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MM/GBSA Binding Energy Prediction on the PDBbind Data Set: Successes, Failures, and Directions for Further Improvement

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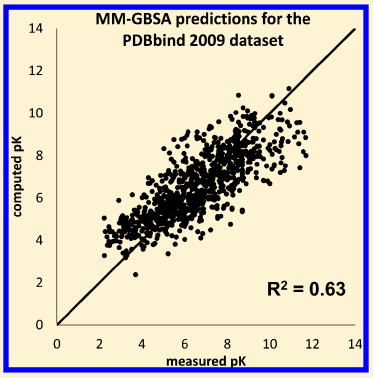
Supporting Information

ABSTRACT: We validate an automated implementation of a combined Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method (VSGB 2.0 energy model) on a large and diverse selection of protein−ligand complexes (855 complexes). Although this data set is diverse with respect to both protein families and ligands, after carefully removing flawed structures, a significant correlation (R^2 = 0.63) between calculated and experimental binding affinities is obtained. Consistent explanations for "outlier" complexes are found. Visual analysis of the crystal structures and recourse to the original literature reveal that neglect of explicit solvent, ligand strain, and entropy contribute to the under- and overestimation of computed affinities. The limits of the Molecular Mechanics/Implicit Solvent approach to accurately estimate protein−ligand binding affinities is discussed as is the influence of the quality of protein−ligand complexes on computed free energy binding values.



INTRODUCTION

In drug design, the reliable estimation of protein−ligand binding free energies is a much sought-after goal. Over the past decades, a number of different approaches for calculating binding free energies, each with individual trade-offs of computational cost versus accuracy, have been developed.[1,2] These range from fast but rather inaccurate scoring functions, which are based on coarse physical approximations, to very compute-intense methods like free-energy perturbation and thermodynamic integration that use less severe approximations to real physics but are often not applicable to systems of interest in drug discovery processes due to the computational costs.[1,2]

The combined Molecular Mechanical/Generalized Born Surface Area (MM/GBSA) approach is the fastest force-field based method that computes the free energy of binding from the difference between the free energies of the protein, ligand, and the complex in solution.[3,4] The advent of faster computers, compute-clusters, and completely automated protein/ligand preparation routines makes the calculation of MM/GBSA model energies feasible on a medium-throughput basis.

The MM/GBSA method is based on the concept that the combination of molecular mechanics (MM) energies, polar and nonpolar solvation terms, and an entropy term can correctly render an approximate free energy of binding of a ligand to a receptor.[3,4] The free energy for each species (ligand, receptor, and complex) is decomposed into a gas-phase MM energy, polar, and nonpolar solvation terms, and an entropy term, as shown in the following equation:

$$\Delta G = \Delta E_{MM} + \Delta G_{solv} - T\cdot\Delta S$$

$$= \Delta E_{bat} + \Delta E_{vdW} + \Delta E_{coul} + \Delta G_{solv,p} + \Delta G_{solv,np} - T\cdot\Delta S$$

E_{MM} is composed of E_{bat} (the sum of bond, angle, and torsion terms in the force field), a van der Waals term, E_{vdW}, and a Coulombic term, E_{coul}. $G_{solv,p}$ is the polar contribution to the solvation free energy, often computed via the Generalized-Born (GB) approximation.[3,4] $G_{solv,np}$ is the nonpolar solvation free energy, usually computed as a linear function of the solvent-accessible surface area (SASA).

In this study we compute protein−ligand binding affinities using the MM/GBSA model known as VSGB 2.0 energy model.[5] In the VSGB 2.0 energy model, the MM energy is calculated with the OPLS protein force-field[6,7] enhanced by several physics-based corrections. These include terms for improved handling of π-stacking and hydrogen bonding interactions. The implicit solvent model is based on a variable dielectric surface Generalized Born (VD-SGB) approach, where the variable dielectric value for each residue was fit to a large number of side-chain and loop predictions. The nonpolar solvation free energy is calculated by a parametrized hydrophobic term.

Various MM/GBSA approaches with different parameters and settings have been used to compute protein−ligand binding affinities. It has been shown that for calculating relative binding affinities, the computationally less demanding Generalized-Born approach for the polar solvation term competes favorably with the more expensive Poisson−Boltzmann method.[8−10] Also, it has been reported that the energies calculated after minimization of the protein−ligand complexes in implicit solvent yield similar correlations with experimental data as the energies calculated from much more time-consuming molecular dynamics simulations in explicit solvent.[8−11] In most studies, the entropy term

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($-T \cdot \Delta S$ in the above equations) is neglected for congeneric series and relative free binding energies.[8−10] While some researchers advocate its usage,[12] others dispute the benefits of including this term, the calculation of which can be a major source of error.[10]

In previous applications relatively small data sets including single protein families with ligands belonging either to diverse[10] or congeneric series have been studied.[13] Publications in which MM/GBSA calculations have been performed for more substantial numbers of complexes include that of Hou et al.[8] They performed calculations for 98 protein−ligand crystal structure complexes and obtained a correlation coefficient R^2 of 0.4 (using the crystallographic ligand pose) between calculated and experimental binding affinities. Brown and Muchmore computed affinities for 308 small ligand protein complexes and obtained correlations with experimental data in the range 0.52−0.69 for three congeneric series.[14] Gao et al.[15] calculated affinities for 233 protein−ligand complexes. The complexes were divided into 16 subsets ranging in size from 5 to 37 members. For 5 out of 16 of the subsets R^2 values of at least 0.49 were obtained. Yang et al.[16] considered 156 complexes belonging to 7 protein families. Correlation between calculated and experimental values (R^2) ranged from 0.3 to 0.6.

