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The amyloid state of proteins in human diseases

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Abstract

Amyloid fibers and oligomers are associated with a great variety of human diseases including Alzheimer's disease and the prion conditions. Here we attempt to connect recent discoveries on the molecular properties of proteins in the amyloid state with observations about pathological tissues and disease states. We summarize studies of structure and nucleation of amyloid and relate these to observations on amyloid polymorphism, prion strains, co-aggregation of pathogenic proteins in tissues, and mechanisms of toxicity and transmissibility. Molecular studies have also led to numerous strategies for biological and chemical interventions against amyloid diseases.

What is the amyloid state?

Many proteins enter the so-called amyloid state, in which they form elongated, fibers, with spines consisting of many-stranded β-sheets. The operational definition of amyloid, which has been adopted by the community of pathologists, is that the fibers are unbranched, usually extracellular, and found *in vivo*; in addition, the fibers bind the dye Congo Red and then show green birefringence when viewed between crossed polarizers (Sipe et al., 2010). By this definition, >25 amyloid-forming proteins have been identified and associated with serious diseases, including amyloid-β peptide (Aβ) with Alzheimer's disease (AD), islet amyloid polypeptide (IAPP) with diabetes type 2, and prion protein (PrP) with the spongiform encephalopathies.

Biophysicists prefer a molecular-based definition, and thus, they have abandoned the requirement that the fibers are usually extra-cellular and disease-associated. The reasons for this change is that the same disease-related proteins form similar fibers *in vitro* and many other proteins form similar fibers when denatured (Fandrich et al., 2003) or during their physiological roles (Chapman et al., 2002; Fowler et al., 2007; Si et al., 2003). Accordingly, biophysicists have adopted a structure-related definition for amyloid fibers, in which amyloid fibers display the *cross-β fiber diffraction pattern* (Figure 1).

This pattern was first observed by the pioneering biophysicist William Astbury (Astbury et al., 1935), who stretched a poached egg white into a fiber in the X-ray beam. Astbury

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reasoned correctly that in such fibers, elongated protein strands must be stacked along the fiber axis, forming protein sheets that are parallel to each other. A decade and a half later, Pauling and Corey built models for these β-sheets, showing that hydrogen bonds hold the strands into sheets.

Proteins may enter the amyloid state when a segment exposes its backbone amide N-H groups and C=O groups, permitting them to couple into hydrogen bonds with other protein chains. Several conditions produce exposed backbone amide groups: denaturation of a normally folded proteins (Chiti et al., 1999); over expression of a protein that overwhelms cellular chaperones and drives it into an inclusion body (Wang et al., 2008); cleavage of a peptide (such as Aβ) from a folded protein; or over production of a natively disordered protein (such as tau or IAPP). Exposure of backbone amide groups is necessary for amyloid formation but not sufficient. In addition, the local concentration of the exposed segment must be sufficiently great to overcome the entropy that opposes formation of ordered fibers. The higher the concentration, the more aggregation is favored. Suppose the concentration of the exposed segment is [P] and that of the aggregated fiber of n units is [nP]. Then (neglecting intermediate states) the free energy change for the process of aggregation is given by:

$$\Delta G=\Delta G^{0}+RT \ln \{[nP]/[P]^{n}\}$$

in which ΔG^{0} is the standard free energy change for the reaction, and RT is the product of the gas constant and the absolute temperature. Because there are tens of thousands of protein molecules in an amyloid fiber, that is n=thousands, $[P]^{n}$ becomes large when the concentration of P goes up, and the log of the ratio becomes extremely negative, meaning that the free energy of amyloid formation is highly favorable.

In the laboratory, scientists can often produce amyloid fibers from a protein at high concentration by partially denaturing it with a destabilizing solvent, a change in pH, heating, or surface denaturation from agitation. In cells and tissues, amyloid formation occurs with abnormally high expression of a protein. In humans, we do not yet understand all causes of amyloid formation, but increased synthesis or reduced degradation of a given protein, leading to an abnormally high concentration, is a factor (Balch et al., 2008).

For amyloid to form, a nucleus must template the bonding pattern of the fiber spine. As described in Section 2, each fibril spine is built on an intricate pattern of hydrogen bonds and steric interactions. From the atomic structure of one such spine, it was suggested that templating the pattern of the spine requires 3 or 4 protein molecules (Nelson et al., 2005). If so, then 3 or 4 molecules must expose their amyloid-forming segments at the same time and must be at high enough concentration for bonding and consequent templating of the fibril pattern. Thus, nucleation is a rare event, but once the nucleus is formed, single molecules can join the growing fibril one at a time, as they open to expose the proper segment and bond at the ends of fibrils. The result is that amyloid fibril formation is characterized by a slow nucleation phase, followed by a more rapid growth phase (Jarrett and Lansbury, 1993). The pathway for amyloid fiber formation is at present an active area of investigation with evidence that fibers can form by nucleated conformational conversion from an oligomeric state (Lee et al., 2011). Because fibrils grow from their ends, breakage of fibrils affects the kinetics of fibrillar growth (Tanaka et al., 2006), and a full description of fibril formation must include rates of nucleation, growth, and breakage (Knowles et al., 2009).

Concepts of nucleation are important in understanding amyloid and prion diseases. Nucleation is stochastic and the chance for forming a nucleus is lowered as the volume of

solution is diminished. For example, a bottle of water can be supercooled to only a few degrees C before ice nucleates. However, as its volume is diminished, water can be increasingly supercooled. Micron-sized drops can be supercooled to -41° C (Kuhns and Mason, 1968). Biological cells are micron sized, and thus, we might expect that amyloid nucleation would be infrequent for intracellular proteins, even when they are at relatively high concentration. Of course if we introduce a nucleus or "seed" from the outside to a supercooled liquid or a supersaturated solution, growth on the nucleus is fast. For instance, try touching an ice chip to the surface of a bottle of supercooled water. Instant crystallization occurs because the ice chip provides the nucleus, or an exact three dimensional pattern, of water molecules in ice. Thus, the concept of seeding is important for understanding propagation of amyloid fibers or prions from cell to cell or organism to organism.

What is the atomic structure of the amyloid spine?

Amyloid fibers share a common "cross-β" spine. In 1959, elongated, unbranched fibrils were reported in electron micrographs of diseased tissues from diverse origins (Cohen and Calkins, 1959), and nine years later X-ray diffraction patterns of such fibrils were identified as Asbury's cross-β type (Eanes and Glenner, 1968). With the advent of synchrotron X-ray radiation, scientists found that amyloid fibers formed from six different proteins; each one was associated with a different clinical syndrome and showed similar cross-β diffraction (Sunde et al., 1997).

Determining the atomic details of the cross-β spine has been slow because the limited order of fibrils, whether isolated from diseased tissues or from in vitro conversion of native proteins to the amyloid state, presents challenges to crystallographic, NMR, and EM methods. But important features have gradually emerged from studies by solid-state NMR (Benzinger et al., 1998; Tycko, 2011), model-building constrained by X-ray fiber and powder diffraction (Makin et al., 2005; Sunde and Blake, 1998), site-directed spin labeling (Serag et al., 2001; Torok et al., 2002), cryo-electron microscopy (Jimenez et al., 1999; Schmidt et al., 2009), scanning mutagenesis (Williams et al., 2004), and single-crystal X-ray diffraction (Nelson et al., 2005). The most general points to emerge are that: (1) In all amyloid fibers, the strongest repeating feature is a set of β-sheets that are parallel to the fibril axis, with their extended strands near perpendicular to the axis; (2) The β-sheets can be either parallel or anti-parallel—that is, adjacent hydrogen-bonded β-strands within a sheet can run in the same direction or in opposite directions; (3) The sheets are usually "in-register," meaning that strands align with each other such that identical sidechains are on top of one another along the fibril axis. In parallel sheets, identical sidechains are separated by an inter-strand distance of 4.8 Å (Figure 1), and in antiparallel sheets, they are separated by 2 ×4.8 Å = 9.6 Å.

The architecture of at least the simplest cross-β amyloid spines has been clarified by determining short segments of amyloid-forming proteins (Apostol et al., 2010; Ivanova et al., 2009; Nelson et al., 2005; Sawaya et al., 2007; Sievers et al., 2011; Wiltzius et al., 2009). The segments examined are those that seem to be the adhesive parts of amyloid proteins. In isolation from the rest of the protein, they form microcrystals and related fibers with morphological similarity to fibers of the entire parent proteins (Balbirnie et al., 2001). The atomic structures of the microcrystals reveal that the motif of the amyloid protofilament consists of a pair of β-sheets that run the length of the fiber-like crystals (Figure 1B). Each sheet is a standard Pauling-Corey β-sheet, in which each strand is hydrogen-bonded to the strand above and below it through its backbone amide groups.

