The Kinetic and Energetic Pull of Chemical Entropy

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Abstract: Single molecule mechanics studies clearly show that the molecular mechanism of muscle contraction is a force-generating myosin motor switch. However, muscle mechanics and energetics cannot be accounted for by summing up the force-generating chemical steps of independent myosin motors – the energetic contribution of the gradient of myosin motors across the force-generating chemical step is required. Chemistry (i.e., the Gibbs free energy equation) describes this energetic contribution as a chemical activity, whereas statistical mechanics describes it as entropic. Here, I show that while mathematically these two energetic terms are similar, physically they are fundamentally different. The entropic interpretation implies a novel thermodynamic model of chemical kinetics in which a chemical reaction is pulled down the entropic energy landscape of an ensemble of molecules rather than being pushed through mass action. I show that the transition from chemical activity to entropy is physical and occurs when thermal fluctuations isolated within N independent molecules become distributed among those molecules. With this transition chemical activity is lost when the N degrees of freedom of independent molecules physically and irreversibly collapse into one ensemble system within which heat is delocalized among the *N* molecules in the form of entropy.

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

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 biomolecules. The question remains, to what extent is biological function the sum of these molecular parts versus an emergent property of systems of molecules? Single molecule structure and mechanic studies clearly demonstrate that the molecular mechanism of muscle contraction is a force-generating molecular switch [1–4]. An ensemble of force-generating molecular switches is a binary mechanical model that accurately describes most aspects of muscle contraction [5,6]. According to this model, muscle power output occurs with the shortening of an entropic spring (the ensemble of switches), and muscle force is the force of that entropic spring. The entropic force is described by the Gibbs free energy equation, where the gradient term is entropic and not a chemical activity [5–7].

Here, I compare the chemical activity and entropic energetic terms, and show that while they are mathematically similar, they describe fundamentally different physical systems. Chemical activities describe thermally isolated molecules, and the entropic energy term describes a molecular ensemble within which heat is delocalized among the molecules. In a molecular ensemble analysis chemical reactions are pulled down a system reaction entropic energy well whereas for thermally isolated molecules chemical reactions are pushed by mass action.

For a system of molecules held at a constant temperature and pressure, the Gibbs free energy, G_i, of chemical state i can in general be written, $G_i = G^o_i + k_BT\cdot\ln\Omega_i$, where G^o_i is the free energy of one molecule in state i, often referred to as the standard free energy, and $k_BT\cdot\ln\Omega_i$ is an emergent system (non-molecular) free energy. Unless otherwise stated, here energy terms (e.g., G_i) have units (k_BT) of energy per molecule, which is related to the molar units (RT) of energy per mol as $RT = N_{AV}k_BT$, where N_{AV} is Avogadro's number. In a conventional chemical analysis, $k_BT\cdot\ln\Omega_i$ is a chemical potential where Ω_i is a chemical activity that is often equated with the number of molecules in state i. In an entropic analysis, $k_B\cdot\ln\Omega_i$ is a system entropy where Ω_i is the number of microstates in chemical state i. Clearly this analysis does not apply to corpuscular models of chemical mechanics in which the system term, $k_BT\cdot\ln\Omega_i$, is arbitrarily removed from the free energy equation for the sole purpose of making the energy of the system a simple sum of the free energy of its molecular (corpuscular) parts, or $G_i = G^o_i$. While corpuscular models have a molecularly explicit appeal, they are non-physical [8] and clearly have little

relevance to the question that is the focus of this physical chemical analysis. Is Ω_i a chemical activity of or the number of microstates within state i.

Figure 1 illustrates a binary mechanical system [6]. 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*, exerted on 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 [1–3]. 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 [2,9]. Moreover we have shown that this binary mechanical model accurately accounts for the basic mechanics and energetics of muscle contraction [5,6].

