Protein Folding, Unfolding and Misfolding: Role Played by Intermediate States

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Abstract: Most proteins fold into their native structure through well defined pathways which involve a limited number of transient intermediates. Intermediates play a relevant role in the folding process; many diseases of genetic nature are in fact coupled with protein misfolding due to formation of stable, inactive intermediate species of the protein. This review deals with a number of diseases associated with protein misfolding and briefly describes the mechanism(s) responsible, at molecular level, for such pathologies. It is also considered the (native ⇄ molten globule) transition, recently observed for some proteins, in which a native protein converts into a stable compact intermediate state able to carry out distinct physiological functions inside the cell. A non-native compact form of cyt *c*, for example, appears to have a role in the programmed cell death (apoptosis) after that the protein is released from the mitochondrion, and non-native forms of the same protein appear involved in some of the disorders attributed to amyloid formation.

Key Words: Protein folding and misfolding, intermediate conformers, genetic diseases, cell apoptosis, Alzheimer.

INTRODUCTION

Defining the mechanism(s) governing protein folding remains a central point in biophysics and molecular biology. To explain how a protein synthesized as a linear unfolded polypeptide rapidly folds into its biologically active structure even though there are a large number of potential conformational states, still remains an intriguing and still unsolved puzzle that has stimulated, over the last years, the interest of many researchers.

At present, a widely accepted view suggests that whereas small proteins fold through a two-state (*unfolded* ⇄ *native*) mechanism, larger proteins (i.e., those with more than 100 residues) fold into the unique native structure through well defined folding pathways which involve a limited number of intermediate species. Therefore, big effort has been lavished to characterize the intermediate species detected during the folding process.

In the last years, a large body of kinetic and equilibrium data have provided extensive information on the protein folding pathway(s) and on the structural properties of intermediate states, thus providing relevant contribution to better understanding the process [1-9]. Its high cooperativity and complexity, however, renders hard the characterization of kinetic intermediates forming during folding, in part due to limitations of the experimental approach [10-12]. Therefore, a valid alternative strategy is represented by the study of partially folded states at equilibrium, which reveal how a non-native state of a protein is structurally organized.

Studies on proteins have outlined the strict correlation between equilibrium and kinetic intermediates [13], and elucidated the properties of the 'molten globule', a non-native compact state considered a major intermediate in protein folding [11,13-15]. A deep knowledge of the properties of intermediate species is important; as it will be described below, stable folding intermediates generated by mutated proteins may cause genetic diseases and facilitate the arising of pathologies in humans.

UNFOLDING/REFOLDING KINETICS

Under physiological-like conditions, a protein folds spontaneously into its native biologically active state following the information encoded in the amino acidic sequence of the polypeptide chain. Most proteins fold through a non-two-state process, which implies the formation of a limited number of intermediate states, as described by the following scheme:

$$U \rightleftarrows n\,(I) \rightleftarrows N$$

where U is the unfolded state, I the intermediate state(s), and N is the native state.

The kinetic investigation of protein folding is generally complicated by the uncertainty concerning the structural characteristics of partly folded states and the role they play during the folding process. An important, not fully clarified point concerns the role played by kinetic intermediates in the route towards formation of the native state. It is not clear yet, for example, whether an intermediate state I is to be treated as an original on-pathway transient species or, instead, as an accumulating off-pathway misfolded form produced by a side-reaction. Recent reports (see below) hypothesize that transient intermediates accumulating as off-pathway forms in protein folding, may induce diseases in humans [11]. Thus, identifying the kinetic mechanism(s) governing protein folding and determining the properties of intermediates forming during the process, remains a priority.

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Protein folding is solely investigated under native-like conditions which favour the rapid formation of the biologically active structure. From a kinetic point of view, intermediates have been classified as 'early' or 'late' intermediates; the 'early' intermediates form rapidly (within a few milliseconds), whereas the 'late' intermediates form just prior to the lowest, rate-limiting step of the folding reaction (see [16] for a detailed description). Being short lived, the 'early' intermediates are very difficult to study, while the 'late' intermediates can be "kinetically trapped"; by decreasing the rate of folding, trapping facilitates the kinetic characterization of intermediate steps.

THE MOLTEN GLOBULE

The molten globule (MG), considered the major intermediate of protein folding, is a non-native compact state of a protein characterized by native-like secondary structure but fluctuating tertiary conformation. This implies that during folding the just synthesized protein collapses into a flexible compact species whose tertiary architecture lacks the tight packing typical of the native state, as shown in Fig. (**1**).

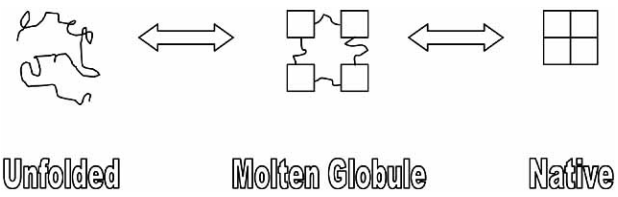

Fig. (1). Molten globule model in the folding pathway of a protein.

