) and non-fermentable (YPG) media after two or three days at 30 °C. Data are representative of three biological replicates.

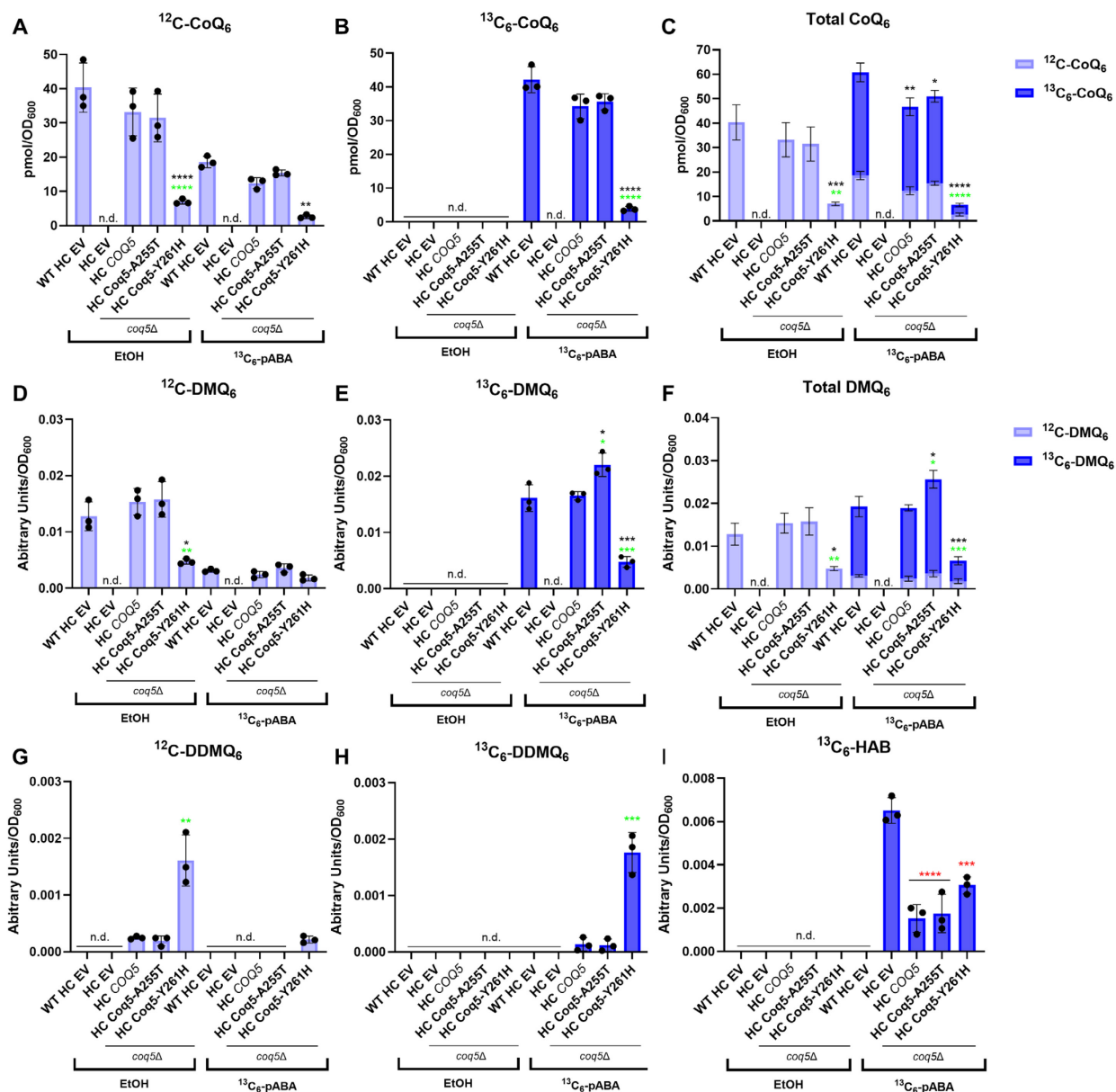


Figure 5. Stable isotope labeling and LC-MS/MS analysis of CoQ and CoQ intermediates in cells expressing high-copy (HC) plasmids. Wild-type yeast transformed with empty vector and *coq5Δ* yeast transformed with the indicated plasmids were labeled at OD₆₀₀ = 0.6 with 8 μg/mL $^{13}\text{C}_6$ -*para*-aminobenzoic acid ($^{13}\text{C}_6$ -pABA) or ethanol as a vehicle control. Lipids were analyzed via LC-MS/MS after 5 hours of labeling. Quantification of ^{12}C -CoQ₆ (A), $^{13}\text{C}_6$ -CoQ₆ (B), total CoQ₆ (C), ^{12}C -DMQ₆ (D), $^{13}\text{C}_6$ -DMQ₆ (E), total DMQ₆ (F), ^{12}C -DDMQ₆ (G), $^{13}\text{C}_6$ -DDMQ₆ (H),

and $^{13}\text{C}_6\text{-HAB}$ (**I**) in the transformants are shown. Total CoQ_6 and DMQ_6 were calculated as the sum of their ^{12}C and $^{13}\text{C}_6$ isotopologues. $^{12}\text{C}\text{-HAB}$ was not quantified due to a co-eluting peak. All data depict mean $\pm$ SD of three biological replicates. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test: black, comparison to wild-type cells expressing the high-copy empty vector (WT HC EV); red, comparison to *coq5* Δ yeast expressing the high-copy empty vector (*coq5* Δ HC EV); green, comparison to *coq5* Δ expressing the high-copy *COQ5* plasmid (HC *COQ5*). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns = not significant. n.d. = peak not detected; n.d. results were excluded from statistical analyses. DMQ_6 , 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinone; DDMQ_6 , 2-methoxy-6-hexaprenyl-1,4-benzoquinone; HAB, 3-hexaprenyl-4-aminobenzoic acid.

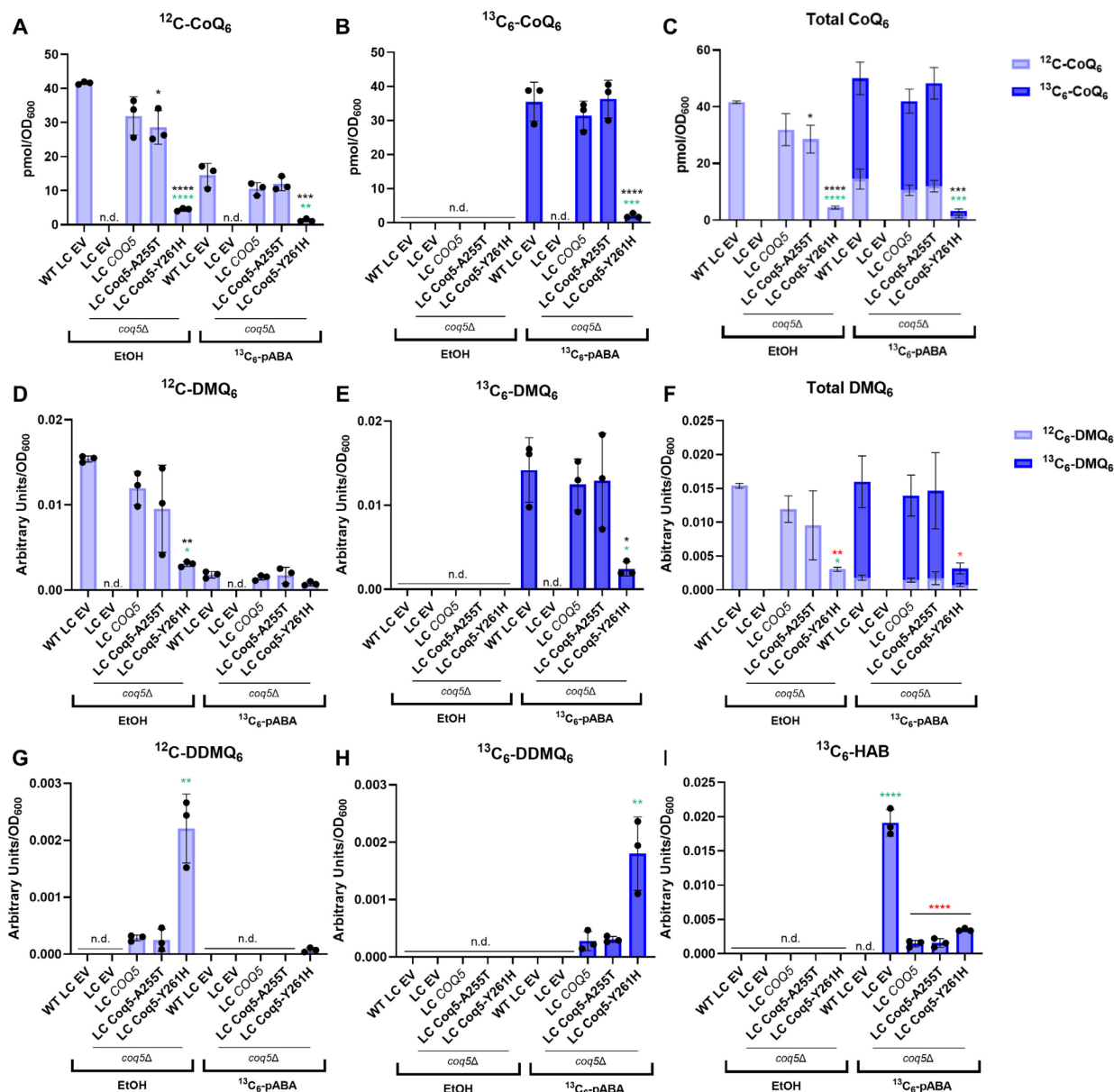


Figure 6. Stable isotope labeling and LC-MS/MS analysis of CoQ and CoQ intermediates in cells expressing low-copy (LC) plasmids. Wild-type yeast transformed with empty vector and *coq5Δ* yeast transformed with the indicated plasmids were labeled with $^{13}\text{C}_6$ -pABA and analyzed as described in Figure 5. Quantification of ^{12}C -CoQ₆ (A), $^{13}\text{C}_6$ -CoQ₆ (B), total CoQ₆ (C), ^{12}C -DMQ₆ (D), $^{13}\text{C}_6$ -DMQ₆ (E), total DMQ₆ (F), ^{12}C -DDMQ₆ (G), $^{13}\text{C}_6$ -DDMQ₆ (H), and $^{13}\text{C}_6$ -HAB (I) in the transformants are shown. All data depict mean $\pm$ SD of three biological replicates.

Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test: black, comparison to wild-type cells expressing the low-copy empty vector (WT LC EV); red, comparison to *coq5Δ* yeast expressing the low-copy empty vector (*coq5Δ* LC EV); green, comparison to *coq5Δ* expressing the low-copy *COQ5* plasmid (LC *COQ5*). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns = not significant. n.d. = peak not detected; n.d. results were excluded from statistical analyses.

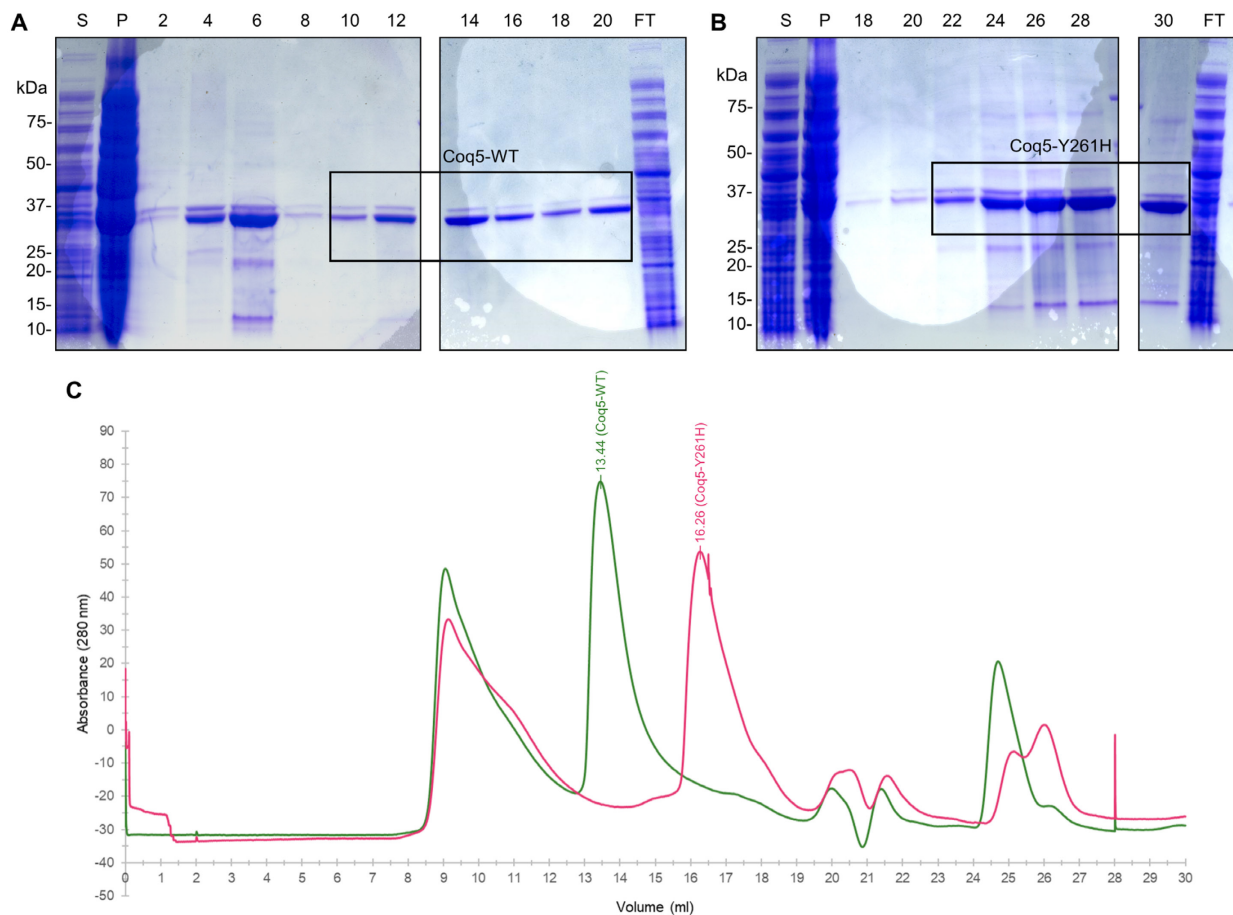


Figure 7. Purification and biophysical characterization of WT and mutant Coq5. (A–B) Coq5-WT and Coq5-Y261H were expressed in *E. coli* and purified from Ni-NTA affinity chromatography. Ni-NTA fractions were analyzed by 4–20% Tris-MOPS SDS-PAGE. Coomassie-stained protein gels of Coq5-WT (**A**) and Coq5-Y261H (**B**) are pictured. S, supernatant; P, pellet; FT, flow-through; E, eluate. Numbers indicate fraction numbers and elution volume (mL). (**C**) Size-exclusion chromatography of pooled and concentrated proteins from Ni-NTA purification of Coq5-WT and Coq5-Y261H. Elution volumes of Coq5-WT (green, ~13.4 mL) and Coq5-Y261H (pink, ~16.3 mL) are consistent with an altered oligomeric state.

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Chapter 5

Perspectives

This work provides an overview of the current functional and structural evidence supporting our understanding of the polypeptides involved in coenzyme Q (CoQ) biosynthesis, with particular focus on the *C*-methyltransferase COQ5 and its homolog in the yeast *Saccharomyces cerevisiae*. The survey begins with the essential role of CoQ in metabolism and the cooperative efforts of the biosynthetic complex termed the CoQ synthome. Subsequent analyses examine the genetic basis of primary CoQ deficiency in humans, highlighting missense single nucleotide variants (SNVs) of potential clinical significance in the genes encoding the core components of the CoQ synthome. We then direct our focus to two functional regions of COQ5—the substrate-binding pocket and the dimer interface—where mutations illuminate mechanistic features. Together, these studies provide new insight into how genetic variation, active-site chemistry, and protein–protein interactions shape CoQ biosynthesis and its pathological disruption.

MACHINE-LEARNING TOOLS FOR VARIANT CLASSIFICATION

The analyses performed in Chapter 2 utilized classical tools such as SIFT (1), PolyPhen-2 (2), and Missense3D (3) to classify missense variants based on sequence conservation and structural features. In particular, structural evidence was interpreted by PolyPhen-2 through annotated functional regions and by Missense3D through local stereochemical features. These mutation classifiers were complementary, and predictions were assessed based on their consensus, which laid the foundation for subsequent biochemical validation. Since then, new machine-learning-based classifiers trained on much larger datasets and incorporating structural context have emerged, including AlphaMissense (4) and PrimateAI-3D (5). In Chapter 3, we incorporated predictions from AlphaMissense for *COQ5* and found that it demonstrated superior performance in identifying deleterious mutations within the substrate-binding pocket compared to our earlier predictions based on consensus of classical tools. A recent study by Rotenberg and colleagues

showed that integrating AlphaMissense with predictors of protein folding stability and residue solvent accessibility greatly improved the classification of deleterious missense variants in p53 (6). Similarly, Tordai and colleagues evaluated AlphaMissense performance across different protein regions, such as membrane-binding, intramembrane, or soluble, and observed a strong correlation with clinical data on SNVs reported in ClinVar (7,8). These results suggest that future analyses of other CoQ synthome components, which are peripherally associated with the inner mitochondrial membrane and interact with hydrophobic CoQ intermediates, would benefit from the incorporation of AlphaMissense.

The release of the AlphaFold Protein Structure Database (9) and the public ColabFold server (10,11) during the writing of Chapter 2 marked a transformative step in the accessibility of high-confidence structural models. With the exception of COQ5 and COQ7–COQ9, no other experimentally determined structures of eukaryotic COQ polypeptides exist (see Chapter 1). Prior studies therefore relied heavily on homology models or *ab initio* tools like Robetta (12–14). AlphaFold excels at single-chain modeling and enables the generation of more accurate models of human COQ polypeptides (15,16). It has also been used to predict novel protein folds (17), which may be applicable to the newly identified yeast Coq11, a polypeptide without a close human homolog (18). In addition, AlphaFold can aid in predicting intrinsically disordered regions (19), which are prevalent across many COQ polypeptides (15).

Beyond single-chain predictions, the development of AlphaFold-Multimer has enabled modeling of protein–protein interactions and multimeric complexes (20). While these predictions remain less reliable than monomeric models, they provide an unprecedented opportunity to investigate assemblies previously inaccessible to structural biology. A notable example is the study by Launay and colleagues, who employed an AlphaFold2-based framework to predict the minimal

functional assembly of the *Escherichia coli* Ubi metabolon, the functional analog of the CoQ synthome (21). This study provides a vital precedent for modeling CoQ biosynthetic complexes using modern machine-learning-based methods. Their results revealed possible arrangements of the soluble complex and provided insights into inter-subunit interfaces. However, this study also highlighted key challenges, including the limited confidence of inter-subunit contacts, difficulty in predicting accurate stoichiometry, and the inability to capture conformational dynamics or assembly states.

Despite its revolutionary impact, AlphaFold has persistent limitations in structural modeling. Firstly, it is insensitive to point mutations (22), which often yield nearly identical predicted protein structures, making it difficult to assess functional effects directly—an issue that is partly mitigated by tools like AlphaMissense. Second, AlphaFold lacks the ability to capture conformational changes or dynamic transitions that are often critical to enzyme function and complex assembly, as AlphaFold2 was trained on experimentally determined structures that are inherently biased toward stable conformations (23). Third, AlphaFold2 and earlier versions were not designed to model complexes containing nucleic acids, small-molecule ligands, ions, or post-translational modifications (23,24). The release of AlphaFold 3, with an updated diffusion-based architecture to jointly predict proteins, DNA, RNA, ligands, ions, and modified residues, mitigates some of these limitations (25,26). Unfortunately, the selection of publicly available ligands is currently limited and does not include prenylquinone intermediates. Finally, AlphaFold predictions do not directly incorporate protein environments, which are important for transmembrane or peripheral membrane proteins (27). While AlphaFold increased the structural coverage of transmembrane proteins, the prediction accuracy was still limited for many protein families (28) and must be treated with caution and in conjunction with transmembrane protein

databases such as the TmAlphaFold database (29). In light of these limitations, researchers using AlphaFold should evaluate complementary approaches in parallel, such as RoseTTAFold All-Atom (RFAA), which supports prediction of structures involving protein–small molecule complexes and assemblies with high fidelity (30). This flexibility makes RoseTTAFold particularly promising for modeling multimeric assemblies and substrate docking scenarios.

While these structure-based tools have significantly advanced the classification of missense variants, they are limited to single-nucleotide substitutions and do not address other variant types that can disrupt protein function. Indels, premature stop codons, and splice site mutations can lead to truncated proteins, altered functional domains, or dysregulated protein expression. To address these gaps, several machine-learning tools have been developed, such as INDELpred (31), MMSplice (32), SplicAI (33), and MAVERICK (34). Incorporating these tools into variant classification pipelines can enhance the interpretation of non-missense variants, providing a more comprehensive understanding of genetic alterations and their potential contributions to disease.

REGULATION OF CoQ AND CoQ INTERMEDIATE REDOX BALANCE

Building on the variant analyses presented above, we next turn to mechanistic studies of CoQ biosynthesis. In our analyses of yeast Coq5 in Chapter 3, it became evident that reduced demethyl-demethoxy-QH₂ (DDMQH₂) is required as a substrate (35). Prior studies similarly demonstrated that Coq3 requires reduced demethyl-QH₂ (DMeQH₂) as a substrate (36). However, since hydroquinones can spontaneously autoxidize, the identity of the oxidoreductase responsible for maintaining these intermediates in the reduced state has not been identified. Recently, human RTN4IP1, which interacts with both COQ3 and COQ5, was proposed to fulfill this role (37). Purified RTN4IP1 was shown to enhance the *O*-methyltransferase activity of purified COQ3 in

the presence of either NADH or NADPH (37). Whether the yeast homolog Yim1 performs the same function, however, remains unclear.

Given the central role of CoQ in mitochondrial electron transport and free radical scavenging (38), regulation of the CoQ redox state is crucial for maintaining cellular homeostasis. Alterations in CoQ redox state can influence the direction of electron flow and the generation of reactive oxygen species (ROS) within the electron transport chain (ETC), as reviewed by Murphy (39) and Scialò and colleagues (40). The redox state of CoQ also contributes to adaptive responses to hypoxia (41) and changes in ETC substrate utilization (42) via complex I, as well as to inflammatory responses mediated by complex II (43,44). These mechanisms are implicated in the pathology of ischemia–reperfusion injury (39,45,46), in which excessive buildup of electrons in the CoQ pool drives ROS production and tissue damage. A recent study by Herrero Martín and collaborators identified a direct link between CoQ production and CoQH₂-mediated reductive stress through the physical association of the CoQ reductase electron-transfer flavoprotein dehydrogenase (ETFDH), the CoQ oxidase complex III, and the CoQ biosynthetic protein COQ2 (47). This complex channels electrons from ETC substrates into the respiratory chain and minimizes electron leakage that leads to ROS production. Through this mechanism, the reductive stress resulting from ETFDH deficiency can be sensed and alleviated by a downregulation of CoQ production, highlighting the role of CoQ in balancing electron flow to prevent both reductive and oxidative stress (47). In yeast, the recently identified atypical short-chain dehydrogenase/reductase, Coq11, was found to associate with the CoQ synthome (18). Deletion of *COQ11* rescues the lipid peroxidation sensitivity of the *coq10Δ* mutant, suggesting that Coq11 may contribute to regulating the CoQ redox state and further reinforcing the connection between CoQ biosynthesis and cellular

redox homeostasis. Whether the redox state of CoQ intermediates can exert similar effects, or whether they are regulated through analogous mechanisms, remains unknown.

Understanding the mechanisms that govern the redox state of CoQ and its biosynthetic intermediates is essential for developing therapeutic strategies targeting CoQ-related disorders. Further research is needed to clarify the functions of proteins such as Yim1 and Coq11 and to explore interventions that can modulate CoQ biosynthesis and redox balance in disease contexts.

CONCLUSION

Together, the studies summarized in this chapter highlight both the progress achieved and the challenges that remain in understanding CoQ biosynthesis. While machine-learning tools are beginning to transform the classification of disease-associated variants, mechanistic insights into how CoQ and its intermediates are regulated remain essential for connecting genotype to phenotype. This work emphasizes the catalytic role of COQ5 and related enzymes and finds implications for redox balance. Despite these advances, many questions in the field of CoQ biology remain unanswered. For example, COQ5 has recently been shown to be toxic to cyanobacteria that possess distant COQ5 homologs but rely on plastoquinone for respiration, thereby raising new questions about substrate selectivity among *C*-methyltransferase homologs (48). As described in Appendix I, yeast Coq5 can also act on a naphthoquinol substrate in addition to its native benzoquinol substrate, DDMQH₂. Appendices II and III extend beyond CoQ biosynthesis, addressing methyltransferase function and mechanistic organic chemistry, respectively. Although distinct in focus, these projects reflect a broader interest in biocatalysis, organocatalysis, and chemical reactivity.

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Appendix I

Yeast Coq5 retains vestigial activity toward naphthoquinol substrates

ABSTRACT

Menaquinone (MK) and ubiquinone (CoQ) are lipid-soluble electron carriers that support anaerobic and aerobic respiration, respectively. A key step in their biosynthesis is the C-methylation of the hydroquinone ring, catalyzed by homologous S-adenosyl-L-methionine (AdoMet)-dependent C-methyltransferases: UbiE/COQ5 in CoQ biosynthesis and MenG in MK biosynthesis. While some bacteria, such as *Escherichia coli*, rely on a single enzyme (UbiE) to act in both pathways, the molecular basis of head group selectivity remains unclear. Here, we combined biochemical assays and molecular docking to probe substrate recognition by *Saccharomyces cerevisiae* Coq5. Remarkably, yeast Coq5 restored MK levels in an *E. coli ubiE* mutant, despite yeast not naturally synthesizing MK. Docking calculations revealed productive binding of the geranylated substrate demethylmenaquinol (DMK₂H₂), stabilized through contacts at the O4' position involving residues E227, R201, and N202. These interactions mirror those observed for benzoquinol-type Coq5 substrates in CoQ biosynthesis. Together, our findings demonstrate that Coq5 retains vestigial activity toward naphthoquinol substrates and identify structural determinants of substrate recognition within the UbiE/MenG C-methyltransferase family. This work provides a framework for understanding how these conserved enzymes distinguish between closely related quinone substrates.

INTRODUCTION

Menaquinone (MK) and coenzyme Q (CoQ, or ubiquinone) are lipid-soluble quinones that function as essential electron carriers in cellular respiration (1,2). MK is the predominant quinone in many prokaryotes and supports anaerobic and facultative respiration, whereas CoQ is broadly distributed across eukaryotes and prokaryotes, where it is required for aerobic respiration (3). Structurally, MK and CoQ differ in the substituents on their head group, with MK having a bulkier naphthoquinone head group and CoQ having a smaller benzoquinone head group. Despite their structural differences, both quinones serve analogous roles in shuttling electrons between primary dehydrogenases and terminal reductases in the respiratory chain (4,5).

The biosynthesis of MK and CoQ proceeds through distinct but related pathways in prokaryotes (Figure 1). A central feature of both molecules is the hydrophobic polyprenyl tail, which anchors the quinone to the membrane. In the yeast *Saccharomyces cerevisiae*, the isoprene units that form this tail are derived from the mevalonate pathway (5), while in *Escherichia coli* they arise from the methylerythritol phosphate pathway (6,7). Tail elongation is catalyzed by *trans*-prenyltransferases IspA and IspB in bacteria and Coq1 in yeast (1,2), which give rise to species-dependent tail lengths—six isoprene units in yeast (forming CoQ₆), and eight isoprene units in *E. coli* (forming CoQ₈ and MK₈). Subsequent attachment of the aromatic quinone headgroup gives rise to early prenylated MK or CoQ intermediates. In particular, the attachment of benzoquinone ring precursors is performed by UbiA in *E. coli* and Coq2 in yeast (1), while the attachment of naphthoquinone ring precursors in *E. coli* is performed by a homologous enzyme named MenA (2). These early intermediates are then further modified by dedicated tailoring enzymes.

Among the head-group-modifying enzymes, UbiE (CoQ C-methyltransferase) and MenG (MK C-methyltransferase) represent a conserved family of *S*-adenosyl-L-methionine (SAM)-

dependent enzymes that install a key *C*-methyl group on the hydroquinone ring, a reaction critical for producing the mature bioactive quinones. In *E. coli*, a single dual-localized enzyme, UbiE, participates in the biosynthesis of both quinones (8), converting demethylmenaquinol (DMKH₂) to menaquinol (MKH₂) and demethyl-demethoxy-QH₂ (DDMQH₂) to demethoxy-QH₂ (DMQH₂; Figure 1). While bacterial UbiE enzymes are clearly bifunctional, it remains unclear whether eukaryotic homologs such as the mitochondrially targeted COQ5 enzymes (9–11) have retained the ability to act on naphthoquinol substrates, or whether this activity was lost during evolution. Moreover, the molecular features that determine substrate specificity in UbiE/MenG homologs are not well understood.

In this work, we employed a combination of biochemical and computational approaches to uncover an unexpected vestigial activity of Coq5, a CoQ-synthesizing higher eukaryotic homolog of UbiE, towards naphthoquinol substrates. Expression of yeast Coq5 restores the levels of MK₈ in an *E. coli ubiE* mutant, highlighting functional conservation between UbiE homologs. Using molecular docking, we identify a hypothesized pre-reactive binding mode of DMKH₂ in yeast Coq5 consistent with residues implicated in the binding and catalysis of the benzoquinol substrate DDMQH₂. These findings highlight the evolutionary conservation of hydroquinone *C*-methyltransferases and provide new insights into the structural basis of substrate promiscuity.

MATERIALS AND METHODS

Bacterial strains and growth medium

Wild-type and *ubiE-401* *E. coli* strains used in this study are in the HW272 and AN70 genetic backgrounds, respectively (Table 1). Growth media were prepared as previously described (8) and included Difco™ Bacto™ brain heart infusion (BHI) medium (Becton Dickinson) or succinate minimal medium (SMM), with selection conferred by the addition of 100 µg/mL of

ampicillin where applicable. Succinate minimal medium was prepared as in (12). Briefly, the following sterile solutions were combined without autoclaving and adjusted to a volume of 1 L: 50 mL 20X phosphates and $(\text{NH}_4)_2\text{SO}_4$ solution, 20 mL 25% sodium succinate solution, 10 mL trace elements solution, 2 mL 8% MgSO_4 solution, 1 mL 1% $\text{Ca}(\text{NO}_3)_2$ solution or molar equivalent of another calcium salt, 0.5 mL 1% thiamine solution, 50 mL of a 4% solution of the complete amino acids mix. The 20X phosphates and $(\text{NH}_4)_2\text{SO}_4$ solution was autoclaved and consisted of 86.92 g/L anhydrous Na_2HPO_4 , 54 g/L KH_2PO_4 , and 20 g/L $(\text{NH}_4)_2\text{SO}_4$. The trace elements solution consists of 5 g/L EDTA, 0.5 g/L FeCl_3 , 0.05 g/L ZnO , 0.01 g/L CuCl_2 , 0.01 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01 g/L of H_3BO_3 and is adjusted to pH 7 before autoclaving. The complete amino acids mix contained 0.8 g adenine, 0.4 g Arg $\cdot$ HCl, 1 g Asp, 0.8 g Cys $\cdot$ HCl, 1g glutamic acid, 0.8 g His $\cdot$ HCl, 0.6 g Ile, 1.2 g Leu, 0.6 g Lys $\cdot$ HCl, 0.8 g Met, 0.6 g Phe, 4 g Ser, 2 g Thr, 0.8 g Trp, 0.4 g Tyr, 0.8 g Uracil, 1.5 g Val. The resulting complete amino acids solution was autoclaved. All other solutions were individually filter-sterilized. Plate medium contained 2% bacto-agar.

Bacterial transformation

All plasmids used in this study are described in Table 2. To generate competent AN70 bacteria, 25 mL cultures were grown in BHI media until $\text{OD}_{600} \sim 0.4$ and chilled on ice for 20–30 min. Cells were harvested by centrifugation at $1900 \times g$ for 10 min at 4 °C. Pellets were resuspended in 25 mL of pre-chilled 10 mM CaCl_2 , incubated on ice for 30 min, then centrifuged again at $1900 \times g$ for 10 min at 4 °C. The supernatant was discarded and pellets were resuspended in 3.8 mL of pre-chilled 100 mM CaCl_2 with 15% glycerol. The resuspension was aliquoted into pre-chilled microcentrifuge tubes, flash frozen with liquid nitrogen, and stored at -80°C for future use.

On the day of transformation, 50 μ L of competent AN70 bacteria were thawed on ice and gently mixed with 50 ng of plasmid DNA and incubated on ice for 30 min on ice followed by a 30-s heat shock at 42 °C and a 15-min recovery period on ice. Bacteria were allowed to recover in SOC Outgrowth medium (New England Biolabs) for 1 h before plating onto Luria-Bertani plate medium (Fisher Scientific) containing 100 μ g/mL ampicillin. Bacteria were kept on BHI plate medium at 4 °C up to a month with periodic verification of respiratory phenotype on SMM plate medium.

Stable isotope labeling and analysis of CoQ and CoQ intermediates

Stable isotope labeling was performed with modifications to previously described protocols (13). Parental or transformed bacterial cells were cultured overnight in 5 mL of BHI media (with 100 μ g/mL ampicillin for transformed cells) at 30 °C with shaking. The following day, pre-cultures were sub-inoculated to $OD_{600} = 0.2$ in 5 mL of fresh media. For analysis of *de novo* CoQ biosynthesis, cultures were treated with either 10 μ g/mL (69.4 μ M) $^{13}C_6$ -4HB (Cambridge Isotope Laboratories) or ethanol as a vehicle control, with a final ethanol concentration of 0.2% (v/v). Cultures were grown at 37 °C with shaking for 4 h to a final OD_{600} of 2–4. For each culture, 2 OD_{600} units of cells (as determined by $OD_{600} \times$ volume in mL) were harvested by centrifugation at $4000 \times g$ for 5 min. Cell pellets were stored in –20 °C until use.

To prepare lipid extracts, cell pellets were thawed on ice, washed in 500 μ L of PBS, and then resuspended in 100 μ L of fresh PBS. 1.2 mL of methanol was added to each resuspension, and cells were lysed by vortexing for 30 s. 100 pmol of a deuterated internal standard, d_9 -CoQ₁₀ (dimethoxy- d_6 , methyl- d_3 ; Sigma-Aldrich), was added to each sample (roughly 10 pmol per injection). Lipids were then extracted twice, each time with 1.8 mL of petroleum ether and 30 s vortexing. A standard curve containing ~0.4 pmol to ~250 pmol of CoQ₉ (Santa Cruz

Biotechnologies) and the same amount of d₉-CoQ₁₀ per sample was resuspended and extracted alongside the experimental samples. Extracted lipids in petroleum ether were dried under N₂ gas and stored at −20 °C.

On the day of analysis, lipids were reconstituted in 200 µL of ethanol containing 1 mg/mL benzoquinone. LC-MS/MS analyses were modified from (13,14). Briefly, 20 µL aliquots of each sample were injected into a Luna PFP(2) Å column (100 × 4.6 mm, 5 µm; Phenomenex) connected to a 4000 QTRAP linear MS/MS spectrometer (Applied Biosystems). The mobile phase consisted of 90% solvent A (methanol:isopropanol, 95:5, 2.5 mM ammonium formate) and 10% solvent B (isopropanol, 2.5 mM ammonium formate). For separation of prenylquinone intermediates, 0.5 min post-injection, the percentage of solvent B increased linearly from 10% to 20% over 5 min, held constant for the next 1.5 min, then decreased linearly back to 10% over the next 0.5 min. The column was re-equilibrated at 10% solvent B for 3 min between each injection. A constant flow rate of 1 mL/min was used. All samples were analyzed in multiple reaction monitoring mode. Precursor-to-product ion transitions are listed in Table 3. Data acquisition and processing was performed using Analyst version 1.4.2 (AB Sciex, Framingham, MA, USA). For each analyte, the relative amount was calculated by summing the peak area of the protonated species and that of its corresponding ammonium adduct, where applicable, corrected for extraction efficiency by the amount of internal standard recovered, then normalized by OD₆₀₀ unit. Relative levels of analytes calculated by this method were reported as Arbitrary Units/OD₆₀₀. The CoQ₉ standard curve was used to verify that analytes were within the linear range of detection of the instrument. One-way ANOVA with Tukey's HSD test was used to compare each strain with every other strain within a treatment group. Where only two strains were present within a treatment group, an unpaired *t*-test was used. Strains that produced no signal above background in any biological replicate were

labeled as ‘n.d.’ (not detected) and excluded from statistical analyses. All statistical analyses and data presentation were performed using GraphPad Prism version 10.4.1 (GraphPad Software, Boston, MA, USA).

Density functional theory calculations

Density functional theory (DFT) calculations were performed using Gaussian 16, Revision A.03 (Gaussian Inc., Wallingford, CT, USA) (15). The geranylated substrate analog, DMK₂H₂, was modeled in GaussView 6.0.16 (Semichem Inc., Shawnee Mission, KS, USA) (16) and optimized using the B3LYP functional (17–20), augmented with Grimme’s D3 empirical dispersion correction (21), and the 6-311+G(d,p) basis set. Solvation effects were included in both optimization and single-point calculations using the CPCM solvation model (22,23) with diethyl ether as the solvent. All atoms were allowed to relax freely during geometry optimizations. Normal vibrational mode analysis was used to confirm the identity of optimized structures as minima (zero imaginary frequencies).

Substrate docking

All docking calculations were performed using AutoDock Vina version 1.1.2 (The Scripps Research Institute, La Jolla, CA, USA) (24). Chain A of the crystal structure of yeast Coq5 in complex with AdoMet (PDB: 4OBW) was used as the target of docking. All waters and other co-crystallized ligands were removed. Polar hydrogens were added using AutoDockTools version 1.5.7 (The Scripps Research Institute, La Jolla, CA, USA) (25) with default settings. The grid box of size 20 Å × 20 Å × 20 Å contained the entire active-site pocket. The optimized structure of DMK₂H₂ was docked to the active site. All rotatable single bonds in DMK₂H₂, as well as the side chains of the following residues lining the active site, were treated as flexible and allowed to rotate freely during docking: R77, Y78, M81, N82, F198, R201, N202, F228, Y263, L264. The

calculation was repeated where E227 was added to the flexible region, but its inclusion did not alter the docking results. This process was repeated three times, and 40 binding poses were generated. The lowest-energy binding mode had an estimated binding affinity of -8.5 kcal/mol as calculated by AutoDock Vina. A two-membered cluster representing a binding intermediate along the substrate-entrance tunnel (-8.1 kcal/mol) and a three-membered cluster in which the hydroquinone head group is rotated 180° (-7.7 kcal/mol) were also observed. All other poses placed the reactive atoms >5 Å apart and were discarded.

RESULTS

Yeast Coq5 restores menaquinone levels in the *E. coli ubiE* mutant

To date, no *in vitro* activity assay exists for purified UbiE/MenG enzymes. To investigate the catalytic activity of yeast Coq5 when expressed in *E. coli*, we introduced either the native *E. coli ubiE* gene or a recombinant gene encoding mature yeast Coq5 (*yCOQ5*) into strain AN70, an *E. coli* mutant defective in the C-methyltransferase step of CoQ and MK biosynthesis. AN70 carries the UbiE^{G142D} mutation (8), which alters a conserved region immediately following methyltransferase motif II and adjacent to several putative substrate-binding residues (26,27). Although UbiE protein levels in AN70 could not be assessed due to the lack of an antibody, the corresponding mutation in yeast Coq5 (*coq5-2*, G199D) was previously shown to yield a stable but catalytically inactive protein (28).

We adapted a previously described LC-MS/MS assay for *de novo* CoQ₈ biosynthesis to simultaneously measure both CoQ₈ and MK₈ production in bacteria. Cells were labeled with ¹³C₆-4HB, which is prenylated by *E. coli* UbiA (29) and decarboxylated by UbiD and its binding partner, the flavin prenyltransferase UbiX (30) to form ¹³C₆-2-octaprenylphenol (¹³C₆-OP), the precursor to all head-group modification steps carried out by the Ubi metabolon (Figure 1).

Subsequent reactions yield hydroxylated late-stage intermediates such as $^{13}\text{C}_6\text{-DDMQ}_8\text{H}_2$, the direct substrate of both UbiE and Coq5. In mutants defective in this step, we expected to observe the accumulation of the diagnostic intermediates $^{12}\text{C-DDMQ}_8$ and $^{13}\text{C}_6\text{-DDMQ}_8$, corresponding to the oxidized quinone form of the substrate. Indeed, the UbiE^{G142D} mutant lacked detectable $^{12}\text{C-CoQ}_8$ and $^{13}\text{C}_6\text{-CoQ}_8$ (Figure 2A, B) and accumulated *de novo*-synthesized $^{13}\text{C}_6\text{-DDMQ}_8$ when labeled (Figure 2D) and $^{12}\text{C-DDMQ}_8$ when unlabeled (Figure 2E). Expression of either *ubiE* or *yCOQ5* restored CoQ₈ biosynthesis (Figure 2A, B) and reduced DDMQ₈ accumulation (Figure 2D, E). The early intermediate OP was also quantified, but no significant difference was observed between the UbiE^{G142D} mutant and rescued strains (Figure 2G, H). Interestingly, the wild-type bacteria produced the highest amounts of OP, though the significance of this finding under the tested conditions remains unclear.

Because *E. coli* UbiE also catalyzes the C-methylation of DMK₈H₂, we asked whether yeast Coq5 could rescue MK₈ biosynthesis in the UbiE^{G142D} mutant. As DMK₈H₂ is a bulkier substrate than DDMQ₈H₂, it was possible that organisms lacking menaquinone had lost this bifunctionality. However, mutants expressing either *ubiE* or *yCOQ5* were able to synthesize MK₈, demonstrating that yeast Coq5 can recognize and methylate naphthoquinol substrates (Figure 2C). Notably, our plasmid encoding *yCOQ5* uses an inducible *lac* promoter (28). Our results demonstrated that basal expression of *yCOQ5* in the absence of an inducer was sufficient to restore MK₈ levels to that of the HW272 wild-type strain. Furthermore, expression of *yCOQ5* depleted the levels of DMK₈ (Figure 2F), indicating efficient catalysis. As MK₈ biosynthesis does not depend on 4HB, no differences were expected between labeled and unlabeled samples.

Molecular docking identifies potential binding modes of DMK₂H₂

To probe the molecular basis of this unexpected activity, we performed docking analyses of a geranylated substrate analog, DMK₂H₂, into the substrate-binding pocket of the AdoMet-bound yeast Coq5 crystal structure (PDB: 4OBW) using AutoDock Vina. Three binding modes were identified, with the lowest-energy binding mode exhibiting a predicted binding affinity of −8.5 kcal/mol (Figure 3A). In agreement with our analyses on the binding of DDMQ₂H₂ outlined in Chapter 3 of this dissertation, the putative catalytic base E227 is positioned adjacent to the O4' hydroxyl group of DMK₂H₂, with R201 forming both a salt bridge with E227 and a hydrogen bond with O4'. Two phenylalanine residues, F198 and F228, lie deep inside the substrate-binding pocket and may engage in π – π stacking with the annulated aromatic ring of DMK₂H₂. The AdoMet methyl carbon and C5 carbon of DMK₂H₂ are positioned within 3.8 Å of each other, suggesting a pre-reactive conformation.

Two alternative binding modes were also identified (Figure 3B, C). The first (−7.7 kcal/mol) resembles a rotated pose observed in previous docking and molecular dynamics of the DDMQ₂H₂ substrate (Chapter 3), with O4' instead forming a hydrogen bond with N82 on the opposite side of the active site. The second alternative binding mode (−8.1 kcal/mol) highlights a role for N202, previously implicated in DDMQ₂H₂ binding (26). Overall, these docking results mirror the binding modes observed for DDMQ₂H₂ and indicate that the Coq5 substrate-binding pocket can accommodate the larger naphthoquinol substrate.

DISCUSSION

We have established that yeast Coq5 can catalyze the methylation of both benzoquinol and naphthoquinol substrates, restoring CoQ₈ and MK₈ levels in an *E. coli ubiE* mutant. Consistent with prior reports that secondary quinones are always detected under both aerobic and anaerobic

conditions (31), bacterial cultures in our study synthesized both CoQ₈ and MK₈ simultaneously. These results suggest that yeast Coq5 can scavenge substrates in *E. coli*, either by associating with the membrane, as proposed for UbiE/MenG during menaquinone biosynthesis (2), or by interacting with the soluble Ubi metabolon (32). In particular, two metabolon components, UbiJ and UbiK, form a subcomplex thought to sequester highly hydrophobic CoQ intermediates and present their head groups to Ubi enzymes within the metabolon (1,32,33). This raises the possibility that the structural and sequence conservation between Coq5 and UbiE may allow Coq5 to participate in similar interactions. However, how cells coordinate the distribution of UbiE to both biosynthetic machineries remains unknown.

Stutts and colleagues recently surveyed the UbiE/MenG benzoquinol/naphthoquinol C-methyltransferase homologs and revealed their phylogenetic segregation into two clades: a bifunctional, mitochondrially targeted clade that acts on both benzoquinol and naphthoquinol substrates, and a monofunctional, plastid-targeted clade that only acts on naphthoquinol substrates (34). They showed that expression of a bifunctional mitochondrial enzyme in plastids or cyanobacteria that do not naturally synthesize CoQ can be cytotoxic. These findings suggest that bifunctionality is tightly regulated during evolution. However, the residues that control substrate specificity in the UbiE/MenG homologs remain undefined. Examination of conserved substrate-binding residues, including those identified in previous studies (26,27) and in Chapter 3 of this dissertation, revealed no clear pattern distinguishing mono- from bifunctional enzymes. While binding affinities calculated by molecular docking suggested a slight preference for DDMQH₂, these numbers are only predictive. Furthermore, our biochemical studies could not reveal the *in vivo* substrate preference of UbiE versus Coq5 due to the lack of commercially available lipid standards for CoQ₈ and MK₈, which prevented absolute quantification of these quinones. Future

work should focus on mutagenesis studies and molecular dynamics simulations, which may help elucidate the molecular mechanism of substrate specificity.

Our observation that yeast Coq5 can bind to and methylate DMK₈H₂ is consistent with recent findings by Basset and coworkers, who showed that several eukaryotic CoQ-synthesizing COQ5 homologs, including yeast Coq5 and human COQ5, restore MK₈ production in an *E. coli* $\Delta ubiE/MenG$ mutant (34). The evolutionary basis of this vestigial substrate promiscuity and whether it holds physiological relevance remain unclear. In humans, menaquinone (vitamin K₂) is obtained from the diet and gut microbiota and plays essential roles in blood clotting and bone metabolism (35–37). In *E. coli*, DMK interacts with several proteins implicated in respiration, such as the nitrate reductase A (NarGHI) (38) and the ArcAB two-component system that controls aerobic–anaerobic transitions (39,40), highlighting the central role of UbiE in metabolism. However, whether dietary or microbiome-derived DMK is absorbed at sufficient levels in humans to interact with human COQ5 or influence metabolism has not been investigated.

In summary, our results demonstrate that yeast Coq5 retains the bifunctionality characteristic of prokaryotic UbiE enzymes, despite its native role being limited to CoQ biosynthesis. By rescuing both CoQ₈ and MK₈ production in an *E. coli ubiE* mutant and accommodating DMK₈H₂ in docking analyses, yeast Coq5 exhibits vestigial substrate promiscuity that may be conserved across eukaryotic homologs. These findings highlight the evolutionary conservation of benzoquinol/naphthoquinol C-methyltransferases and suggest that bifunctionality may persist as a latent property even when no longer required *in vivo*. Future work dissecting the structural determinants of substrate selectivity will be essential to clarify the evolutionary pressures that shaped this division of labor and to assess whether such promiscuity has physiological consequences in eukaryotes.

Table 1. Genotype and source of bacterial strains.

<i>Strain</i>	Genotype	Source
HW272	<i>ubiG</i> ⁺ <i>zei</i> ::Tn10dTet	(41)
AN70	<i>Hfr</i> , <i>metB</i> ⁻ , <i>Str</i> ^R , <i>ubiE</i> -401 (UbiE ^{G142D})	(42)

Table 2. Plasmid constructs used in this study.

<i>Plasmid</i>	Construction Description	Source
pUE6	<i>E. coli</i> JC7623 <i>ubiE</i> open reading frame (ORF) of approximately 756 bp inserted into the MluI site of pQM1, antisense to the yeast <i>CYC1</i> promoter	(8)
pTHQ5-1	Wildtype yeast <i>COQ5</i> ORF segment (+91 to +924) containing the mature Coq5 polypeptide inserted into the BglII site of the pTrcHisB backbone (Invitrogen) for expression in <i>E. coli</i>	(28)

Table 3. Precursor-to-product ion transitions used in this study.

<i>Analyte</i>	<i>m/z</i> [M+H] ⁺	<i>m/z</i> [M+NH ₄] ⁺
OP	639.6/107.1	656.6/107.1
¹³ C ₆ -OP	645.6/113.1	662.6/113.1
DDMQ ₈	683.5/153.1	700.6/153.1
¹³ C ₆ -DDMQ ₈	689.5/159.1	706.6/159.1
CoQ ₈	727.6/197.1	744.6/197.1
¹³ C ₆ -CoQ ₈	733.6/203.1	750.6/203.1
CoQ ₉	795.6/197.1	812.7/197.1
MK ₈	717.6/187.1	734.6/187.1
DMK ₈	703.6/173.1	720.6/173.1
d ₉ -CoQ ₁₀	872.8/206.1	889.8/206.1

FIGURES

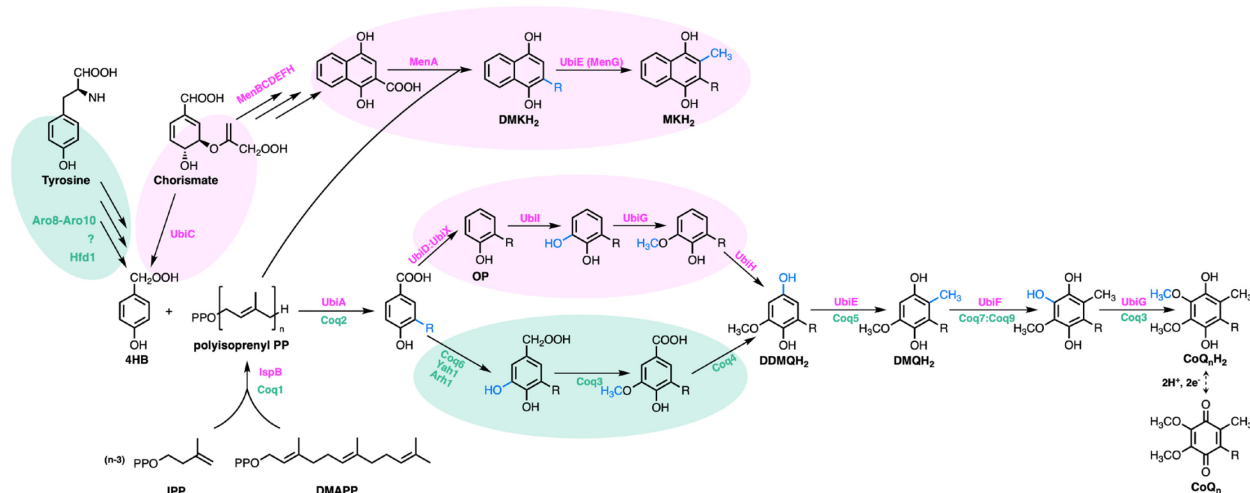


Figure 1. Overview of the CoQ biosynthetic pathway in *S. cerevisiae* and *E. coli*. The prenylquinone biosynthetic pathways in the yeast *S. cerevisiae* (green) and *E. coli* (magenta) share many steps. 4-hydroxybenzoic acid (4HB) is the common aromatic CoQ precursor in yeast and humans, while yeast can additionally utilize *para*-aminobenzoic acid (*p*ABA) (13,43,44). Shown here is the canonical sequence of reactions in yeast and *E. coli*. Intermediates relevant to this study are labeled. IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; DMK₈H₂, demethylmenaquinol-8; MKH₂, menaquinol-8; OP, 2-octaprenylphenol; DDMQH₂, demethyl-demethoxy-ubiquinol; DMQH₂, demethoxy-ubiquinol. Adapted from Pierrel *et al.* (1).

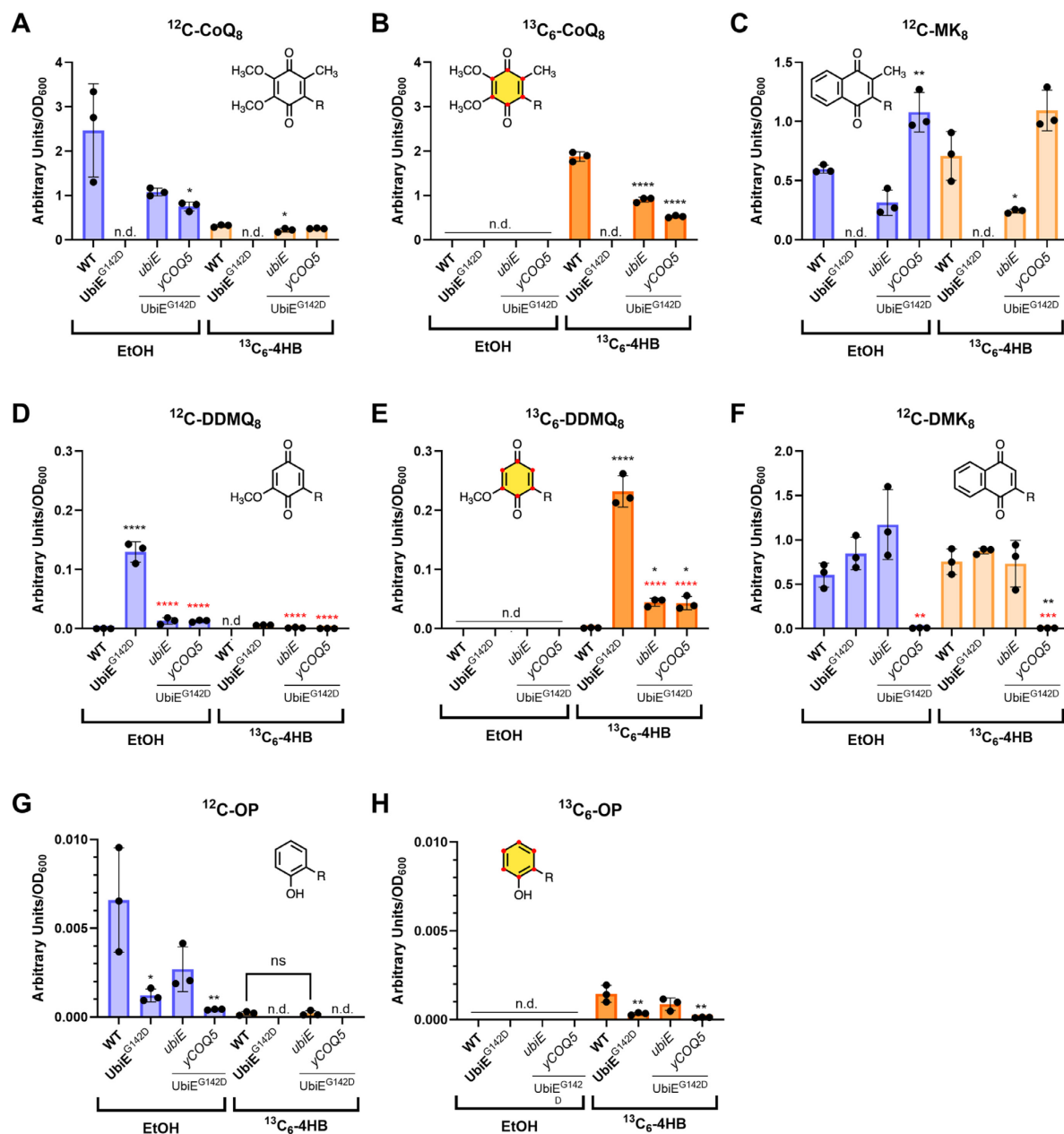


Figure 2. Yeast Coq5 retains the ability to synthesize menaquinol. Wild-type bacteria or bacteria expressing an inactive form of UbiE (UbiE^{G142D}), with or without the indicated plasmids, were labeled at OD₆₀₀ = 0.2 with 10 $\mu\text{g/mL}$ $^{13}\text{C}_6$ -4-hydroxybenzoic acid ($^{13}\text{C}_6$ -4HB) or ethanol as a vehicle control. *ubiE* and *yCOQ5* are plasmids encoding E. coli UbiE polypeptide and recombinant mature yeast Coq5, respectively (see Table 2). Lipids were analyzed via LC-MS/MS after 4 h of labeling. (A–H) Quantification of ^{12}C -CoQ₈ (A), $^{13}\text{C}_6$ -CoQ₈ (B), ^{12}C -MK₈ (C), ^{12}C -

DDMQ₈ (**D**), ¹³C₆-DDMQ₈ (**E**), ¹²C-DMK₈ (**F**), ¹²C-OP (**G**), and ¹³C₆-OP (**H**) in the strains are depicted. MK₈ and DMK₈ are not derived from 4HB and thus ¹³C₆-labeled menaquinone intermediates were not quantified. All data depict mean ± SD of three biological replicates. Statistical significance with respect to various controls within a treatment group was determined by one-way ANOVA with Tukey's HSD test, except for the quantification of ¹²C-OP in ¹³C₆-4HB-labeled cells in (**F**), which was analyzed with an unpaired *t*-test. Black and red asterisks denote comparisons to wild-type bacteria (WT) or bacteria expressing UbiE^{G142D}, respectively. **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001, *****p* ≤ 0.0001, and ns = not significant. n.d. = peak not detected; n.d. results were excluded from statistical analyses. See Materials and Methods for details. DDMQ₈, 2-methoxy-6-octaprenyl-1,4-benzoquinone; DMK₈, demethylmenaquinone-8; OP, 2-octaprenylphenol.

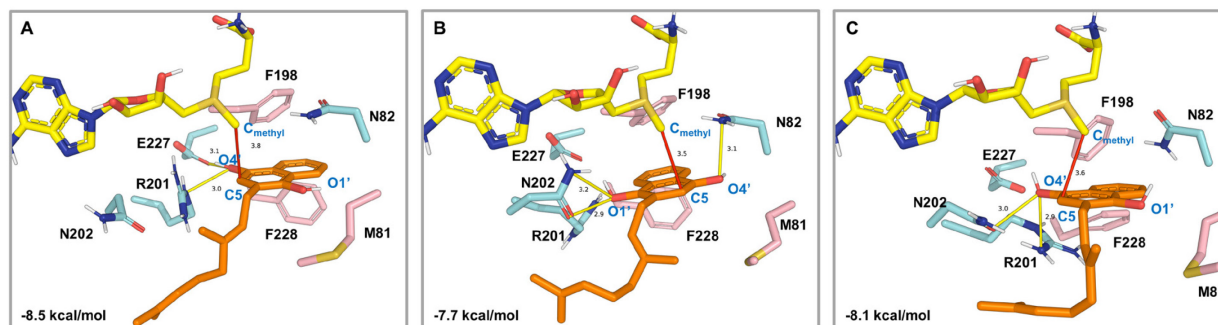


Figure 3. Molecular docking reveals potential binding modes of DMK₂H₂. The geranylated substrate analog, DMK₂H₂, was docked into chain A of the AdoMet-bound crystal structure of yeast Coq5 (PDB: 4OBW) using AutoDock Vina. Representative lowest-energy poses from three clusters where the naphthoquinol head group of DMK₂H₂ (orange) is in close proximity to the methyl group of AdoMet (yellow) are shown along with their estimated binding affinities. (A) The top-ranked pose in which the O4' hydroxyl is positioned next to E227 is pictured. (B) An alternative pose in which the head group is rotated relative to the top-ranked pose is shown. The O4' atom faces away from polar residues E227, R201, and N202. (C) Lowest-energy pose from a cluster likely representing an intermediate along the substrate entry pathway is shown. The head group is less buried in the active site and O4' does not interact with E227. The distance between the reactive atoms (the AdoMet methyl carbon and C5 of DMK₂H₂) is shown in red. Polar contacts within 3.5 Å between side chains and the substrate are shown in yellow. All distances (in Ångströms) are measured between heavy atoms. Atom labels are shown in blue. Polar and nonpolar residues are colored in light blue and pink, respectively. Nonpolar hydrogens are omitted for clarity.

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Appendix II

Computational characterization of hydrogen bonding interactions in methylated arginine residues

ABSTRACT

Arginine residues are post-translationally modified by protein arginine methyltransferases (PRMTs) to form monomethylarginine (MMA), symmetric dimethylarginine (SDMA), or asymmetric dimethylarginine (ADMA). These post-translational modifications (PTMs), thought to be largely irreversible, regulate gene expression and protein interactions and are involved in diverse cellular processes, including epigenetic regulation and mRNA splicing. Yet, their molecular consequences remain poorly understood. Here, we investigate how methylation alters the interaction potential of the guanidinium group, with a focus on noncanonical $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ hydrogen bond-like interactions. Using density functional theory (DFT)-optimized models alongside analyses of experimentally determined protein structures, we characterize the energetics and prevalence of these interactions, finding that they are weaker and longer than conventional $\text{N}-\text{H}\cdots\text{O}$ hydrogen bonds but favored under constrained geometries, potentially contributing to molecular recognition. Finally, a case study of co-crystal structures of the histone H3R2 reader WDR5 bound to arginine in multiple methylation states demonstrates how local binding site flexibility accommodates these contacts, providing a molecular basis for the functional consequences of arginine methylation. These findings support the notion that methylation enables novel contacts rather than simply disrupting hydrogen bonding, offering a rationale for the biological commitment of this PTM in the absence of confirmed demethylases.

INTRODUCTION

Arginine methylation is a widespread post-translational modification (PTM) (1) found in both histone (2) and non-histone proteins (3–5), catalyzed by a family of enzymes known as protein arginine methyltransferases (PRMTs). All members of the PRMT family transfer a methyl group from *S*-adenosyl-L-methionine (AdoMet) to arginine residues to form monomethylarginine (MMA) (2). A second methyl group can then be added either asymmetrically (ADMA) by type I PRMTs (PRMTs 1–4, PRMT 6, PRMT 8) or symmetrically (SDMA) via type II PRMTs (PRMT 5 and PRMT 9) (6). Arginine methylation plays critical roles in the regulation of gene expression (7,8), RNA splicing (4,5,7), protein synthesis and degradation (9), and cellular processes such as proliferation (10,11) and oncogenesis (12). Dysregulation of this modification has been implicated in a range of pathologies, including cancer, cardiovascular disease, and neurological disorders (4,5). Despite its clear biological importance, the structural and molecular consequences of arginine methylation remain poorly understood.

Historically, arginine methylation was regarded as an irreversible modification. Although enzymes such as the Jumonji domain-containing protein JMJD6 (13–15) and peptidyl arginine deiminase 4 (PAD4) (16,17) have been proposed as potential demethylases, evidence on the existence of arginine demethylases remains inconclusive, and removal is generally thought to occur through protein degradation. It is therefore curious what structural advantages or persistent interactions are conferred by methylation to justify this biochemical commitment.

At the molecular level, arginine methylation alters the physical properties of the guanidinium group, increasing its size and altering its hydrophobicity, electrostatic potential, and hydrogen bonding capacity. Addition of one or more bulky hydrophobic methyl groups can modulate local packing and electrostatic interactions by increasing van der Waals contacts or

introducing steric repulsion. Nevertheless, our knowledge of molecular consequences of arginine methylation remains limited due to the lack of direct structural comparisons across different methylation states. Functionally, arginine methylation is known to recruit readers of methylarginine marks (18), modulate protein stability via degron activation or inactivation (9), and influence hydrogen bonding interactions with nucleic acids (19). Despite this functional importance, a comprehensive molecular picture of how methylation modifies interactions with the guanidinium group remains lacking. Methylarginine interactions have been studied extensively in the context of protein design (20,21). In biological systems, however, most attention has centered on arginine methylation's ability to participate in cation- π and π - π interactions, such as those recognized by methylarginine reader domains like Tudor domains (22,23). These interactions are further stabilized by the strong hydrophobic contribution that arises from desolvation of the charged guanidinium group. By contrast, in the context of hydrogen bonding, arginine is primarily seen as a disruptor of hydrogen bonds (24,25), rather than as a potential means of enabling new interactions.

An underexplored molecular consequence is that arginine methylation may allow the formation of $N^+-C-H\cdots O$ hydrogen bond-like interactions. Highly polarized methyl groups may carry partial positive charge and behave as unconventional hydrogen bond donors, as has been demonstrated in the positively charged trimethyllysine (26,27) and the sulfonium methyl group of AdoMet (28,29). Emerging evidence in small molecule systems and computational studies suggests that *N*-methyl groups can engage weak but directional interactions stronger than $C-H-\pi$ contacts (30,31). However, the potential for the delocalized positive charge of the guanidinium group to participate in such interactions remains uncharacterized. Although weaker than canonical hydrogen bonds, these interactions may nevertheless influence methylarginine behavior,

particularly in structurally constrained environments such as protein binding sites. We therefore hypothesize that arginine methylation enables $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ hydrogen bonds inaccessible to unmethylated arginine, which may underlie its distinctive physiological roles.

In this work, we combine density functional theory (DFT) calculations on simplified donor–acceptor complexes with analyses of protein crystal structures to systematically examine the interaction modes of methylated arginine residues. We quantify the energetics of guanidinium N–H donors and $\text{N}^+-\text{C}-\text{H}$ donors, identifying conditions under which $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions become favorable. Although these interactions are weaker and longer than canonical hydrogen bonds, their frequent occurrence and well-defined geometries in protein structures suggest physiological importance. Finally, by analyzing co-crystal structures of the histone H3R2 methylarginine mark reader WDR5 bound to arginine in different methylation states, we show how binding site adaptability and local energetics enable these interactions, providing a molecular framework for understanding the functional consequences of arginine methylation.

COMPUTATIONAL METHODS

Density functional theory-optimized donor and acceptor pairs

Hydrogen bond donors and acceptors were modeled in GaussView 6.0.16 (Semichem Inc., Shawnee Mission, KS, USA) (32). Donors included unmethylated arginine, MMA, ADMA, and SDMA, truncated to a guanidinium head group plus a methyl group in place of $\text{C}\delta$ (Figure 1A). Acceptor residues were modeled as acetate (Asp/Glu), ethanol (Ser/Thr), *p*-methylphenol (Tyr), *N*-methylacetamide (backbone amide) (Figure 1B). Protonation states at physiological pH were used for all calculations. Donor–acceptor complexes were constructed by manual conformational search and subsequent energy minimization. Geometry optimizations, frequency and single-point energy calculations were performed using Gaussian 16, Revision A.03 (Gaussian Inc.,

Wallingford, CT, USA) (33) using the global-meta hybrid-GGA functional M06-2X (34) and split-valence triple-zeta 6-311+G(d,p) (35–38) basis set. Implicit solvation was modeled using the SMD polarizable continuum model (39) for water, diethyl ether, or *N,N*-dimethylformamide (DMF) to approximate polar, nonpolar, and moderately polar environments. Pairwise complexes were classified by donor and acceptor identity, as well as interaction mode (N–H vs. N⁺–C–H). Complexes with the largest interaction energy within each category were used as references for identifying hydrogen bonding in protein structures (see *Interaction energy analysis of experimentally determined protein structures*). A table of DFT-optimized complexes, calculated interaction energies, and interaction distances can be found in Supplementary File 6.

Electrostatic potential maps

Cube files were generated from optimized donor and acceptor structures using Gaussian 16. Electrostatic potential (ESP) maps were visualized using GaussView 6.1.1. ESP values of arginine and its methylated analogues in diethyl ether were extracted with Multiwfn version 3.8 (40,41). Equivalent points were identified by defining vectors along the N_η–H_η bond for N–H donors or the C_θ–H_θ bond for N⁺–C–H donors, then extending the distance to 1.0 Å away from the hydrogen atom.

***Cis–trans* rotational isomerization**

Cis–trans rotational energy profiles of ADMA and SDMA were obtained by relaxed dihedral scans about the N_ε–C_ζ bond in 5° increments over 360°. Geometry optimizations and frequency calculations were performed at the M06-2X/6-311+G(d,p) level of theory using the SMD solvent model for water or diethyl ether. Normal vibrational mode analysis was used to confirm the identity of optimized structures as either minima (zero imaginary frequencies) or transition states (one imaginary frequency). Gibbs free energies were calculated at 298 K.

Interaction energy analysis of experimentally determined protein structures

Protein structures containing ADMA or SDMA were obtained from the RCSB Protein Data Bank (www.rcsb.org, accessed 18 July 2025) (42). X-ray diffraction, electron microscopy, or solution NMR structures were included if heavy atoms were resolved. In total, pairwise interactions between acceptor residues and donor guanidinium in 28 nonredundant proteins structures were studied. For $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions, residues were selected if the acceptor oxygen was within 4 Å of the *N*-methyl carbon. For calculation of interaction energies, residues were truncated as in *Density functional theory-optimized donor and acceptor pairs* (Figure 1), with the exception of threonine, which was truncated as isopropanol. Hydrogens were modeled in GaussView with protonation state at physiological pH and optimized with heavy atoms frozen. Calculations were performed at the M06-2X/6-311+G(d,p) level of theory using the SMD solvent model for diethyl ether or DMF. Interactions were categorized by donor and acceptor identity and interaction mode (N–H vs. $\text{N}^+-\text{C}-\text{H}$ hydrogen bond, electrostatic). Hydrogen bonds were defined as the van der Waals distance between $\text{H}\cdots\text{O}$ in donor–acceptor pairs (≤ 2.7 Å) following Itoh's methods (27). Interactions greater than 2.7 Å were classified as electrostatic. Acceptors forming multiple interactions with the guanidinium received separate categorization. A table of PDB IDs and interaction type, distance, and energy can be found in Supplementary File 7.

Case studies on WDR5

WDR5 WIN-site complexes (PDB IDs 6OI0, 6OI1, 6OI2, and 6OI3) (43) containing unmethylated arginine, MMA, SDMA, and the H3R2me peptide were analyzed. Acceptor residues were selected based on the criteria described in *Interaction energy analysis of experimentally determined protein structures*. Structures were similarly truncated except for adjacent residues S91 and D92, which were treated as one moiety as the backbone carbonyl of S91 is an acceptor and

was truncated at the α -carbon. Constrained geometry optimizations, frequency calculations, and single-point energies were computed at the M06-2X/6-311+G(d,p) level of theory using the SMD polarizable continuum model for diethyl ether or DMF. Complexes were visualized with CYLview20 (Université de Sherbrooke, Sherbrooke, QC, Canada) (44).

RESULTS

Quantum mechanical evaluation of methylated arginine interactions in model complexes

As a basis for subsequent protein structure analysis, DFT-optimized pairwise complexes were used to examine optimal N–H $\cdots$ O hydrogen bonds and N⁺–C–H $\cdots$ O hydrogen bond-like interactions. Initial calculations in diethyl ether ($\epsilon = 4$), chosen to approximate the nonpolar environment of the protein core, yielded large interaction energies that likely overestimate the strengths of such interactions. *N,N*-dimethylformamide (DMF; $\epsilon = 37$) was therefore employed as a more suitable solvent model. The M06-2X functional was chosen for its built-in dispersion corrections to account for non-covalent interactions, while the 6-311G+(d,p) basis set was chosen to provide a balanced description of main-group elements, including polarization and diffuse functions important for charged and non-covalent systems. The use of a single set of diffuse functions was benchmarked against the double-diffuse basis employed by previous studies of N⁺–C–H $\cdots$ O interactions in alkylammonium as reported by Itoh *et al.* (27) and was found to have minimal impact in our system.

