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Citation for the published paper:

Genheden, Samuel; Ryde, Ulf

"Comparison of end-point continuum-solvation methods for the calculation of protein-ligand binding free energies."

Proteins

2012, vol. 80:5, pp. 1326-1342

<http://dx.doi.org/10.1002/prot.24029>

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Comparison of end-point continuum-solvation methods for the calculation of protein–ligand binding free energies

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2013-01-29

Abstract

We have compared the predictions of ligand-binding affinities from several methods based on end-point molecular dynamics simulations and continuum solvation, i.e. methods related to MM/PBSA (molecular mechanics combined with Poisson–Boltzmann and surface area solvation). Two continuum-solvation models were considered, viz. the Poisson–Boltzmann (PB) and generalised Born (GB) approaches. The non-electrostatic energies were also obtained in two different ways, viz. either from the sum of the bonded, van der Waals, non-polar solvation energies, and entropy terms (as in MM/PBSA), or from the scaled protein–ligand van der Waals interaction energy (as in the linear interaction energy approach, LIE). Three different approaches to calculate electrostatic energies were tested, viz. the sum of electrostatic interaction energies and polar solvation energies, obtained either from a single simulation of the complex or from three independent simulations of the complex, the free protein, and the free ligand, or the linear-response approximation (LRA). Moreover, we investigated the effect of scaling the electrostatic interactions by an effective internal dielectric constant of the protein (ϵ_{int}). All these methods were tested on the binding of seven biotin analogues to avidin and nine 3-amidinobenzyl-1*H*-indole-2-carboxamide inhibitors to factor Xa. For avidin, the best results were obtained with a combination of the LIE non-electrostatic energies with the MM+GB electrostatic energies from a single simulation, using $\epsilon_{\text{int}} = 4$. For fXa, standard MM/GBSA, based on one simulation and using $\epsilon_{\text{int}} = 4$ –10 gave the best result. The optimum internal dielectric constant seems to be slightly higher with PB than with GB solvation.

Keywords: Continuum solvation, dielectric constant, ligand-binding affinities, linear interaction energy, MM/PBSA, PDLD/s-LRA/ β

Introduction

The binding of a small molecule (a ligand, L) to a protein or another macromolecular receptor (P) can be described by the reaction



This reaction is governed by several different intermolecular forces, e.g. electrostatic, steric, and hydrophobic effects. In particular, electrostatic effects are essential for protein functions and hence for the ability to accurately predict binding affinities.¹

The most rigorous methods to estimate the binding free energy of the reaction in Eqn. 1 are free-energy perturbation and thermodynamic integration. In these methods, the energy difference is estimated when the interactions between the ligand and the surroundings are turned off. This is typically performed by first turning off the charges of the ligand, giving the electrostatic (polar) contribution to the free energy. Then, the Lennard-Jones parameters are removed, giving the non-electrostatic (non-polar) contribution to the free energy.² These calculations are performed both when the ligand is bound to the protein and when it is free in solution, and the binding energy is the difference between the corresponding two free energies. The disadvantage of these methods is that they give converged results only if the phase spaces of the initial and final states overlap. Therefore, the calculations typically have to be divided into several (typically 10–40) steps, involving unphysical intermediate states, in which the interaction between the ligand and the surroundings is partly removed. This, in combination with the fact that extensive sampling is needed for each step, preferably in the form of many independent simulations, make these methods too computationally demanding to be routinely used in drug design^{3,4} (however, for relative binding energies of ligands differing in only a single group, the convergence is better⁵).

Consequently, more approximate methods to estimate ligand-binding affinities have been developed. One group of methods sample only the end-points of the reaction in Eqn. 1, i.e. the free protein, the free ligand, and the complex, and are thus computationally much cheaper. For the electrostatic contribution, such approaches are often based on the linear-response approximation (LRA),⁶ according to which only the charged and uncharged states of the ligand need to be simulated and the electrostatic contribution can be calculated as

$$\Delta G_{\text{ele}}^{\text{LRA}} = 0.5 \left(\langle E_{\text{ele}}^{L-S} \rangle_{PL} + \langle E_{\text{ele}}^{L-S} \rangle_{PL'} - \langle E_{\text{ele}}^{L-S} \rangle_L - \langle E_{\text{ele}}^{L-S} \rangle_{L'} \right) \quad (2)$$

where E_{ele}^{L-S} is the electrostatic interaction energy between the ligand and the surroundings (S; protein and water), the $\langle \rangle$ brackets indicate ensemble averages, and the subscripts of the brackets indicate the simulation over which the average is calculated: PL and L indicate normal simulations of the complex or of the ligand free in solution, respectively, whereas PL' and L' indicate the corresponding simulations in which the ligand charges have been zeroed.

As the name implies, the LRA is based on the observation that the free-energy change is approximately linear with respect to the ligand charge. Unfortunately, the same does not apply for non-electrostatic part. The linear interaction energy (LIE) method⁷ suggests that the latter part can be calculated from the difference in the average van der Waals interaction energies between the ligand and the surroundings, E_{vdw}^{L-S} , in simulations of the complex and the free ligand. Furthermore, the LIE approach goes beyond the LRA by assuming that the electrostatic energies from the PL' and L' simulations can be neglected. Moreover, the constant of 0.5 in the LRA is often taken as an adjustable parameter, α . Hence, in the LIE method, the total binding free energy is calculated from

$$\Delta G_{\text{bind}}^{\text{LIE}} = \alpha \left(\langle E_{\text{ele}}^{L-S} \rangle_{PL} - \langle E_{\text{ele}}^{L-S} \rangle_L \right) + \beta \left(\langle E_{\text{vdw}}^{L-S} \rangle_{PL} - \langle E_{\text{vdw}}^{L-S} \rangle_L \right) \quad (3)$$

Usually α is between 0.33 and 0.5, depending on the charge and number of hydrogen-bond donors of the ligand, and β is taken to be 0.18,^{8,9} but in many cases, better results are obtained by using different α and β for each drug target.^{10,11}

The LRA and LIE methods are both based on interaction energies obtained from microscopic calculations. Such simulations typically show a slow convergence, meaning that long simulations are needed before the results become stable and reliable.¹ Therefore, many attempts have been made to improve the convergence and speed up the calculations by using continuum-solvation methods. In particular, Warshel and coworkers have suggested the PDLd/s-LRA/ β method (semi-macroscopic protein-dipoles Langevin-dipoles method within a linear-response approximation).^{6,10} In this approach, the LRA (Eqn. 2) is used to calculate the electrostatic contributions, and in the E_{ele}^{L-S} terms, the interaction between the ligand and water is calculated with the semi-microscopic Langevin-dipoles (LD) approach, in which dipoles are placed on a cubic grid (excluding grid points close to the protein or ligand) and their electrostatic response is calculated from the Langevin equation.¹² The non-electrostatic part is borrowed from the LIE method, as is indicated by the symbol β in the abbreviation.

The MM/PBSA method (molecular-mechanics with Poisson–Boltzmann and surface-area solvation) follows a similar approach.^{13,14} In this method, the binding free energy in Eqn. 1 is calculated from

$$\Delta G_{\text{bind}} = \langle G_{PL} \rangle - \langle G_P \rangle - \langle G_L \rangle \quad (4)$$

where the brackets indicate ensemble averages. Each free energy in Eqn. 4 is calculated from

$$G = E_{\text{bnd}} + E_{\text{vdW}} + E_{\text{ele}} + \Delta G_{\text{solv}} + \Delta G_{\text{np}} - TS_{\text{MM}} \quad (5)$$

where E_{bnd} is the bonded molecular mechanics (MM) energy, i.e. the MM energy from the bond, angle, and dihedral terms, E_{vdW} is the MM van der Waals energy, E_{ele} is the MM electrostatic energy, ΔG_{solv} is the polar solvation free energy, calculated by a continuum solvation method, ΔG_{np} is the non-polar solvation free energy, taken from a linear relation to the solvent-accessible surface area, T is the absolute temperature, and S_{MM} the total entropy, consisting of translational, rotational, and conformational contributions. The Poisson–Boltzmann (PB) continuum solvation method can be replaced by any other continuum solvation method and a common choice is the generalised Born method¹⁵ (in which case the method is called MM/GBSA).

Strictly, the averages in Eqn. 4 should be calculated from three separate simulations, i.e. from molecular dynamics (MD) simulations of the complex, the free protein, and the free ligand, i.e.

$$\Delta G_{\text{bind}} = \langle G_{PL} \rangle_{PL} - \langle G_P \rangle_P - \langle G_L \rangle_L \quad (6)$$

where the subscripts of the brackets indicate from what simulation the ensemble average is calculated. This approach will be called three-average MM/PBSA (3A-MM/PBSA) in the following. A much more common approach is to simulate only the complex, whereas the structures of the free protein or the free ligand are obtained by simply deleting either the ligand or the protein from each snapshot of the simulations of the complex.¹¹

$$\Delta G_{\text{bind}} = \langle G_{PL} \rangle_{PL} - \langle G_P \rangle_{PL} - \langle G_L \rangle_{PL} = \langle G_{PL} - G_P - G_L \rangle_{PL} \quad (7)$$

We will call this approach one-average MM/PBSA (1A-MM/PBSA) in the following. It requires fewer simulations, but it also leads to an exact cancellation of E_{bnd} in Eqn. 4 and a strongly increased precision of the method, owing to decreased fluctuations in all the energy terms.

Pearlman has compared the 1A and 3A-MM/PBSA approaches¹⁶ for a set of 16 ligands binding to p38MAP and found that 3A-MM/PBSA in fact gave more accurate results. However, the uncertainty in the binding free energy of the 3A-MM/PBSA was a factor of ~ 5 larger than that in the 1A approach, making it unclear whether the improved accuracy is statistically significant. Swanson et al. also used both approaches, but they did not explicitly compare the final free energies.¹⁷ They argued against the 3A approach, because it introduces noise from insufficient sampling and from errors inherent in the force field and the continuum-solvation method. Instead, they suggested a two-trajectory approach in which both the complex and the free ligand are simulated, because it is easier to obtain complete sampling of the free ligand. Thereby, the reorganisation free energy of the ligand (the difference in E_{bnd} between the bound and free energies) can be obtained. A similar argument has recently been put forward and it was argued that this reorganisation energy is important for obtaining accurate results.¹⁸

Both the PB and GB methods assign a dielectric constant to each point in space. For the bulk solvent, this is a well-defined property, ϵ_{ext} . However, for the protein and the ligand, the internal dielectric constant, ϵ_{int} is not well-defined, because they are not uniform electrostatic media. Usually, $\epsilon_{\text{int}} = 1$ in MM/PBSA calculations, but this value has been much discussed.^{1,19,20,21} Results obtained with $\epsilon_{\text{int}} = 1$ –10 have been reported.^{11,22,23} ϵ_{int} can be considered to be a compensation factor for the interaction that are neglected in the continuum method, and as such, it is more a method-dependent parameter than a physical constant.^{21,24} For instance, if all types of interactions are included in the simulations, ϵ_{int} should be set to 1, as it is in explicit-water simulations.¹ The standard value of 1 in the MM/PBSA method is based on the argument that the calculations should be compatible with the explicit-water simulations.¹⁴ A recent MM/GBSA study using only minimised structures, i.e. without any sampling, attempted a systematic investigation of the value of ϵ_{int} , but it was concluded that $\epsilon_{\text{int}} = 1$ gave the best ranking of the binding affinities.²⁵ On the other hand, a study of the binding of 156 ligands to seven different proteins with full sampling but no entropy term gave the best results with $\epsilon_{\text{int}} = 4$.²² In an investigation of the binding affinities of 59 ligands to six proteins, the results were best with $\epsilon_{\text{int}} = 1$ for three proteins, with $\epsilon_{\text{int}} = 2$ for one protein, and with $\epsilon_{\text{int}} = 4$ for the other two proteins.²³

The PDLd/s-LRA/ β approach also employs ϵ_{int} to make the energies more stable (the electrostatic interactions are scaled down by $1/\epsilon_{\text{int}}$, thereby reducing the fluctuations with the same amount).^{1,21} Typically, a rather low ϵ_{int} (2–4) is recommended for neutral ligands and larger values (~ 25) for charged ligands.^{1,10,24} Warshel has also suggested that MM/PBSA should also gain from scaling down the electrostatic interactions by using $\epsilon_{\text{int}} > 1$,¹⁰ which is supported by the fact that MM/PB(GB)SA often gives a too large range of calculated binding affinities for a set of ligands compared to experiment estimates²⁶ and that MM/PB(GB)SA is missing the scaling factor of electrostatic interaction in LRA and LIE ($\alpha = 0.33$ –0.5).

