Supporting Information

A ratiometric fluorescent probe revealing the abnormality of acetylated tau by visualizing polarity in Alzheimer's disease

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1. Experimental Procedures

Instruments

UV-vis absorbance measurements were recorded on Shimadzu UV-1750. Fluorescence spectra were recorded by a Shimadzu RF-5301 spectrofluorophotometer. Fluorescence quantum yield was recorded by Fluorescence Lifetime & Steady State Spectrometer (FLS920). MS spectra was recorded on a micro TOF II instrument. Fluorescence images were obtained by using a confocal microscope (LSM 900). ^{1}H NMR,and ^{13}C NMR were obtained by Liquid Nuclear Magnetic Resonance Spectrometer (AVANCE III 400).

Computational methodologies

The molecular orbitals (MO) of Cy7-K were calculated using DFT by Gaussian09 package [1] at B3LYP/6-31g (d, p) level. Polarized continuum model (PCM) solvation model was applied to the calculation in different solvents.

Cell Culture

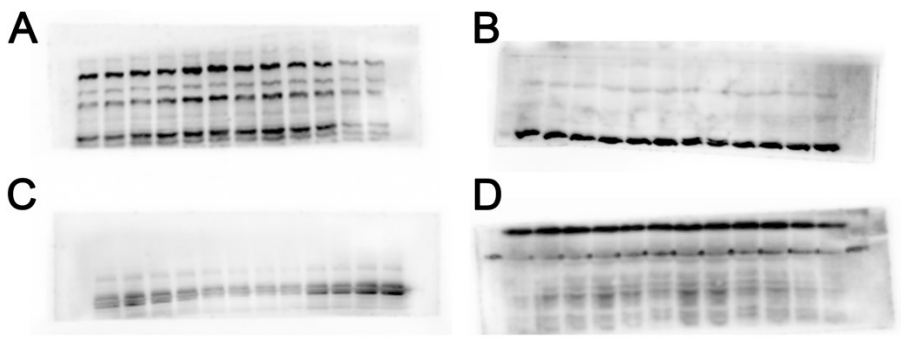
The hippocampal neuronal cell line HT22 and mouse neuroblastoma N2a cells were purchased from the Shanghai Cell Institute of the Chinese Academy of Sciences. The complete medium (pH = 7.4) was composed of DMEM high glucose medium (BASIC DMEM, High Glucose, Gibco, C11965500BT) and 10% fetal bovine serum (Gibco, C0235).

CCK-8 assay

HT22 cells or N2a cells growing at logarithmic phase were cultured in a 96-well plate. Cell density was adjusted to 5×10^4 cells/well. After adherence, the cells were incubated with 0, 2, 5, 10 μM Cy7-K. After 6 h and 24 h, cell viability was measured via CCK-8 assay (Cell Counting Kit-8, Sangon Biotech, E606335). In brief, the cells were incubated with 10 μL CCK-8 for 3 h. The amount of CCK-8 formazan was determined at the reference wavelength of 450 nm by Microplate Reader.

Western blot (WB)

Proteins extract was prepared using RIPA buffer (Solarbio, R0010). After electrophoresis, proteins were electroeluted at a constant current 250 A onto a polyvinylidenedifluoride (PVDF) membrane (Immobilon-P membrane, Millipore). Indicated primary antibodies (Table S3) and secondary antibodieswere used. Protein bands were visualized by an enhanced chemiluminescence assay kit (Chemiluminescent HRP substrate, Millipore). Data analysis using Image J. Western blot analyses were repeated at least three times.



(A) The original picture of AC-Tau. Electrophoresis conditions: constant voltage 60 V for 1 hour and then switch to 120 V for 1 hour. Membrane transfer condition: constant current 250 A for 1 hour. (B) The original picture of Tau. Electrophoresis conditions: constant voltage 60 V for 1 hour and then switch to 120 V for 1 hour. Membrane transfer condition: constant current 250 A for 1 hour. (C) The original picture of HDAC6. Electrophoresis conditions: constant voltage 60 V for 1 hour and then switch to 120 V for 2 hours. Membrane transfer condition: constant current 250 A for 2 hours. (D) The original picture of GAPDH. Electrophoresis conditions: constant voltage 60 V for 1 hour and then switch to 120 V for 1.5 hours. Membrane transfer condition: constant current 250 A for 0.5 hour.

Immunofluorescence (IF)

Cell slides were permeabilised and blocked in 10% goat serum (in 0.3% Triton X-100) at room temperature (RT) for 1 hour, and probed with the primary antibodies diluted in 1% goat serum in PBS for 1 hour at RT and overnight at 4°C. After primary antibody incubation (Table S3), sections were washed with PBS for three times and incubated with appropriate secondary antibodies (Table S3) for 1 hour at RT. Nuclei were counterstained with DAPI (Solarbio, 28718-90-3). Finally, cover slips were sealed with 30% glycerin.

