

Blind prediction of cyclohexane-water distribution coefficients from the SAMPL5 challenge

Caitlin C. Bannan · Kalistyn H. Burley ·
Michael Chiu · Michael K. Gilson ·
David L. Mobley

Received: date / Accepted: date

Abstract describe DC part of SAMPL5 challenge

We analyze submissions and provide reference calculations

Results, range of RMSE or AUE? Better or worse than expected from past sample challenges?

Conclusions: tautomeric enumeration,

Keywords distribution coefficient · blind challenge · free energy

1 Introduction

This year's Statistical Assessment of Modeling of Proteins and Ligand (SAMPL) challenge focuses on prediction of cyclohexane-water distribution coefficients. The inclusion of distribution coefficients replaces the previous focus on hydration free energies which was a fixture of the past five challenges (SAMPL0-4) [1–7]. Due to a lack of ongoing experimental work, hydration free energies are no longer a practical property for blind challenges. It has become increasingly

C. C. Bannan

Department of Chemistry, University of California, Irvine

K. H. Burley

Department of Pharmaceutical Sciences, University of California, Irvine

M. Chiu

Qualcomm Institute, University of California, San Diego

M. K. Gilson

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego

D. L. Mobley

Department of Chemistry and Department of Pharmaceutical Sciences, University of California, Irvine

147 Bison Modular, Irvine, CA 92697 Tel.: +949-824-6383

Fax: +949-824-2949

E-mail: dmobley@mobleylab.org

difficult to find unpublished or obscure hydration free energies and therefore impossible to design a challenge focusing on particular compounds, functional groups or chemical classes. The past SAMPL challenges have driven real improvements in a variety of methods for calculating hydration free energy [1], so we sought to include a similar physical property in SAMPL5. The Drug Design Data Resource (D3R), organizers for SAMPL5, settled on cyclohexane-water distribution coefficients, and thanks to a partnership with Genentech, this led to a series of measurements on drug-like compounds, discussed in detail in this issue. Measurement is also straightforward enough that future distribution coefficient challenges can be deliberately designed to focus on issues that merit attention to move the field forward.

Partition and distribution coefficients are important physical properties which can provide a valuable opportunity for testing computational methods and molecular models. Distribution coefficients describe how all forms of a solute distributes itself across two immiscible solvents. In this case,

$$D = \frac{\sum_i [X_i]_{cyc}}{\sum_i [X_i]_{aq}} \quad (1)$$

where X_i represents a single protonation or tautomeric state of the solute in one of the solvents, and the sum runs over all protonation and tautomeric states. Results are reported as the logarithm of this ratio ($\log D$). These are more complicated than partition coefficients ($\log P$), which are for only a single molecular species of the compound [8]. $\log P$ is proportional to the transfer free energy, and can be calculated from solvation free energies. In contrast, all tautomers will need to be included to accurately calculate $\log D$, which can be estimated from a calculated $\log P$ and the relative populations of tautomers in each solvent. This complexity brings some new complexities to the SAMPL challenge which were avoided in previous hydration free energy challenges. Here, accurate tautomer enumeration in both solvents may be an important part of predicting $\log D$.

Here we discuss results for the SAMPL5 challenge, highlighting difficulties that were common among submissions. We perform a number of error analyses to determine the best methods. We also include details for a set of reference calculations we performed estimating $\log D$ as the cyclohexane/water partition coefficient as well as a series of follow-up studies focusing on the importance of tautomers in estimating $\log D$.

Here we give an overview of the analysis done for the SAMPL5 challenge, including the compounds considered, overall performance of submissions, and the metrics used for analysis. We also include details for sets of reference calculations we performed that include Overall, we believe the outcome of the present SAMPL5 challenge highlights the potential benefits of this type of experimental data to improve computational methods, force fields, sampling algorithms, and treatment of protonation states and tautomers. Many of these issues will be highly relevant for nominally more challenging problems, such as predictions of protein-ligand binding affinities.

2 Challenge Logistics

SAMPL5 began on September 15, 2015 when the specifications for the challenge became available on the D3R website (<http://drugdesigndata.org>), these are also provided in the supporting information. The challenge deadline was February 2, 2016 and experimental results were provided to participants not long after. As in past SAMPL challenges, each group could submit multiple sets of predictions. There was also the option to remain anonymous. A total of 76 prediction sets from 18 participants or participating groups were submitted and assigned a random 2 digit ID number 01 to 76 that will be used throughout this paper. Predictions were analyzed and overview statistics, as well as individual analysis of each submission by various error metrics (as detailed below) were returned to each participant. The challenge culminated with discussions of participants experiences and results at the 1st Annual D3R Workshop at the University of California, San Diego March 9-11, 2016.

For the prediction of distribution coefficients in SAMPL5, a total of 53 molecules were considered (Table 2). Molecules were assigned an identifier in the form SAMPL5_XXX; the complete table can be found below and in the supporting information. The 53 molecules were divided into batches 0, 1, and 2 containing 13, 20, and 20 molecules respectively. We wanted each batch to have a similar dynamic range and for the molecules to increase in size across batches, so on average the smallest molecules are in batch 0 and the largest in batch 2. To ensure each batch had adequate dynamic range, the molecular weight and estimated octanol/water partition coefficient were computed for each compound. These partition coefficients were estimated with OpenEye’s log P calculator. Molecules were then assigned to bins by estimated partition coefficient, and assigned to batches based on molecular weight. Specifically, the smallest molecules from each partition coefficient bin were added to batch 0, then batch 1, and the rest of the molecules comprise batch 2.

Participants could submit partial sets of predictions as long as they included full consecutive batches; that is, they could submit batch 0, batches 0 and 1, or batches 0, 1, and 2. The idea was that all participants should attempt predictions on the full set if at all possible, but grouping into batches would allow people with particularly demanding methods (such as polarizable force fields or methods requiring intensive quantum mechanics) to focus on smaller compounds and still be evaluated. Eight submissions from two participants submitted results for only batch 0, an additional five submissions from two participants provided only batch 0 and batch 1. Here we focus on the results for the complete set of molecules (batches 0, 1, and 2). Separate analysis for the other submission options (batch 0 or batches 0 and 1) is available in the supporting information.

Participants were asked to report a cyclohexane/water distribution coefficient for each molecule. As discussed above, distribution coefficients are the ratio of concentrations for all forms of the solute in cyclohexane and the aqueous layer. During the experimental measurements, the water layer was an aqueous buffer at pH 7.4. We also required participants to provide two esti-

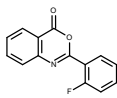
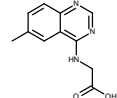
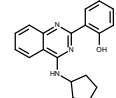
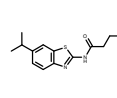
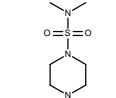
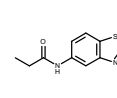
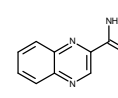
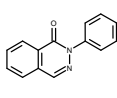
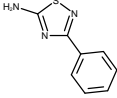
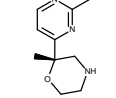
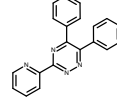
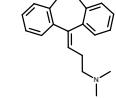
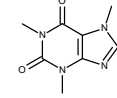
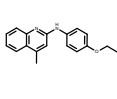
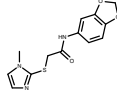
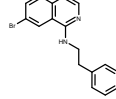
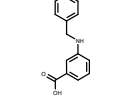
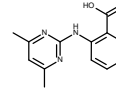
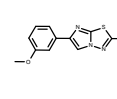
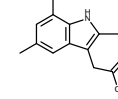
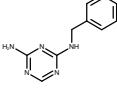
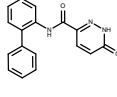
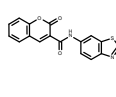
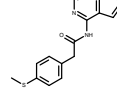
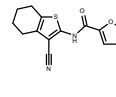
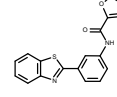
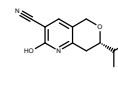
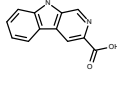
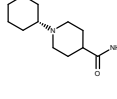
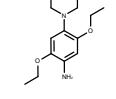
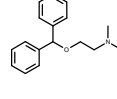
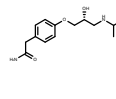
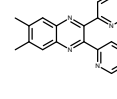
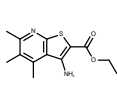
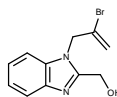
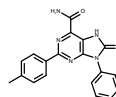
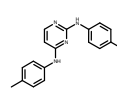
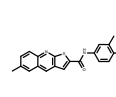
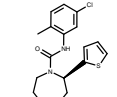
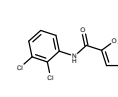
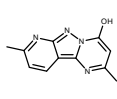
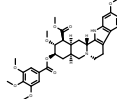
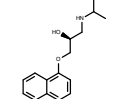
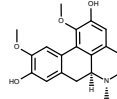
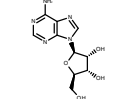
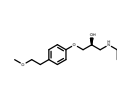
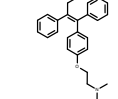
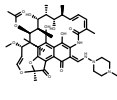
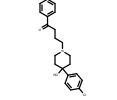
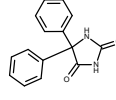
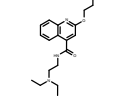
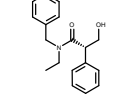
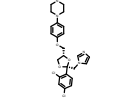
Batch 0						
003: 1.7 ± 0.2	015: 8.8 ± 1.4	017: 3.0 ± 0.3	020: 3.9 ± 0.4	037: 7.5 ± 1.3	045: 1.4 ± 0.2	055: 2.7 ± 0.2
						
