Electronic Supplementary Information

A Novel Two-Photon Ratiometric Fluorescent Probe for Imaging and Sensing of BACE1 in Different Regions of AD Mouse Brain

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Contents

1. Characterization of of Compound 1 and mCyd (Figure S1-S6).

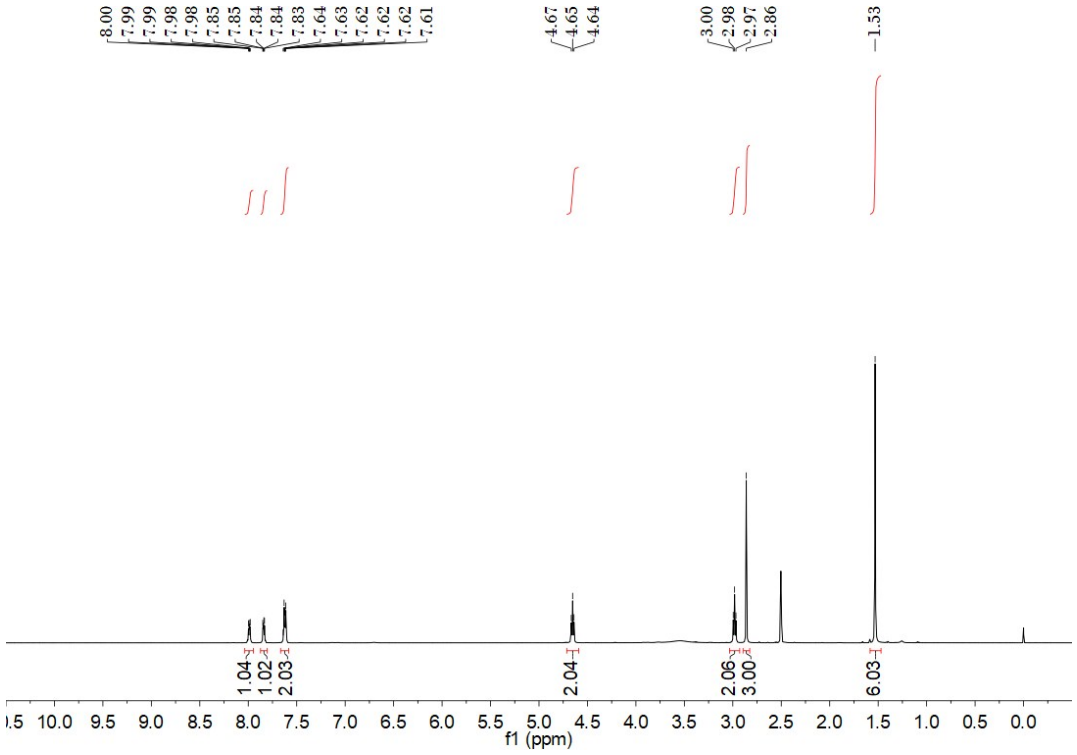


Figure S1. ^{1}H NMR spectrum of Compound 1.

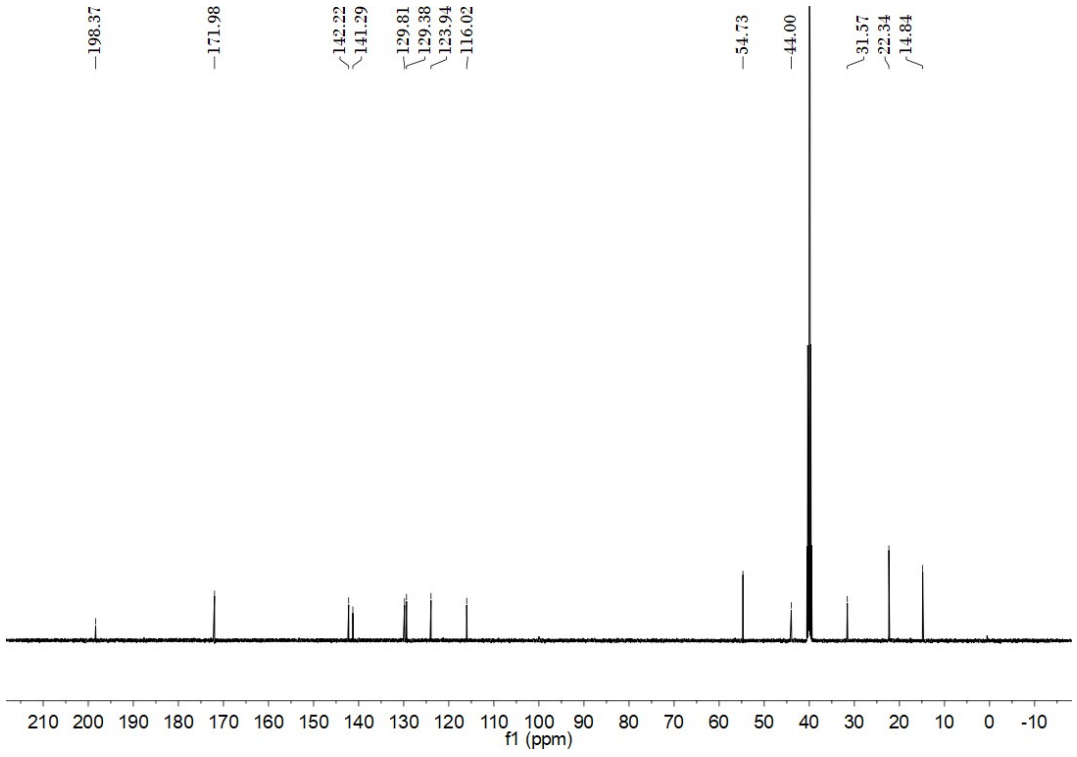


Figure S2. ^{13}C NMR spectrum of Compound 1.