Animals

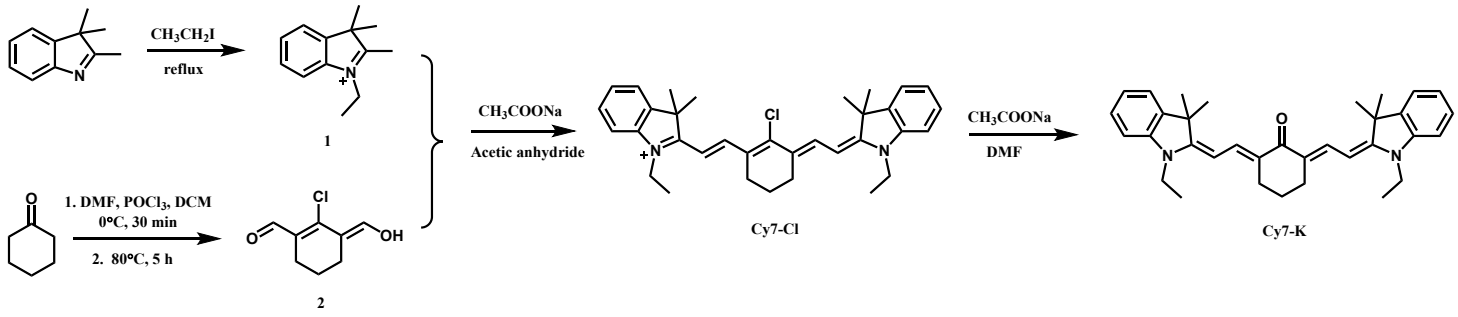
Male APP/PS1 transgenic mice with B6C3 background (Nanjing University-Nanjing Institute of Biomedicine, Cat# N000175) were maintained by mating heterozygous transgenic mice with B6C3 mice. The transgenic mice were identified via PCR analysis of total DNA by using specific primers (Supplementary Table S2). The mice were bred and housed in an animal facility and maintained in air-conditioned rooms at 20 °C–22 °C with a light period of 12 h. This study was conducted in accordance with the recommendations of

the ethical treatment of laboratory animals of the Ministry of Science and Technology of the People's Republic of China. All animal experiments were approved by the Experimental Animal Ethics Committee of Lanzhou University.

Statistical analysis

Data are presented as means ± standard errors of the mean (SEM) or means ± Standard Deviation (SD) as indicated in the Figure legends. For parametric comparison between two groups, F test was conducted to determine the similarity in the variances between the groups that are statistically compared, and statistical significance was analyzed by Student's t-test. For multiple comparisons, Bartlett's test for equal variances was used to determine the variances between the multiple groups and one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test was used to test statistical significance, using GraphPad Prism software. Data was tested for normality using the Shapiro-Wilk test. A P-value of less than 0.05 was considered statistically significant.

Synthesis



Scheme S1. The synthesis procedures of Cy7-K.

Synthesis of Cy7-Cl.

1-Ethyl-2,3,3-triMethylindolenium Iodide, 2-chloro-3-(hydroxyMethylene)cyclohex-1-enecarbaldehyde and Cy7-Cl were prepared according to the reported procedure.[2]

2. Supporting tables

Table S1. Photophysical data for Cy7-K in various solvents.

Solvent	EN T[a]	λ_{abs}/nm [b]	$\varepsilon(10^4\ M^{-1}\ cm^{-1})$ [c]	λ_{em}/nm [d]	$\Delta\lambda$ (nm) [e]	Φ_f [f] (%)
Ether	0.117	481	3.27	502	21	7.16
Dioxane	0.164	495	3.08	517	22	8.93
THF	0.207	491	3.02	514	23	1.44
EA	0.228	488	2.79	518	30	2.48
DMF	0.404	506	2.28	540	34	6.14
DMSO	0.444	516	1.86	557	41	9.05
EtOH	0.654	526	0.67	597	71	24.44
MeOH	0.762	531	0.26	609	78	41.33

(a) Normalized solvent polarity parameters. (b) Maximum absorption wavelength in absorption spectra. (c) Molar extinction coefficients. (d) Maximum emission wavelength in fluorescence spectra. (e) Stocks shift (f) Fluorescence quantum yield.

Table S2. Name of detected gene and primer sequence (Takara Company)

Gene name	primer sequence
APP	GACTGACCACTCGACCAGGTTCTG
PS1	AATAGAGAACGGCAGGAGCA
Reference	CTAGGCCACAGAATTGAAAGATCT

Table S3. Antibody list

Antibodies	Experiment	Dilution ratio	Source	Identifier
Tau (TAU-5)	WB	1:500	Thermo Fisher	MA5-12808
AC-Tau ((Acetyl Lys686)	WB, IF	1:1000/1:100	Abbkine	ABP57444
HDAC6	WB, IF	1:1000/1:100	Solarbio	K002180P
GAPDH (6C5)	WB	1:10000	abcam	Ab8245
Goat Anti-Rabbit IgG	WB	1:500	BOSTER	BA1054
Goat Anti-Mouse IgG	WB	1:500	BOSTER	BA1050
Dylight 594, Goat Anti-Rabbit IgG	IF	1:200	Abbkine	A23420
Goat anti rabbit IgG H&L	IF	1:200	abcam	Ab150079
Dylight 488, Goat Anti-Rabbit IgG	IF	1:200	Abbkine	A23220

3.^{1}H, ^{13}C and MS.

