Relative Contributions of Desolvation, Inter- and Intramolecular Interactions to Binding Affinity in Protein Kinase Systems

PETER A. SIMS,[1] CHUNG F. WONG,[2] DANKA VUGA,[3] J. ANDREW McCAMMON,[4] BARTHOLOMEW M. SEFTON[3]

[1]*Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138*

[2]*Department of Chemistry and Biochemistry, University of Missouri, St. Louis, Missouri 63121*

[3]*Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037*

[4]*Department of Chemistry and Biochemistry, Department of Pharmacology, and Howard Hughes Medical Institute, University of California, San Diego, La Jolla, California 92093-0365*

Received 5 December 2004; Accepted 9 January 2005
DOI 10.1002/jcc.20207
Published online in Wiley InterScience (www.interscience.wiley.com).

Abstract: In several previous studies, we performed sensitivity analysis to gauge the relative importance of different atomic partial charges in determining protein–ligand binding. In this work, we gain further insights by decomposing these results into three contributions: desolvation, intramolecular interactions, and intermolecular interactions, again based on a Poisson continuum electrostatics model. Three protein kinase–inhibitor systems have been analyzed: CDK2–deschloroflavopiridol, PKA–PKI, and LCK–PP2. Although our results point out the importance of specific intermolecular interactions to the binding affinity, they also reveal the remarkable contributions from the solvent-mediated intramolecular interactions in some cases. Thus, it is necessary to look beyond analyzing protein–ligand interactions to understand protein–ligand recognition or to gain insights into designing ligands and proteins. In analyzing the contributions of the three components to the overall binding free energy, the PKA–PKI system with a much larger ligand was found to behave differently from the other two systems with smaller ligands. In the former case, the intermolecular interactions are very favorable, and together with the favorable solvent-mediated intramolecular interactions, they overcome the large desolvation penalties to give a favorable electrostatics contribution to the overall binding affinity. On the other hand, the other two systems with smaller ligands only present modest intermolecular interactions and they are not or are only barely sufficient to overcome the desolvation penalty even with the aid of the favorable intramolecular contributions. As a result, the binding affinity of these two systems do not or only barely benefit from electrostatics contributions.

 J Comput Chem 26: 668–681, 2005

Key words: binding affinity; Poisson model; solvent-mediated intramolecular interactions; CDK2; PKA; LCK; deschloroflavopiridol; PKI; PP2

Introduction

One way to determine the importance of a parameter in affecting a property of interest is to perturb that parameter and observe the resulting change in the property. This technique, known as sensitivity analysis, has been used in a number of disciplines, including our recent applications to biomolecular modeling and simulations.[1–7] In one series of studies, we employed a Poisson model to examine the significance of each atomic partial charge to the binding affinity (or ΔG_{bind}) of a ligand to a receptor.[8–11]

In this study, we try to gain further insight by resolving the intermolecular, intramolecular, and desolvation components of the sensitivity, $\Delta\Delta G_{\text{bind}}$, of a binding affinity to switching off or on

Correspondence to: C. F. Wong; e-mail: wongch@umsl.edu

Contract/grant sponsors: the NIH, NSF, Howard Hughes Medical Institute, Accelrys Inc., National Biomedical Computation Resource, Center for Theoretical Biological Physics (UCSD), and the W. M. Keck Foundation

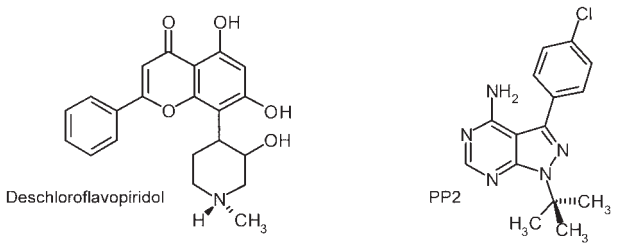

Figure 1. Structures of deschloroflavopiridol and PP2 with the sequence of PKI-(5-24).

each atomic partial charge. In studying protein–ligand interactions, one often focuses on analyzing the direct interactions between protein and ligand atoms/functional groups/residues. However, direct interactions are not the only factors that determine the value of a binding affinity. When a ligand binds to a receptor, the dielectric environments of both the ligand and the receptor are changed. This change in dielectric environments can introduce a desolvation penalty and alter the solvent-mediated intramolecular interactions in the protein and the ligand. For example, consider an atom of the ligand near the protein–ligand interface. It is relatively exposed to solvent before but is less exposed after binding. Therefore, a desolvation penalty results upon binding. Also, because the charge of this atom is less screened by the solvent after binding due to the displacement of solvent molecules in the binding pocket, the strength of its electrostatic interactions with other atoms, not only those with the proteins but also those within the ligand itself, is increased because of the diminished effective dielectric constant. Thus, in studying protein–ligand binding, it is important to examine the change in the solvation energy of each atom, and the change in the solvent-mediated intramolecular interactions within the ligand and within the protein in addition to the direct and solvent-mediated interactions between the protein and the ligand. Here, by using a Poisson model, we quantify the relative magnitude of these contributions in three protein kinase systems.

CDK2–Deschloroflavopiridol System

Deschloroflavopiridol, which appears in Figure 1 with the other three ligands considered in this study, is an analog of the potent CDK inhibitor flavopiridol (which is nearly identical except for the presence of a chloro substituent on the phenyl ring). Synthetic inhibitors of CDK2 are of interest because of the role of this protein kinase in cell cycle regulation.[12] CDK2 forms a complex with Cyclin E during the S-phase of the cell cycle at the restriction point. The CDK2–Cyclin A complex is a requirement for the completion of the S-phase. Cancerous cells are known to underexpress natural inhibitors of CDK2 known as cyclin-dependent kinase inhibitors (CKIs), which compete with ATP.[12] Synthetic inhibitors can therefore help to treat cancers. The IC_{50} of flavopiridol with respect to CDK2 is estimated to be 0.17–0.40 μM.[13] In a previous work, we used sensitivity analysis to identify the most significant atomic partial charges in contributing to binding.[9] Here, we also analyze these results in terms of the desolvation, intermolecular, and intramolecular contributions.

PKA–PKI System

This is another system that we have studied with sensitivity analysis and charge optimization.[8,14] PKI is a much larger ligand than deschloroflavopiridol, giving us an opportunity to examine the influence of ligand size on the relative contributions of the three electrostatic components. The particular form of the PKA–PKI system addressed in this article is known as PKA–PKI(5–24), because the ligand consists of residues 5–24 from a larger peptide. Its sequence is shown in Figure 1.

LCK–PP2 System

We also modeled the electrostatic interactions in a lymphocyte specific kinase (LCK) ligand–receptor system. This protein–tyrosine kinase is only expressed in T cells and has a pivotal role in the T-cell receptor (TCR) signal transduction cascade. LCK phosphorylates several important components that appear early in the TCR cascade.[15] Because of LCK's importance to T-cell activation, it is considered a promising target for inhibitor design against autoimmune diseases.[15] Its structure is also shown in Figure 1.

Methods

Electrostatic Binding Free Energy Change

Our model of receptor–ligand binding involves the computation of the electrostatic binding free energy change from Coulomb's Law and the solution to the Poisson Equation. We can write this electrostatic binding free energy change in matrix–vector form in terms of interaction potentials:[16,17]

$$\Delta G_{\text{elect}} = \frac{1}{2}\mathbf{Q}_L^T L \mathbf{Q}_L + \frac{1}{2}\mathbf{Q}_R^T R \mathbf{Q}_R + \mathbf{Q}_R^T C \mathbf{Q}_L \tag{1}$$

In this expression, $\mathbf{Q}_L$ is the vector containing the ligand atomic partial charges and $\mathbf{Q}_R$ is the vector containing the receptor atomic partial charges. The matrices L and R determine the desolvation penalties of the ligand and receptor, respectively. An element of the matrix L is defined as:[16,17]

$$L_{ij} = \phi_i^{\text{bound}}(\mathbf{r}_j^L) - \phi_i^{\text{unbound}}(\mathbf{r}_j^L) \tag{2}$$

where $\phi_i^{\text{bound}}(\mathbf{r}_j^L)$ and $\phi_i^{\text{unbound}}(\mathbf{r}_j^L)$ are the electrostatic potentials generated by atom i on atom j for the bound and unbound forms, respectively. The elements of matrix R for the receptor can be written in an analogous fashion. Additionally, we can define the elements of the following vector:[16,17]

$$(C^T\mathbf{Q}_R)_i = \sum_{j=1}^{m} q_j^R \phi_i^{\text{bound}}(\mathbf{r}_j^R) \quad (3)$$

which allows one to calculate the electrostatic potential at atom i of the ligand resulting from charges in the receptor. In eq. (3), q_j^R is the point charge on receptor atom j and $\phi_i^{\text{bound}}(\mathbf{r}_j^L)$ is the potential generated at ligand atom i by a unit charge on receptor atom j. m is the number of receptor atoms. This includes both direct Coulombic and solvent-mediated contributions.

All the solvent-mediated terms were computed numerically with the UHBD Poisson–Boltzmann solver[18,19] along with a separate script for calculating direct Coulombic interactions analytically. In all UHBD calculations for the CDK2–deschloroflavopiridol and related systems, we employed a 175 × 250 × 215 grid with 0.3-Å grid spacing. A 240 × 240 × 240 grid was used with 0.4-Å grid spacing for the PKA–PKI system. For the LCK–PP2 and related systems, we used a 180 × 230 × 200 grid with 0.35-Å grid spacing. A solvent dielectric of 78 and a solute dielectric of 2 were used in all applications of UHBD.[18,19]

Calculations of Electrostatic Components

In our previous work, we employed sensitivity analysis to gain insight into the charge utility of individual atoms and functional groups on a ligand and on its receptor active site.[9] In this type of calculation, one estimates the effect of turning off or switching on a charge or group of charges on binding affinity. To measure the effect of turning on a charge, one can calculate the change in binding free energy as:[9]

$$\Delta\Delta G_k = \Delta G(q_k = q_{\text{calc}}) - \Delta G(q_k = 0) \quad (4)$$

In eq. (4), the first term on the right-hand side is the binding energy obtained from a "standard" set of charges while the second term is similar except that the charge of atom k is set to zero. The "standard" set of charges are taken from the CHARMM[20] force field for the protein and from quantum chemical calculations for the ligand.[9] Note that eq. (4) is not meant to imply that ΔG is a function of only one charge; we show explicitly only the charge that is perturbed in the sensitivity analysis. In each case, ΔG is a function of all the ligand and receptor atomic partial charges, the relative positions of all atoms in the system, and the van der Waals radii of all atoms in the system.

