Electronic Supplementary Information

Human serum albumin-mediated recognition of soluble amyloid-β peptides by a time-resolved luminescent probe in plasma

Chengyi Yuan,[a] Weiping Duan,[a] Jian Luo,[a] Yuwang Han,[a] Xiaohui Wang*[a,b]

[a]*College of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing 211816, P. R. China*
[b]*State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, P. R. China*
**Corresponding author. Tel: +86 25 58139537; E-mail address: wangxhui@njtech.edu.cn*

1. Experimental Section

1.1. Reagents and Materials

Common reagents used in the experiments were all of analytical grade and purchased from commercial suppliers unless otherwise stated. Diethylenetriaminepentaacetic acid (DTPA) dianhydride were purchased from Acros Organics. Human Aβ40 was purchased from GL Biochem Ltd. (Shanghai, China). Stock solution of Aβ40 was prepared according to the literature method.[1] The concentration of Aβ40 was immediately measured with a BCA Protein Assay Kit (Pierce). Artificial cerebrospinal fluid was prepared according to the literature,[2] containing NaCl (148 mM), KCl (3 mM), $CaCl_2 \cdot 2H_2O$ (1.4 mM), $MgCl_2 \cdot 6H_2O$ (0.8 mM), $Na_2HPO_4 \cdot 7H_2O$ (0.8 mM), and $NaH_2PO_4 \cdot H_2O$ (0.2 mM). Plasma were purchased from SenBeiJia Biotechnology Ltd. Stock solution of TbL2 (4 mM) was obtained by dissolving the compound in DMSO and filtered using a 0.22 μM filter (organic system). All solutions and buffers were obtained using Milli-Q water, and filtered through a 0.22 μM filter (Millipore) before used.

1.2. Methods

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer. Electrospray ionization mass spectra (ESI-MS) were acquired on an LCQ Fleet electrospray mass spectrometer. UV-vis spectra were measured on a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Time-resolved luminescence and steady-state fluorescence spectra were recorded on a PerkinElmer FL6500 fluorescence spectrometer.

1.3. Synthesis of TbL2

*Synthesis of 2,2'-((((carboxymethyl)azanediyl)bis(ethane-2,1-diyl))bis((2-((4-(benzylamino)phenyl)amino)-2-oxoethyl)azanediyl))diacetic acid (**L2-3H**)*: It was synthesized according to the literature.[3] Briefly, N^1-benzylbenzene-1,4-diamine (2.4 g, 12 mmol) in DMF (25

ml) was added dropwise to DTPA dianhydride (DTPAA) (1.8 g, 5 mmol) in DMF (150 ml) and triethylamine (5 ml) under stirring in nitrogen at 0 ºC. The ice bath was removed after 2 h and the reaction mixture was stirred at 40 ºC for 48 h. The reaction was quenched by H_2O (100 ml) and the solvent was evaporated to 10 ml. Acetone (200 ml) was added to the residue and the resulting precipitate was filtered, washed with anhydrous chloroform and ether, and dried under vacuum to give L2-3H as a deep powder (2.8 g, yield: 75%). ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 7.35-7.28 (br, 12H, benzene), 7.21 (d, J = 6.8 Hz, 2H, benzene), 6.50 (d, J = 8.8 Hz, 4H, benzene), 4.22 (s, 4H, methylene), 3.45-3.41 (br, 4H, NCH_2CON; 4H, terminal NCH_2COOH), 3.04-3.01 (br, 2H, central NCH_2COOH; 4H, NCH_2CH_2N), 2.91 (br, 4 H, NCH_2CH_2N). ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): 172.98, 168.16, 162.29, 145.07, 140.31, 128.19, 127.19, 126.52, 121.02, 112.13, 58.18, 55.50, 52.09, 50.90, 46.77. ESI-MS found (calcd) for $C_{40}H_{47}N_7O_8$ (m/z): 754.50 (754.36) $[M + H]^+$, 766.50 (776.34) $[M + Na]^+$, 792.50 (792.31) $[M + K]^+$. Elemental analysis found (calcd) for $C_{40}H_{47}N_7O_8$ (%): C, 63.83 (63.73); H, 6.75 (6.28); N, 12.69 (13.01).

*Synthesis of Tb(L2)(H_2O) (**TbL2**)*: NaOH (5 M) was added to aqueous solution of L2-3H (0.753 g, 1 mmol, 20 ml) until the pH reached 6. $Tb(NO_3)_3 \cdot 6H_2O$ (0.452 g, 1 mmol) in water (4 ml) was added to the solution of L2-3H slowly and the pH was maintained at 6 by adding aliquots of NaOH (5 M). The mixture was stirred at 45 ºC for 12 h and the solvent was evaporated. The residue was dissolved in distilled water (2 ml). The product was deposited by addition of aqueous solution into 50 ml of acetone and was collected by centrifugation. Brown solid was obtained by vacuum drying after precipitation with acetone for 3 times (0.371 g, yield: 40%). ESI-MS found (calcd) for $C_{40}H_{46}N_7O_9Tb$ (m/z): 932.42 (932.24) $[M - H_2O + Na]^+$. Elemental analysis found (calcd) for $C_{40}H_{46}N_7O_9Tb$ (%): C, 50.95 (51.78); H, 4.91 (5.00); N, 10.85 (10.57).