The MG of several proteins, as carbonic anhydrase, α-lactalbumin, apomyoglobin, cytochrome *c*, has been investigated in high detail during the last years (see, for example, [17-20]); these studies have confirmed the more expanded (less compact) structure of the MG with respect to the native state. The hypothesis asserting that the MG is stabilized mainly by hydrophobic interactions is confirmed from the fact that, although less packed, the non polar groups maintain in the MG their attraction in water. As observed for apomyoglobin the MG is stabilized mainly by hydrophobic interactions, while the native state is instead stabilized by a specific tight packing. About twenty years ago, Ptitsyn and collaborators introduced the "non-uniform model" theory to describe the structural properties of the MG [15, 21]. The theory is based on the hypothesis that in the MG macromolecules undergo a non uniform expansion. This implies that some regions of the macromolecules (those constituting the core of the protein, including α- and β-regions) remain about preserved (the core becomes a little less rigid), whereas other regions, as the loops and the ends of α- and β-regions, result less folded. Thus, although water molecules cannot penetrate relevantly inside the MG core because the non polar groups of α- and β-regions have a very limited capability to expand, the more unfolded regions can be easily penetrated by water. Under this aspect, the MG can be assumed as a "liquid-like" system since the native arrangement of ordered secondary regions is stabilized by ("liquid-like") hydrophobic contacts.

The reason for which loop regions undergo conformational changes as the protein partially unfolds into the MG, is due to the fact that in the native conformation some loops and the protein core are attached one to the other by interactions involving hydrophobic residues. Formation of the MG is coupled with a slight increase of the macromolecule volume, which facilitates release of these hydrophobic amino acids; as a consequence, loops unfold because their stability is significantly reduced without a proper hydrophobic support [15].

At present, there is emerging view that the MG may play a primary role in distinct biological processes. In some cases, proteins were found to undergo conformational changes to perform their physiological (sometimes pathological) functions. Such conformational changes, of tertiary nature, are now becoming recognized as a mechanism potentially used by a protein to achieve structural diversity and, consequently, diversity of functions; to this issue, the MG state is ideally suited to provide the required conformational flexibility. In the case of apolipoprotein E4 (apoE4), a protein that plays an important role in lipid transport in the plasma and in the central nervous system, the enhanced propensity of a common human apoE4 isoform to stabilize the MG state is considered a major risk factor for Alzheimer's disease and atherosclerosis [22]. Also, oleic acid-bound α-lactalbumin, which acquires the properties typical of the MG, was found to induce apoptosis in cancer cells [23].

PROTEIN FOLDING. 'CLASSICAL VIEW' AND 'NEW VIEW'

The mechanism(s) governing protein folding is (are) still far from being fully understood due to the high complexity of the process. Small proteins (those with less than one hundred aminoacidic residues) fold in two-state transitions; in this case, the unfolded polypeptide directly collapses into the compact, biologically active native conformation. Conversely, large proteins (those with more than one hundred aminoacidic residues) fold into the unique, native structure through a limited number of intermediate species. The intermediate states sometimes participate to productive folding (on-pathway intermediates), in other cases they accumulate as compact forms distinct from the native, trapped by non-native intramolecular interactions (off-pathway intermediates).

The "classic view" was initially introduced to tentatively explain the "Levinthal paradox" (proteins fold in a few seconds, although finding the native state among all the possible protein configurations requires a very long time) [24]. The idea is that a protein collapses into the stable, native conformation through well defined folding pathways involving a limited number of intermediate species. Much interest was thus devoted to understand how proteins find the correct folding pathway, avoiding the others. In the last years, kinetic and equilibrium studies have detected an appreciable number of folding intermediates [25, 26]. The characterization of these partially folded forms has provided a precious contribution to a better understanding of the sequence of events carrying the protein from the unfolded to the compact native conformation.

Recently, the introduction of the so-called 'new view' has opened new interesting perspectives. The models on which the 'new view' is based replace single specific 'fold-

ing pathways' with more complex multidimensional 'energy landscapes' (where an 'energy landscape' represents the free energy of a single protein conformation, strictly depending on the degrees of freedom) [7, 27, 28]. On the basis of Anfinsen studies [29], the 'new view' establishes that folding is path-independent and molecule populations and multiple folding pathways, rather than specific structures and pathways, are considered [7, 30, 31]. According to this concept, terms like on- and off-pathways and intermediate states are now viewed as distributions of single chain conformations.

The 'new view' assumes that protein folding to the native state proceeds through multiple routes on funnel-like energy landscapes. Although energy landscapes may be rough, i.e. characterized by non-native minima in which partially folded molecules are trapped, the idea is that the whole protein population finally reaches the native conformation, corresponding to a free energy minimum under the solution conditions [7, 28, 31]. In other words, although single macromolecules follow an own distinct trajectory, the molecule population finally reaches the conformation corresponding to a minimum in free energy, i.e. the native state.

