Electronic Supplementary Information

Fluoro-Substituted Cyanine for Reliable in vivo Labelling Amyloid-β Oligomers and Neuroprotection against Amyloid-β Induced Toxicity

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**Materials and general instruments.** All chemicals for the synthesis of probe were purchased from commercial suppliers and used without further purification. Aβ_{1-40} and Aβ_{1-42} was purchased from r-Peptide (GA, USA). The antibodies were purchased as follows: Aβ oligomer-specific monoclonal antibody (Agirisera, Sweden), Aβ monoclonal antibody (Sigma-Aldrich), Aβ_{1-40} and Aβ_{1-42} polyclonal antibodies (Cell Signaling), or conformation-dependent tau antibody MC1 (Peter Davies), at dilutions from 1:400 to 1:100. The AD transgenic mice model were obtained as the previously described.\(^1\) Stock Aβ_{1-40} solution was prepared by dissolving 1 mg Aβ_{1-40} powder in 400 μL of 1 % ammonium solution without any purification and stored at -20 °C prior to use. To prepare the oligomeric and fibrillar form of Aβ_{1-40}, 50 μM Aβ_{1-40} monomer in 0.1 M phosphate buffer (PB) at 37 °C for 3 hours and 24 hours respectively. All working solutions were prepared by successive dilution of the stock solution with PBS (20 mM, pH 7.4).

\(^1\)H NMR spectra were recorded at 400 MHz, and \(^{13}\)C NMR spectra were recorded at 100 MHz on a Bruker advanced-III 400 NMR spectrometer. Mass spectroscopic (MS) measurements were measured using fast atom bombardment (FAB) on the API ASTAR pulsar I Hybrid Mass Spectrometer or matrix-assisted desorption ionization-time-of-flight (MALDI-TOF) technique. UV-Vis absorption spectra were recorded in 1.0 cm path length quartz cuvettes on a Varian Cary 100-UV-Vis spectrophotometer. Fluorescence emission spectra were measured on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ). Fluorescence images of tissue slices were obtained by using an laser scanning confocal microscope (BX51, Olympus, Japan) equipped with a DP72 digital camera. In vivo images were acquired by using an IVIS Imaging System (Lumina xr).

**Experimental:**

**Synthesis of 3-fluoro-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole (3).** To a solution of 3-bromo-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole (3.23 g, 9.3 mmol) in dry THF (50 mL) was added \(n\)-BuLi (1.6 M, 8.7 mL, 13.9 mmol) at -78 °C. The resulting mixture was stirred for 50 min at -78 °C and then added with N-Fluorobenzenesulfonylimide (5.6 g, 18.6 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h before quenched with ammonia.
chloride solution. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was purified by silica gel column chromatography eluting with 3:1 petroleum ether/ethyl acetate to give compound 4 in 65% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.03 (d, $J$ = 7.6 Hz, 1H), 7.73 (dd, $J$ = 2.4 Hz, $J$ = 8.8 Hz 1H), 7.50-7.44 (m, 2H), 7.39 (dd, $J$ = 4.4 Hz, $J$ = 8.8 Hz, 1H), 7.25-7.17 (m, 2H), 4.49 (t, $J$ = 6.4 Hz, 2H), 3.86 (t, $J$ = 6.4 Hz, 2H), 3.52-3.50 (m, 2H), 3.43-3.41 (m, 2H), 3.32 (s, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 158.6, 156.2, 141.4, 137.1, 126.3, 123.4, 123.3, 122.6, 122.6, 120.6, 119.1, 113.6, 113.3, 109.7, 109.6, 109.2, 106.1, 105.9, 72.1, 71.0, 69.4, 59.2, 43.4. HRMS (MALDI-TOF) m/z Calcd for C$_{17}$H$_{18}$FNO$_2$ 287.1316, Found 287.1314 [M]$^+$

