Supplementary Information for

A mitochondria-targeted fluorescent probe for real-time monitoring of carbon monoxide in living cells and zebrafish

Qinglong Liu,[a] Shihan Ma,[a] Weiying Lin*[,b]

a Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China.

b Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyinglin2013@163.com

*Correspondence to: Weiying Lin, Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyinglin2013@163.com.

Table of Contents

Page

Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All experiments used ultra-pure water. Solvents were purified by standard methods prior. Ultra-pure water (18.2 MΩ·cm) is used by ULPURE. TLC analysis was carried out on silica gel plates, and column chromatography was conducted over silica gel (mesh 200-300); both of them were purchased from Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance III HD 600 MHz NMR spectrometer (United States of America). High-resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope.

Synthesis routine of MNP-CO.

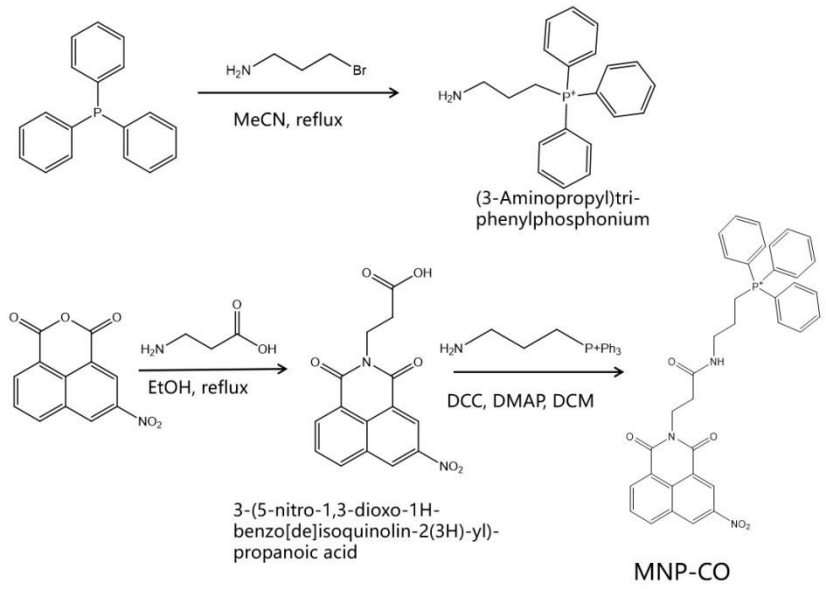


Fig. S1 Synthetic of probe **MNP-CO**

The compounds (3-Aminopropyl)triphenylphosphonium and 3-(5-nitro-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propanoic acid were synthesized according to the methods described in reference[1].

Optical research and analysis

A stock solution (1 mM) of probe **MNP-CO** was initially prepared in dimethyl sulfoxide (DMSO). All spectrometric probes were used at a concentration of 10 μM. The adjunction of 20 μL of stock solution was added to 2.0 mL of different solvent systems to obtain the probe diluent. The solutions of various interfering substances (cations, anions, amino acids and active small molecules) were prepared with twice-distilled water. The providing solutions were mixed well before texting the

spectra. Unless otherwise specified, the required fluorescence spectral measurement is generally an excitation wavelength of 430 nm, an excitation slit width of 5.0 nm, and an emission slit width of 5.0 nm.

Culture and preparation of HeLa cells

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C.

Cytotoxicity assay

HeLa cells were seeded into 96-well plates, and 0, 1, 5, 10, 20, and 30 μM of the probe **MNP-CO** were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Next, MTT (10 μL, 5 mg/mL) was injected into every well and incubated for 4 h. Then, violet formazan was dissolved with DMSO (100 μL). The absorbance of the solution was measured at 492 nm by way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **MNP-CO**.

The cell viability (%) = $(OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100\ \%$.

Confocal imaging of exogenous CO

For the exogenous CO imaging in living cells, HeLa cells were incubated with MNP-CO (10 μM) for 1 h at 37 ℃; After that, cells were washed with PBS. Then, the cells were treated with CORM-2 (100 μM) for 1 h at 37 ℃. Before observation, the cells were washed several times. Fluorescence images were acquired with a 405 nm excitation.

