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Discrete Molecular Dynamics Study of Oligomer Formation by N-Terminally Truncated Amyloid β -Protein

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Abstract

In Alzheimer's disease (AD), amyloid β -protein ($A\beta$) self-assembles into toxic oligomers. Of the two predominant $A\beta$ alloforms, $A\beta_{1-40}$ and $A\beta_{1-42}$, the latter is particularly strongly linked to AD. N-terminally truncated and pyroglutamated $A\beta$ peptides were recently shown to seed $A\beta$ aggregation and contribute significantly to $A\beta$ -mediated toxicity, yet their folding and assembly were not explored computationally. Discrete molecular dynamics (DMD) approach previously captured *in vitro*-derived distinct $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomer size distributions and predicted that the more toxic $A\beta_{1-42}$ oligomers had more flexible and solvent exposed N-termini than $A\beta_{1-40}$ oligomers. Here, we examined oligomer formation of $A\beta_{3-40}$, $A\beta_{3-42}$, $A\beta_{11-40}$, and $A\beta_{11-42}$ by the DMD approach. The four N-terminally truncated peptides showed increased oligomerization propensity relative to the full-length peptides, consistent with *in vitro* findings. Conformations formed by $A\beta_{3-40/42}$ had significantly more flexible and solvent-exposed N-termini than $A\beta_{1-40/42}$ conformations. In contrast, in $A\beta_{11-40/42}$ conformations the N-termini formed more contacts and were less accessible to the solvent. The compactness of the $A\beta_{11-40/42}$ conformations was in part facilitated by Val12. Two single amino acid substitutions that reduced and abolished hydrophobicity at position 12, respectively, resulted in a proportionally increased structural variability. Our results suggest that $A\beta_{11-40}$ and $A\beta_{11-42}$ oligomers might be less toxic than $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers and offer a plausible explanation for the experimentally-observed increased toxicity of $A\beta_{3-40}$ and $A\beta_{3-42}$ and their pyroglutamated forms.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia among the elderly, characterized by an *in vivo* accumulation of extracellular senile plaques containing fibrillar aggregates of amyloid β -protein ($A\beta$). Substantial evidence implicates low molecular weight oligomeric assemblies of $A\beta$ rather than $A\beta$ fibrils, as the proximate neurotoxic agents in AD [1–7]. $A\beta$ is produced through cleavage of the amyloid precursor protein (APP) by β - and γ -secretases [8, 9]. Depending on the position, at which APP is cleaved, different alloforms of $A\beta$ with a sequence length of 36–43 amino acids are generated. The predominant forms are $A\beta_{1-40}$ and $A\beta_{1-42}$, which differ by the presence of two amino acids, Ile41-Ala42, at the C-terminus of the latter. $A\beta_{1-42}$ aggregates faster [10, 11], is genetically more strongly linked to AD than $A\beta_{1-40}$ [12], and forms oligomers that are more toxic to cell cultures than $A\beta_{1-40}$ oligomers [13, 6].

N-terminally truncated $A\beta$ peptides [14–17]: $A\beta_{11(pE)-40/42}$, $A\beta_{11-40/42}$, and $A\beta_{3(pE)-40/42}$, where “pE” stands for the pyroglutamated form of Glu, were shown to increase from 20% up to ~60% in the brain as the disease progresses [18, 19]. Pyroglutamated $A\beta_{3-40/42}$ ($A\beta_{11-40/42}$) peptides are formed after Glu3 (Glu11) undergoes a cyclization process aided by glutaminyl cyclase [14, 20–22, 16]. Näslund et al. showed that $A\beta_{11(pE)-42}$ and $A\beta_{11-42}$

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combined amounted to 20% of the total $A\beta$ in the brain [15]. $A\beta_{11-42}$ was shown to comprise ~10% of the total $A\beta_{X-42}$, whereas $A\beta_{11(pE)-42}$ and $A\beta_{1-42}$ accounted for 15% and 20% of the total $A\beta_{X-42}$, respectively [17], indicating similar amounts of free and pyroglutamated $A\beta_{11-42}$ in the brain. $A\beta_{3(pE)-42(43)}$ was shown to contribute ~25% to the total $A\beta_{X-42/43}$ load [16], whereas much smaller amounts of $A\beta_{3-40/42}$ peptides were reported [23], implying that the majority of $A\beta_{3-4X}$ peptides in the brain are pyroglutamated. $A\beta_{3(pE)-42}$ was found to be the dominant pyroglutamated $A\beta$ isoform [16, 23, 21] and $A\beta_{3(pE)-4X}$ ($X = 0, 2$) peptides in amounts equal or even surpassing the amount of $A\beta_{1-4X}$ peptides were reported *in vivo* [20].

Molecular dynamics (MD) provides tools to structurally characterize $A\beta$ monomer ensembles and oligomeric assemblies, often in detail that surpasses currently available experimental resolution [24, 25]. Most detailed structural information on $A\beta$ folding and oligomer structures can be extracted using fully atomistic MD. Despite numerous all-atom MD studies of $A\beta$ monomers [26–45], the only fully atomistic MD study of $A\beta_{1-40}$ and $A\beta_{1-42}$ dimers was recently reported by Barz and Urbanc [45]. In addition to all-atom MD techniques, two different coarse-grained MD approaches were used to examine $A\beta$ monomers and oligomers [46]. *Ab initio* discrete molecular dynamics (DMD) approach with a four-bead protein model in implicit solvent [47–50] and the implicit solvent OPEP force field combined with replica exchange MD [51, 52]. The DMD approach is currently the only one that successfully captures the experimentally observed features of $A\beta_{1-40}$ and $A\beta_{1-42}$ folding and oligomerization [47–49, 53, 50], such as *in vitro* observed formation of pentamers/hexamers and dodecamers by $A\beta_{1-42}$ but not by $A\beta_{1-40}$ [54, 55] and a quasistable turn at the C-terminus of $A\beta_{1-42}$ monomers that does not occur in $A\beta_{1-40}$ monomers [56–59, 34]. The DMD approach also captured the distinct oligomer size distributions of the full-length Arctic [Glu22Gly] mutants [50], associated with a familial form of AD [60, 61, 13, 62–64], in agreement with available *in vitro* data [65].

Although the chemical modification of Glu resulting in pyroglutamated isoforms alters the chemical properties of Glu, it is not expected to considerably affect the resulting conformations because the terminal amino acids are typically disordered and do not participate significantly in non-local interactions. This assumption is indirectly supported by the *in vitro* observation that N-terminally truncated peptides and their pyroglutamated isoforms have indistinguishable oligomer size distributions [65]. Despite the importance of the N-terminally truncated $A\beta$ peptides, their folding and assembly was not studied *in silico*. Here, we examine folding and oligomer formation by the four naturally occurring N-terminally truncated $A\beta$ peptides, $A\beta_{X-40}$ and $A\beta_{X-42}$ ($X = 3, 11$), using the DMD approach and compare the resulting oligomer formation pathways and structures to the full-length peptides $A\beta_{1-40}$ and $A\beta_{1-42}$ [47, 50, 45].