When the sidechains contain amides (glutamine and asparagine), those amides also form hydrogen bonds to the identical residue in the strands above and below. This creates parallel

arrays of hydrogen bonds running along the fiber axis. The electrostatic interactions of all of these aligned hydrogen bonds mutually polarize one another, producing hydrogen bonds even stronger than those in ice (Tsemekhman et al., 2007). The stability of such interdigitated beta sheets explains the persistence of amyloid fibers and prions.

Within the protofilament, the sidechains emanating from the two sheets are tightly interdigitated, as shown in Figure 1C, like the teeth of a zipper. The interface between the sheets is devoid of water, and hence this motif has been termed the “dry steric zipper.” Dozens of atomic structures of dry steric zipper have been determined by X-ray crystallography and share the following properties: (1) Steric zippers form from self-complementary amino acid sequences, in which their sidechains can mutually interdigitate. The sequences can be polar or non-polar, with large sidechains or small, but they fit together in complementary fashion; (2) Steric zippers have dry interfaces between the two sheets. Thus, the hydrophobic effect contributes to amyloid stability, as does the strong hydrogen bonding; (3) The β-strands are most often in-register, maximizing inter-strand hydrogen bonding, and permitting stacking of glutamine (Gln), asparagine (Asn), and tyrosine (Tyr) residues. Although all steric zippers are expected to be formed from complementary sequences, the sequences do not need to be *self*-complementary. There is strong evidence from solid-state NMR studies (Luhrs et al., 2005; Petkova et al., 2002) that in Aβ, some close interactions are between β-strands that differ in sequence (see Figure 4). Such “hetero-steric zippers” have not yet been observed in X-ray crystal structures.

The most common sheet-to-sheet arrangement for steric zippers is face-to-face (Class 1; Figure 1B), but other arrangements occur (Figure 2). In these other arrangements, the two sheets can be: face-to-back (Classes 2 and 4); pack with opposite edges up rather than both edges up (Class 4); or contain antiparallel strands (Classes 5-8), rather than parallel strands (Classes 1-4) (Sawaya et al. 2007). To date, no examples in Class 3 have been observed.

Some amyloid spines are more complex than single steric zippers. For instance, several different steric zippers all formed by the same protein can occur in the spine. In fact some 13 different steric zippers have been found for the 42-residue sequence of Aβ (Colletier et al., 2011). Many proteins, including the PrP and Sup35 prions, Aβ, and IAPP, have several potential steric-zipper forming segments within their sequences. In a recent report, Lewandowski et al. (2011) provide solid-state NMR evidence that fibers of the yeast prion Sup25 contain three distinct steric zippers (one is shown in Figure 1B).

A second source of increased complexity is the likelihood of hetero-zippers formed from cross-complementary sheets. Hetero-zippers have been found by solid-state NMR in the structure of Het-s, a fungal prion (Wasmer et al., 2008) (Figure 3). This structure, termed a solenoid by its discoverers, consists of a stack of two-layer protein loops. Each loop contains two extended strands with their sidechains interdigitating in a similar manner as those in a steric zipper. Each molecule of Het-s contributes two such loops that stack on top of each other. This pair of loops then stacks on top of, and beneath, pairs of loops from its adjacent molecules in the fiber. The entire structure is amyloid-like. The β-sheet interactions in Het-s have been selected by evolution, in contrast to some interactions that are found in the spontaneous aggregation of disease-related proteins. Hetero-zippers probably are also found in spontaneous aggregates of proteins, such as those of Aβ, but they have not yet been fully defined at high resolution.

What is the structure of protein segments outside of the amyloid spine?

Although we have atomic structures for some amyloid spines, we do not yet have full atomic structures for whole amyloid fibrils. Full amyloid fibrils are more complicated than the

simple spine structures in that some fibrils appear to contain numerous protofilaments and are complex in structure (Lewandowski et al., 2011; White et al., 2009).

The findings about amyloid spines (Section 2) place severe constraints on fibril models. Given that proteins stack in-register with strands spaced 4.8 Å along the fibril axis, the rest of the protein must be flattened out so that each layer is only 4.8Å high or must somehow sit at the periphery of the spine, where it may extend more than 4.8Å along the axis, avoiding overlap with identical domains. A flattened model for Aβ is shown in Figure 4A, based on solid state-NMR measurements (Tycko, 2011). Each Aβ molecule makes a U-turn, called a beta-arch (Kajava et al., 2010). For longer proteins, it has been proposed that U-turns are linked into a serpentine structure, termed a superpleated β-structure (Figure 4B)(Kajava et al., 2010). In a superpleated β-sheet, the entire protein chain is flattened to fit in one 4.8Å layer of the fibril.

Flattening is not necessary for an amyloid-forming protein to retain globular domains. In the model of a designed amyloid of RNase A (Figure 4C), the domains on the periphery of the spine find space to retain their globular structure (Sambashivan et al., 2005). For larger globular domains, a greater circumference of the fibril and a longer protein linker to the steric zipper is required. This means that fibers formed from larger proteins would be expected to have greater diameters. In short, although the spines of amyloid fibers appear similar, fibrils show a great variety of structural complexity.

What is the basis of amyloid cross-seeding?

The observation that amyloid fibrils have spines composed of steric zippers explains why different proteins, when they enter the amyloid state, give fibrils of similar appearance in electron micrographs. The fibrils are all elongated and unbranched, just as their steric zipper spines are elongated and unbranched. The diameters of the fibrils vary because the lengths of the proteins that form them differ and because the number of protofibrils that twist around each other to form the fibril may differ. Thus, we would expect that cross-seeding of amyloid fibril formation is possible in which the seed is formed from another, but similar amyloid fibril. All steric zippers formed from parallel β-strands have one repeat the same: 4.8 Å in the fibril axis direction; similarly, all anti-parallel zippers have one repeat the same: 9.6 Å in the fibril axis (Figure 1). If the seeding steric zipper is complementary in shape to a segment of the seeded protein in solution, we could expect a hetero-steric zipper to form and to serve as a nucleus, as has been shown in vitro for Aβ and IAPP (Andreetto et al., 2010).

In yeast, cross-seeding has been suggested as the mechanism for the observation that one prion can induce the appearance of another (heterologous) prion. However, heterologous prion interactions can also inhibit prion propagation (Bradley et al., 2002). Similar observations have been made with mammalian prions that can either promote or inhibit PrP aggregation (Nilsson et al., 2010). Cross-seeding and cross-inhibition in vivo also has been reported between ApoAII amyloid (AApoAII) and Serum amyloid A (AA) fibrils (Yan et al., 2007). Moreover, AA amyloid can be cross-seeded in susceptible mice with heterologous β-rich proteins (Lundmark et al., 2005). While cross-seeding presumably results from formation of a hetero-zipper, cross-inhibition might result from a capping of fibril ends, a principle used to develop amyloid interfering compounds (Section 7).

In human neurodegenerative diseases, the coexistance of more than one amyloid deposit is a common observation. For example, in Gerstmann-Sträussler-Scheinker Syndrome, colocalisation of APrP and Aβ in the same amyloid plaque has been described (Miyazono et al., 1992). Similarly, in Familial Danish Dementia Aβ and Dan-amyloid (ADan) co-localize in parenchymal and vascular amyloid deposits (Tomidokoro et al., 2005). In Parkinson-related diseases, α-synuclein and tau inclusions can occur in the same cell and form

common inclusion bodies (Giasson et al., 2003). Although cross-seeding provides an attractive explanation for these observations (Giasson et al., 2003; Morales et al., 2010), definitive proof is lacking, and other explanations are possible. For example, (i) two amyloid deposits may simply develop independently of each other; (ii) there may be saturable cellular fractions for the removal of misfolded proteins, and thus one aggregated protein may indirectly stimulate aggregation of further proteins by monopolizing clearance mechanisms; and, (iii) co-localization of two amyloids is only apparent at the light-microscopic level and reflects common cellular niches prone to protein aggregation, while at the ultrastructural level, true coaggreagtion of the two amyloids may not occur. Yet other observations indicate that the interaction of amyloidogenic proteins in human brain can impede, rather than promote, aggregation. For example, cystatin C colocalizes with Aβ-plaques in Alzheimer's disease, but the finding that cystatin C reduces Aβ plaque formation in transgenic mice suggests a mechanism of cross-inhibition rather than cross-seeding (Kaeser et al., 2007).

What are amyloid polymorphisms and amyloid strains?

