Chapter 12
Multiscale Approach to Protein Folding Dynamics

Sebastian Kmiecik, Michał Jamroz, and Andrzej Kolinski

Abstract Dynamic behavior of proteins is a key factor for understanding the functions of a living cell. Description of the conformational transitions of proteins remains extremely difficult for the computational simulation as well as the experimental techniques. No technique is able to span extremely short dynamic events together with long-timescale processes when the most interesting transitions occur. Thus new methods for simulation and utilization of all accessible experimental data are needed. The advances in the development of hybrid models, which attempt to combine a simplified modeling efficiency with atomic resolution accuracy, should provide new opportunities for the use of computer simulation in the integration of different kinds of data to study folding dynamics at relevant timescales. This review outlines the advances in description of protein dynamics and discusses recent applications of the CABS-reduced modeling tool to the studies of protein folding dynamics.

12.1 Introduction

Protein folding and unfolding are among essential processes in a living cell. Recently, attempts to simulate protein dynamics have become very popular, because of basic role of protein flexibility in functions of living organisms, increasing danger of protein misfolding and aggregation diseases (e.g., Alzheimer's, Parkinson's), and thanks to recent advances in experimental and simulation techniques.

Already 40 years ago the problem complexity was highlighted by Levinthal, who pointed out an impossibly long period of time required to fold a protein by a random conformational search (Levinthal 1968). Despite significant technological development from that time, we are still neither able to reliably predict protein structures from their sequences only nor able to monitor protein structure dynamics on relevant

A. Kolinski (✉)
Faculty of Chemistry, University of Warsaw, Warsaw, Poland
e-mail: kolinski@chem.uw.edu.pl

A. Kolinski (ed.), *Multiscale Approaches to Protein Modeling*,
DOI 10.1007/978-1-4419-6889-0_12,

timescales. Recent studies show that a high-resolution structure prediction is possible for small proteins, but requires huge computational resources and the method does not guarantee the prediction success (Bradley et al. 2005b). It is expected that detailed understanding of the folding process may lead to significant improvement of structure prediction algorithms. Thus, characterization of all alternative protein conformations that emerge along the folding pathway, including the unfolded state and partially folded intermediates, is needed.

Protein folding, as the process including both extremely short-timescale subprocesses and long-timescale rearrangements of thousands of atoms, remains extremely difficult not only to simulate but also to study experimentally. Recent reports show progress in the development of experimental techniques allowing for more and more detailed descriptions of very fast processes and short-lived transient conformations. The progress can be also seen in the development of simulation techniques, therefore the number of opportunities for combining experimental results and simulation is growing. Usually general or sparse experimental observations can be interpreted by a simulation or guide the simulation. Theoretical studies already led to better understanding of experimental results, providing easy-to-interpret structural models (Schaeffer et al. 2008). Thus, the role of computational techniques is to deliver all-atom structural models describing the whole process either by utilizing experimental data or by ab initio prediction if possible. Importantly, combining simulation and experiment allows for constant validation of the simulation techniques.

This article begins with a short characterization of experimental techniques providing input for the folding dynamics simulation. Applications of all-atom Molecular Dynamics (MD) and simplified protein models are then briefly discussed. Next, the outcome from coarse-grained, ab initio, long-timescale folding simulations of three protein model systems is described with the focus on comparison of experimental and simulation data. Finally, perspectives of folding dynamics methods and future development needs are summarized.

12.2 Structural Dynamics from Combination of Experiment and Simulation

No single technique, computational or experimental, is able to cover all relevant events of protein folding dynamics (Fig. 12.1). Comprehensive description of the protein dynamics requires integration of different kinds of static and dynamic protein characterizations, at different resolutions (Russel et al. 2009). That can be achieved at atomic level via computational approaches.

