Cross-seeding effects of amyloid β-protein and α-synuclein

Short Communication

Cross-seeding effects of amyloid β-protein and α-synuclein

Kenjiro Ono,[1] Ryoichi Takahashi,[1] Tokuhei Ikeda, and Masahito Yamada

* *Department of Neurology and Neurobiology and Aging, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan*

Address correspondence and reprint requests to Dr. M. Yamada at Department of Neurology & Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8640, Japan. E-mail: m-yamada@med.kanazawa-u.ac.jp

[1] These authors have contributed equally to this study.

Abbreviations used: Aβ, amyloid β-protein; AD, Alzheimer's disease; APP, amyloid precursor protein; APS, ammonium persulfate; αS, α-synuclein; DLB, dementia with Lewy bodies; EM, electron microscopy; fAβ, Aβ fibrils; fαS, αS fibrils; LBD, Lewy body diseases; LBs, Lewy bodies; NAC, non-amyloid component; oligo, cross-linked oligomers; PD, Parkinson disease; PICUP, photo-induced cross-linking of unmodified proteins; Ru(bpy), tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ThS, Thioflavin S; ThT, Thioflavin T.

Amyloid β-protein (Aβ) and α-synuclein (αS) are the primary components of amyloid plaques and Lewy bodies (LBs), respectively. Previous *in vitro* and *in vivo* studies have suggested that interactions between Aβ and αS are involved in the pathogenesis of Alzheimer's disease (AD) and LB diseases (LBD). However, the seeding effects of their aggregates on their aggregation pathways are not completely clear. To investigate the cross-seeding effects of Aβ and αS, we examined how sonicated fibrils or cross-linked oligomers of Aβ40, Aβ42, and αS affected their aggregation pathways using thioflavin T(S) assay and electron microscopy. Fibrils and oligomers of Aβ40, Aβ42, and αS acted as seeds, and affected the aggregation pathways within and among species. The seeding effects of αS fibrils were higher than those of Aβ40 and Aβ42 fibrils in the Aβ40 and Aβ42 aggregation pathways, respectively. We showed that Aβ and αS acted as seeds and affected each other's aggregation pathways *in vitro*, which may contribute to our understanding of the molecular mechanisms of interactions between AD and LBD pathologies.

Key words: α-synuclein, aggregation, Alzheimer's disease, amyloid β-protein, Lewy body diseases, seed

Running head: Cross-seeding effects of Aβ and αS

Amyloid β-protein (Aβ) and α-synuclein (αS) are the primary components of amyloid plaques and Lewy bodies (LBs), respectively. Aggregations of Aβ and αS are considered to be a critical step during neurodegeneration associated with Alzheimer's disease (AD) and Lewy body diseases (LBD), respectively. AD is characterized by the accumulation of Aβ plaques and neurofibrillary tangles. Interestingly, up to 50% of AD cases exhibit significant LB pathology in addition to plaques and tangles (Hamilton 2000). Likewise, patients with dementia with LBs (DLB) frequently exhibit AD pathology, particularly senile plaques (Armstrong *et al.* 1997).

Recent studies suggest that accumulations of oligomers might be the neurotoxic species, rather than fibrils. The progressive accumulation of Aβ oligomers has been identified as a central toxic event during AD that leads to synaptic dysfunction (Ono & Yamada 2011), whereas the formation of αS oligomers that disrupt membrane and mitochondrial activity has been linked to LBD (Kim *et al.* 2009).

It was previously shown that Aβ enhances αS accumulation and neuronal deficits using transgenic mice with neuronal expression of Aβ and αS (Masliah *et al.* 2001). NMR study showed that Aβ and αS might interact directly at a few sites (Mandal *et al.* 2006). A recent *in vitro* study reported that Aβ and αS might interact directly to form hybrid pore-like oligomers that contribute to neurodegeneration (Tsigelny *et al.* 2008). These studies suggest that interactions between Aβ and αS are involved in the pathogenesis of AD and LBD, but the seeding effects of their aggregates on aggregation pathways have not been elucidated. Thus, we determined whether fibrils or cross-linked oligomers of Aβ40, Aβ42, and αS have cross-seeding effects on each other's aggregation pathways *in vitro*.

Materials and methods

Preparation of peptides

Aβ and αS solutions were prepared as described previously (Ono *et al.* 2003, Ono & Yamada 2006). Aβ40 and Aβ42 were purchased from Peptide Institute Inc. (Osaka, Japan). Aβ lyophilizates were dissolved at 25 μM in 10% (v/v) 60 mM NaOH and 90% (v/v) 10 mM

phosphate buffer, pH 7.4. αS was purchased from Recombinant Peptide Technologies (LLC, GA). αS was dissolved at 25 μM in 20 mM Tris buffer, pH 7.4. After sonication for 1 min using a bath sonicator, Aβ and αS solutions were centrifuged for 10 min at 16,000 × *g*.

Preparation of fibrils

The resulting supernatant was incubated at 37°C for 2 (Aβ42) or 10 (Aβ40, αS) days. Fibril preparations were stored as lyophilizates and reconstituted at 25 μM in 10 mM phosphate buffer or 20 mM Tris buffer. After sonication on ice with 30 intermittent pulses using an ultrasonic disruptor, the sonicated Aβ40 fibrils (fAβ40), Aβ42 fibrils (fAβ42), and αS fibrils (fαS) were used for the **seeding** assays.

Thioflavin T (ThT) and thioflavin S (ThS) binding

The reaction mixture contained 5 μM ThT (Wako Chemical Industries Ltd, Osaka, Japan) or ThS (MP Biomedicals, Irvine, CA) and 50 mM of glycine-NaOH buffer, pH 8.5. After vortexing briefly, fluorescence was determined three times at intervals of 10 s using a Hitachi F-2500 fluorometer. Excitation and emission wavelengths of 445 and 490 nm were used for the Aβ40 and Aβ42 assays, respectively. Excitation and emission wavelengths of 440 and 521 nm were used for αS assay, respectively. Fluorescence was determined by averaging three readings and subtracting the ThT or ThS blank readings.

Photo-induced cross-linking of unmodified proteins (PICUP) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Cross-linking of Aβ40, Aβ42, or αS was photo-induced, essentially as previously described (Ono *et al.* 2008, Ono *et al.* 2011b). To 18 μl of 25 μM protein solution, we added 1 μl of 2-4 mM tris(2,2'-bipyridyl)dichlororuthenium(II) (Ru(bpy)) and 1 μl of 40-80 mM ammonium persulfate. The mixtures were irradiated for 1 s with visible light and the reaction was quenched with 1 μl of 1 M dithiothreitol (DTT) (Invitrogen) in ultrapure water. The frequency distribution of monomers and oligomers was determined using SDS-PAGE and silver staining, as described previously (Ono et al. 2008).

