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Thermodynamics of Fragment Binding

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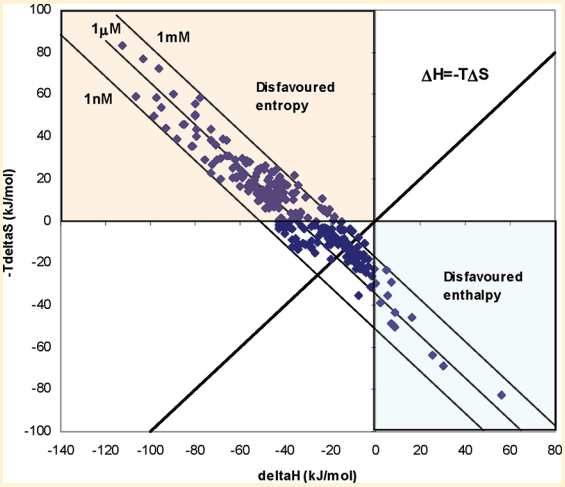
Supporting Information

ABSTRACT: The ligand binding pockets of proteins have preponderance of hydrophobic amino acids and are typically within the apolar interior of the protein; nevertheless, they are able to bind low complexity, polar, water-soluble fragments. In order to understand this phenomenon, we analyzed high resolution X-ray data of protein−ligand complexes from the Protein Data Bank and found that fragments bind to proteins with two near optimal geometry H-bonds on average. The linear extent of the fragment binding site was found not to be larger than 10 Å, and the H-bonding region was found to be restricted to about 5 Å on average. The number of conserved H-bonds in proteins cocrystallized with multiple different fragments is also near to 2. These fragment binding sites that are able to form limited number of strong H-bonds in a hydrophobic environment are identified as hot spots. An estimate of the free-energy gain of H-bond formation versus apolar desolvation supports that fragment sized compounds need H-bonds to achieve detectable binding. This suggests that fragment binding is mostly enthalpic that is in line with their observed binding thermodynamics documented in Isothermal Titration Calorimetry (ITC) data sets and gives a thermodynamic rationale for fragment based approaches. The binding of larger compounds tends to more rely on apolar desolvation with a corresponding increase of the entropy content of their binding free-energy. These findings explain the reported size-dependence of maximal available affinity and ligand efficiency both behaving differently in the small molecule region featured by strong H-bond formation and in the larger molecule region featured by apolar desolvation.



INTRODUCTION

Ligand−protein binding is most widely described by the affinity of the ligand but the value of its more thorough kinetic and thermodynamic characterization has been increasingly recognized. The thermodynamic measure of the affinity is the binding free energy that can be decomposed into enthalpy and entropy components. Relative contribution of these components provides information on the details of the binding event, the type of the interactions, and the quality of the fit between the interacting partners. Interactions of properly oriented polar groups result in favorable change in binding enthalpy. A good fit between the interacting polar groups of the ligand and the protein is essential in order to compensate for breaking interactions of both partners with water upon binding. Moreover, the advantageous binding enthalpy change is typically accompanied with disadvantageous entropy change arising from the decreased configurational entropy of the system (entropy−enthalpy compensation). Advantageous entropy change can be achieved primarily with a fit of the apolar regions of the interacting partners and the geometrical requirements of such an apolar fit is less strict. For this reason, affinity increase can be more easily achieved by adding apolar groups to the ligand and this is the reason why drug discovery programs often result in potent, but large, hydrophobic ligands with entropy driven binding and limited selectivity.[1−3]

These relations between the properties of the interacting partners and the components of the binding free energy might provide guidance to the design of discovery compounds in pharmaceutical research. Preferred compounds bind to their target protein with advantageous binding free energy dominated by enthalpy gain. The prerequisite to the enthalpy gain is the presence of polar groups, and this conveys certain physicochemical properties to these compounds. This finding is in line with well-recognized trends that compound size and polarity of oral drugs are within certain limits as formulated in the Lipinski rules.[4] Since fragments[5] and leadlike compounds[6] could be more easily optimized to Lipinski compliant candidates, the size and polarity of fragments make them ideal starting points for many drug discovery programs.

