Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

NIR fluorescent probe tracing norepinephrine exocytosis and depression occurrence at the cellular level

1. Materials and methods

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

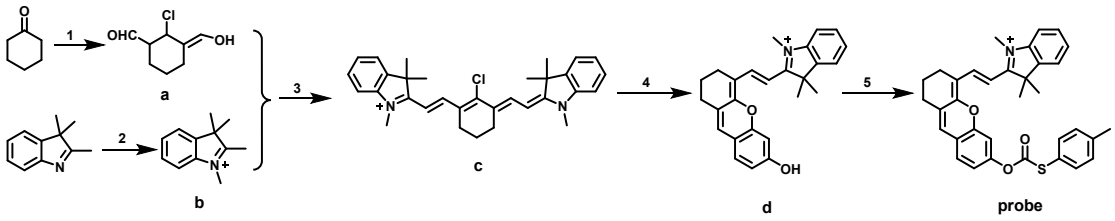
The probe (2 mM) stock solution was prepared by DMSO. The stock solutions of analytes were prepared by PB (pH 5.0) solution containing 50 mM $Na_2S_2O_3$ and 120 mM NaCl. The stock solutions of drugs were prepared by DMEM medium with 0.1% DMSO. The "high-K^+" solution was used to trigger exocytosis that 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. The "Control" solution was used to trigger exocytosis that consisted of 150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM HEPES and 11 mM glucose titrated to pH 7.3 with 1M NaOH.

Instrument. During the spectral test, the instruments used are Hitachi F-7000 fluorescence spectrophotometer and Hitachi U-3900 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 Bruker AVANCE-600 MHz NMR spectrometer. The HRMS data were measured by Thermo Scientific Q Exactive. The cells imaging was carried out by Zeiss LSM880 Airyscan confocal laser scanning microscope. The Beckman Coulter flow cytometry analyzer 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, Prism 8.

2. Synthesis



(1) $POCl_3$, DCM, DMF, r.t., reflux (2) Iodomethane, toluene, 100℃ (3) Potassium acetate, acetic

anhydride, 70℃ (4) Et_3N, DMF, 110℃ (5) Thiophenol, BTC, Et_3N, DCM, r.t.

Scheme S1. Synthesis of probe.

Synthesis of Compound a.

DMF (10 mL) and CH_2Cl_2 (10 mL) were mixed in the ice bath condition, then $POCl_3$ (10 mL) was added drop by drop. After stirring for 30 min, 2.5g cyclohexanone was added and refluxed at 80 °C. After 6 hours, the mixture was poured into the ice water, yellow solid (1.7 g) were precipitated.

Synthesis of Compound b.

2,3,3-Trimethylbenzoindole (5.25 g, 25.0 mmol) and iodoethane (4.0 g, 25.0 mmol) were dissolved in toluene (40 mL). The mixture was stirred at 100 °C for 20 hours and the reaction was cooled to room temperature. The product was filtered, washed with diethyl ether and dried to give a light pink solid (7.95 g).

Synthesis of c.

Compound b (7.3 g, 20 mmol), a (1.7 g, 10 mmol), potassium acetate (2.0 g, 20 mmol) and acetic anhydride (40 mL) were added to the flask. The mixture was heated to 70 °C and stirred for 0.5 h to give a green solution. The mixture was poured into a large amount of ice water, and filtered to afford a green solid (6.2 g).

***Synthesis of d.*[1]**

Compound c (1.0 g, 1.35 mmol), resorcinol (1.7 g, 10 mmol), triethylamine (1.8 mL, 13.5 mmol) were dissolved in dry DMF (15 mL). The mixture was heated to 110°C and stirred for 0.5 h to obtain a blue solution. The reaction mixture was cooled to room temperature, and then was poured into a large amount of ice water. The crude product was filtered and then was purified by silica gel chromatography (CH_2Cl_2: MeOH=10:1) to give a dark blue solid (0.31g, 40%).

Synthesis of Probe.