It has been argued that MM/PBSA is a simplified version of the PDLd/s-LRA/ β , in which the apolar states (PL' and L') have been ignored.¹⁰ The entropy estimate and the lack of a strict theoretical foundation have also been criticised.^{10,27} As both MM/PBSA and PDLd/s-LRA/ β are based on a continuum treatment, it would be of interest to compare their performance. Warshel et al. recently

compared the PDL D/s-LRA/ β , LRA/ β , and LIE methods, and concluded that PDL D/s-LRA/ β was most efficient and did not require any protein-specific parameterisation of β .¹⁰ They also included MM/PBSA in the comparison, but only with literature values, which introduces the risk that the observed differences may come from differences in the force field, simulation set-up, and continuum models used (and also from the large statistical uncertainty in standard MM/PBSA results²⁸), rather than to differences in the approaches. However, the results indicated that MM/PBSA gives a too negative entropy by a factor of ~ 50 kJ/mol. Of course, this is of great importance when estimating absolute binding affinities. However, recent investigations have shown that some additive terms are missing in standard MM/PBSA²⁹ and that different solvation methods give widely different absolute affinities.³⁰ Therefore, MM/PBSA should probably be restricted to calculations of relative affinities of similar ligands with the same charge, for which most available continuum solvation methods give similar results.³¹

In this paper we present a comparison of LRA, LIE, and MM/PBSA-based approaches on a more equal footing, using the same force field and the same simulations. Moreover, we use the same continuum solvation methods, either PB or GB. Thereby, we obtain a new MMPB(GB)-LRA/ β variant of PDL D/s-LRA/ β . Finally, we employ our recent approach to obtain a statistical precision of ~ 1 kJ/mol^{28,32} and use statistical methods to compare the various approaches. For all methods, we test five different values of the internal dielectric constant in order to evaluate the effect of scaling of electrostatic interactions. We also compare the 1A- and 3A- variants of MM/PBSA. Finally, we combine the electrostatic and non-electrostatic parts from different methods in an attempt to identify strengths and weaknesses of the various methods. As test cases we use the binding of biotin and six analogous to the avidin tetramer and the binding of nine 3-amidinobenzyl-1*H*-indol-2-carboxamide inhibitors to factor Xa. These system are well-characterised by X-ray crystallography^{33,34} and experimental binding affinities are available.^{35,36,37,34} They have been extensively studied previously with various theoretical methods.^{27,30,32,38,39,40,41,42,43,44,45,46}

Methods

MM/PB(GB)SA

The MM/PBSA and MM/GBSA energies were calculated from Eqns. 4–7 with infinite cut-off using the same force fields as in the MD simulations. ΔG_{solv} was calculated by solving the Poisson–Boltzmann equation or with a generalised Born method. The Poisson–Boltzmann calculations were performed with the Delphi program.⁴⁷ The calculations employed the Parse radii⁴⁸ for all atoms, a grid spacing of 0.5 Å, a fill ratio of 90%, and a probe radius of 1.4 Å. For the GB calculations, we used the generalised Born method of Onufriev, Bashford, and Case, model I (OBCI)⁴⁹, i.e. with $\alpha = 0.8$, $\beta = 0$, and $\gamma = 2.91$. For these calculations, the second set of modified Bondi radii (mbondi2) was used.⁴⁹ ΔG_{np} was in all cases calculated from a linear relation to the solvent-accessible surface-area (SASA): $\Delta G_{\text{np}} = 0.0227 * \text{SASA}$ (in Å²) – 3.85 kJ/mol. The vibrational entropy was calculated from the harmonic frequencies, calculated at the MM level. They were obtained for a truncated and buffered system (8 + 4 Å), as has been detailed previously to increase the precision.⁴⁶ The temperature (T in Eqn. 5) was set to 300 K.

Sometimes, it is appropriate to divide the MM/PBSA estimates into electrostatic (ΔG_{ele}) and non-electrostatic contributions ($\Delta G_{\text{non-ele}}$) so that

$$\Delta G_{\text{bind}} = \Delta G_{\text{ele}} + \Delta G_{\text{non-ele}} \quad (8)$$

The electrostatic part is then taken to be the sum of the electrostatic energy, E_{ele} , and the polar solvation free energy, ΔG_{solv} , whereas the non-electrostatic part is the sum of the other four terms in Eqn. 5.

It is non-trivial to change the internal dielectric constant when using the mm_pbsa.pl script in Amber. There is no less than three internal dielectric constant that can be set, DIELC for scaling the electrostatic interactions, INDI for scaling the PB energies (when using Delphi), and INTDIEL for scaling the GB energies. DIELC is not necessary to set, because E_{ele} is a linear term that can be scaled afterwards (i.e. only a single calculation is needed). However when changing INDI, DIELC needs to be changed accordingly, whereas when INTDIEL is changed, INDI has no longer any effect. In practice, we obtained E_{ele} and G_{solv} in this paper running the mm_pbsa.pl script twice for each ϵ_{int} . In the first calculation, $G_{\text{solv}}(\text{GB})$ and unscaled E_{ele} were obtained by setting DIELC = 1 and INTDIEL = ϵ_{int} . In the second calculation, $G_{\text{solv}}(\text{PB})$ and scaled E_{ele} were obtained by setting both DIELC and INDI to ϵ_{int} .

MMPB/s-LRA/ β

The MMPB(GB)/s-LRA/ β method tested in this paper is a simple adaptation of the PDLD/s-LRA/ β method by Warshel and coworkers,^{38,24} in which the Langevin-dipole solvation method is replaced by the PB or GB solvation methods. In this approach the binding free energy of a ligand to a protein is divided into electrostatic and non-electrostatic contributions. The electrostatic contribution is calculated within a LRA framework,²⁴ as

$$\Delta G_{\text{ele}}^{\text{LRA/s}} = 0.5 \left(\langle U_{\text{ele}}^{\text{bound}} \rangle_{\text{PL}} + \langle U_{\text{ele}}^{\text{bound}} \rangle_{\text{PL}'} - \langle U_{\text{ele}}^{\text{free}} \rangle_{\text{L}} - \langle U_{\text{ele}}^{\text{free}} \rangle_{\text{L}'} \right) \quad (9)$$

This is identical to Eqn. 2, besides that effective electrostatic potentials U_{ele} are more involved, including continuum solvation and scaling by an effective internal dielectric constant. They can be derived from the thermodynamic cycles in Figure 1 and are calculated as²⁴

$$U_{\text{ele}}^{\text{bound}} = \left(\Delta G_{\text{solv}}^{\text{PL}} - \Delta G_{\text{solv}}^{\text{PL}'} \right) \left(\frac{1}{\epsilon_{\text{int}}} - \frac{1}{\epsilon_{\text{ext}}} \right) + \Delta G_{\text{solv}}^{\text{L}} \left(1 - \frac{1}{\epsilon_{\text{int}}} \right) + \frac{E_{\text{ele}}^{\text{L}}}{\epsilon_{\text{int}}} + \frac{E_{\text{ele}}^{\text{L-S}}}{\epsilon_{\text{int}}} \quad (10)$$

$$U_{\text{ele}}^{\text{free}} = \Delta G_{\text{solv}}^{\text{L}} \left(\frac{1}{\epsilon_{\text{int}}} - \frac{1}{\epsilon_{\text{ext}}} \right) + \Delta G_{\text{solv}}^{\text{L}} \left(1 - \frac{1}{\epsilon_{\text{int}}} \right) + \frac{E_{\text{ele}}^{\text{L}}}{\epsilon_{\text{int}}} \quad (11)$$

where $E_{\text{ele}}^{\text{L-S}}$ and $E_{\text{ele}}^{\text{L}}$ are the intermolecular and intramolecular electrostatic energy of the ligand, respectively (in comparison with Eqn. 5, note that $E_{\text{ele}}^{\text{PL}} = E_{\text{ele}}^{\text{P}} + E_{\text{ele}}^{\text{L}} + E_{\text{ele}}^{\text{L-P}}$, where $E_{\text{ele}}^{\text{P}}$ is the intramolecular electrostatic energy of the protein).

The non-electrostatic part is taken from the LIE method⁴⁹ and is calculated as

$$\Delta G_{\text{non-ele}}^{\text{LIE}} = \beta \left(\langle E_{\text{vdw}}^{\text{L-S}} \rangle_{\text{PL}} - \langle E_{\text{vdw}}^{\text{L-S}} \rangle_{\text{L}} \right) \quad (12)$$

where β is taken to be 0.18.^{8,9}

Binding analysis

Because avidin is a tetramer, three different binding reactions may be studied:



For the first two reactions, there are four different possibilities for PL or PL₃ (there are four different binding sites that can be either filled or empty) and each of them need to be sampled, which is computational demanding. However, for the last reaction, both the P and PL₄ species are unambiguous, so only a single simulation is needed. Therefore, we choose to study that reaction. The binding affinity of a single ligand is obtained by dividing the overall binding affinity by four, assuming that the four binding sites are equivalent (in agreement with experiments^{35,36,37}). For factor Xa (fXa), the first reaction is studied, because only a single ligand binds to fXa.

System preparation

The avidin tetramer and the seven ligands (shown in Figure 2) were prepared as described previously.⁴¹ The simulations were based on the crystal structure of avidin in complex with biotin (Btn1; PDB file 1avd).⁴⁸ The structures of the other ligands were obtained by manually modifying biotin (which in all cases, except one involved the deletion of atoms or conversion of a heavy atom into another).⁴¹ All ionisable residues in the protein were assigned their standard protonation states at pH 7 and the single histidine residue in each subunit was protonated on the NE2 atom.

The fXa protein and the nine inhibitors (also shown in Figure 2) were prepared as described previously.⁴⁶ The simulations were based on the crystal structure of fXa in complex with ligand **125** (PDB file 1lpk).³⁴ Again, the structures of the other ligands were obtained by small manual modifications of ligand **125**. All ionisable residues were assigned their standard protonation states at pH 7 and the histidines were protonated in the following way: Residues 57 and 83 were protonated on the ND1 atoms, residues 91, 145, and 199 on the NE2 atom, and residue 13 on both atoms.

The proteins were described by the Amber99SB force field⁵⁰ and the ligands by the Amber 1999 force field (avidin ligands) or the generalised Amber force field (fXa ligands) with charges derived from RESP calculation.^{51,52,53} The protein–ligand complex, the free protein, and the ligands were all immersed in a truncated octahedral box of TIP4P-Ewald waters extending at least 10 Å from the solute.⁵⁴ These water molecules were stripped off before the energies in Eqns. 5, 10, and 11 were calculated, whereas they were kept when the energies in Eqn. 12 were calculated (the non-electrostatic part of LIE).

Simulations

The MD simulations were based on the following protocol: The system was first energy minimised with 100 cycles of steepest descent, keeping all atoms, except water molecules and hydrogen atoms, restrained to the crystal structure with a harmonic force constant of 418 kJ/mol/Å², followed by a 20 ps *NPT* simulation with the same restraints and a 100 ps unconstrained *NPT* equilibration. This equilibration was followed by a 200 ps unconstrained *NPT* production run, in which snapshots were saved every 5 ps, a conservative estimate of the correlation time of the MM/GBSA energies.^{39,55} The ensemble averages in Eqns. 2, 6, 7, and 9 were calculated from these snapshots. All reported values are averages over 20 independent MD simulations, obtained by using different random starting velocities. Likewise, reported standard errors (SE) are the standard deviations of the results obtained in these 20 simulations, divided by $\sqrt{20}$. This protocol has been shown to give good results for avidin–biotin system.^{44,45} The fXa complexes were simulated in a slightly different way: A single copy of each system was first equilibrated using the procedure described above, except that a 1 ns unconstrained *NPT*

equilibration was used. Thereafter, 40 independent simulations were started from this equilibrated system by assigning different starting velocities. These simulations were equilibrated for another 100 ps, followed by a 200 ps *NPT* production run. This protocol has shown to give good results for the fXa systems.^{44,45}

All MD simulations were run using the sander module of Amber 10.⁵⁶ The SHAKE algorithm⁵⁷ was used to constrain bonds involving hydrogen atoms, allowing a time step of 2 fs. The temperature was kept constant at 300 K in all MD simulations using a Langevin thermostat⁵⁸ with a collision frequency of 2.0 ps⁻¹ and the pressure was kept constant at 1 atm using a weak-coupling isotropic algorithm⁵⁹ with a relaxation time of 1 ps. Particle-mesh Ewald summation,⁶⁰ with a fourth-order B spline interpolation and a tolerance of 10⁻⁵, was used to handle long-range electrostatics. The non-bonded cut-off was 8 Å and the non-bonded pair list was updated every 50 fs.

