Tyrosine Rotamer States in Beta Amyloid: Signatures of Aggregation and Fibrillation

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Supplementary Materials

The supplementary data for the manuscript are divided into 5 paragraphs:

- §S1. Monomer and Dimer System
  Presenting additional data regarding rotamer statistics observed for three monomeric Ab₁-42 both in EI and NEI systems as well as rotamer statistics for dimer in NEI system;
- §S2. Six Peptide Simulations
  Providing details of rotamer statistics for: (i) the six Ab₁-42 peptide simulations, (ii) protofibril in NEI system and beta-sheet hexamer in EI system, as well as structures of aforementioned hexamer;
- §S3. Rotamer Coordinates for Ab₁-42
  Showing the detailed list of all rotamer centres observed within simulations of Ab₁-42 peptide
- §S4. Rotamer Coordinates for Ab₁-40
  Listing all rotamer centres observed within simulations of Ab₁-40 peptide
- §S5. Isolated Ab₁-42 Peptides
  Showing details of Ab₁-42 peptide structural changes during simulation in ionic water only.
§S1. Monomer and Dimer Systems

The complete Tyr rotamer statistics from the three monomeric Ab1-42 simulations discussed in the main text are shown in Figs. S1, S2 and S3. Note how the Tyr appear to prefer the occupation of states 1 and 2. In Fig. S1, we report the results from a simulation of three monomers in an ionic solvent; peptide A was previously reported in Fig 2C of the main text. Basing on the visual analysis and comparing Fig. S1 with Figs. S2 and S3, it appears that the higher concentration of ions in the simulation cell results in more stable monomer structures.

![Figure S1. Rotamer statistics from three Ab1-42 monomers (A, B, C) in EI system.](image)

In Fig. S2, we report the results for three monomers in non-ionic water (NEI system). Tyr of monomer B appears to have an affinity for state 2, with rare movements into states 1 and 3. In contrast, Tyr of monomer A prefers to occupy all states and argueably does not have a preference to any state. This appears to be due to the interactions between the two peptides and can be explained by observing the trajectory. As Tyr of monomer A has freedom to move around it can easily occupy any state and as peptide B comes into close contact for a period, it appears to cause more frequent occupation of states 3 and 4, but as it spends its time as a
monomer it still has a preference for states 1 and 2. The Tyr in peptide B is trapped as during this time, it is interacting directly with the backbone of peptide A, leaving very little room to maneuver, forcing it to predominantly occupy state 2. The results associated with Tyr of monomer C shows a standard result of all four states with a preference to occupy states 1 and 2.

![Figure S2](image)

**Figure S2.** Rotamer statistics from three Ab\textsubscript{1-42} monomers (A, B, C) in NEI system.

In Fig. S3, monomer A is initially close to the other two peptides, but moves away from them by 20ns. After it has become stable Tyr of peptide A shows a distinct preference to states 1 and 2. Once monomer B has aggregated with monomer C, there is a distinct preference to states 3 and 4 for Tyr of B. However, peptide C does not show this same preference, which is caused by the position of the Tyr within the aggregate. It is trapped by peptide B’s backbone, forcing it to take on this position.
Figure S3. Rotamer statistics from an Ab$_{1-42}$ dimer (A and B shows statistics for two peptides in a dimer separately) and monomer (C) in NEI system.

This phenomenon can be seen most clearly in a Ab$_{1-40}$ system we studied (note here that the peptide only has residues 1-40 rather than 1-42). In Fig. S4, two visibly trapped Tyr residues can be seen, one trapped in states 1 and 2, and the other in states 3 and 4. This clearly occurs due to how the Tyrs are orientated with respect to one another in the aggregate, and how they hinder one-another’s movements.

