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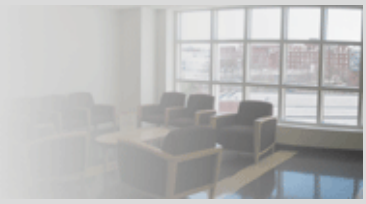

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Dissecting the assembly of Aβ_{16-22} amyloid peptides into antiparallel β-sheets

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Summary

Multiple long molecular dynamics simulations are used to probe the oligomerization mechanism of Aβ_{16-22} (KLVFFAE) peptides. The peptides, in the monomeric form, adopt either a compact random or extended β-strand-like structures. The assembly of the low energy oligomers, in which the peptides form antiparallel β-sheets, occurs by multiple pathways with the formation of an obligatory α-helical intermediate. This observation and the experimental results on fibrillogenesis of Aβ_{1-40} and Aβ_{1-42} peptides suggest that the assembly mechanism (random coil $\rightarrow$ α-helix $\rightarrow$ β-strand) is universal. In Aβ_{16-22} oligomers both interpeptide hydrophobic and electrostatic interactions are critical in the formation of anti-parallel β-sheet structure. Mutations of either hydrophobic or charged residues destabilize the oligomer, which implies that the 16-22 fragments of Arctic(E22G), Dutch (E22Q), and Italian (E22K) mutants are unlikely to form ordered fibrils.

Introduction

According to the amyloid hypothesis Alzheimer's disease (AD) is a result of deposition in brain tissues of Aβ peptides, a normal product in the amyloid precursor protein metabolism [1]. Although the final product in the AD pathogenesis is the insoluble fibril formed as a result of aggregation of Aβ peptides, recent evidence suggests that Aβ oligomers and protofibrils may be the cause of neurotoxicity [2–5]. Aβ oligomers have been envisioned as intermediates in the cascade of events leading to the formation of amyloid fibrils [1]. Beside AD, a number of other neurodegenerative diseases (prion disorders, Parkinson's and Huntington's diseases) are linked to amyloidogenesis [6,7]. It is possible that in all these cases the mobile and soluble oligomers may be the cause of neurodegeneration. Just as in AD, the importance of neurotoxic oligomers has made it urgent to understand, at the molecular level, not only the structure of oligomers, but also the mechanisms of their assembly. In addition, given the similarity in the morphology of fibrils from a variety of peptides and proteins [8], which are unrelated in sequence or length, certain general principles governing

their formation are likely to exist.

Non-crystallinity and insolubility of amyloid fibrils prevent determination of their atomic structures using conventional methods, such as X-ray crystallography or solution NMR. Nevertheless, a detailed picture of the overall architecture of the ordered fibrils is beginning to emerge from a variety of experimental techniques [9,10]. The characteristic silk-like cross-β pattern of *ex vivo* amyloid propagation has been revealed using fibre X-ray diffraction studies [11]. More recently, a number of solid state NMR studies of fibrils of both the full length Aβ peptides and fragments have given valuable insights into the nature of β-sheet organization in amyloids [12,13]. These studies show that in Aβ_{10-35} and Aβ_{1-40} fibrils peptides form in-register parallel β-sheets. On the other hand, antiparallel organization is found for the smaller fragments Aβ_{34-42} [14] and Aβ_{16-22} [15]. The solid state NMR measurements of the structure of amyloid fibrils formed by the fragment N-acetyl-Lys-Leu-Val-Phe-Phe-Ala-Glu-NH_2 (referred to as Aβ_{16-22}) revealed the antiparallel organization of these peptides [15]. This fragment, which is perhaps the shortest one to form amyloid fibrils, contains the crucial central hydrophobic cluster (CHC, residues 17-21 (LVFFA)), which have long been known to be essential for polymerization of the full length peptide [9]. Despite the non-crystalline nature of Aβ_{16-22} fibrils, the NMR lines are unusually sharp, thus providing unambiguous signature of the antiparallel arrangement of β-strands [15].

Aβ_{16-22} is an attractive model system to probe the mechanism of fibril assembly. Unlike the fibres of larger fragments the structure of Aβ_{16-22} fibrils may be anticipated from its sequence alone. The interpeptide interactions must be dominated by favorable contacts between CHC hydrophobic residues. Antiparallel registry confers additional stability by forming interpeptide salt bridges between Lys16 amd Glu22. Although the formation of β-helical fibres [16] even for this short peptide cannot be ruled out, the present study suggests that such structures have higher free energy than the antiparallel structures.

Knowledge of fibril structure, while important, does not provide insights into the assembly mechanism. Therefore, it is essential to study the structural changes in the transition from the monomer to the fibrils at an atomic level. Towards this end, we present, for the

first time, the study of oligomer formation for interacting Aβ_{16-22} peptides and their variants using all-atom molecular dynamics (MD) simulations [17]. Several relatively long MD trajectories were used to establish the kinetics of Aβ_{16-22} assembly into antiparallel β-sheets. Surprisingly, simulations suggest that α-helical structures represent an obligatory intermediates, even though the monomers themselves have very low propensity to form α-helical conformations. We argue that the mechanism outlined here may be general in the oligomerization of Aβ peptides. By dissecting the interactions that drive the oligomerization, we make a number of experimentally testable predictions.

Results

Aβ_{16-22} monomers adopt random coil or β-strand-like structures

To understand the dynamics of assembly of Aβ oligomers it is necessary to first characterize the structure of Aβ monomers. There are no solution structures of Aβ_{16-22} at neutral pH. Solution NMR structure of Aβ_{10-35} (PDB code 1hz3, $T = 283^{\circ}C$, pH=5.6) suggests that it adopts a compact random coil (RC) conformation [18]. According to the DSSP secondary structure assignment none of the residues in the segment 16 to 22 of Aβ_{10-35} is in α-helix or β-strand conformations. A direct probe of the structure of the monomer Aβ_{16-22} is needed, especially because it is the sequence context that determines the nature of secondary structures. Moreover, structural characteristics of the monomer will serve as a suitable reference for the conformational changes that take place in the process of oligomerization.

To characterize the conformational states of the Aβ_{16-22} monomer we generated four 8 ns trajectories and an additional independent set of four 6 ns trajectories. Using the definitions for conformational states of peptides (see Experimental Procedures), we established that RC and β-strand conformations constitute 68 and 29 percents of all monomer structures, respectively. The population of α-helix peptide conformations is negligible (3%). These results are consistent with the simulations of Aβ_{10-35} monomers, in which the CHC was found to have some β-strand propensity [19].

The time dependence of β-strand $< S(t) >$ and α-helix $< H(t) >$ contents (Eq. (1) in Experimental Procedures) are shown in Fig. (1a). The population of RC residue states may be obtained as $< R(t) >= 1 - < S(t) > - < H(t) >$. The time-averaged value of the populations of β-strand and α-helix residues in monomers are 0.33 and 0.11, respectively. Thus, the monomer exists predominantly in the RC or β-strand states. The residue specific α-helix $P_h(i)$ and β-strand $P_s(i)$ propensities show (Fig. (1b)) that β-strand conformations are clearly preferred at Val18, which is consistent with Chou-Fasman prediction [21]. The snapshots of the typical monomer conformations are shown in Fig. (1c).

Interpeptide hydrophobic interactions drive formation of Aβ_{16-22} oligomers

To probe the effect of interpeptide interactions on the dynamics of secondary structure we generated four 11 ns trajectories for the solvated system of three Aβ_{16-22} peptides (Fig. (2a)). Time dependence of the radii of gyration R_{gi} (Fig. (2b)) shows that interpeptide interactions lead to large changes in the size of the peptides. Although the initial values of R_{gi} are below $8\mathring{A}$ (R_g of the monomer is about $8.6\mathring{A}$), interactions between peptides cause dramatic increase in peptide dimensions. For example, by the end of trajectory shown in Fig. (2b) ($t \approx 8ns$) $R_{g2} \approx 11\mathring{A}$, which constitutes nearly a 50 percent increase in about 8 ns. The dimensions of one of the peptides (labeled 3 in Fig. (2b)) do not change as dramatically, which is perhaps a consequence of relatively weak interactions with other peptides. Thus, peptides in oligomers become extended as a result of interpeptide interactions.

The secondary structure changes (Fig. (2c)) accompanying the peptide extension gives a preliminary view of the assembly mechanism of the oligomers. Shortly after initial equilibration α-helical conformations dominate in all peptides (red regions in Fig. (2c)). Subsequently, a rather dramatic increase in the β-strand content (shown in Fig. (2c) in green) is observed indicating $\alpha \rightarrow \beta$ conversion. For example, up to 2 ns none of the residues in peptide 2 (Fig. (2c)) is in the β-strand conformations ($S_2 \approx 0$), while persistent α-helix structure is seen at Val18, Phe19 and, to a lesser extent, at Ala21. In the time interval

from 2 to approximately 4 ns β-strand conformations emerge at positions Leu17, Val18, and subsequently at Phe20, while the α-helical structure survives only at Ala21. Transition to β-strand conformation also occurs later at Phe19 (Fig. (2c)). Consequently, the β-strand content $S_2(t)$ reaches a remarkably high value of 0.8 at about 7 ns. Significantly, $\alpha \rightarrow \beta$ transition is also observed in peptide 1. The amount of β-strand content in the peptide 3 $S_3(t)$ remains small. The average β-structure content in all three peptides in this trajectory approximately doubles. The increase in S_1 and S_2 is consistent with the changes in the radii of gyration R_{g1} and R_{g2} in Fig. (2b). Similar dramatic $\alpha \rightarrow \beta$ transition occurs in other trajectories on similar timescale (data not shown).

To probe the orientations of peptides in Aβ_{16-22} oligomers we computed the functions $d_{ij} = (\hat{u}_i \hat{u}_j)$ for each pair of peptides, where $\hat{u}_i$ is the end-to-end unit vector of i^{th} peptide. Rapid variations in d_{ij} (data not shown) on a time scales as short as 1 ns indicate frequent reorientations of peptides in Aβ oligomers. For this reason, we refer to such trimer structures as disordered oligomers.

Dynamics of $\alpha \rightarrow \beta$ transition

The time dependence of the β-strand (α-helix) structure content in a peptide, $< S(t) >$ ($< H(t) >$), shows a striking behavior (Fig. (3a)). The β-strand content $< S(t) >$ increases monotonically (apart from relatively minor fluctuations), while $< H(t) >$ decreases. Initially, the α-helical content in the peptides is more than four times higher than the β-strand population. In about 11 ns, the β-strand population reaches 0.40, while the α-helix content falls below 0.10 (Fig. (3a)). Thus, in the course of oligomer formation a *dramatic conformational change* in the peptides is observed as illustrated in Fig. (3b).

