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Protein dynamics, folding and misfolding: from basic physical chemistry to human conformational diseases

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Abstract Proteins exhibit a variety of motions ranging from amino acid side-chain rotations to the motions of large domains. Recognition of their conformational flexibility has led to the view that protein molecules undergo fast dynamic interconversion between different conformational substates. This proposal has received support from a wide variety of experimental techniques and from computer simulations of protein dynamics. More recently, studies of the subunit dissociation of oligomeric proteins induced by hydrostatic pressure have shown that the characteristic times for subunit exchange between oligomers and for interconversion between different conformations may be rather slow (hours or days). In such cases, proteins cannot be treated as an ensemble of rapidly interconverting conformational substates, but rather as a persistently heterogeneous population of different long-lived conformers. This is reminiscent of the deterministic behavior exhibited by macroscopic bodies, and may have important implications for our understanding of protein folding and biological functions. Here, we propose that the deterministic behavior of proteins may be closely related to the genesis of conformational diseases, a class of pathological conditions that includes transmissible spongiform encephalopathies, Alzheimer's disease and other amyloidosis.

Key words: Deterministic behavior; Molecular individuality; Conformational disease; Amyloidosis; Prion; Spongiform encephalopathy; Alzheimer's disease

1. Introduction

Following the landmark studies of Anfinsen [1], protein folding has generally been viewed as a process in which a polypeptide chain performs a search in conformational space to achieve the so-called 'native' conformation corresponding to the global free-energy minimum under a given set of physicochemical conditions of the medium. Given the vast size of the conformational space available for folding of even the smallest proteins [2], it was recognized early that folding did not take place by random sampling of all accessible conformations. Instead, the conformational search is currently thought to take place in an energy landscape similar to a multidimensional funnel, the slope of which effectively guides the protein down towards the energy minimum [3,4]. The funnel therefore leads to a huge increase in folding rate (compared to the expected rate for a random diffusional process) and prevents entrapment in partially folded states (local energy minima). Recent results, however, have shown that protein folding to a biologically competent conformation does not always or necessarily lead to a unique state corresponding to the overall free-energy minimum. In some cases, the existence of metastable conformations has been characterized, indicating kinetic rather than thermodynamic control of the folding process [5]. In other cases, folding appears to continue even after the achievement of full biological function (i.e. a slow annealing of the protein structure; [6,7]).

Furthermore, for oligomeric proteins folding is often markedly dependent on protein–protein interactions. The equilibrium of subunit association is generally considered to result from a large number of stochastic association–dissociation events. As a result, the equilibrium is dependent on protein concentration, as expected from the law of mass action. However, recent studies have shown deviations from this expected behavior in the investigation of the equilibrium of subunit association in oligomers ranging from dimers to viral particles. In those cases, an anomalous or complete lack of protein concentration dependence for subunit association was found [8]. This results from high activation free-energy barriers for subunit exchange between oligomers, which leads to very slow rates of exchange during biologically relevant timescales [9,10]. As a result, a persistent (long-lived) conformational heterogeneity is generated in the protein ensemble. This type of behavior has been described as deterministic [8,9] to indicate the persistent heterogeneity or molecular individuality of proteins.

In recent years, studies of protein folding and, in particular, of misfolding and interactions have been stimulated by the realization that misfolding and aggregation are involved in a number of the so-called 'conformational' diseases, of which transmissible spongiform encephalopathies and Alzheimer's disease (AD) are striking examples. The molecular/energetic basis of protein misfolding and aggregation (leading to the formation of amyloid aggregates) in conformational diseases are still largely unknown. Understanding the molecular mechanisms of amyloid formation would represent an important first step in the development of rational strategies to interfere

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Abbreviations: AD, Alzheimer's disease; BSE, bovine spongiform encephalopathy; CJD, Creutzfeld–Jakob disease; DNP, 2,4-dinitrophenol; NP, 3-nitrophenol

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with aggregation and the resulting pathological conditions. Here, we propose that kinetically controlled folding and deterministic behavior may be closely related to the generation of amyloidogenic conformations of proteins.

