Thermodynamics and Kinetics of Folding of a Small Peptide

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Abstract

We study the thermodynamics and kinetics of folding for a small peptide. Our data rely on Monte Carlo simulations where the interactions among all atoms are taken into account. Monte Carlo kinetics is used to study folding of the peptide at suitable temperatures. The results of these canonical simulations are compared with that of a generalized-ensemble simulation. Our work demonstrates that concepts of folding which were developed in the past for minimalist models hold also for this peptide when simulated with an all-atom force field.

The importance of understanding the statistical physics of the protein-folding problem has been recently recognized. The most prominent example is the energy landscape theory and the funnel concept. Originally developed out of analytical and numerical investigations of minimal protein models (which capture only a few, but supposedly dominant parameters in real proteins), such concepts were subsequently also used to probe the folding properties of more realistic models of proteins where the interactions between all atoms

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were taken into account. One example is our previous work on the pentapeptide Met-enkephalin for which we found indeed a funnel-like structure of the free energy landscape and were able to determine its characteristic temperatures of folding. In this paper we complement the above research on the thermodynamic of Met-enkephalin by investigating in addition its kinetics of foldings.

The linear peptide Met-enkephalin has the amino-acid sequence Tyr-Gly-Gly-Phe-Met, and at low temperatures one finds for this peptide two major groups of well-defined compact structures which are characterized (and stabilized) by specific hydrogen bonding patterns. Structure A is the ground-state conformation in ECEEP/2 and has a Type II’ β-turn between the second and last residue, stabilized by two possible hydrogen bonds. The structure B, the second-lowest energy state, is characterized by hydrogen bond between Tyr-1 and Phe-4 resulting in a Type-II β-turn between the first and fourth residue. The overlap of a given configuration with the ground state (structure A) and the second-lowest-energy state (structure B), respectively, allows to distinguish between the various low-energy conformations and defines in a natural way two order parameters for our system.

Simulation of realistic protein models where the interaction between all atoms are taken into account are extremely difficult. Because of the rough energy landscape, simulations based on canonical Monte Carlo or molecular dynamics techniques will get trapped at low temperatures in one of the multitude of local minima separated by high energy barriers. One possibility to overcome these difficulties are generalized-ensemble techniques. Their usefulness for calculation of thermodynamic averages was extensively tested and demonstrated in protein simulations. For a recent review see, for instance, Ref. 16. We have used one of these techniques, described first in Ref. 20, to study the thermodynamics of folding of Met-enkephalin, and utilize it here again to calculate the free energy landscape of the peptide as a function of our two order parameters. However, it is a characteristic of all generalized ensemble algorithms that they do not allow to study the kinetic aspects of folding. This is because the dynamics of these algorithms are artificial ones. While constructed in such a way that the correct distribution of states at a certain temperature can
be recovered (and therefore thermodynamic averages calculated), the time evolution of the system will be different from the one observed in a molecular dynamic simulation at that specific temperature. For this reason, generalized-ensemble algorithms are not suitable for a direct investigation of the kinetics of folding and we had to fall back on canonical Monte Carlo simulations. However, a careful analysis of the free energy landscape of the peptide, as obtained by our generalized-ensemble method, allows to extract indirect information on the kinetic of folding which can then be compared with the outcome of the canonical simulations. Actually, only by using the results of of the generalized-ensemble simulations we were able to find the relevant temperatures on which such research of the kinetics should focus and where at the same time canonical investigations are still feasible. In that sense, our two simulation techniques are complementary.

Our investigation of Met-enkephalin is based on a detailed, all-atom representation of that peptide. The interaction between the atoms is described by a standard force field, ECEPP/2 (as implemented in the KONF90 program) and is given by:

$$E_{\text{tot}} = E_C + E_{\text{LJ}} + E_{\text{HB}} + E_{\text{tor}},$$  

(1)

$$E_C = \sum_{(i,j)} \frac{332 q_i q_j}{r_{ij}},$$  

(2)

$$E_{\text{LJ}} = \sum_{(i,j)} \left( \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^6} \right),$$  

(3)

$$E_{\text{HB}} = \sum_{(i,j)} \left( \frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} \right),$$  

(4)

$$E_{\text{tor}} = \sum_l U_l \left( 1 \pm \cos(n_l \chi_l) \right).$$  

(5)

Here, $r_{ij}$ (in Å) is the distance between the atoms $i$ and $j$, and $\chi_l$ is the $l$-th torsion angle. We further fix the peptide bond angles $\omega$ to their common value $180^\circ$, which leaves us with 19 torsion angles ($\phi$, $\psi$, and $\chi$) as independent degrees of freedom (i.e., $n_F = 19$). In our simulations we did not explicitly include the interaction of the peptide with the solvent and set the dielectric constant $\epsilon$ equal to 2.

