

UC Davis

UC Davis Previously Published Works

Title

Computational Approaches to Predicting the Impact of Novel Bases on RNA Structure and Stability

Permalink

<https://escholarship.org/uc/item/4kd942pn>

Journal

ACS Chemical Biology, 8(11)

ISSN

1554-8929

Authors

Harrison, Jason G

Zheng, Yvonne B

Beal, Peter A

et al.

Publication Date

2013-11-15

DOI

10.1021/cb4006062

Peer reviewed



Published in final edited form as:

ACS Chem Biol. 2013 November 15; 8(11): . doi:10.1021/cb4006062.

Computational approaches to predicting the impact of novel bases on RNA structure and stability

Jason G. Harrison, Yvonne B. Zheng, Peter A. Beal, and Dean J. Tantillo

Department of Chemistry, University of California–Davis, Davis, CA 95616

Peter A. Beal: beal@chem.ucdavis.edu; Dean J. Tantillo: djtantillo@ucdavis.edu

Abstract

The use of computational modelling techniques to gain insight into nucleobase interactions has been a challenging endeavor to date. Accurate treatment requires the tackling of many challenges, but also holds the promise of great rewards. The development of effective computational approaches to predict the binding affinities of nucleobases and analogs can, for example, streamline the process of developing novel nucleobase modifications, which should facilitate the development of new RNAi-based therapeutics, for example. This brief review focuses on available computational approaches to predicting base pairing affinity in RNA-based contexts such as nucleobase-nucleobase interactions in duplexes and nucleobase-protein interactions. The challenges associated with such modelling along with potential future directions for the field are highlighted.

Introduction

Chemical biologists are increasingly captivated by the study of ribonucleic acid (RNA) both for its chemical properties and its diverse biological functions.^(1–5) RNAs can adopt complex three-dimensional shapes and can catalyze a wide range of different chemical reactions.⁽⁶⁾ Functional RNAs can also be small and accessible by total chemical synthesis.⁽⁷⁾ In addition, RNAs have wide-ranging biological properties as essential structural components of cells, information storage and retrieval systems, catalysts and regulators of gene expression. The basic structural component of folded RNAs is the Watson-Crick base-paired double helix (double-stranded RNA, dsRNA) (Figure 1). While dsDNA tends to adopt the familiar B-form helix with well-defined major and minor grooves, dsRNA's structure is different. Duplex RNA favors the A'-form helical structure, where the minor groove is very shallow and wider than the major groove, which is now quite narrow but deep (Figure 1). RNA also regularly adopts structures with loops and single-stranded regions. The base pairs in A'-form RNA are twisted with respect to one another, and are not perpendicular to the primary axis (as in B form DNA). Furthermore, some base pairs in RNA involve non-canonical interactions or protonated bases,^(8, 9) and, in addition to the common four ribonucleosides A, G, C and U, naturally occurring RNAs frequently contain nucleoside analogs (Figure 2).^(10, 11) These modifications of the typical RNA structure extend the functional properties of the RNA beyond those possible without them. Chemists have also introduced nonnatural nucleosides into RNA that impart properties not possible with the native RNA structure alone. This has become even more common recently with increased focus on the therapeutic potential of small RNAs (e.g. siRNAs that induce target knockdown via RNAi) that are easily prepared by chemical synthesis.⁽²⁾ Given the nearly infinite chemical space that could be explored in the development of nucleoside analogs for

use in RNA, there is a need for rapid methods that can be used to filter structures prior to testing. In addition, our fundamental understanding of how changes in nucleoside structure translate into changes in the RNA fold and/or stability is still limited. Thus, the question arises: Can currently available computational methods be helpful in predicting the effects on RNA structure and stability of modified nucleotides, particularly those with novel nucleobase structures that may alter base pairing interactions? Here we review methods that one might consider when trying to address this question and highlight particularly promising approaches.

Available Computational Methods

Bioinformatics

There is a wealth of literature describing attempts to use computational methods to provide understanding of the physical factors that control RNA structure.^(15–17) One approach to predicting various RNA-related phenomena (structures and reactivities),⁽¹⁸⁾ is to make use of statistical/data-mining/informatics methods.^(19–22) These methods, however, are only able to make effective predictions when large enough databases of relevant experimental information are available. Along these lines, much effort has gone toward prediction of the thermodynamics of RNA folding, i.e., predicting secondary structure preferences based on sequences, although the ability to predict secondary structure without the guidance of some experimental data is limited.^(23–29) A recent success in secondary structure prediction is the development of CONTRAfold, which makes use of “fully-automated statistical learning algorithms”⁽³⁰⁾

Explicit Interactions—General Concerns

We focus herein, however, on computational chemistry approaches aimed at predicting base-pairing proficiencies by explicitly taking into account interatomic interactions. The primary challenge in this area is that the systems under investigation are very large (by computational standards), necessitating the use of small model systems (which may unintentionally lack important structural features) or fast computational methods (which may not be able to answer all questions of interest with sufficient accuracy). For example, molecular mechanics (MM) and MM-based molecular dynamics (MD) calculations allow for the investigation of full-sized RNA oligomers, but these methods are based on classical mechanics, and as such, neglect or approximate interactions of quantum mechanical (QM) origin. There are many QM methods that can be used to treat these interactions, but these require considerable computational resources (especially, time).