The equations for sensitivity analysis that we have employed in our studies of the CDK2–deschloroflavopiridol system[9] and in our work with charge optimization[14] can be written in several ways. One formulation that we have found to be quite useful involves writing $\Delta\Delta G$ for an atom k in terms of intermolecular, intramolecular, and desolvation contributions:

$$\Delta\Delta G_k = \Delta\Delta G_{\text{inter},k} + \Delta\Delta G_{\text{intra},k} + \Delta\Delta G_{\text{atom},k} \quad (5)$$

When a ligand binds to a receptor, the interactions between a given ligand atom k and all other ligand atoms change. $\Delta\Delta G_{\text{intra},k}$ is the sum over these intramolecular interaction energy changes. In a fixed conformation model, this is exclusively a solvent-mediated term that can be computed from the solution to the Poisson equation; the direct Coulombic interactions will cancel in the initial and final states in the rigid-conformation model. $\Delta\Delta G_{\text{inter},k}$ is the sum over the intermolecular interactions between atom k and the receptor. Finally, there is a desolvation penalty term $\Delta\Delta G_{\text{atom},k}$, which is a function of the charge on atom k but not of any other charges. A similar formulation applies to protein atoms as well.

We can write all three of these terms as functions of the interaction potentials such that each term can be computed individually. This allows us to analyze the relative contributions of each type of interaction to the overall change in binding affinity. Using the matrix elements defined above:

$$\Delta\Delta G_{\text{intra},k} = q_{L,k} \sum_{\substack{j=1 \\ j\neq k}}^{n} L_{ik} q_{L,j} \quad (6)$$

$$\Delta\Delta G_{\text{inter},k} = q_{L,k} \sum_{j=1}^{m} C_{jk}^T q_{R,j} \quad (7)$$

$$\Delta\Delta G_{\text{atom},k} = \frac{1}{2} L_{kk} q_{L,k}^2 \quad (8)$$

In each of the above equations, $q_{L,j}$ or $q_{R,j}$ refer to the point charges on the jth ligand or receptor atom, respectively. The matrices L and C are those defined in eqs. (2) and (3), respectively. The same mathematical forms apply to analyzing the components of a receptor atom k. One needs only to replace the matrix L with the matrix R and exchange $q_{R,i}$ with $q_{L,i}$ (and vice versa) in eqs. (6), (7), and (8).

We will also apply the relationships in eqs. (6), (7), and (8) to decompose the electrostatic binding affinity itself. Our original matrix–vector expression for ΔG_{elect} given in eq. (1) can be written in terms of the components:

$$\Delta G_{\text{elect}} = \Delta G_{\text{intra,lig}} + \Delta G_{\text{atom,lig}} + \Delta G_{\text{inter}} + \Delta G_{\text{intra,rec}} + \Delta G_{\text{atom,rec}} \Delta G_{\text{elect}} = \frac{1}{2}\sum_{i=1}^{n} \Delta\Delta G_{\text{intra},i}^{\text{lig}} + \sum_{i=1}^{n} \Delta\Delta G_{\text{atom},i}^{\text{lig}} + \sum_{i=1}^{n} \Delta\Delta G_{\text{inter},i}^{\text{lig}} + \frac{1}{2}\sum_{i=1}^{m} \Delta\Delta G_{\text{intra},i}^{\text{rec}} + \sum_{i=1}^{m} \Delta\Delta G_{\text{atom},i}^{\text{rec}} \quad (9)$$

The last two terms for the receptor are more expensive to calculate because the size of matrix R is determined by the number of atoms, which is quite large for the three protein kinases studied here. However, the sum of the two receptor terms can be determined by subtracting the ligand and intermolecular terms from the total electrostatic binding affinity, which can be obtained without first generating the matrix R. We will also show that summation over a sufficiently large shell of receptor atoms in the ligand binding region gives a good approximation of the relative magnitudes of the receptor intramolecular and desolvation terms.

Molecular Modeling

We also study some computationally generated derivatives of deschloroflavopiridol and PP2 whose structures were generated using similar protocols to those outlined in our previous studies.[9,14] Their parent structures were obtained by X-ray crystallography. Briefly, we used the InsightII Builder, Biopolymer, and Discover Modules for making structural modifications and for energy minimization.[21] Hypothetical derivatives of deschloroflavopiridol and PP2 were constructed from the crystal structure coordinates and energy minimized using the InsightII CVFF force field with the steepest descent algorithm to an RMS derivative less than or equal to 0.001 kcal/mol · Å (InsightII, Accelrys Inc., San Diego, 2000). The energy minimizations allowed the modified functional groups on the ligands to relax in the receptor crystal structures with a distance-dependent dielectric (Insight II).

For all ligand–receptor systems, van der Waal radii were taken from the CHARMM27 all-atom force field.[20] Partial charges for CDK2, PKA, PKI, and LCK were taken from this same force field. For PP2, deschloroflavopiridol, and all hypothetical ligand structures, the partial charges were generated from quantum calculations using the Merz–Kollman method with Gaussian 98 (6-31G* basis set).[21–23]

Results and Discussion

Sensitivity Analysis of Receptor Atoms

As we mentioned in the Methods section, eqs. (6), (7), and (8) can also be applied to receptor sensitivity analysis where the effects of turning on protein partial charges are examined. The only necessary modification is the replacement of all occurrences of *L* (ligand) with *R* (receptor). This method was applied to all three systems mentioned in the Introduction, but not all atoms in the three receptors (CDK2, PKA, and LCK) were included to save computational time. In each case, component analysis was only applied to shells of atoms in and around the active sites of the three proteins. A receptor atom is defined to be a member of the shell if it is a member of an amino acid that has at least one atom within 5 Å of any ligand atom in the complex crystal structure (including modeled hydrogens). The structures of the three ligands that accompany each receptor in the complex crystal structures are depicted in Figure 1.

In the case of the CDK2-deschloroflavopiridol system, the shell included 369 receptor atoms. We found that for 112 of the 369 atoms (~30%) $\Delta\Delta G_k$ is dominated by the intramolecular term. For 248 of the 369 atoms (~67%) $\Delta\Delta G_k$ is dominated by the intermolecular term, which leaves the remaining seven atoms (~2%) with the desolvation term dominating. Additionally, we found that for 244 of the 369 receptor atoms (~66%), $\Delta\Delta G_{\text{intra},k}$ was favorable for binding, but that $\Delta\Delta G_{\text{inter},k}$ was favorable for only 144 atoms (~39%). To aid in the comparison of the relative magnitude of the terms, we present plots of the intramolecular and intermolecular components as functions of distance from the closest ligand atom in Figure 2. Figure 3 includes plots of the atom desolvation term and $\Delta\Delta G_k$ as functions of distance from the closest ligand atom. The average magnitude of the intramolecular term is about 0.24 kcal/mol, whereas the intermolecular term has an average magnitude of about 0.37 kcal/mol. The atom desolvation term is much smaller (0.09 kcal/mol on average).

Our thermodynamic model of deschloroflavopiridol binding to CDK2 suggests that the receptor pays a substantial desolvation penalty from its atom term. The receptor atom desolvation term is the fifth term in eq. (9). We did not compute the atom desolvation term for each atom in CDK2, but the sum of the 369 terms that we did compute is 34.4 kcal/mol (which is likely to be close to the actual $\Delta G_{\text{atom,rec}}$ because atoms far away from the receptor–ligand interface are not expected to experience significant desolvation penalty because their immediate dielectric environments change little upon binding). The intramolecular contributions, obtained from half of the sum over the 369 $\Delta\Delta G_{\text{intra},k}$ terms, comes out to −28.3 kcal/mol (an approximation of $\Delta G_{\text{intra,rec}}$). The favorable intramolecular term offsets a large part of the desolvation penalty but is not enough to make binding favorable. The intermolecular term (ΔG_{inter}), which can be computed exactly within this model, favors binding but only contributes −6.6 kcal/mol. We can also compute the ligand intramolecular ($\Delta G_{\text{intra,lig}}$) and the ligand desolvation ($\Delta G_{\text{atom.lig}}$) terms and they were found to be −55.7 kcal/mol and 60.2 kcal/mol, respectively. The total electrostatic contribution to the binding free energy change was found to be 4.5 kcal/mol for CDK2–deschloroflavopiridol. One can see from this example that although the direct and solvent-mediated interactions between the protein and the ligand favor binding, they are overcome by the large desolvation penalty of the protein and ligand atoms, although the penalty is reduced somewhat by the favorable intramolecular terms for the protein and the ligand. Understanding protein–ligand binding affinity thus needs to go beyond analyzing the direct and solvent-mediated interactions between the protein and the ligand.

Figure 2 shows the dependence of the intramolecular contributions of different protein atoms as a function of their distance to the closest ligand atoms. Protein atoms that are closer to the protein–ligand interface are expected to have larger magnitudes for these terms because solvent-mediated interactions are altered to a larger extent upon binding. Figure 2 shows this general trend, although there are some fluctuations, probably because this simple use of distance to estimate the degree of exposure of the protein atoms to the ligand-binding pocket is crude, and that different intramolecular interactions are involved for different protein atoms. Our earlier work used $\Delta\Delta G_k$ as a measure of the charge utility of an atom k in facilitating ligand-receptor binding. It can be decomposed into contributions from intramolecular, intermolecular, and desolvation terms. Typically, desolvation presented a penalty to binding affinity but this penalty is often offset by the receptor intramolecular term, which is more often favorable than unfavorable. As can be seen from Figure 2, the receptor–ligand interaction contribution can also have favorable or unfavorable effects on binding. Whether the charge on an atom is profitable for binding depends on the collective effects of these three terms.

