

PROTEIN FOLDING COOPERATIVITY: BASIC INSIGHTS FROM MINIMALIST MODELS

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Abstract

Basic concepts about two-state, cooperative protein folding and its relation to first-order phase transitions are reviewed. Minimalist models capable of reproducing the required free energy barrier between folded and unfolded macroscopic states are described. A significantly more restrictive “calorimetric” criterion is also discussed, which is based on direct comparison between model and experimental heat capacities with additional assumptions about conformational enthalpy variation in the unfolded state.

key words: lattice model, cooperativity, two-state transition, hydrophobicity, hydrogen bond, structural segregation, first-order phase transition

Introduction

Many small globular proteins fold cooperatively, with no detectable stable intermediates, within a relatively small range of appropriate control parameters, such as temperature, pressure or solvent composition. This “two-state” character of protein folding [1] is consistent with the experimental observation that signals probing different aspects of the molecular three-dimensional organization, such far-UV and near-UV circular dichroism, tryptophane fluorescence, infra-red absorbance, viscosity, etc., vary abruptly and concomitantly within this range. Different experimental probes can reflect, therefore, a single equilibrium constant between two macroscopic states: the ordered, physiologically relevant, folded state and the disordered unfolded state. This hypothesis has been con-

firmly by microcalorimetric measurements which demonstrated the reasonable agreement between the van't Hoff enthalpy, ΔH_{VH} , obtained from the putative equilibrium constant under the assumption of two-state behavior, and the actual enthalpy of denaturation obtained calorimetrically, ΔH_{cal} [2, 3]. Folding cooperativity is biologically very significant since it guarantees that the vast majority of protein molecules will be folded at physiological temperature even though the individual interactions that stabilize the native structure are comparable to $k_{\text{B}}T$. Understanding the physical mechanism of this remarkable behavior is one of the major goals of molecular biophysics and it is clearly the mandatory first step in the construction of any rational framework in which anomalous situations, associated to impaired cooperativity, protein misfolding and disease, can be included.

Two-state protein folding and first-order phase transitions

The significant absorption of heat within a rather small temperature range, implying abrupt changes in enthalpy and entropy (which is the temperature derivative of the Gibbs free energy), suggests a natural analogy between two-state folding and first-order phase transitions, defined in macroscopic systems by a discontinuity in the first derivative of the thermodynamic potential. First-order phase transitions, a very familiar example of which is the melting of ice, are understood in terms of the shape of the microcanonical entropy function,

$$S(E) = k_{\text{B}} \ln \Omega(E), \quad (1)$$

where k_{B} is the Boltzmann constant and $\Omega(E)$ is the density of microstates, or $\Omega(E)dE$ is number of microstates with energy within a small interval between E and $E + dE$. As it is known from standard texts on statistical mechanics (e.g. [4]), the population of microstates of a given “well-behaved” macroscopic system, characterized by a concave microcanonical entropy ($\partial^2 S / \partial E^2 < 0$), at a given absolute temperature T , corresponds to an extremely sharp distribution of energies, $P(E, T)$, around $E = E'(T)$ where $\partial S / \partial E|_{E=E'} = 1/T$. The width of the distribution is proportional to $1/\sqrt{N}$, where N is the number of particles, and it is negligible, for all practical purposes, in a macroscopic system where N is of the order of the Avogadro number $N_{\text{A}} = 6.022 \times 10^{23}$. This probability maximum corresponds to a minimum in the microscopic free energy function defined by

$$F(E, T) = E - TS(E), \quad (2)$$

and minimization of this last function with respect to E provides an equivalent way of determining $E'(T)$. Additionally, since the equilibrium population of microstates, $P(E, T)$, is so sharply distributed around $E'(T)$, any macroscopic property of the system at temperature T , $\langle A \rangle (T)$, defined by the equilibrium average of the corresponding microscopic quantity A over all energy levels, can be considered, to an excellent approximation, to be equal to $A(E'(T))$, i.e.,

$$\langle A \rangle (T) = \int_E P(E, T) A(E, T) dE \approx A(E'(T)), \quad (3)$$

where the brackets $\langle \rangle$ are used to indicate equilibrium average and A is any quantity that can be defined at each energy level, such as energy itself, microcanonical entropy or free energy. The concavity of the microcanonical

entropy function implies that $E'(T)$ and, as a consequence, all macroscopic properties, must vary continuously as a function of T and no phase transition is observed.

