Journal of Alzheimer's Disease 59 (2017) 785–795
DOI 10.3233/JAD-170230
IOS Press

Review

Rosetta Stone for Amyloid Fibrils: The Key Role of Ring-Like Oligomers in Amyloidogenesis

Oxana V. Galzitskaya* and Olga M. Selivanova
Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

Handling Associate Editor: Ewa Bienkiewicz

Accepted 2 June 2017

Abstract. Deeper understanding of processes of protein misfolding, aggregation, formation of oligomers, protofibrils, and fibrils is crucial for the development of future medicine in treatment of amyloid-related diseases. While numerous reports illuminate the field, the above processes are extremely complex, as they depend on many varying parameters, such as the peptide concentration, temperature, pH, presence of metal ions, lipids, and organic solvents. Different mechanisms of amyloid fibril formation have been proposed, but the process of the oligomer−to−fibril transition is the least agreed upon. Our studies of a number of amyloidogenic proteins and peptides (insulin, Aβ peptides, the Bgl2 protein from the yeast cell wall), as well as their amyloidogenic fragments, have allowed us to propose a model of the fibril structure generation. We have found that the main building block of fibrils of any morphology is a ring-like oligomer. The varying models of interaction of ring oligomers with each other revealed in our studies make it possible to explain their polymorphism. Crucially, the amino acid sequence determines the oligomer structure for the given protein/peptide.

Keywords: Alzheimer's disease, amyloid-β, insulin, nucleus, ring-like oligomers, seeds

INTRODUCTION

The number of known diseases associated with an aberrant folding of proteins—amyloidosis—has been growing steadily over the past 30 years. They, in particular, include many neurodegenerative conditions, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementia, and amyotrophic lateral sclerosis, as well as type 2 diabetes. Seemingly, amyloid depositions may occur in any human tissue. It has been established that aberrant protein folding leads frequently to aggregation and generation of fibrils, which at the permissive concentrations form large associations in the form of plaques. But being at the center of attention, solving the problem of fibril formation, and deciphering the mechanism of amyloidogenesis, has been hampered by high polymorphism displayed by the amyloid fibrils. Under similar conditions, the same protein/peptide can form fibrils of varying morphology, which results in the fact that different laboratories actually study different polymorphs. Moreover, samples of the same protein from different sources (for example, from different patients), or those obtained through different methods of sample preparation, exhibit morphologically different fibrils. While the main aim in studying the mechanism of fibril formation is the search for the factors which trigger the process, to be able potentially to prevent it, of great importance are all stages of generation of

*Correspondence to: Oxana V. Galzitskaya, Institute of Protein Research, Institutskaya Street 4, 142290, Pushchino, Russia. E-mail: ogalzit@vega.protres.ru.

ISSN 1387-2877/17/$35.00

elongated polymers from native monomers and into mature fibrils.

Despite the huge number of articles devoted to studying different proteins and peptides, there is no clarity in understanding the molecular level mechanism of fibril formation. Moreover, much attention is focused on studying not only the structure of mature fibrils but also different intermediate states of protein aggregation, including oligomer forms. In this case, not only the structure of different oligomer derivatives is studied, but also their interconnection with disease symptoms. Abundant literature data show the toxicity of oligomer samples from small oligomer formations (dimers) to large supramolecular complexes (annular pores) [1–8]. Indeed, a large number of recent studies clearly show that Aβ oligomers are the initiating and pathologic Aβ species causing Alzheimer's disease and that neither Aβ monomers or fibrillar Aβ (insoluble Aβ) are toxic/pathogenic [9–14]. This allows development of high resolution biological models of protein conformational changes upon amyloid formation.

HOW ARE MOLECULES POLYMERIZED TO FORM FIBRILS?

The cross-β structure of amyloids (the "pleated-sheet") was first revealed in 1968 using the X-ray diffraction analysis of amyloidosis-affected liver and spleen tissues [15]. Since then, X-ray diffraction has become one of the main methods for studying amyloid structures, together with EM visualization, interaction with ThT, and birefringence upon interaction with Congo red. Using X-ray diffraction, it was demonstrated that the presence of the cross-β structure is a common feature of amyloids [10–12].

The cross-β structure suggests that fibrils possess a morphology where the polypeptide chain is organized in such a way that β-layers are formed stretching parallel to the fibril long axis at a distance of about 10 Å from each other. These β-layers are formed of β-strands located at a distance of about 4.7 Å from each other and perpendicular to the fibril long axis. The deciphering of amino acid sequences of first amyloidogenic peptides and proteins [19] gave rise to advanced studies of many proteins/peptides associated with different types of amyloidosis, and, ultimately, to revealing the mechanisms of fibril formation. This has led to the development of a number of models of arrangement of β-sheets in a fibril, and also to creation of a variety of models of fibril formation. Varying descriptions of fibril formation for different proteins/peptides can be found in literature, with particular models being associated with each object of studies (e.g., insulin, amyloid-β (Aβ) peptide and its multiple fragments) [20–22]. Subsequently, no common model of the three-dimensional structure of amyloids currently exists [23].

It is generally believed that fibrils can: 1) be formed of a varying number of filaments (from 2 to 6); 2) associate with each other laterally forming bands of different widths; 3) twist and form bundles of varying diameters; and 4) associate and thus form bundles. The propensity to polymorphism is a general feature of all studied amyloids, being observed not only upon changes in the conditions of fibril formation (pH, ionic strength, temperature), but also under constant conditions. It has been noted that in all amyloid fibrils the diameter of the thinnest polymers is about 10 nm, while their length can reach several micrometers. The question arises: how do the amyloids maintain the typical cross-β structure and similar average parameters, while their amino acid sequences, and the corresponding anticipated ways of the tertiary structure formation are quite different? Besides, taking into account the simplified scheme of fibril formation (destabilized monomer $\longrightarrow$ oligomer $\longrightarrow$ mature fibril), the main unexplained moment in all models is transition from oligomers, frequently possessing a ring-like structure, to fibrils.

