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A semiempirical approach to ligand-binding affinities: Dependence on the Hamiltonian and corrections

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We present a combination of semiempirical quantum-mechanical (SQM) calculations in the conductor-like screening model with the MM/GBSA (molecular-mechanics with generalised Born and surface-area solvation) method for ligand-binding affinity calculations. We test three SQM Hamiltonians, AM1, RM1, and PM6, as well as hydrogen-bond corrections and two different dispersion corrections. As test cases, we use the binding of seven biotin analogues to avidin, nine inhibitors to factor Xa, and nine phenol-derivatives to ferritin. The results vary somewhat for the three test cases, but a dispersion correction is mandatory to reproduce experimental estimates. On average, AM1 with the DH2 hydrogen-bond and dispersion corrections gives the best results, which are similar to those of standard MM/GBSA calculations for the same systems. The total time consumption is only 1.3–1.6 times larger than for MM/GBSA.

Keywords: MM/PBSA, semiempirical calculations, ligand binding, continuum solvation, dispersion, hydrogen-bond corrections.

Introduction

Most drug molecules exert their action by binding to a receptor, typically a protein, forming a complex, as described by the reaction

$$\mathrm{P} + \mathrm{L} \rightarrow \mathrm{PL} \qquad (1)$$

where P is the protein, L the ligand (the drug), and PL the complex. The binding is governed by the binding free energy, ΔG_{bind}. Much effort has been spent on developing computational methods to estimate this quantity.[1,2] If ΔG_{bind} could be accurately calculated, important parts of drug development could be performed in the computer. Thereby, the number of drug candidates that needs to be synthesised could be strongly reduced, which would allow pharmaceutical companies to save vast amounts of money and time.

Computational methods to estimate binding affinities range from statistical scoring functions to simulation-based methods that are exact in theory but require extensive sampling of unphysical intermediate states.[1] An attractive alternative is the so-called end-point methods, which sample only the protein, the ligand, and the complex.[3] One of the most popular end-point methods is MM/GBSA (molecular mechanics with generalised Born and surface-area solvation). This method estimates the binding free energy as the difference in free energy between the complex, the protein, and the ligand, viz., $\Delta G_{\mathrm{bind}} = G(\mathrm{PL}) - G(\mathrm{P}) - G(\mathrm{L})$. Each free energy is estimated from the sum[4,5]

$$G = \langle E_{\mathrm{ele}} + E_{\mathrm{vdW}} + G_{\mathrm{solv}} + G_{\mathrm{np}} - TS_{\mathrm{MM}} \rangle \qquad (2)$$

where the first two terms are the electrostatic and van der Waals energies of the system, estimated at the molecular mechanics (MM) level, G_{solv} is the polar solvation free energy, G_{np} is the non-polar solvation free energy, and the last term is the absolute temperature multiplied by an entropy estimate, obtained at the MM level. The brackets in Eqn. 2 indicate an average over snapshots from a molecular dynamics (MD) or Monte Carlo simulation.

The sampling of snapshots is performed at the MM level because the number of atoms in the system is typically more than 10000, including explicit solvent molecules. However, when the energies in Eqn. 2 are computed, the solvent molecules are removed, normally giving a system of only a few thousands of atoms. Systems of such a size are amenable to semiempirical quantum mechanical (SQM) methods, at least with linear-scaling or divide-and-conquer approaches.[6,7] Indeed, there has been a growing interest of using SQM methods in various scoring schemes.[8,9,10,11]

Such an approach was first introduced by Merz and co-workers.[12] They used crystal structures and supplemented SQM calculations at the AM1[13] level with Poisson–Boltzmann (PB) continuum-solvation energy, a non-polar solvation term from the buried surface area, an entropy estimate from the number of rotable bonds in the ligand, and the dispersion term from the Amber force field. The same approach was applied to ~200 protein–ligand complexes with both the AM1 and PM3[14] methods and it was tested to optimise empirical weights of each of the five terms in the energy function.[15] The same group has also developed a more MM/GBSA-like version of this method, in which the SQM energies (still at the AM1 and PM3 levels and with the Amber dispersion term) were supplemented by PB polar solvation, as well as non-polar solvation and entropy terms from MM/PBSA.[16] The energies were calculated for 50 snapshots from a MD simulation, partly optimised by a SQM/MM approach.

Hobza and co-workers have employed a similar method, using the same MM/GBSA entropy, but they add terms for ligand deformation and ligand non-polar desolvation.[17,18,19] They also employ the more recent PM6[20] method with corrections for dispersion and hydrogen-bond interactions (DH2).[21,22] They use a single minimised structure of the complex,

although they have shown that energies calculated from a minimised structure of the ligand deviates by 13 kJ/mol on average from those calculated on an ensemble of structures from a MD simulation.[23]

Recently, Anisimov and Cavasotto introduced an approach in which they re-evaluate snapshots from MD simulations using the PM3 method, COSMO (conductor-like screening model) solvation,[24] as well as entropy and non-polar solvation from the MM/GBSA approach.[25] They do not add any dispersion correction but they argue that it is necessary to partially minimize the snapshots because the SQM phase-space is different from the simulated. However, it should be noted that a similar argument applies to the standard MM/GBSA method (the simulations are with explicit water, but the energies are calculated in a continuum solvent).

In this paper, we investigate a strict SQM version of MM/GBSA in which we replace the $E_{ele} + E_{vdW} + G_{solv}$ terms in Eqn. 1 by SQM calculations with COSMO solvation. We test three different SQM Hamiltonians, AM1,[13] RM1,[26] and PM6,[20] and investigate the effect of dispersion and hydrogen-bond corrections. We study how the various combinations reproduce experimental binding affinities for three test cases, viz. the binding of seven biotin analogues to avidin, nine inhibitors to factor Xa, and nine phenol-derivatives to ferritin. These test cases have been studied before with theoretical methods[27,28,29,30,31,32,33] and experimental binding affinities are available.[34,35,36,37,38]

Methods

Preparation of protein and ligands. We have studied avidin with seven biotin analogues (Btn1–Btn7), factor Xa (fXa) with nine 3-amidinobenzyl-1H-indole-2-carboxamide inhibitors (C9–C125), and ferritin with nine phenol-derivatives (L01–L09). All the ligands are shown in Figure 1. The preparation of the proteins has been described before.[32,33,39] All ionisable protein residues were assigned their standard protonation state at pH 7. The single histidine residue in each subunit of the tetrameric avidin was protonated on the NE2 atom. For fXa, residues 57 and 83 were protonated on the ND1 atom, residues 91, 145, and 199 on the NE2 atom, and residue 13 on both atoms. In each subunit of ferritin (modelled as a dimer), residues 49, 132, and 147 were doubly protonated, residue 114 was protonated on the ND1 atom, and residue 124 on the NE2 atom. The Amber99SB force field[40] was used to describe the protein atoms in avidin and ferritin, and fXa protein atoms were described with Amber99 force field.[41] The avidin ligands were described using Amber99,[39] and the fXa and ferritin ligands with the general Amber force field.[42] Ligand charges were calculated with the restrained electrostatic potential method,[43] using potentials calculated at the HF/6-31* level and sampled with the Merz–Kollman scheme.[44] Each protein–ligand system was immersed in an octahedral box of TIP4P-Ewald[45] (avidin) or TIP3P[46] (ferritin and fXa) water molecules that extended at least 10 Å outside the protein.

