Finite size effects on thermal denaturation of globular proteins

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Finite size effects on the cooperative thermal denaturation of proteins are considered. A dimensionless measure of cooperativity, \( \Omega_c \), scales as \( N^\zeta \), where \( N \) is the number of amino acids. Surprisingly, we find that \( \zeta \) is universal with \( \zeta = 1 + \gamma \), where the exponent \( \gamma \) characterizes the divergence of the susceptibility for a self-avoiding walk. Our lattice model simulations and experimental data are consistent with the theory. Our finding rationalizes the marginal stability of proteins and substantiates the earlier predictions that the efficient folding of two-state proteins requires \( T_F \approx T_\theta \), where \( T_\theta \) and \( T_F \) are the collapse and folding transition temperatures, respectively.

Single domain globular proteins, which are finite sized systems, undergo remarkably cooperative transitions from an ensemble of unfolded states to well-orderd folded (or native) states as the temperature is lowered (Fig. 1(a)). In many cases, the transition to the native state takes place in an apparent two-state manner, i.e., the only detectable species are the native (more precisely, the conformations belonging to the native basin of attraction (NBA)) or unfolded (U) states. Although the microscopic origin of cooperativity is not fully understood, the transition to the NBA at the folding transition temperature, \( T_F \), is a consequence of the effective interresidue attraction that compensates for the entropy loss. From this perspective the NBA ↔ U transition can be viewed as a phase transition in a finite-sized system. Furthermore, the transition to the NBA at \( T_F \) has the characteristics of a first order phase transition. Many experiments have shown that folded states of globular proteins are only marginally stable below \( T_F \). The free energies of stability of the NBA, relative to the U states, vary within the range of \( (5 - 20)k_BT \) at neutral pH [1b]. Because proteins are polymers we expect that they would also undergo a collapse transition to a compact phase at the temperature \( T_\theta \) suitably modified for finite size systems, when water becomes a poor solvent for the polypeptide. We have previously shown that for protein sequences that fold in an apparent two-state manner \( T_F \approx T_\theta \), which naturally explains the marginal stability of proteins.\(^1\)

The quest to understand, at the molecular level, the cooperative U ↔ NBA transition has lead to a number of computational studies [2b,4-5]. Although considerable effort has been directed to describe the molecular basis of cooperativity, somewhat surprisingly, examination of the finite size effects in the self-assembly of proteins has received little attention. In contrast, scaling theories for finite sized systems undergoing regular first and second order phase transitions have been fully developed. The purpose of this paper is to study the effect of \( N \), the number of amino acid residues in a protein, on the extent of cooperativity in the U ↔ NBA transition.

Thermal denaturation data of wild-type (WT) proteins and lattice models (LMs) of polypeptide chains are used to examine the dependence of the cooperativity on \( N \).

We show that a dimensionless measure of cooperativity, \( \Omega_c \),

\[
\Omega_c = \frac{T_F}{\Delta T} \frac{df_N}{dT}_{T=T_F}
\]

(1)

grows as

\[
\Omega_c \sim N^\zeta
\]

(2)

where \( f_N \) is a measure of occupation of NBA, \( \Delta T \) is the full width at half-maximum of \( df_N/dT \), and \( T_F \) is the folding transition temperature identified with the maximum in \( df_N/dT \). We find that

\[
\zeta = 1 + \gamma
\]

(3)

where \( \gamma \) is the exponent that characterizes the divergence of susceptibility at the critical point for a \( n \)-component ferromagnet with \( n = 0 \), i.e., for a self-avoiding walk. As a byproduct of this study we also show that \( \frac{\Delta T}{T_F} \sim \frac{1}{N} \). The parameter \( \Omega_c \) is a convolution of the sharpness of the transition \( (T_F/\Delta T) \) and the extent to which structure, as measured by \( f_N \), changes around \( T_F \). For infinite systems undergoing sharp transitions, \( \Omega_c \to \infty \), whereas \( \Omega_c \) is small for broad or highly rounded phase transitions. The relationship given in Eq. 3, which can only be valid near \( T_\theta \), establishes the proposal that \( T_F \approx T_\theta \) for two-state folders.

