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Structure-Property Relationship of Amyloidogenic Prion Nanofibrils

Myeongsang Lee, Hyunsung Choi, Jae In Kim,
Gwonchan Yoon and Sungsoo Na

Additional information is available at the end of the chapter

Abstract

The structure and its property for the prion nanofibrils, which exhibit self-assembled steric zipper, amyloid fibrils, are described in this chapter. There is the belief of origin for the infectiousness of the prion can be its molecular structure. It is due to the amyloid toxicity, which is related to its beta sheet rich molecular structure and self-aggregated long fibrils. There is evidence that the difference between PrP^c and PrP^{sc} is transitioned beta sheet from alpha helix to self-assemble and then to the amyloidogenic fibrils. Therefore, the scope of this chapter is the amyloidogenic structural characteristics of prion fibrils and its relationship to the property. The molecular structural characteristics can be changed by properties such as affinity, toxicity, infectivity, and so on, so this is a key factor to understand the origin of prion disease and develop the therapeutic strategy. One of the main properties of amyloid fibrils that we want to describe here is mechanical property such as dynamic property and material property for prion nanofibrils. This chapter can shed light on understanding the infectious characteristics of prion and the relationship of its molecular structures.

Keywords: prion, amyloid fibrils, physiological conditions, heterogeneity interaction, cross seeding, HET-s, singlet, triplet, mechanical characterization, molecular dynamics, coarse-grained model

1. Introduction

The molecular structure and properties of prion nanofibrils, which exhibit self-assembled steric zipper and amyloid fibrils, result in fatal prion diseases such as transmissible spongiform

encephalopathies and neurodegenerative diseases such as Alzheimer's disease. Various origins for the infectious nature of prions have been suggested, including the molecular structure; amyloid diseases exert their toxicity by forming self-aggregated β-sheets and self-assembled long fibrils. For example, hIAPP fibrils induce apoptosis of insulin-secreting β-cells in the pancreas for its hardness and self-propagation characteristics. For the prion, PrP^C and PrP^{Sc} differ because of the transition from an α-helix to a β-sheet, respectively, to self-assemble into amyloidogenic fibrils. This chapter describes the amyloidogenic structural characteristics of prion fibrils and their relationship with their functions. The molecular structural characteristics can be quantified based on affinity, toxicity, and infectivity, among others, which are key factors for understanding the origin of prion diseases and developing therapeutic strategies. We focus on the mechanical properties of amyloid fibrils such as the dynamic and material properties of the HET-s prion.

Prion proteins exist in forms such as fibrils, oligomers, and lattice-like plate structures under different physiological conditions. A common structural feature of prion and amyloid proteins is that prions disrupt normal cell function and exhibit toxic behavior; thus, it is necessary to understand the different conformations of prion proteins under various physiological conditions. Different conformations of prion proteins have been reported under various pH conditions determined using various experimental techniques. Furthermore, some computational groups investigated the development of secondary prion structures as well as protonation status of specific prion residues, which enable secondary structures of prions to form oligomer conformation and grow into fibrillar or plaque structures. Similarly, toxic and nontoxic characteristics of prion proteins for β-helices in the presence of physiological agents were determined through the structural stability and mechanical property analyses.

Various experimental and computational studies have provided insight into the amyloid fibril-forming mechanism. Recently, computational studies were performed to overcome the limitation of experimental methods, which are unable to reveal the detailed molecular structures. For several decades, many studies evaluated the mechanical properties (such as bending and torsional rigidities) and mechanical behaviors of amyloid fibrils. These studies increased the understanding of the mechanism of fibril formation. Most studies evaluated homogeneous amyloid fibrils. However, other experiments revealed that heterogenetic interactions are possible for different amyloid monomers. Alpha-synuclein Aβ, Aβ-amylin, and Aβ-tau are well-known heterogenetic interactions.

Previous experimental and computational studies reported that depending on the conformation of amyloids or prion proteins, 2D material-based amyloids including fibrillar and plaque were deposited, which disrupted normal cell functions. Additionally, 1D materials, based on amyloids such as monomers and oligomers, showed toxic characteristics. These various forms of amyloid and prion proteins are affected by physiological conditions such as thermal fluctuation, internal flow, pH, and ionic strength. For instance, Aβ amyloid proteins exhibit various conformations such as cross-β zipper structures and β-turn-β motif structures. Based on their structural conformation features, the generation and development

of oligomeric and fibrillar Aβ amyloids are different. Specifically, the existence of metal ions including copper, zinc, and aluminum affects the development of oligomeric or fibrillar Aβ amyloids. Therefore, understanding the various conformations of Aβ amyloid and their characteristics under different physiological conditions is necessary for revealing their toxic behaviors.

In this chapter, we introduce the mechanism of formation of amyloid fibrils and diverse molecular structures of prion fibrils. Moreover, the structure-property relationships and mechanical characterization *in silico* are described using HET-s amyloidogenic prion fibrils as an example.

2. Seeding and aggregation mechanisms of prion nanofibrils

In this section, the seeding mechanism and characteristics of prions are introduced with homogeneous/heterogenetic amyloidogenic prion fibrils.

2.1. Prion, amyloid folding, and amyloid fibrils

Prion proteins, known as proteinaceous infectious agents, constitute a subclass of amyloids. Prion infection involves the conversion of proteins from their normal functional conformations, via an unfolded intermediate state, into amyloids. The conversion of the α-helical, isoform of the prion protein (PrP^c) into the insoluble, β-sheet-rich, and infectious form (PrP^{sc}) induces the accumulation of β-sheets and formation of fibrils and bundle structures [1]. After seeding, self-propagation is induced by providing templates for further assemblies [2–4]. This is known as the initial event in prion-inducing disease [5–9]. It is important to understand the propagation and amyloid fibril-forming mechanism for the development of therapeutic agents [10–12]. To understand amyloid fibril formation, numerous experimental and computational studies have been conducted. The molecular and atomic structure of mammalian and fungal prion fibrils should be determined to understand the propensity for polypeptides to form amyloid fibrils. In many cases, mammalian amyloid fibrils, which are known to induce amyloid disease, such as Aβ, α-synuclein, and amylin, do not form structures determined by their amino acid sequences. It is known that for self-propagation, molecular polymorphisms lead to different structures, and various polymorphic structures have been observed under diverse laboratory conditions, as depicted in **Figure 1**. For example, Tycko et al. showed that Aβ$_{1-40}$ has two main polymorphic structures that form fibrils [13]. One is a twofold, and the other is a threefold β-sheet array. According to the interaction directions of the β-sheet layers, templates for fibril structures were determined. Furthermore, self-propagation of HET-s fungal amyloid fibril was studied previously using high-resolution techniques, offering insight into structural changes induced by pH variations [4].

Advanced experimental techniques such as X-ray crystallography, transmission electron microscopy, scanning electron microscopy, and cryo-electron microscopy have revealed

high-resolution amyloid conformations and the self-propagation and fibril formation mechanisms [14–20]. Amyloid fibrils were suggested to aggregate via a dense inherent hydrogen-bond-network and steric-zipper-like interactions between β-sheet layers, resulting in fiber formation along the fibril axis. Amyloid fibrils showed good structural complementarity and highly ordered conformations. However, although the physiochemical characteristics of amyloid proteins have been revealed, the structures and seeding processes remain unclear, and determining the molecular motion of amyloid proteins is challenging.
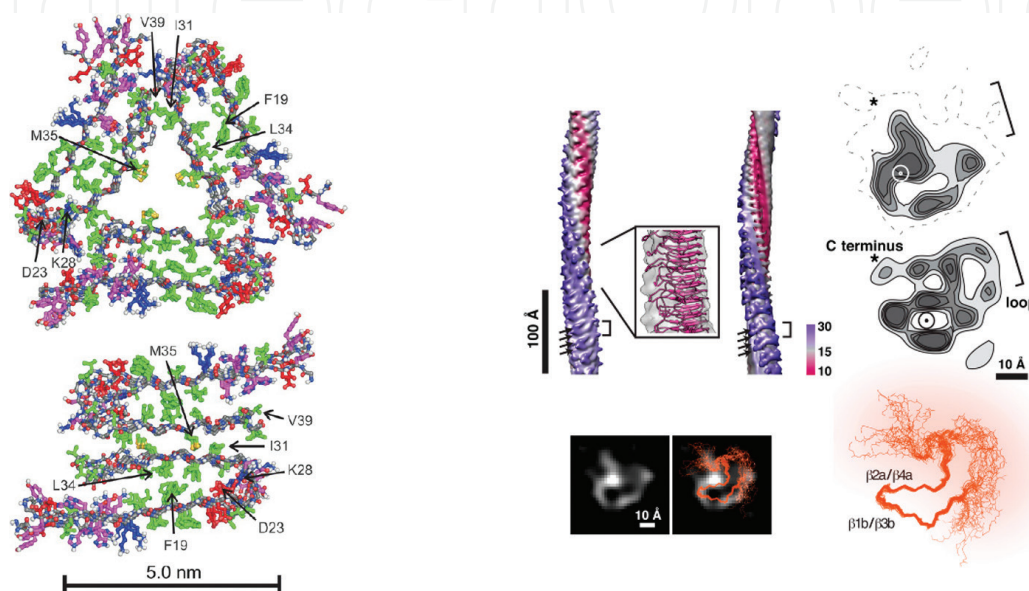

Figure 1. Various conformations for amyloid fibrils along to different cross-sectional area [13] and pH conditions [4].

