Development of novel small-size peptides as putative therapeutic drugs
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**In silico study of full length amyloid β 1-42**

Tri- & penta-oligomers in solution


**Abstract**

Amyloid oligomers are considered to play causal roles in the pathogenesis of amyloid-related degenerative diseases including Alzheimer’s disease. Using MD simulations techniques, we explored the contributions of the different structural elements of trimeric and pentameric full-length Aβ42 aggregates in solution to their stability and conformational dynamics. We found that our models are stable at the temperature of 310 K, and converge toward an interdigitated side-chain packing for intermolecular contacts within the two β-sheet regions of the aggregates: β1 (residues 18-26) and β2 (residues 31-42). MD simulations reveal that the β-strand twist is a characteristic element of Aβ aggregates, permitting a compact, interdigitated packing of side chains from neighboring β-sheets. The β2 portion formed a tightly organised β-helix, whereas the β1 portion did not show such a firm structural organization although it maintained its β-sheet conformation. Our simulations indicate that the hydrophobic core comprising the β2 portion of the aggregate is a crucial stabilizing element in the Aβ aggregation process. Based on these structure-stability findings the β2 portion emerges as an optimal target for further anti-amyloid drug-design.
Proteins can adopt an amazing array of sequence-dependent structures that enable them to perform the myriad of chemical functions critical to life. However, over the past decade, it has become clear that many different proteins can also form misfolded structures leading to insoluble aggregates. With respect to human brain function, protein misfolding can have dramatic consequences and can result in devastating diseases of the brain. There are several known degenerative diseases whose pathogenic mechanism is based on the pathological aggregation of polypeptides. These types of neurodegenerative diseases include Alzheimer’s disease (AD), Parkinson’s disease, Huntington’s disease, prion diseases like Creutzfeldt-Jacob disease, and type II diabetes (Lynn and Meredith 2000; Roclet and Lansbury 2000; Selkoe 2003). It is a common feature to all of the above mentioned diseases that the native structure of the specific peptides has been changed and that during a fast aggregation process, they provide fibrillary products which are toxic to nerve cells or nervous tissue and resistant against enzymatic breakdown. The most extensively studied amyloid-associated disease is AD, which is characterized pathologically by abnormally high numbers of amyloid- or senile plaques in the cerebral cortex and amyloid deposits in the walls of cerebral blood vessels, and by neurofibrillary tangles in dead and dying neurons (Holtzman and Mobley 1991). Amyloid plaques are brain lesions that are composed of amyloid polymers and degenerating neurites accompanied by activated microglia, the inflammatory cells of the brain. The major component of amyloid plaques is a small peptide of 39 to 43 amino acids in length called β-amyloid (Aβ), which is a proteolytic splicing product of the large amyloid precursor protein (APP). Compelling evidence now indicates that factors which increase the production of amyloidogenic variants of Aβ, or which facilitate deposition or inhibit elimination of amyloid deposits, are major risk factors for AD (Selkoe 1999).

Currently, more than 20 different forms of Aβ are known (Westermark 1997), some of which are rare and some of which play central roles in the pathogenesis of diseases affecting millions of patients worldwide. The investigation of amyloid, as a major example of pathology due to protein misfolding, has become a widely studied enterprise (Kisilevsky 2000; Lesne et al. 2006; Sato et al. 2006; Soto et al. 2007; Westermark 2005; Zheng et al. 2007). Consequently, the 3D structure of the amyloid polymers and fibrils is a focus of interest both for a molecular understanding of amyloidogenesis and for the development of innovative therapeutic and diagnostic approaches. Solid-state NMR studies have contributed substantially to the understanding of amyloid fibrils (Ritter et al. 2005; Tycko 2004). However, only a few 3D structures of amyloid fibrils have yet been determined (Luhrs et al. 2005; Nelson et al. 2005), owing in part to the fibrils’ noncrystalline, insoluble, and mesoscopically heterogeneous nature. This makes these structures difficult to access by established structural techniques such as X-ray crystallography or solution-state NMR (Thompson 2003). Since the 1970’s, amyloid has been defined by its main β-sheet fibrillar structure (Eanes and Glenner 1968; Glenner 1980a, b), and by properties associated with this. The unifying structure is an assembled protein fibril in which the sheets are parallel to the fibril direction and where the strands run perpendicular to the fibril. This principal organization is believed to be common to all amyloid fibrils, irrespective of the biochemical nature (Westermark 2005).