Studies carried out over the last two decades have significantly improved our understanding of the process, and led to the formulation of the 'new view' hypothesis. However, further studies are necessary for defining some still unclear aspects of the process. In a recent future the use of sophisticated techniques, such as atomic force microscopy (utilized to unfold proteins mechanically [32]) or single-molecule fluorescence (able to detect the behaviour of single macromolecules, otherwise masked when the whole molecule population is considered) [33] should provide, together with the computational approach, a remarkable contribution for better defining the energy landscapes and probing the 'new view' concept in protein folding.

FOLDING, MISFOLDING AND DISEASES. ROLE OF INTERMEDIATE CONFORMERS

Point mutations in proteins sometimes block the folding pathway at level of stable intermediate states; when this happens, the protein cannot adopt its native conformation and its biological activity results altered or lost. In many cases, the altered function of the mutated protein causes genetic diseases [34]. Cystic fibrosis, a disease characterized by chronic lung diseases and pancreatic dysfunction, is caused by the deletion of a single amino acid (Phe508) in cystic fibrosis trans-membrane conductance regulator (CFTMR) protein (a member of the ATP-binding cassette transporters superfamily) [35]. Wild type (wt) CFTMR is synthesized and folded inside the cell endoplasmic reticulum (ER) as an immature precursor. Through a complex glycosylation process which involves molecular chaperones (proteins that stabilize, through binding, unstable conformers of other proteins [36]), CFTMR then folds into its 'native', biologically active conformation in the Golgi apparatus. The 70 kDa heat-shock protein (Hsp70), a major chaperone present in human cell cytosol, participates to the folding process of CFTMR; it binds to the just synthesized, unfolded CFTMR and holds the protein in a state able to fold, thus permitting its translocation into different cell compartments [37, 38]. Unlike the wt protein, the Phe508del mutant of CFTMR folds into a stable intermediate state which requires a longer time to convert into the native conformation. The enhanced stability of the intermediate alters the mechanism(s) governing the process [37] and, as a consequence, mutated CFTMR does not reach the cell membrane; it is degraded within the ER.

Chronic liver and lung diseases may be caused by events of the same nature. In this case, the disease is originated by an insufficient amount of α-l-antitrypsin (AT), the serine protease inhibitor (see [39] for recent review). The main function of this 394-residues protein (52 kDa), which is synthesized in hepatocytes [40], consists in protecting the lungs against the proteolytic attack of neutrophil eleastase (NE). In its structure AT possesses 3 β-sheets and an exposed loop containing the residues interacting with the proteinase [41, 42]. Mutations within the loop induce conformational changes in the protein; the rigid, native structure converts into a more flexible conformation and the protein becomes inactive. The resulting AT deficiency causes lung disease since the delicate equilibrium between NE and AT (which is crucial for health) is lost; as NE becomes predominant, tissues are destroyed.

Two main types of mutation have been identified in AT: the Glu264Val mutation, which origins the S mutant [43], and the Glu342Lys mutation, which gives rise to the Z mutant [44, 45]. In both cases, AT deficiency is observed. In particular, the Z mutant undergoes aggregation; the accumulation of molecular aggregates inside the liver causes the organ disease. The Glu342Lys mutation is considered responsible for formation of aggregates: under physiological-like conditions, in fact, a partly folded state of the Z mutant is stabilized. In this intermediate, the exposed loop is characterized by enhanced flexibility (caused by the disruption of a salt bridge) and a β-sheet is open; this facilitates the insertion of the exposed loop of another molecule, and dimers are formed. The successive formation of polymers induces the pathology, since they generate inclusion bodies in the endoplasmic reticulum of the liver [46, 47].

The polymerization phenomenon is not restricted to AT; other antiproteinases undergo similar processes. In antithrombin and α-antichimotrypsin, some mutations originate partly folded states characterized by a high tendency to polymerize; this is cause of thrombosis or obstructive pulmonary diseases of genetic origin [48, 49].

On the whole, the examples above described highlight the importance of studies devoted to clarify the properties of intermediate states of proteins, of interest in biochemistry and experimental medicine.

PROTEIN MISFOLDING AND AGGREGATION

Conformational diseases may be induced also by different mechanisms. As described in the previous section, cystic fibrosis and chronic liver diseases arise from the impossibility of a misfolded protein to reach its final cellular destination. In other cases, protein misfolding favours formation of highly ordered aggregates, called amyloid fibrils, which are correlated with a number of pathologies as neurodegenerative disorders (including Alzheimer's disease and Parkinson's disease) or amyloidoses (pathological states due to

formation of extracellular amyloid deposits) (see [50] for recent review). Amyloid fibrils are composed by a number (generally 2-to-6) of filaments, called protofilaments, twisted together; in each filament, the molecules arrangement favours formation of β-sheets running along the fibril main axis [51, 52]. For some proteins, as amylin [53, 54] or the yeast prion protein Sup35p [55], the structural properties of fibrils have been determined in good detail.

