Supplementary Information

NIR-IIb emissive transmembrane voltage nano-indicator for optical monitoring of electrophysiological activities in vivo

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Materials

Y_2O_3 (99.999%), Yb_2O_3 (99.999%), Er_2O_3 (99.999%), anhydrous ethanol, oleylamine (OM), and cyclohexane (99.9%) were purchased from Adamas-beta Co., Ltd. Oleic acid (OA, 90%) and 1-octadecene (ODE, 90%) and kainic acid (KA) were purchased from Sigma Aldrich. Dimethyl sulfoxide (GC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were purchased from Aladdin. Cy7.5 was purchased from DuoFluor and RH237 was purchased from Shanghai Maokang Biotechnology Co., Ltd. Cal-520 was purchased from AAT Bioquest, Inc. calcein-AM/propidium iodide was purchased from Beyotime.

Methods

Preparation of $RE(CF_3COO)_3$ (RE: Yb^{3+}, Y^{3+}, and Er^{3+})

5 mL CF_3COOH (TFA) and 5 mL H_2O were mixed and dropwise into 10 mmol RE_2O_3. Then the solution was stirred at 80°C until the solution became transparent. The remaining H_2O and TFA were evaporated under 100 ℃. The wet $RE(CF_3COO)_3$ was collected and dried at 80 °C overnight. The prepared $RE(CF_3COO)_3$ was stored in a dry dish for future use.

Synthesis of lanthanide nanoparticles $NaYbF_4$:Er or $NaYF_4$

1 mmol $RE(CF_3COO)_3$ [RE: 95% Yb, 5% Er], and 1 mmol Na $(CF_3COO)_3$ were added to a 100 mL three-necked flask. Next, a mixture of 3.2 mL oleic acid (OA), 3.3 mL oleylamine (OM), and 6.4 mL 1-octadecene (ODE) was added to the flask. The temperature was increased to 100°C under an N_2 atmosphere and continuous stirring. Under vacuum and with continuous stirring, the solution was maintained at the temperature for 45 minutes. Then the temperature of the solution increased to 250°C under a nitrogen(N_2) atmosphere. After 45 minutes, the solution was further raised to 260°C and held for 15 minutes. After natural cooling to room temperature, an equal volume of ethanol was added to the solution, and synthesized nanoparticles were obtained through centrifugation at 12500 rpm for 15 minutes. After washing the nanoparticles three times with ethanol: cyclohexane (1:1 volume/volume), the nanoparticles were finally dispersed in 4 mL of cyclohexane. For the synthesis of $NaYF_4$ or $NaYbF_4$ nanoparticles, 1 mmol $RE(CF_3COO)_3$ was replaced with 1 mmol $Y(CF_3COO)_3$ or $Yb(CF_3COO)_3$. The remaining steps followed the process of $NaYbF_4$:Er

Synthesis of lanthanide luminescence nanoparticles $NaYbF_4$:Er@$NaYF_4$ with different shell thickness

2 mL solution of prepared $NaYbF_4$:Er in cyclohexane was mixed with the 6.4 mL OA and 6.4 mL ODE in a 100 mL three-necked flask. To achieve the desired $NaYF_4$ shell thickness (2.0/2.4/3.0/3.5 nm), 0.4/0.8/1.2/1.6 mmol $Na(CF_3COO)_3$ and an equal amount $Y(CF_3COO)_3$ were separately added to the solution. The temperature was increased to 100°C under an N_2 atmosphere and continuous stirring. Under vacuum and with continuous stirring, the solution was maintained at the temperature for 45 minutes. Subsequently, the temperature was increased to 295°C under an N_2 atmosphere and held for 75 minutes, followed by further heating to 300°C and holding for 15 minutes. After natural cooling, an equal volume of ethanol was added to the solution, and core-shell lanthanide nanoparticles were obtained through centrifugation at 12500 rpm for 15 minutes. The core-shell lanthanide nanoparticles were washed three times with ethanol: cyclohexane (1:1 volume/volume) and finally dispersed in 4 mL of cyclohexane.

Surface modification of lanthanide luminescent nanoparticles with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

Cyclohexane solution with 20 mg $NaYbF_4$:Er@$NaYF_4$ or $NaYF_4$ was dropped into the 10 mL of dichloromethane and stirred for 10 minutes. Subsequently, 40 mg of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was added to the solution, and the mixture was stirred overnight. After solvent evaporation, the resulting solid was re-dispersed in 10 mL of deionized water and sonicated for 25 minutes. After centrifugation at 10000 rpm for 15 minutes, the DPPC-modified lanthanide nanoparticles were finally

dispersed in 2 mL of deionized water.

Preparation of transmembrane voltage nano-indicator $NaYbF_4$:Er@$NaYF_4$@Cy7.5@DPPC (Cy7.5-ErNP)

The prepared $NaYbF_4$:Er@$NaYF_4$@DPPC solution was mixed with various molar quantities of Cy7.5 (1 mM in DMSO). After vigorous shaking for 1 minute, the mixture was centrifuged at 10000 rpm for 10 minutes. The obtained Cy7.5-ErNP was dispersed in the PBS buffer for further use.

Dye sensitization of Cy7.5-ErNP with different dye concentrations in the NIR IIb window

1 mL of $NaYbF_4$:Er@$NaYF_4$@DPPC with a concentration of 20 mg/mL was prepared in a polyethylene (PE) tube. The Cy7.5 solution (1 mM, DMSO) with volume of 0/2.5/5/7.5/10/15/20 nmol was added into the PE tube. After vigorous shaking for 1 minute and centrifugation at 10000 rpm for 10 minutes, the Cy7.5-ErNP solution was transferred to a four-sided transparent colorimetric dish for luminescence spectral testing under an 808 nm laser.

FRET efficiency of optimal Cy7.5-ErNP as a transmembrane voltage indicator

The optimal Cy7.5-ErNP was ensured under the conditions of 2 nm $NaYF_4$ inert shell thickness and 5 nmol/mL dye modification concentrations (20 mg $NaYbF_4$:Er@$NaYF_4$@DPPC). The lifetime of Cy7.5 in the Cy7.5-ErNP was tested with the time-resolved fluorescence spectrometer. Secondly, in order to simulate the true state of Cy7.5 on the $NaYF_4$ inert shell without the presence of FRET, Cy7.5 was also

infiltrated into the surface of $NaYF_4$@DPPC with a similar size to $NaYbF_4$:Er@$NaYF_4$@DPPC. The lifetime of Cy7.5 in the $NaYF_4$@Cy7.5@DPPC reflected the true state of Cy7.5 on the $NaYF_4$ inert shell of ErNP but without the interaction of FRET.