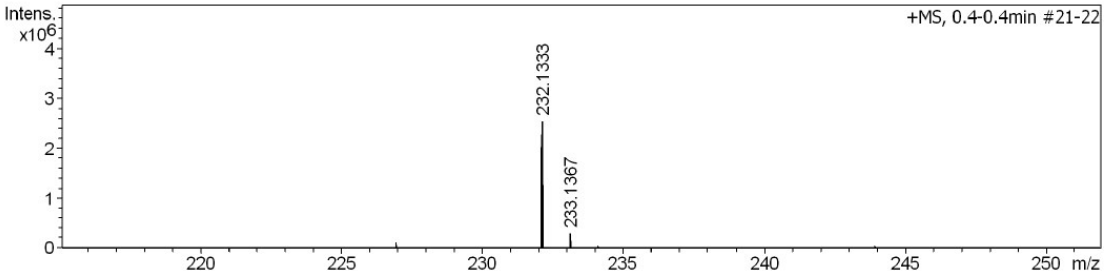


Figure S3. HR-MS of Compound 1.

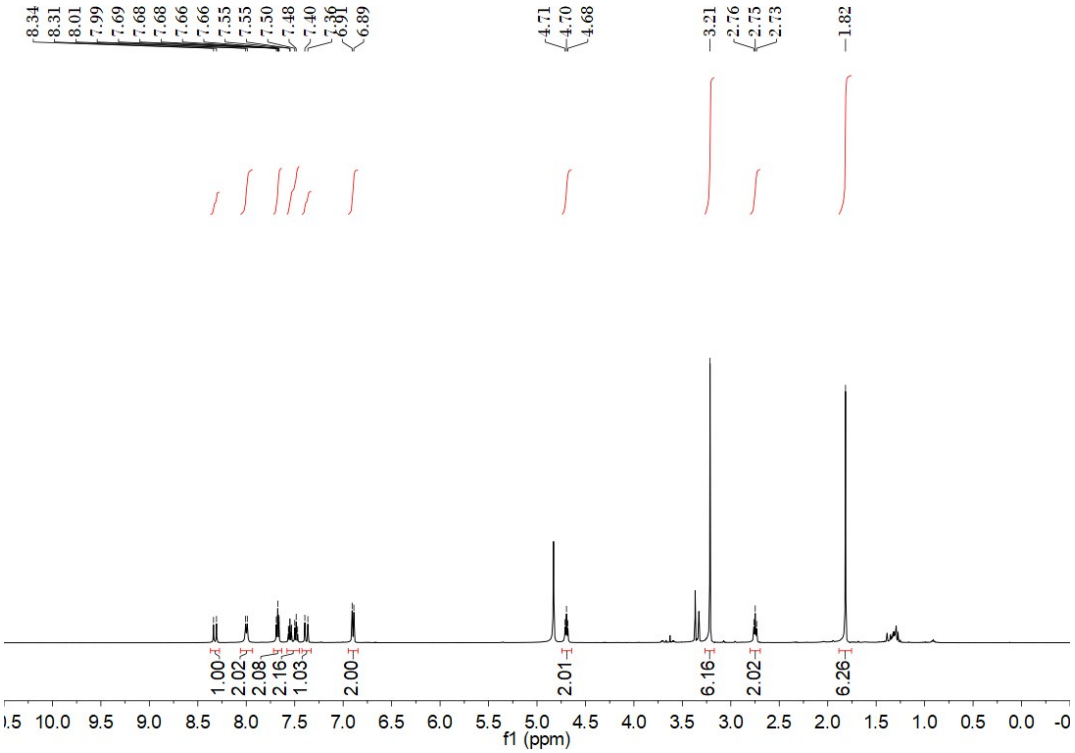


Figure S4. ^{1}H NMR spectrum of mCyd.