2. Protein dynamics: hierarchical conformational substates, folding funnels and metastable conformations

Proteins have long been known to be conformationally dynamic. Since the pioneering studies by Linderström-Lang [11] on the exchange of amide hydrogens of protein backbones with hydrogens of the solvent water, multiple lines of evidence have shown that proteins exhibit a wide range of motions over several orders of magnitude in time. For example, NMR and fluorescence studies of protein motions have provided connections between experimental results and predictions from molecular dynamics simulations (for early perspectives, see [12–15]). Because peptide bond planes can undergo rotations of tens of degrees with activation energies as low as a few kJ/mol, protein motions are thought to occur in a relatively unimpeded way, leading to fast interconversion rates between different conformational states [16].

Studies carried out by Frauenfelder and co-workers have been particularly revealing in characterizing the existence of conformational substates in proteins. They showed that the kinetics of re-binding of CO to myoglobin was markedly non-exponential at low temperatures [17,18]. Non-exponential kinetics originates from the existence of several conformational substates of myoglobin possessing distinct CO binding rates. At room temperature, the rate of interconversion between different conformational substates is fast, due to the low activation barriers between them. With decreasing temperatures, the activation barriers become more difficult to overcome, leading to long-lived occupancy of different substates.

Using time-resolved fluorescence as a probe of protein conformational dynamics, we and other groups have shown that proteins exhibit significant conformational heterogeneity at room temperature (for early examples, see [19–22]). These results indicate that the activation free-energy barriers for interconversion between substates may be sufficiently high to lead to the observation of conformational heterogeneity even at room temperature. These studies further indicate that the model of conformational substates discussed above is generally applicable to describe the dynamics of proteins.

During the past two decades, an impressive amount of information on the energetics, kinetics and pathways of folding of several different proteins has been obtained. At the same time, efforts have been made to incorporate experimental results into conceptual frameworks that could be generally applicable to describe folding. In this regard, the introduction of the idea that proteins fold in a funnel-shaped energy landscape (Fig. 1) [3,4,17] appears particularly useful in explaining some of the key issues of folding. However, the funnel model generally considers that the end-point of folding corresponds to a unique, native state corresponding to the global free-energy minimum of the system [4]. In light of the considerations presented above, it would seem that including the possible existence of multiple conformers at the bottom of the funnel would be more appropriate.

For several proteins, the final outcomes of folding appear to be determined by kinetic rather than thermodynamic factors (reviewed in [5]). These studies indicate that the free-energy barriers in conformational space can be quite high, leading to the persistence of metastable states. In such cases, the final folded state may not correspond to the global free-energy minimum, and the free-energy diagram for folding may be considerably more rugged or complicated than the funnel represented in Fig. 1. The existence of high activation barriers for interconversion between conformational substates may prevent the achievement of the most stable conformations of proteins [5] or lead to the co-existence of long-lived conformers that markedly differ in stability (see below). In either case, differences in biological function may also be expected from different protein conformers.

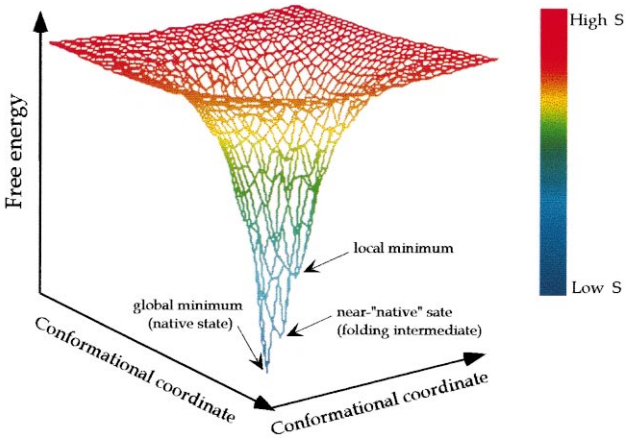

Fig. 1. The protein folding funnel, showing the dependence of the overall free energy of the protein on conformation. The diagram is color-coded according to the configurational entropy level of the polypeptide chain (red for high entropy and blue for low entropy). Folding proceeds from a disordered state to progressively more ordered conformations corresponding to lower energy levels. Possible folding intermediates are represented by local energy minima. The native state, at the bottom of the funnel, is thought to correspond to a unique conformation (or a very homogeneous set of conformations in fast exchange).

