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Structural basis of infectious and non-infectious amyloids

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Abstract

Amyloid fibrils are elongated protein aggregates well known for their association with many human diseases. However, similar structures have also been found in other organisms and amyloid fibrils can also be formed *in vitro* by other proteins usually under non-physiological conditions. In all cases, these fibrils assemble in a nucleated polymerization reaction with a pronounced lag phase that can be eliminated by supplying pre-formed fibrils as seeds. Once formed, the fibrils are usually very stable, except for their tendency to break into smaller pieces forming more growing ends in the process. These properties give amyloid fibers a self-replicating character dependent only on a source of soluble protein. For some systems and under certain circumstances this can lead to infectious protein structures, so-called prions, that can be passed from one organism to another as in the transmissible spongiform encephalopathies and in fungal prion systems. Structural details about these processes have emerged only recently, mostly on account of the inability of traditional high-resolution methods to deal with insoluble, filamentous specimens. In consequence, current models for amyloid fibrils are based on fewer constraints than common atomic-resolution structures. This review gives an overview of the constraints used for the development of amyloid models and the methods used to derive them. The principally possible structures will be introduced by discussing current models of amyloid fibrils from Alzheimer's β -peptide, amylin and several fungal systems. The infectivity of some amyloids under specific conditions might not be due to a principal structural difference between infectious and non-infectious amyloids, but could result from an interplay of the rates for filament nucleation, growth, fragmentation, and clearance.

Introduction

Amyloid fibrils are best known for their association with devastating disorders such as Alzheimer's disease, type II diabetes, and the transmissible spongiform encephalopathies (like Creutzfeldt-Jakob disease and mad cow disease) [1]. But similar structures can be formed by many proteins [2] and small peptides [3]. Conversely, for some proteins, the amyloid fibril even appears to be the structure intended by nature and provide positive functions, e.g. a protective layer for bacteria [4,5]. In medical contexts, the term amyloid is used only for pathologically relevant, extracellular aggregates of human proteins detected by specific histological staining properties [6,7]. Therefore, diseases like Parkinson's disease and Huntington's disease are not considered amyloidoses because, in these cases, aggregation occurs inside of cells. In structural terms, however, all these diseases are very closely related and presently the term 'amyloid fibril' is widely used in structural biology to refer to any structure with the following properties [8]: (i) un-branched protein fibril with diameter in the range from 3 to 10 nm, (ii) high content of β -structure and a cross- β diffraction pattern (see below), and (iii) a core region that is extremely resistant against hydrogen/deuterium exchange, proteases, and chemical denaturation. Most amyloid fibrils also test positive in the traditional

staining assays (e.g. green birefringence with Congo Red), but these tests can give false positives [9].

All amyloid fibrils form in a nucleation polymerization mechanism; i.e. a slow reaction leads to a nucleus (defined as the least stable intermediate) that can further react to form a fibril via the addition of more subunits in a favorable reaction [10]. In many cases agitation can expedite the reaction, most likely by fragmentation of already formed fibrils providing more growing ends [11]. The slow nucleus formation at the beginning of the reaction can lead to extended lag phases, which can be circumvented by the addition of preformed fibrils, referred to as “seeds”. These properties also explain why amyloid fibrils (or seeds) can represent the underlying mechanism of infectious proteins, known as prions. An infectious amyloid may be the underlying agent of the transmissible spongiform encephalopathies, but there is still no final agreement about the exact nature of the neurotoxic and the infectious species in this case [12]. Prions have also been described in some fungal systems [13,14]. Although the fungal prions seem not to have any direct adverse effects to the host cells (comparable to the neurotoxic effects of the mammalian prion protein and many amyloids), the prions in fungi have the tremendous advantage of behaving as non-chromosomal genetic elements (i.e. they are “protein-genes”) and therefore can be studied using the highly developed methods of fungal genetics [15]. Neither the prions nor other amyloid forming proteins show any significant sequence identity to each other, although some of them share unusual features (e.g. large numbers of asparagines and/or glutamines).