The amyloid beta 1-42 peptide (Aβ42) fragment is the dominant Aβ species in the amyloid plaques of AD patients, and compared with Aβ40 it displays a dramatically increased propensity to form amyloid fibrils in vitro (Burdick et al. 1992; Jarrett et al. 1993a, b; Riek et al. 2001). Furthermore, a comparison of the kinetic data
of Aβ_{40} and Aβ_{42} shows that Aβ_{40} adopts more varied conformational structures compared to Aβ_{42}, as seen by the fluctuations of the Tyr signal displayed by Aβ_{40} (Vestergaard et al. 2005). A comparative experimental-theoretical conformational study has been carried out by Sgourakis et al. (Sgourakis et al. 2007), which sheds light on the differential conformational behavior of the monomers in aqueous solution of Aβ_{40} and Aβ_{42}. The presence of metal ions, in particular copper, zinc, and iron, has been reported to enhance Aβ aggregation (Atwood et al. 1999; Atwood et al. 2004; Huang et al. 2004). Several important structural characteristics of Aβ_{42} fibrils have been determined (Luhrs et al. 2005; Nelson et al. 2005; Ritter et al. 2005; Thompson 2003; Tycko 2004), establishing that Aβ_{42} fibrils form a cross-β structure (Kirschner et al. 1986) that contains parallel, in-register β-sheets (Balbach et al. 2002). Most recently it was found that memory deficits in middle-age transgenic AD model mice are caused by the extracellular accumulation of a dodecameric Aβ_{42} soluble assembly (Lesne et al. 2006). While the major toxic species in AD are probably Aβ-oligomers, thus being a principal target of AD drug development, it remains unclear why Aβ-oligomers should be neurotoxic. Plausible mechanisms of toxicity include links to oxidative stress, metal binding, free radical formation (Chauhan and Chauhan 2006) or ion channel formation (Quist et al. 2005), and therefore it may be possible to interact with these processes by applying reducing agents, metal chelators (Bush 2002) or ion channel inhibitors. In general terms an ideal approach would be to interfere with the early phases of molecular pathways that lead to the disease. Consequently, one attractive therapeutic strategy for the treatment of AD is to inhibit Aβ-peptide aggregation since this appears to be one of the first steps in the pathogenic process of amyloidosis that is not associated with some natural biological function (Lansbury 1997). Breaking these amyloid aggregates down by non-peptide or peptide inhibitors seems to be a promising way to combat AD (Doig 2007). To that aim, it is crucial to understand the conformational and dynamic behavior of the structure of the Aβ_{42} aggregates in order to rationally design a putative AD drug. Moreover, the conformational analysis and the investigation of the structure of Aβ_{42} aggregates might lead to a more profound understanding of the primary pathogenesis of AD and comprehend the development at the molecular level of this dramatic disease.

Most computational investigations of the structure and dynamics of Aβ-peptides have focused on monomers (Baumketner et al. 2006; Buchete et al. 2005; Gordon and Meredith 2003; Lazo et al. 2005; Massi et al. 2001; Petkova et al. 2005; Petkova et al. 2006; Sato et al. 2006; Sciarretta et al. 2006a; Sgourakis et al. 2007; Wei and Shea 2006), dimers (Huet and Derreumaux 2006; Tarus et al. 2005; Urbanc et al. 2004), and other low-order oligomers (Buchete and Hummer 2007; Gnanakaran et al. 2006; Hwang et al. 2004; Klimov and Thirumalai 2003; Ma and Nussinov 2006; Rohrig et al. 2006; Zheng et al. 2007). The idea of considering a full-length molecular model of the Aβ aggregates is worthy of consideration, since the majority of the studies mentioned above have employed models using a truncated-sequence molecular model and/or insoluble fibril state. In our present investigation, profiting from the structural information available from recent solid-state nuclear magnetic resonance (ssNMR) experiments (Balbach et al. 2002; Luhrs et al. 2005; Paravaštu et al. 2006; Petkova et al. 2002; Petkova et al. 2005; Petkova et al. 2006) on the parallel cross-β structure of Aβ protofilaments, we perform all-atom/explicit solvent simulations of amyloid aggregates containing three and five peptide units. Every unit with “hairpin” shaped Aβ_{1–42} peptides was located in a plane roughly perpendicular to the fibril axis as it was observed by using ssNMR (Luhrs et al. 2005). Among the different structural topologies possible depending on experimental growth conditions (Paravaštu et al. 2006; Petkova et al. 2005;
Petkova et al. 2006), we focus on Aβ1–42 fibrils grown under the conditions defined as in reference 16. The objectives of our study are twofold: 1) to explore the contributions of the different structural elements to stability of full-length aggregated Aβ42 in solution, and 2) to investigate the most promising, potential structural target for further drug-design based on the structure-stability information of our model. Our reasonably long-time simulations (100 ns) of both tri- and pentameric aggregates at 310 K, in which fibrils are experimentally stable (below ~330 K), permit us to draw conclusions on structurally relevant aggregate characteristics such as the secondary-to-tertiary structural elements (e.g., β-strands, intra- and intermolecular contacts), internal salt-bridges, and the interior hydration. In the present study we analyze the evolution of these elements at 310 K, focusing in particular on the structure and dynamics behavior in solution of the full-length Aβ42 aggregates.