058: 2.2 ± 0.3	059: 1.8 ± 0.2	061: 5.9 ± 1.0	068: 2.7 ± 0.3	070: 5.9 ± 0.8	080: 2.6 ± 0.2	
						
Batch 1						
004: 2.3 ± 0.3	005: 2.6 ± 0.3	007: 3.1 ± 0.4	010: 7.8 ± 1.6	011: 7.1 ± 1.3	021: 2.2 ± 0.2	026: 8.0 ± 1.7
						
027: 3.4 ± 0.3	042: 3.2 ± 0.4	044: 3.7 ± 0.4	046: 2.7 ± 0.4	047: 2.1 ± 0.3	048: 2.7 ± 0.3	056: 3.5 ± 0.3
						
060: 6.7 ± 1.6	063: 6.7 ± 1.0	071: 2.8 ± 0.3	072: 4.9 ± 0.7	081: 6.0 ± 0.8	090: 2.8 ± 0.3	
						
Batch 2						
002: 2.5 ± 0.2	006: 2.7 ± 0.4	013: 3.1 ± 0.3	019: 3.1 ± 0.4	024: 3.0 ± 0.4	033: 3.0 ± 0.3	049: 2.1 ± 0.2
						
050: 5.6 ± 0.4	065: 5.3 ± 0.5	067: 4.5 ± 0.6	069: 3.9 ± 0.5	074: 6.6 ± 0.4	075: 4.8 ± 0.6	082: 5.1 ± 0.6
						
083: 8.4 ± 0.7	084: 3.6 ± 0.5	085: 2.8 ± 0.3	086: 4.5 ± 0.6	088: 2.9 ± 0.4	092: 3.9 ± 0.4	
						

Table 1 A complete list of compounds used in the SAMPL5, sorted by batch. The average unsigned error, reported in log units, was calculated with all submitted predictions for that compound.

mates for uncertainty, a statistical uncertainty for their computational method and a model uncertainty that estimates agreement with experiment. The statistical uncertainty was intended to be the variation expected over repeated calculations of the same value. The model uncertainty, on the other hand, was intended to provide an estimate of how well the calculated value will agree with experiment. For example, in a recent study we computed cyclohexane/water partition coefficients using alchemical solvation free energy calculations in GROMACS where the statistical uncertainties were around 0.05 but the root mean squared error was around 1.4 log units. An important part of creating predictive models is the ability to know when they will be unreliable or fail. Thus, analysis of model uncertainties is an important part of evaluating any model.

3 Analysis of Submission Performance

As in past SAMPL challenges, we considered a variety of error metrics in analyzing all predictions submitted to SAMPL5. Each error metric was calculated for all submissions, by batch, and distributed to challenge participants before the workshop. Here we will focus primarily on six error metrics: the root-mean-squared error (RMSE), average unsigned error (AUE), average signed error (ASE), Pearson’s R (R), Kendall’s tau (tau), and the ‘error slope’ explained in depth below. We also calculated maximum absolute error and percent of predictions with the correct sign which are not included in the analysis here, but were provided to challenge participants and are available in the supporting information. Uncertainty in each metric was calculated as the standard deviation over 1000 bootstrap trials, where each trial consists of creating a ‘new’ dataset by sampling pairs of (predicted, calculated) values and associated uncertainties from the original set, with replacement. As described previously, this bootstrapping technique also included variation in the experimental values based on their reported uncertainties[1].

As discussed above, an important evaluation of a predictive tool is the ability to predict the accuracy of a computational method – that is, how well the calculated values are likely to agree with experiment – not just its statistical error. As in SAMPL4 [1], a quantile-quantile plot (QQ Plot) was created for each prediction set. QQ Plots compare the fraction of a normal distribution within a number of standard deviations to the distribution of errors (calculated minus experiment) that are within that number of model uncertainties. For example, consider the number of predictions within one standard deviation of the expected value; if the samples are drawn from a normal distribution, then 0.68 of the values ought to fall within one standard deviation, so the value on the x-axis is 0.68. The value on the y-axis will represent the fraction of predictions that are within one model uncertainty of the experimental value. If the model uncertainty is accurate, then this ought also to correspond to a value of 0.68. A linear regression analysis helps summarize these results. The ‘error slope’ is the slope of the line comparing the fraction of predictions

within a specified range of experiment to the expected fraction from a normal distribution. An error slope of greater than one indicates that the calculated values are within uncertainty of experiment more often than expected, or in other words the model uncertainty was overestimated. In contrast, an error slope less than one suggests the model uncertainty was underestimated.

We also attempted to identify any individual molecules where most of the methods failed to accurately estimate the distribution coefficient. To accomplish this a data set was created for each molecule consisting of all predictions submitted for that molecule, then all the error metrics discussed above were calculated for each molecule. Here we will primarily focus on just average unsigned error for molecules, but all other error metrics were provided to participants and are available in supporting information.

4 Reference calculations

We calculated distribution coefficients through a few different methods as a reference. KHB, a graduate student in the Mobley group, performed a set of blind calculations estimating the $\log D$ as a partition coefficient between cyclohexane and water calculated from solvation free energies. In addition, CCB and DLM performed calculations after the challenge which were not included with the prediction sets. We considered a null hypothesis where all molecules are assumed to distribute equally between cyclohexane and water. Many fast structural based tools for octanol/water partition coefficients exist, which we compared with no and little correction for cyclohexane. We also included post challenge analysis of protonation and tautomeric states as a correction from calculated partition coefficients to distribution coefficients

4.1 Calculating partition coefficients from solvation free energies

We decided to estimate distribution coefficients, more simplistically, by assuming only a single neutral tautomer of each solute and calculated $\log P$ from a difference in solvation free energies. Before the challenge, each molecule was taken directly as the provided SMILES string. As demonstrated in the literature, partition coefficients are directly proportional to the difference between the solvation free energy for the solute into each solvent. We use previously established and automated protocols [9] to calculate the solvation free energy of each molecule into water and cyclohexane. Then the calculated partition coefficient was reported as an estimate for $\log D$.

To calculate solvation free energies, we used automated tools created by the Mobley lab. Molecular dynamics simulations were performed in GROMACS [10–16] with the General AMBER Force Field (GAFF) [17] with AM1-BCC charges [18,19]. Topology and coordinate files for the solvated boxes with 1 solute molecule and 500 cyclohexane or 1000 water molecules were built using the Solvation Toolkit [9]. These files were then converted to AMBER,

DESMOND, and LAMMPS formats and provided to SAMPL5 participants. The Solvation Toolkit takes advantage of many open source Python modules and is available at <https://github.com/MobleyLab/SolvationToolkit>. It converts SMILES strings or IUPAC names of any mixture of small organic compounds to parameterized molecules and builds topology and coordinate files for a variety of simulation packages. All molecular dynamics parameters are identical to previous studies [20, 4, 9]. The molecule is taken from the solvated box to a non-interacting gas phase in 20 lambda values. Solvation free energies are calculated with Alchemical Analysis tool [21] using the multi-state Bennett acceptance ratio to extract free energy difference between the beginning and end state. The partition coefficient was calculated as the difference between the cyclohexane solvation free energy and the hydration free energy. The statistical uncertainty was reported as the propagated uncertainty from the solvation free energy calculations. The model uncertainty was estimated to be the same for all molecules and reported as the root-mean-squared error from a recent study on calculating cyclohexane/water partition coefficient, specifically 1.4 log units [9]. These were assigned submission ID 39 and included in the error analysis performed on all submissions.

Simulation box size does not affect the calculated solvation free energy. Hydration free energies were previously shown to be independent of box sizes from 2 to 9 nanometers, within calculated uncertainties [22]. Polar solutes are more likely to significantly affect long range interactions so we calculated the dipole moment of each SAMPL5 molecule using the position and charges on atoms in the mol2 files. SAMPL5_024 had the largest dipole moment so it was used as the solute for the box size investigation. The solvation free energy calculations were set up as described above, changing the number of cyclohexane molecules from 100 to 500. Our calculations above are performed with PME coulomb interactions; here we also repeated the solvation free energy calculations with reaction field coulomb interactions assigning the dielectric coefficient for cyclohexane, 2.043 [23]. We found that for 2.5 to 4.5 nm box edges there is no significant change in the calculated solvation free energy for SAMPL5_024 in cyclohexane. The input, results, molecular dynamics parameter files, and tables of solvation free energies are available in the supporting information.

