

# The Generic Nature of Protein Folding and Misfolding

Christopher M. Dobson

## 1. Abstract

The ability of proteins to fold to their functional states is an astonishing example of the power of biological evolution at the molecular level. Despite the large number of different native protein folds, the process of folding can be described in terms of a universal mechanism that appears to be based on the generation of the correct overall topology through interactions involving a relatively small number of residues. Protein misfolding is an intrinsic aspect of normal folding within the complex cellular environment, and its effects are minimized in living systems by the action of a range of protective mechanisms including molecular chaperones and quality control systems. Unfolded and misfolded proteins have a tendency to aggregate to form a variety of species including the highly organized and kinetically stable amyloid fibrils. The latter species represent a generic form of structure resulting from the inherent polymer properties of polypeptide chains, and their formation is associated with a wide range of debilitating human diseases. Amyloid fibrils and their precursors appear to have similar adverse effects on cellular function regardless of the sequence of the component peptide or protein. Our increasing knowledge of the interplay between different forms of protein structure and their generic characteristics provides a platform for rational therapeutic intervention designed to prevent or treat this whole family of diseases.

## 2. Introduction

Virtually every chemical process on which our lives depend is stimulated or controlled by protein molecules (Branden and Tooze, 1999). Different proteins are distinguished by a different order of amino acids in the polymeric sequence of typically 300 such building blocks. Following their synthesis in the cell, the majority of proteins must be converted into tightly folded compact structures in order to function. As many of these structures are astonishingly intricate, the fact that folding is usually extremely efficient is a remarkable testament to the power of evolutionary biology. Although proteins are the most abundant molecules in living systems other than water, the 100,000 or so different types of proteins within our bodies represent only a tiny fraction of all possible sequences. Indeed, as there are 20 different naturally occurring amino acids, the total possible number of different proteins with the average size of those in our bodies is astronomical, much greater than the number of atoms in the universe. Moreover, the properties of natural proteins are not typical of random sequences, but have been selected through evolutionary pressure to have specific characteristics—of which the ability to fold to unique structures and hence to generate enormous selectivity and diversity in their functions—is a particularly important one. As we shall see later, however, under

some conditions even natural proteins can revert to behavior that is typical of polymers that have not been subject to such careful evolutionary selection.

The interior of a cell is a highly crowded environment in which proteins and other macromolecules are present at a concentration that can exceed 300mg/ml (Ellis and Minton, 2003). Within the cells of all living organisms, however, there is an array of auxiliary factors that assist in the folding process, including folding catalysts and molecular chaperones (Hartl and Hayer-Hartl, 2002). These factors serve to enable polypeptide chains to fold efficiently in the complex milieu of the cell, but they do not determine their native structures; the latter are fully encoded by the amino acid sequences. How proteins find their unique native states from the information contained within their sequences is a question at the heart of molecular biology. Furthermore, the reproducibility and complexity of biological self-assembly compared to related processes in nonbiological systems is arguably the most remarkable feature of living systems. Understanding protein folding, perhaps the most fundamental example of biological self-assembly, is therefore a first step on the path to resolving one of the most important questions that can be addressed by modern science (Vendruscolo *et al.*, 2003).

### 3. The Universal Mechanism of Protein Folding

It is only recently that the mechanism by which even the simplest of proteins fold to specific structures has been defined in any detail. There is strong evidence that the native state of a protein corresponds, except in very rare circumstances, to the structure that is the most stable under physiological conditions. Nevertheless, the total number of possible conformations of a polypeptide chain is so large that it would take an enormous length of time to find this particular structure through a systematic search of all conformational space. Recent experimental and theoretical studies have, however, provided a resolution of this problem (Wolynes *et al.*, 1995; Karplus, 1997; Dill and Chan, 1997; Dobson *et al.*, 1998; Dobson, 2003). It is now clear that the folding of a small protein does not involve a series of mandatory steps between well-defined partially folded states, but rather a stochastic search of the many conformations accessible to a polypeptide chain. The conceptual basis of such a mechanism is shown in **Figure 2-1; see color insert**. In essence, the inherent fluctuations in the conformation of an incompletely folded polypeptide chain enable even residues at very different positions in the amino acid sequence to come into contact with one other. Because the correct (native-like) interactions between different residues are on average more stable than the incorrect (nonnative) ones, such a search mechanism is, in principle, able to find the lowest energy structure provided that no substantial barriers develop between different conformations (Wolynes *et al.*, 1995; Karplus, 1997; Dill and Chan, 1997; Dobson *et al.*, 1998; Dobson, 2003). It is evident that this process is extremely efficient for those special sequences that have been selected during evolution to fold to globular structures, and indeed, only a very small number of all possible conformations needs be sampled during the search process.

This stochastic description of protein folding involves the concept of an “energy landscape” for each protein, describing the free energy of the polypeptide chain as a function of its conformational properties. To enable a protein to fold efficiently, the landscape required has been likened to a funnel because the conformational space accessible to the polypeptide chain becomes more restricted as the native state is approached (Wolynes *et al.*, 1995; Dill and Chan, 1997). In essence, the high degree of disorder of the polypeptide chain is reduced as folding progresses because the more favorable enthalpy associated with stable native-like interactions can offset the decreasing entropy of the polypeptide chain as the structure becomes more ordered. The exact manner in which the correct overall fold can be achieved through such a process is emerging primarily from studies of a group

of small proteins—most having less than 100 residues—that fold to their native states without populating significantly any intermediate states (Jackson, 1998). A particularly important experimental strategy has been to use site-directed mutagenesis to probe the roles of individual residues in the folding process (Matouschek *et al.*, 1989; Fersht, 1999, 2000; Vendruscolo *et al.*, 2001). The results of a wide range of studies suggest that the fundamental mechanism of folding can be described as “nucleation-condensation,” in which a folding nucleus of a small number of residues forms, about which the remainder of the structure can then condense (Fersht, 2000).

Detailed insight into how such a generic mechanism can generate unique folds for specific sequences of amino acids has come from a combination of experimental and theoretical studies (Dobson and Karplus, 1999; Fersht and Daggett, 2002). The ultimate objective is to describe complete energy landscapes for folding reactions, and to understand exactly how these are defined by the sequences involved. Recently, experimental data have been incorporated directly in computer simulations of folding, and this approach has allowed structural ensembles representing a variety of important states populated during the folding of particular proteins to be defined in atomic detail as Figure 2-1 illustrates (Vendruscolo *et al.*, 2001; Korzhnev *et al.*, 2004; Vendruscolo and Dobson, 2005). Such studies suggest that a crucial aspect of the transition states of folding reactions is that they possess the same overall topology as the native fold (Debe *et al.*, 1999; Vendruscolo *et al.*, 2001; Dobson, 2003; Makarov and Plaxco, 2003; Lindorff-Larsen *et al.*, 2004). It appears that this topology can result from the acquisition of a native-like environment for a group of residues that constitute the core of the folding nucleus; in essence, these interactions force the chain to adopt a rudimentary native-like architecture (Vendruscolo *et al.*, 2001; Lindorff-Larsen *et al.*, 2004; Vendruscolo and Dobson, 2005). Once this topology has been achieved, the native structure is almost invariably generated when the remainder of the protein coalesces around this nucleus. Conversely, if these key interactions are not formed, the protein cannot fold to a stable globular structure. As all the protein molecules have to pass through the transition state region of the energy landscape prior to achieving their folded state, this mechanism therefore acts also as a “quality control” process by which misfolding can generally be avoided (Davis *et al.*, 2002). Although the details of the way different proteins fold may appear to differ dramatically, in terms of the rates of folding and the type of species populated during the folding process, the essential features of this overall mechanism can be considered to be universal. For example, for proteins that have regions of very high helical propensity, this type of structure may be substantially formed early in the folding process. The transition state may then appear to be as a rather diffuse structure in which a relatively large number of less completely formed interactions define the overall topology (Fersht, 2000; Paci *et al.*, 2004). The folding of such proteins may be well described by a specific mechanism such as “diffusion-collision” (Karplus and Weaver, 1994), but can still be viewed as a special case of the generic mechanism by which all proteins fold (Dobson *et al.*, 1998; Dobson, 2003).

Although the *in vitro* folding of small proteins appears to be predominantly two state, the folding of proteins having more than about 100 residues has been found to involve the significant population of a larger number of species than just the highly unfolded and fully folded states (Dobson *et al.*, 1998; Fersht, 1999). Experiments show that the resulting folding intermediates often correspond to species in which segments of the protein have become highly native-like, while others have yet to achieve a folded state. In other cases the protein may have formed a significant proportion of nonnative interactions, and hence, becomes trapped at least transiently in a misfolded state (Dobson *et al.*, 1998; Capaldi *et al.*, 2002). Indeed, it appears that larger proteins generally fold in modules, that is, that folding takes place largely independently in different segments or domains of the protein (Panchenko *et al.*, 1996; Dobson *et al.*, 1998). In such cases, key interactions are likely to define the fold within local regions or domains, and other specific interactions ensure that these initially folded regions subsequently interact appropriately to form the correct overall structure (Vendruscolo and

Dobson, 2005). The fully native structure is only acquired, however, when all the native-like interactions are formed both within and between the domains; this happens in a final cooperative folding step when all the side chains become locked in their unique close-packed arrangement and water is excluded from the protein core (Cheung *et al.*, 2002). Such a mechanism is appealing because it begins to explain that highly complex structures may be assembled in manageable pieces, each of which achieves its overall architecture by the mechanism described above for small proteins. Moreover, such a principle can readily be extended to describe the assembly of complexes containing a variety of different proteins, and in some cases other macromolecules, notably nucleic acids (Trieber and Williamson, 1999). Thus, even large molecular machines such as the ribosome or the proteasome can be assembled efficiently and with high fidelity.