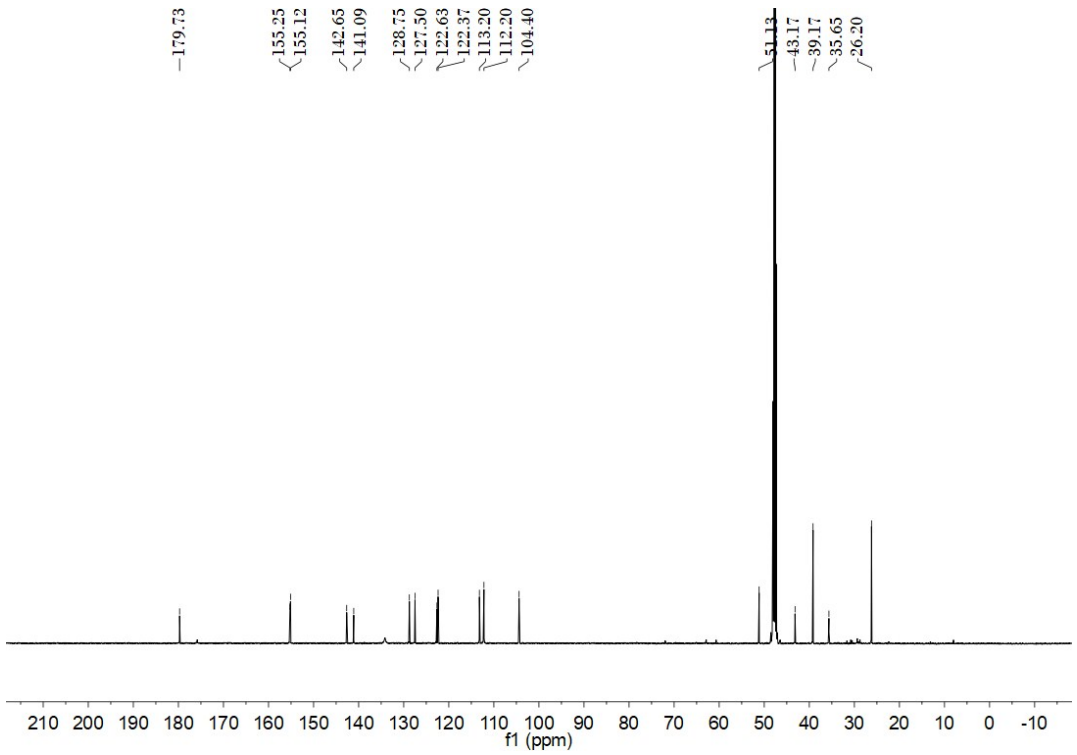


Figure S5. ^{13}C NMR spectrum of mCyd.

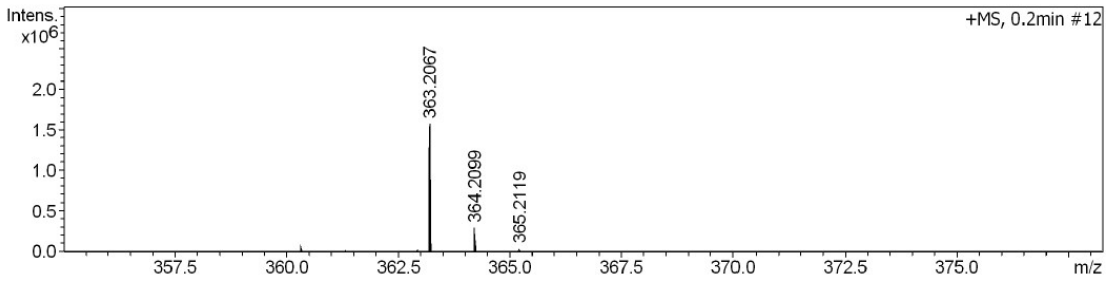


Figure S6. HR-MS of mCyd.

2. Effects of pH on the responses of mCyd, AF633 and AF633mCyd probe (Figure S7).

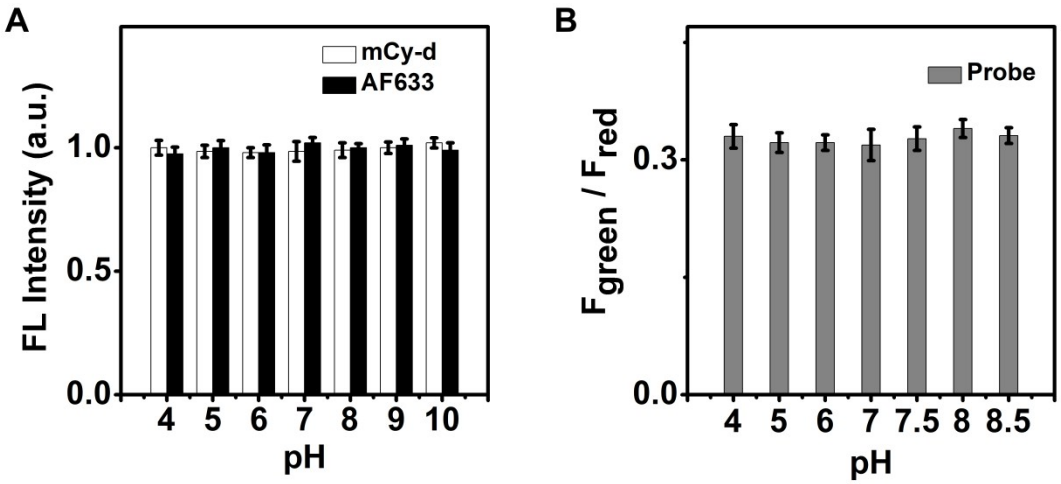


Figure S7. The changes of (A) fluorescence emission peaks ascribed to mCyd (white bars) and AF633 (black bars) dyes at 578 nm and 651 nm, respectively and (B) F_{green}/F_{red} of AF633mCyd probe (gray bars), obtained in different pH values in PBS containing 0.05% DMSO (Error bars, n = 6, SD).