The FRET efficiency of optimal Cy7.5-ErNP was calculated by the following equation:

$$E_{FRET} = \frac{1}{1 + \frac{R^6}{R_0^6}} = 1 - \frac{\tau_{DA}}{\tau_D} \quad (1)$$

where E_{FRET} was the energy transfer efficiency between the donor and acceptor, which could be calculated by the fluorescence lifetime changes of the donor before and after the FRET. R was the distance between the energy donor and the energy acceptor. The R_0 was the Förster radius which corresponds to a distance with 50% transfer efficiency. τ_D and τ_{DA} were individually the lifetime of the donor before and after the occurrence of FRET.

Theoretical molecular calculation of Cy7.5 in the Cy7.5-ErNP for voltage sensing

The structure and properties of Cy7.5 were calculated by the Gaussion 09W software (Revision E.01 WIN64). The LUMO/HOMO visualization results of Cy7.5 were obtained through the VMD software and the quantum chemistry program Multiwfn 3.8.[1] The maximum absorption equation wavelength of Cy7.5 was further corrected by the empirical equation[2]:

$$\lambda\max\,(exp.) = 1.39 * \lambda\max\,(calc.) - 89.68 \quad (2)$$

Validation of Cy7.5-ErNP/Cy7.5/RH237 used for membrane potential sensing under

an external electric field (EEF).

Two silver chloride wires with 0.2 mm diameter were placed in parallel on the bottom of a cell-culture dish with 30 mm diameter and were fixed by the PDMS (Polydimethylsiloxane). Notably, both ends of the silver chloride wires should extend beyond the depth of the dish, which prevented electrical leakage after connection with the power. The modified culture dish was sterilized and HEK293T cells were seeded. Culture medium with Cy7.5-ErNP was added into the dish, and co-incubation with cells continued for 2 hours. Bur for Cy7.5 or RH237, the co-incubation required only 10 minutes. After that, the co-incubation medium was replaced by the fresh medium and the dish was observed under the microscopy. The exposed sections of silver chloride wires were connected to a AC power supply device, which consisted of a programmable signal generator, a voltage amplifier, and an oscilloscope. Series of square wave signal (40% high level; 60% low level) with 100 ms cycle and 100 mV amplitude was applied to stimulate the cells located between the two parallel wires. At the same time, the imaging results of cells tagged by the Cy7.5-ErNP/Cy7.5/RH237 were recorded.

Fluorescence insensitivity of Cy7.5-ErNP/Cy7.5 for ion concentration fluctuation.

Prepared Cy7.5-ErNP was evenly distributed into Na^+ or K^+ solution with concentration of 50/100/150/200/300/400/500 mM. The luminescence intensity of Cy7.5-ErNP under various ion concentration was further compared with the Cy7.5-ErNP in deionized water. Besides, the luminescence of Cy7.5-ErNP under other ions with physiological concentration was also compared with the condition under the deionized water. The ion insensitivity of Cy7.5 was also verified according to the above approach.

Cell culture

Human embryonic kidney (HEK-293T) cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) which consisted of 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), and 0.1% chlamydia-specific additives. HL-1 cell line was cultured in Claycomb Medium with 10% FBS, 1% P/S and 0.1% chlamydia-specific additives. Besides, 100 uM norepinephrine and 2 mM L-glutamine was added to the Claycomb Medium.

MTT assay

Similar number of HL-1 cells were seeded into 96-well plate. Various concentrations (0/100/200/300/400/500 ug/mL) of Cy7.5-ErNP were added to the plate, with 5 replicate samples for each concentration. After 24 hours co-incubation at 37 ℃with 5% CO_2, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well (10 uL, 5 mg/mL, per well) and incubated for 3 hours at 37℃ with 5% CO_2. After removing the culture medium and unmetabolized MTT, the dimethylsulfoxide (DMSO) was carefully added to dissolve the resulting precipitate. The absorbance at 490/570 nm per well was measured by the enzyme-linked immunosorbent assay instrument. The OD (optical density) value per group was compared to determine cellular toxicity.

Live and dead cell staining

The culture medium with Cy7.5-ErNP (300 ug/mL) was incubated with HL-1 cells in a culture dish for 24 hours. After removing the medium and washing with the PBS buffer, Calcein AM (Ex/Em= 494/517 nm) and propidium iodide (PI, Ex/Em=535/617 nm)

solution was added to the dish. After incubation for 30 minutes, the stained cells in the culture dish were observed under the fluorescence microscope.

The mouse model of kainic acid induced mesial temporal lobe epilepsy (mTLE) and electroencephalogram (EEG) monitoring.

Kainic acid-induced mTLE model: The animal procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of ShanghaiTech University (approval No. 20211115001) and was conducted in accordance with the guidelines of the IACUC, ShanghaiTech University. Male C57BL/6 mice with 8 weeks were chosen for kainic acid (KA) intracerebral injection. A mouse was randomly selected from the cage and placed into a chamber filled with isoflurane. After being anesthetized, the head of the mouse was fixed by the stereotaxic apparatus, and continuous anesthetic gas was delivered into the nasal cavity of the mouse. To ensure eye moistening during the surgery, tetracycline eye ointment was applied to the inner surface of the mouse's eyelids. Depilatory cream was applied to the head of the mouse to remove the fur above the skull. Next, the skin above the skull was incised along the midline of the skull using surgical scissors. The skin was pushed aside to expose the skull. Cotton swabs with 10% H_2O_2 were used to remove the period and clean the skull surface.

After confirming the positions of bregma and lambda on the skull surface, a borehole was drilled out with the electric drill at the location of the left hippocampus (AP: -2 mm, ML: -1.5 mm). Using cotton swabs to wipe away a little bleeding, the needle tip of the microsyringe containing KA solution (5 mM in PBS buffer) was inserted into the borehole location. With the assistance of a stereotaxic apparatus, the needle's descent

depth was controlled to 2 mm. Then 0.2 μL KA solution was injected into the left hippocampus. The incised skin was bonded by the biological adhesive. Once the mouse regained consciousness, it was returned to the cage. After about 30 minutes, epileptic symptoms began to be observed on the KA mouse.

