Monomer topology defines folding speed of heptamer

NEIL BASCOS,¹ JESSE GUIDRY,² AND PERNILLA WITTUNG-STAFSHEDE³

¹Molecular and Cellular Biology Graduate Program, Tulane University, New Orleans, Louisiana 70112, USA

²Louisiana State University Health Science Center, Department of Pharmacology, New Orleans, Louisiana 70112, USA

³Biochemistry and Cell Biology and Chemistry Departments, Rice University, Houston, Texas 77251, USA

(RECEIVED December 9, 2003; FINAL REVISION January 27, 2004; ACCEPTED January 27, 2004)

Abstract

Small monomeric proteins often fold in apparent two-state processes with folding speeds dictated by their native-state topology. Here we test, for the first time, the influence of monomer topology on the folding speed of an oligomeric protein: the heptameric cochaperonin protein 10 (cpn10), which in the native state has seven β -barrel subunits noncovalently assembled through β -strand pairing. Cpn10 is a particularly useful model because equilibrium-unfolding experiments have revealed that the denatured state in urea is that of a nonnative heptamer. Surprisingly, refolding of the nonnative cpn10 heptamer is a simple two-state kinetic process with a folding-rate constant in water (2.1 sec^{-1} ; pH 7.0, 20°C) that is in excellent agreement with the prediction based on the native-state topology of the cpn10 monomer. Thus, the monomers appear to fold as independent units, with a speed that correlates with topology, although the C and N termini are trapped in β -strand pairing with neighboring subunits. In contrast, refolding of unfolded cpn10 monomers is dominated by a slow association step.

Keywords: cochaperonin protein; protein folding; protein assembly; contact order; topology

Many small monomeric proteins fold in apparent two-state processes, whereas folding of larger such proteins (of >100 residues) often require involvement of equilibrium and kinetic intermediates (Jackson 1998). Statistical correlations of the folding rates of small single-domain proteins (folding without intermediates) against a number of parameters, such as stability, chain length, transition-state placement, and topology, have been tested. The native-state contact order, a parameter reporting on the degree of local versus nonlocal contacts in the native state (i.e., a measure of the native-state topology), was found to be highly significant (Plaxco et al. 1998, 2000). Contact order is defined as the average sequence separation between contacting residues in the native state. Proteins with lower contact order (greater influence of local interactions, such as α -helices) exhibit an increased folding rate compared with that of proteins with higher contact order (greater influence of nonlocal interactions, such as

β -sheets). Also related to native-state topology, the number of sequence-distant contacts per residue, defined as the long-range order (LRO), correlates as well as contact order with experimental folding rates of small globular proteins (Gromiha and Selvaraj 2001; Gromiha 2003): The more sequence-distant contacts per residue, the slower the folding is.

Several groups have attempted to define mechanistic models that explain the empirical topology-rate correlation. The topomer-search model is a simple mechanistic description that quantitatively accounts for the broad scope of observed two-state folding rates (with one notable exception; Jones and Wittung-Stafshede 2003). This theory stipulates that the search for those unfolded conformations with a grossly correct topology is the rate-limiting step in folding (Makarov and Plaxco 2003). The topomer-search model stems from simulations of the properties of inert Gaussian chains and uses the number of sequence-distant contacts (Q_D) as its variable. Q_D divided by the length of the polypeptide is thus identical to LRO, if the definitions of contacting residues and the cutoff in terms sequence separation are equal. Nonexplicit chain representations, such as the one used in the topomer-search model, are often insufficient in reproducing other hallmarks of two-state protein folding

Reprint requests to: Pernilla Wittung-Stafshede, Biochemistry and Cell Biology Department, MS-140, Rice University, Houston, TX 77251, USA; e-mail: pernilla@rice.edu; fax: (713) 348-5154.

Article published ahead of print. Article and publication date are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.03559504>.

(Karanicolas and Brooks III 2003). Notably, it was recently reported that an explicit chain model, including many-body interactions, exhibited calorimetric cooperativity and linear Chevron plots and also reproduced topology-dependent protein-folding rates (Kaya and Chan 2003). This observation suggests that the empirical topology-rate relationship arises due to an interplay between local conformational preferences and favorable nonlocal interactions (Kaya and Chan 2003).

