Supplemental Data

An Entropic Mechanism to Generate Highly Cooperative and Specific Binding from Protein Phosphorylations

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Mathematical Derivations: The Law of Mass Action

We consider the binary reaction

$$A + B \leftrightharpoons C$$

for A binding to B to form complex C and calculate the fraction of A in complex C at equilibrium. We will not make a distinction between the Helmholtz and Gibbs free energies because the reaction occurs in solution with negligible loss of volume or change in pressure.

Including Only Translational Entropy

The total free energy of the system is

$$F = \sum_i E_i - T \sum_i S_i \tag{1}$$

where the sum is over the interacting molecular species (A, B, and C), and E_i denotes the total energy of species i and S_i its total entropy. For simplicity, we consider an ideal gas (our results do not change for dilute solutions [1]), then E_i obeys

$$E_i = N_i \left(\frac{3}{2} k_B T + \epsilon_i \right) \tag{2}$$

for N_i molecules of species i, all having the same internal energy ϵ_i. The Boltzmann constant is k_B and temperature is T. The translational entropy S_i^{trans} satisfies [1]:

$$S_i^{\text{trans}} = k_B N_i \left[\frac{5}{2} - \log(C_i / c_0) \right] + N_i \sigma_i(T) \tag{3}$$

where C_i is the concentration of species i (number of molecules per unit volume), c_0 is a constant, and σ_i is a function of temperature.

At equilibrium, the free energy is at a minimum [1]: $dF = 0$. From Equation 1, a minimum in F implies

$$\sum_i \frac{\partial E_i}{\partial N_i} dN_i = T \sum_i \frac{\partial S_i}{\partial N_i} dN_i. \tag{4}$$

Every time an A and a B molecule react, the number of A and B molecules both decrease by one and the number of C molecules increases by one. Similarly, if a C molecule decays, the number of C molecules decreases by one and the number of A and B molecules both increase by one. Consequently,

$$dN_A = dN_B = -dN_C. \tag{5}$$

Differentiating Equations 2 and 3 with respect to N_i, and using Equation 5, allows Equation 4 to become

$$\begin{aligned} &\left[-\epsilon_A - \epsilon_B + \epsilon_C - \frac{3}{2} k_B T \right] dN_C \\ &= T \left[-\sigma_A - \sigma_B + \sigma_C + k_B \log\left(\frac{C_A C_B}{c_0 C_C} \right) - \frac{3}{2} k_B \right] dN_C \end{aligned} \tag{6}$$

or, simplifying further,

$$\frac{C_C}{C_A C_B} = c_0^{-1} \exp\left[-\left(\frac{f_C - f_A - f_B}{k_B T} \right) \right] \tag{7}$$

where $f_i = \varepsilon_i - T\sigma_i$. The right-hand side of Equation 7 is the inverse of the equilibrium dissociation constant, K_D, and depends (through the definition of f_i) on both the change in binding energy, $\epsilon_c - \epsilon_A - \epsilon_B$, and on terms originating from the change in translational entropy, $\sigma_c - \sigma_A - \sigma_B$. Having $\epsilon_c < \epsilon_A + \epsilon_B$ favors the formation of C.

Including Both Translational and Conformational Entropies

Assume that molecule A is a disordered protein that becomes ordered on binding to protein B, then the entropy of free A increases by a conformational entropy term:

$$S_A = S_A^{\text{trans}} + S_A^{\text{conf}} \tag{8}$$

Following the derivation given above, the law of mass action changes to

$$\frac{C_C}{C_A C_B} = \frac{e^{-S_A^{\text{conf}}/k_B}}{K_D} \tag{9}$$

If the conformation entropy of free A is large, then the amount of complex C reduces.

Defining $K_{\text{eff}} = K_D / C_B$ to include the concentration of B, then

$$\frac{N_C}{N_A} = \frac{e^{-S_A^{\text{conf}}/k_B}}{K_{\text{eff}}} \tag{10}$$

canceling the volume terms. The sum of the number of A molecules and the number of C molecules is conserved throughout the reaction, $dN_A + dN_C = 0$. This constraint and Equation 10 allows the calculation of the fraction of A molecules in complex C:

$$\frac{N_C}{N_A + N_C} = \frac{1}{1 + K_{\text{eff}}\, e^{S_A^{\text{conf}}/k_B}} \tag{11}$$

A small K_{eff} or a small S_A^{conf} encourages A to form C.

Supplemental References

S1. Landau, L.D., and Lifschitz, E.M. (1984). *Statistical Physics* (London: Butterworth-Heinemann).