SUPPORTING INFORMATION

Interactions between soluble species of β-amyloid and α-synuclein promote oligomerization while inhibiting fibrillization

Jason Candreva[1], Edward Chau[1], Margaret E. Rice[2] and Jin Ryoun Kim[1,]*

[1]Department of Chemical and Biomolecular Engineering, New York University, 6 MetroTech Center, Brooklyn, NY 11201, USA

[2]Departments of Neurosurgery, and Neuroscience and Physiology, New York University School of Medicine, New York, NY 10016, USA

Corresponding Author

*Email: jin.kim@nyu.edu

MATERIALS AND METHODS

Materials

Lyophililzed Aβ containing 40 residues (D1-V40) was purchased from the ERI Amyloid Laboratory (Oxford, CT, USA). Lyophilized Aβ conjugated with HiLyte Fluor 488 at the N-terminus was purchased from AnaSpec (Fremont, CA, USA). Alexa Fluor 647 NHS ester used to conjugate αS was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Aβ sequence-specific antibodies 6E10 and 4G8 were purchased from Covance (Princeton, NJ, USA). Other Aβ sequence-specific antibodies, anti-Aβ22-35 and 5C3, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Enzo Life Sciences (Farmingdale, NY, USA), respectively. FPLC columns used for αS purification were purchased from GE Healthcare (Piscataway, NJ, USA). αS sequence-specific antibodies, F11, 5C2, 211 and D10, were purchased from either Santa Cruz Biotechnology, Inc (Dallas, TX, USA) or Novus International (Saint Charles, MO, USA). Alkaline-phosphatase secondary antibodies and solutions for chemiluminescent development were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated.

Recombinant production and purification of αS

BL21(DE3) cells containing the plasmid pRK172 were used for expression of αS, as described previously.[1, 2] Briefly, cells were grown in LB medium containing ampicillin at 37 °C with shaking at 250 rpm, to an OD600 of ~1.0. Expression of αS was then induced by adding isopropyl-β-D-1-galactopyranoside (IPTG) to a final concentration of 1 mM. Expression was carried out for another 18-20 hours at 25 °C with shaking at 250 rpm. Cells were pelleted by centrifugation at 4,000 rpm

and 4 °C for 20 minutes. Cells were then resuspended and lysed by sonication, and the lysate was clarified by centrifugation at 18,000 rpm and 4 °C for 30 minutes. The clarified lysate was heat treated at 80 °C for 20 minutes and further clarified by additional centrifugation. The soluble αS was purified by passing the lysate through an anion-exchange column (HiTrap Q XL, GE Healthcare), size exclusion column (HiPrep 16/60 Sephacryl S-100, GE Healthcare), and desalting column (HiPrep 26/10, GE Healthcare). Identity of αS was confirmed by SDS-PAGE with Coomassie Blue staining and dot blot assays using αS sequence-specific antibodies, F11, 5C2, 211 and D10. Purity of αS was determined to be > 95% by SDS-PAGE. The purified αS was flash frozen and lyophilized using a Labconco Freezone 6 Freeze Dryer System (Kansas City, MO, USA). Lyophilized samples were stored at -80 ºC until further use.

Fluorescent labeling of αS

αS was labeled with Alexa Fluor 647 according to manufacturer's protocols, using a succinimidyl ester to primary amine reaction. The reaction occurred in aqueous buffer. According to manufacturer's protocols, the labeling pH was set at 7.2 to ensure that N-terminal labeling is favored, as the N-terminal amine has a lower pKa (7.6) than the lysine side group amine (10.5). The reaction was incubated for 1 hour at room temperature with continuous stirring. The labeled αS was separated from unlabeled dye on a HiPrep 16/60 Sephacryl S-100 size exclusion column. The labeled αS was then desalted through a HiPrep 26/10 desalting column, aliquoted into microcentrifuge tubes, lyophilized on a Labconco freeze dry system, and stored at -80 °C. To determine the degree of labeling (DOL), or the dye-to-protein ratio, the following equation was used:

$$DOL = (A_{max} \times MW) / ([protein] \times \varepsilon_{dye}) \quad \text{Eq. 1}$$

where A_{max} is the absorbance at the maximum wavelength of the dye, MW is the molecular weight of the protein, [protein] is the concentration of the protein in mg/ml, and ε_{dye} is the extinction coefficient of the dye at its maximum wavelength. DOL values below 1 were desired, as this increased the probability of only the N-terminus being labeled. Thus, concentrations of proteins and fluorescent dyes were adjusted to achieve typical DOL values of 0.1-0.2. At this range of DOL values, labeling of αS occurred predominantly with a single rather than multiple Alexa Fluor 647 molecule as found in our previous study[1]: the molecular weights of the two major αS forms after the labeling were 14,460 and 15,304 Da, corresponding to those of unlabeled αS and single-labeled αS, respectively. While an exact location for the labeling remains yet to be verified, samples containing αS labeled with Alexa Fluor 647 in this manner was found to exhibit aggregation behaviors similar to those prepared using exclusively unlabeled αS.[1] For Alexa Fluor 647, the maximum absorbed wavelength is 650 nm, and the ε_{dye} is 239,000 $cm^{-1}M^{-1}$. To determine [protein], a correction factor was used to account for the contribution of the dye to the absorbance at 280 nm, using the following equation:

$$A_{protein} = A_{280} - A_{max} \times CF \quad \text{Eq. 2}$$

where $A_{protein}$ is the absorbance at 280 nm used to determine [protein], A_{280} is the measured absorbance at 280 nm, A_{max} is the measured absorbance at the maximum wavelength of the dye, and CF is the correction factor, which is 0.03 for Alexa Fluor 647.

Preparation of Aβ and αS monomers

Aβ samples were prepared according to previously established protocols.[2-6] The lyophilized Aβ powder was pretreated with hexafluoroisopropanol (HFIP) to remove all traces of pre-formed aggregate species. To prepare Aβ monomer samples, the pretreated peptide was redissolved in 20 mM NaOH at a concentration of ~310 μM and incubated at room temperature for 10 minutes. The NaOH treatment was used to promote disaggregation of pre-formed aggregates. The sample was subsequently diluted in phosphate buffered saline with azide (PBSA, final buffer components: 80 mM Na_2HPO_4/NaH_2PO_4, 150 mM NaCl, 0.02% NaN_3, pH ~7.4). Aβ monomer samples were filtered through a 0.45 μm filter to remove any large aggregates, and concentration was determined by absorbance at 280 nm with scattering effects[7] on a Varian Cary 50 UV-Vis Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

αS monomers were prepared according to previously established protocols.[1, 2] Briefly, the lyophilized αS was resuspended to 700 μM in PBSA. The αS monomer solution was filtered through a 0.45 μm filter, and concentration was determined by absorbance at 280 nm with scattering effects. Fluorescently-labeled Aβ and αS monomers were prepared similarly (see below for the ratios of labeled to unlabeled polypeptides in samples).

Preparation of oligomeric Aβ and αS

Aβ oligomeric species were prepared by incubating Aβ monomers, following the established protocol [2, 3]. Briefly, Aβ monomers prepared as described above were incubated at a monomer-equivalent concentration of 230 μM and 37 °C in siliconized tubes. After 2-day incubation, any precipitates present in samples were removed by centrifugation for 5 minutes at 14,000 rpm, and

the supernatant containing Aβ oligomers was then collected. To determine Aβ concentration, a sample of the oligomers was dissolved in 8 M urea, followed by measurements of absorbance at 280 nm with consideration of scattering effects.

