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Advancing predictive modeling through focused development of model systems to drive new modeling innovations

A funding proposal recently submitted to the NIH

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ABSTRACT

This work seeks to advance quantitative methods for biomolecular design, especially for predicting biomolecular interactions, via a focused series of community blind prediction challenges. Physical methods for predicting binding free energies, or “free energy methods”, are poised to dramatically reshape early stage drug discovery, and are already finding applications in pharmaceutical lead optimization. However, performance is unreliable, the domain of applicability is limited, and failures in pharmaceutical applications are often hard to understand and fix. On the other hand, these methods can now typically predict a variety of simple physical properties such as solvation free energies or relative solubilities, though there is still clear room for improvement in accuracy. In recent years, competitions and crowdsourcing have proven an effective model for driving innovations in diverse fields. In our field, blind prediction challenges have played a key role in driving innovations in prediction of physical properties and binding, especially in the form of the SAMPL series of challenges. Here, we will continue and extend SAMPL prediction challenges to include new physical properties, more complicated host-guest binding data, and application to biomolecular systems. Carefully selected systems and novel experimental data will provide challenges of gradually increasing complexity spanning between systems which are now tractable to those which are marginally out of reach of today’s methods but still slightly simpler than those covered by the Drug Design Data Resource (D3R) series of challenges on existing pharmaceutical data. We will work with D3R to run blind challenges on the data we generate and to ensure it is designed to maximally benefit the field.

In **Aim 1**, we will collect new measurements on partitioning, distribution, and protonation of drug-like compounds, in collaboration with partners in the pharmaceutical industry. In **Aim 2**, we leverage our expertise in host-guest binding to generate new data on host-guest binding in cucubiturils and deep cavity cavitands. And in **Aim 3**, we use high-throughput robotic experiments to generate new protein-ligand binding data of biological relevance. **Aim 4** focuses on using this data in the SAMPL series of challenges, applying proven crowdsourcing-based techniques to drive the development of new methods and new understanding of the strengths and weaknesses of existing techniques. We will also run reference calculations with the latest techniques.

This work will ensure the continued success of SAMPL challenges which have already driven considerable innovation in the field and been the focus of 100 different publications (each typically cited 5-50 times) since their inception around 2007, and will play a key role in driving the next several generations of improvements in computational techniques for molecular design. The research proposed here will lead to significant improvements in the predictive power of physical models for drug discovery, molecular design and the prediction of physical properties.

SPECIFIC AIMS

While computational techniques are widely used in pharmaceutical drug discovery, current generation technologies (such as docking) are unsuitable for true molecular design. Specifically, these techniques fail to predict small molecule binding affinities to target and antitarget biomolecules with sufficient accuracy for many applications. While virtual screening techniques can improve enrichment, they lack the accuracy to guide molecular design. A new generation of physical techniques, alchemical free energy calculations, are poised to fill this void by providing quantitative, predictive tools useful in multiple stages of the drug discovery pipeline.

Recent success of alchemical methods in predicting accurate affinities has sparked considerable enthusiasm, but the domain of applicability of these techniques is currently highly limited; broad application and routine use will require further evaluation, refinement, and development. There is a vast gulf between targets within the domain of applicability and those which are outside it, frustrating progress. Bridging this gulf will require focused study of carefully selected systems of intermediate complexity. Without such a bridge, these techniques may encounter the same problems faced by docking and related techniques: routine failure without clear insights into why, and years to decades spent making small methodological modifications without dramatic improvements in predictive power.

We propose a carefully designed, crowdsourcing-based model to drive innovation to bridge this gap. Specifically, we will collect targeted experimental datasets that highlight and focus the community on specific modeling difficulties, fielding blind prediction Challenges to spur new, crowdsourced methodology innovation from the community. This model has been proven to drive progress in a given area, both inside the computational biology community [1–6], and for even more visible projects like Netflix [7] and the XPrizes [8–10]. Here, we design an extension of the Statistical Assessment of Modeling of Proteins and Ligand (SAMPL) series Challenges, which allow a wide variety of different approaches to compete on equal footing on the same data, fostering rapid innovation, quick recognition of the best new technologies, and dissemination of these to the community. The data we generate will allow several Challenges of differing difficulty, between systems tractable with current methodologies and the pharmaceutically-relevant drug targets featured in the NIH-funded D3R effort [11]. Central to this proposal is collecting targeted data and running blind Challenges to drive progress, but the data itself will have an even longer-term impact on the community. Our Aims are:

Aim 1. Collect new physical property datasets to assess accuracy and spur improvements in force fields and modeling of protonation states and tautomers.

We will develop new solution-phase datasets for druglike small molecules. These data can test critical aspects of small molecule modeling (including accounting for interactions and treatment of protonation/tautomeric state) and improve our ability to predict physical properties relevant to drug discovery in new regions of chemical space. We will initially focus on aqueous/nonpolar distribution coefficients and pK_a measurements, advancing to solubilities and membrane permeabilities, while using these data to drive improvements in the modeling of ligand interactions.

Aim 2. Measure affinities of drug-like compounds in supramolecular hosts to challenge quantitative models of binding in systems not plagued by major receptor sampling issues.

We will measure new host-guest binding free energies (using cucurbiturils and deep-cavity cavitands as hosts) to field binding Challenges with varying complexity between physical property prediction and protein-ligand binding. Host-guest systems are some of the simplest cases of molecular recognition, and thus these binding data will drive improvements in modeling of simple binding systems with techniques of relevance to drug discovery.

Aim 3. Develop model protein-ligand systems that isolate specific modeling challenges of drug targets.

We will identify suitable biological protein-ligand model systems that isolate individual modeling challenges (selected to push the limits of physical techniques) and develop these for blind Challenges based on new protein-ligand affinity measurements. While the initial year will feature fragment binding to human serum albumin, subsequent Challenge systems will be selected using a novel informatics platform to focus on timely modeling issues.

Aim 4. Crowdsourcing innovation via community blind Challenges to advance biomolecular design.

The data collected in Aims 1–3 will drive annual SAMPL Challenges, allowing the field to test the latest methods and force fields to assess progress, compare them against one another head-to-head, and perform sensitivity analysis to learn how much different factors (protonation state, tautomer selection, solvent model, force field, sampling method, etc.) affect predictive power. Results will then feed back into improved treatment of these factors for subsequent Challenges, driving cycles of application, learning, and advancement – an approach proven to work in a variety of previous crowdsourcing approaches to science, as well as in prior iterations of SAMPL itself.

Overall, these SAMPL Challenges provide a catalyst to drive the next several generations of computational methodology innovation, development, and assessment for pharmaceutical drug discovery.

SIGNIFICANCE

Physical modeling is poised to transform drug discovery and chemical biology by enabling true molecular design. While modeling is used extensively in drug discovery, it mainly assists in idea generation and filtering large libraries of compounds for screening. Instead, we envision using computational techniques extensively to guide design. Consider a medicinal chemist in the not-too-distant future who has just received potency data for newly-synthesized inhibitors against a desired biomolecular target. Before leaving work, she generates ideas for perhaps 100 new compounds for synthesis, setting her computer to work overnight. By morning, the idea compounds have been prioritized based on reliable predictions of their affinity for the desired target, selectivity against antitargets, solubility, and membrane permeability. Looking through the top few compounds, she selects some for synthesis. If synthesizing and testing each compound takes several days, this workflow could compress roughly a year's work into a few days.

While not yet a reality, significant strides have been made toward accurate binding affinities [113–121], solubilities [122–124], selectivity and drug resistance [121, 125], and membrane permeability [126, 127]. A considerable amount of science and engineering still remains to be done to make this vision a reality, but the question now seems more one of *when* rather than *whether* it is achieved.

Widespread availability of inexpensive graphics processing units (GPUs) provides a 100-fold increase in price-to-performance ratio over CPUs, while advances in automation [128] and sampling protocols have helped simulation-based techniques reach the point where they now begin to be genuinely useful in guiding drug discovery for a limited *domain of applicability* [116–120, 129, 130]. Specifically, in some situations, free energy calculations appear to be capable of achieving RMS errors of 1–2 kcal/mol with current force fields, even in prospective applications, sufficient to drastically reduce the number of molecules that must be synthesized and assayed [117, 121, 131]. As a consequence, pharmaceutical companies have rapidly adopted these methods in active discovery projects [116].

Despite progress, current modeling methods suffer from severe limitations hindering their widespread use in molecular design. For example, even “small” protein conformational changes not gracefully handled by current methodologies can yield errors up to 5 kcal/mol in calculated binding free energies [132], force field limitations still pose major challenges [133], and the inability to treat important chemical effects like protonation and tautomer equilibria drastically limits the domain of applicability. **For many pharmaceutically relevant systems, the most important sources of error—and modeling challenges—are not yet clear.** [116]

These hurdles frustrate progress in the field. Method developers focus on addressing some specific challenges, but **new methods are tested on disparate datasets making it difficult to gauge progress and identify valuable methods when they are developed.** And applications projects tend not to take advantage of the latest methods—in part because their utility is not yet clear. As a result, valuable methods can languish relatively unused in the literature for many years without impacting applications in drug discovery.

We propose an iterated Challenge-based approach to drive methodological innovation via crowdsourcing. A series of community blind prediction Challenges will test and push the limits of predictive techniques, providing a bridge between challenging but tractable problems and pharmacologically relevant but currently intractable problems. Our Statistical Assessment of Modeling of Proteins and Ligands (SAMPL) Challenges take advantage of proven crowdsourcing-based approaches to science (discussed below), allowing head-to-head comparisons of existing methods and fostering development of new methods. Regular, progressive Challenges provide an environment for rapid innovation from “unconventional innovators” [109] from diverse fields who form collaborative teams, applying and integrating distinct approaches [106].

Meaningful Challenges require new targeted data collection. A key focus of this proposal is on generating new data to test methods in prospective Challenges in an unbiased way. New data provides additional value over retrospective tests on literature data: First, we are able to ensure high data quality and ensure we can collect additional data for the same systems as needed. Additionally, retrospective tests do not test predictive power—they easily fall victim to significance hacking or inadvertent overfitting [134]. Retrospective work also may utilize prior knowledge, even if unintentionally. For example, if a ligand binding mode is already known crystallographically, a researcher may use that binding mode in retrospective tests, whereas prospective or design work requires first selecting among candidate binding modes, introducing substantial uncertainty unaccounted for in the retrospective statistics [113, 135, 136]. While discovery projects provide one opportunity for prospective tests, they are not adequate—partly because often, the predicted compounds are in fact never tested [118] or the experimental data necessary to assess prediction quality (binding affinities or crystallography, for example) is absent.

Tractable Challenges inspire rapid progress, while Challenges that are too difficult accomplish little [106].

We require high quality Challenges carefully designed specifically to maximize community learning by pushing the limits of current technology. While the Drug Design Data Resource (D3R [112]) also fields blind Challenges, these rely on contributed experimental protein-ligand datasets from now-defunct pharmaceutical discovery programs [112]. D3R Challenges provide a necessary *benchmark* for real-world performance, but provide only modest new insight to drive improvement because a multitude of issues are responsible for poor performance [137].

Methodology improvement is most rapid when complex goals are achieved via tractable intermediate milestones which can be independently assessed using high quality data [106]. Thus, we focus on using Challenges to drive and evaluate solutions to specific *component* problems that make it difficult to predict biomolecular interactions. The Challenge format allows the entire community to learn from both failure and success. Much as *unit testing* is indispensable for identifying software bugs when complex integration tests fail, Challenges like SAMPL are valuable in pinpointing modeling errors, especially when projects like D3R highlight overall performance problems. Hence, SAMPL Challenges focus on predicting properties that isolate likely sources of error, such as physical properties of small molecules (hydration free energies, aqueous tautomer ratios, partition or distribution coefficients between aqueous and nonpolar phases) or binding in systems of reduced complexity (such as host-guest complexation, or binding of fragments to trypsin and HIV integrase).

The **SAMPL series of Challenges has already proven it can drive dramatic improvements in modeling methodologies**. SAMPL, born out of frustration with the lack of venues for comparing predictive accuracy on a level playing field, was initiated by Anthony Nicholls of OpenEye software in 2007/2008 [139], and has run Challenges approximately every two years since then [138, 140–146]. Governance transitioned to an unfunded academic collaboration among the PI and co-Investigators during SAMPL3 in 2012; this collaboration ran SAMPL4 (2014), SAMPL5 (2016), currently SAMPL6 (2017–2018). The PI of this proposal (Mobley) was a primary organizer of SAMPL4–6 (2014–present). **SAMPL has demonstrated its utility as a valuable community resource, resulting in ~100 publications typically cited 5–50 times or more each** [1–100]. SAMPL datasets are so valuable to the community that they foster retrospective tests long after the Challenges end (e.g. [147–150]).

Until now, **SAMPL has relied on donated data and time**, making it impossible to design Challenges specifically to improve modeling as we propose here. Despite these limitations, our recent survey reveals that SAMPL has already had a significant effect on the field; past participants are overwhelmingly enthusiastic about SAMPL and the plans we propose here [111]. One of our most frequent requests is for modestly larger datasets, which we propose here. Our proposed Challenges bridge the gap between calculations of simple physical properties that isolate force field inaccuracies from slow conformational sampling inherent to protein-ligand systems—such as hydration free energies, which can already be calculated fairly accurately [138]—and the D3R Grand Challenges on protein-ligand binding, which are a major source of community consternation [112, 151–153]. Unless this gap is bridged, there is a very real possibility that modeling will continue to fall far short of expectations in pharmaceutical systems for reasons which remain unclear [116].

Our major goal is to rapidly advance predictive modeling to enable it to guide biomolecular design; extending the SAMPL Challenges will accomplish this. This work will play a vital role in enhancing the work being done on *existing* data by D3R, helping prepare methods for application to D3R's pharmaceutical Challenges.

INNOVATION

This work proposes a five-year plan for fielding SAMPL Challenges as an *innovation engine* for molecular design. Specifically, collaborative scientific competitions or Challenges are a proven model for inducing innovation [105, 106, 109], applying crowdsourcing to finding and identifying robust new methodologies [106]. The proposed series of SAMPL Challenges brings crowdsourcing to bear on molecular interactions in a way which focuses on solving specific, incremental problems that impair our ability to accurately and robustly predict these interactions. Thus, while the proposed work is indeed innovative (as we argue below), the most important innovation comes from participants in SAMPL Challenges, as is the case in all crowdsourcing approaches to innovation [106, 109].

Reliance on others for innovation is a strength of this model. It is not always obvious where the most important

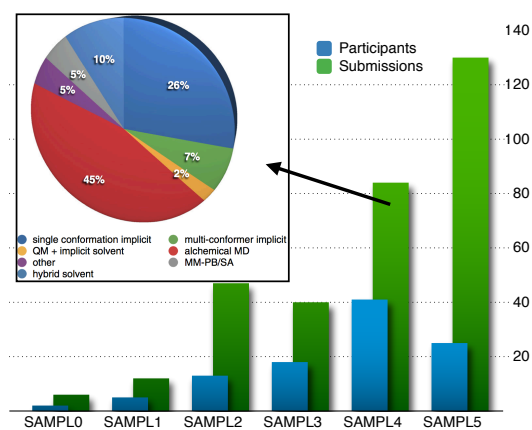


Figure 1. SAMPL historical participation [138]. Historical participation in SAMPL host-guest + solvation/distribution Challenges has climbed rapidly, and we expect this trend to continue. The number of participating groups is shown in blue, and the number of submissions in green. The inset shows the diversity of methods employed for the SAMPL4 hydration Challenge, which is typical for SAMPL. While SAMPL5 had fewer participants, this was because SAMPL4 included an HIV integrase binding prediction Challenge, whereas D3R handled such data after SAMPL4. Despite this shift, the number of submissions in SAMPL5 was the highest yet.

