Equilibrium Collapse and the Kinetic 'Foldability' of Proteins†

Ian S. Millet,[‡,§] Lara E. Townsley,[§,||] Fabrizio Chiti,[⊥] Sebastian Doniach,[‡] and Kevin W. Plaxco*[,||,#]

Department of Applied Physics, Stanford University, Stanford, California 92343, Department of Chemistry and Biochemistry and Biomolecular Science and Engineering Program, University of California, Santa Barbara, Santa Barbara, California 93106, and Dipartimento di Biochimica, Università di Firenze, Viale Morgagni 50, 50134, Firenze, Italy

Received August 17, 2001; Revised Manuscript Received November 8, 2001

ABSTRACT: An important element of protein folding theory has been the identification of equilibrium parameters that might uniquely distinguish rapidly folding polypeptide sequences from those that fold slowly. One such parameter, termed σ, is a dimensionless, equilibrium measure of the coincidence of chain compaction and folding that is predicted to be an important determinant of relative folding kinetics. To test this prediction and improve our understanding of the putative relationship between nonspecific compaction of the unfolded state and protein folding kinetics, we have used small-angle X-ray scattering and circular dichroism spectroscopy to measure the σ of five well-characterized proteins. Consistent with theoretical predictions, we find that near-perfect coincidence of the unfolded state contraction and folding ($\sigma \approx 0$) is associated with the rapid kinetics of these naturally occurring proteins. We do not, however, observe any significant correlation between σ and either the relative folding rates of these proteins or the presence or absence of well-populated kinetic intermediates. Thus, while $\sigma \approx 0$ may be a necessary condition to ensure rapid folding, differences in σ do not account for the wide range of rates and mechanisms with which naturally occurring proteins fold.

Proteins spontaneously fold to their unique native structures many orders of magnitude more rapidly than would be possible were folding an exhaustive, random search of conformational space (*1*). This seemingly paradoxical behavior has led to numerous theoretical models of how naturally occurring proteins achieve their native structures on a biologically relevant time-scale (reviewed in *2*−*5*), many of which have emerged from studies of the simulated folding of simple, protein-like lattice and off-lattice polymers (e.g., *6*−*11*).

A common element among many theoretical approaches has been the effort to identify *equilibrium* parameters that might uniquely give rise to the *kinetic* property of rapid folding. For example, Wolynes, Onuchic, and others have noted that, to ensure the rapid folding of simplified on- and off-lattice polymers, the folding transition temperature (T_m; the temperature below which the native state is stable relative to the unfolded state) must be significantly higher than T_g, the temperature at which local minima dominate kinetics and folding becomes arbitrarily slow (reviewed in *2*; see also *12*). A related criterion, described by Sali, Shakhnovich, Karplus, and others (e.g., *7*), suggests that kinetic "foldability" correlates with the presence of a large gap between the energy of the native state and that of all other states. Finally, Thirumalai and co-workers have predicted that relative folding rates are defined by σ, a dimensionless, equilibrium measure that reflects the ease with which the denatured state undergoes nonspecific collapse relative to the ease with which the protein folds as temperature or denaturant concentration is reduced (*5*, *13*−*18*). Despite the theoretical community's emphasis on uncovering the equilibrium determinants of rapid folding, relatively few experimental investigations of these putative determinants have been reported.

Here we describe the results of an experimental test of whether one of these parameters, σ, is a significant determinant of the relative folding kinetics of real proteins. Two reportedly equivalent measures of σ can be derived. The first, σ_T, is defined in terms thermal unfolding and is related to the characteristic folding (T_m) and collapse (T_θ) temperatures by

$$\sigma_T = (T_\theta - T_m)/T_\theta$$

where T_m is the temperature (in degrees kelvin) at which half of a population of polymers is folded and T_θ is the temperature at which the root-mean-square radius of gyration (R_g) of the ensemble is midway between those of the folded and unfolded states. The second, σ_D, is reported to be an equivalent parameter obtained via chemical denaturation experiments (*17;* D. Thirumalai and D. Klimov, personal communication). It is defined by

† The U.S. Department of Energy and the National Institutes of Health support SSRL. Portions of this work were funded by UCBioSTAR Grant 19991296.

