The Strange Case of Enthalpy-Entropy Compensation

Ponder Lab Group Meeting
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Win some, lose some: enthalpy–entropy compensation in weak intermolecular interactions

Enthalpy–entropy compensation is a general feature of many chemical reactions and processes in biological systems, but its origin has remained obscure. A simple thermodynamic argument suggests that enthalpy–entropy compensation is a general property of weak intermolecular interactions, and that the two contributions to the free energy should nearly balance out for a hydrogen bond at 300 K.

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Searle and Williams [7] have discussed the relevance of enthalpy–entropy compensation to the binding of agonists versus antagonists to a common receptor site. They suggest that binding of agonists is mainly enthalpy driven while that of antagonists is mostly entropy driven, as has indeed been shown experimentally to be the case for the A1 adenosine receptor [8]. Recently, the topic is attracting more attention in the contexts of supramolecular host–guest and biomolecular drug–receptor association reactions [7, 9–11]. Although enthalpy–entropy compensation has in the past been regarded as a ‘ubiquitous property of water’ [1], it appears to be a property of all weak intermolecular interactions
For any 'reasonable' function the quadratic force constant $f$ of the equilibrium structure (the curvature of the function at the equilibrium position) is approximately proportional to the dissociation energy $D_o$. This proportionality is exact for the Morse potential, a mathematical representation of the potential energy curve in Figure 1:

$$V(r-r_o) = D_o \left\{ e^{-2B|r-r_o|} - 2 e^{-B|r-r_o|} \right\}, \quad f = 2B^2 D_o$$

(where $V$ is the potential energy as a function of the interatomic distance $r$, $r_o$ is the equilibrium distance and $B$ is a constant), and for the inverse power potential:

$$V(r) = \frac{D_o}{|1-m|} \left\{ m \left( \frac{r_o}{r} \right)^l - 1 \left( \frac{r_o}{r} \right)^m \right\} \quad l>m, \quad f = \frac{ml}{(r_o)^2 D_o}$$

(where $l$ and $m$ are constants). For a harmonic oscillator, the frequency $\nu$ of the A–B stretching vibration is $\nu = \sqrt{f/\mu}/2\pi$ where $\mu$ is the reduced mass of the system. In other words, for a given mass, the frequency $\nu$ increases as the square root of the force constant and hence as the square root of the dissociation energy $D_o$. Given $\nu$, we can then use the standard statistical mechanical relationship [14]:

$$S_{vib} = R \left( \frac{\nu}{e^\nu - 1} - \ln \left( e^\nu - 1 \right) \right)$$

where $\nu = \hbar \nu/kT = 1.439 \nu/T$ ($\nu$ in cm$^{-1}$, $T$ in K; $R$ is the gas constant), to estimate the corresponding contribution to the vibrational entropy at any temperature.
Fig. 1. The energy of a hydrogen bond depends on the interatomic distance. The curve shows a typical potential energy curve for a O–H⋯O hydrogen bond; vertical scale, energy in kcal mol\(^{-1}\); horizontal scale, H⋯O distance in Å. As the interatomic distance decreases, the bond becomes stronger, but as the atoms approach each other too closely, repulsion forces take over.
Fig. 2. As the frequency $\nu$ increases, the vibrational entropy $S_{\text{vib}}$ decreases. The curve shows the relationship between $\nu$ and $S_{\text{vib}}$ at 300 K. Both $\nu$ and $S_{\text{vib}}$ are dependent on the strength of the bond; for strong bonds, $\nu$ is high and $S_{\text{vib}}$ is small.
Fig. 3. The enthalpy–entropy compensation curve at $T = 300$ K for a simple association process, $A + B \leftrightarrow A\cdot B$. The value of $6TS_{\text{vib}}$, the entropic contribution to the free energy, estimated from our simple model, is plotted against the dissociation enthalpy $D_0 = -\Delta H^0$, both expressed in kcal mol$^{-1}$. For strong covalent bonds, enthalpy predominates, while for very weak associations, entropy predominates. For the intermediate case where $D_0 \approx 5$ kcal mol$^{-1}$, the typical energy of a hydrogen bond, the value of $D_0$ is nearly equal and opposite to the entropic term.
Figure 1. Complex formation between a ligand (containing polar functionalities A and B) and a receptor (containing polar functionalities X and Y) with exchange of four water molecules to the bulk solvent. (Broken lines indicate hydrogen bonds.)
Understanding Noncovalent Interactions: Ligand Binding Energy and Catalytic Efficiency from Ligand-Induced Reductions in Motion within Receptors and Enzymes