THE KEY ROLE OF OLIGOMERS IN THE AMYLOID FORMATION SCENARIO

Oligomers are invariable participants in the process of generation of fibrils (Fig. 1). Studying of many published EM images (even when not mentioned by the authors) reveal ring-like oligomer particles of about 10 nm in diameter [24, 25]. It should be noted that electron microscopy (EM) and atomic-force microscopy (AFM) images found in different publications show oligomer particles similar to that of thin fibrils, and often possessing a cavity in the middle, i.e., they are ring oligomers [24–28]. But even if the authors of the publications pay attention to oligomer structures, they rarely assign to them a role in the fibril formation, through their association into elongated polymers. Nevertheless, Nielsen [29] describes the use of the EM method for studying the process of fibrillogenesis of $A\beta_{1\text{-}42}$ peptide. A new mechanism of α-synuclein fibril formation was discovered [30], according to which a

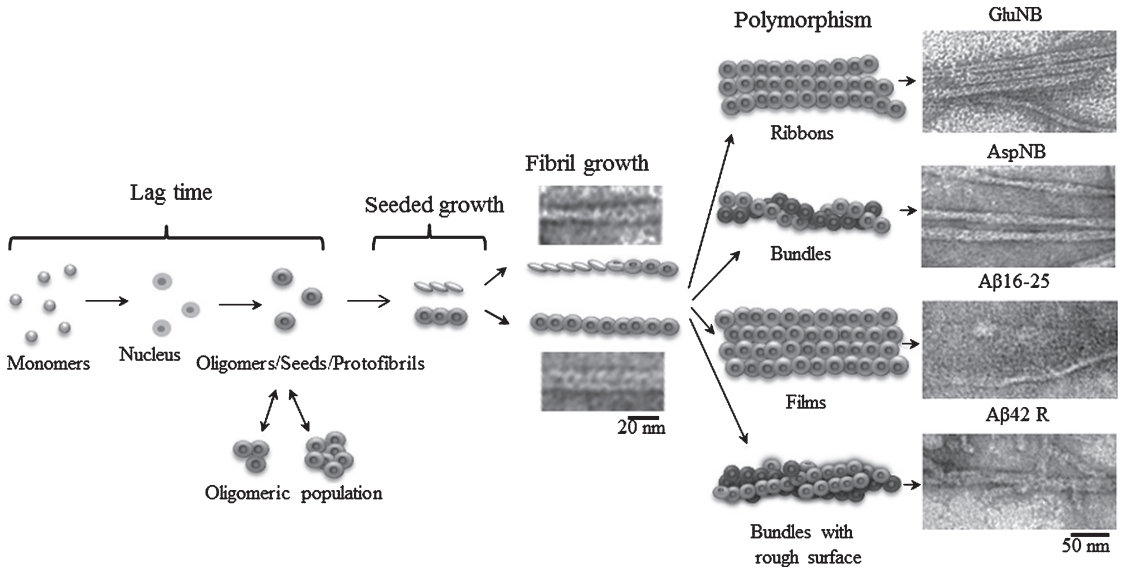

Fig. 1. Schematic representation of fibrillation process. The building block for fibril formation is a ring oligomer. If the ring oligomers interact with each other side-by-side, slightly overlapping each other, then ribbons of different widths (GluNB) [40], bundles of different diameters (AspNB) [40] and films ($A\beta_{16\text{-}25}$) [72] are formed. In the chaotic interaction of ring oligomers fibrils with a rough surface are formed ($A\beta_{42}$ R) [36, 38].

fibril is formed through association of pre-formed oligomer granular structures as a growing unit. In various publications, EM and AFM images frequently show rounded oligomers of approximately the same diameter as that of thin fibrils, the oligomer particles often having an internal cavity, which indicates that they are ring structures [18, 22]. A number of models has been proposed for the process of assembling of amyloid fibrils, in which the interaction of oligomer particles in different ways leads to formation of elongated polymers of various amyloid proteins [18, 25–29]. Our own studies of different amyloidogenic proteins (insulin, Aβ peptides, and their fragments, amyloidogenic fragments of Bgl2 protein) show that at the beginning of fibril formation a large number of oligomer ring-like structures is observed as well as a small number of short thin fibrils with a diameter comparable to that of oligomers [36–40]. As the time of incubation increases, the fraction of oligomer particles decreases, fibrils grow to several micrometers in length, and fibrils of different morphologies are formed. At a high enough magnification, it is possible to see that fibrils are formed from the ring oligomers associated in various ways, which would explain polymorphism of fibrils formed under the same conditions. When the conditions change, some new forms of fibrils can appear. This could also be explained as changes in the way of fibril formation of the same structure blocks (oligomers) under new conditions. Moreover, the apparently similar average diameter of thin fibrils observed for most amyloidogenic small proteins/peptides (about 10 nm) might be the reason for the oligomeric nature of formation of fibrils of different peptides. Nonetheless, every peptide exhibits some peculiarities upon oligomer formation. The diameter of oligomers, their height and internal diameter of the ring, as well as the number and way of packing of molecules within oligomers, can vary [36, 38–40]. It becomes evident how important it is for correct interpretation of the experimental data obtained with EM methods to consider the role of X-ray diffraction (sample preparation and analysis of the diffraction patterns), solid-state nuclear magnetic resonance (ssNMR), as well as final revision of the results using the methods of theoretical biophysics and bioinformatics.

ELECTRON MICROSCOPY OF $A\beta_{1\text{-}40}$ AND $A\beta_{1\text{-}42}$ AMYLOID FIBRILS

Peptide fibrils seem to consist of two filaments which can be twisted with a different period. However, at a large magnification, it can be seen that the finest fibrils consist of rounded ring oligomers (Fig. 2). These ring oligomers have the diameter of about 8–10 nm and frequently interact with each other, either ring-to-ring, or more frequently ring-on-ring with some shift. A similar way of fibrils

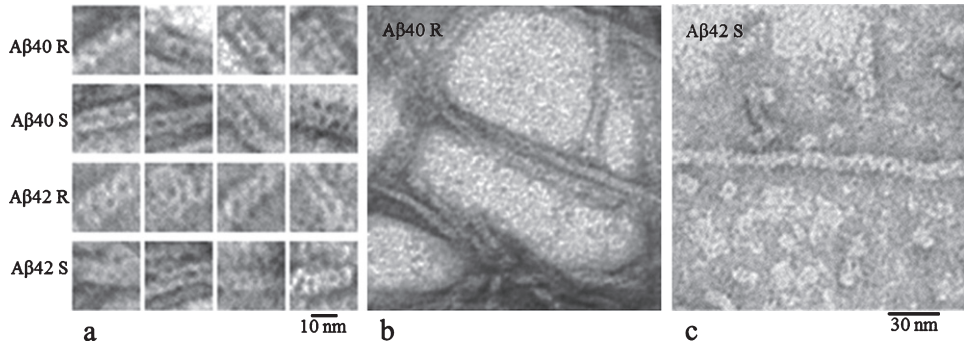

Fig. 2. Electron microscopy images of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ fibrils produced from synthetic (S) and recombinant peptides (R). Conditions for all samples: 5% DMSO, 50 mM Tris-HCl, pH 7.5, incubation for 26 h at 37°C. (a) Gallery of fibril fragments; (b) and (c) are fields of the $A\beta_{40}$ R and $A\beta_{42}$ S fibrils.

