Structural Basis for Aβ1–42 Toxicity Inhibition by Aβ C-Terminal Fragments: Discrete Molecular Dynamics Study

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Received 2 February 2011; received in revised form 12 April 2011; accepted 14 May 2011
Available online 23 May 2011
Edited by D. Case

Keywords: amyloid β protein assembly; toxicity inhibitors; structure-toxicity relationship; discrete molecular dynamics; coarse-grained protein model

Abstract

Amyloid β-protein (Aβ) is central to the pathology of Alzheimer’s disease. Of the two predominant Aβ allotypes, Aβ1–40 and Aβ1–42, the latter forms more toxic oligomers. C-terminal fragments (CTFs) of Aβ were recently shown to inhibit Aβ1–42 toxicity in vitro. Here, we studied Aβ1–42 assembly in the presence of three effective CTF inhibitors and an ineffective fragment, Aβ21–30. Using a discrete molecular dynamics approach that recently was shown to capture key differences between Aβ1–40 and Aβ1–42 oligomerization, we compared Aβ1–42 oligomer formation in the absence and presence of CTFs or Aβ21–30 and identified structural elements of Aβ1–42 that correlated with Aβ1–42 toxicity. CTFs co-assembled with Aβ1–42 into large heterooligomers containing multiple Aβ1–42 and inhibitor fragments. In contrast, Aβ21–30 co-assembled with Aβ1–42 into heterooligomers containing mostly a single Aβ1–42 and multiple Aβ21–30 fragments. The CTFs, but not Aβ21–30, decreased the β-strand propensity of Aβ1–42 in a concentration-dependent manner. CTFs and Aβ21–30 had a high binding propensity to the hydrophobic regions of Aβ1–42, but only CTFs were found to bind the Aβ1–42 region A2–F4. Consequently, only CTFs but not Aβ21–30 reduced the solvent accessibility of Aβ1–42 in region D1–R5. The reduced solvent accessibility of Aβ1–42 in the presence of CTFs was comparable to the solvent accessibility of Aβ1–40 oligomers formed in the absence of Aβ fragments. These findings suggest that region D1–R5, which was more exposed to the solvent in Aβ1–42 than in Aβ1–40 oligomers, is involved in mediating Aβ1–42 oligomer neurotoxicity.

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Introduction

Alzheimer’s disease (AD) is an irreversible, progressive neurodegenerative disorder that is the dominant cause of dementia in the elderly. One of the hallmarks of AD is accumulation of extracellular senile plaques, which contain fibrillar aggregates of amyloid β-protein (Aβ). Genetic, pathologic, and biochemical evidence strongly supports the hypothesis that low-order oligomeric assemblies of Aβ, rather than fibrils, are the proximate neurotoxic...
agents in AD. The majority of Aβ oligomers were found to be neurotoxic, and certain oligomers decreased neuronal viability to 10- to 100-fold more strongly than Aβ fibrils. Aβ1–40 and Aβ1–42, the major Aβ alloforms in the brain, differ by the presence of two amino acids, I41 and A42, at the C-terminus of the latter. Aβ1–42 aggregates faster, forms more toxic assemblies, and is genetically linked to aggregated, early-onset familial forms of AD. Cross-linking studies showed that Aβ1–42 forms pentamers and hexamers (paranuclei) and multiples of paranuclei, including dodecamers and octadecamers, whereas Aβ1–40 exists as a mixture of monomers through tetramers. These observations have been confirmed independently by ion-mobility spectrometry/mass spectrometry. Interestingly, Aβ dodecamers, which putatively form by self-association of two paranuclei, have been detected in vivo in several independent studies. In recent years, an ab initio discrete molecular dynamics (DMD) approach, using a four-bead protein model and residue-specific hydrophobic interactions offered important insights into the folding and assembly of Aβ1–40 and Aβ1–42. This approach recapitulated the essential, experimentally observed differences between the folding and oligomerization of Aβ1–40 and Aβ1–42 and demonstrated that Aβ1–42 but not Aβ1–40 oligomerization was driven primarily through intermolecular interactions involving the C-terminal region (I31–A42). The DMD approach also predicted a quasi-stable turn at the C-terminus of Aβ1–42, which does not occur in Aβ1–40, a prediction that was supported by several experimental studies. In a most recent study, Streltsov et al. reported the first X-ray structure of the Aβ18–41 tetramer encapsulated in a shark Ig new antigen receptor, which resembles the DMD-derived oligomeric structures.

The DMD findings have led to a hypothesis that C-terminal fragments (CTFs) of Aβ1–42 may interfere with Aβ1–42 oligomerization. Recently, we reported that Aβ1–42 CTFs ranging from Aβ29–42 to Aβ39–42, as well as Aβ30–40, attenuated Aβ1–42 neurotoxicity in neuronal cell culture. We also investigated the aqueous solubility, aggregation kinetics, and morphology of CTFs and found that their aggregation propensity correlated with the previously reported tendency to form β-hairpin structures, whereas their ability to inhibit Aβ1–42-induced neurotoxicity correlated with a tendency to form an irregular “coil–turn” structure. Dynamic light-scattering (DLS) data revealed that two Aβ1–42 oligomer populations, which were scarcely populated in the absence of inhibitors, were enhanced by CTFs in an inhibitor-specific manner. In particular, stabilization of the smaller of the two Aβ1–42/CTF heterotypic assembly populations with a hydrodynamic radius of 8–12 nm correlated with the degree of toxicity inhibition. Stabilization of nontoxic Aβ1–42 assemblies might thus be a promising strategy for designing Aβ1–42 toxicity inhibitors. A similar mechanism was found for several other inhibitors, including scyllo-inositol, benzothiazole derivatives, and the polyphenols epigallocatechin-3-gallate, resveratrol, myricetin, and nordihydroguaiaretic acid. However, the mode of interaction of inhibitors with Aβ1–42 and the structural changes in Aβ1–42 that are required for a successful toxicity inhibition are unknown.

Our preliminary DMD study of Aβ1–42 assembly in the presence of Aβ29–42, Aβ31–42, or Aβ39–42, using Aβ1–42/CTF molar concentration ratios of up to 1:2, demonstrated that these CTFs inserted themselves among Aβ1–42 peptides, reducing their intermolecular contacts. Inhibition of Aβ1–42 toxicity by CTFs in a cell culture was concentration dependent and most efficient at the Aβ1–42/CTF molar concentration ratio of ~1:10. Here, we applied the DMD approach to examine the assembly of Aβ1–42 in the presence of three CTFs that efficiently inhibited Aβ1–42 toxicity and a control peptide, Aβ21–30, which had no effect on Aβ1–42 toxicity, at several Aβ1–42/Aβ21–30 concentration ratios, including 1:10. We explored the effects of Aβ31–42, Aβ39–42, and two additional Aβ fragments (Aβ30–40 and Aβ21–30) that were not included into our prior DMD study using an improved, recently reported parameterization of the DMD approach. The aim of the present work was to explore structural elements involved in Aβ1–42 toxicity inhibition by CTFs. To achieve that, we analyzed the Aβ1–42 structures formed in the presence of effective inhibitors and compared them to Aβ1–42 oligomers formed in the absence of inhibitors and in the presence of ineffective Aβ21–30 fragments. We also compared the Aβ1–42 assembly structures formed in the presence of Aβ fragments to Aβ1–40 oligomer populations (formed in the absence of Aβ fragments). Based on the present computational results and previously reported toxicity data, we propose a mechanism in which CTFs inhibit Aβ1–42 toxicity by binding to specific regions of Aβ1–42, reducing its ability to form a β structure, and interrupting putative interactions of Aβ1–42 with its cellular targets.

Results

We selected four AβX–Y fragments to study their effect on Aβ1–42 assembly. Of all the experimentally examined CTFs, Aβ1–41 was chosen because it was the strongest inhibitor of neurotoxicity. Aβ39–42, the shortest of all the CTFs under study, showed surprisingly high inhibition of neurotoxicity. Aβ30–40 was selected because its degree of Aβ1–42 toxicity inhibition was comparable to that of the other two CTFs, and Aβ21–30, which did not inhibit
Aβ_{1-42} toxicity in cell culture, was chosen as a control peptide.

We simulated Aβ_{1-42} assembly in the presence of CTFs or Aβ_{21-30} using a four-bead protein model with backbone hydrogen bonding and amino-acid-specific interactions, as described in Methods (see Supplementary Methods in Supplementary Material). We used the implicit solvent parameters E_H = 0.3 and E_C1 = 0 and physiological temperature estimate T = 0.13, which recently has been shown to match well the in vitro temperature dependence of the average β-strand in Aβ_{1-40} and Aβ_{1-42} monomers24 and the distinct oligomer size distributions of Aβ_{1-40}, Aβ_{1-42}, and their Arctic mutants, [E22G]Aβ_{1-40} and [E22G]Aβ_{1-40}.25 The total number of Aβ_{1-42} and fragment peptides and simulation box sizes (Table I in Supplementary Material) were chosen to correspond to a total molar concentration of ~3 - 4 mM as used in the previously published work.25

Typical molar concentrations in in vitro studies are 10- to 100-fold lower than the total molar concentration in our computational study. Due to limitations in the total number of atoms and time scales that can currently be efficiently studied by computer simulations,43 millimolar concentrations are required for proteins to interact with each other and thus form assemblies and to obtain sufficient statistics on the assembled structures. The effect of Aβ fragments on Aβ_{1-42} assembly and the resulting structures were quantified and compared to unaltered Aβ_{1-42} oligomer structures using data from the recently published work.25

In the description of our results below, the following abbreviations for specific Aβ regions are used: CHC, central hydrophobic cluster (L17–A21); MHR, mid-hydrophobic cluster (I31–M35); and CTR, C-terminal region (V39–A42).

Aβ_{X,Y} and Aβ_{1-42} associate into Aβ_{1-42}/Aβ_{X,Y} heterooligomers

Initially separated monomeric Aβ_{1-42} and toxicity inhibitor Aβ_{30-40}, Aβ_{31-42}, or Aβ_{39-42} (Fig. 1a) associated first into small Aβ_{1-42}/CTF heterooligomers of various sizes and compositions (Fig. 1b) and then into larger heterotypic assemblies (Fig. 1c), and finally, all Aβ_{1-42} and CTF peptides in each trajectory converged into a single large heterotypic assembly (Fig. 1d–e). These results are in qualitative agreement with the DLS observations of increased abundance of Aβ_{1-42} oligomers in the presence of these CTFs relative to Aβ_{1-42} alone.35 Thus, the three toxicity inhibitors (Aβ_{30-40}, Aβ_{31-42}, or Aβ_{39-42}) acted as a “glue” capturing Aβ_{1-42} molecules into an amorphous heterotypic assembly. In contrast, the control peptide Aβ_{21-30} had the opposite effect. In the presence of this fragment, both Aβ_{1-42} and Aβ_{21-30} remained predominantly monomeric. In addition, small heterotypic assemblies comprising an Aβ_{1-42} molecule surrounded by seven to eight Aβ_{30-40} peptides also were observed (Fig. 2a–e, see also Fig. II in Supplementary Material). The size distributions of Aβ_{1-42}/Aβ_{X,Y} assemblies (Fig. II in Supplementary Material) evolved within 5 × 10^6 to 10 × 10^6 simulation steps into a quasi-steady state, after which the temporal changes in the oligomer size distributions were no longer statistically significant up to 20 × 10^6 simulation steps.

