The amyloid state and its association with protein misfolding diseases

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Abstract | The phenomenon of protein aggregation and amyloid formation has become the subject of rapidly increasing research activities across a wide range of scientific disciplines. Such activities have been stimulated by the association of amyloid deposition with a range of debilitating medical disorders, from Alzheimer's disease to type II diabetes, many of which are major threats to human health and welfare in the modern world. It has become clear, however, that the ability to form the amyloid state is more general than previously imagined, and that its study can provide unique insights into the nature of the functional forms of peptides and proteins, as well as understanding the means by which protein homeostasis can be maintained and protein metastasis avoided.

The conversion of normally soluble peptides and proteins into intractable amyloid deposits has emerged in recent years as a subject of fundamental importance in scientific disciplines ranging from physics and chemistry to biology and medicine[1–11]. This explosion of interest has primarily resulted from the recognition that many of the disorders associated with amyloid formation[5] are no longer rare, as they were discovered just a generation or two ago, but are rapidly becoming among the most common and debilitating medical conditions in the modern world[12,13]. This change has occurred because many of these disorders are strongly associated with ageing, such as Alzheimer's disease, or with lifestyle, such as type II diabetes[1,14,15]. Alzheimer's disease has recently been labelled the 'twenty-first century plague', and it has been estimated that there will be over 80 million new cases during the next 40 years[12]. Type II diabetes is already endemic in many countries, with more than 300 million affected individuals at the present time; indeed, its increasing prevalence is predicted to stop the great increases in life expectancy that have occurred in particular during the twentieth century[14].

There are now approximately 50 disorders, with a multitude of disparate symptoms (TABLE 1), which are associated with the misfolding of normally soluble, functional peptides and proteins, and their subsequent conversion into intractable aggregates, of which the archetypal examples are amyloid fibrils (FIG. 1). Such fibrils are thread-like structures, the formation of which is associated both with a loss of function of the proteins involved and with the generation of often toxic intermediates in the process of self-assembly[2,5,7,8,16–25].

The amyloid state, however, is relevant not only in the context of disease, but also because its very existence challenges in many ways our current understanding of the nature, structure and evolution of the functional states of proteins[26–30]. From a wide range of *in vitro* experiments on peptides and proteins we now know that the formation of amyloid structures is not a rare phenomenon associated with a small number of diseases but rather that it reflects a well-defined structural form of the protein that is an alternative to the native state — a form that may in principle be adopted by many, if not all, polypeptide sequences[1,5,8,26,31].

To begin to understand the manner in which proteins either adopt and maintain the specific states that are needed to carry out given functions or instead misfold and form potentially pathogenic aggregates such as amyloid fibrils, it is important to investigate the nature and properties of the various states in which these molecules can be found[1,5]. It is also important to clarify how the conversion of proteins into the amyloid state is generally avoided in living systems. To obtain such understanding, it is necessary to define the specific mechanisms by which aggregation occurs and the manner in which it can induce pathogenic behaviour[1,3,5,7]. Much progress in these areas has come from the introduction of techniques and approaches that were originally developed for studying molecular systems in other areas of science, for example chemical kinetics, nanotechnology and microfluidics[6,32]. Further insights have been provided by comparative studies of the behaviour of a wide range of peptides and proteins, not only those that are associated with disease but also those that do not give rise to any known pathogenic condition[5].

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Table 1 | **Some human diseases associated with protein misfolding and amyloid aggregation***

Disease	Aggregating protein or peptide	Polypeptide length (number of residues)	Structure of protein or peptide
Neurodegenerative diseases			
Alzheimer's disease	Amyloid-β peptide	37–43	Intrinsically disordered
Spongiform encephalopathies	Prion protein or its fragments	230	Intrinsically disordered and α-helical
Parkinson's disease	α-synuclein	140	Intrinsically disordered
Amyotrophic lateral sclerosis	Superoxide dismutase 1	153	β-sheet and Ig-like
Huntington's disease	Huntingtin fragments	Variable	Mostly intrinsically disordered
Familial amyloidotic polyneuropathy	Transthyretin mutants	127	β-sheet
Non-neuropathic systemic amyloidosis			
Amyloid light chain (AL) amyloidosis	Immunoglobulin (Ig) light chains or its fragments	~90	β-sheet and Ig-like
Amyloid A (AA) amyloidosis	Serum amyloid A1 protein fragments	76–104	α-helical and unknown fold
Senile systemic amyloidosis	Wild-type transthyretin	127	β-sheet
Haemodialysis-related amyloidosis	β_2-microglobulin	99	β-sheet and Ig-like
Lysozyme amyloidosis	Lysozyme mutants	130	α-helical and β-sheet
Non-neuropathic localized amyloidosis			
Apolipoprotein A1 (Apo A-1) amyloidosis	Apo A-1 fragments	80–93	Intrinsically disordered
Type II diabetes	Amylin	37	Intrinsically disordered
Injection-localized amyloidosis	Insulin	21 and 30	α-helical and insulin-like

*A selection of diseases associated with extracellular amyloid deposits or intracellular inclusions with amyloid-like characteristics. See REF. 5 for a more comprehensive list of the approximately 50 human protein misfolding diseases and their associated proteins.

In this Review we touch on these different aspects of the amyloid phenomenon by particularly focusing on the molecular nature of amyloid species, the mechanism of their formation, and the nature of their structures and properties in the context of their biological importance in both health and disease.

The multiplicity of protein states

In addition to their functional native structures, proteins also populate various other states, including disordered and partially ordered conformations, and different aggregated assemblies.

Protein structure, folding and solubility. Although it was evident from early studies that both soluble and fibrillar forms of proteins could exist[33], most attention was focused on the soluble states that were found to possess demonstrable biological function. The discovery that at least some such species could crystallize led to the determination by X-ray crystallography of the first structure of a globular protein, myoglobin, in 1958 (REF. 34). Since then, a huge array (now approaching 100,000 entries in the Protein Data Bank[35]) of protein structures has been determined, including in recent times a range of membrane proteins[36], by a series of increasingly sophisticated methods also involving NMR spectroscopy and electron microscopy. It became evident from early studies that the varied and intricate structures of globular proteins are encoded by their amino acid sequences, and that these molecules have an intrinsic ability to fold spontaneously[37]. Much research has been carried out since then into the mechanism of folding, both under carefully controlled conditions in the laboratory and within cellular environments[1,11,38–40].

From these studies we now know that the folding process can be described as a diffusional search on a free energy surface[1,38–40]. A combination of experimental and theoretical studies has clarified how given sequences define specific free energy surfaces that enable folding[38–40]. This problem is, however, challenging, as a free energy surface depends on a very large number of relatively weak interactions, including hydrogen bonds, electrostatic interactions, dispersion forces and interactions with solvent molecules that underlie the hydrophobic effect. Moreover, as we discuss below, in some cases the native state of a given peptide or protein may not be structured in a globular form but disordered. Regardless of its nature, however, the functional native state is likely to only reflect a local free energy minimum at physiological concentrations, as self-association into aggregated protein species may in many cases lower the global free energy[41,42] (BOX 1). Indeed, the maintenance of protein solubility has emerged as a central aspect of the more general topic of protein homeostasis[3,43,44].

Disordered and partially folded states. As noted above, some peptides and proteins, even under normal physiological conditions, do not fold into globular structures, or they do so only in certain regions of their sequences or in the presence of specific binding partners[45,46]. Although such systems are not suitable for conventional X-ray diffraction studies, alternative biophysical

Intrinsic
In the case of a protein, a property that only depends on its amino acid sequence.

