Supplementary Information:

FRET detection of amyloid β-peptide oligomerization using a fluorescent protein probe presenting a pseudo-amyloid structure

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Experimental Section

Preparation of CPCYAB4: The gene encoding superfolder GFP (sfGFP) containing 8 mutations (S30R, Y39N, F64L, S65T, N105T, Y145F, I171V, A206V) was constructed by PCR from pGFPuv (Clonthech). The products were inserted into the *Nde*I and *Eco*RI sites of pET22b(+) (Novagen) to generate the plasmid, pET22-sfGFP. The plasmids encoding cyan and yellow variants, pET22-CFP and pET22-YFP, containing Y66W and N147I mutations and S65G, V68L, Q69K, S72A, and T203Y mutations, respectively, were also constructed by PCR from pET22-sfGFP. The plasmids, pET22-cfP13H and pET22-yfP13H, encoding the engineered fluorescent proteins presenting pseudo-Aβ β-sheet surfaces with L15K, E17V, D19F, D21E, T118H, V120K, R122V, E124F, and K126E mutations were constructed by PCR from pET22-CFP and pET22-YFP. To generate the circular permutant of the engineered YFP, the plasmid, pET22-yfP13H was used as a template for the PCR reaction. The DNAs, dN1-172 and dC173-238, encoding N- and C-terminal fragments corresponding to residues 1-172 and 173-238, respectively, of the fluorescent proteins were amplified by PCR with NF1_Kpn (5'-GGT AGA AGG TAC CGG AAG TAA AGG AGA AGA AC-3') and NR172_Eco (5'-CCA ATC CGA ATT CCC TTC AAC GTT GTG GC-3') for the N-terminal fragment and with CF173_Nde (5'-GGA AGC CAT ATG GAT GGA TCC GTT CAA-3') and CR238_Kpn (5'-CCA CCT GGT ACC ACC ACT TCC TTT GTA GAG CTC ATC C-3') for the C-terminal fragment. The dN1-172 DNA was digested with *Kpn*I and *Eco*RI, and the dC173-238 DNA was digested with *Nde*I and *Kpn*I. These digested DNAs were ligated into the *Nde*I/*Eco*RI site of pET22b(+), and the product was transferred into XL1-blue. The plasmid was purified and sequenced to give pET22-cpyfP13H.

To generate the plasmid encoding CPCYAB4, short DNA corresponding to the linker sequence, $(GGS)_{12}$ between cfP13H and cpyfP13H was designed for inserting after the *Eco*RI site of pET22-cfP13H and before the *Nde*I site of pET22-cpyfP13H. A

dsDNA, N-Eco-GGS (5'-GCA CTG AAT TCG GGA GGT TCG-3', 5'-pACC CGA ACC TCC CGA ATT CAG TGC-3') was ligated with a dsDNA, M-GGS (5'-pGGT GGA TCC GGT GGA TCA GGT GGA TCC GGT GGT AGT-3', 5'-pACC ACT ACC ACC GGA TCC ACC TGA TCC ACC GGA TCC-3'). A dsDNA, C-Nde-GGS (5'-pGGT GGC AGT CAT ATG TCG GCG-3', 5'-CGC CGA CAT ATG ACT GCC-3') was also ligated with M-GGS DNA. These ligation solutions were mixed and further reacted with T4 DNA ligase. The ligation products were amplified by PCR using primers (5'-GCA CTG AAT TCG GGA GGT TCG-3', 5'-CGC CGA CAT ATG ACT GCC-3'). The products were purified by acrylamide gel electrophoresis, and digested with *Nde*I and *Eco*RI. The digested DNAs were subcloned into *Nde*I/*Eco*RI sites of pET22b(+). The plasmids were purified and sequenced. The plasmid encoding $(GGS)_{12}$ sequence was digested with *Nde*I and *Eco*RI, and then the product was purified by acrylamide gel electrophoresis to give the dsDNA, Link-GGS12. The plasmid pET22-cpyfP13H was digested with *Nde*I and *Not*I, and then the fragment encoding the recombinant protein was purified by agarose gel electrophoresis. The fragment DNA was ligated with Link-GGS12 and *Eco*RI/*Not*I-digested pET22-cfP13H. The plasmid was purified and sequenced to give pET22-CPCYAB4 encoding the FRET sensor CPCYAB4.