Size-exclusion chromatography (SEC)

PICUP reagents were removed from cross-linked samples by SEC as described previously (Ono *et al.* 2010). Aβ and αS peptides were eluted in the void volume, whereas Ru(bpy), APS, and DTT entered the column matrix separately from Aβ or αS. Fractions were lyophilized immediately after collection. The lyophilizates were reconstituted at 25 μM.

Seeding activity of fibril and cross-linked oligomers of Aβ40, Aβ42, and αS

Fibrils or cross-linked oligomers (oligo) of Aβ40, Aβ42, or αS were prepared at a concentration of 25 or 5 μM in 10mM phosphate buffer or 20 mM Tris buffer, pH 7.4. For the seeding assays, sonicated fibrils (Fig. 1) or cross-linked peptides (Fig. 1) were added as seeds to un-cross-linked peptides at a ratio of 10% (v/v). The mixtures were incubated at 37°C for 0–7 days.

Electron microscopy (EM)

A 10-μl aliquot of each sample was spotted onto a glow-discharged, carbon-coated formvar grid (Okenshoji Co. Ltd, Tokyo, Japan) and incubated for 20 min. The droplet was displaced with an equal volume of 2.5% (v/v) glutaraldehyde in water and incubated for an additional 5 min. Finally, the peptide was stained with 8 μl of 1% (v/v) uranyl acetate in water (Wako Chemical Industries Ltd). This solution was wicked off and the grid was air-dried. Samples were examined using a JEM-1210 transmission electron microscope.

Statistical analysis

One-way factorial ANOVA followed by Bonferroni *post hoc* comparisons were used to determine statistical significance among data sets. These tests were implemented within GraphPad Prism software (version 4.0a, GraphPad Software, Inc., San Diego, CA). Significance was defined as $p<0.05$.

Results and discussion

We used a well-characterized assay of fibril formation, thioflavin dye binding (LeVine 1999), to determine the effects of fibrils or cross-linked oligomers on peptide assembly. In order to compare their seeding effects precisely, we arranged Aβ40, Aβ42, and αS solutions with the same concentration (25 μM), at which oligo Aβ40 and oligo Aβ42 functioned as seeds during Aβ

assembly (Ono *et al.* 2009, Ono *et al.* 2010). As shown in Fig. 2A, the ThT fluorescence followed a sigmoidal curve when fresh Aβ40 was incubated at 37°C, which was characterized by a ~1 day lag time, a ~5 day period of increasing ThT binding, and a plateau after ~6 days. This curve is consistent with a nucleation-dependent polymerization model (Jarrett & Lansbury 1993, Ono *et al.* 2003). When fresh Aβ40 was incubated with fAβ40 at 37°C, the fluorescence increased hyperbolically without a lag phase, and a plateau occurred after ~4 h (Fig. 2A). This curve is consistent with a first-order kinetic model (Ono *et al.* 2003). Similar effects were observed after the addition of fAβ42 or fαS. The growth rates of fAβ42 and fαS were 27.9 and 64.8 FU/h, respectively, while the rate of fAβ42 was significantly lower ($p < 0.01$) than those of fAβ40 (68.6 FU/h) and fαS. The plateau occurred after ~2 h with fαS, which was earlier than that with fAβ40 (~4 h). Fluorescence also increased hyperbolically without a lag phase when fresh Aβ40 was incubated with oligo Aβ40 at 37°C and a plateau occurred after ~48 h (Fig. 2A). Similar effects were observed after the addition of oligo Aβ42 or oligo αS, though their growth rates (oligo Aβ42, 8.1 FU/h; oligo αS, 6.4 FU/h) were significantly lower ($p < 0.05$) than that produced by oligo Aβ40 (9.6 FU/h). Overall, the seeding effects during Aβ40 assembly were in the order of fαS ≥ fAβ40 > fAβ42 >> oligo Aβ40 > oligo Aβ42 > oligo αS. As shown in Fig. 2B, fAβ40 produced with a fresh Aβ40 solution assumed a nonbranched, helical filament structure of ~7 nm with a helical periodicity of approximately 220 nm, as described previously (Ono *et al.* 2003, Ono *et al.* 2008). A typical fibrillar structure was also observed when Aβ40 was incubated with fAβ40, fAβ42, fαS, oligo Aβ40, oligo Aβ42, or oligo αS (Figs. 2C-H). At lower concentration (5 μM), fαS acted as seeds (growth rate, 9.6 FU/h) although oligo αS did not.

Similar results were obtained for Aβ42 assembly. As shown in Fig. 3A, the ThT fluorescence produced a sigmoidal curve when fresh Aβ42 was incubated at 37°C, which was characterized by a ~2-h lag time, a ~10-h period of increasing ThT binding, and a plateau after ~12 h. The fluorescence increased hyperbolically without a lag phase when fresh Aβ42 was incubated with fAβ42 at 37°C and a binding plateau occurred after ~2 h (Fig. 3A). A similar effect was observed after the addition of fAβ40 or fαS. The growth rates of fAβ42, fAβ40, and fαS were 48.9, 41.5, and 54.2 FU/h, respectively, while the rates of fAβ42 and fαS were significantly higher ($p < 0.01$) than that of fAβ40. The fluorescence also increased hyperbolically without a lag phase when fresh Aβ42 was incubated with oligo Aβ42 at 37°C and a plateau occurred after ~4 h (Fig. 3A). Similar effects were observed after the addition of oligo Aβ40 or oligo αS, although their

growth rates (oligo Aβ40, 24.7 FU/h; oligo αS, 21.5 FU/h) were lower than that of oligo Aβ42 (38.0 FU/h) ($p < 0.01$). Overall, the seeding effects during Aβ42 assembly were in the order of fαS ≥ fAβ42 > fAβ40 >> oligo Aβ42 ≥ oligo Aβ40 > oligo αS. As shown in Fig. 3B, fresh Aβ42 solution formed nonbranched filaments with a width of ~8 nm and varying degrees of helicity, as described previously (Ono *et al.* 2003, Ono *et al.* 2008). We also observed thicker, straight, non-branched filaments with a width of ~12 nm. Typical fibrillar structures were also observed when Aβ42 was incubated with fAβ42, fAβ40, fαS, oligo Aβ42, oligo Aβ40, or oligo αS (Figs. 3C-H). At lower concentration (5 μM), fαS acted as seeds (growth rate, 15.2 FU/h) although oligo αS did not.

