Supporting Information

Chemical Fluorescent Probe for Detection of Aβ Oligomers

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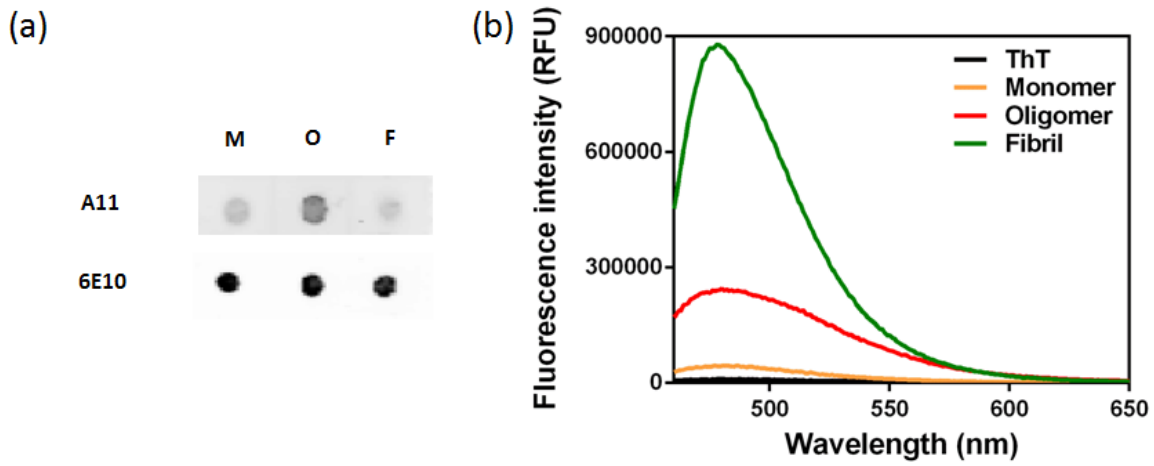


Figure S1. Characterization of monomers, oligomers and fibrils formed from synthetic $A\beta_{1-40}$ peptide. (a) Dot blots of Aβ probed by oligomer-specific A11 and 6E10 antibodies; (b) Emission spectra of **ThT** alone and when incubated with monomers, oligomers and fibrils of Aβ (λ_{ex}=440 nm, dye: 5 μM, Aβ: 20 μM).

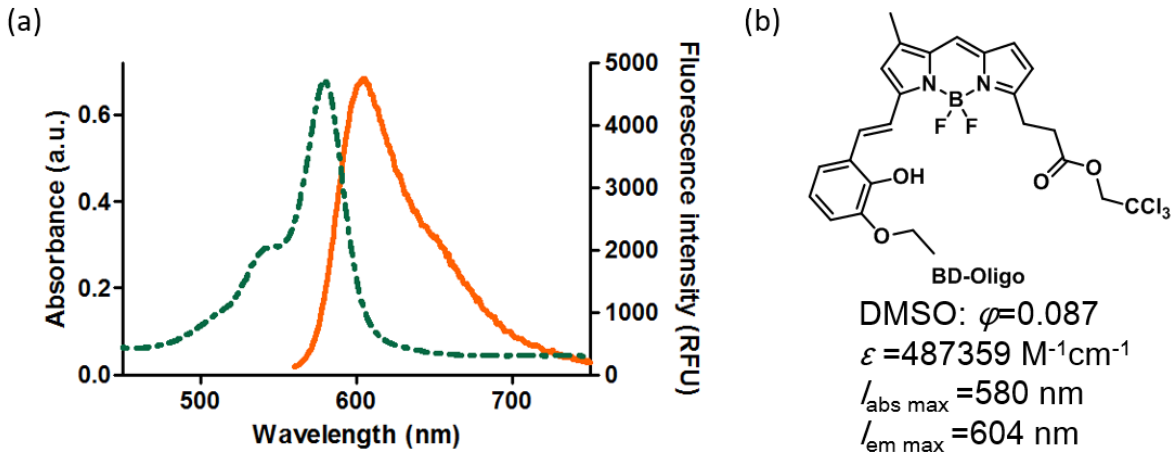


Figure S2. Spectra and spectral information of **BD-Oligo**. (a) absorbance and emission spectra of **BD-Oligo**; (b) absorbance maximum, emission maximum and quantum yield of **BD-Oligo**, measured in DMSO.