Protein solubility
The concentration of the soluble fraction of a protein in equilibrium with the insoluble fraction. This thermodynamic definition, in the presence of high kinetic barriers between the various soluble and insoluble states of a protein, may, however, need to be extended to include kinetic factors.

Protein homeostasis
The ensemble of cellular processes that regulates the behaviour of proteins in terms of their conformations, interactions, concentrations and localizations.

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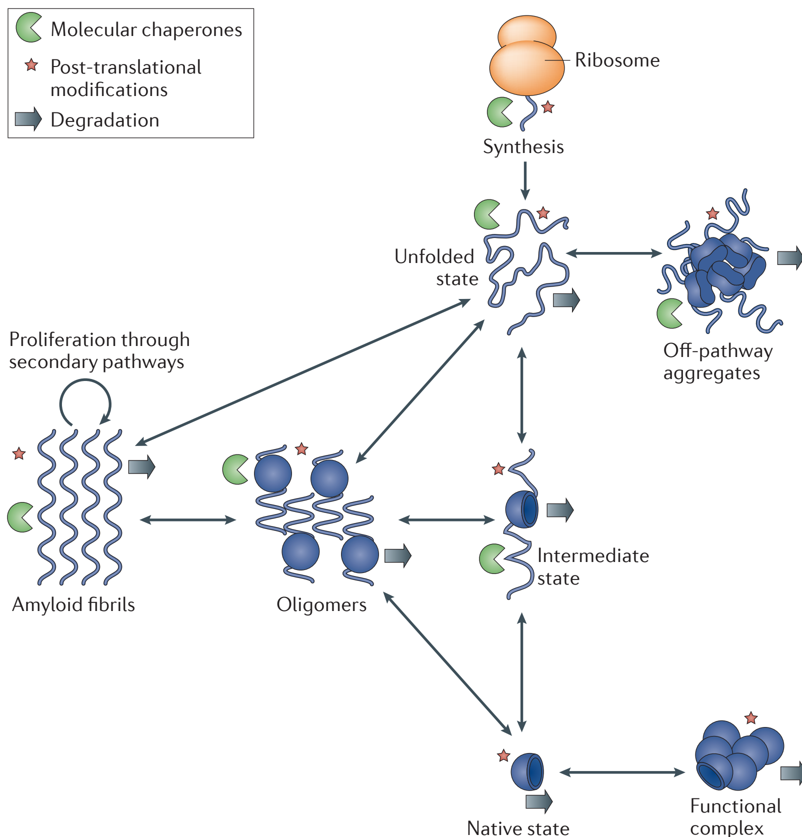

Figure 1 | **A protein can exist in various different states.** The populations of the different states and their interconversion rates are determined by their different thermodynamic stabilities, by the free energy barriers that are associated with the corresponding transitions as well as by the rates of synthesis and degradation, the propensity to interact with chaperones and to undergo post-translational and other chemical modifications. The amyloid state of a protein is a highly ordered form of aggregate in which the polypeptide chains adopt a fibrillar structure, which is capable of self-replication, for example through secondary processes. Amyloid fibrils are rich in β-sheet structure and typically form from unfolded or partially unfolded conformations of proteins and peptides, some of which are fragments of larger proteins. The amyloid state is 'generic' in that its characteristic architecture is not encoded by specific amino acid sequences.

methods — particularly approaches based on NMR spectroscopy — can provide detailed information about the ensembles of structures that exist under specific conditions[47–49]. Such proteins, often described as 'intrinsically disordered' or 'natively unfolded', have important biological roles, notably in signalling and regulation, not least because they are capable of interacting in different ways with multiple partners[45,46]. Intrinsically disordered proteins are not, however, necessarily prone to aggregation, as their sequences have usually evolved to maintain the level of solubility that is required for their optimal function; for example, through the existence of extensive regions that are highly abundant in charged and polar groups that disfavour intermolecular association from a thermodynamic point of view[50]. Moreover, as is discussed below, kinetic barriers to aggregation are crucial in enabling both globular and disordered proteins to maintain their soluble and functional states. Many of the peptides and proteins that are involved in the most common misfolding diseases are intrinsically disordered in their free soluble forms, such as the amyloid-β peptide in Alzheimer's disease[2,7], α-synuclein in Parkinson's disease[51,52] and amylin (also known as IAPP) in type II diabetes[53] (TABLE 1).

Many proteins adopt various other biologically relevant conformational states in addition to their native structures (FIG. 1), and again a range of biophysical techniques has been developed to define their properties[45,46]. Thus, for example, even those proteins that normally function in globular states often adopt intermediate conformations (corresponding to local minima on the free energy surface) before becoming fully folded and incorporated into their biological environments (for example, in the cytosol or within membranes) following their synthesis on the ribosome, particularly those proteins that are large and have complex folds[54]. In addition, even proteins that have folded correctly can subsequently unfold, at least locally, often simply as a consequence of the dynamical fluctuations that occur within protein molecules[55]. These partially folded protein species are particularly vulnerable to misfolding and aggregation from which they must be protected in living systems[56,57]. Partially folded states may, however, also be required for functional reasons, including trafficking to specific cellular locations that require translocation across mitochondrial[58,59] and even nuclear[60,61] membranes.

Aggregated species and the amyloid form of protein structure. Although most common forms of proteins are soluble, and are usually monomeric species or components of well-defined complexes, such as the ribosome and the proteasome, some functional states are insoluble, for example the fibrillar assemblies that form the cellular cytoskeleton[62] or spider silk[6,63]. Proteins are also, however, vulnerable to forming a wide variety of non-functional aggregated species. Indeed, proteins exhibit generic polymeric and colloidal patterns of behaviour, and many non-biological and synthetic polymers show similarly condensed phases, including, for example, filamentous and particulate gel states[64]. Aggregated forms of proteins can be generally amorphous on an ultrastructural level, consisting of more or less disordered assemblies of interacting chains of the same or of different sequences. Of particular fascination, however, because of their remarkable structures and properties, are highly ordered self-associated species of peptides and proteins, notably amyloid fibrils[5,8] and closely related prion-like[65,66] states. The amyloid state was first observed in the context of systemic amylodidosis more than 150 years ago, and indeed the name 'amyloid' means 'starch-like', as the deposits observed in the tissues and organs of patients who died from these conditions contained deposits that stained with iodine, which is used to detect starch[67,68].

Since then, it has been found that the proteinaceous deposits extracted from tissues in misfolding diseases typically have amyloid characteristics[5,69]. Such deposits are usually primarily composed of one protein, although they are typically associated *in vivo* with various other molecules[70–72]. Remarkably, there is no evident similarity in the sequences, native structures or functions of the group of disease-associated proteins[5]. Despite such differences, the corresponding amyloid fibrils all contain a common

Generic
In the case of proteins, a property that is common to most of them, as opposed to 'specific' properties. Such generic properties are often associated with the backbone that is common to all polypeptide molecules, whereas specific properties arise from the variations in the chemistry that is mediated by the side chains.