5.2 Methods

5.2.1 System setup

The human amyloid beta 1-42 peptide (Aβ42) (figure 5.1) was taken as a model system. The calculations were carried out using the structure of the Aβ42 fibrils (PDB entry 2BEG) determined by Lührs and coworkers (Lührs et al. 2005) that was downloaded from the Protein Data Bank (Berman et al. 2002). Due to the fact that residues 1-17 are disordered and lacking a unique and stable conformation we only had experimental structural information from residues 18-42 (Lührs et al. 2005). Therefore, in order to have the completed molecule of Aβ42, the sequence for the residues 1-17 was completed using an arbitrary doubly extended orientation ($\phi \approx 180^\circ$ and $\psi \approx 180^\circ$). Both trimeric and pentameric aggregates were investigated. The trimeric system was obtained by deletion of the edging chains of the experimental structure determined by Lührs and coworkers (Lührs et al. 2005), which is a pentameric aggregate. Protons were added using the program pdb2gmx, in the GROMACS suite of programs, for optimization of the hydrogen bond network.

The peptide aggregates were embedded in a rectangular box containing SPC waters (Berendsen et al.), leaving at least 10 Å between the solutes and the edge of the box. The total number of water molecules varied between 15630 and 25919. Na+ ions were added to the systems by replacing water molecules in random positions, thus making the whole system neutral (table 5.1). In order to equilibrate our systems we carried out an extensive simulating annealing (SA) protocol with repeating heating/cooling cycles covering a broad temperature range (from 310 up to 500 K), in which fibrils are experimentally stable.

**Figure 5.1**: Amino acid sequence of human amyloid beta 1-42 peptide (Aβ42) and schematic representation of a molecule of Aβ42 in a hairpin shape. The residues 1-17 comprise the disordered region. The residues 18-42 comprise the β-sheet region.
(below ~330 K) or fully dissociated (above ~373 K) (Meersman and Dobson 2006; Sasahara et al. 2005). During the whole minimization position restraint was applied for the alpha carbons (Ca) of residues 18-42. As a result of the SA runs, the DR residues showed a large range of conformational sampling. Multiple simulations were performed for each system, starting from different initial random velocity distributions, generating five independent strands of simulations (labelled A-E, see table 5.1). Details of the equilibration procedure can be found in the Supp. Information of chapter 5 in appendix A. For each strand between 20-100 ns production runs were obtained and analyzed. The coordinates were saved every 2 ps.

### 5.2.2 Simulation parameters

All simulated annealing (SA) simulations and molecular dynamics (MD) simulations were performed using the GROMACS 3.3.2 package of programs (Van Der Spoel et al. 2005), with the OPLS-AA force field (Kaminski et al. 2001). The simulations were run under NPT conditions, using Berendsen’s coupling algorithm (Berendsen et al. 1984) for keeping the temperature and pressure constant (P = 1 bar, τ_p = 0.5 ps; T = 310 K or 500 K, τ_T = 0.1 ps). The LINCS algorithm (Hess et al. 1997) was used to constrain the lengths of hydrogen containing bonds; the waters were restrained using the SETTLE algorithm (Miyamoto and Kollman 1992). A force constant of 1000 kJ mol⁻¹ nm⁻¹ was used for position restraints during the SA. For the subsequent unrestrained MD simulations, the same parameters were used as for the restrained MD, except that the temperature was maintained at 310 K and no positional restraints were applied. The time step for the simulations was 0.002 ps and the compressibility 4.8x10⁻⁵ bar⁻¹. Van der Waals forces were treated using a 1.2 nm cutoff. Long-range electrostatic forces were treated using the particle mesh Ewald method (PME) (Darden et al. 1993a).

### 5.2.3 Analysis

The analysis of the simulations was performed using the analysis tools provided in the Gromacs package. The root mean square deviation (RMSD) of backbone atoms, the total and potential energies were calculated. The root mean square fluctuation (RMSF) of the backbone atoms and the hydrophilic, hydrophobic and total Solvent Accessible Surface Area (SASA) were also determined. The total number of hydrogen bonds in the peptide group were quantified by counting acceptor and donor atom pairs that are not further apart than 0.35 nm (Beke et al. 2006). Secondary structure analysis used DSSP (Kabsch and Sander 1983). All molecular graphical presentations were created by VMD (Humphrey et al. 1996) and/or UCSF Chimera (Pettersen et al. 2004a) packages. The standard deviation of every given value is shown in between brackets.

### Table 5.1: Selected parameters used in simulations A-E.

<table>
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<tr>
<th>MD</th>
<th>βA</th>
<th>Na⁺</th>
<th>H₂O</th>
<th># Atoms</th>
<th>Time</th>
</tr>
</thead>
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<td>3</td>
<td>9</td>
<td>17284</td>
<td>53742</td>
<td>20</td>
</tr>
<tr>
<td>B3</td>
<td>3</td>
<td>9</td>
<td>17284</td>
<td>53742</td>
<td>20</td>
</tr>
<tr>
<td>C3</td>
<td>3</td>
<td>9</td>
<td>17302</td>
<td>53796</td>
<td>20</td>
</tr>
<tr>
<td>D3</td>
<td>3</td>
<td>9</td>
<td>15630</td>
<td>48780</td>
<td>20</td>
</tr>
<tr>
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<td>3</td>
<td>9</td>
<td>17284</td>
<td>53742</td>
<td>100</td>
</tr>
<tr>
<td>A5</td>
<td>5</td>
<td>15</td>
<td>25919</td>
<td>80907</td>
<td>20</td>
</tr>
<tr>
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<td>21200</td>
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</tr>
<tr>
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<td>15</td>
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</tr>
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<td>15</td>
<td>20832</td>
<td>65646</td>
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<td>15</td>
<td>20009</td>
<td>63177</td>
<td>100</td>
</tr>
</tbody>
</table>
5.3 Results and Discussion