Protein-protein interactions determine a wide array of protein functions and, therefore, are of fundamental importance in biology. In addition to heterogeneous protein-protein complexes, many proteins are oligomeric due to the association of identical subunits. The folding of oligomeric proteins is often complicated: It involves not only intramolecular interactions as the polypeptide chains interact with themselves (monomer folding) but also the intermolecular interactions as they associate with other monomers (subunit assembly). Equilibrium-unfolding pathways of oligomeric proteins (mostly dimers and tetramers) have been reported, revealing a variety of mechanisms (Ziegler et al. 1993; Mann et al. 1995; Waldburger et al. 1996; Nichtl et al. 1998; Bodenreider et al. 2002). Some proteins display monomeric or dimeric intermediates (Gloss and Matthews 1998; Doyle et al. 2000), whereas others fold in apparent two-state equilibrium reactions in which folding and oligomerization are coupled (Milla and Sauer 1994; Srivastava and Sauer 2000). Kinetic pathways for formation of oligomeric proteins may range from “induced-fit” (assembly before folding) to “lock-and-key” (folding before assembly) types of mechanisms (Jones and Thornton 1996). Recent simulation work suggests that, as in the case of (monomeric) protein folding, the native-state topology is the major factor that governs the choice of assembly mechanism for oligomers (Levy et al. 2004). No experimental study has yet tested the influence of native-state topology (contact order or LRO for the monomeric unit) on the folding rates of polypeptides within oligomeric frameworks.

The homo-heptameric cochaperonin protein 10, cpn10 (Martin et al. 1993; Todd et al. 1995; Burston et al. 1996; Shtilerman et al. 1999), is an attractive model for studies of the interplay between polypeptide folding and protein-protein assembly. The overall structure of cpn10 appears conserved in all organisms: In the heptamer, each cpn10 subunit (polypeptide/monomer) adopts an irregular β -barrel topology. The dominant interaction between subunits is an anti-parallel pairing of the first β -strand in one subunit and the final β -strand in the other subunit (Hunt et al. 1996). The flexibility of the involved side-chains and of the backbone in these regions contributes to high plasticity of the interface. We recently showed that human mitochondrial cpn10 (Fig. 1, inset) dissociates into monomers below a concentration of $\sim 3 \mu\text{M}$ monomer concentration (Guidry et al. 2000). This modest affinity might be linked to a significant

Figure 1. Semilogarithmic plot of unfolding and refolding kinetics for human mitochondrial cpn10 heptamer as a function of urea concentration. Open symbols indicate wild-type cpn10; closed symbols, Phe90Trp cpn10; circles, fluorescence; squares, far-UV CD; squares, unfolding (same for both Phe90Trp and wild-type cpn10). Solid curve is a two-state kinetic fit (combining fast folding phase and unfolding data). (Inset) Figure of the human cpn10 heptamer. The N- and C-terminal β -strands constituting the monomer-monomer interface are highlighted at one of the seven interfaces.

loss of configurational entropy upon oligomerization, which would be the case if folding and assembly processes are coupled. In accord, we have found that monomeric cpn10 adopts a folded structure in solution, but it exhibits only marginal thermodynamic stability (Guidry and Wittung-Stafshede 2002). Chemically induced unfolding of the cpn10 heptamer using GuHCl as the denaturant leads to a monomeric unfolded state (Guidry et al. 2000). In contrast, nonnative heptamers form in a two-state equilibrium transition (midpoint of $\sim 4.6 \text{ M}$ urea; pH 7.0, 20°C) when urea is the denaturant (Guidry et al. 2000). This latter observation gives us the unique opportunity to probe the folding process of an oligomeric protein without the influence of assembly. Remarkably, the nonnative cpn10 heptamer is found to refold in an apparent two-state process with a speed that is in excellent agreement with predictions based on the monomer topology. In contrast, the refolding process of unfolded cpn10 monomers is dominated by a slow association step.

Results and Discussion

The unfolding and refolding kinetics of the cpn10 heptamer was probed by stopped-flow mixing experiments in urea (pH 7.0, 20°C). Progress of the reactions were monitored by far-UV CD (reporting on the presence of secondary structure) and tyrosine emission (reporting on folded monomers and assembly), detection probes also used in earlier equi-

librium-unfolding experiments (Guidry et al. 2000). In Figure 1, we show a semilogarithmic plot of $\ln k_{\text{obs}}$ (k_F and k_U) versus urea concentration. Unfolding is a single-exponential process, there is no missing amplitude, and CD and fluorescence probes give identical kinetic traces. In terms of refolding, there is a fast phase corresponding to ~80% of the CD and fluorescence amplitude changes that depend linearly on the urea concentration. The remaining 20% of the signals change in a slower phase that is not significantly dependent on the urea concentration (Fig. 1). For both phases, CD and fluorescence probes give identical rate constants at each urea condition. Because the amplitude ratio between the two phases is constant throughout the concentration range of urea studied and the ratio is identical for CD and fluorescence changes, it implies that there are two parallel processes occurring (i.e., two fractions of molecules; Oliveberg et al. 1998). Importantly, in none of the kinetic-refolding experiments is there any missing amplitude within the instrument's dead time, and there was no protein-concentration dependence in the fast phase.