Results and Discussion

Electrostatic contributions

We have estimated the binding free energy of seven biotin analogues to the avidin tetramer and nine inhibitors to fXa (Figure 2) using several approximate end-point approaches that employ continuum solvation methods. Each binding-affinity method consists of a electrostatic and a non-electrostatic term (Eqn. 8). For each of the two terms, we test three different variants as is summarised in Table 1. For each variant, two different continuum-solvation methods were used, GB or PB and five different values of the internal dielectric constant of the protein (ϵ_{int}) were tested, viz., 1, 2, 4, 10, and 25.

We start by considering the electrostatic contribution to the binding free energy (ΔG_{ele}). This energy is shown in Table 2 for the avidin test case and the 1A-MM/PB(GB), 3A-MM/PB(GB), and MMPB(GB)/s-LRA methods (cf. Table 1). Btn1–Btn3 have a net charge of $-1 e$, whereas the other four biotin analogues are neutral. As the charge determines the response to the varying dielectric constant, we will start by discussing the charged ligand. Interestingly, the MM/GB and MM/PB results show an opposite dependence on ϵ_{int} : The MM/PB energies become more negative, whereas the MM/GB energies become more positive as ϵ_{int} is increased. For $\epsilon_{\text{int}} \geq 10$, both methods give the same ΔG_{ele} within 3 kJ/mol. The LRA-based methods show another dependence: The MMGB/s-LRA estimates become more negative, whereas the MMPB/s-LRA estimates become more positive (not Btn2). For MM(GB)PB/s-LRA, the GB and PB estimates do not converge to the same value, nor do they converge to the same value as the MM/PB(GB) methods. At $\epsilon_{\text{int}} = 25$, the MM/GB and MMGB/s-LRA estimate differ by more than 60 kJ/mol, whereas the MM/PB and MMPB/s-LRA results differ by 2–12 kJ/mol.

For the uncharged ligands, the trends are less clear, but with PB, all ligands give more negative ΔG_{ele} with increasing ϵ_{int} , except Btn7 with LRA. With GB, six of the cases give the same trend and the other six give the opposite trend. For all methods, the GB and PB results at $\epsilon_{\text{int}} = 25$ agree within 1 kJ/mol for all ligands, except Btn4 and Btn7 with LRA and Btn7 with 1A-MM/PB(GB). The electrostatic contribution is close to 0 kJ/mol at $\epsilon_{\text{int}} = 25$ for all methods, except for the same three cases.

The average standard error (SE) of the electrostatic contributions over the seven biotin analogues is also shown in Table 2 (there is no correlation between the ligand net charge and the SEs for each ligand). For 1A-MM/PB(GB) the SE is 0.1–1 kJ/mol and for MM/PB(GB)/s-LRA, it is 0.5–3 kJ/mol (i.e. typically 2–3 times larger). For the 3A-MM/PB(GB) method, the SE is 3–6 times larger than for the corresponding 1A variant, which is the main reason why the 3A method has been little used. For all methods, PB gives a somewhat higher SE than GB and the SE decreases with increasing

ϵ_{int} .

The electrostatic contributions for the fXa inhibitors are shown in Table S1. All of the inhibitors are positively charged: C39, C57 and C63 have a single positive charge, whereas the others are doubly charged. For all the PB-based method, ΔG_{ele} becomes more negative with increasing ϵ_{int} , with the exception of C49 for MMPB/s-LRA. The 1A-MM/GB method shows the same trend while 3A-MM/GB and MMGB/s-LRA show varying trends. There is no correlation between the net charge and the trends. The SEs are similar to those for the avidin test case, but the 3A approach gives 7–11 times larger SEs than the 1A approach and the SEs of the LRA-based methods increase with ϵ_{int} .

The results in Table 2 show that the 3A-MM/PB(GB) and MMPB(GB)/s-LRA methods sometimes give quite different results. We have tried to understand this difference by comparing them term-wise (Table S2). Unfortunately, it turns out that most of the terms show differences that are much larger than the net difference in the electrostatic energy (e.g. up to 894 kJ/mol, compared to 6–18 kJ/mol difference between 3A-MM/GB and MMGB/s-LRA with $\epsilon_{\text{int}} = 1$), owing to the fact that the MMPB(GB)/LRA terms are based on simulations both with a normal ligand and with a ligand with zeroed charges, and that the solvation terms are scaled by a factor of $(1/\epsilon_{\text{int}} - 1/\epsilon_{\text{ext}})$. Likewise, there is an extensive cancellation of the difference among the terms, perhaps best illustrated by the ΔE_{ele}^P term that is ignored in MMPB(GB)/LRA, although it amounts to 52–433 kJ/mol in 3A-MM/GB.

Non-electrostatic contribution

Next, we turn to the non-electrostatic contribution, for which we can compare three approaches: 1A-MM/SA, 3A-MM/SA, and LIE, i.e. $\Delta E_{\text{bnd}} + \Delta E_{\text{vdW}} + \Delta G_{\text{np}} + T\Delta S_{\text{MM}}$ for MM/SA and $\Delta G_{\text{non-ele}}$ in Eqn. 12 for LIE (cf. Table 1; note that the non-electrostatic energy is independent of the solvation model and the internal dielectric constant). The results are shown in Table 3 for the avidin test case. There is a fair correlation between the 1A- and 3A-MM/SA ($r^2 = 0.74$). The 1A results are 18 kJ/mol more positive on average, but the individual differences range from 42 kJ/mol for Btn2 to –15 kJ/mol for Btn4.

There is also a fair correlation between the 3A-MM/SA and LIE estimates ($r^2 = 0.63$). The 3A-MM/SA results are 70 kJ/mol more negative on average and the individual differences range from –19 kJ/mol for Btn7 to –97 kJ/mol for Btn4. The LIE estimates show a much smaller variation among the seven ligands (–7 to –21 kJ/mol, compared to –26 to –116 kJ/mol), owing to the scaling by $\beta = 0.18$. On the other hand, 3A-MM/SA shows a smaller variation than 1A-MM/SA (–1 to –130 kJ/mol).

The average standard errors over the seven biotin analogues are also shown in Table 3. It can be seen that the 1A-MM/SA estimates have a precision of 1 kJ/mol, i.e. similar to that of the electrostatic contribution. The precision of the 3A-MM/SA estimate is much worse, 7 kJ/mol, which is slightly larger than for the electrostatic contribution. The LIE method has the smallest uncertainties, 0.2 kJ/mol, again owing to the scaling by $\beta = 0.18$.

If we instead look at the fXa test case (results shown in Table S3), the correlation between 1A- and 3A-MM/SA is much worse ($r^2 = 0.22$), but the average difference (26 kJ/mol) is comparable to that in the avidin test case. The correlation between the 3A-MM/SA and the LIE estimates is slightly larger ($r^2 = 0.39$) and the average difference is –100 kJ/mol. The standard errors of the 1A-MM/SA and the LIE methods are similar to those in the avidin test case, whereas those of the 3A-MM/SA results are slightly larger (9 kJ/mol). The largest difference between the two test cases is that the range of the non-electrostatic energies over the seven or nine ligands is much smaller for fXa for the 1A-MM/SA and LIE methods (14 and 3 kJ/mol, compared to 129 and 14 kJ/mol, respectively), whereas for the 3A-MM/SA method, the ranges are similar, 89 and 92 kJ/mol.

The two MM/GBSA methods

The most interesting results are the total binding free energies (i.e. the sum of the electrostatic and non-electrostatic energies, cf. Table 1), which can be compared with experimental data.³⁶ In Table 4, the various methods based on GB solvation, obtained with five different values of the internal dielectric constant are compared, using four different quality measures, viz. the mean absolute deviation (MAD), the MAD after removal of systematic errors (i.e. after subtraction of the signed average; MADtr), Pearson's correlation coefficient (r^2), and the predictive index (PI).⁶¹ The MAD estimates the quality of the absolute binding affinities, whereas the other three measures estimate the quality of the relative affinities within each set of ligands. The latter is normally of the prime interest in ligand design, so we will pay more attention to the latter three measures. The corresponding raw data are shown in Tables S4–S12 in the supporting material.

We start by considering the 1A-MM/GBSA method, which has become the standard MM/GBSA procedure. Figure 3 shows the experimental and calculated free energies for the seven ligands. Detailed results with precision estimates are shown in Table S4. Figure 3 and Table 4 show that the best results are obtained with $\epsilon_{\text{int}} = 1$: Both r^2 and PI decrease drastically if ϵ_{int} is increased, whereas MADtr increases. This is caused mainly by a deterioration of the estimate of Btn4. However, the absolute errors decrease slightly when ϵ_{int} is increased, as is manifested by a decreased MAD. The precision of the free-energy estimates is ~ 1 kJ/mol, and it improves slightly with increased ϵ_{int} . This is expected because as we increase the scaling of the electrostatic terms, we also scale down the uncertainties of these estimates.

Next, we consider the 3A-MM/GBSA approach. This method is seldom used because it requires more simulations (one for the free protein and one for each ligand free in solution) and it gives more noisy data that result in poorer precision.⁴³ However, from a theoretical point of view, it should be more accurate because it explicitly considers ligand and protein reorganisation.^{42,50} The results of this approach are shown in Figure 4 and detailed results can be found in Table S5. From Table 4 it can be seen that a larger systematic error is introduced by using this approach: MAD is larger than for 1A-MM/GBSA, whereas MADtr is slightly smaller. Likewise, both r^2 and PI are better with 3A than with 1A-MM/GBSA. However, the standard error has increased to 7–9 kJ/mol because we have less error cancellation than in the 1A approach. As with 1A-MM/GBSA, the results deteriorates with increased ϵ_{int} , although the precision is somewhat improved with increased ϵ_{int} .

The corresponding results for the fXa test case are shown in Table 5. Unfortunately, this test case gives essentially the opposite result: The 3A-MM/GBSA results are mostly worse than the 1A-MM/GBSA results, especially for low values of ϵ_{int} , and both method becomes better if we increase ϵ_{int} . The MADtr of 1A-MM/GBSA is particularly low for this test case ~ 3 kJ/mol (but MADtr much worse with the 3A-MM/GBSA approach). The reason for this is that the range of the experimental affinities is much smaller for fXa, 20 kJ/mol, compared to 67 kJ/mol for avidin (and eight of the nine fXa inhibitors have affinities within 9 kJ/mol). This means that the null-hypothesis that all ligands have the same affinity actually gives a MADtr of 4 kJ/mol for fXa, but 20 kJ/mol for avidin. Thus, all methods that give a small enough variation among the nine fXa will also give a good MADtr. Therefore, it is more important to consider r^2 and PI, which are not affected by the small range of experimental affinities. From Table 5, it can be seen that 1A-MM/GBSA corresponding result gives a better r^2 than 3A-MM/GBSA, whereas the opposite is true for the PI. Both quality measures are best for $\epsilon_{\text{int}} = 4$ –10.

Comparing Eqns. 6 and 7, it can be seen that the difference between the 1A and 3A approaches comes from two terms $\delta G_{\text{re}}^{\text{P}} = \langle G_{\text{P}} \rangle_{\text{PL}} - \langle G_{\text{P}} \rangle_{\text{P}}$ and $\delta G_{\text{re}}^{\text{L}} = \langle G_{\text{L}} \rangle_{\text{PL}} - \langle G_{\text{L}} \rangle_{\text{L}}$, which have been called the reorganisation free energies of the protein and the ligand, respectively.⁵⁰ The latter term has recently

been investigated for the binding of 31 ligands to the X-linked inhibitor of apoptosis protein and it was concluded that it is important for accurate estimates of binding free energies.⁵⁰ In that study, they obtained ligand reorganisation energies between 0 and 33 kJ/mol. The corresponding results for the biotin analogous are shown in Table 6. It can be seen that $\delta G_{\text{re}}^{\text{L}}$ ranges between 0 and 22 kJ/mol and that the difference is uncorrelated to the size of the ligand. The number of rotatable bonds for the seven ligands are 5, 5, 6, 7, 6, 6, and 0, respectively and there is no correlation to this property either, as noted in Ref. 50 as well. $\delta G_{\text{re}}^{\text{L}}$ is insensitive to of the internal dielectric constant and to the solvation method (variation up to 2 kJ/mol). Most of the reorganisation energy comes from the non-electrostatic part, especially the entropy term. Similar conclusions can be drawn for the fXa test case, as can be seen in Table S12, although there are somewhat larger effects from E_{bnd} and E_{vdW} , as well as from ϵ_{int} (up to 18, 10, and 5 kJ/mol, respectively).

