

Amyloid β -protein monomer structure: a computational and experimental study.

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Abstract

The structural properties of the A β 42 peptide, a main constituent of the amyloid plaques formed in Alzheimer's disease, were investigated through a combination of ion-mobility mass spectrometry and theoretical modeling. Replica exchange molecular dynamics simulations using a fully atomic description of the peptide and implicit water solvent were performed on the -3 charge state of the peptide, its preferred state under experimental conditions. Equilibrated structures at 300K were clustered into three distinct families with similar structural features within a family and with significant root mean square deviations between families. An analysis of secondary structure indicates the A β 42 peptide conformations are dominated by loops and turns but show some helical structure in the C-terminal hydrophobic tail. A second calculation on A β 42 in a solvent-free environment yields compact structures turned "inside out" from the solution structures (hydrophobic parts on the outside, polar parts on the inside). Ion mobility experiments on the A β 42 -3 charge state electrosprayed from solution yield a bimodal arrival time distribution. This distribution can be quantitatively fit using cross-sections from dehydrated forms of the three families of calculated solution structures and the calculated solvent-free family of structures. Implications of the calculations on the early stages of aggregation of A β 42 are discussed.

Introduction

Alzheimer's disease (AD) belongs to a family of neurodegenerative disorders associated with pathological folding and aggregation of peptides and proteins in the brain. A common feature of these diseases is the formation of amyloid fibrils and plaques. The presence of these plaques among AD patients and an understanding of their genetic origin lead to the amyloid hypothesis to explain the progress of the disease (1). This hypothesis posits that amyloid plaques are central to onset and development of AD. The pros and cons of this hypothesis have recently been reviewed (2).

In recent years, it has become apparent that neither the plaques nor the monomeric protein units are the main pathogenic agents in AD (3). What appears to be primarily responsible are small, soluble oligomers termed paranuclei (5 or 6 monomer units) or ADDLs, and protofibrils (4-9). Hence, the research focus on AD has shifted from characterizing the amyloid fibrils to understanding the detailed role, structure, and mechanism of formation of these species (10, 11).

The monomer unit in AD is termed the amyloid β -protein ($A\beta$). $A\beta$ is formed from the large transmembrane amyloid precursor protein (APP) by cleavage by β - and γ -secretase. While a variety of $A\beta$ peptides are produced, the two dominant forms are $A\beta_{40}$ and $A\beta_{42}$, composed of 40 and 42 amino acid residues, respectively. The average molar ratio of $A\beta_{40}$: $A\beta_{42}$ *in vivo* is ~ 10 , but $A\beta_{42}$ is the dominant form found in amyloid plaques and is the more neurotoxic of the two species (3, 5, 12).

While the nature of the amyloid fibrils has been successfully probed using a number of experimental techniques (13-20), the structural characterization of the A β monomer remains difficult due to its tendency to aggregate. A limited number of NMR studies have been reported on fragments of the A β monomer (21-24) as well as on full-length peptides in which the Met35 residue was oxidized (25, 26). Only a single NMR study has been performed to date on unmodified A β 40 and A β 42 monomers in water (26). This study produced important information about regions of localized stability of the peptides, but due to lack of long-range constraints failed to provide a complete atomic characterization of the peptide. In order to understand the aggregation process and why different fragments and mutants aggregate at different rates and apparently by different pathways, it is crucial that monomer structures be obtained. Consequently, the primary goal of the work reported here is to obtain a global tertiary family of structures of wild type A β 42 using a combination of ion mobility methods and molecular modeling. Ion mobility provides cross-section data that, it will be argued, correlate with both solution and solvent-free A β 42 conformations. These cross-sections provide an important benchmark against which the modeling can be assessed. The modeling, on the other hand, provides detailed structural information and information on how environment affects that structure. We focus here on the modeling. A detailed treatment of the experimental portion of the work is currently in press (27).