#### 4. Protein Folding and Misfolding in the Cellular Environment

The folding of some proteins *in vivo* appears to be cotranslational, that is, it begins when the nascent chain is still being synthesized on the ribosome (Hardesty and Kramer, 2001). Electron microscopy and X-ray crystallography are now providing remarkably detailed structures of ribosomes in a variety of different states associated with protein synthesis (Yusopov *et al.*, 2001). Moreover, preliminary experiments have suggested that it might soon be possible to observe the conformational properties of polypeptide chains as they emerge during the synthetic process (Woolhead *et al.*, 2004; Gilbert *et al.*, 2004). Other proteins are thought to undergo the major part of their folding in the cytoplasm only after release from the ribosome, while yet others fold in specific compartments such as the endoplasmic reticulum (ER) following translocation through membranes (Jenni and Ban, 2003). Although many details of the folding of particular proteins will depend on the environment in which it takes place, the fundamental principles of folding derived from *in vitro* studies and discussed above are unlikely to be changed in any significant manner. But as incompletely folded chains expose regions of the polypeptide molecule that are buried in the native state, such species are prone to inappropriate contacts with other molecules within their local environment. There is evidence that in some cases rather extensive nonnative interactions may form transiently to bury highly aggregation prone regions such as exposed hydrophobic surfaces (Hore *et al.*, 1997; Capaldi *et al.*, 2002). But to cope with this problem more generally, living systems have evolved a range of elaborate strategies to prevent interactions with other molecules prior to the completion of the folding process (Hartl and Hayer-Hartl, 2002).

One of the most important of the mechanisms to protect against aggregation is the large number of molecular chaperones that are present in all types of cells and cellular compartments. Despite their similar general role in enabling efficient folding and assembly, their specific functions can differ substantially, and it is evident that many types of chaperone work in tandem with each other (Ellis and Hartl, 1999; Hartl and Hayer-Hartl, 2002). Some molecular chaperones have been found to interact with nascent chains as they emerge from the ribosome, and bind rather nonspecifically to protect aggregation-prone regions rich in hydrophobic residues. Others are involved in assisting at later stages of the folding or assembly process. The most intensively studied molecular chaperone is the bacterial “chaperonin” GroEL and its cochaperone GroES, and many of the details of the mechanism through which this system functions are now well understood (Ellis, 1996; Hartl and Hayer-Hartl, 2002). A remarkable aspect of GroEL is that it contains a cavity in which polypeptide chains can be sequestered during folding and protected from the external environment. In addition to molecular chaperones, there are several types of folding catalyst that accelerate steps in the folding process that might otherwise be extremely slow (Hartl and Hayer-Hartl, 2002). The most important are peptidylprolyl isomerases, that increase the rate of *cis/trans* isomerization of peptide bonds involving proline residues, and protein disulphide isomerases that enhance the rate of formation and reorganization of disulphide bonds within proteins (Balbach and Schmid, 2000; Schiene and Fisher, 2000).

Because of the complexity of folding, misfolding is an inherent feature of the folding process for all proteins, particularly under adverse conditions. Misfolding can broadly be defined as reaching a state that has a significant proportion of nonnative interactions between residues and whose properties differ significantly from those of a similar state having overwhelmingly native-like interactions. The cellular levels of many chaperones are, for example, substantially increased during cellular stress, as their frequent designation as heat-shock proteins (Hsps) indicates (Pelham, 1986). Some molecular chaperones act to capture misfolded proteins, or even some types of aggregates, and provide them with another opportunity to fold correctly (Shorter and Lindquist, 2004). Such active intervention requires energy, and adenosine 5'-triphosphate (ATP) is required for many of the molecular chaperones to function correctly (Ellis and Hartl, 1999). Despite the fact that many molecular chaperones are usually at very high levels only in stressed systems, it is clear that they have a critical role to play in all organisms even when present at lower levels under normal physiological conditions. Moreover, in eukaryotic systems, many proteins that are synthesized in a cell are destined for secretion to an extracellular environment. These proteins are translocated into the ER where folding takes place prior to secretion through the Golgi apparatus. The ER contains a wide range of molecular chaperones and folding catalysts to promote efficient folding, and in addition, the proteins involved are subject to stringent "quality control" prior to secretion **Figure 2-2; see color insert** (Sitia and Braakman, 2003). Unfolded and misfolded proteins detected in this way are then targeted for degradation through the ubiquitin-proteasome pathway (Ellis and Hartl, 1999; Kaufman, 2002). The importance of the quality control process is underlined by the fact that recent experiments indicate that perhaps a third of all polypeptide chains may fail to satisfy the quality control mechanism in the ER, and for some proteins the success rate is even lower (Schubert *et al.*, 2000). Like the "heat-shock response" in the cytoplasm, the "unfolded protein response" in the ER is also upregulated during stress and, as we shall see below, is strongly linked to the avoidance of misfolding diseases.

Because of the importance of proteins in all biological processes, it is not surprising that failure to fold correctly, or to remain correctly folded, will give rise to the malfunctioning of living systems and therefore to disease. Indeed, it is increasingly evident that a large group of human diseases can be directly associated with aberrations in the folding process (Table 2-1) (Thomas *et al.*, 1995;

**Table 2-1.** Representative protein folding diseases

Disease	Protein	Site of folding
Hypercholesterolaemia	low-density lipoprotein receptor	ER
Cystic fibrosis	cystic fibrosis trans-membrane regulator	ER
Phenylketonuria	phenylalanine hydroxylase	cytosol
Huntington's disease	huntingtin	cytosol
Marfan syndrome	fibrillin	ER
Osteogenesis imperfecta	procollagen	ER
Sickle cell anaemia	hemoglobin	cytosol
$\alpha$ 1-antitrypsin deficiency	$\alpha$ 1-antitrypsin	ER
Tay-Sachs disease	$\beta$ -hexosaminidase	ER
Scurvy	collagen	ER
Alzheimer's disease	$\beta$ -amyloid/presenilin	ER
Parkinson's disease	$\alpha$ -synuclein	cytosol
Scrapie/Creutzfeldt-Jakob disease	prion protein	ER
Familial amyloidosis	transthyretin/lysozyme	ER
Retinitis pigmentosa	rhodopsin	ER
Cataracts	crystallins	cytosol
Cancer	p53	cytosol

From Dobson, 2001.

Dobson, 2001). Some of these diseases (e.g., cystic fibrosis) result from the fact that if proteins do not fold correctly they will not be able to exercise their proper function. In other cases, misfolded proteins escape all the protective mechanisms discussed above and form intractable deposits either within cells or in extracellular space. An increasing number of pathological conditions, including Alzheimer's and Parkinson's diseases, the spongiform encephalopathies and type II diabetes, are known to be directly associated with the deposition of specific proteins in a range of organs and tissues (Tan and Pepys, 1994; Pepys, 1995; Thomas *et al.*, 1995; Koo *et al.*, 1999; Dobson, 2001; Selkoe, 2003). As we shall see later, not only does this process result in a loss of function, but may be associated with a "toxic gain of function" in that the proteinaceous aggregates or their precursors can in some cases induce cell damage and cell death. Diseases associated with protein misfolding and aggregation are among the most debilitating, socially disruptive, and costly in the modern world, and they are becoming increasingly prevalent as our lifestyles change and our populations age (Dobson, 2002).

## 5. The Generic Nature of Amyloid Formation

One of the most characteristic features of many of the misfolding diseases is that they often give rise to the deposition of proteins in the form of amyloid fibrils and plaques (Tan and Pepys, 1994; Pepys, 1995; Thomas *et al.*, 1995; Koo *et al.*, 1999; Dobson, 2001; Selkoe, 2003). Such deposits can form in the brain, in other vital organs such as the liver and spleen, or in skeletal tissue, depending on the particular disorder. In the case of neurodegenerative diseases associated with aggregation, the quantities of the deposits can be very small, while in systemic diseases they may involve kilograms of protein. Each amyloid disease involves the aggregation of a specific protein, (Table 2-2) although a range of other components including other proteins and carbohydrates is also found to be associated with the deposits when they form *in vivo*. The soluble forms of the 20 or so proteins involved in the well-defined amyloidoses vary substantially—they range from large globular proteins to small and apparently unstructured peptides—but the aggregated forms have many common characteristics (Sunde and Blake, 1997). Amyloid deposits show specific optical properties, notably birefringence, on binding certain dye molecules such as Congo red; these properties have been used in post mortem diagnosis for more than 100 years. The fibrillar structures that are characteristic of many of the aggregates have very similar morphologies; they can be seen in electron or atomic force microscopy images to be long, unbranched, and often twisted structures, a few nanometers in diameter. Moreover, samples in which the fibrils can be at least partially aligned show a characteristic "cross- $\beta$ " pattern in X-ray fibre diffraction experiments (Sunde and Blake, 1997). The latter indicates that the core structure of the fibrils is made up of  $\beta$ -sheets whose component strands run perpendicular to the fibril axis (**Figure 2-3; see color insert**) (Jiménez *et al.*, 2002). Fibrils having all the characteristics of *ex vivo* deposits can be reproduced *in vitro* from the component proteins under carefully chosen conditions, showing that they can self-assemble in the absence of any additional cellular components.