* To whom correspondence should be addressed. Phone: (805) 893-5558. Fax: (805) 893-4120. E-mail: kwp@chem.ucsb.edu.

‡ Department of Applied Physics, Stanford University.

§ These authors contributed equally to this work.

|| Department of Chemistry and Biochemistry, University of California, Santa Barbara.

⊥ Dipartimento di Biochimica, Università di Firenze.

Biomolecular Science and Engineering Program, University of California, Santa Barbara.

10.1021/bi015695a CCC: $22.00
Published on Web 12/11/2001

$$\sigma_D = (C_\theta - C_m)/C_\theta$$

where C_m is the denaturant concentration at which half of the population of polymers is folded and C_θ is the denaturant concentration at which the average R_g of the ensemble is midway between those of the folded and unfolded states. Because thermal unfolding is often irreversible, σ_D is much more readily accessible experimentally than σ_T.

Several research groups have explored the relationship between σ and the folding kinetics of simplified, protein-like computational models. Thirumalai and co-workers have demonstrated that on- and off-lattice polymer sequences with high σ fold orders of magnitude more slowly than sequences that contract only at conditions nearer the midpoint of the folding transition (see, e.g., *5*). Shakhnovich, Karplus, and co-workers have also investigated the relationship between σ and folding rates. While these authors report that folding kinetics correlate more strongly with the energy gap criterion than with σ (and, indeed, raise questions about the definition of the parameter), they nevertheless observe a statistically significant correlation between σ and folding rates across several simplified protein models (*19*). Chiu and Goldstein, in contrast, have noted that the relationship between σ and folding rates might be significantly more complex than the simple, monotonic relationship described by either of the above groups and might critically depend on the amino acid composition of the proteins in question (*20*). Last, Thirumalai and co-workers have reported that the folding of simplified computational models with high values of σ occurs via well-populated kinetic intermediates, whereas low-σ sequences fold via concerted, two-state kinetics (see, e.g., *5*). Results such as these have lead to the hypothesis that σ may be a determinant of the relative folding kinetics and mechanisms of real proteins (see, e.g., *5, 14*), a prediction that provides a rare opportunity to quantitatively evaluate the correspondence between theory and experiment in protein folding.

The results of previous experimental studies do not adequately constrain σ. When studied using any of a variety of spectroscopic probes, the equilibrium folding of many proteins is well fitted as a two-state process (see, e.g., *21*−*23*), suggesting that their folding is highly cooperative and σ is near zero. Currently employed spectroscopic probes, however, are sensitive to the formation of secondary or tertiary structure but do not provide an unambiguous measure of the nonspecific contraction of the denatured ensemble as conditions are relaxed (*24*). Small-angle X-ray scattering (SAXS),[1] in contrast, provides a direct measure of the average dimensions of a conformational ensemble and thus is supremely well suited as a means of monitoring nonspecific contraction and thus determining σ. To test the predicted relationship between σ and the rates and mechanisms of protein folding, we have employed SAXS and circular dichroism spectroscopy (CD) to measure the parameter experimentally. We have characterized σ_D for five proteins that span a 4 orders of magnitude range of folding rates and fold via both two-state and non-two-state kinetic mechanisms. The relevant simulations suggest that the σ_D of the slowest folding of these proteins should be greater than that of the fastest by at least 0.5 (see, e.g., *5, 15*), a difference well within reach of experimental verification.

[1] Abbreviations: AcP, muscle acylphosphatase; CD, circular dichroism; GuHCl, guanidine hydrochloride; SAXS, small-angle X-ray scattering.

