CONFORMATIONAL DIFFERENCES BETWEEN TWO AMYLOID β OLIGOMERS OF SIMILIAR SIZE AND DISSIMILAR TOXICITY

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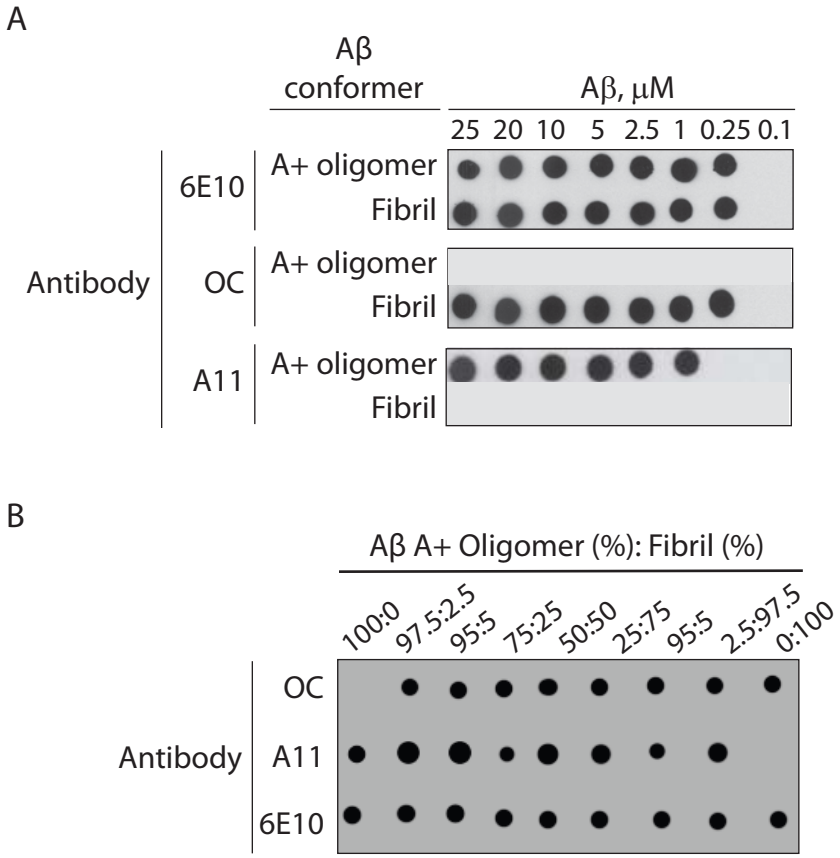

FIGURE S1. Conformation-specific antibody detection sensitivity of Aβ conformers. Aβ42 A+ oligomers and fibrils (25 μM) were deposited (A) alone or (B) as mixtures on nitrocellulose, and detected using conformation-specific antibodies (A11, prefibrillar oligomers; OC, fibrillar conformers) and a sequence-specific antibody (6E10, N-terminus of Aβ).

