Protein–Ligand Interactions: Thermodynamic Basis and Mechanistic Consequences

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It is difficult to imagine any biological process that is not initiated by the binding of some chemical entity (or ‘ligand’) to a protein. Thus, enzymatic catalysis, signal transduction, ion channel activity, immune responses and the various events involved in genetic expression are all obvious examples of events which, at the molecular level, must begin with the binding of some ligand to a specific binding site on a protein molecule. Furthermore, as each one of these processes proceeds along its reaction pathway, modulation of the affinities of such ligands, positive and negative interactions caused by the binding of other ligands and the release of these ligands either in their original or in altered form constitute obligatory steps in the driving forces and controlling restraints that adapt them to their specific tasks. We extend the basic conceptions set forth in this article to a more detailed level focused on the fundamental nature of ligand–ligand interactions of a protein–ligand complex and the energetic mechanistic consequences to which they lead.

Thermodynamics of Binding

The energetics of chemical reactions in solution are expressed in terms of three functions: $\Delta G^\circ$, the Gibbs free energy, which designates the relative probability of existence of any two chemical species; $\Delta H^\circ$, the enthalpy, which specifies their relative heat contents and $\Delta S^\circ$, their entropy, generally interpreted as their temperature-independent degrees of disorder. These three entities are written as capital letters to emphasise that they are ‘state properties’; that is, they express the properties of a system under specific conditions or, as used here, the difference in such properties between two such species, and are completely independent of their previous history and thus of any mechanistic pathway between any two such entities. The three thermodynamic functions are related by eqn 1: See also: Cell Biophysics; GABAα Receptors; Protein–Ligand Interactions: General Description; Substrate Binding to Enzymes; Thermodynamics in Biochemistry

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$  

The superscript ‘‘’ indicates the value of the property of a molar concentration of unity. The free energy function, $\Delta G^\circ$, is defined as

$$\Delta G^\circ = -RT \ln K$$  

where $R$ is the gas constant, $T$ the absolute temperature and $K$ the equilibrium constant for a given chemical reaction under a specified set of experimental conditions. Since $K$ is an experimentally determinable entity, we will use it to discuss the free energy aspects of protein–ligand binding reactions.

Single site binding

Since the formal expression for this, the simplest case of the phenomenon, provides the basis for the treatment of even the most complex case, it is useful to consider its derivation in some detail. Given a reaction

$$P + L \rightleftharpoons PL$$  

where $P$ is a protein with a single site specific for the binding of a ligand, $L$, and PL is the protein–ligand complex, we may define its dissociation constant, $K_D$, as

$$K_D = \frac{[P][L]}{[PL]}$$  

Since $[P]$ is not ordinarily an easily measured entity, we use the conservation equation

$$[P_T] = [P] + [PL]$$
where \([P_T]\) is the molar concentration of the total protein, an experimental quantity. Solving eqn [5] for \([P]\), substituting its equivalent expression for \([P]\) in eqn [4] and rearranging, we find that

\[
[PL] = \frac{[P_T][L]}{K + [L]} \tag{6}
\]

The hyperbolic relationship given in eqn [6] is a fundamentally important one, capable of describing binding behaviour for many systems more complex than that of eqn [3]. Indeed, eqn [6] provides the phenomenologically correct equation for any binding reaction where the binding sites for ligands do not interact with each other, and it can accommodate all such mechanisms without any change in mathematical form. All that is required to account for additional complexity is provided by defining \(K_D\) with a correspondingly complex definition; a point which we will now demonstrate with a few examples.

Specific ligand-binding proteins (such as enzymes and transport proteins) frequently undergo a two-step binding process, where the ligand (L) binds to an open form of the protein (P) to form a relatively weak encounter complex (PL). The presence of the ligand then induces the closing of the binding site cleft to form a much tighter complex (QL). Such a process may be represented as

\[ P + L \rightleftharpoons \text{PL} \rightleftharpoons \text{QL} \tag{7} \]

Applying the same approach as used to derive eqn [6] to this reaction, we obtain

\[
[PL] = \frac{[P_T][L]/(1 + K_1)}{K_D/(1 + K_1) + [L]]; \tag{8}
\]

\[
[QL] = \frac{[P_T][L]K_1/(1 + K_1)}{K_D/(1 + K_1)} + [L]
\]

where \(K_D = [P][L]/[PL]\) and \(K_1 = [QL]/[PL]\).

