Empirical free energy calculations of Human Immunodeficiency Virus Type 1 protease crystallographic complexes. II. Knowledge-based ligand-protein interaction potentials applied to thermodynamic analysis of hydrophobic mutations.

Gennady M. Verkhivker

Agouron Pharmaceuticals, Inc. , 3565 General Atomics Court ,
San Diego, CA 92121-1121 USA

Running title: Empirical free energy analysis of HIV-1 complexes

Key words: *hydrophobic mutations/empirical free energy/ HIV-1 protease complexes/hydrophobicity/ knowledge-based ligand-protein potentials*

Abstract

Empirical free energy calculations of HIV-1 protease crystallographic complexes based on the developed knowledge-based ligand-protein interaction potentials have enabled a detailed thermodynamic analysis. Binding free energies are estimated within an empirical model that postulates that hydrophobic effect, mean field ligand-protein interaction potentials and conformational entropy changes are the dominant forces that determine complex formation. To provide a quantitative framework of the binding thermodynamics contributions the derived knowledge-based potentials have been linked with the hydrophobicity and conformational entropy scales originally developed to explain protein stability. The comparative analysis of studied inhibitors provides reasonable estimates of distinctions in their binding affinity with HIV-1 protease and gives insight into the nature of the binding determinants.

The binding free energy changes upon a simple hydrophobic mutation Ile -> Val in the JG-365, MVT-101 and U75875 inhibitors of HIV-1 protease have been evaluated within a model that includes the effects of solvation, cavity formation, conformational entropy and mean field ligand-protein interactions. In general, free energy changes associated with a particular perturbation of a system can not be rigorously decomposed into separate terms from first principles. We explored the relationships between the changes in hydrophobic contributions and mean field ligand-protein interaction energies in the context of a totally buried and dense area of the binding site. We assume, therefore, that these simple hydrophobic deletions would not induce noticeable conformational changes in the enzyme and can be interpreted with some confidence in the framework of the model. The analysis has revealed the decisive effect of the energetics of ligand-protein interactions on the estimated free energy changes.

The wealth of structural information about HIV-1 protease complexes (Wlodawer and Erickson, 1993 ; Abdel-Meguid, 1993 ; Appelt, 1993) provided the basis for the recent development of a model that evaluates quantitatively the essential steps of the binding process and includes as a critical ingredient knowledge-based ligand-protein interaction potentials (Verkhivker *et al*., 1995). In this work we apply the proposed model to study binding thermodynamics of the acetylpepstatin (Fitzgerald *et al*., 1990 ; Gustchina *et al*., 1991), U75875 (Thanki *et al*., 1992) and SKF107457 (Dreyer *et al.,* 1992 ; Murphy *et al*., 1992) inhibitors with HIV-1 protease (Figure 1).

The inspection of HIV-1 protease-ligand complexes reveals the important structural peculiarities that are determined by thermodynamics of the binding process. A substantial structural change of the protease and the corresponding conformational entropy loss accompany complex formation of all ligands that are bound usually in the extended conformation. The strength of ligand binding to HIV-1 protease is also known to be poorly correlated with the total number of ligand-protein contacts and the number of hydrogen bonds formed in the active site (Appelt, 1993). Thus, binding affinity to HIV-1 protease results not merely from formation of a certain number of hydrogen bonds in the active site and favorable ligand-protein interface contacts, but rather from a delicate energetic balance of a variety of interrelated factors that contribute to thermodynamics of the binding process.

Molecular mechanics analysis (Sansom *et al*., 1992; Gustchina *et al*., 1994) and molecular dynamics simulations of HIV-1 protease complexes (Harte *et al*., 1992 ; Harte *et al*., 1993) necessitate the assignment of protonation states for the active site aspartyl residues. By contrast, we employ mean field ligand-protein interaction potentials that allow us to circumvent this problematic procedure for the studied HIV-1 protease complexes, encompassing all physical forces into one averaged representation.

The described thermodynamic analysis aims to explain the pronounced difference in binding affinity to HIV-1 protease for the acetylpepstatin, U75875 and SKF107457 inhibitors. We examine the contributions of mean field ligand-protein interactions; investigate the energetic factors that determine the balance between unfavorable desolvation of polar groups and their favorable interactions in the active site; and explore the consequences of the enthalpy/entropy compensation effect (Gilli *et al*., 1994) that is believed to be a critical component of molecular recognition process.

Computational model.