To a solution of p-toluenethiol (10 mmol) and triphosgene (0.5 equiv) in CH_2Cl_2 (20 mL) at 0 ℃, pyridine (1 equiv 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 d (1 mmol) and Et_3N (2 equiv) were dissolved in 10 mL CH_2Cl_2. The previous crude product (2 equiv 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 (1/10) as eluent to give probe as a dark purple solid (0.18 g, 21%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.79 (d, J = 15.2 Hz, 1H), 7.70 (d, J = 7.4 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.53 (d, J = 8.3 Hz, 4H), 7.40 (s, 1H), 7.33 (d, J = 7.9 Hz, 2H), 7.29 (s, 1H), 7.16 (d, J = 10.6 Hz, 1H), 6.65 (d, J = 15.2 Hz, 1H), 3.95 (s, 3H), 2.84 – 2.78 (m, 2H), 2.75 (t, J = 5.9 Hz, 2H), 2.42 (s, 3H), 2.02 – 1.92 (m, 2H), 1.84 (s, 6H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 179.6, 168.7, 159.5, 152.9, 146.2, 142.4, 142.1, 140.6, 134.6, 130.5, 130.1, 129.8, 128.8, 128.1, 127.7, 123.0, 122.3, 120.0, 118.2, 114.5, 112.9, 108.8, 105.6, 51.0, 48.0, 47.8, 47.7, 47.5, 47.4, 47.3, 47.1, 31.8, 28.9, 26.4, 23.5, 20.0, 19.9. MS (ESI, m/z): Calcd for [M^+] 534.20974, found: 534.20974. The proposed sensing mechanism of the probe NIR-NE for the detection of NE is shown in Scheme 1, which was determined by HRMS, as expected (Figure S4 in Supporting Information).

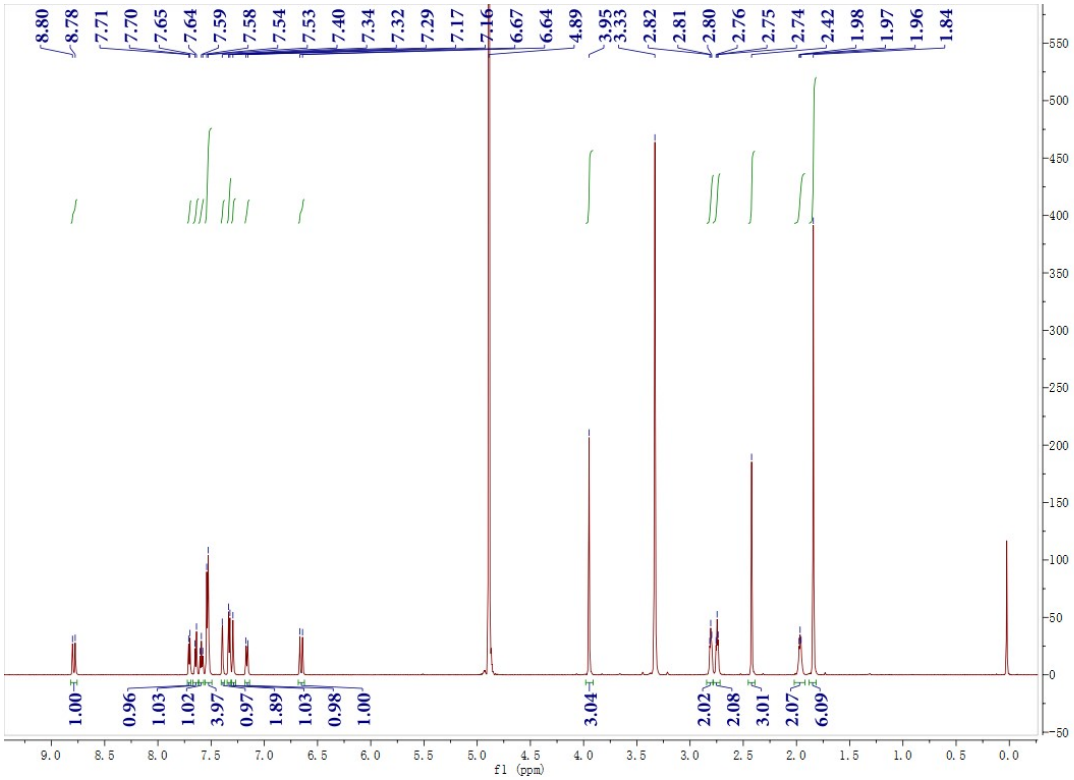


Figure S1. ^{1}H spectra of probe in CD_3OD.