Determination of the folded structure is a priority for the complete biochemical protein characterization. A large number of folded protein structures at atomic resolution were determined by X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. Since protein flexibility is a key determinant of a protein biological function, the derived static structures are frequently insufficient in description of function mechanisms on the molecular level. Crystal or NMR

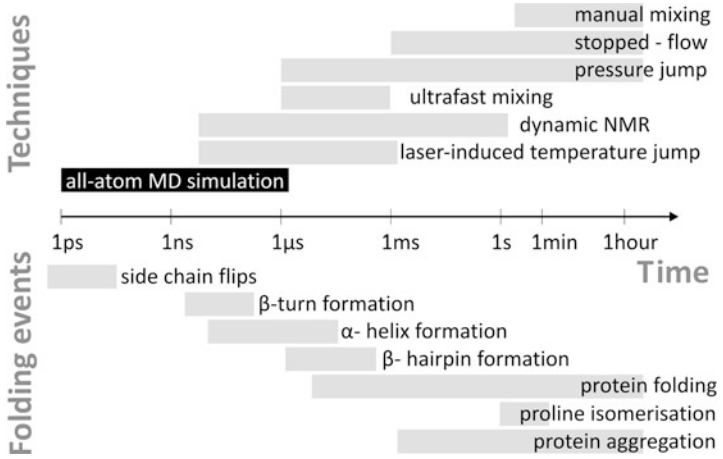

Fig. 12.1 Timescale resolution of various experimental techniques used in studying the protein folding dynamics (above the axis) and timescales of protein folding events (*gray bars* below the axis) compared to the time frame accessible to all-atom MD

structures provide a good starting point for the simulation of native-fold dynamics under conditions close to the physiological ones (Rueda et al. 2007). Computational techniques provide also the opportunity for combining different resolution data for the interpretation of low-resolution data. Namely, X-ray or NMR atomic structure data can be combined with low resolution but valuable results from small-angle X-ray scattering (Forster et al. 2008; Krukenberg et al. 2008) or electron microscopy (DiMaio et al. 2009; Jonic and Venien-Bryan 2009).

Experimental evidence on completely folded conformations is much more vast than data on the key folding states (for definitions, see Table 12.1). Besides static structure determination, NMR spectroscopy is a powerful experimental technique for protein dynamics characterization spanning many timescales and yielding sparse, site-specific spatial information that can be used in simulation (Mittermaier and Kay 2006). Only for a few proteins, the folding intermediates were characterized using protein engineering (Matouschek et al. 1992) and NMR techniques (Udgaonkar and Baldwin 1988; Bycroft et al. 1991). Protein engineering (phi-value analysis) remains the only experimental technique for probing Transition State structures at the level of individual residues (Serrano et al. 1992; Otzen et al. 1994).

Even much less is known, however, about very early folding events. Obviously, it is very important to understand how protein folding is initiated and how the native structure is formed. The denatured state – a highly heterogeneous ensemble of partially folded conformations is very difficult to study, although there have been recent reports of NMR studies of residual structure in denatured proteins. Such structures, along with hydrophobic clusters, were discovered even under highly denaturing

Table 12.1 Some definitions were taken and adapted to protein folding from the review by Rusell et al. on structural dynamics of macromolecular processes

Terms used in the field of protein folding structural dynamics	
State	A state is described by a three-dimensional structure of an assembly at some resolution. The structure may be flexible and its description may be incomplete.
Key state	The set of key states and transitions between them capture the essence of the process. Key states need not be stable and can correspond to Transition States.
Transition/Transition State	A transition occurs between a pair of key states that can interconvert directly without passing through other key states. If not indicated otherwise, the term Transition State usually refers to the state of the highest energy along folding reaction coordinate. This state is thought to consist of a large number of extremely short-lived Transition State structures, partially folded, with equal probability to complete the folding process or unfold again.
Restraint	Restraint restricts geometric and/or temporal properties of an assembly, such as the distance between two components, the overall shape of the complex, or the time interval between two key states. A restraint is a scalar function that quantifies the agreement between a restrained feature and the data.
Protein engineering (Phi-value analysis)	The method relies on the quantity of Phi: Phi $= 0$ suggests the absence of interaction in the Transition State, whereas Phi $= 1$ marks an interaction similar to that in the native state (Matouschek, Kellis et al. 1989). Phi values are the result of conformational folding stability and folding kinetics comparison of the wild-type protein with those of one or more point mutants.
Protein folding pathway	A pathway is represented as a set of key states connected by transitions with associated trajectory and rate information.
Molten globule	Molten globule is known as a stable, collapsed state with partial order that proteins can exhibit under certain conditions (Kuwajima 1989; Ptitsyn 1995). Compared to the native structure molten globule possesses less tightly packed native-like secondary and tertiary structure.

conditions (Kazmirski et al. 2001; Klein-Seetharaman et al. 2002). Moreover, denatured proteins can exhibit a long-range ordering of native-like topology (Shortle and Ackerman 2001). Therefore, the folding process can be directed from the very beginning when starting from a specific structure (Dobson 1994; Blanco et al. 1998). It becomes evident that the denatured state plays a crucial role in all aspects of protein stability and folding mechanisms (Shortle 1996).

