Supporting Information

Nanocage-Confined Electrochemiluminescence for the Detection of Dopamine Released from Living Cells

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S1. Experimental Section

Chemicals and Reagents. All chemicals were used as received without further purification and all aqueous solutions were prepared with ultrapure water (18.2 MΩ cm). Concentrated ammonia aqueous solution (25 wt%), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium hydrogen phthalate (KHP), histamine dihydrochloride (98%), tripropylamine (TPrA) and hexadecyltrimethylammonium chloride (CTAC, 97%) were ordered from Aladdin. Cetyltrimethylammonium bromide (CTAB, ≥98%) and tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride ($Ru(dpp)_3Cl_2$) were bought from Alfa Aesar. Tetraethoxysilane (TEOS, ≥99.0%), triethanolamine (TEA), poly(methylmethacrylate) (PMMA, M_w = 996000), Dulbecco's phosphate buffered saline (DPBS) and Dulbecco's modified Eagle's medium (DMEM) were obtained from Sigma-Aldrich. Fetal bovine serum (FBS) was obtained from Gibco. Poly(dimethylsioxane) (PMDS) was bought from Dow Corning (USA). Indium tin oxide (ITO) coated glasses (surface resistivity < 17 Ω/square, thickness 100 ± 20 nm) were purchased from Zhuhai Kaivo Optoelectronic Technology (China).

Preparation of SSNM/ITO electrode. The SSNM/ITO electrodes were fabricated using the Stöber-solution growth approach as reported previously.[S1] Briefly, the ITO glasses were immersed in a mixture of 70 mL water and 30 mL ethanol containing 0.16 g CTAB, 10 μL concentrated ammonia aqueous solution (25 wt%) and 80 μL TEOS. Then, the SSNM was grown on the surface of ITO glasses at 60 °C for 24 h. After rinsed with abundant water, the ITO glasses were aged at 100 °C overnight.

Preparation of LSNM/ITO electrode. The LSNM/ITO electrodes were prepared by the biphasic-stratification growth method.[S2] Briefly, 2.5 g CTAC and 90 μL TEA were added into 25 mL water and the mixture was sonicated for 15 min. The ITO glasses were put at the bottom of a round bottom flask and subsequently the mixture was transferred to the flask and stirred at 60 °C for 1 h. Then 8.25 mL cyclohexane solution containing 0.75 mL TEOS was added to the above solution drop by drop. Reaction was continued at 60 °C under stirring for 5 h. The ITO glasses were taken out, washed by ethanol and water successively, and aged at 60 °C for 1 h.

Preparation of ENA/ITO Electrode. Before the preparation of ENA/ITO electrode, both the SSNM/ITO and LSNM/ITO electrodes were immersed in the ethanol solution containing 0.1 M HCl under stirring for 15 min to remove the surfactants or surfactants and cyclohexane in nanochannels. A PMMA-assisted transfer approach (as illustrated in **Figure 1**) was employed to exfoliate the SSNM. Typically, a thin layer of PMMA was firstly spin-coated on the top of the SSNM/ITO electrode, which was subsequently immersed in 2 M HCl solution to etch the ITO layer, achieving the free-standing PMMA/SSNM. Then, in a solution containing 70 μM $Ru(dpp)_3^{2+}$, the PMMA/SSNM was fished out with the LSNM/ITO electrode to prepare PMMA/SSNM/LSNM/ITO electrode with $Ru(dpp)_3^{2+}$ trapped inside nanochannels of LSNM. After further heated at 100 °C for 2 h to promote the Si-O chemical bonding reactions between SSNM and LSNM, the electrode was immersed in acetone for 2 h to remove the top PMMA layer, producing eventually the ENA/ITO electrode.

Fabrication of the Detection chip. The detection chip was composed of a PDMS cover and an ENA/ITO electrode. A slice of PDMS was prepared by mixing PDMS monomer and curing agent (10:1, w/w) and baking the mixture at 75 °C for 1.5 h. A circular hole (9 mm in diameter, 100 μL) was then drilled on the slice as the reservoirs, after which the PDMS slice was peeled off and transferred onto the ENA/ITO electrode. A Ag/AgCl wire and a Pt wire were used as the reference electrode and the auxiliary electrode, respectively.