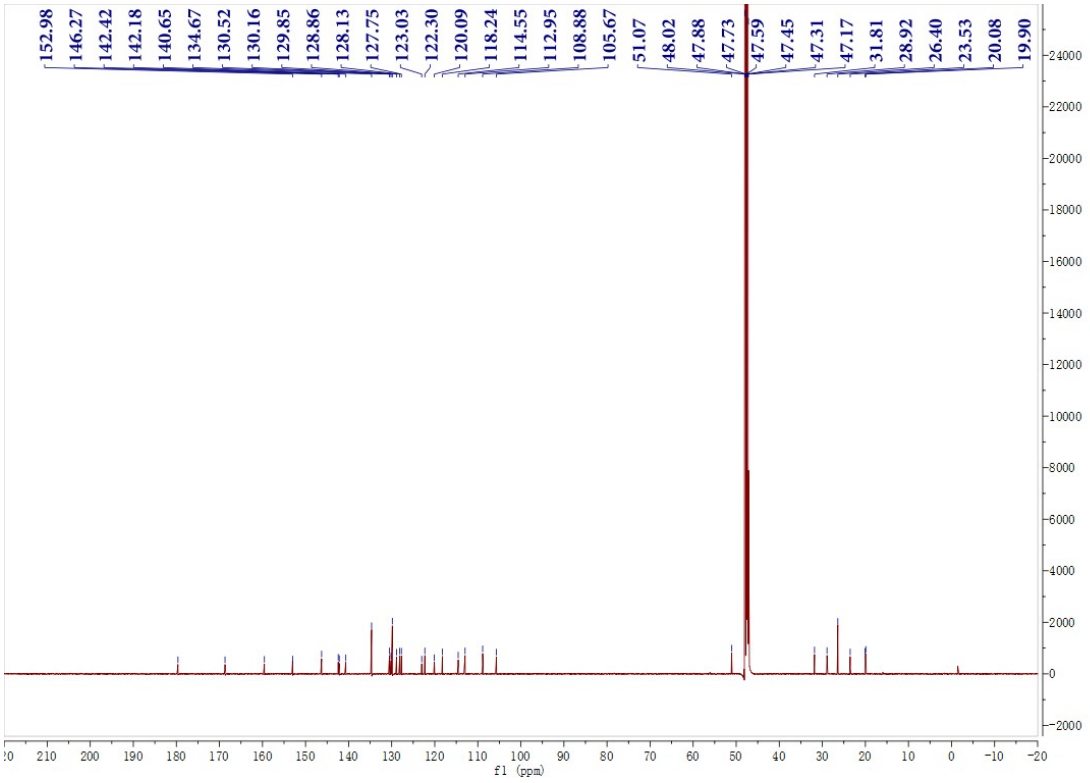


Figure S2. ^{13}C NMR spectra of probe in CD_3OD.

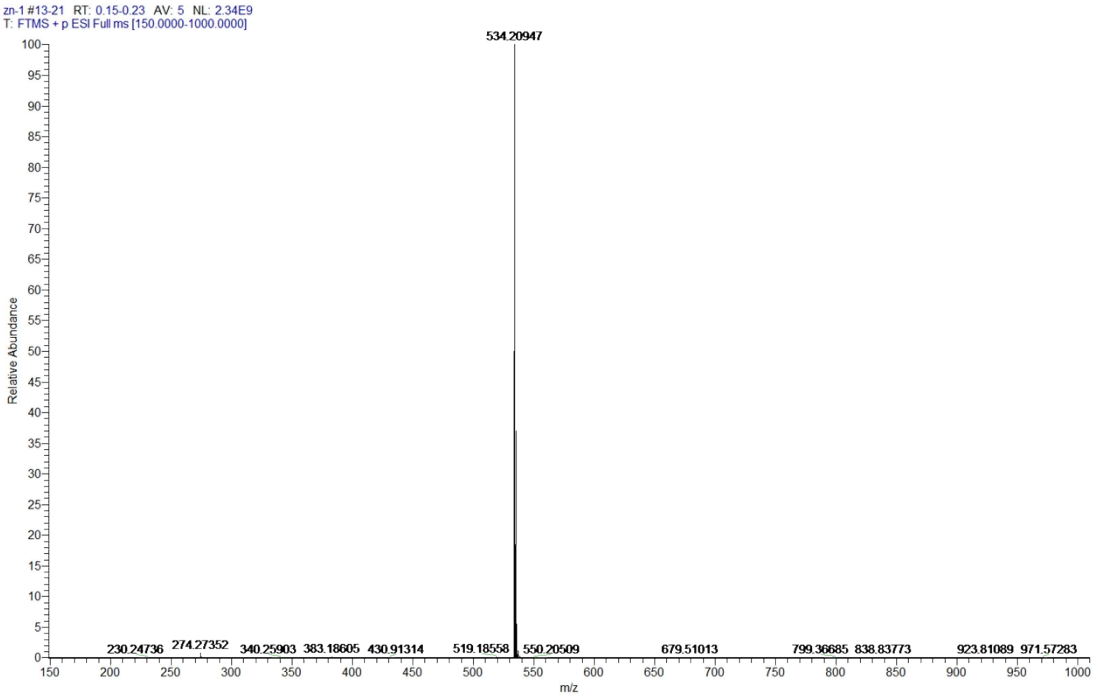
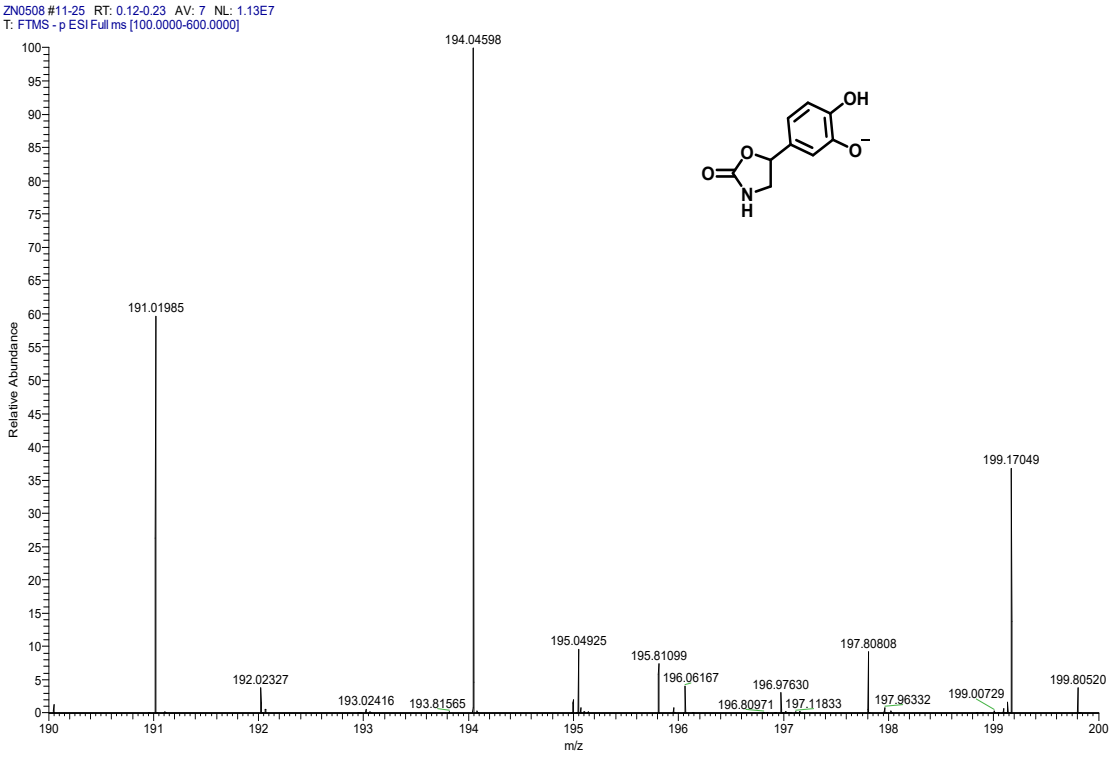