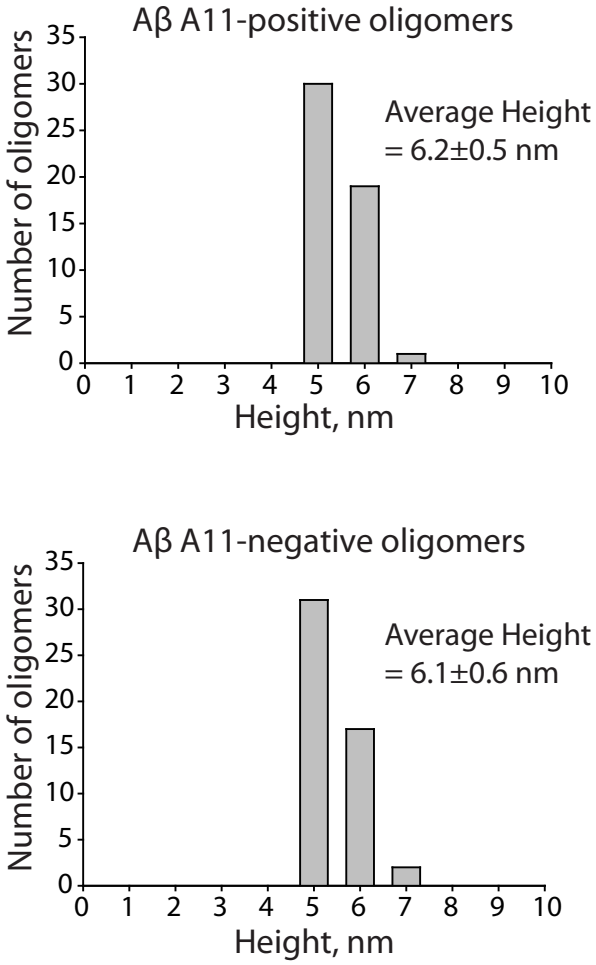

FIGURE S2. Height distributions of Aβ oligomers. Aβ42 oligomers (25 μM) were assembled without agitation, deposited on mica substrates, imaged using AFM, and the heights were measured using MFP 3D software (Asylum Research).

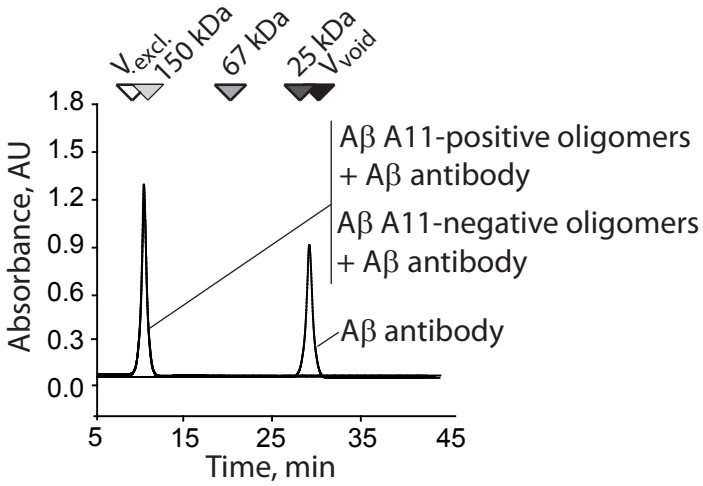

Figure S3. SEC analysis of Aβ oligomers. Aβ42 oligomers (25 μM) were complexed with a domain antibody specific for Aβ, and analyzed using size-exclusion chromatography.

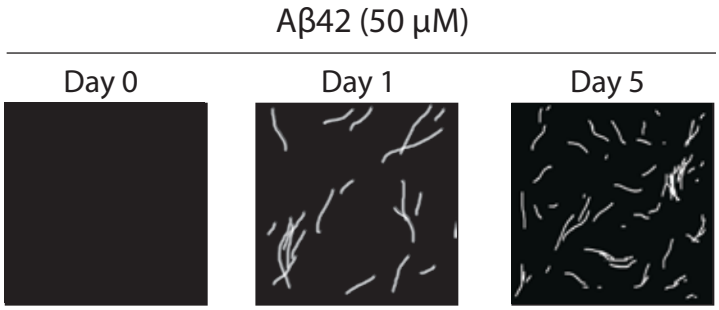

FIGURE S4. AFM analysis of Aβ fibrillization. Aβ42 (50 μM) was assembled without agitation, deposited on mica substrates, and imaged using AFM. Each image is 3x3 μm.