Preparation of CPCYAB4

The plasmid pET22-CPCYAB4 was transformed into an E. coli strain, BL21(DE3)pG-KJE8 containing the plasmid encoding GroES-GroEL and DnaK-DnaJ-GrpE chaperone proteins. Cells were grown on a Luria-Bertani (LB) agar plate containing carbenicillin (150 μg / mL), chloramphenicol (34 μg / mL), and 1% (w/v) glucose at 37°C. An isolated single colony was picked to inoculate 3 mL of LB medium containing carbenicillin, chloramphenicol, and glucose, and then the solution was incubated at 37°C for approximately 8 h. 300 μL of the culture was combined with

300 mL of LB medium containing carbenicillin and chloramphenicol, and the solution was incubated at 20°C for approximately 48 h. Cells were harvested by centrifugation at 4°C and 7,500 rpm for 10 min. The cell pellet was resuspended in 10 mL of lysis buffer (50 mM phosphate buffer, pH 8.0, containing 0.5 M NaCl and 5 mM imidazole) and sonicated. The soluble fraction was collected by centrifugation at 4°C and 8,500 rpm for 60 min. The crude protein was purified with Ni-NTA resin (Qiagen). The soluble fraction was applied on the resin, which was then washed with lysis buffer (10 x volume for resin) and washing buffer (50 mM phosphate buffer, pH 8.0, containing 0.5 M NaCl and 30 mM imidazole) (6 x volume for resion). The proteins were eluted by elution buffer (50 mM phosphate buffer, pH 8.0, containing 0.5 M NaCl and 250 mM imidazole) by checking the color derived from the fluorescent protein. Gel filtration using a Sephadex column (PD10, GE Healthcare) for changing the buffer with incubation buffer (10 mM phosphate buffer, pH 7.5, containing 150 mM NaCl) was carried out. Concentration of CPCYAB4 was determined by absorption at 280 nm (ε = 43600 M^{-1} cm^{-1}).

Synthesis of Aβ1-42 and its mutants

Wild-type Aβ1-42, E22Q, E22G, and A21G peptides were synthesized by the solid phase method by Fmoc strategy with O-(7-azabezotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HATU) as the coupling reagents. To remove the resin and the protecting groups, the peptide resin was treated with trifluoroacetic acid (TFA) containing m-cresol, ethanedithiol, and thioanisole as scavengers for 1 h at room temperature. The product was solidified with diethylether in an ice-bath. Peptides were purified with RP-HPLC by employing a Hitachi 2000 HPLC system. Each peptide was purified on a Develosil Packed column ODS-UG-5 (10 x 250 mm, Nomura Chemical Co.) using a linear gradient of acetonitrile/0.1% NH_4OH. Peptides were identified by MALDI-TOFMS measured on a

Shimadzu MALDI III mass spectrometer using 3,5-dimethoxy-4-hydroxycinnamic acid as a matrix. The molecular ion peaks $(M+H)^+$ were shown as m/z found (calcd): Aβ1-42, 4518.0 (4515.1); E22Q, 4513.9 (4514.1); E22G, 4443.4 (4443.0); A21G, 4501.8 (4501.1). The amounts of the peptide were determined by amino acid analysis performed with the phenyl isothiocyanate (PTC) method on a Wakopak WS-PTC column (Wako Chemical Co.).

Preparation of globulomer

To prepare Aβ1-42 globulomer, 0.25 mg of Aβ1-42 was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and HFIP was removed by freeze dry. The solid was resupended at a concentration of 5 mM in 11 μL of dimethylsulfoxide (DMSO), and then 127 μL of phosphate buffered saline (20 mM NaH_2PO_4, pH 7.4, containing140 mM NaCl) to 400 μM, and 1/10 volume 2% sodium dodecyl sulfate (SDS; in H_2O) (13.8 μL) was added. After 7 h of incubation at 37°C, 422 μL of H_2O was added, and the solution was incubated at 37°C for 18 h. After centrifugation (13,000 rpm at 4°C for 5 min) to remove the precipitate, the supernatant was concentrated by ultrafiltration with a 30 kDa microcon (YM-30, Millipore). The solution was dialyzed against 5 mM NaH_2PO_4 buffer, pH 7.4, containing 35 mM NaCl at 4°C. After the centrifugation at 13,000 rpm at 4°C for 10min, the supernatant was stored at 4°C and the concentration was determined by amino acid analysis.