The objective of this work was **not** to generate a kind of master equation that can be applied for predictions. Such an equation would anyway be protocol-dependent and not generally applicable. It is obvious from a number of recently published data that excellent results for predictions can mainly be obtained from congeneric series of ligands in a single or in closely related receptors. It is less obvious why the method sometimes fails completely or at least produces outliers which lower its reliability in daily drug discovery work. Here, we analyze the results of an automated implementation of the VSGB 2.0 energy model[5] method on a large and diverse data set with the goal to identify reasons for failure.

Using a KNIME-automated[17] protocol we perform calculations for over one thousand protein−ligand crystal structure complexes taken from the refined set of the PDBbind09-CN (henceforth called PDBbind) database.[18,19] This database is a collection of high quality protein−ligand complex crystal structures (resolution ≤2.5 Å) and accompanying K_d or K_i values. It is diverse with respect to both ligands and proteins. There is some consensus that MM/GBSA models only work well for congeneric series.[8,13] However, by using the refined set of the PDBbind database as our data set we challenge this assumption. In contrast to previous studies we do not simply report correlation coefficients. Instead, by visual inspection and recourse to the original literature we try to understand why binding affinities are under- or overestimated with respect to experimental values.

METHODS

Complex Selection and Preparation. We used the same preparation protocol as in an earlier publication[20] on the PDBbind[19] refined set of 1741 entries. In the previous publication all complexes with ligands of a molecular weight larger than 900 (mostly polypeptides), with more than 20 donors and acceptors (mostly polyglycosides), and those with more than one P atom (mostly NADPH and ADP/ATP) were removed. The protein complexes were prepared using Schrödinger's Protein Preparation Wizard[21] with all options switched on, i.e. adding hydrogens, assigning disulfide bonds, removing waters further than 5 Å from the ligand, adjusting charges, capping termini, adding missing side-chains using Prime,[22,23] optimizing hydrogen bond clusters, and performing a restrained minimization using the OPLS2005 force-field[6,7] on the whole protein/ligand complex. During the minimization the heavy atoms were restrained to remain within a 0.3 Å root-mean-square deviation of the original PDB structure. Cases where the protein preparation wizard produced an error were individually inspected and either manually corrected or dropped. All ligands as given in SD files in the PDBbind database were additionally scanned by RDKit,[24] and cases where RDKit reported an error were either corrected or dropped. This left an overall number of 1387 complexes, of which 1137 complexes were processed without error by the KNIME protocol.

For the further analysis all (143) complexes with ions in the binding site were removed (Supporting Information, Table S1). The exclusion of such complexes aims at separating issues associated with the parameterization of metal ions from the assessment of the overall performance of the MM/GBSA approach. In a previous study that analyzes the performance of MM/GBSA on metal-containing proteins, Hou et al.[8] cite the lack of accurate GB parameters for ions as a probable cause of poor correlations for two series of compounds in their MM/GBSA calculations. In another study, Zhang et al.[25] conclude that polarizable force-fields are needed to treat metal ions. Another 139 complexes were removed for miscellaneous reasons. These include cofactors close to the ligand, ligand differing from the ligand reported in the original PDB file, multiple ambiguous ligand binding modes, or incomplete binding site. Some data were removed because the automated procedure to convert PDB file content (especially ligands) into correct structures failed. A full list of all complexes with the reason for exclusion can be found in the Supporting Information (Table S2). In order to fully automate the MM/GBSA calculations starting from pure, unprocessed PDB files as structural input, computational routines will have to be developed that spot such problems. After all exclusions a total of 855 complexes remained (Supporting Information, Table S3). The data preparation workflow is shown in Figure 1.

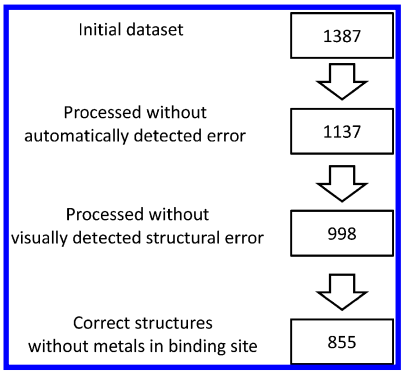

Figure 1. Data preparation workflow. Numbers indicate the number of complexes left.

Going through the initial results, and checking major outliers, we detected that for some of these the automatic preparation procedure had yielded flawed structures. For example, ligands were not detected correctly or phenoxy oxygens were deprotonated. Hence, we visually inspected all processed complexes by comparing the KNIME output crystal structure complexes with the superimposition of the original crystal structures, 2D depictions of ligand structures, and 3D-binding site interactions using the visualization tools available in RCSB PDB.[26] For example, a complex was removed because the incorrect "flipping" of the

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original crystal structure conformation of a glutamine side-chain (GLN 200) had a dramatic impact on the minimized binding mode of the ligand (PDB ID 1elc). 133 structures had to be discarded because they obviously were wrongly recognized or protonated. In some complexes with ambiguous protonation states we referred to the original literature for further information. We also discarded 5 complexes with ligands exhibiting multiple occupancy. The existence of multiple occupancy makes it harder to build good models for ligands. Liebeschuetz et al.[27] estimate that at least 2% of protein−ligand complexes can be expected to exhibit multiple binding modes.