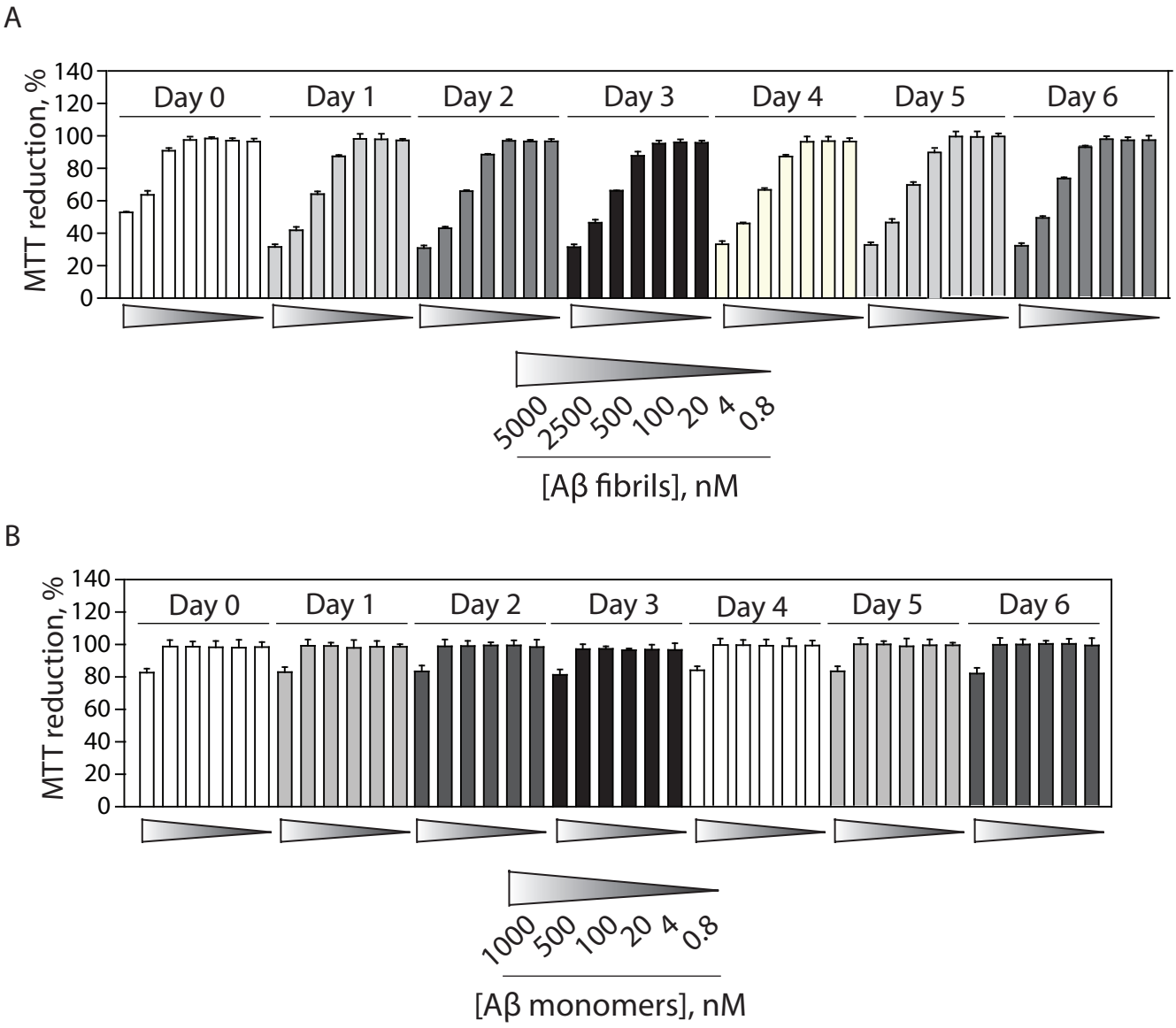

FIGURE S5. Toxicity analysis of Aβ monomers and fibrils. Aβ42 was assembled at (A) 50 μM and (B) 10 μM without agitation, added to PC12 cells at a range of Aβ concentrations (0.8-5000 nM), and the relative toxicity was evaluated (n=3).