formation was reported earlier, when a fibril is generated through interaction of some globular structures (oligomers) [29]. The same observations were also reported in a later publication [41]. Upon staining with uranyl acetate, the staining agent might fill the holes in ring oligomers, and at low magnification it would appear as if fibrils were generated from two filaments. In addition to fibrils with the diameter of up to 10 nm, fibrils of a larger diameter are also seen for both peptides: up to 15 nm for $A\beta_{1\text{-}40}$, and between 15 and 35 nm for $A\beta_{1\text{-}42}$. Such structures could have formed as a result of association of the ring oligomers with each other in a disorderly manner. This mode of fibril formation leads to generation of fibrils of varying diameters. Corroborating this idea, time-lapse AFM studies [42] allowed observing how the sides of early fibrils pick up oligomers through lateral interactions, thus making fibrils thicker. In the case of $A\beta_{1\text{-}42}$ peptide, the diameter of mature fibrils varies greatly, while their surface appears very rough. It is interesting that for the synthetic $A\beta_{1\text{-}42}$ peptide the roughness of its surface is more characteristic. As seen in Fig. 4 of Dovidchenko et al. [43], $A\beta_{1\text{-}42}$ has a propensity for fibril branching, which is a characteristic of the synthetic $A\beta_{1\text{-}42}$ peptide previously noted by Suvorina et al. [44]. Thus, we propose that thickening of fibrils can occur concurrently with their lengthening, because this is more plausible than the search for a partner (filament-to-filament) in solution. Based on the above, we suggest that generation of fibrils of Aβ peptides takes place according to a simplified scheme: a monomer ⟶ a ring oligomer ⟶ a mature fibril, consisting of ring oligomers (see Fig. 2). Using the EM method, we have therefore demonstrated that fibrils of these peptides are formed by association of rounded ring structures. Negatively stained transmission electron microscopy images of fibrils extracted from Alzheimer's disease brain tissues [39] are very similar to our EM results. Moreover, similar structures were described by Lashuel et al. [41]. Looking at the historical chain, it will be found that *in situ* amyloid fibrils appear to be composed of roughly globular subunits: the structure could consist of a series of stacked disks where each disk might consist of five prime subunits arranged in a pentagon [26]. Fifty years later we have obtained the same picture which reveals that the amyloid structure *in vitro* has a ladder-type arrangement of ring-like oligomers [26].

DETERMINATION OF AMYLOIDOGENIC REGIONS INVOLVED IN AMYLOID FIBRIL FORMATION FOR $A\beta_{1\text{-}40}$ AND $A\beta_{1\text{-}42}$ PEPTIDES

Determination of regions responsible for aggregation of peptides and formation of amyloid fibrils is one of the primary aims in studying the formation of such structures. Knowledge of these regions will facilitate the choice of compounds interacting with the given region and thus preventing its aggregation. Therefore, in our study, one of the tasks was to determine amyloidogenic regions in amyloid fibrils formed by $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides and to compare the data with the theoretical prediction.

As known, the core of an amyloid fibril is resistant to the action of proteases. Therefore, it is possible to suggest that regions of the polypeptide chain involved in the core of the amyloid structure will be protected from the action of proteases, while the other parts of the polypeptide chain will be cleaved by proteases. Fibrils formed by $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides were treated with a mixture of proteases

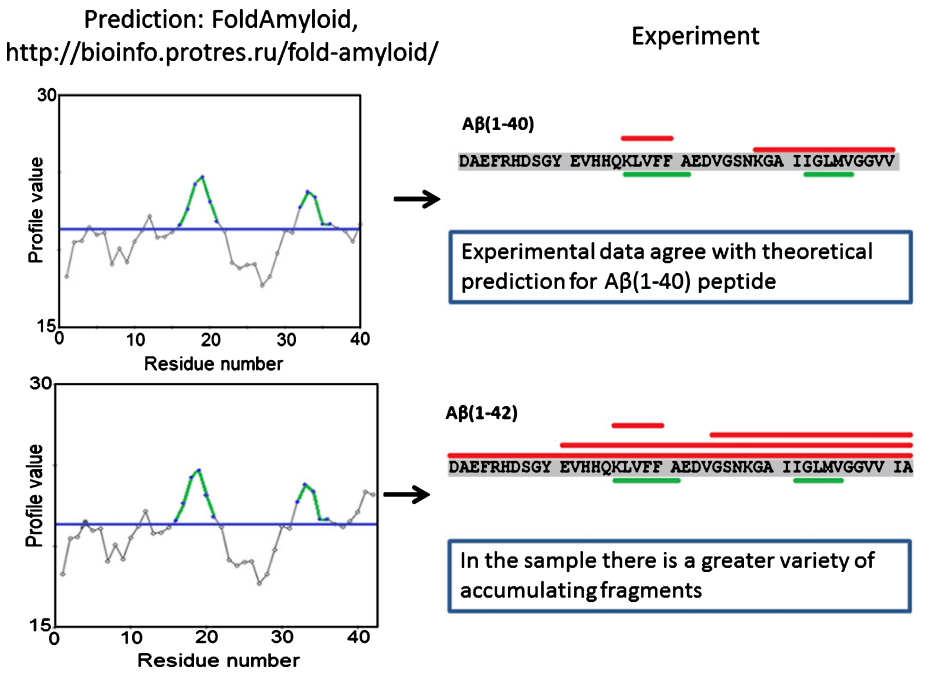

Fig. 3. Amyloidogenic regions predicted by the FoldAmyloid program are underlined by green color. Regions of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ most protected from proteolysis in the amyloid structure are indicated by red lines.

consisting of trypsin, chymotrypsin, and proteinase K. When proteolysis was terminated, the mixture was centrifuged [38, 45]. The cleaved peptides (not included in the core of the amyloid fibril) remained in the supernatant, whereas amyloid structures were precipitated. The precipitate was washed, dried in a vacuum concentrator, and dissolved in formic acid. The resulting peptides were separated by reversed phase chromatography and identified by tandem mass spectrometry. The data obtained was processed using the PEAKS Studio 7 program.