CTFs inhibit β-strand formation in Aβ_{1-42}

The structural basis for Aβ_{1-42} neurotoxicity is as yet unknown. Several studies addressed the relevance of β-strand formation in Aβ-oligomer-mediated toxicity. Neurotoxic pre-fibrillar Aβ assemblies lacking the cross-β structure (which is characteristic of Aβ fibrils with ~45–55% of β-sheet structure) were identified by several studies.13,19,45,46 In contrast, Chimon et al. described neurotoxic Aβ intermediates with parallel β-sheet structures,47 and Wu et al. found fibrillar oligomers that nucleated formation of toxic oligomers but did not form fibrils.48 Our DMD-derived oligomer structures25 are consistent with in vitro data by Kirkita et al. who found Aβ_{1-40} and Aβ_{1-42} oligomers with a relatively low β-strand of 10–20%45 and no cross-β structure. Here, we asked whether changes in the β-strand propensity in Aβ_{1-42} due to the presence of CTFs and Aβ_{21-30} at the stage of initial hydrophobic collapse may correlate with their reported ability to inhibit Aβ_{1-42} toxicity.

To examine the effect of Aβ fragments on β-strand formation in Aβ_{1-42}, we calculated the average β-strand content, ⟨β-strand⟩, of Aβ_{1-42} within Aβ_{1-42}/Aβ_{X,Y} assemblies and compared them to ⟨β-strand⟩ in Aβ_{1-42} oligomers formed in the absence of fragments (Fig. 3a, continuous curves). ⟨β-Strand⟩ in Aβ_{1-42} decreased from 19% in the absence of fragments to ~16% at relative molar concentrations of Aβ_{X,Y} of ~0.5 for all toxicity inhibitors. At the relative Aβ_{X,Y} molar concentrations of ~1, Aβ_{30-40} and Aβ_{31-42} further reduced ⟨β-Strand⟩ in Aβ_{1-42} to ~15%. At this Aβ_{31-42} concentration, ⟨β-Strand⟩ in Aβ_{1-42} was at its lowest value, and at the relative Aβ_{31-42} molar concentration above 2 and up to 10, ⟨β-Strand⟩ remained at a value of ~16% (~16% reduction). ⟨β-Strand⟩ in Aβ_{1-42} above the relative Aβ_{30-40} molar concentration of ~2 decreased with the Aβ_{30-40} concentration and reached a value of ~12% at the relative molar concentration of 10 (37% reduction). Thus, of the two longer toxicity inhibitors, Aβ_{30-40} was significantly more efficient in suppressing the formation of β-strand structure in Aβ_{1-42} than Aβ_{31-42}. The shortest toxicity inhibitor, Aβ_{39-42}, had the most prominent effect on ⟨β-Strand⟩ in Aβ_{1-42} at all relative molar concentrations >0.5 and reduced ⟨β-Strand⟩ to ~10% (48% reduction). In contrast to the three toxicity inhibitors, the control
peptide $\text{A}^\beta_{21-30}$ increased $\langle \beta\text{-strand} \rangle$ in $\text{A}^\beta_{1-42}$ (Fig. 3a, gray continuous curve), and this increase was strongly $\text{A}^\beta_{21-30}$ concentration dependent.

$\langle \beta\text{-strand} \rangle$ propensity in the A$\beta$ fragments themselves was variable. All three toxicity inhibitors showed similar concentration dependencies of $\langle \beta\text{-strand} \rangle$ (Fig. 3a, black, red, and green broken curves), experiencing a slight $\text{A}^\beta_{1-42}$-induced increase in $\langle \beta\text{-strand} \rangle$ about or just below the relative molar CTF concentrations of 0.5, followed by a decrease at higher CTF concentrations. $\text{A}^\beta_{31-42}$ had the highest $\langle \beta\text{-strand} \rangle$ values at all concentrations under study, reaching ~12% at the highest relative $\text{A}^\beta_{31-42}$ molar concentration of 10, followed by

**Fig. 1.** Populations of 16 $\text{A}^\beta_{1-42}$ and (a–d) 256 $\text{A}^\beta_{39-42}$ molecules at four different time frames: (a) $t=0$ (b), $t=0.1 \times 10^6$, $t=1 \times 10^6$, and $t=20 \times 10^6$ simulation steps. $\text{A}^\beta_{39-42}$ molecules are displayed in yellow, and $\text{A}^\beta_{1-42}$ molecules are represented in dark blue with the N-terminal amino acid D1 marked as red spheres. (e) A magnified and rotated final $\text{A}^\beta_{1-42}/\text{A}^\beta_{39-42}$ heterooligomer obtained at $t=20 \times 10^6$ simulation steps. The figure was created using the VMD software package.
$A\beta_{30-40}$ with $\sim 7\%$ and $A\beta_{39-42}$ with $\sim 2\%$. The control peptide $A\beta_{21-30}$ was characterized by the highest $\langle \beta\text{-strand} \rangle$ (ranging from 12.8% at the lowest to 17.6% at the highest $A\beta_{21-30}$ concentration) of all four $A\beta$ fragments. $\langle \beta\text{-strand} \rangle$ in $A\beta$ fragments was at all concentrations lower than $\langle \beta\text{-strand} \rangle$ in $A\beta_{1-42}$ oligomers formed in the absence of fragments (19.1%) and significantly lower than that of $A\beta_{1-42}$ assembling in the presence of $A\beta_{21-30}$ at the 1:10 molar concentration ratio (27.9%). As shown in

![Image](image_url)

Fig. 2. Populations of 16 $A\beta_{1-42}$ and (a–d) 128 $A\beta_{21-30}$ molecules at four different time frames: (a) $t = 0$ (b), $t = 0.1 \times 10^6$, $t = 10^6$, and $t = 20 \times 10^6$ simulation steps. $A\beta_{21-30}$ molecules are displayed in yellow, and $A\beta_{1-42}$ molecules are represented in dark blue with the N-terminal amino acid D1 marked as red spheres. (e) Two magnified $A\beta_{1-42}/A\beta_{21-30}$ heterooligomers obtained at $t = 20 \times 10^6$ simulation steps. The figure was created using the VMD software package.
Fig. 3, the β-strand propensity of Aβ fragments correlated with the average β-strand in Aβ₁₋₄₂ and also with the Aβ-fragment-induced change in the Aβ₁₋₄₂ β-strand propensity. To our knowledge, there are currently no experimental data on the effect of Aβ fragments on the secondary structure of Aβ₁₋₄₂; thus, direct comparison between in silico and in vitro data cannot be made. Nonetheless, our analysis suggests that the Aβ₁₋₄₂ secondary structure changes may be relevant to the Aβ-fragment-induced changes in Aβ toxicity. In addition, we compared β-strand propensities per residue in Aβ₁₋₄₀ and Aβ₁₋₄₂ hexamers formed in the absence of fragments (Fig. 3c, blue and orange curves) with the β-strand propensities per residue in heterotypic assemblies comprising Aβ₁₋₄₂ and Aβ fragments (Fig. 3c, black, red, green, and gray curves). None of the three toxicity inhibitors changed the relative distribution of β-strand propensities along the Aβ₁₋₄₂ sequence. The β-strand propensity per residue in Aβ₁₋₄₂ hexamers was significantly different from the one found in Aβ₁₋₄₀ hexamers, as reported and discussed in our prior work. For example, region A2–F4 in Aβ₁₋₄₀ but not in Aβ₁₋₄₂ hexamers had a high β-strand propensity (Fig. 3c, blue and orange curves). All three toxicity inhibitors reduced the β-strand propensities in region Q15–L17 of Aβ₁₋₄₂, but only Aβ₃₀₋₄₀ and Aβ₃₉₋₄₂ reduced these propensities at R5–V12, A21–S26, and I31–V36. The control peptide Aβ₂₁₋₃₀, on the other hand, substantially increased the β-strand propensity in several Aβ₁₋₄₂ regions: D7–E₁₁, F₁₉–F₂₀, E₂₂–V₂₄, G₂₉–I₃₂, and L₃₄–V₃₆ (Fig. 3c, gray curve).

Aβₓ₋ᵧ fragments alter the tertiary and quaternary structures of Aβ₁₋₄₂

Here, we quantified structural changes in Aβ₁₋₄₂ due to the presence of each fragment and identified...
the specific peptide regions involved in the interaction between $\beta_{1-42}$ and $\beta_{X-Y}$. For this purpose, simulation data corresponding to the $\beta_{1-42}/\beta_{X-Y}$ molar concentration ratio 1:10, which was needed for an efficient toxicity inhibition, were used. In these simulations, six $\beta_{1-42}$ molecules co-assembled with 229 $\beta_{30-40}$, 210 $\beta_{1-42}$, 630 $\beta_{39-42}$, or 252 $\beta_{21-30}$ peptides using a simulation box size of 318 Å.

**Effect of $\beta$ fragments on the tertiary structure of $\beta_{1-42}$**

The tertiary structures of $\beta_{1-42}$ in the absence or presence of $\beta$ fragments are shown in Fig. 4a–e. All three toxicity inhibitors slightly reduced the number and strength of intramolecular contacts relative to $\beta_{1-42}$ hexamers formed in the absence of inhibitors, mostly in the N-terminal region (Fig. 4a–e, box 1). Almost no change in the tertiary structure was observed at the C-terminal region (Fig. 4a–e, box 4). Among the three inhibitors, $\beta_{39-42}$ reduced the intramolecular contacts in $\beta_{1-42}$ the most (Fig. 4d). The control peptide $\beta_{21-30}$ inhibited the $\beta_{1-42}$ intramolecular contacts at the N-terminal region (Fig. 4e, box 1) but, at the same time, increased the number and strengths of intramolecular contacts in all other regions of $\beta_{1-42}$ (Fig. 4e, boxes 2–5) relative to $\beta_{1-42}$ hexamers (Fig. 4a). Overall, the three CTIs decreased—whereas $\beta_{21-30}$ increased—the stability of the $\beta_{1-42}$ tertiary structure. The exception was the N-terminal region (Fig. 4a–e, box 1), where all $\beta_{X-Y}$ reduced intramolecular contacts in $\beta_{1-42}$.