To more clearly present the results obtained with our simulations, we have divided our system into two regions. The first region encompasses the residues 1 to 17, which is very likely to be a region with a large degree of flexibility. We will refer to this region as the “disordered region” (DR). The second region comprises the residues 18 to 42, which is the β-sheet region (βR). The βR presents a β-strand-turn-β-strand motif (hook-like or hairpin shape) that contains two intermolecular, parallel, in-register β-sheets that are formed by residues 18-26 (β₁) and 31-42 (β₂) connected by a turn formed by residues 27-30 (Luhrs et al. 2005) (figure 5.1). Figure 5.2 shows the superposition of five structures collected every 2 ns of SA of the pentameric system, which form the starting structures of the five independent simulations performed (denoted strands A-E). In figure 5.2, a high degree of mobility of the DR is clearly visible. A similar result was observed for the trimeric system. Using the final structures obtained by the SA protocol as starting structures for the MD simulations, we initially generated 20 ns trajectories for each of the strands. Although the potential energies of the systems quickly equilibrate over a period of 100 ps (see figure 5.3S in appendix A), it became clear that 20 ns is too short to reach equilibrium in the overall structural properties of the aggregates, e.g.: β-helix formation.

Therefore we extended two of the simulations, for both the trimeric and the pentameric system, to 100 ns. The first 20 ns of these simulations is considered equilibration time. The results described and discussed in the remaining part of this paper are mainly based on the extended simulations, corresponding to string E, nevertheless, strings A-D have shown similar results. Figure 5.3 shows snapshots of the trimeric and pentameric aggregates at 0 and 100 ns of simulation. Top and lateral views are shown.

The average values of the properties (e.g. RMSD and angles) are calculated only considering the last 80 ns of the simulations. Figure 5.4 displays the evolution of RMSDs and RMSFs for simulations E. As a general feature it can be observed that the trimeric system compared to the pentameric form displays a higher flexibility. Figures 5.4A and 5.4C shows the evolution of the RMSD for the DR and the βR (and its subdivisions; β₁, Turn and β₂) for simulations E₃ and E₅. The portions β₁ and Turn showed the higher

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**Figure 5.2:** Overlapped stereoviews of 5 configurations collected every 2 ns of simulation from the SA of the pentameric aggregate. (A) Top view indicating the Cα’s position restraint zone. (B) Lateral view.

**Figure 5.3:** Snapshots at 0 and 100 ns of simulation of the trimeric and pentameric aggregates. Top and lateral views are shown.
fluctuations of RMSD. This flexibility can also be appreciated in Figure 5.4B and 5.4D which show the RMSF values per residue per chain. It was observed in all simulations (strings A-E) and as a general feature, that the trimeric aggregate is structurally more flexible than the pentamer.

This fact may be attributed to the higher degree of packing that a pentameric aggregate has in comparison with the trimeric one. Especially in the case of the trimeric aggregate, generally large fluctuations in RMSD occurred for the β1 and Turn portions with an average value of 0.43 (0.06) and 0.44 nm (0.08), respectively. On the other hand, it is interesting to note that the β2 portion reached an average RMSD value of 0.34 nm (0.02) after ~12 ns of simulation (Figure 5.4A). This can be correlated with the formation of a β-helix structure in this portion of the aggregate. Nevertheless, in the case of pentameric aggregates generally no large RMSD fluctuations were observed. For pentamers the largest RMSD value was observed for the β1 portion with 0.41 nm (0.03), while the Turn portion revealed the smallest RMSD value of 0.24 nm (0.03). The β2 portion showed an average RMSD value of 0.30 nm (0.03) (Figure 5.4C) which was smoothly reached after 20 ns of simulation, and which can be attributed to the formation of a β-helix structure in this portion of the aggregate. A good correlation with what was observed for RMSD profiles can be appreciated in Figures 5.4B and 5.4D that show the RMSF values for each portion of the trimeric and pentameric aggregate respectively. The highest RMSF values were observed for amino acid residues located in the DR, β1 and turn portions for the trimeric aggregate, while for the pentameric aggregate this was mainly seen in DR and β1 portions (Figures 5.4B and 5.4D). Interestingly, in the vicinity of each Gly residue (residues 9, 25, 29, 33, 37 and 38) RMSF values increased due to...
the natural lack of side chains of this amino acid residue. It is worth noting that the lowest RMDF values, in all simulations for both trimeric and pentameric aggregates, were observed for those amino acid residues located in the $\beta_2$ portion.