2. Results

We examined four N-terminally truncated peptides: $A\beta_{3-4X}$ and $A\beta_{11-4X}$ ($X = 0, 2$) with the following one-letter amino acid sequences:

E³FRHDSGYEVHHQKL¹⁷VFFA²¹EDVGSNKGAI³¹IGLMV³⁶GGVVIA

E¹¹VHHQKLL¹⁷VFFA²¹EDVGSNKGAI³¹IGLMV³⁶GGVVIA

where the two additional amino acids Ile41 and Ala42 at the C-terminus of the two longer peptides are written in bold letters. We refer to the hydrophobic region Leu17-Ala21 as the central hydrophobic cluster (CHC), the region Ile31-Val36 as the mid-hydrophobic region (MHR), and Val39-Val39 or Val39-Ala42 as the C-terminal region (CTR). The four-bead DMD approach with two implicit solvent parameters $E_{HP} = 0.3$ (the strength of effective hydrophobic interactions) and $E_{CH} = 0$ (the strength of effective electrostatic interactions) at

the physiological temperature estimate $T = 0.13$ (expressed in units of E_{HB}/k_B was applied as explained in *Methods*). These values were previously shown to result in *in silico* monomer and oligomer ensemble structures consistent with *in vitro* findings [53, 50].

In addition to the potential energy of each trajectory, which converged after $15 - 20 \times 10^6$ simulation steps, we examined the time evolution of oligomer size distributions for all four truncated peptides up to 40×10^6 simulation steps, checking the distributions at regular intervals of 10×10^6 simulation steps and comparing them to those derived previously for $A\beta_{1-40}$ and $A\beta_{1-42}$ (Fig. 1, Supplementary Material). Size distributions for the N-terminally truncated $A\beta$ peptides converged at $15 - 20 \times 10^6$ simulation steps. Structural analysis on all six peptides was performed by using the time frames between 39×10^6 and 40×10^6 simulation steps, resulting in 10 time frames per trajectory or 80 time frames per peptide. From these 80 time frames, we extracted populations of monomers and oligomers of different sizes. The structural analysis was done either over entire populations or over the resulting ensembles of conformers sorted by their size. The number of resulting monomer and oligomer conformers is given Table 1. The structural analysis described below was performed on monomers and oligomers of selected sizes, trimers and heptamers, that were formed by all six peptides in sufficient amounts to allow a reliable structural characterization (Table 1).

2.1. N-terminally truncated $A\beta$ peptides form larger oligomers

$A\beta_{1-40}$ and $A\beta_{1-42}$ oligomer size distributions, which were derived by photo-induced cross-linking of unmodified peptides (PICUP), followed by SDS-PAGE by Teplov and collaborators [66], were distinct and in some cases strongly affected by a single amino acid substitution or deletion [54, 65]. The DMD approach captured these experimentally observed differences for $A\beta_{1-40}$, $A\beta_{1-42}$, and their Arctic (Glu22Gly) mutants [47, 50]. It is important to note that the comparison between the *in vitro* and *in silico* oligomer size distributions is not straightforward. First, the mapping of the optical density of the silver-stained gel bands to *in silico* data is not clear.¹ Second, there are some intrinsic and unavoidable differences between the conditions under which PICUP/SDS-PAGE experiments and DMD simulations are conducted. Because much higher $A\beta$ concentrations are used *in silico* than *in vitro*, DMD-derived monomers and smaller oligomers are present at much lower numbers than *in vitro*. Third, the SDS-PAGE gel scale depends approximately logarithmically on the oligomer size, resulting in a single smeared band corresponding to $A\beta$ oligomers larger than dodecamers. Fourth, Tyr¹⁰ is essential in radical-forming cross-linking reactions and was shown to critically contribute to the PICUP cross-linking efficiency [66, 65, 67]. Consequently, the PICUP/SDS-PAGE data on $A\beta_{11-40/42}$ peptides are not meaningful [65] and the closest comparison to our DMD-derived $A\beta_{11-40/42}$ distributions could be made using PICUP/SDS-PAGE data on $A\beta_{10-40/42}$ peptides. The comparison of *in silico* and *in vitro* oligomer size distributions, described below, should be viewed with these complications in mind.

We examined the effect of N-terminal truncations on the resulting DMD-derived oligomer size distributions and compared them to the PICUP/SDS-PAGE data [65] (Fig. 1). In addition to $A\beta_{3-40/42}$ and $A\beta_{11-40/42}$, which are naturally occurring isoforms, we also derived the oligomer size distributions of $A\beta_{10-40/42}$ for the sake of a direct comparison to the PICUP/SDS-PAGE data. Whereas dimers and trimers were the most abundant oligomers in the DMD-derived $A\beta_{1-40}$ size distribution, deletion of the first two residues resulted in a decreased number of dimers and trimers and increased number of oligomers larger than

¹The optical density may correspond to the total volume of the oligomers comprising the gel band, their total surface area, or something else.

trimers up to octamers (Fig. 1a; black and red curves), consistent with PICUP/SDS-PAGE data [65] (Fig. 1a; second column). Deletion of the first 9 residues from $A\beta_{1-40}$ resulted in a distribution that lacked dimers and was significantly shifted towards larger oligomers, including trimers through hexamers, decamers through pentadecamers, and also included icosamers and hexacosamers, respectively (Fig. 1a; green curve). Thus, the DMD-derived $A\beta_{10-40}$ oligomer size distribution fitted well the description of the experimentally-observed *irregular* $A\beta_{10-40}$ distribution as reported by Bitan *et al.* [65] (Fig. 1a). Finally, the DMD-derived $A\beta_{11-40}$ distribution shared the irregular character of the $A\beta_{10-40}$ distribution, but displayed a significantly lower number of tetramers, an increased number of octamers, and was also characterized by a significant number of oligomers larger than decamers (Fig. 1a; blue curve).

