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Supplementary information for

A strategy for iron oxide nanoparticles to adhere to the neuronal membrane in the substantia nigra of mice

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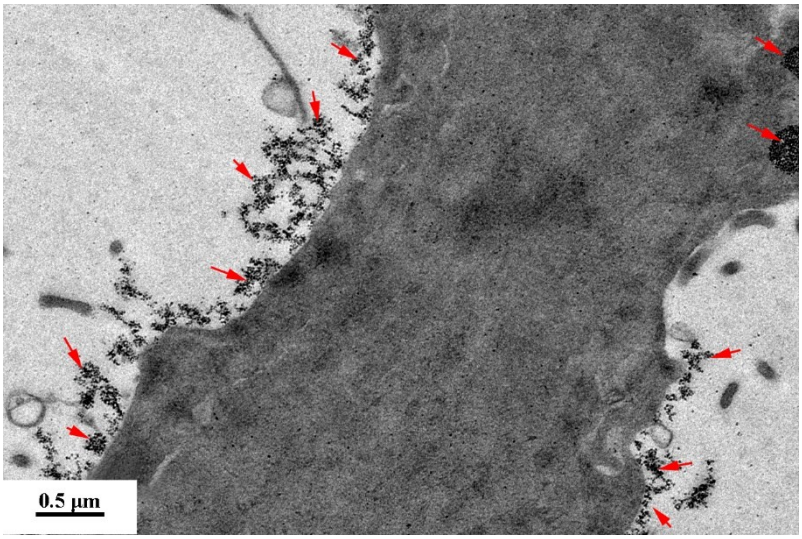

Figure S1. Distribution of SA/PEI-SPIONs on PC-12 cell membrane

SA/PEI-SPIONs were incubated with PC-12 cells which had been firstly cultured in RPMI Medium 1640 containing 0.2 mg/L biotin for 12 h and the cells were observed by transmission electron microscopy (TEM), and SA/PEI-SPIONs were clearly attached on the PC-12 cell membrane, which can be attributed to the specific binding between SA and biotin sites on the cell surface. This work makes possible the biomedical applications that need the nanoparticles to target cell membranes.

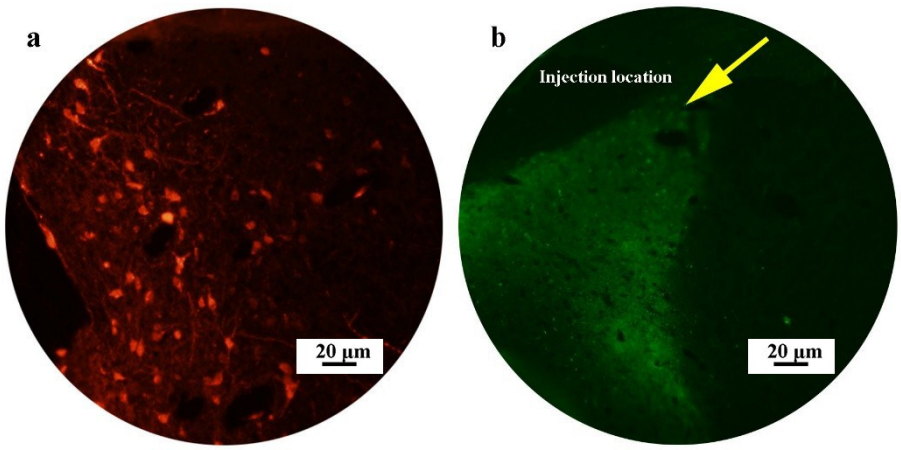

Figure S2. (a) Neurons transfected with *AVV-DIO-ChR2-mCherry*, (b) Substantial nigra after injection of FITC-SA/PEI-SPIONs under general fluorescence microscope (multiple of objective lens: 10×)

