

NEW HORIZONS IN THERMODYNAMIC DECOMPOSITION

Going against much of the current received wisdom in the field, Professor Steve Homans proposes thermodynamic decomposition as an aid to optimising ligand affinity.



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All biological processes depend on specific recognition between molecules with carefully tuned affinities. The Holy Grail for those searching for new chemical entities to inhibit such processes is the ability to do this at will. Unfortunately, this is not straightforward, since many competing thermodynamic processes contribute to binding affinity.

The standard free energy of binding, which determines the strength of the interaction, is not only determined by structure, but also involves the dynamics of the interacting partners (see figure, p100). Thus the static crystal structure of a protein ligand complex, while an excellent starting point, does not contain all the necessary information to predict binding affinity.

The key to a full understanding of the binding process is decomposition of the thermodynamics of binding into enthalpic (structural) and entropic (dynamic) contributions from the protein, the cognate ligand and solvent water.

Recent research has focused on undertaking this kind of thermodynamic decomposition for a protein ligand complex. Such is the complexity of the problem that the best chance of success was a model system.

For this purpose the interaction of the mouse major urinary protein (MUP) with a panel of cognate ligands was selected. MUP is an abundant protein found in male mouse urine that binds pheromones, where subtle recognition of a series of related compounds underlies its biological function.

The protein has a typical lipocalin fold consisting of an eight-stranded β -barrel and a single α -helix, and the barrel interior forms a hydrophobic cavity.

A number of small hydrophobic molecules can bind within

the cavity. The protein is thus an ideal model system for studying the thermodynamics of binding a series of related ligands. A major surprise is that, despite the hydrophobicity of the interacting partners, binding is strongly enthalpy driven, rather than the entropic binding signature typical of such interactions. It was hoped that thermodynamic decomposition would solve this puzzle.

The approach relies heavily on the concept of thermo-dynamic cycles, which takes a rigorous approach to elucidating the thermodynamic contributions from protein, ligand and solvent.

The results of these studies indicate that the principal driving force for the association derives from favourable dispersion interactions between the ligand and the protein. While at first sight this is unsurprising, the general consensus that has developed in recent years would suggest that solute-solute dispersion interactions will not be a primary driving force, since such interactions will be offset by favourable solute-solvent dispersion interactions that exist prior to the association.

Data indicate that this conclusion is incorrect, at least in the case of MUP. The binding pocket of this protein is sub-optimally hydrated, which means that there is an imbalance between solute-solute and solute-solvent dispersion interactions that favour binding. Accordingly, the entropic contribution from desolvation of the binding pocket, which would normally be characterised as the principal driving force for a hydrophobic interaction, is zero within error (see table, p100).

So why is this important for the optimisation of the binding affinity of ligands in general? The errors in the thermodynamic parameters listed in the table are too great to offer a predictive

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estimation of binding affinities. However, knowledge of the principal driving force is adequate help towards the optimisation of the binding affinity. For example, in the case of MUP, the appropriate strategy would be to optimise solute-solute dispersion interactions. (Note that ligand desolvation contributes unfavourably to binding in the table.)

Many might view this approach as a 'no brainer', but it does contradict the conventional assumptions mentioned above. Research showed that primary aliphatic alcohols fit the binding pocket nicely, and are excellent surrogate ligands for MUP.

Looking ahead to the potential for drug development in general, many proteins might possess binding pockets that are sub-optimally hydrated, in which case significant gains in affinity can be achieved by focusing on these parts of the binding pocket to optimise the dispersion contribution to binding.

Thermodynamic decomposition offers an 'on pathway' approach to converging on a high affinity ligand without fruitless 'off pathway' optimisations. At present the approach is too tortuous and time-consuming to include in discovery pipelines. However, by applying the approach to a variety of interactions with different thermodynamic signatures, we aim to assess, and as necessary 'tune', molecular mechanical forcefields with the ultimate aim of thermodynamic decomposition *in silico*. **END**

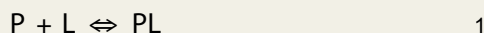
References are available from the author on request.