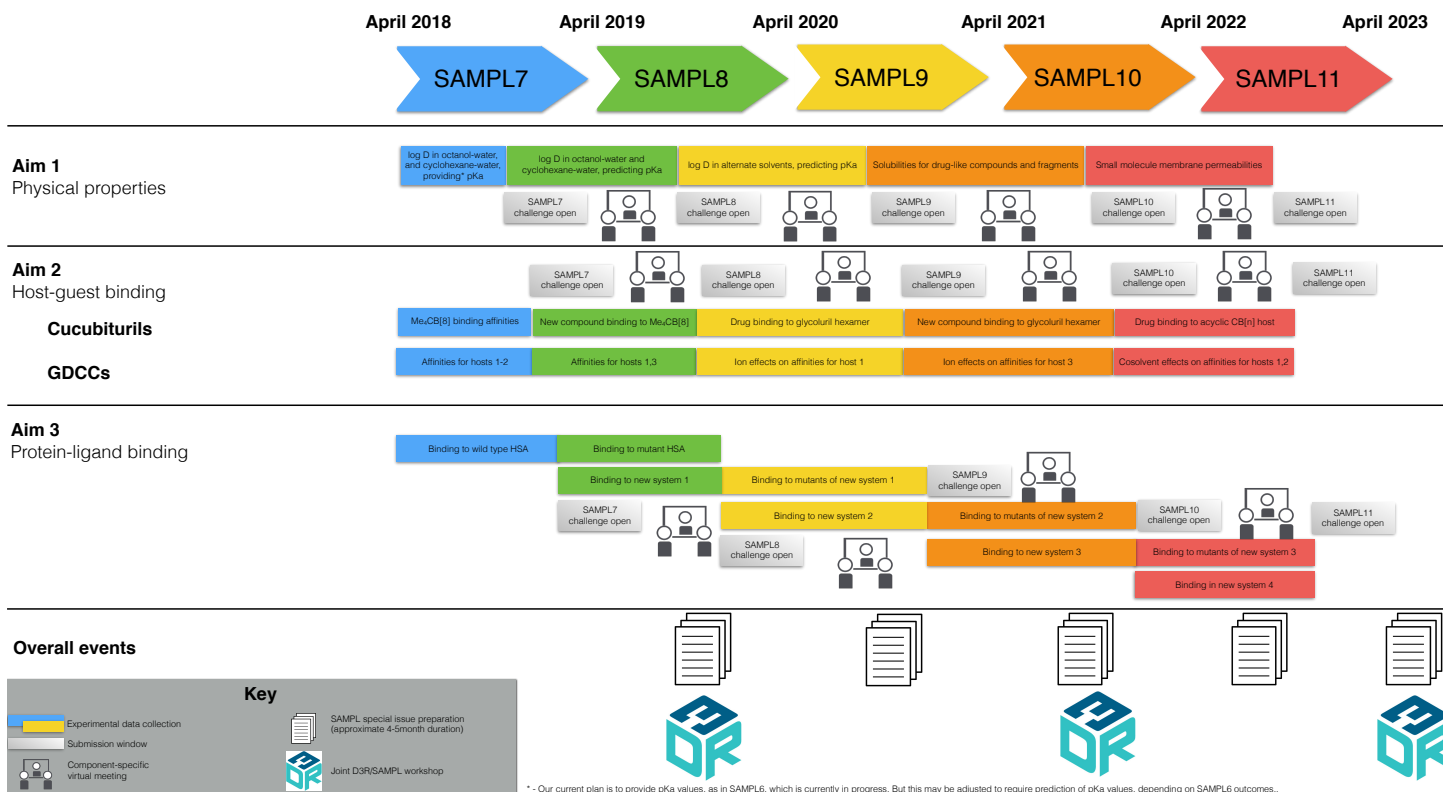


Figure 2. Timeline for SAMPL activities. Activities covered by this grant include data collection and SAMPL Challenges on our three major components (physical properties, host-guest binding, and protein-ligand binding), with each Challenge cycle color-coded separately. Data collection within each Aim is shown by a colored bar indicating what is measured and curated. Data collection/curating is followed by a submission window for that Challenge component, then all results and analysis are returned to participants and posted on the SAMPL website; this also will nucleate more detailed long-term discussion on the relevant Slack channel. At this point, we will also release the data to the public as a high quality benchmark. Each component will then wrap up with a virtual meeting focused on lessons learned and areas which need further exploration; these will be recorded and posted on our website to assist in rapid dissemination of new insights. Virtual meetings precede the submission window for the next SAMPL Challenge, giving the opportunity to incorporate lessons learned for the next Challenge. Submission windows and virtual meetings are staggered across categories so that participants can be involved in all three major areas without multiple simultaneous deadlines. In-person meetings are co-hosted with D3R and will occur every two years, supplemented by effort-wide virtual meetings in between. Special issues of JCAMD, where participants report lessons learned, will have deadlines shortly after the virtual meeting on the protein-ligand Challenge for that year, and a 4-5 month timeline (based on historical experience) from the submission deadline until the special issue appears (with the first papers appearing online substantially sooner). Rapid dissemination is critical for rapid progress, so we encourage the use of preprints and informal reports to supplement the special issue.

innovations may come from, but Challenges draw together teams of diverse entrants, including unconventional innovators who may apply unorthodox, risky, or radical technologies [106, 109]; progress comes both from the usual suspects and unexpected sources. It is not necessary to decide in advance who is best positioned to solve key problems [106]—the Challenge itself drives innovation and allows it to be recognized, regardless of source.

Crowdsourcing models for innovation have an ample track record, with the XPrize driving major headlines [108–110], and the Netflix Prize also familiar to many [107]. While there are other Challenges in the area of biomolecular modeling, such as D3R [112], the pK_a cooperative [154], CAPRI [155] and CASP [101], **no other blind Challenge focuses specifically on datasets collected specifically to drive quantitative protein-ligand modeling.**

SAMPL is designed in the spirit of the wildly successful biomedical DREAM Challenge [104–106] which had more than 800 recent participants. As recommended by the DREAM Challenge organizers [106], SAMPL focuses on high-quality data, decomposing complex problems into tractable but difficult component problems in order to benefit from crowdsourcing, and drawing together diverse teams and methods to allow various solutions to be evaluated.

Our recent survey indicates SAMPL has already been important in advancing protein-ligand modeling [111]; attached testimonials highlight some specific ways SAMPL has advanced science. Several historical examples further serve to highlight how SAMPL can foster innovation (see also Figure 3 and our SAMPL bibliography). While the first SAMPL Challenges focusing on hydration free energies saw heterogeneous performance, they highlighted pitfalls of existing methods and force fields which led to marked improvements in PB models [140, 156, 157], identified some limitations of fixed-charge force fields [158, 159] that spurred their repair [158–160], and helped motivate alternate implicit or hybrid solvent models [161–163]. Shifts in protonation state and tautomer proved particularly important in SAMPL5’s log D Challenge [145, 164], as they will in binding. Host-guest binding Chal-

lenges [165] have highlighted the importance of salt effects [146, 165, 166] and in some cases revealed severe force field limitations [167, 168], pointing the way forward for improving predictive models [165, 169].

This proposal focuses on the development of SAMPL Challenges tailored to progressively advance computational methods for binding (Aim 4), gathering the requisite experimental data to drive these Challenges (Aims 1-3). **While the primary innovation will result from our Challenges, we also innovate on several other fronts.** Aim 3 develops a new informatics platform to facilitate the rapid identification and study of useful protein-ligand systems that are both experimentally tractable for high-throughput biophysical measurement and focus on specific challenges of interest; an innovative, automated wetlab helps produce our high quality data. Additionally, students in our computational groups perform reference calculations to benchmark current state-of-the-art techniques. This provides student training, but also drives innovation by (1) Benchmarking advanced community method entries against our reference “current best practices” calculations; (2) Facilitating learning by allowing others to compare against our reference results to determine how methodology differences impact results; and (3) Focusing the field on key issues by analyzing sensitivity to conditions such as ionic strength, protonation states or tautomers.

APPROACH

A series of data-driven Challenges will spur systematic advances in modeling for biomolecular design.

We collect targeted experimental datasets focused on specific problems spanning a range of complexity (Aims 1–3), and use these to field SAMPL Challenges driving innovation (Aim 4). Data collection brings together multiple laboratories and industrial collaborators, including both theorists and experimentalists: graduate students from the Mobley and Chodera laboratories are paired with well-equipped experimental groups in industry to collect physical property data (Aim 1); Gibb and Isaacs, leading experimentalists in supramolecular chemistry, work with theorist Mobley to perform host-guest affinity measurements (Aim 2); and the Chodera lab applies new automated approaches to identify suitable protein-ligand systems and measure binding (Aim 3).

Aim 1: Collect new physical property datasets to assess accuracy and spur improvements in force fields and modeling of protonation states and tautomers.

Simple physical properties such as solvation, phase partitioning, and protonation equilibria can be calculated precisely (but not necessarily accurately) with physical methods, allowing quantitative comparison between calculations and experiment and revealing and isolating deficiencies in our models. These properties allow us to directly probe force field accuracy and chemical effects like protonation and tautomer handling in the absence of slow conformational changes and other effects which complicate assessment in protein-ligand systems.

Rationale: We will perform new solution-phase physical property measurements for drug-like molecules to motivate improvements in force fields and handling of protonation states and tautomers. This builds on our work on water-cyclohexane distribution coefficients ($\log D$) for SAMPL5 (with Genentech), which revealed major issues with handling of protonation states and tautomers [145] as well as serious force field limitations [160] (Figure 3). Distribution coefficients give the equilibrium ratio of solute concentration between aqueous and nonpolar phases at a given pH. Thus, because these mimic transfer free energies from aqueous to protein- or membrane-like environments, they capture many of the same characteristics as binding but without the associated challenges of receptor conformational sampling and specific ligand-receptor interactions. Many methods performed poorly in predicting $\log D$ in SAMPL5, with even the best methods having accuracies less than would be expected based on hydration free energies [145], yet failures were informative and the major sources of error were issues which also plague ligand-receptor binding. In many respects, distribution coefficients are an ideal SAMPL Challenge, as they are sufficiently difficult for clear failures to be frequent, with ample room for improvement, but not so difficult that reasons for failure were generally unclear. Still, many methods consistently disagreed with experiment for some compounds [145, 160, 164, 171], revealing the impact that targeted follow-up experiments (such as those we will conduct here) can have on improving models. Thus, **targeted Challenges on solution-phase properties address some of the complexities of binding.**

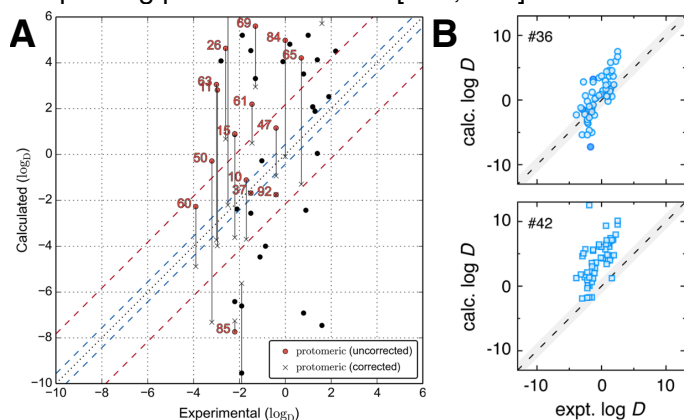


Figure 3. Lessons learned from SAMPL5 $\log D$ predictions. Predictions of $\log D$ values for SAMPL5 provided a number of key lessons. (A) Methods which treated multiple protonation and tautomeric states in their predictions performed dramatically better than those which did not; here, red dots move to x symbols when these effects are treated, improving accuracy in every case [170]. (B) Re-parameterization of a force field to more accurately reproduce pure solvent dielectric constants resulted in dramatically better predictions (top) than the original force field (bottom) [160].

Previous physical property SAMPL Challenges have already driven significant progress, but a frequent request is for modestly larger datasets and follow-up experiments to validate specific measurements [111]. This was impossible with donated data, but here, we will expand the size and quality of Challenge datasets, allowing us to expand to provide greater coverage of the full dynamic range. **Follow-up experiments will become routine, improving our ability to learn from failures.** For example, SAMPL5's lack of dynamic range meant that, when calculated values often spanned a larger dynamic range than experiment, it was unclear if this was due to the choice of compounds, experimental limitations, or force field deficiencies [145, 160, 164, 171].

Physical property datasets will be tailored for synergy with binding datasets. Specifically, ligands studied in host-guest and protein-ligand binding Challenges will be prioritized for inclusion in solution-phase physical property datasets; if physical property data is already public for these, analogs or fragments will be included. Thus, if a binding free energy proves difficult to predict, participants can determine if its solution-phase properties (such as protonation states or tautomers) were similarly difficult, thereby narrowing down the source of the error.

Below, we summarize plans for data collection for the SAMPL7-11 Challenges of Aim 4 (see also Figure 2). These specific datasets were selected to help the field first resolve problems encountered in SAMPL5 and then progress to accurate estimation of additional properties. Datasets will be larger than prior SAMPL Challenges—at least 96 compounds for good statistics—though when possible, a much larger amount of data will be collected.

SAMPL7: Cyclohexane/water and octanol/water distribution coefficients, given pK_a. Building on the success of distribution coefficients at motivating rapid modeling advances [145], we will measure cyclohexane-water distribution coefficients at pH 7.4 for a new batch of commercially-available drug- and fragment-like molecules overlapping with compounds studied in Aims 2 and 3. We will also measure octanol-water distribution coefficients for the same compounds, given indications that their prediction may be computationally tractable [172, 173] despite the heterogeneous structure of the wet octanol phase [174]. Because pK_a prediction was difficult but critical for SAMPL5, we will focus SAMPL7 on force fields and tautomers by measuring and providing pK_a values, revisiting pK_a prediction in SAMPL8 and 9. A similar approach is currently being applied for SAMPL6 (data collection is in process in collaboration with Merck), so plans here may be adjusted modestly depending on SAMPL6 outcomes.

SAMPL8 and SAMPL9: Distribution coefficients and pK_a's for drug-like molecules. Distribution coefficient measurements conflate the challenging issues of protonation state and tautomer prediction, as well as transfer into different environments. After allowing participants to predict distribution coefficients given pK_a values for SAMPL6-7, the SAMPL8-9 datasets will include distribution coefficients and pK_a values, with participants asked to predict both. The datasets will include pK_a values and distribution coefficients for an extensive set of drug-like molecules. SAMPL8 will focus on octanol-water and cyclohexane-water, and SAMPL9 will shift to alternate solvents to ensure that models are capable of handling varied non-water phases. As in SAMPL7, we plan to focus partly on molecules and fragments overlapping with compounds being studied in Aims 2–3.

SAMPL10-11: Solubility prediction and membrane permeability. With solubility predictions now becoming tractable [122–124] (with Schrödinger also working on amorphous solubility), solubility measurements will be a valuable test for SAMPL10, combining the solvation aspects of SAMPL1-8 with a new solid phase component. New computational techniques are targeting membrane permeability [126, 127], and this is experimentally accessible (see support letters from Pfizer and Merck), leading to our interest in permeability for SAMPL11. However, it is likely that both solubility and permeability will still be extremely challenging – so we plan to also measure distribution coefficients and pK_a's for the same compounds to help participants isolate potential points of failure.

Based on community feedback [111], we are also interested in subsequent SAMPL Challenges on hydration and conformational free energies, and will seek suitable sources of reliable experimental data.

Experimental plan: Experimental data will be collected in collaboration with our pharma partners, roughly following the model used for SAMPL5, where Chodera lab student Bas Rustenburg worked with Genentech and adapted an existing workflow to measure cyclohexane-water log D [171]. A similar approach is currently being employed with Merck for SAMPL6. Thus, Mobley and Chodera lab students will visit industry partners collecting targeted datasets. This collaborative approach (see Letters of Collaboration from Genentech, Pfizer, GSK, and Merck) gives us substantial access to equipment and high-throughput measurement workflows—such as the Sirius T3 (which can measure partition/distribution coefficients, pK_a's, and solubilities for molecules with titratable groups)—automation equipment, and compound libraries—for the purposes of rapidly collecting targeted datasets. This approach works [171], and our partners see the value of this data and SAMPL to the community.

Overall, Aim 1 extends prior SAMPL Challenges via data focused on quantitative prediction of physical properties relevant to accurately predicting biomolecular interactions, paving the way to applications in more complex systems.

Aim 2: Measure affinities of drug-like compounds in supramolecular hosts to challenge quantitative models of binding in systems not plagued by major receptor sampling issues.

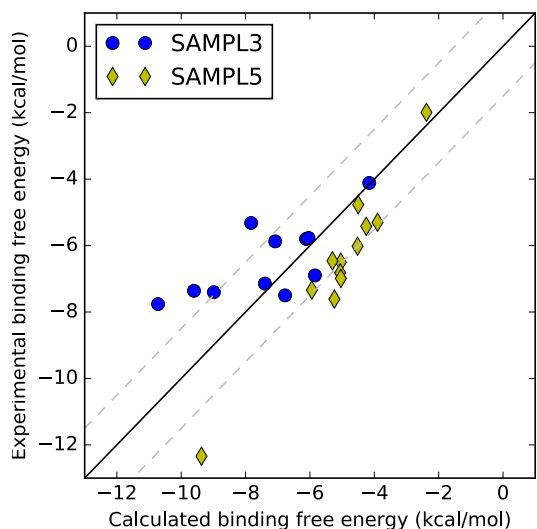


Figure 4. The best host-guest binding predictions of SAMPL3 [175] and SAMPL5 [167]. Binding free energy predictions have shown clear improvements from SAMPL3 to SAMPL5 as the major obstacles become understood and are treated better by models, though a systematic offset remains in the best SAMPL5 predictions (yellow). Dashed lines denote errors of ± 1.5 kcal/mol.

of host-guest systems—cucurbiturils and analogs (SA 2.1) and Gibb’s deep-cavity cavitands (GDCCs, SA 2.2)—both of which build on prior SAMPLs. These two sets of systems exhibit different complexities [165], with the hosts of 2.1 bringing modest co-solvent and ion effects and some receptor sampling problems for the acyclic hosts, and the GDCCs of 2.2 bringing profound ion and co-solvent effects as well as water sampling challenges. Methods which perform well on one class may not perform well on the other [165] because of these distinct challenges. This diversity and complexity is important for Challenges which seek to drive researchers to focus on all of the important features rather than just a subset [106]. As the SAMPL community would like even more diversity in hosts and guests [111], we will also seek to include additional host-guest systems via data donation (as in SAMPL1-5).