3. Persistent conformational heterogeneity and deterministic behavior of proteins

The reversible association of multiple subunits to form an oligomeric protein is expected to obey the law of mass action, being thus dependent on protein concentration. This is a consequence of the fast exchange of subunits between oligomers, leading to energetic/conformational averaging of all the molecules in the ensemble. However, investigation of the reversible subunit dissociation of several oligomers by hydrostatic pressure has revealed significant deviations from the law of mass action [8,23–25]. This anomalous behavior ranges from the reduced dependence on protein concentration for subunit association/dissociation in trimers and tetramers [9,25] to a complete lack of protein concentration dependence for the association of large protein aggregates such as viral particles [26,27]. This behavior has been investigated in detail in the case of dimeric triosephosphate isomerase (TIM) [8,10]. The dissociation of TIM subunits induced by hydrostatic pressure was found to be completely independent of protein concentration, in apparent violation of the law of mass action [8]. In subsequent studies, the kinetics and equilibrium of dissociation/unfolding of TIM induced by guanidine hydrochloride were investigated [10]. These studies showed that the activa-

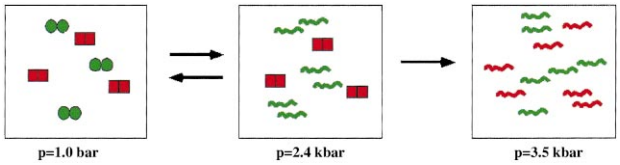

Fig. 2. Molecular individuality of TIM dimers. The scheme is based on results from pressure-induced dissociation of TIM subunits [8]. At atmospheric pressure (1 bar), TIM exists as a heterogeneous ensemble of strong (red) and weak (green) dimers with respect to their pressure stability. Application of an intermediate pressure (2.4 kbar) causes reversible subunit dissociation of the weak dimers only. Decompression to atmospheric pressure causes subunit reassociation and a second cycle of pressure (to 2.4 kbar) only brings about dissociation of the weak dimers again. This indicates lack of conformational exchange between weak and strong dimers during the experimental time-scale (i.e. several hours). Strong dimers can only be dissociated at high pressure (3.5 kbar).

tion free-energy barriers for dissociation of TIM dimers are quite high (≥99 kJ/mol), corresponding to a characteristic time of dissociation of 15 h or longer. As the refolding/reassociation of TIM subunits were found to be fast [10], the characteristic dissociation time is the rate-limiting step in a cycle of dissociation/reassociation. This prevents the exchange of TIM subunits between different dimers in times ranging from several hours to days, preventing conformational/energetic averaging of the molecules in the ensemble. In other words, energetic/conformational differences between individual TIM dimers in solution may remain 'frozen' during biologically relevant time-scales (hours to days). In line with this hypothesis, different populations of TIM dimers exhibiting persistent differences in thermodynamic stability were found in experiments in which dimer dissociation was induced by hydrostatic pressure (Fig. 2) [8] or guanidine hydrochloride (Moreau et al., in preparation). This led us to propose the existence of persistent conformational heterogeneity to explain the behavior of TIM. In practical terms, TIM dimers present a certain degree of 'molecular individuality' and behave as a deterministic system, in analogy with the behavior exhibited by macroscopic objects.

The concepts of molecular individuality and deterministic behavior of proteins may have profound implications in our understanding of the relationships between stability and biological functions. Many biologically important processes such as catalysis, metabolic protein turnover, viral infection and replication, amyloid formation (see below) and many others occur on time-scales that are comparable to or shorter than the slow dynamics of conformational interconversion that lead to deterministic behavior. This means that, under certain circumstances, these biological processes may involve not homogeneous protein populations, but rather persistently heterogeneous ensembles of conformations.

