Supporting information

A dual-site fluorescent probe for tracing the interaction between ATP and NE in neuroinflammation

1. Materials and methods

Materials. All the reagents are analytical grade and purchased from professional reagent suppliers. Experimental water is purified deionized water.

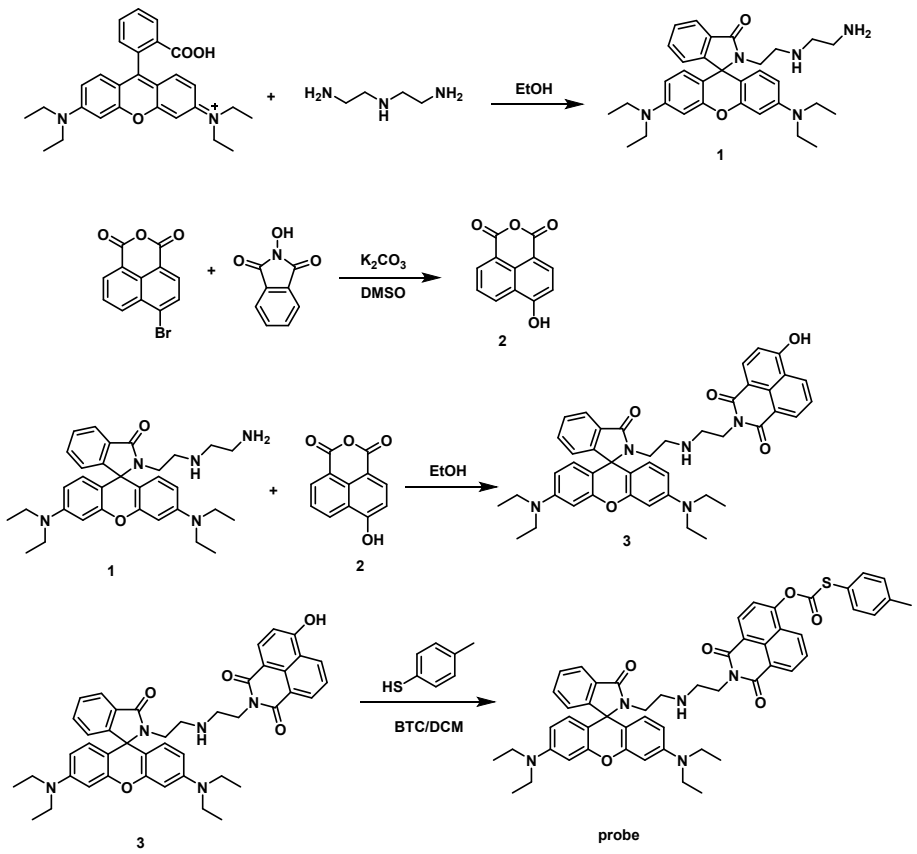
The probe (2 mM) stock solution was prepared by DMSO. The stock solutions of analytes were prepared by PB (pH 5.0) solution. The stock solutions of LPS were prepared by DMEM medium. The "high-K^+" solution was used to trigger exocytosis that consisted of 55 mM NaCl, 100 mM KCl, 5 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose titrated to pH 7.3 with 1M NaOH.

Instrument. During the spectral test, the instruments used are Hitachi F-4700 fluorescence spectrophotometer and Shimadzu UV-2600i ultraviolet visible spectrophotometer. The quartz tube was provided by China Shanghai Huamei Laboratory Instrument Factory. The ^1H NMR and ^{13}C NMR are measured by Quantum-IPlus 400-2 (AS400) 400 MHz and 101 MHz. The HRMS data were measured by Thermo Scientific Q Exactive. The cells imaging was carried out by Zeiss LSM880 Airyscan confocal laser scanning microscope. CytoFLEX S of the Beckman Coulter was used to quantify intracellular fluorescence intensity.

Cell culture. The PC12 cells were grown in DMEM medium with 10% fetal bovine serum and 1% antibiotics in 37 ◦C, 5% carbon dioxide.

Software. Originpro 9.0, Zen 2, Image J.

2. Synthesis



Synthesis of Compound 1

Compound 1 was prepared according to previous reported works [1].

Synthesis of Compound 2

4-Bromo-1,8-naphthalic anhydride (2 mmol), N-hydroxyphthalimide (2.4 mmol), and K_2CO_3 (3 mmol) were added to 100 mL flask. The mixture was refluxed in dimethyl sulfoxide for 6 h. After acidification with hydrochloric acid, filtered and washed 3 times with deionized water to obtain compound 2.

Synthesis of Compound 3

The mixture of compound 1 (2 mmol) and compound 2 (2 mmol) were dissolved in ethanol and refluxed 8 h. After evaporating the solvent under vacuum, the crude product was then purified by chromatography column with methanol/dichloromethane (1/10, v/v) to obtain compound 3.