The concentrations of both [PL] and [QL] as a function of [L] remain strictly hyperbolic, sharing the same apparent dissociation constant \(K_D/(1 + K_1)\), but with different numerator terms which no longer are simply \([P_T][L]\). The observed behaviour of such a system depends on the nature of the experimental signal used to measure the process. If that signal (e.g. protein fluorescence quenching) were to measure only [QL], then the limiting horizontal asymptote reached as \([L] \rightarrow \infty\) will no longer be equal to \([P_T]\). If, however, the signal used measures both [PL] and [QL] indiscriminately, then the numerator simplifies and then

\[
([PL] + [QL]) = \frac{[P_T][L]}{K_D/(1 + K_1)} \tag{9}
\]

Corresponding equations for more complex binding processes can be derived by the same process. In addition, despite their degree of algebraic complexity, they must all follow the same general rule as long as only single site binding occurs: (1) they must be hyperbolic in [L] as long as all other conditions (including constant concentrations of other liganding species) remain constant; (2) the numerators of the equations for the sum of all protein–ligand forms will be \([P_T][L]\). The \(K\) term in the denominator will be an algebraic term independent of \([P]\) and \([L]\), but the complexity of its nature will depend on the specific details of the particular binding scheme used. See also: Binding and Catalysis

\section*{pH dependence}

The formation of protein–ligand complexes involves non-covalent interactions between functional groups on both entities. Many of these functional groups such as lysyl, histidyl and glutamyl side-chain residues have ionisable protons. A ligand will generally have a near-absolute preference for interaction with either a protonated or an ionised form of each such residue. Since a ligand typically interacts with several functional groups and may require a very specific pattern of ionisation states among the residues which comprise its binding site, the pH dependence of ligand-binding processes may be extremely complex and the equations describing that behaviour are correspondingly ponderous. These very properties, however, provide a powerful set of tools for examining structure–function relationships in protein–ligand binding. Examination of the equation describing the simplest case of a pH-dependent binding reaction will serve to illustrate the general principles of the phenomenon and its interpretation.

Given the reaction

\[
H^+ + \text{P} \rightleftharpoons \text{K}_H^+ \quad \text{and} \quad K_L = \frac{[P][H^+]^0}{[H^+][P]} \tag{10}
\]

We find that

\[
[H^+P] = \frac{[P_T][L]}{\left(1 + \frac{K_H}{[H^+]^0}\right)K_L + [L]} \tag{11}
\]

The dependence of the binding versus [L] at any given pH is seen to be hyperbolic. The protein ionisation constant, \(K_H^+\) (usually expressed as \(pK = -\log K_H^+\)) occurs solely in the denominator of the expression and this generalisation holds true for systems of any complexity. The observed \(K_D\) from such a plot actually is expressed by the entire \((1 + K_H^+/[H^+])K_L\) term. The p\(K_H^+\) itself, however, may be resolved by plotting the \(K_{obs}\) versus pH, as in Figure 1, or more usefully, by plotting log\(K_{obs}\) versus pH as in Figure 2. The numerical value of the p\(K\) (which represents the pH at which the functional group is half ionised) is indicated by the
inflection point of the plot in Figure 1 and by the intersection of the two linear portions of the log plot in Figure 2. It may also be noted that the slope of the log plot is unity for the ionisation of a single proton, as is the case for eqn [10], but will correspond numerically to the number of protons that must be ionised in more complex cases. The slope of the line in Figure 2 will be negative for cases such as that in eqn [10] where the ligand-binding site must be protonated and positive for processes which require the loss of a proton.

See also: pH and Buffers

The pH behaviour for a more realistic case involving multiple ionisable sites is shown in Figure 3. A mathematically extreme, but not at all uncommon case, is one where the geometry of the binding site functional groups requires that the group with the lower pK must be unprotonated and the group with the higher pK must be protonated. The pH behaviour of such a system is shown in Figure 4, where it is seen that the inflection points of the curves do not correspond to either intrinsic pK. The existence of such a case can be inferred from the amplitude of the binding plot, which may now be substantially less than the maximum value of [P].