In recent years, studies have significantly improved our understanding of properties common to fibrils; these include the cross-β structure and the frequent hydrophobic (or polar) interactions along the fibril main axis [50]. Further, in fibrils the β-sheets are less twisted than what expected from analysis of β-structure of globular proteins [56, 57]. Protein conversion into fibrils follows a 'nucleated growth' biphasic mechanism, characterized by a lag phase during which the nucleus is formed, and by a rapid phase during which the oligomers, that still miss a fibrillar shape, associate with the nucleus itself [58-60]. The oligomers, unstructured and constituted by 3-6 protein molecules, are in equilibrium with monomeric forms and precede protofibrils formation. Once the critical mass is reached, the oligomers associate and give rise to protofibrils formation. Protofibrils act either as on-pathway [58, 61] or off-pathway [62, 63] intermediates in the kinetics of formation of fibrils. The aggregation process may be inhibited by molecular chaperones or degradation processes, while it is favoured by side chains hydrophobicity, low net charge of the polypeptide and chain propensity to form β-sheet regions.

CYTOCHROME C: NON-NATIVE CONFORMATIONS AND DISTINCT BIOLOGICAL FUNCTIONS

Some authors have addressed the role of cofactors that may enable protein-folding variants to attain new functions. This is the case of α-lactalbumin bound to oleic acid that possesses spectroscopic properties typical of the molten globule and is able to induce apoptosis in cancer cells [23, 64]. Recent findings have demonstrated that this same lipid cofactor (i.e., oleic acid) is able to induce structural changes also in cytochrome *c* (cyt *c*), with formation of a molten globule-like state [65]. Indeed, in recent years several non-native conformers of cyt *c* were characterized and different biological functions, strictly depending on the conformation of the hemoprotein, were proposed [66-70].

Cyt *c*, whose structure is shown in Fig. (**2**), is a single chain hemoprotein of 104 amino acids containing three major and two minor α-helices in the structure, with the prosthetic group lying within a crevice lined with hydrophobic residues. The heme is covalently attached to the polypeptide chain by two thioether bridges with residues Cys 14 and Cys 17, while His 18 and Met 80 are the axial ligands of the six-coordinated low spin heme iron in the native state [71]. As mitochondrial peripheral membrane protein, it functions in between the inner and the outer membrane, mediating electron transfer (eT) between different proteins of the respiratory chain. Studies on the interaction between cyt *c* and various membrane systems indicate that cyt *c* mediates eT between cyt *c* reductase and cyt *c* oxidase as unbound or membrane-bound protein showing a limited number of non-native exchangeable compact conformations [72, 73].

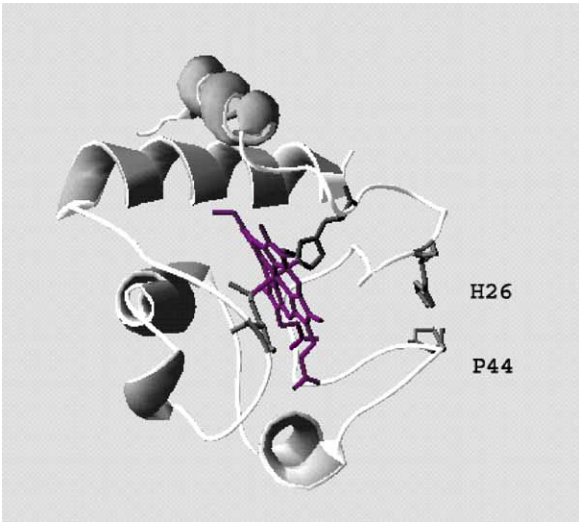

Fig. (2). Ribbon structure of horse cyt *c*. The heme group, bound to H18 (right) and M80 (left), is represented in black. On the right side, residues H26 and P44 are shown. The H-bond between H26 and the carbonyl group of E44 keeps two Ω-loops bound one to the other. The protein structure [85] was visualized with the Swiss-Pdb software [86].