Confocal imaging of endogenous CO

For the endogenous CO imaging, HeLa cells were treated using both heme stimulation and hypoxic cultivation. For heme stimulation, control cells were incubated with **MNP-CO** (10 μM) for 1 h at 37 ℃. The cells were stimulated with heme (100 μM) for 4 h and incubated with **MNP-CO** (10 μM) for 1h in the experimental group. For hypoxic cultivation, HeLa cells of the experimental group were incubated in a hypoxic environment (98% N_2 and 2% O_2). In the control group, HeLa cells were incubated in a normoxic environment (5% CO_2 and 95% air). Both the cells were incubated with **MNP-CO** (10 μM) for 1h in their respective environments and investigated by fluorescence imaging.

Zebrafish Maintenance and Imaging

For the exogenous CO imaging in live zebrafish, zebrafish larvae were divided into two groups for experiments. The larvae were incubated with only **MNP-CO** (10 μM) for 1 h in the control group. In the experimental group, the larvae were incubated with **MNP-CO** (10 μM) for 1 h, and then incubated with CORM-2 (100 μM) for 1 h. Then, zebrafish larvae were investigated by fluorescence imaging. Bright-field and fluorescence images were acquired with a 405 nm excitation.

Characterization of MNP-CO

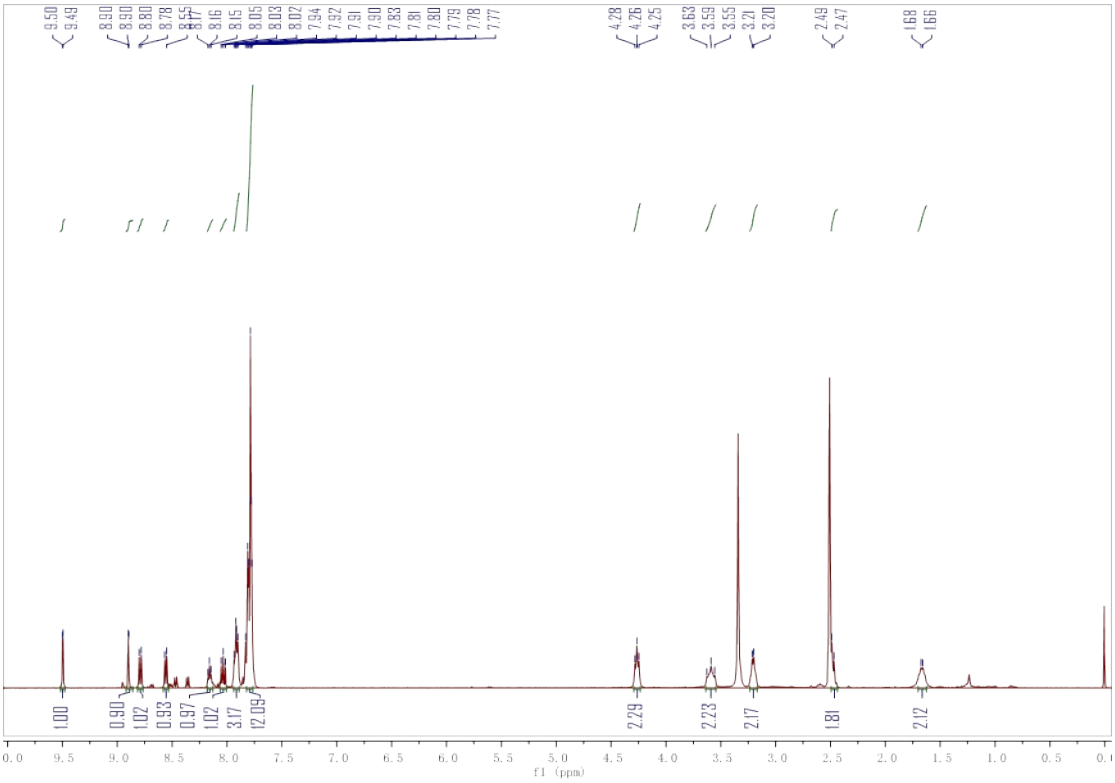


Fig. S2 The ^{1}H NMR spectrum of **MNP-CO** in DMSO-*d6*.

