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An Asymptotic Comparative Analysis of the Thermodynamics of Non-Covalent Association

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Abstract. There is an ambiguity in the theoretical models for computing association constants, the key observable in a laboratory, of non-covalent associations. We show that three different models give unique result asymptotically in the limit of strong associate. For weak associations, the disagreement reflects the nature of ill-defined "associated complex" which can be defined, among various ways, either geometrically or thermodynamically depending on measurement techniques. Furthermore, even when the free energy of association is unique, the corresponding entropy and enthalpy can still be different from different types of measurements – a surprising source of entropy-enthalpy compensation. This work provides a mathematical basis for modeling non-covalent association processes in biology.

1. Introduction

Noncovalent association between proteins and small ligands in aqueous solution is a fundamental physical process in biology. The energetics, more precisely the free energy, associated with such a process, hence, is of central importance in biochemistry [36]. Still, several seemingly different theories of binding exist. In this paper we discuss three different formulations for calculating the binding free energy. In order to elucidate the main features in each formalism, we only consider dilute concentrations for the protein and the ligand, and between them a simple spherical symmetric intermolecular potential $U(r)$ shown in Fig. 1. Using Laplace's method for integrals, we show that these different formulae are asymptotically equivalent in the limit of tight-binding. Since the work of Kramers [21] it has been known that two-state transition with Arrhenius kinetics is only a limiting behavior with a sufficiently large ($\gg kT$) activation energy barrier. Therefore for weak binding at finite temperature, the subtle differences in the various formulae are indeed expected and even biochemically meaningful.

This work is motivated by the recent surging activity in direct measurement of noncovalent intermolecular forces between proteins and ligands in aqueous phase with atomic force microscopy (AFM) [11,23,6,22]. A quantitative theory is now required to correlate these single-molecule measurements with the more traditional measurements, and calculating the binding energy. In return, these

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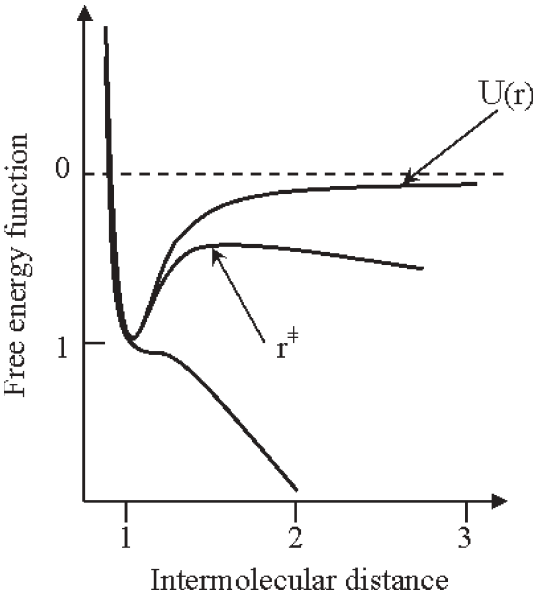

Fig. 1. Intermolecular energy function $U(r)$ and free energy function (the potential of mean force) $F(r)$ between a protein and a ligand as functions of temperature T.From top to bottom: $kT = 0$, $kT/V_0 = 0.5$, and $kT/V_0 = 1.5$.When $T = 0$, $F(r) = U(r)$. The ordinate is in unit V_0, the bond energy, and the abscissa is in unit r_0, the bond length.Both the height and the position ($r^{\ddagger}$) of the free energy barrier decrease with increasing temperature.The barrier disappears all together when $kT/V_0 > 1.5$

measurements provide experimental tests for the intermolecular potentials one uses in computational biochemistry [13,18], and increase the reliability of computing binding free energy based on potential of mean force at a mesoscopic level [12,2].

We show that the differences in free energy of binding, according to different definitions, are asymptotically small for tight-binding. The corresponding differences in enthalpy and entropy of binding – two important components of the binding energy – however, are significantly larger. This result can be understood and further explored in terms of the theory of entropy-enthalpy compensation [29,25]. Mechanistically, we find that the differences are due to factors such as temperature dependence of the location of activation barriers and characteristics of the potential of mean force $U(r)$.