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 force exerted on binary mechanical systems (e.g., muscle, rubber, titin) is entropic [6,10,11], $k_B T \cdot \ln(N_B/N_A)$ is entropic energy. While this is a classic statistical mechanic description of a binary system [12], it is at odds with conventional chemistry where the term $k_B T \cdot \ln(N_B/N_A)$ is assumed to be a chemical activity not entropy. Here, I consider these two fundamentally different descriptions of the term $k_B T \cdot \ln(N_B/N_A)$. In conventional chemical analyses, 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.

Here, I show that even in non-mechanical ($F = 0$) chemical systems, $k_B T \cdot \ln(N_B/N_A)$ is entropic not a chemical activity. This represents a significant change in 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

For a system of N molecules like those in Fig. 2A, the system state, $\{N_A,N_B\}$, describes an ensemble of molecules thermally distributed between states A and B within a probability density function (defined below) centered on $N_A = N - N_B$. The extent of a forward reaction, $\delta\eta$, (the reaction coordinate) changes the state of the system $\{N–\delta\eta,\delta\eta\}$ when both the number of molecules in state B increases by $\delta\eta$ and the system equilibrates within the probability density function for $\{N–\delta\eta,\delta\eta\}$.

The free energy, G, of this system increases with $\delta\eta$ along the reaction coordinate 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, $F\cdot d\cdot\delta\eta$ is the net work performed by $\delta\eta$ displacements, d, of a system spring with a force, F, and S is the system entropy in state$\{\delta\eta,N–\delta\eta\}$. The change in the system free energy, δG, along the reaction coordinate, $\delta\eta$, is the reaction free energy, $\Delta_r G$, which from Eq. 3 is

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

where ΔS is the reaction entropy, $\delta S/\delta\eta$. This is the Gibbs free energy equation. 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.

The same equation (Eq. 4) is obtained in a conventional chemical activity analysis, only in this case N_A and N_B are not reaction coordinates but rather physical numbers of molecules that define the state of the system. In a chemical activity analysis, the chemical potential difference between molecular states, $\mu_B - \mu_A = \Delta G° + k_B T\cdot\ln(N_B/N_A)$, somehow contains these physical numbers of molecules; and $T\Delta S = 0$. In contrast, in an entropic analysis the chemical potential difference between molecular states, $\mu_B - \mu_A = \Delta G°$, is defined within a single molecule as defined in corpuscular models, and $T\Delta S = –k_B T\cdot\ln(N_B/N_A)$. In both cases, $\Delta G°$ is the standard free energy and the reaction 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 here is whether $k_B T\cdot\ln(N_B/N_A) = \mu_B - \mu_A - \Delta G°$ as described in chemical activity models or whether $–k_B T\cdot\ln(N_B/N_A) = T\Delta S$ as described in entropic models (see below).

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

According to Eq. 3, $\mu_B - \mu_A$ describes a change in system free energy with each molecular transition, $\delta\eta$. The change in the free energy of a molecule with a chemical transition is defined by the energy landscape of that molecule (Fig. 2A), which a priori describes changes in free energy along a molecule's conformational coordinate [13]. According to transition state theory, the inverse times to diffuse in both forward, $k°_+$, and reverse, $k°_–$, directions over an activation energy barrier separating states A and B (Fig. 2A) are related to $\Delta G°$ as $k°_+/k°_- = \exp(–\Delta G°/k_B T)$ [14], where $k°_+$ and $k°_–$ are forward and reverse rate constants and $\Delta G°$ is the standard reaction free energy [15]. The standard reaction enthalpy, $\Delta H°$, and entropy, $\Delta S°$, contribute to the standard free energy as $\Delta G° = \Delta H° – T\Delta S°$. However, $\Delta S°$ differs from ΔS in Eq. 4. The former describes the entropic change of a molecule with an A to B transition, whereas the latter describes the entropic change of a system of molecules with a $\{N_A,N_B\}$ to $\{N_A–1,N_B+1\}$ transition. The implication that the definition of entropy changes with scale is discussed below.