αS oligomers were prepared by incubating αS monomers, as reported previously.[2, 8] αS monomers prepared as described above were diluted to 350 μM in PBSA and incubated in a glass vial at 37 °C with gentle shaking at 250 rpm for 6 hours. After incubation, the oligomers were collected by filtration using Amicon Ultra 100 kDa ultracentrifugation filters (MilliporeSigma, Burlington, MA, USA). The flow through (containing monomers) was discarded. The retentate was washed three times with PBSA to remove any remaining monomers. Concentration of αS oligomers was determined similarly as for Aβ oligomers using UV absorbance at 280 nm. Fluorescently-labeled Aβ and αS monomers were incubated and treated similarly to prepare oligomeric species.

Preparation of fibrillar Aβ and αS

Monomers of Aβ and αS prepared at 230 μM and 350 μM, respectively, were incubated in glass vials at 37 °C with stirring at 250 rpm for ~4 weeks. Fibrils were pelleted by centrifugation at 14,000 rpm for 5 minutes, followed by 3 washes with PBSA by resuspending the pellet and centrifugation. Fibril concentrations were determined through back calculation from concentrations of peptides/proteins in wash supernatants measured by UV absorbance at 280 nm with correction for scattering. Fluorescently-labeled Aβ and αS monomers were incubated and treated similarly to prepare fibrillar species.

Preparation of mixtures of Aβ and αS

In order to determine the interactions between Aβ and αS, the two polypeptides were mixed at designated concentrations. Monomers, oligomers and fibrils of each polypeptide were prepared as described above, and mixed in PBSA to a total volume of 400-600 µl. The samples were incubated in siliconized tubes at 37 °C under a quiescent condition for 7 days. Aliquots were used to analyze and characterize the extent of aggregation. In order to selectively monitor Aβ and αS on SDS- and native-PAGE gels, fluorescently-labeled peptides/proteins were also mixed with corresponding unlabeled peptides/proteins at a ratio of 1:1000 for Aβ in all three forms and 1:800 for αS monomers, while αS oligomers and fibrils were prepared at 1:100. The ratios were chosen after multiple rounds of optimization aiming to minimize the fraction of labeled peptides/proteins. This was to ensure aggregation behaviors of samples containing the labeled peptides/proteins similar to those containing exclusively unlabeled proteins while permitting detection of proteins by fluorescence (see below). Fluorescently-labeled samples were mixed to a total volume of 150 µl and covered from light exposure to minimize photodegradation of the fluorophores during incubation.

Thioflavin T fluorescence

Thioflavin T (ThT) fluorescence was used to monitor the kinetics of amyloid aggregation. Twenty microliters of a 0.1 mM stock solution of ThT was added to 10 µl of protein/peptide sample and 170 µl of PBSA. The fluorescence was measured immediately on a Photon Technology International QuantaMaster4 Fluorometer (Horiba, Kyoto, Japan). The excitation wavelength was 440 nm, and emission was monitored at 485 nm.

Circular dichroism

Secondary structures of peptides/protein samples were analyzed with circular dichroism (CD) on a Jasco J-815 spectropolarimeter (Jasco, Easton, MD, USA). The buffer (PBSA) background spectrum in the far-UV range was subtracted from each sample spectrum. Samples were put into a cuvette with a 1 mm pathlength, and wavelength was scanned in the far-UV range.

Native- and SDS-PAGE and in-gel fluorescence imaging

Native- and SDS-PAGE were used to determine aggregation states of samples. For native-PAGE, ~12 µl of total protein sample was loaded onto NativePAGE 4-16% Bis-Tris Gels (Thermo Fisher Scientific) placed into the XCell SureLock Mini-Cell gel running tank (Thermo Fisher Scientific). NativePAGE Running Buffer (Thermo Fisher Scientific) was used in both compartments of the cell, and the gel was run for ~1.5 hours at a constant 150 V.

For SDS-PAGE, 50 µl of sample was centrifuged for 5 minutes at 14,000 rpm to separate insoluble aggregates from soluble species. The supernatant was collected carefully to avoid disturbing the insoluble pellet. Equal volumes (12 µl) of supernatant and total protein sample were denatured with NuPAGE LDS Sample Buffer (4X; Thermo Fisher Scientific) and heated to 95 °C for 10 minutes. Samples were loaded onto NuPAGE 4-12% Bis-Tris Gels (Thermo Fisher Scientific), and the gel was run for ~25 minutes at a constant 200V.

To track locations of Aβ and αS individually on SDS- and native-PAGE gels, the polypeptides were labeled with HiLyte Fluor 488 (green bands) and Alexa Fluor 647 (red bands), respectively.

The gels were imaged using a GE Typhoon Trio phosphoimager in the Scientific Cores & Shared Resources at the NYU Langone Medical Center.

Transmission Electron Microscopy

Five microliter aliquots of sample were pipetted on copper grids and then negatively stained with 1% uranyl acetate solution in deionized water. The samples were imaged on a Phillips CM-12 Transmission Electron Microscope (FEI Corp., Hillsboro, OR, USA) at 120 kV with a 4k × 2.67k GATAN digital camera in the Scientific Cores & Shared Resources at the NYU Langone Medical Center.

Dot blot Immunoassay

Proteins/peptides (1 μg) in aqueous buffer were applied to a nitrocellulose membrane, and allowed to dry at a room temperature. Membrane blocking, washing, incubation with primary antibodies (e.g., A11) and alkaline phosphatase-conjugated secondary antibodies, and development with chemiluminescence were performed according to the manufacturer's protocols.

A competitive binding dot blot assay was used to determine the Aβ binding locations of αS monomers, oligomers, and fibrils. αS monomers, oligomers, and fibrils were incubated with Aβ monomers, oligomers, and fibrils at a 10:1 ratio (230 μM αS:23 μM Aβ) to saturate binding sites on Aβ with αS. Samples of Aβ and αS incubated alone (referred to as Aβ only and αS only) were also included. Solution containing a total of 1 μg Aβ was dotted on a nitrocellulose membrane and allowed to air dry for 15 minutes, followed by blocking, washing, incubation with primary and alkaline-phosphatase secondary antibodies, and chemiluminescent development according to the

manufacturer's protocols. Several Aβ sequence-specific antibodies were used as primary antibodies, including 6E10, 4G8, anti-Aβ (22-35) and 5C3, recognizing Aβ1-16, Aβ17-22, Aβ22-35, and Aβ32-40, respectively.

Dynamic Light Scattering

A z-average hydrodynamic diameter of peptide/protein samples was measured using a Zetasizer Nano ZS 90 instrument (Malvern Instruments Ltd., UK). Reported values represent the average of five measurements. Further information on size distribution was obtained using CONTIN algorithm.

SUPPLEMENTARY RESULTS

Characterization of aggregation of Aβ only samples

When analyzed by fluorescence of ThT, a dye recognizing β-sheet rich amyloid assemblies,[9] aggregation kinetics of Aβ monomer only samples were characterized by a sigmoidal curve typical of nucleation-dependent polymerization, with a lag phase of ~4 days before significant increases of ThT fluorescence (**Fig. S3A**), as reported elsewhere.[4, 10] Transmission electron microscopy (TEM) confirmed the lack of large aggregates in Aβ monomer only samples immediately after preparation and the existence of amyloid fibrils after a 7-day incubation (**Fig. S3B**). Freshly-prepared Aβ monomers were structurally disordered when examined by circular dichroism (CD) (**Fig. S4**). A similar CD spectrum was previously reported for structurally disordered Aβ monomers.[11, 12] Aggregation of Aβ only samples containing HiLyte Fluor 488-labeled Aβ was monitored by SDS-PAGE and native-PAGE, with visualization using in-gel fluorescence imaging (**Figs. S3C-D and S5)**. On SDS-PAGE, both total and soluble fractions were compared to determine if Aβ was lost to insoluble aggregates during incubation. Native-PAGE was used to examine the population distributions between monomer vs. oligomers under a non-denaturing condition. Note that migration of a protein in a native-PAGE gel under a non-denaturing condition depends not only on molecular weight, but also charge and conformation of the protein. Thus, "accurate" molecular weight determination of a protein using native-PAGE based on molecular weight standard proteins is difficult, while relative positions of our samples on a native-PAGE gel are generally consistent with previous reports.[8, 13, 14] Instead, we grouped band positions in native-PAGE into those primarily and commonly found with monomers and oligomers (**Figs. S1B and S3D**). As reported elsewhere,[11, 12] the fresh Aβ monomer samples contained a minor fraction of non-monomeric species, presumably small oligomers, seen in streaking around a major band in native-PAGE (**Fig. S1B; right panel and Fig. S3D; left panel**). The samples were used as Aβ