Because there are only three peptides in our simulations, we expect large fluctuations in the oligomer structure. Although the average β-strand content reaches about 0.4, there are substantial variations in the secondary structure propensities at the residue level. Using the average probabilities to observe β-strand $P_s(i)$ or α-helix $P_h(i)$ conformations at residue

i, we find that $P_s(i)$ for Leu17, Val18, and Phe20 are 0.26, 0.41, and 0.28, respectively (the corresponding $P_h(i)$ are 0.09, 0.31, and 0.17). Other residues (i =Phe19, Ala21) are better accommodated by an α-helical structure ($P_h(i) > P_s(i)$). The ratio $P_s(i)/P_h(i)$ is 1.4, which reflects the general bias towards β-strand conformations in Aβ peptides. The largest β-strand propensity is found for Val18, which may be identified as the initiation site for β-strand structure. We can also surmise that during aggregation of Aβ_{16-22} β-strand formation begins near peptide's N-terminal.

Using the definitions for α-helix and β-strand peptide structures (see Experimental Procedures), we determined that Aβ_{16-22} peptide in oligomer adopts β-strand and α-helix states with the probabilities 0.30 and 0.26, respectively. The probability to find a peptide in a random coil state is 0.44. Thus, β-strand and α-helix states together constitute more than half of all peptide conformations.

The distribution of (ϕ, ψ) states in Fig. (3c) shows that conformational states of individual residues tend to localize near either the α-helix or β-strand states. The plot identifies a region to the left of α-helix state, which also has significant population (a RC state). The α-helix and β-strand states are connected by a path, which is apparently sampled during the α-helix $\rightarrow$ β-strand conversion. This observation is consistent with Fig. (3a), which demonstrates the increase in the β-structure content occurs at the expense of α-helix states.

Assembly of antiparallel β-sheets

Disordered oligomers are stabilized by extensive network of interpeptide hydrophobic interactions. Despite occasional interpeptide contacts between charged Lys and Glu residues a stable antiparallel β-sheet arrangement is not discernible on the time scale of simulations ($\approx 11ns$). To enable the formation of antiparallel β-sheet structures on simulation timescales, we adopted a "fast forward" strategy to probe the assembly of the ordered oligomer (see Experimental Procedures). Our strategy targets the dynamics of "successful" oligomer formation at longer time scales.

There are large variations in the kinetics of oligomer assembly, which are indicative of heterogeneity of assembly pathways. Residues in the peptides 1 and 3 frequently sample β-states, whereas peptide 2 is predominantly α-helical (data not shown). The highest β-strand content is found in the peptide 1 ($< S_1 >= 0.35$). For comparison, in peptide 2 $< S_2 >= 0.05$. In accord with this, the average radii of gyration for the peptides 1 and 3 $< R_{g,1} >= 9.0\mathring{A}$ and $< R_{g,3} >= 8.8\mathring{A}$ are larger than that for the helix-rich peptide 2 ($< R_{g,2} >= 7.7\mathring{A}$). In contrast, in another trajectory for ordered oligomer α-helix structure in the peptide 2 almost completely dissolves after about 1.5 ns and is converted to β-strand conformations. Simultaneously, an increase in R_{g2} from $7.5\mathring{A}$ to $9.0\mathring{A}$ is observed. This structural transition results in small α-helix content ($< H_2 >= 0.14$). The probability to find a peptide in Aβ_{16-22} ordered oligomer in β-strand state (average over four trajectories) is 0.40. Strikingly, the probability of α-helix peptide states is much smaller (0.19). The probability of random coil peptide states (0.41) is comparable to that of β-strand.

Emergence of antiparallel β-sheets is most clearly seen, if one examines $d_{ij} = \hat{u}_i(t)\hat{u}_j(t)$ (see Experimental Procedures). For an ideal antiparallel arrangement of the peptides i and j $d_{ij} = -1$, while $d_{ij} = 1$, if peptides are in parallel conformation. We found that in one of the trajectories two pairs of peptides (labeled 1-2 and 1-3) rapidly (in about 1 ns) adopt antiparallel orientation, while the peptides 2 and 3 are parallel (Fig. (4a)). Once such a structure is formed, it remains mostly stable during the course of simulations ($\approx$ 6 ns). Interpeptide salt bridges between Lys16 and Glu22 confer stability to the pairs of peptides in antiparallel registry. Stable electrostatic contacts between Glu and Lys of the peptides pairs 1-3 and 2-3 (with the probabilities $P_{1-3,Glu22-Lys16} = 0.94$ and $P_{1-2,Glu22-Lys16} = 0.64$) ensures proper orientation in antiparallel β-sheets. An example of antiparallel in-registry packing of peptides 1 and 3 in Aβ_{16-22} oligomers is shown in Fig. (4b).

Antiparallel registry of peptides in Aβ_{16-22} oligomers: Taking into account the most frequent contacts and the functions d_{ij} for each trajectory, we reconstruct preferential orientation of peptides in Aβ_{16-22} oligomers and the network of frequent interactions between the peptides 1 and 2 and the peptides 1 and 3 (Fig. (4c)). This figure illustrates that antiparallel

orientation of Aβ_{16-22} peptides is determined by electrostatic contacts between charged terminals. For the peptides 1 and 2 the contact Glu22-Lys16 stabilizes the formation of (mostly) hydrophobic contacts Phe19-Lys16, Phe19-Phe20, Leu17-Phe20. For the peptides 1 and 3 the contact between charged terminals Lys16-Glu22 (the opposite terminal in the peptide 1) serves to stabilize antiparallel registry of this pair of peptides. Beside the salt bridge, the antiparallel pattern of contacts between peptides 1 and 3 is established by Leu17-Ala21, Leu17-Phe20, Phe19-Ala21, Phe19-Phe20. The electrostatic interactions confer the required specificity to form in-register peptide packing. Because the contacts between the peptides 2 and 3 are weak (their average probability is less than 0.50) and less numerous, peptide 1 acts as a linker between the peptides 2 and 3. We believe that the observed antiparallel pattern represents the initial seed, which in the presence of other peptides may subsequently grow into amyloid fibrils.

Because assembly of Aβ_{16-22} oligomer takes place in the solution, there are considerable fluctuations as compared to the fibrils monitored in solid state NMR [15]. As a result the β-strand content in oligomers is not nearly as large as observed in fibrils [15]. Nevertheless, the tendency to form antiparallel β-sheet with substantial β-strand content is established in our simulations. Although the number of successful formations of antiparallel β-sheets is relatively small, it is clear that the structures of interacting peptides in oligomers resemble those that are formed in fibrils.

β-helix formation for Aβ_{16-22} peptides is unlikely: Based upon electrostatic considerations alone we can assess the formation of a circular arrangement of the Aβ_{16-22} peptides. In fact, such structures, in which the charged terminals of one peptide are in contact with the terminals of two other peptides, were transiently observed in our simulations. Such an oligomer can be a seed for forming a β-helical fibre [16], in which the orientation of each tripeptide unit is the opposite to those of its immediate neighbors. Our current simulations suggest that circular oligomers are unstable, because the interpeptide hydrophobic interactions are compromised. The formation of a circular conformation stabilized by both interpeptide salt bridges and hydrophobic interactions that can propagate to a β-helical fibre structure

requires at least six peptides. Therefore, such arrangement can not be ruled out on the basis of the present simulations alone. However, formation of β-helical structures places like charges at the vertices of the tripeptide triangles and hence β-helices are likely to be unstable. Furthermore, the results of solid state NMR experiments seem to rule out the possibility of forming β-helix fibres for short fragments of Aβ peptides [15].

Interactions contributing to the antiparallel β-structure

Fig. (5) shows that the formation of $(A\beta_{16-22})_3$ is energetically favorable. This follows from the time dependence of $< \delta E_{pot}(t) >= (< E^o_{pot}(t) > -3 < E^m_{pot} >)/3 < E^m_{pot} >$, where $< E^o_{pot}(t) >$ is the potential energy of oligomer averaged over four trajectories and $< E^m_{pot} >$ is the time averaged potential energy of the monomer. This plot clearly shows that due to favorable electrostatic interactions the antiparallel arrangement of peptides provides an additional gain in stability as compared to disordered oligomer [22]. The importance of electrostatic interactions can also be gleaned from the fluctuations in the potential energy $E^o_{pot}(t)$ of the $A\beta_{16-22}$ oligomer. Dramatic fluctuations in $E^o_{pot}(t)$ are associated with the formation and dissolution of contacts between charged residues. A strong correlation (the average correlation factor is 0.8) is observed between $E^o_{pot}(t)$ and the number of interpeptide salt bridges between Lys and Glu. In contrast, no correlation (the average correlation factor is 0.1) is seen between $E^o_{pot}(t)$ and the total number of interpeptide hydrophobic contacts. Therefore, electrostatic interactions play a crucial role in the orientation of the peptides, while hydrophobic interactions provide a non-specific "glue" for binding $A\beta_{16-22}$ peptides together.

The assembly dynamics also suggests that electrostatic and hydrophobic interactions play distinct roles in antiparallel β-sheet formation. The extent to which hydrophobic and electrostatic interactions control the assembly of oligomers is not only relevant for understanding the initial events in $A\beta_{16-22}$ oligomerization, but also in the context of fibrillogenesis of full length Aβ peptides.

To probe the distinct role of electrostatic and hydrophobic interactions we engineered two mutants. In one of them labeled K16G/E22G the charged residues Lys16 and Glu22 are replaced with polar and neutral Gly. This substitution eliminates the possibility of formation of interpeptide salt bridges. In the second mutant, L17S/F19S/F20S, we substituted three hydrophobic residues Leu17, Phe19, Phe20 with polar Ser. These positions are chosen because most of the hydrophobic interpeptide contacts in Aβ_{16-22} oligomers involve these amino acids. By studying the assembly of the mutated peptides the role of electrostatic and hydrophobic interactions can be dissected. For both the mutants, we generated four independent trajectories using the initial wild-type structures for ordered oligomer.