It was widely assumed until recently that the ability to form amyloid fibrils was limited to a relatively small number of proteins, largely those seen in disease states, and that these proteins must possess some specific sequence motifs encoding this apparently aberrant structure. Studies have now shown, however, that the ability of polypeptide chains to form such structures is common, and indeed may be considered a generic feature of polypeptide chains (Chiti *et al.*, 1999; Dobson, 1999). In particular, it has been shown that fibrils can be formed by many proteins that are not associated with disease once they are placed under conditions that destabilize the native structures, including such well-known proteins as myoglobin (Fändrich *et al.*, 2001; Stefani and Dobson, 2003), as Table 2-3 shows. The fact that specific sequences of amino acids are not essential for forming amyloid structures comes from the demonstration that homopolymers such as polythreonine or polylysine can also form

Table 2-2. Examples of diseases associated with amyloid deposition

Clinical syndrome	Fibril component	a: Organ limited b: Systemic
Alzheimer’s disease	Aβ peptide, 1–40, 1–42	a
Spongiform encephalopathies	full-length prion protein or fragments	a
Primary systemic amyloidosis	intact light chain or fragments	b
Secondary systemic amyloidosis	76-residue fragment of amyloid A protein	b
Familial amyloidotic poly neuropathy I	transthyretin variants and fragments	b
Senile systemic amyloidosis	wild-type transthyretin and fragments	b
Hereditary cerebral amyloid angiopathy	fragment of cystatin-C	a
Hemodialysis-related amyloidosis	β2-microglobulin	b
Familial amyloidotic polyneuropathy II	fragments of apolipoprotein A-I	b
Finnish hereditary amyloidosis	71-residue fragment of gelsolin	b
Type II diabetes	islet-associated polypeptide (IAPP)	a
Medullary carcinoma of the thyroid	calcitonin	a
Atrial amyloidosis	atrial natriuretic factor	a
Lysozyme amyloidosis	full-length lysozyme variants	b
Insulin-related amyloidosis	full-length insulin	b
Fibrinogen α-chain amyloidosis	fibrinogen α-chain variants	b

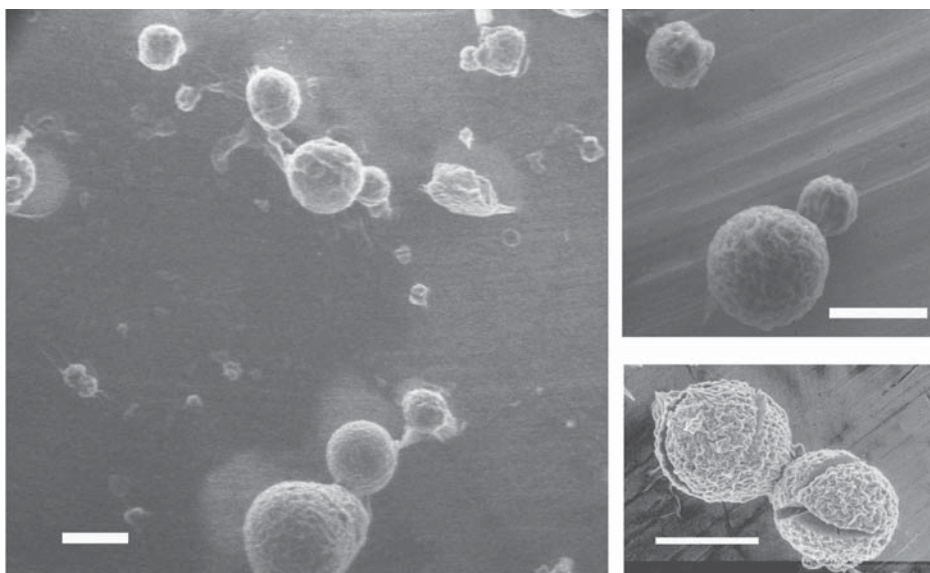
Adapted from Dobson, 2001, and Selkoe, 2003.

Table 2-3. Representative proteins unrelated to disease that form amyloid fibrils *in vitro*

SH3 domain p85 phosphatidylinositol-3-Kinase (bovine)
Fibronectin type III module (murine)
Acylphosphatase (equine)
Monellin ( <i>Dioscoreophyllum camminsii</i> )
Phosphoglycerate kinase (yeast)
Apolipoprotein CII (human)
ADA2H (human)
Met aminopeptidase ( <i>Pyrococcus furiosus</i> )
Apo-cytochrome c ( <i>Hydrogenobacter thermophilus</i> )
HypF N-terminal domain ( <i>Escherichia coli</i> )
Apomyoglobin (equine)
Amphoterin (human)
Curlin CgsA subunit ( <i>Escherichia coli</i> )
V1 domain (murine)
Fibroblast growth factor ( <i>Notophthalmus viridescens</i> )
Stefin B (human)
Endostatin (human)

Adapted from Stefani and Dobson, 2003.





**Figure 2-4.** Images of insulin spherulites recorded by ESEM (environmental scanning electron microscopy). The scale bar is 50  $\mu\text{m}$ . The picture at the bottom right illustrates some spherulites that have fractured. Optical experiments using crosspolarizers show that the structure involves a spherical lamellar array in which the amyloid fibrils are oriented tangentially to the lamellae (Krebs *et al.*, 2004).

very well-defined amyloid fibrils under appropriate conditions (Fändrich and Dobson, 2002). Moreover, fibrils of similar appearance to those containing large proteins can be formed by peptides with just a handful of amino acid residues (Lopez de al Puz *et al.*, 2002). The conditions under which amyloid structures form, rather than, for example, amorphous aggregates, vary with the characteristics of the sequence involved. A search for appropriate conditions, therefore, can involve a systematic screening exercise while varying parameters such as pH and temperature, in much the same way as a search is made for conditions under which the native states of proteins can be crystallized. One can consider that amyloid fibrils are the most highly organized structures adopted by unfolded polypeptide chains, and therefore will form most readily under conditions of relatively slow growth (Zurdo *et al.*, 2001). Indeed, the formation of amyloid fibrils can be considered to be the type of behavior that might be expected if polypeptide chains were to behave as simple polymer molecules. It is now evident, for example, that amyloid fibrils can form higher order assemblies such as spherulites (Figure 2-4), structures with diameters that are typically tens of microns and that have been known for synthetic polymers such as polyethylene for 50 years (Krebs *et al.*, 2004).

A great deal of effort has recently been directed at the determination of the detailed molecular structures of amyloid fibrils (Sunde and Blake, 1997; Jiménez *et al.*, 1999, 2002; Wille *et al.*, 1999; Serpell *et al.*, 2000; Petkova *et al.*, 2002; Jaronec *et al.*, 2004). It is clear that the core structure of the fibrils involves interactions, particularly hydrogen bonds, between polypeptide chains that are largely extended. As only the main chain is common to all polypeptides, the side chains must be packed within the fibrils in whatever way is most favorable for a given sequence. Although the side chains do not determine the core structure, they do affect the details of the fibrillar assembly such



as their exact dimensions and the readiness with which they form (Chamberlain *et al.*, 2000). Moreover, in some cases it appears as if only a small proportion of the total polypeptide chain is incorporated in the core sheet structure, with the remainder of the chain associated in some other manner with the fibrillar assembly. In other cases, particularly small peptides, the whole of the molecule may be arranged in the repetitive  $\beta$ -sheet structure (Jaroniec *et al.*, 2004). The generic amyloid structure contrasts strongly with the thousands of different structures that are adopted by native globular proteins (Branden and Tooze, 1999). In these structures the packing of the side chains must be the dominant factor, rather than the intrinsic preferences of the main chain that are revealed in structures such as amyloid fibrils (Dobson, 1999, Fändrich *et al.*, 2001, Fändrich and Dobson, 2002). The strands and helices so familiar in the structures of native proteins are then the most stable structures that the main chain can adopt in the folds that are primarily defined by interactions between the side chains. Native structures are normally, however, only marginally stable; minor changes in the solution conditions, such as pH and temperature, the structures can unfold and may then, at least under some circumstances, reassemble in the form of amyloid fibrils.

One of the primary features of the “generic model” of amyloid formation is that the ability to form fibrils is common but the relative propensities to form fibrils vary substantially with the polypeptide sequence (DuBay *et al.*, 2004). There is considerable evidence supporting this assumption. Indeed, the mutation of single amino acids in a 100-residue protein can change the rate at which aggregation occurs from in its denatured state by an order of magnitude or more (Chiti *et al.*, 2003). Furthermore, the change in aggregation rate caused by such mutations can be correlated with the predicted changes in properties such as charge, secondary structure propensity, and hydrophobicity (Chiti *et al.*, 2003). This correlation has been found to hold for a wide range of different sequences, a finding that strongly endorses the generic model of aggregation and amyloid formation. Analysis of the aggregation rates of different proteins can also be rationalized using similar ideas, showing that natural proteins vary in their aggregation potentials by factors of  $10^5$  or more (**Figure 2-5; see color insert**) (DuBay *et al.*, 2004). Of particular interest is that the group of proteins that have been found to be partially or completely unfolded even under physiological conditions, usually known as natively unfolded proteins, have sequences that are predicted to have very low aggregation propensities (DuBay *et al.*, 2004; Uversky and Fink, 2004). It is well established that aggregation, like crystallization or indeed protein folding, is a nucleated process (Harper and Lansbury, 1997). Interestingly, it has been suggested that the residues involved in nucleation of the folding of a globular protein could be distinct from those that nucleate its aggregation into amyloid fibrils (Chiti *et al.*, 2002). Such a situation could result from the different nature of the partially folded species that initiate the two processes, and would provide an opportunity for evolutionary pressure to select sequences that favor folding over aggregation.