Simulation Protocol. The molecular dynamics (MD) simulations were run by the sander module in Amber 10.[47] The temperature was kept at 300 K using Langevin dynamics[48] with a collision frequency of 2.0 ps^{-1}. The pressure was kept at 1 atm using a weak-coupling approach[49] with isotropic position rescaling and a relaxation time of 1 ps. Particle-mesh Ewald summation[50] with a fourth-order B-spline interpolation and a tolerance of 10^{-5} was used to treat long-range electrostatics and the long-range van der Waals interaction were treated with a continuum approach. The non-bonded cutoff was 8 Å and the non-bonded pair list was updated every 50 fs. The MD time step was 2 fs and the SHAKE algorithm[51] was used to constrain bond lengths involving hydrogen atoms.

The complexes were simulated as follows: First, the system was optimized by 500 steps of steepest descent minimization, keeping all atoms, except water molecules and hydrogen atoms, restrained to their start positions with a force constant of 418 kJ/mol/Å^2. The minimisation was followed by 20 ps MD equilibration with a constant pressure and the restraining force reduced to 214 kJ/mol/Å^2. Finally, a 300 ps (avidin) or 1200 ps (fXa and ferritin) simulation at a constant pressure, but without any restrains, was performed and the final structure was used for energy calculations. We employed 20 independent simulations for each of the avidin ligands and 40 independent simulations for the other ligands, by assigning different starting velocities to atoms (for the avidin ligand Btn2, 25 simulations were run to obtain a precision similar to that of the other ligands). Avidin is a tetramer with four binding sites. The full tetramer was treated explicitly in the calculations and the binding energy was calculated for each ligand separately, treating the other three ligands as a part of protein. Hence, we performed energy calculations (both with MM and SQM) on 40 snapshots for ferritin and fXa, and $4 \times 20 = 80$ structures for avidin.

MM/GBSA Calculations. ΔG_{bind} was calculated according to Eqn 2. The terms were calculated with Amber 10[47] with all water molecules stripped off and without any periodic boundary conditions, but with an infinite cutoff. The MM energies were estimated using the same force field as in the simulations. The polar solvation energy was calculated by the GB model of Onufriev et al., model I (α = 0.8, β = 0, and γ = 2.91). The non-polar solvation energy was estimated from the solvent-accessible surface area (SASA) according to $G_{\text{np}} = \gamma$ SASA + b, with γ = 0.0227 (kJ/mol)/Å^2 and b = 3.85 kJ/mol.[52] The entropy was estimated by a normal-mode analysis of the harmonic frequencies calculated at the MM level. For this calculation, we used our modification of MM/GBSA to improve the precision:[53] All residues more than 12 Å from any atom in the ligand were deleted and the remaining atoms were minimised, keeping all residues more than 8 Å from ligand fixed (including all water molecules), to ensure that the geometry is as close as possible to the original structure. In the frequency calculations, the fixed buffer region was omitted.

Semiempirical calculations. All SQM calculations were performed with the MOPAC2009 software.[54] Three Hamiltonians were tested AM1,[13] RM1,[26] and PM6.[20] All SQM calculations were performed with the localised molecular-orbital method (mozyme keyword).[55] Dispersion and hydrogen-bond corrections were computed by the transferable SQM DH2[21,22] method as implemented in MOPAC2009. This dispersion correction will be referred to as PDC and the hydrogen-bond corrections as HBC. We also tested the dispersion correction suggested by Merz and coworkers,[12,15] i.e. to take the r^{-6} part of Lennard-Jones potential, calculated with the same force field as used in the MD simulation (denoted ADC in the following).[12] Solvation energies were calculated by COSMO[24] with an external dielectric constant of 80 and with radii of 1.30, 2.00, 1.83, 1.72, and 2.16 Å for H, C, N, O, and S, respectively.[56]

We tested the influence of the radii on the results by also using recently suggested optimised atomic radii.[57] MOPAC2009 requires that all atoms of the same element have the same radii so we used the element-wise radii, optimised separately for AM1 and RM1.[57] No optimised radii were presented for PM6, so for this method, we instead used the radii optimised for PM5. As will be seen below, this did not give significantly different affinities. Therefore, calculations with optimised radii were performed only on the ferritin test case.

To create a SQM version of MM/GBSA, the $E_{\text{ele}} + E_{\text{vdW}} + G_{\text{solv}}$ terms in Eqn. 2 were replaced with the SQM heat of formation in COSMO solvent, H_f^{COSMO}. To this energy, the dispersion (E_{disp}) and hydrogen-bond (E_{HBC}) corrections were optionally added, and to obtain ΔG_{bind}, the MM/GBSA G_{np} and TS_{MM} terms were added, i.e., Eqn 2 was replaced with

$$G = \left\langle H_f^{\mathrm{COSMO}} \left(+ E_{\mathrm{disp}} \right) \left(+ E_{\mathrm{HBC}} \right) + G_{\mathrm{np}} - TS_{\mathrm{MM}} \right\rangle \quad (3)$$

Quality measures. The quality of the results relative to the experimental affinities[34,35,36,37,38] was estimated using the following measures: the mean absolute deviation after the systematic error (i.e. the mean signed error) has been removed (MADtr), the correlation coefficient (r^2), the slope of the best regression line, and Kendall's rank correlation coefficient, calculated for the pairs of ligands for which both the experimental and calculated differences in affinities are statistically significant at the 90% level (τ_{90}).[58] The number of such significant pairs is also given (np_{90}). For avidin and fXa, no experimental uncertainty was reported. Therefore, we assumed a typical experimental uncertainty of 1.7 kJ/mol for these two proteins.[59] The uncertainty of the quality measures was estimated using a parametric bootstrap (using 1000 random samples),[60] utilising the uncertainty of both the experiments and computational predictions.

Results and Discussion

We have estimated the ligand-binding free energy using the MM/GBSA method and by replacing the $E_{\mathrm{ele}} + E_{\mathrm{vdW}}$ and G_{solv} energies with solvated SQM energies. We have studied three different proteins (avidin, factor Xa, and ferritin), using 7–9 ligands for each protein. We will discuss the results for each protein separately.

Avidin. The H_f^{COSMO} results obtained with the three SQM methods are shown in Table 1. For the AM1 and RM1 methods, H_f^{COSMO} is positive (i.e., indicating no binding), whereas PM6 gives negative energies for four of the seven ligands. These heats of formation can be compared to the sum of the corresponding energies in the standard MM/GBSA method ($E_{\mathrm{ele}} + E_{\mathrm{vdW}} + G_{solv}$, where the last term is calculated with GB). It is clear that AM1 and RM1 give trends that are opposite to those of the classical energies with correlation coefficients (r) of –0.83 and –0.48, respectively. However, there is a positive correlation between the PM6 heat of formation and the classical energies, r = 0.45. Adding dispersion (PDC) and hydrogen-bond corrections to the SQM results improves the correlation to r = 0.77–0.86 for all three methods. Employing instead the Amber dispersion energy (ADC) increases the correlation even more (r = 0.95–0.99). The reason for this is that the E_{ele} and G_{solv}(GB) terms nearly cancel, so that $E_{\mathrm{ele}} + E_{\mathrm{vdW}} + G_{\mathrm{solv}}$ is dominated by the E_{vdW} term.

By adding the MM/GBSA non-polar and entropy terms, we get our total SQM estimates of the binding free energies. These estimates are also given in Table 1, with or without the dispersion and hydrogen-bond corrections, and the results are compared to experiments[34,35,36] in Table 2. For the uncorrected energies (i.e., $H_f^{\mathrm{COSMO}} + G_{np} - TS_{MM}$) with AM1 and RM1, positive free energies are obtained, and the ranking of the ligands is poor, as is indicated by negative Kendall's τ_{90} rank correlation coefficients and slopes. The correlation is also negative, although r^2 is positive by definition. For PM6, the binding affinities are still positive, but the correlation is positive, although r^2 = 0.4 is not particularly good. The MADtr of 23 kJ/mol is slightly worse than a null hypothesis that assigns the same affinity to all ligands (giving a MADtr of 20 kJ/mol).