We monitored the kinetics of αS assembly using ThS assay to determine whether αS assembly was affected by homogeneous or heterogeneous fibrils or oligomers. αS produced a sigmoidal curve in the absence of any fibrils or oligomers, which was characterized by a lag time of 12 h, a period of increasing ThS binding for 4.5 days, and a plateau after 5 days (Fig. 4A)—results consistent with the nucleation-dependent polymerization model (Wood *et al.* 1999). The binding curves increased hyperbolically without a lag phase when αS was incubated with fαS and a plateau occurred after ~6 h (Fig. 4A). A similar effect was observed after the addition of fAβ40 or fAβ42, though their growth rates were different. The growth rates of fαS, fAβ40, and fAβ42 were 25.0, 11.3, and 18.9 FU/h, respectively, while the rate of fαS was significantly higher ($p < 0.05$) than that of fAβ40 or fAβ42. The fluorescence also increased hyperbolically without a lag phase when fresh αS was incubated with oligo αS at 37°C and a plateau occurred after ~3 days (Fig. 4A). Similar effects were observed after the addition of oligo Aβ40 or oligo Aβ42, though their growth rates (oligo Aβ40, 3.7 FU/h; oligo Aβ42, 4.7 FU/h) were significantly lower than oligo αS (6.1 FU/h) ($p < 0.01$). Overall, the seeding effects during αS assembly were in the order of fαS > fAβ42 > fAβ40 >> oligo αS > oligo Aβ42 > oligo Aβ40. We observed nonbranched, helical filaments with diameters of 10 nm after the incubation of fresh αS (Fig. 4B). Typical fibrillar structures were also observed when αS was incubated with fαS, fAβ40, fAβ42, oligo αS, oligo Aβ40, or oligo Aβ42 (Figs. 4C-H).

Previously, we reported that sonicated fibrils and cross-linked oligomers functioned as seeds during Aβ and αS assembly (Ono *et al.* 2003, Ono *et al.* 2009, Ono *et al.* 2010, Ono *et al.* 2011a). Our used PICUP-stabilized oligomers have significant biochemical properties such as increased neurotoxic activity and β-sheet ratios of secondary structures with higher oligomer

order (Ono *et al.* 2009, Ono *et al.* 2010, Ono *et al.* 2011a). The current study showed that fibrils and cross-linked oligomers of Aβ40, fAβ42, and fαS had seeding effects on the aggregation pathways of different species and the same species. The seeding effects of oligomers were lower than those of fibrils. Interestingly, the seeding effect of fαS was higher than that of fAβ40 on Aβ40 aggregation. Similarly, the seeding effect of fαS was higher than that of fAβ42 on Aβ42 aggregation. The seeding effects of fAβ42 and fAβ40 on the αS aggregation pathway were lower than that of fαS.

The molecular mechanisms underlying the interactions between Aβ40, Aβ42, and αS need to be considered. The hydrophobic core of Aβ, i.e., residues 17-21, is known to play an important role in the formation and stabilization of amyloid fibrils (Wood *et al.* 1995). Similarly, a central hydrophobic non-amyloid component (NAC) region (residues 61-95) is also essential for αS aggregation (Lucking & Brice 2000). NAC is known to promote Aβ aggregation by binding 81-95 residues of NAC via 25-35 Aβ residues (Yoshimoto *et al.* 1995). A previous NMR study also showed that αS might interact directly with Aβ at a few sites, i.e., G67, G73, and V74, belonging to NAC (Mandal *et al.* 2006). It was reported that Aβ facilitated the formation of more stable αS oligomers via direct interaction with NAC *in vitro* (Tsigelny, *et al.* 2008). In our study, it was interesting that the fibrillar form of αS accelerated the Aβ40 and Aβ42 aggregations more rapidly than fAβ40 and fAβ42. Previously, Naiki's group analyzed the interaction effects of Aβ40 and Aβ42 on the kinetics of *in vitro* fAβs formation using ThT assay (Hasegawa *et al.* 1999). The aggregation of fresh Aβ40 was accelerated by the addition of fAβ42, though the effect was lower compared with the addition of fAβ40 to Aβ40 (Hasegawa *et al.* 1999). The fluorescence increased hyperbolically without a lag phase when Aβ42 was incubated with fAβ40 and it proceeded to equilibrium more rapidly than without fAβ40 (Hasegawa *et al.* 1999). It was also suggested that the hydrophobic core may contribute to the association between Aβ40 and Aβ42 (Hasegawa *et al.* 1999). The hydrophobic cores of Aβ40, Aβ42, and αS may also contribute to their monomer-fibril association with each other. In particular, the hydrophobic core of αS might have stronger promoting effects than those of Aβ40 and Aβ42.

Several *in vivo* studies have focused on understanding the direct interaction of Aβ and αS. Masliah *et al.* (2001) showed that Aβ promotes the *in vivo* aggregation of αS while it exacerbates learning deficits with amyloid precursor protein (APP)/αS double transgenic mice. αS has also been shown to accumulate in the brains of Tg2576 mice (Yang *et al.* 2000) and APP/presenilin-1

double transgenic mice, which produce large amounts of Aβ (Kurata *et al.* 2007). More importantly, several studies using human brains have shown that Aβ contributes to the levels and status of αS aggregation and LB formation (Lippa *et al.* 2005, Pletnikova *et al.* 2005). Lippa *et al.* (2005) compared the amygdala of subjects with pathological aging and AD. The Aβ40 plaque level was higher in cases with secondary αS aggregates. The Aβ42 plaque level was not associated with αS aggregation. Pletnikova *et al.* (2005) examined autopsied brains from 21 cases of LBD. In brains without Aβ deposits, there was little or no LB in the cerebral cortex. In contrast, they observed significant increases in LB in the cerebral cortex and αS immunoreactive lesions in the cingulate cortex of brains with Aβ deposits (Pletnikova *et al.* 2005). Immunoblots of αS from the cingulate cortex of brains with Aβ deposits indicated significantly higher levels of insoluble αS compared with brains without Aβ deposits (Pletnikova *et al.* 2005). Overall, these studies of transgenic mice and human brains support a hypothesis that Aβ and αS interact *in vivo* and that these interactions are of significance for the pathogenesis of the disease. The mechanisms of extracellular Aβ and intracellular αS interaction are unclear, but there have been some significant reports. Agnati *et al.* (2010) suggested that Aβ and αS cannot diffuse via the extracellular space but that they can move from cell to cell via tunneling nanotubes, which may propagate mitochondrial damage and cell degeneration. This was supported by the presence of Aβ and αS within intracellular nanotubes. Indeed, Aβ monomers and oligomers have been detected intracellularly in the brains of AD patients (Walsh *et al.* 2000, Steinerman *et al.* 2008), while αS monomers and oligomers were detected extracellularly in biological fluids, such as the blood plasma and cerebrospinal fluid of LBD patients (El-Agnaf *et al.* 2003, Tokuda *et al.* 2010). Thus, it is possible that the oligomers and monomers of Aβ and αS might promote the aggregation of each other both intracellularly and extracellularly.