Theoretical analysis of thermodynamic aspects of molecular recognition has led to a number of approaches (Searle and Williams, 1991; Williams *et al*., 1991; Horton and Lewis, 1992; Bohm, 1994; Krystek *et al*., 1993) that describe the essential steps of binding process. In general, the decomposition of binding free energy in terms of separate contributions is not allowed by first principles of statistical mechanics (Mark and van Gunsteren, 1994). However, analysis of complex biophysical phenomena such as molecular recognition on the atomic level can be rationalized and advanced on the premises of empirical free energy models that postulate the dominant forces of binding process (see for example, Krystek *et al*., 1993). To study ligand binding with HIV-1 protease we elaborated recently a model of ligand-protein association that contains as a critical component the developed mean field ligand-protein interaction potentials (Verkhivker *et al*., 1995). In this model the total binding free energy consists of the following contributions :

$$\Delta G^{bind} = \Delta G^{ligand-protein}_{interaction} + \Delta G^{ligand-water}_{interaction} + \Delta G^{water-protein}_{interaction} + \Delta G^{nonpolar}_{desolvation} + \Delta G^{polar}_{desolvation} \\ + \Delta G^{ligand}_{conformchange} + \Delta G^{protein}_{conformchange} - T\Delta S^{ligand}_{conform} - T\Delta S^{protein}_{conform} + \Delta G^{ligand-protein}_{rot,transl} + \Delta G^{water}_{rot,transl}$$

It is worth stressing that this model of ligand-protein association assumes for simplicity the contributions to be additive and separable. We analyzed mean field ligand-protein interactions as $\Delta G^{n-n}_{interaction}$ contributions between ligand nonpolar groups and nonpolar groups of the protein and $\Delta G^{n-p,p-p,p-n}_{interaction}$ component that represents the interactions between polar and nonpolar groups of a ligand and the protein. We examined also the desolvation contributions of nonpolar $\Delta G^{nonpolar}_{desolvation}$ and polar groups $\Delta G^{polar}_{desolvation}$, conformational entropy changes $T\Delta S^{protein}_{conform}$ that HIV-1 protease undergoes upon binding and the entropy loss $\Delta G^{ligand-protein}_{rot,transl}$, $\Delta G^{water}_{rot,transl}$ due to a reduction of rotational and translational degrees of freedom of a system composed of ligand, protein and crystallographic water molecules.

In the previous study we derived a set of pairwise ligand-protein interaction potentials from a statistical survey of available HIV-1, HIV-2 and SIV ligand-protein complexes (Verkhivker *et al.,* 1995). The interactions between ligand and protein atoms were defined as contact-specific and individual contact events were counted and used in frequency distributions if the interatomic distance between any non-hydrogen ligand and protein atoms was smaller than sum of their van der Waals radii plus the diameter of a water molecule with the van der Waals radii of the CHARMM_19 version (Brooks et al., 1983). All contacts with crystallographic water molecules, which are located in the active site and satisfy the defined contact event with both ligand and protein hydrophilic atoms, were counted in the frequency distributions. The pairwise potentials at distances greater than the sum of their van der Waals radii and the diameter of a water molecule were not considered as contact-specific. The derived potentials for different ligand-protein atom type pairs represent a mean field energy that corresponds to the probability of observing a given interaction distance in the training set of ligand-protein complexes. For a given ligand atom *a*, protein atom *b* separated by distance *s* the potential is defined by the following expression:

$$\Delta G^{ab}(s) = RTln[1 + m_{ab}\sigma] - RTln[1 + m_{ab}\sigma\frac{f^{ab}(s)}{f(s)}]$$

where m_{ab} is the number of pairs with ligand atom of type *a* and protein atom of type *b*, σ is the weight given to each observation; $f^{ab}(s)$ is the frequency with which this pair of atoms is observed at interatomic distance *s* and $f(s)$ is the total number of atom pairs of all types that are separated by interatomic distance *s*. The probabilities of observing particular distances between pairs of ligand and protein atom types were computed, normalized and then translated into mean force interaction potentials (Verkhivker *et al.*, 1995).

To evaluate the ligand and protein desolvation contributions to binding free energies we used an empirical solvation scale (Eisenberg and and McLachlan *et al*., 1986 ; Nicholls *et al*., 1991 ; Sharp *et al*., 1991) computed with the 1.4 Å probe radius for a water molecule and the atomic solvation parameters (Wesson and Eisenberg, 1992) that were adjusted by Sharp (Sharp *et al*., 1991).