Fragments bind to various sites of proteins, but there are distinguishable ones that are able to bind many different small molecules including fragments and solvents.[7−10] These experimentally observed sites with high propensity for small

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ligands with various properties can also be identified by computational methods.[10,11] The weakly specific and highly efficient (high ligand efficiency) binding of small molecules to proteins is experimentally well-established, but its origin is incompletely understood.

Our analysis will therefore focus on fragment type compounds having not more than 22 heavy atoms and thus a maximal molecular weight around 300 Da. The investigation of fragments will allow us to recognize important properties of fragment−protein binding and then to explain binding properties of larger compounds.

CHARACTERIZATION OF FRAGMENT BINDING HOT SPOTS

Protein−protein interactions occur at large surfaces, nevertheless, a few adjacent residues often make significant contribution to the binding free energy.[12] These sites are known as hot spots[13] consisting mostly polar and conserved residues that is in line with their distinguished role in binding. Hot spots are surrounded by apolar amino acids providing a hydrophobic environment that enhances polar interactions.[12]

Contrary to protein−protein interactions, small molecule binding occurs in pockets having preference for hydrophobic amino acids[14] and are buried in the dominantly apolar protein interior. Polar molecules, however, are able to bind to these basically apolar sites by extremely strong H-bonds[15−17] that prompted us to investigate these interactions in depth in fragment-protein complexes. Consequently, we analyzed complexes with fragment sized ligands from the Protein Data Bank,[18] the richest deposit of experimental structural data of protein−ligand complexes. Although strictly speaking neither free-energy nor enthalpy can be decomposed and assigned to specific interactions, it is well-established, that optimal H-bonds give advantageous contributions to these thermodynamic quantities. On the other hand, suboptimal H-bonds tend to disfavor binding owing to the loss of H-bonds with water and to entropic penalty accompanied by binding. In order to take into account favorable H-bonds we investigated high-resolution complexes in which H-bonded heavy-atoms have optimal geometrical arrangements. All complexes containing ligands with not more than 40 heavy atoms and with $R \leq 1.8$ Å were downloaded from the Protein Data Bank.[18] H-atoms were added with the Reduce program.[19] Protein pockets were identified,[20] and ligands bound outside of the pockets were removed. Finally, 1297 complexes were investigated out of which 666 have fragment sized ligands with not more that 22 heavy atoms. H-bonds were identified where the donor−acceptor distances (N or O-atoms) did not exceed 3 Å and the D−H···A angle was not below 160° (Supporting Information).

The average number of H-bonds in these protein−ligand complexes is presented in Figure 1 as a function of the number of ligand heavy atoms. The curve rises fast at low heavy atom numbers. It is near to 2 above 10 heavy atoms but then it changes slowly and is still around 2 at the fragment limit (22 heavy atoms, indicated by a vertical line in Figure 1). The average number of H-bonds is somewhat higher than 3 at 40 heavy-atoms that corresponds to the size-limit of druglike compounds.

The spatial extents of the ligands (ligand size) and the spatial extent of the part participating in H-bonds (H-bonding size) were analyzed in the same set of PDB complexes. This was done with a simple model in which the largest distance between ligand heavy atoms was considered as ligand size and the largest

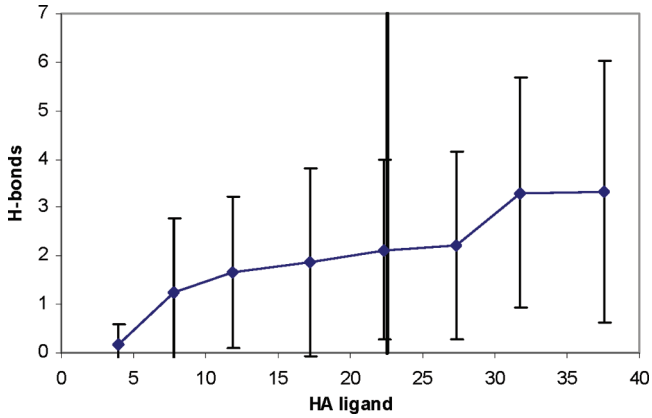

Figure 1. Number of H-bonds in protein−ligand complexes as a function of the number of ligand heavy atoms. Vertical bars represent standard deviations. The vertical line indicates the fragment limit.