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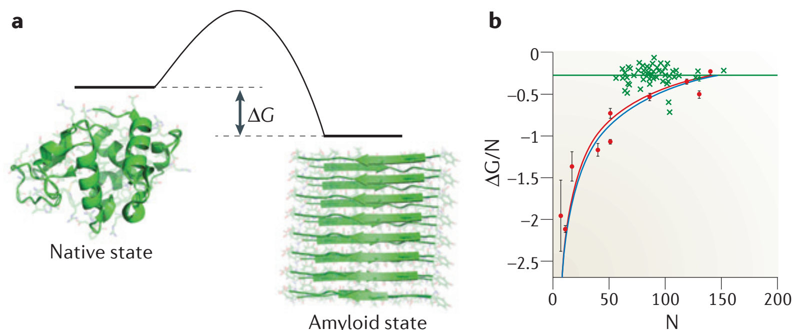
Box 1 | **Thermodynamics of the amyloid state**



In the native state of a protein, most of the interactions between amino acid residues are intramolecular, whereas in the amyloid state intermolecular interactions generally dominate. The native state is thermodynamically stable relative to the amyloid state if the free energy (G) of the peptide or protein molecule is lower in the native state than in the amyloid state. As a protein will not spontaneously transition from a state of lower free energy to a state of higher free energy, the conversion into the amyloid state will only take place when its free energy is lower than that of the native state. As the stability of the amyloid state (ΔG) is dependent on the protein concentration, whereas that of the native state (unless it exists in a functional complex) is to a good approximation independent of the protein concentration, there is a concentration at which the stability of the amyloid state is the same as that of the native state; this is the critical concentration. At concentrations exceeding this critical value, a protein is more stable in the amyloid state than in its native state. In such situations, the native state can only persist if there are high free energy barriers that hinder the transition into the more stable amyloid state (see the figure, part **a**). Under such conditions, the native state is then said to be kinetically metastable[41,42]. See the figure, part **b**, for a depiction of experimentally determined standard free energies per residue (ΔG/N, red dots) for a set of peptides and proteins as a function of the length, N, of the polypeptide chain[41]; for reference, the free energy differences between native and denatured (unfolded) states of proteins with sequence lengths in the same range are also shown (green crosses)[41]. Topological constraints associated with the packing of a long polypeptide chain decrease the free energy gain from the conversion to the amyloid form and for polypeptide chains longer than ~150 residues. The standard free energy associated with amyloid formation is on average comparable or more negative than that associated with protein folding. Part **b** adapted with permission from Baldwin, A. J. *et al.* Metastability of native proteins and the phenomenon of amyloid formation. *J. Am. Chem. Soc.* **133**, 14160–14163 (2011). © (2011) American Chemical Society.

'cross-β' pattern in X-ray fibre diffraction studies that is indicative of the component β-strands being oriented perpendicularly to the fibril axis[5,8,73–75] (FIG. 2). Moreover, in solid-state magic angle spinning NMR spectroscopy studies, the typically high-resolution nature of the amyloid fibril spectra provides direct evidence for extensive regions of highly ordered molecular structures within the fibrillar environment[75–78].

Although the amyloid form was initially identified within the context of disease, it became apparent in the late 1990s that a much wider range of 'ordinary' peptides and proteins (that is, those that are not associated with any known pathogenic misfolding condition) can convert under appropriate laboratory conditions into aggregates with all the characteristics of the amyloid fibrils that are associated with disease[1,26]. These observations, together with a wide variety of biophysical and computational studies, led to the suggestion that the amyloid structure can in principle be adopted by any polypeptide chain[79–82]. Indeed, experiments with a series of homopolymers composed of only one type of amino acid showed that these systems can also form very well-defined amyloid fibrils[31] (FIG. 2). The amyloid state of a protein is therefore generic, as it is accessible to many different polypeptide chains, and, unlike the native state, its essential architecture is not encoded by the amino acid sequence[1,26], although the details of its structure and stability can be markedly sequence-dependent, as we discuss below.

The nature of the amyloid state

Amyloid structures have fundamentally different features from native conformations, which confer unique properties upon them, typically including a very high level of kinetic and thermodynamic stability.

Detailed structure determination. Similar to globular native states, amyloid structures are closely packed and highly ordered. At the same time, however, they fundamentally differ from native states, as they possess a generic architecture that is rich in β-sheet structure[8,74,75,77,78,83]. By contrast, the folds of native states are highly diverse and can range from mostly α-helical to primarily β-sheet structures with a wide variety of different and often highly intricate topologies[1].

Amyloid fibrils from different proteins seem to be remarkably similar at the nanometre length scale[5,73]. Indeed, under electron microscopy or atomic force microscopy (AFM), amyloid fibrils tend to appear as unbranched filamentous structures only a few nanometers in diameter but often micrometres in length (FIG. 3). They are typically observed to consist of multiple protofilaments that twist around each other to form mature fibrils. X-ray fibre diffraction studies indicate that the core of each protofilament adopts a cross-β structure, in which β-strands form effectively continuous hydrogen-bonded β-sheets that run along the length of the fibril[73,84] (FIG. 2). Developments in cryo-electron microscopy[75,76,85] and solid-state NMR spectroscopy[75–78] of the fibrils themselves, and in X-ray diffraction studies of peptide microcrystals[8,74], have resulted in an increasingly detailed knowledge of the molecular structures of amyloid fibrils. These studies confirm the generic nature of the overall structures of different fibrils, which can be attributed to the common properties of the polypeptide backbone that support the hydrogen bonding pattern in the fibril core and to variations that result from the manner in which the different sets of side chains are incorporated into the common fibrillar architecture[5,31].

The cross-β architecture (FIG. 2) provides very great stability to the fibrils, as it allows the formation of a continuous array of hydrogen bonds[86]. Recently, a complete structure of a mature amyloid fibril formed through the hierarchical self-assembly of cross-β filaments, from an 11-residue polypeptide, has been determined by using various biophysical methods, in particular solid-state NMR spectroscopy and cryo-electron microscopy techniques[75] (FIG. 3). This structure shows many of the characteristics that were anticipated from earlier studies[8,73,74,76–79,83,85], including the existence of component protofilaments, each having a cross-β structure composed of pairs of nearly flat β-sheets, which then interact with other protofilaments through specific interactions of side

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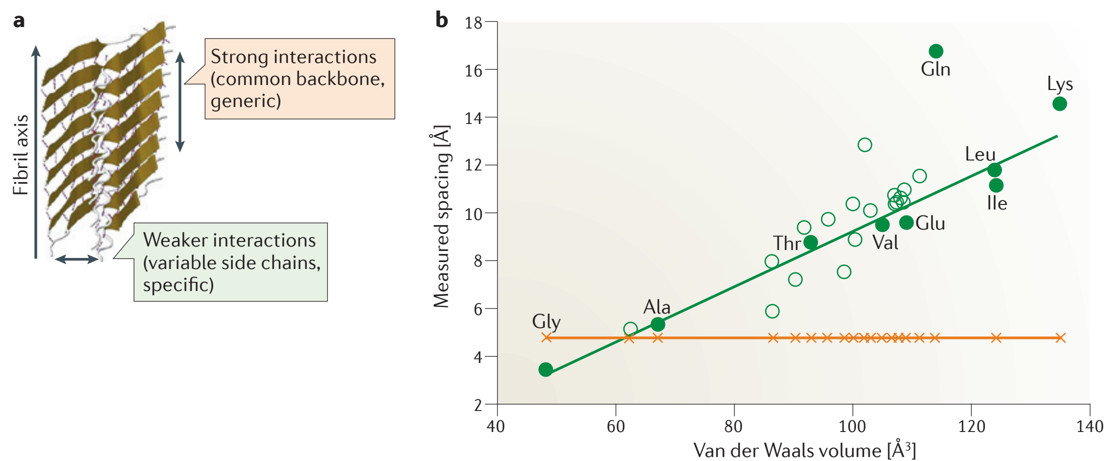

