Calculating the Absolute Free Energy of Association of Netropsin and DNA

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Abstract: Calculations of the absolute free energy of association of netropsin to the Dickerson dodecamer $[d(CGCGAATTGCGC)]_2$ have been carried out, using the double annihilation method and electrostatic decoupling and an all-atom model of DNA and netropsin in a periodic box of water. The calculated free energy of association is −10.3 kcal/mol, in good agreement with the low salt value of −11.5 kcal/mol for a related sequence $[d(GCGAATTCGC)]_2$[1a] and −10.2 kcal/mol for $[d(CGCAAATTGGC)_2]$.[1b] Separate calculation of the electrostatic and van der Waals free energies leads to the conclusion that the predominant contribution to association is the van der Waals energy, despite the +2 charge of netropsin and the polyanionic nature of the DNA. This is because the electrostatic free energy is almost identical for removing the charges of netropsin in water and when bound to DNA. Our calculations show that 14 molecules diffuse into the GAATTC binding site of netropsin, consistent with our determination of the number of water molecules that are present at that site in the crystal structure of $[d(CGCAAATTGGC)_2]$.[2a,b]

Introduction

Ligands that bind in the minor groove of DNA are of importance in understanding the nature of ligand−DNA recognition in aqueous solution, as well as in the development of sequence-selective DNA binders. One of the best characterized of such ligands is netropsin, which is a long, flat molecule that is sterically and electrostatically complementary to DNA (Figure 1). Both thermodynamic and crystallographic data have characterized its insertion into the minor groove of AT-rich sequences of DNA. It has a 10^9 association constant (K_a) for DNA at low salt concentrations[1a] and binds in the minor groove, causing little change in the DNA structure. Its association with DNA is salt dependent and decreases in strength with increasing salt. Thus, there clearly is a significant electrostatic component to DNA−netropsin association, but the question remains: quantitatively, how much does the contribution of complementary electrostatics, given the +2 charge of netropsin and the polyanionic nature of DNA, contribute to the absolute free energy of association of these two molecules?

This is also an interesting question because such a question has been addressed for two protein−ligand interactions by Miyamoto and Kollman.[3] They found that double annihilation, free energy calculations were able to reproduce the facts that biotin−streptavidin had a ΔG association of ∼20 kcal/mol and that of *N*-acetyl tryptophan amide−α-chymotrypsin was ∼5−10 kcal/mol. Separately calculating the van der Waals and electrostatic contributions suggested that the biotin−streptavidin association was predominantly van der Waals in nature and the *N*-acetyl tryptophan amide−α-chymotrypsin free energy predominantly electrostatic, although the other term in each case was of significant magnitude. Interestingly, these component preferences are opposite to what one might initially expect, since biotin has a negative charge and a very polar ureido group with an extensive H-bonding network of protein groups interacting with these functionalities.

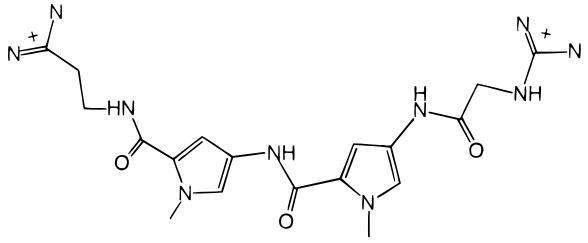

Figure 1. Structure of netropsin.

On the other hand, *N*-acetyl tryptophan is uncharged and α-chymotrypsin is known to prefer hydrophobic groups in its P1 pocket. This makes it particularly interesting to study the netropsin−DNA complex, where a naive view would expect the electrostatic component to dominate the association.

The double annihilation free energy method was proposed by Jorgensen,[4] based on the following cycle (Figure 2), where **A** and **B** are association molecules and **D** is a dummy molecule with no interaction with its surroundings.