2.2. Self-seeding and cross-seeding of amyloids

Diseases related to the toxicity of amyloid proteins are induced by the accumulation of amyloids, which consists of fibrillar forms and tangles. Experimental studies revealed that amyloid fibrils extracted from patients with amyloid disease brain had a fibrillar form and residual nonfibrillar materials [3]. The extracted fibrils showed widths and morphologies similar to those of synthetic amyloid fibrils *in vitro*, but the precise morphologies of the extracted amyloid fibrils are difficult to assess because of the self-association of fibrils and adhesive particles. Aβ amyloid fibrils from amyloid disease brains presumably contained chain length variations and chemical modifications. Previously, experimental and computational studies evaluated the kinetics of amyloid fibril formation. For both extracted and synthetic amyloid fibrils, the growth rate of amyloid fibrils was accelerated and amplified by seeding. However, nonfibrillar particles were not accelerated, and they did not show seeding behavior. Typically, amyloid fibrils are constructed by adding amyloid protein blocks composed of 4 or 5 parallel- or anti-parallel-layered β-sheets. These structures may also be 'tetramers' or 'pentamers', depending on the number of layers present. Monomers referred to as seeds act as "Lego blocks," and single amyloid fibrils are constructed in a specific elongation direction when seed monomers are added. Particularly, single monomers can be regarded as templates for fibril formation. Previous studies evaluated the elongation processes

of uniformly composed monomers to understand the self-propagation process [2, 3, 21]. The process is referred to as "self-seeding" and homogeneous amyloid block monomers form fibrillar conformations. Previous studies also provided insight into the structural and physiochemical characteristics of diverse amyloid fibrils. However, accumulated amyloid agents, which are present in neurodegenerative disease, are not uniform. Thus, better models are needed to understand the polymorphic features of the fibril formation mechanism. Recent studies have evaluated the different types of amyloid block monomers that can bind to each other to form fibrils and tangles through a specific mechanism known as "cross-seeding". The key point of this mechanism is that one block monomer of amyloid proteins can be regarded as a template for other block monomers, even if the blocks have different physiochemical characteristics and are constructed with different conformations and compositions [22–25]. The cross-seeding mechanism can explain the formation of heterogeneous-composed amyloid fibrils and polymorphic fibrils, and these models offered better insight into amyloid accumulation processes. Experimental studies showed that seeding with amyloid-like fibrils made from short synthetic peptides from other amyloid proteins or fibrils of a completely different nature; for example, bacterial curli or Sup35 from *Saccharomyces* may function through other interactions [26]. It appears likely that the β-sheet structure of the seed has a general effect on the seeded material, inducing misfolding, and production of a new seed that can proceed to an amyloid fibril. In addition, Yan et al. examined the effect of coinjection of murine senile apolipoprotein A-II amyloid and reactive protein A amyloid amyloidosis to determine the heterologous transmissible seeding mechanism [22] shown in **Figure 2**.
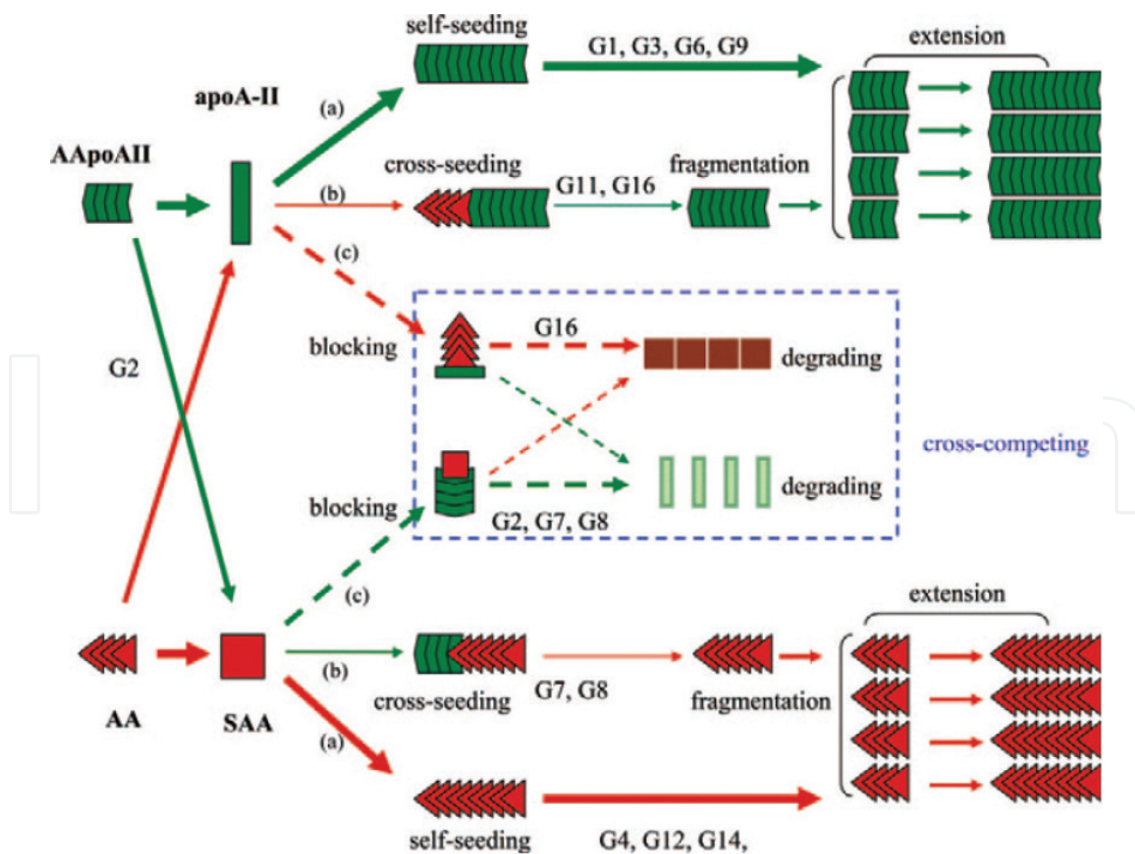

Figure 2. Cross-seeding mechanism of heterogeneous amyloid fibrils [22].

2.3. Cross-seeded fibrils

Recently, computational studies were performed to overcome the limitation of experimental studies, which are unable to determine molecular structures in detail. For several decades, many studies have evaluated the mechanical properties and behaviors of amyloid fibrils [27–32], providing insight into the amyloid fibril formation mechanism. Most studies examined homologous amyloid fibrils. Experimentally, it was revealed that heterogeneous interactions enabled different amyloid monomers to aggregate with each other. Alpha synuclein-Aβ, Aβ-amylin, and Aβ-tau, which are well-known heterologous amyloid fibrils, have been used to understand the effect of structural affinity on amyloid fibril formation [33–38]. Recent studies revealed the structural and interaction features of cross-seeded amyloid oligomers and proto-fibrils. Oligomeric Aβ-tau heterologous amyloid is one example of a cross-seeded amyloid protein. Aβ and U-shaped tau monomers are structural similar in that both have a common U-shaped β-turn-β structure. In addition, Miller et al. determined the structural stabilities of possible compositions of Aβ-tau heterologous oligomers using experimental and computational methods [38], as shown in **Figure 3**. They studied the interactions between heterologous monomers. Furthermore, Choi et al. studied the mechanical and physicochemical features of heterologous oligomers with diverse-mutated Tau monomers [37]. They found that different binding directions showed different structural stabilities. It was observed that amyloid beta monomers and other monomers could act as templates for seeding processes.
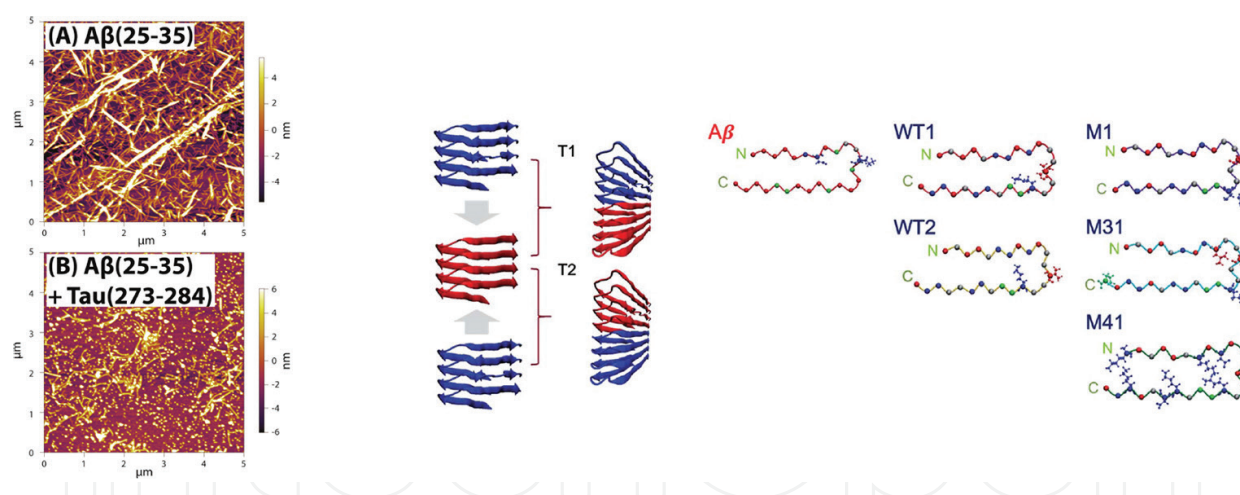

Figure 3. Experimental result for heterogeneous amyloid fibrils (*left panel*) [36] and schematic constraint for simulation of heterogeneous amyloid fibrils (*right panel*) [37].

Many computational studies showed that not only structural similarity but also specific interactions between residues of amyloid proteins play a major role in seeding processes. Elongation of amyloid fibrils along the fibril axis is related to the binding features of the seed amyloids and their structural stabilities. The binding sites on monomers enable the attachment of other monomers [39]. The specific interactions can be classified as intra-layer interactions and inter-layer interactions, which confer structural stability, as depicted in **Figure 4**.
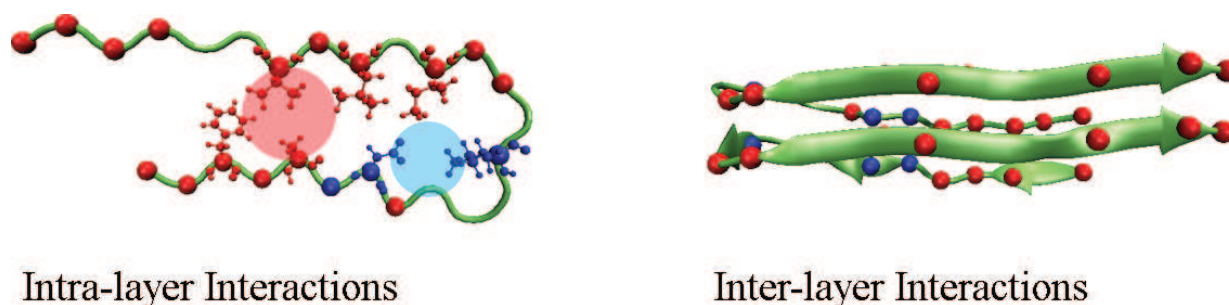
Figure 4. Intra-layer interaction and inter-layer of heterogeneous mixture of amyloid proteins [37].

