The Kinetic and Energetic Pull of Chemical Entropy On Small Ensembles of Proteins

Josh E. Baker[1*]

[1]University of Nevada, Reno School of Medicine: Reno, NV 89521 USA

*Corresponding author. Email: jebaker@unr.edu

Abstract: Proteins in biological systems function at the interface of single molecule and bulk chemistry and thus provide novel insights into the basic physical chemistry of scaling and emergent phenomena. For example, a binary mechanical model that accounts for many energetic and mechanical aspects of muscle contraction provides a novel and testable framework for investigating the relationship between molecular mechanics and chemical thermodynamics in single molecules and small molecular ensembles. The problem addressed here is that the model and supporting experimental data require that entropic forces balance chemical reactions. While this is consistent with statistical mechanics, it is at odds with basic chemistry. Specifically, counter to a classic chemical activity analysis, the mere presence of molecules (chemical activity or mass action) does not physically push a reaction toward equilibrium; rather, the number of microstates, Ω, accessible in a chemical state pulls a reaction down an entropic funnel of increasing Ω toward equilibrium. Here I develop an entropic model of chemical thermodynamics and kinetics and compare it with conventional chemical activity models. I show that an a priori system reaction energy landscape fully describes the chemical kinetics of both equilibrium and non-equilibrium chemical reactions, formally justifying the thermodynamic rate constants required for binary mechanical models of muscle contraction.

Keywords: Entropy, Chemical Thermodynamics, Free Energy, Reaction Energy Landscape, Microstates, Micropathways, Non-Equilibrium, Kinetics, Entropic Funnel

Introduction

Technological advances over the past 70 years have fueled a molecular revolution in the biological sciences, providing remarkably detailed descriptions of the structure and function of a myriad of biomolecules. The question remains, to what extent is biological function the sum of these molecular parts versus an emergent property of systems of molecules? While this is fundamentally a physical chemical question, insights from small molecular ensembles in biological systems are informing new perspectives on this important chemical thermodynamic problem. For example, a binary mechanical model that describes the mechanochemistry of muscle contraction offers a new perspective on entropic forces in enzyme-catalyzed chemical reactions [1,2]. This model provides a clear description of the mechanisms required to contain entropic forces, providing an explicit solution to the Gibbs paradox [3]. The problem addressed here is that the model and supporting experimental data require that chemical reactions are balanced by entropic forces [2]. While this is consistent with the entropy of binary systems in statistical mechanics, it is inconsistent with the conventional assumption in chemistry that chemical reactions are balanced – not by entropy, but – by forces exerted by the mere physical presence of molecules (through chemical activity or mass action). Here, I compare an entropic model of chemical thermodynamics with the conventional chemical activity model and show that while the reaction free energy equations have the same form, the physical meaning of the "concentration" terms differ fundamentally. In an entropic model an a priori system reaction energy landscape fully describes the chemical kinetics of both equilibrium and non-equilibrium chemical reactions, formally justifying the thermodynamic rate equations required in a binary mechanical model of muscle contraction [2].

Figure 1 illustrates a binary mechanical system [2]. The molecular mechanism is a reversible molecular switch (Fig. 1A) that displaces an element external to the switch a distance, d, with a transition from state A to B. If the element is stiff, the molecular switch generates force. An equilibrium ensemble of molecular switches constitutes a binary mechanical system (Fig. 1B) within which a Boltzmann distribution of chemical states, N_B/N_A, decreases exponentially with a force, F, applied to the system as

$$N_B/N_A = \exp[-(\Delta G° + F \cdot d)/k_B T] \qquad \text{Eq. 1}$$

where N_A and N_B are the number of molecules in states A and B, and $\Delta G°$ is the standard free energy for a molecular transition from state A to B.

In muscle, the molecular switch is a myosin motor (oval) that generates force through a discrete conformational change induced by actin binding (solid black line) and driven energetically by the binding free energy, $\Delta G°$. We have directly observed in mechanics experiments on systems ranging from single myosin motors to single muscle cells that myosin motors function as reversible molecular switches [4–6]. We have also shown in muscle fibers that the force-dependence of the distribution between the two states of the molecular switch follows Eq. 1 [5,7]. Moreover we have shown that this binary mechanical model accurately accounts for the basic mechanics and energetics of muscle contraction [1,2].