For example, for virus particles a clear need for a distribution of conformations that can be regarded as 'frozen' in time was pointed out by Weber and co-workers [26,27]. A typical virus shell consists of the non-covalent assembly of dozens of copies of one or a few types of coat proteins. Therefore, the assembly of the capsid should be sharply dependent on protein concentration. In an infected cell, the constituent virus coat proteins are over-expressed, and it is thermodynamically favorable to assemble a virus particle. However, after cell lysis and release of the virus into the environment, the concentration of viral particles is so low that each virus can be considered a single, isolated particle. This means that the stochastic chance of reassociation would be extremely small if a virus particle dissociated under these conditions. Therefore, the rate of subunit dissociation (i.e. capsid disassembly) has to be negligibly slow in order to assure the long-term survival of the assembled virus. Thus, despite the fact that virus association is thermodynamically unstable under these conditions, the viral particle persists as a 'frozen' macromolecular species, rather than as an assembly that is in dynamic chemical equilibrium with its constituent monomers.

For enzymes such as TIM, a benefit that could result from adopting a permanently folded structure (rather than undergoing cycles of dissociation/association) is an increase in resistance to chemical modification such as thiol oxidation, deamidation or proteolytic digestion [8]. Unfolded or unstructured conformations are in general more vulnerable to these covalent modifications. Indeed, the marked resistance of TIM against oxidation and proteolysis is directly correlated with maintenance of its native dimeric structure [8,28,29].

Perhaps one of the most interesting possible implications of deterministic behavior and molecular individuality of proteins is in relation to the genesis of conformational or amyloid diseases, as discussed below.

4. The individuality of protein molecules and conformational diseases

Conformational diseases comprise a class of important human pathologies including AD and Parkinson's diseases and the transmissible spongiform encephalopathies. It is believed that these pathological conditions are related to conformational changes from non-toxic to toxic forms of specific proteins or fragments.

Transmissible spongiform encephalopathies (such as bovine spongiform encephalopathy (BSE) in cattle and Creutzfeld–Jakob disease (CJD) in humans) are associated with the accumulation in the brain of an abnormal protease-resistant form of the prion protein (PrP). Mammalian prion diseases are apparently transmitted directly by PrP and do not involve genetic transmission via nucleic acids [30–32]. It is believed that the disease is caused by a conformational change in PrP from a benign cellular conformation (PrP^{C}) to a neurotoxic form (PrP^{Sc}) that self-propagates by recruiting and inducing the conformational change in additional PrP^{C} molecules.

The economic and public health impact of prion diseases has stimulated substantial research into the biochemistry, cell biology and epidemiology of prion toxicity. However, the molecular/energetic basis of prion transmission are still incompletely understood. For example, little is known on the molecular state of the protein that corresponds to the infectious, self-propagating particle. It is known that one infectious unit corresponds to approximately 10^5 PrP molecules [33]. This could mean that infection is caused by a large aggregate of PrP^{Sc} molecules or, alternatively, that a single one of those molecules is actually infectious.

A puzzling feature of the prion hypothesis regards the existence of prion 'strains', i.e. clinically distinct disease forms within a single animal species that are not associated with mutations in the PrP gene (e.g. eight different strains have been reported that propagate in the hamster; [34]). Thus,

different copies of the same gene product (with identical amino acid sequences) appear to exist in different conformations. These observations imply a persistent, long-lived conformational heterogeneity of prion molecules, which allows different strains to be perpetuated over long periods of time and even when passaged through an intermediate species with a different PrP gene [35]. Such persistent conformational heterogeneity is reminiscent of the situation described above regarding the energetic basis of the molecular individuality of proteins. Thus, the origin of prion strains may reside in the existence of multiple conformational substates of PrP separated by sufficiently high activation free-energy barriers. In this regard, a recent study from Prusiner's group described that different strains of hamster prions exhibit different sensitivities to unfolding by guanidine hydrochloride [34]. This is completely analogous to the behavior we have described for TIM (see above; [8]), and indicates the deterministic nature of prions.