It was revealed that the structural stabilities of intra-layers were affected by steric-zipper-like interactions [40–42] and salt-bridge interactions [37]. Intra-structural stabilities are dominantly affected by specific interactions. Hydrophobic residues in interior regions of amyloid fibrils form steric-zipper-like structures and help maintain cross-sectional structures. Although heterologous monomers contain different residues, hydrophobic residues commonly construct dry-regions and affect structural stabilities. In addition, charged residues such as lysine (K) and aspartic acid (D) form salt-bridge regions and affect intra-stabilities.
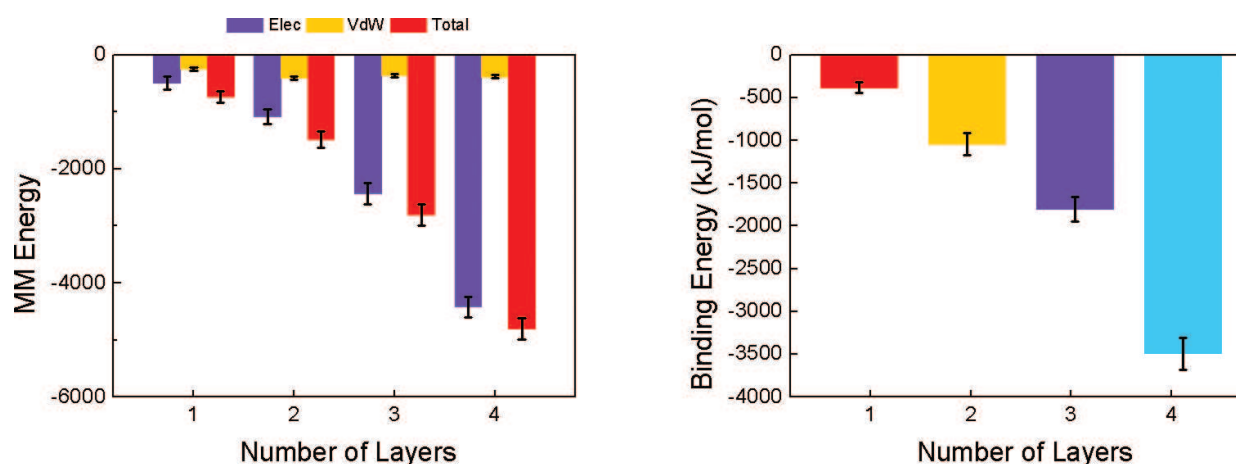

Figure 5. MM and binding energies of Aβ-tau mixtures after 20-ns equilibrium MD simulations [37].

The stabilities of inter-layers are also affected by hydrogen-bonding networks and nonbonding interaction energies, such as Van der Waals and electrostatic energy induced by interactions between residues of layers that face each other. Interactions between single layers are dominantly affected by electrostatic and Van der Waals energies. However, as the number of layers is increased, the effect of electrostatic energies becomes dominant. In the fibril formation processes of heterologous amyloids, binding between monomers is affected by electrostatic energies (**Figure 5**).

3. Molecular structure of prions in various physiological environments

In the previous section, we described the general aggregation and seeding mechanism of amyloid-like prion fibrils based on homogeneous and heterogeneous structures. During the aggregation of prion fibrils in the seeding mechanism, the external environment affects growth of prion-like amyloid fibrils. Here, the effect of the external environment is referred to as the physiological conditions, which have been shown to affect molecular conformational variation in the fibrillary growth of PrP^C, HET-s, and Sup35, in detail.

First, the development of prion proteins is caused by the conversion of native prion protein monomeric PrP^C as misfolded and denatured PrP^{Sc} because of external conditions such as partial mutation, internal flow, pH, ionic strength, and temperature variations. This PrP^C protein is glycosylated and functions similar to components of the extracellular surface of neurons, which play a significant role in signal transduction. This native PrP^C is converted to monomeric PrP^{Sc}, which gradually aggregates to form oligomeric, fibrillar, and plaque structures. These converted monomeric PrP^{Sc} act as seeds at specific concentrations with a lag phase, which induce the aggregation of fibrillar and plaque prions. This conversion is frequently observed in amyloidosis including Alzheimer's disease by Aβ from amyloid precursor protein and cardiovascular diseases by transthyretin (105–115) from native functional transthyretin monomers [6, 43, 44]. As shown in **Figure 6**, aggregation of converted PrP^{Sc} from PrP^C monomer deposited near the brains of human and mouse represents the hallmark of neuropathological features [45].
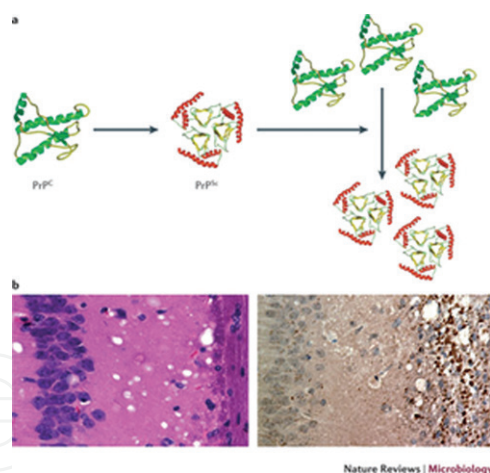

Figure 6. Prion proteins. (a) Conversion mechanism from PrP^C to PrP^{Sc} and (b) experimental result of PrP prion proteins [45].

The other type of prion proteins is the HET-s prion protein. According to Govaerts et al., the left-handed β-rich helical structures containing α-helices are located outside of the β-helical structures of prion fibers and are responsible for their toxicity (refer **Figure 6(a)**) [46]. In contrast, right-handed prion proteins are not toxic based on combined experimental and computational studies. HET-s prion amyloid proteins were also reported to have β-solenoid conformations and triangular hydrophobic cores [47]. In 2008, Wasmer et al. determined detailed structural information of HET-s (219–289) via solid-state nuclear magnetic resonance techniques as shown in **Figure 7**.
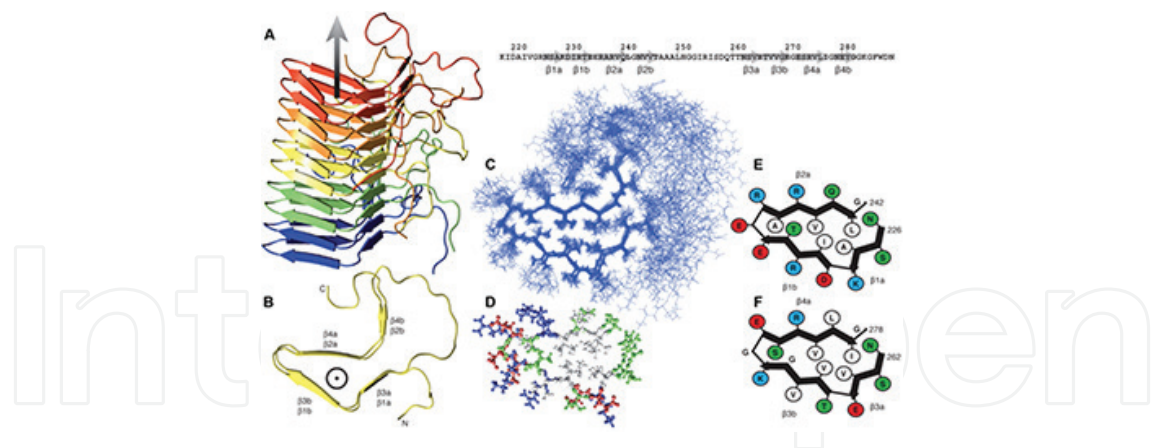
Figure 7. (A) Side view of HET-s prion fibrils (B) Top view (C) NMR structure (D) Type of amino acid for single layer of HET-s (E) Side chain interaction of first layer (F) Side chains for second layer [47].

A third type of prion proteins is known as the Sup35 prion proteins, which exhibit cross-β characteristics with steric zipper structures as shown in **Figure 3** [48, 49] and are similar to other proteins that cause several degenerative diseases such as Alzheimer's diseases, type II diabetes, and dialysis-related amyloidosis by Aβ, human islet polypeptide, and β_2-microglobulin [41]. Specifically, partial prion proteins fragment from the Sup35 prion protein and show polymorphic features including lateral thickness increases (**Figures 8** and **9**) with changes in physiological conditions such as pH, thermal variation, internal flow, and ionic strength [50].
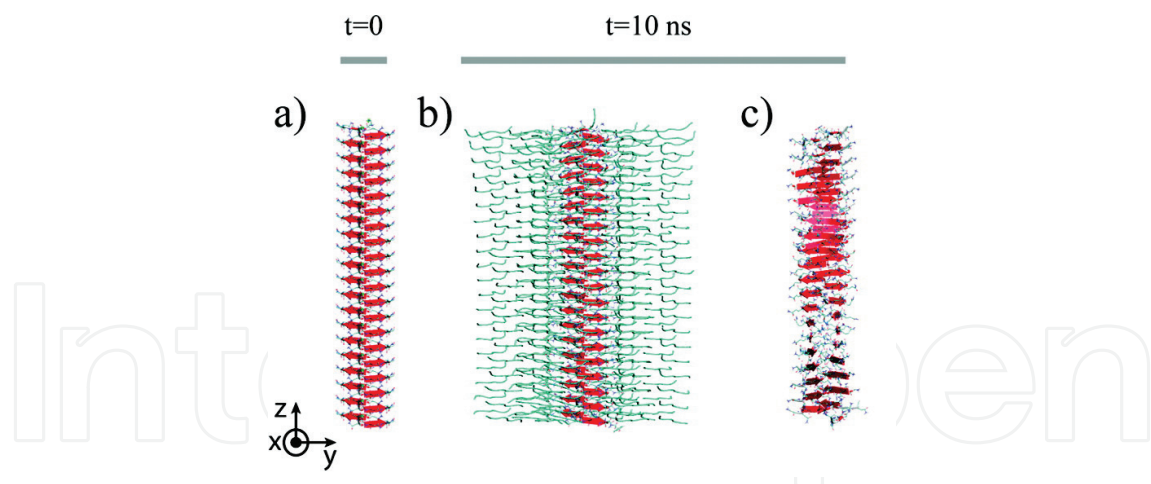

Figure 8. Lateral thickness composition of Sup 35 prion proteins. (a) Unit protofilament crystalline structures, (b) lateral thickness composition of Sup 35 prion fibrils, and (c) equilibrium result of unit protofilament prion protein [51].