distance of heavy atoms participating in an intermolecular H-bond was considered as H-bonding size. Figure 2 shows average ligand sizes and H-bonding sizes as functions of the heavy atom count. This analysis revealed that H-bonds do not extend to the whole ligands. Their size is restricted to about 5 Å on average within the fragment size limit (vertical line in Figure 2), and its average is around 10 Å at 40 heavy atoms.

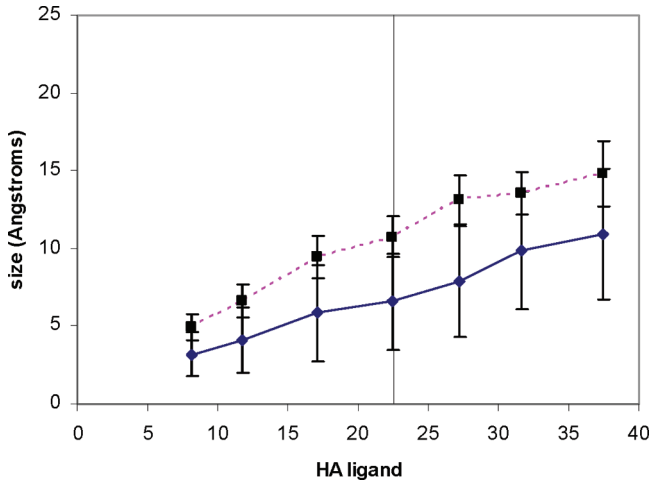

Figure 2. H-bonding size (straight line) and ligand size (dotted line) in protein−ligand complexes as a function of the number of ligand heavy atoms. Vertical bars represent standard deviations. Vertical line indicates fragment limit.

These results suggest that fragments bind to proteins with typically 2 hydrogen bonds or less and that the extent of this binding region is around 5 Å. Fundamental properties of these sites are their ability to create binding in a small region by forming specific interactions, mainly H-bonds and thus representing an ideal site for fragment binding. These sites are often identified as ligand binding hot spots.

Hot spots can be well-characterized by investigating complexes formed by various ligands with the same protein. Orita and co-workers[21] collected 25 examples of fragment optimizations where the structure of the fragment complex and that of a larger compound optimized from the fragment are both available. They concluded that fragments and their extended counterparts exhibit the same binding mode and

Table 1. Averages of Conserved and Nonconserved Hydrogen Bonds in Complexes of Fragments and in Complexes of Optimized Compounds Obtained from the Fragments[a]

fragments				optimized			
HA[b]	H-bonds conserved	H-bonds nonconserved	H-bonds total	HA[b]	H-bonds conserved	H-bonds nonconserved	H-bonds total
14.3	2.28	0.16	2.44	28.3	2.24	1.36	3.60

[a]Based on data in ref 21. [b]Number of heavy atoms.

Table 2. H-Bonds in 813 Fragment Complexes of 63 Proteins: (a) Averaged for Proteins; (b) Averaged for Fragments

(a) Complexes of 63 Proteins							
H-bonds conserved		H-bonds nonconserved		H-bonds total		number of complexes of a protein	
2.97		10.08		13.05		13	
(b) Complexes of 813 Fragments							
H-bonds conserved	H-bonds nonconserved	H-bonds total	HA[a]	HT[b]	clogP[c]	tPSA[d]	distance[e]
2.45	2.86	5.30	15.2	5.5	0.62	86	1.0

[a]Number of heavy atoms. [b]Number of heteroatoms. [c]Logarithm of calculated octanol/water partition coefficient[39] [d]Topological polar surface area.[40] [e]Distance between ligand centers bound to the same protein (Å).