Methods

Experiment

The ion mobility measurements (28, 29) were performed on a homebuilt instrument that has been previously described (30). Ions are formed by nanoelectrospray (nano-ESI) and injected through a small capillary into an ion funnel. The funnel transmits the ions to the entrance of the mobility cell where it stores them. Every 10^{-3} seconds a pulse of stored ions is injected into the mobility cell filled to 5 Torr with helium gas. The ion pulse drifts through the gas under the influence of a weak electric field, exits the cell, passes through a quadrupole mass filter and is detected as a function of time. The arrival time distribution (ATD) contains information regarding the shapes of the ions and whether more than one conformer is present in the mass-selected ion beam. This information can be quantified in the following manner. The velocity, v_D , of an ion drifting through a gas under the influence of a weak electric field E , is proportional to that field;

$$v_D = KE \quad (1)$$

where the proportionality constant K is termed the ion mobility. By making measurements at a variety of electric field strengths, very accurate values of K can be obtained (31). Kinetic theory relates K to the ion-He collision cross-section σ , which in turn can be related to the time the ion packet arrives at the detector, t_A :

$$\sigma = 1.3 \left(\frac{q^2 E^2 T}{\mu k_B p^2 N^2 \ell^2} \right)^{1/2} (t_A - t_0) \quad (2)$$

where q is the ion charge, T the temperature in Kelvin, p the pressure in Torr, μ the reduced mass of the ion-He collision partners, N the He gas density at 273 K and 760 Torr, k_B the Boltzmann constant, ℓ the cell length and t_0 the time the ion spent exiting the

mobility cell until reaching the detector. All of the parameters on the right-hand side of equation 2 are known or can be measured accurately, therefore a precise value of σ can be obtained. When a mass spectrum is desired, the ion funnel is no longer used as storage device and the ions pass continuously through the cell to the detector.

Wild type A β 42 was synthesized by Fmoc (N-(9-fluorenyl) methoxycarbonyl) chemistry, purified by reversed-phase HPLC and characterized by mass spectrometry and amino acid analysis (32). Samples for nano-ESI were prepared by dissolving previously quantified lyophilized peptides in H₂O to produce a concentration of 4mg/mL. To this solution, 0.006 volume of 1 N NaOH was added followed by 1 volume of 20 mM ammonium acetate, pH 7.4, reducing the peptide concentration to 2mg/mL. The solution was sonicated for 1 minute and then 100 μ L were transferred to a filter (Gel Filtration G-10 Macro Spin Column from Nest Group Inc.), previously hydrated and washed with 10 mM ammonium acetate, pH 7.4, and spun for 5 minutes at 2,000g. The filtrate was collected and used immediately. The final peptide concentrations of collected filtrate ranged from 30-40 μ M.

Modeling

At pH7, the wild type A β 42 peptide (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA⁴²) contains six negatively-charged side chains and three positively-charged side chains, producing a charge on the molecule of -3. This is the species modeled here. Modeling was done using the CHARMM all-atom force field (33) with the covalent bonds involving hydrogen kept constant using the SHAKE algorithm (34).

The dynamics were propagated with a time step of 2 fs. The solvent effects were modeled through the GB/SA solvation scheme (35). The simulations were organized according to the replica exchange (REX) protocol (36-39). The protocol considered 20 identical replicas of the peptide, simulated at temperatures spaced exponentially between 250 K and 650 K. Every 1000 simulation time steps, attempts to exchange replicas with adjacent temperatures were made. This allowed for better thermalization of the simulated system compared to conventional constant temperature simulations which are prone to getting trapped in potential energy minima. A total number of 10,000 replica exchanges were attempted. This resulted in a 20 ns simulation time per replica, or 400 ns total simulation time. The simulations were carried out on an AMD Athlon dual-CPU computational cluster with the clock frequency 1.6 GHz. In total, the simulations took more than two months to complete. As the output of the simulations, 355 peptide conformations were collected over the final 10 ns of the trajectory at regular time intervals. To check the statistical uncertainty of the selected conformation, a second set of conformations was chosen at different intervals. The conclusions drawn from the analysis of this second conformational ensemble did not differ from those we reached by using the first ensemble.

Results

A typical nano-ESI mass spectrum is shown in Figure 1. The -3 charge state is largest as expected from the -3 charge on the peptide in solution. Also evident are a -4 charge state, a very small -2 charge state and a feature that appears to have a charge of -5/2. This latter

peak is clearly a dimer or higher-order aggregate and is discussed in detail elsewhere (27). The high-resolution spectrum of the -3 charge state (Figure 1) reveals a series of peaks separated by 0.33 amu, as expected for ^{13}C isotopes of a monomer species with 3 charges. The distribution of the isotope pattern is fit exactly by the distribution expected for the A β 42 peptide. A similar distribution of peaks separated by 0.25 amu is obtained for the -4 charge state (data not shown). Hence, both the -3 and -4 species are dominantly monomers. However because baseline resolution was not obtained in the isotope pattern, some -6 dimer could contribute to the peak labeled -3 in Figure 1.

