Electronic Supplementary Material (ESI) for Molecular BioSystems.

SUPPLEMENTARY INFORMATION

Engineering of a peptide probe for β-amyloid aggregates

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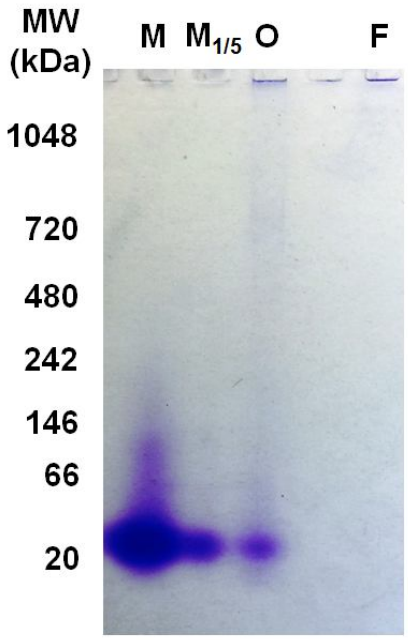

Fig. S1 Native-PAGE of Aβ monomers (M), oligomers (O) and fibrils (F) loaded at 8 μg each. Numbers indicate positions of various molecular weight standard proteins. Note that migration of a protein in a native-PAGE gel depends not only on molecular weight, but also charge and conformation of the protein, and therefore accurate molecular weight determination of a protein using native-PAGE is difficult. In the lane labeled with $M_{1/5}$, 1.6 μg of Aβ monomer samples were loaded. Comparison of M and $M_{1/5}$ lanes demonstrates the presence of Aβ oligomeric contaminants in Aβ monomer samples particularly when a larger mass of Aβ was prepared. Comparison of M and O lanes indicates that ≤ ~ 20 % of Aβ oligomer samples consisted of Aβ monomers.

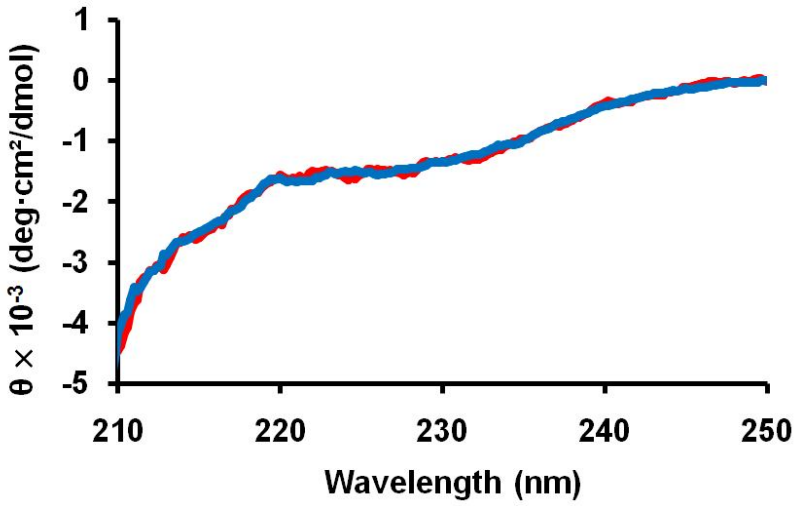

Fig. S2 Circular Dichroism (CD) of Aβ monomer samples at 50 μM (red) and 100 μM (blue) measured at room temperature.