Classical Mechanics

MM and MD methods have been applied successfully to modeling DNA and RNA.^(31–40) Issues facing these approaches, however, include appropriate treatment of nucleobase polarizability and subtle electrostatic effects.^(41, 42) Although well-parameterized MM force-field methods can faithfully reproduce higher order interaction energies,⁽⁴³⁾ there is room for improvement in accurate prediction of RNA macrostructure.⁽⁴⁴⁾ Without a doubt, improvements to these methods will help facilitate the rapid computation of key properties of RNA oligomers.⁽⁴⁵⁾

Quantum Mechanics

Among the QM approaches available for studying RNA, semi-empirical QM methods have been found to be less successful than *ab initio* and density functional theory (DFT) methods for many systems, while providing extremely accurate results for some specific cases.^(46–50) Various high-level quantum mechanical (e.g., post-Hartree Fock) methods can very accurately capture features that depend on, for example, electron correlation and dispersion

effects,^(51, 52) but due to computational expense, most investigations have so far utilized small model systems, containing only one or two base pairs, designed to capture specific effects (e.g., π -stacking,^(53, 54) hydrogen bonding,⁽⁵⁵⁾ sterics/shape⁽⁵⁶⁾, electrostatics),^(51, 57, 58) rather than the combination of these effects that is manifested experimentally. Proper modeling of these combined effects are no small task, and ongoing efforts have been led by the Šponer and Hobza laboratories for both RNA and DNA systems, among others.^(59–61) Even these “small” model systems are large enough to prohibit the use of the highest levels of theory, and studies aimed at determining which methods are appropriate for probing which interactions are ongoing.^(62, 63)

Nonetheless, these studies have provided key insights into the forces that contribute to RNA structure, for example, in the area of π -stacking, an important contributor to the total interaction energies between nucleobases. For example, utilizing very high levels of theory (coupled cluster methods) on the interaction of benzene dimers, Sinnokrot and Sherrill obtained benchmark-quality data to be used in the creation of more accurate force-fields.⁽²⁷⁾ In addition, some DFT methods can reproduce π -stacking interactions with accuracy comparable to that from much higher levels of theory, at least in select cases.⁽⁶⁰⁾

There have also been some successes in the reproduction of base pair affinities using approaches that neglect the sugar-phosphate backbone but focus only on particular free nucleobases. In the case of known base pairs, only small changes to geometric features associated with hydrogen-bonding and π -stacking compared to diffraction data have been found when using such models.⁽⁵⁵⁾ The essential role of hydrogen-bonding in nucleic acid structure has also been amply demonstrated using very high level QM methods.⁽⁶⁴⁾ Nonetheless, further work is needed on larger systems that incorporate structural features beyond the base pair in order to account for distortions (e.g., helical strain, propeller twist) imposed by the larger superstructure of the RNA duplex^(57, 65)—distortions that likely contribute not only to magnitudes of interaction energies between bases, but also to base-pairing specificity and sequence effects.⁽⁶⁶⁾

Recent Successes

Interaction Energies from QM Using Experimentally Determined Nucleobase Geometries

Recently, Znosko, Lewis and co-workers described a study that appears to dispel two myths in the field of RNA (and DNA) structure prediction: 1) average fiber diffraction data does not provide reliable geometries for predicting interaction energies between bases, and 2) stacking energies from QM calculations are not accurate enough to predict RNA structures.⁽⁶⁷⁾ Using nucleobase geometries from average diffraction data and QM (MP2) energy calculations, these researchers were able to demonstrate a correlation between predicted and experimental nearest-neighbor binding energies. Although additional work will be required to make such predictions quantitatively accurate, this approach appears to have promise for the prediction of not only base-pairing partners, but also stacking preferences, however this has not been without debate.^(68, 69)

Kamya and Muchall utilized a similar approach (X-ray crystal structure data for nucleobase geometries and the PBE0 DFT method for energy calculations, along with QTAIM⁽⁷⁰⁾ [Quantum Theory of Atoms In Molecules] analysis of electron density) to quantify hydrogen bonding and π -stacking interactions.⁽⁷¹⁾ On the basis of their results, these researchers suggested that conflicting experimental and computational evidence on the relative strengths of A•T and A•U base pairs⁽⁶⁴⁾ is likely a manifestation of sequence effects, i.e., the context in which such base pairs occur can tip the balance.

Together, these studies demonstrate the benefits of employing experimental structural information in QM-based calculations on base-pairing affinities. By doing so, approximations associated with using MM methods to predict geometries of large systems or using small model systems for geometry optimization with QM methods are avoided. The structural consequences of the many small interactions contributing to stable base pairing are present in the experimentally determined geometries, which can then be analyzed utilizing a reliable QM method. The ever-growing library of experimentally characterized RNA structures will allow such approaches to be further refined, opening the door toward a richer understanding of the fundamental interactions at play.

Automated Docking

While the approaches just described are very useful for teasing out the origins of base pairing affinities, they are still computationally demanding. If one wishes to survey a wide variety of nucleobase analogs (experimentally available or proposed) so as to predict their binding propensities in particular contexts, then a more rapid and computationally tractable approach is required. Automated computational docking may provide such an approach.^(72, 73) Since such methods are often utilized, for example, to screen large libraries of potential drug leads, they are fast. Consequently, these methods generally employ MM force fields, of varying sophistication, to assign scores to small molecule-protein binding orientations (poses). The application of such methods to problems in nucleic acid chemistry,^(74–76) however, is an under-explored area that appears to have potential.