A summary of interaction energies and O $\cdots$ H distances for the analyzed donor–acceptor pairs is presented in Table 1. In diethyl ether, the N⁺–C–H $\cdots$ O interaction energies are approximately 30–55% of the corresponding N–H $\cdots$ O interaction energies with the same acceptor. O $\cdots$ H distances of the observed N⁺–C–H $\cdots$ O interactions are 20–45% longer than that of the corresponding N–H $\cdots$ O interactions. Similar trends were found in DMF, except that N⁺–C–H $\cdots$ O

interactions with acetate and *p*-methylphenol were not observed; these acceptors instead favored N–H $\cdots$ O hydrogen bonding. When comparing the two solvents, N⁺–C–H $\cdots$ O interaction energies in DMF were smaller in magnitude and the distances longer than in diethyl ether, consistent with the differences in their dielectric constants. These results indicate that N⁺–C–H $\cdots$ O interactions do exist and adopt geometries comparable to canonical hydrogen bonds. Although weaker than conventional N–H $\cdots$ O interactions, their magnitudes are within the range of non-covalent interactions known to contribute to protein stability and recognition, suggesting that they may be physiologically relevant, particularly in low-dielectric environments such as the protein interior.

To better understand the potential of methyl groups to participate in hydrogen bond-like interactions, we examined the electrostatic potential (ESP) of each donor and acceptor. ESP maps were generated for the head groups of arginine (**1**), three MMA conformers (**2a–c**), and two conformers each of ADMA (**3a–b**) and SDMA (**4a–b**; Figure 2). Focusing on dimethylarginine, ESP maps of **3a**, **3b**, and **4a** revealed exposed N–H groups suitable for hydrogen bonding while also permitting interactions with *N*-methyl. The unique geometry of **4a** further enables bidentate N⁺–C–H $\cdots$ O interactions in addition to bidentate N–H $\cdots$ O hydrogen bonds. In **4b**, however, adjacent methyl groups increase steric hindrance around the N–H groups, reducing their exposure and limiting canonical hydrogen bonding interactions while enabling N⁺–C–H $\cdots$ O interactions.

ESP maps were also generated for acceptors, showing the anionic acetate (**5**) with the most negative potential (Figure 3A), followed by neutral acceptors *N*-methylacetamide (**8**), *p*-methylphenol (**7**), and ethanol (**6**; Figure 3B). Acetate exhibits the most negative ESP over its carboxylate group, consistent with hydrogen bonding observed with N–H in the DFT-optimized complexes. The oxygen atoms of *N*-methylacetamide and ethanol exhibit less negative ESP values but remain capable of forming interactions with *N*-methyl. The acceptor *p*-methylphenol exhibits

electron density above and below its phenyl ring, enabling favorable π – π and cation– π interactions observed in the DFT-optimized pairs.

To quantify the potential on the donors as experienced by an approaching acceptor in a moderately polar protein binding pocket, we evaluated the ESP calculated in the solvent DMF at points that are just within the van der Waals radii of the donor hydrogen atom. For N–H donors, the ESP was sampled 1.0 Å from the hydrogen atom (H_η), within its van der Waals radius of 1.2 Å. For N^+ –C–H donors, the ESP was sampled at 1.0 Å from the H_θ atom of the methyl group. Comparison of the ESP values in DMF revealed that N–H donors consistently exhibited approximately 39% higher positive potential than N^+ –C–H donors, consistent with the stronger interaction energies observed in the DFT-optimized complexes (Figure 4).

Energetic contributions of N^+ –C–H $\cdots$ O bonds in experimentally determined structures

To assess the biological relevance of N^+ –C–H $\cdots$ O hydrogen bond-like interactions in methylated arginine, experimentally determined structures from the Protein Data Bank (PDB) containing ADMA or SDMA were analyzed. Donor–acceptor pairs were isolated and truncated analogously to our DFT-optimized complexes. Hydrogen atoms, often absent in these structures, were modeled using DFT. When a donor residue interacted with multiple acceptors, the contribution of each acceptor was considered separately whenever possible. Interactions were categorized by donor and acceptor identity and by interaction mode (N–H vs. N^+ –C–H hydrogen bond, electrostatic, or mixed). O $\cdots$ H distances below 2.7 Å (the sum of van der Waals radii of oxygen and hydrogen) were treated as a hydrogen bond; all other interactions were classified as electrostatic, including cation– π or salt-bridge contacts. A summary of interaction energies and distances for the PDB donor–acceptor pairs in diethyl ether and DMF is presented in Table 2.

N-methylacetamide, representing backbone amide acceptors, accounted for the largest fraction of the observed $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions, whereas *p*-methylphenol representing tyrosine residues, accounted for the fewest. In fact, *N*-methylacetamide accounted for all $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions involving ADMA, suggesting that these interactions may preferentially form with backbone carbonyls. Tyrosine, in contrast, may favor alternative interactions such as $\pi-\pi$ or cation- π contacts due to steric hindrance or geometric constraints.

Interaction energies in protein structures were solvent-dependent, and $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions observed in protein structures exhibited smaller magnitudes and longer $\text{O}\cdots\text{H}$ distances compared to canonical $\text{N}-\text{H}\cdots\text{O}$ hydrogen bonds, consistent with trends observed in DFT-optimized complexes. For each interaction type, the geometries and energies of the PDB were compared with those of the corresponding DFT-optimized complexes. A substantial fraction of interactions fell outside the hydrogen-bond distance cutoff ($\text{O}\cdots\text{H}$ distance ≤ 2.7 Å) and were classified as electrostatic. After excluding these interactions, the average $\text{O}\cdots\text{H}$ distances in $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions closely matched those of the DFT-optimized complexes. Interaction energies, however, were consistently lower in protein structures compared to DFT-optimized complexes, indicating that geometric constraints in the binding site do not allow for optimal interaction.

In addition to hydrogen bonding interactions, we observed electrostatic interactions in a stacked conformation between the positively charged guanidinium and the phenyl ring of tyrosine residues or negatively charged carboxylates, which also contribute to arginine binding or recognition. Representative structures of each interaction type are shown in Figure 5.

Interestingly, we observed uneven distribution of ADMA and SDMA conformers in the PDB, which may influence the types of interactions available to each donor upon binding and potentially affect binding affinity. PRMTs may exhibit positional preference for methylation, as

modeled by Yue *et al.* for PRMT5 (45). However, whether these conformers reflect the relative abundance of conformers under physiological conditions is not known. To investigate this, we calculated the relative thermodynamic stability of major ADMA (**3a** and **3b**) and SDMA (**4a** and **4b**) conformers, as well as their *cis*–*trans* rotational isomerization energy barrier (Figure S1). Diethyl ether was used to simulate a highly hydrophobic protein interior, whereas water modeled a surface-exposed amino acid in aqueous solution. In both solvents, **3b** is more thermodynamically stable than **3a** ($\Delta G_{298K, \text{ water}} = -3.5$ kcal/mol, $\Delta G_{298K, \text{ ether}} = -3.4$ kcal/mol), likely due to steric clashes between the methyl groups and the side chain methylene, modeled here as a methyl group (Figure S1A). Similarly, for SDMA, **4b** is more thermodynamically stable than **4a** ($\Delta G_{298K, \text{ water}} = -5.1$ kcal/mol, $\Delta G_{298K, \text{ ether}} = -6.6$ kcal/mol). **4b** displays rotational symmetry in the truncated form with minimal steric clashes, whereas **4a**—capable of forming both bidentate N–H $\cdots$ O and N⁺–C–H $\cdots$ O hydrogen bonds—is less favorable thermodynamically. Although our small sample size in the PDB prevented strong conclusions about the correlation between relative abundance in proteins and calculated stabilities, the energy barrier of rotation at 298 K for ADMA and SDMA ranged from 12.4–16.2 kcal/mol across both solvents, suggesting that these *cis*–*trans* isomers readily interconvert under physiological conditions.

Methylation-state-dependent binding in the WDR5 protein model system

To compare directly the effect of methylation in a single arginine-binding environment, we performed a case study on the human WDR5 protein, a histone H3R2 reader (46–49) involved in transcriptional activation by methylation of H3K4 (46,48,49). With the exception of ADMA, WDR5 recognizes and binds all other methylation states of H3R2 (43). Lorton *et al.* recently crystallized WDR5 with unmethylated arginine (PDB ID 6OI0), MMA (6OI1), and SDMA (6OI2), as well as the H3R2me peptide (6OI3). They further characterized the binding affinities of all

H3R2 methylation states using isothermal titration calorimetry (ITC), demonstrating that WDR5 binds MMA most tightly (-6.82 kcal/mol), followed by SDMA (-6.81 kcal/mol), then unmodified arginine (-6.73 kcal/mol) (43).

From these crystal structures, we extracted the coordinates of H3R2 and all residues containing an oxygen atom within 4 Å, which included the backbone carbonyls of S49, S91, F133, S175, and C261, as well as the charged residue D92. We analyzed pairwise interaction energies analogously to our analysis of other protein structures, as well as total interaction energy with all acceptor residues (Figure 6, left table). Given the number of polar residues in the WIN site of WDR5, we performed our analysis in the moderately polar solvent DMF.

While the magnitudes of computed interaction energies were overestimated compared to experimentally observed binding affinities, the trends observed in total interaction energies between the guanidinium head group and the collection of acceptor residues were broadly consistent with ITC results. Interactions with MMA were the strongest, with a total interaction energy of -18.9 kcal/mol in DMF (Figure 6B), which increased further when analyzing the guanidinium head group interactions in the co-crystal structure of MMA in the H3R2me peptide (-25.4 kcal/mol; Figure 6C). However, our results did not agree with ITC data regarding the relative binding affinities of unmodified arginine and SDMA, as our calculated interaction energies were larger in magnitude for unmodified arginine (-16.8 kcal/mol) as compared to SDMA (-14.1 kcal/mol; Figure 6A, D).

Focusing on individual residues, we observed that interactions with charged residues ranged from -7.0 to -9.5 kcal/mol, while those with uncharged backbone carbonyls ranged from -0.4 to -5.2 kcal/mol. Notably, the moiety of S91 backbone + D92 forms a distinctive interaction with the guanidinium head group. In the complex containing unmodified arginine, the backbone

carbonyl of S91 forms a hydrogen bond with one N–H group, while the carboxylate of D92 forms a salt-bridge contact with a nearest O $\cdots$ H distance of 3.9–4.0 Å. In complexes with MMA, the S91 carbonyl is positioned to interact with two N–H groups. Meanwhile, the added methyl group approaches the carboxylate of D92 with an O $\cdots$ H distance of 2.7 Å in the isolated amino acid (Figure 6B) and 2.31 Å in the H3R2me peptide (Figure 6C), suggestive of a N⁺–C–H $\cdots$ O hydrogen bond-like interaction. Indeed, the interaction energies are highest in the H3R2me peptide (–9.7 kcal/mol), then isolated MMA (–9.5 kcal/mol), and finally unmodified arginine (–7.4 kcal/mol). Complexation with SDMA also led to the formation of a N⁺–C–H $\cdots$ O hydrogen bond-like interaction with the backbone of S91, although the introduction of steric bulk likely reduced the interaction energy (–7.0 kcal/mol; Figure 6D). Similar logic could be extended to explain the lack of binding with ADMA, as it would place two methyl groups near S91 and D92. More broadly speaking, however, we did not observe clear correlation between interaction energy and distance, suggesting that subtle geometric differences are at play.

Taken together, these results indicate that N⁺–C–H $\cdots$ O interactions play a small but meaningful modulatory role in the WDR5 recognition of H3R2. While these N–H $\cdots$ O and N⁺–C–H $\cdots$ O interactions contribute to binding, they are insufficient to fully predict binding strengths in this system. Other interactions formed by aliphatic and aromatic residues not analyzed here, such as hydrophobic and cation– π interactions, may also contribute to binding and recognition.

DISCUSSION

Hydrogen bonding and electrostatic interactions involving dimethylarginine

Our calculations and structural analyses demonstrate that *N*-methylation consistently reduces the strength of guanidinium hydrogen bonding, in agreement with previous studies of methylated donors. In both diethyl ether and DMF models, interactions involving *N*-methyl groups

were ~33–50% weaker than those mediated by unsubstituted N–H groups in ADMA and SDMA, underscoring the electronic penalty imposed by replacing a polar donor with a more hydrophobic substituent. Importantly, DMF-optimized geometries more closely resembled those observed in PDB structures than those optimized in diethyl ether, suggesting that this moderately polar solvent better mimics the mixed electrostatic environment of protein binding pockets. PDB-derived interaction energies covered a broad range, from 5% to 75% of DFT-optimized values, with most clustering between 33–50%. This reflects not only intrinsic differences between idealized models and biological systems, but also the environmental heterogeneity and geometric constraints imposed within proteins.

Within protein binding pockets, multiple weak forces cooperate to generate specificity, rather than a single dominant interaction type. Canonical N–H $\cdots$ O hydrogen bonding remains prevalent, but we also observed isolated cases of N⁺–C–H $\cdots$ O hydrogen bond-like interactions. Notably, some acceptors engaged both N–H and *N*-methyl donors simultaneously, producing additive interaction energies despite longer donor–acceptor distances. Such configurations closely resemble the glutamate–arginine salt bridges seen with the active site double-E loops of PRMTs (50–52). Interestingly, product specificity of PRMT7 can be altered through the mutation of one of its double-E loop glutamates (E181D), which adjusts the position of the catalytic carboxylate that also participates in hydrogen bonding interactions. This mutation enables the dimethylation of arginine residues (53,54) and suggest that such configurations may contribute to dimethylarginine affinity in non-PRMT proteins.

Comparing ADMA and SDMA interaction geometries found in protein structures to those found in DFT-optimized complexes, we find that electrostatic contributions, including cation– π , and *N*-methyl– π interactions, were frequently observed in proteins. In terms of hydrogen bonding

interactions, carboxylates and backbone carbonyls dominated as acceptors, while hydroxyl-bearing side chains were underrepresented near methylated arginine residues. Tyrosine, in particular, more often engaged in aromatic stacking or cation- π interactions than in hydrogen bonding through its hydroxyl, suggesting that the π -system of Tyr competes with or even overrides its H-bonding potential. Furthermore, while electrostatic interactions with serine and threonine were mostly hydrogen bond-like, their interaction distances were often greater than the hydrogen bond cutoff of 2.7 Å. Overall, while DFT-optimized complexes tended to converge on idealized N-H $\cdots$ O geometries, structural data from the PDB reveal that in protein contexts, dimethylarginine participates in a more diverse set of electrostatic contacts.

WDR5 as a model system for methylarginine-dependent binding

The comparison between DFT-optimized models and experimental PDB geometries highlights the strong context-dependence of methylarginine interactions. DFT provides high-resolution insights into intrinsic donor-acceptor preferences, but necessarily neglects solvent effects, steric hindrance, and cooperative effects from multiple residues, limiting their ability to replicate physiological conditions. To probe these context-dependent features, we analyzed the H3R2-binding site of WDR5 as a case study.

Analysis of WDR5's binding to arginine variants reveals the nuanced role of arginine methylation. While methylation decreases opportunities for canonical N-H $\cdots$ O hydrogen bonding, it may modulate binding specificity through electrostatic complementarity and steric fitting. Among the states examined, MMA showed the most favorable interaction energy, consistent with experimental ITC data showing MMA binds WDR5 most tightly. This suggests that the single methyl group optimizes balance between size and polarity, preserving multiple N-H contacts while introducing favorable interactions such as the N⁺-C-H $\cdots$ O interaction with D92.

SDMA interactions were somewhat weaker, comparable to unmethylated arginine, whereas ADMA failed to form a stable complex, likely due to steric clashes within the constrained WIN site (43). Thus, WDR5 illustrates how methylation can either promote or hinder binding, depending on how local geometry and electronic complementarity are altered. Rather than uniformly disrupting binding, methylation tunes the binding landscape in a context-dependent manner.

Structural and biochemical context of dimethylarginine recognition

Our findings align with previous work showing that $N^+-C-H\cdots O$ hydrogen bonds are 33–50% as strong as $N-H\cdots O$ bonds (55–57). Scheiner and others demonstrated similar trends in computational model systems, with $N^+-C-H\cdots O$ bonds weakening in polar solvents like DMF compared to less polar environments. Related interactions, such as $C-H\cdots \pi$ and $N^+-C-H\cdots \pi$, differ in their origins (dispersion vs. electrostatics), directionality (nonpreferential vs. integral), and strength (weak vs. strong) (30,31). Other physiologically relevant, polarized donors, including trimethyllysine (27,29,58) and trimethylsulfonium (29) participate in interactions similar to those observed with methylarginine, which may explain why tyrosine often engages electrostatically rather than through hydrogen bond-like interactions. Multiple studies confirm that methylarginines participate in diverse contacts, including π -stacking and cation– π interactions with aromatic side chains in binding pockets (20,21,59–61).

Notably, WDR5 binds unmethylated arginine, MMA, and SDMA with comparable affinities, but does not accommodate ADMA, suggesting selectivity is based on methylation identity rather than the mere presence of a modification, similar to observations for WDR5 binding to H3K4 (62). Similar trends in methylarginine affinity occur in JMJD5, a lysine demethylase with novel methylarginine-specific protease activity (63). In contrast to WDR5's inability to bind to

ADMA, the UBR-box domain of the E3 ubiquitin ligase UBR2 binds preferentially to ADMA, but less so to unmodified arginine and SDMA (64). The Tudor domain of TDRD3 also shows selectivity for ADMA, mediated primarily by Y566, which participates in an *N*-methyl- π as well as cation- π interactions (59). Comparable binding modes have been described for synthetic ADMA inhibitors (20,21). Together, the diversity in binding preferences suggest that methylarginine recognition is not governed solely by disruption of hydrogen bonding but also by steric and conformational factors that distinguish between methylation states.

Allosteric implications

Allosteric effects have been observed in several histone systems, highlighting how one methylation event can influence subsequent modifications. For instance, PRMT7-mediated monomethylation at H4R17 activates PRMT5-catalyzed methylation of H4R3 (65). Likewise, H3K4me₃ is required for PRC2 activation and trimethylation of H3K27, while H3K36me₃ instead inhibits it (66). In the case of WDR5, its binding to MMA and SDMA marks at H3R2 promotes the methylation of H3K4 via the H3K4 methyltransferase complex MLL (47), of which WDR5 is a member (67), while ADMA inhibits this binding and thus the subsequent methylation of H3K4 (43,68).

Limitations and future directions

The use of static protein structures and model simplifications—such as the use of methyl-capped head groups and a pairwise focus on interaction energies—do not fully capture the conformational dynamics and electronic cooperativity seen in a protein binding site. For example, while DFT-optimized geometries often favored interactions involving N-H donors with highly polar acceptors, PDB-derived pairs revealed a more diverse repertoire, including cation- π and methyl- π contacts as well as longer (>2.7 Å) hydrogen bond-like interactions. Pairwise

calculations also limited our ability to capture the effects on $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions exerted by adjacent residues. A noteworthy example is the possible cation–cation interaction between an unmethylated arginine’s guanidinium and dimethylarginine. These interactions have been reported in aqueous systems (69) and in crystal structures of organic compounds (70), suggesting potential significance in biological contexts. Validation using molecular dynamics simulations and biophysical approaches such as isothermal titration calorimetry or surface plasmon resonance would provide quantitative benchmarks for the computational predictions, particularly regarding ligand-dependent differences in binding affinity. Mutagenesis studies could further delineate the contributions of $\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ contacts relative to other noncovalent forces.

Solvent representation remains another important limitation. Calculations in diethyl ether tended to exaggerate electrostatic stabilization, whereas DMF geometries more closely resembled the mixed polarity of protein interiors. Neither solvent models the hydrogen bonding interactions with water. Incorporating explicit solvent molecules or more advanced continuum models such as IEF-PCM could improve capture of electronic environments.

A final limitation is sampling bias. Of the 28 PDB structures analyzed, only 7 contained ADMA, yielding just 12 measurable interactions. Serine and threonine acceptors were rarely observed, further constraining generalizability. The apparent prominence of MMA—historically regarded as a transient intermediate but now increasingly viewed as functionally significant (71,72)—also suggests the need for deeper study. Expanding both DFT simulations and structural surveys to systematically compare unmethylated arginine, MMA, ADMA, and SDMA across diverse protein families would provide a more complete picture.

Emerging perspectives on reversibility

Recent reports of nonspecific arginine demethylases, particularly within the Jumonji domain-containing protein family (13,15), challenge the notion of methylation as a strictly permanent PTM. However, these findings remain controversial, as many of these activities were observed *in vitro* and strong evidence has not been presented *in vivo* (73). Protein arginine deiminase PAD4 has been proposed to act as an arginine demethylase via demethyelimination (72,74), yet subsequent work yielded conflicting results (16,17,75). In particular, PAD4 is also able to catalyze the conversion of unmodified arginine to citrulline, disputing its role as a strict arginine demethylase (73).

Conclusion

$\text{N}^+-\text{C}-\text{H}\cdots\text{O}$ interactions, though weaker and longer than canonical $\text{N}-\text{H}\cdots\text{O}$ hydrogen bonds, persist in physiological settings and contribute significantly to recognition of dimethylated arginine residues. Methylation reshapes the hydrogen bonding landscape while simultaneously opening new opportunities for complementary electrostatic and steric interactions. As methylarginine emerges as a key regulator of gene expression and disease (5,76–79), understanding its interaction repertoire is increasingly important. A more complete understanding of methylarginine interactions will require integrating weaker noncanonical forces such as van der Waals complementarity, long-range electrostatics, and water displacement effects into models of protein–ligand binding. Case studies, such as WDR5 and related chromatin-associated proteins, highlight how methylation can enhance, impair, or redirect binding depending on the context. Ultimately, this work emphasizes the importance of incorporating subtle interaction modes into binding models and of applying multidisciplinary approaches to fully uncover the biological consequences of post-translational arginine methylation.

Table 1. Summary of interaction energies (ΔE_{int}) and distances of DFT-optimized complexes.

Acceptor	Interaction Type	Average ΔE_{int} (kcal/mol), Diethyl Ether	Average O $\cdots$ H Distance ($\text{\AA}$)	Average ΔE_{int} (kcal/mol), <i>N,N</i> - Dimethylformamide	Average O $\cdots$ H Distance ($\text{\AA}$)
ADMA					
Acetate	COO $\cdots$ H-C-N $^+$	-22.0	2.2	Not Obs.*	N/A*
Acetate	COO $\cdots$ H-N	-39.6	1.5	-15.8	1.6
Ethanol	HO $\cdots$ H-C-N $^+$	-4.3	2.3	-3.1	2.4
Ethanol	HO $\cdots$ H-N	-8.3	1.9	-6.4	1.9
<i>p</i> -methylphenol	HO $\cdots$ H-C-N $^+$	-2.7	2.5	-0.8	2.6
<i>p</i> -methylphenol	HO $\cdots$ H-N	-5.9	2.0	-3.3	1.9
<i>N</i> -methylacetamide	C=O $\cdots$ H-C-N $^+$	-4.4	2.6	-2.2	2.6
<i>N</i> -methylacetamide	C=O $\cdots$ H-N	-13.3	1.8	-7.7	2.0
SDMA					
Acetate	COO $\cdots$ H-C-N $^+$	Not Obs.*	N/A*	Not Obs.*	N/A*
Acetate	COO $\cdots$ H-N	-38.3	1.4	-16.6	1.6
Ethanol	HO $\cdots$ H-C-N $^+$	-3.9	2.3	-2.5	2.4
Ethanol	HO $\cdots$ H-N	-7.9	1.9	-6.3	1.9
<i>p</i> -methylphenol	HO $\cdots$ H-C-N $^+$	-2.1	2.7	Not Obs.*	N/A*
<i>p</i> -methylphenol	HO $\cdots$ H-N	-6.7	2.0	-3.8	2.1
<i>N</i> -methylacetamide	C=O $\cdots$ H-C-N $^+$	-5.3	2.3	-2.7	2.4
<i>N</i> -methylacetamide	C=O $\cdots$ H-N	-13.1	1.8	-7.9	1.8

* Interactions not observed in DFT-optimized donor/acceptor pairs

Hydrogen bonds defined as $d_{\text{O-H}} \leq 2.7 \text{ \AA}$

Table 2. Summary of interaction energies (ΔE_{int}) and distances of pairwise interactions analyzed from experimentally determined protein structures.

Interaction Type	Average ΔE_{int} (kcal/mol), Diethyl Ether	Average ΔE_{int} (kcal/mol), <i>N,N</i> - Dimethylformamide	Average O $\cdots$ H Distance (Å)
ADMA Interactions			
C=O $\cdots$ H–C–N ⁺	–3.0	–0.8	2.5
COO $\cdots$ H–N	–26.5	–9.6	2.1
HO $\cdots$ H–N	–4.2	–2.0	2.3
Electrostatic (phenyl) – guanidinium	–3.1	–2.5	3.5
Electrostatic (carboxylate) – guanidinium	–14.7	–2.9	2.8
Mixed (C=O $\cdots$ H–N and C=O $\cdots$ H–N ⁺)	–5.0	–1.7	2.2, 2.4 ^a
SDMA Interactions			
COO $\cdots$ H–C–N ⁺	–14.9	–2.5	2.5
COO $\cdots$ H–N	–20.9	–6.4	2.2
C=O $\cdots$ H–C–N ⁺	–3.3	–0.9	2.5
C=O $\cdots$ H–N	–8.1	–4.5	2.1
HO $\cdots$ H–C–N ⁺	–2.6	–0.9	2.3
HO $\cdots$ H–N	–5.0	–3.2	2.4
Electrostatic (phenyl) – guanidinium	–3.5	–2.3	3.4
Electrostatic (carboxylate) – guanidinium	–14.2	–2.3	2.8
Electrostatic (carbonyl) – guanidinium	–2.6	–1.7	3.0
Electrostatic (hydroxyl) – guanidinium	–1.0	–0.3	2.9
Mixed (COO $\cdots$ H–N and COO $\cdots$ H–C–N ⁺)	–28.3	–9.2	2.2, 2.6 ^a
Mixed (C=O $\cdots$ H–N and C=O $\cdots$ H–C–N ⁺)	–9.4	–4.5	2.1, 2.6 ^a
Mixed (HO $\cdots$ H–N and HO $\cdots$ H–C–N ⁺)	4.1	–3.6	2.1, 2.7 ^a

^a Average distance for each interaction specified by category

Hydrogen bonds defined as $d_{\text{O-H}} \leq 2.7$ Å

FIGURES

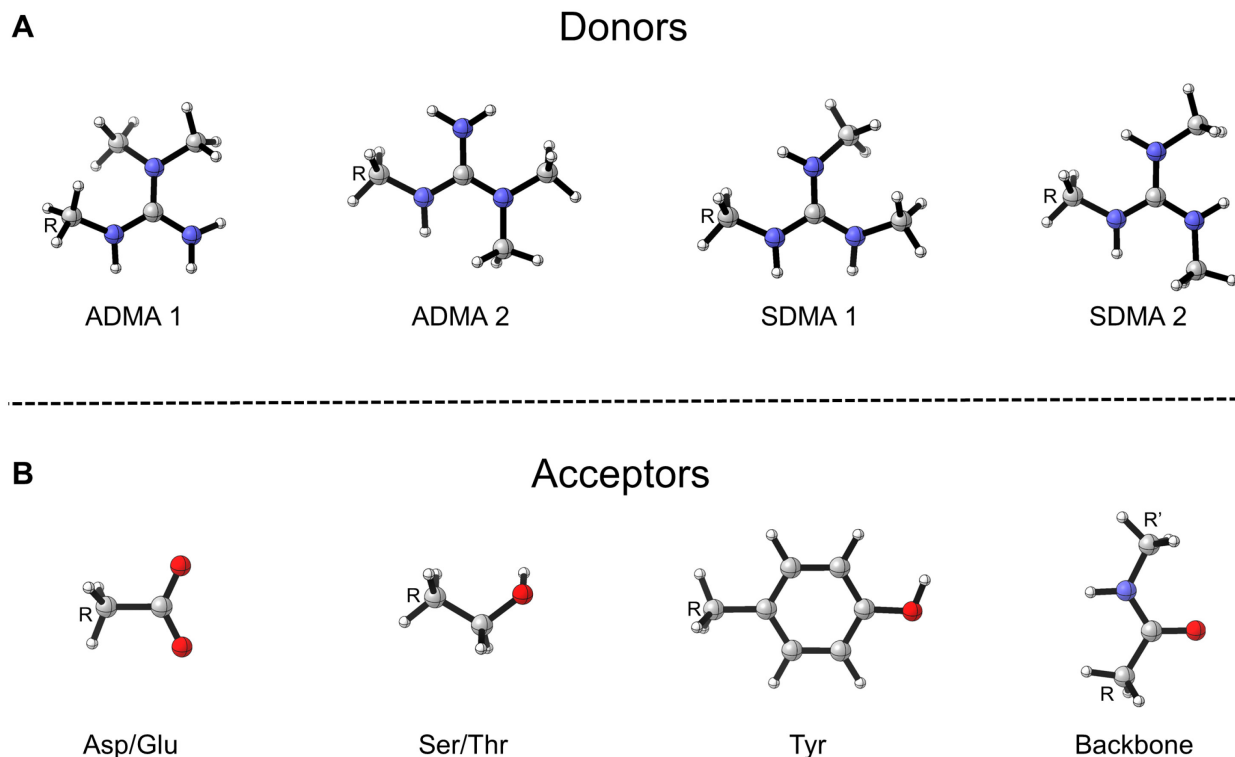


Figure 1. DFT-optimized hydrogen bond donor and acceptor head group models. (A) Hydrogen bond donors representing functional head groups of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are pictured, with two conformers illustrated for each. (B) Hydrogen bond acceptors representing functional head groups for Asp/Glu (modeled as acetate), Ser/Thr (ethanol), Tyr (*p*-methylphenol), and protein backbone (*N*-methylacetamide) are pictured. Methylene groups in amino acid side chains, as well as adjoining residues in the backbone, are truncated and replaced with methyl groups, denoted by R or R'. Geometric optimization was performed using M06-2X/6-311+G(d,p), SMD (diethyl ether).

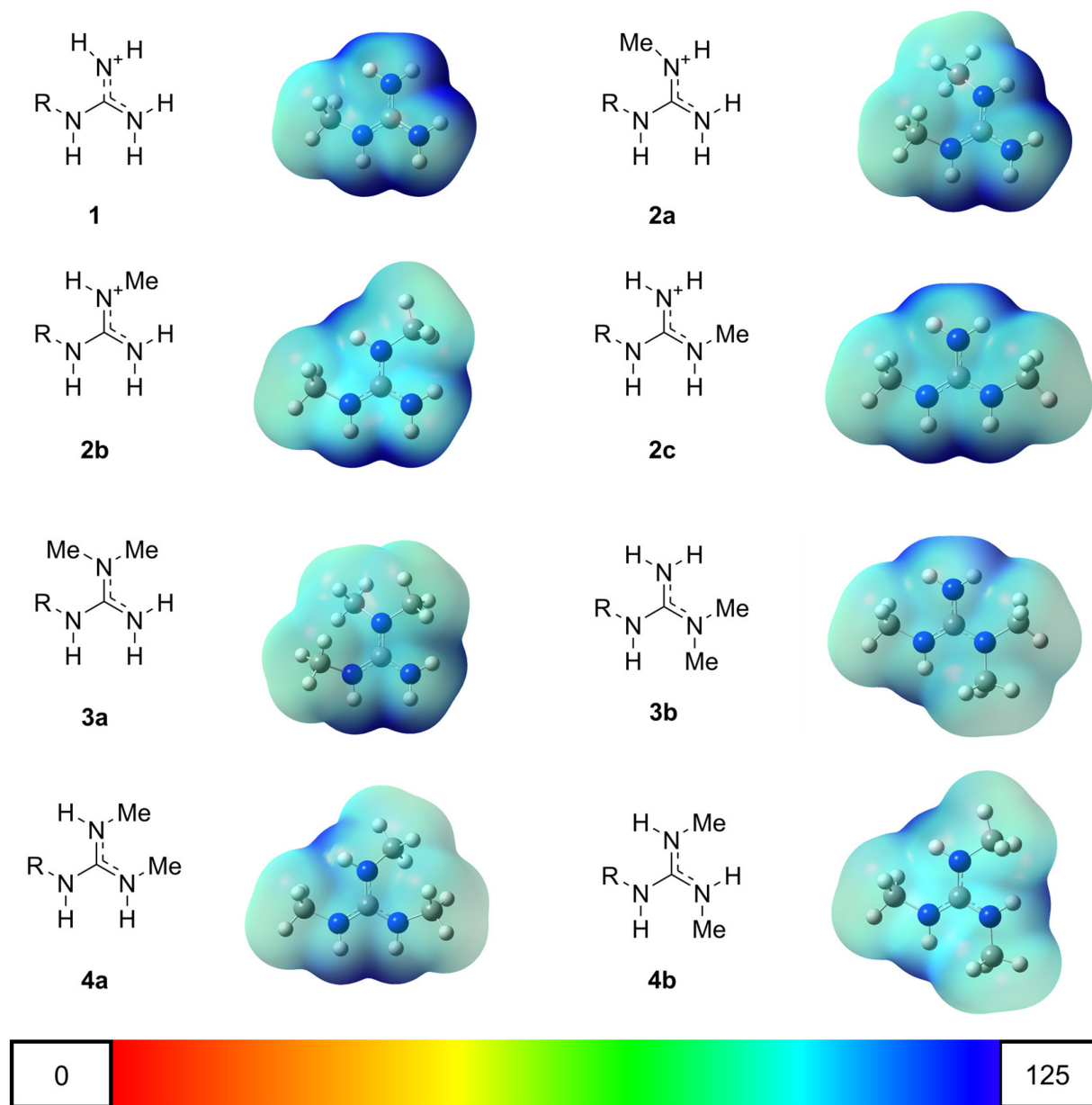


Figure 2. Electrostatic potential maps of DFT-optimized donors. Electrostatic potential (ESP) maps of DFT-optimized models of arginine (**1**), MMA (**2a–c**), ADMA (**3a–b**), SDMA (**4a–b**) optimized with M06-2X/6-311+G(d,p), SMD (diethyl ether) are shown. R denotes the position of the side chain methylene. Values are given in kcal/mol.

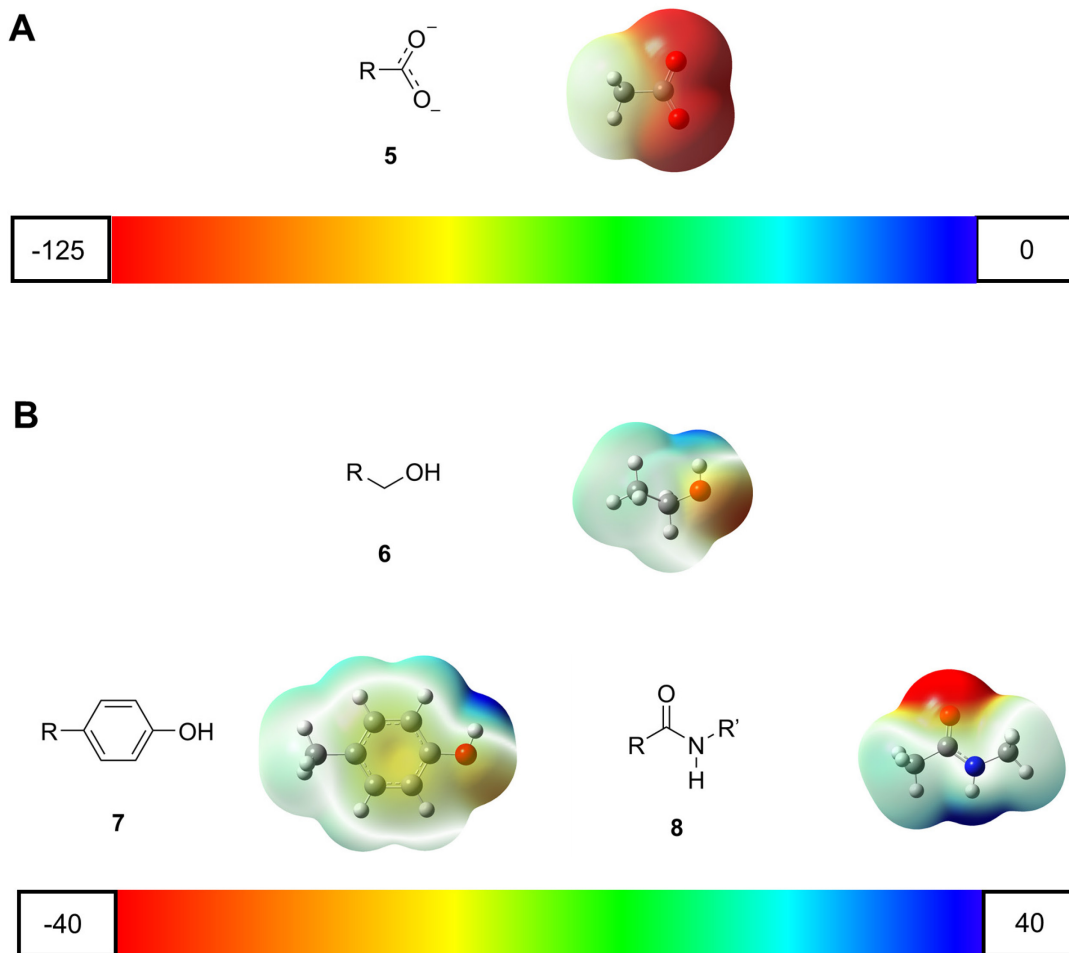


Figure 3. Electrostatic potential maps of DFT-optimized acceptors. ESP maps of DFT-optimized models of (A) Glu/Asp (acetate, **5**). (B) Ser/Thr (ethanol, **6**), Tyr (*p*-methylphenol, **7**) and protein backbone (*N*-methylacetamide, **8**) optimized with M06-2X/6-311+G(d,p), SMD (diethyl ether) are shown. R and R' denote the position of the side chain methylene (**5-7**) or adjoining residues (**8**). Values are given in kcal/mol.

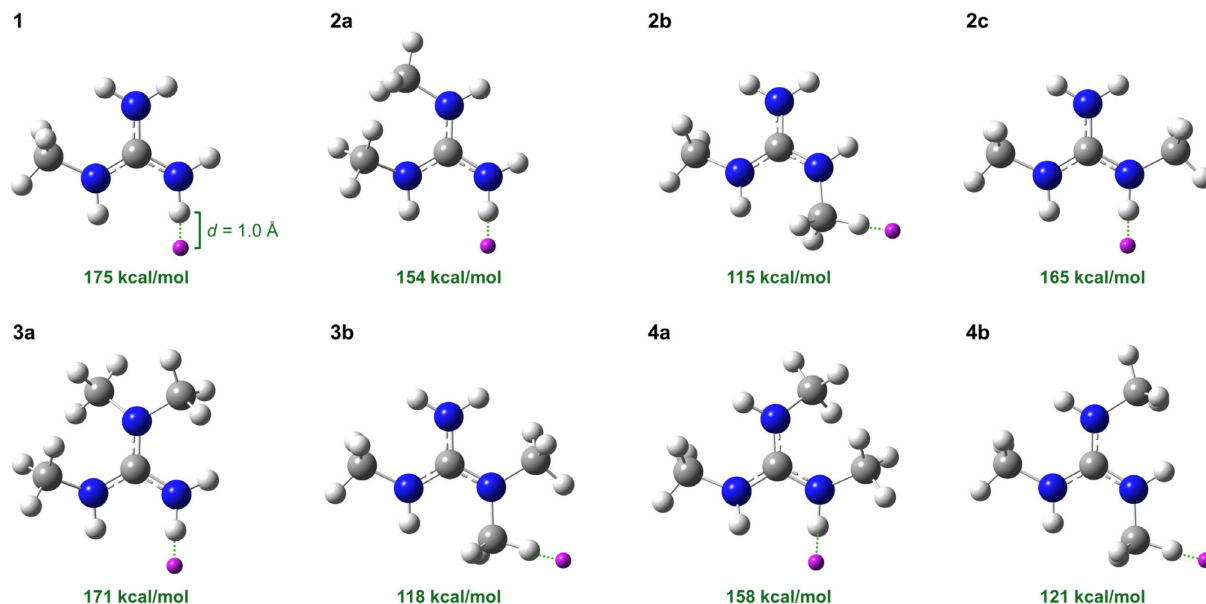


Figure 4. Electrostatic potential of DFT-optimized donors at fixed points. The electrostatic potential of the head group models of arginine (**1**), MMA (**2a–c**), ADMA (**3a–b**), and SDMA (**4a–b**) at a point (purple) 1.0 Å away from the hydrogen atom (H_η) of N–H donors or from the methyl hydrogen atom (H_θ) of N^+ –C–H donors were compared. Values are calculated with M06-2X/6-311+G(d,p), SMD (DMF) and denoted in green. Points are chosen along the $N\eta$ – H_η or $C\theta$ – H_θ bondaxis.

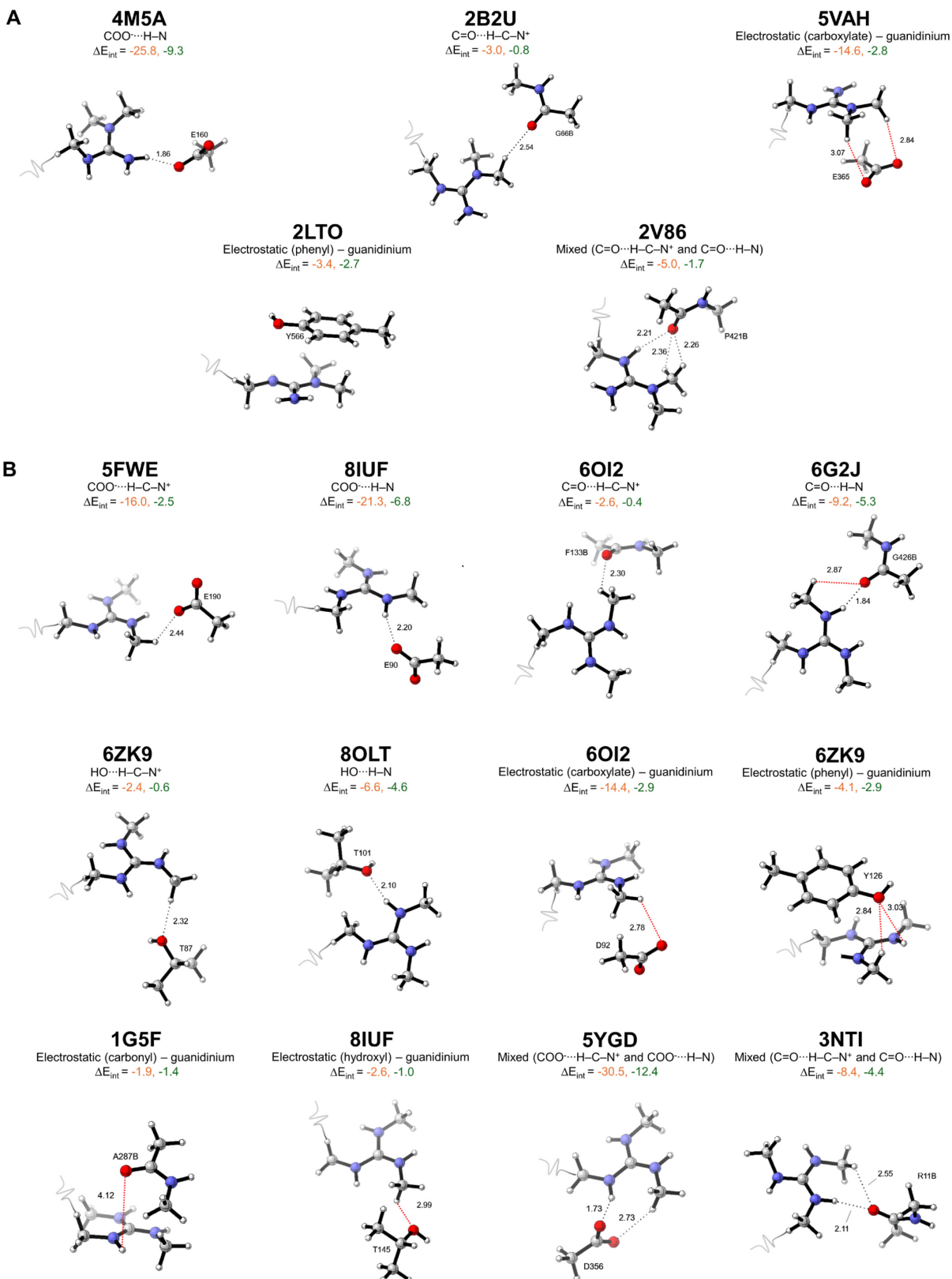


Figure 5. Representative interactions of ADMA and SDMA found in experimentally determined protein structures. Representative examples of interactions with ADMA (**A**) and SDMA (**B**) sampled from the Protein Data Bank (PDB) are pictured. Black dotted lines indicate an interaction ≤ 2.7 Å; red dotted lines indicate an interaction > 2.7 Å. Gray wavy lines denote the position of the donor residue's side chain methylene. Hydrogen positions were optimized and interaction energies (ΔE_{int} , in kcal/mol) were calculated at the M06-2X/6-311+G(d,p) level of theory using the SMD solvation model for diethyl ether (orange) and DMF (green). For clarity, only geometries optimized in diethyl ether are shown.

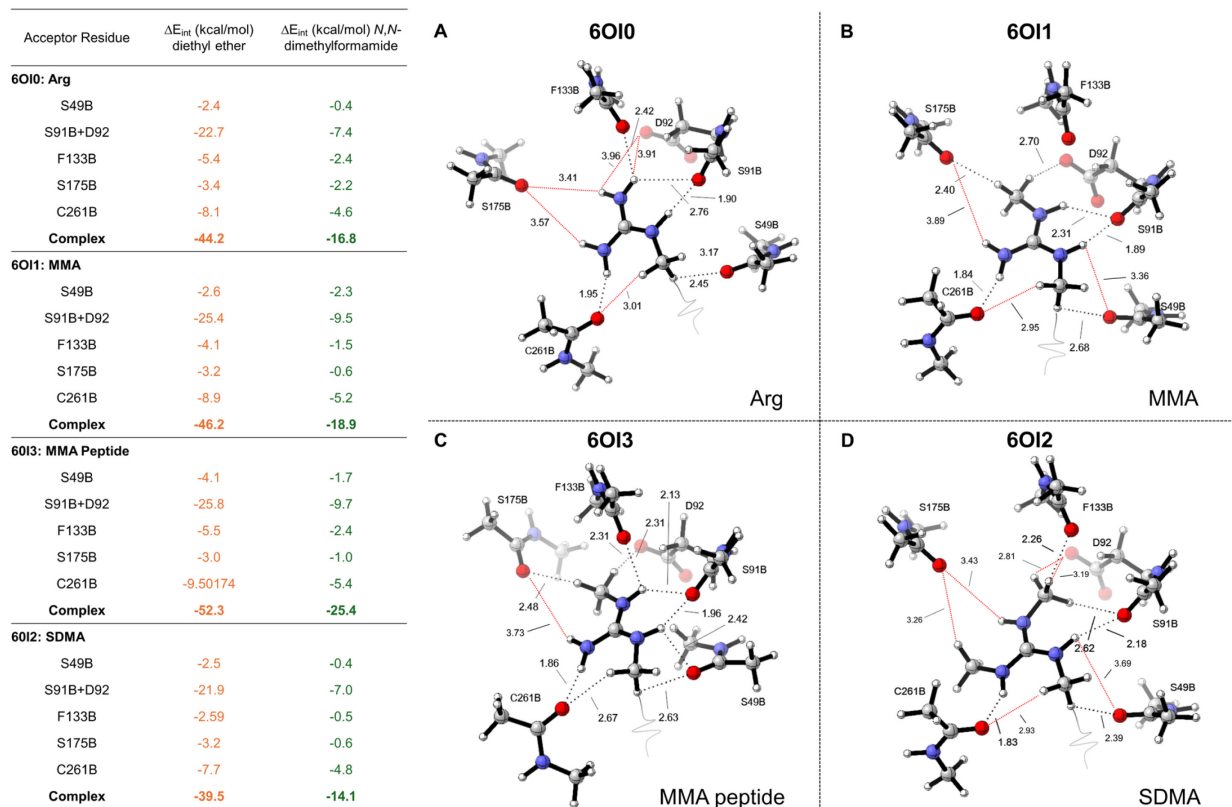


Figure 6. Case study of arginine, MMA and SDMA binding in WDR5. A case study was performed of human WDR5 complexed with arginine (**A**), MMA (**B**), MMA from histone H3R2me peptide (**C**), and SDMA (**D**). Black dotted lines indicate an interaction ≤ 2.7 Å and red dotted lines indicate an interaction > 2.7 Å. Left: ΔE_{int} (kcal/mol) was calculated at the M06-2X/6-311+G(d,p) level of theory using the SMD solvation model for diethyl ether (orange) and DMF (green). Gray wavy lines denote the position of the donor residue's side chain methylene. B denotes the backbone of a residue. For clarity, only geometries optimized in diethyl ether are shown.

SUPPLEMENTAL INFORMATION

Table S1. Computed energies of donor and acceptor head groups in diethyl ether.

Structure	dG _{thermal} (M06-2X/6-311+G(d,p))	E (M06-2X/6-311+G(d,p))	G = dG _{thermal} + E
Arg	0.086241	-245.123662	-245.037421
ADMA 1	0.142831	-323.710353	-323.567521
ADMA Rotational Isomerization TS	0.143021	-323.694207	-323.551186
ADMA 2	0.142016	-323.714955	-323.572939
SDMA 1	0.144010	-323.709871	-323.565861
SDMA Rotational Isomerization TS	0.141832	-323.690050	-323.548217
SDMA 2	0.140672	-323.716555	-323.575884
Acetate	0.022876	-228.575168	-228.552291
Ethanol	0.055107	-155.019062	-154.963955
<i>N</i> -methylacetamide	0.073494	-248.410458	-248.336965
<i>p</i> -methylphenol	0.100770	-346.741066	-346.640296

Table S2. Computed energies of donor and acceptor head groups in DMF.

Structure	dG _{thermal} (M06-2X/6-311+G(d,p))	E (M06-2X/6-311+G(d,p))	G = dG _{thermal} + E
Arg	0.086249	-245.145224	-245.058976
ADMA 1	0.141963	-323.727994	-323.586031
ADMA 2	0.140796	-323.732622	-323.591826
SDMA 1	0.141943	-323.727532	-323.585590
SDMA 2	0.139444	-323.734008	-323.594565
Acetate	0.021434	-228.594942	-228.573509
Ethanol	0.054965	-155.019708	-154.964743
<i>N</i> -methylacetamide	0.073357	-248.413037	-248.339680
<i>p</i> -methylphenol	0.100670	-346.741889	-346.641219

Table S3. Computed energies of *cis*–*trans* rotational isomerization in water.

Structure	dG _{thermal} (M06-2X/6-311+G(d,p))	E (M06-2X/6-311+G(d,p))	G = dG _{thermal} + E
ADMA 1	0.140610	-323.725450	-323.584840
ADMA Rotational Isomerization TS	0.142042	-323.705934	-323.563892
ADMA 2	0.141806	-323.721030	-323.579224
SDMA 1	0.142039	-323.722010	-323.579971
SDMA Rotational Isomerization TS	0.142365	-323.707230	-323.564865
SDMA 2	0.139633	-323.727705	-323.588072

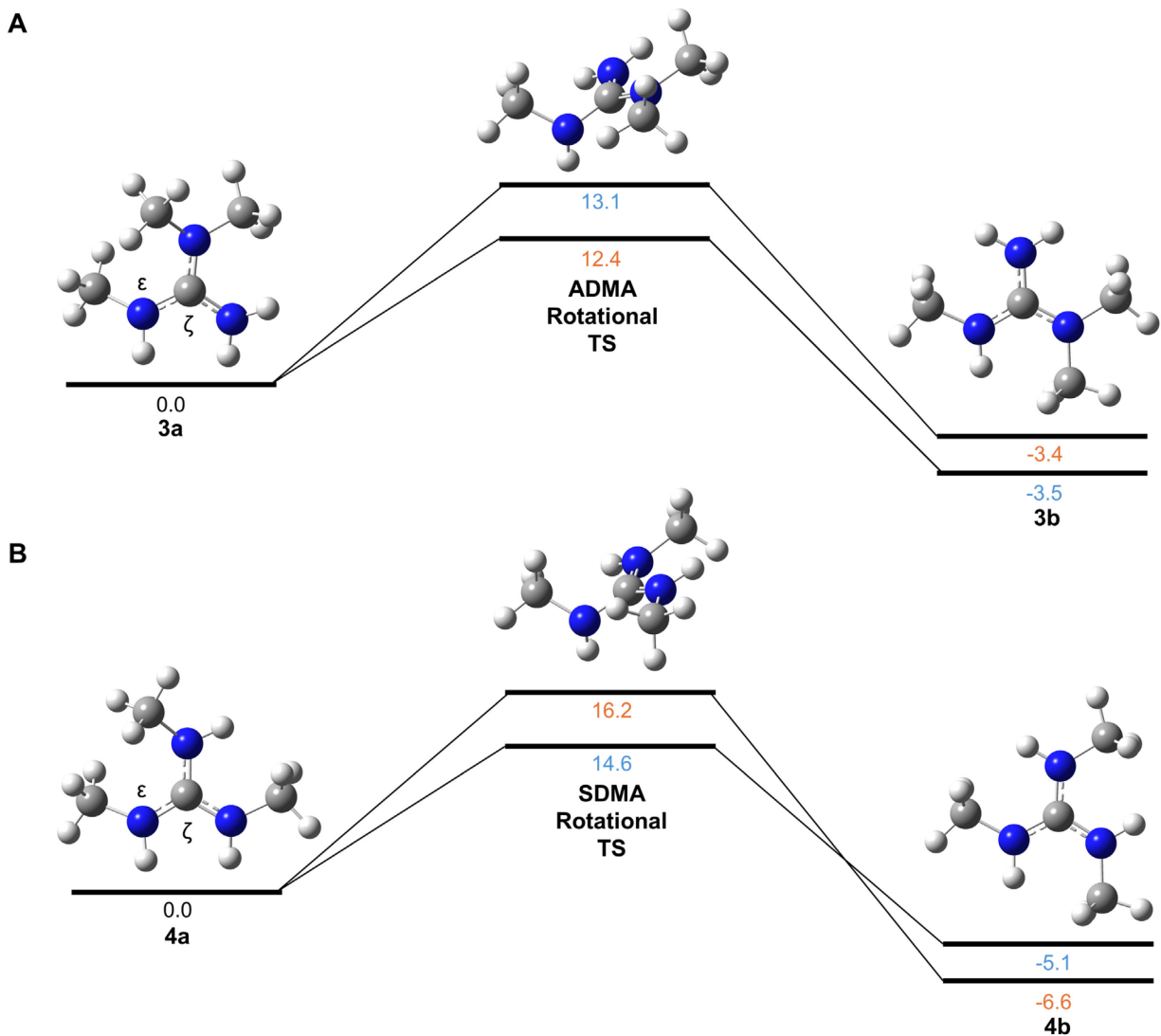


Figure S1. Standard Gibbs free energy of *cis*–*trans* rotational isomerization of ADMA and SDMA. The energy barrier in kcal/mol of a 180° rotation along the N ϵ –C ζ bond of ADMA (**A**) and SDMA (**B**) in diethyl ether (orange) and water (blue). $\Delta G_{298\text{K}}$ and $\Delta G_{298\text{K}}^\ddagger$ are calculated at the M06-2X/6-311+G(d,p) level of theory using the SMD solvation model. For clarity, only geometries optimized in diethyl ether are pictured.

Cartesian Coordinates

Calculations in diethyl ether

Arg

C	2.14605	-0.58454	0.41274
H	1.99535	-1.31429	-0.38571
H	1.82848	-1.00697	1.36875
N	1.42897	0.65046	0.12431
H	1.95090	1.51534	0.08909
C	0.11637	0.72082	-0.05728
N	-0.46190	1.89574	-0.31169
H	0.09080	2.73373	-0.41776
H	-1.45787	1.96202	-0.46130
N	-0.62726	-0.38091	0.01562
H	3.20514	-0.34798	0.47091
H	-0.21972	-1.27826	0.23133
H	-1.62441	-0.33996	-0.13712

MMA 1

C	2.29701	-0.42896	0.57048
H	2.50108	-1.31580	-0.02923
H	1.81071	-0.71620	1.50175
N	1.46719	0.53760	-0.14962
H	1.94488	1.31134	-0.59290
C	0.14319	0.64475	-0.05199
N	-0.43886	1.82366	-0.29652
H	0.10176	2.67609	-0.31450
H	-1.43040	1.88284	-0.47672
N	-0.61529	-0.39771	0.28475
H	3.23898	0.06119	0.80919
H	-1.57509	-0.18842	0.52643
C	-0.28543	-1.80337	0.04604
H	0.35469	-1.89337	-0.83056
H	0.20271	-2.25842	0.90710
H	-1.21825	-2.32827	-0.15114

MMA 2

C	2.15262	-0.67860	0.36888
H	2.00600	-1.30829	-0.51087
H	1.80323	-1.19990	1.26302
N	1.46894	0.59856	0.20781
H	2.02137	1.44239	0.25395
C	0.15870	0.71646	0.01737
N	-0.38844	1.91878	-0.15581
H	-1.39164	1.96846	-0.26337
N	-0.60995	-0.37575	-0.01408
H	3.21462	-0.47552	0.47865
H	-0.23467	-1.29153	0.18123
H	-1.60734	-0.29603	-0.14744
C	0.37018	3.16174	-0.16666
H	1.13351	3.14355	-0.94810
H	-0.32261	3.97134	-0.38066
H	0.83590	3.34854	0.80398

MMA 3

C	2.14778	-0.59023	0.41355
H	2.06423	-1.28927	-0.42157
H	1.77356	-1.05594	1.32822
N	1.43945	0.65063	0.13231
H	1.97687	1.50215	0.04923
C	0.12465	0.73574	-0.05965

N	-0.44171	1.91320	-0.31877
H	0.16078	2.72197	-0.37952
N	-0.62966	-0.36133	0.00492
H	3.19705	-0.34592	0.55844
H	-0.22631	-1.26109	0.21893
H	-1.63154	-0.31271	-0.10541
C	-1.86358	2.08476	-0.58268
H	-2.46026	1.78958	0.28353
H	-2.04106	3.13886	-0.77893
H	-2.17085	1.51082	-1.46021

ADMA 1

C	2.19710	-0.54437	0.42949
H	2.52598	-1.28734	-0.29740
H	1.61272	-1.03011	1.20918
N	1.40864	0.51627	-0.19656
H	1.91911	1.32531	-0.52497
C	0.08066	0.65812	-0.11341
N	-0.43581	1.89333	-0.15135
H	0.16696	2.69867	-0.06637
H	-1.39489	2.05069	-0.42416
N	-0.72533	-0.39366	0.02746
C	-2.11732	-0.22678	0.44257
C	-0.37812	-1.72225	-0.48245
H	-2.20567	0.59054	1.15673
H	-2.76654	-0.04264	-0.41867
H	-2.43328	-1.14794	0.93040
H	-0.16611	-2.41405	0.33452
H	-1.23222	-2.09363	-1.05074
H	0.47624	-1.66435	-1.15194
H	3.07124	-0.08511	0.88934

ADMA 2

C	2.61923	0.25802	-0.00904
H	2.89259	-0.33601	-0.88445
H	2.84973	-0.29459	0.90413
N	1.21238	0.63380	-0.04141
H	0.98863	1.60339	-0.20744
C	0.21197	-0.24901	0.00096
N	0.50086	-1.55712	0.03290
H	1.46096	-1.86521	0.07023
H	-0.20578	-2.23768	0.26819
N	-1.05658	0.15829	-0.00212
C	-2.14282	-0.81860	-0.06654
C	-1.37112	1.58407	0.06518
H	-1.95133	-1.55337	-0.85015
H	-2.27958	-1.32529	0.89294
H	-3.05990	-0.29226	-0.31764
H	-1.14399	2.08685	-0.87922
H	-2.43367	1.68779	0.26753
H	-0.82466	2.06230	0.88032
H	3.20680	1.17247	-0.01332

SDMA 1

C	1.16588	1.80188	-0.20284
H	1.56525	1.38041	-1.12410
H	1.94800	1.83718	0.55565
N	0.00954	1.02282	0.23971
H	-0.76918	1.54218	0.62160

C	-0.19820	-0.26797	-0.01737
N	0.82116	-1.09080	-0.29109
N	-1.44431	-0.74854	-0.02744
H	0.57097	-1.98935	-0.68122
H	-1.55230	-1.75122	0.04025
C	2.17872	-0.96120	0.23963
H	2.54788	-1.96298	0.45238
H	2.16561	-0.39547	1.17024
H	2.84949	-0.48047	-0.47234
C	-2.64287	0.07684	0.02917
H	-2.79022	0.50557	1.02380
H	-3.49548	-0.55631	-0.20527
H	-2.58845	0.87451	-0.71380
H	0.82258	2.81426	-0.40695

SDMA 2

C	-1.66707	1.80226	0.00270
H	-2.20234	1.47314	0.89685
H	-2.20471	1.47781	-0.89177
N	-0.30081	1.29960	0.00031
H	0.45531	1.96796	-0.00638
C	0.00118	0.00046	-0.00015
N	-0.97479	-0.90854	0.00113
N	1.27679	-0.38893	-0.00107
H	-1.93015	-0.58322	-0.00001
H	1.47551	-1.37859	-0.00214
C	-0.73432	-2.34391	0.00278
H	-1.69906	-2.84416	0.00514
H	-0.18333	-2.64680	0.89639
H	-0.18634	-2.64961	-0.89177
C	2.39930	0.53830	0.00055
H	2.39047	1.16652	-0.89367
H	3.31513	-0.04689	0.00241
H	2.38702	1.16719	0.89422
H	-1.62472	2.88837	0.00556

CH₃COO

C	0.20010	0.00042	-0.00010
O	0.80478	-1.09503	0.00003
O	0.69883	1.14917	0.00003
C	-1.34373	-0.05120	0.00001
H	-1.71651	-1.07601	-0.00001
H	-1.72535	0.47380	-0.87988
H	-1.72523	0.47372	0.88001

EtOH

C	-1.20644	-0.24189	-0.02238
C	0.07998	0.55995	0.04714
H	-1.25590	-0.96893	0.79382
H	-2.07374	0.41767	0.06284
H	-1.27100	-0.78146	-0.96979
H	0.12245	1.28535	-0.76721
H	0.13132	1.11682	0.99021
O	1.23524	-0.25700	-0.10600
H	1.22370	-0.92184	0.58958

N-methylacetamide

C	-0.48300	0.14712	-0.00600
O	-0.37289	1.39429	-0.00055
N	0.60571	-0.65834	-0.00620
H	0.48739	-1.65745	-0.00086
C	1.95250	-0.10487	0.00416

H	2.10417	0.55273	-0.85115
C	-1.82454	-0.53274	0.00257
H	-2.44970	-0.09330	-0.77228
H	-2.31115	-0.35318	0.96133
H	-1.75487	-1.60607	-0.16121
H	2.12969	0.47515	0.90991
H	2.66788	-0.92092	-0.04228

p-methylphenol

C	-1.37491	-0.01282	0.00541
C	-0.66402	1.19206	0.00475
C	0.72316	1.21779	0.00059
C	1.43542	0.01960	-0.00166
C	0.75145	-1.19114	0.00073
C	-0.64210	-1.19747	0.00462
H	-1.20960	2.13081	0.00812
H	1.26749	2.15507	0.00133
H	1.30288	-2.12669	0.00167
H	-1.16407	-2.14892	0.00793
O	2.79667	0.09539	-0.00389
H	3.17066	-0.79256	-0.00379
C	-2.88179	-0.01709	-0.00613
H	-3.27276	-1.02231	0.15920
H	-3.26761	0.33973	-0.96535
H	-3.28360	0.63626	0.77211

6OI0 Arg

C	1.96944	0.02200	-0.01750
N	0.66818	-0.61094	0.00255
C	-0.52274	-0.00650	0.01171
N	-0.68807	1.32550	0.02397
N	-1.53002	-0.77923	-0.01255
H	2.25131	0.41008	0.96232
H	2.69916	-0.73181	-0.30816
H	0.65814	-1.60946	0.21415
H	0.09614	1.97465	0.03333
H	-1.61022	1.70371	-0.13225
H	-1.44247	-1.78074	-0.14840
H	-2.47464	-0.43184	0.07408
H	1.99181	0.82500	-0.75809

6OI0 S49B

C	-1.84293	-0.51883	0.02055
C	-0.46183	0.14397	-0.00708
O	-0.36902	1.36827	-0.01013
N	0.60571	-0.65209	-0.06602
C	1.95457	-0.10208	0.03095
H	-2.46178	-0.05062	-0.74475
H	2.14077	0.58329	-0.79684
H	0.47898	-1.64907	0.01672
H	2.08634	0.44103	0.96892
H	2.66687	-0.92368	-0.01613
H	-2.29457	-0.32472	0.99539
H	-1.80322	-1.59604	-0.14675

6OI0 S91BD92

C	-3.44561	-0.32986	-0.39103
C	-2.02276	-0.17658	0.14773
O	-1.52986	-1.07828	0.80392
N	-1.34109	0.93050	-0.15166
C	0.06151	1.05545	0.26647
C	0.95602	0.20569	-0.66993

C	2.30879	-0.14099	-0.08315
O	2.56910	0.14600	1.10696
O	3.12742	-0.74702	-0.81939
H	-4.10713	-0.56813	0.44279
H	1.09949	0.71932	-1.62600
H	0.44271	-0.73066	-0.90199
H	0.15641	0.69016	1.28631
H	-1.76165	1.62859	-0.74593
H	-3.44883	-1.17267	-1.08447
H	0.33434	2.10997	0.24149
H	-3.80872	0.56208	-0.90306

6OI0 F133B

C	-1.81698	-0.54703	0.03767
C	-0.46295	0.15001	-0.03431
O	-0.40451	1.38305	-0.01308
N	0.60150	-0.62972	-0.08833
C	1.96253	-0.10572	0.04764
H	-2.50770	-0.04499	-0.63960
H	2.04725	0.87181	-0.42341
H	0.46729	-1.62821	-0.03594
H	2.65610	-0.79549	-0.43186
H	2.23202	0.00080	1.10206
H	-2.19802	-0.43548	1.05518
H	-1.76699	-1.60842	-0.20954

6OI0 S175B

C	-1.83841	-0.52219	0.00851
C	-0.46604	0.13705	-0.01555
O	-0.39729	1.35950	-0.00177
N	0.60687	-0.65075	-0.01284
C	1.97909	-0.08725	0.00999
H	-2.43077	-0.11101	-0.80926
H	2.14168	0.54446	-0.86352
H	0.48934	-1.65203	-0.02225
H	-2.32419	-0.25369	0.94804
H	-1.80225	-1.60937	-0.07809
H	2.68457	-0.91533	0.00102
H	2.12403	0.51050	0.91042

6OI0 C261B

C	-1.80209	-0.56412	0.01572
C	-0.46707	0.16222	-0.01711
O	-0.42125	1.38721	-0.00483
N	0.60244	-0.61802	-0.03765
C	1.96499	-0.10685	0.02122
H	-1.71261	-1.63720	-0.15290
H	2.14438	0.59045	-0.79827
H	0.46740	-1.61702	-0.01769
H	-2.45796	-0.12990	-0.74028
H	-2.25918	-0.39226	0.99260
H	2.14366	0.41276	0.96474
H	2.65226	-0.94581	-0.06509

6OI1 MMA

C	-2.44746	-0.12032	0.00564
C	2.46238	-0.09991	0.00006
N	1.13679	-0.65048	-0.01390
C	-0.00114	0.06747	-0.00851
N	-0.02845	1.35519	0.00585
N	-1.11458	-0.65259	-0.01409
H	-3.13488	-0.94938	0.17813

H	-2.57694	0.58185	0.83555
H	-2.71407	0.37357	-0.93250
H	2.71759	0.34234	0.96639
H	3.16481	-0.90837	-0.19443
H	0.81391	1.93040	0.04668
H	2.58090	0.65156	-0.78559
H	1.03086	-1.65025	0.14950
H	-1.00837	-1.66033	-0.05310
H	-0.91280	1.84036	-0.03884

6OI1 S49B

C	-1.83045	-0.51797	0.02457
C	-0.46276	0.13602	-0.02737
O	-0.37775	1.36608	-0.00839
N	0.61153	-0.65606	-0.05886
C	1.94478	-0.09316	0.03310
H	-2.48835	-0.00736	-0.67815
H	2.13595	0.57414	-0.80896
H	0.48992	-1.65596	-0.01645
H	-1.80715	-1.58493	-0.20340
H	2.66884	-0.90589	0.01515
H	-2.23092	-0.38144	1.03161
H	2.06362	0.47585	0.95758

6OI1 S91BD92

C	-3.46317	-0.24977	-0.45818
C	-2.02889	-0.20231	0.11363
O	-1.55365	-1.21091	0.64784
N	-1.34840	0.92918	0.00332
C	0.05088	1.00091	0.43911
C	0.95834	0.34116	-0.62866
C	2.32532	-0.12960	-0.09884
O	2.59742	-0.07192	1.11840
O	3.14643	-0.58221	-0.94447
H	-4.14202	-0.54172	0.34416
H	1.12601	1.02689	-1.46482
H	0.45008	-0.53065	-1.04996
H	0.15010	0.46522	1.38081
H	-1.75951	1.70188	-0.49894
H	-3.78106	0.70408	-0.88021
H	0.30579	2.04792	0.59956
H	-3.48711	-1.01991	-1.23052

6OI1 F133B

C	-1.82193	-0.54289	0.04313
C	-0.45747	0.15262	-0.04731
O	-0.38095	1.39471	-0.01280
N	0.59596	-0.63550	-0.09526
C	1.94674	-0.11693	0.05350
H	-2.53710	-0.00385	-0.57747
H	2.05032	0.84610	-0.44313
H	0.45425	-1.63326	-0.04808
H	-1.78460	-1.59012	-0.26067
H	-2.15639	-0.48388	1.08127
H	2.64992	-0.82233	-0.38853
H	2.19544	0.02142	1.10982

6OI1 S175B

C	-1.87931	-0.47291	0.00949
C	-0.45705	0.13750	-0.01711
O	-0.30657	1.34590	-0.00229
N	0.57195	-0.67315	-0.01340

C	1.96769	-0.10876	0.01097
H	-2.45447	-0.03081	-0.80412
H	2.12365	0.52351	-0.86185
H	0.44625	-1.67349	-0.02450
H	-1.87923	-1.55945	-0.08579
H	2.66414	-0.94427	-0.00009
H	2.10586	0.48524	0.91373
H	-2.34524	-0.19075	0.95470

6OI1 C261B

C	-1.81113	-0.57015	0.01840
C	-0.46743	0.17185	-0.01830
O	-0.41477	1.39006	-0.00552
N	0.60525	-0.60581	-0.04548
C	1.96363	-0.11894	0.02506
H	2.14363	0.62334	-0.75408
H	0.46297	-1.60393	-0.01869
H	2.64123	-0.95769	-0.12064
H	2.16698	0.34346	0.99388
H	-2.25481	-0.41626	1.00426
H	-2.47652	-0.12648	-0.72349
H	-1.71248	-1.63879	-0.16960

6OI2 SDMA

C	-2.43322	-0.70760	0.00123
N	-1.33865	0.24683	-0.01102
C	-0.02708	-0.02354	-0.00936
N	0.81227	0.91519	-0.00586
N	0.47334	-1.25535	-0.00076
C	0.58203	2.33061	-0.01006
C	1.89865	-1.53268	0.01252
H	-2.53320	-1.20103	0.96901
H	-3.34806	-0.15379	-0.19934
H	0.07890	2.65473	-0.92168
H	-0.02636	2.62973	0.84850
H	1.53808	2.84245	0.06747
H	2.40183	-1.14406	-0.87804
H	2.37557	-1.12824	0.90970
H	2.02002	-2.61516	0.02808
H	-2.29941	-1.45010	-0.78910
H	-1.59483	1.20943	0.20216
H	1.78730	0.66110	0.00375
H	-0.15094	-2.05239	-0.08303