monomer samples without further separation. Consistent with the ThT fluorescence and TEM data, SDS- and native-PAGE results confirmed that soluble Aβ monomers self-assembled into insoluble aggregates (**Figs. S3C-D; left panels**). Although some of the total fraction of the Aβ monomer only sample also lost fluorescence after incubation (**Fig. S3C; left panel**), this was likely because the resulting fibrils were resistant to denaturation by SDS and/or were too large to enter the gels.

Fresh Aβ oligomer samples (monomer-equivalent concentration of 70 μM) were ThT-positive (**Fig. S3A**), primarily protofibrillar (**Fig. S3B**; top middle panel) and β-sheet structured (**Fig. S4**), as described previously.[5, 15-18] Unlike samples of other conformers, the Aβ oligomer samples were recognizable by A11, an antibody detecting oligomeric conformation of various amyloid proteins (**Fig. S4; inset**).[19] The z-average hydrodynamic diameter of Aβ in the oligomer samples were ~89-92 nm (also see **Fig. S6A**), as judged by dynamic light scattering (DLS). Based on fluorescent labeling followed by SDS-PAGE and native-PAGE (**Figs. S3C-D**; middle panels), our fresh Aβ oligomer samples were soluble and contained a non-negligible fraction of Aβ monomers, as reported for Aβ oligomer samples prepared using similar *in vitro* protocols.[4, 5] The Aβ oligomer samples were used without further separation. After 7 day incubation, ThT fluorescence of the Aβ oligomer samples increased by ~50% (**Fig. S3A**), and was accompanied by conversion of soluble Aβ species to insoluble amyloid fibrils (**Fig. S3B**; bottom middle panel and **Figs. S3C-D**; middle panels), suggesting that the Aβ oligomers are on the fibrillization pathway.

As expected, Aβ fibrils at 70 μM (monomer-equivalent concentration) exhibited steadily high ThT fluorescence (**Fig. S3A**), consistent with the mature fibrillar morphology (**Fig. S3B**), and appeared insoluble during the 7-day incubation (**Figs. S3C-D**; right panels).

Characterization of aggregation of αS only samples

No significant fibrillar aggregation was observed for αS monomer only samples (350 μM) during the 7-day incubation, as judged by ThT fluorescence and TEM (**Fig. S7A-B**). Similar to Aβ monomers, a CD spectrum of αS monomers was indicative of their disordered structure (**Fig. S4**). We note that fibrillar aggregates emerged from αS monomer only samples under other incubation conditions (e.g., with constant shaking or oligomeric seed), as seen in our previous study[2] and below. The SDS- and native-PAGE results show that soluble αS monomers remained monomeric during the incubation (**Fig. S7C-D; left panels**).

Samples containing pre-formed αS oligomers were prepared at the monomer-equivalent concentration of 17 μM, to represent the concentration of αS oligomers typically formed from *in vitro* incubation of 350 μM αS monomers at 37 ºC (~5% yield).[2] The preformed αS oligomers showed negligible ThT fluorescence (**Fig. S7A**) and appeared as globular or pore-like assemblies ~20 nm in diameter on TEM (**Fig. S7B**; top middle panel), as reported previously.[20, 21] Neither an increase in ThT fluorescence nor a morphological change was observed from αS oligomer only samples during 7 day incubation (**Fig. S7A-B**). The preformed αS oligomers were rich in β-sheets (**Fig. S4**). The αS oligomers were A11-positive similar to Aβ oligomers, but in contrast to other αS conformers (**Fig S4; inset**). The z-average hydrodynamic diameter of αS in the oligomer samples was ~26-30 nm (also see **Fig. S6B**), when determined by DLS. SDS- and native-PAGE of fluorescently labeled αS oligomers confirmed that the samples remained mostly soluble and oligomeric during the incubation (**Fig. S7C-D**; middle panels). Two bands were detected with αS oligomers on SDS-PAGE (**Fig. S7C**; middle panel) with the lower band reflecting a minor fraction of the samples, which can be better detected by fluorescence (**Fig. S1A: left panel**). Note that the

presence of 5% αS oligomers accelerated aggregation of αS monomers into fibrils compared to αS monomer only samples (**Figs. S8A-B**), although the dominant fractions of αS remained as monomers and oligomers (data not shown). The implication is that the αS oligomers are intermediates on the fibrillization pathway. Collectively, our αS oligomers displayed molecular and morphological characteristics similar to other αS oligomers, including those shown to be cytotoxic,[20, 22, 23] supporting the biological relevance of our αS oligomer samples.

When incubated alone, αS fibrils (monomer-equivalent concentration of 350 μM) remained highly ThT-positive, insoluble and fibrillar during a 7-day incubation (**Fig. S7A-B, and Fig. S7C-D**; right panels), as expected. ThT fluorescence intensities per monomer-equivalent concentration of αS fibrils (350 μM; **Fig. S7A**) were lower than Aβ fibrils (70 μM; **Fig. S3A**). The difference may be due to variations in local structure and amino acid composition of ThT binding sites within Aβ and αS fibrils, which determine ThT fluorescence.[9] While αS in fibril samples was mostly insoluble (**Fig. S7C**), streaking bands observed at the top (right below a well) of the native-PAGE gel for αS fibrils (**Figs. S1B and S7D; right panel**) may indicate their slight dissociation to smaller aggregates. No such a sign of dissociation was detected with Aβ fibrils **(Figs. S1B and S3D; right panel**), presumably accounting for different levels of stability between Aβ and αS fibrils.[24] The presence of αS monomers, presumably resulting from dissociation, was also detected by native-PAGE from the αS fibril samples unlike αS oligomer samples (**Fig. S7D**). The difference is likely due to a concentration difference between the two samples, as when the same masses of αS were loaded onto a native-PAGE gel, the presence of monomers was detected from both samples by fluorescence (**Fig. S1B**).