In our previous sensitivity analysis study of the CDK2–deschloroflavopiridol system, we computed the charge utilities of 103 receptor atoms in 20 active site amino acids.[9] Our new component analysis of these charge utilities has revealed a more complete picture of where these contributions originate. For example, the backbone N–H group of Leu83 and the backbone carbonyl group

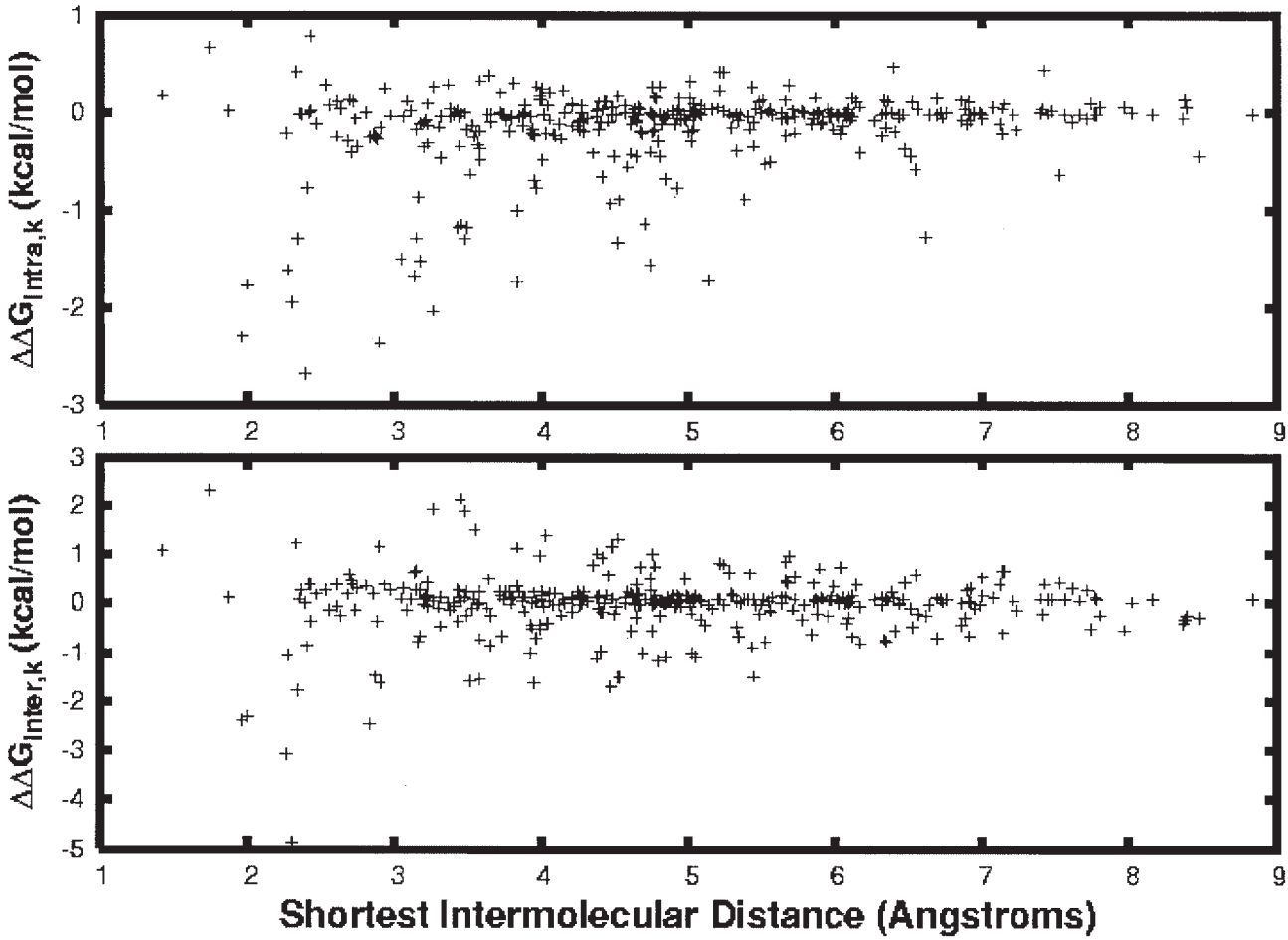

Figure 2. Plot of $\Delta\Delta G_{\text{intra},k}$ vs. distance from the closest ligand atom (top) and $\Delta\Delta G_{\text{inter},k}$ vs. distance from the closest ligand atom (bottom) for the 369 CDK2 atoms in the shell of interest.

of Glu81 were both found to have favorable electrostatic contributions to binding in our previous analysis.[9] In this study, we found that the $\Delta\Delta G_{\text{inter},k}$ term for the hydrogen in Leu83:NH is quite large for a receptor atom (−2.4 kcal/mol). However, the intramolecular contribution is almost as significant (−2.3 kcal/mol). Thus, this hydrogen contributes favorably to binding not only by interacting favorably with the ligand, but also by improving its solvent-mediated intramolecular interactions within the protein upon binding. On the other hand, the carbonyl oxygen has a negligible $\Delta\Delta G_{\text{inter},k}$ term, and its beneficial effects on binding result solely from having a favorable intramolecular term ($\Delta\Delta G_{\text{intra},k} = -2.7$ kcal/mol). This is another example that illustrates that one cannot simply look at the interactions between an atom on the protein and nearby atoms on the ligand to deduce whether the atom is beneficial to binding. In this case, the protein atom makes favorable contributions to binding because its intramolecular interactions with the protein are enhanced upon ligand binding, with little contribution from protein–ligand interactions.

We used the same approach to analyze the PKA–PKI system. In this case a large, 306 atom peptide ligand is bound to a long cleft of protein kinase A in contrast to our previous discussion where a small 49-atom ligand is buried in the CDK2 active site. Using the definition of shell membership described above, we assigned 712 PKA atoms to the protein–ligand interface. In this model, the intramolecular term dominates the charge utility of only 81 of the tested atoms (~11%), whereas the intermolecular term dominates 607 (~85%) of the atoms. The atom desolvation term dominated in the case of seven atoms. These trends are much different from what we computed in the CDK2 case. However, the ratio of atoms that had favorable intramolecular and intermolecular terms is similar to that of the CDK2–deschloroflavopiridol system: 471 (~66%) of the 712 atoms had favorable intramolecular terms and 279 (~39%) atoms had favorable intermolecular terms.

Figures 4 and 5 are analogous to Figures 2 and 3 but for the PKA–PKI receptor analysis. Figure 4 shows that the majority of the large magnitude intramolecular contributions to the charge utility are favorable, and that protein atoms further away from the ligand than in the CDK2 case also present appreciable intramolecular terms. This is probably because of the large size of the PKI ligand, which displaces more water molecules and thus diminishes the solvent-screening effects to a larger extent. In general, the

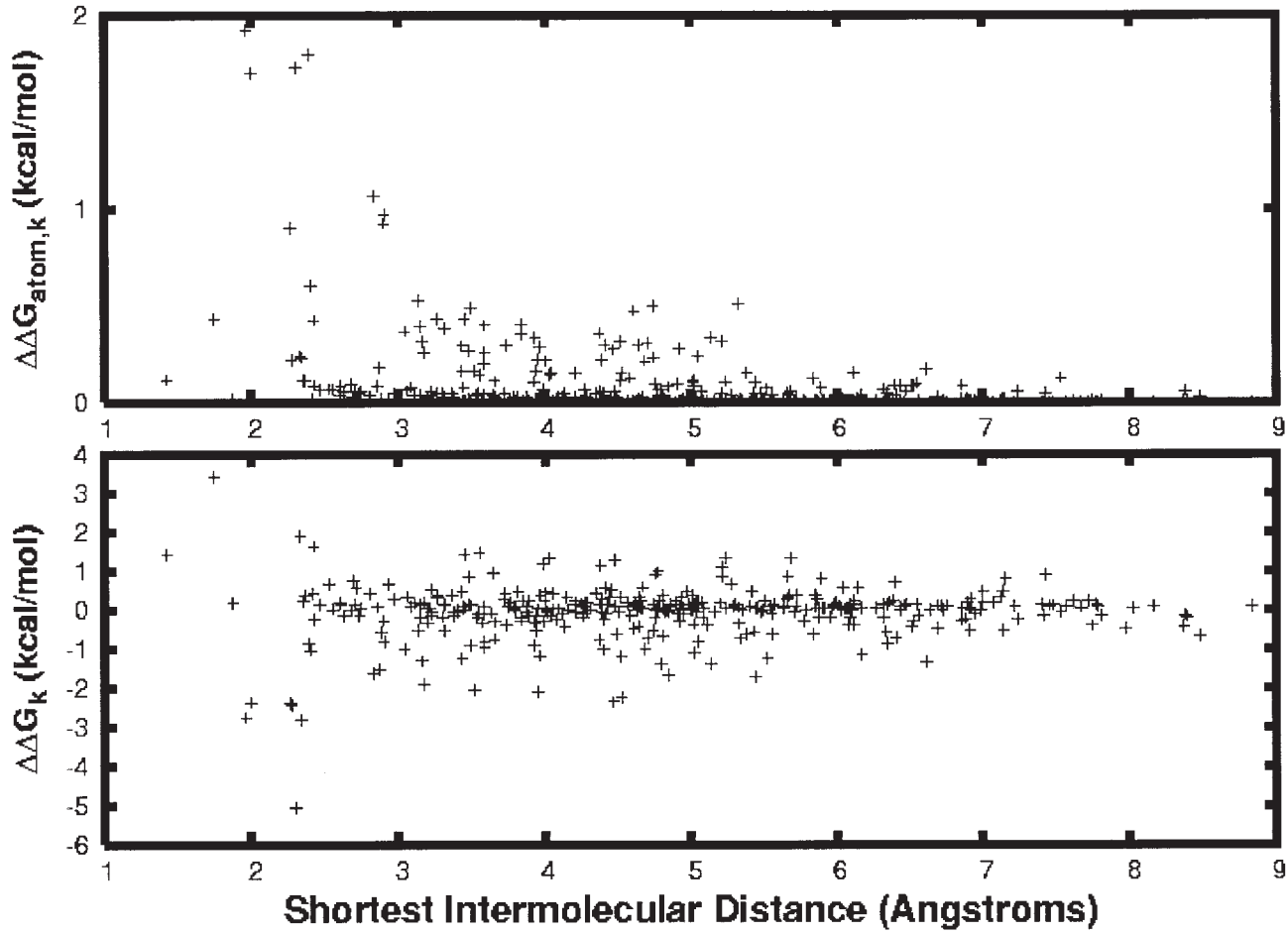

Figure 3. Plot of $\Delta\Delta G_{\text{atom},k}$ vs. distance from the closest ligand atom (top) and $\Delta\Delta G_k$ vs. distance from the closest ligand atom (bottom) for the 369 CDK2 atoms in the shell of interest.