2. Three Formulations and their equivalence for tight-binding

Diffusion formalism of spherical association. The stochastic diffusion of a ligand and its interaction with a protein can be represented by a diffusion equation with the presence of a force field [21]. If the ligand-protein interaction is spherical, then in spherical coordinates, one has

$$\frac{D}{r^2}\frac{d}{dr}r^2\left(\frac{dP(r)}{dr}+\frac{1}{kT}\frac{dU(r)}{dr}P(r)\right)=0. \tag{1}$$

The equilibrium solution to the equation gives the probability distribution of the ligand around the protein:

$$P(r) = Ce^{-U(r)/kT}, \tag{2}$$

where T is temperature in Kelvin, k is the Boltzmann constant, and C is a normalization factor

$$C^{-1} = \int_0^\infty 4\pi r^2 e^{-U(r)/kT} dr.$$

In the spherical coordinates, the probability for r is

$$4\pi r^2 P(r) = C \exp\left[-\frac{U(r) - kT \ln\left(4\pi r^2\right)}{kT} \right] \tag{3}$$

Preferential binding. This approach has its origin in Debye-Hückel's theory for ion binding based on linear Poisson-Boltzmann equation [15]. Later, Kirkwood and Buff [20] and Schellman [33–35] have repeatedly emphasized the importance in viewing molecular association as having *excess* number of ligands in the neighborhood of a protein, and the central role of concentration fluctuations in the theory of weak binding, where the partners in a "complex" need not to be even physically in contact. Rather they are interactive via intermolecular forces. For very weak binding, the conventional stoichiometric binding model should be replaced by the theory of preferential interaction [35]. This theory emphasizes the occupancy of solvent molecules at the binding site, and the ligand binding is accompanied with exchanging ligand with the solvent molecules. The binding should be defined thermodynamically through osmotic pressure. The general relation between the thermodynamics of ligand binding and molecular interaction is formalized in the Kirkwood-Buff theory [20]. This thermodynamic view of binding, which significantly deviates from the traditional thinking for ligand binding, leads to

$$K_1 = \int_0^\infty \left(e^{-U(r)/kT} - 1 \right) 4\pi r^2 dr \tag{4}$$

where the equilibrium association constant K_1 is defined as the concentration ratio $\frac{[PL]}{[P][L]}$ in equilibrium (we use the chemistry convention $[\cdot]$ for the concentration of a chemical species), and $\frac{[PL]}{[P]}$ is the ratio between the probabilities of bound and free proteins at a given ligand concentration $[L]$. K_1 has the dimension of a volume, thus $K_1[L]$ is dimensionless. Without loss of generality, we will assume that the integral in (4) always converges. This is certainly true for most of the $U(r)$ in biochemistry. We observe that K_1 is intimately related to the definition for the species PL, the protein-ligand complex. For weak interaction, different experimental methods can have different operational definitions for a complex.

Kramers' theory with transition state. This second formulation has its origin in the theory of chemical kinetics. It requires precise geometric definitions (as an alternative to the thermodynamic one above) for a bound and a free ligand. This gives rises to the concept of transition state, i.e., there is an energy maximum between the two states of the ligand. The association-dissociation equilibrium is a balance between intermolecular energy and the translational entropy of the ligand. Here one first introduces, as Bjerrum [5] did for salt dissociation, a "free energy function" (Fig. 1)

$$F(r) = U(r) - kT \ln 4\pi r^2 \tag{5}$$

and from which, the transition state $r^{\ddagger}$ is identified as the maximum of $F(r)$. Therefore,

$$K_2[L] = \frac{\int_0^{r^{\ddagger}} e^{-U(r)/kT} 4\pi r^2 dr}{\int_{r^{\ddagger}}^{r_c} e^{-U(r)/kT} 4\pi r^2 dr} \tag{6}$$

where r_c is the mean distance between two ligand molecules. It is inversely related to the concentration of the ligand: $4\pi r_c^3/3 = 1/[L]$. This treatment is known as the cell model for binary solutions [15]. This free energy landscape point of view has been further developed by Hill [17] and Szabo et al. [39] and applied to the study of simple protein folding kinetics [41,9]. More recently, Bjerrum [5] and Landau's [8] approach was also shown to be consistent with the solution of nonlinear Poisson-Boltzmann equation [31].