3. Two-photon action spectra of mCyd and AF633 (Figure S8).

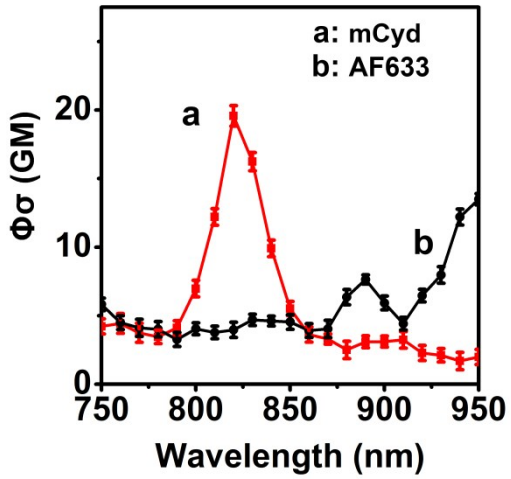
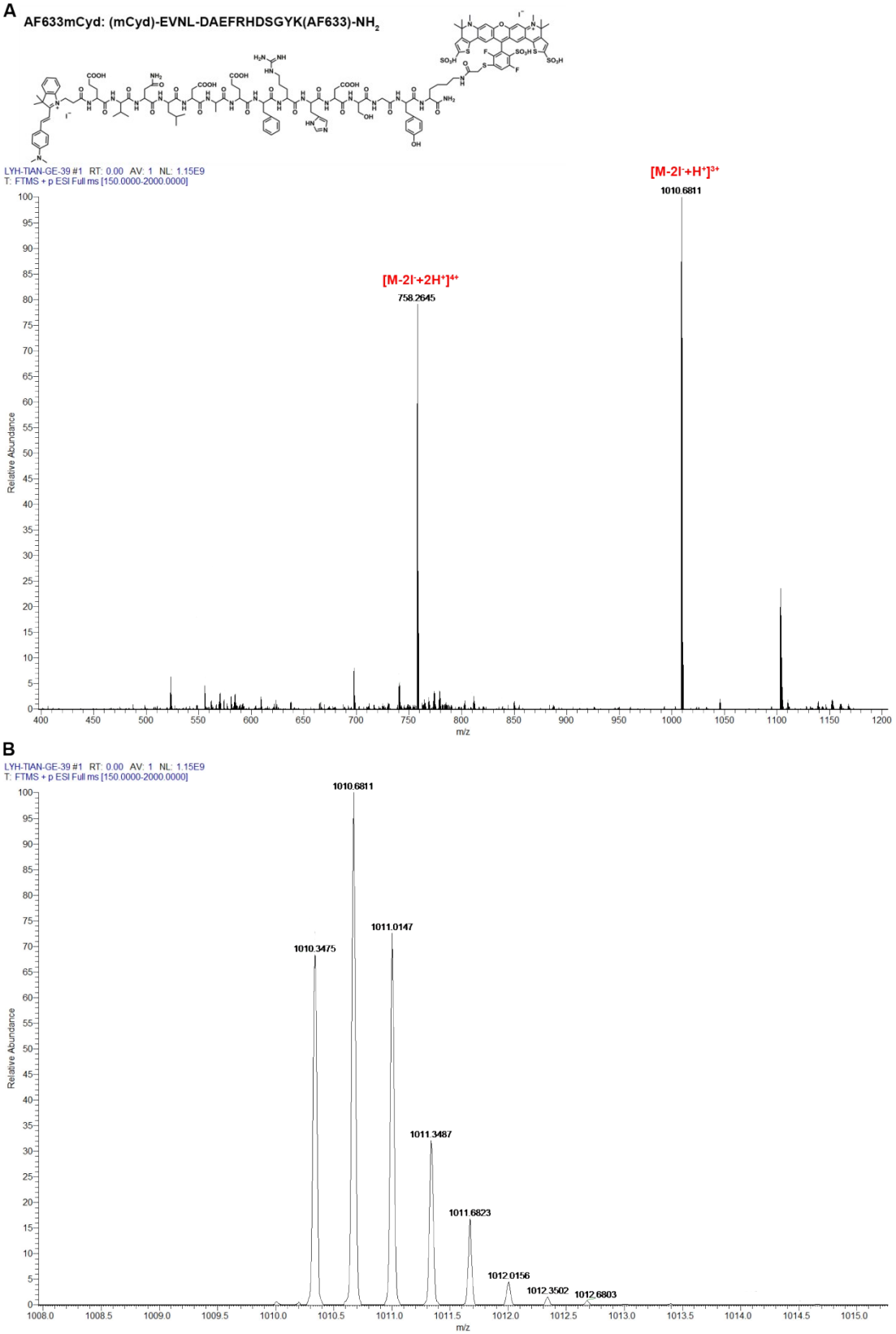


Figure S8. Two-photon action spectra of (a) mCyd and (b) AF633 (Error bars, n = 6, SD). Spectra were measured in 10 mM PBS containing 0.05% DMSO, pH = 4.5.

