**CONTRIBUTORS**

Salah Salah (first author)

(order of others to be determined)

Sonya Hanson - kinase inhibitor cheminformatics and experiments

Bas Rustenburg - constant-pH MD

Greg Ross -

Julie Behr - kinase inhibitor

Andrea Rizzi - Epik calculation automations

Steven Albanese - retrieval of

John Chodera

Marilyn Gunner (corresponding author)

**POTENTIAL TITLES**

The importance of protonation state effects in kinase inhibitor binding

How important are protonation state effects in kinase inhibitor binding?

Protonation state effects in kinase inhibitor binding

Protonation state changes in kinase inhibitor binding

**POTENTIAL JOURNALS**

PNAS? - worth a try

J Med ChemJ Med Chem?

JCAMD - appeals to drug discovery modelers

**OBJECTIVES**

* Educate modelers about protonation state effects of small molecules (mixtures of populations, population shifts)
* Provide the first large-scale assessment of the prevalence of protonation state effects in kinase inhibitor binding
* Categorize the effects observed upon ligand binding into (1) protein protonation state changes, (2) ligand protonation state changes, (3) tautomer shifts, (4) mixtures of protomers/tautomers
* Convey how easy it is for kinase inhibitors to assume different protonation states in solution:  
  Half of all FDA-approved kinase inhibitors are predicted to have multiple protonation states within 6 kT;  
  Many have multiple tautomers easily accessible (same ionization state, different proton location)
* Quantify how large we expect the error to be if protonation state effects are neglected in modeling protein:ligand binding free energies (e.g. PBSA, alchemical free energy calculations, even docking)
* Quantify how much work the protein can do on the ligand to shift populations of protonation states
* Connect with experiment where possible; build on Abl:imatinib data from Seeliger
* Extract simple rules of thumb that indicate whether ligand or kinase will have protonation state effects.   
  Do some ligands always bind in a different protonation state?   
  Are some kinases always likely to have protonation state effects?   
  When are there surprises?  
  Should binding be electroneutral?

**ABSTRACT**

While proteins and small molecules can populate a variety of protonation states and proton tautomers, detailed atomistic modeling (such as docking, molecular dynamics simulations, and free energy calculations) generally lack a dynamic treatment of protonation states.

By omitting these effects, some form of error in introduced in trying to model the energetics of association.

Recently, experimental and computational evidence has emerged that protonation state effects are important in the recognition of the selective kinase inhibitor imatinib by Abl kinase.

As nearly half of all kinase inhibitors have multiple protonation states predicted to be easily accessible, this phenomenon may be widespread in kinase inhibitor recognition.

In this paper, we estimate the prevalence of protonation state effects---and hence the expected frequency of errors---as well as their magnitude and provenance for 50 FDA-approved kinase:inhibitor complexes.

We find that …

We provide guidelines (or rules of thumb) to help the modeler anticipate when protonation state effects may be relevant in kinase inhibitor binding.

**OUTLINE**

Introduction

* Why do people care about selective kinase inibition?
  + 50% of active drug discovery projects
  + Selectivity is difficult to achieve
  + Conformational dynamics / flexibility also challenging
  + Even imatinib required hundreds of compounds to be synthesized
* Abl:imatinib finding that protonation state effects were important
  + Review experimental and computational evidence
* Little attention is often paid to protonation state effects during design; single dominant protonation state assumed

Kinase inhibitors and protonation states

* Imatinib experimental data (50% population of piperazine states at pH 7.4)
* Epik predicted pKas for all FDA-approved inhibitors
  + Compare to available experimental pKa data (where possible)
* Interpretation of populations for ligand in solution and how little it costs to shift protonation states upon binding
* Kinase inhibitors can be up to picomolar potency (38 kT?), so cost to shift protonation states is little by comparison
* Could matter for selectivity [look for specific cases]

Survey of protonation state effects in FDA-approved kinase:inhibitor complexs

* Briefly describe methodology
  + Holo kinase structures with and without ligand
  + Also look at apo kinase structures
* Selection of protein complexes (FDA-approved inhibitors and their targets of therapy from PDB)
* Main Table of summarized charge changes and tautomer populations
* Prevalence: How frequently do we see any kind of protonation state effects?
* Provenance: What classes of protonation state effects do we see?
  + Protein protonation state changes
  + Ligand protonation state changes
  + Both protein and ligand? Why not?
  + Proton tautomer shifts
  + Mixtures of protonation states?

Assessing the error in binding free energies when protonation state effects are neglected?

* How much work does the protein do on the ligand to shift populations?
  + Compute population shifts of ligand protonation states from solution to complex
  + Use KL divergence (and enthalpy difference) to estimate work and free energy difference
* How much error is made in assuming binding free energy is computed for fixed protonation states?
  + Compute DeltaG for confining protonation states to “common” state via MCCE for ligand, protein, and complex
  + Perform a reference YANK calculation with two ligand protonation states as an illustration?
  + PBSA calculation?

Detailed examination of key kinase:inhibitor complexes

* 3D structure
* Binding site 2D interactions
* KLIFS fingerprint
* Examination of implied protonation states from X-ray structure

Experiments?

* Could potentially compute protonation state effects at two different pH
* E.g. ponatinib : DDR1? Abl? (look into Salah’s table) at different pH

Discussion:

* How much should I (a modeller) worry about protonation state effects?
* Caveats:
  + Could be artifacts from rigid experimental structures
  + Promising route for identifying systems to follow up on experimentally
    - Lots of experiments one could do: NMR, ITC, pH-dependent affinity assays
* Can we harness the power of protonation state effects by being clever?
  + pH-targeted inhibition/selectivity (tumor intracellular environment)
  + Diane Barber: Mutations (e.g. His to Arg) may change charge, could exploit this difference to achieve selectivity
  + Solubility control could be important

Detailed Methods:

* Everything we need to do to replicate the study

**FIGURES/TABLES**

**Table: Summary of protonation state effects in ~50 FDA-approved kinase inhibitor complexes**

**Pie chart of fraction of different kinds of effects?**

- (Donut chart?)

- Venn diagram of what kinds of effects we see

**Figure: Kinase perspective: residue protonation state hotspots on consensus structure and sequence alignment**

- Sequence alignment highlighting residues that interact and have Delta pKa

- Consensus structure showing residues that have significant Delta pKa

- Sequence alignment highlighting residues that have significant Delta N\_protons

- Consensus structure showing residues that have significant Delta N\_protons

- Kinome tree illustrating which kinases are considered in this study and which ones have protein or ligand protonation state effects

**Figure: Inhibitor perspective**

- inhibitor orinhibitor class

- binding titration curves for most populated and equilibrium species

- point out where the ligand binds in different protonation states to different kinases; route to slectivity?

**Figure: Binding curves for a few key kinase:inhibitor pairs**

- binding curves: Dominant protomer in solution; dominant inhibitor in complex; ensemble average population

- illustrative energy diagram: energy levels in solution and in bound state

**Figures: Detailed binding interactions for some key kinase:inhibitor complexes**

KLIFS + detailed 2D view of binding interactions for each complex

**SUPPLEMENTARY DATA**

**Table summarizing all 50 kinase:inhibitor MCCE2 calculation results**

Kd of each protomer/tautomer and for ensemble Kd

protein : PDB : apo protein total charge : Delta H+\_protein : inhibitor apo total charge : Delta H+\_ligand : key residues

- Sorted by kinase or organized by inhibitor? Or both? Sort by class of effects?

**Figures for all kinase:inhibitor pairs**

**Datasets for MCCE2 output for all kinase:inhibitor pairs**

**FOLLOW-ON PAPERS**

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