K_2 can be simplified if we assume dilute ligand concentration. With $r_c \to \infty$, we have

$$\begin{aligned}
\int_{r^{\ddagger}}^{r_c} e^{-U(r)/kT} 4\pi r^2 dr &= \frac{4}{3}\pi r_c^3 - \frac{4}{3}\pi (r^{\ddagger})^3 + \int_{r^{\ddagger}}^{r_c} \left(e^{-U(r)/kT} - 1\right) 4\pi r^2 dr \\
&\sim \frac{4}{3}\pi r_c^3 - \frac{4}{3}\pi (r^{\ddagger})^3 + \int_{r^{\ddagger}}^{\infty} \left(e^{-U(r)/kT} - 1\right) 4\pi r^2 dr \\
&= \frac{4}{3}\pi r_c^3 \left\{ 1 + O\left[\left(\frac{r^{\ddagger}}{r_c} \right)^3 \right] \right\}
\end{aligned}$$

Note the integral converges. Therefore,

$$K_2 \approx \int_0^{r^{\ddagger}} e^{-U(r)/kT} 4\pi r^2 dr. \tag{7}$$

Binding with arbitrary cutoff. In reality, a third formulation has been widely used in the computation of protein-ligand binding. It has its origin in the McMillan and Mayer's solution theory, with further development by Hill [14]. For a recent review see [12]:

$$K_3 = \int_0^{r_\xi} e^{-U(r)/kT} 4\pi r^2 dr. \tag{8}$$

One unsatisfying aspect of this formulation is, even conceptually, the arbitrary "cutoff" r_ξ for defining protein-ligand complex. It is well-know, however, that the computation of binding energy is insensitive to this arbitrary cutoff if the binding is tight.This can be shown as follows.

The general requirement for choosing r_ξ is that $r_\xi \gg r_0$, at which the energy function $U(r)$ is at its minimum $U(r_0) = -V_0 < 0$ [14,12]. V_0 and r_0 are usually called bond energy and bond length, respectively.Let's introduce an r_1 such that $r_0 < r_1 < r_\xi$.Then when $V_0/kT \to \infty$ and applying Laplace's method of integration [3,24] (8) becomes

$$\begin{aligned}\int_0^{r_\xi} e^{-U(r)/kT} 4\pi r^2 dr &= \int_0^{r_1} e^{-U(r)/kT} 4\pi r^2 dr + \int_{r_1}^{r_\xi} e^{-U(r)/kT} 4\pi r^2 dr \\ &\sim 4\pi r_0^2 e^{V_0/kT} \sqrt{\frac{2\pi kT}{|U''(r_0)|}} + 4\pi r_1^2 e^{-U(r_1)/kT} \frac{kT}{|U'(r_1)|} \\ &= 4\pi r_0^2 e^{V_0/kT} \sqrt{\frac{2\pi kT}{|U''(r_0)|}} \\ &\quad \times \left[1 + \left(\frac{r_1}{r_0}\right)^3 e^{-\frac{U(r_1)+V_0}{kT}} \sqrt{\frac{kT|U''(r_0)|}{2\pi U'^2(r_1)}}\right]\end{aligned}$$

We see that the second term is exponentially small with increasing large V_0/kT. In other words, K_3 is insensitive to the choice of r_ξ when the binding is tight [12]. Nevertheless, removing this arbitrariness is still desirable, at least conceptually.Compare (8) with (7), we see that r_ξ can be made more precise.

Equivalence proof for tight-binding. We now show the asymptotic equivalence between (4) and (7) in the limit of tight-binding.In fact we note in the limit of $kT \to 0$:

$$\begin{aligned}K_1 &= \int_0^\infty \left(e^{-U(r)/kT} - 1\right) 4\pi r^2 dr \\ &= \int_0^{r^\ddagger} e^{-U(r)/kT} 4\pi r^2 dr - \frac{4}{3}\pi (r^\ddagger)^3 + \int_{r^\ddagger}^\infty \left(e^{-U(r)/kT} - 1\right) 4\pi r^2 dr \\ &= K_2 \left[1 + O\left(\sqrt{kT} e^{-V_0/kT}\right)\right]\end{aligned}$$

That is to say K_1 and K_2 are different only by an exponentially small term $e^{-V_0/kT}$.