The ability to understand some features of the aggregation process that results in amyloid fibrils has prompted investigation of the mechanism by which they are assembled from the precursor species. In the native states of globular proteins the polypeptide main chain is usually buried within the folded structure and, in addition, a variety of more subtle structural features have evolved to inhibit aggregation (Richardson and Richardson, 2002). Conditions that favor formation of amyloid fibrils from such proteins are those that stimulate at least partial unfolding, for example low pH or elevated temperature (Kelly, 1998; Chiti *et al.*, 1999). Because of the dominance of the globular fold in preventing aggregation, mutations that destabilize the native state are commonly involved in familial forms of amyloid disease (Harper and Lansbury, 1997; Chiti *et al.*, 2003). Fragmentation of proteins, through proteolysis or other means, is another mechanism of promoting amyloid formation. Thus, some amyloid disorders, including Alzheimer's disease, result from the aggregation of fragments of larger precursor proteins that are unable to fold in the absence of the remainder of the protein structure (see Table 2-2). Because of the requirement for nucleation of aggregation, experiments *in vitro* indicate that the

formation of fibrils, by appropriately destabilized or fragmented proteins, is generally characterized by a lag phase, followed by a period of rapid growth. By analogy with crystallization, such a lag phase can be eliminated by addition of pre-formed fibrils to fresh solutions, a process known as seeding (Harper and Lansbury, 1997). Although the details of the events taken place during fibril growth are not yet known in detail, the overall kinetic profiles can often be simulated by using relatively simple models that incorporate well-established principles of nucleated processes (Padrick and Miranker, 2002; Chien *et al.*, 2004).

It appears from studies carried out so far that there are many common features in the mechanism of formation of amyloid fibrils by different peptides and proteins (**Figure 2-5; see color insert**) (Harper and Lansbury, 1997; Koo *et al.*, 1999; Nettleton *et al.*, 2000, Caughey and Lansbury, 2003). The first phase of the aggregation process involves the formation of oligomeric species as a result of relatively nonspecific interactions, although in some cases specific structural transitions such as domain swapping (Schlunegger *et al.*, 1997; Rousseau *et al.*, 2004) may be involved if such processes increase the rate of aggregation. At least the smaller oligomers are likely to be relatively soluble, and the earliest species visible by electron or atomic force microscopy as aggregation occurs generally resemble small bead-like structures, sometimes linked to one another, often described as amorphous aggregates or as micelles. These early “prefibrillar aggregates” then appear to transform into species with more distinctive morphologies, sometimes described as “protofibrils” or “protofilaments” (Jiménez *et al.*, 2002; Bitan *et al.*, 2003; Caughey and Lansbury, 2003). These latter structures are commonly short, thin, sometimes curly, fibrillar species that are thought, in some cases at least, to self-assemble into mature fibrils, perhaps by lateral association accompanied by some degree of structural reorganization as indicated in **Figure 2-6; see color insert** (Bouchard *et al.*, 2000; Plakoutsi *et al.*, 2004). The extent to which dissolution and reassembly of monomeric species is involved at the different stages of amyloid assembly is not clear, but such processes could well be important under the conditions of slow growth that frequently generate fibrils with the most regular appearance. The earliest aggregates are likely to be relatively disorganized structures that expose on their surfaces a variety of segments of the protein that are normally buried in the globular state. In other cases these early aggregates appear to be quite distinctive structures, including well-defined “doughnut”-shaped species seen for a number of systems (Lashuel *et al.*, 2002; Malisauskas *et al.*, 2003; Chien *et al.*, 2004). A particularly exciting development that promises to stimulate significant progress in studies of the mechanism of amyloid formation is the recent ability to use single molecule optical techniques to follow fibril growth in real time (Ban *et al.*, 2003).

## 6. Common Features of Protein Self-Assembly

Under particular conditions, a protein can adopt one or a number of more or less distinct states that can be represented schematically, as shown in **Figure 2-7; see color insert** (Dobson, 2003). The populations of the different states under a given set of conditions will depend on their relative thermodynamic stabilities and the rates at which they interconvert. Amyloid fibrils are just one of the types of aggregates that can be formed by a protein. They have particular significance, however, because their highly organized hydrogen-bonded structure is likely to give greater kinetic stability than more amorphous aggregates. This diagram emphasizes the importance for biological systems of controlling and regulating the various states accessible to a given polypeptide chain at given times and under given conditions. Indeed, such regulation is at least as important as the regulation and control of the various chemical transformations that take place in the cell (Dobson, 2003). The latter is achieved primarily through enzymes, and the former by means of the molecular chaperones and

the mechanisms for protein degradation, mentioned above. In a similar way that the aberrant behavior of enzymes can cause metabolic disease, the aberrant behavior of the chaperone and other machinery regulating polypeptide conformations can contribute to misfolding and aggregation diseases (Macario and De Macario, 2002).

The details of specific diseases associated with protein misfolding will be discussed in detail in later articles in this volume. The concepts represented in **Figure 2-7; see color insert**, however, serve as a general framework for understanding the molecular events that underlie such diseases, and indeed the principles that can be used to intervene for therapeutic purposes (Dobson, 2004a). As we have discussed above, it is partially or completely unfolded polypeptides that are particularly highly aggregation-prone. Such species will inevitably be generated during folding, and a variety of molecular chaperones is present in abundance in the cellular compartments wherever such processes occur. It is interesting in this regard that the majority of the deposits associated with aggregation diseases are extracellular; indeed, all the classical amyloidoses involve such aggregates, but in disorders such as Parkinson's and Huntington's diseases similar aggregates are found but here they are located within cells (Selkoe, 2003). It is therefore particularly important that proteins are correctly folded prior to their secretion from the cell; hence, the need for a highly effective system of quality control in the ER. Recently, however, extracellular chaperones have been identified, and these are likely to be of particular interest in the context of amyloid diseases (Wilson and Easterbrook-Smith, 2000). There is, of course, a continuous process of protein degradation in a normally functioning organism to eliminate misfolded as well as redundant proteins. During such processes, which require unfolding and proteolysis of polypeptide chains, aggregation may be a particularly danger. Degradation pathways, such as those of the ubiquitin–proteasome system, are therefore highly regulated to avoid such events (Bence *et al.*, 2001; Sherman and Goldberg, 2001).

In recent years our understanding of the detailed mechanism of all the processes associated with the complete life spans of proteins—from their synthesis to their degradation—has advanced dramatically through progress in both cellular and structural biology. High-resolution structures of ribosomes, proteasomes, molecular chaperones, and other complexes are revealing the details of how these complex molecular machines operate and are regulated. Structural techniques are also providing an increasing amount of information about the various states of the proteins that are processed by these assemblies. This task is particularly challenging as the majority of these species (such as unfolded and partially folded states and the less organized types of aggregates) are ensembles of more or less highly disordered structures, and in addition, may have only a transient existence. Major advances in the structural analysis of nonnative states of proteins are, however, now being made, particularly through the use of new approaches involving NMR spectroscopy (Petkova *et al.*, 2002; Vendruscolo and Dobson, 2003; Jaroniec *et al.*, 2004; Korzhnev *et al.*, 2004). It is therefore becoming possible to identify and characterize the various species that are represented in **Figure 2-7; see color insert** for specific proteins, and hence, to begin to understand the factors determining their behavior in different contexts (Dobson, 2003, 2004a).

To understand misfolding and aggregation diseases we need to know not just how such systems function under normal conditions, but also why they fail to function under other circumstances (Zurdo *et al.*, 2001; Horwich, 2002). The effects of many pathogenic mutations can be particularly well understood from the schematic representation given in **Figure 2-6; see color insert**. Many of the mutations associated with the familial deposition diseases increase the populations of partially unfolded states by decreasing the stability or cooperativity of the native state (Booth *et al.*, 1997; Ramirez-Alvarado *et al.*, 2000; Dumoulin *et al.*, 2003). Cooperativity is perhaps one of the most important characteristics of globular proteins that enables them to resist aggregation (Dobson and Karplus, 1999; Dobson, 1999, 2003). It ensures that even for proteins that are only marginally stable

in their native states, as the vast majority are, the population of unfolded molecules, or of unfolded regions of the polypeptide chain under physiological conditions, is minimal. Other familial amyloid diseases are associated with the accumulation of fragments of native proteins, produced by normal or aberrant processing or incomplete degradation; such species are unable to fold into aggregation-resistant states. Other pathogenic mutations act by enhancing the propensities of such species, or of natively unfolded proteins, to aggregate, for example by increasing their hydrophobicity or decreasing their charge (Chiti *et al.*, 2003). In the case of the transmissible encephalopathies, it is likely that ingestion of preaggregated states of an identical protein (e.g., through consumption of tissue from the same species or through contamination of surgical instruments or even perhaps by blood transfusion) increases dramatically the inherent rate of aggregation, and hence, underlies the mechanism of transmission (Harper and Lansbury, 1997; Prusiner, 1997; Chien *et al.*, 2003). Indeed, it has been shown recently that prion diseases can be induced in laboratory animals by injecting amyloid aggregates produced from purified recombinant prion protein, that no suggesting factors are necessary to generate disease (Legname *et al.*, 2004).

## 7. Generic Aspects of Misfolding Diseases

An extremely important question to be answered in the context of all the misfolding diseases is exactly how protein aggregation generates the clinical manifestations of the various conditions. In the case of systemic amyloid disease, the accumulation of large quantities of insoluble protein aggregates may itself disrupt the functioning of the organs in which they are located (Pepys, 1995). In other cases it may be that the loss of functional protein results in the failure of some crucial cellular process (Caughey and Lansbury, 2003). But for neurodegenerative disorders, such as Alzheimer's disease, it appears likely that the primary symptoms result from the destruction of neurons by mechanisms associated with the aggregation process (Koo *et al.*, 1999; Horwich, 2002; Caughey and Lansbury, 2003; Selkoe, 2003; Stefani and Dobson, 2003). It has recently been found that the early prefibrillar aggregates of proteins associated with neurological diseases can be highly damaging to cells; by contrast, the mature fibrils are often relatively benign (Walsh *et al.*, 2002; Selkoe, 2003). Investigation of the effects of aggregates on cells in culture indicates, however, that the toxic nature of protein aggregates is not restricted to species formed from the peptides and proteins associated with pathological conditions. Indeed, experiments have recently indicated that prefibrillar aggregates of proteins that are not connected with any known diseases can be as toxic to cells as those of the A $\beta$  peptides (Bucciantini *et al.*, 2002).