Synthesis of Compound probe

To a solution of p-toluenethiol (10 mmol) and triphosgene (0.5 *eq.*) in CH_2Cl_2 (20 mL) at 0 °C, pyridine (1 *eq.* in 5 mL of CH_2Cl_2) was added dropwise. The mixture was stirred for 1 h at 0 °C and then poured into 100 mL of ice water. The organic layer was separated, washed with H_2O, dried with sodium sulfate, and concentrated under reduced pressure. The obtained crude product was used for further synthesis directly. Compound 3 (1 mmol) and Et_3N (2 *eq.*) were dissolved in 10 mL CH_2Cl_2. The previous crude product (2 *eq.* in 5 mL of CH_2Cl_2) was added. The mixture was gradually warmed to room temperature and continued to react for 10 h. After that, the solvent was removed under reduced pressure, and the residue was separated by column chromatography using CH_3OH/CH_2Cl_2 (v:v, 1:20) as eluent to give probe as a orange solid (yield: 21%). ^{1}H NMR (400 MHz, Chloroform-*d*): 8.67–8.49 (m, 2H), 8.42–8.29 (m, 1H), 7.95 (d, 2H), 7.79 (s, 1H), 7.47 (s, 2H), 7.13 (s, 1H), 6.71 (s, 2H), 6.61–6.45 (m, 4H), 6.37 (s, 4H), 4.26 (d, 2H), 3.62 (d, 2H), 3.46–3.13 (m, 12H), 2.18 (s, 3H), 1.13 (s, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*): 163.5, 148.9, 135.1, 132.8, 132.4, 131.1, 130.5, 129.0, 127.9, 123.9, 122.8, 108.3, 105.3, 97.9, 65.0, 44.9, 44.4, 37.5, 21.1, 12.6. MS (ESI, m/z): Calcd for [M+] 872.3487, found: 872.34796.

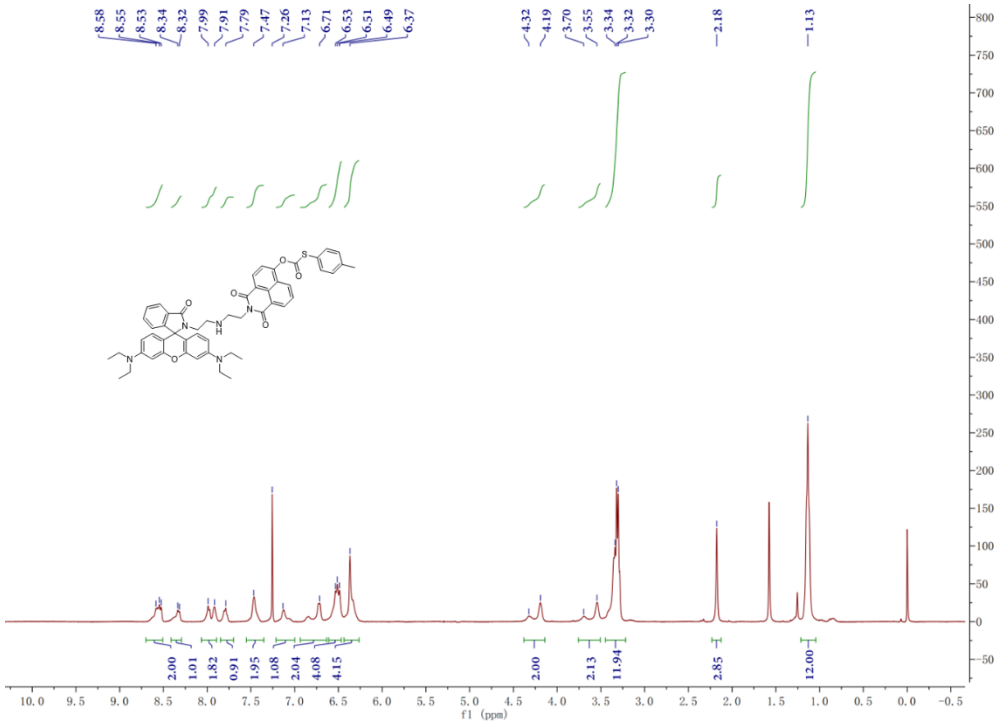


Fig. S1 ^{1}H spectra of RN-NE/ATP in Chloroform-*d*.