The extrapolated speed for the major refolding process in water is $2.1 \pm 0.5 \text{ sec}^{-1}$ (pH 7.0, 20°C). Upon combining this folding-rate constant with the unfolding-rate constant that can be extrapolated to water ($0.000045 \pm 0.000005 \text{ sec}^{-1}$), a free energy of unfolding in water of ~23 kJ/mole is estimated. This value is in excellent agreement with the stability obtained from the equilibrium-unfolding experiments of cpn10 performed in urea, $22 \pm 3 \text{ kJ/mole}$ (Guidry et al. 2000), in support of a two-state kinetic process for this fraction of the molecules. Taken together, the major (faster) phase correlates to refolding of the nonnative heptamer, and this process is a simple two-state kinetic reaction.

We used the crystal structure of heptameric human mitochondrial cpn10 (Landry et al. 1997) to calculate the native-state absolute contact order for one cpn10 monomer to 18.4%. The relative contact order, that is, contact order normalized with the length of the (monomeric) protein, is 18.2% because each cpn10 polypeptide has 101 residues. According to the correlation between folding speeds and relative-contact order for single-domain proteins (Plaxco et al. 2000; Ivankov et al. 2003), this value corresponds to a predicted folding rate in water for the cpn10 monomer of 19 sec^{-1} . A more recent correlation, using absolute contact-order, was shown to be successful in predicting folding rates for multistate folding proteins and small peptides, in addition to two-state folding proteins (Ivankov et al. 2003). By using this correlation, the predicted folding speed in water for the cpn10 monomer is 3.8 sec^{-1} . We also calculated the topomer-search variable Q_D for the cpn10 monomer: Its value is 73, which predicts (Makarov and Plaxco 2003) a folding speed of 7.6 sec^{-1} . Finally, when the LRO value for the cpn10 monomer was inserted in the regression equation given for all- β proteins with a 12-residue separation cutoff (Gromiha and Selvaraj 2001), a predicted folding-rate con-

stant of 21 sec^{-1} was obtained. Given that folding rates for various proteins may span over six orders of magnitude (Jackson 1998; Plaxco et al. 1998), the experimental refolding speed in water for the nonnative cpn10 heptamer (2.1 sec^{-1}) is in excellent agreement with the topology-based predictions.

The minor fraction (20% of molecules) in the urea-refolding experiments may correspond to nonnative cpn10 heptamers that have disassembled (fully or partially) in the unfolded state (or upon dilution to trigger refolding) and thus need to assemble before or during refolding. If the number of native-like contacts determine the speed, as in the topomer-search concept (Makarov and Plaxco 2003), a coupled assembly/folding process will be slower than a folding-only reaction because more contacts have to be made when assembly also has to occur. To test this hypothesis, we performed some time-resolved refolding experiments from the GuHCl-induced unfolded state of cpn10, which, in contrast to the urea-induced state, contains mostly monomeric unfolded polypeptides (Guidry et al. 2000). Because the equilibrium-unfolding transitions induced by GuHCl are protein-concentration dependent due to the coupled dissociation step (Guidry et al. 2000), the kinetic conditions had to be carefully selected and a full Chevron-plot could not be obtained.