Since $\Delta G_{\mathbf{B}} = 0$, $\Delta G_{\mathbf{A}}(\text{association}) = \Delta G_{\mathbf{C}} - \Delta G_{\mathbf{D}}$. The calculation of $\Delta G_{\mathbf{A}}$ can be accomplished by mutating molecule **B** both when bound to the receptor ($\Delta G_{\mathbf{D}}$) and free in solution ($\Delta G_{\mathbf{C}}$) to nothing. Jorgensen and Buckner,[4] Miyamoto and

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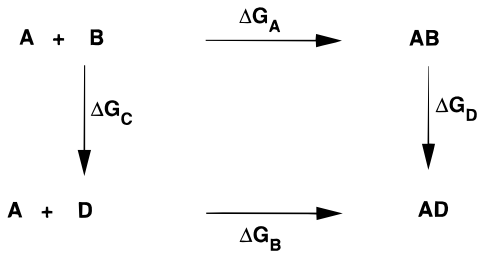

Figure 2. Thermodynamic cycle for an association process.

Kollman,[3] and Merz[5] have shown this approach to give reasonable absolute free energies of association when compared to experiment albeit on very small ligands, CH_4 , K^+, and CO_2. As noted above, Miyamoto and Kollman[3] also showed reasonable agreement with experiment for **B** = biotin and *N*-acetyl tryptophan, at a cost of not being able to study the reversibility as rigorously as in the small ligand cases and with the assumption that the internal energies of free and bound ligand were the same.

All of the above studies did not correctly calculate the free energy cost of restraining ligand **B** as it disappears in the bound **A**:**B** complex. Such restraints are necessary so that **B** does not move significantly from the correct bound position for **B** as it is mutated to **D** (dummy), which would make the calculated $\Delta G_{\mathbf{D}}$ irrelevant. Recently both Hermans and Wang[6] and Gilson et al.[7] showed how to rigorously calculate these restraint energies, although this is still difficult to do for complex ligands with many degrees of freedom. An exciting recent application of absolute free energy methods to a camphor−cytochrome P450 complex,[8] where the camphor is mutated to six water molecules, considers these restraint free energies and is able to calculate the $\Delta G_{\mathbf{A}}$ of camphor in impressive agreement with experiment.

In the work presented below, we use the same methodology employed in the biotin−streptavidin and *N*-acetyl tryptophan−α-chymotrypsin studies. Our goals are less quantitative than in the absolute free energy calculations of the more rigid ligands: (1) Can we calculate an absolute free energy of binding of a similar magnitude to experiment, i.e., within ±5 kcal/mol and (2) is the predominant component in the $\Delta G_{\mathbf{A}}$ for netropsin−DNA electrostatics or van der Waals interactions?

Methods

The starting conformation for our free energy simulations was obtained from the crystal structure of d(CGCGAATTC^{Br}GCG)•d(CGCGAATTC^{Br}GCG) and Netropsin complex (PDB entry 6BNA).[2b] We removed the bromine atoms from the structure and then placed sodium ions 3.6 Å from the phosphate bisector in the initial configuration. The netropsin structure from the complex was used as the starting conformation for isolated drug calculation. The starting structures were solvated by placing three-site TIP3P water molecules[9] around the solute such that the solvent molecules were placed up to 12 Å away for the complex and up to 13 Å away for the isolated drug along each of the axes and as close as 3.0 Å from any given solute atom.