The dispersion correction is between –53 and –156 kJ/mol for PDC, and between –131 and –391 kJ/mol for ADC, and therefore lowers the binding free energies considerably. However, for AM1 and RM1, ΔG_{bind} with PDC is still positive. The AM1 predictions with PDC are still bad with r^2 and the slope close to zero, but the results with ADC are quite good, giving r^2 = 0.6 and τ_{90} = 0.8. In both cases, the MADtr is worse than the null hypothesis. The RM1 predictions are good, irrespectively of the correction. In fact, a perfect τ_{90} = 1.0 is

obtained with ADC. However, the range of the predicted affinities increases considerably with ADC, giving a MADtr of 35 kJ/mol, whereas the MADtr when using PDC is better than the null hypothesis, 15 kJ/mol. On the other hand, RM1 with PDC gives incorrect ranking for two ligand pairs (τ_{90} = 0.7). The PM6 ranking becomes perfect when we add either of the dispersion corrections (τ_{90} = 1.0), although r^2 and MADtr are better with PDC.

The hydrogen-bond correction (HBC) is between –30 and –93 kJ/mol. For AM1, this correction makes the ranking with both dispersion corrections perfect (τ_{90} = 1.0). However, both r^2 and MADtr are better with the PDC correction. In fact, the combination of AM1, PDC, and HBC gives a slope of 1 and a very low MADtr, 8 kJ/mol. These results are compared to the experimental data in Figure 2. The range of the predictions is 59 kJ/mol, i.e., close the experimental range (67 kJ/mol). For RM1, τ_{90} is not affected much by the correction, giving 0.8 and 1.0, for the PDC and ADC corrections, respectively. r^2 and MADtr are better with the PDC correction. Likewise, the PM6 results are not affected much by HBC, still giving a perfect ranking, but slightly better MADtr and r^2 for PDC. All of the predicted affinities are very different from the experimental energies in absolute terms, but we have previously shown that absolute energies are strongly affected by the solvation method.[28]

It is of interest to also compare the SQM approaches to a pure MM approach. Therefore, the MM/GBSA and MM/PBSA results[28] are also included in Tables 1 and 2. For MM/GBSA, τ_{90} = 0.9 and r^2 = 0.7, i.e., significantly worse than the best SQM results. However, the range is on par with the best SQM results and the slope is close to unity, giving a reasonable MADtr of 12 kJ/mol. The MM/PBSA method is even better with τ_{90} = 1.0, r^2 = 0.9, and MADtr = 10 kJ/mol. This is similar to the best SQM method (AM1+PDC+HBC) as can be seen in Figure 2.

When comparing different methods, it is also important to consider the uncertainty of the predictions. The precision of the binding affinities, presented as standard deviations of the mean, i.e., standard errors, are shown in Table 3. All the SQM methods have a similar precision 2–3 kJ/mol, although there is a clear tendency that the standard error increases when the dispersion (PDC by less than 0.1 kJ/mol, ADC by 0.5 kJ/mol on average) and hydrogen-bond corrections are added (by 0.3 kJ/mol on average). Therefore, the uncorrected SQM methods give a similar precision to the MM/GBSA and MM/PBSA methods, whereas the standard error of the SQM results with both corrections is slightly larger. In Table 1, we also list the average uncertainty for the individual terms. It can be seen that the standard errors of the entropy, heat of formation, and $E_{ele} + E_{vdW} + G_{solv}$ terms are similar in size, 1.4 – 2.0 kJ/mol. The standard errors of the electrostatics and polar solvation terms are larger, but they cancel to a large extent. The uncertainty of the non-polar solvation term is negligible. The uncertainty of the HBC term is similar to that of the heat of formation, whereas that of the dispersion corrections is smaller (especially for PDC).

Factor Xa. The results of the fXa test case are shown in Tables 4–6 (raw data, quality metrics, and standard errors, respectively). This protein contains a structural Ca^{2+} ion, which is not parametrised in the RM1 method. Therefore, the Ca^{2+} ion was ignored in all the RM1 calculations. This is a reasonable approximation, because the Ca^{2+} ion is located ~25 Å from the ligand, and the results of PM6 and AM1 did not change much when the ion was removed (cf. Table 5, $MADtr^b$ and $\tau_{90}{}^b$ columns).

For fXa, all three SQM methods give positive and rather similar H_f^{COSMO} results for all nine ligands. The uncorrected energies are strongly anti-correlated to the MM/GBSA $E_{ele} + E_{vdW} + G_{solv}$ energies with r = –0.9. The dispersion corrections are large, –179 to –204 kJ/mol for PDC and –356 to –400 kJ/mol for ADC. With these corrections, the energies for all ligands become negative. However, with PDC, the results are still anti-correlated to the MM/GBSA results (r = –0.9 for RM1 and PM6, and –0.2 for AM1), whereas with ADC, the

AM1 and RM1 results are reasonably correlated to the MM/GBSA result (r = 0.5–0.7), but the PM6 results still shows a weak anti-correlation (–0.2). The HBC is rather small, –34 to –40 kJ/mol.

fXa is a tricky test case because all except one ligand have experimental affinities within 9 kJ/mol (and six of them have experimental affinities within 4 kJ/mol). Moreover, the standard errors of the ΔG_{bind} predictions (Table 6) are appreciably larger than for the avidin test case, 3–7 kJ/mol (dominated by the heat of formation and ADC terms). Together, this means that only very few pairs of ligands have significantly different differences. In fact, all SQM methods give negative correlations, slopes and τ_{90} values, except AM1 and RM1 with ADC. The correlations with ADC and HBC are only mediocre (r^2 = 0.4 and 0.2), but the MADtr of ~5 kJ/mol is equal to that of the null hypothesis and τ_{90} is perfect, although it is based on only very few significant pairs (9 for AM1, but 1 for RM1). The results for the MM/GBSA method is similar as can be seen in Figure 3: τ_{90} = 1.0 (based on 12 pairs) and r^2 = 0.4, and the MADtr is slightly better than that of the null hypothesis, 4 kJ/mol.

Ferritin. The results for ferritin are shown in Tables 7–9 (raw data, quality metrics, and standard errors, respectively). The ferritin ligands interact with the receptor through a single hydrogen bond and extensive hydrophobic interactions in a buried cavity. Therefore, this is a good test case for the dispersion and hydrogen-bond corrections.

For ferritin, again all three SQM methods give positive and similar H_f^{COSMO} results for all nine ligands. The uncorrected energies are almost perfectly anti-correlated to the MM/GBSA $E_{\text{ele}} + E_{\text{vdW}} + G_{\text{solv}}$ energies (r < –0.95). The dispersion corrections are quite large, –51 to –103 for PDC and –101 to –226 kJ/mol. With these corrections, the SQM energies become negative for all ligands and the correlation with the MM/GBSA results is perfect (r > 0.98). This is probably because the MM/GBSA term is dominated by the E_{vdW} term. The HBC is small, –1 to –5 kJ/mol.

From the results in Table 8, it is clear that the SQM methods without any corrections fail to give a proper ranking of the ligands, with negative τ_{90} and slopes for all methods. However, with the dispersion corrections, the ranking becomes perfect (τ_{90} = 1.0) for all methods. The PDC gives a more narrow range of the estimated affinities and a low MADtr, 1–2 kJ/mol, whereas ADC gives a much wider range and a significantly worse MADtr 21 to 22 kJ/mol. The correlation is the same (within statistical uncertainty) for all methods, r^2 = 0.9–1.0. The HBC has an insignificant effect and the three SQM methods give similar results. MM/GBSA gives τ_{90} and r^2 values (1.0 and 0.9, respectively) that are similar to the SQM methods, but MADtr = 9 kJ/mol is significantly worse than that of the best SQM methods and also worse than the null hypothesis (3 kJ/mol). The best SQM methods are compared to MM/GBSA in Figure 4.