MATERIALS AND METHODS

The SAXS data for protein L and cytochrome *c* were adopted from previously published reports (*21, 22*). Horse heart cytochrome *c*, bovine ubiquitin, and hen egg white lysozyme were obtained commercially (Sigma, Inc., Milwaukee, WI) and used without further purification. The C21S mutant of muscle acylphosphatase (AcP) and the Y34W mutant of protein L were purified as previously described (*22, 23*). Sample conditions were as follows: cytochrome *c*, 100 mM sodium phosphate, pH 7, 23 °C; ubiquitin, 50 mM sodium phosphate, pH 7, 25 °C; protein L, 50 mM Tris, pH 7, 5 °C; AcP, 50 mM Tris, pH 7.2, 25 °C; lysozyme, 50 mM Tris, pH 7, 25 °C.

C_m values were determined via CD performed on 0.5−1 mg/mL (near-UV) and 0.1−0.2 mg/mL (far-UV) protein solutions. Ellipticity was monitored at the wavelengths presented (Figure 1, left panels) using an Aviv 202 CD spectrometer temperature controlled to ±0.1 °C. The AcP unfolding curve was generated with urea; all others were generated with guanidine hydrochloride (GuHCl). AcP and lysozyme unfold very slowly, and thus these proteins were monitored using manually mixed solutions equilibrated in excess of 10 h. For all other proteins, the denaturant concentration was varied using an automatic titrator with a 60−300 s equilibration at each concentration prior to data collection. Data from chemical denaturations were fitted to standard two-state transitions with sloping, linear baselines:

$$CD = CD_u + S_u[den] + \frac{CD_f + S_f[den] - CD_u - S_u[den]}{1 + e^{-(mC_m - m[den])/RT}}$$

where R is the gas constant, T is the absolute temperature, CD_f and CD_u represent the CD signals of the folded and unfolded states in water, respectively, S_f and S_u represent the denaturant dependencies of these values, m is the so-called equilibrium m-value (a measure of the degree to which denaturant destabilizes the protein), and C_m is the midpoint of the folding transition. The data were fit in Kaleidagraph (Abelbeck software) using nonlinear least squares regression.

SAXS experiments were conducted at Beam Line 4-2 of the Stanford Synchrotron Radiation Laboratory. All SAXS experiments were conducted at ∼10 mg/mL protein. Lyophilized protein was freshly dissolved in the appropriate denaturant-containing buffer, centrifuged at ∼10000*g*, and then allowed to equilibrate for 10 min to 10 h (depending on the unfolding rate). Ubiqutin and AcP samples also contained 5 mM of the radical scavenger *N-tert*-butyl-α-(4-pyridyl)nitrone *N*′-oxide (Fluka, Inc.), allowing for increased X-ray flux and significantly improved signal-to-noise. For cytochrome *c* and lysozyme, both of which are commercially available in quantity, a flow cell was used, also allowing for significantly increased flux and improved signal-to-noise. The sample cuvette was held at ±∼1 °C of the desired temperature. R_g values were determined using the Guinier approximation (*25*), with S^2 fitting ranges of 0.000018−0.0001 Å^{-1}. Equilibrium R_g values were fit to a linear free energy relationship using the equation:

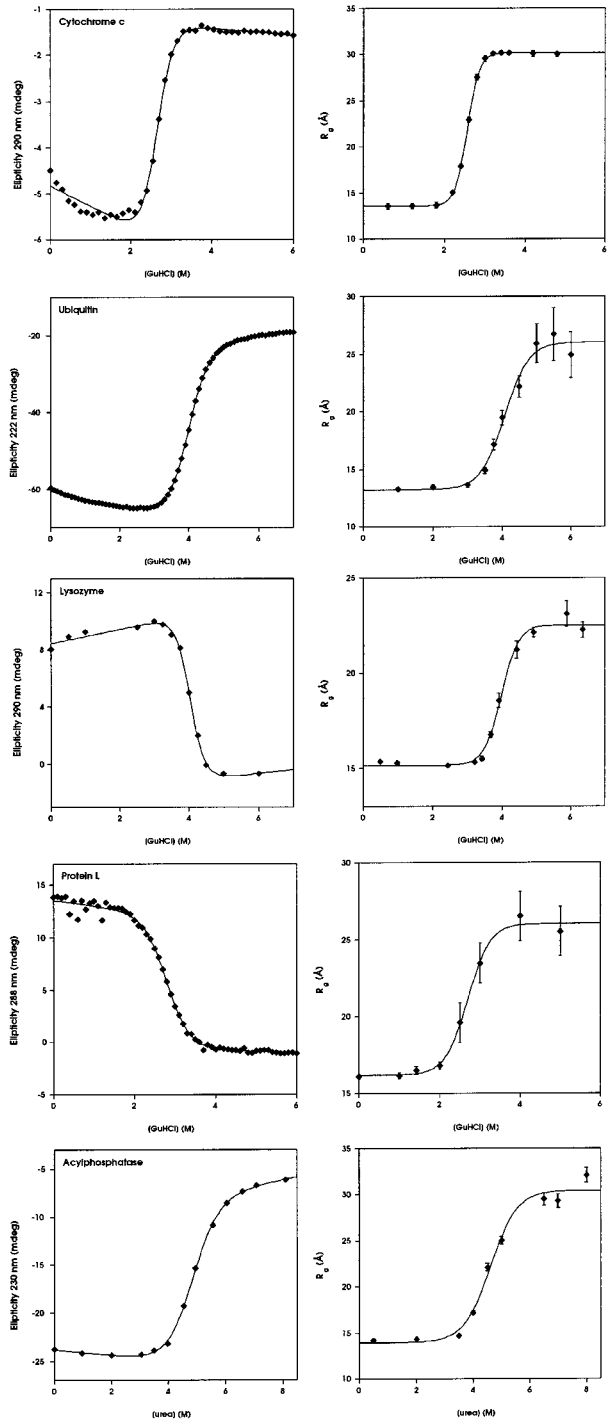

FIGURE 1: Equilibrium folding and collapse as monitored by CD (left panels) and SAXS (right panels), respectively. Two of the five proteins, cytochrome *c* and ubiquitin, are single-domain proteins often reported to fold via burst phase intermediates under the conditions employed (*26*−*29*). Lysozyme is a two-domain protein that folds via one or more well-populated intermediate states under most solvent conditions (*21*, *30*). Protein L and acylphosphatase, are, in contrast, generally reported to fold via two-state kinetics under almost all solvent conditions (*12*, *23*). The equilibrium unfolding of each protein as measured by CD is well fitted ($r^2 > 0.997$) as a fully cooperative, two-state process (solid lines). Similarly, while the X-ray absorbance of the denaturant degrades the accuracy of R_g determined under highly denaturing conditions, the denaturant dependencies of R_g for all five proteins are well fitted ($r^2 > 0.985$) by a two-state linear free energy model lacking sloping baselines (solid lines). For all five proteins, the midpoints of the R_g- and ellipticity-monitored transitions coincide closely, reflecting near-zero σ.

$$R_g = \sqrt{R_d^2 + \frac{R_n^2 - R_d^2}{1 + e^{-(mC_\theta - m[\mathrm{den}])/RT}}}$$

where R_n and R_d are the R_g of native and denatured protein, respectively, m is the equilibrium m-value, and C_θ is the midpoint of the collapse transition. This equation takes into account the geometric additivity of R_g values. The data points and error bars presented (Figure 1, right panels) represent the average and standard deviation, respectively, of two to three independent measurements. Error ranges for fitted paraeters in Table 1 represent estimated 95% confidence intervals.

