Protein aggregation

I. Amyloid assembly is an alternative to protein folding

Proteins fold into native states as a result of specific interactions between amino acids. However, these interactions are not limited to intramolecular contacts - they may also occur between different molecules. Intermolecular amino acid interactions lead to formation of protein aggregates. In many cases, protein aggregates eventually are transformed into amyloid fibrils (Fig. 1).

Fig. 1a Generic structure of amyloid fibril is stabilized by hydrogen bonds (HBs, dashed lines), which are oriented parallel to the fibril axis and perpendicular to β–strands. This arrangement of peptides is called in-registry, because each residue in one chain is exactly matched by the same residues from the neighboring chains.

Fig. 1b Electronic microphotograph of a typical amyloid fibril.

As a rule amyloid fibrils appear as unbranched, long rod- or ribbon-like structures with the typical diameter of about 10 nm and the length reaching up to 1μm and more. Amyloid fibrils are exceptionally stable against chemical denaturants or temperature. Experiments show that they can withstand up to 8M urea or the temperatures as high as 100 °C (prion fibrils). From a macroscopic viewpoint, formation of amyloid fibrils is
essentially irreversible event. Once formed, fibrils cannot dissociate under physiological conditions. Experimental (in vitro) time scales of amyloid formation, which can be as large as days or weeks, far exceed typical folding scales of 1 msec or less.

More than 20 protein sequences sharing no obvious sequence similarity are known to assemble into wild-type amyloid structures. Many more proteins are now shown to form amyloids under appropriate (although, usually non-physiological) conditions. The examples include lysozyme, myoglobin, or SH3 domains. It is important to note that despite dramatic sequence variations all amyloid fibrils have the same internal basic organization represented by β-sheet shown in Fig. 1a. Universality of amyloid structures is based on hydrogen bonds (HBs) between backbone atoms, which can be formed by any polypeptide sequence irrespective of the nature of amino acid side chains. As shown in Fig. 1a HBs stabilize extensive β-sheet structure inside a fibril. Individual β-sheets are laminated to form 3D fibrils. It is likely that formation of amyloid fibrils is a generic feature of a polypeptide chain, which offers a “generic” alternative to native structure.

Because proteins in amyloid fibrils adopt universal conformations drastically different from native ones, aggregation breaks one-to-one unique correspondence between a sequence and a native structure. One immediate consequence of aggregation is a disruption of biological function of aggregated proteins. However, protein amyloids are not merely non-functional, but are also highly cytotoxic and their assembly is the likely cause of many neurogenerative and other diseases, such as Alzheimer’s (amyloids formed by Aβ peptides), Parkinson’s, Creutzfeldt-Jakob disease (a human version of “mad cow” disease), type II diabetes etc. It has also been argued that amyloidogenic diseases represent a natural limit for functioning of living organisms. For example, in the case of humans amyloid related diseases typically occur at old age. For thousands of years human evolution produced no pressure to develop strategies to fight them, because lifespan did not usually exceed 30 years or so. With dramatic increase in an average human lifespan to 70 and more years, we entered the territory, where we cannot rely on naturally developed mechanisms against amyloid-related disorders.

Fig. 2 The sequence of Aβ1–42 peptide. Residue numbering is given below the sequence one-letter code. Positively and negatively charged residues are shown in blue and red. The 16-22 fragment, which includes central hydrophobic cluster (CHC), is boxed. CHC and C-terminal are the two regions, which largely determine the structure and assembly of Aβ amyloids.
II. Structural transition in amyloid assembly: From monomers to fibrils

The following sections describe the structure of amyloidogenic peptides in solution and in fibrils as well as the mechanisms of fibril formation and growth. We will mostly focus on Aβ peptides. However, because of generic nature of fibril structure and assembly many aspects are applicable to other polypeptides as well.