Fibrillar and plaque forms of PrP^{Sc} prion proteins are frequently observed because of their considerable infectivity ability. These fibrillar and plaque types of prion proteins can be generated through repetitive fragmentation and elongation mechanisms. The prion proteins grow by adding monomers or attracting other fragmented prion segments. A previous study showed that fragmented and denatured prion proteins add PrP^{C} to grow PrP^{Sc} based on protein-misfolding cycling amplification techniques [45] (refer to **Figure 10**).

Colby et al. investigated the prion propagation mechanism via repetitive fragmentation and elongation by adding additional prion monomers and evaluated the kinetics study and conducted imaging analysis [15]. Through the repetitive process of fragmentation and elongation mechanism, neuro-toxic prion oligomers could be generated. In this aggregation, after conversion from PrP^C to PrP^{Sc}, prion proteins showed toxic characteristics and affected intercellular processes. Similarly, for the fragmentation and elongation mechanisms of HET-s prion proteins, Mizuno et al. investigated similar fibrillar and plaque proteins such as prion, particularly HET-s, under various pH conditions [4]. Fragmentation and addition of prion seeds generated different types of fibrillar HET-s near pH 2 and 3, while lattice-like HET-s was observed under neutral physiological conditions. Between pH 2 and 3, a tight fibrillar HET-s structure was observed, while fibrillar HET-s exhibited a cavity at pH 3 [4, 37]
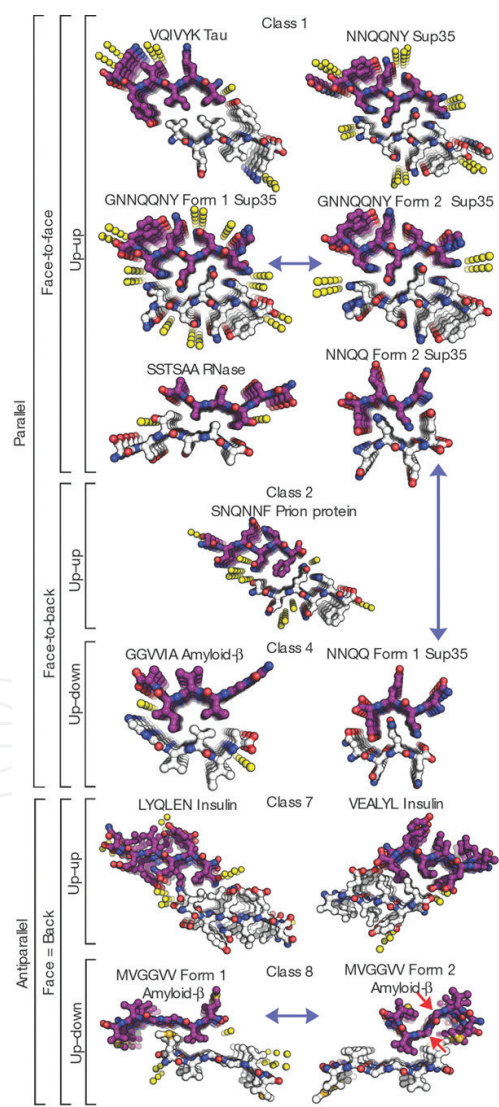

Figure 9. Polymorphic schematic of amyloid fibrils along to eight classes [41].
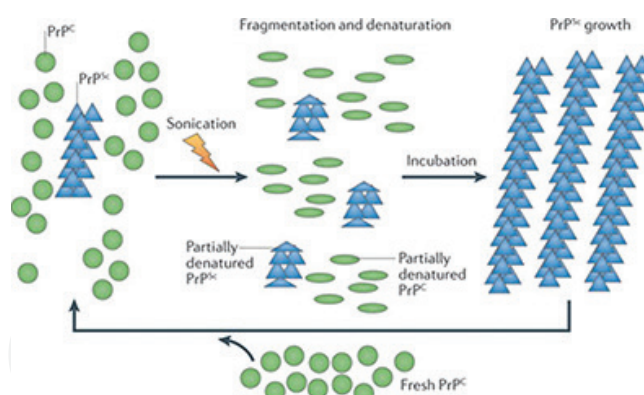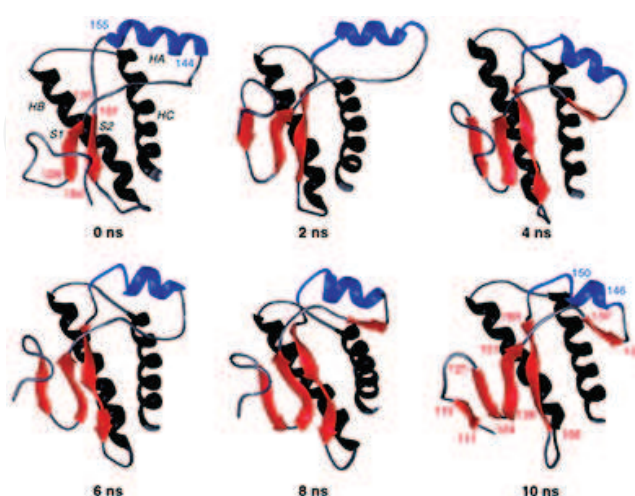
Figure 10. Schematic for protein-misfolding cyclic amplification [45].

To discuss the structural characteristics of plaque and fibrillar prions from converted PrP^{Sc}, in this section, we described the conversion mechanism of PrP^{C} from PrP^{Sc} prion proteins at the atomic scale based on computational results. As described previously, native PrP^{C} proteins varied from misfolded PrP^{C} to PrP^{Sc} based on the physiological conditions [52–56]. In 2001, Daggett et al. reported computational results for the conversion of PrP^{C} when pH conditions were varied from neutral to low pH conditions [56]. They observed that the core region of PrP^{C} was stabilized, while the N-terminus exhibited considerable structural fluctuations compared to the core and C-terminal regions. In addition, they reported the conversion process from PrP^{C} to PrP^{Sc} over time.

As shown in **Figure 11**, additional β-strands were generated at the N-terminal regions with lengthening of the β-strands after 2 ns. Furthermore, they found that Met-129 at the N-terminal region triggered the conversion of the turn-rich N-terminal region into β-strands. Therefore, external physiological factors such as ionic strength and pH altered the native PrP^{C} resulting in misfolding into PrP^{Sc} structures with the additional generation of β-strands, which may be related to the aggregation of PrP^{Sc} structures.

Figure 11. Trajectory variation of PrP^{C} at low pH with respect to time trajectories [56].

Another study observed protofibril formation of scrapie prion protein, which contained numerous β-strand conformation variations [55]. They computationally found that the conversion from monomeric syrian PrP^C segments to PrP^{Sc} occurred because of a point asparagine mutation at the 147th aspartate residue (D147N) under neutral and low pH conditions. Subsequently, they observed that the prion protofibril was stabilized and compared their computational results to the experimental results of DeMarco et al. [55]. The monomeric conversion from D147N PrP^C to PrP^{Sc} occurred near β-strands at the 1st and 2nd β-sheet regions of the original structures. In this procedure, they determined the critical role of additionally generated β-strands in the conversion of PrP^C structures through computational analysis. The salt bridge region between the 147th aspartate and 151st arginine residue was disrupted; this event did not critically alter the structural stability of D147N PrP^C structures, but the D147 mutation affected expansion of the N-terminal region, generating additional β-strands regions. These results support their previous computational results for the role of the Met-129 residue.

Based on the converted PrP^{Sc} unit monomers, they constructed the basic protofibril unit of the converted PrP^{Sc} monomer by connecting adjacent β-strand regions of the monomer. As shown in **Figure 12(a)**, basic trimer structures of prion protofibril units using converted PrP^{Sc} monomer under acidic conditions were constructed and stabilized based on molecular dynamics simulations. By superimposing the trimer structures along the fibril axis as shown in **Figure 12(b)** with a 60° rotation, they replicated the sixfold symmetry of prion fibril structures. Using this computationally constructed prion protofibril model, they compared the results with the previously reported experimental results of Cauhey et al. Electron microscopy images were consistent with the constructed computational model of the prion protofibril.
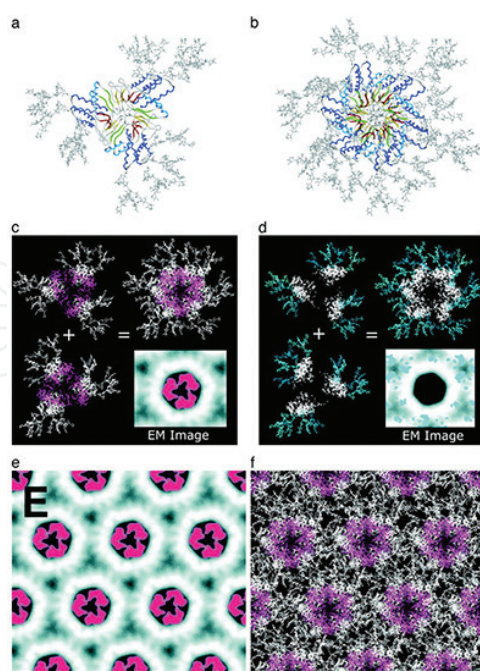

Figure 12. Comparison with simulation results (a–b) and EM images (c–f) of two-dimensional PrP structures [55].