We explicitly mention the visualization step as being important. It is tempting to use automatic routines to set up protein−ligand systems, but they do not work in every case. If wrong structures are included in a validation, the performance cannot be properly analyzed. Although this is a technical rather than a scientific reason for failure, we believe that it is important to point out that one needs to make sure that the correct structures are used in MM/GBSA approach validation. This is not a trivial task. Especially ligand and protein tautomerism and protonation states are not easily deducible,[1] but MM/GBSA results are obviously affected by these states.

In summary, we must accept that any automatic procedure to prepare files for detailed MM/GBSA calculations will produce some incorrect starting structures. In the current work, after visual inspection we discarded erroneous structures that would have required manual interference in order to correct them (Supporting Information, Table S2). In smaller data sets (e.g., in an actual drug-discovery project), users would be required to manually correct such errors.

MM/GBSA Computations. The complexes selected according to the previous section (i.e., cleaned up and energy-refined) were processed by the VSGB 2.0 energy model[5] KNIME workflow. In order to test the influence of water molecules on the calculated energies, two sets were prepared: one for which all water molecules explicitly present in the X-ray structures were removed, and one in which explicit X-ray water molecules close to the ligand were retained. The first set is considered to be less problematic since the choice of which water molecules to keep, and whether they should be regarded as part of the receptor or part of the ligand, is debatable. The second set was used to test if retaining X-ray water molecules (when present) within 3.5 Å from the ligand would be benificial for the overall correlation between calculated and experimental binding affinities. In order to test the influence of the inclusion of ligand strain energies on the final correlations, computations were performed with and without the inclusion of ligand strain for both sets.

The KNIME workflow consisted of a PDB reader node, a molecule writer node (maestro format), and two metanodes. Metanodes are nodes that contain subworkflows. Structure parsing and preparation and MM/GBSA calculations were performed within the metanodes. In the structure parsing and preparation metanode, bond orders, charges, and protonation states were assigned, and selected atoms, ions, or residue types deleted. All metal ions and cofactors were deleted. The actual MM/GBSA calculations (MM/GBSA module version 1.41) were performed within the second metanode. Energy values were computed from single-point calculations using the VSGB2.0 energy model.[5] The ligand strain energy is the difference between two energies: the energy of the ligand as it is in the complex, and the energy of the extracted ligand, minimized, starting from the geometry in the refined complex. Calculations to assess the strain were performed in implicit solvent.

Quality Metrics. We use the residual squared error (RSE), the mean unsigned error (MUE), and the square of Pearson's correlation coefficient ($R^2_{\text{Pearson}} = R^2$). We use R^2 instead of R because R^2 estimates the proportion of the variance of the data set explained and if a Gaussian-distributed error is expected the variance and so the R^2 describe the distribution of errors best. RSE[28] and MUE measure the absolute accuracy of the estimation, i.e., how well the experimental value is reproduced by the correlation. For RSE and MUE, the range of the estimated scores is adjusted to the measured values of the free energy of binding. Since this employs an additional fitting step, there is one less degree of freedom in the data set, and the RSE has to be calculated with dividing by $(N-2)$ in the denominator.

$$\text{MUE} = \frac{1}{N}\sum_{i=1}^{N} |y_{i,comp} - y_{i,meas}|$$

$$\text{RSE} = \sqrt{\frac{1}{N-2}\sum_{i=1}^{N} (y_{i,comp} - y_{i,meas})^2}$$

$$R^2_{\text{Pearson}} = \left(\frac{\sum_{i=1}^{n} (y_{i,meas} - \overline{y_{meas}})(y_{i,comp} - \overline{y_{comp}})}{\sqrt{\sum_{i=1}^{n} (y_{i,meas} - \overline{y_{meas}})^2}\sqrt{\sum_{i=1}^{n} (y_{i,comp} - \overline{y_{comp}})^2}} \right)^2$$

Here, N is the number of data points, $y_{i,comp}$ is the estimated binding energy, and $y_{i,meas}$ is the measured binding energy.

RESULTS AND DISCUSSION

The data set used here is significantly larger than the data sets reported in other studies that use similar methodology. In contrast to other studies,[8] we excluded complexes with ligands that chelate metal ions. Also we did not divide the large diverse data set into protein-specific subsets.[14−16] This allows us to identify common factors associated with complexes that are outliers. The calculated MM/GBSA protein−ligand binding energies for the remaining set of 855 complexes were linearly scaled to the experimental pK. A summary of measured versus computed free energy of binding for all remaining 855 complexes is shown in Table 1.

Table 1. Summary of R^2, RSE, and MUE Values for MM/GBSA Calculations with Different Conditions

conditions	R^2	RSE	MUE
ligand strain/water	0.58	1.29	1.03
ligand strain/no water	**0.63**	**1.20**	**0.96**
no ligand strain/no water	0.61	**1.20**	0.98
no ligand strain/water	0.56	1.32	1.06

The best $R^2 = 0.63$, RSE = 1.20, and MUE = 0.96 values are obtained if ligand strain is included, and explicit water molecules (only waters within 3.5 Å of the ligand were considered) are left out. The computed MM/GBSA energies are best fit (e.g., lowest squared error) to the measured pK values with the equation

$$\textit{computed } pK = -0.0456*\textit{MM-GBSA Energy} + 2.564$$

The exclusion of ligand strain has a smaller detrimental effect on the overall performance ($R^2 = 0.63$ vs $R^2 = 0.61$) than the inclusion of only water molecules within 3.5 Å of the ligand ($R^2 = 0.63$ vs $R^2 = 0.58$). A plot of the measured versus the estimated binding affinity is shown in Figure 2.