3. Enthalpy and entropy of binding

The equilibrium association constant, K, is directly related to the free energy change of the association $\Delta G = -k_B T \ln K$. The free energy can be further decomposed

into an mechanical energy contribution (enthalpy) and an thermal entropy contribution: $\Delta G = \Delta H - T\Delta S$. The formula to compute the enthalpy is based on temperature dependence of K [15],

$$\Delta H = -\frac{d \ln K}{d(1/kT)}. \tag{9}$$

While the above three formalisms for binding constant (free energy of binding) calculation are asymptotically equivalent in the limit of tight-binding, the corresponding enthalpy and entropy calculations can be significantly different because there are several hidden temperature dependence in their computations. For example, both $U(r)$ and $r^{\ddagger}$ are functions of T in general, and their dependence on T contribute significantly to the enthalpy and entropy of binding. Furthermore in statistical mechanic term, the formula in (4) is based on a grand canonical ensemble while (6) is based on canonical ensemble. These different ensembles could lead to different entropy and enthalpy of binding according to the theory of entropy-enthalpy compensation [29,25].

Enthalpy calculation. Formally we can calculate the enthalpies of binding corresponding to K_1 and K_2 in (6).

$$\begin{aligned}\Delta H_1 &= -\frac{d \ln K_1}{d(1/kT)} \\ &= \frac{1}{K_1}\int_0^\infty U(r) e^{-U(r)/kT} 4\pi r^2 dr \\ &\quad -\frac{T}{K_1}\int_0^\infty \frac{\partial U(r,T)}{\partial T} e^{-U(r)/kT} 4\pi r^2 dr\end{aligned} \tag{10}$$

As one can see, the second term which is due to temperature dependent $U(r)$, also contributes to the heat capacity.In the classical chemistry with covalent bonding and/or gas phase association, the temperature dependence of $U(r)$ are usually weak. Hence such systems have very small heat capacity associated with bonding. For weak noncovalent interaction in aqueous solution appeared in biochemisty, the second term is often more important than the fist term in (10).Therefore in reality, one need to also know $U(x)$ as function of T in order to reasonably compute enthalpy and entropy. However, we show here that even when $U(r)$ is T independent, there are still differences in the calculations for enthalpy based on different methods. Similar analysis can be carried out in a straightforward way if one knows $U(x,T)$.

To calculate the enthalpy associated with K_2, one notices that even though the denominator of (6) is approximately equal to 1/[L], their temperature dependences are completely different. Hence we have to use (6) rather than (7) for computing enthalpy change associated with the binding. The enthalpy of the binding

$$\Delta H_2 = -\frac{d \ln K_2}{d(1/kT)} = -\frac{d \ln Q_b}{d(1/kT)} + \frac{d \ln Q_f}{d(1/kT)}$$

in which Q_b and Q_f are numerator and denominator of (6) representing the partition functions for the bound and free states. The enthalpy of the bound ligand is

$$\begin{aligned} H_2^b &= -\frac{d \ln Q_b}{d(1/kT)} \\ &= \frac{1}{Q_b} \int_0^{r^\ddagger} U(r) e^{-U(r)/kT} 4\pi r^2 dr \\ &\quad -\frac{4\pi r^{\ddagger 2} e^{-U(r^\ddagger)/kT}}{K_2} \left(\frac{dr^\ddagger}{d(1/kT)} \right) \end{aligned} \tag{11}$$

where

$$\frac{dr^\ddagger}{d(1/kT)} = \frac{2(kT)^2 r^\ddagger}{2kT - r^{\ddagger 2} U''(r^\ddagger)} \tag{12}$$

is the temperature dependence of the transition state, which is derived from (5) since

$$U'(r^\ddagger) - 2kT/r^\ddagger = 0$$

A similar result can be obtained for the enthalpy of a free ligand H_2^f. Thus we see that for noncovalent binding the transition state changes with temperature, and this contributes to a term in enthalpy of binding. According to this model, the relative change in transition state with respect to a relative change in temperature is

$$\frac{T}{r^\ddagger} \frac{dr^\ddagger}{dT} = \frac{1}{r^{\ddagger 2} U''(r^\ddagger)/2kT - 1}. \tag{13}$$

Translational entropy of a free ligand. When a ligand is dissolved in a solution, the solvation process can be broken down into two parts: to put the ligand in a fixed point in the solution, and then let it free. The interaction between the ligand and solvent molecules is contained in the first part, which includes an entropy term. The second step has a pure entropy from translational motion and mixing of N ligand molecules $k \ln \left(\frac{V}{N\Lambda^3} \right)$ where V is the volume of the solution, N is the number of ligand in the solution, and $\Lambda = \frac{h}{\sqrt{2\pi mkT}}$ is the de Broglie thermal wavelength [4]:

$$\mu_L = \mu_L^0(T, \rho) + kT \ln \left(\rho \Lambda^3 \right)$$

where $\rho = N/V$ is the number concentration (molarity) of the ligand in solution.