HIV-1 protease undergoes a considerable conformational change upon ligand binding and the resulting hydrophobic effect $\Delta G^{protein}_{conform.change}$ from the burial of the protein competes with a concomitant loss of the protein conformational entropy $T\Delta S^{protein}_{conform}$. These gross effects were evaluated with the reconciled hydrophobicity scale (Pickett and Sternberg, 1993) and conformational entropy scale (Sternberg and Chickos, 1994). If any non-hydrogen atom of the protein residue lies within 4.3 Å distance of any non-hydrogen ligand atom, both the backbone and side chain atoms of this residue were assumed to be locked upon binding and are included in conformational entropy evaluations.

In general, the minimum energy conformation of the HIV-1 protease inhibitors may be different in solution than when they are bound to the enzyme. However, the extended conformation of the ligand backbone, observed in crystal structures for studied HIV-1 inhibitors bound in the active site, dominates also the thermodynamic equilibrium of the unbound ligand in solvent (Verkhivker, unpublished data). Therefore, we assume that the gross conformational energy changes $\Delta G^{ligand}_{conform.change}$ between unbound and bound conformations of the inhibitors are negligible compared to the corresponding protein conformational changes $\Delta G^{protein}_{conform.change}$. We evaluate, however, the loss of ligand conformational entropy $T\Delta S^{ligand}_{conform}$ due to freezing of its side-chain rotatable bonds upon binding. This contribution was approximated given 0.6 kcal/mol binding free energy loss per rotatable bond where all side-chain rotatable bonds of the ligands that lead to altered positions of heavy atoms were considered (Pickett and Sternberg, 1993; Sternberg and Chickos, 1994; Krystek *et al*., 1993).

We found that the value of 11 kcal/mol for the loss of rotational and translational degrees of freedom upon ligand-protein association $\Delta G^{ligand-protein}_{rot,transl}$ and the value of 2.4 kcal/mol for the entropy loss of every crystallographic water molecule, firmly bound to the polar groups in the active site, reproduced more accurately the absolute binding free energies for a number of studied HIV-1 protease complexes (Verkhivker *et al.,* 1995) and agree with the estimated range for these constant factors (Finkeltstein and Janin, 1989 ; Horton and Lewis, 1992 ; Dunitz, 1994).

Comparative analysis of the acetylpepstatin, U75875 and SKF107457 inhibitors.

While the crystal structure of the complex with the U75875 inhibitor reveals only a single conformation of the ligand (Thanki *et al*., 1992), acetylpepstatin (Fitzgerald *et al*., 1990) and SKF107457 (Murphy *et al*., 1992 ; Dreyer *et al.,* 1992) have been observed in two orientations. The alternative conformations of these inhibitors are related to each other by the pseudo-2-fold symmetry of the protease dimer with equal occupancy for the SKF107457 inhibitor. In this study, we used the ligand conformation deposited for the HIV-1 protease complex with SKF107457 (Dreyer *et al.,* 1992) and the acetylpepstatin conformation that dominates thermodynamic equilibrium of the protease complex (Fitzgerald *et al*., 1990).

The comparative analysis of binding free energy components (Table I) shows a delicate but clearly dissimilar balance between the values of desolvation contribution, interaction energy and conformational entropy for studied HIV-1 protease complexes. The distribution of interaction energies for different pairs of ligand-protein atom types (Figure 2) reveals that with the exception of the O-O and N-C contributions the SKF107457 inhibitor interacts more weakly with HIV-1 protease. The interaction energy profile across subsites for the SKF107457 inhibitor points to Phe residue as interacting more strongly with HIV-1 protease at the P1 position (-22.8 kcal/mol) than both acetylpepstatin and U75875 (Figure 3), while weaker interactions at all other subsites apparently attenuate the total interaction gain. However, the desolvation and conformational entropy penalties upon binding for the SKF107457 inhibitor are considerably less severe. This supports the enthalpy-entropy compensation paradigm that the favorable gain in interaction energy exists in a balance with a concomitant entropy loss. The results suggest that the extremely weak interactions of SKF107457 at the P1' position (-5.3 kcal/mol) (Figure 3) may be potentially improved by increasing the size of the hydrophobic residue that would not cause a considerable entropy cost. Indeed, the HIV-1 protease complexes with the ligands that represent modifications of the SKF107457 inhibitor where Gly at the P1' position was systematically replaced by methyl, n-propyl and benzyl groups revealed a gradual enhancement of the binding affinity from 4 nM to 3.0, 1.2 and 0.6 nM respectively (Dreyer *et al.,* 1992). The quantitative picture of interactions in different subsites for acetylpepstatin shows a minimum in mean field interaction energy (increase in binding energy) at the P3 , P3' and P1 positions corresponding to -20.6 kcal/mol, -19.7 kcal/mol and -18.7 kcal/mol respectively (Figure 3). Although the interaction energy for acetylpepstatin is lower than that of SKF107457, the unfavorable desolvation contribution of the polar groups offsets the favorable interaction gain. The interaction energy