Figure S3. HR-MS spectrum of probe.



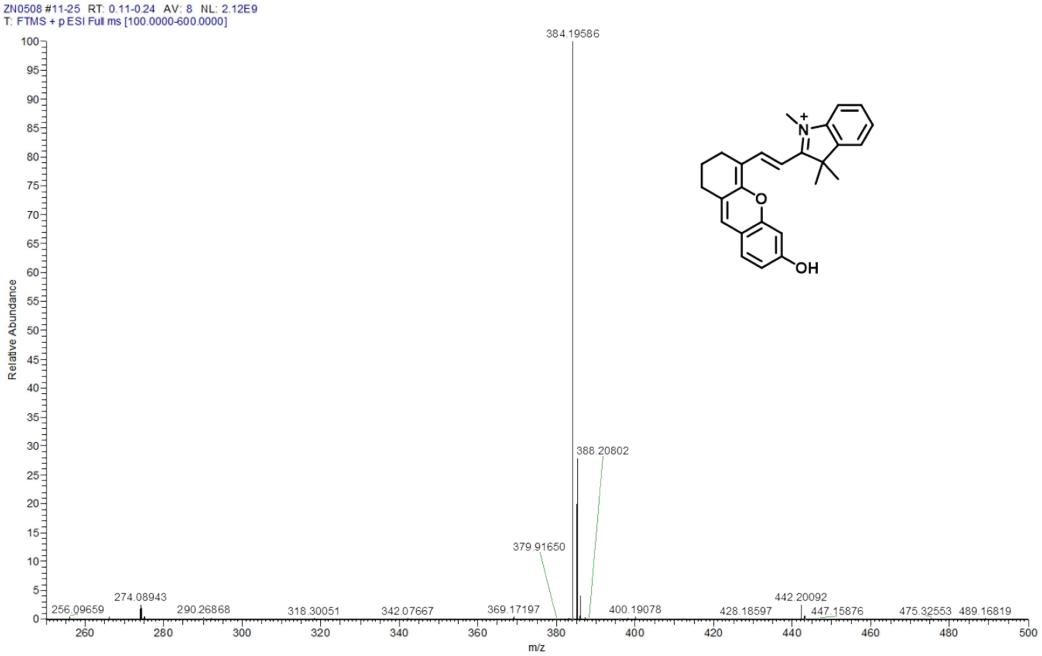


Figure S4. HR-MS spectrum of probe with NE.