**Figure 5.5A** and **5.5B**, illustrate the temporal development of the secondary structure content for the trimeric and pentameric aggregates, along the 100 ns trajectory. In **Table 5.2** the secondary structure content for the DR and $\beta R$ is summarized. For all simulations, both $\beta_1$ and $\beta_2$ portions are stable at room temperature for the duration of the simulations. Even peptides located at the ends of the aggregate present stable $\beta$-strand regions, with the occasional loss of $\beta$-sheet content in the $\beta_1$ portion. A small content of $\alpha$-helix and $3_{10}$-helix structures is infrequently observed for the DR. These kinds of helices are notably present with peptides at the edge of the aggregate. As a general phenomenon, the loss of structure is more pronounced in the trimeric aggregates, indicating that, at least at the finite lengths studied here, they are more structurally flexible than the pentamers. The loss of structure, especially of the $\beta$-strand motifs, of peptides from the aggregate ends seems to start at the $\beta_1$ and in the turn portions, while the core of the aggregate comprised of the $\beta_2$ portion remains largely intact. On the other hand, transient helical conformations in the N-terminal residues (**Figure 5.5**) were also reported in previous fibril formation experiments and simulations (Baumketner et al. 2006; Kirkitadze et al. 2001; Massi et al. 2002; Tarus et al. 2005).

**Figure 5.6** shows the average inter-chain distances of the mass center of the C’s at the various regions of our systems. A large fluctuation can be appreciated for the turn portion in simulation E3, which suggests a great mobility in this portion of the aggregate. The DR showed an average value of 0.77 nm (0.04), which is also slowly reached (after ~40 ns) by the $\beta_1$ portion with an average of 0.72 nm (0.09) indicating that these two parts of the molecule behave similarly with respect to inter-chain mobility.

It is interesting to note the great influence that the DR has over the $\beta_1$ portion. The $\beta_2$ portion showed the lowest fluctuation as compared to the other portions, and also the lowest average value of 0.42 nm (0.03) suggesting a low

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**Table 5.2**: Summarized average percentage of the secondary structural composition of the different regions and portions of the $A\beta$ aggregates in solution. The average values here shown are calculated over the last 80 ns of simulation.

| System | Secondary Structure | DR $\beta_1$ | $\beta R$ $\beta_2$
<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Extended$^a$</td>
<td>15.1(3.1)</td>
<td>24.4(7.0)</td>
</tr>
<tr>
<td></td>
<td>Helical$^b$</td>
<td>4.3(2.9)</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Bend$^c$</td>
<td>28.3(4.7)</td>
<td>7.4(4.0)</td>
</tr>
<tr>
<td></td>
<td>Coil$^e$</td>
<td>52.4(4.5)</td>
<td>68.2(6.4)</td>
</tr>
<tr>
<td>Trimeric</td>
<td>Extended$^a$</td>
<td>5.2(1.3)</td>
<td>29.3(5.3)</td>
</tr>
<tr>
<td></td>
<td>Helical$^b$</td>
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<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Bend$^c$</td>
<td>36.1(3.6)</td>
<td>0.6(1.1)</td>
</tr>
<tr>
<td></td>
<td>Coil$^e$</td>
<td>54.2(2.1)</td>
<td>70.1(5.5)</td>
</tr>
<tr>
<td>Pentameric</td>
<td>Extended$^a$</td>
<td>5.2(1.3)</td>
<td>29.3(5.3)</td>
</tr>
<tr>
<td></td>
<td>Helical$^b$</td>
<td>4.4(3.1)</td>
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<td></td>
<td>Coil$^e$</td>
<td>54.2(2.1)</td>
<td>70.1(5.5)</td>
</tr>
</tbody>
</table>

$^a$Extended = $\beta$-Strand + $\beta$-Bridge

$^b$Helical = $\alpha$-Helix + $3_{10}$-Helix (3-Helix) + $\pi$-Helix (5-Helix)

$^c$Bend = Turns Type I-IV + Bend

$^e$Coil = Unstructured

*The standard deviation is given in between brackets.*
inter-chain mobility and a great compactness in this portion (figure 5.6A). A very similar pattern was observed for the pentameric aggregate of the β2 portion with respect to mobility and compactness. In this case an average value of 0.45 nm (0.01) was rapidly and smoothly reached (figure 5.6B). On the other hand, the DR region showed a general trend towards a larger inter-chain distance (1.21 nm (0.02) for simulation E5), while the β1 portion did not follow a same pattern as the DR which we found for the trimeric aggregate. Therefore, the β1 portion in the pentameric configuration appears to be more compact (0.66 nm (0.04)) than the β1 portion of the trimeric aggregate. Interestingly, the turn portion in the pentamers showed a smaller fluctuation than the same portion in the trimers, suggesting that this as well as the β1 portion, has a lower inter-chain mobility and a higher compactness (0.53 nm (0.02)) in the pentameric aggregate. Very similar inter-chain distances in the center of mass were seen for the Cα’s and Cβ’s of each portion in our system (see figure 5.4S in appendix A). These results, especially the behavior of the β2 portion, are in good agreement with the experimental findings of Balbach et al. (Balbach et al. 2002) for a parallel β-sheet organization, who reported the nearest-neighbor intermolecular distances of 4.8±0.5 Å for carbon sites. At this point we emphasize the high stability observed for the β2 portion, which suggests a possible mechanism for fibril elongation as was previously reported (Buchete and Hummer 2007). Hypothetically, in a first step, the initial monomer addition at the end of a growing aggregate would be driven by strong hydrophobic interactions stabilizing the β2 portion. In a second stage, the less stable β1 portion would form, finally followed by the more flexible turn with its relatively hydrophilic residues adopting the fibril conformation. On the other hand, it was also observed for Aβ42 monomers in solution that the sequence I31GLMVGVVIA42 (namely, the β2 portion) may be responsible for the higher propensity of this peptide to form amyloids (Sgourakis et al. 2007).

