

## **Bifunctional curcumin analogue for two-photon imaging and inhibiting crosslinking of amyloid beta in Alzheimer's disease**

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### **Supplemental Materials**

#### **1. Experimental section**

Reagents used for the synthesis were purchased from Aldrich and used without further purification. Column chromatography was performed on silica gel (SiliCycle Inc., 60 Å, 40-63 mm) slurry packed into glass columns. Synthetic A $\beta$  peptides (1-40/42) were purchased from rPeptide (Bogart, GA, 30622). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at 500 MHz and 125 MHz respectively, and reported in ppm downfield from tetramethylsilane. Fluorescence measurements were carried out using an F-4500 Fluorescence Spectrophotometer (Hitachi). Mass spectra were obtained at Harvard University, Department of Chemistry Instrumentation Facility. Transgenic female APP/PS1 mice were purchased from Jackson Laboratory. **All animal experiments were approved by the Institutional Animal Use and Care Committee at Massachusetts General Hospital.**

**Synthesis of CRANAD-28 and -44:** The syntheses were performed following a modified procedure that was based on our previous reports<sup>[1]</sup>. **CRANAD-28:** 2,2-Difluoro-1,3-dioxaboryl-pentadione (40 mg, 0.25 mmol) was dissolved in acetonitrile (4.0 mL),

followed by the additions of acetic acid (0.1 mL), tetrahydroisoquinoline (20  $\mu$ L), and N-phenyl-3-methyl-1H-pyrazole-carboxaldehyde (93 mg, 0.5 mmol). The resulted solution was stirred at 60 °C for 4 hours. An orange precipitate was formed during the reaction, which was collected by filtrating the reaction mixture and by washing twice with a mixture solvent of ethyl acetate/hexanes (1:5). 90 mg, yield: 74.4%. **m.p. > 250°C**;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ (ppm) 2.46 (s, 6H), 5.95 (s, 1H), 6.50 (d, 2H,  $J = 16\text{Hz}$ ), 7.44 (m, 6H), 7.54 (m, 4H), 7.97 (d, 2H,  $J = 16\text{Hz}$ ), 7.99 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$ (ppm) 11.1, 101.2, 117.6, 117.9, 125.0, 128.6, 129.3, 137.2, 138.7, 139.2, 141.6, 179.0;  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ )  $\delta$ (ppm) 141.2, 141.3; ESI-MS ( $\text{M}^+$ )  $m/z = 484.2$ ; **HRMS (ESI-TOF) ( $\text{M}+\text{H}^+$ )  $m/z = 485.1948$  (calculated: 485.1955, relative Error = 2.4ppm).**

**CRANAD-44:** Yield: 68.6%. **m.p. > 250°C**;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ (ppm) 2.39 (s, 6H), 6.40 (s, 1H), 6.68 (d, 2H,  $J = 16\text{Hz}$ ), 7.83 (d, 2H,  $J = 16\text{Hz}$ ), 8.01 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$ (ppm) 10.2, 100.2, 116.0, 117.3, 138.2, 138.7, 140.5, 179.0;  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ )  $\delta$ (ppm) 141.2, 141.3; ESI-MS ( $\text{M}+\text{H}^+$ )  $m/z = 333.10$ ; **HRMS (ESI-TOF) ( $\text{M}+\text{H}^+$ )  $m/z = 333.1326$  (calculated: 333.1329, relative Error = 1.7ppm).**

**Quantum yield:** The measurements were performed by the procedure described in ref. [2].

**A $\beta$  species preparation:** A $\beta$ 40 aggregates for *in vitro* studies were generated by slow stirring of A $\beta$ 40 in PBS buffer for 3 days at room temperature. A $\beta$ 40/42 monomers were prepared and characterized by following our previously reported procedures [1].

**Fluorescence spectral testing:** The tests of CRANAD-28 and -44 with A $\beta$ 42 monomers, A $\beta$ 40 monomers, and A $\beta$ 40 aggregates were performed following our previously reported procedure [1].