The equilibration protocol adopted in our current simulations is similar to that of Singh et al.[10] This protocol was adopted to relieve bad contacts between the solute and the solvent and to allow the solvent molecules to reorient and make favorable contacts with the solute. In our first step we restrained the starting structure with 25 kcal/mol-residue harmonic restraint and carried out potential energy minimization for about 1000 iterations followed by molecular dynamics at 300 K for about 3 ps. We then released the restraints on the solute in five steps by reducing the restraint by 5 kcal/mol-residue per step followed by 600 iterations of conjugate gradient minimization. Thus in the fifth step the whole system was minimized without harmonic restraints. The system was then heated to 300 K with a temperature coupling of 0.2 ps. All the covalent bonds to the hydrogens were held constant to a tolerance of 0.0005 A by applying the SHAKE routine.[11] We used a 1 fs time step in all our calculations. The temperature of the system was allowed to fluctuate around 300 K with a temperature coupling time of 0.2 ps and the pressure was allowed to fluctuate around 1 bar with a pressure coupling time of 0.6 ps. We implemented cubic periodic boundary conditions with a 10 or 12 Å uniform cutoff and 10 or 12 Å cutoff for the electrostatic and Lennard-Jones potential evaluations for solute−water and water−water interactions. All solute−solute interactions (including sodium ions) were evaluated under the assumption that this will avoid the cutoff artifacts on electrostatic interactions of sodium ions with phosphates without serious consequences to the solute−solvent interactions. The evaluation of all solute−solute interactions in our periodic simulations is possible since we do not generate images for the solute atoms.

All our calculations were carried out with AMBER 4.0.[12] In our free energy perturbation calculations we used the thermodynamic windows method with fixed widths[13] for the electrostatic part and the slow growth method[13] for the van der Waals part. The free energy perturbation was carried out by mutating netropsin → 0 in the complex and in water in the forward direction only ($\lambda = 1 \rightarrow 0$) and the free netropsin in solution was mutated to null in both forward ($\lambda = 1 \rightarrow 0$) and backward ($\lambda = 0 \rightarrow 1$) directions. The force field parameters describing netropsin were changed smoothly and uniformly to null, employing methods described elsewhere.[13]

Results

The structure of the complex stayed stable throughout the equilibration. In our free energy perturbation calculations a variety of conditions were tried to understand their impact on the structure and on the calculated free energy changes. The nature and size of cutoffs had a major consequence on the computed free energy change for the complex but not for the isolated netropsin in water. This is not surprising considering the relative size of netropsin (20 Å) compared to the size of the complex (41 Å × 17 Å).

The electrostatic free energy change for the isolated netropsin in water was about the same for both conditions. The length of the simulation to calculate electrostatic free energy (50 and 100 ps) for both the isolated netropsin and the complex did not change the computed values, indicating that these numbers converge within 50 ps. Similarly we found that the length of simulation (200 and 400 ps) to calculate van der Waals energy did not alter the computed numbers.

When we used a uniform cutoff of 10 or 12 Å for the entire solvated system, the electrostatic free energy change for the complex was twice that of the complex during a simulation with the cutoff applied only to solute−solvent and solvent−solvent interactions. In our van der Waals perturbation calculations we were able to carry out a stable simulation for the complex without any structural disruption only with a 400 ps simulation. The 200 ps simulation for the complex could not be run beyond 171 ps without the disruption of the complex. We used the van der Waals free energy statistics from this 171 ps simulation and

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Table 1. Free Energy Components for Netropsin → 0 in Solution

	electrostatic (ele)			van der Waals (vdW)		
perturbation parameter λ	time (ps)	cutoff (A)	ΔG (kcal/mol)	time (ps)	cutoff (A)	ΔG (kcal/mol)
1 → 0	50	10[a]	235.7			
1 → 0	50	10[b]	233.2	200	10[b]	17.4
1 → 0	100	12[b]	238.6	200	12[b]	19.3
1 → 0	50	12[b]	236.4			
0 → 1	100	12[b]	232.3	200	12[b]	15.9

[a] Cutoff applied uniformly. [b] Cutoff applied to solute−solvent and solvent nonbonded interactions.