Thus, using mass-spectrometry we determined regions of the chain that are protected from the action of proteases in amyloid fibrils formed by the $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides. We demonstrated that for the $A\beta_{1\text{-}40}$ peptide, amino acid regions 16–22 and 28–40 are protected from the action of proteases upon formation of the amyloid structure (Fig. 3). These data are in agreement with the amyloidogenic regions (amino acid residues 16–21 and 32–36) in the sequence of the $A\beta_{1\text{-}40}$ peptide predicted by the FoldAmyloid program (http://bioinfo.protres.ru/fold-amyloid/) [46] (Fig. 3). A more complicated distribution of protected regions of the chain is observed for $A\beta_{1\text{-}42}$. In some molecules, the regions from 16 to 20 and 26 to 42 amino acid residues are also protected, but in other molecules the whole peptide is inaccessible to proteases. These data agree with the EM data showing that the diameter of the $A\beta_{1\text{-}42}$ fibrils varies much greater (8–35 nm) as compared to that of $A\beta_{1\text{-}40}$ (8-9 nm).

SOLID-STATE NMR METHOD ENABLES CONSTRUCTING ATOMIC MODELS OF $A\beta_{1\text{-}42}$ AND $A\beta_{1\text{-}40}$

ssNMR possesses high resolution and reveals some essential details of pure fibers. On the other hand, the method requires highly homogeneous samples. So, in these studies we have refined the sample (in the seeding, reseeding, and other processes) to remove other polymorphic fibers. When it is reported that the fiber structure of one sample differs from that of another sample, it could be due to the variations in the sample preparation procedures. It should be noted that the structural model produced using ssNMR is based upon the algorithm that takes into account the distance constraints. The larger is the number of the distance constraints obtained, the smaller would be the number of alternative structural models which can satisfy these data. In cryo-EM studies and ssNMR different fibril growth conditions are used, therefore different models for amyloid fibrils can be

constructed. Moreover, the chemical shifts in spectra for brain-seeded fibrils are significantly different from the shifts reported previously for fibrils grown *in vitro* [47, 48], and, more importantly, the chemical shifts for brain-seeded fibrils from different patients with Alzheimer's disease (patient I and patient II) are also different [49].

When ssNMR was eventually performed on full-length $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ fibers, these structures were found to be in an in-register parallel β-sheet pattern [50]. It was revealed in the ssNMR study that the secondary structure elements of protofibrils are more closely related to oligomers than to mature Aβ fibrils [51]. The core β-strand–turn–β-strand unit is similar for fibrils formed from $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides. The side chain packing registry within the β-turn-β motif of $A\beta_{1\text{-}42}$ fibrils is similar to that observed previously in $A\beta_{1\text{-}40}$ fibrils [47, 52]. The data are consistent with the previous studies showing that the $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides homogeneously mix in amyloid fibrils, suggesting that $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ fibrils have the same structural architecture (Fig. 2). Phe19 packs against Leu34 in the fibril conformation for both $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides [47, 49, 51, 52]. The *C*-terminus is not accessible to proteases for both $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ fibrils [38, 45]. This allows us to use the obtained structural model for $A\beta_{1\text{-}40}$ fibrils [49] to construct a possible model for $A\beta_{1\text{-}42}$ fibrils (see Fig. 4).

Such model is perfectly consistent with the calculated fibril nuclei sizes, i.e., the trimeric structure of $A\beta_{1\text{-}40}$ fibrils arises from stabilization of unstable dimer aggregates, where in the case of $A\beta_{1\text{-}42}$ the trimeric unstable structure is stabilized by the 4-th peptide. To support this model, one can note the similar width of $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ oligomers (Fig. 2); but $A\beta_{1\text{-}42}$ fibrils are slightly wider than $A\beta_{1\text{-}40}$ fibrils due to lower orderliness of the ring-like oligomers in $A\beta_{1\text{-}42}$ fibrils [36, 38]. As a direct consequence of the square structure, the fibril cross-section must have a hole in the center, which must be larger (∼3 nm) than that inside the triangular structure (∼1 nm). Such cavity within the oligomer structure is consistent with the X-ray data from which suggests the presence of a tubular cylinder with diameter of about 8.5 nm (Figs. 2, 5).

It is very important to study the full length of $A\beta_{1\text{-}42}$ or $A\beta_{1\text{-}40}$ because the fragments of these peptides will have other structures of oligomers as reported earlier [53]. The in-register parallel β-sheet organization was found in Aβ fibrils, but some fragments of the Aβ peptide give fibrils with antiparallel β-strands [54, 55].

INTERPRETATION OF X-RAY DATA IS AN IMPORTANT STEP IN THE MODELLING OF AMYLOID STRUCTURE

At present, no detailed interpretation of all reflections obtained by the X-ray analysis of amyloid preparations is available. But it is noted that a diffraction pattern can be affected by the way of alignment of amyloid fibrils (stretch frame alignment, alignment in a glass capillary, magnetic alignment, thin film alignment) [17, 53, 56, 57], sample concentration [58] and purity, source and method of its obtainment [57, 59]. It was demonstrated that a well aligned preparation has a more complex pattern of clear reflections and yields much more information [57]. In the literature, the existence of additional reflections for amyloid structures is explained in different ways. And much attention is paid to separate meridional and equatorial reflections [17, 53, 56]. Accurate interpretation of X-ray diffraction reflections requires not only a high quality of the obtained diffraction patterns but also

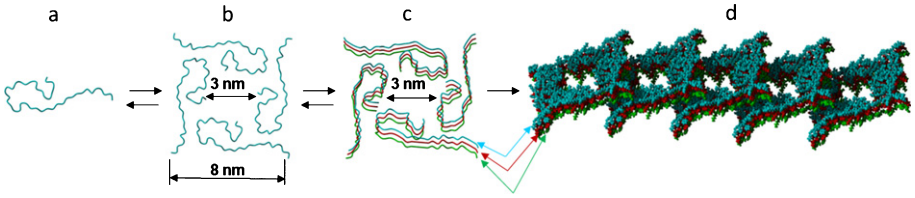

Fig. 4. Schematic representation of a possible mechanism of formation of fibrils by $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides: (a) destabilized monomer; (b) primary oligomer, the basis for formation of an annular oligomer; (c) the ring-like oligomer composed of three primary oligomers or 12 monomers (dodecamer) is the basic building block for formation of fibrils (seed); (d) the fibril build up from ring-like oligomers (protofibrils). Arrows show individual primary oligomers or the protofibril core. PDB entry 2M4J was used for modeling [49]. Arg5-Glu22 inter- and Asp23-Lys28 intra-peptide salt bridges are present in the dodecamer structure, which is the building block of fibrils. Oligomers overlap in the same row and between the rows. The hydrophobic carboxyl-terminal sheet surface in fibrils from residues 26–40(42) is screened by sheet stacking interaction with the same surface from adjacent fibril.