charge utility and all of its components are larger in magnitude as a group than in our previous case of a small ligand-kinase complex. The $\Delta G_{\text{atom,rec}}$ term dominates $\Delta G_{\text{intra,rec}}$ to a large extent for the PKA–PKI system. We estimated these terms to be 110.0 kcal/mol and −79.2 kcal/mol, respectively, from our 712 atom shell. These two numbers sum to 30.8 kcal/mol. (As mentioned earlier, a better estimate comes from subtracting the explicitly calculated ligand intramolecular and intermolecular terms from the total electrostatic binding affinity. This type of calculation gives a value of 31.7 kcal/mol, which is very close to the estimate given by the shell model). Despite this large penalty and the inability for the change in intramolecular interactions to compete with the desolvation penalty, we found the total intermolecular term to be very favorable, −92.3 kcal/mol. As a result, electrostatics effects can still make a favorable contribution to the binding affinity.

For the LCK–PP2 case, the ligand is comparable in size to deschloroflavopiridol. Our model suggests that in this system, the favorable changes in intraprotein interactions compete very effectively with the desolvation term. Figures 6 and 7 include plots of all three charge utility components along with the total charge utility for the 359 shell atoms near the LCK–PP2 interface as functions of distance from the closest ligand atom. The charge utilities of 248 (~69%) LCK shell atoms are dominated by the intramolecular term, whereas the magnitude of the intermolecular term is largest for only 78 (~22%) of the shell atoms. The remaining 31 atoms (~9%) have charge utilities that are dominated by the atom desolvation term. About 74% of the shell atoms have favorable intramolecular terms, and 48% have favorable intermolecular terms. We calculated that the average magnitude of $\Delta\Delta G_{\text{intra},k}$ for the 359 LCK shell atoms to be about 0.17 kcal/mol compared to average magnitudes of 0.07 kcal/mol and 0.09 kcal/mol for $\Delta\Delta G_{\text{inter},k}$ and $\Delta\Delta G_{\text{atom},k}$, respectively.

Using the LCK shell, we can approximate $\Delta G_{\text{intra,rec}}$ and $\Delta G_{\text{atom,rec}}$ to be about −28.3 kcal/mol and 31.1 kcal/mol, respectively. The sum of these two numbers give 2.8 kcal/mol, which is very close to the 2.7 kcal/mol obtained by using the subtraction method mentioned above. The intermolecular contribution to the electrostatic binding affinity is −5.2 kcal/mol. Two particular atoms stand out in the bottom half of Figure 6 for making significant favorable contributions to the intermolecular term. The hydrogen on the backbone amide nitrogen of Met:319 has a $\Delta\Delta G_{\text{inter},k}$ of −2.7 kcal/mol and the backbone oxygen of Glu:317

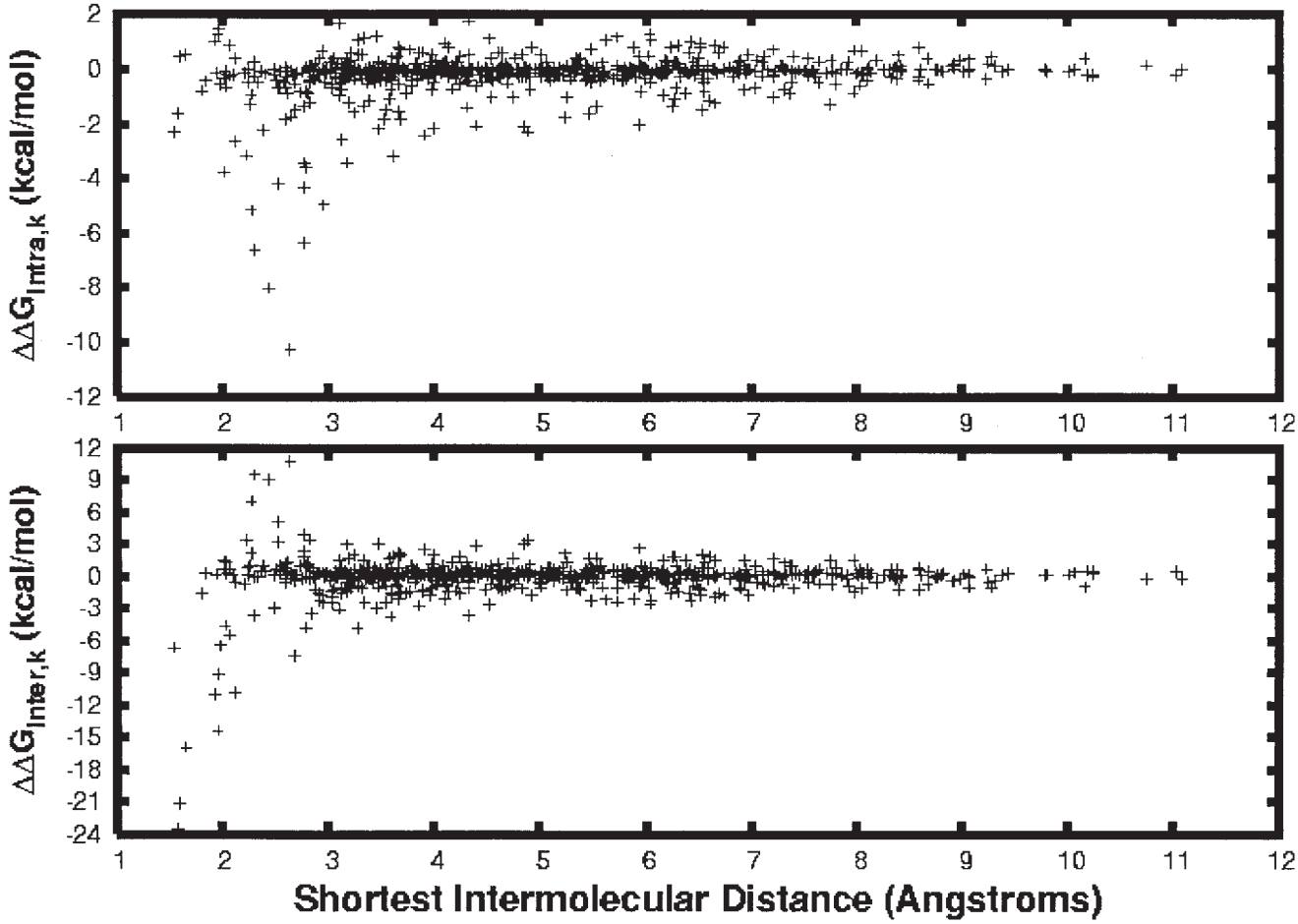

Figure 4. Plot of $\Delta\Delta G_{\text{intra},k}$ vs. distance from the closest ligand atom (top) and $\Delta\Delta G_{\text{inter},k}$ vs. distance from the closest ligand atom (bottom) for the 712 PKA atoms in the shell of interest.

has a $\Delta\Delta G_{\text{inter},k}$ of -2.6 kcal/mol. These two atoms are said to be hydrogen bonding sites in the crystallographic study of this system.[15] We note however, from the lower half of Figure 7 that the charge utilities of these two atoms are very different. This is because the contributions from the intramolecular and atom desolvation terms are different for these two atoms. The oxygen has an intramolecular contribution of -2.5 kcal/mol and an atom desolvation term of 1.4 kcal/mol, whereas the hydrogen's intramolecular and atom desolvation terms are -1.5 and 2.2, respectively. These two important atoms are an example of how the intramolecular competition with the atom desolvation term can ultimately determine their relative charge utility even though they have similar intermolecular contributions.

Sensitivity Analysis on Ligand Atoms: CDK2–Deschloroflavopiridol

Figure 8 shows the intramolecular terms for each ligand atom in the CDK2–deschloroflavopiridol system mapped onto the ligand structure. In our previous computational analysis of this system, we described the key protein–ligand electrostatic interactions in some detail.[9] One consensus that we arrived at with the experimental results for this system was the importance of the hydrogen bonding regions of the ligand that include the carbonyl group and the adjacent hydroxyl group (which appear at the top of Fig. 8).[9] It seems that along with substantial and favorable charge utilities, these atoms also make the most significant contributions to their intraligand terms. The intramolecular terms for the carbonyl group are most noteworthy—the carbon atom (C4) has a $\Delta\Delta G_{\text{intra},k}$ value of -16.5 kcal/mol and the oxygen atom (O4) has a $\Delta\Delta G_{\text{intra},k}$ value of -10.9 kcal/mol. The C5–O5–H21 hydroxyl group atoms have $\Delta\Delta G_{\text{intra},k}$ values of -5.2 kcal/mol, -8.4 kcal/mol, and -8.5 kcal/mol, respectively. In all five of the atoms mentioned thus far, the intramolecular term dominates all charge utility contributions. In fact, the charge utilities of 30 of the 49 ligand atoms ($\sim$61%) are dominated by the intramolecular term and the intermolecular term dominates for only 18 of the 49 ligand atoms ($\sim$37%). The intramolecular term is favorable in 67% of atoms and the intermolecular term is favorable in 57% of atoms. Not only does the intramolecular term dominate the charge utility of the majority of deschloroflavopiridol atoms, it also has the highest average mag-

