

Supplementary Information

In situ growth of cobalt porphyrin-based covalent organic framework on multi-walled carbon nanotubes for ultrasensitive real-time monitoring of living cell-released nitric oxide

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Experiment section

Materials and reagents

All reagents were of analytical reagent grade and used as received without further treatment. All aqueous solutions were prepared with ultrapure water ($> 18.25 \text{ M}\Omega \cdot \text{cm}$), which was obtained from a Lichun water purification system (Jinan, China). Multi-walled carbon nanotubes (MWCNTs, outer diameter (OD): 10 - 20 nm, 95%) were purchased from Xfnano Co. [5,10,15,20-tetrakis (4-aminophenyl) porphinato] (TAPP) were prepared according to the published procedure.¹

Apparatus and measurements

Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-6510A scanning electron microscopy. For SEM imaging, Au (1-2 nm) was sputtered onto these grids to prevent charging effects and to improve the image clarity. Transmission electron microscopy (TEM) images were tested on a Hitachi H-800 microscope (Japan). Energy dispersive X-ray spectroscopy (EDS) analysis was performed on a X-MAX50 energy dispersive spectrometer (Oxford, UK). Fourier transform infrared (FT-IR) spectra were recorded using a Bruker Vertex 70 infrared spectrometer with 2 cm^{-1} resolution. X-ray diffraction (XRD) data were collected on a Bruker D8 Focus X-ray diffractometer using a Cu-K α radiation ($k = 1.5406 \text{ \AA}$). Raman images were obtained using a T64000. A ThermoFisher ESCALAB 250 X-ray photoelectron spectrometer (powered at 150W) by Al K α radiation ($\lambda = 8.357 \text{ \AA}$; spot size, 500 μm) was used to perform X-ray photoelectron spectra (XPS). Electrochemical data were obtained on a CHI760E electrochemical workstation (Shanghai Chenhua Co.) at room temperature.

Synthesis of Co(TAPP)

The synthetic [5,10,15,20-tetrakis (4-aminophenyl) porphinato]-cobalt [Co(TAPP)] was modified slightly according to the steps reported in the literature.^{2,3} In a flask, a mixture of TAPP (85.1 mg) and $\text{Co}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ (157.1 mg) was added in 30 mL of DMF. The mixture was refluxed for 3 h with N_2 protection. Then, the solvent was removed by evaporation after the reaction. The solid was dissolved again in CHCl_3 and filtered to remove excess unreacted $\text{Co}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$. The resulting filtrate was evaporated to dryness under a reduced pressure and dried under vacuum to for 12 h at 60 °C to form Co(TAPP).

Electrochemical measurement

The electrochemical measurements including cyclic voltammetry (CV), linear sweep voltammetry (LSV) and chronoamperometry were performed in a three electrodes cell system. The MWCNTs, COF-366-Co and MWCNTs@COF-366-Co modified on glassy carbon electrode (GCE) or glassy carbon rotating disc electrode were used as the working electrode. Platinum plate was used as the counter electrode, and a saturated calomel electrode (SCE) was the reference electrode. CV measurement was performed at a scan rate of 100 mV/s, and the potential ranged from 0 to 1.2 V versus SCE. Chronoamperometry was tested at a potential of 1.05 V. The background solution in 0.01 M phosphate buffer solution (PBS, pH 7.4) was purged with N_2 gas for 30 min before experiment. The electrochemical impedance spectroscopy was 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. All experiments were carried out in the presence

of N₂. Serial dilutions of NO solution were prepared in a nitrogen filled glove bag with the deaerated PBS and freshly NO saturated solution.

Preparation of NO in solution

NO saturated solution was prepared according to the procedure reported in the literature.⁴ All instruments were carefully degassed with nitrogen for 30 minutes to exclude O₂ before preparing NO saturated solution. Then, gas was generated by dropping 6 M H₂SO₄ into saturated NaNO₂ solution, followed by passing the NO gas sequentially through 2 M NaOH solutions to remove oxygen and other nitrogen oxides. Finally, NO saturated solution was obtained by bubbling NO gas into a sealed brown bottle containing 10 mL of deoxygenated PBS (pH 7.4) for 30 min and keeping under NO atmosphere until use. NO gas was toxic at concentration higher than 100 ppm, so all the preparation procedure was carried out in a fume hood. The saturated NO solution was 1.8 mM at 20 °C and stored in a brown glass bottle at 4 °C.