Our simulations were started from completely random initial conformations (Hot Start) and one Monte Carlo sweep updates every torsion angle of the peptide once. In our
generalized-ensemble algorithm, configurations are updated according to the following probability weight:

\[ w(E) = \left(1 + \frac{\beta (E - E_0)}{n_F}\right)^{-n_F}, \]  

(6)

where \( E_0 \) is an estimator for the ground-state energy, \( n_F \) is the number of degrees of freedom of the system, and \( \beta = 1/k_B T \) is the inverse temperature (\( k_B \) is the Boltzmann constant and \( T \) the temperature of the system). A simulation with such a weight samples a large range of energies, since high energies are suppressed only by a power law. Hence, the thermodynamic average of any physical quantity \( A \) can be calculated over a wide temperature range by

\[ < A >_T = \frac{\int dx \ A(x) w^{-1}(E(x)) e^{-\beta E(x)}}{\int dx \ w^{-1}(E(x)) e^{-\beta E(x)}}, \]  

(7)

where \( x \) stands for configurations. It is known from our previous work that the ground-state conformation for Met-enkephalin has the KONF90 energy value \( E_{GS} = -12.2 \) kcal/mol. We therefore set \( E_0 = -12.2 \) kcal/mol, \( T = 50 \) K (or, \( \beta = 10.1 \) [1 kcal/mol]) and \( n_F = 19 \) in our probability weight factor in Eq. (6). All thermodynamic quantities were then calculated from a single production run of 1,000,000 MC sweeps which followed 10,000 sweeps for thermalization. At the end of every fourth sweep we stored the energies of the conformation, the corresponding (solvent excluded) volume (in Å\(^3\)) which is calculated by the double cubic lattice method and our two “order parameters” (the overlap \( O_A \) of the conformation with the (known) ground state (structure A) and the overlap \( O_B \) of the conformation with conformer B). The overlap was defined by

\[ O(t) = 1 - \frac{1}{90} \frac{1}{n_F} \sum_{i=1}^{n_F} |\alpha_i^{(t)} - \alpha_i^{(RS)}|, \]  

(8)

where \( \alpha_i^{(t)} \) and \( \alpha_i^{(RS)} \) (in degrees) stand for the \( n_F \) dihedral angles of the conformation at \( t \)-th Monte Carlo sweep and the reference state conformation, respectively. Symmetries for the side-chain angles were taken into account and the difference \( \alpha_i^{(t)} - \alpha_i^{(RS)} \) was always projected into the interval \([-180^\circ, 180^\circ]\). Our definition guarantees that we have
Using the results of our generalized-ensemble simulation, we explored for various temperatures the free energies

\[ G(O_A, O_B) = -k_B T \log P(O_A, O_B) . \]  

(10)

Here, \( P(O_A, O_B) \) is the probability to find a peptide conformation with values \( O_A, O_B \) (at temperature \( T \)). We chose the normalization so that the lowest value of \( G(O_A, O_B) \) is set to zero for each temperature.

The generalized-ensemble simulation was complemented by 100 canonical Monte Carlo simulations for each chosen temperature. In these canonical simulations we measured the overlap \( O_A \) with the (known) ground state (Conformer A) after each MC sweep. Once we found a configuration with \( O_A(t) \geq 0.8 \), we identified this configuration with the ground state, stored the number of MC sweeps \( t_{last}^i \) for further analysis, and stopped the MC run. We also stopped the MC run if no ground state was sampled after 200,000 MC sweeps, i.e. \( t_{last} = 200,000 \) indicates that the ground state was not found in that specific run. The frequency \( n_f \) with which the ground state (conformer A) was sampled in all 100 canonical runs and the average minimal folding time \( t_f \) defined by

\[ t_f = \frac{1}{100} \sum_{i=1}^{100} t_{last}^i . \]  