The previously reported DMD-derived $A\beta_{1-42}$ oligomer size distribution was multimodal, in agreement with *in vitro* data [54], comprising three peaks centered at (i) dimers and trimers, (ii) pentamer and hexamers, and (iii) decamers to dodecamers (Fig. 1b, black curve) [50]. Deletion of the first 2 residues resulted in decreased numbers of dimers and trimers, increased numbers of tetramers through decamers and a decreased population of dodecamers and larger oligomers (Fig. 1b, red curve). Despite these changes, $A\beta_{3-42}$ oligomer size distribution was multimodal and similar to the $A\beta_{1-42}$ distribution, as can be more clearly seen in Fig. 1d, (Supplementary Material). Overall, these results were in agreement with *in vitro* data [65] (Fig. 1b, insert). Deletion of the first 9 residues resulted in the DMD-derived $A\beta_{10-42}$ oligomer size distribution with fewer monomers, dimers, and trimers than $A\beta_{1-42}$ and $A\beta_{3-42}$ distributions but with similar abundances of pentamers through heptamers, and an increased abundance of tridecamers through pentadecamers (Fig. 1b, green curve). In addition, we noted the emergence of oligomers of sizes 18 through 20 and an evolving peak at the oligomer size 32 (Fig. 1b, green curve). Considering the limitations of the comparison of *in silico* and *in vitro* oligomer size distributions, the DMD-derived $A\beta_{10-42}$ oligomer size distribution was multimodal and therefore similar to the $A\beta_{1-42}$ and $A\beta_{3-42}$ distributions, consistent with the PICUP/SDS-PAGE findings [65]. Importantly, deletion of the first 10 residues resulted in the $A\beta_{11-42}$ oligomer size distribution that was more irregular than the other distributions, was shifted to larger oligomers relative to $A\beta_{1-42}$, $A\beta_{3-42}$, and $A\beta_{10-42}$ distributions, and displayed increased numbers of hexamers to decamers as well as larger oligomers, including oligomers comprising 25 and 26 peptides.

The above results demonstrate that the N-terminal truncation enhances the $A\beta$ oligomer formation propensity, in particular for the $A\beta$ peptides without the first 10 residues, as also reflected in the total number of oligomers larger than heptamers formed by the six peptides (Table 1). Previous DMD studies already demonstrated that the effective hydrophobicity drives the $A\beta$ assembly into oligomers [47, 50]. N-terminal truncations reduce the length and thus the role of the predominantly hydrophilic N-terminal peptide region, enhancing the contribution of the hydrophobic peptide regions, resulting in an enhanced assembly propensity.

2.2. N-terminal truncation increases the secondary structure differences between $A\beta_{X-40}$ and $A\beta_{X-42}$ ($X = 3, 11$) oligomers

The predominant secondary structure components adopted by all $A\beta$ peptides under study were turns and β -strands, whereas helices formed with a very low propensity, consistent with the collapsed coil oligomer morphology reported by previous DMD studies [47, 49, 50]. We calculated the average β -strand and turn propensities of entire populations of monomers and oligomers of the four truncated peptides and compared them to those of full-length peptides (Table 2). Of the four truncated peptides, $A\beta_{3-42}$ displayed the highest β -strand content, followed by $A\beta_{11-42}$, both comparable to the β -strand content in full-length peptides. Conformations formed by $A\beta_{3-40}$ and especially by $A\beta_{11-40}$ had significantly

lower β -strand content than conformations formed by the other four peptides. Across the six peptides, the average turn content was anti-correlated with the average β -strand content (Table 2) with the correlation coefficient of -0.987 , consistent with a constant sum of the β -strand and turn contents ($62.6\% \pm 1.3\%$). The average coil content was the lowest for $A\beta_{11-40}$ and the highest for $A\beta_{3-42}$ but the differences were not statistically significant (Table 2).

We then calculated the average β -strand and turn propensity per amino acid for monomers, trimers, and heptamers of the six peptides (Figs. II–IV, Supplementary Material). To obtain the β -strand and turn contents per assembly state, we averaged the β -strand, turn, and coil propensities over all amino acids in the peptide (Fig. 2). Conformers formed by $A\beta_{3-40}$ and $A\beta_{3-42}$ displayed β -strand, turn, and coil propensities similar to those formed by full-length $A\beta$ peptides. The β -strand content in $A\beta_{11-42}$ displayed the steepest increase with the oligomer size, starting from a zero and increasing to the β -strand content of $A\beta_{1-42}$ and $A\beta_{3-42}$ for hexamers and heptamers (Fig. 2a), while the turn as well as the coil content decreased upon oligomer formation (Fig. 2b). Of the six peptides, $A\beta_{11-40}$ formed oligomers with the least β -strand and the most turn structure (Fig. 2).

Notably, differences in the average β -strand and turn content between $A\beta_{X-40}$ and $A\beta_{X-42}$ increased with X and were the largest for $A\beta_{11-40}$ versus $A\beta_{11-42}$ conformations. This observation may not be too surprising as the percentage difference in the primary structure increases from 5% for $X = 1$ to 6.7% for $X = 11$. $A\beta_{11-40}$ had the highest turn and the lowest β -strand content of the six peptides and $A\beta_{11-42}$ was characterized by the steepest increase in the β -strand content with the oligomer size.

2.3. N-terminal truncations alter the tertiary and quaternary structure of the resulting conformations

To investigate the effects of the N-terminal truncation on the tertiary and quaternary structure of the resulting conformers, we calculated and analyzed the contact maps (see Supplementary Methods in Supplementary Material) for monomers, trimers, and heptamers of the four truncated peptides and compared them to those derived previously for the full-length $A\beta$ peptides (Figs. V–VIII, Supplementary Material). A detailed description of the effect of the N-terminal truncations on the contact maps of the resulting monomer and oligomer conformations is given in Supplementary Results in Supplementary Material.

To best describe the effect of N-terminal truncations on the tertiary and quaternary structure of the resulting monomer and oligomer conformations, we introduced a new quantity, *the connectivity of a residue X*, which was calculated as the average contact strength between X and the entire peptide. In analogy, *the connectivity of a peptide region R* was calculated as the average contact strength between $X \in R$ and the entire peptide, averaged over all residues X within the peptide region R of interest. Tables 3 and 4 summarize the connectivities of three residues and three peptide regions in $A\beta_{1-40}$ and $A\beta_{1-42}$ conformations and the corresponding conformations of truncated $A\beta_{X-40}$ and $A\beta_{X-42}$ ($X = 3, 11$) peptides, respectively. In all $A\beta_{1-40}$ conformations, Ala2 had significantly higher connectivity than in $A\beta_{1-42}$ conformations, whereas Phe4 had comparable connectivities. In all $A\beta_{3-40}$ and $A\beta_{3-42}$ conformations, Phe4 had a significantly lower tertiary connectivity than in the corresponding $A\beta_{1-40}$ and $A\beta_{1-42}$ conformations, whereas lower quaternary connectivity of Phe4 was noted only in $A\beta_{3-40}$ and $A\beta_{3-42}$ heptamer conformations. The connectivity of Val12 was high on all conformations and comparable to the connectivity of the CHC. Quaternary connectivity of Val12 dramatically increased in all $A\beta_{11-40}$ and $A\beta_{11-42}$ oligomer conformations, indicating the importance of Val12 in stabilizing the $A\beta_{11-40}$ and $A\beta_{11-42}$ oligomer structures. Importantly, the CHC and MHR quaternary connectivities were significantly *higher* in $A\beta_{3-40}$ relative to $A\beta_{1-40}$ trimers and heptamers, whereas in