Figure 2 | **Generic features of the amyloid structure.** Amyloid structures exhibit a range of specific features and possess common characteristics. Representation of the 'cross-β' structure common to amyloid fibrils (part **a**). The spacing between polypeptide chains along the fibril axis is constant to a good approximation even for very different polypeptide sequences, a generic property arising from the common inter-main chain hydrogen bonding constraints (orange line in part **b**). By contrast, the spacing between the β -sheets in the direction perpendicular to the fibril axis is highly dependent on the nature of the side chains and originates from variable packing constraints. Correlation of the inter-sheet spacing (green circles; green line indicates specific properties) with the average van der Waals volumes of the side chains for a range of different amyloid fibrils (part **b**); filled green circles indicate fibrils that are formed by homopolymers (that is, polypeptide repeats of the same type of amino acid residue)[31]. Part **b** reprinted with permission from REF. 31, Wiley.

chains and through water-filled interfaces (FIG. 3). This study has also revealed a specific structural basis for an example of polymorphism[75] that is often observed in amyloid fibrils and has also been associated with the existence of different 'strains' in the context of prion propagation[87,88]. Interestingly, a high-resolution structure of a yeast prion segment has also been determined and has been shown to adopt a β-helical structure that is related to but different from the cross-β architecture. β-helical structures also occur in functional globular proteins and are encoded by the protein sequence, a finding that is in agreement with the idea that fungal prions may have been selected through evolution to carry out specific functions[89].

Physical basis for the amyloid structure. The common cross-β architecture of amyloid structures originates from the universal propensity of polypeptide chains to form backbone hydrogen bonding — a propensity that, in a condensed state, can be most readily accommodated through extended intermolecular β-sheets. The lateral packing of such β-sheets, however, relies on specific patterns of interactions between side chains that depend on the amino acid sequences of the component proteins[8,74] (FIG. 2). In this context, it has been observed that mixed fibrils, composed of different polypeptide chains, are rare, and indeed contiguous domains in multidomain proteins tend to differ in sequence to reduce their propensity to aggregate[90].

An important question is why the assembly of a large number of polypeptide chains should generate this type of higher order structure[8,75]. A series of theoretical studies has indicated how the amyloid characteristics arise from the inherent properties of polypeptide chains, including their persistence length (that is, the local stiffness of the polypeptide chain) and the chirality of the α-carbon atoms[81,82]. The 'one-dimensional' pseudo-crystalline nature of the cross-β fibrils may thus be the most highly organized structure that can be formed by an otherwise flexible polypeptide chain, as completely ordered three-dimensional cross-β type crystals are probably only accessible (at least on kinetic grounds) to peptides of fewer than approximately ten residues[91]. The uniform width of the resulting fibrils can be understood to be the result of a competition between the intrinsic tendency to form twisted structures, which originates from the chirality of the protein building blocks and the increasing energy penalty that results from the elastic deformation of thick filaments into twisted structures[91]. These fibrillar structures have remarkable mechanical properties, including a high Young's modulus and tensile strength[86], which arise in large part from the hydrogen bonding network of the cross-β structure and are intermediate between those of covalent materials, such as steel and carbon nanotubes, and non-covalently bonded filaments, such as actin and tubulin[86,92].

Stability of the amyloid state. There has been much speculation, and recently increasing evidence, that the amyloid state might be thermodynamically more stable than the functional native states of many protein molecules even under physiological conditions[41,42]. As the conversion of proteins from their soluble states to the amyloid form involves the formation of intermolecular contacts, the thermodynamic stability of the amyloid state is increasingly favoured at higher concentrations (BOX 1). Remarkably, the critical concentration above which the stability of the amyloid state exceeds that of the soluble state may in some cases be lower than the physiological concentration of a given protein *in vivo*, which suggests the intriguing possibility that some, and perhaps many, proteins might routinely function at

Chirality
The characteristic of a molecular structure that does not have mirror symmetry.

Young's modulus
A measure of the elastic properties of a material, defined as the ratio between stress and strain along a given axis.

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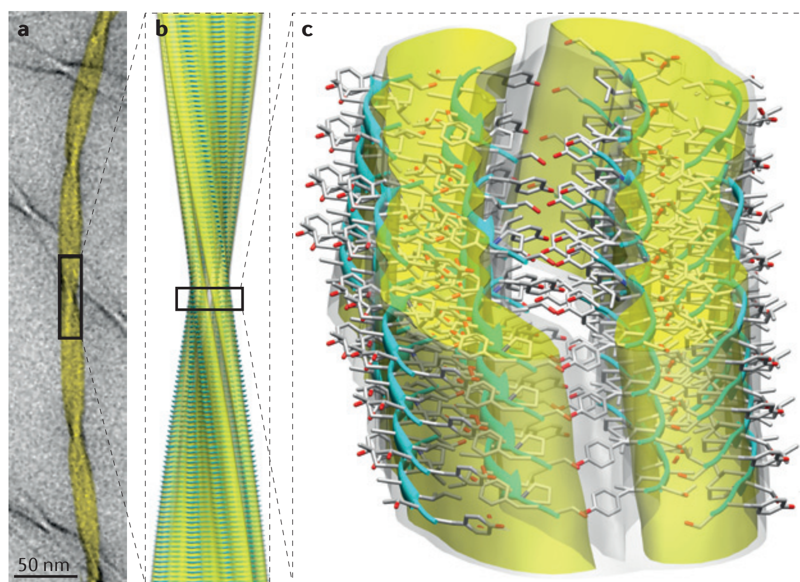

Figure 3 | **Structure of an amyloid fibril at atomic resolution.** The structure shown is of one of several polymorphs of the amyloid fibrils that are formed from an 11-residue fragment of transthyretin[75]. The combination of cryo-electron microscopy imaging (part **a**) with solid-state NMR analysis has enabled the determination of an atomic-level structure (part **b**). A more detailed view (part **c**) shows the hierarchical organization of this amyloid fibril in which the three filaments that form the mature fibril illustrated here are in turn formed by pairs of cross-β protofilaments, which are each composed of pairs of β-sheets[75]. The fibril surfaces are shown as electron density maps, and the constituent β-sheets are shown in a ribbon representation; oxygen, carbon, and nitrogen atoms are shown in red, gray and blue, respectively. Adapted with permission from Fitzpatrick, A. W. P. *et al.* Atomic structure and hierarchical assembly of a cross-β amyloid fibril. *Proc. Natl Acad. Sci.* USA **110**, 5468–5473 (2013).

concentrations that exceed the conventional definition of their thermodynamic solubility[41,93].

An analysis of a series of amyloid fibrils suggests that these types of structure are likely to become thermodynamically unstable relative to globular native structures, and indeed even relative to unstructured states, for polypeptide chains of more than ~150 residues because of the topological constraints that are associated with the packing of a long polypeptide chain into the fibril core[41]. Biology may have exploited this feature by evolving proteins with 300–500 residues on average to minimize the risk of amyloid formation. In accordance with this view, the amyloid fibrils that are known to be associated with disease are all composed of relatively short peptides or proteins, or of proteolytic fragments of larger precursor proteins (TABLE 1).

These observations, therefore, have led to the remarkable conclusion that, at the concentrations present in living systems, the native states may not always represent the absolute free energy minima of the corresponding polypeptide chains — the native form of a protein could in some cases simply be a metastable monomeric (or functionally oligomeric) state that is separated from its polymeric amyloid form by high kinetic barriers[41,42] (BOX 1). As much evidence reveals that the amyloid state is very resistant to denaturants and proteases, and mechanically robust[5,43], such a state could represent, at least under some conditions, a highly structured alternative to the native state but one that under normal biological conditions, although not under some disease-associated ones, may be kinetically inaccessible. Understanding such kinetic factors therefore becomes a major target for developing an understanding of the circumstances in which amyloid assemblies occur in living systems.

