Supporting Information

Fradinger *et al.* 10.1073/pnas.0807163105

SI Text

CTF Synthesis. CTFs were synthesized by Fmoc chemistry using automated Applied Biosystems 433A synthesizers. The synthesis scale was between 0.20 and 0.25 mmol. The coupling and deprotection cycles were extended from the manufacturer's recommended times, 30 and 10 min, to 60 and 30 min, respectively. Coupling cycles were performed by using 4 eq. of incoming amino acid, 4 eq. of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, and 4 eq of *N*,*N*-diisopropylethyamine in *N*-methylpyrrolidone (NMP). Fmoc deprotection was done by using 20% piperidine in NMP. The ε-NH_2 group of Lys was protected by *tert*-butoxycarbonyl (BOC). Cleavage of the peptide from the resin and side-chain deprotection (where appropriate) were performed by using 10 ml of the following mixtures: (*i*) 9.5:0.5 trifluoroacetic acid (TFA):H_2O; (*ii*) 9.25:0.5:0.25 TFA:H_2O:ethanedithiol (EDT); (*iii*) 87.5:0.5:0.5:0.25 TFA:H_2O:thioanisole:EDT; or (*iv*) 9.5:0.25:0.25 TFA:EDT:triisopropylsilane. After filtration of the resin, several methods were used for isolation and purification of the crude peptides. For complete details, see ref. 1.

Cell Culture. Rat pheochromocytoma (PC-12) cells were maintained in F-12 nutrient mixture with Kaighn's modification (F-12K) with 15% heat-inactivated horse serum and 2.5% FBS at 37°C in an atmosphere of 5% CO_2. For cell viability assays, cells were plated in 96-well plates at a density of 30,000 cells per well in differentiation media (F-12K, 0.5% FBS, 100 μM nerve growth factor) and maintained for 48 h.

Primary mouse hippocampal cultures were generated from pregnant C57 black mice (E18). Embryos were removed from the uterus, and hippocampi were dissected and placed in sterile Hanks' buffered salt solution. Hippocampi were cut into 1-mm pieces, added to 1 ml of 0.05% trypsin and 0.001% DNase (Sigma), and incubated at 37°C for 15 min, agitating every 2–3 min to break apart clumps. Then, 3 ml of neurobasal media (Invitrogen) containing 10% FBS was added and the tissue was centrifuged for 5 min at 200 $\times$ *g* to gently pellet the cells. The cells were resuspended in neurobasal media by gentle trituration with a polished Pasteur pipette. Cells were plated at a density of 1–3 $\times$ 10^6 cells per milliliter on acid-treated, polyL-lysine (0.1 mg/ml)-coated 12-mm glass coverslips in 6-cm plastic culture dishes with 7 ml of growth media (neurobasal media, 2% B27 supplement, 500 μM glutamine, and 0.5% antibiotics). Cells were maintained for 2 weeks with changing half of the volume of the media twice weekly. After 2 weeks in culture, the cells had established synaptic connections and were ready for electrophysiological recordings.

Cell Viability Assays. To assess the biological activity of the CTFs themselves, peptide solutions were prepared by dissolving the CTFs in DMSO and diluting into F-12K media to yield concentrations of 1–200 μM. Aliquots of 10 μl were added to differentiated PC-12 cells to yield final concentrations of 0.1, 1, 5, 10, and 20 μM and incubated for 15 h. Negative controls included DMSO at the same concentration as in the peptide solutions and media alone. A positive control was 1 μM staurosporine for full kill. The staurosporine control was used to establish the dynamic range of the experiment and represented a 100% reduction in cell viability, based on which the percentage viability of all of the experimental conditions was calculated. The toxicity of all of the CTFs was studied at concentrations up to 20 μM because this is a concentration at which we observe a robust toxic effect for Aβ42. We did not study higher CTF concentrations because inhibition assays using higher CTF concentrations demonstrated a rescue of Aβ42-induced toxicity, supporting that the CTFs remained nontoxic even at the highest concentration used. Cell viability was assessed qualitatively by visual observation and quantitatively by the CellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega). Briefly, 15 μl of dye solution was incubated with the cells for 3 h. Then 100 μl of solubilization/stop solution was added and the plates were incubated overnight in the dark to ensure complete solubilization. Plates were read by using a Synergy HT microplate reader (BioTek), and the absorbance at 570 nm (formazan product) minus the absorbance at 630 nm (background) was recorded. Corrected absorbance was used to calculate the percent cell viability from the experimental change ($A_{\text{media}} - A_{\text{experimental}}$) over the dynamic range ($A_{\text{media}} - A_{\text{staurosporine}}$). At least three independent experiments with six replicates ($n \geq 18$) were carried out, and the results were averaged.