SAMPL6 is currently in progress and includes components on GDCCs and cucurbiturils, so our plans in this section may be adjusted somewhat depending on the outcomes of SAMPL6.

Subaim 2.1: Cucurbituril-based receptors: model binding systems

Cucurbituril derivatives for host-guest binding. Building on previous success with cucurbit[*n*]uril (CB[*n*]) experiments for SAMPL [178–180], we will conduct a series of new experiments on these receptors, with experimental work conducted by co-investigator Isaacs, an expert on these systems who provided data for previous SAMPLs. CB[*n*] receptors are particularly well suited to our goals because they exhibit: (1) strong binding affinities in water, comparable to protein-ligand affinities (routinely μM to nM; occasionally pM to fM) [181–187], (2) high selectivities between structurally related guests which translate into large $\Delta\Delta G$ values [188], (3) low molecular weights (1–2 kDa) permitting high levels of theory to be used, and (4) highly restricted conformational degrees of freedom, reducing conformational sampling challenges often seen in protein-ligand binding. For SAMPL7-11, we will resynthesize a series of CB[*n*]-type receptors of increasing complexity, measure K_a values, and determine host-guest stoichiometry and geometry toward pharmaceutically relevant guests (selected drugs) in order to stringently test methods for predicting binding. Figure 5 shows the chemical structures of three hosts—Me₄CB[8] [189], glycoluril hexamer [190], and acyclic CB[*n*]-type receptors [191–196] which span a range in terms of level of preorganization and formal charge.

Moving beyond solution-phase physical properties, we want Challenge data which introduces some of the additional complexity of binding in a controlled manner. Aim 1 focuses on the behavior of small molecules in different environments, in the absence of receptors and the associated potential for slow sampling, strong specific interactions, and other challenges like salt effects. Binding in host-guest systems (Aim 2) retains many of these same challenges and introduces strong specific interactions and other issues like salt effects [165], while still avoiding many of the issues with slow sampling (of protein conformational changes, ions, and ligand binding modes) seen in biomolecular interactions. **Thus, binding in host-guest systems introduces new challenges relevant to biomolecular interactions, but without the full complexity of protein-ligand interactions**, as recently reviewed [165].

Already, host-guest SAMPL Challenges have provided key tests for modeling of binding interactions [165], resulting in new attention paid to how co-solvents and ions modulate binding (resulting in errors of up to 5 kcal/mol when these effects are neglected) and the importance of adequately sampling water rearrangements [144, 146, 165, 176]. This new attention has resulted in clear improvements (Figure 4), though host-guest binding remains difficult to model accurately [177], in part due to force field limitations (resulting in new force field work [169]).

Here, we design a series of SAMPL Challenges focused on two classes of host-guest systems—cucurbiturils and analogs (SA 2.1) and Gibb’s deep-cavity cavitands (GDCCs, SA 2.2)—both of which build on prior SAMPLs. These two sets of systems exhibit different complexities [165], with the hosts of 2.1 bringing modest co-solvent and ion effects and some receptor sampling problems for the acyclic hosts, and the GDCCs of 2.2 bringing profound ion and co-solvent effects as well as water sampling challenges. Methods which perform well on one class may not perform well on the other [165] because of these distinct challenges. This diversity and complexity is important for Challenges which seek to drive researchers to focus on all of the important features rather than just a subset [106]. As the SAMPL community would like even more diversity in hosts and guests [111], we will also seek to include additional host-guest systems via data donation (as in SAMPL1-5).

drug	features
memantine	adamantane; 1:1
saxagliptin	adamantane; 1:1
premarin	steroid
pancuronium	steroid
varenicline	1:1 vs 1:2
valsartan	pK_a 4.37
omeprazole	pK_a 4.77
ranolazine	pK_a 7.17; epitopes
pradaxa	pK_a 3.87; epitopes
nilotinib	epitopes; pK_a 6.3
sensipar	epitopes; folding
vyvance	diamine; epitopes; folding
minocycline	tetracyclin; amino aniline

Table 1. **Selected drugs whose binding to CB[*n*] hosts will be assayed for SAMPL7, 9, and 11 Challenges (SA 2.1).** These drugs bind to the cucurbituril-based host systems considered here, some at high affinity, so measuring their affinities provides a way to test methods for predicting binding interactions absent complexities present in protein-ligand systems.

SAMPL7-11 cucurbituril Challenges.

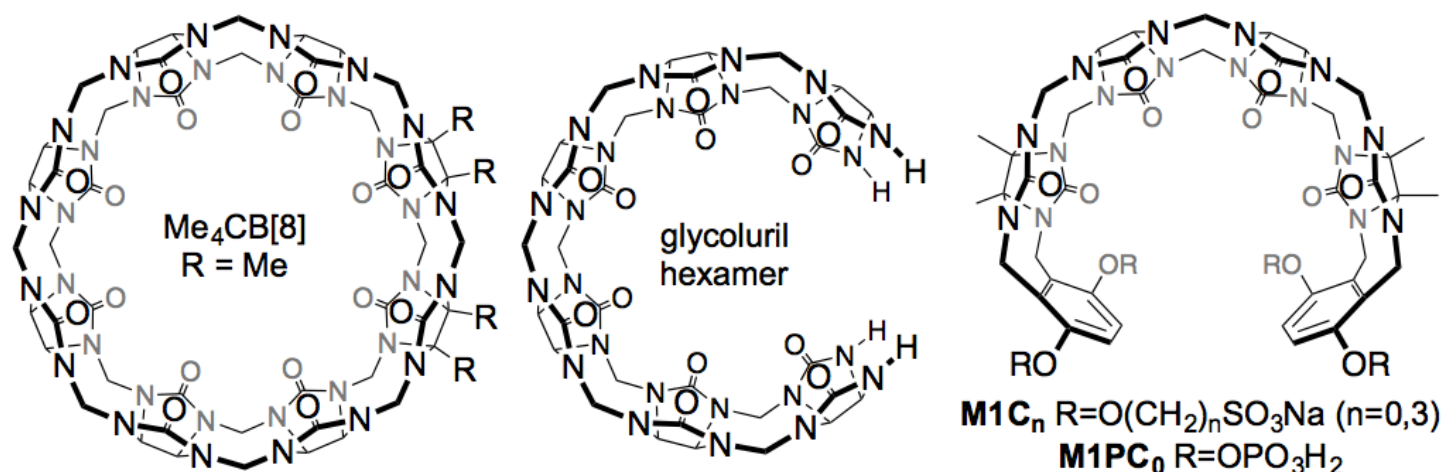


Figure 5. SAMPL7-11 host-guest Challenges will feature cucurbituril hosts and analogs, including Me₄CB[8], glycoluril hexamer, and acyclic CB[n]-type receptors (SA 2.1). These receptors bind a variety of drug-like molecules, some with high affinity.

For SAMPL7, we will measure K_a and ΔH values, stoichiometry, and geometry for the interaction of Me₄CB[8] (a soluble CB[8] derivative) with 15 guests (selected top drugs, Table 1) by either direct or competition isothermal titration calorimetry (ITC), UV/Vis or fluorescence indicator displacement assay, or NMR competition experiments, as previously [180–182, 197]. Our selection of Me₄CB[8] binding to top drugs allows us to modulate the computational complexity by: 1) changing host flexibility (e.g. Me₄CB[8] can exhibit ellipsoidal deformation) [189], 2) allowing the possibility of binary or ternary (e.g. 1:1 and/or 1:2 host:guest) complexes [198–200], 3) using drugs with several potential binding epitopes or modes to induce sampling issues. Host:guest stoichiometry and geometry (e.g., which binding epitope is complexed) will be addressed by ITC n values, Job plots monitored by UV/Vis or NMR [201], and by ¹H NMR complexation induced changes in chemical shifts [202]. All studies will be conducted in phosphate buffered saline (pH 7.4 with physiological salt) which introduces its own complexities due to salt competition for binding [165, 203]. SAMPL8 will revisit the same host, but use 15 different guests be selected from commercial sources on the basis of reference calculations (on a larger set of guests) to ensure that they cover substantial dynamic range and/or exhibit affinities that depend substantially on the force field or water model, thus effectively testing our force fields and methods. For SAMPL9, we will focus on binding of the same 15 drugs (Table 1), but to glycoluril hexamer. This host introduces the complication of increased conformational dynamics, and influences the number and energy of solvating (and unusually coordinated) water molecules implicated in the high binding constants for CB[n]-guest complexes [187, 204]. The selected drugs include several with p*K_a* values in the 3.8 to 7.4 range; given that CB[n]-type receptors (like biomolecular receptors) can induce p*K_a* shifts in their guests of up to 4 p*K_a* units [205–207], this will test how well models can predict these effects. Additionally, it will couple nicely with the focus on p*K_a* values in Aim 1 – especially so given that Aim 1 compounds will include some of the same chemical functionality. SAMPL10 will revisit glycoluril hexamer with the same 15 guests from SAMPL7. SAMPL11 will shift to acyclic CB[n]-type receptors (e.g. M1C₃, M1C₀, and M1PC₀ that contain anionic solubilizing groups attached via different linker lengths. As in SAMPL3 [175], these acyclic CB[n]-type receptors introduce conformational complexity, and water interactions play a key role.

Subaim 2.2. Gibb deep cavity cavitands for host-guest studies

History of GDCC SAMPL Challenges. During SAMPL4 [208] and SAMPL5 [209] we focused on two specific GDCC hosts: the octa-acid **1** (R = H) and another octa-acid variant with four methyl groups at the portal of the binding pocket (**1**, R = Me). These studies used ITC to measure the thermodynamics of (1) host **1** (R = H) binding a range of 9 carboxylate guests, and (2) the binding of 6 carboxylate and trimethylammonium guests to both hosts (**1**, R = H and Me; Figure 6). In both cases ¹H-NMR titration was also used to confirm ITC-derived free energies of binding. Relative to cucurbiturils, the GDCCs introduce new complexities because of their tight exit portal, modest issues with host conformational sampling, slow water rearrangements, salt/buffer condition-dependence, and protonation state complexities [146, 165]. Thus GDCC-oriented Challenges are particularly important since these issues complicate protein-ligand interactions as well.

Novel deep cavity hosts probe the effects of binding site charge constellations. For future GDCC datasets, we will expand on the range of hosts by including **2** and **3** in our ITC studies (Figure 6). Like cavitand **1**, host **2** is an octa-acid derivative. However, the four benzoate groups are relocated from the extreme exterior in the case of **1**, to the rim of the binding pocket in **2**. This is expected to have a direct effect on the binding of charged guests as well as

an indirect effect on guest complexation via changes to the solvation of the empty host. Octa-trimethylammonium cavitand (“positand” **3**) has the same overall architecture as host **1**, but inverts the charges on the water solubilizing exterior coat. While it is not yet clear if this switch in groups relatively remote from the pocket will directly affect guest complexation, results from related systems suggest it can (unpublished).

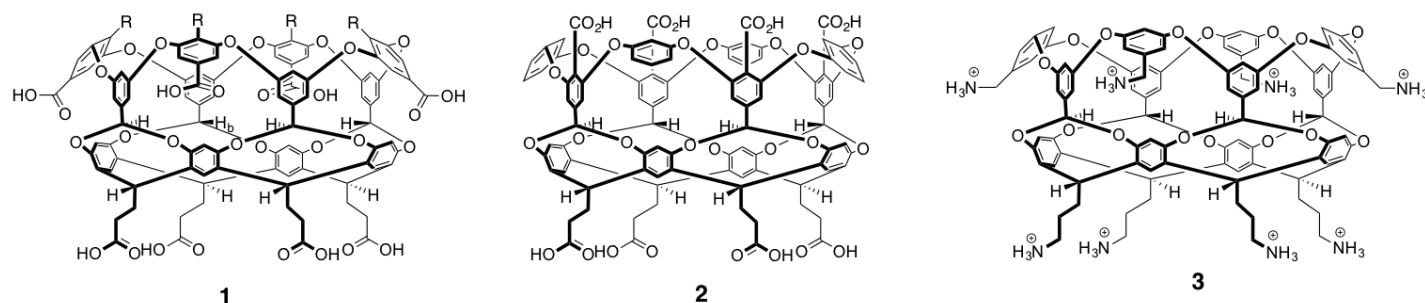


Figure 6. Gibb deep cavity cavitands for the SAMPL7-11 datasets (SA 2.2). These hosts bind a variety of carboxylate and trimethylammonium guests in a strongly salt-dependent manner, providing a stringent test of our ability to model salt-dependent binding.

SAMPL7-11 deep cavity cavitand datasets. Data for SAMPL7 will focus on how well the effect of host carboxylate substituent location can be predicted, and will involve hosts **1** and **2** with a set of at least five previously uninvestigated guests. Guests will be selected from commercial sources on the basis of reference calculations in a similar manner to SAMPL8 in Subaim 2.1, specifically picking guests which have broad dynamic range and, here, have marked differences in affinities between hosts. SAMPL8 will provide a second iteration of this experiment to test algorithmic improvements in predictive modeling following SAMPL7 by comparing hosts **1** and **3** with a different set of guests. We anticipate that because of the relative remoteness of the charged groups in these two hosts, the effects of switching charges will be subtler than the differences between **1** and **2**. SAMPL9 will consider the effect of common biologically-relevant counterions/salts on guest binding, comparing the effects of NaCl and NaI on the complexation of at least five guests to **1**. We have previously shown that iodide has a weak affinity for the binding pocket of **1**, while sodium ions have an affinity for the outer carboxylates [210], requiring modeling to capture the differential affinities of these ions in addition to guest affinities to successfully model the observed affinities. SAMPL10 will follow up on this by examining the effects of these same two salts on the complexation of at least five guests to **3**, again giving the modeling community time to incorporate algorithmic improvements following SAMPL9. While we have not yet quantified salt affinities to host **3**, we expect the iodide to have affinity for both the pocket and the positively charged solubilizing groups. For SAMPL11 we will consider the effects of co-solvents on the binding of five guests to **1** and **2** to probe the effect of co-solvent competition for the binding site, as well as effects co-solvents may have on weakening the hydrophobic effect. Participants frequently request larger datasets, so every effort will be made to include additional guests beyond the minimal number proposed if time allows or if human time can be reduced (such as via automated calorimetry). Regardless, the total number of binding affinities measured for the family is substantial, so the data will be of considerable value as a benchmark [165].

Aim 3: Develop model protein-ligand systems that isolate specific modeling challenges of drug targets.

To drive real improvement in quantitative modeling of protein-ligand interactions, we need to be able to revisit the same systems again and again in order to gauge and drive progress—in much the same way as participants in the Netflix Challenge had to genuinely improve their ability to predict user feedback to succeed [107]. While D3R [112] evaluates the accuracy of current protein-ligand binding methods, performance in each Challenge iteration varies widely depending on the nature of the donated pharmaceutical data and difficulty of the target. The high complexities of D3R targets make it difficult to identify clear points of failure [112, 151–153]. For example, while kinases are targets of great interest to drug discovery, blind Challenges involving kinase targets conflate issues of slow protein conformational dynamics [211], protonation state effects of protein [212] and ligand [213, 214], phosphorylation, charged ligands, and other challenges; failures on such targets are thus often unexplained.

Only by revisiting the same systems repeatedly will we benefit from the wisdom of the crowds in identifying specific problems and validating solutions to these problems. Thus, here, **we identify and develop model protein-ligand systems to isolate specific accuracy-limiting effects in a series of SAMPL Challenges.** By developing *model binding systems*—biological protein-ligand systems comprised of single-domain proteins binding to a simple ligand series free of complex phenomena—we can study systems of complexity intermediate between completely artificial systems (like the valuable T4 lysozyme L99A model binding site [135, 165, 215]) and complex pharmaceutical targets where multiple modeling challenges make it difficult to learn from failure (Figure 8A). This allows Challenges focused on identifying and evaluating multiple solutions to selected accuracy-limiting effects (such as how to deal with ligand and protein protonation-state issues [216], slow protein conformational dynamics, etc.), with the ability to revisit the same systems repeatedly as needed to drive innovation.