**Effect of $\beta$ fragments on the quaternary structure of $\beta_{1-42}$**

The quaternary structures of $\beta_{1-42}$ in the absence or presence of $\beta$ fragments are shown in Fig. 4f–j. All four $\beta$ fragments reduced both the number and the strengths of intermolecular contacts among $\beta_{1-42}$ molecules in a concentration-dependent way (Fig. IV, Supplementary Material). The remaining intermolecular $\beta_{1-42}$ contacts were $\beta_{X-Y}$ specific. An almost complete absence of specific intermolecular contacts among $\beta_{1-42}$ was observed in the presence of $\beta_{30-40}$ and $\beta_{21-30}$. The interpretation of this reduction was, however, different for the two peptides. In the case of $\beta_{21-30}$, $\beta_{1-42}$ molecules did not interact because they belonged to different heterotypic assemblies, whereas in the case of $\beta_{30-40}$, all six $\beta_{1-42}$ molecules belonged to the same $\beta_{1-42}/\beta_{30-40}$ heterooligomer but were spatially separated from each other, resulting in the reduction in all intermolecular contacts. Similar to $\beta_{30-40}$, $\beta_{21-30}$ and $\beta_{39-42}$ spatially separated individual $\beta_{1-42}$ molecules within heterooligomers. Interestingly, none of the four fragments completely inhibited the intermolecular $\beta_{1-42}$ contacts. In the presence of $\beta_{31-42}$ or $\beta_{39-42}$, the intermolecular contacts among $\beta_{1-42}$ molecules were more abundant than in the presence of $\beta_{30-40}$. In the presence of $\beta_{39-42}$, the intermolecular contacts among the CHC, MHR, and CTR regions of $\beta_{1-42}$ were more abundant than in the presence of $\beta_{31-42}$. $\beta_{31-42}$ induced a few new intermolecular contacts involving the N-terminal region G9–V12 of $\beta_{1-42}$ (Fig. 4h, box 5).

**Interaction regions between $\beta_{1-42}$ and $\beta$ fragments**

Intermolecular contacts between $\beta_{1-42}$ and each fragment are shown in Fig. 4k–n. Boxes 1–4 indicate the regions of $\beta_{1-42}$ that most strongly interacted with all four fragments: the A2–F4 region, CHC, MHR, and CTR. Of the four fragments, $\beta_{39-42}$ formed the most frequent contacts with $\beta_{1-42}$, likely due to its highly hydrophobic nature and its short length (Fig. 4m). $\beta_{30-40}$ and $\beta_{31-42}$ displayed comparable average numbers and strengths of intermolecular contacts (Fig. 4k and l), though the strengths were slightly increased in $\beta_{30-40}$ relative to $\beta_{31-42}$. The intermolecular contacts between the control $\beta_{21-30}$ peptide and $\beta_{1-42}$ were comparable to those observed for $\beta_{30-40}$ and $\beta_{31-42}$ in CHC, MHR, and CTR (Fig. 4n, boxes 2–4). There were no contacts between $\beta_{21-30}$ and the A2–F4 region of $\beta_{1-42}$ (Fig. 4n, box 1). Instead, region R5–V12 of $\beta_{1-42}$ formed an antiparallel β-sheet with $\beta_{21-30}$ (Fig. 4n, region between boxes 1 and 2). These contacts were specific to $\beta_{21-30}$ interaction with $\beta_{1-42}$ and were not observed for any other $\beta$ fragment. Thus, the interaction between $\beta_{21-30}$ and $\beta_{1-42}$ was stronger than would have been expected based on the strongly hydrophilic nature of $\beta_{21-30}$ relative to CTIs.

**Binding propensity of $\beta_{X-Y}$ to $\beta_{1-42}$**

To quantify the degree of the interaction between $\beta_{1-42}$ and $\beta_{X-Y}$, we calculated the binding propensity by averaging the intermolecular $\beta_{1-42}/\beta_{X-Y}$ contact maps shown in Fig. 4k–n over the specified regions. The results are shown in Table 1. For comparison, the binding propensity of $\beta_{1-42}$ to itself in hexamers was included. We found that $\beta_{39-42}$ had 2- to 4-fold higher binding propensity than the other three fragments to the four regions of $\beta_{1-42}$ under study (Table 1). $\beta_{21-30}$ had a binding propensity
similar to that of CHC, MHR, and CTR as Aβ30–40 and Aβ31–42 despite its predominant hydrophilic nature. Importantly, Aβ21–30 had at least 5-fold lower binding propensity to the A2–F4 region of Aβ1–42 relative to the CTFs, highlighting a possible relevance of the A2–F4 region of Aβ1–42 in mediating toxicity.

**Fig. 4.** Intramolecular (a–e) and intermolecular (f–j) contact maps of Aβ1–42 assemblies formed in the absence and at 1:10 molar concentration ratio of the three toxicity inhibitors (Aβ30–40, Aβ31–42, and Aβ39–42) and the control peptide Aβ21–30. The intramolecular and intermolecular contact maps of Aβ1–42 hexamers are shown in (a) and (f), respectively. The intermolecular maps that elucidate regions of contact between Aβ1–42 and each of the four Aβ fragments are shown in (k) to (n). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner. The boxes mark regions of concentrated contacts.
Table 1. Binding propensity of Aβ30–40, Aβ31–42, Aβ39–42, and Aβ21–30 to specific regions of Aβ1–42 at an Aβ1–42/AβX–Y molar concentration ratio of 1:10

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<tr>
<td>A2–F4</td>
<td>0.59±0.03</td>
<td>0.51±0.03</td>
<td>1.69±0.19</td>
<td>0.09±0.02</td>
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<td>CHC</td>
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<td>0.65±0.03</td>
<td>2.45±0.16</td>
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<tr>
<td>MHR</td>
<td>0.70±0.04</td>
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<td>1.89±0.13</td>
<td>0.62±0.09</td>
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<tr>
<td>CTR</td>
<td>0.74±0.05</td>
<td>0.72±0.05</td>
<td>2.27±0.18</td>
<td>0.74±0.14</td>
<td>0.18±0.01</td>
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Binding propensities of Aβ1–42 to itself in unaltered Aβ1–42 hexamers are given for comparison.

* Aβ31–42 binding propensities that were significantly different from Aβ30–40 binding propensities (nonoverlapping error bars).

* Aβ39–42 binding propensities that were two to four times larger than those for any other fragment.

* Aβ21–30 binding propensity to the A2–F4 region more than five times lower than that for any other fragment. The error bars correspond to standard error of the mean.

**CTFs reduce solvent exposure of the N-terminal region of Aβ1–42**

Aβ1–42 oligomers presumably mediate neurotoxicity through interaction with cell membranes and/or other cellular components.49–52 Important insights into the specific Aβ1–42 regions participating in these interactions may be gleaned from studying which regions are most exposed to the environment within homotypic and heterotypic assemblies. In our recent study, we showed that the solvent-accessible surface area (SASA) in the N-terminal region was significantly lower in Aβ1–40 hexamers than in Aβ1–42 hexamers (see Fig. S5b in Supporting Information of Ref. 25). To check whether this difference between Aβ1–40 and Aβ1–42 was specific to hexamers or a more general feature, we used here data from our previous study25 to calculate SASA per residue using the entire Aβ1–40 and Aβ1–42 populations of monomers and oligomers. The results demonstrated that the distinct SASA profiles in the N-terminal region D1–R5 between Aβ1–40 and Aβ1–42 were characteristic not only for hexamers but also for entire Aβ1–40 and Aβ1–42 populations (Fig. 5, blue and orange curves). The regions of Aβ that on average were most exposed to the solvent were mostly hydrophilic residues (D1, R5–S8, H13–Q15, E22–D23, and S26–K28), whereas the hydrophobic regions (CHC, MHR, and CTR) had low SASA values.

To quantify the effect of Aβ fragments on the solvent exposure of Aβ1–42, we calculated SASA per residue using the simulation data acquired at the Aβ1–42/AβX–Y molar concentration ratio 1:10 (Fig. 5). For Aβ1–42 assembled in the presence of the three toxicity inhibitors, the SASA in region D1–R5 was reduced relative to Aβ1–42 assembled in the absence of inhibitors. For all three CTFs, the SASA values almost overlapped with the SASA values derived for Aβ1–40 assemblies formed in the absence of Aβ fragments (Fig. 5). In contrast, Aβ21–30 induced an increased SASA in region D1–R5 of Aβ1–42 (Fig. 5, gray curve). The observed correlation between solvent exposure of the N-terminal region of Aβ1–42 and the degree of toxicity suggests that the N-terminal region D1–R5 of Aβ1–42 is involved in Aβ1–42-mediated toxicity, likely through interaction with cellular targets.

**Discussion**

Our recent in vitro studies of a series of CTFs (AβX–42, X=29–39; Aβ30–40) and the control peptide Aβ21–30 were studied for aqueous solubility, aggregation propensity, and morphology...
characteristics. None of these properties directly correlated with the ability of CTFs to inhibit toxicity.\textsuperscript{33} Rather, the degree of toxicity inhibition by CTFs, in particular, $\alpha_{31-42}$, $\alpha_{39-42}$, and $\alpha_{30-40}$, correlated with stabilization of the smaller and attenuation of the larger of the two oligomer populations with hydrodynamic radii of 8–12 nm and 20–60 nm, respectively, as measured by DLS.\textsuperscript{35} These findings are difficult to reconcile using a simple explanation of how the inhibitors affect $\alpha_{1-42}$ assembly but, rather, demonstrate that (i) CTFs do not prevent $\alpha_{1-42}$ self-assembly, (ii) CTFs bind to $\alpha_{1-42}$ and subtly affect its assembly, and (iii) CTFs and $\alpha_{1-42}$ co-assemble into oligomeric structures that are not grossly different from those formed in the absence of inhibitors (i.e., they have similar hydrodynamic radii\textsuperscript{35} and similar morphologies) (H.L. and G.B., unpublished results).

Two plausible mechanisms by which inhibitors could reduce $\alpha_{1-42}$ toxicity are as follows: (a) CTFs modulate $\alpha_{1-42}$ oligomer structure by inducing subtle structural changes or (b) CTFs mask $\alpha_{1-42}$ groups or regions that interact with cellular targets. These mechanisms are not mutually exclusive and may be operating together. Our purpose in the present study was to gain insight into these mechanisms from the relatively high resolution data provided by the DMD approach. Thus, we examined the structures and structural changes occurring during early stages of $\alpha_{1-42}$ assembly in the presence of three selected toxicity inhibitors ($\alpha_{30-40}$, $\alpha_{31-42}$, and $\alpha_{39-42}$) or a control peptide ($\alpha_{21-30}$). $\alpha_{1-42}$ was found to co-assemble with all three CTFs under study into large heterooligomers. In contrast, $\alpha_{1-42}$ and $\alpha_{21-30}$ formed small heterotypic assemblies comprising mostly one $\alpha_{1-42}$ and seven to eight $\alpha_{21-30}$ peptides. Thus, in our simulations, toxicity inhibitors but not control peptides induced formation of large heterooligomers.

Mechanisms by which toxicity inhibitors interact with $\alpha_{1-42}$ to reduce toxicity are not well understood. Liu et al. showed that a simple disaccharide, trehalose, inhibited oligomer formation and reduced toxicity of $\alpha_{30-40}$ but not that of $\alpha_{39-42}$.\textsuperscript{53} This study suggests that trehalose induced alloform-specific structural changes that disrupted specific interactions of $\alpha_{30-40}$ but not those of $\alpha_{39-42}$, with its environment. Similarly, here, we found specific interactions of different inhibitors with $\alpha_{1-42}$.