The protein reorganisation energy $\delta G_{\text{re}}^{\text{P}}$, which is shown in Table 7 for avidin, is much larger, ranging from -218 to 19 kJ/mol. Six of the ligands have a large negative protein reorganisation energy, whereas Btn4 has a much smaller and positive reorganisation energy. For the charged ligands, the largest contributions come from the non-electrostatic part, with only a modest contribution from the electrostatic part. For Btn5 and Btn6, there is a much larger contribution from the electrostatic part (for small ϵ_{int}). For the fXa test case (shown in Table S13), the electrostatic part dominates the protein reorganisation energy for six of the ligands, but the opposite is true for the other three.

MMGB/s-LRA/ β

The results of the MMGB/s-LRA/ β method for avidin are shown in Figure 5 and details can be found in Tables 4 and S6. It can be seen that for the optimum $\epsilon_{\text{int}} = 1$, the method performs slightly better than the MM/GBSA methods, with $\text{MAD}_{\text{tr}} = 8$ kJ/mol, $r^2 = 0.85$, and $\text{PI} = 0.89$. The standard error is 0.5–2 kJ/mol, which is similar to that of the 1A-MM/GBSA method.

For the fXa test case, shown in Table 5, the MMGB/s-LRA/ β results are worse. The best results are still obtained with $\epsilon_{\text{int}} = 1$, but they are always worse than for 1A-MM/GBSA. In fact, for $\epsilon_{\text{int}} > 1$ MMGB/s-LRA/ β even gives negative PIs and negative correlations. It is also notable that the standard error increases with ϵ_{int} , in variance to the MM/GBSA methods.

Singh and Warshel recently compared the results of the PDL/D/s-LRA/ β method with literature values of MM/PBSA for two biotin analogues (Btn2 and Btn3) and five HIV1-RT inhibitors, and argue that the former results are superior.⁴⁰ They fit β for each target protein (i.e. to two values for avidin) and use $\epsilon_{\text{int}} = 4$ for neutral ligands and $\epsilon_{\text{int}} = 25$ for charged ligands. However, β has a quite small effect on the quality estimates, as can be seen in Table S14. On the other hand, the results for the two common ligands differ considerably between our calculations and those in Ref. 40. For MMGB/s-LRA/ β with $\epsilon_{\text{int}} = 25$ and $\beta = 0.33$, we obtain -189 and -116 kJ/mol for Btn2 and Btn3, respectively, (and -66 and -48 kJ/mol with PB solvation) whereas they obtained -61 and -59 kJ/mol. This shows that the results strongly depend on the system preparation, simulation protocol, and continuum-solvation method. Moreover, the results for fXa show that the performance of the various methods depends on the test system.

Other GB-based method

Both the MM/GBSA and MMGB/s-LRA/ β methods involve separate electrostatic and non-electrostatic terms. Therefore, additional methods can be constructed by combining the various terms as is shown in Table 1. For instance, we can combine the non-electrostatic terms from 3A-MM/GBSA with the

electrostatic terms from 1A-MM/GBSA (which show a better precision than the corresponding 3A terms). This approach will be called 1A-MM/GB-3A-MM/SA and the detailed results are collected in Table S7. From the quality measures in Tables 4 and 5, it can be seen that the accuracy is similar to that of 3A-MM/GBSA, which implies that the accuracy of the 3A-MM/GBSA method is limited by the non-electrostatic part. As expected, the precision is improved compared to the 3A-MM/GBSA method, but only marginally (to 7 kJ/mol for avidin and 9 kJ/mol for fXa), showing that the precision of the two terms is similar.

Furthermore, we can abandon the non-electrostatic part of MM/GBSA and replace it with the corresponding term from LIE. Depending on the number of averages used for the electrostatic part, we will call these approaches 1A-MM/GB β or 3A-MM/GB β . Detailed results of these methods can be found in Tables S8 and S9, and quality measures are given in Tables 4 and 5. For avidin, the best results are obtained for intermediate values of ϵ_{int} (4–10). For these values, the two approaches give similar results, and these are the best obtained for any method in this investigation, viz. MADtr = 7–9 kJ/mol, r^2 = 0.86–0.89, and PI = 0.92–0.99. However, for fXa, the best results are obtained with ϵ_{int} = 25 and the 1A-MM/GB β approach is better, but the results are only mediocre, e.g. with r^2 = 0.38.

Two additional methods can be formed by combining the electrostatic part from MMGB/s-LRA/ β with non-electrostatic part from either 1A-MM/GBSA or 3A-MM/GBSA. We will call these methods MMGB/s-LRA/1A-MM/SA and MMGB/s-LRA/3A-MM/SA. Detailed results of these methods can be found in Tables S10 and S11, and quality measures are shown in Tables 4 and 5. Both methods show only mediocre results compared to the other methods. For fXa, the best results are obtained with ϵ_{int} = 1–2, whereas for avidin, the various quality measures give differing trends. In general MMGB/s-LRA/3A-MM/SA gives better results than the 1A variant.

PB-based methods

Quality measures of all methods based on PB solvation are shown in Table 8 for the avidin test case (detailed results are given in Table S4–S12). It can be seen that the results are rather similar to those obtained with GB solvation (Table 4): The MMPB/s-LRA/MMSA methods give the best results with ϵ_{int} = 1, but they are worse than for some other methods. The MM/PBSA methods also give the best results at ϵ_{int} = 1, but with PB solvation, the 1A approach actually gives slightly better results than the 3A approach. All the other method gain from the use of a larger ϵ_{int} and the best results are typically obtained with a slightly larger value than with GB.

As with GB solvation, the best results are obtained with the 1A-MM/PB β and 3A-MM/PB β methods (with ϵ_{int} = 10), with MADtr = 7–8 kJ/mol, r^2 = 0.85–0.89, and PI = 0.91–0.99, i.e. similar to the best results obtained with GB solvation. The 1A-MM/PBSA and MMPB/s-LRA/ β methods (with ϵ_{int} = 1 and 2, respectively) give only slightly worse results, with MADtr = 9–10 kJ/mol, r^2 = 0.76–0.90, and PI = 0.90–0.96.

The SE of the various methods is consistently slightly higher with PB solvation than with GB solvation. However, the difference is quite small, 0.5 kJ/mol on average for ϵ_{int} = 1, but less than 0.1 kJ/mol ϵ_{int} > 4. Moreover, the trends are the same as with GB solvation. In particular, all methods involving 3A estimates have quite large SEs of 7–10 kJ/mol, whereas the other methods have SEs of less than 3 kJ/mol. All SEs are reduced when ϵ_{int} is increased, but the 3A-MM/PB β method gains most from the scaling and gives at ϵ_{int} = 25 a SE of 0.5 kJ/mol, which is lower than for all the other methods, except 1A-MM/PB β .

For the fXa test case (shown in Table 9), none of the methods give reasonable results, except the three MM/PBSA methods at high ϵ_{int} , with r^2 = 0.41–0.56 and PI = 0.55–0.73. However, only 1A-

MM/PBSA gives a low MAD_{tr} (4 kJ/mol). This is similar to the GB results, except that the 1A-MM/GB β method also gave reasonable results.

Conclusions

In this paper, we have investigated five related issues of great interest for the calculation of ligand-binding affinities with end-point methods involving continuum solvation models. First, we have compared the 1A and 3A approaches to obtain MM/PB(GB)SA binding affinities. Our results show that the performance of the two approaches depends on the test case and the solvation model: For avidin and GB solvation, the 3A-MM/GBSA approach gave better results than 1A-MM/GBSA. However, with PB solvation and for fXa, the opposite was observed. However, in all cases, the 3A approach gave 4–5 times larger standard errors than the 1A approach, meaning that 16–25 as many independent simulations are needed to reach a similar precision.

Second, we have compared the MM/PB(GB)SA approach to obtain non-electrostatic energies (involving bonded, van der Waals, SASA, and entropy terms from harmonic frequencies) with the LIE approach. The results in Tables 4 and 8 quite clearly show that for the binding of seven biotin analogues to avidin, the LIE approach gives better results than the MM/PB(GB)SA approach. It is possible that the entropy in MM/PBSA deteriorates the results, as has recently been suggested by Warshel.⁴⁰ However, for fXa, with its small (but more typical) range of binding affinities, the MM/PB(GB)SA approach gives the better results. Of course, the LIE results could be improved by fitting the β parameter for each drug target, as has been done before,⁴⁰ but then we start to move away from physics to statistical QSAR method.

Third, we have compared the MM/PB(GB) approach to obtain electrostatic binding energies from the electrostatic interactions and the continuum solvation energies with the stricter LRA approach. Interestingly, our results indicate that for both avidin and fXa, the former approach gives better results, implying that the MM/PB(GB) β combinations give the best results for avidin, whereas the pure MM/PB(GB)SA methods give the best results for fXa. The MM/PB(GB) approach also requires fewer simulations than the LRA approach. On the other hand, 1A-MM/PB β is the method with the best precision.

Fourth, we have studied the effect of varying the internal dielectric constant of the protein, ϵ_{int} . For our test cases, the MMPB(GB)/s-LRA/ β and MMPB(GB)/s-LRA/3A-MMSA methods seem to prefer low values of ϵ_{int} (1–2), whereas the two MM/PB(GB) β methods prefer large values of ϵ_{int} (4–25). For the other four methods, the preferred value depends on the test case or the solvation model. The optimum value of ϵ_{int} is typically slightly higher for PB than for GB solvation. In almost all cases, the precision is improved with increased ϵ_{int} , because we scale the two terms with lowest precision. The effect is especially pronounced for the 3A-MM/PB β method.

Finally, we have compared the results obtained with two continuum solvation methods, PB calculated with Delphi⁴⁷ and the GB^{OBCI} method in Amber.⁴⁹ These two methods gave the best PB and GB results in a previous investigation of more PB and GB methods with the 1A-MM/PB(GB)SA approach for the avidin test case (and the PB results were appreciably better than the GB results).³⁹ In this paper, we show that PB gives somewhat better results than GB for avidin and most methods (not 1A-MMPB-3A-MMSA). However, for fXa, GB gives better r^2 and PI for all methods (with optimum ϵ_{int}). PB also gives slightly larger standard errors than GB. The raw data in Tables S3–S12 show that the absolute binding affinities strongly depend on the continuum solvation method used for all methods (e.g. by up to 123 kJ/mol for MMPB(GB)/s-LRA/ β), in accordance with our previous results.³⁹ This shows that it is quite meaningless to discuss absolute binding energies for methods that contain a

continuum-solvation estimate.

The results in Tables 4 and 9 show that best results for avidin are obtained with the MMGB/s-LRA/ β , 1A-MM/PB(GB) β , 3A-MM/PB β , and 1A-MMGB-3A-MMSA methods. With optimum values of ϵ_{int} , all five methods give $\text{MAD}_{\text{tr}} = 7\text{--}8$ kJ/mol, $r^2 = 0.85\text{--}0.89$, and $\text{PI} = 0.89\text{--}0.99$, but there is a systematic error (MAD) of $17\text{--}23$ kJ/mol. However, for the more demanding fXa test case, only the 1A-MM/PB(GB)SA approach gives reasonable results ($\text{MAD}_{\text{tr}} = 3\text{--}4$ kJ/mol, $r^2 = 0.56\text{--}0.64$, and $\text{PI} = 0.55\text{--}0.72$).

The various methods have different computational demands. 1A-MM/PBSA requires a single MD simulation (of the complex), whereas 3A-MM/PBSA requires three MD simulations, viz. also of the free protein and the free ligand, although the former is the same for all ligands and the latter involves smaller simulated systems than the complex. The LIE approach involves two simulations (the complex and the free ligand), whereas the LRA approximation involves two additional simulations (the complex and the free ligand with zeroed protein charges). Therefore, the computational effort of the methods increases in the order 1A-MM/PB(GB)SA (1) < 1A-MM/PB(GB) β (2) < 3A-MM/PB(GB)SA = 1A-MMPB(GB)-3A-MMSA = 3A-MM/PB(GB) β (3) < MMPB(GB)/s-LRA/ β = MMPB/s-LRA/1A-MMSA = MMPB/s-LRA/3A-MMSA(4).