1.4. Time-resolved luminescence recognition of Aβ.

For the experiments in Tris-HCl buffer and artificial CSF, TbL2 (25 μM) was pre-incubated with HSA (100 μM) and then different concentrations of Aβ40 monomer were added to the complex of TbL2 and HSA. For the experiments in plasma, plasma was pre-diluted by Tris-HCl buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) to different concentration (v/v, 100%, 80%, 50%, 20%, and 10%). The time-resolved luminescence responses of TbL2 (25 μM) to different concentration of Aβ monomer were measured in 20% plasma. Experiments were performed in duplicate and errors are reported as standard deviation. The detection limit (3σ/slope) of TbL2 for Aβ according to the literature method.[4] A linear regression curve was fitted according to the

emission intensity at 546 nm in the range of 0 − 10 μM, and the slope of curve was obtained using Origin 8.5.

1.5. ThT competition assay

Aβ40 (40 μM) was pre-incubated in buffer at 37 °C for 48 h. ThT solution (ThT final concentration 10 μM) was added to Aβ40 solution and incubated in the dark at 37 °C for 5 min. Aliquots of TbL2 (0.5 μL, 2 mM) were then added to the ThT–Aβ40 system at 25 °C and the fluorescence emission spectra of ThT (λ_{ex} = 440 nm) were measured in the range of 470 – 660 nm.

1.6. Determination of the binding constant of TbL2 for Aβ40 or HSA

Increasing concentration of probe TbL2 was titrated against a fixed concentration of Aβ40 or HSA (20 μM), then time-resolved luminescence intensity at 546 nm was recorded (λ_{ex} = 265 nm). Each curve was fit to the nonlinear equation:[5]

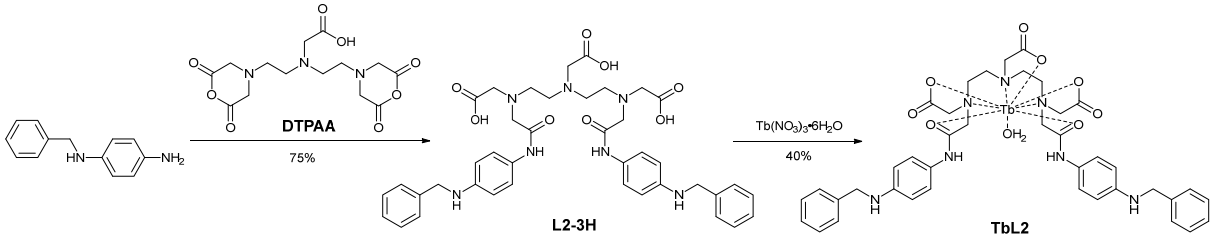
$$Y = B_{max} \times X/(K_a + X) \quad \text{(S1)}$$

where X is concentration of TbL2 and Y is change in luminescence intensity, B_{max} is the maximum specific binding has the same units as Y, K_a is the equilibrium binding constant.

1.7. Fluorescence spectra of HSA in the presence of TbL2

The steady-state fluorescence spectra of HSA (λ_{ex} = 280 nm) in the presence of TbL2 were measured at 25 ºC. For synchronous fluorescence measurement, the scanning interval between excitation and emission wavelengths (Δλ) was stabilized at 15 and 60 nm, respectively. The synchronous fluorescence spectra of the resulting solutions were measured with each Δλ at 25 ºC. For denaturation experiment, HSA (10 μM) was mixed with different concentrations of GnHCl (1.5 and 3.0 M), then incubated in the dark at 37 °C for 5 min in Tris-HCl buffer.

2. Supplementary Scheme, Figures, and Tables



Scheme S1 Synthetic route to TbL2:

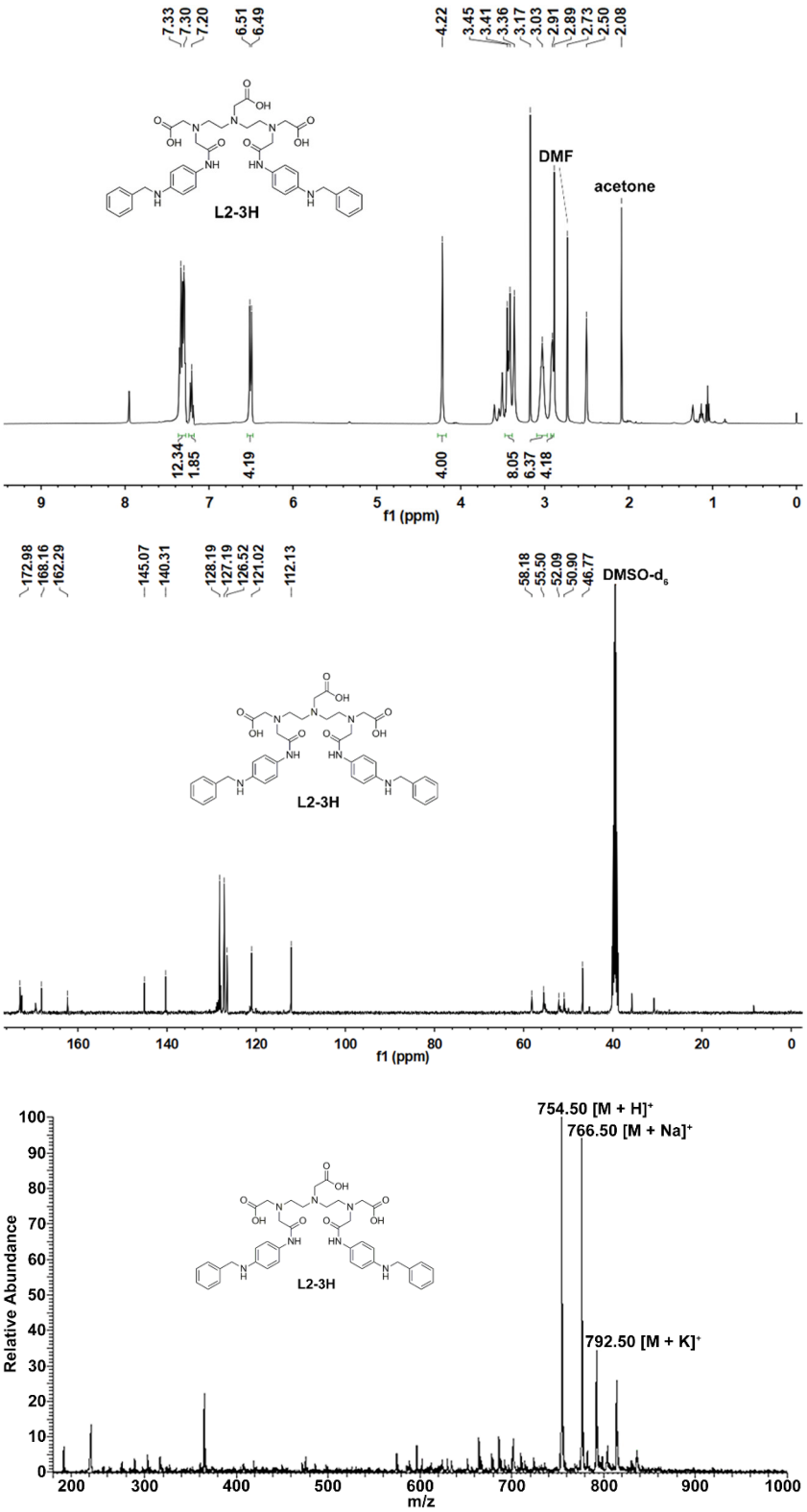


Fig.S1 ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and ESI-MS spectra for L2-3H.

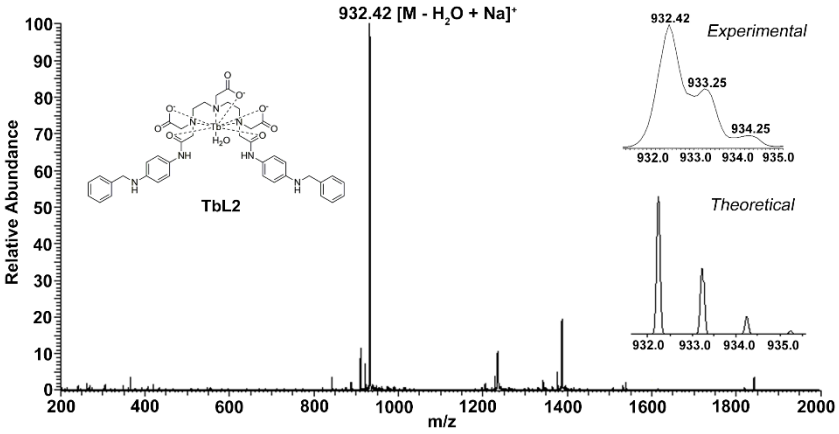


Fig.S2 ESI-MS spectra for TbL2.

