Supplementary information

Characterization of Aβ samples

In our Aβ40 and Aβ42 low molecular weight species (LMWS) samples, the lack of large aggregates was confirmed by transmission electron microscopy (TEM) (Fig S2). Aβ40 and Aβ42 LMWS samples displayed no significant fluorescence when mixed with Thioflavin T (ThT) (data not shown), a dye exhibiting fluorescence upon binding to fibrillar and protofibrillar Aβ aggregates [1-4]. Aβ40 and Aβ42 LMWS samples were not recognized by an antibody A11 (data not shown), known to recognize specific conformational structures found in soluble Aβ oligomers[5]. The presence of largely irregular structures in Aβ40 and Aβ42 LMWS samples was confirmed by CD spectroscopy (Fig S5).

Aβ40 soluble oligomer samples contained predominantly long protofibrils (Fig S2) and were recognized by A11 in the dot blot assay[6]. ThT fluorescence intensities displayed by these samples were ~ 17% of those displayed by Aβ40 fibrils at the same mass concentrations. Size exclusion chromatography (SEC) analyses indicated that a major fraction (~80%) of these Aβ40 oligomer samples at 23 μM was oligomeric (>70 kDa) with the minor fraction (~20%) consisting of Aβ40 LMWS [6].

The primary Aβ42 soluble oligomer samples consisted of small globular and short protofibrillar species (Fig S2). These Aβ42 oligomers were not recognized by A11 (data not shown) and this finding is consistent with the previous A11 tests with Aβ42 oligomer samples prepared similarly [7-8]. A predominant fraction (~70%) of these Aβ42 oligomer samples at 23 μM was oligomeric (>70 kDa) with the remainder comprising Aβ42 LMWS, as determined by SEC. ThT fluorescence intensities displayed by these Aβ42 oligomer samples were ~23% of those displayed by Aβ42 fibrils at the same mass concentration. The

secondary Aβ42 soluble oligomer samples contained slightly longer protofibrils displaying a greater morphological homogeneity, when compared to the primary Aβ42 oligomer samples (compare Figs S2 and S3). The secondary Aβ42 oligomer samples displayed β sheet structures (Fig S5) and were A11-positive (data not shown). The primary and secondary Aβ42 oligomer samples exhibited similar population profiles at 23 μM: ~70 and ~30 % of Aβ molecules existed at oligomeric and low molecular weight (LMW) states, respectively, as determined by SEC. The secondary Aβ42 oligomer samples displayed ThT fluorescence intensities, which corresponded to ~ 35 % of those displayed by Aβ42 fibrils at the same mass concentration.

Aβ40 and Aβ42 insoluble fibril samples exhibited much higher (>3-fold) ThT fluorescence intensities compared to the other Aβ samples, and were A11-negative (data not shown). The presence of mature fibrils in these samples was confirmed by TEM (Fig S2).

Characterization of PG44 oligomers at 10 μM.

When freshly prepared, PG44 in solution was found to exist mostly (90%) oligomeric (>70 kDa) at 10 μM (which is a monomer-equivalent concentration), as determined by SEC (see Fig 2). This size of PG44 oligomers corresponds to > 14-mers of monomeric PG44. No visible precipitate was detected from PG44 solutions at 10 μM for at least 2 hrs. PG44 oligomers formed at 10 μM were found to be dissociated into predominantly monomers when SDS was added to solutions, as determined by SDS-PAGE (data not shown), indicating that oligomeric structures formed by PG44 at this concentration were SDS-labile.

Note that 10 μM in monomer-equivalent concentration of PG44 in solution represents < 0.7 μM in oligomer-equivalent concentration given consideration of the apparent molecular weight (i.e., >70 kDa)

of PG44 oligomers. When FlAsH fluorescence of PG44 at 10 μM was measured with increasing concentrations of FlAsH, fluorescence intensities increased and then leveled off at ~0.8 μM of FlAsH in the presence and absence of Aβ oligomers at 23 μM (data not shown). These findings indicate that the majority of tetra-cysteine motifs in signal domains of PG44 at 10 μM in monomer-equivalent concentration were not accessible to FlAsH whether or not Aβ oligomers were co-present in solution, presumably because of the oligomeric nature of PG44 at this concentration.

Since PG44 at 10 μM in monomer-equivalent concentration was predominantly oligomeric, multiple tetra-cysteine motifs might be present in close proximity at this concentration. As such, a single FlAsH molecule might be bound to four proximal cysteine residues originated from multiple PG44 molecules. To examine whether FlAsH is covalently bound to a single or multiple PG44 molecule(s), oligomeric PG44 solutions with or without 1.6 μM of FlAsH were run on SDS-PAGE. Oligomeric PG44 dissociated into predominantly monomers in SDS-PAGE as described above and no significant difference was observed whether FlAsH was present or absent in PG44 solutions at 10 μM (data not shown). This result indicates the lack of significant inter-molecular covalent association among PG44 molecules mediated by FlAsH under our experimental setup. Similar findings were reported with PG46 oligomers[6]. Our observations with PG44 and PG46 are also consistent with the previous findings that binding affinity of FlAsH to a continuous tetra-cysteine motif is greater (>10-fold) than discontinuous tetra-cysteine motifs under a similar experimental condition (i.e., in the presence of 1 mM tris(2-carboxyethyl)phosphine (TCEP)+100 μM 1,2-ethanedithiol (EDT))[9]. Taken together, our results suggest that one FlAsH molecule was bound to a single, accessible PG44 or PG46 molecule.