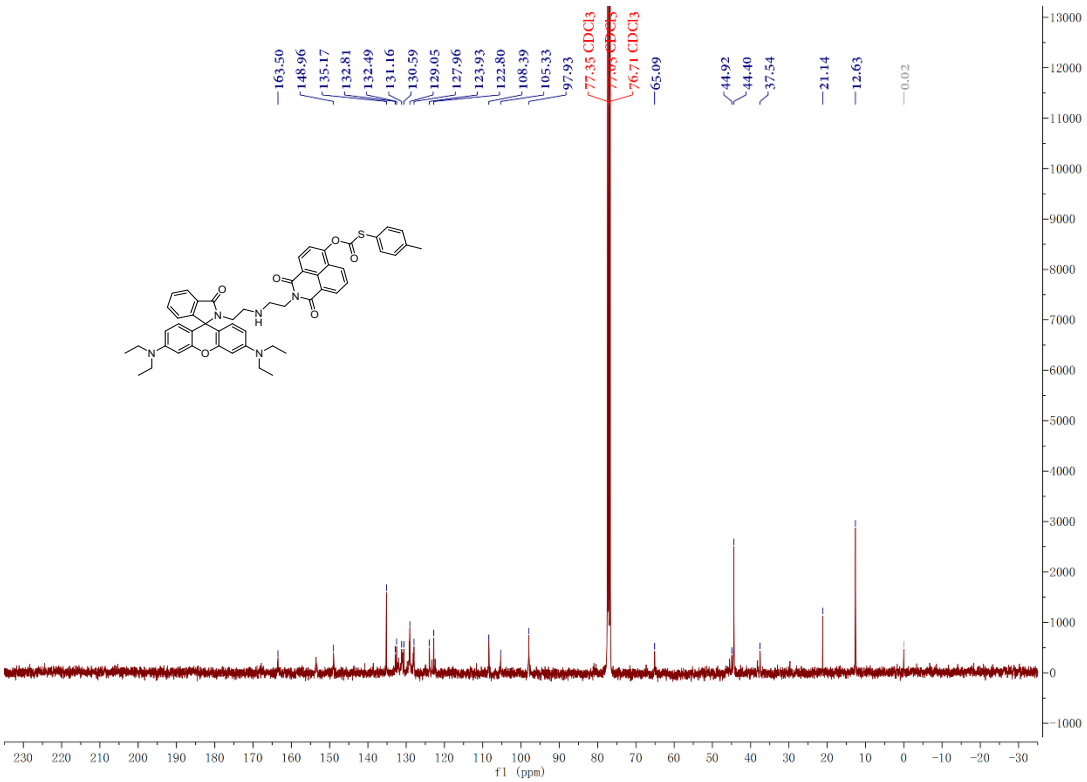


Fig. S2 ^{13}C NMR spectra of RN-NE/ATP in Chloroform-*d*.

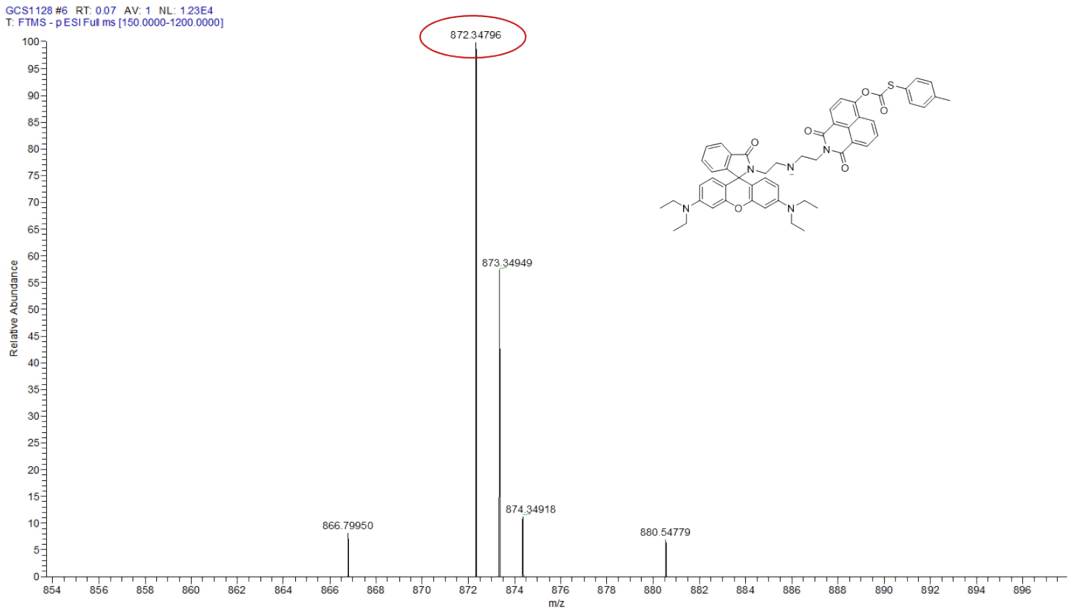


Fig. S3 HR-MS spectrum of RN-NE/ATP.

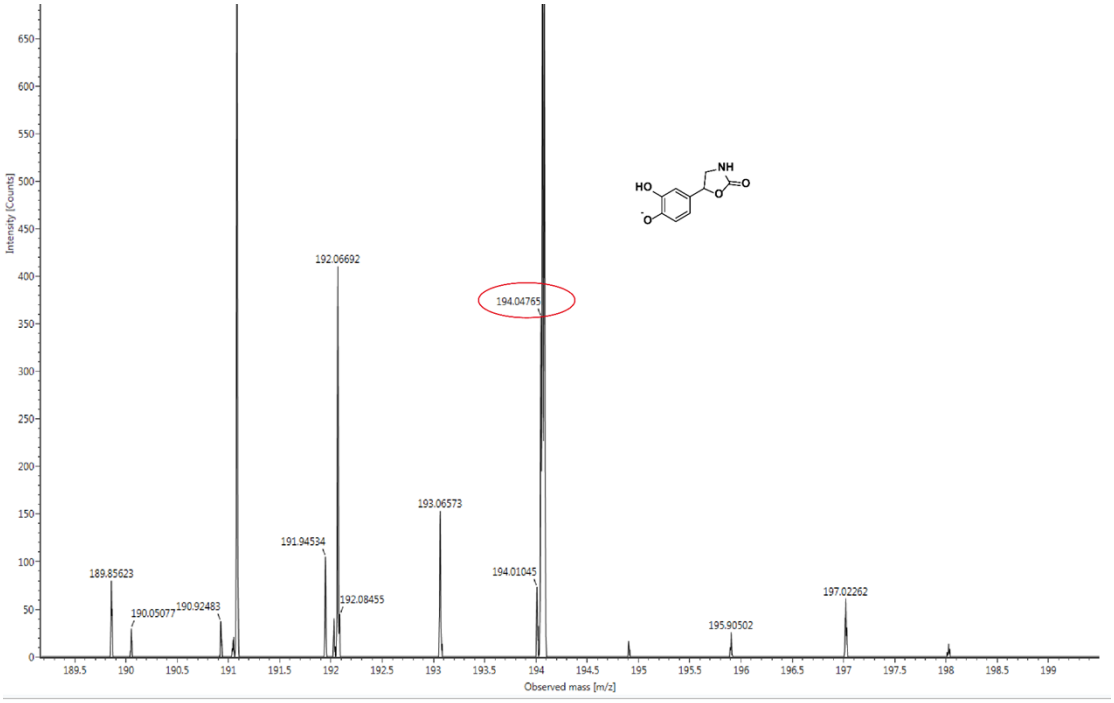


Fig. S4 HR-MS spectrum-1 of RN-NE/ATP with NE.