RESULTS

The five proteins under study have previously been the subjects of extensive kinetic characterization. Four are simple, single-domain proteins lacking disulfide bonds. Two of these, protein L and AcP, are reported to fold via two-state kinetics under a wide variety of solvent conditions (*12*, *23*). In contrast, cytochrome *c* and ubiquitin exhibit burst-phase kinetics and/or kinetic roll-over under the conditions employed here (*26*, *27*). Although the exact interpretation of these observations remains controversial (*28*, *29*), they are usually taken as an indication of well-populated folding intermediates. The fifth protein, lysozyme, is a two-domain protein containing four disulfide bonds. Exhaustive time-resolved spectroscopic and SAXS studies have demonstrated that lysozyme folds via one or more well-populated intermediate states under a wide variety of solvent conditions (see, e.g., *21*, *30*) including those employed here (L.E.T. and K.W.P., unpublished observations). The folding rates of the proteins employed here have been adopted from the literature and are reported in Table 1.

The determination of C_m requires a spectroscopic probe that unambiguously discriminates between the native state and all other configurations. Near-UV CD, which measures aromatic side chain packing, is a direct and unmistakable indicator of native state population and was used to monitor the folding of protein L, cytochrome *c*, and lysozyme. The two remaining proteins, AcP and ubiquitin, exhibit extremely weak near-UV signals, and thus their folding was monitored via far-UV CD. While this provides a far less direct measure of the population of native state (some highly specific "collapsed" states exhibit secondary structure and are thus far-UV CD-active), the far-UV CD unfolding curves of these proteins coincide closely with unfolding curves derived via fluorescence (*27*, *31*), suggesting that these spectral changes also coincide with the formation of native core packing. The CD-based unfolding curves of all five proteins are well fitted via simple, two-state unfolding models (Figure 1, left panels), producing C_m (Table 1) quite similar to previously reported values (*27*, *31*−*33*).

The determination of C_θ requires a means of directly monitoring the average dimensions of a heterogeneous ensemble of conformations as the concentration of denaturant is decreased and the denatured state contracts and folds. We have used SAXS to measure R_g and thus monitor this equilibrium conformational change. The denaturant dependencies of R_g for the five proteins investigated here are well fitted ($r^2 > 0.985$) by a two-state linear free energy model

Table 1: Equilibrium and Kinetic Folding Parameters[a]

protein	folding rate[b] (s^{-1})	σ_D	C_m (M)	C_θ (M)	denaturant
cyt *c*	6300	-0.02 ± 0.03	2.65 ± 0.04	2.61 ± 0.06	GuHCl
ubiquitin	1550[c]	0.05 ± 0.04	3.97 ± 0.01	4.19 ± 0.18	GuHCl
lysozyme	37[d]	-0.02 ± 0.02	4.03 ± 0.06	3.96 ± 0.06	GuHCl
protein L	10[e]	0.00 ± 0.04	2.76 ± 0.03	2.76 ± 0.10	GuHCl
AcP	0.22	-0.01 ± 0.05	4.82 ± 0.07	4.78 ± 0.24	urea

[a] Error ranges for fitted parameters represent estimated 95% confidence intervals. [b] Extrapolated rates of folding to the native state, in water under the conditions employed for the determination of σ_D (*23, 29, 32, 33;* L.E.T. and K.W.P., unpublished experiments). Actual folding rates may be lower due to kinetic roll-over, which is particularly notable for cytochrome *c* (*26*) and lysozyme (*30*). [c] Kinetic data for F45W mutation (*29*). The refolding rate of the wild-type protein (for which σ was determined) is similar (S. Jackson, personal communication). [d] L.E.T. and K.W.P., unpublished data collected at 25 °C, pH 7. [e] Reported is the estimated folding rate of protein L at 5 °C, the temperature at which σ_D was determined. At 22.5 °C, the estimated folding rate is 62 s^{-1} (*33*).

without sloping baselines (Figure 1, right panels), producing well-constrained values of C_θ (Table 1). We observe no statistically significant evidence in favor of denatured state contraction as the denaturant concentration is reduced to C_θ.