Cpn10 refolding from the GuHCl-induced unfolded state was dominated by a (>67% of CD and fluorescence amplitudes) slow phase that could be approximated with a mono-exponential decay equation: $k_{\text{obs}} = 0.0011 \text{ sec}^{-1}$ and 0.0082 sec^{-1} for 15 and 25 μM final cpn10 concentration, respectively, at 1 M GuHCl (Fig. 2). These rates are roughly similar to those for the slow fraction in the cpn10-refolding experiments in urea ($k_{\text{obs}} \sim 0.01$ to 0.05 sec^{-1} for 30 μM final cpn10 concentration; Fig. 1). Because this slow phase is protein-concentration dependent (the higher the cpn10 concentration, the faster is the process), it correlates with an assembly process involving formation of interprotein interactions as the rate-limiting step. To address the order of this reaction, which relates to the number of cpn10 molecules that must associate in the rate-limiting step, the kinetic traces were analyzed in terms of initial rates (v_{init}) as a function of total protein concentration ($v_{\text{init}} = k \times [\text{cpn10}]^X$; where k is a rate constant and X is the order of the reaction). From the slope of the logarithmic plot of initial rates versus protein concentration ($\log[v_{\text{init}}] = \text{constant} + X \times \log[\text{cpn10}]$), a reaction order of 4 ± 1 was estimated, implying that more than half of the seven polypeptides need to interact in the transition-state complex. The remaining fraction ($\leq 33\%$) of the cpn10 molecules in the refolding experiments in GuHCl exhibited faster, mono-exponential kinetics ($k_{\text{obs}} = 0.33 \text{ sec}^{-1}$ for 15 μM cpn10 at 1 M GuHCl; Fig. 2, inset). The speed of this process is similar to that observed for the major fraction of the nonnative cpn10 heptamers when compared at similar denaturant strength. Generally, the concentration

Figure 2. Kinetic reactions monitored by fluorescence at 308 nm (excitation at 280 nm) upon mixing GuHCl-unfolded wild-type cpn10 with buffer to a final GuHCl-concentration of 1 M (gray indicates 25 μ M; black, 15 μ M final cpn10 concentration, respectively). (Inset) Short timescale kinetics upon mixing GuHCl-unfolded cpn10 with buffer to a final GuHCl concentration of 1 M (15 μ M final cpn10 concentration). The short timescale change (inset) corresponds to roughly one-third of the total amplitude change in both fluorescence and CD signals; the long timescale change to the remaining two-thirds of the total changes.

of urea is scaled by a factor of two to give equal strength as GuHCl (Moczygemba et al. 2000); therefore, the 1 M GuHCl data were compared with the 2 M urea data. It thus appears that the minor fraction in the GuHCl experiments is unfolded cpn10 monomers that refold (with a speed matching that of the nonnative heptamer) before they assemble.

In summary, we here tested for the first time the influence of monomer topology on the folding speed of an oligomeric protein. We discovered that refolding of nonnative cpn10 heptamers is a simple two-state process with a speed that agrees with that predicted based on the native-state topology of each monomer. The data suggest that the monomers fold independently, with a topology-defined speed, although the C and N termini peptides are trapped in β -strand pairing with neighboring subunits. Nonnative heptameric cpn10 may be considered a multidomain monomeric protein that folds with a speed determined by the topology of one domain (i.e., of one polypeptide chain). We propose that folding rates of real single-chain multidomain proteins may also be defined by the topology of only one of the domains. This minimal topomer would be the module with the highest native-state contact order in case the subdomains differ in structure. Studies on additional oligomeric and multidomain systems are clearly desired to test this hypothesis.

From a biological perspective, the *in vivo* starting point for formation of functional cpn10 heptamers is most likely random-coil-like monomers that have just left the ribosome. We found that *in vitro* several such molecules need to

assemble before the reaction to the native oligomer can proceed successfully. Because this association step is slower than polypeptide folding by several orders of magnitude, it becomes rate limiting for the overall reaction (Fig. 3). That assembly facilitates polypeptide folding is in accord with the low thermodynamic stability of folded monomers (Guidry and Wittung-Stafshede 2002) and the fact that $\sim 90\%$ of the overall heptamer stability is governed by interface interactions (Guidry et al. 2000). Protein-protein interfaces that are in principle “glued together” may have functional relevance in the case of cpn10. This implies that folded (nonfunctional) cpn10 monomers will never be present in living systems; instead, cpn10 will always exist as (functional) heptamers that will assure efficient cycling on and off the cpn60 tetradecamer.

Materials and methods

Escherichia coli expression and purification of wild-type and Phe90Trp human mitochondrial cpn10 was performed as previously described (Landry et al. 1997; Steede et al. 2000). The equilibrium-unfolding behavior of Phe90Trp cpn10 is identical to that for wild-type cpn10 (Guidry and Wittung-Stafshede 2002), making this variant an additional cpn10 molecule, native-like but with increased fluorescence, that can be used for studies of the refolding dynamics. Protein concentrations were determined from $\epsilon_{280} = 6,900$ and $9,800 \text{ M}^{-1} \text{ cm}^{-1}$ for wild-type and Phe90Trp cpn10, respectively (Guidry and Wittung-Stafshede 2002).