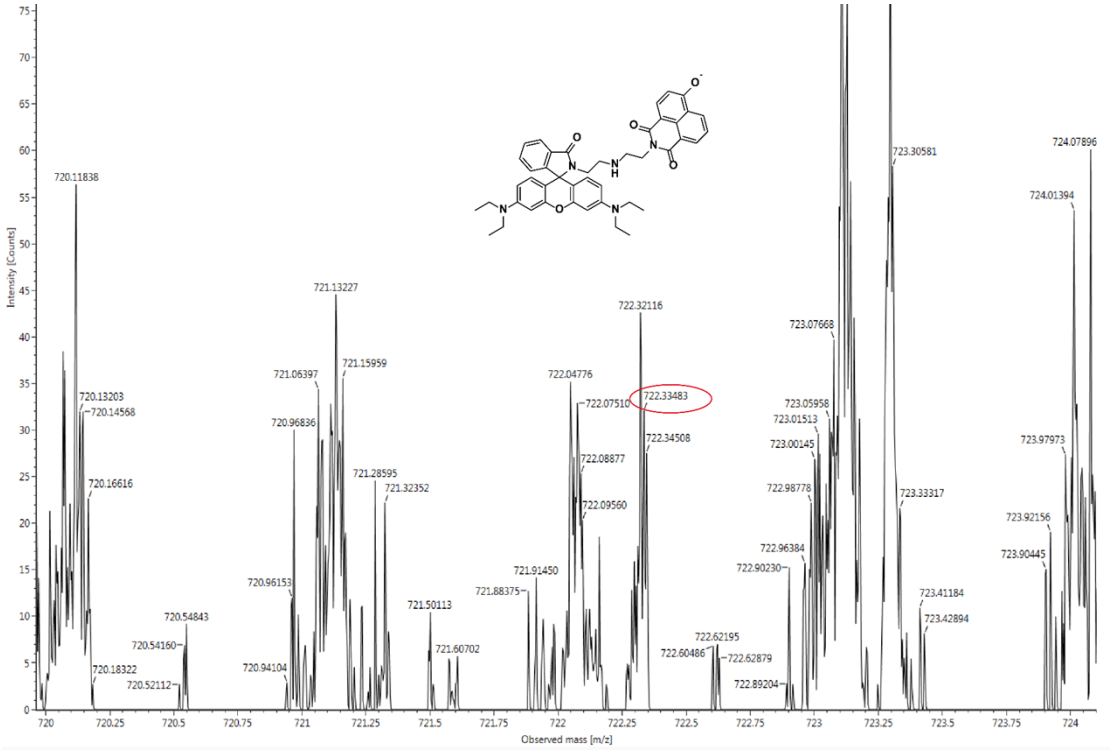


Fig. S5 HR-MS spectrum-2 of RN-NE/ATP with NE.

3. Spectroscopic Reaction behavior.

The stock solution of RN-NE/ATP (2 mM) was prepared in DMSO and stored at 4 ^{o}C. All spectroscopic study of probe was carried out in test solution of RN-NE/ATP (10 μM) in 2 mL PB/acetonitrile (v:v 1:1, pH 5.0) system, which was prepared by adding 10 μL RN-NE/ATP stock solution into buffer in a quartz cuvette. After measuring the emission and absorption spectra of the test solution, the response behavior of probe to NE and ATP were determined by adding NE stock solution or ATP stock solution into the test solution of probe. The temporal tracking of the RN-NE/ATP reaction with NE was carried out by adding NE into probe test solution in PB/ acetonitrile (v:v 1:1, pH 5.0) system followed by recording UV-vis and fluorescence spectra at ambient temperature every 20 minute with mixing. The temporal tracking of the probe reaction with NE was carried out by adding ATP into RN-NE/ATP test solution in PB/ acetonitrile (v:v 1:1, pH 5.0) system followed by recording UV-vis and fluorescence spectra at ambient temperature every 5 minute with mixing. The other analyte reaction behavior of probe was determined in a similar procedure.

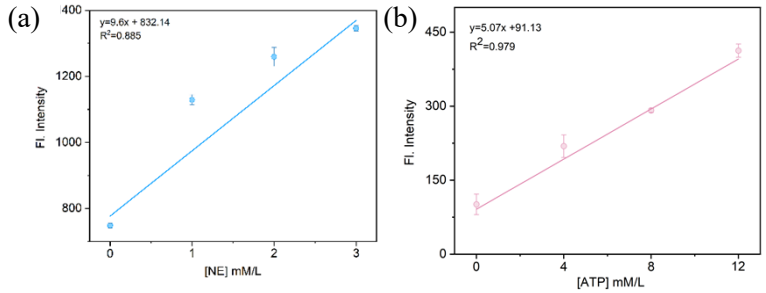


Fig. S6 (a) Quantitative fluorescent intensities at 480 nm upon addition of 0 mM, 1 mM, 2 mM, 3 mM NE to 10 μM RN-NE/ATP -containing system. The fluorescent intensity data were obtained 180 min after NE addition, error bars represent standard deviations, n=3, λ_{ex}: 400 nm; (b) Quantitative fluorescent intensities at 580 nm upon addition of 0 mM, 4 mM, 8 mM, 12 mM NE to 10 μM RN-NE/ATP -containing system. The fluorescent intensity data were obtained 60 min after NE addition, error bars represent standard deviations, n=3, λ_{ex}: 560 nm.