$A\beta_{3-42}$ heptamers the CHC and MHR had *lower* connectivities than in $A\beta_{1-42}$ heptamers. In most $A\beta_{11-40}$ and $A\beta_{11-42}$ conformations, the CHC and MHR tertiary and quaternary connectivities were significantly higher than in their corresponding $A\beta_{1-40}$ and $A\beta_{1-42}$ conformations. The tertiary connectivity of the CTR was significantly higher in $A\beta_{11-42}$ conformations relative to $A\beta_{1-42}$ conformations. Both $A\beta_{11-40}$ and $A\beta_{11-42}$ heptamers displayed higher CTR quaternary connectivities than the corresponding $A\beta_{1-40}$ and $A\beta_{1-42}$ heptamers.²

Overall, the effect of the N-terminal truncation on the tertiary and quaternary structure of the resulting monomer and oligomer conformations was stronger for $A\beta_{11-4X}$ than for $A\beta_{3-4X}$ ($X = 0, 2$) conformations. *The truncations either reduced (in $A\beta_{3-4X}$ conformations) or enhanced (in $A\beta_{11-4X}$ conformations) the average number of and strength of contacts that the N-terminal residues (and to a lesser degree also the CHC and MHR) formed with either the rest of the peptide or intermolecularly.*

2.4. Flexibility and solvent exposure of the N-termini is increased in $A\beta_{3-4X}$ and decreased in $A\beta_{11-4X}$ ($X = 0, 2$) oligomers

To gain additional structural insights and identify the peptide regions of N-terminally truncated oligomers that are most likely to interact with the surroundings, we calculated two quantities: the coarse grained solvent accessible surface area (CG-SASA) per residue and the distance from the center of mass per residue (see Supplementary Methods in Supplementary Material). Previous studies [47, 50] reported that $A\beta_{1-42}$ oligomers were characterized by a larger solvent exposure of the N-terminal region Asp1-Asp7 than $A\beta_{1-40}$ oligomers, which was postulated to be the cause for an increased toxicity of $A\beta_{1-42}$ oligomers relative to $A\beta_{1-40}$ oligomers [47, 68]. Both quantities, the CG-SASA and distance from the center of mass, revealed interesting structural differences between $A\beta_{3-4X}$ and $A\beta_{11-4X}$ conformations relative to $A\beta_{1-40}$ and $A\beta_{1-42}$ conformations (Figs. 3, 4, and 5).

In all conformations, the CHC and MHR had the lowest CG-SASA values below $25 \text{ \AA}^2$ (Figs. 3a,c; Figs. 4a,c; and 5a,c). The C-terminal residues Val39 (for the $A\beta_{X-40}$, $X = 1, 3, 11$, peptides) and the region Val39-Ile41 (for the $A\beta_{X-42}$, $X = 1, 3, 11$, peptides) also had low CG-SASA values. The solvent exposure of the hydrophobic regions (CHC, MHR, CTR) was not affected by the N-terminal truncation neither in monomers, nor in trimers or heptamers. In contrast, the solvent exposure of the N-terminal region of the truncated peptides was strongly affected. In $A\beta_{3-40}$ and $A\beta_{3-42}$ conformations, we observed an increase in the CG-SASA values as well as the distance from the center of mass in the region Glu3-Arg5, indicating an increased flexibility of this region in $A\beta_{3-4X}$ ($X = 0, 2$) conformations. Although the first amino acid was the most exposed to the solvent in conformations formed by all six peptides, the amino acids with the largest distance from the center of mass in all $A\beta_{1-40}$ conformations and in $A\beta_{3-40}$ monomers and trimers were Asp7 and Ser8 (Figs. 3b,d; Figs. 4b,d; and 5b,d). In $A\beta_{3-40}$ heptamers, however, Glu3 was the most distant from the center of mass, demonstrating that the N-terminal flexibility increases with the oligomer size, a conclusion also supported by the CG-SASA data (Fig. 5). The amino acid the most distant from the center of mass in $A\beta_{1-42}$ conformations was Asp1 (monomers and heptamers) or Ser8 (trimers). In all $A\beta_{3-42}$ conformations, the N-terminal Glu3 was the most distant from the center of mass, demonstrating an increased flexibility of N-termini upon the truncation. The largest distance from the center of mass for $A\beta_{1-4X}$ and $A\beta_{3-4X}$ monomers (trimers) was 11–12 Å (17–18 Å), which allowed us to estimate a

²Note that the CTR region in $A\beta_{1-42}$ conformations had a slightly lower connectivity than in $A\beta_{1-40}$ conformations, however, the CTR region comprised 4 residues in $A\beta_{1-42}$ and only 2 residues in $A\beta_{1-40}$ conformations, so that the sum of intra- and intermolecular CTR contacts was significantly larger in $A\beta_{1-42}$ than in $A\beta_{1-40}$ conformations, consistent with the CTR of $A\beta_{1-42}$ dominating both folding and oligomer formation [47, 50].

monomer (trimer) diameter to $\sim 23\text{\AA}$ ($\sim 35\text{\AA}$). In a similar way, a diameter of $A\beta_{1-4X}$ and $A\beta_{3-4X}$ ($X = 0, 2$) heptamers was estimated to $50\text{--}55\text{\AA}$ with the longer alloforms ($X = 2$) forming slightly larger heptamers. Notably, none of the heptamer conformations of the six peptides had residues that were closer than $\sim 10\text{\AA}$ from the center of mass, indicating that heptamers adopted less spherical, more elongated shapes than the corresponding trimers. This feature was the most pronounced for $A\beta_{1-40}$ heptamers and was a consequence of the particular $A\beta_{1-40}$ assembly through formation of intermolecular β -strands involving the region Ala2-Phe4, which resulted in “dumbbell”-like oligomers [50]. Thus, heptamer conformations that displayed a departure from the quasi-spherical shape, represented an onset of elongated, protofibril-like assemblies comprising two or more quasi-spherical oligomers.

Deletion of the first 10 residues from $A\beta_{1-40}$ and $A\beta_{1-42}$ had an opposite effect on the flexibility of the N-termini. The CG-SASA values for $A\beta_{11-4X}$ ($X = 0, 2$) trimers and heptamers mostly followed the CG-SASA values for the full-length $A\beta$ oligomers except for Glu11, which had as an N-terminal amino acid significantly higher solvent exposure (Figs. 4a,c and 5a,c). Interestingly, in $A\beta_{11-40}$ (but not $A\beta_{11-42}$) monomers, the CHC was more exposed to the solvent than in any other conformations, elucidating the importance of the CHC in $A\beta_{11-40}$ oligomer formation (Fig. 3a,c). In $A\beta_{11-4X}$ conformations, the distance of the N-terminal residues from the center of mass remained similar to that in $A\beta_{1-4X}$ ($X = 0, 2$) conformations (Fig. 3b,d; Fig. 4b,d; and Fig. 5b,d). Because the $A\beta_{11-4X}$ peptides lacked the Asp1-Tyr10 region, which was the region most exposed to the solvent in full-length $A\beta$ conformations, $A\beta_{11-4X}$ ($X = 0, 2$) heptamers had significantly more compact structure than $A\beta_{1-4X}$ ($X = 0, 2$) heptamers, as indicated by a reduced distance from the center of mass in the former (Fig. 5b,d). In $A\beta_{11-40}$ and $A\beta_{11-42}$ monomers, amino acids Glu22 and Asn27, respectively, were the farthest ($\sim 10\text{\AA}$) from the center of mass, resulting in monomer diameters of $\sim 20\text{\AA}$ (Fig. 3b,d). The amino acids the most distant from the center of mass ($14\text{--}15\text{\AA}$ in trimers and $16\text{--}20\text{\AA}$ in heptamers) were Ser26 and Asn27, resulting in trimer/heptamer conformations with diameters of $28\text{--}30\text{\AA}/32\text{--}40\text{\AA}$, respectively, which was slightly less than the diameters of conformations formed by the longer peptides (Figs. 4b,d and 5b,d). The distance from the center of mass per residue assumed the lowest values for heptamers of $A\beta_{11-40}$ and $A\beta_{11-42}$, demonstrating that these heptamers were more spherical and compact than heptamers formed by the longer peptides.