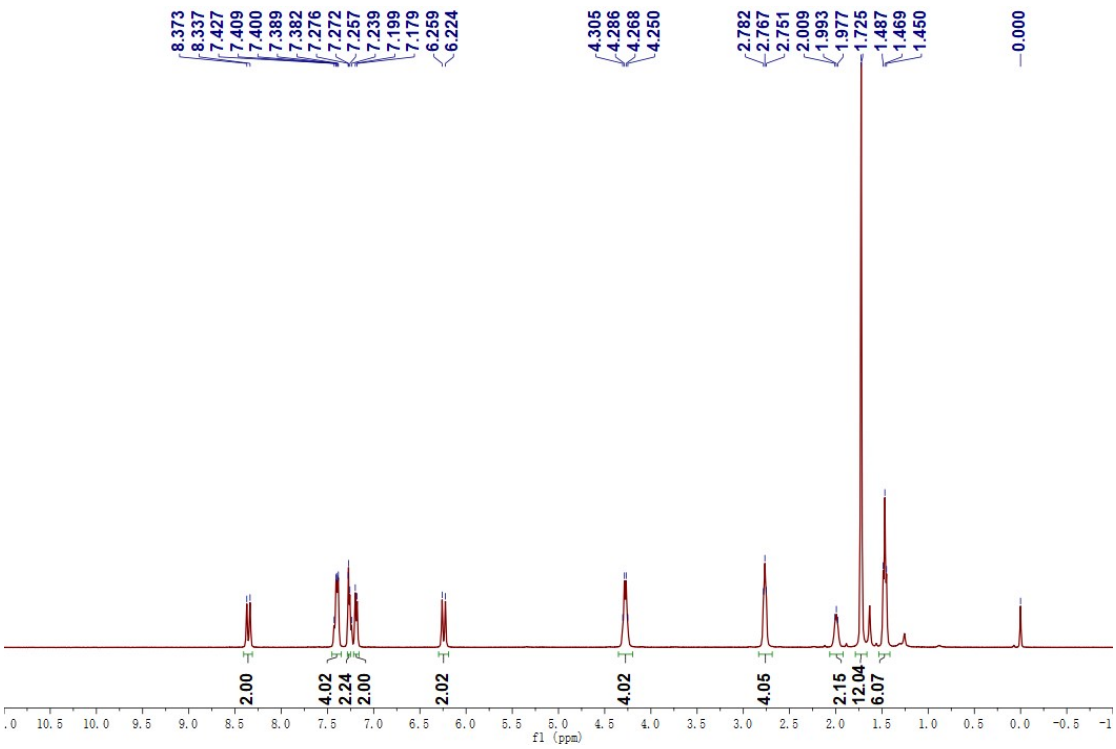


Fig. S1. The ^{1}H NMR of Cy7-Cl.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.35 (d, J = 14.1 Hz, 2H), 7.47 – 7.35 (m, 4H), 7.31 – 7.23 (m, 2H), 7.19 (d, J = 7.9 Hz, 2H), 6.24 (d, J = 14.1 Hz, 2H), 4.28 (q, J = 7.3 Hz, 4H), 2.77 (t, J = 6.1 Hz, 4H), 1.99 (t, J = 6.4 Hz, 2H), 1.73 (s, 12H), 1.47 (t, J = 7.2 Hz, 6H).