Solution conformation of monomers: Amyloidogenic proteins and peptides show no structural homology of their native states, some of them are even natively unstructured. For example, under normal conditions Alzheimer’s Aβ monomers adopt in water a random coil structure containing no significant amounts of α-helices or β-strands (Fig. 3). In the absence of interpeptide interactions it is difficult to correlate amyloidogenic propensity and the properties of unstructured monomeric conformations. The experiments show that Aβ1-42 peptide, which differs from the more abundant Aβ1-40 by having two additional C-terminal residues (Fig. 2), forms fibrils significantly faster than Aβ1-40. Nevertheless, their solution structures are very similar. Molecular dynamics (MD) simulations show that wild-type Aβ10-35 fragment and highly amyloidogenic Dutch E22G mutant adopt almost identical monomeric conformations in water (Protein Science 11, 1639 (2002)). Dutch mutant forms fibrils twice as fast than the wild-type peptide.

Overall, it appears that the solution structures of Aβ and other short amyloidogenic peptides have little long-range order or easily identifiable elements of secondary structure. However, their study is very important, because one needs to know the initial conformations of peptides as they start the long process of amyloid assembly.

![Solution structure of Aβ10-35 monomer is disordered (PDB code 1hz3). Hydrophobic, polar, positively and negatively charged residues are colored in green, orange, blue, and red, respectively.](image)

Structural characteristics of fibrils: As the concentration of amyloidogenic peptides increases, they show a propensity to form amyloid fibrils. The Aβ amyloids were one of the first, for which detailed structural information was obtained (Biochemistry 42, 3151 (2003); Biochemistry 45, 498 (2006)). It was established that individual Aβ40 peptides in β-sheets are in parallel, in-registry conformation (as shown in Fig. 1a). Experiments also suggest that within a fibril Aβ peptides make a turn, which is stabilized by interpeptide
electrostatic interactions (Fig. 4). The proposed structural model for Aβ fibril organization suggests that Aβ peptides form a laminated structure (protofilament) consisting of four β-sheet layers (Fig. 4). The structural unit of a protofilament, which is replicated along a fibril axis, includes two Aβ peptides, of which one contributes its β-strands to the upper pair of β-sheets and the second to the lower pair of β-sheets. The interesting feature of the structure in Fig. 4 is a staggering shift of β2-sheet relative to the β1-sheet. The average distance between backbone atoms in the β-sheet is about 5 Å, whereas a typical distance between β-sheets is about 9 Å. (These distances between β-strands and β-sheets are typical for other amyloid fibrils composed of different sequences.)

![Fig. 4. 3D structure of Aβ10-40 fibril protofilament derived from solid-state NMR measurements. Each peptide contributes its two β-strands, β1 and β2, either to upper or lower pairs of β-sheets. In hexamer fragment shown, three Aβ1-40 peptides form the upper pair of β-sheets (in red/pink) and three form the lower pair of β-sheets (in yellow/orange). Aβ peptides from the upper and lower β-sheet pairs interact via antiparallel docking of their β-strands. Due to shift of β-sheets along the fibril axis the interactions between β1 and β2 are intermolecular and the left and right edges of the protofilament are distinct.](image)

Although extensive β–sheet structure is always present in a fibril independent on particular sequence, other details of fibril organization are sequence dependent. For example, short fragments of Aβ sequences (such as Aβ16-22 and Aβ34-42) have antiparallel, in-registry orientation within β–sheets and there is no evidence that these individual peptides form any turns in the fibrils. The study of Aβ peptides suggests that fibril structure utilizes both inter- and intrapeptide interactions to maximize its stability.

Recent experimental study of the structure of amyloid fibrils for Sup35 fragment GNNQQNY showed that two β-sheets are laminated together to form a dry interface between them. Interestingly, Sup35 peptide is highly hydrophilic and to stabilize amyloid fibril the hydrogen bonds are formed not only between peptides’ backbones, but between in-registry positioned hydrophilic side chains (such as Asn, Gln, and Tyr) within β-

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1. *An antiparallel in-registry arrangement means that a residue i from one peptide is matched with the residue N-i+1 from the other (N is the total number of residues).*
sheets. The tight packing of laminated β-sheets is achieved through a steric “fit” and vander-Waals interactions (Nature 435, 773 (2005)). The first actual structure of the peptide in a fibril has been reported. The 11-mer peptide YTIALLLSPYS from transthyretin (residues 105-115) adopts a perfectly planar, extended β-strand conformation (Fig. 5).

