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Ligand affinities predicted with the MM/PBSA method: dependence on the simulation method and the force field

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Abstract

The free energy of binding between avidin and seven biotin analogues has been calculated with the molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) method. We have studied how the force field and the method to generate geometries affects the calculated binding free energies. Four different force fields were compared, but we saw no significant difference in the results. However, it is not recommendable to mix the force fields used for the geometry generation and energy calculations. In the molecular dynamics simulations, explicit water molecules must be used, but the size of the simulated system and the boundary conditions are less important. In fact, non-periodic simulations with a fixed protein outside a relatively small simulated system (18 Å) seems to be a proper approach. The mean absolute error was 9–19 kJ/mole, with a standard error of 5–15 kJ/mole, which arises mainly from the entropy term.

Introduction

Almost all biological processes depend on the interaction and binding of molecules. A typical example is the binding of a small molecule (a ligand, *L*) to a macromolecule (the receptor, *R*, typically a protein or a nucleic acid), forming a complex (*RL*):

$$R + L \rightarrow RL \tag{1}$$

Such a reaction is of particular interest in medicinal chemistry, because the action of most drugs (inhibition, activation, etc.) is caused by the binding of the drug to its target receptor. Therefore, an important goal of theoretical chemistry is to develop accurate methods to predict the free energy of this reaction, the binding affinity, ΔG_{bind}.[1,2]

The most accurate and stringent theoretical method to predict ligand affinities is free energy perturbation (FEP).[3] In this method, a free energy change is calculated by slowly changing a system to another via a set of unphysical mixed states, using molecular dynamics (MD) or Monte Carlo simulations. Unfortunately, the results converge only for small changes. Therefore, this method has mainly been used to calculate the relative binding affinities of similar drugs to the same protein.[1,3,4,5] However, recently it has been shown that accurate absolute binding affinities can also be obtained by FEP, but only at a very large computational effort (~6 000 CPU-days per ligand).[6]

Therefore, more approximate methods to estimate ligand affinities have been developed, e.g. the linear interaction energy (LIE) method[7] and the molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) method.[8] Both method restrict the simulations to the states before and after binding. Other methods are also available, which estimate binding energies without any simulations. They are based on physical or structural quantities, obtained from statistical regression analyses of receptor–ligand complexes.[1] Unfortunately, none of these more approximate methods provide a uniform and high accuracy for all types of receptors.[1] Therefore, improved methods for predicting ligand affinities are strongly needed.

Among the approximate methods, the MM/PBSA approach is attractive, because it does

not contain any parameters that vary for different ligand–receptor systems and it involves a set of physically well-defined terms: The binding affinity is estimated from the free energies of the three reactants,[8]

$$\Delta G_{bind} = G(RL) - G(R) - G(L) \quad (2)$$

where all the reactants are assumed to be in water solution. The free energy of each of the reactants is estimated as a sum of four terms:

$$G = \langle E_{MM} \rangle + \langle G_{Solv} \rangle + \langle G_{np} \rangle - T\langle S_{MM} \rangle \quad (3)$$

where G_{Solv} is the polar solvation energy of the molecule, estimated by the solution of the Poisson–Boltzmann (PB) equation,[9] G_{np} is the non-polar solvation energy, estimated form the solvent-accessible surface area of the molecule,[10] T is the temperature, S_{MM} is the entropy of the molecule, estimated from a normal-mode analysis of harmonic frequencies calculated at the molecular mechanics (MM) level, and E_{MM} is the MM energy of the molecule, i.e. the sum of the internal energy of the molecule (i.e. bonded terms, E_{int}) and the electrostatics (E_{es}) and van der Waals interactions (E_{vdW}):

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

All the terms in Eqn. (3) are averages of energies obtained from a number of snapshots taken from MD simulations. In order to reduce the time-consumption and to obtain stable energies, the same geometry is normally used for all three reactants (complex, ligand and receptor), i.e. only the *RL* complex is simulated by MD.[11] Thereby, E_{int} cancels out in the calculation of ΔG_{bind}. The MM/PBSA method has successfully been applied on several different systems.[8,12,13,14,15,16,17]

This means that we can try to improve the MM/PBSA method by improving each of the five (non-cancelling) energy terms in Eqns. (3–4). However, the most time-consuming part of the MM/PBSA method is the generation of the snapshots (geometries) employed in the energy calculations, i.e. the MD simulations. In this article, we compare ligand affinities calculated with the MM/PBSA method on snapshots obtained with different simulation methods, ranging

from simulations with a large number of explicit water molecules and periodic boundary conditions or reaction-field corrections, via Generalised Born (GB) implicit solvent models,[18] to vacuum simulations with a constant or distance-dependent dielectric constant. In addition, we compare calculations (both geometry and energy) with four different force fields, viz. the Amber force fields ff94,[19] ff99,[20] ff03,[21] and ff02,[22] which differ mainly in the treatment of electrostatic interactions (ff94, ff99, and ff03 are non-polarisable, with charges obtained with different quantum mechanical (QM) methods, whereas ff02 is a polarisable force field). In addition, we also try to calculate energies with charges calculated with QM methods on the actual conformation of all residues in the protein and in all snapshots.

For such an investigation, it is necessary to have a good test system. We have selected the avidin–biotin complex, because it is well characterised by X-ray crystallography,[23,24,25,26] a wide range of experimental binding free energies for a number of ligands (biotin analogues) is available,[27,28,29] and the system has been investigated by several different theoretical methods, including FEP,[4,5] LIE,[5] and MM/PBSA.[12,17,30] Biotin is a member of the vitamin B group and is needed for growth. Avidin is a protein found in egg white, where it is believed to protect the chicken embryos from disease-causing organisms by binding biotin very strongly – this interaction is among the strongest known in nature.[23] Seven ligands were studied in this investigation (shown in Figure 1), with binding affinities (ΔG_{bind}) ranging from –85 to –19 kJ/mole.[28]

Methods

Biotin analogues and neutral arginine

The seven biotin analogues (BTN1–BTN7) studied in this investigation are shown in Figure 1. MM parameters for these molecules were obtained in the following way: The molecules were optimised with the Hartree–Fock method (HF) and the 6-31G** basis set. The electrostatic potential (ESP) was then calculated with a single-point calculation, using three different methods depending on the intended force field: HF/6-31G* for ff94 and ff99,[19,20]

B3LYP/cc-pVTZ for ff02,[22] and B3LYP/cc-pVTZ with solvent effects treated with the integral equation formalism polarised continuum method (IEFPCM) method[31] and a dielectric constant of 4 for ff03.[21] The points at which the ESPs were calculated were selected according to the Merz–Kollman scheme,[32] but using a higher than default density of points (10 concentric layers with 17 points/Å^2). All QM calculations were performed with the Gaussian 03 software.[33]

Atomic charges were then fitted to the ESPs using the RESP (restrained electrostatic potential fit) procedure,[34] as implemented in the Amber 8.0 antechamber module.[35] The RESP procedure for the ff02 simulations differs slightly from that for the other force fields:[22] To take in account the self-polarisation in the RESP procedure, the ESPs around the molecule owing to induced dipoles (ESP_{ind}) were calculated. Then, a new set of points charges were fitted to the difference between ESP_{QM} and ESP_{ind}. The new charges were used in the next iteration to self-polarise the molecule and to calculate the new ESP_{ind}. This was repeated iteratively until the charges did not change. All atoms in the seven biotin analogues were assigned standard Amber atom types.

The BTN2 and BTN5 biotin analogues include a guanidinium group that is positively charged in neutral aqueous solution. However, it has been shown that it is the neutral form of the ligand that binds to the protein.[27,28] Therefore, we simulated only the neutral form of these molecules (as in previous investigations[12]) and the corresponding experimental binding affinities were corrected for the fact that only the neutral form of the ligand binds to the protein.[27,28]

Charges for a neutral arginine residue were also determined by the same methods for ff94 and ff03. The RESP calculations were based on the full dipeptide, optimised at the HF/6-31G* level. The resulting charges of the biotin analogues and the neutral arginine residues are given in Tables S1– S4 in the supporting information.

The protein

All calculations in this investigation are based on the X-ray structure of biotin complexed with avidin (PDB accession code 1avd).[30] The seven different biotin analogues were built into the crystal structure using Spartan.[36] The building was trivial (deletion or replacement of existing atoms) for all molecules, except for BTN4, for which a $CH_3OOC–$ group had to be built into the crystal structure.