Fersht (1985) has noted the fact that pK values derived from kinetic rate measurements generally contain reaction rate constants and thus may differ substantially from their thermodynamic counterparts.

Multiple site binding

The simplest case of this class is that of a protein which has multiple sites, all of which have the same intrinsic KD values. This class of independent multisite binding reactions also includes the not uncommon occurrence of very weak and nonspecific binding of small ligands (such as simple...
The binding of a ligand to a protein cannot be considered mathematically complex and the fitting of such equations to experimental data from such a system are much more directly interpretable. This advantage is due to the fact that in a reaction where one molecule of ligand A and one molecule of ligand B can bind simultaneously to a protein molecule, either [A] or, alternatively, [B] can be held constant while the other ligand is systematically varied. In the corresponding homotrophic case, the experimenter has neither control over nor knowledge of whether a system contains PL₄, 2PL₂ or 4PL. Since the simple form of the heterotrophic system has important implications for multisubstrate enzyme systems as well as for the binding of activators and noncompetitive inhibitors, we will describe the energetics of such a case here in some detail. See also: Enzyme Activity: Reversible Inhibition

Our discussion is framed by the following five axioms proposed by Weber (1975):

- The study of the interactions of proteins and small ligands provides a means of relating biological function to fundamental physical chemical principles.
- Structural information must be accompanied by corresponding energetic studies if it is to lead to appropriate physical interpretation.
- The binding of a ligand to a protein cannot be considered as an event apart from the changes in that protein induced by that binding.
- We never deal with the binding of a single ligand to a protein.
- Therefore, it is impossible for the binding of two ligands to the same protein to be really independent.

Weber also replaced the elaborate chemical potential binding treatment of Wyman (1948) with the much simpler but still rigorous Gibbs free energy (\(\Delta G\)) approach. Based on the relationship, \(\Delta G = -RT \ln K\), where \(K\) is the equilibrium constant for a ligand-binding event; and given the simple algebraic additivity of \(\Delta G\) function, the consequences of Weber’s axioms are most clearly expressed in the form of the classic ‘thermodynamic square’

\[
\begin{align*}
\text{A} + \text{E} &\rightarrow \text{EA} + \text{B} \\
\text{A} + \text{E} + \text{B} &\rightarrow \text{EAB}
\end{align*}
\]

where equilibrium constants, \(K_1, K_2, K_3\) and \(K_4\), are assigned to the corresponding numbered steps, and \(\Delta G_\text{tot} = -RT \ln K\) are again defined for each step.

We define an interaction parameter \(\Delta G_I\) as the difference between the free energy of formation of the ternary complex from E, A and B and that of the sum of the free energies of formation of the two binary complexes

\[
\Delta G_I = \Delta G_1 + \Delta G_4 - (\Delta G_1^* + \Delta G_2^*)
\]
Since
\[ \Delta G^o_i + \Delta G^o_j = \Delta G^o_k + \Delta G^o_l \]  
and \( \Delta G^o_i = \Delta G^o_j - \Delta G^o_k = \Delta G^o_l - \Delta G^o_i \).

Substituting \(-RT \ln K_n\) for each \( \Delta G^o_i \) and rearranging, we define free energy interaction parameter, \( \Delta G^i \), as
\[ \Delta G^i = -RT \ln \frac{K_i}{K_2} = -RT \ln \frac{K_3}{K_1} \]  

In other words, the \( \Delta G^i \) expresses the difference in the free energy of A binding to the EB complex over that of A binding to free E.

Of course, the effect of the presence of bound B on the binding of A must be identical in sign and magnitude to that of the presence of bound A on the binding of B. It should be noted that \( \Delta G^i \) may be either positive or negative depending on whether A and B mutually decrease or increase each other’s affinity for the enzyme.

Most ligand-binding reactions are studied by measuring the dependence of some physical property of the P–L complex on the concentration of a ligand, thus obtaining the values of \( K_s \) and \( \Delta G^i \) for each step around the square. However, since \( \Delta G^i \) is itself the resultant of two independent terms, one enthalpic and the other entropic; \( \Delta G^i = \Delta H^i - T \Delta S^i \) at a given absolute temperature \( T \), we may properly go on to define the other interaction parameters \( \Delta H^i \), \( \Delta S^i \) and \( \Delta C_p^i \) (the change in the heat of capacity, \( d\Delta H/dt \)). The examination of the parameters for each step around the square provides more details of the causative forces of a given \( \Delta G^i \) and thereby extends our understanding of the nature of the binding process. The requirements for measuring and interpreting these parameters lead us to the consideration of the effects of temperature on chemical equilibria.