EEG monitoring: The mouse skull was exposed by the operation which was similar to the previous description. In addition to creating the borehole for KA, two other boreholes were drilled at another location in the hippocampus (AP: -2.8 mm, ML: -3.0 mm) and at the contralateral occipital bone of the KA-injection borehole. Before inserting the needle tip of the microsyringe, two EEG electrodes were individually inserted into two other boreholes and secured by the composite resin cement. Subsequent steps were similar to those described previously. The EEG from the electrode in the hippocampus reflected the electrophysiological signals originating from the epileptic focus induced by KA. The EEG from the electrode in the occipital bone reflected the electrophysiological signals from the mouse brain cortex.

Optical recording of brain electrophysiological activities in mTLE mice using Cy7.5-ErNP

During the establishment of mTLE mouse model, two additional boreholes were carefully drilled adjacent to the KA injection site. These boreholes were individually located at the hippocampus (AP: -2.8 mm, ML: -3.0 mm) and the motor cortex (AP: -0.8 mm, ML: 0.8 mm). After injecting 0.2 μL of KA solution (5 mM in PBS buffer), the Cy7.5-ErNP solution (5 uL, 20 mg/mL) was injected into both the hippocampus and motor cortex. After sealing the incision, the Cy7.5-ErNP labeled KA mouse was placed

under the NIR-II imaging system with a 1500 nm long-pass filter. To minimize the motion interference, the mouse was securely positioned. When the mouse regained consciousness and displayed a stiffened and raised tail, the NIR IIb imaging result of Cy7.5-ErNP began to be recorded.

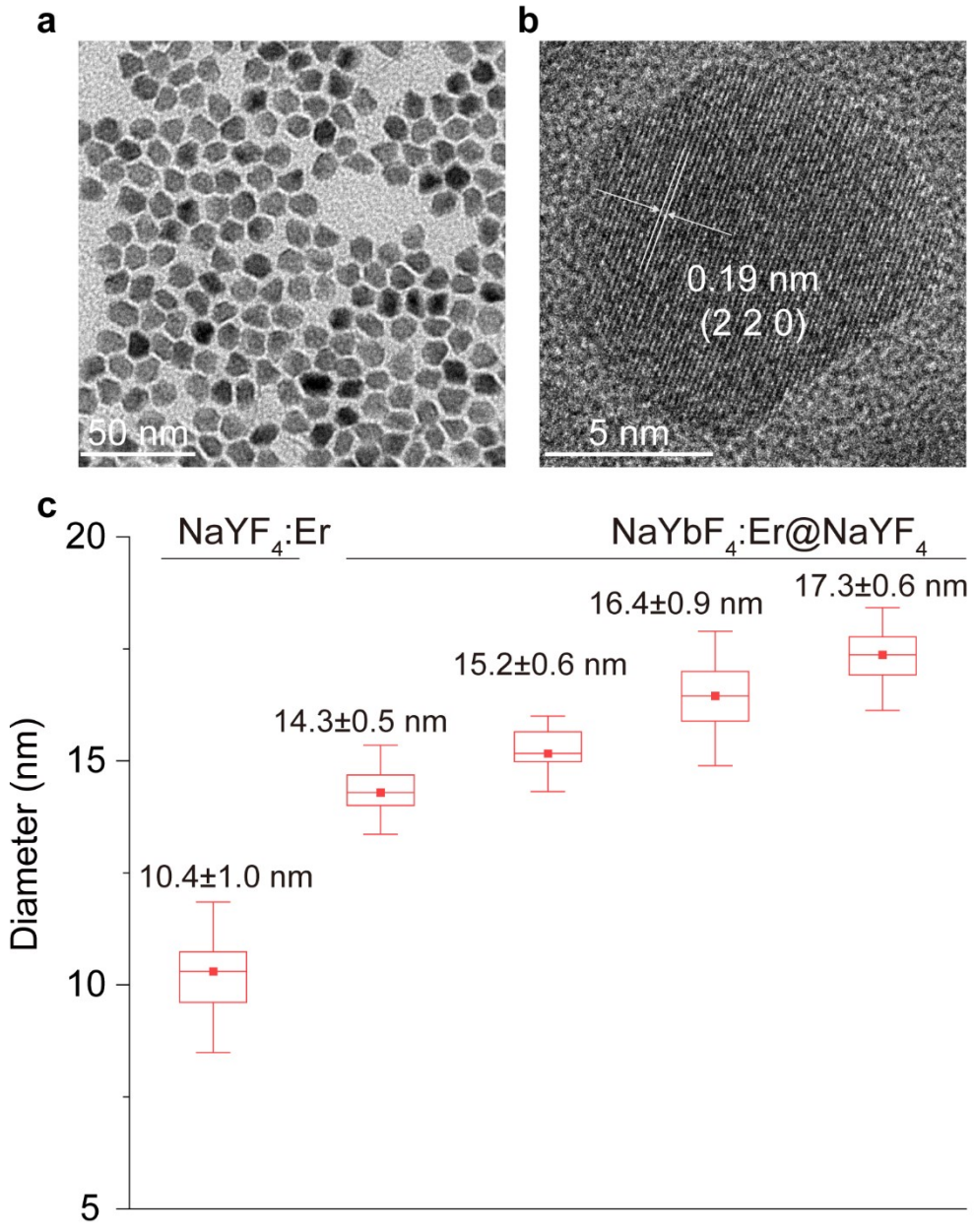


Figure S1. a Transmission Electron Microscopy (TEM) imaging of $NaYbF_4$:Er. **b** High-

resolution TEM image of the $NaYbF_4$:Er lanthanide nanoparticle. **c** The diameter statistical result of $NaYbF_4$:Er and $NaYbF_4$:Er@$NaYF_4$ with different inert shell thickness. The data were presented as mean values based on two hundred of nanoparticles counted in each group (n = 200). The inert shell thickness was calculated by the equation (3):

$$shell\ thickness = \frac{Diameter_{core-shell}}{2} - \frac{Diameter_{core}}{2} \quad (3)$$

And the standard deviation (SD) of inert shell thickness was calculated by the equation (4):

$$\sigma_{thickness} = \sqrt{\left(\frac{\sigma_{core-shell}}{2}\right)^2 + \left(\frac{\sigma_{core}}{2}\right)^2 - \left(\frac{\sigma_{(core-shell)(core)}}{2}\right)} \quad (4)$$