MD is a well-established and powerful method for studying dynamics of complex molecular systems. However, there is a gap between the timescales of the classical MD simulation and the timescales of protein folding (Fig. 12.1). Recent advances in algorithms scalability and computer hardware have made the microsecond-timescale simulations with tens of thousands of atoms practical (Klepeis et al. 2009). An average protein folds slower by orders of magnitude. Thus, simulations of real-size proteins are limited to high-temperature unfolding simulations (whose results

are questionable due to dramatic, highly non-physiological conditions) or dynamics of the experimental structure.

For larger proteins, the all-atom simulations of the entire folding process, from random coil to native state, are so far possible only for Go models. There have been a number of Go potential-based studies of protein G using a simplified model (Prieto et al. 2005), an all-atom model (Shimada and Shakhnovich 2002) or with a weak Go-like contribution to the applied force field (Lee et al. 2004). In Go models, only native interactions are taken into account. Consequently, the lowest energy of the native conformation is guaranteed. The obvious weak point of such approach is that the knowledge of the native structure is needed to construct the Go potential. Significant shortcoming comes also from neglecting the non-native interactions, thereby ignoring their sometimes important role in the folding mechanisms (Rothwarf and Scheraga 1996; Blanco et al. 1997).

12.3 Protein Dynamics by a High-Resolution Reduced Modeling

Due to the timescale limitations of the all-atom molecular mechanics, the reduced models offer the most promising possibilities to study large-scale protein rearrangements during the folding process (Kolinski and Skolnick 2004). The simplest models of protein-like systems with highly idealized protein chain representation and interaction scheme led to the understanding of the basic rules governing protein folding (Chan and Dill 1990). Addressing more specific problems like some aspects of folding kinetics is possible, with a more complex interaction scheme and still very simple chain representation (Thirumalai and Klimov 1999). The most advanced reduced models (high-resolution models employing complex interactions) enable the folding studies of real proteins. The simulations can be successfully performed using various sampling and interaction schemes like Monte Carlo search with the knowledge-based statistical potentials (CABS) (Kmiecik and Kolinski 2007a, 2008) or Langevin dynamics with the physics-based united-residue force field (UNRES) (Liwo et al. 2005).

12.3.1 Paradigm Systems of Protein Folding Studies by a High-Resolution De Novo Modeling

Numerous experimental and simulation studies established small proteins: barnase (experimental structure PDB code: 1BNR), chymotrypsin inhibitor (2CI2), and b1 domain of protein G (2GB1) as model systems for folding studies. From early 1990s, as a result of extensive and pioneering protein engineering analyses, barnase and chymotrypsin inhibitor were being presented as complementary variants of protein folding mechanism (for general observations from experiments, see Table 12.2).

Table 12.2 General experimental findings on the model system structure dynamics

Proteins	Barnase	2GB1	2CI2
Number of aa	110	56	64
Number of domains and hydrophobic cores in native	2 domains, 3 cores	Single domain, single core	
Folding kinetics	3-state (through at least one intermediate) (Fersht 1993)	2-state (Jackson 1998; Krantz et al. 2002) or 3-state (Park et al. 1999; Roder et al. 2006), presence of an intermediate is under debate	2-state (no intermediates detected) (Jackson and Fersht 1991)
Folding mechanism	Variants of nucleation–condensation mechanism (Daggett and Fersht 2003a,b)		
	The more tendency for stable secondary structure in the denatured state, the folding is more hierarchical, and TS is assembled from pre-formed elements of secondary structure.	As the propensity for the stable secondary structure decreases, consolidation of the secondary and tertiary structure is less separated and occurs simultaneously during condensation from extended nucleus formed in TS.	
Denatured state	Considerable amount of residual structure (Arcus, Vuilleumier et al. 1995; Freund et al. 1996)	Highly unstructured	
		Different from the fully unfolded random coil state: restricted motions in native helical and second β-hairpin areas (Kuszewski et al. 1994; Frank et al. 1995)	Very slight tendency for the native helical structure and a minor hydrophobic clustering near the center of the chain (de Prat Gay et al. 1995)