Table 2. Free Energy Components for Netropsin → 0 in Solvated Complex

	electrostatic (ele)			van der Waals (vdW)		
perturbation parameter λ	time (ps)	cutoff (A)	ΔG (kcal/mol)	time (ps)	cutoff (A)	ΔG (kcal/mol)
1 → 0	50	12[a]	274.4			
1 → 0	50	12[b]	238.8	171 ($\lambda = 1 \rightarrow 0.145$)	10[b]	26.4[c]
1 → 0	100	12[b]	234.6	400	12[b]	26.3

[a] Cutoff applied uniformly. [b] Cutoff applied to solute−solvent and solvent nonbonded interactions. [c] Estimated from extrapolating $\lambda = 0.855$ to $\lambda = 0.0$ from data similar to the complex shown in Figure 4.

Table 3. Free Energy Changes in kcal/mol of Netropsin → 0

isolated drug ΔG_C (kcal/mol)		complex ΔG_D (kcal/mol)	
ele	vdW	ele	vdW
235.2 ± 2.23[a]	17.5 ± 1.7[a]	236.7 ± 2.0[b]	26.3 ± 0.2[b]

ΔG_A (kcal/mol)			
ele[a]	vdW[a]	total	exptl[b]
−1.5	−8.8	−10.3	−11.5

[a] $\Delta G_A = \Delta G_C - \Delta G_D$. total: $\Delta G_A = \Delta G_{Ele} + \Delta G_{vdW}$. [b] [d(GC-GAATT-CGC)]$_2$,[1a] [d(CGCAAATTGGC)$_2$].[1b]

estimated the free energy change to be 26.4 kcal/mol, which is very close to the value (26.3 kcal/mol) computed from the 400 ps van der Waals perturbation simulation.

The free energy changes for the annihilation of the isolated netropsin molecule in water and in the complex are given in Table 3. The total free energy change of 10.3 kcal/mol, a sum of individual electrostatic and van der Waals calculations, is about the same magnitude as the low salt value of 11.5 kcal/mol.[1]

The free energy components for netropsin → 0 in solution are given in Table 1. The electrostatic component of the free energy change for the isolated netropsin in solution was calculated from four forward simulations and one backward simulation. The free energy change shown in Table 3 is an average of the these four forward and one backward free energy perturbation calculations. The van der Waals component of the free energy change for isolated netropsin was calculated in two forward and one backward slow growth simulation and they are also given in Table 1. The van der Waals free energy shown in Table 3 is an average of these two forward and one backward free energy perturbation calculations.

The free energy components for netropsin → 0 in solvated complex are given in Table 2. The average electrostatic free energy change for the complex shown in Table 3 is calculated from two forward simulations ($\lambda = 1 \rightarrow 0$) and the calculated numbers are 238.75 kcal/mol (50 ps, $\lambda = 1 \rightarrow 0$) and 234.64 kcal/mol (100 ps, $\lambda = 1 \rightarrow 0$). In this averaging we did not include the computed number (274.4 kcal/mol) from the first simulation with 12 Å uniform cutoff because it truncates long-range electrostatic interactions between the DNA and the sodium ions and therefore leads to an unstable structure and unreliable free energy statistics. We observed similar cutoff artifacts in the calculation of free energy change from cytsoine to uracil in a RNA hairpin loop.[15] The average van der Waals energy shown in Table 1 for DNA−netropsin → 0 ($\lambda = 1 \rightarrow 0$) is calculated from two forward simulations which are 26.64 kcal/mol (400 ps, $\lambda = 1 \rightarrow 0$) and 26.45 kcal/mol (171 ps, $\lambda = 1 \rightarrow 0.145$) when estimated from extrapolating $\lambda = 0.855$ to $\lambda = 0.0$ from the data similar to the one shown in Figure 4 for DNA−netropsin solvated complex.