Temperature dependence

Any chemical reaction may be written in the form
\[ \text{A} + \text{B} \rightleftharpoons \text{C} + \text{D} + q \]  
where \( q \) is the amount of heat released from the reaction to its surroundings. Le Chatelier’s principle states that if \( q \) is positive the reaction will be pushed back as the temperature is raised, and will be pulled forward with increasing temperature if \( q \) is negative. It should be noted that \( q \) itself is not a state property. However, if a reaction is carried out reversibly, \( q \) mol\(^{-1}\) = \( \Delta H^c \), the molar enthalpy of the reaction. The fundamental relationship governing the temperature dependence of a chemical reaction, which follows from combining eqns [1] and [2], is the van’t Hoff equation. See also: Thermodynamics in Biochemistry
\[ \frac{\partial \Delta G^o}{\partial T} = \frac{\partial \ln K}{\partial T} = \frac{\Delta H^o}{RT^2} \]  

The experimentally applicable form of this equation is
\[ \ln K = -\frac{\Delta H^o}{RT} \]  
Thus, if one plots \( \ln K \) for a reaction versus \( 1/T \), as in Figure 5, the slope of the resulting curve equals \( \Delta H^o/\text{R} \). If \( \Delta H^c \) is itself independent of temperature, a straight line will be obtained. However, such plots for protein–ligand binding reactions, when accurately measured over a sufficient temperature range, are typically nonlinear, resulting in temperature-dependent \( \Delta H \) values. Such behaviour in a system signifies only the existence of a finite ‘heat capacity’, defined as
\[ \Delta C_p = \frac{dH^c}{dT} \]  

Attempts to interpret such nonlinear van’t Hoff plots as two asymptotic straight lines intersecting at a ‘break-point’ continue to occur in the literature. Such portrayals are a misleading result of the logarithmic nature of such plots. In the commonly (and quite properly) used plots of pH-dependent functions, a similarly inherent sigmoidal curve now also appears as a pair of intersecting straight lines.

The several thermodynamic functions which may be derived by appropriate measurements of \( q \) are listed in Table 1 for five different cases of their dependence on \( T \), each determined by a different experimental method. It can be seen from the table that curvature in a van’t Hoff plot (or its kinetic counterpart, the Arrhenius plot) serves only to rule out case 1 behaviour, showing the existence of a finite \( \Delta C_p \) it provides no further distinction between cases 2, 3, 4 or 5. If the ligand-binding step involves a conformational change in either the E–P complex or the free protein P itself, as in the reaction

\[ \text{E} \rightleftharpoons \text{E}^\prime \]  

\[ \text{EL} \]
the calorimetrically determined $\Delta H$ observed will take the form shown in Figure 6. Only direct calorimetric measurements of $\Delta H$ itself can reveal the nature of the $\Delta C_p$. If such a study indicates a temperature dependence of the $\Delta C_p$ itself, then a further examination using a differential scanning calorimeter (DSC) may reveal the presence of a peak indicating a protein conformational change as shown in Figure 7. These successive relationships are described in detail in Fisher (1988) and Eftink and Biltonen (1980).

It is clear from Figure 6 that where a $\Delta C_p$ of substantial magnitude exists, the particular value of the $\Delta H$ for a given ligand-binding reaction measured either from the values of $K_D$ at two different temperatures or, alternatively, from a calorimetric determination at a single temperature, has no real physically interpretable significance. It has been found that protein–ligand binding reactions are characterised quite generally by having substantial negative $\Delta C_p$ values usually falling in the range from $-200$ to $-600 \text{ cal mol}^{-1} \text{ deg}^{-1}$. Over a 20° range in temperature the observed $\Delta H$, then, may change by as much as 12 kcal mol$^{-1}$ and may pass from a positive value through zero to a negative value as the temperature is raised. In such a case, then it is only the $\Delta C_p$ itself which has significance. The physical basis of the $\Delta C_p$ values accompanying protein binding has been

