

Accuracy of macroscopic and microscopic pK_a predictions of small molecules evaluated by the SAMPL6 blind prediction challenge

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Abstract

Complete abstract.

- number of submissions [1]
- summary of analysis
- difficulties observed

0.1 Keywords

SAMPL · blind prediction challenge · acid dissociation constant · pK_a · small molecule · macroscopic pK_a · microscopic pK_a · macroscopic protonation state · microscopic protonation state

0.2 Abbreviations

SAMPL Statistical Assessment of the Modeling of Proteins and Ligands

pK_a $-\log_{10}$ acid dissociation equilibrium constant

SEM Standard error of the mean

RMSE Root mean squared error

MAE Mean absolute error

τ Kendall's rank correlation coefficient (Tau)

R² Coefficient of determination (R-Squared)

1 Introduction

Complete introduction section: - Importance of small molecule pKa prediction for pharmaceutical efforts. - Definition of pKa - Acid dissociation equilibrium constant - Add pKa equation - Add free energy of protonation state equation - Definition of microscopic and macroscopic pKas - Introduce linear protonation state free energy diagram [Cite Gunner et al 2019 paper] FIGURE: linear plot of free energy vs pH

Importance of small molecule pKa prediction for pharmaceutical efforts.

Explain why we are doing a pKa challenge and connect to past and previous challenges

SAMPL (Statistical Assessment of the Modeling of Proteins and Ligands). About SAMPL challenges: Collectively, these challenges have assessed the effects of force field accuracy, solvation models, pKa and tautomer predictions.

During the SAMPL5 challenge, log D predictions experienced difficulties predicting log D values accurately, unless protonation states and tautomers were taken into account.

For this iteration of the SAMPL challenge, we have taken one step back and isolated just the problem of predicting solvent protonation states.

This is the first time a blind pKa prediction challenge has been fielded as part of SAMPL. In this first iteration of the challenge, we aimed to assess the performance of current pKa prediction methods and isolate potential causes of inaccurate pKa estimates, with the aim of determining how pKa prediction inaccuracies might impact predicted affinities for drug-like molecules. For example, for both logD and binding affinity predictions, any error in predicting the free energy of accessing a minor protonation state in solution that becomes dominant in the complex will directly add to the error in the predicted transfer or binding free energy.

Challenge goal: determining how pKa prediction inaccuracies might impact predicted affinities for drug-like molecules. For example, for both logD and binding affinity predictions, any error in predicting the free energy of accessing a minor protonation state in solution that becomes dominant in the complex will directly add to the error in the predicted transfer or binding free energy.

Reason for blind pKa challenge: - Impact on binding affinity predictions - Impact on logD predictions (SAMPL6) - Drug-like molecules are especially challenging.

Protonation state effects were a dominant accuracy-limiting factor for logD from SAMPL5, and should also be accuracy-limiting in binding free energy predictions. Errors in pKa predictions can cause modeling the wrong charge, protonation and tautomerization states which affect hydrogen bonding opportunities and overall dipole moment of the ligand.

Explain the physics of the predicted property

EQUATION: pKa equation

EQUATION: free energy of protonation state equation

Introducing linear protonation state free energy diagram

FIGURE: linear plot of free energy vs pH

FIGURE: a diagram illustrating the ways in which the pKa errors can influence prediction errors for binding affinities

Overview of kinds of pKa prediction methods available (ML, QM, empirical methods ...)

Explain challenge design.

Experimental macroscopic pKa values were measured using a UV-metric assay performed using a Sirius T3 [cite exp. paper] supported by Merck, MRL, Rahway NJ.

Communicate concepts behind challenge design and why we made specific choices: Explain why we have types I, II, III Explain why we preenumerated microstates

Participants had the option to submit predictions in one of 3 categories: Microscopic pKa values (type I), microscopic state populations (type II), or macroscopic pKa values (type III).

The comparison between macroscopic and microscopic pKa values is not always a straightforward one.

Overview of available pKa prediction methods and methods that participated in SAMPL6. [Reminder to cite all papers here.]

Explain future direction for this challenge

Challenge path: predict pKas, give people pKas to predict logDs on same molecules, then predict for new set of compounds logDs without provided pKas.

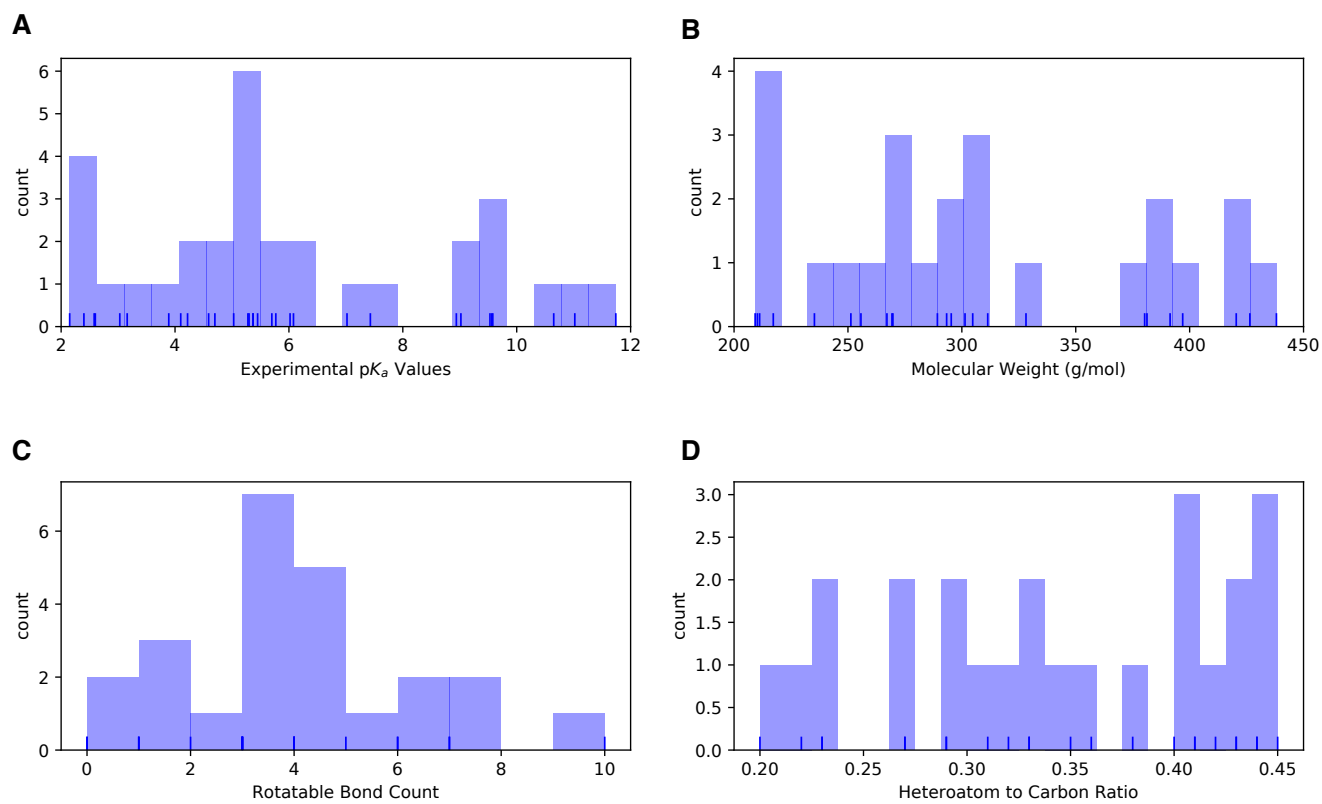


Figure 1. Distribution of molecular properties of 24 compounds in SAMPL6 pK_a Challenge. **A** Histogram of spectrophotometric pK_a measurements collected with Sirius T3 [1]. Overlaid carpet plot indicates the actual values. Five compounds have multiple measured pK_a s in the range of 2-12. **B** Histogram of molecular weights of compounds in SAMPL6 set. Molecular weights were calculated by neglecting counter ions. **C** Histogram of the number of non-terminal rotatable bonds in each molecule. **D** The histogram of the ratio of heteroatom (non-carbon heavy atom) count to the number of carbon atoms.