To establish the results given above we used thermal and chemical denaturation data together with the LMs of a polypeptide chain to compute the growth of \( \Omega_c \) with \( N \). In the LM each amino acid is represented as a single bead confined to the vertices of a cubic lattice [2c]. The energy of a conformation specified by the positions, \( \{ \vec{r}_i \} (i = 1, 2, \ldots, N) \), is \( E \{ \vec{r}_i \} = \sum_{i<j} \epsilon_{ij} \delta_{r_{ij},a} \), where \( a \) is the lattice spacing, \( r_{ij} = |\vec{r}_i - \vec{r}_j| \), \( \delta_{r_{ij},a} \) is the Kronecker delta function. The contact energies \( \epsilon_{ij} = -1 \), if the interaction between beads \( i \) and \( j \) in a given conformation is also present in the native state (i.e., the lowest energy conformation for a given sequence), and is zero, otherwise. Even though simple LMs do not quantitatively capture the cooperativity of folding transitions in proteins, they are useful for obtaining global folding properties. The precise choice of \( \epsilon_{ij} \) should not affect the predicted universal scaling of \( \Omega_c \) with \( N \). Our purpose
in undertaking LM Monte Carlo (MC) simulations is to show that Eqs. (1)-(3) should be valid for any model of proteins that exhibits a cooperative $U \leftrightarrow \text{NBA}$ transition.

To calculate $\Omega_c$, for LMs we employ the temperature dependence of the overlap function

$$
\chi = 1 - \frac{1}{N^2 - 3N + 2} \sum_{i<j+1}^N \delta_{ij} r_{ij}^0
$$

(4)

where $r_{ij}^0$ is the distance between beads $i$ and $j$ in the native conformation. The overlap function $\chi$ is an order parameter that distinguishes the NBA and U states. The folding transition temperature $T_F$ can be estimated from the location of the maximum in $d < \chi > /dT$, where $< ... >$ indicates a thermal average. For LMs $< \chi > \approx 1 - f_N$. Therefore, Eq. (1) may be evaluated using

$$
\Omega_c = \frac{T_F^2}{\Delta T} \left( \frac{d < \chi >}{dT} \right)_{T=T_F}
$$

(5)

Analysis of experimental and simulation data: To establish the results given above we first analyzed thermal denaturation data for WT proteins. As an example we show in Fig. (1) the plot of $f_N(T)$ and $df_N(T)/dT$ for villin ($N = 35$) and ADA 2h ($N = 80$). In accord with Eq. (2) we find that the thermal denaturation of ADA 2h is more cooperative than that of villin headpiece.

From Fig. 2 we find that $\frac{\Delta T}{T_F}$, from thermal denaturation data for 32 WT proteins scales as $N^{-\lambda}$ with $\lambda = 1.08 \pm 0.04$. Given that the data for these proteins are obtained under varying experimental conditions and using different methods for computing the enthalpy and entropy changes at $T_F$, the agreement between the predicted and observed behavior is excellent. For LMs $\Delta T/T_F \sim N^{-\lambda}$ with $\lambda = 1.14 \pm 0.06$ (Fig. 2). The small deviation of $\lambda$ from unity in LMs is, in all likelihood, due to the simplicity of the $\alpha$-carbon representation of the polypeptide chain that does not capture the crucial role of side chains. Inclusion of side chains, which are tightly packed in native conformations, is expected to reduce fluctuations. Moreover, for $N < 40$ most of the beads are on the surface, which also leads to considerable conformational fluctuations. Therefore, the expected relation $\frac{\Delta T}{T_F} \sim N^{-1}$ holds nearly quantitatively.