3. Spectroscopic Reaction behavior.

The stock solution of probe (2 mM) was prepared in DMSO and stored at 4 ºC. All spectroscopic study of probe was carried out in test solution of probe (10 μM) in 2 mL PB/DMSO (v:v 1:1, pH 5.0) system, which was prepared by adding 20 μL probe 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 norepinephrine were determined by adding norepinephrine stock solution into the test solution of probe. The temporal tracking of the probe reaction with norepinephrine was carried out by adding norepinephrine into probe test solution in PB/DMSO (v:v 1:1, pH 5.0) system followed by recording UV-vis and fluorescence spectra at ambient temperature every 10 minute with mixing. The concentration for probe and norepinephrine is 20 μM and 5 mM, respectively. The other analytes reaction behavior of probe were determined in a similar procedure.

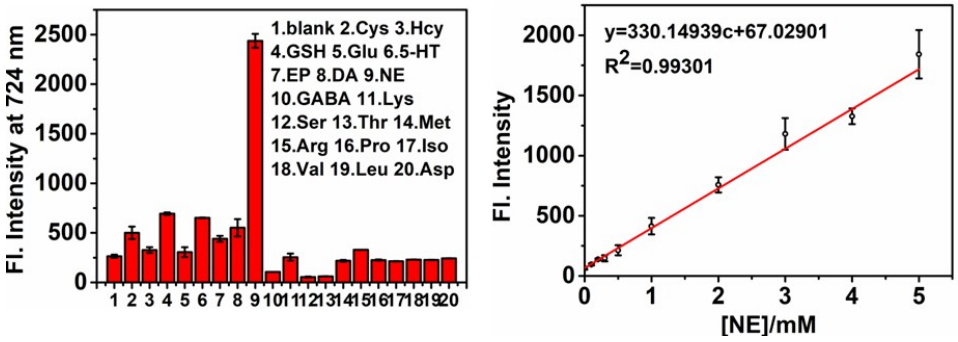


Figure S5. The selectivity of 20 μM probe upon 5 mM neurotransmitters (DA, EP, NE, 5-HT, GABA) and GSH, 500 μM amino acids (Lys, Ser, Thr, Met, Arg, Pro, Iso, Val, Lue, Asp), 100 μM Cys and 10 μM Hcy; Quantitative fluorescent intensities at 724 nm upon addition of 0 μM, 100 μM, 200 μM, 300 μM, 500 μM, 1 mM, 2 mM, 3 mM, 4 mM and 5 mM NE to 10 μM probe-containing system, respectively. The fluorescent intensity data were obtained 300 min after NE addition, error bars represent standard deviations, n=3, λ_{ex}: 650 nm, slit: 5 nm/ 5 nm.

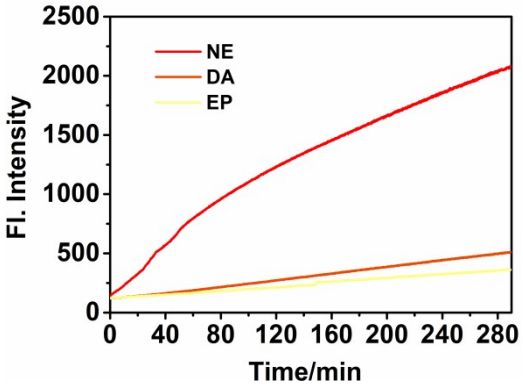


Figure S6. The time dependent of 20 μM probe upon 5 mM NE, DA and EP in 0-300 min.

4. Cell imaging.

Establishment of cell depression model. PC12 cells were cultivated in DMEM at 37℃ in a humidified atmosphere containing 5% CO_2 at 37℃ for 12h. 50 μM, 100 μM, 200 μM, 400 μM corticosterone was added to the dish for 12 h, respectively. The CCK-8 method was carried out to evaluate cell viability.

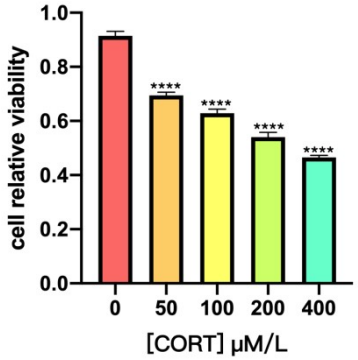


Figure S7. The CCK-8 assay of different concentrations of CORT on PC12 cells.

Cell imaging of endogenous norepinephrine in PC12 cells. PC12 cells were cultivated in DMEM at 37℃ in a humidified atmosphere containing 5% CO_2 at 37℃ for 12h. Probe (20 μM) was added to the dish and the cells were incubated for temporal tracking. The probe reaction with endogenous norepinephrine in PC12 cells was carried out by recording Zeiss LSM880 Airyscan confocal laser scanning microscope at ambient temperature every 2 minute.