The first 3D structure of the engineered peptide KFFEAADKFFFE was recently solved using X-ray and electron diffraction. This structure reveals several interesting features (Fig. 6). Similar to short Aβ peptides, these peptides form antiparallel in-registry β-sheets, which are stabilized by interpeptide HBs and salt bridges. Individual β-sheets are organized in a layered, brick-like structure “glued” together by the attractive interactions between hydrophobic phenylalanines and π-stacking interactions between their side chains.

Fig. 5 Fibril conformation adopted by the 105-115 transthyretin peptide (PDB code 1rws) facilitates interpeptide backbone HBs between carboxyl oxygens (in red) and amide hydrogens (in pale blue). Side chains are oriented perpendicularly with respect to the figure plane and are engaged in interactions between β-sheets (Proc. Natl. Acad. Sci USA 101, 711 (2004)).

Fig. 6 The 3D structure of KFFEAADKFFFE peptide in amyloid fibril (PDB code 2bfi). Red and blue colors distinguish layers of peptides. The peptides shown on a background (in yellow and green) interact with the front-end peptides (in blue and red) through HBs and salt bridges (between E and K residues). The magnified image displays the hydrophobic and π-stacking interactions between laminated β-sheets (through the contacts between phenylalanine side chains). This view is parallel to the fibril axis and interpeptide hydrogen bonds (Proc. Natl. Acad. Sci. USA 102, 315 (2005)).
The analysis of fibril conformations share several common characteristics:

1. Stability of fibrils is derived from the delicate balance between hydrogen bonding and side chain interactions (hydrophobic interactions, salt bridges, side chain hydrogen bonding, and stacking interactions).
2. Polypeptide chains in fibrils tend to maximize favorable hydrophobic and electrostatic interactions. Long chains take advantage of intra-peptide interactions.
3. Fibrils in their core are highly dehydrated.

III. Mechanism of amyloid formation.

Formation of fibrils is preceded by oligomer assembly: Amyloid fibrils are the final product of a long chain of molecular events, which starts with individual proteins or peptides (monomers) (Fig. 7). Due to intermolecular interactions monomers associate into mobile, soluble spherical oligomers. This process is extremely slow and even in vitro, at highly elevated micromolar concentration, spans the timescale of hours to days.

The composition of Aβ oligomers depends on the peptide concentration and particular peptide variant. At nanomolar concentrations Aβ1-40 dimers form, whereas at micromolar concentrations a range of oligomer sizes is observed for Aβ1-40 and Aβ1-42. For example, the Aβ1-42 oligomers are composed of five or six monomers with the typical diameter of 6 to 7 nm. Because oligomers appear to exist in equilibrium with monomers, the formation of oligomers is reversible.

Fig. 7. Assembly mechanism for amyloids (Proc. Natl. Acad. Sci. USA 100, 330 (2003)).
The importance of oligomers is not only related to their key role in the amyloid assembly, but also to their pronounced cytotoxicity. Interestingly, recent findings suggest generic mechanisms of their formation. Glabe and coworkers (Science 300, 486 (2003)) showed that oligomers formed by diverse polypeptide sequences, from Alzheimer’s Aβ to fragments of prion proteins (in all, seven different sequences), share the same basic structure. Therefore, identifying a generic structure of oligomers may be relevant for devising the methods of blocking amyloidogenesis in general.

Experiments also reveal that amyloid assembly is a nucleation-driven process. The yield of amyloid fibrils plotted as a function of time typically shows a classical sigmoid curve with a characteristic time lag. The existence of the lag is due to the kinetic process of assembly of nuclei (oligomers). Once nuclei are formed, they trigger rapid increase in the amount of assembled fibrils.