profile for the U75875 inhibitor reveals the strongest interaction gain of -19.3 kcal/mol at the P1 position, while at the P1’ position the interaction is clearly weaker due to apparently the steric hindrance between the hydroxyl group at this position and the carbonyl oxygen of Asp 125.
The $\Delta G^{protein}_{conform.change}$ contributions result from the gross effects of HIV-1 protease rearrangements upon binding and the corresponding large changes of solvent-accessible surface area. Although HIV-1 protease complexes manifest a general topological "pattern" of the protein structure, there is a noticeable dissimilarity in the side-chains within this common topological fold (Wlodawer and Erickson, 1993) that is reflected in the observed differences for the protein isomerization energies (Table I). On the basis of structural and thermodynamic data for ligand-protein binding it has been concluded (Spolar and Record, 1994) that local protein folding events and disorder-order transitions could couple to the binding process, leading to a substantial conformational entropy contribution. Our results suggest that for all studied inhibitors binding free energy has a significant entropic component as a result of partial folding transition of HIV-1 protease.
The values of rotational and translational entropy loss due to fixation of crystallographic water molecules in the active site (Table I) are to be considered only in the context of their favorable interactions with both the ligands and the protein. Since a different number of these water molecules satisfy the contact event for studied complexes, we obtain $\Delta G^{water}_{rot,transl}$ of 14.4 and 21.6 kcal/mol for acetylpepstatin and U75875 respectively. However, the total interaction energy of water molecules is -15.4 kcal/mol for acetylpepstatin and -18.2 kcal/mol for U75875. Thus, water molecules in the ligand-protein interface contribute marginally to the net binding energy. Nevertheless, our studies support the notion that hydrogen bonds to the bridged water molecule and to the active-site aspartates are apparently required for high potency and are necessary to guarantee recognition of the inhibitor, facilitating the rest of ligand-protein contacts to come in favorable proximity. It appears that formation of these specific hydrogen bonds is overall energetically favorable, for example for SKF107457, since the desolvation penalty for them does not compromise their interaction energy (Table I).
The performed analysis shows that the evaluated binding free energy differences result from both the effect of specific interactions ($\Delta G^{polar}_{desolvation}$, $\Delta G^{n-p,p-p,p-n}_{interaction}$) and the hydrophobic effect ($\Delta G^{nonpolar}_{desolvation}$, $\Delta G^{n-n}_{interaction}$). The studied HIV-1 protease inhibitors appeared to exploit different strategies to achieve their binding free energy (Table I). The low conformational entropy and desolvation penalties balance the relatively weak interaction strength of the SKF107457 inhibitor. The U75875 inhibitor is a highly potent due to both favorable interactions and moderate desolvation penalty of its polar groups, although at higher conformational entropy cost. By contrast, the low binding affinity of the acetylpepstatin inhibitor results from a large desolvation energy of the polar groups that offsets favorable interactions with the protein.
The proposed binding free energy model considers the important steps of the association process, common in current approaches (Horton and Lewis, 1992 ; Krystek *et al*., 1993 ; Bohm, 1994), while also reflecting the precise energetic strength of interactions, specific for HIV-1 protease complexes. A simple transfer of the derived interaction potentials to a totally different class of proteins needs to be considered with great caution because we may attenuate the accuracy of ligand-protein interactions derived for HIV-1 protease. In fact, the extracted resolution of specific intermolecular interactions in the active site of HIV-1 protease seems to be critical for understanding the relative importance of the binding determinants . Therefore a simple compilation of general enzyme-ligand interactions (Klebe, 1994) may not be sufficient to differentiate between binding affinity of tightly bound HIV-1 protease inhibitors. However, the derived mean field potentials could be transferred to the class of structurally similar proteins such as HIV-2, SIV and FIV proteases (Verkhivker, work in progress).