**Synthesis of 3-bromo-6-fluoro-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole (4).** To a solution of compound 3-fluoro-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole (1.06 g, 3.71 mmol) in chloroform (20 mL) was added NBS (0.66 g, 3.71 mmol) batch wise in an ice-water bath. After addition, the reaction mixture was allowed to warm to room temperature slowly and stirred overnight. The reaction mixture was washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give compound 5 in 85% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.09-8.06 (m, 1H), 7.63-7.60 (m, 1H), 7.52-7.50 (m, 1H), 7.36-7.26 (m, 2H), 7.21-7.16 (m, 1H), 4.40 (d, $J$ = 5.6 Hz, 2H), 3.82-3.80 (m, 2H), 3.49-3.46 (m, 2H), 3.40-3.38 (m, 2H), 3.29 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 158.7, 156.3, 140.1, 137.4, 128.9, 124.2, 124.2, 123.2, 122.3, 122.2, 114.4, 114.1, 111.8, 110.8, 110.0, 109.9, 106.2, 106.0. HRMS (MALDI-TOF) m/z Calcd for C$_{17}$H$_{17}$BrFNO$_2$ 366.0499, Found 366.0502 [M]$^+$

**Synthesis of 6-bromo-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole-3-carbaldehyde (5).** To a solution of 3-bromo-6-fluoro-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole (3.4 g, 9.3 mmol) in dry THF (50 mL) was added n-BuLi (1.6 M, 8.7 mL, 13.9 mmol) at -78 $^\circ$C. The resulting mixture was stirred for 50min at -78 $^\circ$C and then added with N-formylmorpholine (1.86 mL, 18.6 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2h before quenched with ammonia chloride solution. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was purified by silica gel column chromatography eluting with 2:1 petroleum ether/ethyl acetate to give compound 6 in 60% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.08 (s, 1H), 8.53 (s, 1H), 8.02-8.00 (m, 1H), 7.80-7.77 (m,
1H), 7.54 (d, J = 8.8 Hz, 1H), 7.45 (dd, J = 4.0 Hz, J = 9.2 Hz 1H), 7.28-7.23 (m, 1H), 4.52 (t, J = 5.6 Hz, 2H), 3.88 (t, J = 5.6 Hz, 2H), 3.52-3.50 (m, 2H), 3.40 (d, J = 2.8 Hz, 2H), 3.28 (s, 3H). 13C NMR (100 MHz, CDCl3) δ 191.8, 159.3, 156.9, 145.2, 137.8, 128.8, 127.5, 124.4, 123.7, 123.7, 123.7, 122.8, 114.8, 114.5, 110.6, 110.5, 109.8, 106.7, 106.4, 72.1, 71.0, 69.5, 59.2, 43.9. HRMS (MALDI-TOF) m/z Calcd for C18H18FNO3 316.1343, Found 316.1340[M]+

**Synthesis of (E)-4-(2-(6-fluoro-9-(2-(2-methoxyethoxy) ethyl)-9H-carbazol-3-yl)vinyl)-1-(2-hydroxyethyl)-quinolin-1-ium chloride (F-SLOH).** A solution mixture of 6 (0.25 g, 0.8 mmol), 6 (0.25 g, 1.1 mmol) and piperidine (0.1 mL) in ethanol (40 mL) was heated to reflux overnight. After cooling down to room temperature, the organic solvent was removed. The residue was purified by precipitation from methanol and ethyl acetate to afford F-SLOH in 57% yield. 

1H NMR (400 MHz, DMSO-d6) δ 9.23 (d, J = 6.8 Hz, 1H), 9.16 (d, J = 8.4 Hz, 1H), 8.91 (s, 1H), 8.59 (d, J = 8.8 Hz, 1H), 8.54 (d, J = 6.8 Hz, 1H), 8.41 (d, J = 3.6 Hz, 2H), 8.24 (t, J = 7.6 Hz, 1H), 8.15-8.13 (m, 1H), 8.08-8.03 (m, 2H), 7.79 (d, J = 8.8 Hz, 1H), 7.73 (dd, J = 4.4 Hz, J = 8.8 Hz 1H), 7.40-7.35 (m, 1H), 5.34 (s, 1H), 5.08-5.05 (m, 2H), 4.65-4.62 (m, 2H), 3.94-3.92 (m, 2H), 3.84-3.81 (m, 2H), 3.46-3.50 (m, 2H), 3.30-3.27 (m, 2H), 3.31-3.29 (m, 2H), 3.10 (s, 3H). 13C NMR (100 MHz, CDCl3) δ 158.3, 155.9, 153.2, 147.9, 144.7, 142.9, 138.2, 137.5, 134.8, 128.8, 127.9, 126.8, 126.7, 126.6, 122.8, 122.7, 122.4, 122.2, 119.3, 116.6, 115.0, 114.1, 113.8, 111.6, 111.5, 110.7, 106.2, 106.0, 71.3, 69.8, 69.0, 59.0, 58.1, 43.5, 22.1. HRMS (MALDI-TOF) m/z Calcd for C36H30FN2O4 [M]+ 485.2211, Found 485.2235.