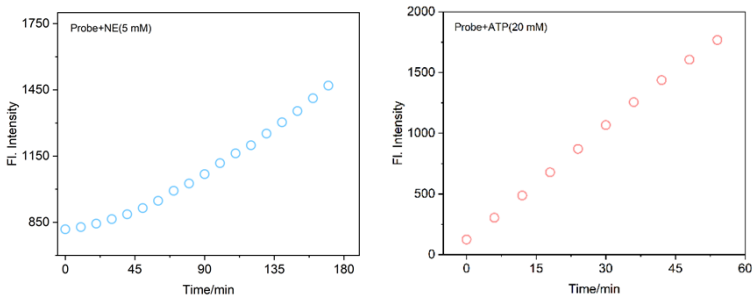


Fig. S5 The time dependent of 10 μM RN-NE/ATP upon 5 mM NE and 20 mM ATP.

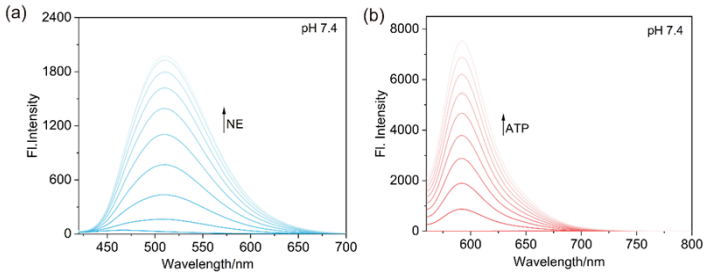


Fig. S7 (a) The fluorescent response of 10 μM RN-NE/ATP upon 5 mM NE in PBS (pH 7.4) /acetonitrile (v:v 1:1) system in 0-300 min (λ_{ex}: 400 nm); (b) The fluorescent response of 10 μM RN-NE/ATP upon 20 mM ATP in PBS (pH 7.4) /acetonitrile (v:v 1:1) system in 0-60 min (λ_{ex}: 560 nm).

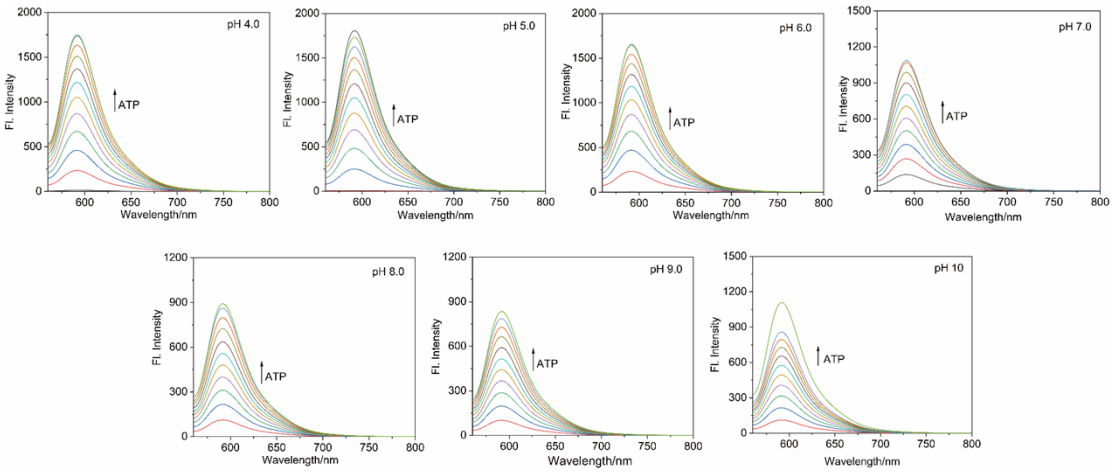


Fig. S8 The fluorescent response of 10 μM RN-NE/ATP upon 20 mM ATP in PB/acetonitrile (v:v 1:1, pH :4-10) system in 0-60 min (λ_{ex}: 560 nm).