In systems displaying first-order phase transitions the microcanonical entropy $S(E)$ contains a convex interval ($\partial^2 S/\partial E^2 > 0$), in such a way that there can be up to three energy values, E' , for a given absolute temperature T , satisfying the condition $\partial S/\partial E|_{E=E'} = 1/T$ (Fig. 1). All these three energy values, one inside the convex interval, $E'_2(T)$, and the other two in each of the adjacent concave regions, $E'_1(T)$ and $E'_3(T)$ with $E'_1(T) < E'_2(T) < E'_3(T)$, correspond to extrema in the probability distribution $P(E, T)$ but while for $E'_1(T)$ and $E'_3(T)$ the extrema are maxima, for $E'_2(T)$ the extremum is a minimum. In terms of free energy the situation is reversed, since probability maxima correspond to free energy minima and vice versa. Again, the energy probability distribution is sharply peaked around both maxima and any macroscopic quantity $\langle A \rangle (T)$ can be expressed, to an excellent approximation, as

$$\langle A \rangle (T) \approx P(E'_1(T))A(E'_1(T)) + P(E'_3(T))A(E'_3(T)). \quad (4)$$

At a single critical absolute temperature $T = T_c$, determined from the inclination of the double tangent to $S(E)$, the two probability maxima are equivalent, i.e. $P(E'_1(T_c)) = P(E'_3(T_c)) = 1/2$ and $\langle A \rangle (T)$ becomes the average between $A(E'_1(T_c))$ and $A(E'_3(T_c))$. In macroscopic systems $P(E'_1(T), T)$ becomes essentially unity for any temperature even slightly below T_c , in which case

$$\langle A \rangle (T < T_c) \approx A(E'_1(T), T), \quad (5)$$

and, similarly,

$$\langle A \rangle (T > T_c) \approx A(E'_3(T), T), \quad (6)$$

for any $T > T_c$. As the temperature increases from a value slightly below T_c to a value slightly above it, there is no discontinuity in the macroscopic free energy, $\langle F \rangle (T)$, because $F(E'_1(T_c)) = F(E'_3(T_c))$, but the macroscopic energy and entropy do change discontinuously from $\langle E \rangle (T < T_c) \approx E'_1(T_c)$ to $\langle E \rangle (T > T_c) \approx E'_3(T_c)$ and from $S(E'_1(T_c))$ to $S(E'_3(T_c))$. These discontinuities, represented by thick arrows in Fig. 1, define the first order phase transition.

Two-state protein folding corresponds to a situation in which eq. 4 is a valid approximation, implying the existence of a convex region in the microcanonical entropy function. Because single protein molecules are many orders of magnitude smaller than typical macroscopic systems, however, the approximations shown in eqs. 5 and 6 are not valid within a detectable interval around the folding transition temperature, T_f . Enthalpy and entropy, as well as other macroscopic properties of the system, do change abruptly around T_f , but not discontinuously as in the case of first-order phase transitions. The experimentally observed heat capacity of protein folding, the temperature derivative of the energy (or enthalpy), displays, therefore, a sharp peak around T_f but it does not diverge to infinity as in the case of melting ice. It has been natural therefore to consider a convex microcanonical entropy or, equivalently, a free energy barrier between folded and unfolded macroscopic states, as the defining characteristic of two-state folding behavior [5–8]. Much insight on how this convex microcanonical entropy can

