BEST PRACTICES

Applying Schrödinger solutions for small molecule protonation state enumeration and pK$_a$ prediction

Executive Summary

- The pK_a of a drug is a key physicochemical property to consider in the drug discovery process given its importance in determining the ionization state of a molecule at physiological pH
- Schrödinger provides several solutions for predicting pK_a values, protonation state distribution, and derived properties that can be applied across a range of drug discovery stages, from screening through lead optimization.

Background

Small molecules can undergo ionization in solution where they either lose or gain protons (H$^+$) at different ionizing sites. The measure of the propensity of a site or molecule to ionize by the association/dissociation of one or more protons is quantified by a pK_a value. If the pKa value refers to a particular site ionizable site the value is a microscopic pK_a (micro-pK_a), and it is a macroscopic pK_a (macro-pK_a) if the value refers to the entire molecule. The specific arrangement of protons around the ionizing sites constitutes a protonation state, and different protonation states of the same charge level are called tautomers. Each protonation state is in thermodynamic equilibrium with the others and therefore has a free energy associated with its population within this collection of protonation states, which may be derived either from micro-pK_a values through thermodynamic equations or obtained directly by comparing the free energies of the states. In drug design, understanding the different protonation states of a molecule is critical, since they will drive properties including solubility, membrane permeability, and activity.

Updated: October 3, 2024

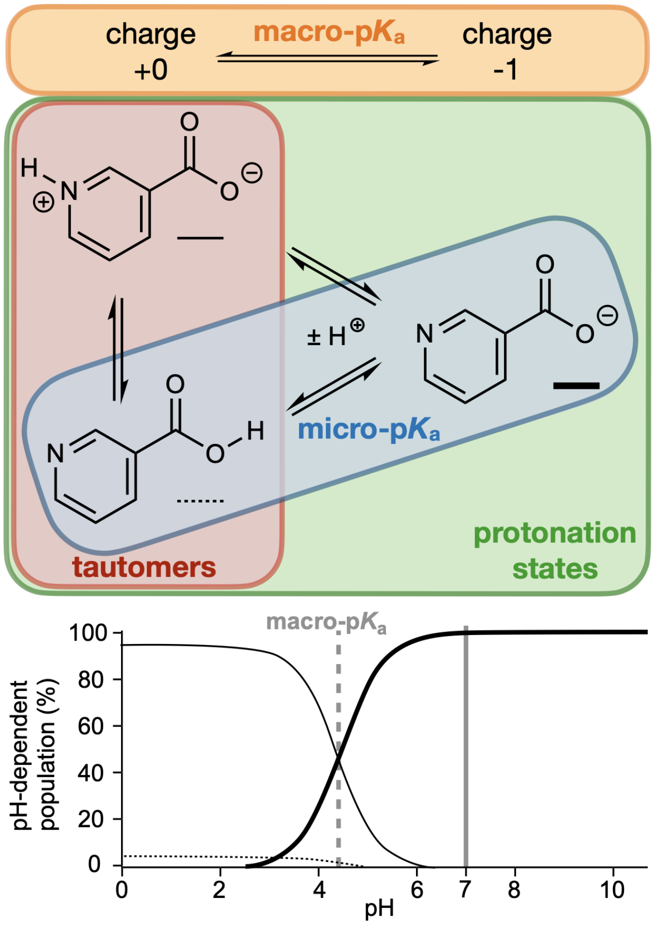


Figure 1. Relationships between macro-pK_a, micro-pK_a, protonation states, and tautomers and the corresponding speciation diagram.

Challenges of pK_a prediction

Determining which states predominate at a given pH and by how much is a challenging task both experimentally and computationally. Predicting pK_a values is an important step to calculating state distributions, which in turn enables prediction of important related quantities that would otherwise be inaccessible.

Schrödinger’s Solutions

Epik Classic

Epik Classic, previously known simply as Epik[1], is an expert system for rapidly and accurately predicting the micro-pK_a values and the most populated protonation states for a ligand at a given pH. The underlying pK_a prediction technology is the empirical Hammett-Taft linear free energy relationship (LFER), which identifies an ionizing group, takes its root pK_a value, perturbs it by the bonded chemical fragments, and applies charge spreading to arrive at its effective micro-pK_a value. Epik Classic then uses the predicted pK_a values to enumerate a ligand’s protonation states, rank them by energy, and then return the most populated states. Because Epik Classic uses SMARTS patterns-based rules, it is fast enough for high-throughput, although at the expense of being unaware of both conformational and stereochemical effects.