To test for inhibitory effect the CTFs on Aβ42-induced toxicity, solutions of Aβ42 and CTF at a ratio of 1:10 were prepared. Control experiments with Aβ42 alone showed that 5 μM Aβ42 caused a robust ($\approx$40%) reduction in cell viability. CTFs were dissolved in DMSO and diluted with Aβ42 solutions. The mixtures were subjected to a brief centrifugation to remove preformed aggregates, and then aliquots of 10 μl were immediately added to cells to yield final concentrations of 50 μM CTF and 5 μM Aβ42. Although the solutions were all clear to the eye, it is possible that some aggregation occurred upon dilution in the medium. As a result, it is possible that the actual soluble CTF concentration was lower than the nominal concentration. Cell viability was determined by the MTT assay as described above. At least three independent experiments with six replicates ($n \geq 18$) were carried out, and results were averaged. CTFs that showed strong inhibition of Aβ42-induced toxicity were studied further to determine their dose-dependent activity.

Dose-dependence MTT experiments with Aβ(31–42) and Aβ(39–42) were conducted as described above with final Aβ42:CTF ratios of 1:0, 1:1, 1:2, 1:5, and 1:10 for both CTFs and 1:20, 1:15 for Aβ(39–42) only. Three independent experiments with five to six replicates ($n \geq 15$) were carried out, and results were averaged.

In addition, dose dependence LDH release experiments were performed using the CytoTox-ONE Homogenous Membrane Integrity Assay (Promega). Control experiments with Aβ42 alone identified a concentration of 10 μM that yielded robust cell death. Aβ42 was mixed with either Aβ(31–42) or Aβ(39–42) at ratios from 1:1 to 1:10. Because DMSO somewhat permeabilizes cell membranes and causes high background in this assay, all peptides were dissolved initially in 60 mM NaOH and then diluted into media. [In separate MTT experiments, the toxicity of Aβ solubilized by either DMSO or 60 mM NaOH was compared. The data showed slight (<10%) increase in the toxicity of DMSO-solubilized Aβ42 relative to peptide that was solubilized in 60 mM NaOH (P. Maiti and G.B., unpublished results).] A 10-μl aliquot of each mixture was added to the cells to yield final concentrations of 10–50 μM [Aβ(31–42)] or 10–100 μM [Aβ(39–42)] and 10 μM Aβ42. Cells were incubated with peptide mixtures for 48 h and then assayed for cell death. Briefly, cells were incubated at room temperature for 20 min, then 100 μl of dye solution were added and incubated for 10 min at room temperature. Fifty microliters of stop solution were added, and the plates were read at an excitation wavelength of

560 nm and emission wavelength of 590 nm. Three individual experiments of five to six replicates were carried out ($n \geq 15$), and the results were averaged.