Avidin is a tetramer, composed of four identical subunits. The four biotin sites are independent of each other, so the calculations were made on one of the sites (subunit A), whereas the other three sites were considered as a part of the protein.[12]

All Asp and Glu residues were assumed to be negatively charged and all Lys and Arg residues were positively charged (if not otherwise stated). From a detailed study of the hydrogen-bond structure and the solvent exposure, it was decided that the single His residue in each subunit of avidin is protonated on the $N^{\delta 1}$ atom. All the other residues were assumed to be neutral. The first and last residues in the protein were omitted in the calculations, because they are not visible in the crystal structure.

MM/PBSA

ΔG_{bind} of each of the seven biotin analogues were calculated according to Eqns. (2–4) for all snapshots. The energies were automatically obtained using the mm_pbsa module of Amber 8.0.[35] The electrostatic and van der Waals energies were calculated by the sander module. The polar solvation energy was calculated with the finite-difference PB equation solver DelPhi II[37] (results denoted ΔG_{PB} in the following), because the Amber pbsa module gave dubious results for the charged ligands (more than 80 kJ/mole too negative binding affinities). These calculations employed a grid spacing of 0.5 Å and the grid size was adapted so that the longest linear dimension extended 10% outside the protein. Test calculations, as well as earlier studies,[12] have shown that these parameters give stable and converged energies. Parse radii[38] and the standard Amber charges (different for the four force fields tested) were

employed for all atoms. For comparison, the solvation energy was also calculated by the default Generalised Born method in Amber 8.0, viz. GB^{OBC} with α, β, and γ set to 1.0, 0.8, and 4.85, respectively.[39]

The entropy was estimated by a normal-mode analysis of the vibration frequencies, calculated with the Amber nmode module. This is very memory demanding and therefore the entire protein could not be used. Instead, only residues with any atom within 8 Å of the ligand in the last snapshot were included in these calculations.[12] The truncated systems were minimised using a distance-dependent dielectric constant of $\varepsilon = 4r$ and the entropies were then calculated using classical statistical formulas.[40]

The non-polar solvation energy was calculated from the solvent-accessible surface area (SASA), obtained with the Amber molsurf module using a probe radius of 1.4 Å. G_{np} was obtained from the SASA according to:

$$G_{np} = \gamma \, \mathrm{SASA} + b \qquad (5)$$

with $\gamma = 0.0227$ kJ/mole/Å^2 and $b = 3.85$ kJ/mole.[12]

The MM/PBSA procedure and all parameters in this investigation were chosen to reproduce as closely as possible previous MM/PBSA calculations,[8] especially those for the avidin–biotin complexes.[12] MM/PBSA energy calculations were performed with the Amber ff94,[19] ff99,[20] ff03,[21] and ff02[22] force fields. The calculations are denoted 94, 99, 03, and 02, in the following. The four force fields differ mainly in the treatment of the electrostatics (not ff94 and ff99, which employ the same charges).

If not otherwise stated, all residues in the protein had their standard charge (specified above). However, this gives a net charge of +18. In reality, these positive charges are compensated by counter ions. To determine if the net charge of the protein affects ΔG_{bind}, the complex was neutralised in some calculations by turning off the minimum number of lysine residues that were furthest away from the ligand site. These calculations are denoted 03n below.

Another common way to deal with a charged protein is to scale the charges (i.e. to assume that all interactions are damped by a dielectric constant). In a few calculations, we evaluated the effect of such a treatment by scaling all charges in the protein by a factor of 4. These calculations are denoted 03s.

QM charges

For one set of snapshots, we tested to recalculate the charges on the atoms with QM methods (denoted QM below): Charges for all residues in the complex were calculated with the HF/6-31G* method based on the snapshots from the 94oh simulation. For each snapshot, a separate set of charges were calculated. The protein was divided into dipeptides (i.e. each residue was capped by $CH_3CO–$ and $–NHCH_3$ groups) and the charges were calculated for these using the standard Merz–Kollman method[43] (the RESP method will only deteriorate the charges when the correct conformation is used, owing to its additional restraint of the charges towards zero[41]). The ESP charges on the capping groups were then discarded, whereas the charge on the C^{α} atom was adapted so that the whole residue had an integer charge. The calculations took ~150 CPU-days in total for the 70 000 calculations of all residues in all complexes and snapshots (~3 CPU-minutes per residue on the average).

MM/PBSA with ff02

The use of the polarisable ff02 force field in the MM/PBSA calculations is not straight-forward because standard programs to calculate PB solvation energies (e.g. DelPhi II,[54] pbsa,[35] or Mead[42]) cannot not include the effect of polarisation in a self-consistent way. A primitive but simple solution to this problem is to simulate the induced dipole moments by a pair of nearby charges: We can use two charges (q_1 and q_2) to reproduce both the Amber charge (q) and the induced dipole moment (μ) by

$$q_1 - q_2 = \mu/\mathrm{r} \tag{6}$$

$$q_1 + q_2 = q \tag{7}$$

where the vector between the two charges, *r*, has the same direction as the dipole moment. After some test calculations, we decided to set the length of *r* to 0.01 Å. The dipole moments were taken from a self-consistent energy calculation on each snapshot, including all the explicit water molecules in the simulation. This gives a correct description of the polarisation in the *RL* complex, but not in the isolated protein, because the water molecules are not allowed to reorganise after the removal of the ligand and empty ligand-binding site is not filled with water molecules. Similar problems apply to the isolated ligand. We currently try to implement a solvent model that may incorporate polarisation in a self-consistent way.

Geometries

The main goal of this investigation was to compare different methods to obtain the geometries (snapshots) at which the MM/PBSA energies are calculated. These are typically obtained by MD simulations and the MD calculations normally dominate the computer time consumption (e.g. 55 h for the 03oh simulation of one complex, compared to ~0.5 h for the corresponding MM/PBSA energy calculation). Therefore, we tested several different ways to generate the geometries.

In all simulations, the SHAKE algorithm[43] was used to constrain all bond length and the simulations were run by the Amber 8.0 sander module.[31] The temperature was kept constant at 300 K using the Berendsen weak-coupling algorithm[44] with a time constant of 1 ps. The MD time step was 2 fs and the non-bonded cut-off was 8 Å for the periodic-boundary simulations and 17 Å in the other simulations. The non-bonded pair list was updated every 50 ps.

If not otherwise stated, we used the following protocol: The complex was first optimised by 1000 steps of minimisation, keeping all atoms, except water molecules (if present) and hydrogen atoms, restrained to their crystal position with a force constant of 418 kJ/mole/Å^2. This was followed by a 20 ps MD equilibration with a constant pressure (isotropic pressure scaling with a force constant of 1 ps) and the restraining force constant reduced to 214 kJ/mole/Å^2, a 50 ps equilibration with constant pressure and no restraints, a 200 ps MD

equilibration with constant volume, and finally a 200 ps MD simulation with coordinates saved every 10 ps for a total of 20 snapshots. In calculations without periodic boundary conditions, only the 20 and 200 ps MD simulations were run.

In some previous MM/PBSA applications, longer MD simulations and more snapshots (3–500 ps, and 50 snapshots) have been used.[12,15] However, it was recently suggested that this gives only a little gain in accuracy compared to a shorter simulations with fewer snapshots.[30] Therefore, we used only 20 snapshots in these calculations, except in two calculations, which were prolonged to 2000 ps and 200 snapshots (94ohl and 03ohl). Five different simulation methods were tested, as described below.

Periodic boundary simulations (oh)

The complex was solvated in an octahedral box with water molecules extending at least 10 Å outside the protein on all sides. The electrostatics were treated with particle-mesh Ewald method[45] with a grid size of 80^3 Å, a fourth-order B-spline interpolation, and a tolerance of 10^{-5}. Simulations were performed with the ff03 (03oh), ff99 (99oh), and ff94 (94oh) force fields. With the polarisable ff02 force field, two simulations were performed, one with the non-polarisable TIP3P[46] water model (used in the ff094 and ff03 calculations; 02oht) and one with polarisable POL3[47] water model (02ohp). Two additional simulations with ff03 were performed to test the influence of the total charge of the protein on the results: one in which the charge of the complex was compensated by the addition of the proper number of negative counter ions (14 or 18 chloride ions, depending on whether the ligand was charged or not; 03ohc), and one in which the proper number of positively charged residues far from the ligand were neutralised (03ohn) (in all the other periodic-boundary calculations, Amber automatically neutralise the protein by adding a uniform neutralising plasma to the system).