However, the precision of the methods needs also to be considered, because fewer independent simulations are needed for methods with a low standard error. For the five best methods, the standard error with the optimum ϵ_{int} are 0.8, 2.0, 0.3, 0.9, and 0.3 kJ/mol for 1A-MM/PB(GB)SA ($\epsilon_{\text{int}} = 10$ and 25 for GB and PB, respectively, for fXa), MMGB/s-LRA/ β ($\epsilon_{\text{int}} = 1$ for avidin), 1A-MM/(PB)GB β ($\epsilon_{\text{int}} = 4$ and 10 for GB and PB, respectively, for avidin), 3A-MM/PB β ($\epsilon_{\text{int}} = 10$ for avidin), and 1A-MMGB-3A-MMSA ($\epsilon_{\text{int}} = 1$ for avidin), respectively. This means that if a precision of 1 kJ/mol is intended, the various methods would require 13, 80, 2, 16, and 2 independent simulations, respectively. Finally, we should consider that the GB calculation is about three times faster than the corresponding PB calculation. Therefore, for the avidin test case, the best method in terms of accuracy and computational efficiency seems to be 1A-MM/GB β with $\epsilon_{\text{int}} = 4$. For fXa, 1A-MM/GBSA works best. On the other hand, the 3A and LRA approaches are expected to be theoretically more accurate, although it is not reflected in our results.

Acknowledgements

This investigation was supported by grants from the Swedish research council (grant 2010-5025) and from the FLÅK research school in pharmaceutical science at Lund University. It was also supported by computer resources of Lunarc at Lund University, HPC2N at Umeå University, and C3SE at Chalmers Technical University.

References

- 1 Warshel A, Sharma PK, Kato M, Parson WW. Modeling electrostatic effects in proteins. *Biochim Biophys Acta* 2006; 1764:1647-1676.
- 2 Deng Y, Roux B. Computations of Standard Binding Free Energies with Molecular Dynamics Simulations. *J Phys Chem B* 2009; 113:2234-2246.
- 3 Aleksandrov A, Thompson D, Simonson T. Alchemical free energy simulations for biological complexes: powerful but temperamental... *J Mol Recognit* 2010; 23: 117-127.
- 4 Chipot C, Rozanska X, Dixit SB. Can free energy calculations be fast and accurate at the same time? Binding of low-affinity, non-peptide inhibitors to the SH2 domain of the src protein. *J Comput-Aided Mol Design* 2005; 19: 765-770.
- 5 Zeevaart JG, Wang L, Thakur VV, Leung CS Tirado-Rives J, Bailey CM, Domaoal RA, Anderson KS, Jorgensen WL.

-
- Optimization of azoles as anti-human immunodeficiency virus agents guided by free-energy calculations. *J Am Chem Soc* 2008; 130:9492-9499.
- 6 Lee FS, Chu ZT, Bolger MB, Warshel A. Calculations of antibody antigen interactions – microscopic and semimicroscopic evaluation of the free-energies of binding of phosphorylcholine analogs to Mcpc603. *Protein Eng* 1992; 5:215-228.
 - 7 Åqvist J, Medina C, Samuelsson JE. A new method for predicting binding affinity in computer-aided drug design. *Prot Eng* 1994; 7:385-391.
 - 8 Hansson T, Marelius, J, Åqvist J. Ligand binding affinity prediction by linear interaction energy methods. *J Comput-Aided Mol Design* 1998; 12:27-35
 - 9 Almlöf M, Brandsdal B O, Åqvist J. Binding Affinity Prediction with Different Force Fields: Examination of the Linear Interaction Energy Method. *J Comput Chem* 2004; 25: 1242-1254.
 - 10 Singh N, Warshel A. Absolute binding free energy calculations: On the accuracy of computational scoring of protein–ligand interactions. *Proteins* 2010; 78:1705-1723.
 - 11 Foloppe N, Hubbard R. Towards Predictive Ligand Design With Free-Energy Based Computational Methods? *Curr Med Chem* 2006; 13:3583-3608.
 - 12 Warshel A, Levitt M Theoretical studies of enzymic reactions: dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme. *J Mol Biol* 1976; 103:227–249
 - 13 Srinivasan J, Cheatham III TE, Cieplak P, Kollman PA, Case DA. Continuum Solvent Studies of the Stability of DNA, RNA, and Phosphoramidate–DNA Helices. *J Am Chem Soc* 1998; 120:9401-9809.
 - 14 Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L, Lee M, Lee T, Duan Y, Wang W, Donini O, Cieplak P, Srinivasan J, Case DA, Cheatham III TE. Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Acc Chem Res* 2000; 33:889-897.
 - 15 Still WC, Tempczyk A, Hawley RC, Hendrickson T. Semianalytical treatment of solvation for molecular mechanics and dynamics. *J Am Chem Soc* 1990; 112:6127-6129.
 - 16 Pearlman D A. Evaluating the Molecular Mechanic Poisson–Boltzmann Surface Area Free Energy Method Using a Congeneric Series of Ligands to p38 MAP Kinase. *J Med Chem* 2005; 48: 7796-7807.
 - 17 Swanson J M J, Henchman R H, McCammon J A. Revisiting Free Energy Calculations: A Theoretical Connection to MM/PBSA and Direct Calculation of the Association Free energy. *Biophys J* 2004; 86: 67-74.
 - 18 Yang C-Y, Sun H, Chen J, Nikolovska-Coleska Z, Wang S. Importance of Ligand reorganisation Free Energy in Protein–Ligand Binding-affinity Prediction. *J Am Chem Soc* 2009; 131:13709-13721.
 - 19 Sharp K A, Honig B. Electrostatic interactions in macromolecules: theory and applications. *Annu Rev Biophys Biophys Chem* 1990; 19:301-32.
 - 20 Naim M, Bhat S, Rankin K N, Dennis S, Chowdhury S F, Siddiqi I, Drabik P, Sulea T, Bayly C I, Jakalian A, Purisma E O. Solvated Interaction Energy (SIE) for Scoring Protein–Ligand Binding Affinities. 1. Exploring the Parameter Space. *J Chem Inf Model*, 2007; 47:122-133.
 - 21 Schutz C N, Warshel A. What Are the Dielectric “Constants” of Proteins and How To Validate Electrostatic Models? *Proteins: Struct Funct Genet* 2001; 44: 400-417.
 - 22 Yang T, Wu JC, Yan C, Wang Y, Luo R, Gonzales MB, Dalby KN, Ren P. Virtual screening using molecular simulations. *Proteins* 2011; 79:1940-1951.
 - 23 Hou T, Wang J, Li Y, Wang W. Assessing the performance of the MM/PBSA and MM/GBSA methods. 1. The accuracy of binding free energy calculations based on molecular dynamics simulations. *J Chem Inf Model* 2011; 51:69-82
 - 24 Sham YY, Chu ZT, Tao H, Warshel A. Examining methods for calculations of binding free energies: LRA, LIE, PDLD-LRA, and PDLS/S-LRA calculations of ligands binding to an HIV protease. *Proteins: Struct Funct Genet* 2000; 39:393-407.
 - 25 Rastelli G, Del Rio A, Degliesposti G, Sgobba M. Fast and accurate predictions of binding free energies using MM-PBSA and MM-GBSA. *J Comput Chem* 2010; 31:797-810.
 - 26 Mikulskis P, Genheden S, Rydberg P, Sandberg L, Olsen L, Ryde U. Binding affinities of the SAMPL3 trypsin and host-guest blind tests estimated with the MM/PBSA and LIE methods, *J Comput-Aided Mol Design* 2011, submitted.
 - 27 Kuhn B, Gerber P, Schultz-Gash T, Stahl M. Validation and Use of the MM-PBSA Approach for Drug Discovery. *J Med Chem* 2005; 48: 4040–4048.
 - 28 Genheden S, Ryde U. How to obtain statistically converged MM/GBSA results. *J Comput Chem* 2010; 31:837-846
 - 29 Gohlke H, Case DA. Converging free energy estimates: MM-PB(GB)SA studies on the protein-protein complex Ras-Raf. *J Comput Chem* 2004; 25:238-250
 - 30 Genheden S, Luchko T, Gusarov S, Kovalenko A, Ryde U. An MM/3D-RISM approach for ligand-binding affinities. *J Phys Chem B* 2010; 114:8505-8516.

-
- 31 Kongsted J, Söderhjelm P, Ryde U. How accurate are continuum solvation models for drug-like molecules? *J Comp-Aided Mol Design*, 2009; 23:395-409
- 32 Kongsted J, Ryde U. An improved method to predict the entropy term with the MM/PBSA approach. *J Comput-Aided Mol Design*, 2009; 23:63-71
- 33 Pugliese L, Coda A, Malcovati M, Bolognesi M. Three-dimensional Structure of the Tetragonal Crystal Form of Egg-white Avidin in its functional Complex with Biotin at 2.7 Å Resolution. *J Mol Biol* 1993; 231:698-710.
- 34 Matter R R, Defossa E, Heinelt U, Blohm P-M, Schneider D, Müller A, Hreok S, Schreuder H, Liesum A, Brachvogel V, Lönze P, Walser A, Al-Obeidi F, Wildgoose P. Design and Quantitative Structure–Activity Relationship of 3-Amidinobenzyl-1H-indole-2-carboxamides as Potent, Nonchiral, and Selective Inhibitors of Blood Coagulation Factor Xa. *J Med Chem* 2002; 45:2749-2769.
- 35 Green N M. Thermodynamics of the binding of biotin and some analogues by avidin. *Biochem J* 1966; 101: 774–790.
- 36 Green N M. Avidin. *Adv Protein Chem* 1975; 29: 85–133.
- 37 Green N M. Avidin and streptavidin. *Methods Enzymol* 1990; 184: 51–67.
- 38 Miyamoto S, Kollman, P A. Absolute and relative binding free energy calculations of the interaction of biotin and its analogs with streptavidin using molecular dynamics/free energy perturbation approaches. *Proteins: Struct Funct Genet* 1993; 16: 226–245.
- 39 Wang J, Dixon R, Kollman P A. Ranking ligand binding affinities with avidin: a molecular dynamics-based interaction energy study. *Proteins, Struct Funct Genet* 1999; 34: 69–81.
- 40 Kuhn B, Kollman P A. Binding of a Diverse Set of Ligands to Avidin and Streptavidin: An Accurate Quantitative Prediction of Their Relative Affinities by a Combination of Molecular Mechanics and Continuum Solvent Models. *J Med Chem* 2000; 43: 3786–3791.
- 41 Weis A, Katebzadeh K, Söderhjelm P, Nilsson I, Ryde U. Ligand Affinities Predicted with the MM/PBSA Method: Dependence on the Simulation Method and the Force Field. *J Med Chem* 2006; 49:6596-6606.
- 42 Brown S P, Muchmore S W. High-Throughput Calculation of Protein–Ligand Binding Affinities: Modification and Adaptation of the MM-PBSA Protocol to Enterprise Grid Computing. *J Chem Inf Model* 2006; 46: 999–1005.
- 43 Söderhjelm P, Ryde U. Conformational dependence of charges in protein simulations. *J Comput Chem*, 2009; 30:750-760
- 44 Söderhjelm P, Kongsted J, Ryde U. Ligand Affinities Estimated by Quantum Chemical Calculations. *J Chem Theory Comput* 2010; 6:1726-1737.
- 45 Genheden S, Ryde U. A Comparison of Different Initialization Protocols to Obtain Statistically Independent Molecular Dynamics Simulation. 2011; 32:187–195.
- 46 Genheden S, Nilsson I, Ryde U. Binding affinities of factor Xa inhibitors estimated by TI and MM/GBSA. *J Chem Inf Model* 2010; 51:947-958
- 47 Rocchia W, Alexov E, Honig B. Extending the Applicability of the Nonlinear Poisson–Boltzmann Equation: Multiple Dielectric Constants and Multivalent Ions. *J Phys Chem B* 2001; 105:6507-6514.
- 48 Sitkoff D, Sharp KA, Honig B. Accurate Calculation of Hydration Free Energies Using Macroscopic Solvent Models. *J Phys Chem* 1994; 98:1978-1988.
- 49 Onufriev A, Bashford D, Case DA. Exploring protein native states and large-scale conformational changes with a modified generalized Born model. *Proteins* 2004; 55:383-394.
- 50 Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins: Struct Funct Bioinform* 2006; 65:712-725.
- 51 Wang J, Cieplak P, Kollman PA. How well does a restrained electrostatic potential (RESP) model perform in calculating conformational energies of organic and biological molecules?. *J Comput Chem* 2001; 21:1074-1074.
- 52 Bayly CI, Cieplak P, Cornell WD, Kollman PA. A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model. *J Phys Chem* 1993; 97:10269-10280.
- 53 Wang J M, Wolf R M, Caldwell K W, Kollman P A, Case D A. Development and testing of a general amber force field. *J Comput Chem* 2004; 25:1157-1174.
- 54 Horn HW, Swope WC, Pitera JW, Madura JD, Dick TJ, Hura G, Head-Gordon T. Development of an improved four-site water model for biomolecular simulations: TIP4P-Ew. *J Chem Phys* 2004; 120:9665-9678.
- 55 Genheden S, Ryde U. Comparison of the efficiency of the LIE and MM/GBSA methods to calculate ligand-binding energies. *J Chem Theory Comput*, 2011, in press (doi 10.1021/ct200163).
- 56 Case DA, Darden TA, Cheatham III TE, Simmerling CL, Wang J, Duke RE, Luo R, Crowley M., Walker RC, Zhang W, Merz KM, Wang B, Hayik S, Roitberg A, Seabra G, Kolossvary I, Wong KF, Paesani F, Vanicek J, Wu X, Brozell SR, Steinbrecher T, Gohlke H, Yang L, Tan C, Mongan J, Hornak V, Cui G, Mathews DH, Seetin MG, Sagui C, Babin V, Kollman PA. Amber 10, University of California, San Francisco, 2008.