The molecular energy landscape (Fig. 2A) describes all molecular contributions to the system free energy, which is to say $\mu_B - \mu_A = \Delta G°$, as described in an entropic analysis and proposed by T.L. Hill [16]. The energy $k_B T \cdot \ln(N_B/N_A)$ (whether physical numbers of molecules or system entropy) is not contained within the energy landscape of a molecule, and so the system free energy does not increase by $k_B T \cdot \ln(N_B/N_A)\delta\eta$ with $\delta\eta$ molecular transitions (Eq. 3), as assumed in a conventional chemical activity analysis.

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, $\{N_A,N_B\}$ [12,17]. 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 forward molecular transitions, Eq. 6

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

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

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

$\Delta_r G = \Delta G° + k_B T \cdot \ln[(N_B + 1)/(N_A)]$ for a forward molecular transition Eq. 7

$\Delta_r G = \Delta G° + k_B T \cdot \ln[(N_A + 1)/(N_B)]$ for a reverse molecular transition

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

While these equations are consistent with a conventional chemical activity analysis, I show that 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 balance chemical reactions. Instead, 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. 2B and 3 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 $\{N_A-1,N_B+1\}$ gives the equations for ΔS above,

$$\Delta S = -k_B\cdot\ln[((N_A - 1)!(N_B + 1)!)/(N_A!N_B!)] = -k_B\cdot\ln[(N_B + 1)/N_A] \text{ for a forward step, and}$$

$$\Delta S = -k_B\cdot\ln[((N_A + 1)!(N_B - 1)!)/(N_A!N_B!)] = -k_B\cdot\ln[(N_A + 1)/N_B] \text{ for a reverse step.}$$

It is not insignificant that in both cases because of the "+ 1" in the numerator ΔS is defined when $N_B = 0$ for a forward reaction and when $N_A = 0$ for a reverse reaction. This is not true for a chemical activity analysis where the free energy change in the forward direction, $k_BT\cdot\ln[N_A/N_B]$, is not defined when $N_B = 0$, and the free energy change in the reverse direction, $k_BT\cdot\ln[N_B/N_A]$, is not defined when $N_A = 0$. According to these equations, as required for a reversible step, the net ΔS for a forward step from $\{N_A,N_B\}$ to $\{N_A-1,N_B+1\}$ followed by a reverse step from $\{N_A-1,N_B+1\}$ to $\{N_A,N_B\}$ is zero.

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

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

which defines an average change in system entropy, $k_BT\cdot\ln[(N_B + \delta\eta/2)/(N_A - \delta\eta/2)]$, over $\delta\eta$ steps. The change in system free energy, δG, with $\delta\eta$ steps is then

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

Here the reaction is driven both by the change in molecular free energy,·$\Delta G°$, summed over $\delta\eta$ molecular transitions from A to B and by the average change in system entropy 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 purportedly pushing 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 and $F = 0$. 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 Δ_rG 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. 5 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 the 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 ($\Delta_rG = 0$), the probability of finding the system in state $\{N_A+1,N_B–1\}$ relative to $\{N_A,N_B\}$ is $\exp[–\Delta G°/k_BT + \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})]$, where $\ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB}) = T\Delta S/k_BT$. When $\Delta G° = 0$, $\Omega_{NA,NB}$ is the probability density function, $P_{NA,NB}$. The equilibrium constant, $K = (\Omega_{NA,NB}/\Omega_{NA-1,NB+1})_{eq} = \exp[–\Delta G°/k_BT]$ 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 $k_BT\cdot\ln[(\Omega_{NA-1,NB+1}/\Omega_{NA,NB})]$], the free energy equation becomes

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

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 [6].

Equilibrium Kinetics

Chemical kinetics, like energetics, have both molecular and system components. The molecular component is the molecular kinetic rate constants, $k°_-$ and $k°_+$, defined a priori (independent of the occupancy of the landscape) by the molecular energy landscape in Fig. 2A. Here I show that the system kinetic component is the number of microstates into which the system is pulled, defined a priori (independent of the occupancy of the landscape) by the system energy landscape in Figs. 2B and 3. This contrasts with a chemical activity analysis in which the system kinetic component is a physical number of molecules that occupy and push molecules out of a state in a molecular landscape. I show that the two approaches are mathematically similar, but only the former entropic analysis allows one to simulate non-equilibrium kinetics in a thermodynamic system.