Also shown in Figure 1 is the ATD for the -3 charge state. Since the isotope pattern indicates this peak is primarily composed of monomer, the two dominant features in the ATD must correspond to two distinct, non-interconverting monomer families of conformers on the millisecond timescale. Average cross-sections for these two features were obtained in the usual manner (30) yielding $635 \text{ \AA}^2$ for the earlier peak and $700 \text{ \AA}^2$ for the later peak. The ATD expected for a system with two species of $635 \text{ \AA}^2$ and $700 \text{ \AA}^2$ should show two relatively well-resolved peaks narrower than those seen experimentally, indicating that the ATD contains more than two features. The fact the cross-sections of the two main features vary by 10% and that they don't interconvert on the millisecond time scale of the experiment indicates the two conformers differ significantly in structure. It should also be mentioned that a detailed analysis of this ATD, and one from the [Pro¹⁹]A β 42 alloform, indicate that a small amount of oligomer contributes to a third feature at even shorter time (27). This point, while a minor complicating factor, does not impact the structural determination made here.

Two independent replica exchange molecular dynamics calculations of A β 42 were carried out – one in implicit water and one solvent free. To compare with experiment, the implicit water structures were instantaneously dehydrated through energy-minimization in vacuum. The cross-sections of the hydrated, dehydrated, and solvent-free results are shown as panels (a), (b) and (c) in Figure 2. The hydrated peptide(s) exhibit the largest cross sections because water solvation extends the peptide by replacing intramolecular charge solvation and hydrogen bonding with interactions with water. The radii of gyration for the solvated peptides range from 11 Å to 15 Å, indicating that the peptide exists as a mixture of larger coils and smaller globular conformations. The average cross section for this distribution of peptides, 858 Å², is larger than the experimentally-determined cross-sections. However, simply dehydrating and energy-minimizing A β 42 reduces its average size to 765 Å² bringing the calculated results into the range of experimentally observed cross sections. Finally, the calculation in a solvent-free environment yields structures that are notably more compact (679 Å²) than the dehydrated and solvated conformations, with radii of gyration not exceeding 10 Å.

A comparison of the theoretical and experimental results suggests that the two families of structures seen experimentally (Figure 2d) correspond to the families of solvent-free (Figure 2c) and dehydrated (Figure 2b) structures found theoretically. Representative conformers of solvent-free and dehydrated structures are shown in Figure 3. The solvent-free conformations are characterized by a quasi-spherical shape with the hydrophilic residues clustered in the center and the hydrophobic residues exposed. Structures

obtained by dehydrating solution-phase conformations are organized in the opposite manner, with hydrophilic residues on the surface and hydrophobic residues in the center. Conversion between the two families would involve “flipping the structure inside out,” a highly unfavored process energetically. While both the solvent-free and dehydrated model peptide cross-sections are slightly larger than experiment ($\sim 7\%$), the difference between these two cross-sections agrees well with the experimentally observed difference. The small differences in the experimental and model absolute cross-sections may be due to an insufficient number of exchanges in the modeling, inaccuracies in the minimized structures formed by “instantaneous” dehydration of the hydrated model peptides, or difficulties in converting model structures into cross sections in the A β 42 size range.

The agreement between experiment and theory suggests that the modeling methods employed yield biophysically meaningful results and therefore that further analysis of the solution theoretical results would produce new insight into A β folding. However, with 355 structures, there are too much data to analyze on a case-by-case basis. Therefore to see if there are clusters of conformations with structural similarities, a principal coordinate (PC) analysis was run (40). This analysis clusters like structures according to their pair-wise root mean square (RMS) atomic deviations. Principal coordinates are then sought through the matrix of data that best clusters the data. A two-dimensional plot of the first two of these coordinates is given in Figure 4. This analysis suggests there are three families of conformers within the 355 structures in our sample. Family 3 is the most compact (i.e., structurally similar) while families 1 and 2 are more diverse. Similar