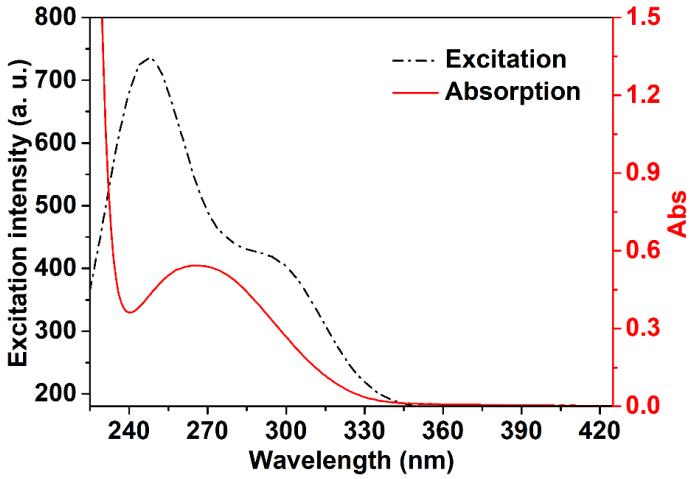


Fig. S3 Excitation (λ_{em} = 546 nm) and absorption of TbL2 (25 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, 6.25‰ v/v DMSO, pH 7.4).

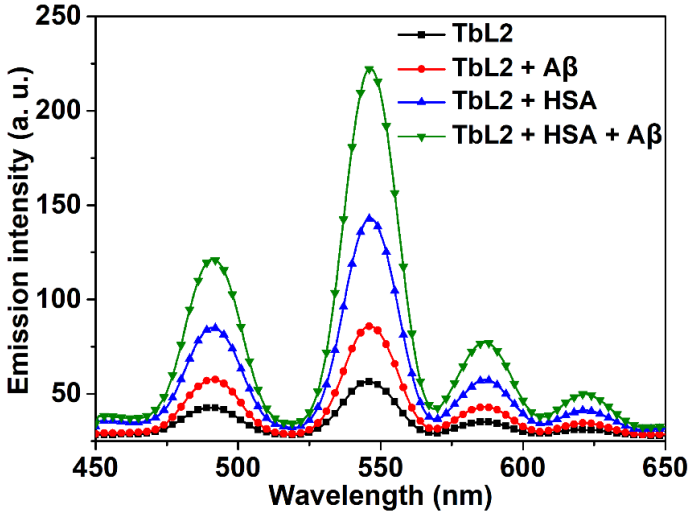


Fig. S4 Time-resolved luminescence spectra of TbL2 (25 μM, λ_{ex} = 265 nm) in the absence and presence of Aβ (20 μM) or/and HSA (100 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, 6.25‰ v/v DMSO, pH 7.4).

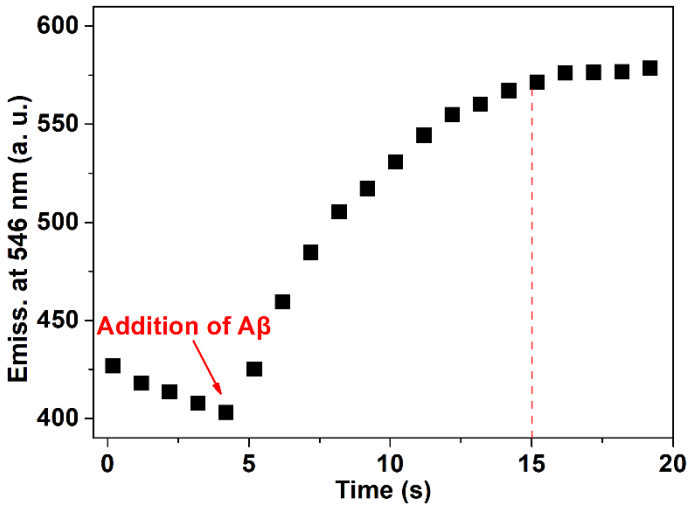


Fig. S5 Time course of luminescence intensity of TbL2 (25 μM, λ_{ex} = 265 nm) at 546 nm after addition of 40 μM Aβ in buffer (5 mM Tris-HCl, 50 mM NaCl, 6.25‰ v/v DMSO, pH 7.4).

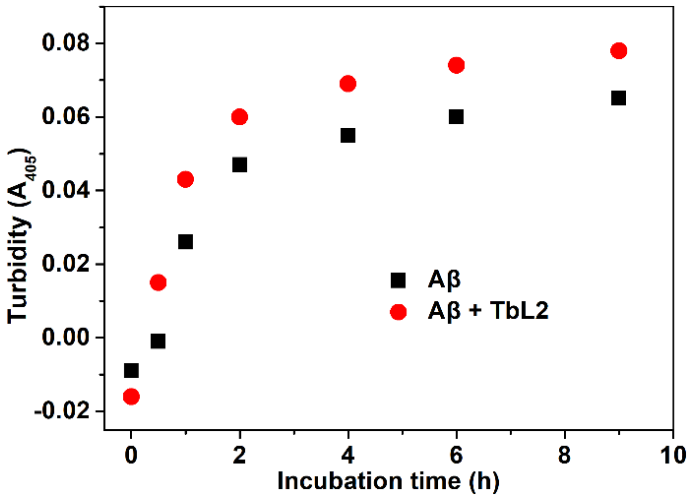


Fig. S6 Turbidity (A_{405})-monitored kinetics of Aβ40 (40 μM) aggregation in the absence or presence of TbL2 (25 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, 6.25‰ v/v DMSO, pH 7.4).