According to Eq. 1, when $\Delta G° = 0$,

$$F = -k_B T \cdot \ln(N_B/N_A)/d. \qquad \text{Eq. 2}$$

Because the passive force exerted on binary mechanical systems (e.g., muscle, rubber, titin) is entropic [2,8,9], $k_B T \cdot \ln(N_B/N_A)$ is entropic energy. While this is a classic statistical mechanic description of a binary system [10], it is at odds with conventional chemistry where the term $k_B T \cdot \ln(N_B/N_A)$ is assumed to be part of a chemical potential not entropy. Thus, statistical mechanics and chemistry offer two fundamentally different descriptions of the term $k_B T \cdot \ln(N_B/N_A)$. In conventional chemistry, N_A and N_B are numbers of molecules that physically exist in states A and B (chemical activities), which through their mere presence physically push a reaction (mass action) toward equilibrium. In an entropic analysis, N_A and N_B describe a system reaction coordinate $\{N_A,N_B\}$ that is defined independent of the physical presence of molecules in states A and B. Rather than being pushed by physical quantities (or a mass) of molecules, in an entropic analysis the reaction is pulled toward states of higher system entropy.

While this problem is highlighted by the entropic force of a binary mechanical system (Eq. 2), it is not limited to mechanical systems. Here, I show that even in non-mechanical ($F = 0$) chemical systems, $k_B T \cdot \ln(N_B/N_A)$ is entropic energy not a chemical potential. This represents a radical transformation of our basic understanding of chemical energetics and kinetics (the model

implies that the physical presence of molecules, N_A and N_B, has no effect on the kinetics or energetics of a chemical reaction) and leads to an entropic formulation of chemical kinetics in which non-equilibrium kinetics are fully defined by an a priori system reaction energy landscape.

Reaction Free Energy

In a given system state $\{N_A,N_B\} = \{\delta\eta,N–\delta\eta\}$, the free energy, G, for the reaction in Fig. 1B is expressed in terms of the extent of the reaction, $\delta\eta$, as

$$G = (\mu_B - \mu_A)\cdot\delta\eta + F\cdot d\cdot\delta\eta - TS, \qquad \text{Eq. 3}$$

where $(\mu_B - \mu_A)$ is the chemical potential difference between states A and B, $\underline{F}\cdot d\cdot\delta\eta$ is the net work performed by $\delta\eta$ displacements, d, of a system spring with a force, $\underline{F}$, and S is the system entropy in state$\{\delta\eta,N–\delta\eta\}$. When one molecule transitions from state A to B the change, Δ, in system energy is described by the reaction free energy

$$\Delta_r G = \delta G/\delta\eta = (\mu_B - \mu_A) + F\cdot d - T\Delta S, \qquad \text{Eq. 4}$$

where ΔS is the reaction entropy, $\delta S/\delta\eta$. The terms on the right hand side of Eq. 4 are the chemical forces that when $\Delta_r G < 0$ energetically drive a reaction from $\{N_A,N_B\}$ to $\{N_A–1,N_B+1\}$, or when $\Delta_r G > 0$ energetically drive a reaction from $\{N_A–1,N_B+1\}$ to $\{N_A,N_B\}$, or when $\Delta_r G = 0$ balance the reaction.

Early chemists were averse to the concept of a reaction entropy, ΔS, in part because of their limited mathematical backgrounds but also because they did not understand Gibbs [11]. Instead, with a more Newtonian worldview, they were convinced by the general arguments of Waage and Guldberg in 1864 that the mere presence (number or mass) of molecules in a state i physically pushes molecules out of that state through a mechanism often referred to as mass action [11]. Because this mechanism needed an energetic basis, chemists defined the chemical potential, μ_i, of chemical state, i, as a function of the number of molecules in that state, $\mu_i = \mu^\circ_i + k_B T\cdot\ln N_i$, such that when N_i increases μ_i increases, energetically pushing molecules out state i. According to this analysis, $\mu_B - \mu_A = \Delta G^\circ + k_B T\cdot\ln(N_B/N_A)$, where ΔG° is the standard

free energy, which when substituted into Eq. 4 describes an equilibrium force, $\underline{F}$, in Eq. 2 that is balanced against the force of mass action, $\mu_B - \mu_A$, not entropy, ΔS.

While entropic contributions to the standard free energy, $\Delta G° = \Delta H° - T\Delta S°$, are often considered, $\Delta S°$ differs from ΔS. The standard entropic change, $\Delta S°$, describes the entropic change of a molecule with an A to B transition, whereas the reaction entropy, ΔS, describes the entropic change of a system of molecules with a $\{N_A,N_B\}$ to $\{N_A-1,N_B+1\}$ transition. The reaction entropy, ΔS, continues to be ignored by chemists despite the fact that the force exerted on a binary system (Eq. 2) is entropic, $\Delta S = -k_B \cdot \ln(N_B/N_A)$.

In both a chemical activity and entropic analysis, the free energy equation (Eq. 4) is

$$\Delta_r G = \Delta G° + F \cdot d + k_B T \cdot \ln(N_B/N_A). \qquad \text{Eq. 5}$$

The question at hand is whether $k_B T \cdot \ln(N_B/N_A)$ is a component of a chemical potential, $\mu_B - \mu_A$, where N_A and N_B describe the physical presence (chemical activity or mass) of molecules in states A and B, or whether $k_B T \cdot \ln(N_B/N_A)$ is a system entropic energy, $T\Delta S$, where N_A and N_B describe an a priori system reaction coordinate $\{N_A,N_B\}$ that is defined independent of the physical presence of molecules.