Though all three CTFs decreased the tendency of $\alpha_{1-42}$ to form $\beta$-strands, $\alpha_{39-42}$ was the most efficient and $\alpha_{31-42}$, the least efficient among the three. The ability to disrupt $\beta$-strand formation was negatively correlated with the $\beta$-strand content in CTFs themselves. In contrast to CTFs, $\alpha_{21-30}$ significantly increased the propensity of $\alpha_{1-42}$ to form the $\beta$-strands.

Of the four $\alpha_{1-42}$ fragments, $\alpha_{39-42}$ interacted most strongly with $\alpha_{1-42}$. Interestingly, the predominantly hydrophilic $\alpha_{21-30}$ interacted with $\alpha_{1-42}$ as strongly as $\alpha_{30-40}$ or $\alpha_{31-42}$, though the location and nature of these interactions were distinct from those between $\alpha_{1-42}$ and the two CTFs. These results suggest that peptide length affects the $\alpha_{1-42}/\alpha_{X-Y}$ interaction more than does its hydrophobic versus hydrophilic nature. As expected, the self-assembly of $\alpha_{X-Y}$ was strongly affected by the hydrophobic versus hydrophilic character of the fragments. In contrast to the CTFs, $\alpha_{21-30}$ did not self-assemble. Importantly, unlike the three toxicity inhibitors, $\alpha_{21-30}$ did not interact with the A2–F4 region of $\alpha_{1-42}$.

An involvement of the A2–F4 region in $\alpha_{1-40}$ but not $\alpha_{1-42}$ folding and oligomer formation was demonstrated in previous DMD studies.\textsuperscript{22,24,25} In $\alpha_{1-40}$, the A2–F4 region had a relatively high $\beta$-strand propensity,\textsuperscript{22,24,25} resulting in less favorable $\alpha_{40}$ hexamer formation, which included a slow dock-and-lock intermolecular interaction involving the A2–F4 $\beta$ strand regions.\textsuperscript{25} Intermolecular contacts among the A2–F4 regions caused the N-terminal region to be more shielded from the solvent in $\alpha_{1-40}$ than in $\alpha_{1-42}$ oligomers. Similar to the differences between $\alpha_{1-40}$ and $\alpha_{1-42}$, our present analysis showed that region D1–R5 of $\alpha_{1-42}$ was significantly less exposed to the solvent in heterooligomers formed in the presence of toxicity inhibitors than in $\alpha_{1-42}$ oligomers formed in the absence of $\alpha_{3}$ fragments. In contrast, in the presence of $\alpha_{21-30}$, the solvent exposure of the N-terminal region D1–R5 of $\alpha_{1-42}$ significantly increased relative to unaltered $\alpha_{1-42}$ oligomers.

Our hypothesis that the N-terminus of $\alpha_{1-42}$ mediates $\alpha_{1-42}$-induced toxicity is consistent with findings of two recent studies. Luheishi et al. who used a Drosophila model of AD demonstrated that (a) an A2F substitution in $\alpha_{1-42}$ increased its toxicity, and (b) whereas an E22G substitution in $\alpha_{1-42}$ dramatically increased its toxicity, a double substitution, E22G/F4D, led to a significantly reduced toxicity.\textsuperscript{54} In addition, Jin et al. used $\alpha_{1-42}$ dimers isolated from the cortex of AD patients and showed that they mediate toxicity by directly inducing Tau phosphorylation. They further demonstrated that the monoclonal antibodies that bind to the N-terminal DI, but not the antibody that binds to the C-terminus of $\alpha_{1-42}$, inhibited this $\alpha_{1-42}$-mediated toxicity.\textsuperscript{55}

In summary, our present results offer mechanistic insights into processes involved in $\alpha_{1-42}$ assembly in the presence of $\alpha_{1-42}$ fragments and provide an insight into the putative mechanism(s) by which the CTFs inhibit toxicity. We identified two structural elements, increased $\beta$-strand propensity and increased solvent exposure at the N-terminus of $\alpha_{1-42}$ that correlated with $\alpha_{1-42}$-induced toxicity...
Table 2. Summary of the cell viability data for $\beta_{1-42}$-induced toxicity in cell cultures extracted from Fradinger et al.$^{32}$ and for $\beta_{1-42}$ in the presence of $\beta$ fragments extracted from Li et al.$^{33}$ (column 2) alongside the main structural changes in $\beta_{1-42}$ during its assembly at the highest concentration of $\beta$ fragments as observed here (columns 3–5).

<table>
<thead>
<tr>
<th>$\beta_{X-Y}$</th>
<th>Cell viability (%)</th>
<th>Assembly state</th>
<th>$\Delta$($\beta$-strand) (%)</th>
<th>$\Delta$SASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_{1-42}$</td>
<td>62 ± 4</td>
<td>Oligomers</td>
<td>0</td>
<td>±0</td>
</tr>
<tr>
<td>+$\beta_{30-40}$</td>
<td>98 ± 7</td>
<td>Large H-O</td>
<td>–7</td>
<td>&lt;0</td>
</tr>
<tr>
<td>+$\beta_{1-42}$</td>
<td>105 ± 5</td>
<td>Large H-O</td>
<td>–3</td>
<td>&lt;0</td>
</tr>
<tr>
<td>+$\beta_{19-42}$</td>
<td>89 ± 5</td>
<td>Large H-O</td>
<td>–9</td>
<td>&lt;0</td>
</tr>
<tr>
<td>+$\beta_{21-30}$</td>
<td>63 ± 7</td>
<td>Ms/small H-Os</td>
<td>9</td>
<td>&gt;0</td>
</tr>
</tbody>
</table>

H-O, heterooligomer; M, monomer.

(2 Table 2). This work delineates plausible structure–toxicity relationships amenable to in vitro and in vivo testing and provides structural information of potential importance for drug design.

Methods

The DMD approach

DMD is a form of molecular dynamics that utilizes interparticle potentials in a form of a single or a combination of square wells, which simplifies calculations of individual trajectories and makes DMD orders of magnitude faster than molecular dynamics.$^{36}$ Here, DMD is combined with a four-bead protein model, in which each amino acid is described by up to four beads (representing the amino $N$, $\alpha$-carbon $C1$, carboxyl $C$, and $\beta$-carbon $C3$ groups).$^{57,58}$ This four-bead protein model is based on geometric properties and the backbone hydrogen bonding introduced by Ding et al.$^{58}$ The lengths of bonds and the angular constraints are determined phenomenologically by calculating their distributions using the known folded protein structures of ~7700 proteins from the Protein Data Bank.$^{58,59}$ Effective backbone hydrogen bonds are implemented between the nitrogen atom $N$ of the $i$-th amino acid and the carbon atom $C$ of the $j$-th amino acid.$^{58}$ The absolute value of the potential energy of the backbone hydrogen bond interaction, $E_{HB}$, represents a unit of energy, and the temperature is given in units of $E_{HB}/k_B$. We implemented the amino-acid-specific interactions due to hydrophathy and charge of individual side chains that are critical$^{22}$ to distinguish between $\beta_{1-40}$ and $\beta_{1-42}$ folding and assembly pathways.$^{20,22,25}$ The ab initio DMD approach with a four-bead protein model and implicit solvent used in this study is the same as that used in the previous studies of $\beta$ folding and assembly.$^{22,25}$ and has been described in detail by Urbanc et al.$^{20}$ Relative to earlier DMD studies,$^{22,23,32}$ a present DMD parametrization includes a more accurate estimate of physiological temperature and two times longer simulation time, which was recently shown to best account for the distinct oligomer size distributions of $\beta_{1-40}$, $\beta_{1-42}$, and their Arctic mutants.$^{24,25}$

The DMD simulation protocol

Keeping the number of $\beta_{1-42}$ molecules fixed (either 16 or 6), we varied the number of $\beta_{X-Y}$ molecules to obtain a desired $\beta_{1-42}/\beta_{X-Y}$ concentration ratio. The total molar concentration was kept constant across simulations and equal to the one used in our previous study of oligomer formation of full-length $\beta$ peptides.$^{29}$ Initially, the centers of mass of unstructured monomeric $\beta_{1-42}$ and $\beta_{X-Y}$ molecules were arranged in the simulation box into a cubic lattice. Distinct initial configurations for multiple trajectories per each $\beta_{1-42}/\beta_{X-Y}$ concentration were obtained by performing short, high-temperature DMD simulation runs and saving configurations every 0.1×10$^6$ simulation steps. This protocol resulted in randomly placed and mixed peptides of each type that were used as initial populations for production runs (Fig. 1 in Supplementary Material). Multiple trajectories at each $\beta_{1-42}/\beta_{X-Y}$ concentration ratio were acquired at physiological temperature ($T=0.13$) using the implicit solvent parameters $E_{BP}=0.3$ and $E_{CH}=0$ that best described the initial hydrophobic collapse into globular $\beta_{1-42}$ oligomers. For each of the four $\beta_{X-Y}$, five different molar concentration ratios were studied, resulting in 20 systems. For each system, eight trajectories of 20×10$^6$ simulation steps were acquired. The resulting $\beta_{1-42}$ structures formed in the presence of $\beta_{X-Y}$ molecules were quantified in terms of their secondary, tertiary, and quaternary structures and solvent exposure and compared to unaltered $\beta_{1-42}$ oligomer structures derived previously.$^{29}$ Supplementary Methods in Supplementary Material provides a more detailed description of our computational approach and its limitations.

Acknowledgements

This research was supported by National Institutes of Health grants AG027818 and AG023661, Larry L. Hillblom Foundation grant 20052E, a generous gift from the Turken Family, and the National Science Foundation through TeraGrid resources provided by Purdue University. We thank Dr. Bogdan Barz for his help with the VMD graphics.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmb.2011.05.021

References


Supplementary Material for *Structural basis for $A\beta_{1-42}$ toxicity inhibition by $A\beta$ C-terminal fragments: Discrete molecular dynamics study*

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Preprint submitted to Elsevier May 29, 2011
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Supplementary Methods

Discrete Molecular Dynamics. DMD is a form of MD that can be applied when the interparticle potentials are approximated by one or more square wells [1]. The force between two particles is then non-zero only at discrete distances, at which the particles collide, rendering DMD event-driven. Between events/collisions, particles move along straight lines with constant speeds, which simplifies calculations of individual trajectories and makes DMD orders of magnitude faster than MD, which is important for studying protein assembly at biologically relevant time scales [2]. In our DMD approach, the simulation volume and number of particles are fixed, the temperature is held constant by the Berendsen thermostat [3], and periodic boundary conditions are implemented [4].