The entropy of ligand-solvent interaction is $-\partial \mu_L^0 / \partial T$. To estimate this term, one often develops simple models for the interaction.[1] has shown that model based on hard spheres, (scaled particle theory [32, 27]) can be used as a reasonable estimation for this part of the entropy.In our above analysis, this term is hidden in the potential of mean force $U(r) = \mu_{PL}(T, r) - \mu_L^0$.

Tethered protein ligand association. In a set of recent experiments, ligands and proteins attached by molecular linkers (e.g., a disulfide bond or polymer chain) are studied [40, 19]. Such linker introduces an additional intermolecular potential between the protein and the ligand:

$$U(r) = U_{intermolecular}(r) + U_{linker}(r)$$

The important contribution from the linker is that the ligand is now being physically constrained around the protein.In mathematical terms:

$$\lim_{r\to\infty} U_{linker}(r) = \infty, \qquad \text{while} \qquad \lim_{r\to\infty} U_{intermolecular}(r) = 0.$$

The r_c in (6) now is no longer determined by the concentration of the ligand, rather it is determined by the length of the linker, which can be stochastic in the case of the polymer chain [26]. The situation of these experiments are completely analogous to that of protein-ligand interaction under an external force [37, 38]: Instead of pulling apart, the additional force pushing the ligand toward the receptor. It has been shown that for some U_{linker} the overall $U(r)$ has two energy wells separated by an energy barrier. For such a system one expects to observe a two-state transition. For other U_{linker}, there will be no energy barrier. In the latter case, bound and free states are not defined and relaxation kinetics is not first-order. See the original papers for more discussion.

4. A model calculation based on Lennard-Jones 6–12 potential

Recent measurements on protein-ligand association and dissociation by AFM has provided detailed intermolecular force (and potential of mean force) for non-covalent interaction [11, 23, 10, 37, 22, 30]. It should be now possible to compare the calculated binding constant, and even entropy and enthalpy of association, based on measured $U(r)$, and the traditional measured binding thermodynamics [6, 7]. Here we present a simple example for calculating thermal energetics of binding based on Lennard-Jones 6–12 (LJ6-12) intermolecular potential. The LJ6-12 representation provides a first-order approximation for the more realistic analysis in the future. The advantage of using this functional form is that the intermolecular interaction is parameterized in terms of two distinct physical quantities r_0 and V_0, a "bond length" and a "bond energy", respectively:

$$U(r) = -V_0\left[2\left(\frac{r_0}{r}\right)^6 - \left(\frac{r_0}{r}\right)^{12}\right] \tag{14}$$

Experimental measurements on streptavidin-biotin complex by AFM have shown that this functional form captures the essence of the intermolecular potential between streptavidin and biotin [6].

We calculate the equilibrium association constant according to K_2 in (6).

$$K = \kappa\frac{\int_0^{r^\ddagger} 4\pi r^2 e^{-U(r)/kT}dr}{\int_{r^\ddagger}^{r_c} 4\pi r^2 e^{-U(r)/kT}dr} \tag{15}$$

in which $r^{\ddagger}$ defines transition state, r_c is inversely related to ligand concentration $\rho = 3/4\pi r_c^3$. κ is a dimensionless ligand orientational factor which is always smaller than unity [16]. Thus, the free energy, enthalpy and entropy of the equilibrium association are:

$$\Delta G = -kT \ln K = \Delta H - T\Delta S \tag{16}$$

where the enthalpy for the association:

$$\Delta H = \int_0^{r^{\ddagger}} 4\pi r^2 U(r) P_b(r) dr - \int_{r^{\ddagger}}^{r_c} 4\pi r^2 U(r) P_f(r) dr \tag{17}$$

in which $P(r)$'s are the pair distribution functions:

$$P_b(r) = \frac{e^{-U(r)/kT}}{\int_0^{r^{\ddagger}} 4\pi r^2 e^{-U(r)/kT} dr}, \qquad P_f(r) = \frac{e^{-U(r)/kT}}{\int_{r^{\ddagger}}^{r_c} 4\pi r^2 e^{-U(r)/kT} dr} \tag{18}$$

The entropy for the protein-ligand binding is:

$$\begin{aligned} \Delta S = k \ln \kappa &- k \int_0^{r^{\ddagger}} 4\pi r^2 dr\, P_b(r) \ln P_b(r) \\ &+ k \int_{r^{\ddagger}}^{r_c} 4\pi r^2 dr\, P_f(r) \ln P_f(r). \end{aligned} \tag{19}$$