The free energy change, ΔG°, for a molecular transition from state A to B

The standard free energy, $\Delta G°$, for a reaction is a molecular property. For example, $\Delta G°$ for the hydrolysis of ATP to ADP and P_i can be physically defined for one molecule as the change in free energy associated with breaking a single phosphoanhydride bond.

Figure 2A illustrates an energy landscape for a single molecule that reversibly switches between molecular states A and B with forward and reverse rate constants, $k°_+$ and $k°_-$, and a free energy difference of $\Delta G°$ [12]. This energy landscape describes changes in free energy along the reaction (or conformational) coordinate of a molecule [13]. According to kinetic theory, the inverse time to diffuse in both forward, $k°_+$, and reverse, $k°_-$, directions over an activation energy barrier separating A and B (Fig. 2A) are related to $\Delta G°$ as $k°_+/k°_- = \exp(-\Delta G°/k_B T)$ [14]. Thus, for an isolated single molecule, the ratio of the time-averaged occupancy of molecular states B and A is

$$N_B/N_A = \exp(-\Delta G^o/kT). \qquad \text{Eq. 6}$$

This equation describes an energetic balance between ΔG^o and the energy term – $k_B T \cdot \ln(N_B/N_A)$, which evident from this example does not describe a chemical activity (mass action) since there is only one molecule diffusing in the molecular energy landscape. While this energy term emerges from the molecular energy landscape, it is clearly not part of it. It is an ensemble, system energy that is defined independent of the physical presence of molecules in states A and B (Eq. 6). As proposed by T.L. Hill [15], when one molecule undergoes a transition from A to B, the only contribution of that molecular transition to the system free energy ($\mu_B - \mu_A$) is ΔG^o not $\Delta G^o + k_B T \cdot \ln(N_B/N_A)$.

Free energy changes for system transitions from chemical state $\{N_A,N_B\}$ to $\{N_A–1,N_B+1\}$

For an ensemble of N such molecules contained in a solution held at constant T, P, V, and $F = 0$, the reaction occurs within a system energy landscape along a system reaction coordinate, $\{N_A,N_B\}$ (Fig. 2B). System energy landscapes can be plotted as changes in the reaction free energy of the system, $\Delta_r G$, (Eq. 4, Fig. 3A) or as changes in the free energy of the system, G, (Eq. 3, Fig. 3B) both along the same system reaction coordinate, $\{N_A,N_B\}$ [10,16]. In addition to the incremental molecular free energy changes, ΔG^o, that contribute to the system free energy with each molecular step from A to B, $\Delta_r G$ accounts for non-molecular (non-ΔG^o) changes in the free energy of the system with each chemical step from $\{N_A,N_B\}$ to $\{N_A–1,N_B+1\}$. These system contributions to free energy include PV work, non-PV work ($F \cdot d$) and ΔS in Eq. 4.

Reaction (system) entropy, ΔS

Using elementary statistical mechanic arguments, the reaction entropy, ΔS , for any reversible two-state reaction (Figs. 1 and 2) is shown below to be

$\Delta S = –k_B \cdot \ln[(N_B + 1))/(N_A)]$ for molecular transitions and

$\Delta S = –R \cdot \ln([B]/[A])$ for molar transitions,

resulting in molecular and molar reaction free energy equations ($\Delta_r G = \Delta G° – T\Delta S$)

$$\Delta_r G = \Delta G° + k_B T \cdot \ln[(N_B + 1))/(N_A)] \quad \text{and} \qquad \text{Eq. 7}$$

$$\Delta_r G = \Delta G° + RT \cdot \ln([B]/[A]).$$

While these equations are consistent with a conventional chemical activity analysis, here N_A, N_B, [A], and [B] are unrelated to chemical activities. That is, they are not physical quantities

of molecules that through their mere presence (mass action) balance chemical reactions. Clear from the analysis below, N_A, and N_B are system reaction coordinates that describe the reaction entropy, $\Delta S(N_A,N_B)$ in chemical state $\{N_A,N_B\}$; and [A] and [B] are molar reaction coordinates that describe the molar reaction entropy, $\Delta S([A],[B])$ in chemical state {[A],[B]}.

At equilibrium, $k_BT\cdot\ln(N_B/N_A) = -\Delta G°$ not because $N_A + N_B$ molecules equilibrate within the energy landscape of one molecule [i.e., when $\mu_B - \mu_A = 0$], but because N_A and N_B describe the point along the system reaction coordinate, $\{N_A,N_B\}$, at which the system equilibrates [i.e., the point at which $T\Delta S(N_A,N_B) = -\Delta G°$]. This is illustrated in Figs. 1B and 2 for small numbers of molecules and derived in general as follows.