6OI2 S49B

C	-1.84483	-0.52849	0.02135
C	-0.46304	0.14159	-0.00456
O	-0.39226	1.36790	-0.01029
N	0.61839	-0.64877	-0.07252
C	1.96606	-0.09420	0.03238
H	-2.44252	-0.10214	-0.78436
H	2.13901	0.61998	-0.77338
H	0.49489	-1.64420	0.03304
H	-2.32005	-0.28288	0.97262
H	-1.79839	-1.61275	-0.08920
H	2.68193	-0.90974	-0.05143
H	2.10535	0.41649	0.98768

6OI2 S91BD92

C	3.43913	-0.02905	0.54454
C	2.04389	-0.23384	-0.03588
O	1.61948	-1.37803	-0.18151

N	1.33009	0.84953	-0.34205
C	-0.06574	0.72674	-0.78989
C	-1.00782	0.63840	0.42340
C	-2.32131	-0.08241	0.13976
O	-2.58958	-0.55858	-0.98241
O	-3.11639	-0.18178	1.10288
H	4.15370	-0.57977	-0.06889
H	-1.23890	1.63533	0.81166
H	-0.50366	0.10463	1.23373
H	-0.15004	-0.18226	-1.38113
H	1.70389	1.75972	-0.11890
H	3.73515	1.01978	0.59449
H	3.44959	-0.45569	1.54871
H	-0.29727	1.57959	-1.42847

6OI2 F133B

C	-1.83742	-0.53164	0.03558
C	-0.46447	0.14848	-0.04062
O	-0.38205	1.38082	-0.01045
N	0.59642	-0.64504	-0.07641
C	1.97037	-0.10690	0.04333
H	-2.51673	-0.02847	-0.65216
H	2.01684	0.89910	-0.36780
H	0.46229	-1.64381	-0.03344
H	-2.21765	-0.39919	1.05061
H	-1.79965	-1.59695	-0.19672
H	2.27314	-0.06523	1.09259
H	2.65239	-0.75638	-0.50441

6OI2 S175B

C	-1.85455	-0.51494	0.00669
C	-0.46438	0.14370	-0.01030
O	-0.36993	1.36152	-0.00168
N	0.59271	-0.64647	-0.01181
C	1.98268	-0.10308	0.00812
H	-2.42900	-0.11821	-0.83080
H	2.14783	0.53041	-0.86329
H	0.46884	-1.64721	-0.01395
H	2.13978	0.48590	0.91202
H	2.67080	-0.94522	-0.00982
H	-1.81707	-1.60328	-0.05734
H	-2.35322	-0.22339	0.93217

6OI2 C261B

C	-1.82144	-0.56812	0.01887
C	-0.46947	0.16520	-0.02169
O	-0.42042	1.38804	-0.00433
N	0.61183	-0.61050	-0.04220
C	1.97477	-0.11273	0.02407
H	-1.72661	-1.64688	-0.10245
H	2.16661	0.59101	-0.78798
H	0.47443	-1.60959	-0.02018
H	2.15752	0.39720	0.97241
H	2.65436	-0.95726	-0.06788
H	-2.45646	-0.16221	-0.77031
H	-2.29234	-0.34921	0.97890

6OI3 MMA H3R2me Peptide

C	-2.45521	-0.05842	0.05220
C	2.44250	-0.05926	0.08218
N	1.14898	-0.68794	-0.12989
C	0.00392	0.00157	-0.05100

N	0.00929	1.33306	-0.03039
N	-1.14020	-0.67593	0.01450
H	-3.19921	-0.84832	0.14522
H	-2.66396	0.52487	-0.84888
H	-2.54508	0.58964	0.92968
H	2.52107	0.38120	1.07894
H	3.20430	-0.82922	-0.01700
H	0.86526	1.88441	-0.02088
H	2.62373	0.70938	-0.67173
H	1.06670	-1.66059	0.16112
H	-0.86582	1.83327	-0.06900
H	-1.08071	-1.67229	-0.16734

6OI3 S49B

C	-1.82134	-0.52874	0.03871
C	-0.47439	0.15269	-0.02868
O	-0.37189	1.37475	-0.01448
N	0.60595	-0.64986	-0.09853
C	1.94432	-0.10418	0.04852
H	-2.47826	-0.09692	-0.71740
H	2.19139	0.53836	-0.79950
H	0.47604	-1.64738	-0.03035
H	2.00780	0.48656	0.96626
H	2.66119	-0.92040	0.11844
H	-2.25333	-0.31932	1.01989
H	-1.76289	-1.60848	-0.10308

6OI3 S91BD92

C	-3.50303	-0.26811	-0.38132
C	-2.11147	-0.13154	0.18522
O	-1.73884	-0.82840	1.13357
N	-1.30759	0.77768	-0.38641
C	0.08615	0.94135	0.02716
C	0.99433	-0.08549	-0.63940
C	2.41707	-0.14365	-0.03769
O	2.69163	0.69090	0.85082
O	3.16021	-1.04283	-0.49910
H	-4.22606	-0.14500	0.42666
H	1.08385	0.12284	-1.71182
H	0.54320	-1.08030	-0.56540
H	0.12522	0.82177	1.10686
H	-1.64252	1.27214	-1.20012
H	0.39388	1.95933	-0.21176
H	-3.71395	0.45341	-1.17063
H	-3.61276	-1.28066	-0.77501

6OI3 F133B

C	-1.82068	-0.54095	0.00945
C	-0.47868	0.15669	-0.00712
O	-0.39027	1.37963	-0.00194
N	0.60692	-0.63986	-0.02729
C	1.95887	-0.10496	0.01057
H	-2.37723	-0.24853	-0.88285
H	2.08972	0.69771	-0.71597
H	0.47488	-1.63808	0.02126
H	2.65930	-0.90864	-0.21526
H	2.18687	0.29827	1.00225
H	-2.37990	-0.19612	0.88059
H	-1.73697	-1.62730	0.03911

6OI3 S175B

C	1.81753	-0.54696	0.01304
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C	0.47811	0.15705	-0.00860
O	0.39918	1.38042	-0.00376
N	-0.60981	-0.63639	-0.03523
C	-1.96025	-0.10316	0.01886
H	2.33183	-0.27948	0.93840
H	-2.29854	0.09458	1.04014
H	-0.48142	-1.63534	0.01426
H	1.73313	-1.63211	-0.05083
H	2.41887	-0.18065	-0.82033
H	-2.64209	-0.82262	-0.43445
H	-1.99885	0.82545	-0.55030

6OI3 C261B

C	-1.82641	-0.52737	0.00292
C	-0.47827	0.15060	-0.00606
O	-0.36763	1.37657	-0.00030
N	0.60177	-0.64661	-0.00389
H	-1.75548	-1.61462	-0.02153
H	0.47122	-1.64647	-0.00712
H	-2.36213	-0.21939	0.90255
C	1.95121	-0.10720	0.00320
H	-2.39779	-0.18200	-0.86040
H	2.12700	0.50267	-0.88493
H	2.11134	0.51421	0.88622
H	2.65533	-0.93686	0.01441

ADMA – CH₃COO 1

C	-1.28920	0.09674	-0.23580
N	-2.50077	-0.39631	0.09788
N	-0.34774	-0.66432	-0.76195
H	0.76739	-0.45345	-0.76553
H	-0.60162	-1.62106	-0.95991
N	-1.06513	1.41294	-0.06143
H	-1.66222	1.90769	0.58306
C	0.20304	2.04243	-0.40797
H	0.47285	1.78252	-1.43192
H	0.06541	3.12057	-0.34358
H	1.00443	1.72624	0.26248
C	2.78498	-0.27473	0.23621
O	2.25195	-0.02950	1.32467
O	2.16317	-0.41511	-0.87332
C	4.29642	-0.43293	0.14917
H	4.53380	-1.44939	-0.17426
H	4.69543	0.24952	-0.60434
H	4.76645	-0.23816	1.11210
C	-2.72774	-1.83623	0.08035
H	-1.92308	-2.36024	0.60044
H	-3.65790	-2.04570	0.60334
H	-2.80965	-2.22095	-0.94143
C	-3.58902	0.49484	0.47974
H	-3.47020	0.86822	1.50254
H	-3.65273	1.34048	-0.20795
H	-4.52475	-0.05633	0.42245

ADMA – CH₃COO 2

C	1.40355	-0.17165	-0.21003
N	0.49076	-1.02099	-0.64499
N	1.16746	1.14922	-0.21008
N	2.60016	-0.60613	0.25036
H	3.35770	0.05892	0.28019
C	2.95471	-2.01379	0.30750
H	3.00844	-2.46781	-0.68709

H	3.93133	-2.09572	0.78017
H	2.23437	-2.56237	0.91734
H	0.76651	-1.99205	-0.67929
C	-0.00447	1.70482	-0.88026
H	0.26235	2.68817	-1.26917
H	-0.29547	1.06680	-1.71270
H	-0.84460	1.78823	-0.18756
O	-2.01809	-0.74966	-0.76975
C	-2.61532	-0.27021	0.25574
O	-2.05472	0.21382	1.24518
C	-4.13541	-0.32138	0.19172
H	-4.48122	0.22696	-0.68766
H	-4.46026	-1.35801	0.07674
H	-4.58000	0.10542	1.08958
H	-0.62666	-0.82032	-0.66919
C	2.02869	2.07504	0.51209
H	2.36962	1.63503	1.45035
H	2.89468	2.37880	-0.08625
H	1.44657	2.96303	0.75356

ADMA – CH₃COO 3

C	-1.59213	0.38046	-0.01305
N	-1.61570	-0.94139	-0.23211
N	-2.67916	0.95657	0.55477
H	-2.70613	1.95914	0.65331
H	-3.57221	0.48981	0.52538
N	-0.53630	1.10684	-0.32686
H	0.45872	0.67557	-0.48587
C	-0.51665	2.54519	-0.12718
H	-0.61175	2.81516	0.93002
H	0.44544	2.90831	-0.48418
H	-1.30458	3.04387	-0.69923
C	-2.68486	-1.76592	0.31756
H	-2.36050	-2.80369	0.29726
H	-2.88864	-1.49838	1.35637
H	-3.60583	-1.67993	-0.26884
C	-0.50907	-1.59245	-0.93100
H	-0.17040	-0.96737	-1.75715
H	0.33093	-1.76909	-0.25536
H	-0.87462	-2.53525	-1.33426
C	2.48232	-0.18410	0.23064
O	1.90649	0.43606	-0.72371
O	1.90803	-0.68434	1.20825
C	3.99695	-0.30368	0.10648
H	4.24063	-0.87852	-0.79048
H	4.43607	0.68898	-0.01456
H	4.42556	-0.79353	0.97983

ADMA – CH₃COO 4

C	1.91223	0.26200	0.02459
N	2.48969	1.27865	0.69460
N	0.70598	0.40049	-0.49860
N	2.61093	-0.86956	-0.13986
H	2.15476	-1.64381	-0.59675
C	3.94230	-1.08170	0.40446
H	3.93866	-1.06035	1.49784
H	4.28063	-2.06362	0.08235
H	4.64283	-0.33458	0.02416
H	3.30188	1.10097	1.26573
C	-0.05092	1.63643	-0.29359
H	-1.04119	1.49113	-0.71994
H	-0.18854	1.82847	0.77266

H	0.45754	2.47895	-0.77276
O	-1.89955	-0.39248	0.92257
C	-2.90840	-0.19317	0.20827
O	-2.91667	0.31839	-0.94178
C	-4.27698	-0.60465	0.76956
H	-4.75067	-1.31749	0.08969
H	-4.18814	-1.04962	1.76078
H	-4.92709	0.27266	0.82097
H	1.93419	2.08046	0.95009
C	0.11717	-0.68552	-1.28136
H	0.71036	-0.86933	-2.18275
H	0.04374	-1.59454	-0.68092
H	-0.89784	-0.39396	-1.54209

ADMA – CH₃COO 5

C	-1.59196	0.38043	-0.01283
N	-2.67922	0.95640	0.55488
N	-1.61525	-0.94148	-0.23162
N	-0.53618	1.10685	-0.32647
H	0.45900	0.67572	-0.48566
C	-0.51675	2.54526	-0.12722
H	-0.61224	2.81553	0.92986
H	0.44542	2.90838	-0.48402
H	-1.30454	3.04372	-0.69968
H	-2.70663	1.95910	0.65219
C	-2.68454	-1.76620	0.31744
H	-2.35914	-2.80370	0.29928
H	-2.89023	-1.49735	1.35551
H	-3.60465	-1.68194	-0.27059
O	1.90660	0.43667	-0.72363
C	2.48212	-0.18396	0.23060
O	1.90758	-0.68426	1.20803
C	3.99674	-0.30407	0.10663
H	4.24031	-0.87922	-0.79018
H	4.43630	0.68837	-0.01451
H	4.42502	-0.79398	0.98011
H	-3.57229	0.48974	0.52404
C	-0.50906	-1.59228	-0.93136
H	-0.17097	-0.96705	-1.75766
H	0.33139	-1.76902	-0.25630
H	-0.87478	-2.53502	-1.33462

ADMA – CH₃COO 6

C	1.40348	-0.17163	-0.21001
N	0.49082	-1.02099	-0.64526
N	1.16730	1.14920	-0.20998
N	2.60002	-0.60615	0.25050
H	3.35754	0.05893	0.28057
C	2.95460	-2.01380	0.30754
H	3.00857	-2.46769	-0.68709
H	3.93111	-2.09577	0.78045
H	2.23415	-2.56248	0.91716
H	0.76650	-1.99208	-0.67927
C	-0.00449	1.70478	-0.88044
H	0.26235	2.68824	-1.26908
H	-0.29513	1.06688	-1.71311
H	-0.84485	1.78796	-0.18800
O	-2.05423	0.21384	1.24495
C	-2.61514	-0.27019	0.25566
O	-2.01821	-0.74950	-0.77005
C	-4.13525	-0.32153	0.19223
H	-4.46003	-1.35824	0.07777

H	-4.57953	0.10553	1.09013
H	-4.48147	0.22646	-0.68720
H	-0.62642	-0.82031	-0.66949
C	2.02843	2.07509	0.51221
H	2.36965	1.63498	1.45031
H	2.89422	2.37918	-0.08625
H	1.44613	2.96288	0.75399

ADMA – CH₃COO 7

C	-1.46565	-0.17952	0.02183
N	-0.43177	-0.99692	0.05267
N	-1.29462	1.15149	-0.03202
N	-2.73353	-0.65857	0.02408
H	-3.46382	-0.03307	0.32878
C	-3.03095	-2.08044	0.05726
H	-2.70296	-2.55208	0.98894
H	-4.10858	-2.20094	-0.03346
H	-2.55946	-2.58483	-0.78809
H	-0.63563	-1.97366	0.20813
C	0.01130	1.75821	0.21040
H	-0.14369	2.68860	0.75904
H	0.63929	1.09873	0.80854
H	0.52332	1.97733	-0.73053
O	2.61363	0.01645	1.26532
C	2.82265	-0.26761	0.08094
O	1.92378	-0.61028	-0.76425
C	4.23873	-0.22108	-0.47442
H	4.27767	0.47157	-1.31824
H	4.94876	0.08966	0.29058
H	4.51337	-1.20888	-0.85162
H	0.63295	-0.75050	-0.26242
C	-2.40310	2.05140	-0.31615
H	-3.07810	1.61643	-1.05435
H	-2.96527	2.30448	0.58958
H	-1.99561	2.97013	-0.73586

ADMA – CH₃COO 8

C	-1.64079	0.39311	0.00081
N	-2.79076	1.10553	-0.14084
N	-1.72634	-0.94427	0.03291
N	-0.48882	1.01461	0.13794
H	0.45459	0.52619	0.40750
C	-0.37373	2.45642	0.00983
H	-0.90985	2.98318	0.80579
H	0.68405	2.70193	0.08989
H	-0.73214	2.80756	-0.96230
H	-2.70797	2.09482	-0.32011
C	-3.02235	-1.60597	0.12215
H	-2.86196	-2.62266	0.47435
H	-3.67111	-1.09943	0.83927
H	-3.52218	-1.65231	-0.85124
O	2.24143	-0.34037	-1.28348
C	2.60233	-0.15581	-0.11212
O	1.83837	0.20610	0.84318
C	4.06081	-0.37776	0.27004
H	4.12155	-1.18227	1.00716
H	4.66045	-0.63653	-0.60160
H	4.45958	0.52407	0.73967
H	-3.58528	0.66108	-0.57647
C	-0.51676	-1.76388	0.05825
H	0.25848	-1.31867	-0.56716
H	-0.13434	-1.87575	1.07651

H	-0.76782	-2.74482	-0.34271
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ADMA – CH₃COO 9

C	-1.59215	0.38059	-0.01308
N	-2.67905	0.95691	0.55480
N	-1.61595	-0.94127	-0.23209
N	-0.53617	1.10672	-0.32694
H	0.45876	0.67517	-0.48600
C	-0.51603	2.54504	-0.12716
H	-0.61059	2.81495	0.93011
H	0.44599	2.90793	-0.48460
H	-1.30408	3.04398	-0.69882
H	-2.70569	1.95947	0.65357
C	-2.68531	-1.76555	0.31754
H	-2.36133	-2.80343	0.29691
H	-2.88884	-1.49819	1.35645
H	-3.60634	-1.67910	-0.26869
O	1.90656	0.43588	-0.72381
C	2.48233	-0.18426	0.23059
O	1.90794	-0.68467	1.20805
C	3.99699	-0.30371	0.10666
H	4.24079	-0.87904	-0.78997
H	4.43604	0.68891	-0.01487
H	4.42554	-0.79310	0.98030
H	-3.57222	0.49040	0.52536
C	-0.50932	-1.59248	-0.93085
H	-0.17096	-0.96779	-1.75743
H	0.33087	-1.76855	-0.25528
H	-0.87476	-2.53557	-1.33353

ADMA – CH₃COO 10

C	1.31841	-0.16330	0.24231
N	0.41704	-0.95584	0.74971
N	1.06819	1.17092	0.11819
N	2.52587	-0.59207	-0.24489
H	3.27806	0.08096	-0.24237
C	2.92222	-1.98498	-0.14922
H	2.20965	-2.61727	-0.68252
H	3.89432	-2.09562	-0.62692
H	2.99934	-2.33202	0.88713
H	0.76284	-1.90099	0.87139
C	0.01136	1.80374	0.89248
H	0.38002	2.75786	1.27610
H	-0.88109	1.99555	0.28718
H	-0.26609	1.17219	1.73341
O	-2.16316	-0.60963	0.97636
C	-2.79056	-0.33311	-0.15261
O	-3.97527	-0.08516	-0.17001
C	-1.94871	-0.35810	-1.41015
H	-2.57161	-0.11706	-2.26869
H	-1.12710	0.35852	-1.33489
H	-1.50609	-1.34847	-1.53915
H	-1.14952	-0.73907	0.85745
C	1.92267	2.05237	-0.65921
H	2.78013	2.41861	-0.08073
H	2.28218	1.55046	-1.55794
H	1.33248	2.91393	-0.97284

SDMA – CH₃COO 1

C	1.50543	0.46006	-0.11099
N	0.70669	-0.49198	-0.53524
N	2.78426	0.21398	0.27065

H	3.35610	1.03533	0.40561
N	1.09241	1.73906	-0.09219
H	1.56981	2.36378	0.54083
C	-0.17180	2.19046	-0.65836
H	-1.01264	1.92369	-0.01610
H	-0.11670	3.27189	-0.77517
H	-0.31493	1.73499	-1.63857
C	1.16988	-1.79130	-1.00028
H	0.43641	-2.15547	-1.71939
H	2.13601	-1.70340	-1.49983
H	1.24702	-2.52187	-0.19217
H	-0.38151	-0.36392	-0.57879
C	3.19096	-0.97918	1.01161
H	2.35475	-1.37782	1.58708
H	3.57883	-1.75784	0.35369
H	3.97727	-0.68942	1.70713
O	-1.85190	-0.51564	-0.74994
C	-2.50589	-0.25468	0.31316
O	-2.02424	0.23139	1.34690
C	-3.99421	-0.57839	0.25662
H	-4.12643	-1.64569	0.06389
H	-4.49130	-0.31056	1.18802
H	-4.45330	-0.03894	-0.57505

SDMA – CH₃COO 2

C	-1.50546	0.46005	-0.11093
N	-0.70659	-0.49173	-0.53556
N	-2.78418	0.21355	0.27082
H	-3.35630	1.03470	0.40583
N	-1.09269	1.73910	-0.09190
H	-1.57034	2.36370	0.54105
C	0.17143	2.19082	-0.65802
H	0.31435	1.73605	-1.63858
H	0.11636	3.27234	-0.77403
H	1.01237	1.92357	-0.01609
C	-1.16959	-1.79105	-1.00078
H	-0.43660	-2.15460	-1.72069
H	-1.24572	-2.52200	-0.19292
H	-2.13618	-1.70335	-1.49947
H	0.38153	-0.36351	-0.57913
C	-3.19044	-0.97980	1.01171
H	-3.57939	-1.75801	0.35389
H	-2.35363	-1.37899	1.58590
H	-3.97574	-0.69010	1.70840
O	1.85214	-0.51499	-0.75033
C	2.50580	-0.25455	0.31307
O	2.02385	0.23105	1.34690
C	3.99410	-0.57855	0.25688
H	4.49135	-0.30931	1.18779
H	4.12598	-1.64623	0.06604
H	4.45324	-0.04072	-0.57579

SDMA – CH₃COO 3

C	1.50542	0.46006	-0.11100
N	0.70672	-0.49203	-0.53522
N	2.78426	0.21404	0.27066
H	3.35607	1.03543	0.40558
N	1.09234	1.73904	-0.09224
H	1.56968	2.36379	0.54081
C	-0.17185	2.19039	-0.65848
H	-1.01269	1.92376	-0.01617
H	-0.11671	3.27180	-0.77549

H	-0.31500	1.73475	-1.63860
C	1.16994	-1.79135	-1.00021
H	0.43645	-2.15561	-1.71924
H	2.13603	-1.70342	-1.49984
H	1.24720	-2.52186	-0.19206
H	-0.38150	-0.36400	-0.57874
C	3.19100	-0.97908	1.01164
H	2.35485	-1.37765	1.58724
H	3.57877	-1.75780	0.35374
H	3.97740	-0.68930	1.70705
O	-2.02431	0.23160	1.34685
C	-2.50590	-0.25467	0.31317
O	-1.85185	-0.51580	-0.74985
C	-3.99420	-0.57843	0.25662
H	-4.49128	-0.31075	1.18807
H	-4.45330	-0.03884	-0.57495
H	-4.12640	-1.64569	0.06371

SDMA – CH₃COO 4

C	1.63240	0.39900	0.02230
N	0.55671	-0.21017	0.45650
N	2.81300	-0.24640	-0.15789
H	3.61082	0.35270	-0.31400
N	1.61611	1.72439	-0.21862
H	2.32068	2.08560	-0.84434
C	0.50591	2.59723	0.13347
H	0.16170	2.36393	1.14153
H	0.86678	3.62429	0.10944
H	-0.33868	2.48499	-0.54778
C	0.57039	-1.50323	1.12633
H	-0.29595	-1.53141	1.78639
H	0.47866	-2.33351	0.42551
H	1.47788	-1.61665	1.72211
H	-0.45141	0.22576	0.34195
C	2.91785	-1.60860	-0.67883
H	3.01674	-2.34619	0.11839
H	2.04691	-1.85461	-1.28706
H	3.80308	-1.65842	-1.31154
O	-1.85745	0.64297	0.26337
C	-2.58275	-0.31874	-0.16551
O	-2.17066	-1.45007	-0.44953
C	-4.06204	0.01068	-0.32558
H	-4.62404	-0.85989	-0.66105
H	-4.46359	0.36599	0.62608
H	-4.17594	0.82134	-1.04941

SDMA – CH₃COO 5

C	-1.57471	0.45189	-0.01494
N	-0.65405	-0.47055	0.14597
N	-2.87497	0.13781	-0.22645
H	-3.46721	0.89621	-0.53154
N	-1.24543	1.75500	-0.00869
H	-1.98075	2.41602	0.19456
C	0.12572	2.24611	-0.05393
H	0.71680	1.61858	-0.72325
H	0.10522	3.26548	-0.43704
H	0.59183	2.23695	0.93350
C	-0.76918	-1.83242	-0.35677
H	0.23645	-2.14598	-0.63921
H	-1.15674	-2.52360	0.39480
H	-1.40495	-1.86833	-1.24250
H	0.35178	-0.23272	0.48908

C	-3.53927	-1.02222	0.35997
H	-3.57175	-1.86919	-0.32697
H	-3.03163	-1.32468	1.27643
H	-4.56004	-0.73703	0.61155
O	1.78073	-0.23853	0.96681
C	2.57638	-0.27494	-0.02809
O	2.22984	-0.23427	-1.21882
C	4.05732	-0.36315	0.32149
H	4.66758	-0.46817	-0.57455
H	4.35340	0.54117	0.85925
H	4.22907	-1.20963	0.98975

SDMA – CH₃COO 6

C	1.23299	0.19856	0.05364
N	2.57022	0.34037	0.15907
N	0.67894	-1.00890	-0.07197
H	-0.35097	-1.01725	-0.26475
N	0.44866	1.26999	0.06281
H	-0.58856	1.11913	0.19167
C	0.94188	2.62891	-0.05129
H	1.61906	2.73595	-0.90312
H	0.08475	3.28099	-0.21077
H	1.45413	2.96013	0.85805
C	3.54881	-0.58809	-0.39842
H	4.41863	-0.01159	-0.71012
H	3.86864	-1.33662	0.32765
H	3.13675	-1.08929	-1.27466
H	2.90392	1.26761	0.37499
C	1.24254	-2.23114	0.48669
H	1.84395	-2.78356	-0.23811
H	1.85159	-2.01715	1.36686
H	0.41014	-2.86443	0.79326
O	-2.19005	0.94540	0.37807
C	-2.67264	-0.12681	-0.07254
O	-1.99892	-1.09604	-0.50277
C	-4.18626	-0.26824	-0.06088
H	-4.51704	-0.97374	-0.82243
H	-4.48575	-0.65479	0.91774
H	-4.66605	0.70024	-0.20123

SDMA – CH₃COO 7

C	1.10737	0.07519	0.48253
N	1.63894	0.55197	-0.64569
N	0.32734	0.82021	1.26569
H	-0.29442	0.31465	1.88226
N	1.39503	-1.17459	0.86826
H	1.11957	-1.43607	1.80358
C	1.74139	-2.23142	-0.07442
H	2.79100	-2.17371	-0.37407
H	1.58238	-3.18685	0.42203
H	1.08123	-2.16525	-0.94148
C	0.94552	1.46692	-1.56158
H	1.15890	1.13293	-2.57579
H	-0.13134	1.39660	-1.40380
H	1.30065	2.49172	-1.44307
H	2.33628	-0.04441	-1.06744
C	0.14219	2.26234	1.17882
H	1.06824	2.72973	0.84727
H	-0.67649	2.50923	0.50125
H	-0.08792	2.62863	2.17877
C	-1.80198	-0.37900	-0.47654
C	-2.45356	-1.18411	0.66124

H	-3.26954	-1.78127	0.24294
H	-1.73596	-1.88202	1.10103
H	-2.87261	-0.53026	1.42808
O	-0.96065	-0.99364	-1.17629
O	-2.16245	0.81285	-0.60867

ADMA – EtOH 1

C	-1.76212	0.28201	-0.00122
N	-2.28256	1.41127	0.49535
N	-0.48999	0.24608	-0.39623
N	-2.53872	-0.80039	-0.09738
H	-2.16409	-1.61510	-0.55874
C	-3.93281	-0.83462	0.32275
H	-4.54029	-0.13597	-0.25773
H	-4.30588	-1.84155	0.15392
H	-4.02230	-0.60952	1.38780
H	-3.24083	1.44737	0.80735
C	0.31339	1.46971	-0.38311
H	1.30316	1.22711	-0.76234
H	-0.13520	2.23617	-1.02061
H	0.42381	1.85645	0.63399
C	0.07389	-0.99230	-0.93610
H	-0.01527	-1.80484	-0.20996
H	-0.42251	-1.27408	-1.86950
H	1.13020	-0.82730	-1.13413
H	-1.70721	2.22117	0.66396
O	3.28562	-0.03707	-0.88997
H	3.94733	0.31410	-1.49383
C	3.80034	0.02133	0.44285
H	3.88493	1.06544	0.76522
H	4.79812	-0.42703	0.47806
C	2.85652	-0.73739	1.35028
H	2.76853	-1.77689	1.02640
H	3.23450	-0.72590	2.37484
H	1.86163	-0.28423	1.35013

ADMA – EtOH 2

C	1.34133	0.48538	-0.03851
N	2.21337	1.41890	0.37186
N	1.68891	-0.80361	-0.02409
N	0.12994	0.85574	-0.44727
H	-0.59052	0.15373	-0.61094
C	-0.29657	2.24437	-0.53443
H	0.35881	2.81254	-1.19883
H	-1.30015	2.25134	-0.95444
H	-0.32550	2.72087	0.44931
H	1.96138	2.39539	0.35433
C	2.98252	-1.21392	0.51807
H	2.96949	-2.29182	0.65505
H	3.80001	-0.95710	-0.16167
H	3.15400	-0.75488	1.49369
C	0.78843	-1.80377	-0.59453
H	-0.09866	-1.94327	0.02844
H	0.47963	-1.51440	-1.60171
H	1.32353	-2.74724	-0.65921
H	3.18801	1.19825	0.50398
O	-2.30647	-0.53556	-0.87833
H	-2.67888	-0.91711	-1.67970
C	-3.26876	-0.61664	0.18271
H	-3.51019	-1.66545	0.37990
H	-4.18581	-0.09997	-0.11499
C	-2.67072	0.03169	1.41072

H	-2.43172	1.08034	1.21771
H	-3.38407	-0.00942	2.23623
H	-1.76046	-0.48726	1.72170

ADMA – EtOH 3

C	-1.23014	-0.14793	-0.16327
N	-0.21260	-0.91851	-0.55150
N	-1.07566	1.17439	-0.05833
N	-2.40861	-0.71023	0.13086
H	-3.20240	-0.10376	0.26855
C	-2.65141	-2.14582	0.09671
H	-1.96929	-2.66640	0.77187
H	-3.66913	-2.31825	0.43769
H	-2.54992	-2.54679	-0.91508
H	-0.35214	-1.90986	-0.67519
C	0.15943	1.81316	-0.50912
H	-0.03273	2.87650	-0.63119
H	0.96615	1.67752	0.21661
H	0.46996	1.41081	-1.47406
C	-2.13821	1.99771	0.51363
H	-2.95828	2.14175	-0.19590
H	-2.51856	1.54839	1.43274
H	-1.71898	2.96872	0.76344
H	0.74158	-0.57360	-0.66425
O	2.54919	-0.41140	-0.96616
H	2.94259	-0.21761	-1.82329
C	3.47928	-0.07395	0.07250
H	3.66493	1.00507	0.06370
H	4.42754	-0.58828	-0.10677
C	2.88508	-0.50724	1.39327
H	2.68985	-1.58194	1.39072
H	3.58069	-0.28482	2.20479
H	1.94854	0.01868	1.59531

SDMA – EtOH 1

C	1.73256	0.25690	0.10496
N	1.30422	-0.94715	0.47888
N	0.93410	1.32783	0.15534
H	1.26727	2.14474	-0.33836
N	2.98112	0.40137	-0.35192
H	3.36161	1.33715	-0.37334
C	3.86581	-0.70660	-0.68215
H	3.34909	-1.42416	-1.32150
H	4.71547	-0.30496	-1.22941
H	4.23568	-1.21148	0.21439
C	-0.09360	-1.37986	0.52233
H	-0.12980	-2.41548	0.18674
H	-0.49980	-1.31829	1.53274
H	-0.69317	-0.77302	-0.15531
C	-0.16975	1.52775	1.09619
H	-1.12909	1.29193	0.63562
H	-0.01714	0.91384	1.98298
H	-0.15976	2.57359	1.39957
H	2.00756	-1.66144	0.60756
O	-2.41983	0.36604	-1.18002
H	-2.50634	0.84900	-2.00750
C	-3.72715	0.06983	-0.68226
H	-4.30046	-0.47789	-1.43718
H	-4.26107	0.99921	-0.45621
C	-3.57406	-0.76664	0.56875
H	-3.00878	-0.22344	1.33038
H	-4.55595	-1.00806	0.98105

H	-3.05553	-1.70218	0.34489
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SDMA – EtOH 2

C	-1.35017	0.48173	0.01659
N	-0.41421	-0.27787	-0.54003
N	-2.57530	0.00094	0.27853
H	-3.27522	0.68870	0.52150
N	-1.07976	1.75886	0.30739
H	-1.70194	2.23163	0.94713
C	0.11413	2.46528	-0.13593
H	0.26437	2.31560	-1.20650
H	-0.03950	3.52605	0.05014
H	1.00511	2.13525	0.40359
C	-0.66338	-1.47161	-1.34440
H	0.12992	-1.53316	-2.08772
H	-0.65440	-2.38194	-0.74423
H	-1.61930	-1.38416	-1.86013
C	-2.86607	-1.38891	0.63150
H	-3.14438	-1.98271	-0.23916
H	-2.00334	-1.84163	1.12038
H	-3.69679	-1.38436	1.33487
H	0.54766	0.06402	-0.54906
O	2.40524	0.06409	-0.79300
H	2.91556	0.45269	-1.51074
C	3.29302	-0.37737	0.24312
H	3.86557	0.47352	0.62473
H	3.99403	-1.11182	-0.16334
C	2.45668	-0.99298	1.34161
H	1.87575	-1.83464	0.95629
H	3.10361	-1.35935	2.14111
H	1.77104	-0.25677	1.76899

SDMA – EtOH 3

C	-0.97480	0.20698	-0.20013
N	-2.24746	0.51101	0.06392
N	-0.53487	-1.05794	-0.14923
H	0.46843	-1.15413	-0.27900
N	-0.10885	1.17607	-0.49931
H	0.81090	0.87989	-0.81371
C	-0.38844	2.59707	-0.36659
H	-0.80284	2.82059	0.61928
H	0.55161	3.13326	-0.47675
H	-1.07654	2.94638	-1.14114
C	-3.16510	-0.30157	0.86135
H	-3.84863	0.37739	1.36734
H	-3.74354	-0.98812	0.24381
H	-2.61178	-0.85991	1.61564
C	-1.34859	-2.23529	-0.45803
H	-1.76203	-2.69258	0.44093
H	-2.15748	-1.97528	-1.14061
H	-0.70315	-2.95973	-0.95182
H	-2.53057	1.46398	-0.11644
O	2.29077	-0.45892	-0.87592
H	2.66566	-0.75362	-1.71355
C	3.33203	-0.41255	0.11823
H	4.12350	0.26258	-0.21782
H	3.75576	-1.41270	0.24275
C	2.72078	0.07609	1.41095
H	1.91976	-0.59181	1.73840
H	3.48188	0.10243	2.19310
H	2.31594	1.08436	1.29457

SDMA – EtOH 4

C	-1.31333	0.01363	-0.12436
N	-0.18876	-0.59076	-0.49537
N	-2.42131	-0.69883	0.10183
H	-2.36440	-1.70255	0.02068
N	-1.34012	1.34096	0.02514
H	-2.21829	1.77647	0.26299
C	-0.18570	2.19588	-0.21045
H	0.15444	2.12199	-1.24598
H	-0.48619	3.22165	-0.01187
H	0.63706	1.93458	0.45868
C	-0.06876	-2.03147	-0.65419
H	0.94055	-2.23830	-1.00315
H	-0.22508	-2.55276	0.29436
H	-0.77485	-2.40581	-1.39982
C	-3.67978	-0.11489	0.54100
H	-3.56953	0.37799	1.51000
H	-4.05909	0.59819	-0.19487
H	-4.40351	-0.91983	0.64154
H	0.66143	-0.04484	-0.63393
O	2.47222	0.28742	-0.85522
H	2.91131	0.70737	-1.60180
C	3.39428	0.16707	0.23733
H	3.70768	1.16306	0.56524
H	4.28032	-0.38317	-0.09200
C	2.70106	-0.57032	1.36104
H	2.38439	-1.56389	1.03458
H	3.38628	-0.68901	2.20270
H	1.82443	-0.01961	1.71099

SDMA – EtOH 5

C	1.30491	0.01568	-0.13147
N	2.43432	-0.65473	0.11641
N	1.28447	1.34695	-0.01891
H	2.14861	1.82187	0.19229
N	0.20047	-0.63688	-0.48039
H	-0.66305	-0.12239	-0.65261
C	0.12151	-2.08608	-0.56757
H	0.29546	-2.55371	0.40561
H	-0.88225	-2.33846	-0.90245
H	0.83625	-2.47787	-1.29596
C	3.67815	-0.02266	0.53183
H	4.42288	-0.80423	0.65894
H	4.03698	0.67605	-0.22778
H	3.55417	0.49906	1.48401
C	0.09798	2.15370	-0.26648
H	-0.23316	2.06284	-1.30372
H	-0.71742	1.86187	0.39934
H	0.35561	3.19151	-0.06943
H	2.41453	-1.65966	0.02958
O	-2.48009	0.15039	-0.89611
H	-2.92928	0.51072	-1.66746
C	-3.36468	0.18255	0.23283
H	-4.27843	-0.36938	-0.00460
H	-3.63520	1.21866	0.45954
C	-2.65232	-0.45349	1.40551
H	-1.75968	0.11432	1.67982
H	-3.31670	-0.48227	2.27138
H	-2.35670	-1.47761	1.16472

ADMA_1 – NMA 1

C	3.52295	-1.53638	1.25515
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H	3.13305	-2.51343	0.96932
H	2.82116	-1.04976	1.93102
N	3.76788	-0.68390	0.09216
H	4.71058	-0.67544	-0.27302
C	2.97861	0.32231	-0.31007
N	3.54567	1.38613	-0.89532
H	4.54740	1.50585	-0.87175
H	3.00691	2.01547	-1.47190
N	1.66343	0.29235	-0.12408
C	0.86041	1.50431	-0.29209
C	0.91463	-0.96471	-0.01705
H	1.38369	2.36361	0.12635
H	0.63584	1.68193	-1.34883
H	-0.08033	1.35611	0.23478
H	0.63511	-1.17053	1.01758
H	0.00117	-0.85671	-0.60111
H	1.50326	-1.78830	-0.41624
H	4.46899	-1.67077	1.77734
C	-3.28939	0.27911	0.36447
O	-2.08537	0.23927	0.12387
N	-4.17235	-0.48424	-0.30828
H	-5.15186	-0.41954	-0.07755
C	-3.74841	-1.39576	-1.35453
H	-3.26651	-0.85268	-2.17024
C	-3.85684	1.18622	1.42810
H	-3.60485	2.21800	1.17691
H	-3.38054	0.94765	2.38050
H	-4.93773	1.09565	1.53306
H	-3.04192	-2.13039	-0.96253
H	-4.62456	-1.91384	-1.73991

ADMA_1 – NMA 2

C	-0.98284	2.18834	0.12710
H	0.06708	1.97575	0.32777
H	-1.55813	2.14680	1.05097
N	-1.53986	1.24314	-0.84055
H	-1.44742	1.48407	-1.81777
C	-2.33996	0.21277	-0.56029
N	-3.22154	-0.19234	-1.48729
H	-3.37786	0.36575	-2.31374
H	-3.58875	-1.13261	-1.47739
N	-2.30830	-0.39441	0.62604
C	-3.39940	-1.26626	1.05143
C	-1.08818	-0.47259	1.43394
H	-4.35270	-0.89092	0.68314
H	-3.24471	-2.29295	0.70468
H	-3.42222	-1.26561	2.14098
H	-1.19569	0.10570	2.35380
H	-0.93018	-1.52185	1.69309
H	-0.22478	-0.12615	0.86956
H	-1.08112	3.18890	-0.29303
C	2.98914	0.20806	0.05920
O	1.78436	0.44630	0.01254
N	3.49004	-0.98202	-0.32363
H	4.48513	-1.13541	-0.26772
C	2.63227	-2.04809	-0.80522
H	1.90633	-2.33447	-0.04122
C	3.98404	1.22750	0.55403
H	3.75841	1.45655	1.59729
H	3.86251	2.14510	-0.02327
H	5.01579	0.88464	0.47726
H	2.08966	-1.73288	-1.69874

H	3.25090	-2.90969	-1.04906
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ADMA_1 – NMA 3

C	-1.66730	2.22871	-0.53054
H	-2.04317	2.88645	0.25450
H	-2.48659	1.95093	-1.19290
N	-1.03049	1.03460	0.02052
H	-0.01608	1.06013	0.11430
C	-1.55053	-0.19603	0.00158
N	-0.71313	-1.24115	-0.06716
H	0.28618	-1.04659	-0.11010
H	-1.00762	-2.14218	0.27955
N	-2.87215	-0.40101	0.01390
C	-3.42534	-1.71459	-0.30205
C	-3.80257	0.53148	0.65170
H	-2.83773	-2.19655	-1.08208
H	-3.46298	-2.35441	0.58560
H	-4.43971	-1.57198	-0.67342
H	-4.44319	1.01627	-0.08730
H	-4.42735	-0.03733	1.34300
H	-3.26260	1.28291	1.22161
H	-0.92014	2.76747	-1.11266
C	2.93104	0.40568	0.05855
O	1.70576	0.22291	0.02740
N	3.78355	-0.62240	0.03691
H	4.77506	-0.43413	0.05621
C	3.32370	-1.99895	-0.02749
H	2.69779	-2.23425	0.83581
C	3.51184	1.78987	0.12478
H	3.15744	2.27340	1.03713
H	3.14235	2.36444	-0.72611
H	4.60102	1.78871	0.11611
H	2.74960	-2.16945	-0.94064
H	4.19204	-2.65439	-0.02609

ADMA_1 – NMA 4

C	1.22747	1.98746	0.86302
H	1.43858	2.81174	0.18046
H	2.11239	1.78306	1.46497
N	0.80934	0.78227	0.14958
H	-0.19422	0.61171	0.08676
C	1.55914	-0.31568	-0.00069
N	0.94520	-1.50440	-0.04885
H	-0.06564	-1.52912	0.06765
H	1.39904	-2.29290	-0.48536
N	2.89266	-0.24948	-0.07188
C	3.70639	-1.45557	0.05270
C	3.58984	0.92706	-0.59342
H	3.26839	-2.13613	0.78159
H	3.81769	-1.96278	-0.91107
H	4.69320	-1.15934	0.40749
H	4.14967	1.43453	0.19450
H	4.28649	0.59158	-1.36384
H	2.88524	1.61781	-1.04939
H	0.41945	2.28154	1.53203
C	-2.94609	-0.53315	0.02200
O	-1.71147	-0.54789	0.12603
N	-3.60259	0.59903	-0.24739
H	-4.60904	0.57397	-0.31639
C	-2.91038	1.86274	-0.43093
H	-2.39004	2.15399	0.48444
C	-3.75778	-1.78547	0.19465

H	-3.58535	-2.17544	1.19941
H	-3.40330	-2.53018	-0.51988
H	-4.82470	-1.62116	0.04798
H	-2.18593	1.78723	-1.24467
H	-3.64391	2.62675	-0.67905

ADMA_1 – NMA 5

C	4.38182	-0.66018	-0.37287
H	4.94721	-0.65328	0.55948
H	4.30806	0.35589	-0.75847
N	3.04451	-1.22339	-0.19548
H	2.94480	-2.21375	-0.37025
C	1.90199	-0.51892	-0.17622
N	0.78522	-1.06804	-0.65469
H	0.84746	-1.94577	-1.15055
H	-0.14968	-0.74891	-0.37039
N	1.88280	0.73194	0.29168
C	0.74882	1.61164	0.01895
C	2.80639	1.19447	1.32808
H	0.34923	1.40676	-0.97258
H	-0.04040	1.48284	0.76492
H	1.10942	2.63972	0.04835
H	3.51797	1.91933	0.92757
H	2.21869	1.67123	2.11440
H	3.34115	0.35478	1.76524
H	4.91172	-1.26896	-1.10441
C	-2.80503	0.14552	-0.32361
O	-1.83878	-0.48751	0.11517
N	-4.04511	-0.08400	0.12586
H	-4.81112	0.44789	-0.25943
C	-4.31759	-1.08023	1.14789
H	-4.02026	-2.07310	0.80580
C	-2.64077	1.22155	-1.36538
H	-2.02587	0.83657	-2.18018
H	-2.11536	2.06411	-0.90937
H	-3.59213	1.57295	-1.76396
H	-3.77083	-0.84925	2.06366
H	-5.38551	-1.07742	1.35615

ADMA_1 – NMA 6

C	4.17293	-0.86489	-0.33890
H	4.72342	-0.81842	0.60121
H	4.23731	0.09823	-0.84407
N	2.77114	-1.22621	-0.13356
H	2.53922	-2.20632	-0.21746
C	1.73392	-0.37598	-0.22511
N	0.56774	-0.82848	-0.68573
H	0.54470	-1.71987	-1.15914
H	-0.32126	-0.33492	-0.54309
N	1.87127	0.90246	0.12873
C	0.88011	1.90455	-0.25931
C	2.84265	1.33683	1.13344
H	0.43376	1.64803	-1.21793
H	0.09072	1.98978	0.49139
H	1.39422	2.86113	-0.35432
H	3.65887	1.89929	0.67575
H	2.32402	1.98259	1.84407
H	3.24050	0.48272	1.67597
H	4.62830	-1.61989	-0.97852
C	-3.07359	0.25860	-0.10496
O	-1.91217	0.48221	-0.45973
N	-3.42705	-0.90831	0.45383

H	-4.38911	-1.04940	0.72302
C	-2.46880	-1.97826	0.66098
H	-2.05459	-2.31311	-0.29323
C	-4.15314	1.29140	-0.28266
H	-4.21403	1.55090	-1.34077
H	-3.86804	2.19082	0.26590
H	-5.12821	0.95008	0.06371
H	-1.65082	-1.64561	1.30387
H	-2.97785	-2.81336	1.13769

ADMA_1 – NMA 7

C	-4.35628	-0.76028	-0.11609
H	-4.97086	-0.12094	-0.75059
H	-4.27431	-0.31441	0.87402
N	-3.02318	-0.96845	-0.67896
H	-2.90359	-1.78325	-1.26503
C	-1.88989	-0.42016	-0.21015
N	-0.74997	-1.10776	-0.29061
H	-0.79010	-2.07946	-0.56262
H	0.17339	-0.65107	-0.28495
N	-1.90288	0.78964	0.35360
C	-0.76577	1.26232	1.14115
C	-2.87967	1.81456	-0.01778
H	-0.31412	0.43426	1.68540
H	-0.01133	1.73241	0.50534
H	-1.13967	1.99115	1.86008
H	-3.57920	2.00641	0.79832
H	-2.33390	2.73307	-0.24055
H	-3.42492	1.51807	-0.91068
H	-4.84002	-1.73134	-0.01726
C	2.83947	-0.47823	0.09391
O	1.80871	0.01786	-0.37476
N	4.03037	0.10759	-0.07522
H	4.84793	-0.32367	0.32924
C	4.17675	1.34755	-0.81842
H	3.79475	1.23045	-1.83369
C	2.81052	-1.76821	0.87115
H	2.43831	-2.56024	0.21771
H	2.11367	-1.66227	1.70461
H	3.78985	-2.05384	1.25398
H	3.62965	2.15648	-0.33083
H	5.23361	1.60286	-0.85869

ADMA_1 – NMA 8

C	1.22742	1.98744	0.86295
H	1.43857	2.81165	0.18032
H	2.11232	1.78307	1.46494
N	0.80928	0.78218	0.14961
H	-0.19428	0.61157	0.08690
C	1.55912	-0.31574	-0.00067
N	0.94521	-1.50449	-0.04871
H	-0.06562	-1.52923	0.06791
H	1.39903	-2.29300	-0.48520
N	2.89262	-0.24949	-0.07201
C	3.70643	-1.45552	0.05257
C	3.58969	0.92709	-0.59362
H	3.26852	-2.13608	0.78151
H	3.81770	-1.96276	-0.91118
H	4.69324	-1.15922	0.40730
H	4.14945	1.43467	0.19427
H	4.28639	0.59162	-1.36399
H	2.88502	1.61773	-1.04965

H	0.41939	2.28161	1.53189
C	-2.94610	-0.53312	0.02209
O	-1.71149	-0.54799	0.12625
N	-3.60245	0.59910	-0.24746
H	-4.60889	0.57415	-0.31657
C	-2.91008	1.86274	-0.43099
H	-2.38981	2.15397	0.48442
C	-3.75794	-1.78533	0.19480
H	-3.58590	-2.17501	1.19975
H	-3.40323	-2.53030	-0.51934
H	-4.82480	-1.62100	0.04771
H	-2.18556	1.78711	-1.24465
H	-3.64350	2.62681	-0.67921

ADMA_2 – NMA 1

C	-5.04507	-0.60450	-0.21888
H	-5.34302	0.12413	-0.97617
H	-5.42005	-0.29607	0.76003
N	-3.59943	-0.77704	-0.19640
H	-3.22711	-1.69226	-0.39703
C	-2.73988	0.21131	0.06561
N	-3.22050	1.44122	0.29286
H	-4.21409	1.60065	0.35467
H	-2.61327	2.18759	0.59181
N	-1.42810	-0.01081	0.08351
C	-0.49977	1.09384	0.33035
C	-0.91001	-1.35470	-0.17538
H	-0.62274	1.87857	-0.42156
H	-0.65097	1.51459	1.32876
H	0.51282	0.70078	0.26929
H	-1.15125	-1.67503	-1.19311
H	0.17118	-1.33005	-0.06342
H	-1.31798	-2.07047	0.54314
H	-5.48974	-1.56398	-0.47051
C	3.51445	-0.49064	0.06066
O	2.29921	-0.63796	0.17549
N	4.05405	0.70937	-0.22510
H	5.05579	0.79183	-0.30588
C	3.22893	1.88854	-0.40698
H	2.50483	1.73057	-1.20933
C	4.47356	-1.63952	0.24080
H	4.17306	-2.45323	-0.42073
H	4.39447	-1.99820	1.26885
H	5.50849	-1.36720	0.03397
H	2.68792	2.13018	0.51131
H	3.87258	2.72613	-0.66913

ADMA_2 – NMA 2

C	-0.51014	2.22831	-0.70737
H	-0.74133	2.87140	0.14640
H	-1.05582	2.57724	-1.58802
N	-0.80534	0.83279	-0.42119
H	-0.00137	0.19884	-0.41247
C	-2.02986	0.38536	-0.16488
N	-3.04893	1.25909	-0.08172
H	-2.90586	2.22406	-0.33785
H	-4.00158	0.92989	-0.08992
N	-2.25971	-0.91385	0.04081
C	-3.58864	-1.36423	0.45074
C	-1.16514	-1.87526	-0.08188
H	-3.92453	-0.82879	1.34194
H	-4.31815	-1.23260	-0.35393

H	-3.53090	-2.42203	0.69054
H	-0.46019	-1.78381	0.74875
H	-1.58892	-2.87618	-0.07748
H	-0.62820	-1.73283	-1.02111
H	0.55477	2.29875	-0.91968
C	2.80625	-0.32285	-0.24527
O	1.66148	-0.41546	-0.69778
N	3.04383	0.19511	0.96934
H	3.99609	0.25157	1.29784
C	1.97120	0.67119	1.82391
H	1.47977	1.54466	1.38815
C	3.99637	-0.79017	-1.03833
H	4.02617	-0.23588	-1.97804
H	3.86095	-1.84593	-1.27843
H	4.93913	-0.65643	-0.50871
H	1.22631	-0.11300	1.97411
H	2.39265	0.94865	2.78795

ADMA_2 – NMA 3

C	-0.47193	1.94778	-0.41624
H	0.21112	1.43934	-1.09954
H	-0.03573	1.96478	0.58513
N	-1.77598	1.29994	-0.40020
H	-2.58294	1.85411	-0.63821
C	-1.95853	0.03644	-0.00252
N	-0.89556	-0.69608	0.33048
H	0.06730	-0.37341	0.19461
H	-1.00985	-1.66416	0.58734
N	-3.19168	-0.47721	0.07192
C	-3.36708	-1.88075	0.43910
C	-4.34897	0.33870	-0.28750
H	-2.87880	-2.54058	-0.28312
H	-2.97293	-2.07485	1.43996
H	-4.42961	-2.10565	0.44264
H	-4.34195	0.59082	-1.35186
H	-5.24987	-0.22975	-0.07421
H	-4.38167	1.25399	0.30878
H	-0.61412	2.97069	-0.75683
C	2.90891	0.20979	0.21728
O	1.85576	-0.38748	-0.02469
N	4.09810	-0.33913	-0.06363
H	4.93712	0.17879	0.14974
C	4.21073	-1.65614	-0.66614
H	3.78370	-2.41740	-0.01067
C	2.91959	1.58759	0.82635
H	2.31827	1.57644	1.73670
H	2.45894	2.28432	0.12248
H	3.92430	1.93695	1.06309
H	3.68543	-1.68502	-1.62208
H	5.26463	-1.87263	-0.82854

ADMA_2 – NMA 4

C	3.24575	-2.27870	-0.36308
H	2.77850	-2.58429	-1.30226
H	2.77819	-2.80659	0.47177
N	3.16622	-0.83594	-0.18499
H	4.02982	-0.32686	-0.08102
C	2.01022	-0.16596	-0.09133
N	0.85974	-0.83896	-0.15100
H	0.88137	-1.82928	-0.34105
H	-0.06163	-0.38828	-0.21613
N	2.00915	1.15891	0.07679

C	0.73600	1.86936	0.19234
C	3.26852	1.89777	0.10398
H	0.17655	1.83556	-0.74531
H	0.12401	1.43765	0.98678
H	0.94524	2.90584	0.44248
H	3.84707	1.71866	-0.80582
H	3.04247	2.95866	0.15644
H	3.86603	1.62941	0.97998
H	4.29760	-2.55144	-0.39874
C	-2.70886	-0.25391	0.36751
O	-1.79020	-0.01686	-0.42441
N	-3.99047	-0.12327	0.00455
H	-4.71059	-0.33464	0.67910
C	-4.36922	0.30093	-1.33255
H	-3.95797	1.28746	-1.55231
C	-2.43752	-0.68815	1.78402
H	-1.94257	0.13022	2.31155
H	-1.75547	-1.53982	1.76865
H	-3.34450	-0.95999	2.32296
H	-3.99935	-0.40468	-2.07852
H	-5.45505	0.34410	-1.38603

SDMA_1 – NMA 1

C	-0.90310	-0.16448	1.91197
H	-1.22862	0.81687	2.25447
H	0.15956	-0.15113	1.67090
N	-1.70305	-0.56689	0.75687
H	-1.87370	-1.55649	0.64096
C	-2.29908	0.25584	-0.10071
N	-1.89414	1.52434	-0.24118
N	-3.34806	-0.17352	-0.81090
H	-2.53381	2.14004	-0.72448
H	-3.60662	0.36654	-1.62485
C	-0.50964	1.98354	-0.10251
H	-0.30394	2.67215	-0.92171
H	0.17338	1.13715	-0.17617
H	-0.35536	2.50371	0.84427
C	-4.05410	-1.42142	-0.55975
H	-3.48108	-2.28781	-0.89958
H	-4.99368	-1.39125	-1.10682
H	-4.27312	-1.52221	0.50463
H	-1.08773	-0.88531	2.70711
C	2.80461	-0.75756	-0.20775
O	1.58645	-0.63804	-0.09641
N	3.61985	0.31429	-0.23728
H	4.61423	0.17372	-0.32906
C	3.09976	1.66512	-0.14210
H	2.44044	1.88591	-0.98467
C	3.46704	-2.10823	-0.30374
H	2.97250	-2.68662	-1.08497
H	3.32505	-2.63282	0.64333
H	4.53318	-2.04126	-0.51952
H	2.53695	1.79650	0.78514
H	3.93563	2.36217	-0.15279

SDMA_1 – NMA 2

C	-4.08577	-0.33731	0.44214
H	-3.73130	-0.88524	1.31504
H	-4.47392	-1.03831	-0.29670
N	-3.01154	0.49444	-0.09811
H	-3.25100	1.44735	-0.33187
C	-1.70805	0.19842	-0.04886

N	-1.28963	-1.06860	0.06382
N	-0.80264	1.17643	-0.09590
H	-0.28140	-1.16674	0.16582
H	0.17125	0.88437	-0.16902
C	-2.00168	-2.22509	-0.47989
H	-1.25300	-2.94589	-0.80417
H	-2.59984	-1.94009	-1.34614
H	-2.64157	-2.69956	0.26421
C	-1.11887	2.58972	0.03116
H	-1.60714	2.97658	-0.86712
H	-0.18502	3.12987	0.17023
H	-1.75683	2.77026	0.89924
H	-4.88947	0.32666	0.75477
C	2.72792	-0.62892	0.07558
O	1.50283	-0.46469	-0.00944
N	3.56866	0.40754	0.01002
H	4.56047	0.23746	0.08806
C	3.09182	1.77044	-0.14466
H	2.51973	1.87332	-1.06933
C	3.32448	-1.99655	0.25008
H	3.06327	-2.60337	-0.61896
H	2.88081	-2.46201	1.13172
H	4.40785	-1.96968	0.35986
H	2.46054	2.05270	0.70116
H	3.95092	2.43680	-0.18330

SDMA_1 – NMA 3

C	3.86549	-0.94591	-0.33389
H	3.32710	-1.57489	-1.04248
H	4.14939	-1.53607	0.53763
N	3.05195	0.21302	0.02944
H	3.52663	1.10383	0.06703
C	1.71590	0.24582	0.05425
N	0.99602	-0.87485	0.19794
N	1.07747	1.40786	-0.08324
H	-0.01017	-0.72821	0.12621
H	0.07653	1.39725	0.09810
C	1.43545	-2.04117	0.96368
H	0.55548	-2.47407	1.43685
H	2.13494	-1.74899	1.74756
H	1.89791	-2.79672	0.32861
C	1.71595	2.65420	-0.47461
H	2.32443	3.06751	0.33391
H	0.93186	3.36933	-0.71267
H	2.33546	2.51081	-1.36285
H	4.76706	-0.57598	-0.81889
C	-2.80104	0.31957	0.15655
O	-1.56749	0.39732	0.24067
N	-3.38909	-0.73697	-0.41282
H	-4.39674	-0.76989	-0.45601
C	-2.61919	-1.83871	-0.96367
H	-1.91626	-1.47431	-1.71525
C	-3.69139	1.40799	0.68574
H	-3.49203	2.32441	0.12667
H	-3.43602	1.58998	1.73071
H	-4.74886	1.15938	0.60677
H	-2.06251	-2.35299	-0.17650
H	-3.30570	-2.54306	-1.42830

SDMA_1 – NMA 4

C	1.17530	-1.41564	1.21913
H	2.00233	-1.31290	1.92218

H	1.29322	-2.33947	0.65136
N	1.08960	-0.23952	0.35751
H	0.13730	0.12195	0.21471
C	2.12335	0.50462	-0.01355
N	3.37135	0.00772	-0.03567
N	1.93630	1.78538	-0.34953
H	4.11112	0.68758	-0.14241
H	2.67382	2.23977	-0.86961
C	3.71247	-1.38325	-0.33171
H	4.65911	-1.38362	-0.86934
H	2.95394	-1.83177	-0.97332
H	3.82408	-1.97873	0.57441
C	0.68427	2.50551	-0.15874
H	-0.07191	2.20189	-0.88545
H	0.88903	3.56744	-0.27709
H	0.29923	2.33058	0.84658
H	0.24639	-1.46618	1.78623
C	-2.40013	-0.51144	-0.26683
O	-1.63634	0.31319	0.24808
N	-3.72829	-0.35852	-0.20892
H	-4.31805	-1.05565	-0.63801
C	-4.34143	0.76554	0.47812
H	-4.07587	0.76115	1.53675
C	-1.88264	-1.74198	-0.96635
H	-1.50825	-2.43995	-0.21325
H	-1.05147	-1.46250	-1.61507
H	-2.65122	-2.24220	-1.55538
H	-4.01028	1.70820	0.03979
H	-5.42166	0.68328	0.37819

SDMA_1 – NMA 5

C	1.06902	-1.25141	1.44521
H	1.91136	-1.07935	2.11574
H	1.16994	-2.23340	0.98130
N	0.97513	-0.17747	0.46018
H	0.02971	0.19112	0.31184
C	2.00824	0.48818	-0.03968
N	3.24073	-0.04547	-0.06099
N	1.83780	1.72680	-0.51343
H	3.99158	0.59418	-0.28008
H	2.56130	2.09561	-1.11369
C	3.52953	-1.47175	-0.20756
H	4.45497	-1.56503	-0.77296
H	2.73342	-1.96396	-0.76669
H	3.65713	-1.96475	0.75641
C	0.62383	2.51274	-0.33662
H	-0.17881	2.17017	-0.99300
H	0.86028	3.54832	-0.57156
H	0.28482	2.45460	0.69813
H	0.14940	-1.23081	2.02852
C	-2.82395	0.03833	0.13561
O	-1.73886	0.49833	0.50265
N	-2.89640	-0.99756	-0.71390
H	-3.80328	-1.35173	-0.97815
C	-1.70445	-1.63666	-1.24072
H	-1.08224	-0.91179	-1.77086
C	-4.12164	0.62710	0.61844
H	-4.16956	1.66866	0.29539
H	-4.12468	0.61589	1.70952
H	-4.99615	0.09382	0.24696
H	-1.11564	-2.08284	-0.43521
H	-2.00703	-2.41866	-1.93401

SDMA_2 – NMA 1

C	4.34100	0.12570	0.50274
H	4.26434	0.52583	1.51726
H	4.59488	0.92927	-0.19220
N	3.11058	-0.53753	0.09886
H	3.12936	-1.53739	-0.03441
C	1.94112	0.09616	-0.04632
N	1.86527	1.40386	0.21646
N	0.86480	-0.56183	-0.46134
H	2.68616	1.86685	0.57568
H	-0.04653	-0.09533	-0.50195
C	0.66082	2.19511	0.00555
H	0.90722	3.23460	0.20837
H	-0.14209	1.88427	0.67731
H	0.31523	2.10807	-1.02635
C	0.88468	-1.96411	-0.84394
H	1.60297	-2.14224	-1.64796
H	-0.10798	-2.21639	-1.21123
H	1.11763	-2.61240	0.00579
H	5.14034	-0.61108	0.48285
C	-2.90047	0.04693	-0.31865
O	-1.78740	0.20024	-0.82907
N	-3.04750	-0.50114	0.89744
H	-3.97642	-0.60592	1.27693
C	-1.90805	-0.94198	1.68108
H	-1.35225	-1.72088	1.15351
C	-4.15165	0.47280	-1.03760
H	-4.18642	-0.03369	-2.00338
H	-4.09537	1.54639	-1.22603
H	-5.05982	0.25037	-0.47803
H	-1.23280	-0.10774	1.88600
H	-2.27036	-1.34309	2.62534

SDMA_2 – NMA 2

C	-4.50801	0.05754	0.19103
H	-4.72955	0.85183	-0.52602
H	-4.51802	0.46260	1.20584
N	-3.23425	-0.57986	-0.10719
H	-3.23369	-1.56178	-0.33796
C	-2.06124	0.06431	-0.10672
N	-2.02404	1.36602	0.19106
N	-0.93839	-0.58315	-0.39724
H	-2.89359	1.83429	0.39367
H	-0.03377	-0.09518	-0.38683
C	-0.79845	2.15228	0.18748
H	-1.06515	3.18320	0.40706
H	-0.30965	2.11346	-0.78798
H	-0.10168	1.79726	0.94954
C	-0.90550	-1.98859	-0.76608
H	-1.25260	-2.62637	0.05162
H	0.12829	-2.24358	-0.99102
H	-1.50541	-2.17859	-1.65975
H	-5.28493	-0.69943	0.11645
C	2.50582	-0.25086	0.34747
O	1.71537	0.23015	-0.47228
N	3.82664	-0.07625	0.22327
H	4.43944	-0.48362	0.91352
C	4.39793	0.68726	-0.87335
H	4.12471	0.24361	-1.83210
C	2.02512	-1.05245	1.52987
H	1.42373	-1.89003	1.16946

H	1.38336	-0.41920	2.14628
H	2.84264	-1.43346	2.14113
H	4.03829	1.71735	-0.85178
H	5.48086	0.68212	-0.77035

SDMA_2 – NMA 3

C	-0.90539	-1.98851	-0.76614
H	-1.50536	-2.17848	-1.65976
H	-1.25238	-2.62638	0.05154
N	-0.93836	-0.58311	-0.39718
H	-0.03375	-0.09509	-0.38668
C	-2.06124	0.06427	-0.10663
N	-3.23421	-0.57997	-0.10723
N	-2.02413	1.36596	0.19126
H	-3.23356	-1.56188	-0.33801
H	-2.89369	1.83410	0.39412
C	-4.50806	0.05736	0.19075
H	-5.28496	-0.69958	0.11570
H	-4.72935	0.85184	-0.52616
H	-4.51841	0.46215	1.20567
C	-0.79860	2.15230	0.18771
H	-0.10174	1.79723	0.94965
H	-1.06536	3.18318	0.40746
H	-0.30988	2.11367	-0.78780
H	0.12840	-2.24342	-0.99118
C	2.50585	-0.25072	0.34751
O	1.71537	0.23029	-0.47220
N	3.82668	-0.07631	0.22313
H	4.43950	-0.48365	0.91338
C	4.39794	0.68696	-0.87367
H	4.12450	0.24323	-1.83232
C	2.02517	-1.05210	1.53007
H	1.42359	-1.88962	1.16984
H	1.38359	-0.41866	2.14649
H	2.84270	-1.43317	2.14128
H	4.03849	1.71711	-0.85219
H	5.48089	0.68165	-0.77083

ADMA_1 – PHE 1

C	-4.12685	0.70885	-0.28005
C	-2.82216	1.20300	-0.33352
C	-1.72276	0.37811	-0.12390
C	-1.92317	-0.97106	0.14267
C	-3.21018	-1.49210	0.19529
C	-4.29768	-0.65072	-0.01589
H	-2.65949	2.25499	-0.54458
H	-0.71475	0.77503	-0.17366
H	-3.36276	-2.54854	0.39339
H	-5.29977	-1.06521	0.02421
O	-0.81182	-1.75816	0.34096
H	-1.07923	-2.66132	0.54930
C	-5.31269	1.61699	-0.47491
H	-6.13789	1.08933	-0.95659
H	-5.05109	2.47946	-1.09028
H	-5.67616	1.99251	0.48616
C	1.68595	0.10631	1.87742
H	1.26255	1.10835	1.95406
H	2.68385	0.09976	2.31482
N	1.73716	-0.36634	0.49462
H	0.96481	-0.96276	0.21065
C	2.82156	-0.37498	-0.28369
N	2.93526	-1.32836	-1.22133

H	2.25188	-2.07067	-1.26358
H	3.50814	-1.17917	-2.03923
N	3.80189	0.51693	-0.13196
C	5.10937	0.30948	-0.75075
C	3.55974	1.86007	0.39951
H	5.36114	-0.74946	-0.75937
H	5.13354	0.70854	-1.76937
H	5.85080	0.83514	-0.14997
H	3.95390	1.96378	1.41193
H	4.06608	2.57174	-0.25415
H	2.49668	2.08782	0.38931
H	1.05532	-0.58333	2.43689

ADMA_1 – PHE 2

C	4.17942	-0.59088	0.30426
C	2.92967	-1.19429	0.47207
C	1.75889	-0.55741	0.08161
C	1.83161	0.70902	-0.48706
C	3.05660	1.33310	-0.66926
C	4.21958	0.67817	-0.27125
H	2.87059	-2.18174	0.91865
H	0.79387	-1.03440	0.21621
H	3.10649	2.32259	-1.11334
H	5.17534	1.17185	-0.41151
O	0.64375	1.31305	-0.85119
H	0.82058	2.18009	-1.23817
C	5.43820	-1.30334	0.72399
H	6.29340	-0.62631	0.71810
H	5.66094	-2.13267	0.04689
H	5.33712	-1.71792	1.72946
C	-2.72665	1.72277	1.50515
H	-2.81517	1.36764	2.53208
H	-3.72075	1.87752	1.08730
N	-1.96752	0.79111	0.67030
H	-0.98964	1.01530	0.52508
C	-2.49753	-0.03608	-0.24009
N	-1.77459	-0.33103	-1.32733
H	-0.90632	0.16425	-1.49528
H	-1.96338	-1.16244	-1.86734
N	-3.72021	-0.54762	-0.08941
C	-4.40836	-1.18930	-1.20750
C	-4.31315	-0.77506	1.23032
H	-4.17199	-0.68074	-2.14119
H	-4.14344	-2.24867	-1.28022
H	-5.48040	-1.10763	-1.03139
H	-5.14215	-0.08920	1.41365
H	-4.68637	-1.80009	1.25708
H	-3.56222	-0.66535	2.00882
H	-2.20000	2.67601	1.50626

ADMA_1 – PHE 3

C	4.22663	0.60173	-0.29908
C	3.07572	0.65055	-1.09087
C	1.98232	-0.16612	-0.83273
C	2.02999	-1.05241	0.23863
C	3.16127	-1.12575	1.03963
C	4.24936	-0.30182	0.76250
H	3.03696	1.33605	-1.93163
H	1.09979	-0.13390	-1.46156
H	3.19657	-1.82479	1.86956
H	5.13283	-0.36724	1.38877
O	0.91956	-1.83248	0.46770

H	1.10091	-2.46282	1.17561
C	5.39525	1.50828	-0.58466
H	6.28167	1.19192	-0.03320
H	5.16950	2.53855	-0.29513
H	5.63788	1.51228	-1.64955
C	-4.23174	-1.71091	-0.68307
H	-4.79756	-1.47915	-1.58579
H	-4.79320	-1.38608	0.19196
N	-2.91183	-1.08193	-0.69079
H	-2.14695	-1.63961	-1.04638
C	-2.56337	0.00026	0.01838
N	-1.30401	0.10770	0.45165
H	-0.67345	-0.68945	0.44044
H	-0.92160	1.00515	0.71222
N	-3.45519	0.94936	0.30607
C	-3.18898	1.94349	1.34242
C	-4.60492	1.23149	-0.55553
H	-2.60674	1.50368	2.15067
H	-2.66347	2.81158	0.93300
H	-4.14704	2.27130	1.74452
H	-5.53826	0.92691	-0.07847
H	-4.63143	2.30764	-0.73503
H	-4.49522	0.72808	-1.51284
H	-4.08967	-2.78883	-0.62103

ADMA_1 – PHE 4

C	3.87362	-0.56453	-0.40909
C	2.94799	-0.06814	-1.33063
C	1.90297	0.75871	-0.93593
C	1.76944	1.09354	0.40654
C	2.67162	0.61216	1.34634
C	3.71571	-0.20934	0.93071
H	3.04910	-0.32741	-2.37961
H	1.19256	1.14815	-1.65742
H	2.56255	0.87587	2.39401
H	4.41942	-0.58122	1.66828
O	0.70351	1.89003	0.76042
H	0.79796	2.17839	1.67675
C	5.02453	-1.42785	-0.85497
H	5.36192	-2.08173	-0.04900
H	5.87531	-0.81144	-1.15996
H	4.74435	-2.04866	-1.70787
C	-4.95019	-0.54161	-0.99758
H	-5.69672	-0.76385	-0.23459
H	-4.37948	-1.44177	-1.22040
N	-4.04823	0.53089	-0.58006
H	-4.30817	1.46962	-0.85084
C	-2.77360	0.37481	-0.19497
N	-1.89948	1.35116	-0.45770
H	-2.15913	2.09220	-1.09272
H	-1.02536	1.45037	0.05257
N	-2.36825	-0.73443	0.42364
C	-0.94526	-1.03996	0.55849
C	-3.26716	-1.55525	1.23708
H	-0.39782	-0.70202	-0.31991
H	-0.52465	-0.58104	1.45842
H	-0.84070	-2.12189	0.63466
H	-3.45750	-2.51860	0.76011
H	-2.78434	-1.72565	2.20101
H	-4.20587	-1.03642	1.41257
H	-5.45785	-0.22170	-1.90664