The dependence of $\Omega_c$ on $N$ for WT proteins and LMs shows that $\Omega_c \sim N^\gamma$ (Fig. (3)). From the linear fit to the log-log plot of the data we find $\gamma \approx 2.17 \pm 0.09$ for WT proteins and $\approx 2.33 \pm 0.08$ for LMs. The 5th order $\epsilon$ expansion for polymers using $n$-component $\phi^4$ theory with $n = 0$ gives $\gamma = 1.22$. Thus, from Eq. (3) we predict that $\gamma \approx 2.22$. Thus, the data for WT proteins and LMs are consistent with the theoretical prediction (Eq. (4)). We should emphasize that the robustness of the fit has been checked using different fitting procedures. The remarkable finding relating the critical exponent $\gamma$ to thermal denaturation of proteins gives further credence to the proposal that efficient folding is achieved at $T_F \approx T_{p\lambda}$. It also suggests that $U \leftrightarrow \text{NBA}$ transition is only weakly first order, thus explaining the marginal stability of globular proteins.

Most folding experiments are performed by titrating with denaturants (urea or guanidine hydrochloride). At denaturant concentrations above the midpoint $C_m$ (at which the populations of the folded and unfolded states are equal) proteins are denaturated. Thus, phase transitions to the NBA occur by varying denaturant concentration. In analogy with Eq. (1) we computed, for 33 WT proteins $\Omega_c = \frac{C_m^2}{\Delta C} \left| \frac{df_N}{dC} \right|_{C=C_m}$ and $\Delta C/C_m$, where $\Delta C$ is the full width at half-maximum of $df_N/dC$. The plots of $\ln \Omega_c$ and $\ln \Omega_c$ as a function of $\ln N$ yield $\lambda \approx 1.22 \pm 0.14$ and $\gamma \approx 2.45 \pm 0.29$, respectively (see the insets to Figs. (2.3)). Thus, the scaling of $\Omega_c$ and $\Delta C/C_m$ remains essentially unchanged even though the chemical and thermal denaturation mechanisms are vastly different. This result also suggests that $\gamma$ is universal. However, the dependence of $\ln \Omega_c$ on $\ln N$ has a correlation coefficient of only about 0.6 compared to 0.95 for thermal denaturation. We believe that larger uncertainties result from greater drift in the experimental signals in denaturant-induced unfolding compared to thermal denaturation.
The sharpness of the folding transition $\Delta T/T_F$ as a function of $N$. Open circles represent the results from LM simulations with the corresponding fit (dotted line) $\Delta T/T_F \sim N^{-\lambda}$ with $\lambda = 1.14 \pm 0.08$. The linear fit (solid line) to the experimental data for 32 WT proteins (solid circles) gives $\lambda = 1.08 \pm 0.04$. The correlation coefficient for $\ln \Delta T/T_F$ and $\ln N$ is 0.95. For clarity LM data are shifted up by 0.4. Inset shows the dependence of the width of folding transition $\ln \Delta C/C_m$ for chemical denaturation on $\ln N$. The linear fit to the data points collected for 33 WT proteins yields $\lambda = 1.22 \pm 0.14$ (the correlation factor is 0.59).

FIG. 3: Plot of $\ln \Omega_c$ as a function of $\ln N$. Symbols are the same as in Fig. (2). The dotted line is a fit to the LM data, which gives $\zeta = 2.33 \pm 0.08$. The solid line is a fit to the experimental values of $\Omega_c$ with the exponent $\zeta = 2.17 \pm 0.09$. The correlation coefficient for $\ln \Omega_c$ and $\ln N$ is 0.95. The LM data are shifted down by 0.7. The dependence of the folding cooperativity on $N$ for chemical denaturation is plotted in the inset. The linear fit to experimental data (solid line) results in the exponent $\zeta = 2.45 \pm 0.29$ (the correlation factor is 0.59). Both sets of experimental data rule out $\zeta = 2$.