Figure S4. Ab$_{1-40}$ Dimer showing movements of trapped Tyr side-chains. MD picture taken after frame 1500.
§S2. Six Peptide Simulations

For completeness, in Figure S5 we show the full set of rotamer statistics from the simulation with six \( \text{Ab}_{1-42} \) peptides aggregating into a dimer and a tetramer as discussed in the main text (peptides A, B, C and E have been shown in Fig. 6).

\[ \text{(A)} \quad \begin{array}{c}
\text{Figure S5. Rotamer statistics from the six Ab}_{1-42} \text{ peptide simulations Figures A – F shows the statistic for peptides A-F respectively.}
\end{array} \]

In Figure S6 we show the full set of results for the protofibril, NEI system; Tyr A, C, E and F were displayed in Figure 8 of the main text.
Figure S6. Rotamer statistics from the protofibril simulation in NEI system. Figures A – F show the statistics for different peptides.
Figure S7. Beta-sheet hexamer with in EI system.

In Figure S7, we show snapshots of the protofibril simulation using the ionic solution (the 110ns structure was shown in Figure 7C). The structure appears more stable with the screening ions creating a more compact conformation.

In Figure S8, it can also be seen that there are less instances of Rotamer states 5 and 6, although they still are present.

Figure S8. Beta-sheet hexamer (in EI system) rotamer response. Each figure A – F shows the statistic for different peptide in the hexamer.
§S3. Rotamer Coordinates for Ab₁₋₄₂

The coordinates of each Tyr’s rotamer states from all our Ab₁₋₄₂ simulations are shown in Table S1 and displayed graphically in the main text Figure 9. This figure depicts the variation in the state and shows that this variation does not noticeably change when the system is part of a monomer, amorphous dimer or tetramer, or protofibril hexamer. It should also be noted that there is more variation along the X₂ and a relatively narrower range for X₁. Outliers are expected and are seen for states 1, 3 and 6. This is because the sample pool associated with that particular peptide and rotamer is too small to get a genuinely long-time average.

**Table S1.** Rotamer (X₁;X₂) coordinates for all Tyr in our Ab₁₋₄₂ simulations

| System       | Peptide  | R1   | R2   | R3       | R4       | R5 | R6 |
|--------------|----------|------|------|----------|----------|----|----|
| Three Peptides | A - Monomer | (67,239) | (74,81) | (166;116) | (168;291) | -  | -  |
|              | B - Monomer | (66,230) | (72,108) | (164,112) | (166,277) | -  | -  |
|              | C - Monomer | (69,270) | (73,67) | (168,117) | (166,305) | -  | -  |
| Amorphous    | A - Monomer | (74,250) | (74,63) | (164,90)  | (161,271) | -  | -  |
|              | B - Monomer | (83,350) | (72,74) | (159,115) | -         | -  | -  |
|              | C - Monomer | (71,257) | (76,97) | (156,124) | (156,277) | -  | -  |
| (EI)         | A - Monomer | (64,249) | (75,72) | (168,89)  | (171,267) | -  | -  |
|              | B - Dimer  | (71,236) | (68,58) | (165,79)  | (171,273) | -  | -  |
|              | C - Dimer  | (65,276) | (74,58) | (168,116) | -         | -  | -  |
| Three Peptides | A - Dimer 1 | (70,247) | (67,81) | (164,117) | (158,262) | -  | -  |
|              | B - Dimer 1 | (93,305) | (72,63) | (168,91)  | (169,266) | -  | -  |
|              | C - Dimer 2 | (72,276) | (62,66) | (164,104) | (163,270) | -  | -  |
|              | D - Dimer 2 | (75,284) | (72,74) | (162,85)  | (161,270) | -  | -  |
| Amorphous    | A - Hexamer | (69,237) | (73,89) | (126,112) | -         | -  | -  |
|              | B - Hexamer | (65,265) | (70,73) | (175,88)  | (170,293) | -  | -  |
|              | C - Hexamer | (62,257) | (67,63) | (161,98)  | (155,266) | -  | -  |
|              | D - Hexamer | -         | (71,78) | (163,95)  | (165,287) | -  | -  |
|              | E - Hexamer | (69,265) | (67,78) | (160,102) | (171,294) | -  | -  |
|              | F - Hexamer | (67,244) | (53,50) | -         | -         | -  | -  |
| Six Peptides | A - Hexamer | (80,248) | (72,90) | (169,89)  | (165,270) | -  | -  |
|              | B - Hexamer | (78,271) | (73,81) | (163,90)  | (168,267) | -  | -  |
|              | C - Hexamer | (69,269) | (79,89) | (171,89)  | (172,270) | -  | -  |
|              | D - Hexamer | (80,273) | (77,91) | (166,90)  | (166,273) | (284,59) | (296,342) |
|              | E - Hexamer | (66,269) | (78,86) | (189,88)  | (166,268) | (285,91) | (297,275) |
|              | F - Hexamer | (76,263) | (70,64) | (163,90)  | (174,270) | -  | -  |
| Protofibril  | A - Hexamer | (83,237) | (91,120) | (171,91)  | (167,270) | (276,80) | -  |
|              | B - Hexamer | (104,294) | -       | (169,94)  | (162,270) | -  | -  |
|              | C - Hexamer | (81,271) | (77,71) | (168,89)  | (173,278) | -  | (279,251) |
|              | D - Hexamer | (72,270) | (57,90) | (159,108) | (165,246) | -  | -  |
|              | E - Hexamer | (66,270) | (72,90) | (162,92)  | (164,268) | (286,98) | (284,265) |
|              | F - Hexamer | (88,254) | (83,69) | (169,91)  | (174,269) | (287,77) | (285,260) |

