Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A long-wavelength mitochondria-targeted CO fluorescent probe for living cells and zebrafish imaging

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1. Materials and instruments

All of the chemicals were commercially purchased and used without any further purification. Freshly prepared ultrapure water was used throughout the experiment. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument (the supplier from Shimadzu, Japan). ^{1}H and ^{13}C NMR data were obtained by Bruker AV-400 NMR spectrometer (the supplier from Shimadzu, Japan). Absorption spectra were obtained by UV-3101PC spectrophotometer (the supplier from Shimadzu, Japan). Fluorescence spectra were obtained by Horiba FluoroMax-4 spectrophotometer (the supplier from HORIBA Scientific, America). Fluorescence imaging of Cys in live cells and zebrafish was carried out on an Olympus FV1000-IX81 confocal fluorescence microscope (the supplier from Olympus Corporation, Japan).

2. Determination of the detection limit

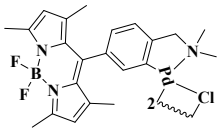
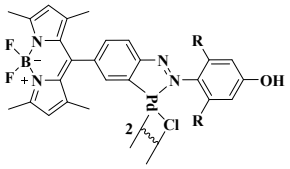
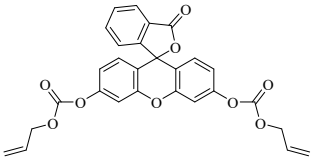
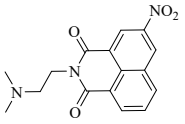
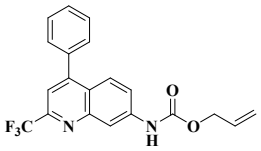
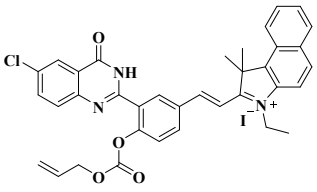
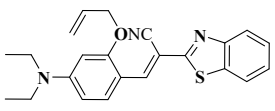
The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectra of probe **Rh-CO** were measured by ten times and the standard deviation of blank measurement was obtained. To gain the slope, the fluorescence intensities at 630 nm were plotted as the increasing concentrations of CORM-2. So the detection limit was calculated with the following equation (1):

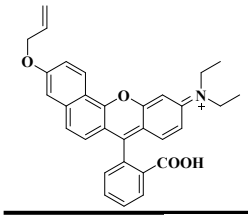
$$\text{Detection limit} = 3\sigma/k \qquad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus the concentrations of CORM-2.

3. Comparison of the properties of probes for CO

Table S1. Comparison of the properties of probes for CO

Probe	Detection limit (nM)	Emission peak (nm)	Test system	Endogenous experiment	Targeting of organelle	Ref.
	---	503	Aqueous solution	---	---	J. Am. Chem. Soc., 2012, 134, 15668-15671
	720	512	30% DMSO	Hypoxia, OGD/R	---	Anal. Chem., 2016, 88, 11154-11159
	37	520	0.5% DMSO	Heme	---	Anal. Chem., 2016, 88, 10648-10653
	19.8	538	10% DMSO	Heme	---	Sens. Actuators B Chem., 2021, 344 130245
	41	520	0.5% DMSO	Heme, LPS	Golgi	Sens. Actuators B Chem. 2021, 347, 130631
	30	605	Aqueous solution	Heme	---	Sens. Actuators B Chem., 2021, 340, 129920
	30.8	546	5% DMSO	---	---	Anal. Chem., 2019, 91, 9388-9392

	9.4	630	Aqueous solution	Heme, Glucose induction	mitochondria	this work

4. The response time of Rh-CO to CORM-2

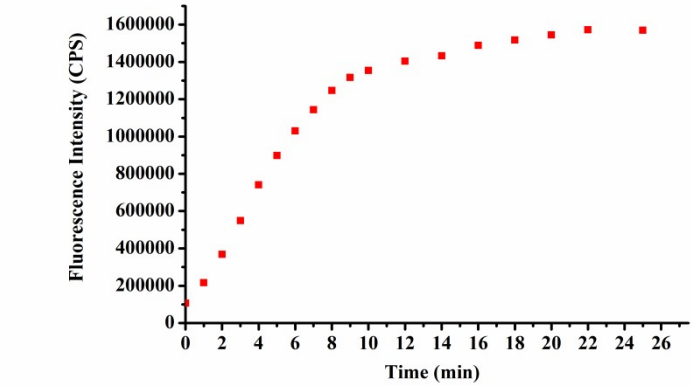


Fig. S1. Fluorescence intensity of probe solution (5 μM **Rh-CO** + 5 μM $PdCl_2$) after adding the CORM-2 (100 μM) for different time.