Epik

Epik[2] is a complete redesign of Epik that leverages Schrödinger’s powerful machine learning (ML) technology for more accurate results across broader chemical space. Ionizing groups are initially identified by SMARTS patterns and are then used to enumerate the protonation states for a range of ionizations. The micro-pK_a values of each site in each state are predicted with 3-layer atomic graph convolutional neural networks (GCNNs) extending out radially six bonds from the ionizing atom. The predicted pK_a values for the states are then used to predict the relative energies of the states to both allow determination of the most populated states at a pH and calculation of macro-pK_a values. The topological nature of the ML approach means that Epik, like previous versions, is rapid but agnostic to 3D geometry and stereochemistry.

Jaguar pK_a

Jaguar pK_a takes a third, more physics-based approach to predicting micro-pK_a values for a ligand. This workflow calculates the pKa values at the user-defined ionizing sites in a query ligand by first generating the conjugate pair, on which are then executed conformational searches to locate the lowest energy structures,[3,4] followed by density functional theory (DFT) based geometry optimizations and single-point energy evaluations. These resulting conformationally-averaged, “raw” micro-pK_a values are then corrected using empirically-parametrized relationships to give accurate predictions. Jaguar pK_a performs best on non-tautomerizable structures. Being physics-based, it does take into account geometric and stereochemical effects, but at the expense of speed.

Macro-pK_a

Macro-pK_a[4] follows the same philosophy as Jaguar pK_a by combining physics-based DFT calculations with empirical corrections, but extends its applicability to enable calculation of tautomerizable ligands and uses a more sophisticated ML model for the corrections. Macro-pK_a automatically identifies ionizing sites, enumerates the protonation states for a range of charges including conformer generation, and calculates the associated micro-pK_a values. These micro-pK_a values are aggregated into experimentally-observable macro-pK_a predictions and an estimate of the pH-dependent populations of each protonation state, highlighting the most populated states. As with Jaguar pK_a, the micro-pK_a values are computed from DFT-based geometry optimizations and single-point energy evaluations, but these values are then used as part of the input to an ML model similar in architecture to the one in Epik to generate the final micro-pK_a predictions. For simple systems, Macro-pK_a is similar in cost to Jaguar pK_a with a comparable accuracy, but as the number of protonation states increases, the exhaustiveness of this approach requires more computational resources than Jaguar pK_a.

Use Case Examples

Note: Each use case example below could be approached with any of the listed solutions within that section. The dataset presented highlights the applicability of just one of the possible solutions.

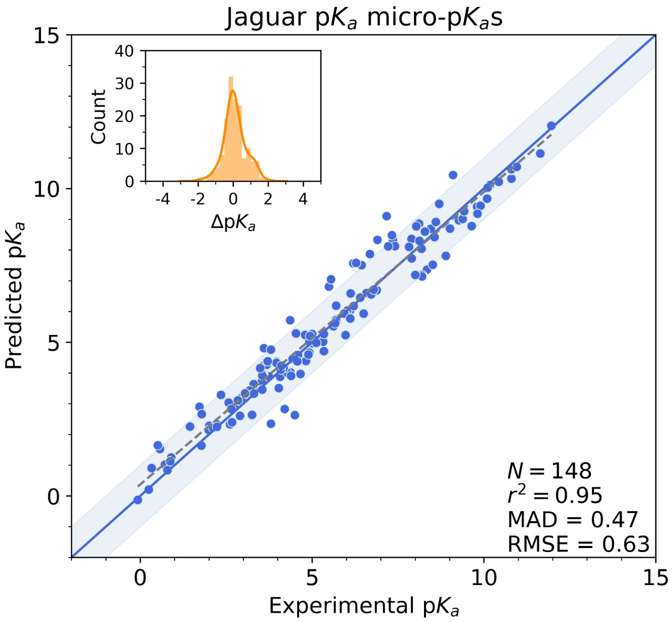
Querying microscopic pK_a values

Applicable Solutions:

- Epik Classic
- Epik
- Jaguar pK_a

When investigating the binding modes of a ligand, the micro-pK_a value of an ionizing site is an indicator of the propensity for it to become ionized at a given pH. The ionization state of the ligand directly influences how it interacts with another molecule such as a protein, e.g., whether or not it can participate in a salt bridge.