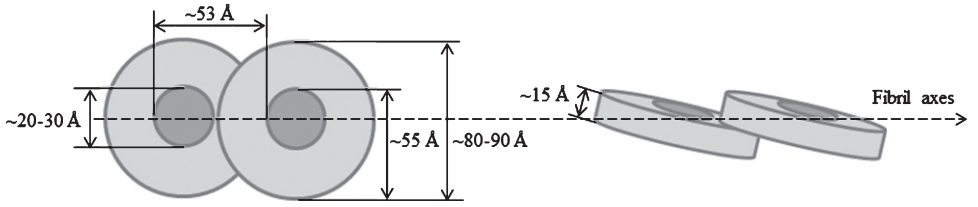

Fig. 5. Schematic representation of the arrangement of ring oligomers (the short tubular cylinders) in a fibril. These parameters correspond to a number of reflexes according to X-ray data [53, 56]. Equatorial reflexes are marked perpendicular, and meridional are parallel to the axis of the fibril.

explanation of the fibril structure on the molecular level. Bragg spacings for fragments of the Aβ peptide are summarizes in Table 1 of Inouye et al. [53]. The meridional reflection at 53 Å for $A\beta_{1\text{-}40}$ indicates a periodic structure along the H-bonding direction. Models that could account for the low-angle meridional reflections in Aβ peptides 9–28 and 1–40 can be: (a) periodic arrangement of discrete objects along the fibril axis; (b) staggered arrangement of subfibrils; and (c) twisting of the fibril. Case (a) supports our model both for $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ [37, 38, 43] and for the short peptides of 10 residues long [40] having a ladder-type arrangement of oligomers (see Figs. 4 and 5).

For fragment $A\beta_{1\text{-}28}$, a series of small-angle equatorial maxima were consistent with a tubular fibril having a mean diameter of 86 Å and a wall composed of pairs of cross-β pleated sheets. The data may also be consistent with pairs of cross-β sheets that are centered 71 Å apart [60]. A reasonable concurrence was obtained between the observed and calculated positions of the intensity maxima (Table 1 in [53]) for either the tubular cylinder model or the slab-walls model and in which the fibril walls consist of pairs of β-pleated sheets.

X-ray diffraction pattern from $A\beta_{1\text{-}40}$ was obtained in papers [53, 56]. The equatorial intensity maximum at 49.2 Å is derived, rather, from cylindrically averaged interference between objects that are separated by a distance of 55 Å. Namely this distance corresponds to our model (see Fig. 5). As the authors wrote, the 28-Å-wide tubular structure would be a protofilament of the fiber, but in our model this size of tubular structure corresponds to the size of the hole in the short tubular structure (oligomer) (Fig. 5).

After 18 years, scientists forgot this very important data obtained from the X-ray analysis: the meridional reflection at 53 Å and equatorial at 49.2 Å for $A\beta_{1\text{-}40}$. It should be noted that equatorial reflection at 55 Å was obtained for insulin also [61]. Now practically every month new models of Aβ fibrils are suggested [62, 63]. But to construct a relevant model it is necessary to use direct structural methods (X-ray with high resolution EM). The conclusion made by Perutz in his last paper is that the tubular cylindrical β-sheets are the only structures consistent with some of the X-ray and EM data [27]. He underlined that for Aβ fragment 11–25 optical reconstruction shows cylinders of 57 Å in diameter with 37-Å-thick walls surrounding the central hole of 19.5 Å in diameter.

HISTORACAL PATHWAY OF RECONSTRUCTION OF AMYLOID FORMATION

Provided our hypothesis is correct and the main structural block of amyloid proteins/peptides upon fibrillogenesis is a ring oligomer, it should be explained why previously the prevailing interpretations of the results and models did not actually consider the role of oligomers, while most of the attention was focused on the nucleation mechanism of fibril formation (incidentally, in this case a ring oligomer can be interpreted as a seed). We see from Economou et al. [64] that small Aβ oligomers act as seeds. All available models suggest the presence of several filaments in a fibril. Each filament has a cross-β structure on the molecular level. The cross-β structure of fibrils suggests that along the whole fibril and perpendicular to its axis there passes a β-sheet formed of β-strands positioned perpendicular to the fibril axis. Purely mechanistic considerations make this molecular structure disputable, because it cannot compellingly explain some main properties of fibrils, such as polymorphism, fragmentation, branching, spontaneous fibril breaking, and rejoining [65]. The discrepancies between H/D exchange and ssNMR results [66], the presence of water in the holes of ring-like oligomers [27], affinity of specific antibodies to protofibrils and oligomers [67] further exacerbate the explanations.

When did the conception of fibrils as structures formed of several filaments change? It began

in the 1930s when the structure of polymers was studied [68]. In the mid 1950s, it was already established that proteins were polymerized with the formation of β-sheets which, in their turn, interacted with each other forming piles [69]. When investigations of tissues damaged by amyloidosis began (there were yet no names of corresponding proteins/peptides responsible for this, and the amino acid sequences of the studied peptides were not deciphered) with the use of the EM method, it became possible to obtain data on the structure of amyloids isolated from the deposits in the brain, spleen and liver tissues. Thus, for example, the studies of components isolated from fibrous formations of brain tissues from Alzheimer's disease patients (Aβ peptide) allowed obtaining valuable data on the structure of amyloids using the EM method [70]. However, the concept on the structure of polymers and the existence in some fibril formations of specific reflections for polymers [15] led to erroneous interpretations of the EM data. The EM images clearly show oligomer structures with a diameter coinciding with that of thin fibrils. But the fibril images were interpreted as polymers with a diameter of about 75–80 Å, consisting of 5-6 amyloid protofibrils lying parallel to each other along the fibril axis, and having the diameter of about 25–35 Å. In the cross section of a fibril, such packing is seen as a ring structure, the internal diameter of the ring being about 15–20 Å [70]. Surprisingly, it is possible to see exactly the same morphology of Aβ peptide fibrils using the EM method [36–38, 44]. However, the knowledge accumulated during the recent years and the remaining unsolved problems of the structure organization as well as our data on amyloid peptides and their fragments allow us to propose another view on the process of fibrillogenesis, and the structural organization of amyloid fibrils. After destabilization of amyloid proteins/peptides, ring oligomers are formed of monomeric molecules. Varying association of these oligomers with each other (ring-to-ring, ring-to-ring with some overlapping, and probably ring-on-ring or ring-to-ring in a random way) provokes generation of fibrils. In this case, the main structural elements of fibrils are ring oligomers of varying morphology (ring parameters, the number of protein/peptide molecules within an oligomer). Such organization of fibrils explains the complex diffraction pattern of both polymorphism of fibrils and their ability to branching and fragmentation upon external influence. Our explanation does not conflict with the data of the X-ray analysis, because the ring oligomeric structures according to our model contain both β-strands and β-sheets. Importantly, in such organization of fibrils the amino acid sequence itself determines the oligomer structure for the given protein/peptide.

