Journal of Computer-Aided Molecular Design manuscript No.
(will be inserted by the editor)

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

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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. This focus on distribution coefficients replaces the focus on hydration free energies which has been a fixture of the past five challenges (SAMPL0-4) [18, **?**,**?**,12,**?**,**?**,**?**]. Due to a lack of ongoing experimental work, hydration free energies are no longer a practical property for blind challenges. It has become

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increasingly difficult to find unpublished or obscure hydration free energies and therefore impossible to design a challenge focusing on particular compounds, functional groups or chemical classes.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 [18], so we sought to include a similar physical property in SAMPL5. The organizers settled on cyclohexane-water distribution coefficients, and thanks to a partnership with Genetech, were able to conduct 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,

$$D = \frac{\sum_i [X_i]_{cyc}}{\sum_i [X_i]_{aq}} \tag{1}$$

where X_i represents a single tautomeric state in one of the solvents and results are reported as the logarithm of this ratio ($\log D$). These are more complicated than partition coefficients ($\log P$), which are limited to a single, typically neutral tautomer [17]. $\log P$ is proportional to the transfer free energy, which can be calculated from solvation free energies. All tautomers will need to be included to accurately calculate $\log D$, introducing some new complexities avoided in previous hydration free energy studies in SAMPL. Accurate tautomer enumeration in both solvents will 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 $\log D$. 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 1, 2016 and experimental results were provided to participants not

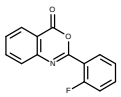
long after. As in past SAMPL challenges, each group could submit multiple sets of predictions. There was also the option to remain anonymous. A total of 76 prediction sets from 18 participants or participating groups were submitted and assigned a 2 digit ID number 01 to 76 that will be used throughout 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.

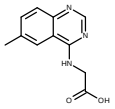
For the prediction of distribution coefficients in SAMPL5, a total of 53 molecules were considered. Molecules were assigned an identifier in the form SAMPL5_XXX; the complete table can be found below and in the supporting information. The 53 molecules were divided into batches 0, 1, and 2 containing 13, 20, and 20 molecules respectively. We wanted each batch to have a similar dynamic range and for the molecules to increase in size, so on average the smallest molecules are in batch 0 and the largest in batch 2. To control for dynamic range, molecules were grouped by calculated octanol/water partition coefficient and then by molecular weight. The smallest molecules from each partition coefficient group were added to batch 0, then batch 1, the rest of the molecules compromise batch 2.

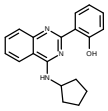
Participants could submit just batch 0, batches 0 and 1, or batches 0, 1, and 2. The idea was that all participants should attempt predictions on the full set if at all possible, but grouping into batches would allow people with particularly demanding methods (such as polarizable force fields or methods 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. Included in the challenge information was the SMILES string for each molecule as well as mol2 and SDfiles. All information provided to challenge participants is included in supporting information.

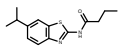
Participants were asked to report a cyclohexane/water distribution coefficient for each molecule. As discussed above, distribution coefficients are the ratio of concentrations for all forms of the solute in cyclohexane and the aqueous layer. During the experimental measurements, the water layer was an aqueous buffer at pH 7.4. We also required participants to provide two estimates for uncertainty, a statistical uncertainty for their computational method and a model uncertainty that estimates agreement with experiment. The statistical uncertainty should be the variation expected from repeated computational calculations. The model uncertainty, on the other hand, is 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