Cell Culture. PC12 cells obtained from BeNa Culture Collection were cultured in DMEM supplemented with 10% FBS. Cultures were incubated in the cell culture dish at 37 °C in a 5% CO_2 atmosphere, and the growth medium was exchanged every 2 days. To detect dopamine released from cells, 200 μL of cell solution was seeded in a 96-well plate the night before detection. Prior to experiment, the culture medium was discarded and the cells were rinsed three times with DPBS. Then the cells were treated with histamine at a final concentration of 20 μM for 20 min in the incubator, after which the supernatant was collected and detected by the ENA sensor.

Instrumentation and Measurements. Voltammetry characterization was carried out on the CHI920C electrochemical workstation (CH Instruments, Shanghai) using a three-electrode configuration, consisting of the working electrode (ENA/ITO electrode), the reference electrode (Ag/AgCl (saturated KCl) electrode) and the auxiliary electrode (Pt wire). The

parameters of differential pulse voltammetry (DPV) were as follows: increment potential 0.01 V, amplitude 0.05 V, pulse width 0.05 s and pulse period 0.5 s. The fluorescence (FL) and ECL spectra were obtained on the spectrofluorophotometer (RF-5301pc, Shimadzu) and the QEpro spectrophotometer (Ocean Optics), respectively. The potential-dependent ECL intensity measurement was performed on the MPI-E ECL analytical system (Remex Analysis Instrument, Xi'an). ECL images were captured by the Tanon 5200 chemiluminescent imaging system (Tanon Science & Technology Co., Ltd., Shanghai) equipped with Macro Zoom lens and CCD camera. The potentiostatic control was supplied by the CHI812 electrochemical workstation (CH Instrument, Shanghai). The exposure time of CCD was 5 s. The gray values of ECL images were analyzed by ImageJ software.

Scanning electron microscopy (SEM) images were obtained on the SU8010 field-emission scanning electron microscope (Hitachi, Japan) at an accelerating voltage of 5.0 kV. Transmission electron microscopy (TEM) images were captured on the HT7700 electron microscope (Hitachi, Japan) operated at an accelerating voltage of 100 kV. The specimen for TEM was prepared by mechanically scraping SSNM or LSNM from the surface of ITO electrode, dispersing them in ethanol, and then dropping the dispersion on the copper grids.

S2. Molecular Structure of $Ru(dpp)_3^{2+}$

Figure S1 shows the molecular structure of $Ru(dpp)_3^{2+}$. It has a diameter of 2 nm, thus being able to permeate LSNM but not SSNM.

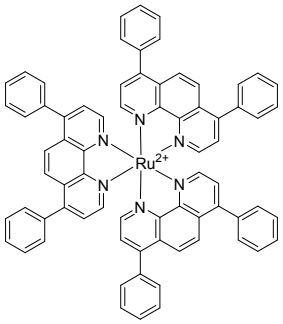

Figure S1. Molecular structure of $Ru(dpp)_3^{2+}$.

S3. Voltammetric Characterization of SSNM and LSNM

Figure S2 shows the CV responses of SSNM/ITO (a) and LSNM/ITO (b) electrodes before and after removal of the surfactants. In the case of as-prepared SSNM and LSNM, in which the surfactants were retained, no apparent faradaic current was observed for $Fe(CN)_6^{3-}$ (blue lines), indicating both SSNM and LSNM were crack-free. Only after excluding surfactants from the nanochannels, current was observed. Because of the negatively charged silica surface, the access of $Fe(CN)_6^{3-}$ was repelled, leaving suppressed current signals (red lines). The CV curves of bare ITO electrode (black lines) are shown for comparison.