4. Sensing mechanism of the AF633mCyd probe (Figure S9-S10).



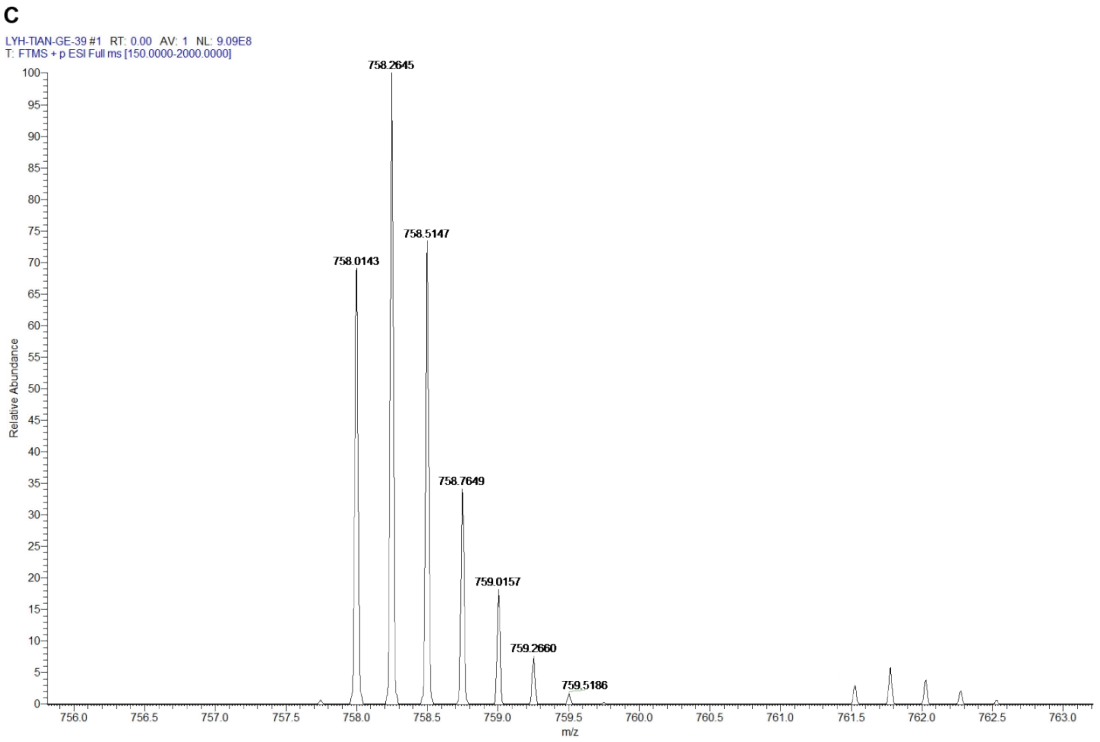
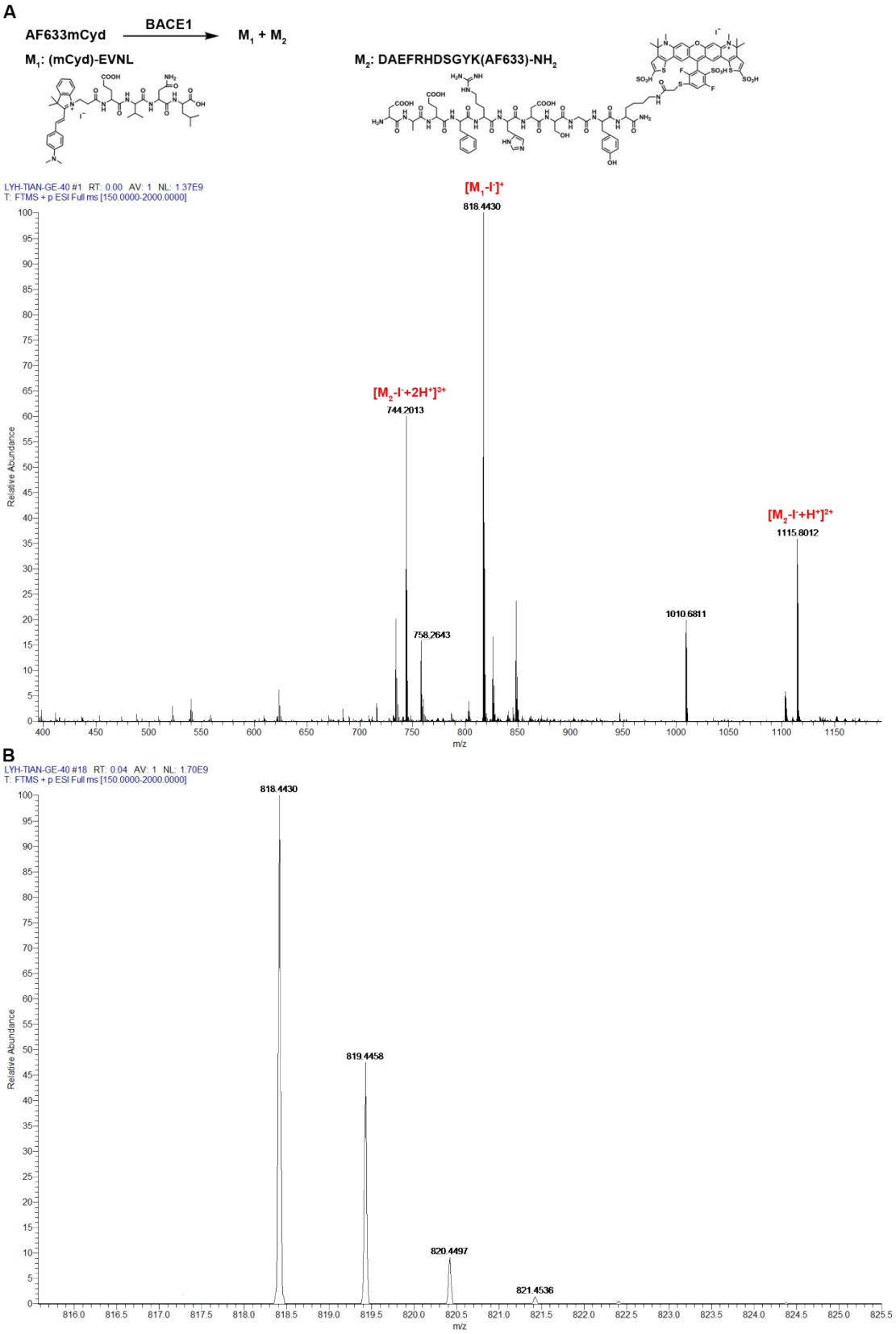


Figure S9. HR-MS of AF633mCyd probe.