Explain potential benefits of these challenge

Improving computational methods...

1.1 Motivation for a blind pKa challenge

why we are doing a pKa challenge and connect to past and previous challenge?

SAMPL (Statistical Assessment of the Modeling of Proteins and Ligands). About SAMPL challenges: Collectively, these challenges have assessed the effects of force field accuracy, solvation models, pKa and tautomer predictions.

During the SAMPL5 challenge, log D predictions experienced difficulties predicting log D values accurately, unless protonation states and tautomers were taken into account.

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This is the first time a blind pKa prediction challenge has been fielded as part of SAMPL. In this first iteration of the challenge, we aimed to assess the performance of current pKa prediction methods and isolate potential causes of inaccurate pKa estimates, with the aim of determining how pKa prediction inaccuracies might impact predicted affinities for drug-like molecules. For example, for both logD and binding affinity predictions, any error in predicting the free energy of accessing a minor protonation state in solution that becomes dominant in the complex will directly add to the error in the predicted transfer or binding free energy.

Challenge goal: determining how pKa prediction inaccuracies might impact predicted affinities for drug-like molecules. For example, for both logD and binding affinity predictions, any error in predicting the free energy of accessing a minor protonation state in solution that becomes dominant in the complex will directly add to the error in the predicted transfer or binding free energy.

Reason for blind pKa challenge: 1. Impact on binding affinity predictions 2. Impact on logD predictions (SAMPL6) 3. Drug-like molecules are especially challenging.

Future challenge direction Challenge path: predict pKas, give people pKas to predict logDs on same molecules, then predict for new set of compounds logDs without provided pKas. Potential benefits of these challenges: 1. Improving computational methods 2. Detecting hidden contributors to error

1.2 Approaches to predict pKas

Overview of kinds of pKa prediction methods available (ML, QM, empirical methods ...)

2 Methods

2.1 Structure and logistics of the SAMPL6 pKa prediction challenge

Describe the structure of SAMPL6 pKa challenge

Experimental macroscopic pKa values were measured using a UV-metric assay performed using a Sirius T3 [cite exp. paper] supported by Merck, MRL, Rahway NJ.

Communicate concepts behind challenge design and why we made specific choices: 1. Explain why we have types I, II, III 2. Explain why we pre-enumerated microstates

Participants had the option to submit predictions in one of 3 categories: Microscopic pKa values (type I), microscopic state populations (type II), or macroscopic pKa values (type III).

The comparison between macroscopic and microscopic pKa values is not always a straightforward one.

- When instructions and input files were made available

- Challenge dates

- Input files

- What to predict? Three type of submissions.

- Multiple submissions allowed

- Predicting the pKa values of the whole set wasn't a requirement.

- 2nd D3R/SAMPL Workshop took place in La Jolla, San Diego on Feb 22-23, 2018.

Referece Figure ?? Drug-like molecules are often larger and more complex than the ones used in this study.

124 2.2 Enumeration of requested prediction microscopic protonation states

125 1. OpenEye (filter out resonance structures), Epik

126 2. Participant supplied structures

127 Microstate pairs: Only +/-1 charge change transitions are allowed. List of allowed transitions. +2 transitions are not consid-
128 ered.

129 2.3 Evaluation approaches

130 2.3.1 Statistical metrics for submission performance

131 - Root mean squared error (RMSE)

132 - Mean absolute error (MAE)

133 - Mean Error (ME)

134 - Square of Pearson Correlation Coefficient (R^2)

135 - Slope of prediction vs. experimental value linear fit

136 Uncertainty in each performance statistic was calculated by bootstrapping (10,000) to estimate 95% confidence intervals.

137 2.3.2 Matching algorithms for pairing predicted and experimental pKas

138 Explain why it is necessary due to lacking structural information. Cite recommendations from article such as preserving sequence.
139 Experimental data doesn't inform protonation site and overall charge of species. Experimental data doesn't capture the whole
140 picture. We don't know charge and we don't know tautomers. We don't know the charge state of macrostates, this causes a
141 matching problem

142 Explain Hungarian method for matching experimental and predicted pKas

143 Explain Closest method for matching experimental and predicted pKas

144 Explain microstate based matching.

145 2.4 Reference calculations

146 Schrodinger Epik Schrodinger Jaguar Chemicalize MoKa

147 3 Results and Discussion

148 A paragraph to explain the submission methods. Define method categories: DL, LFER, QSPR/ML, QM, QM+LEC, and QM+MM, Blind pre-
149 dictions, Reference calculations, Null model (pKa prospector lookup)

149 Submissions spanning different method categories were made to the SAMPL6 pK_a Challenge: database lookup (DL), linear
150 free energy relationship (LFER), quantitative structure property relationship (QSPR), machine learning (ML), quantum mechanics
151 (QM) models with and without linear empirical correction (LEC), and combined quantum mechanics and molecular mechanics
152 (QM+MM). Unique submission IDs were assigned to each submission. Table 1 matches method names with submission IDs.
153 Unique IDs were also assigned when multiple submissions exists for different submission types of the same method such as
154 microscopic pK_a(type I) and macroscopic pK_a (type III).

155 3.1 Analysis of macroscopic pK_a predictions (Type III)

156 MI: Methods are indicated by submission IDs.

157 Refer to SI TABLE: Error statistics for all participants. Refer to SI FIGURE: Error distribution ridge plots for each method
158 (exp-pred macroscopic pK_a). Which methods tend to overestimate and which methods tend to underestimate?

159 MI: SI TABLE: Error statistics for all participants

160 Describe number of missing and extra pKa for each method. Report in total for all molecules how many predicted pKas are
161 there and how many experimental pKas. Refer to FIGURE: missing and extra pKa counts.

162 MI: SI TABLE: Missing and extra pKa counts

163 Describe overall performance comparison of different methods, grouped by methods class.

164 Explain rationale behind how we analyze the data and determine success/failure

Table 1. Submission IDs, names, category, and type for all the pK_a prediction sets. Reference calculations are labeled as *nb###*. The method name column lists the names provided by each participant in the submission file. The “type” column indicates if submission was or a post-deadline reference calculation, denoted by “Blind” or “Reference” respectively. The table is not ordered by performance.