Molecular modeling has also indicated that the octapeptide repeat region located in the N-terminal domain of PrP is capable of adopting several different conformations with identical stabilities upon binding copper ions [36]. Furthermore, PrP^{C} in solution appears to have a predominantly α-helical fold [37–40], despite the fact that most of the amino acid residues in PrP have a preference for β-conformation [36]. Thus, it is possible that PrP exists in a balance between the native α-helical fold determined by the docking of side-chains and the β-structure that would otherwise be preferred by structural propensity [36]. High free-energy barriers, leading to slow interconversion between them, likely separate these two largely different conformations. Finally, NMR measurements have shown that a loop between β-strand 2 and α-helix 2 in PrP exhibits relatively slow conformational fluctuations (i.e. slower than the limits of detection by NMR) [40]. Interestingly, this loop is also one of the regions of greater variability in charge distribution on the surface of PrP from different species, suggesting that it may be involved in the species barrier for transmission [40].

The hypothesis presented above of the deterministic behavior of prions (and its relationship to strain formation) can be considered in light of some of the known features of prion biochemistry. In vitro, prions have been shown to form amyloid fibrils [41,42]. In a persistently heterogeneous ensemble, conceivably only a fraction of the molecules is in the 'appropriate' conformation for aggregation into fibrils. Thus, the initial rate of fibril formation is slow. After formation of a certain amount of amyloid 'seeds', the speed of fibrillization increases, which has been interpreted as indicative of template-induced conversion of additional PrP molecules. Alternatively, we propose that the seeds might select from the solution those PrP molecules that already exhibit the aggregation-prone conformation (Fig. 3). It is important to note that both processes (template-induced polymerization or selection of specific molecules from the ensemble) would give rise to the same type of kinetics of amyloid formation. Thus, future investigation of the deterministic hypothesis will probably require detailed characterization of the existence of persistent conformational heterogeneity in the ensemble of PrP molecules prior to amyloid aggregation.

Using the Sup35 yeast prion model, Chien and Weissman [43] have recently proposed that the conformational diversity of prion molecules is related to the species barrier for transmission. They showed that a chimera containing the prion

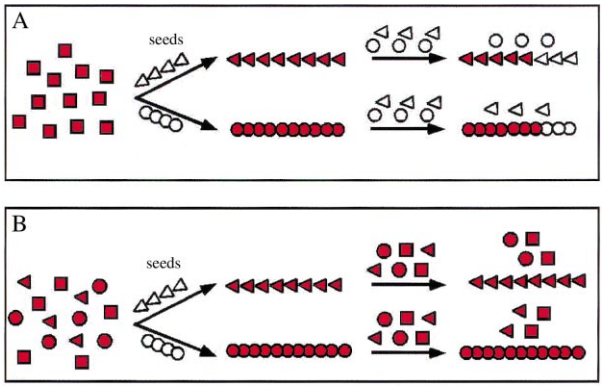

Fig. 3. Alternative hypothesis for prion strain formation. A: Shows the 'template-induced conformational change' model (based on [43]). The original population of chimeric prion molecules (red squares) is believed to be homogeneous and sufficiently plastic to conform to templates ('seeds') consisting of either one of two parental types (open circles or triangles). The growing fibril of a given conformation, however, only accepts the addition of parental prions of the same conformation. B: Shows the 'deterministic selection' model. The original population of prions is supposed to exist as a persistently heterogeneous ensemble of conformations (either due to intrinsic properties of the prion molecule or to stabilization of different conformers by interaction with different modulators; see text). Fibril growth and strain propagation, thus, involve selection of the 'appropriate' conformer from the solution.

domains of Sup35 from *Candida albicans* and *Saccharomyces cerevisiae* could be seeded to form amyloid fibrils using both Sup35 'parental' species. However, a species transmission barrier prevented direct cross-seeding of *C. albicans* or *S. cerevisiae* prions. These results led them to propose that the chimera was sufficiently flexible to undergo template-induced conversion to either type of parental amyloid fibril (Fig. 3A). Similar observations were made in the transmission of human prions to mice expressing a chimera of mouse and human prions [44]. On the basis of the previous discussion, an alternative explanation for these results is that the seeds select the specific amyloidogenic PrP (or chimeric Sup35) conformations amongst multiple conformations present in the ensemble (Fig. 3B).

