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Estimates of ligand-binding affinities supported by quantum mechanical methods

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Abstract

In this paper, we review our efforts to use quantum mechanical (QM) methods to improve free-energy estimates of the binding of drug candidates to their receptor proteins. First, we have tested the influence of various implicit solvation models on predictions of the ligand-binding affinity. The accuracy of implicit solvation models strongly depend on the parameterisation, but also on the magnitude of the solvation energy (i.e. their accuracy should be discussed in relative terms). However, if only relative solvation energies within a series of similar drug molecules with the same net charge are considered, nearly all methods tested give a comparable accuracy of 2–5 kJ/mol. Second, we have studied the conformational dependence of QM charges and their influence on ligand-binding affinities. The conformational dependence is significant, but it is to a large extent cancelled by solvation energies. Third, we have estimated the effect and range of electrostatic interactions beyond a point-charge model. The results show that multipoles up to octupoles and anisotropic polarisabilities have a significant influence on energies for residues up to 10–15 Å from the active site and that different sets of point-charge models may give strongly varying results. However, if only relative energies are considered, the effect is to a large extent cancelled. Fourth, we have tried to develop an accurate QM-based molecular mechanics potential, in which not only the electrostatic terms are improved, but also the dispersion and repulsion. However, even with quite sophisticated expressions, it seems difficult to reduce the average error below 2–3 kJ/mol per interaction (e.g. a hydrogen bond), compared to the full QM treatment. Finally, we have developed a new method, PMISP (polarised multipole interaction with supermolecular pairs), for the calculation of accurate interaction energies. It employs an accurate force field for electrostatics and induction, including multipoles up to octupoles and anisotropic polarisabilities calculated by QM methods on amino-acid fragments of the protein in each conformation observed in snapshots from a molecular dynamics simulation, whereas short-range interactions are estimated by high-level QM calculations for all pairs of the ligand with near-by residues. We show that this approach allows us to go far beyond the current accuracy of molecular mechanics methods, down to an error of 5–10 kJ/mol for a full protein–ligand complex. It can be combined with estimates of solvation, entropy, and dynamic effects to give estimates of binding affinities. However, several problems remain to be solved before any significant improvement in the accuracy can be seen.

Key Words: Ligand-binding affinities, MM/PBSA, implicit solvation methods, electrostatics, polarisation, quantum mechanical methods.

Introduction

During the latest decades, theoretical methods have become a powerful complement to experiments for the study of reactions in biological systems. In particular, it has repeatedly been shown that quantum mechanical (QM) calculations can provide structures of protein active sites with an accuracy similar or better than X-ray and NMR structures (Neese 2006, Ryde 2007). However, accurate energies are much harder to obtain (Ryde 2009), even if it has been shown that QM activation energies can be used to select the most probable reaction mechanisms of enzyme reactions (Siegbahn & Borowski 2006, Himo 2006).

One reaction of particular interest is the binding of a ligand (L) to a receptor (R), forming the complex (RL):

$$L + R \rightarrow RL \quad (1)$$

The free energy of this reaction, ΔG_{bind}, is the binding affinity of the ligand to the receptor. Such an energy is interesting because most drugs exert their action by binding to a receptor protein or nucleic acid. If it could be accurately calculated, drug development could be performed by computers, thereby saving an enormous amount of money.

Therefore, much effort have been devoted to calculate ligand-binding affinities with various methods (Gohlke & Klebe 2003). The most accurate results are normally obtained with free-energy perturbations (FEP; Beveridge & Dicapua 1989). Unfortunately, these are extremely time-consuming, their results typically converge only if the difference in binding affinity of similar ligands are considered, and most of the time is spent on simulations of non-physical states. Therefore, cheaper but more approximate approaches have been developed, which typically study only the reactants and products in Eqn. 1. Two examples of such approaches are the linear interaction energy (LIE, Åqvist 1994, Hansson 1998) and the MM/PBSA methods (molecular mechanics, combined with Poisson–Boltzmann and surface-area solvation models; Kollman et al. 2000).

Owing to the size of the receptor, such studies have traditionally been performed with molecular mechanical (MM) methods. However, as the computers have become more powerful, there has been a growing interest to improve the results of the affinity predictions by using quantum mechanical (QM) calculations (Raha et al. 2007). Such approaches range from using QM to calculate a few MM parameters of the ligand to treating the entire receptor–ligand complex with QM.

In this paper, we will review our efforts in this area, including tests of the accuracy of implicit solvation methods, the conformational dependence of MM charges, the importance of induction and a more accurate description of electrostatics, and the development of an accurate and effective way to treat the complete receptor–ligand complex with QM methods. It will be seen that the effect of using the more accurate QM calculations is unexpectedly small if only relative energies are considered and that the accuracy of ligand-bining estimates in general is not limited by the accuracy of the MM force field.

The MM/PBSA approach

We first shortly describe the standard MM/PBSA approach (Kollman et al. 2000), because it shows what terms are normally considered as important for ligand-binding affinities and it provides an appropriate framework for including QM calculations in binding-affinity calculations.

In the MM/PBSA approach, the binding free energy is calculated as the difference in free energy of the three reactants in Eqn 1:

$$\Delta G_{bind} = \langle G_{RL} \rangle - \langle G_R \rangle - \langle G_L \rangle \quad (2),$$

where each free energy is estimated as a sum, according to

$$G = E_{\text{MM}} + G_{\text{solv}} + G_{\text{np}} - TS_{\text{MM}} \quad (3),$$

where E_{MM} is the molecular mechanics gas-phase energy of the reactant, consisting of the internal energy (from bonds, angles, and dihedral angles), as well as the non-bonded

electrostatic and van der Waals energies:

$$E_{MM} = E_{int.} + E_{el.} + E_{vdW} \quad (4)$$

G_{solv} is the polar part of the solvation energy, which is traditionally calculated by solving the Poisson–Boltzmann (PB) equation (Sharp & Honig 1990). G_{np} is the non-polar part of the solvation energy (the cavitation energy, as well as the dispersion and exchange repulsion between the solute and the solvent), which normally is calculated by a relation to the solvent accessible surface area (SASA; Hermann 1972). The last term TS_{MM} is the product of the absolute temperature and the entropy, which is calculated from a normal-mode analysis of a truncated system at the molecular-mechanics level (Kollman et al. 2000). The averages in Eqn. 2 are calculated from a set of snapshots extracted from a molecular dynamics simulation to include the effects of dynamics. Each of the three free energies in Eqn. (2) should in principle be calculated from an individual simulation, but it is more common to simulate only the complex and then calculate all three free energies in Eqn. 2 from this simulation (Kollman et al. 2000, Swanson et al. 2004). Thereby, the precision of the method is improved and the internal MM energy (E_{int}) cancels.

Traditionally, only rather few snapshots have been used in the energy calculations, ~20, taken from a single molecular dynamics (MD) simulation. However, recent studies in our group have shown that this gives high statistical uncertainties in the prediction (Genheden & Ryde 2009a). The precision of the method can be improved by a factor of ~3 if a buffer of fixed atoms is used in the calculation of the entropy term (Kongsted & Ryde 2009). Moreover, it is more favourable to run several short independent simulations than a single long MD simulation (Genheden & Ryde 2009a). However, to obtain a standard error (i.e. the standard deviation of the mean value) of 1 kJ/mol for the final affinity estimates (which is needed to compare different methods with a statistical confidence), typically 1600–12800 energy calculations or 3–24 ns total simulation time is needed. Interestingly, it seems to be enough to obtain the independent simulations by simply varying the initial velocities of the simulations – changing the solvation, the conformation of residues not clearly discernible in crystal structures, the geometry of rotable groups, the protonation state of histidine residues, or even the net charge of the protein did not change the results significantly (Genheden & Ryde 2009b). Likewise, different simulation protocols (spherical, octahedral, or truncated simulation boxes) give similar results, but implicit solvation methods during the MD simulations typically give poor results (Weiss et al. 2006). On the other hand, several studies have indicated that dynamical effects are less important – in fact, minimised structures often give as good (and much cheaper) results as MD simulations (Kuhn et al. 2005, Rastelli et al. 2009).