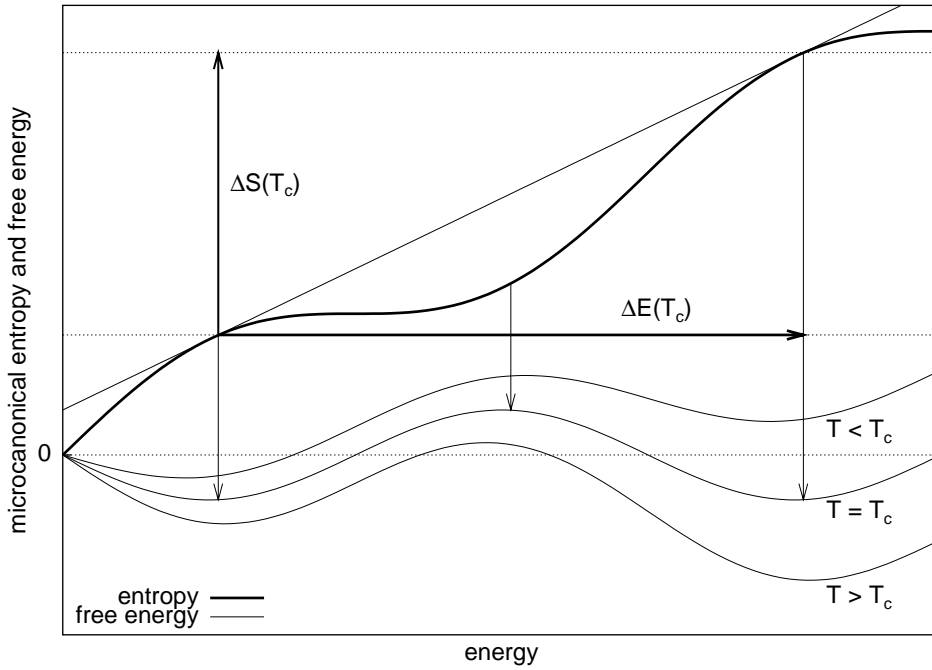


Figure 1: Microcanonical entropy, $S(E)$, and free energy, $F(E) = E - TS(E)$, for a hypothetical macroscopic system displaying a first-order phase transition. The inclination of the double-tangent to the entropy curve is $1/T_c$ and the contact points determine the energies of two probability maxima at this temperature, $E'_1(T_c)$ and $E'_3(T_c)$ or, equivalently, the positions of two free energy minima. The point in the entropy curve inside the convex region at which the derivative is also $1/T_c$ determines $E'_2(T_c)$, the energy of minimal probability or maximal free energy. Downward arrows are intended to emphasize the correspondence between these three points in the microcanonical entropy and their counterparts in the free energy function. Note $F(E'_1(T_c)) = F(E'_3(T_c))$ while $F(E'_1(T))$ becomes smaller (larger) than $F(E'_3(T))$ when T is smaller (larger) than T_c . Thick arrows represent the discontinuities in the macroscopic energy and entropy functions associated with the first-order phase transition, i.e., $\Delta E(T_c) \approx E'_3(T_c) - E'_1(T_c)$ and $\Delta S(T_c) \approx S(E'_3(T_c)) - S(E'_1(T_c))$.

arise on heteropolymeric systems has been provided by minimalist protein models.

Minimalist models reproduce two-state folding behavior

Minimalist models represent protein molecules in a very simplified manner, with one or a few beads corresponding to each amino acid and chain conformation often restricted to a regular lattice (Fig. 2). To each model conformation an “energy” can be computed, usually as a sum over contacts of sequence-dependent pairwise interactions. Model energies are actually intended to represent, also in a very simplified manner, a potential of mean force, or a free energy averaged over all degrees of freedom not explicitly included in the model [6]. The resulting overall simplicity, which permits very detailed computational analysis either by Monte Carlo simulations or even, in some situations, by complete enumeration of conformational space, constitutes the major advantage of these models over more realistic models where all protein heavy atoms are represented in continuous space. The explicit consideration of essential heteropolymeric characteristics, such as chain connectivity, excluded volume and a sequence-dependent potential of mean force, permits, on the other hand, a more reliable generalization to real proteins than results obtained on more abstract, purely analytical studies.