Thermodynamic analysis of hydrophobic mutations in HIV-1 protease inhibitors.

The quantitative understanding of hydrophobic effects at individual sites of proteins have been greatly improved based on the recent progress in protein engineering, the increasing number of crystal structures of mutants and thermodynamic measurements of protein stability (Matthews, 1991 ; Kellis *et al*., 1988 ; Shortle *et al*., 1990) The hydrophobic effect that is believed to be one of the major driving forces of protein folding process (Dill, 1990) and ligand-protein binding (Miyamoto and Kollman, 1993) implies the release of nonpolar groups from solvent environment and subsequent packing of these groups either in the protein interior or in the active site of a ligand-protein complex. Hydrophobic interactions have been shown to contribute directly to thermal stability of proteins (Matsumura *et al*., 1988) compromising packing interactions and hydrophobic stabilization with the opposing strain energy. The latter depends on the protein mobility at the mutation site (Karpusas *et al*., 1989) and can be relaxed through concerted shifts of both backbone and side chains (Lim *et al*., 1994). The changes in protein stability that occur when a hydrophobic residue is replaced by a smaller one have been rationalized (Eriksson *et al*., 1992) as consisting of two terms. The first term depends only on the amino acid identity and corresponds to the transfer free energy of the residue from solvent to the protein interior. The second term is associated with the extent of the protein response and proportional to the cavity size created by this mutation. It has been observed that there is a good correlation between the changes in protein stability upon the deletion of a hydrophobic group and the number of methyl/methylene groups within a 6 Å radius from the deleted group (Serrano *et al*., 1992). Local packing density at the immediate region of mutation was argued to play an important role in the effects of hydrophobic mutations on protein stability. The observed free energy changes have been rationalized as a combination of packing interactions, reorganization energy and cavity formation where the reorganization term provides the balance between maximum packing energy and minimum torsional strain. A thermodynamic model was proposed recently to explain the observed upper and lower limits of hydrophobic deletion on protein stability (Lee, 1993). It was suggested that the rigidity of the mutation site determines the free energy change while the strength of packing interactions in the protein interior is the same as that in solvent. However, it is generally accepted that the packing interactions in a protein are stronger that in a nonpolar liquid and a solid-like model is more appropriate to describe the protein interior. Computational free energy perturbation studies of mutations of a hydrophobic residue to another residue of a smaller size showed that the loss of favorable packing interactions in the mutation site, rather than unfavorable interactions with water, may be the dominant contribution of the hydrophobic effect (Sneddon and Tobias, 1992). The free energy differences between the folded and unfolded states of wild-type and mutant proteins obtained from protein engineering studies have established an empirical scale of the effect of hydrophobic mutations on protein stability (Serrano *et al*., 1992). In particular, the deletion of completely buried methyl groups in the protein interior may lead to free energy changes of 1.5 kcal/mol with a standard deviation of 0.6 kcal/mol (Serrano *et al*., 1992).

These experiments inspired us to analyze the thermodynamic consequences of hydrophobic mutations in some HIV-1 protease inhibitors on their binding affinity to the protease. In the absence of experimental information it was challenging to predict the effect of a simple hydrophobic mutation Ile->Val in the JG-365, MVT-101 and U75875 inhibitors on their binding affinity to HIV-1 protease. Free energy perturbation studies based on molecular dynamics simulations are highly demanding computationally and entail the protonation state assignment for the HIV-1 protease active site aspartyl residues. We elected instead to utilize an empirical free energy model that decomposes the binding free energy differences and the overall hydrophobic effect on solvation contribution, conformational entropy and mean field ligand-protein interactions (Pickett and Sternberg, 1993 ; Krystek *et al*., 1993 ; Jackson and Sternberg, 1994 ; Morton *et al*., 1995 ; Verkhivker *et al*., 1995). We chose the hydrophobic mutation Ile->Val at the P2' position of the U75875 and JG-365 inhibitors and at the P2 position for the MVT-101 inhibitor. The Ile residue of the HIV-1 protease inhibitors is completely buried in the active site of the protease for all studied complexes and is surrounded by a dense protease environment. We assume, therefore, that the designed hydrophobic deletion would not induce noticeable conformational changes in the enzyme, only removing interactions in the active site without creating new ones (Serrano *et al*., 1992). We evaluated the packing density of the ligand-protein interface in the vicinity of the mutation site by computing the number of protein side-chain methyl/methylene groups in a radius of 6 Å from the group deleted in the wild-type ligand-protein complex (Table II). This density appeared to be similar to the analogous estimates in the protein interior (Serrano *et al*., 1992) and is more reminiscent of a solid environment than a liquid. As a result, the reorganization energy of the protein upon this mutation must be similar and minimal for all studied complexes and would result in an additional "hole" in the active site of the protease. We elucidate the binding free energy changes upon the designed mutation within the following empirical free energy model :