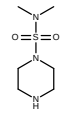
Batch 0

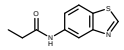
003: 1.7 ± 0.2 

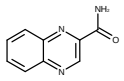
015: 8.8 ± 1.4 

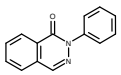
017: 3.0 ± 0.3 

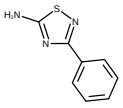
020: 3.9 ± 0.4 

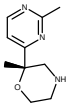
037: 7.5 ± 1.3 

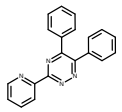
045: 1.4 ± 0.2 

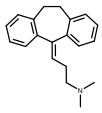
055: 2.7 ± 0.2 

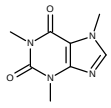
058: 2.2 ± 0.3 

059: 1.8 ± 0.2 

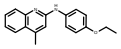
061: 5.9 ± 1.0 

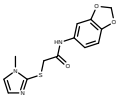
068: 2.7 ± 0.3 

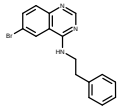
070: 5.9 ± 0.8 

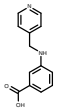
080: 2.6 ± 0.2 

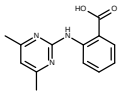
Batch 1

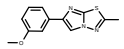
004: 2.3 ± 0.3 

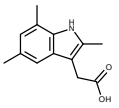
005: 2.6 ± 0.3 

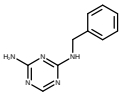
007: 3.1 ± 0.4 

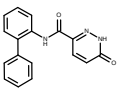
010: 7.8 ± 1.6 

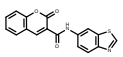
011: 7.1 ± 1.3 

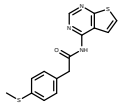
021: 2.2 ± 0.2 

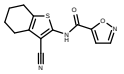
026: 8.0 ± 1.7 

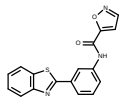
027: 3.4 ± 0.3 

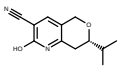
042: 3.2 ± 0.4 

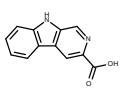
044: 3.7 ± 0.4 

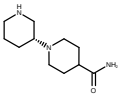
046: 2.7 ± 0.4 

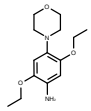
047: 2.1 ± 0.3 

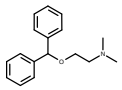
048: 2.7 ± 0.3 

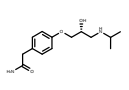
056: 3.5 ± 0.3 

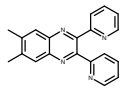
060: 6.7 ± 1.6 

063: 6.7 ± 1.0 

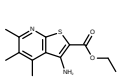
071: 2.8 ± 0.3 

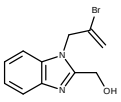
072: 4.9 ± 0.7 

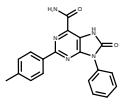
081: 6.0 ± 0.8 

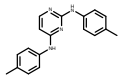
090: 2.8 ± 0.3 

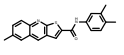
Batch 2

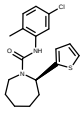
002: 2.5 ± 0.2 

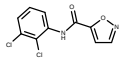
006: 2.7 ± 0.4 

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 predictions for that compound.

units. An important part of creating predictive models is the ability to know when it will fail. Analysis of model uncertainties then, is an important part of evaluating any model.

3 Error metrics

Similar to past SAMPL challenges, we considered a large number 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 available in the supporting information. Uncertainty in each metric was calculated as the standard deviation in 1000 bootstrap trials. As described previously, this bootstrapping technique included variation in the experimental values based on their reported uncertainties[18].

As discussed above, an important evaluation of a predictive tool is the ability to estimate how well and when a computational method will agree with experiment. As in SAMPL4 [18], a quantile-quantile plot (QQ Plot) was created for each prediction set. QQ Plots compare a normal distribution with how well a set of predictions agree with experiment according to the model uncertainty. For example, consider the number of predictions within one standard deviation of the expected value, the value on the x-axis represents the normal distribution (0.68) and the value on the y-axis will represent the fraction of predictions that are within one model uncertainty of the experimental value. A regression analysis helps summarize these results. The 'error slope' is the slope of the line comparing the fraction of predictions within range of experiment to the expected fraction in 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 over estimated. Oppositely, an error slope less than one suggests the model uncertainty was underestimated.

The last goal of prediction analysis was 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 available in supporting information.