Electrophysiological Studies. On the day of the experiment, a coverslip of hippocampal culture was transferred to the recording chamber (Warner RC-25F) of an inverted microscope (DIAPHOT 300; Nikon), and cells were perfused with an "extracellular solution" (130 mM NaCl/5.4 mM KCl/1.8 mM $CaCl_2$/0.8 mM $MgCl_2$/10 mM D-glucose/10 mM Hepes/0.02 mM bicuculline methiodide/0.1 mM tetrodotoxin, pH 7.4). Spontaneous mEPSCs were measured by using whole-cell recordings. Glass microelectrodes filled with a solution composed of 105 mM CsCl, 2.5 mM $MgCl_2$, 10 mM EGTA, 40 mM Hepes, 5 mM D-glucose, 4 mM Mg-ATP, and 0.5 mM Na-GTP (pH 7.2). The mEPSCs were recorded at a holding potential of −70 mV in cultured neurons with a membrane seal of 3–5 MΩ by using a patch-clamp amplifier model Axopatch 200A (Axon Instruments) and digitized by using a Digidata 1322A Interface (Axon Instruments). Signals were filtered at 2 kHz and sampled at 10 kHz. Continuous recording and analysis of mEPSCs were performed with Minianalysis software (Synaptosoft). Cells were perfused at a flow rate of 0.4–0.5 ml/min with peptide samples of 3 μM Aβ42, 1:1 or 1:10 Aβ42:CTF, or vehicle control (extracellular solution with DMSO). To calculate mEPSC amplitude and frequency, events were analyzed for 1 min before and every 5 min during the application of peptide samples. Data are presented as mean ± SE. Statistical significance was assessed by using Student's t test.

Dynamic Light Scattering (DLS). Aβ42:CTF mixtures containing 30 μM nominal concentration each of Aβ42 and Aβ(31–42) or Aβ(39–42) were studied by using an in-house-built system with a He-Ne laser (wavelength 633 nm, power 50 mW; Coherent) as a light source and an arrangement of collection optics that is optimized for maximal sensitivity. Light scattered at 90° was collected by using image transfer optics and detected by an avalanche photodiode built into a 256-channel correlator (Precision Detectors). The size distribution of scattering particles was reconstructed from the scattered light correlation function by using PrecisionDeconvolve deconvolution software (Precision Detectors) based on the regularization method by Tikhonov and Arsenin (2).

Two-hundred-microliter samples were lyophilized, stored at −20°C, and then reconstituted in 200 μl of water. The solutions were sonicated for 60 s and filtrated through a syringe filter (20-nm pore size; Whatman) before DLS measurements. The actual concentration was measured by amino acid analysis (AAA). Two replicates with similar concentrations each of Aβ42 and CTF were measured for each condition.

Photo-Induced Cross-Linking of Unmodified Proteins (PICUP). CTFs were dissolved in 60 mM NaOH and diluted into 10 mM sodium phosphate (pH 7.4; 1:10 vol/vol) to yield a nominal concentration of 200 μM. Preformed aggregates were removed by filtration through a 20-nm pore size filter. Low-molecular-weight (LMW) Aβ42 was prepared by ultrafiltration as described previously (3). The actual concentration of each solution was determined *post facto* by AAA. Each CTF was mixed with LMW Aβ42 at an ≈1:1 concentration ratio, and the mixtures were immediately subjected to PICUP as described previously (4, 5). Only experiments yielding similar (±10%) concentrations were each of Aβ42 and CTF were taken into account. All of the CTFs contain only amino acid residues with low reactivity in PICUP chemistry (5); therefore, cross-linking occurred only among Aβ42 molecules. The cross-linked peptide mixtures were analyzed by SDS/PAGE, and Aβ42 hexamer abundance was quantified by densitometric analysis using One-Dscan (Scanalytic) as described previously (6, 7). Hexamer intensity was normalized to the intensity of the entire lane for each CTF.