Binding assay of CPCYAB4 and globulomer

Fluorescence measurements were recorded on a Hitachi F2500 Fluorescent Spectrophotometer using a 3 x 3 mm quartz cell. The fluorescence spectra of CPCYAB4 (18 nM) were measured in assay buffer (10 mM phosphate buffer, pH 7.5, containing 150 mM NaCl and 0.005% Tween 20) at 25°C. Excitation wavelength was 435 nm, and the emission intensities at 480 nm (F_{480}) and 527 nm (F_{527}) were collected. Aβ1-42

globulomer was titrated with CPCYAB4 solution. The dissociation constant of CPCYAB4 and globulomer was calculated by the equation assumed as a 1 : 3 stoichiometry (monomer concentration of Aβ) using Kaleida Graph;

$$F = 1+ (Fb - Ff)([CPCYAB4]_0 + [A\beta]_0/3 + K_d - ((([CPCYAB4]_0 + [A\beta]_0/3 + K_d)^2 - 4([CPCYAB4]_0[A\beta]_0/3)^{1/2}) / (2[CPCYAB4]_0) \quad (1)$$

where F, Fb, and Ff represent the FY/FC values (fluorescence ratios of intensities of cpYFP at 527 nm to those of CFP at 480 nm) of each solution, bound CPCYAB4, and free CPCYAB4, respectively, and $[CPCYAB4]_0$ and $[A\beta]_0$ represent the initial concentrations of CPCYAB4 and Aβ1-42. K_d is the dissociation constant between CPCYAB4 and globulomer.

Detection of oligomer formation of Aβ peptides by FRET assay

Each Aβ1-42 peptide was dissolved in HFIP to monomerize, and then the solvent was evaporated by N_2 gas. DMSO was added and mixed by voltex, and then incubation buffer (10 mM phosphate buffer, pH 7.5, containing 150 mM NaCl) was added. Using this solution, Aβ peptides (40 μM) were incubated at 37°C, 25°C, and 15°C for several hours, and the sampling solutions at various incubation time were assessed by the fluorescence of CPCYAB4 and size-exclusion chromatography (SEC) using a Superdex 75 5/150 column (GE Healthcare) in assay buffer. Concentrations of CPCYAB4 and Aβ for fluorescence measurement were 18 nM and 200 nM, respectively. SEC was performed in incubation buffer, and the peak areas at 1.15 mL of elution volume corresponding to the oligomeric assemblies were plotted.

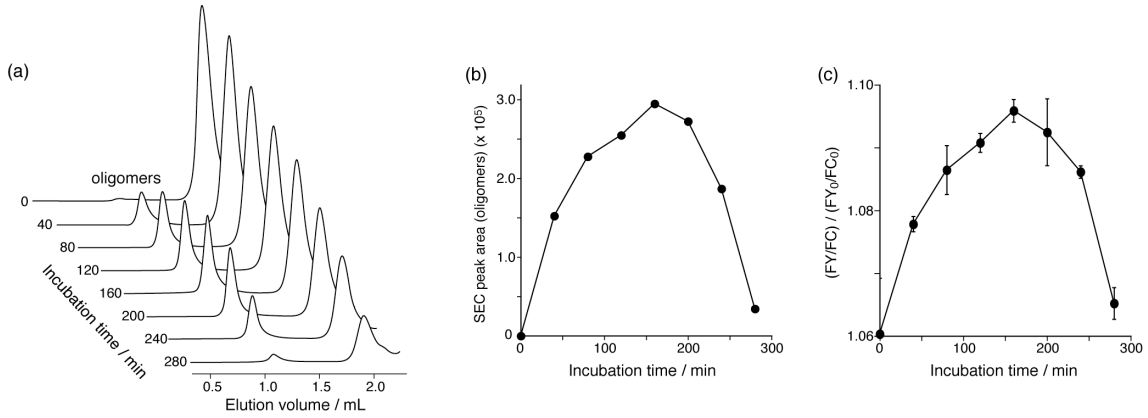

Fig. S1 (a) SEC chromatogram of Aβ1-42 wild-type incubated at 37°C. (b) SEC peak areas corresponding to oligomers and protofibrils as a function of incubation time of Aβ1-42. (c) FRET changes of CPCYAB4 in the presence of Aβ1-42 as a function of incubation time. Ab1-42 (40 mM) was incubated at 37°C in 10 mM phosphate (pH 7.5) containing 150 mM NaCl. SEC was performed on a Superdex 75 column with 10 mM phosphate (pH 7.5) containing 150 mM NaCl. The Peak near the void volume was chosen for oligomers and protofibrils.Fluorescence spectra of CPCYAB4 (18 nM) were measured in the presence of Aβ1-42 (200 nM) in 10 mM phosphate (pH 7.5) containing 150 mM NaCl and 0.005% (v/v) Tween 20.