Cell culture and real-time determination

For the purpose of real-time measurement, the level of NO secreted by living cells, human umbilical vein endothelial cells (HUVECs) were cultivated in RPMI-1640 media supplemented with 10% fetal bovine serum in a humidified 5% CO₂ incubator at 37 °C. The media in the cultures was changed regularly to provide adequate supplements for the growing cells and maintain a stable pH value until a 90% confluency was achieved. The obtained cells were then removed from the petri dishes with the aid of trypsinization and rinsed thoroughly with dulbecco's PBS (pH 7.4) to wipe off residual serum or trypsin. Subsequently, the resulting cells were suspended in

a certain volume of dulbecco's PBS (pH 7.4) to obtain a solution with 1.0×10^7 cells per Milliliter. During the amperometric test of NO released from the cells, they were stimulated by injecting L-arginine (L-Arg) and N ω -nitro-L-arginine methyl ester hydrochloride (L-NAME) as stimulant and inhibitor, respectively. The amount of NO released from HUVECs was sensitively detected via employing the fabricated MWCNTs@COF-366-Co as identification unit.

Results and discussion

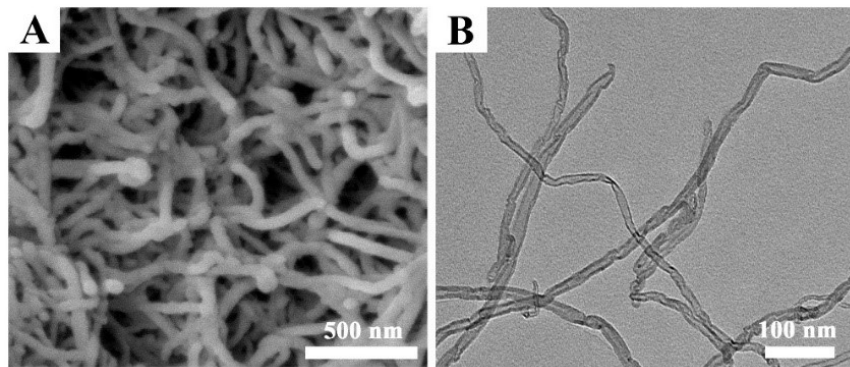


Fig. S1 (A) SEM and (B) TEM images of MWCNTs.

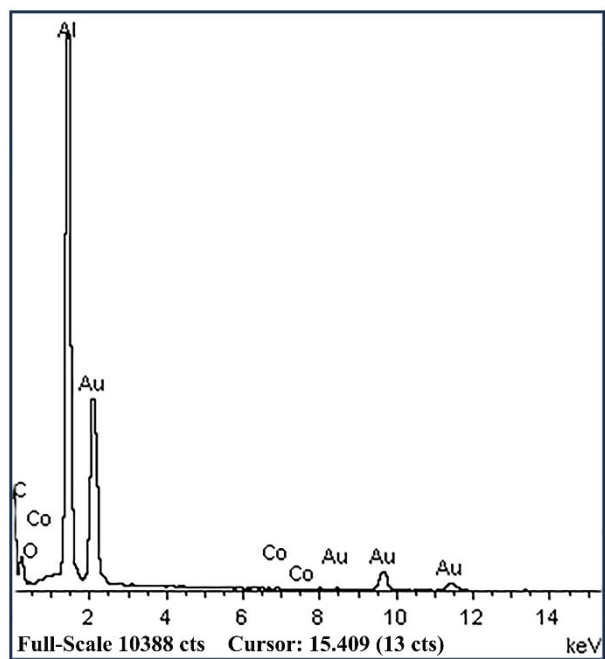


Fig. S2 EDS of MWCNTs@COF-366-Co.