In one recent application, Brenk and co-workers used a computational docking approach (DOCK 3.5.54 with small modifications for RNA) to predict the binding of a variety of nucleobase analogs to adenine-binding riboswitches whose structures had been determined using X-ray crystallography.⁽⁷⁷⁾ Binding modes for analog-riboswitch complexes that had already been determined experimentally were successfully reproduced via docking. The docking calculations were also able to distinguish known binders from structurally related decoy molecules, although not infallibly. A strong correlation between docking scores and known dissociation constants was not observed, but this is a problem that is not particular to docking with RNA receptors. Some successful predictions of commercially available compounds that might bind to these riboswitches were also made. Studies of this type show that docking of nucleobases and nucleobase analogs into RNA binding sites is feasible, even using docking software not designed specifically for use with RNA.^(57, 65, 66, 78–82)

Desirable Data

The types of calculations highlighted above require experimental structures as starting points limiting the number of systems that can be explored using computational methods. There is obviously a need for additional structural data if new nucleobase analogs with desired functions are to be designed rationally or obtained from molecular docking screens. For instance, recent reports of the structure of human Ago2, the key nuclease of the RNAi pathway, bound to guide strand RNA provide an opportunity for siRNA optimization using computational screening for nucleoside analogs that bind well to this protein (Figure 3).⁽⁸⁵⁾ At this point, although relatively few structures are available, different duplex RNA structures have been reported, including some that contain nucleobase analogs.⁽⁵⁾ For instance, structural studies and UV melting (T_M) data for duplex RNAs containing the uridine mimic ribo-2,4-difluorotoluy indicate that this modification is destabilizing opposite A and induces numerous local structural changes that subtly effect the geometry of the RNA duplex; this modified nucleotide does, however, appear to bind more tightly to G, in which case less structural distortion is observed (Figure 4).^(83, 84) This experimental work fed directly into computational work, as this study also described QM (MP2) calculations on difluorotoluy-containing base pairs; these calculations reproduced the order of base pair

stabilities determined experimentally.⁽⁷⁶⁾ The availability of additional structural and thermodynamic data of this type will no doubt facilitate computational approaches aimed at identifying new nucleoside analogs that can be used to deepen our understanding of these fundamental interactions.

Computational Approaches for the Future

While the work of Brenk and coworkers showed that computational docking could be applied to RNA to discover new ligands for an RNA-based receptor, the question remains whether such tools can be used to predict how well a nucleobase analog attached *covalently* in place of a particular nucleobase will bind to its neighbors. One can envision many possible computational approaches to this problem. For example, a layered approach could be used in which the geometries of the analog and the nucleobases interacting directly with it are optimized with QM methods, leaving the rest of the duplex frozen to an experimentally determined geometry. Šponer and co-workers recently have made methodological advances toward this approach.⁽⁶⁶⁾ Alternatively, one could utilize the recently described fragment-based⁽⁸⁶⁾ QM approaches developed by Raghavachari and co-workers (and others) for treating large molecules such as DNA oligomers.⁽⁸⁷⁾ A third option would be to repurpose existing computational docking software to predict the binding proficiency of a nucleobase analog. Finally, with better coding and faster computers, high-level dynamical approaches will become more viable. While there are challenges to overcome with each of these methods, computational (bio)chemistry will likely play an important role in the future development of new nucleobase analogs capable of stable and specific pairing in RNA.

Acknowledgments

P.A.B. acknowledges the National Institutes of Health for financial support in the form of grant R01-GM080784. J.G.H. acknowledges a Graduate Assistance in Areas of National Need (GAANN) fellowship.

Keywords

Nucleobase	structural component of a nucleic acid often involved in base pairing
RNAi	RNA interference, the process of cleavage and ultimately degradation of a specifically targeted RNA induced by an added RNA duplex containing a strand matching the target sequence
Ago2	nuclease responsible for cleavage of target RNA in RNAi, binds one of the strands of a short interfering RNA duplex and uses it as a guide for target identification
Riboswitch	an RNA species involved in regulation of gene expression containing a ligand-binding aptamer domain and expression platform
DFT	Density functional theory, a quantum mechanical method that uses the electron density to calculate molecular properties, as opposed to calculation of the wavefunction in <i>ab initio</i> methods
Classical Mechanics	Method for description of objects, including molecules, using Newton's laws of motion. This could be a description using potential energy (MM) only, or potential and kinetic energy together (MD)
Quantum Mechanics	Class of methods for describing the properties of molecules using the principles of quantum physics

Automated Docking

Computational methods (usually molecular mechanics-based) designed to describe and predict interactions between small and large molecules