***Ab Initio* Discrete Molecular Dynamics (DMD).** In DMD, all interparticle potentials are replaced by a square-well or a combination of square-well potentials. The resulting dynamics is driven by collisions between particles, which are otherwise moving along straight lines with constant velocities. The Berendsen thermostat algorithm (8) is periodically applied to keep the temperature of the system constant. We use a four-bead protein model in which the backbone is modeled by three atoms/beads (corresponding to the amide N, the α-carbon C_α, and the carbonyl C' groups), and the side chain is represented by one bead, C_β (with exception of G, which has no side-chain bead) (9). An effective backbone hydrogen bond is introduced between the nitrogen atom N_i of the ith amino acid and the carbon atom C'_j of the jth amino acid (9). Effective hydropathic interactions among side-chain atoms are introduced to mimic the solvent effects (10). The relative strength of hydropathic interactions between pairs of side-chain beads is based on the Kyte–Doolittle hydropathy scale (11). In our model, the hydrophobic amino acids are A, C, F, L, M, I, and V, and hydrophilic amino acids are D, E, H, K, N, Q, and R. The side chains of the remaining amino acids G, P, S, T, W, and Y interact only through steric repulsion. All model parameters are set to the same values as used in the previous study (10). We set the potential energy of the hydrogen bond, E_{HB}, to unit energy ($E_{HB} = 1$) and the maximal absolute value of the potential energy of the hydropathic interactions $E_{HP} = 0.3$, such that the potential energy of two interacting Ile side chain beads is −0.3. Using the unit of temperature E_{HB}/k_B where k_B is Boltzmann's constant, we use $T = 0.15$ as a reasonable estimate of physiological temperatures.

Contact Maps. By definition, two beads are in contact if they are at a distance equal to or smaller than 7.5 Å. 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: intramolecular 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. The contact map of each assembly is normalized by the number of contributing peptide molecules. Because in our model each amino acid is represented by up to four beads, 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. All contact maps were first calculated separately for each heterooligomer and then averaged over all assemblies under consideration.

1. Condron MM, Monien BH, Bitan G (2008) Synthesis and purification of highly hydrophobic peptides derived from the C-terminus of amyloid β-protein. *Open Biotechnol J* 2:87–93.
2. Tikhonov AN, Arsenin VY (1977) *Solution of Ill-Posed Problems* (Halsted, Washington, DC).
3. Bitan G, Teplow DB (2005) Preparation of aggregate-free, low molecular weight amyloid-β for assembly and toxicity assays. *Methods Mol Biol* 299:3–9.
4. Bitan G, Teplow DB (2004) Rapid photochemical cross-linking—A new tool for studies of metastable, amyloidogenic protein assemblies. *Acc Chem Res* 37:357–364.
5. Bitan G (2006) Structural study of metastable amyloidogenic protein oligomers by photo-induced cross-linking of unmodified proteins. *Methods Enzymol* 413:217–236.
6. Bitan G, *et al.* (2003) Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways. *Proc Natl Acad Sci USA* 100:330–335.

7. Bitan G, Lomakin A, Teplow DB (2001) Amyloid β-protein oligomerization: Prenucleation interactions revealed by photo-induced cross-linking of unmodified proteins. *J Biol Chem* 276:35176–35184.
8. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. *J Chem Phys* 8:3684–3690.
9. Ding F, Borreguero JM, Buldyrey SV, Stanley HE, Dokholyan NV (2003) Mechanism for the α-helix to β-hairpin transition. *Proteins* 53:220–228.
10. Urbanc B, *et al.* (2004) *In silico* study of amyloid β-protein folding and oligomerization. *Proc Natl Acad Sci USA* 101:17345–17350.
11. Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 157:105–132.

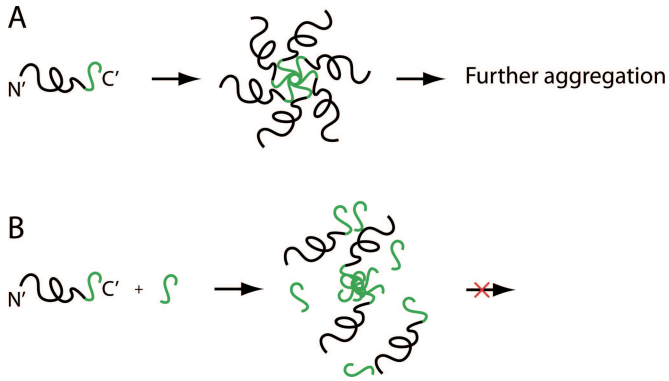


Fig. S1. Schematic representation of Aβ42 CTFs as inhibitors of full-length Aβ42 oligomerization. (*A*) The C termini (green) of several Aβ42 molecules are hypothesized to form the hydrophobic core of oligomers. (*B*) CTFs derived from the C terminus of Aβ42 coassemble with the C terminus of the full-length peptide, leading to disruption of oligomerization.