Additionally, they observed conformational variation in the full-length bovine PrP prion at low pH using molecular dynamics [53]. The conformational variation of native full-length bovine PrP prion structures was found to be PrP^{Sc} with additional generation of β-strands; the role of the Met-129 residue was determined by conformational and secondary structure analysis, as shown in **Figure 13**.
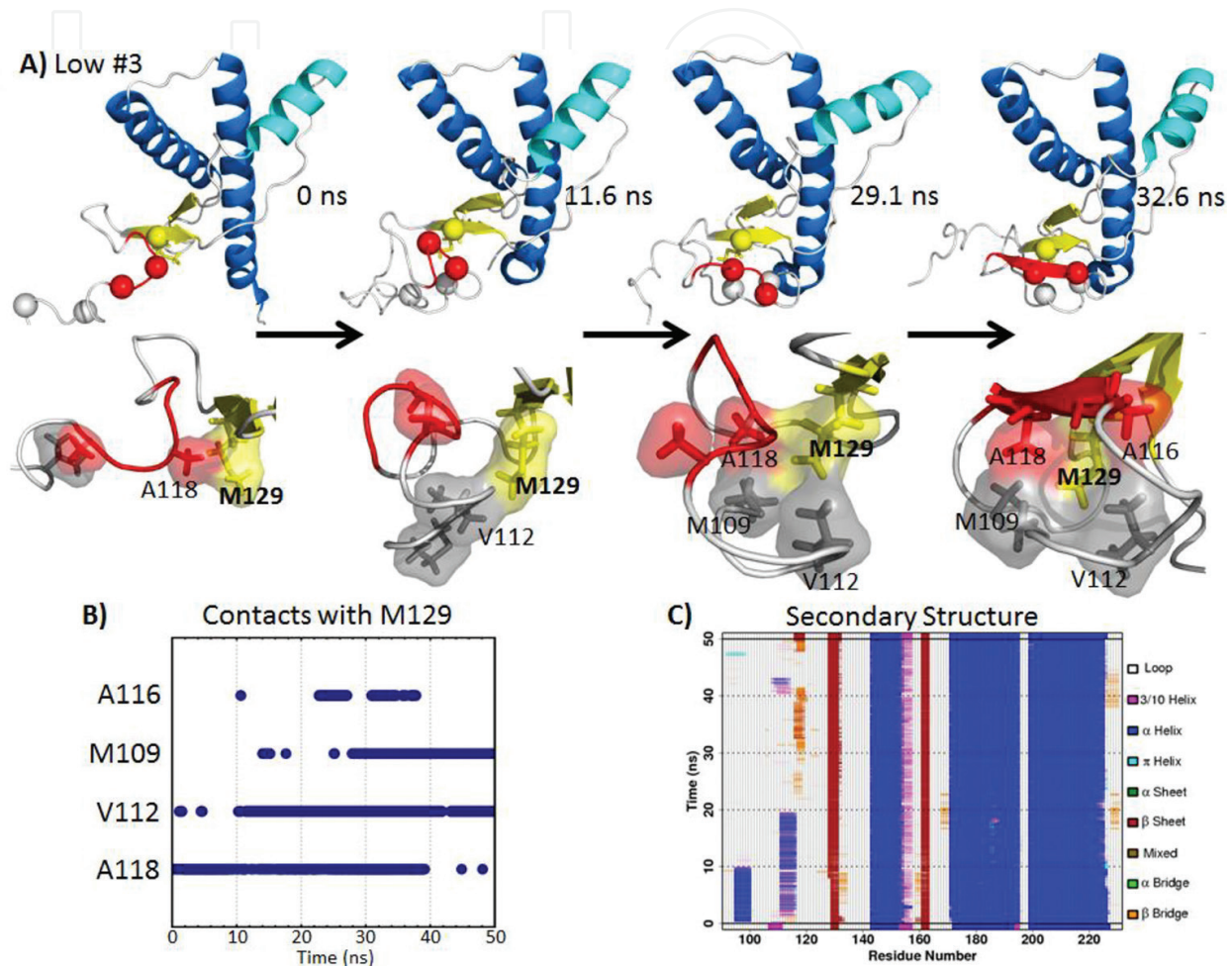

Figure 13. Representation of β-strands formation at the N-terminal region due to the N-terminal hydrophobic contacts with M129 in low pH. (a) Top panel describes the bovine PrP with crucial hydrophobic residue described as sphere, (b) hydrophobic contact with M129 respect whole time trajectories, and (c) secondary structure analysis of PrP [54].

Based on computational studies of prion proteins, physiological conditions such as pH, ionic strength, point mutation, and temperature variation have a considerable effect on structural variations in HET-s and native PrP^C structures as PrP^{Sc} structures; some conditions enlarge the N-terminal region and cause formation of additional β-strands, which are similar to reported experimental results. Converted PrP^{Sc} with additional β-strands can connect to protofibril structures. Therefore, understanding prion proteins under external physiological conditions may be useful for preventing the generation of fibril structures and their toxic behaviors.

4. Characterization of amyloid molecular structure by mechanical testing

Above, we introduced the generation of amyloid-like prion fibrils via self-aggregation and seeding mechanisms based on previously reported experimental and computational studies. Specifically, we described the aggregation mechanism from native PrP^C segments to denatured PrP^{Sc} segments under physiological conditions, where pH variation caused partially disordered N-terminal regions of PrP^C to convert to β-strands at the N-terminal region of PrP^{Sc}. These denatured PrP^{Sc} segments aggregated as fibrillar prion amyloids, which are toxic to cell function and delete the lipid bilayer of membranes. In this section, we explain the role of the β-sheet-rich prion fibrils and their toxic characteristics from a mechanical perspective determined through experimental and computational studies.

4.1. Importance of mechanical properties of amyloid fibrils

4.1.1. Disease—breakage and infections

Amyloid fibrils are disease-related proteins found in various types of neurodegenerative and degenerative diseases. Aggregated fibrils were detected in diseased cells. Understanding the mechanical behavior of amyloid fibrils is important because it is related to fibril breakage. Misfolded amyloids do not initially form long fibrils. Single amyloid monomers inside the body begin to aggregate. They stack into small oligomers, forming aggregates, which grow into the fibrillar form. These fibers may be up to 100-nm long [5]. In humans, amyloid fibrils eventually break. In our surroundings, breakage of an item is not a good thing. For example, if a ruler is broken into two pieces, we do not use it anymore. Because we cannot measure the length which it was made to measure. However, Long amyloid fibrils breakage creates small oligomers, which become seeds for new fibrils. Additionally, in environments containing both fibrils and oligomers, aggregation occurs more rapidly than in environments containing only oligomers [5]. Thus, breakage of amyloid fibrils generates additional fibrils. Understanding when the fibril will break may be determined by measuring mechanical properties of amyloid fibrils.

4.1.2. Bio-materials

Several studies have compared the mechanical properties (e.g., Young's modulus) of amyloid fibrils with those of other bio-materials. Amyloid fibrils exhibit a Young's modulus comparable to those of wood and silk, and its strength is comparable to those of metals such as aluminum or steel (**Figure 14**) [57]. Comparable mechanical properties of amyloid fibrils with these materials indicate that amyloid fibril is a potentially useful bio-material. Compared to proteinaceous biomaterials such as actin filaments, amyloid fibrils exhibit longer persistence length [19]. Persistence length is a basic mechanical property quantifying the stiffness of a polymer. Materials with shorter lengths than its persistence length behave as elastic materials and form longer polymers. The mechanical properties and behaviors of elastic materials and polymers are very different. Polymers are materials that act as soft chains, making them unsuitable for use as support materials. Proteins are composed of poly-peptide chains and are polymers. The poly-peptide chain folds as a globular protein (or misfolds as an amyloid), and

the structure is maintained by noncovalent interactions between amino-acids. Amyloid fibrils or actin filaments are large structures constructed by large numbers of proteins composed of polypeptide chains.
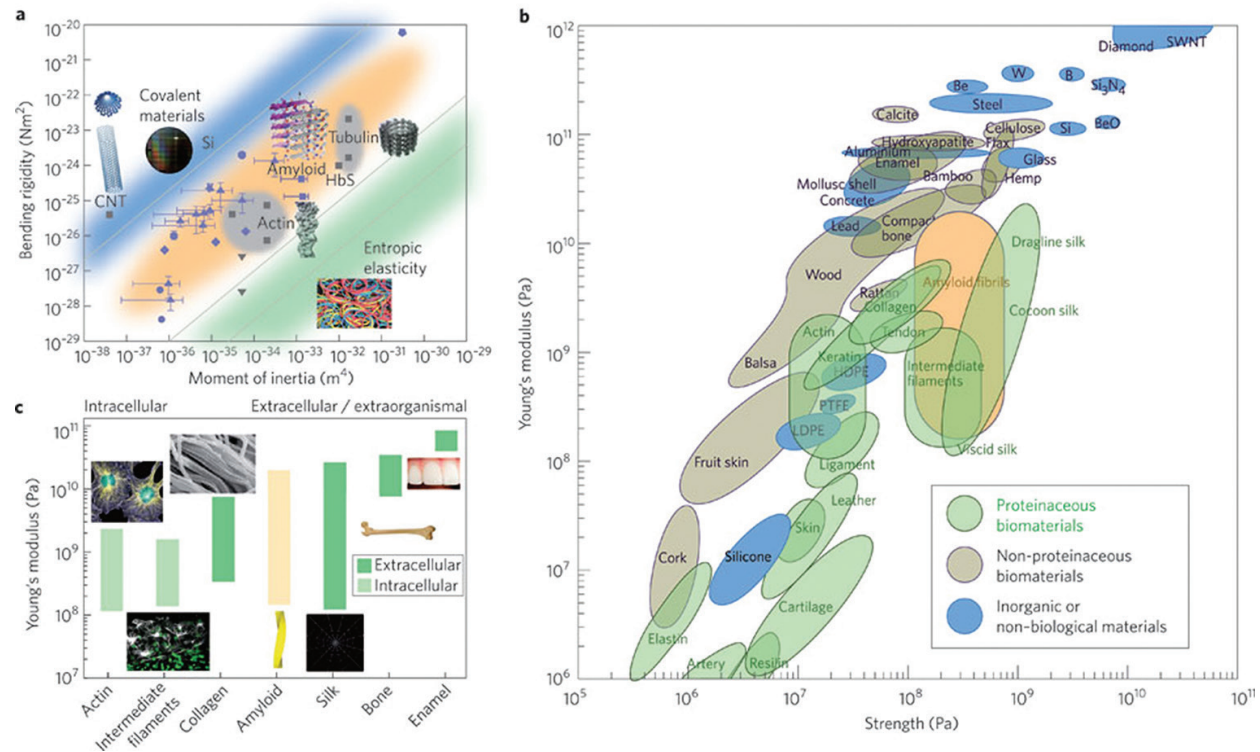

Figure 14. (a) Bending rigidity of amyloid fibrils compared to other materials (b) Young's modulus of amyloid fibrils (c) Young's modulus of biomaterials [57].