The finding that cyt *c* plays a role in the programmed cell death (i.e., in cell apoptosis) after its release from the mitochondrion, has recently renewed interest in this protein [74, 75]. It has been proposed that in cytoplasm cyt *c* binds to the apoptosis protease activation factor (APAf-1) to form a complex that activates pro-caspase 9; this gives rise to an enzymatic reaction cascade leading to the execution of apoptosis in cells. For formation of the complex, called "apoptosome", the presence of ATP or dATP is required [76]. Evidence for structural changes of cyt *c* in apoptosis has been provided by Jemmerson *et al.* [77] who demonstrated, for the first time, that membrane-bound cyt *c* is involved in apoptotic activity and represents a relevant factor for caspase activation. The structural changes of cyt *c* in cell apoptosis have been related with changes occurring in the complex formed with phospholipid vesicles; in the complex, conformations of cyt *c* distinct from the native are formed. The dissociation of Met80 from the sixth coordination position of the heme iron is a peculiar characteristic of this non-native state. The changes also include alteration of the tertiary structure and perturbation of the heme crevice [77, 78]. Protein partial unfolding, together with weakening of the Met80-Fe(III) coordination bond, allow small molecules, as H_2O_2, to get access into the heme site of cyt *c* [79]. The peroxidase activity acquired by phospholipid membrane-bound cyt *c* appears critical in the early stages of apoptosis. In mitochondria, the phospholipid cardiolipin (CL) is about 25% of all lipids; recent findings have demonstrated that the cytc/CL complex acts as powerful CL-specific peroxidase and generates CL hydroperoxides that play a role in cyt *c* (and other pro-apoptotic factors) release from mitochondrial membrane [80, 81]. Thus, whereas the native fold as well as the low-spin hexacoordination of the heme iron are important for cyt *c* to function as electron carrier, a non-native compact conformation induces peroxidase activity in the protein, of relevance for the execution of the apoptotic program.

Table 1. Biological Effects of Altered Folding Mechanism and Related Diseases

Protein	Structural Mechanism	Biological Effect and Related Disease
Apolipoprotein E4[a]	Molten globule stabilization	Alzheimer's disease
Alpha-lactalbumin[b]	Molten globule stabilization by interaction with lipids	Apoptosis in cancer cells
Cystic fibrosis Transmembrane regulator (CFTR)[c]	Phe508 deletion, misfolding, retention in the endoplasmic reticulum and degradation	Cystic fibrosis
Alpha-1-antitrypsin[d]	Aminoacid mutations, conformational changes and aggregation	Alpha-1-antitrypsin deficiency
Prion protein[e]	Misfolding and aggregation in brain	Creutzfeldt-Jacob disease
Beta-amyloid[f]	Misfolding and aggregation in brain	Alzheimer 's disease
Cytochrome *c*[g]	Interaction with phospholipids and non-native intermediates with peroxidase activity	Apoptosis, Parkinson's disease and amyloidosis

[a]Ref. 22; [b]Refs. 23,54; [c]Refs. 34-38; [d]Refs.41-47; [e]Refs. 50,55; [f]Refs. 50-54; [g]Refs. 65-67, 79-84

An additional role for the peroxidase activity of cyt c has been recently proposed in the study of the oxidative stress-induced aggregation of α-synuclein, a "natively unfolded" protein constituting the major component of intracellular inclusions in several neurodegenerative disorders, such as Parkinson's disease [82, 83]. Recently, some authors have observed the formation of millimeter-length fibers of phospholipid vesicles-bound cyt *c* displaying amyloid (ß-sheet) characteristics [84]; this implies that the cyt *c*-phospholipids interaction may play a role in some of the disorders attributed to amyloid formation, such as AA-amyloidosis and Alzheimer's disease.

PERSPECTIVES

The present review has pointed out the important role played by intermediate states in protein folding, and indicated how dramatic consequences may be generated by protein misfolding (see Table **1**, for a schematic view). This justifies the wide body of studies devoted to determine the structural and functional properties of forms having structure intermediate between the native and fully unfolded state, and aiming to clarify how non-native compact states may influence protein folding and misfolding.

Most of the human diseases described in this review are of genetic nature, being generated by point mutations which favour accumulation of non-native compact forms of the protein. This outlines how dramatic consequences may derive from a point mutation occurring in a protein.

Studies on mutants have significantly improved our knowledge on the role of side chains (in particular, the invariant ones) in terms of structural stabilization, folding and functionality. However, such studies are not limited to elucidate specific aspects of protein misfolding and relative diseases; they also provide a precious contribution to better understanding the route of processes in which a native protein converts into the more flexible MG state. As said, a protein acquiring a non-native compact conformation may accomplish different functions when involved in distinct processes. In the case of cyt *c*, the protein was proposed to be released from the mitochondrium as a MG state, during cell apoptosis. Work from this laboratory provided significant contribution to better elucidate this point, showing that oleic acid-bound cyt *c* acquires a MG character and is able to bind ATP, a molecule which actively participates to the apoptotic process [65]. Studies have also revealed that the residue H26 is critical for stabilization of cyt *c*. The H26Y mutant of yeast cyt *c* shows a MG character: flexibility higher than the native form, decreased stability and altered heme crevice, where M80 is displaced by a lysine from the sixth coordination position of the heme-iron [14, 78]. The H26Y variant of cyt *c* possess high potentialities for investigation on protein folding and for defining the role played by the MG in the apoptotic process. To shed deeper light on these points, work is in progress in this laboratory.