K16G/E22G Aβ_{16-22}: The principal result obtained for this mutant is that oligomer becomes unstable. In three (out of four) trajectories dissolution of peptides is observed. As an example, we display in Fig. (6a) the distances between peptides centers of mass R_{ij}^{CM} as a function of time for one of trajectories. Shortly before 1.5 ns peptide 1 breaks away as the distances R_{12}^{CM} and R_{13}^{CM} sharply increase. Accordingly, the number of interpeptide contacts $C_{12}(t)$ and $C_{13}(t)$ drop to zero (data not shown). The breakage of the peptide 1 is "permanent", because the contacts with other peptides are not restored (Fig. (6b)). Similar events take place in two other trajectories as well. Although we cannot rule out aggregation of AβK16G/E22G on much larger time scales, the stability of such a structure would be considerably less than that for the wild-type oligomers. Dynamics of $d_{ij}(t)$ shows that deletion of charged terminals significantly increases the fluctuations in the orientations of peptides in Aβ_{16-22} oligomer (Fig. (6c)). The peptides frequently change their orientations relative to each other and, in many instances, reverse it by 180°. Thus, replacing charged terminals with polar residues produces a drastic destabilizing effect on Aβ_{16-22} oligomers.

L17S/F19S/F20S Aβ_{16-22}: In all the trajectories we observed partial dissolution of amyloid oligomers for this mutant. For example, in one of the trajectories peptide 3 breaks away at about 0.9 ns as the distances between the centers of mass R_{13}^{CM} and R_{23}^{CM} exceed 15$\mathring{A}$ (Fig. (7a)) and the number of contacts, which the peptide 3 forms with other chains drops to zero. After peptide 3 separates from the trimer the distance between the peptides 1 and

2 R_{12}^{CM} also gradually increases up to 20 $\mathring{A}$. At this point the only contact (between charged terminals, Glu22 and Lys16) remains intact between these peptides. Separation of peptides from the oligomer is also observed in all other trajectories.

The simulations of L17S/F19S/F20S mutant provide strong evidence that the removal of three hydrophobic residues makes Aβ_{16-22} oligomers unstable. Not only did we observe individual peptides separating from oligomers, but the entire oligomer complex itself became loosely formed and, in few instances, appeared to be on a brink of disintegration. Overall, we registered four events, in which Aβ_{16-22} peptides break away from oligomers on approximately 13.9 ns total timescale.

Replacement of three bulky hydrophobic residues with a relatively compact polar Ser drastically affects the β-strand and α-helix propensities as well. The average β-strand residue content in this mutant $< S >= 0.19$ is smaller than the population of α-helix residues ($< H >= 0.28$). The α-helix propensity is especially large at the positions Ser19 and Ser20 ($P_h(Ser19) = 0.54$ and $P_h(Ser20) = 0.46$). The corresponding β-strand propensities are about 0.1. The dominance of α-helix structures is the direct consequence of sequence mutation, which reduce steric constraints.

By comparing the results for the wild-type and the two mutants we draw two important conclusions: (1) Both hydrophobic and electrostatic interactions are crucial for the assembly of Aβ_{16-22} into an ordered oligomer; (2) The initial driving force of oligomer assembly is the favorable interpeptide interactions between the LVFFA cluster. However, the ordered (antiparallel) orientation is only obtained upon the formation of salt bridges. The latter imparts the stability to antiparallel conformations of peptides as evidenced by $d_{ij}(t) \approx -1$ (Fig. (4a)). In accord with this we find that the orientational fluctuations are considerably less for the AβL17S/F19S/F20S mutant than in AβK16G/E22G.

So far we have focused on the contributions of salt bridges and hydrophobic interactions to the stability of peptides in Aβ_{16-22} oligomers. On the other hand, by using ^{13}C and ^{15}N labeled Aβ_{16-22} peptide samples, Balbach *et al.* [15] concluded that hydrogen bond is estab-

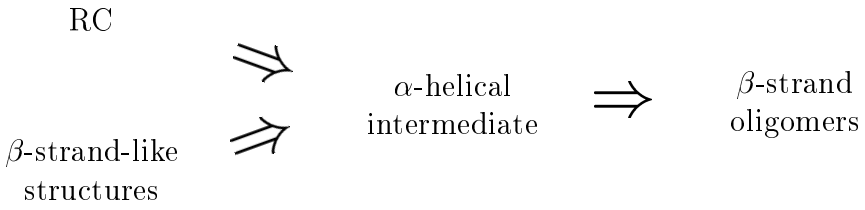
lished between CO(Leu17) and NH(Ala21). We investigated hydrogen bonding in Aβ_{16-22} oligomers and found that the hydrogen bond NH(Leu17)-CO(Ala21), which has the highest probability of occurring in the simulations, is also formed between these residues. When this hydrogen bond is present, the average distance between nitrogen and carbonyl atoms is 4.4 $\mathring{A}$, which is consistent with the antiparallel arrangement of peptides [15]. However, because simulations are performed in water, we observe frequent disruptions in hydrogen bonding. The few interpeptide hydrogen bonds that are frequently formed are largely localized near the stable interpeptide sidechain contacts.

Discussion

Assembly mechanism - road to antiparallel β-sheet is through α-helical intermediate: Multiple long molecular dynamics simulations of interacting Aβ_{16-22} peptides yield novel insights into the plausible mechanisms governing oligomerization. The conformations of Aβ_{16-22} peptides, in a monomeric form, partition into two distinct sets of structures. The first consists of RC conformations with the mean end-to-end distance typical of collapsed peptides. The second is best described by an extended β-strand-like conformations. The small size of this peptide allows for frequent transitions between those structures on the simulation time scale. The strongest propensity to form β-strand is found for Val18. Somewhat surprisingly the α-helical structures are rarely sampled by monomers.

Relatively little is known about the mechanisms of oligomerization. The time scale for forming detectable oligomers (or fibres) of even short fragments of Aβ peptides is too long to be directly probed by MD simulations. Nevertheless, MD can give a glimpse of the initial events in the assembly of Aβ peptides into ordered structures. Out of the four trajectories totaling more than 40 ns for the wild-type an antiparallel arrangement of Aβ peptides is clearly found in only one. Multiple simulations starting from this structure established that this arrangement, once formed, is stable. However, in all the trajectories a profound conformational transition from α-helical to β-strand structures is observed, which is driven

by interpeptide interactions. Even for this short fragment the α-helical conformations are (initially) preferentially populated. Significantly, such a structural transition is not seen for $A\beta_{16-22}$ monomers. Our simulations clearly reveal a gain in β-strand content and a transient increase in α-helix content (Fig. (8)). These observations are reminiscent of the aggregation dynamics of full length Aβ peptides [11]. Because α-helical populations is always detected for *interacting* peptides regardless of the initial conditions, we propose that the α-helical structure is an obligatory intermediate in the process of oligomerization. Thus, the plausible kinetic mechanism for the assembly of Aβ oligomers, which involves multiple pathways, may be described by the scheme



The mobile β-strand oligomers can grow to form insoluble fibres either by nucleated polymerization [23] or templated assembly [24].

To get further insight into the structure of $(A\beta_{16-22})_3$ we have computed the radial distribution of water molecules around $A\beta_{16-22}$ oligomers. Surprisingly, we found that the density of water is substantially reduced in the interior of oligomer as compared to the bulk value (data not shown). Moreover, near its center the $A\beta_{16-22}$ oligomer is effectively dehydrated. Eisenberg and coworkers [25] have shown that a peptide from yeast prion Sup35 also forms a dry β-sheet amyloids. Therefore, it appears that expulsion of water does not represent a rate-limiting step in the assembly of oligomers for these relatively short fragments.

Recently, by monitoring secondary structure changes by circular dichroism, Teplow and coworkers showed [11] that at the first stages of assembly of amyloids $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides adopt helical conformations. Only subsequently the transition $\alpha \rightarrow \beta$ takes place.

These findings suggest that α-helical conformations may be "on-pathway" intermediate to fibrillization. Their detailed experimental observations and our MD simulations (on much shorter timescales) suggest that, at least in this class of peptides, multiple routes to amyloid fibrils with obligatory α-helical intermediate may represent a general mechanism. A plausible rationalization for this conclusion can be given as follows. Our simulations clearly show that the major driving force for oligomerization is the hydrophobic interactions that serve as a "glue" for the peptides. Initially, a given peptide interacting with the others find itself in a confined region that is predominantly hydrophobic, i.e., the peptide experiences a membrane-like environment. This naturally renders the hydrophobic region of the peptide to be helical. Because the helical structures cannot pack efficiently to maximize favorable hydrophobic interactions, an $\alpha \rightarrow \beta$ transition occurs.

This argument suggests that the degree of "membrane-like" environment felt by Aβ peptides depends on the peptide concentration. As a result the extent of α-helix formation and the time scale (t_{max} in the study of Teplow and coworkers [11]), at which the maximum helicity is observed, will depend on the peptide concentration.

It is interesting to speculate on the nature of the plausible intermediate that may be found for Aβ_{1-40} and Aβ_{1-42} peptides based on our studies. The N-terminal of these peptides is largely hydrophilic, whereas the C-terminal and 17-21 (LVFFA) CHC region are hydrophobic. The CHC region is connected to the C-terminal terminal by the VGSN turn (residues 24-27). Assuming that hydrophobic forces drive oligomerization, we propose that the structure in the intermediate is of the form RC-α-T-α, where random coil is restricted to hydrophilic N-terminal (residues 1-10 or 12) and the turn T corresponds to the VGSN segment. The transition from this structure to a nucleus composed of β-strand may be the rate limiting step in fibrillization.

Predictions for related Aβ_{16-22} fragments: One of the important results of our study is the distinct roles of the hydrophobic and electrostatic interactions in the formation and stabilization of antiparallel β-sheet structure of Aβ_{16-22} oligomers. The initial driving force is the non-specific association between the CHC residues. Formation of the salt bridges, Lys-Glu,

not only enhances the oligomer stability, but also produces the specific antiparallel registry of Aβ_{16-22} peptides. These observations can be used to predict the plausible outcomes of oligomer formation for the sequence KLVFFAX, where X is a substitute residue. Such fragments occur in alloforms of Aβ peptides. For example, the alloforms with X=Gly, Gln, and Lys are referred to as a Arctic (E22G), Dutch (E22Q), and Italian (E22K) mutants, respectively. The simulations with X=Gly and Lys also replaced with Gly show that the oligomer is unstable in the absence of favorable interpeptide salt bridges. The same line of reasoning leads us to predict that in the alloforms mentioned above fibril formation with antiparallel registry of the strands is unlikely in the 16-22 fragment.