ADMA_1 – PHE 5

C	3.75198	-0.59572	-0.43748
C	2.92377	0.07579	-1.34244
C	1.93168	0.94657	-0.91047
C	1.75097	1.14909	0.45396
C	2.55575	0.49465	1.37584
C	3.55132	-0.36805	0.92301
H	3.06306	-0.08105	-2.40748
H	1.29778	1.47052	-1.61753
H	2.40822	0.65470	2.43952
H	4.18052	-0.87288	1.64855
O	0.72745	1.98471	0.84289
H	0.81226	2.19375	1.78143
C	4.83840	-1.51687	-0.92739
H	5.25762	-2.10146	-0.10725
H	5.65262	-0.94829	-1.38515
H	4.45724	-2.20922	-1.68141
C	-4.84119	-0.58699	-1.05644
H	-5.59750	-0.83845	-0.31222
H	-4.24191	-1.46884	-1.27791
N	-3.97596	0.50283	-0.60779
H	-4.25914	1.43938	-0.86203
C	-2.70760	0.37233	-0.19369
N	-1.84906	1.37047	-0.42495
H	-2.11026	2.11247	-1.05828
H	-0.99491	1.49006	0.11388
N	-2.29302	-0.73572	0.42135
C	-0.86779	-1.01491	0.58424
C	-3.19219	-1.57857	1.21138
H	-0.30800	-0.65771	-0.27859
H	-0.47657	-0.55663	1.49759
H	-0.74322	-2.09528	0.65347
H	-3.35347	-2.54287	0.72587
H	-2.72621	-1.74457	2.18436
H	-4.14423	-1.07887	1.36907
H	-5.33780	-0.26939	-1.97241

ADMA_1 – PHE 6

C	3.00454	0.77059	-0.04128
C	2.87568	-0.27732	0.87687
C	2.05092	-1.36611	0.62669
C	1.32737	-1.42249	-0.56299
C	1.43389	-0.39082	-1.48913
C	2.27000	0.69210	-1.22319
H	3.44100	-0.24573	1.80319
H	1.96971	-2.18226	1.33578
H	0.87226	-0.43368	-2.41751
H	2.35126	1.48803	-1.95664
O	0.52003	-2.50844	-0.76334
H	0.18986	-2.50061	-1.66913
C	3.93193	1.92529	0.23398
H	3.76322	2.74120	-0.47033
H	4.97640	1.61436	0.14184
H	3.79409	2.31139	1.24652
C	-3.24232	2.27845	-0.29883
H	-2.46612	2.94345	-0.67800
H	-3.23013	2.28840	0.79058
N	-3.07818	0.90930	-0.78866
H	-3.61978	0.65321	-1.60310
C	-2.57831	-0.11222	-0.07953
N	-3.04796	-1.34566	-0.31223
H	-3.88937	-1.47730	-0.85380

H	-2.52080	-2.16633	-0.05191
N	-1.64283	0.07514	0.84726
C	-1.35741	-0.95354	1.84846
C	-0.71006	1.20528	0.80584
H	-2.27882	-1.44868	2.15293
H	-0.64509	-1.69026	1.46708
H	-0.92482	-0.46025	2.71773
H	-0.96640	1.95263	1.55929
H	0.28984	0.81771	1.00554
H	-0.70819	1.65656	-0.18329
H	-4.21295	2.63904	-0.63600
C	3.00454	0.77059	-0.04128
C	2.87568	-0.27732	0.87687
C	2.05092	-1.36611	0.62669
C	1.32737	-1.42249	-0.56299
C	1.43389	-0.39082	-1.48913
C	2.27000	0.69210	-1.22319
H	3.44100	-0.24573	1.80319
H	1.96971	-2.18226	1.33578
H	0.87226	-0.43368	-2.41751
H	2.35126	1.48803	-1.95664
O	0.52003	-2.50844	-0.76334
H	0.18986	-2.50061	-1.66913
C	3.93193	1.92529	0.23398
H	3.76322	2.74120	-0.47033
H	4.97640	1.61436	0.14184
H	3.79409	2.31139	1.24652
C	-3.24232	2.27845	-0.29883
H	-2.46612	2.94345	-0.67800
H	-3.23013	2.28840	0.79058
N	-3.07818	0.90930	-0.78866
H	-3.61978	0.65321	-1.60310
C	-2.57831	-0.11222	-0.07953
N	-3.04796	-1.34566	-0.31223
H	-3.88937	-1.47730	-0.85380
H	-2.52080	-2.16633	-0.05191
N	-1.64283	0.07514	0.84726
C	-1.35741	-0.95354	1.84846
C	-0.71006	1.20528	0.80584
H	-2.27882	-1.44868	2.15293
H	-0.64509	-1.69026	1.46708
H	-0.92482	-0.46025	2.71773
H	-0.96640	1.95263	1.55929
H	0.28984	0.81771	1.00554
H	-0.70819	1.65656	-0.18329
H	-4.21295	2.63904	-0.63600

ADMA_1 – PHE 7

C	2.15374	1.40906	0.02400
C	1.83857	0.87939	-1.23194
C	1.86070	-0.48950	-1.47170
C	2.20476	-1.36528	-0.44394
C	2.52530	-0.86220	0.81637
C	2.49703	0.51239	1.03977
H	1.57375	1.55218	-2.04148
H	1.61639	-0.89197	-2.44789
H	2.79885	-1.54149	1.61827
H	2.75135	0.89229	2.02434
O	2.19877	-2.69585	-0.72180
H	2.51460	-3.19331	0.04139
C	2.15792	2.89654	0.26882
H	1.74302	3.14202	1.24932

H	1.58493	3.42523	-0.49562
H	3.17810	3.28991	0.24214
C	-0.95572	-1.44207	1.54507
H	-0.76297	-2.37649	1.01597
H	-1.95979	-1.46226	1.96708
N	-0.80408	-0.28324	0.67093
H	0.12590	0.11680	0.60587
C	-1.78933	0.45135	0.15273
N	-1.55067	1.74471	-0.11111
H	-0.67754	2.16156	0.18074
H	-2.09777	2.23724	-0.80151
N	-3.00138	-0.05520	-0.08161
C	-4.14083	0.82190	-0.34151
C	-3.20656	-1.46690	-0.41478
H	-4.07016	1.72677	0.25997
H	-4.20571	1.08244	-1.40271
H	-5.04670	0.28747	-0.05716
H	-3.75138	-1.98162	0.37852
H	-3.79154	-1.51091	-1.33518
H	-2.25417	-1.95999	-0.59329
H	-0.23996	-1.34108	2.36077

ADMA_1 – PHE 8

C	2.21768	1.36773	0.06269
C	1.89076	0.88469	-1.20888
C	1.85464	-0.47747	-1.48446
C	2.15127	-1.39409	-0.47781
C	2.48095	-0.93778	0.79785
C	2.51172	0.43031	1.05710
H	1.66393	1.58833	-2.00369
H	1.60226	-0.84225	-2.47330
H	2.71472	-1.64838	1.58473
H	2.77397	0.77235	2.05345
O	2.08960	-2.71586	-0.78995
H	2.38319	-3.24563	-0.03978
C	2.29319	2.84610	0.34438
H	3.33115	3.19015	0.32757
H	1.88753	3.08660	1.32977
H	1.74720	3.42028	-0.40698
C	-0.96469	-1.36199	1.58838
H	-0.78772	-2.32571	1.10848
H	-1.96376	-1.34889	2.02196
N	-0.80947	-0.25597	0.64839
H	0.12333	0.13203	0.56039
C	-1.78919	0.47490	0.11446
N	-1.52613	1.74872	-0.21696
H	-0.62306	2.14204	0.01120
H	-2.06996	2.21505	-0.92821
N	-3.01607	-0.01448	-0.07129
C	-4.13877	0.87278	-0.36771
C	-3.25932	-1.43948	-0.30656
H	-4.04042	1.80724	0.18216
H	-4.21064	1.07573	-1.44084
H	-5.05215	0.37560	-0.04295
H	-3.78584	-1.89269	0.53519
H	-3.87665	-1.52984	-1.20169
H	-2.32309	-1.96256	-0.48489
H	-0.23849	-1.22473	2.38937

ADMA_2 – PHE 1

C	3.47327	0.48404	-0.59972
C	2.46062	-0.17845	-1.29779

C	1.46575	-0.88814	-0.63526
C	1.47529	-0.93877	0.75455
C	2.47054	-0.29105	1.47536
C	3.46144	0.40927	0.79332
H	2.45422	-0.14894	-2.38278
H	0.69254	-1.41190	-1.18704
H	2.47784	-0.33692	2.56006
H	4.24145	0.90544	1.36144
O	0.45554	-1.62757	1.37212
H	0.63675	-1.70985	2.31651
C	4.53281	1.26774	-1.32899
H	5.45261	1.32357	-0.74462
H	4.19785	2.29147	-1.52052
H	4.76576	0.81058	-2.29233
C	-2.19220	-2.30177	-0.75439
H	-2.06592	-2.17527	-1.83257
H	-3.17740	-2.72652	-0.54456
N	-1.99815	-1.04242	-0.05006
H	-1.23072	-1.00620	0.61201
C	-2.73839	0.04411	-0.26493
N	-3.74641	-0.01718	-1.14606
H	-4.01236	-0.89689	-1.56080
H	-4.36160	0.76675	-1.29568
N	-2.47477	1.18691	0.37175
C	-3.28153	2.37417	0.09621
C	-1.32568	1.26585	1.27229
H	-3.20490	2.66609	-0.95436
H	-4.32900	2.20220	0.35665
H	-2.90956	3.19172	0.70647
H	-0.38866	1.11183	0.72919
H	-1.30888	2.25563	1.71972
H	-1.41361	0.53162	2.07742
H	-1.43692	-2.99706	-0.39444

ADMA_2 – PHE 2

C	3.80529	-0.46788	-0.44220
C	2.59397	-0.63768	-1.11500
C	1.47310	0.12200	-0.79713
C	1.55573	1.06679	0.21862
C	2.74828	1.25648	0.90730
C	3.85983	0.49151	0.57057
H	2.52431	-1.37425	-1.90892
H	0.53940	-0.01248	-1.33181
H	2.80830	1.99517	1.70077
H	4.78873	0.64542	1.11018
O	0.41572	1.77532	0.52589
H	0.61907	2.46199	1.17282
C	5.02519	-1.26946	-0.81392
H	5.60899	-1.53144	0.07064
H	5.67640	-0.69741	-1.48118
H	4.74909	-2.19106	-1.32879
C	-0.76957	-1.33037	1.49332
H	-0.53421	-2.20619	0.88324
H	-1.30400	-1.63738	2.39592
N	-1.53877	-0.35332	0.73498
H	-1.12414	0.56481	0.61582
C	-2.74738	-0.59965	0.23190
N	-3.30914	-1.79912	0.44027
H	-2.78646	-2.53495	0.89030
H	-4.16870	-2.06087	-0.01641
N	-3.40382	0.33599	-0.45701
C	-4.75574	0.06566	-0.94271

C	-2.77763	1.63603	-0.69265
H	-4.75165	-0.72718	-1.69582
H	-5.41688	-0.21248	-0.11865
H	-5.14454	0.97046	-1.39996
H	-1.81597	1.51893	-1.19742
H	-3.42999	2.21969	-1.33599
H	-2.63402	2.18041	0.24522
H	0.16623	-0.85919	1.78672

ADMA_2 – PHE 3

C	4.57807	-0.30640	-0.34459
C	3.37804	-0.88992	-0.76082
C	2.14985	-0.43229	-0.30212
C	2.10214	0.63374	0.59249
C	3.28152	1.23366	1.01880
C	4.50440	0.76114	0.54858
H	3.40511	-1.72066	-1.45917
H	1.22624	-0.89343	-0.63236
H	3.24673	2.06824	1.71275
H	5.41801	1.23916	0.88672
O	0.87107	1.05511	1.01625
H	0.97881	1.77049	1.65343
C	5.90264	-0.83264	-0.83260
H	6.70051	-0.10915	-0.65831
H	5.86926	-1.05429	-1.90148
H	6.17075	-1.75706	-0.31271
C	-3.71083	2.32004	-0.95900
H	-4.19090	2.77904	-0.09109
H	-4.44685	2.16377	-1.75026
N	-3.05924	1.06714	-0.60284
H	-2.05180	1.03453	-0.63739
C	-3.71362	-0.00106	-0.14084
N	-5.04016	0.07684	0.03109
H	-5.54232	0.91257	-0.22708
H	-5.58992	-0.74442	0.23023
N	-3.06053	-1.12410	0.15704
C	-3.77122	-2.24954	0.76344
C	-1.62076	-1.21527	-0.08815
H	-4.32940	-1.92558	1.64470
H	-4.44970	-2.72324	0.04881
H	-3.03694	-2.98331	1.08307
H	-1.06152	-0.55475	0.58031
H	-1.30818	-2.24048	0.09052
H	-1.39163	-0.96791	-1.12801
H	-2.94662	2.99780	-1.33075

ADMA_2 – PHE 4

C	-3.79298	1.11164	0.15408
C	-2.46586	1.11879	0.59412
C	-1.65243	0.00063	0.46863
C	-2.16418	-1.16057	-0.10642
C	-3.47940	-1.19323	-0.55319
C	-4.28067	-0.06200	-0.41783
H	-2.06267	2.01774	1.05028
H	-0.62819	0.01466	0.82526
H	-3.87934	-2.09923	-0.99840
H	-5.30778	-0.10054	-0.76529
O	-1.32904	-2.24138	-0.20846
H	-1.80960	-2.98985	-0.58140
C	-4.65564	2.33901	0.29331
H	-5.69538	2.11780	0.04820
H	-4.31477	3.13568	-0.37378

H	-4.62209	2.72837	1.31361
C	4.88115	1.76194	-0.71162
H	5.79048	1.17523	-0.55909
H	4.76429	2.48192	0.10128
N	3.70915	0.90385	-0.81607
H	3.24084	0.84940	-1.70749
C	3.28313	0.11425	0.17317
N	3.96249	0.10572	1.32621
H	4.77290	0.69232	1.45215
H	3.64958	-0.43150	2.11907
N	2.20494	-0.65419	0.01790
C	1.80337	-1.57508	1.08326
C	1.47642	-0.63759	-1.25154
H	2.60585	-2.28249	1.30859
H	1.52781	-1.03057	1.99106
H	0.93200	-2.12866	0.74456
H	2.10617	-1.00014	-2.06943
H	0.61545	-1.29412	-1.15898
H	1.11822	0.36968	-1.47953
H	4.97500	2.31082	-1.64512

ADMA_2 – PHE 5

C	4.00479	0.51092	0.46259
C	2.78865	0.51296	1.14890
C	1.68223	-0.17767	0.66808
C	1.78425	-0.88360	-0.52494
C	2.97906	-0.89680	-1.23409
C	4.07660	-0.20263	-0.73452
H	2.70443	1.06079	2.08194
H	0.74483	-0.17635	1.21320
H	3.05386	-1.44304	-2.16967
H	5.00720	-0.21762	-1.29238
O	0.66236	-1.54671	-0.96967
H	0.87520	-2.06309	-1.75675
C	5.20878	1.22916	1.01327
H	5.86819	1.56495	0.21105
H	5.78927	0.56919	1.66468
H	4.91347	2.09906	1.60269
C	-3.10006	2.66015	-0.85396
H	-2.96055	2.50067	-1.92560
H	-2.18426	3.06581	-0.41788
N	-3.49667	1.42795	-0.18781
H	-4.38528	1.41356	0.28764
C	-2.74136	0.32577	-0.15970
N	-1.55892	0.33217	-0.78107
H	-1.23491	1.16469	-1.25048
H	-0.94118	-0.47340	-0.79684
N	-3.16065	-0.76781	0.48065
C	-2.30367	-1.95145	0.53022
C	-4.45927	-0.77122	1.15150
H	-2.10002	-2.33141	-0.47378
H	-1.35984	-1.73537	1.03694
H	-2.82456	-2.72314	1.08929
H	-5.26700	-0.57174	0.44237
H	-4.61793	-1.75758	1.57799
H	-4.48716	-0.03639	1.96124
H	-3.89890	3.38424	-0.71484

ADMA_2 – PHE 6

C	-4.10212	0.47673	0.47875
C	-2.85225	0.63381	1.08275
C	-1.71145	0.03880	0.55756

C	-1.81413	-0.73212	-0.59498
C	-3.04387	-0.90830	-1.21586
C	-4.17529	-0.30564	-0.67380
H	-2.76922	1.22905	1.98642
H	-0.74788	0.16131	1.04010
H	-3.11921	-1.51537	-2.11296
H	-5.13370	-0.45121	-1.16116
O	-0.66279	-1.30644	-1.08448
H	-0.86776	-1.85013	-1.85501
C	-5.32496	1.15070	1.04391
H	-6.23522	0.63019	0.74218
H	-5.39883	2.18302	0.68943
H	-5.29099	1.17776	2.13472
C	3.47537	2.56484	-0.81151
H	2.58595	3.07403	-0.43231
H	3.38137	2.40800	-1.88845
N	3.69401	1.30354	-0.11757
H	4.54478	1.20040	0.41282
C	2.82665	0.28679	-0.13312
N	1.68023	0.41698	-0.80709
H	1.46015	1.28276	-1.27572
H	0.98581	-0.32201	-0.85871
N	3.10042	-0.84724	0.51559
C	2.12468	-1.93624	0.50355
C	4.35765	-0.98465	1.24804
H	1.17809	-1.62183	0.94902
H	1.94568	-2.28727	-0.51579
H	2.52250	-2.76123	1.08747
H	4.40378	-0.28356	2.08626
H	4.41915	-1.99542	1.64035
H	5.21360	-0.82606	0.58710
H	4.33993	3.19785	-0.62816

ADMA_2 – PHE 7

C	-4.10409	-0.47441	-0.50985
C	-2.82253	-0.76826	-0.98270
C	-1.68827	-0.21944	-0.39724
C	-1.82883	0.64399	0.68388
C	-3.09003	0.95768	1.17275
C	-4.21452	0.39826	0.57222
H	-2.70818	-1.44011	-1.82736
H	-0.69894	-0.45432	-0.77423
H	-3.19604	1.63654	2.01336
H	-5.19685	0.65042	0.95776
O	-0.68310	1.16807	1.23680
H	-0.90730	1.75374	1.97007
C	-5.32148	-1.10171	-1.13707
H	-6.22083	-0.52744	-0.90946
H	-5.47132	-2.11836	-0.76208
H	-5.21845	-1.16246	-2.22224
C	3.61889	-2.47879	0.81925
H	2.72951	-3.02451	0.49483
H	3.57279	-2.30123	1.89603
N	3.76194	-1.22667	0.08949
H	4.57685	-1.11110	-0.49268
C	2.85400	-0.24608	0.11876
N	1.74701	-0.40755	0.84888
H	1.60301	-1.25764	1.37249
H	1.03156	0.30750	0.93305
N	3.04562	0.87880	-0.57357
C	2.01896	1.92039	-0.55325
C	4.27091	1.05776	-1.34971

H	1.06348	1.53657	-0.91770
H	1.88849	2.32211	0.45482
H	2.33438	2.72808	-1.20745
H	4.33172	0.32972	-2.16339
H	4.26255	2.05405	-1.78213
H	5.15426	0.97327	-0.71118
H	4.49412	-3.08777	0.60723

ADMA_2 – PHE 8

C	-4.08541	0.47709	0.52577
C	-2.79907	0.79225	0.96737
C	-1.66930	0.25858	0.35722
C	-1.82064	-0.61053	-0.71670
C	-3.08886	-0.94499	-1.17568
C	-4.20696	-0.40148	-0.55168
H	-2.67505	1.46851	1.80693
H	-0.67612	0.51215	0.71104
H	-3.20379	-1.62779	-2.01180
H	-5.19413	-0.67034	-0.91332
O	-0.68059	-1.12116	-1.29395
H	-0.91281	-1.70488	-2.02627
C	-5.30163	1.08571	1.17319
H	-6.14920	0.39870	1.14267
H	-5.59968	2.00212	0.65528
H	-5.10543	1.34236	2.21556
C	3.68643	2.43884	-0.81321
H	2.79632	3.00540	-0.52887
H	3.67693	2.24748	-1.88860
N	3.77891	1.19419	-0.06221
H	4.57068	1.06810	0.54934
C	2.85333	0.23104	-0.11582
N	1.77882	0.40708	-0.88963
H	1.66552	1.26219	-1.41214
H	1.04462	-0.28760	-0.98252
N	2.99589	-0.89123	0.59211
C	1.95546	-1.91756	0.53153
C	4.18194	-1.08290	1.42344
H	0.99042	-1.51759	0.85109
H	1.86396	-2.32225	-0.47962
H	2.22903	-2.72579	1.20323
H	4.21656	-0.35096	2.23510
H	4.14041	-2.07646	1.86048
H	5.09500	-1.01538	0.82649
H	4.56448	3.03468	-0.57695

SDMA_1 – PHE 1

C	-1.09149	-0.91970	-0.85656
H	-0.66738	-0.19056	-0.16883
H	-0.82477	-0.66422	-1.88242
N	-2.54242	-0.97295	-0.67300
H	-2.99072	-1.86685	-0.81984
C	-3.28677	-0.01433	-0.12370
N	-2.88465	1.26078	-0.12081
N	-4.44836	-0.33376	0.45628
H	-3.38103	1.88165	0.50383
H	-5.10700	0.41499	0.61964
C	-2.02493	1.88636	-1.12581
H	-2.43496	2.86988	-1.35087
H	-2.03321	1.29561	-2.04034
H	-1.00347	1.99722	-0.76167
C	-4.88269	-1.69860	0.71950
H	-5.19247	-2.20860	-0.19649

H	-5.73269	-1.65468	1.39658
H	-4.08354	-2.26288	1.20245
H	-0.68453	-1.90095	-0.61660
C	4.09503	-0.48915	-0.19320
C	3.17373	0.05932	-1.09066
C	2.01136	0.67808	-0.65035
C	1.74812	0.75503	0.71527
C	2.64614	0.21528	1.62799
C	3.80887	-0.39855	1.16788
H	3.37342	0.00647	-2.15646
H	1.30766	1.11126	-1.35275
H	2.44197	0.27412	2.69275
H	4.50507	-0.81416	1.88894
O	0.58192	1.36123	1.10050
H	0.54294	1.40815	2.06276
C	5.36227	-1.13485	-0.68987
H	5.86008	-1.68785	0.10803
H	6.06273	-0.38273	-1.06378
H	5.15676	-1.82752	-1.50916

SDMA_1 – PHE 2

C	2.18894	1.50170	-0.29395
C	1.46536	0.83416	-1.28532
C	1.43672	-0.55464	-1.35551
C	2.14161	-1.30682	-0.41911
C	2.86612	-0.66423	0.58244
C	2.88447	0.72621	0.63539
H	0.91938	1.41096	-2.02568
H	0.88252	-1.06634	-2.13457
H	3.41499	-1.24764	1.31558
H	3.45533	1.21584	1.41822
O	2.07490	-2.66517	-0.52191
H	2.65743	-3.07157	0.13014
C	2.24699	3.00674	-0.24787
H	2.29445	3.37004	0.78073
H	1.37427	3.45083	-0.73037
H	3.13538	3.37702	-0.76786
C	-1.46640	-2.15221	0.33158
H	-1.76131	-2.21701	1.37796
H	-0.37925	-2.16996	0.24946
N	-2.04058	-0.94923	-0.26946
H	-2.34264	-1.01910	-1.23124
C	-2.37438	0.16285	0.38293
N	-1.80970	0.46936	1.55714
N	-3.30406	0.97508	-0.12908
H	-2.25719	1.20764	2.08403
H	-3.35261	1.91345	0.24302
C	-0.43827	0.12379	1.93849
H	-0.00550	0.98402	2.44741
H	0.15347	-0.08077	1.04783
H	-0.41025	-0.73772	2.60665
C	-4.15969	0.63456	-1.25747
H	-3.60561	0.64125	-2.19993
H	-4.95044	1.37893	-1.31433
H	-4.61507	-0.34539	-1.10494
H	-1.87492	-3.01221	-0.19672

SDMA_1 – PHE 3

C	4.07742	0.28177	-0.61272
C	2.78940	0.42602	-1.13643
C	1.66165	0.12271	-0.38503
C	1.81340	-0.33187	0.92159

C	3.08013	-0.48081	1.46914
C	4.19929	-0.17409	0.69890
H	2.66638	0.78311	-2.15411
H	0.66807	0.23756	-0.80522
H	3.19336	-0.83207	2.49043
H	5.18591	-0.29311	1.13398
O	0.67401	-0.61749	1.63521
H	0.90518	-0.94661	2.51219
C	5.28732	0.59777	-1.45266
H	6.19424	0.60830	-0.84650
H	5.18689	1.57286	-1.93478
H	5.41900	-0.14732	-2.24223
C	-4.54399	-1.38290	-0.73730
H	-4.29827	-1.95266	0.15802
H	-4.22074	-1.93028	-1.62273
N	-3.93801	-0.05440	-0.66182
H	-4.50222	0.72217	-0.97790
C	-2.80944	0.24315	-0.01550
N	-1.88107	-0.68724	0.22438
N	-2.60785	1.49122	0.41851
H	-1.14348	-0.44238	0.87871
H	-1.66637	1.73836	0.69138
C	-1.60403	-1.84406	-0.62653
H	-0.53094	-2.02947	-0.58253
H	-1.87728	-1.63087	-1.66008
H	-2.12791	-2.73766	-0.28654
C	-3.63367	2.52325	0.47373
H	-3.87793	2.90356	-0.52167
H	-3.24788	3.34517	1.07227
H	-4.53538	2.13718	0.95218
H	-5.62334	-1.24971	-0.77733

SDMA_1 – PHE 4

C	4.05458	-0.33723	-0.49608
C	2.81804	-0.82656	-0.92712
C	1.62411	-0.31682	-0.43335
C	1.66000	0.70208	0.51179
C	2.87120	1.20529	0.96300
C	4.05751	0.68145	0.45539
H	2.78809	-1.62170	-1.66526
H	0.66961	-0.70122	-0.77637
H	2.89223	1.99893	1.70379
H	5.00253	1.07749	0.81137
O	0.45233	1.18247	0.97920
H	0.60694	1.88860	1.61972
C	5.33829	-0.88582	-1.06115
H	6.19742	-0.55581	-0.47557
H	5.48453	-0.54873	-2.09121
H	5.32900	-1.97800	-1.07083
C	-4.66975	-0.45220	-1.25120
H	-4.04261	-0.19469	-2.10402
H	-5.38665	0.34765	-1.06591
N	-3.83469	-0.72274	-0.08147
H	-4.09172	-1.51907	0.48525
C	-2.64565	-0.17190	0.16675
N	-2.29934	1.00547	-0.36953
N	-1.76555	-0.81097	0.93992
H	-1.33028	1.26860	-0.23192
H	-0.96987	-0.27367	1.26604
C	-3.23224	2.08634	-0.69048
H	-2.71252	3.02813	-0.52412
H	-4.09951	2.05036	-0.03112

H	-3.56042	2.04706	-1.72880
C	-1.89943	-2.19445	1.37185
H	-2.65234	-2.30237	2.15673
H	-0.93805	-2.51311	1.76870
H	-2.15523	-2.83574	0.52642
H	-5.21267	-1.36586	-1.48531

SDMA_1 – PHE 5

C	4.14434	-0.57046	-0.43945
C	2.85191	-1.03087	-0.70908
C	1.72979	-0.29506	-0.35208
C	1.89213	0.92929	0.28943
C	3.16278	1.41136	0.57008
C	4.27619	0.65918	0.20281
H	2.72105	-1.98426	-1.21136
H	0.73208	-0.65958	-0.57211
H	3.28558	2.36772	1.06929
H	5.26640	1.04398	0.42256
O	0.75711	1.62962	0.62538
H	0.99556	2.45552	1.06351
C	5.34787	-1.38646	-0.83268
H	6.27275	-0.85379	-0.60730
H	5.33661	-1.61131	-1.90204
H	5.36672	-2.33909	-0.29670
C	-4.84931	-1.36299	-0.31265
H	-4.48020	-1.49474	-1.32898
H	-5.73642	-0.72950	-0.32186
N	-3.79272	-0.81314	0.53569
H	-3.69485	-1.21716	1.45652
C	-2.79664	-0.02622	0.12593
N	-2.91472	0.71380	-0.98581
N	-1.65876	0.01163	0.81783
H	-2.06005	1.13933	-1.31965
H	-0.99870	0.76042	0.62809
C	-4.16636	1.28464	-1.48551
H	-3.93388	2.25174	-1.92742
H	-4.86494	1.44120	-0.66404
H	-4.62840	0.65336	-2.24449
C	-1.31480	-0.92024	1.88205
H	-1.91292	-0.74563	2.78045
H	-0.26652	-0.76417	2.13059
H	-1.44393	-1.95131	1.54694
H	-5.11689	-2.33887	0.08794

SDMA_2 – PHE 1

C	-3.60683	-0.73050	-0.33865
C	-2.45765	-0.70795	-1.13429
C	-1.45790	0.23643	-0.93906
C	-1.59725	1.17732	0.07624
C	-2.72736	1.17864	0.88311
C	-3.72181	0.22776	0.66772
H	-2.34572	-1.43928	-1.92871
H	-0.57422	0.25285	-1.56752
H	-2.83206	1.91304	1.67578
H	-4.60326	0.23454	1.30043
O	-0.56248	2.06629	0.26369
H	-0.82248	2.74525	0.89855
C	-4.69456	-1.74258	-0.58628
H	-5.37900	-1.79939	0.26124
H	-4.27630	-2.73682	-0.75718
H	-5.27890	-1.47485	-1.47122
C	2.69115	2.01260	-0.91219

H	2.55666	2.58150	0.01089
H	1.73418	1.92586	-1.43155
N	3.26242	0.70103	-0.63845
H	4.14588	0.46792	-1.06633
C	2.66990	-0.21226	0.13491
N	1.49655	0.06061	0.70293
N	3.25754	-1.39356	0.34082
H	1.03874	0.94076	0.48757
H	2.77670	-2.06852	0.91602
C	0.77053	-0.88268	1.54177
H	-0.13086	-0.38781	1.89733
H	1.36871	-1.17378	2.40900
H	0.47561	-1.77114	0.97678
C	4.54940	-1.76378	-0.21756
H	4.52328	-1.75641	-1.31008
H	4.77840	-2.77343	0.11328
H	5.33707	-1.09480	0.13764
H	3.38622	2.54958	-1.55278

SDMA_2 – PHE 2

C	3.42380	-0.82877	-0.39247
C	2.37276	-0.53900	-1.26841
C	1.45768	0.46888	-0.99546
C	1.58326	1.20461	0.17936
C	2.61544	0.93910	1.06850
C	3.52793	-0.07188	0.77334
H	2.27450	-1.10784	-2.18774
H	0.65236	0.69554	-1.68563
H	2.70946	1.51322	1.98523
H	4.33523	-0.27128	1.47018
O	0.62879	2.16487	0.42751
H	0.89310	2.70529	1.18231
C	4.41386	-1.91623	-0.71832
H	5.12768	-2.05522	0.09472
H	4.97637	-1.67159	-1.62319
H	3.90788	-2.86875	-0.89416
C	-1.00240	-0.53780	1.97390
H	-1.05115	0.46816	2.39867
H	-0.10820	-0.63620	1.35314
N	-2.20421	-0.84153	1.20924
H	-2.76537	-1.63001	1.49471
C	-2.58963	-0.14716	0.13669
N	-1.91019	0.93530	-0.23608
N	-3.65061	-0.54537	-0.56994
H	-1.11007	1.23772	0.30928
H	-3.96351	0.04521	-1.32584
C	-2.19574	1.68436	-1.45022
H	-1.45800	2.48014	-1.52361
H	-3.18861	2.13850	-1.41390
H	-2.11693	1.04610	-2.33409
C	-4.42966	-1.73563	-0.26221
H	-3.79640	-2.62530	-0.27080
H	-5.18651	-1.84616	-1.03494
H	-4.93157	-1.64573	0.70399
H	-0.93923	-1.25247	2.79087

SDMA_2 – PHE 3

C	-4.67507	-0.35241	0.30036
C	-3.48068	-0.97973	0.66825
C	-2.24987	-0.51421	0.22644
C	-2.19319	0.60306	-0.60354
C	-3.36608	1.24324	-0.98550

C	-4.59255	0.76154	-0.53261
H	-3.51526	-1.85253	1.31305
H	-1.33011	-1.01105	0.51353
H	-3.32524	2.11258	-1.63512
H	-5.50190	1.26857	-0.83829
O	-0.95859	1.02734	-1.01277
H	-1.05764	1.78211	-1.60428
C	-5.99956	-0.86644	0.80159
H	-6.82948	-0.35536	0.31145
H	-6.09763	-0.71032	1.87950
H	-6.10171	-1.93812	0.61467
C	1.50740	-1.49080	-0.19532
H	0.91436	-0.73179	-0.71116
H	1.21750	-1.53821	0.85762
N	2.93434	-1.22328	-0.32495
H	3.51128	-1.91147	-0.78487
C	3.52074	-0.11752	0.13501
N	2.78955	0.82480	0.73125
N	4.83908	0.04503	0.00649
H	1.79167	0.68649	0.79265
H	5.24823	0.90253	0.34754
C	3.34587	2.05771	1.26772
H	2.52713	2.63746	1.68624
H	3.82191	2.64852	0.48129
H	4.06512	1.84951	2.06287
C	5.71818	-0.94081	-0.60650
H	5.67497	-1.89030	-0.06746
H	6.73442	-0.55924	-0.55245
H	5.46121	-1.09903	-1.65666
H	1.30801	-2.45670	-0.65289

Calculations in N,N-dimethylformamide

Arg

C	2.14597	-0.58373	0.41184
H	2.01262	-1.30547	-0.39702
H	1.81063	-1.02229	1.35438
N	1.42891	0.65104	0.12798
H	1.95341	1.51384	0.06682
C	0.11540	0.72125	-0.04879
N	-0.46393	1.89829	-0.29225
H	0.09768	2.72877	-0.41669
H	-1.45332	1.95200	-0.48864
N	-0.62979	-0.37844	0.02905
H	3.20193	-0.33990	0.49401
H	-0.22074	-1.28099	0.22150
H	-1.62789	-0.32916	-0.12028

MMA 1

C	2.29514	-0.42961	0.57253
H	2.47027	-1.33171	-0.01348
H	1.82655	-0.69250	1.52041
N	1.46805	0.53764	-0.14789
H	1.94571	1.33270	-0.55346
C	0.14344	0.64374	-0.04889
N	-0.43918	1.82061	-0.29253
H	0.11100	2.66627	-0.34769
H	-1.43533	1.87921	-0.45003
N	-0.61411	-0.39917	0.29178
H	3.25042	0.04966	0.77797
H	-1.58604	-0.19064	0.48360
C	-0.28283	-1.80221	0.04420
H	0.34578	-1.89057	-0.84143

H	0.21805	-2.25887	0.89741
H	-1.21800	-2.32757	-0.13996

MMA 2

C	2.14780	-0.67741	0.39092
H	2.00978	-1.33560	-0.46977
H	1.78031	-1.17097	1.29305
N	1.47325	0.59737	0.18602
H	2.03844	1.43435	0.15976
C	0.15968	0.71764	0.02347
N	-0.38859	1.91883	-0.15134
H	-1.38988	1.96153	-0.28576
N	-0.61770	-0.36929	0.05895
H	3.20879	-0.47327	0.50919
H	-0.21636	-1.29557	0.06451
H	-1.60441	-0.28675	-0.14284
C	0.36801	3.16170	-0.15848
H	1.10506	3.16714	-0.96558
H	-0.33491	3.97440	-0.32252
H	0.87115	3.32009	0.79832

MMA 3

C	2.14399	-0.58675	0.42526
H	2.02724	-1.30796	-0.38702
H	1.78722	-1.02663	1.35950
N	1.44030	0.65184	0.12876
H	1.97298	1.50896	0.06295
C	0.12563	0.73311	-0.06725
N	-0.43903	1.90961	-0.33465
H	0.16744	2.71657	-0.39487
N	-0.62818	-0.36281	0.01053
H	3.19910	-0.34809	0.53210
H	-0.21843	-1.26676	0.19537
H	-1.62249	-0.32475	-0.16099
C	-1.86322	2.08733	-0.57481
H	-2.44788	1.77965	0.29535
H	-2.03785	3.14490	-0.75537
H	-2.18743	1.52297	-1.45248

ADMA 1

C	2.19354	-0.54385	0.42312
H	2.52676	-1.28914	-0.29966
H	1.60654	-1.02987	1.20120
N	1.40680	0.51311	-0.21057
H	1.91637	1.33832	-0.49996
C	0.07987	0.65740	-0.11049
N	-0.43805	1.89177	-0.13035
H	0.17963	2.69166	-0.10934
H	-1.39662	2.04711	-0.40966
N	-0.72295	-0.39666	0.03702
C	-2.11600	-0.22809	0.44380
C	-0.37718	-1.72216	-0.47914
H	-2.20730	0.58901	1.15785
H	-2.75776	-0.03794	-0.42170
H	-2.43637	-1.15065	0.92640
H	-0.17518	-2.41947	0.33585
H	-1.22806	-2.08587	-1.05751
H	0.48426	-1.66301	-1.13930
H	3.06505	-0.08096	0.88414

ADMA 2

C	2.61451	0.25978	-0.01019
H	2.86787	-0.32896	-0.89504

H	2.86500	-0.30497	0.89060
N	1.20825	0.63603	-0.01295
H	0.98364	1.61598	-0.10061
C	0.20925	-0.24880	0.00255
N	0.50133	-1.55587	0.00942
H	1.46043	-1.86076	0.09002
H	-0.21317	-2.24842	0.17788
N	-1.06051	0.15872	-0.00776
C	-2.14235	-0.82441	-0.02158
C	-1.36533	1.58780	0.01778
H	-2.02797	-1.51467	-0.86038
H	-2.17477	-1.38862	0.91450
H	-3.08268	-0.29423	-0.14090
H	-0.99902	2.08397	-0.88518
H	-2.44384	1.70656	0.06384
H	-0.92730	2.06339	0.89866
H	3.20037	1.17530	-0.01962

SDMA 1

C	1.16730	1.79922	-0.21073
H	1.55309	1.39578	-1.14625
H	1.95926	1.81084	0.53828
N	0.00937	1.02329	0.22887
H	-0.77640	1.54842	0.58834
C	-0.19718	-0.26800	-0.02538
N	0.82376	-1.09030	-0.30053
N	-1.44192	-0.74832	-0.03198
H	0.56365	-2.00994	-0.63352
H	-1.55165	-1.75277	0.01254
C	2.17414	-0.95652	0.24743
H	2.55978	-1.96073	0.41367
H	2.14598	-0.43374	1.20323
H	2.84078	-0.43115	-0.43651
C	-2.63846	0.07761	0.03468
H	-2.77172	0.51288	1.02841
H	-3.49247	-0.55849	-0.18599
H	-2.59203	0.87443	-0.70977
H	0.82881	2.81885	-0.38479

SDMA 2

C	-1.66754	1.80492	0.00089
H	-2.20658	1.48042	0.89431
H	-2.20684	1.47705	-0.89111
N	-0.30304	1.29864	0.00122
H	0.45254	1.96849	0.00498
C	0.00077	-0.00011	0.00172
N	-0.97325	-0.91127	0.00203
N	1.27714	-0.38693	0.00110
H	-1.92993	-0.58794	0.00461
H	1.47763	-1.37658	0.00323
C	-0.73435	-2.34705	0.00154
H	-1.70182	-2.84263	0.00105
H	-0.18316	-2.65257	0.89422
H	-0.18274	-2.65167	-0.89114
C	2.39977	0.53904	-0.00110
H	2.38652	1.17000	-0.89322
H	3.31314	-0.05026	-0.00407
H	2.39151	1.16867	0.89206
H	-1.61672	2.89073	-0.00126

CH₃COO

C	0.19547	-0.00000	-0.01137
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O	0.75516	-1.12111	0.00198
O	0.75307	1.12218	0.00212
C	-1.34448	-0.00099	-0.00448
H	-1.74306	-0.89725	-0.48253
H	-1.74350	0.89044	-0.49117
H	-1.68523	0.00422	1.03607

EtOH

C	-1.20596	-0.24272	-0.02259
C	0.07879	0.56047	0.04719
H	-1.24990	-0.97345	0.79055
H	-2.07249	0.41699	0.06946
H	-1.27474	-0.77679	-0.97321
H	0.12000	1.28770	-0.76575
H	0.12993	1.11389	0.99187
O	1.23619	-0.25670	-0.10643
H	1.22070	-0.92124	0.59085

N-methylacetamide

C	-0.48297	0.14609	-0.00419
O	-0.37688	1.39722	-0.00036
N	0.60521	-0.65484	-0.00417
H	0.48689	-1.65496	-0.00181
C	1.95329	-0.10530	0.00285
H	2.11665	0.53207	-0.86602
C	-1.82212	-0.53609	0.00171
H	-2.42974	-0.13627	-0.80849
H	-2.33276	-0.31501	0.93905
H	-1.74345	-1.61492	-0.11075
H	2.12719	0.49253	0.89772
H	2.66458	-0.92554	-0.01981

p-methylphenol

C	-1.37535	0.01275	-0.00526
C	-0.66529	-1.19302	-0.00475
C	0.72281	-1.21907	-0.00066
C	1.43579	-0.02064	0.00164
C	0.75259	1.19127	-0.00082
C	-0.64138	1.19775	-0.00466
H	-1.21131	-2.13159	-0.00820
H	1.26466	-2.15817	-0.00138
H	1.30576	2.12583	-0.00173
H	-1.16322	2.14941	-0.00817
O	2.79685	-0.09499	0.00394
H	3.16897	0.79561	0.00419
C	-2.88205	0.01885	0.00601
H	-3.27028	1.02611	-0.15351
H	-3.26807	-0.34345	0.96317
H	-3.28411	-0.63119	-0.77492

6OI0 Arg

C	1.96931	0.02383	-0.01878
N	0.66877	-0.61042	0.00574
C	-0.52276	-0.00720	0.01698
N	-0.68944	1.32464	0.02741
N	-1.52930	-0.78102	-0.00335
H	2.20167	0.51765	0.92606
H	2.71136	-0.75430	-0.18482
H	0.66069	-1.61535	0.18344
H	0.09629	1.97304	-0.00612
H	-1.61354	1.69952	-0.13508
H	-1.44369	-1.77743	-0.17561

H	-2.47251	-0.41868	0.03320
H	2.03014	0.74338	-0.83890

6OI0 S49B

C	-1.84298	-0.51901	0.02131
C	-0.46200	0.14402	-0.00690
O	-0.36941	1.36832	-0.01106
N	0.60569	-0.65191	-0.06510
C	1.95445	-0.10157	0.03139
H	-2.45531	-0.06843	-0.76011
H	2.14950	0.57422	-0.80260
H	0.47954	-1.65109	0.00462
H	2.08246	0.45036	0.96513
H	2.66492	-0.92528	-0.00232
H	-2.30516	-0.30461	0.98710
H	-1.79735	-1.59905	-0.12246

6OI0 S91BD92

C	-3.44541	-0.33172	-0.39076
C	-2.02264	-0.17677	0.14775
O	-1.52969	-1.07660	0.80645
N	-1.34110	0.92959	-0.15456
C	0.06145	1.05589	0.26340
C	0.95620	0.20372	-0.67058
C	2.30894	-0.14118	-0.08272
O	2.56905	0.14909	1.10666
O	3.12767	-0.74908	-0.81723
H	-4.10649	-0.56766	0.44421
H	1.09933	0.71710	-1.62642
H	0.44576	-0.73490	-0.90196
H	0.15443	0.69827	1.28597
H	-1.77041	1.63778	-0.73244
H	-3.45026	-1.17592	-1.08273
H	0.33242	2.11057	0.22980
H	-3.80452	0.56067	-0.90408

6OI0 F133B

C	-1.81676	-0.54760	0.03747
C	-0.46304	0.15006	-0.03434
O	-0.40512	1.38312	-0.01256
N	0.60175	-0.62919	-0.08882
C	1.96260	-0.10461	0.04721
H	-2.50532	-0.05404	-0.64863
H	2.05245	0.85847	-0.45109
H	0.46858	-1.62895	-0.03469
H	2.65690	-0.81342	-0.40259
H	2.22142	0.02847	1.10183
H	-2.20170	-0.42711	1.05268
H	-1.76036	-1.61110	-0.19732

6OI0 S175B

C	-1.83846	-0.52234	0.00860
C	-0.46622	0.13714	-0.01549
O	-0.39768	1.35961	-0.00168
N	0.60686	-0.65049	-0.01282
C	1.97895	-0.08667	0.00994
H	-2.42555	-0.12500	-0.82020
H	2.15011	0.52742	-0.87506
H	0.49016	-1.65298	-0.02167
H	-2.33067	-0.24113	0.94120
H	-1.79726	-1.61032	-0.06230
H	2.68271	-0.91602	0.02128

H	2.11834	0.52583	0.90155
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6OI0 C261B

C	-1.80215	-0.56447	0.01578
C	-0.46734	0.16224	-0.01708
O	-0.42186	1.38725	-0.00479
N	0.60240	-0.61769	-0.03765
C	1.96480	-0.10614	0.02118
H	-1.70761	-1.63786	-0.14533
H	2.15714	0.56728	-0.81575
H	0.46854	-1.61797	-0.02007
H	-2.45500	-0.13570	-0.74628
H	-2.26185	-0.38739	0.99071
H	2.13503	0.43687	0.95325
H	2.65007	-0.94920	-0.03395

6OI1 MMA

C	-2.44771	-0.12095	0.00807
C	2.46225	-0.09990	0.00160
N	1.13664	-0.65024	-0.01188
C	-0.00129	0.06730	-0.00710
N	-0.02872	1.35510	0.00568
N	-1.11459	-0.65260	-0.01070
H	-3.13498	-0.95814	0.13520
H	-2.58790	0.55666	0.85633
H	-2.69991	0.40896	-0.91466
H	2.69088	0.40255	0.94472
H	3.16711	-0.91988	-0.12015
H	0.81568	1.93126	0.02131
H	2.60428	0.60163	-0.82528
H	1.03284	-1.65422	0.12596
H	-1.00928	-1.65907	-0.07852
H	-0.91150	1.84573	-0.04202

6OI1 S49B

C	-1.83044	-0.51829	0.02510
C	-0.46288	0.13598	-0.02715
O	-0.37830	1.36611	-0.00900
N	0.61158	-0.65584	-0.05832
C	1.94469	-0.09260	0.03337
H	-2.48119	-0.02801	-0.69894
H	2.14156	0.57045	-0.81104
H	0.49058	-1.65729	-0.02374
H	-1.79917	-1.59037	-0.17463
H	2.66718	-0.90670	0.02101
H	-2.24424	-0.35586	1.02295
H	2.06242	0.47923	0.95673

6OI1 S91BD92

C	-3.46295	-0.25257	-0.45775
C	-2.02868	-0.20227	0.11396
O	-1.55359	-1.20824	0.65311
N	-1.34839	0.92877	-0.00147
C	0.05082	1.00272	0.43416
C	0.95852	0.33809	-0.63041
C	2.32546	-0.13005	-0.09815
O	2.59739	-0.06663	1.11883
O	3.14676	-0.58651	-0.94154
H	-4.14120	-0.53879	0.34737
H	1.12586	1.02183	-1.46789
H	0.45296	-0.53675	-1.04941
H	0.14801	0.47539	1.38061

H	-1.76790	1.70776	-0.48929
H	-3.77639	0.70038	-0.88412
H	0.30381	2.05157	0.58452
H	-3.48993	-1.02728	-1.22564

6OI1 F133B

C	-1.82168	-0.54354	0.04299
C	-0.45756	0.15266	-0.04709
O	-0.38164	1.39480	-0.01235
N	0.59623	-0.63489	-0.09569
C	1.94679	-0.11577	0.05315
H	-2.53449	-0.01443	-0.58929
H	2.04402	0.85088	-0.43690
H	0.45565	-1.63406	-0.04849
H	-1.77707	-1.59434	-0.24587
H	-2.16148	-0.47292	1.07885
H	2.64792	-0.81980	-0.39462
H	2.19964	0.01035	1.11061

6OI1 S175B

C	-1.87935	-0.47308	0.00945
C	-0.45724	0.13761	-0.01684
O	-0.30693	1.34605	-0.00214
N	0.57195	-0.67285	-0.01355
C	1.96758	-0.10820	0.01087
H	-2.44996	-0.04444	-0.81496
H	2.13002	0.51083	-0.87077
H	0.44627	-1.67433	-0.02263
H	-1.87426	-1.56057	-0.07057
H	2.66223	-0.94516	0.01397
H	2.10269	0.49549	0.90787
H	-2.35107	-0.17828	0.94812

6OI1 C261B

C	-1.81120	-0.57051	0.01851
C	-0.46775	0.17190	-0.01816
O	-0.41535	1.39014	-0.00568
N	0.60518	-0.60545	-0.04541
C	1.96342	-0.11816	0.02508
H	2.16951	0.57221	-0.79513
H	0.46405	-1.60486	-0.02414
H	2.63861	-0.96788	-0.05007
H	2.14588	0.40133	0.96889
H	-2.25702	-0.41221	1.00292
H	-2.47400	-0.13150	-0.72878
H	-1.70731	-1.63942	-0.16291

6OI2 SDMA

C	-2.43243	-0.71026	0.00282
N	-1.33887	0.24530	-0.00970
C	-0.02702	-0.02372	-0.00807
N	0.81139	0.91585	-0.00473
N	0.47468	-1.25506	0.00065
C	0.57971	2.33104	-0.00904
C	1.90026	-1.53089	0.01384
H	-2.45551	-1.28848	0.92776
H	-3.35846	-0.14453	-0.06834
H	0.08203	2.65818	-0.92270
H	-0.03719	2.63085	0.84349
H	1.53670	2.84040	0.07355
H	2.40030	-1.15272	-0.88266
H	2.37765	-1.11289	0.90385

H	2.02334	-2.61282	0.04334
H	-2.36567	-1.38146	-0.85672
H	-1.59832	1.21404	0.16450
H	1.78749	0.66162	-0.00539
H	-0.14585	-2.05188	-0.12154

6OI2 S49B

C	-1.84489	-0.52853	0.02240
C	-0.46320	0.14170	-0.00433
O	-0.39259	1.36803	-0.01163
N	0.61836	-0.64859	-0.07122
C	1.96594	-0.09370	0.03304
H	-2.43838	-0.11401	-0.79293
H	2.15295	0.60216	-0.78598
H	0.49560	-1.64699	0.01556
H	-2.32781	-0.27115	0.96686
H	-1.79435	-1.61375	-0.07329
H	2.67957	-0.91332	-0.02463
H	2.09747	0.43614	0.97931

6OI2 S91BD92

C	3.43884	-0.03512	0.54529
C	2.04366	-0.23371	-0.03752
O	1.61927	-1.37632	-0.19521
N	1.33003	0.85285	-0.33244
C	-0.06572	0.73484	-0.78182
C	-1.00804	0.63387	0.43029
C	-2.32152	-0.08384	0.13886
O	-2.58961	-0.54821	-0.98829
O	-3.11680	-0.19325	1.10073
H	4.15544	-0.56480	-0.08449
H	-1.23759	1.62805	0.82610
H	-0.50664	0.09145	1.23694
H	-0.14846	-0.16600	-1.38536
H	1.71439	1.76352	-0.12480
H	3.72515	1.01459	0.61990
H	3.45698	-0.48820	1.53783
H	-0.29573	1.59750	-1.40752

6OI2 F133B

C	-1.83721	-0.53216	0.03537
C	-0.46455	0.14855	-0.04068
O	-0.38263	1.38091	-0.00998
N	0.59666	-0.64453	-0.07692
C	1.97041	-0.10588	0.04292
H	-2.51759	-0.03003	-0.65245
H	2.01549	0.89667	-0.37594
H	0.46325	-1.64456	-0.03164
H	-2.21629	-0.40106	1.05123
H	-1.79537	-1.59715	-0.19592
H	2.27088	-0.05906	1.09308
H	2.65217	-0.76343	-0.49566

6OI2 S175B

C	-1.85459	-0.51508	0.00672
C	-0.46454	0.14380	-0.01026
O	-0.37030	1.36164	-0.00152
N	0.59270	-0.64619	-0.01188
C	1.98256	-0.10255	0.00804
H	-2.42372	-0.13228	-0.84128
H	2.15531	0.51529	-0.87359
H	0.46919	-1.64804	-0.01245

H	2.13541	0.49932	0.90434
H	2.66882	-0.94630	0.00782
H	-1.81267	-1.60382	-0.04162
H	-2.35936	-0.21101	0.92502

6OI2 C261B

C	-1.82146	-0.56842	0.01920
C	-0.46969	0.16526	-0.02156
O	-0.42096	1.38811	-0.00448
N	0.61183	-0.61016	-0.04192
C	1.97463	-0.11200	0.02417
H	-1.72058	-1.64765	-0.08970
H	2.17506	0.57299	-0.80219
H	0.47510	-1.61041	-0.02422
H	2.15142	0.41537	0.96430
H	2.65221	-0.96018	-0.04439
H	-2.45126	-0.17289	-0.77980
H	-2.29799	-0.34009	0.97443

6OI3 MMA H3R2me Peptide

C	-2.45535	-0.05870	0.05237
C	2.44236	-0.05939	0.08217
N	1.14884	-0.68810	-0.12990
C	0.00377	0.00137	-0.05092
N	0.00909	1.33285	-0.03021
N	-1.14032	-0.67618	0.01458
H	-3.19817	-0.85153	0.12263
H	-2.65628	0.54102	-0.83952
H	-2.54974	0.57927	0.93706
H	2.52168	0.37985	1.07932
H	3.20464	-0.82824	-0.02174
H	0.86624	1.88443	-0.02739
H	2.62193	0.71091	-0.67020
H	1.06732	-1.65986	0.16627
H	-0.86480	1.83740	-0.06226
H	-1.08080	-1.67299	-0.16712

6OI3 S49B

C	-1.82141	-0.52855	0.03769
C	-0.47446	0.15294	-0.02901
O	-0.37198	1.37500	-0.01395
N	0.60590	-0.64954	-0.09923
C	1.94424	-0.10394	0.04841
H	-2.49638	-0.05825	-0.67834
H	2.19079	0.54456	-0.79561
H	0.47388	-1.64725	-0.02298
H	2.01142	0.47858	0.97177
H	2.65837	-0.92310	0.10748
H	-2.22707	-0.37072	1.04025
H	-1.76666	-1.59973	-0.15897

6OI3 S91BD92

C	-3.50292	-0.26841	-0.38184
C	-2.11140	-0.13242	0.18493
O	-1.73870	-0.83054	1.13233
N	-1.30763	0.77770	-0.38542
C	0.08607	0.94100	0.02842
C	0.99442	-0.08479	-0.63955
C	2.41716	-0.14358	-0.03788
O	2.69157	0.68977	0.85180
O	3.16043	-1.04202	-0.50051
H	-4.22523	-0.14473	0.42701

H	1.08298	0.12644	-1.71137
H	0.54576	-1.08100	-0.56758
H	0.12624	0.82541	1.10868
H	-1.64880	1.28826	-1.18773
H	0.39069	1.95870	-0.21557
H	-3.71080	0.45448	-1.17043
H	-3.61390	-1.27988	-0.77852

6OI3 F133B

C	-1.82058	-0.54129	0.00921
C	-0.47876	0.15669	-0.00726
O	-0.39065	1.37966	-0.00186
N	0.60704	-0.63958	-0.02760
C	1.95886	-0.10434	0.01032
H	-2.39173	-0.22112	-0.86421
H	2.08284	0.70709	-0.70729
H	0.47559	-1.63899	0.01963
H	2.65743	-0.90669	-0.22571
H	2.19250	0.28604	1.00616
H	-2.36500	-0.22544	0.90102
H	-1.73284	-1.62755	0.00488

6OI3 S175B

C	1.81748	-0.54711	0.01276
C	0.47822	0.15721	-0.00875
O	0.39958	1.38060	-0.00391
N	-0.60989	-0.63598	-0.03526
C	-1.96020	-0.10243	0.01897
H	2.32140	-0.29803	0.94925
H	-2.27825	0.13545	1.03810
H	-0.48216	-1.63612	0.01100
H	1.73117	-1.63063	-0.07055
H	2.42729	-0.16600	-0.80788
H	-2.64429	-0.84527	-0.39137
H	-2.01562	0.80161	-0.58837

6OI3 C261B

C	-1.82648	-0.52759	0.00269
C	-0.47848	0.15064	-0.00613
O	-0.36808	1.37663	-0.00021
N	0.60172	-0.64636	-0.00402
H	-1.75242	-1.61399	-0.03804
H	0.47169	-1.64738	-0.00661
H	-2.35497	-0.23405	0.91176
C	1.95105	-0.10669	0.00321
H	-2.40360	-0.16939	-0.85174
H	2.13075	0.49733	-0.88852
H	2.11101	0.51827	0.88401
H	2.65357	-0.93748	0.02040

ADMA – CH₃COO 1

C	-1.33838	0.07905	-0.20266
N	-2.56368	-0.37111	0.11392
N	-0.40085	-0.74188	-0.66567
H	0.64368	-0.51830	-0.68943
H	-0.64208	-1.71399	-0.78958
N	-1.06754	1.38489	-0.08214
H	-1.73898	1.97647	0.38210
C	0.20509	1.97654	-0.47263
H	0.43256	1.73008	-1.51147
H	0.10467	3.05612	-0.38007
H	1.01769	1.62379	0.16519

C	2.86848	-0.27485	0.22742
O	2.36695	0.04283	1.32048
O	2.22135	-0.50289	-0.84075
C	4.38670	-0.38619	0.11524
H	4.66038	-1.32023	-0.37953
H	4.75717	0.43372	-0.50655
H	4.86248	-0.33180	1.09403
C	-2.84325	-1.80351	0.08192
H	-2.10938	-2.35681	0.67258
H	-3.82458	-1.97023	0.51792
H	-2.84672	-2.18770	-0.94244
C	-3.62440	0.56789	0.46720
H	-3.43075	1.04475	1.43302
H	-3.73376	1.33844	-0.29997
H	-4.55975	0.01919	0.53465

ADMA – CH₃COO 2

C	1.45366	-0.17075	-0.18080
N	0.50271	-0.99983	-0.59765
N	1.24587	1.15115	-0.16525
N	2.64090	-0.65123	0.22972
H	3.41262	-0.00544	0.30477
C	2.95457	-2.07065	0.26779
H	2.95509	-2.51518	-0.73159
H	3.94700	-2.17932	0.69939
H	2.24208	-2.60378	0.90052
H	0.72839	-1.98401	-0.62781
C	0.03963	1.71570	-0.76797
H	0.24244	2.75541	-1.01841
H	-0.20804	1.17864	-1.68383
H	-0.80810	1.65288	-0.08186
O	-2.09651	-0.66826	-0.79144
C	-2.72482	-0.25279	0.23158
O	-2.20184	0.16453	1.27907
C	-4.24687	-0.27852	0.12028
H	-4.56089	0.35806	-0.71084
H	-4.58136	-1.29424	-0.10390
H	-4.71939	0.06643	1.03941
H	-0.54067	-0.75588	-0.63781
C	2.21950	2.05221	0.44165
H	2.55130	1.66961	1.40885
H	3.08879	2.20198	-0.20669
H	1.73698	3.01288	0.60590

ADMA – CH₃COO 3

C	-1.63629	0.36168	-0.00511
N	-1.62481	-0.96312	-0.19112
N	-2.74177	0.93876	0.51117
H	-2.78799	1.94489	0.57435
H	-3.62893	0.45894	0.46501
N	-0.57653	1.10000	-0.30256
H	0.38632	0.67510	-0.45030
C	-0.57156	2.54271	-0.12511
H	-0.70435	2.82746	0.92346
H	0.39755	2.90701	-0.46098
H	-1.34796	3.02217	-0.72691
C	-2.73565	-1.78581	0.27717
H	-2.41717	-2.82515	0.26917
H	-3.00775	-1.52193	1.30092
H	-3.61306	-1.68227	-0.36885
C	-0.49364	-1.59826	-0.86491
H	-0.20845	-1.02136	-1.74596

H	0.36868	-1.67416	-0.19831
H	-0.80566	-2.59013	-1.18502
C	2.55055	-0.14742	0.22595
O	1.95074	0.45318	-0.71766
O	2.01098	-0.59903	1.25191
C	4.05471	-0.33110	0.03950
H	4.22750	-1.02906	-0.78452
H	4.51800	0.61878	-0.23519
H	4.52257	-0.72366	0.94191

ADMA – CH₃COO 4

C	-2.07728	0.24228	-0.01500
N	-2.70052	1.36992	-0.39208
N	-0.79600	0.26684	0.33950
N	-2.77974	-0.89396	0.02854
H	-2.31495	-1.73383	0.33900
C	-4.18295	-0.98841	-0.34539
H	-4.33184	-0.71446	-1.39268
H	-4.49124	-2.02206	-0.21004
H	-4.80299	-0.35196	0.29013
H	-3.62046	1.32348	-0.80516
C	-0.05356	1.52806	0.33391
H	0.97869	1.30246	0.59461
H	-0.05870	1.97496	-0.66322
H	-0.48358	2.23248	1.05245
O	2.31848	-0.17558	-1.08097
C	3.17134	-0.11232	-0.16738
O	2.96092	0.23574	1.02360
C	4.62160	-0.47785	-0.51602
H	5.01441	-1.19023	0.21325
H	4.70289	-0.89697	-1.51929
H	5.24073	0.42205	-0.45640
H	-2.17205	2.21290	-0.55883
C	-0.15129	-0.94367	0.84826
H	-0.63531	-1.27991	1.77058
H	-0.18713	-1.74284	0.10380
H	0.89304	-0.70557	1.03875

ADMA – CH₃COO 5

C	-1.63629	0.36173	-0.00493
N	-2.74170	0.93883	0.51149
N	-1.62493	-0.96305	-0.19112
N	-0.57649	1.10000	-0.30234
H	0.38631	0.67506	-0.45023
C	-0.57140	2.54269	-0.12470
H	-0.70409	2.82730	0.92392
H	0.39772	2.90696	-0.46059
H	-1.34779	3.02229	-0.72638
H	-2.78784	1.94496	0.57476
C	-2.73587	-1.78568	0.27703
H	-2.41753	-2.82507	0.26882
H	-3.00791	-1.52197	1.30084
H	-3.61328	-1.68191	-0.36895
O	1.95072	0.45317	-0.71773
C	2.55062	-0.14757	0.22573
O	2.01111	-0.59948	1.25160
C	4.05480	-0.33099	0.03923
H	4.22771	-1.02848	-0.78517
H	4.51798	0.61909	-0.23493
H	4.52269	-0.72397	0.94145
H	-3.62890	0.45909	0.46528
C	-0.49383	-1.59817	-0.86505

H	-0.20879	-1.02128	-1.74616
H	0.36858	-1.67401	-0.19856
H	-0.80586	-2.59005	-1.18509

ADMA – CH₃COO 6

C	1.45368	-0.17079	-0.18086
N	0.50279	-0.99982	-0.59805
N	1.24588	1.15110	-0.16532
N	2.64079	-0.65133	0.22993
H	3.41257	-0.00561	0.30503
C	2.95437	-2.07078	0.26802
H	2.95519	-2.51524	-0.73139
H	3.94664	-2.17953	0.69995
H	2.24162	-2.60389	0.90047
H	0.72837	-1.98402	-0.62801
C	0.03986	1.71568	-0.76845
H	0.24264	2.75552	-1.01838
H	-0.20724	1.17893	-1.68464
H	-0.80819	1.65249	-0.08278
O	-2.20179	0.16538	1.27884
C	-2.72477	-0.25261	0.23160
O	-2.09646	-0.66808	-0.79139
C	-4.24684	-0.27913	0.12062
H	-4.58088	-1.29497	-0.10362
H	-4.71935	0.06551	1.03988
H	-4.56134	0.35742	-0.71034
H	-0.54052	-0.75581	-0.63801
C	2.21933	2.05212	0.44193
H	2.55090	1.66940	1.40916
H	3.08879	2.20198	-0.20617
H	1.73676	3.01276	0.60618

ADMA – CH₃COO 7

C	-1.50258	-0.16416	0.03586
N	-0.51229	-1.04510	0.12218
N	-1.25073	1.14964	-0.02749
N	-2.78038	-0.58330	-0.00037
H	-3.50476	0.08797	0.20907
C	-3.15746	-1.98783	0.00488
H	-2.88524	-2.48138	0.94230
H	-4.23683	-2.04178	-0.11833
H	-2.68765	-2.51205	-0.82936
H	-0.76129	-2.01661	0.24097
C	0.06731	1.69806	0.27917
H	-0.07253	2.60082	0.87629
H	0.66338	0.98738	0.84955
H	0.60395	1.95608	-0.63751
O	2.69186	-0.10630	1.27794
C	2.85234	-0.32783	0.06559
O	1.94412	-0.70091	-0.74111
C	4.23429	-0.12677	-0.54911
H	4.19904	0.72801	-1.23033
H	4.98682	0.05882	0.21663
H	4.51349	-1.00229	-1.13889
H	0.49902	-0.83907	-0.16851
C	-2.30917	2.10707	-0.32539
H	-2.96783	1.72515	-1.10635
H	-2.89885	2.34649	0.56549
H	-1.84301	3.02019	-0.69180

ADMA – CH₃COO 8

C	1.68312	0.37733	0.01012
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N	2.84033	1.06692	0.12526
N	1.73310	-0.95963	-0.02612
N	0.53638	1.02947	-0.09819
H	-0.38420	0.56029	-0.34370
C	0.45701	2.47694	0.00658
H	1.00942	2.97543	-0.79561
H	-0.59396	2.74788	-0.07824
H	0.82769	2.83255	0.97184
H	2.79579	2.05942	0.30482
C	3.01976	-1.64513	-0.11159
H	2.83234	-2.68866	-0.35156
H	3.63974	-1.21711	-0.90256
H	3.56180	-1.59907	0.83764
O	-2.31326	-0.40637	1.27775
C	-2.65863	-0.14655	0.11115
O	-1.89061	0.27976	-0.80529
C	-4.11695	-0.34650	-0.29292
H	-4.17176	-1.05103	-1.12643
H	-4.71363	-0.71961	0.53886
H	-4.53173	0.60247	-0.64173
H	3.66317	0.59972	0.47738
C	0.50503	-1.74617	-0.10861
H	-0.27509	-1.30733	0.51542
H	0.14431	-1.81120	-1.13911
H	0.72236	-2.74773	0.25834

ADMA – CH₃COO 9

C	-1.63628	0.36171	-0.00500
N	-2.74171	0.93880	0.51138
N	-1.62489	-0.96307	-0.19115
N	-0.57650	1.10000	-0.30242
H	0.38633	0.67508	-0.45025
C	-0.57145	2.54270	-0.12484
H	-0.70417	2.82736	0.92377
H	0.39766	2.90698	-0.46072
H	-1.34785	3.02224	-0.72656
H	-2.78789	1.94493	0.57460
C	-2.73579	-1.78572	0.27706
H	-2.41742	-2.82510	0.26887
H	-3.00782	-1.52199	1.30086
H	-3.61321	-1.68199	-0.36891
O	1.95073	0.45319	-0.71768
C	2.55058	-0.14752	0.22582
O	2.01104	-0.59936	1.25170
C	4.05476	-0.33103	0.03936
H	4.22764	-1.02870	-0.78489
H	4.51798	0.61898	-0.23501
H	4.52264	-0.72383	0.94166
H	-3.62891	0.45904	0.46516
C	-0.49376	-1.59820	-0.86503
H	-0.20865	-1.02128	-1.74610
H	0.36861	-1.67408	-0.19849
H	-0.80579	-2.59006	-1.18512

ADMA – CH₃COO 10

C	-1.35047	-0.12524	-0.23958
N	-0.49410	-0.99494	-0.76382
N	-1.02022	1.16955	-0.12023
N	-2.54977	-0.53532	0.20559
H	-3.26777	0.16134	0.34301
C	-2.96828	-1.92854	0.19764
H	-2.25396	-2.54409	0.74777

H	-3.93246	-1.98823	0.69736
H	-3.07381	-2.31624	-0.81950
H	-0.81194	-1.94585	-0.88600
C	0.07122	1.76534	-0.88523
H	-0.28972	2.69687	-1.32544
H	0.92746	1.98384	-0.24196
H	0.39026	1.09817	-1.68160
O	2.09409	-0.67853	-0.92436
C	2.78065	-0.37054	0.10235
O	3.97986	-0.06180	0.09856
C	2.03043	-0.38781	1.43759
H	2.71003	-0.21936	2.27220
H	1.26566	0.39536	1.44028
H	1.51839	-1.34433	1.57004
H	0.56676	-0.82418	-0.80950
C	-1.85293	2.09280	0.64097
H	-2.70570	2.44690	0.05229
H	-2.21102	1.62257	1.55722
H	-1.23881	2.94880	0.91694

SDMA – CH₃COO 1

C	1.55003	0.46752	-0.09923
N	0.74196	-0.51735	-0.44766
N	2.84466	0.24885	0.22051
H	3.40470	1.08365	0.32970
N	1.10643	1.73090	-0.09542
H	1.66986	2.41928	0.38391
C	-0.16819	2.15234	-0.65871
H	-1.00239	1.84507	-0.02524
H	-0.14814	3.23738	-0.74299
H	-0.29794	1.72074	-1.65258
C	1.19361	-1.82310	-0.91050
H	0.42533	-2.20910	-1.57999
H	2.12748	-1.73411	-1.46679
H	1.32831	-2.53408	-0.09314
H	-0.31062	-0.37797	-0.48949
C	3.31843	-0.93039	0.94556
H	2.52794	-1.33898	1.57601
H	3.67935	-1.70755	0.27144
H	4.14057	-0.61436	1.58562
O	-1.89053	-0.57359	-0.70825
C	-2.58813	-0.27015	0.30731
O	-2.14823	0.21443	1.36559
C	-4.09020	-0.50843	0.17254
H	-4.28017	-1.50192	-0.23827
H	-4.59782	-0.39923	1.13058
H	-4.50120	0.22097	-0.53138

SDMA – CH₃COO 2

C	-1.55008	0.46748	-0.09918
N	-0.74193	-0.51738	-0.44751
N	-2.84473	0.24874	0.22047
H	-3.40472	1.08355	0.32988
N	-1.10649	1.73086	-0.09531
H	-1.67026	2.41937	0.38342
C	0.16797	2.15238	-0.65890
H	0.29768	1.72050	-1.65266
H	0.14769	3.23739	-0.74350
H	1.00230	1.84544	-0.02544
C	-1.19350	-1.82307	-0.91058
H	-0.42536	-2.20878	-1.58040
H	-1.32784	-2.53428	-0.09337

H	-2.12752	-1.73408	-1.46659
H	0.31067	-0.37791	-0.48935
C	-3.31833	-0.93050	0.94564
H	-3.67906	-1.70781	0.27161
H	-2.52780	-1.33890	1.57619
H	-4.14056	-0.61455	1.58560
O	1.89055	-0.57379	-0.70812
C	2.58816	-0.27012	0.30737
O	2.14831	0.21487	1.36549
C	4.09022	-0.50850	0.17255
H	4.59786	-0.39996	1.13064
H	4.28020	-1.50164	-0.23909
H	4.50124	0.22148	-0.53078

SDMA – CH₃COO 3

C	1.55006	0.46752	-0.09918
N	0.74204	-0.51749	-0.44748
N	2.84473	0.24902	0.22052
H	3.40471	1.08386	0.32970
N	1.10619	1.73076	-0.09531
H	1.67016	2.41950	0.38285
C	-0.16826	2.15210	-0.65909
H	-1.00268	1.84424	-0.02619
H	-0.14847	3.23717	-0.74277
H	-0.29740	1.72098	-1.65326
C	1.19371	-1.82319	-0.91041
H	0.42532	-2.20927	-1.57975
H	2.12742	-1.73413	-1.46693
H	1.32864	-2.53418	-0.09311
H	-0.31045	-0.37813	-0.48941
C	3.31863	-0.93016	0.94567
H	2.52827	-1.33860	1.57638
H	3.67936	-1.70742	0.27158
H	4.14092	-0.61398	1.58545
O	-2.14830	0.21498	1.36532
C	-2.58817	-0.27013	0.30727
O	-1.89061	-0.57398	-0.70818
C	-4.09026	-0.50847	0.17280
H	-4.59762	-0.39990	1.13105
H	-4.50151	0.22147	-0.53042
H	-4.28035	-1.50166	-0.23865