The term "strain" is used by microbiologists to denote structurally or functionally variant microbes within a given species. Similarly, prion strains in mammals have been assumed to be variants of PrP aggregates that exhibit characteristic biological properties (Aguzzi et al., 2007; Colby and Prusiner, 2011; Collinge and Clarke, 2007). Whereas the genotype of a microbial strain is encoded by its nucleic acid sequence, that of a prion strain is encoded by the "conformation" of PrP (Colby and Prusiner, 2011; Collinge and Clarke, 2007; Prusiner, 1982). The formation of structurally distinct, polymorphic protein fibers is now recognized as a common property of amyloid-forming proteins (Chiti and Dobson, 2006; Goldsbury et al., 2005; Tanaka et al., 2006). For example, synthetic Aβ can aggregate into structurally diverse amyloid fibrils, which retain their conformational properties and cellular toxicities after repeated passage (Petkova et al., 2005).

Hypotheses for the conformational basis of amyloid strains

Three models for the molecular basis of prion strains and amyloid poymorphs have been proposed on the basis of atomic structures of amyloid-like fibers (Figure 2). The models suggest that strains are based in distinct steric zipper spines of the associated amyloid fibers. The first model is termed *packing polymorphism*, and is illustrated in Figure 2 by the pairs of zippers connected by double-headed arrows. In packing polymorphism, an amyloid segment packs in two or more distinct ways, producing fibrils with different structures and distinctive properties. The simplest form of packing polymorphism is a registration shift in which the two sheets forming the steric zipper in the second polymorph shift their interdigitation from that in the zipper of the first polymorph. Because the nature and position of the sidechains on the outer surface of the fibers differ in the two polymorphs, the properties of the fibers must be different (Greenwald and Riek, 2010; Wiltzius et al., 2009). Thus, in packing polymorphism, one sequence forms two or more "conformations".

The second structural model for strains is termed *segmental polymorphism*. In segmental polymorphism, two or more different segments of an amyloid protein are capable of forming steric zipper spines. Figure 2 shows several segments from Aβ which form different steric zippers. Fibrils formed with different steric-zipper spines will each have distinctive properties. Proteins particularly rich in different segments able to form steric zippers include Aβ (Colletier et al., 2011), IAPP (Wiltzius et al., 2009) and PrP (Sawaya et al., 2007).

In a third type of amyloid polymorphism, *heterosteric zippers*, the zipper is formed from the interdigitation of non-identical beta sheets. Though not yet seen in X-ray structures at the atomic level, heterotypic interactions between sheets are observed in the constrained models

derived from solid-state NMR and cyro-EM (Greenwald and Riek, 2010; Schmidt et al., 2009; Tycko, 2011). The existence of such *hetero*amyloid spines, in addition to self-complementary spines, greatly increases the number of potential amyloid polymorphs and prion strains.

The hypothesis that distinct steric zipper structures are at the basis of amyloid fiber polymorphism and prion strains is consistent with other observations about steric zippers. Steric zippers can be extremely stable. For example the steric-zipper formed by the segment of Sup35 with amino acid sequence GNNQQNY could not be dissolved by 5% SDS nor 4M urea; dissolution required 100% formic acid, 4M guanidinium hydrocholoride, or 0.5 M NaOH (Balbirnie et al., 2001). Thus, steric zippers share with prion strains robust "conformations" that can conceivably be transmitted from cell to cell or organism to organism. Another similarity between steric zippers and prion strains is that environmental conditions seem to affect the formation of both. For example, incubation of the yeast prion Sup35 at either 4° or 37° produces different prion strains in yeast (Tanaka et al., 2004). Similarly, the differing steric zippers formed from the same protein segment in Figure 2 were created by incubating the segments under different solution conditions.

Amyloid morphotypes

In the brain, Aβ deposits are heterogeneous in histopathological appearance and biochemical composition, both within and among brain regions and patients (Maarouf et al., 2008; Tekirian et al., 1998; Thal et al., 2006). Aβ aggregation can occur in association with the vasculature or in the brain parenchyma as amyloid plaques. Point mutations within the Aβ sequence can either lead to vascular amyloid, amyloid plaques, or both (Herzig et al., 2006). Vascular and parenchymal Aβ deposits differ in the ratio of deposited Aβ ending at amino acid 40 to Aβ ending at amino acid 42 (Herzig et al., 2006). Plus, the Aβ40 : Aβ42 ratio has been linked to different neurotoxicities and clinical Alzheimer's disease onset (Duering et al., 2005; Kumar-Singh et al., 2006; Kuperstein et al., 2010). In addition, Aβ displays length variations due to truncations at the N terminus (e.g., Aβ starting at residue 3, 11, or 17) and variations in posttranslational modifications (e.g., isomerization, pyroglutamyl formation, phosphorylation, nitration). All these factors can profoundly influence Aβ aggregation and histopathological appearance of the amyloid (De Strooper, 2010; Kumar et al., 2011; Kummer et al., 2011; Miravalle et al., 2005; Tekirian et al., 1998). A predominance of N-truncated and post-translationally modified Aβ distinguishes Aβ-deposits in Alzheimer's disease compared to normal aging and mouse models (Kuo et al., 2001; Piccini et al., 2005). Although it remains difficult to study the conformational state of Aβ in vivo, indirect measures using luminescent conjugated polythiophene probes that detect particular amyloid conformations suggest the occurrence of conformationally distinct Aβ deposits in brain (Nilsson et al., 2007) (Figure 5). Luminescent conjugated polythiophenes have also been used to discriminate prion strains (Sigurdson et al., 2007).

Different Aβ morphotypes in the brain may indicate that local factors influence the Aβ aggregates. They may also represent various stages in the disease process (Thal et al., 2006) or reflect the templated propagation of conformationally distinct seeds (Levine and Walker, 2010). Although these possibilities are not mutually exclusive, the third explanation has gained momentum by the finding that Aβ morphotypes can be transmitted to transgenic mice overexpressing the Aβ precursor protein (AβPP) in vivo (Meyer-Luehmann et al., 2006). These observations are reminiscent of previous transmission studies using transgenic mice that overexpress PrP and deposit PrP amyloid (Peretz et al., 2002), suggesting that the characteristics of prion strains may also apply to multimeric Aβ. However, the link between Aβ conformational variants and distinct clinical subtypes of β-amyloidoses is still lacking.

Hetereogeneous amyloid morphotypes are also observed in other amyloidoses. Transthyretin (TTR) amyloid deposits show variations in fibrils made of full-length versus C-terminal fragments of TTR (Bergstrom et al., 2005), and in familial cases, TTR amyloid deposition varies in the ratio of incorporated wildtype versus mutant TTR (Ihse et al., 2011; Ihse et al., 2008). Strikingly, such amyloid heterogeneity is associated with the organ tropism (i.e., that the amyloid preferentially deposits in particular organs) and clinical manifestation of TTR-amyloidoses (Westermark and Westermark, 2010). Similarly, length variants of the AA protein characterize two different histopathological AA amyloid patterns in the kidney with distinct clinical phenotypes (Westermark et al., 1989). In the brain, tau and α-synuclein inclusions reveal histopathological heterogeneity that is diagnostic of the various tauopathies and α-synucleinopathies, respectively (Goedert et al., 2010). Consistently, α-synuclein and tau fibrils in vitro exhibit conformational diversity (Frost et al., 2009; Heise et al., 2005). Although recent studies have reported the remarkable transmission of disparate proteopathic lesions in transgenic mice (see Section 8), solid evidence for the hypothesis that the heterogeneous disease phenotypes are the result of the (prion-like) templated conversion of conformationally distinct TTR, AA, tau and α-synuclein seeds is still lacking.

Amyloid toxicities and animal models

Not all amyloids are toxic. First described in bacteria, fungi, and yeast, and more recently in mammals, amyloids can function in the formation of biofilms, the binding and storage of peptide hormones, the formation of melanin formation, or the launch of an antiviral innate immune response (Chapman et al., 2002; Fowler et al., 2007; Hou et al., 2011; Maji et al., 2009). The type of amyloid and the controlled growth conditions may account for the lack of toxicity of so-called *functional amyloids* (Greenwald and Riek, 2010; Watt et al., 2011).

However, most amyloid formation in mammals occurs with aging and is associated with diseases commonly referred to as *protein misfolding diseases, aggregation diseases, proteopathies*, or more specifically *amyloid diseases* or *amyloidoses* (Chiti and Dobson, 2006; Selkoe, 2003). An association of a given amyloid with a disease does not necessarily denote causality. However, a causal relationship between the amyloid formation and amyloid toxicity is suggested from familial cases in which a pathogenic mutation leads to an overproduction of the amyloidogenic protein or enhances the propensity of the protein to aggregate (Table 1). It remains unclear which step of the amyloid formation cascade is toxic, and this step may be different for the various amyloid diseases.