4 Reference calculations from the Mobley group

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

Partition coefficients are the ratio of concentrations of a solute in a single tautomeric state distributed between two solvents. Before the challenge, each molecule was taken directly as the provided SMILES string with no further tautomer enumeration. 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 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 [1–7] with the General AMBER Force Field (GAFF) [8] with AM1-BCC charges[9,10]. 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. 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 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 [11,12]. 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 [13] 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

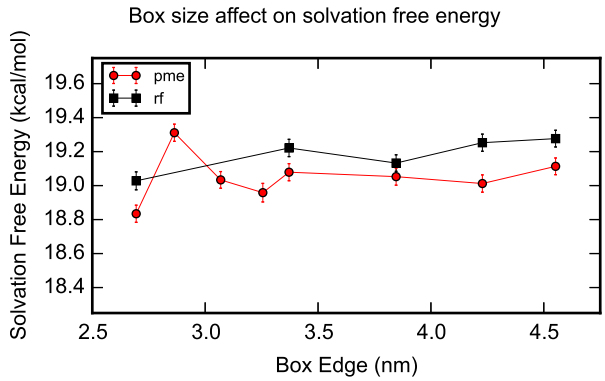


Fig. 1 This is a figure for the box size study

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. 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 [14]. 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 [**?**]. 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 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 different from partition coefficients in that they include all forms of the solute in both solvents. A common way to correct between experimentally measured distribution coefficients and partition coefficients is with pKa values for the solute. This is a simple

correction using the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{X}{HX} \tag{2}$$

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

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

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

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

We use Schrödinger's Epik tool to calculate pKa values for each molecule according to experimental conditions. We then estimated a $\log D$ using the equations above. 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 $\log D$:

$$\log D = \log P + \frac{-E_{statepenalty}}{k_B T \ln(10)} \tag{5}$$

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. Therefor 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 [15,16] 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 [17]. 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

Cyclohexane/water distribution coefficients were predicted by a broad range of methods for the SAMPL5 challenge. A large portion of predictions used alchemical molecular dynamics simulations to estimate the solvation free energy in explicit solvent using fixed-charge all-atom force fields, all-atom/course-grained hybrid force fields, and polarizing force fields. Other methods include 3D-RISM, EC-RISM, COSMO-RS, QM/MM, ...

SAMPL5 is the first 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 [18], 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.

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 group, we also created a plot comparing their predictions to experimental results. A few example plots are provided (Fig. 2) these represent a typical submission, in that these groups 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 broken down by batch.

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 show the sorted methods, in reality there are many submissions that are not significantly different from one another.

In considering the results for the error slope analysis, participants generally tend to do poorly estimating model uncertainty. The top three submissions are the only 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 over-

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].