Method Category	Method	Microscopic pK_a (Type I) Submission ID	Macroscopic pK_a (Type III) Submission ID	Submission Type	Ref.
DL	Substructure matches to experimental data in pKa OpenEye pKa Prospector Database v1.0		<i>5nm4j</i>	Null	[2]
DL	OpenEye pKa-Prospector 1.0.0.3 with Analog Search ion identification algorithm		<i>pwn3m</i>	Null	[2]
LFER	ACD/pKa GALAS (ACD/Percepta Kernel v1.6)	<i>v8qph</i>	<i>37xm8</i>	Blind	[3]
LFER	ACD/pKa Classic (ACD/Percepta Kernel, v1.6)		<i>xmyhm</i>	Blind	[4]
LFER	Epik Scan (Schrodinger v2017-4)		<i>nb007</i>	Reference	[5]
LFER	Epik Microscopic (Schrodinger v2017-4)	<i>nb008</i>	<i>nb010</i>	Reference	[5]
QSPR/ML	OpenEye Gaussian Process	<i>6tvf8</i>	<i>hytjn</i>	Blind	[6]
QSPR/ML	OpenEye Gaussian Process Resampled		<i>q3pfp</i>	Blind	[6]
QSPR/ML	S+pKa (ADMET Predictor v8.5, Simulations Plus)	<i>hdiyq</i>	<i>gyuhx</i>	Blind	[7]
QSPR/ML	Chemicalize v18.23 (ChemAxon MarvinSketch v18.23)		<i>nb015</i>	Reference	[8]
QSPR/ML	MoKa v3.1.3	<i>nb016</i>	<i>nb017</i>	Reference	[9, 10]
QM	Adiabatic scheme with single point correction: SMD/M06-2X//6-311++G(d,p)//M06-2X/6-31+G(d) for bases and SMD/M06-2X//6-311++G(d,p)//M06-2X/6-31G(d) for acids + thermal corrections	<i>ko8yx</i>	<i>ryzue</i>	Blind	[11]
QM	Direct scheme with single point correction: SMD/M06-2X//6-311++G(d,p)//M06-2X/6-31+G(d) for bases and SMD/M06-2X//6-311++G(d,p)//M06-2X/6-31G(d) for acids + thermal corrections	<i>w4z0e</i>	<i>xikp8</i>	Blind	[11]
QM	Adiabatic scheme: thermodynamic cycle that uses gas phase optimized structures for gas phase free energy and solution phase geometries for solvent phase free energy. SMD/M06-2X/6-31+G(d) for bases and SMD/M06-2X/6-31G(d) for acids + thermal corrections	<i>wcvnu</i>	<i>5byn6</i>	Blind	[11]
QM	Vertical scheme: thermodynamic cycle that uses only gas phase optimized structures to compute gas phase and solvation free energy. SMD/M06-2X/6-31+G(d) for bases and SMD/M06-2X/6-31G(d) for acids + Thermal corrections	<i>arcko</i>	<i>w4iyd</i>	Blind	[11]
QM	Direct scheme: solution phase free energy is determined by solution phase geometries without thermodynamic cycle SMD/M06-2X/6-31+G(d) for bases and SMD/M06-2X/6-31G(d) for acids + thermal corrections	<i>wexjs</i>	<i>y75vj</i>	Blind	[11]
QM + LEC	Jaguar (Schrodinger v2017-4)	<i>nb011</i>	<i>nb013</i>	Reference	[12]
QM + LEC	CPCM/B3LYP/6-311+G(d,p) and global fitting	<i>y4wws</i>	<i>35bdm</i>	Blind	[13]
QM + LEC	CPCM/B3LYP/6-311+G(d,p) and separate fitting for neutral to negative and for positive to neutral transformations	<i>qsicn</i>	<i>p0jba</i>	Blind	[13]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P3NI-q-noThiols-2par	<i>kxztz</i>	<i>ds62k</i>	Blind	[14]
QM + LEC	EC-RISM/MP2/cc-pVTZ-P2-q-noThiols-2par	<i>ftc8w</i>	<i>2ii2g</i>	Blind	[14]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P2-phi-all-2par	<i>ktpj5</i>	<i>nb001</i>	Blind*	[14]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P2-phi-noThiols-2par	<i>wuuvv</i>	<i>nb002</i>	Blind*	[14]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P3NI-phi-all-2par	<i>2umai</i>	<i>nb003</i>	Blind*	[14]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P3NI-phi-noThiols-2par	<i>cm2yq</i>	<i>nb004</i>	Blind*	[14]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P2-phi-all-1par	<i>z7fhp</i>	<i>nb005</i>	Blind*	[14]
QM + LEC	EC-RISM/MP2/6-311+G(d,p)-P3NI-phi-all-1par	<i>8toyp</i>	<i>nb006</i>	Blind*	[14]
QM + LEC	EC-RISM/MP2/cc-pVTZ-P2-phi-noThiols-2par	<i>epvmk</i>	<i>tjd0</i>	Blind	[14]
QM + LEC	EC-RISM/MP2/cc-pVTZ-P2-phi-all-2par	<i>xnoe0</i>	<i>mkhqa</i>	Blind	[14]
QM + LEC	EC-RISM/MP2/cc-pVTZ-P3NI-phi-noThiols-2par	<i>4o0ia</i>	<i>mpwiy</i>	Blind	[14]
QM + LEC	EC-RISM/B3LYP/6-311+G(d,p)-P3NI-q-noThiols-2par	<i>nxaaw</i>	<i>ad5pu</i>	Blind	[14]
QM + LEC	EC-RISM/B3LYP/6-311+G(d,p)-P3NI-phi-noThiols-2par	<i>0xi4b</i>	<i>f0gew</i>	Blind	[14]
QM + LEC	EC-RISM/B3LYP/6-311+G(d,p)-P2-phi-noThiols-2par	<i>cywyk</i>	<i>np6b4</i>	Blind	[14]
QM + LEC	PCM/B3LYP/6-311+G(d,p)	<i>gdqeg</i>	<i>yc70m</i>	Blind	[14]
QM + LEC	COSMOtherm_FINE17 (COSMOtherm C30_1701, BP/TZVPD/FINE//BP/TZVP/COSMO)	<i>t8ewk</i>	<i>0hxtm</i>	Blind	[15, 16]
QM + LEC	DSD-BLYP-D3(BJ)/def2-TZVPD//PBEh-3c[DCOSMO-RS] + RRHO(GFN-xTB[GBSA]) + Gsolv(COSMO-RS[TZVPD]) and linear fit		<i>xvxzd</i>	Blind	[17]
QM + LEC	ReSCoSS conformations // DSD-BLYP-D3 reranking // COSMOtherm pKa: DSD-BLYP-D3(BJ)/def2-TZVPD// PBE-D3(BJ)/def2-TZVP/COSMO + RRHO(GFN-xTB + GBSA-water) + Gsolv[COSMO-RS(FINE17/TZVPD)] level and COSMOtherm pKa applied at the single conformer pair level (COSMOthermX17.0.5 release and BP-TZVPD-FINE-C30-1701 parameterization)	<i>eyetm</i>	<i>8xt50</i>	Blind	[17]
QM + LEC	ReSCoSS conformations // COSMOtherm pKa: DSD-BLYP-D3(BJ)/def2-TZVPD// PBE-D3(BJ)/def2-TZVP/COSMO + RRHO(GFN-xTB + GBSA-water) + Gsolv[COSMO-RS(FINE17/TZVPD)] level and COSMOtherm pKa was applied directly on the resulting conformer sets with at least 5% Boltzmann weights for each microspecies (COSMOthermX17.0.5 release and BP-TZVPD-FINE-C30-1701 parameterization)	<i>ccpmw</i>	<i>yqkga</i>	Blind	[17]
QM + MM	M06-2X/6-31G*(for bases) or 6-31+G*(for acids) for gas phase, solvation free energy using TI with explicit solvent and GAFF, solvation free energy of proton -265.6 kcal/mol	<i>0wfzo</i>		Blind	[18]
QM + MM	M06-2X/6-31G*(for bases) or 6-31+G*(for acids) for gas phase, solvation free energy using TI with explicit solvent and GAFF, solvation free energy of proton -271.88 kcal/mol	<i>z3btx</i>		Blind	
QM + MM	M06-2X/6-31G*(for bases) or 6-31+G*(for acids) + thermal state correction for gas phase, solvation free energy using TI with explicit solvent and GAFF, solvation free energy of proton -265.6 kcal/mol	<i>758j8</i>		Blind	
QM + MM	M06-2X/6-31G*(for bases) or 6-31+G*(for acids) + thermal state correction for gas phase, solvation free energy using TI with explicit solvent and GAFF, solvation free energy of proton -271.88 kcal/mol	<i>hgn83</i>		Blind	