Spherical system with a reaction-field correction (94sr and 03sr)

In these simulation, we used a spherical system (43.4 Å radius) filled with explicit water

molecules instead. A numerical PB solver was used to calculate the reaction field outside the water sphere.[48] The dielectric constant was 1 inside and 80 outside the sphere. The force constant for keeping the water molecules inside the sphere was set to 41.8 kJ/mole/Å^2.

Simulations of a small spherical system (94k and 03k)

Two simulations were run in a way to reproduce the earlier MM/PBSA calculations on the avidin–biotin complex[12] as closely as possible. The complex was neutralised by turning off the minimum number of arginine and lysine residues that were furthest away from the ligand. A 20-Å sphere of water molecules was added to the complex, centred on the ligand. The system was then minimised for 1000 steps and equilibrated by a 30 ps MD simulation with everything except solvent and hydrogen atoms fixed in position. Next, the complex was minimised by 4×1000 steps, keeping atoms more than 18 Å from the ligand fixed and with a restraint on everything except water molecules to the crystal structure. The restraint force constant was successively reduced in the four simulations: 105, 75, 46 and 21 kJ/mole/Å^2, respectively. All solvent water molecules were allowed to move freely. This was followed by a 30 ps MD equilibration with atoms more than 15.5 Å from the ligand restrained towards the crystal structure with a force constant of 84 kJ/mole/Å^2. After that, additional water molecules were added to fill the sphere better and the system was equilibrated by a 30 ps MD simulation. Finally, a 300 ps MD simulation was run, during which coordinates were saved every 6 ps for a total of 50 snapshots.

Implicit water models (03gb, 03cd and 03dd)

In an attempt to reduce the time consumption of the MD simulations, we run four simulations without explicit solvent molecules. In the 03gb simulation, the Generalised Born GB^{OBC} method was employed (model II, igb = 5).[39] We tried both a calculation without any explicit water molecules and a calculation with a sphere of explicit water molecules around the ligand (with a radius of 20 Å, 03gbs).

Another way to avoid explicit water molecules is to use a dielectric constant. We tested two calculations of this type, one with a fixed dielectric constant of 4 (03cd) and one with a distance-dependent dielectric constant ($\varepsilon = 4r$; 03dd). The latter approach is used in the calculation of entropy in the MM/PBSA scheme.[12]

Minimised structures (94mm and 03mm)

Recently, it has been suggested that more stable results are actually obtained if a single minimised structure is used, instead of snapshots from a MD simulation.[31] Therefore, we also tested to simply optimise the crystal structure (with explicit water molecules in an octahedral box from the 94oh and 03oh simulations) by 1000 steps of minimisation. To test how much the results depend on the starting structure, we also minimised all the 20 snapshots from the 03oh simulations.

Nomenclature

To simplify the discussion throughout this article, we denote the various calculations by *g*/*e*, where *g* specifies how the geometries (snapshots) were obtained and *e* specifies what force field was used for the MM/PBSA energy calculations. The geometry generation methods are periodic boundary simulations in an truncated octahedral box (oh), a spherical system with a reaction-field correction (sr), a smaller spherical system without any corrections for long-range interactions (k), implicit solvent calculations with the generalised Born method (gb), or with a dielectric function, which was either a constant of 4 (cd) or distance-dependent (dd), or by minimised structures (rather than snapshots from MD simulations for the other methods, mm). An "l" appended to the geometry method means that longer simulations were used (2000 ps), whereas "n" means that the protein was neutralised and "c" means that counter-ions were used in the simulations to neutralise the protein. The methods were described in the previous section and they can be performed with any of the four tested force fields (ff94, ff99, ff03, or ff02). The 02oht and 02ohp simulations were

performed with the non-polarisable TIP3P or the polarisable POL3 water models, respectively.

Likewise, the energy calculations can be performed with any of the four force fields or with QM charges calculated on the actual conformation of each amino acid in the proteins (QM). An "n", "s", and "b" appended to the energy force field indicates that the protein was neutralised before the energy calculation, that all charges where scaled by a dielectric constant of 4, or that the solvation calculations were performed at an ionic strength of 0.1 M, respectively. A "g" denotes that the generalised Born solvation energies were used instead of the PB energies. Thus, 03oh/94 means the geometries were obtained using ff03 with periodic boundary conditions in an octahedral box and the energies were calculated using ff94.

Results and discussion

Energy terms and uncertainty

The results of a typical set of MM/PBSA calculations (03oh/03) for the binding of the seven biotin analogues to avidin are given in Table 1. The Table shows the five energy terms in Eqns. (3–4), as well as the total binding energy and the experimental results.[28] The total ΔG_{bind} is the sum of the first five terms (because we list $-T\Delta S_{MM}$ in the Table). It can be seen that the electrostatics, van der Waals, and non-polar solvation terms are favourable for the binding, whereas the polar solvation and entropy terms are unfavourable for the binding in all the seven complexes.

The energies are dominated by the electrostatics and polar solvation terms, especially for the three first biotin analogues (BTN1–BTN3), which have a net charge of –1 (the other four molecules are neutral). However, these two energies nearly cancel, as can be seen in the forth last row. This is a manifestation of the dielectric screening of the solvent. The van der Waals term is also rather large (–49 to –200 kJ/mole) and it correlates with the size of the molecule. It has been argued that the net binding of biotin to avidin is dominated by this term.[4] The non-

polar solvation term is always small (–11 to –21 kJ/mole) and also correlates with the size of the ligand, whereas the entropy term is intermediate in size (28–96 kJ/mole). It is dominated by the nearly constant translational and rotational contributions (~90 kJ/mole), whereas the variation is caused by the vibrational contribution.

The last two columns show how the various energy terms contribute to the statistical uncertainty of the calculations by giving the standard deviation of the various terms (the differences) in the 20 snapshots. Three terms show the largest standard deviations: E_{el}, G_{Solv}, and S_{MM}. However, owing to the cancellation of the former two terms, the sum of these two terms have a much smaller standard deviation (~20 kJ/mole). Therefore, the standard deviation of ΔG_{bind} is dominated by the contribution from the entropy and it is quite large (47–67 kJ/mole for the seven biotin analogues). Thus, efforts to increase the precision of the MM/PBSA method should concentrate on that term. However, it should be noted that the standard deviation of the mean value for ΔG_{bind} of course is lower by a factor of $\sqrt{20}=4.5$ (5–15 kJ/mole) and this value can be improved by studying more snapshots (the standard deviations of the mean value for ΔG_{bind} in the longer 03ohl/03 simulations are only 3–4 kJ/mole).

The difference between the calculated and experiment values for ΔG_{bind} vary from –47 to +20 kJ/mole, giving an average deviation of 0.5 kJ/mole and a mean absolute deviation (MAD) of 16 kJ/mole, i.e. close to the statistical precision of the method. The relation between the calculated and estimated binding affinities is plotted in Figure 2, showing that there is no systematic error in the method (i.e. that the absolute binding energies are almost as accurate as the relative energies (the best regression line gives a MAD of 12 kJ/mole). In addition, the points are rather scattered.

Our results are quite similar to those reported in the original MM/PBSA investigation,[12] especially the 94k/94 calculations, which was designed to be as similar as possible. However some details differ; for example, the charges used for the six biotin analogues are not

available and had to be recalculated. Therefore, the individual energy terms may differ by up to 45 kJ/mole. However, the MAD for the two calculations are quite similar, 16 kJ/mole and 13 kJ/mole. This shows that our method works properly and reproduces the original MM/PBSA method closely.

GB solvation energies

The last three rows in Table 1 show the results using an alternative method to calculate ΔG_{Solv}, the generalised Born GB^{OBC} method,[39] (thus, actually representing a MM/GBSA method; 03oh/03g). These calculations are somewhat faster than the PB calculations, but they give much worse results: The MAD is 16 kJ/mole with the PB method, but 35 kJ/mole with the GB method. Moreover, the estimated ΔG_{bind} with the GB is consistently lower than the experimental data (by 8–71 kJ/mole), whereas the results with the PB method is rather well scattered around the experimental values (average error –0.5 kJ/mole). Of course, this means that the relative binding affinities with GB is better than the absolute ones: If the average error of the calculated binding affinities subtracted from all the affinities, a much smaller MAD (TR MAD) is obtained with GB, 19 kJ/mole, but this is still larger than for the PB results (16 kJ/mole for both MAD and TR MAD). Moreover, the PB results gives a better correlation line to the experimental results than the GB results (correlation coefficients, r^2 of 0.65 and 0.53). Similar results are obtained also with the other methods (e.g. MAD = 16 and 55 kJ/mole, TR MAD = 15 and 16 kJ/mole, and r^2 = 0.88 and 0.73 for 94oh/94 and 94oh/94g, respectively). Therefore, we conclude that the PB solvation energies give better results than the GB energies, and all results in the rest of this article are obtained with ΔG_{Solv} calculated with the PB method.