High resolution structural information on amyloid fibrils would be important in understanding the mechanistic details of amyloid and prion formation. However, for full-length proteins, such data are very scarce and are currently almost exclusively restricted to amyloid fibrils formed by small peptides. This is mostly due to the inability of x-ray crystallography and solution nuclear magnetic resonance (NMR), the usual high-resolution methods, to address insoluble, filamentous specimens. Therefore, models are fundamental to our understanding of amyloids and prions. Structural constraints (e.g. overall dimensions, distance between specific atoms, etc.) are used together with known structural principles of polypeptides to develop high-resolution models by molecular dynamics similar to model building in solution NMR [16]. While solution NMR structural models are usually made with hundreds of constraints, generally much fewer constraints are used for amyloid models resulting in uncertainties at all structural levels (secondary, tertiary, and quaternary). This review discusses possible experimental constraints that can be used for amyloid fibril models and gives some examples of models suggested for well-studied systems. Finally, the difference between infectious and non-infectious amyloids will be discussed.

Structural information constrains amyloid fibril models at multiple levels

Some large-scale constraints are the overall dimension (i.e. the diameter of the fibril), number and arrangement of protofilaments, mass-per-unit-length, repeat distance, handedness, and polarity of amyloid fibrils. The diameter of fibrils (and protofilaments) can be determined to a reasonable accuracy directly from negatively stained or cryo-electron micrographs. Whereas the extended conformation of short peptides fits into the most commonly observed diameter for amyloid fibrils (3-10 nm), for longer peptides and proteins the fully extended conformation of the complete peptide is unlikely. For example a 40 amino acid peptide spans about 15 nm in the extended conformation, a value that goes far beyond the diameter usually observed for amyloid fibrils. Therefore turn/loop regions are an essential part of amyloid models for larger peptides.

For fibrils with evident helical twist the repeat length can usually be measured from micrographs and the handedness can be obtained from uni-directionally heavy metal

shadowing [17-20]. The handedness can also be determined by atomic force microscopy [19]. Knowledge of repeat length and handedness provides information about the amount and direction of average twist between β -strands in the cross- β structure.

The number of protofilaments is sometimes directly evident from electron micrographs [21], but in other cases the resolution of negatively stained specimens is not high enough and analysis of cryo-specimens is necessary [20]. In a twisted fibril, parts of the structure that have a longer distance to the twist axis undergo stronger distortions, and this must be taken into account in model building. By virtue of these distortions, the twist is thought to restrict the diameter of amyloid fibrils [22]. Generally, helical reconstruction or reconstruction using single particle methods [20,23] can also be used to obtain a low to medium-resolution electron density map of the fibrils that can be further used in adapting possible models.

The mass-per-length of an amyloid fibril can be measured directly by scanning transmission electron microscopy and be used to derive the number of monomers per unit length – an important number for model building [24]. Polarity (directionality) of amyloid fibrils can sometimes be determined directly from micrographs [19]. Another often more accessible way to check for polarity is measuring the growth rates at the two ends of the fibrils [25-28]. Different growth rates indicate a polar fibril (with two different ends), whereas equal growth rates are not necessarily proof of symmetric fibrils; in such cases further studies would be necessary.

One of the defining features of amyloid fibrils is a polypeptide conformation rich in β -structure in a cross- β arrangement. This means that β -strands are perpendicular to the fibril long-axis and arranged in β -sheets running parallel to the fibril axis (Fig. 1 a). Whereas spectroscopic methods, such as circular dichroism and infrared spectroscopy, are used to estimate the overall amount of β -structure, x-ray fiber diffraction is commonly used to show the cross- β arrangement. X-ray diffraction results in a strong meridional 0.47-0.48 nm reflection interpreted as the distance between β -strands and a weaker ~ 1.0 nm reflection commonly interpreted as the distance between β -sheets packed against each other in the fibril [29] (Fig. 1 b). Recently, electron diffraction was used to analyze some amyloid fibrils providing similar results [30-33].