(11)

are other quantities which we recorded for each temperature. Note that \( t_f \) is only a lower bound for the folding time since we stopped our runs after 200,000 sweeps if no ground state was found in a MC run. For each temperature, we also measured the frequency of unfolded states \( P_{uf}(t) \) as a function of time (in Monte Carlo sweeps). This quantity is defined by

\[ P_{uf}(t) = \frac{1}{100} \sum_{i=1}^{100} \theta_i(t, t_{last}^i) \]  

(12)

with

\[ \theta_i(t, t_{last}^i) = \begin{cases} 1 & t \leq t_{last}^i \\ 0 & \text{otherwise} \end{cases} \]  

(13)
In addition to the above quantities we finally measured the frequency \( n_f^{noB} \) with which the simulations went straight into the ground state (conformer A) (i.e. without being trapped first in conformer B); the frequency \( n_f^B \) of runs which were first trapped in conformer B before finally finding the ground state \( (n_f^B) \); and the corresponding folding times \( t_f^{noB} \) and \( t_f^B \). All 6 quantities are listed in Table 1.

For each temperature, we allowed at least 10 of the 100 canonical runs to finish the whole 200,000 sweeps independently on whether the ground state was found or not. For these 10 runs we stored the “time series” of conformations for a more detailed analysis. This allowed us to calculate the escape (or “life”) time \( \tau_{es} \) for conformer A and conformer B. For this we recorded the frequency \( n_{es}^A(t) \) \( (n_{es}^B(t)) \) with which the simulations remained in state A (B) for \( t \) MC sweeps. For three out of our four temperatures these frequencies could be fitted by a single exponential fit

\[
n_{es}(t) = A \times e^{-t/\tau_{es}} \tag{14}
\]

and the resulting escape times \( \tau_{es} \) are listed in Table 2.

We start our analyses with the results of our generalized-ensemble simulation. In earlier work we could determine the collapse temperature \( T_\theta = 295 \pm 30 \) K and the folding temperature \( T_f = 230 \pm 30 \) K\(^\text{13}\). While these two characteristic temperature are important to understand the mechanism of folding of Met-enkephalin, the essence of the funnel landscape idea is competition between the tendency towards the folded state and trapping due to ruggedness of the landscape. One way to measure this competition is by the ratio:\(^\text{26}\)

\[
Z = \frac{E - E_0}{\sqrt{\langle E^2 \rangle - \bar{E}^2}}, \tag{15}
\]

where the bar denotes averaging over compact configurations. The landscape theory asserts that good folding protein sequences are characterized by large values of \( Z \).\(^\text{26}\) Using the results of our previous simulations and defining a compact structure as one where \( V(i) \leq 1380 \text{A}^3 \), we find \( E - E_0 = 40.7(1.0) \) Kcal/mol, \( \langle E^2 \rangle - \bar{E}^2 = 15.7(2.0) \) (Kcal/mol)^2, from which we estimate for the above ratio \( Z = 10.3(1.1) \). This value indicates that Met-enkephalin is a good folder.
Another way of characterizing the competition between tendency towards the folded state and roughness of the energy landscape relies on knowledge of the glass temperature $T_g$. It is expected that for a good folder the glass transition temperature, $T_g$, where glass behavior sets in, has to be significantly lower than the folding temperature $T_f$, i.e. a good folder can be characterized by the relation

$$\frac{T_f}{T_g} > 1.$$  \hspace{1cm} (16)

We can calculate a crude estimate of the glass transition temperature by using the approximation

$$T_g = \sqrt{\frac{\langle E^2 \rangle - \bar{E}^2}{2k_B S_0}};$$  \hspace{1cm} (17)

where the bar indicates again averaging over compact structures and $S_0$ is the entropy of these states estimated by the relation

$$S_0 = \log \bar{n}_{compact} - C$$  \hspace{1cm} (18)

Here, $C$ is chosen such that the entropy of the ground state becomes zero. The results of our simulation leads to a value of $s_0 = 55(4)$. Together with the above quoted value for $\langle E^2 \rangle - \bar{E}^2 = 15.7(2.0)$ (in Kcal/mol)$^2$ one finds as an estimate for the glass transition temperature

$$T_g = 190(20) \text{ K}.$$  \hspace{1cm} (19)

This result is in good agreement with a recent estimate $T_g = 180 \pm 30 \text{ K}$ for that peptide determined from the change in the fractal dimension of the free energy landscape with temperature. Since it was stated in earlier work that $T_f = 230(30)$ K, it is obvious that the ratio $T_f/T_g > 1$, and again one finds that Met-enkephalin has good folding properties. We remark that our results are consistent with an alternative characterization of folding properties by Thirumalai and collaborators as was pointed out in detail in Ref. [13].