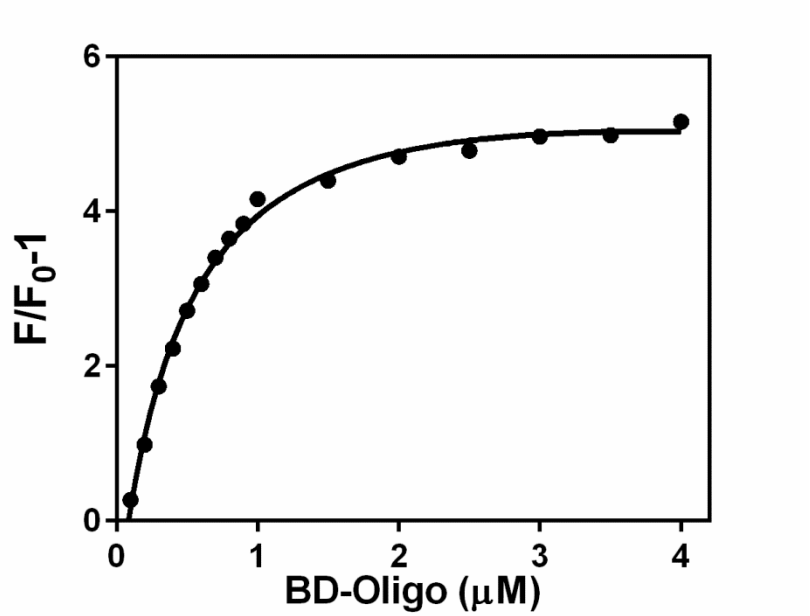


Figure S3. **BD-Oligo** binding constant (Aβ oligomers: 20 μM, λ_{ex} = 530 nm), F is the fluorescence intensity of **BD-Oligo** at 580 nm after binding with Aβ oligomers; F_0 is the fluorescence intensity of **BD-Oligo** at 580 nm before binding with Aβ oligomers..

Detailed statistics for Figure S3

Best-fit values	
Bmax	7.886
Kd	0.4819
NS	-0.2092
Background	-1.172
Std. Error	
Bmax	0.3667
Kd	0.07957
NS	0.09903
Background	0.2375
95% Confidence Intervals	
Bmax	7.088 to 8.685
Kd	0.3085 to 0.6553
NS	-0.4250 to 0.006613
Background	-1.690 to -0.6546
Goodness of Fit	
Degrees of Freedom	12
R2	0.9958
Absolute Sum of Squares	0.1423
Sy.x	0.1089
Number of points	
Analyzed	16

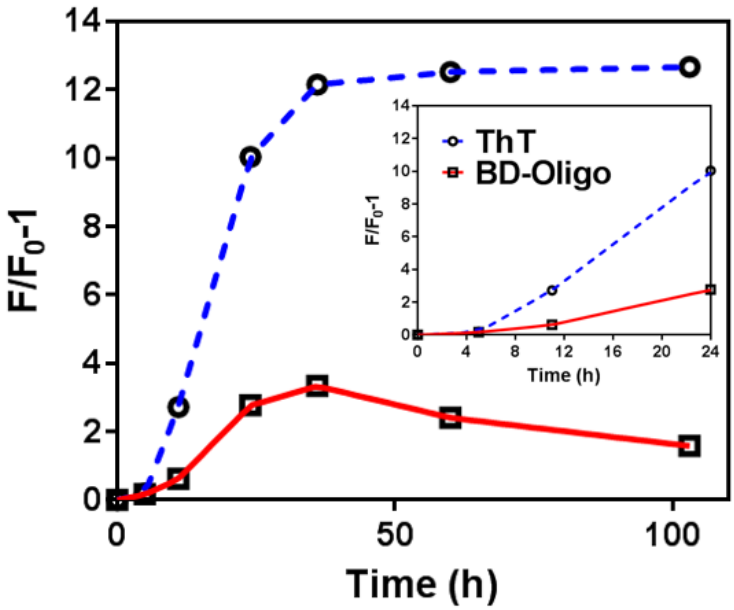


Figure S4. Time-dependent fibril formation of Aβ was monitored by **ThT**, whereas **BD-Oligo** detects on-fibril pathway oligomers (dye: 5 μM, Aβ: 20 μM). F is the fluorescence intensity of BD-Oligo at 580 nm after binding with Aβ oligomers; F_0 is the fluorescence intensity of BD-Oligo at 580 nm before binding with Aβ oligomers.