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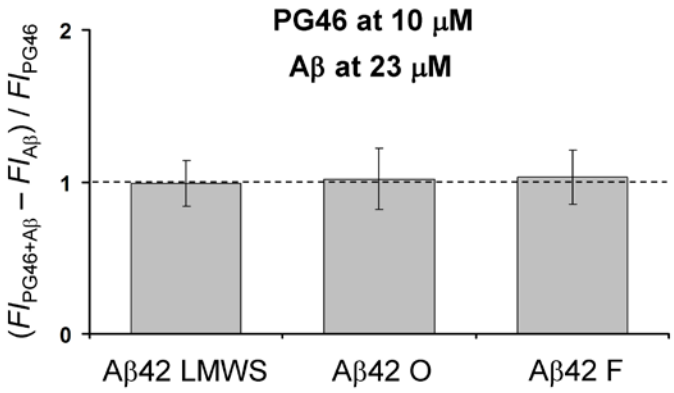

Figure S1. FlAsH fluorescence signals of PG46 when mixed with Aβ42 species in various aggregation states. Samples containing Aβ42 at 23 μM were mixed with PG46 at 10 μM followed by addition of FlAsH at 1.6 μM. Samples were excited with light at 508 nm and fluorescence intensities were measured at 536 nm. The data obtained were used for calculation of FlAsH fluorescence signals, i.e., the ratio of (FlAsH fluorescence intensity of a mixture of PG46 plus Aβ – FlAsH fluorescence intensity of Aβ only) / (FlAsH fluorescence intensity of PG46 only). As such, this ratio must be 1 for PG46 only samples. Note that the value of this ratio was 1.9 ± 0.2 for FlAsH fluorescence signals of PG46 when mixed with Aβ40 oligomers under similar conditions[6]. A dotted line for the value of this ratio = 1 is shown for better comparison. The errors were evaluated by the propagation of error method. LMWS, O and F represent low molecular weight species, soluble oligomers and insoluble fibrils, respectively. Molar concentrations of PG46 and Aβ are monomer-equivalent concentrations.

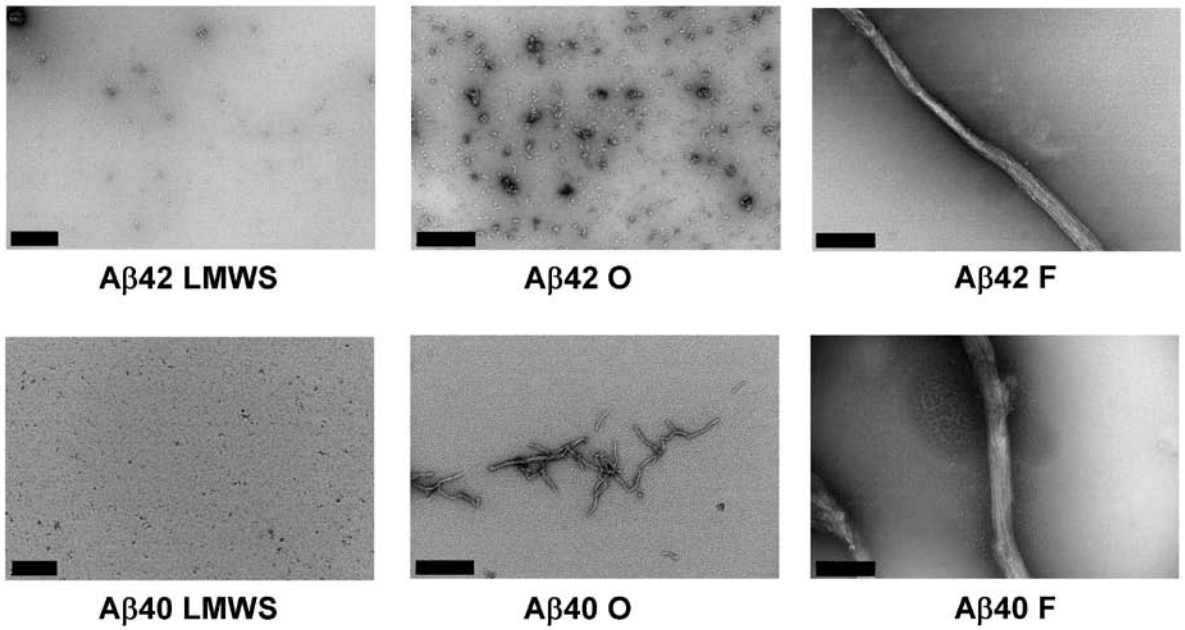

Figure S2. Representative transmission electron microscopy (TEM) images of Aβ42 and Aβ40 samples prepared *in vitro*. LMWS, O and F represent low molecular weight species, soluble oligomers and insoluble fibrils, respectively. Scale bars: 200 nm.