An advantage of the generalized-ensemble approach is that it allows us to observe directly the folding funnel of Met-enkephalin. Our results are compared with that of the canonical
runs. In Fig. 1 we show the free energy landscape as a function of both the overlap $O_A$ with the ground state and the overlap $O_B$ with structure B in the high-temperature situation (at $T = 1000$ K). The free energy has its minimum at small values of the overlap indicating that both conformers appear with only very small frequency at high temperature. We have superimposed on the free-energy landscape, as calculated from the generalized-ensemble simulation, the folding trajectory of a canonical Monte Carlo simulation (marked by dots) at the same temperature. However, we did not connect the dots, for otherwise the plot would become unreadable. It is obvious that the concentration of the dots marks the time the simulation spent in a certain region of the landscape. We see that this time is strongly correlated with the free-energy as calculated from the generalized-ensemble simulation. For instance, we have no dots for $O_A \approx 1$ (i.e. the ground state region), a region of the energy landscape suppressed by many $k_B T$. Actually, the folded state was found even at this high temperature, in 47 of the 100 canonical runs within the available 200,00 sweeps. However, the ground state is not stable at this high temperature. We found that the average escape time out of this state was about $\tau_{es} = 9$ MC sweeps, which one has to compare with an average minimal folding time of $t_f = 145,599$ MC sweeps. Hence, the probability to find folded states at such a high temperature in canonical simulations is negligible. This results is consistent with the displayed free energy landscape calculated from generalized-ensemble simulation.

At $T = 300 K$, which is essentially the collapse temperature $T_\theta = 295 \pm 30$ K of Ref. [13], a large part of the space of possible configurations lies within the $2k_B T$ contour as is clear from Fig. 2. Correspondingly, the dots, which mark the folding trajectory of a canonical simulation at this temperature, are equally distributed over the whole plot. We remark that at this temperature the folded conformation was found in all of the 100 canonical simulations. Compared with the high temperature $T = 1000$ K the average folding time decreased by an order of magnitude to $t_f = 19864$ MC sweeps and the escape time increased by an order of magnitude towards $\tau_{es} = 2000$ MC sweeps enhancing the probability to find that state at $T = 300$ K.
At the folding temperature $T_f = 230$ K a funnel in the energy landscape appears with a gradient towards the ground state, but Fig. 3 shows that there are various other structures, the most notable of which is Conformer B (where $O_B \approx 1$), with free energies $3 \, k_B T$ higher than the ground-state conformation but separated from each other and the ground state only by free energy barriers less than $1 \, k_B T$. No other long-lived traps are populated. Hence, the funnel at $T_f$ is reasonably smooth. Folding routes include direct conversion from random-coil conformations into Conformer A or some short trapping in Conformer B region before reaching Conformer A region, but at the folding temperature it is possible to reach the ground state from any configuration without getting kinetically trapped. This was indeed observed by us in the 100 canonical runs we performed at this temperature. Some of the runs went directly from the unfolded state to the folded conformation (state A), while in other runs we saw first short trapping in the region of conformer B before folding into the ground-state structure. The folding trajectory displayed in the figure is an example for the later case. We found as escape time out of conformer B roughly $\tau_{es}^B = 5000$ MC sweeps. Due to such trapping only 86 of 100 MC runs found the folded state within 200,000 sweeps, leading to an average minimal folding time of $t_f = 77,230$ MC sweeps, which is 4 times as long as for the collapse temperature $T_\theta = 300$ K. However, the escape time for the folded state also increased to $\tau_{es}^A = 19430$ MC sweeps, about 10 times as long as for $T = 300$ K. Hence, the interplay of folding time and life time of the folded state leads to the increases probability of the folded state in the free energy plot for this temperature.