The kinetics of amyloid formation

The study of the kinetics of the processes that lead to the formation of the amyloid state is increasingly revealing the complex mechanisms involved in this transition.

The transition from soluble to fibrillar states. The transition of a protein from its functional soluble state to the amyloid state is a complex phenomenon that results from the interplay between various elementary processes that involve multiple precursor species, including small assemblies detectable by electron microscopy and AFM methods, as well as protofibrillar structures, which are generally much smaller in length and width than those of mature fibrils[94].

Many studies, in particular by microscopic and mass spectrometric techniques[95–97] and by single-molecule optical methods[17,98], have revealed that the initial stages of the aggregation process involve the formation of a heterogeneous array of oligomeric species. In some cases, including those of α-synuclein[16] and yeast prions[66], these oligomers have been shown to undergo slow transitions from relatively disorganized species to more compact structures with a rudimentary cross-β structure and hence are probably able to grow into fibrillar species (BOX 2).

Analysis of aggregation kinetics. Major advances in our understanding of the ways in which amyloid fibrils are formed have come from careful experimental kinetic studies together with the realization that the formalism of chemical kinetics provides an opportunity to use such studies in a quantitative manner to shed light on the molecular mechanisms that underlie protein aggregation[99,100]. In this context, despite the established role of chemical kinetics as a means of testing reaction mechanisms in many biologically important processes, ranging from enzyme kinetics to protein folding, its application to the process of amyloid formation has been highly challenging. This situation has arisen because of the difficulties in obtaining reproducible experimental kinetic data and because of the nonlinear nature of the differential equations that describe the protein aggregation reactions; this nonlinear nature leads to mathematical complications in establishing integrated rate laws of the type required for a complete kinetic analysis of a reaction (BOX 2). However, recent progress, both experimental and theoretical, has shown that these obstacles can be overcome, which indicates that the methods of chemical kinetics will be able to have a role in elucidating the mechanisms of protein aggregation, as they have done in other areas of physical and biological chemistry[18,100].

One of the crucial advances already emerging from the application of integrated rate laws to the analysis of the process of amyloid formation involves the interpretation

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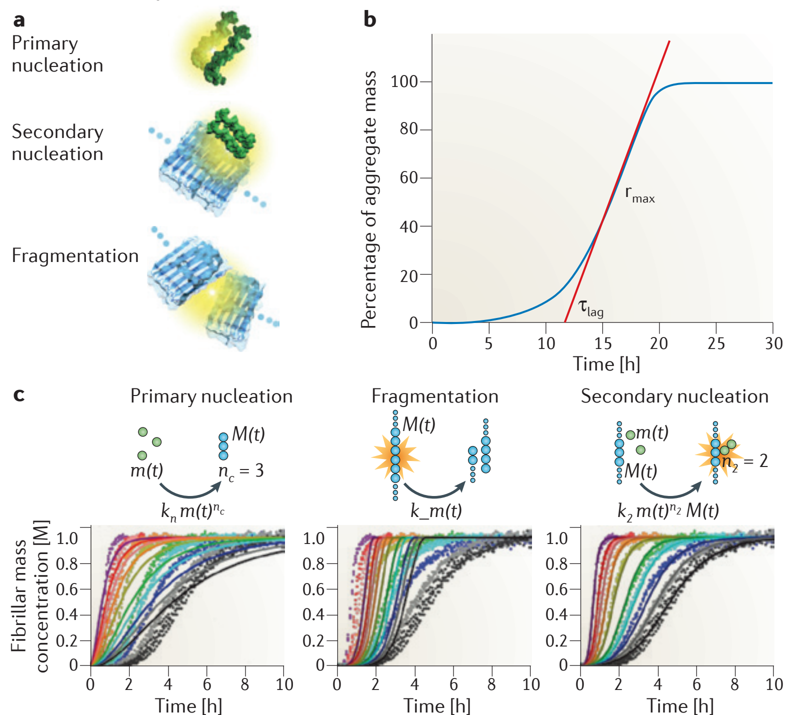
Box 2 | Kinetics of amyloid formation

Whereas thermodynamics describes whether or not a transition from one state to another is spontaneous and can occur without an external driving force, kinetics addresses the question of how fast such a transformation will take place. Kinetic measurements have historically been one of the main tools for elucidating reaction mechanisms for both small-molecule reactions and for enzymology[174]. As aggregate populations observed during amyloid formation are heterogeneous, a convenient tool for describing the kinetics of amyloid formation is the master equation formalism[99,100]. This formalism connects the microscopic steps that underlie amyloid formation with their macroscopic manifestations by considering the different ways in which new aggregates can be formed: for example, from monomer through primary nucleation (see the figure, part **a**; top; monomers are shown in green); from existing fibrils through fragmentation (see the figure, part **a**; bottom; fibrils are shown in blue); or from a combination of both monomeric and aggregated species through secondary nucleation (see the figure, part **a**; centre). Integrated rate laws that are obtained for filament growth processes that occur under the action of these various microscopic processes commonly take the form of sigmoidal functions (see the figure, part **b**; blue line), which are characterized by a lag time (τ_{lag}) and a maximal growth rate (r_{max}; red line). Comparison of such integrated rate laws with experimental kinetic measurements enables the relative importance of specific microscopic processes to be tested. In this example (see the figure, part **c**) the aggregation kinetics for increasing concentrations (see the figure, part **c**; coloured lines) of the amyloid-β peptide are compared with integrated rate laws that contain primary nucleation (see the figure, part **c**; left), fragmentation (see the figure, part **c**; centre) and monomer-dependent secondary nucleation (see the figure, part **c**; right). The data show that secondary nucleation is the dominant process under these conditions (n_c and n_2 indicate the sizes of the critical nuclei for primary and secondary nucleation, respectively; k_n and k_- indicate the rates of primary nucleation and fragmentation, respectively; and m and M indicate the concentrations of the monomers and the fibril mass, respectively), at time t. To avoid overfitting, global analysis approaches have emerged as powerful tools[99,100]. In this strategy, a known parameter, most commonly the concentration of a monomeric protein or peptide at the beginning of the aggregation reaction is varied in a known manner, and then the entire data set in terms of both time and concentration is fitted to a single rate law. More details can be found in the literature[99,100]. Parts **a** and **c** adapted with permission from Cohen, S. I. A. *et al.* Proliferation of amyloid-β 42 aggregates occurs through a secondary nucleation mechanism. *Proc. Natl Acad. Sci. USA* **110**, 9758–9763 (2013). Part **a** © (2005) National Academy of Sciences, USA. Lührs, T. *et al.* 3D structure of Alzheimer's amyloid-β(1-42) fibrils. *Proc. Natl Acad. Sci. USA* **102**, 17342–17347 (2005). Part **b** from Knowles, T. P. J. *et al.* An analytical solution to the kinetics of breakable filament assembly. *Science* **326**, 1533–1537 (2009). Reprinted with permission from AAAS.



of its typical sigmoidal reaction time course. This type of behaviour, in which a lag phase is observed before a rapid growth phase, is a feature of nucleated polymerization[66,101]; in cases in which the total quantity of protein is limited, the growth phase is followed by a plateau phase in which the reaction rate declines as a result of the depletion of the soluble species that is being monitored as it converts into fibrils (BOX 2). When monomers add to the ends of the fibrils, they adopt the cross-β conformation to match that of the peptides already present in the aggregate that hence function as templates[66,102]. Indeed, when pre-formed aggregates are added to a solution during its lag phase, rapid fibril formation can take place as a result of seeding, a process analogous to that familiar from studies of crystallization phenomena[101]. Thus, the conversion of a protein molecule from its soluble state into the amyloid form can be triggered by nucleation, as well as by templating or seeding[4,103] from existing aggregates.