* Microscopic pK_a submissions were blind, however, participant requested a correction after blind submission deadline for macroscopic pK_a submissions. Therefore, these were assigned submission IDs in the form of *nb###*.

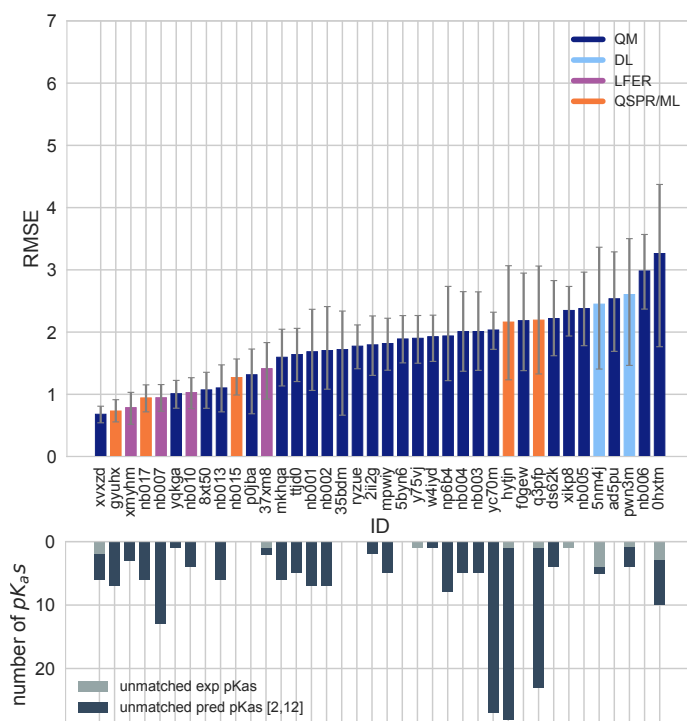


Figure 2. RMSE and unmatched pK_a counts vs. submission ID plots for macroscopic pK_a predictions based on Hungarian matching. Methods are indicated by submission IDs. RMSE is shown with error bars denoting 95% confidence intervals obtained by bootstrapping over challenge molecules. Lower bar plots show the number of unmatched experimental pK_a s (light grey, missing predictions) and the number of unmatched pK_a predictions (dark grey, extra predictions) for each method between pH 2 and 12. Submission IDs are summarized in Table 1. Submission IDs of the form *nb###* refer to non-blinded reference methods computed after the blind challenge submission deadline. All others refer to blind, prospective predictions. Submissions are colored by their method categories. Light blue colored database look up methods are utilized as the null prediction method.

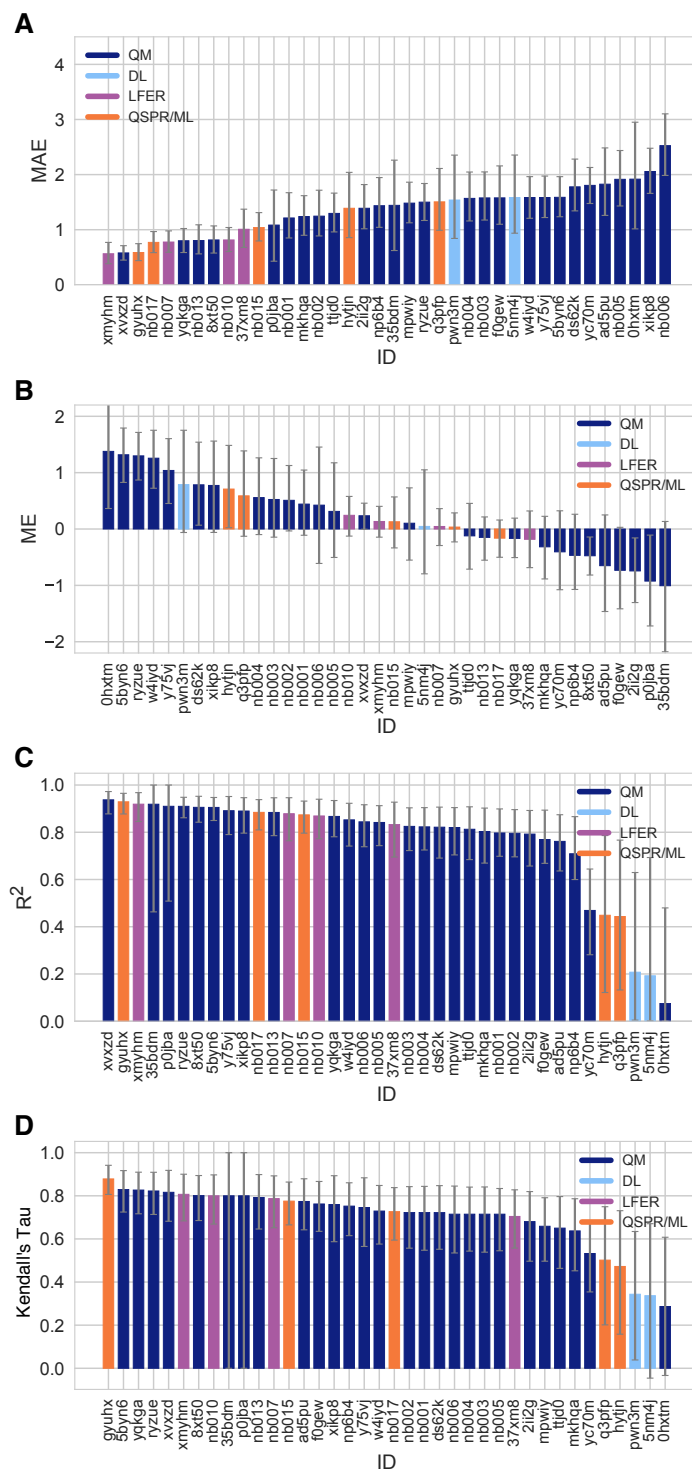


Figure 3. Additional performance statistics for macroscopic pK_a predictions based on Hungarian matching. Methods are indicated by submission IDs. Mean absolute error (MAE), mean error (ME), Pearson's R^2 , and Kendall's Rank Correlation Coefficient Tau (τ) are shown, with error bars denoting 95% confidence intervals obtained by bootstrapping over challenge molecules. Refer to Table 1 for submission IDs and method names. Submissions are colored by their method categories. Light blue colored database look up methods are utilized as the null prediction method.