Amyloid toxicity can result from losing the function of a protein or from the sequestration or mislocation of other proteins (Olzscha et al., 2011). This latter mechanism may be the toxicity mechanism for the RNA-binding proteins TDP-43 and FUS (Mackenzie et al., 2010) although their classification as amyloids is uncertain. However, for most amyloid diseases, a gain of toxic function remains a favored hypothesis.

In search of the toxic amyloid species

Despite the longstanding knowledge that amyloids are associated with disease (Cohen and Calkins, 1959), we still lack a clear understanding of how amyloids lead to dysfunction, aside from the instances in which amyloids disrupt tissue structure and organ function via simple mass action (Pepys, 2001; Westermark, 2005). This mass action mode of toxicity may well be the most important one for most systemic amyloidoses and for the amyloid associated with the cerebral vessels (cerebral amyloid angiopathy, CAA) (Figure 6). CAA of various types (Aβ, ADan, British amyloid [ABri], Cystatin C amyloid [ACys]) all result in a thickening of the vascular basal lamina, loss of smooth muscle cells, perivascular inflammation, and eventually vessel wall rupture and hemorrhages (Revesz et al., 2009). Similar appearances and toxicities of CAA (independent of the amyloid type) are also seen

in Aβ- and ADan-transgenic mouse models (Calhoun et al., 1999; Coomaraswamy et al., 2010). Moreover, correlations between CAA severity and hemorrhage frequency was found in humans and mouse models, suggesting that the mass of amyloid fibrils may be the most important parameter mediating vascular toxicity (Dierksen et al., 2010; Maeda et al., 1993; Winkler et al., 2001).

Other amyloid deposits may not be the predominant toxic entity per se. In Alzheimer's disease autopsy material, the soluble Aβ species correlate more strongly with the degree of dementia than does the mass of Aβ plaques (Haass and Selkoe, 2007). Indeed, a variety of soluble Aβ multimeric species (e.g., dimers, timers, dodecamers and larger oligomers) have been isolated from the Alzheimer's disease brain, and they induce synaptic toxicity and dysfunction, both in cell culture and when injected into the rodent brain (Lesne et al., 2006; Shankar et al., 2008). Similarly, synthetic, multimeric Aβ appears to be more toxic than Aβ monomers or fibrils (Haass and Selkoe, 2007; Lambert et al., 1998; Ono et al., 2009), but it is often unclear how the synthetic Aβ species relate to the in vivo counterparts (Meyer-Luehmann et al., 2006; Paravastu et al., 2009). Also, for tau and α-synuclein, soluble oligomeric species appear to be more toxic than the corresponding amyloid fibrils (Haass and Selkoe, 2007; Spires-Jones et al., 2011; Winner et al., 2011).

The physicochemical properties of the toxic oligomeric species are not well understood, and a consistent nomenclature is needed (Glabe, 2008). It is generally assumed that the greater toxicities of oligomers are mediated by their unique structural features (Campioni et al., 2010). The higher relative toxicity of small soluble oligomeric species, however, may also mirror the greater diffusion capability of such small aggregates through the tissue and into various compartments. Along the same lines, the relatively lower toxicity of amyloid fibrils may reflect the fact that many of the toxic structural entities of the fibril are buried in the amyloid mass (Haass and Selkoe, 2007; Keshet et al., 2010).

For Aβ toxicity, both receptor-mediated interactions and non-receptor-mediated membrane interactions have been described (Roychaudhuri et al., 2009; Yankner and Lu, 2009). The most significant toxicity of Aβ is towards the synapse. This is consistent with the profound loss of synapses in the Alzheimer's disease brain and the observation that oligomeric Aβ species inhibit LTP, an electrophysiological correlate of memory formation (Shankar and Walsh, 2009). Soluble Aβ species bind to post-synaptic structures and interact with various putative ligands, such as PrP, NMDA receptor, EphB2, or downstream signaling events (Cisse et al., 2011; Lacor et al., 2007; Lauren et al., 2009; Snyder et al., 2005; Wei et al., 2010), but their in vivo relevance for Alzheimer's disease pathogenesis is still unclear. Non-receptor membrane cytotoxicity for Aβ has been suggested through the insertion of Aβ oligomers into membranes, resulting in membrane disruption, possibly with the formation of cation-sensitive ion channels and dysregulation of calcium homeostasis (Glabe and Kayed, 2006; Roychaudhuri et al., 2009). Similar observations have been made with other oligomeric amyloid intermediates, suggesting that membrane disruption may be a more general mechanism in which amyloidogenic proteins exert their toxicity (Glabe and Kayed, 2006; Hebda and Miranker, 2009; Stefani, 2010).

Nevertheless, bona fide amyloid lesions in the brain, such as Aβ plaques, neurofibrillary tangles, and Lewy bodies, are not benign and are probably in equilibrium with their soluble proteinaceous constituents. For example Aβ plaques are responsible for local neuritic dystrophy (Figure 6), gliosis, and eventually can lead to disturbed neural network activity (Hedden et al., 2009; Tsai et al., 2004). Aβ plaques may also serve as a source of the more toxic and soluble Aβ assemblies, consistent with the view that a dynamic continuum of the various amyloid intermediates, not a given protein entity, elicits toxicity (Jan et al., 2011; Martins et al., 2008; Selkoe, 2011; Wogulis et al., 2005)

The value of animal models of human amyloid diseases

Spontaneous amyloidoses do not occur in typical laboratory animals, with the exception of ApoAII amyloidoses in some genetically-defined mouse strains.(Higuchi et al., 1993). Aged non-human primates and some other higher order mammals can develop β-amyloidosis and tau fibrillary inclusions in the brain; however, these animals do not develop the clinical signs of the human diseases (Jucker, 2010). Aged vervet monkeys, but not other non-human primates, can spontaneously develop TTR-amyloidosis. In vervet monkeys, spontaneous amyloidosis has been linked to the TTR lle122 allelic variant, which is also a disease-causing mutation in humans (Ueda et al., 2011). AA amyloidosis caused by inflammation or infection does occur in animals (Gruys, 2004).

The animals models of amyloidoses currently used are largely confined to genetically engineered mouse models. Most models bear transgenes that overexpress the amyloidogenic human protein, or, more often, its mutant counterpart that characterizes the familial forms of the disease (Table 1). This transgene approach has successfully modeled most cerebral amyloidoses (including tauopathies, α-synucleinopathies, prion diseases) and systemic amyloidoses (such as TTR-, gelsolin-, and IAPP-amyloidosis) (Buxbaum, 2009; Jucker, 2010; Page et al., 2009; Wadsworth et al., 2010). In all cases, overexpression of the amyloidogenic human protein appeared necessary because mice with a simple knock-in of the human amyloidogenic proteins do not spontaneously develop amyloid.

These mouse models are extremely valuable for the understanding of the amyloid aspect of the diseases although the models rarely fully recapitulate the clinical phenotype and neurodegeneration observed in humans. Nevertheless, the models have been successfully used to evaluate preclinical amyloid-modifying therapies (Jucker, 2010). Moreover, the incompleteness of most mouse models in recapitulating the entire spectra of the human disease has often been helpful for deciphering and understanding the complexity of the pathomechanisms of the diseases (Jucker, 2010).

Can amyloid formation be inhibited?

Chemical interventions

Discovery of chemical interventions against amyloid conditions have taken at least four different paths. One of the most promising paths is stabilization of the structure of the soluble form of a protein, diminishing the rate at which it is likely to undergo conversion to the amyloid state. The pioneering demonstration of this strategy was on TTR. TTR is a homotetramer that carries serum retinol binding protein and thyroid hormones, such as thyroxine. In several amyloid diseases, one of many mutations can destabilize TTR, leading to fibrous deposits in the heart and peripheral nerves. Using structure-based design, several potent and specific binders to the TTR hormone pocket have been described that inhibit fibril formation (Klabunde et al., 2000). The same strategy could be applied to other amyloid forming proteins that have a stable native structure.

A second approach is to screen for small molecules that disrupt fibril and oligomer formation. This enormous topic is worthy of a review on its own. Writing in 2007, Necula et al. (Necula et al., 2007) list 16 screening studies for molecules that inhibit fibrils of Aβ, and they go on to study molecules that inhibit formation of Aβ oligomers. In a recent study using small-molecule microarrays (Chen et al., 2010), 79 compounds were discovered that rescue cells from cytotoxicity. The authors suggested that a mechanism of rescue is that a compound can accelerate Aβ aggregation past an early-forming toxic oligomer. Screening for compounds that inhibit fibrils of tau is also an active area (Pickhardt et al., 2005). Despite this huge effort, we are unaware of compounds that have yet been found to be efficacious in treating Alzheimer's disease.