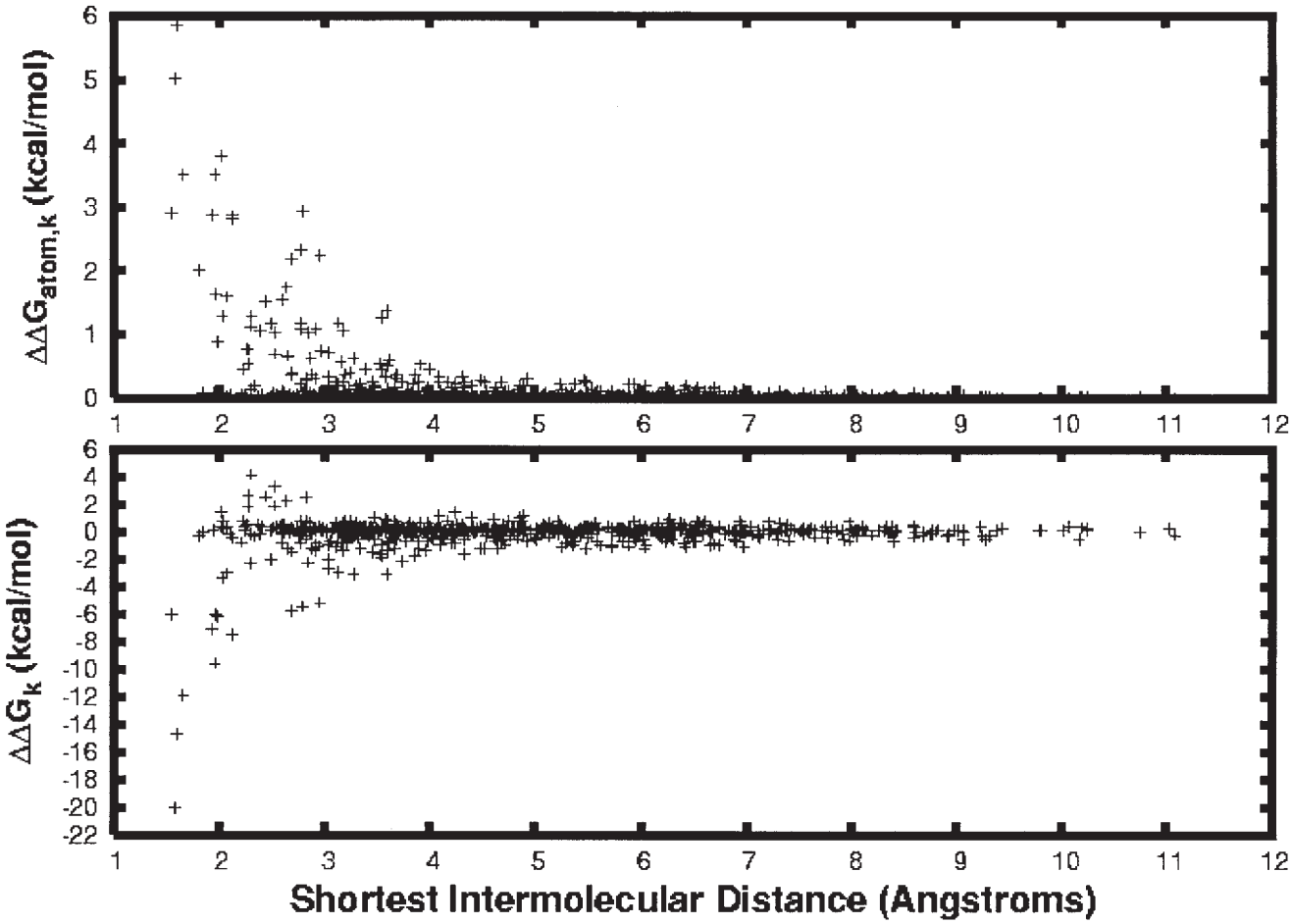

Figure 5. Plot of $\Delta\Delta G_{\text{atom},k}$ vs. distance from the closest ligand atom (top) and $\Delta\Delta G_k$ vs. distance from the closest ligand atom (bottom) for the 712 PKA atoms in the shell of interest.

nitude of the three terms. The average magnitudes of the intramolecular, intermolecular, and atom desolvation terms are 2.5 kcal/mol, 1.1 kcal/mol, and 1.2 kcal/mol, respectively. Thus, it takes more than simple hydrogen bond interactions between the protein and the ligand to explain the useful contributions of these atoms to binding; the intramolecular terms are also quite significant.

The ligand desolvation penalty for this system (4.6 kcal/mol) is less than that of the receptor. $\Delta G_{\text{intra,lig}}$ (−55.7 kcal/mol) competes more effectively with $\Delta G_{\text{atom.lig}}$ (60.2 kcal/mol) than $\Delta G_{\text{intra,rec}}$ does with the receptor atom desolvation term for this system. Nevertheless, the sum of the intramolecular and desolvation terms is unfavorable for both the protein and the ligand and their combined effects overwhelm the favorable intermolecular interactions between the protein and the ligand. As a result, the total electrostatic contribution to the binding affinity of deschloroflavopiridol for CDK2 is a 4.5 kcal/mol penalty.

To gain further insights, we have constructed two analogs of deschloroflavopiridol A and B as shown in Figure 9. The two hypothetical structures were modeled using the modeling protocol described in the Methods section and in our previous computational study of this system.[9] We applied the same techniques for ligand sensitivity analysis to the two modeled structures that we used to analyze the CDK2–deschloroflavopiridol system. Together, the three structures show very little variation in their electrostatic contributions to the binding affinity. $\Delta\Delta G_{\text{elect}}$ for structures A and B differs from that of deschloroflavopiridol by only about 0.6 kcal/mol and 0.1 kcal/mol, respectively. As we mentioned earlier, the change in intraligand interactions plays a major role in competing with the ligand desolvation penalty in the CDK2–deschloroflavopiridol system. Because the matrix elements that govern these changes (along with the partial charges) as defined in eq. (2) are functions only of the system's spatial parameters, there is very little change in these matrix elements when a slightly different atom is substituted at a given position. For example, structure B involves the replacement of the key deschloroflavopiridol hydroxyl group with a thiol group. The sulfur atom in structure B is only slightly larger than the oxygen in deschloroflavopiridol such that the ligand desolvation penalty matrix for structure B is similar to that of deschloroflavopiridol. However, the partial charges of the atoms in structure B are distributed differently than in deschloroflavopiridol. We see that although this gives structure B a much smaller intramolecular contribution to the

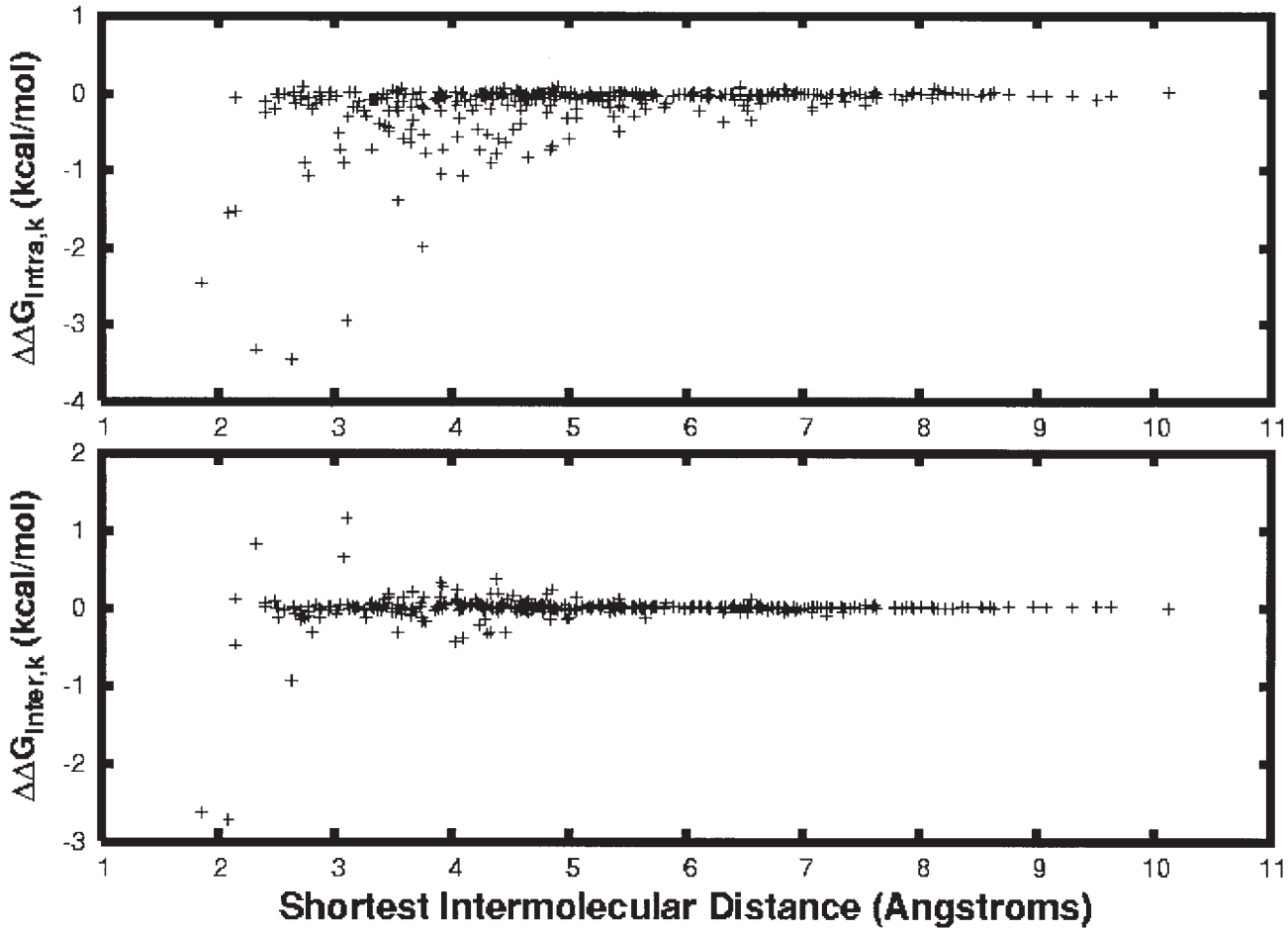

Figure 6. Plot of $\Delta\Delta G_{\text{intra},k}$ vs. distance from the closest ligand atom (top) and $\Delta\Delta G_{\text{inter},k}$ vs. distance from the closest ligand atom (bottom) for the 359 LCK atoms in the shell of interest.