The Four-Bead Protein Model with Amino Acid-Specific Interactions. In the four-bead protein model, the backbone is represented by three beads, corresponding to the amide (N), the α-carbon (Cα), and the carbonyl (C) groups. Each side-chain is represented by one side-chain bead (Cβ). The aqueous solvent is implemented by introducing effective amino acid-specific hydrophatic interactions among Cβ atoms [5] using the hydropathy scale derived by Kyte and Doolittle [6]. At neutral pH, the amino acids I, V, L, F, C, M, and A are considered hydrophobic, N, Q, and H are considered non-charged hydrophilic, and R, K, D, and E are considered charged hydrophilic. The remaining amino acids with absolute values of hydropathies below threshold values are treated as neutral. Each Cβ atom is associated with a hydropathy parameter normalized between −1 (for the most hydrophobic residue) to +1 (for the most hydrophilic residue) and neutral Cβ atoms are assigned a zero hydropathy parameter. Attractive or repulsive Cβ–Cβ interactions are implemented within the group of hydrophobic residues or within the group of hydrophilic residues, respectively. The remaining Cβ–Cβ interactions are due to the excluded volume effect. The effective hydrophatic interaction is modeled by a single square-well potential with the absolute value of the potential energy (or the strength of the interaction) equal to $E_{HP}$ and the interaction range of 0.75 nm. When the Cβ–Cβ distance of two hydrophobic/hydrophilic side-chain atoms decreases from above to below 0.75 nm, the two atoms experience an attractive/repulsive interaction, equal to $E_{HP}$ multiplied by an average of their respective hydropathy parameters.

The implicit solvent parameter $E_{HP}$, which strongly depends on the
presence or absence of water in the solvent, is set to $E_{HF} = 0.3$ (in units of $E_{HH}$) [5, 7, 8]. The interaction between two hydrophobic side chains is attractive, to mimic the tendency of each hydrophobic residue to decrease its solvent exposure. The interaction between two hydrophilic side chains is repulsive, to mimic the tendency of the two hydrophilic residues to maximize their total solvent exposure. The effective electrostatic interactions, as discussed in detail in our recent work [8], are not expected to play a significant role at the early stages of $\alpha\beta$ assembly, thus the corresponding implicit solvent parameter was set to zero, $E_{CH} = 0$. Under such conditions, the charged amino acids R, K, D, and E are considered purely hydrophilic.

**Limitations of the DMD Approach.** The present DMD simulations are limited by: (i) the use of high molar concentrations (about 100-fold higher than those used *in vitro*), resulting in assembly populations that lack $\alpha\beta_{1-42}$ monomers; and (ii) the modeling of the early, hydrophobic-collapse stage of the assembly during which effective electrostatic interactions may not play a key role. With regard to the high concentrations used in the simulations, we note that the *in vitro* oligomer distributions are actually quite insensitive to the concentration for concentrations above a threshold of $\sim$1-3 $\mu$M under which large oligomers, e.g. pentamers and hexamers, begin to fall apart into dimers and trimers (G. Bitan, unpublished data). In contrast, the aggregation kinetics is sensitive to the concentration. The rate at which the oligomers eventually assemble into larger aggregates and fibrils is accelerated as the concentration increases, but the oligomer distribution itself does not change appreciably. Due to these limitations as well as the limited number of peptide chains that can be simulated, our approach is tuned to simulate a single population of $\alpha\beta_{1-42}$/CTF heterooligomers and cannot account for the larger of the two assembly populations observed by DLS [9, 10]. The initial hydrophobic collapse and a much slower aggregation into fibrils occur at two well-separated time scales and likely involve distinct assembly mechanisms. The DMD approach captures oligomer formation and resulting structures but cannot be used, in its current form, to predict the ability of heterotypic assemblies to aggregate into fibrils.

**Simulation Parameters.** In prior work, we reported results of preliminary simulations of $\alpha\beta_{1-42}$ in the presence of $\alpha\beta_{29-42}$, $\alpha\beta_{31-42}$, or $\alpha\beta_{39-42}$ [9]. These preliminary simulations were performed at a temperature of $T = 0.15$ and the corresponding trajectories were each $10 \times 10^6$ simulation steps long. Here, new simulation trajectories, each $20 \times 10^6$ sim-
ulation steps long, were acquired at a better estimate of the physiological temperature of $T = 0.13$, which was recently shown to result in a closer agreement between in silico and in vitro findings [11, 8]. In addition, the box sizes (volumes) in the present simulations were adjusted to the $A\beta$ fragment length and the concentration ratio $A\beta_{1-42}$:CTF to keep the total molar concentration approximately the same for all systems under study across all $A\beta$ fragment concentrations and equal to the molar concentration used in our previous work with 32 $A\beta_{1-42}$ molecules in a cubic simulation box of size 250 Å [5, 7, 8].

In the first set of simulations reported here, we studied $A\beta_{1-42}$ oligomerization in the presence of each of the three CTFs at four different relative $A\beta_{1-42}$:CTF number concentrations\(^4\): 1:1 (1:2), 1:2 (1:4), 1:4 (1:8), and 1:8 (1:16), where the number concentrations in parentheses correspond to $A\beta_{1-42}$:$A\beta_{39-42}$ (Table I, column 1). The same number concentration ratios, 1:1, 1:2, 1:4, and 1:8, were explored in control simulations of $A\beta_{1-42}$ assembly in the presence of $A\beta_{21-30}$. Keeping the number of $A\beta_{1-42}$ molecules fixed at 16, we varied the number of $A\beta_{30-40}$, $A\beta_{31-42}$, or $A\beta_{21-30}$ molecules from 16 to 128 and the number of $A\beta_{39-42}$ molecules from 32 to 256. The size of the cubic simulation box for each CTF type and concentration is reported in Table I. Initially, the centers of mass of unstructured monomeric $A\beta_{1-42}$ and $A\beta$ fragments were arranged in the simulation box into a cubic lattice. Within the simulation box, $A\beta_{1-42}$ and $A\beta$ fragments were segregated with $A\beta_{1-42}$ molecules on one side, and $A\beta_{X-Y}$ peptides on the other side (Fig. Ia). Distinct initial configurations\(^5\) for 8 trajectories per $A\beta$ fragment per number concentration ratio were obtained by performing short high-temperature ($T = 4$) DMD simulation runs and saving configurations

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\(^3\)The total molar concentration is a sum of the the $A\beta_{1-42}$ and CTF molar concentrations. The molar concentration of $A\beta_{1-42}$ is equal to the number concentration of $A\beta_{1-42}$ multiplied by its molar mass. The molar mass of each of the three CTFs, expressed in terms of the molar mass of $A\beta_{1-42}$, is estimated based on the sequence length relative to $A\beta_{1-42}$: (a) 11/42 $\approx$ 0.262 (for $A\beta_{30-40}$); (b) 12/42 $\approx$ 0.286 (for $A\beta_{31-42}$); (c) 4/42 $\approx$ 0.095 (for $A\beta_{39-42}$); and (d) 10/42 $\approx$ 0.238 (for $A\beta_{21-30}$). This is an approximation that is consistent with our four-bead protein model, in which all amino acids (except glycine) have the same mass, and so within this model the number of amino acids in the sequence is a good estimate of the mass of the peptide sequence.

\(^4\)The relative $A\beta_{1-42}$:CTF number concentration specifies the number of CTF molecules for each $A\beta_{1-42}$ molecule used in simulations. The relative number concentrations do not depend on the molar masses of $A\beta_{1-42}$ and CTF molecules.

\(^5\)We use the term “configuration” to refer to a population of all conformers of different compositions and sizes present in a simulation at a particular time frame.
every $0.1 \times 10^6$ simulation steps. This protocol resulted in randomly-placed peptides of each of the two types, as expected based on entropy considerations (Fig. Ib). We conducted eight productions runs per A$\beta_{X-Y}$ per concentration ratio, in total acquiring 128 trajectories, each $20 \times 10^6$ simulation steps long.

The purpose of the first set of simulations described above was to examine heterotypic assemblies formed by A$\beta_{1-42}$ and A$\beta_{X-Y}$ and quantify the changes in structural properties of A$\beta_{1-42}$ with increasing relative concentration of A$\beta$ fragments. The relative molar concentrations of A$\beta_{X-Y}$ explored in the first set of simulations (Table I) were low relative to the A$\beta_{1-42}$:CTF molar concentration $\sim 1:10$, which was required to inhibit A$\beta_{1-42}$ toxicity in cell cultures [9]. To delve into structural characteristics of heterooligomers at the molar concentration ratios relevant to toxicity inhibition, we acquired a second set of simulations of 6 A$\beta_{1-42}$ assembling in the presence of (a) 229 A$\beta_{30-40}$, (b) 210 A$\beta_{31-42}$, (c) 630 A$\beta_{39-42}$, and (d) 252 A$\beta_{21-30}$ molecules. The simulation box in all three cases was selected to match the overall molar concentration in the first set of simulations, 318 Å. The total molar concentration used was 10-100 fold larger than in typical in vitro conditions. Such a high molar concentration is needed to increase the probability of intermolecular interactions, making the assembly process fast enough to be observed and quantified in silico. In this second set of simulations, we acquired for each of the four A$\beta$ fragments 8 trajectories of $20 \times 10^6$ simulation steps, in total 32 trajectories, following the same protocol, temperature, and implicit solvent parameters as in the first set of simulations.

**Structural Analysis.**

**Probability Distributions of Assembly Sizes.** The acquired DMD trajectories contained populations of A$\beta_{1-42}$ and A$\beta_{X-Y}$ monomers, A$\beta_{1-42}$ and A$\beta_{X-Y}$ oligomers, and heterotypic assemblies comprising both A$\beta_{1-42}$ and A$\beta_{X-Y}$ peptides. To identify the connections between individual chains within each assembly we used the criterion that was introduced in the previous studies [5, 7, 8], in which two chains A and B are considered to be part of the same assembly if there exists a pair of atoms, one from chain A and one from chain B, such that the distance between the center of masses of the two atoms is less than 0.75 nm.

In total, eight trajectories of A$\beta_{1-42}$ assembly in the presence of A$\beta$ fragments were produced for each A$\beta_{X-Y}$ and each A$\beta_{1-42}$:A$\beta_{X-Y}$ concent-
tration ratio. The size distributions of resulting assemblies were calculated by taking into account populations of assemblies at time frames of 19, 19.5, and $20 \times 10^6$ simulation steps to facilitate comparison to the Aβ$_{1-42}$ oligomer size distribution obtained in the absence of Aβ fragments in our previous work [8]. For each of these 24 assembly populations, the probability distributions of Aβ$_{1-42}$ and Aβ$_{X-Y}$ assemblies of all sizes within the heterotypic assemblies were calculated. This was achieved by considering the spatial coordinates of either only Aβ$_{1-42}$ peptides or only Aβ$_{X-Y}$ peptides and applying the above criterion to determine whether two peptide chains belonged to the same assembly. The final normalized probability size distributions of Aβ$_{1-42}$ and Aβ$_{X-Y}$ assemblies within the Aβ$_{1-42}$/Aβ$_{X-Y}$ heterooligomers and the standard errors of the mean (SEM) were calculated as averages over 24 individual probability distributions.