5. Targeting the stability of amyloid fibrils as a possible strategy in amyloidosis

Insight into the energetics of amyloid fibril formation may provide guidance to the development of strategies to interfere with aggregation and to prevent amyloid-related toxicity.

In a recent study [45], we have investigated the stability of amyloid fibrils of the β-amyloid peptide (Aβ) involved in amyloid formation and deposition in senile plaques in the brains of AD patients. Aβ plays a central role in the neuropathology of AD [46–49]. Although recent studies have shown that nonfibrillar Aβ may be involved in neurodegeneration, considerable evidence indicates that Aβ aggregation and amyloid deposition are related to AD neurotoxicity ([50–52] and refs. therein). Thus, agents capable of interfering with aggregation have the potential to prevent or diminish the toxicity of Aβ.

In the lack of detailed molecular structures of either soluble or fibrillar Aβ (which precludes a structure-based drug design approach), our strategy to identify potential anti-amyloidogenic compounds initially relied on an investigation of the stability of amyloid fibrils. To this end, amyloid fibrils formed

in vitro by Aβ peptides of different chain-lengths were investigated in denaturant solutions. We found that the stability of Aβ fibrils in guanidine hydrochloride solutions was markedly increased when the peptide contained a cluster of non-polar amino acids (residues 29–42) in its C-terminal region. Furthermore, cooling caused reversible and nearly complete disaggregation of Aβ fibrils, suggesting that a significant contribution to the stability of Aβ fibrils comes from entropy-driven hydrophobic interactions (which are known to be destabilized at low temperatures). These observations led to the hypothesis that hydrophobic compounds could be effective in destabilizing and disaggregating amyloid fibrils.

After examining a number of moderately hydrophobic compounds (i.e. sufficiently hydrophobic to interfere with amyloid aggregation but still maintaining good solubility in aqueous media), we found that nitrophenols (e.g. 2,4-dinitrophenol, DNP, or 3-nitrophenol, NP) prevent amyloid aggregation in vitro and cause the disassembly of pre-aggregated fibrils [45]. Of greater interest, nitrophenols block the neurotoxicity of Aβ to rat hippocampal neurons in primary culture, and cause a marked reduction in the area occupied by amyloid deposits in rat brains [45]. These results indicate that nitrophenols and their derivatives should be explored as possible drug candidates or lead compounds for the development of drugs to prevent amyloid aggregation and neurotoxicity in AD.

6. Conclusions

The notion that proteins exist as ensembles of molecules in different conformational substates explains the origin of fast intramolecular protein motions [17]. Furthermore, it may be generally expanded to explain apparently 'anomalous' protein–protein interactions in oligomeric assemblies, provided that activation barriers of the appropriate magnitude are considered for interconversion between different conformational substates [10]. The deterministic behavior of protein ensembles caused by high free-energy barriers for conformational interconversion may be directly related to strain formation in prion diseases and to amyloid aggregation in other amyloidosis.

A problem shared by all in vitro studies of amyloidogenesis is that the in vitro environment probably does not reproduce the in vivo milieu. Conceivably, amyloidogenic proteins interact in vivo with a number of intracellular or extracellular 'modulators' (e.g. other proteins, glycosaminoglicans) that shift the equilibrium of conformations towards conformers that are more or less amyloidogenic. Ageing, which normally favors the manifestation of amyloid diseases, may cause changes in the availability of such modulators that eventually lead to stabilization of amyloid-prone conformers. Such modulators may also cause changes in activation free-energy barriers between states, leading to changes in the rates of conformational interconversion. In this context, it is important to consider that the 'induced conformational change' model for amyloid formation proposed on the basis of extensive in vitro studies has yet to be proved for amyloidogenesis in vivo. As an alternative hypothesis, one should consider that different conformational substates of a potentially amyloidogenic protein may be kinetically or thermodynamically stabilized in vivo by interactions with different types of modulators exhibiting distinct affinities for different conformational states of the protein. This would lead to a deterministic scenario in which amyloid formation could be viewed as a slow process of selection and aggregation of the amyloidogenic conformation amongst all possible existing conformers.