All experiments were performed at 20°C (pH 7.0) in 5 mM phosphate buffer with denaturants (urea or GuHCl) added as indicated. Urea and GuHCl were of highest purity, and the urea-stocks were prepared immediately before use. Stopped-flow mixing was performed on a PiStar (Applied PhotoPhysics), monitoring fluorescence at 308 nm (wild-type cpn10) or 330 nm (Phe90Trp cpn10) upon excitation at 280 nm, and far-UV CD at 220 nm, in a 2-mm cell. One-to-five protein-to-buffer/urea mixing was used with final protein (monomer) concentration of 30 μ M. For urea-induced unfolding, the kinetic traces were best fit to single-exponential equations, and there was no missing amplitude in either fluorescence or CD. For refolding in urea (starting with unfolded cpn10 in 5 or 7 M urea did not change the kinetics), the data at each urea concentration were best fit to biexponential equations (80% of CD and fluorescence amplitudes in a faster phase; 20% of

Figure 3. Scheme of the kinetic folding and assembly pathway of the cpn10 heptamer. Association of four or more unfolded monomers is rate limiting for the overall process and precedes the polypeptide-folding step. The latter reaction is defined by the native-state topology of each monomer, although the N and C termini are constrained in β -strand pairing interactions. U indicates unfolded; F, folded.

the amplitudes in a slower phase). For refolding experiments of wild-type cpn10 from the GuHCl-induced unfolded state, both stopped-flow and manual mixing experiments were performed (monitored by far-UV CD at 220 nm and tyrosine fluorescence at 308 nm) to capture all kinetic steps.