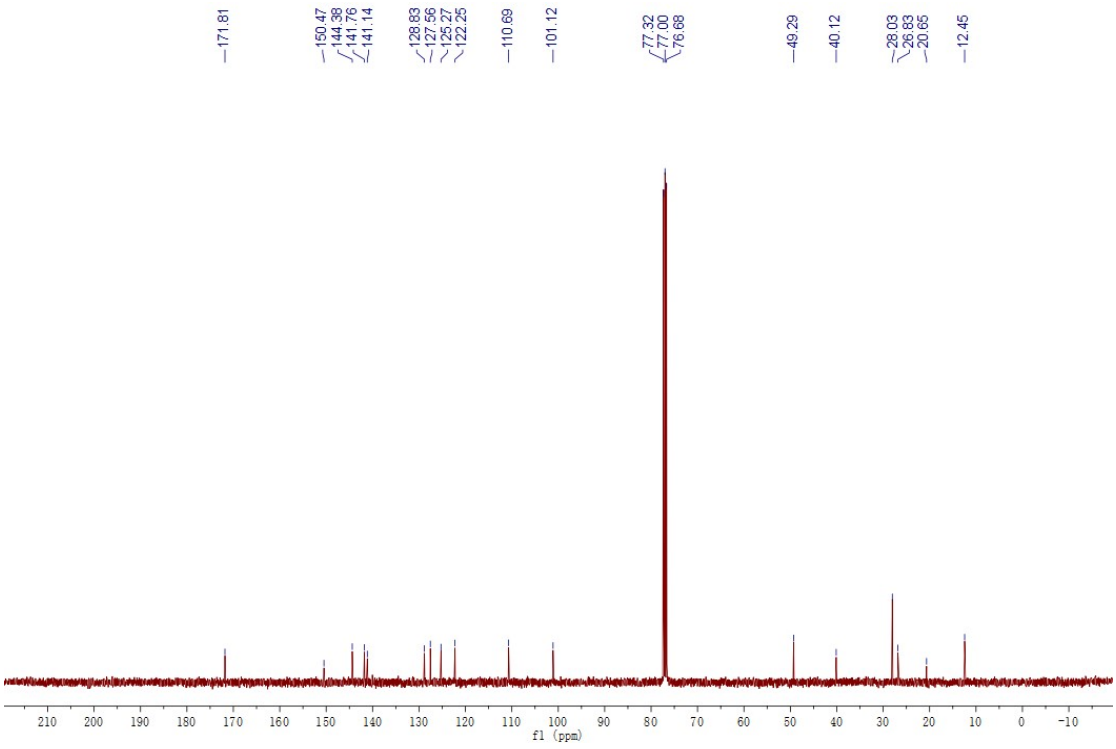


Fig. S2. The ^{13}C NMR of Cy7-Cl.

^{13}C NMR (101 MHz, Chloroform-d) δ 171.8, 150.5, 144.4, 141.8, 141.1, 128.8, 127.6, 125.3, 122.3, 110.7, 101.1, 49.3, 40.1, 28.0, 26.8, 20.7, 12.5.

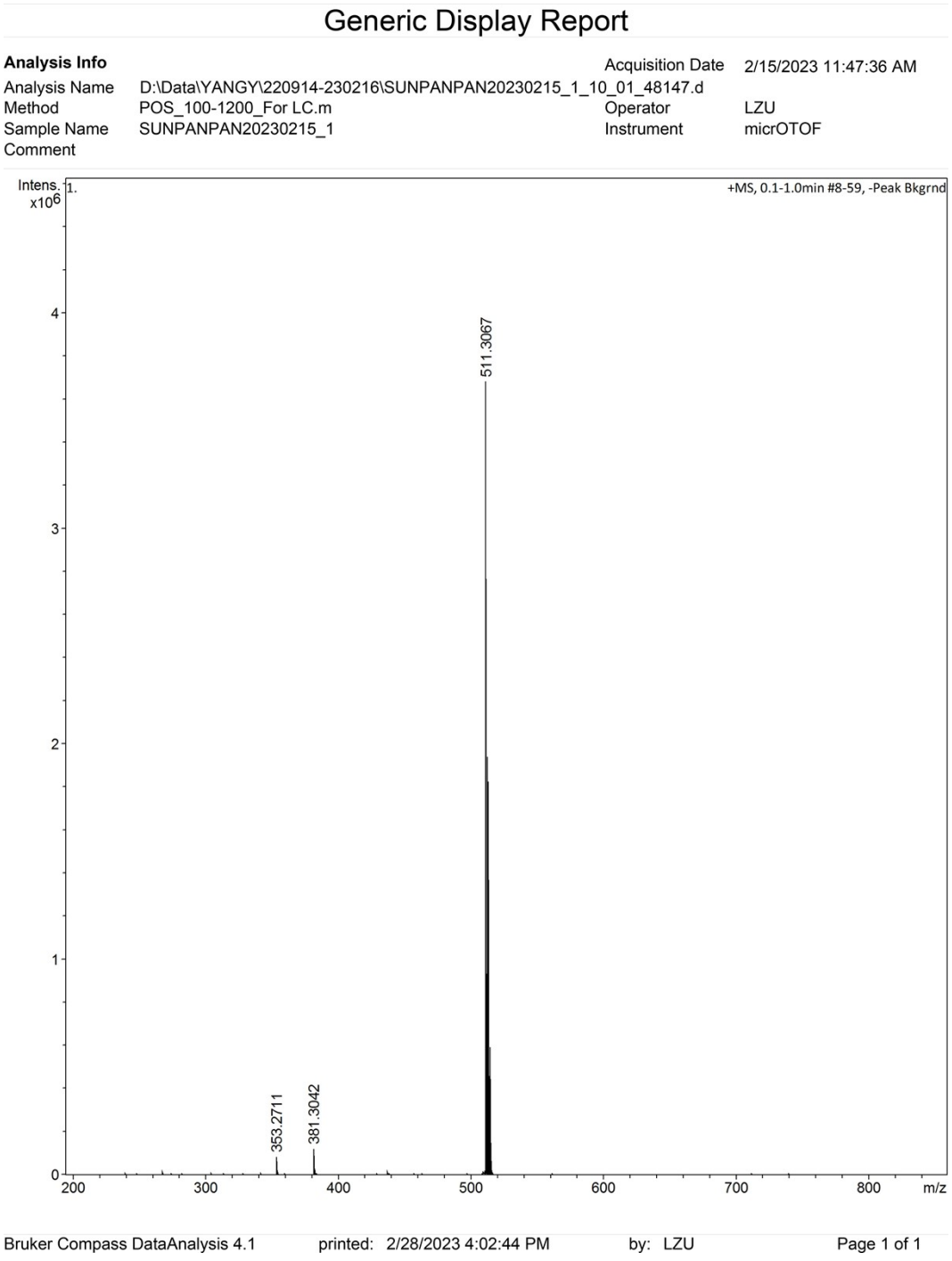


Fig. S3. The MS of Cy7-Cl.

