Critical aspects of hierarchical protein folding

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We argue that the first order folding transitions of proteins observed at physiological chemical conditions end in a critical point for a given temperature and chemical potential of the surrounding water. We investigate this critical point using a hierarchical Hamiltonian and determine its universality class. This class differs qualitatively from those of other known models.

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Proteins are complex macromolecular objects whose structure is determined by the evolutionary process that formed them. In particular this means that the folding of proteins into its three-dimensional structure must be efficient. There have been several attempts to grasp aspects of the protein folding process, in enumeration of configurations, in description of folding pathways and in discussing the influence of water on protein structure.

Presumably proteins evolved by subsequently adding properties to simpler structures which also fold. This has led us to propose a hierarchical description of the folding process, where each added subunit folds conditionally on the folding of structures above it in the hierarchy. Formulating this in the framework of statistical mechanics we suggest that the folding can be parametrized in a number of variables \( \varphi_1, ..., \varphi_N \) each taking the value 0 or 1 where 1 corresponds to a correctly folded substructure. The hierarchy is implemented through the Hamiltonian

\[
H = - \epsilon_0 (\varphi_1 + \varphi_1 \varphi_2 + \varphi_1 \varphi_2 \varphi_3 + \cdots + \varphi_1 \varphi_2 \cdots \varphi_N) .
\]

(1)

The interactions between the protein and the surrounding water may be taken into account by adding to this Hamiltonian a coupling parametrized through the water variables \( w_1, w_2, ..., w_N \). These variables couple to the unfolded protein degrees of freedom because they expose hydrophobic amino acids to the water. The resulting Hamiltonian is

\[
H = - \epsilon_0 (\varphi_1 + \varphi_1 \varphi_2 + \varphi_1 \varphi_2 \varphi_3 + \cdots + \varphi_1 \varphi_2 \cdots \varphi_N) - [(1 - \varphi_1)w_1 + (1 - \varphi_1 \varphi_2)w_2 + \cdots + (1 - \varphi_1 \varphi_2 \cdots \varphi_N)w_N]
\]

(2)

where the hydrophobic effect is taken into account by letting each of the \( w \)'s take a value from the set, \( \epsilon_{\text{min}} + s \Delta \), \( s = 0, 1, ..., g - 1 \). \( \Delta \) is the spacing of the energy levels of the water-protein interactions.

The partition function is

\[
Z = \left(e^{\epsilon_0/T} \right)^N \left( \frac{1}{2} \frac{r^{-N} - 1}{1 - r} + 1 \right)
\]

(3)

The variable \( r \) is the ratio of statistical weights of unfolded to folded state, per variable:

\[
r = \frac{g}{2} e^{-\mu/T} \frac{1 - e^{-\Delta/T}}{1 - e^{-g\Delta/T}}
\]

with \( \mu = -\epsilon_0 - \epsilon_{\text{min}} \) being the chemical potential of the surrounding water.

The physical meaning of this model is that the water molecules in contact with an unfolded portion of the protein has lower entropy than when not in contact (thus in the our model, hydrophobicity is caused by ordering of water and not by repulsive potentials, as is usually believed). In the model one finds that a first order transition takes place when the parameter \( r \) switches between \( r < 1 \) and \( r > 1 \). Plotting \( r \) against \( T \) one obtains a non-monotonic
function which for small \( \mu \) values passes \( r = 1 \) twice, corresponding to unfolding at both low and high temperature, as indeed seen in experiments \([11,12]\). The mechanism for the transitions is the following. At high temperature the entropy gain of the protein chain causes the unfolding. As temperature is lowered the system gains more entropy by shielding the hydrophobic residues from the water. This leads to folding. As the temperature is lowered even further the cold unfolding transition occurs. Below this transition entropy is insignificant and the dominating effect is the attractive coupling between the water and the unfolded protein.

For an intermediate value of the chemical potential, \( r \) just touches the line \( r = 1 \), that is \( d r / d T = 0 \) when \( r = 1 \), corresponding to a merging of two first order transitions. This defines a critical point. Around this point, \( r \) varies quadratically in \( T - T_c \) and linearly in \( \mu - \mu_c \), as seen from expanding Eq. (1). In experiments of protein folding this point is accessible by changing the pH value of the solution. In fact, Privalov’s data on low pH values indeed indicate that such a critical point exists. The scaling properties around this point thus opens for a possibility to gain insight into the nature of the folding process, in particular whether the hierarchical scheme we suggest can be falsified.