5. UV−Vis spectra of probe Rh-CO for sensing CORM-2

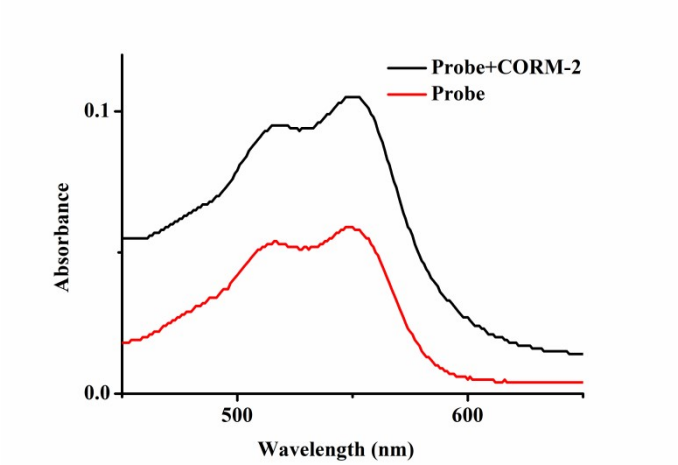


Fig. S2. The UV-vis absorption spectrum of probe solution (5 μM **Rh-CO**+5 μM $PdCl_2$) in the presence of CORM-2 (100 μM) in PBS buffer of pH 7.4.

6. The pH effect on probe + $PdCl_2$ before and after adding CORM-2

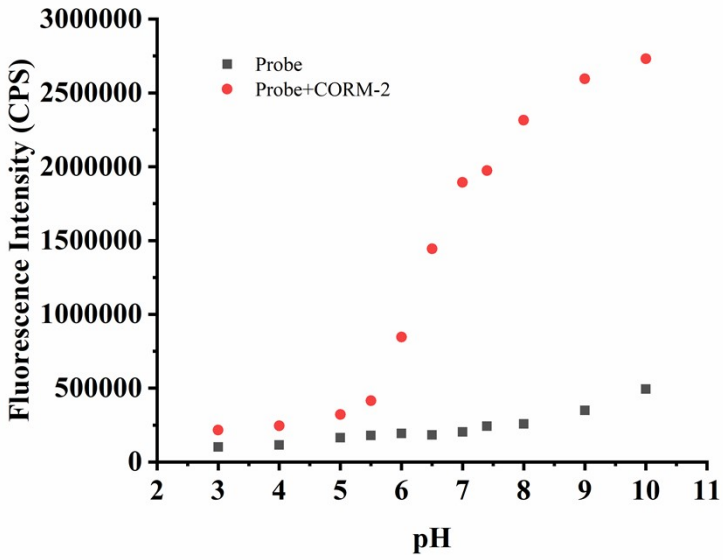


Fig. S3. The pH effect on probe + $PdCl_2$ before and after adding CORM-2 (70 μM).

7. Spectral experiments of probe Rh-CO

In the fluorescence titration experiment, the probe solution with probe **Rh-CO** concentration of 5 μM was prepared and divided into several groups. According to a certain concentration gradient, CORM-2 (0-70 μM) was added to the solution. When the reaction between CORM-2 and probe **Rh-CO** was complete, we recorded and saved all spectral data.

In the time-dependent fluorescence experiment, we first prepared a probe solution with probe **Rh-CO** concentration of 5 μM, and then the spectral data of the solution were collected at 630 nm. When CORM-2 was not added, the spectrum data of the blank probe solution was recorded. After the addition of CORM-2 (100 μM), the spectrum data was recorded every minute. After 20 minutes, the fluorescence intensity was basically unchanged, and then the spectral data was recorded and saved.

In the selective experiment, we first prepared a series of analyte solutions, such as K^+, Na^+, Zn^{2+}, Ca^{2+}, Al^{3+}, Mg^{2+}, Fe^{2+}, Cl^-, NO_2^-, SO_4^{2-},HCO_3^-, HSO_3^{--}, SCN^-, Br^-, Thr, His, Leu, Arg, Pro, Gly, Met, Ser, Asn, TBHP, ·TBHP, H_2O_2,·OH, KO_2, NaClO, GSH, Hcy, Cys, CORM-2. Then we prepared a probe solution with probe solution (5 μM **Rh-CO**+5 μM $PdCl_2$), and the solution was divided into several groups. The above analytes were added to the probe solution. After that, we collect the spectral data at 630 nm.