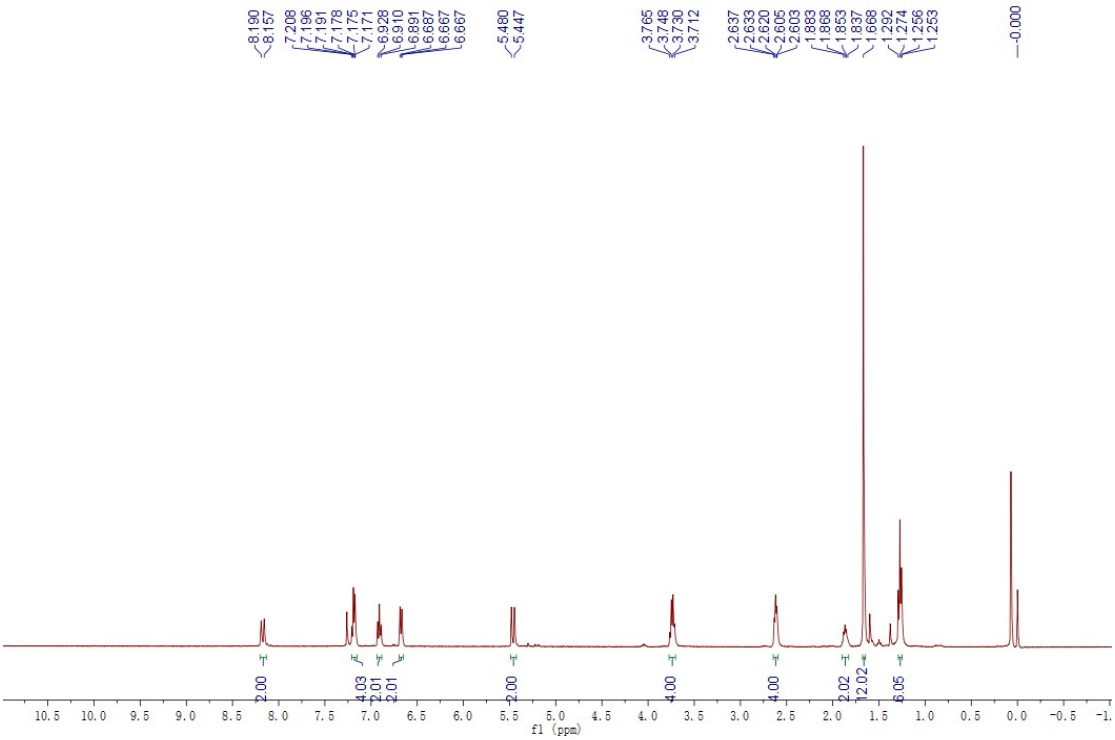


Fig. S4. The ^{1}H NMR of Cy7-K.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.17 (d, J = 13.2 Hz, 2H), 7.21 – 7.15 (m, 4H), 6.92-6.89 (m, 2H), 6.68 (d, J = 8.1 Hz, 2H), 5.46 (d, J = 13.2 Hz, 2H), 3.74 (q, J = 7.1 Hz, 4H), 2.72 – 2.56 (m, 4H), 1.86 (q, J = 6.1 Hz, 2H), 1.67 (s, 12H), 1.27 (t, J = 7.2 Hz, 6H).