In Fig. 3b, we show heat capacity as a function of temperature for chemical potential below, at and above the critical value \( \mu = \mu_c \). For the chosen values of \( \mathcal{E}_b = 1 \) and level density \( \Delta = 0.02 \) and \( g = 350 \) the critical point is situated at \( T_c = 1.33303 \ldots, \mu_c = 1.2838 \ldots \). That is, it is situated at a minimum of the heat capacity curve. This is at first sight surprising, usually heat capacity has a pronounced increase at the critical point. The minimum reflects a partial ordering of the hierarchy, as envisioned in Fig. 4b where we show the degree of folding, counted by the average number of folded variables \( \varphi_i = 1, i = 1, \ldots, n \) from \( i = 1 \) until the first variable \( i = n + 1 \) which takes value \( \varphi_{n+1} = 0 \). The average value of this \( \langle n \rangle \) is \( N/2 \) at the critical point, reflecting that the system is on average half ordered at this point. Correspondingly the heat capacity dips to a value in between the value of an unfolded and a completely folded state.

To characterize the functional form of the dip in the heat capacity, we investigate analytically \( C_{\text{sing}}(T) = C(T, \mu) - C(T, \mu_c) \) with \( \mu >> \mu_c \) for different values of hierarchy size \( N \). For finite \( N \) we may express the singular part of the heat capacity in the form:

\[
C_{\text{sing}} = |T_c - T|^{-\alpha} g \left( (T_c - T) N^{1/\nu} \right)
\]

(5)

where \( g(x) \to \text{const} \) when \( x \to \infty \) and \( g(x) \propto x^\alpha \) when \( x \to 0 \). We find analytically \( \alpha = \nu = 2 \) from differentiating the partition function \([3]\). Fig. 2b demonstrate this finite size scaling. Similarly we in Fig. 2b show the behavior of the order parameter \( \langle n \rangle \) as function of \( T - T_c \) and \( N \):

\[
\langle n \rangle = |T - T_c|^\beta f \left( (T - T_c) N^{1/\nu} \right)
\]

(6)

with \( f(x) \to \text{const} \) when \( x \to \infty \) and \( f(x) \propto x^{-\delta} \) when \( x \to 0 \) where exponents \( \beta = -2 \), also found analytically. It may be surprising that \( \beta \) is negative, but this reflect in part the unusual use of an extensive (in \( N \)) order parameter, in part that for \( \mu = \mu_c \) then the order parameter only obtains a non-zero value at \( T = T_c \) when \( N \to \infty \).

Likewise, we find that the susceptibility \( \chi = d\langle n \rangle / d\mu \) scales as \( |T - T_c|^{-\gamma} \) where \( \gamma = 4 \) and that \( \langle n \rangle \propto (\mu - \mu_c)^{1/\delta} \) for \( \mu > \mu_c \) where \( \delta = -1 \). Thus the usual exponent relations, \( \alpha + 2 \beta + \gamma = 2, \alpha + \beta (\delta + 1) = 2, \) and \( \gamma (\delta + 1) = (2-\alpha)(\delta - 1) \) are fulfilled \([3]\). However the hyperscaling relation \( \nu = 2 - \alpha \), where \( \nu \) is the dimensionality of the system, is not fulfilled. However, this relation has no meaning, as there are no spatial degrees of freedom.