Effect of protein net charge

The calculated binding free energies for the various tested methods (listed as the errors compared to the experimental values[28]) are shown in Table 2, together with the MAD, as well

as the slope, intercept, and correlation coefficient (r^2) for the relation between the calculated and experimental values for each method, and the MAD of the calculated affinities corrected by their average error (TR MAD). The latter results were included to check if the methods may have systematic errors, but still give a good correlation (r^2 close to 1 and a low TR MAD) between calculated and experimental data. We have seen that the largest variation comes from the $T\Delta S_{MM}$ term. This variation is so large that it sometimes obscures the results. Therefore, we also give in Table 2 results that were obtained with the same value of $T\Delta S_{MM}$ in all calculations (cf. the legend of Table 2). These results are called MAD* and r^2* in Table 2.

We started to investigate the effect of the protein net charge on the results. As mentioned above, the 03oh/03 method gave a MAD of 16 kJ/mole and $r^2 = 0.65$. In these calculations, the MD simulations were performed on a protein that was neutralised by the addition of a uniform neutralising plasma, whereas the energy calculations were performed on the fully charged protein. When the complex was neutralised in the energy calculations (03oh/03n), the binding free energies for the various ligands change by at the most 3 kJ/mole. This shows that charges far away from the binding site have a minor effect on the binding affinity, in agreement with measurements on other systems.[49] Consequently, the treatment of surface charges in the energy calculations seems to be of minor importance for the results. This is also supported by the results obtained when the polar solvation energies were calculated with an ionic strength of 0.1 (03oh/03b; MAD = 16 kJ/mole, $r^2 = 0.65$). Likewise, similar results were obtained if also the MD simulations are run with a neutralised protein (03ohn/03; MAD = 13 kJ/mole, $r^2 = 0.92$).

On the other hand, if all the charges were scaled by a dielectric constant of 4 (again, only in the energy calculations, 03oh/03s), the MAD was almost doubled (31 kJ/mole) and the correlation became worse ($r^2 = 0.35$). Thus, a uniform scaling of all charges seems to be a poor method to treat the protein. Likewise, addition of counter ions in the MD simulations gave appreciably worse results (03ohc/03n; MAD = 27 kJ/mole, $r^2 = 0.61$). However, the

latter deterioration seems to arise entirely from the $T\Delta S_{MM}$ term: MAD* and r^2* are 12 kJ/mole and 0.67, i.e. similar to the 03oh/03 calculations (11 kJ/mole and 0.71).

Methods to generate geometries

After this investigation of the effect of net charges in the simulations and energy calculations, we turned to the main topic of this investigation: the influence of various simulation methods on the accuracy of the predicted binding affinities. In this investigation, we kept the full charge of the protein (not in 03k) and concentrated on simulations and energy calculations with ff03.

As mentioned above, the periodic 03oh/03 calculations gave a MAD of 16 kJ/mole. Interestingly, the non-periodic 03sr/03 simulations (with a spherical system and a reaction-field correction) gave the same MAD (Table 2). However, the linear fits of the 03sr/03 method gave a much better correlation (r^2 = 0.98, compared to 0.65), but this is not a general result: With ff94, 94oh/94 actually gives slightly better MAD and r^2 than 94sr/94 (14 kJ/mole and 0.96, compared to 19 kJ/mole and 0.83). Therefore, the two methods seem to give results of a similar accuracy.

Next, we studied the effect of simulating only a relatively small part of the protein: In the 03k simulations, water molecules were added as a spherical cap of 20 Å around the centre of the ligand (compared to 43.4 Å for the full system in the 03sr calculations) and only amino acids within 18 Å of the ligand were allowed to move. This is the same protocol used in the original MM/PBSA calculations on the biotin–avidin complex.[12] From Table 2, it can be seen that such a protocol (03k/03) gives slightly worse MAD than for the 03oh/o03 and 03sr/03 calculations (18, compared to 16 kJ/mole), but a similar correlation (r^2 = 0.74). Likewise, the 94k/94 calculations give similar results (MAD = 16 kJ/mole, and r^2 = 0.88) to the 94oh/94 and 94sr/94 calculations.

The importance of the modelling of the solvent was also tested. The geometries of the 03oh, 03sr, and 03k simulations were obtained with explicit water molecules, but in the

03gb/03 simulation, the implicit GB^{OBC} method was used instead. Interestingly, it turned out that the avidin tetramer separated into two dimers in five of the seven GB simulations, indicating that the GB^{OBC} method has severe problems to describe intermolecular interactions. If we ignore this problem and calculate the MM/PBSA energies for the dimer that included the ligand, the results were quite poor, with a MAD of 41 kJ/mole and $r^2 = 0.42$ (Table 2). This is much worse than for the calculations with explicit water molecules.

We also tested one set of calculations with the GB^{OBC} method, in which we included a small solvent cap (20 Å) around the ligand. However, in these simulations, the water cap either moved away from the ligand, or if the cap was forced to stay around the ligand, the ligand and the cap dissociated from the protein. Therefore, no energies were calculated from these structures.

In addition, we performed two sets of simulations without explicit and implicit water molecules. Instead, the electrostatics interactions were scaled by a dielectric function, which was either a constant of 4 (the 03cd/03 simulations) or distance dependent ($\varepsilon = 4r$; the 03dd/03 simulations). The latter is the same method employed in the entropy calculations in MM/PBSA.[8,12] Interestingly, both methods gave very poor results, with MADs of 145 and 108 kJ/mole, respectively (Table 2). Thus, we can conclude that an explicit solvent model is essential for the generation of geometries, even if all energies are calculated with the same implicit solvent PB method. Currently, no implicit solvent model seems to give any proper geometries.

Recently, it has been suggested[30] that a single minimised structure might be used instead of the time consuming MD simulation. In the 03mm/03 calculations, the crystal structure with added explicit solvent water molecules (as in the 03oh calculations) was minimised and the energy calculated on the single minimised structure (using explicit solvent molecules in a periodic box, i.e. the same type as in the 03oh simulations). From Table 2, it can be seen that such a treatment in most cases gave good results, but for some inhibitors, completely

erroneous energies were obtained (especially for the problematic BTN4 molecule, but also for BTN5, and sometimes BTN6).

Moreover, these calculations were quite unstable: If the minimisations were started from different structures, quite different results were obtained, as can be seen in Table 3 (the minimisations were started from the 20 snapshots of the 03oh simulation; standard deviation of 50 kJ/mole and errors of up to 142 kJ/mole). However, it can also be seen that the variation in the calculated ΔG_{bind} arises almost entirely from the solute entropy: The sum of the other four terms (second last column) has a standard deviation of only 13 kJ/mole. If the best value of $T\Delta S_{MM}$ is added to this estimate (giving a vanishing average error), a MAD of 10 kJ/mole is obtained. This indicates that the method to estimate ΔS needs to be improved.

When comparing the performance of the various simulation methods, it is also important to take into account their respectively computer time consumption. The CPU time consumption (on 64-bit AMD Opteron 148 processor at 2.2 GHz with 1 GB memory) for the MD simulations of BTN1 with the various protocols are shown in Figure 3. This figure shows that 03sr is the most expensive method, requiring almost five times as much computer time as the 03oh method (which can use a smaller non-bonded cut-off). Another factor of two can be saved by using the 03k simulations. Considering that these three simulation methods gave similar results, the 03k simulation seems to be best choice in time of computer time, although the other two approaches are theoretically more accurate.

The calculations without an explicit solvent (03dd and 03cd) are another factor of two faster than the 03k calculations, but they gave very poor results. Of course, the MM calculations were fastest, 25 times faster than the corresponding MD simulation. If it is possible to identify and correct the problematic cases with MM structures, this is undoubtedly the best choice as a compromise between accuracy and time consumption. Otherwise, the original 03k method is probably the best choice. Interestingly, the generalised Born GB^{OBC} method consumed three times as much time as the 03oh simulation and gave a very poor

results. Undoubtedly, this is the poorest choice of simulation method.