A
AF633mCyd
BACE1
M1 + M2
M1: (mCyd)-EVNL
M2: DAEFRHDSGYK(AF633)-NH2
LYH-TIAN-GE-40 #1 RT: 0.00 AV: 1 NL: 1.37E9
T: FTMS + p ESI Full ms [150.0000-2000.0000]
[M1-I-]+
818.4430
[M2-I-+2H+]3+
744.2013
[M2-I-+H+]2+
1115.8012
1010.6811
758.2643
Relative Abundance
m/z
B
LYH-TIAN-GE-40 #18 RT: 0.04 AV: 1 NL: 1.70E9
T: FTMS + p ESI Full ms [150.0000-2000.0000]
818.4430
819.4458
820.4497
821.4536
Relative Abundance
m/z

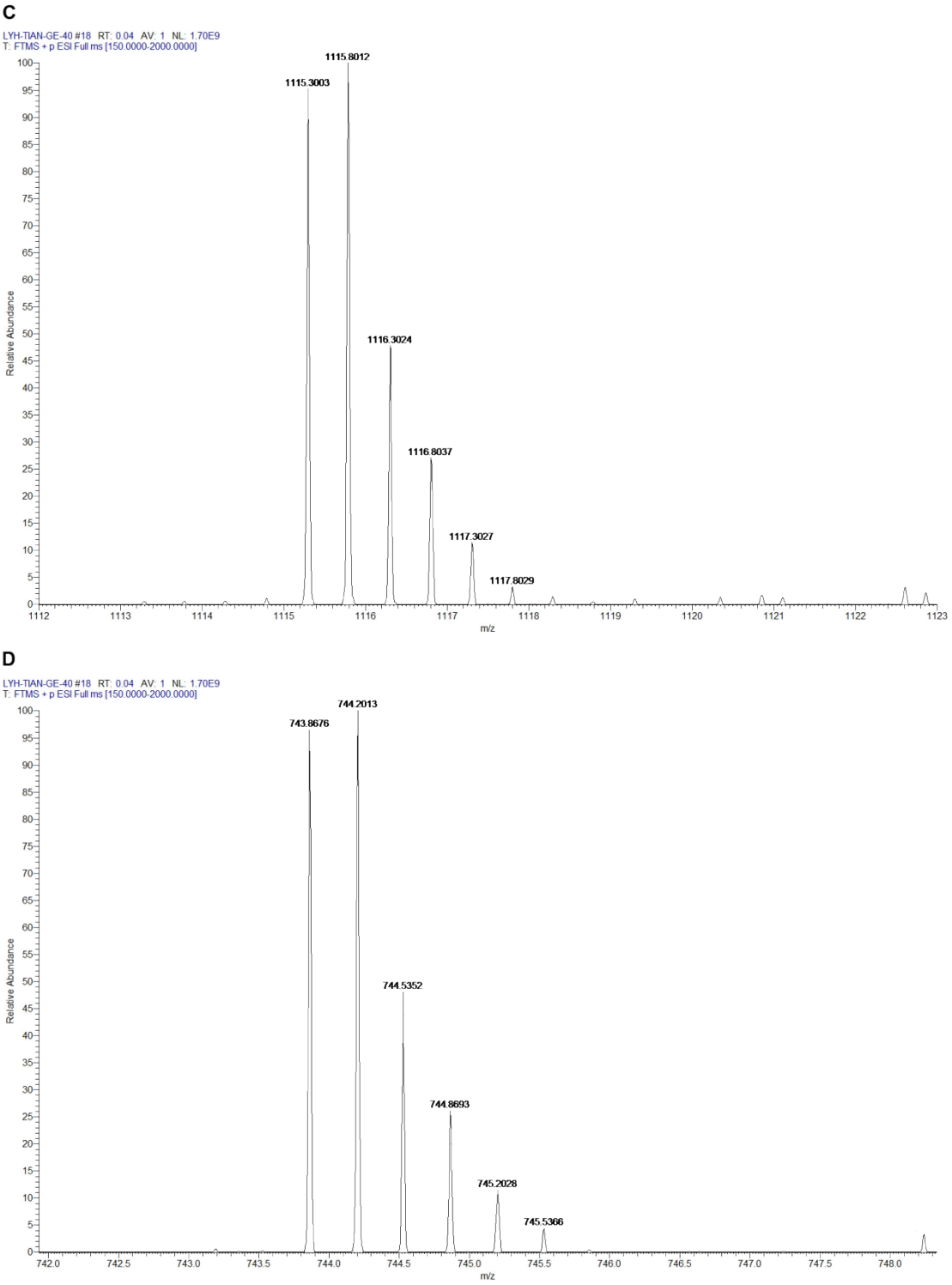


Figure S10. HR-MS of N-terminus (M_1) and C-terminus (M_2) fragments of the AF633mCyd probe cleaved at the β site by BACE1.

5. Selectivity and competition tests of the AF633mCyd probe toward determination of BACE1 (Figure S11).

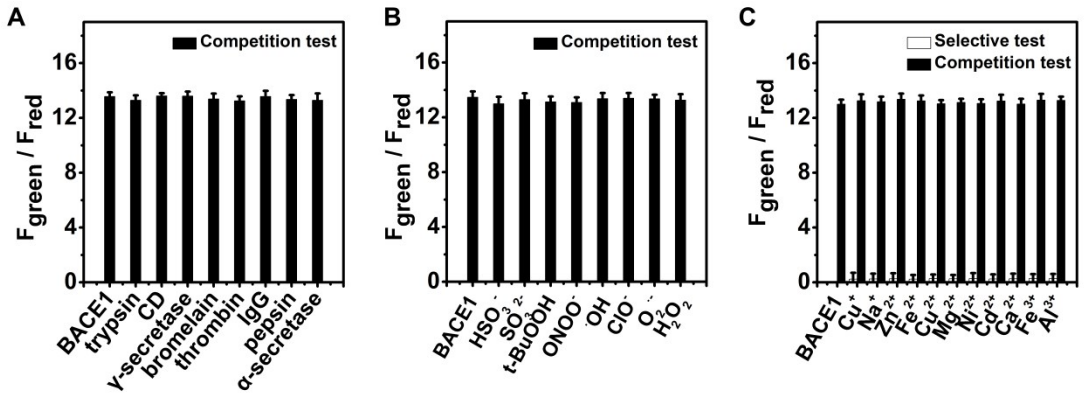


Figure S11. Competition tests of 5.0 μM AF633mCyd probe toward (A) proteins (500.0 nM for each), (B) ROS and other anions (1.0 mM for each). (C) Selectivity (white bars) and competition tests (black bars) of 5.0 μM AF633mCyd probe toward metal anions (1.0 mM for each) (Error bars, n = 6, SD).