An important step towards the construction of a model is the assignment of secondary structure regions (strands and turns/loops) in the protein. This can be done by hydrogen/deuterium exchange (strand regions should exchange slower compared to turn regions while disordered regions should exchange very fast) followed by analysis in mass spectrometry or solution NMR [34-36]. Another more direct way is to analyze chemical shifts in solid-state NMR experiments of amyloid fibrils with magic angle spinning [35,37,38]. In this case, due to the specific properties of solid-state NMR, often only the very highly ordered strand regions are observed in the spectra and not the more mobile loop/turn regions [39]. Mutation to cysteine and using spin labels in combination with electron paramagnetic resonance spectroscopy (EPR) or fluorescent probes can be used to explore the environment of the mutation site, giving indications whether it is in the tightly folded core structure or in loop or disordered regions [40,41]. In some cases mutational analysis (e.g. mutation to β -structure breaking amino acids like proline or even deletions of regions) can provide clues about secondary structure regions [35,42].

In several cases (especially for large proteins), the amyloid fibril is formed by only part of the protein while the rest of the protein is still attached, often with no obvious changes in its normal structure. This phenomenon has been observed for most prions [21,43,44]. In these cases, the region of fibril formation (in prions called the “prion domain”) can often be defined by limited

protease digestion [45-48], and for fungal prions, by genetic methods [49] and transformation of amyloid fibrils into cells [46,50-52].

The next important step in model formation is to find the general arrangement of β -strands with respect to each other in the β -sheets. Important questions here are: Are the sheets arranged parallel, antiparallel, or mixed? Are the interactions between strands along the hydrogen-bonding direction (i.e. along the fibril axis) intermolecular or intramolecular? If they are mostly intermolecular are they in register or out of register? Fourier transform infrared spectroscopy has been used to decide whether β -structure is mainly parallel or antiparallel, but it has recently been clarified that the distinction cannot be made using this method [53]. Clearer answers to those questions come from solid-state NMR and EPR [37,40,54]. Fluorescence labeled proteins have also been used, but with somewhat lower resolution [41].

In the final step of model building, which is to define the exact topology of the peptide chain (how are the β -strands connected by the peptide chain?), direct measurements of molecular contacts are absolutely required (at this step mostly side chain contacts because main chain interactions have been discussed above). The best sources for this are direct distance measurements from solid-state NMR [37]. Spin labels and FRET measurements with fluorescence labels can also be used [55], but both methods have a lower resolution and introduce bulky groups in the structure. For the structure of A β peptide mutational analysis has been used to identify side-chain interactions [56,57]. Mutations to cysteine residues have also been used to judge solvent accessibility and environment of the mutation site [28,35,41,58].

Other challenges for amyloid fibril models

For most amyloid systems, in addition to direct structural constraints, there are also some properties that the proposed models should plausibly explain. These properties are usually less restrictive than direct structural constraints and can be explained in different ways. One of these properties, high stability, could be explained by large intermolecular contact surfaces between subunits, but could also be explained by normal sized contacts with high complementarity (in shape, charge, etc.). Nucleated polymerization is another of these properties. An important feature that has been observed in some systems is self-propagating polymorphism [59,60]. In these cases, the protein can adopt structurally different amyloid forms that, once formed, can seed themselves, but do not usually induce other forms. This phenomenon leads to so-called variants and species barriers in some of the prion systems [59]. In prion systems, each variant can lead to slightly different phenotypes (in fungal systems this is mostly determined by the amount of residual soluble protein). This phenomenon points to a molecular imprinting mechanism in which a protein subunit adopts the structure of the specific variant while being added at the end of the fibril. Models should explain how this is achieved on the structural level. Another constraint is the observation that complete scrambling of amino acids in the amyloid-forming domain of some yeast prion proteins neither abolished *in vivo* prion formation nor *in vitro* amyloid fibril formation [61,62].

Amyloid fibril structures and possible models

High-resolution information on amyloid fibrils and related structures is only available for small peptides (usually ~10 residues) [3,63-65]. Griffin and coworkers described the exact conformations of an 11-residue peptide fragment of the amyloidogenic protein transthyretin in amyloid fibrils using solid-state NMR [63]. This includes all main chain dihedral angles and all side chains conformations, but the structure does not provide information about the packing of peptides into a fibril. All other structures have been obtained from microcrystals of small peptides [3,64,65]. The exact structural relationship between amyloid fibrils and microcrystals

is not clear [66] and it has been shown for one system that the peptide structures in amyloid fibrils and microcrystal are significantly different [67].