Force fields

In this investigation, we have also compared the performance in the MM/PBSA method with four different force fields available in the Amber software, the Amber ff94, ff99, ff03, and ff02 force fields. All four force fields use mostly the same parameters for the bonded and van der Waals interactions and differ mainly in the treatment of the electrostatics.

In the Cornell et al. (ff94) force field,[19] charges are derived using the quantum mechanical Hartree–Fock (HF) method with the 6-31G* basis set. Such a method exaggerates the dipole moment of most residues by 10–20%, thereby building in an average way in the effect of polarisation that would be expected in aqueous solutions. The Amber ff99[20] is a relatively minor modification of this force field, in which some of the dihedral terms are improved (but the charges and van der Waals parameters are not changed).

The Duan et al. (2003) force field[21] is a modification of the ff99 force field. The main change is that all the charges have been recalculated using the density functional B3LYP method with the cc-pVTZ basis set, including solvent effects with the IEFPCM method[44] and a dielectric constant of 4. Thus, an improved QM method is used to calculate the charges, which should give essentially correct gas-phase dipole moment, and then solvation effects in a protein-like continuum environment are explicitly introduced by the IEFPCM method.

Finally, the Amber ff02 force field is a polarisable variant of ff99 (it uses the same bonded and van der Waals parameters). The charges were determined by vacuum B3LYP/cc-pVTZ calculations.[22] Then, induction effects are explicitly included in the MM calculations by the use of isotropic dipole polarisabilities on all atoms. These give rise to an induced dipole moment on all atoms, which are treated as a dynamic variable in the MD simulations, but are solved self-consistently for the MM/PBSA energy calculations.

Interestingly, the results in Table 2 show no significant difference between ff94, ff99, and ff03: They all give MADs around 16 kJ/mole (14–19 kJ/mole) for the best simulation methods

(oh, sr, and k) and also similar (but more varying) correlation coefficients (r^2 = 0.64–0.98). Thus, there is no significant improvement of the newer ff03. There are at least two possible explanations to this somewhat unexpected result: First, the B3LYP/cc-pVTZ calculations undoubtedly gives better gas-phase properties than HF/6-31G*, but there is no guarantee that the IEFPCM continuum calculations with ε = 4 should reproduce the strongly inhomogeneous dielectric environment in a protein better than the HF/6-31G* calculations – both methods are very crude solutions to the induction problem. Second, the three force fields share the same van der Waals parameters, which were developed for ff94. Therefore, it is likely that they perform better with ff94 than ff03. This illustrates that it is important to optimise all the non-bonded parameters in a force field, not only the charges.

A few calculations were also performed with the polarisable ff02 force field. When using (non-polarisable) TIP3P water model (02oht/02), BTN2 and BTN3 gave large errors, leading to a high MAD of 55 kJ/mole. By changing the water to the polarisable POL3 model, this problem could be avoided and the MAD was reduced to 18 kJ/mole, i.e. similar to the other force fields. However, the MAD* and r^2* are worse than for the other force fields, 23 kJ/mole and 0.17. Thus, the ff02 force field, although theoretically more accurate, is not yet a better alternative to the cheaper non-polarisable ff94 and ff03. The reason for this is probably that the MM/PBSA energy calculations were not performed in a fully self-consistent manner. Moreover, the MD simulations with ff02 took approximately three times as much computer time as the corresponding ff03 (and ff94 and ff09) calculations (Figure 3). Calculations with the polarisable water model was only 11% slower than the calculations with TIP3P, so there is no reason not to use polarisable water.

Mixing force fields

Recently, there has been a growing interest of calculating ligand binding affinities using pure QM methods.[50,51] A problem with such an approach is that it is prohibitively expensive to generate geometries with these methods (perform MD simulations or energy minimisations).

A possible solution would be to generate the geometries (snapshots) with one method (e.g. MM) and calculate the energies with a more expensive and accurate method. As a simple test of such an approach, we performed a number of calculations in which we mixed the various Amber force fields (i.e. we used one force field for the MD simulations and one for the MM/PBSA energy calculations).

Interestingly, the results in Table 2 indicate that this is not fully straight forward: Most of these mixed calculations gave worse results than the corresponding calculations with the same force field in both the geometry and energy calculations. This is most evident for the MAD* values: Only two of the ten mixed calculations (94k/03 and 02ohp/94; disregarding the 94oh/99 calculation, which differs from the 94oh/94 calculation only in the $T\Delta S_{MM}$ term) gave a MAD* within the range of the oh, sr, or k calculations with the same force field in both calculations (7–11 kJ/mole). All the others gave MADs* of 12–23 kJ/mole. However, no correlation between the simulation method or force fields and the energies can be seen. Moreover, the mixed force-field calculations gave similar correlation coefficients as those with a single force field, indicating that the error is mainly in the absolute energy.

This indicates that energies obtained with a different method than the geometries may be quite inaccurate, which it is somewhat unexpected considering that it is a common practice in quantum chemistry to use a cheap method to obtain the geometries and then calculate energies with more expensive and accurate methods.[52] The problem with mixing force fields is that the ensemble generated by the MD simulations with one force field is not valid for the other force field. This is probably related to the non-bonded parameters: If they are too dissimilar, geometries with interactions on the steep repulsive side of the potential will be encountered, which may give rise to large and rapidly varying energy terms. This also explains the larger variation in the results of the mixed force-field calculations.

Recalculation of the charges

It is well known that atomic charges obtained from QM calculations depend quite

strongly on the conformation of the molecule.[41,53] The RESP method tries to compensate for this effect by restraining the charges towards zero and the Amber charges are furthermore obtained by averaging over several different conformations.[21,38,53] A more attractive (but computationally much more demanding) solution to this problem is to recalculate all charges for the right conformation (which is different for all residues in the protein and in all snapshots). We tested this suggestion by recalculating the ff94 charges on exactly the conformations encountered in each snapshot from the 94oh simulation (70 000 calculations on dipeptides, taking a total of ~150 CPU-days). Unfortunately, this did not improve the result: The MAD for the 94oh/QM calculations was slightly larger than for the original 94oh/94 calculations (20 compared to 16 kJ/mole) and r^2 was also lower (0.74 compared to 0.88). Again, the reason for this is probably that we used different charges in the geometry generation and in the energy calculation.

Therefore, we also tried to run a new set of MD simulations, using the QM charges from the last snapshot of the previous MD simulation. Unfortunately, all these calculations failed owing to problems with the bond-length constraints. This indicates that the new charges are too large, compared to the van der Waals parameters, giving rise to unstable trajectories. This is another function of the RESP procedure in Amber, viz. to reduce the size of ill-determined charges.[38]

Longer simulations

Finally, for two systems, 03oh/03 and 94oh/94, we continued the MD simulations for a total of 2000 ps and sampled ten times more snapshots, for which we calculated MM/PBSA energies the normal way. The results of these calculations are also included in Table 2 (03ohl/03, and 94ohl/94). It can be seen that these prolonged simulations gave similar results to those of the shorter simulations: the MADs are 9 and 16 kJ/mole, compared to 16 and 14 kJ/mole. Likewise, r^2 are 0.92 and 0.97 kJ/mole, compared to 0.65 and 0.96. This gives us a feeling of the uncertainties in these values.

On the other hand, the calculated binding affinities for the seven biotin analogues vary more: Most of them change by less than 9 kJ/mole, but four change by 12–18 kJ/mole and in one case, the change is as large as 41 kJ/mole. However, all the large changes in energies are connected with major changes in the conformation of the ligand, or its interactions with the protein. Thus, longer equilibrations (than 250 ps used here) seem to be needed for stable results.

Conclusions

In this paper, we have studied whether it is possible to improve the predictions of ligand binding affinities obtained by the MM/PBSA method by using different force fields and whether it is possible to save computer time by using more approximate simulation methods. We have used as a test case the binding of biotin-analogues to avidin. This complex has the advantage of not involving any metals and showing little change in the structure of the protein upon binding. This makes the predictions in this investigation more reliable, avoiding the problem that the MM/PBSA method gives rather poor results if the complex is quite different from the free receptor (because the complex geometry is used also for the free receptor). Moreover, the biotin ligand is rather rigid (although the carboxylate tail is fully flexible). This allows us to concentrate on the terms of main interest in this investigation, viz. the electrostatics, solvation, and van der Waals terms. Furthermore, avidin has a high net charge, allowing us to investigate how such a charge is best treated, and the ligands are both neutral and charged. Finally, the biotin–avidin complexes show an unusually large spread in binding affinities, which reduces the risk of obtaining trends by chance.