Performance comparison of different methods, grouped by methods class

Method comparison based on statistical metrics. Explain the numerical matching methods used. Explain rationale behind how we analyze the data and determine success/failure. Method comparison according to different statistics: RMSE, MAE, ME, R2, m, Kendall's tau.

3.1.1 Consistently well performing methods for macroscopic pK_a prediction

Table 2. Four consistently well-performing prediction methods for macroscopic pK_a prediction based on consistent ranking within the Top 10 according to various statistical metrics. Submissions were ranked according to RMSE, MAE, R^2 , and τ . Consistently well-performing methods were selected as the ones that rank in the Top 10 in each of these statistical metrics. These methods also have less than 2 unmatched experimental pK_a s and less than 7 unmatched predicted pK_a s according to Hungarian matching. Performance statistics are provided as mean and 95% confidence intervals.

Submission ID	Method Name	RMSE	MAE	R^2	Kendall's Tau (τ)	Unmatched Exp. pK_a Count	Unmatched Pred. pK_a Count [2,12]
<i>xvxzd</i>	Full quantum chemical calculation of free energies and fit to experimental pK_a	0.68 [0.54, 0.81]	0.58 [0.45, 0.71]	0.94 [0.88, 0.97]	0.82 [0.68, 0.92]	2	4
<i>gyuhx</i>	S+pKa	0.73 [0.55, 0.91]	0.59 [0.44, 0.74]	0.93 [0.88, 0.96]	0.88 [0.8, 0.94]	0	7
<i>xmyhm</i>	ACD/pKa Classic	0.79 [0.52, 1.03]	0.56 [0.38, 0.77]	0.92 [0.85, 0.97]	0.81 [0.68, 0.9]	0	3
<i>8xt50</i>	ReScOSS conformations // DSD-BLYP-D3 reranking // COSMOtherm pK_a	1.07 [0.78, 1.36]	0.81 [0.58, 1.07]	0.91 [0.84, 0.95]	0.80 [0.68, 0.89]	0	0

Check if top few performing methods are consistent between error metrics.

3.1.2 Which chemicals are harder to predict?

For physical prediction methods sulfur containing heterocycles, amide next to aromatic heterocycles, compounds with iodo and bromo domains have lower pK_a prediction accuracy.

Prediction performance of individual molecules

Which chemical structures make pK_a predictions more difficult?

SAMPL6 pK_a set consisted of only 24 small molecules which limits our ability to do statistical analysis to determine which chemical substructures contribute to greater errors in pK_a predictions.

Illustration/explanation of effects where microscopic pK_a s and macroscopic pK_a s can differ

Are there any correlations between molecular descriptors and pK_a errors?

What can we learn from failures? Which physical effects are driving failures?

MI: FIGURE: Molecular MAE comparison across methods.

Does molecular descriptors explain errors/performance? We looked for correlation with descriptors, and potential explanation for errors. Keep spurious correlations in mind if we have many descriptors. No correlation observed. Reference the SI Figure of correlations.

Comparison of errors/performance against molecular descriptors. Look for correlation with descriptors, and potential explanation for errors. Keep spurious correlations in mind if we have many descriptors.

MI: Figure SI: correlation between prediction error and molecular descriptors

Are pK_a predictions better in middle region? No correlation between pK_a value and error was seen. Reference the SI Figure.

MI: Figure: Ridge plots of Delta pK_a error to identify compounds that were frequently mispredicted

Compare ME of molecules across methods. Are there molecules often overestimated or underestimated?

No correlation of macroscopic pK_a number to the errors? But we have low representation of multiprotic compounds

3.1.3 Comparing microscopic pK_a predictions directly to macroscopic experimental pK_a values leads to underestimation of errors

Discussion of matching experimental and predicted values

Difficulty of assessing predicted pK_a s using experimental data: matching problem

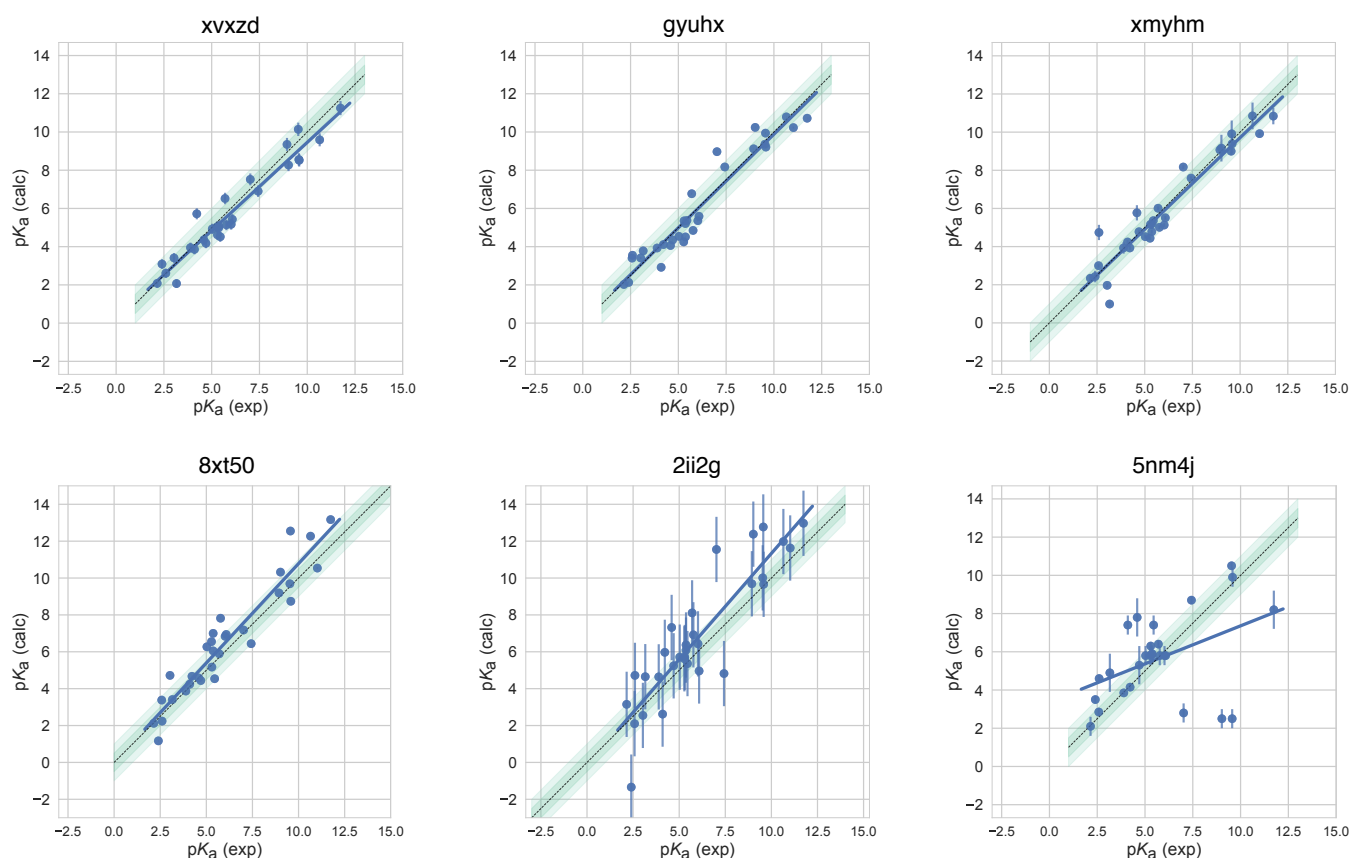


Figure 4. Predicted vs. experimental value correlation plots of 4 consistently well-performing methods, a representative method with average performance (*2ii2g*), and the null method (*5nm4j*). Dark and light green shaded areas indicate 0.5 and 1.0 units of error. Error bars indicate standard error of the mean of predicted and experimental values. Experimental pK_a SEM values are too small to be seen under the data points. EC-RISM/MP2/cc-pVTZ-P2-q-noThiols-2par method (*2ii2g*) was selected as the representative method with average performance because it is the method with the highest RMSE below the median.