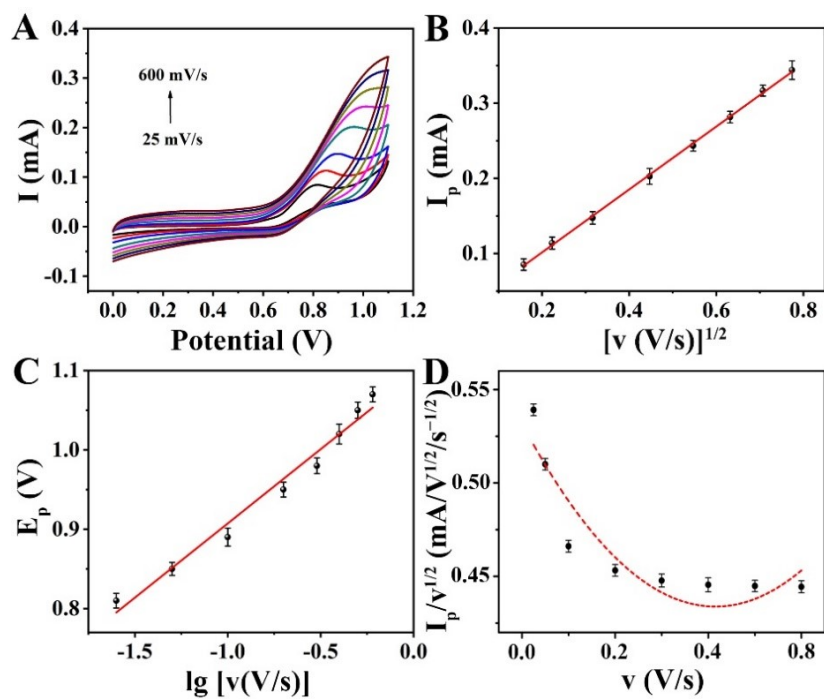


Fig. S3 CV curves of 216 μM NO of MWCNTs@COF-366-Co based sensor at different scan rates (25, 50, 100, 200, 300, 400, 500, and 600 mV/s). Dependence of (B) I_p on $v^{1/2}$, (C) E_p on $\lg v$, and (D) $I_p/v^{1/2}$ on v .