According to Boltzmann, the system entropy in state $\{N_A,N_B\}$ is $S = k_B\cdot\ln(\Omega_{NA,NB})$, where $\Omega_{NA,NB}$ is the number of microstates available to the system in chemical state $\{N_A,N_B\}$. Specifically, $\Omega_{NA,NB} = N!/(N_A!N_B!)$. The change in entropy with a single chemical step from $\{N_A,N_B\}$ to $\{N_A-1,N_B+1\}$ is then

$$\Delta S = -k_B\cdot\ln[((N_A - 1)!(N_B + 1)!)/(N_A!N_B!)] = -k_B\cdot\ln[(N_B + 1))/(N_A)].$$

The change in system entropy associated with $\delta\eta$ chemical steps from $\{N_A,N_B\}$ to $\{N_A-\delta\eta,N_B+\delta\eta\}$ is

$$\Delta S = -k_B\cdot\ln[((N_A - \delta\eta)!(N_B + \delta\eta)!)/(N_A!N_B!)] \approx -\delta\eta\cdot k_B\cdot\ln[(N_B + \delta\eta/2))/(N_A - \delta\eta/2)],$$

and so

$$\Delta_rG = \delta\eta\cdot\Delta G° + \delta\eta\cdot k_BT\cdot\ln[(N_B + \delta\eta/2)/(N_A - \delta\eta/2)]. \qquad \text{Eq. 8}$$

Here the reaction is driven both by the change in molecular free energy,$\cdot\Delta G°$, summed over $\delta\eta$ molecular transitions from A to B and by the average change in system entropy, $k_BT\cdot\ln[(N_B + \delta\eta/2)/(N_A - \delta\eta/2)]$, summed over $\delta\eta$ chemical steps from $\{N_A,N_B\}$ to $\{N_A-\delta\eta,N_B+\delta\eta\}$.The change in entropy, ΔS, relative to $S = 0$ occurs from $\{N/2,N/2\}$ to $\{N/2-\delta\eta, N/2+\delta\eta\}$, or

$$\Delta S = -\delta\eta\cdot k_B\cdot\ln[(N/2 + \delta\eta/2))/(N/2 - \delta\eta/2),$$

or

$$\Delta S = -\delta\eta \cdot k_B \cdot \ln[(1 + \delta\eta/N)/(1 - \delta\eta/N)], \qquad \text{Eq. 9}$$

where $\delta\eta/N$ is a fractional change in the extent of the reaction relative to unity. Assuming $\delta\eta = 1$ mol, Eq. 9 can be written in its molar form

$$\Delta S = -R \cdot \ln([B]/[A]).$$

Entropic changes along a system reaction coordinate.

Figure 2B illustrates entropic changes along the reaction coordinate for a system of $N = 5$ molecules each occupying either molecular state A or B. When the system is in state {5,0}, $\Omega_{5,0} = 1$. Unlike in chemical activity models where $k_B \cdot \ln(0/5)$ is undefined, here the system entropy, $k_B \cdot \ln(1)$, is zero. After one net forward step, the system enters state {4,1}, increasing the number of microstates from 1 to 5 and increasing the system entropy from 0 to $k_B \cdot \ln(5)$. This increase in the number of microstates physically pulls the reaction forward since there are five-times more micropathways in the forward direction than in the reverse direction. This contrasts with a chemical activity of molecules that purportedly pushes a reaction forward. When $\Delta G^o = 0$, the reaction continues until a maximum system entropy is reached along the reaction coordinate, equilibrating in system state {3,2} when $(N_B + 1)/N_A = \exp(0)$ (Eq. 7).

Figure 3A is a plot of the reaction free energy (Eq. 5) along the system reaction coordinate, $\{N_A,N_B\}$, for $N = 10$ molecules. The reaction entropy, $k_BT \cdot \ln[N_B+1,N_A]$, increases logarithmically with each step whereas the molecular free energy, ΔG^o, is a constant offset (it is the same for each step). Figure 3A shows that when ΔG^o decreases from 0 to –1.5 k_BT (down arrow) the equilibrium state of the system changes from {5.5,4.5} to {2,8} (right arrow) corresponding to a change from $(N_B + 1)/N_A = \exp(0)$ to $(N_B + 1)/N_A = \exp(1.5)$. Because $\Delta_r G$ is the change in free energy, G, of the system with a chemical step (Eqs. 3 and 4), the free energy, G, of the system can be obtained by integrating Eq. 4 over the reaction coordinate. In Fig. 3B, the two plots in Fig. 3A are integrated and replotted as free energy, G. These are the system free energy landscapes described by Gibbs within which the reaction equilibrates at a point along the reaction coordinate $\{N_A,N_B\}$ where G is a minimum. Figure 3 clearly shows that entropic forces, $T\Delta S$, (Fig. 3A) drive a reaction down an entropic energy funnel, TS (Fig. 3B) toward equilibrium.

