Amino acid substitutions [K16A] and [K28A] distinctly affect amyloid β -protein oligomerization

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Abstract Amyloid β -protein ($A\beta$) assembles into oligomers that play a seminal role in Alzheimer's disease (AD), a leading cause of dementia among the elderly. Despite undisputed importance of $A\beta$ oligomers, their structure and the basis of their toxicity remain elusive. Previous experimental studies revealed that the [K16A] substitution strongly inhibits toxicity of the two predominant $A\beta$ alloforms in the brain, $A\beta_{40}$ and $A\beta_{42}$, whereas the [K28A] substitution exerts only a moderate effect. Here, folding and oligomerization of [A16] $A\beta_{40}$, [A28] $A\beta_{40}$, [A16] $A\beta_{42}$, and [A28] $A\beta_{42}$ are examined by discrete molecular dynamics (DMD) combined with a four-bead implicit solvent force field, DMD4B-HYDRA, and compared to $A\beta_{40}$ and $A\beta_{42}$ oligomer formation. Our results show that both substitutions promote $A\beta_{40}$ and $A\beta_{42}$ oligomerization and that structural changes to oligomers are substitution- and alloform-specific. The [K28A] substitution increases solvent-accessible surface area of hydrophobic residues and the intrapeptide N-to-C terminal distance within oligomers more than the [K16A] substitution. The [K16A] substitution decreases the overall β -strand content, whereas the [K28A] substitution exerts only a modest change. Substitution-specific tertiary and quaternary structure changes indicate that the

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[K16A] substitution induces formation of more compact oligomers than the [K28A] substitution. If the structure-function paradigm applies to A β oligomers, then the observed substitution-specific structural changes in A β ₄₀ and A β ₄₂ oligomers are critical for understanding the structural basis of A β oligomer toxicity and correct identification of therapeutic targets against AD.

Keywords Alzheimer's disease · Protein folding and assembly · Oligomerization · Oligomer structure · Amyloid β -protein · Structure-toxicity relationship

1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by amyloid plaques, neurofibrillary tangles, and neuronal loss [1]. The onset of AD is strongly associated with aggregation of amyloid β -protein (A β), which is the primary proteinaceous component of amyloid plaques. 39–43 amino-acid-residue-long A β is formed through cleavage of the amyloid precursor protein (APP) by β - and γ - secretases, which cleave A β at the N- and C-terminal, respectively. Of the two predominant alloforms in the human brain, A β ₄₀ and A β ₄₂, A β ₄₂ is genetically more strongly associated with AD, and is one of the most sensitive cerebrospinal fluid biomarkers of the disease [2]. The exact cause of AD is still unknown. The amyloid cascade hypothesis initially postulated that amyloid plaques exert their toxic function leading to massive neuronal loss in AD [3] but was later revised due to accumulated evidence that implicated soluble low molecular weight (LMW) A β oligomers in triggering the observed neuropathology in AD [4]. Whereas the central role of A β oligomers in AD pathology is well known, the A β oligomer structure has not been determined experimentally and the structural aspects/elements that mediate A β oligomer toxicity are not well understood.

Unlike the A β oligomer structure, the parallel cross- β structure [5] of A β fibrils formed by A β ₄₀ [6–11] and A β ₄₂ [12–15] has been characterized by several NMR techniques and electron cryomicroscopy. A recurrent motif of the A β fibril organization is a turn/loop structure within the decapeptide region A21–A30, which is flanked by two β -strands, whereby the β -sheets along the fibrillar axis are stabilized by lateral hydrophobic attraction and salt bridges formed between the side chains of D23 and K28 [6–8, 16]. It appears that K28 may play an important role already at the stage of A β folding. Folding dynamics of the decapeptide fragment, A β _{21–30}, derived from both experimental [17–19] and computational [20–26] studies elucidated the importance of the hydrophobic contact between V24 and K28 in stabilization of the folded structure, which is believed to nucleate folding of full-length A β . K28 and nearby residues are a common binding site for inhibitors of A β toxicity [27], which suggests that K28 is at least partially exposed to the solvent at the early assembly stage, which produces toxic oligomers, consistent with its charged hydrophilic nature. The A β sequence contains another lysine residue, K16, adjacent to the central hydrophobic cluster L17–A21, a region which plays an important role in A β fibrillogenesis [28, 29]. K16 was reported to be involved in salt bridge formation within A β fibrils [7, 30, 31] but may be predominantly exposed to solvent [32, 33] at earlier assembly stages, allowing K16 to interact with inhibitors of fibril formation [34].

Full-length A β contains three residues that are positively charged at neutral pH, arginine at position 5 and two lysines at positions 16 and 28, respectively. These three positively

charged residues are likely to interact with a cellular membrane because they can participate in both effective electrostatic interactions with negatively charged phospholipid head groups and effective hydrophobic interactions with lipid hydrocarbon groups [35–39]. Substitutions of positively charged residues R5, K16, and K28 with alanine were reported to significantly reduce A β -induced toxicity to human embryonic kidney (HEK293) cells [40]. Molecular tweezers (CLR01), which predominantly bind to lysines, were demonstrated to inhibit A β aggregation and protect against A β toxicity in cell cultures [41]. The effect of single amino acid substitutions, where alanine was used in place of K16 or K28, on in vitro aggregation and toxicity of A β_{40} and A β_{42} was recently examined [42]. [A16]A β_{40} and [A16]A β_{42} displayed significantly reduced toxicity to cell cultures, whereas the [K28A] substitution had only a modest effect on A β induced cell toxicity. Circular dichroism (CD) spectroscopy revealed a decrease in the rate of secondary structure evolution in the [K16A] and [K28A] analogues relative to that of wild type (WT) peptides. The results of photoinduced cross-linking of unmodified proteins (PICUP) combined with gel electrophoresis (SDS-PAGE) showed that the [K28A] substitution in A β_{42} caused a shift of oligomer size distribution toward larger oligomers. Electron micrographs of [A28]A β_{40} and [A28]A β_{42} revealed abundant short fibrils, suggesting that the [A28] substitution facilitates nucleation while decreasing the elongation rate.

Discrete molecular dynamics (DMD) simulations using the four-bead protein model with amino-acid-specific interactions (DMD4B-HYDRA) can reproduce experimentally observed [43] oligomer propensities of A β_{40} and A β_{42} [44], their Arctic mutants [G22]A β_{40} and [G22]A β_{42} involved in the early onset familial AD [45], and naturally occurring N-terminally truncated A β_{40} and A β_{42} isoforms [46]. These simulations revealed globular to elongated oligomeric assemblies, characterized by a variable degree of disorder, in particular at the N-terminal region, which appears to be significantly more disordered and solvent accessible in A β_{42} oligomers than in A β_{40} oligomers [44]. These observations, which were confirmed by explicit-solvent fully atomistic MD simulations [47], led to a hypothesis that the N-terminal flexibility and solvent exposure of N-terminal residues are associated with the ability of A β oligomers to mediate toxicity through interactions with a cellular membrane. Interestingly, [G22]A β_{40} oligomers also possess highly flexible and solvent-exposed N-termini and are thus structurally more similar to A β_{42} than to A β_{40} oligomers [45]. Similarly, the physiologically relevant oligomers formed by both A β_{3-40} and A β_{3-42} , which have been shown to be more toxic than their WT counterparts, have even more flexible and solvent-accessible N-termini than those formed by WT peptides [46]. The predictions of DMD4B-HYDRA simulations, including the effect of toxicity inhibitors of the A β_{42} assembly structure [48], provide a basis for a plausible structure-toxicity relationship that can be further examined. To elucidate the role of lysines at early stages of A β_{40} and A β_{42} assembly and complement the existing experimental findings [42], we here apply the DMD4B-HYDRA approach to investigate the effect of lysine substitutions with alanine, K16A and K28A, on A β oligomer assembly dynamics and resulting structures.

2 Methods

A more detailed explanation of the DMD4B-HYDRA approach, simulation protocol, and methods of structural analysis are provided in [Supporting Information](#).

3 Results

Here we examine the effect of two single amino acid substitutions, [K16A] and [K28A], on oligomer formation and structure of $A\beta_{40}$ and $A\beta_{42}$ monomer and oligomer ensembles using the DMD4B-HYDRA approach, which allows characterization of assembly pathways from spatially separated unstructured peptides to quasi steady state monomer and oligomer populations and has been successfully applied to studies of $A\beta_{40}$ and $A\beta_{42}$ and their physiologically relevant isoforms [44–46, 49]. For each of the four mutant peptides under study, [A16] $A\beta_{40}$, [A16] $A\beta_{42}$, [A28] $A\beta_{40}$, and [A28] $A\beta_{42}$, eight DMD trajectories, each comprising 32 initially unstructured and spatially separated peptides, were acquired at physiological temperature and structurally analyzed following the previously published protocol [45] (see also Methods in [Supporting Information](#)).

The primary structure of $A\beta_{42}$ is

DAEFRHDSGY EVHHQ**K**₁₆LVFF AEDVGSN**K**₂₈GA IIGLMVGGVV IA.

The two lysines, K16 and K28, which are being replaced by alanine in our study, are shown in bold font. The primary structure of $A\beta_{40}$ includes the N-terminal 40 amino acids of the $A\beta_{42}$ sequence and differs from $A\beta_{42}$ by the absence of the C-terminal amino acids IA. At neutral pH, both $A\beta_{40}$ and $A\beta_{42}$ have 6 negatively charged and 3 positively charged amino acids, giving both WT peptides a net charge of -3 . In each of the four $A\beta$ analogues, [A16] $A\beta_{40}$, [A16] $A\beta_{42}$, [A28] $A\beta_{40}$, and [A28] $A\beta_{42}$, a positively charged lysine is substituted by an uncharged alanine, thereby reducing the net charge of each mutant peptide to -4 . An experimental study by Sinha et al. revealed a puzzling effect of the two single amino acid substitutions, [K16A] and [K28A], on $A\beta_{40}$ and $A\beta_{42}$ toxicity to cell cultures [42]. Whereas the [K16A] substitution significantly reduced $A\beta_{40}$ and $A\beta_{42}$ toxicity, the [K28A] substitution did not have a strong effect [42]. As the two substitutions change the overall charge of WT peptides in the same way, the difference in the resulting ability to mediate toxicity may be of a structural origin. In the following, the oligomerization pathways and structures of [A16] $A\beta_{40}$, [A16] $A\beta_{42}$, [A28] $A\beta_{40}$, and [A28] $A\beta_{42}$ are analyzed and compared to the corresponding pathways and structures of WT peptides.