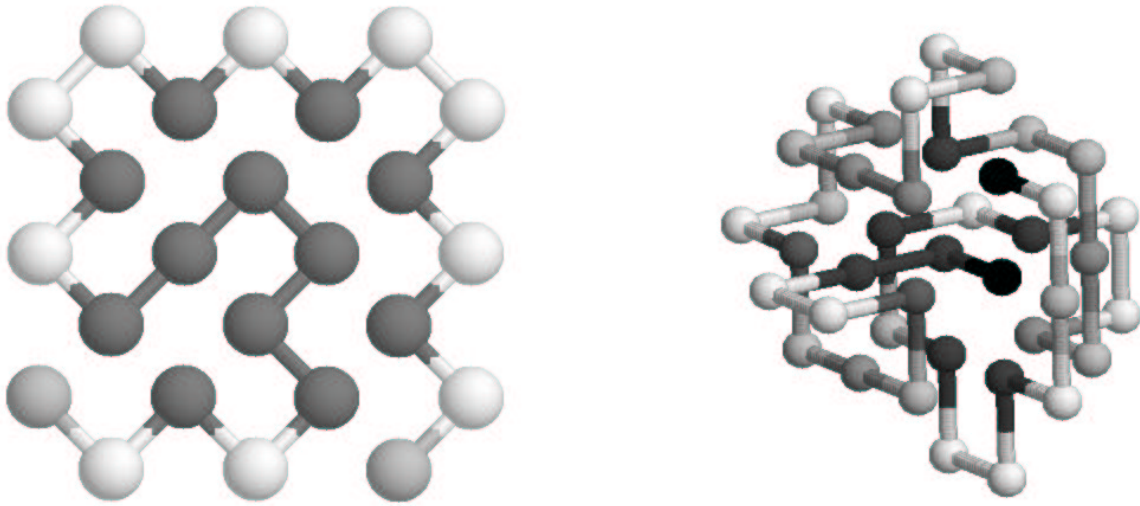


Figure 2: Hydrophobic minimalist model in the square lattice (left) [9] and a three-dimensional version in the cubic lattice (right) [10]. Internal monomers are more hydrophobic and are shown in darker tones of gray. Both models display a two-state folding transition to these structurally segregated native conformations with an energy function intended to mimic the hydrophobic effect.

Shakhnovich and Finkelstein [11] have considered the possibility that folding cooperativity could be related to the ordering of amino acid sidechains in a transition from a “molten-globule” hypothetical intermediate [12], where the overall native topology would already be present while mobile sidechains would not be closely packed, to the native structure with rigid, tightly packed sidechains. The initial transition from the completely unfolded state to the molten globule intermediate was considered to be not two-state and similar to the hydrophobic collapse of homopolymers under poor solvent conditions. Simulations of minimalist models with sidechains performed by Dill and coworkers have later shown that the balance between entropy and enthalpy (or energy) reductions during sidechain freezing does not imply necessarily the existence of a free energy barrier and a two-state transition [7, 13]. Furthermore, many simulations of minimalist models with no sidechains performed by different groups (reviewed in [6, 7, 14, 15]) have displayed two-state protein-like behavior, indicating that even without sidechains the heteropolymeric transition from a disordered macroscopic state to a native state dominated by a single structure is qualitatively different from a homopolymeric collapse.

In order to reproduce protein-like folding behavior an adequate minimalist model must be able, at the least, to rapidly fold during a computer simulation from any arbitrary conformation to the model native state, consisting of one or a few similar structures, at a temperature where the native state is thermodynamically stable. Shakhnovich and coworkers have suggested that this behavior is reproduced when the native conformation corresponds to a sufficiently deep global minimum in the surface of model energies, as can be quantified by a sufficiently negative Z -score,

$$Z = \frac{E^* - \bar{E}}{\sigma_E}, \quad (7)$$

where E^* is energy of the native structure while \overline{E} and σ_E are the average energy and corresponding standard deviation over unfolded conformations [16, 17]. Monte Carlo simulations of sequences designed to have very negative Z -scores for arbitrary maximally compact conformations in the cubic lattice resulted in rapid folding at the temperature of thermodynamic stability. Furthermore, the equilibrium population of conformations was bimodal along the energy coordinate at the folding transition midpoint, indicating the existence of a free energy barrier between folded and unfolded macroscopic states (reviewed in [6]). Onuchic, Wolynes and collaborators have rationalized this combination between thermodynamic stability with kinetic accessibility in terms of a rugged energy surface with the overall shape of a funnel [14, 18, 19]. Protein-like folding behavior should arise when the ruggedness, which results from energetic frustration and can be quantified by a glass transition temperature T_g , happens to be small when compared to the overall funnel bias towards the native structure which can be measured by the folding transition temperature T_f . It is actually not hard to show, at least for the case of a Gaussian density of unfolded microstates, that T_f/T_g maximization and Z -score minimization are closely related (e.g. [20–22]).