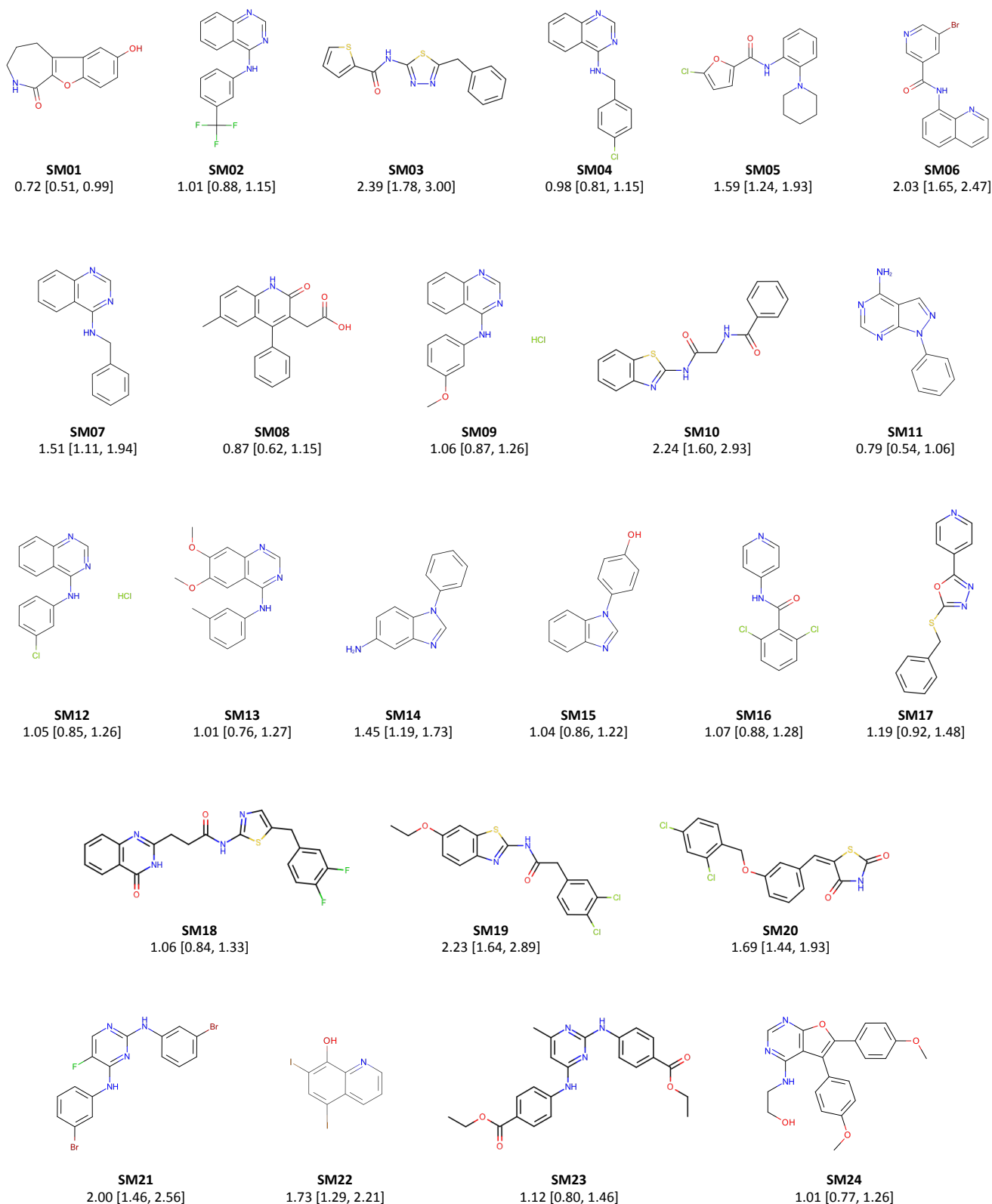


Figure 5. Molecules of SAMPL6 Challenge with MAE calculated for all macroscopic pK_a predictions. MAE calculated considering all prediction methods indicate which molecules had the lowest prediction accuracy in SAMPL6 Challenge. MAE values calculated for each molecule include all the matched pK_a values, which could be more than one per method for multiprotic molecules (SM06, SM14, SM15, SM16, SM18, SM22). Hungarian matching algorithm was employed for pairing experimental and predicted pK_a values. MAE values are reported with 95% confidence intervals.