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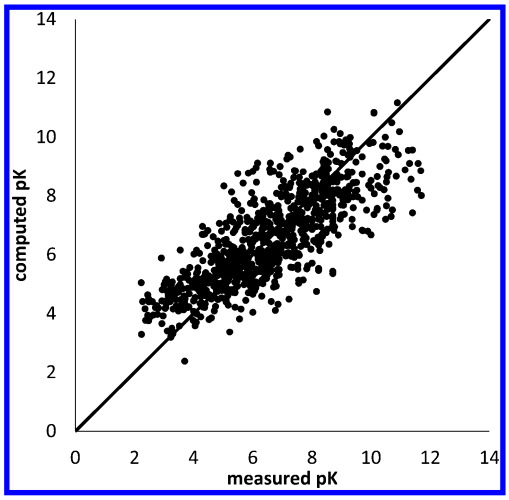

Figure 2. Measured versus computed binding affinities. $R^2 = 0.63$, RSE = 1.2, MUE = 0.96.

In order to verify that the predictive performance results from the MM/GBSA approach and not just due to overly simple correlations in the data set, we calculated the correlation coefficient between the measured affinities and basic ligand properties. Both molecular weight and the heavy atom count correlate with the measured affinities with $R^2 = 0.44$, whereas the logP[29] correlates with the measured affinities with $R^2 = 0.27$ (Plots can be found in the Supporting Information.). This is much lower than the correlation with the computed MM/GBSA affinities.

Checa et al. have previously found that the correlation with measured binding affinities gets worse if explicit solvent is used.[30] In a study involving the calculation of the relative binding affinities of trypsin inhibitors, they recommend to use a "cap" of water molecules to properly balance the solute−solvent interactions. They obtained the best correlations with experimental data with distance-dependent dielectrics (crystallographic water molecules excluded). The water cap produced an equivalent correlation. Retaining only selected crystallographic water molecules however produced significantly weaker correlations. In this study we kept all water molecules within 3.5 Å of the ligand. However, this cutoff value may truncate extended water networks. The correct identification of the most tightly bound water molecules is a nontrivial task that currently engages many researchers.[31−34] Well-ordered water molecules tend to be located in highly polar cavities, and they make three or four hydrogen bonds with the protein and the ligand.[35] In addition, the number of identified crystallographic waters is sensitive to the resolution of the crystal structure.

Quality of Protein−Ligand Structures. In order to investigate how the performance depends on one marker of the quality of the X-ray structure, we split the analysis according to the resolution of the X-ray structures. There was a modest trend for binding affinities of crystal structure complexes with lower resolutions to be estimated worse than those with higher resolution. The R^2 for the best performing combination was 0.6 for crystal structure complexes with resolutions between 2 and 2.5 Å (370 complexes) and 0.65 for crystal structures with resolutions better than 2 Å (485 complexes).

However, we do not give too much emphasis to these results because, as discussed by Warren et al.,[36] resolution alone is not a sufficient measure of the quality of an X-ray crystal structure. The resulting prototype database (Iridium) that emerged from their work is divided into data sets classified as being non-trustworthy, of medium trust, and highly trustworthy. Twenty of the complexes in the Iridium database overlap with those used in this present study. These are summarized in Table 2. Generally

Table 2. Summary of Overlap of Complexes with Those in the Iridium Database[36] and Difference of the Calculated p*K* as Compared to the Experimental Value (Δp*K*)

PDB ID	trust category	associated comment	Δp*K*[a]
1ai5	high		+0.6
1aj7	non	very poor electron density for ligand	+1.7
1br6	high	poor density for deposited ligand	+1.5
1ezq	high		−1.2
1f0u	high	possible alternate conformation for ligand carboxyl	−0.27
1fcx	high	possible alternate conformation for ligand carboxyl	+2.2
1fcz	high		+0.2
1fh8	high		−1.0
1fh9	high		+0.12
1fhd	high		−0.56
1fl3	high		+0.1
1h1p	high		+0.5
1h1s	high		−1.9
1l2s	high		+0.6
1lpz	high		+0.6
1mq6	high		−2.3
1n2v	high		+1.1
1n46	high		−2.1
1v48	medium	packing effects	−1.5
2tpi	medium	alternative ligand conformation	+1.5

[a]Like in the text, + and − values for Δp*K* stand here for overestimated and underestimated p*K* values, respectively.

complexes with ligands that have either poor ligand electron density or alternative ligand conformations show larger deviations between calculated and experimental p*K* values than complexes in the high-trust category with no associated comment. However, there are three exceptions (PDB ID 1h1s, 1mq6, 1n46) to this observation, all of which had underestimated binding affinities. The factor Xa (FXa) complex PDB ID 1mq6 is discussed in more detail in the section "Problems Arising from the Neglect of Water". With respect to the thyroid hormone receptor-beta complex PDB ID 1n46, chain A was used for calculations. In hindsight chain B would likely have given a better protein−ligand interaction energy, as R282 is in a conformation to form a hydrogen bond with the ligand which it is not in chain A. This highlights the extreme sensitivity of the MM/GBSA method as implemented here to the initial coordinates without molecular dynamics. In this particular example, even a short MD trajectory might have readjusted the side-chain conformation of R282 in chain A also. In the case of CDK2 kinase PDB ID 1h1s, the underestimation of binding affinity may arise from the failure to account for water-mediated hydrogen bonding between the purine-based inhibitor and the catalytic lysine.