Finally, Fig. 4 shows the situation for $T = 150$ K where we expect onset of glassy behavior. Again one sees a funnel-like bias toward the ground state, however, the funnel is no longer smooth and the free energy landscape is rugged. Free energy barriers of many $k_B T$ now separate different regions and would act as long-lived kinetic traps in a canonical simulation rendering folding at this temperature extremely difficult. This can be seen for the folding trajectory we display in that figure: the simulation got trapped in a region of the landscape far away from the folded state and never reached the folded state within the 200,000 sweeps of the simulation. Actually, only in 19 out of 100 Monte Carlo simulations of
200,000 sweeps we found the folded state and the minimal folding time is at least $t_f = 172866$ MC sweeps. Our data did not allow a single exponential fit to calculate the escape times for conformer A or conformer B at this temperature, however, we found that the average folding time for folding trajectories which did not go through the region of conformer B increased only modestly to $t_f^{noB} = 51,145$ MC sweeps from $t_f^{noB} = 40384$ MC sweeps at the folding temperature $T_f = 230K$, while the folding time for trajectories going through the region of conformer B increased from $t_f^B = 104,499$ MC sweeps to at least $t_f^B = 173,856$ MC sweeps. This demonstrates that with increasing glassiness of the system, it becomes more and more difficult to escape the now much longer living traps.

To further understand the folding kinetics we studied the time evolution of the fraction of unfolded states $P_{uf}(t)$ (as defined in Eq. [12]) versus time (in MC sweeps) for the four chosen temperatures. These fractions were calculated from the time series of the 100 canonical Monte Carlo simulations of up to 200,000 sweeps for each of the four temperatures. Fig. 5 displays this quantity as a function of Monte Carlo time on a log-log scale. It is obvious that the time evolution of that quantity cannot be described by a power law (which would imply a straight line in the plot) indicating that the folding has to be described by a combination of exponentials or, numerically simpler, a stretched exponential. Indeed we found that for our two highest temperatures, the observed curves can be described by a single exponential fit

$$P_{unfolded} = A \times e^{-t/\tau_1},$$  \hspace{1cm} (20)

while for the folding temperature $T_f = 230K$ and our lowest temperature $T = 150 K$, which is below the glass temperature, we needed stretched exponential fit:

$$P_{unfolded} = A \times e^{-(t/b)^c}$$  \hspace{1cm} (21)

to describe our data. The lines in Fig. 5 mark the fits through our data and we see that the chosen functional forms describe well our data. Table 3 lists the coefficients of the chosen fits. It is obvious from these kinetic data that above the folding temperature no long living
traps exist. This is in agreement with the smoothness of the energy landscape which we observe in Fig. 1 and 2. As the temperature is lowered, around at temperature $T_f \approx 230$ K, non-exponential behavior started to be observed. In the beginning this mechanism can be described by a few exponentials indicating that only small number of traps start to play a role. For instance, at the folding temperature $T_f = 230$ K, the kinetics could also be fitted well with a two exponential form (fit not shown). This is consistent with Fig. 3 where we observe indeed only few local traps in the free energy landscape. However, as the temperature gets lower and lower, the number of traps substantially increases and glassy-like dynamics is observed. Fig. 4 shows that the many local minima separated by free energy barriers of many $k_B T$. As a result, the residence time in some local traps becomes of the order of the folding event. Folding dynamics is now non-exponential (since different traps have different escape times). In this regime stretched exponentials are much more appropriate. Such a behavior was predicted from studies of minimal protein models and is now verified by us for a realistic protein model.

To summarize, we have studied the thermodynamics and kinetics of the peptide Met-enkephalin, using a combination of generalized-ensemble techniques and canonical Monte Carlo. Generalized-ensemble techniques introduce an artificial dynamics and therefore do not allow to study directly the the kinetics of proteins. However, these sophisticated techniques enable reconstruction of the free-energy landscape of a protein. We have shown that a careful analyses of these landscapes leads to indirect information on the folding kinetics. For this purpose, we compared our generalized-ensemble results with dynamical Monte Carlo simulations at appropriate temperatures. This demonstrates the usefulness of generalized-ensemble techniques in investigations of the mechanism and kinetics of folding. Combining generalized-ensemble results with dynamical Monte Carlo simulations, the present study provide evidence that the concepts of folding that were developed in the past for minimalist models hold also for our peptide when simulated with an all-atom force field.
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REFERENCES