Cell imaging of exogenous norepinephrine in PC12 cells. PC12 cells were cultivated in DMEM at 37℃ in a humidified atmosphere containing 5% CO_2 at 37℃ for 12h. Probe (10 μM) was added to the dish and the cells were incubated 1 mM, 2 mM, 3 mM NE 15 min respectively. The reactions were carried out by recording Zeiss LSM880 Airyscan confocal laser scanning microscope.

Imaging of cell exocytosis. Probe (20 μM) was added to the dish and the cells were incubated for another 30min. After being washed for 3 times with PBS, stimulated with 200 μL high-K^+ solution/ 200 μL the Control solution the cells were ultimately imaged. The temporal tracking was carried out by Zeiss LSM880 Airyscan confocal laser scanning microscope at ambient temperature every 10 s, form 0 to 510 s. The same experiment was performed on the depression model group.

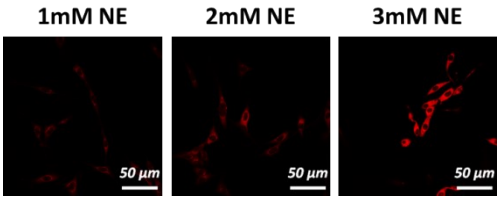


Figure S8. PC12 cells with the probe (20 μM) and different concentrations of norepinephrine cultured in red channel. (λ_{ex}: 633 nm, bar: 50 μm).

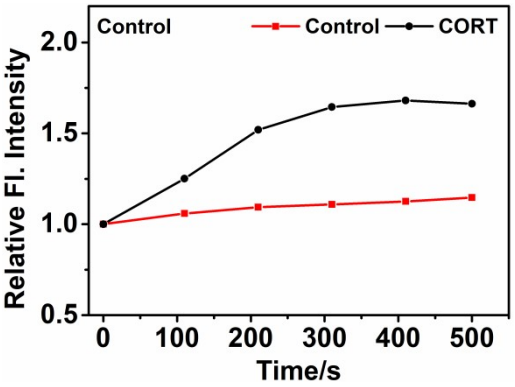


Figure S9. The time dependent of probe loaded normal/ depressed PC12 cells with the Control solution. (200 μL).

Cell imaging of antidepressant drug intervention. PC12 cells were cultivated in DMEM at 37℃ in a humidified atmosphere containing 5% CO_2 at 37℃ for 12h. Then 400 μM corticosterone and 10 μM drug solution were added to the dish for 12 h. Probe (10 μM) was added to the dish and the cells were incubated for 30 min. Then stimulated with 200 μL high-K^+ solution, the cells were imaged. The reactions were carried out by recording Zeiss LSM880 Airyscan confocal laser scanning microscope.

Cell imaging of drug intervention (2). PC12 cells were cultivated in DMEM at 37℃ in a humidified atmosphere containing 5% CO_2 at 37℃ for 12h. Then 400 μM corticosterone was added to the dish for 12 h. Before the Probe (10 μM) added to the dish, the cells were incubated Yohimbine/ Metoprolol (10 μM) for 15 min. The reactions were carried out by recording Zeiss LSM880 Airyscan confocal laser scanning microscope. Then stimulated with 200 μL high-K^+ solution, the cells were imaged.

5. Flow cytometry

PC12 cells were cultivated in DMEM at 37℃ in a humidified atmosphere containing

5% CO2 at 37℃ for 12h. Then 400 μM corticosterone was added to the dish for 12 h. After being washed with PBS, the cells were incubated probe (10 μM) for 30 min. The cells were then dispersed using trypsin digestion solution without EDTA. Filter with 100 mesh nylon before sample injection. The same experiment was performed on the control group.