The seeded proliferation of misfolded proteins that occurs during prion disease may explain the pathogenesis of AD and LBD. The pathological similarities between prion disease and AD have long engendered the speculation that AD might be inducible in a prion-like manner (Gajdusek 1994). The Jucker group reported that the seeding of Aβ deposition was ineffective in nontransgenic mice, while the phenotype of induced Aβ deposits in transgenic mice mirrored that of deposits in the extract, thereby implicating an Aβ-templating mechanism (Meyer-Luehmann *et al.* 2006, Eisele *et al.* 2009). It was also shown that soluble extracts of Aβ deposits could act as amyloid-inducing seeds (Langer *et al.* 2011). Increasing evidence implicates the templated

corruption of disease-specific proteins in other neurodegenerative diseases such as LBD. A seeding-like process in αS lesions was bolstered by the observation that fetal dopaminergic neural transplants in the striatum of PD patients can eventually lead to the presence of αS-positive LBs in some cells, suggesting that αS seeds propagate from the host to the graft (Li *et al.* 2008, Kordower *et al.* 2008). *In vitro* cross-seeding effects have never been reported before, but our *in vitro* study indicated that aggregates of Aβ and αS acted as seeds and promoted the aggregation of each other.

In conclusion, the fibrils and oligomers of Aβ40, Aβ42, and αS functioned as seeds and promoted each other's aggregation pathways, though the oligomers were less efficient than fibrils. The cross-seeding effects observed in this study may provide new insights into the molecular mechanisms that underlie the interactions between AD and LBD pathogenesis.

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References

Agnati, L. F., Guidolin, D., Baluska, F., Leo, G., Barlow, P. W., Carone, C. and Genedani, S. (2010) A new hypothesis of pathogenesis based on the divorce between mitochondria and their host cells: possible relevance for Alzheimer's disease. *Curr Alzheimer Res,* **7,** 307-322.

Armstrong, R. A., Cairns, N. J. and Lantos, P. L. (1997) β-Amyloid (Aβ) deposition in the medial temporal lobe of patients with dementia with Lewy bodies. *Neurosci Lett,* **227,** 193-196.

Eisele, Y. S., Bolmont, T., Heikenwalder, M. et al. (2009) Induction of cerebral β-amyloidosis: intracerebral versus systemic Abeta inoculation. *Proc Natl Acad Sci U S A,* **106,** 12926-12931.

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E. et al. (2003) α-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J,* **17,** 1945-1947.

Gajdusek, D. C. (1994) Spontaneous generation of infectious nucleating amyloids in the transmissible and nontransmissible cerebral amyloidoses. *Mol Neurobiol,* **8,** 1-13.

Hamilton, R. L. (2000) Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using α-synuclein immunohistochemistry. *Brain Pathol,* **10,** 378-384.

Hasegawa, K., Yamaguchi, I., Omata, S., Gejyo, F. and Naiki, H. (1999) Interaction between Aβ(1-42) and Aβ(1-40) in Alzheimer's β-amyloid fibril formation in vitro. *Biochemistry,* **38,** 15514-15521.

Jarrett, J. T. and Lansbury, P. T., Jr. (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell,* **73,** 1055-1058.

Kim, H. Y., Cho, M. K., Kumar, A. et al. (2009) Structural properties of pore-forming oligomers of α-synuclein. *J Am Chem Soc,* **131,** 17482-17489.

Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B. and Olanow, C. W. (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med,* **14,** 504-506.

Kurata, T., Kawarabayashi, T., Murakami, T. et al. (2007) Enhanced accumulation of phosphorylated α-synuclein in double transgenic mice expressing mutant β-amyloid

precursor protein and presenilin-1. *J Neurosci Res,* **85,** 2246-2252.

Langer, F., Eisele, Y. S., Fritschi, S. K., Staufenbiel, M., Walker, L. C. and Jucker, M. (2011) Soluble Aβ seeds are potent inducers of cerebral β-amyloid deposition. *J Neurosci,* **31,** 14488-14495.

LeVine, H., 3rd (1999) Quantification of β-sheet amyloid fibril structures with thioflavin T. *Methods Enzymol,* **309,** 274-284.

Li, J. Y., Englund, E., Holton, J. L. et al. (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med,* **14,** 501-503.

Lippa, S. M., Lippa, C. F. and Mori, H. (2005) α-Synuclein aggregation in pathological aging and Alzheimer's disease: the impact of β-amyloid plaque level. *Am J Alzheimers Dis Other Demen,* **20,** 315-318.

Lucking, C. B. and Brice, A. (2000) α-synuclein and Parkinson's disease. *Cell Mol Life Sci,* **57,** 1894-1908.

Mandal, P. K., Pettegrew, J. W., Masliah, E., Hamilton, R. L. and Mandal, R. (2006) Interaction between Aβ peptide and α synuclein: molecular mechanisms in overlapping pathology of Alzheimer's and Parkinson's in dementia with Lewy body disease. *Neurochem Res,* **31,** 1153-1162.

Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M. and Mucke, L. (2001) β-amyloid peptides enhance α-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc Natl Acad Sci U S A,* **98,** 12245-12250.

Meyer-Luehmann, M., Coomaraswamy, J., Bolmont, T. et al. (2006) Exogenous induction of cerebral β-amyloidogenesis is governed by agent and host. *Science,* **313,** 1781-1784.

Ono, K., Condron, M. M., Ho, L., Wang, J., Zhao, W., Pasinetti, G. M. and Teplow, D. B. (2008) Effects of grape seed-derived polyphenols on amyloid β-protein self-assembly and cytotoxicity. *J Biol Chem,* **283,** 32176-32187.

Ono, K., Condron, M. M. and Teplow, D. B. (2009) Structure-neurotoxicity relationships of amyloid β-protein oligomers. *Proc Natl Acad Sci U S A,* **106,** 14745-14750.

Ono, K., Condron, M. M. and Teplow, D. B. (2010) Effects of the English (H6R) and Tottori (D7N) familial Alzheimer disease mutations on amyloid β-protein assembly and toxicity. *J Biol Chem,* **285,** 23186-23197.

Ono, K., Ikeda, T., Takasaki, J. and Yamada, M. (2011a) Familial Parkinson disease mutations influence α-synuclein assembly. *Neurobiol Dis,* **43,** 715-724.

Ono, K., Mochizuki, H., Ikeda, T., Nihira, T., Takasaki, J. I., Teplow, D. B. and Yamada, M. (2011b) Effect of melatonin on α-synuclein self-assembly and cytotoxicity. *Neurobiol Aging*, in press.

Ono, K. and Yamada, M. (2006) Antioxidant compounds have potent anti-fibrillogenic and fibril-destabilizing effects for α-synuclein fibrils in vitro. *J Neurochem,* **97,** 105-115.

Ono, K. and Yamada, M. (2011) Low-n oligomers as therapeutic targets of Alzheimer's disease. *J Neurochem,* **117,** 19-28.

Ono, K., Yoshiike, Y., Takashima, A., Hasegawa, K., Naiki, H. and Yamada, M. (2003) Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. *J Neurochem,* **87,** 172-181.

Pletnikova, O., West, N., Lee, M. K., Rudow, G. L., Skolasky, R. L., Dawson, T. M., Marsh, L. and Troncoso, J. C. (2005) Aβ deposition is associated with enhanced cortical α-synuclein lesions in Lewy body diseases. *Neurobiol Aging,* **26,** 1183-1192.