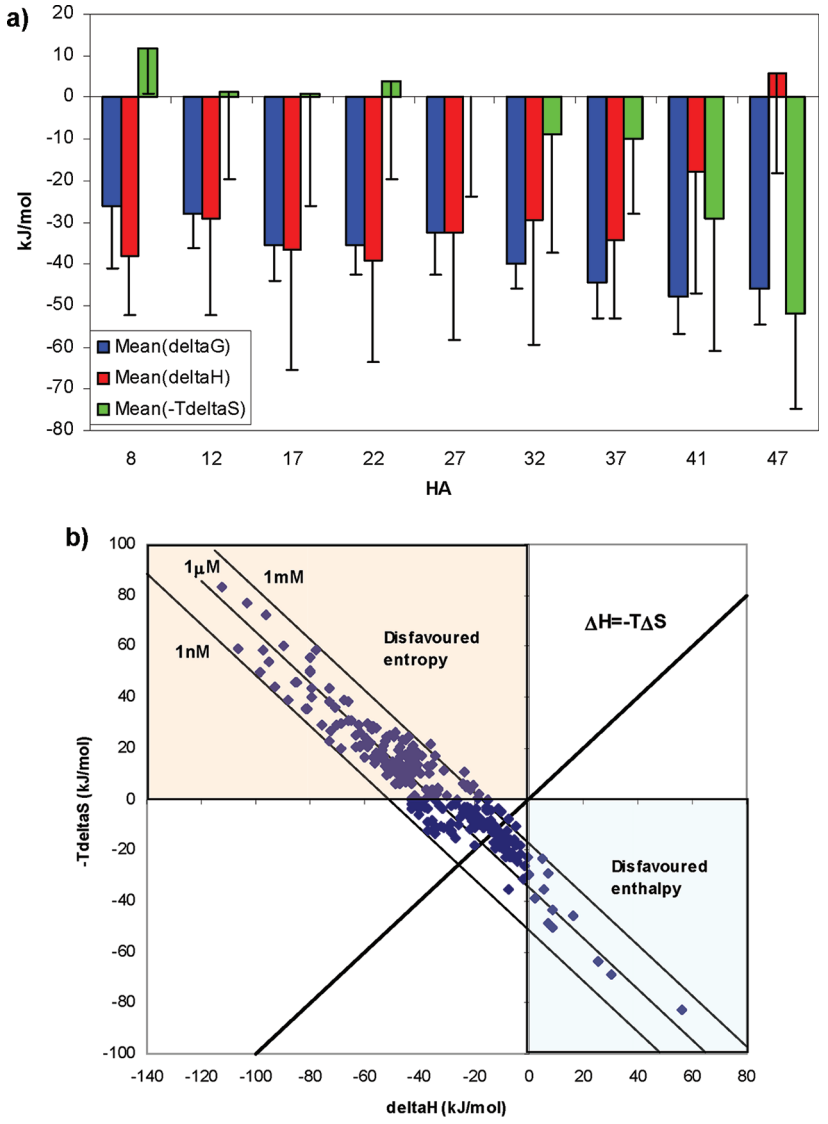

Figure 3. (a) Average free-energy (blue) enthalpy (red) and entropy (green) of ligand binding versus number of heavy atoms (HA). (b) Enthalpic and entropic components of binding for 284 fragment−protein complexes.

this is reflected by the number of conserved H-bonds. The average number of H-bonds for fragments in Table 1 is near 2, and it agrees well with the number of H-bonds found in the analysis of the large set of PDB complexes (Figure 1). In the

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Orita data set, the majority of the H-bonds are conserved thus showing the privileged role of the hot spot and its hydrogen bonds in the binding of fragments and also of larger optimized compounds.