In summary, $A\beta_{3-40}$ and $A\beta_{3-42}$ conformations displayed more flexible (disordered) N-termini than $A\beta_{1-40}$ and even $A\beta_{1-42}$ conformations, whereas $A\beta_{11-40}$ and $A\beta_{11-42}$ formed compact monomer and oligomer conformations with N-terminal regions in contact with other peptide regions. The flexibility of the N-termini in $A\beta_{3-40}$, $A\beta_{1-42}$, and $A\beta_{3-42}$ oligomer conformations increased with the oligomer size.

2.5. Effects of N-terminal truncations on the free energy landscape of $A\beta$ conformational ensembles

The free energy landscapes of conformational ensembles of the six peptides were derived as described in Supplementary Methods (Supplementary Material). The end-to-end (NC) distance of each peptide in the conformational ensemble was chosen as the first reaction coordinate and the sum of CG-SASA values over all hydrophobic residues along the sequence (hydrophobic CG-SASA) was selected as the second reaction coordinate. The resulting free energy landscapes displayed distinct isoform-dependent features (Fig. 6). $A\beta_{1-42}$ conformations were distributed within a shallower and wider free energy minimum than $A\beta_{1-40}$ conformations, indicating a higher degree of conformational disorder in the former (Fig. 6a,d), consistent with all-atom MD results [45]. The $A\beta_{3-40}$ and $A\beta_{3-42}$ ensembles were significantly more spread along the NC-distance coordinate (Fig. 6b,e). Whereas the free energy minima of $A\beta_{1-40/42}$ conformations ($12\text{--}17\text{ nm}^2$) and $A\beta_{3-40/42}$

conformations ($11 - 16 \text{ nm}^2$) populated similar hydrophobic CG-SASA values, the free energy minima of $A\beta_{11-40/42}$ conformations were shifted to significantly lower hydrophobic CG-SASA values of $6 - 11 \text{ nm}^2$ (Fig. 6c,f), indicating relatively compact $A\beta_{11-40}$ and $A\beta_{11-42}$ conformations with hydrophobic residues more efficiently shielded from the solvent than in the conformations formed by the other four peptides.

We explored the degree to which an addition of a single amino acid at the N-terminus (Tyr10) and the hydrophobic character of Val12 affected the $A\beta_{11-40}$ and $A\beta_{11-42}$ free energy landscapes. In addition to DMD trajectories of $A\beta_{10-40/42}$ assembly, the assembly of $A\beta_{11-40}$ and $A\beta_{11-42}$ peptides, modified by two single amino acid substitutions, Val12Ala and Val12Ser, was examined. These substitutions decreased (Val12Ala) and abolished (Val12Ser) the hydrophobicity of the residue at position 12. The comparison of the free energy landscapes of the $A\beta_{10-40/42}$, $A\beta_{11-40/42}$, [Ala12]- $A\beta_{11-40/42}$, and [Ser12] $A\beta_{11-40/42}$, analogous to those in Fig. 6 (Fig. IX; Supplementary Material), showed that whereas the additional amino acid at the N-terminus (Tyr10) did not alter the resulting conformational ensembles (Fig. IX a,e and b,f; Supplementary Material), the two amino acid substitutions Val12Ala and Val12Ser resulted in a wider spread and increase of the NC distances, respectively. The Val12Ser substitution in addition resulted in monomer and oligomer conformations, in which hydrophobic residues were less efficiently shielded from the solvent as indicated by a shift towards larger hydrophobic CG-SASA values in the free energy landscapes (Fig. IX d,h and b,f; Supplementary Material). None of these three modifications, however, caused the degree of a conformational disorder observed in $A\beta_{3-40/42}$ conformational ensembles (Fig. 6b,e).

2.6. Assembly pathways

We monitored the assembly starting from a monomer peptide through a dimer, a larger quasi-spherical oligomer into an elongated protofibril-like assembly for all six peptides (Figs. 7 and 8). These pathways showed distinct structural characteristics of $A\beta_{3-40}$ (Fig. 7b), $A\beta_{1-42}$ (Fig. 8a), and $A\beta_{3-42}$ (Fig. 8b) conformations displaying flexible and solvent exposed N-termini and significantly more compact $A\beta_{11-40}$ (Fig. 7c) and $A\beta_{11-42}$ (Fig. 8c) conformations. Figs. 7 and 8 also indicate that the amount of β -strand content increased with the assembly size, consistent with data in Fig. 2a. The DMD-predicted assembly pathways are dominated by a fast hydrophobic collapse into small globular oligomeric structures, which self-assemble further to form elongated, curvilinear assemblies as hypothesized earlier by Teplow and collaborators [66, 54]. Recently, Kelly and collaborators examined $A\beta$ assembly *in vitro* and postulated a nucleated structural conversion from elongated protofibrillar assemblies into fibrils with a cross- β structure [69]. Our DMD-derived assembly pathways are consistent with this postulate although the DMD simulation times are still one or more orders of magnitude too short to observe the nucleated structural conversion from elongated, curvilinear protofibril-like assemblies into fibrils with a cross- β structure.

3. Conclusions and Discussion

Several studies demonstrated that the N-terminally truncated forms of $A\beta$, $A\beta_{3-4X}$ and $A\beta_{11-4X}$ ($X = 0, 2$) exist in substantial amounts in the AD brain and might play an important role in the AD pathology [14–17], yet folding and assembly of these isoforms were not explored *in silico*. Here, we examined monomer folding and oligomer formation by $A\beta_{3-4X}$ and $A\beta_{11-4X}$ ($X = 0, 2$) peptides using the four-bead protein model combined with efficient DMD and structurally characterized the resulting DMD-derived populations of monomers and oligomers. Our results revealed that the oligomer size distributions of the truncated $A\beta$ peptides, especially the $A\beta_{11-42}$ oligomer size distribution, were shifted towards larger oligomers relative to the oligomer size distributions of full-length peptides. This observation

is consistent with several experimental reports on higher aggregation propensities of the N-terminally truncated peptides [70, 71].