Steinerman, J. R., Irizarry, M., Scarmeas, N., Raju, S., Brandt, J., Albert, M., Blacker, D., Hyman, B. and Stern, Y. (2008) Distinct pools of β-amyloid in Alzheimer disease-affected brain: a clinicopathologic study. *Arch Neurol,* **65,** 906-912.

Tokuda, T., Qureshi, M. M., Ardah, M. T. et al. (2010) Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease. *Neurology,* **75,** 1766-1772.

Tsigelny, I. F., Crews, L., Desplats, P. et al. (2008) Mechanisms of hybrid oligomer formation in the pathogenesis of combined Alzheimer's and Parkinson's diseases. *PLoS One,* **3,** e3135.

Walsh, D. M., Tseng, B. P., Rydel, R. E., Podlisny, M. B. and Selkoe, D. J. (2000) The oligomerization of amyloid β-protein begins intracellularly in cells derived from human brain. *Biochemistry,* **39,** 10831-10839.

Wood, S. J., Wetzel, R., Martin, J. D. and Hurle, M. R. (1995) Prolines and amyloidogenicity in fragments of the Alzheimer's peptide β/A4. *Biochemistry,* **34,** 724-730.

Wood, S. J., Wypych, J., Steavenson, S., Louis, J. C., Citron, M. and Biere, A. L. (1999) α-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of

Parkinson's disease. *J Biol Chem,* **274,** 19509-19512.

Yang, F., Ueda, K., Chen, P., Ashe, K. H. and Cole, G. M. (2000) Plaque-associated α-synuclein (NACP) pathology in aged transgenic mice expressing amyloid precursor protein. *Brain Res,* **853,** 381-383.

Yoshimoto, M., Iwai, A., Kang, D., Otero, D. A., Xia, Y. and Saitoh, T. (1995) NACP, the precursor protein of the non-amyloid β/A4 protein (Aβ) component of Alzheimer disease amyloid, binds Aβ and stimulates Aβ aggregation. *Proc Natl Acad Sci U S A,* **92,** 9141-9145.

Figure Legends

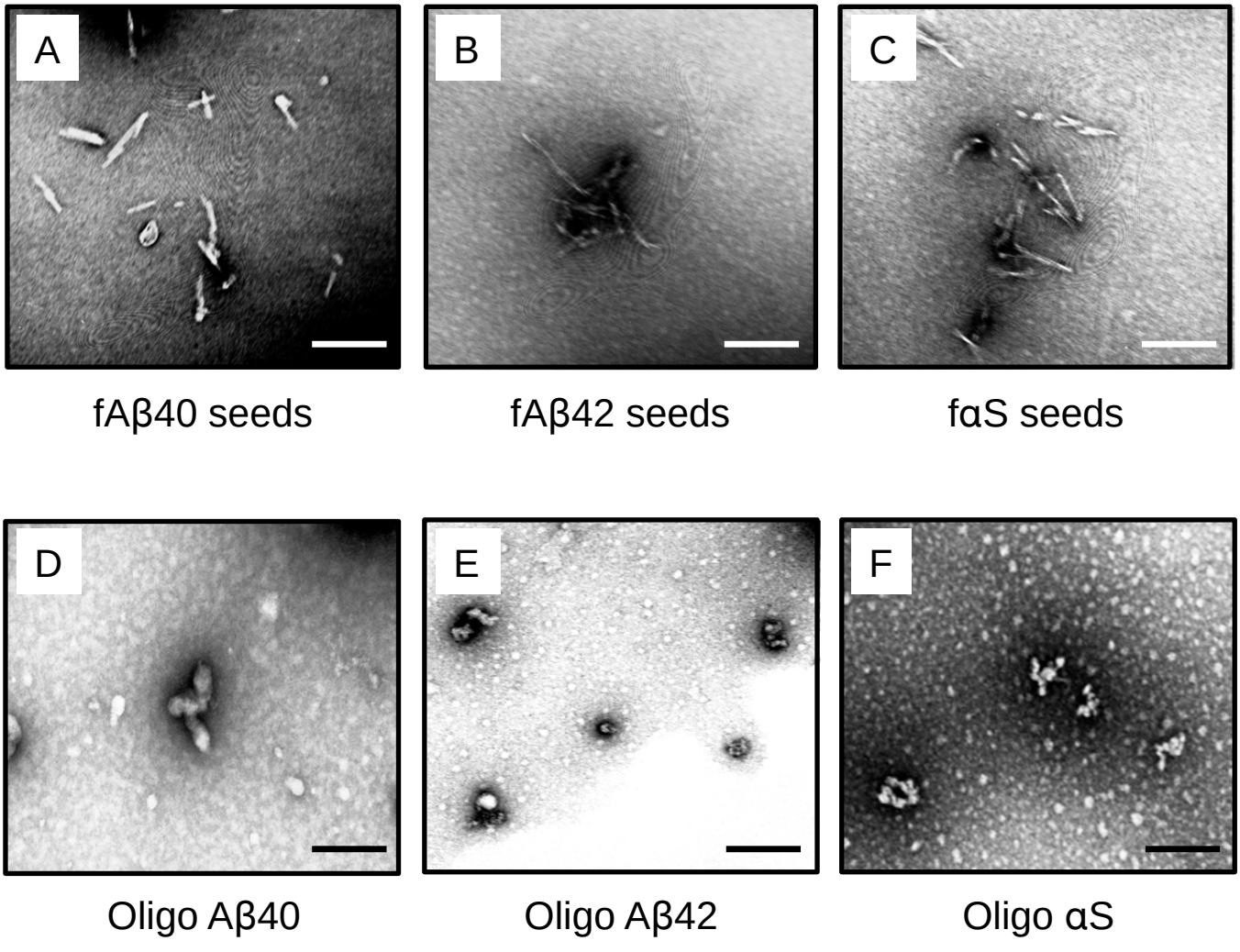
Fig. 1. Morphology of sonicated fibrils and oligomers of Aβ40, Aβ42, and αS. EM was used to determine the morphologies of fAβ40 (A), fAβ42 (B), fαS (C), oligo Aβ40 (D), oligo Aβ42 (E), and oligo αS (F). Scale bars indicate 100 nm.

Fig. 2. Cross seeding effects of Aβ40 with Aβ42 or αS. (A) ThT binding. A 25-μM Aβ40 solution was incubated without (◇) or with 10% (v/v) fAβ40 (○), fAβ42 (△), fαS (□), cross-linked oligomers (oligo) of Aβ40 (●), Aβ42 (▲), or αS (□). Binding is expressed as the mean fluorescence (FU) ± S.E. (B-H) Aβ40 assembly morphology. EM was used to determine the morphologies of assemblies of Aβ40 without (B) or with fAβ40 (C), fAβ42 (D), fαS (E), oligo Aβ40 (F), oligo Aβ42 (G), or oligo αS (H). Scale bars indicate 100 nm.