The model proteins folding from the denatured to the native state occurs in millisecond timeframe, therefore remaining inaccessible to all-atom MD. Our research group has recently attempted to characterize their full folding process, performing unbiased, ab initio simulation in a high-resolution, reduced representation space (Kmiecik and Kolinski 2007a, 2008), using the CABS model (Kolinski 2004). The use of the reduced representation of polypeptide chain led to a significant reduction

of the conformational space. Thus simulated system evolution from a highly denatured to a near-native state was possible in a reasonable timescale. Compared to the experimental results, we have obtained a similar sequence of folding events and have identified the interactions critical for the folding process. For the simulation results and comparison with other experimental findings, see Table 12.3.

It is particularly interesting to compare GB1 with CI2, which are of similar single-domain characteristics (Table 12.3). The CABS simulation observations (Table 12.3, Fig. 12.2) are in slight disagreement with the interpretation of the experimental data summarized by Daggett and Fersht (2003a,b). According to them, CI2 folds via nucleation collapse around an extended nucleus – similar to what has been observed for GB1. Indeed, in the case of GB1, all nuclei residues take part in the nucleation event at very early stages of folding. To the contrary, CI2 folds via assembly of distinct cooperative subunits (Kmiecik and Kolinski 2007a). At the folding transition, only the native tertiary interactions are observed between two central strands – β3–β4. Consolidation of the α-helix and β3–β4 takes place at lower temperatures.

Very interestingly, a comparison of the CABS energy (or radius of gyration) as a function of temperature shows a very similar exponential thermal dependence for all three proteins what means that the stepwise formation of cooperative subunits in CI2 case does not affect the characteristics of these observables (Kmiecik and Kolinski 2007a, 2008). What is important, differences in the folding pathways observed in the simulations of GB1 and CI2 are actually in agreement with the available experimental data (particularly in revealing important long-range interactions being consistent with the phi-value analysis).

The simulations described here provide a detailed insight into the folding mechanism on the level of individual residues. Since procedures for protein chain reconstruction to all-atom representation exist (Gront et al. 2007), smooth and fully automated transition to atomic resolution is feasible (Kmiecik 2007b). Such a hierarchical methodology was successfully applied to protein structure prediction during a community-wide testing experiment of the prediction methods (Kolinski and Bujnicki 2005). The approach ranked second best in general as well as in the new fold category – the critical test for ab initio methods – after ROSETTA, the recombination of short fragments extracted from known protein structures (Bradley et al. 2005a).

The presented approach goes far beyond the simple analytical or Go models or all-atom MD enabling the study of complete unfolding/folding pathways. Physically realistic folding mechanisms observed in the CABS simulations imply that the interactions in the denatured state are very similar to those in the native structures. Consequently, the knowledge-based potentials from native structures are a good approximation of the interactions in the denatured state. Moreover, proposed Monte Carlo dynamics and a sampling scheme mimic the qualitative features of the continuous long-time dynamics of proteins. Therefore, the suggested model may be a useful tool for qualitative studies of entire folding pathways of large proteins and macromolecular assemblies.