-
- 57 Ryckaert JP, Ciccotti G, Berendsen HJC. Numerical Integration of the Cartesian Equations of Motion of a System with Constraints: Molecular Dynamics of n-Alkanes. *J Comput Phys* 1977; 23:327-341.
- 58 Wu X, Brooks BR. Self-guided Langevin dynamics simulation method . *Chem Phys Lett* 2003, 381:512-518.
- 59 Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR. Molecular dynamics with coupling to an external bath. *J Chem Phys* 1984; 81:3684–3690.
- 60 Darden T, York D, Pedersen L. Particle Mesh Ewald-an N.Log(N) method for Ewald sums in large systems. *J Chem Phys* 1993; 98:10089-10092.
- 61 Pearlman DA, Charifson PS. Are Free Energy Calculations Useful in Practice? A Comparison with Rapid Scoring Functions for the p38 MAP Kinase Protein System. *J Med Chem* 2001; 44:3417-3423

Table 1. Summary of the various methods used in the article. The first part of the table defines the various electrostatic and non-electrostatic methods. The second part of the table defines the various binding-affinity methods, which consist of one electrostatic and one non-electrostatic method (in this part of the table, only methods based on GB solvation are shown, although each method also have a variant with the PB method instead). The tables in which the results for the various methods are presented are also given, besides Tables 4, 5, 8, and 9, which contain results for all binding-affinity methods. For all electrostatic methods, calculations with $\epsilon_{int} = 1, 2, 4, 10,$ and 25 were performed.

Method	Terms	Eqns.	Tables
<i>Electrostatic methods</i>			
1A-MM/GB	$E_{ele} + \Delta G_{solv}(GB)$	5, 7	2, S1
1A-MM/PB	$E_{ele} + \Delta G_{solv}(PB)$	5, 7	2, S1
3A-MM/GB	$E_{ele} + \Delta G_{solv}(GB)$	5, 6	2, 6, 7, S12, S13
3A-MM/PB	$E_{ele} + \Delta G_{solv}(PB)$	5, 6	2, 6, 7, S12, S13
MMGB/s-LRA	$\Delta G_{ele}^{LRA/s} + \Delta G_{solv}(GB)$	9–11	2, S1
MMPB/s-LRA	$\Delta G_{ele}^{LRA/s} + \Delta G_{solv}(PB)$	9–11	2, S1
<i>Non-el methods</i>			
1A-MM/SA	$E_{vdW} + \Delta G_{np} - TS_{MM}$	5, 7	3, S3
3A-MM/SA	$E_{bnd} + E_{vdW} + \Delta G_{np} - TS_{MM}$	5, 6	3, 6, 7, S3, S12, S13
LIE (β)	$\Delta G_{non-ele}^{LIE}$	12	3, S3, S14
Binding-affinity method	Electrostatic method	Non-electrostatic method	Tables
1A-MM/GBSA	1A-MM/GB	1A-MM/SA	S4
3A-MM/GBSA	3A-MM/GB	3A-MM/SA	S5
MMGB/s-LRA/ β	MMGB/s-LRA	LIE (β)	S6
1A-MMGB-3A-MMSA	1A-MM/GB	3A-MM/SA	S7
1A-MM/GB β	1A-MM/GB	LIE (β)	S8
3A-MM/GB β	3A-MM/GB	LIE (β)	S9
MMGB/s-LRA/1A-MMSA	MMGB/s-LRA	1A-MM/SA	S10
MMGB/s-LRA/3A-MMSA	MMGB/s-LRA	3A-MM/SA	S11

Table 2. Electrostatic contributions (ΔG_{ele} in kJ/mol) to the free energy of the binding the seven biotin analogues to avidin for the 1A-MM/PB(GB)SA, 3A-MM/PB(GB)SA, and MMPB(GB)/s-LRA/ β methods, with either PB or GB solvation and calculated with five different values of the internal dielectric constant, ϵ_{int} . SE is the average standard error for the seven biotin analogues.

	1A-MM/GB					1A-MM/PB				
ϵ_{int}	1	2	4	10	25	1	2	4	10	25
Btn1	-45.5	-31.0	-23.7	-19.4	-17.6	4.3	-14.1	-20.7	-21.9	-20.0
Btn2	-35.9	-26.2	-21.4	-18.5	-17.4	13.2	-9.5	-18.4	-21.1	-19.8
Btn3	-47.6	-31.9	-24.1	-19.4	-17.5	2.7	-14.3	-20.5	-21.6	-19.7
Btn4	18.0	7.8	2.8	-0.3	-1.5	118.3	54.9	23.8	6.0	-0.2
Btn5	7.0	2.8	0.7	-0.5	-1.0	82.3	38.5	16.9	4.4	-0.4
Btn6	12.7	5.8	2.3	0.2	-0.6	83.0	38.9	17.4	4.9	0.4
Btn7	-16.6	-8.8	-4.9	-2.6	-1.7	12.1	4.8	1.1	-1.0	-8.0
SE	1.0	0.5	0.3	0.1	0.1	1.3	0.6	0.3	0.2	0.2
	3A-MM/GB					3A-MM/PB				
ϵ_{int}	1	2	4	10	25	1	2	4	10	25
Btn1	-51.7	-33.7	-24.7	-19.3	-17.2	5.0	-9.0	-15.3	-18.0	-17.9
Btn2	-40.5	-27.9	-21.6	-17.8	-16.3	19.7	-1.5	-11.5	-16.4	-17.0
Btn3	-42.0	-28.4	-21.7	-17.6	-16.0	20.3	-0.7	-10.6	-15.5	-16.3
Btn4	10.8	4.6	1.5	-0.4	-1.1	73.9	34.8	15.4	4.1	-0.2
Btn5	-16.5	-9.3	-5.7	-3.5	-2.6	60.2	27.0	10.8	1.5	-1.6
Btn6	-9.0	-5.2	-3.3	-2.2	-1.7	57.8	26.7	11.4	2.5	-0.7
Btn7	-19.9	-10.8	-6.2	-3.5	-2.4	7.2	2.4	-0.2	-1.7	-2.1
SE	6.4	3.3	1.7	0.8	0.4	7.8	3.9	1.9	0.8	0.5
	MMGB/s-LRA					MMPB/s-LRA				
ϵ_{int}	1	2	4	10	25	1	2	4	10	25
Btn1	-33.9	-58.0	-70.0	-77.2	-80.1	-19.5	-14.5	-12.0	-10.5	-9.9
Btn2	-24.1	-89.7	-122.5	-142.2	-150.1	-14.4	-21.2	-24.6	-26.7	-27.5
Btn3	-31.4	-58.1	-71.4	-79.5	-82.7	-18.4	-16.3	-15.2	-14.5	-14.3
Btn4	6.2	6.2	6.1	6.1	6.1	31.8	8.9	-2.5	-9.4	-12.1
Btn5	3.1	1.3	0.4	-0.1	-0.3	18.5	9.3	4.6	1.9	0.7
Btn6	-2.7	-1.5	-0.8	-0.5	-0.3	7.8	4.2	2.3	1.3	0.8
Btn7	-4.5	-2.6	-1.7	-1.1	-0.9	-2.1	3.8	6.7	8.5	9.2
SE	2.0	1.0	0.7	0.5	0.5	2.6	1.3	0.8	0.6	0.6

Table 3. Non-electrostatic contribution to the free energy of the binding the seven biotin analogues to avidin in kJ/mol ($\Delta G_{\text{non-ele}}$) for the 1A-MM/SA, 3A-MM/SA, and LIE methods. SE is the average standard error over the seven biotin analogues.

	1A-MM/SA	3A-MM/SA	LIE
Btn1	-69.7	-96.5	-19.9
Btn2	-67.8	-109.6	-21.2
Btn3	-55.6	-86.4	-18.1
Btn4	-130.2	-115.6	-18.5
Btn5	-75.1	-73.6	-16.2
Btn6	-77.4	-94.7	-11.1
Btn7	-1.4	-26.3	-7.0
SE	0.7	6.9	0.2

Table 4. Quality measures of the methods based on GB solvation for the binding of seven biotin analogues to avidin.^a

	1A-MM/GBSA	3A-MM/GBSA	MMGB/s-LRA/ β	1A-MMGB-3A-MMSA	1A-MM/GB β	3A-MM/GB β	MMGB/s-LRA/1A-MMSA	MMGB/s-LRA/3A-MMSA
$\epsilon_{\text{int}} = 1$								
MAD	38.9	65.3	16.5	56.6	17.0	8.1	39.4	53.6
MADtr	15.2	14.2	7.9	16.2	14.2	9.5	22.0	19.3
r^2	0.60	0.72	0.85	0.78	0.71	0.75	0.29	0.59
PI	0.85	0.87	0.89	0.87	0.78	0.87	0.65	0.87
SE	1.3	9.4	2.0	7.0	1.1	6.4	2.1	7.3
$\epsilon_{\text{int}} = 2$								
MAD	37.3	57.0	19.7	52.8	17.3	13.1	56.3	70.1
MADtr	19.9	17.1	19.6	18.1	8.5	8.1	25.5	26.6
r^2	0.33	0.58	0.68	0.64	0.80	0.84	0.53	0.62
PI	0.69	0.87	0.85	0.87	0.87	0.87	0.80	0.87
SE	0.9	7.6	1.1	6.9	0.6	3.3	1.3	7.0
$\epsilon_{\text{int}} = 4$								
MAD	36.5	52.8	25.9	50.9	19.2	17.3	64.8	78.3
MADtr	24.6	19.2	28.1	19.1	7.0	7.9	30.0	33.7
r^2	0.21	0.49	0.63	0.52	0.86	0.89	0.56	0.61
PI	0.65	0.80	0.91	0.80	0.99	0.99	0.87	0.87
SE	0.8	7.1	0.7	6.9	0.3	1.7	1.0	6.9
$\epsilon_{\text{int}} = 10$								
MAD	36.1	50.3	31.0	49.8	20.3	19.8	69.9	83.2
MADtr	27.3	20.5	33.2	20.4	7.6	8.7	33.7	39.4
r^2	0.15	0.43	0.61	0.45	0.88	0.89	0.57	0.60
PI	0.44	0.64	0.82	0.80	0.92	0.99	0.87	0.87
SE	0.7	7.0	0.6	6.9	0.2	0.8	0.9	6.9
$\epsilon_{\text{int}} = 25$								
MAD	35.9	49.3	33.0	49.3	20.8	20.8	72.0	85.2
MADtr	28.5	21.0	35.3	21.1	8.5	9.4	35.2	41.7
r^2	0.13	0.40	0.60	0.42	0.87	0.89	0.57	0.60
PI	0.44	0.64	0.82	0.64	0.92	0.92	0.87	0.87
SE	0.7	6.9	0.5	6.9	0.2	0.5	0.9	6.9

^a The quality measures are the mean absolute deviation (MAD), the MAD after removal of systematic errors (i.e. after subtraction of the signed average; MADtr), Pearson's correlation coefficient (r^2), and the predictive index (PI).⁶¹ Both MAD and MADtr are in kJ/mol. The best result for each value of ϵ_{int} is highlighted in **bold face**. SE is the standard error of the binding affinities, averaged over the seven biotin analogues.

