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Binding energies of tyrosine kinase inhibitors: Error assessment of computational methods for imatinib and nilotinib binding

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ABSTRACT

The binding energies of imatinib and nilotinib to tyrosine kinase have been determined by quantum mechanical (QM) computations, and compared with literature binding energy studies using molecular mechanics (MM). The potential errors in the computational methods include these critical factors:

- Errors in X-ray structures such as structural distortions and steric clashes give unrealistically high van der Waals energies, and erroneous binding energies.
- MM optimization gives a very different configuration to the QM optimization for nilotinib, whereas the imatinib ion gives similar configurations
- Solvation energies are a major component of the overall binding energy. The QM based solvent model (PCM/SMD) gives different values from those used in the implicit PBSA solvent MM models. A major error in inhibitor—kinase binding lies in the non-polar solvation terms.
- Solvent transfer free energies and the required empirical solvent accessible surface area factors for nilotinib and imatinib ion to give the transfer free energies have been reverse calculated. These values differ from those used in the MM PBSA studies.
- An intertwined desolvation—conformational binding selectivity process is a balance of thermodynamic desolvation and intramolecular conformational kinetic control.
- The configurational entropies ($T\Delta S$) are minor error sources.

1. Introduction

Protein kinases have been the focus for many drug-based cancer treatments. Tyrosine kinases are enzymes responsible for the activation of many proteins by signal transduction cascades. The proteins are activated by adding a phosphate group to the protein (phosphorylation). Tyrosine kinase inhibitors can compete with adenosine triphosphate (ATP), the phosphorylating entity, the substrate, or both, or can act in an allosteric fashion, by binding to a site outside the active site, affecting its activity by a conformational change. Targeting the tyrosine kinases that regulate cell growth and proliferation has been very productive, as witnessed by the success of inhibitors such as imatinib mesylate, or Gleevec, and nilotinib, or Tasigna, for the treatment of both chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST). BCR-ABL is the oncogenic protein-tyrosine kinase responsible for the pathogenesis of chronic myelogenous leukemia (American Cancer Society, 2013; Okuno, 2011). Development of new tyrosine kinase inhibitors to overcome resistance, or improved efficacy, requires an understanding of the binding efficiency and effectiveness of the inhibitor with the tyrosine kinase. Nilotinib is a second generation inhibitor which is similar to imatinib, but 30 times more effective in treating CML.

The computation of binding free energies between small molecule ligands and proteins is a difficult task since the binding energy usually involves small differences amongst large enthalpies and entropies related to the free and bound states of protein and the ligand. Inter-atomic forces are strong and short ranged, resulting in steep energy functions that are strongly dependent on molecular conformation. Proteins and ligands are usually very flexible, having many degrees of freedom, making conformational energy profiles very dominant contributing factors. Solvation effects can be very large for both free and bound protein and ligand, including protonation and salt effects. Dispersion or van der Waals and hydrophobic effects between ligands and proteins can also be

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large. It is also conceivable that small configurational changes (including molecular strain) in bond lengths and angles might occur during binding, which might have significant energy impacts (Gilson and Zhou, 2007; Mobley and Dill, 2009; Kuriyan et al., 2008; Bissantz et al., 2010). These effects will have large first order effects in any calculation. Second order effects include translational, rotational, vibrational, and repulsive effects, which are known to be smaller.

Enthalpic contributions to binding free energy are driven by the strength and directed specificity of ionic, polar, hydrogen bond, electrostatic (coulombic), van der Waals and polarization interactions. These interactions usually have small entropy contributions. Changes in binding entropy include small configurational translational and rotational processes, somewhat larger conformational processes, or much larger solvent effects (which include desolvation or rearrangement during binding). The enthalpy changes (in vacuo or gas phase) between the protein and ligand before and after binding closely approximates the free energy of binding interaction, less any configurational entropy change, which may be significant. Gilson has suggested that configurational entropy ($T\Delta S$) between the free and bound ligand can be a loss of ca. 25 kcal/mol (vibrational entropy and conformational entropy differences of 24.6 and 1.8 kcal/mol, respectively) on amprenavir ligand binding to HIV protease, using a M2 mining minima method (Gilson and Zhou, 2007, Eq. (7)). The M2 method is based on calculating binding free energies, potential energy wells and solvation free energies, assuming molecules are rigid rotator/harmonic oscillators, and using force field energies. It is not known what errors are involved in all these assumptions, and the use of empirical force field energies which have no electrons, so cannot evaluate excited electronic or delocalized states. Often force field parameters have to be generated for specific inhibitor molecules during an investigation, when using molecular mechanics methods calibrated for protein molecules. In addition, various conformational states of the studied inhibitor would need specific force field parameters to be generated for the different states for each calculation which might be used in a molecular dynamics simulation (Mackerell, 2004). Real large molecules are far from rigid rotators/harmonic oscillators, and it has been shown (Carpinelli et al., 2015) that anharmonic, not-separable, ro-vibrational states must be considered. The contribution of electronically excited states, which have their own ro-vibrational states, have important roles.