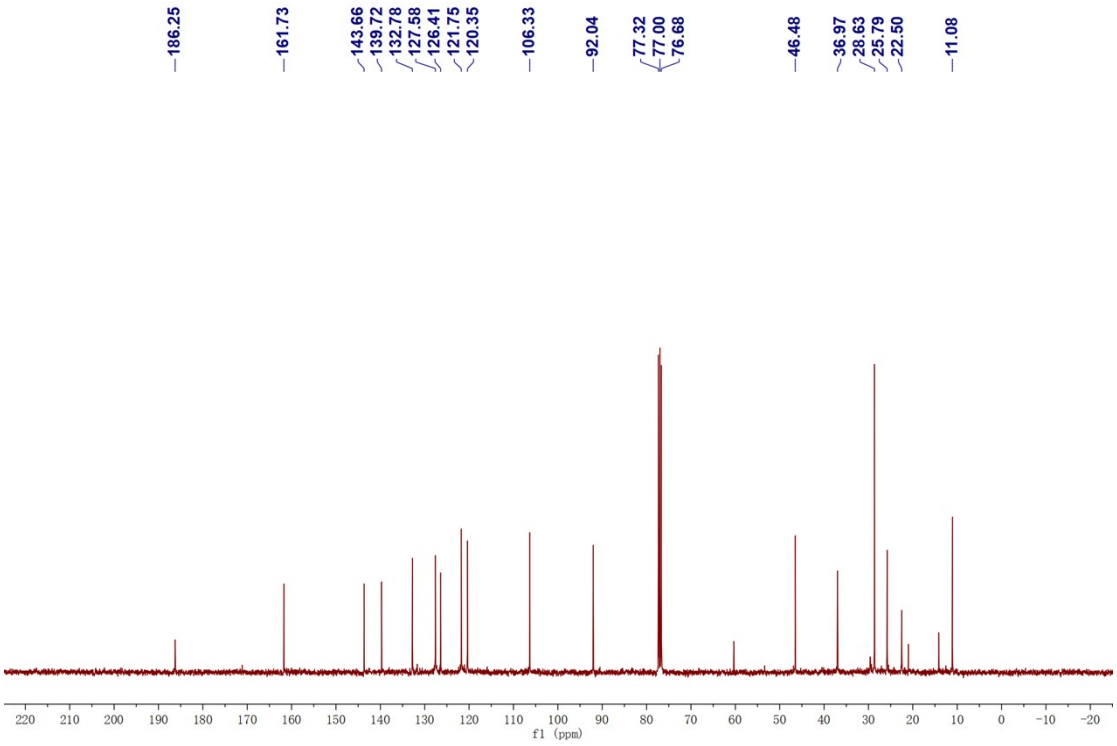


Fig. S5. The ^{13}C NMR of Cy7-K.

^{13}C NMR (101 MHz, Chloroform-d) δ 186.3, 161.7, 143.7, 139.7, 132.8, 127.6, 126.4, 121.8, 120.4, 106.3, 92.0, 46.5, 37.0, 28.6, 25.8, 22.5, 11.1.

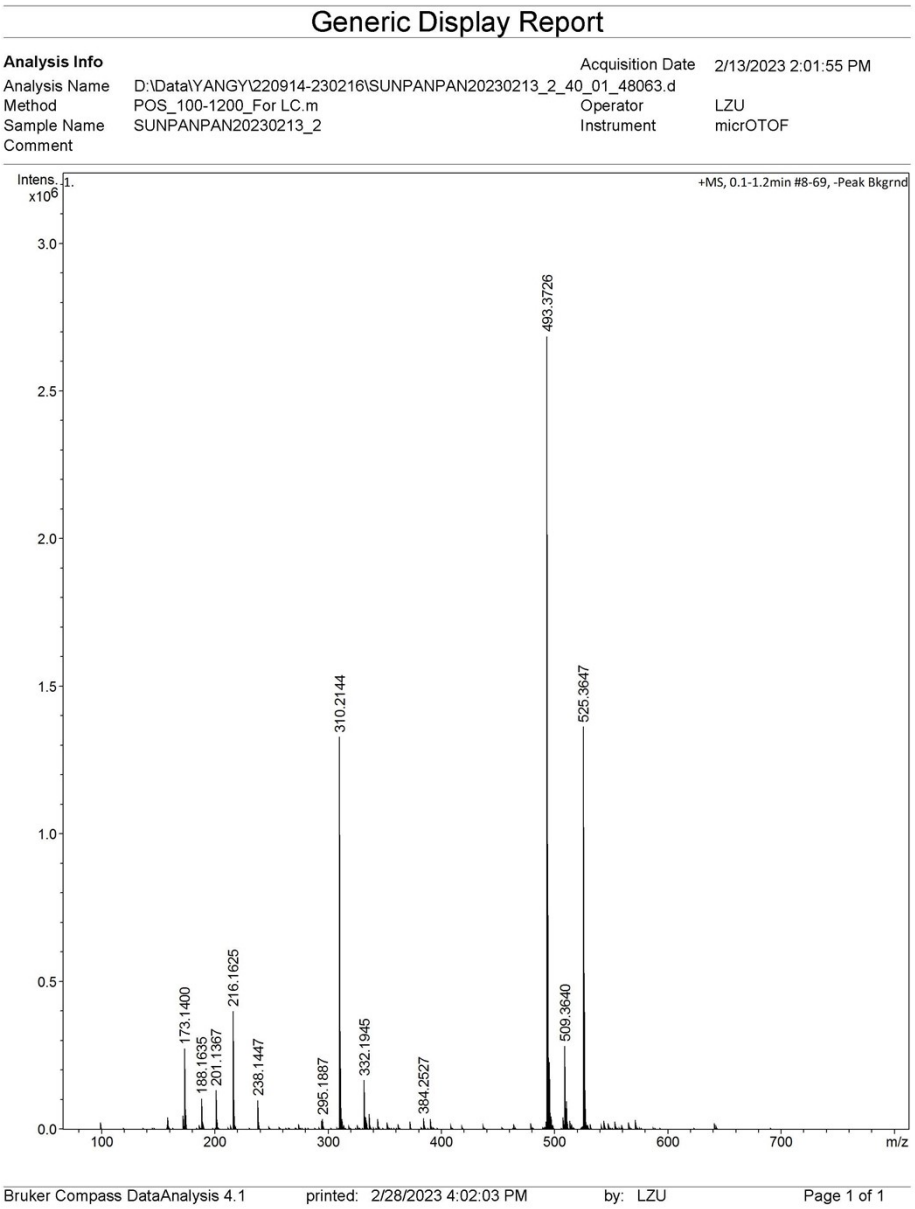


Fig. S6. The MS of Cy7-K.

