Glass Transition Temperature and Fractal Dimension of Protein Free Energy Landscapes

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Abstract
The free-energy landscape of two peptides is evaluated at various temperatures and an estimate for its fractal dimension at these temperatures calculated. We show that monitoring this quantity as a function of temperature allows to determine the glass transition temperature.

Keywords: Energy landscape, folding funnel, fractal dimension, protein folding, generalized-ensemble simulations

It is well known that a large class of proteins folds spontaneously into unique, globular shape which is determined solely by the sequence of amino acids (the monomers) \cite{1}. Folding of a protein into this three-dimensional structure (in which it is biologically active) is now often described by energy landscape theory and the funnel concept \cite{2}. In this “new view” of folding \cite{3} it is assumed that the energy landscape of a protein is rugged, however, unlike for a random hetero-polymer, there is a sufficient overall slope so that the numerous folding routes converge towards the native structure. The particulars of the funnel-like energy landscape determine the transitions between the different thermodynamic states \cite{2, 4}. For instance, a common scenario for folding may be that first the protein collapses from a random coil to a compact state. This coil-to-globular transition is characterized by the collapse transition temperature \(T_\theta\). In the second stage, a set of compact structures is explored. The final stage involves a transition, characterized by the folding temperature \(T_f \leq T_\theta\), from one of the many local minima in the set of compact structures to the native conformation.

However, the essence of the funnel landscape idea is competition between the tendency towards the folded state and trapping due to ruggedness of the landscape.

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It is expected that for a good folder the temperature, $T_g$, where glass behavior sets in, has to be significantly lower than the folding temperature $T_f$ \[4\]. It follows that it is important to calculate these two temperatures when examining the folding of a protein in computer simulations. While the folding temperature $T_f$ can be easily determined by monitoring the changes of a suitable order parameter with temperature, the situation is less obvious for the glass transition temperature $T_g$ which is normally determined from the slowing down of folding times with temperature in kinetic simulations \[2\]. To measure $T_g$ from equilibrium properties of the protein one can use the intimate connection between “roughness” and fractality. Especially, we expect that the fractal dimension of the folding funnel will increase with increasing roughness of the free energy landscape. We conjecture that the glass transition temperature $T_g$ is associated with a change of the fractal dimension of the folding funnel and propose to measure $T_g$ by calculating the fractal dimension of the protein free energy landscape as a function of temperature. We remark that our approach differs from Ref. \[5\] where recently for some peptides the fractal properties of the time series of the potential energy were studied.

While landscape theory and funnel concept were developed from studies of minimalistic protein models without reference to specific proteins, we intend to probe our assumption for two distinct peptides. The first peptide is Met-enkephalin, which has the amino acid sequence Tyr-Gly-Gly-Phe-Met. In previous work \[3,4\] evidence was presented that the folding of this peptide can be described within the funnel concept. Estimators for the collapse temperature $T_\theta = 295 \pm 30$ K and the folding temperature $T_f = 230 \pm 30$ K were presented \[4\]. As the second peptide we choose poly-alanine of chain length $N = 20$, which undergoes at $T = 508(5)$ K a sharp transition between a completely ordered helical state and a random (coil) state \[8,9\]. Hence, we expect no finite glass transition temperature for this polypeptide; and the thermal behavior of the fractal dimension should differ significantly from that of Met-enkephalin.

Our simulations of both peptides relied on a detailed, realistic, description of the intramolecular interactions. Such simulations are known to be notoriously difficult. This is because at low temperatures simulations based on canonical Monte Carlo or molecular dynamics techniques will get trapped in one of the multitude of local minima separated by high energy barriers, and physical quantities cannot be calculated accurately. Only recently, with the development of generalized-ensemble techniques such as multicanonical sampling \[10\] and simulated tempering \[11\], calculation of accurate low-temperature thermodynamic quantities became feasible in protein simulations \[12,8,9\]. Hence, the use of one of these novel techniques, multicanonical sampling \[10\], was crucial for our project.

In a multicanonical algorithm \[10\] conformations with energy $E$ are assigned a weight $w_{mu}(E) \propto 1/n(E)$, $n(E)$ being the density of states. A simulation with this weight generates a random walk in the energy space and a large range of energies is sampled. Hence, one can use the re-weighting techniques \[13\] to calculate the
free energy $G(x)$ as a function of the chosen reaction coordinate $x$ over a wide temperature range by

$$G(x, T) = -k_B T \log[P(x)w_{mu}^{-1}(E(x))] e^{-E(x)/k_B T} - C.$$  \hspace{1cm} (1)

Here, $P(x)$ is the distribution of $x$ as obtained by our multicanonical simulation and $C$ is chosen so that the lowest value of $G(x)$ is set to zero for each temperature. Unlike in a canonical simulation the weight $w_{mu}$ is not a priori known, and estimators have to be calculated using the procedures described in Refs. \[12, 14\].