The connection at the molecular level between the mechanism of the nucleation process and the duration of the lag phase[104] has, however, proved to be surprisingly complicated[99,100]. In classic nucleated polymerization reactions a primary nucleation step is responsible for the initial formation of aggregates from soluble precursor species, which is followed by an elongation of the fibrils through the addition of precursor species. In the case of amyloid formation, however, the nucleation process can also involve secondary steps that depend on the behaviour of the aggregates that form during the polymerization reaction[18,100] (BOX 2).

Among these secondary processes, fibril fragmentation (BOX 2) is an important example, as each fragmentation event increases the number of fibril ends that generate growth through the attachment of soluble protein molecules, and can therefore result in an exponential proliferation of fibrillar species[18,100,105]. In such cases, the lag phase may not be strongly dependent on the time required for the nucleation of growth-competent aggregates but rather on the time taken for the initial filaments, formed through primary nucleation, to multiply through fragmentation to a level that can be detected in established assays[99,100]. Indeed, secondary processes, such as fragmentation or surface catalysed secondary nucleation, which we discuss below, can considerably contribute to the observed lag phase and subsequent rapid growth processes[99,100].

Specific mechanisms of amyloid formation. The availability of integrated rate laws that describe protein aggregation makes it possible to extract the rates of the component microscopic processes from macroscopic experimental measurements[99,100]. Phenomenological descriptions of the aggregation process, in terms of parameters such as lag times and growth rates, can thus now be complemented by mechanistic descriptions based on specific molecular steps, such as the rates of the individual primary and secondary processes[99,100]. For example, a recent *in vitro* study of the 42-residue variant of the amyloid-β peptide has revealed that once an aggregate concentration of ~10 nM is reached, the subsequent rapid proliferation of aggregates is dominated

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by secondary nucleation that is catalysed by the fibrillar surfaces — an observation that could be highly important with regard to the progression of disease[18] (BOX 2). An interesting finding emerging from this type of analysis is that the microscopic mechanisms of amyloid formation can be very different even for relatively small modifications, such as single-residue substitutions, of the peptide or protein under investigation[106].

Another important development in this area has been the application of microfluidic techniques to the study of amyloid assembly, enabling fundamental events to be directly characterized[32,107]. These events include the spatial propagation of the aggregation process once it has started in a given location as a result of a nucleation event. This *in vitro* propagation process could therefore markedly contribute to the mechanisms of spreading of amyloid species within and between cells[108–110]. This behaviour has been described as a prion-like phenomenon (involving non-infectious prion-like particles)[111,112], as spreading was initially observed with prions[87,88], but has more recently been seen for other disease-associated proteins, including α-synuclein, amyloid-β and tau[113,114]; indeed, such a phenomenon is inherent in the physical descriptions of protein aggregation[110]. In particular, although diffusion itself can be a very slow process, the fact that the aggregates are able to multiply in number through secondary nucleation greatly favours their spreading in space and can lead to reaction–diffusion type phenomena that generate waves of spreading either directly or through specific biological transport mechanisms[32,107].

It has also been realized that by systematically reducing the volume of the microfluidic droplets it is possible to monitor individual nucleation events, hence revealing the initial events responsible for the initiation[32,107] and subsequent proliferation of the aggregation process[32,107]. These microfluidic-based studies have also revealed that molecular confinement within small volumes, characteristic of the intracellular environment, can result in a major statistical suppression of the nucleation step of protein aggregation. The consequent increases in the kinetic stability of the soluble states of peptides and proteins could have a major role in the avoidance of aggregation within biological systems in which compartmentalization is ubiquitous[32], as well as slowing the propagation of protein misfolding and aggregation once it has started in a specific cellular or subcellular compartment.

Finally, it is becoming increasingly recognized that measurements of kinetic and thermodynamic parameters *in vitro* are of fundamental importance not only for analysing experimental measurements in terms of reaction mechanisms but also for predicting aggregation behaviour under conditions in which such parameters cannot be readily measured, most importantly at low concentrations *in vivo*. This possibility is particularly important, as neurodegenerative diseases commonly develop over timescales of years or decades, whereas laboratory experiments to define the mechanisms of aggregate formation are restricted to timescales that are several orders of magnitude shorter and are therefore commonly carried out at concentrations much greater than those found in living systems.

Nucleation
In the transition from a fluid phase to a condensed phase, a process that generates species within the fluid phase that are capable of growing into the condensed phase.

Templating
A phenomenon in which structured aggregates promote the conversion of soluble protein species into similar aggregates.

Seeding
A phenomenon in nucleated growth processes by which nuclei of the aggregated phase promote the formation of larger aggregates.

Primary nucleation
A nucleation process that takes place by the spontaneous assembly of monomeric species.

Secondary nucleation
A process by which the formation of new nuclei in the aggregated phase is catalysed by existing aggregates.

Spreading
In the context of neurodegeneration, the spatial propagation of amyloid assemblies from cell to cell by a series of diffusion or transport mechanisms that are coupled with seeding or templating processes.

Cooperativity
A property of a system that results from its collective behaviour, but it is not exhibited by its component parts.

Molecular chaperones
Proteins that assist the protein-folding process, the maintenance of the soluble state of proteins and more generally contribute to generating and preserving protein homeostasis.

The amyloid state in health and disease

The healthy state of a cell is characterized by a detailed balance between the different states that proteins can populate. Perturbations to such balance, unless they are kept under strict control, can lead to deleterious events and disease.

The maintenance of biological functionality. As proteins in their functional forms can be thermodynamically and chemically metastable[41], mechanisms have evolved to maintain their solubility *in vivo* for prolonged periods of time and to avoid their conversion into non-functional amyloid states. Exceptions are cases in which the amyloid state is functional[5,30,115]. Thus, for example, Pmel17 is a highly aggregation-prone protein that forms functional amyloid structures that are involved in melanosome biogenesis[30], and it has been found that certain peptide and protein hormones in secretory granules of the endocrine system are stored in cross-β conformations[115]. Rather few such examples have, however, been observed, particularly in mammalian systems, and functional amyloid formation is undoubtedly very carefully regulated[30,115].

Many of the characteristics of proteins that enable the avoidance of aggregation, and amyloid formation in particular, are encoded by their amino acid sequences[116]. The elucidation of this code has enabled the identification of factors that determine the intrinsic aggregation propensity of these molecules[117–119]. Hence, it has been realized that globular proteins fold into structures that sequester aggregation-prone regions in their interior; in addition, typical features of the folding process, such as very high cooperativity, generate considerable kinetic barriers to the conversion of folded proteins into aggregation-prone species[50,120]. Furthermore, specific patterns of residues, such as alternating hydrophobic–hydrophilic stretches[50,121], that tend to favour the amyloid state are commonly selected against during evolution[119,121,122] or are otherwise neutralized by the insertion of highly aggregation-resistant residues, which are known as 'gatekeepers' (REFS 50,123).