In general, 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. Again, this is not the rate at which molecules transition between states with units of number of molecules per second, it is the rate at which the system transitions between ensemble chemical states along the reaction coordinate $\{N_A,N_B\}$ of the system energy landscape (Figs. 2B and 3) with units of chemical states per second. If $k_+ > k_-$, the reaction progresses left to right along the reaction coordinate of system energy landscape at a positive rate $k_+ - k_-$. If $k_+ < k_-$, the reaction progresses right to left along the reaction coordinate of the system energy landscape at a positive rate $k_- - k_+$. If $k_+ = k_-$, the reaction is at equilibrium.

At equilibrium, the probability of finding the system in state $\{N_A+1,N_B-1\}$ relative to state $\{N_A,N_B\}$ is

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

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

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, ΔG°. It can also be understood kinetically as

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

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

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

Recall that

$\Delta S = -k_B \cdot \ln(\Omega_{NA,NB}/\Omega_{NA-1,NB+1}) = -k_B \cdot \ln [(N_{B(eq)} + 1)/ N_{A(eq)})$ for a forward step and

$\Delta S = -k_B \cdot \ln(\Omega_{NA-1,NB+1}/\Omega_{NA,NB}) = -k_B \cdot \ln [(N_{A(eq)} + 1)/ N_{B(eq)})$ for a reverse step

and on comparison,

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

which is mathematically consistent with a chemical activity analysis, only here again $N_{A(eq)}$ and $N_{B(eq)}$ are not physical numbers of molecules but 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

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

where $N_A(t)$ 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 $\{N_A,N_B\}_o$.

Figure 4 summarizes the energetic and kinetic differences between a collection of 5 independent molecules (Fig. 4A) and an ensemble of 5 molecules (Fig. 4B). Figure 4A illustrates five independent molecular switches (Fig. 1A), where thermal fluctuations are isolated within each independent molecule and occur within five independent molecular energy landscapes (Fig. 2A). In this case, each molecule is its own independent thermodynamic system. Figure 4B illustrates an ensemble of five molecular switches where thermal fluctuations are delocalized among the five molecules and occur within one ensemble molecular energy landscape (Fig. 3B). In this case, the five molecules are contained within one thermodynamic system.

Figure 4C illustrates a single state transition from A to B of one switch out of five independent switches. The forward and reverse rates for this reaction, $4k°_{+}$ and $3k°_{-}$, depend on the number of molecules in states A and B. The ratio of these rates is $(4/3)\cdot(k°_{+}/k°_{-})$, and the five independent switches in Fig. 4C are at equilibrium only if $k°_{+}/k°_{-}$ equals ¾.

Figure 4D illustrates a single state transition from A to B of one switch in an ensemble of five switches. The forward and reverse rates for this reaction do not depend on the number of molecules in states A and B, and according to Eq. 10 the ratio of these rates is $(10/5)\cdot(k°_{+}/k°_{-})$, which is at equilibrium only if $k°_{+}/k°_{-}$ equals ½.

There is clearly a physical difference between the five switches in Figs. 4A and the five switches in 4B. It is then no surprise that the transition from the five independent molecules in Fig. 4A to the single ensemble of five molecules in Fig. 4B is a physical transition. This transition occurs when thermal fluctuations are no longer localized within individual molecules but are delocalized among those molecules in the form heat contained within (an increase in entropy of) the ensemble. This increase in entropy [a gain of $\Omega_{NA,NB}$] results from an irreversible collapse in the $N = N_A + N_B$ degrees of freedom [a loss of N_A and N_B] when the single ensemble of five molecules is formed from five thermally isolated molecules.