Notes and references

1. Armitage BA. Imaging RNA in live cells. *Curr Opin Chem Biol.* 2011; 15:806–812. [PubMed: 22055496]
2. Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. *Chem Biol.* 2012; 19:60–71. [PubMed: 22284355]
3. Guan L, Disney MD. Recent advances in developing small molecules targeting RNA. *ACS Chem Biol.* 2012; 7:73–86. [PubMed: 22185671]
4. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Lindahl T, Pan T, Yang YG, He C. N⁶-methyladenosine in nuclear RnA is a major substrate of the obesity-associated FTO. *Nature Chem Biol.* 2011; 7:885–887. [PubMed: 22002720]
5. Phelps K, Morris A, Beal PA. Novel Modifications in RNA. *ACS Chem Biol.* 2012; 7:100–109. [PubMed: 22148740]
6. Doudna JA, Cech TR. The chemical repertoire of natural ribozymes. *Nature.* 2002; 418:222–228. [PubMed: 12110898]
7. Beaucage SL. Solid-phase synthesis of siRNA oligonucleotides. *Curr Opin Drug Discov Dev.* 2008; 11:203–216.
8. Nagaswamy ULS, Maia, Hury James, Collins Shakkala, Zhang Zhengdong, Zhao Qin, Fox George E. NCIR: a database of non-canonical interactions in known RNA structures. *Nuc Acids Res.* 2002; 30:395–397.
9. Leontis NB, Lescoute A, Westhof E. *Curr Opin Struc Biol.* 2006; 16:279–287.
10. Cantara WA, Crain PF, Rozenski J, McCloskey JA, Harris KA, Zhang X, Vendeix FA, Fabris D, Agris PF. The RNA Modification Database, RNAMDB: 2011 update. *Nucleic Acids Res.* 2011; 39:D195–201. [PubMed: 21071406]
11. Genesilico. [Accessed Sept. 15th 2013] Modomics: A Database of RNA Modification Pathways. <http://modomics.genesilico.pl/>
12. Wedler HB, Cohen SR, Davis RL, Harrison JG, Siebert MR, Willenbring D, Hamann CS, Shaw JT, Tantillo DJ. Applied Computational Chemistry for the Blind and Visually Impaired. *J Chem Educ.* 2012; 89:1400–1404.
13. Keqiong Ye TJ, Malinina L, Patel DJ. Recognition of small interfering RNA by a viral suppressor of RNA silencing. *Nature.* 2003; 426:874–878. [PubMed: 14661029]
14. Voorhees RM, Mandal D, Neubauer C, Kohrer C, RajBhandary UL, Ramakrishnan V. The structural basis for specific decoding of AUA by isoleucine tRNA on the ribosome. *Nature Struct Mol Biol.* 2013; 20:641–643. [PubMed: 23542153]
15. Li L, Xu JZ, Yang DY, Tan XR, Wang HF. Computational approaches for microRNA studies: a review. *Mamm Genome.* 2010; 21:1–12. [PubMed: 20012966]
16. Šponer J.; Lankas, F. Computational studies of RNA and DNA. Springer; Dordrecht, The Netherlands: 2006.
17. Šponer J, Šponer JE, Petrov AI, Leontis NB. Quantum Chemical Studies of Nucleic Acids: Can We Construct a Bridge to the RNA Structural Biology and Bioinformatics Communities? *J Phys Chem B.* 2010; 114:15723–15741. [PubMed: 21049899]
18. Bundschuh R. Computational prediction of RNA editing sites. *Bioinformatics.* 2004; 20:3214–3220. [PubMed: 15231535]
19. Agius P, Bennett KP, Zuker M. Comparing RNA secondary structures using a relaxed base-pair score. *RNA-Publ RNA Soc.* 2010; 16:865–878.
20. Samuel, CF.; Yaqi, WAN.; Rick, R.; Russ, BA. Biocomputing 2010. WORLD SCIENTIFIC; 2009. Predicting RNA Structure by Multiple Template Homology Modeling; p. 216-227.
21. Gerlach W, Giegerich R. GUUGle: a utility for fast exact matching under RNA complementary rules including G-U base pairing. *Bioinformatics.* 2006; 22:762–764. [PubMed: 16403789]