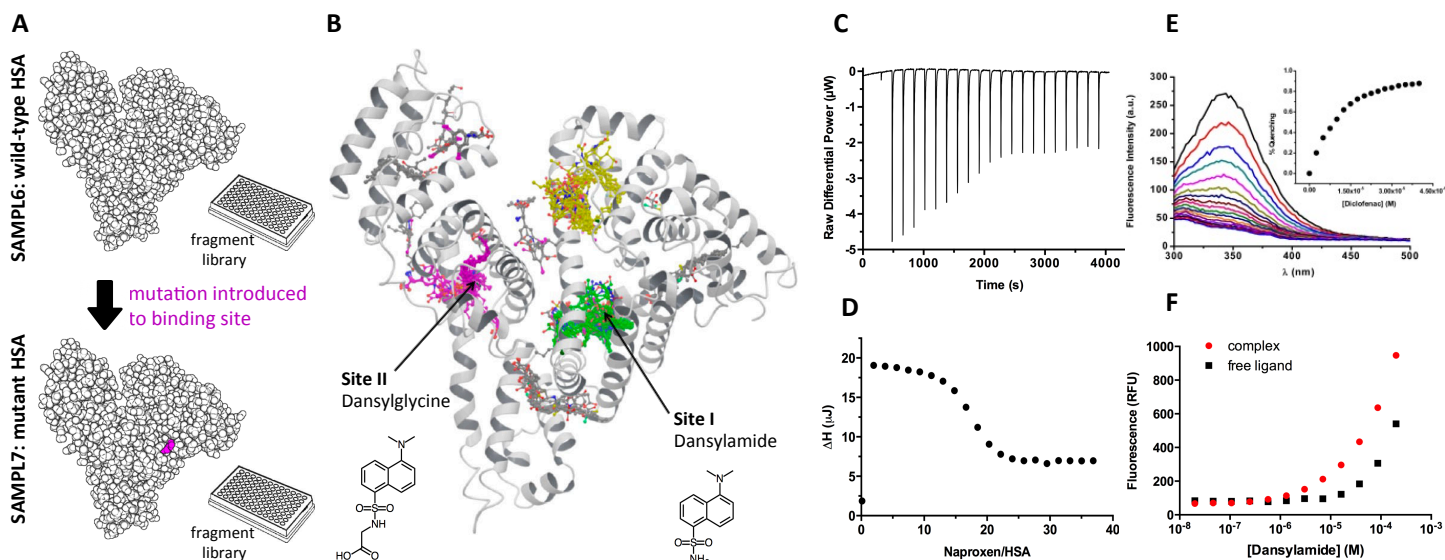


Figure 7. The SAMPL7/8 protein-ligand Challenge focuses on soluble drug fragment binding to human serum albumin (HSA) (Aim 3). (A) SAMPL7 will study binding of a library of at least 96 small soluble druglike fragments to recombinant HSA, with an engineered HSA mutant used for SAMPL8. (B) HSA has at least eight known binding sites, with two major well-characterized sites (green, Sudlow's Site I; purple, Site II) that bind a variety of drugs (figure from [217]). Two fluorescent probes—dansylamide and dansylglycine—bind with $\sim\mu\text{M}$ affinity and high selectivity to Site I and Site II, respectively; both exhibit binding-enhanced fluorescence at 480 nm, and can be used to site-specifically probe ligand affinities by competition. (C, D) Binding affinities of soluble molecules can be measured by isothermal titration calorimetry (ITC); here, we show the (C) differential power and (D) integrated injection heats for the ITC titration of HSA by naproxen sodium collected using the Chodera lab automation pipeline; (E) HSA tryptophan fluorescence quenching can also be used to measure ligand binding affinity; here, HSA titration by diclofenac is shown, with the inset plot showing percent quenching at 346 nm [218, 219]. (F) Direct fluorescence binding assay of Dansylamide (fluorescent ligand) and HSA collected on the Chodera lab automation system. The binding curve can be constructed based on binding-induced fluorescence emission at 480 nm.

Crowdsourced innovation enhances the potential of model systems for driving progress. For example, SAMPL3 focused on charged fragments binding to trypsin [220], and rapidly focused the field on the deficiencies of current alchemical free energy methodologies in treating the binding of charged ligands. Within two years, multiple laboratories developed solutions to this problem [221–223].

SAMPL7-11 model protein-ligand Challenges. We will introduce a new model protein-ligand system each SAMPL (revisiting the prior SAMPL's system if this becomes too difficult), with multiple Challenges on each system (Figure 2) to allow iterative improvement and assessment. Our SAMPL7 data will focus on the binding of small soluble drug fragments to one particular protein (below), with fragments also studied in Aim 1. However, maximizing gains in this area requires adapting subsequent Challenges based on deficiencies identified by prior Challenges, so our new informatics platform (below) will aid in system selection.

SAMPL7: Assessing predictive modeling of binding to multiple weak sites via measuring fragment binding to human serum albumin (HSA). HSA, the most abundant blood plasma protein, has a remarkable ability to bind a variety of small molecule drugs in multiple binding sites (Figure 7B) [224]. Using HSA as a model receptor isolates the challenge of multiple weak ligands binding to a stable rigid protein that is also pharmacologically relevant because of its dramatic modulation of drug pharmacokinetics [217]. HSA has at least *eight* known binding sites, with numerous crystal structures available for drugs binding to two predominant sites (Site I and II) [217]. Small soluble molecules resembling drug fragments are highly likely to bind to HSA ($\geq 90\%$ of such fragments, as detected by SPR [225]), providing an experimentally-tractable diverse set of ligands spanning several orders of magnitude in affinity [225]. As many current physical modeling methods assume a single well-defined binding site with high affinity [226], this Challenge will allow the isolation of the effect of weak multiple binding from the majority of other confounding factors in protein-ligand binding. HSA is relatively rigid, and computational methods already show some promise in computing binding affinities to HSA [217, 227, 228], making this an optimal model system.

Recombinant HSA will be expressed in *E. coli* and purified via refolding from inclusion bodies [229], then defatted at low pH [230] to ensure the resulting protein is free of the glycosylation and bound fatty acids found in plasma-isolated HSA [230]. Recombinant expression will allow mutant forms of HSA (engineered via single-primer mutagenesis) to be fielded for SAMPL8 (Figure 7). We will obtain a diverse library of at least 96 soluble drug-fragment-like molecules in pre-plated format as dry compound, and use our automated ITC pipeline (Figure 7C,D) to characterize overall binding affinities to HSA. The same ligands pre-plated in DMSO format will be used to conduct a separate set of fluorescence titration assays (monitoring tryptophan fluorescence quenching, Figure 7D)

and competition assays with site-specific fluorescent probes (Figure 7B) to resolve site-specific affinities to Sites I and II. We will field several levels of Challenges, including Challenges focused on affinities to Sites I and II, as well as Challenges focused on predicting overall affinity and stoichiometry.

SAMPL8-11: Rapid development of new tailored model systems using a novel informatics platform. We are developing a novel informatics platform, TargetExplorer, to identify protein targets that can be rapidly developed into tractable model systems focusing on individual challenges (Figure 8). This tool filters all known protein targets with structural data available in the PDB, first selecting for experimental tractability, then annotating experimentally tractable targets to determine which targets possess (or are likely to be free of) specific challenges for physical modeling. This will allow us to select systems isolating specific modeling challenges.

The Chodera lab has developed an automated wetlab to facilitate the development of such systems using bacterial expression (see Equipment and Facilities). Potential targets matching desired challenge criteria will be screened for bacterial expression using high-throughput cloning, transformation, and expression testing, with purity and yield assessed by capillary electrophoresis on a Caliper GXII. Targets will be screened for stability in various buffers using Thermofluor thermal shift assays [233]. Ligands identified via TargetExplorer as spanning a wide dynamic range of binding affinities will be purchased as dry powder stocks and prepared for assay by highly accurate gravimetric solution preparation techniques using a Quantos automated balance. Our lab has access to a wide variety of biophysical techniques for measuring protein-ligand binding affinities, including fluorescence (if fluorescent probe ligands are available), absorption (e.g. Soret band shifts), automated isothermal titration calorimetry (provided ligands are sufficiently soluble), surface plasmon resonance, microscale thermophoresis (MST), luminescence, and alphascreen; all except MST are fully automated.

We take a twofold approach to developing Challenge datasets: First, we will purchase and assay small molecules similar to known ligands, presuming that these molecules are likely to have measurable affinities. Second, using single-primer quick-change mutagenesis, we will introduce site-directed mutants to modulate the binding affinities of known ligands. This can be performed and screened for expression in 96-well format. Thus, datasets will consist of a matrix of protein mutants and ligands, providing opportunity to deeply explore the effects of interest.

Aim 4: Crowdsourcing innovation via community blind Challenges to advance biomolecular design.

The heart of our work lies here – driving innovation via community blind SAMPL Challenges centered around the datasets of Aims 1–3 (Figure 2). This approach utilizes crowdsourcing (see Innovation, above) to advance science. Our Challenges test the state of the art, provoke new methodological and force field innovations, allow comparative evaluation of methods so that advances are quickly recognized and spread, and drive downstream improvements. Our SAMPL Challenges are progressive, building on one another so that for success in later Challenges, participants must build on lessons learned from prior Challenges. Each iteration will likely yield its own incremental benefits (e.g., as in Figure 3) for molecular design, in addition to contributing to progress towards accurate prediction of biomolecular interactions.

SAMPL blind Challenges. Yearly SAMPL Challenges following our timeline (Figure 2) serve to advance the state-of-the-art. As is critical for such Challenges [106], we will advertise aggressively via press releases, commentaries

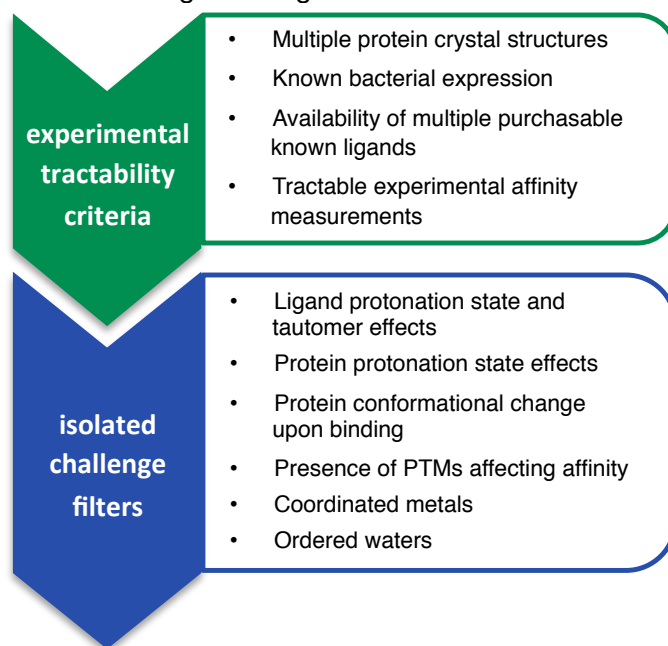


Figure 8. Mining model protein-ligand systems to focus on individual modeling challenges via a structural and chemical informatics platform (Aim 3). We are developing a structural and chemical informatics system called TargetExplorer [<https://github.com/choderalab/targetexplorer>] that applies successive filters to all potential protein-ligand systems for which structural data is available. Suitable model systems should meet all experimental tractability criteria (green box) and possess only a few challenging properties, ideally only one (blue box). Tractability of experimental affinity measurements includes properties like known ligands with potentially fluorescent scaffolds (for fluorescence competition assays), highly soluble ligands (for ITC), or ligands above a minimal mass (for SPR or MST). Additional filters annotate experimentally tractable systems with their potential computational challenges, including charged ligands or potential ligand protonation state or tautomer effects [231] (deduced from predicted aqueous protonation/tautomer energies); potential protein protonation state effects (deduced from MCCE2 calculations [232]); protein conformational changes (deduced from variation in protein conformation or the presence of unresolved loops in protein-ligand crystal structures); the presence of post-translational modifications that may affect affinity (deduced from Uniprot annotations); coordinated metals (identified in crystal structures); and ordered waters (present in multiple crystal structures).

in relevant journals, CCL, e-mail, and Twitter. As experimental data for each component becomes available and is curated, input files and Challenge details will be made available at least six months prior to the Challenge deadline; data not yet available at that time will be held for a subsequent Challenge (with the exception of three months for year 1 due to startup timescales). Advertising will feature our full timeline (which will also be available at <https://drugdesigndata.org/about/sampl> along with details of plans) allowing planning of participation. Challenge datasets will now also be larger than prior SAMPLs, as frequently requested [111].

As in prior SAMPLs, submissions will be handled by a web upload service on the SAMPL website which validates submissions to ensure that they meet format standards for automated processing and analysis. Analysis will be conducted by our automated Python framework, and results returned automatically online. All participant submissions and methodology descriptions will (as before) be made available publicly on the website, along with participant information (except for participants who specifically request to remain anonymous prior to submission). Aggregate statistics and historical performance will also be made available on our website, along with a record linking publications to historical submissions.

As the project progresses, we will push to transition towards submission of *methods* rather than of *predictions*. Specifically, as automated workflow engines such as OpenEye's Orion, AutoDesk's MDT, and others begin to be adopted on a larger scale, we will push participants to submit their methods in a standardized package, and automatically apply their submitted method to the Challenge data via cloud computing resources. This is already done in some Challenge platforms [106] but will be new to SAMPL, resolving one major hurdle for participation. Participants, while enthusiastic about SAMPL and how it benefits science, often note the burden of participating [111]; submission of methods rather than results will solve this problem, allowing participation with minimal investment of human time. This will also allow for easy archival of methods and a focus on testing workflows rather than human expertise. We will work with the NSF-funded Molecular Sciences Software Sustainability Institute (MoISSI) to identify best practices in this respect. One hurdle will be obtaining computer time, but we will seek donations of time from cloud computing vendors, support from pharma, and adapt our workflows so we can run on NSF supercomputing resources where we will seek grants. In the worst case we would ask participants submitting methods for a modest budget to run their methods, but having this level of automation results in substantial savings in human time which can itself result in monetary savings. This migration will also facilitate post-Challenge follow-up and method comparison, by allowing us to archive reproducible methods. Technology already allows this sort of archival and reproducibility, such as via Docker [234] containers with the participants' executable programs [106].

We will leverage SAMPL Challenges to identify modeling failures and identify potential solutions. The learning process begins prior to each Challenge and continues afterwards. In advance, we provide guidance to participants as to what known modeling issues we expect may be relevant. For a host-guest system, for example, we might highlight known buffer/salt effects, protonation state challenges, and point out previous work on sampling challenges, with pointers to the relevant experimental work and to modeling work from past SAMPL Challenges and elsewhere [165]. This helps participants design their approach. **Additionally, we will run reference calculations using current best practices.** This serves several purposes: It provides a test of the current methods and force fields; it helps facilitate learning—we announce what calculations we plan to perform, make input files available in a wide variety of formats [145, 146, 235], and others can repeat our calculations with with method or force field variations to test protocol differences; it provides training to our students who are involved (as these reference calculations provide them familiarity with the latest methods and make them instrumental in community learning); and it allows sensitivity analysis, as by varying the conditions (protonation state, tautomer, etc., [145]) we can see how much this impacts calculated values and thereby how important each factor is. Reference calculations have, for example, helped us highlight the importance of a small amount of water in cyclohexane for accurately calculating log D values, show how an incorrect tautomer could affect calculated values by many log units [145], and discover that small force field modifications could significantly improve results on hydration free energies [138].

Dissemination of Challenge results. The most critical phase of each Challenge occurs after results are released. Typically, some participants uncover specific problems or solutions which are missed by others, so dissemination of these insights becomes critical to drive improvements. SAMPL workshops will occur after Challenges every two years (years 1, 3, and 5), co-hosted with D3R Grand Challenge workshops (see support letter) During the off years, discussion and dissemination of results will be via asynchronous means (below) and a “virtual workshop” consisting of talks and interaction over Google Hangouts or YouTube Live. For all workshops, virtual or otherwise, talks will be selected from abstracts written to describe what each participant learned from participating—allowing us to prioritize presentations from those who have learned a great deal or done exceptionally well, rather than those who are simply testing an existing method with no new insight gained. If the D3R effort is not renewed, we will run SAMPL workshops independently, controlling costs via the model we use for our Workshops on Free Energy Methods in Drug Design—specifically, most participants will pay their own way to the workshop, and we

will seek pharmaceutical and software industry sponsorships to defray costs.

Rapid dissemination of results is critical so that new insights can be used in subsequent Challenges. We will continue to publish special issues of the *Journal of Computer Aided Molecular Design* (JCAMD) collecting publications related to each year's SAMPL Challenges (see Letter of Support). As requested by participants [111], we will also begin providing summaries of the key results and insights from each SAMPL that are suitable for a broad, non-technical audience – either in JCAMD or as commentary in another suitable journal. To ensure immediate availability of reports, we will strongly encourage prepublication sharing of results and analysis, including both slides and posters from SAMPL meetings (via F1000 Research) and paper preprints (via bioRxiv).