6. FACS and MTT measurements of the AF633mCyd probe in live cells (Figure S12-S14).

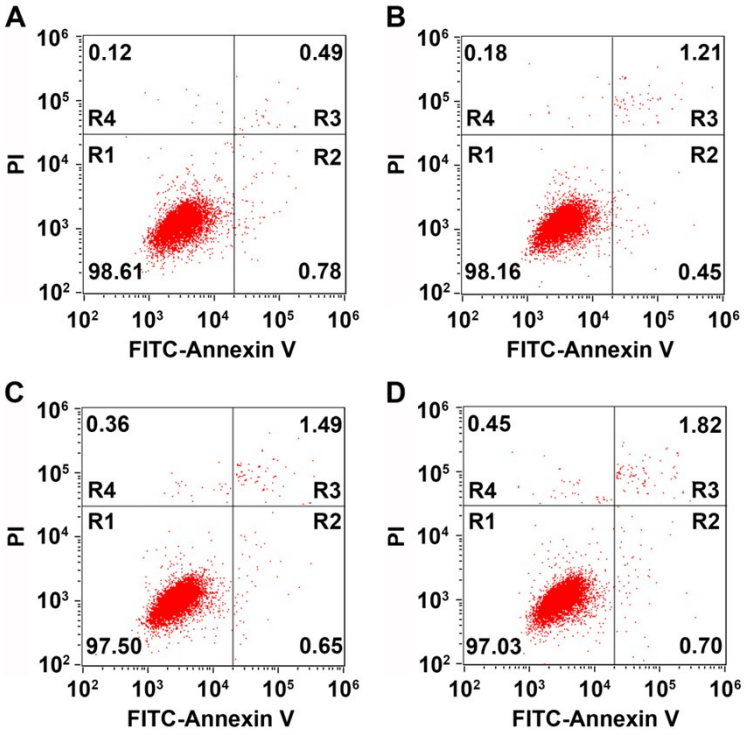


Figure S12. Apoptosis assay of the AF633mCyd probe at concentrations of (A) 0.0, (B) 20.0, (C) 40.0 and (D) 80.0 μM incubated with neurons for 24 h. The quadrants of R1, R2, R3 and R4 show the live, early apoptotic, late apoptotic and dead cells, respectively.

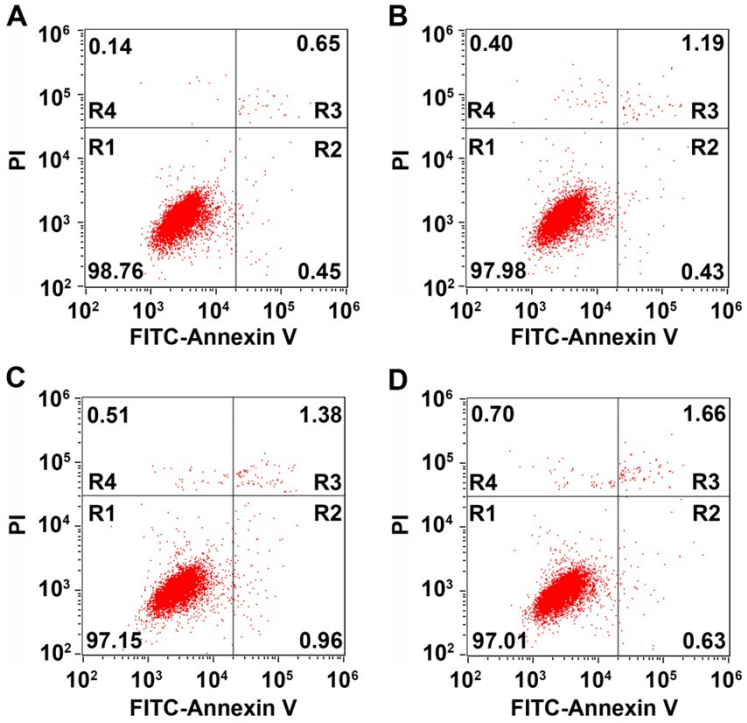


Figure S13. Apoptosis assay of the AF633mCyd probe at concentrations of (A) 0.0, (B) 20.0, (C) 40.0 and (D) 80.0 μM incubated with SHSY-5Y cells for 24 h. The quadrants of R1, R2, R3 and R4 represent the live, early apoptotic, late apoptotic and dead cells, respectively.

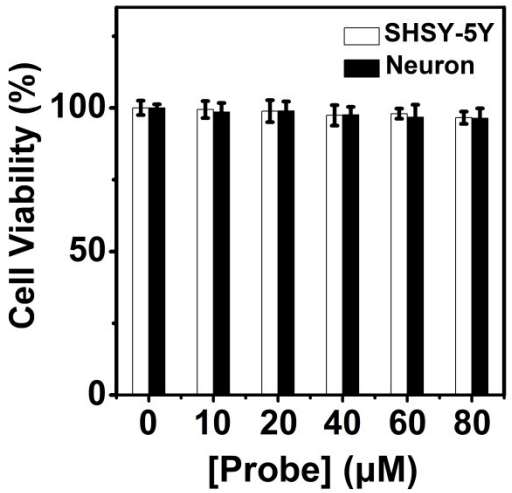


Figure S14. MTT assay of the AF633mCyd probe at concentrations of 0.0, 10.0, 20.0, 40.0, 60.0 and 80.0 μM incubated with SHSY-5Y cells (white bars) and neurons (black bars) for 24 h (Error bars, n = 6, SD).