22. Cao S, Chen SJ. Predicting RNA folding thermodynamics with a reduced chain representation model. *RNA-Publ RNA Soc.* 2005; 11:1884–1897.
23. Butcher SE, Pyle AM. The Molecular Interactions That Stabilize RNA Tertiary Structure: RNA Motifs, Patterns, and Networks. *Acc Chem Res.* 2011; 44:1302–1311. [PubMed: 21899297]
24. Capriotti E, Marti-Renom MA. Computational RNA structure prediction. *Curr Bioinf.* 2008; 3:32–45.
25. Laing C, Schlick T. Computational approaches to 3D modeling of RNA. *J Phys Cond Matt.* 2010; 22:283101.
26. Laing C, Schlick T. Computational approaches to RNA structure prediction, analysis, and design. *Curr Opin Struct Biol.* 2011; 21:306–318. [PubMed: 21514143]
27. Nam KH, Gaot JL, York DM. Quantum mechanical/molecular mechanical simulation study of the mechanism of hairpin ribozyme catalysis. *J Am Chem Soc.* 2008; 130:4680–4691. [PubMed: 18345664]
28. Shi BY, Cho SS, Thirumalai D. Folding of Human Telomerase RNA Pseudoknot Using Ion-Jump and Temperature-Quench Simulations. *J Am Chem Soc.* 2011; 133:20634–20643. [PubMed: 22082261]
29. Weeks KM, Mauger DM. Exploring RNA Structural Codes with SHAPE Chemistry. *Acc Chem Res.* 2011; 44:1280–1291. [PubMed: 21615079]
30. Do CB, Woods DA, Batzoglou S. CONTRAfold: RNA secondary structure prediction without physics-based models. *Bioinformatics.* 2006; 22:e90–e98. [PubMed: 16873527]
31. Reha D, Hocek M, Hobza P. Exceptional thermodynamic stability of DNA duplexes modified by nonpolar base analogues is due to increased stacking interactions and favorable solvation: Correlated ab initio calculations and molecular dynamics simulations. *Chem - Eur J.* 2006; 12:3587–3595. [PubMed: 16502452]
32. Bevilacqua PC, SantaLucia J. The biophysics of RNA. *ACS Chem Biol.* 2007; 2:440–444. [PubMed: 17649965]
33. Ditzler MA, Otyepka M, Sponer J, Walter NG. Molecular Dynamics and Quantum Mechanics of RNA: Conformational and Chemical Change We Can Believe In. *Acc Chem Res.* 2010; 43:40–47. [PubMed: 19754142]
34. Cheatham, TE., III; Case, DA. Using Amber to Simulate DNA and RNA. In: Jiri Sponer, FL., editor. *Computational studies of RNA and DNA.* Springer; Dordrecht, The Netherlands: 2006. p. 45-72.
35. McDowell SE, Spackova N, Sponer J, Walter NG. Molecular dynamics simulations of RNA: An in silico single molecule approach. *Biopolymers.* 2007; 85:169–184. [PubMed: 17080418]
36. Vendeix FAP, Munoz AM, Agris PF. Free energy calculation of modified base-pair formation in explicit solvent: A predictive model. *RNA-Publ RNA Soc.* 2009; 15:2278–2287.
37. Špa ková, Na; Cheatham, T., III; Šponer, J. Molecular Dynamics Simulations of Nucleic Acids. In: Šponer, J.; Lankaš, F., editors. *Computational Studies of RNA and DNA.* Springer; Netherlands: 2006. p. 301-325.
38. Auffinger, P.; ACV. *Molecular Dynamics Simulations of RNA Systems.* WILEY-VCH Verlag GmbH & Co., KGaA; Weinheim: 2005.
39. Krepl M, Zgarbová M, Stadlbauer P, Otyepka M, Banáš P, Ko a J, Cheatham TE, Jure ka P, Šponer J. Reference Simulations of Noncanonical Nucleic Acids with Different χ Variants of the AMBER Force Field: Quadruplex DNA, Quadruplex RNA, and Z-DNA. *J Chem Theory Comput and Comp.* 2012; 8:2506–2520.
40. Sanbonmatsu, K.; Blanchard, S.; Whitford, P. Molecular Dynamics Simulations of the Ribosome. In: Dinman, JD., editor. *Biophysical approaches to translational control of gene expression.* Springer; New York: 2013. p. 51-68.
41. Neidle, S. *Principles of Nucleic Acid Structure.* Academic Press; New York: 2008. 6 - RNA Structures and Their Diversity; p. 204-248.
42. Cooper VR, Thonhauser T, Puzder A, Schroder E, Lundqvist BI, Langreth DC. Stacking interactions and the twist of DNA. *J Am Chem Soc.* 2008; 130:1304–1308. [PubMed: 18163624]