Implicit solvation methods

We have studied the effect of various solvation models on the ligand-binding affinities. For example, it is possible that the binding-affinity estimates could be improved by calculating the solvation energy of the ligand by a QM method. To a first approximation, the net solvation contribution to the ligand-binding affinities comes mainly from the solvation energy of the ligand (because that of the complex and the free receptor nearly cancel, at least for a buried binding site). Therefore, we first concentrated on the solvation energy of the isolated ligand and calculated it with four different types of solvation models (Kongsted et al. 2009):

- The polarised continuum method (PCM), in which the interface between the solvent and the solute is described by a surface formed by small area elements and the reaction field from the solvent is described as a charge on each area element (Tomasi et al. 1999).
- The Langevin dipole method (LD), in which the solvent is described by an array of explicit dipoles on a grid, which is affected by the electrostatic field of the solute and

all the other dipoles according to the Langevin equation (Warshel 1979, Florián & Warshel 1999).

- The Poisson–Boltzmann (PB) method, in which the solute and solvent are assigned different dielectric constants, and the solute is described by a set of atomic charges. The potential in any point of the system can then be obtained by solving the Poisson–Boltzmann equation, possibly in the presence of ions (Sharp & Honig 1990).
- The generalised Born (GB) method, which approximates the solvation energy by a pair-potential between each charge, employing a specific screening function between each pair, which depends on the geometry of the solute (Still et al. 1990).

Each of these methods give only the polar solvation energy, so they need to be combined with a method to calculate also the non-polar part of the solvation energy. For the PCM methods, which typically are connected with QM calculations, sophisticated expressions for cavitation, dispersion, and exchange repulsion are used (Tomasi et al. 1999), whereas the PB and GB methods have traditionally been combined by a simple linear relation to the SASA (Hermann 1972). However, this has started to change (Tan et al. 2007). Moreover, all methods require a set of charges for each atom. These can either be MM point charges, e.g. from some of the Amber force fields, they can be calculated by QM methods, or the solvation energy can be calculated directly from the electronic distribution, within the QM calculation. Finally, all methods also require a set of radii, defining the extent of the solute. These are normally obtained by a more or less thorough parametrisation. Typically, the more parameters that are included in the model, the better can the results be (Nicholls et al. 2008). In total, we tested 24 variants of implicit solvation models, in which the four solvation models mentioned above were combined with different sets of charges, radii, and non-polar solvation expressions (Kongsted et al. 2009).

First, we studied a set of 20 neutral and 20 singly charged ions for which experimental data are available, to test the general performance of the methods. For the neutral molecules, the PCM methods performed best with MADs (mean absolute deviations) down to 1.3 kJ/mol. The LD and the best GB methods gave twice as large errors, 3.5 kJ/mol, whereas the best PB method had a MAD of 5.6 kJ/mol. Maximum errors ranged from 5 to 22 kJ/mol. For the ions, the results were appreciably worse with error ranging from 16 to 25 kJ/mol, with again PCM best, but GB worst. The reason for this large quantitative difference is simply that the solvation energies of the ions are much larger; in relative terms the errors of the ions are actually smaller (0.05–0.07) than for the neutral molecules (0.09–0.34).

This becomes even more apparent if neutral drug-like molecules are considered. For these, the experimental solvation energies are not known, so instead the spread among the various methods was studied. It turned out to be ~4 times larger for the drug-like molecules than for the small neutral molecules. However, the size of the solvation energy was also ~4 times larger and there was a good correlation between the size of the solvation energy and the spread among the various methods as can be seen in Figure 1. Thus, the accuracy of implicit solvation methods should be discussed in relative, rather than absolute terms.

Further details can be obtained if we use a weighted average of the results of all methods as a measure of the true solvation energy (Kongsted et al. 2009). Naturally, the details of the results depend on the weighting of the average, but the general result is stable, viz. that the absolute solvation energies vary quite extensively, but if only drug candidates within analogous series and with the same net charge are compared, all 24 methods except two gave the same relative solvation energies within 2–5 kJ/mol. Even if this corresponds to a factor of 2–7 in the binding constant, it shows that there is little gain of using QM-based methods to calculate solvation energies of the ligand, especially as this may reduce the cancellation of errors if the receptor and the complex are treated with other (MM-based) methods. This study also shows that implicit solvation methods are far from quantitative and that there is little hope that they will give accurate binding affinities for diverse sets of drug candidates.

Further studies have shown that if several conformations of the ligands are considered,

e.g. in the sampling of the MM/PBSA method, the difference among the various methods increase somewhat (to 5–8 kJ/mol for the neutral ligands; Genheden et al. 2009b). Moreover, there are also significant effects from the surrounding protein, even for a buried ligand site. Different implicit solvation methods give widely different absolute binding affinities with MM/PBSA-like approaches, as can be seen in Figure 2, and as has also been shown before (Gohlke & Case 2004, Rastelli et al. 2009). Unfortunately, it is not a matter of a simple translation of the curves, because the various methods give MADs of 12–43 kJ/mol (with a standard error of ±1 kJ/mol) even after such a translation. In fact, the slope varies among the methods, so that those that give a poor MAD have a good correlation coefficient (r^2 = 0.59–0.91; standard error ±0.03) and vice versa. Again, there is no indication that more advanced and computationally expensive methods give better results.

Conformational dependence of charges

Most MM force fields for biological macromolecules treat electrostatic interactions by the Coulomb interaction between point charges on each atom in the system. The atomic charges are normally obtained by QM calculations. For example, the charges in the Amber force fields are obtained by restrained electrostatic potential (RESP) method (Bayly et al. 1993), according to which the electrostatic potential (ESP) is calculated in a large number of points around the molecule of interest and atomic charges are fitted to reproduce these potentials (Bachrach 1994, Sigfridsson & Ryde 1998). Such ESP fits are unstable in that charges on atoms that are buried inside the molecule (mostly carbon atoms) are poorly determined and therefore may have large and counter-intuitive values, which may give problems in simulations. In the RESP approach, this buried-charge problem is cured by restraining the charges towards zero using a hyperbolic restraint.

In all common force fields, the charges on the macromolecules are predetermined and available in force-field libraries. This is a potential problem, because it is well-known that QM charges depend on the conformation of the molecule (Williams 1990, Reynolds et al. 1992). For example, it has been shown that the interaction energy between a small molecule and water molecules in the first solvation shell may vary by up to 20 kJ/mol for a charged molecule (Stouch & Williams 1993) and up to 13 kJ/mol for a neutral molecule (3 kJ/mol on average; Sigfridsson & Ryde 2002). Therefore, for accurate results, new QM charges should be calculated for each conformation of both the receptor and ligand, which of course would be extremely time-consuming in a molecular dynamics simulation.

However, with an MM/PBSA approach, in which the ligand-binding affinity is calculated only for a limited number of snapshots, it is possible to recalculate all charges within the *RL* complex. We have done this for a study of the binding affinities of seven biotin analogues (shown in Figure 3) to avidin (Weis et al. 2006): The protein was divided into CH_3CO– and –$NHCH_3$ capped residues, for which QM ESP charges were calculated according to the Merz–Kollman scheme (Besler et al. 1990) at the HF/6-31G* level of theory, i.e. the same level as for the Amber 1994 and 1999 force fields (Cornell et al. 1995, Wang et al. 2000). The calculations took 3 minutes per residue on average and were trivially parallel. Charges for all residues in this tetrameric protein were calculated for 20 snapshots for each ligand, giving 1 095 760 distinct charges in total.

However, when these QM charges were used in a MM/PBSA prediction of the binding affinities, no improvement in the affinities was observed (Weis et al. 2006). On the contrary, the MAD from the experimental data increased from 14 to 20 kJ/mol and the correlation coefficient (r^2) decreased from 0.96 to 0.74, compared to calculations with the Amber-94 force field, which was used for the MD simulations. However, considering that only 20 snapshots were used, taken from the same simulation, and the standard deviation in the individual MM/PBSA estimates among the 20 snapshots was very high, 40–70 kJ/mol, these differences are probably not statistically significant (Genheden & Ryde 2009a).

A detailed study of the differences between the QM and Amber charges in these simulations (Söderhjelm & Ryde 2009b) showed that the total electrostatic energies are totally different for the two charge sets (–103 and –66 MJ/mol), but this is mainly caused by a constant offset of the energies. However, even the relative energies in a MD simulation, i.e. the conformational energies of the protein, differed by up to 150 kJ/mol or 17 % of the total variation in electrostatic energy. The electrostatic ligand-binding energies differed by 43 and 8 kJ/mol on average for the charged and neutral ligands, respectively (3–4 % of the total energy). However, these differences are to a great extent compensated by solvation effects. For the sum of the electrostatic and the polar solvation interaction energy, the difference between the Amber and QM charges is reduced to 7 and 3 kJ/mol, respectively. This shows that the conformational dependence of the charges is highly significant, but it is largely cancelled by solvation effects, explaining why fixed MM charges works reasonably well in simulations.