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3. An Equation for the Estimation of Binding Constants

Binding constants in aqueous solution can be estimated from Equation (3), which is derived from the above considerations.

\[ \Delta G = \Delta G_{t+r} + n \Delta G_r + A \Delta G_h + \sum \Delta G_p \]  

(3)
Figure 7. Plots of the approximate unfavorable translational plus rotational free energy of binding \( \Delta G_{(\text{trans}+\text{rot})} \) for binding to a receptor of mass >2000 Daltons as a function of the mass of the ligand (■ ■ ■, aqueous medium; ▲▲▲, nonpolar medium).
Table 2. Entropy changes accompanying cyclization at 298°K

<table>
<thead>
<tr>
<th>System*</th>
<th>$-\Delta S^o$ (cal deg^{-1} mol^{-1})</th>
<th>$-\Delta S^o$/ (no. int. rot.)</th>
<th>$-\Delta S^o_{corr}$/ (no. int. rot.)</th>
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<tr>
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<tr>
<td></td>
<td>7.7</td>
<td>3.85</td>
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<tr>
<td></td>
<td>10.9</td>
<td>3.63</td>
<td>4.90</td>
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<td></td>
<td>10.3</td>
<td>5.14</td>
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<tr>
<td></td>
<td>13.3</td>
<td>3.32</td>
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<td></td>
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<td>4.25</td>
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<td></td>
<td>21.0</td>
<td>5.25</td>
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<tr>
<td></td>
<td>19.8</td>
<td>3.30</td>
<td>3.72</td>
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<td></td>
<td>19.6</td>
<td>3.92</td>
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<tr>
<td></td>
<td>19.0</td>
<td>2.71</td>
<td>3.91</td>
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<tr>
<td></td>
<td>18.8</td>
<td>3.13</td>
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Entropic Penalty for Restricting Bond Rotation:

Table 1: Average values for the parameters of Equation 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Physical process</th>
<th>Value (kJ mol(^{-1}))</th>
<th>Factor[^{[a]}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta G_{t+r})</td>
<td>energy cost of bimolecular association</td>
<td>+5.4</td>
<td>(ca. 10)</td>
</tr>
<tr>
<td>(\Delta G_r)</td>
<td>energy cost of restriction of an internal rotor</td>
<td>+1.4</td>
<td>(ca. 2[^{[b]}])</td>
</tr>
<tr>
<td>(\Delta G_h)</td>
<td>benefit of the hydrophobic effect (per Å(^2) of buried hydrocarbon)</td>
<td>−0.17 (Å(^{-2}))</td>
<td>(ca. 1[^{[c]}])</td>
</tr>
<tr>
<td>(\Delta G_p)</td>
<td>benefit of making a neutral hydrogen bond of ideal geometry</td>
<td>−4.7</td>
<td>ca. 7</td>
</tr>
<tr>
<td>(\Delta G_{ionic})</td>
<td>benefit of making an ionic hydrogen bond of ideal geometry</td>
<td>−8.3</td>
<td>ca. 28</td>
</tr>
</tbody>
</table>