43. Kolar M, Berka K, Jurecka P, Hobza P. On the Reliability of the AMBER Force Field and its Empirical Dispersion Contribution for the Description of Noncovalent Complexes. *ChemPhysChem*. 2010; 11:2399–2408. [PubMed: 20629063]
44. Yildirim I, Turner DH. RNA challenges for computational chemists. *Biochem*. 2005; 44:13225–13234. [PubMed: 16201748]
45. Auffinger, P. Molecular Dynamics Simulations of RNA Systems: Importance of the Initial Conditions. In: Šponer, J.; Lankaš, F., editors. *Computational Studies of RNA and DNA*. Springer; Netherlands: 2006. p. 283-300.
46. Hobza P, Kabelac M, Šponer J, Mejzlik P, Vondrasek J. Performance of empirical potentials (AMBER, CFF95, CVFF, CHARMM, OPLS, POLTEV), semiempirical quantum chemical methods (AM1, MNDO/M, PM3), and ab initio Hartree-Fock method for interaction of DNA bases: Comparison with nonempirical beyond Hartree-Fock results. *J Comp Chem*. 1997; 18:1136–1150.
47. McNamara JP, Hillier IH. Semi-empirical molecular orbital methods including dispersion corrections for the accurate prediction of the full range of intermolecular interactions in biomolecules. *Phys Chem Chem Phys*. 2007; 9:2362–2370. [PubMed: 17492099]
48. Radak BK, Harris ME, York DM. Molecular Simulations of RNA 2'-O-Transesterification Reaction Models in Solution. *The Journal of Physical Chemistry B*. 2012; 117:94–103. [PubMed: 23214417]
49. Giese TJ, Gregersen BA, Liu Y, Nam K, Mayaan E, Moser A, Range K, Faza ON, Lopez CS, Lera ARd, Schaftenaar G, Lopez X, Lee TS, Karypis G, York DM. QCRNA 1.0: A database of quantum calculations for RNA catalysis. *Journal of Molecular Graphics and Modelling*. 2006; 25:423–433. [PubMed: 16580853]
50. Lee, T-S.; Giambra u, GM.; York, DM. Chapter 10 - Insights into the Role of Conformational Transitions and Metal Ion Binding in RNA Catalysis from Molecular Simulations. In: Ralph, AW., editor. *Annual Reports in Computational Chemistry*. Elsevier; 2010. p. 168-200.
51. Riley KE, Pito ák M, Jure ka P, Hobza P. Stabilization and Structure Calculations for Noncovalent Interactions in Extended Molecular Systems Based on Wave Function and Density Functional Theories. *Chem Rev*. 2010; 110:5023–5063. [PubMed: 20486691]
52. Riley KE, Hobza P. Noncovalent interactions in biochemistry. *Wiley Interdisciplinary Reviews: Computational Molecular Science*. 2011; 1:3–17.
53. Hobza P, Šponer J. Toward True DNA Base-Stacking Energies: MP2, CCSD(T), and Complete Basis Set Calculations. *J Am Chem Soc*. 2002; 124:11802–11808. [PubMed: 12296748]
54. Villani G. Theoretical Investigation of the Coupling between Hydrogen-Atom Transfer and Stacking Interaction in Adenine–Thymine Dimers. *ChemPhysChem*. 2013; 14:1256–1263. [PubMed: 23494877]
55. Fonseca Guerra C, Bickelhaupt FM, Snijders JG, Baerends EJ. Hydrogen Bonding in DNA Base Pairs: Reconciliation of Theory and Experiment. *J Am Chem Soc*. 2000; 122:4117–4128.
56. Pallan PS, Greene EM, Jicman PA, Pandey RK, Manoharan M, Rozners E, Egli M. Unexpected origins of the enhanced pairing affinity of 2'-fluoro-modified RNA. *Nucleic Acids Res*. 2011; 39:3482–3495. [PubMed: 21183463]
57. Schneider, B.; Berman, H. Basics of Nucleic Acid Structure. In: Šponer, J.; Lankaš, F., editors. *Computational Studies of RNA and DNA*. Springer; Netherlands: 2006. p. 1-44.
58. Šponer, J.; Jure ka, P.; Hobza, P. Base Stacking and Base Pairing. In: Šponer, J.; Lankaš, F., editors. *Computational Studies of RNA and DNA*. Springer; Netherlands: 2006. p. 343-388.
59. Hobza P, Šponer J. Structure, Energetics, and Dynamics of the Nucleic Acid Base Pairs: Nonempirical Ab Initio Calculations. *Chem Rev*. 1999; 99:3247–3276. [PubMed: 11749516]
60. Hobza P. Calculations on Noncovalent Interactions and Databases of Benchmark Interaction Energies. *Acc Chem Res*. 2012; 45:663–672. [PubMed: 22225511]
61. Šponer J, Jure ka P, Marchan I, Luque FJ, Orozco M, Hobza P. Nature of Base Stacking: Reference Quantum-Chemical Stacking Energies in Ten Unique B-DNA Base-Pair Steps. *Chem — Eur J*. 2006; 12:2854–2865. [PubMed: 16425171]
62. Sinnokrot MO, Sherrill CD. High-Accuracy Quantum Mechanical Studies of π - π Interactions in Benzene Dimers. *J Phys Chem A*. 2006; 110:10656–10668. [PubMed: 16970354]