Fibrils grow via template assisted mechanism: The mechanism of deposition of peptides on preformed fibrils is fundamentally different from the one described above (Ann. Rev. Biomed. Eng. 4, 155 (2002)). Experiments have shown that Aβ peptides deposit individually as monomers under physiological conditions (Fig. 8). The two-stage “dock-lock” mechanism of fibril growth was proposed. During the first stage a disordered monomeric peptide docks to the fibril. Within the second stage a monomer becomes locked in the fibril state due to structural reorganization. The template assisted elongation of Aβ1-40 fibrils was visualized experimentally in real-time. The study showed that the fibrils extend at their ends with a constant rate.

Fig. 8 A growth of existing Aβ16-22 fibril (in cartoon representation) by monomer deposition. In the incoming disordered monomer hydrophobic side chains are shown in green, charged terminals are colored in blue (Lys) and red (Glu).

IV. Factors favoring protein aggregation

Cellular environment is crowded with the variety of molecules, such as proteins, ribosomes, RNAs, lipids, ions etc (Fig. 9). It is estimated that on an average the volume fraction of all these molecules is about 0.3. Therefore, the possibility of forming protein
aggregated state is very high and cells employ different strategies to fight aggregation. Among these are molecular chaperones discussed in the previous lecture, which sequester and rescue misfolded proteins and often allow them a second chance to fold. Another class of “helpers” includes protein folding catalysists, such as peptidylprolyl isomerases, which facilitate slow structural transitions in amino acids. In addition, cells have developed a remarkable “quality-control” system based on glycosylation and deglycosylation capable of distinguishing folded from misfolded proteins. Proteins, which fail to pass these “tests”, are subject to degradation by ubiquitin proteasome systems.

Fig. 9 Schematic view of the crowded cellular environment. Shapes of different color represent various proteins, RNAs, lipids, etc. A given protein enclosed in a circle may interact with these surrounding molecules. Intermolecular interactions may profoundly change the structure and the properties of a protein.

In most cases, these strategies are effective in preventing aggregation. There are, however, several factors, which increase its risks.

1. Folded proteins bury their hydrophobic residues inside the native structures limiting the exposure of these residues to solvent. However, protein may start folding before completion of synthesis on ribosome. Folded structures of parts of protein sequences are unlikely to bury hydrophobic residues to the same degree as complete sequences.

2. Some external factors such as temperature (resulting in “heat shock”) may increase the probability of unfolding of native conformations. This would also increase the probability of exposing hydrophobic residues to solvent.

3. Certain mutations introduced in a sequence may reduce the stability of a folded state, again increasing the chances of transient unfolding.
In all these situations hydrophobic residues become exposed that make them susceptible to intermolecular interactions. Therefore, one would expect a kinetic competition between folding and aggregation in a cell. Under normal conditions proteins fold reliably enough and quickly “hide” hydrophobic residues in the native structure. If, however, the concentration of misfolded or partially folded proteins increases, aggregation may overcome folding.

It is now well established that destabilization of native state is one of the main conditions for protein aggregation. Let us consider several examples.

1. Lysozyme is one of the best characterized proteins and it does not normally form amyloid (aggregate) state. However, specific mutations are known to lead to systemic amyloidogenesis for this protein (Nature Structural Biology 9, 308 (2002)). For example, replacing aspartic amino acid with histidine at the sequence position 67 results in formation of lysozyme amyloids. Experiments show that this mutation significantly unfolds part of lysozyme native structure (Fig. 10) and creates a misfolded intermediate. Hydrogen exchange data suggest that the probability of misfolded state increases 60 times for the mutant as compared to the wild-type lysozyme.

![Fig. 10 Native structure of lysozyme. α-helix shown in green and β-domain constitute parts of the structure, which become unfolded due to the mutation.](image)

2. An interesting example comes from myoglobin, which is known as highly soluble protein with no propensity to form aggregated (amyloid) states (Fig. 11). However, it has been recently shown that, if pH is increased to 9 and temperature is raised to 65 °C, this protein readily form amyloid structures (Nature 410, 165 (2001)). The myoglobin fibrils bear all the standard characteristics of amyloid
fibrils. As for almost all other proteins, formation of myoglobin amyloids is associated with radical structural transition from the native conformation. In the native state myoglobin contains only $\alpha$-helices and no $\beta$-strands (Fig. 11), whereas upon deposition into amyloid fibrils myoglobin adopts exclusively $\beta$-strand structure.

