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**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 **← Elizabeth’s vote**

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 lacks a dynamic treatment of protonation states.

By omitting these effects, some form of error is 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**

Selective kinase inhibition is a key problem in contemporary rational drug design. Kinase-mediated protein phosphorylation is the primary method by which eukaryotic cells regulate division, cell fate, and metabolism. **[citation needed]** Dysfunctions in these pathways are responsible for a vast array of cancers, autoimmune disorders, and diseases of excessive inflammation. **[citation needed]** Kinases present an ideal target for rational drug design because of their diversity and selectivity in these varied cell processes. The human kinome consists of 512 distinct enzymes, representing nearly two percent of the total library of proteins produced in the body. **[citation needed]** Even isoforms of the same kinase have been shown to have diverse structures and specificity for radically different cell functions. **[I could get into more detail on this but it could be a tangent]** Developing pharmaceuticals to influence cell activity by modifying kinase activity is a proven approach to effective treatment of dysfunction.

Although kinase-inhibiting compounds are the focus of half of all active drug discovery projects, several aspects of their discovery present significant challenges given the current design standards. It is difficult to achieve selectivity. **[Why?]** Accurately modeling the conformational dynamics of a kinase with its inhibitor presents unique challenges. **[Why?]** Modeling flexibility is also difficult using the current available algorithms. **[Why?]** Together, these shortcomings of the rational drug design process result in a high failure rate of promising compounds. Even in a straightforward case like the development of imatinib as a treatment for chronic myeloid leukemia, the *in silico* investigation could not weed out the single *in vitro* winner without requiring the synthesis of hundreds of other compounds. Thus, developing more accurate methods of simulating these enzyme-ligand interactions is a prime opportunity to make significant improvements in rational drug discovery.

Protonation state effects have been shown to be important in understanding the mechanism by which imatinib binds to its target protein, Bcr-Abl tyrosine kinase (Abl). Seeliger et al. found that protonation state effects **[finish this sentence]**. **[Summarize the rest of the experimental and computational evidence for role of protonation state in mediating kinase inhibitor interactions]** We also know that many kinase inhibitors have multiple protonation states at physiologic pH and also at the acidified pH environment common in many cancers. This body of evidence suggests that taking protonation states into account may be a key factor in developing more accurate drug screening processes.

Little attention is paid to protonation states during design, however. Most rational drug design experiments assume a single dominant protonation state in the target ligand, and do not explore the potential mechanisms of alternative protonation states in the interaction between enzyme and ligand. Historically, it has been computationally expensive to perform this type of analysis; **[why else have people not been simulating multiple protonation states?]**.

Our analysis of a library of 50 kinase:inhibitor interactions shows that neglecting protonation states in drug design simulations is an oversight which can hide the presence of physiologically relevant interactions between enzyme and ligand, or overstate the affinity of a seemingly promising compound. We propose a new approach that can accurately reproduce these multiple protonation states in a computationally inexpensive yet theoretically robust manner.

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

**Results**

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**

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? *Depends on how other scientists are likely to use the data. Do others in the field tend to focus on one kinase and many inhibitors, one inhibitor and multiple kinases, or class of effects for a set of kinases with their inhibitors?*

**Figures for all kinase:inhibitor pairs**

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

**FOLLOW-ON PAPERS**

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