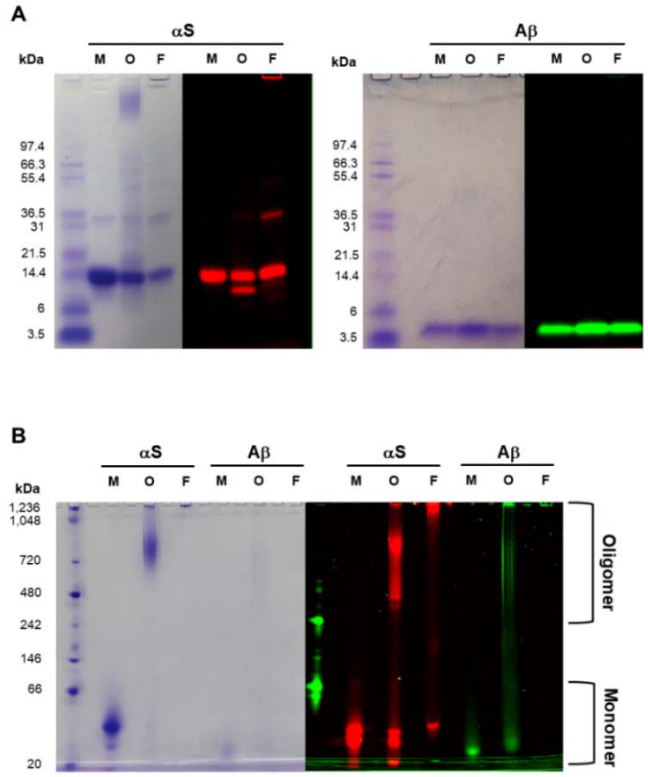

Figure S1. (A) SDS-PAGE and (B) Native-PAGE analyses of freshly prepared monomer (M), oligomer (O) and fibril (F) only samples containing labeled Aβ and αS visualized by (left) Coomassie staining or (right) fluorescence imaging. The leftmost lane in each gel contains molecular weight standard proteins with their respective molecular weights shown alongside. In (A), when resolved by SDS-PAGE, the primary bands of Aβ and αS in their respective fresh monomer, oligomer and fibril samples appeared as expected according to their molecular weights (Aβ: ~4 kDa and αS: ~14 kDa). The αS oligomer samples contained a minor fraction shown in the upper portion of an SDS-PAGE gel, indicative of their high SDS-resistance, after Coomassie staining. No such smear bands were detectable by fluorescence, implying that the labeled αS may not readily be incorporated into SDS-resistant oligomeric assemblies. Due to the undesired artifact of SDS on aggregation states, [25, 26] oligomeric states of samples were examined by native-PAGE. Another minor band of the αS oligomer samples was detected below the major band, when visualized by fluorescence imaging. In (B), migration of a protein in a native-PAGE gel under a non-denaturing condition depends not only on molecular weight, but also charge and conformation of the protein. Thus, "accurate" molecular weight determination of a protein using native-PAGE based on molecular weight standard proteins is difficult, while relative positions of our samples on a native-PAGE gel are generally consistent with previous reports.[8, 13, 14] Instead, we grouped band positions in native-PAGE into those primarily and commonly found with monomers and oligomers of Aβ and αS, which are shown in the right. The boundaries of the two groups were chosen as rather broad. αS oligomer and fibril samples contained minor fractions, which were more readily detectable by fluorescence imaging. Note that regular molecular weight standards for native-PAGE can not directly be used for fluorescence imaging, and no fluorescently-labeled molecular weight standards are currently available. Overall, in (A) and (B), the major bands on SDS- and native-PAGE gels are located at the same positions whether visualized by Coomassie staining or fluorescence imaging.

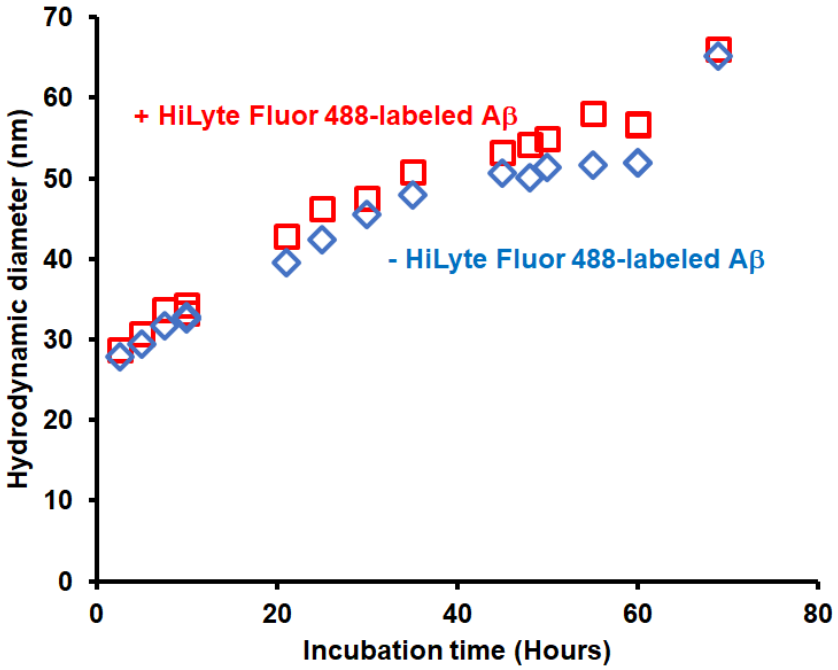

Figure S2. Time-course change in z-average hydrodynamic diameter of samples containing the labeled Aβ (red squares) and exclusively unlabeled Aβ (blue diamonds) during incubation under the condition used to prepare oligomer samples.