3. Polyaminoacid peptides are the peptides composed of only one type of amino acids. Polylysine (PK) adopts random structure under normal physiological conditions. If pH is adjusted to 11 (at which positive charge on lysine side chain is neutralized) and the temperature is increased to 52 °C, PK forms amyloid fibrils (*The EMBO Journal* 21, 5682 (2002)). Similar behavior was observed for polyglutamic acid (PE), which carries positive charge under normal conditions. Again, adjusting pH to 4, which makes PE side chains neutral, induces the formation of amyloid fibrils.

![Fig. 11 Native structure of myoglobin. Numbers indicate $\alpha$-helices.](image)

4. Assembly of transthyretin (TTR) into amyloid fibrils is related to the onset of several amyloidogenic diseases. In the native state TTR forms tetrameric structure, in which four identical units are docked together (Fig. 12 shows a native TTR dimer, which constitutes only half of the full native structure). Experiments show that low pH values induce unfolding of the $\beta$-strands C and D that, in turn, exposes the $\beta$-strand B. This strand is capable of binding to the strands B of other TTR monomers. (It is also possible that the strand A participates in aggregation, too). As a result TTR dimer in Fig. 12 turns into elementary building block of amyloid fibril by aggregating head-to-head and tail-to-tail.
Fig. 12 Native structure of TTR dimer. Letters indicate β-strands. The β-stands CBEFF’E’B’C’ and DAGHH’G’A’D’ comprise the first and the second β-sheets, respectively, which form a β-sandwich.

V. Preventing amyloid formation through protein design

In addition to molecular chaperones and folding catalysts, proteins utilize additional strategies designed to prevent aggregation, which are based on specific elements in local structure. These strategies were recently catalogued by Richardson and Richardson (Proceedings National Academy Sciences USA 99, 2754 (2002)). They considered structures and sequences of so called edge β-strands in the proteins deposited to PDB database. Edge strands are those, which are found on the edges of β-sheets and, thus, they may be potentially exposed to interactions with other proteins. Therefore, upon aggregation edge strands are placed at intermolecular interface. One would expect that proteins have developed certain ways to protect edge β–strands against aggregation. The survey of protein structures led to the following conclusions.

1. Edge β–strands tend to have minimum number of dangling or unsatisfied hydrogen bonds. This implies that most of the backbone donors and acceptors are engaged in intrachain hydrogen bonds, which stabilize the native fold. In order to minimize unsatisfied hydrogen bonds a β–sheet may be wrapped around itself as a β–barrel, in which two edge β–strands of the sheet meet. Alternatively, other elements of protein structure such as short α-helices or loops may be docked to an edge β–strand. Because these elements do not usually have a regular pattern of acceptor and donor atoms, further propagation of β–sheet is discouraged.
2. Edge β–strands often contain twists or bulges that also violate regular pattern of donor and acceptor atoms.

3. Edge β–strands of a β–sandwich may have charged amino acids, whose side chains are pointed inward the β–sandwich. As long as such β–strand remains on the surface of the protein it is well solvated. However, upon aggregation such charged residue becomes buried that is energetically unfavorable.

Consider several examples of proteins, which use these features to protect themselves from aggregation.

**Designed β–sheet proteins:** Recently Hecht and coworkers designed β–sheet proteins, which contain six or eight identical seven residue repeats of the type ○●○●○●○, where ○ and ● correspond to hydrophilic and hydrophobic residues, respectively (Proceedings National Academy Sciences USA 99, 2760 (2002)). Even though the native state of these polypeptide chains is a perfect β–sandwich similar to that in Fig. 12, the proteins readily form amyloid fibrils, because their edge β–strands contain none of the features outlined above. However, if a hydrophobic residue in the edge strand is substituted to positively charged lysine (○●○K○●○, where K denotes lysine), amyloid formation is completely blocked. This design strategy falls into the category 3 described above.