The standard errors of the predictions, listed in Table 9, are similar to the avidin results, which is somewhat unexpected, considering that the avidin binding affinities are based on twice as many individual estimates (half as many MD simulations were used, but each simulation gives rise to four affinity estimates for the tetrameric avidin protein). This indicates that the precision depends quite strongly on the exposure on the binding site: In ferritin, the binding site is completely buried in the protein, whereas that of avidin is somewhat more exposed and more flexible. The binding site of fXa is a partly solvent-exposed cleft. From the average uncertainties of the individual terms in Table 7, it is clear that the entropy term dominates the uncertainty for this test case.

For ferritin, we tested also to use COSMO radii recently optimised for the AM1, RM1, and PM5 methods.[57] The results of these calculations are also included in Table 7. It can be seen that the optimised radii change the net H_f^{COSMO} by less than 2 kJ/mol (1.1 kJ/mol on average). With a single exception, the results are always slightly smaller with the optimised

radii. The differences are smaller with AM1 than with the other two methods. However, these differences are so small that they are not statistically significant and they do not change any of the quality measures significantly, as is shown in Table 8 for the calculations with PDC+HBC. This shows that the results are robust with respect to the COSMO radii. Therefore, calculations with optimised COSMO radii were not performed for the other two proteins.

Time consumption. A SQM calculation on a single snapshot of the complex on an Intel Xeon 2.26 GHz computer takes ~2200 s for AM1 and RM1, and ~4100 s for PM6. This is 40–70 times longer than the corresponding MM/GBSA calculation, which takes ~60 s. On the other hand, the cost for the entropy and MD simulations, which are common to both approaches, are ~1200 and ~5800 s for each snapshot, respectively. This shows that in practice, the SQM calculations only increase the total time for the calculations by a factor of 1.3–1.6.

Conclusions

We have evaluated a SQM variant of MM/GBSA, in which the MM energy and polar solvation terms are replaced by a SQM energy in COSMO continuum solvent. We have studied three different proteins with 7–9 ligands each. As often observed in ligand-binding studies, the three proteins give somewhat different results. However, we still obtain much useful information on at least five issues.

First, we can conclude that the dispersion correction is important for the SQM method. From the results in Tables 2, 5, and 8, it is quite clear that in the great majority of cases, most of the quality measures are improved when a dispersion correction is included. This is especially clear for fXa, for which 93% of the quality measures (MADtr, r^2, τ_{90}, slope, and range for two dispersion corrections and three SQM methods) improve with the dispersion correction. For ferritin, PDC always improve the results, whereas only r^2 and τ_{90} are improved with ADC. For avidin, the AM1 and RM1 results are improved, whereas only r^2 and τ_{90} are improved for PM6. Moreover, nearly all affinities without the dispersion corrections are positive.

Second, we have compared two different dispersion corrections, PDC and ADC. For two of the proteins, avidin and ferritin (i.e. the two proteins in which the ligand binds in a cavity), the best results are obtained with PDC, whereas for fXa (in which the ligand binds to a more solvent-exposed cleft), ADC gives the better results. For all three proteins, the ADC terms are larger than the PDC terms. For avidin and ferritin, this gives too large ranges of the predicted affinities and therefore also large MADtr.

Third, we have studied the effect of the hydrogen-bond correction. Again, the results are somewhat varying, although in a less systematic way, often because the correction is rather small. However, 71% of the quality measures are improved by the HBC correction, so therefore we recommend the HBC, which is also strongly supported by calculations on small intermolecular test cases, for which both dispersion and hydrogen-bond corrections are indispensable for accurate results.[21,22,61]

Fourth, we have compared three different SQM Hamiltonians, AM1, RM1, and PM6. For fXa, AM1 gives the best results, whereas for the other two proteins, the results are more varying. For avidin, PM6 gives the best correlations and rankings, whereas AM1 often is better for the other quality measures. In particular, with the preferred PDC and HBC corrections, AM1 gives the best results for all of the quality measures (although PM6 gives the same r^2 and τ_{90}). For ferritin, RM1 often gives the better results, but the differences are not statistically significant. Therefore, we tend to recommend the AM1 method, although the PM6-DH2 method gave slightly better results on smaller molecules.[22,61]

Finally, we can compare the SQM results with those obtained with the standard MM/GBSA method obtained with the same MD snapshots. For avidin, the preferred

AM1+PDC+HBC method gives significantly better results than MM/GBSA for all five quality measures. On the other hand, MM/PBSA gives results that are better than MM/GBSA and similar to those of AM1+PDC+HBC (within statistical uncertainty). For fXa, MM/GBSA gives clearly better result than AM1+PDC+HBC, because PDC gives poor results for this protein. On the other hand, the best SQM method, AM1+ADC+HBC, gives results of the same quality as MM/GBSA. For ferritin, the best SQM methods (including AM1+PDC+HBC) give significantly better MADtr, slope and ranges than MM/GBSA, but similar r^2 and τ_{90}.

The results of the AM1+PDC+HBC approach, as well as the best alternative SQM approach and MM/GBSA, for the three proteins are shown in Figures 2–4. It can be seen that the performance of the SQM methods depends strongly on the protein and ligands studied. Moreover, the absolute binding affinities are often quite poor and depend on both the solvation method (as has been observed before[28]) and the dispersion correction. This indicates that the accuracy of MM/GBSA is not primarily limited by the accuracy of the MM force field, but rather by its underlying approximations, e.g. the use of only end-point structures (the complex, protein, and ligand), all taken from the complex simulation, the continuum description of the solvent, and the normal-mode entropy term.[62]

However, considering the rather modest increase in the total amount of computation time and the fact that the SQM calculations have the theoretical advantage of including polarisation and charge-transfer effects in a consistent way, we think that the SQM-COSMO/SA method employed here, especially using AM1, PDC, and HBC, is a competitive alternative to MM/(PB)GBSA calculations for ligand binding. However, it remains to investigate why PDC does not work properly for fXa and whether the performance depends on the solvent-exposure of the binding site.

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Table 1. Energies for the avidin ligands in kJ/mol.[a]