The systematic investigation has provided several interesting results:

- Mixing of force fields, i.e. to use one force field in the MD simulations and another in the MM/PBSA energy calculations, may give inaccurate energies. In particular, charges calculated for the correct conformation in each snapshot did not give any improved results. This may pose a problem in investigations based on very accurate energy estimates, e.g.

QM calculations of the whole protein.[50,51]

- The generalised Born GB^{OBC} method gives poor simulated structures at a high computational cost and also poor absolute binding affinities compared to the PB solvation model. This is somewhat surprising, considering that GB^{OBC} is considered as one of the most accurate GB methods and it has been shown to perform well for several different proteins.[39,54,55] However, GB models are still quite approximate[56] and apparently, their performance on different proteins varies.
- An explicit solvent model in the MD simulation is essential for accurate results. However, the three methods with explicit water (periodic boundary conditions and electrostatics treated by the particle-mesh Ewald method, a spherical system with a reaction-field correction outside the sphere, and a restricted spherical simulated system, without any corrections for long-range effects and parts of the protein fixed) give similar results. Therefore, calculations with only a small simulated system is recommended, because it is 2–10 times faster than the other two methods, even if it is theoretically more approximate. Similar indications have also been obtained for free energy perturbations.[57]
- Simulations without explicit water molecules give poor results.
- The three non-polarisable force fields tested (the Amber ff94, ff99, and ff03 force fields) give similar results. Better methods are needed for the use of a polarisable force field in MM/PBSA, giving a more consistent treatment of the solvation energy.
- The MM/PBSA method is insensitive to the total charge of the protein and to surface charges far from the ligand-binding site. No special care to neutralising the protein is needed. Likewise, the method works well both for neutral and charged ligands (using the PB solver DelPhi, but not with the default pbsa method in Amber 8.0).
- It is often possible to obtain good results with single geometries obtained by energy minimisation, instead of averages from several structures obtained from snapshots in MD simulations. However, in some cases, entirely wrong affinities are obtained. Some filtering

is needed before such a method becomes reproducible and useful.

- The estimated entropy has the largest variation among the five energy terms in MM/PBSA. This is also the term that needs most effort in terms of human and computational resources. Therefore, this term should be improved.
- The MM/PBSA method with the best simulation methods give MADs of 9–19 kJ/mole, which is close to standard deviation of the results (5–15 kJ/mole). The maximum error in the best calculations is 14–47 kJ/mole. The absolute affinities are almost as accurate as the relative affinities for the biotin–avidin complex.
- MD simulations longer than 450 ps seem to be needed for stable results.

In conclusions, the MM/PBSA method is an attractive method to calculate absolute ligand binding affinities, especially for cases where the expected spread of the affinities is quite high. However, it is less useful for fine-tuning a drug candidate, because the standard deviation of the MM/PBSA energies is quite high, 5–15 kJ/mole. This is the reason why Pearlman recently obtained poor results with MM/PBSA for 16 ligand of p38 MAP kinase, which had a spread of the binding affinities of only 10 kJ/mole.[57]

However, many details of MM/PBSA undoubtedly remain to be improved. In particular, we have seen that the entropy term should be made less time consuming and more stable. Moreover, methods to calculate solvation energies with a polarisable force field (and also with higher-order multipole moments) are needed. We currently work along these lines.

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Supporting Information Available: Charges for the seven biotin analogues and the four force fields and charges for a neutral Arg residue with the ff94 and ff03 force fields.

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Table 1. Results of the 03oh/03 calculations, showing the various energy terms, as well as the standard deviation of the results for the simulations of BTN1 and BTN7 (kJ/mole). $\Delta\Delta G$ is the deviation between the calculated and experimental values for ΔG_{bind}.

	Energy terms (kJ/mole)							Standard deviation	
Ligand	BTN1	BTN2	BTN3	BTN4	BTN5	BTN6	BTN7	BTN1	BTN7
ΔE_{el}	-1224.4	-1295.3	-1286.8	-173.6	-83.3	-50.5	-108.5	45.8	20.8
ΔE_{vdW}	-147.9	-149.5	-131.5	-199.5	-127.9	-128.2	-49.0	15.8	11.2
ΔG_{np}	-16.9	-16.9	-16.8	-20.8	-16.4	-16.4	-10.6	0.2	0.2
ΔG_{PB}	1224.2	1321.3	1259.4	265.7	146.4	122.8	123.9	30.1	13.3
$-T\Delta S_{MM}$	81.4	96.2	69.7	81.5	66.8	65.5	28.0	45.6	57.1
ΔG_{bind} (PB)	-83.7	-44.1	-105.9	-46.7	-14.4	-6.7	-16.2	47.1	62.4
ΔG_{bind} (*e*xp.[28])	-85.4	-59.8	-58.6	-36.8	-34.3	-20.9	-18.8		
$\Delta\Delta G$ (PB)	1.6	15.7	-47.4	-9.9	20.0	14.2	2.6		
$\Delta E_{el} + \Delta G_{PB}$	-0.3	26.1	-27.4	92.1	63.2	72.3	15.4	22.4	19.5
ΔG_{GB}	1194.8	1291.6	1245.0	204.8	103.0	68.9	113.0	11.3	14.4
ΔG_{bind} (GB)	-113.1	-73.8	-120.4	-107.7	-57.8	-60.6	-27.2	47.1	60.3
$\Delta\Delta G$ (GB)	-27.7	-14.0	-61.8	-70.9	-23.5	-39.7	-8.3		

Table 2. The results of the MM/PBSA calculations: Errors for calculated values of ΔG_{bind} for the various biotin analogues (compared to the experimental data[28]), the mean absolute deviation (MAD), as well as the correlation coefficient (r^2) for a fit of the calculated values to the experimental data. Finally, the MAD of the calculated affinities, translated by their average error (TR MAD) is also included, as well as the MAD and r^2 obtained when the same value of $T\Delta S_{MM}$ is used for all calculations: 90.9, 107.4, 105.1, 78.3, 50.4, 56.1, and 33.5 kJ/mole, for the seven biotin analogues, respectively, obtained as the average deviations of the calculated binding affinities without the $T\Delta S_{MM}$ term from experimental values for the 15 best calculations, viz. the oh, sr, and k calculations with ff94, ff99, and ff03; other choices gave similar, but slightly worse, results (MAD* and r^2*). All energies are in kJ/mole.

	Kuhn[8]	03oh/03	03oh/03g	03oh/03n	03ohn/03	03oh/03b	03oh/03s	03ohc/03
BTN1	11.3	1.6	-27.7	3.7	-4.4	1.7	47.4	15.8
BTN2	-2.9	15.7	-14	17.3	-23.8	15.7	68.4	-27.9
BTN3	9.6	-47.4	-61.8	-44.2	-12.2	-47.3	0.0	-47.7
BTN4	18.0	-9.9	-70.9	-10.2	2.1	-9.9	13.1	39.2
BTN5	3.8	20.0	-23.5	20.8	17.7	20.0	36.8	28.5
BTN6	15.5	14.2	-39.7	11.2	17.8	14.3	17.9	8.5
BTN7	30.1	2.6	-8.3	3.3	14.9	2.6	30.3	20.2
MAD	**13.0**	**15.9**	**35.1**	**15.8**	**13.3**	**15.9**	**30.6**	**26.8**
r^2	0.92	0.65	0.53	0.64	0.92	0.65	0.35	0.61
TR MAD	7.7	16.1	19.1	15.7	13.0	16.1	17.4	24.6
MAD*	19.8	11.4	59.0	11.3	8.8	11.4	33.2	11.9
r^2*	0.58	0.71	0.54	0.69	0.80	0.71	0.18	0.67