**Binding constant (Kd) measurement:** To PBS solution (1.0 mL) of CRANAD-28 (25 nM), various amounts of A $\beta$ s (aggregates, oligomers, dimers, and monomers respectively) were added to final A $\beta$ s concentrations of 25.0, 50.0, 75.0, 100.0, 125.0, and 150 nM, and their fluorescence intensities at 580 nm were recorded (Ex: 498 nm). Kd calculation was based on the fluorescence decrease ( $\Delta\text{FI} = F_0 - F_{(C(A\beta))}$ ), where  $F_0$  is the fluorescence intensity of CRANAD-28 without A $\beta$ s, and  $F_{(C(A\beta))}$  is the fluorescence intensity of CRANAD-28 with a tested concentration of A $\beta$ s. The Kd binding curve was

generated using Prism 5.0 software with nonlinear one-site binding regression (SI Fig.1b).

**Histological staining of brain slice:** A 30-micron brain slice from an 18-month old APP/PS1 mouse was fixed with 4% formalin for 5 min, and washed with distilled water twice for 5 min. Then the slice was incubated with CRANAD-28 solution (1.0  $\mu$ M in 20% ethanol and 80% dd water) for 15 minutes, and then washed with 20% ethanol followed by washing with dd water. After drying, the slice was covered with VectaShield mounting media. Thioflavin S and CRANAD-44 staining were followed using a similar procedure. Florescence images were observed using Nikon Eclipse 50i microscope.

**Thinning skull surgery:** An APP/PS1 mouse (9 months old) was anesthetized with Ketamine/xylazine (70 mg/kg) and a thin-skull imaging window was surgically prepared as described in ref. [3].

**Two-Photon imaging:** CRANAD-28 (2.0 mg/kg in a fresh solution containing 15% cremorphor, 15% DMSO and 70% PBS) was injected intravenously at time 0 min by a bolus injection during image acquisition. Two-photon fluorescence excitation was accomplished with a 900-nm laser (Prairie Ultima). Imaging was performed using a two-photon microscope (Prairie Technologies) equipped with a 20x water immersion objective (N.A. 1.0, Zeiss). Images were collected for 15 seconds per frame 512x512  $\mu$ m matrix for 60 min. Texas-red dextran (70,000 MW) (50  $\mu$ L) was injected in the tail vein at 60 min after CRANAD-28 injection, and images outlining the blood vessels were obtained. In BBB penetration study, a wild type mouse (C57BL6) was imaged for 5 min to obtain pre-injection background. CRANAD-28 (2.0 mg/kg in a fresh solution containing 15% cremorphor, 15% DMSO and 70% PBS) was then injected in the tail vein, and images were collected for 20 seconds per frame 512x512  $\mu$ m matrix for 30 min. Images were analyzed with ImageJ software using 2 ROIs for each data group.

**Gel electrophoresis and Western blotting:** Samples were separated on 4–20% gradient Tris-glycine mini gels (Invitrogen). For FAM-A $\beta$ 42 gels, the images were acquired using IVIS®Spectrum (Perkin Elmer) with excitation = 465 nm, and emission = 520 nm. For native A $\beta$ 42 gels, the gel was transferred onto a nitrocellulose membrane in a cooled transferring buffer and the membrane was blocked at room temperature for 2 hours. After blocking, the membrane was incubated in a solution of 6E10 anti-A $\beta$  primary antibody