Liebeschuetz et al.[27] recently assessed the model quality of ligand structures in protein crystal structures. This was done by comparing the geometries of the protein-bound ligands with the geometries seen in small crystal structures. As a result of their analysis they recommend that only the very highest resolution (<1.3 Å) protein−ligand structures should be used for evaluating ligand strain. The ligand structure in lower resolution crystal structures may represent a conformation that fits the electron

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density reasonably well, but it may not be the lowest energy conformation that is able to fit the electron density. Performing high level computations starting from the crystal structure ligand conformation may result in artificially high strain energies.[37] Here we use a rather conservative protocol for calculating ligand strain. That is, we compare the energy of the ligand in the minimized complex, with that of the extracted ligand which is only subjected to a local minimization. With this conservative estimate of ligand strain, a slightly better correlation between experimental values and calculated MM/GBSA values is obtained when we include strain in our calculations.

One of the crystal structures mentioned by Liebeschuetz et al.[27] as having questionable geometry is the HIV-1 protease complex PDB ID 2fgv. This corresponds to one of our overestimated affinities. With respect to thrombin, Senger et al.[38] discuss sulfonamide-related conformational effects. They found the conformational energy penalty for binding thrombin to be significantly smaller for the ligand in the crystal structure PDB ID 2jh6 as compared to PDB ID 2jh5. The only difference between the inhibitors is the order of a carbon−carbon-bond. They performed torsion scans on the torsions (C═C)-(S═O) and (C−C)-(S═O) by means of *ab initio* calculations. A window of 1.4 p*K* units exists in the experimental binding affinities of these complexes. The calculated energy window is only 0.1 p*K* units, even with the inclusion of ligand strain. This may be a reflection of the fact that current force-fields are unequal to the task of assessing relative energies for conformers of drug-size molecules.[37]

Problems Arising from the Neglect of Water. Visual inspection of binding site interactions suggests that some of the complexes with an underestimated binding affinity of more than 1 p*K* unit (Supporting Information, Table S4) involve ligands with important water-mediated hydrogen bonds. These include five proteins for which specific water molecules have been identified as being crucial for tight binding affinity (HIV-1 protease, FXa, scytalone dehydratase, oligo-peptides, and trypsin).[31] The interactions of these bridging waters are depicted in the publication of Barillari et al.[31] We discuss specific examples for HIV-1 protease, *5′-methylthioadenosine/S-adenosylhomocysteine nucleosidase* (MTA/AdoHcy), lipocalin, FXa, and scytalone dehydratase for which literature precedence exists.

HIV-1 Protease. With respect to HIV-1 protease, the binding free energy of WAT301 in the crystal structure PDB ID 1hpx (Figure 3) has been calculated to be −3.3 kcal/mol by Hamelberg and McCammon.[40] In our calculations, this complex was underestimated by −1.7 p*K* units as compared to the experimental binding affinity. This may be a consequence of our neglect of explicit solvent. The complex PDB ID 3djk is underestimated by −1.8 p*K* units. The ligand is described as forming a unique water-mediated interaction with the NH of G48.[41]

5′Methylthioadenosine/S-Adenosylhomocysteine Nucleosidase. The protein MTA/AdoHcy nucleosidase has two complexes (PDB ID 1y6q, 1y6r) whose binding affinities are underestimated by −3.7 and −2 p*K* units respectively. WAT3 (HOH234 PDB ID 1y6r and HOH418 PDB ID 1y6q) is described as being crucial for tight affinity between inhibitor and protein[43] (see Figure 4).

Lipocalin. The imidazole group of histamine is stabilized by two residues, Ser-83 and Glu-103, via hydrogen bonds involving bridging water molecules (PDB ID 3bu1, Figure 5). The mutations, S83L and E103L, resulted in 75- and 43-fold decreases in binding affinity respectively, indicating the importance of the hydrogen bonding network to ligand binding. 5-HT (PDB ID 3brn) makes similar water-mediated interactions. The binding

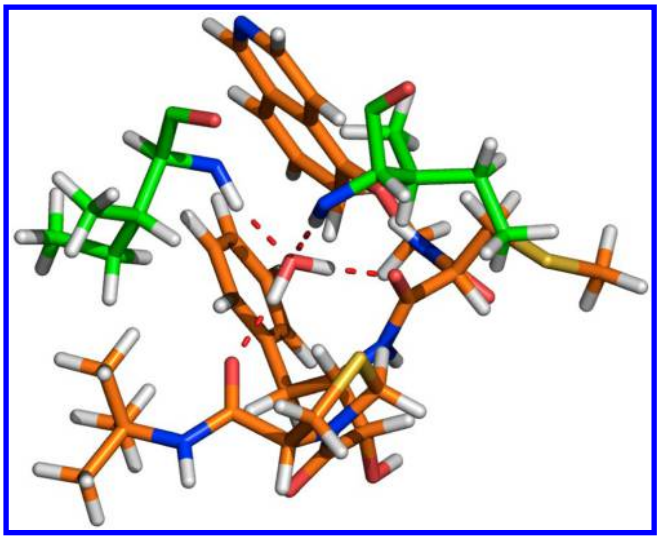

Figure 3. Water-mediated hydrogen bond between HIV-1 protease and ligand KNI272 (WAT 301 in PDB ID 1hpx). The ligand is colored in orange and the protein residues (I50′ and I50) are colored in green. Hydrogen bonds are represented by red dashed lines. All figures have been generated with PyMOL.[42]