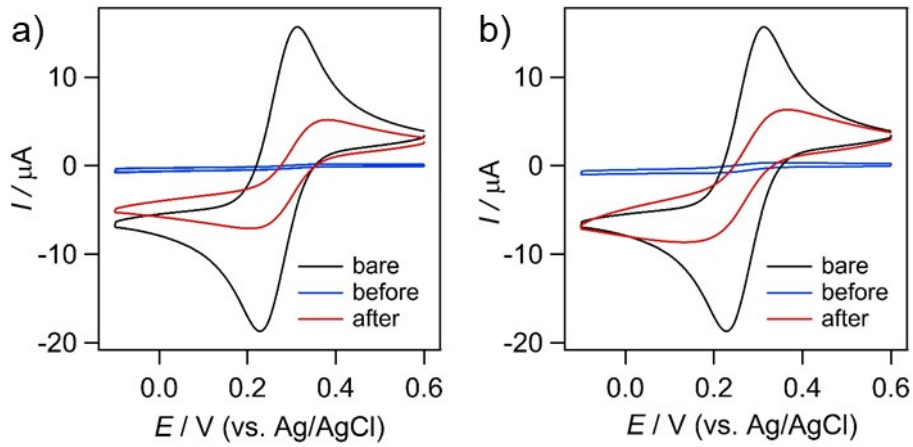

Figure S2. CV responses obtained with bare ITO electrode (black), SSNM/ITO (a) or LSNM/ITO (b) electrode before (blue) and after (red) removal of the surfactants in the nanochannels. The solution was 0.05 M KHP (pH = 4) buffer containing 0.5 mM $Fe(CN)_6^{3-}$. The scan rate was 0.05 V s^{-1}.

S4. Voltammetry of $Ru(dpp)_3^{2+}$ at SSNM/ITO and LSNM/ITO Electrodes

As compared in **Figure S3**, the current response associated with $Ru(dpp)_3^{2+}$ was only observed with the LSNM/ITO electrode (blue lines) but not the SSNM/ITO electrode (black lines). It suggests that the compact film of SSNM blocked the access of $Ru(dpp)_3^{2+}$ to the underlying electrode surface because of size-exclusion effect.

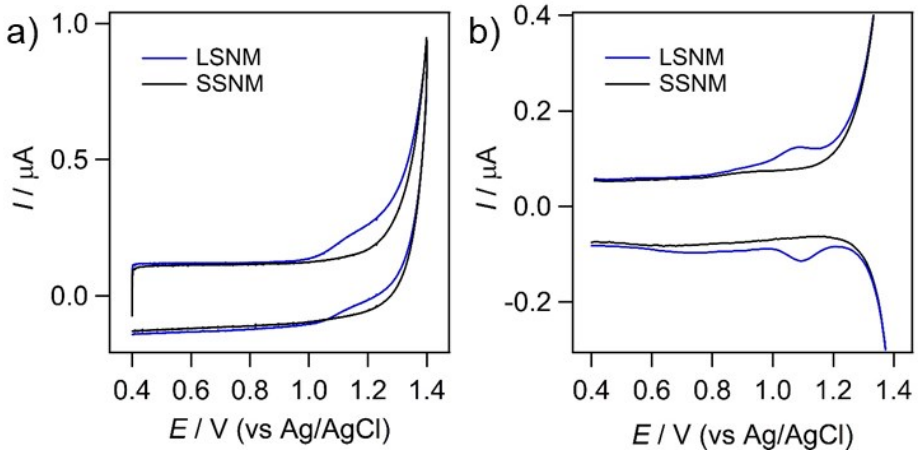

Figure S3. CV (a) and DPV (b) curves obtained with SSNM/ITO (black) and LSNM/ITO (blue) electrodes in DPBS containing 100 μM $Ru(dpp)_3^{2+}$. The scan rate was 0.05 V s^{-1}.

Figure S4 shows the voltammetry of the BNA/ITO electrode in DPBS in the absence and presence of 100 μM $Ru(dpp)_3^{2+}$. No redox currents were observed in both cases, indicating that $Ru(dpp)_3^{2+}$ cannot permeate SSNM.