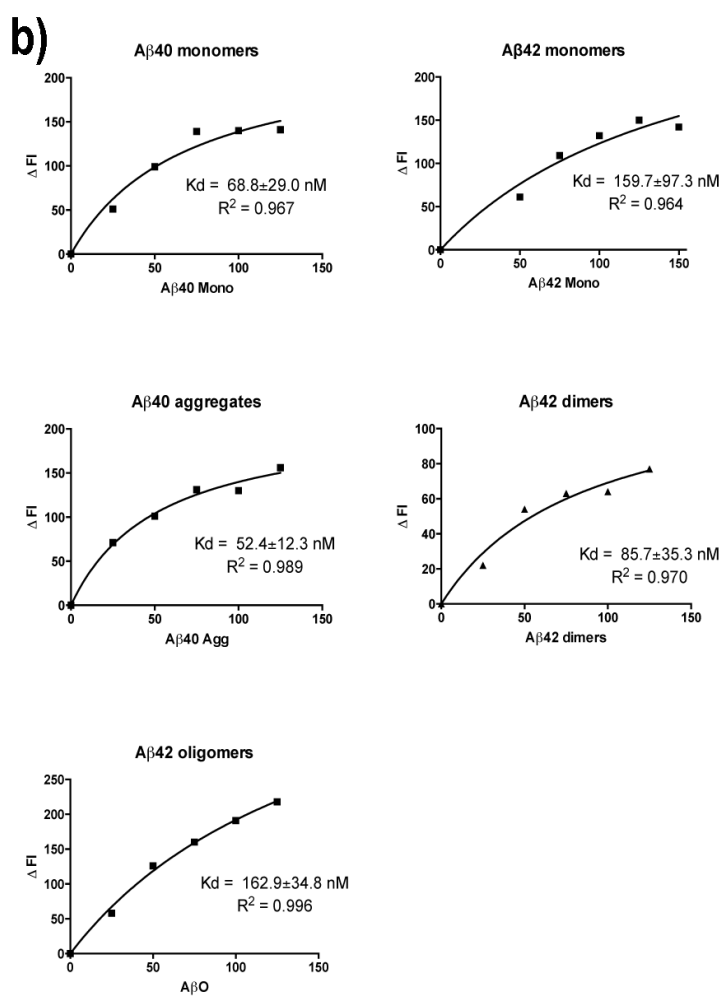
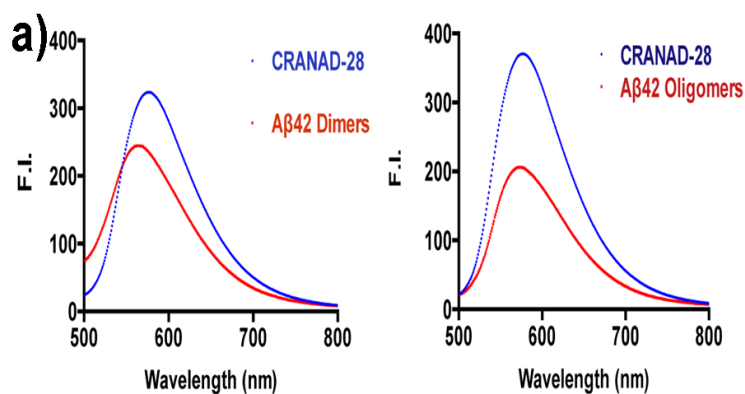
(1:2000 dilution, Covance, Dedham, MA) at 4°C overnight. After washing with TBS buffer, the membrane was incubated with the secondary antibody for 2 hours at room temperature. Western Breeze Chemiluminescent kit (Invitrogen) was used to visualize the bands. The images were acquired using IVIS®Spectrum under a bioluminescence imaging setting. SeeBlue®plus2 (Invitrogen)(4-250KD) was used as a molecular weight marker.

All the samples used for SDS-PAGE gel and Western blot were prepared using the same procedure as described below. A 5 µL HFIP (hexafluoroisopropanol) solution (25 µM) of native Aβ42 or FAM-Aβ42 was added to a 1.5 mL eppendorf tube (Protein LoBind). After evaporating the organic solvent under vacuum, a 5 µL DMSO or DMSO solution of CRANAD-28 (25 µM) was added to the tube, followed by the addition of 15 µL of Vitamin C solution in PBS (33.3 µM) and 5 µL of copper sulfate solution in PBS (12.5 µM). The resulting mixture was incubated at 37°C for 4 hours, and was then subjected to gel electrophoresis. For natural crosslinking, no Vc and Copper were added. The resulting mixture was incubated at 37°C for 24 hours.

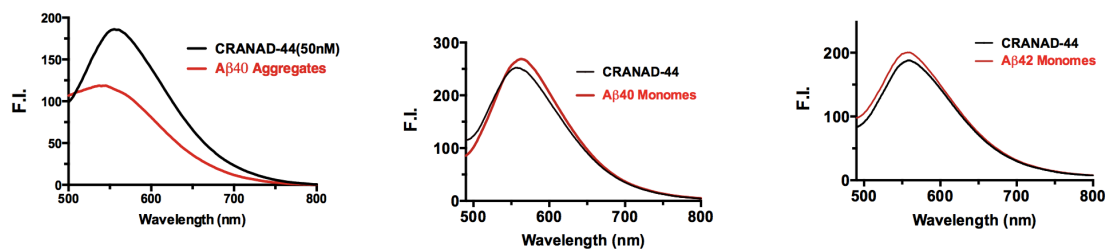
**Reference:**

- [1] a) C. Ran, X. Xu, S. B. Raymond, B. J. Ferrara, K. Neal, B. J. Bacskai, Z. Medarova, A. Moore, *J Am Chem Soc* **2009**, *131*, 15257-15261; b) C. Ran, Zhao, W., Moir, RD, Moore, A. , *PLoS One* **2011**, *6*, e19362.
- [2] S. Fery-Forgues, Lavabre, D., *J. Chem. Edu.* **1999**, *76*, 1260-1264.
- [3] D. F. Marker, M. E. Tremblay, S. M. Lu, A. K. Majewska, H. A. Gelbard, *JoVE* **2010**, *43*, pii: 2059.