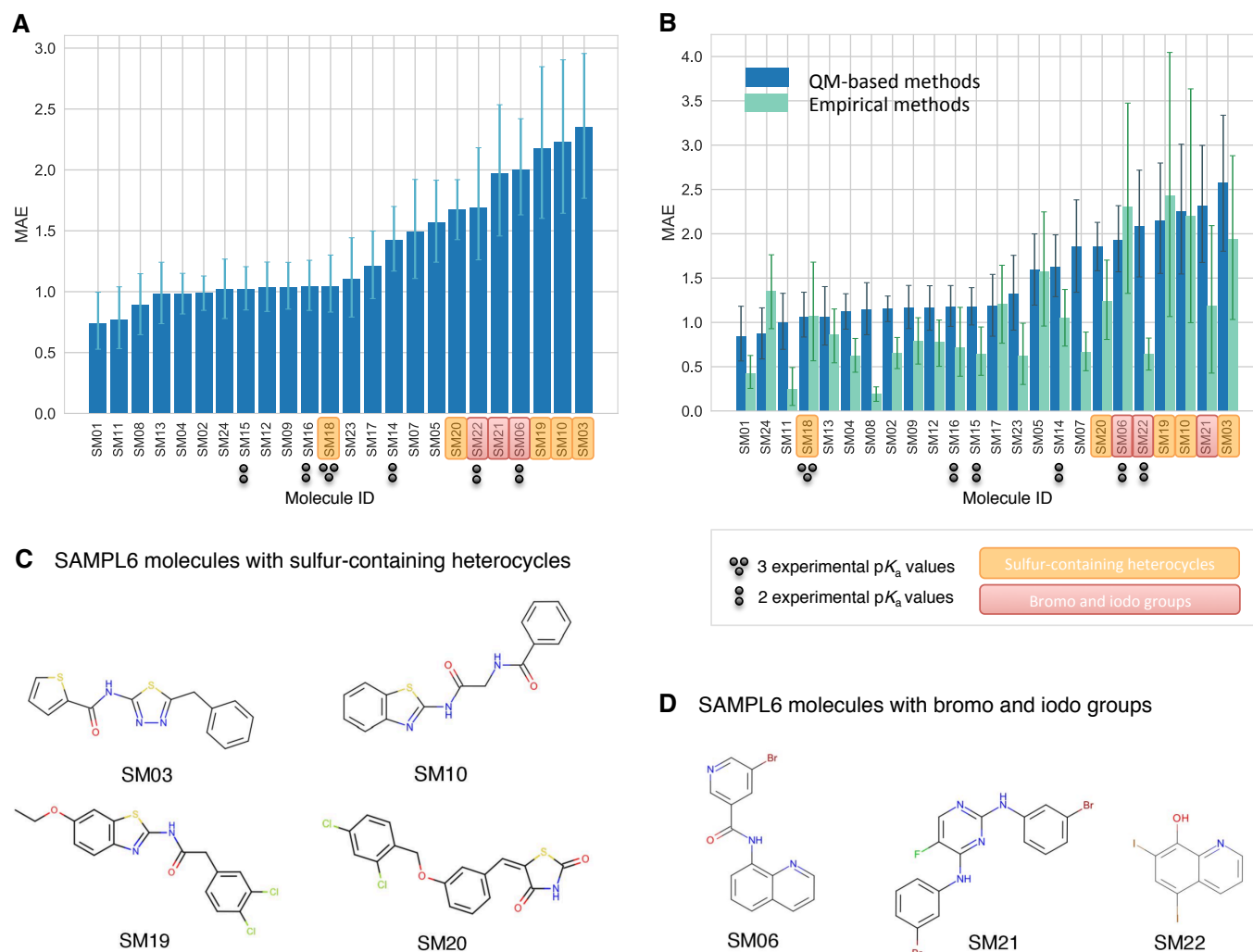


Figure 6. Average prediction accuracy calculated over all prediction methods was lower for molecules with sulfur-containing heterocycles, bromo, and iodo groups. (A) MAE calculated for each molecule as an average of all methods. **(B)** MAE of each molecule broken out by method category. QM-based methods (blue) include QM predictions with or without linear empirical correction. Empirical methods (green) include QSAR, ML, DL, and LFER approaches. **(C)** Depiction of SAMPL6 molecules with sulfur-containing heterocycles. **(D)** Depiction of SAMPL6 molecules with iodo and bromo groups.

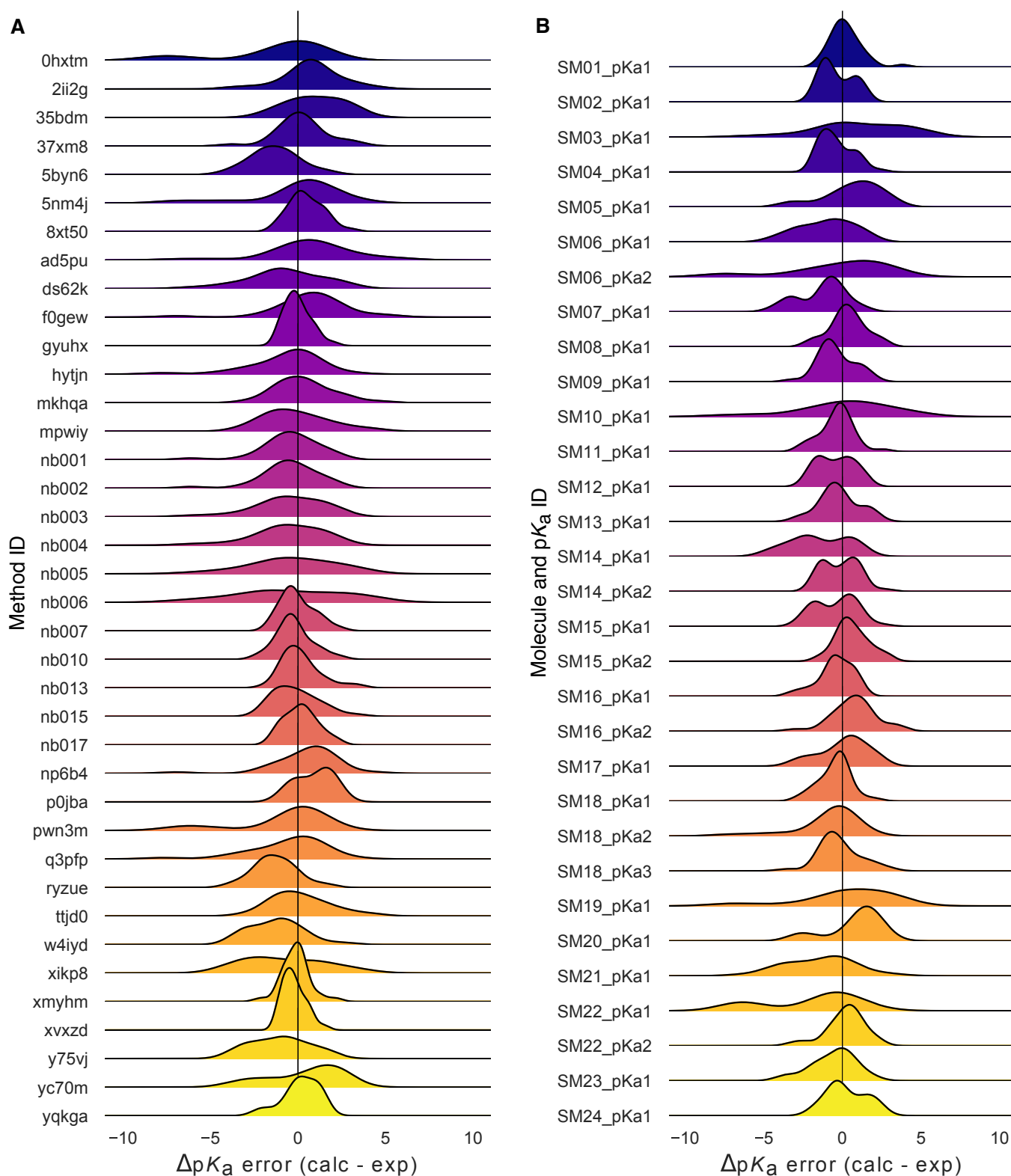


Figure 7. Prediction error distribution plots show how prediction accuracy varies across methods and individual molecules. (A) pK_a prediction error distribution for each submission for all molecules according to Hungarian matching. **(B)** Error distribution for each SAMPL6 molecule for all prediction methods according to Hungarian matching. For multiprotic molecules, pK_a ID numbers (pKa1, pKa2, and pKa3) were assigned in the direction of increasing experimental pK_a value.

195 Explain rationale behind how we analyze the data and determine success/failure

196 Compare experimental data to microscopic pKa predictions, assuming experimental pKas are titrations of distinguishable
197 sides and therefore equal to microscopic pKas. Molecules with only 1 pKa or well separated multiple pKas (more than 3 pKa
198 units apart) SM14 and SM18 were excluded from this analysis, since their experimental pKa values don't satisfy these criteria.

199 Errors computed by microstate-based matching are larger compared to numerical matching algorithms. Microscopic pKa
200 analysis with numerical matching algorithms may mask errors due to higher number of guesses made.

201 MI: FIGURE Type I analysis, comparing analysis of 22 molecules (Hungarian vs Microstate matching)

202 3.2 Analysis of microscopic pK_a predictions using microstates determined by NMR (8 molecules)

203 MI: FIGURE: Assign experimental pKa to microscopic transitions observed by NMR.

204 Conclusions will only be about 4-aminoquinazoline series and benzimidazole (8 molecules, 10 pKas) Refer to SI figure of
205 dominant microstates.