	Btn1	Btn2	Btn3	Btn4	Btn5	Btn6	Btn7	SE[b]
E_{ele}	-1273.0	-1272.2	-1249.3	-163.4	-106.8	-74.2	-105.3	3.8
E_{vdW}	-155.7	-155.2	-142.8	-205.0	-136.0	-136.3	-57.6	1.2
G_{solv} (GB)	1223.0	1237.9	1203.3	181.8	107.4	88.3	85.6	3.0
$E_{ele} + E_{vdW} + G_{solv}$ (GB)	-205.7	-189.5	-188.9	-186.6	-135.4	-122.2	-77.3	1.6
G_{np}	-16.8	-16.8	-16.8	-21.1	-16.3	-16.1	-10.5	0.0
$-TS_{MM}$	-97.7	-104.4	-99.5	-98.2	-81.3	-74.9	-65.8	1.7
E_{PDC}	-136.1	-141.2	-129.0	-155.9	-113.6	-103.3	-52.6	0.4
E_{ADC}	-360.9	-365.2	-344.1	-391.3	-260.2	-245.6	-130.7	1.0
E_{HBC}	-89.9	-87.0	-92.5	-42.1	-35.7	-30.4	-42.6	1.8
H_f^{COSMO} (AM1)	72.3	93.5	69.3	102.5	66.2	59.1	26.1	2.0
H_f^{COSMO} (RM1)	57.8	61.4	49.7	121.2	53.8	61.5	31.1	1.4
H_f^{COSMO} (PM6)	-74.3	-53.9	-78.8	40.8	17.0	19.9	-17.3	1.7
ΔG_{bind}(AM1)	153.3	181.1	152.0	179.6	131.2	117.8	81.4	
ΔG_{bind}(AM1/PDC)	17.2	39.9	23.0	23.7	17.6	14.5	28.8	
ΔG_{bind}(AM1/ADC)	-207.6	-184.0	-192.1	-211.7	-129.0	-127.8	-49.3	
ΔG_{bind}(AM1/PDC/HBC)	-72.7	-47.0	-69.5	-18.3	-18.1	-15.9	-13.8	
ΔG_{bind}(AM1/ADC/HBC)	-297.5	-271.0	-284.6	-253.7	-164.7	-158.2	-92.0	
ΔG_{bind}(RM1)	138.8	149.0	132.4	198.3	118.8	120.2	86.4	
ΔG_{bind}(RM1/PDC)	2.7	7.8	3.4	42.4	5.2	16.9	33.8	
ΔG_{bind}(RM1/ADC)	-222.1	-216.2	-211.7	-193.0	-141.4	-125.4	-44.3	
ΔG_{bind}(RM1/PDC/HBC)	-87.2	-79.2	-89.1	0.3	-30.5	-13.5	-8.8	
ΔG_{bind}(RM1/ADC/HBC)	-312.1	-303.2	-304.2	-235.1	-177.1	-155.8	-87.0	
ΔG_{bind}(PM6)	6.7	33.6	3.9	117.9	82.1	78.7	38.0	
ΔG_{bind}(PM6/PDC)	-129.4	-107.6	-125.1	-38.0	-31.5	-24.7	-14.6	
ΔG_{bind}(PM6/ADC)	-354.3	-331.5	-340.2	-273.4	-178.2	-166.9	-92.8	
ΔG_{bind}(PM6/PDC/HBC)	-219.3	-194.5	-217.6	-80.1	-67.2	-55.1	-57.3	
ΔG_{bind}(PM6/ADC/HBC)	-312.1	-303.2	-304.2	-235.1	-177.1	-155.8	-87.0	
ΔG_{bind}(MM/GBSA)	-124.7	-101.9	-106.2	-109.5	-70.3	-63.5	-22.0	
ΔG_{bind}(MM/PBSA)	-73.5	-55.4	-49.2	-10.0	-28.7	5.6	10.7	
ΔG_{bind}(Exp)[34,35,36]	-85.4	-59.8	-58.6	-36.8	-34.3	-20.9	-18.8	

[a] In all cases, PL – P – L energies are listed.
[b] Average standard error over the seven ligands.

Table 2. Quality measures of ΔG_{bind} predicted with the various methods for the avidin test case. [a]

SQM	HBC	Disp.	MADtr	r^2	τ_{90}	np_{90}	slope	range
AM1	no	no	45.0	0.38	-0.63	16	-0.9	99.7
		PDC	22.4	0.01	-0.33	9	0.0	25.5
		ADC	30.0	0.55	0.75	16	1.8	162.3
	yes	PDC	7.5	0.86	1.00	13	1.0	58.9
		ADC	50.4	0.76	1.00	17	2.8	205.6
RM1	no	no	40.0	0.11	-0.53	17	-0.5	111.9
		PDC	15.1	0.38	0.69	13	0.4	39.7
		ADC	35.1	0.69	1.00	17	2.3	177.8
	yes	PDC	15.6	0.77	0.76	17	1.4	89.4
		ADC	55.4	0.82	1.00	17	3.3	225.1
PM6	no	no	23.2	0.40	0.60	15	1.1	113.9
		PDC	26.1	0.88	1.00	17	2.0	114.8
		ADC	70.2	0.81	1.00	18	3.8	261.5
	yes	PDC	51.6	0.86	1.00	17	3.0	164.3
		ADC	55.4	0.82	1.00	17	3.3	225.1
MM/GBSA[28]			12.3	0.69	0.88	17	1.2	102.7
MM/PBSA[28]			9.7	0.93	1.00	17	1.3	84.2
Average SE[b]			1.2	0.03	0.01		0.1	

[a] The quality measures are the mean absolute deviation when the systematic error has been removed (MADtr in kJ/mol), the correlation coefficient (r^2), Kendall's rank correlation coefficient, calculated for the pairs of ligands for which both the experimental and calculated differences in affinities are statistically significant at the 90% level (τ_{90}; assuming an experimental uncertainty of 1.7 kJ/mol[59]), the number of such significant pairs (np_{90}; the number of experimentally significant pairs is 18), the slope of best regression line, and the range of affinities (kJ/mol).

[b] Standard error for the various quality measures obtained by a parametric bootstrap and averaged over all the 16 methods in the table.

Table 3. Standard errors of ΔG_{bind} predicted with the various methods for the avidin test case (kJ/mol).

SQM	HBC	Disp.	Btn1	Btn2	Btn3	Btn4	Btn5	Btn6	Btn7	Average
AM1	no	no	2.8	2.5	3.1	3.3	2.3	2.5	2.3	2.7
		PDC	2.8	2.5	3.0	3.3	2.4	2.6	2.3	2.7
		ADC	2.8	2.9	3.0	3.8	2.7	2.8	2.3	2.9
	yes	PDC	3.0	3.0	3.3	3.5	2.5	2.8	2.6	3.0
		ADC	3.2	3.4	3.5	4.1	2.9	3.1	2.7	3.3
RM1	no	no	2.3	2.4	2.5	2.7	2.5	2.0	1.4	2.3
		PDC	2.4	2.5	2.5	2.6	2.5	2.1	1.3	2.3
		ADC	3.1	3.1	3.0	3.1	2.6	2.4	1.5	2.7
	yes	PDC	2.9	3.0	3.0	2.9	2.5	2.4	1.7	2.6
		ADC	3.6	3.7	3.8	3.5	2.8	2.8	1.9	3.2
PM6	no	no	2.5	2.5	2.7	2.8	2.2	2.1	1.9	2.4
		PDC	2.7	2.7	2.7	2.9	2.3	2.2	1.9	2.5
		ADC	3.6	3.6	3.6	3.8	2.8	2.8	2.1	3.2
	yes	PDC	3.3	3.2	3.5	3.4	2.5	2.7	2.3	3.0
		ADC	3.6	3.7	3.8	3.5	2.8	2.8	1.9	3.2
MM/GBSA			2.5	2.5	2.4	2.9	2.3	2.0	1.7	2.3
MM/PBSA			3.3	3.1	2.9	3.0	2.7	2.3	1.8	2.7

Table 4. Energies of fXa ligands in kJ/mol. [a]