It appears that the precursor aggregates of amyloid fibrils can readily pass through cell membranes and trigger a series of biochemical events that leads to cell death by apoptosis or necrosis (Bucciantini *et al.*, 2004). It may well be the case that small aggregates are toxic because they contain highly disordered polypeptide chains that display a large variety of amino acids, including those with hydrophobic side chains, on their surfaces resulting in aberrant interactions with a variety of cellular components including membranes (Polverino *et al.*, 2003; Stefani and Dobson, 2003; Bucciantini *et al.*, 2004). The generic nature of such aggregates and their effects on cells has recently been reinforced through experiments with polyclonal antibodies raised against small aggregates of A $\beta$  peptides. These antibodies have been found to crossreact with early aggregates of different peptides and proteins and, moreover, to inhibit their toxicity in cell assays (Kayed *et al.*, 2003). That antibodies are able to recognize the common features of the early aggregates of different polypeptides may at first sight be surprising, particularly as these antibodies recognize neither the monomeric proteins nor the mature fibrils (Dumoulin and Dobson, 2005). But, as we have noted earlier, molecular chaperones appear to be able to recognize common features of misfolded proteins, such as segments of high hydrophobicity

(Hartl and Hayer-Hartl, 2002). Moreover, antibodies have been found that recognize mature fibrils formed by a range of different proteins (O'Nuallian and Wetzel, 2002). Moreover, *in vivo*, most amyloid deposits are associated with serum amyloid protein (SAP), a protein that is thought to act to inhibit the clearance of these deposits within the body; indeed, SAP labeled with radioactive isotopes is used to image amyloid deposits for clinical purposes (Pepys *et al.*, 2002).

Such findings raise the question as to how cellular systems are normally able to deal with the intrinsic tendency of incompletely folded proteins to aggregate. The answer is likely to be that the molecular chaperones and other "housekeeping" mechanism are remarkably efficient in ensuring that such potentially toxic species are destroyed or otherwise rendered harmless under normal circumstances (Bucciantini *et al.*, 2002; Hartl and Hayer-Hartl, 2002). Molecular chaperones of various types are able to shield hydrophobic regions of unfolded proteins, whether monomeric or present as small oligomers, to solubilize some forms of aggregates, or to alter the partitioning between different forms of aggregates (Hartl and Hayer-Hartl, 2002). The latter mechanism, for example, could, in addition to suppressing toxicity, prevent the precursors of amyloid fibrils converting into intractable species, thereby allowing them be refolded or disposed of by the cellular degradation systems. Indeed, evidence has been obtained that such a situation occurs with polyglutamine sequences associated with Huntington's disease where the precursor species appear to be diverted into amorphous aggregates by the combined action of two molecular chaperones, Hsp70 and Hsp40 (Muchowski *et al.*, 2000). If all such protective processes fail, it may still be possible for potentially harmful species to be sequestered in relatively harmless forms such as inclusion bodies in bacteria or aggresomes in eukaryotic systems. Indeed, it has been suggested that the formation of mature amyloid fibrils and plaques, whose toxicity appears to be much lower than that of their precursors as we have discussed above, may itself represent a protective mechanism under some circumstances (Koo *et al.*, 1999; Caughey and Lansbury, 2003; Stefani and Dobson, 2003).

Most of the aggregation diseases are, however, not associated with specific genetic mutations or infectious agents but with old age. The ideas summarized in this article offer a qualitative explanation of why this could be the case. We have seen that all proteins have an inherent tendency to aggregate unless they are maintained in a highly regulated and controlled environment. There is evidence from a variety of studies of protein sequences that selective pressure during evolution has resulted in avoidance of patterns of amino acid residues that are known to promote  $\beta$ -sheet formation and aggregation (Broome and Hecht, 2000). But just as random mutations tend to decrease protein stability, they are likely to enhance aggregation propensities, with the results that sequences evolve to be sufficiently stable or aggregation-resistant for their biological functions, but not to have such characteristics optimized further. We can see, therefore, that our recent ability to prolong life for a high proportion of the human population well beyond the normal reproductive age, that is, beyond the time that evolutionary pressure is significant, could itself lead to the proliferation of these diseases (Dobson, 1999, 2002). In old age it is likely that the probability of misfolded or aggregated species is increased and that the efficacy of the protective mechanisms is reduced (Csermely, 2001; Dobson, 2002; Macario and de Macario, 2002). It is intriguing, however, to speculate, that favorable mutations in aggregation-prone proteins might be the reason that some people do not readily succumb even in extreme old age to disorders such as Alzheimer's disease (Chiti *et al.*, 2003).

In addition to extended life spans, many other risk factors associated with sporadic forms of amyloid diseases are also associated with relatively recent changes of the life styles associated with human societies (Dobson, 2002). The transmissible prion diseases such as scrapie and bovine spongiform encephalopathy (BSE) are associated with intensive farming where the population density of animals is higher than in the wild, and particularly with modern practices of feeding animals with protein rendered from old animals of the same species. Chronic wasting disease, affecting large proportions of some of the deer and elk populations of North America, appears to be a very recent

phenomenon, perhaps associated with increasing numbers in the wild or the practice of deer farming (Miller and Williams, 2003). Similarly, the present dramatic increases in type II diabetes, estimated to affect up to 15% of the populations of some countries, is associated with obesity due to changing diets and reduced exercise (Höppener *et al.*, 2002). Some modern medical procedures have also been found to result in transmission of prion diseases, for example, Creutzfeldt-Jakob disease (CJD) cases have been shown to arise from treatment with contaminated growth hormone extracted from cadavers (Prusiner, 1997), or the amyloid deposition associated with hemodialysis, where the concentration of  $\beta$ -2 microglobulin in serum increases as the result of its failure to be cleared by the kidneys (Gejyo *et al.*, 1986). Indeed, it seems probable that in many countries of the world, health problems associated with amyloid disorders will soon be more costly, in both economic and social terms, than either heart disease or cancer.

## 8. Common Strategies for Therapeutic Intervention

Many of the amyloid diseases have no effective treatment at the present time, and those therapies that do exist can require radical intervention, for example, transplantation of organs such as the liver in some of the systemic amyloidoses (Stangou *et al.*, 1999). Some promising potential pharmaceuticals for specific amyloid diseases have arisen through serendipity or large-scale screening, but a better understanding of the underlying origins of amyloid disorders and their relatives gives hope that more rational approaches may soon bear fruit (Cohen and Kelly, 2003; Dobson, 2004a). The number of distinct steps in the process of converting a normally soluble polypeptide chain into toxic species or intractable deposits suggests that there is a variety of targets for possible therapeutic intervention. An important development in this regard is improved methodology for detecting amyloid deposits within living systems. Although the large-scale deposits of proteins associated with systemic diseases can be imaged, as described above, by use of radiolabeled SAP, the detection of aggregates involved in neurodegenerative conditions has proven to be particularly challenging. There have, however, been recent developments that show great promise, particularly involving analogues of the dye molecules that are widely used to monitor the formation of amyloid structures *in vitro* or their presence in tissue sections taken from patients during post mortem examination. By appropriate isotopic labeling of such compounds it has been possible to detect amyloid deposits in living subjects using positron emission tomography (Klunk *et al.*, 2004). Such developments should enable the efficacy of potential drugs to be monitored much more rapidly and quantitatively than in the past, at least if the drugs are designed to inhibit the aggregation process that leads directly to amyloid deposition.

The diagram shown in **Figure 2-8; see color insert** shows schematically the specific steps at which intervention could be envisaged on the basis of the ideas discussed earlier in this article (Dobson, 2004a). Indeed, it is reassuring that almost all of the current strategies for preventing or treating the amyloid disorders can be rationalized on such a picture. Step A, for example, involves the stabilization of the native state of an amyloidogenic protein to reduce its ability to expose aggregation-prone regions of the polypeptide chain to the outside world. Examples of this strategy include the use of analogues of the hormone thyroxine, the natural ligand for transthyretin, the protein associated with particularly prevalent types of amyloidoses (Hammarstrom *et al.*, 2003). Such ligands have been found to reduce the rate at which disease-associated mutational variants of this protein aggregate *in vitro*. Similarly, specific antibodies raised against wild-type lysozyme prevent the *in vitro* formation of amyloid fibrils by pathogenic forms of this protein (Dumoulin *et al.*, 2003; Dumoulin and Dobson, 2005). Of particular interest is quinacrine, an early antimalarial drug that was found to inhibit the replication of the pathogenic forms of the prion protein in cell cultures during screening



trials of approved pharmaceuticals known to cross the blood–brain barrier (May *et al.*, 2003). There is evidence that quinacrine acts to stabilize the soluble form of the prion protein, and clinical trials have been initiated to investigate its potential in treating CJD (Cohen and Kelly, 2003).