206 Choosing molecules with right protonation state is important. Do people predict the correct sequence of dominant mi-
207 crostates? " Even if your pKa prediction is correct, protonation state prediction can be wrong." Analyze which state has lowest
208 free energy for each charge group (The sequence of "experimentally visible states")

209 3.2.1 Accuracy of predicted pKa values when microstate matching is used

210 Assessment of individual methods by each of our analysis methods

211 Performance comparison of different methods, grouped by methods class

212 MI: FIGURE: Ranking of microscopic pKa prediction error statistics for all participants (8 mol, microstate match).

213 MI: FIGURE: Violin plots of Delta pKa error to identify compounds that were frequently mispredicted (microstate match)

214 3.2.2 Dominant microstate prediction accuracy of methods

215 Calculate relative free energy of microstates to determine dominant microstate of each charge Compare predicted and experi-
216 mental dominant microstates and calculate accuracy of each method

217 MI: FIGURE: Dominant microstate accuracy vs method plot. Charges together and separate.

218 What percent of the time predictions capture the dominant protonation state correctly? Match by microstate and calculate
219 RMSE and MAE. If you know the microstates, can you predict the value of the pKa right?

220 Does top 3 methods predict the same dominant microstate sequence? How differently do different methods predict microscopic transi-
tions? (method vs method correlation plot to see if methods predict the same microstate pairs or not)

221 3.2.3 Which molecules caused lower dominant microstate prediction accuracy?

222 Which molecule has more errors in predicting the major microstates?

223 MI: FIGURE: Dominant microstate Accuracy vs Molecule ID plot, all charges and separate charges. Also think about plotting accuracy of
QM and empirical methods separately.

224 Comment on consensus prediction accuracy. Comparison of predicted microstates using consensus set of transitions of high accuracy
prediction methods

225 3.2.4 Demonstrate how numerical matching often masks the error

226 Match by Hungarian and calculate accuracy of microstate prediction overall. When matched by pKa value, do people come with
227 the same transition pairs?

228 MI: FIGURE: [accuracy-of-microstates-based-on-numeric-matching] For most methods the microstate pair of Hungarian predicted pKa
does not match experimentally determined microstate pair.

3.3 Analyzing microscopic pKa prediction from the perspective of thermodynamics

Explain linearity relative free energy of protonation states with respect to pH. Free energy perspective simplifies data capturing and analysis. Reference Marilyn's paper.

Thermodynamic cycle closure checking allows evaluation of microscopic pKas without experimental data. Checking for thermodynamic consistency

3.3.1 Cycle closure error

Marilyn observed very good cycle closure results and very bad one that are up to 10 kcal/mol

She suggesting checking the cycle with maximum cycle closure error for each method and reporting that for each method. An histogram of max cycle closure error will help us bin these results into 3 categories: 1. good agreement 2. moderate 3. severe

"We think thermodynamic cycles of protonation states need to be closed" Message: Methods need to be checked for cycle closure errors. There can be information there that can be used to correct pKa predictions. When cycles are not closed it may be used as an indicator of prediction uncertainty.

3.4 How would pKa errors affect protein-ligand binding affinity predictions?

How do accuracy limitations in small molecule pKa prediction translate into modeling errors in ligand affinity prediction?

MI: FIGURE: a diagram illustrating the ways in which the pKa errors can influence prediction errors for binding affinities (A) When minor aqueous protonation state binds (B) When multiple protonation states can bind the complex

3.5 Lessons learned from SAMPL6 pKa Challenge

Do any methods predict within experimental accuracy (how is the field doing overall)?

Common challenging factors for accurate pKa predictions. Tautomers, Heterocycles etc.

Overall results: Do any methods predict within experimental accuracy (how is the field doing overall)? Common challenging factors for accurate pKa predictions. Tautomers, Heterocycles etc.

Discussion of matching problem between experimental and predicted values. Difficulty of assessing predicted pKas using experimental data: matching problem Explain rationale behind how we analyze the data and determine success/failure.

Conclusion about prediction performance of individual molecules: SAMPL6 pKa set consisted of only 24 small molecules which limits our ability to do statistical analysis to determine which chemical substructures contribute to greater errors in pKa predictions. Which chemical structures make pKa predictions more difficult?

What can we learn from failures? Which physical effects are driving failures? Cycle closure errors

3.6 Suggestions for future challenges

Discuss what can be done to further improve future challenges

How can we maximize what we learn? What should we have people predict? How should we select compounds / measure pKas?

Suggestions about challenge construction

Enumeration of protonation states before predictions (which states does one need to consider?)

Suggestions about challenge analysis

NMR experimental techniques could be used to validate microstate information in future challenges

Reporting microscopic pKa predictions with charges, microstate free energies is better Experimental dataset with microstate information is more helpful.

What can be done to further improve future challenges How can we maximize what we learn? What should we have people predict? How should we select compounds / measure pKas? NMR experimental techniques could be used to validate microstate information in future challenges

Suggestions about challenge construction Enumeration of protonation states before predictions (which states does one need to consider?) Suggestions about challenge analysis

270 4 Conclusion

271 5 Code and data availability

- 272 • SAMPL6 pK_a challenge instructions, submissions, experimental data and analysis is available at <https://github.com/samplchallenges/SAMPL6>

273 6 Overview of supplementary information

274 Organized in SI document:

- 275 • TABLE SI 1: ???

276 Extra files:

- 277 • Any extra files

278 7 Author Contributions

279 Conceptualization, MI, JDC, CB, DLM ; Methodology, MI, JDC ; Software, MI, AR, ASR ; Formal Analysis, MI, ASR, AR ; Investigation, MI ; Resources, JDC; Data Curation, MI ; Writing-Original Draft, MI, JDC; Writing - Review and Editing, MI, ASR, AR, CB, DLM, JDC; Visualization, MI, AR ; Supervision, JDC, DLM, CB, ASR ; Project Administration, MI ; Funding Acquisition, JDC, DLM.

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290 Mike Chui

291 9 Disclosures

292 JDC is a member of the Scientific Advisory Board for Schrödinger, LLC. DLM is a member of the Scientific Advisory Board of
293 OpenEye Scientific Software.

294 Table ref: [3, 4, 7, 8, 10] trial: [], +, -, *, #

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10 Supplementary Information

MI: Figure [typeIII-error-dist-by-method] Distribution of prediction errors for each method in SAMPL6 Challenge. Analyses was performed based on Hungarian matching algorithm. Y-axis labels indicate submission IDs of each method.

MI: [pKa-error-vs-pKa-value]. Error in pKa predictions does not correlate with the true value of pKa. Left figure was constructed using closest match between experimental and predicted pKas. Y-axis is absolute residuals of the pKa prediction.

MI: FIGURE [desc-vs-MAE-correlation]. There is no clear correlation between molecular descriptors and mean absolute error for each molecule when calculated for all methods.

MI: SI Table: Type I collection

MI: SI Table: Type III collection

MI: SI Figure: type I correlation plots of each method

MI: SI Figure: type III correlation plots of each method

MI: TABLE: InChI and SMILES for chemicals

MI: TABLE: Statistics based on hungarian matching

MI: TABLE: Statistics based on microstate matching

MI: TABLE: NMR determined microstates of 8 molecules