**Physical Measurements.** Background subtractions were routinely performed in all these assay measurements. The error bars are obtained by calculating three groups experiment data to get the “Mean ± S.D.”

**Preparation of Aβ monomer, oligomer and fibril**

Monomeric Aβ_{1-40} was purchased from r-peptide. The Aβ_{1-40} stock solution was prepared by dissolving 1 mg Aβ powder in 100% hexafluoroisopropanol, dried under vacuum and redispersed in 400 μL of 1% ammonium solution. The Aβ oligomer was prepared by diluting the Aβ_{1-40} monomer to 50 μM with phosphate buffer (PB) and incubated at 37°C for 3 h; while the Aβ fibril was prepared by incubating 50 μM Aβ_{1-40} monomer at 37°C for 24 h.
**In-Vitro Binding Assays.** To obtain the apparent binding constants ($K_d$), a solution of 500 μL containing Aβ species (1.0 μM) with the addition of different concentrations of F-SLOH or SLOH was measured by a fluorescence spectrometer after incubation for 3 min at room temperature with excitation wavelength of 470 nm. The fluorescence intensity maximum ($F_{\text{max}}$) corresponding to the concentration of F-SLOH or SLOH were recorded. Then, the $F_{\text{max}}$ were calculated according to the linear regression of $1/(x\text{-intercept})$ to get the $K_d$ value.

**Circular Dichroism (CD) measurements.** The solution of Aβ monomers, oligomers and fibrils (20 μM) was prepared with PBS respectively. CD characteristic spectra of 20 μM F-SLOH and, Aβ solutions with different forms in the presence or absence of 20 μM F-SLOH was then scanned from 180 to 320 nm with 1 nm bandwidth for three times by Jasco J-810 Circular Dichroism Spectropolarimeter after incubation for 3 mins.

**Inhibition effect of F-SLOH on Aβ$_{1-40}$ fibrillation.** To study the inhibitory effect of F-SLOH, F-SLOH solution (50 μM) was co-incubated with 50 μM Aβ monomers in 20 mM PBS at 37 °C for 24 hr. The resultant mixtures were then labeled with ThT (molar ratio of ThT/Aβ is 1:2) for visualizing under a prism-type TIRFM system.

The oligomerization of Aβ$_{1-42}$ was inhibited by F-SLOH, as revealed by SDS-PAGE. Aβ$_{1-42}$ was first dissolved to 1 mM in 100% hexafluoroisopropanol (HFIP). The HFIP was removed under vacuum and the resulting peptide was stored at -20 °C. To prepare oligomeric Aβ$_{1-42}$, the peptide was first resuspended in a dry DMSO, to which a 5 mM F-12 (without phenol red) culture media was added to bring the peptide to a final concentration of 100 μM. The peptide solution was incubated at 4 °C for 24 h to form oligomers, as a control. The Aβ$_{1-42}$ monomer was mixed with an appropriate amount of cyanines at a concentration of either 100 or 500 μM and incubated at 4 °C for 24 h to study the inhibitory effect of F-SLOH. All samples were treated with PICUP prior to performing SDS-PAGE and Coomassie Blue staining.