\[^{[a]}\] Factor by which binding is promoted (opposed) at RT. \[^{[b]}\] Per rotor. \[^{[c]}\] Upon burial of 33 Å\(^2\) of the hydrocarbon.
Figure 4. Schematic representation of a receptor that binds ligands X, Y, and Z with affinities $\Delta G_X$, $\Delta G_Y$, and $\Delta G_Z$, respectively. a) Binding of Z results in a structure with an intermolecular distance $d_0$. b) When Y and Z are connected by a rigid, strain-free linker (Y–Z) they bind to the receptor with positive cooperativity ($\Delta G_{Y,Z}$ more negative than $\Delta G_Y + \Delta G_Z$) and there is structural tightening ($d_1 < d_0$). c) If X is connected to Y–Z by a rigid, strain-free linker to form X–Y–Z then further structural tightening will occur ($d_2 < d_1$) leading to a further cooperative enhancement. d) The shorter linker between Y and Z does not allow both these binding interactions to occur with optimal geometry. Y–Z binds the receptor with negative cooperativity ($\Delta G_{Y,Z}$ more positive than $\Delta G_Y + \Delta G_Z$) and there is structural loosening ($d_3 > d_0$).
Figure 5. The binding interaction between the glycopeptide antibiotic vancomycin and the peptide ligand N-α-acetyl-Lys-(N-ε-acetyl)-d-Ala-d-Ala. Hydrogen bonds are indicated by dotted lines. The binding is also promoted by hydrophobic interactions, notably of the Ala methyl groups to the aromatic rings of the antibiotic. H² discussed in the text is indicated.
Entropy–enthalpy compensation: Fact or artifact?

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Abstract

The phenomenon of entropy–enthalpy (S-H) compensation is widely invoked as an explanatory principle in thermodynamic analyses of proteins, ligands, and nucleic acids. It has been suggested that this compensation is an intrinsic property of either complex, fluctuating, or aqueous systems. The questions examined here are whether the observed compensation is extra-thermodynamic (i.e., reflects anything more than the well-known laws of statistical thermodynamics) and if so, what does it reveal about the system? Compensation is rather variably defined in the literature and different usages are discussed. The most precise and interesting one, which is considered here, is a linear relationship between $\Delta H$ and $\Delta S$ for some series of perturbations or changes in experimental variable. Some recent thermodynamic data on proteins purporting to show compensation is analyzed and shown to be better explained by other causes. A general statistical mechanical model of a complex system is analyzed to explore whether and under what conditions extra-thermodynamic compensation can occur and what it reveals about the system. This model shows that the most likely behavior to be seen is linear S-H compensation over a rather limited range of perturbations with a compensation temperature $T_c = d\Delta H/d\Delta S$ within 20% of the experimental temperature. This behavior is insensitive to the details of the model, thus revealing little extra-thermodynamic or causal information about the system. In addition, it will likely be difficult to distinguish this from more trivial forms of compensation in real experimental systems.
Fig. 1. Entropy (expressed as TS at 298K)–enthalpy compensation plots for (A) calcium binding to proteins, (B) protein unfolding, (C) hydrogen exchange in cytochrome c, and (D) alkane solvation. Using experimental ΔG and ΔH data taken from Kuroki et al. (1992), Privalov and Gill (1988), Milne et al. (1999), and Ben-Naim and Marcus (1984), respectively (■). Using randomly generated ΔH values with experimental ΔG’s (○).
In summary, if the range of $\Delta G$’s measured in a series of experiments is much smaller than the range of $\Delta H$’s, then with respect to $\Delta H$, $\Delta G \approx \text{Constant}$. Linear $dH-dS$ compensation follows immediately from the relationship $\Delta G = \Delta H - T\Delta S$. The question then is whether this arises from (1) larger errors in determining $\Delta H$ than $\Delta G$, (2) some extra-experimental constraint that a priori restricts the range of observable $\Delta G$s, or (3) some extra-thermodynamic mechanism of $\Delta H-\Delta S$ compensation. For the three data sets examined here, the statistical tests strongly suggest, although they cannot prove, the first explanation. I argue that this is because extra-experimental constraints a priori restrict the range of observable $\Delta G$s to less than the precision in $dH$ measurements, even though the latter may be carefully measured. Nevertheless, without knowing the molecular origin of the entropy and enthalpy components and from statistical tests alone, one cannot rule out some type of extra-thermodynamic compensation of the type seen in the model presented here.
Figure 1

