Supporting Information for

The development of a hemicyanine-based ratiometric CO fluorescent probe with long emission and its applications for imaging CO *in vitro* and *in vivo*

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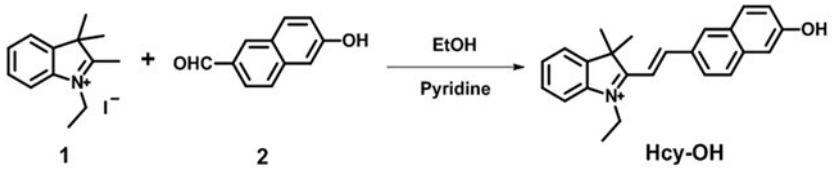
1. Materials and apparatus.

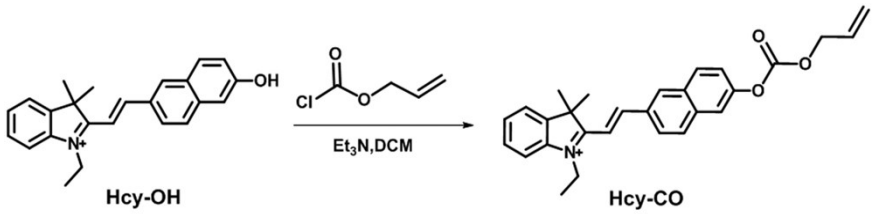
NMR data were obtained with an AVANCE III 400 MHz Digital NMR spectrometer with tetramethylsilane (TMS) as an internal standard. High Resolution Mass Spectrometric (HRMS) results were obtained at an Agilent 1100 HPLC/MSD spectrometer. The pH experiments were conducted using a Mettler-Toledo Delta 320 pH meter. Absorption experiments were carried out on a Shimadzu UV-2700 spectrometer. Photoluminescent spectra were harvested with a HITACHI F4600 fluorescence spectrophotometer. Biological imaging experiments were accomplished using Nikon A1 fluorescence microscopy with a femtosecond laser.

2. Synthesis of Hcy-OH



The synthesis of **Hcy-OH** was according to previously reported method[1--3]. Compound **1**(441 mg,1.4 mmol)[2,3] and compound **2** (200 mg, 1.16 mmol) was dissolved in absolute ethanol (40 mL), then added four drops of pyridine to the solution, after the mixture was refluxed for 4 hours under the nitrogen atmosphere. The mixture was cooled to room temperature, product precipitated from solution. Then the solvent was removed by reduced pressure and the residue purified by silica gel chromatography (DCM: MeOH = 50: 1) to acquire compound 3 as an orange-red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 – 8.57 (m, 2H), 8.31 (dd, J = 9.0, 2.0 Hz, 1H), 7.99 – 7.84 (m, 4H), 7.77 – 7.59 (m, 3H), 7.29 – 7.19 (m, 2H), 4.75 (q, J = 7.3 Hz, 2H), 1.85 (s, 6H), 1.49 (t, J = 7.2 Hz, 3H).

3. Synthesis of Hcy-CO.



At 0°C, Hcy-OH (140 mg, 0.3 mmol) and triethylamine (0.15 mL) were dissolved in anhydrous dichloromethane (15 mL), then dropwise allyl chloroformate (35.7mg, 0.3mmol). under the nitrogen atmosphere, the mixture was stirred at room temperature for 12 hours, and purified by column chromatography (DCM: MeOH = 50: 1) to acquire target probe (**Hcy-CO**). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.02 (s, 1H), 8.42 – 8.26 (m, 3H), 8.10 (d, *J* = 16.1 Hz, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.72 – 7.57 (m, 5H), 7.41 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.06 (ddt, *J* = 16.5, 10.4, 5.9 Hz, 1H), 5.49 (dt, *J* = 17.1, 1.4 Hz, 1H), 5.40 (dd, *J* = 10.4, 1.3 Hz, 1H), 5.15 (q, *J* = 7.3 Hz, 2H), 4.82 (dt, *J* = 5.9, 1.4 Hz, 2H), 2.65 (s, 1H), 2.12 (s, 1H), 1.91 (s, 6H), 1.68 (t, *J* = 7.3 Hz, 3H), 1.28 (s, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 181.35 , 154.99 , 151.29 , 143.53 , 136.50 , 134.43 , 132.06 , 131.25 , 131.17 , 130.93 , 130.05 , 129.78 , 129.05 , 126.75 , 122.84 , 121.80 , 119.82 , 118.07 , 114.89 , 112.81 , 77.26 , 69.46 , 52.60 , 44.57 , 27.09 , 14.61 .