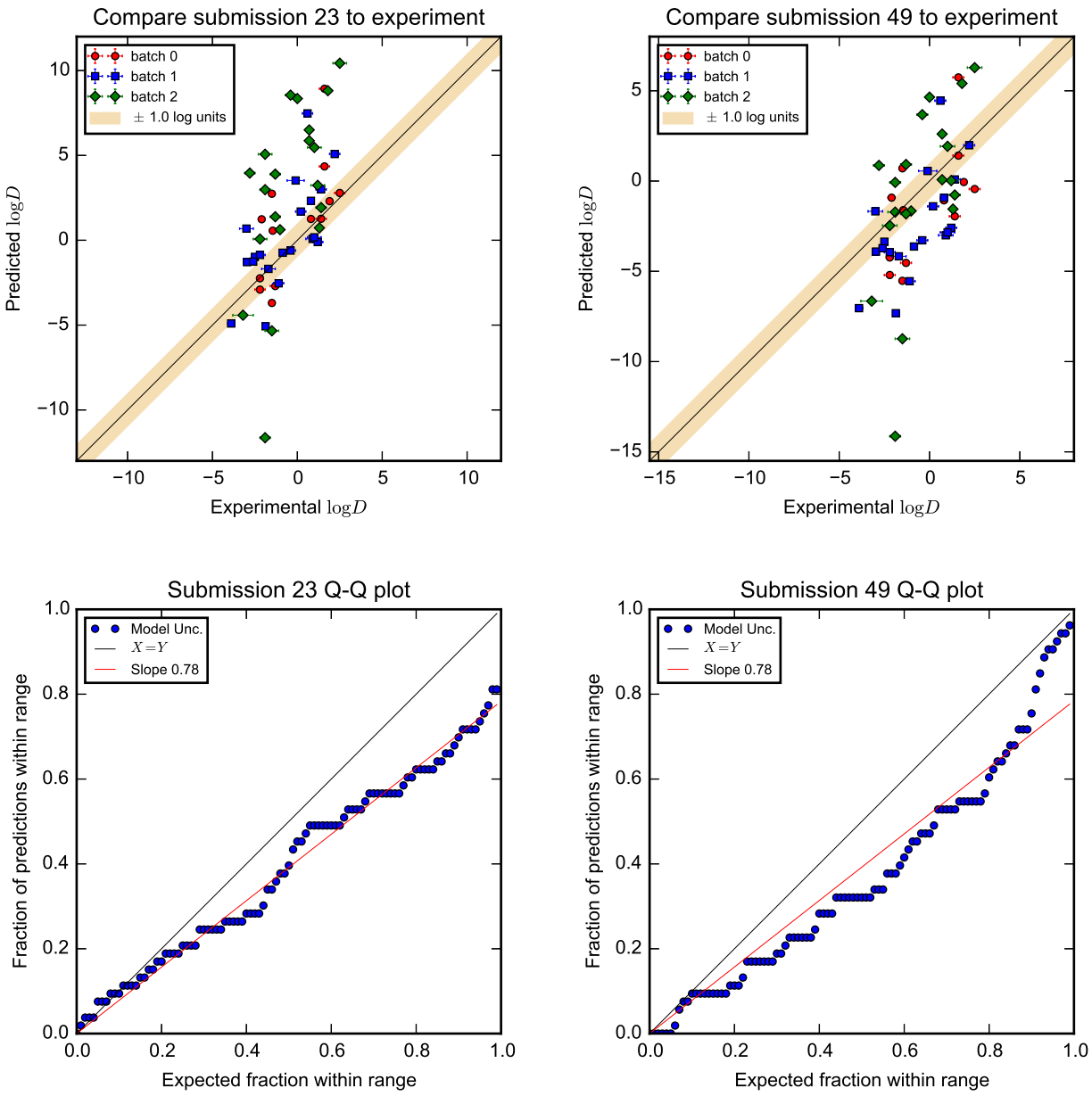


Fig. 2 These are examples of plots created for each set of predictions. They were chosen to try to represent the average submissions, those that were in the middle by most error metrics. a and b) comparison plots showing how predicted distribution coefficients compared to experiment for both groups. c,d) QQ Plots showing how their actual predictions were distributed compared to expectations given the model uncertainty.