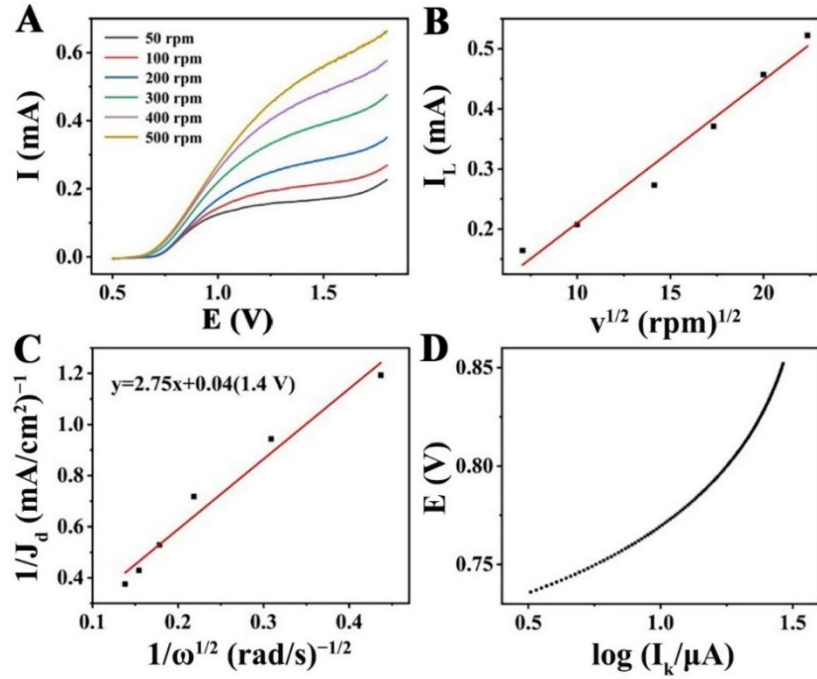


Fig. S4 (A) Linear sweep voltammetry of the MWCNTs@COF-366-Co based sensor for 432 μM NO oxidation at different rotation rates. (B) Plot of the plateau currents vs. the square root of rotation rate of the electrode. (C) Koutecky-Levich plots of MWCNTs@COF-366-Co based sensor at 1.4 V. (D) Tafel plots obtained from rotating-disk data for the oxidation of NO (432 μM) in PBS (pH 7.4) on MWCNTs@COF-366-Co based sensor.

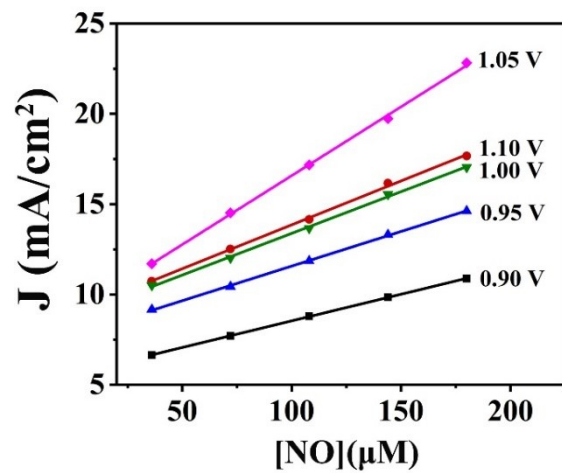


Fig. S5 Amperometric response of MWCNTs@COF-366-Co based sensor toward addition of NO at different operating potential.

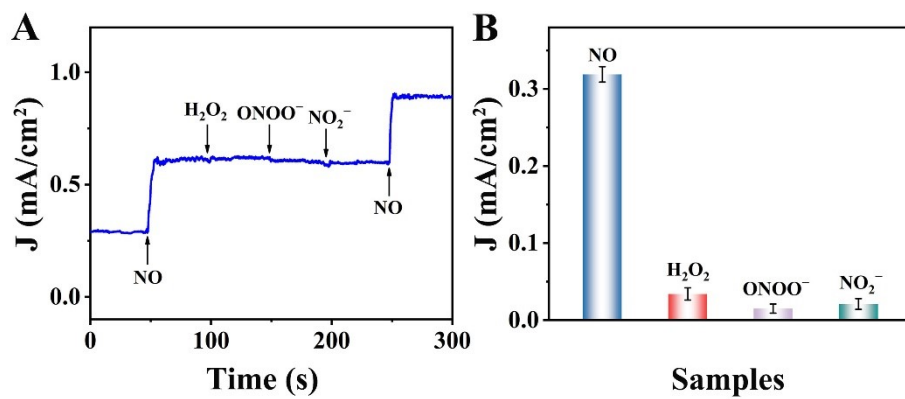


Fig. S6 (A) Selectivity of MWCNTs@COF-366-Co based sensor towards NO and interfering substances and (B) profiles.

Table S1 Analytical properties comparison of various NO sensors based on different materials.

Modified electrodes	Sensitivity ($\mu\text{A}\cdot\mu\text{M}^{-1}\cdot\text{cm}^{-2}$)	Linear range (μM)	LOD (μM)	Ref.
Nanostructured-Au/PDMS-film electrode	6.16	0.01-1.295	0.01	5
CuTAPc-MCOF@AgNPs	29.1	0.18-17.1	0.0126	6
N-G/FePc/Nafion/PLL ITO electrode	0.21	0.18-400	0.18	7
p-bpy-COF/GCE	-	0.36-44.3	0.0173	8
CNTs/AgNWs/PDMS	-	0.01-60.94	0.0025	9
RGO-Co ₃ O ₄ @Pt GCE	0.37	10-650	1.73	10
MWCNTs@COF-366-Co	8.9	0.18-400	0.016	This work

References

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