Consistent with ssNMR data on amyloid fibrils (Petkova et al. 2002; Petkova et al. 2005; Petkova et al. 2006), all our starting fibril conformations have Asp23/Lys28 salt bridge contacts in the loop region. Other studies also showed that charged residues are important for the dynamics of protein aggregation and the stability of β-sheet structures (Dima and Thirumalai 2004; Ma and Nussinov 2006; Massi et al. 2002; Thirumalai et al. 2003). Consistent with previous results (Buchete and Hummer 2007; Buchete et al. 2005), our trajectories of both trimeric and pentameric aggregates reveal that the Asp23/Lys28 bridges are maintained at 310 K. Figure 5.7 shows the distance between the center of mass of the carboxyl group of Asp23 and the center of mass of the NH3+ group of Lys28, as well as a simplified image of the starting configuration and the configuration after 100 ns of simulation for the pentameric case.

![Figure 5.6: Average inter-chain distances of the mass center of the Cα’s versus time of the different regions and portions of the trimeric (A) and pentameric (B) system.](image-url)
It can be appreciated that the positive and negative charges alternate along the fibril axis as in a one-dimensional ionic crystal. We observed that both trimeric and pentameric aggregates form intra-chain salt bridges (Asp$^{23}$/Lys$^{28}$) and inter-chain salt bridges (Asp$^{23}$/Lys$^{28}_1$), where $n$ stands for the chain number. Moreover, the majority of the salt bridges were maintained during the whole simulation time reaching an average distance between the center of mass of the carboxyl group of Asp$^{23}$ and the center of mass of the NH$_3^+$ group of Lys$^{28}$ of 0.32 nm (0.03) for simulation E$_5$, and of 0.38 nm (0.19) for simulation E$_3$. Also, as illustrated in Figure 5.6 it can be concluded that for those salt bridges where Asp$^{23}$ took part in, an average distance of 0.58 nm (0.15) was reached after approximately 32 ns, which was kept until the end of the simulation. On the other hand, salt bridges involving Asp$^{23}_5$ were broken (~43 ns, Asp$^{23}_5$/Lys$^{28}_5$, Figure 5.7C) or displayed large fluctuations (~50 ns, Asp$^{23}_5$/Lys$^{28}_4$, Figure 5.7D) since after a certain amount of simulation time this negatively charged amino acid residue was totally solvated by molecules of water. Figure 5.5S, in Appendix A, shows the distance between the center of mass of the carboxyl group of Asp$^{23}$ and the center of mass of the NH$_3^+$ group of Lys$^{28}$ for the trimeric system. In this case, the intra-chain salt bridges and the inter-chain salt bridges were formed and broken over the entire simulation time. In case of trimeric aggregates large fluctuations occurred, salt bridges being formed and broken over the entire simulation time, which may explain, at least in part, the high-
er flexibility of the trimeric system compared to the pentameric conformation (Figure 5.5S in Appendix A). Also, as reported before for Aβ$_{40}$ fibrils (Buchete and Hummer 2007; Buchete et al. 2005), we find narrow water channels solvating the Aβ$_{21}$/Lys$_{28}$ salt bridges within the aggregates. Indirect experimental evidence for interior hydration of Aβ fibrils comes from differential scanning calorimetry (Sasahara et al. 2005), although ssNMR data do not indicate large structural differences between lyophilized and wet fibrils (Paravaštu et al. 2006; Petkova et al. 2006). Visual inspection reveals the formation of narrow water channels solvating the interior Aβ$_{21}$/Lys$_{28}$ salt bridges into the aggregates. Recent simulations of fibrils with up to 32 Aβ$_{16–22}$ peptides in antiparallel β-strands have also shown similar water channels hydrating the Lys$_{28}$/Glu$_{36}$ side chains (Rohrig et al. 2006). In several instances we noted an exchange in the salt bridge partners from the initial Aβ$_{21}$/Lys$_{28}$ pair to Glu$_{36}$/Lys$_{28}$ as the end peptides begin to dissociate from the aggregates (Figure 5.6S).