In all cases, the complete peptide adopts an extended β -strand conformation and is arranged in a cross- β fashion and almost all interactions (main chain hydrogen bonding and side chain interactions) are intermolecular. Most peptides are arranged in-register in parallel β -sheets, while some are arranged in register in antiparallel β -sheets. But there are considerable variations in the packing of the β -sheets against each other [3]. One striking observation is a staggered arrangement of β -strands in neighboring β -sheets, i.e. neighboring sheets are staggered by 0.24 nm (half the distance of strands in a sheet) to optimize the packing of side-chains at the interface between β -sheets (Fig 1 c and d).

Models of amyloid fibrils of larger peptides and proteins can be roughly divided into two classes based on the number of intra- versus intermolecular contacts. Models with mostly intermolecular interactions have been proposed for A β (see below) and others. These are principally similar to the structures observed for small peptides, but include more than one extended β -strand and turns connecting these strands. In most of these structures, proteins were observed to be in a parallel in-register arrangement. A favorable and stabilizing interaction between like residues has been suggested as explanation for this arrangement [37,68].

Other models assume larger numbers of intramolecular interactions, effectively resulting in fibrils that involve globular folded subunits interacting with each other through a small number of β -strand contacts. In some of these models, a subunit fold similar to the native structure of the proteins has been suggested [69-73]; another often suggested subunit fold is the parallel β -helix (or, more general, a β -solenoid structure). In these models the protein folds into a β -solenoid structure that then interacts at the edges to form amyloid fibrils basically comprised of a continuous β -solenoid structure (cf. model for HET-s fibrils below). It has been suggested that domain swapping in native-like parts of a protein decorating a cross- β spine might stabilize amyloid structures [74,75]. It is likely that not one single general model applies to all amyloid fibrils but that many of these models are employed in different systems.

Amyloid fibrils of amyloid- β peptide

Amyloid fibrils of the amyloid- β peptide (A β) are found in senile plaques in the brains of Alzheimer's disease patients [76,77]. A β is a 40-42 amino acid residue peptide produced by cleavage from a larger precursor protein. In structural terms, A β fibrils are arguably one of the best studied amyloid fibrils with structural constraints available from many different methods, including electron microscopy, x-ray fiber diffraction, hydrogen/deuterium exchange, limited proteolysis, mutational studies, solid-state NMR, and spin labeling [19,42,56-58,60,78-90]. A β (1-40) fibrils appear usually as a polymorphic mixture of fibrils [60,78,79]. All types of fibrils seem to be formed of ~ 5 nm diameter protofilaments [79]. Twists are left-handed [19, 78] and the mass-per-unit-length was determined to be minimally two A β molecules per 0.47 nm, but fibrils with three, four or five A β molecules per 0.47 nm were also observed [78,79]. Two different types of fibrils (straight and twisted) have been shown to have distinct structural differences on the atomic level and to be able to self-replicate by seeding [60]. These specific types of fibrils have a mass-per-unit-length of two or three molecules of A β per 0.47 nm for the straight and twisted forms, respectively [60]. Solid-state NMR and EPR have shown that all main chain hydrogen bonds are intermolecular and the arrangement of peptides is parallel and in-register [83,90]. Secondary structure assignments vary in the precise residues, possibly due to different polymorphs of A β fibrils, but agree largely about the number of secondary structure elements. They predict that while the ~ 10 N-terminal residues are largely disordered, there exist two extended β -strand regions from ~ 10 to ~ 22 and ~ 30 to ~ 40 , and a loop region between the two strands [42,89]. A model for one particular fibril type has been developed

mainly based on the previously discussed constraints and direct distance measurements from solid-state NMR [86,89] (Fig. 2 a and b). In this model, the two β -strands of one A β molecule each form a β -sheet by intermolecular contacts and the two sheets are packed against each other interacting only via side chains. Using the term “ β -hairpin” to describe the conformation of one A β molecule seems inappropriate because there is no main chain hydrogen bonding between the two β -strands within the molecule. The term “ β -arch” has been proposed for principally similar conformations in β -solenoid structures [91]. In this nomenclature, the β -arch includes the β -strands and the connecting loop would be the “ β -arc”. β -arches can stack on top of each other to form a “ β -arcade” [91]. The two β -strands within one A β molecule have been found to be staggered by 0.24 nm [56,86], similar to interacting β -sheets in crystal structures of small peptides [3].