Sufficiently negative Z -scores can be obtained in minimalist models with different $L \times L$ symmetric interaction matrices containing $L(L - 1)/2$ independent parameters, where L is the number of monomer types or “letters”. Not surprisingly, it is easier to obtain an appropriate combination of sequence, structure and energy matrix when L is large. In the limiting case where L is the same as the number of monomers, N , native contacts can be made attractive while non-native contacts remain neutral or repulsive, like potentials of the type introduced by Go [23], and the native structure becomes a very deep global energy minimum by construction. In more realistic interaction schemes L must be smaller than N and it is also unlikely that different pairwise combinations of monomer types should correspond to completely independent energies. If hydrophobicity is assumed to be the dominant factor in the stabilization of protein structures [24], the number of independent parameters in the matrix should correspond to the number of monomer hydrophobicities, i.e. L , and not $L(L - 1)/2$. In HP models, which have been extensively studied by Dill, Chan and coworkers (reviewed in [7]) there are only hydrophobic (H) and polar (P) monomer types and some small HP sequences do display two-state folding thermodynamics in the two-dimensional square lattice. Attempts to design longer HP sequences to fold to maximally compact conformations in the three-dimensional cubic lattice have failed, however, basically because Z -scores could not be made sufficiently negative [17, 25]. More recently, a combination of the Z -score criterion with a different energy function also intended to mimic the hydrophobic effect resulted in an interesting structural restriction on conformations that should have a higher probability of corresponding to sufficiently negative Z -scores [9]. Appropriate native structures for the hydrophobic energy function were suggested to be not maximally compact, as the ones used in previous studies, but structurally segregated, with most of its monomers being either completely buried or completely exposed to the solvent (Fig. 2). This suggestion was corroborated by Monte Carlo simulations [9, 10, 26] and complete enumeration [27] in the square lattice and by Monte Carlo simulations in the cubic lattice [28].

Based on detailed analyses of folding trajectories of their cubic lattice model with optimized sequences, Shakhnovich and coworkers have suggested that the free energy barrier for folding arises from the search of a

specific set of native contacts, which they called the “folding nucleus” [29, 30]. Hao and Scheraga have compared a cubic lattice model with an optimized contact potential with no explicit backbone interactions, similar in spirit to the one used by Shakhnovich and coworkers, to a more complicated model in a high coordination (210) lattice (for a recent review on high coordination lattice models see [31]) with both sidechain and backbone interactions. Both models displayed a two-state folding transition but the origin of the free energy barrier appeared to be different in each case. For the cubic lattice model the free energy barrier appeared near the native energy when the chain was already compact while for the high coordination model the free energy barrier occurred at an intermediate energy level dominated by semi-open conformations. While for the first model the rate limiting step appeared to be the concerted formation of some native contacts, as had been observed by Shakhnovich and coworkers, for the other model the barrier was related to the relative orientation of locally structured units [8]. Partial characterization of the transition state ensemble can also be performed experimentally by site-directed mutagenesis and Φ -value analysis [32]. Comparison between experimental and simulation results have been particularly useful in the interpretation of experimental data and in the construction of plausible folding mechanisms.

More restrictive criteria for protein-like folding behavior

It has become apparent that even being two-state by the criterion of a free energy barrier, models can display different degrees of cooperativity and it can be very insightful to compare the folding cooperativity of minimalist models to the ones observed in real proteins. Thirumalai and coworkers have proposed an adimensional cooperativity index, Ω_c , computable from the temperature derivative $d\langle A \rangle / dT$ of any normalized (i.e., ranging from 0 to 1) macroscopic quantity $\langle A \rangle$ used as an order parameter,

$$\Omega_c = \frac{T_{\max}^2 \left[\frac{d\langle A \rangle}{dT} \right]_{T=T_{\max}}}{\Delta T}, \quad (8)$$

where T_{\max} is the temperature at which the derivative peaks, $\left[\frac{d\langle A \rangle}{dT} \right]_{T=T_{\max}}$ is the height of this peak and ΔT is the width at its half-maximal height [33]. They have obtained Ω_c values below 10 for a cubic lattice model with sidechains while for real two-state proteins it ranges from around 10 to 100. Ω_c is a direct measure of the transition sharpness and it tends to infinity for a macroscopic first-order phase transition. Protein-like cooperativity, as measured by Ω_c , does not appear to impose drastic restrictions on minimalist models, however. As can be seen in Table II of reference [34], many three-dimensional minimalist models can display Ω_c values within the range observed for real proteins or even higher. Importantly, non two-state transitions can have Ω_c values comparable to the ones observed for small globular proteins [22, 35].