	C9	C39	C47	C49	C50	C53	C57	C63	C125	SE[b]
E_{ele}	-280.2	-231.1	-259.6	-274.1	-241.2	-254.4	-204.5	-229.9	-271.6	9.6
E_{vdW}	-185.7	-172.4	-186.6	-186.2	-188.9	-182.9	-181.8	-173.4	-183.8	2.0
G_{solv} (GB)	302.9	261.2	290.4	307.4	278.0	281.9	241.0	261.9	301.5	8.7
$E_{ele} + E_{vdW} + G_{solv}$ (GB)	22.7	30.1	30.8	33.3	36.7	27.5	36.5	32.0	29.8	2.5
G_{np}	-163.0	-142.3	-155.8	-153.0	-152.2	-155.5	-145.4	-141.4	-154.0	0.1
$-TS_{MM}$	-24.7	-24.5	-24.6	-24.9	-24.5	-24.4	-24.0	-23.8	-24.5	2.8
E_{PDC}	116.8	112.0	116.4	118.1	119.2	112.3	113.1	116.1	114.8	1.4
E_{ADC}	-392.6	-356.0	-387.5	-394.5	-400.2	-392.7	-373.6	-357.5	-388.9	4.0
E_{HBC}	-35.5	-34.6	-36.1	-40.1	-38.3	-36.9	-33.7	-34.0	-36.7	0.8
H_f^{COSMO} (AM1)	146.2	119.2	140.6	149.5	143.4	142.0	131.4	123.5	141.0	3.4
H_f^{COSMO} (RM1)	164.8	134.8	164.9	173.3	165.9	167.2	147.7	135.9	160.7	3.2
H_f^{COSMO} (PM6)	134.6	96.8	131.6	140.2	128.5	130.9	96.9	95.3	128.2	3.5
ΔG_{bind}(Exp)[37]	-46.2	-27.3	-46.8	-41.9	-46.2	-44.3	-38.0	-37.4	-43.4	

[a] In all cases, PL – P – L energies are listed. All results were obtained without the Ca^{2+} ion.
[b] Average standard error over the nine ligands.

Table 5. Quality measures of ΔG_{bind} predicted with the various methods for the fXa test case.[a]

SQM	HBC	Disp.	MADtr	MADtr[b]	r^2	τ_{90}[b]	τ_{90}	np_{90}	slope	range
AM1	no	no	14.1	14.3	0.76	-1.00	-1.00	17	-1.6	35.9
		PDC	6.9	7.5	0.26	-1.00	-1.00	3	-0.4	14.2
		ADC	4.7	4.4	0.37	1.00	1.00	7	0.6	21.1
	yes	PDC	5.6	6.2	0.12	-1.00	-1.00	2	-0.2	12.1
		ADC	4.7	4.3	0.41	1.00	1.00	9	0.8	24.6
RM1	no	no	17.4		0.74		-0.89	19	-2.1	44.1
		PDC	10.0		0.58		-1.00	13	-0.9	22.4
		ADC	4.6		0.05			0	0.2	11.6
	yes	PDC	8.7		0.64		-1.00	7	-0.7	16.9
		ADC	4.5		0.19		1.00	1	0.3	14.6
PM6	no	no	21.6	21.8	0.67	-0.89	-0.89	18	-2.6	49.1
		PDC	14.2	14.4	0.46	-0.88	-0.88	17	-1.3	36.9
		ADC	8.3	8.7	0.05	-0.20	-0.20	5	-0.3	26.5
	yes	PDC	12.8	13.0	0.46	-0.86	-0.86	14	-1.1	31.7
		ADC	7.4	7.9	0.01	-1.00	-1.00	2	-0.1	21.0
MM/GBSA			4.3		0.43		1.00	12	0.7	21.7
Average SE[c]			1.4		0.14		0.09		0.3	

[a] The quality measures are the same as in Table 2. The number of pairs with experimentally significant differences in the binding affinities is 22, assuming a typical experimental uncertainty of 1.7 kJ/mol.[59] All results were obtained without the Ca^{2+} ion, except the MADtr and τ_{90} columns marked by [b].

[c] Standard error for the various quality measures obtained by a parametric bootstrap and averaged over all the 16 methods in the table.

Table 6. Standard errors of ΔG_{bind} predicted by the various methods for the fXa test case (kJ/mol). [a]

SQM	HBC	Disp.	C9	C39	C47	C49	C50	C53	C57	C63	C125	Average
AM1	no	no	3.3	4.3	5.2	4.1	3.9	5.2	3.8	3.4	4.1	4.1
		PDC	3.1	3.7	5.1	4.4	3.5	5.3	4.2	3.9	4.4	4.2
		ADC	4.5	4.5	5.3	5.2	4.3	5.9	5.9	5.5	5.2	5.1
	yes	PDC	3.2	4.0	5.3	4.6	3.6	5.4	4.1	3.9	4.4	4.3
		ADC	4.5	4.9	5.5	5.5	4.6	6.1	5.8	5.6	5.2	5.3
RM1	no	no	3.9	3.8	4.6	4.3	3.6	5.2	3.8	3.6	3.7	4.1
		PDC	3.4	3.6	4.5	4.7	3.2	5.3	4.3	3.6	4.0	4.1
		ADC	4.6	4.7	4.9	5.7	4.5	6.2	6.0	5.1	5.1	5.2
	yes	PDC	3.6	4.1	4.8	4.8	3.4	5.5	4.4	3.6	4.2	4.3
		ADC	4.7	5.3	5.3	5.9	4.8	6.4	6.1	5.2	5.3	5.4
PM6	no	no	3.4	4.0	4.7	4.6	3.6	5.3	4.4	4.1	4.3	4.3
		PDC	3.5	4.0	4.7	5.1	3.6	5.8	5.3	4.6	4.8	4.6
		ADC	5.4	5.7	5.6	6.2	5.5	7.1	7.4	6.6	6.4	6.2
	yes	PDC	3.6	4.5	5.1	5.2	3.8	5.9	5.3	4.8	5.0	4.8
		ADC	5.5	6.1	5.9	6.5	5.8	7.3	7.5	6.8	6.6	6.4
MM/GBSA			3.5	3.2	3.9	4.1	3.5	3.5	4.6	3.8	4.2	3.8

[a] All results were obtained without the Ca^{2+} ion.

Table 7. Energies of ferritin ligands in kJ/mol. [a]

	L01	L02	L03	L04	L05	L06	L07	L08	L09	SE[b]
E_{ele}	-7.7	-8.8	-9.1	-8.5	-15.9	-16.0	-11.6	-17.3	-17.6	1.4
E_{vdW}	-124.1	-137.4	-128.5	-124.6	-108.1	-97.8	-97.8	-88.1	-61.7	1.1
G_{solv} (GB)	45.1	45.3	44.6	45.5	45.1	46.4	41.8	45.0	41.0	0.8
$E_{ele} + E_{vdW} + G_{solv}$ (GB)	-86.7	-100.8	-92.9	-87.6	-79.0	-67.4	-67.6	-60.3	-38.3	1.2
G_{np}	-17.0	-18.8	-18.2	-17.2	-15.7	-14.6	-14.5	-13.7	-12.2	0.1
$-TS_{MM}$	66.8	74.2	65.9	68.7	63.0	62.1	59.0	57.3	50.9	2.2
E_{PDC}	-91.8	-102.8	-98.1	-94.9	-81.7	-76.5	-74.1	-67.3	-51.4	0.6
E_{ADC}	-202.3	-225.6	-212.5	-208.5	-175.8	-161.6	-156.2	-135.7	-100.6	2.2
E_{HBC}	-1.0	-1.0	-1.1	-1.7	-2.9	-2.7	-2.5	-3.3	-4.8	0.4
H_f^{COSMO} (AM1)	46.0	49.5	50.9	49.3	41.1	41.9	40.0	37.5	33.2	1.1
H_f^{COSMO} (AM1)[c]	45.3	49.3	49.9	48.7	41.2	41.2	39.3	36.8	32.5	1.1
H_f^{COSMO} (RM1)	50.2	53.0	53.5	51.2	44.9	41.2	39.6	36.6	31.3	1.0
H_f^{COSMO} (RM1)[d]	48.4	51.4	51.8	49.8	43.9	40.0	38.0	35.1	29.9	1.0
H_f^{COSMO} (PM6)	40.8	43.9	46.4	41.9	36.8	33.1	31.3	32.5	26.0	1.4
H_f^{COSMO} (PM6)[e]	39.4	42.7	45.0	40.6	35.9	31.8	29.9	31.0	24.7	1.4
ΔG_{bind}(Exp)[38]	-30.5	-30.1	-32.7	-30.6	-28.2	-26.0	-27.5	-22.8	-18.7	