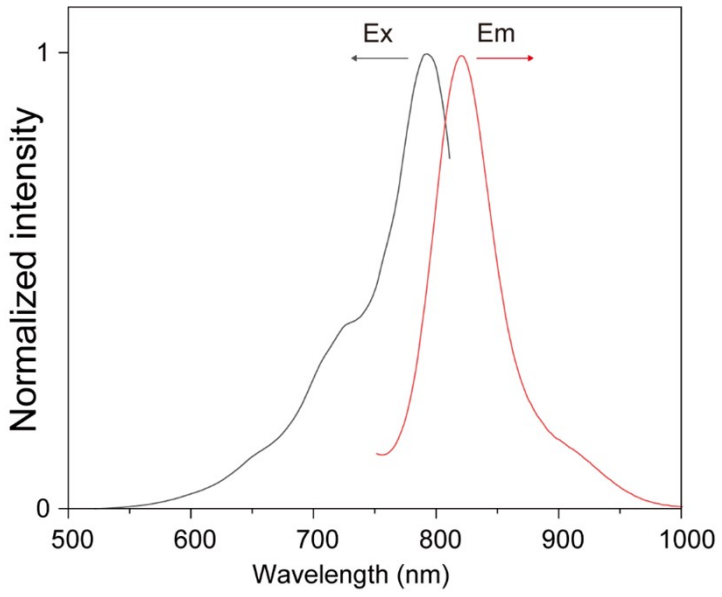


Figure S2. The normalized excitation and fluorescence spectra of Cy7.5 in DMSO.

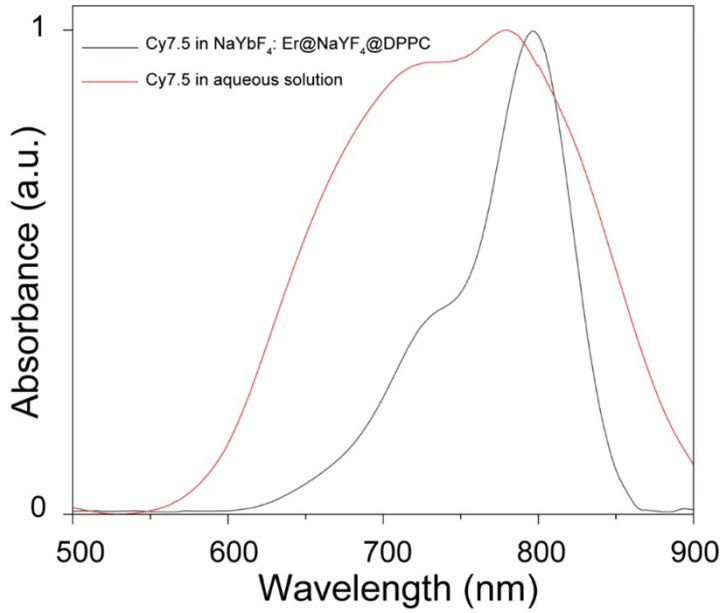


Figure S3. The H-aggregation comparison of Cy7.5 dissolved in aqueous solution and infiltrated into the $NaYbF_4$:Er@$NaYF_4$@DPPC.

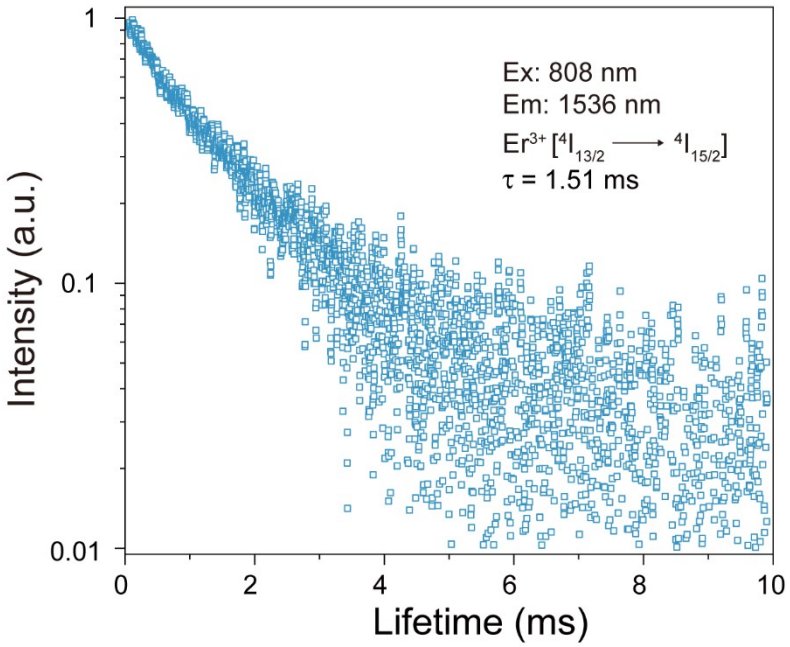


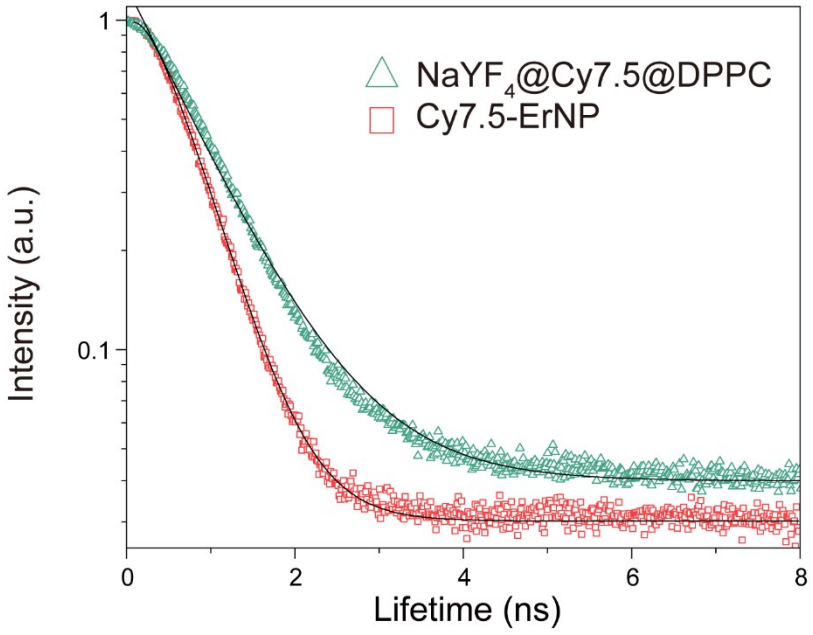
Figure S4. Luminescence lifetime measurement of $NaYbF_4$:Er@$NaYF_4$@Cy7.5@DPPC (Cy7.5-ErNP) at 1536 nm under the excitation of an 808 nm laser.