Using the C_m and C_θ values obtained via CD and SAXS, respectively, we have determined σ_D for the five proteins described (Table 1). All five exhibit near-zero σ_D; four of the five σ_D are within 95% confidence intervals of zero, and the fifth exhibits a small, nonzero σ (0.05 ± 0.04) easily equivalent to zero when possible systematic experimental errors are taken into account (for example, SAXS sample cell temperature control is only accurate to ~1 °C). The near-zero values of σ_D provide additional support of the claim that, irrespective of their folding rates, none of the proteins exhibit any signs of denatured state contraction prior to folding to the native state. We observe no statistically significant evidence that σ is either quantitatively correlated with folding rates or qualitatively correlated with the population of kinetic intermediates.

DISCUSSION

Studies of the simulated folding of protein-like on- and off-lattice polymers have led to the prediction that σ must be low in order to ensure rapid folding and that differences in σ might partially account for the range of rates and intermediates associated with the folding of naturally occurring proteins. While the measured σ described here are consistent with the prediction that $\sigma \approx 0$ is advantageous with regard to generating rapid folding, we observe no evidence in support of the suggestion that differences in σ account for any of the dispersion in known protein folding rates or mechanisms.

Despite the diversity of their folding kinetics, all five proteins we have investigated fold with equivalently low σ. Consistent with this, σ_D is not correlated with their folding rates; even the most slowly folding of the proteins studied exhibits a σ_D within experimental error of zero, significantly lower than the predicted value of $\sim 0.5 + \sigma_{fastest}$. σ appears similarly uncorrelated with the presence or absence of well-populated kinetic intermediates, as even lysozyme—which folds with clearly non-two-state kinetics—exhibits a σ_D within error of zero. Counter to the behavior of some simplified on- and off-lattice protein models, the relative folding kinetics of real proteins are not defined by this measure of their equilibrium properties.

Two questions arise concerning the validity of the results presented here. The first regards the correspondence between the experimentally observed σ_D, and σ_T, the factor predicted by theory to be related to folding kinetics. Consistent with theory (e.g., *17*), however, additional experimental data suggests that the two parameters are effectively equivalent. Arai and Hirai have followed the thermal unfolding of lysozyme via SAXS and differential scanning calorimetry (*34*). These data allow us to estimate that, like σ_D, σ_T is approximately zero for this protein at neutral pH. The second question regards whether the proteins described are representative of all proteins. Experimental evidence demonstrates that they are not, as some proteins have been observed with significantly nonzero σ (*35, 36*). Indeed, even the σ of lysozyme differs significantly from zero at low pH (*37, 38*). The results reported here and elsewhere (*39*−*41*), however, demonstrate that σ values of zero are quite common and that σ is not an accurate predictor of the relative folding kinetics of real proteins.

The observation that $\sigma \approx 0$ is common for naturally occurring proteins is consistent, however, with the suggestion that concomitant collapse and folding is advantageous with regard to generating rapid, biologically relevant folding rates (*5, 42*). Biopolymers exhibiting near-zero σ are thought to exhibit a near-perfect balance between local and nonlocal interactions (*42*), which optimizes progress toward the native state without enhancing non-native interactions that can potentially retard the folding process. Thus, while the absolute folding rates of proteins for which σ is zero would be rapid (compared to proteins that populate kinetically trapped, collapsed states), their relative folding rates would be defined by structural or thermodynamic parameters other than σ.

One such alternative parameter is the difficulty of the diffusive search for the gross native topology, the so-called topomer search problem *(43, 44)*. This model is based on the observation that, if the native state is formed in a highly cooperative fashion, only those unfolded configurations with the same overall topology as the native state would be able to collapse rapidly into a stable well. The results presented here are among many that demonstrate that the folding of the native state is highly cooperative. For example, truncation mutations demonstrate that 90−95% of a protein's native interactions must be present before its free energy drops significantly below zero *(45, 46)*. The difficulty of the diffusive search for those rare unfolded conformations capable of forming 90−95% of native interactions (to become trapped in the native well) without undergoing significant, rate-limiting rearrangements may be the dominant determinant of relative folding rates. This suggestion is consistent with the observation that native state topology is highly correlated with the folding rates of simple proteins *(44, 48)*. Were non-native, collapsed configurations stable (i.e., were $\sigma > 0$), then the energy surface of folding would presumably be dominated by these local, collapsed minima, and kinetic barriers other than the topomer search would determine the rate-limiting step in the process.