**Tranthyretin (TTR):** As discussed above this protein forms a tetrameric native state by burying highly hydrophobic β–strand H (Fig. 12). Under denaturing conditions TTR forms amyloid fibrils, in which the elementary unit being propagated is made of (BEFF’E’B’) strands (and, presumably, (AGHH’G’A’) as well). Formation of this unit requires unfolding of the edge β–strands C, D, C’, and D’, which protect the β–strands B and B’, A and A’. Analysis of the structure of these edge strands shows that they are highly twisted and short flanked by the loops (categories 1 and 2 above). Irregular conformation of these β–strands disrupts the pattern of hydrogen bond donors and acceptors in the protein backbone.

**Protein S6:** S6 is a two-state folding ribosomal protein, which does not normally aggregate. It has been shown that, if four charged residues in the β–strand 2 (two glutamic acids at the positions 41 and 42 and two arginines at the positions 46 and 47 in Fig. 13) are replaced with hydrophobic residues, the mutant S6 readily forms aggregated tetramers (Proceedings National Academy Sciences USA 97, 9907 (2000)). Note that S6 aggregated state is formed as a result of interaction between β2 strands of S6 monomers. Oliveberg and coworkers termed these charged residues as “gatekeepers”, because they are designated to prevent aggregation of S6. Two pairs of charged residues placed in tandem in β2 make interactions between β2 strands of different S6 molecules highly unfavorable. Furthermore, sharp twist and bend near the end of β2 strand also help in preventing aggregation. In terms of classification of “anti-aggregation” strategies S6 uses the categories 2 and 3.
Fig. 13 Native structure of S6. Different types of native structure, helix and strands, are color coded in yellow and blue, respectively. Side chains of several charged amino acid “gatekeepers” are shown in blue (positively charged) and red (negatively charged).

**β-helix:** Carbonic anhydrase has a native state in form of a three-strand β-helix. In order to protect the edges of β-helix from aggregation this protein uses a loop and a short α-helix (Fig. 14). The advantage of placing α-helix near the edge β-strand is that α-helix uses almost all its backbone acceptors and donors to form internal hydrogen bonds. As a result the number of unsatisfied dangling hydrogen bonds is kept to a minimum (category 1).

Fig 14 Native structure of carbonic anhydrase takes the form of β-helix. Short α-helix covering edge β-strand is shown in orange (*Proceedings National Academy of Sciences* 99, 2754 (2002)).
VI. Conclusions

The assembly of proteins into aggregated structures (such as amyloids) poses a serious challenge to monomeric protein folding. The danger of protein aggregation is always present for two main reasons. First, cellular environment is highly crowded with various types of molecules making intermolecular interactions highly probable. Second, it appears that any protein sequence may inherently form amyloid fibrils, which represent the most generic form of protein aggregated states. Formation of aggregated (fibril) structures of proteins is usually accompanied by a drastic conformational change. As a result proteins can no longer perform biological functions. Furthermore, fibril structures are often cytotoxic that leads to a class of diseases associated with protein misfolding and aggregation. Proteins have evolved to use different design strategies to minimize the risks of aggregation.

VII. Molecular dynamics simulations of the assembly of Aβ oligomers

Oligomers formed by amyloidogenic peptides play a crucial role in the assembly of amyloid fibrils. However, due to their transient nature the experimental characterization of oligomers poses extreme challenge. Computational studies involving molecular dynamics (MD) simulations offer an opportunity to probe these elusive species. The advantages of MD are

1. the detailed microscopic dynamics of individual molecules can be probed
2. the contributions of various interactions can be dissected
3. various sequence mutations or external conditions (such as changes in temperature, pH value, or the concentration of chemical denaturants) can be examined

Recent MD simulations for Aβ16-22 peptides probed the initial stages in the oligomer formation (Structure 11, 295 (2003); Proc. Natl. Acad. Sci. USA 101, 14760 (2004)).