binding affinity (−30.1 kcal/mol as compared to −55.7 kcal/mol for deschloroflavopiridol), the atom desolvation term $\Delta G_{\text{atom,lig}}$ is also much lower in magnitude (35.0 kcal/mol vs. 60.2 kcal/mol for deschloroflavopiridol). The sum of these two contributions for structure B is about 4.9 kcal/mol (vs. 4.7 kcal/mol for deschloroflavopiridol). Furthermore, the spatial parameters of structure B result in a somewhat smaller unfavorable receptor contribution than in the case of CDK2–deschloroflavopiridol. Recall that the latter case gives an unfavorable receptor contribution of about 6.6 kcal/mol, whereas structure B gives 5.8 kcal/mol in our model. Although the intermolecular interaction energy suffers somewhat (by 0.6 kcal/mol) for structure B due to the loss of the hydroxyl group, the less unfavorable collective effects of the protein and ligand intramolecular and desolvation contributions allows this hypothetical ligand to limit its electrostatic penalty to the overall binding affinity. As a result, CDK2–deschloroflavopiridol and CDK2–structure B have very similar electrostatic binding affinity penalties of about 4.5 kcal/mol and 4.6 kcal/mol, respectively.

We observed the same phenomenon for CDK2–structure A. The collective effects of the intramolecular and desolvation terms for the ligand and the receptor introduce a smaller penalty for structure A than for deschloroflavopiridol (4.5 kcal/mol vs 6.0 kcal/mol). As a result, although the chloro group severely damages the intermolecular interactions between structure A and CDK2 relative to deschloroflavopiridol, the electrostatic penalty to the binding affinity increases only slightly (5.2 kcal/mol).

PKA–PKI Ligand Sensitivity Analysis

Despite having very large unfavorable combined contributions from the ligand and receptor intramolecular and desolvation terms, the PKA–PKI complex actually has a very favorable electrostatic contribution to the total binding affinity (−27.5 kcal/mol). This is largely due to the many favorable intermolecular interactions formed between the peptide ligand and the long PKA cleft. We mentioned earlier that the unfavorable intramolecular and desolvation penalty for the receptor was calculated to be 31.7 kcal/mol. The corresponding penalty for the ligand is even worse at about 33.2 kcal/mol. Although it appears that PKI has a more favorable change in intramolecular interaction energies than the receptor, it has a severe atom desolvation penalty of about 119.8 kcal/mol. Nevertheless, for this large ligand system, the intermolecular con-

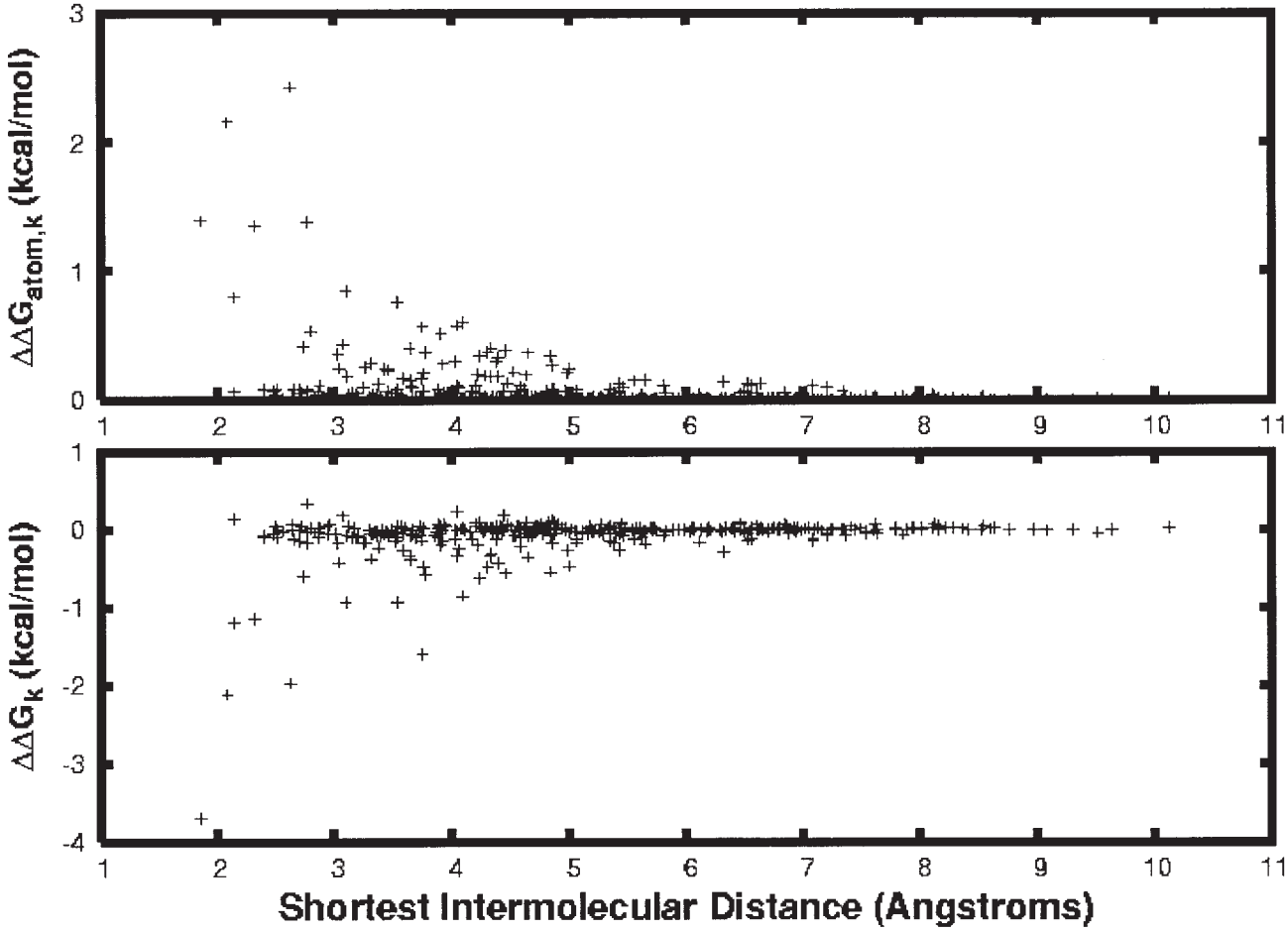

Figure 7. Plot of $\Delta\Delta G_{\text{atom},k}$ vs. distance from the closest ligand atom (top) and $\Delta\Delta G_k$ vs. distance from the closest ligand atom (bottom) for the 359 LCK atoms in the shell of interest.

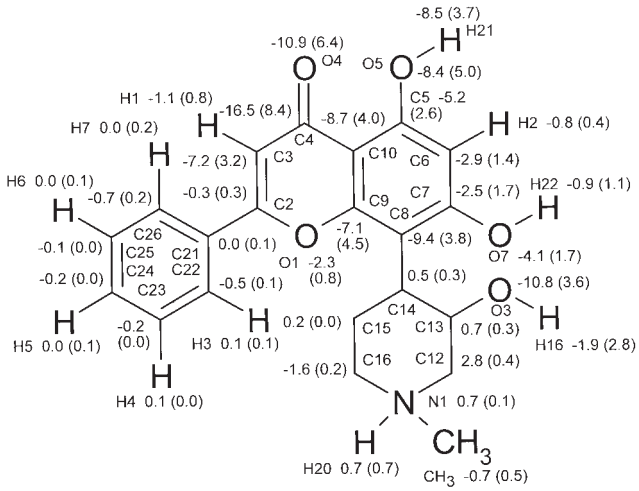

Figure 8. $\Delta\Delta G_{\text{intra},k}$ and $\Delta\Delta G_{\text{atom},k}$ (in parentheses) for atoms and functional groups in deschloroflavopiridol (bound to CDK2) in kcal/mol. In the cases where functional groups are labeled with values, the numbers represent the sum of the $\Delta\Delta G_{\text{intra},k}$ or $\Delta\Delta G_{\text{atom},k}$ values for all atoms in the group.

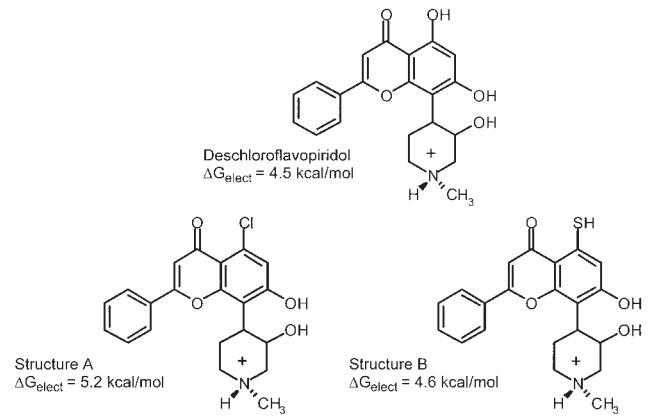

Figure 9. Deschloroflavopiridol along with two hypothetical, modeled structures A and B and their computed ΔG_{elect} values for binding to CDK2.