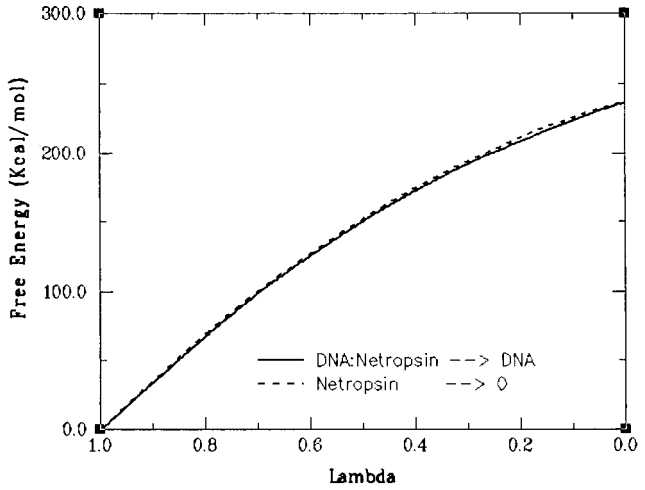

Figure 3. Free energy change with electrostatic decoupling for the netropsin → 0 change in solvated complex and isolated in solution in 100 ps forward simulations. The electrostatic free energy plotted as a function of λ (perturbation paramater). The solid line represents the electrostatic free energy change for mutating netropsin → 0 in the complex and the dashed line represents the free energy change for netropsin → 0 in solution. The free energy change is plotted at an interval of $\Delta\lambda = 0.005$.

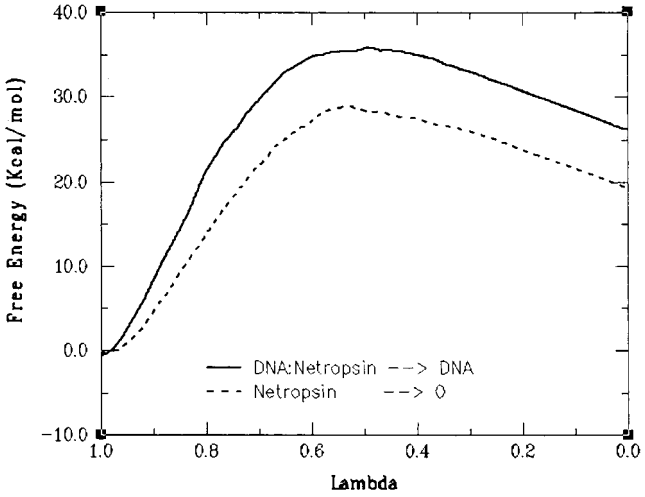

Figure 4. Free energy change with electrostatic decoupling for the netropsin → 0 change in solvated complex and isoalted drug in solution in 400 ps forward simulations. The van der Waals free energy change as a function of λ (perturbation parameter). The solid line represents the van der Waals free energy change for mutating netropsin → 0 in the complex and the dashed line represents the free energy change for netropsin → 0 in solution. The free energy change is plotted at an interval of $\Delta\lambda = 0.00001$.

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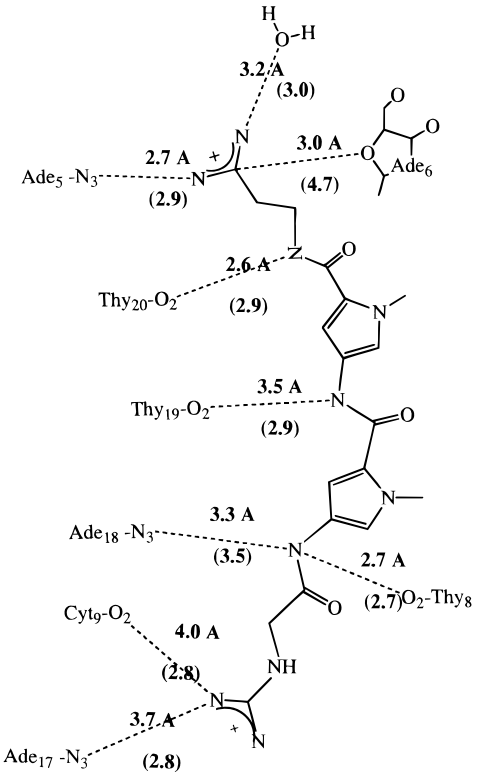

Figure 5. A schematic representation of DNA−netropsin interactions. The distances between potential hydrogen bond donors and acceptors are shown in the X-ray structure (above the dotted line) and in the structure after 10 ps of equilibration (below the dotted line in parentheses). The water shown at the top is the only crystallographic water that was within the vicinity of the ligand.