Figure 5.6S in Appendix A displays the distance between the center of mass of the carboxyl group of Glu$_{28}$ and the center of mass of the NH$_{2}$ group of Lys$_{28}$ for the intra-chain salt bridges (Glu$_{n,28}$/Lys$_{n,28}$) and the inter-chain salt bridges (Glu$_{n,28}$/Lys$_{n+1,28}$) for the pentameric case. The presence of Glu$_{28}$/Lys$_{28}$ salt bridges has been suggested for small Aβ peptide aggregates, Aβ monomers in solution (Baumketner et al. 2006; Borreguero et al. 2005; Cruz et al. 2005; Lazo et al. 2005), and Aβ$_{40}$ fibrils (Buchete and Hummer 2007). The Glu$_{28}$/Lys$_{28}$ ion pair might play an important role in the aggregate elongation (Buchete and Hummer 2007). Overall, based on our own and other previous studies the main driving force for fibril elongation appears to be the formation of C-terminal β-sheets (our β2 portion). This hypothesis seems to be supported by recent experiments showing that perturbing the hydrogen bonds in the β1 and β2 portions through selective N-methylation affects both fibril growth and structure (Sciarretta et al. 2006a). Disrupting the backbone hydrogen bonds of the β1 portion resulted in relatively slow growth of fibrils with a blurred boundary, while derangement of the β2 hydrogen bonds, had less effects on fibril growth and yielded fibrils with a sharply defined surface. These experimental results are consistent with our simulation findings.

Figure 5.8 displays the formation of a β-helix structure occurring especially on the β2 portion of the pentamers. In order to quantify the twist of this β-helix motif, two dihedral angles were defined, namely γ and δ. Using as a pivot the amino acid residue Val$_{36}$, which encompasses the β2 portion of our model, the definition of the γ angle involves the Ca’s of residues Ile$_{n+1}$, Val$_{36}$, Val$_{38}$ and Ile$_{n+1}$, while the definition of the δ angle involves residues Ala$_{n-42}$, Val$_{36}$, Val$_{36}$ and Ala$_{n-42}$. Figures 5.8A and 5.8B show a spatial view of the β2 portion at 0 ns and 100 ns of simulation time respectively. Top views (Figures 8A1 and 8B1) and lateral views (Figures 8A2 and 8B2) are also shown. Figures 8C and 8D illustrate the temporal development of the γ and δ angles, respectively.

The β-helix is a plausible structural motif for amyloid fibrils, since it is primarily a β-sheet structure with the proper cross-β orientation (Esposito et al. 2006; Ferguson et al. 2006; Zheng et al. 2006a; Zheng et al. 2006b) and known to occur in bona fide proteins. As described above, in our model of Aβ$_{40}$ aggregates in solution the formation of a β-helix structure occurred especially on the β2 portion (Figure 5.8). The model used by us is an elaboration of the classical cross-β molecular structure, which permits the incorporation of the favorable twisted β-sheet structures. This kind of helical structures enables the hydrogen bonding between the β-strands to be extended over the total length of the amyloid fibrils, thereby accounting for their characteristic rigidity and stability (Tycko 2004). Increased fibril twisting could be caused by the loss of directional hydrogen bonding, and a gain in packing interactions (Chothia and Janin 1982).
Figure 5.8: Simplified images of the starting configuration (A1, top view and A2 lateral view) and the final configuration (B1, top view and B2 lateral view) after 100 ns of the β2 portion for the pentameric system is shown. The γ (C) and δ (D) dihedral angles versus time are also shown. The definition of the γ dihedral angle involves the Cα’s of residues Ile$^{n31}$, Val$^{n36}$, Val$^{n-1}_{36}$ and Ile$^{n-1}_{31}$, while the definition of the δ dihedral angle involves residues Ala$^{n42}$, Val$^{n36}$, Val$^{n-1}_{36}$ and Ala$^{n-1}_{42}$, where $n$ stands for the number of the peptide chain.

A schematic definition of those angles is shown in Figure 5.8A. In general, after approximately 20 ns of simulation an average value of -8.95° (0.97) and -9.40° (1.93) was reached for γ and δ, respectively. In Figure 5.8C a great fluctuation for the δ angle can be observed. This may be caused by the fact that the definition of this angle involves the Cα of the terminal residues Ala$^{n42}$, which are in direct contact with the solvent and therefore leading to a greater mobility. Since twisted β-sheets optimize the hydrogen bonds, side-chain stacking, and electrostatic interactions, it is commonly accepted that twisted sheets are more stable than flat ones. This result is in good agreement with those reported by Periole et al. that shows the formation of a cross-β by the peptide GNNQQNY with a twist ranging from 0 to 12 degrees per peptide around its axis when fully solvated (Periole et al. 2009).