Table 12.3 General observations on the model system structure dynamics from the computer simulation

Proteins	Barnase	2GB1	2CI2
Insights from all-atom simulations	*MD simulations* of the denatured state (Bond et al. 1997; Wong et al. 2000), intermediate state (Li and Daggett 1998), and Transition State *with restraints from Phi-values* (Salvatella et al. 2005) provided the models and enabled the interpretation of NMR and Phi-value data.	*MC simulations with Go potential* (Shimada and Shakhnovich 2002) *and with restraints from Phi values* (Hubner et al. 2004) identified six residues forming the folding nucleus.	*MC simulations with Go potential combined with Phi-value analyses* revealed that β3–β4 should be the last element to unfold (Li and Shakhnovich 2001). *MD unfolding* showed sequential or parallel unfolding of substructures, preference of β3–β4 as the last and β1–β5 as the first to unfold could be noted (Lazaridis and Karplus 1997; Ferrara et al. 2000a,b; Reich and Weikl 2006).
High-resolution reduced model simulation results (de novo by CABS) (Kmiecik and Kolinski 2007a, 2008)	Near-perfect correspondence between most persistent long-range contacts in the simulated denatured state and the folding nuclei described in protein engineering studies of barnase (Matouschek et al. 1992), 2GB1 (McCallister et al. 2000), and CI2 (Jackson et al. 1993). Nucleation–condensation mechanism consistent with the experimental observations and mentioned above simulations. Heat capacity peak involved with completion of a nucleus formation and transition to molten globule. Nucleus consists of long-range native-like hydrophobic interactions. Nucleus initiation sites are early formed portions of secondary structure: single β-hairpin and α-helix, adjacent to each other in the nucleus core.		Sequential assembly of cooperative subunits, identical to those obtained in MD studies (see above). Heat capacity peak involved with transition from highly unstructured state to hydrophobic clustering around β3–β4.

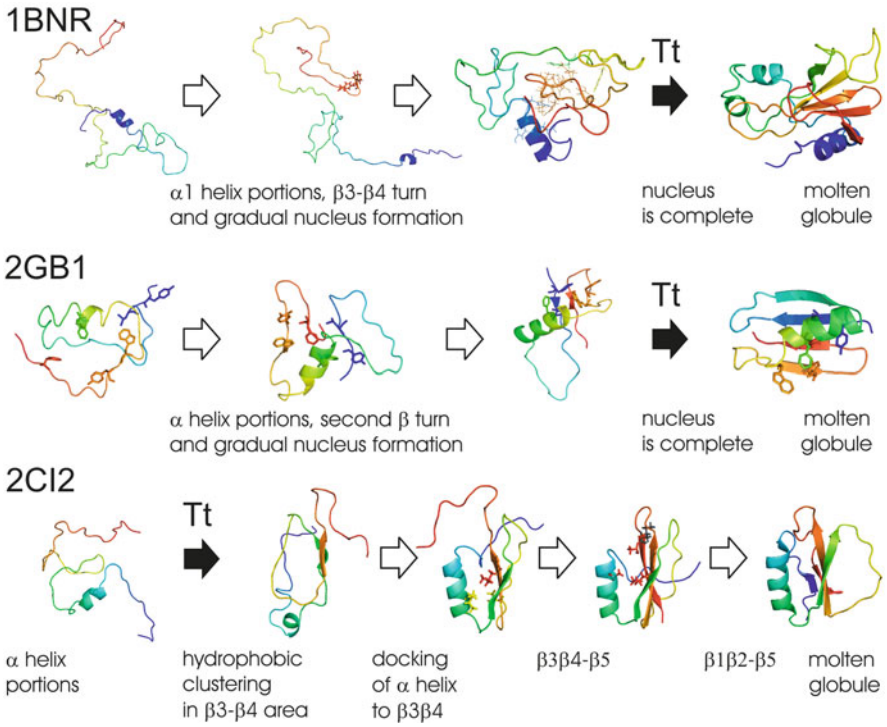

Fig. 12.2 Folding pathways explored by CABS – a comparison of key states of barnase, b1 domain of protein G, and chymotrypsin inhibitor in a highly denaturing state just before T_t and below T_t (Kmiecik and Kolinski 2007a, 2008). The transition temperature (T_t) is identified by the steepest drop of the energy and the peak of the heat capacity. T_t cannot be strictly identified with the major Transition State. Sometimes, as for CI2, conformations observed at T_t may be relatively unstructured, with some features of a molten globule state

12.4 Summary

Molecular level characterization of protein folding and folding in the context of protein interactions is crucial for understanding basic mechanisms of life. Most of existing models of protein folding transient structures and their complexes have been obtained by custom-designed methodologies for integration of experimental results, using tools not suited to handle large-scale objects equipped with different incompatible force fields. Accessing higher efficiency and precision would require new methods for simulation and utilization of all accessible experimental data (Russel et al. 2009).