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**Table 1** The expression of thermodynamic functions by various thermometric methods

<table>
<thead>
<tr>
<th>Case</th>
<th>Method:</th>
<th>Parameter:</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>van’t Hoff</td>
<td>$\Delta H^\circ = 0$</td>
<td>$\Delta C_p^\circ = 0$</td>
</tr>
<tr>
<td>2</td>
<td>$\Delta H^\circ$ finite</td>
<td>$\Delta C_p^\circ = 0$</td>
<td>$\frac{d\Delta C_p^\circ}{dT} = 0$</td>
</tr>
<tr>
<td>3</td>
<td>$\Delta H^\circ$ finite</td>
<td>$\Delta C_p^\circ$ finite</td>
<td>$\frac{d\Delta C_p^\circ}{dT} = 0$</td>
</tr>
<tr>
<td>4</td>
<td>$\Delta H^\circ$ finite</td>
<td>$\Delta C_p^\circ$ finite</td>
<td>$\frac{d\Delta C_p^\circ}{dT}$ finite</td>
</tr>
<tr>
<td>5</td>
<td>$\Delta H^\circ$ finite</td>
<td>$\Delta C_p^\circ$ finite</td>
<td>$\frac{d\Delta C_p^\circ}{dT}$ finite</td>
</tr>
</tbody>
</table>

**Source:** Reproduced from Fisher (1988). Reprinted with permission of Wiley.
ascribed to a variety of sources, including displacement of bound water molecules by the ligand (Sturtevant, 1977). It has also been noted, however (Brandts et al., 1974; Eftink and Biltonen, 1980; Fisher et al., 1986) that the scheme shown in eqn [14] will generate an apparent $\Delta C_p$, even if no individual step in the scheme actually has a finite $\Delta C_p$.

See also: Protein–DNA Complexes: Nonspecific; Protein–Ligand Interactions: Energetic Contributions and Shape Complementarity

Entropy–Enthalpy Compensation

This phenomenon was first described by Lumry and Rajender (1970) who noted the fact that in many binding reactions the small changes in $\Delta G^\circ$ ($\pm 3$ kcal mol$^{-1}$) resulted from the difference between two much larger forces, typically a large ($\sim 20$ kcal mol$^{-1}$) favourable negative $\Delta H^\circ$ driving force largely or totally offset by an opposing $\Delta S$ component. Furthermore, because of the temperature factor of $\Delta S$, a ligand A which enhances the binding of ligand B at one temperature may inhibit that binding at a different temperature. An example of the compensation phenomenon in the case of mammalian glutamate dehydrogenase is shown in Figure 8.

A striking example of entropy–enthalpy compensation is the case of the hydrophobic bond, a term coined by Kauzmann (1959) to describe the binding of a nonpolar moiety to a corresponding hydrophobic patch on a protein. This interaction exhibits a positive $\Delta H^\circ$ term in the $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ equation and thereby a repulsive deterrent to binding. However, a nonpolar moiety in aqueous solution tends to be surrounded by a hydrogen-bonded cage of water molecules (termed ‘icebergs’ by Klotz (1953)). On encounter with the nonpolar protein patch this structured water is disrupted leading to a positive increase in $\Delta S$, a compensating negative ($-\Delta S$) term in the Gibbs equation and a negative $\Delta G^\circ$ of binding.

Given their broadly general probability of occurrence, the quantitatively significant and directionally disparate effects of the two different secondarily binding ligands on $\Delta G^\circ$ illustrated in Figure 8 have important implications for biological activity at all levels of organisation. Any binary P–L complex whether in a cell, a subcellular entity or simply in an in vitro reaction is subject to the secondary binding of a wide variety of potential ligands, which may either increase or decrease its activity. These include specific substrates, products and modifiers as well as any of a host of anions and, of greatest importance, the ubiquitous

Figure 6 The temperature dependence of the observed $\Delta H$ for a coupled, hidden two-state system: $\Delta H_0 \rightarrow \Delta H_1 \rightarrow \Delta H_2$. It is assumed that [L] is presented in saturating conditions. Reproduced from Fisher (1988). Reprinted with permission of Wiley.