Entropy-enthalpy compensation as a general phenomenon in thermodynamics. Three examples of compensating entropic and enthalpic contributions to the free energy as a function of temperature in general thermodynamic phenomena. The free energy ($\Delta G$) of the process as a function of temperature is shown, along with enthalpic ($\Delta H$) and entropic ($T\Delta S$) contributions. (a) Transfer of neopentane from its neat phase to water (data from figure 3 of Reference 59), (b) myoglobin unfolding (data from table 2 of Reference 65), and (c) protein-protein association (data from figure 3b of Reference 15). In all three cases, $\Delta H$ and $T\Delta S$ change substantially whereas $\Delta G$ remains almost constant, suggesting substantial entropy-enthalpy compensation.
Figure 2
Examples of severe compensation reported in the calorimetry literature. Several cases are shown in which ligand modifications lead to large changes in the enthalpic and entropic contributions to binding whereas the overall binding free energy remains essentially unchanged. (a) Severe compensation in HIV-1 protease inhibitors (data from table 1 of Reference 58), (b) para-substituted benzamidinium trypsin inhibitors binding to trypsin (data from table 1 of Reference 96), and (c) nonpolar ring expansions in arylsulfonamide trypsin inhibitors (data from table S3 of Reference 91). Quantities in parentheses denote one standard error of least significant digit.
Figure 4
Typical isothermal titration calorimetry (ITC) experimental configuration and data. (a) A typical experimental configuration for power-compensating ITC. (b) Typical data from an ITC experiment showing applied power as a function of time (top) and integrated heats of injection with fit to thermodynamic parameters (bottom) (reproduced from figure 2 of Reference 15, with permission).
Figure 3
Compensation behavior in calorimetry data. All plots show apparent compensation behavior between enthalpic ($\Delta H$) and entropic ($T\Delta S$) components of free energy of binding. (a) Apparent compensation behavior from isothermal titration calorimetry (ITC) measurements of Ca$^{2+}$ to calcium-binding proteins (black circles) with linear fit [red dashed line, slope = 0.92(5), $R^2 = 0.96(3)$ by bootstrap] (data from figure 3 of Reference 56). (b) Meta-analysis of ITC measurements of protein-ligand complexes (circles) selected from the BindingDB database (62) with linear fit [dashed line, slope = 0.93(3), $R^2 = 0.91(2)$ by bootstrap] (data from figure 1 of Reference 81). (c) Apparent (but fallacious) compensation over a wide range of energies. The figure shows independent ITC measurements performed in different laboratories using identical samples of ligand and protein [4-carboxy-benzencesulfonylamine (CBS) binding to bovine carbonic anhydrase II] from the ABRF-MIRG’02 (Molecular Interactions Research Group of the Association of Biomolecular Resource Facilities) assessment (71). Here reported measurement errors (error bars representing one standard error) are much smaller than the actual variation among independent measurements (computed from table 3 of Reference 71). Linear fit denoted by dashed line [slope = 0.99(2), $R^2 = 0.997(1)$ by bootstrap].
(d) Instrumental limitations on binding affinities measurable by ITC generally restrict the measurable range of $\Delta G$ (but not $\Delta H$) to the unshaded region, inducing a linear correlation in $\Delta H$ and $T\Delta S$ due to the window effect as described in the text (data from figure 1 of Reference 72).
A simple model system illustrating weak entropy-enthalpy compensation. An idealized protein and ligand interact via a Morse potential that is strengthened or weakened to simulate ligand modifications. (a) Intermolecular Morse potential $U(r) = D_e [1 - e^{-a(r-r_0)}]^2$, with $r_0 = 2.8$ Å, $a = 1/(0.5$ Å), and well depth $D_e$ varying from 2 to 10 kcal mol$^{-1}$. (b) Potential of mean force $F(r) = U(r) - k_B T \ln 4\pi r^2$ between protein and ligand as a function of intermolecular distance $r$ for temperature $T = 25$C. (c) Standard entropic ($T\Delta S$) and enthalpic ($\Delta H$) contributions to the binding free energy for different well depths $D_e$, computed from classical statistical mechanics. Note that, although some entropy-enthalpy compensation is apparent, it is not linear or severe.
Figure 6
Distribution of published binding free energies and correlation with enthalpy. (a) Distribution of binding free energies computed from ChEMBL pKᵢ activity data (data from figure 4 of Reference 53). (b) Poor correlation of enthalpy (ΔH) with free energy (ΔG) of binding from meta-analysis of isothermal titration calorimetry (ITC) measurements selected from the BindingDB database (62) (all ITC measurements from the BindingDB as of this writing are shown in a manner similar to that in figure 2a of Reference 81). Whereas aldose reductase and HIV-1 protease show some correlation between enthalpy and the free energy of binding, correlation is generally poor for other systems, such as renin, and enthalpies span a much broader range than free energies.
Entropy-Enthalpy Compensation: Role and Ramifications in Biomolecular Ligand Recognition and Design