Biological Implications

Growing evidence shows that oligomers of Aβ peptides might cause neurotoxicity even though the final product of amyloidogenesis is the deposition of plaques in the brain. These observations make it important to understand, at an atomic level, the kinetics of polymerization of Aβ peptides. To shed light on this issue, we have simulated oligomer formation for the fragment Aβ_{16-22} peptides, which have been observed to form ordered fibrils. Molecular dynamics simulations presented here show that the route to the ordered oligomer, which is an intermediate step in the formation of the fibril, occurs through an "on-pathway" α-helical intermediate just as in the fibrillogenesis of the full length Aβ-peptides [11]. These results not only indicate a common mechanism of fibrillization in this class of peptides, but also suggests that therapeutic agents that destabilize the helical intermediates might prevent oligomerization. Stability of the ordered antiparallel β-sheets depends both on electrostatic and hydrophobic interactions. While interpeptide hydrophobic interactions promote non-specific association, the formation of salt bridges confers the precise antiparallel registry of the β-stands. Therefore, those mutations, which destroy the salt bridges or weaken the net hydrophobic interactions, can also inhibit fibril formation. Furthermore, the architecture of the amyloid fibrils in Aβ peptides is determined by maximizing the number of hydrophobic

and electrostatic interactions. The use of this rule and the propensities of residues at specific locations might be useful in modeling the structure of amyloid fibrils.

Experimental Procedures

Simulation details: Molecular dynamics simulations using the MOIL program [26] were performed to probe the mechanism of oligomerization of Aβ_{16-22} peptides. Specifically, we simulate the assembly of (Aβ_{16-22})$_3$ oligomer from three Aβ_{16-22} peptides. The amino acid sequence of Aβ_{16-22} is Lys-Leu-Val-Phe-Phe-Ala-Glu and is capped with uncharged acetyl and amide groups. The Aβ_{16-22} sequence includes LVFFA central hydrophobic cluster from Aβ_{1-42} and its terminal residues are oppositely charged (positive charge on lysine and negative charge on glutamic acid).

The initial conformation for Aβ_{16-22} monomer was extracted from the solution NMR structure for Aβ_{10-35} peptide (PDB access code 1hz3) [18]. For reference, we performed simulations to characterize the structural characteristics and fluctuations of the Aβ_{16-22} monomer. The initial conformations of the trimer were obtained by replicating the individual Aβ_{16-22} monomer structures in random orientations. The simulations were carried out using the microcanonical ensemble. The systems of peptides and water were enclosed in a cubic box. The number of water molecules was about 1,300 and depended slightly on the initial orientations of Aβ peptides. The density of water in the simulation box with the volume 41781.9 Å^3 is approximately $0.98 g/cm^3$ at 300K. After a short relaxation of the positions of water molecules, the energy of the system was minimized using the conjugate gradient algorithm for 1000 steps. Particle Mesh Ewald method was used to compute electrostatic interactions [27]. The cut-off distances for direct electrostatic and van-der-Waals interactions were 12 and 9 Å, respectively. The dielectric constant was set to 1 and periodic boundary conditions were used for water. Starting with energy minimized structure the system was linearly heated to 300K during 300 *ps* simulations. After the heating stage the system was equilibrated for an additional 300 *ps* at 300K. The integration step of 1 fs was used

in all MD simulations. At the heating and equilibration stages velocities were rescaled every interaction step. Rescaling was turned off during production runs. Conformational snapshots were saved with 1 ps interval.

Because we expect the time scales for oligomer formation to be relatively slow, we employed the following novel approach to facilitate interactions between the peptides. The positions of peptides were constrained by harmonic coupling (the spring constant $k_c = 0.02 kcal/(mol\mathring{A}^2)$) between the center of the water box and the oligomer center of mass. The peptide concentration corresponds, within an order of magnitude, to that estimated experimentally for $A\beta_{16-22}$ amyloid deposits. We have checked that addition of the constraining potential does not alter in any significant way the potential energy of the system. More importantly, the individual peptides are given sufficient volume for efficient conformational sampling in MD simulations. This is reflected in multiple reorientations of individual peptides in $A\beta_{16-22}$ oligomers. Similar, although less general, method of facilitating chain aggregation has been recently used [28].

To establish the general validity of our results we generated multiple (eight) independent trajectories for both the monomer and the trimer systems. The total simulation time for the monomer system is 56 ns, while the wild type $(A\beta_{16-22})_3$ oligomer was simulated for 68 ns. For the $(A\beta_{16-22})_3$ oligomer two independent sets of MD simulations were performed, which differ with respect to the initial orientations of peptides. The centers of mass of the peptides in the initial conformation in the first set of simulations were separated by about 7 Å. Starting with this conformation and after energy minimization four independent heating and equilibration trajectories were obtained. Their final conformations served as initial structures for four 10.7 ns production trajectories. These simulations target structural changes that occur upon interpeptide interactions and formation of disordered oligomers.

To probe oligomer ordering on longer time scales, the following computational strategy was implemented. Four independent heating and equilibration trajectories were generated starting with the initial structure different from that used in the first set of simulations. Then four preliminary production trajectories were initiated, in which we monitored the

population of α- and β-structure in each peptide. These trajectories were terminated as soon as in one of those (i) the average β-structure content in two peptides approached 40% and (ii) a pair of peptides adopted and maintained approximately antiparallel orientation. The structure satisfying these conditions was used as the starting point for four independent production trajectories of 6.4 ns each.

The rationale for using such computational strategy is as follows. From the first set of simulations we observed an accumulation of β-strand structure and formation of salt bridges between peptides. However, the timescale of formation of β-strand structure with antiparallel orientation of peptides is too long for direct MD simulations. Thus, we sought to "fast forward" the oligomer kinetics by picking up the snapshot with high β-strand content and roughly antiparallel peptide orientation and use it as a starting point for new simulations.

Probes for amyloid formation: We used several quantities to characterize structural changes in Aβ_{16-22} oligomers. To characterize relative orientation of peptides as a function of time, the scalar product of end-to-end unit vectors $d_{ij}(t) = \hat{u}_i(t)\hat{u}_j(t)$ for a pair of peptides i and j was computed. The interpeptide interactions were probed by the distance between the centers of mass of peptides i and j, $R_{ij}^{CM}(t)$ and by the number of interpeptide contacts between sidechains $C_{ij}(t)$. (For calculating C_{ij} sidechains are assumed to be in contact, if their centers of mass are less than 6.5 $\mathring{A}$ apart.) We also computed peptide radii of gyration $R_{g,i}(t)$. Emergence of stable contacts was evaluated by computing the contact maps as a function of time and the probabilities of formation of individual contacts. The conformational energies of (Aβ_{16-22})$_3$ oligomer $E_{pot}^{o}(t)$ and Aβ_{16-22} monomer $E_{pot}^{m}(t)$ were monitored as a function of time.

Secondary structure probes: Using two definitions of β-strand and α-helix we calculated S_i (H_i), the fraction of residues in a peptide i, whose dihedral angles ϕ and ψ satisfy the definition of local β-strand (α-helix) structure. Following [29] we assumed ("strict definition") that for a β-strand $-150^\circ \leq \phi \leq -90^\circ$ and $90^\circ \leq \psi \leq 150^\circ$ and for an α-helix $-80^\circ \leq \phi \leq -48^\circ$ and $-59^\circ \leq \psi \leq -27^\circ$. The other ("broad") definition [30,31] assumes

that, if ϕ and ψ angles are discretized into 20 intervals of 18°, β-strand conformations correspond to the vertices of the polygon (-180,180),(-180,126),(-162,126),(-162,108),(-144,108),(-144,90),(-50,90), (-50,180) on the Ramachandran plot; α-helix structure is confined to the polygon (-90,0),(-90,-54),(-72,-54),(-72,-72),(-36,-72),(-36,-18),(-54,-18),(-54,0). Both definitions provide qualitatively similar results, although the amount of secondary structure identified with the "strict" definition is predictably smaller. Throughout this study we use the "broad" definition for β-strand and α-helix. Both ϕ and ψ angles are defined only for five inner residues.

The time dependence of β-strand content is obtained using

$$< S(t) >= \frac{1}{3M} \sum_{k=1}^{M} \sum_{i=1}^{3} S_i^k(t), \tag{1}$$

where i is the peptide index, k is the trajectory index, and M is the number of trajectories. The average β-strand content is computed as $< S >= \frac{1}{T} \int_0^T < S(t) > dt$, where T is the simulation time. The α-helix contents ($< H(t) >$ and $< H >$), radius of gyration $< R_g(t) >$, and potential energies $< E_{pot}(t) >$ were computed in a similar way.

To characterize the distribution of Aβ_{16-22} peptide structures we classify a conformation to be β-strand (or α-helix) if (a) the (ϕ, ψ) angles of any two consecutive residues are in the corresponding (β-strand or α-helix) Ramachandran regions and (b) no two consecutive residues are in the α-helix (β-strand) conformations. If neither β-strand or α-helix conformation is assigned, then a conformation is classified as RC.

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Figure legends

Fig. (1) (a) The average β-strand $< S(t) >$ (in green) and α-helix $< H(t) >$ (in red) contents in Aβ_{16-22} monomers as a function of time. $< S(t) >$ and $< H(t) >$ give the probability to observe a residue in a β-strand or α-helix conformations when averaged the ensemble of eight independent trajectories.

(b) The β-strand $P_s(i)$ and α-helix $P_h(i)$ propensities as a function of sequence position i in Aβ_{16-22} monomers. $P_s(i)$ and $P_h(i)$ are the probabilities to observe β-strand or α-helix structure at i, which are averaged over eight trajectories.

(c) Representative snapshots of monomer structures. The structure on the left is in RC conformation with zero β-strand or α-helix contents. The other two structures are in β-strand states. Specifically, four out of five residues are in β-strand conformations in the center snapshot, while all residues in the right snapshot are in the β-strand conformations. If all the residues in Aβ_{16-22} are in β-strand conformations, the end-to-end distance $r_{1N} \approx 23\mathring{A}$. Because of large conformational fluctuations, r_{1N} for β-strand-like structures is typically smaller than $23\mathring{A}$. The charged sidechains are shown in blue (Lys16) and red (Glu21) and the hydrophobic (CHC) sidechains are given in green. Program RasMol v2.6 [20] has been used to visualize molecular structures in this and other figures.

Fig. (2) (a) The snapshot of solvated (Aβ_{16-22})$_3$ oligomer. Coloring of residues is the same as in Fig. (1c). The oligomer is stabilized by mostly hydrophobic interactions between CHC residues (in green). Because salt bridges are rare, the peptides do not show any preferential orientations in the oligomer. We refer to such oligomer as disordered as opposed to that observed on longer time scales (Fig. (4b)).

(b) The radii of gyration of peptides R_{gi} as a function of time for one of the (Aβ_{16-22})$_3$ trajectories. Colors encode peptides as indicated in the plot.