Acknowledgments

We thank Kevin Plaxco and Jonathan Kohn (University of California, Santa Barbara, CA) and Dmitrii E. Makarov (University of Texas at Austin, TX) for calculating contact order and number of sequence distance contacts for the cpn10 monomer. This work was supported by the NIH (GM59663). P.W.-S. is an Alfred P. Sloan Research Fellow.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Bodenreider, C., Kellershohn, N., Goldberg, M.E., and Mejean, A. 2002. Kinetic analysis of R67 dihydrofolate reductase folding: From the unfolded monomer to the native tetramer. *Biochemistry* **41**: 14988–14999.
- Burston, S.G., Weissman, J.S., Farr, G.W., Fenton, W.A., and Horwich, A.L. 1996. Release of both native and non-native proteins from a *cis*-only GroEL ternary complex. *Nature* **383**: 96–99.
- Doyle, S.M., Braswell, E.H., and Teschke, C.M. 2000. SecA folds via a dimeric intermediate. *Biochemistry* **39**: 11667–11676.
- Gloss, L.M. and Matthews, C.R. 1998. Mechanism of folding of the dimeric core domain of *Escherichia coli* trp repressor: A nearly diffusion-limited reaction leads to the formation of an on-pathway dimeric intermediate. *Biochemistry* **37**: 15990–15999.
- Gromiha, M.M. 2003. Importance of native-state topology for determining the folding rate of two-state proteins. *J. Chem. Inf. Comput. Sci.* **43**: 1481–1485.
- Gromiha, M.M. and Selvaraj, S. 2001. Comparison between long-range interactions and contact order in determining the folding rate of two-state proteins: Application of long-range order to folding rate prediction. *J. Mol. Biol.* **310**: 27–32.
- Guidry, J., Moczygamba, C., Steede, K., Landry, S., and Wittung-Stafshede, P. 2000. Reversible denaturation of oligomeric human chaperonin 10: Denatured state depends on chemical denaturant. *Protein Sci.* **9**: 2109–2117.
- Guidry, J.J. and Wittung-Stafshede, P. 2002. Low stability for monomeric human chaperonin protein 10: Interprotein interactions contribute majority of oligomer stability. *Arch. Biochem. Biophys.* **405**: 280–282.
- Hunt, J.F., Weaver, A.J., Landry, S.J., Gierasch, L., and Deisenhofer, J. 1996. The crystal structure of the GroES co-chaperonin at 2.8 Å resolution. *Nature* **379**: 37–45.
- Ivankov, D.N., Garbuzynskiy, S.O., Alm, E., Plaxco, K.W., Baker, D., and Finkelstein, A.V. 2003. Contact order revisited: Influence of protein size on the folding rate. *Protein Sci.* **12**: 2057–2062.
- Jackson, S.E. 1998. How do small single-domain proteins fold? *Fold. Des.* **3**: R81–R91.
- Jones, S. and Thornton, J.M. 1996. Principles of protein–protein interactions. *Proc. Natl. Acad. Sci.* **93**: 13–20.
- Jones, K. and Wittung-Stafshede, P. 2003. The largest protein observed to fold by two-state kinetic mechanism does not obey contact-order correlation. *J. Am. Chem. Soc.* **125**: 9606–9607.
- Karanicolas, J. and Brooks III, C.L. 2003. The importance of explicit chain representation in protein folding models: An examination of Ising-like models. *Proteins* **53**: 740–747.
- Kaya, H. and Chan, H.S. 2003. Contact order dependent protein folding rates: Kinetic consequences of a cooperative interplay between favorable nonlocal interactions and local conformational preferences. *Proteins* **52**: 524–533.
- Landry, S.J., Steede, N.K., and Maskos, K. 1997. Temperature dependence of backbone dynamics in loops of human mitochondrial heat shock protein 10. *Biochemistry* **36**: 10975–10986.
- Levy, Y., Wolynes, P.G., and Onuchic, J.N. 2004. Protein topology determines binding mechanism. *Proc. Natl. Acad. Sci.* **101**: 511–516.
- Makarov, D.E. and Plaxco, K.W. 2003. The topomer search model: A simple, quantitative theory of two-state protein folding kinetics. *Protein Sci.* **12**: 17–26.
- Mann, C.J., Shao, X., and Matthews, C.R. 1995. Characterization of the slow folding reactions of trp aporepressor from *Escherichia coli* by mutational analysis of prolines and catalysis by a peptidyl-prolyl isomerase. *Biochemistry* **34**: 14573–14580.
- Martin, J., Geromanos, S., Tempst, P., and Hartl, F.U. 1993. Identification of nucleotide-binding regions in the chaperonin proteins GroEL and GroES. *Nature* **366**: 279–282.
- Milla, M.E. and Sauer, R.T. 1994. P22 Arc repressor: Folding kinetics of a single-domain, dimeric protein. *Biochemistry* **33**: 1125–1133.
- Moczygamba, C., Guidry, J., and Wittung-Stafshede, P. 2000. Heme orientation affects holo-myoglobin folding and unfolding kinetics. *FEBS Lett.* **470**: 203–206.
- Nichtl, A., Buchner, J., Jaenicke, R., Rudolph, R., and Scheibel, T. 1998. Folding and association of β -galactosidase. *J. Mol. Biol.* **282**: 1083–1091.
- Oliveberg, M., Tan, Y.J., Silow, M., and Fersht, A.R. 1998. The changing nature of the protein folding transition state: Implications for the shape of the free-energy profile for folding. *J. Mol. Biol.* **277**: 933–943.
- Plaxco, K.W., Simons, K.T., and Baker, D. 1998. Contact order, transition state placement and the refolding rates of single domain proteins. *J. Mol. Biol.* **277**: 985–994.
- Plaxco, K.W., Simons, K.T., Ruczinski, I., and David, B. 2000. Topology, stability, sequence, and length: Defining the determinants of two-state protein folding kinetics. *Biochemistry* **39**: 11177–11183.
- Shtilerman, M., Lorimer, G.H., and Englander, S.W. 1999. Chaperonin function: Folding by forced unfolding. *Science* **284**: 822–825.
- Srivastava, A.K. and Sauer, R.T. 2000. Evidence for partial secondary structure formation in the transition state for arc repressor refolding and dimerization. *Biochemistry* **39**: 8308–8314.
- Steede, N.K., Guidry, J.J., and Landry, S.J. 2000. Preparation of recombinant human Hsp10. *Methods Mol. Biol.* **140**: 145–151.
- Todd, M.J., Boudkin, O., Freire, E., and Lorimer, G.H. 1995. GroES and the chaperonin-assisted protein folding cycle: GroES has no affinity for nucleotides. *FEBS Lett.* **359**: 123–125.
- Waldburger, C.D., Jonsson, T., and Sauer, R.T. 1996. Barriers to protein folding: Formation of buried polar interactions is a slow step in acquisition of structure. *Proc. Natl. Acad. Sci.* **93**: 2629–2634.
- Ziegler, M.M., Goldberg, M.E., Chaffotte, A.F., and Baldwin, T.O. 1993. Refolding of luciferase subunits from urea and assembly of the active heterodimer: Evidence for folding intermediates that precede and follow the dimerization step on the pathway to the active form of the enzyme. *J. Biol. Chem.* **268**: 10760–10765.