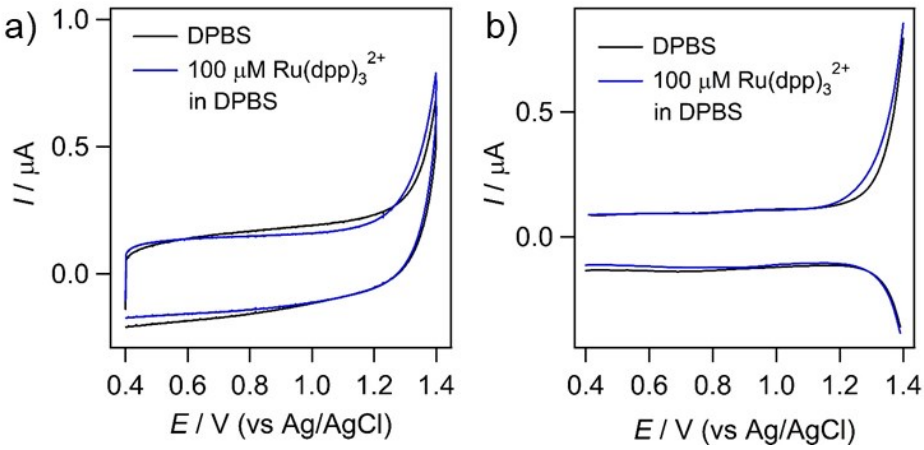

Figure S4. CV (a) and DPV (b) curves of BNA/ITO electrode in DPBS (black) or DPBS containing 100 μM $Ru(dpp)_3^{2+}$ (blue). The scan rate was 0.05 V s^{-1}.

S5. Voltammetry of ENA/ITO and BNA/ITO Electrodes

Figure S5 compares CVs of BNA/ITO and ENA/ITO electrode in DPBS. No redox current was observed with BNA/ITO electrode, while a small current signal was displayed at ~1.10 V with ENA/ITO electrode, indicating that $Ru(dpp)_3^{2+}$ was successfully trapped in the nanocages.

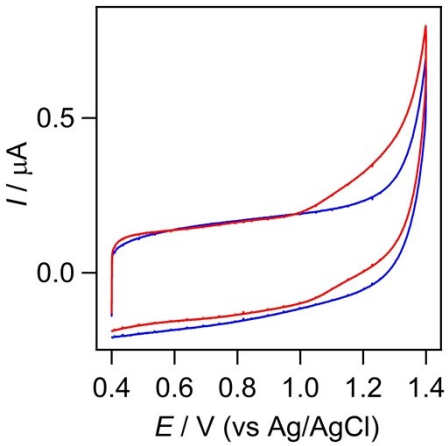

Figure S5. CV curves of BNA/ITO (blue) and ENA/ITO (red) electrodes in DPBS. The scan rate was 0.05 V s^{-1}.

S6. Long-term Stability of the ENA/ITO Electrode

To demonstrate the long-term stability of ENA/ITO electrode, the electrode was immersed in DPBS solution in a petri dish for up to 41 hours, during which the electrode was taken out at specific time intervals and the solid-state FL spectra were captured. As shown in **Figure S6**, the solid-state FL intensity dropped quickly in the first 5 hours, which might be attributed to the desorption of the luminophores adsorbed on the ENA surface during electrode fabrication. The FL intensity kept the same from 5 hours to 41 hours, confirming that the leakage was successfully avoided and demonstrating the long-term stability of the sensor.