3.1 K→A substitutions promote $A\beta$ oligomerization

The convergence of 40×10^6 time-units-long DMD trajectories to quasi steady state populations was determined by monitoring time evolution of the potential energy and oligomer size distributions. Our data showed that the potential energy converged after $\sim 20 \times 10^6$ time units. For each peptide under study, time evolution of oligomer sizes was quantified by calculating the oligomer size distribution every 10×10^6 time units (Fig. S1). The starting configuration contained only monomers as reflected in a peak at the oligomer order of 1 at time 0. Oligomerization started immediately and the oligomer size distributions evolved significantly in the first 10×10^6 time units. Between 10×10^6 and 20×10^6 time units oligomerization slowed down for all $A\beta$ analogues and after 20×10^6 time units the changes in oligomer size distributions were mostly due to statistical fluctuations. The DMD4B-HYDRA-derived quasi steady state oligomer size distributions of all studied $A\beta$ analogues are depicted in Fig. 1. The effect of the [K16A] and [K28A] mutations on the quasi steady state oligomer size distribution of $A\beta_{40}$ is statistically significant ($p < 0.01$) as verified by a multidimensional χ^2 test. Unlike $A\beta_{40}$ solubility, which does not appear to be strongly affected by the two K→A substitutions, the two substitutions decrease $A\beta_{42}$ solubility. This decrease is particularly significant for [A28] $A\beta_{42}$ (Fig. 1b).

Fig. 1 Oligomer size distribution of (a) A β_{40} , [A16]A β_{40} , [A28]A β_{40} and (b) A β_{42} , [A16]A β_{42} and [A28]A β_{42} . The error bars correspond to the SEM values

For dimers through tetramers, the [A16]A β_{40} and [A28]A β_{40} oligomer size distributions qualitatively follow the trend of the A β_{40} oligomer size distribution (Fig. 1a). In all three A β_{40} analogues, dimers represent the most abundant oligomers. Relative to A β_{40} , the mutant peptides display significantly increased propensity for pentamer through decamer formation. It is well known that hydrophobicity drives protein folding and assembly and A β_{42} , which has two additional hydrophobic amino acids at the C-terminus, forms on average larger oligomers than A β_{40} , a feature captured by the DMD4B-HYDRA approach [45]. Because alanine is more hydrophobic than lysine, increased propensity to form larger oligomers in A β_{40} mutants (relative to A β_{40}) is observed as expected (Fig. 1a). Comparing simulated quasi steady state oligomer size distributions of the three A β_{40} analogues to the corresponding PICUP/SDS-PAGE data [42], a qualitative agreement for low-order oligomers up to tetramers is observed. In our simulations, however, both [A16]A β_{40} and [A28]A β_{40} display increased propensities to form larger oligomers (Fig. 1a), which was not detected by PICUP/SDS-PAGE, possibly due to a lower cross-linking efficiency and diminished resolution for detection of larger oligomers. The increased self-assembly propensity of [A28]A β_{40} in our simulations is consistent with electron microscopy images, which revealed abundant short fibrils, implying a high nucleation rate for this peptide [42].

In contrast to the two A β_{40} mutants, for which the low-order oligomer propensities follow the qualitative trend of A β_{40} oligomer propensities, the [A16]A β_{42} and [A28]A β_{42} oligomer size distributions differ significantly from the A β_{42} oligomer size distribution (Fig. 1b), which is in a general agreement with PICUP/SDS-PAGE results [42]. In our simulations, [A16]A β_{42} dimer and trimer propensities decrease, whereas the tetramer propensity increases relative to the case of A β_{42} (Fig. 1b), which is in a qualitative agreement with the the PICUP/SDS-PAGE data [42]. [A28]A β_{42} trimers are the most abundant [A28]A β_{42} oligomers in our simulations. Relative to A β_{42} , the oligomer size distribution of [A16]A β_{42} exhibits significantly fewer oligomers larger than nonamers (Fig. 1b). Similar to the case of [A28]A β_{40} , the [A28]A β_{42} oligomer size distribution displays increased tetramer through octamer propensity compared to the WT distribution, which again was not captured by PICUP/SDS-PAGE. The shift toward larger [A28]A β_{42} oligomers observed in our simulations (Fig. 1b), which is a result of increased hydrophobicity of the mutant peptide, is

consistent with AFM images of A β fibril morphology, suggesting an increased nucleation rate of [A28]A β_{42} relative to the WT peptide [42].

3.2 [K16A] and [K28A] distinctly affect the free energy landscapes of A β_{40} and A β_{42} conformational ensembles

Free energy landscapes of entire ensembles of conformations (irrespective of their assembly state) of all six A β peptides are shown in Fig. 2. The two reaction coordinates, N-to-C distance and hydrophobic CG-SASA are chosen as measures of a degree of peptide extension within the conformation and an ability of peptides to shield hydrophobic residues from the solvent, respectively, as they are expected to be sensitive to the K $\rightarrow$ A substitutions. A comparison of six free energy landscapes in Fig. 2 shows that the conformational ensembles of both A β_{40} and A β_{42} are affected by the two substitutions. To quantify the K $\rightarrow$ A-induced changes in A β_{40} and A β_{42} free energy landscapes, 1D projections of the free energy to the two reaction coordinates are shown in Fig. 3.

The results in Fig. 3, including the Mann-Whitney U test comparisons of distributions for each mutant peptide to those of the WT peptide with the resulting *p*-values (caption of Fig. 3), confirm that the two substitutions, [K16A] and [K28A], exert statistically significant effects on A β_{40} and A β_{42} conformational ensembles. Both [A16]A β_{40} and [A28]A β_{40} conformations display increased N-to-C distances (Fig. 3a) and higher CG-SASA values (Fig. 3b) than A β_{40} conformations. This effect is more pronounced for [A28]A β_{40} than for [A16]A β_{40} (Fig. 3a,b). Whereas the two substitutions induce similar changes to the A β_{40} conformational ensembles (increased N-to-C distances and higher CG-SASA values), the two substitutions affect A β_{42} conformational ensembles in a substitution-specific way (Fig. 3c,d). The N-to-C distance distribution of for the [A16]A β_{42} ensemble is shifted toward smaller values relative to the WT distribution, while the N-to-C distance distribution of [A28]A β_{42} is indistinguishable (in a statistical sense) from the WT distribution (Fig. 3c). Flexibility and solvent accessibility of the N-terminal region of A β_{42} , reflected in increased N-to-C distances, has been postulated to be the key structural feature that mediates

Fig. 2 Free energy landscapes of entire A β_{40} , [A16]A β_{40} , [A28]A β_{40} , A β_{42} , [A16]A β_{42} and [A28]A β_{42} populations with respect to two reaction coordinates: the distance between the N- and C-terminal C α beads and the sum of CG-SASA values over all hydrophobic amino acids. The color scale on the right side is expressed in $k_B T$. For clarity, all conformations with the free energy values larger than $6k_B T$ (occurrence probability smaller than 2.5×10^{-3}) are excluded

Fig. 3 1D projections of free energy landscapes onto each of the two reaction coordinates: **(a,c)** the N-to-C distance and **(b,d)** the hydrophobic CG-SASA for entire **(a–b)** A β_{40} , [A16]A β_{40} , and [A28]A β_{40} and **(c–d)** A β_{42} , [A16]A β_{42} , and [A28]A β_{42} ensembles. A Mann-Whitney U test comparison of [A16]A β_{40} and [A28]A β_{40} N-to-C distance **(a)** and hydrophobic CG-SASA **(b)** distributions to the respective A β_{40} distributions results in $p < 10^{-3}$ for both reaction coordinates and for both mutants. A similar comparison of [A16]A β_{42} and [A28]A β_{42} N-to-C distance distributions to that of A β_{42} results in $p < 10^{-3}$ and $p = 0.11$, respectively **(c)**. Comparing [A16]A β_{42} and [A28]A β_{42} hydrophobic CG-SASA distributions to that of A β_{42} , p -values of 0.02 and $< 10^{-3}$ are found **(d)**

toxicity [48]. The distinct effect of the two substitutions on N-to-C distances may explain why [A16]A β_{42} (reduced N-to-C distances) displays significantly lower toxicity to cell cultures, whereas cell culture toxicity of [A28]A β_{42} (no significant effect on N-to-C distances) is not strongly affected [42]. Both substitutions increase CG-SASA values of A β_{40} conformations, implying increased aggregation propensities of both [A16]A β_{40} and [A28]A β_{40} (Fig. 3b). The distribution of CG-SASA values for the [A16]A β_{42} ensemble is slightly shifted to lower values. On the other hand, an opposite effect is noted for the CG-SASA distribution of the [A28]A β_{42} ensemble (Fig. 3d). These substitution-specific changes to the CG-SASA distribution of A β_{42} ensembles are consistent with a smaller number of oligomers larger than decamers for [A16]A β_{42} (relative to A β_{42}) as well as increased oligomerization propensity of [A28]A β_{42} compared to that of A β_{42} (Fig. 1b).