A third approach uses the self-assembling property of amyloid fibers to poison the growth of amyloid fibers with peptides (Sciarretta et al., 2006). A biological system which apparently uses this strategy is Het-S, a native inhibitor of the HET-s prion (Wasner et al. 2008). Adoption of this principle for chemical design is based on our understanding that β-sheets are the fundamental organizing principle of amyloid fibrils and that fibrils grow by addition of new strands to the sheets. The fiber is poisoned or "capped" by adding a peptide that acts as a new strand via hydrogen bonding to the sheet at the fibril's growing edge but prevents the subsequent addition of another amyloid molecule. Early on it was shown that the segment of Aβ with sequence KLVFF inhibits Aβ aggregation (Tjernberg et al., 1996), but this peptide itself forms steric-zipper fibrils (Colletier et al., 2011). More recent work has emphasized modifications of the blocking peptide, both to inhibit fibrillation of the target protein and to prevent self-fibrillation of the blocker (for review see Sciarretta et al., 2006). Depending on the system it has been found that blocking fiber formation could either increase or diminish the concentration of toxic oligomers (Sciarretta et al., 2006). A protein domain that has been found to inhibit fiber assembly of Aβ is the N-terminal domain of myelin basic protein (Liao et al., 2010).

The fourth approach is a variation of the third: inhibit fiber growth by the structure-based design of peptides targeted to block the ends of fibrils. This approach has become possible by the determination of the atomic structures of steric zippers, and it has been shown to be effective for inhibition in vitro of two different amyloid fibers (Sievers et al., 2011). Based on the structure of the steric zipper segment of the tau protein with sequence VQIVYK, an all D-amino acid inhibitor was designed to cap the ends of VQIVYK fibrils. This 6-residue all D-peptide was found to inhibit fibrillation of both VQIVYK fibers and constructs of tau. The fact that this blocker designed to cap steric zippers also blocks fibrillation of the parent protein of the zipper strengthens the hypothesis that steric zippers form the essential spine of amyloid fibrils.

Biological interventions

Amyloid formation depends on the concentration of the amyloid-forming proteins. Thus, inhibiting the generation of amyloidogenic proteins or of their precursors is a primary therapeutic strategy. For example, suppression of the inflammatory process responsible for serum amyloid A protein (SAA) overproduction is a therapeutic option for AA amyloid and elimination of B cell clones that overproduce immunoglobulin light chains represents a therapeutic option for AL amyloid (Pepys, 2001). Likewise, genetic variability in the expression of amyloidogenic proteins at slightly higher levels than normal may contribute to the risk of amyloidoses (Singleton et al., 2004). However, because of the incomplete mechanistic understanding of such genetic variability, no therapeutic strategies to reduce protein expression at the genetic levels have so far been developed.

Some amyloid-forming proteins are derived from longer precursor proteins that need cleavage to become amyloidogenic. The best-known example is AβPP that is sequentially cleaved by β-secretase and γ-secretase to release the Aβ peptide (De Strooper, 2010). Secretase inhibitors are currently in clinical trials, but current inhibitors may need refinement to avoid unwanted side effects, i.e. blocking cleavage to other substrates (De Strooper, 2010). Other amyloids (e.g. AA, AApoAII, ACys) also consist of protein fragments of larger precursors; however, it is not always clear whether such fragmentation is necessary for the amyloidoses or whether truncation is a secondary event without physiological significance (Westermark, 2005). While the relationship between posttranslational modification of amyloids and disease pathogenesis in general remains ill defined, inhibiting pyroglutamyl formation is pursued as a therapeutic target for Alzheimer's disease (Schilling et al., 2008).

The finding that vaccination of AβPP transgenic mice can prevent and reduce cerebral β-amyloidosis has stimulated the development of antibody-based immunotherapeutics for Alzheimer's disease (Brody and Holtzman, 2008). Although mechanistically still unclear, antibodies directed toward Aβ gain access to the brain where they bind to soluble and/or deposited Aβ species and promote their degradation. Phagocytosis of microglia as well as other mechanisms have been proposed for amyloid removal (Brody and Holtzman, 2008). Subsequent human immunotherapy trials showed also a reduction of Aβ deposits in brains of Alzheimer's disease patients, as predicted from the preclinical mouse work (Jucker, 2010). However, unwanted side effects and lack of cognitive improvements in "immunized" Alzheimer's disease patients must be overcome in future trials by early preventative, rather than therapeutic, interventions (Golde et al., 2011; Selkoe, 2011). Immunization against other amyloids, such as PrP^{sc}, Tau, and α-synuclein have also been reported in transgenic mouse models (Aguzzi and O'Connor, 2010; Chai et al., 2011; Masliah et al., 2011). Along the same line, immunological depletion (in addition to pharmacological depletion) of serum amyloid P component (SAP) has been developed as a therapeutic strategy. SAP is claimed to stabilize amyloid fibrils and to be associated with most amyloids (Bodin et al., 2010; Pepys et al., 2002).

8. Are amyloid diseases transmissible?

In vitro assembly of amyloid fibrils can be initiated or accelerated by the addition of an amyloid seed (nucleus). Although originally suggested for PrP and Aβ (Jarrett and Lansbury, 1993), nucleated protein aggregation is likely a phenomenon that is common to all amyloids. Thus, from the view of a structural biologist, the features that define the amyloid state, in themselves render amyloids as 'transmissible', i.e., an amyloid nucleus can template the aggregation of a homologous protein. Nevertheless, only prions have unequivocally been shown to be infectious at the level of organisms, in which an exogenous, proteinaceous agent (the prion) initiates disease (Aguzzi and Rajendran, 2009). Why is this, and are we sure that infectivity at the organism level is restricted to prions?

A prion-like infectious cycle has been reported for AA amyloid (Westermark and Westermark, 2010). AA amyloid can be transmitted to susceptible hosts through a variety of inoculation routes. Transmission of AA amyloidosis between organisms (horizontal transmission, or 'infectivity' in the view of microbiologists) has not been proven unequivocally, but it appears to occur in captive cheetahs through fecal-oral transmission (Zhang et al., 2008). For mouse ApoAII amyloidosis, mother-to-offspring transmission (vertical transmission) has been demonstrated under experimental conditions (Korenaga et al., 2006).

In age-related neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, disease-specific proteopathic deposits occur in predictable region-specific and temporal patterns (Jucker and Walker, 2011). Such observations raise the possibility that the occurrence of the deposits occurs via prion-like propagation and spreading. Indeed, intracerebral inoculation of young, human-sequence AβPP-transgenic mice with brain extract from Alzheimer's disease patients or aged AβPP-transgenic mice induces β-amyloidosis in a time- and concentration-dependent manner. The induced Aβ deposits first occur in the inoculated brain region, and then spread into neighboring areas often along anatomical and neuronal pathways. After prolonged incubation periods β-amyloid induction spreads throughout most of the entire brain (Hamaguchi et al., 2012; Jucker and Walker, 2011). The amyloid-inducing factor in the brain extract is likely aggregated Aβ in a conformation or polymorph that is most easily generated in the brain, because synthetic Aβ does not show comparable seeding activity in vivo (Langer et al., 2011; Meyer-Luehmann et al., 2006). Similar inoculation experiments have also been performed with α-synuclein- and

tau-containing brain extracts; indeed, prion-like propagation spreading of the induced lesions in susceptible mice has also been suggested (Clavaguera et al., 2009; Mougenot et al., 2011). The mechanism by which intracytoplasmic lesions propagate remains puzzling, and its elucidation may provide new insights into basic cell biological processes (Aguzzi and Rajendran, 2009).

More recently, it has been shown that Aβ-containing brain extract can also induce cerebral β-amyloidosis if the extract is applied intraperitoneally (Eisele et al., 2010). These remarkable findings imply that the proteopathic seed can travel from the periphery to the brain. AA amyloid also has been suggested to spread among organs, possibly via the blood (Westermark and Westermark, 2010). Thus, in susceptible hosts and under experimental conditions, transmission, propagation, and spreading of amyloid seeds within and between organs appears possible.