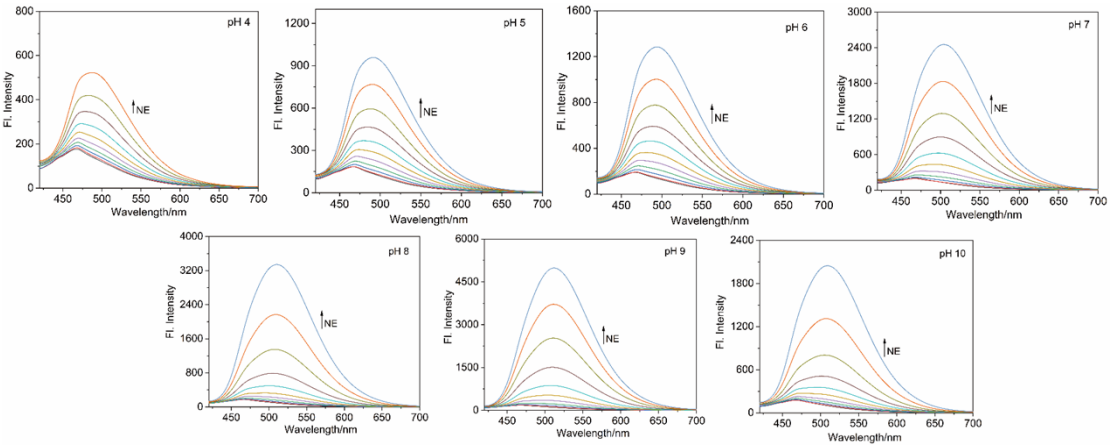


Fig. S9 The fluorescent response of 10 μM RN-NE/ATP upon 5 mM NE in PB/acetonitrile (v:v 1:1, pH :4-10) system in 0-300 min (λ_{ex}: 400 nm).

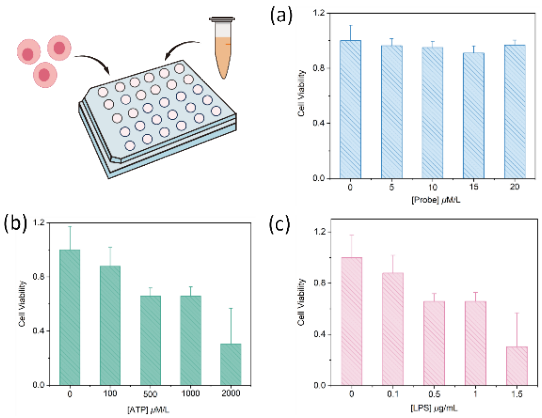
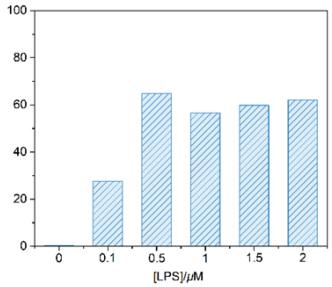


Fig. S10 (a) The CCK-8 assay of different concentrations of RN-NE/ATP on PC12 cells for 6 h; (b) The CCK-8 assay of different concentrations of ATP on PC12 cells for 6 h; (C) The CCK-8 assay of different concentrations of LPS on PC12 cells for 12 h.



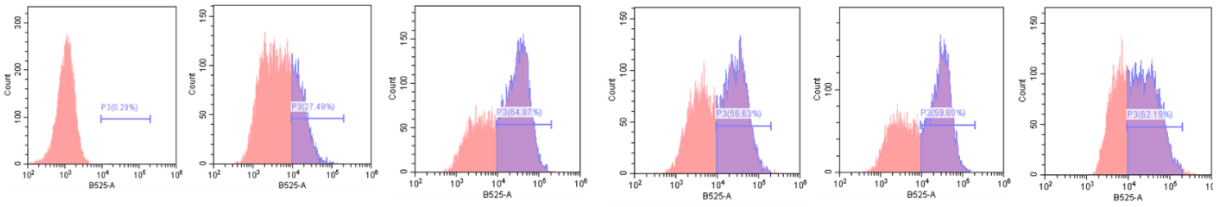


Fig. S11 Flow cytometry detection of ROS in PC12 cells using DCFH.

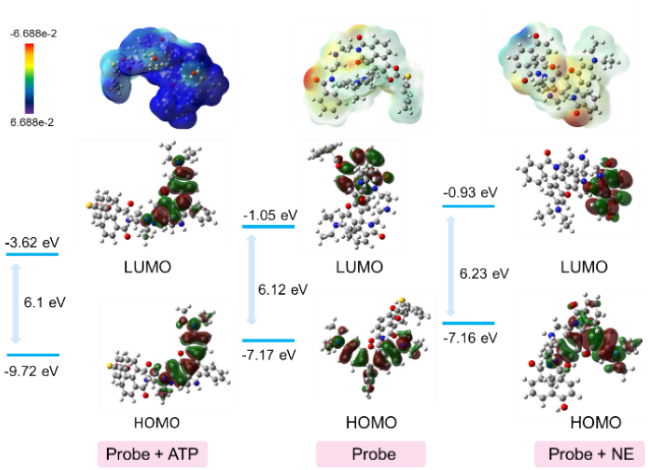


Fig. S12 The density functional theory (DFT) calculations combined with frontier orbital theory for RN-NE/ATP and the react products of RN-NE/ATP with ATP and NE.

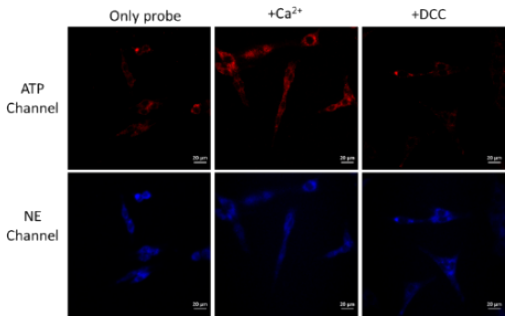


Fig. S13 PC12 cells with the RN-NE/ATP (20 μM) cultured; RN-NE/ATP (20 μM) pre-cultured PC12 cells with Ca^{2+} and DCC in red channel and blub channel. (λ_{ex}: 405 nm, bar: 20 μm)