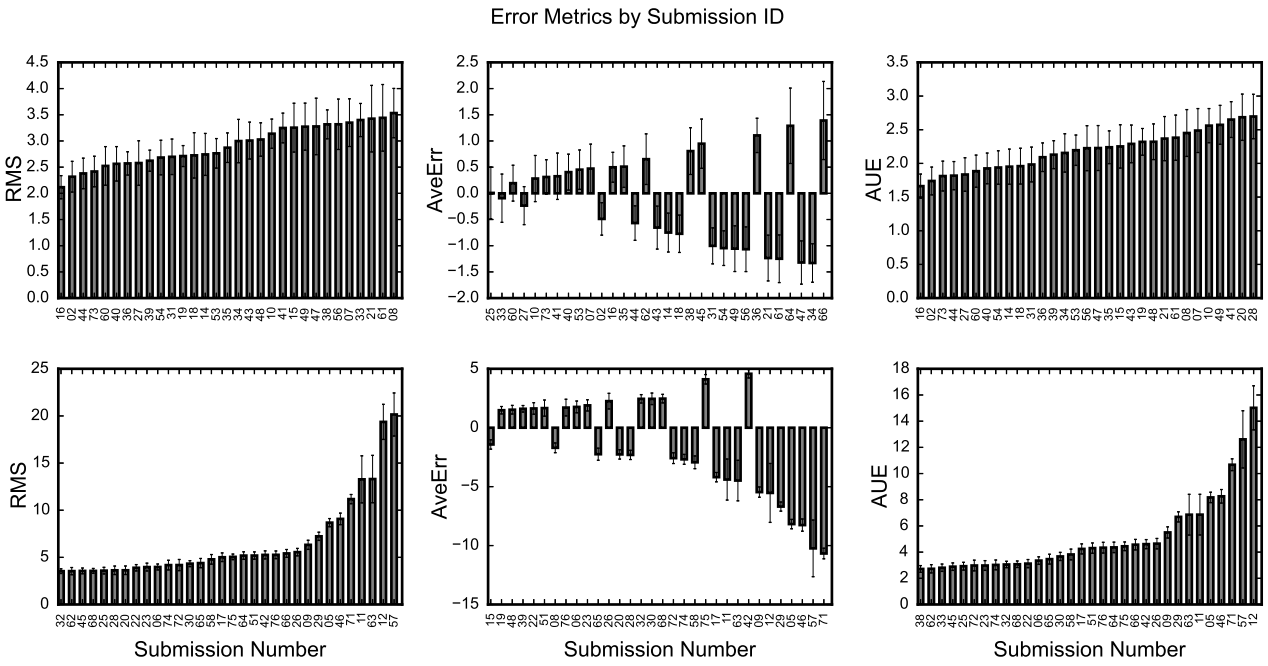


Fig. 3

estimated their model uncertainty. All other submissions have an error slope below one, indicating a significant underestimation of the model uncertainty.

5.1 Top performing submissions

In order to determine which submissions performed the best, we will group error metrics into two categories. How well the predictions directly agree with experiment (RMSE and AUE) and then how well correlated the predictions are with experiment (tau and R). Unlike past SAMPL challenges, there does appear to be one submission which performs the best by all of these metrics, submission 16. There are two additional submissions which performed in the top by these four metrics, 14 and 36. Each of these submission results compared to experiment are shown in figure 5. For submission 16, Andreas Klamt from COSMOlogic used Conductor-Like-Screening Model for Real Solvents (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, zwitter ions, and tautomers were performed in COSMO-RS for relevant molecules. For submission 14, Frank Pickard from the National Institute of Health used a quantum mechanical calculation in implicit solvent For submission 36, Christopher Fennell from Oklahoma State University estimated