**Lethal dose 50 (LC$_{50}$) investigation.** The cytotoxicity assay was performed with human neuroblastoma SH-SY5Y cell line. The cells were cultured in Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium (DMEM/F12, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), incubated in a humidified incubator at 37 °C with 5 % CO$_2$. The cells were seeded
at a density of $5 \times 10^4$ cells/well in a cell culture medium on day before treatment. At the next day, the cells were incubated with fresh medium containing F-SLOH at final concentrations between 1 nM to 200 µM for 2, 6 and 24 h under 37 °C. The medium was replaced with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution of 250 µg/mL for 3 h incubation. The MTT was then replaced with dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. The cell plate was shaken for 15 min and the absorbance was measured at 540 nm with a reference at 690 nm by an absorbance microplate reader (BioTek, ELx800™). Results were expressed as a percentage of control and the cytotoxicity was calculated by $\frac{\text{Abs}_{540/690} \text{of F-SLOH}}{\text{Abs}_{\text{control}}} \times 100\%$. An average result of triplicate was found in each condition and the results were express as mean ± SEM.

**Primary Cell Isolation and Culture.** Hippocampus neuronal cells were isolated from 7 day-old Sprague-Dawley rats that were obtained from the Chinese University of Hong Kong and all animal handling experiments were approved by the Hong Kong Baptist University Committee on the Use of Human and Animal Subjects in Teaching and Research. The hippocampus were dissected out and washed with cold Hank's Balanced Salt Solution (HBSS, Gibco). The cells were resuspended in DMEM/F12 medium containing 10 % FBS and papain solution (Sigma-Aldrich) in 2 mg/ml, incubated at 37 °C for 20 min under gentle shaking. The solution was purified by a 0.4 µm nylon filter (BD falcon) and the filtrate was further subjected to centrifugation at 1000 rpm for 5 min. The hippocampus neuronal cells stayed in the pellet and was resuspended in neurobasal medium (Gibco) with 2 % B27 (B-27® Supplement 50X, Gibco) 0.25 % penicillin (PSN, Gibco) and 0.25 % glutamax (GlutaMAX-I 100X, Gibco) as a complete medium. The centrifugation step was repeated and the cells were finally seeded on a poly-D-lysine (Sigma-Aldrich) coated 96 well plate (Nunc) at a density of $5\times10^4$ cells/well. Cells were incubated at 37 °C, under 80 % humidity and 5 % CO₂. After the day of isolation, cytosine of 1 µM was added into the culture medium and the neurons were treated after 7 days in culture.

**Neuroprotection assay.** The neuroprotection assay was performed with primary hippocampus neuronal cells. Fresh complete medium containing 10 µM of Aβ (monomeric, monomeric with seeding and fibrillar forms) and Aβ/F-SLOH (1:1) separately, incubated for 24 h. The medium
was then replaced with MTT solution for 3 h and further replaced by DMSO before absorption measurement as mentioned in cytotoxicity assay.

**Reactive oxygen species (ROS) measurement.** This assay was performed with hippocampus cells. The cells were firstly treated with 100 μM Carboxy-DCFDA (5-(and-6)-Carboxy-2',7'-Dichlorofluorescein Diacetate) (DCFH-DA) in dark for 30 min. After that, the cells were washed with PBS twice before incubating with PBS containing 10 μM of Aβ (monomeric, monomeric with seeding and fibrillar forms) and Aβ/F-SLOH (1:1) separately. The fluorescence intensity at 530 nm was measured by microplate reader (Biotek) under an excitation of 485 nm. All values were expressed as the percent of that of control.

**Calcium imaging.** The cell imaging performed with primary hippocampus cells. Cells were plated at 8000 cells/dish on confocal dishes 7 d before imaging. Then, the cells were treated with F-SLOH of 10 μM, fluo-4 am (Invitrogen) of 3 μM and HBSS successively with 30 min each at 37 °C in the dark. The calcium influx induced by Aβ1-42 monomer was monitored by the fluorescence intensity change at 506 nm under Leica confocal laser scanning microscope (TCS SP8) coupled a 20× water-oil objective (HC PL APO 20×/0.75 Imm) with an excitation at 488 nm. The signal was collected for 15 min in 1 frame/3s and the data analysis was performed with Leica Application Suite X (LAS X).

**Animal Experiments.** In this study, all animal experiments were performed in compliance with the NIH Guidelines for the Use of Animals in Research and approved by the Institutional Animal Care and Use Committee at the Shanghai University of Medicine & Health Sciences. We have taken great efforts to reduce the number of animal used and to minimize animal suffering.