The original QM charges could not be used directly in MD simulations, probably because the buried-charge problem makes some of the charges unphysically high (Weis et al. 2006, Söderhjelm & Ryde 2009b). However, by averaging the charges, either over the 7×20 snapshots or also over all occurrences of the same residue anywhere in the sequence (giving a single charge for each atom in each residue, like the Amber force field, providing an alternative way to obtain fixed MM charges), much more stable charges could be obtained, which could be used in MD simulations (Söderhjelm & Ryde 2009b). However, no significant improvement of the results were seen in MM/PBSA estimates of the ligand affinities.

Recently, similar calculations of QM charges have been performed on four other proteins (Genheden et al. 2009a). The results show that averaged charges are very similar even if obtained for different proteins ($r^2 > 0.99$). The charges are similar to the Amber RESP charges, but in general of a slightly higher magnitude, indicating that the arbitrary constraint in the RESP method is too strong. Moreover, a few of the Amber charges seem to be improper, especially for N atoms in amino terminals. When these averaged QM charges are used in MM/GBSA (i.e. MM/PBSA with the PB solvation model replaced by GB) estimates of the ligand binding affinities, with a strongly increased sampling, a slight but significant improvement of the results is seen for the correlation coefficient (from 0.60±0.01 to 0.68±0.01; a different solvation model and a much larger number of snapshots were used in these estimates, explaining the difference from the estimates mentioned above).

Improved models of electrostatics

Even when using charges derived from quantum chemistry and considering the conformational dependence of charges, the functional form of standard force fields is still insufficient to describe all aspects of intermolecular interactions. An important step towards a better description of the interaction energy between the ligand and the protein is to improve the description of the electrostatic interactions. One way is to recognise that point charges are only the first term in a series expansion of the electrostatic interactions, which can be continued by dipoles, quadrupoles, and higher multipoles (Stone & Alderton 1985, Engkvist et al. 2000). A second way is to include an explicit treatment of polarisation. There has been an interest to develop polarisable force fields for proteins for a long time, but still only a few general-purpose polarisable force fields are available, and the great majority of protein simulations are still performed with non-polarisable force fields (Halgren & Damm 2001, Cieplak 2001, Maple et al. 2005, Gresh et al. 2007, Warshel et al. 2007). Although it is well-known that electronic polarisation typically contributes 10–30% of the total electrostatic interaction energy (Söderhjelm & Ryde 2009a), the use of a polarisable force field often does not give results that justify the increase in computer load. For example, for the binding of seven analogues to avidin, no improvement in the binding affinities was observed for MM/PBSA with the polarisable Amber 2002 force field, compared to non-polarisable force

fields (Weis et al. 2006).

Non-standard accurate force fields are available that take into account both higher-order multipoles and anisotropic polarisabilities, e.g. NEMO, Amoeba, SIBFA, and EFP (Engkvist et al. 2000, Ren & Ponder 2002, Gresh et al. 2007, Day et al. 1996). These could in principle give very accurate interaction energies, especially if the multipoles and polarisabilities are calculated for the correct conformation of each molecule or residue. The SIBFA method has been used to calculate ligand-binding affinities (Gresh et al. 2005, Miller Jenkins et al. 2007, Roux et al. 2007).

One of the problems with assessing the importance of higher multipoles and polarisation is that there are many different ways to derive them from a QM calculation. In contrast to point-charges, multipoles are commonly derived directly from a partitioning of the electron density into local contributions, although fitting techniques may also be used. The partitioning can either be performed in real space (e.g. the atoms-in-molecules approach; Bader 1990) or in terms of the basis set (i.e. related to the Mulliken analysis; Mulliken 1955), and a large variety of methods have been proposed (Náray-Szabó & Ferenczy 1995). For polarisabilities, the methods are even more diverse, because there are also many ways to apply the perturbing field (Dehez et al. 2001, Williams & Stone 2003, Gagliardi et al. 2004).

We have compared the multipoles and polarisabilities obtained from two basis-space partitioning methods with regard to the ability to reproduce the electrostatic potential from the corresponding QM calculation, with and without an applied electric field (Söderhjelm et al. 2007). We found that a method based on an orthogonal but localized basis set (Gagliardi et al. 2004) gave better results than a Mulliken-like approach, especially when the basis set included diffuse functions. We also tested the dependence of the error on the distance from the molecular surface and found for typical interaction distances that there is no gain in using a higher multipole level than octupoles. In fact, the quadrupole level might be a more balanced choice when considering the performance of the polarisabilities. Empirically, we have also found that ESP-fitted point charges usually give an accuracy that is slightly better than a density-based multipole expansion truncated after dipoles.

Next, we investigated how polarisation models perform for realistic intermolecular interactions. It is known that the point-polarisability model benefits from error cancellation between the lack of Pauli effects and the approximate use of the homogeneous electric field at each polarisable point (Masia et al. 2005). In perturbational QM calculations, which are needed to address these effects separately, the lack of Pauli effects (as in e.g. Kitaura–Morokuma decomposition; Kitaura & Morokuma 1976) favours over-polarisation, because nothing prevents the electrons from floating over to the surrounding molecules. This is also a problem in traditional QM/MM methods (Laio et al. 2002). On the other hand, the use of homogeneous fields effectively limits the polarization. We performed a systematic study of this cancellation for a wide range of interactions (Söderhjelm et al. 2008), primarily dimers between amino-acid side-chains and water. Supermolecular calculations at the HF/cc-pVTZ or HF/ANO-L level were used as a reference, and the same level was used in the approximate models. For all considered systems (at the most favourable interaction distance), the error of a model that included the exact field but no Pauli effects was significantly larger than the error of the point polarisability model. In average, the relative errors in the induced electrostatic potential were 16% and 10%, respectively, but the maximum errors (for the formamide dimer) were 161% and 94%, respectively. This indicates that the polarisability model works well in general, but that it is unsuitable for modeling strong interactions, although the problem can be reduced by using a damping function (Masia et al. 2005). By using several intermediate models, we also found that all other approximations in the polarisability model are of less importance, and thus, the only way to significantly improve current polarisation models is to simultaneously include field derivatives and coupling between polarisation and repulsion. A promising way to achieve this is by employing repulsive pseudopotentials (Söderhjelm & Öhrn 2009).

As a first practical test of the importance, conformation- and distance dependence of the improved electrostatics and polarisation, we have studied their influence on the electronic spectrum of the retinal chromophore in rhodopsin (Söderhjelm et al. 2009a). Even if this is not ligand-binding energies, it is most likely that the results will be closely similar also for binding affinities. A full NEMO model was calculated for the whole protein (two different crystal structures were tested), by performing QM calculations on each capped amino acid, both at the HF/6-31G* and B3LYP/aug-cc-pVDZ levels, which took 17 and 250 CPU days, respectively. The multipoles and polarisabilities were obtained by the LoProp method (Gagliardi et al. 2004). The results show that the absolute energies strongly depend on the electrostatic description. For example, the excitation energies calculated with the non-polarisable Amber-94 and Amber-03 force fields (Cornell et al. 1995, Duan et al. 2003) could differ by up to 16 kJ/mol (and by 8 kJ/mol for the difference in the calculated excitation energies between various mutants). Polarisabilities had an even stronger influence of up to 46 kJ/mol. Anisotropic polarisabilities are important for residues within 6–10 Å of the chromophore. The polarisable Amber-02 force field gave errors of up to 10 kJ/mol, indicating the conformational dependence, but it can be used as a good approximation for interaction more than 10 Å from the chromophore. The quadrupoles contribute by up to 26 kJ/mol, but their interactions level off already at 6 Å from the chromophore. Quite unexpectedly, multipoles and polarisabilities calculated at the HF/6-31G* level gave energies that differed by less than 8 kJ/mol from those obtained at the B3LYP/cc-pVDZ level and the results converged within 8 Å from the chromophore.