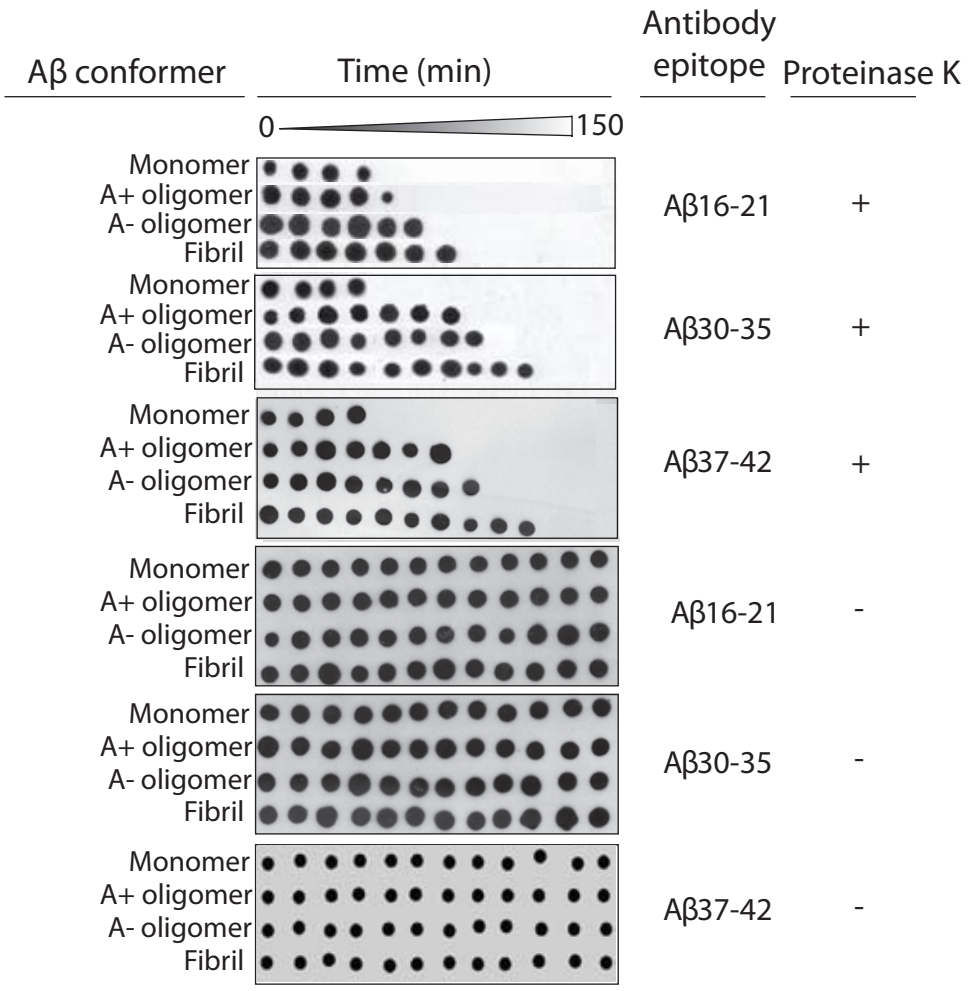

FIGURE S6. Rate of proteolytic fragmentation of Aβ peptide segments within Aβ oligomers. Aβ42 (25 μM) was incubated with Proteinase K (0.5 μg/mL), deposited periodically on nitrocellulose (every 10-30 min), and probed with antibodies specific for central (Aβ16-21) and C-terminal (Aβ30-35 and Aβ37-42) Aβ peptide segments. The time intervals were 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120 and 150 min.