CONCLUSION

An opinion exists that upon future development of some therapeutic agents for treatment of amyloidosis, a personalized approach will be required in each case, because the fibril formations can differ morphologically between patients [71]. In connection with this, the unified way of formation of fibrils from oligomers, which we have discovered, could facilitate development of relevant fields of medicine of a generalized mode of action. However, we believe that the key attention should be focused not on the processes of polymer formation in the form of fibrils, and not even upon oligomer aggregates, but rather on physiological, hereditary and other possible causes leading to destabilization of native protein/peptide molecules, thus triggering the process of fibril generation. Therefore of great importance are bioinformatics approaches for revealing amyloidogenic regions in problematic proteins/peptides, experimental approaches for studying different mutant forms of the latter—occurring in patients with hereditary amyloidosis, as well as in experimental models. Such multi-discipline studies will shed more light on the problem of amyloidosis treatment at the molecular level, involving the known and not yet well understood mechanisms of cell self-healing, with participation of molecular chaperons, degradosomes, and other intracellular systems. It is also important to compare the processes of fibril formation with the mechanisms of molecular self-assembly, as well as the functioning of a number of cytoskeleton components, which can be considered as variants of physiological amyloid structures, together with numerous examples of physiological amyloid structures, such as those in secretory granules of the endocrine system, or in biofilms of yeasts and bacteria.

ACKNOWLEDGMENTS

We are grateful to T.B. Kuvshinkina and N. Lissin for assistance in preparation of the manuscript, A.V. Glyakina, E.I. Grigorashvili for their help in obtaining images. The studies were supported by the Russian Science Foundation (grant number 14-14-00536).

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/17-0230r2).

REFERENCES

[1] Jan A, Adolfsson O, Allaman I, Buccarello A-L, Magistretti PJ, Pfeifer A, Muhs A, Lashuel HA (2011) Abeta42 neurotoxicity is mediated by ongoing nucleated polymerization process rather than by discrete Abeta42 species. *J Biol Chem* **286**, 8585-8596.

[2] Stroud JC, Liu C, Teng PK, Eisenberg D (2012) Toxic fibrillar oligomers of amyloid-β have cross-β structure. *Proc Natl Acad Sci U S A* **109**, 7717-7722.

[3] Hong S, Ostaszewski BL, Yang T, O'Malley TT, Jin M, Yanagisawa K, Li S, Bartels T, Selkoe DJ (2014) Soluble Aβ oligomers are rapidly sequestered from brain ISF in vivo and bind GM1 ganglioside on cellular membranes. *Neuron* **82**, 308-319.

[4] Jin M, Selkoe DJ (2015) Systematic analysis of time-dependent neural effects of soluble amyloid β oligomers in culture and in vivo: Prevention by scyllo-inositol. *Neurobiol Dis* **82**, 152-163.

[5] Breydo L, Kurouski D, Rasool S, Milton S, Wu JW, Uversky VN, Lednev IK, Glabe CG (2016) Structural differences between amyloid beta oligomers. *Biochem Biophys Res Commun* **477**, 700-705.

[6] Kreutzer AG, Hamza IL, Spencer RK, Nowick JS (2016) X-ray crystallographic structures of a trimer, dodecamer, and annular pore formed by an Aβ17-36 β-hairpin. *J Am Chem Soc* **138**, 4634-4642.

[7] Truex NL, Nowick JS (2016) Coassembly of peptides derived from β-sheet regions of β-amyloid. *J Am Chem Soc* **138**, 13891-13900.

[8] Truex NL, Wang Y, Nowick JS (2016) Assembly of peptides derived from β-sheet regions of β-amyloid. *J Am Chem Soc* **138**, 13882-13890.

[9] Zempel H, Thies E, Mandelkow E, Mandelkow E-M (2010) Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. *J Neurosci* **30**, 11938-11950.

[10] Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe DJ (2011) Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. *Proc Natl Acad Sci U S A* **108**, 5819-5824.

[11] Zempel H, Luedtke J, Kumar Y, Biernat J, Dawson H, Mandelkow E, Mandelkow E-M (2013) Amyloid-β oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin. *EMBO J* **32**, 2920-2937.

[12] Hayden EY, Teplow DB (2013) Amyloid β-protein oligomers and Alzheimer's disease. *Alzheimers Res Ther* **5**, 60.

[13] Goure WF, Krafft GA, Jerecic J, Hefti F (2014) Targeting the proper amyloid-beta neuronal toxins: A path forward for Alzheimer's disease immunotherapeutics. *Alzheimers Res Ther* **6**, 42.

[14] Viola KL, Klein WL (2015) Amyloid β oligomers in Alzheimer's disease pathogenesis, treatment, and diagnosis. *Acta Neuropathol (Berl.)* **129**, 183-206.

[15] Eanes ED, Glenner GG (1968) X-ray diffraction studies on amyloid filaments. *J Histochem Cytochem* **16**, 673-677.

[16] Glenner GG, Eanes ED, Bladen HA, Linke RP, Termine JD (1974) Beta-pleated sheet fibrils. A comparison of native amyloid with synthetic protein fibrils. *J Histochem Cytochem* **22**, 1141-1158.

[17] Sunde M, Serpell LC, Bartlam M, Fraser PE, Pepys MB, Blake CC (1997) Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J Mol Biol* **273**, 729-739.

[18] Sunde M, Blake C (1997) The structure of amyloid fibrils by electron microscopy and X-ray diffraction. *Adv Protein Chem* **50**, 123-159.