Table 5. Quality measures of the methods based on GB solvation for the binding of nine inhibitors to fXa.^a

	1A- MM/GBSA	3A- MM/GBSA	MMGB/s- LRA/ β	1A-MMGB- 3A-MMSA	1A- MM/GB β	3A- MM/GB β	MMGB/s-LRA/ 1A-MMSA	MMGB/s-LRA/ 3A-MMSA
$\epsilon_{\text{int}} = 1$								
MAD	16.2	66.2	21.2	43.3	57.6	46.1	52.6	78.5
MADtr	3.9	32.5	6.3	21.8	5.6	44.8	5.1	21.2
r^2	0.38	0.14	0.08	0.41	0.01	0.00	0.36	0.47
PI	0.58	0.42	0.32	0.72	0.09	0.04	0.66	0.72
SE	1.1	12.4	1.1	9.3	0.9	8.3	1.3	9.3
$\epsilon_{\text{int}} = 2$								
MAD	36.5	74.5	28.3	62.4	37.4	27.3	45.5	71.4
MADtr	2.9	20.4	13.2	21.2	4.5	23.9	12.2	17.5
r^2	0.59	0.35	-0.56	0.42	0.05	0.00	-0.21	0.22
PI	0.62	0.65	-0.76	0.72	0.24	0.04	-0.55	0.40
SE	0.9	10.2	1.0	9.2	0.5	4.3	1.3	9.3
$\epsilon_{\text{int}} = 4$								
MAD	46.6	78.7	32.1	72.5	27.2	21.1	42.0	67.9
MADtr	3.0	18.0	19.7	20.9	4.3	13.4	18.7	18.8
r^2	0.64	0.46	-0.49	0.42	0.14	0.00	-0.29	0.07
PI	0.72	0.75	-0.76	0.72	0.61	0.03	-0.55	0.44
SE	0.8	9.5	1.5	9.2	0.3	2.3	1.7	9.4
$\epsilon_{\text{int}} = 10$								
MAD	46.6	78.7	32.1	72.5	27.2	21.1	42.0	67.9
MADtr	3.0	18.0	19.7	20.9	4.3	13.4	18.7	18.8
r^2	0.64	0.46	-0.49	0.42	0.14	0.00	-0.29	0.07
PI	0.72	0.75	-0.76	0.72	0.61	0.03	-0.55	0.44
SE	0.8	9.5	1.5	9.2	0.3	2.3	1.7	9.4
$\epsilon_{\text{int}} = 25$								
MAD	55.1	82.2	35.8	81.0	18.7	17.6	39.4	64.9
MADtr	3.2	20.2	25.1	20.8	4.1	5.1	24.1	22.4
r^2	0.64	0.43	-0.46	0.42	0.38	0.02	-0.32	0.01
PI	0.68	0.73	-0.76	0.81	0.70	0.09	-0.68	0.10
SE	0.8	9.3	2.0	9.2	0.2	0.8	2.2	9.5

^a The quality measures are the same as in Table 4. A negative sign of r^2 indicates that r is negative.

Table 6. Ligand reorganisation free energies ($\delta G_{\text{re}}^{\text{L}}$ in kJ/mol) for the binding of seven biotin analogues to avidin, calculated at different values of ϵ_{int} . The corresponding MM/GBSA components in Eqn. 5 are also given (note that $\Delta G_{\text{non-ele}} = E_{\text{bnd}} + E_{\text{vdW}} + \Delta G_{\text{np}} - TS_{\text{MM}}$). Av, Max, Min, and Range are the average, maximum, minimum, and range among the seven biotin analogues.

	E_{bnd}	E_{vdW}	ΔG_{np}	$-TS_{\text{MM}}$	$\Delta G_{\text{non-ele}}$	$E_{\text{ele}} + \Delta G_{\text{solv(GB)}}$					$\delta G_{\text{re}}^{\text{L}}$
ϵ_{int}						1	2	4	10	25	1
Btn1	2.5	-1.5	0.1	5.1	6.2	1.8	0.8	0.4	0.1	0.0	7.9
Btn2	1.2	1.3	0.1	6.6	9.1	-1.1	-0.6	-0.3	-0.2	-0.1	7.9
Btn3	-3.0	3.6	0.1	18.6	19.4	2.4	1.2	0.6	0.2	0.1	21.8
Btn4	-3.7	-2.0	0.2	8.1	2.5	0.2	0.0	-0.1	-0.1	-0.1	2.7
Btn5	5.4	0.1	0.0	8.7	14.2	-0.1	-0.1	0.0	0.0	0.0	14.1
Btn6	-2.3	-0.2	0.1	2.4	-0.1	1.1	0.5	0.3	0.1	0.0	1.0
Btn7	-0.4	0.0	0.0	-0.4	-0.8	0.7	0.3	0.2	0.1	0.0	-0.1
Av	-0.1	0.2	0.1	7.0	7.2	0.7	0.3	0.1	0.0	0.0	7.9
Max	5.4	3.6	0.2	18.6	19.4	2.4	1.2	0.6	0.2	0.1	21.8
Min	-3.7	-2.0	0.0	-0.4	-0.8	-1.1	-0.6	-0.3	-0.2	-0.1	-0.1
Range	9.1	5.6	0.2	19.0	20.2	3.5	1.8	0.9	0.4	0.2	21.9

Table 7. Protein reorganisation free energies ($\delta G_{\text{re}}^{\text{P}}$ in kJ/mol) and MM/GBSA energy components for the binding of seven biotin analogues to avidin, calculated at different values of ϵ_{int} . The terms are the same as in Table 6.

	E_{bnd}	E_{vdW}	ΔG_{np}	$-TS_{\text{MM}}$	$\Delta G_{\text{non-ele}}$	$E_{\text{ele}} + \Delta G_{\text{solv(GB)}}$					$\delta G_{\text{re}}^{\text{P}}$
ϵ_{int}						1	2	4	10	25	1
Btn1	-46.3	-65.9	-1.9	-17.7	-131.9	-32.2	-14.4	-5.6	-0.3	1.9	-164.0
Btn2	-102.5	-90.8	-1.6	-8.5	-203.4	-14.2	-4.3	0.6	3.5	4.7	-217.6
Btn3	-124.2	-52.1	0.0	-24.8	-201.0	13.1	9.2	7.3	6.1	5.6	-187.9
Btn4	-10.4	106.6	11.1	-59.0	48.4	-29.1	-13.0	-5.0	-0.1	1.8	19.3
Btn5	-24.2	-1.6	1.5	-26.6	-50.9	-93.9	-48.2	-25.4	-11.7	-6.2	-144.8
Btn6	-74.8	36.6	3.9	-34.6	-68.9	-91.0	-46.1	-23.7	-10.2	-4.8	-159.9
Btn7	-52.3	-37.8	-2.4	-3.9	-96.3	-15.9	-9.2	-5.8	-3.8	-3.0	-112.2
Av	-62.1	-15.0	1.5	-25.0	-100.6	-37.6	-18.0	-8.2	-2.3	0.0	-138.2
Max	-10.4	106.6	11.1	-3.9	48.4	13.1	9.2	7.3	6.1	5.6	19.3
Min	-124.2	-90.8	-2.4	-59.0	-203.4	-93.9	-48.2	-25.4	-11.7	-6.2	-217.6
Range	113.8	197.5	13.5	55.1	251.8	107.1	57.5	32.7	17.8	11.9	236.9

Table 8. Quality measures of the methods based on PB solvation for the binding of seven biotin analogues to avidin.^a

	1A- MM/PBSA	3A- MM/PBSA	MMPB/s- LRA/ β	1A-MMPB- 3A-MMSA	1A- MM/PB β	3A- MM/PB β	MMPB/s-LRA/ 1A-MMSA	MMPB/s-LRA/ 3A-MMSA
$\epsilon_{\text{int}} = 1$								
MAD	21.9	12.3	29.5	23.5	74.1	63.8	27.1	40.6
MADtr	9.9	9.9	12.6	23.0	30.7	15.2	18.5	17.4
r^2	0.90	0.78	0.62	0.71	0.38	0.41	0.35	0.62
PI	0.96	0.90	0.77	0.70	0.82	0.69	0.65	0.82
SE	1.5	10.4	2.6	7.0	1.3	7.8	2.7	7.5
$\epsilon_{\text{int}} = 2$								
MAD	15.8	29.8	25.3	27.0	43.1	40.3	33.3	44.8
MADtr	13.9	15.2	8.5	15.8	16.4	9.6	25.7	21.7
r^2	0.64	0.67	0.76	0.76	0.59	0.67	0.18	0.46
PI	0.88	0.87	0.92	0.87	0.82	0.82	0.44	0.80
SE	1.0	7.9	1.4	6.9	0.7	3.9	1.5	7.1
$\epsilon_{\text{int}} = 4$								
MAD	28.6	41.1	23.1	41.2	28.9	29.0	37.0	47.0
MADtr	18.8	18.2	9.3	18.0	10.1	7.7	29.3	24.7
r^2	0.37	0.55	0.68	0.66	0.75	0.83	0.13	0.37
PI	0.65	0.80	0.83	0.87	0.87	0.87	0.44	0.64
SE	0.8	7.2	0.9	6.9	0.4	1.9	1.1	7.0
$\epsilon_{\text{int}} = 10$								
MAD	35.1	47.4	21.9	48.3	21.8	22.7	39.2	48.5
MADtr	24.0	20.0	10.6	19.6	7.2	7.7	31.6	26.4
r^2	0.22	0.46	0.57	0.53	0.85	0.89	0.11	0.33
PI	0.65	0.80	0.83	0.80	0.91	0.99	0.36	0.64
SE	0.7	7.0	0.7	6.9	0.3	0.9	1.0	6.9
$\epsilon_{\text{int}} = 25$								
MAD	35.6	49.1	21.4	50.8	19.3	21.0	40.0	49.2
MADtr	26.3	20.8	11.2	19.9	7.9	8.7	32.4	27.1
r^2	0.15	0.42	0.53	0.46	0.85	0.89	0.10	0.31
PI	0.44	0.64	0.67	0.80	0.91	0.92	0.36	0.64
SE	0.8	6.9	0.6	6.9	0.3	0.5	0.9	6.9

^a The quality measures are the same as in Table 4.

Table 9. Quality measures of the methods based on PB solvation for the binding of nine inhibitors to fXa.^a

	1A- MM/PBSA	3A- MM/PBSA	MMPB /s-LRA/ β	1A-MMPB- 3A-MMSA	1A- MM/PB β	3A- MM/PB β	MMPB/s-LRA/ 1A-MMSA	MMPB/s-LRA/ 3A-MMSA
$\epsilon_{\text{int}} = 1$								
MAD	116.0	42.1	69.6	90.1	189.8	141.1	14.2	30.1
MADtr	20.1	44.9	15.9	14.6	21.4	56.4	13.7	15.3
r^2	-0.64	-0.02	-0.58	0.01	-0.81	-0.14	-0.39	0.16
PI	-0.80	0.08	-0.77	0.13	-0.92	-0.44	-0.79	0.36
SE	1.7	13.8	1.4	9.3	1.5	10.2	1.6	9.3
$\epsilon_{\text{int}} = 2$								
MAD	22.6	30.2	50.5	17.0	96.4	74.0	23.3	49.2
MADtr	10.6	18.5	19.1	16.7	11.8	29.3	17.9	15.6
r^2	-0.37	0.06	-0.71	0.23	-0.81	-0.13	-0.50	0.05
PI	-0.71	0.14	-0.85	0.49	-0.95	-0.47	-0.69	0.34
SE	1.1	10.6	1.1	9.3	0.7	5.2	1.4	9.3
$\epsilon_{\text{int}} = 4$								
MAD	22.3	58.1	41.0	48.2	51.5	41.7	32.8	58.8
MADtr	6.2	13.5	22.5	18.6	7.3	15.8	21.5	18.9
r^2	0.00	0.37	-0.57	0.34	-0.77	-0.11	-0.40	0.02
PI	-0.14	0.66	-0.76	0.72	-0.95	-0.41	-0.68	0.26
SE	0.9	9.6	1.6	9.2	0.4	2.7	1.8	9.4
$\epsilon_{\text{int}} = 10$								
MAD	46.8	76.1	36.3	72.7	27.0	23.6	38.6	64.5
MADtr	4.1	16.4	24.9	20.2	5.0	7.8	23.9	21.5
r^2	0.37	0.44	-0.50	0.40	-0.13	-0.05	-0.35	0.01
PI	0.50	0.73	-0.76	0.72	-0.30	-0.08	-0.68	0.10
SE	0.8	9.3	1.9	9.2	0.3	1.3	2.1	9.4
$\epsilon_{\text{int}} = 25$								
MAD	54.5	81.9	35.3	80.4	19.3	17.9	40.9	66.8
MADtr	3.5	19.3	25.9	20.7	4.3	5.2	24.9	22.6
r^2	0.56	0.43	-0.47	0.41	0.18	0.00	-0.34	0.00
PI	0.55	0.73	-0.76	0.72	0.60	-0.01	-0.68	0.10
SE	0.8	9.3	2.1	9.2	0.2	0.8	2.2	9.5

^a The quality measures are the same as in Table 4.