Deletion of the first two residues resulted in monomer and oligomer structures with more flexible and solvent exposed N-termini than the corresponding $A\beta_{1-40}$ and $A\beta_{1-42}$ conformations. Tertiary and quaternary contacts among the CHC, MHR, and CTR were not much affected by this truncation. In contrast, deletion of the first 10 residues resulted in an increased tertiary and quaternary connectivity of $A\beta_{11-4X}$ conformations, consistent with their compact quasi-spherical structure, in which Val12 forms strong intra- and intermolecular contacts. Klimov and collaborators recently examined the role of the N-terminal truncation by comparing $A\beta_{1-40}$ and $A\beta_{10-40}$ monomers, dimers, and tetramers using fully-atomistic replica exchange MD in implicit solvent [72, 73, 28]. In their study, truncation did not significantly affect the resulting monomer and dimer conformations and they noted that Tyr10 played a key role in intermolecular interactions [72, 73]. In our simulations, $A\beta_{10-4X}$ and $A\beta_{11-4X}$ ($X = 0, 2$) peptides formed more compact monomer and oligomer conformations with enhanced tertiary and quaternary contacts. In agreement with the $A\beta_{1-40}$ and $A\beta_{10-40}$ conformations predicted by Takeda and Klimov [72], we observed that $A\beta_{11-40}$ conformations had significantly less β -strand content than $A\beta_{1-40}$ conformations and that both peptides formed globular conformations.

We elucidated assembly pathways of the six peptides under study by showing that a hydrophobic collapse drives the self-assembly into quasi-spherical oligomers, followed by an elongation into curvilinear, unstructured protofibril with β -strand content slowly but steadily increasing with time. Based on the results of the PICUP/SDS-PAGE oligomer characterization and other *vitro* observations, Teplow and collaborators concluded that $A\beta$ assembly proceeds through formation of micelle-like oligomers that assemble further into curvilinear protofibrils, which finally convert into fibrils with the cross- β structure [66, 54]. Kelly and collaborators recently examined early $A\beta$ assembly stages *in vitro* and postulated a nucleated structural conversion from elongated protofibrillar assemblies into cross- β fibrils [69], which can be experimentally monitored by Thioflavin T (ThT) fluorescence spectroscopy [74]. The structural conversion from unstructured protofibrils into cross- β fibrils would require a cooperative structural re-arrangement of more than one peptide, which is associated with a loss of conformational entropy and thus a high free energy activation barrier. DMD-derived assembly $A\beta$ pathways described here are consistent with the postulated nucleated structural conversion from curvilinear, relatively unstructured elongated protofibrils into an ordered cross- β fibrils.

In vivo, truncated forms with Glu as the N-terminal amino acid undergo the cyclization process that involves an interaction of the N-terminal region with glutaminyl cyclases, and results in pyroglutamated isoforms $A\beta_{3(pE)-4X}$ and $A\beta_{11(pE)-4X}$ ($X = 0, 2$). Our structural data demonstrates that $A\beta_{3-4X}$ but not $A\beta_{11-4X}$ ($X = 0, 2$) conformations are characterized by more flexible and solvent exposed N-termini. Consequently, if the hydrophobic collapse into oligomeric structures occurs prior to the cyclization process, $A\beta_{3-4X}$ conformations with flexible, solvent exposed N-termini would interact with peptidases more efficiently than $A\beta_{11-4X}$ conformations, providing a plausible explanation for a much larger amount of $A\beta_{3(pE)-4X}$ relative to the “free” $A\beta_{3-4X}$ and similar amounts of $A\beta_{11-4X}$ and $A\beta_{11(pE)-4X}$ observed in the AD brain [15, 14, 17, 16].

Previously, the DMD approach predicted structural differences at the N-termini of conformations formed by $A\beta_{1-40}$ and $A\beta_{1-42}$, linking the more flexible and solvent exposed N-termini in $A\beta_{1-42}$ conformations to their increased ability to mediate toxicity [47, 50, 45]. This hypothesis was further extended by Urbanc *et al.* to explain the effect of peptide inhibitors of $A\beta_{1-42}$ toxicity [75–77] on the resulting $A\beta_{1-42}$ conformations by

demonstrating that the efficient peptide inhibitors (but not the inefficient control peptide) interacted with (see also [78]) and reduced the solvent exposure at the N-termini of $A\beta_{1-42}$ conformations [68]. Recently, two $A\beta$ mutations at position 2 (Ala2Val and Ala2Thr) were found to significantly alter the resulting $A\beta$ toxicity, thereby supporting this hypothesis [79, 80]. The present study predicts that $A\beta_{3-4X}$ conformations with even more flexible and solvent exposed N-termini than their full-length $A\beta$ counterparts would be even more toxic *in vivo*. This conjecture is supported by experimental findings that demonstrate $A\beta_{3(pE)-42}$ oligomer-induced neuronal apoptosis in mice [81], $A\beta_{3(pE)-4X}$ toxicity in cell cultures [82], and suggested that the N-terminus of $A\beta_{3(pE)-4X}$ oligomers interacts with cellular targets and mediates toxicity [83]. Although $A\beta_{11(pE)-4X}$ deposition in AD brains was clearly demonstrated [17], to our knowledge, $A\beta_{11(pE)-4X}$ or $A\beta_{11-4X}$ toxicity was not characterized yet. The results of the present study elucidate unique structural properties of monomers and oligomers formed by the N-terminally truncated $A\beta$ peptides, reveal the mechanisms underlying their folding and assembly, which are consistent with available experimental data [54, 69], and provide further evidence of a plausible correlation between the oligomer structures and $A\beta$ peptide-specific toxicity.

4. Methods

4.1. The DMD Approach

In DMD the interactions between two particles are defined by square-well potentials [84], which makes DMD orders of magnitude faster than MD. Here, DMD was combined with a four-bead protein model, in which each amino acid is represented by the amino group (N), α -carbon group (C^α), carbonyl group (C) and the side-chain group (C^β) [85]. Geometric properties of the peptide and backbone hydrogen bonding parameters introduced by Ding *et al.* were determined phenomenologically by statistical analysis of ~7700 proteins with known folded structures from the Protein Data Bank (PDB) [86]. The backbone hydrogen bond is implemented between the amide group of amino acid i (N_i) and the carbonyl group of amino acid j (C_j) and the bond is defined by the distance between N_i and C_j and by auxiliary bond lengths involving the nearest-neighboring atoms of N_i and C_j [85]. The potential energy associated with formation of one hydrogen bond is E_{HB} . E_{HB} represents a unit of energy in our approach and thus temperature is expressed in units of E_{HB}/k_B . The amino-acid-specific interactions due to the hydrophathy and charge of side chains were implemented into the four-bead protein model [47, 48] by using the Kyte-Doolittle hydrophathy scale [87]. This implicit-solvent DMD approach with a four-bead protein model is described in detail in previous studies [47, 48, 53, 50].