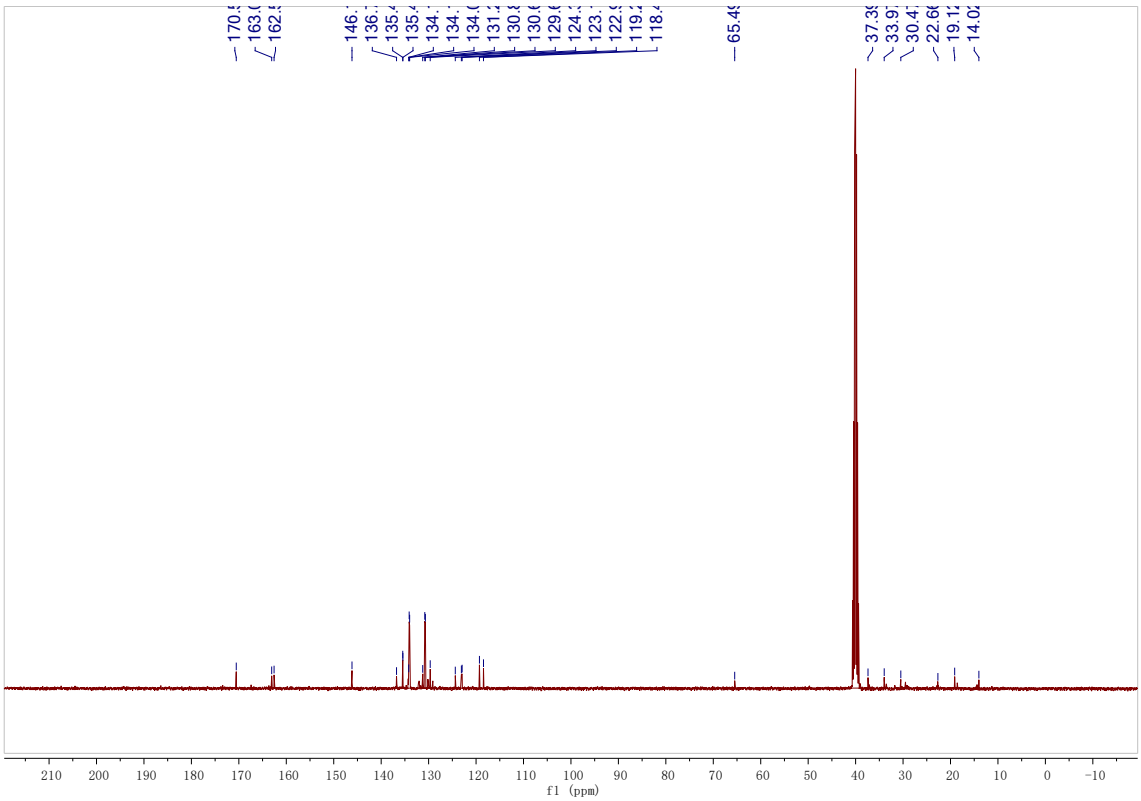


Fig. S3 The ^{13}C NMR spectrum of **MNP-CO** in DMSO-*d6*.