[a] In all cases, PL – P – L energies are listed.
[b] Average standard error. over the nine ligands.
[c] Results obtained with optimised AM1 COSMO radii.[57]
[d] Results obtained with optimised RM1 COSMO radii.[57]
[e] Results obtained with optimised PM5 COSMO radii.[57]

Table 8. Quality measures of ΔG_{bind} predicted by the various methods for ferritin test case. [a]

SQM	HBC	Disp.	MADtr	r^2	τ_{90}	np_{90}	slope	range
AM1	no	no	11.5	0.82	-0.93	27	-2.2	33.0
		PDC	2.0	0.94	1.00	21	1.4	20.0
		ADC	21.6	0.93	1.00	29	6.7	91.9
	yes	PDC	1.3	0.92	1.00	17	1.1	16.3
		PDC[b]	1.3	0.93	1.00	15	1.2	16.7
		ADC	20.7	0.93	1.00	29	6.4	88.2
RM1	no	no	13.2	0.85	-0.86	29	-2.6	38.4
		PDC	1.0	0.93	1.00	13	1.0	15.6
		ADC	19.7	0.93	1.00	30	6.3	86.6
	yes	PDC	1.2	0.89	1.00	7	0.7	11.9
		PDC[c]	1.3	0.87	1.00	9	0.7	12.2
		ADC	18.7	0.93	1.00	30	6.0	82.8
PM6	no	no	12.2	0.82	-0.93	27	-2.3	34.6
		PDC	1.3	0.97	1.00	15	1.3	17.4
		ADC	20.6	0.93	1.00	29	6.6	90.4
	yes	PDC	0.6	0.97	1.00	12	1.0	13.7
		PDC[d]	0.6	0.97	1.00	11	1.0	13.8
		ADC	19.7	0.93	1.00	29	6.3	86.6
MM/GBSA			8.7	0.93	1.00	28	3.3	45.8
Average SE[e]			0.9	0.08	0.03		0.3	

[a] The quality measures are the same as in Table 2. The number of pairs with experimentally significant differences in the binding affinities is 33.
[b] Results obtained with optimised AM1 COSMO radii.[57]
[c] Results obtained with optimised RM1 COSMO radii.[57]
[d] Results obtained with optimised PM5 COSMO radii.[57]
[e] Standard error for the various quality measures obtained by a parametric bootstrap and averaged over all the 16 methods in the table.

Table 9. Standard errors of ΔG_{bind} predict by the various methods for the ferritin test case (kJ/mol).

SQM	HBC	Disp.	L01	L02	L03	L04	L05	L06	L07	L08	L09	Average
AM1	no	no	2.5	2.9	3.0	3.0	2.5	2.2	2.0	2.2	3.2	2.6
		PDC	2.6	2.8	3.1	2.9	2.7	2.2	2.1	2.3	3.2	2.7
		ADC	3.4	3.7	4.3	3.3	3.8	3.4	2.9	2.9	3.4	3.4
	yes	PDC	2.6	2.8	3.2	2.9	2.8	2.4	2.0	2.5	3.3	2.7
		ADC	3.4	3.7	4.4	3.3	3.9	3.5	2.8	3.2	3.6	3.5
RM1	no	no	2.6	2.9	2.8	3.1	2.3	2.1	1.7	2.2	2.9	2.5
		PDC	2.7	2.8	2.9	2.9	2.5	2.1	1.8	2.3	3.0	2.5
		ADC	3.2	3.4	3.9	3.0	3.3	3.0	2.5	2.7	3.2	3.1
	yes	PDC	2.7	2.8	2.9	2.9	2.5	2.1	1.8	2.4	3.1	2.6
		ADC	3.2	3.4	4.0	3.1	3.4	3.1	2.5	2.9	3.5	3.2
PM6	no	no	2.6	3.1	3.2	3.0	2.7	2.3	1.8	2.4	3.1	2.7
		PDC	2.9	3.1	3.4	2.9	2.9	2.5	2.1	2.5	3.2	2.8
		ADC	3.8	4.2	4.8	3.5	4.0	3.8	3.0	3.1	3.7	3.8
	yes	PDC	2.9	3.1	3.6	3.0	3.0	2.6	2.1	2.7	3.5	2.9
		ADC	3.8	4.2	4.9	3.5	4.1	3.9	3.1	3.4	4.0	3.9
MM/GBSA			2.6	2.9	2.7	3.2	2.7	2.6	1.9	2.2	2.9	2.6

Figure 1. Ligands used in this study: the seven biotin analogues Btn1–Btn7, the nine fXa inhibitors C9–C125, and the nine phenol derivatives L1–L9 (sBu and iPr denote secondary butyl and isopropyl groups, respectively).