These observations were further validated by analyzing proteins complexed with at least six different fragments (813 PDB complexes of 63 proteins, see the Supporting Information for details). We first identified the H-bonds and then classified them to be conserved if they appear at least in 80% of the complexes formed by the various ligands with the same protein. In contrast to the analysis of the PDB presented in Figure 1, and in line with ref 21, complexes were not restricted to high-resolution structures and H-bonds were identified when appropriate heteroatom distances are below 3.3 Å. The use of lower resolution structures is consistent with a less stringent definition of H-bonding geometry. However, the assumption of a favorable contribution to binding free-energy based on optimal H-bonding geometry is no longer possible. On the other hand, the conservation in various complexes is adopted as an indication of favorable H-bonds. The averaged number of conserved H-bonds in a protein is near 3 (Table 2a) and their average for a ligand is near 2.5 (Table 2b). The somewhat higher numbers with respect to those in Table 1 are the consequences of the polar nature of the fragments as it is indicated by their descriptors (clogP, number of heteroatoms, and tPSA). The average distance between the centers of the fragments bound to the same protein is 1.0 Å (Table 2b). This clearly shows that the majority of the compounds bind to the same site that gives further support to the observation that fragments bind to the hot spot located in the inner cavity of the proteins.

Summarizing our analyses of fragment complexes, we conclude that proteins bind fragment sized compounds at a small site that is capable to form energetically favorable H-bonds. The number of H-bonds is around 2, and the dimension of this H-bond forming site is around 5 Å on average. These small volume sites contribute significantly to binding free energy and consequently can be identified as the hot spot of the proteins. These hot spots could therefore provide sufficient free-energy to bind appropriate fragments.

BINDING TO HOT SPOTS IS ENTHALPY DOMINATED

Fragments typically bind to well-defined sites of proteins that were identified as hot spots and whose size and H-bonding ability seems to be limited. Here, we analyze the binding thermodynamics of fragments in order to further characterize hot spots.

An analysis of the binding enthalpy and entropy of 756 compounds from ref 30 shows that the binding of fragment sized compounds is typically the result of enthalpy gain (Figure 3a). This data set contains 284 fragment-like complexes (at most 22 ligand heavy atoms) whose binding enthalpy and entropy are shown in Figure 3b. Although entries of this set fulfill the size criteria of fragment based drug discovery the binding affinity of these molecules is typically in the low micromolar range (average pK_d = 5.53). Consequently, their biological testing does not require extremely high water solubility. In contrast to fragment libraries routinely used in discovery settings, this set is therefore not expected to be biased toward polar compounds. 83% of these fragments show enthalpy dominated binding ($\Delta H < -T\Delta S$), and all but 12 bind with favorable enthalpy ($\Delta H < 0$). Similarly, thermodynamic data of a set of 624 Astex fragments revealed that 90% exhibit enthalpy dominated binding and all but three fragments bind with favorable enthalpy.[22] These data clearly show that fragment binding is overwhelmingly enthalpically favored and most often entropically unfavored. The few exceptions in the publicly available data set from ref 30 include negatively charged ions (pyrophosphates, bisphosphonates, and benzoates) interacting with metal cations. The formation of these charge−charge interactions is accompanied by large enthalpical desolvation penalty that results in an overall unfavorable enthalpy change. This finding is in accord with the reported observation that salt-bridges in proteins do not give important contribution to protein stability.[23,24]

The dominance of enthalpy in the binding of fragments to hot spots is in line with the finding that they are able to create favorable H-bonds. Although hot spots are typically located in apolar cavities we argue below that they need to form H-bonds and cannot exclusively rely on hydrophobic interactions.

The contribution of hydrophobic interactions to the binding free energy is commonly estimated with the burial of apolar surface area upon complex formation. In order to estimate the maximum binding free energy from apolar desolvation the surface area and the apolar surface area of 571 distinct ligands from the 1297 high resolution complexes extracted from PDB (see above for details) were calculated.[25] The straight line in Figure 4 shows the maximal surface area as the function of the number of heavy atoms (HA) and indicates that the maximal available surface is around 20 A^2/HA. Taking into account that