Figure 2. Jaguar pK_a micro-pK_a predictions for a dataset of small molecules.



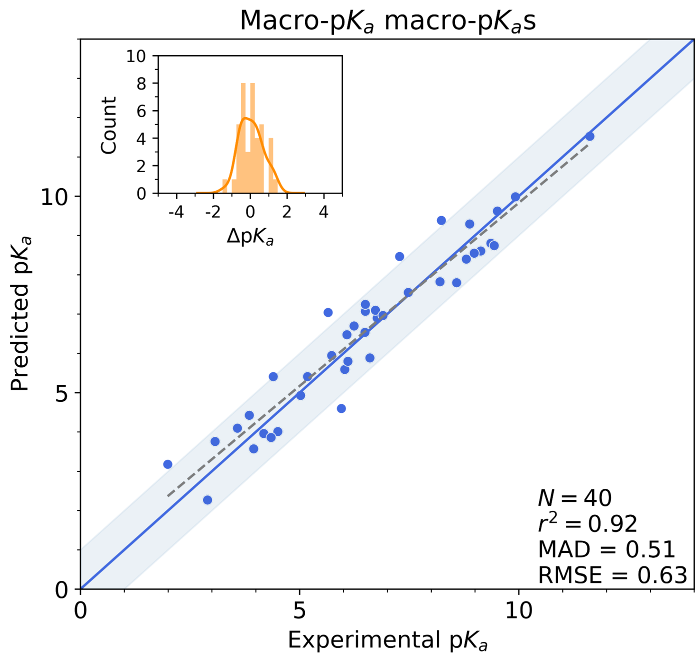
Querying apparent or macroscopic pK_a values

Applicable Solutions:

- Epik
- Macro-pK_a

For monoprotic or polyprotic compounds with a single dominant tautomer at each charge level, micro-pK_as may very closely match the apparent or macro-pK_a value that is most commonly obtained through titration experiments. However, for compounds or ionization states with multiple competitive tautomers, the micro-pK_a value of a single tautomer may not fully reproduce the experimentally observed macroscopic value. To obtain this apparent value, all states' must first be enumerated and evaluated so that all their micro-pK_a values are considered in the macro-pK_a calculation.

Figure 3. Macro-pK_a macro-pK_a predictions for a dataset of tautomeric molecules.



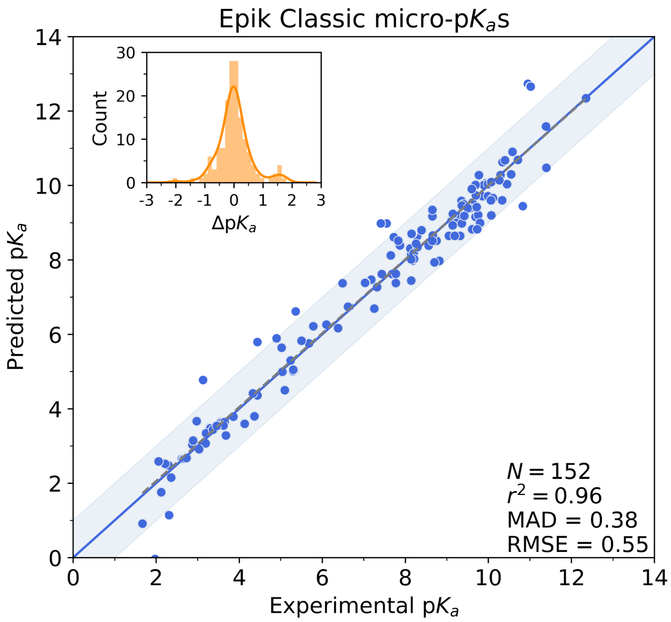
Ligand preparation and high-throughput screening

Applicable Solutions:

- Epik Classic
- Epik

Physics-based simulations typically require specification of all atoms in the simulation system, including all hydrogen atoms. Thus, structure-based simulations including Glide docking, molecular dynamics, and free energy perturbation with FEP+ should be performed using an ensemble of the highly-populated protonation states of a ligand. Therefore, a crucial first step in any structure-based screen of a small molecule ligand library is to prepare the ligands by obtaining the most populated protonated states. Epik Classic and Epik are integrated with our automated ligand preparation workflow, LigPrep, to allow preparation of large ligand libraries for high-throughput screening. Additionally, both Epik Classic and Epik and their LigPrep implementations allow for the generation and scoring of additional states that may potentially bind to metal ions in the pocket.