Topic contribution	Description	Value, kJ/mol	Enthalpic contribution	Description	Value kJ/mol
$T\Delta S_i^0$	Protein degrees of freedom	-0.8 ± 3.8^a	ΔH_i^0	New solute-solute interactions	~ -76
	Ligand degrees of freedom	~ -37		Changes in ligand/protein structure	~ 0
$-T\Delta S_{\text{solVL}}^0$	Ligand desolvation	$+26.7 \pm 8.4$	$-\Delta H_{\text{solVL}}^0$	Ligand desolvation	$+43.8 \pm 8.2$
$T\Delta S_{\text{solVPL}}^0 - T\Delta S_{\text{solVP}}^0$	Desolvation of protein/complex	$+0.4 \pm 9.2$	$\Delta H_{\text{solVPL}}^0 - \Delta H_{\text{solVP}}^0$	Desolvation of protein/complex	-12.3 ± 8.4
$T\Delta S_b^0$	Observed entropy	-10.7 ± 0.5^a	ΔH_b^0	Observed enthalpy	-44.5 ± 0.4^a

Decomposition of the thermodynamics of binding ligand 2-methoxy-3-isopropylpyrazine to mouse major urinary protein.

Binding thermodynamics for a ligand-protein association

The interaction of a ligand with a protein can be written in the form of a standard chemical equilibrium:



The association constant K_a (or equivalently, the reciprocal of the dissociation constant $1/K_d$) for this reaction is related to the standard free energy of binding as follows:

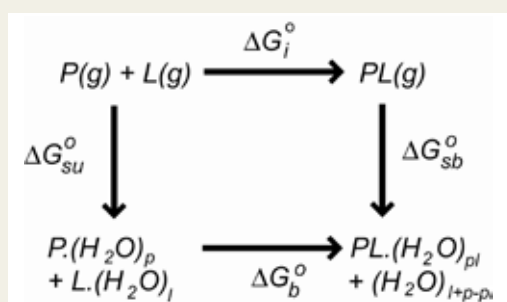
$$\Delta G_b^0 = -RT \ln K_a \quad 2$$

Note that the standard free energy of binding ΔG_b^0 must not be confused with the free energy of binding ΔG_b , which by definition is zero at equilibrium. The standard free energy of binding in turn comprises the standard enthalpy of binding ΔH^0 and the standard entropy of binding ΔS^0 (multiplied by the absolute temperature T):

$$\Delta G_b^0 = \Delta H_b^0 - T\Delta S_b^0 \quad 3$$

The enthalpy can be loosely defined as the static, structural component of the association, whereas the entropy is related to the dynamics of the interacting partners.

The formulation of the interaction in terms of equation 1 is in fact a gross oversimplification of the binding process, since it ignores solvent water. A complete formalism involves a thermodynamic cycle as shown, where we account for the fact that the ligand and protein are associated with H_2O .



Here, ΔG_b^0 is the observed standard free energy of binding, ΔG_i^0 is the 'intrinsic' solute-solute interaction in the absence of solvent, and the terms ΔG_{sb}^0 and ΔG_{su}^0 are related to the solvation of the complex and the free species, respectively. Since G is a state function, the sum over the cycle is zero, and we can thus write:

$$\Delta G_b^0 = \Delta G_i^0 + \{ \Delta G_{sb}^0 - \Delta G_{su}^0 \} \quad 4$$

We can equate ΔG_{sb}^0 with the solvation free energy of the complex $\Delta G_{\text{solVPL}}^0$, whereas ΔG_{su}^0 comprises the sum of the solvation free energies of the free protein and free ligand, $\Delta G_{\text{solVP}}^0 + \Delta G_{\text{solVL}}^0$. Thus, finally we can write:

$$\Delta G_b^0 = \Delta G_i^0 + \{ \Delta G_{\text{solVPL}}^0 - \Delta G_{\text{solVP}}^0 - \Delta G_{\text{solVL}}^0 \} \quad 5$$

A similar equation can be written for the enthalpy and entropy of binding since these are also state functions. The thermodynamic decomposition approach involves the determination of each component on the right-hand side of equation 5, as shown in the table above.