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§S4. Rotamer Coordinates for Ab\textsubscript{1-40}

The overall results for Ab\textsubscript{1-40} simulations are very similar to the Ab\textsubscript{1-42} simulations. The Tyr rotamer locations fall into the same categories. As seen in Table S2, the time-averaged occupation probabilities show the same pattern as with Ab\textsubscript{1-42}, with a preference towards states 1 and 2 as monomers, and states 3 and 4 when a part of an amorphous aggregate.

Table S2. Proportion of time spent in the various rotamer states in different Ab\textsubscript{1-40} simulated structures.

|          | Ab\textsubscript{1-40} Monomer | Ab\textsubscript{1-40} Amorphous Aggregate |
|----------|-------------------------------|---------------------------------------------|
| State 1  | 0.500                         | 0.286                                      |
| State 2  | 0.333                         | 0.286                                      |
| State 3  | 0.167                         | 0.214                                      |
| State 4  | -                             | 0.214                                      |
| State 5  | -                             | -                                          |
| State 6  | -                             | -                                          |

§S5. Single Ab\textsubscript{1-42} Peptides

50 ns trajectories for Ab\textsubscript{1-42} peptide in ionic solvent (monomer with ions and water) as well as in water only with no excess ions added (monomer Only) indicate that although the RMSD calculated with respect to the initial structure is relatively high (Fig. S9), it plateaus towards the end of the simulations.

Figure S9. RMSD in Å calculated with respect to the starting structure for monomeric Ab\textsubscript{1-42} simulation in water with excess ions (Monomer with Ions) and in water without excess ions (Monomer Only).
High values of the RMSD can be explained by the high flexibility of the peptide that has no tertiary stabilization. The peptide comprises two α-helices connected by a flexible linker. Even a small conformational change of this linker leads to a substantial change in the relative orientations of the helices, as illustrated in Fig. S10. This is a main source of the high RMSD values observed in Fig. S9, the others are: small alterations of the shorter helix structure; and the flexibility of the unstructured part of the peptide. A lack of abnormal folding in the single monomers suggests that the force field employed is parametrized well and is suitable for the simulation of the peptide.

Figure S10. Structure overlap of Ab$_{1-42}$ peptide. The initial, reference structure is shown in red while the final structure, after a 50ns trajectory in water with excess ions, is shown in blue.