7. MTT measurement of $O_2^{\bullet-}$ toward live cells (Figure S15).

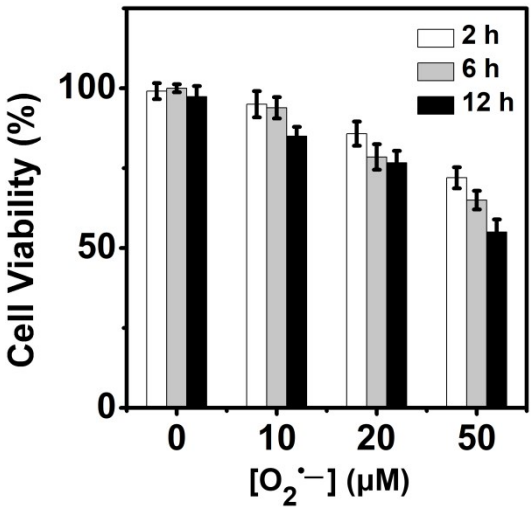


Figure S15. MTT assay of $O_2^{\bullet-}$ at concentrations of 0.0, 10.0, 20.0 and 50.0 μM toward neurons for 2 h (white bars), 6 h (gray bars) and 12 h (black bars) (Error bars, n = 6, SD).

8. Picture of mouse brain slice (Figure S16).

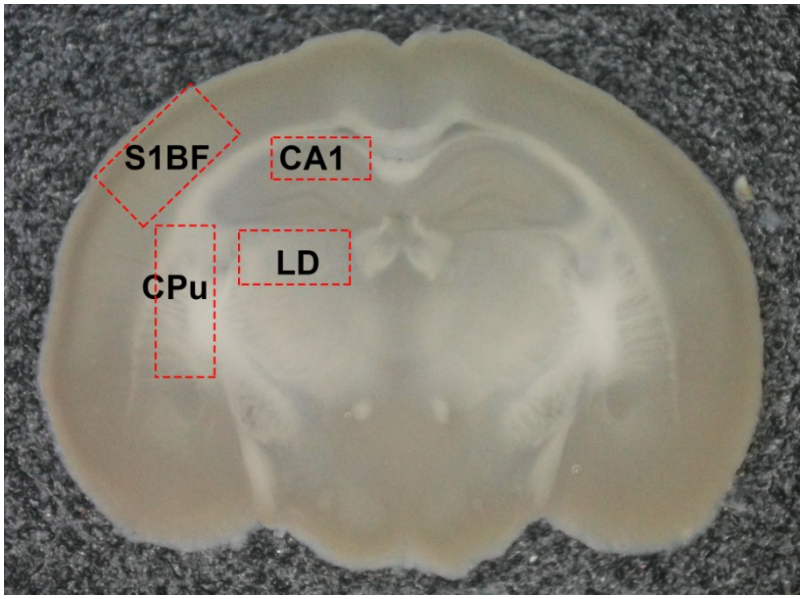


Figure S16. Picture of the mouse brain slice about -1.2 mm from the bregma.

9. Western Blot images of BACE1 in different regions of mouse brain (Figure S17).

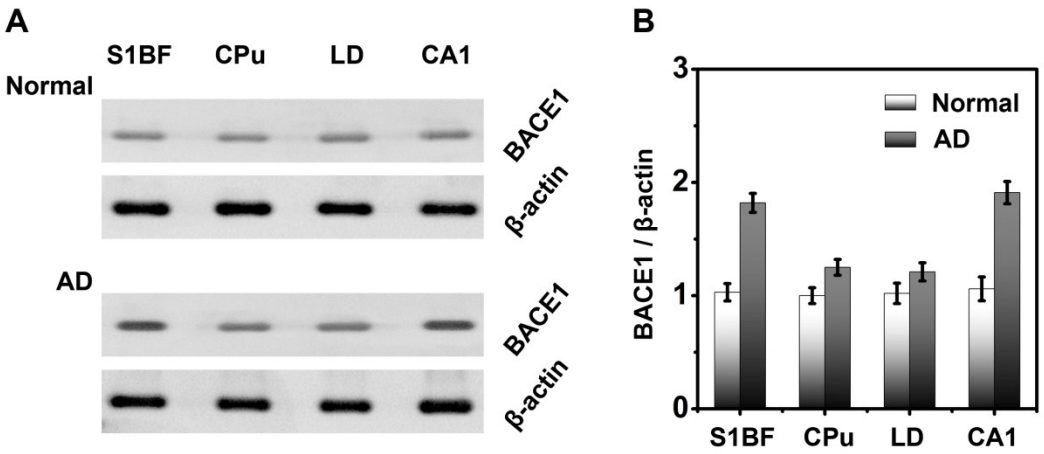


Figure S17. (A) Western Blot images of BACE1 and β-actin proteins in the S1BF, CPu, LD and CA1 regions of AD and normal mouse brain. (B) Ratios of band density changes in A. The band densities were calculated by NIH ImageJ software. More than 20 mice were measured for the statistical analyses (Error bars, n = 20, SD). Proteins were visualized with BACE1 and β-actin specific antibodies, respectively.