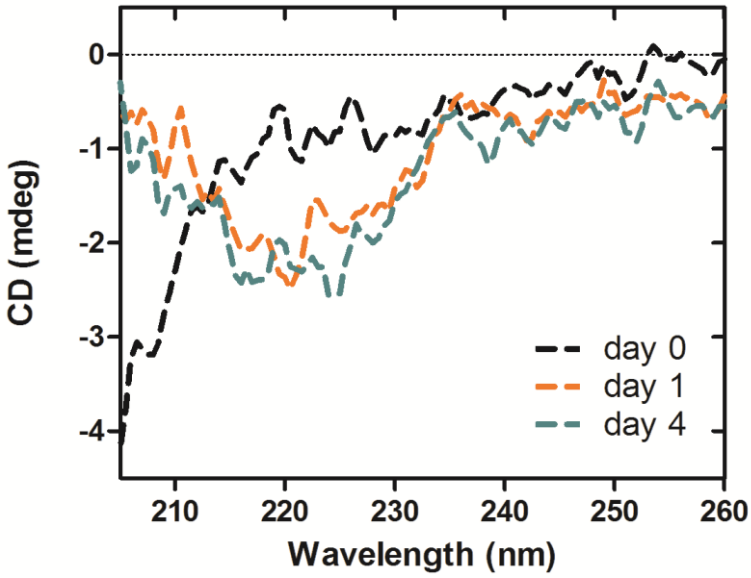


Figure S5. Biophysical characterization of oligomer-specific response. CD spectra for Aβ at various time-points, after fibril formation time course is initiated.

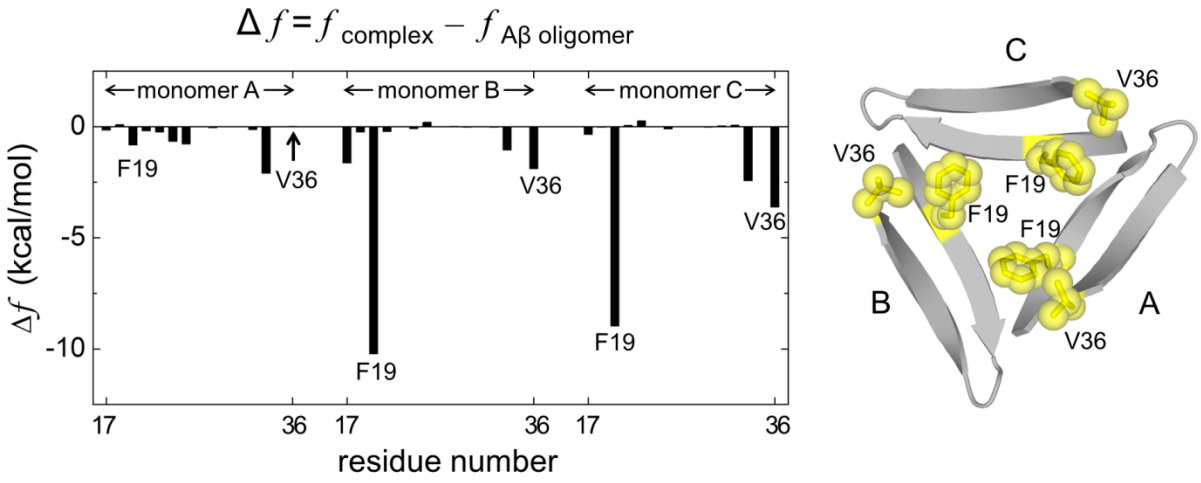


Figure S6. Site-directed thermodynamics analysis of the **BD-Oligo** complex with Aβ oligomer ($A\beta_{17\text{-}36}$). Residue-specific free energy values (Δf) are plotted for the free energy of Aβ oligomer with **BD-Oligo** binding (f_{complex}) relative to that of Aβ oligomer without **BD-Oligo** ($f_{A\beta\ \text{oligomer}}$) for each residue.