$$\Delta\Delta G^{binding}_{ile \to val} = \Delta G^{binding}_{mutant(val)} - \Delta G^{binding}_{native(ile)} =$$

$$\Delta G^{complex}_{ile \to val} - \Delta G^{uncomplexed}_{ile \to val} = \Delta\Delta G^{interaction}_{ile \to val} + \Delta\Delta G^{cavity}_{ile \to val} - \Delta(T\Delta S)_{ile-val}$$

$$\Delta\Delta G^{interaction}_{ile \to val} = \Delta G^{interaction}_{ile \to val} + \Delta G^{desolvation}_{ile \to val}$$

To evaluate the desolvation free energy contribution $\Delta G^{desolvation}_{ile \to val}$ we determine first the buried solvent-accessible surface area of the wild-type ligand residue as the difference in side-chain solvent accessibility (Lee and Richards, 1971) of this residue in the uncomplexed state and in the complex with the protein. A theoretical mutation is then performed by deleting the hydrophobic group from the Ile residue and the change in the buried solvent-accessible surface area for the mutated residue is also calculated. The desolvation free energy difference $\Delta G^{desolvation}_{ile \to val}$ can be computed then from the loss of the buried solvent-accessible surface area in the complex upon mutation, using solvation scales for alkanes : 12.0 cal/mol Å^2 (Wesson and Eisenberg, 1992) , 8.5 cal/mol Å^2 (Giensen *et al*., 1994) or 6.0 cal/mol Å^2 (Simonson *et al*., 1994). The most significant feature of Ile->Val uncomplexed free energy change is that the desolvation free energy contributions (Table II), obtained with a solvation scaling factor of 12.0 cal/mol Å^2 (Wesson and Eisenberg, 1992), are small and similar for all the inhibitors studied, resulting from the corresponding changes in buried solvent-accessible surface area. There has been a considerable controversy (Ben-Naim and Mazo, 1993 ; Giensen *et al*., 1994 ; Simonson *et al*., 1994) regarding the necessity of a molecular volume correction term (Sharp *et al*., 1991 ; Wesson and Eisenberg, 1992) in solvation free energy calculations. It was reconciled recently that the amino acides transfer from water to vacuum should not involve these correction terms (Chan and Dill , 1994 ; Kumar *et al*., 1995). However, it is clear from our results (Table II) that the appropriate modifications of the solvation scale (Giensen *et al*., 1994 ; Simonson *et al*., 1994) would not affect noticeably the predicted binding free energy changes. The changes in the energy of cavity formation $\Delta\Delta G^{cavity}_{ile \to val}$ were determined from the computed cavity size left in the complexes upon the deletion of a hydrophobic group with the proportional factor of 24.0 cal/mol Å^{-3}, (Eriksson *et al.,* 1992). The values of $\Delta\Delta G^{cavity}_{ile \to val}$ for studied HIV-1 protease inhibitors agree with the estimated loss of van der Waals interactions resulting from cavity formation per methyl or methylene group (Nicholls *et al*., 1991 ; Pickett and Sternberg, 1993). The favorable conformational entropy contribution $\Delta(T\Delta S)_{ile \to val}$ to the binding free energy changes (Table II) was determined from the corresponding loss in side-chain mobility upon binding for the Ile and Val residues (Pickett and Sternberg, 1993).