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References

[1] Anfinsen, C.B. (1973) Science 181, 223–230.
[2] Levinthal, C. (1968) J. Chim. Phys. 65, 44–45.
[3] Onuchic, J.N., Wolynes, P.G., Luthey-Schulten, Z. and Socci, N.D. (1995) Proc. Natl. Acad. Sci. USA 92, 3626–3630.
[4] Wolynes, P.G., Onuchic, J.N. and Thirumalai, D. (1995) Science 267, 1619–1620.
[5] Baker, D. and Agard, D.A. (1994) Biochemistry 33, 7505–7509.
[6] Subramanian, V., Steel, D.G. and Gafni, A. (1996) Protein Sci. 5, 2089–2094.
[7] Subramanian, V., Bergenhem, N.C., Gafni, A. and Steel, D.G. (1995) Biochemistry 34, 1133–1136.
[8] Rietveld, A.W. and Ferreira, S.T. (1996) Biochemistry 35, 7743–7751.
[9] Erijman, L. and Weber, G. (1991) Biochemistry 30, 1595–1599.
[10] Rietveld, A.W. and Ferreira, S.T. (1998) Biochemistry 37, 933–937.
[11] Linderström-Lang, K. (1958) in: Symposium on Protein Structure (Neuberger, A., Ed.), Methuen, London.
[12] Karplus, M. and McCammon, J.A. (1983) Annu. Rev. Biochem. 52, 263–300.
[13] Careri, G. and Gratton, E. (1984) in: The Fluctuating Enzyme (Welch, G.R., Ed.), Chapter 10, Wiley and Sons, New York.
[14] Wüthrich, K. (1981) Biochem. Soc. Symp. 46, 17–37.
[15] Wüthrich, K. (1989) Science 243, 45–50.
[16] Frauenfelder, H. and Gratton, E. (1986) Methods Enzymol. 127, 207–216.
[17] Frauenfelder, H., Sligar, S.G. and Wolynes, P.G. (1991) Science 254, 1598–1603.
[18] Ansari, A., Berendzen, J., Bowne, S.F., Frauenfelder, H., Iben, T.E., Sauke, T.B., Shyamsunder, E. and Young, R.D. (1985) Proc. Natl. Acad. Sci. USA 82, 5000–5004.
[19] Alcala, J.R., Gratton, E. and Prendergast, F.G. (1987) Biophys J. 51, 925–936.
[20] Ferreira, S.T. (1989) Biochemistry 28, 10066–10072.
[21] Mei, G., Rosato, N., Silva, N., Rusch, R., Gratton, E., Savini, I. and Finazzi-Agro, A. (1992) Biochemistry 31, 7224–7230.
[22] Ferreira, S.T., Stella, L. and Gratton, E. (1994) Biophys. J. 66, 1185–1196.
[23] Weber, G. (1992) Protein Interactions, Chapman and Hall, New York.
[24] Silva, J.L. and Weber, G. (1993) Annu. Rev. Phys. Chem. 44, 89–113.
[25] Pedrosa, C. and Ferreira, S.T. (1994) Biochemistry 33, 4046–4055.
[26] Da Poian, A.T., Oliveira, A.C., Gaspar, L.P., Silva, J.L. and Weber, G. (1993) J. Mol. Biol. 231, 999–1008.
[27] Weber, G., Da Poian, A.T. and Silva, J.L. (1996) Biophys. J. 70, 167–173.
[28] Waley, S.G. (1973) Biochem. J. 135, 165–172.
[29] Sun, A.Q., Yuksel, K.U. and Gracy, R.W. (1992) J. Biol. Chem. 267, 20168–20174.
[30] Alper, T., Cramp, W.A., Haig, D.A. and Clarke, M.C. (1967) Nature 214, 764–766.
[31] Griffith, J.S. (1967) Nature 215, 1043–1044.
[32] Prusiner, S.B. (1998) Proc. Natl. Acad. Sci. USA 95, 13363–13383.
[33] Bolton, D.C., McKinley, M.P. and Prusiner, S.B. (1982) Science, 1309–1311.