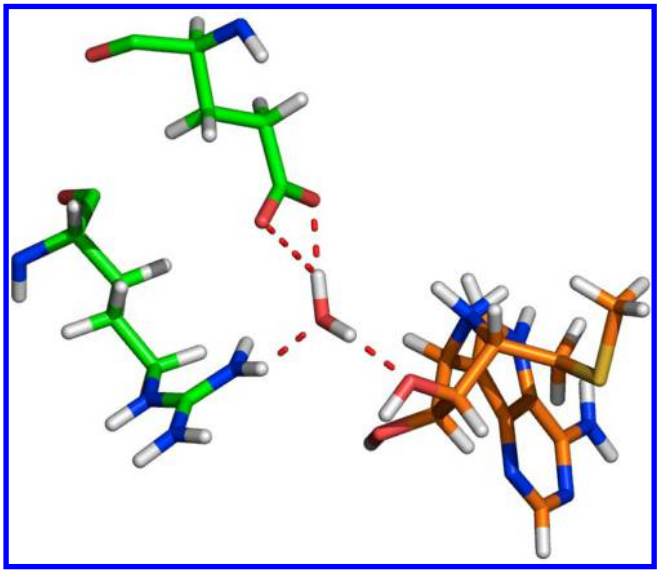

Figure 4. Crucial water-mediated hydrogen bond (HOH 234 PDB ID 1y6r) between 5′-methylthio-immucillin A (MT-ImmA) and MTA/AdoHcy nucleosidase. MT-ImmA is colored in orange and the protein residues (E12 and R173) are colored in green.

affinities of these complexes are underestimated by −3.4 and −3.3 p*K* units respectively according to our MM/GBSA protocol.[44]

Scytalone Dehydratase. Two bridging water molecules (Figure 6) are present in the binding site of scytalone dehydratase (PDB ID 5std, 6std, 7std). The binding affinities of these complexes are underestimated by −3.3, −0.7, and −3.2 p*K* units respectively. Barillari et al.[31] have calculated the binding free energies of these waters to range between −2.1 and −5.7 kcal/mol. The binding affinity of a fourth scytalone dehydratase complex (PDB ID 2std) is underestimated by −2.3 p*K* units.

HIV-1 Protease, FXa, and Ornithine-Binding Protein: Water Displacement. Release of a well ordered water into bulk solvent can lead to an increase in binding affinity due to a favorable gain in entropy.[35] Cyclic ureas (HIV-1 protease) displace a conserved

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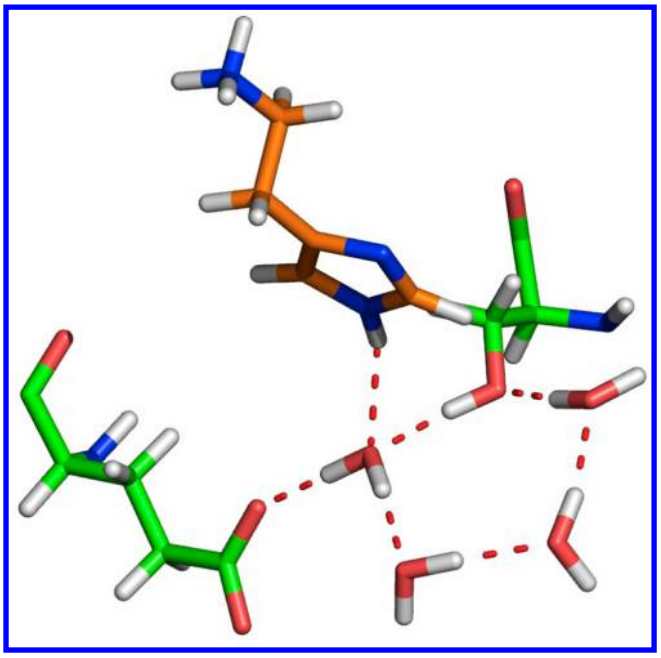

Figure 5. Interaction between histamine and bridging water molecules with monomine (PDB ID 3bu1). Histamine is colored in orange and the protein residues (S83 and E103) are colored in green.

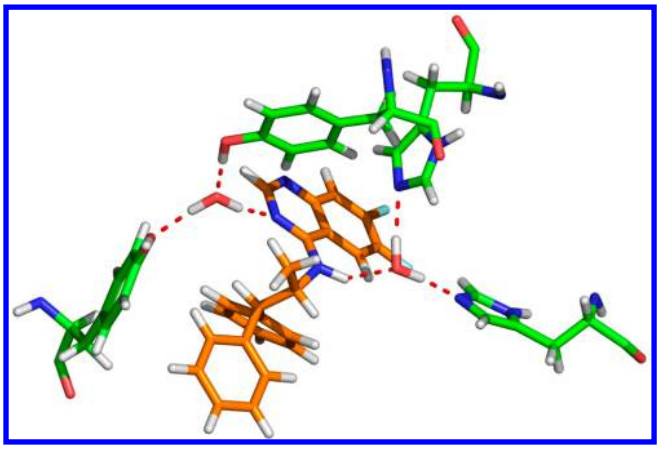

Figure 6. Water-mediated interactions between scytalone dehydratase and an inhibitor (PDB ID 5std). The inhibitor is colored in orange and the protein residues are colored in green (H110, H85, Y30, and Y50).

water molecule[32] (e.g., PDB ID, 1dmp, 1qbs, 1bv7, and 1ajx). The calculated MM/GBSA binding affinities underestimate the affinity of most of these complexes (1dmp: −1.7; 1qbs: −1.3; 1ajx: −0.1 p*K* units) with the exception of 1bv7(+0.7 p*K* units). Barillari et al.[31] did not find a correlation between the binding free energies of water molecules and the change in binding affinity of ligands displacing waters, at least not for non-congeneric ligand pairs. They found cases where displacing a loosely bound water resulted in a large increase in binding affinity, whereas displacement of a tightly bound water led to a decrease in binding affinity. They conclude that this finding underlines the complex nature of the binding process where water displacement constitutes only one part.