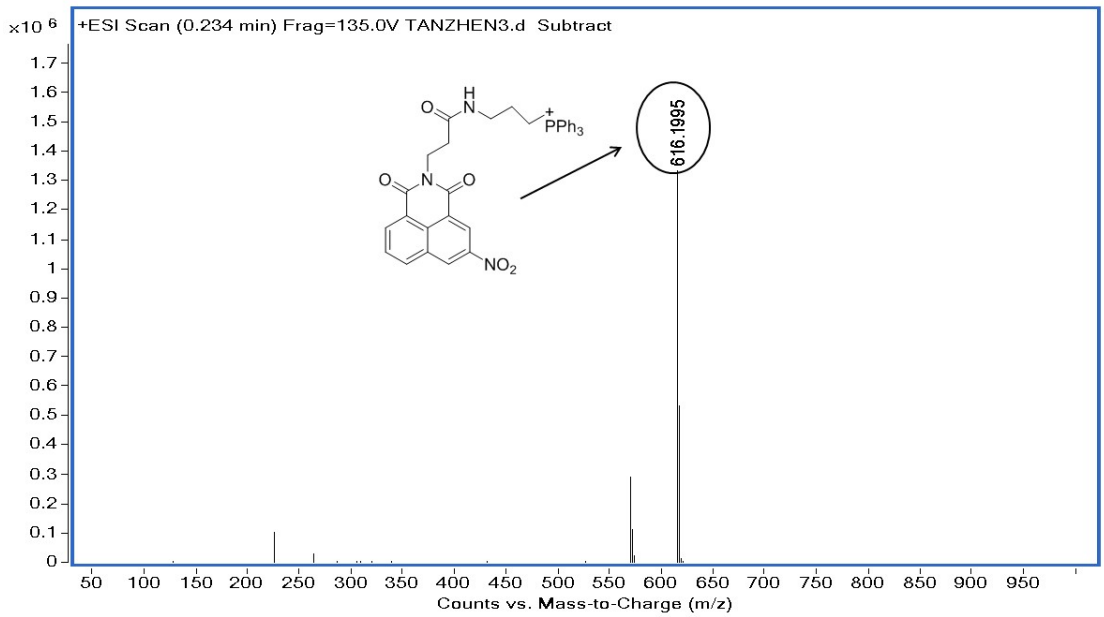


Fig. S4 The HRMS spectrum of **MNP-CO**.

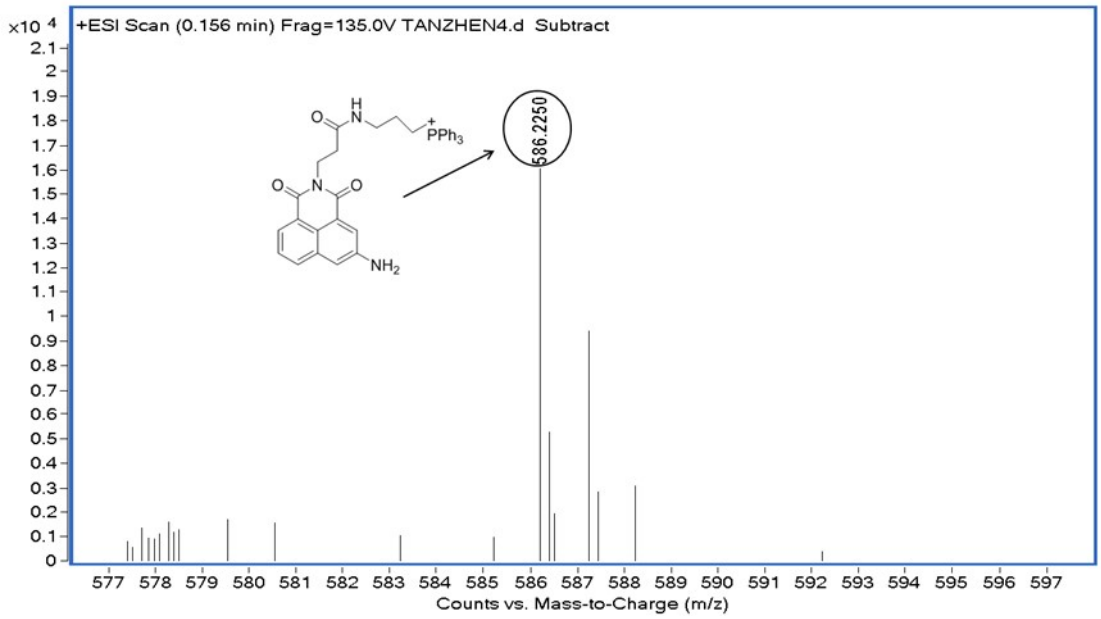


Fig. S5 Mass spectrum of **MNP-CO** reacted with CO.