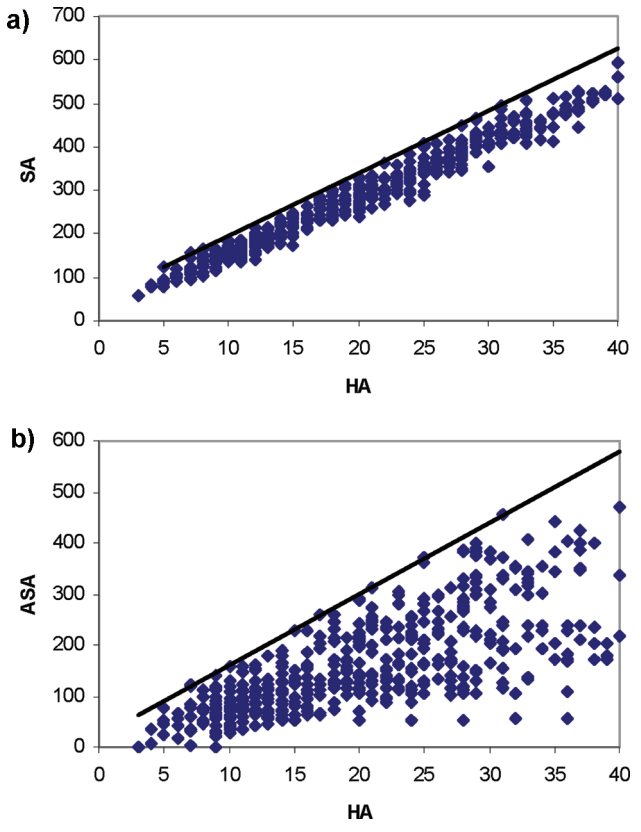

Figure 4. (a) Surface area (SA) versus number of heavy atoms (HA) for the 571 distinct ligands of 1297 complexes from PDB (see text for the selection of complexes). (b) Apolar surface area (ASA) versus number of heavy atoms (HA) for 571 distinct ligands of 1297 complexes from PDB (see text for the selection of complexes).

the estimated free energy gain of 20 A^2 corresponds to 1 kJ/mol,[26] we see that no more than 1 kJ/mol per heavy atom can be gained by desolvation. As rigid-body entropy loss upon binding is estimated to be 15−20 kJ/mol,[27] it is clear that apolar desolvation alone is unable to ensure the binding of fragments. The role of the hot spot is to provide specific polar interactions and thus to ensure higher affinity binding. As stabilization by H-bonds is enthalpy driven, the dominant contribution to the binding-free energy of fragments is enthalpy as it is observed for the majority of the fragments.

Fragment screening experiments on multiple targets have demonstrated that fragments exhibit lower selectivity than do large compounds.[28] It was also observed that more hydrophobic fragments tend to be less selective.[29] As polar compounds form H-bonds that are associated with enthalpy, it is expected that improved selectivity goes together with an increased favorable enthalpic content of the binding free-energy.

While fragment binding to hot spots is typically driven by favorable enthalpy contributions, this does not hold for larger molecules.[30] The free-energy of ligand binding and especially its components show significant dependence on ligand size (Figure 3a). We found that binding enthalpy—the driving force of small molecule binding—does not increase with ligand size while entropy, which does not contribute favorably to binding of small ligands, becomes increasingly important for larger compounds. We showed above that the dominantly entropic apolar dehydration can contribute to binding by about 1 kJ/mol per heavy atom. The binding of fragments, however, typically requires H-bonds that are dominantly enthalpic and can give a favorable contribution of over 5 kJ/mol.[31] Larger ligands, on the other hand, favor entropic binding and this suggests that apolar desolvation has a more significant contribution to binding than do polar interactions including H-bonds. A reason why larger ligands do not achieve high binding free-energy by increasing their polar interactions was proposed by Hann et al.[32] suggesting that an increasing number of polar interaction points has a reduced probability for a good matching. This is supported by the plot of available enthalpy vs ligand heavy atoms for compounds from ref 30. Figure 5 shows no increase of available favorable binding enthalpy with increasing ligand size. Another possible reason for disfavoring an increased number of H-bonds is the increasing entropic