The parallel and in-register arrangement (i.e. each residue interacts directly with the same residue in the next subunit) explains directly the observation of self-replicating variants. Slight differences in the structure (e.g. length of β -strand, register of β -strands to each other, position of loop, conformation of loop, etc.) will be automatically self-replicating due to the complementary stacking of the next A β molecule assembling on the end of the amyloid fibril (Fig. 2 e). Models principally very similar to this one have been suggested for amyloid fibrils of a 22-residue fragment of β 2-microglobulin and for human CA150 amyloid fibrils [92,93].

Amyloid fibrils of amylin

Human amylin, also known as islet amyloid polypeptide (IAPP), is a peptide hormone of 37 residues with a disulfide bond between cysteine residues 2 and 7. Under normal circumstances, amylin is released into the circulation and is excreted via the kidney [94]. But amyloid fibrils of amylin are the major component of pancreatic amyloid deposits found in many patients (>90%) with non-insulin-dependent (type II) diabetes [95]. The amyloid fibrils may be the causative factor for this disease, but there is also evidence that the pathogenic component is a smaller oligomer of amylin [96]. Amylin fibrils are polymorphic, but all seem to be rope-like bundles composed of about 5 nm protofilaments [17,97]. The predominant type of fibril contains three protofilaments in a left-handed coil with a pitch of 25 - 50 nm [97]. X-ray fiber diffraction has been used to show that amylin fibrils have well ordered cross- β structure [98]. The mass-per-length of the 5 nm protofilaments has been shown to be about 10 kDa/nm corresponding to ~ 1.2 subunits per 0.47 nm fibril length [17]. EPR of spin-labeled amylin fibrils shows that the peptides are arranged parallel in-register and indicate that the N-terminal residues might be somewhat less ordered [99] but direct secondary structure assignment has not been performed on amylin fibrils.

Based on the experimental data, a model has been suggested for amylin in which the individual peptides from residues 9 to 37 have a planar S-shaped fold with three β -strands (residues 12-17, 22-27, and 31-37) [100] (Fig. 2 c and d). The molecules are stacked in register with a 0.47 nm axial rise and a small rotational twist per step. In this model, the N-terminal peptide containing the disulfide bond is found outside of the core structure decorating the protofilaments.

Amyloid fibrils of HET-s

Amyloid fibrils of the HET-s protein in *Podospora anserina* are the [Het-s] prion [13]. Only in the prion form is this protein active in a self/non-self recognition system called heterokaryon incompatibility [101]. The C-terminal prion domain - HET-s(218-289) - is necessary and sufficient for prion formation and prion propagation *in vivo* and for formation of amyloid fibrils *in vitro* [43,47]. It has also been shown to be infectious when transformed back into fungal cells [46]. HET-s(218-289) fibrils appear smooth-sided and are approximately 4.5 nm wide [33]. They show a strong 0.47 nm meridional reflection by electron diffraction indicating a

cross- β structure and STEM measurements resulted in a mass-per-unit-length of about 1 subunit per 0.94 nm [33]. Hydrogen/deuterium exchange and solid-state NMR chemical shifts were used to assign four β -strands comprising residues 266-234 (β 1), 237-245 (β 2), 262-270 (β 3), and 273-282 (β 4) [35,102]. β 1- β 2 and β 3- β 4 show significant sequence similarity to each other and therefore a possible hydrogen bonding interaction between β 1 and β 3 and β 2 and β 4 has been suggested [35], but has not been directly observed yet. A β -solenoid model for HET-s(218-289) fibrils (Fig. 3 a) that agrees well with these experimental constraints has been proposed [35]. In the model, β 1 forms half of its hydrogen bonds intramolecular to β 3, and assuming that subunits are all equivalent in the fiber, the other half is formed intermolecular with β 3 of another subunit. However, neither intra- nor intermolecular contacts have been observed directly. Solid-state NMR has shown that the connecting loops seem to be relatively flexible and might not have a well-defined structure even in amyloid fibrils [39]. One interesting feature of this model is that without the in-register parallel arrangement, the implementation of self-replicating variants seems much less straightforward. [Het-s] variants have not been observed to date, and sharp peaks in solid-state NMR suggest quite homogeneous fibril preparations in contrast to the polymorphic preparations that have been observed for most other amyloid systems (see above) [35,37,103]. In contrast with most other prions and amyloids, [Het-s] seems to have an evolved function only in the prion form and therefore the prion conformation can be considered the native fold of the HET-s protein. This would be a plausible explanation for lack of polymorphism *in vivo*. In spite of this, HET-s(218-289) was found to form different types of fibrils at pH 2, which did not show infectivity when transformed into fungal cells [104]. Future work has to establish whether different variants of [Het-s] exist.