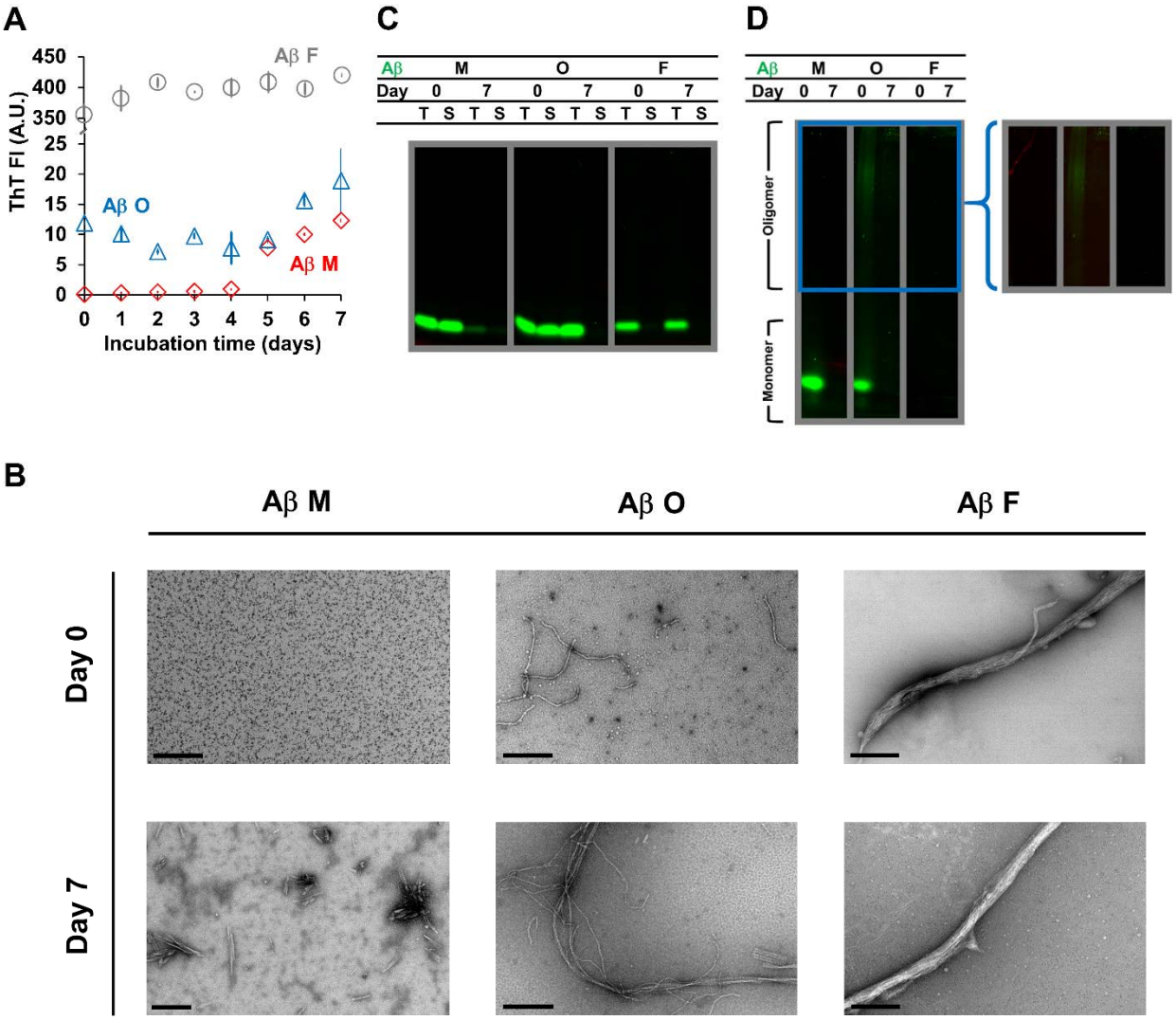

Figure S3. Characterizations of Aβ monomers (M), Aβ oligomers (O) and Aβ fibrils (F) incubated for 7 days at 37 °C, as examined by (A) ThT fluorescence, (B) TEM, and (C-D) in-gel fluorescence imaging of (C) SDS-PAGE and (D) native-PAGE. In (A-D), the concentration of Aβ in each sample was 70 μM. Concentrations of oligomers and fibrils were monomer-equivalent concentrations. In (A), data on Aβ monomers, Aβ oligomers and Aβ fibrils are shown with red diamonds, blue triangles and gray circles, respectively. Error bars: 1 standard deviation of triplicates. In (B), representative TEM images of Aβ samples on Day 0 (top panels) and Day 7 (bottom panels) are shown with scale bars of 200 nm. In (C-D), Aβ samples contained HiLyte Fluor 488-labeled Aβ (green). Each panel was taken from a bigger gel image (see **Figure S5**) and reassembled for better presentation. In (C), T: total fraction and S: soluble fraction. In (D), the images in the right provide brighter upper portions of the native-PAGE gels – enclosed by a blue box in the left – obtained by renormalizing the brightness from the monomer to the oligomer bands, which often contained lower percentages of fluorophores.

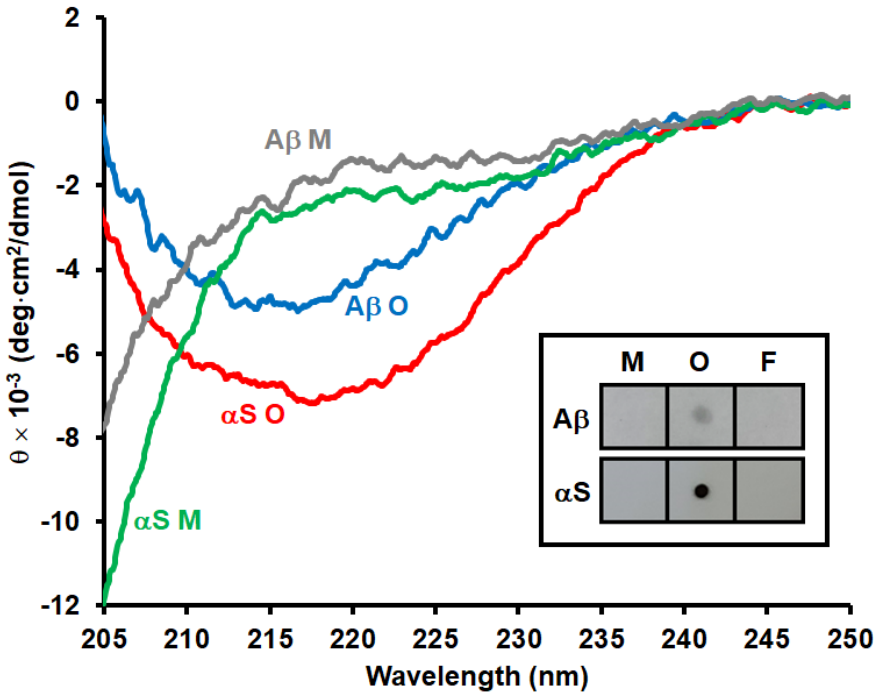

Figure S4. Circular dichroism (CD) spectra of Aβ monomers (Aβ M, gray), Aβ oligomers (Aβ O, blue), αS monomers (αS M, green) and αS oligomers (αS O, red). The spectra shown here are largely similar to those previously reported for structurally disordered monomers and β-sheet structured oligomers of Aβ and αS.[11, 12, 20] Inset: dot blot assays of monomers (M), oligomers (O) and fibrils (F) of Aβ and αS using A11.

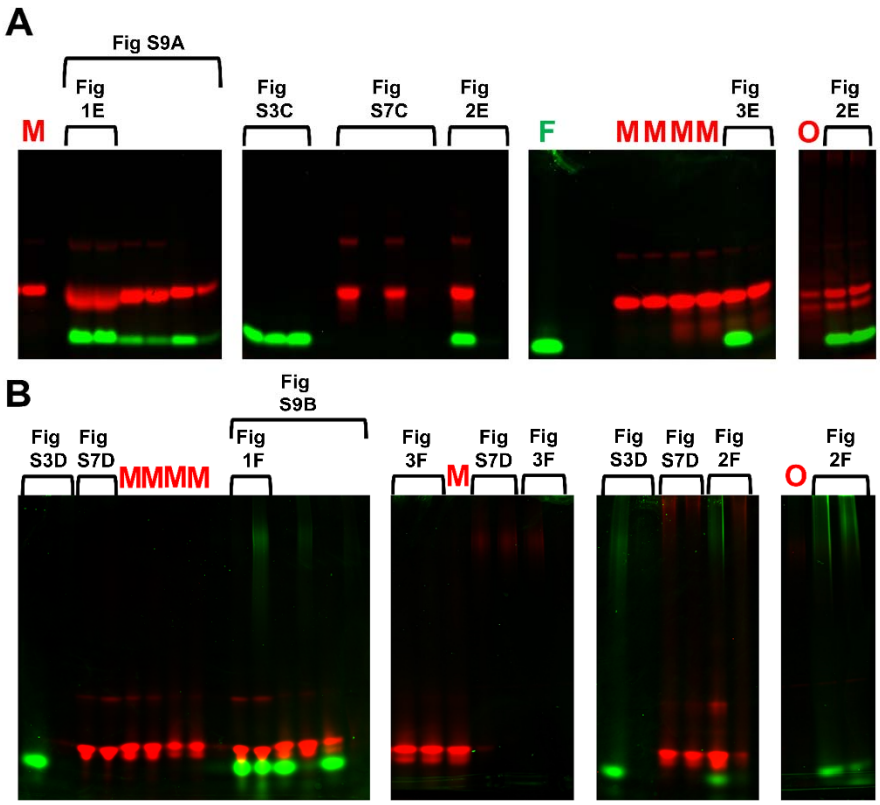

Figure S5. Representative bigger gel images of (A) SDS-PAGE and (B) native-PAGE from which panels in Figures 1, 2, 3, S3, S7 and S9 were taken. The gel images also contain additional control samples, such as Aβ fibrils (F), αS monomers (M) and αS oligomers (O), whose images are not shown in Figures 1, 2, 3, S3, S7 and S9. The gel images demonstrate that the relative positions of the major bands on both SDS- and native-PAGE gels appeared consistent across all individual and mixture samples of Aβ and αS.