Finally, we have compared isotropic polarisabilities obtained by the NEMO approach on the correct conformation for two proteins with those used in standard polarisable force fields (Söderhjelm et al. 2009d). The results show that the Amber-02 force field uses quite crude polarisabilities (only 1–3 values for each element; Cieplak et al. 2001), as can be seen in Figure 4. Thus, the performance of polarisable force fields can probably be improved by using distinct polarisabilities for each atom in the protein, in the same way as for point charges.

Improved models of non-electrostatic terms

When studying ligand binding, it is not sufficient to only improve the electrostatics and polarisation, because binding energies also include significant contributions from dispersion and repulsion. It is not a good solution to simply take these contributions from a standard force field, because the latter is usually parameterised for a certain choice of electrostatics.

There are two main approaches to construct a consistent intermolecular potential that reproduces the QM potential. In the first approach, used by e.g. SIBFA and EFP, one tries to reproduce each term of a QM energy decomposition method, such as the restricted variational space (RVS) method (Bagus et al. 1984, Stevens & Fink 1987). In the second approach, used by e.g. NEMO, one of the force field terms, usually the repulsion, serves as a remainder term, which is fitted for a wide range of interactions in such a way that the total interaction energy is recovered. The advantage of the first approach is that it has a clearer connection to the physics involved and therefore can be expected to be more transferable when applied to new systems. The advantage of the second approach is that one can use simpler expressions for the various terms without necessarily compromising the accuracy. For example, to model the true electrostatic energy, it is necessary to go beyond the multipole approximation to capture charge-penetration effects. This can be done by using damping functions (Freitag et al. 2000, Piquemal et al. 2003) or density fitting techniques (Cisneros et al. 2005). On the other hand, in the second approach, one can stay within the multipole approximation and instead absorb the charge penetration into the fitted repulsion term, because these terms have approximately the same dependence on the molecular overlap.

To separate the problem of choosing a suitable functional form for the repulsion from the issue of transferability, we have performed a large fit to Hartree–Fock exchange-repulsion energies (i.e. the first approach), in which we employed several expressions based on the

well-known near-proportionality between the exchange repulsion and the squared molecular overlap (Söderhjelm et al. 2006). In particular, we compared expressions based on the orbital overlap (possibly weighted by the orbital energies), which depends on all the occupied orbitals of the interacting molecules, and the much simpler density overlap, which only depends on the electron density. A large data set of molecular dimers was used, including all types of normal hydrogen bonds in proteins, but also e.g. CH–O and OH–? interactions. Some key results are given in the Exrep column of Table 1. When using only one parameter (i.e. assuming proportionality), there is no significant difference between the various models, but we found that it was possible to substantially improve the orbital overlap results by adding a second parameter modeling the deviation from proportionality (the exact functional form was found to be of less importance), whereas much more parameters were needed to get a similar improvement in the case of density-overlap expressions. Thus, we concluded that the orbital overlap is preferable for describing the exchange repulsion, if one wants a stable model with few parameters.

We spent three years trying to develop a NEMO potential for amino acids and drug molecules with such high accuracy as has previously been achieved for smaller molecules (Engkvist et al. 2000). To illustrate the transferability problems we encountered, we have revisited the fitting expressions and data set of the previous work (Söderhjelm et al. 2006), but plugged in the remainder terms from NEMO instead of the exchange repulsion. The results are shown in Table 1 (note that the errors now reflect the accuracy of the full potential, not only the repulsion). As expected, the NEMO errors are much larger than the Exrep errors. This reflects that errors from the other terms are included in the fitting error. In particular, the errors increase by 33–56% when going from the HF level to the MP2 level, where also the dispersion contributes. Interestingly, the advantage of using the orbital overlap has completely disappeared in the NEMO fits, even when using an additional parameter. The energy-weighted orbital overlap still provides a reasonable description, but the simpler density overlap is equally good.

The most surprising result of this investigation was obtained when we deliberately made the potential worse (see Table 1). When the level of multipoles was reduced, the error increased, showing that higher multipoles (in this case quadrupoles) are important. However, when the polarisation was completely neglected, the error actually decreased. The reason is simply that the reference repulsion energy becomes smaller in magnitude (it does not have to counteract the polarisation). Nevertheless, this result indicates that the errors cumulated in the various NEMO terms are so large that they obscure the more obvious error of neglecting the polarisation. This is probably the reason why the use of polarisable force fields sometimes does not give as great improvement as one would expect, especially when the remaining force-field terms are not simultaneously improved.

In conclusion, we have constructed a general potential where all parameters are derived directly from QM calculations, besides one fitted parameter per element for the dispersion and one universal parameter for the repulsion. However, it seems difficult to reduce the average error below 2–3 kJ/mol for each interaction (e.g. a hydrogen bond). Although this accuracy is better than that of a standard force field, it is probably not sufficient for useful predictions of binding affinities, considering that a protein–ligand complex sometimes includes ~20 contacts or more.

The PMISP method

Owing to the problems with constructing a full accurate molecular-mechanics potential, we decided to follow a different approach. The problems in NEMO were connected to the short-range terms, whereas the long-range electrostatics and inductions can be expected to be well described by the multipole expansion and the anisotropic polarisation. Therefore, we decided to simply calculate the short-range interactions by QM calculations. This would

involve QM calculations of the ligand with all protein groups within 4–7 Å. For typical protein–ligand complexes, this corresponds to 300–800 atoms. Unfortunately, it is very hard to estimate dispersion accurately with theoretical methods – high-level methods, like MP2 or CCSD(T), are needed, together with large basis sets (Jurecka et al. 2006). Therefore, these calculations need to be performed with some sort of fractionation approach. We decided to simply calculate all pairs of the ligand together with a nearby group from the protein.

Thus, the interaction energy of this method, which we call PMISP (polarised multipole interaction with supermolecular pairs; Söderhjelm & Ryde 2009a), is calculated from:

$$E_{\mathrm{PMISP}}(RL) = E_{\mathrm{el}}(RL) + E_{\mathrm{ind}}(RL) + E_{\mathrm{nc}}(RL) \quad (5),$$

where E_{el} and E_{ind} are the electrostatic and induction interaction energies, respectively (note that all energies in Eqn. 5 are interaction energies between *L* and *R*, not the energies of the *RL* complex*)*. E_{el} is calculated from a multicentre–multipole expansion up to quadrupoles in all atoms and bond centres in the protein and the ligand. Likewise, E_{ind} is calculated from anisotropic dipole polarisabilities in the same centres in a self-consistent manner. E_{nc} is the non-classical term, containing mainly dispersion and exchange repulsion, but also short-range corrections to the classical terms, e.g. charge penetration. It is estimated by

$$E_{nc}(RL) = \sum_{i=1}^{n} c_i \left(E_{QM}(R_i L) - E_{el}(R_i L) - E_{ind}(R_i L) \right) \quad (6)$$

where the receptor has been divided into a number of fragments (R_i), using the molecular fractionation with conjugate caps (MFCC) method (Zhang & Zhang 2003). The fragments are composed of amino acids, capped with CH_3CO– and –$NHCH_3$ groups. The caps from neighbouring fragments are joined to form one $CH_3CONHCH_3$ conjugate cap (concap) for each amino acid and the energies of these concaps are subtracted ($c_i = -1$ in Eqn. 3) from the energies of the capped amino-acid fragments ($c_i = 1$). $E_{\mathrm{QM}}(R_iL)$ is the counterpoise-corrected QM interaction energy of the R_i–*L* pair. A similar formula is used to derive properties (multipoles and polarisabilities) for the whole protein from fragment calculations (Söderhjelm & Ryde 2009a). The multipoles and polarisabilities are calculated from monomer calculations on each capped amino acid in the protein.

For a large protein, only a few fragments R_i are in close contact with the ligand, so the direct use of Eqn. 5 would be very inefficient. Therefore, much time can be saved without compromising the accuracy by using a QM/MM approach, PMISP/MM (Söderhjelm et al. 2009b): For residues close to the ligand (*M*), the full PMISP approach is used, whereas for more distant residues, E_{nc} is approximated by the Lennard–Jones term from a classical force field, E_{LJ}:

$$E_{\mathrm{PMISP/MM}}(RL) = E_{\mathrm{el}}(RL) + E_{\mathrm{ind}}(RL) + E_{\mathrm{nc}}(ML) + E_{\mathrm{LJ}}(RL) - E_{\mathrm{LJ}}(ML) \quad (7)$$

Thus, we use the same accurate multipole–polarisability model for the whole protein as in PMISP.