Spectroscopic response of MNP-CO to CO

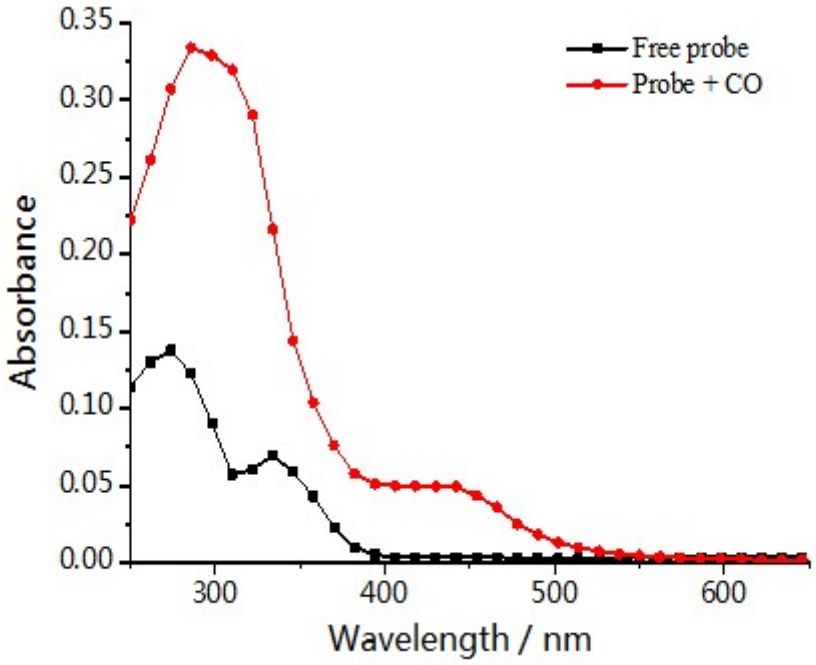


Fig. S6 Absorption spectrum of **MNP-CO** (black line) in the presence of CO (red line).

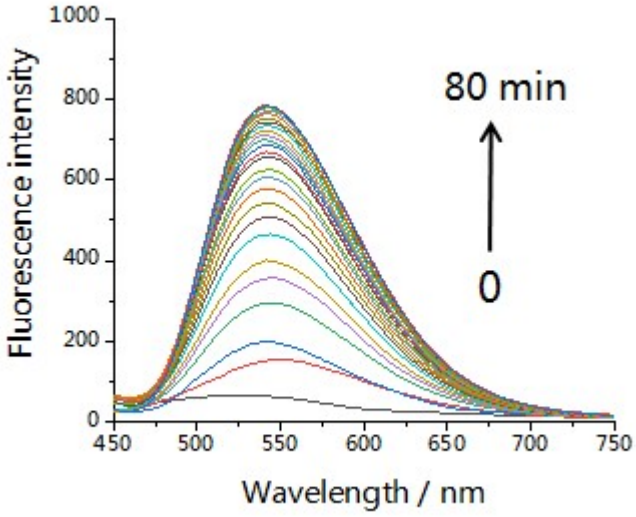


Fig. S7 Time-dependent fluorescence spectra of **MNP-CO** (10 μM) with CORM-2 (100 μM)(λ_{ex}=430 nm).