Figure 4. Epik Classic micro-pK_a predictions for a dataset of 152 drug molecules.



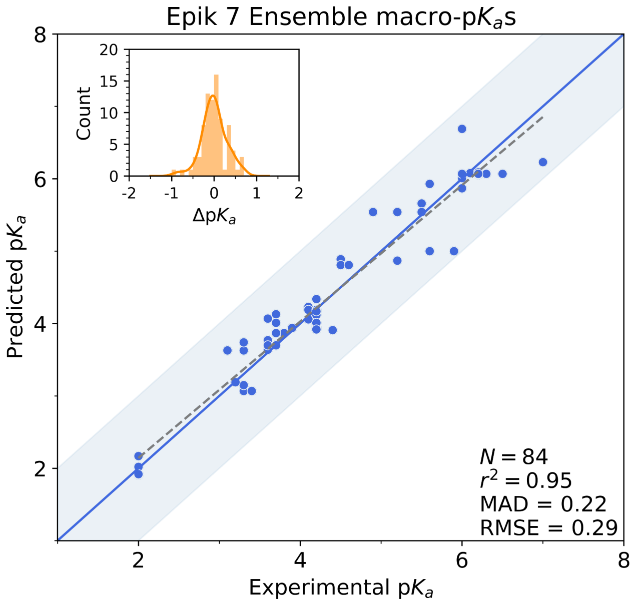
Hit-to-lead optimization

Applicable Solutions:

- Epik Classic
- Epik

Once hits are identified, a series of analogs are synthesized to explore the relevant chemical space in greater detail to arrive at improved behavior. It is important to be able to screen potential candidates rapidly and accurately to assess which to optimize further. The < 0.5 log unit accuracy and sub-second calculation speed of Epik Classic and Epik make them excellent tools for rapid idea generation and testing. In addition to pK_a value and protonation state distribution prediction, they have been implemented in other ADMET or property predictors, such as for membrane permeability and solvation energy.

Figure 5. Epik macro-pK_a predictions for a dataset of congeneric tricyclic thrombin inhibitors.



Early-stage lead optimization

Applicable Solutions:

- Epik
- Jaguar pK_a
- Macro-pK_a

Optimizing the many physical characteristics required can be laborious and costly, from ideation, through synthesis and assay. In this environment, where high quality property predictions are required and time permits, Schrödinger's physics-based predictors, Jaguar pK_a and Macro-pK_a, take into account more molecular characteristics, including conformational and stereochemical effects to improve pK_a prediction accuracy.

Additionally, Macro-pK_a and Epik both offer detailed speciation reports for a queried ligand. These are especially helpful for understanding the distribution of tautomeric states across the pH spectrum.

Figure 6. A Macro-pK_a report detailing the macro-pK_a value and the distribution of protonation states across a pH range.