Figure 7 The temperature dependence of the apparent $\Delta C_p$, generated by the system portrayed in Figure 5. Curves are shown for four different $\Delta H_0$ values.

Figure 8 Interaction parameters for the enzyme–NADPH–L-glutamate complex (a positive interaction) and for the enzyme–NADPH–ADP complex (a negative interaction of bovine liver glutamate dehydrogenase. Reproduced from Fisher (1988). Reprinted with permission of Wiley.
supply of protons present in any aqueous medium. Following the initial axiom that essentially all biological processes must be initiated by a protein–ligand binding event, it follows that the enthalpy–entropy compensation phenomenon will continue to affect the same protein as it undergoes further chemical and structural changes along its reaction pathway. Further, the sum of these effects will inevitably be passed on to the succeeding levels of any major process, each component of which will be subject to further modification. It would appear, then that \( \Delta H - \Delta S \) compensation is one of nature’s most basic tools for the vast and intricate patterns of regulation that are only now beginning to be discerned. See also: Enzyme Activity: Control

**Binding energy transduction**

In 1946, Pauling suggested that the energy of formation of a reactive enzyme–substrate complex must in some manner be passed along the reaction coordinate in such a way as to provide a driving force for the catalytic step (Pauling, 1946; Jencks, 1975; Gavish, 1986); each produced elegant formulations proving that the concept was indeed theoretically possible, but their conclusions included terms which could not be accessed by direct experiment. Lumry and Gregory (1986) proposed a testable mechanism for such a process in which they dissected the free energy profile of an enzyme reaction into two components: one expressing the energy of the protein itself and the other reflecting that of the substrate process. If the activation barriers of the two energetic components are unrelated, along their reaction coordinates (as shown in Figure 9a), then little energetic driving force will be transmitted. If, however, the free energy profiles are complementary to one another (as shown Figure 9b), then the activation barrier may be substantially reduced. Experimental evidence in support of this notion has been obtained based on fine-grained isothermal calorimetric determination of the temperature dependence of the \( \Delta H \) of binding in a number of intermediate complexes in the bovine 1-glutamate dehydrogenase catalysed reaction (Fisher and Singh, 1991).

**Molecular Crowding Effects on Solute Diffusion in Cells**

The rate of diffusion defined as the movement in space of a solute is one of the principal hydrodynamic parameters customarily used to define the shape of a macromolecule. Such measurements are customarily made in homogenous aqueous solutions at low macromolecular concentrations. However, cells and subcellular organelles are known to be filled with high concentrations of proteins, polynucleotides, metabolites among other solutes in addition to membranes and skeletal structures. On this basis, it has been generally assumed that such ‘crowding’ must significantly lower the diffusion rates of macromolecules. If so, this effect could have a significant impact on ligand binding. The issue remains highly contentious, however, due in large part to the experimental difficulties of measuring events in such complex and heterogeneous systems. Physics itself distinguishes two distinct classes of diffusive behaviour, based not on the rate of the process but only on the exponential dependence of that rate. Based on Einstein’s equations for Brownian motion (Einstein, 1955), the rate of the mean square displacement, \( \bar{r^2} \), of a solute particle is

\[
\bar{r^2} = 6Dt
\]

where the diffusion constant \( D \) is \( D = kT/\eta \), \( k \) the Boltzman’s constant, \( T \) the absolute temperature and \( \eta \) the frictional coefficient represents a shape factor (asphericity) of the solute. Any phenomenon obeying this equation is termed ‘normal’ (Brownian) diffusion, regardless of its actual rate, as expressed by the value of \( D \).

Solute diffusion that does not conform to the \( \bar{r^2} \) dependence of eqn [21] has been defined by Bouchaud and Georges (1990) as ‘abnormal diffusion’, a description ascribed to any of the various ‘crowding’ factors described in the preceding text. Dix and Verkman (2008) have critically assessed the available experimental evidence in terms of this behavioural duality in cellular aqueous compartments. Surprisingly, they concluded that even in the most crowded and geometrically constrained cases, normal Brownian behaviour is generally observed. The relatively few well-established cases of abnormal diffusion appear to be due to lipid–protein and protein–protein interactions. Dix and Verkman conclude that ‘the notion of universally anomalous diffusion in cells as a consequence of molecular crowding is not correct and that slowing of diffusion in cells is less marked than has been generally assumed’.