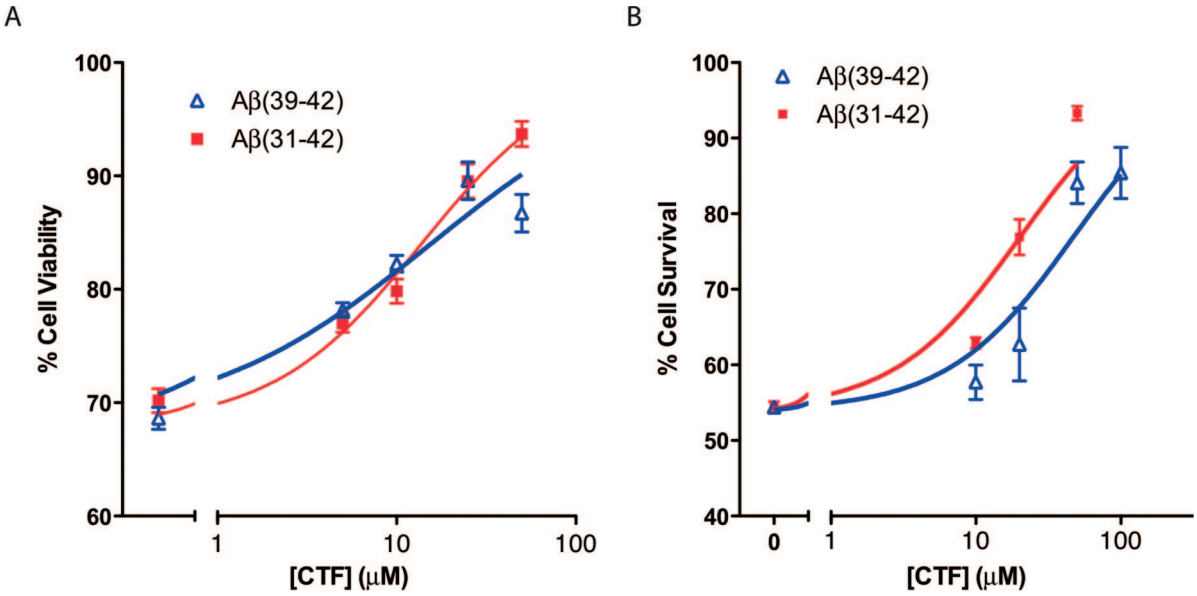


Fig. S2. Dose-dependent inhibitory activity of Aβ(31–42) and Aβ(39–42). (*A*) Cell viability determined by the MTT assay in differentiated PC-12 cells in the presence of 5 μM Aβ42 and Aβ42:CTF concentration ratios ranging from 1:0 to 1:10. The data were normalized to full-kill and media controls and reported as mean $\pm$ SD (n = 15–18). (*B*) Cell death determined by the LDH release assay in differentiated PC-12 cells in the presence of 10 μM Aβ42 and Aβ42:CTF concentration ratios ranging from 1:0 to 1:5 for Aβ(31–42) and 1:10 for Aβ(39–42). The data were normalized to full-kill and media controls and are reported as mean $\pm$ SD (n = 18–24).