cross-sections are found within a family and they differ significantly between families. Family 1 (206 structures) has the largest number of members and displays an average cross section of $769 \text{ \AA}^2$ after dehydration. Families 2 and 3 have average dehydrated cross-sections of $718 \text{ \AA}^2$ and $814 \text{ \AA}^2$, respectively. Figure 5 shows representative solvated conformations belonging to each family and a breakdown of secondary structure, as computed from the STRIDE algorithm (41). Clusters #1 and #3 are most homogeneous with respect to the secondary structure of their constituents. Both clusters are composed mostly of loops and turns in the N-terminal part of the sequence. They differ in the hydrophobic C-terminal region in which cluster #1 exhibits a greater propensity for helix formation and cluster #3 is more prone to forming β -strands. In contrast, cluster #2 contains conformations in which both helices and β -strands appear in the C-terminal region. The amount of ordered α - or β -structure observed in the conformations from all clusters is small. At most, the ordered residues constitute 10-20% of the total sequence for any conformation.

Is the existence of three distinctly different families of solution structures consistent with our experimental data? Figure 2e shows the ATD expected for a mixture of solvent-free and dehydrated A β 42 molecules. The percentages of solvent-free and dehydrated structures, 31% and 69%, respectively, is chosen to best match experiment. The population of families #1, #2, and #3 within the dehydrated structures is assumed to be 58%, 23%, and 19%, reflecting the percentages of the three families of structures obtained in the calculation. In constructing the model ATD, we assumed that there was rapid interconversion between structures within a given family but no interconversion

between families on the timescale of the experiment (~1 millisecond). Kinetic theory (31) was used to obtain the model line shapes. The shape of the theoretical ATD agrees remarkably well with experiment (Figure 2d). The presence of the smaller cluster families 2 and 3 provides a reasonable explanation for the broadening of the two main peaks observed in the experiment.

Discussion and Conclusions

By drawing on the complementary strengths of ion-mobility mass spectrometry and theoretical modeling, we were able to shed new light on the conformational states of the monomeric A β 42 peptide, the building block of amyloid fibers. Our simulations reveal that A β 42 in aqueous solution adopts both extended chain as well as collapsed-coil structures. A β 42 does not display a unique fold, as would be the case for a typical protein, but rather comprises a mixture of rapidly interconverting conformations. The monomeric conformations could be separated into three distinct families whose members displayed mostly coil-like structure, with few helical or strand elements present. Results of our simulations are consistent with solution NMR experiments (based on NOE constraints, chemical shift indices, and *J*-scalar couplings) and circular dichroism (CD) spectroscopy. The absence of regular α - or β -structure has been demonstrated in NMR studies of A β 10-35 (22, 23), A β 40, A β 42 (26, 42) and oxidized alloforms A β 40 and A β 42 (25, 26). The percentage of ordered residues in soluble A β 42 found in our simulations agrees reasonably well with percentages determined by NMR and CD (43-46) that estimate the amount of unordered structure to be 60-80%, of α -helices to be <

10% and of β -sheets to be ~ 10 -20%. CD measurements are expected to yield higher percentages of ordered secondary structure elements as they provide an average picture taken over all monomers and oligomers (dimers, trimers, etc.) that exist in dynamic equilibrium *in vitro*, while here only the secondary structure of monomers is assessed. Oligomers are likely to contain elements of secondary structure not present in the monomeric form. The paucity of regular secondary structure elements in the monomeric peptide in solution contrasts with its mostly helical form in a membrane-like environment (47) and its β -sheet structure when incorporated in the amyloid fibril (13).

Previous solution NMR studies (22, 23, 25, 26) indicated that A β can possess localized regions of well-defined structure. In particular, the central hydrophobic residues Leu17-Ala21 (Leu-Val-Phe-Phe-Ala) (23) of the A β 10-35 peptide were found to be well-structured. This region, known as the central hydrophobic cluster (CHC), has been shown to be critical for fibril formation (48, 49). The CHC also was found to be well-structured in A β 40^{ox} and A β 42^{ox} (25, 26), peptides in which Met35 is oxidized, as well as in the A β 40 and A β 42 peptides (26). Molecular dynamics simulations by Straub and co-workers (50) on the A β 10-35 fragment indicate that the CHC residues underwent considerably smaller structural fluctuations than the rest of the peptide during a one ns run. In the present study, all conformations corresponding to the clustered Family 3 (Figure 5c) display a structured CHC, with an rmsd of < 0.6 Å from the experimental structure obtained on the A β 10-35 fragment (23). Conformations from Families 1 and 2 show less well-structured CHCs. However, Family 3 comprises only 15% of our cohort

of structures, indicating the full-length peptide exhibits less structural stability in the CHC than the 10-35 fragment.