(c) Dynamics of secondary structure in Aβ_{16-22} peptides forming oligomer at residue level (same trajectory as in (a)). The secondary structure is assigned according to the values of dihedral angles ϕ and ψ (see Experimental Procedures). β-strand, α-helix, and RC

conformations are represented in green, red, and blue, respectively. A dramatic conversion of α-helix structure into β-strand in peptide 2 is correlated with its extension (see text for details).

Fig. (3) (a) The average β-strand $< S(t) >$ (in green) and α-helix $< H(t) >$ (in red) contents in Aβ_{16-22} peptides in disordered oligomers as a function of time. $< S(t) >$ and $< H(t) >$ give the probability to observe a residue in a β-strand or α-helix conformations averaged over the ensemble of four independent trajectories. The plot shows $\alpha \rightarrow \beta$ conversion, which is driven by extensive (mostly hydrophobic) interpeptide interactions.

(b) The backbone traces of Aβ_{16-22} oligomers illustrate the $\alpha \rightarrow \beta$ structural transition shown in Fig. (3a). Approximately two-thirds of residues in the left oligomer are in α-helix conformations and none adopt β-strand states (other residues are in RC conformations). The structure is taken at 43 ps (soon after the start of production run). In the snapshot on the right recorded about 10 ns later two-thirds of residues are already in β-strand conformations, whereas the fraction of α-helix residues is dropped to less than 0.1.

(c) Distribution of (ϕ, ψ) dihedral angles in the disordered Aβ_{16-22} oligomers. The 18° interval grid is shown by white dashed lines. The α-helix and β-strand states are contoured with solid white lines. Residue conformations in Aβ_{16-22} oligomers are restricted to α-helix, β-strand, and RC (next to α-helix) states. The path connecting the α-helix and β-strand regions is attributed to $\alpha \rightarrow \beta$ conversion.

Fig. (4) (a) Orientations of peptides in ordered Aβ_{16-22} oligomer characterized by the time dependence of $d_{ij} = (\hat{u}_i \hat{u}_j)$ (see Experimental Procedures for definition). Colors code peptide pairs. Analysis shows that peptides 1 and 3 maintain an almost perfect antiparallel in-registry orientation for at least 5 ns. Note that the interaction between peptides 1 and 2 is weak.

(b) The conformational snapshot for the peptides 1 and 3, which are locked in antiparallel in-registry packing. Two salt bridges between charged terminals Lys and Glu (in blue and green, respectively) are formed in this structure. Coloring of side chains is the same as in Fig. (1c).

(c) The emerging antiparallel registry of peptides in ordered Aβ_{16-22} oligomers illustrated through the network of most frequent interpeptide contacts (grey dashed lines). The interpeptide interactions propagate from the anchoring contacts between charged side chains Lys16 and Glu22, which establish antiparallel orientation of peptides.

Fig. (5) The time dependence of the relative potential energy $< \delta E_{pot}(t) >$ (see text for definition) for the disordered (left panel) and ordered (right panel) oligomers. The dashed baseline indicates the relative potential energy of a Aβ_{16-22} monomer. The panels show that formation of oligomers is energetically favorable. Additional gain in stability due to electrostatic interactions lowers the average $< \delta E_{pot}(t) >$ for an ordered oligomer as compared to disordered one.

Fig. (6) (a) The distances between the centers of mass of K16G/E22G Aβ_{16-22} peptides R_{ij}^{CM} as a function of time. Sharp increase in R_{12}^{CM} and R_{13}^{CM} reflect breaking of peptide 1 from K16G/E22G Aβ_{16-22} oligomer after $\approx$ 1.5 ns. Color codes are the same as in Fig. (4a).

(b) Snapshot of the final conformation for the trajectory shown in Fig. (6a). After 2 ns the distance between peptide 1, which escaped from Aβ oligomer at about 1.5 ns, is increased to about 25 $\mathring{A}$. Hydrophobic side chains are given in green.

(c) The time dependence of $d_{ij} = (\hat{u_i}\hat{u_j})$ (see Experimental Procedures for definition) for K16G/E22G Aβ_{16-22} oligomer. d_{ij} quantifies the orientations of peptides for the trajectory, in which oligomer integrity was retained. Frequent variations in d_{ij} are in sharp contrast with almost constant values of d_{ij} seen for the ordered oligomer (Fig. (4a)). On an average, the fluctuations in d_{ij} are twice as large for K16G/E22G as compared to the wild-type. Color codes are the same as in Fig. (4a).

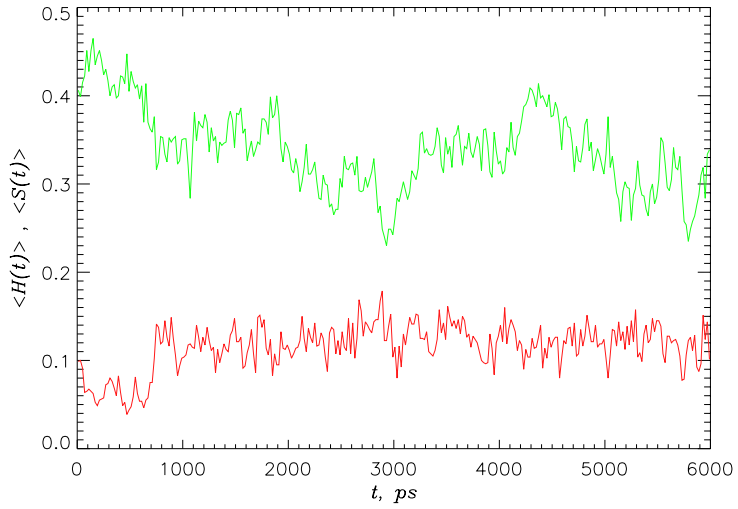
Fig. (7) (a) The distances between the centers of mass of L17S/F19S/F20S Aβ_{16-22} peptides R_{ij}^{CM} as a function of time. An increase in R_{ij}^{CM} reveals partial dissolution of oligomer starting with peptide 3 separating from oligomer at $\approx$ 0.9 ns. By the end the trajectory the interactions between peptides 1 and 2 are also weakened. Color codes are the same as in Fig. (4a).

(b) Snapshot of the final conformation in the trajectory shown in Fig. (7a). By 4 ns peptide

3 is $\gtrsim 35\mathring{A}$ apart from two other peptides, which are only linked by a single salt bridge. Coloring of side chains is the same as in Fig. (1c), except for Ser side chains shown in orange.

Fig. (8) The distribution of Aβ_{16-22} peptide conformational states for monomer, disordered and ordered oligomers. The plot demonstrates that monomer conformations are predominately RC (in blue) or β-strand (in green). In the course of oligomer assembly the share of β-strand conformations increases, whereas the fraction of the α-helical states (in red) reaches maximum in disordered oligomers, and declines with oligomer ordering (propagation of antiparallel registry). The RC states become less populated with the progress in oligomer assembly. The occurrence of α-helical intermediate and accumulation of β-strand structures are consistent with recent experiments [11].

(a)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1000
2000
3000
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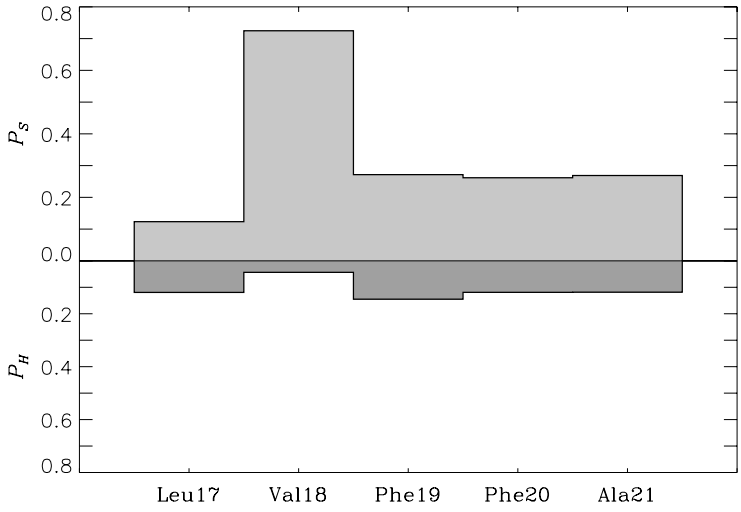
6000
t, ps

(b)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Ala21

(c)

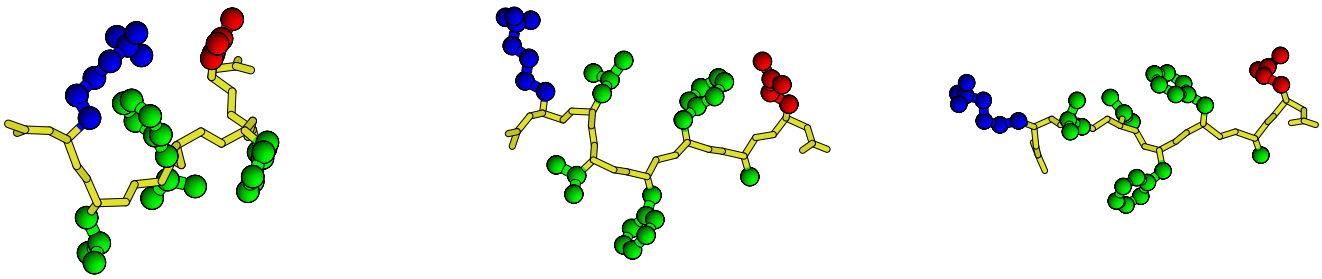


Fig. (1)

(a)