Figure 3B illustrates how changes in $\Delta G°$ affect the free energy, G, landscape. Changes in N_A and N_B, however, have no effect on the energy landscape because they are reaction coordinates, not chemical activities. In this way, an entropic chemical analysis radically transforms our understanding of chemical kinetics and energetics. Because chemical kinetics and energetics are defined by free energy landscapes, the physical presence of molecules, N_A and N_B, has no effect on the kinetics or energetics of a chemical reaction.

Equilibrium Energetics

At equilibrium ($\Delta_r G = 0$), the probability of finding the system in state $\{N_A+1, N_B-1\}$ relative to $\{N_A, N_B\}$ is $\exp[-\Delta G°/k_B T + \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})]$, where $\ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB}) = T\Delta S/k_B T$. Thus, when $\Delta G° = 0$, $\Omega_{NA,NB}$ is the probability density function, $P_{NA,NB}$. For comparison, according to chemical activity models the probability of finding a molecule in state B relative to state A is $\exp[-\Delta G°/k_B T]$, and when $\Delta G° = 0$, $P_{NA,NB}$ is a binomial distribution. The equilibrium constant, $K = (\Omega_{NA,NB}/\Omega_{NA-1,NB+1})_{eq} = \exp[-\Delta G°/k_B T]$ is $K = (N_{B(eq)} + 1)/N_{A(eq)}$ and describes the point along the reaction coordinate $\{N_A, N_B\}_{eq}$ at which the system equilibrates (Fig. 3). For comparison, in chemical activity models the equilibrium constant $K = N_{B(eq)}/N_{A(eq)}$ describes the physical distribution of N molecules within the energy landscape of a single molecule (Fig. 2A).

Non-Equilibrium Energetics

When the system is perturbed from equilibrium by a change in the internal energy of the system, δE, [i.e., a change in $\Delta G°$ or $k_B T \cdot \ln[(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})]$], the free energy equation becomes

$$\Delta_r G = [\Delta G° - k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})]_{eq} + \delta E = \delta E,$$

where δE is a non-equilibrium (ne) perturbation to any of the energy term on the right-hand side of Eq. 5. Here,

$$\delta E = [\Delta G° - k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})]_{ne}.$$

As illustrated in Fig. 3, if the system is perturbed by a change in $\Delta G°$, the system relaxes to a new equilibrium state, $\{N_A, N_B\}_{eq}$, as $k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})$ approaches the new $\Delta G°$.

Through this process, $\Delta_r G$ returns to zero and δE remains in the system in the form of a change in system entropy. If the system perturbation is a change in $k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})$ [an irreversible transfer of molecules between molecular states] the system relaxes back to the original equilibrium state, $\{N_A,N_B\}_{eq}$, as $k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})_{ne}$ approaches $\Delta G°$. Through this process the entropic δE is lost from the system as heat. This analysis becomes more complex when non-$P\Delta V$ work is performed, in which case, in addition to exchanges between $k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})$ and $\Delta G°$, δE can be exchanged with internal mechanical potentials; and in addition to being lost from the system as heat or stored in the system as entropy, δE can be lost from the system as work performed on the surroundings [2].

Equilibrium Kinetics

Chemical kinetics, like energetics, have both molecular and system components. The net rate at which the system transitions from chemical state $\{N_A,N_B\}$ to chemical state $\{N_A–1,N_B+1\}$ is

$$d\{N_A,N_B\}/dt = k_+ - k_-,$$

where k_+ and k_- are the forward and reverse transition rates between these states. At equilibrium, the probability of finding the system in state $\{N_A+1,N_B–1\}$ relative to state $\{N_A,N_B\}$ is

$$k_{+(eq)}/k_{-(eq)} = \exp[(-\Delta G° + k_B T \cdot \ln[\Omega_{NA-1,NB+1}/\Omega_{NA,NB}]_{eq})/k_B T] \text{ or}$$

$$k_{+(eq)}/k_{-(eq)} = (k°_+/k°_-) \cdot \exp[k_B T \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})_{eq}/k_B T].$$

Here, $k°_+$ and $k°_-$ are molecular contributions to k_+ and k_- and $\exp[-T\Delta S/k_B T]$ is the system contribution to k_+ and k_-. The influence of $-\Delta S/k_B$ on kinetics can be understood energetically as a tilt of the system landscape that adds to the tilt of the molecular landscape, $\Delta G°$. It can also be understood kinetically as

$$k_{+(eq)}/k_{-(eq)} = (k°_+/k°_-) \cdot (\Omega_{NA-1,NB+1}/\Omega_{NA,NB})_{eq}$$

where the factor $\Omega_{NA-1,NB+1}/\Omega_{NA,NB}$ describes the number of micropathways available for the forward reaction relative to the reverse reaction. For example, in Fig. 2B there are five micropathways from {5,0} to {4,1} and one micropathway back from {4,1} to {5,0}, which means that the forward reaction is entropically five-fold more likely than the reverse reaction ($\Omega_{NA-1,NB+1}/\Omega_{NA,NB} = 5$). This implies forward and reverse rate constants of

$$k_{+(eq)} = k^\circ_{+} \cdot \Omega_{NA-1,NB+1(eq)}$$
$$k_{-(eq)} = k^\circ_{-} \cdot \Omega_{NA,NB(eq)}.$$