Chan has recently proposed a significantly more restrictive criterion for protein-like cooperativity [22]. Assuming that temperature independent model energies could be equated to real protein enthalpies, he has suggested that model quantities analogous to $\Delta H_{\text{vH}} / \Delta H_{\text{cal}}$ should be close to unity. One such quantity directly computable from model heat capacity curves, $C_V(T)$, is

$$\kappa_2 = \sqrt{\frac{\Delta H_{\text{vH}}}{\Delta H_{\text{cal}}}} = \frac{2T_f \sqrt{C_V(T_f)}}{\Delta H_{\text{cal}}}. \quad (9)$$

Interestingly, minimalist models that were considered to display two-state, protein-like thermodynamics by the free energy barrier criterion were found to have $\Delta H_{\text{VH}}/\Delta H_{\text{cal}}$ far below unity and should not be considered “calorimetrically” two-state [22, 34]. This conclusion deserves some explanation since, as discussed above, the experimentally observed agreement between ΔH_{VH} and ΔH_{cal} is actually considered the major experimental confirmation of a two-state folding transition. So how can it be that a model having a convex interval in its microcanonical entropy function and being two-state by definition can have a $\Delta H_{\text{VH}}/\Delta H_{\text{cal}}$ significantly smaller than unity? The main reason resides in the calorimetric enthalpy for the model, ΔH_{cal} , which is assumed to correspond not to the area of the model heat capacity peak after baseline subtractions, as it is done in real proteins, but to the area of the whole heat capacity curve from $T = 0$ to $T = \infty$ with no baseline subtractions (for a discussion on the effect of baseline subtractions see [36]). Minimalist models with additive energy functions, even in the case of very specific Go-type potentials, tend to have a non-negligible heat capacity after unfolding due to residual favorable interactions that are gradually broken as the temperature increases and a significant amount of heat is absorbed by the system at temperatures higher than T_f . Since ΔH_{VH} in a two-state transition equals the difference in enthalpy (or model energy) between native and unfolded states at T_f , it must be smaller than ΔH_{cal} (Fig. 3 (left)). The major restriction imposed by this calorimetric criterion is therefore not on the two-state nature of the transition itself but on the heat capacity of the unfolded state, which should be negligible. The motivation for this requirement is that the significant heat capacity increase observed in real proteins after unfolding can be mainly accounted for by the organization of water molecules around exposed hydrophobic surfaces [37] and should not occur in minimalist models where solvent molecules are not included explicitly.