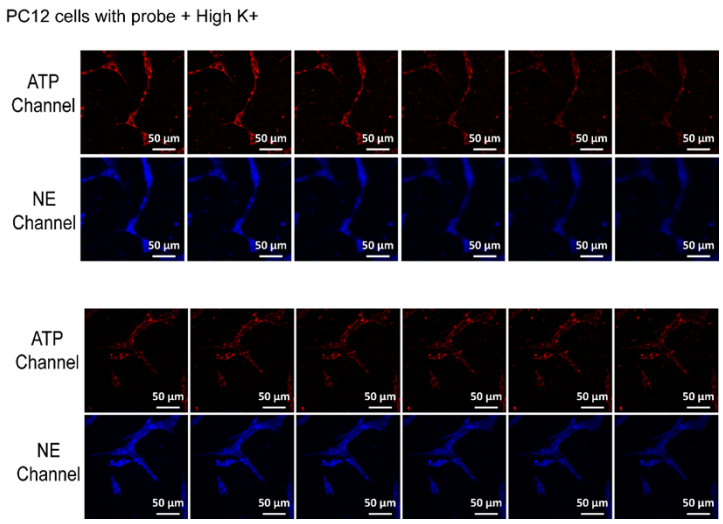


Fig. S14 RN-NE/ATP loaded normal PC12 cells with the high K^+ solution in 120s.

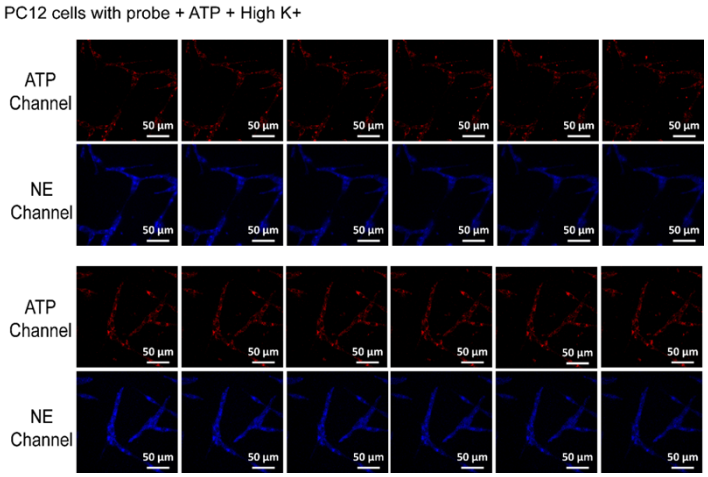


Fig. S15 RN-NE/ATP loaded normal PC12 cells with the high K^+ solution in 120s.

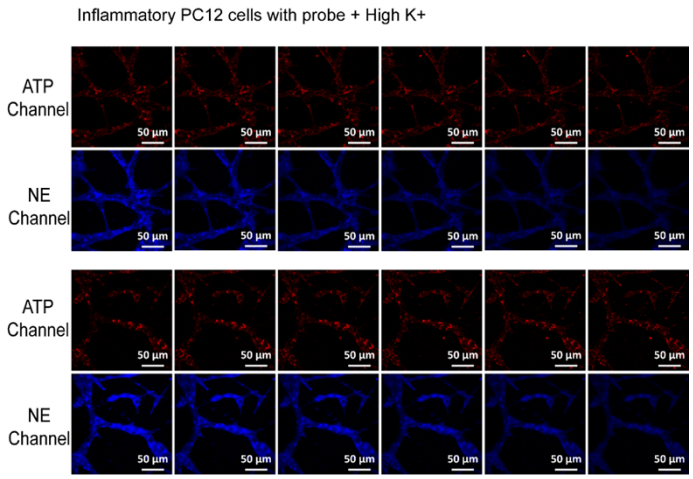


Fig. S16 RN-NE/ATP loaded inflammatory PC12 cells with the high K^+ solution in 120s.

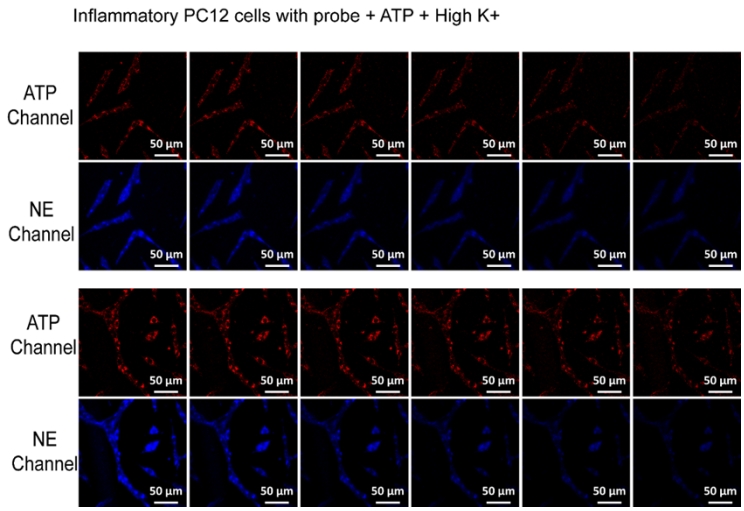


Fig. S17 RN-NE/ATP loaded inflammatory PC12 cells with the high K^+ solution in 120s.