1 J.N. Onuchic, H. Nymeyer, A.E. Garcia, J. Chahine and N.D. Socci, Adv. Prot. Chem. 43, 87 (2000)

2 K.A. Dill and H.S. Chan, Nature Structural Biology 4, 10 (1997).

3 E.I. Shakhnovitch, Curr. Opin. Struc. Biol. 7, 29 (1997).

4 J.D. Bryngelson, J.N. Onuchic, N.D. Socci and P.G. Wolynes, Proteins 21, 167 (1995).

5 M. Gruebele, Ann Rev Phys Chem. 50, 485 (1999).

6 J.-E. Shea and C.L. Brooks, III, Ann. Rev. Phys. Chem. 52, 499 (2001).

7 Z. Y. Guo and D. Thirumalai, Biopolymers 36, 83 (1995).

8 J.D. Bryngelson and P.G. Wolynes, Proc. Natl. Acad. Sci. (USA) 84, 7524 (1987).

9 P.E. Leopold, M. Montal and J.N. Onuchic, Proc. Natl. Acad. Sci. (USA) 89, 8721 (1992).

10 J.N. Onuchic, Z. Luhey-Schulten and P.G. Wolynes, Annu. Rev. Phys. Chem. 48, 545 (1997).

11 E.M. Boczko and C.L. Brooks III, Science 269, 393 (1995).

12 F.B. Sheinerman and C.L. Brooks III, Proteins 29, 193 (1997).

13 U.H.E. Hansmann, M Masuya, and Y. Okamoto, Proc. Natl. Acad. Sci. (U.S.A.) 94, 10652 (1997).

14 U.H.E. Hansmann, Y. Okamoto and J.N. Onuchic, Proteins 34, 472 (1999).

15 U.H.E. Hansmann and Y. Okamoto, J. Comp. Chem. 18, 920 (1997).

16 U.H.E. Hansmann and Y. Okamoto, in: Stauffer, D. (ed.) “Annual Reviews in Computational Physics VI” (Singapore: World Scientific), p.129. (1998).

17 U.H.E. Hansmann and Y. Okamoto, J. Comp. Chem. 14, 1333 (1993).
18 Y. Okamoto and U.H.E. Hansmann, J. Phys. Chem. 99, 11276 (1995).

19 U.H.E. Hansmann and Y. Okamoto, J. Phys. Chem. 103, 1595 (1999).

20 U.H.E. Hansmann and Y. Okamoto, Phys. Rev. E 56, 2228 (1997).

21 M.J. Sippl, G. Némethy, and H.A. Scheraga, J. Phys. Chem. 88, 6231 (1984), and references therein.

22 H. Kawai, Y. Okamoto, M. Fukugita, T. Nakazawa, and T. Kikuchi, Chem. Lett. 1991, 213 (1991); Y. Okamoto, M. Fukugita, T. Nakazawa, and H. Kawai, Protein Engineering 4, 639 (1991).

23 A.M. Ferrenberg and R.H. Swendsen, Phys. Rev. Lett. 61, 2635 (1988); Phys. Rev. Lett. 63, 1658(E) (1989), and references given in the erratum.

24 Hansmann, U.H.E. and Okamoto, Y., Physica A, 212, 415 (1994).

25 F. Eisenhauber, P. Lijnzaad, P. Argos, C. Sander and M. Scharf, J. Comp. Chem. 16, 273 (1995).

26 R.A. Goldstein, Z.A. Luthey-Schulten and P.G. Wolynes, Proc. Natl. Acad. Sci. U.S.A. 89 4918 (1992).

27 N.D. Socci, J.N. Onuchic and P.G. Wolynes, Proteins 32, 136 (1998).

28 N.A. Alves and U.H.E. Hansmann, Int. J. Mod. Phys. C 11, 301 (2000)

29 C.J. Camacho and D. Thirumalai, Proc. Natl. Acad. Sci. USA 90, 6369 (1993).

30 D.K. Klimov and D. Thirumalai, Proteins 26, 411 (1996).
Tables:

TABLE I. Number of times that the ground state configuration was found in less than 200,000 MC sweeps and the lower limit for the average folding time (in MC sweeps). We further distinguish between the case that the ground state was found without first visiting conformer ‘B’ and the case where the ground state was found only after visiting conformer ‘B’

| $T$ [K] | $n_f$ | $t_f$ [MC sweeps] | $n_f^{noB}$ | $t_f^{noB}$ [MC sweeps] | $n_f^B$ | $t_f^B$ [MC sweeps] |
|--------|-------|-------------------|-------------|------------------------|--------|-------------------|
| 1000   | 47    | 145599 (14286)    | 40          | 80696 (12562)          | 7      | 176145 (32753)    |
| 300    | 100   | 19864 (1141)      | 45          | 8570 (759)            | 55     | 29104 (2852)      |
| 230    | 86    | 77230 (7489)      | 47          | 40384 (5505)          | 39     | 104499 (14479)    |
| 150    | 19    | 172866 (16964)    | 14          | 51145 (13132)         | 5      | 173856 (34871)    |