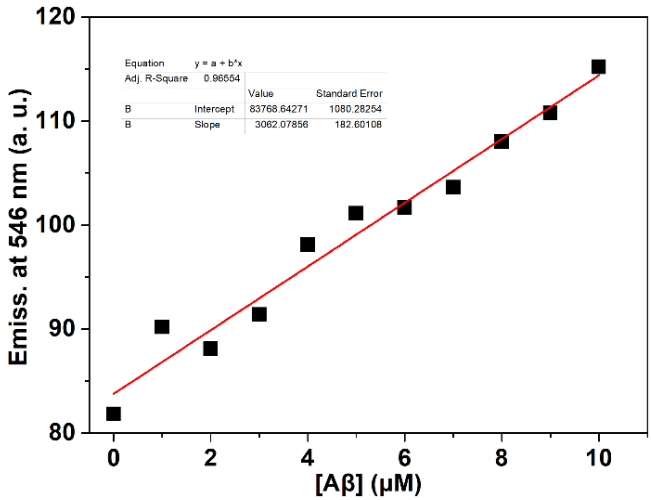


Fig. S7 Plot of luminescence intensity of TbL2 (25 μM, λ_{ex} = 265 nm) at 546 nm as a function of Aβ40 concentration in the range of 0 − 10 μM in buffer (5 mM Tris-HCl, 50 mM NaCl, 6.25‰ v/v DMSO, pH 7.4).

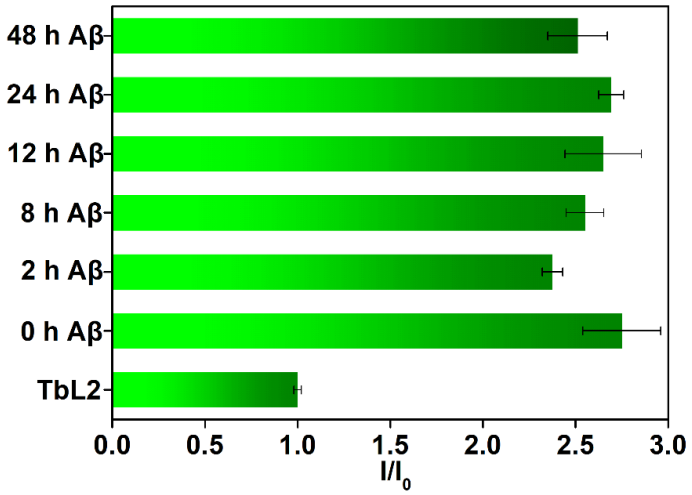


Fig. S8 The emission intensity ratio (I/I_0) of TbL2 (25 μM, λ_{ex} = 265 nm) at 546 nm with or without Aβ40 (50 μM) with different pre-incubation time in the presence of HSA (100 μM) in buffer (5mM Tris-HCl, 50 mM NaCl, 6.25‰ v/v DMSO, pH 7.4). Error bars indicate means ± s.d. (n = 3).

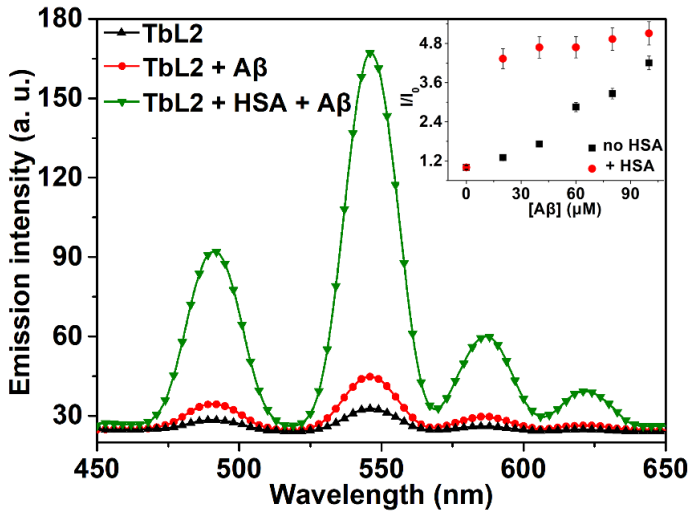


Fig. S9 Time-resolved luminescence spectra of TbL2 in the absence and presence of Aβ (20 μM) and HSA (100 μM). Inset shows emission intensity ratio (I/I_0) of TbL2 at 546 nm versus the concentration of Aβ40 in the absence or presence of HSA (100 μM). All spectra were measured in artificial CSF; [TbL2] = 25 μM; λ_{ex} = 265 nm. Error bars indicate means ± s.d. (n = 3).