4.2. Determination of mechanical properties

4.2.1. Experiments

Studies have focused on the relationship between amyloid fibril mechanical properties and behaviors in biology. One of the pioneer studies of mechanical property measurement of amyloid fibril was conducted by Smith et al. in 2006 [58]. In their study, the mechanical properties of insulin fibrils (not prions) were measured using atomic fore microscopy to determine ultimate strength, Young's modulus, persistence length, bending rigidity, shear modulus, and torsional rigidity (**Figure 15**). Young's modulus and persistence length were measured to be 3.3 ± 0.4 GPa and 22 ± 3 μm, respectively. Several studies revealed that amyloid fibril's mechanical properties are comparable to those of silk, a well-known and broadly used biomaterial [57–59]. Interestingly, amyloid fibrils share some structural characteristics with silk: (1) frequently repetitive primary sequences; (2) stable and irreversible β-sheet-enriched state; (3) structural similarity with β-sheet rich structure; (4) self-assembling behavior to fibrillary structures in solution; and (5) hydrogen bonding between β-sheets. Comparable mechanical properties and structural features indicate that amyloid fibrils can also be used in various applications [59]. The Young's modulus of various amyloid fibrils ranged from 10^9 to 10^{10} Pa, which is similar to that of silk [57].
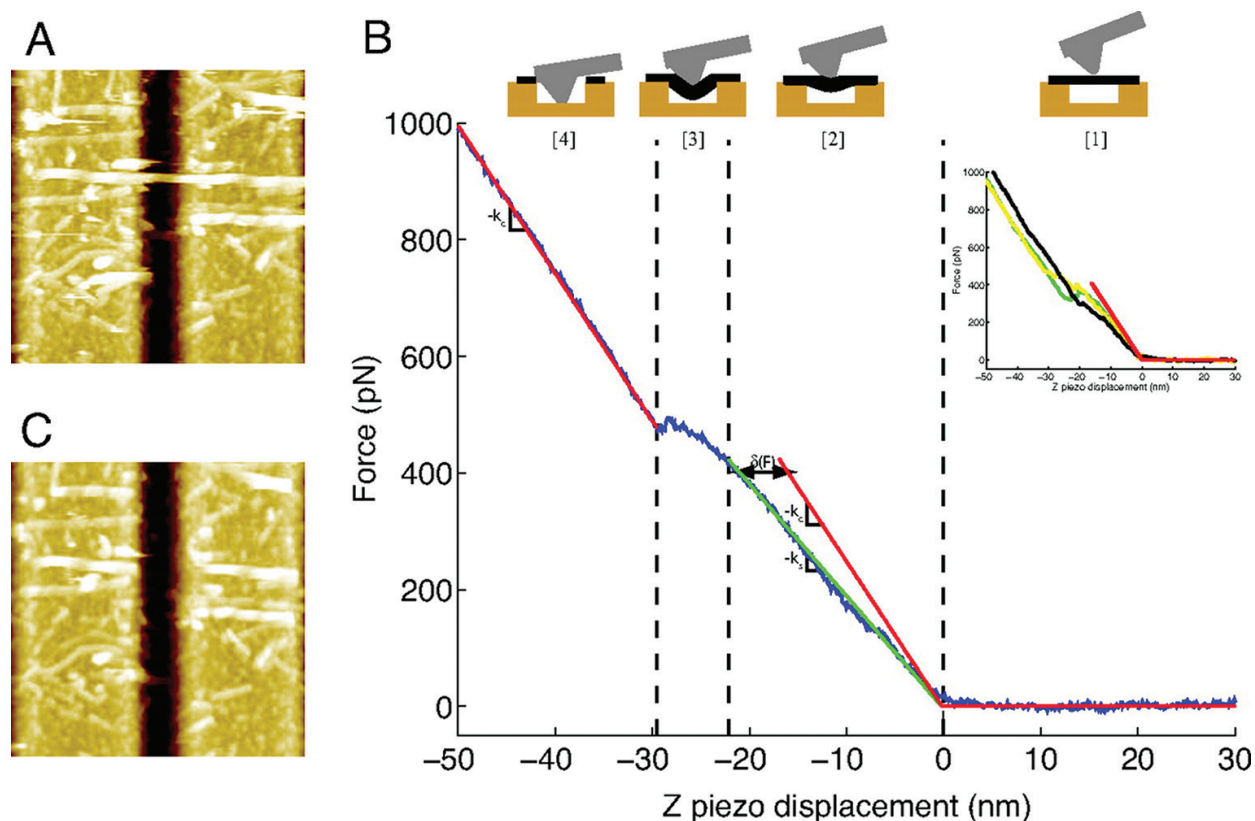
Figure 15. (A) Image of insulin amyloid fibrils before breakage (B) Force displacement curve for bending with atomic force microscope (C) Image after breakage [58].

The mechanical properties of amyloid fibrils are not affected by covalent bonds constructing the β-sheets, but rather by noncovalent bonds between β-sheets. Hydrogen bonds are important for maintaining amyloid fibrils and their mechanical properties; other non-covalent bonds (e.g., electrostatic interactions, π-π interactions, etc.) can also affect strength depending on the amyloid fibril structures.

4.2.2. Simulations

Experimental studies have revealed that various types of amyloid fibrils exist. Not only disease-related fibril types such as Alzheimer's disease, Jakob-Cruetzfeld disease, type 2 diabetes, and Parkinson's disease, among others but also those that form amyloid fibrils in same phenotypes of amyloids are important. For example, human islet amyloid polypeptide (hIAPP), which is found in cells of type 2 diabetes patients, forms eight different fibril types by stacking in different directions [17, 41]. Differences in the composition of amyloid fibrils lead to different mechanical behaviors and fractions in the body. Small fraction structures are more difficult to form than large fraction structures. Simulation methods constitute an easier approach for evaluating these variations in amyloid fibrils. A common structural characteristic of the amyloid fibril is its repetitive structure of β-sheets. Structural information determined experimentally may differ from that determined computationally.

Detailed structural information (distance between β-sheets, twist angle between layers, etc.) of amyloid fibrils is clearly needed to rapidly and effectively build the fibrils [17]. An important simulation study evaluated the size-dependent mechanical properties of $A\beta_{1-40}$ [60]. In this study, two different types of Aβ amyloid fibrils were constructed: a twofold symmetric structure and threefold symmetric structure. A computational model was prepared using an elastic network model (ENM), and normal mode analysis (NMA) was applied to calculate eigenvalue eigenvectors of the structure. Torsional modulus, bending rigidity, and Young's modulus were calculated for different types of amyloid fibrils, with the twofold symmetric structure showing better mechanical properties. Yoon et al. analyzed four configurations of hIAPP amyloid fibrils. They also applied ENM to calculate the mechanical properties of hIAPP amyloid fibrils. Despite this detailed chemical interaction information, each structure exhibited different mechanical properties related to differences in H-bond interactions [61]. Additionally, the effects of mutations on the mechanical properties of amyloid fibrils [62] or multi-strand effects can be calculated [63].

4.3. Structural effect of prion

4.3.1. Structural variation depending on environment and its properties

Proteins functioning in living bodies interact with various surroundings such as water, ion, proteins, ligands, and DNA. Protein conformation, which is determined by sequence and length of the polypeptide chain, is important in the interactions with the surroundings of a protein. Some environmental conditions may favor amyloid formation (which may lead to an unhealthy condition). Studies of prions revealed that prions form a certain shape or structure under specific conditions, indicating that amyloid structure depends on the surroundings [4]. In this study, prions were exposed to different pH conditions, including pH 2, 3, 4, and 7. In each state, prions formed triplets, singlets and triplets, an angled-layer aggregate, and bundles at pH 2, 3, 4, and 7, respectively. The stability of each conformation changed when the pH was changed (surroundings). For example, a triplet structure formed at pH 2 and 3 but with different conformations. Additionally, a singlet was detected at pH 3. When the pH was changed to 4, the triplet fibril disappeared while the angled-layer structure remained. These structures may be related to the mechanical properties or interactions between fibrils. Triplet prion fibrils are stable and noninfectious (pH 2). However, when pH is increased, the triplet fibril structure is broken into singlets (pH 3) and become infectious. These singlet fibrils can easily break and form new fibrils from new seeds, forming an angled-layered structure (pH 4) as shown in **Figure 16**.

4.3.2. Simulation methods

Changes in pH affect the chemical interactions of prion amyloid fibrils. Although simulation researches do not reveal chemical interaction, they provide other useful information.