Conclusion

A change in thermal scale is an irreversible physical process through which a thermodynamic system is redefined on a different spatial scale. On the thermal scale of a protein, an individual protein, while composed of many structural elements, is a thermodynamic system defined energetically by that protein's entropic energy well (Fig. 2A). On a smaller thermal scale, the structural elements of that protein when isolated are each thermodynamic systems defined energetically by their own entropic energy wells [18]. On a larger thermal scale, a protein ensemble is a thermodynamic system defined energetically by its own entropic energy well (Fig. 3B).

On any given thermal scale, the energy landscape of the system is a priori defined independent of the occupancy of the states of its constituent parts. In a single protein system, the kinetics and energetics of the protein are described by constants ($\Delta G°$, $k°_{+}$ and $k°_{-}$) that are defined in terms of its reaction energy landscape (Fig. 2A) independent the occupancy of the states of the constituent structural elements. Likewise, in an ensemble protein system, the

423 kinetics and energetics of the protein ensemble are described by constants
424 [$\Delta G° - k_B T \cdot \ln(N!/N_A!N_B!)$, k_+ and k_-] that are defined a priori in terms of its reaction energy
425 landscape (Fig. 3B) independent of the occupancy of the states of proteins.
426 The transition between thermal scales is not a reversible mental exercise. It is an
427 irreversible physical process that occurs when thermal fluctuations isolated within the energy
428 landscapes of the individual constituent parts of a system become thermal fluctuations among
429 those constituent parts in the system, at which point the energy landscapes of the constituent
430 parts are no longer defined. In other words, these landscapes physically collapse into the
431 landscape of the system of constituent parts defined on the larger thermal scale. This collapse
432 involves the loss of the degrees of freedom (the landscapes) of the constituent parts as well as a
433 corresponding gain in entropic energy associated with heat delocalized among the constituent
434 parts in the larger system. The entropic gain describes the number of ways (i.e., the number of
435 microstates) constituent states can be occupied in a given system state. A transition to a larger
436 thermal scale is thermodynamically irreversible because of the associated increase in entropy.
437 The cognitive challenge associated with the irreversibility of increasing scale is embodied by
438 both Maxwell's demon (on the energy scale of heat) and Schrodinger's cat (on the energy scale
439 of light).
440 Two thermal scales cannot exist at the same time. Either thermal fluctuations are isolated
441 within individual molecules or thermal fluctuations occur among those molecules. A collection
442 of individual molecules irreversibly becomes an ensemble of molecules when thermal
443 fluctuations isolated within individual molecules become thermal fluctuations among those
444 molecules (Fig. 4). In biological systems this occurs across all thermal scales from amino acids
445 to macromolecular structures, which implies that the formation of macromolecular structures is
446 entropically driven [18]. Thus, this analysis provides a new approach for studying biological
447 processes ranging from protein folding to self-assembly to macromolecular function (e.g.,
448 muscle contraction). It is easy to understand how thermal fluctuations among structural
449 components occur when those components are contained within a common macromolecular
450 structure. It is also possible, however, that thermal fluctuations among system components occur
451 in general when those components are contained within the same macroscopic compartment,
452 where thermal energy is exchanged through the solution within or through the walls of that
453 compartment.

In addition to $k_B T \cdot \ln(N!/N_A!N_B!)$, other non-molecular (non-$\Delta G°$) free energies that tilt the system reaction free energy landscape can be incorporated into Eq. 10. For example, a binary mechanical model of muscle includes a work term, $F(t) \cdot d$, in Eq. 10, and these non-equilibrium kinetic rates accurately describe many aspects of both transient and steady state mechanics of active muscle [6,19,20]. Under near-equilibrium conditions, these simulations converge with the thermodynamic relationship between $\Delta G°$, N_A, N_B, and F described by Eq. 5 at $\Delta_r G = 0$ [5,6].

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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.