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REFERENCES

[1] Sosnick, T.R; Mayne, L.; Hiller, R.; Englander, S.W. *Nat. Struct. Biol*., **1994**, *1*, 149.
[2] Sali, A.; Shakhnovich, E.I.; Karplus, M. *Nature,* **1994**, *369*, 248.
[3] Baldwin, R.L. *J. Biomol. NMR,* **1995**, *5*, 103.
[4] Bai, Y.; Sosnick, T.R.; Mayne, L.; Englander, S.W. *Science,* **1995**, *269*, 192.
[5] Ewbank, J.J.; Creighton, T.E., Hayer-Hartle, M.K.; Hartle, F.U. *Struct. Biol.,* **1995**, *2*, 10.
[6] Privalov, P.L. *J. Mol. Biol.,* **1996**, *258*, 707.
[7] Dill, K.A.; Chan, H.S. *Nat. Struct. Biol.,* **1997**, *4*, 10.
[8] Baldwin, R.L. *Nat. Struct. Biol.,* 1997, **4**, 965.
[9] Yeh, S-R; Rousseau, D.L. *Nat. Struct. Biol.,* **1998**, *5*, 222.
[10] Creighton, T. E. *Biochem. J*., **1990**, *270*, 1.
[11] Kim, P.S.; Baldwin, R.L. *Annu. Rev. Biochem*., **1990**, *59*, 631.
[12] Chan, H.S.; Dill, K.A. *Proteins,* **1998**, *30*, 2.
[13] Dobson, C.M. *Curr. Opin. Struct. Biol*., **1992**, *2*, 6.
[14] Kuwajima, K. *Proteins: Struct. Funct. Genet.,* **1989**, *6*, 87.
[15] Ptitsyn, O.B. In *Protein Folding*; Creighton, T.E. Ed.; Freeman and Company, New York, **1992**, pp. 243-300.
[16] Schmid, F.X. In *Protein Folding*; Creighton, T.E. Ed.; Freeman and Company, New York, **1992**, pp. 197-242.