At equilibrium, $k_{+(eq)} = k_{-(eq)}$, or

$$k^\circ_{+} \cdot \Omega_{NA-1,NB+1(eq)} = k^\circ_{-} \cdot \Omega_{NA,NB(eq)}.$$

To demonstrate consistency with the chemical activity approach,

$$k_{+(eq)} = k^\circ_{+} \cdot N_{A(eq)}$$
$$k_{-(eq)} = k^\circ_{-} \cdot (N_{B(eq)} + 1)$$

where here again $N_{A(eq)}$ and $N_{B(eq)}$ are not physical, active numbers of molecules, they are the system reaction coordinate $\{N_A,N_B\}_{eq}$ at which the system equilibrates.

Non-Equilibrium Kinetics

Chemical kinetics is fully determined from the molecular and system tilt of the system energy landscape. It follows that non-equilibrium kinetics is fully determined by the non-equilibrium tilt, δE, of these landscapes, where a non-equilibrium perturbation to ΔG° affects the molecular rates k°_{+}/k°_{-}, and an irreversible transfer of molecules between molecular states affects $\Omega_{NA,NB}/\Omega_{NA-1,NB+1}$. In general,

$$k_{+}(t)/k_{-}(t) = (k^\circ_{+}/k^\circ_{-})(\Omega_{NA-1,NB+1}(t)/\Omega_{NA,NB}(t)) = \exp[-\delta E(t)/k_B T]. \qquad \text{Eq. 10}$$

According to Eq. 10, when $\delta E(t) < 0$, $k_{+}(t) > k_{-}(t)$, consistent with the requirement that a negative $\delta E(t)$ drives the reaction forward. This forward reaction increases $T\Delta S$ (Fig. 3) until

$\delta E(t) = 0$, at which point $k_+(t) = k_-(t)$. When $\delta E(t) > 0$, $k_+(t) > k_-(t)$, consistent with the requirement that a positive $\delta E(t)$ drives the reaction backward. This reaction decreases $T\Delta S$ (Fig. 3) until $\delta E(t) = 0$, at which point $k_+(t) = k_-(t)$.

The non-equilibrium rate equation is the same as the equilibrium rate equation

$$d\{N_A,N_B\}/dt = k^\circ_+ \cdot \Omega_{NA-1,NB+1} - k^\circ_- \cdot \Omega_{NA,NB}(t).$$

For comparison with a chemical activity analysis, from above

$$dN_A/dt = k^\circ_+ \cdot N_A(t) - k^\circ_- \cdot (N_B(t) + 1) \qquad \text{Eq. 11}$$

where one last time, $N_A(t)$ and $N_B(t)$ describe the time dependence of steps along the system reaction coordinate $\{N_A,N_B\}$, not the time-dependence of the physical numbers of molecules in different states. Changes in $N_B(t) + 1$ are equal and opposite changes in $N_A(t)$ along the system reaction coordinate, and so

$$dN_A/dt = -N_A(t) \cdot [k^\circ_- + k^\circ_+]$$

which means that $N_A(t)$ decreases exponentially with time as

$$N_A(t) = N_{Ao} \cdot \exp(-(k^\circ_+ + k^\circ_-)t),$$

with a relaxation rate, $k^\circ_+ + k^\circ_-$, where N_{Ao} is the initial position on the reaction coordinate $\{N_A,N_B\}_o$.

Conclusion

In summary, a molecular energy landscape (Fig. 2A) a priori defines ΔG° and rate constants, $k°_+$ and $k°_-$, for an isolated molecule (Fig. 2A) independent of the physical presence of that molecule [13]. Similarly, an entropic energy landscape (Fig. 2B) a priori defines system energetics and rates, k_+ and k_-, for N molecules (Figs. 2B and 3) independent of the physical presence of those molecules. Together ΔG° and system entropy create a system energy landscape within which a reaction is energetically pulled down an entropic funnel (Fig. 3) until at equilibrium entropic forces are balanced against ΔG° (Eq. 5).

I have shown that non-equilibrium chemical kinetic equations (Eq. 10) can be derived from chemical thermodynamics, and that these equations have the same form as kinetic equations shown to accurately describe non-equilibrium kinetics. Claims that non-equilibrium kinetic equations cannot be derived from chemical thermodynamics are based on a chemical activity analysis in which N_A and N_B are not coordinates along the reaction energy landscape but are instead physical quantities of molecules that through their mere presence balance chemical reactions.