Other protective mechanisms against amyloid formation are associated with properties of the cellular environment, including the location of proteins within specific compartments[124,125], and the presence of a multitude of molecular chaperones and degradation processes, such as the ubiquitin–proteasome[126–128] and the autophagy[129–131] systems, which function to prevent the formation and accumulation of misfolded and aggregated polypeptide chains[11,132]. Indeed, the major genetic risk factor for late-onset Alzheimer's disease is the presence of an apolipoprotein E variant that reduces the ability of cells to degrade the amyloid-β peptide[133].

'Housekeeping' mechanisms that are capable of suppressing protein aggregation are thus essential at all stages of protein life cycles. For example, molecular chaperones that function within the cellular environment, where synthesis and folding take place, can target specific steps in the process that leads to amyloid fibrils, notably specifically to inhibit either primary or secondary nucleation processes[134], as shown, for example, in the case of heat shock protein 70 (Hsp70) that can prolong the lag phase in the aggregation of the yeast protein Ure2p[135]. Other molecular

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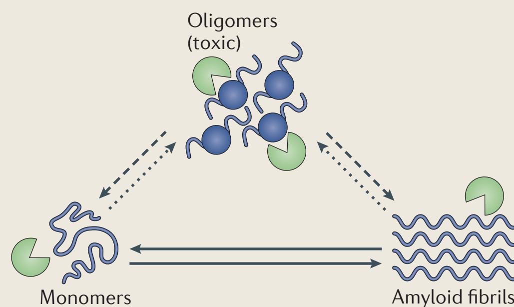
Box 3 | **Strategies for therapeutic intervention**



The maintenance of protein homeostasis and the avoidance of protein metastasis can be achieved by regulating the concentrations of the different states of all the proteins that are contained within a living system and by controlling the rates of conversion between them. To this end, it is particularly important to reduce the population of oligomeric species (pre-fibrillar species) by disrupting the processes of their formation (see the figure; dashed arrows) or by promoting the pathways of their removal (dotted arrows). These strategies can be implemented in various ways[166]; for example, through the modulation of production processes (targeting synthesis or proteolysis)[175], degradation (targeting the ubiquitin–proteasome[126,127] and the autophagy[129,130] systems) or stability (usually targeting the native state)[167,168]. For example, one can use the ability of antibodies[176,177], or artificially-generated analogues such as affibodies[178], to selectively bind to the native states of aggregation-prone proteins, as binding generally results in increased stability and hence to a reduction in aggregation propensity. In some cases, it might be possible to use antibody-based immunotherapy approaches to reduce the level of highly aggregation-prone species (such as amyloid-β) by stimulating their clearance[179,180]. Antibodies and their analogues also offer other possibilities, one of which could be to mimic the action of natural molecular chaperones by targeting the aberrant misfolded species that give rise to cellular damage. If such 'artificial chaperones' can be developed and can be targeted to the appropriate location (for example, by enhancing their ability to cross the blood–brain barrier), then they could represent a highly effective therapy[3,43]. Furthermore, it is also becoming evident that small molecules can also function to suppress the early stages of protein aggregation, for example by binding to specific amyloidogenic species and by reducing the risk of nucleation and proliferation of pathogenic agents[168–172].

chaperones, such as clusterin[98,136], can function in the extracellular space and sequester aggregation-prone species to inhibit their proliferation and growth to prevent protein aggregation. Indeed, clusterin, together with ~20 other proteins, has been found in multiple genome-wide association studies to have a strong genetic link with late-onset Alzheimer's disease[137–139]. Indeed, it is possible that different steps in the process of amyloid formation will be regulated by different types of chaperones that therefore function together to generate a series of defences against the formation and proliferation of aggregated and potentially pathogenic forms of proteins.

The origins of pathogenicity. As most of our detailed knowledge of the structures and of the thermodynamic and kinetic properties of amyloid fibrils has been obtained from *in vitro* studies, it is of great importance to relate these findings to the events that occur within living systems and hence to begin to explore the molecular basis of the pathogenicity of protein aggregates[16,19,22,25,140–142]. In principle, it is possible to carry out the analysis of reaction kinetics in living systems to identify the specific microscopic processes that are primarily responsible for the proliferation of the aggregates using strategies similar to those described above for *in vitro* experiments. For instance, analysis of data from studies of transgenic mice indicates that a dominant contribution to mammalian prion propagation results from secondary processes such as fragmentation[100] (BOX 2). Studies of amyloid formation within simpler model organisms, such as fruitflies and nematode worms, also demonstrate that links can be made between the findings of detailed *in vitro* experiments and the events that take place *in vivo*[143–146]. In such efforts, it is becoming increasingly possible to apply a wide range of biophysical approaches and imaging techniques to living systems[147–149] to compare the events that occur in the different environments.

Systemic amyloidoses
A group of diseases characterized by the widespread deposition of amyloid aggregates in organs and tissues.

Despite the fact that it has been clear for many years that there is a close association between the appearance of amyloid deposits and the onset of pathological events in protein misfolding diseases[2], much remains to be understood about the specific mechanisms underlying these events[7,22,150,151]. In the case of systemic amyloidoses, it seems likely that the primary cause of disease is simply the presence of large quantities, in some cases even kilograms, of amyloid deposits in vital organs, including the liver, spleen and kidney[5]. In neurodegenerative disorders, by contrast, there are in many cases no detectable correlations between the overall quantity of fibrillar aggregates and the stage of disease advancement[7,18,151,152], which suggests the possibility that these human disorders could be associated with misfolding events that induce cellular damage but that result in few persistent or readily detectable aggregates[69].

Indeed, a view has emerged over the past 15 years that pre-fibrillar species, rather than mature amyloid fibrils, are likely to represent the primary pathogenic agents in non-systemic conditions, notably neurodegenerative diseases and other organ-specific conditions such as type II diabetes[5,7,8,16–25,69,153] (BOX 3). Indeed, increasing evidence suggests that the oligomeric assemblies that are almost universally observed as intermediates during the aggregation process are 'generically' damaging to cells[7,16,17,19–25]. This phenomenon has been observed both for proteins that are associated with disease and for those that are not linked to any known pathology[21,153].

The origin of the toxicity of the oligomers may arise from their inherently misfolded nature, as they display on their surfaces chemical groups that under normal physiological conditions would not be accessible within the cellular environment, notably from hydrophobic side chains[20,154,155]. Such surfaces can interact inappropriately with many functional cellular components, ranging from other proteins to nucleic acids and lipid membranes[156,157]. As aggregation produces an array of different oligomers, various types of toxic species can be expected — in fact, almost any misfolded species is likely to have the potential to generate at least some level of toxicity or cellular dysfunction[69], depending on the nature of the cells in which they are formed or to which they are exposed[17,20].

It is also evident that in some cases, including α-synuclein[17], the oligomers that initially appear may be relatively disordered species, as their formation is likely to involve coalescence around hydrophobic groups. A subsequent conformational change can then result in other types of oligomer with at least a rudimentary amyloid-like core, composed of hydrogen bonded β-sheets and which possess increased stability and a larger hydrophobic

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surface than the initial oligomers[17,20,66,82,154]. Such secondary oligomers could be precursors of the cross-β structure in amyloid fibrils, and their highly hydrophobic surfaces are likely to generate particularly damaging aberrant interactions with other cellular components. After further growth, which ultimately leads to the formation of mature fibrils, these oligomeric species become less capable of generating pathological effects, as their surface-to-volume ratio decreases together with the extent to which hydrophobic regions are exposed[82,154].