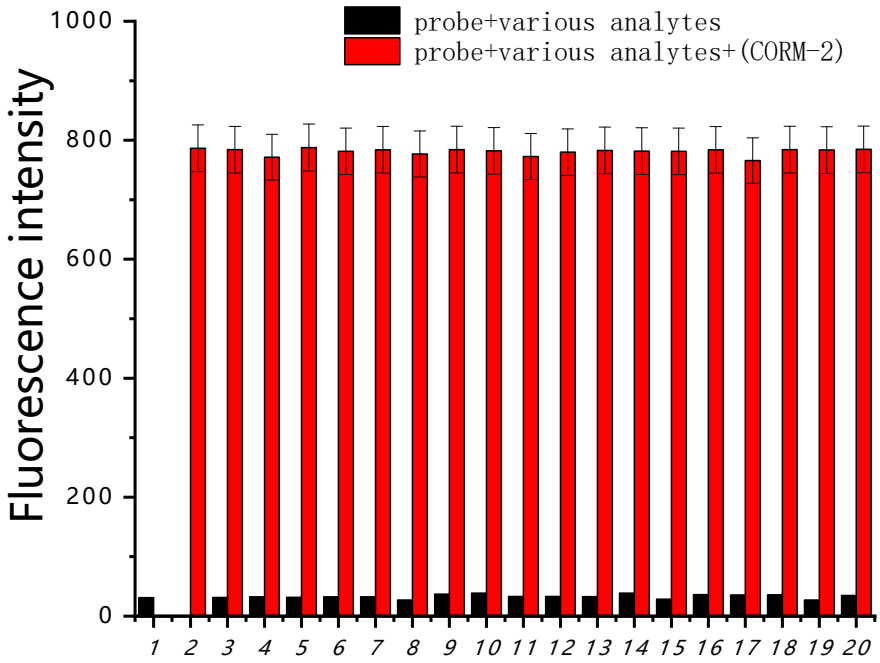


Fig. S8. Competitive binding experiments of **MNP-CO** (10 μM) upon addition of CORM-2 (100 μM) and other analytes (100 μM) (black bars). Emission intensities of **MNP-CO** (black) and **MNP-CO** + CO + analyte (red): (1) Free probe, (2) CORM-2, (3) $NaNO_2$, (4) NO, (5) $ONOO^-$, (6) H_2O_2, (7) $^1O_2$, (8) Na_2S, (9) NaHS, (10) $NaHSO_3$, (11)$CaCl_2$, (12) KCl, (13) NaClO, (14) $ZnCl_2$, (15) Hcy, (16) Cys, (17) GSH, (18) Na_2CO_3, (19) $NaHCO_3$, (20) FA.

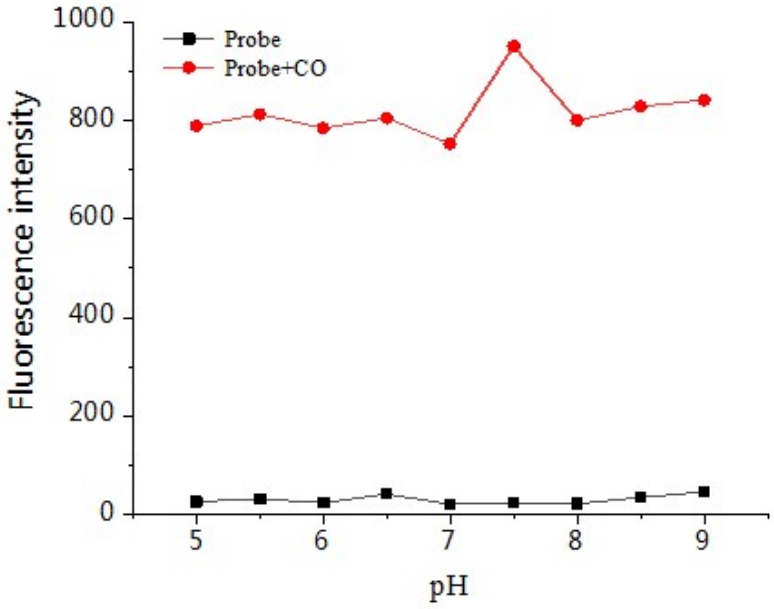


Fig. S9 Effect of pH on the fluorescence of **MNP-CO** (10 μM) before and after the addition of CORM-2 (100 μM). $\lambda_{ex}/\lambda_{em}$ = 430/520 nm.