SDMA – CH₃COO 4

C	1.69387	0.39730	0.02254
N	0.58254	-0.23914	0.33767
N	2.88483	-0.23814	-0.05116
H	3.68462	0.36924	-0.16883
N	1.67569	1.72002	-0.19645
H	2.47245	2.12306	-0.66947
C	0.52678	2.57186	0.06667
H	0.11595	2.35262	1.05321
H	0.86724	3.60520	0.04238
H	-0.26295	2.42987	-0.67306
C	0.54114	-1.53112	1.01004
H	-0.39738	-1.57136	1.56150
H	0.55950	-2.36742	0.31032
H	1.37155	-1.62528	1.71158
H	-0.37415	0.21419	0.22019
C	3.04982	-1.60262	-0.55331
H	3.06199	-2.33627	0.25306
H	2.25537	-1.85388	-1.25684
H	4.00220	-1.64600	-1.07913

O	-1.91241	0.63966	0.23895
C	-2.66953	-0.28785	-0.18835
O	-2.28849	-1.37952	-0.64252
C	-4.16726	-0.00066	-0.10651
H	-4.74866	-0.77655	-0.60385
H	-4.46411	0.05003	0.94471
H	-4.38691	0.97095	-0.55409

SDMA – CH₃COO 5

C	1.60353	0.45424	0.02021
N	0.67810	-0.46794	-0.16970
N	2.89162	0.12588	0.25439
H	3.49126	0.89433	0.52273
N	1.27608	1.75375	0.01834
H	2.02646	2.41928	-0.10656
C	-0.09230	2.25025	0.00409
H	-0.71604	1.63795	0.65810
H	-0.07937	3.27582	0.36933
H	-0.51688	2.23108	-1.00221
C	0.77925	-1.83946	0.31231
H	-0.23503	-2.16235	0.54896
H	1.20066	-2.51482	-0.43460
H	1.38162	-1.88842	1.22038
H	-0.30098	-0.20994	-0.48514
C	3.55653	-1.03631	-0.33224
H	3.55225	-1.89384	0.34145
H	3.07908	-1.31555	-1.27215
H	4.58895	-0.75844	-0.53843
O	-1.83808	-0.25075	-0.96776
C	-2.61518	-0.26971	0.03546
O	-2.25496	-0.20816	1.22527
C	-4.10527	-0.35974	-0.28382
H	-4.69962	-0.48416	0.62089
H	-4.41697	0.55543	-0.79464
H	-4.29111	-1.19252	-0.96552

SDMA – CH₃COO 6

C	1.25108	0.19888	0.04100
N	2.58241	0.33344	0.15108
N	0.68465	-1.00616	-0.08295
H	-0.33730	-1.00335	-0.27803
N	0.46977	1.27620	0.03938
H	-0.55531	1.13057	0.18133
C	0.97419	2.63434	-0.05802
H	1.65304	2.74234	-0.90761
H	0.12265	3.29553	-0.20958
H	1.49284	2.94569	0.85409
C	3.56452	-0.61599	-0.36566
H	4.44692	-0.04966	-0.65900
H	3.85462	-1.35590	0.38099
H	3.17117	-1.12514	-1.24579
H	2.92472	1.26085	0.35887
C	1.23297	-2.23257	0.48643
H	1.84689	-2.78532	-0.22671
H	1.82543	-2.02070	1.37794
H	0.39208	-2.86236	0.77667
O	-2.21052	0.93897	0.41028
C	-2.69849	-0.11688	-0.07026
O	-2.02977	-1.06783	-0.54841
C	-4.21184	-0.26934	-0.03740
H	-4.55891	-0.88266	-0.86904
H	-4.48426	-0.77671	0.89310

H	-4.70456	0.70265	-0.05128
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SDMA – CH₃COO 7

C	-1.10337	0.17357	-0.44161
N	-1.52591	0.67899	0.72075
N	-0.28105	0.85095	-1.24165
H	0.16778	0.31763	-1.97470
N	-1.52338	-1.03681	-0.82522
H	-1.30749	-1.31778	-1.77205
C	-1.97348	-2.05497	0.11442
H	-2.99742	-1.86979	0.44798
H	-1.94853	-3.01424	-0.39887
H	-1.29200	-2.08388	0.96716
C	-0.71720	1.53994	1.58822
H	-0.92664	1.24992	2.61693
H	0.34155	1.36892	1.39206
H	-0.97381	2.59263	1.46106
H	-2.25314	0.14946	1.18091
C	-0.00700	2.27993	-1.19104
H	-0.88721	2.81603	-0.83808
H	0.84652	2.49565	-0.54607
H	0.21496	2.61056	-2.20474
C	1.79807	-0.56042	0.43954
C	2.13384	-1.29587	-0.86783
H	2.90581	-2.04412	-0.66016
H	1.25704	-1.82558	-1.24892
H	2.51996	-0.61142	-1.62512
O	0.98300	-1.13185	1.20367
O	2.37447	0.53448	0.64323

ADMA – EtOH 1

C	-1.31590	2.28955	-0.33513
H	-1.60718	2.78735	0.59044
H	-2.20993	1.98078	-0.87585
N	-0.45237	1.13685	-0.08483
H	0.54858	1.29244	-0.17157
C	-0.82929	-0.14333	-0.18714
N	0.08286	-1.04750	-0.56819
H	1.01160	-0.72288	-0.81836
H	-0.03855	-2.02356	-0.33801
N	-2.08522	-0.52534	0.05292
C	-2.54401	-1.85775	-0.33077
C	-2.95806	0.19653	0.98016
H	-2.09745	-2.15487	-1.27855
H	-2.30382	-2.59570	0.44079
H	-3.62552	-1.81515	-0.45488
H	-3.77426	0.68902	0.44856
H	-3.37495	-0.52991	1.67987
H	-2.39051	0.92916	1.54814
H	-0.76158	2.99348	-0.95490
C	3.95938	-0.99503	0.31611
C	3.14973	0.24342	0.63793
H	4.70468	-0.78216	-0.45519
H	4.48447	-1.34018	1.20990
H	3.30993	-1.80008	-0.03707
H	2.39691	0.03051	1.39960
H	3.78875	1.04822	1.01077
O	2.41419	0.70903	-0.50557
H	3.03730	0.99293	-1.18541

ADMA – EtOH 2

C	-3.70827	-0.22710	-0.20258
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H	-4.02315	0.33694	-1.08128
H	-3.61959	0.44789	0.64778
N	-2.44131	-0.92125	-0.42624
H	-2.50480	-1.88788	-0.71818
C	-1.25537	-0.54991	0.07648
N	-0.35933	-1.49067	0.38717
H	-0.64406	-2.46019	0.35687
H	0.64128	-1.29224	0.42637
N	-0.97286	0.73738	0.28708
C	0.15812	1.13760	1.11939
C	-1.57874	1.81048	-0.50129
H	0.33795	0.39843	1.89799
H	1.06274	1.26826	0.51911
H	-0.09423	2.08814	1.59044
H	-2.28693	2.38771	0.09631
H	-0.77645	2.46962	-0.83723
H	-2.07747	1.40467	-1.37798
H	-4.46442	-0.97663	0.02704
C	3.96704	0.74736	-0.28115
C	3.01679	-0.32609	-0.76989
H	4.82562	0.29973	0.22773
H	4.34151	1.32951	-1.12656
H	3.46441	1.42455	0.41339
H	2.15705	0.11551	-1.27940
H	3.51690	-0.99508	-1.47684
O	2.47100	-1.09017	0.31092
H	3.19268	-1.46977	0.82526

ADMA – EtOH 3

C	2.81682	-1.89154	0.33708
H	2.60115	-2.60889	-0.45526
H	1.98438	-1.86682	1.03918
N	3.05999	-0.55245	-0.19649
H	4.02488	-0.31431	-0.38737
C	2.21670	0.48272	-0.09957
N	2.72112	1.71845	-0.00042
H	3.71567	1.84637	0.12497
H	2.17168	2.52398	-0.26448
N	0.89693	0.30128	-0.07037
C	-0.00235	1.37296	0.35159
C	0.24458	-0.83597	-0.72224
H	0.46825	1.98056	1.12308
H	-0.28533	2.00421	-0.49665
H	-0.89488	0.90527	0.76635
H	-0.21143	-1.49871	0.01409
H	-0.53781	-0.44181	-1.37397
H	0.95581	-1.38384	-1.33520
H	3.71238	-2.20830	0.87022
C	-4.79511	-0.16104	-0.43551
C	-3.39497	0.29185	-0.06966
H	-5.43427	-0.19717	0.45170
H	-5.24502	0.53574	-1.14712
H	-4.77215	-1.15487	-0.88841
H	-2.75937	0.32705	-0.95683
H	-3.42112	1.29957	0.36157
O	-2.75306	-0.61035	0.83015
H	-3.30856	-0.69005	1.61368

SDMA – EtOH 1

C	1.67579	0.28771	0.05326
N	2.14899	1.46995	-0.35994
N	0.43010	0.18746	0.52001

N	2.47323	-0.78140	0.00798
H	2.13104	-1.65140	0.38749
C	3.84242	-0.74690	-0.48502
H	4.46190	-0.07645	0.11538
H	4.24374	-1.75437	-0.41042
H	3.87350	-0.43604	-1.53158
H	3.06074	1.53633	-0.78869
C	-0.40193	1.38523	0.63788
H	-1.35634	1.09038	1.06634
H	0.06841	2.12410	1.29193
H	-0.58704	1.83183	-0.34322
C	-0.06287	-1.09793	1.01502
H	0.00940	-1.86483	0.23938
H	0.49580	-1.41845	1.89919
H	-1.11189	-0.98057	1.27459
H	1.54310	2.27185	-0.44524
O	-3.33274	-0.33362	0.77370
H	-3.99624	0.05019	1.35666
C	-3.55032	0.16366	-0.54853
H	-3.38835	1.24754	-0.57386
H	-4.58332	-0.03101	-0.85492
C	-2.58966	-0.53603	-1.48433
H	-2.73245	-1.61836	-1.43856
H	-2.76533	-0.20771	-2.51133
H	-1.55156	-0.31062	-1.22879

SDMA – EtOH 2

C	1.35548	0.48203	-0.05649
N	2.24042	1.41774	0.31920
N	1.68977	-0.81025	-0.01653
N	0.13638	0.85435	-0.44150
H	-0.57682	0.15335	-0.63398
C	-0.31028	2.23881	-0.45577
H	0.29558	2.84247	-1.13591
H	-1.33738	2.24376	-0.81410
H	-0.28343	2.67746	0.54534
H	2.02780	2.39564	0.18370
C	3.01630	-1.20730	0.45135
H	3.06817	-2.29218	0.45096
H	3.79763	-0.82328	-0.21002
H	3.19344	-0.85308	1.46990
C	0.70742	-1.82208	-0.39775
H	-0.14896	-1.81692	0.28307
H	0.35968	-1.66053	-1.42119
H	1.18420	-2.79683	-0.34854
H	3.21105	1.18215	0.46317
O	-2.31736	-0.47289	-0.94584
H	-2.50526	-1.10567	-1.64800
C	-3.09526	-0.81380	0.21045
H	-2.81682	-1.81270	0.56165
H	-4.15614	-0.82601	-0.05561
C	-2.82961	0.22210	1.27947
H	-3.09781	1.21939	0.92286
H	-3.42952	0.00065	2.16479
H	-1.77678	0.22400	1.57288

SDMA – EtOH 3

C	-1.70209	0.25573	-0.12219
N	-1.31709	-0.97098	-0.47101
N	-0.86592	1.29722	-0.21102
H	-1.18367	2.14927	0.23287
N	-2.93705	0.45388	0.34581

H	-3.26878	1.40856	0.38332
C	-3.85564	-0.61082	0.72132
H	-3.35826	-1.32840	1.37633
H	-4.68400	-0.15819	1.26131
H	-4.24996	-1.13045	-0.15582
C	0.06513	-1.44787	-0.50664
H	0.06717	-2.47773	-0.15192
H	0.47412	-1.41939	-1.51742
H	0.68340	-0.84594	0.15872
C	0.21507	1.43069	-1.18767
H	1.17784	1.15692	-0.75508
H	0.01147	0.81290	-2.06180
H	0.24653	2.47243	-1.50321
H	-2.04187	-1.67114	-0.55441
O	2.37315	0.31074	1.25811
H	2.41944	0.98057	1.94809
C	3.64764	0.22863	0.61710
H	4.41727	-0.04222	1.34769
H	3.91513	1.20001	0.18621
C	3.56221	-0.82170	-0.46830
H	2.83739	-0.53369	-1.23334
H	4.53616	-0.94375	-0.94755
H	3.26194	-1.78455	-0.04696

SDMA – EtOH 4

C	1.35895	0.48419	-0.00596
N	0.39901	-0.27971	0.50451
N	2.59371	0.00575	-0.22522
H	3.28533	0.69768	-0.48313
N	1.10284	1.76482	-0.28853
H	1.76623	2.25174	-0.87570
C	-0.12184	2.45537	0.08563
H	-0.32545	2.31957	1.14960
H	0.02337	3.51530	-0.11051
H	-0.97766	2.10208	-0.49562
C	0.61027	-1.49882	1.27983
H	-0.22601	-1.58946	1.97125
H	0.64343	-2.38757	0.64899
H	1.53259	-1.42531	1.85581
C	2.89479	-1.37700	-0.59820
H	3.13818	-1.98977	0.26977
H	2.05441	-1.81999	-1.13270
H	3.75506	-1.35470	-1.26472
H	-0.55445	0.08232	0.51187
O	-2.41552	0.08281	0.80703
H	-2.90402	0.63855	1.42393
C	-3.27908	-0.30126	-0.27092
H	-3.70435	0.59112	-0.74055
H	-4.09976	-0.91107	0.11863
C	-2.45959	-1.08804	-1.26859
H	-1.99677	-1.95366	-0.78727
H	-3.10182	-1.44547	-2.07625
H	-1.67408	-0.46656	-1.70564

SDMA – EtOH 5

C	-0.97943	0.20141	-0.18761
N	-2.25193	0.53459	0.03285
N	-0.56259	-1.06920	-0.08550
H	0.44050	-1.18784	-0.19657
N	-0.08930	1.14633	-0.49277
H	0.82634	0.82914	-0.79783
C	-0.34838	2.57446	-0.40062

H	-0.76816	2.82874	0.57513
H	0.60125	3.09186	-0.51870
H	-1.02856	2.91094	-1.18732
C	-3.20342	-0.23674	0.83032
H	-3.89325	0.47062	1.28660
H	-3.76989	-0.94148	0.22167
H	-2.68130	-0.77309	1.62201
C	-1.38924	-2.23857	-0.38834
H	-1.86535	-2.64381	0.50441
H	-2.15226	-1.99136	-1.12681
H	-0.73422	-2.99883	-0.81047
H	-2.51366	1.48738	-0.18136
O	2.26900	-0.55503	-0.85090
H	2.61238	-0.92360	-1.67337
C	3.34220	-0.44312	0.10045
H	4.13863	0.17535	-0.32227
H	3.74631	-1.43778	0.30792
C	2.78736	0.18302	1.35905
H	1.98232	-0.42947	1.77368
H	3.57666	0.26240	2.10945
H	2.40239	1.18624	1.15938

ADMA_1 – NMA 1

C	3.36757	-1.81302	0.78014
H	2.77698	-2.63003	0.36529
H	2.87038	-1.41929	1.66606
N	3.58204	-0.74355	-0.19444
H	4.48768	-0.72521	-0.64559
C	2.89999	0.40908	-0.23248
N	3.53161	1.51994	-0.63469
H	4.52313	1.48345	-0.82745
H	3.01125	2.29970	-1.01119
N	1.62513	0.47210	0.14404
C	0.97396	1.76613	0.34256
C	0.73354	-0.69072	0.07559
H	1.65694	2.46152	0.82940
H	0.63412	2.18533	-0.60947
H	0.10678	1.60910	0.98103
H	0.59938	-1.14491	1.05956
H	-0.23373	-0.33797	-0.28210
H	1.12802	-1.42885	-0.62063
H	4.34506	-2.19431	1.07103
C	-3.43260	0.30976	0.05255
O	-2.28053	0.73714	0.08323
N	-3.70681	-0.97139	-0.26046
H	-4.66776	-1.27944	-0.27242
C	-2.65570	-1.92101	-0.57397
H	-2.07466	-1.58570	-1.43623
C	-4.61540	1.19133	0.36683
H	-4.60089	2.04919	-0.30771
H	-4.50887	1.56542	1.38689
H	-5.56874	0.67200	0.27160
H	-1.97784	-2.04584	0.27386
H	-3.11492	-2.88012	-0.80623

ADMA_1 – NMA 2

C	0.63693	-0.45160	1.97693
H	-0.37429	-0.16448	1.68427
H	1.18740	0.43267	2.29801
N	1.35072	-1.11163	0.88317
H	1.34902	-2.12314	0.89284
C	2.28894	-0.54749	0.11409

N	3.27992	-1.31707	-0.35830
H	3.34611	-2.27908	-0.05553
H	3.77601	-1.05960	-1.19979
N	2.27266	0.75603	-0.16054
C	3.43807	1.41289	-0.74755
C	1.03752	1.54340	-0.17047
H	4.35686	0.97915	-0.35571
H	3.42579	1.33419	-1.83874
H	3.40192	2.46537	-0.46873
H	1.03908	2.27207	0.64293
H	0.99024	2.07547	-1.12291
H	0.16013	0.90679	-0.08877
H	0.58991	-1.15182	2.81000
C	-3.12414	0.24764	-0.02731
O	-2.17444	0.85460	0.46428
N	-2.94188	-0.76242	-0.89893
H	-3.74734	-1.23557	-1.28075
C	-1.61310	-1.19040	-1.29302
H	-1.07995	-0.38584	-1.80600
C	-4.55176	0.58732	0.31950
H	-4.71732	1.64797	0.12389
H	-4.69923	0.41635	1.38783
H	-5.27569	-0.00307	-0.24209
H	-1.02998	-1.48952	-0.41861
H	-1.70778	-2.03973	-1.96718

ADMA_1 – NMA 3

C	1.73030	2.26247	0.56274
H	2.17076	2.91230	-0.19441
H	2.50830	1.93059	1.24967
N	1.05404	1.11196	-0.03376
H	0.04501	1.18749	-0.13876
C	1.51873	-0.14338	-0.02278
N	0.63560	-1.14989	0.01473
H	-0.35469	-0.90987	0.04006
H	0.89982	-2.06262	-0.32846
N	2.82861	-0.40526	-0.01302
C	3.31298	-1.75319	0.26951
C	3.81253	0.50236	-0.60398
H	2.70067	-2.22392	1.03734
H	3.31456	-2.37089	-0.63407
H	4.33355	-1.66916	0.64135
H	4.44667	0.94902	0.16406
H	4.43539	-0.07822	-1.28682
H	3.31811	1.28349	-1.17540
H	0.98950	2.82929	1.12585
C	-2.97636	0.39109	-0.07233
O	-1.73671	0.41135	-0.06466
N	-3.64799	-0.76101	0.00245
H	-4.65768	-0.73947	-0.00440
C	-2.96715	-2.04037	0.09913
H	-2.32055	-2.19912	-0.76717
C	-3.77819	1.65809	-0.16332
H	-3.48916	2.18926	-1.07191
H	-3.52587	2.28997	0.69033
H	-4.85176	1.47429	-0.17461
H	-2.36092	-2.08801	1.00680
H	-3.71680	-2.82813	0.13128

ADMA_1 – NMA 4

C	1.11530	1.99191	0.72127
H	1.27421	2.77024	-0.02641

H	2.01770	1.87924	1.32119
N	0.74987	0.71663	0.10915
H	-0.24584	0.49916	0.06202
C	1.54615	-0.35444	0.02758
N	0.98305	-1.57128	0.05971
H	-0.02831	-1.62848	0.14475
H	1.46511	-2.35721	-0.35386
N	2.87519	-0.23944	-0.04945
C	3.73482	-1.40478	0.13952
C	3.52471	0.92705	-0.64939
H	3.33095	-2.05320	0.91576
H	3.84863	-1.96800	-0.79214
H	4.71383	-1.04899	0.45883
H	4.06691	1.50413	0.10200
H	4.23059	0.56750	-1.40042
H	2.79347	1.56017	-1.14534
H	0.30013	2.29493	1.37780
C	-2.96252	-0.52675	-0.01749
O	-1.73961	-0.69420	0.09885
N	-3.47768	0.69320	-0.19365
H	-4.47869	0.79382	-0.28343
C	-2.63908	1.87761	-0.25114
H	-2.07214	1.99211	0.67608
C	-3.91770	-1.68533	0.03141
H	-3.76795	-2.22040	0.97072
H	-3.67753	-2.36758	-0.78630
H	-4.95865	-1.37425	-0.04939
H	-1.93975	1.81777	-1.08899
H	-3.27892	2.74717	-0.38455

ADMA_1 – NMA 5

C	-4.37973	-0.65995	0.21531
H	-4.91749	-0.51030	-0.72176
H	-4.30869	0.28839	0.74671
N	-3.04405	-1.21006	-0.00604
H	-2.95565	-2.21581	0.05962
C	-1.89658	-0.52456	0.09847
N	-0.79412	-1.15409	0.51148
H	-0.88069	-2.10340	0.84795
H	0.14528	-0.82556	0.25674
N	-1.85173	0.78156	-0.17758
C	-0.70992	1.59134	0.23816
C	-2.74950	1.41067	-1.14593
H	-0.33254	1.24127	1.19758
H	0.09053	1.56074	-0.50656
H	-1.05423	2.61940	0.35057
H	-3.45240	2.08379	-0.65121
H	-2.13824	1.98517	-1.84441
H	-3.29357	0.65638	-1.70861
H	-4.93454	-1.36396	0.83422
C	2.76444	-0.01451	0.36632
O	1.84390	-0.54630	-0.26540
N	4.01643	-0.00211	-0.10803
H	4.74182	0.43344	0.44293
C	4.36013	-0.59414	-1.38853
H	4.13162	-1.66160	-1.39397
C	2.53186	0.67325	1.68521
H	1.84563	0.07532	2.28624
H	2.06238	1.64136	1.49202
H	3.45862	0.83665	2.23502
H	3.80462	-0.11486	-2.19690
H	5.42639	-0.45572	-1.55582

ADMA_1 – NMA 6

C	4.15287	-0.81051	-0.27071
H	4.62454	-0.87321	0.71064
H	4.25703	0.20276	-0.65688
N	2.74065	-1.18441	-0.21953
H	2.52572	-2.14848	-0.43847
C	1.71136	-0.32877	-0.31865
N	0.58506	-0.73284	-0.90800
H	0.58520	-1.61955	-1.39344
H	-0.31889	-0.27962	-0.73907
N	1.81487	0.91791	0.14759
C	0.83945	1.94065	-0.21936
C	2.69846	1.26730	1.25972
H	0.47765	1.77353	-1.23227
H	-0.00737	1.94432	0.47172
H	1.34113	2.90761	-0.17871
H	3.52838	1.89050	0.92078
H	2.11117	1.82462	1.99189
H	3.08228	0.37093	1.74035
H	4.65792	-1.49468	-0.95154
C	-3.06748	0.19013	-0.12471
O	-1.97705	0.45319	-0.64365
N	-3.26089	-0.92939	0.58570
H	-4.16927	-1.09793	0.99283
C	-2.20394	-1.90298	0.79034
H	-1.85219	-2.29931	-0.16518
C	-4.24209	1.11974	-0.26858
H	-4.48157	1.21586	-1.32936
H	-3.95315	2.10523	0.10074
H	-5.12281	0.77294	0.27109
H	-1.35886	-1.45280	1.31641
H	-2.60017	-2.72063	1.38900

ADMA_1 – NMA 7

C	-4.30531	-0.79190	0.00974
H	-4.97475	-0.15370	-0.56814
H	-4.16029	-0.36041	0.99951
N	-3.01468	-0.96955	-0.65304
H	-2.91422	-1.79963	-1.22251
C	-1.86551	-0.38911	-0.27469
N	-0.71611	-1.04273	-0.45014
H	-0.75049	-2.01223	-0.73475
H	0.19149	-0.56268	-0.50249
N	-1.86691	0.81963	0.29333
C	-0.68051	1.32580	0.97801
C	-2.89754	1.81744	0.00567
H	-0.15894	0.51312	1.48189
H	-0.00059	1.81981	0.27870
H	-1.01127	2.04635	1.72586
H	-3.53791	1.98404	0.87406
H	-2.39490	2.75188	-0.25029
H	-3.50048	1.50998	-0.84530
H	-4.76251	-1.77403	0.12359
C	2.72833	-0.43282	0.13196
O	1.88268	0.03222	-0.64218
N	4.00024	-0.01740	0.10655
H	4.65905	-0.42581	0.75345
C	4.46035	0.99545	-0.82672
H	4.31259	0.66681	-1.85720
C	2.37478	-1.49374	1.14072
H	2.00685	-2.37414	0.60922

H	1.56751	-1.12251	1.77546
H	3.22248	-1.77748	1.76340
H	3.91851	1.93139	-0.67819
H	5.52113	1.16512	-0.65387

ADMA_1 – NMA 8

C	1.11525	1.99188	0.72101
H	1.27444	2.77017	-0.02664
H	2.01743	1.87921	1.32127
N	0.74995	0.71657	0.10885
H	-0.24572	0.49891	0.06191
C	1.54631	-0.35446	0.02733
N	0.98328	-1.57134	0.05975
H	-0.02808	-1.62851	0.14463
H	1.46534	-2.35731	-0.35372
N	2.87530	-0.23950	-0.04988
C	3.73490	-1.40482	0.13938
C	3.52491	0.92723	-0.64922
H	3.33109	-2.05291	0.91593
H	3.84850	-1.96843	-0.79207
H	4.71398	-1.04899	0.45838
H	4.06666	1.50425	0.10254
H	4.23120	0.56796	-1.39999
H	2.79380	1.56031	-1.14541
H	0.29986	2.29496	1.37723
C	-2.96272	-0.52673	-0.01730
O	-1.73982	-0.69424	0.09900
N	-3.47782	0.69326	-0.19343
H	-4.47884	0.79402	-0.28289
C	-2.63911	1.87760	-0.25076
H	-2.07251	1.99224	0.67665
C	-3.91793	-1.68528	0.03142
H	-3.76809	-2.22063	0.97056
H	-3.67788	-2.36732	-0.78651
H	-4.95890	-1.37418	-0.04919
H	-1.93946	1.81755	-1.08833
H	-3.27883	2.74719	-0.38460

ADMA_2 – NMA 1

C	-5.04325	-0.59146	-0.24238
H	-5.30283	0.10970	-1.03869
H	-5.44106	-0.22990	0.70869
N	-3.60283	-0.78861	-0.16747
H	-3.24430	-1.71812	-0.32669
C	-2.73534	0.19954	0.06207
N	-3.20271	1.44590	0.21570
H	-4.19599	1.60678	0.29774
H	-2.59379	2.18979	0.52100
N	-1.42550	-0.03844	0.11152
C	-0.48556	1.06329	0.32349
C	-0.91972	-1.39522	-0.09365
H	-0.57655	1.81059	-0.46951
H	-0.65403	1.54015	1.29346
H	0.52054	0.65055	0.30747
H	-1.15973	-1.75455	-1.09841
H	0.16111	-1.37403	0.02208
H	-1.33817	-2.08134	0.64747
H	-5.49591	-1.55460	-0.46448
C	3.55166	-0.47309	0.04723
O	2.36097	-0.72142	0.23530
N	3.97811	0.77009	-0.23928
H	4.96411	0.92886	-0.38524

C	3.05787	1.88700	-0.34387
H	2.31836	1.70695	-1.12750
C	4.60834	-1.54508	0.13056
H	4.34532	-2.34912	-0.55882
H	4.60714	-1.95664	1.14182
H	5.60559	-1.17510	-0.10662
H	2.53189	2.04766	0.60038
H	3.62702	2.78132	-0.59043

ADMA_2 – NMA 2

C	-0.69508	2.33970	-0.65000
H	-0.90527	2.88872	0.27214
H	-1.34102	2.71008	-1.44969
N	-0.85590	0.90541	-0.46721
H	0.00208	0.34981	-0.50598
C	-2.01781	0.33901	-0.15285
N	-3.09673	1.11285	0.05216
H	-3.04706	2.10034	-0.15215
H	-4.02013	0.70581	0.07271
N	-2.11524	-0.98502	-0.01054
C	-3.36652	-1.58089	0.45288
C	-0.95577	-1.83252	-0.27816
H	-3.70335	-1.10520	1.37655
H	-4.14952	-1.49873	-0.30642
H	-3.18758	-2.63321	0.65479
H	-0.19305	-1.71969	0.49738
H	-1.28871	-2.86675	-0.30126
H	-0.51642	-1.58848	-1.24713
H	0.34004	2.51756	-0.93412
C	2.78787	-0.23376	-0.23594
O	1.73209	-0.10724	-0.86531
N	2.88467	0.10044	1.05830
H	3.77073	-0.01949	1.52717
C	1.76091	0.64207	1.80135
H	1.39816	1.56275	1.33807
C	4.02963	-0.76782	-0.89715
H	4.30875	-0.09221	-1.70806
H	3.80084	-1.74073	-1.33512
H	4.86531	-0.86694	-0.20515
H	0.94032	-0.07792	1.84467
H	2.09221	0.86163	2.81430

ADMA_2 – NMA 3

C	-0.47284	1.94210	-0.35044
H	0.19546	1.45816	-1.06599
H	-0.02210	1.91009	0.64403
N	-1.78266	1.30831	-0.34460
H	-2.58580	1.87777	-0.56303
C	-1.96967	0.02912	-0.00654
N	-0.90758	-0.72189	0.29249
H	0.05310	-0.38930	0.18145
H	-1.02407	-1.69950	0.51160
N	-3.20297	-0.48452	0.04314
C	-3.38743	-1.89248	0.38844
C	-4.35705	0.35716	-0.26403
H	-2.89107	-2.54262	-0.33655
H	-3.00354	-2.10337	1.38991
H	-4.45109	-2.11152	0.37460
H	-4.32213	0.71057	-1.29815
H	-5.25804	-0.23500	-0.13233
H	-4.40735	1.21432	0.41258
H	-0.60880	2.98074	-0.64260

C	2.91275	0.21573	0.19246
O	1.87377	-0.42508	0.00225
N	4.11520	-0.31779	-0.06248
H	4.94133	0.23669	0.10892
C	4.26375	-1.66686	-0.57800
H	3.85077	-2.39584	0.12209
C	2.89494	1.63045	0.70756
H	2.28194	1.67168	1.60919
H	2.43341	2.27243	-0.04638
H	3.89408	2.00454	0.92885
H	3.74888	-1.77287	-1.53476
H	5.32421	-1.86650	-0.71796

ADMA_2 – NMA 4

C	3.36780	-2.12534	-0.39464
H	2.98663	-2.39817	-1.38118
H	2.87893	-2.73690	0.36794
N	3.17710	-0.70549	-0.13913
H	3.98738	-0.16323	0.12052
C	1.97762	-0.11253	-0.12913
N	0.88392	-0.84415	-0.35259
H	0.98024	-1.83173	-0.53959
H	-0.05618	-0.44696	-0.45770
N	1.87647	1.19838	0.10657
C	0.55548	1.82024	0.16077
C	3.07799	2.00133	0.31552
H	0.05063	1.75853	-0.80593
H	-0.06769	1.34161	0.92006
H	0.68256	2.86633	0.42497
H	3.76033	1.91023	-0.53322
H	2.78140	3.04235	0.40544
H	3.59671	1.70752	1.23249
H	4.43643	-2.32411	-0.36935
C	-2.59873	-0.41670	0.25731
O	-1.83316	-0.13568	-0.67262
N	-3.90212	-0.11668	0.20224
H	-4.49338	-0.36893	0.98077
C	-4.48853	0.55288	-0.94506
H	-4.01201	1.52100	-1.11019
C	-2.10637	-1.10831	1.50151
H	-1.34136	-0.48518	1.97028
H	-1.64023	-2.05383	1.21676
H	-2.90402	-1.29966	2.21856
H	-4.37185	-0.05122	-1.84692
H	-5.54830	0.70399	-0.75000

SDMA_1 – NMA 1

C	-0.71720	0.04472	1.76273
H	-0.98320	1.07142	2.01152
H	0.30628	0.00214	1.38941
N	-1.66285	-0.49044	0.78606
H	-1.91596	-1.46433	0.88599
C	-2.34411	0.21865	-0.11098
N	-1.93807	1.43640	-0.49186
N	-3.47477	-0.27746	-0.62078
H	-2.60386	1.96103	-1.04468
H	-3.84107	0.16546	-1.45281
C	-0.54058	1.86938	-0.56239
H	-0.42022	2.44733	-1.47815
H	0.12287	1.00507	-0.60433
H	-0.27309	2.49702	0.28906
C	-4.13858	-1.47461	-0.12737

H	-3.57427	-2.37814	-0.37279
H	-5.11446	-1.53246	-0.60369
H	-4.27728	-1.41477	0.95363
H	-0.79982	-0.56201	2.66313
C	2.88550	-0.73604	-0.28394
O	1.67652	-0.72101	-0.50831
N	3.54838	0.36954	0.10559
H	4.54122	0.31240	0.27689
C	2.87353	1.64298	0.27593
H	2.43572	1.98131	-0.66653
C	3.70428	-1.99415	-0.42900
H	3.61417	-2.35176	-1.45639
H	3.28797	-2.75866	0.22951
H	4.75692	-1.84499	-0.18961
H	2.07756	1.56271	1.01984
H	3.60239	2.37751	0.61349

SDMA_1 – NMA 2

C	-4.04831	-0.24920	0.54747
H	-3.67761	-0.81076	1.40471
H	-4.51337	-0.93176	-0.16389
N	-2.96354	0.52004	-0.05900
H	-3.16855	1.48443	-0.28160
C	-1.67628	0.16568	-0.06781
N	-1.30725	-1.11713	0.06888
N	-0.73162	1.09682	-0.19861
H	-0.30130	-1.25835	0.11349
H	0.22326	0.75742	-0.30847
C	-2.09401	-2.25482	-0.40974
H	-1.39180	-3.02643	-0.72072
H	-2.70023	-1.97221	-1.27130
H	-2.73853	-2.66303	0.36864
C	-0.98072	2.52764	-0.13003
H	-1.52729	2.88141	-1.00831
H	-0.01715	3.03154	-0.09593
H	-1.54240	2.78184	0.77188
H	-4.79550	0.46157	0.89530
C	2.75938	-0.62373	0.03980
O	1.53343	-0.64451	-0.14365
N	3.42543	0.53167	0.12144
H	4.42315	0.51170	0.27768
C	2.74924	1.81289	0.02109
H	2.22776	1.89912	-0.93566
C	3.55215	-1.89283	0.17326
H	3.43570	-2.47335	-0.74388
H	3.13684	-2.47742	0.99607
H	4.61032	-1.70859	0.35486
H	2.02590	1.93205	0.83208
H	3.49349	2.60330	0.09109

SDMA_1 – NMA 3

C	-3.78254	-0.96749	0.55472
H	-3.22935	-1.37655	1.40005
H	-3.99481	-1.76038	-0.16216
N	-3.03853	0.13617	-0.05008
H	-3.56239	0.98150	-0.23070
C	-1.70723	0.24196	-0.08279
N	-0.91947	-0.83810	0.00786
N	-1.14185	1.44552	-0.19317
H	0.07676	-0.62153	0.03277
H	-0.13173	1.46849	-0.30306
C	-1.28540	-2.17103	-0.47068

H	-0.37298	-2.65132	-0.82086
H	-1.98377	-2.10509	-1.30586
H	-1.72113	-2.78466	0.31793
C	-1.86697	2.70068	-0.07473
H	-2.52891	2.86238	-0.92945
H	-1.13511	3.50479	-0.04791
H	-2.45295	2.72792	0.84685
H	-4.72390	-0.56178	0.92027
C	2.80183	0.40343	0.04766
O	1.58125	0.56658	-0.09482
N	3.32818	-0.82183	0.13085
H	4.32585	-0.91980	0.25487
C	2.50319	-2.01454	0.05269
H	1.79399	-2.05197	0.88404
C	3.74178	1.57235	0.13359
H	3.44218	2.19730	0.97689
H	3.64274	2.16620	-0.77687
H	4.78034	1.26748	0.25791
H	1.94748	-2.03328	-0.88771
H	3.15144	-2.88683	0.10057

SDMA_1 – NMA 4

C	1.21453	-1.41840	1.22880
H	2.10821	-1.36568	1.85073
H	1.25128	-2.32035	0.61635
N	1.08719	-0.20672	0.42459
H	0.13518	0.16781	0.33953
C	2.10742	0.51938	-0.01751
N	3.33760	-0.00706	-0.13887
N	1.92350	1.80725	-0.32132
H	4.07463	0.65801	-0.33384
H	2.64468	2.26572	-0.86164
C	3.60875	-1.39589	-0.51201
H	4.50514	-1.39984	-1.12970
H	2.78367	-1.80158	-1.09781
H	3.77948	-2.02670	0.36046
C	0.71011	2.55071	-0.01637
H	-0.11774	2.25595	-0.66460
H	0.92194	3.60701	-0.16681
H	0.42156	2.39278	1.02428
H	0.34244	-1.46930	1.87988
C	-2.36774	-0.53357	-0.17668
O	-1.67529	0.34669	0.34892
N	-3.69297	-0.39381	-0.30275
H	-4.22237	-1.13613	-0.73656
C	-4.39042	0.78505	0.17932
H	-4.25708	0.89931	1.25687
C	-1.76512	-1.81650	-0.68665
H	-1.43001	-2.40627	0.17002
H	-0.89285	-1.58351	-1.30018
H	-2.47302	-2.40724	-1.26750
H	-4.01534	1.68303	-0.31516
H	-5.44995	0.67118	-0.04063

SDMA_1 – NMA 5

C	1.07163	-1.28929	1.44922
H	1.93104	-1.15002	2.10551
H	1.15121	-2.25320	0.94505
N	0.96467	-0.17893	0.50810
H	0.01783	0.18986	0.38052
C	1.98875	0.49015	-0.00697
N	3.22455	-0.03599	-0.04399

N	1.80189	1.72657	-0.47809
H	3.95468	0.60424	-0.32785
H	2.52919	2.11536	-1.06295
C	3.51340	-1.45932	-0.22504
H	4.43642	-1.53402	-0.79702
H	2.71644	-1.94384	-0.78995
H	3.64815	-1.97272	0.72693
C	0.57649	2.49042	-0.29882
H	-0.23605	2.10066	-0.91649
H	0.78139	3.51842	-0.58957
H	0.26826	2.47281	0.74775
H	0.16603	-1.28381	2.05441
C	-2.82372	-0.01856	0.16199
O	-1.78140	0.42324	0.65726
N	-2.80769	-0.88531	-0.86025
H	-3.68452	-1.22024	-1.23249
C	-1.56689	-1.36274	-1.44338
H	-0.97527	-0.52907	-1.82916
C	-4.17197	0.39926	0.68377
H	-4.26312	1.48235	0.58310
H	-4.22400	0.15575	1.74640
H	-4.99612	-0.08378	0.15987
H	-0.97187	-1.90355	-0.70300
H	-1.80862	-2.03494	-2.26417

SDMA_2 – NMA 1

C	4.34141	0.08690	0.36344
H	4.26790	0.45080	1.39121
H	4.59583	0.91659	-0.30017
N	3.10661	-0.55755	-0.05724
H	3.13504	-1.54304	-0.27439
C	1.93129	0.07309	-0.13995
N	1.84952	1.36478	0.19002
N	0.84617	-0.57586	-0.55305
H	2.68696	1.83490	0.50053
H	-0.06643	-0.11304	-0.54832
C	0.62753	2.14558	0.06915
H	0.86110	3.16870	0.35373
H	-0.15255	1.76401	0.73161
H	0.25881	2.13839	-0.95884
C	0.85623	-1.97824	-0.93658
H	1.54811	-2.15560	-1.76364
H	-0.14868	-2.23393	-1.26638
H	1.12304	-2.62339	-0.09469
H	5.13496	-0.65463	0.31244
C	-2.90121	0.10342	-0.20944
O	-1.84455	0.18136	-0.84496
N	-2.95055	-0.42442	1.02167
H	-3.83997	-0.46559	1.49794
C	-1.77047	-0.94940	1.68523
H	-1.30872	-1.73708	1.08530
C	-4.19746	0.60001	-0.79051
H	-4.38376	0.07140	-1.72708
H	-4.09158	1.66190	-1.01996
H	-5.04266	0.45668	-0.11810
H	-1.03501	-0.15976	1.85602
H	-2.07213	-1.36501	2.64459

SDMA_2 – NMA 2

C	-4.47987	0.10877	0.22203
H	-4.71114	0.86495	-0.53236
H	-4.45239	0.57509	1.20953

N	-3.22344	-0.56374	-0.07154
H	-3.25300	-1.54714	-0.29798
C	-2.04363	0.06118	-0.13497
N	-1.97814	1.37463	0.09790
N	-0.93705	-0.62022	-0.41738
H	-2.83839	1.87489	0.26688
H	-0.03109	-0.14300	-0.47658
C	-0.73662	2.13206	0.05749
H	-0.97594	3.16932	0.27899
H	-0.27320	2.07794	-0.93009
H	-0.03036	1.76507	0.80576
C	-0.92475	-2.05760	-0.63611
H	-1.25920	-2.59842	0.25313
H	0.10127	-2.34618	-0.85596
H	-1.55098	-2.33724	-1.48732
H	-5.26595	-0.64241	0.21489
C	2.45598	-0.20291	0.33925
O	1.75746	0.11913	-0.62967
N	3.78516	-0.04672	0.32192
H	4.32038	-0.32405	1.13181
C	4.47819	0.50390	-0.82926
H	4.30479	-0.11123	-1.71423
C	1.85201	-0.79065	1.58792
H	1.35035	-1.72633	1.32988
H	1.09798	-0.10159	1.97451
H	2.59571	-0.98270	2.36063
H	4.13398	1.51876	-1.03760
H	5.54390	0.52510	-0.61091

SDMA_2 – NMA 3

C	-0.92416	-2.05743	-0.63588
H	-1.55020	-2.33723	-1.48717
H	-1.25858	-2.59835	0.25331
N	-0.93690	-0.62005	-0.41713
H	-0.03109	-0.14255	-0.47625
C	-2.04371	0.06105	-0.13490
N	-3.22336	-0.56418	-0.07163
N	-1.97861	1.37453	0.09792
H	-3.25262	-1.54759	-0.29807
H	-2.83901	1.87454	0.26688
C	-4.48003	0.10802	0.22164
H	-5.26592	-0.64337	0.21436
H	-4.71133	0.86410	-0.53285
H	-4.45289	0.57441	1.20911
C	-0.73729	2.13229	0.05770
H	-0.03103	1.76547	0.80608
H	-0.97691	3.16948	0.27920
H	-0.27370	2.07831	-0.92980
H	0.10197	-2.34570	-0.85559
C	2.45608	-0.20245	0.33936
O	1.75742	0.12004	-0.62933
N	3.78534	-0.04700	0.32160
H	4.32067	-0.32473	1.13129
C	4.47831	0.50323	-0.82981
H	4.30433	-0.11183	-1.71471
C	1.85219	-0.79001	1.58815
H	1.35089	-1.72595	1.33032
H	1.09787	-0.10112	1.97446
H	2.59588	-0.98159	2.36099
H	4.13458	1.51826	-1.03806
H	5.54410	0.52388	-0.61177

ADMA_1 – PHE 1

C	-0.92416	-2.05743	-0.63588
H	-1.55020	-2.33723	-1.48717
H	-1.25858	-2.59835	0.25331
N	-0.93690	-0.62005	-0.41713
H	-0.03109	-0.14255	-0.47625
C	-2.04371	0.06105	-0.13490
N	-3.22336	-0.56418	-0.07163
N	-1.97861	1.37453	0.09792
H	-3.25262	-1.54759	-0.29807
H	-2.83901	1.87454	0.26688
C	-4.48003	0.10802	0.22164
H	-5.26592	-0.64337	0.21436
H	-4.71133	0.86410	-0.53285
H	-4.45289	0.57441	1.20911
C	-0.73729	2.13229	0.05770
H	-0.03103	1.76547	0.80608
H	-0.97691	3.16948	0.27920
H	-0.27370	2.07831	-0.92980
H	0.10197	-2.34570	-0.85559
C	2.45608	-0.20245	0.33936
O	1.75742	0.12004	-0.62933
N	3.78534	-0.04700	0.32160
H	4.32067	-0.32473	1.13129
C	4.47831	0.50323	-0.82981
H	4.30433	-0.11183	-1.71471
C	1.85219	-0.79001	1.58815
H	1.35089	-1.72595	1.33032
H	1.09787	-0.10112	1.97446
H	2.59588	-0.98159	2.36099
H	4.13458	1.51826	-1.03806
H	5.54410	0.52388	-0.61177

ADMA_1 – PHE 2

C	4.21049	-0.57062	0.32833
C	2.96539	-1.18019	0.51346
C	1.78885	-0.56586	0.10398
C	1.84908	0.68490	-0.50269
C	3.06977	1.31618	-0.69836
C	4.23899	0.68346	-0.28081
H	2.91453	-2.15495	0.98841
H	0.82796	-1.04731	0.25150
H	3.10918	2.29378	-1.16902
H	5.19034	1.18259	-0.43340
O	0.66007	1.26090	-0.88945
H	0.82603	2.12123	-1.29897
C	5.47623	-1.26186	0.76326
H	6.32395	-0.57524	0.74496
H	5.70860	-2.10157	0.10195
H	5.37741	-1.66051	1.77551
C	-2.77128	1.83631	1.35257
H	-2.86380	1.57399	2.40686
H	-3.76431	1.94365	0.91741
N	-2.00091	0.83974	0.60931
H	-1.02045	1.05070	0.46299
C	-2.51990	-0.05844	-0.23825
N	-1.78873	-0.43675	-1.29337
H	-0.91875	0.04225	-1.49175
H	-1.96802	-1.31416	-1.76055
N	-3.74315	-0.55736	-0.05885
C	-4.41257	-1.30008	-1.12384
C	-4.35111	-0.67355	1.26791

H	-4.17421	-0.86924	-2.09526
H	-4.12869	-2.35657	-1.10603
H	-5.48675	-1.21937	-0.96116
H	-5.18108	0.02598	1.38223
H	-4.72592	-1.69289	1.37412
H	-3.60962	-0.50013	2.04369
H	-2.25100	2.78961	1.26911

ADMA_1 – PHE 3

C	4.07944	0.65873	-0.24827
C	2.96060	0.65714	-1.08724
C	1.92818	-0.25734	-0.91926
C	2.00497	-1.19396	0.10845
C	3.10700	-1.21814	0.95456
C	4.13344	-0.29489	0.76803
H	2.89719	1.38281	-1.89223
H	1.06887	-0.25754	-1.58097
H	3.16584	-1.95477	1.75004
H	4.99283	-0.32218	1.43016
O	0.95800	-2.07034	0.24624
H	1.14654	-2.69775	0.95686
C	5.17892	1.67188	-0.43272
H	6.07378	1.38414	0.12135
H	4.86501	2.65704	-0.07536
H	5.44457	1.77575	-1.48712
C	-4.43070	-1.55347	-0.46691
H	-5.00621	-1.32599	-1.36482
H	-4.90122	-1.08077	0.39416
N	-3.04296	-1.10928	-0.58451
H	-2.37904	-1.79652	-0.91767
C	-2.51493	-0.04672	0.03691
N	-1.22815	-0.08514	0.39650
H	-0.69757	-0.94658	0.31574
H	-0.71308	0.76843	0.56178
N	-3.25564	1.02601	0.32041
C	-2.77065	2.04638	1.24599
C	-4.41379	1.41179	-0.48733
H	-2.20561	1.58735	2.05583
H	-2.14759	2.78288	0.72950
H	-3.63797	2.55165	1.66944
H	-5.34199	1.28529	0.07286
H	-4.29880	2.46364	-0.75493
H	-4.45145	0.82783	-1.40325
H	-4.42555	-2.63149	-0.31138

ADMA_1 – PHE 4

C	3.58127	-0.76569	-0.35654
C	2.79933	-0.17694	-1.35535
C	1.88940	0.83205	-1.06448
C	1.74692	1.26385	0.25105
C	2.50935	0.69676	1.26463
C	3.41976	-0.31044	0.95222
H	2.90415	-0.51401	-2.38193
H	1.28423	1.28279	-1.84357
H	2.38852	1.03322	2.29007
H	4.01119	-0.75133	1.74818
O	0.80474	2.23098	0.49949
H	0.84766	2.50042	1.42699
C	4.58246	-1.83951	-0.69371
H	4.87644	-2.39505	0.19834
H	5.48660	-1.40508	-1.13047
H	4.17340	-2.54411	-1.42098

C	-4.58268	-0.97402	-1.07658
H	-5.44297	-0.95049	-0.40686
H	-3.98225	-1.85758	-0.86389
N	-3.76229	0.22912	-0.94952
H	-3.96315	0.98142	-1.59614
C	-2.57294	0.29307	-0.33881
N	-1.65491	1.15228	-0.79558
H	-1.83063	1.64480	-1.66072
H	-0.91471	1.50601	-0.19726
N	-2.28352	-0.50302	0.69192
C	-0.90519	-0.66552	1.14672
C	-3.31219	-1.00987	1.60140
H	-0.22147	-0.64552	0.29866
H	-0.63338	0.11190	1.86814
H	-0.82633	-1.63740	1.63330
H	-3.45015	-2.08564	1.47820
H	-2.98348	-0.80718	2.62205
H	-4.25482	-0.49383	1.43662
H	-4.93466	-1.03226	-2.10566

ADMA_1 – PHE 5

C	3.58136	-0.76580	-0.35620
C	2.79928	-0.17765	-1.35525
C	1.88928	0.83141	-1.06484
C	1.74687	1.26388	0.25048
C	2.50946	0.69741	1.26428
C	3.41994	-0.30986	0.95232
H	2.90405	-0.51524	-2.38167
H	1.28403	1.28170	-1.84413
H	2.38870	1.03443	2.28954
H	4.01150	-0.75025	1.74847
O	0.80462	2.23105	0.49851
H	0.84766	2.50100	1.42585
C	4.58257	-1.83975	-0.69290
H	4.87732	-2.39424	0.19955
H	5.48629	-1.40559	-1.13080
H	4.17314	-2.54527	-1.41908
C	-4.58251	-0.97457	-1.07625
H	-5.44279	-0.95085	-0.40652
H	-3.98199	-1.85798	-0.86321
N	-3.76224	0.22870	-0.94968
H	-3.96302	0.98062	-1.59677
C	-2.57292	0.29296	-0.33894
N	-1.65484	1.15192	-0.79605
H	-1.83049	1.64411	-1.66140
H	-0.91475	1.50599	-0.19777
N	-2.28361	-0.50264	0.69221
C	-0.90533	-0.66498	1.14721
C	-3.31241	-1.00908	1.60177
H	-0.22155	-0.64538	0.29919
H	-0.63354	0.11277	1.86830
H	-0.82654	-1.63665	1.63422
H	-3.45041	-2.08489	1.47897
H	-2.98380	-0.80603	2.62238
H	-4.25498	-0.49306	1.43666
H	-4.93451	-1.03325	-2.10530

ADMA_1 – PHE 6

C	2.96355	0.80045	-0.04268
C	2.89766	-0.27073	0.85562
C	2.11043	-1.38606	0.60026
C	1.36649	-1.44935	-0.57787

C	1.41851	-0.39918	-1.48865
C	2.21377	0.71230	-1.21470
H	3.47486	-0.23097	1.77451
H	2.06688	-2.21253	1.30094
H	0.84181	-0.45016	-2.40757
H	2.24677	1.52609	-1.93229
O	0.60963	-2.56499	-0.78904
H	0.14805	-2.48643	-1.63351
C	3.80534	2.01168	0.26446
H	3.89352	2.65873	-0.60970
H	4.81035	1.72262	0.58096
H	3.36385	2.59901	1.07499
C	-3.15557	2.30905	-0.30362
H	-2.36502	2.94999	-0.69454
H	-3.13080	2.32487	0.78518
N	-3.03523	0.93460	-0.79085
H	-3.61774	0.69104	-1.58176
C	-2.58821	-0.10384	-0.06995
N	-3.12613	-1.31061	-0.28427
H	-3.93860	-1.39919	-0.87840
H	-2.65103	-2.15644	-0.00453
N	-1.64136	0.04557	0.85410
C	-1.36793	-1.01623	1.82162
C	-0.68258	1.15306	0.82686
H	-2.29821	-1.48485	2.14166
H	-0.69875	-1.77227	1.40010
H	-0.89079	-0.56227	2.68882
H	-0.92758	1.90222	1.58236
H	0.30532	0.74148	1.03637
H	-0.66022	1.61118	-0.15906
H	-4.11951	2.69402	-0.63315

ADMA_1 – PHE 7

C	2.17404	1.40436	0.02995
C	1.86994	0.88369	-1.23202
C	1.88725	-0.48481	-1.47852
C	2.21136	-1.36784	-0.45078
C	2.51908	-0.87401	0.81621
C	2.49796	0.49980	1.04533
H	1.60719	1.56179	-2.03787
H	1.64240	-0.87854	-2.45850
H	2.76726	-1.56052	1.62020
H	2.73165	0.87216	2.03789
O	2.20479	-2.69906	-0.73404
H	2.43572	-3.20030	0.05824
C	2.16390	2.88889	0.28742
H	1.73268	3.11632	1.26496
H	1.58906	3.41533	-0.47686
H	3.18048	3.29326	0.27592
C	-0.95744	-1.40660	1.56542
H	-0.75389	-2.35359	1.06318
H	-1.96436	-1.42571	1.98054
N	-0.80927	-0.27129	0.65907
H	0.10632	0.16307	0.63342
C	-1.80197	0.45854	0.14264
N	-1.57195	1.75066	-0.12886
H	-0.69320	2.16905	0.14655
H	-2.12341	2.23496	-0.82315
N	-3.00987	-0.05954	-0.08446
C	-4.15119	0.80609	-0.37015
C	-3.20742	-1.47864	-0.38475
H	-4.09236	1.71987	0.21924

H	-4.20116	1.05370	-1.43490
H	-5.05593	0.26788	-0.08963
H	-3.73718	-1.98067	0.42674
H	-3.80398	-1.54588	-1.29617
H	-2.25348	-1.96822	-0.56428
H	-0.24747	-1.27722	2.38222

ADMA_1 – PHE 8

C	2.19944	1.38441	0.05482
C	1.89244	0.89045	-1.21721
C	1.88851	-0.47383	-1.48720
C	2.19389	-1.37935	-0.47341
C	2.50411	-0.91226	0.80306
C	2.50496	0.45739	1.05561
H	1.64405	1.58634	-2.01240
H	1.64159	-0.84654	-2.47482
H	2.73655	-1.61649	1.59630
H	2.74095	0.80891	2.05520
O	2.16665	-2.70534	-0.77904
H	2.38740	-3.22349	0.00521
C	2.21297	2.86412	0.33809
H	3.23643	3.25078	0.34295
H	1.77601	3.08213	1.31519
H	1.65446	3.41344	-0.42222
C	-0.95042	-1.33506	1.60789
H	-0.74880	-2.30381	1.14793
H	-1.95419	-1.33639	2.03087
N	-0.81015	-0.24316	0.64823
H	0.10502	0.18963	0.59598
C	-1.80569	0.46928	0.11331
N	-1.57141	1.74784	-0.21545
H	-0.67895	2.16366	0.01697
H	-2.12413	2.20194	-0.92892
N	-3.01900	-0.05093	-0.07618
C	-4.15822	0.80688	-0.39290
C	-3.22807	-1.48181	-0.30424
H	-4.08896	1.74733	0.15161
H	-4.21657	1.00335	-1.46778
H	-5.06302	0.28789	-0.07848
H	-3.75004	-1.94038	0.53747
H	-3.83659	-1.59032	-1.20370
H	-2.27916	-1.98538	-0.47099
H	-0.23488	-1.16742	2.41279

ADMA_2 – PHE 1

C	3.39575	0.47612	-0.63268
C	2.45370	-0.33513	-1.27192
C	1.49145	-1.03575	-0.55401
C	1.46252	-0.92788	0.83333
C	2.38930	-0.13215	1.49630
C	3.34808	0.55874	0.75938
H	2.47479	-0.42558	-2.35342
H	0.76927	-1.66854	-1.05869
H	2.36305	-0.05314	2.57895
H	4.07224	1.17438	1.28323
O	0.47759	-1.61274	1.50186
H	0.60450	-1.52178	2.45592
C	4.41420	1.25163	-1.42677
H	5.28155	1.50241	-0.81376
H	3.98714	2.18794	-1.79845
H	4.75549	0.68003	-2.29219
C	-2.13514	-2.32027	-0.70923

H	-1.96546	-2.22634	-1.78453
H	-3.13653	-2.71950	-0.52982
N	-1.94596	-1.04351	-0.03678
H	-1.23848	-1.00759	0.68747
C	-2.67997	0.03924	-0.29574
N	-3.66516	-0.04321	-1.19791
H	-3.82101	-0.90004	-1.70798
H	-4.16875	0.77496	-1.50452
N	-2.44494	1.18859	0.34129
C	-3.25799	2.36616	0.04327
C	-1.30742	1.29100	1.25287
H	-3.08945	2.71504	-0.97928
H	-4.31893	2.14855	0.18472
H	-2.97798	3.15802	0.73199
H	-0.36643	1.10965	0.72540
H	-1.28571	2.29747	1.66080
H	-1.40854	0.58951	2.08546
H	-1.40591	-3.01677	-0.30120

ADMA_2 – PHE 2

C	3.62030	-0.53311	-0.46999
C	2.43303	-0.54463	-1.20929
C	1.37768	0.30435	-0.90085
C	1.49924	1.18366	0.17200
C	2.66642	1.21657	0.92490
C	3.71575	0.36059	0.59636
H	2.33229	-1.23070	-2.04462
H	0.46114	0.29123	-1.48061
H	2.75496	1.90028	1.76387
H	4.62432	0.39020	1.18901
O	0.41753	1.97888	0.46304
H	0.64040	2.58716	1.18057
C	4.76163	-1.44665	-0.83379
H	5.50717	-1.47812	-0.03763
H	5.25850	-1.10502	-1.74648
H	4.40786	-2.46408	-1.01543
C	-0.62536	-1.14618	1.63918
H	-0.28680	-2.01277	1.06524
H	-1.17830	-1.48167	2.51994
N	-1.43581	-0.25747	0.81851
H	-1.09126	0.68413	0.67013
C	-2.60599	-0.61244	0.28787
N	-3.09949	-1.83169	0.54714
H	-2.52481	-2.51462	1.01925
H	-3.88895	-2.18648	0.02787
N	-3.29517	0.24124	-0.47074
C	-4.61464	-0.13469	-0.97463
C	-2.74318	1.56207	-0.76285
H	-4.54190	-0.94539	-1.70455
H	-5.27028	-0.43811	-0.15531
H	-5.05293	0.73172	-1.46140
H	-1.75536	1.47732	-1.22152
H	-3.40500	2.05922	-1.46625
H	-2.67486	2.17076	0.14343
H	0.24748	-0.58795	1.97222

ADMA_2 – PHE 3

C	-4.61796	-0.34638	0.27785
C	-3.43074	-1.03312	0.55382
C	-2.19406	-0.52911	0.17329
C	-2.12339	0.69042	-0.49853
C	-3.28952	1.39333	-0.78335

C	-4.52182	0.87167	-0.39353
H	-3.47528	-1.98254	1.07876
H	-1.28018	-1.06954	0.39313
H	-3.23621	2.34441	-1.30477
H	-5.42465	1.43011	-0.61950
O	-0.88684	1.14912	-0.85001
H	-0.97452	1.99200	-1.31259
C	-5.95107	-0.91935	0.68367
H	-6.74563	-0.17987	0.57131
H	-5.93590	-1.25191	1.72428
H	-6.20847	-1.78581	0.06757
C	3.74898	2.33858	0.85640
H	4.21910	2.75407	-0.03760
H	4.49750	2.22272	1.64394
N	3.09545	1.07196	0.55909
H	2.11673	0.98419	0.78881
C	3.74255	-0.00510	0.10846
N	5.06738	0.06113	-0.07565
H	5.55614	0.93664	0.04116
H	5.56040	-0.67736	-0.55521
N	3.08645	-1.13925	-0.13903
C	3.82134	-2.32439	-0.57796
C	1.63473	-1.19217	0.02885
H	4.25035	-2.17626	-1.57283
H	4.61586	-2.57185	0.12954
H	3.12749	-3.15916	-0.62021
H	1.14319	-0.42577	-0.57470
H	1.28964	-2.16702	-0.30382
H	1.35621	-1.06367	1.07899
H	2.98381	3.03045	1.19918

ADMA_2 – PHE 4

C	1.79087600	1.74044900	-0.30432200
C	1.29726200	0.83064400	-1.24552700
C	1.60791500	-0.52180800	-1.18278000
C	2.43389700	-0.99272700	-0.16329700
C	2.94822000	-0.10562100	0.77752000
C	2.62361000	1.24728500	0.70021800
H	0.65357900	1.18799700	-2.04414000
H	1.21939300	-1.21942700	-1.91638900
H	3.59647100	-0.47054400	1.56868200
H	3.02940800	1.92941700	1.44061500
O	2.70613300	-2.32896900	-0.13605100
H	3.28061600	-2.52631100	0.61437200
C	1.41257300	3.19749400	-0.36969200
H	2.05091300	3.79833200	0.28018300
H	0.37578100	3.34613400	-0.05222100
H	1.49991900	3.58174500	-1.38858500
C	-3.72106000	1.69352700	0.20187600
H	-4.68033000	1.20067300	0.37382800
H	-3.64716300	1.99819600	-0.84504300
N	-2.61806000	0.82187300	0.58088000
H	-1.91394500	1.20069500	1.19722000
C	-2.40122900	-0.37741600	0.03488300
N	-3.22278100	-0.81538400	-0.92878300
H	-4.03348900	-0.27175600	-1.18774500
H	-3.22006300	-1.78619400	-1.20565400
N	-1.36548500	-1.12019200	0.42497600
C	-1.08063800	-2.39048500	-0.23948800
C	-0.54189400	-0.68873800	1.55303200
H	-1.80395300	-3.15882600	0.04906700
H	-1.09242200	-2.26662100	-1.32397300

H	-0.08455400	-2.71320600	0.05346700
H	-1.16673300	-0.46233800	2.42037800
H	0.12679400	-1.50346900	1.81840900
H	0.05837700	0.18814600	1.29190000
H	-3.67057800	2.57942100	0.82999800

ADMA_2 – PHE 5

C	3.98093	0.50110	0.51537
C	2.75212	0.43348	1.17820
C	1.66336	-0.22944	0.62413
C	1.79500	-0.83830	-0.62027
C	3.00278	-0.78040	-1.30598
C	4.08279	-0.11369	-0.73302
H	2.64299	0.90768	2.14850
H	0.71481	-0.27964	1.14752
H	3.09999	-1.25060	-2.27985
H	5.02249	-0.07172	-1.27435
O	0.69432	-1.47989	-1.13203
H	0.91290	-1.88440	-1.98219
C	5.16238	1.19263	1.14410
H	5.88243	1.50652	0.38647
H	5.67993	0.52351	1.83815
H	4.84821	2.07262	1.70894
C	-3.19682	2.62556	-0.84893
H	-3.08736	2.45206	-1.92189
H	-2.27883	3.06656	-0.45346
N	-3.52988	1.39276	-0.15070
H	-4.40040	1.35977	0.35809
C	-2.74357	0.31351	-0.14348
N	-1.57905	0.35315	-0.79785
H	-1.30784	1.18335	-1.30439
H	-0.96104	-0.44800	-0.85983
N	-3.11000	-0.78961	0.51273
C	-2.21947	-1.94831	0.53457
C	-4.38589	-0.82500	1.22409
H	-2.04720	-2.32994	-0.47499
H	-1.26255	-1.69901	1.00027
H	-2.69432	-2.73027	1.12015
H	-5.21962	-0.65059	0.53928
H	-4.50365	-1.81142	1.66279
H	-4.40995	-0.08243	2.02639
H	-4.01466	3.32412	-0.69126

ADMA_2 – PHE 6

C	-4.10976	0.47503	0.48733
C	-2.85436	0.63197	1.08153
C	-1.71630	0.04335	0.54247
C	-1.82661	-0.72196	-0.61445
C	-3.06258	-0.89773	-1.22556
C	-4.19118	-0.30088	-0.66959
H	-2.76379	1.22411	1.98674
H	-0.74762	0.16710	1.01446
H	-3.14325	-1.50018	-2.12534
H	-5.15373	-0.44607	-1.14946
O	-0.68103	-1.28987	-1.11327
H	-0.88408	-1.80371	-1.90625
C	-5.32927	1.14344	1.06653
H	-6.24098	0.62299	0.76818
H	-5.40801	2.17801	0.71905
H	-5.28470	1.16551	2.15723
C	3.50788	2.55481	-0.80507
H	2.62619	3.07121	-0.41811

H	3.39989	2.40283	-1.88139
N	3.71881	1.28886	-0.11817
H	4.56687	1.18094	0.41734
C	2.83924	0.28403	-0.12885
N	1.69049	0.42742	-0.79681
H	1.49037	1.28403	-1.29214
H	1.00231	-0.31443	-0.86135
N	3.10088	-0.85213	0.52156
C	2.11186	-1.92801	0.51993
C	4.36214	-1.00534	1.24321
H	1.17046	-1.59545	0.96390
H	1.92800	-2.28860	-0.49540
H	2.50067	-2.75110	1.11273
H	4.43570	-0.28651	2.06396
H	4.39958	-2.00854	1.65742
H	5.21426	-0.87982	0.57030
H	4.38243	3.17535	-0.62664

ADMA_2 – PHE 7

C	4.10551	0.37805	-0.59411
C	2.82048	0.60167	-1.09744
C	1.68662	0.21384	-0.39368
C	1.83040	-0.41461	0.83990
C	3.09544	-0.65406	1.36298
C	4.21948	-0.25731	0.64259
H	2.70247	1.08893	-2.06024
H	0.69422	0.39157	-0.79404
H	3.20217	-1.15050	2.32276
H	5.20434	-0.44954	1.05597
O	0.68768	-0.78505	1.50253
H	0.91077	-1.20363	2.34443
C	5.32345	0.83127	-1.35603
H	6.21483	0.29627	-1.02423
H	5.50084	1.90049	-1.20620
H	5.20011	0.66625	-2.42841
C	-3.89294	2.41219	0.53570
H	-3.03729	3.01329	0.21965
H	-3.89754	2.32610	1.62490
N	-3.87080	1.10027	-0.09449
H	-4.66064	0.83781	-0.66402
C	-2.88213	0.21814	0.07303
N	-1.84507	0.54628	0.84928
H	-1.81307	1.45058	1.29664
H	-1.06289	-0.07775	1.01207
N	-2.92488	-0.97379	-0.52674
C	-1.84139	-1.93049	-0.30862
C	-4.06560	-1.32848	-1.36888
H	-0.88709	-1.52951	-0.65929
H	-1.75903	-2.19454	0.74872
H	-2.06444	-2.83169	-0.87245
H	-4.17090	-0.62771	-2.20099
H	-3.89172	-2.31987	-1.77702
H	-4.99205	-1.34984	-0.78881
H	-4.80516	2.91298	0.22130

ADMA_2 – PHE 8

C	4.10362	0.38646	-0.59000
C	2.81808	0.62445	-1.08457
C	1.68485	0.22552	-0.38543
C	1.83021	-0.42849	0.83429
C	3.09633	-0.68342	1.34815
C	4.21925	-0.27561	0.63280

H	2.69866	1.13184	-2.03669
H	0.69190	0.41454	-0.77910
H	3.20426	-1.20054	2.29682
H	5.20479	-0.48011	1.03871
O	0.68849	-0.80823	1.49334
H	0.91307	-1.24629	2.32485
C	5.32175	0.85340	-1.34325
H	6.20195	0.27117	-1.06523
H	5.53523	1.90408	-1.12462
H	5.17481	0.76542	-2.42156
C	-3.88557	2.40896	0.56428
H	-3.03049	3.01287	0.25206
H	-3.88603	2.30978	1.65238
N	-3.86749	1.10454	-0.08147
H	-4.65857	0.85081	-0.65324
C	-2.88006	0.21881	0.07378
N	-1.83883	0.53801	0.84818
H	-1.80586	1.43558	1.30882
H	-1.06339	-0.09412	1.01183
N	-2.92711	-0.96700	-0.53766
C	-1.84269	-1.92638	-0.33630
C	-4.07484	-1.31377	-1.37346
H	-0.88906	-1.51794	-0.68009
H	-1.75970	-2.20927	0.71616
H	-2.06459	-2.81746	-0.91647
H	-4.18580	-0.60657	-2.19920
H	-3.90545	-2.30218	-1.79064
H	-4.99671	-1.33858	-0.78629
H	-4.79849	2.91446	0.25959

SDMA_1 – PHE 1

C	-0.68783	-0.57314	0.80565
H	-0.67700	0.41034	1.27523
H	0.07213	-0.61705	0.02499
N	-2.02384	-0.86280	0.28478
H	-2.36961	-1.80256	0.42430
C	-2.90270	0.04147	-0.14480
N	-2.50943	1.25444	-0.55323
N	-4.20497	-0.25303	-0.14703
H	-3.25380	1.92104	-0.71270
H	-4.81742	0.35658	-0.67256
C	-1.21861	1.55992	-1.17206
H	-1.39166	2.32207	-1.93022
H	-0.81131	0.67469	-1.66016
H	-0.50040	1.94117	-0.44567
C	-4.77998	-1.43932	0.47016
H	-4.53491	-2.34386	-0.09239
H	-5.86034	-1.31582	0.48016
H	-4.42847	-1.54271	1.49832
H	-0.46345	-1.32193	1.56353
C	2.94317	-1.21153	-0.32526
C	2.61983	-0.23710	-1.27603
C	2.22075	1.03956	-0.90148
C	2.13293	1.36535	0.45120
C	2.45094	0.41454	1.41602
C	2.85047	-0.86087	1.02138
H	2.68016	-0.48313	-2.33195
H	1.96527	1.78660	-1.64509
H	2.37730	0.66490	2.47042
H	3.09037	-1.59636	1.78278
O	1.71436	2.62366	0.77191
H	1.69655	2.72367	1.73209

C	3.39362	-2.58495	-0.75075
H	3.36028	-3.28462	0.08606
H	4.42095	-2.56048	-1.12610
H	2.76253	-2.97568	-1.55228

SDMA_1 – PHE 2

C	2.07017	1.55462	-0.33913
C	1.40183	0.80942	-1.31482
C	1.47876	-0.57860	-1.34985
C	2.24173	-1.25110	-0.39715
C	2.92306	-0.52992	0.58090
C	2.83278	0.85954	0.60057
H	0.80542	1.32372	-2.06240
H	0.95437	-1.14874	-2.10902
H	3.52171	-1.05135	1.32207
H	3.36714	1.41080	1.36810
O	2.28664	-2.61215	-0.46890
H	2.84188	-2.95556	0.24244
C	1.93548	3.05376	-0.27834
H	1.07166	3.34066	0.33014
H	1.79223	3.47717	-1.27436
H	2.82022	3.51110	0.16847
C	-1.36000	-2.17190	0.37719
H	-1.67716	-2.23903	1.41729
H	-0.27089	-2.14117	0.32687
N	-1.97116	-1.00404	-0.25447
H	-2.29606	-1.12250	-1.20492
C	-2.36416	0.09960	0.38220
N	-1.81365	0.45193	1.55103
N	-3.33197	0.85672	-0.13799
H	-2.27651	1.20519	2.04427
H	-3.44028	1.78918	0.23806
C	-0.42854	0.17231	1.93272
H	-0.03108	1.06126	2.42120
H	0.16782	-0.02977	1.04441
H	-0.36106	-0.67299	2.61902
C	-4.16308	0.46916	-1.26828
H	-3.60151	0.49167	-2.20579
H	-4.98445	1.17883	-1.33466
H	-4.57292	-0.53047	-1.11320
H	-1.71454	-3.05441	-0.15276