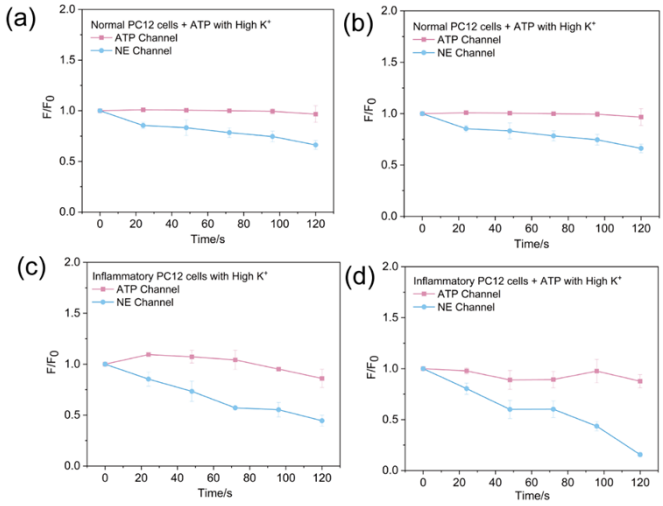


Fig. S18 (a, b) the blue and red channel fluorescent intensity curves in normal PC12 cells; (c, d) the blue and red channel fluorescent intensity curves in inflammatory PC12 cells.

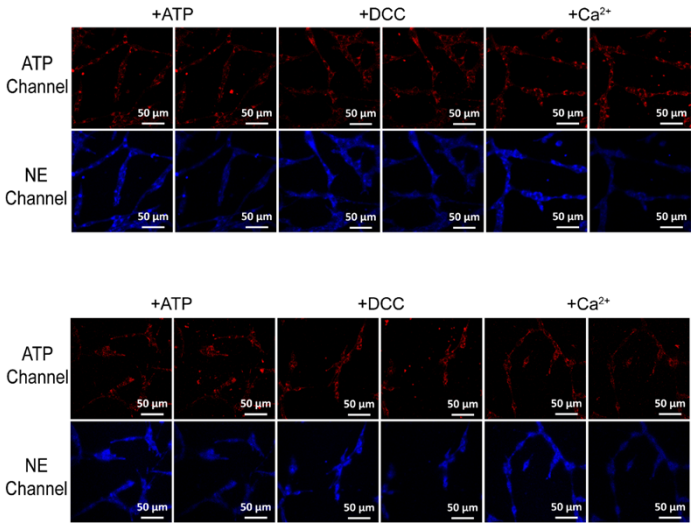


Fig. S19 ATP- RN-NE/ATP pre-incubated normal PC12 cells, DCC- RN-NE/ATP pre-incubated cells and Ca^{2+}- RN-NE/ATP pre-incubated cells with the high K^{+} solution in red and blue channel.

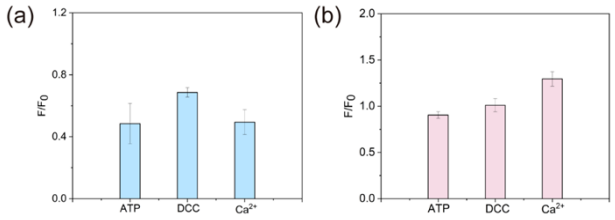


Fig. S20 The blue and red channel fluorescent intensity curves in normal PC12 cells with ATP, DCC and Ca^{2+}.

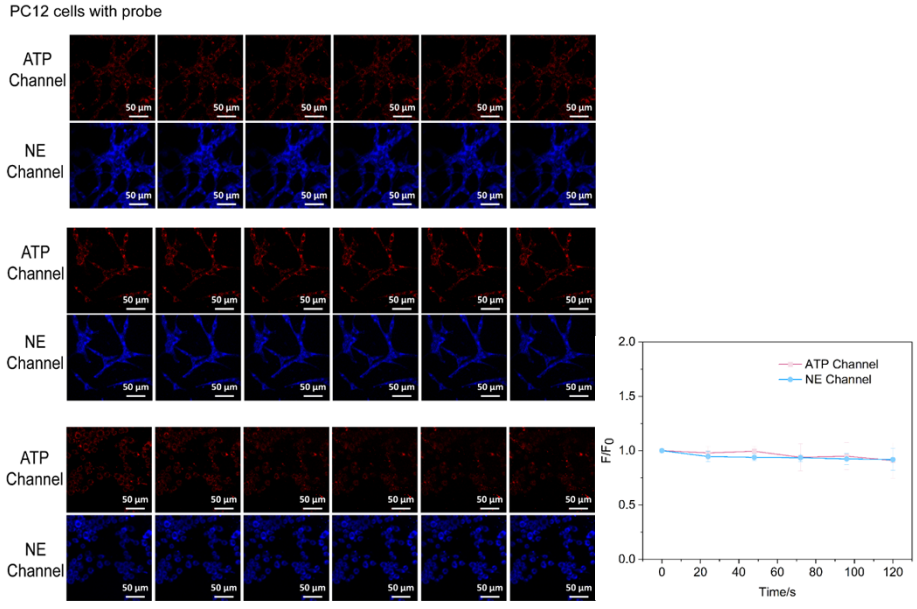


Fig. S21 RN-NE/ATP loaded PC12 cells in 120s.

References:

[1] L. Yao, W. Zhang, C. Yin, Y. Zhang and F. Huo, *Analyst*, 2022,**147**, 4222-4227.

[2] L. Zhang, X. A. Liu, K. D. Gillis, T. E. Glass, *Angew. Chem.*, 2019, **131**, 7693-7696.

[3] N. Zhou, F. J. Huo, Y. K. Yue, C. X. Yin, *J. Am. Chem. Soc.*, 2020, **142**, 17751-17755.