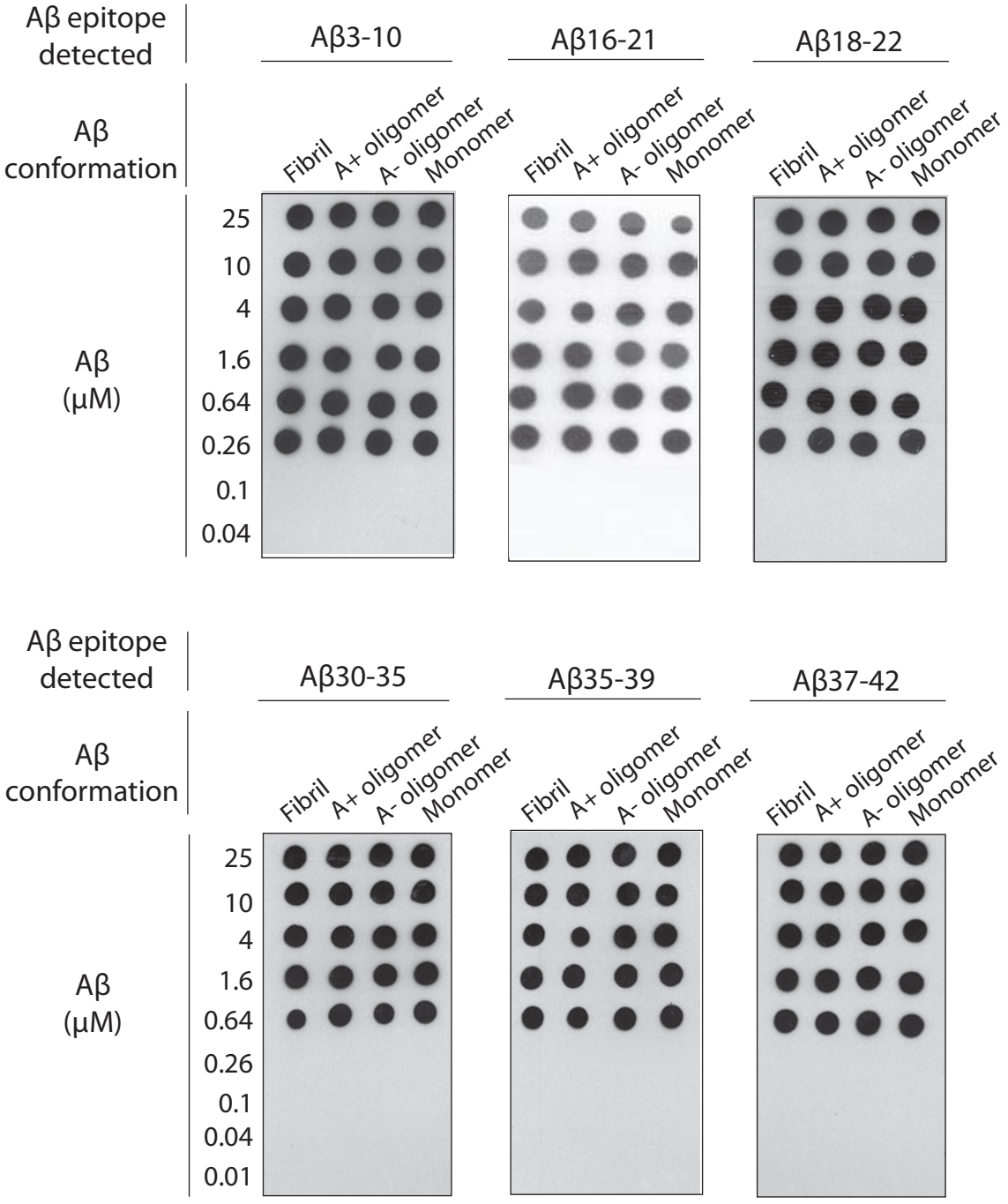

FIGURE S7. Sequence-specific antibody detection sensitivity of Aβ conformers. Aβ42 (25 μM) was diluted and deposited on nitrocellulose, and then detected using sequence-specific antibodies against epitopes distributed throughout Aβ.