TABLE II. Escape times for conformer A and B as function of temperature. For $T = 150$ K no single exponential fit was possible.

| $T$ [K] | $\tau_{eq}^A$ [MC sweeps] | $\tau_{eq}^B$ [MC sweeps] |
|--------|---------------------------|---------------------------|
| 1000   | 9.0 (5)                   | 6.9 (6)                   |
| 300    | 2022 (17)                 | 789 (16)                  |
| 230    | 19430 (230)               | 4830 (50)                 |
| 150    | -                         | -                         |
TABLE III. Coefficients for a stretched exponential fit $P_{\text{unfolded}} = A \times e^{-(t/b)^c}$ of the frequency of unfolded conformations as a function of time (in MC sweeps) for 100 canonical Monte Carlo runs. For the case of $T = 1000$ K and $T = 300$ K we added for comparison the coefficients for a single-exponential fit (i.e. $c = 1$) and mark these coefficients by a $\ast$.

| $T$ [K] | a         | b               | c  |
|---------|-----------|-----------------|----|
| 1000    | $1.002(3)$| $310,000(3,000)$| 0.95(2) |
| 1000    | 1(∗)     | 304,250 (760) (∗) | 1 (∗) |
| 300     | $1.004(8)$| 18,000(3,000)   | 0.91(15) |
| 300     | 1 (∗)    | 20,200 (100) (∗) | 1 (∗) |
| 230     | 0.97(2)  | 90000(4,000)    | 0.76(4) |
| 150     | 1.005(1) | 6,613,000(20,000) | 0.43(1) |
Figure Captions:

1. Fig. 1: Free energy $G(O_A, O_B)$ as a function of both overlaps $O_A$ and $O_B$ (as defined in the text) for $T = 1000$ K. The data rely on a generalized-ensemble simulation of 1,000,000 sweeps. The contour lines are spaces $1k_B T$. Superimposed is the folding trajectory of a canonical simulation of 200,000 sweeps at the same temperature. We marked with “U” the region of random unfolded conformers.

2. Fig. 2: Free energy $G(O_A, O_B)$ as a function of both overlaps $O_A$ and $O_B$ (as defined in the text) for $T = T_\theta = 300$ K. The data rely on a generalized-ensemble simulation of 1,000,000 sweeps. The contour lines are spaces $1k_B T$. Superimposed is the folding trajectory of a canonical simulation of 200,000 sweeps at the same temperature. “U” marks random, unfolded conformers and “A” marks conformer A.

3. Fig. 3: Free energy $G(O_A, O_B)$ as a function of both overlaps $O_A$ and $O_B$ (as defined in the text) for $T = T_f = 230$ K. The data rely on a generalized-ensemble simulation of 1,000,000 sweeps. The contour lines are spaces $1k_B T$. Superimposed is the folding trajectory of a canonical simulation of 200,000 sweeps at the same temperature. “U” marks random, unfolded structures, “A” conformer A and “B” marks conformer B.

4. Fig. 4: Free energy $G(O_A, O_B)$ as a function of both overlaps $O_A$ and $O_B$ (as defined in the text) for $T = 150$ K, well below the glass transition temperature $T_g = 190\pm20$. The data rely on a generalized-ensemble simulation of 1,000,000 sweeps. The contour lines are spaces $1k_B T$. Superimposed is the folding trajectory of a canonical simulation of 200,000 sweeps at the same temperature. The minimum corresponding to the ground state (conformer A) is marked by “A”.

5. Fig. 5: Probability of unfolded configurations as a function of Monte Carlo time on a log-log plot. The lines mark our fits (see text) through the data points.
$T = 1000 \text{ K}$
$T = 300 \text{ K}$
T = 230 K