Post-Challenge analysis also drives progress, and often occurs outside of formal workshops and meetings. While this has happened in the past—for example, when participants using similar methods work together after the SAMPL meeting to identify the origin of these discrepancies [146, 165, 176, 236, 237]—we hope to accelerate this kind of collaboration. To facilitate rapid exchange of ideas and open communication between the community, we will use collaboration software—such as Slack, which facilitates scientific communication for the NASA/JPL Mars Rover teams and NSF antarctic scientific research teams—to build a community discussion platform, facilitating a process of learning from one another more rapidly than normal publication channels. Given the overlap between molecules/fragments used in Aim 1 and those studied in Aims 2–3, insights from one Challenge component will have implications for predictions in the other components, making this rapid exchange key. The students supported in this project will also play a role, working to understand the methods employed and bringing together researchers using similar methods to try and understand performance differences, including by running follow-up calculations.

Each Challenge will have lasting effects. SAMPL datasets are collected primarily to drive Challenges, but both the datasets and the lessons learned have lasting value. The lessons learned will have the most immediate near-term impact and are critical for progress because each Challenge builds on prior iterations. However, the datasets themselves are also key. In the past, due to the unfunded nature of SAMPL, we primarily emphasized compilation of the datasets and conducting Challenges. However, SAMPL datasets achieved a life of their own outside of SAMPL, becoming standard test sets [147, 148, 165, 238, 239]. This is one way SAMPL Challenges achieve their lasting effect, so we will perform additional curation on datasets post-Challenge, with follow-up experiments (as often requested by participants [111]) when needed, then release the data publicly as standard benchmark sets [165]. We will make the data (including primary data, processed data, and our analysis of Challenge submissions) available freely and publicly with permanent, citeable DOIs; ensure relevant data is deposited in standard community repositories (e.g. BindingDB [240]); and guarantee data longevity via backup hosting on library archival facilities (such as the UC's DASH (<https://dash.cdlib.org/>)).

COLLABORATION MANAGEMENT PLAN

We have a strong previous history of successful collaboration, with Mobley and Chodera having co-authored roughly a dozen publications and organized several workshops and other initiatives. Mobley, Isaacs, and Gibb have also worked together to coordinate past SAMPL Challenges, and Mobley and Gibb a previous NSF workshop. PI Mobley will oversee the project, with teams for the other aims (Aim 1: Mobley & Chodera; Aim 2.1: Isaacs; Aim 2.2: Gibb; Aim 3: Chodera; Aim 4: Mobley & Chodera) involving the other co-investigators as needed. Meetings will consist of a monthly Google Hangout and a yearly in-person planning meeting. Chodera and Mobley will communicate more frequently due to the interlinked nature of their work. Publications are expected to be largely dictated by the overall Timeline, with an experimental publication associated with each Challenge component being prepared for distribution to participants along with their results. Conflict resolution is expected to be straightforward, but if any serious difficulties arise, Michael Gilson (UCSD) will arbitrate given our close connections with D3R.

OUTLOOK

Physical methods have been slow to achieve their promise in binding prediction, in part because truly significant innovations are so hard to recognize due to a lack of standard tests and benchmarks, and in part because of an “applications first” approach which seems to plague our community where we rush to apply our methods to problems of pharmaceutical relevance without ensuring they can tackle simpler, better-understood problems first. Here, we extend the successful series of SAMPL blind Challenges to crowdsource innovation focused on a series of key component problems centered around our major goal, quantitative prediction of biomolecular interactions. Collection of high quality, focused datasets will spur method innovation, beginning with relatively simple physical property prediction and progressing to challenging problems in biomolecular recognition via a series of carefully designed intermediate steps. Building on SAMPL's strong prior track record, the proposed series of tailored Challenges will focus community innovation on critical problems we can resolve in the near term, resulting in dramatic improvements in computational molecular design.

Full List of SAMPL References

- [1] Monroe, J. I. and Shirts, M. R.: Converging free energies of binding in cucurbit[7]uril and octa-acid host-guest systems from SAMPL4 using expanded ensemble simulations. J Comput Aided Mol Des. 28(4): 401–415, March 2014.
- [2] Muddana, H. S., Yin, J., Sapra, N. V., Fenley, A. T., and Gilson, M. K.: Blind prediction of SAMPL4 cucurbit[7]uril binding affinities with the mining minima method. J Comput Aided Mol Des. 28(4): 463–474, February 2014.
- [3] Gallicchio, E., Chen, H., Chen, H., Fitzgerald, M., Gao, Y., He, P., Kalyanikar, M., Kao, C., Lu, B., Niu, Y., Pethe, M., Zhu, J., and Levy, R. M.: BEDAM binding free energy predictions for the SAMPL4 octa-acid host challenge. J Comput Aided Mol Des. 29(4): 315–325, March 2015.
- [4] Mikulskis, P., Cioloboc, D., Andrejić, M., Khare, S., Brorsson, J., Genheden, S., Mata, R. A., Söderhjelm, P., and Ryde, U.: Free-energy perturbation and quantum mechanical study of SAMPL4 octa-acid host-guest binding energies. J Comput Aided Mol Des. 28(4): 375–400, April 2014.
- [5] Hsiao, Y.-W. and Söderhjelm, P.: Prediction of SAMPL4 host-guest binding affinities using funnel metadynamics. J Comput Aided Mol Des. 28(4): 443–454, February 2014.
- [6] Bhakat, S. and Söderhjelm, P.: Resolving the problem of trapped water in binding cavities: Prediction of host-guest binding free energies in the SAMPL5 challenge by funnel metadynamics. J Comput Aided Mol Des. 31(1): 119–132, 2017.
- [7] Pal, R. K., Haider, K., Kaur, D., Flynn, W., Xia, J., Levy, R. M., Taran, T., Wickstrom, L., Kurtzman, T., and Gallicchio, E.: A combined treatment of hydration and dynamical effects for the modeling of host-guest binding thermodynamics: The SAMPL5 blinded challenge. Journal of Computer-Aided Molecular Design. 31(1): 29–44, 2017.
- [8] Yin, J., Henriksen, N. M., Slochower, D. R., and Gilson, M. K.: The SAMPL5 Host-Guest Challenge: Computing Binding Free Energies and Enthalpies from Explicit Solvent Simulations by the Attach-Pull-Release (APR) Method. J Comput Aided Mol Des. 31(1): 133–145, 2017.
- [9] Bosisio, S., Mey, A. S. J. S., and Michel, J.: Blinded predictions of host-guest standard free energies of binding in the SAMPL5 challenge. J Comput Aided Mol Des. 31(1): 61–70, 2017.
- [10] Tofoleanu, F., Lee, J., Pickard IV, F. C., König, G., Huang, J., Baek, M., Seok, C., and Brooks, B. R.: Absolute binding free energy calculations for octa-acids and guests. J Comput Aided Mol Des. 31(1): 107–118, 2017.
- [11] Mobley, D. L., Wymer, K. L., Lim, N. M., and Guthrie, J. P.: Blind prediction of solvation free energies from the SAMPL4 challenge. J Comput Aided Mol Des. 28(3): 135–150, March 2014.
- [12] Muddana, H. S., Fenley, A. T., Mobley, D. L., and Gilson, M. K.: The SAMPL4 host-guest blind prediction challenge: An overview. J Comput Aided Mol Des. 28(4): 305–317, March 2014.
- [13] Sullivan, M. R., Sokkalingam, P., Nguyen, T., Donahue, J. P., and Gibb, B. C.: Binding of carboxylate and trimethylammonium salts to octa-acid and TEMOA deep-cavity cavitands. J Comput Aided Mol Des. 31(1): 1–8, 2017.
- [14] Deng, N., Forli, S., He, P., Perryman, A., Wickstrom, L., Vijayan, R. S. K., Tiefenbrunn, T., Stout, D., Gallicchio, E., Olson, A. J., and Levy, R. M.: Distinguishing Binders from False Positives by Free Energy Calculations: Fragment Screening Against the Flap Site of HIV Protease. J. Phys. Chem. B. 119(3): 976–988, January 2015.
- [15] Li, L., Dill, K. A., and Fennell, C. J.: Testing the semi-explicit assembly model of aqueous solvation in the SAMPL4 challenge. J Comput Aided Mol Des. 28(3): 259–264, January 2014.
- [16] Paranahebage, S. S., Gierhart, C. S., and Fennell, C. J.: Predicting water-to-cyclohexane partitioning of the SAMPL5 molecules using dielectric balancing of force fields. J Comput Aided Mol Des. 30(11): 1059–1065, August 2016.

- [17] Klamt, A., Eckert, F., Reinisch, J., and Wichmann, K.: Prediction of cyclohexane-water distribution coefficients with COSMO-RS on the SAMPL5 data set. *J Comput Aided Mol Des.* 30(11): 959–967, July 2016.
- [18] Tielker, N., Tomazic, D., Heil, J., Kloss, T., Ehrhart, S., Güssregen, S., Schmidt, K. F., and Kast, S. M.: The SAMPL5 challenge for embedded-cluster integral equation theory: Solvation free energies, aqueous pKa, and cyclohexane–water log D. *J Comput Aided Mol Des.* 30: 1035–1044, August 2016.
- [19] König, G., Pickard, F. C., Huang, J., Simmonett, A. C., Tofoleanu, F., Lee, J., Dral, P. O., Prasad, S., Jones, M., Shao, Y., Thiel, W., and Brooks, B. R.: Calculating distribution coefficients based on multi-scale free energy simulations: An evaluation of MM and QM/MM explicit solvent simulations of water-cyclohexane transfer in the SAMPL5 challenge. *J Comput Aided Mol Des.* 30(11): 989–1006, August 2016.
- [20] Luchko, T., Blinov, N., Limon, G. C., Joyce, K. P., and Kovalenko, A.: SAMPL5: 3D-RISM partition coefficient calculations with partial molar volume corrections and solute conformational sampling. *J Comput Aided Mol Des.* 30(11): 1–13, September 2016.
- [21] Santos-Martins, D., Fernandes, P. A., and Ramos, M. J.: Calculation of distribution coefficients in the SAMPL5 challenge from atomic solvation parameters and surface areas. *J Comput Aided Mol Des.* 30(11): 1079–1086, September 2016.
- [22] Perryman, A. L., Santiago, D. N., Forli, S., Santos-Martins, D., and Olson, A. J.: Virtual screening with AutoDock Vina and the common pharmacophore engine of a low diversity library of fragments and hits against the three allosteric sites of HIV integrase: Participation in the SAMPL4 protein–ligand binding challenge. *J Comput Aided Mol Des.* 28(4): 429–441, February 2014.
- [23] König, G., Pickard, F. C., Mei, Y., and Brooks, B. R.: Predicting hydration free energies with a hybrid QM/MM approach: An evaluation of implicit and explicit solvation models in SAMPL4. *J Comput Aided Mol Des.* 28(3): 245–257, February 2014.
- [24] Voet, A. R. D., Kumar, A., Berenger, F., and Zhang, K. Y. J.: Combining in silico and in cerebro approaches for virtual screening and pose prediction in SAMPL4. *J Comput Aided Mol Des.* 28(4): 363–373, January 2014.
- [25] Park, H.: Extended solvent-contact model approach to SAMPL4 blind prediction challenge for hydration free energies. *J Comput Aided Mol Des.* 28(3): 175–186, February 2014.
- [26] Rustenburg, A. S., Dancer, J., Lin, B., Feng, J. A., Ortwine, D. F., Mobley, D. L., and Chodera, J. D.: Measuring experimental cyclohexane-water distribution coefficients for the SAMPL5 challenge. *Journal of Computer-Aided Molecular Design.* 30(11): 945–958, July 2016.
- [27] Reinisch, J. and Klamt, A.: Prediction of free energies of hydration with COSMO-RS on the SAMPL4 data set. *J Comput Aided Mol Des.* 28(3): 169–173, January 2014.
- [28] Muddana, H. S., Sapra, N. V., Fenley, A. T., and Gilson, M. K.: The SAMPL4 hydration challenge: Evaluation of partial charge sets with explicit-water molecular dynamics simulations. *J Comput Aided Mol Des.* 28(3): 277–287, January 2014.
- [29] Manzoni, F. and Söderhjelm, P.: Prediction of hydration free energies for the SAMPL4 data set with the AMOEBA polarizable force field. *J Comput Aided Mol Des.* 28(3): 235–244, March 2014.
- [30] Sandberg, L.: Predicting hydration free energies with chemical accuracy: The SAMPL4 challenge. *J Comput Aided Mol Des.* 28(3): 211–219, February 2014.
- [31] Brini, E., Paranaheewage, S. S., Fennell, C. J., and Dill, K. A.: Adapting the semi-explicit assembly solvation model for estimating water-cyclohexane partitioning with the SAMPL5 molecules. *J Comput Aided Mol Des.* 30(11): 1067–1077, September 2016.
- [32] Kamath, G., Kurnikov, I., Fain, B., Leontyev, I., Illarionov, A., Butin, O., Olevanov, M., and Pereyaslavets, L.: Prediction of cyclohexane-water distribution coefficient for SAMPL5 drug-like compounds with the QMPFF3 and ARROW polarizable force fields. *J Comput Aided Mol Des.* 30(11): 977–988, September 2016.

- [33] Diaz-Rodriguez, S., Bozada, S. M., Phifer, J. R., and Paluch, A. S.: Predicting cyclohexane/water distribution coefficients for the SAMPL5 challenge using MOSCED and the SMD solvation model. *J Comput Aided Mol Des.* 30(11): 1007–1017, August 2016.
- [34] Kenney, I. M., Beckstein, O., and Iorga, B. I.: Prediction of cyclohexane-water distribution coefficients for the SAMPL5 data set using molecular dynamics simulations with the OPLS-AA force field. *J Comput Aided Mol Des.* 30(11): 1–14, August 2016.
- [35] Caldararu, O., Olsson, M. A., Riplinger, C., Neese, F., and Ryde, U.: Binding free energies in the SAMPL5 octa-acid host–guest challenge calculated with DFT-D3 and CCSD(T). *J Comput Aided Mol Des.* 31(1): 87–106, 2017.
- [36] Genheden, S. and Essex, J. W.: All-atom/coarse-grained hybrid predictions of distribution coefficients in SAMPL5. *J Comput Aided Mol Des.* 30(11): 969–976, July 2016.
- [37] Chung, K.-C. and Park, H.: Extended solvent-contact model approach to blind SAMPL5 prediction challenge for the distribution coefficients of drug-like molecules. *J Comput Aided Mol Des.* 30(11): 1019–1033, July 2016.
- [38] Koziara, K. B., Stroet, M., Malde, A. K., and Mark, A. E.: Testing and validation of the Automated Topology Builder (ATB) version 2.0: Prediction of hydration free enthalpies. *J Comput Aided Mol Des.* 28(3): 221–233, January 2014.
- [39] Yin, J., Henriksen, N. M., Slochower, D. R., Shirts, M. R., Chiu, M. W., Mobley, D. L., and Gilson, M. K.: Overview of the SAMPL5 host–guest challenge: Are we doing better? *J Comput Aided Mol Des.* 31(1): 1–19, 2017.
- [40] Bannan, C. C., Burley, K. H., Chiu, M., Shirts, M. R., Gilson, M. K., and Mobley, D. L.: Blind prediction of cyclohexane–water distribution coefficients from the SAMPL5 challenge. *J Comput Aided Mol Des.* 30(11): 1–18, September 2016.
- [41] Lee, J., Tofoleanu, F., Pickard, F. C., König, G., Huang, J., Damjanović, A., Baek, M., Seok, C., and Brooks, B. R.: Absolute binding free energy calculations of CBClip host–guest systems in the SAMPL5 blind challenge. *J Comput Aided Mol Des.* 31(1): 71–85, 2017.
- [42] Jones, M. R., Brooks, B. R., and Wilson, A. K.: Partition coefficients for the SAMPL5 challenge using transfer free energies. *J Comput Aided Mol Des.* 30(11): 1129–1138, September 2016.
- [43] Pickard, F. C., König, G., Tofoleanu, F., Lee, J., Simonett, A. C., Shao, Y., Ponder, J. W., and Brooks, B. R.: Blind prediction of distribution in the SAMPL5 challenge with QM based protomer and pKa corrections. *J Comput Aided Mol Des.* 30(11): 1–14, September 2016.
- [44] Cao, L. and Isaacs, L.: Absolute and relative binding affinity of cucurbit[7]uril towards a series of cationic guests. *Supramolecular Chemistry.* 26(3-4): 251–258, March 2014.
- [45] Muddana, H. S. and Gilson, M. K.: Prediction of SAMPL3 host–guest binding affinities: Evaluating the accuracy of generalized force-fields. *J Comput Aided Mol Des.* 26(5): 517–525, January 2012.
- [46] Gibb, C. L. D. and Gibb, B. C.: Binding of cyclic carboxylates to octa-acid deep-cavity cavitand. *J Comput Aided Mol Des.* 28(4): 319–325, November 2013.
- [47] Klimovich, P. V. and Mobley, D. L.: Predicting hydration free energies using all-atom molecular dynamics simulations and multiple starting conformations. *Journal of Computer-Aided Molecular Design.* 24(4): 307–316, April 2010.
- [48] Mobley, D. L., Liu, S., Cerutti, D. S., Swope, W. C., and Rice, J. E.: Alchemical prediction of hydration free energies for SAMPL. *Journal of Computer-Aided Molecular Design.* 26(5): 551–562, PMC3583515, May 2012.
- [49] Muddana, H. S., Varnado, C. D., Bielawski, C. W., Urbach, A. R., Isaacs, L., Geballe, M. T., and Gilson, M. K.: Blind prediction of host–guest binding affinities: A new SAMPL3 challenge. *J Comput Aided Mol Des.* 26(5): 475–487, February 2012.