3.3 [K16A] and [K28A] distinctly affect β -strand content of A β_{40} and A β_{42}

To quantify [K16A]- and [K28A]-induced secondary structure changes in A β_{40} and A β_{42} conformations (irrespective of the assembly state), we calculated the average turn and β -strand (as the two most prominent secondary structure elements) propensities per amino acid (Fig. 4). In addition to a local increase in the β -strand content at positions 16 and 28 in [A16]A β_{40} , [A16]A β_{42} , [A28]A β_{40} , and [A28]A β_{42} conformations (relative to WT data), larger secondary structure changes at more distant regions are observed. Overall, a decrease in β -strand content is associated with an increase in turn propensities and *vice versa*.

Fig. 4 (a,c) Turn and (b,d) β -strand propensity per amino acid for monomers through hexamers of A β ₄₀, [A16]A β ₄₀, [A28]A β ₄₀, A β ₄₂, [A16]A β ₄₂ and [A28]A β ₄₂

In A β ₄₀ ensembles, the most notable K→A-induced change in the secondary structure occurs at the N-terminus: whereas A β ₄₀ ensembles are characterized by high β -strand content in the A2–F4 region, this region lacks any β -strand structure in [A16]A β ₄₀ and [A28]A β ₄₀ ensembles (Fig. 4b). Instead, [A16]A β ₄₀ and [A28]A β ₄₀ ensembles display elevated β -strand content in the R5–V12 region, a feature in common with ensembles of A β ₄₂ and its two analogues (Fig. 4b,d). [A16]A β ₄₀ and [A28]A β ₄₀ conformations also display lower β -strand propensities in the C-terminal region L34–V39 than A β ₄₀ ensembles (Fig. 4b). Because the C-terminal region participates in fibril formation, an overall decrease in β -strand content in [A16]A β ₄₀ and [A28]A β ₄₀ ensembles may result in a slower conversion into the β -sheet structure. Indeed, time evolution of the CD spectra revealed a slower conformational transition into a β -sheet structure for [A16]A β ₄₀ and an even more delayed if not incomplete transition for [A28]A β ₄₀ in comparison to A β ₄₀ [42]. [A16]A β ₄₀ (but not [A28]A β ₄₀) conformations display decreased β -strand content in the H13–H14 region (Fig. 4b). Only [A28]A β ₄₀ conformations exhibit decreased β -strand and concomitantly shifted and increased turn propensities in the V24–G29 region (Fig. 4a,b), implying a more stable loop/turn structure in [A28]A β ₄₀ conformations.

In A β ₄₂ ensembles, [K16A] and [K28A]-induced changes in the turn and β -strand propensities are less pronounced than in A β ₄₀ ensembles (Fig. 4c,d). Of the two substitutions, [K16A] has a larger effect on the secondary structure of A β ₄₂ than [K28A]. Relative to A β ₄₂, [A16]A β ₄₂ (but not [A28]A β ₄₂) ensembles exhibit reduced β -strand content within the N-terminal region (Y10–H14), central hydrophobic cluster (L17–F19) and C-terminal region (M35–V40) as shown in Fig. 4d. Because the ability to form β -strand structure has been associated with an ability to mediate toxicity, reduced β -strand content in [A16]A β ₄₂ conformations is consistent with the observed reduced toxicity of this peptide in cell cultures [42]. [A28]A β ₄₂ (but not [A16]A β ₄₂) conformations show decreased β -strand content in the V24–S26 region and shifted/increased turn propensity in this region (Fig. 4c,d), similar to [K28A]-induced changes in A β ₄₀ conformations.

Average turn and β -strand propensities in A β_{40} and A β_{42} peptides were also calculated for each assembly state separately, for monomers through hexamers. Results are displayed in Fig. S2 for A β_{40} , [A16]A β_{40} , [A28]A β_{40} conformations and in Fig. S3 for A β_{42} , [A16]A β_{42} , [A28]A β_{42} conformations. The above observations of K $\rightarrow$ A-induced secondary structure changes appear to be mostly independent of the assembly state.

3.4 [K16A] and [K28A] substitutions induce alloform- and oligomer order-specific changes to free energy landscapes

Free energy landscapes for populations of monomers through hexamers of all six peptides are displayed in Fig. 5. All A β peptides feature monomer ensembles with high hydrophobic CG-SASA values, whereas dimers and in particular larger oligomers populate lower CG-SASA values, reflecting their ability to shield hydrophobic residues from the solvent, resulting in a decreased population of conformations with large (>5 nm²) hydrophobic CG-SASA values. [A16]A β_{40} and [A28]A β_{40} dimers and trimers have slightly increased hydrophobic CG-SASA values when compared to A β_{40} dimers and trimers. Notably, [A16]A β_{40} and [A28]A β_{40} trimer populations contain conformations with increased N-to-C distance relative to A β_{40} trimer populations. [A16]A β_{40} and [A28]A β_{40} display increased populations of pentamers and hexamers relative to those of A β_{40} . [A16]A β_{40} and [A28]A β_{40} trimers through hexamers exhibit similar free energy landscapes. Relative to A β_{40} , A β_{42} dimers populate increased N-to-C distances and hydrophobic CG-SASA values. [A16]A β_{42} and [A28]A β_{42} dimer and trimer ensembles exhibit increased hydrophobic CG-SASA values relative to those of A β_{42} dimers and trimers, respectively. Unlike

Fig. 5 Free energy landscapes of A β_{40} , [A16]A β_{40} , [A28]A β_{40} , A β_{42} , [A16]A β_{42} and [A28]A β_{42} monomers through hexamers conformations in the space of two reaction coordinates: the distance between the N- and C-terminal C α beads and the sum of CG-SASA values over all hydrophobic amino acids. The color scale on the right side is expressed in units of $k_B T$. The black rectangle in each landscape is a visual guide and corresponds to the area covered by A β_{42} dimer conformations

[A28] $A\beta_{42}$ trimers, [A16] $A\beta_{42}$ trimers shows a shift toward smaller N-to-C distances relative to those of $A\beta_{42}$ trimers. Both [A16] $A\beta_{42}$ and [A28] $A\beta_{42}$ tetramer ensembles are significantly larger than the $A\beta_{42}$ tetramer ensemble. [A16] $A\beta_{42}$ tetramers (but not [A28] $A\beta_{42}$ tetramers) exhibit smaller N-to-C distances. As trimers and tetramers are the most abundant [A16] $A\beta_{42}$ and [A28] $A\beta_{42}$ oligomers in our simulations, these substitution-specific structural changes might be relevant to understanding significantly decreased cell culture toxicity of [A16] $A\beta_{42}$ (but not [A28] $A\beta_{42}$) relative to $A\beta_{42}$ toxicity [42].

Figure 5 shows an elevated free energy at large N-to-C terminal distances (the reaction coordinate on the x -axis) for oligomers larger than trimers of all $A\beta$ peptides with an exception of $A\beta_{40}$. A previous DMD4B-HYDRA study, which compared $A\beta_{40}$ and $A\beta_{42}$ oligomer structures, reported an increased N-terminal flexibility and solvent accessibility of $A\beta_{42}$ relative $A\beta_{40}$ oligomers [44]. This structural plasticity of N-termini in $A\beta_{42}$ oligomers is reflected in an increased population of conformations with increased N-to-C terminal distances observed in Fig. 5. Interestingly, both K→A substitutions increase structural plasticity at N-termini of $A\beta_{40}$ oligomers, which is concomitant with the loss of the β -strand in the A2–F4 region (Fig. 4b). The effect of the two K→A substitutions on N-terminal plasticity of $A\beta_{42}$ oligomers depends on oligomer order and is substitution-specific. The hydrophobic CG-SASA (the reaction coordinate on the y -axis) measures solvent exposure of hydrophobic residues within oligomers and thus their propensity to assemble into larger oligomers. To better visualize the effect of the two substitutions on solvent exposure, the dependence of the hydrophobic CG-SASA on oligomer order is displayed in Fig. S4 for $A\beta_{40}$ and $A\beta_{42}$ analogues (panels A and B, respectively). Both K→A substitutions significantly increase the solvent exposure of $A\beta_{40}$ monomers through tetramers. [K28A] $A\beta_{40}$ dimers, tetramers, pentamers, and hexamers are characterized by larger hydrophobic CG-SASA values than their [K16A] $A\beta_{40}$ counterparts. The [K16A] substitution increases the hydrophobic CG-SASA of monomers, dimers, trimers, and pentamers of $A\beta_{42}$, whereas the [K28A] substitution increases the hydrophobic CG-SASA of all $A\beta_{42}$ oligomers other than tetramers. Of the two substitutions, [K28A] causes a significantly larger increase in the hydrophobic CG-SASA in $A\beta_{42}$ oligomers than [K16A]. These substitution-specific changes in the hydrophobic CG-SASA provide insight into why the two substitutions exert distinct effects on the oligomer size distribution (Fig. 1). Because oligomers formed by [A28] $A\beta$ analogues have an increased hydrophobic CG-SASA relative to those formed by [A16] $A\beta$ analogues, [A28] $A\beta_{40}$ ([A28] $A\beta_{42}$) is prone to form larger oligomers than [A16] $A\beta_{40}$ ([A16] $A\beta_{42}$), which can be verified in Fig. 1 (red versus blue bars in panels A and B). These findings show that despite identical amino acid compositions (albeit distinct sequences), [A16] $A\beta_{40}$ ([A16] $A\beta_{42}$) and [A28] $A\beta_{40}$ ([A28] $A\beta_{42}$) form oligomers with distinct structural features. These findings highlight the importance of the amino acid sequence and show that the local amino acid environment strongly affects oligomer formation and resulting structures.