SDMA_1 – PHE 3

C	3.42527	0.57086	-0.71845
C	2.28740	1.28518	-0.32793
C	1.42095	0.79545	0.64040
C	1.68345	-0.43647	1.23539
C	2.80895	-1.16469	0.87068
C	3.66955	-0.65439	-0.09975
H	2.07711	2.24520	-0.78942
H	0.54503	1.35875	0.94399
H	3.01123	-2.12409	1.33696
H	4.54713	-1.22843	-0.37899
O	0.77875	-0.89556	2.15754
H	1.08381	-1.73340	2.53018
C	4.34961	1.11766	-1.77492
H	5.20146	0.45400	-1.93049
H	4.73063	2.10204	-1.49104
H	3.82869	1.23215	-2.72934
C	-3.73570	-1.32784	-1.59012
H	-3.69105	-2.11193	-0.83496
H	-3.11101	-1.60139	-2.44057

N	-3.33716	-0.04846	-1.00657
H	-3.89320	0.75699	-1.26144
C	-2.48184	0.10612	0.00375
N	-1.55666	-0.82135	0.27975
N	-2.55496	1.19555	0.77178
H	-1.05411	-0.70122	1.15315
H	-1.76566	1.39038	1.37324
C	-0.92358	-1.69003	-0.71156
H	0.10200	-1.86597	-0.38826
H	-0.89677	-1.20069	-1.68546
H	-1.43166	-2.65054	-0.79834
C	-3.64502	2.15854	0.72923
H	-3.61204	2.76376	-0.18043
H	-3.54111	2.81548	1.58955
H	-4.60714	1.64684	0.79146
H	-4.76620	-1.22591	-1.92512

SDMA_1 – PHE 4

C	4.06072	-0.34103	-0.49505
C	2.82224	-0.81215	-0.94267
C	1.62917	-0.29587	-0.45282
C	1.66602	0.71140	0.50653
C	2.88014	1.19728	0.97284
C	4.06578	0.66731	0.46807
H	2.78948	-1.59889	-1.68995
H	0.67361	-0.66668	-0.80790
H	2.90250	1.98110	1.72397
H	5.01196	1.04956	0.83695
O	0.46232	1.19328	0.96929
H	0.61429	1.89549	1.61682
C	5.34364	-0.89820	-1.05423
H	6.20001	-0.58637	-0.45435
H	5.50544	-0.54998	-2.07846
H	5.31956	-1.99001	-1.07986
C	-4.67684	-0.45198	-1.24658
H	-4.05384	-0.19745	-2.10347
H	-5.38764	0.35254	-1.05767
N	-3.83803	-0.72689	-0.08171
H	-4.08994	-1.53130	0.47702
C	-2.64912	-0.17685	0.16176
N	-2.30374	0.99964	-0.38030
N	-1.76698	-0.81438	0.93392
H	-1.33688	1.26800	-0.23871
H	-0.97631	-0.27606	1.26841
C	-3.23957	2.08369	-0.68189
H	-2.71670	3.02265	-0.50860
H	-4.10204	2.04017	-0.01662
H	-3.57645	2.05480	-1.71799
C	-1.91518	-2.18923	1.38674
H	-2.68753	-2.27936	2.15498
H	-0.96297	-2.50313	1.80866
H	-2.16000	-2.84245	0.54718
H	-5.22589	-1.36369	-1.47427

SDMA_1 – PHE 5

C	3.58486	-0.78714	-0.35709
C	2.42991	-0.72938	-1.14482
C	1.51646	0.30901	-1.01882
C	1.74922	1.31498	-0.08374
C	2.88999	1.28585	0.70865
C	3.79723	0.23759	0.56412
H	2.24274	-1.51252	-1.87315

H	0.62436	0.34764	-1.63447
H	3.06697	2.07071	1.43805
H	4.68553	0.22060	1.18725
O	0.79846	2.29662	0.03878
H	1.10142	2.96930	0.66322
C	4.56455	-1.92069	-0.51500
H	5.36967	-1.84700	0.21766
H	5.01162	-1.91468	-1.51284
H	4.07035	-2.88683	-0.38485
C	-4.13947	-1.85775	-0.02598
H	-3.90774	-1.84714	-1.09059
H	-5.15163	-1.48556	0.13281
N	-3.14707	-1.07792	0.71106
H	-2.79804	-1.48602	1.56780
C	-2.47748	-0.02936	0.23255
N	-2.96227	0.68927	-0.79059
N	-1.29876	0.29885	0.76174
H	-2.32610	1.37336	-1.17898
H	-0.89250	1.19165	0.50021
C	-4.38545	0.91732	-1.04665
H	-4.48715	1.92498	-1.44537
H	-4.95465	0.85335	-0.11929
H	-4.78745	0.20914	-1.77116
C	-0.56682	-0.54089	1.69888
H	-1.08356	-0.61173	2.65940
H	0.40757	-0.08547	1.86602
H	-0.42138	-1.54211	1.28713
H	-4.07898	-2.88336	0.33291

SDMA_2 – PHE 1

C	-3.51886	-0.77159	-0.34614
C	-2.37479	-0.71128	-1.14805
C	-1.41211	0.27418	-0.96690
C	-1.58449	1.22074	0.03939
C	-2.71088	1.18461	0.85241
C	-3.66733	0.19176	0.65179
H	-2.23454	-1.44854	-1.93250
H	-0.52944	0.31620	-1.59584
H	-2.83815	1.92115	1.64022
H	-4.54400	0.16848	1.29104
O	-0.59164	2.15438	0.20716
H	-0.85492	2.79580	0.88074
C	-4.56464	-1.83147	-0.57533
H	-5.23726	-1.90990	0.28034
H	-4.10467	-2.80762	-0.74419
H	-5.16915	-1.59707	-1.45650
C	2.73770	1.96791	-0.96956
H	2.64451	2.56712	-0.06075
H	1.77163	1.91975	-1.47802
N	3.24104	0.63706	-0.66347
H	4.06571	0.31683	-1.15053
C	2.61974	-0.21240	0.15715
N	1.48789	0.15690	0.75749
N	3.13859	-1.41985	0.38551
H	1.06387	1.03973	0.49473
H	2.62542	-2.05514	0.97956
C	0.73306	-0.70962	1.65040
H	-0.10143	-0.13428	2.04647
H	1.35252	-1.03681	2.48850
H	0.33976	-1.58249	1.12161
C	4.38451	-1.88803	-0.20386
H	4.31408	-1.92991	-1.29359

H	4.57113	-2.89066	0.17248
H	5.21839	-1.24377	0.08386
H	3.45568	2.44859	-1.62941

SDMA_2 – PHE 2

C	3.41611	-0.83621	-0.39391
C	2.37713	-0.52536	-1.27622
C	1.46740	0.48803	-0.99874
C	1.58727	1.20715	0.18707
C	2.60945	0.91954	1.08371
C	3.51413	-0.09654	0.78515
H	2.27737	-1.08593	-2.20040
H	0.66551	0.72308	-1.69037
H	2.69499	1.47877	2.01061
H	4.30901	-0.31753	1.49021
O	0.64406	2.17297	0.43893
H	0.86823	2.64625	1.25151
C	4.41184	-1.91742	-0.72332
H	4.90550	-2.28417	0.17821
H	5.18662	-1.54012	-1.39751
H	3.92677	-2.76030	-1.21985
C	-1.01528	-0.50258	1.98293
H	-1.06285	0.51188	2.38730
H	-0.11715	-0.61621	1.36996
N	-2.21206	-0.81984	1.21793
H	-2.76539	-1.61370	1.50697
C	-2.58567	-0.15108	0.12514
N	-1.89352	0.91710	-0.26895
N	-3.64440	-0.55786	-0.57758
H	-1.11325	1.23832	0.29258
H	-3.93687	0.00242	-1.36528
C	-2.19003	1.66592	-1.48019
H	-1.45663	2.46533	-1.55952
H	-3.18709	2.11127	-1.43798
H	-2.11312	1.02835	-2.36437
C	-4.43816	-1.72919	-0.23832
H	-3.82320	-2.63187	-0.24641
H	-5.21548	-1.83078	-0.99154
H	-4.91073	-1.61728	0.74045
H	-0.96013	-1.20095	2.81443

SDMA_2 – PHE 3

C	3.00022	-0.79646	-0.14581
C	2.45739	-0.02367	-1.17567
C	1.73579	1.13556	-0.91177
C	1.53860	1.54071	0.40785
C	2.08241	0.79453	1.45074
C	2.80722	-0.35961	1.16578
H	2.59908	-0.33357	-2.20652
H	1.32483	1.73415	-1.71857
H	1.94090	1.11563	2.47842
H	3.22483	-0.93453	1.98640
O	0.80781	2.67164	0.61782
H	0.73255	2.83807	1.56599
C	3.72783	-2.08159	-0.44184
H	4.42800	-2.33074	0.35751
H	4.28214	-2.01463	-1.38005
H	3.02060	-2.91223	-0.53496
C	-0.38145	-1.85578	-0.08994
H	0.25783	-1.17213	0.47445
H	-0.12455	-1.80265	-1.15142
N	-1.78713	-1.54301	0.12727

H	-2.39577	-2.27705	0.45921
C	-2.32102	-0.34953	-0.13720
N	-1.55549	0.62795	-0.62270
N	-3.61757	-0.13353	0.08946
H	-0.57881	0.42888	-0.79453
H	-3.99494	0.77782	-0.12737
C	-2.04497	1.96487	-0.92142
H	-1.21377	2.53723	-1.32511
H	-2.40455	2.46242	-0.01756
H	-2.84503	1.93148	-1.66507
C	-4.51886	-1.14287	0.62458
H	-4.58551	-2.00406	-0.04460
H	-5.50433	-0.69251	0.71232
H	-4.19464	-1.47232	1.61444
H	-0.20891	-2.86923	0.26447

Calculations in water

Arg

C	2.14597	-0.58373	0.41184
H	2.01262	-1.30547	-0.39702
H	1.81063	-1.02229	1.35438
N	1.42891	0.65104	0.12798
H	1.95341	1.51384	0.06682
C	0.11540	0.72125	-0.04879
N	-0.46393	1.89829	-0.29225
H	0.09768	2.72877	-0.41669
H	-1.45332	1.95200	-0.48864
N	-0.62979	-0.37844	0.02905
H	3.20193	-0.33990	0.49401
H	-0.22074	-1.28099	0.22150
H	-1.62789	-0.32916	-0.12028

MMA 1

C	2.29514	-0.42961	0.57253
H	2.47027	-1.33171	-0.01348
H	1.82655	-0.69250	1.52041
N	1.46805	0.53764	-0.14789
H	1.94571	1.33270	-0.55346
C	0.14344	0.64374	-0.04889
N	-0.43918	1.82061	-0.29253
H	0.11100	2.66627	-0.34769
H	-1.43533	1.87921	-0.45003
N	-0.61411	-0.39917	0.29178
H	3.25042	0.04966	0.77797
H	-1.58604	-0.19064	0.48360
C	-0.28283	-1.80221	0.04420
H	0.34578	-1.89057	-0.84143
H	0.21805	-2.25887	0.89741
H	-1.21800	-2.32757	-0.13996

MMA 2

C	2.14780	-0.67741	0.39092
H	2.00978	-1.33560	-0.46977
H	1.78031	-1.17097	1.29305
N	1.47325	0.59737	0.18602
H	2.03844	1.43435	0.15976
C	0.15968	0.71764	0.02347
N	-0.38859	1.91883	-0.15134
H	-1.38988	1.96153	-0.28576
N	-0.61770	-0.36929	0.05895
H	3.20879	-0.47327	0.50919
H	-0.21636	-1.29557	0.06451

H	-1.60441	-0.28675	-0.14284
C	0.36801	3.16170	-0.15848
H	1.10506	3.16714	-0.96558
H	-0.33491	3.97440	-0.32252
H	0.87115	3.32009	0.79832

MMA 3

C	2.14399	-0.58675	0.42526
H	2.02724	-1.30796	-0.38702
H	1.78722	-1.02663	1.35950
N	1.44030	0.65184	0.12876
H	1.97298	1.50896	0.06295
C	0.12563	0.73311	-0.06725
N	-0.43903	1.90961	-0.33465
H	0.16744	2.71657	-0.39487
N	-0.62818	-0.36281	0.01053
H	3.19910	-0.34809	0.53210
H	-0.21843	-1.26676	0.19537
H	-1.62249	-0.32475	-0.16099
C	-1.86322	2.08733	-0.57481
H	-2.44788	1.77965	0.29535
H	-2.03785	3.14490	-0.75537
H	-2.18743	1.52297	-1.45248

ADMA 1

C	2.19354	-0.54385	0.42312
H	2.52676	-1.28914	-0.29966
H	1.60654	-1.02987	1.20120
N	1.40680	0.51311	-0.21057
H	1.91637	1.33832	-0.49996
C	0.07987	0.65740	-0.11049
N	-0.43805	1.89177	-0.13035
H	0.17963	2.69166	-0.10934
H	-1.39662	2.04711	-0.40966
N	-0.72295	-0.39666	0.03702
C	-2.11600	-0.22809	0.44380
C	-0.37718	-1.72216	-0.47914
H	-2.20730	0.58901	1.15785
H	-2.75776	-0.03794	-0.42170
H	-2.43637	-1.15065	0.92640
H	-0.17518	-2.41947	0.33585
H	-1.22806	-2.08587	-1.05751
H	0.48426	-1.66301	-1.13930
H	3.06505	-0.08096	0.88414

ADMA 2

C	2.61451	0.25978	-0.01019
H	2.86787	-0.32896	-0.89504
H	2.86500	-0.30497	0.89060
N	1.20825	0.63603	-0.01295
H	0.98364	1.61598	-0.10061
C	0.20925	-0.24880	0.00255
N	0.50133	-1.55587	0.00942
H	1.46043	-1.86076	0.09002
H	-0.21317	-2.24842	0.17788
N	-1.06051	0.15872	-0.00776
C	-2.14235	-0.82441	-0.02158
C	-1.36533	1.58780	0.01778
H	-2.02797	-1.51467	-0.86038
H	-2.17477	-1.38862	0.91450
H	-3.08268	-0.29423	-0.14090
H	-0.99902	2.08397	-0.88518
H	-2.44384	1.70656	0.06384

H	-0.92730	2.06339	0.89866
H	3.20037	1.17530	-0.01962

SDMA 1

C	1.16730	1.79922	-0.21073
H	1.55309	1.39578	-1.14625
H	1.95926	1.81084	0.53828
N	0.00937	1.02329	0.22887
H	-0.77640	1.54842	0.58834
C	-0.19718	-0.26800	-0.02538
N	0.82376	-1.09030	-0.30053
N	-1.44192	-0.74832	-0.03198
H	0.56365	-2.00994	-0.63352
H	-1.55165	-1.75277	0.01254
C	2.17414	-0.95652	0.24743
H	2.55978	-1.96073	0.41367
H	2.14598	-0.43374	1.20323
H	2.84078	-0.43115	-0.43651
C	-2.63846	0.07761	0.03468
H	-2.77172	0.51288	1.02841
H	-3.49247	-0.55849	-0.18599
H	-2.59203	0.87443	-0.70977
H	0.82881	2.81885	-0.38479

SDMA 2

C	-1.66754	1.80492	0.00089
H	-2.20658	1.48042	0.89431
H	-2.20684	1.47705	-0.89111
N	-0.30304	1.29864	0.00122
H	0.45254	1.96849	0.00498
C	0.00077	-0.00011	0.00172
N	-0.97325	-0.91127	0.00203
N	1.27714	-0.38693	0.00110
H	-1.92993	-0.58794	0.00461
H	1.47763	-1.37658	0.00323
C	-0.73435	-2.34705	0.00154
H	-1.70182	-2.84263	0.00105
H	-0.18316	-2.65257	0.89422
H	-0.18274	-2.65167	-0.89114
C	2.39977	0.53904	-0.00110
H	2.38652	1.17000	-0.89322
H	3.31314	-0.05026	-0.00407
H	2.39151	1.16867	0.89206
H	-1.61672	2.89073	-0.00126

CH₃COO

C	0.19547	-0.00000	-0.01137
O	0.75516	-1.12111	0.00198
O	0.75307	1.12218	0.00212
C	-1.34448	-0.00099	-0.00448
H	-1.74306	-0.89725	-0.48253
H	-1.74350	0.89044	-0.49117
H	-1.68523	0.00422	1.03607

EtOH

C	-1.20596	-0.24272	-0.02259
C	0.07879	0.56047	0.04719
H	-1.24990	-0.97345	0.79055
H	-2.07249	0.41699	0.06946
H	-1.27474	-0.77679	-0.97321
H	0.12000	1.28770	-0.76575
H	0.12993	1.11389	0.99187

O	1.23619	-0.25670	-0.10643
H	1.22070	-0.92124	0.59085

N-methylacetamide

C	-0.48297	0.14609	-0.00419
O	-0.37688	1.39722	-0.00036
N	0.60521	-0.65484	-0.00417
H	0.48689	-1.65496	-0.00181
C	1.95329	-0.10530	0.00285
H	2.11665	0.53207	-0.86602
C	-1.82212	-0.53609	0.00171
H	-2.42974	-0.13627	-0.80849
H	-2.33276	-0.31501	0.93905
H	-1.74345	-1.61492	-0.11075
H	2.12719	0.49253	0.89772
H	2.66458	-0.92554	-0.01981

p-methylphenol

C	-1.37535	0.01275	-0.00526
C	-0.66529	-1.19302	-0.00475
C	0.72281	-1.21907	-0.00066
C	1.43579	-0.02064	0.00164
C	0.75259	1.19127	-0.00082
C	-0.64138	1.19775	-0.00466
H	-1.21131	-2.13159	-0.00820
H	1.26466	-2.15817	-0.00138
H	1.30576	2.12583	-0.00173
H	-1.16322	2.14941	-0.00817
O	2.79685	-0.09499	0.00394
H	3.16897	0.79561	0.00419
C	-2.88205	0.01885	0.00601
H	-3.27028	1.02611	-0.15351
H	-3.26807	-0.34345	0.96317
H	-3.28411	-0.63119	-0.77492

6OI0 Arg

C	1.96931	0.02383	-0.01878
N	0.66877	-0.61042	0.00574
C	-0.52276	-0.00720	0.01698
N	-0.68944	1.32464	0.02741
N	-1.52930	-0.78102	-0.00335
H	2.20167	0.51765	0.92606
H	2.71136	-0.75430	-0.18482
H	0.66069	-1.61535	0.18344
H	0.09629	1.97304	-0.00612
H	-1.61354	1.69952	-0.13508
H	-1.44369	-1.77743	-0.17561
H	-2.47251	-0.41868	0.03320
H	2.03014	0.74338	-0.83890

6OI0 S49B

C	-1.84298	-0.51901	0.02131
C	-0.46200	0.14402	-0.00690
O	-0.36941	1.36832	-0.01106
N	0.60569	-0.65191	-0.06510
C	1.95445	-0.10157	0.03139
H	-2.45531	-0.06843	-0.76011
H	2.14950	0.57422	-0.80260
H	0.47954	-1.65109	0.00462
H	2.08246	0.45036	0.96513
H	2.66492	-0.92528	-0.00232
H	-2.30516	-0.30461	0.98710

H -1.79735 -1.59905 -0.12246

6OI0 S91BD92

C -3.44541 -0.33172 -0.39076
C -2.02264 -0.17677 0.14775
O -1.52969 -1.07660 0.80645
N -1.34110 0.92959 -0.15456
C 0.06145 1.05589 0.26340
C 0.95620 0.20372 -0.67058
C 2.30894 -0.14118 -0.08272
O 2.56905 0.14909 1.10666
O 3.12767 -0.74908 -0.81723
H -4.10649 -0.56766 0.44421
H 1.09933 0.71710 -1.62642
H 0.44576 -0.73490 -0.90196
H 0.15443 0.69827 1.28597
H -1.77041 1.63778 -0.73244
H -3.45026 -1.17592 -1.08273
H 0.33242 2.11057 0.22980
H -3.80452 0.56067 -0.90408

6OI0 F133B

C -1.81676 -0.54760 0.03747
C -0.46304 0.15006 -0.03434
O -0.40512 1.38312 -0.01256
N 0.60175 -0.62919 -0.08882
C 1.96260 -0.10461 0.04721
H -2.50532 -0.05404 -0.64863
H 2.05245 0.85847 -0.45109
H 0.46858 -1.62895 -0.03469
H 2.65690 -0.81342 -0.40259
H 2.22142 0.02847 1.10183
H -2.20170 -0.42711 1.05268
H -1.76036 -1.61110 -0.19732

6OI0 S175B

C -1.83846 -0.52234 0.00860
C -0.46622 0.13714 -0.01549
O -0.39768 1.35961 -0.00168
N 0.60686 -0.65049 -0.01282
C 1.97895 -0.08667 0.00994
H -2.42555 -0.12500 -0.82020
H 2.15011 0.52742 -0.87506
H 0.49016 -1.65298 -0.02167
H -2.33067 -0.24113 0.94120
H -1.79726 -1.61032 -0.06230
H 2.68271 -0.91602 0.02128
H 2.11834 0.52583 0.90155

6OI0 C261B

C -1.80215 -0.56447 0.01578
C -0.46734 0.16224 -0.01708
O -0.42186 1.38725 -0.00479
N 0.60240 -0.61769 -0.03765
C 1.96480 -0.10614 0.02118
H -1.70761 -1.63786 -0.14533
H 2.15714 0.56728 -0.81575
H 0.46854 -1.61797 -0.02007
H -2.45500 -0.13570 -0.74628
H -2.26185 -0.38739 0.99071
H 2.13503 0.43687 0.95325
H 2.65007 -0.94920 -0.03395

6OI1 MMA

C -2.44771 -0.12095 0.00807
C 2.46225 -0.09990 0.00160
N 1.13664 -0.65024 -0.01188
C -0.00129 0.06730 -0.00710
N -0.02872 1.35510 0.00568
N -1.11459 -0.65260 -0.01070
H -3.13498 -0.95814 0.13520
H -2.58790 0.55666 0.85633
H -2.69991 0.40896 -0.91466
H 2.69088 0.40255 0.94472
H 3.16711 -0.91988 -0.12015
H 0.81568 1.93126 0.02131
H 2.60428 0.60163 -0.82528
H 1.03284 -1.65422 0.12596
H -1.00928 -1.65907 -0.07852
H -0.91150 1.84573 -0.04202

6OI1 S49B

C -1.83044 -0.51829 0.02510
C -0.46288 0.13598 -0.02715
O -0.37830 1.36611 -0.00900
N 0.61158 -0.65584 -0.05832
C 1.94469 -0.09260 0.03337
H -2.48119 -0.02801 -0.69894
H 2.14156 0.57045 -0.81104
H 0.49058 -1.65729 -0.02374
H -1.79917 -1.59037 -0.17463
H 2.66718 -0.90670 0.02101
H -2.24424 -0.35586 1.02295
H 2.06242 0.47923 0.95673

6OI1 S91BD92

C -3.46295 -0.25257 -0.45775
C -2.02868 -0.20227 0.11396
O -1.55359 -1.20824 0.65311
N -1.34839 0.92877 -0.00147
C 0.05082 1.00272 0.43416
C 0.95852 0.33809 -0.63041
C 2.32546 -0.13005 -0.09815
O 2.59739 -0.06663 1.11883
O 3.14676 -0.58651 -0.94154
H -4.14120 -0.53879 0.34737
H 1.12586 1.02183 -1.46789
H 0.45296 -0.53675 -1.04941
H 0.14801 0.47539 1.38061
H -1.76790 1.70776 -0.48929
H -3.77639 0.70038 -0.88412
H 0.30381 2.05157 0.58452
H -3.48993 -1.02728 -1.22564

6OI1 F133B

C -1.82168 -0.54354 0.04299
C -0.45756 0.15266 -0.04709
O -0.38164 1.39480 -0.01235
N 0.59623 -0.63489 -0.09569
C 1.94679 -0.11577 0.05315
H -2.53449 -0.01443 -0.58929
H 2.04402 0.85088 -0.43690
H 0.45565 -1.63406 -0.04849
H -1.77707 -1.59434 -0.24587

H	-2.16148	-0.47292	1.07885
H	2.64792	-0.81980	-0.39462
H	2.19964	0.01035	1.11061

6OI1 S175B

C	-1.87935	-0.47308	0.00945
C	-0.45724	0.13761	-0.01684
O	-0.30693	1.34605	-0.00214
N	0.57195	-0.67285	-0.01355
C	1.96758	-0.10820	0.01087
H	-2.44996	-0.04444	-0.81496
H	2.13002	0.51083	-0.87077
H	0.44627	-1.67433	-0.02263
H	-1.87426	-1.56057	-0.07057
H	2.66223	-0.94516	0.01397
H	2.10269	0.49549	0.90787
H	-2.35107	-0.17828	0.94812

6OI1 C261B

C	-1.81120	-0.57051	0.01851
C	-0.46775	0.17190	-0.01816
O	-0.41535	1.39014	-0.00568
N	0.60518	-0.60545	-0.04541
C	1.96342	-0.11816	0.02508
H	2.16951	0.57221	-0.79513
H	0.46405	-1.60486	-0.02414
H	2.63861	-0.96788	-0.05007
H	2.14588	0.40133	0.96889
H	-2.25702	-0.41221	1.00292
H	-2.47400	-0.13150	-0.72878
H	-1.70731	-1.63942	-0.16291

6OI2 SDMA

C	-2.43243	-0.71026	0.00282
N	-1.33887	0.24530	-0.00970
C	-0.02702	-0.02372	-0.00807
N	0.81139	0.91585	-0.00473
N	0.47468	-1.25506	0.00065
C	0.57971	2.33104	-0.00904
C	1.90026	-1.53089	0.01384
H	-2.45551	-1.28848	0.92776
H	-3.35846	-0.14453	-0.06834
H	0.08203	2.65818	-0.92270
H	-0.03719	2.63085	0.84349
H	1.53670	2.84040	0.07355
H	2.40030	-1.15272	-0.88266
H	2.37765	-1.11289	0.90385
H	2.02334	-2.61282	0.04334
H	-2.36567	-1.38146	-0.85672
H	-1.59832	1.21404	0.16450
H	1.78749	0.66162	-0.00539
H	-0.14585	-2.05188	-0.12154

6OI2 S49B

C	-1.84489	-0.52853	0.02240
C	-0.46320	0.14170	-0.00433
O	-0.39259	1.36803	-0.01163
N	0.61836	-0.64859	-0.07122
C	1.96594	-0.09370	0.03304
H	-2.43838	-0.11401	-0.79293
H	2.15295	0.60216	-0.78598
H	0.49560	-1.64699	0.01556

H	-2.32781	-0.27115	0.96686
H	-1.79435	-1.61375	-0.07329
H	2.67957	-0.91332	-0.02463
H	2.09747	0.43614	0.97931

6OI2 S91BD92

C	3.43884	-0.03512	0.54529
C	2.04366	-0.23371	-0.03752
O	1.61927	-1.37632	-0.19521
N	1.33003	0.85285	-0.33244
C	-0.06572	0.73484	-0.78182
C	-1.00804	0.63387	0.43029
C	-2.32152	-0.08384	0.13886
O	-2.58961	-0.54821	-0.98829
O	-3.11680	-0.19325	1.10073
H	4.15544	-0.56480	-0.08449
H	-1.23759	1.62805	0.82610
H	-0.50664	0.09145	1.23694
H	-0.14846	-0.16600	-1.38536
H	1.71439	1.76352	-0.12480
H	3.72515	1.01459	0.61990
H	3.45698	-0.48820	1.53783
H	-0.29573	1.59750	-1.40752

6OI2 F133B

C	-1.83721	-0.53216	0.03537
C	-0.46455	0.14855	-0.04068
O	-0.38263	1.38091	-0.00998
N	0.59666	-0.64453	-0.07692
C	1.97041	-0.10588	0.04292
H	-2.51759	-0.03003	-0.65245
H	2.01549	0.89667	-0.37594
H	0.46325	-1.64456	-0.03164
H	-2.21629	-0.40106	1.05123
H	-1.79537	-1.59715	-0.19592
H	2.27088	-0.05906	1.09308
H	2.65217	-0.76343	-0.49566

6OI2 S175B

C	-1.85459	-0.51508	0.00672
C	-0.46454	0.14380	-0.01026
O	-0.37030	1.36164	-0.00152
N	0.59270	-0.64619	-0.01188
C	1.98256	-0.10255	0.00804
H	-2.42372	-0.13228	-0.84128
H	2.15531	0.51529	-0.87359
H	0.46919	-1.64804	-0.01245
H	2.13541	0.49932	0.90434
H	2.66882	-0.94630	0.00782
H	-1.81267	-1.60382	-0.04162
H	-2.35936	-0.21101	0.92502

6OI2 C261B

C	-1.82146	-0.56842	0.01920
C	-0.46969	0.16526	-0.02156
O	-0.42096	1.38811	-0.00448
N	0.61183	-0.61016	-0.04192
C	1.97463	-0.11200	0.02417
H	-1.72058	-1.64765	-0.08970
H	2.17506	0.57299	-0.80219
H	0.47510	-1.61041	-0.02422
H	2.15142	0.41537	0.96430

H	2.65221	-0.96018	-0.04439
H	-2.45126	-0.17289	-0.77980
H	-2.29799	-0.34009	0.97443

6OI3 MMA H3R2me Peptide

C	-2.45535	-0.05870	0.05237
C	2.44236	-0.05939	0.08217
N	1.14884	-0.68810	-0.12990
C	0.00377	0.00137	-0.05092
N	0.00909	1.33285	-0.03021
N	-1.14032	-0.67618	0.01458
H	-3.19817	-0.85153	0.12263
H	-2.65628	0.54102	-0.83952
H	-2.54974	0.57927	0.93706
H	2.52168	0.37985	1.07932
H	3.20464	-0.82824	-0.02174
H	0.86624	1.88443	-0.02739
H	2.62193	0.71091	-0.67020
H	1.06732	-1.65986	0.16627
H	-0.86480	1.83740	-0.06226
H	-1.08080	-1.67299	-0.16712

6OI3 S49B

C	-1.82141	-0.52855	0.03769
C	-0.47446	0.15294	-0.02901
O	-0.37198	1.37500	-0.01395
N	0.60590	-0.64954	-0.09923
C	1.94424	-0.10394	0.04841
H	-2.49638	-0.05825	-0.67834
H	2.19079	0.54456	-0.79561
H	0.47388	-1.64725	-0.02298
H	2.01142	0.47858	0.97177
H	2.65837	-0.92310	0.10748
H	-2.22707	-0.37072	1.04025
H	-1.76666	-1.59973	-0.15897

6OI3 S91BD92

C	-3.50292	-0.26841	-0.38184
C	-2.11140	-0.13242	0.18493
O	-1.73870	-0.83054	1.13233
N	-1.30763	0.77770	-0.38542
C	0.08607	0.94100	0.02842
C	0.99442	-0.08479	-0.63955
C	2.41716	-0.14358	-0.03788
O	2.69157	0.68977	0.85180
O	3.16043	-1.04202	-0.50051
H	-4.22523	-0.14473	0.42701
H	1.08298	0.12644	-1.71137
H	0.54576	-1.08100	-0.56758
H	0.12624	0.82541	1.10868
H	-1.64880	1.28826	-1.18773
H	0.39069	1.95870	-0.21557
H	-3.71080	0.45448	-1.17043
H	-3.61390	-1.27988	-0.77852

6OI3 F133B

C	-1.82058	-0.54129	0.00921
C	-0.47876	0.15669	-0.00726
O	-0.39065	1.37966	-0.00186
N	0.60704	-0.63958	-0.02760
C	1.95886	-0.10434	0.01032
H	-2.39173	-0.22112	-0.86421

H	2.08284	0.70709	-0.70729
H	0.47559	-1.63899	0.01963
H	2.65743	-0.90669	-0.22571
H	2.19250	0.28604	1.00616
H	-2.36500	-0.22544	0.90102
H	-1.73284	-1.62755	0.00488

6OI3 S175B

C	1.81748	-0.54711	0.01276
C	0.47822	0.15721	-0.00875
O	0.39958	1.38060	-0.00391
N	-0.60989	-0.63598	-0.03526
C	-1.96020	-0.10243	0.01897
H	2.32140	-0.29803	0.94925
H	-2.27825	0.13545	1.03810
H	-0.48216	-1.63612	0.01100
H	1.73117	-1.63063	-0.07055
H	2.42729	-0.16600	-0.80788
H	-2.64429	-0.84527	-0.39137
H	-2.01562	0.80161	-0.58837

6OI3 C261B

C	-1.82648	-0.52759	0.00269
C	-0.47848	0.15064	-0.00613
O	-0.36808	1.37663	-0.00021
N	0.60172	-0.64636	-0.00402
H	-1.75242	-1.61399	-0.03804
H	0.47169	-1.64738	-0.00661
H	-2.35497	-0.23405	0.91176
C	1.95105	-0.10669	0.00321
H	-2.40360	-0.16939	-0.85174
H	2.13075	0.49733	-0.88852
H	2.11101	0.51827	0.88401
H	2.65357	-0.93748	0.02040

ADMA – CH₃COO 1

C	-1.33838	0.07905	-0.20266
N	-2.56368	-0.37111	0.11392
N	-0.40085	-0.74188	-0.66567
H	0.64368	-0.51830	-0.68943
H	-0.64208	-1.71399	-0.78958
N	-1.06754	1.38489	-0.08214
H	-1.73898	1.97647	0.38210
C	0.20509	1.97654	-0.47263
H	0.43256	1.73008	-1.51147
H	0.10467	3.05612	-0.38007
H	1.01769	1.62379	0.16519
C	2.86848	-0.27485	0.22742
O	2.36695	0.04283	1.32048
O	2.22135	-0.50289	-0.84075
C	4.38670	-0.38619	0.11524
H	4.66038	-1.32023	-0.37953
H	4.75717	0.43372	-0.50655
H	4.86248	-0.33180	1.09403
C	-2.84325	-1.80351	0.08192
H	-2.10938	-2.35681	0.67258
H	-3.82458	-1.97023	0.51792
H	-2.84672	-2.18770	-0.94244
C	-3.62440	0.56789	0.46720
H	-3.43075	1.04475	1.43302
H	-3.73376	1.33844	-0.29997
H	-4.55975	0.01919	0.53465

ADMA – CH₃COO 2

C	1.45366	-0.17075	-0.18080
N	0.50271	-0.99983	-0.59765
N	1.24587	1.15115	-0.16525
N	2.64090	-0.65123	0.22972
H	3.41262	-0.00544	0.30477
C	2.95457	-2.07065	0.26779
H	2.95509	-2.51518	-0.73159
H	3.94700	-2.17932	0.69939
H	2.24208	-2.60378	0.90052
H	0.72839	-1.98401	-0.62781
C	0.03963	1.71570	-0.76797
H	0.24244	2.75541	-1.01841
H	-0.20804	1.17864	-1.68383
H	-0.80810	1.65288	-0.08186
O	-2.09651	-0.66826	-0.79144
C	-2.72482	-0.25279	0.23158
O	-2.20184	0.16453	1.27907
C	-4.24687	-0.27852	0.12028
H	-4.56089	0.35806	-0.71084
H	-4.58136	-1.29424	-0.10390
H	-4.71939	0.06643	1.03941
H	-0.54067	-0.75588	-0.63781
C	2.21950	2.05221	0.44165
H	2.55130	1.66961	1.40885
H	3.08879	2.20198	-0.20669
H	1.73698	3.01288	0.60590

ADMA – CH₃COO 3

C	-1.63629	0.36168	-0.00511
N	-1.62481	-0.96312	-0.19112
N	-2.74177	0.93876	0.51117
H	-2.78799	1.94489	0.57435
H	-3.62893	0.45894	0.46501
N	-0.57653	1.10000	-0.30256
H	0.38632	0.67510	-0.45030
C	-0.57156	2.54271	-0.12511
H	-0.70435	2.82746	0.92346
H	0.39755	2.90701	-0.46098
H	-1.34796	3.02217	-0.72691
C	-2.73565	-1.78581	0.27717
H	-2.41717	-2.82515	0.26917
H	-3.00775	-1.52193	1.30092
H	-3.61306	-1.68227	-0.36885
C	-0.49364	-1.59826	-0.86491
H	-0.20845	-1.02136	-1.74596
H	0.36868	-1.67416	-0.19831
H	-0.80566	-2.59013	-1.18502
C	2.55055	-0.14742	0.22595
O	1.95074	0.45318	-0.71766
O	2.01098	-0.59903	1.25191
C	4.05471	-0.33110	0.03950
H	4.22750	-1.02906	-0.78452
H	4.51800	0.61878	-0.23519
H	4.52257	-0.72366	0.94191

ADMA – CH₃COO 4

C	-2.07728	0.24228	-0.01500
N	-2.70052	1.36992	-0.39208
N	-0.79600	0.26684	0.33950
N	-2.77974	-0.89396	0.02854

H	-2.31495	-1.73383	0.33900
C	-4.18295	-0.98841	-0.34539
H	-4.33184	-0.71446	-1.39268
H	-4.49124	-2.02206	-0.21004
H	-4.80299	-0.35196	0.29013
H	-3.62046	1.32348	-0.80516
C	-0.05356	1.52806	0.33391
H	0.97869	1.30246	0.59461
H	-0.05870	1.97496	-0.66322
H	-0.48358	2.23248	1.05245
O	2.31848	-0.17558	-1.08097
C	3.17134	-0.11232	-0.16738
O	2.96092	0.23574	1.02360
C	4.62160	-0.47785	-0.51602
H	5.01441	-1.19023	0.21325
H	4.70289	-0.89697	-1.51929
H	5.24073	0.42205	-0.45640
H	-2.17205	2.21290	-0.55883
C	-0.15129	-0.94367	0.84826
H	-0.63531	-1.27991	1.77058
H	-0.18713	-1.74284	0.10380
H	0.89304	-0.70557	1.03875

ADMA – CH₃COO 5

C	-1.63629	0.36173	-0.00493
N	-2.74170	0.93883	0.51149
N	-1.62493	-0.96305	-0.19112
N	-0.57649	1.10000	-0.30234
H	0.38631	0.67506	-0.45023
C	-0.57140	2.54269	-0.12470
H	-0.70409	2.82730	0.92392
H	0.39772	2.90696	-0.46059
H	-1.34779	3.02229	-0.72638
H	-2.78784	1.94496	0.57476
C	-2.73587	-1.78568	0.27703
H	-2.41753	-2.82507	0.26882
H	-3.00791	-1.52197	1.30084
H	-3.61328	-1.68191	-0.36895
O	1.95072	0.45317	-0.71773
C	2.55062	-0.14757	0.22573
O	2.01111	-0.59948	1.25160
C	4.05480	-0.33099	0.03923
H	4.22771	-1.02848	-0.78517
H	4.51798	0.61909	-0.23493
H	4.52269	-0.72397	0.94145
H	-3.62890	0.45909	0.46528
C	-0.49383	-1.59817	-0.86505
H	-0.20879	-1.02128	-1.74616
H	0.36858	-1.67401	-0.19856
H	-0.80586	-2.59005	-1.18509

ADMA – CH₃COO 6

C	1.45368	-0.17079	-0.18086
N	0.50279	-0.99982	-0.59805
N	1.24588	1.15110	-0.16532
N	2.64079	-0.65133	0.22993
H	3.41257	-0.00561	0.30503
C	2.95437	-2.07078	0.26802
H	2.95519	-2.51524	-0.73139
H	3.94664	-2.17953	0.69995
H	2.24162	-2.60389	0.90047
H	0.72837	-1.98402	-0.62801

C	0.03986	1.71568	-0.76845
H	0.24264	2.75552	-1.01838
H	-0.20724	1.17893	-1.68464
H	-0.80819	1.65249	-0.08278
O	-2.20179	0.16538	1.27884
C	-2.72477	-0.25261	0.23160
O	-2.09646	-0.66808	-0.79139
C	-4.24684	-0.27913	0.12062
H	-4.58088	-1.29497	-0.10362
H	-4.71935	0.06551	1.03988
H	-4.56134	0.35742	-0.71034
H	-0.54052	-0.75581	-0.63801
C	2.21933	2.05212	0.44193
H	2.55090	1.66940	1.40916
H	3.08879	2.20198	-0.20617
H	1.73676	3.01276	0.60618

ADMA – CH₃COO 7

C	-1.50258	-0.16416	0.03586
N	-0.51229	-1.04510	0.12218
N	-1.25073	1.14964	-0.02749
N	-2.78038	-0.58330	-0.00037
H	-3.50476	0.08797	0.20907
C	-3.15746	-1.98783	0.00488
H	-2.88524	-2.48138	0.94230
H	-4.23683	-2.04178	-0.11833
H	-2.68765	-2.51205	-0.82936
H	-0.76129	-2.01661	0.24097
C	0.06731	1.69806	0.27917
H	-0.07253	2.60082	0.87629
H	0.66338	0.98738	0.84955
H	0.60395	1.95608	-0.63751
O	2.69186	-0.10630	1.27794
C	2.85234	-0.32783	0.06559
O	1.94412	-0.70091	-0.74111
C	4.23429	-0.12677	-0.54911
H	4.19904	0.72801	-1.23033
H	4.98682	0.05882	0.21663
H	4.51349	-1.00229	-1.13889
H	0.49902	-0.83907	-0.16851
C	-2.30917	2.10707	-0.32539
H	-2.96783	1.72515	-1.10635
H	-2.89885	2.34649	0.56549
H	-1.84301	3.02019	-0.69180

ADMA – CH₃COO 8

C	1.68312	0.37733	0.01012
N	2.84033	1.06692	0.12526
N	1.73310	-0.95963	-0.02612
N	0.53638	1.02947	-0.09819
H	-0.38420	0.56029	-0.34370
C	0.45701	2.47694	0.00658
H	1.00942	2.97543	-0.79561
H	-0.59396	2.74788	-0.07824
H	0.82769	2.83255	0.97184
H	2.79579	2.05942	0.30482
C	3.01976	-1.64513	-0.11159
H	2.83234	-2.68866	-0.35156
H	3.63974	-1.21711	-0.90256
H	3.56180	-1.59907	0.83764
O	-2.31326	-0.40637	1.27775
C	-2.65863	-0.14655	0.11115

O	-1.89061	0.27976	-0.80529
C	-4.11695	-0.34650	-0.29292
H	-4.17176	-1.05103	-1.12643
H	-4.71363	-0.71961	0.53886
H	-4.53173	0.60247	-0.64173
H	3.66317	0.59972	0.47738
C	0.50503	-1.74617	-0.10861
H	-0.27509	-1.30733	0.51542
H	0.14431	-1.81120	-1.13911
H	0.72236	-2.74773	0.25834

ADMA – CH₃COO 9

C	-1.63628	0.36171	-0.00500
N	-2.74171	0.93880	0.51138
N	-1.62489	-0.96307	-0.19115
N	-0.57650	1.10000	-0.30242
H	0.38633	0.67508	-0.45025
C	-0.57145	2.54270	-0.12484
H	-0.70417	2.82736	0.92377
H	0.39766	2.90698	-0.46072
H	-1.34785	3.02224	-0.72656
H	-2.78789	1.94493	0.57460
C	-2.73579	-1.78572	0.27706
H	-2.41742	-2.82510	0.26887
H	-3.00782	-1.52199	1.30086
H	-3.61321	-1.68199	-0.36891
O	1.95073	0.45319	-0.71768
C	2.55058	-0.14752	0.22582
O	2.01104	-0.59936	1.25170
C	4.05476	-0.33103	0.03936
H	4.22764	-1.02870	-0.78489
H	4.51798	0.61898	-0.23501
H	4.52264	-0.72383	0.94166
H	-3.62891	0.45904	0.46516
C	-0.49376	-1.59820	-0.86503
H	-0.20865	-1.02128	-1.74610
H	0.36861	-1.67408	-0.19849
H	-0.80579	-2.59006	-1.18512

ADMA – CH₃COO 10

C	-1.35047	-0.12524	-0.23958
N	-0.49410	-0.99494	-0.76382
N	-1.02022	1.16955	-0.12023
N	-2.54977	-0.53532	0.20559
H	-3.26777	0.16134	0.34301
C	-2.96828	-1.92854	0.19764
H	-2.25396	-2.54409	0.74777
H	-3.93246	-1.98823	0.69736
H	-3.07381	-2.31624	-0.81950
H	-0.81194	-1.94585	-0.88600
C	0.07122	1.76534	-0.88523
H	-0.28972	2.69687	-1.32544
H	0.92746	1.98384	-0.24196
H	0.39026	1.09817	-1.68160
O	2.09409	-0.67853	-0.92436
C	2.78065	-0.37054	0.10235
O	3.97986	-0.06180	0.09856
C	2.03043	-0.38781	1.43759
H	2.71003	-0.21936	2.27220
H	1.26566	0.39536	1.44028
H	1.51839	-1.34433	1.57004
H	0.56676	-0.82418	-0.80950

C	-1.85293	2.09280	0.64097
H	-2.70570	2.44690	0.05229
H	-2.21102	1.62257	1.55722
H	-1.23881	2.94880	0.91694

SDMA – CH₃COO 1

C	1.55003	0.46752	-0.09923
N	0.74196	-0.51735	-0.44766
N	2.84466	0.24885	0.22051
H	3.40470	1.08365	0.32970
N	1.10643	1.73090	-0.09542
H	1.66986	2.41928	0.38391
C	-0.16819	2.15234	-0.65871
H	-1.00239	1.84507	-0.02524
H	-0.14814	3.23738	-0.74299
H	-0.29794	1.72074	-1.65258
C	1.19361	-1.82310	-0.91050
H	0.42533	-2.20910	-1.57999
H	2.12748	-1.73411	-1.46679
H	1.32831	-2.53408	-0.09314
H	-0.31062	-0.37797	-0.48949
C	3.31843	-0.93039	0.94556
H	2.52794	-1.33898	1.57601
H	3.67935	-1.70755	0.27144
H	4.14057	-0.61436	1.58562
O	-1.89053	-0.57359	-0.70825
C	-2.58813	-0.27015	0.30731
O	-2.14823	0.21443	1.36559
C	-4.09020	-0.50843	0.17254
H	-4.28017	-1.50192	-0.23827
H	-4.59782	-0.39923	1.13058
H	-4.50120	0.22097	-0.53138

SDMA – CH₃COO 2

C	-1.55008	0.46748	-0.09918
N	-0.74193	-0.51738	-0.44751
N	-2.84473	0.24874	0.22047
H	-3.40472	1.08355	0.32988
N	-1.10649	1.73086	-0.09531
H	-1.67026	2.41937	0.38342
C	0.16797	2.15238	-0.65890
H	0.29768	1.72050	-1.65266
H	0.14769	3.23739	-0.74350
H	1.00230	1.84544	-0.02544
C	-1.19350	-1.82307	-0.91058
H	-0.42536	-2.20878	-1.58040
H	-1.32784	-2.53428	-0.09337
H	-2.12752	-1.73408	-1.46659
H	0.31067	-0.37791	-0.48935
C	-3.31833	-0.93050	0.94564
H	-3.67906	-1.70781	0.27161
H	-2.52780	-1.33890	1.57619
H	-4.14056	-0.61455	1.58560
O	1.89055	-0.57379	-0.70812
C	2.58816	-0.27012	0.30737
O	2.14831	0.21487	1.36549
C	4.09022	-0.50850	0.17255
H	4.59786	-0.39996	1.13064
H	4.28020	-1.50164	-0.23909
H	4.50124	0.22148	-0.53078

SDMA – CH₃COO 3

C	1.55006	0.46752	-0.09918
N	0.74204	-0.51749	-0.44748
N	2.84473	0.24902	0.22052
H	3.40471	1.08386	0.32970
N	1.10619	1.73076	-0.09531
H	1.67016	2.41950	0.38285
C	-0.16826	2.15210	-0.65909
H	-1.00268	1.84424	-0.02619
H	-0.14847	3.23717	-0.74277
H	-0.29740	1.72098	-1.65326
C	1.19371	-1.82319	-0.91041
H	0.42532	-2.20927	-1.57975
H	2.12742	-1.73413	-1.46693
H	1.32864	-2.53418	-0.09311
H	-0.31045	-0.37813	-0.48941
C	3.31863	-0.93016	0.94567
H	2.52827	-1.33860	1.57638
H	3.67936	-1.70742	0.27158
H	4.14092	-0.61398	1.58545
O	-2.14830	0.21498	1.36532
C	-2.58817	-0.27013	0.30727
O	-1.89061	-0.57398	-0.70818
C	-4.09026	-0.50847	0.17280
H	-4.59762	-0.39990	1.13105
H	-4.50151	0.22147	-0.53042
H	-4.28035	-1.50166	-0.23865

SDMA – CH₃COO 4

C	1.69387	0.39730	0.02254
N	0.58254	-0.23914	0.33767
N	2.88483	-0.23814	-0.05116
H	3.68462	0.36924	-0.16883
N	1.67569	1.72002	-0.19645
H	2.47245	2.12306	-0.66947
C	0.52678	2.57186	0.06667
H	0.11595	2.35262	1.05321
H	0.86724	3.60520	0.04238
H	-0.26295	2.42987	-0.67306
C	0.54114	-1.53112	1.01004
H	-0.39738	-1.57136	1.56150
H	0.55950	-2.36742	0.31032
H	1.37155	-1.62528	1.71158
H	-0.37415	0.21419	0.22019
C	3.04982	-1.60262	-0.55331
H	3.06199	-2.33627	0.25306
H	2.25537	-1.85388	-1.25684
H	4.00220	-1.64600	-1.07913
O	-1.91241	0.63966	0.23895
C	-2.66953	-0.28785	-0.18835
O	-2.28849	-1.37952	-0.64252
C	-4.16726	-0.00066	-0.10651
H	-4.74866	-0.77655	-0.60385
H	-4.46411	0.05003	0.94471
H	-4.38691	0.97095	-0.55409

SDMA – CH₃COO 5

C	1.60353	0.45424	0.02021
N	0.67810	-0.46794	-0.16970
N	2.89162	0.12588	0.25439
H	3.49126	0.89433	0.52273
N	1.27608	1.75375	0.01834
H	2.02646	2.41928	-0.10656

C	-0.09230	2.25025	0.00409
H	-0.71604	1.63795	0.65810
H	-0.07937	3.27582	0.36933
H	-0.51688	2.23108	-1.00221
C	0.77925	-1.83946	0.31231
H	-0.23503	-2.16235	0.54896
H	1.20066	-2.51482	-0.43460
H	1.38162	-1.88842	1.22038
H	-0.30098	-0.20994	-0.48514
C	3.55653	-1.03631	-0.33224
H	3.55225	-1.89384	0.34145
H	3.07908	-1.31555	-1.27215
H	4.58895	-0.75844	-0.53843
O	-1.83808	-0.25075	-0.96776
C	-2.61518	-0.26971	0.03546
O	-2.25496	-0.20816	1.22527
C	-4.10527	-0.35974	-0.28382
H	-4.69962	-0.48416	0.62089
H	-4.41697	0.55543	-0.79464
H	-4.29111	-1.19252	-0.96552

SDMA – CH₃COO 6

C	1.25108	0.19888	0.04100
N	2.58241	0.33344	0.15108
N	0.68465	-1.00616	-0.08295
H	-0.33730	-1.00335	-0.27803
N	0.46977	1.27620	0.03938
H	-0.55531	1.13057	0.18133
C	0.97419	2.63434	-0.05802
H	1.65304	2.74234	-0.90761
H	0.12265	3.29553	-0.20958
H	1.49284	2.94569	0.85409
C	3.56452	-0.61599	-0.36566
H	4.44692	-0.04966	-0.65900
H	3.85462	-1.35590	0.38099
H	3.17117	-1.12514	-1.24579
H	2.92472	1.26085	0.35887
C	1.23297	-2.23257	0.48643
H	1.84689	-2.78532	-0.22671
H	1.82543	-2.02070	1.37794
H	0.39208	-2.86236	0.77667
O	-2.21052	0.93897	0.41028
C	-2.69849	-0.11688	-0.07026
O	-2.02977	-1.06783	-0.54841
C	-4.21184	-0.26934	-0.03740
H	-4.55891	-0.88266	-0.86904
H	-4.48426	-0.77671	0.89310
H	-4.70456	0.70265	-0.05128

SDMA – CH₃COO 7

C	-1.10337	0.17357	-0.44161
N	-1.52591	0.67899	0.72075
N	-0.28105	0.85095	-1.24165
H	0.16778	0.31763	-1.97470
N	-1.52338	-1.03681	-0.82522
H	-1.30749	-1.31778	-1.77205
C	-1.97348	-2.05497	0.11442
H	-2.99742	-1.86979	0.44798
H	-1.94853	-3.01424	-0.39887
H	-1.29200	-2.08388	0.96716
C	-0.71720	1.53994	1.58822
H	-0.92664	1.24992	2.61693

H	0.34155	1.36892	1.39206
H	-0.97381	2.59263	1.46106
H	-2.25314	0.14946	1.18091
C	-0.00700	2.27993	-1.19104
H	-0.88721	2.81603	-0.83808
H	0.84652	2.49565	-0.54607
H	0.21496	2.61056	-2.20474
C	1.79807	-0.56042	0.43954
C	2.13384	-1.29587	-0.86783
H	2.90581	-2.04412	-0.66016
H	1.25704	-1.82558	-1.24892
H	2.51996	-0.61142	-1.62512
O	0.98300	-1.13185	1.20367
O	2.37447	0.53448	0.64323

ADMA – EtOH 1

C	-1.31590	2.28955	-0.33513
H	-1.60718	2.78735	0.59044
H	-2.20993	1.98078	-0.87585
N	-0.45237	1.13685	-0.08483
H	0.54858	1.29244	-0.17157
C	-0.82929	-0.14333	-0.18714
N	0.08286	-1.04750	-0.56819
H	1.01160	-0.72288	-0.81836
H	-0.03855	-2.02356	-0.33801
N	-2.08522	-0.52534	0.05292
C	-2.54401	-1.85775	-0.33077
C	-2.95806	0.19653	0.98016
H	-2.09745	-2.15487	-1.27855
H	-2.30382	-2.59570	0.44079
H	-3.62552	-1.81515	-0.45488
H	-3.77426	0.68902	0.44856
H	-3.37495	-0.52991	1.67987
H	-2.39051	0.92916	1.54814
H	-0.76158	2.99348	-0.95490
C	3.95938	-0.99503	0.31611
C	3.14973	0.24342	0.63793
H	4.70468	-0.78216	-0.45519
H	4.48447	-1.34018	1.20990
H	3.30993	-1.80008	-0.03707
H	2.39691	0.03051	1.39960
H	3.78875	1.04822	1.01077
O	2.41419	0.70903	-0.50557
H	3.03730	0.99293	-1.18541

ADMA – EtOH 2

C	-3.70827	-0.22710	-0.20258
H	-4.02315	0.33694	-1.08128
H	-3.61959	0.44789	0.64778
N	-2.44131	-0.92125	-0.42624
H	-2.50480	-1.88788	-0.71818
C	-1.25537	-0.54991	0.07648
N	-0.35933	-1.49067	0.38717
H	-0.64406	-2.46019	0.35687
H	0.64128	-1.29224	0.42637
N	-0.97286	0.73738	0.28708
C	0.15812	1.13760	1.11939
C	-1.57874	1.81048	-0.50129
H	0.33795	0.39843	1.89799
H	1.06274	1.26826	0.51911
H	-0.09423	2.08814	1.59044
H	-2.28693	2.38771	0.09631

H	-0.77645	2.46962	-0.83723
H	-2.07747	1.40467	-1.37798
H	-4.46442	-0.97663	0.02704
C	3.96704	0.74736	-0.28115
C	3.01679	-0.32609	-0.76989
H	4.82562	0.29973	0.22773
H	4.34151	1.32951	-1.12656
H	3.46441	1.42455	0.41339
H	2.15705	0.11551	-1.27940
H	3.51690	-0.99508	-1.47684
O	2.47100	-1.09017	0.31092
H	3.19268	-1.46977	0.82526

ADMA – EtOH 3

C	2.81682	-1.89154	0.33708
H	2.60115	-2.60889	-0.45526
H	1.98438	-1.86682	1.03918
N	3.05999	-0.55245	-0.19649
H	4.02488	-0.31431	-0.38737
C	2.21670	0.48272	-0.09957
N	2.72112	1.71845	-0.00042
H	3.71567	1.84637	0.12497
H	2.17168	2.52398	-0.26448
N	0.89693	0.30128	-0.07037
C	-0.00235	1.37296	0.35159
C	0.24458	-0.83597	-0.72224
H	0.46825	1.98056	1.12308
H	-0.28533	2.00421	-0.49665
H	-0.89488	0.90527	0.76635
H	-0.21143	-1.49871	0.01409
H	-0.53781	-0.44181	-1.37397
H	0.95581	-1.38384	-1.33520
H	3.71238	-2.20830	0.87022
C	-4.79511	-0.16104	-0.43551
C	-3.39497	0.29185	-0.06966
H	-5.43427	-0.19717	0.45170
H	-5.24502	0.53574	-1.14712
H	-4.77215	-1.15487	-0.88841
H	-2.75937	0.32705	-0.95683
H	-3.42112	1.29957	0.36157
O	-2.75306	-0.61035	0.83015
H	-3.30856	-0.69005	1.61368

SDMA – EtOH 1

C	1.67579	0.28771	0.05326
N	2.14899	1.46995	-0.35994
N	0.43010	0.18746	0.52001
N	2.47323	-0.78140	0.00798
H	2.13104	-1.65140	0.38749
C	3.84242	-0.74690	-0.48502
H	4.46190	-0.07645	0.11538
H	4.24374	-1.75437	-0.41042
H	3.87350	-0.43604	-1.53158
H	3.06074	1.53633	-0.78869
C	-0.40193	1.38523	0.63788
H	-1.35634	1.09038	1.06634
H	0.06841	2.12410	1.29193
H	-0.58704	1.83183	-0.34322
C	-0.06287	-1.09793	1.01502
H	0.00940	-1.86483	0.23938
H	0.49580	-1.41845	1.89919
H	-1.11189	-0.98057	1.27459

H	1.54310	2.27185	-0.44524
O	-3.33274	-0.33362	0.77370
H	-3.99624	0.05019	1.35666
C	-3.55032	0.16366	-0.54853
H	-3.38835	1.24754	-0.57386
H	-4.58332	-0.03101	-0.85492
C	-2.58966	-0.53603	-1.48433
H	-2.73245	-1.61836	-1.43856
H	-2.76533	-0.20771	-2.51133
H	-1.55156	-0.31062	-1.22879

SDMA – EtOH 2

C	1.35548	0.48203	-0.05649
N	2.24042	1.41774	0.31920
N	1.68977	-0.81025	-0.01653
N	0.13638	0.85435	-0.44150
H	-0.57682	0.15335	-0.63398
C	-0.31028	2.23881	-0.45577
H	0.29558	2.84247	-1.13591
H	-1.33738	2.24376	-0.81410
H	-0.28343	2.67746	0.54534
H	2.02780	2.39564	0.18370
C	3.01630	-1.20730	0.45135
H	3.06817	-2.29218	0.45096
H	3.79763	-0.82328	-0.21002
H	3.19344	-0.85308	1.46990
C	0.70742	-1.82208	-0.39775
H	-0.14896	-1.81692	0.28307
H	0.35968	-1.66053	-1.42119
H	1.18420	-2.79683	-0.34854
H	3.21105	1.18215	0.46317
O	-2.31736	-0.47289	-0.94584
H	-2.50526	-1.10567	-1.64800
C	-3.09526	-0.81380	0.21045
H	-2.81682	-1.81270	0.56165
H	-4.15614	-0.82601	-0.05561
C	-2.82961	0.22210	1.27947
H	-3.09781	1.21939	0.92286
H	-3.42952	0.00065	2.16479
H	-1.77678	0.22400	1.57288

SDMA – EtOH 3

C	-1.70209	0.25573	-0.12219
N	-1.31709	-0.97098	-0.47101
N	-0.86592	1.29722	-0.21102
H	-1.18367	2.14927	0.23287
N	-2.93705	0.45388	0.34581
H	-3.26878	1.40856	0.38332
C	-3.85564	-0.61082	0.72132
H	-3.35826	-1.32840	1.37633
H	-4.68400	-0.15819	1.26131
H	-4.24996	-1.13045	-0.15582
C	0.06513	-1.44787	-0.50664
H	0.06717	-2.47773	-0.15192
H	0.47412	-1.41939	-1.51742
H	0.68340	-0.84594	0.15872
C	0.21507	1.43069	-1.18767
H	1.17784	1.15692	-0.75508
H	0.01147	0.81290	-2.06180
H	0.24653	2.47243	-1.50321
H	-2.04187	-1.67114	-0.55441
O	2.37315	0.31074	1.25811

H	2.41944	0.98057	1.94809
C	3.64764	0.22863	0.61710
H	4.41727	-0.04222	1.34769
H	3.91513	1.20001	0.18621
C	3.56221	-0.82170	-0.46830
H	2.83739	-0.53369	-1.23334
H	4.53616	-0.94375	-0.94755
H	3.26194	-1.78455	-0.04696

SDMA – EtOH 4

C	1.35895	0.48419	-0.00596
N	0.39901	-0.27971	0.50451
N	2.59371	0.00575	-0.22522
H	3.28533	0.69768	-0.48313
N	1.10284	1.76482	-0.28853
H	1.76623	2.25174	-0.87570
C	-0.12184	2.45537	0.08563
H	-0.32545	2.31957	1.14960
H	0.02337	3.51530	-0.11051
H	-0.97766	2.10208	-0.49562
C	0.61027	-1.49882	1.27983
H	-0.22601	-1.58946	1.97125
H	0.64343	-2.38757	0.64899
H	1.53259	-1.42531	1.85581
C	2.89479	-1.37700	-0.59820
H	3.13818	-1.98977	0.26977
H	2.05441	-1.81999	-1.13270
H	3.75506	-1.35470	-1.26472
H	-0.55445	0.08232	0.51187
O	-2.41552	0.08281	0.80703
H	-2.90402	0.63855	1.42393
C	-3.27908	-0.30126	-0.27092
H	-3.70435	0.59112	-0.74055
H	-4.09976	-0.91107	0.11863
C	-2.45959	-1.08804	-1.26859
H	-1.99677	-1.95366	-0.78727
H	-3.10182	-1.44547	-2.07625
H	-1.67408	-0.46656	-1.70564

SDMA – EtOH 5

C	-0.97943	0.20141	-0.18761
N	-2.25193	0.53459	0.03285
N	-0.56259	-1.06920	-0.08550
H	0.44050	-1.18784	-0.19657
N	-0.08930	1.14633	-0.49277
H	0.82634	0.82914	-0.79783
C	-0.34838	2.57446	-0.40062
H	-0.76816	2.82874	0.57513
H	0.60125	3.09186	-0.51870
H	-1.02856	2.91094	-1.18732
C	-3.20342	-0.23674	0.83032
H	-3.89325	0.47062	1.28660
H	-3.76989	-0.94148	0.22167
H	-2.68130	-0.77309	1.62201
C	-1.38924	-2.23857	-0.38834
H	-1.86535	-2.64381	0.50441
H	-2.15226	-1.99136	-1.12681
H	-0.73422	-2.99883	-0.81047
H	-2.51366	1.48738	-0.18136
O	2.26900	-0.55503	-0.85090
H	2.61238	-0.92360	-1.67337
C	3.34220	-0.44312	0.10045

H	4.13863	0.17535	-0.32227
H	3.74631	-1.43778	0.30792
C	2.78736	0.18302	1.35905
H	1.98232	-0.42947	1.77368
H	3.57666	0.26240	2.10945
H	2.40239	1.18624	1.15938

ADMA_1 – NMA 1

C	3.36757	-1.81302	0.78014
H	2.77698	-2.63003	0.36529
H	2.87038	-1.41929	1.66606
N	3.58204	-0.74355	-0.19444
H	4.48768	-0.72521	-0.64559
C	2.89999	0.40908	-0.23248
N	3.53161	1.51994	-0.63469
H	4.52313	1.48345	-0.82745
H	3.01125	2.29970	-1.01119
N	1.62513	0.47210	0.14404
C	0.97396	1.76613	0.34256
C	0.73354	-0.69072	0.07559
H	1.65694	2.46152	0.82940
H	0.63412	2.18533	-0.60947
H	0.10678	1.60910	0.98103
H	0.59938	-1.14491	1.05956
H	-0.23373	-0.33797	-0.28210
H	1.12802	-1.42885	-0.62063
H	4.34506	-2.19431	1.07103
C	-3.43260	0.30976	0.05255
O	-2.28053	0.73714	0.08323
N	-3.70681	-0.97139	-0.26046
H	-4.66776	-1.27944	-0.27242
C	-2.65570	-1.92101	-0.57397
H	-2.07466	-1.58570	-1.43623
C	-4.61540	1.19133	0.36683
H	-4.60089	2.04919	-0.30771
H	-4.50887	1.56542	1.38689
H	-5.56874	0.67200	0.27160
H	-1.97784	-2.04584	0.27386
H	-3.11492	-2.88012	-0.80623

ADMA_1 – NMA 2

C	0.63693	-0.45160	1.97693
H	-0.37429	-0.16448	1.68427
H	1.18740	0.43267	2.29801
N	1.35072	-1.11163	0.88317
H	1.34902	-2.12314	0.89284
C	2.28894	-0.54749	0.11409
N	3.27992	-1.31707	-0.35830
H	3.34611	-2.27908	-0.05553
H	3.77601	-1.05960	-1.19979
N	2.27266	0.75603	-0.16054
C	3.43807	1.41289	-0.74755
C	1.03752	1.54340	-0.17047
H	4.35686	0.97915	-0.35571
H	3.42579	1.33419	-1.83874
H	3.40192	2.46537	-0.46873
H	1.03908	2.27207	0.64293
H	0.99024	2.07547	-1.12291
H	0.16013	0.90679	-0.08877
H	0.58991	-1.15182	2.81000
C	-3.12414	0.24764	-0.02731
O	-2.17444	0.85460	0.46428

N	-2.94188	-0.76242	-0.89893
H	-3.74734	-1.23557	-1.28075
C	-1.61310	-1.19040	-1.29302
H	-1.07995	-0.38584	-1.80600
C	-4.55176	0.58732	0.31950
H	-4.71732	1.64797	0.12389
H	-4.69923	0.41635	1.38783
H	-5.27569	-0.00307	-0.24209
H	-1.02998	-1.48952	-0.41861
H	-1.70778	-2.03973	-1.96718

ADMA_1 – NMA 3

C	1.73030	2.26247	0.56274
H	2.17076	2.91230	-0.19441
H	2.50830	1.93059	1.24967
N	1.05404	1.11196	-0.03376
H	0.04501	1.18749	-0.13876
C	1.51873	-0.14338	-0.02278
N	0.63560	-1.14989	0.01473
H	-0.35469	-0.90987	0.04006
H	0.89982	-2.06262	-0.32846
N	2.82861	-0.40526	-0.01302
C	3.31298	-1.75319	0.26951
C	3.81253	0.50236	-0.60398
H	2.70067	-2.22392	1.03734
H	3.31456	-2.37089	-0.63407
H	4.33355	-1.66916	0.64135
H	4.44667	0.94902	0.16406
H	4.43539	-0.07822	-1.28682
H	3.31811	1.28349	-1.17540
H	0.98950	2.82929	1.12585
C	-2.97636	0.39109	-0.07233
O	-1.73671	0.41135	-0.06466
N	-3.64799	-0.76101	0.00245
H	-4.65768	-0.73947	-0.00440
C	-2.96715	-2.04037	0.09913
H	-2.32055	-2.19912	-0.76717
C	-3.77819	1.65809	-0.16332
H	-3.48916	2.18926	-1.07191
H	-3.52587	2.28997	0.69033
H	-4.85176	1.47429	-0.17461
H	-2.36092	-2.08801	1.00680
H	-3.71680	-2.82813	0.13128

ADMA_1 – NMA 4

C	1.11530	1.99191	0.72127
H	1.27421	2.77024	-0.02641
H	2.01770	1.87924	1.32119
N	0.74987	0.71663	0.10915
H	-0.24584	0.49916	0.06202
C	1.54615	-0.35444	0.02758
N	0.98305	-1.57128	0.05971
H	-0.02831	-1.62848	0.14475
H	1.46511	-2.35721	-0.35386
N	2.87519	-0.23944	-0.04945
C	3.73482	-1.40478	0.13952
C	3.52471	0.92705	-0.64939
H	3.33095	-2.05320	0.91576
H	3.84863	-1.96800	-0.79214
H	4.71383	-1.04899	0.45883
H	4.06691	1.50413	0.10200
H	4.23059	0.56750	-1.40042

H	2.79347	1.56017	-1.14534
H	0.30013	2.29493	1.37780
C	-2.96252	-0.52675	-0.01749
O	-1.73961	-0.69420	0.09885
N	-3.47768	0.69320	-0.19365
H	-4.47869	0.79382	-0.28343
C	-2.63908	1.87761	-0.25114
H	-2.07214	1.99211	0.67608
C	-3.91770	-1.68533	0.03141
H	-3.76795	-2.22040	0.97072
H	-3.67753	-2.36758	-0.78630
H	-4.95865	-1.37425	-0.04939
H	-1.93975	1.81777	-1.08899
H	-3.27892	2.74717	-0.38455

ADMA_1 – NMA 5

C	-4.37973	-0.65995	0.21531
H	-4.91749	-0.51030	-0.72176
H	-4.30869	0.28839	0.74671
N	-3.04405	-1.21006	-0.00604
H	-2.95565	-2.21581	0.05962
C	-1.89658	-0.52456	0.09847
N	-0.79412	-1.15409	0.51148
H	-0.88069	-2.10340	0.84795
H	0.14528	-0.82556	0.25674
N	-1.85173	0.78156	-0.17758
C	-0.70992	1.59134	0.23816
C	-2.74950	1.41067	-1.14593
H	-0.33254	1.24127	1.19758
H	0.09053	1.56074	-0.50656
H	-1.05423	2.61940	0.35057
H	-3.45240	2.08379	-0.65121
H	-2.13824	1.98517	-1.84441
H	-3.29357	0.65638	-1.70861
H	-4.93454	-1.36396	0.83422
C	2.76444	-0.01451	0.36632
O	1.84390	-0.54630	-0.26540
N	4.01643	-0.00211	-0.10803
H	4.74182	0.43344	0.44293
C	4.36013	-0.59414	-1.38853
H	4.13162	-1.66160	-1.39397
C	2.53186	0.67325	1.68521
H	1.84563	0.07532	2.28624
H	2.06238	1.64136	1.49202
H	3.45862	0.83665	2.23502
H	3.80462	-0.11486	-2.19690
H	5.42639	-0.45572	-1.55582

ADMA_1 – NMA 6

C	4.15287	-0.81051	-0.27071
H	4.62454	-0.87321	0.71064
H	4.25703	0.20276	-0.65688
N	2.74065	-1.18441	-0.21953
H	2.52572	-2.14848	-0.43847
C	1.71136	-0.32877	-0.31865
N	0.58506	-0.73284	-0.90800
H	0.58520	-1.61955	-1.39344
H	-0.31889	-0.27962	-0.73907
N	1.81487	0.91791	0.14759
C	0.83945	1.94065	-0.21936
C	2.69846	1.26730	1.25972
H	0.47765	1.77353	-1.23227