63. Waller MP, Robertazzi A, Platts JA, Hibbs DE, Williams PA. Hybrid density functional theory for π -stacking interactions: Application to benzenes, pyridines, and DNA bases. *J Comp Chem*. 2006; 27:491–504. [PubMed: 16444702]
64. Pérez A, Šponer J, Jurecka P, Hobza P, Luque FJ, Orozco M. Are the Hydrogen Bonds of RNA (A \oplus U) Stronger Than those of DNA (A \oplus T)? A Quantum Mechanics Study. *Chem – Eur J*. 2005; 11:5062–5066. [PubMed: 15977281]
65. Schlick, T. RNA: The Cousin Left Behind Becomes a Star. In: Šponer, J.; Lankaš, F., editors. *Computational Studies of RNA and DNA*. Springer; Netherlands: 2006. p. 259-281.
66. Mládek A, Šponer JE, Kulhánek P, Lu XJ, Olson WK, Šponer J. Understanding the Sequence Preference of Recurrent RNA Building Blocks Using Quantum Chemistry: The Intrastrand RNA Dinucleotide Platform. *J Chem Theory Comput*. 2011; 8:335–347. [PubMed: 22712001]
67. Johnson CA, Bloomingtondale RJ, Ponnusamy VE, Tillinghast CA, Znosko BM, Lewis M. Computational Model for Predicting Experimental RNA and DNA Nearest-Neighbor Free Energy Rankings. *J Phys Chem B*. 2011; 115:9244–9251. [PubMed: 21619071]
68. Šponer J, Morgado CA, Svozil D. Comment on “Computational Model for Predicting Experimental RNA and DNA Nearest-Neighbor Free Energy Rankings”. *J Phys Chem B*. 2012; 116:8331–8332. [PubMed: 22686484]
69. Johnson CA, Bloomingtondale RJ, Ponnusamy VE, Tillinghast CA, Znosko BM, Lewis M. Reply to “Comment on ‘Computational Model for Predicting Experimental RNA and DNA Nearest-Neighbor Free Energy Rankings’”. *J Phys Chem B*. 2012; 116:8333–8334.
70. Bader RFW. A quantum theory of molecular structure and its applications. *Chem Rev*. 1991; 91:893–928.
71. Kamy PRN, Muchall HM. Revisiting the Effects of Sequence and Structure on the Hydrogen Bonding and π -Stacking Interactions in Nucleic Acids. *J Phys Chem A*. 2011; 115:12800–12808. [PubMed: 21721560]
72. Cummings MD, DesJarlais RL, Gibbs AC, Mohan V, Jaeger EP. Comparison of Automated Docking Programs as Virtual Screening Tools. *J Med Chem*. 2005; 48:962–976. [PubMed: 15715466]
73. Moitessier N, Westhof E, Hanessian S. Docking of Aminoglycosides to Hydrated and Flexible RNA. *J Med Chem*. 2006; 49:1023–1033. [PubMed: 16451068]
74. Detering C, Varani G. Validation of Automated Docking Programs for Docking and Database Screening against RNA Drug Targets. *J Med Chem*. 2004; 47:4188–4201. [PubMed: 15293991]
75. Li Y, Shen J, Sun X, Li W, Liu G, Tang Y. Accuracy Assessment of Protein-Based Docking Programs against RNA Targets. *J Chem Inf Model*. 2010; 50:1134–1146. [PubMed: 20481574]
76. Morley SD, Afshar M. Validation of an empirical RNA-ligand scoring function for fast flexible docking using RiboDock®. *J Comput Aided Mol Des*. 2004; 18:189–208. [PubMed: 15368919]
77. Daldrop P, Reyes FE, Robinson DA, Hammond CM, Lilley DM, Batey RT, Brenk R. Novel Ligands for a Purine Riboswitch Discovered by RNA-Ligand Docking. *Chem Biol*. 2011; 18:324–335. [PubMed: 21439477]
78. Barbault F, Ren B, Rebehmed J, Teixeira C, Luo Y, Smila-Castro O, Maurel F, Fan B, Zhang L, Zhang L. Flexible computational docking studies of new aminoglycosides targeting RNA 16S bacterial ribosome site. *Eur J of Med Chem*. 2008; 43:1648–1656. [PubMed: 18096272]
79. Fuchs JE, Spitzer GM, Javed A, Biela A, Kreutz C, Wellenzohn B, Liedl KR. Minor Groove Binders and Drugs Targeting Proteins Cover Complementary Regions in Chemical Shape Space. *J Chem Inf Model*. 2011; 51:2223–2232. [PubMed: 21819135]
80. Krüger DM, Bergs J, Kazemi S, Gohlke H. Target Flexibility in RNA-Ligand Docking Modeled by Elastic Potential Grids. *ACS Med Chem Lett*. 2011; 2:489–493.
81. Šponer J, Jurecka P, Hobza P. Accurate Interaction Energies of Hydrogen-Bonded Nucleic Acid Base Pairs. *J Am Chem Soc*. 2004; 126:10142. [PubMed: 15303890]
82. Johnson CA, Bloomingtondale RJ, Ponnusamy VE, Tillinghast CA, Znosko BM, Lewis M. Computational Model for Predicting Experimental RNA and DNA Nearest-Neighbor Free Energy Rankings. *J Phys Chem B*. 2011; 115:9244. [PubMed: 21619071]
83. Li F, Pallan PS, Maier MA, Rajeev KG, Mathieu SL, Kreutz C, Fan Y, Sanghvi J, Micura R, Rozners E, Manoharan M, Egli M. Crystal structure, stability and in vitro RNAi activity of

oligoribonucleotides containing the ribo-difluorotoluy nucleotide: insights into substrate requirements by the human RISC Ago2 enzyme. *Nucleic Acids Res.* 2007; 35:6424–6438. [PubMed: 17881374]

84. Xia J, Noronha A, Toudjarska I, Li F, Akinc A, Braich R, Frank-Kamenetsky M, Rajeev KG, Egli M, Manoharan M. Gene Silencing Activity of siRNAs with a Ribo-difluorotoluy Nucleotide. *ACS Chem Biol.* 2006; 1:176–183. [PubMed: 17163665]
85. Schirle NT, MacRae IJ. The Crystal Structure of Human Argonaute2. *Science.* 2012; 336:1037–1040. [PubMed: 22539551]
86. Gordon MS, Fedorov DG, Pruitt SR, Slipchenko LV. Fragmentation Methods: A Route to Accurate Calculations on Large Systems. *Chem Biol.* 2011; 112:632–672.
87. Mayhall NJ, Raghavachari K. Molecules-in-Molecules: An Extrapolated Fragment-Based Approach for Accurate Calculations on Large Molecules and Materials. *J Chem Theory Comput.* 2011; 7:1336–1343.