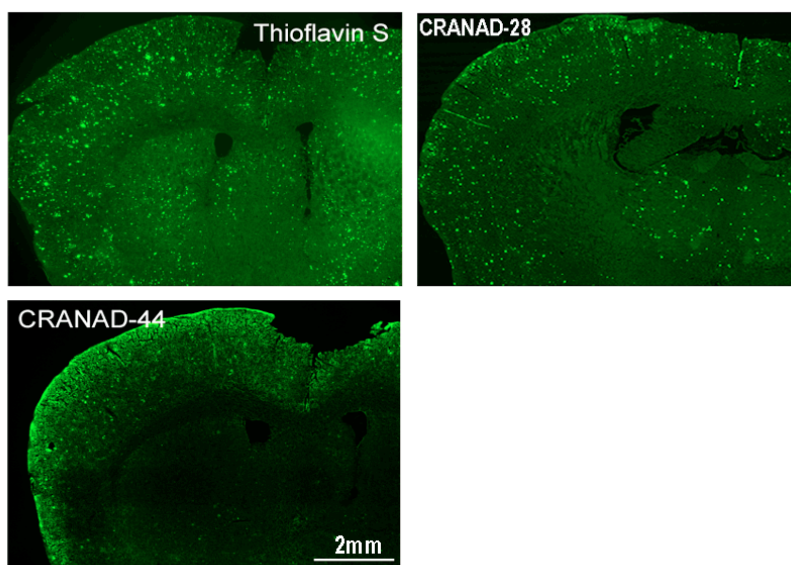
## 2. Supplemental Figures



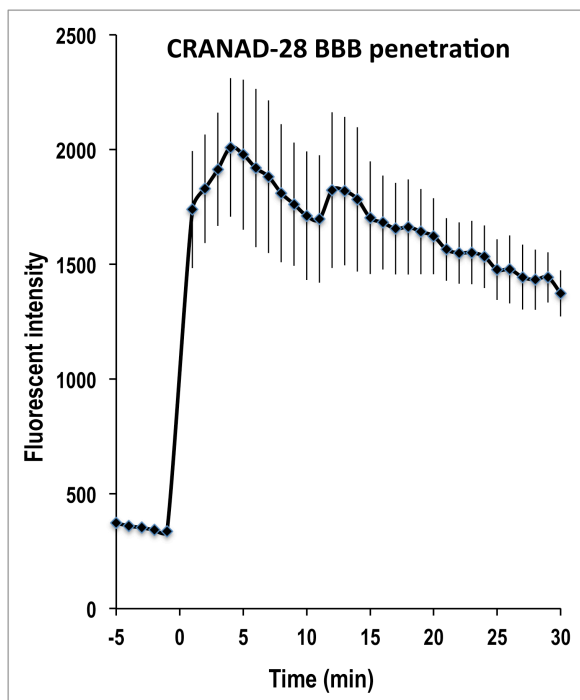
**SI Fig.1** (a) Fluorescence properties changes upon interaction of CRANAD-28 with Aβ42 dimers and Aβ42 oligomers. (b)  $K_d$  measurement of CRANAD-28 with various Aβs.



**SI Fig.2** Fluorescence properties changes upon interaction of CRANAD-44 with A $\beta$ 40 aggregates, A $\beta$ 40 monomers and A $\beta$ 42 monomers. No significant changes were observed for CRANAD-44 with A $\beta$ 40/42 monomers.



**SI Fig.3** Histological staining of consecutive brain slices from an 18-month old APP/PS1 AD mouse. CRANAD-28 showed excellent staining capability, which was similar to Thioflavin S, a standard staining agent for A $\beta$  plaques. However, compared to Thioflavin S and CRANAD-28, a lesser number of plaques were observed with CRANAD-44.



**SI Fig.4** BBB penetration of CRANAD-28 observed by two-photon imaging in a wild type mouse. The intensities of two ROIs nearby blood vessels were measured and averaged.