**Figure 9** The Lumry ‘free energy complementarity’ concept. (a) An example where no alteration in the activation free energy of the substrate process occurs. (b) An example where free energy changes in the protein itself are partially complementary to those of the substrate system. The dashed line represents the level of ligand-binding energy store in the protein molecule itself. Reproduced from Lumry and Gregory (1986). Reprinted with permission of Wiley.
Conformational Landscapes

These entities are a variety of recently proposed three-dimensional maps of the free energy distribution of the various conformations available to a protein molecule and the barriers that separate them on one axis and some sequence of events on the other. An example for the case of an enzyme reaction is shown in Figure 10.

The concept underlying such landscapes, that a protein fluctuates rapidly between an array of readily accessible conformations and that the distribution of the occupancy of the array changes upon the binding of a ligand, has lain dormant for three decades since its formulation in the nine-authored treatise ‘The Fluctuating Enzyme’ (Welch, 1986). Its sudden rediscovery in the past few years may be attributed to the convergence of experimental evidence from four lines of investigation each seeking to answer a different question. The first impetus leading to the landscape concept may have been provided by the investigation of the protein-folding problem. Here the only conceivable solution to reducing the immense number of conformations of a free peptide chain down to the single unique form required by its specific function took the form of a landscape in which successive steps sequentially funnelled the number of free energy conformer minima into a single deep pit with a jagged surface (Dill et al., 2008). Meanwhile Monad et al. (1965) had formulated the concept of ‘allostery’ initially involving a landscape of only two conformational states and four subunits linked by a set of rigid rules governing their interconversion induced by ligand-binding events. Over the years, allostery has developed into a sophisticated field involving broad systems, multiple states and complex rules best expressed by a form of a landscape. Enzyme chemists, pursuing the mystery of enzymatic catalysis with their own experimental tools and theoretical bases, have finally become aware of the existence of multiple conformations of a given complex and the significant shifts in the distribution of occupancy of those states induced by ligand binding. An early hint to the occurrence of such behaviour in enzymatic reactions was provided by the isotope tracer studies by Rose (1998) of the fumarase reaction shown in Figure 11. Though the chemical event in this reaction appears to involve nothing more than the addition of the elements of water to a carbon–carbon double bond, Rose found it to comprise 10 different complexes and 3 different enzyme conformations. Experimental evidence supporting the existence of such conformational landscapes in enzyme catalysed reactions has been presented by Swint-Kruse and Fisher (2008) for the glutamate dehydrogenase reaction and by Benkovic et al. (2008) for the dihydrofolate reductase reaction. A trio of papers in the journal Science/AAS expressed views of various aspects of dynamic signalling in terms of energy landscape. Smock and Giersch (2009) emphasised the remodelling of free energy landscapes in molecular signal transmission. Tokuriki and Tawfik (2009) relate protein conformational diversity to their ability to evolve. Engler et al. (2009) carry the theme on the effects of soluble factors on the dynamic cellular adhesion factors required for tissue differentiation. Recently, the new field of computational

![Figure 10](image-url) The multiple reaction coordinates of an enzyme reaction plotted on a surface whose vertical axis is ΔG° of the protein component of the reaction with the various conformations available to the protein on one horizontal axis and the successive reaction intermediates on the other. (This portrayal of the energetics of an enzyme reaction is in contrast to the conventional two-dimensional forms shown in Figure 9.) The red lines trace the conformational and kinetic interconversions accessible to the enzyme in its various ligand-bound forms. I indicates a dead-end inhibitor complex and T indicates a ‘tunnelling’ step. An important feature of the landscape is that the barrier heights between adjacent conformation change with each reaction step as the ligands (substrates and products) undergo chemical changes. Reproduced from Swint-Kruse and Fisher (2008).
biology has turned its attention to the various facets of multiconformational states and, hopefully, may ultimately provide a unifying viewpoint for understanding what must be a general phenomenon, currently expressed by each of these subdisciplines in their own historically based scientific dialects. In any case, it is clear that the gaining of a deeper understanding of ligand-binding effects is an essential requirement for progress in a wide variety of biological processes of current interest. See also: Protein–Ligand Interactions: Induced Fit

References


Further Reading