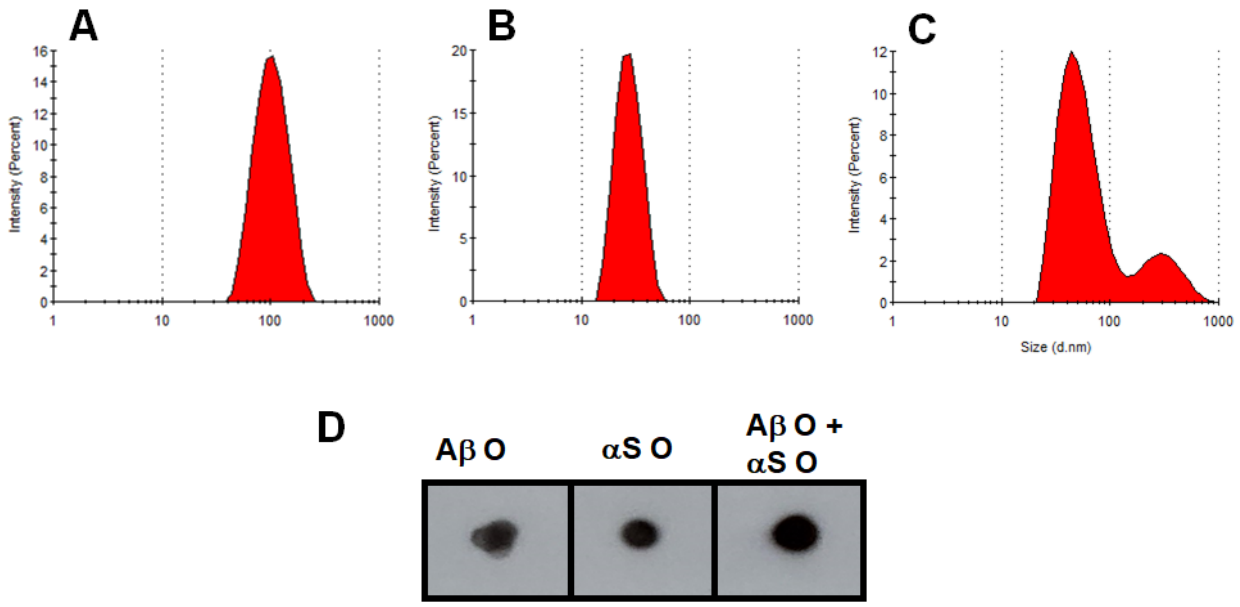

Figure S6. (A-C) Size distribution as determined by CONTIN analysis of dynamic light scattering data for samples of (A) Aβ oligomers (Aβ O) at 70 μM on Day 0, (B) αS oligomers (αS O) at 17.5 μM on Day 0, and (C) the mixture of Aβ O at 70 μM and αS O at 17.5 μM on Day 7 during incubation at 37 ºC. (D) Dot blot assays of samples containing (left) Aβ O on Day 0, (middle) αS O on Day 0 and (right) the mixture of Aβ O and αS O on Day 7 during incubation at 37 ºC. The oligomer concentrations are monomer-equivalent concentrations.

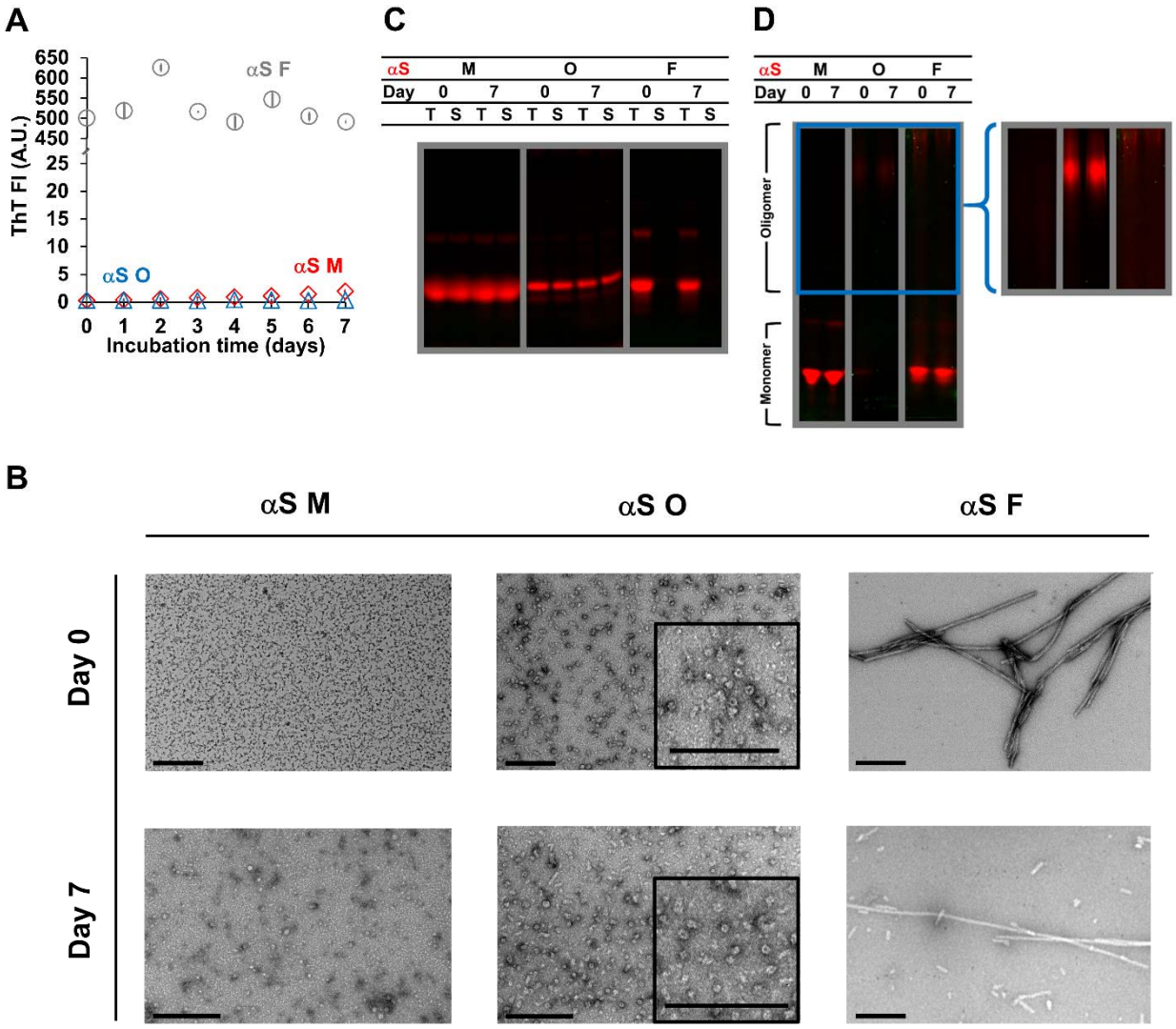

Figure S7. Characterizations of αS monomers (M), αS oligomers (O) and αS fibrils (F) incubated for 7 days at 37 °C, as examined by (A) ThT fluorescence, (B) TEM, and (C-D) in-gel fluorescence imaging of (C) SDS-PAGE and (D) native-PAGE. In (A-D), the concentrations of αS monomers and αS fibrils were 350 μM and that of αS oligomers was 17 μM. Concentrations of oligomers and fibrils were monomer-equivalent concentrations. In (A), data on αS monomers, αS oligomers and αS fibrils are shown with red diamonds, blue triangles and gray circles, respectively. Error bars: 1 standard deviation of triplicates. In (B), representative TEM images of αS samples on Day 0 (top panels) and Day 7 (bottom panels) are shown with scale bars of 200 nm. In (C-D), αS samples contained Alexa Fluor 647-labeled αS (red). Each panel was taken from a bigger gel image (see **Figure S5**) and reassembled for better presentation. In (C), T: total fraction and S: soluble fraction. In (D), the images in the right provide brighter upper portions of the native-PAGE gels – enclosed by a blue box in the left – obtained by renormalizing the brightness from the monomer to the oligomer bands, which often contained lower percentages of fluorophores.