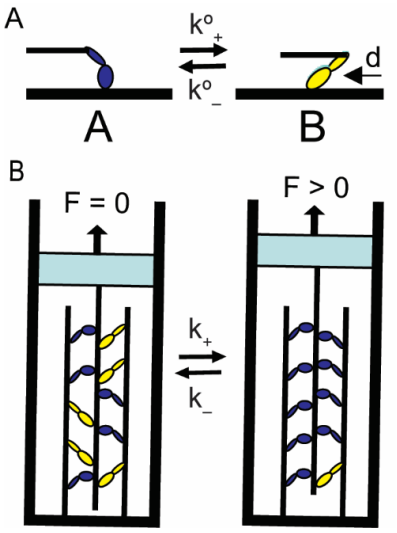

Fig. 2. The molecular and system contributions to the reaction free energy. (A) The molecular contribution to the reaction free energy is described by a molecular energy landscape. The free energy difference between the two metastable states, A and B, is $\Delta G°$. The rate, $k°_{+}$, at which a molecule 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}.

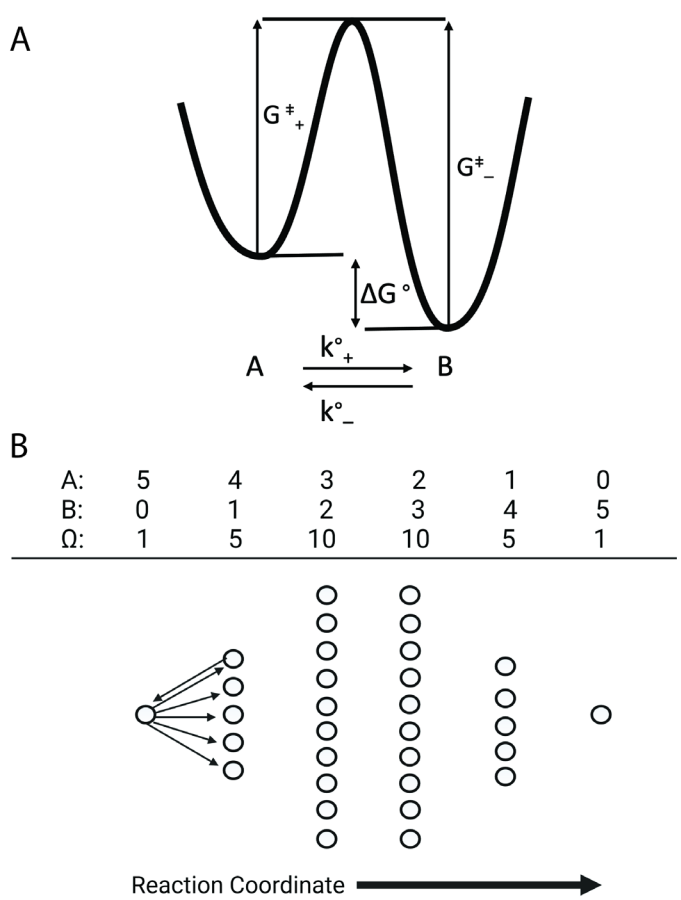

Fig. 3. **Change in Free energy along a reaction coordinate. (A)** Equation 5 (N = 10 and F = 0) is plotted for a ΔG° of 0 (green line) and –1.5 k_BT (blue line). For ΔG° = 0, the reaction equilibrates (Δ_rG = 0) at {5.5,4.5} (x-axis = 4.5, y-axis = 0). When ΔG° decreases from 0 to –1.5 k_BT (down arrow) the system re-equilibrates (right arrow) at {2,8} (x-axis = 8, y-axis = 0). **(B)** The system free energy, G, (integrals of Δ_rG in panel A over the reaction coordinate) is plotted for ΔG° of 0 (green line) and –1.5 k_BT (blue line). The same trajectory in panel A describing re-equilibration following a decrease in ΔG° is illustrated in panel B (arrows).