In the case of amyloid diseases that are associated with peptide fragments of larger proteins (see Table 2-3), a possible therapeutic approach is to reduce the levels of the aggregation-prone species by inhibiting the enzymes that generate them from their precursor protein, that is, by intervening at Step 2 of the schematic picture in **Figure 2-8; see color insert**. This approach has been investigated in particular for Alzheimer's disease where the 40 or 42 residue A $\beta$  peptide forms the pathogenic aggregates. The strategy here is the development of inhibitors of the secretase enzymes that cleave the A $\beta$  peptide from the membrane associated amyloid precursor protein (APP), and a number of promising candidates have been identified (Wolfe, 2002). Another approach in the future could be to use techniques such as gene therapy or stem cells to replace polypeptides whose aggregation is the origin of disease by mutational variants with lower aggregation propensities (Step C in **Figure 2-8; see color insert**). As we discussed above, studies of the kinetics of aggregate formation by a series of different peptides and proteins enables the prediction of mutations that are expected to decrease the rate of amyloid formation (Chiti *et al.*, 2003). This approach can be considered as a molecular analogue of liver transplantation for those familial amyloidoses where the therapeutic benefit arises because the mutant proteins associated with disease are primarily produced by this organ (Stangou *et al.*, 1999).

Another strategy to reduce the levels of aggregation-prone species in an organism is to increase the rate at which they are cleared (Step D in **Figure 2-8; see color insert**). One method of achieving this objective could be to generate antibodies to the polypeptides involved in the aggregation process. One particularly exciting approach has involved immunization with A $\beta$  peptides to treat Alzheimer's disease (Schenk, 2002). Immunization has been shown to result in extensive clearance of amyloid deposits in a mouse model of the disease, and indeed, there is some evidence that similar effects occurred in human patients in initial clinical trials. Although these trials were terminated because of an inflammatory response in some of the patients, the results provide optimism that this therapeutic strategy has real potential either with improved vaccines or through passive immunotherapy involving infusion into patients of antibodies generated by external means. Another approach designed to induce enhanced clearance of amyloid deposits has involved the design of inhibitors of SAP, the protein discussed earlier in the context of the protection that it affords to amyloid deposits in the body (Pepys *et al.*, 2002). The more general principle of using rationally designed small molecules to target specific proteins for degradation is an exciting prospect.

An alternative strategy, represented as Step E in **Figure 2-8; see color insert**, involves prevention of the growth of fibrils by their amyloidogenic precursors rather than trying to decrease the levels of the latter within the body. A variety of peptides and peptide-analogues, often designated as  $\beta$ -breakers, have been designed to bind tightly to the ends of growing fibrils and to inhibit further growth. Again, the major target of such strategies has been Alzheimer's disease, and *in vitro* studies look very promising (Soto, 2003). Such species will need to be designed carefully so as not to generate increased concentrations of the oligomers and other fibril precursors that, as discussed above, appear to be the most damaging species in at least some of the neurodegenerative diseases. Last, but by no means least, is the possibility that the generic character of amyloid structures and the mechanism by which they are formed is sufficient that a strategy to address one of these diseases might be effective in preventing or treating others. At the very least one can be optimistic that the overall approach to developing effective therapeutics will be applicable to more than one of these diseases. More speculatively, it is at least possible that the same compounds could be successful in reducing the probability of becoming afflicted with any of the amyloid diseases. Compelling evidence that such a universal strategy based on the generic character of amyloid formation might be viable at least



in principle comes from the finding the antibodies raised against small aggregates of the A $\beta$  peptides to recognize similar aggregates of other proteins (Kayed *et al.*, 2003).

## 9. Concluding Remarks

Proteins have evolved to fold efficiently and usually to remain correctly folded and soluble, despite the inherent tendency of polypeptide chains to aggregate. This remarkable achievement is undoubtedly a result of the natural selection of sequences and the coevolution of the environments in which they function. It is clear that as we develop further our knowledge of the mechanism of protein folding, and of the way that it is enhanced and regulated within the cellular environment, we shall be able to answer with increasing conviction the more general question of how evolution has enabled even the most complex biological systems to self-assemble with astonishing fidelity. Such knowledge will represent a very significant step towards understanding at a molecular level one of the most fascinating and fundamental characteristics of life itself (Vendruscolo *et al.*, 2003). Over the past century, however, we have begun to see the limitations of evolution in the context of the rapidity with which our environments and lifestyles are changing. Under such conditions one can see the inability of proteins to remain in their evolved and functional structures and their progressive conversion into the generic structures that reflect their fundamental polymeric nature. The mechanisms that have evolved to recognize, and render harmless, misfolded and aggregation-prone species are astonishingly efficient during the life spans of most individuals until the onset of old age (Dobson, 1999; Stefani and Dobson, 2003). This finding, along with evidence of common features in the manner that proteins convert into pathogenic species, suggest that it might be possible to enhance our natural defenses against aggregation in a variety of rational ways. If this possibility can be brought to fruition, it will provide an opportunity of improving significantly the quality of life in our aging societies, not only by preventing the suffering of those afflicted with these debilitating and disturbing disorders but also by reducing the burden of care that inevitably falls on the remainder of the population.

## 10. Abbreviations

A $\beta$	Amyloid $\beta$ peptides
APP	Amyloid precursor protein
ATP	Adenosine 5'-triphosphate
BSE	Bovine spongiform encephalopathy
CJD	Creutzfeldt-Jakob disease
ER	Endoplasmic reticulum
Hsps	Heat-shock proteins
SAP	Serum amyloid protein

## Acknowledgments

The ideas in this article have emerged from extensive discussions with many students, research fellows, and colleagues over some 20 years. I am most grateful to all of them; they are too numerous to mention here, but the names of many appear in the list of references. This article is a substantially revised version of earlier reviews (Dobson, 2003, 2004a, 2004b). The research of CMD discussed in this article has been supported by Programme Grants from the Wellcome Trust and the Leverhulme Trust, and by the BBSRC, EPSRC, and MRC.

## References

- Balbach, J., and Schmid, F.X. (2000). Proline isomerization and its catalysis in protein folding. In: Pain, R.H. (Ed.), *Protein folding*, 2nd ed. Oxford: Oxford University Press, pp. 212–249.
- Ban, T., Hamada, D., Hasegawa, K., Naiki, H., and Goto, Y. (2003). Direct observation of amyloid fibril growth monitored by thioflavin T fluorescence. *J. Biol. Chem.* 278:16462–16465.
- Bence, N.F., Sampat, R.M., and Kopito, R.R. (2001). Impairment of the ubiquitin–proteasome system by protein aggregation. *Science* 292:1552–1555.
- Bitan, G., Kirkitadze, M.D., Lomakin, A., Vollers, S.S., Benedek, G.B., and Teplow, D.B. (2003). Amyloid beta-protein (A $\beta$ ) assembly: A $\beta$ 40 and A $\beta$ 42 oligomerize through distinct pathways. *Proc. Natl. Acad. Sci. USA* 100: 330–335.
- Booth, D.R., Sunde, M., Bellotti, V., Robinson, C.V., Hutchinson, W.L., Fraser, P.E., Hawkins, P.N., Dobson, C.M., Radford, S.E., Blake, C.C.F., and Pepys, M.B. (1997). Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. *Nature* 385:787–793.
- Bouchard, M., Zurdo, J., Nettleton, E.J., Dobson, C.M., and Robinson, C.V. (2000). Formation of insulin amyloid fibrils followed by FTIR simultaneously with CD and electron microscopy. *Protein Sci.* 9:1960–1967.
- Branden, C., and Tooze, J. (1999). *Introduction to protein structure*. 2nd ed. New York: Garland Publishing.
- Broome, B.M., and Hecht, M.H. (2000). Nature disfavors sequences of alternating polar and non-polar amino acids: implications for amyloidogenesis. *J. Mol. Biol.* 296:961–968.
- Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C.M., and Stefani, M. (2002). Inherent cytotoxicity of aggregates implies a common origin for protein misfolding diseases. *Nature* 416:507–511.
- Bucciantini, M., Calloni, G., Chiti, F., Formigli, L., Nosi, D., Dobson, C.M., and Stefani, M. (2004). Pre-fibrillar amyloid protein aggregates share common features of cytotoxicity. *J. Biol. Chem.* 279:31374–31382.
- Capaldi, A.P., Kleanthous, C., and Radford, S.E. (2002). Im7 folding mechanism: misfolding on a path to the native state. *Nat. Struct. Biol.* 9:209–216.
- Caughey, B., and Lansbury, P.T., Jr. (2003). Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu. Rev. Neurosci.* 26:267–298.
- Chamberlain, A.K., MacPhee, C.E., Zurdo, J., Morozova-Roche, L.A., Hill, H.A., Dobson, C.M., and Davis, J.J. (2000). Ultrastructural organization of amyloid fibrils by atomic force microscopy. *Biophys. J.* 79:3282–3293.
- Cheung, M.S., Garcia, A.E., and Onuchic, J.N. (2002). Protein folding mediated by solvation: water expulsion and formation of the hydrophobic core occur after the structural collapse. *Proc. Natl. Acad. Sci. USA* 99:685–690.
- Chien, P., DePace, A.H., Collins, S.R., and Weissman, J.S. (2003). Generation of prion transmission barriers by mutational control of amyloid conformations. *Nature* 424:948–951.
- Chien, P., Weismann, J.S., and DePace, A.H. (2004). Emerging principles of conformation-based prion inheritance. *Annu. Rev. Biochem.* 73:617–656.
- Chiti, F., Webster, P., Taddei, N., Clark, A., Stefani, M., Ramponi, G., and Dobson, C.M. (1999). Designing conditions for *in vitro* formation of amyloid protofilaments and fibrils. *Proc. Natl. Acad. Sci. USA* 96:3590–3594.
- Chiti, F., Taddei, N., Baroni, F., Capanni, C., Stefani, M., Ramponi, G., and Dobson, C.M. (2002). Kinetic partitioning of protein folding and aggregation. *Nat. Struct. Biol.* 9:137–143.
- Chiti, F., Stefani, M., Taddei, N., Ramponi, G., and Dobson, C.M. (2003). Rationalisation of mutational effects on peptide and protein aggregation rates. *Nature* 424:805–808.
- Cohen, F.E., and Kelly, J.W. (2003). Therapeutic approaches to protein-misfolding diseases. *Nature* 426:905–909.
- Csermely, P. (2001). Chaperone overload is a possible contributor to “civilization diseases.” *Trends Gen.* 17:701–704.
- Davis, R., Dobson, C.M., and Vendruscolo, M. (2002). Determination of the structures of distinct transition state ensembles for a  $\beta$ -sheet peptide with parallel folding pathways. *J. Chem. Phys.* 117:9510–9517.
- Debe, D.A., Carlson, M.J., and Goddard, W.A., III. (1999). The topomer-sampling model of protein folding. *Proc. Natl. Acad. Sci. USA* 96:2596–2601.
- Dill, K.A., and Chan, H.S. (1997). From Levinthal to pathways to funnels. *Nat. Struct. Biol.* 4:10–19.
- Dobson, C.M. (1999). Protein misfolding, evolution and disease. *Trends Biochem. Sci.* 24:329–332.
- Dobson, C.M. (2001). The structural basis of protein folding and its links with human disease. *Philos. Trans. R. Soc. Lond. B* 356:133–145.
- Dobson, C.M. (2002). Getting out of shape—protein misfolding diseases. *Nature* 418:729–730.
- Dobson, C.M. (2003). Protein folding and misfolding. *Nature* 426:884–890.