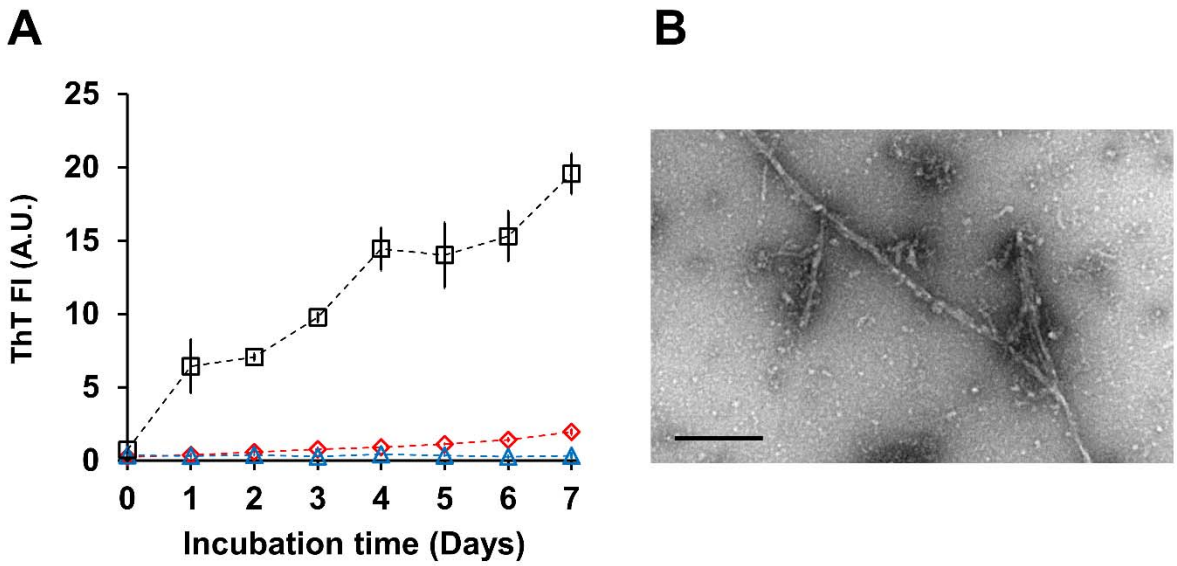

Figure S8. (A) Time-course Thioflavin T (ThT) fluorescence of αS monomers at 333 μM seeded with αS oligomers at 17 μM (black squares), αS monomers at 350 μM (red diamonds) and αS oligomers at 17 μM (blue triangles) and (B) Representative transmission electron microscopy (TEM) image of αS monomers at 333 μM seeded with αS oligomers at 17 μM after 7 day incubation at 37 ºC. The oligomer concentration is monomer-equivalent concentration. In (A), error bars: 1 standard deviation of triplicates. In (B), scale bars: 200 nm.

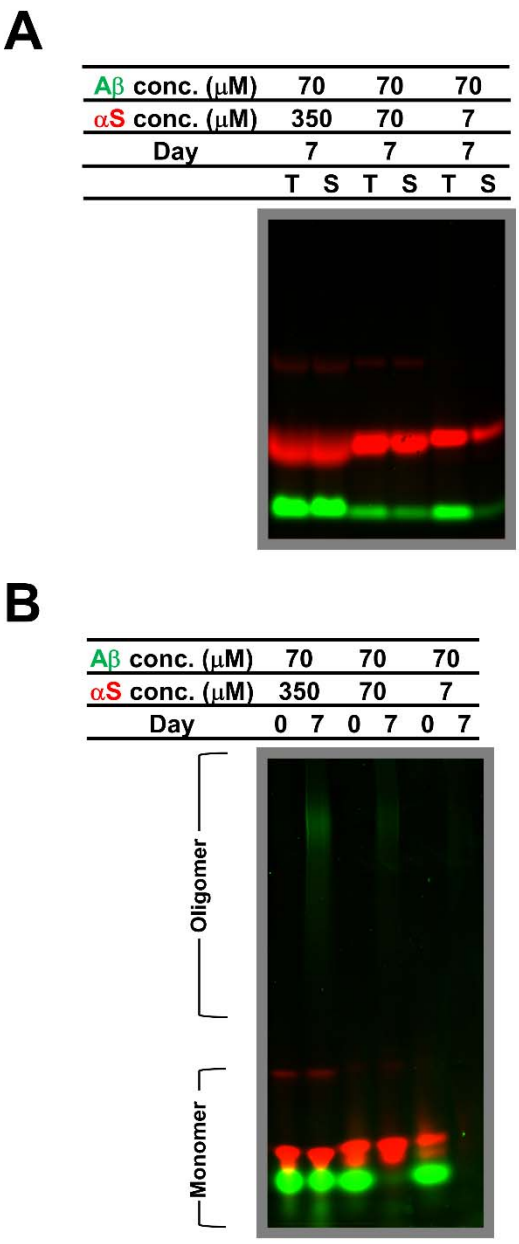

Figure S9. In-gel fluorescence imaging of (A) SDS-PAGE and (B) native-PAGE at the beginning and/or end of 7-day incubation at 37 °C of Aβ monomers at 70 μM mixed with αS monomers at various concentrations (7, 70 and 350 μM). In (A-B), samples contained HiLyte Fluor 488-labeled Aβ (green) and Alexa Fluor 647-labeld αS (red). In (A), T and S represent total and soluble fractions of samples, respectively.

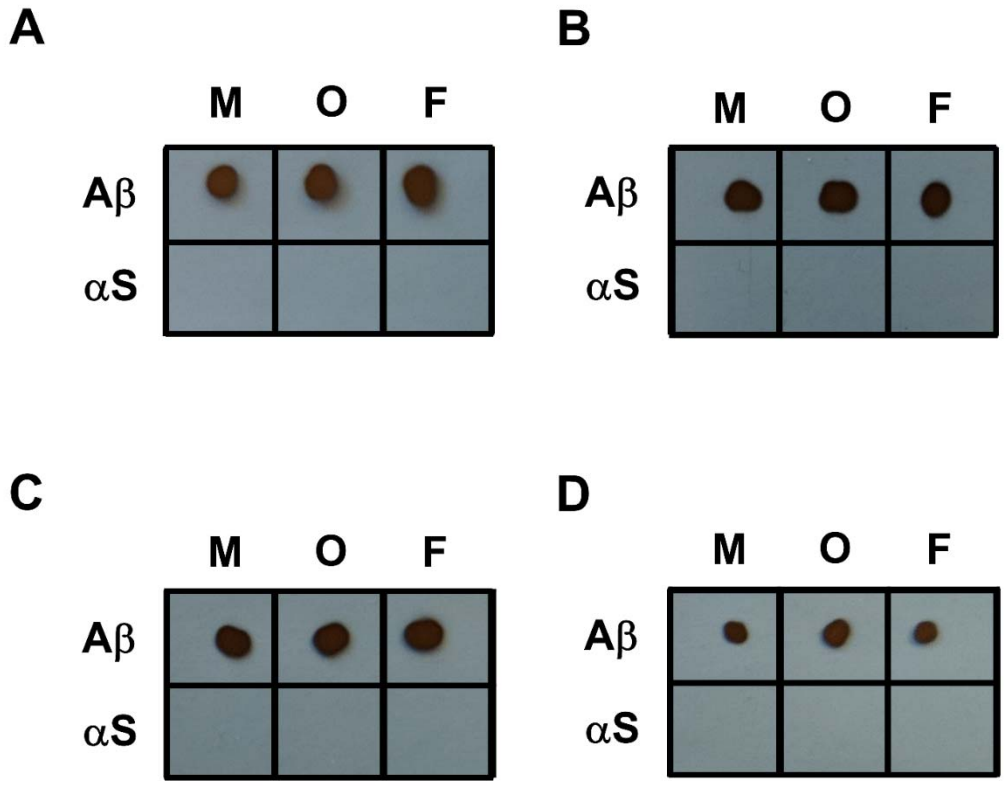

Figure S10. Dot blot assay using Aβ sequence-specific antibodies of (A) 6E10 recognizing Aβ1-16, (B) 4G8 recognizing Aβ17-22, (C) Anti-Aβ (22-35) recognizing Aβ22-35, and (D) 5C3 recognizing Aβ32-40 with Aβ only samples (top row) and αS only samples (bottom row) in their respective monomeric (left column), oligomeric (middle column) and fibrillar forms (right column). M, O and F represent monomers, oligomers and fibrils, respectively.