3.5 [K16A] and [K28A] alter the β -strand content in alloform- and oligomer order-specific way

Figure 6 shows the average turn, strand, and coil content of each of the six peptides for monomers through hexamers. Overall, turns (30–40%) dominate the average secondary structure, followed by coil (~30%), and β -strand (~20%) content. Because dimers and trimers of $A\beta_{40}$ and its two analogues represent the most abundant oligomers, we here comment on the secondary structure changes in monomers, dimers, and trimers of $A\beta_{40}$ due to [K16A] and [K28A] substitutions. [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$ monomers display an

Fig. 6 The average (a,d) turn, (b,e) β -strand, and (c,f) content in A β_{40} , [A16]A β_{40} , [A28]A β_{40} , A β_{42} , [A16]A β_{42} and [A28]A β_{42} populations in dependence on the oligomer order. First, the average turn and β -strand content were calculated for each conformation in the monomer ensemble or in the ensemble of oligomers of specific order. Then, the average over the entire ensemble and the corresponding SEM values were derived

increased coil content relative to A β_{40} monomers (Fig. 6c). In comparison to A β_{40} dimers, [A16]A β_{40} dimers exhibit increased turn and decreased β -strand content (Fig. 6a–c). Similarly, [A28]A β_{40} dimers exhibit an increased coil and concomitantly decreased β -strand content (Fig. 6a–c). Increased turn and decreased β -strand content characterizes also [A16]A β_{40} and [A28]A β_{42} trimers. Additionally, [A28]A β_{42} trimers (but not [A16]A β_{40} trimers) display increased coil content. Thus, the ability to form the β -strand structure in [A16]A β_{40} and [A28]A β_{40} dimers and trimers is reduced. [A16]A β_{42} monomers, dimers, and trimers exhibit significantly increased turn (Fig. 6d) and decreased β -strand content relative to A β_{42} monomers, dimers, and trimers (Fig. 6e), whereas coil content is not strongly affected (Fig. 6f). In contrast, [A28]A β_{42} monomers and trimers display decreased turn (Fig. 6d) and increased β -strand (Fig. 6e) content relative to A β_{42} monomers and trimers, however, the effect of [K28A] substitution on the secondary structure of A β_{42} oligomers is not as large as that of [K16A] substitution. While [A16]A β_{42} pentamers exhibit decreased β -strand content, [A28]A β_{42} hexamers show significantly increased ability to form β -strand structure than either A β_{42} or [A16]A β_{42} hexamers. If the ability to form β -strand oligomers is associated with the ability of A β oligomers to exert toxicity, then the decrease in β -strand in [A16]A β_{42} dimers, trimers, and pentamers may be linked to observed significantly lower toxicity of [A16]A β_{42} relative to A β_{42} [42].

3.6 [K16A] and [K28A] affect tertiary and quaternary structure of oligomers

Figures S5 and S7 show tertiary contact maps of monomers through hexamers formed by A β_{40} and A β_{42} analogues, respectively. Oligomer state-specific quaternary contact maps for A β_{40} and A β_{42} analogues are displayed in Figs. S6 and S8, respectively. Overall, tertiary and quaternary contacts do not depend strongly on the oligomer state. This feature appears to be specific to A β as other proteins, such as stefin B-var2 form oligomers with oligomer order-specific tertiary and quaternary contacts [50]. Tertiary contacts of [A16]A β_{40} and

[A28] $A\beta_{40}$ conformations are not affected by K $\rightarrow$ A substitutions (Fig. S5) and similarly, tertiary contacts of [A16] $A\beta_{42}$ and [A28] $A\beta_{42}$ conformations show only small deviations from tertiary contacts of $A\beta_{42}$ conformations (Fig. S7). In contrast, quaternary contacts are significantly affected by K $\rightarrow$ A substitutions in a substitution- and alloform-specific way but the substitution-induced changes are similar for dimers through hexamers of each of the six peptides (Figs. S6 and S8).

Dimerization is the earliest assembly event in $A\beta$ assembly. It is thus important to understand in which way the two substitutions affect the interactions among residues and dimer structure. Because the two substitutions exert distinct effects on cell toxicity, we here also focus on substitution-specific structural changes in $A\beta_{40}$ and $A\beta_{42}$ dimers. Figures 7 and 8

Fig. 7 (a) turn and β -strand propensity of dimers of $A\beta_{40}$, [A16] $A\beta_{40}$, [A28] $A\beta_{40}$. (b) free energy landscape of dimers of $A\beta_{40}$, [A16] $A\beta_{40}$, [A28] $A\beta_{40}$ in the space of two reaction coordinates: the distance between the N- and C-terminal C_α beads and the sum of CG-SASA values over all hydrophobic amino acids. The color scale on the right side is expressed in $k_B T$. (c) intramolecular and intermolecular contact maps of dimers of $A\beta_{40}$, [A16] $A\beta_{40}$, [A28] $A\beta_{40}$. C_α - C_α contacts are displayed in the upper left triangle and C_β - C_β contacts in the lower right triangle. The color scale is displayed on the right

Fig. 8 (a) turn and β -strand propensity of dimers of A β_{42} , [A16]A β_{42} , [A28]A β_{42} . (b) free energy landscape of dimers of A β_{42} , [A16]A β_{42} , [A28]A β_{42} in the space of two reaction coordinates: the distance between the N- and C-terminal C α beads and the sum of CG-SASA values over all hydrophobic amino acids. The color scale on the right side is expressed in $k_B T$. (c) intramolecular and intermolecular contact maps of dimers of A β_{42} , [A16]A β_{42} , [A28]A β_{42} . C α -C α contacts are displayed in the upper left triangle and C β -C β contacts in the lower right triangle. The color scale is displayed on the right

show the extent to which the two substitutions alter the secondary, tertiary, and quaternary structure of A β_{40} and A β_{42} dimers, respectively.

3.6.1 Characterization of [A16]A β_{40} and [A28]A β_{40} dimers

[A16]A β_{40} dimers exhibit significantly increased turn propensities at the N-terminal region G9–Q15 and at the C-terminal region V36–V40 when compared to [A28]A β_{40} dimers and even more so when compared to A β_{40} dimers (Fig. 7a, left panel). [A28]A β_{40} dimers display enhanced turn and decreased β -strand propensities in the central folding region A21–N27 (Fig. 7a). Unlike A β_{40} dimers, [A16]A β_{40} and [A28]A β_{40} dimers do not form a

β -strand at A2–F4. Instead, dimers formed by [A16]A β_{40} and [A28]A β_{40} exhibit increased β -strand content in the N-terminal region R5–H13 (Fig. 7a, right panel). Relative to [A16]A β_{40} dimers, [A28]A β_{40} dimers have increased β -strand content in regions E11–Q15, L17–V18, and L34–V36 and decreased β -strand content in the central folding region A21–N27 (Fig. 7a, right panel).

Free energy landscapes of [A16]A β_{40} and [A28]A β_{40} dimers are statistically distinct from that of A β_{40} dimers in hydrophobic CG-SASA distribution ($p < 10^{-3}$, Mann-Whitney U test). Hydrophobic residues of [A16]A β_{40} dimers and even more so of [A28]A β_{40} dimers are significantly more exposed to the solvent than those of A β_{40} dimers (Fig. 7b). The N-to-C distances in [A16]A β_{40} and [A28]A β_{40} dimers are shifted to larger values relative to those of A β_{40} dimers ($p = 0.06$ and $p < 10^{-3}$, respectively).

Figure 7c shows tertiary and quaternary contacts in A β_{40} , [A16]A β_{40} , and [A28]A β_{40} dimers. Whereas tertiary contacts in dimers formed by the two mutant A β_{40} peptides do not appear to be affected by either of the two substitutions (Fig. 7c, top maps), quaternary contacts are strongly affected in a substitution-specific way (Fig. 7c, bottom maps). This result is interesting by itself as it demonstrates that although these two substitutions do not strongly affect folding, they affect dimerization in a substitution-specific way. The key quaternary contacts in A β_{40} dimer formation are contacts between central hydrophobic clusters (L17–A21) of the two peptides in a dimer, followed by contacts between the central hydrophobic cluster of one and the C-terminal dipeptide region V39–V40 of the other peptide (Fig. 7c, bottom left map). In [A16]A β_{40} dimers, the latter quaternary contacts are even stronger and quaternary contacts between the central hydrophobic cluster of one and the dipeptide region I31–I32 of the other peptide are strongly enhanced (Fig. 7c, bottom center map). The same, albeit less enhanced, quaternary contacts between the central hydrophobic cluster of one and the dipeptide region I31–I32 of the other peptide are observed in [A28]A β_{40} dimers, which display additional enhanced quaternary contacts between C-terminal regions V40–V41 of the two peptides in a dimer (Fig. 7c, bottom right map). Thus, [A16]A β_{40} and [A28]A β_{40} dimers feature enhanced quaternary contacts with the central hydrophobic cluster and with C-terminal region, respectively. Overall stronger quaternary contacts in [A16]A β_{40} and [A28]A β_{40} dimers (relative to A β_{40} dimers) result in an increased free energy needed for a structural conversion into fibrils, resulting in slower aggregation kinetics, which was indeed reported by Sinha et al. in their in vitro study [42].

3.6.2 Characterization of [A16]A β_{42} and [A28]A β_{42} dimers

Of dimers formed by the three A β_{42} analogues, [A16]A β_{42} dimers display overall the largest turn and the lowest β -strand propensities (Fig. 8a, left panel), indicating increased compactness of [A16]A β_{42} dimer conformations in comparison to A β_{42} as well as [A28]A β_{42} dimers. In comparison to A β_{42} dimers, [A16]A β_{42} dimers show strongly increased turn propensities in several regions along the sequence D7–N8, H13–Q15, A21–V24, and V36–G37 (Fig. 8a, left panel). Relative to A β_{42} dimers, [A28]A β_{42} dimers exhibit notably decreased turn propensities at the N-terminal region Q15–L17, and the C-terminal region V36–G38, whereas regions within or close to the central folding region V24–S26 and A30–I31 feature increased turn propensities (Fig. 8a, left panel). [A16]A β_{42} dimers display either similar or lower β -strand propensities than A β_{42} dimers throughout the sequence except in the dipeptide region S26–N27 (Fig. 8a, right panel). β -Strand propensities of [A28]A β_{42} dimers are overall higher than those of [A16]A β_{42} dimers except in regions R5–H6, G9–Y10, and G25–N27 (Fig. 8a, right panel). Importantly, the C-terminal region

G33–G38 is characterized by the highest β -strand propensity in [A28]A β_{42} dimers, followed by A β_{42} dimers, and [A16]A β_{42} dimers, which exhibit the lowest β -strand content. Free energy landscapes of the three A β_{42} analogues are shown in Fig. 8b.