[34] Peretz, D., Scott, M.R., Groth, D., Williamson, R.A., Burton, D.R., Cohen, F.E. and Prusiner, S.B. (2001) Protein Sci. 10, 854–863.
[35] Bruce, M., Chree, A., McConnell, I., Foster, J., Pearson, G. and Fraser, H. (1994) Phil. Trans. R. Soc. Lond. B343, 405–411.
[36] Clarke, A.R., Jackson, G.S. and Collinge, J. (2001) Phil. Trans. R. Soc. Lond. B356, 185–195.
[37] Riek, R., Hornemann, S., Wider, G., Billeter, M., Glockshuber, R. and Wüthrich, K. (1996) Nature 382, 180–182.
[38] James, T.L., Liu, H., Ulyanov, N.B., Farr-Jones, S., Zhang, H., Donne, D.G., Kaneko, K., Groth, D., Mehlhorn, I., Prusiner, S.B. and Cohen, F.E. (1997) Proc. Natl. Acad. Sci. USA 94, 10086–10091.
[39] Zahn, R., Liu, A., Lührs, T., Riek, R., von Schroetter, C., Garcia, F.L., Billeter, M., Calzolai, L., Wider, G. and Wüthrich, K. (2000) Proc. Natl. Acad. Sci. USA 97, 145–150.
[40] Garcia, F.L., Zahn, R., Riek, R. and Wüthrich, K. (2000) Proc. Natl. Acad. Sci. USA 97, 8334–8339.
[41] Jackson, G.S., Hosszu, L.L.P., Power, A., Hill, A.F., Kenney, J., Saibil, H., Craven, C.J., Waltho, J.P., Clarke, R. and Collinge, J. (1999) Science 283, 1935–1937.
[42] Swietnicki, W., Morillas, M., Chen, S.G., Gambetti, P. and Surewicz, W.K. (2000) Biochemistry 39, 424–431.
[43] Chien, P. and Weissman, J.S. (2001) Nature 410, 223–227.
[44] Telling, G.C., Parchi, P., DeArmond, S.J., Cortelli, P., Montagna, P., Gabizon, R., Mastrianni, J., Lugaresi, E., Gambetti, P. and Prusiner, S.B. (1996) Science 274, 2079–2082.
[45] De Felice, F.G., Houzel, J.C., Garcia-Abreu, J., Louzada, P.R.F, Jr., Afonso, R.C., Meirelles, M.N.L., Lent, R., Moura Neto, V. and Ferreira, S.T. (2001) FASEB J., Express article 10.1096/fj.00-0676fje.
[46] Haass, C. and De Strooper, B. (1999) Science 286, 916–919.
[47] Selkoe, D.J. (1994) J. Neuropathol. Exp. Neurol. 53, 438–447.
[48] Yankner, B.A. (1996) Neuron 16, 921–932.
[49] Verbeek, M.M., Ruiter, D.J. and de Waal, R.M.W. (1997) Biol. Chem. 378, 937–950.
[50] Pike, C.J., Burdick, D., Walencewicz, A.J., Glabe, C.G. and Cotman, C.W. (1993) J. Neurosci. 13, 1676–1687.
[51] Lorenzo, A. and Yankner, B.A. (1994) Proc. Natl. Acad. Sci. USA 91, 12243–12247.
[52] Geula, C., Wu, C.K., Saroff, D., Lorenzo, A., Yuan, M. and Yankner, B.A. (1998) Nat. Med. 4, 827–831.