A study conducted in 2013 compared the mechanical properties of prion amyloid fibrils (left-handed turn) and nonprion fibrils (right-handed turn) using ENM and NMA [27]. Basic structural units were very similar β-sheets, but mechanical properties such as elastic modulus and bending rigidity differed for both fibrils as shown in **Figure 17**. In fact, a toxic prion fibril exhibited higher mechanical properties. Additionally, left-handed prion fibrils had more local contacts than nonprion fibrils, which are consistent with the results of a previous study analyzing hydrogen bonds.
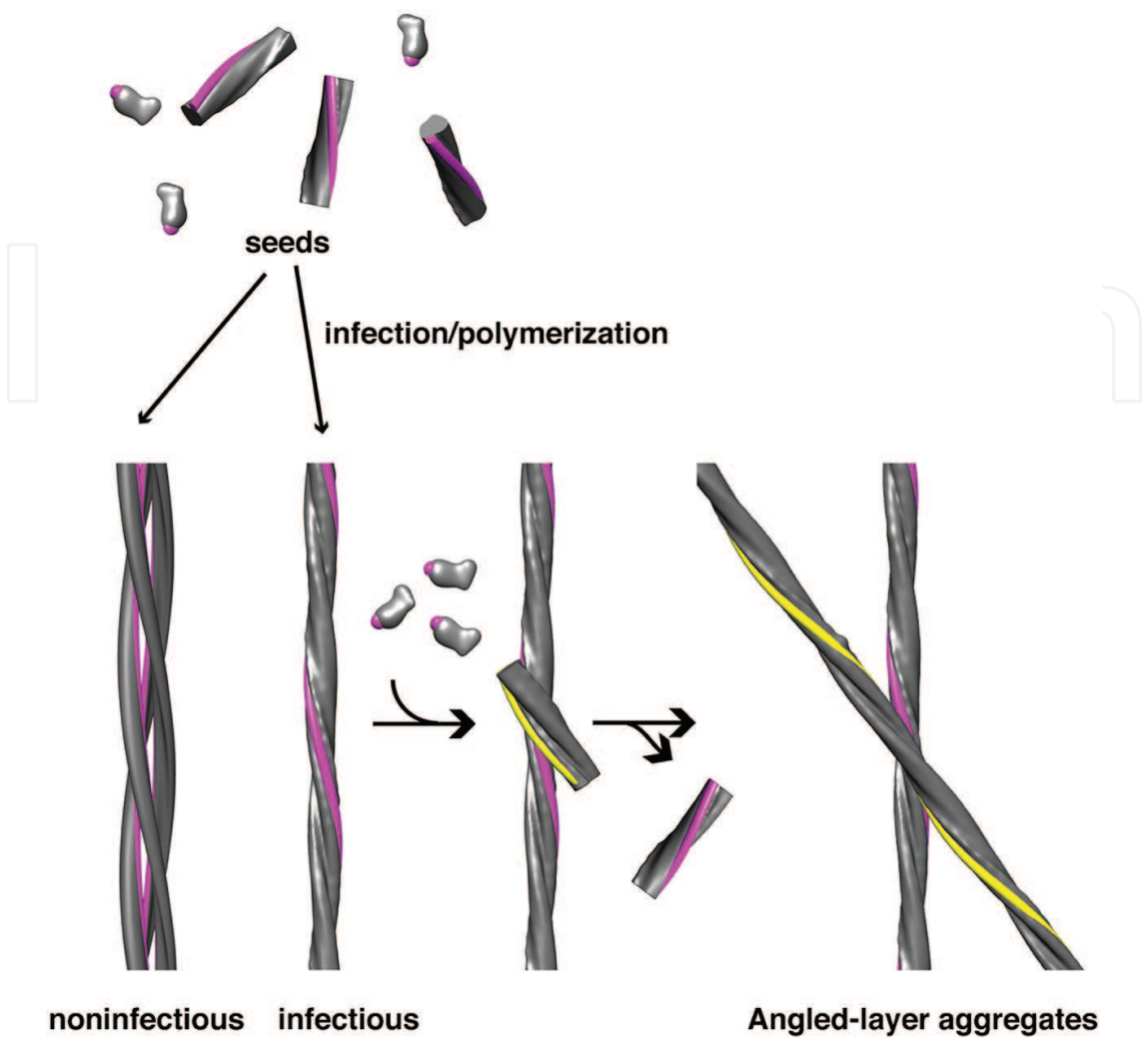
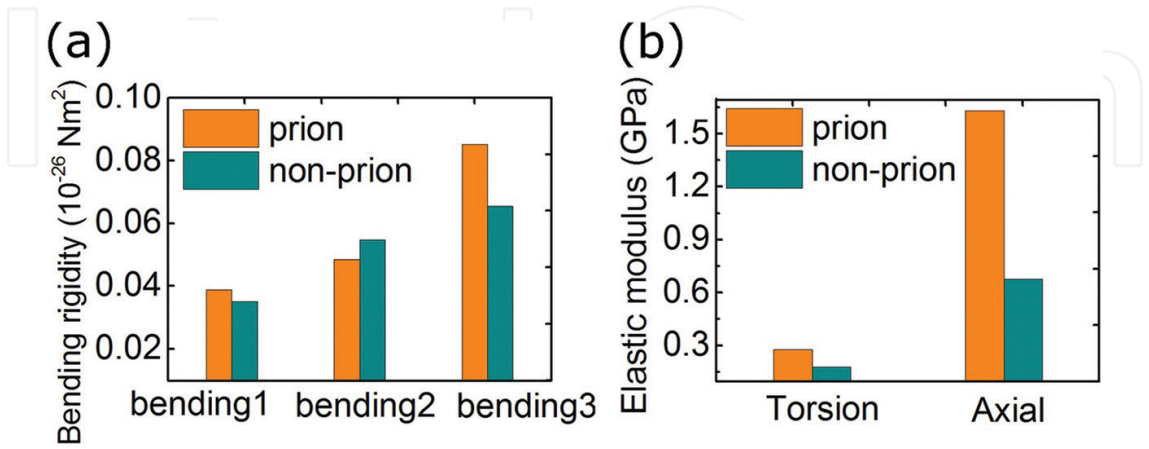**Figure 16.** Surface seeding model for assembly of HET-s prion disease fibrils [4].

Figure 17. Difference of mechanical properties between prion fibril (orange) and non-prion fibril (a) Bending rigidities (b) Torsional and axial elastic modulus [27].

Triplet fibrils were also examined by ENM and NMA [64]. In this study, prion triplet fibrils were compared at pH 2 and 3. Mechanical properties such as bending rigidity and torsional modulus were calculated for both models; fibrils for the pH 2 model showed better values for both parameters. This indicates that the pH 2 model was more stable than the pH 3 model, which is known to be noninfectious [4]. However, the pH 3 triplet showed a bending rigidity of 0.6×10^{-26} Nm^2, which is much larger than the value of 0.3×10^{-26} Nm^2 determined for a singlet fibril in a previous study [27]. The structure and mechanical properties of prion fibrils are related, explaining their toxicities. Moreover, in the previous study, the potential for conformational changes between the pH 2 and 3 models was observed. NMA provides results in eigenvalues and eigenvectors, which are connected to natural frequencies and normal modes. A normal mode corresponds to a single natural frequency, and the low frequency normal mode describes large motions of structures (i.e., for amyloid fibril, bending, twisting, and extension). Comparison of the normal mode calculated from the pH 2 model and direction vector between the pH 2 and 3 model showed that low-frequency normal modes up to the 23rd mode described conformational changes in triplet fibril caused by pH change. These results indicate that conformational changes are caused by pH changes, but the conformational change direction can reveal structure information (**Figure 18**).
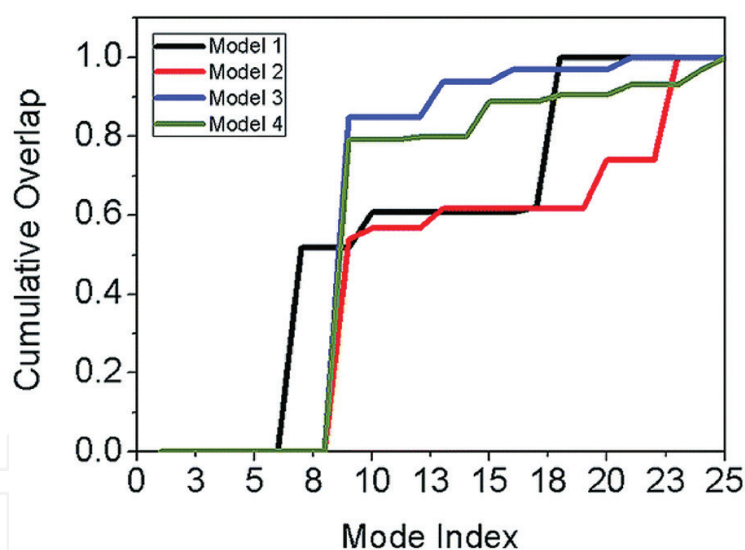

Figure 18. Cumulative overlap between normal modes calculated from pH 2 model and direction vector between pH 2 and pH 3 model [64].

5. Summary

In this chapter, the formation of amyloidogenic prion nanofibrils and the relationship between molecular structure and its properties are presented. In the first section, the seeding mechanisms of prions for the homogenous and heterogeneous fibrils are introduced, and the

molecular structure differences in the assembly process of amyloid fibrils under environmental conditions are described in the second section. Finally, the mechanical characterization of amyloid fibrils *in vitro* and *in silico* is explained. It remains challenging to determine the infectiousness and toxicity of prions and amyloids. Therefore, additional studies are needed to determine the molecular structure, aggregation kinetics, and properties of prions and amyloids. Increasing the understanding of these molecules will aid in the development of therapeutic strategies for prion diseases.

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References

[1] Stöhr J, Weinmann N, Wille H, Kaimann T, Nagel-Steger L, Birkmann E, et al. Mechanisms of prion protein assembly into amyloid. Proceedings of the National Academy of Sciences. 2008;105:2409–2414.

[2] Kihara M, Chatani E, Sakai M, Hasegawa K, Naiki H, Goto Y. Seeding-dependent maturation of β2-microglobulin amyloid fibrils at neutral pH. Journal of Biological Chemistry. 2005;280:12012–12018.

[3] Paravastu AK, Qahwash I, Leapman RD, Meredith SC, Tycko R. Seeded growth of β-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. Proceedings of the National Academy of Sciences. 2009;106:7443–7448.

[4] Mizuno N, Baxa U, Steven AC. Structural dependence of HET-s amyloid fibril infectivity assessed by cryoelectron microscopy. Proceedings of the National Academy of Sciences. 2011;108:3252–3257.

[5] Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. New England Journal of Medicine. 2003;349:583–596.

[6] Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nature Reviews Neuroscience. 2003;4:49–60.

[7] Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annual Review of Biochemistry. 2006;75:333–366.

[8] Pepys MB. Amyloidosis. Annual Review of Medicine. 2006;57:223–241.

[9]   Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. Cell. 2012;148: 1188–1203.

[10] Merlini G, Seldin DC, Gertz MA. Amyloidosis: pathogenesis and new therapeutic options. Journal of Clinical Oncology. 2011;29:1924–1933.

[11] Wang C, Yang A, Li X, Li D, Zhang M, Du H, et al. Observation of molecular inhibition and binding structures of amyloid peptides. Nanoscale. 2012;4:1895–1909.

[12] Baral Pravas K, Swayampakula M, Rout Manoj K, Kav Nat NV, Spyracopoulos L, Aguzzi A, et al. Structural basis of prion inhibition by phenothiazine compounds. Structure. 2014;22:291–303.

[13] Tycko R, Wickner RB. Molecular structures of amyloid and prion fibrils: consensus versus controversy. Accounts of Chemical Research. 2013;46:1487–1496.

[14] Kad NM, Myers SL, Smith DP, Alastair Smith D, Radford SE, Thomson NH. Hierarchical assembly of β2-microglobulin amyloid *in vitro* revealed by atomic force microscopy. Journal of Molecular Biology. 2003;330:785–797.

[15] Madine J, Jack E, Stockley PG, Radford SE, Serpell LC, Middleton DA. Structural Insights into the polymorphism of amyloid-like fibrils formed by region 20–29 of amylin revealed by solid-state NMR and X-ray fiber diffraction. Journal of the American Chemical Society. 2008;130:14990–15001.