Materials	Gradient	relative QY
Cy7.5	4490613.08	-
$NaYF_4$@Cy7.5@DPPC	2439492.41	4.60%
Cy7.5-ErNP	914541.05	1.72%

Figure S5. The relative quantum yields (relative QY) of Cy7.5 in $NaYF_4$@Cy7.5@DPPC and Cy7.5-ErNP. The absolute quantum yield (QY) of Cy7.5 in dimethylformamide (DMF) under the 808 nm excitation was 9.04 %. The relative QY of Cy7.5 in different conditions were calculated according to the equation (5):

$$\Phi_x = \Phi_{ST}\left(\frac{Gradient_x}{Gradient_{ST}}\right)\left(\frac{\eta_x}{\eta_{ST}}\right) \quad (5)$$

where Φ_{ST} means the QY of Cy7.5, $Gradient_{ST}$ means the gradient between the Cy7.5 absorbance and fluorescence spectra, and η_{ST} means the refractive index of Cy7.5 solution. Φ_x, $Gradient_x$, η_x individually represented the QY, gradient, and refractive index of the tested samples.



	τ_1 (ns)	B_1	τ_2 (ns)	B_2	τ_{ave} (ns)
$NaYF_4$@Cy7.5@DPPC	0.787	0.999	28.729	0.001	0.815
Cy7.5-ErNP	0.327	0.460	0.397	0.540	0.365

Figure S6. Fluorescence lifetime measurement of Cy7.5 in both $NaYF_4$@Cy7.5@DPPC and Cy7.5-ErNP under the excitation of an 808 nm laser. The fluorescence data was fitted by the double exponential function: $I(t) = B_1 \cdot e^{\left(\frac{-t}{\tau_1}\right)} + B_2 \cdot e^{\left(\frac{-t}{\tau_2}\right)}$ The average lifetime ($\tau_{ave} = \frac{\tau_1 \cdot B_1 + \tau_2 \cdot B_2}{B_1 + B_2}$) of Cy7.5 in $NaYF_4$@Cy7.5@DPPC and Cy7.5-ErNP were 0.815 ns and 0.365 ns, respectively.

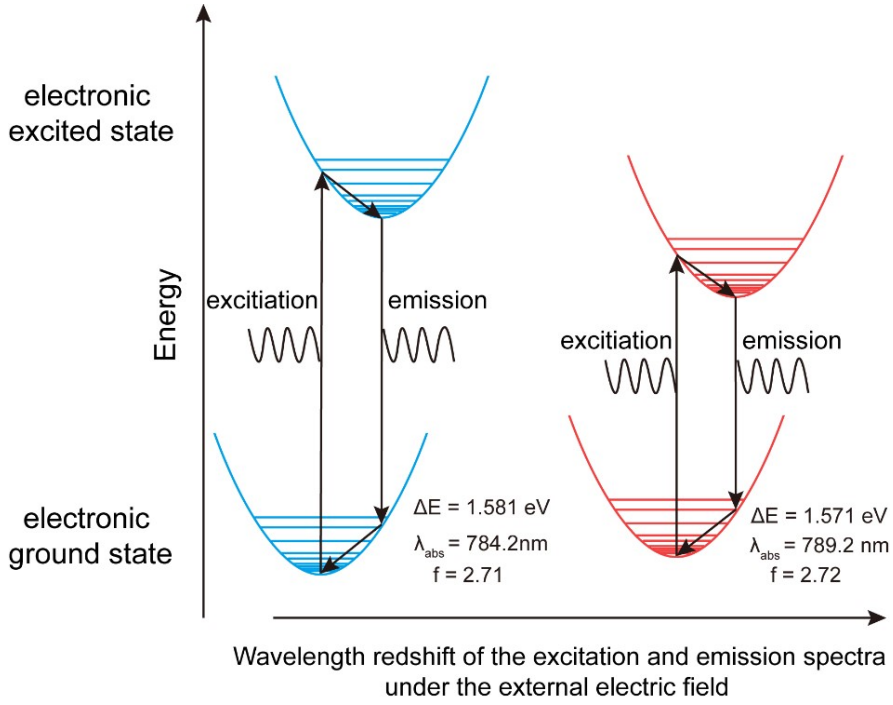


Figure S7. Franck–Condon energy diagram showed the spectra shift of the Cy7.5 molecule in the absence and presence of the EEF. With the EEF, the excitation energy of Cy7.5 became 1.571 eV from the original 1.581 eV, which represents the redshift of 5 nm in both the calculated absorbance and emission spectra.

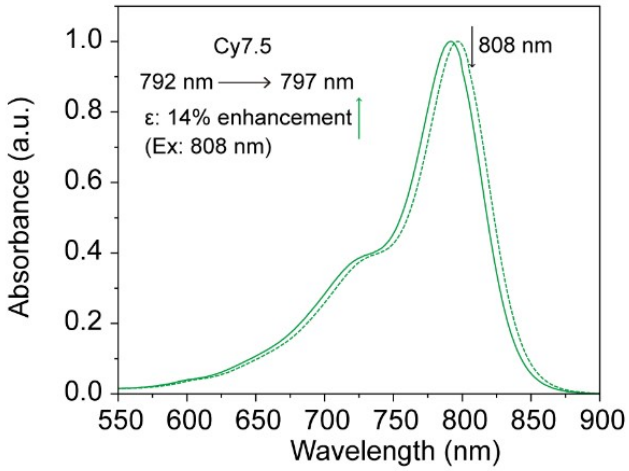


Figure S8. Absorption coefficient (ε) changes of Cy7.5 at 808 nm. The peak of the Cy7.5 absorbance spectrum was at 792 nm. In presence of the EEF, the ε of Cy7.5 at 808 nm achieved the enhancement of 14 %