John D. Chodera\textsuperscript{1} and David L. Mobley\textsuperscript{2}

SUMMARY POINTS

1. While a weak form of entropy-enthalpy compensation is likely common, evidence of a severe or pervasive form of compensation is poor.

2. Measurement and calculation of enthalpies and entropies is more difficult than measuring or computing free energies.

3. Entropic and enthalpic contributions are difficult to interpret and are unlikely to be useful in rational ligand design.

4. When intuition fails in proposing modifications that lead to affinity gains, schemes that compute binding free energies directly are poised to be of high utility.
Arylsulfonamide Ligands for Human Carbonic Anhydrase II

Figure 1. (a) Structures of the partially fluorinated ligands used in this study, and their abbreviations. The abbreviation of each ligand indicates the number of fluorine atoms on the benzo-extension (e.g., F₂BTA contains two fluorine atoms). (b) An overlay of the heavy atoms of the H₄BTA and F₄BTA ligand from (aligned) crystal structures of the two HCA-ligand complexes. (c) Diagram of the amino acid residues of HCA that form contacts with the benzothiazole (H₄BTA) and perfluorobenzothiazole (F₄BTA) sulfonamide ligands, determined previously from crystal structures of each complex. Favorable ligand–protein interactions are represented with a blue dashed line, and unfavorable interactions, with a red dashed line. These interactions between ligand and protein were deemed favorable or unfavorable on the basis of the work of Diederich and colleagues.
Figure 3. Diagram of the pK\textsubscript{a}-corrected thermodynamic results for $\Delta \Delta J^\circ_{\text{bind}}$ (compared to H\textsubscript{4}BTA)—where $J = G$ (blue), $H$ (green), and $S$ (red)—obtained from ITC measurements at 298.15 K. The relative differences in the enthalpy and entropy of binding (i.e., mutual $H/S$ compensation) result in indistinguishable values of $\Delta G^\circ_{\text{bind}}$ for 4-F\textsubscript{1}BTA, 5,6-F\textsubscript{2}BTA, 4,7-F\textsubscript{2}BTA, 4,6-F\textsubscript{2}BTA, 4,5,6-F\textsubscript{3}BTA, and F\textsubscript{4}BTA. The gray region demarcates the 95% confidence interval (i.e., two standard deviations) of $\Delta \Delta G^\circ_{\text{bind}}$ for H\textsubscript{4}BTA.
Figure 5. Hydration site thermodynamics for H₄BTA and three of the partially fluorinated variants (4,6-F₂BTA, 5,6-F₂BTA, and 6,7-F₂BTA). The colors of the hydration sites range from green (favorable) to red (unfavorable). The range for ΔH°ₘᵦₜ (left panel) is [−5.0 to +5.0 kcal mol⁻¹] whereas for −TΔS°ₘᵦₜ (right panel) the range is [0.0 to +5.0 kcal mol⁻¹]. WaterMap computes all values relative to bulk solvent. The black arrow indicates an additional localized hydration site in 5,6-F₂BTA that is not observed in other χ-F₂BTA variants. The dashed oval indicates a cluster of hydration sites that is entropically unfavorable in 5,6-F₂BTA and 6,7-F₂BTA relative to 4,6-F₂BTA.