Because of the unusual nature of proteopathic agents and their obligatory relationships to the host, the infectivity of protein-based diseases between individuals is refractory to verification by Koch's postulates, which were designed to assess the infectivity of microbes (Walker et al., 2006). Hence, it has been suggested that the postulates be modified to account for the physicochemical characteristics of the infectious protein and to recognize the importance of the host in governing susceptibility to the disease (Walker et al., 2006). Naturally, epidemiological evidence also should be brought to bear on this issue. According to these criteria, compelling evidence for the infectivity of prion diseases has been presented (Colby and Prusiner, 2011), but for non-prion amyloidoses such evidence is still largely lacking. Thus, in future studies, there is a need for the epidemiological assessment of the relevance of the experimental amyloid transmission studies. Moreover, there is a need to study host susceptibility traits that allow proteopathic seeds to become "infectious." It may well turn out that, although mechanistically similar to prionoses at the molecular level, the susceptibility of humans to the horizontal transmission of non-prion amyloid diseases is clinically insignificant under normal conditions. Nevertheless, the remarkable prevalence of this pathogenic principle suggests that common therapeutic strategies might be directed toward a variety of currently untreatable diseases.

9. Outlook

Although the research reviewed here portrays only part of the rapidly advancing knowledge about amyloid diseases, it may be sufficient to define some of the critical questions for the next phase of work.

At the molecular level, we still lack high resolution knowledge of amyloid oligomers in all but the simplest fibers. Recent work has begun to reveal the structural basis of prion strains. Now we need to establish whether amyloid strains play a physiologically significant role in other amyloid diseases and if so, we need a fuller view of amyloid polymorphism. Furthermore, we need a better molecular understanding of the assembly pathways from functional proteins to amyloid oligomers and fibers and their pathways for disassembly. At the level of cellular biology, we need to learn which biological cofactors stabilize and destabilize amyloid structures, and we need to fill in more details of metabolic and signaling pathways that regulate degradation and disposal of amyloids.

An urgent need is the further development of structural and physiochemical techniques that permit the analysis of aggregated proteins in cells and living tissues, as opposed to extracted amyloid or recombinant amyloid. A remaining mystery is the enormously greater potency of seeding by amyloid and prions extracted from tissues compared to recombinant amyloids. Is this greater potency due to undetected biological cofactors in the extracted material, or has the extracted protein been templated into some structure in vivo which the recombinant,

apparently identical, material cannot achieve? Can biological factors be discovered which can convert recombinant proteins to forms that are as potent as extracted amyloid?

Another mystery involves the mechanisms and pathways for cellular toxicity of amyloid. Are there common mechanisms of toxicities, or do mechanisms differ between systemic and cerebral amyloid diseases? What are the toxic structures? Are oligomers distinct from small fibers, and what accounts for their toxicity? Why can toxicity of PrP be recapitulated in animal models whereas the toxicity of Aβ in animal models is comparatively modest? What is different about functional amyloids that render them non-toxic?

Finally, the implications for disease of the recently reported experimental transmission of non-prion amyloids need to be established. Are similar or different structures responsible for toxicity and transmission? Can amyloid in the environment seed human diseases, and if so, what protective measures are necessary?

As answers to these questions emerge, a class of diseases that afflict and kill millions will be understood and perhaps controlled by preventative and therapeutic interventions.

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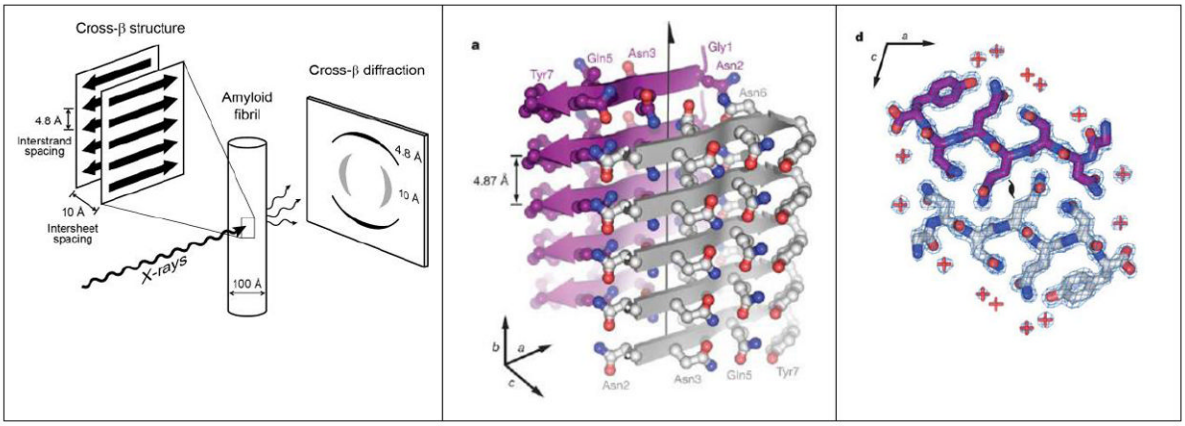

Figure 1. Properties of Amyloid Fibers

(**A**) The characteristic cross-β diffraction pattern observed when X-rays are directed on amyloid fibers. The diffuse reflection at 4.8 Å spacing along the meridian (vertical) shows extended protein chains running roughly perpendicular to the fibril and spaced 4.8 Å apart. The even more diffuse reflection at ~10 Å spacing along the equator (horizontal) shows that the extended chains are organized into sheets spaced ~10 Å apart. For less well oriented fibrils, both reflections blur into circular rings. (**B**) The steric zipper structure of the sequence segment GNNQQNY from the yeast prion Sup35. Five layers of β-strands are shown of the tens of thousands in a typical fibril or microcrystal. The front sheet shows the protein backbones of the strands as gray arrows; the back sheet is in purple. Protruding from each sheet are the sidechains. The arrow marks the fibril axis. (**C**) The two interdigitating β-sheets are viewed down the axis. Water molecules, shown by red + signs are excluded from the tight interface between the sheets. Red carbonyl groups and blue amine groups form hydrogen bonds up and down between the layers of the sheet (Nelson et al., 2005). Panels B and C reprinted from Nelson et al. 2005.

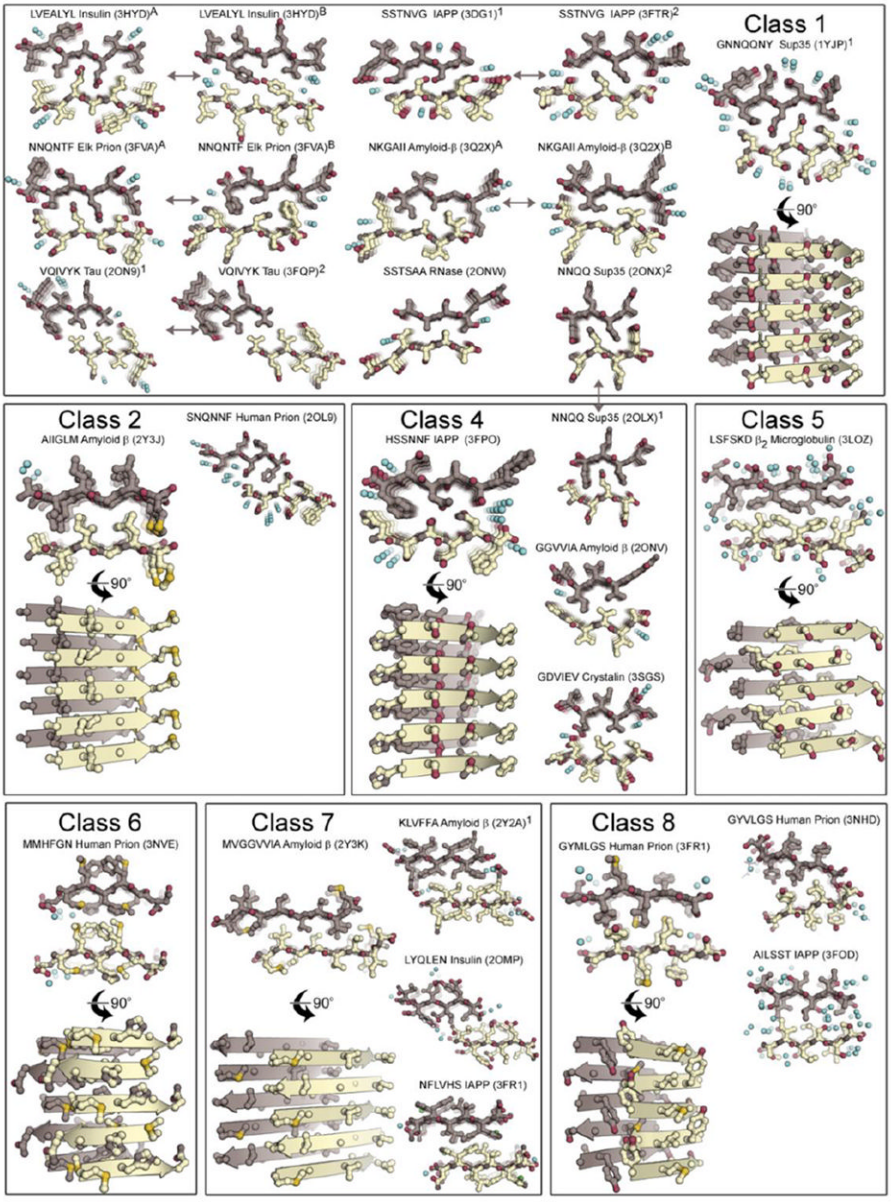

Figure 2. Steric-zipper protofilaments

Twenty-eight atomic structures of steric-zipper protofilaments from amyloid-forming proteins, determined by X-ray diffraction. All are viewed projected down the protofilament axis, revealing the two sheets (one gold and one purple) with their interdigitated sidechains. Selected zippers are also viewed perpendicular to the protofilament axis, with five layers of β-strands shown with backbones as arrows. Water molecules are shown as aqua spheres; notice their absence from the interfaces between the paired sheets.