The free energies involved in solvation processes before and after binding can be calculated using the well established PCM/SMD quantum mechanical solvation model to compare solvation energies in vacuo and water (Marenich et al., 2009). This approach would be more rigorous than the solvent models typically used in molecular mechanics based methods (PBSA, GBSA) used to investigate protein-inhibitor binding, which comprise an electrostatic component and a non-polar component based on an arbitrary solvent accessible surface area (SASA) factor usually somewhere between 5 and 45 cal/mol/Å^2. Explicit solvent models require empirical interaction potentials between the solvent and solute, and between solvent molecules.

The computational methodologies used (Gilson and Zhou, 2007; Mobley and Dill, 2009) vary considerably, from docking and scoring techniques to those using advanced force fields, incorporating electronic polarization, i.e., iteratively adjusting partial atomic charges from quantum mechanical calculations during docking. Molecular mechanics force field calculations can handle large molecular systems, like proteins, but due to the empirical nature of force fields, the neglect of electrons, electron polarization and charge transfer are not accounted for explicitly. This can limit the accuracy with which interactions are calculated and consequently the free energies obtained. Ideally ab initio quantum chemistry approaches should be used as these explicitly include electrons, however quantum mechanics is not practicable for large proteins.

Calculating the conformational energies vary from docking methods using one conformation to more sophisticated molecular mechanics—molecular dynamics techniques where the free ligand, free protein, and the complexed ligand–protein conformations are simulated using an explicit solvent model. The difference between energy minimized snapshots of free and bound molecules can be found in molecular dynamics calculations. This approach has to account for many possible low energy conformations by seeding the calculations with best guesses. Molecular mechanics force fields may not give the lowest energy conformation, due to their empirical shortcomings and difficulties with parameter optimization particularly for conformational states (Mackerell, 2004). Force field energy minimizations can result in strained torsional angles, as torsion angles are the softest conformational parameters, and have the largest effects on molecular geometries (Brameld et al., 2008). Molecules which have significant ability to maximize π electronic delocalization over multiple aromatic rings, as found in nilotinib and imatinib, could show large conformational energy differences by using quantum mechanical energy minimization compared to force field energies.

The conformational structure of the protein binding pocket is a crucial factor which defines inhibitor effectiveness (Seeliger et al., 2007; Aleksandrov and Simonson, 2010; Lin et al., 2013). Imatinib is known to bind 2400 times less tightly (experimentally measured 4.6 kcal/mol penalty) to the c-SRC form (DFG-in) of tyrosine kinase than to the closely related c-ABL form (DFG-out), even though the X-ray crystal structures of both complexes are very similar. It has been shown that c-SRC can adopt the inactive ABL conformation which gives strong binding, but the free energy between these conformational states for the c-SRC is the dominant factor. The 4.6 kcal/mol difference in binding energies between the c-SRC and c-ABL forms has been shown to almost the same (4.4 kcal/mol) as the difference between the DGF-out and DFG-in conformations of the c-SRC kinase, suggesting that *conformational selection is the main source of imatinib binding selection* (Aleksandrov and Simonson, 2010).

Electrostatic interactions in molecular mechanics force fields are commonly calculated from quantum mechanical Merz Kollman (MK)/RESP partial charges. Comparisons of MK, RESP and CHELPG methods show very similar performances (Sigfridsson and Ryde, 1998). However Sigfridsson and Ryde (1998) has shown that the MK method showed a significant rotational dependence on the orientation of the grid coordinate system (0.04–0.05 e), whereas CHELPG showed a lesser rotational dependence, as it was specifically designed to remove such dependence (Breneman and Wiberg, 1990). Hence use of MK/RESP charges may induce some errors when calculating electrostatic interactions as inputs into force field calculations.

The starting point for any calculation of binding energies is an X-ray structure of the complexed ligand–protein complex. However it has been shown (Davis et al., 2008) that published X-ray structures suffer from many problems, including poor fits, such that conformations and configurations (bond lengths, angles etc.) can be unrealistic and highly distorted. Amides can be *cis* or non-planar, and even planar aromatic rings can be deformed. There can be severe steric clashes in the X-ray structure which are not easily apparent. It has been shown (Thompson and Day, 2014) that molecular strain can be induced by intermolecular interactions in single-component crystal structures of molecules with no intramolecular hydrogen bonding, resulting in some molecules being distorted by up to 5 kcal/mol by crystal packing forces. As intermolecular hydrogen bonding is involved in inhibitor—protein binding, large distortions may occur due to crystal packing forces.