[19] Glenner GG, Terry W, Harada M, Isersky C, Page D (1971) Amyloid fibril proteins: Proof of homology with immunoglobulin light chains by sequence analyses. *Science* **172**, 1150-1151.

[20] Finder VH, Glockshuber R (2007) Amyloid-beta aggregation. *Neurodegener Dis* **4**, 13-27.

[21] Vestergaard B, Groenning M, Roessle M, Kastrup JS, van de Weert M, Flink JM, Frokjaer S, Gajhede M, Svergun DI (2007) A helical structural nucleus is the primary elongating unit of insulin amyloid fibrils. *PLoS Biol* **5**, e134.

[22] Meisl G, Yang X, Hellstrand E, Frohm B, Kirkegaard JB, Cohen SIA, Dobson CM, Linse S, Knowles TPJ (2014) Differences in nucleation behavior underlie the contrasting aggregation kinetics of the Aβ40 and Aβ42 peptides. *Proc Natl Acad Sci U S A* **111**, 9384-9389.

[23] Smaoui MR, Poitevin F, Delarue M, Koehl P, Orland H, Waldispühl J (2013) Computational assembly of polymorphic amyloid fibrils reveals stable aggregates. *Biophys J* **104**, 683-693.

[24] Goldsbury C, Frey P, Olivieri V, Aebi U, Müller SA (2005) Multiple assembly pathways underlie amyloid-beta fibril polymorphisms. *J Mol Biol* **352**, 282-298.

[25] Goldsbury CS, Wirtz S, Müller SA, Sunderji S, Wicki P, Aebi U, Frey P (2000) Studies on the in vitro assembly of a beta 1-40: Implications for the search for a beta fibril formation inhibitors. *J Struct Biol* **130**, 217-231.

[26] Benditt EP, Eriksen N (1966) Amyloid. 3. A protein related to the subunit structure of human amyloid fibrils. *Proc Natl Acad Sci U S A* **55**, 308-316.

[27] Perutz MF, Finch JT, Berriman J, Lesk A (2002) Amyloid fibers are water-filled nanotubes. *Proc Natl Acad Sci U S A* **99**, 5591-5595.

[28] Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, Kagan B, Ghiso J, Lal R (2005) Amyloid ion channels: A common structural link for protein-misfolding disease. *Proc Natl Acad Sci U S A* **102**, 10427-10432.

[29] Nielsen EH, Nybo M, Svehag SE (1999) Electron microscopy of prefibrillar structures and amyloid fibrils. *Methods Enzymol* **309**, 491-496.

[30] Bhak G, Lee J-H, Hahn J-S, Paik SR (2009) Granular assembly of alpha-synuclein leading to the accelerated amyloid fibril formation with shear stress. *PLoS One* **4**, e4177.

[31] Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem* **274**, 25945-25952.

[32] Kumar S, Udgaonkar JB (2009) Conformational conversion may precede or follow aggregate elongation on alternative pathways of amyloid protofibril formation. *J Mol Biol* **385**, 1266-1276.

[33] Morris AM, Watzky MA, Finke RG (2009) Protein aggregation kinetics, mechanism, and curve-fitting: A review of the literature. *Biochim Biophys Acta* **1794**, 375-397.

[34] Roychaudhuri R, Yang M, Hoshi MM, Teplow DB (2009) Amyloid beta-protein assembly and Alzheimer disease. *J Biol Chem* **284**, 4749-4753.

[35] Jeong JS, Ansaloni A, Mezzenga R, Lashuel HA, Dietler G (2013) Novel mechanistic insight into the molecular basis of amyloid polymorphism and secondary nucleation during amyloid formation. *J Mol Biol* **425**, 1765-1781.

[36] Dovidchenko NV, Glyakina AV, Selivanova OM, Grigorashvili EI, Suvorina MY, Dzhus UF, Mikhailina AO, Shiliaev NG, Marchenkov VV, Surin AK, Galzitskaya OV (2016) One of the possible mechanisms of amyloid fibrils formation based on the sizes of primary and secondary folding nuclei of Aβ40 and Aβ42. *J Struct Biol* **194**, 404-414.

[37] Grigorashvili EI, Selivanova OM, Dovidchenko NV, Dzhus UF, Mikhailina AO, Suvorina MY, Marchenkov VV, Surin AK, Galzitskaya OV (2016) Determination of size of folding nuclei of fibrils formed from recombinant Aβ(1-40) peptide. *Biochemistry (Mosc)* **81**, 538-547.

[38] Selivanova OM, Surin AK, Marchenkov VV, Dzhus UF, Grigorashvili EI, Suvorina MY, Glyakina AV, Dovidchenko NV, Galzitskaya OV (2016) The mechanism underlying amyloid polymorphism is opened for Alzheimer's disease amyloid-β peptide. *J Alzheimers Dis* **54**, 821-830.

[39] Selivanova OM, Suvorina MY, Surin AK, Dovidchenko NV, Galzitskaya OV (2017) Insulin and lispro insulin: What is common and different in their behavior? *Curr Protein Pept Sci* **18**, 57-64.

[40] Selivanova OM, Glyakina AV, Gorbunova EY, Mustaeva LG, Suvorina MY, Grigorashvili EI, Nikulin AD, Dovidchenko NV, Rekstina VV, Kalebina TS, Surin AK, Galzitskaya OV (2016) Structural model of amyloid fibrils for amyloidogenic peptide from Bgl2p–glucantransferase of S. cerevisiae cell wall and its modifying analog. New morphology of amyloid fibrils. *Biochim Biophys Acta* **1864**, 1489-1499.

[41] Lashuel HA, Hartley DM, Petre BM, Wall JS, Simon MN, Walz T, Lansbury PT (2003) Mixtures of wild-type and a pathogenic (E22G) form of Abeta40 in vitro accumulate protofibrils, including amyloid pores. *J Mol Biol* **332**, 795-808.

[42] Jansen R, Dzwolak W, Winter R (2005) Amyloidogenic self-assembly of insulin aggregates probed by high resolution atomic force microscopy. *Biophys J* **88**, 1344-1353.

[43] Dovidchenko NV, Finkelstein AV, Galzitskaya OV (2014) How to determine the size of folding nuclei of protofibrils from the concentration dependence of the rate and lag-time of aggregation. I. Modeling the amyloid protofibril formation. *J Phys Chem B* **118**, 1189-1197.

[44] Suvorina MY, Selivanova OM, Grigorashvili EI, Nikulin AD, Marchenkov VV, Surin AK, Galzitskaya OV (2015) Studies of polymorphism of amyloid-β 42 peptide from different suppliers. *J Alzheimers Dis* **47**, 583-593.