4.2 Consideration of tautomers after SAMPL5

To help understand how the results from our partition coefficient calculations could have been improved, we considered corrections for changes in the solutes' protonation or tautomeric states. Distribution coefficients differ from partition coefficients in that they include all forms of the solute in both solvents. A common way to convert between experimentally measured distribution coefficients and partition coefficients is with pKa values for the solute. This is a simple

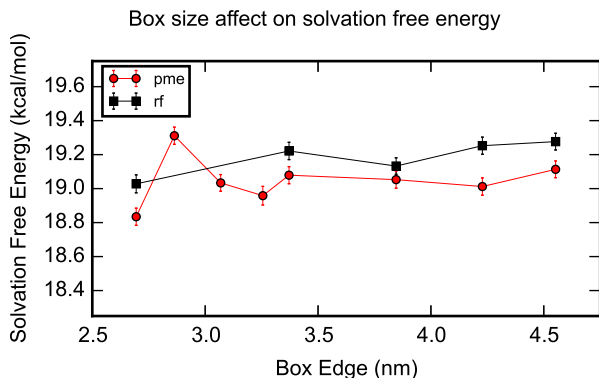


Fig. 1

correction using the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[X]}{[HX]} \quad (2)$$

to relate the concentration of neutral species to the charged species at a given pH. This correction will depend on if the neutral solute is acidic or basic. The equation used to calculate a distribution coefficient ($\log D$) from a partition coefficient ($\log P$) for a basic solute (or X in eqn. 2) is below

$$\log D = \log P - \log(1 + 10^{pK_a - pH}) \quad (3)$$

Alternatively for an acid solute (or HX in eqn. 2) we would instead use:

$$\log D = \log P - \log(1 + 10^{pH - pK_a}) \quad (4)$$

We use Schrödinger's Epik tool to estimate pKa values for each molecule according to experimental conditions. We then estimated $\log D$ using the equations above, accounting for just one change in protonation state. For acidic solutes, the smallest acidic pKa was used with equation 4, oppositely for basic solutes the largest basic pKa was used with equation 3 to estimate $\log D$ from $\log P$. Using pKa values only accounts for one change in protonation, whereas a correct distribution coefficient should include all relevant tautomers and protonation states of the molecule in both solvents. To correct for all other tautomer states we used Schrödinger's LigPrep to enumerate tautomers for each molecule in the aqueous solution. The results of the enumeration includes a energetic "state penalty" which relates the population of that tautomer to all others. This state penalty can be converted into log units and used as a correction term to convert $\log P$ to $\log D$:

$$\log D = \log P + \frac{-E_{state\ penalty}}{k_B T \ln(10)} \quad (5)$$

where k_B is Boltzmann constant and T is temperature. LigPrep can only perform the tautomer enumeration with water or DMSO as a solvent, so we were unable to predict tautomers in cyclohexane. Therefore both of these corrections account for the protonation or tautomer states only in the aqueous layer and assume the tautomer remains fixed in cyclohexane as the one used in the initial simulation.

4.3 Estimating distribution coefficients with a fast, structural based partition coefficient calculator

Many structural based tools exist for octanol/water partition coefficients; they are very fast and generally accurate. However, these tools are all trained on empirical data, meaning they are limited by the training data. We chose the OpenEye tool OEXlogP [24,25] as an example of such a tool. Two post prediction sets were prepared with the OEXlogP tool. First, the predicted octanol/water partition coefficient was considered an estimate for $\log D$. In the second set, we calculated a correction for the bias between the calculated XlogP values and a set of experimental cyclohexane/water partition coefficients [8]. For the rest of this paper we will refer to the octanol/water partition coefficient set as $XlogP_{oct}$ and the bias corrected set as $XlogP_{corr}$.

5 Results and Discussion

A broad range of methods were used for the 76 submissions predicting cyclohexane/water distribution coefficients for the SAMPL5 challenge. Many of these predictions used alchemical molecular dynamics simulations to estimate the solvation free energy in explicit solvent using several classes of force fields, including fixed-charge all-atom force fields, all-atom/coarse-grained hybrid force fields, and polarizable force fields. A variety of quantum mechanics (QM) methods were also used including QM/molecular mechanics (QM/MM) with implicit or explicit solvent, QM with non-Boltzmann Bennett free energy calculations, and QM energy calculations with a single optimized molecular geometry. Two participants used variations on the reference interaction-site model (RISM). One participant uses QM calculations to derive parameters to tune an empirical model for activity coefficients and used these to estimate distribution coefficients. A few submissions used empirically trained methods for calculating solvation free energies. A particularly successful submission, which will be discussed again below, employed the Conductor-Like-Screening Model for Real Solvents (COSMO-RS).

SAMPL5 is the first SAMPL challenge to include distribution coefficients, but we can estimate how well we expect submissions to do based on past SAMPL challenges which included hydration free energies. Distribution coefficients can be related to transfer free energy between solvents, which allows us to estimate an expected performance from root-mean-squared errors (RMSE)

in past hydration free energy calculations. In SAMPL4 [1], the average RMSE for the best half of submissions was about 1.5 kcal/mol which would correspond to 1.54 log units error in a distribution coefficient. In contrast, only five submissions had an RMSE less than 2.5 log units in SAMPL5. There are many reasons for this perceived change in accuracy, such as a more complex set of molecules and the complexity of estimating tautomer populations, discussed in depth below. It is also likely the case that since this is the first challenge on predicting distribution coefficients, participants had not yet developed good protocols to deal with many of these challenges, meaning that somewhat less accuracy ought to be expected. It took several challenges focused on hydration before a range of methods could achieve the success noted in SAMPL4.

As discussed above, we calculated root-mean-squared error (RMSE), average unsigned error (AUE), average signed error (ASE), Pearson's R (R), Kendall's tau (tau), and the slope from the QQ plot (error slope) for each set of predictions. These are reported for all submissions (Table 5), but the rest of the analysis will focus only on submissions that reported For each submission, we also created a plot comparing the predicted and experimental values. Some example plots are provided (Fig. 2); these represent a typical submission, in that these submissions were in the middle of the pack by most error metrics. Comparison and QQ plots for every submission are available in the supporting information as well as error metric tables by batch rather than just for the full set.

To help visualize all of the error metrics, the data was compiled into a histogram where results are sorted from best to worst for that metric (closest to 1 for error slope for example). These metrics are split into measurements of deviation from experiment (Fig. 3) and correlation with experiment (Fig. 4) distinctions which helped in identifying high performing groups. This analysis included only submissions that included data for all molecules; the other submissions were indicated in Table 5 and generally fall in the middle of the pack on most metrics. In comparing methods by all of the error metrics, it is important to keep in mind the uncertainty in these error metrics. While Figures 3 and 4 methods ordered by performance in some sense, the reality is that there are many submissions that are not significantly different from one another.

In the error slope analysis, the slopes are often substantially different from 1, indicating that participants generally provided poor estimates of model uncertainty. Only the top three submissions are within uncertainty of 1. Andrew Paluch from Miami University used conservative estimates based on results in previous calculations for solubility and hydration free energy for submissions 53 and 60. Gerhard Koenig from Max-Planck-Institut fuer Kohlenforschung provided no explicit discussion of model uncertainty with submission 43. Only submission 40 significantly overestimated their model uncertainty. All other submissions have an error slope below one, indicating a significant underestimation of the model uncertainty. This suggests that analysis and prediction of model uncertainty remains a key frontier for predictive molecular simulations, and much further effort is needed in that area.