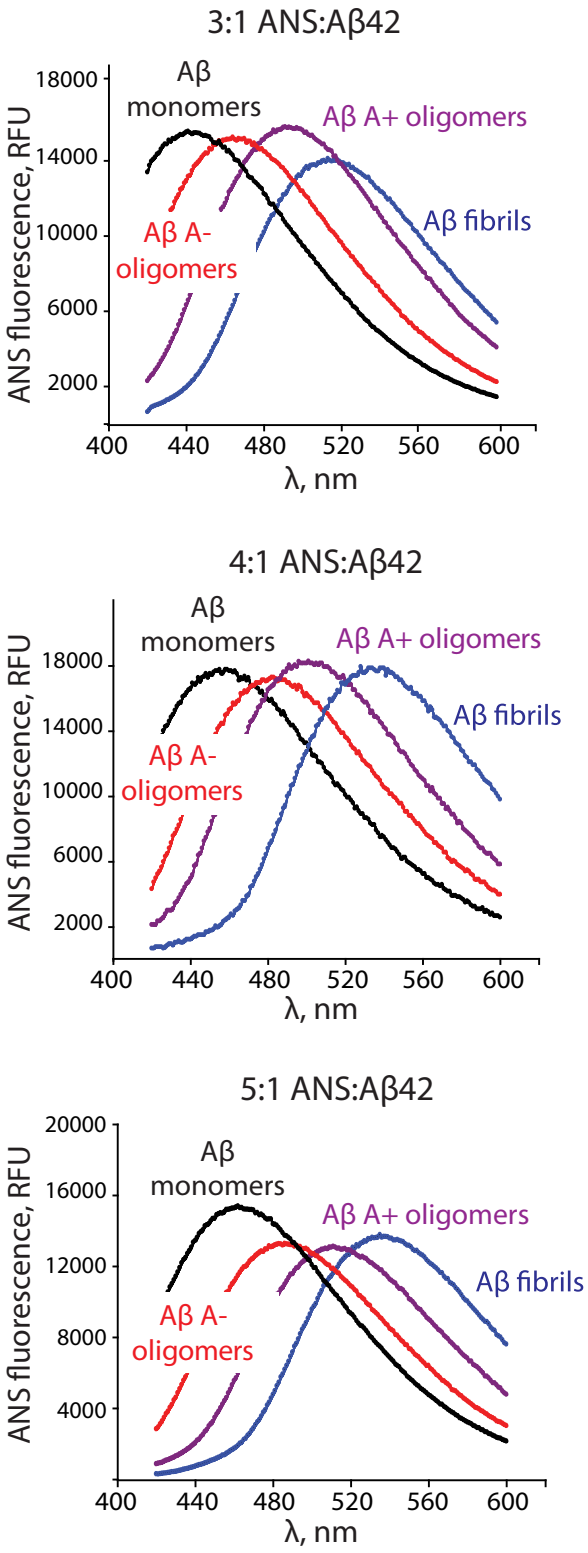

FIGURE S8. ANS fluorescence analysis of Aβ conformers. Aβ42 (2.5 μM) was combined with 8-anilinonaphthalene-1-sulfonate (ANS) in three molar ratios (3:1 to 5:1 ANS:Aβ), and the fluorescence spectra of each Aβ conformer was evaluated. The excitation wavelength was 380 nm.

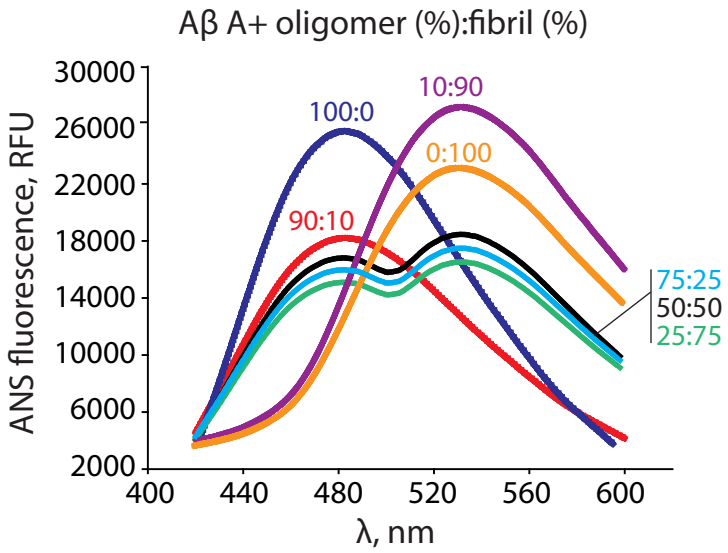

FIGURE S9. ANS fluorescence analysis of the homogeneity of Aβ conformers. Aβ42 A+ oligomers and fibrils were combined in different ratios, and their ANS spectra was measured (excitation at 380 nm). The molar ratio of ANS:Aβ was 4:1.

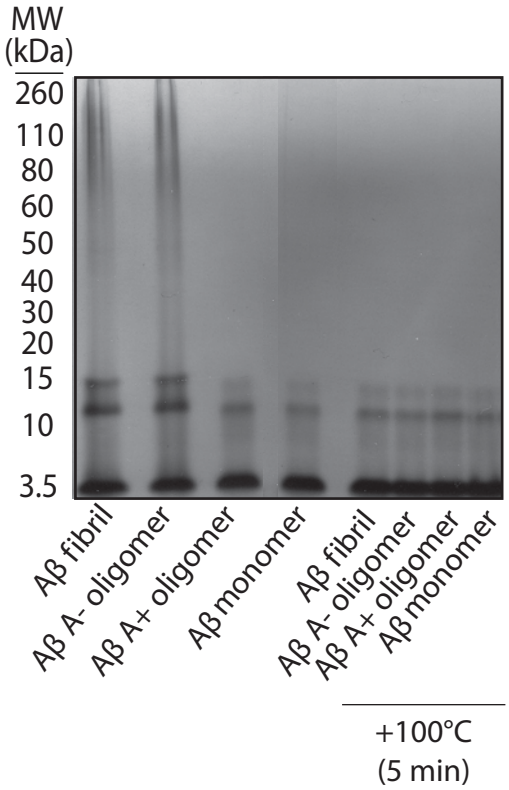

FIGURE S10. SDS-PAGE analysis of Aβ conformers. Aβ42 (25 μM) was added to LDS sample buffer, and analyzed via SDS-PAGE (10% bis-tris gels). As a loading control, each Aβ conformer was heated (100 °C, 5 min) prior to SDS-PAGE analysis. Aβ was visualized via silver stain.