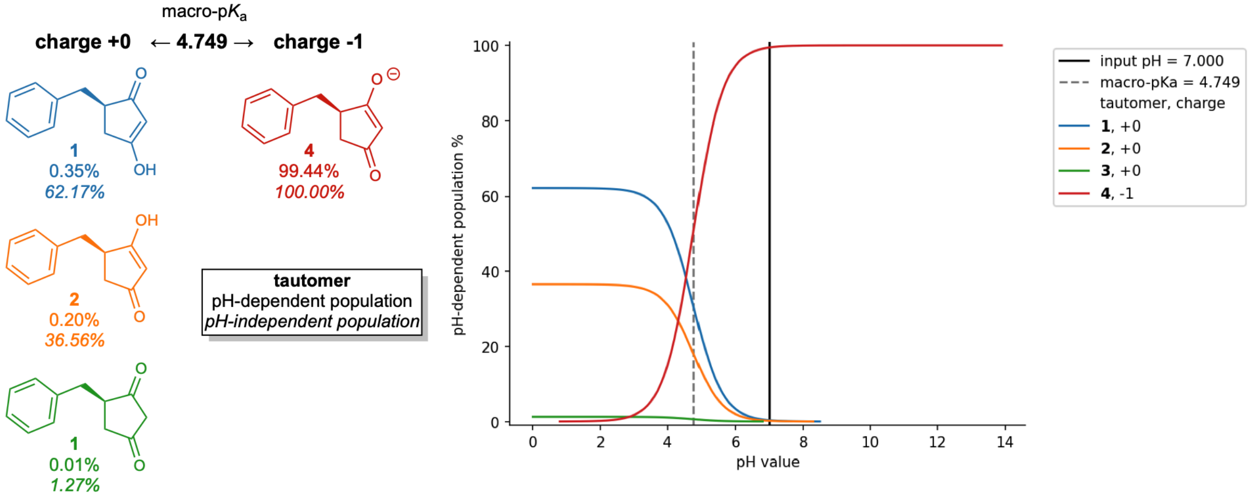


Table 1. Comparison of features of the small molecule protonation state enumeration and pK_a prediction technologies.

Feature\Program	**Epik Classic**	**Epik**	**Jaguar pK_a**	**Macro-pK_a**
Technology	Hammett-Taft LFERs	ML	DFT + Empirical Fitting	DFT + ML
Year Introduced	2007	2022	2005	2023
Ionization Enumeration Distance	±5	±2[a]	±1	±2[a]
Average Single Ligand Run Time	0.2 s	0.4 s	1 h	1 d
Practical Maximum Ligand Size	150 atoms	200 atoms	100 atoms[b]	100 atoms[b]
Predicts micro-pK_a values	Yes	Yes	Yes	Yes
Predicts macro-pK_a values	No	Yes	No	Yes
Enumerates States	Yes	Yes	No	Yes
Predicts Populations	Yes	Yes	No	Yes
Accurate pK_a values	Yes	Yes	Yes	Yes
Remote Intramolecular Interactions	No	No	Yes	Yes
Conformational Effects	No	No	Yes	Yes
Stereochemistry	No	No	Yes	Yes
Pseudochirality	No	No	No	Yes
DMSO Solvent	Yes	No	Yes	No
Metal Binding States	Yes	Yes	No	No
Trainable	No	Yes[c]	Yes	Yes[c]
Panel	Yes	Yes	Yes	Yes
Speciation Report	No	Yes	No	Yes
LigPrep Integration	Yes	Yes	No	No

[a] Easily adjustable; [b] Strongly influenced by the number of conformers (and tautomers in Macro-pK_a); [c] Only by internal experts at this time.

References

(1) Shelley, J. C.; Cholleti, A.; Frye, L. L.; Greenwood, J. R.; Timlin, M. R.; Uchimaya, M. Epik: A Software Program for pKaprediction and Protonation State Generation for Drug-like Molecules. *J. Comput. Aided Mol. Des.* **2007**, *21* (12), 681–691. https://doi.org/10.1007/s10822-007-9133-z.

(2) Johnston, R. C.; Yao, K.; Kaplan, Z.; Chelliah, M.; Leswing, K.; Seekins, S.; Watts, S.; Calkins, D.; Chief Elk, J.; Jerome, S. V.; Repasky, M. P.; Shelley, J. C. Epik: pKa and Protonation State Prediction through Machine Learning. *J. Chem. Theory Comput.* **2023**, *19* (8), 2380–2388. https://doi.org/10.1021/acs.jctc.3c00044.

(3) Bochevarov, A. D.; Watson, M. A.; Greenwood, J. R.; Philipp, D. M. Multiconformation, Density Functional Theory-Based pKa Prediction in Application to Large, Flexible Organic Molecules with Diverse Functional Groups. *J. Chem. Theory Comput.* **2016**, *12* (12), 6001–6019. https://doi.org/10.1021/acs.jctc.6b00805.

(4) Cao, Y.; Balduf, T.; Beachy, M. D.; Bennett, M. C.; Bochevarov, A. D.; Chien, A.; Dub, P. A.; Dyall, K. G.; Furness, J. W.; Halls, M. D.; Hughes, T. F.; Jacobson, L. D.; Kwak, H. S.; Levine, D. S.; Mainz, D. T.; Moore, K. B., III; Svensson, M.; Videla, P. E.; Watson, M. A.; Friesner, R. A. Quantum Chemical Package Jaguar: A Survey of Recent Developments and Unique Features. *J. Chem. Phys.* **2024**, *161* (5), 052502. https://doi.org/10.1063/5.0213317.