Free energy landscapes of [A16]A β_{42} and [A28]A β_{42} dimers are shifted to higher hydrophobic SASA values ($p < 10^{-3}$ for both mutant peptides, Mann-Whitney U test comparison of 1D distributions along the hydrophobic CG-SASA reaction coordinate) when compared to that of A β_{42} dimers. The two substitutions also induce statistically significant effects on the N-to-C distance distribution ($p < 10^{-3}$ for both mutant peptides, Mann-Whitney U test comparison of 1D distributions along the N-to-C distance reaction coordinate). [A16]A β_{42} dimers and even more so [A28]A β_{42} dimers display decreased populations of dimers with very small distances < 1 nm (Fig. 8b). [A16]A β_{42} dimers exhibit the lowest population of the largest N-to-C distances > 2.5 nm. The absence of these largest N-to-C distances in [A16]A β_{42} dimers is consistent with their more compact structure, which imposes restrictions on the N-to-C distance distribution. The more compact structure of [A16]A β_{42} relative to [A28]A β_{42} dimers may underlie the reduced toxicity of the former but not much of the latter analogue to cell cultures [42].

Figure 8c shows tertiary and quaternary contact maps for dimers of the three A β_{42} analogues. Unlike the tertiary maps for A β_{40} analogues, which are not affected by the two substitutions, tertiary contacts between the central hydrophobic cluster and C-terminal region are enhanced in particular in [A28]A β_{42} dimers (Fig. 8c, upper right map). Quaternary contacts play a dominant role in A β_{42} assembly as reported previously [44, 51] (Fig. 8c, lower left map). Notably, these quaternary contacts are significantly weaker in [A16]A β_{42} dimers (Fig. 8c, lower center map) but not in [A28]A β_{42} dimers (Fig. 8c, lower right map). Compared to A β_{42} dimers, both [A16]A β_{42} and [A28]A β_{42} dimers exhibit stronger quaternary contacts between the central hydrophobic clusters of the two peptides in a dimer and between the central hydrophobic cluster of one peptide and C-terminal region of the other peptide in a dimer. However, [A28]A β_{42} dimers are more similar to A β_{42} dimers with respect to the dominant role of the C-terminal region in dimerization (Fig. 8c, lower left and right maps). In contrast, the quaternary structure of [A16]A β_{42} dimers is dominated by the central hydrophobic cluster, which is the key region in [A16]A β_{42} dimer formation (Fig. 8c, lower center map), a feature that is observed in the quaternary structure of A β_{40} dimers (Fig. 7c, lower left map). The dominance of the C-terminal region in A β_{42} but not A β_{40} assembly is structurally linked to increased flexibility and solvent exposure of the N-terminal region in A β_{42} relative to A β_{40} oligomers, which was postulated to underlie distinct abilities of the two peptides to mediate toxicity [44, 48]. As the C-terminal region dominates quaternary contacts of A β_{42} and [A28]A β_{42} dimers but not [A16]A β_{42} dimers, this same hypothesis thus provides a plausible explanation of why the [K16A] substitution but not the [K28A] substitution inhibits A β_{42} toxicity [42].

3.7 [K16A] and [K28A] substitutions alter solvent exposure and amino acid arrangement in A β oligomers

To gain insight into solvent exposure of individual residues and their arrangement within oligomers of different orders, the CG-SASA and distance from the center of mass per amino acid are displayed in Figs. S9 and S10 for A β_{40} and A β_{42} analogues, respectively. In addition to decreased CG-SASA and distance values at positions 16 and 28, in [A16]A β and [A28]A β analogues, respectively, which are expected upon replacing a solvent-exposed charged residue by a hydrophobic residue, additional non-local changes in CG-SASA and distance values are noted. Relative to A β_{40} conformations, [A16]A β_{40} and [A28]A β_{40}

conformations exhibit increased CG-SASA and distance values in the N-terminal region for monomers through hexamers, indicating an increased solvent exposure and flexibility of the N-terminal region, in particular at A2 and F4 (Fig. S9). This effect is slightly more pronounced in [A28]A β ₄₀ than in [A16]A β ₄₀ conformations and is the strongest in trimers and tetramers (Fig. S9). The exception is H6, which is significantly more shielded from the solvent in all [A16]A β ₄₀ and [A28]A β ₄₀ conformations. The two substitutions induce less pronounced changes to CG-SASA and distance from the center of mass in A β ₄₂ analogues; however, most [A16]A β ₄₂ conformations have lower N-terminal CG-SASA and distance values than [K28A]A β ₄₂ conformations (Fig. S10). Specifically, [A16]A β ₄₂ tetramers and pentamers have significantly decreased CG-SASA and distance values at the N-terminal relative to [A28]A β ₄₂ and A β ₄₂ tetramers and pentamers, which exhibit overlapping values.

3.8 [K16A] and [K28A] substitutions affect oligomer growth and morphology

Morphology of oligomers can be further quantified by three principal moments of inertia, I_1 , I_2 , and I_3 , whereby $I_1 = I_2 = I_3$ would correspond to perfectly spherical objects and $I_1 \approx I_2 > I_3$ would characterize elongated objects (beam- or cigar-shaped). We calculated the principal moments of inertia for monomers through decamers of all six A β peptides to examine the oligomer morphologies and find out whether or not the two substitutions affect the scaling of the principal moments of inertia with oligomer order. We recently reported the results of a similar analysis applied to scaling of oligomers formed by a globular amyloidogenic protein, stefin B-var2 [50].

We assume that the principal moments of inertia I_i ($i = 1, 2, 3$) scale with oligomer order n as $I_i \propto n^{\alpha_i}$ ($i = 1, 2, 3$). To verify our assertion and find scaling exponents α_i for each of the six A β peptides, we calculated the three principal moments of inertia for monomers through decamers of each of the six A β peptides and display data in a log-log plot (Fig. 9). These plots demonstrate that (i) oligomers are cylindrically elongated structures ($I_1 \approx I_2 > I_3$) and (ii) the three moments of inertia scale with oligomer order if data for monomers and dimers are excluded. Fitting the remaining data (for trimers through decamers) to a straight line within each log-log plot results in six sets of α_i ($i = 1, 2, 3$) as reported in Table 1 along with their SEM values. Note that α_1 and α_2 for A β ₄₀ tend to be larger than the corresponding exponents for A β ₄₂ whereas α_3 is smaller for A β ₄₀ than for A β ₄₂, indicating that A β ₄₂ oligomers are in morphology closer to quasi-spherical objects and A β ₄₀ are more elongated as lateral dimensions of A β ₄₂ conformations grow with oligomer order faster than lateral dimensions of A β ₄₀ conformations. The two substitutions induce statistically significant changes to all three scaling exponents α_i ($i = 1, 2, 3$) corresponding to A β ₄₀ conformations. In contrast, only α_3 for [A16]A β ₄₂ oligomers is significantly different from α_3 for A β ₄₂ oligomers. All three scaling exponents for [A28]A β ₄₂ are statistically indistinguishable from respective scaling exponents for A β ₄₂ (Table 1).

To gain more intuitive insight into the meaning of the scaling exponents, we take into account that oligomers are cylindrically elongated beam-like objects, such that their two principal moments of inertia, $I_1 \approx I_2 \sim ML^2$ and $I_3 \sim MD^2$, where L and D are the length and thickness of the rod, respectively, and the mass of each assembly M is proportional to the number of peptides, $M = M_0n$ (where M_0 is the mass of a monomer). Defining new scaling exponents, α , β , γ , for scaling of the length $L \sim n^\alpha$, thickness $D \sim n^\beta$, and volume $V \sim n^\gamma$, we can express α , β , γ by α_i ($i = 1, 2, 3$) to find $\alpha = (\alpha_1 + \alpha_2)/4 - 1/2$, $\beta = (\alpha_3 - 1)/2$, and $\gamma = (\alpha_1 + \alpha_2)/4 - 1/2 + \alpha_3 - 1$ (Table 1). Notably, $\alpha > \beta$ for all A β

Fig. 9 (a-f) Log-log plots of principal moments of inertia as a function of oligomer order for Aβ₄₀, [A16]Aβ₄₀, [A28]Aβ₄₀, Aβ₄₂, [A16]Aβ₄₂ and [A28]Aβ₄₂. A straight line fit includes trimers through decamers. The SEM values are smaller than the data point size

peptides, indicating that elongation is faster than lateral growth for oligomers up to decamers, consistent with an assembly process through protofibril formation. A comparison of scaling exponents γ across the six peptides shows that $\gamma \approx 1.25$ for all six peptides, which implies similar (fractal) scaling of mass with volume ($\gamma^{-1} \approx 0.83$). A similar value of γ was previously reported for scaling of the volume of stefin B-var2 oligomers with oligomer order (oligomer mass) [50]. The elongation exponent α and the thickness exponent β are the largest and smallest, respectively, for Aβ₄₀, which features the most elongated oligomer morphology. These two exponents are significantly different for Aβ₄₂, which forms more spherically-shaped oligomers than Aβ₄₀. Both [A16]Aβ₄₀ and [A28]Aβ₄₀ are characterized by smaller α and larger β than Aβ₄₀ (Table 1), indicating that both substitutions result in formation of less elongated assemblies. In contrast, only [A16]Aβ₄₂ exhibits