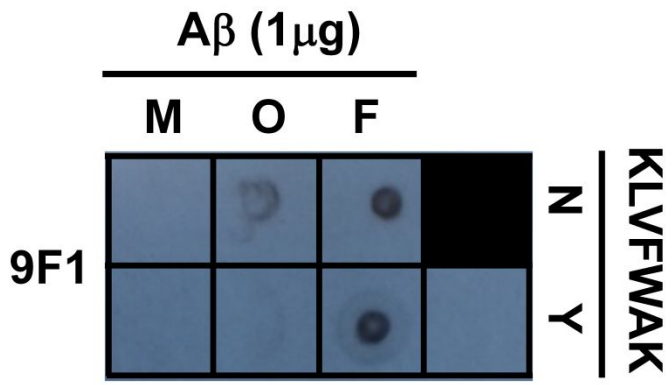

Fig. S3 Dot blot assays of Aβ samples (monomers (M), oligomers (O) and fibrils (F) in the first, second and third columns, respectively) in the absence (N, the first row) and presence (Y, the second row) of FITC-KLVFWAK using 9F1. The results shown here and Fig. 5 for competitive binding assays with an antibody 9F1 represent those obtained from two independent experiments. The molar concentration ratio of [Aβ]/[KLVFWAK] = 1:10 for the second row.

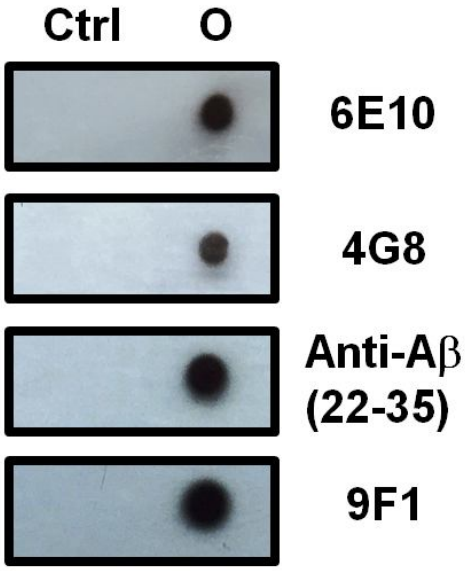

Fig. S4 A11 dot blot assays of Aβ oligomer (O) samples after competitive binding assays. Aβ oligomer samples were first blotted onto membranes for competitive binding assays with sequence specific antibodies (i.e., 6E10, 4G8, Anti-Aβ (22-35) or 9F1). Then, membranes were incubated in stripping buffers to remove sequence specific antibodies, followed by additional incubation with an amyloid oligomer-specific antibody A11. Subsequently, chemiluminescent signals were developed using alkaline phosphatase-conjugated secondary antibodies. As negative controls (Ctrl), Aβ monomer samples were blotted and treated in the same fashion in each membrane. Each panel is labeled with a name of sequence-specific antibody used for competitive binding assays prior to A11 dot blot assays.

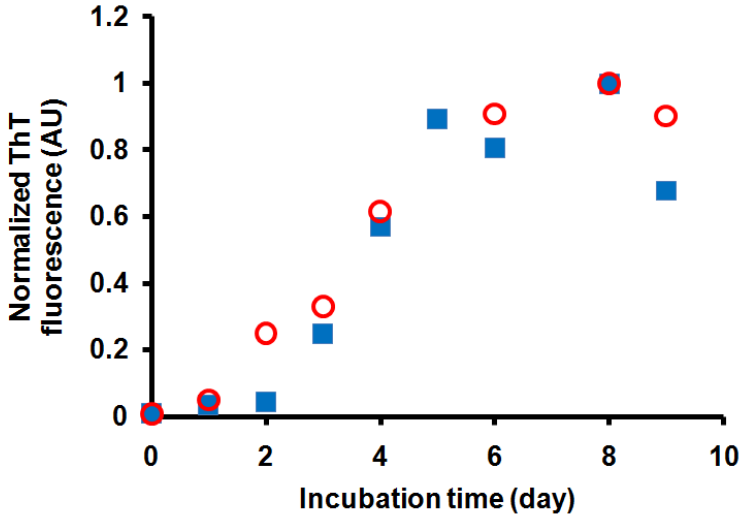

Fig. S5 Aggregation kinetics of two Aβ samples independently prepared at 50 μM monitored by ThT fluorescence. Aβ samples were incubated at 37 ^{o}C under a quiescent condition and aliquots withdrawn at designated time points during incubation.