Abel et al.[45] have calculated the energy for a ligand to displace solvent for congeneric pairs of fXa inhibitors (WaterMap approach). Some of the matched pair complexes used in their study are also present in our data set. The pairs are PDB ID (1nfu and 1nfy), (1mq5 and 1mq6), and (2bq7 and 2boh). The affinities of the inhibitors that do not displace water are relatively well predicted in our study (within 1.1 p*K* units of the experimental value). However, the affinities of the inhibitors anticipated to displace water are underestimated by up to −2.3 p*K* units relative to experimental values. The complex PDB ID 1z6e is also expected to displace a water molecule from the active site. Its calculated binding affinity is underestimated by −2.9 p*K* units.

In the primary PDB publications describing the relevant crystal structures, we find several additional complexes with underestimated affinities for which relocation of protein-bound water impacts on binding affinity, the ornithine-binding protein complexes PDB ID, 1lag and 1lah.[46] These complexes are underestimated by −1.7 and −1.9 p*K* units respectively.

Thus, the MM/GBSA protocol may fail to produce estimated affinities close to experimental values when the displacement of water is a key contributor to protein−ligand binding affinity. In such cases, tools investigating the importance of water distribution and free energy (e.g., WaterMap,[45,47] SZMAP[48−50] or 3D-RISM[51−54]) are required to get a more complete picture of the complex formation.

Problems Arising from the Neglect of Entropy. *HIV-1 Protease.* HIV-1 protease mutants can be classified into active-site and nonactive site mutants. Both kinds of mutations can have an effect on the binding affinity of an inhibitor to the receptor. Modulation of binding affinity is achieved either by direct interactions between residues of the active site and the inhibitor or indirectly by alteration of the dynamic response of the protein to the inhibitor.[55] Experimental[56] and molecular dynamics simulations[57] show that certain mutations alter the equilibrium between the open and closed forms of the protease relative to the wild type. Flap dynamics are involved in the modulation of inhibitor binding affinity by influencing both the association and dissociation rates. The mutations L90M, G48V and L90M/G48V decrease the affinity of inhibitors relative to the wild type.[56] The increase in dissociation rates may be due to an increase in flap opening rates, decrease in flap closing rates, or both. Molecular dynamics simulations[57] indicate that the V82F/I48V mutation shifts the equilibrium between closed and semiopen forms; the mutant favors the semi-open conformation more than the wild type. Therefore, inhibitors have to pay a larger energetic cost to close the mutant flap.[57]

One of the underlying assumptions in structure-based drug design is that the entropy change upon binding of a ligand to the protein is a constant and therefore independent of the nature of the ligand and the protein. Marshall[55] states that this should not be taken as a given. By means of rigidity analysis of a data set of 206 high resolution structures of HIV-1 protease, Heal et al.[58] have found that inhibitors fall into two classes. Some have a significant effect on flap rigidity (flap flexibility score tends to one), and others do not (flap flexibility score tends to zero). Some of the HIV-1 proteases that are outliers in our study were included in the investigation of Heal et al.[58] There is a clear trend for an outlier complex to fall into the class of inhibitor that does not rigidify the flap. In contrast, well estimated (calculated p*K* within 1 p*K* unit of the experimental value) complexes are those that are calculated to rigidify the flap. It is expected that inhibitors/mutations that rigidify the flaps will bias the structure to the closed flap conformation, and this bias will be smaller for inhibitors that do not rigidify the flaps.[58] Five complexes (PDB ID 1sdt, 1mtr, 2bpv, 2bpy, 3bgc) with 1.1 to 3.4 p*K* units too high estimated binding affinities have flap flexibility scores that tend to zero (≤0.008). These inhibitors have to pay a larger enthalpic

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cost to close the flaps. This is not accounted for in our calculations however. Some corroboration that the binding of this group of inhibitors is entropy driven is given by the binding affinity data for PDB ID 1sdt. The enthalpic component of the binding affinity is positive.[59] Twelve complexes with well estimated binding affinities (PDB ID 1ajx, 1ajv, 1g35, 1g2k, 1ebw, 2pk5, 2cen, 1w5x, 1w5w, 1w5v, 1msm, 1msn) have flap flexibility scores that tend to 1 (≥0.4). The binding affinity of the inhibitor in complex PDB ID 2pk5 is enthalpy driven.[12] The enthalpic and entropic components of the binding affinity of the inhibitor in complex PDB ID 1msn are more balanced.[60] Li et al.[61] have assembled a calorimetry data set, including some data for which protein−ligand crystal structure complexes exist. They note that especially for HIV-1 protease inhibitors, the enthalpy and entropy contributions can vary significantly, even though the total binding energies are similar. They state that their isothermal titration calorimetry measurements on a diverse set of ligands validate the entropy-enthalpy compensation effect.