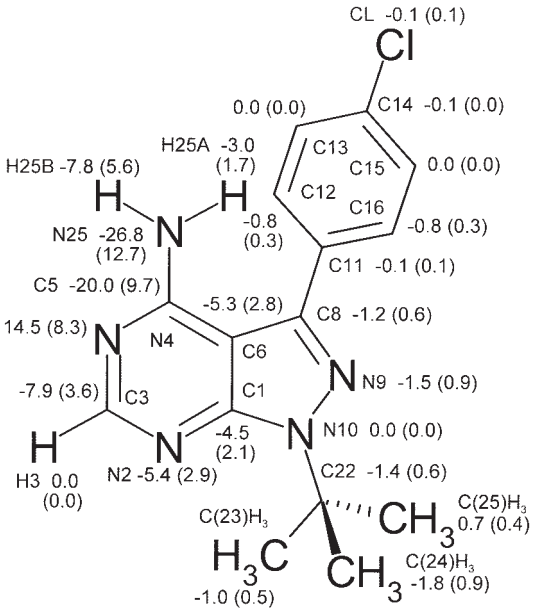

Figure 10. $\Delta\Delta G_{\text{intra},k}$ and $\Delta\Delta G_{\text{atom},k}$ (in parentheses) for atoms and functional groups in PP2 (bound to LCK) in kcal/mol. In the cases where functional groups are labeled with values, the numbers represent the sum of the $\Delta\Delta G_{\text{intra},k}$ or $\Delta\Delta G_{\text{atom},k}$ values for all atoms in the group.

tribution dominates, with a favorable contribution of −92.3 kcal/mol, giving a favorable overall electrostatic contribution to the binding affinity.

The intermolecular term dominates charge utilities on an atom-by-atom basis in the ligand sensitivity analysis. Of the 306 charge utilities computed for PKI, 247 of them are dominated by their intermolecular term (~81%). Only 53 atoms had charge utilities dominated in magnitude by the intramolecular term, and only four are dominated by the atom term. Furthermore, the average magnitude of $\Delta\Delta G_{\text{inter},k}$ for the 306 ligand atoms was 1.5 kcal/mol compared to 0.8 kcal/mol and 0.4 kcal/mol for the average intramolecular and atom desolvation terms. It appears that the electrostatics of at least this large receptor–peptide complex is much different from what we observe in the CDK2–deschloroflavopiridol system and what we will discuss for the LCK–PP2 ligand sensitivity analysis.

LCK–PP2 Ligand Sensitivity Analysis

Figure 10 maps the $\Delta\Delta G_{\text{intra},k}$ values for the LCK–PP2 ligand sensitivity analysis onto each atom of the PP2 structure. This figure highlights the intramolecular contributions of several noteworthy atoms. First, we should note that a crystallographic study of the LCK–PP2 system suggests that this ligand forms three major

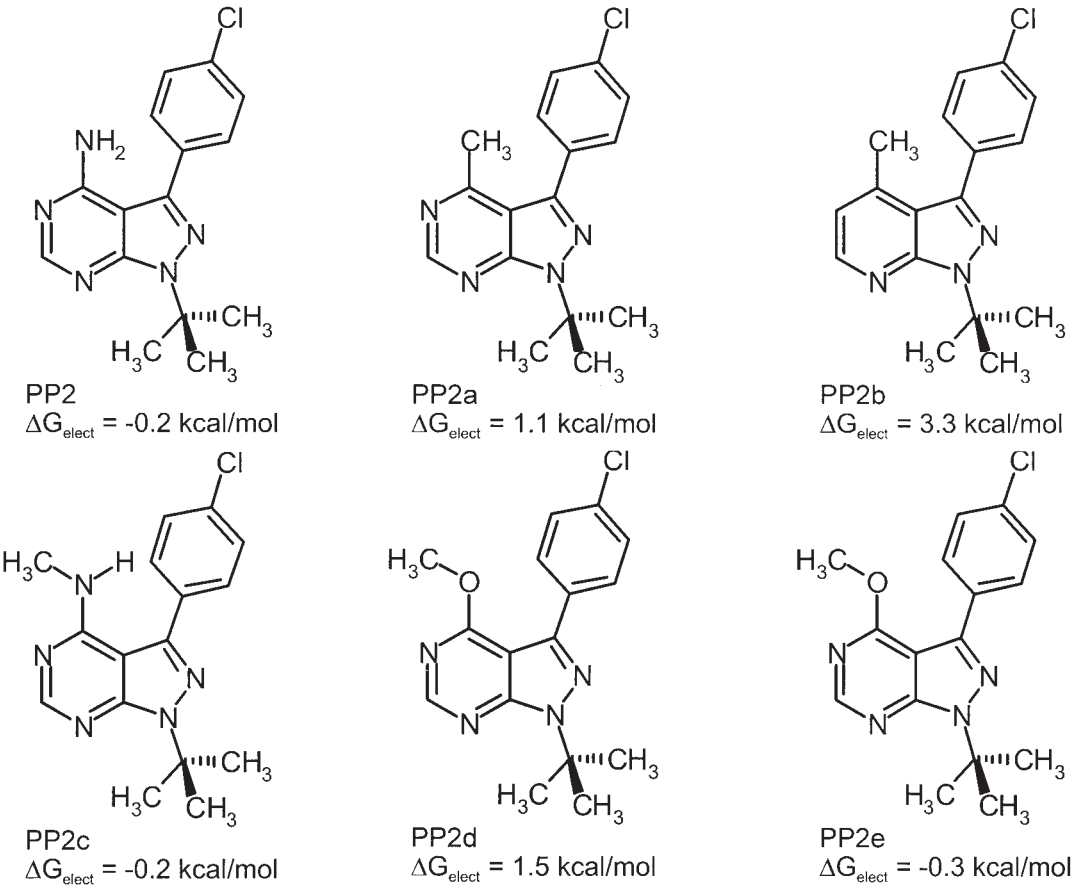

Figure 11. PP2 along with five hypothetical modeled structures and their computed ΔG_{elect} values for binding to LCK.

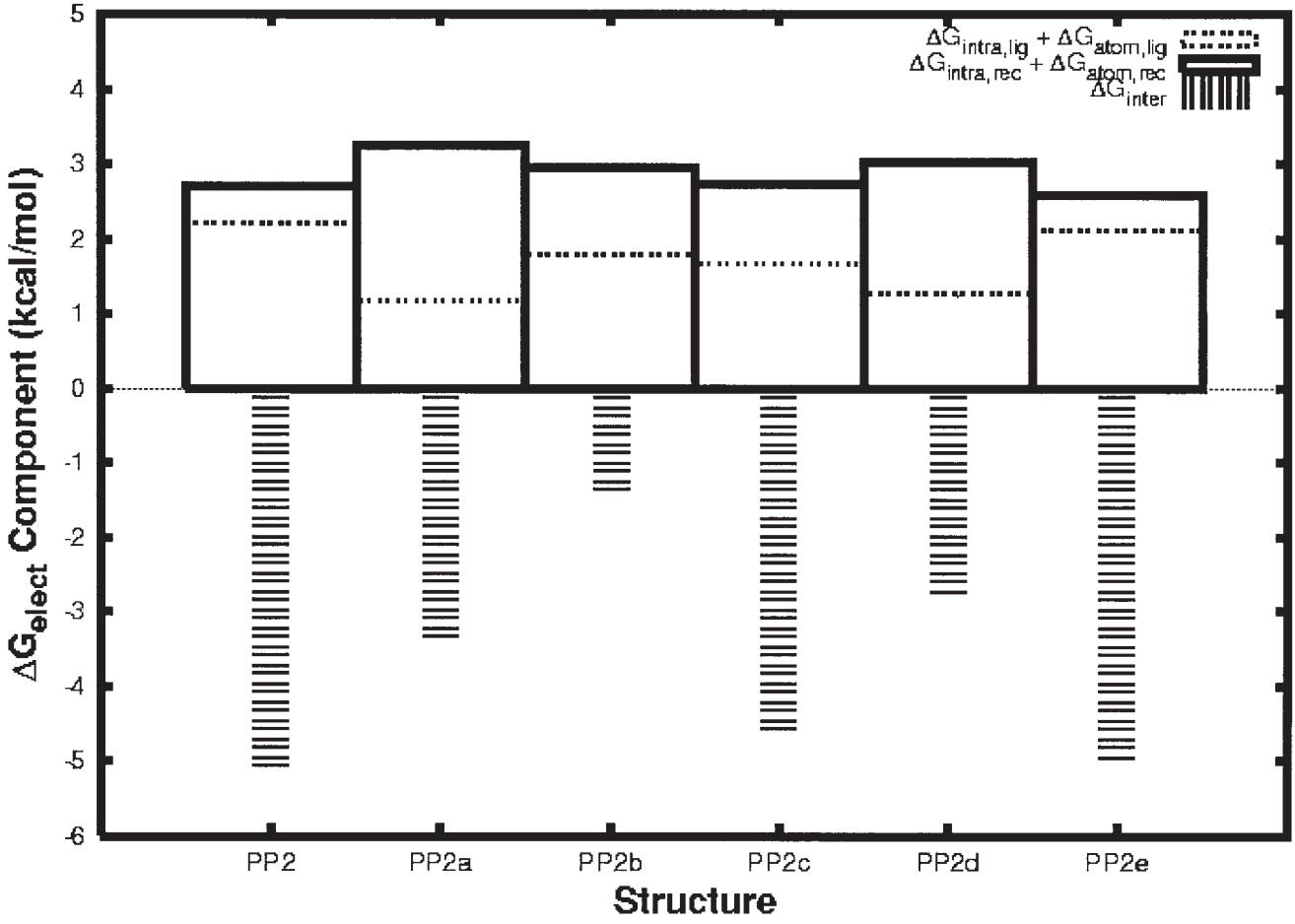

Figure 12. Plot of $\Delta G_{\text{intra,lig}}$ plus $\Delta G_{\text{atom,lig}}$, $G_{\text{intra,rec}}$, and $\Delta G_{\text{atom,rec}}$, and ΔG_{inter} for PP2 and five hypothetical modeled structures for binding to LCK.

hydrogen bonds with the receptor.[15] The first of these is between the amino group on PP2 and the carbonyl oxygen of Glu:317 on LCK.[15] We computed the interaction energies between the three amino ligand atoms (N25, H25A, and H25B) and the carbonyl oxygen of Glu:317 and found the sum of the three energies to be −1.3 kcal/mol. A second hydrogen bond is said to form between the N4 heterocyclic nitrogen of PP2 and the backbone N–H group of Met:319 on LCK.[15] The sum of the interaction energies between the N–H atoms of Met:319 and the N4 heterocyclic nitrogen of PP2 comes to −2.4 kcal/mol in our model. A third hydrogen bonding interaction is said to exist between the PP2 amino group and the hydroxyl group of Thr:316 on LCK.[15] The total interaction energy between the amino group of PP2 and the hydroxyl group on LCK is about 0.5 kcal/mol, not a favorable one. Taking other intermolecular interactions into account gives an overall protein–ligand interaction term, ΔG_{inter}, for the LCK–PP2 system to be −5.15 kcal/mol. The ligand suffers a smaller intramolecular/desolvation penalty (2.2 kcal/mol) than the protein in this case (which we reported previously to be 2.7 kcal/mol). This results from a favorable intramolecular contribution of −52.8 kcal/mol and an atom desolvation penalty of 55.0 kcal/mol. Overall, the favorable intermolecular contributions edge over the intramolecular/desolvation penalty of the protein and the ligand to give a slightly favorable electrostatic contribution to the binding affinity.

