Is there a link between selectivity and binding thermodynamics profiles?

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Thermodynamics of ligand binding is influenced by the interplay between enthalpy and entropy contributions of the binding event. The impact of these binding free energy components, however, is not limited to the primary target only. Here, we investigate the relationship between binding thermodynamics and selectivity profiles by combining publicly available data from broad off-target assay profiling and the corresponding thermodynamics measurements. Our analysis indicates that compounds binding their primary targets with higher entropy contributions tend to hit more off-targets compared with those ligands that demonstrated enthalpy-driven binding.

Introduction

High on-target affinity and designed selectivity against off-targets are usually the key points in the target product profile of many discovery programs and, consequently, these are among the most desired objectives of multiparameter medicinal chemistry optimizations. Potency optimizations are generally carried out by introducing apolar or polar substituents and subsequently monitoring the binding affinity (expressed in K_i or IC_{50} values). High specificity, however, does not demand high affinity [1]. Improving the binding affinity can be achieved by enthalpy- or entropy-driven optimization that covers substantially different thermodynamic profiles. Apparently, enthalpy and entropy changes are linked by the widely observed enthalpy–entropy compensation, although its impact has been recently challenged [2]. Binding affinity shows the quantity of the ligand–protein interactions via the Gibbs free energy of binding, whereas the corresponding thermodynamic profile describes the quality of the interactions.

The relationship between the knowledge encoded in Gibbs free energy of binding and its components, enthalpy and entropy, can be explained by the analogy of the projection. Constellations of stars such as the Cassiopeia are plane projections having a graph-type pattern. Stars, however, are not located at the same distance, some stars are much closer than the others and therefore the projection has a hidden dimension. Taking this distance dimension into account makes the plane a 3D object. Constellations were used for efficient navigation for hundreds of years, and improving the binding free energy drove medicinal chemistry programs in the past decades. Space travelers, however, should use the information from the third dimension for successful navigation and, similarly, thermodynamic profiles provide beneficial information on the interactions for medicinal chemists.

Ligand–protein interactions involve attractive forces and hydration effects. Properly positioned polar groups contribute to specific interactions, such as H-bonds, salt-bridges, polar–polar interactions and nonclassical interactions such as σ-hole-mediated halogen bonding that result in enthalpy gain. To exploit this enthalpy reward the binding partners should be in optimal orientation, because the binding energy is highly sensitive to the distance and the angle of the interacting atoms [3,4]. Nonpolar groups typically form weaker, less-oriented and less-specific interactions such as van der Waals contacts and π–π stacking [5]. Changes in desolvation entropy are favorable in both cases, but the desolvation of polar groups is associated with unfavorable desolvation enthalpy. For example, the desolvation enthalpies of OH and NH functionalities are 36.4 kJ/mol and 33.0 kJ/mol, respectively – in the range of the enthalpy gain realized with polar interactions – whereas for a methyl group the corresponding value is only 2.4 kJ/mol [6,7]. If the interactions with binding-site water molecules do not override the primary ligand–protein interactions the affinity gain achieved by the introduction of polar groups is generally enthalpy biased.

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By contrast, introduction of nonpolar substituents typically results in entropic reward that is mainly mediated by desolvation effects. Suboptimal positioned polar moieties would not be exploited in terms of enthalpy gain. The positional sensitivity of enthalpic optimization can be exemplified with a HIV-1 protease inhibitor pair. Saquinavir and TMC126 have the same number of polar groups; however saquinavir binding is associated with unfavorable (~5 kJ/mol) binding enthalpy but TMC126 binding is significantly more enthalpic ($\Delta H \sim -50$ kJ/mol) owing to the better orientation of its polar groups [7]. It should also be noted that binding-site water molecules have a complex influence on thermodynamics signatures [8–10]. Orientation of polar groups largely influences their specific interactions compared with nonpolar functional groups that are introduced to fill apolar cavities. The latter types of interactions show less dependence on distance and are less sensitive to orientation. As a result, optimization of binding affinity is more straightforward by hydrophobic moieties. Noninteracting or suboptimal positioned polar atoms are charged by the unfavorable desolvation enthalpy and thus generally result in decreased affinity. Accordingly, enthalpy-driven optimization is considered to be significantly more challenging compared with the entropy-driven process. Favorable binding energy can be achieved by entropy-driven approaches such as the introduction of nonpolar groups around apolar protein surfaces.