Presently, the all-atom MD is a well-established technique for the protein dynamics simulation, reaching longer than one microsecond timescales for tens of thousands of atoms, using the most advanced software and hardware infrastructures. Over the last 3 years, the maximum simulation speed recorded for an all-atom MD simulation has increased by roughly an order of magnitude, largely due to

more efficient parallelization over large numbers of multicore processing nodes. During the same time period, the capacity of individual high-end processor cores has increased by only about 50% (Klepeis et al. 2009). In practice, all-atom MD with explicit solvent allows for folding pathway simulations of peptides or very small, fast-folding proteins. Obviously, the throughput accessible to MD will continue to increase. However, the gap between the needed and the achievable is still huge and will remain so in the years ahead.

Access to longer timescales and larger system sizes would require combination of all-atom MD with reduced modeling techniques employing either a reduced geometrical representation of modeled systems and/or simplified models of motion, reduced interaction schemes, and implicit solvent models. In parallel with efficiency, present efforts concentrate on the accuracy of tools. Currently, force-field improvement of both, all-atom and reduced models, as well as the water potentials is a priority due to precision issues (Scheraga et al. 2007). Development of hybrid models that attempt to combine reduced modeling efficiency with high accuracy at atomic resolution, together with experimental results, will be critical. The reduced modeling tool developed in our lab provides a high-throughput element of such systems and the unique combination of solutions applied there can serve as an inspiration for design of novel, more efficient tools.

References

Arcus VL, Vuilleumier S et al (1995) A comparison of the pH, urea, and temperature-denatured states of barnase by heteronuclear NMR: implications for the initiation of protein folding. J Mol Biol 254:305–321

Blanco FJ, Ortiz AR et al (1997) Role of a nonnative interaction in the folding of the protein G B1 domain as inferred from the conformational analysis of the alpha-helix fragment. Fold Des 2:123–133

Blanco FJ, Serrano L et al (1998) High populations of non-native structures in the denatured state are compatible with the formation of the native folded state. J Mol Biol 284:1153–1164

Bond CJ, Wong KB et al (1997) Characterization of residual structure in the thermally denatured state of barnase by simulation and experiment: description of the folding pathway. Proc Natl Acad Sci USA 94:13409–13413

Bradley P, Malmstrom L et al (2005a) Free modeling with ROSETTA in CASP6. Proteins 61 (S 7):128–134

Bradley P, Misura KM et al (2005b) Toward high-resolution de novo structure prediction for small proteins. Science 309:1868–1871

Bycroft M, Ludvigsen S et al (1991) Determination of the three-dimensional solution structure of barnase using nuclear magnetic resonance spectroscopy. Biochemistry 30:8697–8701

Chan HS, Dill KA (1990) Origins of structure in globular proteins. Proc Natl Acad Sci USA 87:6388–6392

Daggett V, Fersht AR (2003a) The present view of the mechanism of protein folding. Nat Rev Mol Cell Biol 4:497–502

Daggett V, Fersht AR (2003b) Is there a unifying mechanism for protein folding? Trends Biochem Sci 28:18–25

de Prat Gay, Ruiz-Sanz GJ et al (1995) Conformational pathway of the polypeptide chain of chymotrypsin inhibitor-2 growing from its N terminus in vitro. Parallels with the protein folding pathway. J Mol Biol 254:968–979

DiMaio F, Tyka MD et al (2009) Refinement of protein structures into low-resolution density maps using ROSETTA. J Mol Biol 392:181–190

Dobson CM (1994) Protein folding. Solid evidence for molten globules. Curr Biol 4:636–640.