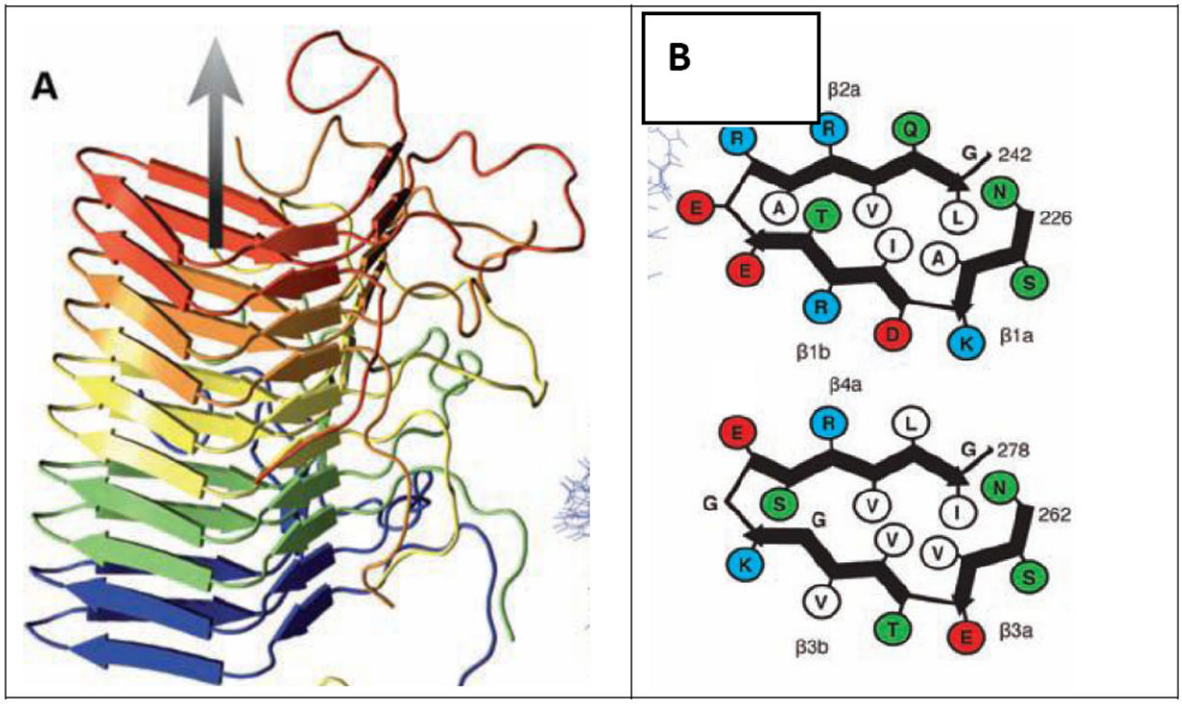

Figure 3. Structure of a hetero-zipper

The solid-state NMR-derived structure of Het-s shows hetero-zippers (Wasmer et al., 2008). (**A**) The protein chain of each molecule (in a single color) contains six β-strands, organized in double loops. The double loops of adjacent molecules sit on top of one another, hydrogen bonded up and down. (**B**) The two layers are shown schematically with sidechains represented as circles. Each layer may be regarded as a hetero-zipper, in which the sidechains of opposing strands interdigitate. Figure reprinted from Wasmer et al. 2008.

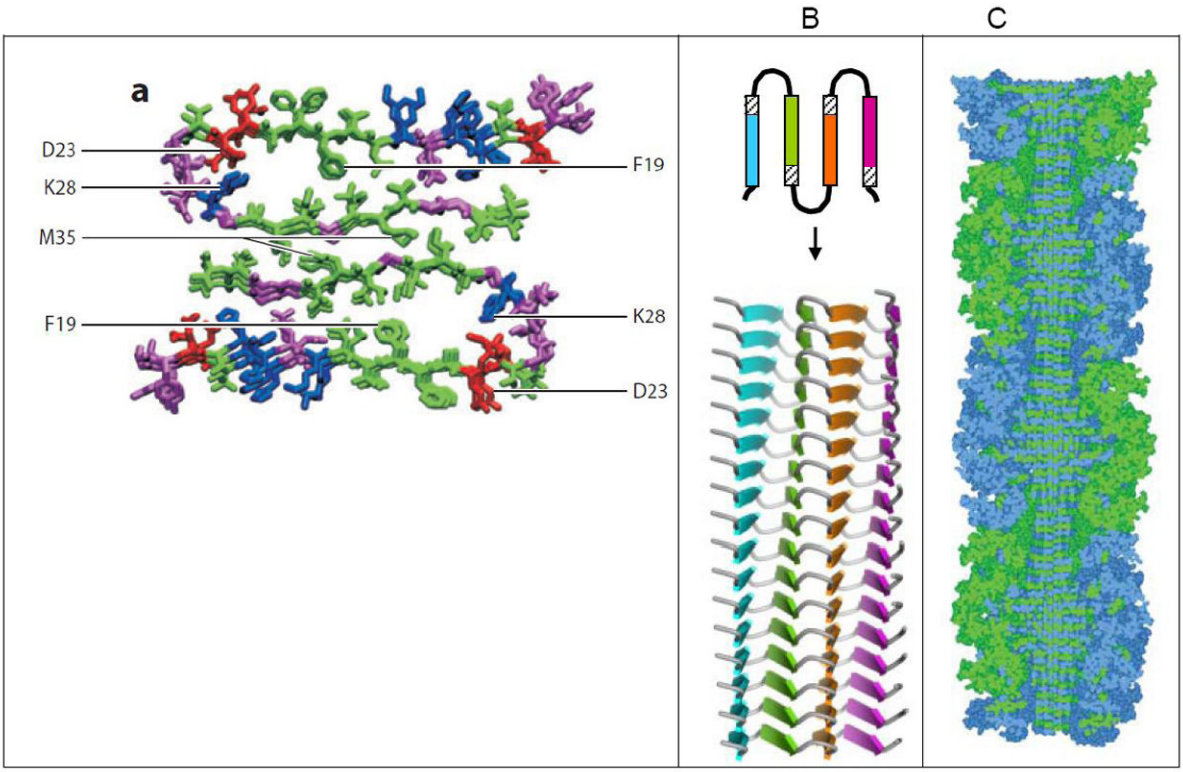

Figure 4. Models for amyloid fibrils larger than a single steric-zipper spine

(**A**) Model for Aβ1-40 based on solid-state NMR data with additional constrains from electron microscopy (Tycko, 2011). The view is down the fibril axis, showing two molecules of Aβ, each with a U-turn or "β-arch". Where the green segments of the two molecules abut, they appear to form a homo-steric zipper (Class 1 in Figure 2), and a hetero-zipper could exist between the two arms of each U. Both types of steric zipper need to be confirmed by higher resolution structures. (**B**) A proposed structure for longer amyloid proteins is a "superpleated β-structure" (Kajava et al., 2010), in which the protein chain forms several U-turns/beta-arches. The view of the upper diagram is down the fibril axis; the view of the lower is perpendicular to the fibril axis. In the lower diagram, each protein chain is hydrogen bonded to the ones above and below (not shown). Hetero-zippers may exist between pairs of differently colored β-strands. This type of structure has been proposed for several proteins in the amyloid state including Ure2p, Sup35p, and α-synuclein. (**C**) A model for a designed amyloid of ribonuclease A with ten glutamine residue inserted between the core and C-terminal domains (Sambashivan et al., 2005) based on X-ray and electron microscopy data and steric constraints. The view is perpendicular to a cut-away of the fibril. The twisting steric zipper can be seen at the center. Globular subunits of ribonuclease A, which are essentially in their native conformation, are at the periphery. The amyloid-like fibrils of this designed amyloid show enzymatic activity, confirming that ribonuclease molecules retain native-like structure.