It is interesting to consider how the structure of the A β 42 monomers determines possible pathways for its self-assembly. It has been shown experimentally that the first stage of self-assembly, oligomerization, may involve the transient appearance of α -helices on route to fibril formation. For example, Kirkitadze *et al.* (46) reported a transitory increase in α -helical content during fibrillogenesis of A β 42, as well as A β 40 and all clinically relevant A β alloforms. CD measurements were used to show that the relative amount of α -helices in water solutions of A β 42 increased from 3% to about 20% after 4 days of incubation, coincident with peptide oligomerization. Because fibrils are rich in β -strands, and the C-terminal part of the A β is embedded in cell membrane in an α -helical form an $\alpha \rightarrow \beta$ structural transition must occur in the process of A β secretion and amyloid assembly. Our simulations reveal modest, but statistically significant, amounts of helical structure in the hydrophobic C-terminal region of the peptide, a region with high propensity for forming helices in apolar media. We propose that the experimental increase in α -structure reported at intermediate observation times during fibrillogenesis results from association of unstructured A β monomers into oligomers in such a way that the hydrophobic tails of the peptides become shielded from the solvent by the rest of the peptides. This creates a local apolar micro-environment which promotes growth of helical structure from the pre-existing seeds. This hypothesis, shown schematically in Figure 6,

is currently being tested through simulations and ion mobility mass spectrometry studies on higher oligomers.

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Figure Legends:

Figure 1: Negative ion ESI mass spectrum of A β 42 solution showing charge states -2 through -4. The peak labeled -5/2 has an m/z-value formally corresponding to a dimer of charge -5. The expanded view on the left shows an expanded mass scale for the -3 peak. The expanded view on the right shows an ATD of the -3 species.

Figure 2: Scatter plot of radius of gyration (R_g) versus cross section (σ) of (a) hydrated, (b) dehydrated, and (c) solvent-free theoretical A β 42 structures. (d) Experimental ATD of A β 42 charge state -3. (e) Theoretical ATD of solvent-free (sf) and dehydrated A β 42 structures consisting of families #1, #2, and #3.

Figure 3: Typical A β 42 structures obtained by simulations (a) in a solvent-free environment and (b) in implicit water with subsequent dehydration (minimization in solvent-free environment). Hydrophobic residues are shown in white.

Figure 4: Result of principal coordinate analysis displaying best clustering of the three families of hydrated A β 42 structures. Each family is separated from another family by an rmsd of ~ 10 Å.

Figure 5: Typical representatives of (a) family #1, (b) family #2, and (c) family #3 of solvated A β 42 structures. Hydrophobic residues are shown in white. Helical sections are indicated by fat cylinders. (d) Secondary structure analysis of all structures of families

#1, #2, and #3 using the STRIDE algorithm. (We note that in the CHARMM 22 force field, π - and α -helices have comparable energies).

Figure 6: Schematic representation of A β 42 conformations in the early stages of aggregation. Monomers without helical structure are in equilibrium with monomers containing small amounts of helical structure at the C-terminus. Five or six monomers assemble to form a paranucleus (43) where the hydrophobic C-termini are shielded by the more hydrophilic parts of the peptides. Hydrophobic residues are marked in black.