ID	Ave. err.	RMS	AUE	tau	R	Err. slope
01 ^b	2.3 ± 0.8	5.1 ± 0.5	4.3 ± 0.5	0.13 ± 0.13	0.20 ± 0.18	0.44 ± 0.09
02	-0.5 ± 0.3	2.3 ± 0.3	1.7 ± 0.2	0.48 ± 0.07	0.63 ± 0.07	0.69 ± 0.07
03 ^b	-7.6 ± 3.4	21.3 ± 2.6	15.9 ± 2.4	0.52 ± 0.10	0.59 ± 0.12	-0.00 ± 0.00
04 ^a	1.6 ± 0.5	2.5 ± 0.6	1.9 ± 0.4	0.77 ± 0.12	0.87 ± 0.05	0.77 ± 0.13
05	-8.2 ± 0.4	8.7 ± 0.4	8.2 ± 0.4	0.29 ± 0.08	0.39 ± 0.11	0.21 ± 0.04
06	1.8 ± 0.5	4.0 ± 0.3	3.4 ± 0.3	0.46 ± 0.09	0.61 ± 0.10	0.58 ± 0.07
07	0.5 ± 0.5	3.3 ± 0.5	2.5 ± 0.3	0.34 ± 0.08	0.51 ± 0.11	0.33 ± 0.07
08	-1.7 ± 0.4	3.5 ± 0.5	2.5 ± 0.3	0.58 ± 0.06	0.70 ± 0.06	0.60 ± 0.07
09	-5.5 ± 0.4	6.3 ± 0.5	5.5 ± 0.4	0.29 ± 0.08	0.40 ± 0.10	0.26 ± 0.05
10	0.3 ± 0.4	3.1 ± 0.3	2.6 ± 0.3	0.51 ± 0.07	0.69 ± 0.08	0.79 ± 0.07
11	-4.4 ± 1.7	13.3 ± 2.5	6.9 ± 1.6	0.45 ± 0.09	0.53 ± 0.09	0.39 ± 0.07
12	-5.5 ± 2.5	19.4 ± 1.9	15.0 ± 1.7	0.37 ± 0.09	0.39 ± 0.12	-0.00 ± 0.00
13 ^a	-11.1 ± 5.0	21.0 ± 5.0	12.2 ± 4.8	0.56 ± 0.17	0.43 ± 0.22	0.59 ± 0.17
14	-0.7 ± 0.4	2.7 ± 0.4	2.0 ± 0.3	0.57 ± 0.06	0.72 ± 0.06	0.66 ± 0.08
15	-1.4 ± 0.4	3.3 ± 0.5	2.3 ± 0.3	0.57 ± 0.07	0.70 ± 0.06	0.61 ± 0.07
16	0.5 ± 0.3	2.1 ± 0.2	1.7 ± 0.2	0.73 ± 0.04	0.84 ± 0.03	0.46 ± 0.08
17	-4.2 ± 0.4	5.0 ± 0.5	4.2 ± 0.4	0.36 ± 0.08	0.51 ± 0.10	0.50 ± 0.06
18	-0.8 ± 0.4	2.7 ± 0.4	2.0 ± 0.3	0.47 ± 0.08	0.60 ± 0.07	0.62 ± 0.08
19	1.5 ± 0.3	2.7 ± 0.2	2.3 ± 0.2	0.54 ± 0.06	0.75 ± 0.06	0.83 ± 0.06
20	-2.3 ± 0.4	3.6 ± 0.5	2.7 ± 0.3	0.55 ± 0.07	0.70 ± 0.06	0.48 ± 0.07
21	-1.2 ± 0.4	3.4 ± 0.6	2.4 ± 0.3	0.44 ± 0.08	0.45 ± 0.16	0.58 ± 0.07
22	1.6 ± 0.5	3.9 ± 0.3	3.1 ± 0.3	0.29 ± 0.09	0.48 ± 0.11	0.68 ± 0.08
23	1.9 ± 0.5	4.0 ± 0.4	3.0 ± 0.4	0.42 ± 0.07	0.58 ± 0.07	0.78 ± 0.08
24 ^a	2.3 ± 0.7	3.3 ± 0.8	2.5 ± 0.6	0.77 ± 0.13	0.88 ± 0.05	0.67 ± 0.15
25	0.0 ± 0.5	3.6 ± 0.4	2.9 ± 0.3	0.53 ± 0.07	0.70 ± 0.07	0.71 ± 0.07
26	2.3 ± 0.7	5.6 ± 0.4	4.6 ± 0.4	0.25 ± 0.08	0.37 ± 0.11	0.46 ± 0.07
27	-0.2 ± 0.4	2.6 ± 0.4	1.8 ± 0.2	0.49 ± 0.07	0.61 ± 0.08	0.66 ± 0.07
28	-2.3 ± 0.4	3.6 ± 0.5	2.7 ± 0.3	0.54 ± 0.07	0.69 ± 0.07	0.47 ± 0.07
29	-6.7 ± 0.4	7.2 ± 0.4	6.7 ± 0.4	0.33 ± 0.08	0.45 ± 0.11	0.28 ± 0.04
30	2.5 ± 0.5	4.3 ± 0.3	3.7 ± 0.3	0.39 ± 0.10	0.52 ± 0.12	0.53 ± 0.07
31	-1.0 ± 0.3	2.7 ± 0.3	2.0 ± 0.3	0.56 ± 0.07	0.72 ± 0.06	0.63 ± 0.08
32	2.5 ± 0.4	3.5 ± 0.2	3.1 ± 0.2	0.47 ± 0.07	0.64 ± 0.07	0.25 ± 0.06
33	-0.1 ± 0.5	3.4 ± 0.3	2.8 ± 0.3	0.53 ± 0.07	0.71 ± 0.07	0.73 ± 0.07
34	-1.3 ± 0.4	3.0 ± 0.4	2.2 ± 0.3	0.56 ± 0.06	0.69 ± 0.07	0.61 ± 0.07
35	0.5 ± 0.4	2.9 ± 0.3	2.2 ± 0.2	0.36 ± 0.08	0.54 ± 0.09	0.35 ± 0.07
36	1.1 ± 0.3	2.6 ± 0.2	2.1 ± 0.2	0.57 ± 0.07	0.75 ± 0.06	0.50 ± 0.07
37 ^a	-7.1 ± 4.9	19.6 ± 4.1	13.9 ± 3.7	0.59 ± 0.16	0.41 ± 0.22	-0.00 ± 0.00
38	0.8 ± 0.4	3.3 ± 0.3	2.7 ± 0.3	0.41 ± 0.08	0.58 ± 0.08	0.78 ± 0.07
39	1.6 ± 0.3	2.6 ± 0.2	2.1 ± 0.2	0.49 ± 0.08	0.65 ± 0.10	0.63 ± 0.08
40	0.4 ± 0.3	2.6 ± 0.3	1.9 ± 0.2	0.48 ± 0.07	0.61 ± 0.08	1.16 ± 0.05
41	0.3 ± 0.4	3.2 ± 0.3	2.7 ± 0.3	0.53 ± 0.07	0.69 ± 0.07	0.77 ± 0.07
42	4.6 ± 0.4	5.3 ± 0.4	4.6 ± 0.3	0.50 ± 0.08	0.61 ± 0.11	0.15 ± 0.05
43	-0.7 ± 0.4	3.0 ± 0.4	2.3 ± 0.3	0.51 ± 0.08	0.67 ± 0.08	0.94 ± 0.07
44	-0.6 ± 0.3	2.4 ± 0.3	1.8 ± 0.2	0.47 ± 0.07	0.63 ± 0.07	0.70 ± 0.07
45	0.9 ± 0.5	3.6 ± 0.3	2.9 ± 0.3	0.38 ± 0.08	0.58 ± 0.10	0.71 ± 0.07
46	-8.3 ± 0.5	9.1 ± 0.6	8.3 ± 0.5	0.23 ± 0.08	0.31 ± 0.10	0.14 ± 0.03
47	-1.3 ± 0.4	3.3 ± 0.5	2.2 ± 0.3	0.58 ± 0.06	0.71 ± 0.06	0.62 ± 0.08
48	1.5 ± 0.4	3.0 ± 0.3	2.3 ± 0.3	0.38 ± 0.07	0.55 ± 0.08	0.42 ± 0.07
49	-1.1 ± 0.4	3.3 ± 0.4	2.6 ± 0.3	0.42 ± 0.07	0.58 ± 0.07	0.78 ± 0.07
50 ^b	-7.1 ± 2.7	16.6 ± 3.2	9.2 ± 2.4	0.60 ± 0.09	0.66 ± 0.08	0.38 ± 0.10
51	1.7 ± 0.7	5.2 ± 0.4	4.3 ± 0.4	0.31 ± 0.08	0.46 ± 0.11	0.46 ± 0.08
52 ^a	-3.5 ± 1.1	5.4 ± 0.6	4.8 ± 0.7	0.56 ± 0.14	0.59 ± 0.14	0.23 ± 0.10
53	0.5 ± 0.4	2.8 ± 0.3	2.2 ± 0.2	0.44 ± 0.09	0.58 ± 0.10	1.00 ± 0.06
54	-1.0 ± 0.3	2.7 ± 0.3	1.9 ± 0.2	0.56 ± 0.07	0.70 ± 0.06	0.65 ± 0.08
55 ^b	-11.6 ± 3.3	22.3 ± 3.0	13.7 ± 3.1	0.59 ± 0.09	0.61 ± 0.11	0.38 ± 0.09
56	-1.1 ± 0.4	3.3 ± 0.5	2.2 ± 0.3	0.57 ± 0.06	0.71 ± 0.06	0.67 ± 0.08
57	-10.2 ± 2.4	20.2 ± 2.3	12.6 ± 2.2	0.43 ± 0.09	0.42 ± 0.12	0.38 ± 0.07
58	-2.9 ± 0.5	4.8 ± 0.5	3.8 ± 0.4	0.30 ± 0.09	0.44 ± 0.11	0.55 ± 0.08
59 ^a	-4.2 ± 1.0	5.6 ± 0.6	5.2 ± 0.6	0.54 ± 0.15	0.55 ± 0.14	0.13 ± 0.07
60	0.2 ± 0.3	2.5 ± 0.4	1.9 ± 0.2	0.49 ± 0.08	0.60 ± 0.08	1.02 ± 0.06
61	-1.2 ± 0.5	3.4 ± 0.6	2.4 ± 0.3	0.44 ± 0.08	0.45 ± 0.16	0.53 ± 0.07
62	0.7 ± 0.5	3.5 ± 0.4	2.7 ± 0.3	0.27 ± 0.09	0.38 ± 0.12	0.73 ± 0.08
63	-4.5 ± 1.7	13.3 ± 2.5	6.9 ± 1.6	0.45 ± 0.09	0.52 ± 0.08	0.41 ± 0.07
64	1.3 ± 0.7	5.2 ± 0.4	4.4 ± 0.4	0.35 ± 0.08	0.51 ± 0.10	0.43 ± 0.07
65	-2.2 ± 0.5	4.4 ± 0.5	3.5 ± 0.4	0.24 ± 0.10	0.35 ± 0.12	0.61 ± 0.08
66	1.4 ± 0.7	5.4 ± 0.4	4.6 ± 0.4	0.34 ± 0.08	0.51 ± 0.10	0.41 ± 0.07
67 ^a	-5.0 ± 3.1	11.9 ± 4.5	6.2 ± 2.9	0.59 ± 0.17	0.58 ± 0.13	0.56 ± 0.17
68	2.5 ± 0.4	3.6 ± 0.3	3.1 ± 0.2	0.47 ± 0.07	0.64 ± 0.07	0.25 ± 0.06
69 ^a	-5.1 ± 2.9	11.9 ± 4.4	6.2 ± 2.8	0.59 ± 0.16	0.57 ± 0.12	0.59 ± 0.16
70 ^b	-7.0 ± 2.6	16.5 ± 3.2	9.2 ± 2.4	0.60 ± 0.09	0.67 ± 0.08	0.36 ± 0.10
71	-10.7 ± 0.4	11.2 ± 0.5	10.7 ± 0.4	0.22 ± 0.08	0.29 ± 0.11	0.16 ± 0.03
72	-2.6 ± 0.5	4.2 ± 0.6	3.0 ± 0.4	0.56 ± 0.06	0.70 ± 0.06	0.45 ± 0.07
73	0.3 ± 0.3	2.4 ± 0.3	1.8 ± 0.2	0.48 ± 0.08	0.64 ± 0.08	0.50 ± 0.08
74	-2.7 ± 0.4	4.2 ± 0.5	3.0 ± 0.4	0.56 ± 0.07	0.70 ± 0.06	0.44 ± 0.07
75	4.1 ± 0.4	5.1 ± 0.3	4.4 ± 0.3	0.23 ± 0.09	0.34 ± 0.12	0.29 ± 0.06
76	1.7 ± 0.7	5.3 ± 0.4	4.3 ± 0.4	0.32 ± 0.08	0.47 ± 0.10	0.47 ± 0.08