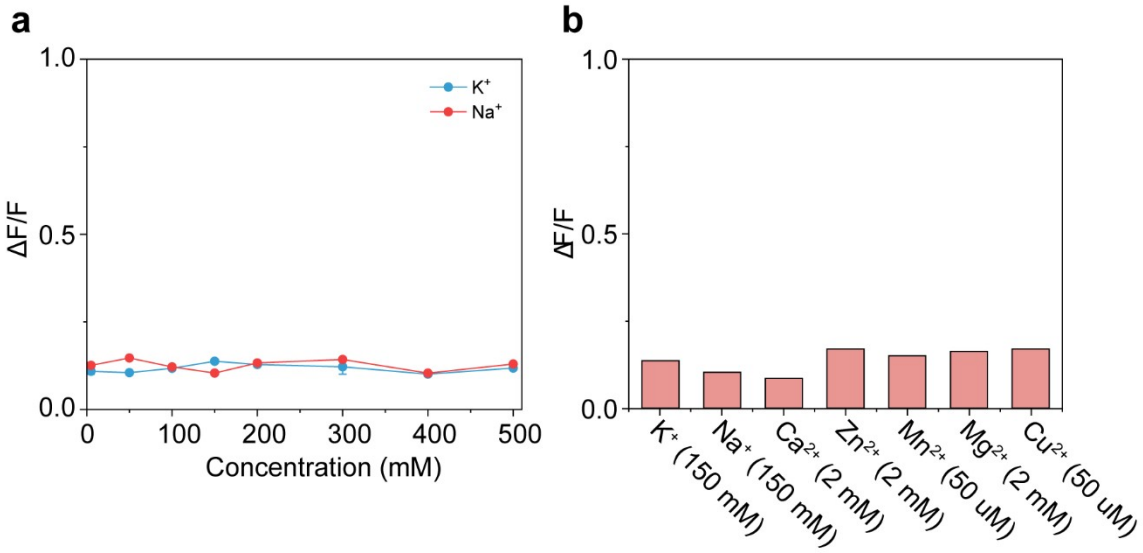


Figure S9. a Fluorescence insensitivity of Cy7.5 for the variation of Na^+ or K^+ concentration. **b** ΔF/F comparison of Cy7.5 under the various physiological cations.

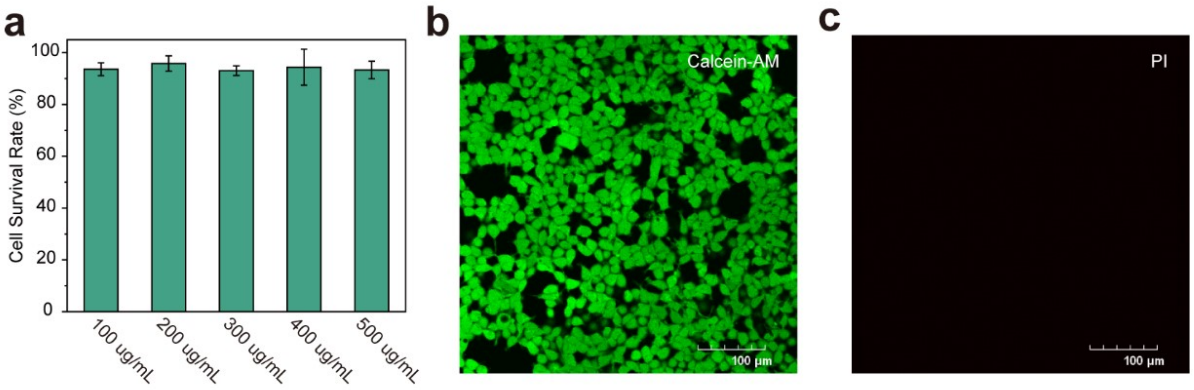


Figure S10. a The MTT assay result of Cy7.5-ErNP which co-incubated with HL-1 cells for 24 hours. Five samples in each group (n = 5). **b** and **c** The staining result of HL-1 cells with calcein-AM and propidium iodide (PI) after co-incubation with Cy7.5-ErNP for 24 hours.

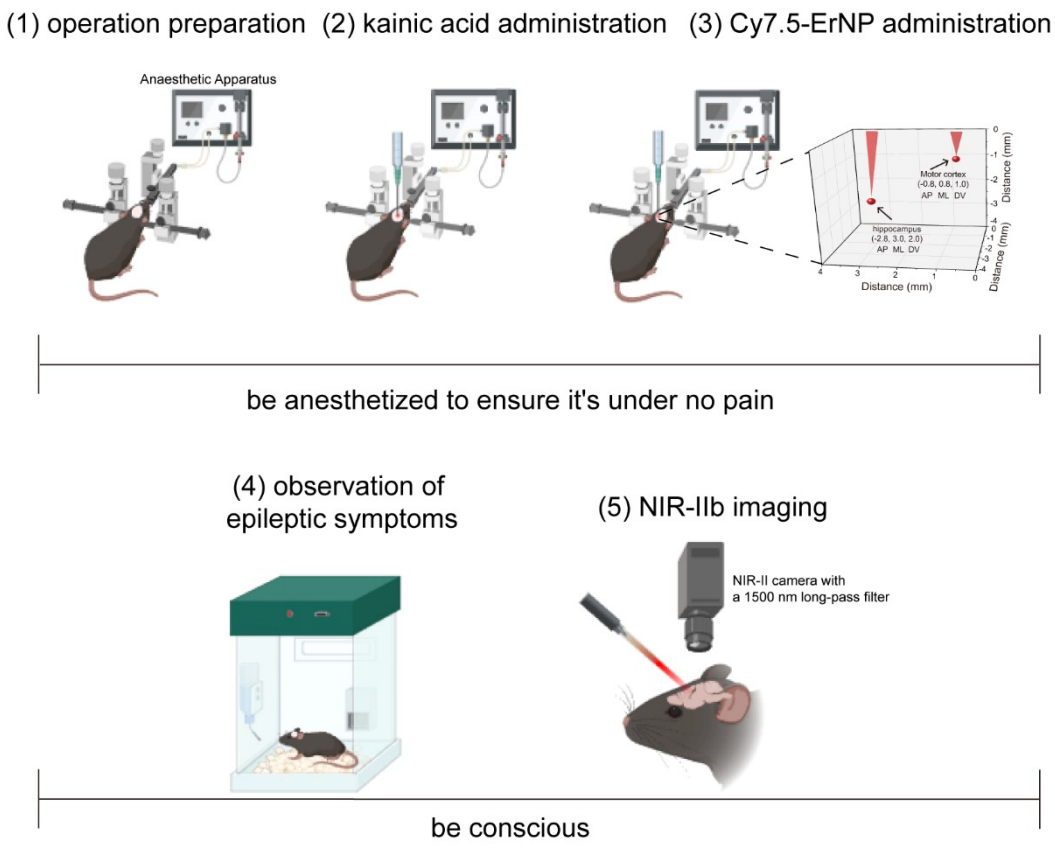


Figure S11. The schematic diagram of Cy7.5-ErNP administrated kainic acid-injected (KA) mouse modeling (created with BioRender.com). The mouse became anesthetized throughout the entire surgical procedure. In brief, the operation preparation consisted of pre-anesthesia, fur and skin removal, and drilling holes in the skull. After that, the KA solution and Cy7.5-ErNP were successively administrated. The locations of Cy7.5-ErNP in the KA-mouse brain were as follows: hippocampus region (ML 3.0 mm, AP -2.8 mm, DV 3.0 mm) and the motor cortex (ML 0.8 mm, AP -0.8 mm, DV 1.0 mm). After the mouse became conscious and exhibited the epileptic symptoms, the NIR-IIb imaging for monitoring electric activity in vivo started optical recording.