In addition to ΔS, other non-molecular (non-ΔG°) free energies that tilt the system reaction free energy landscape are easily incorporated into Eq. 10. For example, we have added the work term, F(t)d, to δE(t) in Eq. 10, and have shown that simulations of a binary mechanical model that incorporate these non-equilibrium kinetic rates accurately describe both the transient and steady state mechanics of active muscle. Under near-equilibrium conditions, these simulations converge with the thermodynamic relationship between ΔG°, N_A, N_B, and F described by Eq. 5 at $\Delta_r G = 0$. In short, the analysis presented herein provides a transformative entropic interpretation of reaction free energies that unites chemical kinetics and thermodynamics.

References and Notes

[1] J. E. Baker and D. D. Thomas, *A Thermodynamic Muscle Model and a Chemical Basis for A.V. Hill's Muscle Equation*, J Muscle Res Cell Motil. **21**, 335 (2000).

[2] J. E. Baker, *Thermodynamics and Kinetics of a Binary Mechanical System: Mechanisms of Muscle Contraction*, Langmuir **38**, 15905 (2022).

[3] J. E. Baker, *Cells Solved the Gibbs Paradox by Learning How to Contain Entropic Forces*, Arxiv **arXiv:2305**, (2023).

[4] J. E. Baker, I. Brust-Mascher, S. Ramachandran, L. E. LaConte, and D. D. Thomas, *A Large and Distinct Rotation of the Myosin Light Chain Domain Occurs upon Muscle Contraction.*, Proc. Natl. Acad. Sci. U. S. A. **95**, 2944 (1998).

[5] J. E. Baker, L. E. W. LaConte, I. Brust-Mascher, and D. D. Thomas, *Mechanochemical Coupling in Spin-Labeled, Active, Isometric Muscle.*, Biophys. J. **77**, 2657 (1999).

[6] J. E. Baker, C. Brosseau, P. B. Joel, and D. M. Warshaw, *The Biochemical Kinetics Underlying Actin Movement Generated by One and Many Skeletal Muscle Myosin Molecules.*, Biophys. J. **82**, 2134 (2002).

[7] J. E. Baker and D. D. Thomas, *Thermodynamics and Kinetics of a Molecular Motor Ensemble.*, Biophys. J. **79**, 1731 (2000).

[8] J. C. Phys and D. E. Hanson, *The Molecular Kink Paradigm for Rubber Elasticity : Numerical Simulations of Explicit Polyisoprene Networks at Low to Moderate Tensile Strains of Explicit Polyisoprene Networks at Low to Moderate Tensile Strains*, **135**, 054902 (2011).

[9] M. S. Kellermayer, S. B. Smith, H. L. Granzier, and C. Bustamante, *Folding-Unfolding Transitions in Single Titin Molecules Characterized with Laser Tweezers*, **276**, 1112 (1997).

[10] C. Kittel and H. Kroemer, *Thermal Physics* (1980).

[11] J. Quílez, *On the Early Thermodynamic and Kinetic Deductions of the Equilibrium Constant Equation*, Found. Chem. **23**, 85 (2021).

[12] L. Styer, *Biochemistry* (W.H. Freeman and Company, New York, 1995).

[13] H. Frauenfelder, S. G. Sligar, P. G. Wolynes, H. Frauenfelder, S. G. Sligar, and P. G. Wolynes, *The Energy Landscapes and Motions of Proteins*, **254**, 1598 (1991).

[14] J. Howard, *Mechanics of Motor Proteins and the Cytoskeleton*, 1st ed. (Sinauer Associates, 2001).

[15] T. L. Hill, *Free Energy Transduction and Biochemical Cycle Kinetics* (Springer-Verlag, New York, 1989).

[16] J. Gibbs, *Elementary Principles in Statistical Mechanics Developed with Especial Reference to the Rational Foundation of Thermodynamics* (1902).

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Fig. 1. Entropic Force in a Binary Mechanical System. (A) A reversible molecular switch undergoes a discrete conformational change from chemical state A to B displacing compliant elements external to the switch a distance, *d*. The switch occurs with forward and reverse rates, $k°_{+}$ and $k°_{-}$. **(B)** An ensemble of molecular switches constitutes a binary mechanical system where, according to Le Chatelier's principle, a force applied to the system reverses the force generating chemistry of the molecular switches.