8. Probe Rh-CO responds for CORM-2 in the presence of other analytes

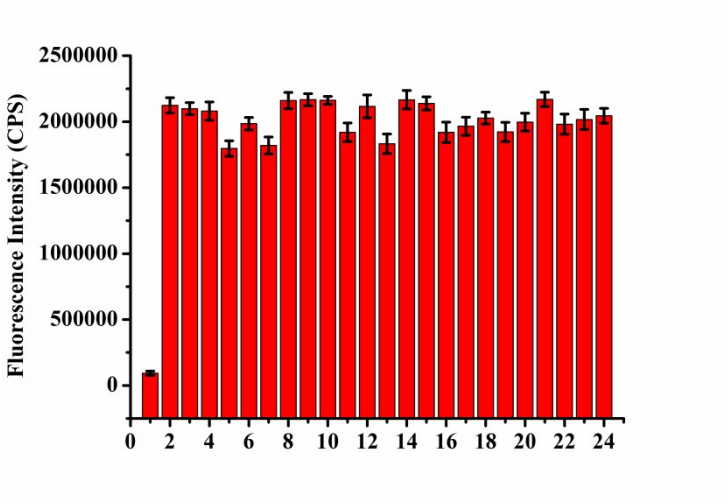


Fig. S4. The fluorescence responses of probe solution (5 μM **Rh-CO** +5 μM $PdCl_2$) toward CORM-2 in the presence of 1. Blank, 2. Na^+, 3. Ca^{2+}, 4. Zn^{2+}, 5. K^+, 6. Mg^{2+}, 7. Al^{3+}, 8. Fe^{2+}, 9. Cl^-, 10. HCO_3^-, 11. SCN, 12. HSO_3^{--}, 13. Br^-, 14. I^-, 15. Thr, 16. Leu, 17. Gly, 18. Arg, 19. Pro, 20. His, 21. Met, 22. Ser, 23. Asn, 24. CORM-2. (the concentration of the substances is 100 μM).

9. Toxicity of the probe Rh-CO

The cell viability of HeLa cells, treated with probe **Rh-CO**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly,

HeLa cells, seeded at a density of 1 × 10^6 cells·mL^{-1} on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. Then the live HeLa cells were incubated with various concentrations (0, 5, 10, 20 and 30 μM) of probe **Rh-CO** suspended in culture medium for 12 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 550 nm was measured.

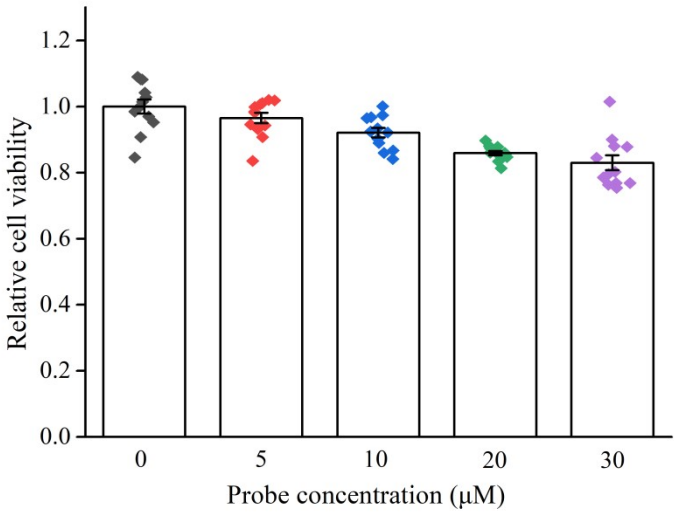


Fig. S5. Viability of HeLa cells with different concentration of probe **Rh-CO**.

10. Cell imaging experiments

The HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO2 at 37 °C humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin.

Firstly, control cells were imaged. Then, HeLa cells were incubated with probe **Rh-CO** (10 μM) for 30 min for imaging. Then, HeLa cells were incubated with probe **Rh-CO** (10 μM) and the $PdCl_2$ (10 μM) for 30 min and for imaging. Next, the other groups of cells were incubated with **Rh-CO** (10 μM) and $PdCl_2$ (10 μM) for 30 min,

washed with culture water, followed by culturing with CORM-2 (20, 30 μM) for 30 min for imaging.

11. Culture of zebrafish

Healthy male and female zebrafish (AB stain) were maintained in different tanks with a 14 h light / 10 h dark cycle at 28 °C. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28 °C.