Ferrara P, Apostolakis J et al (2000a) Computer simulations of protein folding by targeted molecular dynamics. Proteins 39:252–260

Ferrara P, Apostolakis J et al (2000b) Targeted molecular dynamics simulations of protein unfolding J Phys Chem B 104:4511–4518

Fersht AR (1993) The sixth Datta Lecture. Protein folding and stability: the pathway of folding of barnase. FEBS Lett 325(1–2):5–16

Forster F, Webb B et al (2008) Integration of small-angle X-ray scattering data into structural modeling of proteins and their assemblies. J Mol Biol 382:1089–1106

Frank MK, Clore GM et al (1995) Structural and dynamic characterization of the urea denatured state of the immunoglobulin binding domain of streptococcal protein G by multidimensional heteronuclear NMR spectroscopy. Protein Sci 4:2605–2615

Freund SM, Wong KB et al (1996) Initiation sites of protein folding by NMR analysis. Proc Natl Acad Sci USA 93:10600–10603

Gront D, Kmiecik S et al (2007) Backbone building from quadrilaterals: A fast and accurate algorithm for protein backbone reconstruction from alpha carbon coordinates. J Comput Chem 28:1593–1597

Hubner IA, Shimada J et al (2004) Commitment and nucleation in the protein G transition state. J Mol Biol 336:745–761

Jackson SE (1998) How do small single-domain proteins fold? Fold Des 3:R81–R91

Jackson SE, el Masry N et al (1993) Structure of the hydrophobic core in the transition state for folding of chymotrypsin inhibitor 2: a critical test of the protein engineering method of analysis. Biochemistry 32:11270–11278

Jackson SE, Fersht AR (1991) Folding of chymotrypsin inhibitor 2. 1. Evidence for a two-state transition. Biochemistry 30:10428–10435

Jonic S, Venien-Bryan C (2009) Protein structure determination by electron cryo-microscopy. Curr Opin Pharmacol 9:636–642

Kazmirski SL, Wong KB et al (2001) Protein folding from a highly disordered denatured state: the folding pathway of chymotrypsin inhibitor 2 at atomic resolution. Proc Natl Acad Sci USA 98:4349–4354

Klein-Seetharaman J, Oikawa M et al (2002) Long-range interactions within a nonnative protein. Science 295(5560):1719–1722

Klepeis JL, Lindorff-Larsen K et al (2009) Long-timescale molecular dynamics simulations of protein structure and function. Curr Opin Struct Biol 19:120–127

Kmiecik S, Gront D et al (2007b) Towards the high-resolution protein structure prediction. Fast refinement of reduced models with all-atom force field. BMC Structural Biology 7:43

Kmiecik S, Kolinski A (2007a) Characterization of protein-folding pathways by reduced-space modeling. Proc Natl Acad Sci USA 104:12330–12335

Kmiecik S, Kolinski A (2008) Folding pathway of the B1 domain of protein G explored by multiscale modeling. Biophys J 94:726–736

Kolinski A (2004) Protein modeling and structure prediction with a reduced representation. Acta Biochim Pol 51:349–371

Kolinski A, Bujnicki JM (2005) Generalized protein structure prediction based on combination of fold-recognition with de novo folding and evaluation of models. Proteins 61: 84–90.

Kolinski A, Skolnick J (2004) Reduced models of proteins and their applications. Polymer 45: 511–524

Krantz BA, Mayne L et al (2002) Fast and slow intermediate accumulation and the initial barrier mechanism in protein folding. J Mol Biol 324:359–371

Krukenberg KA, Forster F et al (2008) Multiple conformations of *E. coli* Hsp90 in solution: insights into the conformational dynamics of Hsp90. Structure 16:755–765