[16] Mylonas E, Hascher A, Bernadó P, Blackledge M, Mandelkow E, Svergun DI. Domain conformation of tau protein studied by solution small-angle X-ray scattering. Biochemistry. 2008;47:10345–10353.

[17] Nielsen JT, Bjerring M, Jeppesen MD, Pedersen RO, Pedersen JM, Hein KL, et al. Unique identification of supramolecular structures in amyloid fibrils by solid-state NMR spectroscopy. Angewandte Chemie. 2009;121:2152–2155.

[18] Adamcik J, Jung J, Flakowski J, Rios De Los P, Dietler G, Mezzenger R. Understanding amyloid aggregation by statistical analysis of atomic force microscopy images. Nature Nanotechnology. 2010;5:423–428.

[19] Sachse C, Grigorieff N, Fändrich M. Nanoscale flexibility parameters of alzheimer amyloid fibrils determined by electron cryo-microscopy. Angewandte Chemie International Edition. 2010;49:1321–1323.

[20] Xu LP, Liu Y, Zhang X. Interfacial self-assembly of amino acids and peptides: scanning tunneling microscopy investigation. Nanoscale. 2011;3:4901–4915.

[21] Yamaguchi KI, Takahashi S, Kawai T, Naiki H, Goto Y. Seeding-dependent propagation and maturation of amyloid fibril conformation. Journal of Molecular Biology. 2005;352:952–960.

[22] Yan J, Fu X, Ge F, Zhang B, Yao J, Zhang H, et al. Cross-seeding and cross-competition in mouse apolipoprotein A-II amyloid fibrils and protein A amyloid fibrils. The American Journal of Pathology. 2007;171:172–180.

[23] Ono K, Takahashi R, Ikeda T, Yamada M. Cross-seeding effects of amyloid β-protein and α-synuclein. Journal of Neurochemistry. 2012;122:883–890.

[24] Seeliger J, Evers F, Jeworrek C, Kapoor S, Weise K, Andreetto E, et al. Cross-amyloid interaction of Aβ and IAPP at lipid membranes. Angewandte Chemie International Edition. 2012;51:679–683.

[25] Morales R, Moreno-Gonzalez I, Soto C. Cross-seeding of misfolded proteins: implications for etiology and pathogenesis of protein misfolding diseases. PLoS Pathogens. 2013;9:e1003537.

[26] Lundmark K, Westermark GT, Olsén A, Westermark P. Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: cross-seeding as a disease mechanism. Proceedings of the National Academy of Sciences. 2005;102:6098–6102.

[27] Yoon G, Kab Kim Y, Eom K, Na S. Relationship between disease-specific structures of amyloid fibrils and their mechanical properties. Applied Physics Letters. 2013;102:011914.

[28] Kim JI, Lee M, Baek I, Yoon G, Na S. The mechanical response of hIAPP nanowires based on different bending direction simulations. Physical Chemistry Chemical Physics. 2014;16:18493–18500.

[29] Lee M, Baek I, Chang HJ, Yoon G, Na S. The bond survival time variation of polymorphic amyloid fibrils in the mechanical insight. Chemical Physics Letters. 2014;600:68–72.

[30] Choi B, Yoon G, Lee SW, Eom K. Mechanical deformation mechanisms and properties of amyloid fibrils. Physical Chemistry Chemical Physics. 2015;17:1379–1389.

[31] Lee M, Chang HJ, Kim D, Lee Y, Suh H, Ahn N, et al. Relationship between structural composition and material properties of polymorphic hIAPP fibrils. Biophysical Chemistry. 2015;199:1–8.

[32] Lee M, Kwon J, Na S. Mechanical behavior comparison of spider and silkworm silks using molecular dynamics at atomic scale. Physical Chemistry Chemical Physics. 2016;18:4814–4821.

[33] Guo JP, Arai T, Miklossy J, McGeer PL. Aβ and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America. 2006;103:1953–1958.

[34] Li DW, Mohanty S, Irbäck A, Huo S. Formation and growth of oligomers: a monte carlo study of an amyloid tau fragment. PLoS Computational Biology. 2008;4:e1000238.

[35] Raz Y, Miller Y. Interactions between Aβ and mutated tau lead to polymorphism and induce aggregation of Aβ-mutated tau oligomeric complexes. PLoS One. 2013; 8:e73303.

[36] Do TD, Economou NJ, Chamas A, Buratto SK, Shea JE, Bowers MT. Interactions between Amyloid-β and tau fragments promote aberrant aggregates: implications for amyloid toxicity. The Journal of Physical Chemistry B. 2014;118:11220–11230.

[37] Choi H, Lee M, Park HS, Na S. The effect of structural heterogeneity on the conformation and stability of A[small beta]-tau mixtures. RSC Advances. 2016;6:52236–52247.

[38] Miller Y, Ma B, Nussinov R. Synergistic Interactions between repeats in tau protein and Aβ amyloids may be responsible for accelerated aggregation via polymorphic states. Biochemistry. 2011;50:5172–5181.

[39] Peralta MDR, Karsai A, Ngo A, Sierra C, Fong KT, Hayre NR, et al. Engineering amyloid fibrils from β-solenoid proteins for biomaterials applications. ACS Nano. 2015;9:449–463.

[40] Jie Z, Buyong M, Ruth N. Consensus features in amyloid fibrils: sheet–sheet recognition via a (polar or nonpolar) zipper structure. Physical Biology. 2006;3:P1.

[41] Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, et al. Atomic structures of amyloid cross-[bgr] spines reveal varied steric zippers. Nature. 2007;447:453–457.

[42] Matthes D, Gapsys V, Daebel V, de Groot BL. Mapping the conformational dynamics and pathways of spontaneous steric zipper peptide oligomerization. PLoS One. 2011;6:e19129.

[43] Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid [beta]-peptide. Nature Reviews Molecular Cell Biology. 2007;8:101–112.

[44] Xue Q, Zheng QC, Zhang JL, Cui YL, Chu WT, Zhang HX. Mutation and low pH effect on the stability as well as unfolding kinetics of transthyretin dimer. Biophysical Chemistry. 2014;189:8–15.

[45] Colby DW, Prusiner SB. De novo generation of prion strains. Nature Reviews Microbiology. 2011;9:771–777.

[46] Govaerts C, Wille H, Prusiner SB, Cohen FE. Evidence for assembly of prions with left-handed β-helices into trimers. Proceedings of the National Academy of Sciences of the United States of America. 2004;101:8342–8347.

[47] Wasmer C, Lange A, Van Melckebeke H, Siemer AB, Riek R, Meier BH. Amyloid fibrils of the HET-s(218–289) prion form a β solenoid with a triangular hydrophobic core. Science. 2008;319:1523–1526.

[48] Schmidt A, Annamalai K, Schmidt M, Grigorieff N, Fändrich M. Cryo-EM reveals the steric zipper structure of a light chain-derived amyloid fibril. Proceedings of the National Academy of Sciences. 2016;113:6200–6205.

[49] Esposito L, Pedone C, Vitagliano L. Molecular dynamics analyses of cross-β-spine steric zipper models: β-sheet twisting and aggregation. Proceedings of the National Academy of Sciences. 2006;103:11533–11538.

[50] Krone MG, Hua L, Soto P, Zhou R, Berne BJ, Shea JE. Role of water in mediating the assembly of Alzheimer amyloid-β Aβ16–22 protofilaments. Journal of the American Chemical Society. 2008;130:11066–11072.

[51] Periole X, Rampioni A, Vendruscolo M, Mark AE. Factors that affect the degree of twist in β-sheet structures: a molecular dynamics simulation study of a cross-β filament of the GNNQQNY peptide. The Journal of Physical Chemistry B. 2009;113:1728–1737.

[52] Chen W, van der Kamp Marc W, Daggett V. Structural and dynamic properties of the human prion protein. Biophysical Journal. 2014;106:1152–1163.

[53] Cheng C, Daggett V. Molecular dynamics simulations capture the misfolding of the bovine prion protein at acidic pH. Biomolecules. 2014;4:181.

[54] van der Kamp MW, Daggett V. Pathogenic mutations in the hydrophobic core of the human prion protein can promote structural instability and misfolding. Journal of Molecular Biology. 2010;404:732–748.

[55] DeMarco ML, Daggett V. From conversion to aggregation: Protofibril formation of the prion protein. Proceedings of the National Academy of Sciences of the United States of America. 2004;101:2293–2298.

[56] Alonso DOV, DeArmond SJ, Cohen FE, Daggett V. Mapping the early steps in the pH-induced conformational conversion of the prion protein. Proceedings of the National Academy of Sciences. 2001;98:2985–2989.

[57] Knowles TPJ, Buehler MJ. Nanomechanics of functional and pathological amyloid materials. Nature Nanotechnology. 2011;6:469–479.

[58] Smith J, Knowles T, Dobson C, MacPhee C, Welland M. Characterization of the nanoscale properties of individual amyloid fibrils. Proceedings of the National Academy of Sciences. 2006;103:15806–15811.

[59] Cherny I, Gazit E. Amyloids: not only pathological agents but also ordered nanomaterials. Angewandte Chemie International Edition. 2008;47:4062–4069.

[60] Xu Z, Paparcone R, Buehler MJ. Alzheimer's Aβ(1–40) amyloid fibrils feature size-dependent mechanical properties. Biophysical Journal. 2010;98:2053–2062.

[61] Yoon G, Kwak J, Kim JI, Na S, Eom K. Mechanical characterization of amyloid fibrils using coarse-grained normal mode analysis. Advanced Functional Materials. 2011;21:3454–3463.

[62] Yoon G, Lee M, Kim JI, Na S, Eom K. Role of sequence and structural polymorphism on the mechanical properties of amyloid fibrils. PLoS One. 2014;9:e88502.

[63] Gwonchan Y, Myeongsang L, Kyungwoo K, Jae In K, Hyun Joon C, Inchul B, et al. Morphology and mechanical properties of multi-stranded amyloid fibrils probed by atomistic and coarse-grained simulations. Physical Biology. 2015;12:066021.

[64] Choi H, Chang HJ, Shin Y, Kim JI, Park HS, Yoon G, et al. The molecular mechanism of conformational changes of the triplet prion fibrils for pH. RSC Advances. 2015;5:49263–49269.