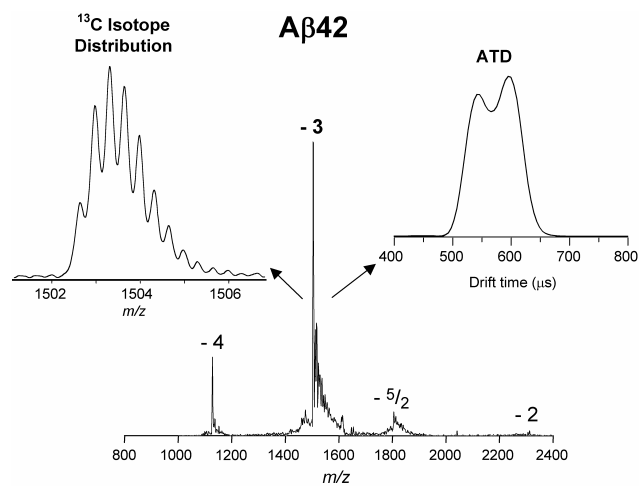


Figure 1

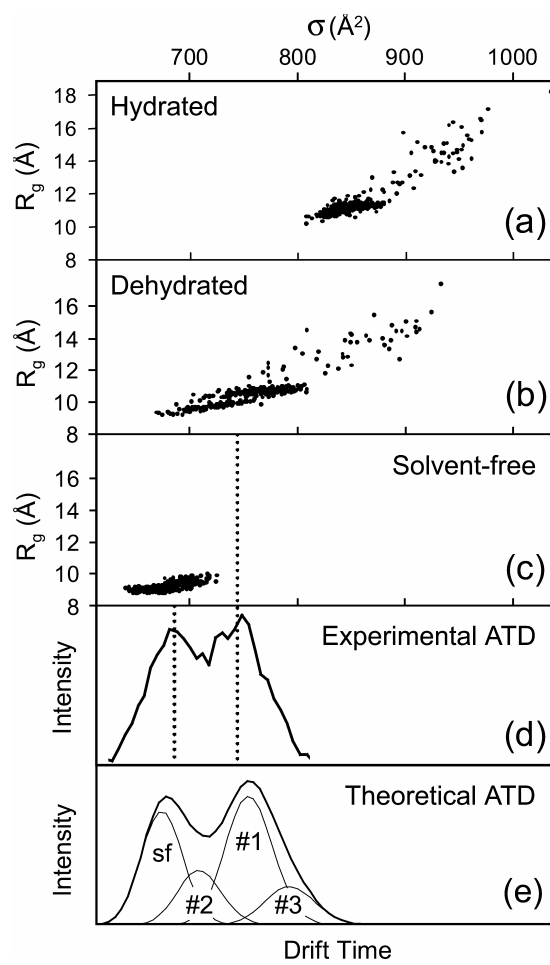


Figure 2

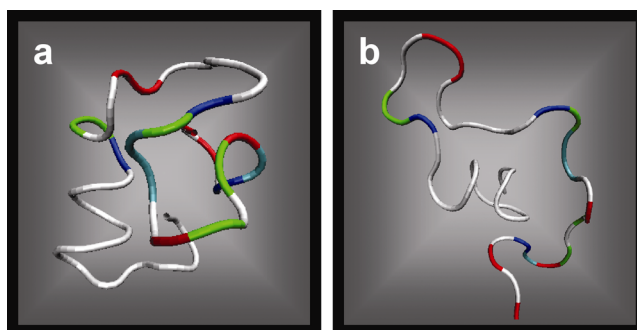


Figure 3