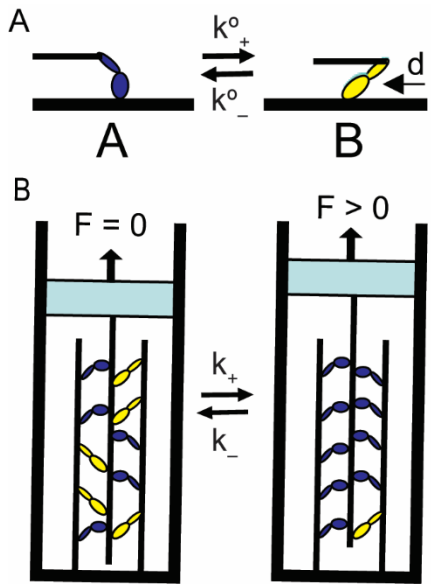

Fig. 2. The molecular and system contributions to the reaction free energy. (A) The molecular contribution to the reaction free energy is described by a molecular energy landscape. The free energy difference between the two metastable states, A and B, is $\Delta G°$. The rate, $k°_{+}$, at which a molecule transitions from molecular state A to B varies exponentially with $-G^{\ddagger}_{+}$, where $G^{\ddagger}_{+}$ is the height of the activation barrier in the forward direction. The rate, $k°_{-}$, at which a molecule transitions from B to A varies exponentially with $-G^{\ddagger}_{-}$, where $G^{\ddagger}_{-}$ is the height of the activation energy barrier in the reverse direction. (**B**) The system contribution to the reaction free energy is entropic. For $N = 5$, there are 6 system states, $\{N_A,N_B\}$, along the reaction coordinate each with $\Omega_{NA,NB} = N!/(N_A!N_B!)$ microstates, illustrated with circles, and entropy $k_B \cdot \ln(\Omega_{NA,NB})$. There are five micropathways (right arrows) by which a molecule in state {5,0} can transition to state {4,1}, and one micropathway (left arrow) by which a molecule in state {4,1} can transition to state {5,0}.

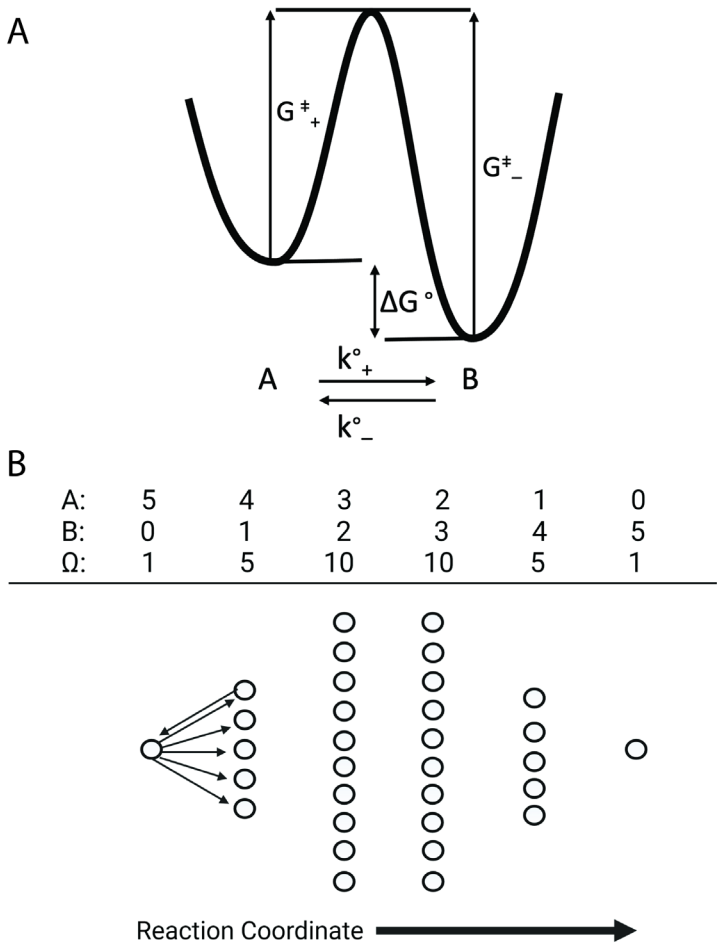
A
G ‡+
G‡−
ΔG °
A
k°+
B
k°−
B
A: 5 4 3 2 1 0
B: 0 1 2 3 4 5
Ω: 1 5 10 10 5 1
Reaction Coordinate

Fig. 3. **Change in Free energy along a reaction coordinate. (A)** For N = 10, Eq. 3 is plotted for a $\Delta G°$ of 0 (green line) and –1.5 k_BT (blue line). For $\Delta G° = 0$, the reaction equilibrates ($\Delta_r G = 0$) at {5.5,4.5}. When $\Delta G°$ is decreased from 0 to –1.5 k_BT (down arrow) the system re-equilibrates (right arrow) at {2,8}. **(B)** The system free energy, G, (the integral of $\Delta_r G$ over the reaction coordinate) is calculated and plotted for $\Delta G°$ of 0 (green line) and –1.5 k_BT (blue line) values in panel A. The same trajectory in panel A describing re-equilibration following a decrease in $\Delta G°$ is illustrated in panel B (arrows).