12. HRMS proof for response mechanism

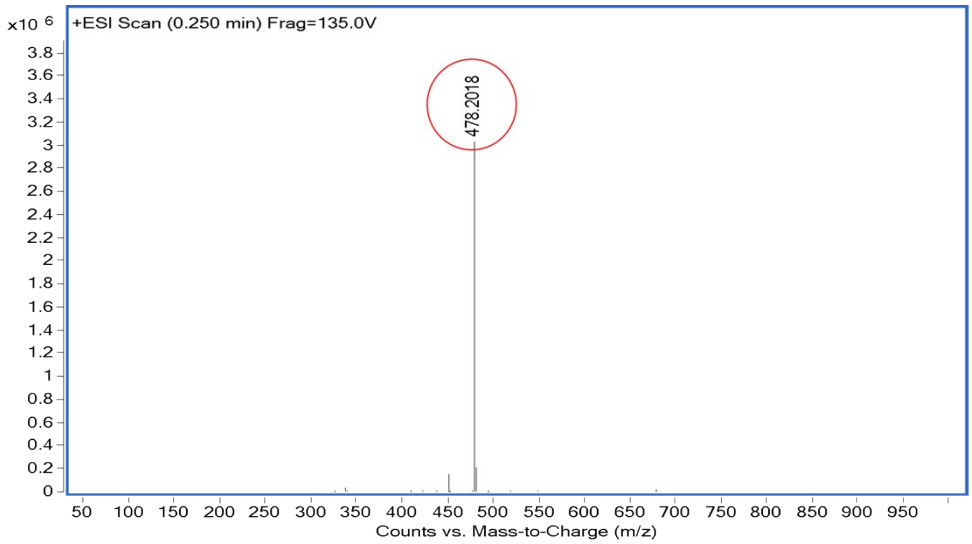


Fig. S6. HRMS of probe **Rh-CO**.

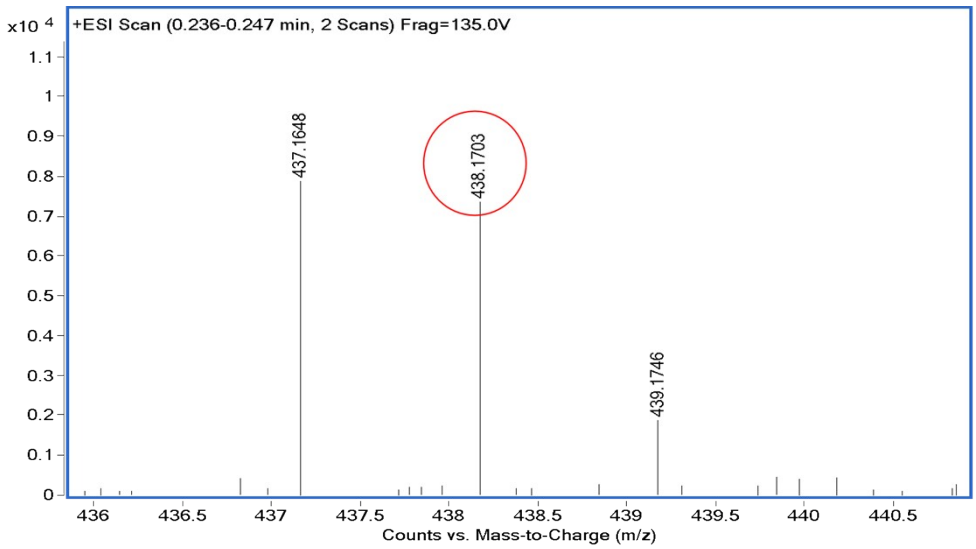


Fig. S7. HRMS of the reaction products of probe **Rh-CO** and CORM-2.