Table S1 Properties of the representative ratiometric CO fluorescent probes

Probe	Em1/ nm	Em2/ nm	Reference
Hcy-CO	515 nm	600 nm	This work
Cou-Flavone	555 nm	475 nm	Chem. Commun., 55(2019),8987-8990
Probe 1	472 nm	545 nm	Sensors & Actuators: B., 251(2017), 389-395
Ratio-CO	455 nm	545 nm	New J. Chem., 42(2018), 14417-14423
Probe 1	510 nm	566 nm	Tetrahedron Letters, 55(2014),2537-2540
NIR-Ratio-CO	592 nm	655 nm	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 227 (2020) 117657
QCy7-CO	570 nm	715 nm	Sensors & Actuators: B., 301(2019), 127075

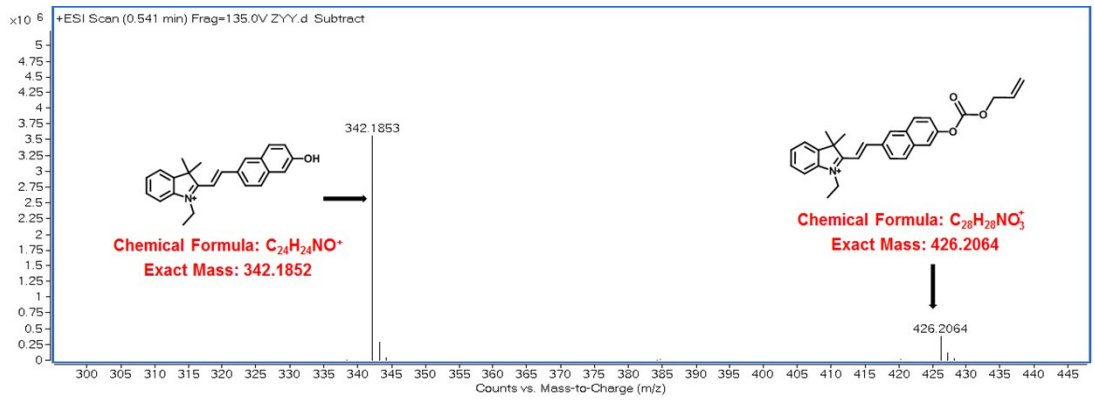


Fig. S1. The HRMS mechanism for **Hcy-CO** (10 μM), $PdCl_2$ (10 μM) in the presence of CORM-2 (70 μM).

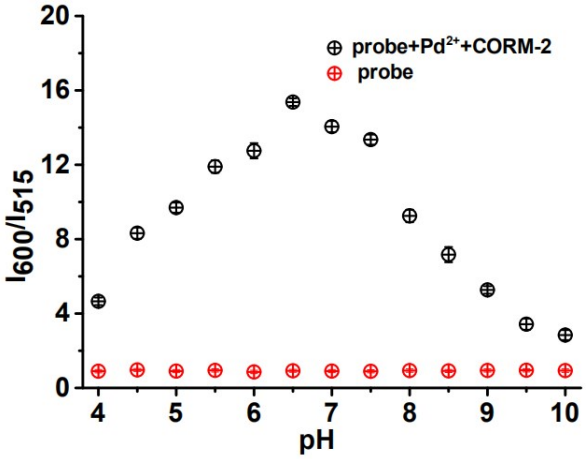


Fig. S2. Fluorescence intensity ratio changes (I_{600}/I_{515}) of **Hcy-CO** (10 μM) in the absence and presence of CORM-2 (70 μM) at various pH conditions.