For tight-binding with large V_0, the distribution in the bound state, $P_b(r)$, can be approximated by a Gaussian distribution (which is equivalent to a harmonic approximation for the energy well):

$$P_b(r) \approx \sqrt{\frac{36V_0}{r_0^2 \pi kT}} e^{-36V_0(r-r^*)^2/r_0^2 kT} \tag{20}$$

and the distribution in the free state is approximately uniform:

$$P_f(r) \approx \frac{3}{4\pi(r_c^3 - r^{\ddagger 2})}. \tag{21}$$

Therefore we have,

$$\Delta H \approx -V_0 + \frac{kT}{2}, \qquad \Delta S \approx k \ln \kappa + \frac{k}{2}\left(1 + \ln \frac{\pi kT r_0^2}{36V_0}\right) + k \ln \rho \tag{22}$$

where ρ is the number concentration of the ligand. ΔH is independent of the "bond length", while ΔS contains a $k \ln r_0$ term. Fig 2 compares the exactly calculations based on Eqs 17–19 and those by the Gaussian approximation (Eq. 22).

The transition state $r^{\ddagger}$ which separates the bound from the free ligand can be determined from the free energy function (Eq. 5):

$$F(r) = U(r) - kTln(4\pi r^2) \tag{23}$$

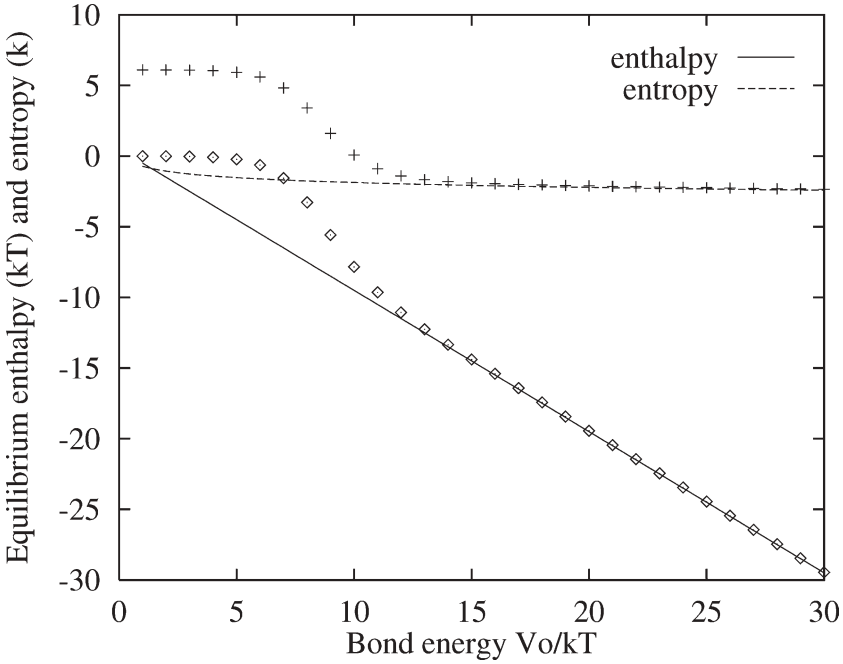

Fig. 2. Enthalpy and entropy for the association of a protein and a ligand. The symbols are numerical calculations based on the LJ6-12 type energy function (Eqs 17–19), and the lines are results based on the Gaussian approximations (Eq. 22)

which is illustrated in Fig 1. The free energy barrier ($\ddagger$) and free energy well ($*$) are the maximum and the minimum of the function, respectively. They satisfy the equation:

$$6\frac{V_0}{kT}\left[\left(\frac{r_0}{r}\right)^6 - \left(\frac{r_0}{r}\right)^{12}\right] = 1 \tag{24}$$

This equation has no real solution for $3V_0/2kT < 1$, indicating that the activation barrier disappears all together at high temperature [28]. For $3V_0/2kT > 1$, the equation yields the positions for the barrier ($r^\ddagger$) and the well (r^*):

$$\left(\frac{r_0}{r^\ddagger}\right)^6 = \frac{1-\sqrt{1-2kT/3V_0}}{2}, \qquad \left(\frac{r_0}{r^*}\right)^6 = \frac{1+\sqrt{1-2kT/3V_0}}{2} \tag{25}$$