Material and Method

Reagents and Solvents

The chemicals, including aldehydes and solvents, were purchased from Sigma Aldrich, Fluka, MERCK, Acros and Alfa Aesar. All the chemicals were directly used without further purification. Normal phase column chromatography purification was carried out using MERCK silica Gel 60 (Particle size: 230-400 mesh, 0.040-0.063 mm).

Measurements and Analysis

HPLC-MS was taken on an Agilent-1200 with a DAD detector and a single quadrupole mass spectrometer (6130 series). The analytical method, unless indicated, is A: H_2O (0.1% HCOOH), B: CH_3CN (0.1% HCOOH), gradient from 10 to 90% B in 10 minutes; C18 (2) Luna column (4.6 × 50 mm^2, 3.5 μm particle size).

Spectroscopic and quantum yield data were measured on a SpectraMax M2 spectrophotometer (Molecular Devices). Compounds in solvent (100 μL) in 96-well polypropylene plates was for fluorescence measurement. Data analysis was performed using Graph Prism 5.0.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AMX500 (500 MHz) spectrometers, and chemical shifts are expressed in parts per million (ppm) and coupling constants are reported as a *J* value in Hertz (Hz).

Quantum Yield Measurements

Quantum yields for **BD-Oligo** were measured by dividing the integrated emission area of their fluorescent spectrum against the area of Rhodamine B in EtOH excited at 490 nm ($\Phi_{rho\text{-}B}$ = 0.7).[1] Quantum yields were then calculated using equation (1), where *F* represents the integrated emission area of fluorescent spectrum, η represents the refractive index of the solvent, and *Abs* represents absorbance at excitation wavelength selected for standards and samples. Emission was integrated from 530 nm to 750 nm.

$$\Phi_{flu}^{sample} = \Phi_{flu}^{reference}\left(\frac{F^{sample}}{F^{reference}}\right)\left(\frac{\eta^{sample}}{\eta^{reference}}\right)\left(\frac{Abs^{reference}}{Abs^{sample}}\right) \quad (1)$$

CD Spectroscopy

CD measurements were made using an Aviv model 62 DS CD spectrometer (Aviv Associates Inc., Lakewood, NJ) at 25 ℃ with a 1-mm path length quartz cuvette, a spectral bandwidth of 1 nm, a signal averaging time of 1 s, and a data interval of 0.5 nm. The spectra presented are the averages of five measurements and corrected using a reference solution lacking Aβ.

Computational Methods

Quantum mechanical calculations

The geometry optimization for **BD-Oligo** compound was performed by using density functional theory at the B3LYP/6-31G* level[2] at the gas phase as well as an aquaous phase using Gaussian 09 program[3]. Vibrational frequency analyses were executed to verify the identity of each stationary point as an energy minimum.

Molecular docking search and molecular dynamics (MD) simulations

BD-Oligo docking search with Aβ oligomer were executed by using AutoDock 4.0 software package[4]. The docking simulations were carried out with a box centered on the Aβ oligomer and employing 50 × 50 × 50 grid points. For the Aβ oligomer structure, we used X-ray (4NTR) determined Aβ trimers derived from the β-amyloid peptide as a working model for toxic Aβ oligomer associated with Alzheimer's disease[5]. We used the Lennard-Jones (LJ) parameter of carbon for boron atom due to the absent of LJ parameter for boron. This is not a harsh substitution since boron atom has four coordination number in **BD-Oligo**[6, 7]. Based on the global docking search, the most energy-minimized complex structure of **BD-Oligo** with Aβ oligomer was used as an initial structure for MD simulations. We performed all-atom, explicit-water MD simulations using AMBER 14 package[8] with the ff99SB force field[9] for the