Amyloid fibrils of Ure2p and Sup35p prion proteins

Amyloid fibrils of Ure2p and Sup35p are the cause of the yeast prions [URE3] and [PSI⁺], respectively [14]. For both proteins, an N-terminal prion domain (Ure2p1-90 and Sup35p1-123) is necessary and sufficient for prion formation and propagation [49]. In the case of Sup35p, recent findings indicate that at least part of the so-called M domain (Sup35p125-253) might also be necessary for certain variants [105]. The sequences of both prion domains are quite unusual and highly enriched with small polar residues (mostly asparagines in Ure2p and mostly glutamines in Sup35p). The C-terminal domains of Ure2p and Sup35p are mainly responsible for the biological functions of the proteins, which are regulation of nitrogen catabolism and transcription termination, respectively [49]. Amyloid fibrils of Ure2p1-89 and Sup35p1-253 have been shown to be infectious when transformed into yeast [50-52]. If attached, the C-terminal domains are an integral part of the prion filament, but seem to retain their native structure [21,44,106,107] and are not necessary for infectivity [50-52]. Experiments with Ure2p have shown that the C-terminal domain can be replaced with unrelated enzymes and resulting fibrils show the same architecture and infectivity [21,45,50].

Full-length Ure2p filaments are about 20 nm in diameter and fibrils made from only the prion domain are about 5 nm, but have a strong tendency to bundle laterally [48]. The C-terminal moieties seem to decorate the amyloid fibrils made of the N-terminal prion domain, thereby preventing its aggregation [45]. The mass-per-unit-length of Ure2p fibrils has been determined to be approximately 1 subunit per 0.47 nm independently of the C-terminal moiety [45]. Similarly, the mass-per-unit-length of Sup35p1-61-GFP has been determined to about 1 subunit per 0.47 nm, although slight differences have been detected depending on the variant used to seed the preparations (leading to different homogenous fibril conformations) [108]. Both Ure2p and Sup35p fibrils have been shown to be polar structures through determination of growth rates on the two different ends of fibrils [25-28]. Experiments with cysteine mutants, fluorescence labels, and chemical cross-linking of Sup35p1-253 fibrils suggest that the regions from residues 25 to 38 and from 91 to 106 in one molecule are in close proximity to the same regions of their neighbors while no evidence was found that other regions in the molecule form

similar intermolecular contacts [41]. Similar results were obtained with spin labels and ESP measurements on Sup35p1-253 fibrils [109]. Solid-state NMR measurements on Sup35p1-253 have shown that the fibrils are arranged parallel in-register [110]. Interestingly, the solid-state NMR results for Sup35p indicate that at least part of the highly charged M domain is also involved in intermolecular contacts and arranged parallel in-register [110]. Solid-state NMR experiments on Ure2p10-39 show that these fibrils are arranged parallel in-register [68]. However, Ure2p10-39 fibrils were not infectious when transformed into yeast cells [50]. Solid-state NMR experiments with infectious Ure2p1-89 fibrils also show parallel in-register arrangement [103]. However, assignments of secondary structure regions have not yet been obtained for Ure2p or Sup35p.