Table 1 Scaling exponents of the three principal moments of inertia α_1 , α_2 , α_3 and the resulting oligomer elongation exponents α , β , γ with their respective SEM values

Peptide	α_1	α_2	α_3	α	β	γ
Aβ ₄₀	2.44 ± 0.15	2.51 ± 0.15	1.48 ± 0.02	0.74 ± 0.08	0.24 ± 0.01	1.22 ± 0.10
[A16]Aβ ₄₀	2.13 ± 0.08*	2.13 ± 0.09*	1.66 ± 0.04*	0.57 ± 0.04*	0.33 ± 0.02*	1.23 ± 0.08
[A28]Aβ ₄₀	2.20 ± 0.10*	2.20 ± 0.13*	1.78 ± 0.09*	0.60 ± 0.06*	0.39 ± 0.05*	1.38 ± 0.15
Aβ ₄₂	2.24 ± 0.08	2.18 ± 0.08	1.71 ± 0.08	0.61 ± 0.04	0.36 ± 0.04	1.32 ± 0.12
[A16]Aβ ₄₂	2.23 ± 0.06	2.26 ± 0.07	1.56 ± 0.04*	0.62 ± 0.03	0.28 ± 0.02*	1.18 ± 0.07
[A28]Aβ ₄₂	2.15 ± 0.05	2.17 ± 0.07	1.60 ± 0.07	0.58 ± 0.03	0.30 ± 0.04	1.18 ± 0.10

significantly decreased β relative to that of $A\beta_{42}$. The [K16A] substitution in $A\beta_{42}$ does not affect the scaling exponent α (Table 1). Scaling exponents are not affected by the [K28A] substitution in $A\beta_{42}$ in a statistically significant way (Table 1). If the β/α ratio is used as a measure of sphericity, then the most spherical assemblies are formed by [A28] $A\beta_{40}$, followed by $A\beta_{42}$, [A16] $A\beta_{40}$, [A28] $A\beta_{42}$, [A16] $A\beta_{42}$, and $A\beta_{40}$ with the most elongated assemblies.

3.9 Oligomerization pathways of $A\beta_{40}$ and $A\beta_{42}$ and their K→A analogues

Characteristic assembly pathways for $A\beta_{40}$ and $A\beta_{42}$ analogues are displayed in Figs. 10 and 11, respectively. Oligomer lifetimes are monitored along the assembly pathways to quantify structural stability of each oligomer conformation. The conformations along the assembly pathways are selected based on three criteria. First, on-pathway oligomers should be relatively stable, which is reflected in their lifetimes that need to be sufficiently long. If an intermediate, for example a tetramer, breaks into two conformations, which immediately self-assemble back, e.g., 4-mer $\rightarrow$ 3-mer + 1-mer $\rightarrow$ 4-mer, where the resulting trimer and monomer have lifetimes not longer than 0.1×10^6 time units, this process is attributed to statistical fluctuations and the two intermediates (trimer and monomer) are not considered as on-pathway oligomers. Second, four on-pathway oligomer species are selected for each peptide, starting from a monomer. Third, pathways with a reasonably long-lived final oligomeric species are chosen to ensure that the final oligomers are also stable. $A\beta_{40}$, [A16] $A\beta_{40}$, and [A28] $A\beta_{40}$ pathways show a progression from a monomer through dimer and trimer into tetramer (Fig. 10). $A\beta_{42}$, [A16] $A\beta_{42}$, and [A28] $A\beta_{42}$ pathways show a progression from a monomer through dimer and trimer or tetramer into pentamer or hexamer (Fig. 11). All displayed conformations include an initial monomer to visualize conformational changes that the monomer (dark blue) undergoes upon dimerization and further assembly. The assemblies with the longest lifetimes in Fig. 10 are $A\beta_{40}$, [A16] $A\beta_{40}$,

Fig. 10 Characteristic oligomerization pathways of $A\beta_{40}$, [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$ from a monomer through a dimer and trimer into a tetramer. The lifetimes of all monomers are $\leq 0.1 \times 10^6$ time units. Dimer lifetimes are 0.1×10^6 , 0.9×10^6 , and 19.6×10^6 time units for $A\beta_{40}$, [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$, respectively. Trimer lifetimes are 10.9×10^6 , 7.9×10^6 , and 0.1×10^6 time units for $A\beta_{40}$, [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$, respectively. Tetramers have the longest lifetimes with 28.9×10^6 , 31.1×10^6 , and 20.2×10^6 time units for $A\beta_{40}$, [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$, respectively. The initial monomer is depicted in dark blue. Red and black spheres indicate positions of the N-terminal and C-terminal C_α beads, respectively

Fig. 11 Characteristic oligomerization pathways of A β_{42} , [A16]A β_{42} and [A28]A β_{42} from a monomer through a dimer and larger oligomers. The lifetimes of all monomers are $\leq 0.1 \times 10^6$ time units. Dimer lifetimes are 1.1×10^6 , 0.9×10^6 , and 0.4×10^6 time units for A β_{42} , [A16]A β_{42} , and [A28]A β_{42} , respectively. Lifetimes of A β_{42} and [A28]A β_{42} trimers are 6.9×10^6 , and 0.5×10^6 time units, respectively. The lifetime of [A16]A β_{42} tetramer is 31.8×10^6 time units. Lifetimes of A β_{42} and [A28]A β_{42} pentamers are 31.9×10^6 , and 38.0×10^6 time units, respectively. The lifetime of [A16]A β_{42} hexamer is 7.2×10^6 time units. The initial monomer is depicted in dark blue. Red and black spheres indicate positions of the N-terminal and C-terminal C $_{\alpha}$ beads, respectively

and [A28]A β_{40} tetramers. The assemblies with the longest lifetimes in Fig. 11 are A β_{42} and [A28]A β_{42} pentamers and [A16]A β_{42} tetramers. Long lifetimes of oligomers of a specific order hint at their structural stability, which contributes to their increased abundance, which is reflected in the oligomer size distribution. There is a qualitative difference between pathways of A β_{40} analogues and A β_{42} analogues. Whereas for A β_{40} analogues oligomer formation proceeds through monomer attachment (monomer + monomer $\rightarrow$ dimer, dimer + monomer $\rightarrow$ trimer, trimer + monomer $\rightarrow$ tetramer) also modular self-assembly (dimer + dimer $\rightarrow$ tetramer, trimer + dimer $\rightarrow$ pentamer, tetramer + dimer $\rightarrow$ hexamer) as displayed in Fig. 11. This observation is consistent with A β_{40} versus A β_{42} oligomer size distributions, of which only the latter is multimodal as reported previously [44, 45].

4 Conclusions and discussion

Recently, Sinha et al. reported that two amino acid substitutions, [K16A] and [K28A], exert profound and distinct effects on in vitro aggregation and cell culture toxicity of the two predominant A β alloforms, A β_{40} and A β_{42} , associated with AD [42]. This experimental study provided new insights into [K16A]- and [K28A]-induced changes in in vitro aggregation, morphology, secondary structure and cell culture toxicity of A β_{40} and A β_{42} but it did not provide much information about substitution-induced structural differences in oligomers, which are postulated to be the proximate toxic species in AD [4]. Here, the effect of K $\rightarrow$ A mutations on A β_{40} and A β_{42} oligomer formation is examined by means of the DMD4B-HYDRA approach, which was previously used to characterize structural differences between A β_{40} and A β_{42} and several of their naturally occurring variants [44–

46, 49, 52]. This approach allowed us to elucidate oligomerization pathways of $A\beta_{40}$ and $A\beta_{42}$ and their analogues, in which lysine at either position 16 or 28 is replaced by alanine. The resulting oligomer structures of $A\beta_{40}$ and $A\beta_{42}$ analogues are then compared to those formed by WT peptides to identify key structural changes induced by the two substitutions. Our simulations capture early stages of $A\beta$ oligomer formation at incubation times smaller than a second, which are inaccessible experimentally, thus complementing the experimental study of Sinha et al., which examined aggregation dynamics at much longer incubation times [42]. Nonetheless, oligomer size distributions as determined by PICUP/SDS-PAGE can be qualitatively compared to those derived by the DMD4B-HYDRA approach, although simulations are by necessity conducted at significantly higher peptide concentrations than those used in vitro. Our results show that both K→A substitutions promote oligomerization in $A\beta_{40}$ analogues as observed through the effect of each substitution on the oligomer size distribution. The populations of [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$ dimers and trimers as the most represented $A\beta_{40}$ oligomer species in our simulations decrease upon each substitution, whereas the populations of pentamers and larger oligomers increase relative to $A\beta_{40}$ populations. The overall composition of oligomeric species in the system is in agreement with the available PICUP/SDS-PAGE data [42]. In our simulations, both substitutions also affect $A\beta_{42}$ size distributions. [A16] $A\beta_{42}$ exhibits a decreased dimer population and an increased tetramer population, in qualitative agreement with the PICUP/SDS-PAGE results [42].