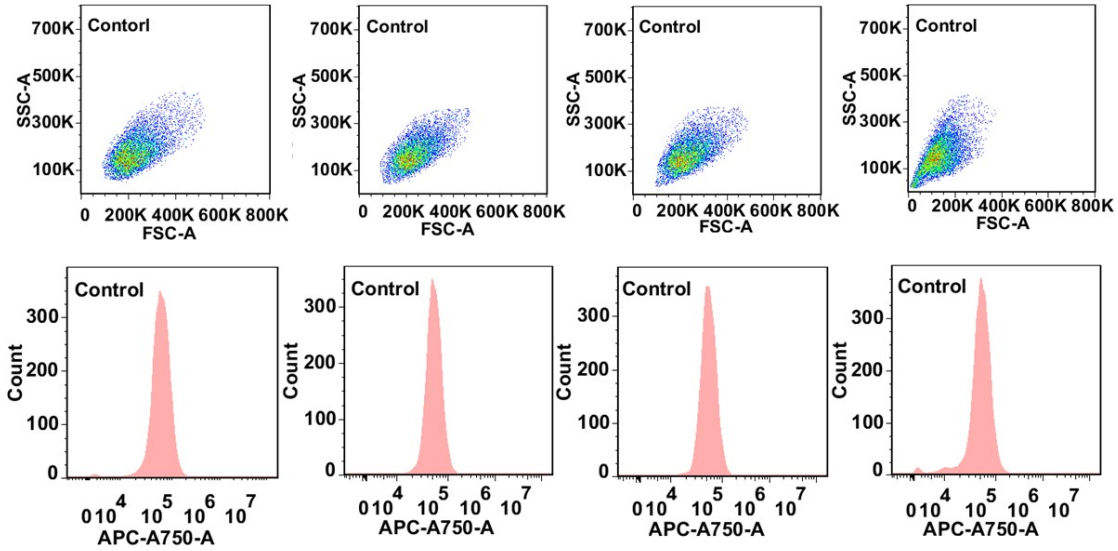


Figure S10. Flow cytometry detection of NE in normal cells using the probe. (λ_{ex}: 640 nm, Alexa Fluor 750, Number of cells collected: 1000).

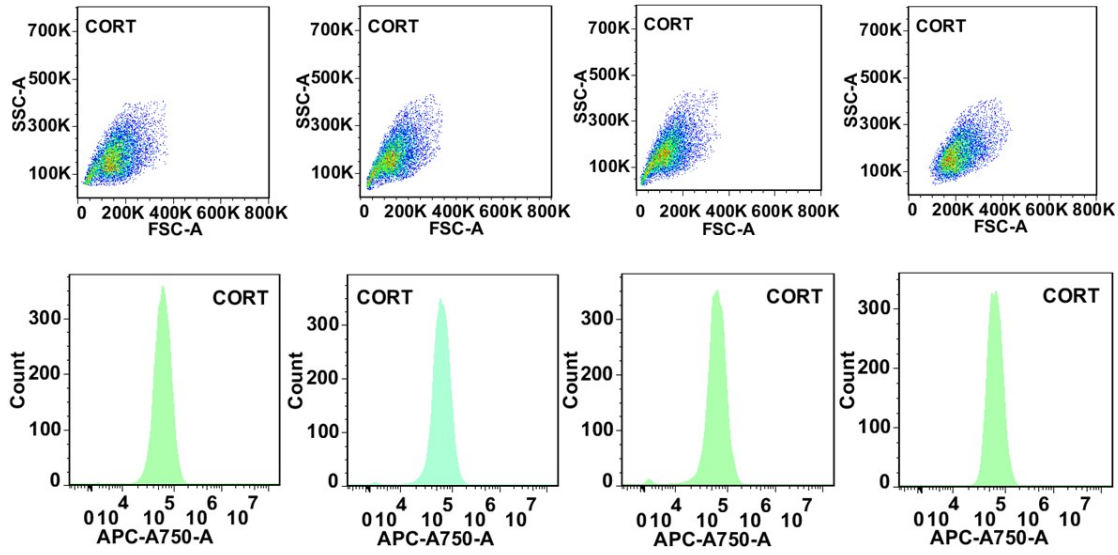


Figure S11. Flow cytometry detection of NE in depressed cells using the probe. (λ_{ex}: 640 nm, Alexa Fluor 750, Number of cells collected: 1000).

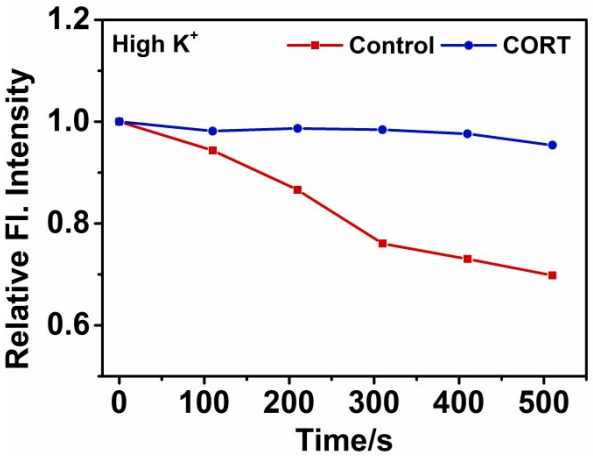


Figure S12. The time dependent of probe loaded normal/ depressed PC12 cells with the high K^+ (200 μL).