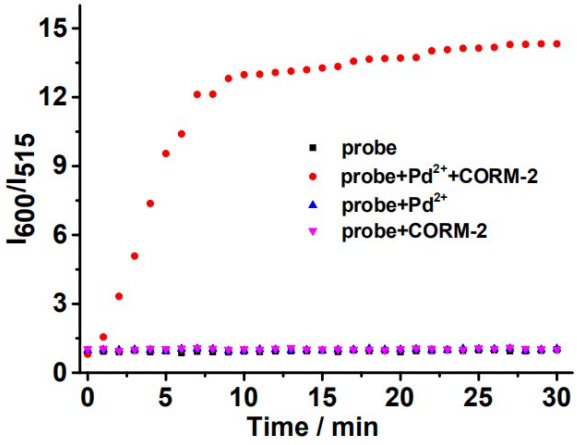


Fig.S3. Fluorescent stability change of **Hcy-CO** (10 μM)+$PdCl_2$ (10 μM) with or without CORM-

2 (70 μM) under photon irradiation.

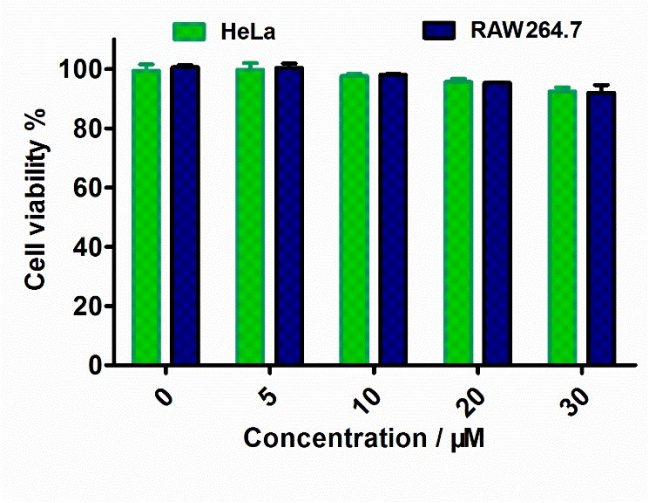


Fig. S4. Survival of HeLa cells and RAW 264.7 cells in the presence of **Hcy-CO** at various concentrations measured using MTT assay.