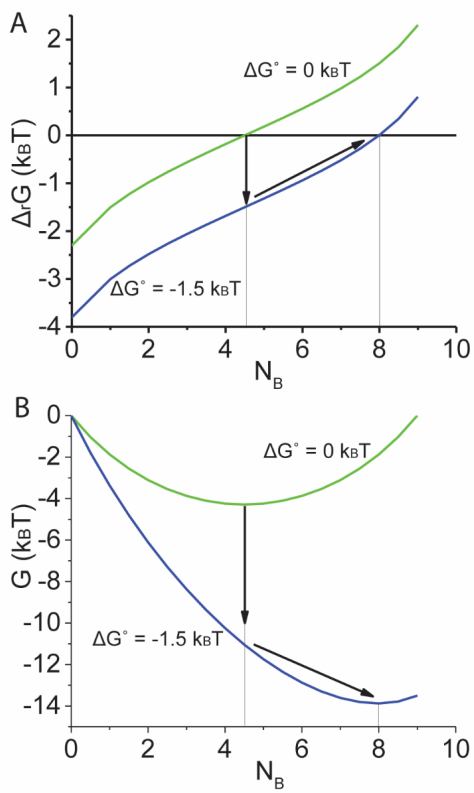
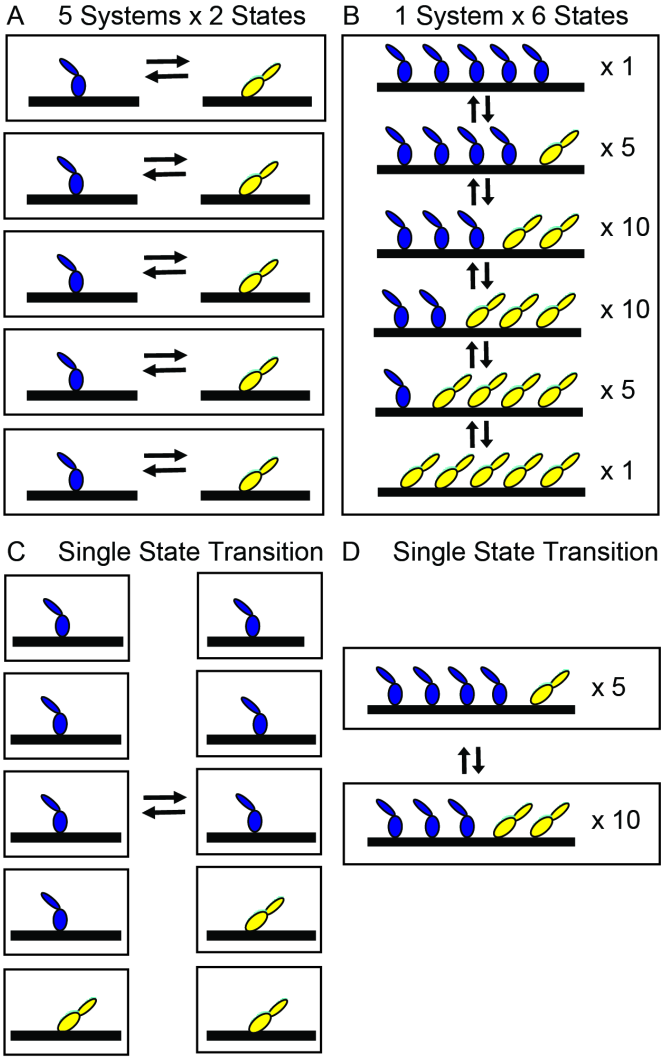
Fig. 4. **Five independent molecular switches versus an ensemble of five molecular switches.** **(A)** Thermal fluctuations isolated within five independent molecular switches (Figs. 1A and 2A) are described as five thermodynamic systems each with two states. **(B)** Thermal fluctuations among five molecular switches (Figs. 1A and 3B, only with five molecules) are described as one ensemble thermodynamic system having six states within which there are 1, 5, or 10 microstates (right). (C) A single state transition for five independent molecular switches. (D) A single state transition for an ensemble of five molecular switches.