4. Supporting Figures

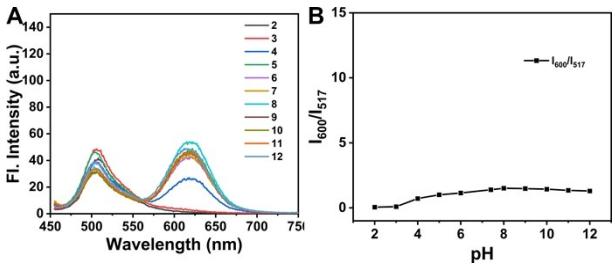


Fig. S7. The pH stability of Cy7-K.

(A) The fluorescence emission spectra of Cy7-K in different pH values. (B) The fluorescence emission ratio of Cy7-K at 600 nm and 517 nm in different pH values.

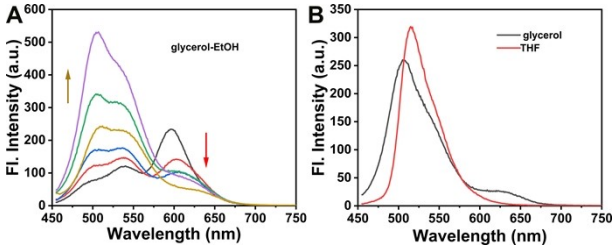


Fig. S8. The viscosity interference of Cy7-K.

(A) The fluorescence spectra of Cy7-K (10 μM) under different viscosity in ethanol -glycerol system with the fraction of glycerol (fw) from 0 to 100%. (B) The fluorescence spectra of Cy7-K in THF and glycerol. Since the fluorescence intensity of the probe Cy7-K in THF is higher than that of glycerol under the same test parameters, the reduced ratio may be caused by the different polarity of glycerol and ethanol.

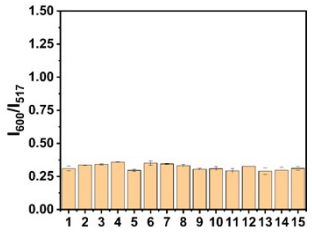


Fig. S9 the selectivity test of Cy7-K.

The ratio of the fluorescence intensities at 600 nm and 517 nm of Cy7-K toward various species in PBS buffer (10 mM, 40%EtOH, pH = 7.4). The metal anions (1 mM) and molecules (100 μM) are as follows: (1) $CuSO_4$•$5H_2O$; (2) $NaNO_2$; (3) $ZnCl_2$; (4) $CaCl_2$; (5) $FeCl_3$•$6H_2O$; (6)$MgSO_4$; (7) $FeCl_2$•$4H_2O$; (8) cysteine; (9) glutathione; (10) glycine; (11) L-alanine; (12) lysine; (13) arginine; (14) serine; (15) ascorbic acid.

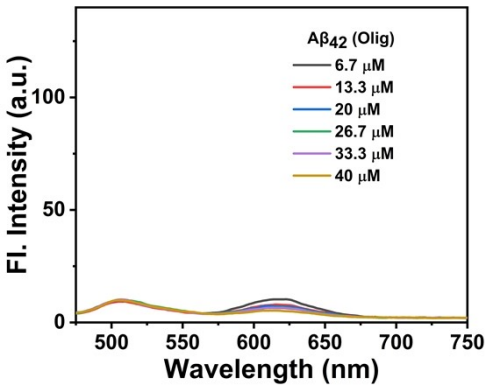


Fig. S10. The fluorescence emission spectra of probe Cy7-K (10 μM) in PBS buffer (10 mM, 40%EtOH, pH = 7.4) with increasing concentration of $A\beta_{42}$ oligomers (6.7, 13.3, 20, 26.7, 33.3 and 40.0 μM).

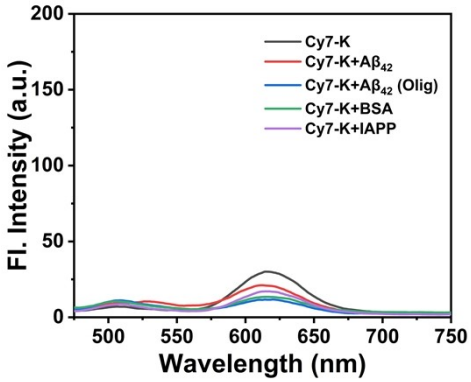


Fig. S11. The fluorescence emission spectra of probe Cy7-K (10 μM) in PBS buffer (10 mM, 40%EtOH, pH = 7.4) upon interaction with $A\beta_{42}$ (50 μM), oligomers of $A\beta_{42}$ (50 μM), hydrophobic protein bovine serum albumin (BSA) (0.9 mg/mL) and amylin (IAPP) (50μM).

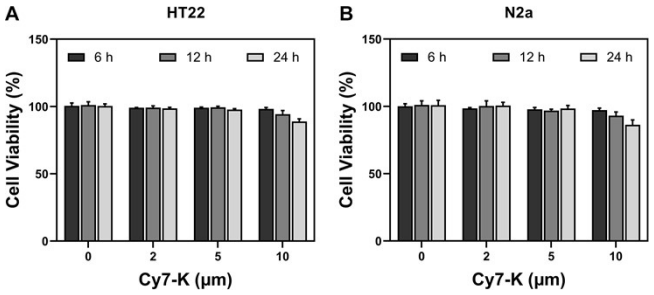


Fig. S12. CCK-8 assay.