The accuracy of the PMISP method has been tested for a 216-atom model of the protein avidin, interacting with seven biotin ligands (Figure 3) in ten different conformations, taken from snapshots of a MD simulation (Söderhjelm & Ryde 2009a). For this model, full QM calculations at the MP2/6-31G* level are possible. The results show that the MFCC fragmentation works excellently, with errors of only ~1 kJ/mol. The PMISP approach gave errors of ~10 kJ/mol for charged ligands and ~6 kJ/mol for neutral ligands, both at the HF and MP2 levels. This is much better than MFCC and similar pairwise additive approaches (Zhang & Zhang 2003) that only sum up the QM pair energies, without taking any account of many-body effects (28 kJ/mol error for a charged ligand). In fact, PMISP was even found to be more accurate than the more advanced fragment molecular orbital (FMO) method (error ~14 kJ/mol; Kitaura et al. 1999; the evaluation used the same systems, fragmentation scheme, and QM level, so that only the treatment of many-body effects differed). The FMO method is in fact more computationally demanding (by a factor of ~5) than PMISP for interaction energies between rigid molecules, because it requires the computation of all fragment dimers. However, the ability of FMO to treat conformational changes at a consistent level is of course

a great advantage that is not easily realisable with PMISP.

Interestingly, the PMISP results can be considered as the best possible results obtainable with a method that assumes pairwise additivity of all energy terms except induction, e.g. as in all normal polarisable or non-polarisable MM force fields. The remaining errors seem to arise from the coupling between polarisation and exchange repulsion, and they can be reduced by doing also trimer calculations (expensive, but accurate), by including multipoles and polarisabilities of all the other fragments in the dimer calculations, or by subtracting the error at the HF level from the PMISP result at the MP2 level (Söderhjelm & Ryde 2009a).

Next, we tested the PMISP/MM approach in Eqn. 7 for the full biotin−avidin complex (Söderhjelm et al. 2009b). We first showed that it is meaningless to use a level like HF/6-31G* for protein−ligand interaction energies − the energy difference to a more realistic MP2/aug-cc-pVTZ calculation (Riley & Hobza 2007) is ~160 kJ/mol, mainly owing to the short-range dispersion. Interestingly, estimates with the polarisable Amber-02 and the non-polarisable Amber-94 force fields also differ by 91 kJ/mol. We also investigated how the PMISP calculations can be sped up by applying approximations either in all calculations or in the classical terms outside a certain distance from the ligand (see Table 2; the errors are computed using the corresponding calculation without the approximation as a reference). Truncation of the multipole expansion at the quadrupole level might cause errors of 7 kJ/mol, although the effect is short-ranged. Even quadrupoles can be omitted outside of 5 Å. Polarisabilities are important and the use of isotropic polarisabilities may give an error of 34 kJ/mol and has a long-range effect. In fact, the result converges faster if anisotropic polarisabilities are used, but their coupling are ignored (i.e. the induction is calculated without iteration). On the other hand, the multipoles and polarisabilities can to a good approximation be calculated with density functional theory, rather than at the more expensive MP2 level − this gives errors of less than 3 kJ/mol. One can also use a smaller basis set to compute the properties outside of ~12 Å. Another possible way to save computer time is to use the polarisable Amber-02 force field; if it is used for residues more than 15 Å from the ligand, their error is less than 3 kJ/mol. For a neutral ligand, the errors are smaller and more short-ranged. For example, the error from using the Amber-02 force field for residues more than 10 Å from the ligand is less than 2 kJ/mol.

Of particular interest is the accuracy of the PMISP/MM approximation and how large the QM system (*M*) needs to be, as this directly affects the efficiency of the method. Our results indicate that the QM system should involve all groups within 6 Å of the ligand, or 275−421 atoms, depending on the size of the ligand. Then, the truncation errors are below 3 kJ/mol and the CPU time for a single-point energy at the MP2/aug-cc-pVTZ level is ~20 days (with the calculations being trivially parallelisable). At 4 Å, the error is 16 kJ/mol for a charged ligand, but it comes mainly from two groups. If these could be identified beforehand, the calculations could be sped up by a factor of two.

Finally, we have combined PMISP/MM with the MM/PBSA approach to obtain actual ligand-binding affinities with all important effects included (Söderhjelm et al. 2009c). This is simply done by replacing E_{MM} in Eqn. 3 with the corresponding PMISP/MM energy, while keeping the other terms. E_{QM} was calculated at the MP2/cc-pVTZ level, whereas the multipoles and polarisabilities were calculated at the B3LYP/6-31G* level. The E_{LJ} term was taken from the Amber 1994 force field (Cornell et al. 1995). The calculations were performed on ten snapshots from a MD simulation with the polarisable Amber-02 force field and the binding of seven biotin analogues to avidin was studed. Unfortunately, the standard PB method cannot take into account multipoles and polarisabilities. Therefore, we instead calculated $G_{solv} + G_{np}$ with the PCM method implemented for EFP and FMO in the GAMESS software (Li et al. 2003).

Recently, there has been a great interest of developing ligand-binding methods that are based on quantum mechanical (QM) methods (Raha et al. 2007). Such methods are typically based either on semiempirical calculations (Raha & Merz 2004, 2005) or on fractionation

approaches, e.g. FMO (Fukuzawa et al. 2006, Nakanishi et al. 2007) or MFCC and related methods (Zhang et al. 2003, 2004, Zhang & Zhang 2005, Mei et al. 2005, He et al. 2005, Wu et al. 2007, Bettens et al. 2007). However, as was discussed above, it is well-known that theoretical calculations of dispersion effects require a very high level of theory (Jurecka et al. 2006). Likewise, accurate predictions of polarisation effects require a large basis set (Giese & York 2004). Only one of these studies (Bettens et al. 2007) has been performed at a level (MP2/6-311(+)G(2d,p) at which there is a hope that dispersion and polarisation effects are satisfactorily treated. On the other hand, that study calculates only interaction energies, and ignores solvation, entropy, and dynamical effects. Therefore, our study seems to be the first one that include all important effects and perform the QM calculations at a proper level of theory.

Unfortunately, the results of the full PMISP+PCM+T?S were rather poor (Söderhjelm et al. 2009c): As can be seen in Figure 5, it gave predicted binding affinities that were positive for all ligands, with a correlation coefficient (r^2) of only 0.27 and a MAD of 19 kJ/mol (after translation by the average signed error). This is appreciably worse than for the MM/PBSA performed with the Amber-02 force field, which gave an MAD of 13 kJ/mol without any systematic error and with $r^2 = 0.65$. The poor result of PMISP+PCM+T?S seems to be mainly caused by the non-polar part of the solvation energy from the PCM model: The net SASA term in standard MM/PBSA is always negative and rather small –11 to –20 kJ/mol, whereas the corresponding PCM term is much larger and positive, 52–155 kJ/mol. This may be related to the fact that PCM uses a van der Waals surface for the cavitation energy (Cossi et al. 1996), whereas the other terms are based on the SASA or the related solvent-excluded surface area. For small molecules, for which PCM was calibrated (Barone et al. 1997), there is little difference between these two surface definitions, but for a protein, the former will give rise to a large number of small cavities within the protein, giving differences in the area of more than a factor of two (Söderhjelm et al. 2009c). These effects were already seen for the largest drug-like molecules (Kongsted et al. 2009). Interestingly, the 3D-RISM solvation method give non-polar energies similar to that of the PCM model (Genheden et al. 2009b; 3D-RISM is the three-dimensional reference interaction site model and it uses statistical mechanical methods to yield the radial pair correlation functions between atomic sites constituting the molecules of liquid; Chandler & Andersen 1972, Kovalenko & Hirata 2000). If we instead use the non-polar SASA estimate for the binding affinity, the absolute PMISP energies are appreciably improved, as can be seen in Figure 5; the MAD is 19 kJ/mol and r^2= 0.52, even if standard MM/PBSA is still better.

Moreover, the non-classical PMISP estimate may be somewhat too large, owing to the limited basis set used in the calculation of E_{QM}. However, if this term is replaced by the corresponding Amber van der Waals term, the MAD increases to 30 kJ/mol. In conclusion, this investigation illustrates that it is hard to improve calculated ligand-binding affinities and that a more physical method not necessarily gives improved results.