The barrier height, i.e., the activation free energy for dissociation:

$$\Delta F^\ddagger = F(r^\ddagger) - F(r^*) = \Delta H^\ddagger - T\Delta S^\ddagger \tag{26}$$

which has an enthalpic and an entropic term:

$$\Delta H^\ddagger = U(r^\ddagger) - U(r^*) = V_0\sqrt{1-2kT/3V_0} \tag{27}$$

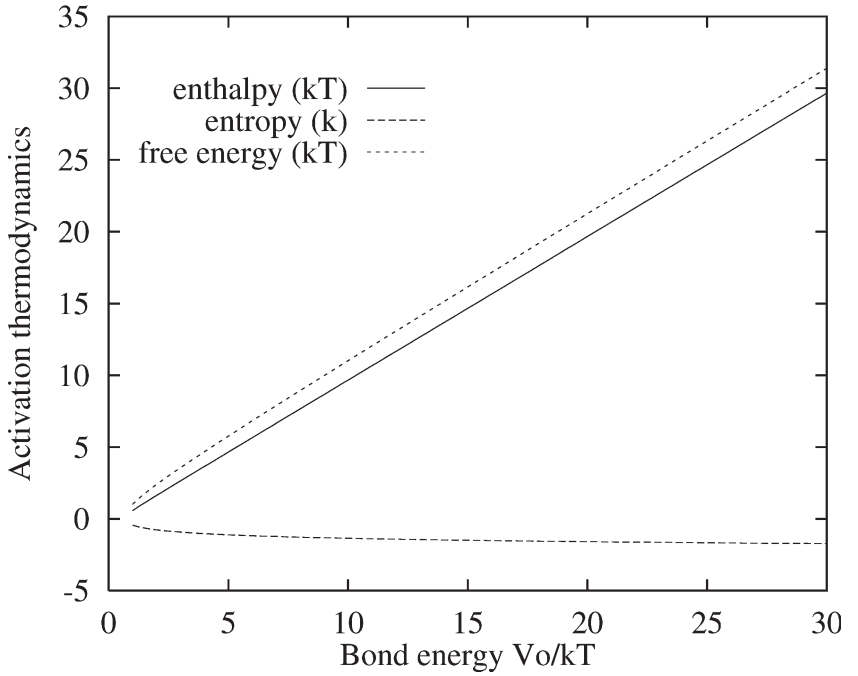

Fig. 3. Free energy, enthalpy, and entropy of the activation barrier for dissociation, in units of kT, as function of the bond energy V_0/kT

$$\Delta S^{\ddagger} = 2k \ln\left(\frac{r^{\ddagger}}{r^*}\right) = \frac{k}{3} \ln\left(\frac{1+\sqrt{1-2kT/3V_0}}{1-\sqrt{1-2kT/3V_0}}\right) \tag{28}$$

Fig. 3 shows that activation free energy, enthalpy and entropy as function of V_0/kT. The simple calculation indicates that, when $V_0 \gg kT$, there is a linear correlation between the ΔH, $\Delta H^{\ddagger}$, and V_0.This correlation has been observed in the experimental measurements on streptavidin-biotin dissociation for a series of structurally perturbed streptavidin [6,7].

5. Discussion

The present work provides a sound mathematical basis for modeling the equilibrium as well as kinetics of non-covalent association.Non-covalent association is one of the most important physical processes in biological cells.The process should and can be understood and quantitatively modeled by interaction forces in terms of thermodynamics via configurational integral.More precisely, the process is a stochastic diffusion in a potential force field, $-\nabla U$, *à la* Kramers' theory [21].In the laboratories, however, such association is usually measured in terms of an association constant K – a concept originated from classical chemistry. However, the K is intimately dependent on the laboratory operational definition for the associated molecular complex. In the present work, we emphasize that for weak non-covalent

association, the concept of K is expected to be mathematically ambiguous.And by an asymptotic analysis, we further show that for strong association the ambiguity is removed.

Our observation that the entropic and enthalpic components of a binding free energy can be dependent on the methods for measurement is surprising.This result needs to be further explored and validated. In this paper we show that, at least in principle, it is possible to experimentally probe the temperature dependence of the transition state for a protein-ligand association through comparitive studies of entropies and enthalpies from different type of laboratory measurements. This result also provides a new perspective on the theory of entropy-emthalpy compensation in biochemical processes [29, 25].

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