Because toxicity is mediated by oligomers forming at early incubation times rather than fibrils that appear typically after hours of incubation [53–55], structural information on LMW oligomers is critical for elucidation of potential structural elements associated with toxicity. It is puzzling that of the two substitutions, [K16A] and [K28A], the former inhibits $A\beta$ toxicity significantly more than the latter, although they both change the overall charge of $A\beta_{40}$ and $A\beta_{42}$ in the same way [42]. We thus systematically examined the effect of the [K16A] and [K28A] substitutions on $A\beta_{40}$ and $A\beta_{42}$ oligomer secondary, tertiary, and quaternary structure as well as their morphology and assembly pathways. In our DMD4B-HYDRA force field, alanine is treated as a hydrophobic residue whereas lysine is modeled as an exclusively hydrophilic residue. Both substitutions in our study thus increase the overall hydrophobicity of the peptides, resulting in a statistically significant increase in hydrophobic CG-SASA values for conformations formed by the four $A\beta_{40}$ and $A\beta_{42}$ analogues compared to those formed by WT peptides. Our results show that relative to $A\beta_{40}$ dimers, [A16] $A\beta_{40}$ and [A28] $A\beta_{40}$ dimers lack the β -strand in the N-terminal region A2–F4, which characterizes $A\beta_{40}$ oligomers. Of the two analogues, [A16] $A\beta_{40}$ dimers exhibit lower β -strand content than [A28] $A\beta_{40}$ dimers and also more compact dimer structures as quantified by N-to-C distance distributions. These structural differences may underlie distinct abilities of [A16] $A\beta_{40}$ versus [A28] $A\beta_{40}$ dimers to mediate cell culture toxicity. Interestingly, [A16] $A\beta_{42}$ but not [A28] $A\beta_{42}$ dimers show reduced β -strand content and also reduced N-to-C distances, consistent with [A16] $A\beta_{42}$ forming more compact oligomer conformations than [A28] $A\beta_{42}$. Our findings demonstrate that peptides of identical composition albeit different sequences are able to exert distinct effects on oligomer formation and structure, consistent with the sequence-to-structure paradigm.

The complexity of the relationship between the protein sequence and oligomer structure was elucidated in previous studies. The first DMD4B-HYDRA study of $A\beta_{40}$ and $A\beta_{42}$ oligomer formation showed that $A\beta_{42}$ oligomers are characterized by increased plasticity in the N-terminal region relative to $A\beta_{40}$ oligomers [44], the feature that was hypothesized to be linked to increased toxicity of $A\beta_{42}$ relative to $A\beta_{40}$ oligomers [48]. This result was unexpected because the sequences of $A\beta_{40}$ and $A\beta_{42}$ differ by two additional

amino acids, IA, at the C-terminus of the latter, so that structural differences would be expected to occur in the C-terminal region. In A β_{42} oligomers, however, the more hydrophobic C-terminus appears to interact with the central hydrophobic cluster (L17–A21) in a way that excludes the N-terminus, while in A β_{40} oligomers, the C-terminus is not hydrophobic enough to match the hydrophobicity of the central hydrophobic cluster, which allows the N-terminus to interact with the central hydrophobic cluster, resulting in more compact oligomer structures. The β -strand structure at A2–F4 in A β_{40} oligomers vanishes in oligomers formed by the Arctic mutant; [G22]A β_{40} and [G22]A β_{40} oligomers adopt significantly less structured N-termini than A β_{40} oligomers, which was proposed to be linked to an earlier onset of the disease in carriers of the Arctic mutation [45]. The complexity of the sequence-structure relationship is reflected in the fact that a single point mutation at position 22 affects the secondary structure propensities in the N-terminal region (A2–F4). Truncation of the first two amino acids from the A β_{40} sequence was also shown to result in oligomers with significantly more flexible and solvent-exposed N-termini [46, 52]. In the present study, A β_{40} is the only variant that forms oligomers, which lack the conformations with extended N-to-C distances, whereby both substitutions, [K16A] and [K28A], cause changes in the N-terminal secondary structure of A β_{40} oligomers that are similar to those induced by previously reported A β_{40} sequence modifications described above. The question that remains to be explored is whether or not increased plasticity at the N-termini of A β_{42} oligomers plays a role in A β_{42} -mediated toxicity [48].

There are at least three mechanisms through which the [K16A] and [K28A] substitutions could affect the resulting A β oligomer toxicity. First, replacing one lysine by alanine makes the peptide more negatively charged, which increases electrostatic repulsion between oligomers and a negatively charged membrane and potentially reduces the affinity of oligomers to interact with and disrupt membranes. Second, instead of three positively charged amino acid residues of WT peptides (R5, K16, K28), each mutant peptide only has two positively charged residues that could favorably interact with the membrane, thereby reducing the multiplicity (entropy) of peptide-membrane interactions, resulting in an increased free energy barrier for A β -membrane binding. Although weaker interactions between A β and a membrane described by the two mechanisms above likely contribute to the observed reduced toxicity of [A16]A β_{40} , [A28]A β_{40} , [A16]A β_{42} , and [A28]A β_{42} , neither mechanism accounts for the experimental observation that of the two substitutions, [K16A] is significantly more efficient in toxicity inhibition [42]. Third, it is possible that distinct local environments at positions 16 and 28 play a role in A β -membrane binding affinity with a binding advantage of K16, adjacent to the strongly hydrophobic central hydrophobic cluster, over K28, which exists in a significantly more hydrophilic region of A β . Alternatively, the two substitutions might induce substitution-specific structural changes in A β oligomers, which additionally alter their ability to interact with and disrupt cellular membranes. Indeed, computational results reported here demonstrate that [K16A]- and [K28A]-induced changes in A β_{40} and A β_{42} oligomer formation are both substitution- and alloform-specific and elucidate the structural foundation of A β oligomer toxicity, which is required for a correct identification of therapeutic targets against AD.

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References

1. Selkoe, D.J.: Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* **81**(2), 741–766 (2001)
2. Shaw, L.M., Vanderstichele, H., Knapik-Czajka, M., Clark, C.M., Aisen, P.S., Petersen, R.C., Blennow, K., Soares, H., Simon, A., Lewczuk, P., Dean, R., Siemers, E., Potter, W., Lee, V.M.Y., Trojanowski, J.Q., Initi, A.D.N.: Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann. Neurol.* **65**(4), 403–413 (2009)
3. Hardy, J.A., Higgins, G.A.: Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184–185 (1992)
4. Hardy, J.: The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J. Neurochem.* **110**, 1129–1134 (2009)
5. Serpell, L.C.: Alzheimer's amyloid fibrils: structure and assembly. *Biochim. Biophys. Acta* **1502**(1), 16–30 (2000)
6. Petkova, A.T., Ishii, Y., Balbach, J.J., Antzutkin, O.N., Leapman, R.D., Delaglio, F., Tycko, R.: A structural model for Alzheimer's β -amyloid fibrils based on experimental constraints from solid state NMR. *Proc. Natl. Acad. Sci. USA* **99**(26), 16742–16747 (2002)
7. Petkova, A.T., Leapman, R.D., Guo, Z., Yau, W.M., Mattson, M.P., Tycko, R.: Self-propagating, molecular-level polymorphism in Alzheimer's β -amyloid fibrils. *Science* **307**, 262–265 (2005)
8. Sciarretta, K.L., Gordon, D.J., Petkova, A.T., Tycko, R., Meredith, S.C.: A β 40-Lactam(D23/K28) models a conformation highly favorable for nucleation of amyloid. *Biochemistry* **44**(16), 6003–6014 (2005)
9. Paravastu, A., Leapman, R., Yau, W.M., Tycko, R.: Molecular structural basis for polymorphism in Alzheimer's β -amyloid fibrils. *Proc. Natl. Acad. Sci. USA* **105**, 18349–18354 (2008)
10. Paravastu, A.K., Qahwash, I., Leapman, R.D., Meredith, S.C., Tycko, R.: Seeded growth of β -amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. *Proc. Natl. Acad. Sci. USA* **106**, 7443–7448 (2009)
11. Tycko, R.: Solid-state NMR studies of amyloid fibril structure. *Annu. Rev. Phys. Chem.* **62**, 279–299 (2011)
12. Luhrs, T., Ritter, C., Adrian, M., Riek-Loher, D., Bohrmann, B., Dobeli, H., Schubert, D., Riek, R.: 3D structure of Alzheimer's amyloid- β (1–42) fibrils. *Proc. Natl. Acad. Sci. USA* **102**, 17342–17347 (2005)
13. Schmidt, M., Sachse, C., Richter, W., Xu, C., Faendrich, M., Grigorieff, N.: Comparison of Alzheimer A β (1–40) and A β (1–42) amyloid fibrils reveals similar protofilament structures. *Proc. Natl. Acad. Sci. USA* **106**(47), 19813–19818 (2009)
14. Xiao, Y., Ma, B., McElheny, D., Parthasarathy, S., Long, F., Hoshi, M., Nussinov, R., Ishii, Y.: A β (1–42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease. *Nat. Struct. Mol. Biol.* **22**(6), 499–505 (2015)
15. Schmidt, M., Rohou, A., Lasker, K., Yadav, J.K., Schiene-Fischer, C., Faendrich, M., Grigorieff, N.: Peptide dimer structure in an A β (1–42) fibril visualized with cryo-EM. *Proc. Natl. Acad. Sci. USA* **112**(38), 11858–11863 (2015)
16. Petkova, A.T., Yau, W.M., Tycko, R.: Experimental constraints on quaternary structure in Alzheimer's β -amyloid fibrils. *Biochemistry* **45**(2), 498–512 (2006)
17. Lazo, N.D., Grant, M.A., Condrón, M.C., Rigby, A.C., Teplow, D.B.: On the nucleation of amyloid β -protein monomer folding. *Protein Sci.* **14**(6), 1581–1596 (2005)
18. Grant, M.A., Lazo, N.D., Lomakin, A., Condrón, M.M., Arai, H., Yamin, G., Rigby, A.C., Teplow, D.B.: Familial Alzheimer's disease mutations alter the stability of the amyloid β -protein monomer folding nucleus. *Proc. Natl. Acad. Sci. USA* **104**, 16522–16527 (2007)
19. Fawzi, N., Phillips, A., Ruscio, J., Doucleff, M., Wemmer, D., Head-Gordon, T.: Structure and dynamics of the A β (21–30) peptide from the interplay of NMR experiments and molecular simulations. *J. Am. Chem. Soc.* **130**, 6145–6158 (2008)
20. Borreguero, J.M., Urbanc, B., Lazo, N.D., Buldyrev, S.V., Teplow, D.B., Stanley, H.E.: Folding events in the 21–30 region of amyloid β -protein (A β) studied in silico. *Proc. Natl. Acad. Sci. USA* **102**(17), 6015–6020 (2005)
21. Cruz, L., Urbanc, B., Borreguero, J.M., Lazo, N.D., Teplow, D.B., Stanley, H.E.: Solvent and mutation effects on the nucleation of amyloid β -protein folding. *Proc. Natl. Acad. Sci. USA* **102**(51), 18258–18263 (2005)
22. Baumketner, A., Bernstein, S.L., Wyttenbach, T., Lazo, N.D., Teplow, D.B., Bowers, M.T., Shea, J.E.: Structure of the 21–30 fragment of amyloid β -protein. *Protein Sci.* **15**(6), 1239–1247 (2006)
23. Chen, W., Mousseau, N., Derreumaux, P.: The conformations of the amyloid- β (21–30) fragment can be described by three families in solution. *J. Chem. Phys.* **125**(8), 084911 (2006)