Kuszewski J, Clore GM et al (1994) Fast folding of a prototypic polypeptide: the immunoglobulin binding domain of streptococcal protein G. Protein Sci 3:1945–1952
Kuwajima K (1989) The molten globule state as a clue for understanding the folding and cooperativity of globular-protein structure. Proteins 6:87–103
Lazaridis T, Karplus M (1997) "New view" of protein folding reconciled with the old through multiple unfolding simulations. Science 278(5345):1928–1931
Lee SY, Fujitsuka Y et al (2004) Roles of physical interactions in determining protein-folding mechanisms: molecular simulation of protein G and alpha spectrin SH3. Proteins 55: 128–138
Levinthal C (1968) Mossbauer Spectroscopy in Biological Systems. In: Debrunner P, Tsibris J, Munck E (eds) Proceedings of a meeting held at Allerton house, Monticello, Illinois. University of Illinois Press, Urbana, Illinois, pp 22–24
Li A, Daggett V (1998) Molecular dynamics simulation of the unfolding of barnase: characterization of the major intermediate. J Mol Biol 275:677–694
Li L, Shakhnovich EI (2001) Constructing, verifying, and dissecting the folding transition state of chymotrypsin inhibitor 2 with all-atom simulations. Proc Natl Acad Sci USA 98:13014–13018
Liwo A, Khalili M et al (2005) Ab initio simulations of protein-folding pathways by molecular dynamics with the united-residue model of polypeptide chains. Proc Natl Acad Sci USA 102:2362–2367
Matouschek A, Kellis JT Jr et al (1989) Mapping the transition state and pathway of protein folding by protein engineering. Nature 340(6229):122–126
Matouschek A, Serrano L et al (1992) The folding of an enzyme. IV. Structure of an intermediate in the refolding of barnase analysed by a protein engineering procedure. J Mol Biol 224:819–835
McCallister EL, Alm E et al (2000) Critical role of beta-hairpin formation in protein G folding. Nat Struct Biol 7:669–673
Mittermaier A, Kay LE (2006) New tools provide new insights in NMR studies of protein dynamics. Science 312(5771):224–228
Otzen DE, Itzhaki LS et al (1994) Structure of the transition state for the folding/unfolding of the barley chymotrypsin inhibitor 2 and its implications for mechanisms of protein folding. Proc Natl Acad Sci USA 91:10422–10425
Park SH, Shastry MC et al (1999) Folding dynamics of the B1 domain of protein G explored by ultrarapid mixing. Nat Struct Biol 6:943–947
Prieto L, de Sancho D et al (2005) Thermodynamics of Go-type models for protein folding. J Chem Phys 123:154903
Ptitsyn OB (1995) Molten globule and protein folding. Adv Protein Chem 47:83–229
Reich L, Weikl TR (2006) Substructural cooperativity and parallel versus sequential events during protein unfolding. Proteins 63:1052–1058
Roder H, Maki K et al (2006) Early events in protein folding explored by rapid mixing methods. Chem Rev 106:1836–1861
Rothwarf DM, Scheraga HA (1996) Role of non-native aromatic and hydrophobic interactions in the folding of hen egg white lysozyme. Biochemistry 35:13797–13807
Rueda M, Ferrer-Costa C et al (2007) A consensus view of protein dynamics. Proc Natl Acad Sci USA 104:796–801
Russel D, Lasker K et al (2009) The structural dynamics of macromolecular processes. Curr Opin Cell Biol 21:97–108
Salvatella X, Dobson CM et al (2005) Determination of the folding transition states of barnase by using PhiI-value-restrained simulations validated by double mutant PhiIJ-values. Proc Natl Acad Sci USA 102:12389–12394
Schaeffer RD, Fersht A et al (2008) Combining experiment and simulation in protein folding: closing the gap for small model systems. Curr Opin Struct Biol 18:4–9
Scheraga HA, Khalili M et al (2007) Protein-folding dynamics: overview of molecular simulation techniques. Annu Rev Phys Chem 58:57–83
Serrano L, Matouschek A et al (1992) The folding of an enzyme. III. Structure of the transition state for unfolding of barnase analysed by a protein engineering procedure. J Mol Biol 224:805–818

Shimada J, Shakhnovich EI (2002) The ensemble folding kinetics of protein G from an all-atom Monte Carlo simulation. Proc Natl Acad Sci USA 99:11175–11180

Shortle D (1996) The denatured state (the other half of the folding equation) and its role in protein stability. Faseb J 10:27–34

Shortle D, Ackerman MS (2001) Persistence of native-like topology in a denatured protein in 8 M urea. Science 293(5529):487–489

Thirumalai D, Klimov DK (1999) Deciphering the timescales and mechanisms of protein folding using minimal off-lattice models. Curr Opin Struct Biol 9:197–207

Udgaonkar JB, Baldwin RL (1988) NMR evidence for an early framework intermediate on the folding pathway of ribonuclease A. Nature 335(6192):694–699

Wong KB, Clarke J et al. (2000) Towards a complete description of the structural and dynamic properties of the denatured state of barnase and the role of residual structure in folding. J Mol Biol 296:1257–1282