**Co-staining brain tissues of Tg mice in vitro.** The triple transgenic model mice were deeply anesthetized and transcardially perfused with PBS, followed by 8% formaldehyde in PBS (pH 7.4). After excising, the brain section was embedded in optimum cutting temperature compound (OCT) and then frozen-sectioned (14 μm thickness) with a microtome. Next, free-floating sections were blocked in a blocking solution containing 10% goat serum, 1% BSA and 0.4% Triton X-100. For staining by ThT, sections were incubated with 1.0 μM ThT solution for 5 min, after washing with 40% ethanol for 2 min and rinsing with PBS for 3 min, the sections were
further incubated with 1.0 μM F-SLOH for 5 min. For the colocalization studies using antibodies, the sections were further incubated with the primary antibodies at 4°C overnight. After rinsing, sections were incubated in the solution of goat anti-mouse or goat anti-rabbit secondary antibody conjugated with Alexa 488 (Molecular Probes, Carlsbad, CA, USA) for 2 h at room temperature. The images for Aβ or tau immunofluorescent reactivity followed by F-SLOH (1.0 μM) for Aβ oligomers were taken sequentially using Olympus Fluorescence microscopy (BX51).

**In vivo NIRF imaging of F-SLOH.** Firstly, the head of Tg mice (7-month old, male) and the age-matched wild-type mice as control were shaved for background imaging. Then, the mice were anesthetized under isoflurane gas (2.0 mL min\(^{-1}\)) supplemented with oxygen (1.0 mL min\(^{-1}\)) to be motionless, and 100 μL of F-SLOH (5 mg/kg, 20% DMSO, 80% propylene glycol) was injected via the tail vein. Fluorescence imaging from the brain section were acquired using an IVIS Imaging System at different time points. And the images were obtained using a filter set (excitation wavelength around 480 nm and emission around 620 nm) with an exposure time of 1 s, and were further analyzed with Living Image software. The data were analyzed by normalizing the fluorescence intensity of brain sections which were obtained from radiant efficiency to the background fluorescence of each mouse [i.e., F(t)/F(pre)] by selecting the ROI, where F(t) was the fluorescence intensity of the time point of interest and F(pre) was the background fluorescence signal.

**In vivo labeling and ex vivo imaging of F-SLOH.** In vivo probe labeling was performed using APP/PS1 (SXFAD) transgenic mice (7-18 months old), APP/PS1/Tau triple transgenic mice or WT mice (12 months old). The brain tissues of Tg mice or WT mice were collected 2, or 4 hours after injecting with F-SLOH. Then, the brain sections were processed and imaged, and incubated with antibodies as described in vitro.

**References**

Reagents and conditions: a, ClCH₂CH₂OCH₂CH₂OCH₃, NaH, DMF, 75 °C; b, NBS, DCM, 0 °C to r.t.; c, n-BuLi, NFSi, THF, -78 °C to r.t.; d, NBS, DCM, 0 °C to r.t.; e, n-BuLi, DMF, THF, -78 °C to r.t.; f, MeCN, ClCH₂CH₂OH, sealed tube, reflux; g, piperidine, EtOH, reflux.

Scheme S1. The synthetic route for F-SLOH.

Fig. S1 Absorption (dotted line) and fluorescence spectra (solid line) of F-SLOH in PBS (20 mM, pH 7.4, blue line) and in DMSO solution (red line).
**Fig. S2** The fluorescence spectra of SLOH (1.0 µM) in the presence of 10.0 µM Aβ\(_{1-40}\) species (monomers, oligomers and fibrils).

**Fig. S3** The fluorescence spectra of F-SLOH (1.0 µM) only (purple line), the fluorescence spectra of F-SLOH (1.0 µM) in a solution of water-glycerol system with viscosity of 4.5 CP (red line) and then addition of 20 µM Aβ\(_{1-40}\) oligomers (blue line).
**Fig. S4** The fluorescence spectra of 1.0 µM $\text{A}_\text{β}_{1-40}$ species (from left to right: monomers, oligomers and fibrils) upon addition of increasing F-SLOH concentrations.

**Fig. S5** Saturation binding curves of F-SLOH to $\text{A}_\text{β}_{1-40}$ species.
**Fig. S6** The fluorescence titration spectra of F-SLOH (1.0 µM) with various concentrations of Aβ_{1-40} oligomers.