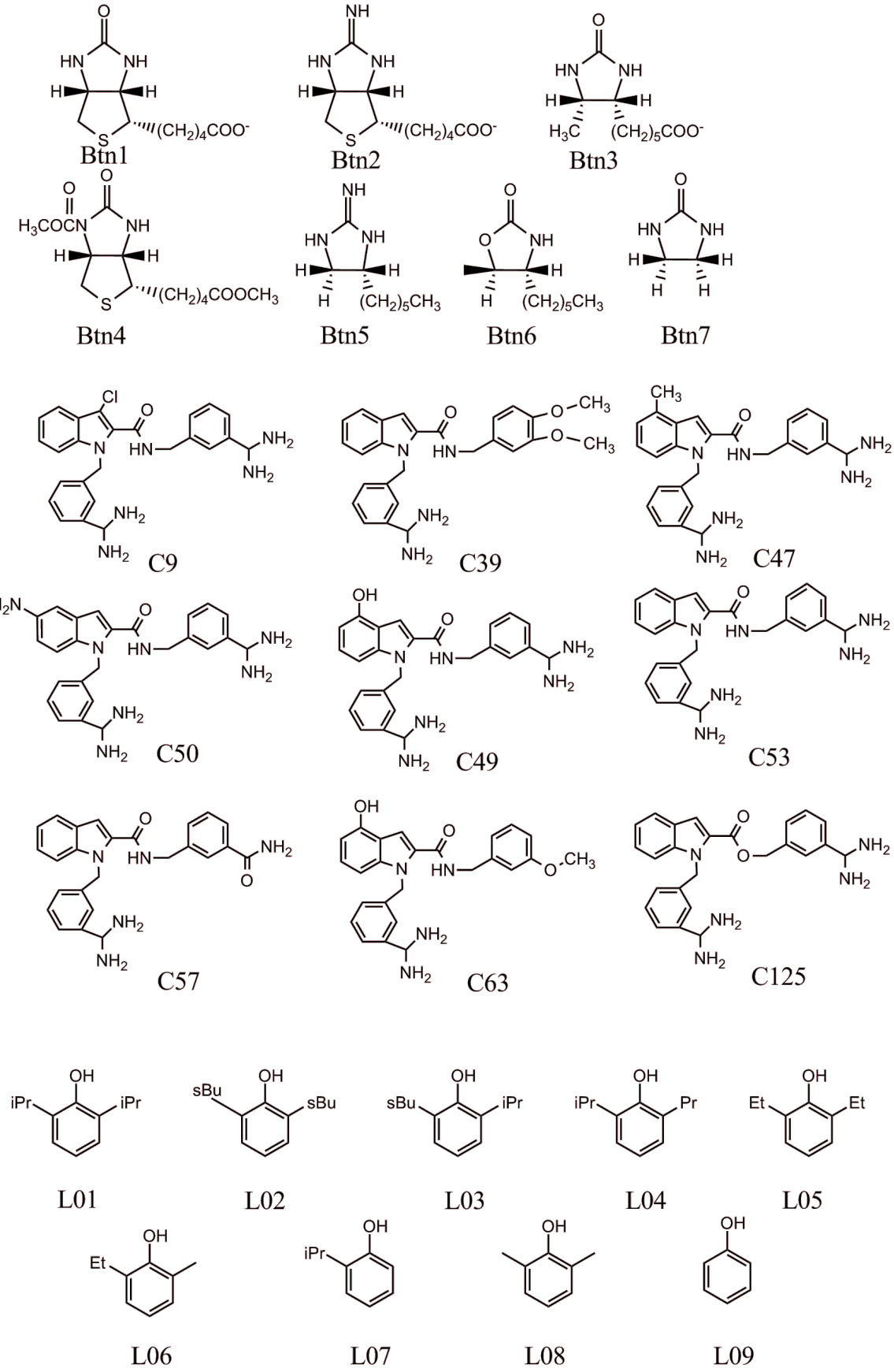

Figure 2. Binding affinities for the seven avidin ligands obtained with the AM1+PDC+HBC, MM/GBSA, and MM/PBSA methods compared to experimental data.[34,35,36]

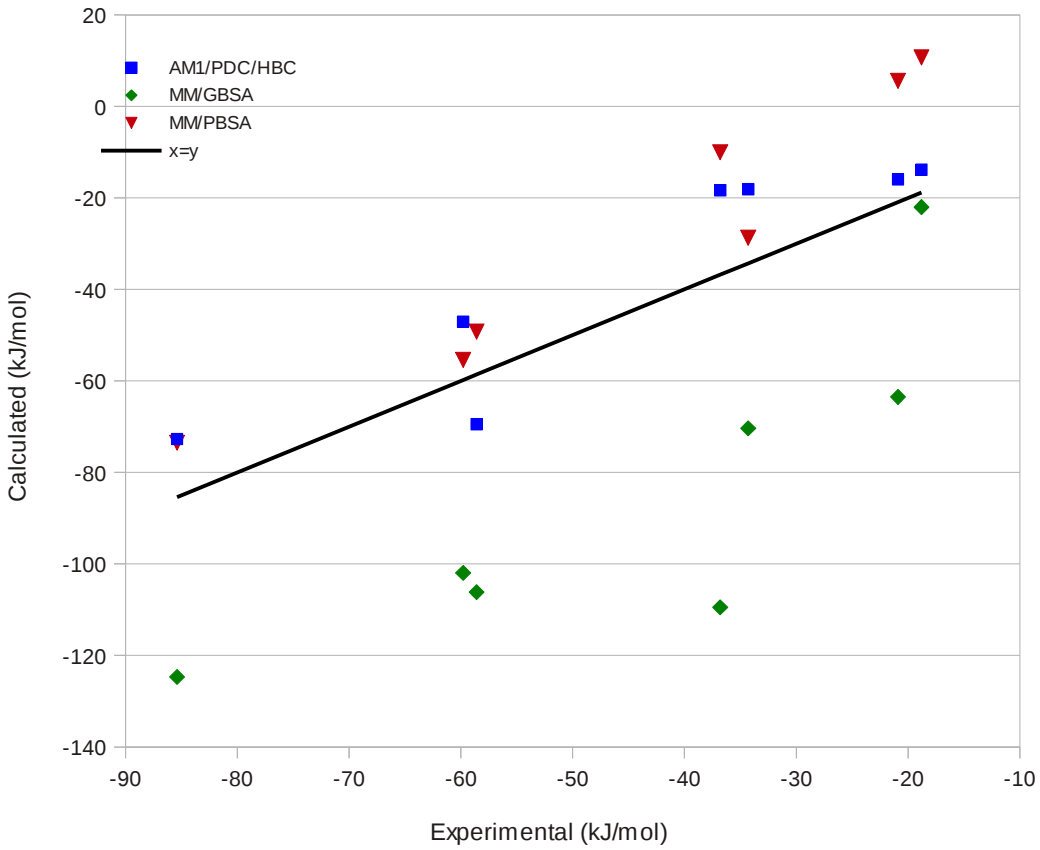

Figure 3. Binding affinities for the nine fXa ligands obtained with the AM1+PDC+HBC, AM1+ADC+HBC, and MM/GBSA methods compared to experimental data.[37]

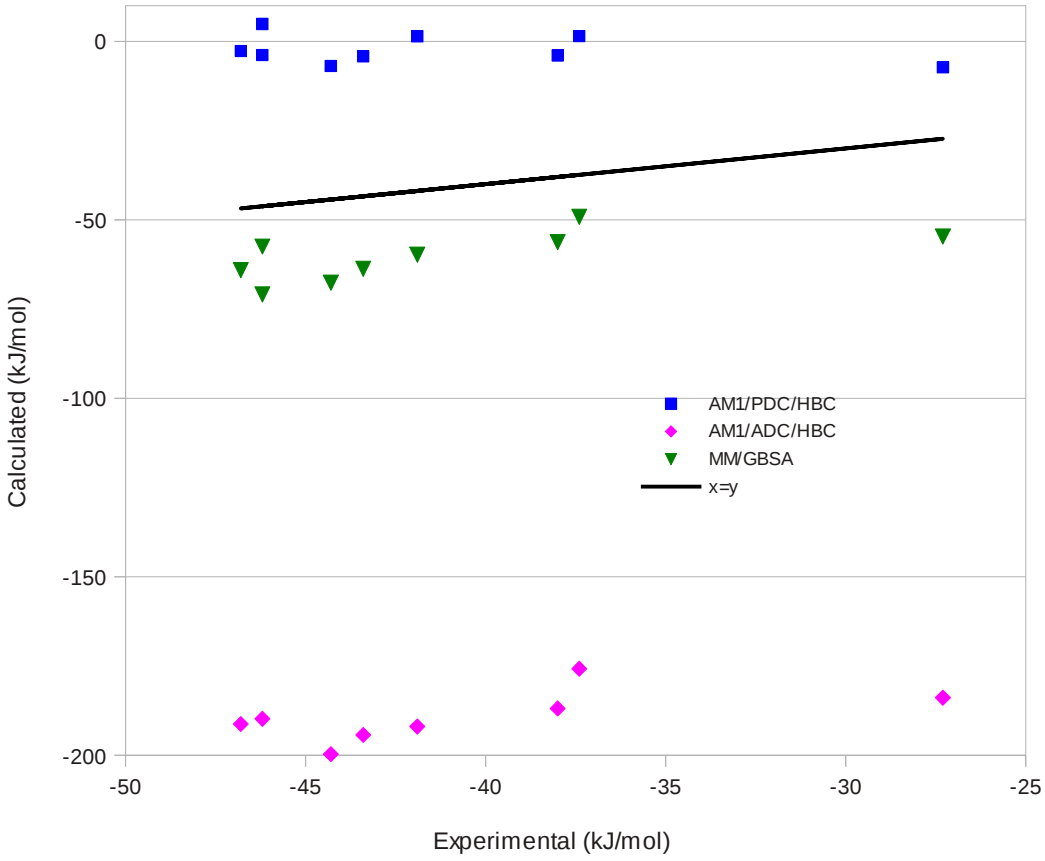

Figure 4. Binding affinities for the nine ferritin ligands obtained with the AM1+PDC+HBC, PM6+PDC+HBC, and MM/GBSA methods compared to experimental data.[38]