From protein homeostasis to protein metastasis. The generation of aggregation-prone misfolded species is a constant danger for proteins, and, indeed, as we discuss above, these hazardous oligomeric species are primary targets for various molecular chaperones[11,98,132] and degradation pathways[126,127,129,130]. Thus, cellular damage and death are likely to become widespread only when misfolded and aggregation-prone species reach levels that can overwhelm the defensive housekeeping systems[1,3,11,158]. This breakdown of protein homeostasis[3,43,132] can lead to a situation that is known as protein metastasis, in which initial aggregation events trigger a cascade of pathological processes that mark the progression of disease.

Support for this general concept comes from the observation that, although proteins are sufficiently soluble to avoid aggregation under normal physiological conditions, many of them are close to their solubility limits[41,93] or are indeed supersaturated[158]. Therefore, even small perturbations in protein homeostasis — for example, as a result of mutations, post-translational modifications, changes in concentration or quality control mechanisms compromised by age, disease or stress[159–162] — can increase the probability of protein aggregation that eventually results in pathogenicity. Indeed, it has been recently shown that proteins that exceed their solubility limits form a metastable 'subproteome' that can undergo widespread aggregation upon impaired protein homeostasis[158]. As such events are usually followed by a substantial transcriptional response[163,164], a better understanding of these changes may clarify the manner in which our natural cellular defences against aggregation can be increased by pharmacological intervention.

Studies of the history of medicine reveal that major advances in prevention and treatment of a given disease generally require a deep understanding of its underlying causes[165]. Without such knowledge, therapies only result from serendipity, chance, or trial and error. As misfolding diseases are 'modern', and only emerged as a major threat to human health and welfare within the last few decades of the twentieth century, there is little or no previous knowledge to build on. This situation is in marked contrast to the history of discoveries that led to the development of antibiotics and vaccines[165]. It is of vital importance, therefore, that we learn as much as possible about the whole family of amyloid-related diseases; that is, about the process that we define as protein metastasis whereby the functional soluble states of proteins convert into self-propagating aberrant molecular assemblies that initiate a cascade of cytotoxic processes. In addition, as the pathogenic agents in the amyloid-related disorders completely differ from those of well-established pathogens, such as bacteria and viruses, the strategies for their suppression are also likely to fundamentally differ from those that have been so successful in other types of disease. Ironically, it is our tremendous success in devising methods of therapeutic intervention in these long-established diseases[166] that has resulted in the greatly increased incidence of neurodegenerative disorders that are associated with old age, including Alzheimer's disease and Parkinson's disease[12,13,15].

It is increasingly clear that the most effective therapeutic strategies for amyloid-related diseases are likely to be those that involve the prevention of the initiation of protein metastasis; after our natural defence mechanisms are overwhelmed by the cascade of pathological events that follow the beginnings of protein aggregation, other processes such as spreading can occur through the migration of pathogenic protein species that are able to seed aggregation and to induce neuronal damage at locations outside the vicinity of the initial nucleation events[18], as we discuss above (BOX 3). The approach of the prevention of protein metastasisn has been recently demonstrated in the case of clinial-related conditions[167]. These conditions are associated with the aggregation of transthyretin, a protein that is involved in the transport of the thyroid hormone thyroxin[167]. Familial mutations can destabilize the homotetrameric native state of this protein, and hence release highly aggregation-prone monomers that can initiate various systemic and neurological disorders (TABLE 1). By binding a substrate analogue, however, the native tetrameric form of the protein can be stabilized, thereby reducing its propensity to aggregate[167]. This strategy has resulted in the development of a small-molecule drug recently approved for clinical use[168,169].

More generally, there is increasing evidence that the interaction of therapeutic agents with other amyloidogenic proteins, including natively disordered species such as α-synuclein and the amyloid-β peptide[170–173], can perturb the early events in protein aggregation, and this approach clearly holds great promise for the future. Indeed, we are optimistic that our increasing knowledge of the mechanistic details of the processes that are associated with amyloid formation will provide a range of new targets for the prevention of the development and proliferation of this whole class of misfolding disorders, and enable intervention at specific points in the complex but increasingly well-understood mechanisms that would otherwise lead to protein metastasis and its pathological consequences[167–172] (BOX 3).

Looking to the future

We are at a turning point in research directed at the amyloid phenomenon, as we are finally becoming capable of defining in great detail the structures and properties of the different states that are involved in the process of protein aggregation and the complex microscopic mechanisms by which they form. With these advances, we now have unprecedented opportunities to intervene in a rational manner to reduce the risk and limit the consequences of the loss of protein homeostasis, which we describe in this Review as protein metastasis.

Protein metastasis
The ensemble of molecular processes whereby the functional soluble states of proteins convert into self-propagating aberrant assemblies that initiate a cascade of cytotoxic events.

Supersaturated
A condition in which a soluble substance is concentrated to a level above its critical value but kinetic barriers delay its transition to an insoluble state.

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A range of exciting developments is under way and we briefly mention here some specific topics that we believe are of particular importance for advancing our knowledge of the amyloid phenomenon. These topics include the greater use of innovative biophysical techniques to characterize in increasing detail the microscopic processes involved in amyloid formation; the further development of robust approaches to define the structures of the protein species formed during such processes; the development of more advanced computational methods that are able to simulate the transitions between the different conformational states that are involved in the process of amyloid formation; the wider use of high-resolution and super-resolution techniques for the *in vivo* and *in vitro* imaging of the events that are associated with amyloid formation and spreading; the generalization to *in vivo* situations of our current molecular understanding of the aggregation processes taking place *in vitro*; and the introduction of increasingly effective series of small and large molecules that are capable of perturbing these processes through screening and rational design.

In terms of the specific development of effective therapies, we identify the following: the development of improved diagnostic methods and more specific and reliable biomarkers of disease; the ability to more rapidly identify potential drug leads and to more effectively monitor their effects; and the opportunity to test on patients potential drugs at or even before the earliest onset of the symptoms of disease. Perhaps of greatest importance, however, is the development of an ever increasing degree of insight into the characteristics of the functional states of proteins, the environments in which they function, and the ways that our natural defences normally function so effectively together to maintain protein homeostasis and to avoid protein metastasis until we reach ages that were almost unprecedented until recent times[1]. Such knowledge will not only provide deep insights into the manner by which biological systems function and have evolved but will also undoubtedly bring new opportunities for rational therapeutic intervention of the type that we have seen in the case of the transthyretin-related diseases[168,169].

As we have discussed, our current understanding of the fundamental mechanisms of protein aggregation, together with our current knowledge of the mode of action of our natural defence mechanisms such as molecular chaperones, indicates that the most effective procedures for the prevention and treatment of misfolding diseases are likely to be those that address the earliest events in their development. Indeed, by analogy to the strategy of using statins to prevent cardiovascular disease[173], the most favourable opportunities for effective therapy against amyloid-related diseases could well be the development of drugs that are able to reduce the risks of aggregation by decreasing the concentrations and formation rates of aberrant protein assemblies or that enable our natural defences to maintain their efficacy for longer periods of time. Underlying any rational approaches to such drug discovery processes, however, there must be a detailed understanding of the mechanism of amyloid formation under various conditions, both *in vitro* and *in vivo*. In this Review, we have tried to show how much progress has been made towards this essential objective and to emphasize that, in addition to providing a basis for therapeutic advances, our increasing knowledge of the amyloid phenomenon has led to great advances in our understanding of the general nature and evolution of protein molecules and the environments in which they normally function so efficiently and so reliably.

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Competing interests statement

The authors declare no competing interests.

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