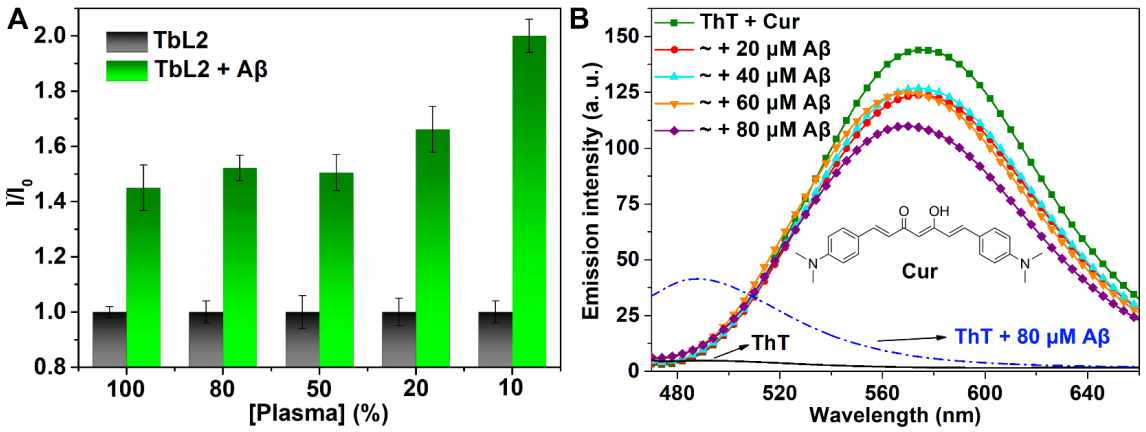


Fig. S10 (A) Emission intensity ratio (I/I_0) of TbL2 (25 μM, λ_{ex} = 265 nm) at 546 nm with Aβ40 monomer (100 μM) in various concentrations of plasma (v/v, 100%, 80%, 50%, 20%, and 10%). Error bars indicate means ± s.d. (*n* = 3). (B) The fluorescence spectra of ThT (10 μM, λ_{ex} = 440 nm) in the absence or presence of Aβ with or without Cur (40 μM) in 20% plasma.

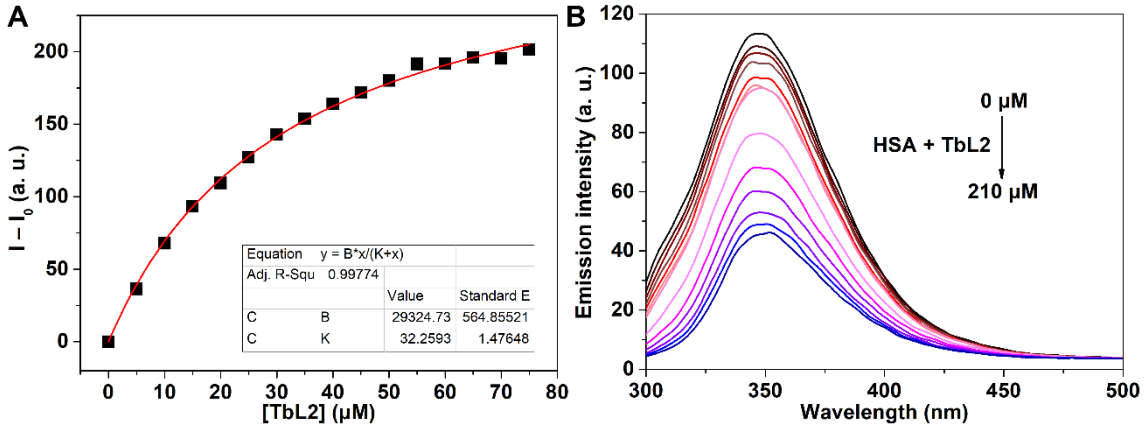


Fig. S11 (A) Plot of the difference in time-resolved luminescence intensity ($I–I_0$) of TbL2 (λ_{ex} = 265 nm) as a function of the concentration of TbL2 in the presence of HSA (20 μM). (B) Fluorescence spectra of HSA (100 μM, λ_{ex} = 280 nm) upon addition of increasing amounts of TbL2 (0, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, and 210 μM).