**Contact Maps.** For contact map analysis and all the other analyses described below, realizations at eleven time frames between 19 and $20 \times 10^6$ simulation steps (at $10^5$-step intervals) were considered. A contact map is a matrix in which the value of each (i,j) element is equal to an average number of contacts between amino acids i and j. We consider two types of contact maps: intra- and intermolecular contact maps. If amino acids i and j belong to the same peptide, the corresponding contacts contribute to the intramolecular contact map, otherwise, to the intermolecular contact map. For a mixture of heterotypic assemblies, we calculate two intra- and three intermolecular contact maps, including the “cross” intermolecular contact map. The contact map of each assembly is normalized by the number of contributing peptide molecules. We normalize intermolecular contact maps between Aβ$_{1-42}$ and Aβ$_{X-Y}$ molecules by the number of Aβ$_{1-42}$ molecules so that the average strength of a contact at (i,j) corresponds to the average number of contacts between amino acid i belonging to Aβ$_{1-42}$ and amino acid j belonging to Aβ$_{X-Y}$. In the four-bead protein model each amino acid is represented by up to four beads, thus the maximal number of intramolecular contacts between residues i and j is $4 \times 4 = 16$. The maximal number of intermolecular contacts between residues i and j can be larger than 16 because amino acid i of one molecule can be surrounded by several amino acids j from multiple molecules. We represent the final average contact maps on a color scale between 0 (dark blue) and 1 (pink), where 0 means no significant contacts and 1 corresponds to 16 contacts, i.e. to one full contact between two amino acids. All colors other than dark blue denote significant non-zero contacts between amino acids, for which the average
number of contacts is larger than the corresponding SEM.

**Binding Propensity.** To quantify the degree of intermolecular interactions between each Aβ fragment and Aβ₁₋₄₂, we calculated a binding propensity, defined as an average contact strength, i.e., the average number of contacts between two specified regions of the peptides in contact. Binding propensity as defined here is a dimensionless quantity, normalized in the same way as the contact maps, with the value of 1 corresponding to one full contact between two amino acids. Its value can be larger than 1 because any amino acid belonging to Aβ₁₋₄₂ can be in contact with the another amino acid involving multiple Aβₓ₋ₐ peptides.

**Secondary Structure.** Amino acid-specific propensities for the secondary structure formation were calculated using the STRIDE program [12, 13]. The secondary structure propensities included several types of α-helical, β-strand, turn, and bridge structures. Here we analyzed in detail the dominant secondary structure element, the β-strand.

**Solvent Accessible Surface Area.** We calculated the solvent accessible surface area per amino acid (SASA) by using the Visual Molecular Dynamics (VMD) software package [14]. This calculation uses a spherical surface around each of the four beads (three in the case of glycine), 1.4 Å away from the atom’s van der Waals surface. The joint “free” surface area for all atoms in an amino acid is then calculated by taking into account surfaces of all other atoms in the assembly. We also consider the backbone carbonyl oxygen and amide hydrogen involved in the hydrogen bond formation. The “free” surface area is defined as the area free of contact with other atoms, which would thus be exposed to the solvent. Amino acids that are buried inside an assembly have small SASA values, whereas amino acids on the surface assume high SASA values.

**Supplementary Results**

**Distributions of Aβ₁₋₄₂ and Aβₓ₋ₐ within Heterotypic Assemblies.** We examined the differences in morphology among the heterotypic assemblies Aβ₁₋₄₂/Aβ₃₀₋₄₀, Aβ₁₋₄₂/Aβ₃₁₋₄₂, Aβ₁₋₄₂/Aβ₃₉₋₄₂, and Aβ₁₋₄₂/Aβ₂₁₋₃₀ by studying distribution of peptides within the final heterotypic assemblies. We calculated the probability size distributions of Aβ₁₋₄₂ molecules (Fig. IIa-d) as well as Aβₓ₋ₐ molecules (Fig. IIe-g) within the heterooligomers.
at four different $A\beta_{1-42}:A\beta_{X-Y}$ concentration ratios, to elucidate the spatial distribution of the two molecule types within the heterotypic assemblies. The size distributions of $A\beta_{1-42}$ versus $A\beta_{X-Y}$ assemblies were examined to elucidate the preference of $A\beta_{1-42}$ (or $A\beta_{X-Y}$) to either self-assemble of associate with $A\beta_{X-Y}$ (or $A\beta_{1-42}$). Our results showed that $A\beta_{1-42}$ and $A\beta_{X-Y}$ size distributions strongly depended on the $A\beta_{1-42}:A\beta_{X-Y}$ concentration ratio.

At low $A\beta_{X-Y}$ concentrations that corresponded to molar concentration ratios 1:MC with $MC < 1$ (Table I), the three toxicity inhibitors promoted $A\beta_{1-42}$ assembly by enhancing self-association of $A\beta_{1-42}$ molecules that resulted in large connected $A\beta_{1-42}$ structures as demonstrated by a dominant peak at $n = 16$ in the corresponding probability distributions (Fig. IIa-c, orange and green curves). At these same concentration ratios, the CTF molecules within heterooligomers formed connected structures of characteristic sizes that depended on the CTF type (Fig. IIe-g, orange and green curves). At the two lowest CTF concentrations, the characteristic sizes of the connected CTF structure were:

\begin{itemize}
    \item $A\beta_{30-40}$: $n = 4 (P = 0.31$ for 1:1), $n = 8 (P = 0.15$ for 1:1 and $P = 0.20$ for 1:2) and $n = 24 (P = 0.20$ for 1:2);
    \item $A\beta_{31-42}$: $n = 2 (P = 0.28$ for 1:1), $n = 5 (P = 0.13$ for 1:1), $n = 12 (P = 0.13$ for 1:1 and $P = 0.19$ for 1:2) and $n = 20 (P = 0.17$ for 1:2);
    \item $A\beta_{39-42}$: $n = 3 (P = 0.21$ for 1:2) and $n = 8 (P = 0.14$ for 1:4),
\end{itemize}

where 1:1, 1:2, and 1:4 refer to the $A\beta_{1-42}$:CTF number concentration ratio and $P$ is the probability of occurrence. Only the low molecular weight ($n \leq 24$) structures with $P > 0.1$ were considered. Interestingly, $A\beta_{30-40}$ self-assembled into tetramers and octamers, $A\beta_{31-42}$ into dimers, pentamers, and dodecamers (resembling the oligomer size distribution of $A\beta_{1-42}$), and $A\beta_{39-42}$ into trimers and octamers. Thus, at low CTF concentrations each of the three types of CTFs were characterized by a particular pattern of self-assembly and the characteristic $A\beta_{30-40}$ and $A\beta_{39-42}$ assemblies were more similar to each other than to $A\beta_{31-42}$.

At CTF concentrations that corresponded to relative molar concentrations larger than 1:2 (see Table I), CTF molecules were numerous enough to insert themselves in-between $A\beta_{1-42}$ molecules and consequently break
the large connected Aβ1−42 structures into smaller ones, resulting in a significant decrease of the peak at n = 16 and increased probabilities at small Aβ1−42 assembly sizes (Fig. IIa-c, blue and magenta curves). Interestingly, at Aβ1−42:Aβ30−40 number concentration ratio 1:4, the peak at n = 16 was replaced by a peak at n = 2 and another peak at n = 16−2 = 14, suggesting that Aβ30−40 caused dissociation of larger Aβ1−42 oligomers into Aβ1−42 dimers (Fig. IIa, the blue curve). A similar tendency was observed for Aβ39−42 (Fig. IIc, the blue curve) but not for Aβ31−42 (Fig. IIb, the blue curve). At the Aβ1−42:Aβ31−42 number concentration ratio 1:4, the peak at n = 16 was replaced by a peak at n = 2 and another peak at n = 16−2 = 14, suggesting that Aβ30−40 caused dissociation of larger Aβ1−42 oligomers into Aβ1−42 dimers (Fig. IIa, the blue curve). A similar tendency was observed for Aβ39−42 (Fig. IIc, the blue curve) but not for Aβ31−42 (Fig. IIb, the blue curve). At the Aβ1−42:Aβ31−42 number concentration ratio 1:4, the peak at monomers (n = 1) and another peak at n = 16 − 1 = 15 suggested that Aβ31−42 caused dissociation of Aβ1−42 assemblies into Aβ1−42 monomers (Fig. IIb, the blue curve). This result implied that at these concentrations only Aβ31−42 (but not Aβ30−40 and Aβ39−42) dissociated Aβ1−42 dimers into monomers.

At Aβ1−42:Aβ30−40 and Aβ1−42:Aβ31−42 number concentration ratio 1:8, Aβ1−42 monomers dominated the size distributions (Fig. IIa-b, magenta curves), whereas at Aβ1−42:Aβ39−42 number concentration 1:16 Aβ1−42 monomers as well as pentamers were the most numerous together with Aβ1−42 structures with n = 16 − 1 = 15 and n = 16 − 5 = 11 molecules, suggesting that the presence of Aβ39−42 stabilized both Aβ1−42 monomers and pentamers. At the highest two CTF concentrations, most of the CTFs of the three types formed a single connected structure within the heterooligomers (Fig. IIe-g, blue and magenta curves). The exception was Aβ30−40, which at the Aβ1−42:Aβ30−40 number concentration ratio of 1:4 formed with equal probability one or two clusters of the same size (Fig. IIe, the blue curve).

The control Aβ fragment that was previously shown not to affect Aβ1−42 toxicity in cell cultures, Aβ21−30, had quite a different effect on Aβ1−42 assembly (Fig. IId,h). In a concentration-dependent way, Aβ21−30 efficiently inhibited Aβ1−42 assembly, such that at the 1:8 number concentration ratio, ~90% of Aβ1−42 peptides were spatially separated from each other. Interestingly, increased Aβ21−30 concentration promoted the association of Aβ1−42 into small heterotypic Aβ1−42/Aβ21−30 assemblies. Among these, a typical heterooligomer comprised one Aβ1−42 molecule associated with 7 to 8 Aβ21−30 peptides (Fig. 2e).

**Effect of Aβ Fragment Concentration on Aβ1−42 Tertiary Structure.** As demonstrated in our recent work [8], the tertiary structure within Aβ1−42 hexamers formed in the absence of Aβ fragments included four turn regions, of which the central folding region centered at G25-S26
was associated with the largest number of strong contacts, followed by the turn region centered at G37-G38 (Fig. IIIa, boxes 3,4). Additional two turns were centered at S8-G9 and Q15-K16 (Fig. IIIa, boxes 1,2). These turns were flanked by regions of increased $\beta$-strand propensity, resulting in bands of contacts perpendicular to the main diagonal (Fig. IIIa, boxes 1-4). The regions most involved in intramolecular contacts were the CHC, MHR, and CTR. In the absence of CTFs, A$\beta_{1-42}$ but not A$\beta_{1-40}$ monomers were characterized by a turn centered at G37-G38 [5], and the CTR of A$\beta_{1-42}$ oligomers was involved in significantly more and stronger contacts than the CTR of A$\beta_{1-40}$, resulting in the group of intramolecular contacts between the CHC and MHR/CTR [8] (Fig. IIIa, box 5).