Concluding Remarks

In this paper we have reviewed our efforts to improve calculated ligand-binding affinities using QM methods, involving all important terms according to the MM/PBSA approach. The basic conclusion for these studies is that it is difficult to improve the accuracy of ligand-binding affinities from the level obtained with standard MM methods. There are many reasons for this.

- First, it is hard to detect unambiguous differences: The results must be converged to a high precision (~1 kJ/mol) to discern in a statistically valid way the small differences among different methods (Genheden & Ryde 2009a). Moreover, a large number of ligands and targets need to be studied before any conclusive results can be obtained. Unfortunately, QM-based methods are so expensive that neither of these requirements

typically can be fulfilled.

- Ligand-binding affinities depend on a large number of factors, including electrostatics, van der Waals interactions, polar- and non-polar solvation, entropy, dynamics, strain, ionic strength, protonation, displacement of water molecules from the binding site, etc. (Gohlke & Klebe 2002). All these terms contribute to the binding affinity and they are probably treated in a rather crude way, so it is not enough to improve a single term to get a significant improvement. Furthermore, many of these terms are correlated, so it is often possible to get a reasonable correlation to experimental results even when omitting physically important terms.
- Many of the terms are large and cancel to a large extent. Therefore, calculated binding affinities are typically too large (often by a factor of 10; Gilson & Zhou 2007) and it is hard to get results that are significantly better than the null-hypothesis that all ligands have the same affinity.
- In particular, there is a major cancellation between electrostatics and solvation. Therefore, details of the method of calculating electrostatic interactions become of less importance. For example, even if the conformational dependence of point charges is significant, giving differences in the electrostatic interaction energy of over 40 kJ/mol for a charged ligand, these differences are reduced to 7 kJ/mol in a solvent (Söderhjelm & Ryde 2009b).
- Likewise, if only relative binding energies are studied, many errors and differences cancel. For example, absolute solvation energies for drug-like molecules may differ by over 200 kJ/mol, but if only relative energies are considered for similar ligands with the same charge, essentially all implicit solvation methods predict the solvation energy within 2–5 kJ/mol (Kongsted et al. 2009).
- QM calculations are typically performed as single-point energy calculations on structures obtained by MM. It is likely that this may deteriorate the results if the MM potentials are different, because MM may sample structures that are high up on the repulsive side of the QM potential (Weis et al. 2006). This may be solved by QM dynamics or minimisations.

Unfortunately, this leads to the rather sad conclusion that currently there seems to be little gain of using advanced methods to calculate ligand-binding affinities. On the contrary, even the cheapest MM-based methods give results of a similar quality as advanced QM-based methods. Of course, that does not mean that we should stop trying more advanced methods, it only means that larger and better validations are needed and that we do not know yet what currently limits binding-affinity estimates.

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Abbreviations

Amoeba: An accurate polarisable force field (Ren & Ponder 2002)
Amber: Program package and force field for molecular mechanics simulations
ANO-L: A large QM basis set
aug-cc-pVDZ: A medium-sized, diffuse QM basis set
aug-cc-pVTZ: A large, diffuse QM basis set
B3LYP: A density-functional theory QM method

cc-pVDZ: A medium-sized QM basis set
cc-pVTZ: A large QM basis set
EFP: Empirical fragment potential, an accurate polarisable force field from QM calculations (Day et al. 1996)
ESP: Electrostatic potential or atomic charges fitted to the ESP (Bachrach 1994)
FEP: Free-energy perturbation (Beveridge & Dicapua 1989)
FMO: Fragment molecular orbital QM method for large systems (Kitaura et al. 1999
GAMESS: A software for QM calculations
GB: Generalised Born (GB) method for calculation of solvation energies (Still et al. 1990)
HF: The Hartree–Fock QM method
L: A ligand
LD: Langevin-dipole method for calculation of solvation energies (Warshel 1979, Florián & Warshel 1999)
LIE: Linear interaction energy method for binding energies (Åqvist 1994, Hansson 1998)
MAD: Mean absolute difference
MD: Molecular dynamics
MFCC: Molecular fractionation with conjugate caps QM method for large systems (Zhang & Zhang 2003)
MM: Molecular mechanics
MM/GBSA: Same as MM/PBSA, but with the PB solvation model replaced by GB
MM/PBSA: A combination of MM, PB, and SA methods to obtain ligand-binding affinities (Kollman et al. 2000)
MP2: Møller–Plesset, second-order perturbation theory, the cheapest correlated QM method
NEMO: An accurate polarisable force field for intermolecular interactions from QM calculations (Engkvist et al. 2000)
PB: Solvation energies obtained by solving the Poisson–Boltzmann equation (Sharp & Honig 1990)
PCM: Polarised continuum method for calculation of solvation energies (Tomasi et al. 1999)
RESP: Restrained electrostatic potential method to obtain atomic charges (Bayly et al. 1993)
PMISP: Polarised multipole interaction with supermolecular pairs, an accurate method to obtain ligand interaction energies from a combination of NEMO and QM calculations (Söderhjelm & Ryde 2009a)
PMISP/QM: The QM/MM variant of PMISP (Söderhjelm et al. 2009b)
QM: Quantum mechanics
QM/MM: A combination of QM and MM, where QM is used for a small system and MM for the rest
R: A macromolecular receptor
RL: A receptor–ligand complex
RVS: Restricted variational space method to obtain QM energy components (Bagus et al. 1984, Stevens & Fink 1987)
r^2: The coefficient of determination (the square of Pearson's correlation coefficient)
SA: Surface area
SASA: Solvent-accessible surface area
SIBFA: An accurate polarisable force field (Gresh et al. 2007)
?*S*: The difference in entropy upon ligand binding
3D-RISM: The three-dimensional reference interaction site model to obtain solvation energies (Chandler & Andersen 1972, Kovalenko & Hirata 2000)
6-31G*: A medium-sized QM basis set
6-311(+)G(2d,p): A rather large QM basis set

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Table 1. Average errors in kJ/mol when fitting the exchange-repulsion energy using four different expressions (Eqns. 12–16 of Söderhjelm et al. 2006). The reference energies are either the first-order exchange-repulsion energies from a Kitaura–Morokuma analysis (Exrep) or the remainder terms (NEMO) defined by $E_{QM} - E_{el} - E_{ind} - E_{disp}$, using a multipole expansion up to quadrupoles and anisotropic polarisabilities for the electrostatic and induction terms and a simple r^{-6} fit for the dispersion. In the NEMO case, results are given at the Hartree–Fock (HF; no dispersion) and MP2 levels, as well as for models neglecting quadrupoles and polarisation, respectively. The data set included 26 molecular dimers of amino-acid side-chains and water in 2621 different geometries (Söderhjelm et al. 2006).

Expression	Exrep	NEMO (HF)	NEMO (MP2)	NEMO (HF) no quadrupoles	NEMO (HF) no polarisation
Orbital overlap	1.2	2.4	3.2	2.4	1.8
Orbital overlap (2 parameters)	0.7	1.9	2.7	2.3	1.6
Energy-weighted orbital overlap	1.1	1.7	2.6	2.2	1.4
Density overlap	1.2	1.8	2.6	2.1	1.5

Table 2. The effect of various approximations in the PMISP/MM calculation of the avidin–biotin interaction energy (Söderhjelm et al. 2009b; the total interaction energy is –1419 kJ/mol). The approximations are applied either for the full system (including also the fragment–ligand dimer calculations) or outside a certain distance from the ligand (excluding the dimer calculations). The approximate convergence distance (Conv) is the distance outside of which the approximation gives an error of less than 4 kJ/mol. The corresponding convergence distance for a neutral biotin analogue is given in brackets.

	Error (kJ/mol)			Conv (Å)
	Full system	From 4 Å	From 10 Å	
No octupoles	6.7	0.1	0.3	2 (3)
No quadrupoles	19.3	4.5	1.4	5 (5)
No induction	-19.2	-23.7	-25.5	20 (9)
Isotropic polarisabilities	34.4	-6.3	-12.9	20 (3)
Non-iterated induction	16.3	10.7	8.5	15 (8)
B3LYP properties[a]	2.7	2.5	1.6	3
6-31G* properties[b]	1.1	-3.2	-5.1	12 (3)
Amber 1994 force field[c]		6.5	-27.4	20 (8)
Amber 2002 force field[d]		30.4	-3.9	15 (7)

[a]versus the MP2 reference
[b]versus the aug-cc-pVTZ reference
[c]using charges
[d]using charges and polarisabilities

Figure 1. The relation between the standard deviation of various implicit solvation methods (either all 24 investigated methods of the PCM, LD, PB, or GB types, or only those of PCM type) and the average solvation energy (again either over all 24 methods or over only the PCM methods) for 20 small molecules and 18 drug-like molecules (Kongsted et al. 2009).