- [50] Skillman, A. G.: SAMPL3: Blinded prediction of host–guest binding affinities, hydration free energies, and trypsin inhibitors. J Comput Aided Mol Des. 26(5): 473–474, May 2012.
- [51] Newman, J., Dolezal, O., Fazio, V., Caradoc-Davies, T., and Peat, T. S.: The DINGO dataset: A comprehensive set of data for the SAMPL challenge. J Comput Aided Mol Des. 26(5): 497–503, December 2011.
- [52] Gallicchio, E., Deng, N., He, P., Wickstrom, L., Perryman, A. L., Santiago, D. N., Forli, S., Olson, A. J., and Levy, R. M.: Virtual screening of integrase inhibitors by large scale binding free energy calculations: The SAMPL4 challenge. J Comput Aided Mol Des. 28(4): 475–490, February 2014.
- [53] Klamt, A. and Diedenhofen, M.: Blind prediction test of free energies of hydration with COSMO-RS. J Comput Aided Mol Des. 24(4): 357–360, April 2010.
- [54] Fennell, C. J., Kehoe, C. W., and Dill, K. A.: Modeling aqueous solvation with semi-explicit assembly. PNAS. 108(8): 3234–3239, February 2011.
- [55] Ellingson, B. A., Skillman, A. G., and Nicholls, A.: Analysis of SM8 and Zap TK calculations and their geometric sensitivity. J Comput Aided Mol Des. 24(4): 335–342, April 2010.
- [56] Surpateanu, G. and Iorga, B. I.: Evaluation of docking performance in a blinded virtual screening of fragment-like trypsin inhibitors. J Comput Aided Mol Des. 26(5): 595–601, December 2011.
- [57] Purisima, E. O., Corbeil, C. R., and Sulea, T.: Rapid prediction of solvation free energy. 3. Application to the SAMPL2 challenge. J Comput Aided Mol Des. 24(4): 373–383, April 2010.
- [58] König, G. and Brooks, B. R.: Predicting binding affinities of host-guest systems in the SAMPL3 blind challenge: The performance of relative free energy calculations. J Comput Aided Mol Des. 26(5): 543–550, December 2011.
- [59] Kehoe, C. W., Fennell, C. J., and Dill, K. A.: Testing the semi-explicit assembly solvation model in the SAMPL3 community blind test. J Comput Aided Mol Des. 26(5): 563–568, December 2011.
- [60] Kumar, A. and Zhang, K. Y. J.: Computational fragment-based screening using RosettaLigand: The SAMPL3 challenge. J Comput Aided Mol Des. 26(5): 603–616, January 2012.
- [61] Meunier, A. and Truchon, J.-F.: Predictions of hydration free energies from continuum solvent with solute polarizable models: The SAMPL2 blind challenge. J Comput Aided Mol Des. 24(4): 361–372, March 2010.
- [62] Genheden, S., Martinez, A. I. C., Criddle, M. P., and Essex, J. W.: Extensive all-atom Monte Carlo sampling and QM/MM corrections in the SAMPL4 hydration free energy challenge. J Comput Aided Mol Des. 28(3): 187–200, February 2014.
- [63] Beckstein, O., Fourrier, A., and Iorga, B. I.: Prediction of hydration free energies for the SAMPL4 diverse set of compounds using molecular dynamics simulations with the OPLS-AA force field. J Comput Aided Mol Des. 28(3): 265–276, February 2014.
- [64] Coleman, R. G., Sterling, T., and Weiss, D. R.: SAMPL4 & DOCK3.7: Lessons for automated docking procedures. J Comput Aided Mol Des. 28(3): 201–209, February 2014.
- [65] Hogues, H., Sulea, T., and Purisima, E. O.: Exhaustive docking and solvated interaction energy scoring: Lessons learned from the SAMPL4 challenge. J Comput Aided Mol Des. 28(4): 417–427, January 2014.
- [66] Reinisch, J., Klamt, A., and Diedenhofen, M.: Prediction of free energies of hydration with COSMO-RS on the SAMPL3 data set. J Comput Aided Mol Des. 26(5): 669–673, May 2012.
- [67] Kulp, J. L., Blumenthal, S. N., Wang, Q., Bryan, R. L., and Guarnieri, F.: A fragment-based approach to the SAMPL3 Challenge. J Comput Aided Mol Des. 26(5): 583–594, January 2012.
- [68] Klamt, A. and Diedenhofen, M.: Some conclusions regarding the predictions of tautomeric equilibria in solution based on the SAMPL2 challenge. J Comput Aided Mol Des. 24(6-7): 621–625, April 2010.

- [69] Fu, J., Liu, Y., and Wu, J.: Fast prediction of hydration free energies for SAMPL4 blind test from a classical density functional theory. J Comput Aided Mol Des. 28(3): 299–304, March 2014.
- [70] Hamaguchi, N., Fusti-Molnar, L., and Wlodek, S.: Force-field and quantum-mechanical binding study of selected SAMPL3 host-guest complexes. J Comput Aided Mol Des. 26(5): 577–582, February 2012.
- [71] Colas, C. and Iorga, B. I.: Virtual screening of the SAMPL4 blinded HIV integrase inhibitors dataset. J Comput Aided Mol Des. 28(4): 455–462, January 2014.
- [72] Ellingson, B. A., Geballe, M. T., Wlodek, S., Bayly, C. I., Skillman, A. G., and Nicholls, A.: Efficient calculation of SAMPL4 hydration free energies using OMEGA, SZYBKI, QUACPAC, and Zap TK. J Comput Aided Mol Des. 28(3): 289–298, March 2014.
- [73] Sulea, T. and Purisima, E. O.: Predicting hydration free energies of polychlorinated aromatic compounds from the SAMPL-3 data set with FiSH and LIE models. J Comput Aided Mol Des. 26(5): 661–667, December 2011.
- [74] Geballe, M. T., Skillman, A. G., Nicholls, A., Guthrie, J. P., and Taylor, P. J.: The SAMPL2 blind prediction challenge: Introduction and overview. J Comput Aided Mol Des. 24(4): 259–279, May 2010.
- [75] Ribeiro, R. F., Marenich, A. V., Cramer, C. J., and Truhlar, D. G.: Prediction of SAMPL2 aqueous solvation free energies and tautomeric ratios using the SM8, SM8AD, and SMD solvation models. J Comput Aided Mol Des. 24(4): 317–333, April 2010.
- [76] Skillman, A. G., Geballe, M. T., and Nicholls, A.: SAMPL2 challenge: Prediction of solvation energies and tautomer ratios. J Comput Aided Mol Des. 24(4): 257–258, April 2010.
- [77] Gallicchio, E. and Levy, R. M.: Prediction of SAMPL3 host-guest affinities with the binding energy distribution analysis method (BEDAM). J Comput Aided Mol Des. 26(5): 505–516, February 2012.
- [78] Mikulskis, P., Genheden, S., Rydberg, P., Sandberg, L., Olsen, L., and Ryde, U.: Binding affinities in the SAMPL3 trypsin and host–guest blind tests estimated with the MM/PBSA and LIE methods. J Comput Aided Mol Des. 26(5): 527–541, December 2011.
- [79] Geballe, M. T. and Guthrie, J. P.: The SAMPL3 blind prediction challenge: Transfer energy overview. J Comput Aided Mol Des. 26(5): 489–496, April 2012.
- [80] Guthrie, J. P.: SAMPL4, a blind challenge for computational solvation free energies: The compounds considered. J Comput Aided Mol Des. 28(3): 151–168, April 2014.
- [81] Nicholls, A., Wlodek, S., and Grant, J. A.: SAMPL2 and continuum modeling. J Comput Aided Mol Des. 24(4): 293–306, April 2010.
- [82] Soteras, I., Orozco, M., and Luque, F. J.: Performance of the IEF-MST solvation continuum model in the SAMPL2 blind test prediction of hydration and tautomerization free energies. J Comput Aided Mol Des. 24(4): 281–291, March 2010.
- [83] Lawrenz, M., Wereszczynski, J., Ortiz-Sánchez, J. M., Nichols, S. E., and McCammon, J. A.: Thermodynamic integration to predict host-guest binding affinities. J Comput Aided Mol Des. 26(5): 569–576, February 2012.
- [84] Sulea, T., Hogues, H., and Purisima, E. O.: Exhaustive search and solvated interaction energy (SIE) for virtual screening and affinity prediction. J Comput Aided Mol Des. 26(5): 617–633, December 2011.
- [85] Beckstein, O. and Iorga, B. I.: Prediction of hydration free energies for aliphatic and aromatic chloro derivatives using molecular dynamics simulations with the OPLS-AA force field. J Comput Aided Mol Des. 26(5): 635–645, December 2011.
- [86] Benson, M. L., Faver, J. C., Ucisik, M. N., Dashti, D. S., Zheng, Z., and Merz, K. M.: Prediction of trypsin/molecular fragment binding affinities by free energy decomposition and empirical scores. J Comput Aided Mol Des. 26(5): 647–659, April 2012.

- [87] Kast, S. M., Heil, J., Güssregen, S., and Schmidt, K. F.: Prediction of tautomer ratios by embedded-cluster integral equation theory. J Comput Aided Mol Des. 24(4): 343–353, March 2010.
- [88] Mobley, D. L., Bayly, C. I., Cooper, M. D., and Dill, K. A.: Predictions of Hydration Free Energies from All-Atom Molecular Dynamics Simulations. J Phys Chem B. 113: 4533–4537, January 2009.
- [89] Newman, J., Fazio, V., Caradoc-Davies, T., Branson, K., and Peat, T. S.: Practical Aspects of the SAMPL Challenge: Providing an Extensive Experimental Data Set for the Modeling Community. Journal of Biomolecular Screening. 14(10): 1245, January 2009.
- [90] Klamt, A., Eckert, F., and Diedenhofen, M.: Prediction of the Free Energy of Hydration of a Challenging Set of Pesticide-Like Compounds†. J Phys Chem B. January 2009.
- [91] Guthrie, J. P.: A Blind Challenge for Computational Solvation Free Energies: Introduction and Overview. J Phys Chem B. 113(14): 4501–4507, January 2009.
- [92] Marenich, A. V., Cramer, C. J., and Truhlar, D. G.: Performance of SM6, SM8, and SMD on the SAMPL1 Test Set for the Prediction of Small-Molecule Solvation Free Energies. J. Phys. Chem. B. 113(14): 4538–4543, April 2009.
- [93] Sulea, T., Wanapun, D., Dennis, S., and Purisima, E. O.: Prediction of SAMPL-1 Hydration Free Energies Using a Continuum Electrostatics-Dispersion Model. J. Phys. Chem. B. 113(14): 4511–4520, April 2009.
- [94] Nicholls, A., Wlodek, S., and Grant, J. A.: The SAMP1 Solvation Challenge: Further Lessons Regarding the Pitfalls of Parametrization. J. Phys. Chem. B. 113(14): 4521–4532, April 2009.
- [95] Nicholls, A., Mobley, D. L., Guthrie, J. P., Chodera, J. D., Bayly, C. I., Cooper, M. D., and Pande, V. S.: Predicting Small-Molecule Solvation Free Energies: An Informal Blind Test for Computational Chemistry. J. Med. Chem. 51(4): 769–779, February 2008.
- [96] Chamberlin, A. C., Cramer, C. J., and Truhlar, D. G.: Performance of SM8 on a Test To Predict Small-Molecule Solvation Free Energies. J. Phys. Chem. B. 112(29): 8651–8655, July 2008.
- [97] Gosink, L. J., Overall, C. C., Reehl, S. M., Whitney, P. D., Mobley, D. L., and Baker, N. A.: Bayesian Model Averaging for Ensemble-Based Estimates of Solvation-Free Energies. J. Phys. Chem. B. 121(15): 3458–3472, April 2017.
- [98] Yang, X., Lei, H., Gao, P., Thomas, D. G., Mobley, D., and Baker, N. A.: Atomic radius and charge parameter uncertainty in biomolecular solvation energy calculations. arXiv:1705.10035 [q-bio]. May 2017.
- [99] Shirts, M. R., Klein, C., Swails, J. M., Yin, J., Gilson, M. K., Mobley, D. L., Case, D. A., and Shirts, M. R.: Lessons learned from comparing molecular dynamics engines on the SAMPL5 dataset. J Comput Aided Mol Des. 31(1): 147–161, 2017.
- [100] Bansal, N., Zheng, Z., Cerutti, D. S., and Merz, K. M.: On the fly estimation of host–guest binding free energies using the movable type method: Participation in the SAMPL5 blind challenge. J Comput Aided Mol Des. 31(1): 47–60, January 2017.

Bibliography and References Cited

- [101] Moult, J., Fidelis, K., Kryshtafovych, A., Schwede, T., and Tramontano, A.: Critical assessment of methods of protein structure prediction (CASP) — round X. Proteins. 82: 1–6, February 2014.
- [102] Monastyrskyy, B., D'Andrea, D., Fidelis, K., Tramontano, A., and Kryshtafovych, A.: New encouraging developments in contact prediction: Assessment of the CASP11 results. Proteins. 84: 131–144, September 2016.
- [103] Moult, J., Fidelis, K., Kryshtafovych, A., Schwede, T., and Tramontano, A.: Critical assessment of methods of protein structure prediction: Progress and new directions in round XI. Proteins. 84: 4–14, September 2016.
- [104] Prill, R. J., Saez-Rodriguez, J., Alexopoulos, L. G., Sorger, P. K., and Stolovitzky, G.: Crowdsourcing Network Inference: The DREAM Predictive Signaling Network Challenge. Sci. Signal. 4(189): mr7–mr7, September 2011.
- [105] Eisenstein, M.: Crowdsourced contest identifies best-in-class breast cancer prognostic. Nat Biotech. 31(7): 578–580, July 2013.
- [106] Saez-Rodriguez, J., Costello, J. C., Friend, S. H., Kellen, M. R., Mangravite, L., Meyer, P., Norman, T., and Stolovitzky, G.: Crowdsourcing biomedical research: Leveraging communities as innovation engines. Nat Rev Genet. 17(8): 470–486, August 2016.
- [107] Bell, R. M., Koren, Y., and Volinsky, C.: All Together Now: A Perspective on the Netflix Prize. CHANCE. 23(1): 24–29, January 2010.
- [108] XPRIZE. <http://www.xprize.org/>.
- [109] Kay, L.: The effect of inducement prizes on innovation: Evidence from the Ansari XPrize and the Northrop Grumman Lunar Lander Challenge. R&D Manage. 41(4): 360–377, September 2011.
- [110] X Prize Foundation, June 2017. Page Version ID: 784796426.
- [111] Mobley, D. L., Chodera, J. D., and Gilson, M. K.: Results of the 2017 Roadmap survey of the Statistical Assessment of Modeling of Proteins and Ligands (SAMPL) challenge community. eScholarship. June 2017.
- [112] Gathiaka, S., Liu, S., Chiu, M., Yang, H., Stuckey, J. A., Kang, Y. N., Delproposto, J., Kubish, G., Dunbar, J. B., Carlson, H. A., Burley, S. K., Walters, W. P., Amaro, R. E., Feher, V. A., and Gilson, M. K.: D3R grand challenge 2015: Evaluation of protein–ligand pose and affinity predictions. J Comput Aided Mol Des. 30(9): 651–668, September 2016.
- [113] Mobley, D. L. and Klimovich, P. V.: Perspective: Alchemical free energy calculations for drug discovery. J. Chem. Phys. 137(23): 230901, January 2012.
- [114] Christ, C. D. and Fox, T.: Accuracy Assessment and Automation of Free Energy Calculations for Drug Design. J. Chem. Inf. Model. 54(1): 108–120, January 2014.
- [115] Deng, N., Forli, S., He, P., Perryman, A., Wickstrom, L., Vijayan, R. S. K., Tiefenbrunn, T., Stout, D., Gallicchio, E., Olson, A. J., and Levy, R. M.: Distinguishing Binders from False Positives by Free Energy Calculations: Fragment Screening Against the Flap Site of HIV Protease. J. Phys. Chem. B. 119(3): 976–988, January 2015.
- [116] Sherborne, B., Shanmugasundaram, V., Cheng, A. C., Christ, C. D., DesJarlais, R. L., Duca, J. S., Lewis, R. A., Loughney, D. A., Manas, E. S., McGaughey, G. B., Peishoff, C. E., and van Vlijmen, H.: Collaborating to improve the use of free-energy and other quantitative methods in drug discovery. J Comput Aided Mol Des. 30(12): 1139–1141, December 2016.
- [117] Wang, L., Wu, Y., Deng, Y., Kim, B., Pierce, L., Krilov, G., Lupyan, D., Robinson, S., Dahlgren, M. K., Greenwood, J., Romero, D. L., Masse, C., Knight, J. L., Steinbrecher, T., Beuming, T., Damm, W., Harder, E., Sherman, W., Brewer, M., Wester, R., Murcko, M., Frye, L., Farid, R., Lin, T., Mobley, D. L., Jorgensen, W. L., Berne, B. J., Friesner, R. A., and Abel, R.: Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field. J Am Chem Soc. 137(7): 2695–2703, February 2015.