Penicillopepsin. Failure to account for a reduced entropy penalty in conformationally preorganized inhibitors as compared to more flexible analogues leads to underestimated affinities. An example in the data set presented here are the complexes PDB ID 1bxo and 1bxq,[62] which refer to the cyclic and acyclic analogues of a ligand. We cannot reproduce the 400-fold difference in binding affinity of the cyclic ligand with respect to the acyclic analogue.

In such cases, the MM/GBSA protocol does not take into account the entropy loss of flexible ligands binding in a single conformation into the receptor, as compared to a very similar, but structurally constrained (e.g., cyclized) analogue.[62] This represents a circumstance in which the MM/GBSA protocol used here may fail to accurately estimate binding affinity due to the lack of an entropy correction.

Influence of Internal Dielectric Constant on Computed Binding Affinities. Hou et al.[9] suggested that computed binding affinities for formally charged ligands (ions) tend to be overestimated by MM/GBSA, especially when using an internal dielectric constant of 1. In their studies, the correlation between calculated and experimental binding affinities of the neuraminidase and α-thrombin data sets showed a marked improvement upon increasing the value of the dielectric constant from 1 to 4. Hou et al.[8] in another study found an internal dielectric constant of 2 to be optimal for the data set of 98 ligands. Yang et al.[16] acknowledge that a global internal dielectric constant is a crude treatment to diminish the strength of electrostatic interactions. They found that they obtained the best correlation between calculated and experimental binding affinities with a value of 4.

We looked into the influence of ligand formal charges on computed binding affinities by separating our data set into charged and neutral ligands. The data set is well balanced with respect to the number of ligands having a formal charge (417) and those that are overall neutral (438). The VSGB 2.0 energy model[5] uses a variable dielectric which is dependent on the amino-acid side-chain varying from 1 for neutral side-chains, up to a maximum of 4 for charged side-chains. If we consider as "well estimated" calculated affinities within one log unit of the experimental ones, we have 513 complexes falling into that category. Among these, 248 are charged and 265 are neutral, showing that neutral and charged ligands are equally well handled in this data set by the MM/GBSA protocol used in this work. It could be that this protocol is more suitable for calculating binding affinities for data sets containing multiple protein families than others. We cannot directly compare our results with those from other studies, as this is the first instance of the use of the energy model VSGB 2.0,[5] for calculating binding affinities for a large number of protein−ligand complexes that we are aware of. Ravindranathan et al.[63] have also investigated the use of a variable dielectric model based on residue types to simulate electrostatic shielding effects. Four data sets were studied separately and not combined. The use of a variable dielectric over a standard dielectric model improved the correlation between calculated and experimental binding affinities.

CONCLUSION

Using an automated MM/GBSA protocol (energy model VSGB 2.0),[5] we have computed 855 protein−ligand binding energies using crystal structure complexes obtained from the PDBbind09-CN database.[19] By lieu of using such a heterogeneous data set, in contrast to congeneric series with single target proteins, we are susceptible to outliers due to data coming from different sources, and the inherent variability of experimental measurements.[64,65] Kramer et al.[64] have recently calculated the experimental uncertainty of heterogeneous public K_i data for public databases to have a standard deviation of 0.54 log K_i units. From integrating the normal distribution with $\sigma = 0.54$ between −infinity to −1 and +1 to +infinity follows, that for the present data set of 855 compounds, the published affinity for 55 compounds should be expected to be more than 1 log unit off its true value (i.e., the average affinity from multiple independent measurements). Nevertheless, we find that a significant correlation ($R^2 = 0.63$) between calculated MM/GBSA energies and experimental data in the form of K_d or K_i values can be obtained for structurally diverse crystal structure complexes. The R^2 values obtained in this study are in the range to those reported by other studies (0.3−0.69).[8,14−16] Whereas previous studies also used relatively large data sets (approximately 100−300 complexes), data were always divided into protein family specific subsets and analyzed separately for each family.[14−16] Our results with a variable internal dielectric constant show that in principle the MM/GBSA approach can yield values that are comparable even among different targets. In practice, however, it probably is more reliable for individual series, given the current difficulty in automatic data preparation.

Outlier complexes have made us aware of some of the shortcomings of the MM/GBSA approach as implemented here. Testing the effect of including, excluding ligand strain and crystallographic water molecules (only those within 3.5 Å of the ligand) in the energy calculation, we find that the inclusion of water molecules deteriorates the predictive quality, while inclusion of ligand strain slightly improves the overall accuracy. Entropy effects represented a recurring theme, especially for some protein families.

There are several main scoring schemes of different computational complexity for calculating protein−ligand binding affinities such as classic scoring functions,[66,67] MM/GBSA,[3,4] and free energy perturbation (FEP).[2] Ultimately they all face the common challenge of how to deal with complexes which contain ligands coordinated to metal ions, water-bridged interactions, the energetics of water displacement from the binding site, entropy, and highly strained ligands. Therefore, the developers and the users of scoring schemes must take the responsibility for gaining an understanding of the terms included in the scheme, the biological target, and the nature of the ligands. Knowing the limits of a scoring scheme may highlight the potential causes of results that are hitherto considered to be outliers.

■ ASSOCIATED CONTENT

Supporting Information

File of PDB codes, experimental values, and calculated MM/GBSA (VSGB2.0 energy model) protein−ligand affinity values for 855 data points. File of PDB IDs of removed compounds including reasons for exclusion. Plots of measured affinity versus molecular weight, heavy atom count, and logP. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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