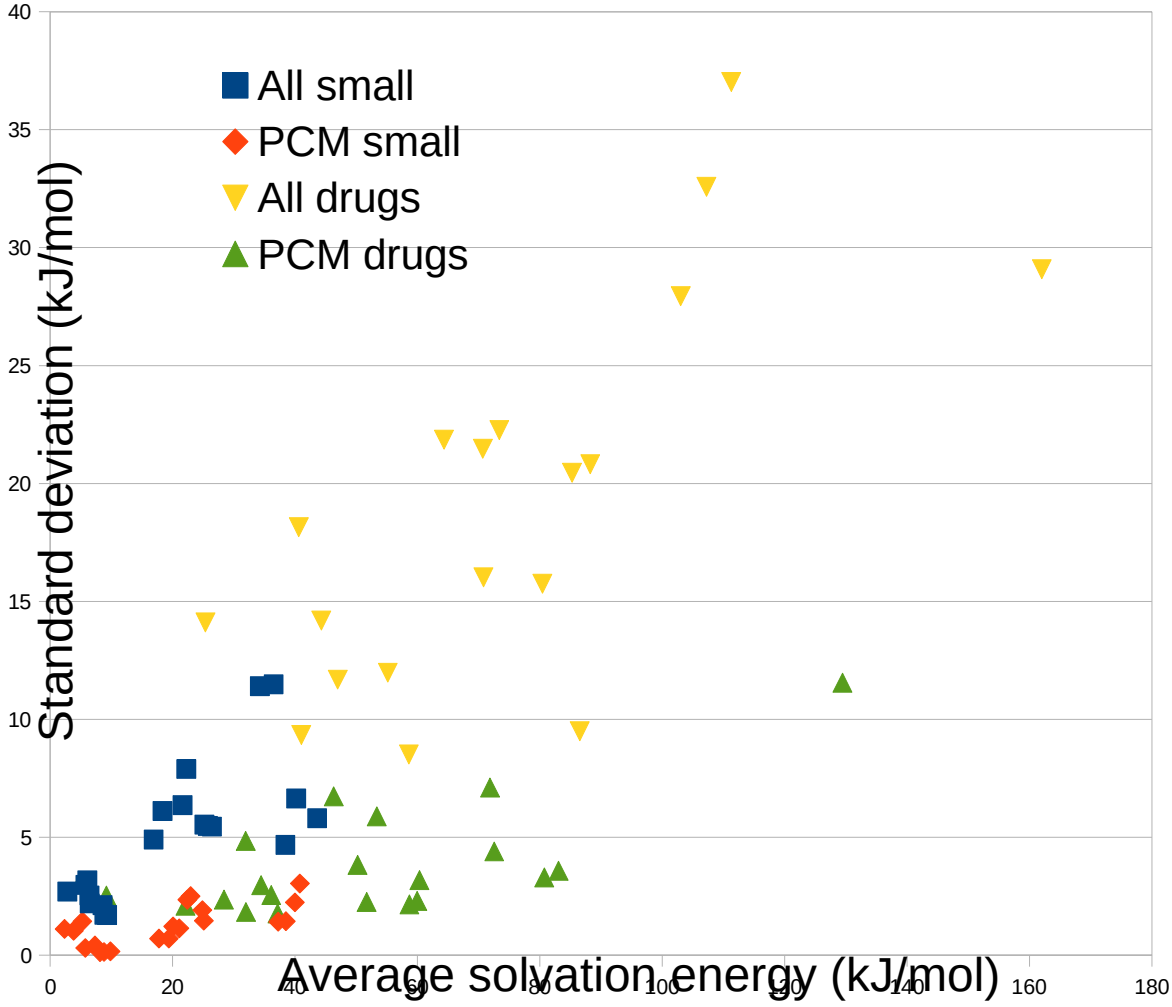

Figure 2. The calculated binding energies of seven biotin analogues to avidin for MM/PBSA-like approaches with several different implicit solvation methods (Genheden et al. 2009b), viz. 3D-RISM with or without the Gaussian fluctuation correction (Kovalenko & Hirata 2000), PB, and four variants of GB (Hawkins et al. 1996, Onufriev et al. 2004, Mongan et al. 2007).

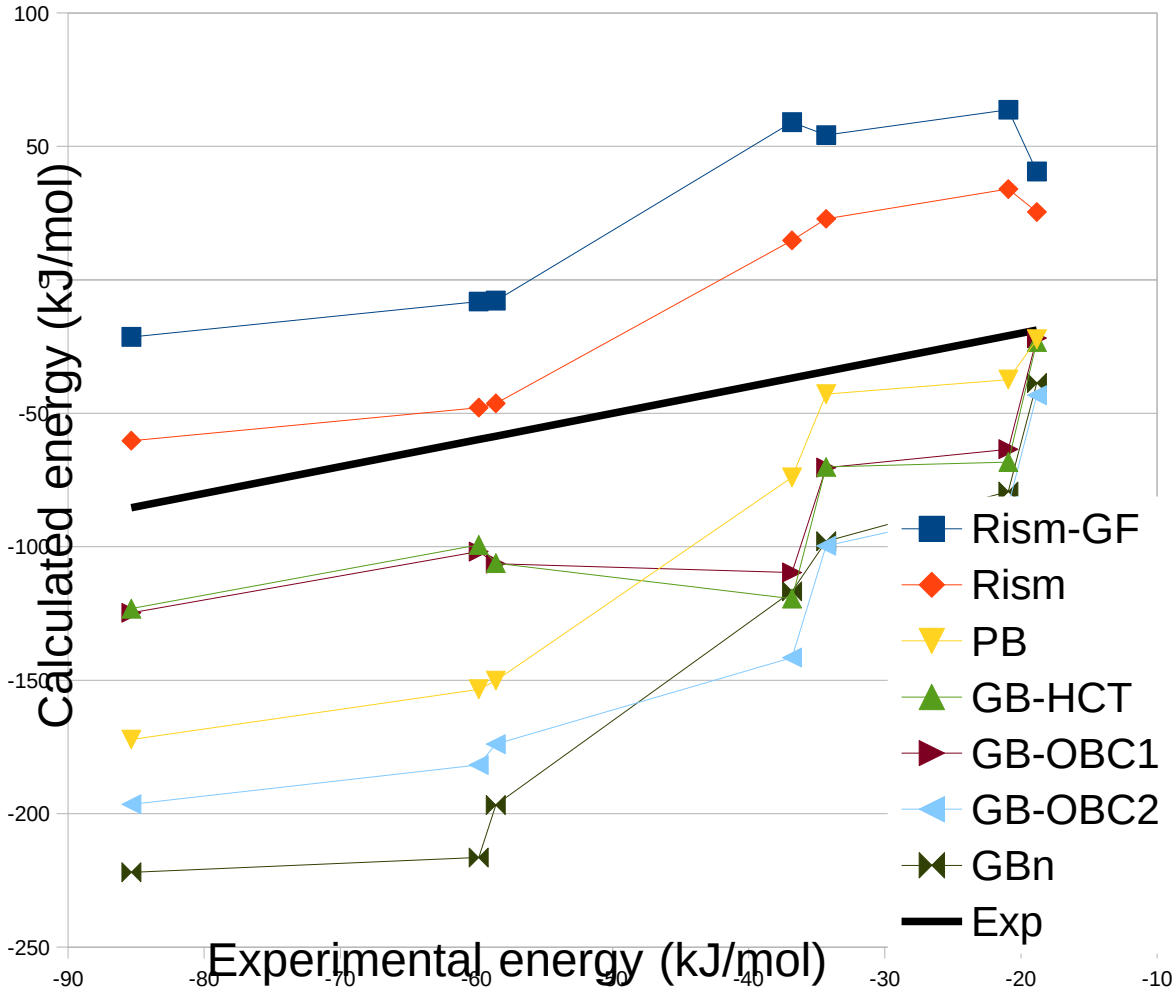

Figure 3. The seven biotin analogues used extensively in our work as a test case (Genheden & Ryde 2009a, Genheden et al. 2009ab, Kongsted & Ryde 2009, Söderhjelm & Ryde 2009ab, Söderhjelm et al. 2009bcd, Weis et al. 2006). a) Btn1 (biotin), b) – g) Btn2–Btn7.