Biological assays

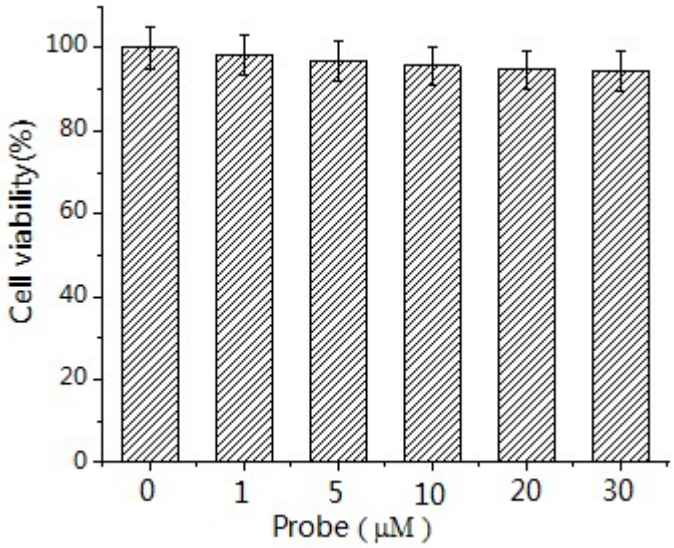
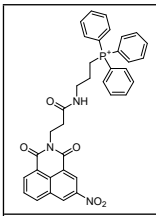
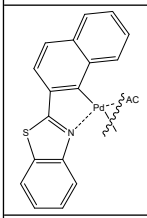
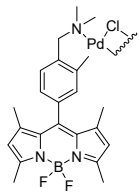
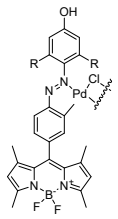
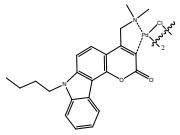
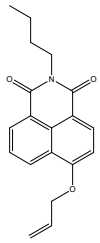
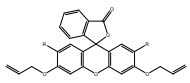
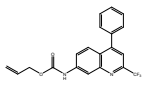
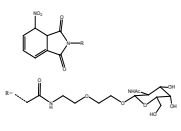
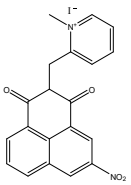
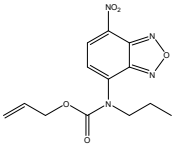
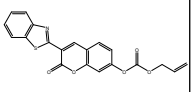
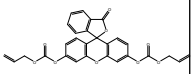
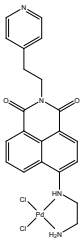


Fig. S10 Cell viability of HeLa cells treated with different concentrations of **MNP-CO** for 24 h.

Table S1. Comparing properties of the probe with representative CO fluorescent probes.

Probe structure	Emission (nm)	Enhancement ratio	Detection limit	Response time	Imaging application	Reference

	541	19-fold	0.97 μM	70 min	cells (exogenous /endogenous), zebrafish	This work
	510	10-fold	0.2 μM	150 min	cells (exogenous /endogenous), zebrafish	Chem. Eng.J., 2021, 419, 129538
	503	10-fold	1 μM	60 min	cells (exogenous)	J. Am. Chem. Soc. 2012, 134, 15668-15671
	512	10-fold	0.72 μM	20 min	cells (exogenous /endogenous)	Anal. Chem., 2016, 88, 11154-11159
	400-500	11-fold	0.65 μM	40 min	cells (exogenous), tissues	Chem. Sci., 2014, 5, 3439-3448.
	545	10-fold	17.9 nM	20 min	cells (exogenous)	New J. Chem., 2018, 42, 14417-14423
	516/527	/	46 nM/29 nM	20 min	cells (exogenous /endogenous)	Anal. Chem., 2017, 89, 3754-3760
	520	60-fold	41 nM	30 min	cells (exogenous /endogenous), zebrafish	Sens. Actuators, B, 2021, 347, 130631
	508	80-fold	21 nM	40 min	cells (exogenous /endogenous), zebrafish	Sens. Actuators, B, 2021, 344, 130177

	520	55-fold	0.18 μM	45 min	cells (exogenous)	Chem. Res. Toxicol., 2020, 33, 651-656
	549	75-fold	36.3 nM	30 min	cells (exogenous)	Tetrahedron Lett., 2016, 57, 2927-2930
	490	42-fold	25 nM	15 min	cells (exogenous)	Sens. Actuators, B, 2017, 240, 625-630
	520	100-fold	37 nM	15 min	cells (exogenous/endogenous)	Anal. Chem., 2016, 88, 10648-10653
	527	/	0.95 μM	15 min	cells (exogenous)	Inorg. Chem., 2021, 60, 7108-7114

References:

1 A. Xu, Y. Tang, and W. Lin, *New J. Chem.* 2018, **42**, 8325-8329.