[17] Semisotnov, G.V.; Kutyshenko, V.P.; Ptitsyn, O.B. *Mol. Biol. USSR,* **1989**, *23*, 808.
[18] Hughson, F.M.; Wright, P.E.; Baldwin, R.L. *Science,* **1990**, *249*, 1544.
[19] Dobson, C.M.; Hanley, C.; Radford, S.E.; Baum, J.A.; Evans, P.A. In *Conformations and Forces in Protein Folding*; Nall, B.T. and Dill, K.A. Eds; AAAS, Washington DC, **1991**, pp. 175-181.
[20] Jeng, M.-F.; Englander, S.W. *J. Mol. Biol*., **1991**, *221*, 1045.
[21] Damashun, G.; Gernat, C.; Damashun, H.; Bychkova, V.E.; Ptitsyn, O.B. *Int. J. Biol. Macromol*., **1986**, *8*, 226.
[22] Hatters, D.M.; Peters-Libeu, C.A.; Weisgraber, K.H. *Trends Biochem Sci*., **2006**, *31*, 445.
[23] Svensson, M.; Sabharwal, H.; Hakansson, A.; Mossberg, A.K.; Lipniunas, P.; Leffler, H.; Svanborg, C.; Linse, S. *J. Biol. Chem*., **1999**, *274*, 6388.
[24] Levinthal, C. *J. Chem. Phys*., **1968**, *65*, 44.
[25] Baldwin, R.L. *Chemtracts-Biochem Mol. Biol*., **1991**, *2*, 79.
[26] Englander, S.W. *Annu. Rev. Biophys. Biomol. Struct*., **2000**, *29*, 213.
[27] Bryngelson, J.D.; Onuchic, J.N.; Socci, N.D.; Wolynes, P.G. *Proteins,* **1995**, *21*, 167.
[28] Alm, E.; Baker, D. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*, 11305.
[29] Anfinsen, C.B. *Science,* **1973**, *181*, 223.
[30] Radford, S.E. *Trends Biochem. Sci*., **2000**, *25*, 611.
[31] Jahn, T.R.; Radford, S.E. *FEBS J.,* **2005**, *272*, 5962.
[32] Fisher, T.E.; Oberhauser, A.F.; Carrion-Vazquez, M.; Marszalek, P.E.; Fernandez, J.M. *Trends Biochem. Sci*., **1999,** *24*, 379.
[33] Weiss, S. *Nat. Struct. Biol*., **2000**, *7*, 724.
[34] Bychkova, V.E.; Ptitsyn, O.B. *FEBS Lett*., **1995**, *359*, 6.
[35] Amaral, M.D. *J. Inherit. Metab. Dis*., **2006**, *29*, 477.
[36] Hendrick, J.P.; Hartl, F.U. *Annu. Rev. Biochem*., **1993**, *62*, 349.
[37] Denning, G.M.; Anderson, M.P.; Amara, J.F.; Marshall, J.; Smith, A.E.; Welsh, M.J. *Nature,* **1992**, *358*, 761.
[38] Farinah, C.M.; Nogueira, P.; Mendes, F.; Penque, D.; Amaral, M.D. *Biochem. J*., **2002**, *366*, 797.
[39] Lomas, D.A.; Parfrey, H. *Thorax,* **2004**, *59*, 529.
[40] Eriksson, S.; Alm, R.; Astedt, B. *Biochim. Biophys. Acta,* **1978**, *542*, 496.
[41] Elliott, P.R.; Lomas, D.A.; Carrel, R.W.; Abrahams, J.P. *Nat. Struct. Biol*., **1996**, *3*, 676.
[42] Kim, S.; Woo, J.; Seo, E.J.; Yu, M.; Ryu, S. *J. Mol. Biol*., **2001**, *306*, 109.
[43] Owen, M.C.; Carrell, R.W.; Brennan, S.O. *Biochim. Biophys. Acta,* **1976**, *453*, 257.
[44] Jeppsson, J. *FEBS Lett*., **1976**, *65*, 195.
[45] Yoshida, A.; Lieberman, J.; Gaidulis, L.; Ewing, C. *Proc. Natl. Acad. Sci. USA,* **1976**, *73*, 1324.
[46] Dafforn, T.R.; Mahadeva, R.; Elliott, P.R.; Sivasothy, P.; Lomas, D.A. *J. Biol. Chem*., **1999**, *274*, 9548.
[47] Sivasothy, P.; Dafforn, T.R.; Gettins, P.G.; Lomas, D.A. *J. Biol. Chem*., **2000**, *275*, 33663.
[48] Potempa, J.; Karzus, E.; Travis, J. *J. Biol. Chem*., **1994**, *269*, 15957.
[49] Silverman, G.A.; Bird, P.I.; Carrell, R.W.; Church, F.C.; Coughlin, P.B.; Gettins, P.G.; Irving, J.A.; Lomas, D.A.; Luke, C.J.; Moyer, R.W.; Pemberton, P.A.; Remold-O'Donnell, E.; Salvesen, G.S.; Travis, J.; Whisstock, J.C. *J. Biol. Chem*., **2001**, *276*, 33293.
[50] Chiti, F.; Dobson, C.M. *Annu. Rev. Biochem*., **2006**, *75*, 333.
[51] Serpell, L.C.; Sunde, M.; Benson, M.D.; Tennent, G.A.; Pepys, M.B.; Fraser, P.E. *J. Mol. Biol*., **2000**, *300*, 1033.
[52] Sunde, M.; Blake, C. *Adv. Protein Chem*., **1997**, *50*, 123.
[53] Kajava, A.V.; Aebi, U. ; Steven, A.C. *J. Mol. Biol*., **2005**, *348*, 247.
[54] Makin, O.; Serpell, L.C. *J. Mol. Biol*., **2004**, *335*, 1279. 45.
[55] Krishnan, R.; Lindquist, S.L. *Nature,* **2005**, *435*, 765.
[56] Jimenez, J.L.; Guijarro, J.I.; Orlova, E.; Zurdo, J.; Dobson, C.M.; Sunde, M.; Saibil, H.R. *EMBO J*., **1999**, *18*, 815.