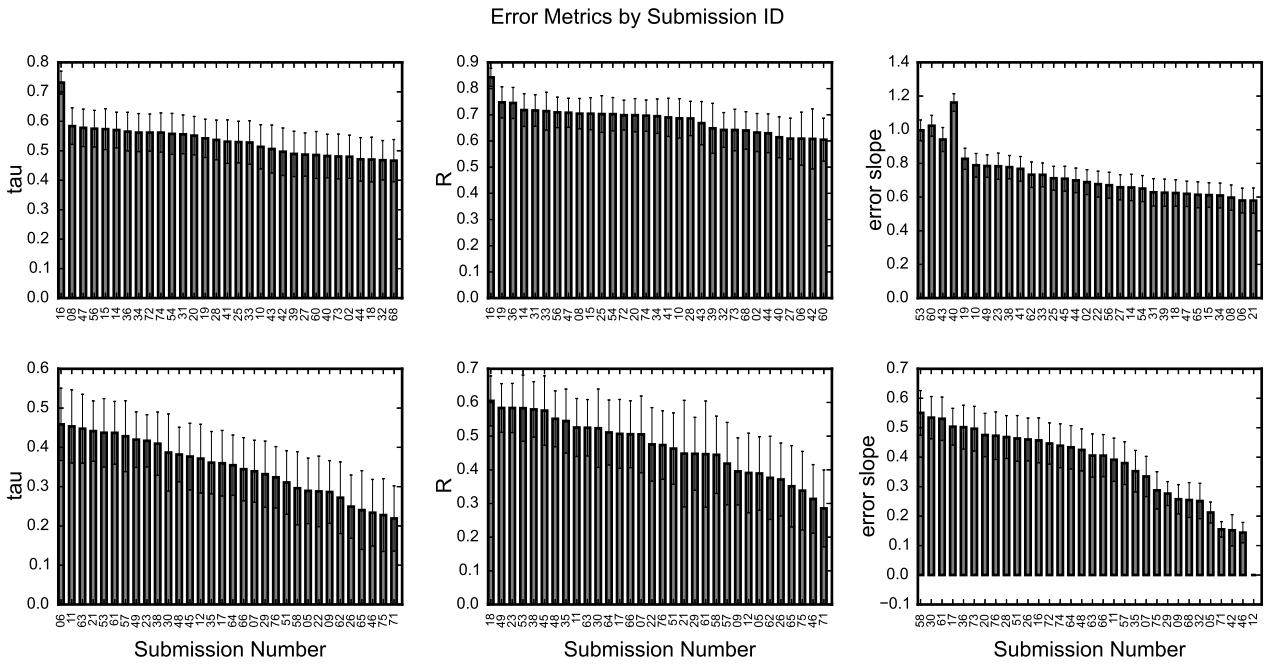


Fig. 4

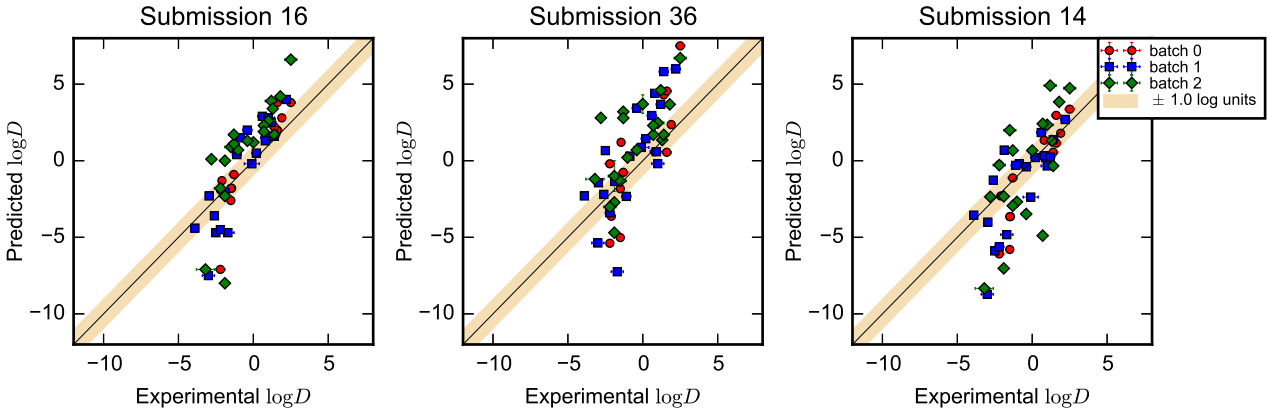


Fig. 5

log D as a partition coefficient, calculated from the difference in alchemical solvation free 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. Fur-

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

ther details for each of these submissions can be found in this issue so only a brief explanation of each method was provided here.

5.2 'other methods'

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 a $\log D = 0$. We performed all possible error analyses discussed above on this pretend data set as a point of comparison We calculated RMSE, AUE, and Ave. Err. for this pretend data set as a point of comparison (Table 5.2). The null hypothesis would have been within the top three submissions for both RMSE and AUE. While a null hypothesis has no actual predictive power, it is an indicator that improvements must be made in our other methods.

There are many structure based and empirically trained prediction methods for octanol/water partition coefficients. We used OEXlogP from Openeye ($XlogP_{oct}$) to represent 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 [17], $XlogP_{corr}$ 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, instead, this indicates a need for improvement for the more physical methods.

5.3 Mobley group prediction results

We submitted a set of blind predictions (39) to the challenge. Solvation free energies were calculated using GROMACS with GAFF and AM1-BCC charges. The initial set of predictions were partition coefficients, determined from the difference in solvation free energies without correcting for variation in tautomers. 39 was within the top 15 submissions for all error metrics.