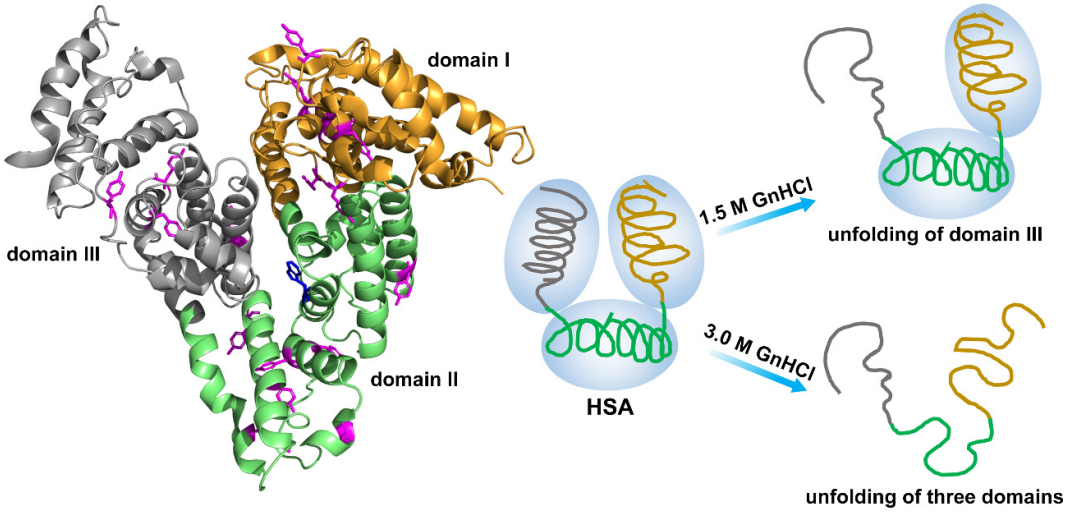


Fig. S12 Left: The distribution of Trp (blue) and Tyr (magentas) residues in the structure of HSA (PDB ID: 1UOR) created by Pymol. Trp214 and Tyr30, 84, 138, 140, 148, 150, 161, 263, 319, 332, 334, 341, 353, and 370 are located in domain I (bright orange) and II (green); Tyr401, 411, 452, and 497 are located in domain III (gray). Right: Schematic illustration of the mechanism of GnHCl-induced unfolding of HSA.

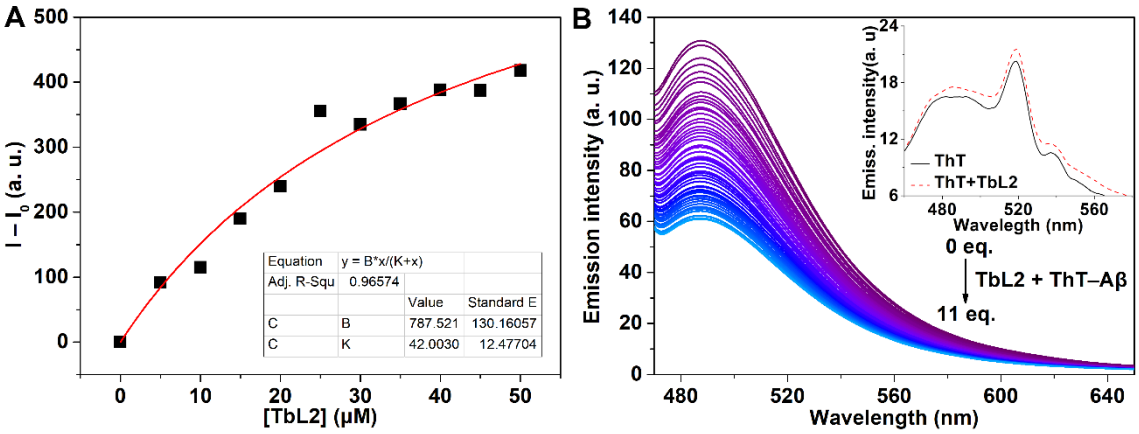


Fig. S13 (A) Plot of the difference in time-resolved luminescence intensity ($I–I_0$) of TbL2 (λ_{ex} = 265 nm) as a function of the concentration of TbL2 in the presence of Aβ40 (20 μM) in (5mM Tris-HCl, 50 mM NaCl, pH 7.4). (B) Fluorescence spectra of ThT (10 μM, λ_{ex} = 440 nm) upon addition of increasing amounts of TbL2 in the presence of Aβ40 (40 μM) with 48 h pre-incubation in buffer (5mM Tris-HCl, 50 mM NaCl, pH 7.4). Inset shows the emission intensity of ThT (10 μM, λ_{ex} = 440 nm) at 547 nm with or without TbL2 (25 μM).