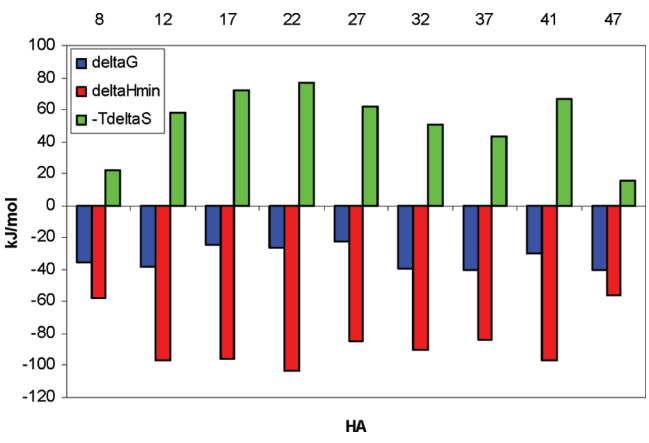

Figure 5. Free-energy (blue) enthalpy (red) and entropy (green) of minimum enthalpy ligand binding versus number of heavy atoms (HA).

penalty that arises from the reduced mobility of the partners interacting with several H-bonds. Indeed, the average number of H-bonds only slowly increases with ligand size as shown in Figure 1.

These considerations also imply that the enthalpy driven binding of a ligand to its target protein tends to decrease the chance of high affinity binding of this ligand to other proteins owing to their unlikely structural fit. For this reason, enthalpy driven binding is associated with selectivity. On the other hand, entropy gain is primarily realized by shape fitting of apolar groups that expel water molecules from their solvation spheres. This requires less stringent geometrical fit, and thus, entropy driven binding is associated with promiscuity. This relation between entropy dominated binding and promiscuity is in agreement with the observation that compounds with high logP tend to be promiscuous,[33,34] and as high logP comes together with large apolar surface area (ASA), these compounds bind with significant entropy component as it is shown by the correlation between ASA and binding entropy.[26]

The conservation of H-bonds in multiple complexes of the same protein demonstrated above indicates that the binding mode to the hot spot is conserved. Larger ligands, however, bind also beyond the hot spot and the interactions realized outside the hot spot are expected to contribute to the observed relation between the enthalpy content of the binding and selectivity.[1−3]

■ LIGAND EFFICIENCY CULMINATES AT HOT SPOTS

The switch from enthalpy dominated binding of small ligands to entropy dominated binding of large ligands can be rationalized by recognizing the limited number of H-bonds created by relatively few atoms compared to desolvation with the participation of several atoms. This view is supported by the relation between ligand size and binding free-energy (affinity). As it was first pointed out by Kuntz et al.[35] and is shown for the set of complexes of ref 30 in Figure 6, the maximum available

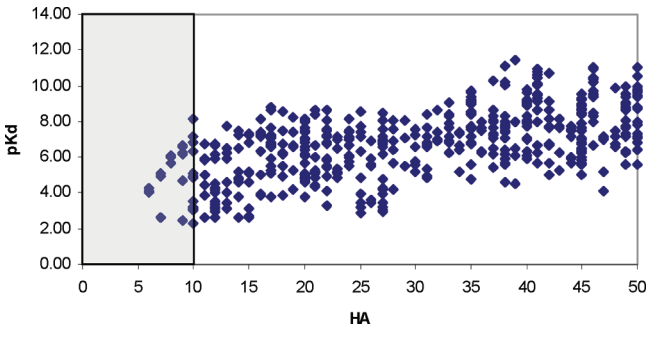

Figure 6. Ligand affinity (pK_d) versus number of heavy atoms (HA). The H-bond forming region with steep increase of pK_d is shaded. (see text for discussion).

binding affinity expressed as pK_d, the negative logarithm of the dissociation constant of the protein−ligand complex, increases sharply with increasing ligand size for small molecules and the increase attenuates for larger size molecules. The increase is the steepest under 10 heavy atoms (H-bond dominating region, shaded in Figure 6) and is almost plateaued for large compounds (apolar desolvation dominating region).