REFERENCES

(1) Hernandez, M., Golbert, S., Zhang, L. G., and Kim, J. R. (2011) Creation of aggregation-defective alpha-synuclein variants by engineering the sequence connecting beta-strand-forming domains. *Chembiochem 12*, 2630-2639.

(2) Hernandez, M., Hu, Y., and Kim, J. R. (2013) A conformation-switching fluorescent protein probe for detection of alpha synuclein oligomers. *Chem. Commun. (Camb) 49*, 10712-10714.

(3) Hu, Y., Su, B., Zheng, H., and Kim, J. R. (2012) A peptide probe for detection of various beta-amyloid oligomers. *Mol. Biosyst. 8*, 2741-2752.

(4) Hu, Y., Zheng, H., Su, B., Hernandez, M., and Kim, J. R. (2012) Modulation of beta-amyloid aggregation by engineering the sequence connecting beta-strand forming domains. *Biochim. Biophys. Acta 1824*, 1069-1079.

(5) Hu, Y., Su, B., Kim, C. S., Hernandez, M., Rostagno, A., Ghiso, J., and Kim, J. R. (2010) A strategy for designing a peptide probe for detection of beta-amyloid oligomers. *Chembiochem 11*, 2409-2418.

(6) Aoraha, E., Candreva, J., and Kim, J. R. (2015) Engineering of a peptide probe for beta-amyloid aggregates. *Mol. Biosyst. 11*, 2281-2289.

(7) Mach, H., Volkin, D. B., Burke, C. J., and Middaugh, C. R. (1995) Ultraviolet absorption spectroscopy. *Methods Mol. Biol. 40*, 91-114.

(8) Celej, M. S., Sarroukh, R., Goormaghtigh, E., Fidelio, G. D., Ruysschaert, J. M., and Raussens, V. (2012) Toxic prefibrillar alpha-synuclein amyloid oligomers adopt a distinctive antiparallel beta-sheet structure. *Biochem. J. 443*, 719-726.

(9) Biancalana, M., and Koide, S. (2010) Molecular mechanism of Thioflavin-T binding to amyloid fibrils. *Biochim. Biophys. Acta 1804*, 1405-1412.

(10) Williams, A. D., Portelius, E., Kheterpal, I., Guo, J.-t., Cook, K. D., Xu, Y., and Wetzel, R. (2004) Mapping Aβ Amyloid Fibril Secondary Structure Using Scanning Proline Mutagenesis. *J. Mol. Biol. 335*, 833-842.

(11) Zhang, A., Qi, W., Good, T. A., and Fernandez, E. J. (2009) Structural differences between Abeta(1-40) intermediate oligomers and fibrils elucidated by proteolytic fragmentation and hydrogen/deuterium exchange. *Biophys. J. 96*, 1091-1104.

(12) Maji, S. K., Ogorzalek Loo, R. R., Inayathullah, M., Spring, S. M., Vollers, S. S., Condron, M. M., Bitan, G., Loo, J. A., and Teplow, D. B. (2009) Amino acid position-specific contributions to amyloid beta-protein oligomerization. *J. Biol. Chem. 284*, 23580-23591.

(13) Paik, S. R., Lee, J. H., Kim, D. H., Chang, C. S., and Kim, J. (1997) Aluminum-induced structural alterations of the precursor of the non-A beta component of Alzheimer's disease amyloid. *Arch. Biochem. Biophys. 344*, 325-334.

(14) Klug, G. M., Losic, D., Subasinghe, S. S., Aguilar, M. I., Martin, L. L., and Small, D. H. (2003) Beta-amyloid protein oligomers induced by metal ions and acid pH are distinct from those generated by slow spontaneous ageing at neutral pH. *Eur. J. Biochem. 270*, 4282-4293.

(15) Garai, K., and Frieden, C. (2013) Quantitative analysis of the time course of Abeta oligomerization and subsequent growth steps using tetramethylrhodamine-labeled Abeta. *Proc. Natl. Acad. Sci. USA 110*, 3321-3326.

(16) Huang, D., Zimmerman, M. I., Martin, P. K., Nix, A. J., Rosenberry, T. L., and Paravastu, A. K. (2015) Antiparallel beta-Sheet Structure within the C-Terminal Region of 42-

Residue Alzheimer's Amyloid-beta Peptides When They Form 150-kDa Oligomers. *J. Mol. Biol. 427*, 2319-2328.
(17) Harper, J. D., Wong, S. S., Lieber, C. M., and Lansbury, P. T. (1997) Observation of metastable Abeta amyloid protofibrils by atomic force microscopy. *Chem. Biol. 4*, 119-125.
(18) Ahmed, M., Davis, J., Aucoin, D., Sato, T., Ahuja, S., Aimoto, S., Elliott, J. I., Van Nostrand, W. E., and Smith, S. O. (2010) Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nat. Struct. Mol. Biol. 17*, 561-567.
(19) Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., and Glabe, C. G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science 300*, 486-489.
(20) Chen, S. W., Drakulic, S., Deas, E., Ouberai, M., Aprile, F. A., Arranz, R., Ness, S., Roodveldt, C., Guilliams, T., De-Genst, E. J., Klenerman, D., Wood, N. W., Knowles, T. P., Alfonso, C., Rivas, G., Abramov, A. Y., Valpuesta, J. M., Dobson, C. M., and Cremades, N. (2015) Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation. *Proc. Natl. Acad. Sci. USA 112*, E1994-2003.
(21) Gallea, J. I., and Celej, M. S. (2014) Structural insights into amyloid oligomers of the Parkinson disease-related protein alpha-synuclein. *J. Biol. Chem. 289*, 26733-26742.
(22) Giehm, L., Svergun, D. I., Otzen, D. E., and Vestergaard, B. (2011) Low-resolution structure of a vesicle disrupting α-synuclein oligomer that accumulates during fibrillation. *Proc. Natl. Acad. Sci. USA 108*, 3246-3251.
(23) Kim, H. Y., Cho, M. K., Kumar, A., Maier, E., Siebenhaar, C., Becker, S., Fernandez, C. O., Lashuel, H. A., Benz, R., Lange, A., and Zweckstetter, M. (2009) Structural properties of pore-forming oligomers of alpha-synuclein. *J. Am. Chem. Soc. 131*, 17482-17489.
(24) Poma, A. B., Guzman, H. V., Li, M. S., and Theodorakis, P. E. (2019) Mechanical and thermodynamic properties of Abeta42, Abeta40, and alpha-synuclein fibrils: a coarse-grained method to complement experimental studies. *Beilstein J. Nanotechnol. 10*, 500-513.
(25) Jensen, P. H., Hojrup, P., Hager, H., Nielsen, M. S., Jacobsen, L., Olesen, O. F., Gliemann, J., and Jakes, R. (1997) Binding of Abeta to alpha-and beta-synucleins: identification of segments in alpha-synuclein/NAC precursor that bind Abeta and NAC. *Biochem. J. 323*, 539-546.
(26) Breydo, L., and Uversky, V. N. (2015) Structural, morphological, and functional diversity of amyloid oligomers. *FEBS Lett. 589*, 2640-2648.