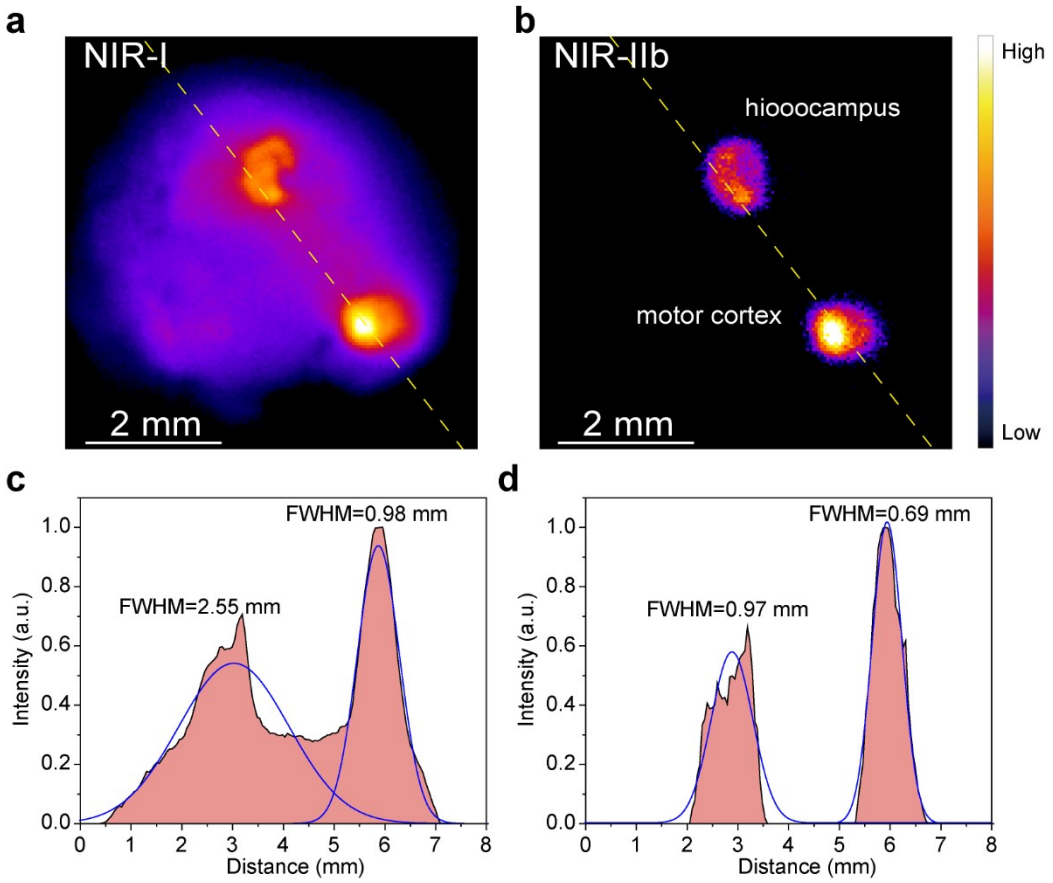


Figure S12. a NIR imaging under a 900 nm long-pass filter and **b** NIR-IIb imaging under a 1500 nm long-pass filter after Cy7.5-ErNP was injected intracranially into the KA mouse. **c** and **d** The intensity profiles of the hippocampus and motor cortex regions in **a** and **b**, respectively.

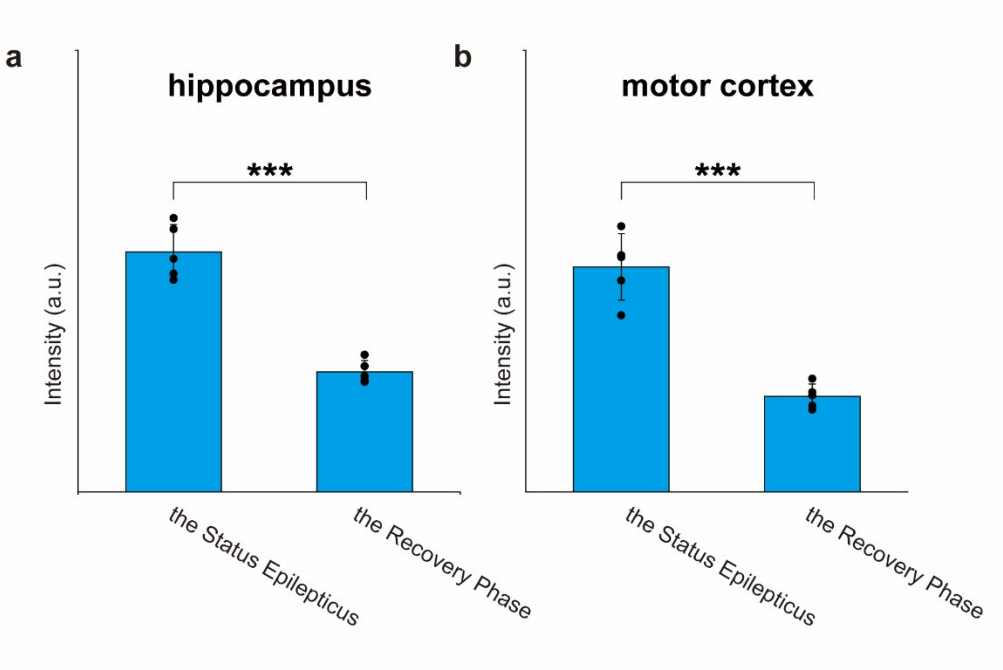


Figure S13 The statistical results of luminescence intensity from Cy7.5-ErNP intracranially administrated into the KA mouse during both the Status Epilepticus and the Recovery Phase. The result of hippocampus was in **a** and the result of motor cortex was in **b**. The significant differences between different groups were determined using Student's t-test. Five regions in each group (n = 5, *P<0.05, **P<0.01, and ***P<0.001).