ACKNOWLEDGMENT

We acknowledge Professor D. Thirumalai for the many informative conversations that shaped this research project, D. Segel for providing the cytochrome *c* SAXS data, and Professor G. Ramponi in whose laboratory the AcP was purified.

REFERENCES

1. Levinthal, C. (1969) in *Mossbauer Spectroscopy in Biological Systems, Proceedings* (Debrunner, P., Tsibris, J. C. M., and Munck, E., Eds.) University of Illinois Bulletin 67, pp 22−25.
2. Onuchic, J. N., Luthey-Schulten, Z., and Wolynes, P. G. (1997) *Annu. Rev. Phys. Chem. 48*, 545−600.
3. Chan, H. S., and Dill, K. A. (1998) *Proteins: Struct., Funct., Genet. 30*, 2−33.
4. Pande, V. S., Grosberg, A., Tanaka, T., and Rokhsar, D. S. (1998) *Curr. Opin. Struct. Biol. 8*, 68−79.
5. Thirumalai, D., and Klimov, D. K. (1999) *Curr. Opin. Struct. Biol. 9*, 197−207.
6. Ueda, Y., Taketomi, H., and Go, N. (1978) *Biopolymers 17*, 1531−1548.
7. Sali, A., Shakhnovich, E., and Karplus, M. (1994) *J. Mol. Biol. 235*, 1614−1636.
8. Dill, K. A., Bromberg, S., Yue, K., Fiebig, K. M., Yee, D. P., Thomas, P. D., and Chan, H. S. (1995) *Protein Sci. 4*, 561−602.
9. Onuchic, J. N., Wolynes, P. G., Luthey-Schulten, Z., and Socci, N. D. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 3626−3630.
10. Nymeyer, H., García, A. E., and Onuchic, J. N. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 5921−5928.
11. Pande, V. S., and Rokhsar, D. S. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 1273−1278.
12. Gillespie, B., and Plaxco, K. W. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 12014−12019.
13. Camacho, C. J., and Thirumalai, D. (1996) *Europhys. Lett. 35*, 627−632.
14. Veitshans, T., Klimov, D., and Thirumalai, D. (1996) *Fold. Des. 2*, 1−22.
15. Klimov, D. K., and Thirumalai, D. (1996) *Phys. Rev. Lett. 76*, 4070−4073.
16. Klimov, D. K., and Thirumalai, D. (1996) *Proteins: Struct., Funct., Genet. 26*, 411−441.
17. Klimov, D. K., and Thirumalai, D. (1998) *Fold. Des. 3*, 127−139.
18. Klimov, D. K., and Thirumalai, D. (1998) *J. Chem. Phys. 109*, 4119−4125.
19. Dinner, A. R., Abkevich, V., Shakhnovich, E., and Karplus, M. (1999) *Proteins: Struct., Funct., Genet. 35*, 34−40.
20. Chiu, T.-L., and Goldstein, R. A. (1997) *J. Chem. Phys. 107*, 4408−4415.
21. Segel, D. J., Bachmann, A., Hofrichter, J., Hodgson, K. O., Doniach, S., and Kiefhaber, T. (1999) *J. Mol. Biol. 288*, 489−499.
22. Plaxco, K. W., Millett, I. S., Segel, D. J., Doniach, S., and Baker, D. (1999) *Nat. Struct. Biol. 6*, 554−556.
23. Van Nuland, N. A. J., Chiti, F., Taddei, N., Raugei, G., Ramponi, G., and Dobson, C. M. (1998) *J. Mol. Biol. 283*, 883−891.