[57] Zandomeneghi, G.; Krebs, M.R.; McCammon, M.G.; Fandrich, M. *Protein Sci*., **2004**, *13*, 3314.
[58] Serio, T.R.; Cashikar, A.G.; Kowal, A.S.; Sawicki, G.J.; Moslehi, J.J.; Serpell, L.; Arnsdorf, M.F.; Lindquist, S.L. *Science,* **2000**, *289*, 1317.
[59] Uversky, V.N.; Li, J.; Souillac, P.; Millett, I.S.; Doniach, S.; Jakes, R.; Goedert, M.; Fink, A.L. *J. Biol. Chem*., **2002**, *277*, 11970.
[60] Pedersen, J.S.; Christensen, G.; Otzen, D.E. *J. Mol. Biol*., **2004**, *341*, 575.
[61] Harper, J.D.; Lieber, C.M.; Lansbury, P.T. Jr. *Chem. Biol*., **1997**, *4*, 951.
[62] Morozova-Roche, L.A.; Zamotin, V.; Malisauskas, M.; Ohman, A., Chertkova, R.; Lavrikova, M.A.; Kostanyan, I.A.; Dolgikh, D.A.; Kirpichnikov, M.P. *Biochemistry,* **2004**, *43*, 9610.
[63] Gosal, W.S.; Morten, I.J.; Hewitt, E.W.; Smith, D.A.; Thomson, N.H.; Radford, S.E. *J. Mol. Biol*., **2005**, *351*, 850.
[64] Svensson, M.; Mossberg, A.K.; Pettersson, J.; Linse, S.; Svanborg, C. *Protein Sci.,* **2003**, *12*, 19356.
[65] Sinibaldi, F.; Mei, G.; Ponticelli, F.; Piro, M.C.; Howes, B.D.; Smulevich, G.; Santucci, R.; Ascoli, F.; Fiorucci, L. *Protein Sci.,* **2005**, *14*, 1049.
[66] Cortese, J.D.; Voglino, A.L.; Hackenbrock, C.R. *Biochemistry,* **1998**, *37*, 6402.
[67] Jemmerson, R.; Liu, J.; Hausauer, D.; Lam, K.P.; Mondino, A.; Nelson, R.D. *Biochemisty,* **1999**, *38*, 3599.
[68] Nantes, I.L.; Zucchi, M.R.; Nascimento, O.R.; Faljoni-Alario, A. *J. Biol. Chem.,* **2001**, *276,* 153.
[69] Tuominem, E.K.; Wallace, C.J.; Clark-Lewis, I.; Craig, D.B.; Rytomaa, M.; Kinnunen, P.K. *J. Biol. Chem.,* **2002**, *277,* 8822.
[70] Sivakolundu, S.G.; Mabrouk, P.A. *J. Biol. Inorg. Chem.,* **2003**, *8,* 527.
[71] Moore, G.R.; Pettigrew, G.W. *Cytochromes c. Evolutionary, structural and physicochemical aspects*, Springer-Verlag; Heidelberg, **1990**.
[72] Bayir, H.; Fadeel, B.; Palladino, M.J.; Witasp, E.; Kurnikov, I.V.; Tyurina, Y.Y., Tyurin, V.A.; Amoscato, A.A.; Jiang, J.; Kochanek, P.M.; DeKosky, S.T.; Greenberger, J.S.; Shvedova, A.A.; Kagan, V.E. *Biochim. Biophys. Acta,* **2006**, *1757*, 648.
[73] Berezhna, S.; Wohlrab, H.; Champion, P.M. *Biochemistry,* **2003**, *42*, 6149.
[74] Liu, X.; Kim, C.N.; Yang, J.; Jemmerson, R.; Wang, X. *Cell,* **1996**, *86*, 147.
[75] Kluk, R.M.; Bossy-Wetzel, E.; Green, D.R.; Newmeyer, D.D. *Science,* **1997**, 275, 1132.
[76] Green, D.R.; Reed, J.C. *Science,* **1998**, *281,* 1309.
[77] Sinibaldi, F.; Piro, M.C.; Howes, B.D.; Smulevich, G.; Ascoli, F.; Santucci, R. *Biochemistry,* **2003**, *42,* 7604.
[78] Sinibaldi, F.; Howes, B.D.; Piro, M.C.; Caroppi, P.; Mei, G.; Ascoli, F.; Smulevich, G.; Santucci , R. *J. Biol. Inorg. Chem.,* **2006**, *11,* 52.
[79] Vladimirov, Y.A.; Proskurnina, E.V.; Izmailov, D.Y.; Novikov, A.A.; Brusnichkin, A.V.; Osipov, A.N., Kagan, V.E. *Biochemistry (Moscow),* **2006**, *71*, 998.
[80] Kagan, V.E.; Tyurin, V.A.; Jiang, J.; Tyurina, Y.Y.; Ritov, V.B.; Amoscato, A.A.; Osipov, A.N.; Belikova, N.A.; Kapralov, A.A.; Kini, V.; Vlasova, I.I.; Zhao, Q.; Zou, M.; Di, P.; Svistunenko, D.A.; Kurnikov, I.V.; Borisenko, G.G. *Nat. Chem. Biol*., **2005**, *1*, 223.
[81] Belikova, N.A.; Vladimirov, Y.A.; Osipov, A.N.; Kapralov, A.A.; Tyurin, V.A.; Potapovich, M.V.; Basova, L.V.; Peterson, J.; Kurnikov, I.V.; Kagan, V.E. *Biochemistry,* **2006**, *45*, 4998.
[82] Olteanu, A.; Pielak, G.J. *Protein Sci.,* **2004**, *13*, 2852.
[83] Cookson, M.R. *Annu. Rev. Biochem*., **2005**, *74*, 29.
[84] Alakoskela, J.M.; Jutila, A.; Simonsen, A.C.; Pirneskoski, J.; Pyhajoki, S.; Turunen, R.; Marttila, S.; Mouritsen, O.G.; Goormaghtigh, E.; Kinnunen, P.K.J. *Biochemistry,* **2006**, *45*, 13447.
[85] Louie, G.V.; Brayer, G.D. *J. Mol. Biol*., **1990**, *214*, 527.
[86] Guex, N.; Peitsch, M.C. *Electrophoresis,* **1997**, *18*, 2714.

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