Although our model is a full-length model for Aβ$_{42}$ aggregate in solution, it is of interest to compare our results to other proposed truncated and/or full-length models for Aβ$_{40}$ structures. The so-called Tycko’s model (Petkova et al. 2006), and the LECB model (Ma and Nussinov 2002) are similar to our model, specifically regarding the hairpin shape of the aggregates. There
is a slight difference between our model, and the Tycko's model. Our model forms a hydrophobic core with Leu\textsuperscript{14} facing the interior of the aggregate in the β2 portion, whereas in Tycko's model the Leu\textsuperscript{14} faces outside. In our model it was observed that the residues Leu\textsuperscript{14} and Val\textsuperscript{16} of the portion β2 formed an inner hydrophobic core in the aggregates. Regarding this matter, our model is more similar to the LECB model than Tycko's. The internal Asp\textsuperscript{31}/Lys\textsuperscript{28} salt bridge interaction is supported by experimental and theoretical data(Luhrs et al. 2005; Ma and Nussinov 2002, 2006; Petkova et al. 2002; Petkova et al. 2006). This interaction has been observed in our model, as well as in the above mentioned models. We note that the position of the turn in our model differs with the position of the same moiety in the LECB model. The turn portion encompasses the residues Asn\textsuperscript{27}-Ala\textsuperscript{36} in our model, while the same portion encompasses the residues Val\textsuperscript{24}-Asn\textsuperscript{27} in the LECB model. As a result of this organization the Asp\textsuperscript{31}/Lys\textsuperscript{28} salt bridge shows a slight difference between the LECB model and our model. In the LECB model the Asp\textsuperscript{31} residue is located in one of the β-strands of the peptide, and the Lys\textsuperscript{28} residue is located in the other β-strand segment, therefore, the Asp\textsuperscript{31}/Lys\textsuperscript{28} interaction occurs in between the two β-strand portion of this model. In our model, the Asp\textsuperscript{31} is located in the β1 portion, while the Lys\textsuperscript{28} is located in the turn portion. A closely related structural model for Aβ\textsubscript{40} fibrils has been proposed by Wetzel et al.(Shivaprasad and Wetzel 2004; Williams et al. 2004). In agreement with our model, the Wetzel's model places the side chains of Phe\textsuperscript{29}, Ile\textsuperscript{32}, Leu\textsuperscript{14}, and Val\textsuperscript{16} in the interior of a single molecular layer. In contrast to our model, side chains of Asp\textsuperscript{31} and Lys\textsuperscript{28} are on the exterior of a single molecular layer. A highly similar molecular organization has been described for another peptide with amyloidogenic properties. The molecular structural organization of the CA150.WW2 protofilament(Ferguson et al. 2006) is stabilized by interdigitated hydrophobic regions, as well as, by the presence of an intra-chain salt bridge between the Glu\textsuperscript{7} and Arg\textsuperscript{24} in a parallel β-sheet arrangement. This structural model has similarities to those reported for aggregates of the Aβ1–40(Petkova et al. 2002), Aβ1–42 peptides(Luhrs et al. 2005) and the structure reported for the peptide Sup35(Zheng et al. 2006b). Beyond the stabilization effect due to the long-range interactions (e.g.: salt bridges formation) depending on sequence composition, length, and environmental conditions, the interdigitating hydrophobic side chains interactions of the aggregates seems to be a commonly recurring feature of these aggregates.

In summary, the contributions of the different structural elements of the full-length Aβ\textsubscript{42} aggregates in solution to their stability and conformational dynamics were explored. Using multiple 20–100 ns long MD simulations of fibril systems of ~60,000 atoms, we studied aggregate models that differ in the quantity of peptide chains, notably pentameric and trimeric aggregates. We found that our models are stable at the temperature of 310 K, and converge towards an interdigitated side-chain packing for intermolecular contacts within the two parallel β-sheet regions of the aggregates, β1 (residues 18–26) and β2 (residues 31–42). In spite of the fact that those chains at the edge of the aggregates showed a higher mobility than those chains near to the aggregate core, the β-sheet region kept its arrangement (β1-turn-β2 / hairpin shape) during the entire simulation time. The β1 portion showed to be more flexible than the β2 portion, which may well be induced by the proximity of the disordered region to the β1 portion. This provides good arguments to study the full-length Aβ models, since the dynamic behavior of a determinate portion of the aggregate can affect the behavior of its surroundings. The Asp\textsuperscript{31}/Lys\textsuperscript{28} salt bridges maintain a stable and relatively rigid interdigitated structure. However, during the initial stages of fibril dissociation the Asp\textsuperscript{31}/Lys\textsuperscript{28} contacts in Aβ peptides at the aggregates ends can break to form the competing Glu\textsuperscript{22}/Lys\textsuperscript{28} interaction. Thus, it suggests
that the loss of Glu$^{22}$/Lys$^{28}$ contacts could be an important indicator for the transition of the Aβ peptides from their solution structures towards the aggregated conformations (Baumketner et al. 2006; Buchete and Hummer 2007). Our whole molecule simulations reveal that the β-strand twist is a characteristic element of Aβ aggregates, permitting a compact, interdigitated packing of side chains from neighboring β-sheets. The β2 portion formed a very well organized β-helix, whereas the β1 portion did not reach such a high level of organization although it maintained its β-sheet conformation.

5.4 Conclusions

This study demonstrates the importance of studying the full-length Aβ aggregates in solution in order to have a more general view of how each part of the aggregate influences the conformational behavior of the whole system. Moreover, these simulations suggest that the hydrophobic core comprising the β2 portion of the aggregate is a crucial stabilizing element in the process of aggregation and possibly in the elongation of Aβ aggregates. For that reason the β2 portion appears to be a promising target for further drug-design based on the structure-stability information, such as new potential “amyloid inhibitors” capable to interact specifically with this portion of the aggregates.

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“I learn to bribe, I learn to say please.
Oh won’t you lick the pavement for me.
I learn to bribe, I learn to say please.
I like you best when you’re on your knees.”

(Garbage)