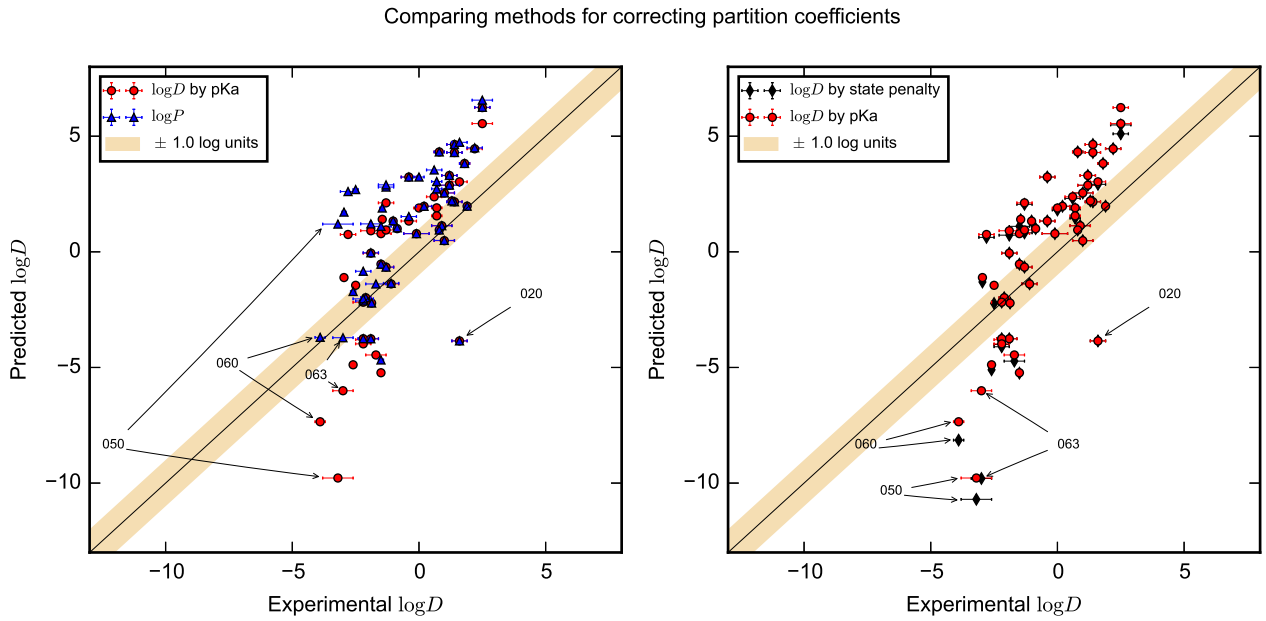


Fig. 6 Plots showing our predictions compared to experiment. a) submission 39 to SAMPL5, with no tautomer correction. b) distribution coefficient corrected from calculated partition coefficient based on pKas. c) distribution coefficient correct from calculated partition coefficient with state penalties

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 correcting for protonation states would have affected the our initial 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 equation 4 for acidic solutes and equation 3 for basic. We assumed only one change in protonation state occurred so only one pK_a was used either the most acidic or basic. This does not account for zwitterions or other neutral tautomers. This 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 concentration in cyclohexane.

For the next set of corrections, we used Schrödinger's Ligprep tool to calculate a state penalty, which gives the relative population of tautomers in water at the given pH 7.4. The state penalty was used to correct the concentration in

the aqueous layer, according to equation 5. State penalties 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 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 also affect the concentration in cyclohexane as well. Outliers and Molecules with 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 during 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 we did not correct for.

5.4 Examining individual molecules

With only 53 molecules, it is difficult to find statistically significant trends; 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 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. 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 a lack of accurate or proper attention given to the affect 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 the reason some of these molecules may have been difficult to accurately predict a distribution coefficient.

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

	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 Simulations with different tautomers...

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.

We reran these tautomers to calculate solvation free energies, used state penalty

Generally hard to tell if its tautomer enumeration that isn't good or the solvation free energies

5.5 Considering how solvent interactions could possibly affect results

6 Conclusions

Overall, range of methods and performance

Compare to dGhydration in past SAMPL challenges? using average errors, possibly what methods/FF are top ranked?

Tautomer and/or pKa predictions are going to be an important part of improving these

We, as a community, need to improve error estimation, both how we do and how we evaluate it...

logP/logD seem to be good options for future blind challenges

Acknowledgements John Chodera and Bas MSKCC Andreas Klamt Chris Fennell Samuel Genheden Frank Pickard Michael Shirts - reference calculation and input format conversion Schrödinger people D3R team people who set up the automated submission system 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...

6.1 Available in supporting info

things provided to participants all scripts used for error analysis all participant files? Can we include the anonymous one? 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

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