Table 2 Error metrics were calculate for each set of predictions, including root-mean-squared error (RMSE), average unsigned error (AUE), average signed error (ASE), Kendall's tau (tau), and Pearson's R (R). Error slope refers to the slope of data in a QQ Plot. Indicated submissions only included batch 0^a or batches 0 and 1^b.

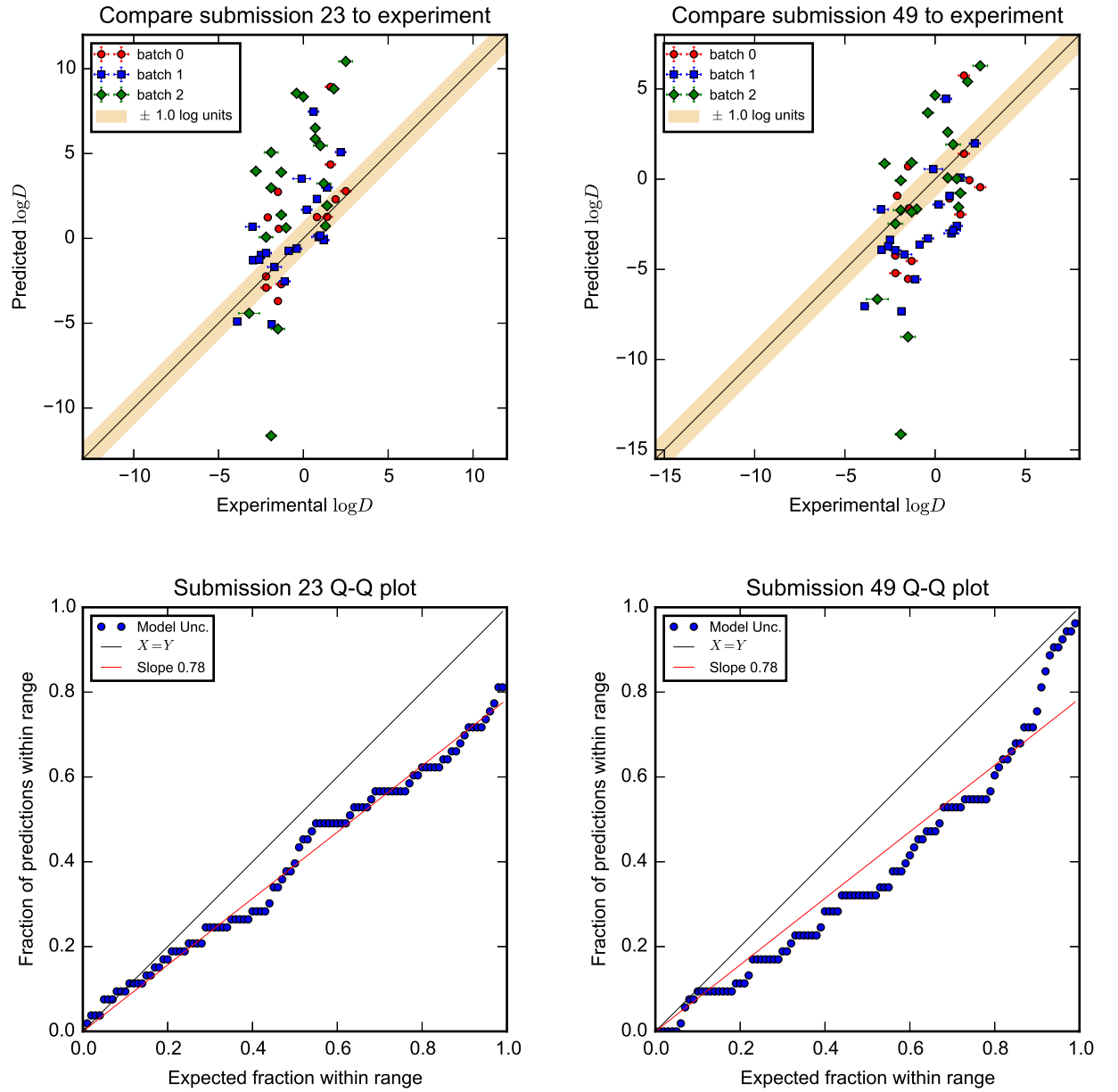


Fig. 2 Examples plots created for each set of predictions. These were chosen to try to represent average submissions, those that were in the middle by most error metrics. Comparison plots, in a) and b), show how predicted distribution coefficients compared to experiment for both submissions. QQ Plots, shown in c,d), show how errors in the predictions were distributed compared to expectations given the model uncertainty.

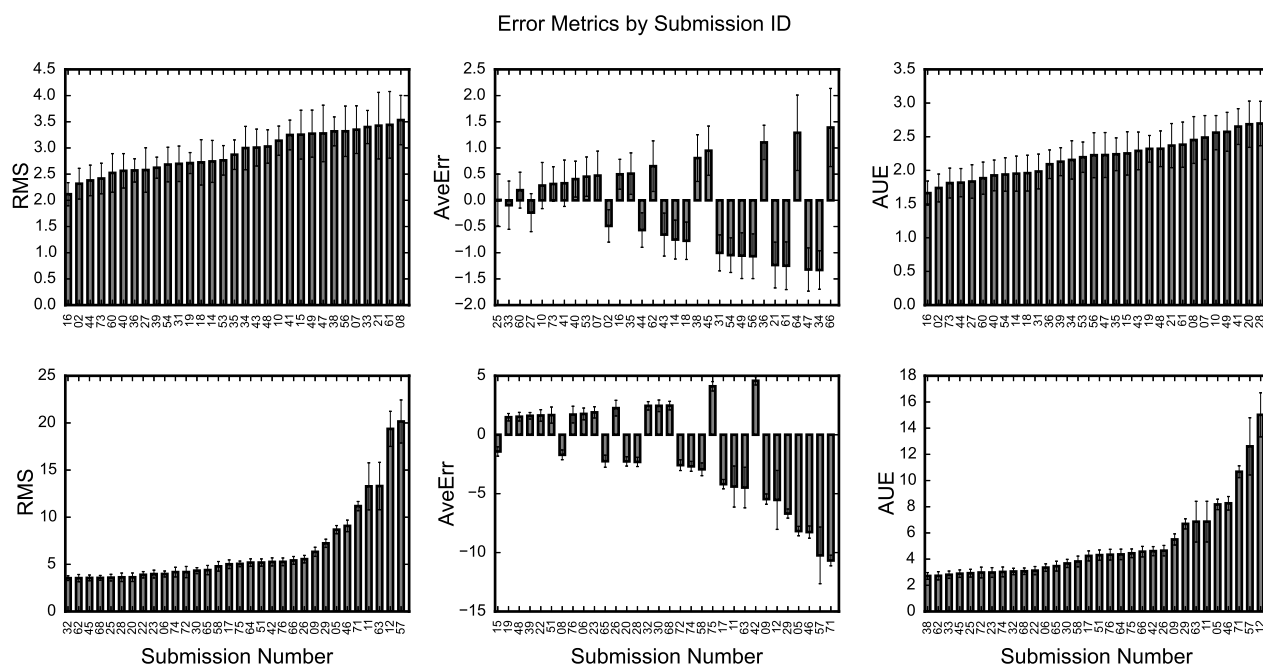


Fig. 3

5.1 Top performing submissions

In order to determine which submissions performed the best, we group error metrics into two categories. The first category describes typical error relative to experiment, and includes metrics RMSE and AUE. The second category describes how well correlated the experimental values are with the experimental values, and includes Kendall τ and Pearson R . Unlike past SAMPL challenges, there does appear to be one submission which performs best by all of these metrics, submission 16, and for most metrics it is better by a statistically significant amount. There are two additional submissions which performed in the top by these four metrics, 14 and 36. Predictions from each of these submissions are compared to experiment in figure 5. For submission 16, Andreas Klamt from COSMOlogic used COSMO-RS to compute a partition coefficient for each solute from the difference in chemical potentials for the solute in each solvent. To find distribution coefficients, calculations for the formation of different protonation states, zwitterions, and tautomers were performed in COSMO-RS for relevant molecules. For submission 14, Frank Pickard from the National Institute of Health calculated solvation free energies from QM calculations with SMD implicit solvent in Gaussian. Absolute pK_a calculations were used to account for additional ionization states. For submission 36, Christopher Fennell from Oklahoma State University estimated $\log D$ as a partition coefficient, calculated from the difference in alchemical solvation free