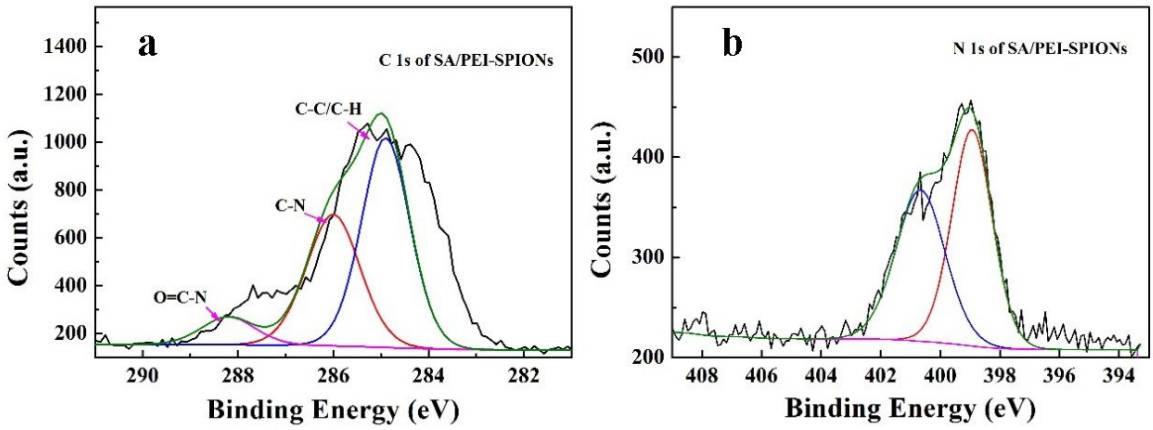

Figure S3. XPS spectra: C 1s of (a) SA/PEI-SPIONs, (b) N 1s of SA/PEI-SPIONs

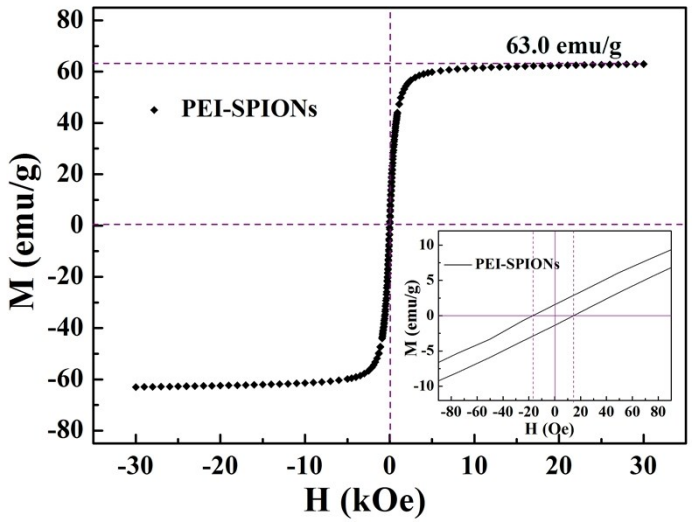

Figure S4. Hysteresis Loop of PEI-SPIONs at 300 K

Frozen section: Brain was immobilized in a centrifugal tube containing 4% paraformaldehyde solution (adding 0.5% glutaraldehyde solution) for 24 h. Then 30% sucrose solution was added for dehydration treatment, and the solution was changed every 24 h until the brain sank into the bottom of the centrifugal tube to prove that the dehydration was completed. The liquid on the surface of the brain was sucked with filter paper, and the brain was fixed on the tissue bracket with O.C.T embedding agent. The brain was cooled in a freezing microtome (-20 ℃) and cut into 10 μm slices. The

cover glass was cleaning overnight with PBS buffer to ensure that the bubbles on the glass are completely discharged.

H-E staining: After the bubbles were removed from the brain slices that spread smoothly on the slide, the brain slices were stained with hematoxylin for 5 min, then washed immediately with deionized water, then differentiated once with alcohol hydrochloride (1:100), and cleaned again. The slices were dyed with eosin for 1 min, then rinsed, dried and sealed using neutral resin seals.

Cytotoxicity of the nanoparticles: PC-12 cells were cultured in about 5 mL RPMI 1640 complete medium (85% RPMI medium 1640, 5% FBS, 10% HI-HS) with 1% penicillin-streptomycin at 37 ℃, in the incubator containing 5% carbon dioxide and 95% oxygen. PC-12 cells were incubated on 96-well plates with 8×10^3 cells per hole in 100 μL RPMI 1640 complete medium for 24, 48, 72 h. After incubation, the RPMI 1640 complete medium was replaced by fresh one, then 100 μL PEI-SPIONs and SA/PEI-SPIONs with different concentrations (0, 5, 10, 25, 50, 100, 200 μg/mL) in PBS were added. After 24, 48, 72 h respectively, the SPIONs in the culture solution were discarded by cleaning with 0.01 M PBS buffer. The cytotoxicity of SPIONs in vitro was evaluated by CCK-8 kit assay.

The preparation of the TEM samples: The substantia nigra samples were fixed with 2.5% glutaraldehyde and 0.1 M sodium cacodylate for 1 h. The samples were then rinsed with sodium cacodylate, fixed again with 1% osmium tetroxide for 1 h. After dehydrated by rinsing with 50%, 70%, 80%, 90% and 100% ethanol, then dehydration was continued with acetone and ethanol with volume ratio of 1:1 and pure acetone,

respectively. The samples were impregnated in a 1:1 mixture of acetone and Epon 812 at 37 ℃ for 1 h, followed by 1:3 mixture of acetone and Epon 812 at 37 ℃ for 3 h. Then the samples were embedded in Epon 812 resin at 37 ℃ for one night, and at 45 ℃ for 12 h, and finally at 60 ℃ for 48 h. The embedded samples were cut into ultra-thin section (60~70 nm thick) with a PT-XL ultramicrotome (RMC, Boeckeler Instruments, USA) using the glass knife. Sections were then transferred to a copper grid coated with a carbon film.

ICP-OES measurement: 0.1 mL of SPIONs dispersed in pure water was dropped into a test tube, followed by the addition of 1 mL of concentrated nitric acid and 0.4 mL of concentrated hydrochloric acid, and the tube was heated in a 65 ℃ water bath for 3 h. Subsequently, the sample was diluted in a 500 mL volumetric flask for measuring of the iron concentration ($C_{(Fe)}$).

Calculation of the concentration of iron oxide ($C_{(SPIONs)}$) by the following formular：

$$\frac{3C_{(SPIONs)}}{232} = \frac{C_{(Fe)}}{56} \cdot n \ \ (n = dilution\ factor)$$