CCK-8 assay of HT22 cells and N2a cells treated with Cy7-K with different concentrations (0, 2, 5, 10 μM). Data are presented as means ± SD.

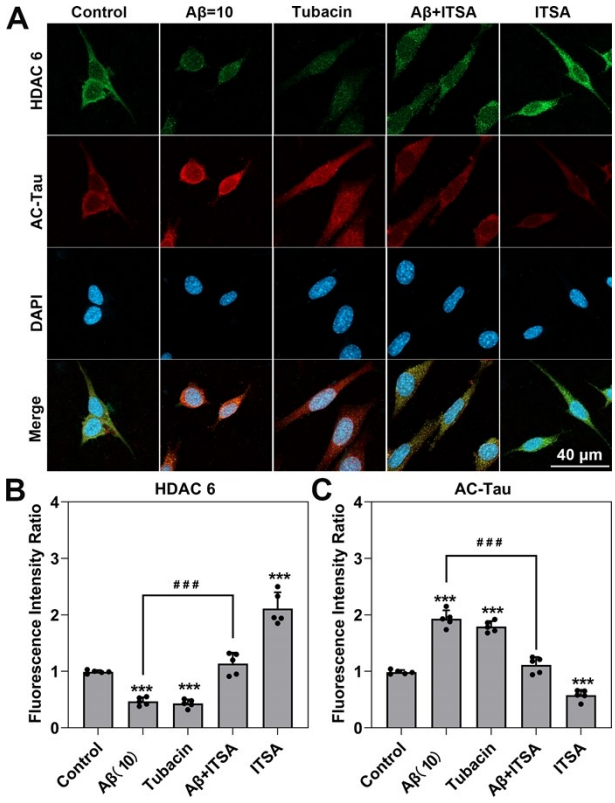


Fig. S13. Representative immunofluorescence pictures of HDAC6 (green) and AC-Tau (red) from HT22 treated with Aβ (10 μM), tubacin (HDAC6 inhibitor), Aβ (10 μM) + ITSA (HDAC6 activator) and ITSA, respectively. Data are presented as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control group. ###P < 0.001 compared with Aβ (10 μM) group. Error bars are ± SD (n = 3).

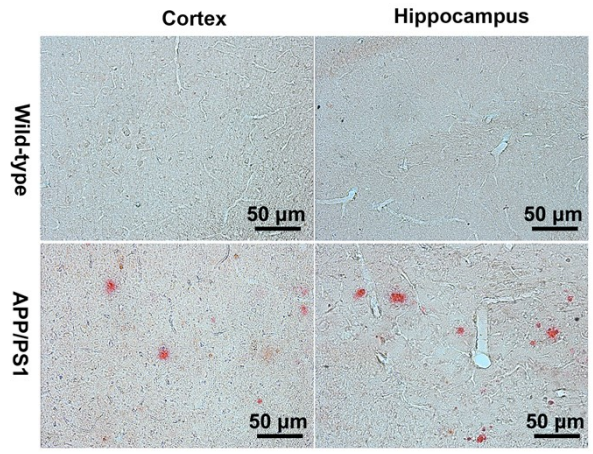


Fig. S14. The cortex and hippocampus of wild mice and APP/PS1 mice were stained with Congo red.

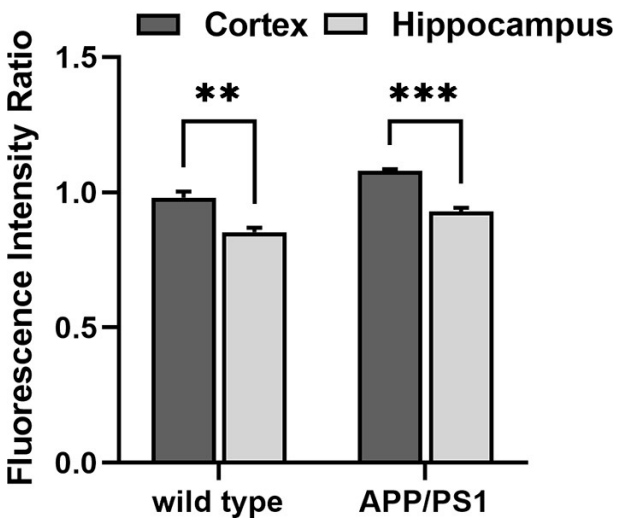


Fig. S15. The relative ratio of fluorescence intensity in hippocampus and cortex of APP/PS1mice and wild-type mice. Statistical analyses of Fig. 6 were performed with image J, *P < 0.05, **P < 0.01, ***P < 0.001 compared with wild-type group. Error bars are ± SEM (n = 3).

References

1. Frisch, M. J. et al. *Gaussian 09, Revision D.01.* (Gaussian Inc., Wallingford CT, 2013).

2. Y. Tan, L. Zhang, K. H. Man, R. Peltier, G. Chen, H. Zhang, L. Zhou, F. Wang, D. Ho, S. Q. Yao, Y. Hu and H. Sun, *ACS Applied Materials & Interfaces,* **2017**, *9*, 6796-6803.