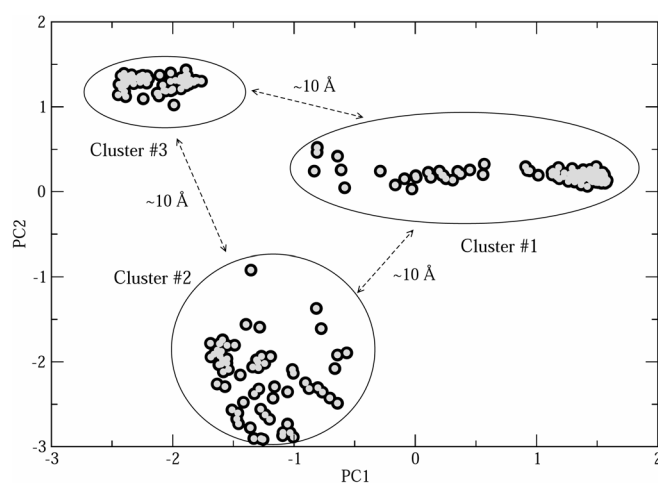


Figure 4

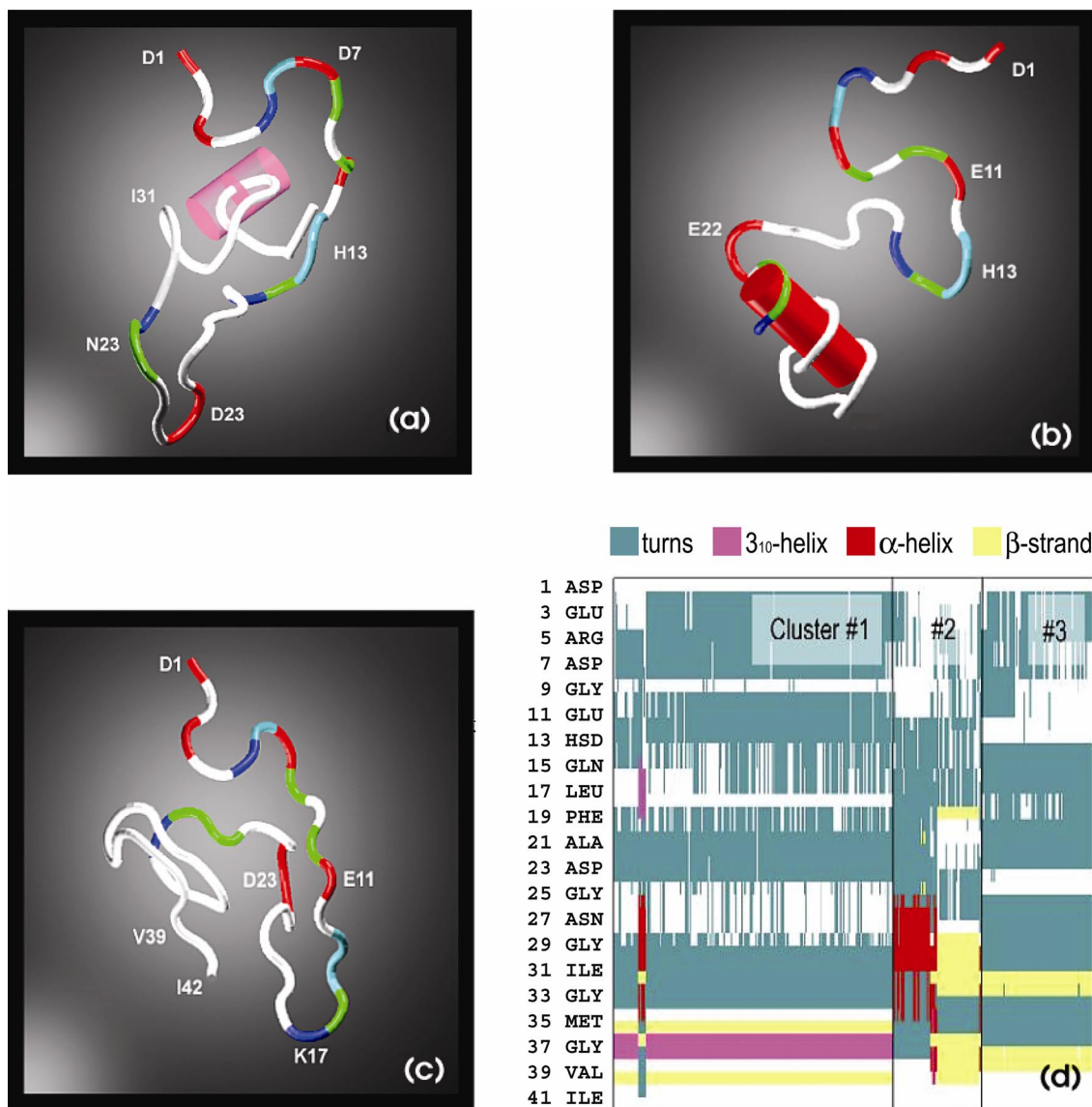


Figure 5

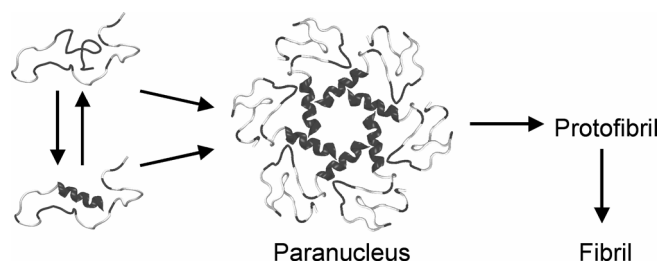


Figure 6