	03sr/03	03k/03	03gb/03	03dd/03	03cd/03	03mm/03	03ohl/03
BTN1	-36.7	4.1	61.3[a]	200.5	117.8	6.9	-3.8
BTN2	-22.9	-12.4	47.3	166.4	107.9	5.5	2.9
BTN3	-24.6	-42.6	2.1	192.3	152.0	-14.2	-6.3
BTN4	-13.9	17.2	86.8[a]	144.5	135.9	322.5	-13.5
BTN5	5.5	13.7	61.0[a]	127.9	99.8	124.6	5.8
BTN6	5.6	-1.3	61.9[a]	116.3	70.8	-8.6	13.7
BTN7	2.1	35.1	17.7[a]	70.2	75.1	47.3	14.4
MAD	**15.9**	**18.1**	**48.3**	**145.4**	**108.5**	**75.7**	**8.6**
r^2	0.98	0.74	0.42	0.48	0.06	0.19	0.92
TR MAD	14.2	17.8	22.2	35.1	22.9	88.2	4.6
MAD*	6.6	7.3	52.7	141.1	106.2	94.9	6.0

r^2*	0.93	0.94	0.38	0.67	0.14	0.01	0.89	
	94oh/94	94ohl/94	94oh/94g	94ohn/94	94sr/94	94k/94	94mm/94	99oh/99
BTN1	-28.9	-25.6	-50.4	-18.5	-2.1	-29.3	25.2	17.2
BTN2	-21.6	-23.0	-70.1	-28.2	-44.8	-6.3	96.6	-14.9
BTN3	-11.6	-11.3	-58.2	-50.1	-13.5	-6.1	-24.4	12.9
BTN4	13.4	0.0	-80.4	40.7	4.1	3.6	310.0	17.9
BTN5	8.5	17.7	-64.7	36.4	14.8	33.4	160.2	17.9
BTN6	-0.6	17.5	-41.7	2.7	24.1	14.2	92.1	25.3
BTN7	10.1	16.1	-17.7	-2.2	29.9	21.4	8.6	10.6
MAD	**13.5**	**15.9**	**54.7**	**25.6**	**19.0**	**16.3**	**102.4**	**16.7**
r^2	0.96	0.97	0.73	0.69	0.83	0.95	0.21	0.84
TR MAD	14.0	16.1	15.5	25.3	18.8	15.9	80.1	8.3
MAD*	8.7	7.4	30.5	11.4	10.0	6.9	91.6	11.9
r^2*	0.91	0.95	0.30	0.86	0.76	0.92	0.05	0.60
	02ohp/02	02oht/02	03oh/94	03ohl/94	03sr/94	03k/94		
BTN1	12.8	-50.9	21.5	22.1	1.0	12.4		
BTN2	8.6	-129.5	29.2	10.2	0.5	1.4		
BTN3	-26.9	-112.9	-6.5	-26.9	21.8	-11.1		
BTN4	-45.4	-47.2	34.6	48.9	15.9	38.9		
BTN5	3.8	-15.1	37.4	49.5	49.5	45.2		
BTN6	11.5	-23.9	48.8	17.0	45.2	12.3		
BTN7	-15.7	10.9	54.4	19.9	23.9	37.1		
MAD	**17.8**	**55.2**	**33.2**	**27.8**	**22.5**	**22.6**		
r^2	0.43	0.68	0.88	0.63	0.89	0.80		
TR MAD	18.9	38.4	14.2	17.2	14.6	18.0		
MAD*	22.7	49.7	19.0	15.6	12.1	23.2		
r^2*	0.17	0.77	0.82	0.90	0.84	0.92		
	94oh/03	94ohl/03	94oh/99	94oh/QM	94sr/03	94k/03	02oht/03	02ohp/94
BTN1	-65.5	-36.8	-17.3	13.8	-7.1	-21.5	10.3	15.6
BTN2	-15.9	-25.5	-22.9	-23.9	-51.3	-32.6	-25.4	5.3
BTN3	-29.3	-26.2	-0.3	-9.6	-9.3	-35.1	-42.6	-9.4
BTN4	27.1	-4.9	19.0	27.3	10.4	3.7	16.0	8.2
BTN5	3.3	-13.3	14.1	36.3	-9.6	19.0	44.0	2.1
BTN6	10.1	2.3	8.7	18.7	5.9	-0.8	-1.4	47.3
BTN7	6.3	15.5	0.5	9.0	6.2	19.2	16.4	20.3
MAD	**22.5**	**17.8**	**11.8**	**19.8**	**14.3**	**18.8**	**22.3**	**15.5**

r^2	0.93	0.98	0.91	0.74	0.78	0.89	0.63	0.80
TR MAD	23.8	14.6	11.8	15.8	13.3	19.3	21.9	12.8
MAD*	17.2	15.2	8.8	11.1	13.8	6.7	12.7	8.2
r^2*	0.93	0.95	0.91	0.73	0.68	0.97	0.65	0.76

[a] One or two subunits dissociate from the other subunits during the simulation.

Table 3. Energy contributions (kJ/mole) to the binding free energy obtained with the 03mm/03 method, starting the minimisation from the twenty snapshots from the 03oh/03 simulations of the BTN1 ligand. $\Delta\Delta G$ is the error in ΔG_{bind} compared to the experimental results.[28] $\Delta G_{bind,no\ S} = \Delta G_{bind} + T\Delta S_{MM}$, and $\Delta\Delta G_{best\ S}$ is the corresponding error if the best value of $T\Delta S_{MM}$ is added to this estimate (giving a vanishing average error).

snapshot	ΔE_{el}	ΔE_{vdW}	ΔG_{np}	ΔG_{PB}	$T\Delta S_{MM}$	ΔG_{bind}	$\Delta\Delta G$	$\Delta G_{bind,no\ S}$	$\Delta\Delta G_{best\ S}$
1	-1195	-166	-17	1242	-148	12	**97**	-137	-5
2	-1174	-169	-18	1250	-24	-86	**-1**	-110	22
3	-1208	-183	-17	1274	-15	-120	**-34**	-134	-2
4	-1181	-175	-17	1269	-90	-15	**70**	-105	27
5	-1209	-172	-17	1259	-133	-7	**79**	-140	-8
6	-1194	-173	-17	1244	-107	-33	**52**	-140	-9
7	-1195	-175	-17	1262	-70	-55	**30**	-126	6
8	-1189	-176	-17	1259	-41	-82	**3**	-124	8
9	-1193	-169	-17	1272	-160	53	**138**	-107	25
10	-1235	-166	-17	1286	-41	-92	**-7**	-133	-1
11	-1251	-172	-17	1297	-34	-110	**-25**	-144	-12
12	-1204	-174	-17	1267	-59	-69	**17**	-128	4
13	-1209	-180	-17	1272	-138	4	**89**	-134	-3
14	-1198	-171	-17	1262	-92	-32	**54**	-124	8
15	-1230	-179	-17	1289	-152	15	**101**	-136	-4
16	-1248	-177	-17	1303	-98	-42	**44**	-140	-8
17	-1214	-180	-17	1269	-200	57	**142**	-143	-11
18	-1247	-182	-17	1314	-87	-46	**39**	-133	-1
19	-1267	-179	-17	1315	-117	-32	**54**	-149	-17
20	-1232	-175	-17	1274	-85	-65	**20**	-150	-18
average	-1214	-175	-17	1274	-95	-37	**48**	-132	0
stdev.	26	5	0	21	50	50	**50**	13	13

Figure 1. Structures of the seven biotin analogues studied: a) biotin (BTN1), b) 2'-iminobiotin (BTN2), c) desthiobiotin (BTN3), d) 1'-N-methoxycarbonylbiotin methyl ester (BTN4), e) *D*-4-*n*-hexyl-2-iminoimidazolidine (BTN5), f) *D*-4-*n*-hexyloxazolidone (BTN6), and g) imidazolidone (BTN7).