The free energy calculations were performed with electrostatic decoupling, and electrostatic and van der Waals contributions to the free energy change of netropsin → 0 in the complex and in solution were calculated. The electrostatic free energy change computed during each interval ($\Delta\lambda = 0.005$) is plotted in Figure 3. The free energy changes for both the complex and the isolated netropsin are identical, indicating that the electrostatic interactions for netropsin in solution and in complex are similar.

The van der Waals free energy change was computed with the slow growth method at the $\Delta\lambda = 0.0000025$ interval but plotted at an interval of $\Delta\lambda = 0.00001$ (see Figure 4). The van der Waals free energy profiles for the complex and the isolated drug are similar but have different magnitudes. The free energy difference starts to diverge for the isolated drug right from the beginning of the simulation and continues to do so until $\Delta\lambda \sim 0.5$ and then maintains that difference until the end of the simulation. In the first half of the simulation, the divergence in the vdW energy accumulation curve might be accounted for by the difference in the change in cavity size in the isolated drug versus the complex.

The interactions between netropsin and DNA in the starting structure and after 10 ps of equilibration are shown in Figure 5. The figure shows that almost all the hydrogen bonding interactions present in the crystal structure were maintained. In addition, three new hydrogen bonds are made with O2 of Thy_{19}, O2 of Cyt_9, and N3 of Ade_{17}. A similar analysis at the end of the simulation would be meaningless because the drug is annihilated.

At the end of the van der Waals simulation and total annihilation of netropsin, 14 water molecules from the bulk solvent have moved into the minor groove, making hydrogen bonding interactions with the polar functionalities of the base pairs. Similarly, in the case of the isolated drug, 18 water molecules have moved into the cavity created by the annihilated drug.

Since there is no direct experimental evidence for the number of water molecules netropsin displaces upon binding to the dodecamer, we carried out the following analysis with crystal structures of d(CGCGAATTC^{Br}GCG)·d(CGCGAATTC^{Br}GCG) uncomplexed (PDB entry code 4BNA)[2a] and in complex with netropsin (6BNA)[2b] along with water molecules. We superimposed the two structures using the sugar and base atoms of the GAATTC segment of the dodecamer. We extracted netropsin out of the complex (6BNA) and used it to define its binding site relative to the uncomplexed structure (4BNA). Since any water which is closer than that permitted by hydrogen bonding to the ligand would be displaced we extracted waters from 4BNA structure which are within 2.8 Å of any given atom of netropsin. This resulted in 14 water molecules (water residue numbers in 4BNA: 32, 35, 37, 40, 42, 45, 46, 65−67, 72, 105, 118, 139) that occupy positions similar to that of netropsin in the uncomplexed solvated DNA (4BNA), which is equal to the number of water molecules that diffuse into the cavity created by netropsin in our calculations.

Discussion

The computed total free energy of −10.3 kcal/mol (Table 1) agrees quite well with the experimentally determined free energy of binding of −11.5 kcal/mol for netropsin binding to $[d(GCGAATTCGC)]_2$[1a] and −10.2 kcal/mol for netropsin binding to $[d(CGCAAATTGGC)_2]$.[1b] Of course, the error bar of the computed value is significant and in the range of ±3 kcal/mol (Table 3). To our knowledge this is the first attempt to calculate absolute free energy of binding of a drug to DNA by using a fully detailed atomic model for both solvent and solute. On the other hand, continuum electrostatics calculations by Mishra and Honig[14] suggest, as we do, that although the electrostatic contribution to binding is large (ΔG_D, see Figure 1), it is almost fully balanced by loss of solvation upon binding (ΔG_C). In our calculations the van der Waals part is making most of the contribution to the absolute free energy of binding, with the electrostatic part, despite being very large in magnitude, nearly identical for the complex and the isolated drug. This is contrary to the conventional ideas of dominant forces responsible for the binding of cationic ligands to polyanionic DNA. It is quite conceivable that the electrostatic interactions, which are long-range interactions, can contribute to the binding of the netropsin and orient it for binding.