4.2. The DMD simulation protocol

Following the same simulation protocol as proposed in the previous study on $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomer formation by Urbanc *al.* (implicit solvent parameters set to $E_{HP}=0.3$ and $E_{CH}=0$) [50], we prepared 32 spatially separated and non-interacting peptides of $A\beta_{3-40}$, $A\beta_{3-42}$, $A\beta_{11-40}$, and $A\beta_{11-42}$ in a cubic box of edge length 25nm. This choice of the system size resulted in a molar concentration of about 3–4 mM, which is 10–100 fold higher than typical *in vitro* concentrations. As previously reported and discussed, such large concentrations are typically used in simulations and are required to observe the assembly *in silico* [47, 50]. Each of the four systems of 32 peptides (for each $A\beta_{3-40}$, $A\beta_{3-42}$, $A\beta_{11-40}$, and $A\beta_{11-42}$) was subjected to 10^6 steps-long, high-temperature ($T = 4$) DMD simulations, resulting in eight replicas of the system for each of the four peptides, which defined the initial conformations for eight DMD trajectories per peptide. In total, 32 DMD trajectories, each 40×10^6 simulation steps long, were acquired. The configurations containing ensembles of monomers and oligomers of different sizes were recorded every 100,000 steps

and upon completion of simulations structurally analyzed. Supplementary Methods in Supplementary Material provide a more detailed description of our methods and analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Oligomer size distributions for (a) $A\beta_{X-40}$ and (b) $A\beta_{X-42}$ ($X = 1, 3, 11$) derived in silico using the DMD approach with the four-bead protein model and the corresponding SDS-PAGE/PICUP results for $A\beta_{X-40/42}$ ($X = 1, 3, 10$) reported previously by Bitan et al. [65]. Distributions were calculated by averaging the populations at time frames 39, 39.5, and 40×10^6 simulation steps of all 8 trajectories of each peptide. The error bars represent the SEM values.

Figure 2.

The average (a) β -strand and (b) turn propensities for monomers and oligomers up to including heptamers of A β_{X-40} and A β_{X-42} ($X = 1, 3, 11$). The error bars correspond to the SEM values. The number of monomer and oligomer conformations for each of the six peptides are reported in Table 1.

Figure 3. (a,c) Coarse-grained (CG) SASA per residue and (b,d) the relative distance of each residue from the center of mass of (a,b) Aβ_{X-40} and (c,d) Aβ_{X-42} monomers. The error bars represent the SEM values. The number of monomer conformations for each of the peptides are reported in Table 1.

Figure 4.

(a,c) Coarse-grained (CG) SASA per residue and (b,d) the relative distance of each residue from the center of mass of (a,b) $\text{A}\beta_{X-40}$ and (c,d) $\text{A}\beta_{X-42}$ trimers. The error bars represent the SEM values. The number of trimer conformations for each of the peptides are reported in Table 1.

Figure 5. (a,c) Coarse-grained (CG) SASA per residue and (b,d) the relative distance of each residue from the center of mass of (a,b) Aβ_{X-40} and (c,d) Aβ_{X-42} heptamers. The error bars represent the SEM values. The number of heptamer conformations for each of the peptides are reported in Table 1.

Figure 6.
(a–f) PMF plots showing the free energy landscape using two reaction coordinates, the N-terminal to C-terminal distance and the sum of SASA values over all hydrophobic residues. The color scale is shown in units of $k_B T$.

Figure 7.

Assembly pathways through quasi-spherical oligomers into elongated protofibril-like assemblies of (a) $A\beta_{1-40}$ (monomer $\rightarrow$ dimer $\rightarrow$ trimer $\rightarrow$ pentamer), (b) $A\beta_{3-40}$ (monomer $\rightarrow$ dimer $\rightarrow$ pentamer $\rightarrow$ octamer), and (c) $A\beta_{11-40}$ (monomer $\rightarrow$ dimer $\rightarrow$ tetramer $\rightarrow$ icosamer). The initial monomer (first panel) is plotted in red in the subsequent assembly states. The N-terminal D1 residues are shown in magenta. Turns and loops are colored in cyan, β -strands are depicted as yellow ribbons, and coil (lack of secondary structure) is shown in silver.

Figure 8.

Assembly pathways through quasi-spherical oligomers to elongated protofibril-like assemblies of (a) $A\beta_{1-42}$ (monomer $\rightarrow$ dimer $\rightarrow$ hexamer $\rightarrow$ dodecamer), (b) $A\beta_{3-42}$ (monomer $\rightarrow$ dimer $\rightarrow$ tetramer $\rightarrow$ nonamer), and (c) $A\beta_{11-42}$ (monomer $\rightarrow$ dimer $\rightarrow$ hexamer $\rightarrow$ pentacosamer). The initial monomer (first panel) is plotted in red in the subsequent assembly states. The N-terminal D1 residues are shown in magenta. Turns and loops are colored in cyan, β -strands are depicted as yellow ribbons, and coil (lack of secondary structure) is shown in silver.

Table 1

Total number of conformers from monomers ($n = 1$) to oligomers of order $n = 2$ through $n = 7$ used in the structural analysis. The conformers were obtained using all eight trajectories per peptide and 10 time frames between 39 and 40×10^6 time steps per trajectory.

Number of Conformers	n=1	n=2	n=3	n=4	n=5	n=6	n=7	n=8
$A\beta_{1-40}$	86	333	306	125	14	23	18	7
$A\beta_{1-42}$	64	185	146	50	55	40	27	68
$A\beta_{3-40}$	71	174	176	175	61	64	8	21
$A\beta_{3-42}$	38	62	139	78	69	47	46	76
$A\beta_{11-40}$	8	4	21	15	41	23	20	160
$A\beta_{11-42}$	5	1	21	9	1	2	38	129

Table 2

The average β -strand and turn propensities and the SEM values for the entire populations of monomers and oligomers of the six peptides. The β -strand and turn propensities per amino acid were calculated first for all 32 peptides in the configuration, independent of the assembly state of the peptide, followed up by averaging over all amino acids in the peptide.