Figure 1. The thermodynamic cycle used to derive the MMPB(GB)/s-LRA terms.^{38,51} The binding free energy is calculated by the central cycle, which is broken down into electrostatic and non-electrostatic contributions, $\Delta G_{bind} = \Delta G_{ele}^{bound} - \Delta G_{ele}^{free} + \Delta G_{non-ele}$. The non-electrostatic term is calculated by Eqn. 12, whereas the electrostatic terms are further broken down. ΔG_{ele}^{bound} is described by the right, outer cycle: $\Delta G(A) = \Delta G_{solv}^{PL} \left(\frac{1}{\epsilon_{int}} - \frac{1}{\epsilon_{ext}} \right)$, $\Delta G(B) = \Delta G_{solv}^L \left(1 - \frac{1}{\epsilon_{int}} \right) + \frac{E_{ele}^L}{\epsilon_{int}} + \frac{E_{ele}^{L-P}}{\epsilon_{int}}$, and $\Delta G(C) = \Delta G_{solv}^{PL'} \left(\frac{1}{\epsilon_{ext}} - \frac{1}{\epsilon_{int}} \right)$. ΔG_{ele}^{free} is calculated by the left, outer cycle: $\Delta G(D) = (\Delta G_{solv}^L + \Delta G_{solv}^P) \left(\frac{1}{\epsilon_{int}} - \frac{1}{\epsilon_{ext}} \right)$, $\Delta G(E) = \Delta G_{solv}^L \left(1 - \frac{1}{\epsilon_{int}} \right) + \frac{E_{ele}^L}{\epsilon_{int}}$, and $\Delta G(F) = \Delta G_{solv}^{P'} \left(\frac{1}{\epsilon_{ext}} - \frac{1}{\epsilon_{int}} \right)$. Eqn. 10 is the sum of the free energies A, B, and C and Eqn. 11 is the sum of the free energies D, E, and F. L is the ligand with full charge, whereas the ligand charges have been zeroed in L' .

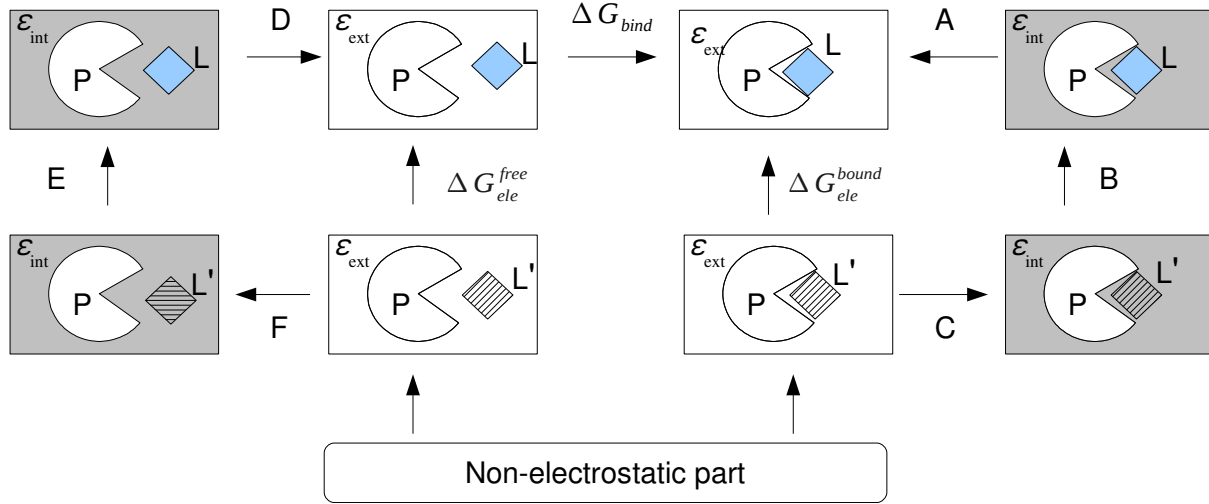


Figure 2. The seven avidin ligands (biotin, Btn1, and Btn2–Btn7) and the nine factor Xa ligands (C9–C125) used in this study. The experimental binding free energies are given to right in kJ/mol.^{34,36}

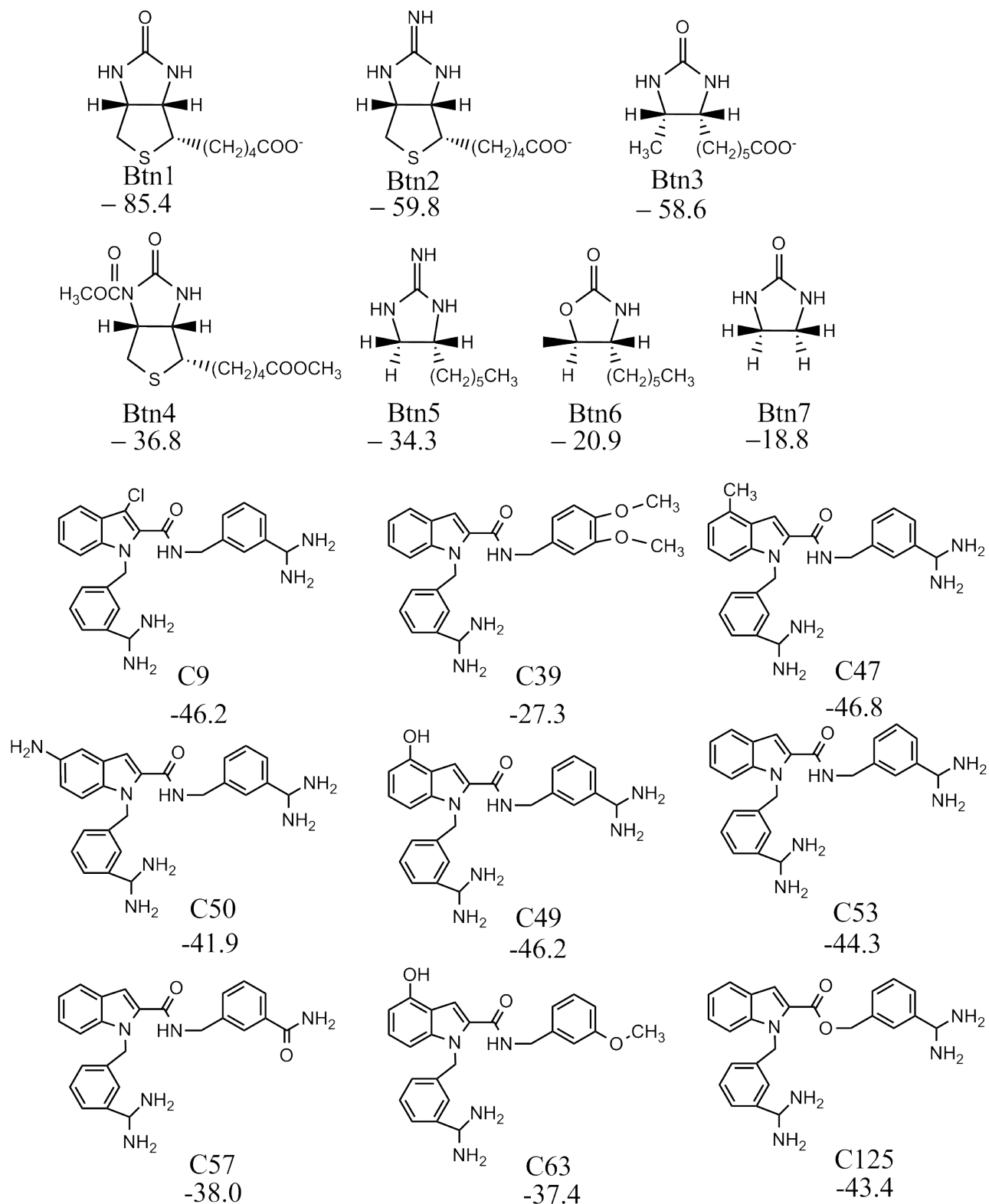


Figure 3. Results of the 1A-MM/GBSA method with different values of ϵ_{int} for the binding of seven biotin analogues to avidin.

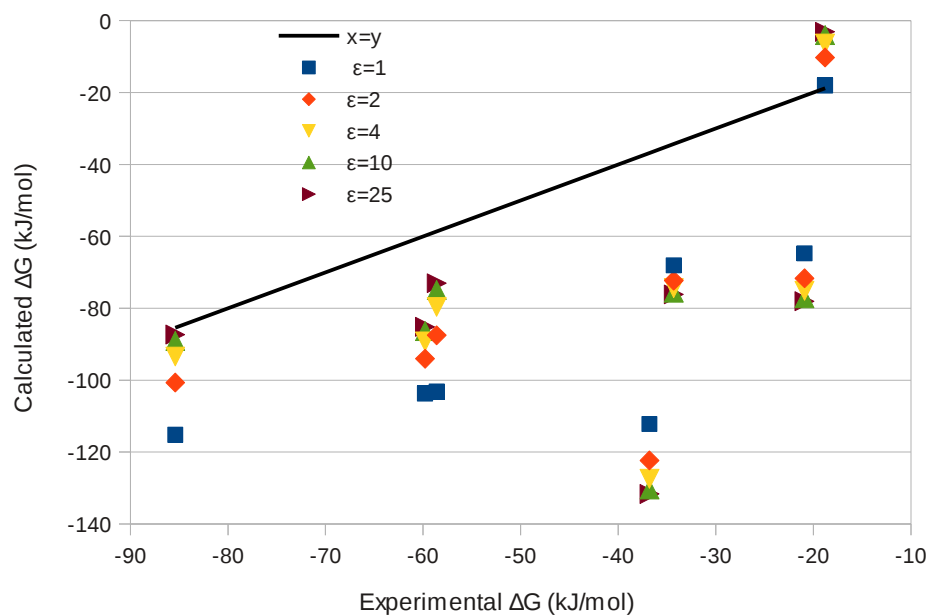


Figure 4. Results of the 3A-MM/GBSA method with different values of ϵ_{int} for the binding of seven biotin analogues to avidin.

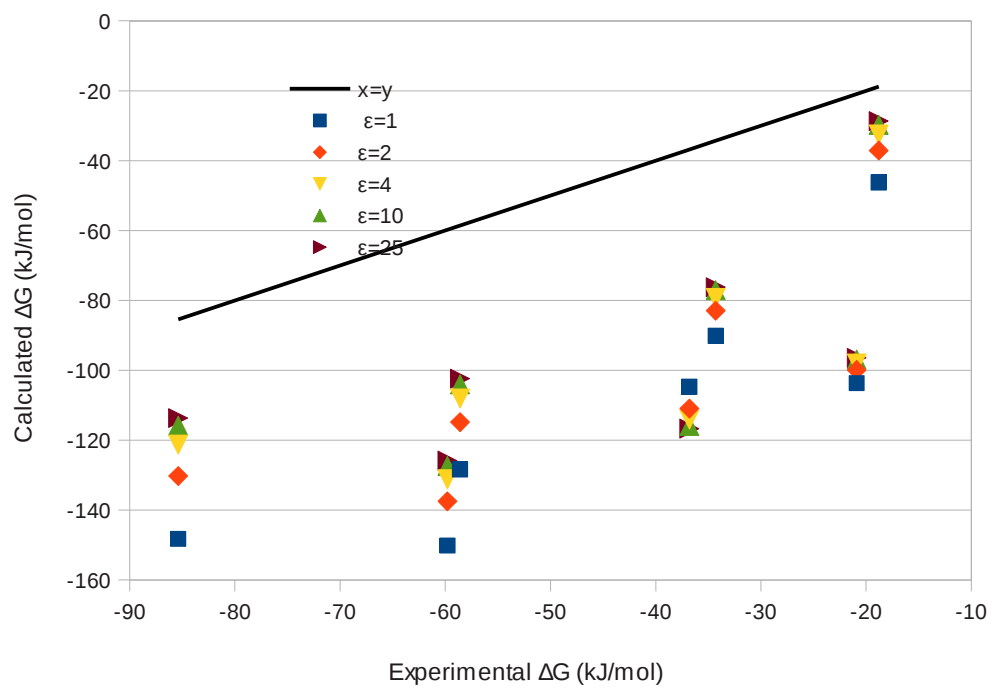


Figure 5. Results of the MMGB/s-LRA/ β method with different values of ϵ_{int} for the binding of seven biotin analogues to avidin.