The main problem in characterizing the roughness of the high-dimensional folding funnel of a protein is the choice of an appropriate reaction coordinate. We choose for Met-enkephalin the overlap with the (known) ground state, $O$, defined by \[6\]

$$O = 1 - \frac{1}{90} \sum_{i=1}^{n_F} |\alpha_i - \alpha_i^{(GS)}|,$$  \hspace{1cm} (2)

where $\alpha_i^{(GS)}$ stand for the $n_F$ dihedral angles of the ground state conformation. Similar, we chose as order parameter for poly-alanine the helicity

$$q = \frac{\tilde{n}_H}{N - 2},$$  \hspace{1cm} (3)

which allows us to distinguish between helical and coil configurations of poly-alanine. Here $\tilde{n}_H$ is the number of helical residues in a conformation, without counting the first and last residues which can move freely and will not be part of a helical segment.

The fractal dimension of the free energy landscape can be calculated from different definitions, and different definitions can yield different information about the graph under study \[15\]. From a theoretical point of view the Hausdorff-Besicovitch definition \[15, 16\] is the proper one to characterize the geometrical complexity, however, it is very difficult to evaluate numerically. More widely used techniques to obtain a dimension of an arbitrary set are box-counting (and its generalized version) \[15, 16, 17\] and the method introduced by Higuchi \[18\], which we used for our analysis.

To define a fractal dimension, Higuchi considers a finite set of observations $X(j), j = 1, 2, ..., N$ taken at a regular interval $k$ in the reaction coordinate, and evaluates the length $L_m(k)$ of the corresponding graph for different interval length $k$ obtained from sequences

$$X_k^m : X(m), X(m + k), X(m + 2k), ..., X(m + \left[\frac{N - m}{k}\right]k),$$  \hspace{1cm} (4)

where $m = 1, 2, ..., k$ and $\left[\frac{N - m}{k}\right]$ denotes the integer part of $(N - m)/k$. The length of the graph is calculated as

$$L_m(k) = \frac{N - 1}{k} \sum_{i=1}^{\left[\frac{N - m}{k}\right]} \frac{|X(m + ik) - X(m + (i - 1)k)|}{k \left[\frac{N - m}{k}\right]}.$$  \hspace{1cm} (5)
If the behavior of the graph has fractal characteristics over the available range \( k \) then

\[
< L(k) > \propto k^{-d},
\]

(6)

where \( d \) is the fractal dimension and \( < L(k) > \) is the average value over \( k \) partial lengths of the graph.

We start now presenting our results which rely on 2,000,000 sweeps for both Met-enkephalin and poly-alanine. The potential energy function \( E_{\text{tot}} \) that we used is given by the sum of electrostatic term \( E_C \), Lennard-Jones term \( E_{LJ} \), and hydrogen-bond term \( E_{hb} \) for all pairs of atoms in the peptide together with the torsion term \( E_{\text{tors}} \) for all torsion angles. The parameters for the energy function were adopted from ECEPP/2 [19] (as implemented in the KONF90 program [20]). We further fix the peptide bond angles \( \omega \) to their common value 180°, and do not explicitly include the interaction of the peptide with the solvent and set the dielectric constant \( \epsilon \) equal to 2.

In Fig. 1 we show the free energy landscape of Met-enkephalin as a function of the overlap with the ground state for \( T = 230 \) K. The funnel towards the ground state is clearly visible in this plot. In previous work [7] it was found that at this temperature no long-living traps exist and therefore the funnel is relatively smooth. In Fig. 2 we show the corresponding logarithm of the averaged curve length \( < L(k) > \) over the interval length \( k \), for this temperature. The straight line corresponds to the least-square fit to the linear model obtained from Eq. (6). The error bars are the standard deviations obtained from \( k \) sets \( L_m(k) \) for the all statistics. Here we present the fractal dimension obtained from the whole statistics instead of introducing any binning procedure of our data. Hence, the errors reported here for the final estimates of \( d \) are related to the deviation of the linear behavior of \( \ln < L(k) > \) in Eq. (6).

Repeating the above analysis for various temperatures, we obtain a plot of the fractal dimension as a function of temperature which is displayed for Met-enkephalin in Fig. 3. Various distinct regions can be observed in this graph. For the high temperature region the fractal dimension seems to be constant and only slightly deviating from a one-dimensional graph: we find \( d \approx 1.15 \). In Refs. [3, 7] it was shown that this temperature region is dominated by extended coil structures with little resemblance to the ground state. Hence the energy landscape is a rapidly increasing function of the overlap, with the minimum at small values of that order parameter. With decreasing temperature the fractal dimension of the free energy landscape increases till it reaches a local maximum of \( d_1 = 1.33 \pm 0.05 \) for \( T = 280 \pm 40 \) K (the quoted uncertainty in the temperature is an upper estimate and given by the range of temperatures for which the measured fractal dimension \( d \) lies within the errorbars of \( d_1 \)). This temperature seems to correspond to \( T_\theta = 295 \pm 20 \) K, the collapse temperature found in earlier work [1] for Met-enkephalin. At \( T_\theta \) both extended coil structures and an ensemble of collapsed structures can exist, and the free energy landscape reflects the large fluctuations at this temperature. In Ref. [7] it was shown that this temperature a funnel-like structure of the landscape
starts to appear, which becomes clearly visible at the folding temperature \( T_f = 230 \pm 30 \) K. We find in our plot of the fractal dimension no indication for this folding transition, presumably because that temperature seems to be within the same peak. Instead we observe that the fractal dimension decreases again with further decreasing temperature. This is consistent with our previous results [6] and indicates that as the temperature decreases the ground state structure becomes more and more favored in the ensemble of compact structures. Actually, the folding temperature is defined by the condition that half of the observed configurations are ground state-like, and that temperature, \( T_f = 230 \pm 30 \) K corresponds roughly to the mid point of the fractal dimension plot between its maximum of \( d = 1.33 \pm 0.05 \) for \( T = 280 \pm 40 \) K and the low-temperature minimum of \( d = 1.25 \pm 0.02 \) at a temperature \( T = 180 \pm 30 \) K. Below that temperature, the fractal dimension increases rapidly again, indication the onset of glassy behavior and the appearance of long-living traps. Hence, we identify this temperature as the glass temperature and find for Met-enkephalin