**Fig. S7** The effect of pH on the fluorescence property of F-SLOH (1.0 µM).
Fig. S8 TEM images of Aβ$_{1-40}$ after incubation 5 hours and 24 hours.

Fig. S9 Hydrodynamic diameters of Aβ during the growth process as measured by DLS.
**Fig. S10** CD spectra of Aβ$_{1-40}$ monomers, oligomers and fibrils in the absence and presence of F-SLOH.

**Fig. S11** TIRFM images of Aβ$_{1-40}$ fibril growth in the absence (left) and the presence (right) of 50 μM F-SLOH.
**Fig. S12** Inhibition of aggregation by F-SLOH as demonstrated by ThT fluorescence assay. The plots of the fluorescence intensity of ThT recorded at 490 nm after incubation of Aβ1-40 monomers for different time periods in the absence (▲) and the presence (●) of F-SLOH, respectively.

**Fig. S13** Instantaneous inhibitory effect of F-SLOH on the seed-mediated growth of Aβ1-40. Length of the fibril measured from TIRFM images after 1 h seed-mediated incubation of Aβ monomer with F-SLOH (50 μM) added at different time points (0, 10, 20, 40 and 60 min) within the one hour-incubation (bottom axis, bars). In the control experiment, the length of Aβ1-40 fibrils were measured at 0, 10, 20, 40 and 60 min without the addition of F-SLOH (top axis, scatter point).
**Fig. S14** SDS-PAGE images for visible observing the inhibition effect of F-SLOH. Lane 1, the molecular weight markers; Lane 2, HFIP-treated monomeric Aβ\textsubscript{1-42}; Lane 3, oligomeric Aβ\textsubscript{1-42} by incubating monomer at 4 °C for 24 h; Lane 4 and 5, in the presence of F-SLOH, at 1:1 and 5:1 molar ratio of F-SLOH to monomers, respectively.

**Fig. S15** SLOH and F-SLOH complex with Aβ oligomers. (A, B) Optimized SLOH and F-SLOH structure at the B3LYP/6-31G* level. (C) Aβ oligomer from X-ray (4NTR). F19 and V36 residues are displayed in yellow and green, respectively. Simulated complex structures of SLOH (D, E) and F-SLOH (F, G) with Aβ oligomer.
**Fig. S16** *In vitro* imaging of F-SLOH in the brain of AD mouse model and wild-type control. Scale bar: 500 µm.

**Fig. S17** F-SLOH labelling *in vitro* displays an age-dependent pattern, as counterstained with Thio-S, in the brains of AD transgenic mice (7-month and 10-month old). Scale bar: 200 µm.
**Fig. S18** *In vitro* double labeling of SLOH or F-SLOH with thioflavin-S (Thio-S) in the brain slices of triple transgenic AD mouse model. As compared with F-SLOH staining, SLOH labeling shows smaller, less sharp staining and more co-localizations with Thio-S. Scale bar: 100 µm.
**Fig. S19** (A) *In vivo* fluorescence images of 7-month old Tg mice and age-matched WT mice at different time points before and after IV injection of 100 µL F-SLOH (5 mg/kg). The fluorescence images were captured at 600–640 nm under excitation around 480 nm. (B) The relative fluorescence signal \([F(t)/F(pre)]\) in the brain regions of Tg and WT mice after IV injection of F-SLOH. The \([F(t)/F(pre)]\) of Tg mouse was significantly higher than that of WT mouse \((p < 0.05)\).

**Fig. S20** F-SLOH labelling *in vivo* in AD double (APP/PS1) transgenic mice which was examined 2 or 4 hours after single injection of F-SLOH. Scale bar: 500 µm.
Fig. S21 In vivo F-SLOH labelling and colocalization analyses with Aβ/tau immunofluorescence in AD transgenic mouse models. F-SLOH was systemically administrated in the APP/PS1 double transgenic or APP/PS1/Tau triple transgenic mice, then in vivo F-SLOH labelled brain sections were incubated with primary antibodies (4G8, pAβ, MC1), followed by secondary antibodies conjugated with Alexa 488. Scale bar: 200 µm.