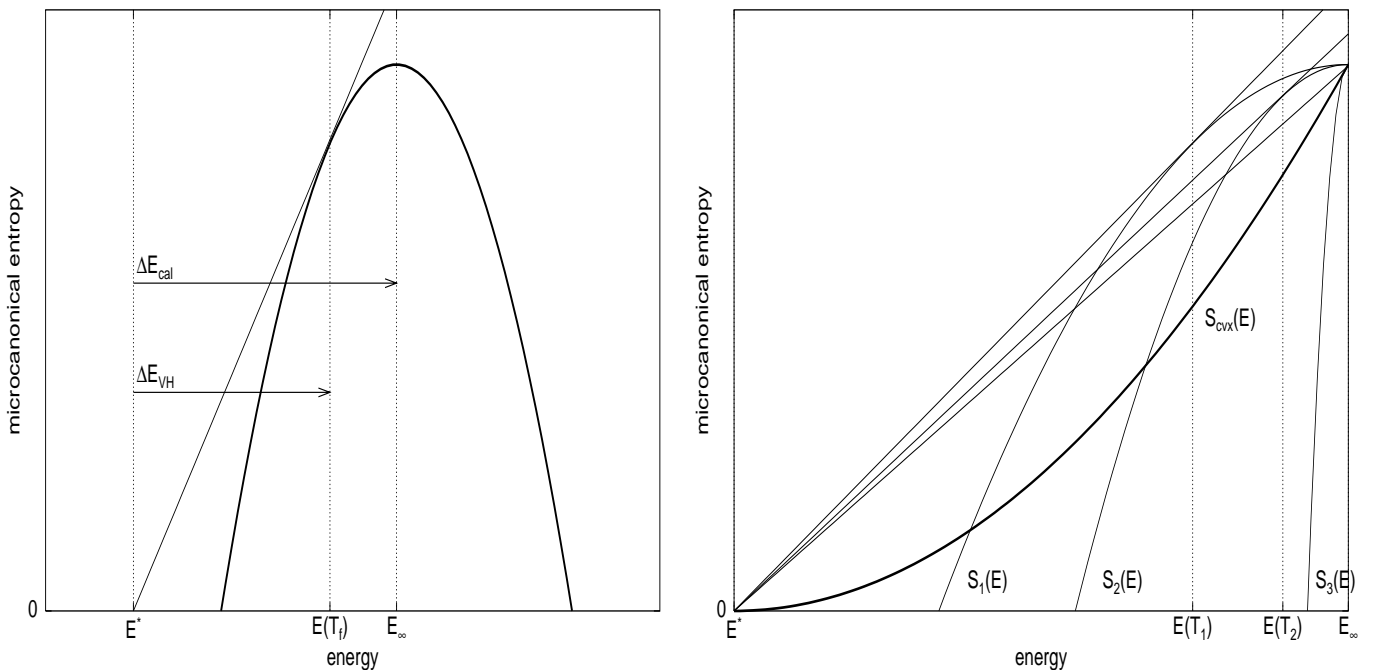


Figure 3: Hypothetical microcanonical entropy for a protein model with a Gaussian distribution of energies for the unfolded state (left). Arrows indicate the difference between van’t Hoff and calorimetric energies (or enthalpies). The difference will decrease if the distribution of unfolded energies becomes sharper as in $S_3(E)$ or, alternatively, if the microscopic entropy becomes convex as in $S_{\text{cvx}}(E)$ (right).

Independently of any possible semantic discussion on whether the calorimetric criterion should be called a two-state criterion or simply a criterion for a protein-like unfolded state, it is clear that the physical assumption on which it is based imply severe restrictions on minimalist models. Considering a Gaussian density of microstates for the unfolded state Chan has concluded that the calorimetric criterion requires that essentially all unfolded conformations must have the same enthalpy [34] (Fig. 3 (right)). In other words, the macroscopic two-state character of the transition would result essentially from the existence of only two microscopic enthalpy levels. It is important to note that Chan's conclusion is based on a Gaussian density of enthalpic microstates, implying a parabolic concave entropy for the unfolded state. The same macroscopic behavior would arise from a convex microcanonical entropy increasing continually from the native to unfolded enthalpies, as also shown in Fig. 3 (right). Chan and coworkers have found that the calorimetric criterion can be satisfied by "capillarity" models consisting of *a priori* cooperative units [22], which were inspired by studies of Freire and coworkers [38]. They have also found that calorimetric cooperativity can arise in a cubic lattice model with an appropriate balance of environment-dependent local and nonlocal interactions [39], although the physical basis for these interactions remains to be addressed. The relation between calorimetric cooperativity and folding kinetics has also been investigated [40]. It has also been very recently observed that calorimetric cooperativity can be significantly improved in hydrophobic minimalist models with an effective reduction in lattice coordination upon chain collapse, a physically motivated scheme intended to reproduce the purely entropic effect of the requirement that polar backbone groups must form hydrogen bonds when they become buried inside the protein hydrophobic core (Pereira de Araújo and Barbosa, unpublished results). In any case it appears that in order to satisfy the calorimetric criterion the energetic and/or entropic contributions for particular interactions must somehow be dependent on context, implying that calorimetric cooperativity arises from many-body interactions not accounted for in usual additive minimalist potentials. A very interesting source of nonadditivity can arise from a more detailed consideration of solvation and a non trivial distance dependence of hydrophobic interactions. The potential effect of this more explicit consideration of solvent degrees of freedom on folding cooperativity is being investigated by different groups and was recently reviewed in [41, 42].

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