H	-0.00737	1.94432	0.47172
H	1.34113	2.90761	-0.17871
H	3.52838	1.89050	0.92078
H	2.11117	1.82462	1.99189
H	3.08228	0.37093	1.74035
H	4.65792	-1.49468	-0.95154
C	-3.06748	0.19013	-0.12471
O	-1.97705	0.45319	-0.64365
N	-3.26089	-0.92939	0.58570
H	-4.16927	-1.09793	0.99283
C	-2.20394	-1.90298	0.79034
H	-1.85219	-2.29931	-0.16518
C	-4.24209	1.11974	-0.26858
H	-4.48157	1.21586	-1.32936
H	-3.95315	2.10523	0.10074
H	-5.12281	0.77294	0.27109
H	-1.35886	-1.45280	1.31641
H	-2.60017	-2.72063	1.38900

ADMA_1 – NMA 7

C	-4.30531	-0.79190	0.00974
H	-4.97475	-0.15370	-0.56814
H	-4.16029	-0.36041	0.99951
N	-3.01468	-0.96955	-0.65304
H	-2.91422	-1.79963	-1.22251
C	-1.86551	-0.38911	-0.27469
N	-0.71611	-1.04273	-0.45014
H	-0.75049	-2.01223	-0.73475
H	0.19149	-0.56268	-0.50249
N	-1.86691	0.81963	0.29333
C	-0.68051	1.32580	0.97801
C	-2.89754	1.81744	0.00567
H	-0.15894	0.51312	1.48189
H	-0.00059	1.81981	0.27870
H	-1.01127	2.04635	1.72586
H	-3.53791	1.98404	0.87406
H	-2.39490	2.75188	-0.25029
H	-3.50048	1.50998	-0.84530
H	-4.76251	-1.77403	0.12359
C	2.72833	-0.43282	0.13196
O	1.88268	0.03222	-0.64218
N	4.00024	-0.01740	0.10655
H	4.65905	-0.42581	0.75345
C	4.46035	0.99545	-0.82672
H	4.31259	0.66681	-1.85720
C	2.37478	-1.49374	1.14072
H	2.00685	-2.37414	0.60922
H	1.56751	-1.12251	1.77546
H	3.22248	-1.77748	1.76340
H	3.91851	1.93139	-0.67819
H	5.52113	1.16512	-0.65387

ADMA_1 – NMA 8

C	1.11525	1.99188	0.72101
H	1.27444	2.77017	-0.02664
H	2.01743	1.87921	1.32127
N	0.74995	0.71657	0.10885
H	-0.24572	0.49891	0.06191
C	1.54631	-0.35446	0.02733
N	0.98328	-1.57134	0.05975
H	-0.02808	-1.62851	0.14463
H	1.46534	-2.35731	-0.35372

N	2.87530	-0.23950	-0.04988
C	3.73490	-1.40482	0.13938
C	3.52491	0.92723	-0.64922
H	3.33109	-2.05291	0.91593
H	3.84850	-1.96843	-0.79207
H	4.71398	-1.04899	0.45838
H	4.06666	1.50425	0.10254
H	4.23120	0.56796	-1.39999
H	2.79380	1.56031	-1.14541
H	0.29986	2.29496	1.37723
C	-2.96272	-0.52673	-0.01730
O	-1.73982	-0.69424	0.09900
N	-3.47782	0.69326	-0.19343
H	-4.47884	0.79402	-0.28289
C	-2.63911	1.87760	-0.25076
H	-2.07251	1.99224	0.67665
C	-3.91793	-1.68528	0.03142
H	-3.76809	-2.22063	0.97056
H	-3.67788	-2.36732	-0.78651
H	-4.95890	-1.37418	-0.04919
H	-1.93946	1.81755	-1.08833
H	-3.27883	2.74719	-0.38460

ADMA_2 – NMA 1

C	-5.04325	-0.59146	-0.24238
H	-5.30283	0.10970	-1.03869
H	-5.44106	-0.22990	0.70869
N	-3.60283	-0.78861	-0.16747
H	-3.24430	-1.71812	-0.32669
C	-2.73534	0.19954	0.06207
N	-3.20271	1.44590	0.21570
H	-4.19599	1.60678	0.29774
H	-2.59379	2.18979	0.52100
N	-1.42550	-0.03844	0.11152
C	-0.48556	1.06329	0.32349
C	-0.91972	-1.39522	-0.09365
H	-0.57655	1.81059	-0.46951
H	-0.65403	1.54015	1.29346
H	0.52054	0.65055	0.30747
H	-1.15973	-1.75455	-1.09841
H	0.16111	-1.37403	0.02208
H	-1.33817	-2.08134	0.64747
H	-5.49591	-1.55460	-0.46448
C	3.55166	-0.47309	0.04723
O	2.36097	-0.72142	0.23530
N	3.97811	0.77009	-0.23928
H	4.96411	0.92886	-0.38524
C	3.05787	1.88700	-0.34387
H	2.31836	1.70695	-1.12750
C	4.60834	-1.54508	0.13056
H	4.34532	-2.34912	-0.55882
H	4.60714	-1.95664	1.14182
H	5.60559	-1.17510	-0.10662
H	2.53189	2.04766	0.60038
H	3.62702	2.78132	-0.59043

ADMA_2 – NMA 2

C	-0.69508	2.33970	-0.65000
H	-0.90527	2.88872	0.27214
H	-1.34102	2.71008	-1.44969
N	-0.85590	0.90541	-0.46721
H	0.00208	0.34981	-0.50598

C	-2.01781	0.33901	-0.15285
N	-3.09673	1.11285	0.05216
H	-3.04706	2.10034	-0.15215
H	-4.02013	0.70581	0.07271
N	-2.11524	-0.98502	-0.01054
C	-3.36652	-1.58089	0.45288
C	-0.95577	-1.83252	-0.27816
H	-3.70335	-1.10520	1.37655
H	-4.14952	-1.49873	-0.30642
H	-3.18758	-2.63321	0.65479
H	-0.19305	-1.71969	0.49738
H	-1.28871	-2.86675	-0.30126
H	-0.51642	-1.58848	-1.24713
H	0.34004	2.51756	-0.93412
C	2.78787	-0.23376	-0.23594
O	1.73209	-0.10724	-0.86531
N	2.88467	0.10044	1.05830
H	3.77073	-0.01949	1.52717
C	1.76091	0.64207	1.80135
H	1.39816	1.56275	1.33807
C	4.02963	-0.76782	-0.89715
H	4.30875	-0.09221	-1.70806
H	3.80084	-1.74073	-1.33512
H	4.86531	-0.86694	-0.20515
H	0.94032	-0.07792	1.84467
H	2.09221	0.86163	2.81430

ADMA_2 – NMA 3

C	-0.47284	1.94210	-0.35044
H	0.19546	1.45816	-1.06599
H	-0.02210	1.91009	0.64403
N	-1.78266	1.30831	-0.34460
H	-2.58580	1.87777	-0.56303
C	-1.96967	0.02912	-0.00654
N	-0.90758	-0.72189	0.29249
H	0.05310	-0.38930	0.18145
H	-1.02407	-1.69950	0.51160
N	-3.20297	-0.48452	0.04314
C	-3.38743	-1.89248	0.38844
C	-4.35705	0.35716	-0.26403
H	-2.89107	-2.54262	-0.33655
H	-3.00354	-2.10337	1.38991
H	-4.45109	-2.11152	0.37460
H	-4.32213	0.71057	-1.29815
H	-5.25804	-0.23500	-0.13233
H	-4.40735	1.21432	0.41258
H	-0.60880	2.98074	-0.64260
C	2.91275	0.21573	0.19246
O	1.87377	-0.42508	0.00225
N	4.11520	-0.31779	-0.06248
H	4.94133	0.23669	0.10892
C	4.26375	-1.66686	-0.57800
H	3.85077	-2.39584	0.12209
C	2.89494	1.63045	0.70756
H	2.28194	1.67168	1.60919
H	2.43341	2.27243	-0.04638
H	3.89408	2.00454	0.92885
H	3.74888	-1.77287	-1.53476
H	5.32421	-1.86650	-0.71796

ADMA_2 – NMA 4

C	3.36780	-2.12534	-0.39464
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H	2.98663	-2.39817	-1.38118
H	2.87893	-2.73690	0.36794
N	3.17710	-0.70549	-0.13913
H	3.98738	-0.16323	0.12052
C	1.97762	-0.11253	-0.12913
N	0.88392	-0.84415	-0.35259
H	0.98024	-1.83173	-0.53959
H	-0.05618	-0.44696	-0.45770
N	1.87647	1.19838	0.10657
C	0.55548	1.82024	0.16077
C	3.07799	2.00133	0.31552
H	0.05063	1.75853	-0.80593
H	-0.06769	1.34161	0.92006
H	0.68256	2.86633	0.42497
H	3.76033	1.91023	-0.53322
H	2.78140	3.04235	0.40544
H	3.59671	1.70752	1.23249
H	4.43643	-2.32411	-0.36935
C	-2.59873	-0.41670	0.25731
O	-1.83316	-0.13568	-0.67262
N	-3.90212	-0.11668	0.20224
H	-4.49338	-0.36893	0.98077
C	-4.48853	0.55288	-0.94506
H	-4.01201	1.52100	-1.11019
C	-2.10637	-1.10831	1.50151
H	-1.34136	-0.48518	1.97028
H	-1.64023	-2.05383	1.21676
H	-2.90402	-1.29966	2.21856
H	-4.37185	-0.05122	-1.84692
H	-5.54830	0.70399	-0.75000

SDMA_1 – NMA 1

C	-0.71720	0.04472	1.76273
H	-0.98320	1.07142	2.01152
H	0.30628	0.00214	1.38941
N	-1.66285	-0.49044	0.78606
H	-1.91596	-1.46433	0.88599
C	-2.34411	0.21865	-0.11098
N	-1.93807	1.43640	-0.49186
N	-3.47477	-0.27746	-0.62078
H	-2.60386	1.96103	-1.04468
H	-3.84107	0.16546	-1.45281
C	-0.54058	1.86938	-0.56239
H	-0.42022	2.44733	-1.47815
H	0.12287	1.00507	-0.60433
H	-0.27309	2.49702	0.28906
C	-4.13858	-1.47461	-0.12737
H	-3.57427	-2.37814	-0.37279
H	-5.11446	-1.53246	-0.60369
H	-4.27728	-1.41477	0.95363
H	-0.79982	-0.56201	2.66313
C	2.88550	-0.73604	-0.28394
O	1.67652	-0.72101	-0.50831
N	3.54838	0.36954	0.10559
H	4.54122	0.31240	0.27689
C	2.87353	1.64298	0.27593
H	2.43572	1.98131	-0.66653
C	3.70428	-1.99415	-0.42900
H	3.61417	-2.35176	-1.45639
H	3.28797	-2.75866	0.22951
H	4.75692	-1.84499	-0.18961
H	2.07756	1.56271	1.01984

H	3.60239	2.37751	0.61349
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SDMA_1 – NMA 2

C	-4.04831	-0.24920	0.54747
H	-3.67761	-0.81076	1.40471
H	-4.51337	-0.93176	-0.16389
N	-2.96354	0.52004	-0.05900
H	-3.16855	1.48443	-0.28160
C	-1.67628	0.16568	-0.06781
N	-1.30725	-1.11713	0.06888
N	-0.73162	1.09682	-0.19861
H	-0.30130	-1.25835	0.11349
H	0.22326	0.75742	-0.30847
C	-2.09401	-2.25482	-0.40974
H	-1.39180	-3.02643	-0.72072
H	-2.70023	-1.97221	-1.27130
H	-2.73853	-2.66303	0.36864
C	-0.98072	2.52764	-0.13003
H	-1.52729	2.88141	-1.00831
H	-0.01715	3.03154	-0.09593
H	-1.54240	2.78184	0.77188
H	-4.79550	0.46157	0.89530
C	2.75938	-0.62373	0.03980
O	1.53343	-0.64451	-0.14365
N	3.42543	0.53167	0.12144
H	4.42315	0.51170	0.27768
C	2.74924	1.81289	0.02109
H	2.22776	1.89912	-0.93566
C	3.55215	-1.89283	0.17326
H	3.43570	-2.47335	-0.74388
H	3.13684	-2.47742	0.99607
H	4.61032	-1.70859	0.35486
H	2.02590	1.93205	0.83208
H	3.49349	2.60330	0.09109

SDMA_1 – NMA 3

C	-3.78254	-0.96749	0.55472
H	-3.22935	-1.37655	1.40005
H	-3.99481	-1.76038	-0.16216
N	-3.03853	0.13617	-0.05008
H	-3.56239	0.98150	-0.23070
C	-1.70723	0.24196	-0.08279
N	-0.91947	-0.83810	0.00786
N	-1.14185	1.44552	-0.19317
H	0.07676	-0.62153	0.03277
H	-0.13173	1.46849	-0.30306
C	-1.28540	-2.17103	-0.47068
H	-0.37298	-2.65132	-0.82086
H	-1.98377	-2.10509	-1.30586
H	-1.72113	-2.78466	0.31793
C	-1.86697	2.70068	-0.07473
H	-2.52891	2.86238	-0.92945
H	-1.13511	3.50479	-0.04791
H	-2.45295	2.72792	0.84685
H	-4.72390	-0.56178	0.92027
C	2.80183	0.40343	0.04766
O	1.58125	0.56658	-0.09482
N	3.32818	-0.82183	0.13085
H	4.32585	-0.91980	0.25487
C	2.50319	-2.01454	0.05269
H	1.79399	-2.05197	0.88404
C	3.74178	1.57235	0.13359

H	3.44218	2.19730	0.97689
H	3.64274	2.16620	-0.77687
H	4.78034	1.26748	0.25791
H	1.94748	-2.03328	-0.88771
H	3.15144	-2.88683	0.10057

SDMA_1 – NMA 4

C	1.21453	-1.41840	1.22880
H	2.10821	-1.36568	1.85073
H	1.25128	-2.32035	0.61635
N	1.08719	-0.20672	0.42459
H	0.13518	0.16781	0.33953
C	2.10742	0.51938	-0.01751
N	3.33760	-0.00706	-0.13887
N	1.92350	1.80725	-0.32132
H	4.07463	0.65801	-0.33384
H	2.64468	2.26572	-0.86164
C	3.60875	-1.39589	-0.51201
H	4.50514	-1.39984	-1.12970
H	2.78367	-1.80158	-1.09781
H	3.77948	-2.02670	0.36046
C	0.71011	2.55071	-0.01637
H	-0.11774	2.25595	-0.66460
H	0.92194	3.60701	-0.16681
H	0.42156	2.39278	1.02428
H	0.34244	-1.46930	1.87988
C	-2.36774	-0.53357	-0.17668
O	-1.67529	0.34669	0.34892
N	-3.69297	-0.39381	-0.30275
H	-4.22237	-1.13613	-0.73656
C	-4.39042	0.78505	0.17932
H	-4.25708	0.89931	1.25687
C	-1.76512	-1.81650	-0.68665
H	-1.43001	-2.40627	0.17002
H	-0.89285	-1.58351	-1.30018
H	-2.47302	-2.40724	-1.26750
H	-4.01534	1.68303	-0.31516
H	-5.44995	0.67118	-0.04063

SDMA_1 – NMA 5

C	1.07163	-1.28929	1.44922
H	1.93104	-1.15002	2.10551
H	1.15121	-2.25320	0.94505
N	0.96467	-0.17893	0.50810
H	0.01783	0.18986	0.38052
C	1.98875	0.49015	-0.00697
N	3.22455	-0.03599	-0.04399
N	1.80189	1.72657	-0.47809
H	3.95468	0.60424	-0.32785
H	2.52919	2.11536	-1.06295
C	3.51340	-1.45932	-0.22504
H	4.43642	-1.53402	-0.79702
H	2.71644	-1.94384	-0.78995
H	3.64815	-1.97272	0.72693
C	0.57649	2.49042	-0.29882
H	-0.23605	2.10066	-0.91649
H	0.78139	3.51842	-0.58957
H	0.26826	2.47281	0.74775
H	0.16603	-1.28381	2.05441
C	-2.82372	-0.01856	0.16199
O	-1.78140	0.42324	0.65726
N	-2.80769	-0.88531	-0.86025

H	-3.68452	-1.22024	-1.23249
C	-1.56689	-1.36274	-1.44338
H	-0.97527	-0.52907	-1.82916
C	-4.17197	0.39926	0.68377
H	-4.26312	1.48235	0.58310
H	-4.22400	0.15575	1.74640
H	-4.99612	-0.08378	0.15987
H	-0.97187	-1.90355	-0.70300
H	-1.80862	-2.03494	-2.26417

SDMA_2 – NMA 1

C	4.34141	0.08690	0.36344
H	4.26790	0.45080	1.39121
H	4.59583	0.91659	-0.30017
N	3.10661	-0.55755	-0.05724
H	3.13504	-1.54304	-0.27439
C	1.93129	0.07309	-0.13995
N	1.84952	1.36478	0.19002
N	0.84617	-0.57586	-0.55305
H	2.68696	1.83490	0.50053
H	-0.06643	-0.11304	-0.54832
C	0.62753	2.14558	0.06915
H	0.86110	3.16870	0.35373
H	-0.15255	1.76401	0.73161
H	0.25881	2.13839	-0.95884
C	0.85623	-1.97824	-0.93658
H	1.54811	-2.15560	-1.76364
H	-0.14868	-2.23393	-1.26638
H	1.12304	-2.62339	-0.09469
H	5.13496	-0.65463	0.31244
C	-2.90121	0.10342	-0.20944
O	-1.84455	0.18136	-0.84496
N	-2.95055	-0.42442	1.02167
H	-3.83997	-0.46559	1.49794
C	-1.77047	-0.94940	1.68523
H	-1.30872	-1.73708	1.08530
C	-4.19746	0.60001	-0.79051
H	-4.38376	0.07140	-1.72708
H	-4.09158	1.66190	-1.01996
H	-5.04266	0.45668	-0.11810
H	-1.03501	-0.15976	1.85602
H	-2.07213	-1.36501	2.64459

SDMA_2 – NMA 2

C	-4.47987	0.10877	0.22203
H	-4.71114	0.86495	-0.53236
H	-4.45239	0.57509	1.20953
N	-3.22344	-0.56374	-0.07154
H	-3.25300	-1.54714	-0.29798
C	-2.04363	0.06118	-0.13497
N	-1.97814	1.37463	0.09790
N	-0.93705	-0.62022	-0.41738
H	-2.83839	1.87489	0.26688
H	-0.03109	-0.14300	-0.47658
C	-0.73662	2.13206	0.05749
H	-0.97594	3.16932	0.27899
H	-0.27320	2.07794	-0.93009
H	-0.03036	1.76507	0.80576
C	-0.92475	-2.05760	-0.63611
H	-1.25920	-2.59842	0.25313
H	0.10127	-2.34618	-0.85596
H	-1.55098	-2.33724	-1.48732

H	-5.26595	-0.64241	0.21489
C	2.45598	-0.20291	0.33925
O	1.75746	0.11913	-0.62967
N	3.78516	-0.04672	0.32192
H	4.32038	-0.32405	1.13181
C	4.47819	0.50390	-0.82926
H	4.30479	-0.11123	-1.71423
C	1.85201	-0.79065	1.58792
H	1.35035	-1.72633	1.32988
H	1.09798	-0.10159	1.97451
H	2.59571	-0.98270	2.36063
H	4.13398	1.51876	-1.03760
H	5.54390	0.52510	-0.61091

SDMA_2 – NMA 3

C	-0.92416	-2.05743	-0.63588
H	-1.55020	-2.33723	-1.48717
H	-1.25858	-2.59835	0.25331
N	-0.93690	-0.62005	-0.41713
H	-0.03109	-0.14255	-0.47625
C	-2.04371	0.06105	-0.13490
N	-3.22336	-0.56418	-0.07163
N	-1.97861	1.37453	0.09792
H	-3.25262	-1.54759	-0.29807
H	-2.83901	1.87454	0.26688
C	-4.48003	0.10802	0.22164
H	-5.26592	-0.64337	0.21436
H	-4.71133	0.86410	-0.53285
H	-4.45289	0.57441	1.20911
C	-0.73729	2.13229	0.05770
H	-0.03103	1.76547	0.80608
H	-0.97691	3.16948	0.27920
H	-0.27370	2.07831	-0.92980
H	0.10197	-2.34570	-0.85559
C	2.45608	-0.20245	0.33936
O	1.75742	0.12004	-0.62933
N	3.78534	-0.04700	0.32160
H	4.32067	-0.32473	1.13129
C	4.47831	0.50323	-0.82981
H	4.30433	-0.11183	-1.71471
C	1.85219	-0.79001	1.58815
H	1.35089	-1.72595	1.33032
H	1.09787	-0.10112	1.97446
H	2.59588	-0.98159	2.36099
H	4.13458	1.51826	-1.03806
H	5.54410	0.52388	-0.61177

ADMA_1 – PHE 1

C	-0.92416	-2.05743	-0.63588
H	-1.55020	-2.33723	-1.48717
H	-1.25858	-2.59835	0.25331
N	-0.93690	-0.62005	-0.41713
H	-0.03109	-0.14255	-0.47625
C	-2.04371	0.06105	-0.13490
N	-3.22336	-0.56418	-0.07163
N	-1.97861	1.37453	0.09792
H	-3.25262	-1.54759	-0.29807
H	-2.83901	1.87454	0.26688
C	-4.48003	0.10802	0.22164
H	-5.26592	-0.64337	0.21436
H	-4.71133	0.86410	-0.53285
H	-4.45289	0.57441	1.20911

C	-0.73729	2.13229	0.05770
H	-0.03103	1.76547	0.80608
H	-0.97691	3.16948	0.27920
H	-0.27370	2.07831	-0.92980
H	0.10197	-2.34570	-0.85559
C	2.45608	-0.20245	0.33936
O	1.75742	0.12004	-0.62933
N	3.78534	-0.04700	0.32160
H	4.32067	-0.32473	1.13129
C	4.47831	0.50323	-0.82981
H	4.30433	-0.11183	-1.71471
C	1.85219	-0.79001	1.58815
H	1.35089	-1.72595	1.33032
H	1.09787	-0.10112	1.97446
H	2.59588	-0.98159	2.36099
H	4.13458	1.51826	-1.03806
H	5.54410	0.52388	-0.61177

ADMA_1 – PHE 2

C	4.21049	-0.57062	0.32833
C	2.96539	-1.18019	0.51346
C	1.78885	-0.56586	0.10398
C	1.84908	0.68490	-0.50269
C	3.06977	1.31618	-0.69836
C	4.23899	0.68346	-0.28081
H	2.91453	-2.15495	0.98841
H	0.82796	-1.04731	0.25150
H	3.10918	2.29378	-1.16902
H	5.19034	1.18259	-0.43340
O	0.66007	1.26090	-0.88945
H	0.82603	2.12123	-1.29897
C	5.47623	-1.26186	0.76326
H	6.32395	-0.57524	0.74496
H	5.70860	-2.10157	0.10195
H	5.37741	-1.66051	1.77551
C	-2.77128	1.83631	1.35257
H	-2.86380	1.57399	2.40686
H	-3.76431	1.94365	0.91741
N	-2.00091	0.83974	0.60931
H	-1.02045	1.05070	0.46299
C	-2.51990	-0.05844	-0.23825
N	-1.78873	-0.43675	-1.29337
H	-0.91875	0.04225	-1.49175
H	-1.96802	-1.31416	-1.76055
N	-3.74315	-0.55736	-0.05885
C	-4.41257	-1.30008	-1.12384
C	-4.35111	-0.67355	1.26791
H	-4.17421	-0.86924	-2.09526
H	-4.12869	-2.35657	-1.10603
H	-5.48675	-1.21937	-0.96116
H	-5.18108	0.02598	1.38223
H	-4.72592	-1.69289	1.37412
H	-3.60962	-0.50013	2.04369
H	-2.25100	2.78961	1.26911

ADMA_1 – PHE 3

C	4.07944	0.65873	-0.24827
C	2.96060	0.65714	-1.08724
C	1.92818	-0.25734	-0.91926
C	2.00497	-1.19396	0.10845
C	3.10700	-1.21814	0.95456
C	4.13344	-0.29489	0.76803

H	2.89719	1.38281	-1.89223
H	1.06887	-0.25754	-1.58097
H	3.16584	-1.95477	1.75004
H	4.99283	-0.32218	1.43016
O	0.95800	-2.07034	0.24624
H	1.14654	-2.69775	0.95686
C	5.17892	1.67188	-0.43272
H	6.07378	1.38414	0.12135
H	4.86501	2.65704	-0.07536
H	5.44457	1.77575	-1.48712
C	-4.43070	-1.55347	-0.46691
H	-5.00621	-1.32599	-1.36482
H	-4.90122	-1.08077	0.39416
N	-3.04296	-1.10928	-0.58451
H	-2.37904	-1.79652	-0.91767
C	-2.51493	-0.04672	0.03691
N	-1.22815	-0.08514	0.39650
H	-0.69757	-0.94658	0.31574
H	-0.71308	0.76843	0.56178
N	-3.25564	1.02601	0.32041
C	-2.77065	2.04638	1.24599
C	-4.41379	1.41179	-0.48733
H	-2.20561	1.58735	2.05583
H	-2.14759	2.78288	0.72950
H	-3.63797	2.55165	1.66944
H	-5.34199	1.28529	0.07286
H	-4.29880	2.46364	-0.75493
H	-4.45145	0.82783	-1.40325
H	-4.42555	-2.63149	-0.31138

ADMA_1 – PHE 4

C	3.58127	-0.76569	-0.35654
C	2.79933	-0.17694	-1.35535
C	1.88940	0.83205	-1.06448
C	1.74692	1.26385	0.25105
C	2.50935	0.69676	1.26463
C	3.41976	-0.31044	0.95222
H	2.90415	-0.51401	-2.38193
H	1.28423	1.28279	-1.84357
H	2.38852	1.03322	2.29007
H	4.01119	-0.75133	1.74818
O	0.80474	2.23098	0.49949
H	0.84766	2.50042	1.42699
C	4.58246	-1.83951	-0.69371
H	4.87644	-2.39505	0.19834
H	5.48660	-1.40508	-1.13047
H	4.17340	-2.54411	-1.42098
C	-4.58268	-0.97402	-1.07658
H	-5.44297	-0.95049	-0.40686
H	-3.98225	-1.85758	-0.86389
N	-3.76229	0.22912	-0.94952
H	-3.96315	0.98142	-1.59614
C	-2.57294	0.29307	-0.33881
N	-1.65491	1.15228	-0.79558
H	-1.83063	1.64480	-1.66072
H	-0.91471	1.50601	-0.19726
N	-2.28352	-0.50302	0.69192
C	-0.90519	-0.66552	1.14672
C	-3.31219	-1.00987	1.60140
H	-0.22147	-0.64552	0.29866
H	-0.63338	0.11190	1.86814
H	-0.82633	-1.63740	1.63330

H	-3.45015	-2.08564	1.47820
H	-2.98348	-0.80718	2.62205
H	-4.25482	-0.49383	1.43662
H	-4.93466	-1.03226	-2.10566

ADMA_1 – PHE 5

C	3.58136	-0.76580	-0.35620
C	2.79928	-0.17765	-1.35525
C	1.88928	0.83141	-1.06484
C	1.74687	1.26388	0.25048
C	2.50946	0.69741	1.26428
C	3.41994	-0.30986	0.95232
H	2.90405	-0.51524	-2.38167
H	1.28403	1.28170	-1.84413
H	2.38870	1.03443	2.28954
H	4.01150	-0.75025	1.74847
O	0.80462	2.23105	0.49851
H	0.84766	2.50100	1.42585
C	4.58257	-1.83975	-0.69290
H	4.87732	-2.39424	0.19955
H	5.48629	-1.40559	-1.13080
H	4.17314	-2.54527	-1.41908
C	-4.58251	-0.97457	-1.07625
H	-5.44279	-0.95085	-0.40652
H	-3.98199	-1.85798	-0.86321
N	-3.76224	0.22870	-0.94968
H	-3.96302	0.98062	-1.59677
C	-2.57292	0.29296	-0.33894
N	-1.65484	1.15192	-0.79605
H	-1.83049	1.64411	-1.66140
H	-0.91475	1.50599	-0.19777
N	-2.28361	-0.50264	0.69221
C	-0.90533	-0.66498	1.14721
C	-3.31241	-1.00908	1.60177
H	-0.22155	-0.64538	0.29919
H	-0.63354	0.11277	1.86830
H	-0.82654	-1.63665	1.63422
H	-3.45041	-2.08489	1.47897
H	-2.98380	-0.80603	2.62238
H	-4.25498	-0.49306	1.43666
H	-4.93451	-1.03325	-2.10530

ADMA_1 – PHE 6

C	2.96355	0.80045	-0.04268
C	2.89766	-0.27073	0.85562
C	2.11043	-1.38606	0.60026
C	1.36649	-1.44935	-0.57787
C	1.41851	-0.39918	-1.48865
C	2.21377	0.71230	-1.21470
H	3.47486	-0.23097	1.77451
H	2.06688	-2.21253	1.30094
H	0.84181	-0.45016	-2.40757
H	2.24677	1.52609	-1.93229
O	0.60963	-2.56499	-0.78904
H	0.14805	-2.48643	-1.63351
C	3.80534	2.01168	0.26446
H	3.89352	2.65873	-0.60970
H	4.81035	1.72262	0.58096
H	3.36385	2.59901	1.07499
C	-3.15557	2.30905	-0.30362
H	-2.36502	2.94999	-0.69454
H	-3.13080	2.32487	0.78518

N	-3.03523	0.93460	-0.79085
H	-3.61774	0.69104	-1.58176
C	-2.58821	-0.10384	-0.06995
N	-3.12613	-1.31061	-0.28427
H	-3.93860	-1.39919	-0.87840
H	-2.65103	-2.15644	-0.00453
N	-1.64136	0.04557	0.85410
C	-1.36793	-1.01623	1.82162
C	-0.68258	1.15306	0.82686
H	-2.29821	-1.48485	2.14166
H	-0.69875	-1.77227	1.40010
H	-0.89079	-0.56227	2.68882
H	-0.92758	1.90222	1.58236
H	0.30532	0.74148	1.03637
H	-0.66022	1.61118	-0.15906
H	-4.11951	2.69402	-0.63315

ADMA_1 – PHE 7

C	2.17404	1.40436	0.02995
C	1.86994	0.88369	-1.23202
C	1.88725	-0.48481	-1.47852
C	2.21136	-1.36784	-0.45078
C	2.51908	-0.87401	0.81621
C	2.49796	0.49980	1.04533
H	1.60719	1.56179	-2.03787
H	1.64240	-0.87854	-2.45850
H	2.76726	-1.56052	1.62020
H	2.73165	0.87216	2.03789
O	2.20479	-2.69906	-0.73404
H	2.43572	-3.20030	0.05824
C	2.16390	2.88889	0.28742
H	1.73268	3.11632	1.26496
H	1.58906	3.41533	-0.47686
H	3.18048	3.29326	0.27592
C	-0.95744	-1.40660	1.56542
H	-0.75389	-2.35359	1.06318
H	-1.96436	-1.42571	1.98054
N	-0.80927	-0.27129	0.65907
H	0.10632	0.16307	0.63342
C	-1.80197	0.45854	0.14264
N	-1.57195	1.75066	-0.12886
H	-0.69320	2.16905	0.14655
H	-2.12341	2.23496	-0.82315
N	-3.00987	-0.05954	-0.08446
C	-4.15119	0.80609	-0.37015
C	-3.20742	-1.47864	-0.38475
H	-4.09236	1.71987	0.21924
H	-4.20116	1.05370	-1.43490
H	-5.05593	0.26788	-0.08963
H	-3.73718	-1.98067	0.42674
H	-3.80398	-1.54588	-1.29617
H	-2.25348	-1.96822	-0.56428
H	-0.24747	-1.27722	2.38222

ADMA_1 – PHE 8

C	2.19944	1.38441	0.05482
C	1.89244	0.89045	-1.21721
C	1.88851	-0.47383	-1.48720
C	2.19389	-1.37935	-0.47341
C	2.50411	-0.91226	0.80306
C	2.50496	0.45739	1.05561
H	1.64405	1.58634	-2.01240

H	1.64159	-0.84654	-2.47482
H	2.73655	-1.61649	1.59630
H	2.74095	0.80891	2.05520
O	2.16665	-2.70534	-0.77904
H	2.38740	-3.22349	0.00521
C	2.21297	2.86412	0.33809
H	3.23643	3.25078	0.34295
H	1.77601	3.08213	1.31519
H	1.65446	3.41344	-0.42222
C	-0.95042	-1.33506	1.60789
H	-0.74880	-2.30381	1.14793
H	-1.95419	-1.33639	2.03087
N	-0.81015	-0.24316	0.64823
H	0.10502	0.18963	0.59598
C	-1.80569	0.46928	0.11331
N	-1.57141	1.74784	-0.21545
H	-0.67895	2.16366	0.01697
H	-2.12413	2.20194	-0.92892
N	-3.01900	-0.05093	-0.07618
C	-4.15822	0.80688	-0.39290
C	-3.22807	-1.48181	-0.30424
H	-4.08896	1.74733	0.15161
H	-4.21657	1.00335	-1.46778
H	-5.06302	0.28789	-0.07848
H	-3.75004	-1.94038	0.53747
H	-3.83659	-1.59032	-1.20370
H	-2.27916	-1.98538	-0.47099
H	-0.23488	-1.16742	2.41279

ADMA_2 – PHE 1

C	3.39575	0.47612	-0.63268
C	2.45370	-0.33513	-1.27192
C	1.49145	-1.03575	-0.55401
C	1.46252	-0.92788	0.83333
C	2.38930	-0.13215	1.49630
C	3.34808	0.55874	0.75938
H	2.47479	-0.42558	-2.35342
H	0.76927	-1.66854	-1.05869
H	2.36305	-0.05314	2.57895
H	4.07224	1.17438	1.28323
O	0.47759	-1.61274	1.50186
H	0.60450	-1.52178	2.45592
C	4.41420	1.25163	-1.42677
H	5.28155	1.50241	-0.81376
H	3.98714	2.18794	-1.79845
H	4.75549	0.68003	-2.29219
C	-2.13514	-2.32027	-0.70923
H	-1.96546	-2.22634	-1.78453
H	-3.13653	-2.71950	-0.52982
N	-1.94596	-1.04351	-0.03678
H	-1.23848	-1.00759	0.68747
C	-2.67997	0.03924	-0.29574
N	-3.66516	-0.04321	-1.19791
H	-3.82101	-0.90004	-1.70798
H	-4.16875	0.77496	-1.50452
N	-2.44494	1.18859	0.34129
C	-3.25799	2.36616	0.04327
C	-1.30742	1.29100	1.25287
H	-3.08945	2.71504	-0.97928
H	-4.31893	2.14855	0.18472
H	-2.97798	3.15802	0.73199
H	-0.36643	1.10965	0.72540

H	-1.28571	2.29747	1.66080
H	-1.40854	0.58951	2.08546
H	-1.40591	-3.01677	-0.30120

ADMA_2 – PHE 2

C	3.62030	-0.53311	-0.46999
C	2.43303	-0.54463	-1.20929
C	1.37768	0.30435	-0.90085
C	1.49924	1.18366	0.17200
C	2.66642	1.21657	0.92490
C	3.71575	0.36059	0.59636
H	2.33229	-1.23070	-2.04462
H	0.46114	0.29123	-1.48061
H	2.75496	1.90028	1.76387
H	4.62432	0.39020	1.18901
O	0.41753	1.97888	0.46304
H	0.64040	2.58716	1.18057
C	4.76163	-1.44665	-0.83379
H	5.50717	-1.47812	-0.03763
H	5.25850	-1.10502	-1.74648
H	4.40786	-2.46408	-1.01543
C	-0.62536	-1.14618	1.63918
H	-0.28680	-2.01277	1.06524
H	-1.17830	-1.48167	2.51994
N	-1.43581	-0.25747	0.81851
H	-1.09126	0.68413	0.67013
C	-2.60599	-0.61244	0.28787
N	-3.09949	-1.83169	0.54714
H	-2.52481	-2.51462	1.01925
H	-3.88895	-2.18648	0.02787
N	-3.29517	0.24124	-0.47074
C	-4.61464	-0.13469	-0.97463
C	-2.74318	1.56207	-0.76285
H	-4.54190	-0.94539	-1.70455
H	-5.27028	-0.43811	-0.15531
H	-5.05293	0.73172	-1.46140
H	-1.75536	1.47732	-1.22152
H	-3.40500	2.05922	-1.46625
H	-2.67486	2.17076	0.14343
H	0.24748	-0.58795	1.97222

ADMA_2 – PHE 3

C	-4.61796	-0.34638	0.27785
C	-3.43074	-1.03312	0.55382
C	-2.19406	-0.52911	0.17329
C	-2.12339	0.69042	-0.49853
C	-3.28952	1.39333	-0.78335
C	-4.52182	0.87167	-0.39353
H	-3.47528	-1.98254	1.07876
H	-1.28018	-1.06954	0.39313
H	-3.23621	2.34441	-1.30477
H	-5.42465	1.43011	-0.61950
O	-0.88684	1.14912	-0.85001
H	-0.97452	1.99200	-1.31259
C	-5.95107	-0.91935	0.68367
H	-6.74563	-0.17987	0.57131
H	-5.93590	-1.25191	1.72428
H	-6.20847	-1.78581	0.06757
C	3.74898	2.33858	0.85640
H	4.21910	2.75407	-0.03760
H	4.49750	2.22272	1.64394
N	3.09545	1.07196	0.55909

H	2.11673	0.98419	0.78881
C	3.74255	-0.00510	0.10846
N	5.06738	0.06113	-0.07565
H	5.55614	0.93664	0.04116
H	5.56040	-0.67736	-0.55521
N	3.08645	-1.13925	-0.13903
C	3.82134	-2.32439	-0.57796
C	1.63473	-1.19217	0.02885
H	4.25035	-2.17626	-1.57283
H	4.61586	-2.57185	0.12954
H	3.12749	-3.15916	-0.62021
H	1.14319	-0.42577	-0.57470
H	1.28964	-2.16702	-0.30382
H	1.35621	-1.06367	1.07899
H	2.98381	3.03045	1.19918

ADMA_2 – PHE 4

C	1.79087600	1.74044900	-0.30432200
C	1.29726200	0.83064400	-1.24552700
C	1.60791500	-0.52180800	-1.18278000
C	2.43389700	-0.99272700	-0.16329700
C	2.94822000	-0.10562100	0.77752000
C	2.62361000	1.24728500	0.70021800
H	0.65357900	1.18799700	-2.04414000
H	1.21939300	-1.21942700	-1.91638900
H	3.59647100	-0.47054400	1.56868200
H	3.02940800	1.92941700	1.44061500
O	2.70613300	-2.32896900	-0.13605100
H	3.28061600	-2.52631100	0.61437200
C	1.41257300	3.19749400	-0.36969200
H	2.05091300	3.79833200	0.28018300
H	0.37578100	3.34613400	-0.05222100
H	1.49991900	3.58174500	-1.38858500
C	-3.72106000	1.69352700	0.20187600
H	-4.68033000	1.20067300	0.37382800
H	-3.64716300	1.99819600	-0.84504300
N	-2.61806000	0.82187300	0.58088000
H	-1.91394500	1.20069500	1.19722000
C	-2.40122900	-0.37741600	0.03488300
N	-3.22278100	-0.81538400	-0.92878300
H	-4.03348900	-0.27175600	-1.18774500
H	-3.22006300	-1.78619400	-1.20565400
N	-1.36548500	-1.12019200	0.42497600
C	-1.08063800	-2.39048500	-0.23948800
C	-0.54189400	-0.68873800	1.55303200
H	-1.80395300	-3.15882600	0.04906700
H	-1.09242200	-2.26662100	-1.32397300
H	-0.08455400	-2.71320600	0.05346700
H	-1.16673300	-0.46233800	2.42037800
H	0.12679400	-1.50346900	1.81840900
H	0.05837700	0.18814600	1.29190000
H	-3.67057800	2.57942100	0.82999800

ADMA_2 – PHE 5

C	3.98093	0.50110	0.51537
C	2.75212	0.43348	1.17820
C	1.66336	-0.22944	0.62413
C	1.79500	-0.83830	-0.62027
C	3.00278	-0.78040	-1.30598
C	4.08279	-0.11369	-0.73302
H	2.64299	0.90768	2.14850
H	0.71481	-0.27964	1.14752

H	3.09999	-1.25060	-2.27985
H	5.02249	-0.07172	-1.27435
O	0.69432	-1.47989	-1.13203
H	0.91290	-1.88440	-1.98219
C	5.16238	1.19263	1.14410
H	5.88243	1.50652	0.38647
H	5.67993	0.52351	1.83815
H	4.84821	2.07262	1.70894
C	-3.19682	2.62556	-0.84893
H	-3.08736	2.45206	-1.92189
H	-2.27883	3.06656	-0.45346
N	-3.52988	1.39276	-0.15070
H	-4.40040	1.35977	0.35809
C	-2.74357	0.31351	-0.14348
N	-1.57905	0.35315	-0.79785
H	-1.30784	1.18335	-1.30439
H	-0.96104	-0.44800	-0.85983
N	-3.11000	-0.78961	0.51273
C	-2.21947	-1.94831	0.53457
C	-4.38589	-0.82500	1.22409
H	-2.04720	-2.32994	-0.47499
H	-1.26255	-1.69901	1.00027
H	-2.69432	-2.73027	1.12015
H	-5.21962	-0.65059	0.53928
H	-4.50365	-1.81142	1.66279
H	-4.40995	-0.08243	2.02639
H	-4.01466	3.32412	-0.69126

ADMA_2 – PHE 6

C	-4.10976	0.47503	0.48733
C	-2.85436	0.63197	1.08153
C	-1.71630	0.04335	0.54247
C	-1.82661	-0.72196	-0.61445
C	-3.06258	-0.89773	-1.22556
C	-4.19118	-0.30088	-0.66959
H	-2.76379	1.22411	1.98674
H	-0.74762	0.16710	1.01446
H	-3.14325	-1.50018	-2.12534
H	-5.15373	-0.44607	-1.14946
O	-0.68103	-1.28987	-1.11327
H	-0.88408	-1.80371	-1.90625
C	-5.32927	1.14344	1.06653
H	-6.24098	0.62299	0.76818
H	-5.40801	2.17801	0.71905
H	-5.28470	1.16551	2.15723
C	3.50788	2.55481	-0.80507
H	2.62619	3.07121	-0.41811
H	3.39989	2.40283	-1.88139
N	3.71881	1.28886	-0.11817
H	4.56687	1.18094	0.41734
C	2.83924	0.28403	-0.12885
N	1.69049	0.42742	-0.79681
H	1.49037	1.28403	-1.29214
H	1.00231	-0.31443	-0.86135
N	3.10088	-0.85213	0.52156
C	2.11186	-1.92801	0.51993
C	4.36214	-1.00534	1.24321
H	1.17046	-1.59545	0.96390
H	1.92800	-2.28860	-0.49540
H	2.50067	-2.75110	1.11273
H	4.43570	-0.28651	2.06396
H	4.39958	-2.00854	1.65742

H	5.21426	-0.87982	0.57030
H	4.38243	3.17535	-0.62664

ADMA_2 – PHE 7

C	4.10551	0.37805	-0.59411
C	2.82048	0.60167	-1.09744
C	1.68662	0.21384	-0.39368
C	1.83040	-0.41461	0.83990
C	3.09544	-0.65406	1.36298
C	4.21948	-0.25731	0.64259
H	2.70247	1.08893	-2.06024
H	0.69422	0.39157	-0.79404
H	3.20217	-1.15050	2.32276
H	5.20434	-0.44954	1.05597
O	0.68768	-0.78505	1.50253
H	0.91077	-1.20363	2.34443
C	5.32345	0.83127	-1.35603
H	6.21483	0.29627	-1.02423
H	5.50084	1.90049	-1.20620
H	5.20011	0.66625	-2.42841
C	-3.89294	2.41219	0.53570
H	-3.03729	3.01329	0.21965
H	-3.89754	2.32610	1.62490
N	-3.87080	1.10027	-0.09449
H	-4.66064	0.83781	-0.66402
C	-2.88213	0.21814	0.07303
N	-1.84507	0.54628	0.84928
H	-1.81307	1.45058	1.29664
H	-1.06289	-0.07775	1.01207
N	-2.92488	-0.97379	-0.52674
C	-1.84139	-1.93049	-0.30862
C	-4.06560	-1.32848	-1.36888
H	-0.88709	-1.52951	-0.65929
H	-1.75903	-2.19454	0.74872
H	-2.06444	-2.83169	-0.87245
H	-4.17090	-0.62771	-2.20099
H	-3.89172	-2.31987	-1.77702
H	-4.99205	-1.34984	-0.78881
H	-4.80516	2.91298	0.22130

ADMA_2 – PHE 8

C	4.10362	0.38646	-0.59000
C	2.81808	0.62445	-1.08457
C	1.68485	0.22552	-0.38543
C	1.83021	-0.42849	0.83429
C	3.09633	-0.68342	1.34815
C	4.21925	-0.27561	0.63280
H	2.69866	1.13184	-2.03669
H	0.69190	0.41454	-0.77910
H	3.20426	-1.20054	2.29682
H	5.20479	-0.48011	1.03871
O	0.68849	-0.80823	1.49334
H	0.91307	-1.24629	2.32485
C	5.32175	0.85340	-1.34325
H	6.20195	0.27117	-1.06523
H	5.53523	1.90408	-1.12462
H	5.17481	0.76542	-2.42156
C	-3.88557	2.40896	0.56428
H	-3.03049	3.01287	0.25206
H	-3.88603	2.30978	1.65238
N	-3.86749	1.10454	-0.08147
H	-4.65857	0.85081	-0.65324

C	-2.88006	0.21881	0.07378
N	-1.83883	0.53801	0.84818
H	-1.80586	1.43558	1.30882
H	-1.06339	-0.09412	1.01183
N	-2.92711	-0.96700	-0.53766
C	-1.84269	-1.92638	-0.33630
C	-4.07484	-1.31377	-1.37346
H	-0.88906	-1.51794	-0.68009
H	-1.75970	-2.20927	0.71616
H	-2.06459	-2.81746	-0.91647
H	-4.18580	-0.60657	-2.19920
H	-3.90545	-2.30218	-1.79064
H	-4.99671	-1.33858	-0.78629
H	-4.79849	2.91446	0.25959

SDMA_1 – PHE 1

C	-0.68783	-0.57314	0.80565
H	-0.67700	0.41034	1.27523
H	0.07213	-0.61705	0.02499
N	-2.02384	-0.86280	0.28478
H	-2.36961	-1.80256	0.42430
C	-2.90270	0.04147	-0.14480
N	-2.50943	1.25444	-0.55323
N	-4.20497	-0.25303	-0.14703
H	-3.25380	1.92104	-0.71270
H	-4.81742	0.35658	-0.67256
C	-1.21861	1.55992	-1.17206
H	-1.39166	2.32207	-1.93022
H	-0.81131	0.67469	-1.66016
H	-0.50040	1.94117	-0.44567
C	-4.77998	-1.43932	0.47016
H	-4.53491	-2.34386	-0.09239
H	-5.86034	-1.31582	0.48016
H	-4.42847	-1.54271	1.49832
H	-0.46345	-1.32193	1.56353
C	2.94317	-1.21153	-0.32526
C	2.61983	-0.23710	-1.27603
C	2.22075	1.03956	-0.90148
C	2.13293	1.36535	0.45120
C	2.45094	0.41454	1.41602
C	2.85047	-0.86087	1.02138
H	2.68016	-0.48313	-2.33195
H	1.96527	1.78660	-1.64509
H	2.37730	0.66490	2.47042
H	3.09037	-1.59636	1.78278
O	1.71436	2.62366	0.77191
H	1.69655	2.72367	1.73209
C	3.39362	-2.58495	-0.75075
H	3.36028	-3.28462	0.08606
H	4.42095	-2.56048	-1.12610
H	2.76253	-2.97568	-1.55228

SDMA_1 – PHE 2

C	2.07017	1.55462	-0.33913
C	1.40183	0.80942	-1.31482
C	1.47876	-0.57860	-1.34985
C	2.24173	-1.25110	-0.39715
C	2.92306	-0.52992	0.58090
C	2.83278	0.85954	0.60057
H	0.80542	1.32372	-2.06240
H	0.95437	-1.14874	-2.10902
H	3.52171	-1.05135	1.32207

H	3.36714	1.41080	1.36810
O	2.28664	-2.61215	-0.46890
H	2.84188	-2.95556	0.24244
C	1.93548	3.05376	-0.27834
H	1.07166	3.34066	0.33014
H	1.79223	3.47717	-1.27436
H	2.82022	3.51110	0.16847
C	-1.36000	-2.17190	0.37719
H	-1.67716	-2.23903	1.41729
H	-0.27089	-2.14117	0.32687
N	-1.97116	-1.00404	-0.25447
H	-2.29606	-1.12250	-1.20492
C	-2.36416	0.09960	0.38220
N	-1.81365	0.45193	1.55103
N	-3.33197	0.85672	-0.13799
H	-2.27651	1.20519	2.04427
H	-3.44028	1.78918	0.23806
C	-0.42854	0.17231	1.93272
H	-0.03108	1.06126	2.42120
H	0.16782	-0.02977	1.04441
H	-0.36106	-0.67299	2.61902
C	-4.16308	0.46916	-1.26828
H	-3.60151	0.49167	-2.20579
H	-4.98445	1.17883	-1.33466
H	-4.57292	-0.53047	-1.11320
H	-1.71454	-3.05441	-0.15276

SDMA_1 – PHE 3

C	3.42527	0.57086	-0.71845
C	2.28740	1.28518	-0.32793
C	1.42095	0.79545	0.64040
C	1.68345	-0.43647	1.23539
C	2.80895	-1.16469	0.87068
C	3.66955	-0.65439	-0.09975
H	2.07711	2.24520	-0.78942
H	0.54503	1.35875	0.94399
H	3.01123	-2.12409	1.33696
H	4.54713	-1.22843	-0.37899
O	0.77875	-0.89556	2.15754
H	1.08381	-1.73340	2.53018
C	4.34961	1.11766	-1.77492
H	5.20146	0.45400	-1.93049
H	4.73063	2.10204	-1.49104
H	3.82869	1.23215	-2.72934
C	-3.73570	-1.32784	-1.59012
H	-3.69105	-2.11193	-0.83496
H	-3.11101	-1.60139	-2.44057
N	-3.33716	-0.04846	-1.00657
H	-3.89320	0.75699	-1.26144
C	-2.48184	0.10612	0.00375
N	-1.55666	-0.82135	0.27975
N	-2.55496	1.19555	0.77178
H	-1.05411	-0.70122	1.15315
H	-1.76566	1.39038	1.37324
C	-0.92358	-1.69003	-0.71156
H	0.10200	-1.86597	-0.38826
H	-0.89677	-1.20069	-1.68546
H	-1.43166	-2.65054	-0.79834
C	-3.64502	2.15854	0.72923
H	-3.61204	2.76376	-0.18043
H	-3.54111	2.81548	1.58955
H	-4.60714	1.64684	0.79146

H	-4.76620	-1.22591	-1.92512
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SDMA_1 – PHE 4

C	4.06072	-0.34103	-0.49505
C	2.82224	-0.81215	-0.94267
C	1.62917	-0.29587	-0.45282
C	1.66602	0.71140	0.50653
C	2.88014	1.19728	0.97284
C	4.06578	0.66731	0.46807
H	2.78948	-1.59889	-1.68995
H	0.67361	-0.66668	-0.80790
H	2.90250	1.98110	1.72397
H	5.01196	1.04956	0.83695
O	0.46232	1.19328	0.96929
H	0.61429	1.89549	1.61682
C	5.34364	-0.89820	-1.05423
H	6.20001	-0.58637	-0.45435
H	5.50544	-0.54998	-2.07846
H	5.31956	-1.99001	-1.07986
C	-4.67684	-0.45198	-1.24658
H	-4.05384	-0.19745	-2.10347
H	-5.38764	0.35254	-1.05767
N	-3.83803	-0.72689	-0.08171
H	-4.08994	-1.53130	0.47702
C	-2.64912	-0.17685	0.16176
N	-2.30374	0.99964	-0.38030
N	-1.76698	-0.81438	0.93392
H	-1.33688	1.26800	-0.23871
H	-0.97631	-0.27606	1.26841
C	-3.23957	2.08369	-0.68189
H	-2.71670	3.02265	-0.50860
H	-4.10204	2.04017	-0.01662
H	-3.57645	2.05480	-1.71799
C	-1.91518	-2.18923	1.38674
H	-2.68753	-2.27936	2.15498
H	-0.96297	-2.50313	1.80866
H	-2.16000	-2.84245	0.54718
H	-5.22589	-1.36369	-1.47427

SDMA_1 – PHE 5

C	3.58486	-0.78714	-0.35709
C	2.42991	-0.72938	-1.14482
C	1.51646	0.30901	-1.01882
C	1.74922	1.31498	-0.08374
C	2.88999	1.28585	0.70865
C	3.79723	0.23759	0.56412
H	2.24274	-1.51252	-1.87315
H	0.62436	0.34764	-1.63447
H	3.06697	2.07071	1.43805
H	4.68553	0.22060	1.18725
O	0.79846	2.29662	0.03878
H	1.10142	2.96930	0.66322
C	4.56455	-1.92069	-0.51500
H	5.36967	-1.84700	0.21766
H	5.01162	-1.91468	-1.51284
H	4.07035	-2.88683	-0.38485
C	-4.13947	-1.85775	-0.02598
H	-3.90774	-1.84714	-1.09059
H	-5.15163	-1.48556	0.13281
N	-3.14707	-1.07792	0.71106
H	-2.79804	-1.48602	1.56780
C	-2.47748	-0.02936	0.23255

N	-2.96227	0.68927	-0.79059
N	-1.29876	0.29885	0.76174
H	-2.32610	1.37336	-1.17898
H	-0.89250	1.19165	0.50021
C	-4.38545	0.91732	-1.04665
H	-4.48715	1.92498	-1.44537
H	-4.95465	0.85335	-0.11929
H	-4.78745	0.20914	-1.77116
C	-0.56682	-0.54089	1.69888
H	-1.08356	-0.61173	2.65940
H	0.40757	-0.08547	1.86602
H	-0.42138	-1.54211	1.28713
H	-4.07898	-2.88336	0.33291

SDMA_2 – PHE 1

C	-3.51886	-0.77159	-0.34614
C	-2.37479	-0.71128	-1.14805
C	-1.41211	0.27418	-0.96690
C	-1.58449	1.22074	0.03939
C	-2.71088	1.18461	0.85241
C	-3.66733	0.19176	0.65179
H	-2.23454	-1.44854	-1.93250
H	-0.52944	0.31620	-1.59584
H	-2.83815	1.92115	1.64022
H	-4.54400	0.16848	1.29104
O	-0.59164	2.15438	0.20716
H	-0.85492	2.79580	0.88074
C	-4.56464	-1.83147	-0.57533
H	-5.23726	-1.90990	0.28034
H	-4.10467	-2.80762	-0.74419
H	-5.16915	-1.59707	-1.45650
C	2.73770	1.96791	-0.96956
H	2.64451	2.56712	-0.06075
H	1.77163	1.91975	-1.47802
N	3.24104	0.63706	-0.66347
H	4.06571	0.31683	-1.15053
C	2.61974	-0.21240	0.15715
N	1.48789	0.15690	0.75749
N	3.13859	-1.41985	0.38551
H	1.06387	1.03973	0.49473
H	2.62542	-2.05514	0.97956
C	0.73306	-0.70962	1.65040
H	-0.10143	-0.13428	2.04647
H	1.35252	-1.03681	2.48850
H	0.33976	-1.58249	1.12161
C	4.38451	-1.88803	-0.20386
H	4.31408	-1.92991	-1.29359
H	4.57113	-2.89066	0.17248
H	5.21839	-1.24377	0.08386
H	3.45568	2.44859	-1.62941

SDMA_2 – PHE 2

C	3.41611	-0.83621	-0.39391
C	2.37713	-0.52536	-1.27622
C	1.46740	0.48803	-0.99874
C	1.58727	1.20715	0.18707
C	2.60945	0.91954	1.08371
C	3.51413	-0.09654	0.78515
H	2.27737	-1.08593	-2.20040
H	0.66551	0.72308	-1.69037
H	2.69499	1.47877	2.01061
H	4.30901	-0.31753	1.49021

O	0.64406	2.17297	0.43893
H	0.86823	2.64625	1.25151
C	4.41184	-1.91742	-0.72332
H	4.90550	-2.28417	0.17821
H	5.18662	-1.54012	-1.39751
H	3.92677	-2.76030	-1.21985
C	-1.01528	-0.50258	1.98293
H	-1.06285	0.51188	2.38730
H	-0.11715	-0.61621	1.36996
N	-2.21206	-0.81984	1.21793
H	-2.76539	-1.61370	1.50697
C	-2.58567	-0.15108	0.12514
N	-1.89352	0.91710	-0.26895
N	-3.64440	-0.55786	-0.57758
H	-1.11325	1.23832	0.29258
H	-3.93687	0.00242	-1.36528
C	-2.19003	1.66592	-1.48019
H	-1.45663	2.46533	-1.55952
H	-3.18709	2.11127	-1.43798
H	-2.11312	1.02835	-2.36437
C	-4.43816	-1.72919	-0.23832
H	-3.82320	-2.63187	-0.24641
H	-5.21548	-1.83078	-0.99154
H	-4.91073	-1.61728	0.74045
H	-0.96013	-1.20095	2.81443

SDMA_2 – PHE 3

C	3.00022	-0.79646	-0.14581
C	2.45739	-0.02367	-1.17567
C	1.73579	1.13556	-0.91177
C	1.53860	1.54071	0.40785
C	2.08241	0.79453	1.45074
C	2.80722	-0.35961	1.16578
H	2.59908	-0.33357	-2.20652
H	1.32483	1.73415	-1.71857
H	1.94090	1.11563	2.47842
H	3.22483	-0.93453	1.98640
O	0.80781	2.67164	0.61782
H	0.73255	2.83807	1.56599
C	3.72783	-2.08159	-0.44184
H	4.42800	-2.33074	0.35751
H	4.28214	-2.01463	-1.38005
H	3.02060	-2.91223	-0.53496
C	-0.38145	-1.85578	-0.08994
H	0.25783	-1.17213	0.47445
H	-0.12455	-1.80265	-1.15142
N	-1.78713	-1.54301	0.12727
H	-2.39577	-2.27705	0.45921
C	-2.32102	-0.34953	-0.13720
N	-1.55549	0.62795	-0.62270
N	-3.61757	-0.13353	0.08946
H	-0.57881	0.42888	-0.79453
H	-3.99494	0.77782	-0.12737
C	-2.04497	1.96487	-0.92142
H	-1.21377	2.53723	-1.32511
H	-2.40455	2.46242	-0.01756
H	-2.84503	1.93148	-1.66507
C	-4.51886	-1.14287	0.62458
H	-4.58551	-2.00406	-0.04460
H	-5.50433	-0.69251	0.71232
H	-4.19464	-1.47232	1.61444
H	-0.20891	-2.86923	0.26447

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Appendix III

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

The work described in this chapter has been reproduced from:

Solea, A. B., Wang, S., Xue, X.-S., Crochet, A., Fromm, K. M., Houk, K. N., Mamula, O., and Allemann, C. (2021) Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids. *Org. Biomol. Chem.* 19, 8025–8029. doi: 10.1039/D1OB01343B.

Excerpts from the Supporting Information relevant to computational methods and analyses performed by the author are attached at the end of this chapter.

COMMUNICATION

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View Journal | View Issue



Cite this: *Org. Biomol. Chem.*, 2021, **19**, 8025

Received 30th July 2021,

Accepted 27th August 2021

DOI: 10.1039/d1ob01343b

rsc.li/obc

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids†

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A straightforward one-pot method for the synthesis of unreported pyrido-[2,1-*a*]isoindolones in excellent yield is described. Two novel isoindolones were synthesized and fully characterized. The alkyl substituents on the pyridine play an important role in the outcome of the reaction. The mechanism, investigated through DFT calculations, features an unprecedented intramolecular cyclization reaction involving a carboxylic acid activated by tosyl chloride and an electron-poor pyridinic nitrogen. This protocol completes the known strategies to obtain functionalized isoindolones.

The isoindolone structure is an important structural motif found in various natural products or compounds of pharmaceutical interest.¹ In particular, derivatives of pyrido-[2,1-*a*]isoindolones are present in natural alkaloids such as (±)-nuevamine, or biologically-active compounds such as valmerin² or urotesin-II receptor antagonists³ (Fig. 1). When the isoindolone structure is polyconjugated, interesting emissive properties are obtained, making such compounds promising organic dyes for bioimaging applications.^{4,5} Such a case is batracylin⁶ which is an antitumor agent and also contains the structural motif of various emissive materials due to its polyconjugated structure.⁷

Several methods for the synthesis of functionalized isoindolones have been reported, but the yields are rather low to moderate. For example, the typical reaction pathways for isoindolone synthesis are based on Diels-Alder or anionic cyclizations,^{8,9} intramolecular cyclization¹⁰ the Heck reac-

tion,¹¹ Mannich-type reaction,¹² radical cyclization of enamine or ynamide derivatives^{13,14} sulfa-Michael-triggered tandem reaction¹⁵ or olefin metathesis.¹⁶ Besides these intermolecular methods, two examples of intramolecular attack from a pyridinic N atom towards either a biacetate,¹⁷ or an aldehyde^{5,18–21} have been reported. These are rare cases of *N*-acyliminium ion formation from an electron-poor heterocycle like pyridine,²² so any such observation is of major interest from a synthetic point of view.

We are reporting here this type of intramolecular cyclization in which the pyridinic nitrogen atom is performing a nucleophilic attack towards the tosylated carboxylic acid derivative, followed by deprotonation of the annealed ring. The novelty of

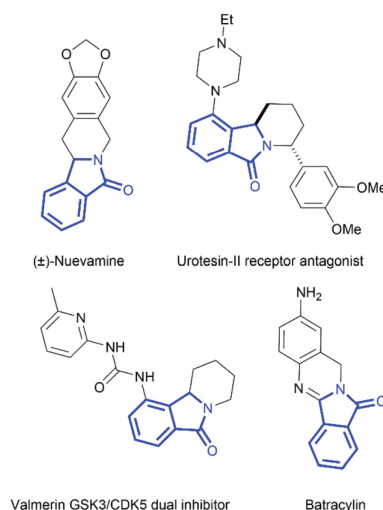


Fig. 1 Selected examples of bioactive molecules with an isoindolone core.

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† Electronic supplementary information (ESI) available: Experimental section, photophysical characterisation, copies of NMR spectra, computational details, X-ray tables. CCDC 2036677–2036679. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d1ob01343b

Communication

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our work lies in the participation of a carboxylic acid instead of the aldehyde that Mamane and coworkers used in their work.¹⁸ Moreover, the *N*-acylamonium intermediate does not undergo rearrangement but it is stabilized by the deprotonation of the carbon at the β position of the nitrogen atom. Indeed, the typical conditions leading to anhydride formation (TsCl in basic media) did not give the expected product (**SA**, see Scheme 1), but a chiral isoindolone *via* an unprecedented cyclization reaction. Two novel isoindolones were fully characterized in solution and solid state and a mechanism based on DFT calculations is proposed.

The brominated precursor **1** was obtained through a reported procedure²³ and subsequently underwent lithiation and carboxylation with gaseous CO₂ to give the chiral pinene-pyridine carboxylic acid **2** (Scheme 1). The formation of **2** was confirmed in solution by NMR and HRMS and in the solid state by single crystal X-ray diffraction (Fig. 2). Compound **2** crystallizes in the *P*₂₁₂₁₂ (no. 18) space group with two molecules in the asymmetric unit. The two molecules interact through middle strength intermolecular H-bonding between the N of the pyridine atom and the carboxylic acid group (Fig. S1†). Deprotonation and tosylation of **2**, following a modified version of a reported procedure,²⁴ did not give the expected anhydride. Instead, the orange compound **3** was isolated in excellent yield.

The structure of **3** was confirmed by single crystal X-ray diffraction as shown in Fig. 2. The compound crystallizes with one molecule in the asymmetric unit in the *P*₂₁₂₁ (no. 19)

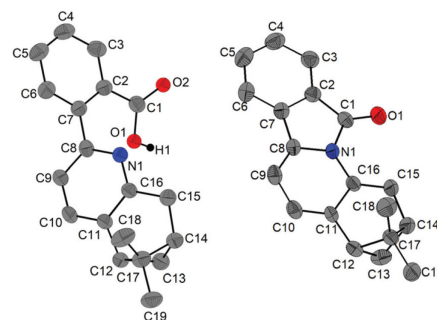
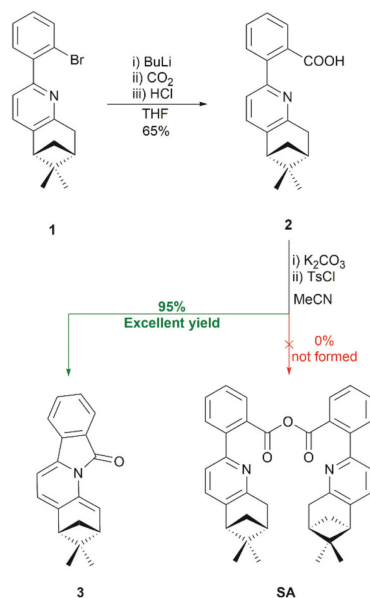


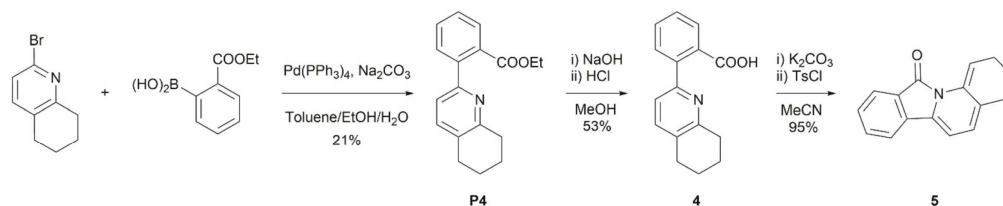
Fig. 2 Molecular structure of **2** (left) and **3** (right) determined by single-crystal X-ray diffraction with ellipsoids at 30% probability with atom numbering scheme. The H atoms were omitted for clarity.

space group. The bond lengths C8–C9, C10–C11 and C15–C16 are in the expected range for double bonds (1.340(5) 1.338(5) and 1.331(5) Å, respectively), whereas the bond length C9–C10 is between a single and a double bond (1.439(5) Å). In comparison, the bond lengths C8–C9, C9–C10 and C10–C11 in compound **2** are typical for aromatic, conjugated structures (1.382(8), 1.391(8), 1.380(8) Å, respectively), while the C15–C16 bond from the pinene unit is clearly a single bond, with a length of 1.496(7) Å. More precisely, for compound **3**, the bond orders for C8–C9 and C10–C11 are indicative of a double bond (1.7093 and 1.7716, respectively), whereas the bond C9–C10 indicates that this bond has more of a single bond character (1.0913), suggesting decreased delocalization in the pyridine ring of the precursor **2** (Fig. S2; details for bond order calculation are provided in the ESI†). Each molecule is interacting with a neighbouring one through strong face-to-face π – π stacking interactions between the phenyl and the lactam rings, with centroid–centroid distances of 3.6 Å (Fig. S3†). The structure of **3** was further confirmed in the solid state through IR, where a strong band at 1660 cm^{−1} was observed, corresponding to the C=O stretch typical for an isoindolonic structure (Fig. S4†). In solution, an interesting feature of the ¹H-NMR spectrum of **3** is the downfield shift of the vinyl proton H15 at 7.41 ppm, in contrast to the doublet at 3.23 ppm observed for the protons in β position to the pyridinic N-atom in the starting material **2** (Fig. S5†). The formation of the isoindolone ring is also visible on the UV-Vis and emission spectra. Whereas compound **2** does not absorb in the visible region nor have any visible emission, compound **3** shows a strong absorption band with a maximum at 474 nm (Fig. S6†). Moreover, when excited at this wavelength, compound **3** emits in the visible region, with a λ_{em} = 588 nm (Fig. S7†).

The same reaction conditions used to obtain **3** were applied to the achiral, cyclohexyl-pyridine analogue **4**, which was obtained through a Suzuki coupling (Scheme 2), followed by hydrolysis of the ethyl ester **P4**. This reaction gave the isoindolone **5** in excellent yield. The pyrido-[2,1-*a*]isoindolonic struc-



Scheme 1 Synthetic steps for obtaining **3**.



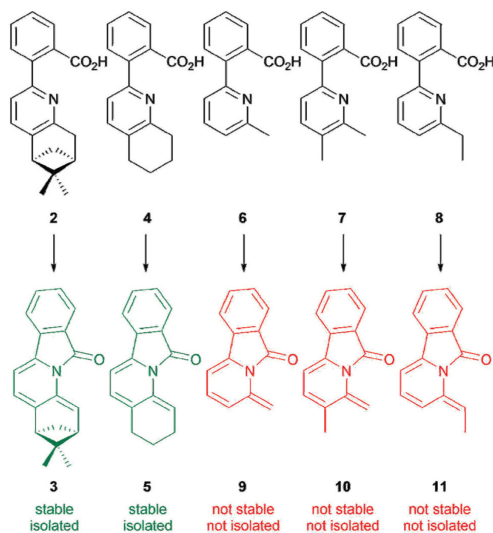
Scheme 2 Synthesis of the achiral analogue 5.

ture of **5** was confirmed in solution by NMR, UV-Vis and emission spectroscopy, HRMS and by IR in solid state. As in the case of compound **3**, the $^1\text{H-NMR}$ spectrum of **5** shows a strong downfield shift for the proton in β -position with respect to the N atom at 7.40 ppm, as well as shifts around 6–6.5 ppm for the vinylic atoms from the former pyridine ring (Fig. S8†). The photophysical properties of **5** are similar to the ones of **3**, with a similar UV-Vis spectrum and a broad absorption band with a maximum at $\lambda_{\text{abs}} = 472$ nm (Fig. S9†) and a maximum of emission at $\lambda_{\text{em}} = 567$ nm (Fig. S10†). The IR spectrum of **5** is also very similar to the IR spectrum of **3**, with a strong band at 1681 cm^{-1} (Fig. S4†), stemming from the C=O stretching from the isindolone moiety. The sterically less hindered, but also more electron-deficient, *ortho*-methyl, dimethyl, and ethyl derivatives **6**, **7**, **8** (Scheme 3) were also used as substrates. The corresponding isindolones **9**, **10**, and **11** could not be isolated. As the substrates **6**, **7**, and **8** were not soluble in acetonitrile, the reaction was done in acetone. NMR titration experi-

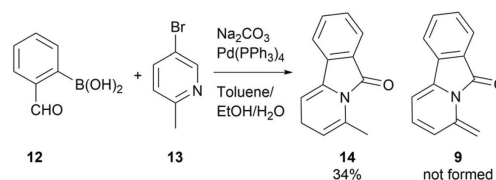
ments showed signals corresponding to isindolones (**9–11**, Fig. S11–S16†). Moreover, ESI-MS spectra (Fig. S17 and S18†) of the reaction mixture showed molecular peaks of the isindolones. Thus, the scope of this reaction is limited to cyclic substituent on the pyridinic moiety. Substrates with aliphatic substituents like ethyl or methyl at the *ortho* position form the isindolone ring but the resulting products are unstable and degrade before isolation. Moreover, in the original solvent (acetonitrile), these substrates are not soluble and mass transfer is most probably limiting the reaction.

In order to check the stability of **9** and to investigate a possible deprotonation of the *ortho* methyl unit, we performed a previously reported reaction which also involves a nucleophilic attack from a pyridinic nitrogen towards an aldehyde group.¹⁸ Instead of starting from the carboxylic acid derivative of a bipyridine, we have made a Suzuki cross-coupling of 2-formylphenylboronic acid **12** and 2-bromo-6-methylpyridine **13** under the reactions conditions described by Mamane and co-workers.¹⁸ As shown in Scheme 4, the desired isindolone **9** was not obtained. Instead of the triene **9**, a stable diene **14** has been easily isolated and purified by column chromatography. We can conclude, that the reaction discussed in this paper is significantly different compared with the one described by Mamane and Igeta. Moreover, the oxidation of the pyridino isindolones with Ag_2O ²⁵ reported by Mamane and co-workers leads to the formation of an ester moiety, indicating thus a difference in oxidation states between our product and theirs.