A general model for both fibrils has been proposed, termed the “parallel superpleated sheet model” [18] (Fig. 3 b and c). It suggests that β -strands connected by turns zig-zag in a planar, serpentine arrangement. Likely positions for β -strands and turns have been proposed for both fibrils, but since no experimental constraints are available, this assignment is tentative. In addition, it is likely that the exact position of strands and turns and even the parts of the prion domain directly involved in amyloid formation might be distinct in different variants [18, 111]. A more general version of the superpleated sheet principal has been suggested by introducing the idea of staggered β -strands [37] (Fig. 3 d). As discussed above, staggering of β -strands was found in all crystal structures of small peptides [3,65] and for A β fibrils [56, 86]. For longer peptides, this would allow for an almost completely random arrangement of β -strands and their connecting loops, while still keeping a parallel in-register arrangement of the whole peptide [37].

Alternative β -solenoid models have been suggested for Sup35p amyloid fibrils [41,112]. Krishnan & Lindquist mainly used their above mentioned fluorescence data to suggest a β -solenoid fold for Sup35p subunits with “head-to-head” and “tail-to-tail” interactions in the fibril [41]. The resulting model suggests symmetric fibrils. Yet, the model contradicts experimental constraints in two ways. First, Sup35p fibrils were shown to be polar [25,27, 28], and second, Sup35p fibrils were shown to have in-register parallel arrangement [110]. These findings have rendered β -solenoid models unlikely.

What makes amyloid fibrils infectious?

Recently, brain extracts from Alzheimer's patients have been shown to exhibit infectious properties when injected into brains of transgenic mice expressing A β precursor protein [113]. These studies further strengthen previous observations of *in vivo* seeding of A β [114, 115]. *In vivo* cross-seeding by heterologous amyloid peptides also appears to induce protein A amyloidosis in mouse models [116,117]. Efficient *in vivo* seeding is one important feature of infectious amyloids and might be enough to produce infectious proteins in fungal systems. For a *bona fide* infectious protein in higher organisms, pathways to travel from one organism to another are necessary. However, the observation that non-infectious amyloids can have infectious properties under certain conditions indicates that the distinction between infectious and non-infectious amyloid fibrils might not be very sharp and may be a question of the interaction of the amyloid fibril with its environment [118].

For all amyloid fibrils nucleation seems to be a relatively rare event. Nevertheless, fibrils are very stable once they reach a significant length (and can act as seeds). This is especially true for the *in vivo* situation in which chaperones could play an important role in preventing amyloid nucleation, but might be unable to reverse the process once it reaches a certain threshold [119]. Therefore, the most evident considerations are the nucleation rate and the fibril growth rate, and these might, for example, influence the age of onset for amyloid-related diseases. However, in all systems (especially for infectious proteins) the fibril scission rate also plays

an important role in sustainable propagation [118,120]. Fragmentation to shorter pieces enables the amyloid fibrils to form more growing ends and allows sustainable propagation of infectious prions against diluting effects like cell-division in fungal systems and proteolytic clearance in higher organisms. Even for non-infectious amyloids, the fragility of fibrils may be important in countering the effect of natural clearance. The scission rate of amyloid fibrils not only depends on fibril fragility, but also on the environment of the fibrils and could even be different in different hosts [118]. In the case of yeast prions, chaperones are involved in fibril scission [49].

Based on experimental data from the [*PSI*⁺] yeast prion, a theoretical model has been developed showing that a specific balance between four rate constants has to be achieved for fungal prions to become infectious entities (Fig 4) [120]. Similar models would apply for other prions including the mammalian prion protein.

In light of these considerations, the main difference between infectious and non-infectious amyloid results from a very low nucleation rate of infectious amyloids (they seldom occur spontaneously and therefore rely on incoming seeds) combined with a high enough growth and scission rate to create sustainable propagation. A non-infectious amyloid, in contrast, could have a higher rate of nucleation but a much slower scission rate, which produces only few growing ends, resulting in material that has little *in vivo* seeding capacity.

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Abbreviations

NMR, nuclear magnetic resonance; EPR, electron paramagnetic resonance; FRET, Förster resonance energy transfer.

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Figure 1.