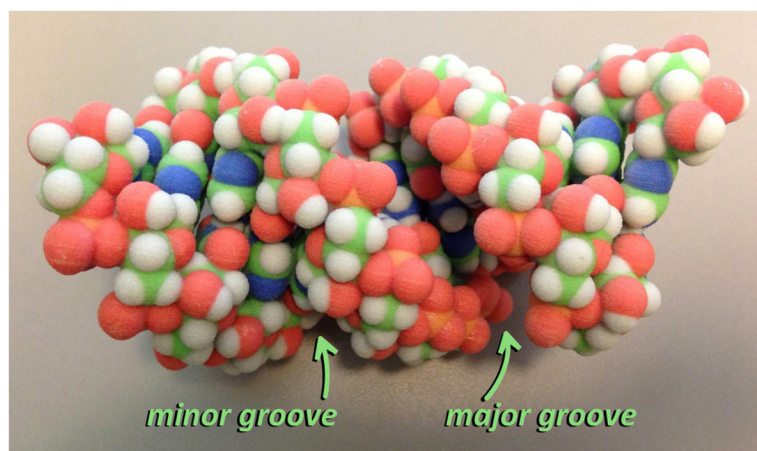


Figure 1. A 3D model⁽¹²⁾ of dsRNA, showing the minor and major grooves (PDB ID: 1R9F).⁽¹³⁾

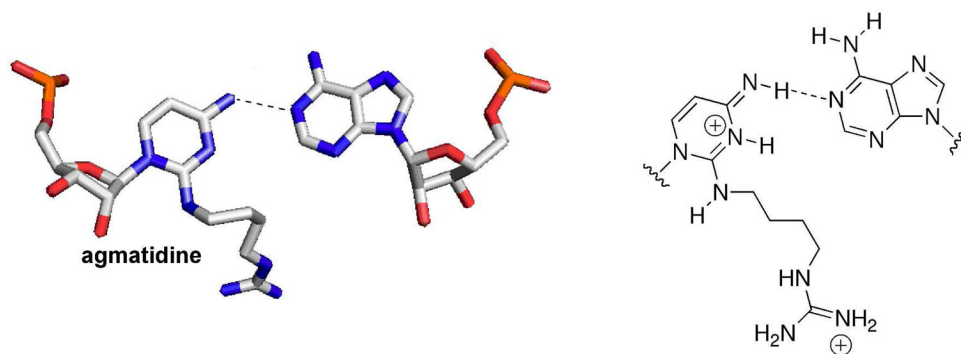


Figure 2. A naturally occurring nucleobase analog with unique base-pairing properties. The cytidine analog agmatidine preferentially base pairs with adenosine (over guanosine) in the archaeal tRNA₂^{Ile}-mRNA duplex formed during decoding in the ribosome.⁽¹⁴⁾

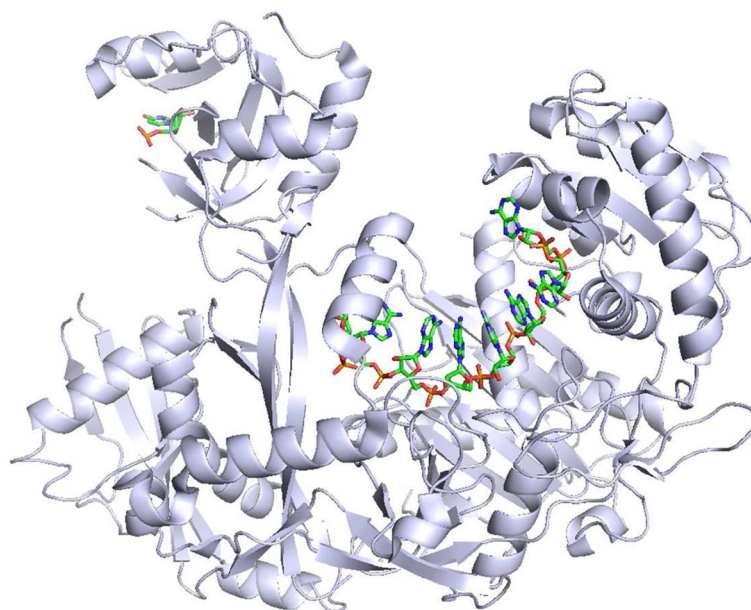


Figure 3.
Crystal structure of human Ago2 bound to guide RNA (PDB ID: 4EI1).⁽⁸⁵⁾

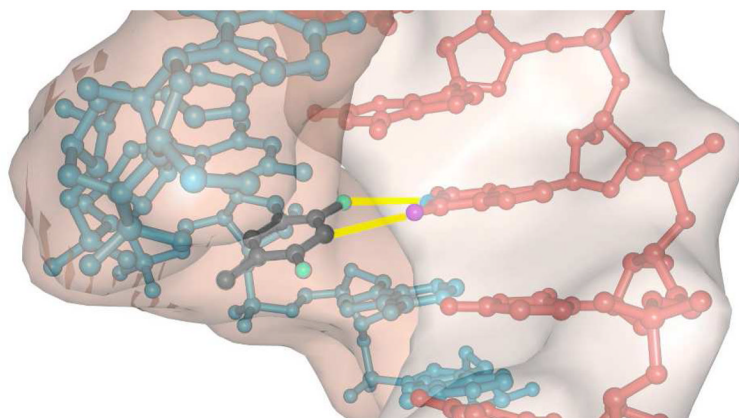


Figure 4. Portion of crystal structure of an RNA dodecamer duplex containing a difluorotoluyl•••G base pair (F atoms highlighted in green; putative F•••HN and CH•••O interactions highlighted in yellow), which affects silencing despite not displaying major disruptions to the duplex structure (PDB ID: 2Q10).⁽⁷⁶⁾