Fig. 3. Cross seeding effects of Aβ42 with Aβ40 or αS. (A) ThT binding. A 25-μM Aβ42 solution was incubated without (◇) or with 10% (v/v) fAβ42 (△), fAβ40 (○), fαS (□), oligo Aβ42 (▲), oligo Aβ40 (●), or oligo αS (□). Binding is expressed as the mean fluorescence (FU) ± S.E. (B-H) Aβ42 assembly morphology. EM was used to determine the morphologies of assemblies of Aβ42 without (B) or with fAβ42 (C), fAβ40 (D), fαS (E), oligo Aβ42 (F), oligo Aβ40 (G), or oligo αS (H). Scale bars indicate 100 nm.

Fig. 4. Cross seeding effects of αS with Aβ40 or Aβ42. (A) ThS binding. A 25-μM αS solution was incubated without (◇) or with 10% (v/v) fαS (□), fAβ40 (○), fAβ42 (△), oligo αS (□), oligo Aβ40 (●), or oligo Aβ42 (▲). Binding is expressed as the mean fluorescence (FU) ± S.E. (B-H) αS assembly morphology. EM was used to determine the morphologies of assemblies of αS without (B) or with fαS (C), fAβ40 (D), fAβ42 (E), oligo αS (F), oligo Aβ40 (G), or oligo Aβ42 (H). Scale bars indicate 100 nm.

Fig. 1.

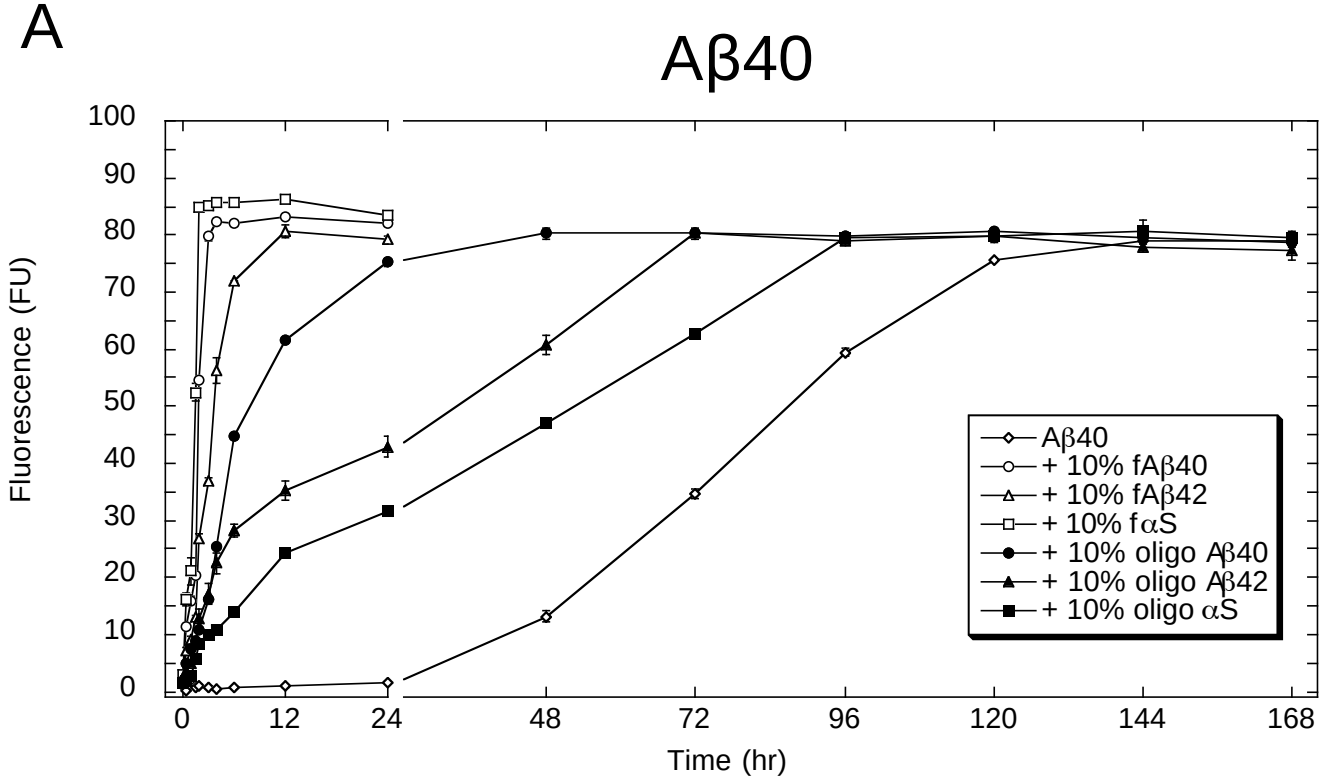


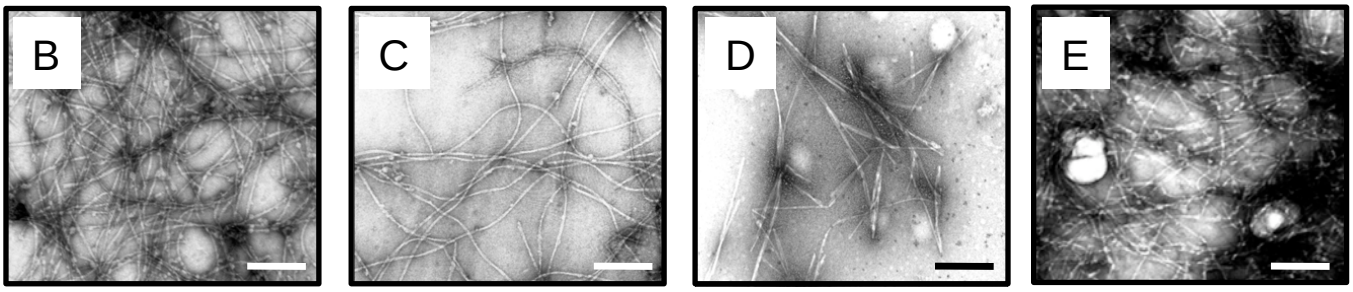
fAβ40 seeds fAβ42 seeds fαS seeds

Oligo Aβ40 Oligo Aβ42 Oligo αS

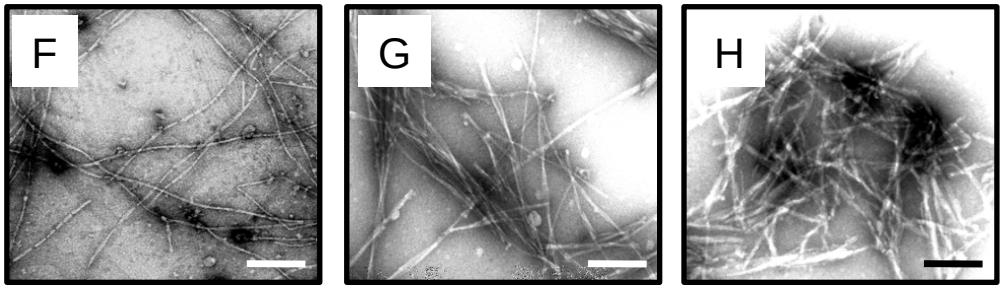
Fig. 2.