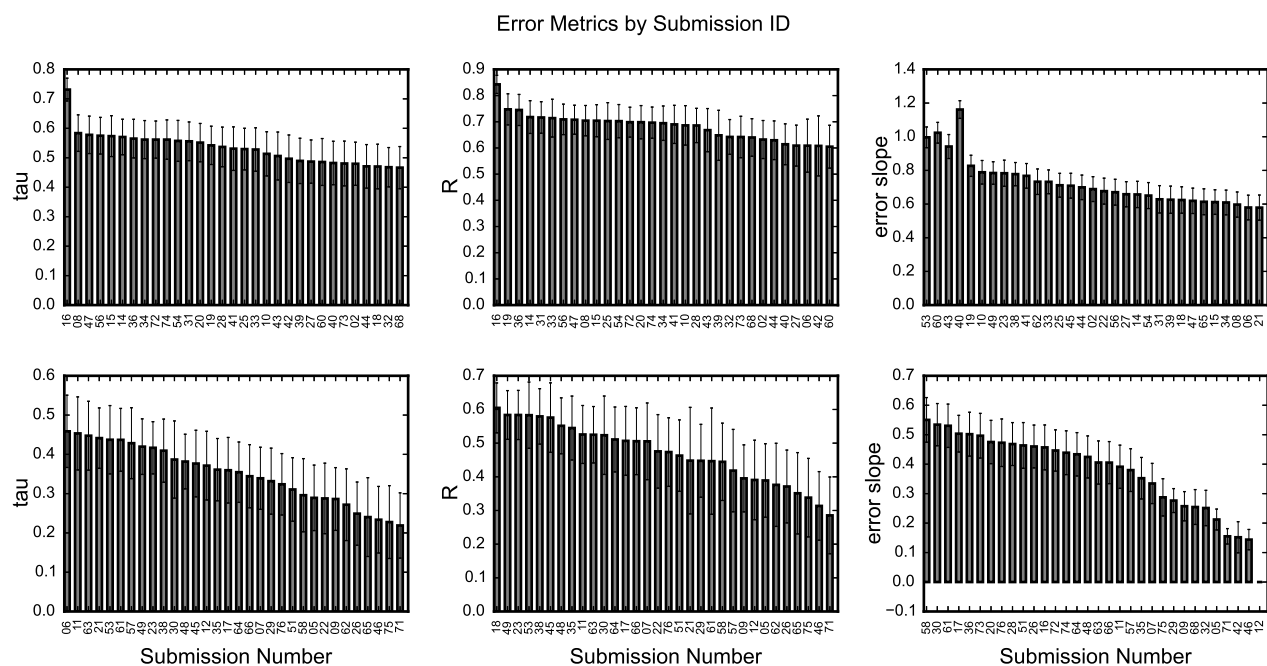


Fig. 4

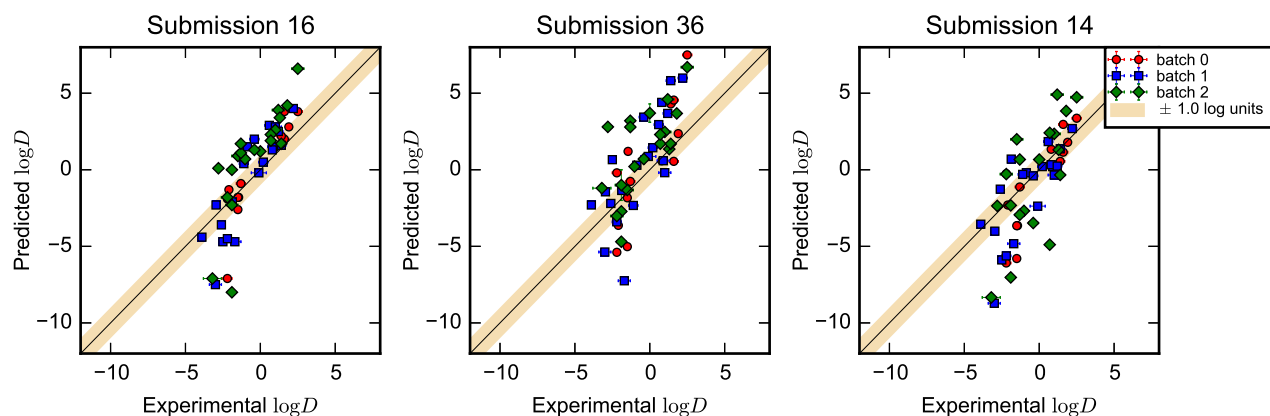


Fig. 5

energies where the solute was parameterized with the dielectrically corrected GAFF force field, water was the dielectrically corrected H2O-DC model, and cyclohexane was a specially optimized united-atom model. Further details for each of these submissions can be found in this issue so only a brief explanation of each method was provided here.

Metric	Null	$XlogP_{oct}$	$XlogP_{corr}$
AveErr	1.5 ± 0.2	2.8 ± 0.2	1.4 ± 0.2
RMS	2.3 ± 0.2	3.1 ± 0.1	1.8 ± 0.1
AUE	1.8 ± 0.2	2.8 ± 0.2	1.6 ± 0.1
tau	N/A	0.62 ± 0.05	0.62 ± 0.05
R	N/A	0.78 ± 0.04	0.78 ± 0.04

Table 3 Null hypothesis corresponds to $\log D = 0$ for all molecules. $XlogP_{oct}$ is a calculation octanol/water partition coefficient for each molecule and $XlogP_{corr}$ includes a bias correction.

5.2 Comparisons to simple empirical models

One way of evaluating predictive models is to compare them to a null hypothesis, or default result of some kind. In the case of distribution coefficients, we chose a null hypothesis where we assume all solute molecules distribute equally between cyclohexane and water, corresponding to $\log D = 0$, as suggested by Christopher Fennell [ref]. We performed all our standard error analyses discussed above on this simple model as a point of comparison, and specifically we calculated RMSE, AUE, and Ave. Err. (Table 5.2). The null hypothesis would have been within the top three submissions for both RMSE and AUE. While this null hypothesis has no actual predictive power and could not be used to rank compounds, the fact that it performs quite well in terms of error statistics is a challenge for the other methods. It may also provide commentary on the dataset, which contains a reasonably large percentage of $\log D$ values that are not that far off from zero.

There are many structure-based and/or empirically trained prediction methods for octanol/water partition coefficients, and to a first approximation, one might imagine that cyclohexane/water partition coefficients would follow similar trends to those in octanol/water. Therefore, we used OEXlogP from Openeye ($XlogP_{oct}$) to examine the possibility of estimating cyclohexane/water distribution coefficients with such a tool, and used a simple correction factor to adjust for a bias difference between octanol and cyclohexane ($XlogP_{corr}$). $XlogP_{oct}$ would be in the top few submissions by tau and R, but in the bottom half by all other metrics (Table 5.2). However, with a simple correction factor trained on experimental cyclohexane/water partition coefficients [8], $XlogP_{corr}$ has a better RMSE and AUE than any other submission. We do not wish to suggest that regression-trained tools are the best mechanism for predicting distribution coefficients; rather, this indicates the potential for cyclohexane/water distribution data to help drive improvements in our physical models, as clearly there are a range of physical effects here which are not yet well described by our models.