Cross- β structure and staggering of β -sheets. (a) Schematic representation of cross- β structure. The fibril axis is vertical; each bar represents a β -strand, with one β -sheet shown in black and one in white. (b) X-ray fiber diffraction pattern of A β (1-42) fibrils [121]. The fibrils were aligned in the vertical direction; note the typical cross- β diffraction signals at 0.47 nm on the meridian and 1.0 nm on the equator. Panel (b) has been reprinted with permission from Wiley Interscience from [121]. (c) and (d) Pair of β -sheets from the crystal structure of the amyloid forming Sup35p peptide GNNQQNY [65]. (c) Each peptide in extended conformation is shown as arrow with side chains protruding. (d) Stick model of the same crystal structure, with carbon shown in purple or grey, oxygen in red, and nitrogen in blue. Note the vertical staggering of one sheet relative to the other, allowing interdigitation of the side chains emanating from each sheet. Amide side chains forming hydrogen-bonded stacks are shaded in grey and pale red in the center and the outside, respectively. Panels (c) and (d) have been reprinted by permission from Macmillan Publishers Ltd. from [65], copyright 2005.

Figure 2.

Models for human amyloid fibrils and self-replicating polymorphism. (a) and (b) model of A β (1-40) fibrils produced with gentle agitation [86]. The long axis of the fibrils is perpendicular to the page in both panels. (a) All-atom representation of a pair of A β molecules. Double-headed arrows indicate contacts established by solid-state NMR [86]. (b) Cartoon representation with residues 12-21 in red and residues 30-40 in blue and an imposed slight left-handed twist between molecules along the fibril axis. Panels (a) and (b) have been reproduced from [37]. (c) and (d) Model of amylin fibrils [100]. (c) All-atom representation of one amylin molecule, with β -strand regions colored in purple. The fibril long axis is perpendicular to the page. (d) Electron micrograph of an amylin fibril prepared by unidirectional shadowing showing left-handed coiling and a crossover spacing of 37 nm alongside a fibril model formed

by three protofilaments having the conformation shown in (c). Panels (c) and (d) have been reproduced with permission from Elsevier from [100]. (e) Schematic representation of the templated assembly on two different variants of amyloid fibrils formed by the same peptide with in-register, parallel arrangement. Preexisting filaments (seeds) are shown in green. In both cases an additional molecule (red), initially natively unfolded in solution, adds to the growing filament. Note that the principle of this scheme is correct for all parallel in-register amyloid structures.

Figure 3.

Models of fungal amyloid fibrils. (a) Model for HET-s(218-289) fibrils, with β -strands shown as arrows. Panel (a) has been reprinted by permission from Macmillan Publishers Ltd. from [35], copyright 2005. (b) Model of Ure2p prion fibrils [18]. The fibril axis is approximately vertical. Top - the space-filled model shows the amyloid core fibril with six stacked β -serpentine in blue. The surrounding globular domains have the folds of C-terminal domain monomers and they are connected to the fibril backbone by extended linker shown in yellow. No significance is attached to the conformation of the linkers other than that their length does not exceed that of a 25-residue polypeptide or to the positions and orientations of the C-terminal domains other than that they should not overlap with each other or with the fibril. Bottom - diagram of proposed β -serpentine fold for the Ure2p prion domain. Charged residues are circled. (c) Model of Sup35p1-253 fibrils [110]. The N domain (blue) is largely β -sheet, whereas the M domain (green) consists of both β -sheet and non- β -sheet regions. The model is schematic and does not predict specific locations, number, or lengths of β -strands or loops, which may depend on the prion variant [110]. Panel (c) has been reproduced from [110]. (d) One possible mode of arrangement of β -strands and loops for a four-layered, parallel, and in-register β -sheet structure. Panel (d) has been reproduced from [37].

Figure 4.

Interplay of rate constants in amyloid systems. Graphical representation of the effect of growth and fragmentation rates on variants of the $[PSI]$ prion [120]. Aggregation states of Sup35p are represented by a gradient with grey being completely soluble and white being completely aggregated. Curved lines indicate states that have the same number of fibrils per cell. The left straight line represents a threshold below which stable prion propagation is not possible. The right straight line illustrates the border between low or high amounts of soluble Sup35p in the cell, resulting in strong or weak color phenotypes, respectively. The black curved line represents the border between large versus small numbers of fibrils per cell resulting in stable versus unstable prions, as indicated. This panel has been adapted by permission from Macmillan Publishers Ltd. from [120], copyright 2006.