24. Plaxco, K. W., and Dobson, C. M. (1996) *Curr. Opin. Struct. Biol. 6*, 630−636.
25. Glatter, O., and Kratky, O. (1982) *Small-angle X-ray Scattering*, Academic Press, London.
26. Elove, G. A., Chaffotte, A. F., Roder, H., and Goldberg, M. E. (1992) *Biochemistry 31*, 6876−6883.
27. Khorasanizadeh, S., Peters, I. D., Butt, T. R., and Roder, H. (1993) *Biochemistry 32*, 7054−7063.
28. Sosnick, T. R., Shtilerman, M. D., Mayne, L., and Englander, S. W. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8545−8550.
29. Krantz, B. A., and Sosnick, T. R. (2000) *Biochemistry 39*, 11696−11701.
30. Matagne, A., and Dobson, C. M. (1998) *Cell. Mol. Life Sci. 54*, 363−371.
31. Chiti, F., Van Nuland N. A. J., Taddei N., Magherini F., Stefani M., Ramponi, G., and Dobson, C. M. (1998) *Biochemistry 37*, 1447−1455.
32. Mines, G. A., Pascher, T., Lee, S. C., Winkler, J. R., and Gray, H. B. (1996) *Chem. Biol. 3*, 491−497.
33. Scalley, M. L., and Baker, D. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 10636−10640.
34. Arai, S., and Hirai, M. (1999) *Biophys. J. 76*, 2192−2197.
35. Gualfetti, P. J., Iwakura, M., Lee, J. C., Kihara, H., Bilsel, O., Zitzewitz, J. A., and Matthews, C. R. (1999) *Biochemistry 38*, 13367−13378.
36. Koide, S., Bu, Z. M., Risal, D., Pham, T. N., Nakagawa, T., Tamura, A., and Engelman, D. M. (1999) *Biochemistry 38*, 4757−4767.
37. Chen, L. L., Hodgson, K. O., and Doniach, S. (1996) *J. Mol. Biol. 261*, 658−671.
38. Hirai, M., Arai, S., Iwase, H., and Takizawa, T. (1998) *J. Phys. Chem. B, 102*, 1308−1313.
39. Zhou, J. M., Fan, Y. X., Kihara, H., Kimura, K., and Amemiya, Y. (1997) *FEBS Lett. 415*, 183−185.
40. Semisotnov, G. V., Kihara, H., Kotova, N. V., Kimura, K., Amemiya, Y., Wakabayashi, K., Serdyuk, I. N., Timchenko, A. A., Chiba, K., Nikaido, K., Ikura, T., and Kuwajima, K. (1996) *J. Mol. Biol. 262*, 559−574.
41. Smith, C. K., Bu, Z. M., Anderson, K. S., Sturtevant, J. M., Engelman, D. M., and Regan, L. (1996) *Protein Sci. 5*, 2009−2019.
42. Thirumalai, D. (1995) *J. Phys. I 5*, 1457−1467.
43. Debe, D. A., and Goddard, W. A. (1999) *J. Mol. Biol. 294*, 619−625.
44. Makarov, D. E., Keller, C. A., Plaxco, K. W., and Metiu, H. (2001) *Proc. Natl. Acad. Sci. U.S.A.* (submitted for publication).
45. Flanagan, J. M., Kataoka, M., Shortle, D., and Engelman, D. M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 748−752.
46. Ladurner, A. G., Itzhaki, L. S., Gay, G. D., and Fersht, A. R. (1997) *J. Mol. Biol. 273*, 317−329.
47. Neira, J. L., and Fersht, A. R. (1999) *J. Mol. Biol. 287*, 421−432.
48. Plaxco, K. W., Simons, K. T., and Baker, D. (1998) *J. Mol. Biol. 277*, 985−994.

BI015695A