- [118] Christ, C. D. Binding affinity prediction from molecular simulations: A new standard method in structure-based drug design? [dx.doi.org/10.7490/f1000research.1112651.1](https://doi.org/10.7490/f1000research.1112651.1), May 2016.
- [119] Cui, G. Affinity Predictions with FEP+: A Different Perspective on Performance and Utility. [dx.doi.org/10.7490/f1000research.1112773.1](https://doi.org/10.7490/f1000research.1112773.1), May 2016.
- [120] Verras, A. Free Energy Perturbation at Merck: Benchmarking against Faster Methods. http://www.alchemistry.org/wiki/images/c/c3/Vertex_FreeEnergyWorkshop2016_AV.pdf, May 2016.
- [121] Aldeghi, M., Heifetz, A., Bodkin, M. J., Knapp, S., and Biggin, P. C.: Predictions of Ligand Selectivity from Absolute Binding Free Energy Calculations. *J. Am. Chem. Soc.* 139(2): 946–957, January 2017.
- [122] Schnieders, M. J., Baltrusaitis, J., Shi, Y., Chattree, G., Zheng, L., Yang, W., and Ren, P.: The Structure, Thermodynamics, and Solubility of Organic Crystals from Simulation with a Polarizable Force Field. *J. Chem. Theory Comput.* 8(5): 1721–1736, May 2012.
- [123] Park, J., Nessler, I., McClain, B., Macikenas, D., Baltrusaitis, J., and Schnieders, M. J.: Absolute Organic Crystal Thermodynamics: Growth of the Asymmetric Unit into a Crystal via Alchemy. *J. Chem. Theory Comput.* 10(7): 2781–2791, July 2014.
- [124] Liu, S., Cao, S., Hoang, K., Young, K. L., Paluch, A. S., and Mobley, D. L.: Using MD Simulations To Calculate How Solvents Modulate Solubility. *Journal of Chemical Theory and Computation.* 12(4): 1930–1941, February 2016.
- [125] Leonis, G., Steinbrecher, T., and Papadopoulos, M. G.: A Contribution to the Drug Resistance Mechanism of Darunavir, Amprenavir, Indinavir, and Saquinavir Complexes with HIV-1 Protease Due to Flap Mutation I50V: A Systematic MM–PBSA and Thermodynamic Integration Study. *J. Chem. Inf. Model.* 53(8): 2141–2153, August 2013.
- [126] Lee, C. T., Comer, J., Herndon, C., Leung, N., Pavlova, A., Swift, R. V., Tung, C., Rowley, C. N., Amaro, R. E., Chipot, C., Wang, Y., and Gumbart, J. C.: Simulation-Based Approaches for Determining Membrane Permeability of Small Compounds. *J. Chem. Inf. Model.* 56(4): 721–733, April 2016.
- [127] Comer, J., Schulten, K., and Chipot, C.: Calculation of Lipid-Bilayer Permeabilities Using an Average Force. *J. Chem. Theory Comput.* 10(2): 554–564, February 2014.
- [128] Liu, S., Wu, Y., Lin, T., Abel, R., Redmann, J. P., Summa, C. M., Jaber, V. R., Lim, N. M., and Mobley, D. L.: Lead optimization mapper: Automating free energy calculations for lead optimization. *J. Comput. Aided Mol. Des.* 27(9): 755–770, September 2013.
- [129] Mikulskis, P., Genheden, S., and Ryde, U.: A Large-Scale Test of Free-Energy Simulation Estimates of Protein–Ligand Binding Affinities. *J. Chem. Inf. Model.* 54(10): 2794–2806, October 2014.
- [130] Homeyer, N., Stoll, F., Hillisch, A., and Gohlke, H.: Binding Free Energy Calculations for Lead Optimization: Assessment of Their Accuracy in an Industrial Drug Design Context. *J. Chem. Theory Comput.* 10(8): 3331–3344, August 2014.
- [131] Shirts, M. R., Mobley, D. L., and Brown, S. P. Free-energy calculations in structure-based drug design. In Merz, , Kenneth MJ., Ringe, D., and Reynolds, C. H. (Eds.): *Drug Design: Structure and Ligand-Based Approaches*. Cambridge University Press, January 2010.
- [132] Lim, N. M., Wang, L., Abel, R., and Mobley, D. L.: Sensitivity in binding free energies due to protein reorganization. *Journal of Chemical Theory and Computation.* 12(9): 4620–4631, July 2016.
- [133] Rocklin, G. J., Boyce, S. E., Fischer, M., Fish, I., Mobley, D. L., Shoichet, B. K., and Dill, K. A.: Blind Prediction of Charged Ligand Binding Affinities in a Model Binding Site. *J. Mol. Biol.* 425(22): 4569–4583, November 2013.
- [134] Nuzzo, R.: How scientists fool themselves – and how they can stop. *Nature.* 526(7572): 182–185, October 2015.
- [135] Mobley, D. L., Graves, A. P., Chodera, J. D., McReynolds, A. C., Shoichet, B. K., and Dill, K. A.: Predicting absolute ligand binding free energies to a simple model site. *J. Mol. Biol.* 371(4): 1118–1134, August 2007.

- [136] Boyce, S. E., Mobley, D. L., Rocklin, G. J., Graves, A. P., Dill, K. A., and Shoichet, B. K.: Predicting ligand binding affinity with alchemical free energy methods in a polar model binding site. *J. Mol. Biol.* 394(4): 747–763, December 2009.
- [137] D3R | What We Have Learned. <https://drugdesigndata.org/about/what-we-have-learned>.
- [138] Mobley, D. L., Wymer, K. L., Lim, N. M., and Guthrie, J. P.: Blind prediction of solvation free energies from the SAMPL4 challenge. *J Comput Aided Mol Des.* 28(3): 135–150, March 2014.
- [139] Nicholls, A., Mobley, D. L., Guthrie, J. P., Chodera, J. D., Bayly, C. I., Cooper, M. D., and Pande, V. S.: Predicting Small-Molecule Solvation Free Energies: An Informal Blind Test for Computational Chemistry. *J. Med. Chem.* 51(4): 769–779, February 2008.
- [140] Nicholls, A., Wlodek, S., and Grant, J. A.: The SAMP1 Solvation Challenge: Further Lessons Regarding the Pitfalls of Parametrization. *J. Phys. Chem. B.* 113(14): 4521–4532, April 2009.
- [141] Mobley, D. L., Bayly, C. I., Cooper, M. D., and Dill, K. A.: Predictions of Hydration Free Energies from All-Atom Molecular Dynamics Simulations. *J Phys Chem B.* 113: 4533–4537, January 2009.
- [142] Geballe, M. T., Skillman, A. G., Nicholls, A., Guthrie, J. P., and Taylor, P. J.: The SAMPL2 blind prediction challenge: Introduction and overview. *J Comput Aided Mol Des.* 24(4): 259–279, May 2010.
- [143] Geballe, M. T. and Guthrie, J. P.: The SAMPL3 blind prediction challenge: Transfer energy overview. *J Comput Aided Mol Des.* 26(5): 489–496, April 2012.
- [144] Muddana, H. S., Fenley, A. T., Mobley, D. L., and Gilson, M. K.: The SAMPL4 host–guest blind prediction challenge: An overview. *J Comput Aided Mol Des.* 28(4): 305–317, March 2014.
- [145] Bannan, C. C., Burley, K. H., Chiu, M., Shirts, M. R., Gilson, M. K., and Mobley, D. L.: Blind prediction of cyclohexane–water distribution coefficients from the SAMPL5 challenge. *J Comput Aided Mol Des.* 30(11): 1–18, September 2016.
- [146] Yin, J., Henriksen, N. M., Slochower, D. R., Shirts, M. R., Chiu, M. W., Mobley, D. L., and Gilson, M. K.: Overview of the SAMPL5 host–guest challenge: Are we doing better? *J Comput Aided Mol Des.* 31(1): 1–19, 2017.
- [147] Yang, X., Lei, H., Gao, P., Thomas, D. G., Mobley, D., and Baker, N. A.: Atomic radius and charge parameter uncertainty in biomolecular solvation energy calculations. [arXiv:1705.10035 \[q-bio\]](https://arxiv.org/abs/1705.10035). May 2017.
- [148] Gosink, L. J., Overall, C. C., Reehl, S. M., Whitney, P. D., Mobley, D. L., and Baker, N. A.: Bayesian Model Averaging for Ensemble-Based Estimates of Solvation-Free Energies. *J. Phys. Chem. B.* 121(15): 3458–3472, April 2017.
- [149] Harger, M., Li, D., Wang, Z., Dalby, K., Lagardère, L., Piquemal, J.-P., Ponder, J., and Ren, P.: Tinker-OpenMM: Absolute and relative alchemical free energies using AMOEBA on GPUs. *J. Comput. Chem.* pp n/a–n/a, 2017.
- [150] Bradshaw, R. T. and Essex, J. W.: Evaluating Parametrization Protocols for Hydration Free Energy Calculations with the AMOEBA Polarizable Force Field. *J. Chem. Theory Comput.* 12(8): 3871–3883, August 2016.
- [151] Ignjatović, M. M., Caldararu, O., Dong, G., Muñoz-Gutierrez, C., Adasme-Carreño, F., and Ryde, U.: Binding-affinity predictions of HSP90 in the D3R Grand Challenge 2015 with docking, MM/GBSA, QM/MM, and free-energy simulations. *J Comput Aided Mol Des.* pp 1–24, August 2016.
- [152] Deng, N., Flynn, W. F., Xia, J., Vijayan, R. S. K., Zhang, B., He, P., Menten, A., Gallicchio, E., and Levy, R. M.: Large scale free energy calculations for blind predictions of protein–ligand binding: The D3R Grand Challenge 2015. *J Comput Aided Mol Des.* pp 1–9, August 2016.
- [153] Sunseri, J., Ragoza, M., Collins, J., and Koes, D. R.: A D3R prospective evaluation of machine learning for protein-ligand scoring. *J Comput Aided Mol Des.* pp 1–11, September 2016.

- [154] Nielsen, J. E., Gunner, M. R., and García-Moreno E., B.: The pKa Cooperative: A collaborative effort to advance structure-based calculations of pKa values and electrostatic effects in proteins. Proteins. 79(12): 3249–3259, December 2011.
- [155] Janin, J.: Assessing predictions of protein–protein interaction: The CAPRI experiment. Protein Science. 14(2): 278–283, February 2005.
- [156] Ellingson, B. A., Skillman, A. G., and Nicholls, A.: Analysis of SM8 and Zap TK calculations and their geometric sensitivity. J Comput Aided Mol Des. 24(4): 335–342, April 2010.
- [157] Ellingson, B. A., Geballe, M. T., Wlodek, S., Bayly, C. I., Skillman, A. G., and Nicholls, A.: Efficient calculation of SAMPL4 hydration free energies using OMEGA, SZYBKI, QUACPAC, and Zap TK. J Comput Aided Mol Des. 28(3): 289–298, March 2014.
- [158] Mobley, D. L., Liu, S., Cerutti, D. S., Swope, W. C., and Rice, J. E.: Alchemical prediction of hydration free energies for SAMPL. Journal of Computer-Aided Molecular Design. 26(5): 551–562, PMC3583515, May 2012.
- [159] Fennell, C. J., Wymer, K. L., and Mobley, D. L.: A Fixed-Charge Model for Alcohol Polarization in the Condensed Phase, and Its Role in Small Molecule Hydration. J. Phys. Chem. B. 118(24): 6438–6446, June 2014.
- [160] Paranahegawage, S. S., Gierhart, C. S., and Fennell, C. J.: Predicting water-to-cyclohexane partitioning of the SAMPL5 molecules using dielectric balancing of force fields. J Comput Aided Mol Des. 30(11): 1059–1065, August 2016.
- [161] Sulea, T. and Purisima, E. O.: Predicting hydration free energies of polychlorinated aromatic compounds from the SAMPL-3 data set with FiSH and LIE models. J Comput Aided Mol Des. 26(5): 661–667, December 2011.
- [162] Li, L., Dill, K. A., and Fennell, C. J.: Testing the semi-explicit assembly model of aqueous solvation in the SAMPL4 challenge. J Comput Aided Mol Des. 28(3): 259–264, January 2014.
- [163] Brini, E., Paranahegawage, S. S., Fennell, C. J., and Dill, K. A.: Adapting the semi-explicit assembly solvation model for estimating water-cyclohexane partitioning with the SAMPL5 molecules. J Comput Aided Mol Des. 30(11): 1067–1077, September 2016.
- [164] Klamt, A., Eckert, F., Reinisch, J., and Wichmann, K.: Prediction of cyclohexane-water distribution coefficients with COSMO-RS on the SAMPL5 data set. J Comput Aided Mol Des. 30(11): 959–967, July 2016.
- [165] Mobley, D. L. and Gilson, M. K.: Predicting Binding Free Energies: Frontiers and Benchmarks. Annual Review of Biophysics. 46(1): 531–558, 2017.
- [166] Muddana, H. S., Yin, J., Sapra, N. V., Fenley, A. T., and Gilson, M. K.: Blind prediction of SAMPL4 cucurbit[7]uril binding affinities with the mining minima method. J Comput Aided Mol Des. 28(4): 463–474, February 2014.
- [167] Yin, J., Henriksen, N. M., Slochower, D. R., and Gilson, M. K.: The SAMPL5 Host-Guest Challenge: Computing Binding Free Energies and Enthalpies from Explicit Solvent Simulations by the Attach-Pull-Release (APR) Method. J Comput Aided Mol Des. 31(1): 133–145, 2017.
- [168] Muddana, H. S., Sapra, N. V., Fenley, A. T., and Gilson, M. K.: The SAMPL4 hydration challenge: Evaluation of partial charge sets with explicit-water molecular dynamics simulations. J Comput Aided Mol Des. 28(3): 277–287, January 2014.
- [169] Yin, J., Fenley, A. T., Henriksen, N. M., and Gilson, M. K.: Toward Improved Force-Field Accuracy through Sensitivity Analysis of Host-Guest Binding Thermodynamics. The Journal of Physical Chemistry B. 119(32): 10145–10155, August 2015.
- [170] Pickard, F. C., König, G., Tofoleanu, F., Lee, J., Simmonett, A. C., Shao, Y., Ponder, J. W., and Brooks, B. R.: Blind prediction of distribution in the SAMPL5 challenge with QM based protomer and pKa corrections. J Comput Aided Mol Des. 30(11): 1–14, September 2016.