The analysis of the energy components shows that the packing density for JG-365 and

U75875 is nearly identical, while the interaction loss with a deleted group is quite different for these inhibitors. The energetics of ligand-protein interactions are environment-dependent and vary from 0.82 kcal/mol for U75875 to 1.54 kcal/mol for MVT-101. However, the differences in buried hydrophobic surface area, cavity formation energy and packing density are considerably smaller. Hence, the desolvation and cavity formation components of the hydrophobic effect provide only a slight modulation on the binding free energy differences. By contrast, the resulting hydrophobic effect and the predicted free energy changes are clearly dominated by the loss of favorable ligand-protein interactions and indicate that the interactions of the perturbed group with the protease is stronger than with solvent. In a comprehensive study of the binding energetics for ligands with a different shape, size and polarity in a buried nonpolar cavity of T4 lysozyme (Morton *et al*., 1995 ; Matthews and Morton *et al*., 1995) the differences in binding free energies were also found to be dominated by the interactions between the ligands and the site rather than by reorganization energies and hydrophobicities of the ligands. The magnitudes of the resulting effect of hydrophobic mutation on binding affinity determined in this study remarkably agree with those detected for changes in protein stabilization energies (Serrano *et al*., 1992) upon Ile -> Val mutation in the protein core. The results suggest that the energetics of hydrophobic interactions in the active site of HIV-1 protease may be similar to the strength of packing interactions in the protein core. We probed the effect of the designed mutation within a simple yet physically meaningful thermodynamic model that rapidly provides useful information about the importance of hydrophobic interactions at various subsites of the ligand - HIV-1 protease interface. It should be emphasized that this model may be valid when the effects of protein reorganization are minimal. Nevertheless, this procedure develops empirical free energy relationships that can be used to estimate the strength of hydrophobic interactions in the active site of HIV-1 protease. This information may be further used as guidance for possible ligand modifications in structure-based drug design. Combined with experimental thermodynamic measurements, the results form the basis for the refinement of the empirical binding free energy model and the knowledge-based ligand-protein interaction potentials (Verkhivker *et al*., work in progress), leading to better understanding and a more statistically valid description of the binding process within this important class of proteases.

References

Abdel-Meguid, S.S. (1993) *Medicinal Research Reviews.*, **13**, 731-778.
Appelt, K. (1993) *Perspectives in Drug Discovery and Design*, **1**, 23-48.
Ben-Naim, A. and Mazo, R.M. (1993) *J. Phys. Chem.*, **97**, 10829-10834.
Bohm, H-J. (1994) *J. Comput. Aided Mol. Design*, **8**, 243-256.
Brooks, B.R., Bruccoleri, R.E., Olafson,D., States, D., Swaminathan, S. and Karplus, M. (1983) *J. Comput. Chem.*, **4**, 184-217.
Chan, H.S. and Dill, K.A. (1994) *J. Chem. Phys.*, **101**, 7007-7026.
Dill, K.A. (1990) *Biochemistry*, **29**, 7133-7155.
Dreyer, G.B., Lambert, D.M., Meek T.D., Carr, T.J., Tomaszek, T.A., Fernandez, A.V., Bartuz, H., Cacciavillani, E., Hassel, A.M., Minnich, M., Petteway, S.R. and Metcalf, B.W. (1992) *Biochemistry*, **31**, 6646-6659.
Dunitz, J. (1994) *Science*, **264**, 670.
Eisenberg, D. and McLachlan, A.D. (1986) *Nature*, **319**, 199-203.
Eriksson, A.E., Baase, W.A., Zhang, X.-J., Heinz, D.W., Blaber, M., Baldwin, E.P. and Matthews, B.W. (1992) *Science*, **255**, 178-183.
Finkeltstein, A. and Janin, J. (1989) *Protein Engng.*, **3**, 1-3.
Fitzgerald, P.M.D., McKeever, B.M., VanMiddlesworth, J.E., Springer, J.P., Heimbach, J.C., Leu, C-T., Herber, W.K., Dixon, R.A.F. and Darke, P.L. (1990) *J. Biol. Chem.*, **265**, 14209-14219.
Giensen, D.J., Cramer, C.J. and Truhlar, D.G. (1994) *J. Phys. Chem.*, **98**, 4141-4147.
Gilli, P., Ferretti, V., Gilli, G. and Bore, P.A. (1994) *J. Phys. Chem.*, **98**, 1515-1518.
Gustchina A., and Weber, I.T. (1991) *Proteins : Struct., Funct., Genet.,* **10**, 325-339.
Gustchina, A., Sansom, C., Prevost, M., Richelle, J., Wodak, S.Y. and Weber, I.T. (1994) *Protein Engng.*, **7**, 309-317.
Harte, W.E., Swaminathan, S. and Beveridge, D.L. (1992) *Proteins : Struct., Funct.,Genet.*, **13**, 175-194.
Harte, W.E. and Beveridge, D.L. (1993) *J. Am. Chem. Soc.*, **115**, 3883-3886.
Horton, N. and Lewis, M. (1992) *Protein Sci.*, **1**, 169-181.
Jackson, R.M. and Sternberg, M.J.E. (1994) *Protein Engng.*, **7**, 371-383.
Karpusas, M., Baase, W.A., Matsumura, M. and Matthews, B.W. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 8327-8241.
Kellis, J., Nyberg, K., Sali, D. and Fersht, A.R. (1988) *Nature*, **333**, 784-786.
Klebe, G. (1994) *J. Mol. Biol.*, **237**, 212-235.
Krystek, S., Stouch, T. and Novotny, J. (1993) *J. Mol. Biol.*, **234**, 661-679.
Kumar. S.K., Szleifer, I., Sharp, K. Rossky, P.J., Friedman, R. and Honig, B. (1995) *J. Phys. Chem.*, **99**, 8382-8391.
Lee, B., (1993) *Protein Science*, **2**, 733-738.
Lee, B. and Richards, F.M. *J. Mol. Biol.*, **5**, 379-400.
Lim, W.A., Hodel, A., Sauer, R. and Richards, F.M. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 423-427.
Mark, A.E. and W. van Gunsteren (1994) *J. Mol. Biol.*, **240**, 167-176.
Matthews, B.W. (1991) *Curr. Opin. Struc. Biol.*, **1**, 17-19.
Matsumura, M., Becktel, W.J. and Mattwews, B.W. (1988) *Nature*, **334**, 406-410.
Morton, A., Baase, W.A. and Matthews, B.W. (1995) *Biochemistry*, **34**, 8564-8575.
Morton, A. and Matthews, B.W. (1995) *Biochemistry*, **34**, 8576-8588.
Miyamoto, S. and Kollman, P. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 8402-8406.
Murphy, K.H.M., Winborne E.L., Minnich,M., Culp, J. and Debouck, C. (1992) *J. Biol. Chem.*, **32**, 22770-22778.
Nicholls, A., Sharp, K.A. and Honig, B. (1991) *Proteins : Struct., Funct., Genet.,* **11**, 281-296.
Pickett, S.,D. and Sternberg, M.J.E. (1993) *J. Mol. Biol.*, **231**, 825-839.
Sansom,C.E., Wu,J., and Weber,I.T., (1992) *Protein Engng..*, **5**, 659-667.