Miyamoto and Kollman[3] also observed that the van der Waals component made most of the contribution to the absolute free energy of binding of anionic biotin to avidin. The van der Waals forces in the DNA minor groove are more favorable than those in water for the reason that preorganized DNA has already paid the price of forming the cavity for netropsin hence has positive contributions from dispersion attraction and no negative contributions from exchange repulsion. However, in water the dispersion attraction causes the water to form favorable contacts with netropsin but have exchange repulsion due to the close proximity of water molecules. The specific hydrogen bond interactions with the base pairs are favorable since the preorganized DNA has paid the energetic cost. However, the water molecules have to reorient in order to make hydrogen bonds with netropsin. It is possible that the size and shape of the cavity in the minor groove versus that in the bulk solvent may control the number of water molecules that diffuse into the cavity

created by the annihilation of netropsin. In our calculations, annihilation of netropsin in bulk solvent causes 18 water molecules to diffuse into the cavity created by it, whereas in the complex only 14 water molecules diffuse into the minor groove. This number of water molecules is encouragingly consistent with 14 water molecules that are associated with the netropsin binding site determined from the crystal structures of $[d(CGCAAATTGGC)_2]$ free[2a] and in complex[2b] with netropsin.

We emphasize that our calculations did not employ the most rigorous technology to determine the translational and rotational contributions to binding (Hermans and Wang, 1997). On the other hand, we expect that the use of the Weiner et al.[16] force field will, if anything, underestimate the magnitude of van der Waals interactions compared to the Cornell et al. (1995)[17] force field since the nonbonded parameters were mostly derived to reproduce liquid properties in the latter. In any case, inclusion of restraint terms would lead to less favorable free energies of binding and stronger van der Waals interactions would lead to more favorable free energires. It is less likely that the electrostatic free energies will be as force field dependent in this case because they are dominated by the net +2 charge of the ligand. We emphasize that the goal here was to calculate the correct magnitude for the binding free energy, which was a very challenging goal because the ΔG_{elec} was hundreds of kcal/mol, and to compare the magnitudes of $\Delta\Delta G_{elec}$ and $\Delta\Delta G_{vdW}$. Notwithstanding the approximations noted above, our goal was reached.

Conclusion

Our computed absolute free energy of binding of ~10 kcal/mol is in good agreement with the experimentally determined binding free energy of ~11 kcal/mol.[1a,b] Our calculations show that the van der Waals interactions provide the driving force for binding because they contribute significantly more in the complex (−26 kcal/mol) than in water (−17 kcal/mol). On the other hand, the very favorable electrostatic free energy on binding (−236 kcal/mol) is almost equal to the very favorable electrostatic solvation free energy (−235 kcal/mol) of netropsin in water. Thus, despite the electrostatic energies being an order of magnitude larger than the van der Waals energies, the van der Waals free energies contribute more to the binding free energies, just as was found for biotin−streptavidin complexation.[3] These results are consistent with continuum electrostatics studies by Mishra and Honig[14] on intercalation of a cationic drug into DNA where the electrostatic effect was large but comparable in both complex and isolated molecules. In our calculations we find that the annihilation of netropsin causes 14 water molecules to diffuse into its binding site, which is equal to the number of water molecule found to be associated with the GAATTC netropsin binding site of the uncomplexed $d[CGCGAATTCGCG]_2$ crystal structure.[2a]

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