The rationalization of the affinity change with heavy atom number explains also the size-dependency of ligand efficiency (LE) as documented by Reynolds and Nissink.[36,37] Here, we

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define LE as affinity per number of heavy atoms (pK_d/HA). Alternative definitions with similar meaning also exist,[35,36,38] and they all share the feature of decreasing LE with increasing heavy atom number. We find the same overall character of the LE function; however, there is a peak apparent at low heavy atom numbers in Figure 7. Although this may be a consequence

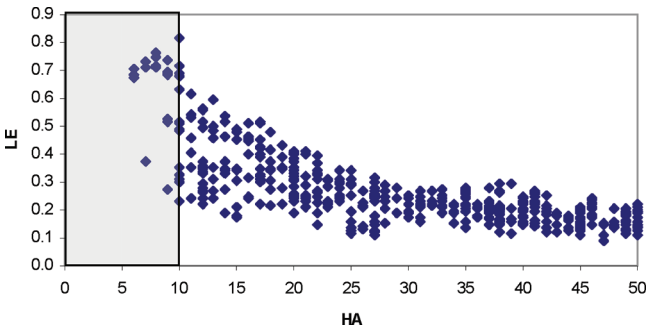

Figure 7. Ligand efficiency (LE) versus number of heavy atoms (HA). LE is defined as pK_d per number of ligand heavy atoms. The H-bond forming region with increasing LE is shaded. (see text for discussion).

of an insufficient sampling of complexes with small ligands this also reflects the sharp increase in the binding affinity of fragments with introducing heteroatoms forming few strong H-bonds.

The best ligand efficiency can be achieved with small compounds binding to the hot spot as the latter is able to form strong interactions in a small region of the protein. Thus, a characteristic feature of hot spots is that they make a binding with high ligand efficiency possible.

CONCLUSION

The binding of ligands to proteins occurs most often to dominantly hydrophobic binding pockets that are embedded in the apolar interior of the proteins. Starting from the observation that polar fragments are able to bind to these pockets, we found that fragments have a strong tendency to form a limited number of H-bonds with the binding pocket. On average, the number of H-bonds does not exceed 2 at the fragment size limit (22 heavy atoms). This conclusion was reached first with the analyses of high resolution complex structures with near to optimal H-bonding geometry (short donor−acceptor separation and near to linear D−H···A angle) and second, with the identification of conserved H-bonds in complexes formed by several ligands with the same protein. Both optimal geometry and conserved H-bonds are likely to favorably contribute to protein−ligand binding. These fragment binding sites are identified as hot spots. Their characteristic feature is their ability to form few H-bonds whose strength is significantly enhanced by hydrophobic embedding and this makes hot spots suitable to bind polar fragments. The linear extent of hot spots was found not to exceed 10 Å on average, and the extent of the H-bonding part of the hot spot was found not to exceed 5 Å on average.

It is argued that apolar desolvation that can contribute at most 1 kJ/mol per heavy atom can not provide enough free energy of binding for small molecules. On the other hand, optimal H-bonds can give a favorable contribution to binding over 5 kJ/mol.[31] This is the reason why H-bonds are used by hot spots to bind fragments. H-bonds realize free-energy gain primarily by favorable enthalpy that makes fragment binding enthalpy driven. Since there is a higher chance to optimize high enthalpy compounds to balanced leads,[30] this finding provides a thermodynamic rationale for fragment based drug discovery.

The advantage of H-bonds over apolar desolvation disappears with increasing ligand size, and there are two reasons thought to be responsible for this. First, with increasing number of H-bonds, the probability of optimal geometrical fit decreases,[32] and second, the entropic penalty arising from the reduced mobility of the interacting partners increases with increasing number of H-bonds. Thus, the enthalpy dominated binding of fragments becomes entropy dominated for large molecules.

The above arguments rationalize the previously recognized size-dependence of maximal available ligand affinity. This quantity increases with increasing ligand size in the fragment region and in particular under 10 heavy atoms in line with the appearance of favorable H-bonds. Beyond the fragment size limit, the change in the available affinity attenuates as apolar desolvation becomes dominating with its less steep size dependence. Ligand efficiency (LE) defined as per atom affinity achieves high values when optimal fragments bind to the hot spot and it exhibits a maximum around 10 heavy atoms. Whether LE improvement in the small molecule region is the consequence of the rapid decrease of binding free-energy or comes from the inadequate sampling is yet to be determined.

ASSOCIATED CONTENT

Supporting Information

H-bonds in 1297 complexes and conserved H-bonds of 63 proteins complexed with multiple ligands (813 complexes) were identified. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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