In the presence of all A$\beta$ fragments, the A$\beta_{1-42}$ tertiary structure preserved all characteristic turn regions (Fig. IIIb-r), even though the strength of the strongest contacts decreased relative to pure A$\beta_{1-42}$ hexamers in a concentration-dependent manner. Simultaneously, we observed that the presence of the three toxicity inhibitors resulted in minor spreading of the “native” tertiary A$\beta_{1-42}$ contacts onto the neighboring amino acids, similar to a conformational change from the native to the molten globule state observed during denaturation [15]. This conformational change in the A$\beta_{1-42}$ tertiary structure was consistent with the reduced $\beta$-strand propensity observed in the presence of toxicity inhibitors (Fig. 3a, black, red and green solid curves). In contrast, the control peptide A$\beta_{21-30}$ increased the strengths of tertiary contacts involving the CHC, MHR, and CTR and thus stabilized the A$\beta_{1-42}$ tertiary fold while simultaneously increasing its $\beta$-strand propensity (Fig. 3a, grey curve).

The presence of the three toxicity inhibitors, almost independently of their concentration, reduced the number and strengths of intramolecular contacts in the central folding region of A$\beta_{1-42}$ (Fig. IIIb-d,f-h,j-l,n-p, box 3), which was hypothesized to nucleate A$\beta$ folding [16]. The control peptide A$\beta_{21-30}$, in contrast, increased the strengths of intramolecular A$\beta_{1-42}$ contacts in the central folding region relative to A$\beta_{1-42}$ hexamers formed in the absence of fragments (Fig. IIIe,i,m,r, box 3). The three toxicity inhibitors also reduced the intramolecular contacts between the CHC and MHR as well as the CHC and CTR (Fig. IIIb-d,f-h,j-l,n-p, box 5), whereas the control peptide had the reverse effect (Fig. IIIe,i,m,r, box 5). Again, for all A$\beta$ fragments, the effect was concentration dependent. The reduction of intramolecular contacts in this region was most pronounced for A$\beta_{39-42}$ (Fig. IIIp, box 5). Of the four A$\beta$ fragments, only the highest concentra-
tion of Aβ30−40 slightly reduced the number of contacts centered at S8-G9 (Fig. IIIIn, box 1), whereas the other three fragments did not significantly affect this region even at the highest concentrations (Fig. IIIo-r, box 1).

**Effect of AβX−Y Fragment Concentration on Aβ1−42 Quaternary Structure.** The quaternary structure of Aβ1−42 hexamers formed in the absence of Aβ fragments was characterized by several intermolecular contact regions [8] (Fig. IVa, boxes 1-4). The strongest contacts were among the CTRs and between the CTR and MHR (Fig. IVa, box 4), between the CHC and MHR/CTR (Fig. IVa, box 3), and among the CHC regions (Fig. IVa, box 2). The intermolecular contacts in Aβ1−42 oligomers were dominated by the CTR, as opposed to the predominant role of the CHC in Aβ1−40 oligomers [7]. Relative to the quaternary structure of these pure Aβ1−42 hexamers, (Fig. IVa), the Aβ1−42 intermolecular contacts were drastically reduced in strengths within the Aβ1−42/AβX−Y heterotypic assemblies in an Aβ fragment concentration-dependent manner (Fig. IVb-r). The quaternary structure of Aβ1−42 molecules in heterotypic assemblies was significantly affected even at the lowest Aβ fragment concentrations (Fig. IVb,c,d,e). In the presence of the three toxicity inhibitors, but not the control peptide Aβ21−30, the intermolecular contacts were reduced in strength and were spread to the neighboring amino acids, akin to denaturation-induced changes from native to molten globule contacts. Interestingly, at low Aβ1−42:AβX−Y number concentration ratios, the intermolecular contacts among N-terminal regions, A2-F4, became stronger in the presence of the three CTFs but not in the presence of Aβ21−30 (Fig. IVb,c,d,e) relative to those in pure Aβ1−42 hexamers (Fig. IVa). We recently identified parallel, in-register intermolecular contacts involving the A2-F4 regions as a characteristic feature of Aβ1−40 but not of Aβ1−42 hexamers [8]. In the presence of Aβ39−42, additional antiparallel intermolecular contacts among the A2-Y10 regions were observed, suggesting that the presence of Aβ39−42 resulted in increased heterogeneity of Aβ1−42 association (Fig. IVd).

The reduction of Aβ1−42 intermolecular contacts in the presence of the control peptide Aβ21−30 was not surprising as the assembly size distributions demonstrated that the presence of Aβ21−30 stabilized Aβ1−42 monomers. Why was there a concentration-dependent decrease in Aβ1−42 intermolecular contacts in the presence of the three toxicity inhibitors, which promoted Aβ1−42 co-assembly with CTFs? The first reason for this decrease was the insertion of CTF molecules in-between the Aβ1−42 molecules, which resulted in diminished contacts among Aβ1−42 peptides within heterooligomers. This
interpretation was consistent with \( A\beta_{1-42} \) assembly size distributions (Fig. IIa-c), which evolved from a single peak at \( n = 16 \) at the lowest CTF concentration to an increased number of \( A\beta_{1-42} \) monomers and small assemblies (\( n < 16 \)) at higher CTF concentrations. However, the displacement of \( A\beta_{1-42} \) molecules within heterooligomers themselves was not sufficient to explain an almost complete absence of intermolecular contacts at the highest \( A\beta_{30-40} \) concentration (Fig. IVn) because at that concentration the \( A\beta_{1-42} \) assembly size distribution still showed considerable numbers of \( A\beta_{1-42} \) assemblies of orders \( n = 2, 3, 4, 8 \), which should have contributed to intermolecular contacts. The second reason for the absence of \( A\beta_{1-42} \) intermolecular contacts in the presence of a large concentration of \( A\beta_{30-40} \) was the loss of well-defined contact regions among \( A\beta_{1-42} \) molecules induced and mediated by \( A\beta_{30-40} \). The lack of specified intermolecular contact regions among \( A\beta_{1-42} \) due to \( A\beta_{30-40} \)-mediated disorder in \( A\beta_{1-42} \) association resulted in an averaged–out contact map and was consistent with the reduction in the \( \beta \)-strand propensity in \( A\beta_{1-42} \) within \( A\beta_{1-42}/A\beta_{30-40} \) heterooligomers. \( A\beta_{1-42} \) assemblies within heterooligomers thus were more diverse both in terms of the specificity of interacting regions within a single heterooligomer and among different heterooligomers. We concluded that \( A\beta_{1-42} \) assemblies within heterooligomers had a more disordered structure than \( A\beta_{1-42} \) oligomers formed in the absence of CTFs.

A decrease in the number and strengths of \( A\beta_{1-42} \) intermolecular contacts, though not as pronounced as in the case of \( A\beta_{30-40} \), was observed also at the highest concentration of \( A\beta_{31-42} \) (Fig. IVo). The \( A\beta_{1-42} \) intermolecular contacts among the CTRs, between the CTR and MHR (Fig. IVo, box 4), and between the CTR and CHC (Fig. IVo, box 3) were still present, though at reduced numbers and weaker strengths relative to pure \( A\beta_{1-42} \) hexamers. Even though \( A\beta_{30-40} \) and \( A\beta_{31-42} \) showed similar ability to displace \( A\beta_{1-42} \) molecules, as demonstrated by the size distributions of \( A\beta_{1-42} \) assemblies (Fig. IIa,c), the intermolecular contact maps suggested that the contact regions among \( A\beta_{1-42} \) molecules within \( A\beta_{1-42}/A\beta_{31-42} \) heterooligomers were better defined (more closely resembling the contacts characteristic for \( A\beta_{1-42} \) hexamers formed in the absence of fragments) than within \( A\beta_{1-42}/A\beta_{30-40} \) heterooligomers. In addition, we observed new \( A\beta_{1-42} \) intermolecular contacts (not existing in the pure \( A\beta_{1-42} \) hexamers) corresponding to an antiparallel structure involving E11-L17 in \( A\beta_{1-42}/A\beta_{31-42} \) heterooligomers (Fig. IVo, box 5).

The highest concentration of \( A\beta_{30-42} \), similar to the highest concentra-
tion of Aβ$_{30-40}$, strongly inhibited most of the Aβ$_{1-42}$ intermolecular contacts (Fig. IVp) with the exception of the parallel, in-register intermolecular contacts among the A2-F4 regions that represented the strongest intermolecular Aβ$_{1-42}$ contacts within Aβ$_{1-42}$/Aβ$_{39-42}$ heterooligomers (Fig. IVp, box 1). This result, combined with the Aβ$_{1-42}$ assembly size distribution within Aβ$_{1-42}$/Aβ$_{39-42}$ heterooligomers, which was characterized by considerable numbers of non-monomeric Aβ$_{1-42}$ structures (Fig. IIc), suggested that Aβ$_{39-42}$ molecules, rather than merely displacing Aβ$_{1-42}$ peptides, mediated disordered intermolecular contacts among Aβ$_{1-42}$ molecules that involved the N-terminal region A2-F4 of two or more monomers. Aβ$_{21-30}$ at the highest number concentration ratio of 1:8 inhibited all intermolecular contacts among Aβ$_{1-42}$ molecules (Fig. IVr).

**Tertiary Structure of Aβ Fragments.** Tertiary contacts within all four Aβ fragments remained unaffected by the variable Aβ fragment concentration (Fig. Vb-r). Aβ$_{39-42}$ was unstructured (Fig. Vd,h,l,p), whereas Aβ$_{30-40}$ (Fig. Vb,f,j,n) and Aβ$_{31-42}$ (Fig. Vc,g,k,o) showed characteristic intramolecular contacts. The control peptide Aβ$_{21-30}$ displayed a few intramolecular contacts between V24 and its C-terminal residues (Fig. Ve,i,m,r).

We identified representative tertiary structures of Aβ$_{30-40}$, Aβ$_{31-42}$, Aβ$_{39-42}$, and Aβ$_{21-30}$ within the Aβ$_{1-42}$/Aβ$_{X-Y}$ heterotypic assembly populations (data not shown). Aβ$_{30-40}$ formed two turn regions, one centered around G33 and the other around G37-G38. Aβ$_{31-42}$ adopted an asymmetric hairpin conformation centered at G37-G38 and in addition its N-terminal amino acids I31-I32 formed a few intramolecular contacts with V34-M35. Because the turn at G37-G38 is closer to the center of the sequence in Aβ$_{31-42}$ than in Aβ$_{30-40}$, Aβ$_{31-42}$ had a higher β-strand propensity than Aβ$_{30-40}$ at all concentrations (Fig. 3a, black and red dashed curves). The β-strand propensities in all Aβ fragments depended only slightly on the relative Aβ$_{1-42}$:Aβ$_{X-Y}$ concentration (Fig. 3a, dashed curves). The three strongest intramolecular contacts in Aβ$_{30-40}$ were V36-V39, I31-L34, and V36-V40 (Fig. Vb,f,j,n). The four strongest intramolecular contacts in Aβ$_{31-42}$ were V36-V39, V36-V40, I31-I41, and I31-V34 (Fig. Vc,g,k,o). Consistent with the lack of any tertiary structure in Aβ$_{39-42}$, its β-strand propensity was minimal, ~1-2%, at all Aβ fragment concentrations (Fig. 3a, green dashed curve). The tertiary structure of the control Aβ$_{21-30}$ was variable and comprised several loop structures but also included more extended structures that contributed to an increased amount of β-strand relative to the other three fragments. The folded structures of Aβ$_{31-42}$ and Aβ$_{39-42}$,
in a series of $\text{A}_\beta_{X-42}$ ($X = 29 - 31$), were recently studied by Wu et al. using replica exchange MD in the presence and absence of the solvent [17]. The $\beta$-hairpin tertiary structure of $\text{A}_\beta_{31-42}$ and the lack of tertiary structure in $\text{A}_\beta_{39-42}$ within $\text{A}_\beta_{1-42}/\text{A}_\beta_{X-Y}$ assembly populations derived by our DMD approach are consistent with the reported $\text{A}_\beta_{31-42}$ and $\text{A}_\beta_{39-42}$ folded conformations in the solvent [17].