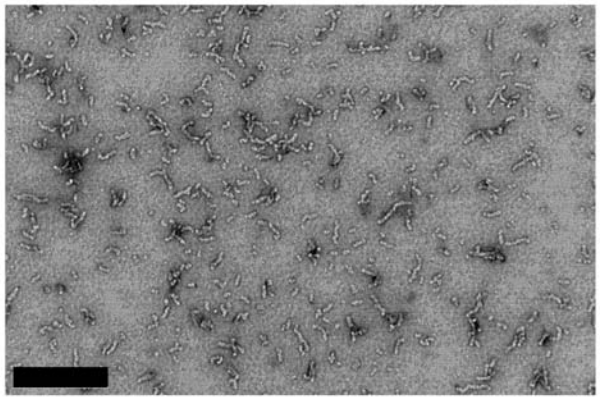

Figure S3. A representative TEM image of the secondary Aβ42 oligomer samples prepared *in vitro*. Scale bar: 200 nm

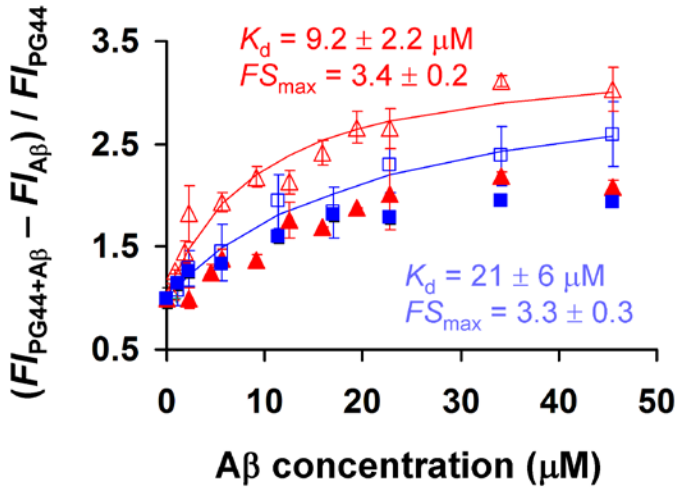

Figure S4. FlAsH fluorescence signals of PG44 at 1 μM (empty symbols) and 10 μM (filled symbols) as a function of concentrations of the primary Aβ42□ oligomers (red triangles) and secondary Aβ42□ oligomers (blue squares). Samples containing Aβ42 oligomers at a designated concentration were mixed with PG44 at 1 or 10 μM followed by addition of FlAsH at 1.6 μM. Samples were excited with light at 508 nm and fluorescence intensities were measured at 536 nm. The data obtained were used for calculation of FlAsH fluorescence signals, i.e., the ratio of (FlAsH fluorescence intensity of a mixture of PG44 plus Aβ – FlAsH fluorescence intensity of Aβ only) / (FlAsH fluorescence intensity of PG44 only). As such, this ratio must be 1 for PG44 only samples. The errors were evaluated by the propagation of error method. Values of the relative K_d and the maximal FlAsH fluorescence signals (FS_{max}) for detection by PG44 at 1 μM are also shown for the primary Aβ42 oligomers (red) and secondary Aβ42 oligomers (blue). Concentrations of PG44 and Aβ are monomer-equivalent molar concentrations.

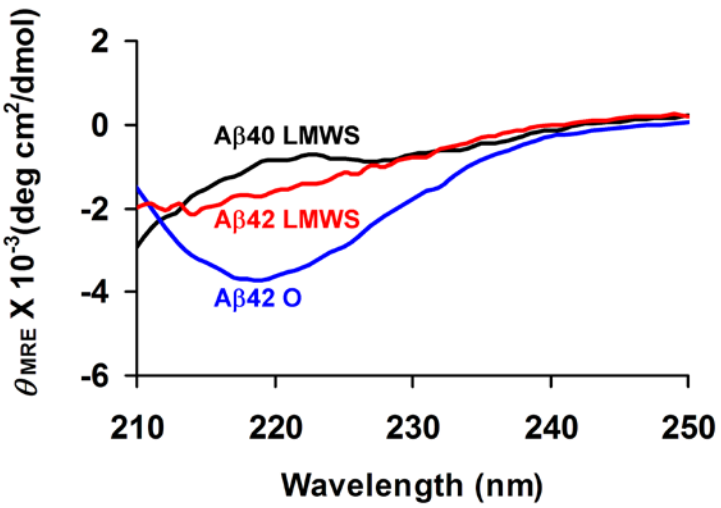

Figure S5. CD spectra of samples containing Aβ40 LMWS (black), Aβ42 LMWS (red) and the secondary Aβ42 oligomers (blue).