Replacement of unstable water molecules within hydrophobic pockets is mostly driven by entropy changes, although enthalpy gain coupled with water replacement by apolar moieties has also been reported [8,9]. The effect of binding-site waters has been recently reviewed by using WaterMap for solvation energetic calculations [11]. Selectivity between dopamine D2 and D3 receptors and kinase targets was also successfully rationalized by the analysis of binding-site water molecules [12,13]. Therefore, computational approaches can significantly facilitate the design of selective compounds, if high-quality crystal structures are available. Furthermore, the combination of experimental and computational approaches can rationalize unique cases where apolar contacts contribute to the favorable binding enthalpy in a protein-binding site occluded from solvent water [14].

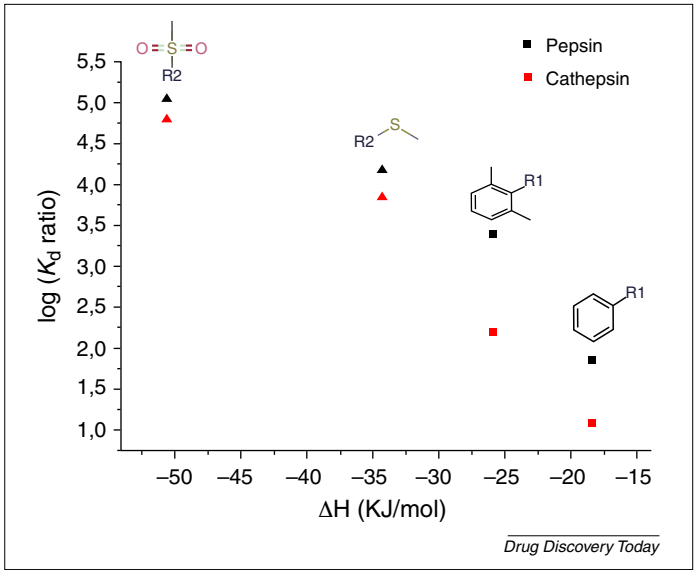

FIGURE 1

Correlation between binding-free-energy difference and binding enthalpy for HIV-1 protease inhibitors.

The quality of interactions and the accompanying binding thermodynamics profile impact selectivity against off-targets [15]. Enthalpically optimized compounds possess carefully positioned ligand-binding-site atom pairs to achieve the desired gain in binding enthalpy. Considering a different binding pocket presented in an off-target protein, the designed interactions will not be able to yield the enthalpic contribution to binding free energy because of the improper orientation of the ligand. Since the very same desolvation penalty of the polar atoms must be paid, the off-target affinity of the ligand will be limited. By contrast, entropically optimized compounds have fewer positional constraints and desolvation of the apolar moieties can result in entropy gain as a result of the lower dependence from the binding environment. These compounds have therefore higher propensity to form attractive interactions with off-targets. In this review, we investigate this hypothesis by analyzing the thermodynamic and selectivity profiles of optimized compounds and marketed drugs.

Binding thermodynamics and selectivity optimization

HIV-1 protease

The relationship between the binding thermodynamics properties of a closely related pairs of compounds published by Kawasaki and Freire serves as an illustrative example of the impact of thermodynamics on selectivity [15]. The thermodynamics profile was measured on the primary target HIV-1 protease, and cathepsin D and pepsin were monitored as antitargets. In the first case, a subtle change such as the introduction of two methyl groups into a phenyl moiety resulted in −11.2 kJ/mol gain in binding free energy owing to the more favorable enthalpy contribution of the methylated derivative (Fig. 1). This effect is a result of the optimal occupancy of a small cavity around the aryl moiety that is well oriented and the methyl groups can form desirable contacts. The selectivity toward pepsin and cathepsin D increased from 12 to 157 and 72 to 2464, respectively. In the second pair the thioether moiety was replaced by the sulfonyl-methyl group that resulted in a 1.2 kJ/mol decrease in binding free energy. However, the binding enthalpy improved from −34.3 kJ/mol to −50.6 kJ/mol, and the entropy contribution decreased by 11.2 kJ/mol. The introduced sulfonyl group establishes a strong H-bond with Asp30 of the protease, as is evident in the crystal structure. The selectivity against pepsin and cathepsin D increased by seven and ninefold, respectively. The authors suggested that maximal selectivity can be achieved by introducing a few very strong H-bonds toward the primary target protein. H-bonds have very rigorous distance and angular constraints. Consequently, suboptimal H-bonds formed with the off-target protein are penalized and this results in a larger decrease in the corresponding binding free energy. The overall picture of the four compounds suggests that, as the enthalpy contribution to binding free energy is increased, the compounds are more specific to the primary target. It is interesting to note that, among these four compounds, the highest affinity one does not have the highest selectivity; instead it is the one with the most favorable binding enthalpy. Although there is no theoretical background to support the linear correlation between these quantities, linear correlation coefficients (r^2) between $\Delta H_{protease}$ and $\Delta\Delta G$ values obtained for pepsin and cathepsin D were significant