- [171] Rustenburg, A. S., Dancer, J., Lin, B., Feng, J. A., Ortwine, D. F., Mobley, D. L., and Chodera, J. D.: Measuring experimental cyclohexane-water distribution coefficients for the SAMPL5 challenge. Journal of Computer-Aided Molecular Design. 30(11): 945–958, July 2016.
- [172] Bhatnagar, N., Kamath, G., and Potoff, J. J.: Prediction of 1-octanol–water and air–water partition coefficients for nitro-aromatic compounds from molecular dynamics simulations. Physical Chemistry Chemical Physics. 15(17): 6467, 2013.
- [173] Bannan, C. C., Calabró, G., Kyu, D. Y., and Mobley, D. L.: Calculating Partition Coefficients of Small Molecules in Octanol/Water and Cyclohexane/Water. Journal of Chemical Theory and Computation. 12(8): 4015–4024, August 2016.
- [174] Kollman, P. A.: Advances and continuing challenges in achieving realistic and predictive simulations of the properties of organic and biological molecules. Accounts of Chemical Research. 29(10): 461–469, 1996.
- [175] Muddana, H. S., Varnado, C. D., Bielawski, C. W., Urbach, A. R., Isaacs, L., Geballe, M. T., and Gilson, M. K.: Blind prediction of host–guest binding affinities: A new SAMPL3 challenge. J Comput Aided Mol Des. 26(5): 475–487, February 2012.
- [176] Bhakat, S. and Söderhjelm, P.: Resolving the problem of trapped water in binding cavities: Prediction of host-guest binding free energies in the SAMPL5 challenge by funnel metadynamics. J Comput Aided Mol Des. 31(1): 119–132, 2017.
- [177] Henriksen, N. M., Fenley, A. T., and Gilson, M. K.: Computational Calorimetry: High-Precision Calculation of Host–Guest Binding Thermodynamics. Journal of Chemical Theory and Computation. 11(9): 4377–4394, September 2015.
- [178] Ma, D., Glassenberg, R., Ghosh, S., Zavalij, P. Y., and Isaacs, L.: Acyclic cucurbituril congener binds to local anaesthetics. Supramolecular Chemistry. 24(5): 325–332, May 2012.
- [179] Cao, L. and Isaacs, L.: Absolute and relative binding affinity of cucurbit[7]uril towards a series of cationic guests. Supramolecular Chemistry. 26(3-4): 251–258, March 2014.
- [180] She, N., Moncelet, D., Gilberg, L., Lu, X., Sindelar, V., Briken, V., and Isaacs, L.: Glycoluril-Derived Molecular Clips are Potent and Selective Receptors for Cationic Dyes in Water. Chem. Eur. J. pp n/a–n/a, August 2016.
- [181] Cao, L., Šekutor, M., Zavalij, P. Y., Mlinarić-Majerski, K., Glaser, R., and Isaacs, L.: Cucurbit[7]uril-Guest Pair with an Attomolar Dissociation Constant. Angew. Chem. Int. Ed. 53(4): 988–993, January 2014.
- [182] Liu, S., Ruspic, C., Mukhopadhyay, P., Chakrabarti, S., Zavalij, P. Y., and Isaacs, L.: The Cucurbit[n]uril Family: Prime Components for Self-Sorting Systems. Journal of the American Chemical Society. 127(45): 15959–15967, November 2005.
- [183] Mock, W. L. and Shih, N. Y.: Structure and selectivity in host-guest complexes of cucurbituril. The Journal of Organic Chemistry. 51(23): 4440–4446, November 1986.
- [184] Assaf, K. I. and Nau, W. M.: Cucurbiturils: From synthesis to high-affinity binding and catalysis. Chem Soc Rev. 44(2): 394–418, January 2015.
- [185] Moghaddam, S., Yang, C., Rekharsky, M., Ko, Y. H., Kim, K., Inoue, Y., and Gilson, M. K.: New Ultrahigh Affinity Host-Guest Complexes of Cucurbit[7]uril with Bicyclo[2.2.2]octane and Adamantane Guests: Thermodynamic Analysis and Evaluation of M2 Affinity Calculations. Journal of the American Chemical Society. 133(10): 3570–3581, March 2011.
- [186] Shetty, D., Khedkar, J. K., Park, K. M., and Kim, K.: Can we beat the biotin–avidin pair?: Cucurbit[7]uril-based ultrahigh affinity host–guest complexes and their applications. Chem. Soc. Rev. 44(23): 8747–8761, 2015.
- [187] Biedermann, F., Uzunova, V. D., Scherman, O. A., Nau, W. M., and De Simone, A.: Release of High-Energy Water as an Essential Driving Force for the High-Affinity Binding of Cucurbit[n]urils. J. Am. Chem. Soc. 134(37): 15318–15323, September 2012.

- [188] Isaacs, L.: Stimuli Responsive Systems Constructed Using Cucurbit[n]uril-Type Molecular Containers. Acc. Chem. Res. 47(7): 2052–2062, July 2014.
- [189] Vinciguerra, B., Zavalij, P. Y., and Isaacs, L.: Synthesis and Recognition Properties of Cucurbit[8]uril Derivatives. Org. Lett. 17(20): 5068–5071, October 2015.
- [190] Lucas, D., Minami, T., Iannuzzi, G., Cao, L., Wittenberg, J. B., Anzenbacher, P., and Isaacs, L.: Templated Synthesis of Glycoluril Hexamer and Monofunctionalized Cucurbit[6]uril Derivatives. J. Am. Chem. Soc. 133(44): 17966–17976, November 2011.
- [191] Ma, D., Zhang, B., Hoffmann, U., Sundrup, M. G., Eikermann, M., and Isaacs, L.: Acyclic Cucurbit[n]uril-Type Molecular Containers Bind Neuromuscular Blocking Agents In Vitro and Reverse Neuromuscular Block In Vivo. Angew. Chem. Int. Ed. 51(45): 11358–11362, November 2012.
- [192] Ma, D., Hettiarachchi, G., Nguyen, D., Zhang, B., Wittenberg, J. B., Zavalij, P. Y., Briken, V., and Isaacs, L.: Acyclic cucurbit[n]uril molecular containers enhance the solubility and bioactivity of poorly soluble pharmaceuticals. Nat Chem. 4(6): 503–510, June 2012.
- [193] Zhang, B. and Isaacs, L.: Acyclic Cucurbit[n]uril-type Molecular Containers: Influence of Aromatic Walls on their Function as Solubilizing Excipients for Insoluble Drugs. J. Med. Chem. 57(22): 9554–9563, November 2014.
- [194] Gilberg, L., Zhang, B., Zavalij, P. Y., Sindelar, V., and Isaacs, L.: Acyclic cucurbit[n]uril-type molecular containers: Influence of glycoluril oligomer length on their function as solubilizing agents. Org. Biomol. Chem. 13(13): 4041–4050, 2015.
- [195] Sigwalt, D., Moncelet, D., Falcinelli, S., Mandadapu, V., Zavalij, P. Y., Day, A., Briken, V., and Isaacs, L.: Acyclic Cucurbit[n]uril-Type Molecular Containers: Influence of Linker Length on Their Function as Solubilizing Agents. ChemMedChem. 11(9): 980–989, May 2016.
- [196] Zhang, B., Zavalij, P. Y., and Isaacs, L.: Acyclic CB[n]-type molecular containers: Effect of solubilizing group on their function as solubilizing excipients. Org. Biomol. Chem. 12(15): 2413–2422, 2014.
- [197] Ma, D., Zavalij, P. Y., and Isaacs, L.: Acyclic Cucurbit[n]uril Congeners Are High Affinity Hosts. J. Org. Chem. 75(14): 4786–4795, July 2010.
- [198] Ko, Y. H., Kim, E., Hwang, I., and Kim, K.: Supramolecular assemblies built with host-stabilized charge-transfer interactions. Chem. Commun. (13): 1305–1315, 2007.
- [199] Barrow, S. J., Kaseira, S., Rowland, M. J., del Barrio, J., and Scherman, O. A.: Cucurbituril-Based Molecular Recognition. Chem. Rev. 115(22): 12320–12406, November 2015.
- [200] Urbach, A. R. and Ramalingam, V.: Molecular Recognition of Amino Acids, Peptides, and Proteins by Cucurbit[n]uril Receptors. Isr. J. Chem. 51(5-6): 664–678, May 2011.
- [201] Connors, K. A.: Binding Constants. New York, NY, John Wiley & Sons, 1987.
- [202] Masson, E., Ling, X., Joseph, R., Kyeremeh-Mensah, L., and Lu, X.: Cucurbituril chemistry: A tale of supramolecular success. RSC Adv. 2(4): 1213–1247, 2012.
- [203] Márquez, C., Hudgins, R. R., and Nau, W. M.: Mechanism of Host-Guest Complexation by Cucurbituril. J. Am. Chem. Soc. 126(18): 5806–5816, May 2004.
- [204] Biedermann, F., Nau, W. M., and Schneider, H.-J.: The Hydrophobic Effect Revisited—Studies with Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force. Angew. Chem. Int. Ed. 53(42): 11158–11171, October 2014.
- [205] Saleh, N., Koner, A., and Nau, W.: Activation and Stabilization of Drugs by Supramolecular pKa Shifts: Drug-Delivery Applications Tailored for Cucurbiturils. Angewandte Chemie. 120(29): 5478–5481, July 2008.
- [206] Nau, W. M., Florea, M., and Assaf, K. I.: Deep Inside Cucurbiturils: Physical Properties and Volumes of their Inner Cavity Determine the Hydrophobic Driving Force for Host–Guest Complexation. Isr. J. Chem. 51(5-6): 559–577, May 2011.

- [207] Ghosh, I. and Nau, W. M.: The strategic use of supramolecular pKa shifts to enhance the bioavailability of drugs. Advanced Drug Delivery Reviews. 64(9): 764–783, June 2012.
- [208] Gibb, C. L. D. and Gibb, B. C.: Binding of cyclic carboxylates to octa-acid deep-cavity cavitand. J Comput Aided Mol Des. 28(4): 319–325, November 2013.
- [209] Sullivan, M. R., Sokkalingam, P., Nguyen, T., Donahue, J. P., and Gibb, B. C.: Binding of carboxylate and trimethylammonium salts to octa-acid and TEMOA deep-cavity cavitands. J Comput Aided Mol Des. 31(1): 1–8, 2017.
- [210] Carnegie, R. S., Gibb, C. L. D., and Gibb, B. C.: Anion Complexation and The Hofmeister Effect. Angew. Chem. 126(43): 11682–11684, October 2014.
- [211] Lin, Y.-L., Meng, Y., Jiang, W., and Roux, B.: Explaining why Gleevec is a specific and potent inhibitor of Abl kinase. Proc. Natl. Acad. Sci. 110(5): 1664–1669, January 2013.
- [212] Shan, Y., Seeliger, M. A., Eastwood, M. P., Frank, F., Xu, H., Jensen, M. Ø., Dror, R. O., Kuriyan, J., and Shaw, D. E.: A conserved protonation-dependent switch controls drug binding in the Abl kinase. PNAS. 106(1): 139–144, June 2009.
- [213] Szakács, Z., Béni, S., Varga, Z., Örfi, L., Kéri, G., and Noszál, B.: Acid-Base Profiling of Imatinib (Gleevec) and Its Fragments. Journal of Medicinal Chemistry. 48(1): 249–255, January 2005.
- [214] Grante, I., Actins, A., and Orola, L.: Protonation effects on the UV/Vis absorption spectra of imatinib: A theoretical and experimental study. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 129: 326–332, August 2014.
- [215] Merski, M., Fischer, M., Balias, T. E., Eidam, O., and Shoichet, B. K.: Homologous ligands accommodated by discrete conformations of a buried cavity. PNAS. 112(16): 5039–5044, April 2015.
- [216] Onufriev, A. V. and Alexov, E.: Protonation and pK changes in protein–ligand binding. Quarterly Reviews of Biophysics. 46(02): 181–209, May 2013.
- [217] Hall, M. L., Jorgensen, W. L., and Whitehead, L.: Automated Ligand- and Structure-Based Protocol for *in Silico* Prediction of Human Serum Albumin Binding. Journal of Chemical Information and Modeling. 53(4): 907–922, April 2013.
- [218] Epps, D. E., Raub, T. J., Caiolfa, V., Chiari, A., and Zamai, M.: Determination of the Affinity of Drugs toward Serum Albumin by Measurement of the Quenching of the Intrinsic Tryptophan Fluorescence of the Protein. Journal of Pharmacy and Pharmacology. 51(1): 41–48, January 1999.
- [219] Bou-Abdallah, F., Sprague, S. E., Smith, B. M., and Giffune, T. R.: Binding thermodynamics of Diclofenac and Naproxen with human and bovine serum albumins: A calorimetric and spectroscopic study. The Journal of Chemical Thermodynamics. 103: 299–309, December 2016.
- [220] Newman, J., Dolezal, O., Fazio, V., Caradoc-Davies, T., and Peat, T. S.: The DINGO dataset: A comprehensive set of data for the SAMPL challenge. J Comput Aided Mol Des. 26(5): 497–503, December 2011.
- [221] Rocklin, G. J., Mobley, D. L., Dill, K. A., and Hünenberger, P. H.: Calculating the binding free energies of charged species based on explicit-solvent simulations employing lattice-sum methods: An accurate correction scheme for electrostatic finite-size effects. The Journal of Chemical Physics. 139(18): 184103, 2013.
- [222] Lin, Y.-L., Aleksandrov, A., Simonson, T., and Roux, B.: An Overview of Electrostatic Free Energy Computations for Solutions and Proteins. J. Chem. Theory Comput. 10(7): 2690–2709, July 2014.
- [223] Reif, M. M. and Oostenbrink, C.: Net charge changes in the calculation of relative ligand-binding free energies via classical atomistic molecular dynamics simulation. Journal of Computational Chemistry. 35(3): 227–243, January 2014.

- [224] Fasano, M., Curry, S., Terreno, E., Galliano, M., Fanali, G., Narciso, P., Notari, S., and Ascenzi, P.: The extraordinary ligand binding properties of human serum albumin. IUBMB Life (International Union of Biochemistry and Molecular Biology: Life). 57(12): 787–796, December 2005.
- [225] Elinder, M., Geitmann, M., Gossas, T., Kallblad, P., Winquist, J., Nordstrom, H., Hamalainen, M., and Danielson, U. H.: Experimental Validation of a Fragment Library for Lead Discovery Using SPR Biosensor Technology. Journal of Biomolecular Screening. 16(1): 15–25, January 2011.
- [226] Gilson, M., Given, J., Bush, B., and McCammon, J.: The statistical-thermodynamic basis for computation of binding affinities: A critical review. Biophysical Journal. 72(3): 1047–1069, March 1997.
- [227] Lexa, K. W., Dolgih, E., and Jacobson, M. P.: A Structure-Based Model for Predicting Serum Albumin Binding. PLoS ONE. 9(4): e93323, April 2014.
- [228] Evoli, S., Mobley, D. L., Guzzi, R., and Rizzuti, B.: Multiple binding modes of ibuprofen in human serum albumin identified by absolute binding free energy calculations. Phys. Chem. Chem. Phys. 18: 32358–32368, November 2016.
- [229] Latta, M., Knapp, M., Sarmientos, P., Bréfort, G., Becquart, J., Guerrier, L., Jung, G., and Mayaux, J.-F.: Synthesis and Purification of Mature Human Serum Albumin from *E. Coli*. Bio/Technology. 5(12): 1309–1314, December 1987.
- [230] Lang, B. E. and Cole, K. D.: Unfolding properties of recombinant human serum albumin products are due to bioprocessing steps. Biotechnology Progress. 31(1): 62–69, January 2015.
- [231] Martin, Y. C.: Let's not forget tautomers. Journal of Computer-Aided Molecular Design. 23(10): 693–704, October 2009.
- [232] Song, Y., Mao, J., and Gunner, M. R.: MCCE2: Improving protein pKa calculations with extensive side chain rotamer sampling. Journal of Computational Chemistry. pp NA–NA, 2009.
- [233] Reinhard, L., Mayerhofer, H., Geerlof, A., Mueller-Dieckmann, J., and Weiss, M. S.: Optimization of protein buffer cocktails using Thermofluor. Acta Crystallographica Section F Structural Biology and Crystallization Communications. 69(2): 209–214, February 2013.
- [234] Docker Inc., . What is Docker? <https://www.docker.com/what-docker>, 2015-05-14T16:17:40-07:00.
- [235] Shirts, M. R., Klein, C., Swails, J. M., Yin, J., Gilson, M. K., Mobley, D. L., Case, D. A., and Shirts, M. R.: Lessons learned from comparing molecular dynamics engines on the SAMPL5 dataset. J Comput Aided Mol Des. 31(1): 147–161, 2017.
- [236] Monroe, J. I. and Shirts, M. R.: Converging free energies of binding in cucurbit[7]uril and octa-acid host–guest systems from SAMPL4 using expanded ensemble simulations. J Comput Aided Mol Des. 28(4): 401–415, March 2014.
- [237] Bosisio, S., Mey, A. S. J. S., and Michel, J.: Blinded predictions of host-guest standard free energies of binding in the SAMPL5 challenge. J Comput Aided Mol Des. 31(1): 61–70, 2017.
- [238] Mobley, D. L. and Guthrie, J. P.: FreeSolv: A database of experimental and calculated hydration free energies, with input files. J Comput Aided Mol Des. 28(7): 711–720, PMC4113415, June 2014.
- [239] Duarte Ramos Matos, G., Kyu, D. Y., Loeffler, H. H., Chodera, J. D., Shirts, M. R., and Mobley, D. L.: Approaches for Calculating Solvation Free Energies and Enthalpies Demonstrated with an Update of the FreeSolv Database. J. Chem. Eng. Data. 62(5): 1559–1569, May 2017.
- [240] Liu, T., Lin, Y., Wen, X., Jorissen, R. N., and Gilson, M. K.: BindingDB: A web-accessible database of experimentally determined protein–ligand binding affinities. Nucl. Acids Res. 35(suppl 1): D198–D201, January 2007.