A





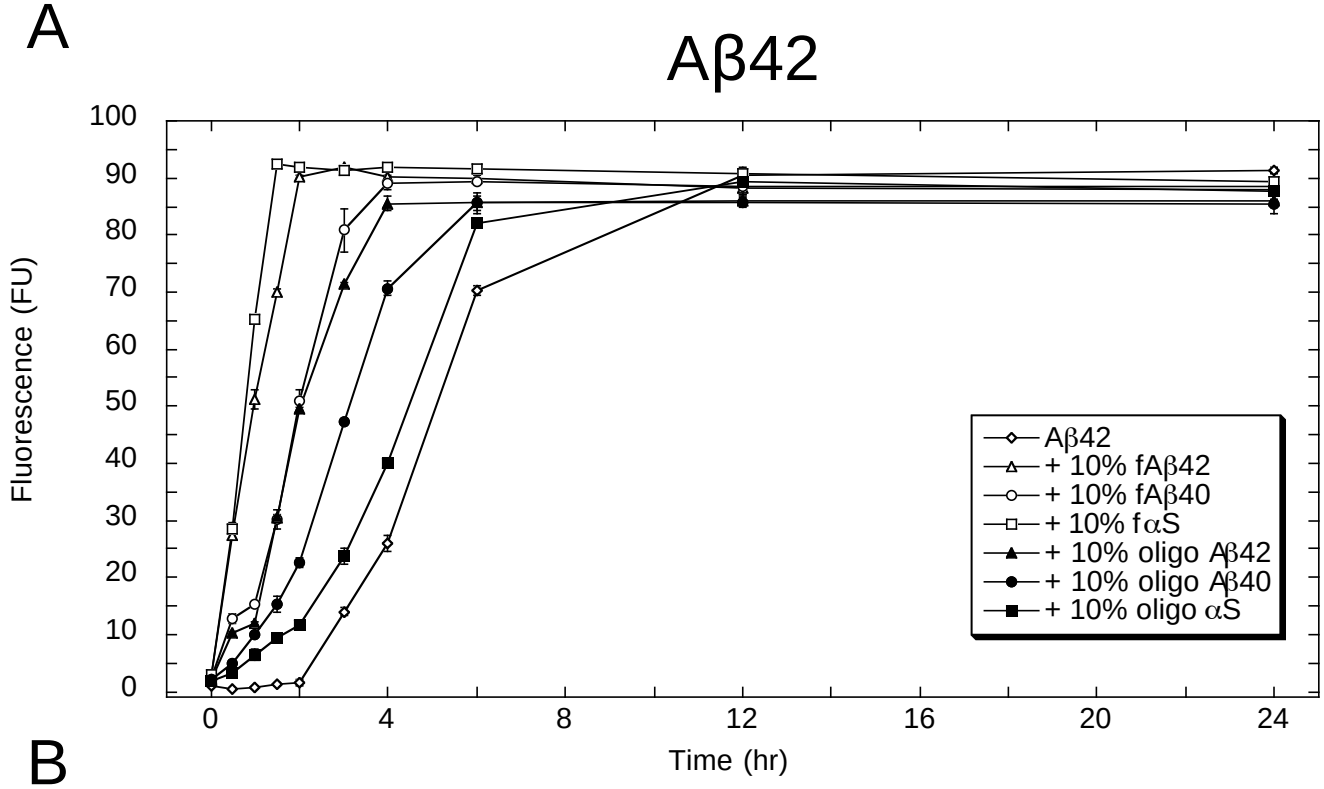
Aβ40 | Aβ40 + fAβ40 | Aβ40 + fAβ42 | Aβ40 + fαS



Aβ40 + oligo Aβ40 | Aβ40 + oligo Aβ42 | Aβ40 + oligo αS

Fig. 3.

A


Aβ42
Fluorescence (FU)
Time (hr)
Aβ42
+ 10% fAβ42
+ 10% fAβ40
+ 10% fαS
+ 10% oligo Aβ42
+ 10% oligo Aβ40
+ 10% oligo αS