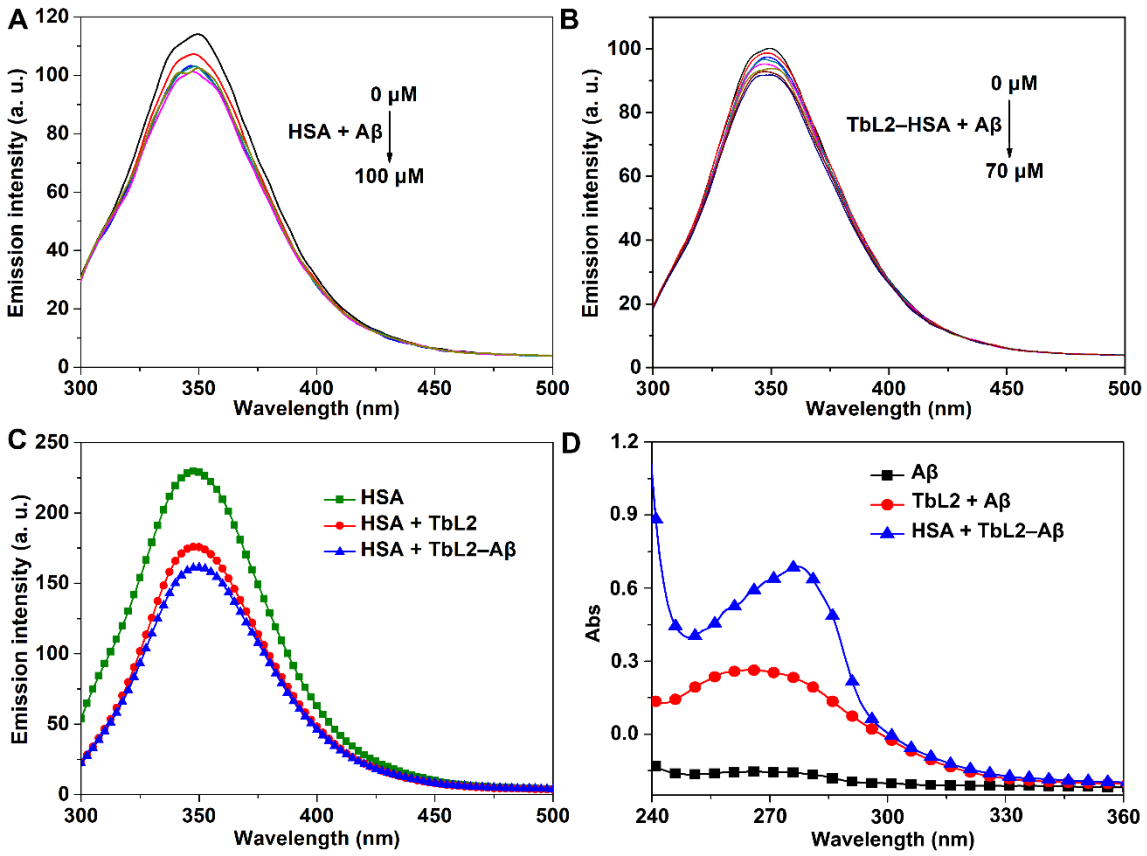


Fig. S14 (A) Fluorescence spectra of HSA (100 μM, λ_{ex} = 280 nm) in the presence of Aβ (0, 20, 40, 60, 80, and 100 μM) in buffer (5mM Tris-HCl, 50 mM NaCl, pH 7.4). (B) Fluorescence spectra of HSA (100 μM, λ_{ex} = 280 nm) pre-incubated with TbL2 (25 μM) upon addition of increasing concentration of Aβ in buffer (5mM Tris-HCl, 50 mM NaCl, pH 7.4). (C) Fluorescence spectra of HSA (100 μM, λ_{ex} = 280 nm) upon addition of TbL2 (60 μM) or 100 μM Aβ-treated TbL2.

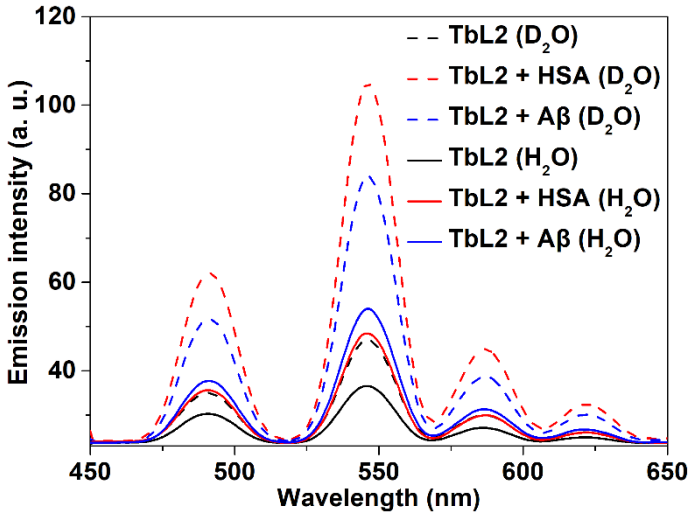


Fig. S15 Time-resolved luminescence spectra of TbL2 (25 μM, λ_{ex} = 265 nm) in the absence or presence of Aβ40 (40 μM) or HSA (40 μM) determined in H_2O and D_2O.

3 References

[1] T. Chen, X. Wang, Y. He, C. Zhang, Z. Wu, K. Liao, J. Wang and Z. Guo, *Inorg. Chem.*, 2009, **48**, 5801-

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[2] C.G. Taylor, G. Meisl, M.H. Horrocks, H. Zetterberg, T.P.J. Knowles and D. Klenerman, *Anal. Chem.,* 2018, **90**, 10385-10393.
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[4] X. Wang, X. Wang, S. Cui, Y. Wang, G. Chen and Z. Guo, *Chem. Sci.*, 2013, **4**, 3748.
[5] W. Fu, C. Yu, Z. Guo, J. Zhang, H. Zhang, H. Tian and W. -H. Zhu, *J. Am. Chem. Soc.*, 2019, **141**, 3171-3177.