5.3 Results of reference calculations

We performed a set of blind reference calculations (submission 39) for the SAMPL5 challenge, calculating $\log P$ for the provided neutral tautomers of

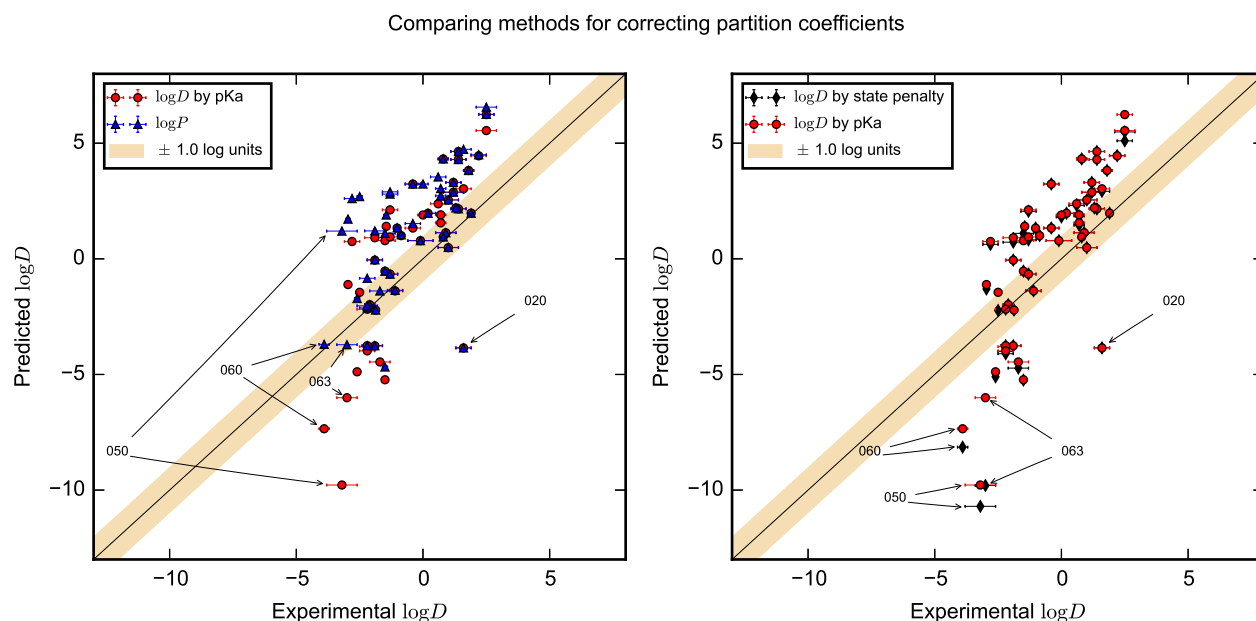


Fig. 6 Plots showing our predictions (reference calculations) compared to experiment. a) submission 39 to SAMPL5, with no tautomer correction. b) distribution coefficient corrected from calculated partition coefficient based on pKas. c) distribution coefficient correct from calculated partition coefficient with state penalties

all solutes. Our protocol for these calculations was announced in advance, and parameter and coordinate files for the calculations were made available (as described above) in formats for a variety of simulation packages. Participants were encouraged to perform their own set of reference calculations for the full set, or at the very least several specified reference compounds, using these files. This would allow differences in performance to be traced back to methodological differences rather than force field differences. Unfortunately, no participants actually reported results of reference calculations, so this type of analysis has thus far been impossible. However, the results of our reference calculations are still helpful for understanding the challenges facing SAMPL5 participants.

For our reference calculations, solvation free energies were calculated using GROMACS with GAFF parameters and AM1-BCC charges. Our reference calculations yielded partition coefficients, determined from the difference in solvation free energies without correcting for variation in tautomers. These calculations were done blindly, and submitted as Submission 39, which was in the top quarter of submissions by most error metrics (Table 5) although, there is a slight bias for concentration in the cyclohexane layer, evident by the average error (1.6 ± 0.3).

Metric	$\log P$	pK_a	state
AveErr	1.6 ± 0.3	0.7 ± 0.3	0.5 ± 0.4
RMSE	2.6 ± 0.2	2.4 ± 0.2	2.6 ± 0.3
AUE	2.1 ± 0.2	2.0 ± 0.2	2.1 ± 0.2
tau	0.49 ± 0.08	0.65 ± 0.07	0.65 ± 0.06
R	0.6 ± 0.1	0.78 ± 0.07	0.77 ± 0.06

Table 4

After the challenge we explored how including protonation and deprotonation would have affected the initial partition coefficient predictions. The first set of corrections involved calculating the pKa for each molecule using Schrödinger’s Epik tool. Next, $\log D$ was calculated using the pKa and partition coefficient determined in submission 39 using equations 4 and 3 for acidic and basic solutes, respectively. We assumed only one change in protonation state occurred so only one pK_a was used. This does not account for zwitterions or alternate neutral tautomers. This correction (labeled the “ pK_a ” correction) showed a slight improvement by most error metrics (Table 5.3) including a decrease in the average error from 1.6 ± 0.3 to 0.7 ± 0.3 indicating less bias toward overly high concentration in cyclohexane.

For the next set of corrections, we used Schrödinger’s Ligprep tool to enumerate tautomers and calculate a state penalty, which gives the relative population of each tautomer in water at pH 7.4. The state penalty was used to correct the concentration in the aqueous layer, according to equation 5. This correction (labeled “state” for “state penalty correction”) results in improvements from the original partition coefficient coefficients for tau (0.49 ± 0.08 to 0.65 ± 0.06) and R (0.6 ± 0.1 to 0.77 ± 0.06), but no significant change in RMSE or AUE. Both of these correction methods only adjust the concentration in the aqueous layer; however, there may be tautomer affects that would change the concentration in cyclohexane as well. Outliers and molecules with particularly significant changes in $\log D$ were indicated by number in figure ???. SAMPL.050 for example, had an initial $\log P$ value of 1.20 ± 0.04 which was decreased significantly to -9.78 with the pKa correction and 10.70 with the state penalty correction compared to the experimental value -3.2 ± 0.6 . SAMPL.060 and SAMPL.060 also changed by more than 3 log units due to these corrections. One explanation for these extreme examples is that the solute might have other neutral tautomers that would affect the concentration in cyclohexane, which these corrections do not account for.

5.4 Examining individual molecules

With only 53 molecules, it is difficult to find any statistically significant trends in terms of functional groups which are well- or poorly-predicted in general; compared to past SAMPL challenges, this set of molecules is much more complex. They are on average larger, more flexible, and contain multiple functional groups per compound. For each molecule, we organized a data set of predicted

	SAMPL5_050		SAMPL5_083	
	tautomer 1	tautomer 2	tautomer 1	tautomer 2
$\Delta G_{hydration}$	11.45 ± 0.04	21.50 ± 0.03	33.98 ± 0.07	32.68 ± 0.1
$\Delta G_{cyclohexane}$	13.09 ± 0.04	13.25 ± 0.04	35.6 ± 0.1	36.1 ± 0.2
$\log P_{cyc/wat}$	1.20 ± 0.04	-6.04 ± 0.03	1.21 ± 0.09	2.5 ± 0.2
Correction	-11.902	-0.453	-0.488	-6.53
$\log D_{cyc/water}$	-10.70 ± 0.04	-6.50 ± 0.03	0.72 ± 0.09	-4.0 ± 0.2
experimental $\log D$	-3.2 ± 0.6		-1.9 ± 0.4	

Table 5

distribution coefficients and compared them to the experimental values, calculating the average unsigned error for each (Table 2). In general, molecules with an AUE less than 2.0 log units (SAMPL5_003, 045, and 059) are slightly more rigid than other molecules and they are all in batch 0. We tried grouping molecules by functional group, molecular mass, and estimated number of tautomers to see if size, presence or absence of particular functional groups, or number of tautomers played a role in how difficult each compound was in general. The only trend found in this process was that all five carboxylic acids (SAMPL5_010, 011, 015, 026, and 060) are in the bottom ten molecules by AUE and RMSE. This could be due to poor treatment of the effects of of protonation state changes. Among the bottom compounds, perhaps unsurprisingly, was SAMPL5_083 which is a large macrocycle and SAMPL5_050, both have many tautomeric forms. Most submissions had significant errors in predicting SAMPL5_074, despite the fact that it is relatively small, rigid, and has no other significant tautomers. Below we will explore why some of these molecules may have had distribution coefficients which were particularly difficult to predict.

Provided SMILES strings may not be the most populated tautomeric form of the molecule. From our tautomer enumeration and discussions with other SAMPL5 participants it became clear that accurately estimating $\log D$ for molecules with many tautomers was difficult. If we could perfectly calculate solvation free energies and tautomer populations in both solvents, the starting tautomer should not effect the final calculated distribution coefficient. Our initial solvation free energy calculations used provided SMILES strings without any consideration of other tautomers. To explore how this may have affected our $\log D$ calculation, we decided to repeat a few solvation free energy calculations with different tautomers. We used SAMPL5_050 and SAMPL5_083 as examples since both have other neutral tautomers that could be present in both the water and cyclohexane solutions. For both SAMPL5_050 and SAMPL5_083 there were significant changes in their calculated solvation free energies and partition coefficients for the two different tautomers (Table 5.4). Distribution coefficients were calculated from the $\log P$ and state penalties calculated with Schrödinger’s LigPrep tool. In both cases the $\log D$ is still significantly different from the experimental values. Since both the calculated solvation free energies and the tautomer/protomer populations are needed to estimate the distribu-

	Original	Water in Cyclohexane
$\Delta G_{hydration}$	21.90 ± 0.04	
$\Delta G_{cyclohexane}$	16.77 ± 0.04	19.54 ± 0.04
$\log D_{cyc/wat}$	-3.76 ± 0.04	-1.73 ± 0.04
experimental $\log D$	-1.9 ± 0.3	

Table 6

tion coefficient, it is impossible for us to know which calculation introduces more error into our estimates.