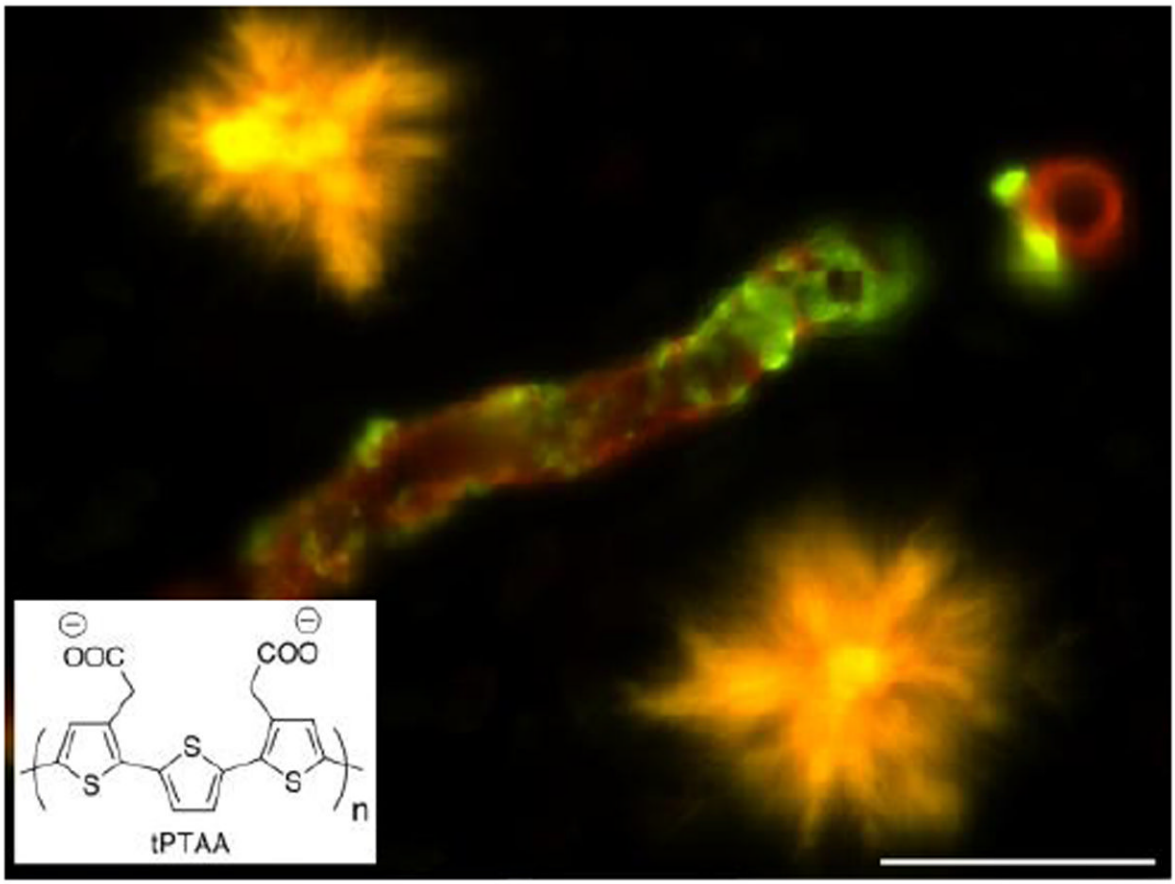

Figure 5. Rainbow amyloid

Novel amyloid dyes can be used as surrogate probes of the supramolecular structure of protein aggregates. Shown are Aβ plaques (yellow) and Aβ amyloid angiopathy (green) in an AβPP transgenic mouse (carrying the AβPP Swedish and AβPP Dutch mutation). Note the different spectral signatures upon staining with the luminescent conjugated polythiophene tPTAA (bottom left). The image was recorded using a combination of green and red filters. Scale bar = 20um.

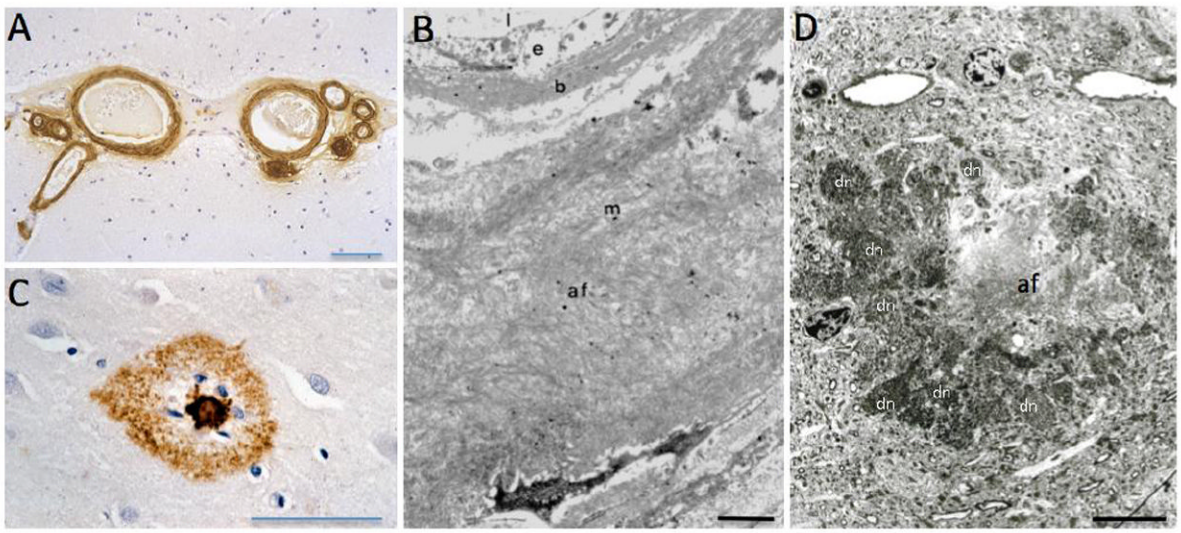

Figure 6. Histopathology of cerebral β-amyloidosis

(A) Aβ immunostaining (brown) reveals severe cerebral amyloid angiopathies (CAA) in superficial cortical vessels in a human case. (B) Ultrastructural analysis of Aβ fibrils (af) in the vessel wall of an arteriole with CAA. Note that the amyloid has displaced nearly the entire vascular wall, disrupting normal vessel-neuron communications (b, basal lamina; e, endothelial cells; l, lumen; m, media; (reprinted with permission from Yamada et al., J Neurol 234: 371-6, 1987). (C) Aβ-immunostaining (brown) of an amyloid plaque in a human Alzheimer's disease case. Note the dense core and glial nuclei (blue) surrounded by a halo of diffuse Aβ immunostaining. (D) Ultrastructure of an Aβ plaque. Note the dense amyloid core with the amyloid fibrils (af) surrounded by numerous dystrophic neuritis (some are labeled with "dn"). The Aβ plaque is from an AβPP transgenic mouse brain due to better tissue preservation compared to postmortem human tissue. Scale bars = 100 um (A), 1 um (B), 50 um (C) 5 um (D).

Table 1

Familial human amyloid diseases in which the mutations promote the formation of amyloid

Disease	Variant protein	Amyloid
Alzheimer's disease	AβPP, PS1/2	Aβ
Hereditary cerebral hemorrhage with amyloidosis, Dutch type	AβPP	Aβ
Hereditary cerebral hemorrhage with amyloidosis, Icelandic type	Cystatin C	ACys
Familial British Dementia	BriPP	ABri
Familial Danish Dementia	BriPP	ADan
Parkinson's disease	α-synuclein	ASyn
Frontotemporal lobar degeneration (FTLD)-tau	Tau	ATau
Gerstmann-Sträussler-Scheinker	PrP	APrP
Greutzfeldt-Jacob Disease	PrP	APrP
Amyotrophic lateral sclerosis	SOD1	ASOD1
Transthyretin familial amyloidosis	TTR	ATTR
Hereditary lysozyme amyloidosis	Lysozyme	ALys
Hereditary fibrinogen A α-chain amyloidosis	Fibrinogen α-chain	AFib
Hereditary ApoAI/II amyloidoses	Apolipoprotein AI/II	AApoAI/II
Finnish hereditary amyloidosis	Gelsolin	AGel