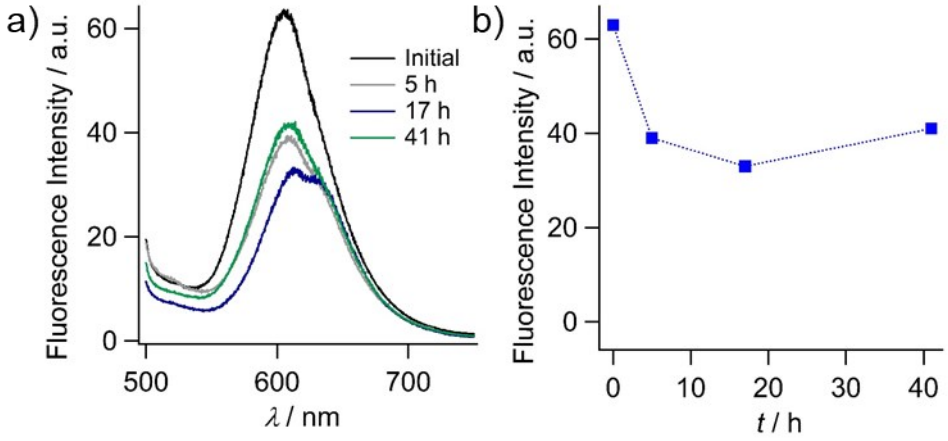

Figure S6. Solid-state FL spectra (a) and peak intensity (b) of the ENA/ITO electrode at different time intervals.

S7. Intensity-based ECL measurement

As shown in **Figure S7**, the analytical performance of the ENA/ITO sensor was evaluated by intensity-based measurement as well. With the addition of DA into the solution, a reduced ECL signal was observed (**Figure S7**a). A calibration curve with two dynamic ranges was plotted for the ECL intensity and the DA concentration (**Figure S7**b), namely 0.500 – 1.22 μM and 1.22 – 25.0 μM with a LOD of 0.0886 μM. The result is comparable with that obtained by imaging-based method.

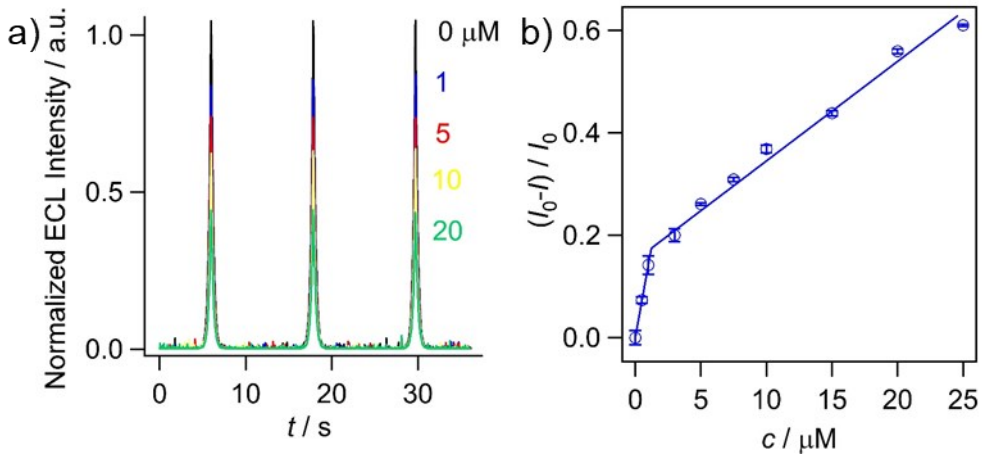

Figure S7. (a) ECL responses of the ENA/ITO electrode to different concentrations of dopamine. (b) The linear dependence of the relative variation of peak ECL intensity on the concentration of dopamine. I_0 is the peak ECL intensity in the absence of DA and I is that in the presence of DA.

S8. Determination of DA in Real Sample

Table S1. Recovery of DA in real sample.

Labeled / μM	Added / μM	Found / μM	Recovery / %
0.989	2.00	3.06	103
8.59	6.00	14.3	95.6

References

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S2 Y. Liu, D. Shen, G. Chen, A. A. Elzatahry, M. Pal, H. Zhu, L. Wu, J. Lin, D. Al-Dahyan, W. Li and D. Zhao, *Adv. Mater.*, 2017, **29**, 1702274-1702281.

S3 X. Lin, Q. Yang, L. Ding and B. Su, *ACS Nano*, 2015, **9**, 11266-11277.