Solvents are not completely immiscible. Though it is very small, ... M, the concentration of water in cyclohexane may affect how a solute is distributed across the two solvents. This will be particularly important for solutes with many polar groups; and may be one reason it was difficult to accurately estimate the $\log D$ for SAMPL5_074. We performed a new calculation of solvation free energy of SAMPL5_074 into cyclohexane with water also in the solution. This simulation was set-up with Solvation Toolkit as described above with 1 solute, 150 cyclohexane, and 7 water molecules. This is roughly 100 times more water in the cyclohexane phase than is measured experimentally. With this amount of water in cyclohexane, it results in a significant change in the computed solvation free energy for SAMPL5_074 into cyclohexane with water (Table 5.4). This dramatically improves the estimation for $\log D$, proving that the presence of water in the cyclohexane layer could have dramatic effects on the calculated distribution coefficients. However, as noted, this was done by dramatically overestimating the amount of water in cyclohexane. However, it is sufficient to show that the presence of water in the cyclohexane phase can dramatically affect the computed distribution coefficient, depending on the affinity of the solute for water. Further simulations would be needed to conclude how varying the concentration of water in cyclohexane would affect these calculations.

Other experimental solvents may affect distribution coefficients. The two phases for the distribution coefficients were cyclohexane and an aqueous buffer, however dimethyl sulfoxide (DMSO) and acetonitrile were used in the experimental procedures. While DMSO and acetonitrile were at very low concentrations, their presence in either solvent layer may affect how a solute distributes between phases. We created topology and coordinate files for a system with 780 water, 130 cyclohexane, 4 DMSO, and 2 acetonitrile molecules using SolvationToolkit. The system was minimized and equilibrated following our procedure above and then a 5 ns constant pressure and temperature production simulation was run. The trajectory from this simulation was visualized with VMD and the movie for it is available in the supplementary information. Both DMSO and acetonitrile spend most of their time near the solvent interface, with very little movement into the bulk of the water or cyclohexane. A detailed understanding of the implications of this for both calculated and measured distribution coefficients may require further study.

6 Conclusions

Past SAMPL challenges often involved a broad range of methods for hydration free energies. Here, in our first SAMPL on cyclohexane/water distribution coefficients, we saw a similarly diverse set of methods. The overall accuracy of the predicted values was reasonable, with the best methods showing 2-2.5 log units of error in $\log D$, but showed clear room for improvement.

This SAMPL5 set was substantially more complex, flexible, and poly-functional than typical molecules in SAMPL hydration challenge sets. Additionally, protonation state and tautomer were not always clear for the compounds, and some compounds likely had multiple relevant protonation states and tautomers, and shifts in protonation state/tautomer on transferring between phases. These issues are especially important given that the challenge focused on distribution coefficients rather than partition coefficients ($\log P$). Given these complexities, it is perhaps not surprising that we saw drop in performance relative to the accuracy that would have been expected for $\log P$ values if participants predicted $\log P$ based on solvation free energies in two solvents that could be computed as accurately as hydration free energies in previous SAMPL challenge (yielding an expected accuracy of about 1.5 log units). In large part, this is probably because of the additional complexities of distribution coefficients such as the need to account for other protonation states and tautomers. Accurately accounting for tautomers appears to be a vital part of accurately calculating $\log D$, and better methods for treating protonation and especially tautomeric states in non-aqueous environments are needed.

We asked participants to estimate two forms of uncertainty, statistical uncertainty and model uncertainty, the latter of which should predict how well their calculation will agree with experiment. This latter uncertainty estimate is particularly key, as it would allow practitioners to predict how reliable their calculations are likely to be in applications. Here, we find that almost every participant dramatically underestimated their model uncertainty. The importance to improve error estimations as a community has been addressed in past SAMPL challenges [1].

Overall, the results of this challenge strongly suggest these types of measurements will be extremely helpful for driving improvements to physical modeling to help guide pharmaceutical drug discovery. The major challenges encountered here are all very likely to occur when attempting to predict binding affinities or other biomolecular properties of interest to drug discovery. Specifically, protonation states and tautomeric states were found to be difficult to predict, uncertain, and potentially environment-dependent in ways that can dramatically affect computed properties, and these same scenarios are likely to apply to biomolecular binding. Thus it seems likely that an improved treatment of these effects within the context of SAMPL or similar challenges will drive improvements to prediction of binding and related properties such as solubility.

Thus, overall, distribution coefficients have been an extremely valuable part of this year’s SAMPL challenge, and, since they can be measured in a relatively straightforward way, seem to be a promising potential source of future data for blind challenges. Additionally, this data highlights important issues, such as tautomer enumeration, that need better treatment in many of our models. The ability to create new, completely blind data sets make distribution coefficients a great option for future challenges.

Acknowledgements

John Chodera and Bas MSKCC

Andreas Klamt at COSMOLogic

Christopher Fennell at Oklahoma State University

Samuel Genheden

Frank Pickard at the National Institute of Health

Michael Shirts - reference calculation and input format conversion

Schrödinger people

You mentioned open eye in your comments, everything I did with openeye I got from scripts you gave me or with their python cookbooks online

D3R team

Maybe something at the end like and all D3R Workshop participants? I bounced ideas back and forth with a lot of people while we were there... not all of them people submitted predictions and not all of them are people’s who’s name I remember...

Should we also thank Genetech people who let Bas do measurements there?

people who set up the automated submission system

6.1 Available in supporting info

things provided to participants all scripts used for error analysis all participant files renamed with submission numbers triple check no names/e-mails/institutions/etc in the final submitted data all plots not in the paper all input/output files for Schrödinger calculations all input files and results files for 'logP' calculations, tautomer redos, box size simulations example MDP and run scripts

References

1. D.L. Mobley, K.L. Wymer, N.M. Lim, J.P. Guthrie, *Journal of Computer-Aided Molecular Design* **28**(3), 135 (2014)
2. M.T. Geballe, J.P. Guthrie, *Journal of Computer-Aided Molecular Design* **26**(5), 489 (2012)
3. M.T. Geballe, A.G. Skillman, A. Nicholls, J.P. Guthrie, P.J. Taylor, *Journal of Computer-Aided Molecular Design* **24**(4), 259 (2010)
4. P.V. Klimovich, D.L. Mobley, *Journal of Computer-Aided Molecular Design* **24**(4), 307 (2010)
5. D.L. Mobley, C.I. Bayly, M.D. Cooper, K.A. Dill, *The Journal of Physical Chemistry B* **113**(14), 4533 (2009)
6. D.L. Mobley, S. Liu, D.S. Cerutti, W.C. Swope, J.E. Rice, *Journal of Computer-Aided Molecular Design* **26**(5), 551 (2012)
7. A. Nicholls, D.L. Mobley, J.P. Guthrie, J.D. Chodera, C.I. Bayly, M.D. Cooper, V.S. Pande, *Journal of Medicinal Chemistry* **51**(4), 769 (2008)
8. A. Leo, C. Hansch, D. Elkins, *Chemical Reviews* **71**(6), 525 (1971)
9. C.C. Bannan, G. Calabró, D.Y. Kyu, D.L. Mobley, in review (2016)

10. H.J.C. Berendsen, D. Van Der Spoel, R. van Drunen, *Comput. Phys. Commun.* **91**(1-3), 43 (1995)
11. B. Hess, C. Kutzner, D. van der Spoel, E. Lindahl, *J. Chem. Theory Comput.* **4**(3), 435 (2008)
12. E. Lindahl, B. Hess, D. van der Spoel, *J. Mol. Model.* **7**(8), 306 (2001)
13. D. van der Spoel, E. Lindahl, B. Hess, G. Groenhof, A.E. Mark, H.J.C. Berendsen, *J. Comput. Chem.* **26**(16), 1701 (2005)
14. S. Pronk, S. Páll, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M.R. Shirts, J.C. Smith, P.M. Kasson, D. van der Spoel, B. Hess, E. Lindahl, *Bioinformatics (Oxford, England)* **29**(7), 845 (2013)
15. S. Páll, M.J. Abraham, C. Kutzner, B. Hess, E. Lindahl, in *Solving Software Challenges for Exascale*, vol. 8759 (Springer International Publishing, Stockholm, Sweden, 2014), pp. 3–27
16. M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, E. Lindahl, *SoftwareX* **1-2**, 19 (2015)
17. J. Wang, R.M. Wolf, J.W. Caldwell, P.A. Kollman, D.A. Case, *Journal of Computational Chemistry* **25**(9), 1157 (2004)
18. A. Jakalian, B.L. Bush, D.B. Jack, C.I. Bayly, *Journal of Computational Chemistry* **21**(2), 132 (2000)
19. A. Jakalian, D.B. Jack, C.I. Bayly, *Journal of Computational Chemistry* **23**(16), 1623 (2002)
20. S. Liu, S. Cao, K. Hoang, K.L. Young, A.S. Paluch, D.L. Mobley, *Journal of Chemical Theory and Computation* **12**(4), 1930 (2016)
21. P.V. Klimovich, M.R. Shirts, D.L. Mobley, *Journal of Computer-Aided Molecular Design* **29**(5), 397 (2015)
22. S. Parameswaran, D.L. Mobley, *Journal of Computer-Aided Molecular Design* **28**(8), 825 (2014)
23. W.M. Haynes, *CRC handbook of chemistry and physics* (CRC press, 2014)
24. R. Wang, Y. Fu, L. Lai, *Journal of Chemical Information and Modeling* **37**(3), 615 (1997)
25. R. Wang, Y. Gao, L. Lai, *Perspectives in Drug Discovery and Design* **19**(1), 47 (2000)