The rationale for Eqs. (2,3) is based on the following arguments. (1) By analogy with magnetic systems $\Delta \chi$ is similar to susceptibility and should be given by $\Delta \chi = T \partial < \chi > / \partial h$, where $h$ is a "magnetic" or an ordering field conjugate to $\chi$. Because $\Delta \chi$ is dimensionless, we expect that the ordering field $h \sim T$ and thus $T d < \chi > / dT \sim T$. Because the transition at $T_0$ is usually second order, while the one at $T_F$ is first order [2c,11], the $T_F \approx T_0$ condition implies that folding of two-state globular proteins occurs near a tricritical point. Therefore, the critical exponents that control the behavior of the polypeptide chain at $T_0$ should manifest itself in the $U \leftrightarrow \mbox{NBA}$ phase transition. Using these arguments we can obtain the $N$ dependence of $\Omega_c$ in the following way. In general, we expect that close to $T \approx T_0 \approx T_F$ the Flory radius $R_F \sim \Delta T^{-\nu} \sim N^\nu$ ($R_F$ is the analogue of the correlation length in magnetic systems). This implies that $\Delta T/T_F \sim N^{-1}$. Because of the analogy to magnetic susceptibility, we expect $T d < \chi > / dT \sim N^\nu$. Using Eq. (2) we obtain the expected relationship $\Omega_c \sim N^{1+\gamma}$, which directly follows from the hypothesis that $T_F \approx T_0$ for efficient two-state folders.

The scaling $\Omega_c \sim N^{\zeta}$ with $\zeta$ clearly different from 2 may appear to be at odds with the idea that the structures in the NBA are sequence-specific. However, the global characteristics embodied in the growth of $\Omega_c$ with $N$ are valid only at $T \approx T_F$. In the neighborhood of this temperature the general characteristics of the $U \leftrightarrow \mbox{NBA}$ transition are governed by the properties of the unfolded states as $T_F$ is approached from above. It has been shown that in the denaturated states ($T > T_F$) the global properties like the gyration radius $R_g \sim N^\nu$ with $\nu \approx 0.59$ as expected for homopolymer. Similarly, the homopolymeric nature around $T_F$ is reflected in the growth of $\Omega_c$ with $N$.

The finding that the folding transition at $T_F$ occurs at a tricritical point suggests that the native states of natural proteins are only marginally stable. Because biological functions require transitions between different states, it is logical to postulate that natural foldable proteins have evolved to ensure $T_0 \approx T_F$. The coil-globular transition at $T_0$ is likely to be a second order transition involving no discontinuity in the free energy. At $T_F$ the transition is of the first order. The closeness of $T_F$ and $T_0$ implies that the discontinuity of the free energy at $T_F$ cannot be large. As a result the folded state is expected to be only marginally stable with respect to the ensemble of denatured states. As argued elsewhere, this condition is also equivalent to maximizing the ratio $T_F/T_0$, where $T_0$ is a glass transition temperature. Marginality condition may also be a requirement for robustness of the folded state. This may explain why small single domain proteins can tolerate a large number of mutations without substantial changes in the native state. It is also likely, as recently shown, that evolution has led to
marginally stable proteins that have maximum sequence-structure compatibility.\textsuperscript{19,20}

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10  We generated between 12 to 20 distinct maximally compact native structures for a given value of \( N \) (27 \( \leq N \) \( \leq 80 \)). For example, all native structures for \( N = 80 \) were confined to the vertices of a 4x4x5 cube. In the Go model each structure corresponds to a distinct sequence. We used, depending on \( N \), between 50 and 100 Monte Carlo trajectories to collect states and applied multiple histogram method\textsuperscript{14} to compute \( \langle \chi(T) \rangle \), \( d < \chi > /dT \), and \( \Omega_c \) using Eq. (5). For the Go models (and other highly optimized sequences) \( T_F \), identified with the location of the maximum in \( d < \chi > /dT \), coincides with \( T_\theta \).\textsuperscript{11}

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15  A list of WT proteins together with the values of parameters for thermal and chemical denaturation is available at www.biotheory.umd.edu/ScalingDB.html or binf.gmu.edu/dklimov/Publications/ScalingDB.html. In both datasets the list of proteins spans the whole range of topologies, from peptides with \( \alpha \)-helical or \( \beta \)-hairpin structures to all \( \alpha \), all \( \beta \), or \( \alpha/\beta \) proteins. Because of spectrum of topologies there is very little sequence similarity. The larger error in \( \zeta \) and \( \lambda \) for chemical denaturation data is due to the shorter span of \( N \).

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