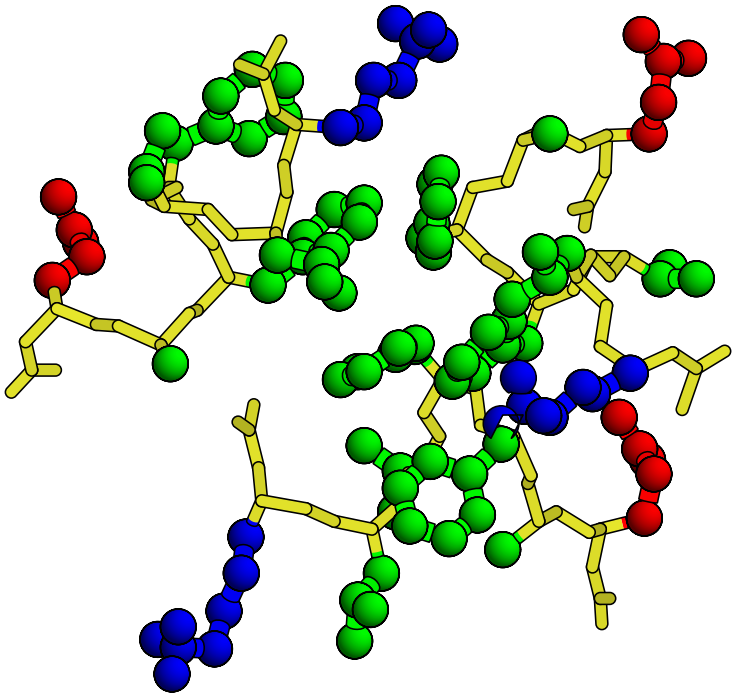
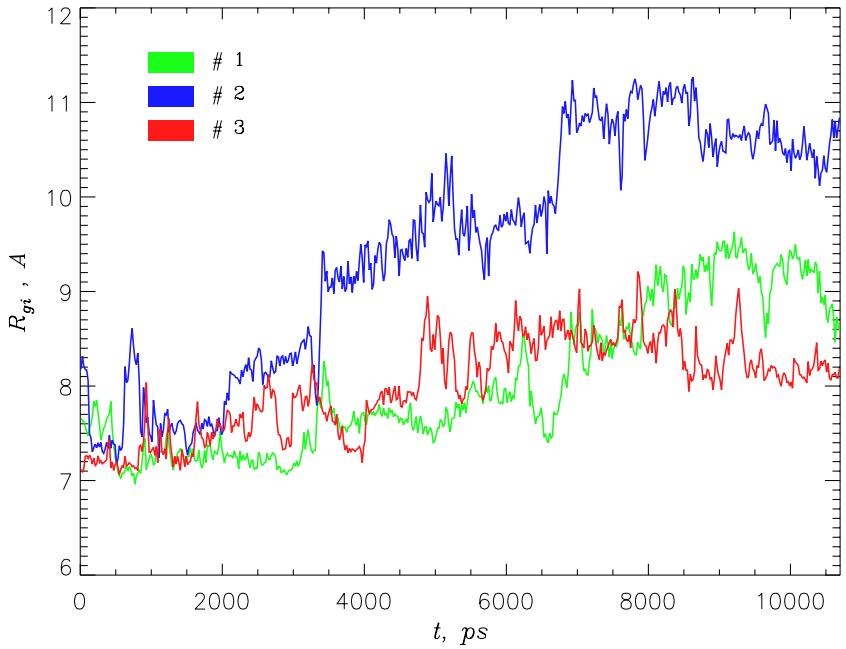


Fig. (2)

(b)



(c)

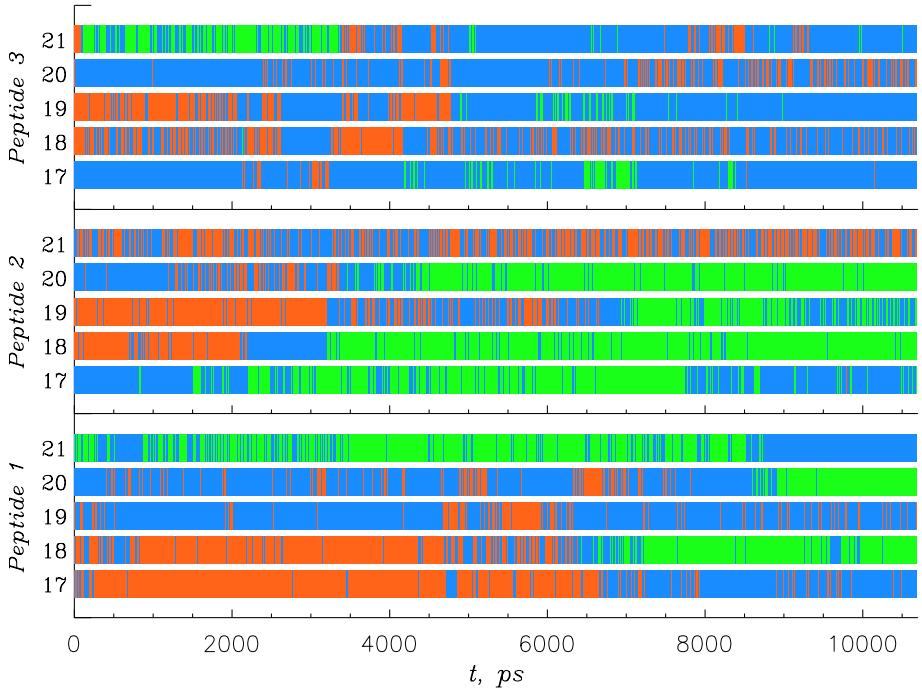
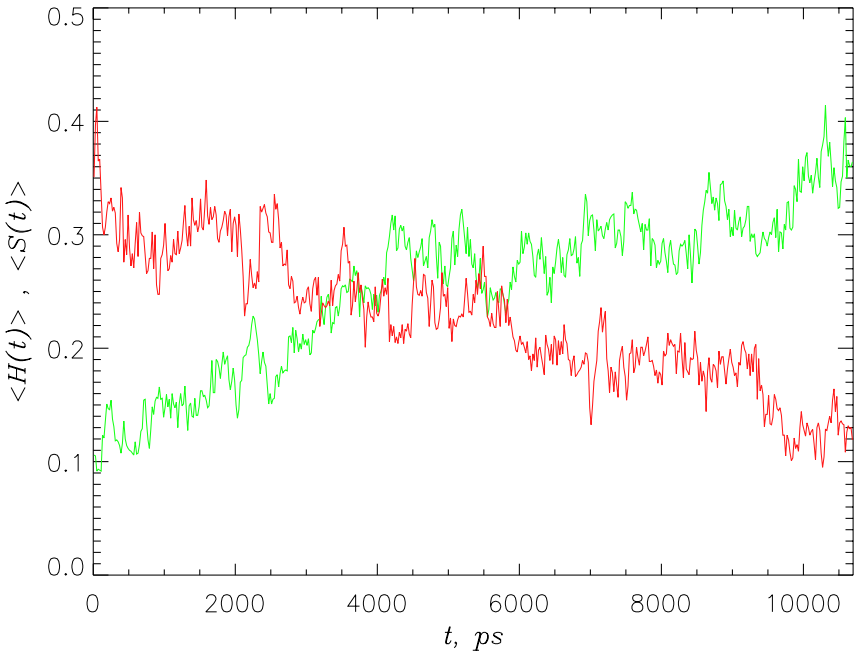


Fig. (2)

(a)



(b)

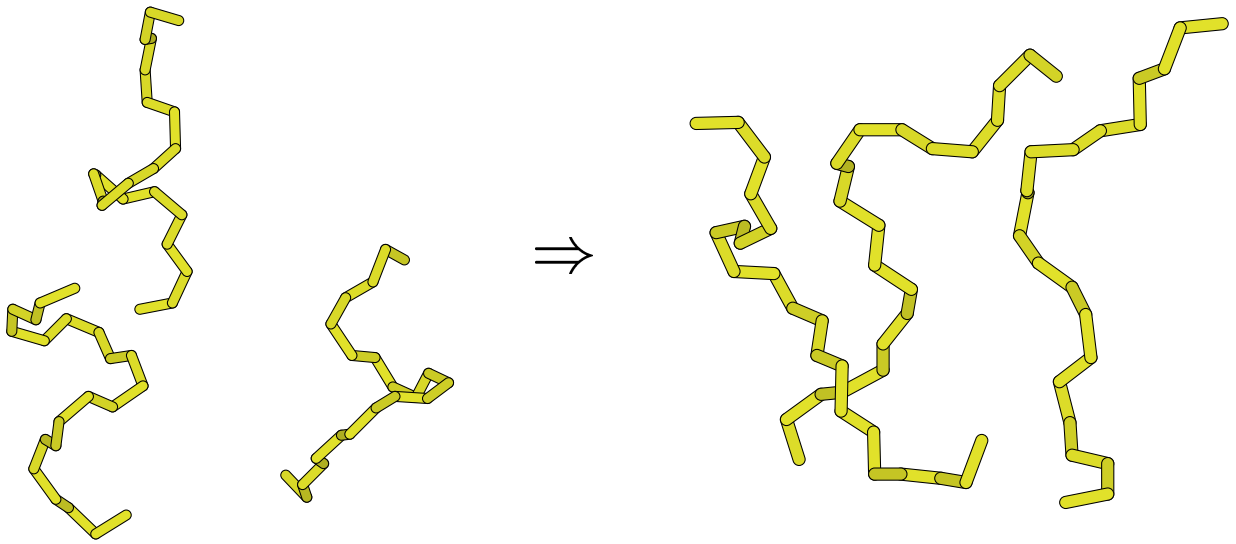


Fig. (3)

(c)