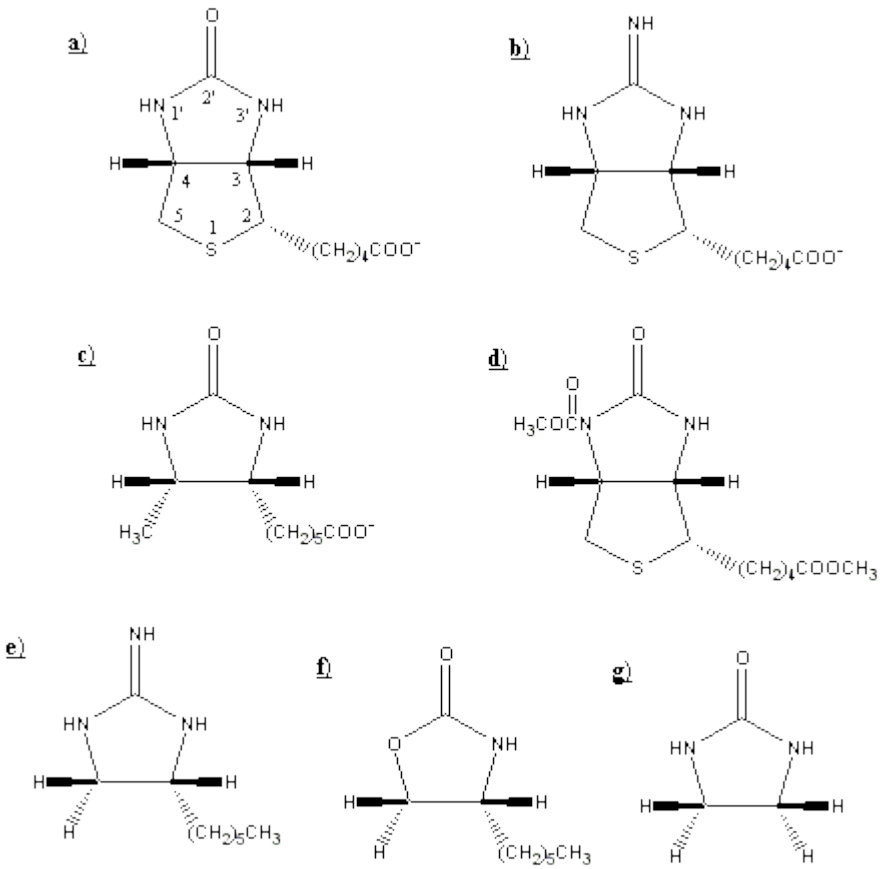


Figure 2. The relation between the experimental and calculated ΔG_{bind} for the 03oh/03 calculations, together with the best regression line. The dotted line is $y = x$.

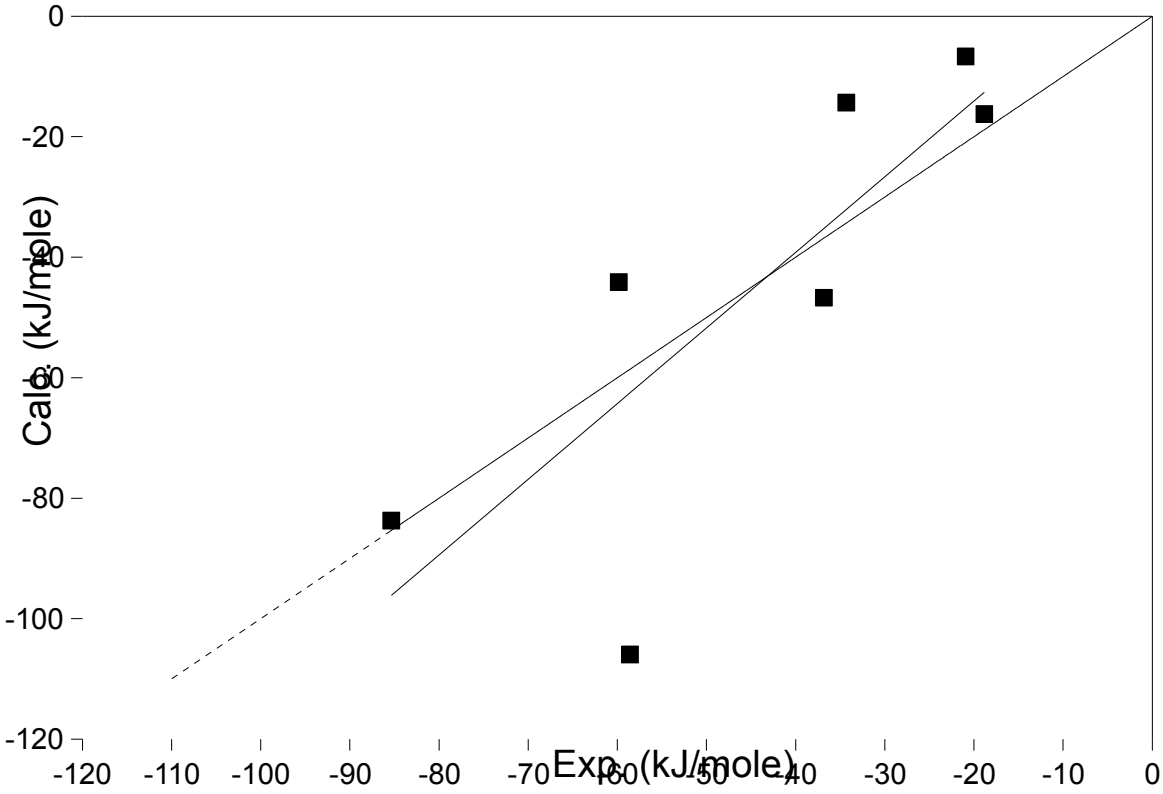
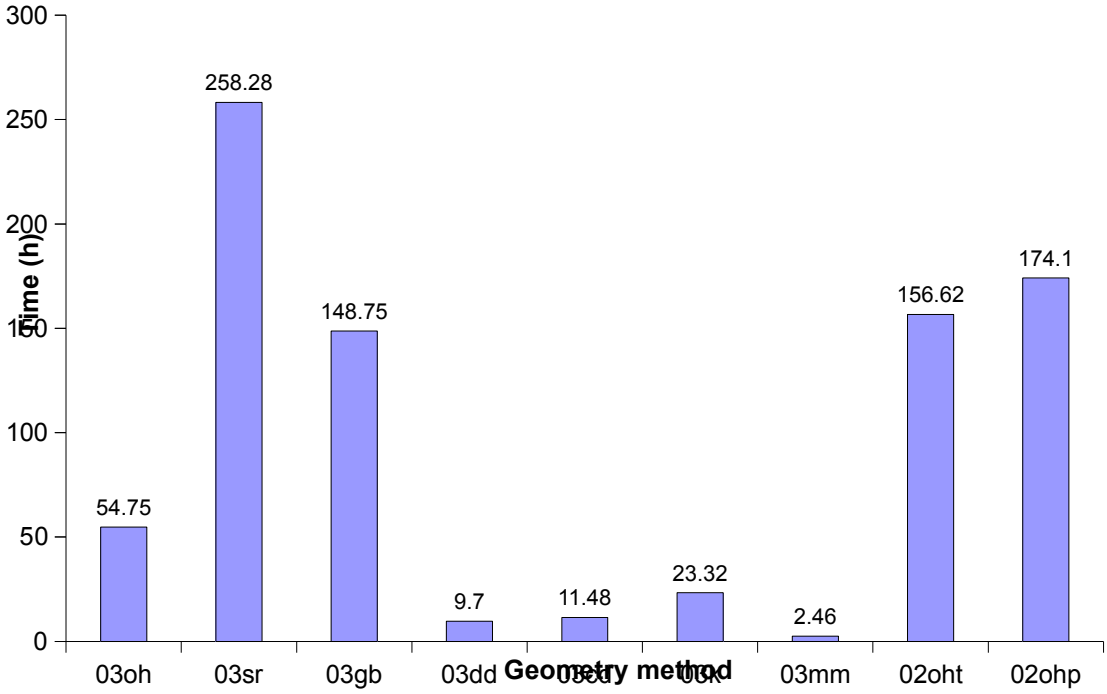
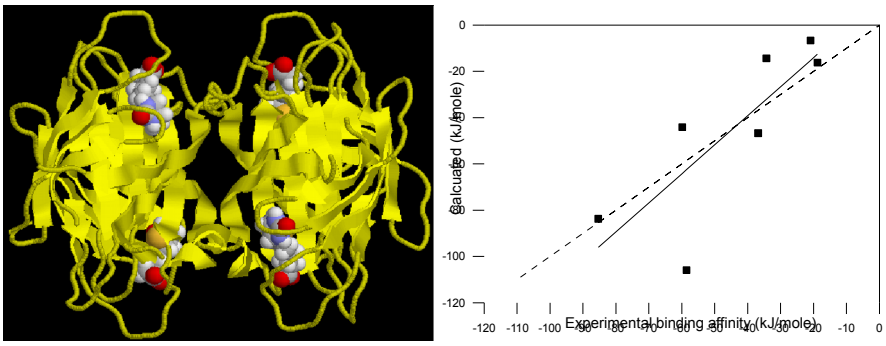


Figure 3. The computer time used by the various geometry methods to generate all the snapshots for BTN1.



TOC graphic


0
-20
-40
-100
-120
Calculated (kJ/mole)
-120 -110 -100 -90 -80 -70 -60 -50 -40 -30 -20 -10 0
Experimental binding affinity (kJ/mole)