B

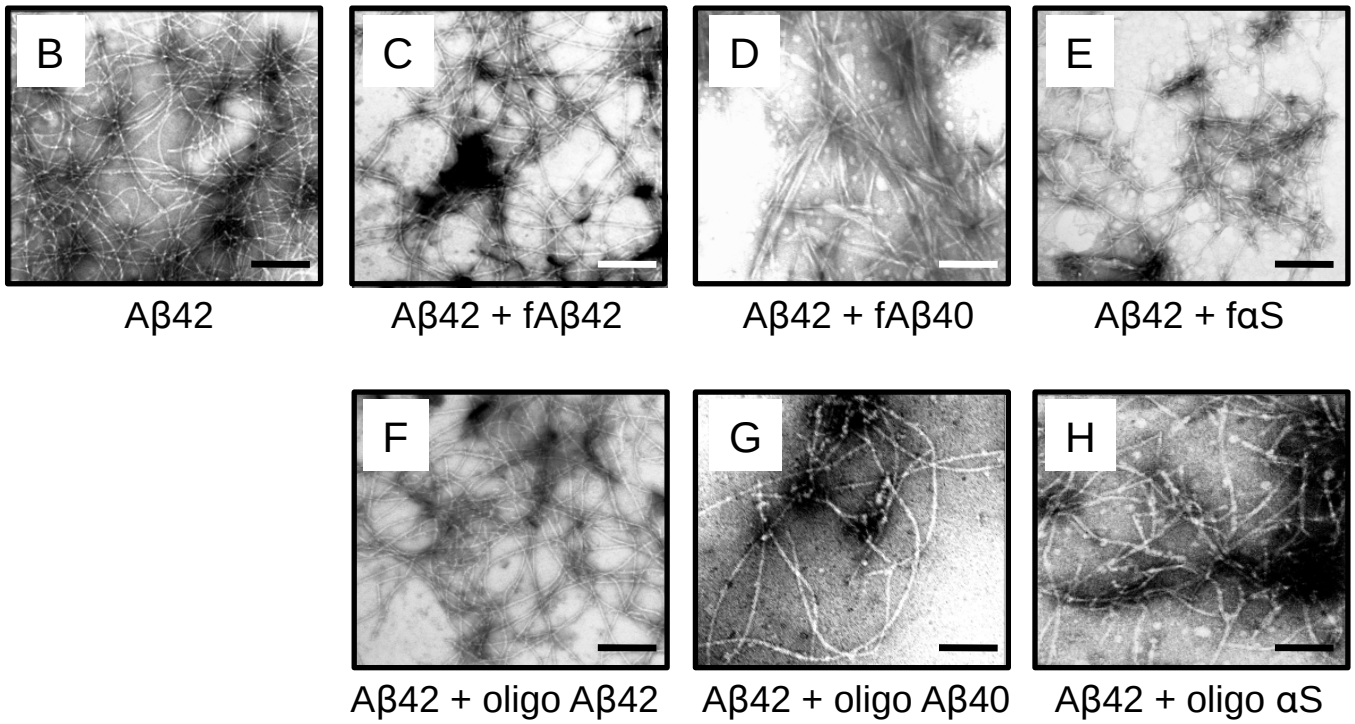
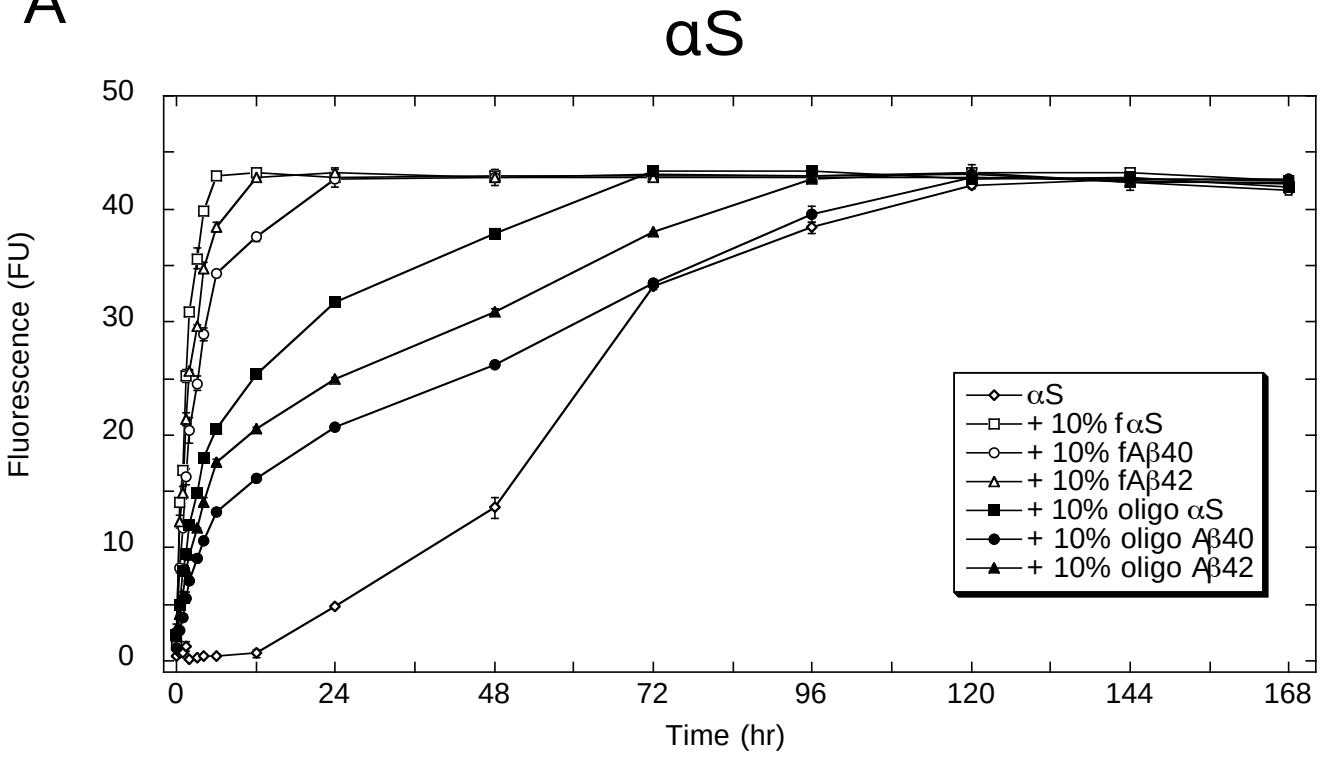
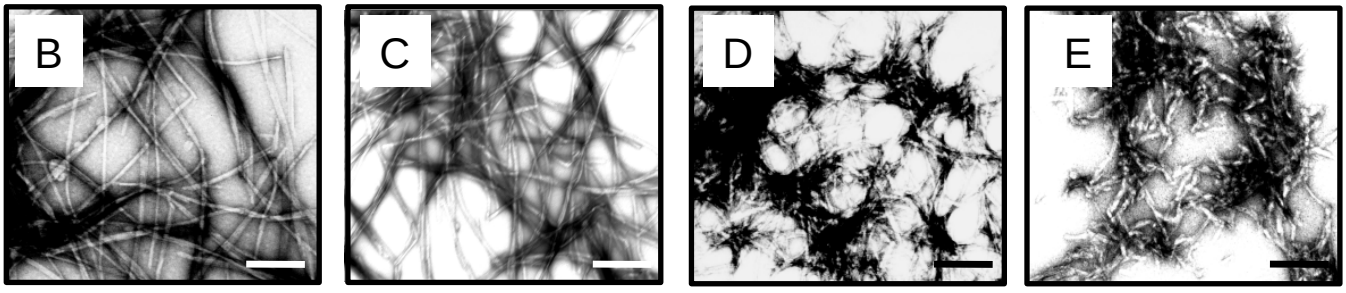

B
C
D
E
F
G
H
Aβ42
Aβ42 + fAβ42
Aβ42 + fAβ40
Aβ42 + fαS
Aβ42 + oligo Aβ42
Aβ42 + oligo Aβ40
Aβ42 + oligo αS

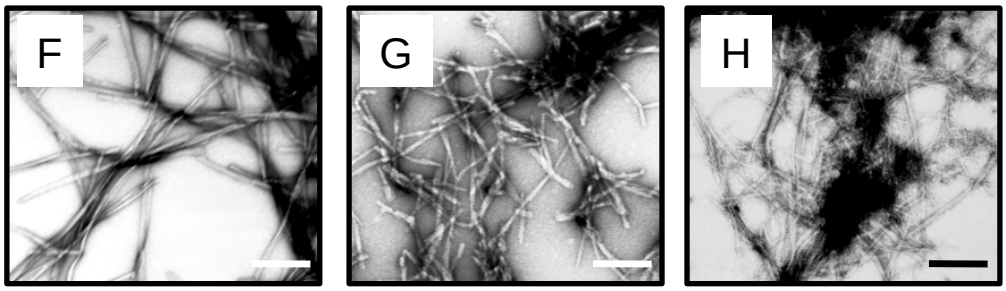
Fig. 4.

A





αS αS + fαS αS + fAβ40 αS + fAβ42



αS + oligo αS αS + oligo Aβ40 αS + oligo Aβ42