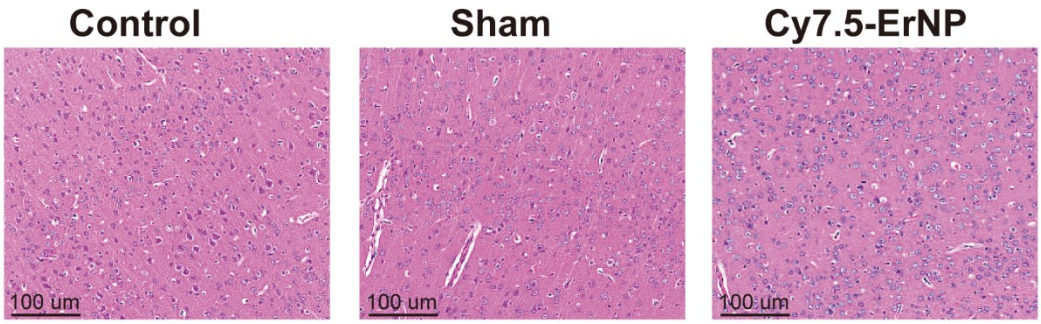


Figure S14. The H&E staining of brain slices one week after the administration of Cy7.5-ErNP.

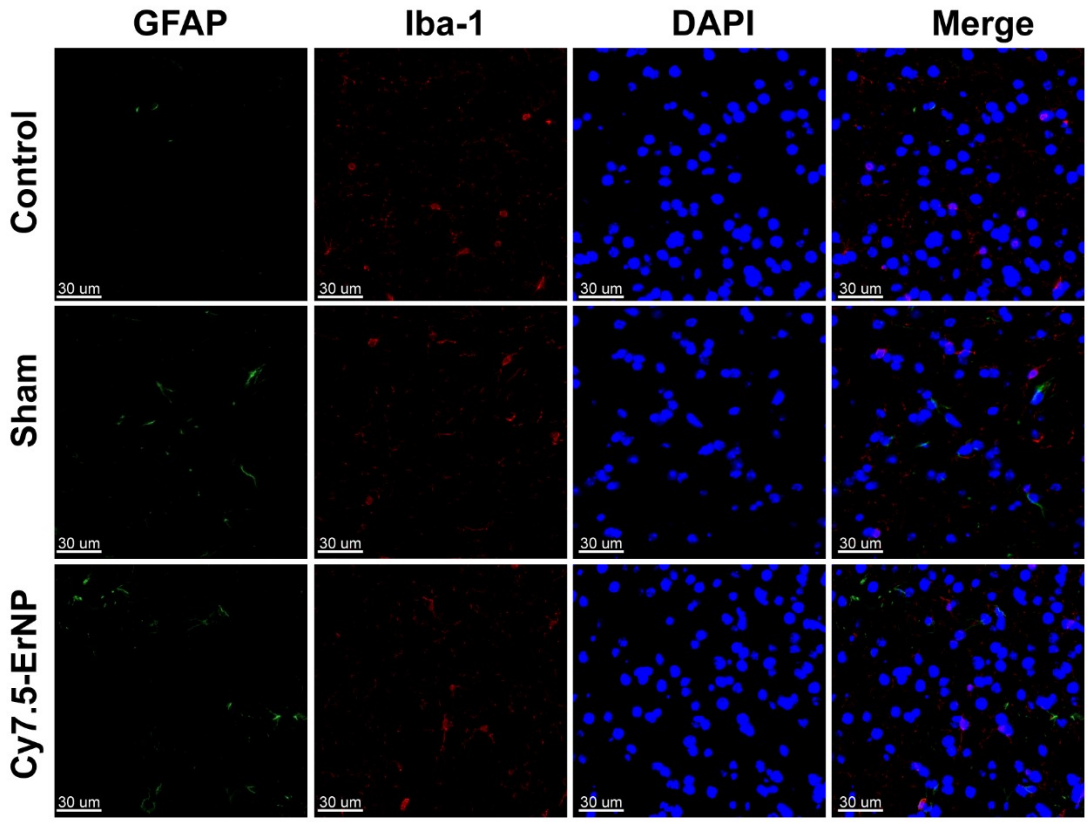


Figure S15. The immunofluorescence staining of brain slices with glial fibrillary acidic protein, and ionized calcium-binding adapter molecule 1 (GFAP/Iba-1). There are three groups as follows: brain slice one week after the Cy7.5-ErNP administration (Cy7.5-ErNP), brain slice one week after similar operation without Cy7.5-ErNP (Sham), and

brain slice without operation (Control).

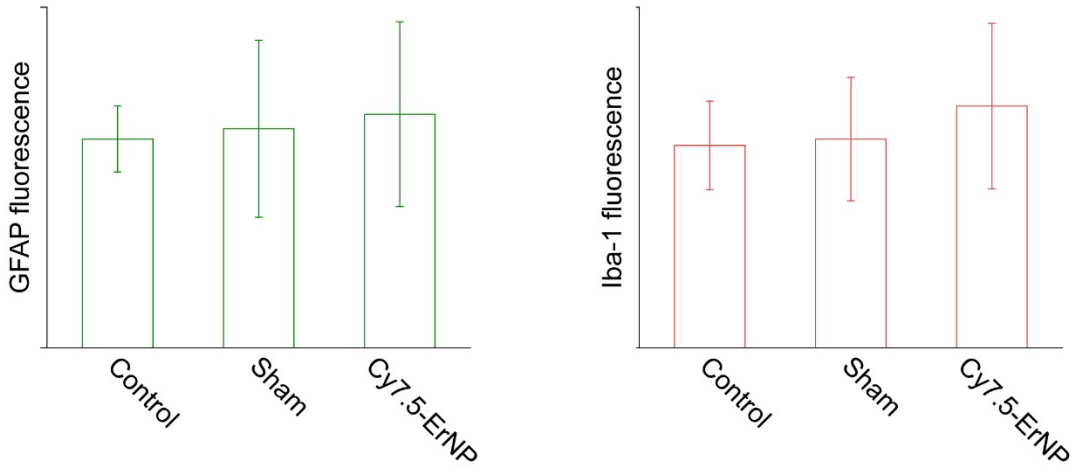


Figure S16. The statistical results of average fluorescence intensity in Figure S15. The result of GFAP staining was on the left and the result of Iba-1 staining was on the right. Five regions in each group (n = 5).

References

1. T. Lu and F. Chen, *J. Comput. Chem.*, 2011, **33**, 580-592.

2. K. Nakano, T. Konishi and Y. Imamura, *Chem. Phys.*, 2019, **518**, 15-24.