- Dobson, C.M. (2004a). In the footsteps of alchemists. *Science* 295:1719–1722.
- Dobson, C.M. (2004b). Principles of protein folding, misfolding and aggregation. *Semin. Cell Dev. Biol.* 15:3–16.
- Dobson, C.M., and Karplus, M. (1999). The fundamentals of protein folding: bringing together theory and experiment. *Curr. Opin. Struct. Biol.* 9:92–101.
- Dobson, C.M., Sali, A., and Karplus, M. (1998). Protein folding: a perspective from theory and experiment. *Angew. Chem. Int. Ed. Eng.* 37:868–893.
- DuBay, K.F., Pawar, A.P., Chiti, F., Zurdo, J., Dobson, C.M., and Vendruscolo, M. (2004). Prediction of the absolute aggregation rates of amyloidogenic polypeptide chains. *J. Mol. Biol.* 341:1317–1326.
- Dumoulin, M., and Dobson, C.M. (2005). Probing the origins, diagnosis and treatment of amyloid disease using antibodies. *Biochimie.* 86:589–600.
- Dumoulin, M., Last, A.M., Desmyter, A., Decanniere, K., Canet, D., Spencer, A., Archer, D.B., Muyldermans, S., Wyns, L., Matagne, A., Redfield, C., Robinson, C.V., and Dobson, C.M. (2003). A camelid antibody fragment inhibits amyloid fibril formation by human lysozyme. *Nature* 424:783–788.
- Ellis, R.J. (Ed.). (1996). *The chaperonins*. San Diego, CA: Academic Press.
- Ellis, R.J., and Hartl, F.U. (1999). Principles of protein folding in the cellular environment. *Curr. Opin. Struct. Biol.* 9:102–110.
- Ellis, R.J., and Minton, A.P. (2003). Join the crowd. *Nature* 425:27–28.
- Fändrich, M., and Dobson, C.M. (2002). The behaviour of polyamino acids reveals an inverse side-chain effect in amyloid structure formation. *EMBO J.* 21:5682–5690.
- Fändrich, M., Fletcher, M.A., and Dobson, C.M. (2001). Amyloid fibrils from muscle myoglobin. *Nature* 410:165–166.
- Fersht, A.R. (1999). *Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding*. New York: W.H. Freeman.
- Fersht, A.R. (2000). Transition-state structure as a unifying basis in protein-folding mechanisms: contact order, chain topology, stability, and the extended nucleus mechanism. *Proc. Natl. Acad. Sci. USA* 97:1525–1529.
- Fersht, A.R. and Daggett, V. (2002). Protein folding and unfolding at atomic resolution. *Cell* 108:573–582.
- Gejyo, F., Homma, N., Suzuki, Y., and Arakawa, M. (1986). Serum levels of  $\beta$ 2-microglobulin as a new form of amyloid protein in patients undergoing long-term hemodialysis. *N. Engl. J. Med.* 314:585–586.
- Gilbert, R.J.C., Fucini, P., Connell, S., Fuller, S.D., Nierhaus, K.H., Robinson, C.V., Dobson, C.M., and Stuart, D.I. (2004). Three-dimensional structures of translating ribosomes by cryo-EM. *Mol. Cell.* 14:57–66.
- Hammarstrom, P., Wiseman, R.L., Powers, E.T., and Kelly, J.W. (2003). Prevention of transthyretin amyloid disease by changing protein misfolding energetics. *Science* 299:713–716.
- Hardesty, B., and Kramer, G. (2001). Folding of a nascent peptide on the ribosome. *Prog. Nucleic Acid Res. Mol. Biol.* 66:41–66.
- Harper, J.D., and Lansbury, P.T., Jr. (1997). Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu. Rev. Biochem.* 66:385–407.
- Hartl, F.U., and Hayer-Hartl, M. (2002). Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295:1852–1858.
- Höppener, J.W.M., Nieuwenhuis, M.G., Vroom, T.M., Ahrén, B., and Lips, C.J.M. (2002). Role of islet amyloid in type 2 diabetes mellitus: consequence or cause? *Mol. Cell. Endocrinol.* 197:205–212.
- Hore, P.J., Winder, S.L., Roberts, C.H., and Dobson, C.M. (1997). Stopped-flow photo-CIDNP observation of protein folding. *J. Am. Chem. Soc.* 119:5049–5050.
- Horwich, A. (2002). Protein aggregation in disease: a role for folding intermediates forming specific multimeric interactions. *J. Clin. Invest.* 110:1221–1232.
- Jackson, S.E. (1998). How do small single-domain proteins fold? *Fold. Des.* 3:R81–R91.
- Jaroniec, C.P., MacPhee, C.E., Bajaj, V.S., McMahon, M.T., Dobson, C.M., and Griffin, R.G. (2004). High resolution molecular structure of a peptide in an amyloid fibril determined by magic angle spinning NMR spectroscopy. *Proc. Natl. Acad. Sci. USA* 101:711–716.
- Jenni, S., and Ban, N. (2003). The chemistry of protein synthesis and voyage through the ribosomal tunnel. *Curr. Opin. Struct. Biol.* 13:212–219.
- Jiménez, J.L., Guijarro, J.I., Orlova, E., Zurdo, J., Dobson, C.M., Sunde, M. and Saibil, H.R. (1999). Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. *EMBO J.* 18:815–821.
- Jiménez, J.L., Nettleton, E.J., Bouchard, M., Robinson, C.V., Dobson, C.M., and Saibil, H.R. (2002). The protofilament structure of insulin amyloid fibrils. *Proc. Natl. Acad. Sci. USA* 99:9196–9201.

- Karplus, M. (1997). The Levinthal paradox, yesterday and today. *Fold. Des.* 2:569–576.
- Karplus, M., and Weaver, D.L. (1994). Folding dynamics: the diffusion-collision model and experimental data. *Protein Sci.* 3:650.
- Kaufman, R.J. (2002). The unfolded protein response in nutrient sensing and differentiation. *Nat. Rev. Mol. Cell Biol.* 3:411–421.
- Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W., and Glabe, C.G. (2003). Common structure of soluble amyloid oligomers implies common mechanisms of pathogenesis. *Science* 300:486–489.
- Kelly, J. (1998). Alternative conformation of amyloidogenic proteins and their multi-step assembly pathways. *Curr. Opin. Struct. Biol.* 8:101–106.
- Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., et al. (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann. Neurol.* 55:306–319.
- Koo, E.H., Lansbury, P.T., Jr., and Kelly, J.W. (1999). Amyloid diseases: abnormal protein aggregation in neurodegeneration. *Proc. Natl. Acad. Sci. USA* 96:9989–9990.
- Korzhev, D.M., Salvatella, X., Vendruscolo, M., Di Nardo, A.A., Davidson, A.R., Dobson, C.M., and Kay, L.E. (2004). Low populated folding intermediates of the Fyn SH3 domain characterized by relaxation dispersion NMR. *Nature* 430:586–590.
- Krebs, M.R.H., MacPhee, C.E., Miller, A.F., Dunlop, I.E., Dobson, C.M., and Donald, A.M. (2004). The formation of spherulites by amyloid fibrils of bovine insulin. *Proc. Natl. Acad. Sci. USA* 101:14420–14424.
- Lashuel, H.A., Hartley, D., Petre, B.M., Walz, T., Lansbury, P.T., Jr. (2002). Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* 418:291.
- Legname, G., Baskakov, I.V., Nguyen, H.O., Riesner, D., Cohen, F.E., DeArmond, S.J., and Prusiner, S.J. (2004). Synthetic mammalian prions. *Science* 305:673–676.
- Lindorff-Larsen, K., Vendruscolo, M., Paci, E., and Dobson, C.M. (2004). Transition states for protein folding have native topologies despite high structural variability. *Nat. Struct. Mol. Biol.* 11:443–439.
- Lopez de la Paz, M., Goldie, K., Zurdo, J., Lacrois, E., Dobson, C.M., Hoenger, A., and Serrano, L. (2002). *De novo* designed peptide-based amyloid fibrils. *Proc. Natl. Acad. Sci. USA* 99:16052–16057.
- Macario, A.J.L., and de Macario, E.C. (2002). Sick chaperones and ageing: a perspective. *Ageing Res. Rev.* 1:295–311.
- Makarov, D.E., and Plaxco, K.W. (2003). The topomer search model: a simple, quantitative theory of two-state protein folding kinetics. *Protein Sci.* 12:17–26.
- Malisauskas, M., Zamotin, V., Jass, J., Noppe, W., Dobson, C.M., and Morozova-Roche, L.A. (2003). Amyloid protofibrils from the calcium-binding protein equine lysozyme: formation of ring and linear structures depends on pH and metal ion concentration. *J. Mol. Biol.* 330:879–890.
- Matouschek, A., Kellis, J.T., Jr., Serrano, L., and Fersht, A.R. 1989. Mapping the transition state and pathway of protein folding by protein engineering. *Nature* 342:122.
- May, B.C., Fafarman, A.T., Hong, S.B., Rogers, M., Deady, L.W., Prusiner, S.B., and Cohen, F.E. (2003). Potent inhibition of scrapie prion replication in cultured cells by bisacridines. *Proc. Natl. Acad. Sci. USA* 100:3416–3421.
- Miller, M.W., and Williams, E.S. (2003). Horizontal prion transmission in male deer. *Nature* 425:35–36.
- Muchowski, P.J., Schaffar, G., Sittler, A., Wanker, E.E., Hayer-Hartl, M.K., and Hartl, F.U. (2000). Hsp70 and Hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc. Natl. Acad. Sci. USA* 97:7841–7846.
- Nettleton, E.J., Tito, P., Sunde, M., Bouchard, M., Dobson, C.M., and Robinson, C.V. (2000). Characterization of the oligomeric states of insulin in self-assembly and amyloid fibril formation by mass spectrometry. *Biophys. J.* 79: 1053–1065.
- O'Neill, B., and Wetzel, B. (2002). Conformational Abs recognizing a generic amyloid fibril epitope. *Proc. Natl. Acad. Sci. USA* 99:1485–1490.
- Paci, E., Friel, C., Lindorff-Larsen, K., Radford, S., Karplus, M., and Vendruscolo, M. (2004). Comparison of the transition states ensembles for folding of Im7 and Im9 determined using all-atom molecular dynamics simulations with  $\Phi$  value restraints. *Proteins* 54:513–525.
- Padrick, S.B., and Miranker, A.D. (2002). Islet amyloid phase partitioning and secondary nucleation are central to the mechanism of fibrillogenesis. *Biochemistry* 41:4694–4703.
- Panchenko, A.R., Luthey-Schulter, Z., and Wolynes, P.G. (1996). Foldons, protein structural modules, and exons. *Proc. Natl. Acad. Sci. USA* 93:2008–2013.
- Pelham, H.R. 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. *Cell* 46: 959–961.