The major sources of favorable intramolecular interaction energy changes in the PP2 ligand come from the same atoms that participate in intermolecular hydrogen bonding interactions. The amino nitrogen of PP2 has a $\Delta\Delta G_{\text{intra},k}$ of −26.8 kcal/mol and the two amino hydrogens contribute −7.8 kcal/mol and −3.0 kcal/mol, respectively. The carbon to which the amino group is attached has a $\Delta\Delta G_{\text{intra},k}$ of −20.0 kcal/mol and the adjacent heterocyclic nitrogen has a $\Delta\Delta G_{\text{intra},k}$ of −14.5 kcal/mol.

The PP2 ligand seems to change its intramolecular interaction energy with drastic alterations in a few atoms. Unlike deschloroflavopiridol, the majority of the PP2 charge utilities are dominated by the intermolecular term (26 out of 37 atoms or about 70%) as opposed to the intramolecular term (11 out of 37 atoms or about 30%). However, the average magnitudes of $\Delta\Delta G_{\text{intra},k}$, $\Delta\Delta G_{\text{inter},k}$, and $\Delta\Delta G_{\text{atom},k}$ for PP2 are 2.9, 1.2, and 1.5 kcal/mol, respectively, giving them the same ordering that we computed for deschloroflavopiridol.

We also explored the effects of modifying key groups of the PP2 ligand just as we did for deschloroflavopiridol. Figure 11 depicts five structures, in addition to PP2, that were modeled into the active site of LCK using the PP2 coordinates as a template and refined with energy minimization. Two of these five structures have electrostatic contributions to the binding affinity that are favorable overall. The other three have unfavorable ΔG_{elect}, but they are still less penalizing than the electrostatic contribution in the CDK2–deschloroflavopiridol system.

In hypothetical structure PP2a, we substituted a methyl group in place of the amino group. The electrostatic binding affinity is about 1.3 kcal/mol less favorable than PP2 according to our model. We note that this modification would effectively remove two hydrogen bonds between the ligand and the receptor compared with the original ligand. Although the unfavorable combined contributions from the intramolecular and desolvation terms are 1.2 kcal/mol, better than that of PP2, the corresponding penalty from the receptor increases to 3.3 kcal/mol. Overall, the total electrostatic effects make PP2a a less favorable binder than PP2.

Structure PP2c is particularly interesting because, despite our modifications, the electrostatic contribution to the binding affinity remains the same as in the original ligand. In this hypothetical structure, a methyl group converts the amino group present in PP2 into a methylamine substituent. This change damages at least one of the two hydrogen bonding interactions that form between the amino group and the LCK active site (ΔG_{inter} actually increases by more than 0.5 kcal/mol). Despite the weakening of some favorable intermolecular interactions, the intramolecular and desolvation penalty of the ligand suffers less than that of PP2 by about 0.6 kcal/mol (the receptor contributions are nearly identical for the two systems).

PP2e is a somewhat unusual case because the modifcation involves the removal of a large portion of the original ligand. We simply replaced the bulky, hydrophobic *tert*-butyl group with a single hydrogen and found that the electrostatic contribution to the overall binding affinity remained favorable within this model. Although the modified LCK–PP2e complex suffers some loss in intermolecular interactions, the dramatic change in ligand shape diminishes the unfavorable combined contributions of the intramolecular and atom desolvation terms for both the ligand and the receptor (which we found to be 2.1 and 2.6 kcal/mol, respectively).

Figure 12 is a plot of the computed components of the electrostatic binding affinity for the five hypothetical derivatives of PP2 along with PP2 itself. It demonstrates that there is little variation in the receptor $\Delta G_{\text{intra,rec}} + \Delta G_{\text{atom,rec}}$ contributions compared with the corresponding contributions from the ligand and from the intermolecular interaction terms. The ligand $\Delta G_{\text{intra,lig}} + \Delta G_{\text{atom,lig}}$ contributions also vary significantly less than the intermolecular interaction term. As a result, the intermolecular interactions roughly describe the trend for the binding affinity of these six molecules, although the other two terms need to be included to distinguish the binding affinity of the three molecules (PP2, PP2c, and PP2e) having comparable intermolecular terms. Therefore, in general, it is necessary to look beyond direct and solvent-mediated protein–ligand interactions to understand protein–ligand binding or to design proteins or ligands. Desolvation effects and intramolecular interactions may also play a significant role.

Conclusions

In several previous studies,[8–11] we carried out sensitivity analysis to study the relative importance of different atomic partial charges in determining protein–ligand binding affinity using a continuum solvent model based on solving the Poisson equation. Here, we also analyze the results in terms of three components: desolvation effects, intramolecular interactions (solely solvent-mediated in the fixed-conformation model used here), and intermolecular interactions (both direct and solvent-mediated). We found that intermolecular interactions do not necessarily dominate the contributions to binding affinity. In some cases, the favorable effects of an atomic charge on binding arise from its improved intramolecular interactions. This change in the solvent-mediated intramolecular interactions results from the displacement of solvent molecules from the protein and the ligand surfaces upon binding. Thus, it is necessary to look beyond protein–ligand interactions to understand protein–ligand recognition and to design proteins or inhibitors.

In comparing the results for the three systems (CDK–deschloropiridol, PKA–PKI, and LCK–PP2) studied here, we find that the case of PKA–PKI with a significantly larger ligand is quite different from the cases of CDK–deschloroflavopiridol and LCK–PP2, in which the ligands are much smaller. In the PKA–PKI case, the intermolecular interactions are very strong, and their favorable interactions help to overcome the unfavorable desolvation effects to give a very favorable electrostatic contribution to binding free energy (-27.5 kcal/mol). On the other hand, although the intermolecular interactions in the other two cases also have favorable contributions to binding, they are not sufficient to overcome the desolvation penalty. As a result, the overall electrostatics contribution to binding free energy is either unfavorable (in the CDK2–deschloroflavopiridol system) or negligible (in the LCK–PP2 system).

Acknowledgments

We thank Professor Sung Hou Kim for providing coordinates of the CDK2–deschloroflavopiridol complex. PAS is the recipient of a Barry M. Goldwater Scholarship at UCSD and an NSF predoctoral fellowship at Harvard.

References

1. Bleil, R. E.; Wong, C. F.; Rabitz, H. J Phys Chem 1995, 99, 3379.
2. Wong, C. F.; Rabitz, H. J Phys Chem 1991, 95, 9628.
3. Wong, C. F.; Thacher, T.; Rabitz, H. In Reviews in Computational Chemistry; Lipkowitz, K. B.; Boyd, D. B., Eds.; Wiley-VCH: New York, 1998, p. 281.
4. Zhang, H.; Wong, C. F.; Thacher, T.; Rabitz, H. Proteins 1995, 23, 218.
5. Zhu, S.-B.; Wong, C. F. J Chem Phys 1993, 99, 9047.
6. Zhu, S.-B.; Wong, C. F. J Chem Phys 1993, 98, 8892.
7. Zhu, S.-B.; Wong, C. F. J Phys Chem 1994, 98, 4695.
8. Gould, C.; Wong, C. F. Pharmacol Ther 2002, 93, 169.
9. Sims, P.; Wong, C. F.; McCammon, J. A. J Med Chem 2003, 46, 3314.
10. Toendel, K.; Wong, C. F.; McCammon, J. A. J Theoret Comput Chem 2003, 2, 43.

11. Wong, C. F.; Hünenberger, P. H.; Akamine, P.; Narayana, N.; Diller, T.; McCammon, J. A.; Taylor, S.; Xuong, N. H. J Med Chem 2001, 44, 1530.
12. Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. J Med Chem 2000, 43, 1.
13. Schoepfer, J.; Fretz, H.; Chaudhuri, B.; Muller, L.; Seeber, E.; Meijer, L.; Lozach, O.; Vangrevelinghe, E.; Furet, P. J Med Chem 2002, 45, 1741.
14. Sims, P.; Wong, C.; McCammon, J. J Comput Chem 2004, 25, 1416.
15. Zhu, X. T.; Kim, J. L.; Newcomb, J. R.; Rose, P. E.; Stover, D. R.; Toledo, L. M.; Zhao, H. L.; Morgenstern, K. A. Structure 1999, 7, 651.
16. Kangas, E.; Tidor, B. J Chem Phys 1998, 109, 7522.
17. Lee, L. P.; Tidor, B. J Chem Phys 1997, 106, 8681.
18. Davis, M. E.; Madura, J. D.; Luty, B. A.; McCammon, J. A. Comput Phys Commun 1991, 62, 187.
19. Madura, J. D.; Briggs, J. M.; Wade, R. C.; Davis, M. E.; Luty, B. A.; Ilin, A.; Antosiewicz, J.; Gilson, M. K.; Bagheri, B.; Scott, L. R.; McCammon, J. A. Comput Phys Commun 1995, 91, 57.
20. MacKerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. J Phys Chem B 1998, 102, 3586.
21. Besler, B. H.; Merz, K. M.; Kollman, P. A. J Comput Chem 1990, 11, 431.
22. Singh, U. C.; Kollman, P. A. J Comp Chem 1984, 5, 129.
23. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pornelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, J. B.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, A.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head–Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian Inc.: Pittsburgh, 1998.