24. Tarus, B., Straub, J.E., Thirumalai, D.: Structures and Free-Energy Landscapes of the wild type and mutants of the A β_{21-30} peptide are determined by an interplay between intrapeptide electrostatic and hydrophobic interactions. *J. Mol. Biol.* **379**(4), 815–829 (2008)
25. Krone, M.G., Baumketner, A., Bernstein, S.L., Wyttenbach, T., Lazo, N.D., Teplow, D.B., Bowers, M.T., Shea, J.E.: Effects of familial mutations on the folding nucleation of the Alzheimer amyloid β -protein. *J. Mol. Biol.* **381**, 221–228 (2008)
26. Cruz, L., Rao, J.S., Teplow, D.B., Urbanc, B.: Dynamics of metastable β -hairpin structures in the folding nucleus of amyloid β -protein. *J. Phys. Chem. B* **116**, 6311–6325 (2012)
27. Keshet, B., Gray, J.J., Good, T.A.: Structurally distinct toxicity inhibitors bind at common loci on β -amyloid fibril. *Protein Sci.* **19**, 2291–2304 (2010)
28. Esler, W.P., Stimson, E.R., Ghilardi, J.R., Lu, Y.A., Felix, A.M., Vinters, H.V., Mantyh, P.W., Lee, J.P., Maggio, J.E.: Point substitution in the central hydrophobic cluster of a human β -amyloid congener disrupts peptide folding and abolishes plaque competence. *Biochemistry* **35**(44), 13914–13921 (1996)
29. de Groot, N.S., Aviles, F.X., Vendrell, J., Ventura, S.: Mutagenesis of the central hydrophobic cluster in A β_{42} Alzheimer's peptide. Side-chain properties correlate with aggregation propensities. *FEBS J.* **273**, 658–668 (2006)
30. Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., van Duinen, S.G., Bots, G.T.A.M., Luyendijk, W., Frangione, B.: Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. *Science* **248**, 1124–1126 (1990)
31. Tycko, R., Sciarretta, K., Orgel, J., Meredith, S.: Evidence for novel β -sheet structures in Iowa mutant β -amyloid fibrils. *Biochemistry* **48**, 6072–6084 (2009)
32. Zhang, S.S., Casey, N., Lee, J.P.: Residual structure in the Alzheimer's disease peptide—probing the origin of a central hydrophobic cluster. *Fold. Des.* **3**, 413–422 (1998)
33. Zhang, S., Iwata, K., Lachenmann, M.J., Peng, J.W., Li, S., Stimson, E.R., Lu, Y., Felix, A.M., Maggio, J.E., Lee, J.P.: The Alzheimer's peptide A β adopts a collapsed coil structure in water. *J. Struct. Biol.* **130**(2–3), 130–141 (2000)
34. Chen, Z., Krause, G., Reif, B.: Structure and orientation of peptide inhibitors bound to β -amyloid fibrils. *J. Mol. Biol.* **354**, 760–776 (2005)
35. Terzi, E., Hölzemann, G., Seelig, J.: Reversible random coil- β -sheet transition of the Alzheimer β -amyloid fragment (25–35). *Biochemistry* **33**, 1345–1350 (1994)
36. Hertel, C., Terzi, E., Hauser, N., Jakob-Rotne, R., Seelig, J., Kemp, J.A.: Inhibition of electrostatic interaction between β -amyloid peptide and membranes prevents β -amyloid-induced toxicity. *Proc. Natl. Acad. Sci. USA* **94**, 9412–9416 (1997)
37. Chauhan, A., Ray, I., Chauhan, V.P.: Interaction of amyloid β -protein with anionic phospholipids: possible involvement of Lys28 and C-terminus aliphatic amino acids. *Neurochem. Res.* **25**, 423–429 (2000)
38. Bokvist, M., Lindstrom, F., Watts, A., Grobner, G.: Two types of Alzheimer's β -amyloid (1–40) peptide membrane interactions: aggregation preventing transmembrane anchoring versus accelerated surface fibril formation. *J. Mol. Biol.* **335**, 1039–1049 (2004)
39. Williamson, M., Suzuki, Y., Bourne, N., Asakura, T.: Binding of amyloid β -peptide to ganglioside micelles is dependent on histidine-13. *Biochem. J.* **397**, 483–490 (2006)
40. Yoshiike, Y., Akagi, T., Takashima, A.: Surface structure of amyloid- β fibrils contributes to cytotoxicity. *Biochemistry* **46**, 9805–9812 (2007)
41. Sinha, S., Lopes, D.H.J., Du, Z., Pang, E.S., Shanmugam, A., Lomakin, A., Talbiersky, P., Tennstaedt, A., McDaniel, K., Bakshi, R., Kuo, P.Y., Ehrmann, M., Benedek, G.B., Loo, J.A., Klärner, F.G., Schrader, T., Wang, C., Bitan, G.: Lysine-specific molecular tweezers are broad-spectrum inhibitors of assembly and toxicity of amyloid proteins. *J. Am. Chem. Soc.* **133**(42), 16958–16969 (2011)
42. Sinha, S., Lopes, D.H.J., Bitan, G.: A key role for lysine residues in amyloid β -protein folding, assembly, and toxicity. *ACS Chem. Neurosci.* **3**(6), 473–481 (2012)
43. Bitan, G., Kirkitadze, M.D., Lomakin, A., Vollers, S.S., Benedek, G.B., Teplow, D.B.: Amyloid β -protein (A β) assembly: A β_{40} and A β_{42} oligomerize through distinct pathways. *Proc. Natl. Acad. Sci. USA* **100**(1), 330–335 (2003)
44. Urbanc, B., Cruz, L., Yun, S., Buldyrev, S.V., Bitan, G., Teplow, D.B., Stanley, H.E.: In silico study of amyloid β -protein folding and oligomerization. *Proc. Natl. Acad. Sci. USA* **101**(50), 17345–17350 (2004)
45. Urbanc, B., Betnel, M., Cruz, L., Bitan, G., Teplow, D.B.: Elucidation of amyloid β -protein oligomerization mechanisms: discrete molecular dynamics study. *J. Am. Chem. Soc.* **132**, 4266–4280 (2010)
46. Meral, D., Urbanc, B.: Discrete molecular dynamics study of oligomer formation by N-terminally truncated amyloid β -protein. *J. Mol. Biol.* **425**, 2260–2275 (2013)

47. Barz, B., Urbanc, B.: Dimer formation enhances structural differences between amyloid β -protein (1–40) and (1–42): an explicit-solvent molecular dynamics study. *PLoS One* **7**(4), e34345 (2012)
48. Urbanc, B., Betnel, M., Cruz, L., Li, H., Fradinger, E., Monien, B.H., Bitan, G.: Structural basis of $A\beta_{1-42}$ toxicity inhibition by $A\beta$ C-terminal fragments: discrete molecular dynamics study. *J. Mol. Biol.* **410**, 316–328 (2011)
49. Lam, A., Teplow, D.B., Stanley, H.E., Urbanc, B.: Effects of the Arctic (E22→G) mutation on amyloid β -protein folding: discrete molecular dynamics study. *J. Am. Chem. Soc.* **130**, 17413–17422 (2008)
50. Žganec, M., Žerovnik, E., Urbanc, B.: Self-assembly of globular protein stefin B into polymorphic oligomers probed by discrete molecular dynamics. *J. Chem. Theory Comput.* **11**, 2355–2366 (2015)
51. Yun, S., Urbanc, B., Cruz, L., Bitan, G., Teplow, D.B., Stanley, H.E.: Role of electrostatic interactions in amyloid β -protein ($A\beta$) oligomer formation: a discrete molecular dynamics study. *Biophys. J.* **92**, 4064–4077 (2007)
52. Meral, D., Urbanc, B.: Erratum to discrete molecular dynamics study of oligomer formation by N-terminally truncated amyloid β -protein. *J. Mol. Biol.* **427**, 2726–2729 (2015)
53. Williams, T.L., Johnson, B.J., Urbanc, B., Jenkins, T.A., Connell, S.D., Serpell, L.C.: Alzheimer's $A\beta_{42}$ oligomers but not fibrils simultaneously bind to and cause damage to ganglioside containing lipid membranes. *Biochem. J.* **439**, 67–77 (2011)
54. Williams, T.L., Urbanc, B., Marshall, K.E., Vadukul, D.M., Jenkins, A.T.A., Serpell, L.C.: Europium as an inhibitor of amyloid β (1–42) induced membrane permeation. *FEBS Lett.* **589**, 3228–3236 (2015)
55. Williams, T.L., Serpell, L.C., Urbanc, B.: Stabilization of native amyloid β -protein oligomers by Copper and Hydrogen peroxide Induced Cross-linking of Unmodified Proteins (CHICUP). *Biochim. Biophys. Acta* **1864**, 249–259 (2016)