[45] Surin AK, Grigorashvili EI, Suvorina MY, Selivanova OM, Galzitskaya OV (2016) Determination of regions involved in amyloid fibril formation for Aβ(1-40) peptide. *Biochemistry (Mosc)* **81**, 762-769.

[46] Garbuzynskiy SO, Lobanov MY, Galzitskaya OV (2010) FoldAmyloid: A method of prediction of amyloidogenic regions from protein sequence. *Bioinformatics* **26**, 326-332.

[47] Paravastu AK, Leapman RD, Yau W-M, Tycko R (2008) Molecular structural basis for polymorphism in Alzheimer's beta-amyloid fibrils. *Proc Natl Acad Sci U S A* **105**, 18349-18354.

[48] Petkova AT, Leapman RD, Guo Z, Yau W-M, Mattson MP, Tycko R (2005) Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* **307**, 262-265.

[49] Lu J-X, Qiang W, Yau W-M, Schwieters CD, Meredith SC, Tycko R (2013) Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. *Cell* **154**, 1257-1268.

[50] Toyama BH, Weissman JS (2011) Amyloid structure: Conformational diversity and consequences. *Annu Rev Biochem* **80**, 557-585.

[51] Scheidt HA, Morgado I, Rothemund S, Huster D, Fändrich M (2011) Solid-state NMR spectroscopic investigation of Aβ protofibrils: Implication of a β-sheet remodeling upon maturation into terminal amyloid fibrils. *Angew Chem Int Ed* **50**, 2837-2840.

[52] Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliott JI, Van Nostrand WE, Smith SO (2010) Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nat Struct Mol Biol* **17**, 561-567.

[53] Inouye H, Fraser PE, Kirschner DA (1993) Structure of beta-crystallite assemblies formed by Alzheimer beta-amyloid protein analogues: Analysis by x-ray diffraction. *Biophys J* **64**, 502-519.

[54] Lansbury PT, Costa PR, Griffiths JM, Simon EJ, Auger M, Halverson KJ, Kocisko DA, Hendsch ZS, Ashburn TT, Spencer RG (1995) Structural model for the beta-amyloid fibril based on interstrand alignment of an antiparallel-sheet comprising a C-terminal peptide. *Nat Struct Biol* **2**, 990-998.

[55] Petkova AT, Buntkowsky G, Dyda F, Leapman RD, Yau W-M, Tycko R (2004) Solid state NMR reveals a pH-dependent antiparallel beta-sheet registry in fibrils formed by a beta-amyloid peptide. *J Mol Biol* **335**, 247-260.

[56] Malinchik SB, Inouye H, Szumowski KE, Kirschner DA (1998) Structural analysis of Alzheimer's beta(1-40) amyloid: Protofilament assembly of tubular fibrils. *Biophys J* **74**, 537-545.

[57] Makin OS, Serpell LC (2005) X-ray diffraction studies of amyloid structure. *Methods Mol Biol* **299**, 67-80.

[58] Fraser PE, Nguyen JT, Surewicz WK, Kirschner DA (1991) pH-dependent structural transitions of Alzheimer amyloid peptides. *Biophys J* **60**, 1190-1201.

[59] Serpell LC, Fraser PE, Sunde M (1999) X-ray fiber diffraction of amyloid fibrils. *Methods Enzymol* **309**, 526-536.

[60] Kirschner DA, Inouye H, Duffy LK, Sinclair A, Lind M, Selkoe DJ (1987) Synthetic peptide homologous to beta protein from Alzheimer disease forms amyloid-like fibrils in vitro. *Proc Natl Acad Sci U S A* **84**, 6953-6957.

[61] Burke MJ, Rougvie MA (1972) Cross- protein structures. I. Insulin fibrils. *Biochemistry (Mosc.)* **11**, 2435-2439.

[62] Colvin MT, Silvers R, Ni QZ, Can TV, Sergeyev I, Rosay M, Donovan KJ, Michael B, Wall J, Linse S, Griffin RG (2016) Atomic resolution structure of monomorphic Aβ42 amyloid fibrils. *J Am Chem Soc* **138**, 9663-9674.

[63] Wälti MA, Ravotti F, Arai H, Glabe CG, Wall JS, Böckmann A, Güntert P, Meier BH, Riek R (2016) Atomic-resolution structure of a disease-relevant Aβ(1-42) amyloid fibril. *Proc Natl Acad Sci U S A* **113**, E4976-E4984.

[64] Economou NJ, Giammona MJ, Do TD, Zheng X, Teplow DB, Buratto SK, Bowers MT (2016) Amyloid β-protein assembly and Alzheimer's disease: Dodecamers of Aβ42, but not of Aβ40, seed fibril formation. *J Am Chem Soc* **138**, 1772-1775.

[65] Binger KJ, Pham CLL, Wilson LM, Bailey MF, Lawrence LJ, Schuck P, Howlett GJ (2008) Apolipoprotein C-II amyloid fibrils assemble via a reversible pathway that includes fibril breaking and rejoining. *J Mol Biol* **376**, 1116-1129.

[66] Tycko R, Wickner RB (2013) Molecular structures of amyloid and prion fibrils: Consensus versus controversy. *Acc Chem Res* **46**, 1487-1496.

[67] Glabe CG (2008) Structural classification of toxic amyloid oligomers. *J Biol Chem* **283**, 29639-29643.

[68] Astbury WT, Dickinson S, Bailey K (1935) The X-ray interpretation of denaturation and the structure of the seed globulins. *Biochem J* **29**, 2351-2360.1.

[69] Pauling L, Corey RB (1953) Two rippled-sheet configurations of polypeptide chains, and a note about the pleated sheets. *Proc Natl Acad Sci U S A* **39**, 253-256.

[70] Shirahama T, Cohen AS (1967) High-resolution electron microscopic analysis of the amyloid fibril. *J Cell Biol* **33**, 679-708.

[71] Qiang W, Yau W-M, Lu J-X, Collinge J, Tycko R (2017) Structural variation in amyloid-β fibrils from Alzheimer's disease clinical subtypes. *Nature* **541**, 217-221.

[72] Selivanova OM, Gorbunova EY, Mustaeva LG, Grigorashvili EI, Suvorina MY, Surin AK, Galzitskaya OV (2016) Peptide Aβ(16-25) forms nanofilms in the process of its aggregation. *Biochemistry (Mosc)* **81**, 755-761.