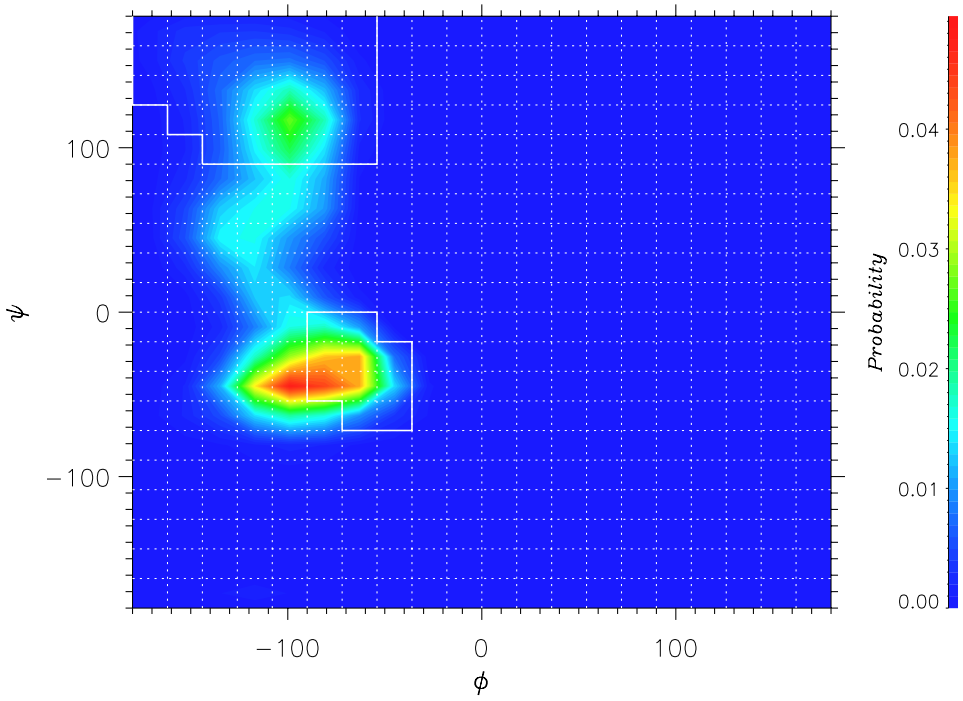
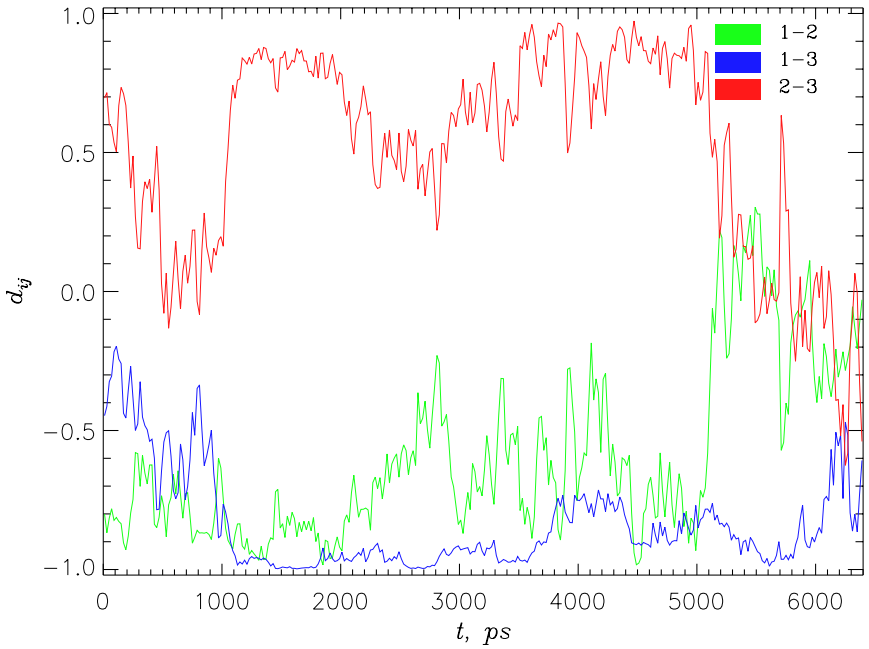


Fig. (3)

(a)



(b)

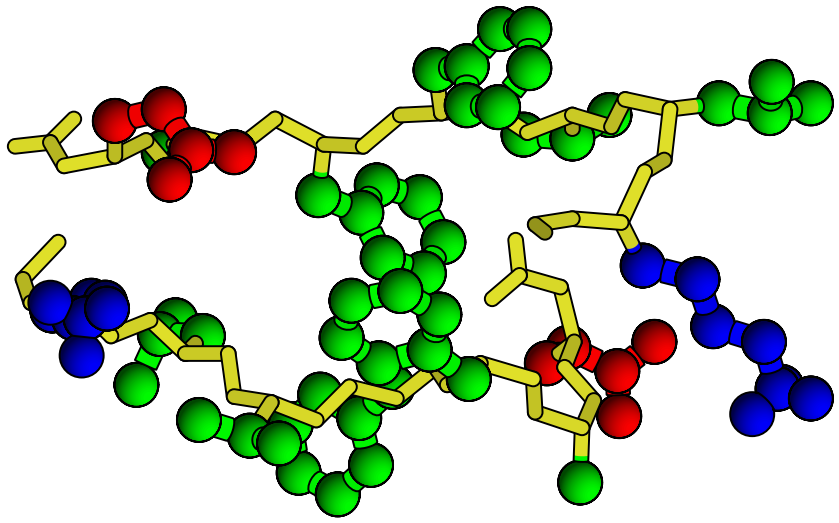


Fig. (4)

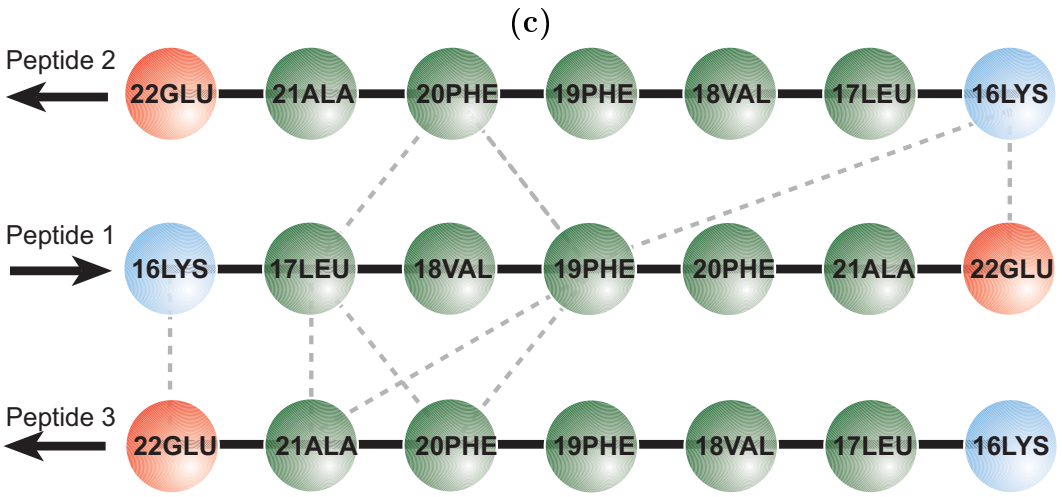

(c)
Peptide 2
22GLU
21ALA
20PHE
19PHE
18VAL
17LEU
16LYS
Peptide 1
16LYS
17LEU
18VAL
19PHE
20PHE
21ALA
22GLU
Peptide 3
22GLU
21ALA
20PHE
19PHE
18VAL
17LEU
16LYS

Fig. (4)

Disordered Aβ_{16-22} oligomers **Ordered Aβ_{16-22} oligomers**

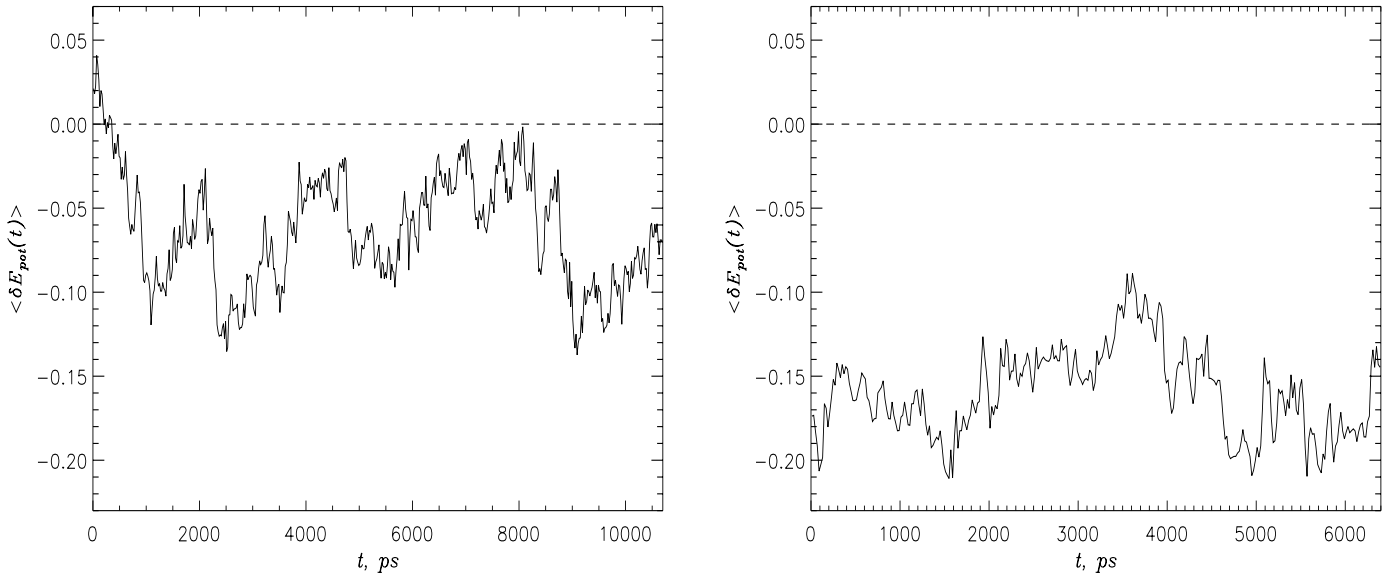
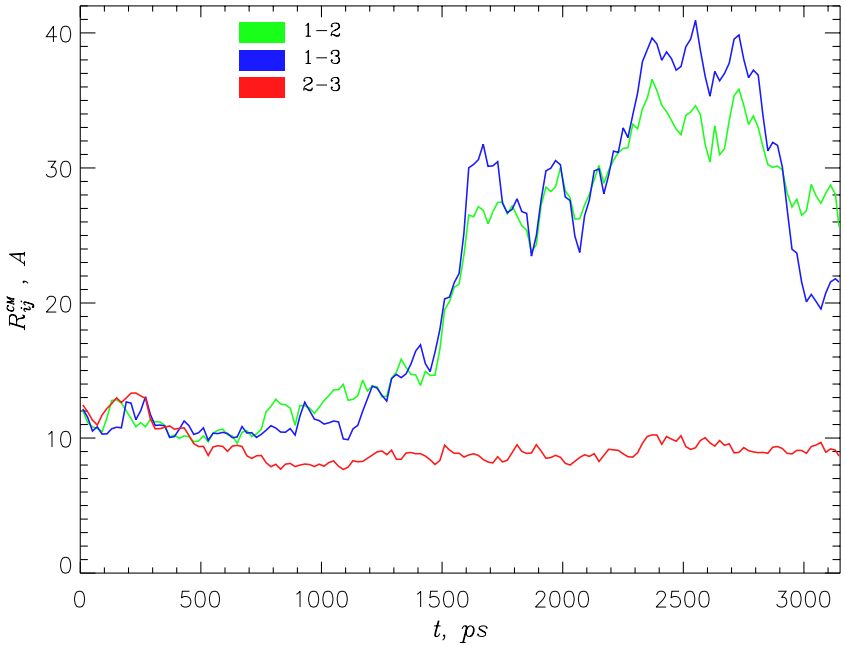


Fig. (5)

(a)



(b)