The results of this study are as follows:

1. The formation of oligomers is accompanied by significant structural changes. Non-interacting monomers adopt random coil structures with small to negligible contents of β-strand or α-helix structure. The distribution of the monomer end-to-end distance $P(r_{1N})$ is consistent with the random coil structure (Fig. 15). Interpeptide interactions induce dramatic structural transitions in Aβ16-22 monomers (Fig. 16a), which result in peptide extension and accumulation of β-strand structure (Fig. 16a,b). Upon the formation of Aβ16-22 oligomers peptides transiently adopt conformations with high α-helix content (Fig. 16b).
Fig. 15 The distribution of the end-to-end distance $r_{1N}$ for Aβ16-22 monomer. The maximum at $r_{1N} \approx 11\textnormal{Å}$ corresponds to random coil states.

Fig. 16. Top: The radii of gyration for three peptide $R_{g,i}$ as a function of time (index $i$ indicates a color coded peptide). A sharp increase in $R_{g,i}$ reveals peptides’ extension. Bottom: The time dependence of the $\alpha$-helix (in red) and $\beta$-strand (in green) contents $H$ and $S$ measured by the fraction of respective residues in peptides. A steady rise in $S$ reflects an accumulation of $\beta$-strand structure and $\alpha \rightarrow \beta$ transition.
2. Oligomer assembly is initially driven by hydrophobic interactions, which result in the formation of disordered oligomers (Fig. 17a). The term *disordered* is associated with the lack of peptide orientation with respect to each other. Computation of the most probable (> 30%) interpeptide side chain contacts reveals that the establishment of structural order is due to electrostatic contacts between Lys and Glu (Fig. 17b). The antiparallel registry of peptides in oligomers is similar to that in Aβ16-22 amyloid fibrils. In ordered oligomers the antiparallel orientation of peptides is maintained throughout the length of simulations (Fig. 18a).

The stability of ordered oligomers is confirmed by the computation of the accessible surface area, which varies within less than 10% (Fig. 18b). This implies that the hydrophobic core in ordered oligomer remains protected from water. Hence, the
nascent elements of fibril structural organization are evident in oligomers even on nano- to microsecond timescale.

Fig. 18. Top: The orientation of peptides in ordered oligomer is characterized by the scalar product $d_{ij}$ of the normalized end-to-end vectors for a pair of peptides $i$ and $j$. The values of $d_{ij} = -1$ and 1 correspond to antiparallel and parallel orientation of peptides, respectively. The structural arrangement of peptides is consistent with the sketch of peptide interactions shown in Fig. 17b. Bottom: The accessible surface area (in Å$^2$) for the ordered oligomer.

3. The MD simulations showed that the stability of oligomers is determined by both, hydrophobic and electrostatic, interactions. Their contribution may be directly probed by sequence mutations, which eliminate or weaken these interactions. For example, substitution of three hydrophobic residues with polar serine (L17S/F19S/F20S) destabilizes the ordered oligomer and leads to its rapid disintegration (Fig. 19). Similar effect is observed when oppositely charged terminals Lys and Glu are replaced with neutral glycines.
Fig. 19 The time dependence of the distance between the centers of mass $R_{ij}^{CM}$ for pairs of peptides $i$ and $j$ in the oligomer composed of the mutant peptides L17S/F19S/F20S. The increase in $R_{ij}^{CM}$, which is due to reduced hydrophobic interactions, indicates dissolution of the mutant oligomer. Color codes different pairs of peptides.

4. MD simulations suggest that oligomers derive their stability exclusively from side chain interaction, whereas interchain hydrogen bonds are still weak (Fig. 20). The finding is in contrast with the fact that the stability of amyloid fibrils comes, to a large extent, from the hydrogen bond network. These MD observations imply that the formation of oligomers depends critically on a sequence composition and different sequence must show varying rates of amyloid assembly.

Fig. 20 The average number of interpeptide hydrogen bonds $N_{HB}$ (lower curve) and side chain contacts $C$ (upper curve) in Aβ16-22 oligomer. Both quantities are given per a generic peptide pair.