Searle, M.S. and Williams, D.H. (1991) *J. Am. Chem. Soc.*, **114**. 10690-10697.
Serrano, L., Kellis,J., Cann, P., Matouschek, A. and Fersht, A.R. (1992) *J. Mol. Biol*., **224**, 783-804.
Sharp, K.A., Nicholls, A., Friedman, R. and Honig B. (1991) *Biochemistry*, **30**, 9686-9697.
Shortle, D., Stites, W. and Meeker, A. (1990) *Biochemistry*, **29**, 8033-8041.
Simonson, T. and Brunger, A.T. (1994) *J. Phys. Chem.*, **98**, 4683-4694.
Sneddon, S.F. and Tobias, D.J. (1992) *Biochemistry*, **31**, 2842-2846.
Spolar, R.S. and Record M.T., Jr. (1994) *Science*, **263**, 777-784.
Sternberg, M.J.E., Chickos, J. (1994) *Protein Engng*., **7**, 149-155.
Thanki, N., Rao, J.K.M., Foundling, S.I., Howe, W.J., Moon, J.B., Hui, J.O., Tomasseli, A.G., Heinrickson, R.L., Thaisrivongs, S. and Wlodawer, A. (1992) *Protein Sci.,* **1**, 1061-1072.
Verkhivker, G., Appelt, K., Freer, S.T. and Villafranca, J.E. (1995) *Protein Engng*., **8**, 677-691.
Wesson, L. and Eisenberg, D. (1992) *Protein Sci.*, **1**, 227-235.
Williams, D.H., Cox, J.P.L., Doig, A.J., Gardner. M., Gerhard, U., Kaye, P.T., Lal, A.R., Nicholls, I.A., Salter, C.J. and Mitchell, R.C. (1991) *J. Am. Chem. Soc.*, **113**, 7020-7030.
Willmans, M., and Eisenberg, D. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 1379-1383.
Wlodawer, A. and Erickson, J.W. (1993) *Annu. Rev. Biochem.*, **62**, 543-585.