**Quaternary Structure of A$\beta$ Fragments.** The strengths of intermolecular contacts among CTFs within $\text{A}_\beta_{1-42}$/CTF heterooligomers increased with the CTF concentration (Fig. VIIb-d,f,h,j,l,n-p). At the lowest $\text{A}_\beta_{1-42}$/CTF concentrations (Fig. VIIb-d), $\text{A}_\beta_{30-40}$ was characterized by the weakest and $\text{A}_\beta_{39-42}$ by the strongest homotypic intermolecular contacts. Among the three toxicity inhibitors, $\text{A}_\beta_{39-42}$ formed the strongest intermolecular contacts at all $\text{A}_\beta_{39-42}$ concentrations, presumably due to its short length and strongly hydrophobic nature, resulting in high self-association propensity of the dipeptide V40-I41 (Fig. VId,h,l,p). The two longer CTFs, $\text{A}_\beta_{30-40}$ and $\text{A}_\beta_{31-42}$, had similar self-assembly propensities and their intermolecular contacts showed concentration-dependent increase in strengths (Fig. VIIb,f,j,n and c,g,k,o). $\text{A}_\beta_{30-40}$ self-assembly was dominated by the region I31-I32, whereas $\text{A}_\beta_{31-42}$ self-assembly was dominated by the region V40-I41, however all hydrophobic amino acids (I31, I32, L34, M35, V36, V39, V40, I41), excluding the terminal ones, were involved in self-assembly of both CTFs. In contrast to the three toxicity inhibitors, the control peptide $\text{A}_\beta_{21-30}$ had a very low self-assembly propensity that was independent of its concentration (Fig. VIe,i,m,r). The few weak contacts existed among A21 and V24, however, most of $\text{A}_\beta_{21-30}$ peptides adopted monomeric states and did not form quaternary structure.

**Regions of Contact between A$\beta_{1-42}$ and A$\beta$ Fragments.** The analysis of the quaternary structure of $\text{A}_\beta_{1-42}/\text{A}_\beta_{X-Y}$ heterotypic assemblies elucidated the regions of contacts between $\text{A}_\beta_{1-42}$ and $\text{A}_\beta_{X-Y}$ molecules (Fig. VIIb-r, boxes 1-4). While all intermolecular contact maps between $\text{A}_\beta_{1-42}$ and A$\beta$ fragments displayed concentration-dependent increase in contact strengths, $\text{A}_\beta_{39-42}$ showed the strongest contacts as well as the sharpest $\text{A}_\beta_{39-42}$ concentration-dependent increase in contact strengths. The regions of $\text{A}_\beta_{1-42}$ that most strongly interacted with A$\beta$ fragments were: the CTR (Fig. VIIb-r, box 4) followed by the CHC (Fig. VIIb-r, box 2), MHR (Fig. VIIb-r, box 3), and the region A2-F4 (Fig. VIIb-r, box 1). Interestingly, unlike its low self-assembly propensity, the control pep-
Aβ_{21−30} coassembled with Aβ_{1−42} and the corresponding intermolecular contacts involving the CHC, MHR, and CTR of Aβ_{1−42} displayed a concentration-dependent increase in contact strengths (Fig. VIIe,i,m,r, boxes 2-4). There were no intermolecular contacts between Aβ_{21−30} and the A2-F4 region of Aβ_{1−42} (Fig. VIIe,i,m,r, box 1). Instead, Aβ_{21−30} formed an antiparallel strand with the R5-E11 region of Aβ_{1−42} (Fig. VIIe,i,m,r, region between boxes 1 and 2).
TABLE CAPTIONS

Table I: Sizes of the cubic simulation boxes each containing 16 $A\beta_{1-42}$ molecules and various numbers of $A\beta$ fragments, corresponding to four different number concentration ratios $A\beta_{1-42}:A\beta_{X-Y}$ (first column). In the parentheses of the first column are the numbers of simulations with $A\beta_{39-42}$. The ratios in the parentheses of columns 2-4 show the molar concentration ratios, calculated from the number concentration ratios, taking into account the length of peptides as an approximation to their masses.
<table>
<thead>
<tr>
<th>Peptide Concentration</th>
<th>Aβ&lt;sub&gt;1-42&lt;/sub&gt;:Aβ&lt;sub&gt;X-Y&lt;/sub&gt;</th>
<th>Aβ&lt;sub&gt;30-40&lt;/sub&gt;</th>
<th>Aβ&lt;sub&gt;31-42&lt;/sub&gt;</th>
<th>Aβ&lt;sub&gt;39-42&lt;/sub&gt;</th>
<th>Aβ&lt;sub&gt;21-30&lt;/sub&gt;</th>
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<td>16:16 (32)</td>
<td>214 Å [1:0.26]</td>
<td>217 Å [1:0.29]</td>
<td>210 Å [1:0.19]</td>
<td>213 Å [1:0.24]</td>
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<tr>
<td>16:32 (64)</td>
<td>228 Å [1:0.53]</td>
<td>231 Å [1:0.57]</td>
<td>221 Å [1:0.38]</td>
<td>226 Å [1:0.48]</td>
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<tr>
<td>16:64 (128)</td>
<td>252 Å [1:1.05]</td>
<td>256 Å [1:1.14]</td>
<td>240 Å [1:0.76]</td>
<td>248 Å [1:0.95]</td>
<td></td>
</tr>
</tbody>
</table>

Table I: Peptide Concentrations
FIGURE CAPTIONS

**Figure I:** (a) The initial cubic lattice arrangement of 16 Aβ1−42 (blue chains with D1 as red spheres) and 16 Aβ31−42 (yellow chains) peptides in the simulation box. (b) The population of conformers obtained from (a) after a short (t = 0.1 × 10^6 simulation steps) high-temperature (T = 4) DMD simulation trajectory resulting in monomeric, non-structured, and randomly placed Aβ1−42 and Aβ31−42 peptides. Populations of conformers akin to (b) were used as starting conformer populations (t = 0) in all production runs.

**Figure II:** Assembly size distributions each averaged over 8 trajectories and three time frames (19 × 10^6, 19.5 × 10^6 and 20 × 10^6). (a-d) Aβ1−42 distributions in the absence of Aβ fragments (black curves) and within heterooligomers formed by Aβ1−42 and (a) Aβ30−40, (b) Aβ31−42, (c) Aβ39−42, or (d) Aβ21−30 at four different number concentrations. (e-h) Aβ fragment distributions within heterooligomers formed by Aβ1−42 and (e) Aβ30−40, (f) Aβ31−42, (g) Aβ39−42, or (h) Aβ21−30 at four different number concentrations. The error bars correspond to SEM.

**Figure III:** Intramolecular contact map of Aβ1−42 assemblies formed (a) in the absence and (b-r) presence of CTFs. In (a), the intramolecular contact map of Aβ1−42 hexamers is shown. In (b-r), the intramolecular contact maps of Aβ1−42 within the heterotypic assemblies (in the time window 19-20 × 10^6) for each of the four Aβ fragment concentrations and for each of the four different fragments (Aβ30−40, Aβ31−42, Aβ39−42, Aβ21−30) are depicted. Different number concentrations of Aβ fragments are marked on the left (the value in the parentheses refers to the corresponding Aβ39−42 concentration). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner. Boxes 1-5 mark regions of concentrated intramolecular contacts.

**Figure IV:** Intermolecular contact map of Aβ1−42 assemblies formed (a) in the absence or (b-r) presence of Aβ fragments. In (a), the intermolecular contact map of Aβ1−42 hexamers is shown. In (b-r), the intermolecular contact maps of Aβ1−42 within the heterotypic assemblies (in the time window 19-20 × 10^6) for each of the four Aβ fragment concentrations and for each of the four Aβ fragments (Aβ30−40, Aβ31−42, Aβ39−42, and Aβ21−30) are depicted. Different number concentration ratios of Aβ fragments are marked.
on the left (the value in the parentheses refers to the corresponding $A\beta_{39-42}$ concentration). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner. Boxes 1-5 mark regions of intermolecular contacts that are of interest.

Figure V: (b-r) Intramolecular contact map of A\beta fragments within heterotypic assemblies calculated within the time window $19-20 \times 10^6$ for each of the four A\beta fragment concentrations and for each of the four different A\beta fragments: $A\beta_{30-40}, A\beta_{31-42}, A\beta_{39-42},$ and $A\beta_{21-30}$. Different number concentration ratios of A\beta fragments are marked on the left (the value in the parentheses refers to the corresponding $A\beta_{39-42}$ concentration). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner.

Figure VI: (b-r) Intermolecular contact map of A\beta fragments within heterotypic assemblies (in the time window $19-20 \times 10^6$) for each of the four A\beta fragment concentrations and for each of the four different A\beta fragments: ($A\beta_{30-40}, A\beta_{31-42}, A\beta_{39-42},$ and $A\beta_{21-30}$). Different number concentration ratios of A\beta fragments are marked on the left (the value in the parenthesis refers to the corresponding $A\beta_{39-42}$ concentration). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner.

Figure VII: (b-r) Intermolecular contact maps between $A\beta_1-42$ and A\beta fragments within heterotypic assemblies calculated in the time window $19-20 \times 10^6$ for each of the four A\beta fragment concentrations and for each of the four different A\beta fragments: $A\beta_{30-40}, A\beta_{31-42}, A\beta_{39-42},$ and $A\beta_{21-30}$. Different number concentration ratios of A\beta fragments are marked on the left (the value in the parenthesis refers to the corresponding $A\beta_{39-42}$ concentration). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner. Boxes 1-4 mark regions of concentrated intermolecular contacts common to all four A\beta fragments.
Figure I: *Initial Populations of Peptides*
Figure II: Size Distributions of $A\beta_{1-42}$ and $A\beta_{X-Y}$ within $A\beta_{1-42}/A\beta_{X-Y}$ assemblies.
Figure III: Intramolecular Contact Maps of $A\beta_{1-42}$
Figure IV: Intermolecular Contact Maps of Aβ₁₋₄₂
Figure V: Intramolecular Contact Maps of $A\beta_{X-Y}$
Figure VI: Intermolecular Contact Maps of $A\beta_{X-Y}$
Figure VII: Intermolecular Contact Maps between $\alpha_{\beta 1-42}$ and $\alpha_{\beta X-Y}$
References