Supplementary Information for

A bimodal probe for fluorescence and synchrotron X-ray fluorescence imaging of dopaminergic neurons in brain

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Materials and methods

Erythrosine B (EB), 1,4-dioxane, triethylamine, isobutyl chloroformate, dialysis membrane (MWCO=8000-14000) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai); immunoglobulin G (IgG) was purchased from Bioss Biotechnology Co., Ltd (Beijing); Sephadex G25 column were purchased from GE healthcare.

Preparation of dendrimer EB/IgG conjugates

Isobutyl chloroformate (10 μL) and triethylamine (1 μL) were added in 5.20 mg of EB (MW=835.9 g/mol) dissolved in 360 μL 1,4-dioxane at -10 ℃ for 1 h. Supernatants were centrifugated for 5 min with 3000 rpm. 0.622 mg of IgG (MW=150,000 g/mol) was dispersed in 60 μL 0.01mol/L PBS buffer and 240 μL Na_2CO_3 buffer in pH 9.0 or so. 4 μL EB supernatant was reacted with IgG solution above 8.0 pH at 4 ℃ staying overnight. The reaction mixture was dialyzed with MWCO=8000-14000 of dialysis membrane (PBS buffer with 1 L for every 12 h) to wipe off organic solvents and centrifugated 3000 rpm for 30 min to get rid of bulky grains. Then, desalination process was efficiently performed by sephadex G25 desalting column and the particle size less than 10 kDa was eliminated by ultrafiltration repeatedly. The obtained probe was concentrated to 1mg/mL and stored at -20 ℃.

Fluorescence spectrum determination

In order to determine the emission spectrum of EB and EB/IgG, the wavelength of maximum absorption (usually the same as the excitation maximum) is determined. The fluorescence excitation and emission spectrum were obtained by Edinburgh FS5 spectrofluorometer.

PAGE analysis

SDS-PAGE Sample Loading Buffer was melted in water bath less than 37 ℃ and placed at room temperature. SDS-PAGE Sample Loading Buffer was added into IgG or EB/IgG. The mixtures were boiling above 95 ℃ for 10min to sufficiently denature IgG and EB/IgG. The samples cooling to room temperature were loaded in 10% SDS-PAGE gel away from light in the process. Electrophoresis could stop until the blue dye reaching the bottom of the gel. The gel was rinsed by double distilled water and stained by Coomassie brilliant blue staining solution for 30-60 min and decolored with double distilled water for thrice. The gel was captured by SYBR imaging system and Blue Light Gel Imager under 340 nm ultraviolet light.

Size distribution and Zeta potential determination

The size distribution and zeta potential of IgG and EB/IgG were characterization by Zetasizer Nano S90 with well-established standard operation procedure. The concentration of IgG and EB/IgG was set at 1mg/mL in PBS buffer with pH 7.4.

Immunofluorescence staining

Male C57BL/6 mice of 6-8 weeks were used in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The mouse brain was removed under anesthesia

after perfusion through the left ventricle. Then the fresh brain was fixed by 4% paraformaldehyde staying overnight and dehydrated in gradient sucrose solution from 10% to 30%. Then, the brain was embedded by optimal cutting temperature (OCT) compound and sliced into 30 μm thick frozen coronal slices by freezing microtome (Leica CM1950). Slices were put in 24-pore plate, rinsed by PBS buffer, treated by 0.3% H_2O_2 for 30 min to eliminate the endogenous peroxidase activity, 6% BSA to block off nonspecific binding antigen and 0.25% triton X-100 for 1 h to enhance the membrane permeability. Next, rinse the slices and incubate with anti-tyrosine hydroxylase antibody, LCN1 (MAB318, 1:200, Millipore) overnight. At length, rinse the slices and co-incubate with EB/IgG and goat anti-mouse IgG H&L (Alexa Fluor@ 488) (ab150113, Abcam, 1:1000) for 2 h and DAPI for 15min. The tissue slices were sealed by anti-fluorescence quencher. The images were obtained by Leica confocal sp8 microscope and Zeiss Imager Axioskop 2.

Synchrotron radiation X-ray fluorescence (SXRF) experiments

The SRXF analysis was performed at the Shanghai Synchrotron Radiation Facility in China. The entire X-ray spectrum was obtained at each pixel by an energy dispersive germanium detector and the pixel step size was set to 1 μm. SRXF analysis was obtained from the BL15U1 beam line of Shanghai Synchrotron Radiation Facility. An incident photon energy of 10 keV was chosen to ensure excitation of the K-line of Iodine, and the sample was raster scanned through the beam at 298K under a helium atmosphere. The sample were positioned onto a kinematic specimen holder for X-ray fluorescence capture. The holder was then set up on a light microscope (Leica DMXRE). Target neurons imaged by standard fluorescence microscopy were located on the grid relative to a reference point using a high spatial resolution motorized x/y stage (Ludl Electronic Products, Hawthorne, NY). Coordinates were determined and precisely located the target neuron once the grid was transferred to the EB/IgG.

Statistical analysis

All data were analyzed by Origin pro 9.0 or GraphPad prism 8.0 software.

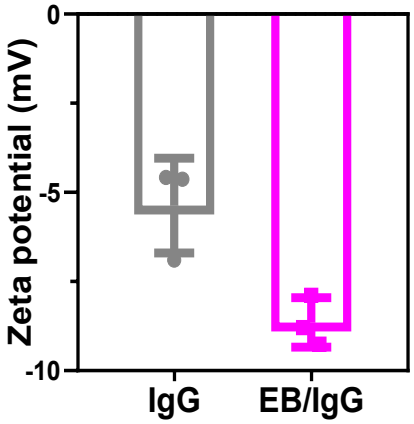

Fig. S1. Zeta potential of IgG and EB/IgG.