Based on the experimental data, we propose the following three-part mechanism: (A) the carboxylic acid is first activated by TsCl; (B) the activated acid then undergoes intramolecular cyclization through an *N*-nucleophilic attack; (C) the resulting intermediate is deprotonated either by the resulting $^- \text{OTs}$



Scheme 3 Substrates and products that have been investigated. Reaction conditions: K_2CO_3 , TsCl in acetonitrile (for products in green), or Et_3N , TsCl in acetone- d_6 (for products in red).



Scheme 4 Synthesis of the methylated derivatives under Mamane and Igeta's reaction conditions.¹⁸

from (B) or HCO_3^- . To evaluate the plausibility of our proposed mechanism, we performed density functional theory (DFT) calculations (Fig. 3). Our computational studies indicated a substantially exergonic pathway. The intramolecular cyclization step (TS1) is a facile step due to the leaving group ability of $^- \text{OTs}$, with an activation barrier of $7.3 \text{ kcal mol}^{-1}$. Following cyclization, the deprotonation by HCO_3^- (TS2b; overall $\Delta G^\ddagger = 10.0 \text{ kcal mol}^{-1}$) proceeds with a more favorable activation barrier compared to that by $^- \text{OTs}$ (TS2a; $\Delta G^\ddagger = 19.1 \text{ kcal mol}^{-1}$). The release of H_2CO_3 is mildly endergonic by our calculations, although this process is expected to be entropically

favorable. This can be explained by the breakage of a strong hydrogen bond between the product and H_2CO_3 (complex-2b), which is enthalpically unfavorable. Overall, our calculations indicate that an intramolecular cyclization followed by HCO_3^- deprotonation leads to a free energy change of $-34.9 \text{ kcal mol}^{-1}$, in line with our hypothesis. The energy of the expected anhydride product was also calculated, and to our surprise, the anhydride was very stable ($\Delta G = -47.4 \text{ kcal mol}^{-1}$, relative to the reactant). The anhydride was not detected in the reaction mixture, thus its formation is presumed to be kinetically unfavorable due to its bimolecular nature. Details of compu-

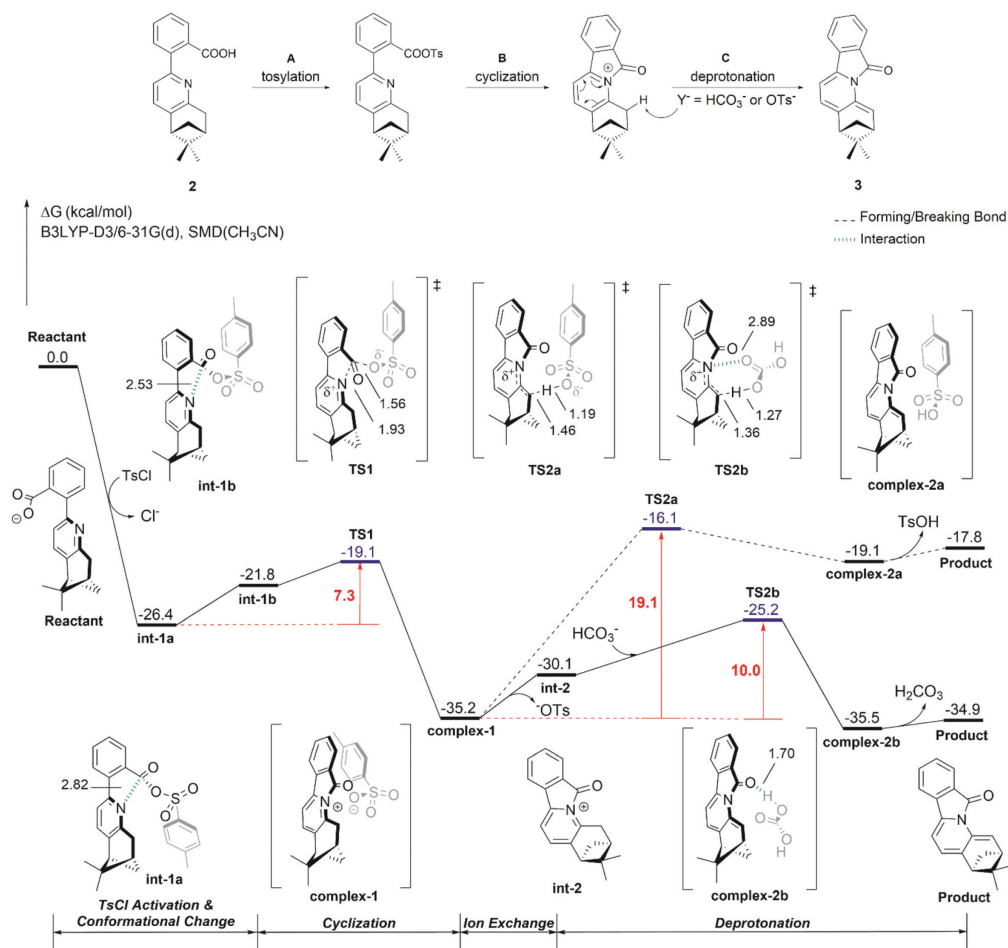


Fig. 3 Overview of the pinene-pyrido[2,1-a]isoindolone 3 formation (top) and free energy profile (in kcal mol^{-1}) for the proposed mechanism calculated at the B3LYP-D3/6-31G(d), SMD(CH_3CN) level of theory (bottom). All intermediate structures were obtained from IRC, with the exception of int-1a and int-2 (conformational search, see ESI†). Ion exchange pertains to the displacement of $^- \text{OTs}$ by HCO_3^- . Interatomic distances are marked in Ångströms.

tational methodologies used in this study are provided in the ESI.†

Conclusions

In conclusion, a new synthetic pathway for obtaining unsaturated pyrido-[2,1-*a*]isoindolones from electron-poor heterocycles was developed. With our procedure, we successfully synthesized chiral and achiral isoindolone derivatives annelated to deprotonated pinene (3) and cyclohexyl (5) rings in excellent yield. Through DFT calculations, the mechanism of the reaction was elucidated.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors wish to thank Dr Albert Ruggi (University of Fribourg) for measuring the Mass Spectra and Sandrine Aeby (HEIA-FR) for assisting with the precursor synthesis.

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Computational study

Computational methodologies

The bond orders for component **3** have been calculated with MOPAC2016⁴ with the AM1 Hamiltonian and a Molecular Mechanics correction. The interface Mercury⁵ has been used. Calculations have been performed on the x-ray crystal structure without structure optimization. All density functional theory (DFT) calculations were performed using Gaussian 16, Revision C.01.⁶ Geometry optimizations, as well as frequency and single-point energy calculations, were performed using the B3LYP⁷ functional augmented with Grimme's D3⁸ empirical dispersion term and the 6-31G(d) basis set. Solvation effects were included in the optimization and single-point calculations using the SMD⁹ solvation model with acetonitrile as the solvent. Gibbs free energies were obtained by summing the thermal correction and single point energies obtained from DFT. No scaling was applied for the ΔG values. Normal vibrational mode analysis was used to verify the structures of minima (zero imaginary frequencies) and transition states (single imaginary frequency). Intrinsic reaction coordinate (IRC) calculations were also used to verify transition state structures, and that they indeed connected the reactants and products. Mixed torsional/Low-Mode conformational sampling in gas phase was performed for the reactant, key intermediates, and transition states, where applicable, using the Merck molecular force field (MMFF94) implemented in MacroModel,¹⁰ followed by DFT optimization. Conformers obtained from each run that fell within 10 kcal/mol of the lowest energy conformer were selected and reoptimized using DFT. GaussView 6.0.16 was used to render optimized structures as well as construct the initial structures used in our calculations.¹¹ Conformational search calculations were set up and rendered in the Maestro GUI.¹² 3D renderings of minima and transition state structures were generated with CYLview 1.0.¹³



Supplemental Figures

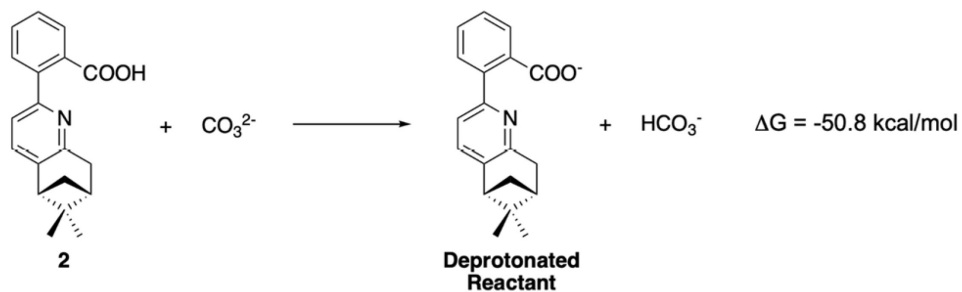


Figure S1. Schematic and energetics of the deprotonation of 2.

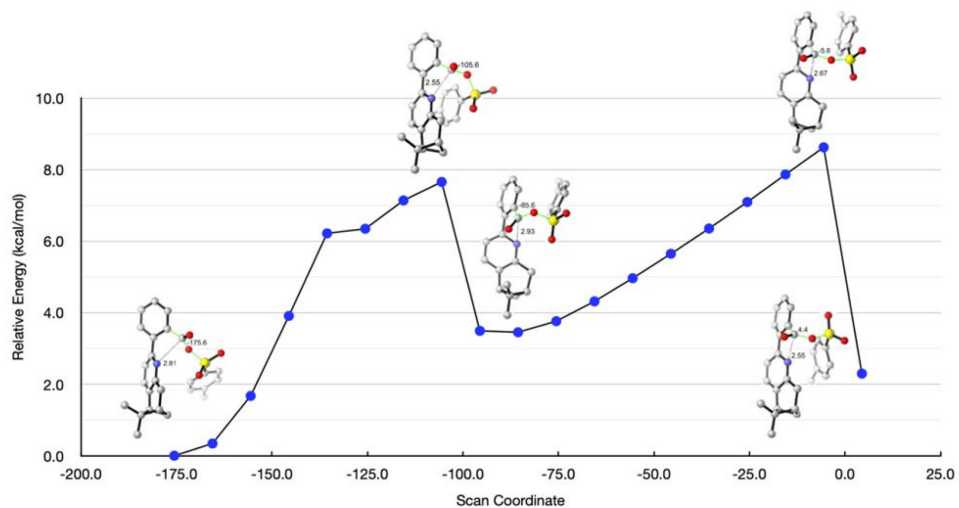
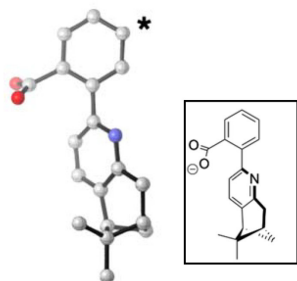


Figure S2. Scan of rotation about the tosyl ester bond in int-1a. Energies shown are single point energies; scan coordinate refers to dihedral angles.

B3LYP-D3/6-31G(d), SMD(CH₃CN) Calculated Cartesian Coordinates and Thermal Corrections

Reactant (deprotonated)



* For all 3D renderings, hydrogens are omitted for clarity.

C	4.69131300	-0.08823100	0.24013900
C	3.36663400	0.35636400	0.11599700
C	2.34401200	-0.60398700	-0.06415300
C	2.68028300	-1.96996400	-0.07632700
C	3.99868100	-2.39515300	0.07974000
C	5.01350400	-1.44579700	0.23578200
C	3.09893200	1.86509700	0.24756000
O	3.57749800	2.58411200	-0.66867800
O	2.44916000	2.20842100	1.26457500
C	0.92066800	-0.22278300	-0.28560700
C	0.56895400	0.91304900	-1.03113600
N	-0.01793000	-1.05684400	0.21513400
C	-0.77843900	1.19150900	-1.25481400
C	-1.30698600	-0.78306300	-0.00429900
C	-1.74865500	0.33503400	-0.74076300
C	-2.35912800	-1.70491000	0.59360500
C	-3.23783100	0.48705000	-0.91827700
C	-3.77489300	-1.23864500	0.20814900
C	-3.96731700	0.29967300	0.47092800
C	-3.35039700	0.92610100	1.72273000
C	-5.44322900	0.71713800	0.40255700
C	-3.79644000	-0.91650200	-1.31247100
H	5.47823700	0.65381000	0.35717500
H	1.88856900	-2.69994300	-0.22018300
H	4.23313700	-3.45668700	0.06665500
H	6.04799100	-1.76194000	0.35008900
H	1.34288700	1.55844400	-1.43185600
H	-1.07150200	2.06427100	-1.83440200
H	-2.23689500	-1.73319900	1.68456900
H	-2.19065200	-2.72955600	0.23556600
H	-3.50209600	1.35994700	-1.52416300
H	-4.53989300	-1.91261200	0.60952100
H	-3.87276000	0.57107600	2.62058000

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	-2.28794900	0.70117300	1.84547500
H	-3.45289800	2.01868600	1.69035500
H	-5.52852100	1.81061500	0.36216400
H	-5.96990600	0.31206100	-0.46663600
H	-5.97427400	0.37433700	1.30004400
H	-3.16508900	-1.53077300	-1.96394600
H	-4.80413100	-0.87395000	-1.73016400

Zero-point correction= 0.326923 (Hartree/Particle)

Thermal correction to Energy= 0.345038

Thermal correction to Enthalpy= 0.345982

Thermal correction to Gibbs Free Energy= 0.280336

Sum of electronic and zero-point Energies= -939.915887

Sum of electronic and thermal Energies= -939.897772

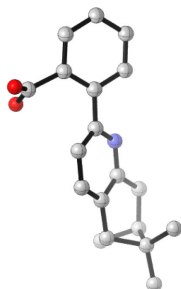
Sum of electronic and thermal Enthalpies= -939.896828

Sum of electronic and thermal Free Energies= -939.962475

Single point energy: -940.242810398

Total free energy: -939.962474398

Number of imaginary frequencies: 0

Reactant (deprotonated) alternative conformations

Thermal correction to Gibbs Free Energy= 0.281174 (Hartree/Particle)

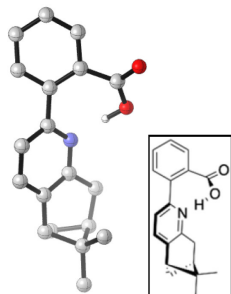
Single point energy: -940.242823439

Total free energy: -939.961649439

ΔG (relative to lowest energy conformer): 0.5 kcal/mol



Reactant (protonated)



C	4.54987400	0.52007200	0.07190900
C	3.14793800	0.56197100	-0.01966100
C	2.42422200	-0.66236100	-0.00470000
C	3.15369500	-1.86103300	0.11637700
C	4.54089800	-1.87777900	0.22799500
C	5.24753800	-0.67566700	0.20658300
H	5.08987600	1.45960500	0.03267800
H	2.61239300	-2.80131800	0.14550000
H	5.06181900	-2.82498300	0.33598100
H	6.33036100	-0.66672600	0.29211500
C	2.60291000	1.97048000	-0.18131400
O	1.42668600	2.28547400	0.35362600
O	3.26874100	2.82792700	-0.74183600
C	0.94382800	-0.80452900	-0.12561700
C	0.37111500	-1.82343700	-0.89608800
N	0.16467900	0.05359200	0.56811300
C	-1.01788400	-1.95836400	-0.92749900
H	0.99961600	-2.48946800	-1.47661700
C	-1.16489800	-0.06809300	0.53793600
C	-1.81258600	-1.08289200	-0.19403300
H	-1.47788300	-2.73874100	-1.52840200
C	-2.01176700	0.94464200	1.28466100
H	-1.73531500	0.93618500	2.34701700
H	-1.78168700	1.95033400	0.90861200
C	-3.31655600	-1.10267500	-0.12567400
H	-3.75128400	-1.93108500	-0.69305200
C	-3.50675700	0.61499900	1.12285100
H	-4.13075000	1.32377500	1.67716700
C	-3.87948700	0.35332000	-0.38197500
C	-3.23802600	1.21604400	-1.47057100
H	-3.62923400	2.24012700	-1.41885000
H	-2.14859400	1.27471000	-1.40089300
H	-3.48223500	0.81688600	-2.46346200
C	-5.39759600	0.34318700	-0.60872300
H	-5.79187900	1.36612200	-0.55713900
H	-5.63060700	-0.05480200	-1.60445300

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	-5.94325500	-0.25826600	0.12426200
C	-3.70884200	-0.90571900	1.37252800
H	-4.74631400	-1.17437000	1.57771900
H	-3.06157200	-1.38197200	2.11706300
H	0.93466800	1.45863300	0.68372700

Zero-point correction= 0.340325 (Hartree/Particle)

Thermal correction to Energy= 0.358049

Thermal correction to Enthalpy= 0.358994

Thermal correction to Gibbs Free Energy= 0.294897

Sum of electronic and zero-point Energies= -940.398315

Sum of electronic and thermal Energies= -940.380590

Sum of electronic and thermal Enthalpies= -940.379646

Sum of electronic and thermal Free Energies= -940.443742

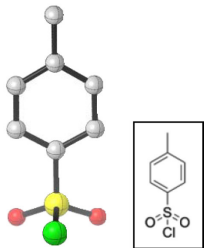
Single point energy: -940.738639147

Total free energy: -940.443842147

Number of imaginary frequencies: 0



TsCl



C	2.16224000	-1.21115300	0.01675500
C	2.87086600	-0.00220100	0.10628700
C	2.16273700	1.20763500	0.00703900
C	0.78482700	1.22326900	-0.17967500
C	0.11915800	-0.00229100	-0.27450400
C	0.78320500	-1.22681900	-0.17015900
H	2.69675700	-2.15359000	0.09473100
H	2.69868800	2.15003100	0.07737000
H	0.24344900	2.16001800	-0.25481400
H	0.24129300	-2.16382300	-0.23790300
S	-1.63608200	-0.00234300	-0.51096800
O	-2.07741500	1.26842300	-1.08069500
O	-2.07940700	-1.27823800	-1.06748000
Cl	-2.36580000	0.00871300	1.52935200
C	4.36629400	0.00316600	0.28273600
H	4.86377300	0.13765200	-0.68716900
H	4.68740900	0.82700700	0.92897800
H	4.72315200	-0.93905000	0.70983900

Zero-point correction= 0.129064 (Hartree/Particle)

Thermal correction to Energy= 0.139872

Thermal correction to Enthalpy= 0.140816

Thermal correction to Gibbs Free Energy= 0.090888

Sum of electronic and zero-point Energies= -1279.638496

Sum of electronic and thermal Energies= -1279.627689

Sum of electronic and thermal Enthalpies= -1279.626745

Sum of electronic and thermal Free Energies= -1279.676672

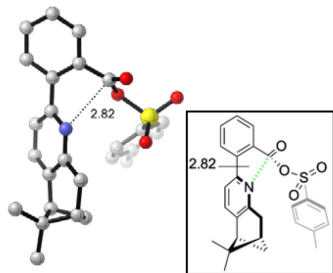
Single point energy: -1279.76756048

Total free energy: -1279.67667248

Number of imaginary frequencies: 0



Int-1a



C	-0.32001500	3.11661100	1.39070000
C	0.38675600	4.32115600	1.55606600
C	1.46648800	4.59087900	0.70342300
C	1.84187800	3.69162500	-0.29505800
C	1.11251700	2.51179600	-0.43418600
C	0.03066600	2.20951100	0.39862500
S	1.54814500	1.36343900	-1.71455300
O	0.35768800	0.92600700	-2.44103500
O	2.72814800	1.82972100	-2.43914800
C	-0.02752500	5.30334000	2.62113800
O	2.01454900	0.11571500	-0.66958200
C	2.33175300	-1.14034200	-1.17641900
O	2.48960500	-1.36092200	-2.35077900
C	2.60218700	-2.07763800	-0.05077900
C	3.83093800	-2.74705200	-0.08757500
C	1.71681200	-2.25517200	1.03587300
C	4.21771200	-3.56695000	0.97178700
C	2.12499500	-3.08519000	2.08946000
C	3.36498500	-3.72533300	2.06598700
C	0.34943600	-1.67532500	1.01864000
C	-0.27712700	-1.21010000	2.17997700
N	-0.26167400	-1.64998200	-0.18557300
C	-1.57961700	-0.71387300	2.09107300
C	-1.50534700	-1.17514900	-0.26559600
C	-2.21761300	-0.68814600	0.85461200
C	-2.20176000	-1.17314600	-1.61499700
C	-3.60357900	-0.15834500	0.59433200
C	-3.60948000	-0.56136900	-1.49806300
C	-4.41178700	-1.18324100	-0.29754900
C	-4.28240900	-2.67999600	-0.00885300
C	-5.90534200	-0.83300200	-0.35572200
C	-3.51450500	0.75164900	-0.67191600
H	-1.15357900	2.88665300	2.04912300
H	2.02526900	5.51501200	0.82215000
H	2.68245700	3.90191500	-0.94740500
H	-0.51631400	1.28213000	0.28324200
H	0.74639400	6.05644100	2.79768200

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	-0.94519800	5.82792800	2.32392800
H	-0.24079600	4.79302200	3.56723300
H	4.48941400	-2.59887600	-0.93820500
H	5.17697200	-4.07508000	0.94234000
H	1.44729900	-3.25954200	2.91984800
H	3.65387100	-4.36555800	2.89478000
H	0.24768100	-1.21947700	3.12961900
H	-2.08736000	-0.33883800	2.97636200
H	-2.24756300	-2.20117200	-1.99772100
H	-1.59340100	-0.59770800	-2.32244100
H	-4.08236200	0.22629900	1.50043100
H	-4.11177800	-0.52984800	-2.47102100
H	-4.76408300	-3.26108500	-0.80575100
H	-3.24779000	-3.02339100	0.07191700
H	-4.78594300	-2.92968500	0.93412400
H	-6.39712400	-1.41371700	-1.14672000
H	-6.39309300	-1.08414100	0.59478200
H	-6.09673700	0.22529400	-0.55635700
H	-2.60583800	1.35170500	-0.78577200
H	-4.38501600	1.39921900	-0.78984400

Zero-point correction= 0.457872 (Hartree/Particle)

Thermal correction to Energy= 0.485954

Thermal correction to Enthalpy= 0.486898

Thermal correction to Gibbs Free Energy= 0.397716

Sum of electronic and zero-point Energies= -1759.245146

Sum of electronic and thermal Energies= -1759.217064

Sum of electronic and thermal Enthalpies= -1759.216119

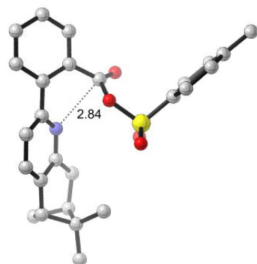
Sum of electronic and thermal Free Energies= -1759.305301

Single point energy: -1759.70301746

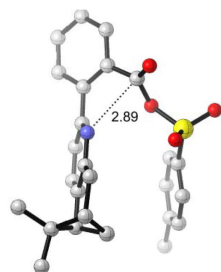
Total free energy: -1759.30530146

Number of imaginary frequencies: 0

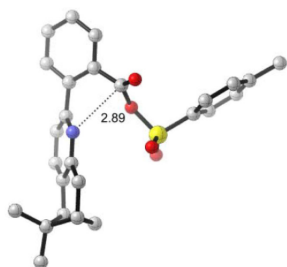


Int-1a alternative conformations

Thermal correction to Gibbs Free Energy=	0.397753 (Hartree/Particle)
Single point energy:	-1759.70214757
Total free energy:	-1759.30439457
ΔG (relative to lowest energy conformer):	0.6 kcal/mol



Thermal correction to Gibbs Free Energy=	0.397522 (Hartree/Particle)
Single point energy:	-1759.70156833
Total free energy:	-1759.30404633
ΔG :	0.8 kcal/mol



Thermal correction to Gibbs Free Energy=	0.397442 (Hartree/Particle)
Single point energy:	-1759.70136351
Total free energy:	-1759.30392151
ΔG :	0.9 kcal/mol



Cl⁻

Cl 0.00000000 0.00000000 0.00000000

Zero-point correction= 0.000000 (Hartree/Particle)

Thermal correction to Energy= 0.001416

Thermal correction to Enthalpy= 0.002360

Thermal correction to Gibbs Free Energy= -0.015023

Sum of electronic and zero-point Energies= -460.360860

Sum of electronic and thermal Energies= -460.359444

Sum of electronic and thermal Enthalpies= -460.358500

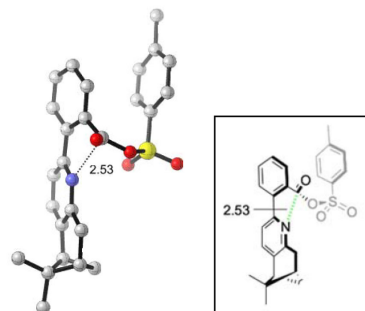
Sum of electronic and thermal Free Energies= -460.375883

Single point energy: -460.360860002

Total free energy: -460.375883002

Number of imaginary frequencies: 0



Int-1b

C	0.66742100	-0.18468200	1.80184600
O	0.39614900	-0.24089700	2.97190100
C	1.28431000	1.00270700	1.13095600
C	2.55599400	1.35657400	1.59865100
C	0.64853900	1.76417500	0.12721300
C	3.23760700	2.43564700	1.04089100
C	1.34889400	2.85543000	-0.41174400
C	2.62997000	3.18182600	0.02829300
C	-0.72910500	1.42115000	-0.29428900
C	-1.31928600	1.87953200	-1.47738800
N	-1.37898600	0.58687900	0.53888500
C	-2.60394800	1.44027000	-1.80143400
C	-2.59667700	0.15546300	0.22412400
C	-3.26359900	0.55430600	-0.95435900
C	-3.30878900	-0.79214500	1.17312500
C	-4.62355000	-0.04692800	-1.19341100
C	-4.67795500	-1.19926100	0.59962600
C	-5.49736700	0.05405600	0.12001000
C	-5.44607500	1.34016700	0.94663900
C	-6.96991500	-0.28678300	-0.14855100
C	-4.49770000	-1.57573400	-0.89783400
H	3.02034200	0.76139600	2.37879200
H	4.23248600	2.69003800	1.39395900
H	0.87815600	3.47439900	-1.16830800
H	3.14756200	4.02997700	-0.41076600
H	-0.78482200	2.53795200	-2.15312500
H	-3.08097000	1.77291800	-2.72014900
H	-3.41501800	-0.31106800	2.15415600
H	-2.68281100	-1.68097800	1.32768400
H	-5.07021000	0.28416000	-2.13623300
H	-5.19066000	-1.90875000	1.25802400
H	-5.96802500	1.19691500	1.90152200
H	-4.43035500	1.67587300	1.17127600
H	-5.95074200	2.15528100	0.41186300
H	-7.50260800	-0.42721100	0.80081000

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	-7.45898300	0.53478700	-0.68728300
H	-7.10472400	-1.19718400	-0.74011000
H	-3.55457100	-2.05434500	-1.18400600
H	-5.32955400	-2.15686900	-1.29961800
C	4.50330300	0.14353600	-1.44119300
C	5.44817600	-0.36178800	-0.53555400
C	5.04174400	-1.33780700	0.38961700
C	3.72730000	-1.79291000	0.42475900
C	2.80912100	-1.25282100	-0.48010900
C	3.18069800	-0.29149300	-1.42067500
S	1.12373800	-1.80563400	-0.43063400
O	1.06519400	-3.26548900	-0.39767900
O	0.62474200	-1.42688100	1.12333900
C	6.86081300	0.16234200	-0.53001600
O	0.33168300	-1.06660900	-1.40987600
H	4.80328300	0.89627400	-2.16495100
H	5.76445500	-1.74427100	1.09175600
H	3.42270200	-2.54420000	1.14640100
H	2.44837600	0.11869300	-2.10641500
H	7.18245700	0.45617800	-1.53459100
H	6.93199800	1.05242700	0.10982500
H	7.56365200	-0.58038400	-0.13918500

Zero-point correction= 0.457440 (Hartree/Particle)

Thermal correction to Energy= 0.485454

Thermal correction to Enthalpy= 0.486399

Thermal correction to Gibbs Free Energy= 0.397883

Sum of electronic and zero-point Energies= -1759.238527

Sum of electronic and thermal Energies= -1759.210513

Sum of electronic and thermal Enthalpies= -1759.209568

Sum of electronic and thermal Free Energies= -1759.298084

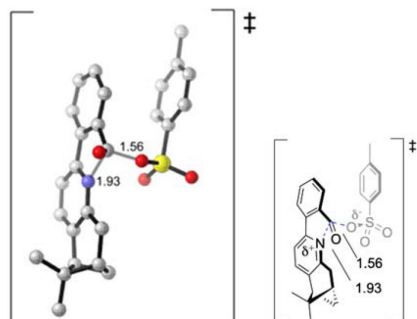
Single point energy: -1759.69596697

Total free energy: -1759.29808397

Number of imaginary frequencies: 0



TS1



C	0.16842500	0.13962600	1.63006300
O	-0.26534700	0.10227800	2.75710500
C	1.16953500	1.13113800	1.10372200
C	2.42613000	1.28380900	1.68277800
C	0.76832800	1.93558300	0.02792700
C	3.30359400	2.23568100	1.15673300
C	1.64605400	2.88999200	-0.49356800
C	2.91448100	3.03083200	0.07210900
C	-0.60104900	1.65085700	-0.42439400
C	-1.31393600	2.22487400	-1.47177400
N	-1.15055800	0.67489300	0.32051500
C	-2.60128600	1.74454000	-1.73674500
C	-2.36435200	0.19425500	0.08948500
C	-3.14193100	0.71694400	-0.96688200
C	-2.92808500	-0.91380400	0.95271400
C	-4.48528900	0.06928000	-1.17479200
C	-4.31919600	-1.32025600	0.43562400
C	-5.24040500	-0.06687400	0.20751800
C	-5.18112400	1.09410200	1.20194600
C	-6.71121400	-0.46050300	0.01358700
C	-4.25117700	-1.47458500	-1.10966800
H	2.72143400	0.65670900	2.51822400
H	4.29284700	2.35406300	1.58907700
H	1.34438000	3.51541500	-1.32843700
H	3.60380700	3.76606500	-0.33304400
H	-0.87476500	3.01382700	-2.07260000
H	-3.18012500	2.16619100	-2.55413200
H	-2.96455000	-0.57721100	1.99534600
H	-2.24192900	-1.76868600	0.92716500
H	-5.02965700	0.49757800	-2.02151400
H	-4.73263900	-2.14441900	1.02585700
H	-5.61035200	0.78817900	2.16452000
H	-4.16825100	1.45856600	1.39316900
H	-5.76999500	1.94225400	0.82971900



Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	-7.28745400	0.39411800	-0.36278400
H	-6.85087300	-1.28908200	-0.68693000
H	-7.14848500	-0.76183200	0.97400700
H	-3.31219200	-1.84653800	-1.53426400
H	-5.08277600	-2.04695200	-1.52360800
C	4.40880000	-0.05064400	-1.41600800
C	5.31868900	-0.54652300	-0.47193400
C	4.85524900	-1.45222400	0.49695100
C	3.52048800	-1.84424500	0.53475900
C	2.63490000	-1.31843400	-0.41032000
C	3.06696700	-0.42841800	-1.39244500
S	0.91906500	-1.80066400	-0.35359100
O	0.83809800	-3.26419800	-0.27492200
O	0.41144900	-1.30607700	1.09856800
C	6.75476900	-0.08805000	-0.47004600
O	0.20514200	-1.11212700	-1.43557600
H	4.75114800	0.64797100	-2.17481400
H	5.54972100	-1.85219000	1.23115300
H	3.17293100	-2.54102800	1.29100600
H	2.36375900	-0.02892600	-2.11415900
H	7.42582600	-0.87228500	-0.10403400
H	7.08134000	0.21262300	-1.47092700
H	6.87679500	0.78125900	0.19043600

Zero-point correction= 0.456855 (Hartree/Particle)

Thermal correction to Energy= 0.484192

Thermal correction to Enthalpy= 0.485137

Thermal correction to Gibbs Free Energy= 0.397928

Sum of electronic and zero-point Energies= -1759.234696

Sum of electronic and thermal Energies= -1759.207359

Sum of electronic and thermal Enthalpies= -1759.206414

Sum of electronic and thermal Free Energies= -1759.293623

Single point energy: -1759.69155109

Total free energy: -1759.29362309

Number of imaginary frequencies:

1

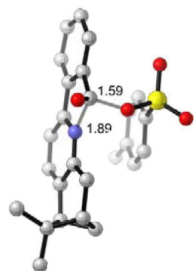
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Red. masses -- 10.5269

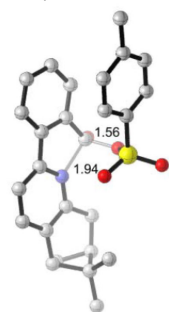
Frc consts -- 0.1789

IR Inten -- 289.0098

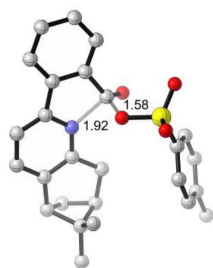


TS1 alternative conformations

Thermal correction to Gibbs Free Energy= 0.399527 (Hartree/Particle)
 Single point energy: -1759.69309030
 Total free energy: -1759.2935633
 ΔG (relative to lowest energy conformer): 0.04 kcal/mol



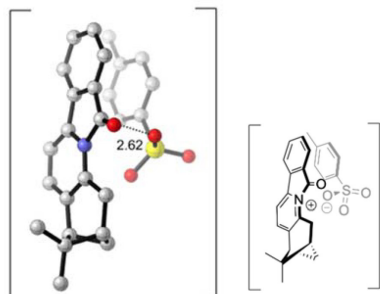
Thermal correction to Gibbs Free Energy= 0.400004 (Hartree/Particle)
 Single point energy: -1759.69313692
 Total free energy: -1759.29313292
 ΔG : 0.3 kcal/mol



Thermal correction to Gibbs Free Energy= 0.399603 (Hartree/Particle)
 Single point energy: -1759.69271515
 Total free energy: -1759.29311215
 ΔG : 0.3 kcal/mol



Complex-1



C	0.20748800	-1.73477700	1.23474000
O	0.81515100	-2.02125700	2.22924600
C	-1.15107000	-2.06225400	0.78435700
C	-2.17488400	-2.68285300	1.48878700
C	-1.29957100	-1.68206800	-0.55741200
C	-3.37811500	-2.91282100	0.81230700
C	-2.48944700	-1.91598500	-1.23541800
C	-3.52898400	-2.53501900	-0.52899500
C	-0.05522200	-1.05664900	-1.00238600
C	0.32626500	-0.52557800	-2.21306300
N	0.82568300	-1.05055800	0.05336200
C	1.61105100	0.02363400	-2.32653000
C	2.04471300	-0.48314800	-0.00410600
C	2.46719000	0.06499500	-1.23235700
C	2.94595900	-0.40166500	1.19861600
C	3.82416000	0.71686800	-1.22298400
C	4.23440700	0.35504500	0.83261500
C	4.87192400	-0.20845800	-0.48828200
C	4.84524400	-1.71563800	-0.75069600
C	6.30826600	0.29037400	-0.69524600
C	3.86044700	1.63757800	0.03991400
H	-2.04321900	-2.97140500	2.52678300
H	-4.20471200	-3.38781500	1.33144900
H	-2.61488300	-1.62157100	-2.27184100
H	-4.47270900	-2.72284200	-1.03236400
H	-0.36489500	-0.53640900	-3.04777200
H	1.93497900	0.44291400	-3.27443200
H	3.16090000	-1.40856100	1.57057700
H	2.39232400	0.11149500	1.99402500
H	4.08749200	1.12625700	-2.20201300
H	4.88481900	0.44647400	1.70794300
H	5.51546600	-2.23094500	-0.05127700
H	3.85443000	-2.16740100	-0.64875000
H	5.19709900	-1.92829100	-1.76812500
H	6.98662600	-0.22776800	-0.00575700

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	6.64152400	0.07488100	-1.71812500
H	6.42357600	1.36502900	-0.52743700
H	2.92239100	2.13395000	0.30212100
H	4.66957200	2.36808400	-0.00014700
C	-3.43555600	1.58621400	-1.66369600
C	-4.52134600	1.04202600	-0.96496400
C	-4.36061900	0.76378700	0.40245500
C	-3.15245700	1.01095600	1.04924700
C	-2.07922200	1.55216000	0.33400000
C	-2.22136400	1.84342900	-1.02276200
S	-0.52861100	1.90172000	1.18815000
O	-0.75426000	3.14682200	1.97126600
O	-0.29893500	0.70042300	2.05470700
C	-5.81656800	0.71330600	-1.66541300
O	0.49036900	2.04312300	0.10644500
H	-3.53815600	1.80603500	-2.72401800
H	-5.18924600	0.33327600	0.96004500
H	-3.03270700	0.76609500	2.10017100
H	-1.37864100	2.25055400	-1.57166000
H	-5.90803300	1.24834800	-2.61639100
H	-5.87735100	-0.36187900	-1.88329300
H	-6.68337900	0.96424700	-1.04333200

Zero-point correction= 0.458872 (Hartree/Particle)

Thermal correction to Energy= 0.486720

Thermal correction to Enthalpy= 0.487664

Thermal correction to Gibbs Free Energy= 0.400634

Sum of electronic and zero-point Energies= -1759.261117

Sum of electronic and thermal Energies= -1759.233269

Sum of electronic and thermal Enthalpies= -1759.232325

Sum of electronic and thermal Free Energies= -1759.319355

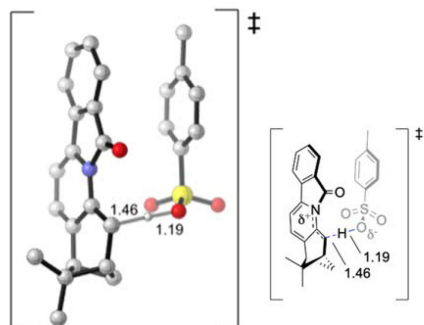
Single point energy: -1759.7199889

Total free energy: -1759.3193549

Number of imaginary frequencies: 0



TS2a



C	-0.55741800	1.01665700	-1.26998900
O	-0.35546700	0.48412100	-2.34272200
C	-1.75350400	1.70013700	-0.75942000
C	-2.97840300	1.90358800	-1.38652400
C	-1.50444000	2.14546100	0.54833100
C	-3.97156400	2.57160500	-0.66631100
C	-2.49605800	2.80636300	1.27056900
C	-3.73073800	3.01099000	0.64585100
C	-0.13633700	1.78273400	0.89816900
C	0.62185600	2.02420500	2.00032800
N	0.40575600	1.09347600	-0.19701100
C	1.98053200	1.58796900	2.01131800
C	1.68889600	0.58772800	-0.20060700
C	2.50675700	0.89009200	0.95935700
C	2.22905400	-0.22263600	-1.21505000
C	3.92937500	0.40292200	0.86813800
C	3.70667900	-0.55661300	-1.01583600
C	4.52390800	0.71879200	-0.56524600
C	4.22253700	2.07201000	-1.20944700
C	6.04136300	0.49492700	-0.61233500
C	3.88711300	-1.09528100	0.43329100
H	-3.15147900	1.54660900	-2.39674100
H	-4.94209400	2.74653900	-1.12012000
H	-2.31934900	3.14918100	2.28531300
H	-4.52136300	3.52159000	1.18808600
H	0.19846500	2.56445600	2.83934200
H	2.60639500	1.82537000	2.86671500
H	1.84811000	-0.09853500	-2.22441400
H	1.46860200	-1.43689000	-0.93457400
H	4.51476000	0.69423100	1.74535100
H	4.10010000	-1.14578900	-1.84938100
H	4.60122400	2.09305600	-2.23928700
H	3.15714100	2.31398900	-1.24594600
H	4.72436800	2.87468600	-0.65380500

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	6.38738900	0.49956800	-1.65384800
H	6.56187500	1.30484500	-0.08532800
H	6.35988200	-0.44999900	-0.16369900
H	3.07508800	-1.69971700	0.84188100
H	4.83889500	-1.60212800	0.59684200
C	-3.51099400	-0.88609600	1.02956500
C	-4.13616000	-1.07428200	-0.21087800
C	-3.41101700	-1.69992100	-1.23766800
C	-2.10636400	-2.14323100	-1.03333500
C	-1.50958700	-1.94914900	0.21503800
C	-2.20367800	-1.31757900	1.24920100
S	0.12200700	-2.60492900	0.55339600
O	-0.00786800	-4.03821300	0.86905900
O	0.88537400	-2.46785100	-0.80738200
C	-5.55550400	-0.61977800	-0.44036800
O	0.73081000	-1.73807000	1.58216400
H	-4.04702500	-0.38125600	1.82870400
H	-3.87310300	-1.83430600	-2.21252000
H	-1.54991300	-2.61264500	-1.83757200
H	-1.72015900	-1.15008200	2.20596400
H	-6.25738800	-1.45190700	-0.29374300
H	-5.83747900	0.17721500	0.25452700
H	-5.69595400	-0.25312200	-1.46281200

Zero-point correction= 0.452782 (Hartree/Particle)

Thermal correction to Energy= 0.480177

Thermal correction to Enthalpy= 0.481121

Thermal correction to Gibbs Free Energy= 0.395635

Sum of electronic and zero-point Energies= -1759.231733

Sum of electronic and thermal Energies= -1759.204338

Sum of electronic and thermal Enthalpies= -1759.203394

Sum of electronic and thermal Free Energies= -1759.288879

Single point energy: -1759.68451445

Total free energy: -1759.28887945

Number of imaginary frequencies:

1

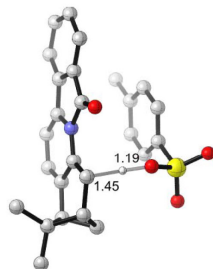
Frequencies -- -1428.0717

Red. masses -- 1.2064

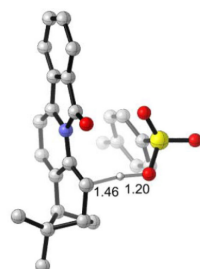
Frc consts -- 1.4496

IR Inten -- 10320.3909

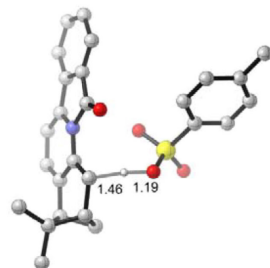


TS2a alternative conformations

Thermal correction to Gibbs Free Energy= 0.394365 (Hartree/Particle)
 Single point energy: -1759.6829728
 Total free energy: -1759.2886078
 ΔG (relative to lowest energy conformer): 0.2 kcal/mol



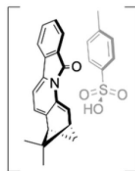
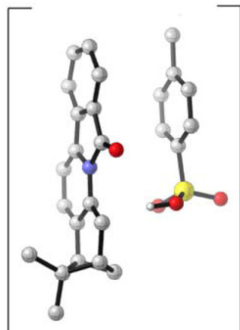
Thermal correction to Gibbs Free Energy= 0.393034 (Hartree/Particle)
 Single point energy: -1759.68058554
 Total free energy: -1759.28755154
 ΔG : 0.8 kcal/mol



Thermal correction to Gibbs Free Energy= 0.391846 (Hartree/Particle)
 Single point energy: -1759.67888435
 Total free energy: -1759.28703835
 ΔG : 1.2 kcal/mol



Complex-2a



C	0.49346000	-1.02897700	-1.34253200
O	0.26929900	-0.50470000	-2.42694000
C	1.70770400	-1.70579600	-0.85633800
C	2.90862700	-1.94508000	-1.51888200
C	1.50202400	-2.11171300	0.47341600
C	3.91582400	-2.61197600	-0.81957600
C	2.51310900	-2.76899000	1.17670100
C	3.71775800	-3.01396000	0.51348000
C	0.15646700	-1.71491300	0.85820700
C	-0.54583600	-1.85332000	2.00866900
N	-0.42034800	-1.08428600	-0.26722000
C	-1.89742200	-1.36637700	2.07387600
C	-1.74095200	-0.60342300	-0.26048200
C	-2.47618000	-0.76328400	1.00077000
C	-2.39952300	-0.04740600	-1.32247100
C	-3.89204900	-0.25132000	0.95800000
C	-3.83398500	0.37093000	-1.07580100
C	-4.62503500	-0.78903900	-0.34002200
C	-4.40085700	-2.24075800	-0.76224800
C	-6.13710500	-0.53346200	-0.29385400
C	-3.88306000	1.15089100	0.27342500
H	3.05040100	-1.61778600	-2.54434900
H	4.86573700	-2.81676300	-1.30415900
H	2.36901900	-3.08096600	2.20676700
H	4.51969100	-3.52523900	1.03853200
H	-0.08696000	-2.33884100	2.86284000
H	-2.46214800	-1.49509600	2.99266800
H	-1.96917600	-0.02282800	-2.31452700
H	-1.18159300	1.54798500	-1.04025100
H	-4.39765200	-0.37539200	1.92055000
H	-4.29154200	0.81659600	-1.96325500
H	-4.86474900	-2.42687100	-1.73935200
H	-3.34598900	-2.51448700	-0.83810500
H	-4.86994000	-2.91866400	-0.03758600

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	-6.57687000	-0.71380700	-1.28309400
H	-6.61564800	-1.22307300	0.41330200
H	-6.40376300	0.48399500	0.00471900
H	-3.03942700	1.80565000	0.49973100
H	-4.81602000	1.69335800	0.42990900
C	3.40060200	0.78727900	1.24903700
C	4.15707500	0.89586800	0.07445900
C	3.57992400	1.52536600	-1.04127400
C	2.29552100	2.05693600	-0.98523700
C	1.57077100	1.94210000	0.20527800
C	2.10915300	1.30687100	1.32545800
S	-0.01168500	2.74340700	0.36322000
O	0.16809700	4.19636100	0.38275600
O	-0.73675900	2.44207200	-1.05403100
C	5.56481600	0.36194000	0.00882200
O	-0.76047900	2.09216300	1.44398000
H	3.82225300	0.28278700	2.11370500
H	4.14528600	1.59618100	-1.96664000
H	1.85752400	2.53510100	-1.85493300
H	1.52589900	1.20981800	2.23462900
H	6.28779600	1.16149300	0.22006300
H	5.72568200	-0.43339100	0.74275900
H	5.79535000	-0.03175400	-0.98631700

Zero-point correction= 0.456821 (Hartree/Particle)

Thermal correction to Energy= 0.485038

Thermal correction to Enthalpy= 0.485982

Thermal correction to Gibbs Free Energy= 0.397438

Sum of electronic and zero-point Energies= -1759.234295

Sum of electronic and thermal Energies= -1759.206078

Sum of electronic and thermal Enthalpies= -1759.205134

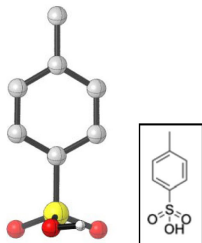
Sum of electronic and thermal Free Energies= -1759.293678

Single point energy: -1759.6911159

Total energy: -1759.2936779

Number of imaginary frequencies: 0



TsOH

C	-1.94852400	1.20745700	0.00835600
C	-2.66550800	0.00040300	-0.02081300
C	-1.95198900	-1.20817500	-0.00219400
C	-0.56008600	-1.22227100	0.04643500
C	0.12135800	-0.00357000	0.07333400
C	-0.55726400	1.21756300	0.05699000
H	-2.48780700	2.15075600	-0.00585300
H	-2.49340700	-2.15002300	-0.02469100
H	-0.01408000	-2.15946300	0.06557300
H	-0.00902900	2.15331400	0.08450300
S	1.89474700	-0.00492000	0.12296000
O	2.38582300	-1.27914000	0.65570600
O	2.17879300	0.03023800	-1.49620400
C	-4.17255800	0.00485400	-0.03995000
H	-4.56822300	0.05136300	0.98361000
H	-4.56221700	0.87436000	-0.57966300
H	-4.57007800	-0.90317900	-0.50463800
O	2.39114700	1.24612900	0.70469800
H	3.15020700	0.10620400	-1.63275000

Zero-point correction= 0.141777 (Hartree/Particle)

Thermal correction to Energy= 0.152755

Thermal correction to Enthalpy= 0.153699

Thermal correction to Gibbs Free Energy= 0.102371

Sum of electronic and zero-point Energies= -895.250978

Sum of electronic and thermal Energies= -895.240001

Sum of electronic and thermal Enthalpies= -895.239057

Sum of electronic and thermal Free Energies= -895.290385

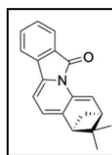
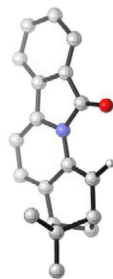
Single point energy: -895.392755863

Total free energy: -895.290384863

Number of imaginary frequencies: 0



Product



C	1.46781800	-1.33075700	-0.10098800
O	1.14001300	-2.51351700	-0.13736600
C	2.81499000	-0.74096800	0.02075500
C	4.05144700	-1.37532000	0.11786900
C	2.70162500	0.66047000	0.02625200
C	5.18902400	-0.57334400	0.22340000
H	4.11999900	-2.45927600	0.11087200
C	3.83995500	1.46311200	0.13223000
C	5.08032400	0.82939700	0.23064600
H	6.16982800	-1.03367900	0.30105200
H	3.76466600	2.54658300	0.13837600
H	5.98087100	1.43149300	0.31446600
C	1.29085300	0.98968800	-0.09488500
C	0.63337100	2.17323200	-0.14137300
N	0.58857100	-0.23741300	-0.16984000
C	-0.80019200	2.18453000	-0.27613600
H	1.19373000	3.09991400	-0.08026900
C	-0.81971000	-0.28272400	-0.29049400
C	-1.49837300	1.02005000	-0.35533200
H	-1.32213600	3.13620000	-0.31764200
C	-1.58781500	-1.40338300	-0.31547600
H	-1.16136400	-2.39303900	-0.24034900
C	-2.99365800	0.92607600	-0.50919200
H	-3.45726100	1.91450700	-0.58857100
C	-3.07835500	-1.19842500	-0.46061100
H	-3.62761400	-2.14366100	-0.49576500
C	-3.59024000	-0.08954500	0.54877400
C	-3.30056600	-0.15138100	-1.59735900
H	-2.62013200	-0.20552100	-2.45234000
H	-4.32948000	-0.09930300	-1.95642700
C	-3.04897500	-0.06124800	1.97793300
H	-3.35922400	0.86500100	2.47850400
H	-3.45673700	-0.90157500	2.55442600
H	-1.95958000	-0.12046800	2.02982600
C	-5.12152200	-0.01753500	0.61824600
H	-5.61048800	-0.03061100	-0.35968900

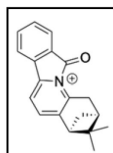
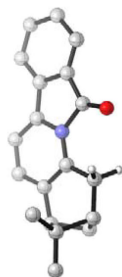


H	-5.51065500	-0.86802400	1.19261400
H	-5.43452400	0.89991900	1.13316700

Zero-point correction=	0.313078 (Hartree/Particle)
Thermal correction to Energy=	0.329128
Thermal correction to Enthalpy=	0.330072
Thermal correction to Gibbs Free Energy=	0.270566
Sum of electronic and zero-point Energies=	-863.958802
Sum of electronic and thermal Energies=	-863.942751
Sum of electronic and thermal Enthalpies=	-863.941807
Sum of electronic and thermal Free Energies=	-864.001313
Single point energy:	-864.27187935
Total free energy:	-864.00131335
Number of imaginary frequencies:	0



Int-2



C	1.47317700	-1.33764200	-0.09716600
O	1.08596600	-2.47391500	-0.14301300
C	2.80912300	-0.75179700	0.02128200
C	4.03679200	-1.39847800	0.11020400
C	2.70266500	0.64778500	0.03097500
C	5.17966400	-0.59773500	0.21166900
H	4.10087900	-2.48177600	0.10036800
C	3.83470600	1.44553400	0.13066000
C	5.07652400	0.79988800	0.22139200
H	6.15732400	-1.06358000	0.28315000
H	3.76432200	2.52819800	0.13876800
H	5.97897800	1.39845300	0.30063300
C	1.28917300	1.00567600	-0.08092600
C	0.64699900	2.22205500	-0.11245800
N	0.55352100	-0.15629300	-0.16036700
C	-0.75022800	2.23626900	-0.22902600
H	1.22209300	3.13822600	-0.04770300
C	-0.79319600	-0.18166900	-0.27962400
C	-1.47573800	1.05095000	-0.31703300
H	-1.27739200	3.18511600	-0.25304100
C	-1.57706000	-1.46766800	-0.35363500
H	-1.21870800	-2.06003000	-1.20338600
H	-1.37489800	-2.06617200	0.54190600
C	-2.97019000	0.96866800	-0.47039900
H	-3.43035600	1.95885800	-0.51323900
C	-3.07689000	-1.15624600	-0.50712800
H	-3.64965200	-2.08466500	-0.58709800
C	-3.55650100	-0.09345100	0.54440400
C	-3.00128600	-0.15002700	1.96846800
H	-3.32039000	0.73326400	2.53545300
H	-3.38793600	-1.03730600	2.48483500
H	-1.90947300	-0.19123500	2.01965900
C	-5.08669100	-0.00844800	0.62676000
H	-5.48029100	-0.88285800	1.15969000
H	-5.38672100	0.88675600	1.18524000
H	-5.57597200	0.03073200	-0.35061000



Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

C	-3.25263300	-0.06977100	-1.60167900
H	-2.55310800	-0.08584800	-2.44402000
H	-4.27151800	-0.00412900	-1.98436000

Zero-point correction= 0.326595 (Hartree/Particle)

Thermal correction to Energy= 0.342659

Thermal correction to Enthalpy= 0.343603

Thermal correction to Gibbs Free Energy= 0.284170

Sum of electronic and zero-point Energies= -864.423613

Sum of electronic and thermal Energies= -864.407549

Sum of electronic and thermal Enthalpies= -864.406605

Sum of electronic and thermal Free Energies= -864.466039

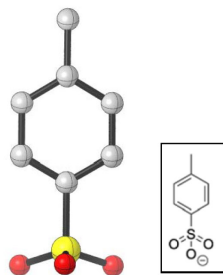
Single point energy: -864.750208299

Total free energy: -864.466038299

Number of imaginary frequencies: 0



OTs



C	1.93358500	-1.20057900	-0.00250900
C	2.65441400	0.00442200	0.01233000
C	1.93333700	1.20632400	-0.00290700
C	0.53699600	1.20978100	-0.03021800
C	-0.16443700	0.00266700	-0.04203800
C	0.53974800	-1.20566100	-0.03008500
H	2.47373100	-2.14524100	0.00383600
H	2.47158100	2.15177300	0.00328800
H	-0.01166900	2.14646200	-0.05485300
H	-0.00752600	-2.14326900	-0.05537400
S	-1.97373000	-0.00084900	0.00579600
O	-2.32350500	-0.04771200	1.45653000
O	-2.37890800	1.27061200	-0.66092500
C	4.16401700	-0.00324000	0.01722200
H	4.56040300	-0.28798800	-0.96674500
H	4.56982200	0.98334100	0.26432600
H	4.55812800	-0.72622200	0.74139900
O	-2.37518100	-1.22884400	-0.74052600

Zero-point correction= 0.131292 (Hartree/Particle)

Thermal correction to Energy= 0.141209

Thermal correction to Enthalpy= 0.142154

Thermal correction to Gibbs Free Energy= 0.094920

Sum of electronic and zero-point Energies= -894.808888

Sum of electronic and thermal Energies= -894.798970

Sum of electronic and thermal Enthalpies= -894.798026

Sum of electronic and thermal Free Energies= -894.845259

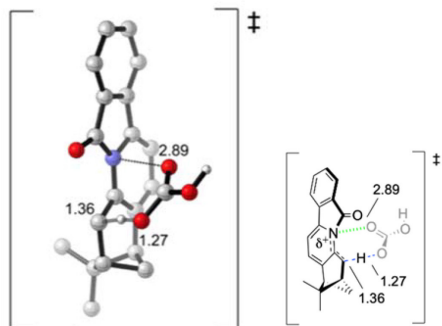
Single point energy: -894.940179852

Total free energy: -894.845259852

Number of imaginary frequencies: 0



TS2b



C	-1.44187800	0.20405900	1.14797900
O	-1.07564000	0.93581100	2.04119300
C	-2.79640300	-0.21616900	0.75823700
C	-4.01949300	0.14191200	1.31601000
C	-2.70550800	-1.10877800	-0.32035500
C	-5.17313000	-0.41629200	0.75886900
C	-3.85370200	-1.66779100	-0.87651800
C	-5.08691700	-1.30829000	-0.32231900
C	-1.29599000	-1.26409900	-0.66223300
C	-0.66901500	-1.96840900	-1.64458500
N	-0.55660800	-0.48670100	0.23171100
C	0.74726700	-1.88058400	-1.74672400
C	0.80171100	-0.32353500	0.13642400
C	1.46879300	-1.07545600	-0.90245200
C	1.56867400	0.54760000	0.95582000
C	2.96609900	-0.92107500	-0.93225400
C	3.07317200	0.45305500	0.68479400
C	3.55338600	-1.04122900	0.53331000
C	3.28069700	0.60463900	-0.84932200
H	-4.06878100	0.83276200	2.15215400
H	-6.14707500	-0.15890700	1.16412700
H	-3.79595000	-2.35858500	-1.71198400
H	-5.99799200	-1.72921500	-0.73777300
H	-1.24787300	-2.57697500	-2.32966200
H	1.26328800	-2.46144900	-2.50579800
H	1.24405500	0.64790800	1.98914500
H	1.21952100	1.79429100	0.52620200
H	3.42203300	-1.52548900	-1.72195900
H	3.63914100	1.10433100	1.35779500
O	1.03739000	3.00105200	0.17049500
C	0.02212300	3.04926200	-0.62517500
O	-0.69594100	2.12263300	-1.00990400
O	-0.21616500	4.33013900	-1.05214800
H	-0.99143900	4.27012800	-1.64037700

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	2.59718300	1.28198500	-1.37053100
H	4.30681600	0.83727000	-1.13694900
C	2.98755400	-2.10740000	1.47080600
H	3.25328000	-3.10963000	1.11034100
H	1.89939200	-2.07031400	1.56910200
H	3.41358500	-1.99123600	2.47553500
C	5.08215200	-1.17414800	0.52026900
H	5.37418700	-2.18139900	0.19640100
H	5.47953400	-1.02164800	1.53190600
H	5.57863800	-0.45761700	-0.14021800

Zero-point correction= 0.348519 (Hartree/Particle)

Thermal correction to Energy= 0.369391

Thermal correction to Enthalpy= 0.370335

Thermal correction to Gibbs Free Energy= 0.298212

Sum of electronic and zero-point Energies= -1128.928226

Sum of electronic and thermal Energies= -1128.907354

Sum of electronic and thermal Enthalpies= -1128.906410

Sum of electronic and thermal Free Energies= -1128.978533

Single point energy: -1129.2767451

Total free energy: -1128.9785331

Number of imaginary frequencies:

1

Frequencies -- -1454.8392

Red. masses -- 1.1677

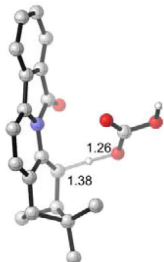
Frc consts -- 1.4562

IR Inten -- 11090.9759

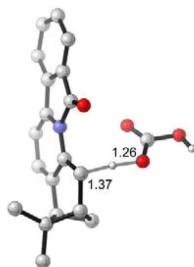


TS2b alternative conformations

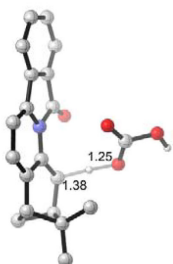
Thermal correction to Gibbs Free Energy= 0.299894 (Hartree/Particle)



Single point energy: -1129.27787337
 Total free energy: -1128.97797938
 ΔG (relative to lowest energy conformer): 0.3 kcal/mol

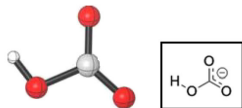


Thermal correction to Gibbs Free Energy= 0.298347 (Hartree/Particle)
 Single point energy: -1129.2760669
 Total free energy: -1128.9777199
 ΔG : 0.5 kcal/mol



Thermal correction to Gibbs Free Energy= 0.299873 (Hartree/Particle)
 Single point energy: -1129.27721464
 Total free energy: -1128.97734164
 ΔG : 0.7 kcal/mol



HCO₃⁻

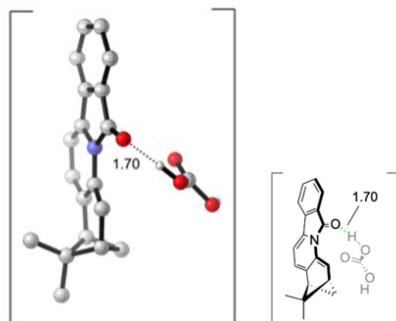
C	0.14740900	0.06222800	0.00004700
O	1.19921300	-0.60448400	-0.00004500
O	-0.04828700	1.30083300	0.00001100
O	-1.04176800	-0.73354300	0.00006700
H	-1.75771700	-0.07581600	-0.00054000

Zero-point correction= 0.026197 (Hartree/Particle)
 Thermal correction to Energy= 0.029718
 Thermal correction to Enthalpy= 0.030662
 Thermal correction to Gibbs Free Energy= 0.000425
 Sum of electronic and zero-point Energies= -264.494550
 Sum of electronic and thermal Energies= -264.491029
 Sum of electronic and thermal Enthalpies= -264.490085
 Sum of electronic and thermal Free Energies= -264.520322
 Single point energy: -264.520746874
 Total free energy: -264.520321874

Number of imaginary frequencies: 0



Complex-2b



C	-1.53407300	0.42830700	0.95883300
O	-1.19012200	1.35643800	1.71111000
C	-2.89212000	-0.02641800	0.64241500
C	-4.11757000	0.44270600	1.11340000
C	-2.80997300	-1.07594400	-0.29199300
C	-5.27538100	-0.16842500	0.63196500
C	-3.97061100	-1.68396800	-0.77617600
C	-5.19845700	-1.21965600	-0.30114900
C	-1.40448700	-1.29172800	-0.58244700
C	-0.76683100	-2.09301900	-1.46923900
N	-0.67567000	-0.38935900	0.23524700
C	0.66433400	-2.02110900	-1.59939300
C	0.73739300	-0.34383400	0.20458600
C	1.38685100	-1.17437100	-0.81751700
C	1.53444400	0.35131600	1.05635900
C	2.88480700	-1.03129700	-0.86240300
C	3.02100100	0.28921100	0.79928200
C	3.48794700	-1.21082800	0.59212400
C	3.23400700	0.48462600	-0.73498000
H	-4.16074200	1.25724000	1.83041400
H	-6.24835200	0.16821100	0.97802100
H	-3.92081400	-2.49341700	-1.49850300
H	-6.11546400	-1.67852100	-0.66023100
H	-1.34730900	-2.76708600	-2.09004500
H	1.16347900	-2.64868000	-2.33169100
H	1.13965500	0.92628500	1.88070600
H	-0.23990800	2.57617300	1.00375800
H	3.32583000	-1.61772200	-1.67432200
H	3.59019900	0.90778500	1.49842400
O	0.35334000	3.26607200	0.59480300
C	0.37526200	3.06033600	-0.71551600
O	-0.31293400	2.27360700	-1.33971600
O	1.29018900	3.87680200	-1.27406100
H	1.26476100	3.70790700	-2.23535000

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

H	2.56948400	1.19496200	-1.23514800
H	4.26716700	0.69920600	-1.01089000
C	2.91749600	-2.30643700	1.49151800
H	3.18720300	-3.29439400	1.09674300
H	1.82968800	-2.26809000	1.58233300
H	3.34052000	-2.22575700	2.50101900
C	5.01475000	-1.35597900	0.56931000
H	5.29359600	-2.35067600	0.19854900
H	5.41491400	-1.25623400	1.58634900
H	5.51928100	-0.61597700	-0.05782600

Zero-point correction= 0.354004 (Hartree/Particle)

Thermal correction to Energy= 0.375203

Thermal correction to Enthalpy= 0.376148

Thermal correction to Gibbs Free Energy= 0.303617

Sum of electronic and zero-point Energies= -1128.944450

Sum of electronic and thermal Energies= -1128.923251

Sum of electronic and thermal Enthalpies= -1128.922307

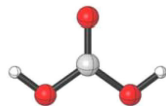
Sum of electronic and thermal Free Energies= -1128.994837

Single point energy: -1129.29845416

Total free energy: -1128.99483716

Number of imaginary frequencies: 0



H₂CO₃

O	1.08628900	-0.68312400	-0.00002900
H	1.86609700	-0.09493400	-0.00002900
C	0.00000000	0.10107500	-0.00009000
O	-1.08628800	-0.68312600	0.00006900
H	-1.86609500	-0.09493500	0.00015700
O	-0.00000100	1.31417700	0.00001200

Zero-point correction= 0.039122 {Hartree/Particle}

Thermal correction to Energy= 0.042862

Thermal correction to Enthalpy= 0.043806

Thermal correction to Gibbs Free Energy= 0.013206

Sum of electronic and zero-point Energies= -264.966649

Sum of electronic and thermal Energies= -264.962909

Sum of electronic and thermal Enthalpies= -264.961965

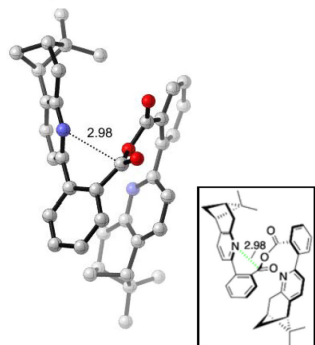
Sum of electronic and thermal Free Energies= -264.992565

Single point energy: -265.005771132

Total free energy: -264.992565132

Number of imaginary frequencies: 0



Anhydride intermediate

C	-0.13606400	-1.17922700	2.49518200
O	-0.33962700	-1.26989800	3.68138000
C	0.70670900	-2.11347600	1.69950800
C	1.79954800	-2.66740000	2.38115900
C	0.50495600	-2.39756800	0.32973000
C	2.71634800	-3.47856500	1.71672500
C	1.43602200	-3.22034000	-0.31965000
C	2.53361100	-3.75101200	0.35978700
C	-0.68918900	-1.91769000	-0.41290500
C	-0.57658500	-1.39691900	-1.70615200
N	-1.87524000	-2.01851000	0.22482800
C	-1.73473700	-0.98720100	-2.37035600
C	-2.97507500	-1.61493500	-0.41191200
C	-2.96373900	-1.10243600	-1.72913900
C	-4.30508100	-1.67958500	0.32004200
C	-4.29975800	-0.73875100	-2.32378100
C	-5.45311100	-1.22253800	-0.59839200
C	-5.12136500	0.14361300	-1.30255600
C	-4.38742900	1.22661000	-0.51385600
C	-6.35847900	0.77417300	-1.95642300
C	-5.28165500	-1.90487400	-1.98457400
O	-0.51577400	-0.08561700	1.73853100
C	-1.46277600	0.82494800	2.15617900
O	-2.36342400	0.53574900	2.90978900
C	-1.30439300	2.14523800	1.48415400
C	-2.41917000	2.99662700	1.59529600
C	-0.19485900	2.54398600	0.69727600
C	-2.47246600	4.20986800	0.92083100
C	-0.28412200	3.76043100	0.00144500
C	1.09733300	1.80358600	0.57043000
C	-1.40215800	4.58507400	0.10462600
C	1.86278400	1.46224700	1.69114000
N	1.52205100	1.57600400	-0.68779700
C	3.09327800	0.83468700	1.50238300

Efficient synthesis of isoindolones by intramolecular cyclisation of pyridinylbenzoic acids

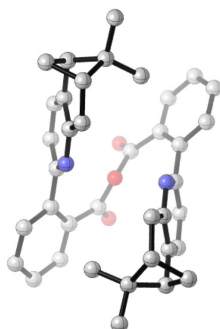
C	2.70600300	0.97871700	-0.86161200
C	3.53366400	0.57606600	0.20627900
C	3.20483600	0.75592100	-2.28003700
C	4.83505400	-0.08821400	-0.16117700
C	4.58191200	0.06718200	-2.27122800
C	5.57310600	0.76490400	-1.27019000
C	4.52359500	-1.12874000	-1.28221400
C	5.57099700	2.29061200	-1.15785800
C	7.02199200	0.30071900	-1.47509100
H	1.93447900	-2.43073800	3.43174100
H	3.56628800	-3.89072300	2.25239400
H	1.28092200	-3.46691200	-1.36587400
H	3.23899000	-4.38408000	-0.17098000
H	0.39737100	-1.30106100	-2.17523400
H	-1.67800200	-0.57786400	-3.37608800
H	-4.24171600	-1.05659900	1.22230000
H	-4.48225100	-2.70766700	0.66232300
H	-4.21734100	-0.38784700	-3.35738000
H	-6.42229500	-1.30148100	-0.09398600
H	-5.03874500	1.63665400	0.26839400
H	-3.47719300	0.86852300	-0.03058700
H	-4.10067200	2.05493000	-1.17430000
H	-7.01952200	1.19128100	-1.18574500
H	-6.06084400	1.59568000	-2.62052500
H	-6.94712000	0.06669000	-2.54799000
H	-4.86516700	-2.91815400	-1.99703800
H	-6.18961300	-1.88557700	-2.59004200
H	-3.25779600	2.66943700	2.19969900
H	-3.34559100	4.84831100	1.01677900
H	0.55588400	4.06363700	-0.61577500
H	-1.43270300	5.52255800	-0.44370800
H	1.49354500	1.67714700	2.68903200
H	3.70590600	0.55309800	2.35535900
H	3.24599400	1.72122800	-2.80173000
H	2.47988800	0.13721800	-2.82659300
H	5.41015300	-0.39964300	0.71657600
H	4.94432100	-0.11220300	-3.28938200
H	3.57267600	-1.66234800	-1.20272300
H	5.32785100	-1.85256500	-1.42635100
H	5.98212500	2.73729400	-2.07234500
H	4.57700100	2.71662300	-0.99911300
H	6.20345000	2.61057900	-0.31948600
H	7.43571700	0.74864900	-2.38781100
H	7.64769800	0.62205700	-0.63264500
H	7.12202100	-0.78488000	-1.56640700

Zero-point correction= 0.654790 (Hartree/Particle)
Thermal correction to Energy= 0.690528



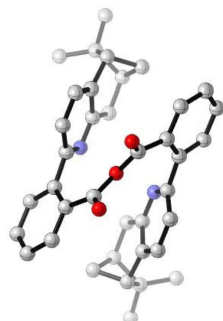
Thermal correction to Enthalpy=	0.691472
Thermal correction to Gibbs Free Energy=	0.585613
Sum of electronic and zero-point Energies=	-1804.386831
Sum of electronic and thermal Energies=	-1804.351093
Sum of electronic and thermal Enthalpies=	-1804.350149
Sum of electronic and thermal Free Energies=	-1804.456008
Single point energy:	-1805.04162086
Total free energy:	-1804.45600786
Number of imaginary frequencies:	0

Anhydride intermediate alternative conformations

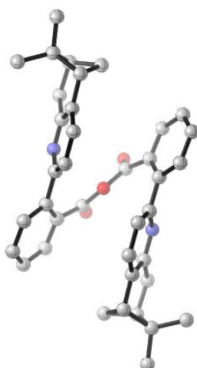


Thermal correction to Gibbs Free Energy=	0.588524 (Hartree/Particle)
Single point energy:	-1805.04376414
Total free energy:	-1804.45524014
ΔG (relative to lowest energy conformer):	0.5 kcal/mol





Thermal correction to Gibbs Free Energy= 0.587995 (Hartree/Particle)
Single point energy: -1805.04268603
Total free energy: -1804.45469103
 ΔG : 0.8 kcal/mol



Thermal correction to Gibbs Free Energy= 0.587094 (Hartree/Particle)
Single point energy: -1805.04170734
Total free energy: -1804.45461334
 ΔG : 0.9 kcal/mol



References

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2. A. V. Malkov, M. Bell, M. Vassieu, V. Bugatti, P. Kočovský *J. Mol. Catal. A: Chem.* 2003, **196**, 179–186.
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