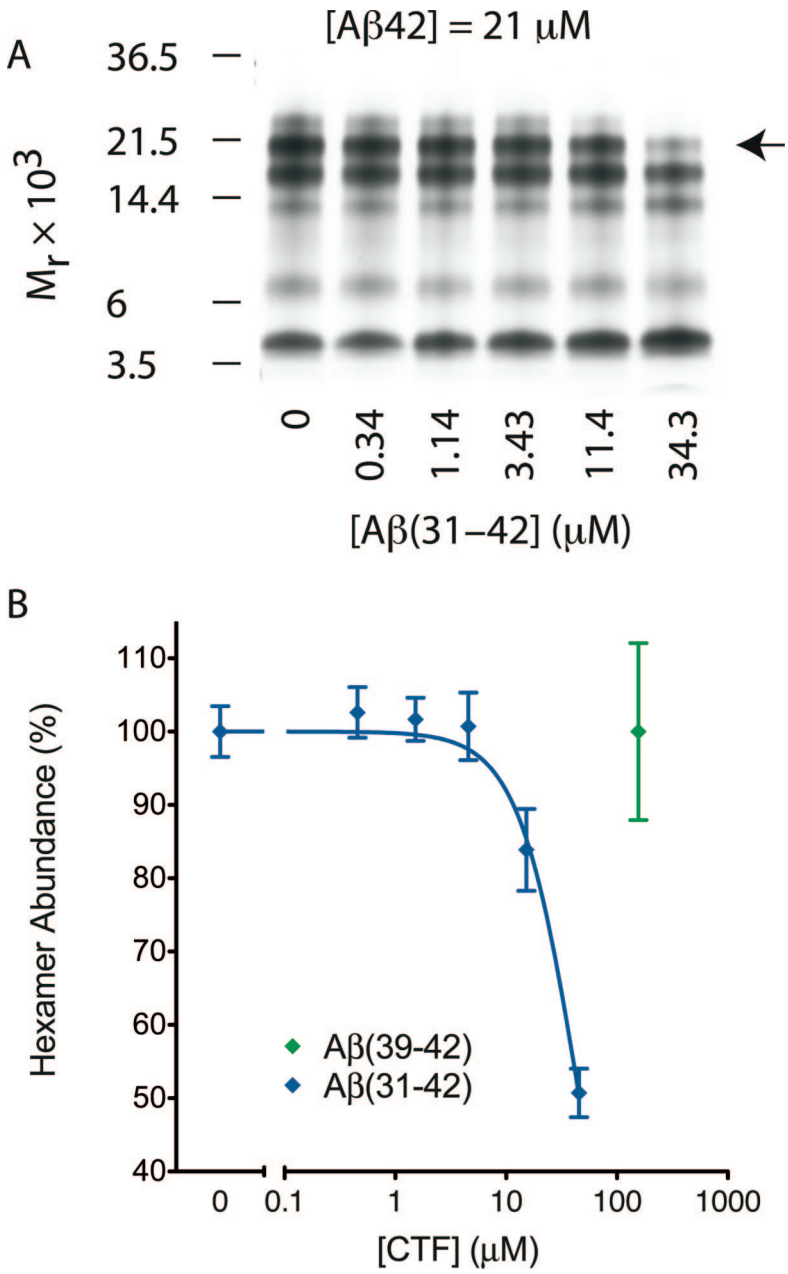
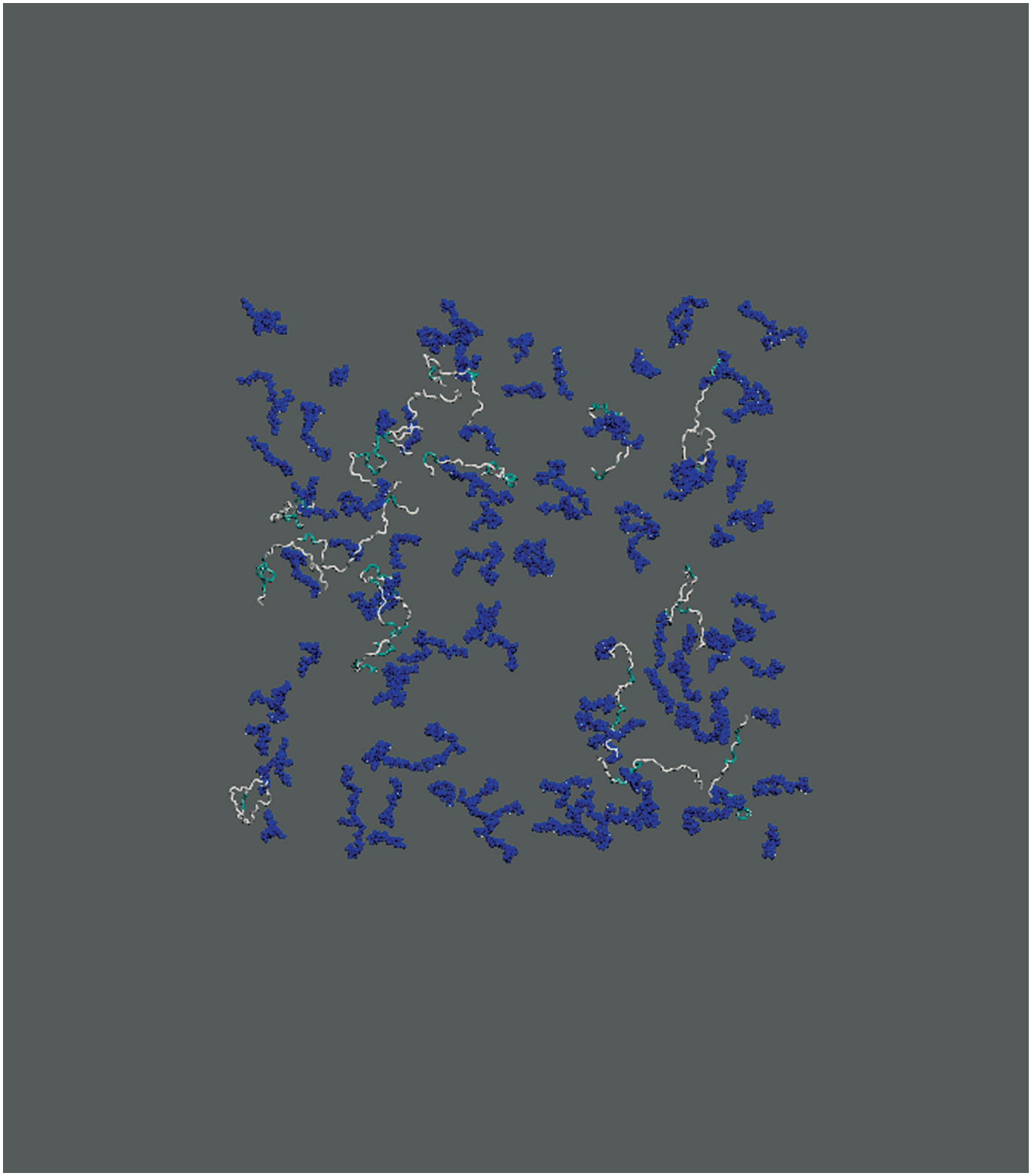


Fig. S3. PICUP analysis. (*A*) A representative experiment is shown. LMW Aβ42 was mixed with increasing concentrations of Aβ(31–42) and photo-cross-linked immediately. The mixtures were fractionated by SDS/PAGE and silver-stained. Positions of molecular weight markers are shown on the left. Aβ(31–42) concentration is given at the bottom of each lane. (*B*) Densitometric analysis of hexamer abundance normalized to the entire lane in experiments similar to those shown in *A* for Aβ(31–42). Data measured at a single concentration (155 $\pm$ 10 μM) is shown for Aβ(39–42). The data are presented as mean $\pm$ SE measured in three independent experiments.



Movie S1. Simulation of the interaction between 16 Aβ42 molecules and 128 Aβ(31–42) molecules between 0 and 10-million simulation steps.

Movie S1 (GIF)