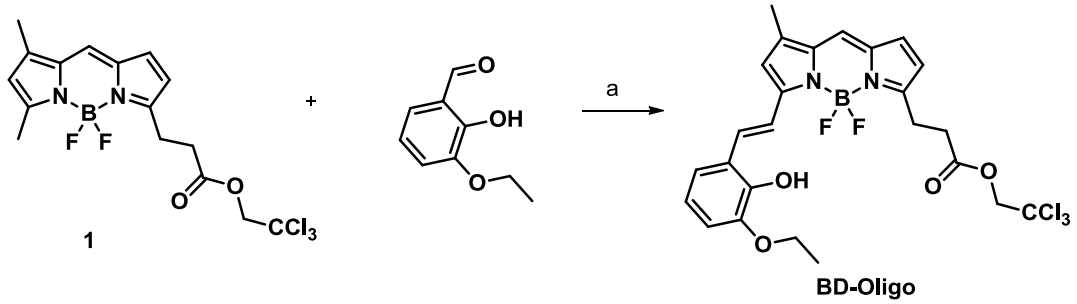
Aβcomplex and the TIP4P-Ew model[10] for water. The 5,329 water molecules were added to the simulation box. The particle mesh Ewald (PME) method[11] was applied for dealing long-range electrostatic interactions while 10 Å cutoff was used for the short-range non-bonded interactions. The system was initially subjected to 500 steps of steepest descent minimization followed by 500 steps of conjugate gradient minimization while the complex structure was constrained by 500 kcal/(mol•Å^2) harmonic potential. Then, the system was minimized using 1,000 steps of steepest descent minimization followed by 1,500 steps of conjugate gradient minimization without harmonic restraints. The system was subsequently subjected to a 20 ps equilibration process in which the temperature was gradually raised from $T = 0$ to 310 K with a constant volume. This was followed by a 200 ps constant-pressure (NPT) ensemble simulation at $T = 310$ K and $P = 1$ bar. We then carried out a 2 ns production run at $T = 310$ K and $P = 1$ bar.

Thermodynamics calculations

We used the three-dimensional reference interaction site model (3D-RISM) theory[12, 13] to compute the solvation free energy ΔG_{solv} of the **BD-Oligo** complex with Aβ oligomer structure. This theory provides the equilibrium water distribution function around a given protein structure, with which ΔG_{solv} can be computed by using the Kirkwood charging formula[14]. The internal energy (Eu) was directly computed from the force field used for the simulations. By combining the internal energy and the solvation free energy, we obtain a binding free energy ($f = E_u + G_{\mathrm{solv}}$). To obtain a residue-specific contribution to the binding free energy, we used an exact decomposition method[15] which provides the site-directed thermodynamic contributions to the free energy upon complexation. In Figure S8, each bar represents the free energy difference (Δf) for each residue obtained from the free energy of Aβ oligomer with **BD-Oligo** ($f$complex) relative to Aβ oligomer without **BD-Oligo** (fAβ oligomer).

Synthesis and Characterization

Scheme S1. Synthetic scheme and structures of **BD-Oligo**[a].



[a]Reagents and conditions: (a) pyrrolidine, acetic acid, ACN, 90 ℃, 5 min.

Procedure for BD-Oligo synthesis

Compound **1** (20 mg, 47 μmol) and aldehyde (94 μmol, 2 equiv) were dissolved in acetonitrile (3 mL), with 6 equiv. of pyrrolidine (23.5 μL, 282 μmol) and 6 equiv. of AcOH (16.1 μL, 282 μmol). The condensation reaction was performed by heating to 90 ℃ for 5 min. The reaction mixture was cooled down to room and concentrated under vacuum, and purified by short silica column (EtOAc / Hexane =2 : 3). Yield: 17.1 mg (63.8 %).

Characterization of BD-Oligo

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.70 (s, 2H), 7.28 (dd, *J*=7.6 Hz, 1.0, 1H), 7.02 (s, 1H), 6.82 (m, 4H), 6.28 (d, *J*=3.9 Hz, 1H), 4.78 (s, 2H), 4.20 – 4.04 (m, 2H), 3.39 (t, *J*=7.5 Hz, 2H), 2.96 (t, *J*=7.5 Hz, 2H), 2.25 (s, 3H), 1.45 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): 171.05, 157.99, 155.12, 145.96, 144.73, 143.09, 136.88, 133.60, 133.52, 126.81, 122.40, 121.88, 119.73, 119.43, 118.84, 116.97, 116.29, 112.13, 94.89, 74.02, 64.72, 33.03, 23.68, 14.81, 11.30.

HRMS *m/z* ($C_{25}H_{24}BCl_3F_2N_2O_4$) calculated: 570.0863, found: 593.0775 $(M+Na)^+$.

References

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