13. ^{1}H NMR and ^{13}C NMR of probe Rh-CO

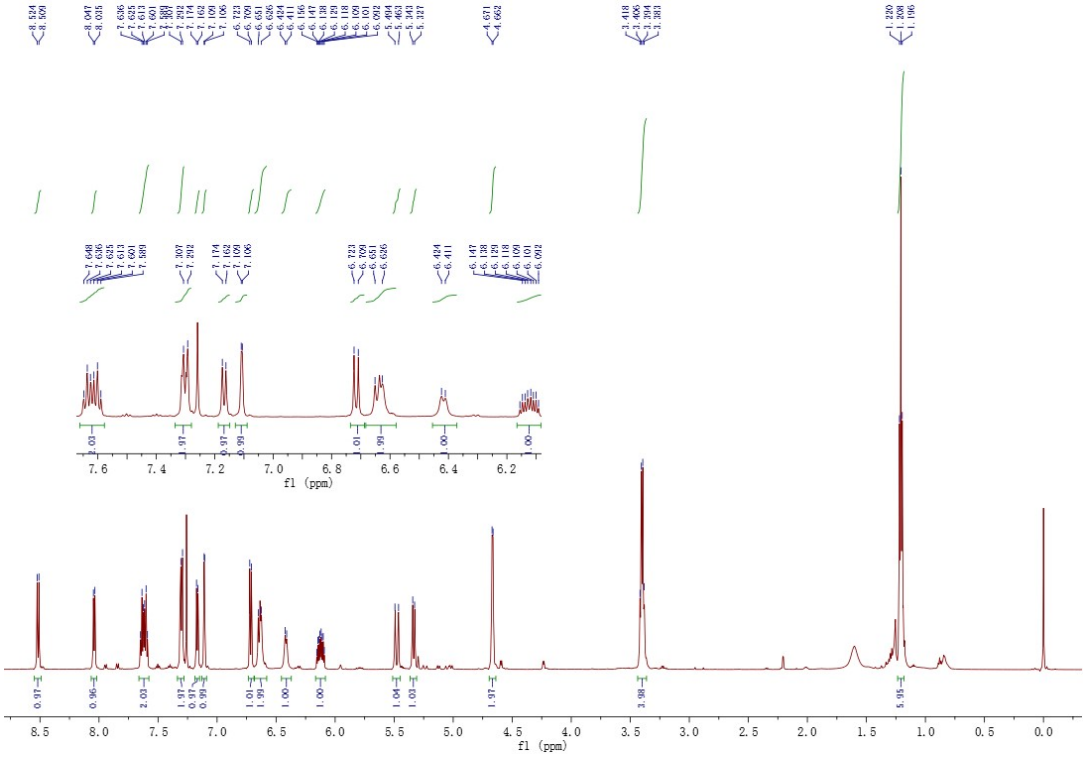


Fig. S8. The ^{1}H-NMR data of probe **Rh-CO**.

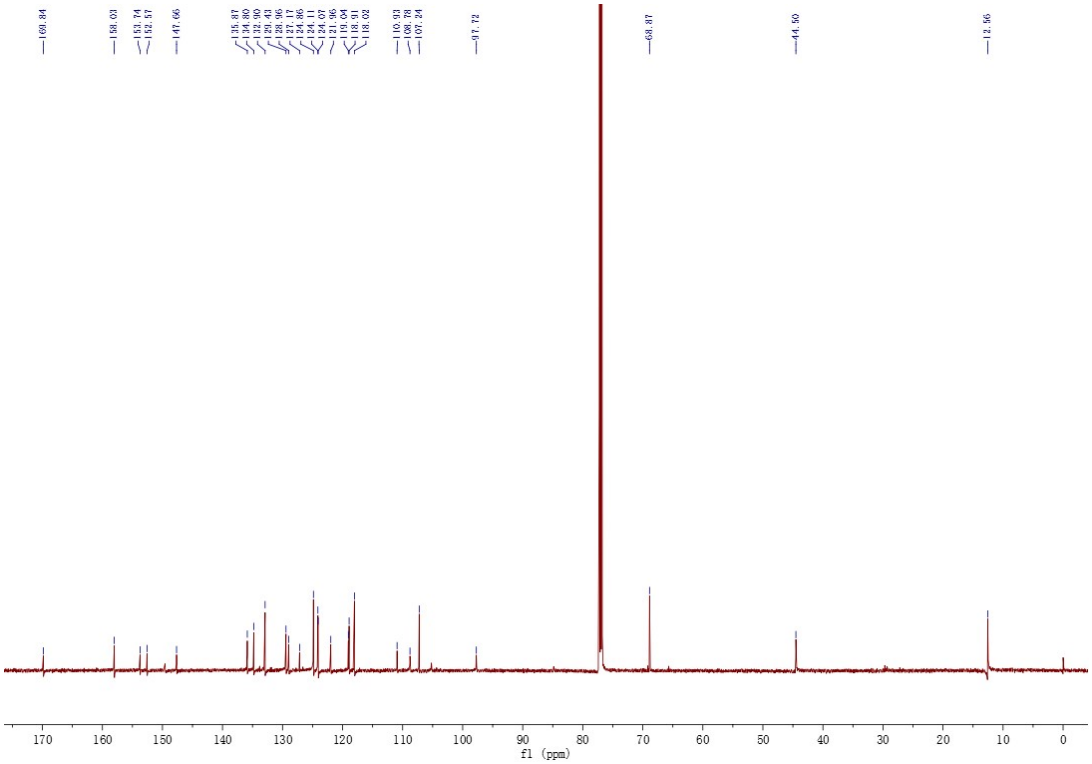


Fig. S9. The ^{13}C-NMR data of probe **Rh-CO**.