- Pepys, M.B. (1995). Amyloidosis. In: Weatherall, D.J., Ledingham, J.G.G., and Warrell, D.A. (Eds.). *The Oxford textbook of medicine*, 3rd ed. Oxford: Oxford University Press, pp.1512–1524.
- Pepys, M.B., et al. (2002). Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature* 417:254–259.
- Petkova, A.T., Ishii, Y., Balbach, J.J., Antzutkin, O.N., Leapman, R.D., Delaglio, F., and Tycko, R. (2002). A structural model for Alzheimer's  $\beta$ -amyloid fibrils based on experimental constraints from solid state NMR. *Proc. Natl. Acad. Sci. USA* 99:16742–16747.
- Plakoutsi, G., Taddei, N., Stefani, M., and Chiti, F. (2004). Aggregation of the acylphosphatase from *Sulfolobus solfataricus*: the folded and partially unfolded states can both be precursors for amyloid formation. *J. Biol. Chem.* 279:14111–14119.
- Polverino de Lauro, P., Taddei, N., Frare, E., Capanni, C., Constantini, S., Zurdo, J., Chiti, F., Dobson, C.M., and Fontana, A. (2003). Protein aggregatin and amyloid fibril formation by an SH3 domain probed by limited proteolysis. *J. Mol. Biol.* 334:129–141.
- Prusiner, S. (1997). Prion diseases and the BSE crisis. *Science* 278:245–251.
- Ramirez-Alvarado, M., Merkel, J.S., and Regan, L. (2000). A systematic exploration of the influence of the protein stability on amyloid fibril formation *in vitro*. *Proc. Natl. Acad. Sci. USA* 97:8979–8984.
- Richardson, J.S., and Richardson, D.C. (2002). Natural  $\beta$ -sheet proteins use negative design to avoid edge-to-edge aggregation. *Proc. Natl. Acad. Sci. USA* 99:2754–2759.
- Rousseau, F., Schymkowitz, J.W., Wilkinson, H.R., and Itzhaki, L.S. (2004). Intermediates control domain swapping during folding of p13suc1. *J. Biol. Chem.* 279:8368–8377.
- Schenck, D. (2002). Amyloid- $\beta$  immunotherapy for Alzheimer's disease: the end of the beginning. *Nat. Rev. Neurosci.* 3:824–828.
- Schiene, C., and Fisher, G. (2000). Enzymes that catalyse the restructuring of proteins. *Curr. Opin. Struct. Biol.* 10:40.
- Schlunegger, M.P., Bennett, M.J., and Eisenberg, D. (1997). Oligomer formation by 3D domain swapping: a model for protein assembly and misassembly. *Adv. Protein Chem.* 50:61–122.
- Schubert, U., Anton, L.C., Gibbs, J., Orbyry, C.C., Yewdell, J.W., and Bennink, J.R. (2000). Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 404:770–774.
- Selkoe, D.J. (2003). Folding proteins in fatal ways. *Nature* 426:900–904.
- Serpell, L.C., Blake, C.C.F., and Fraser, P.E. (2000). Molecular structure of a fibrillar Alzheimer's A $\beta$  fragment. *Biochemistry* 39:13269–13275.
- Sherman, M.Y., and Goldberg, A.L. (2001). Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* 29:15–32.
- Shorter, J., and Lindquist, S. (2004). Hsp104 catalyzes formation and elimination of self-replicating Sup35 pion conformers. *Science* 304:1793–1797.
- Sitia, R., and Braakman, I. (2003). Quality control in the endoplasmic reticulum folding factory. *Nature* 426:891–894.
- Soto, C. (2003). Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat. Rev. Neurosci.* 4:49–60.
- Stangou, A.J., Hawkins, P.N., Booth, D.R., O'Grady, J., Jewitt, D., Rela, M., Pepys, M.B., and Heaton, N.D. (1999). Liver transplantation for non-Met30 TTR associated familial amyloid polyneuropathy. *Hepatology* 30:576.
- Stefani, M., and Dobson, C.M. (2003). Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J. Mol. Med.* 81:678–699.
- Sunde, M., and Blake, C.C. (1997). F, the structure of amyloid fibrils by electron microscopy and X-ray diffraction. *Adv. Protein Chem.* 50:123–159.
- Tan, S.Y., and Pepys, M.B. (1994). Amyloidosis. *Histopathology* 25:403–414.
- Thomas, P.J., Qu, B.H., and Pedersen, P.L. (1995). Defective protein folding as a basis of human disease. *Trends Biochem. Sci.* 20:456–459.
- Trieber, D.K., and Williamson, J.R. (1999). Exploring the kinetic traps in RNA folding, *Curr. Opin. Struct. Biol.* 9:339–345.
- Uversky, V.N., and Fink, A.L. (2004). Conformational constraints for amyloid fibrillation: the importance of being unfolded. *Biochim. Biophys. Acta* 1698:131–153.
- Vendruscolo, M., and Dobson, C.M. (2005). Towards complete descriptions of the free energy landscapes of proteins. *Philos. Trans. R. Soc. Lond. A* 363:433–452.
- Vendruscolo, M., Paci, E., Dobson, C.M., and Karplus, M. (2001). Three key residues form a critical contact network in a transition state for protein folding. *Nature* 409:641–646.
- Vendruscolo, M., Zurdo, J., MacPhee, C.E., and Dobson, C.M. (2003). Protein folding and misfolding: a paradigm of self-assembly and regulation in complex biological systems. *Philos. Trans. R. Soc. Lond. A* 361:1205–1222.

- Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., and Selkoe, D.J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 416:535–539.
- Wille, H., Michelitsch, M.D., Guenebaut, V., Supattapone, S., Serban, A., Cohen, F.E., Agard, D.A., and Prusiner, S.B. (1999). Structural studies of the scrapie prion protein by electron crystallography. *Proc. Natl. Acad. Sci. USA* 99: 3563–3568.
- Wilson, M.R., and Easterbrook-Smith, S.B. (2000). Clusterin is a secreted mammalian chaperone. *Trends Biochem. Sci.* 25:95–98.
- Wolfe, M.S. (2002). Secretase as a target for Alzheimer's disease. *Curr. Top. Med. Chem.* 2:371–383.
- Wolynes, P.G., Onuchic, J.N., and Thirumalai, D. (1995). Navigating the folding routes. *Science* 267:1619–1623.
- Woolhead, C.A., McCormick, P.J., and Johnson, A.E. (2004). Nascent membrane and secretory proteins differ in FRET-detected folding far inside the ribosome and in their exposure to ribosomal proteins. *Cell* 116:725–736.
- Yusupov, M.M., Yusupova, G.Z., Baucom, A., Lieberman, K., Earnest, T.N., Cate, J.H., and Noller, H.F. (2001). Crystal structure of the ribosome at 5.5 Å resolution. *Science* 292:883–896.
- Zurdo, J., Guijarro, J.I., Jiménez, J.L., Saibil, H.R., and Dobson, C.M. (2001). Dependence on solution conditions of aggregation and amyloid formation by an SH3 domain. *J. Mol. Biol.* 311:325–340.

Protein Misfolding, Aggregation and Conformational  
Diseases

Part A: Protein Aggregation and Conformational  
Diseases

Uversky, V.N.; Fink, A. (Eds.)

2006, XVIII, 419 p., Hardcover

ISBN: 978-0-387-25918-5