Peptide	$\langle \beta\text{-Strand} \rangle$	$\langle \text{Turn} \rangle$	$\langle \text{Coil} \rangle$
A β_{1-40}	0.186 $\pm$ 0.017	0.451 $\pm$ 0.032	0.269 $\pm$ 0.035
A β_{1-42}	0.195 $\pm$ 0.015	0.417 $\pm$ 0.031	0.299 $\pm$ 0.038
A β_{3-40}	0.152 $\pm$ 0.014	0.479 $\pm$ 0.027	0.276 $\pm$ 0.034
A β_{3-42}	0.214 $\pm$ 0.016	0.400 $\pm$ 0.030	0.303 $\pm$ 0.040
A β_{11-40}	0.095 $\pm$ 0.008	0.548 $\pm$ 0.038	0.249 $\pm$ 0.043
A β_{11-42}	0.176 $\pm$ 0.013	0.440 $\pm$ 0.035	0.274 $\pm$ 0.042

Table 3

The *intramolecular* (tertiary structure) and *intermolecular* (quaternary structure) connectivity of selected residues (Ala2, Phe4, Val12) and peptide regions (CHC, MHR, CTR) in monomers ($N=1$), trimers ($N=3$), and heptamers ($N=7$) formed by $A\beta_{X-40}$ ($X=1, 3, 11$). The error bars correspond to SEMs. The connectivity values for $A\beta_{3-40}$ and $A\beta_{11-40}$ conformations that differ significantly from those for corresponding $A\beta_{1-40}$ conformations are marked by *.

	$A\beta_{1-40}$			$A\beta_{3-40}$			$A\beta_{11-40}$		
Regions	Tertiary	Quaternary		Tertiary	Quaternary		Tertiary	Quaternary	
Ala2 (N = 1)	0.39±0.07	—		—	—		—	—	
Ala2 (N = 3)	0.35±0.05	0.34±0.05		—	—		—	—	
Ala2 (N = 7)	0.26±0.06	0.34±0.05		—	—		—	—	
Phe4 (N = 1)	0.43±0.08	—		0.28±0.06*	—		—	—	
Phe4 (N = 3)	0.41±0.07	0.30±0.05		0.29±0.04*	0.26±0.06		—	—	
Phe4 (N = 7)	0.36±0.06	0.28±0.04		0.16±0.05*	0.15±0.05*		—	—	
Val12 (N = 1)	0.61±0.11	—		0.58±0.12	—		0.60±0.21	—	
Val12 (N = 3)	0.52±0.09	0.23±0.03		0.49±0.09	0.26±0.05		0.42±0.11	0.60±0.13*	
Val12 (N = 7)	0.55±0.14	0.31±0.08		0.34±0.08	0.47±0.12		0.76±0.15	0.50±0.11*	
CHC (N = 1)	0.55±0.05	—		0.51±0.05	—		0.48±0.08	—	
CHC (N = 3)	0.54±0.05	0.47±0.04		0.48±0.05	0.56±0.05*		0.71±0.09*	0.79±0.11*	
CHC (N = 7)	0.55±0.05	0.46±0.05		0.40±0.06*	0.73±0.08*		0.62±0.08*	1.04±0.10*	
MHR (N = 1)	0.56±0.05	—		0.52±0.05	—		0.89±0.09*	—	
MHR (N = 3)	0.52±0.05	0.43±0.03		0.50±0.05	0.48±0.04*		0.83±0.08*	0.50±0.06	
MHR (N = 7)	0.52±0.05	0.50±0.04		0.36±0.05*	0.59±0.05*		0.78±0.08*	0.70±0.06*	
CTR (N = 1)	0.71±0.11	—		0.60±0.10	—		0.87±0.17	—	
CTR (N = 3)	0.61±0.10	0.64±0.08		0.62±0.10	0.66±0.08		0.62±0.13	0.81±0.12	
CTR (N = 7)	0.61±0.11	0.69±0.09		0.56±0.11	0.75±0.11		0.62±0.13	0.98±0.11*	

Table 4

The *intramolecular* (tertiary structure) and *intermolecular* (quaternary structure) connectivity of selected residues (Ala2, Phe4, Val12) and peptide regions (CHC, MHR, CTR) in monomers ($N=1$), trimers ($N=3$), and heptamers ($N=7$) formed by $A\beta_{X-42}$ ($X=1, 3, 11$). The error bars correspond to SEMs. The connectivity values for $A\beta_{3-42}$ and $A\beta_{11-42}$ conformations that differ significantly from those for corresponding $A\beta_{1-42}$ conformations are marked by *. Additionally, the connectivity values for $A\beta_{X-42}$ ($X=1, 3, 11$) conformations that differ significantly from those for corresponding $A\beta_{X-40}$ ($X=1, 3, 11$) conformations are marked by #.

	$A\beta_{1-42}$			$A\beta_{3-42}$			$A\beta_{11-42}$		
Regions	Tertiary	Quaternary		Tertiary	Quaternary		Tertiary	Quaternary	
Ala2 (N = 1)	0.24±0.04 [#]	—		—	—		—	—	
Ala2 (N = 3)	0.23±0.04 [#]	0.22±0.04 [#]		—	—		—	—	
Ala2 (N = 7)	0.17±0.03 [#]	0.23±0.04 [#]		—	—		—	—	
Phe4 (N = 1)	0.44±0.08	—		0.20±0.04 [*]	—		—	—	
Phe4 (N = 3)	0.38±0.07	0.26±0.05		0.21±0.03 ^{**#}	0.22±0.04		—	—	
Phe4 (N = 7)	0.24±0.05 [#]	0.31±0.07		0.12±0.02 [*]	0.15±0.03 [*]		—	—	
Val12 (N = 1)	0.57±0.09	—		0.48±0.12	—		0.75±0.23 [*]	—	
Val12 (N = 3)	0.55±0.09	0.19±0.04		0.50±0.11	0.30±0.05		0.59±0.14	0.49±0.10 [*]	
Val12 (N = 7)	0.49±0.09	0.17±0.04 [#]		0.44±0.08	0.27±0.04 [#]		0.45±0.11 [#]	0.49±0.11 [*]	
CHC (N = 1)	0.55±0.05	—		0.48±0.05 [#]	—		0.69±0.09 ^{**#}	—	
CHC (N = 3)	0.51±0.05	0.42±0.03		0.51±0.05	0.48±0.04		0.58±0.06	0.69±0.07 [*]	
CHC (N = 7)	0.52±0.05	0.48±0.04		0.49±0.05	0.41±0.03 ^{**#}		0.55±0.05	0.49±0.06 ^{**#}	
MHR (N = 1)	0.52±0.05	—		0.43±0.05	—		0.89±0.09 [*]	—	
MHR (N = 3)	0.50±0.04	0.42±0.03		0.49±0.04	0.42±0.03		0.83±0.08 [*]	0.50±0.06	
MHR (N = 7)	0.43±0.04 [#]	0.49±0.04		0.46±0.04 [#]	0.38±0.03 ^{**#}		0.78±0.08 [*]	0.70±0.06 [*]	
CTR (N = 1)	0.59±0.07	—		0.63±0.07	—		0.83±0.10 [*]	—	
CTR (N = 3)	0.59±0.06	0.56±0.05		0.60±0.06	0.57±0.06		0.80±0.09 [*]	0.65±0.09	
CTR (N = 7)	0.47±0.05	0.73±0.07		0.54±0.06	0.51±0.05 ^{**#}		0.64±0.08 [*]	0.98±0.11 [*]	