In terms of experiments on proteins, the relevant scaling behaviour is the how the degree of folding (order parameter) and the heat capacity behaves as function of temperature, when one changes chemical potential away from its critical value. The qualitative prediction is that the width of the singular part of the heat capacity has a minimum at the critical value \( \mu = \mu_c \). The broadening of the heat capacity is

\[
C_{\text{sing}}(T - T_c)^2 = h \left( \frac{T - T_c}{\Delta \mu^{1/2}} \right) \quad \text{for} \quad \mu > \mu_c
\]

(7)

where \( h(x) \propto x^{-2} \) for \( x \to \infty \) and \( h(x) = \text{const} \) for \( x \to 0 \) and where \( \Delta \mu = \max(\mu - \mu_c, \Delta \mu_{\text{min}}) \) with \( \Delta \mu_{\text{min}} \propto 1/N \) takes into account the finite size sensitivity of the scaling. We show in Fig. 3a, an example of such a data collapse. These predictions are experimentally accessible through the use of standard calorimetric techniques, where one should seek to obtain a data collapse above the critical point, i.e. the point of minimal width. The heat capacity below the critical \( \mu \) is complicated by the merging of two first order transitions. However, the distance between these moves away from each other in \( T \) as \( \Delta \mu^{1/2} \).

Likewise, we expect the degree of folding \( \langle n \rangle \) to show data collapse of the form
\[ \langle n \rangle (T - T_c)^2 = k \left( \frac{T - T_c}{\Delta \mu^{1/2}} \right) \text{ for } \mu > \mu_c \] (8)

where \( k(x) \) behaves asymptotically as \( h \). We show this in Fig. 3b. This quantity can be observed experimentally through fluorescence measurements.

In summary, we have proposed that the hierarchical ordering of folding pathways implies a critical point with a diminishing heat capacity at criticality. We have determined all critical exponents, and proposed two experiments that could confirm or falsify the concept of hierarchical folding.

[1] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts and J. D. Watson, *Molecular biology of the cell* (Garland Publ., New York, 1994).
[2] H. Neurath, Science, 224, 350 (1984).
[3] W. Gilbert, Science, 228, 823 (1985).
[4] K. A. Dill, S. Bromberg, K. Yue, K. M. Fiebig, D. P. Yee, P. D. Thomas and H. S. Chan, Protein Science 4, 561 (1995).
[5] P. A. Lindgård and H. Bohr, Phys. Rev. E 56, 4497 (1997).
[6] K. A. Dill, K. M. Fiebig and H. S. Chan, Proc. Natl. Acad. Sci. 90 1942 (1993).
[7] H. Li, C. Tang and N. S. Wingreen, NEC preprint (1997).
[8] A. Hansen, M. H. Jensen, K. Sneppen and G. Zocchi, Physica A, in press (1998).
[9] A. Hansen, M. H. Jensen, K. Sneppen and G. Zocchi, submitted to Proc. Natl. Acad. Sci.
[10] J. T. Edsall, J. Am. Soc. 57 1506 (1935).
[11] P. L. Privalov, Biochem. and Molecul. Biol. 25, 281 (1990).
[12] G. I. Makhatadze and P. L. Privalov, Adv. Prot. Chem. 47, 307 (1995).
[13] H. E. Stanley, *Phase Transitions and Critical Phenomena* (Cambridge Univ. Press, Cambridge, 1971).

FIG. 1. a) Heat capacity, \( C \), as a function of \( T \). b) Degree of folding, \( \langle n \rangle \), as a function of \( T \). Here \( g = 350, \Delta = 0.02 \) and \( N = 100 \). The value \( N = 100 \) has been chosen as to be close to realistic values for this parameter.

FIG. 2. a) Finite size scaling of the heat capacity for \( \mu = \mu_c, g = 350 \) and \( \Delta = 0.02 \). Here \( \alpha = 2 \) and \( \nu = 2 \). b) Finite size scaling of degree of folding, \( \langle n \rangle \). Here \( \beta = -2 \).

FIG. 3. a) \( C_{sing}(T - T_c)^2 \) vs. \( (T - T_c)/\Delta \mu^{1/2} \). b) \( \langle n \rangle(T - T_c)^2 \) vs. \( (T - T_c)/\Delta \mu^{1/2} \). We have chosen \( N = 100, g = 350 \) and \( \Delta = 0.02 \). Note the good quality of the data collapse in spite of smallness of the system.
Figure 1a
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Figure 1b

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*Critical aspects of hierarchical protein folding*
Figure 2a
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*Critical aspects of hierarchical protein folding*
Figure 2b
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*Critical aspects of hierarchical protein folding*
Figure 3a
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*Critical aspects of hierarchical protein folding*
Figure 3b
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