\[
T_g = 180 \pm 30 K. \tag{7}
\]

This estimate is consistent with \( T_g = 160 \pm 30K \) as determined by an approximate calculation in Ref. [22].

As mentioned above, it is expected that for a protein \( T_f > T_g \), i.e. a good folder can be characterized by the relation [4]

\[
\frac{T_f}{T_g} > 1. \tag{8}
\]

The result for \( T_f = 230 \pm 30 \) K (as quoted in Ref. [3]) and our new estimate for the glass transition temperature \( T_g \) lead indeed to \( T_f/T_g = 1.28 > 1 \). This value of the ratio clearly demonstrates that Met-enkephalin is a good folder according to the above criterion. Our result is consistent with an alternative characterization of folding properties. Thirumalai and collaborators [21] have pointed out that the kinetic accessibility of the native conformation can be classified by the parameter

\[
\sigma = \frac{T_\theta - T_f}{T_\theta}, \tag{9}
\]

i.e., the smaller \( \sigma \) is, the more easily a protein can fold. With central values \( T_\theta = 295 \) K and \( T_f = 230 \) K, taken from Ref. [3], we have for Met-enkephalin \( \sigma \approx 0.2 \) which implies reasonably good folding properties according to Ref.[21]. Hence, we see that there is a strong correlation between the folding criterion \( (T_f/T_g > 1) \) proposed by Bryngelson and Wolynes [4] and the one by Thirumalai and co-workers [21].

It is interesting to compare the above graph with the behavior of the fractal dimension for poly-alanine with its sharp helix-coil transition. Following the receipt described for Met-enkephalin, we obtain from an analysis of free energy landscapes \( G(q,T) \) (with \( q \) defined in Eq. [3]) a plot of the fractal dimension as a function of
temperature for poly-alanine chains of length \( N = 20 \). The graph is displayed in Fig. 4.

Here we also observe an almost flat curve for high temperatures with a small value \( (d \approx 1) \) of the fractal dimension of the free energy landscape as a function of the helicity, which is our order parameter for this system. The small value for the fractal dimension indicates again the relative smooth landscape of the peptide in this temperature region which is dominated by coil structures. Again the fractal dimension increases with decreasing temperature till a maximum of \( d = 1.6 \pm 0.3 \) is reached at \( T = 510 \pm 30 \) K for \( N = 20 \).

This temperature corresponds to the helix-coil-transition temperature \( T_c = 508 \pm 5 \) K of Ref. [9]. Below that temperature the fractal dimension decreases again rapidly, but unlike for Met-enkephalin it does not increase again at some lower temperature. The rapid decrease in \( d \) reflects the observation that below \( T_c \) the system exist almost exclusively in a single configuration, namely as a single extended \( \alpha \)-helix, and that the transition between coil and helix states is either first-order-like or can be described as a strong second order transition [9]. Since the ground state structure is so strongly energetically favored for poly-alanine, we find no indication for a glass transition at lower temperatures. Such a behavior would be expected for pronounced transitions of the above type.

Let us summarize our results. We have used generalized-ensemble simulations to calculate the free-energy landscapes of two peptides as a function of a suitable reaction coordinate for a large temperature range. We have measured the fractal dimension of these energy landscapes and studied its thermal behavior. Our results show that the fractal dimension \( d(T) \) as a function of temperature is sensitive to thermodynamic transitions in the molecules. Especially, it is possible to determine estimators for the glass transition temperature \( T_g \) from this quantity.

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Figure Captions:

1. Free energy of Met-enkephalin as a function of the overlap with the (known) ground state $O$ for $T = 230$ K. The results are calculated from a generalized-ensemble simulation of 2,000,000 Monte Carlo sweeps.

2. Linear regression for $\ln < L(k) >$ (as defined in Eq. (3)) for $T = 230$ K.

3. Fractal dimension of the free-energy landscape of Met-enkephalin as function of temperature.

4. Fractal dimension of the free-energy landscape of Poly-alanine (with chain length $N = 20$) as a function of temperature.
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