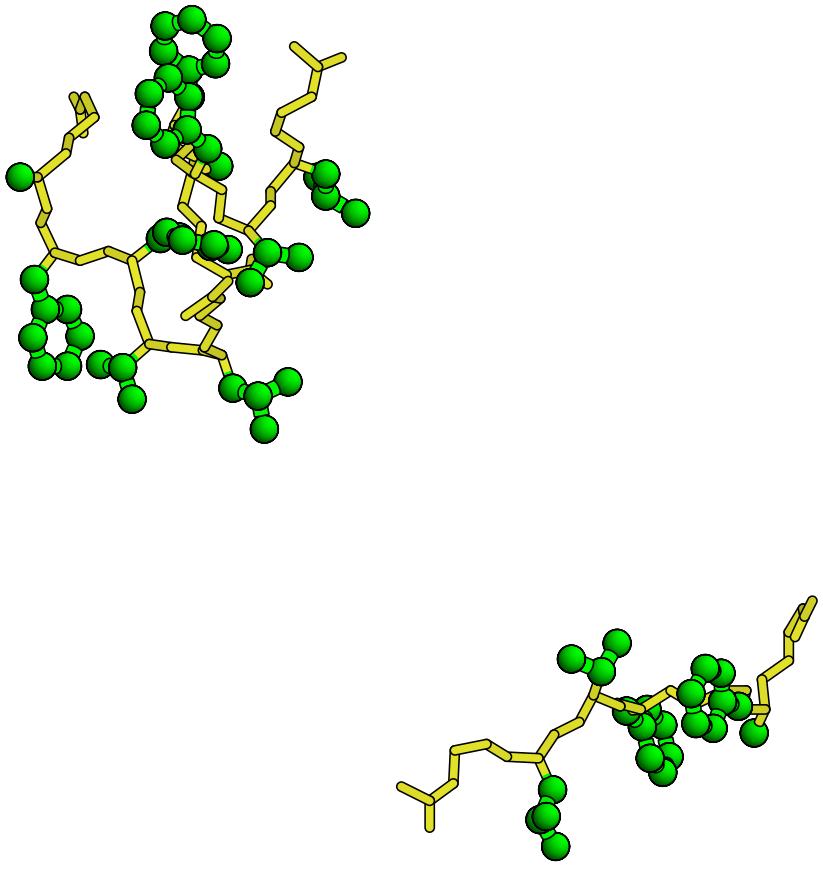


Fig. (6)

(c)

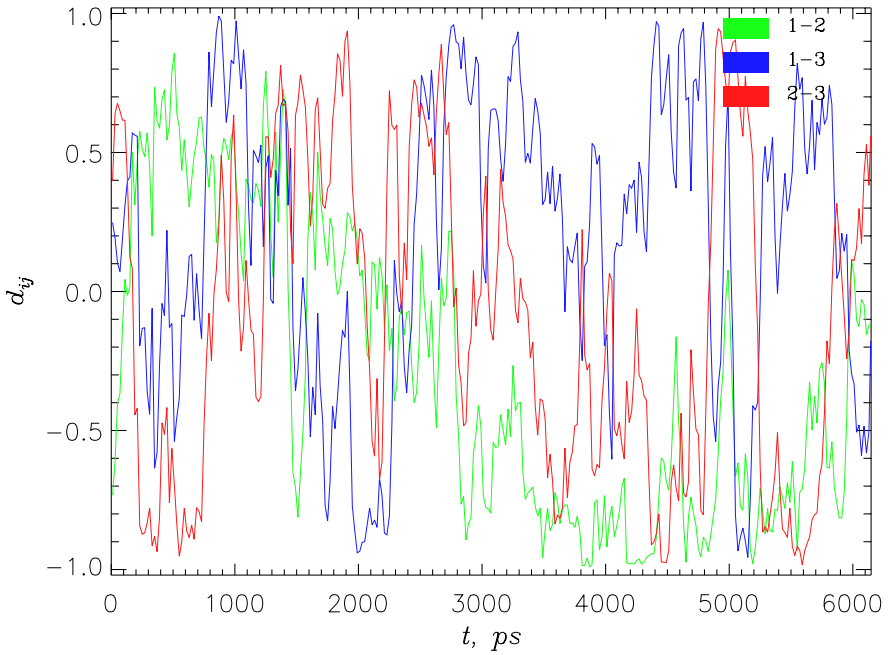
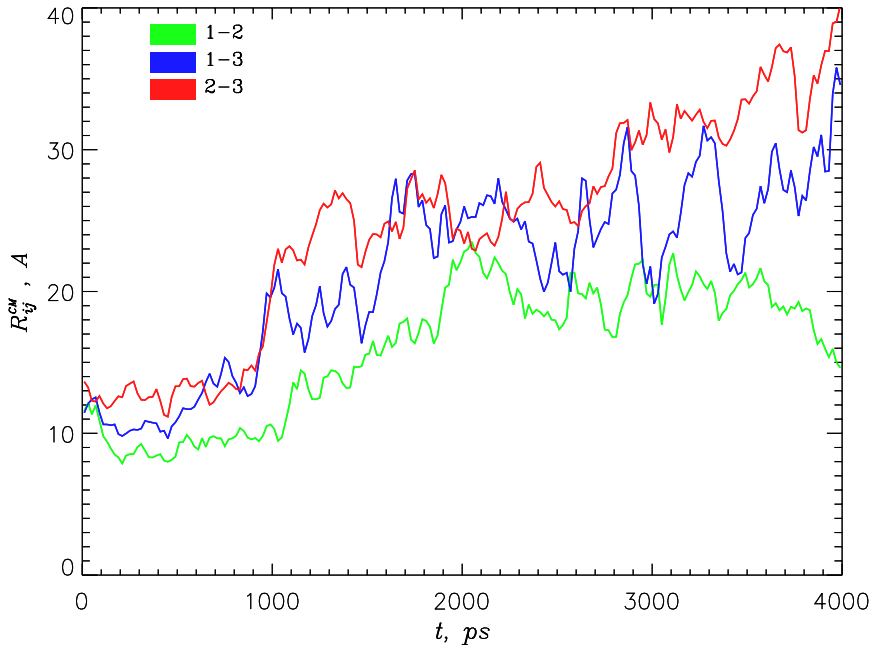


Fig. (6)

(a)



(b)

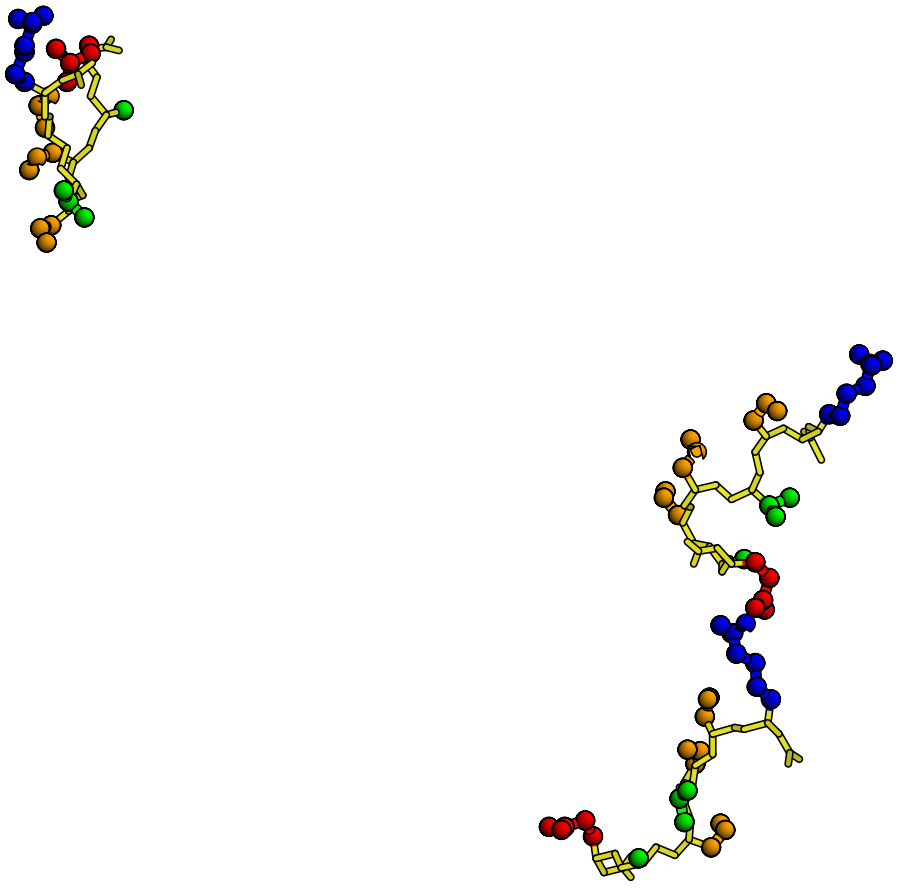


Fig. (7)

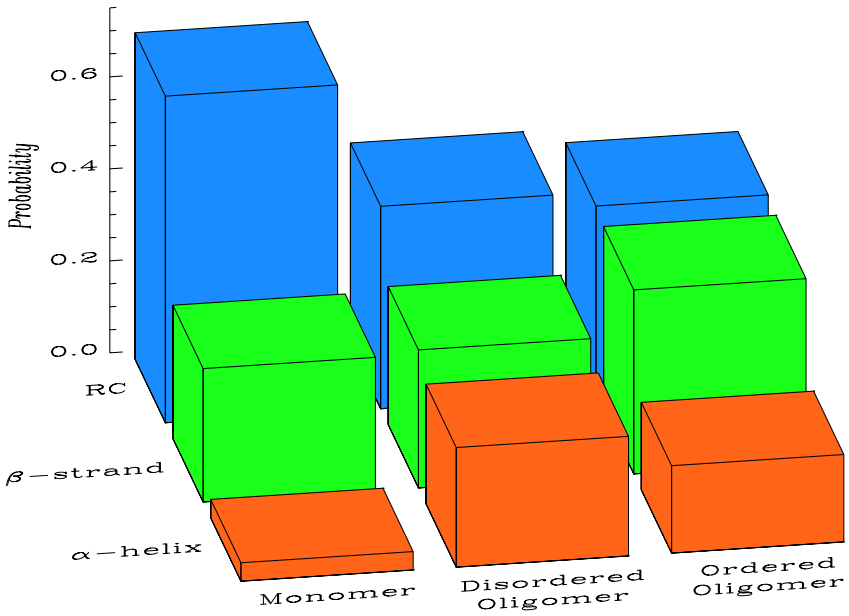

Probability
0.6
0.4
0.2
0.0
RC
β−strand
α−helix
Monomer
Disordered
Oligomer
Ordered
Oligomer

Fig. (8)