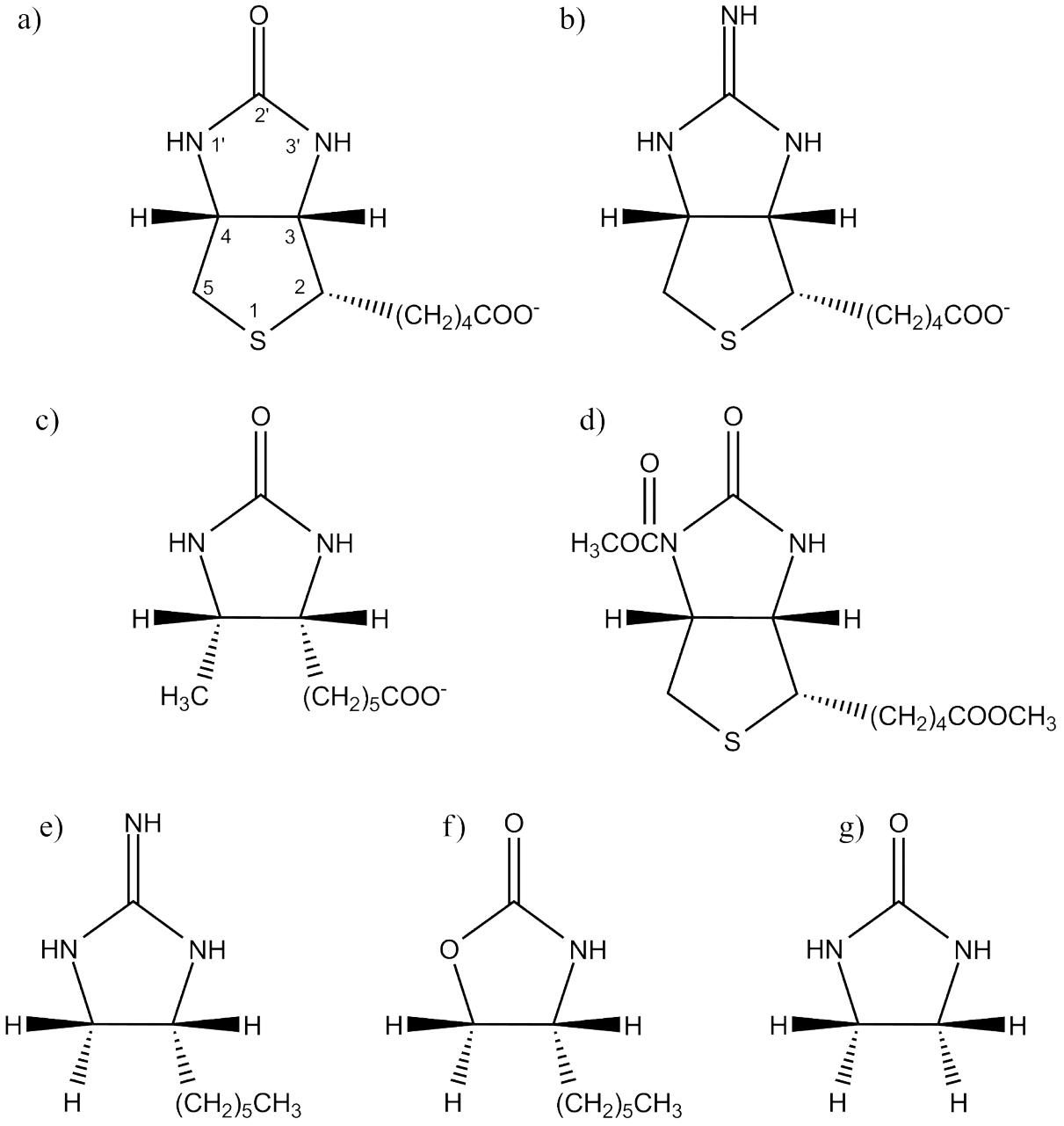

Figure 4. Correlation between the NEMO and Amber-02 polarisabilities (in units of Å^3), coded after the elements (Söderhjelm et al. 2009d). The line marks $x = y$.

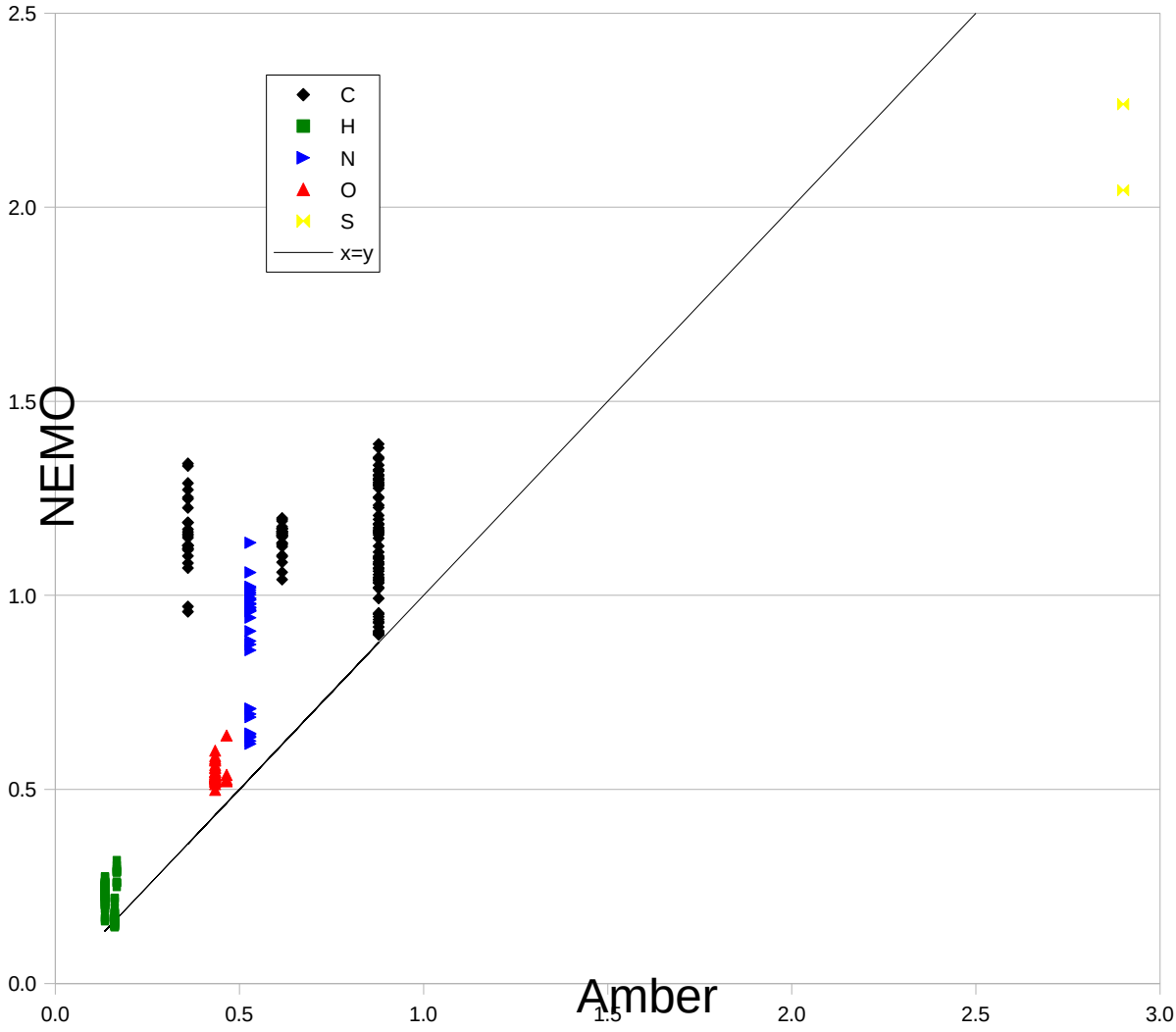

Figure 5. The results of the PMISP+PCM+T?S, PMISP+SASA+T?S, PMISP+SASA+T?S+E_{vdW}, and MM/PBSA (with the Amber-02 force field) methods for the binding of seven biotin analogues to avidin (Söderhjelm et al. 2009c).