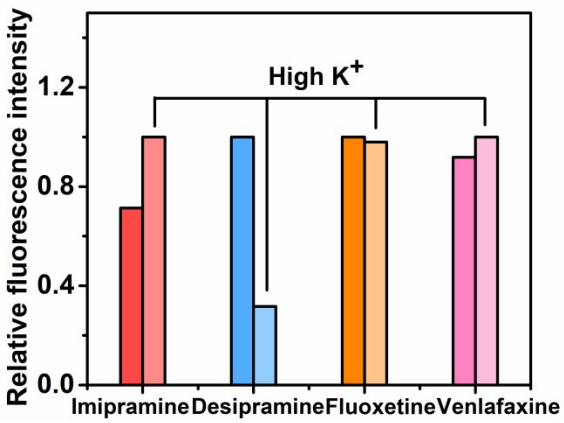


Figure S13. Probe loaded antidepressant treating PC12 cells with the high K+ (200 μL) solution in red channel (λ_{ex}: 633 nm, bar: 50 μm).

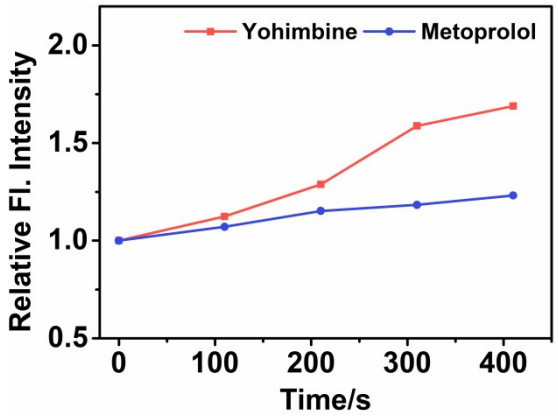


Figure 14. Probe loaded Yohimbine/ Metoprolol treating PC12 cells with the high K+ (200 μL) solution in red channel (λ_{ex}: 633 nm, bar: 50 μm).

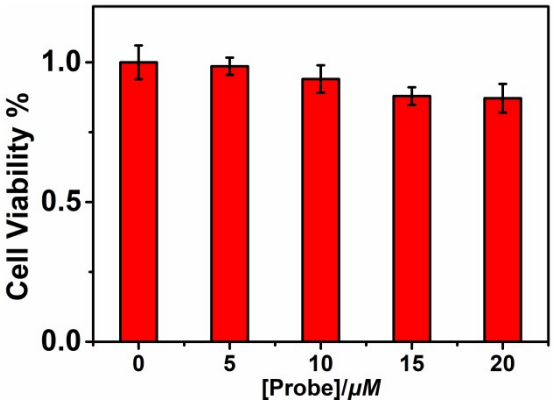


Figure S15. The CCK-8 detection kits to verify the acute toxicity of probes (10 h).

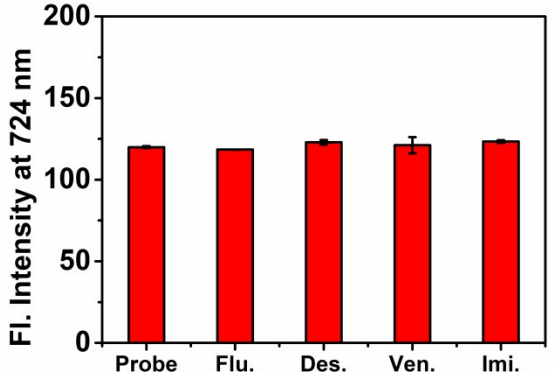


Figure S16. Fluorescence intensity of system at 724 nm upon addition of 100 μM Imipramine, Desipramine, Fluoxetine, Venlafaxine in presence of probe (10 μM), respectively.

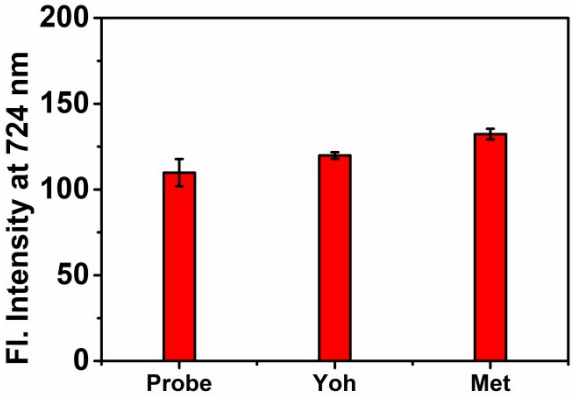


Figure S17. Fluorescence intensity of system at 724 nm upon addition of 100 μM Yohimbine and Metoprolol in presence of probe (10 μM), respectively.

[1]Y.T. Yang, T.T. Zhou, M. Jin, K.Y. Zhou, D.D. Liu, X. Li, F.J. Huo, W. Li, C.X. Yin, J. Am. Chem. Soc., 2020, 142, 1614-1620.