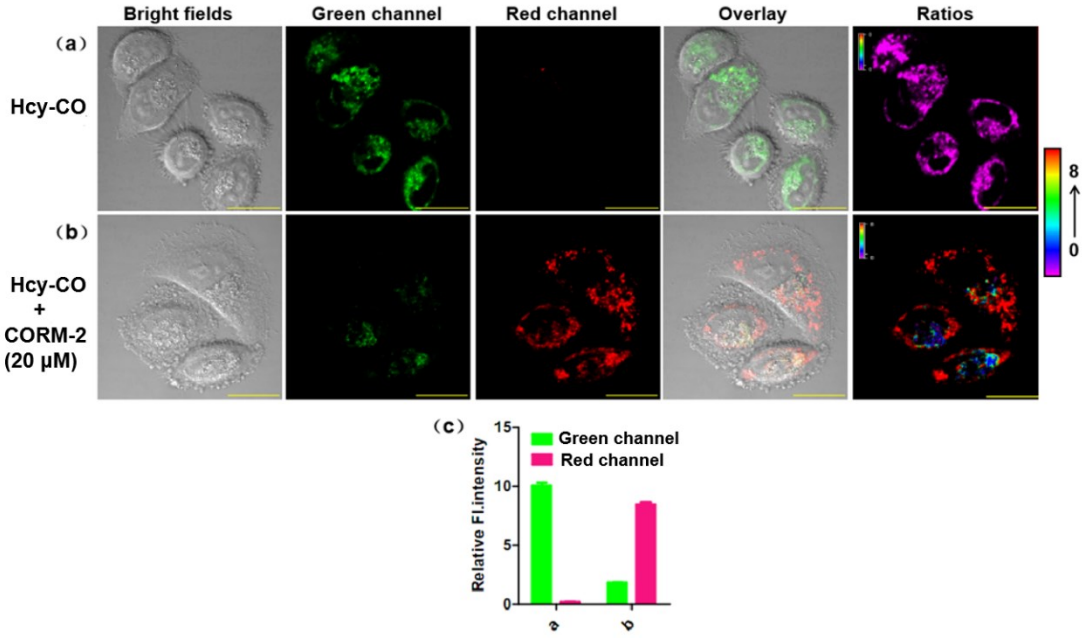


Fig. S5. Fluorescent imaging of **Hcy-CO** in HeLa cells for detecting exogenous CO. (a) RAW 264.7cells were treated with 10 μM **Hcy-CO**+$PdCl_2$ (10 μM) for 30 min. (b) HeLa cells were pre-treated with probe system (**Hcy-CO**+$PdCl_2$, 10 μM each) for 30 min and subsequently incubated with 20 μM CORM-2 for 30 min. (c) Quantified fluorescence intensities of cellsl in the green channel and red channel respectively. λ_{ex} = 405 nm, λ_{em1} (Green channel) = 500−540 nm; λ_{em2} (Red channel) = 570−620 nm. Scale bar = 20 μm.

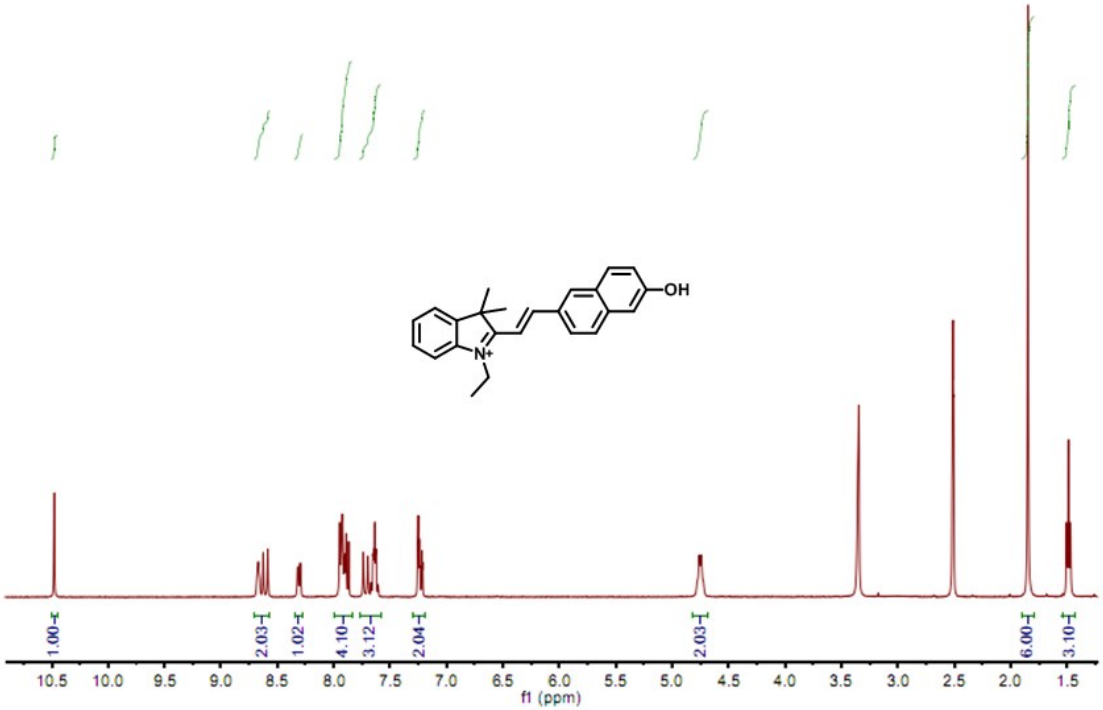


Fig. S6. [1]HNMR spectrum of **Hcy-OH** in DMSO-d_6.

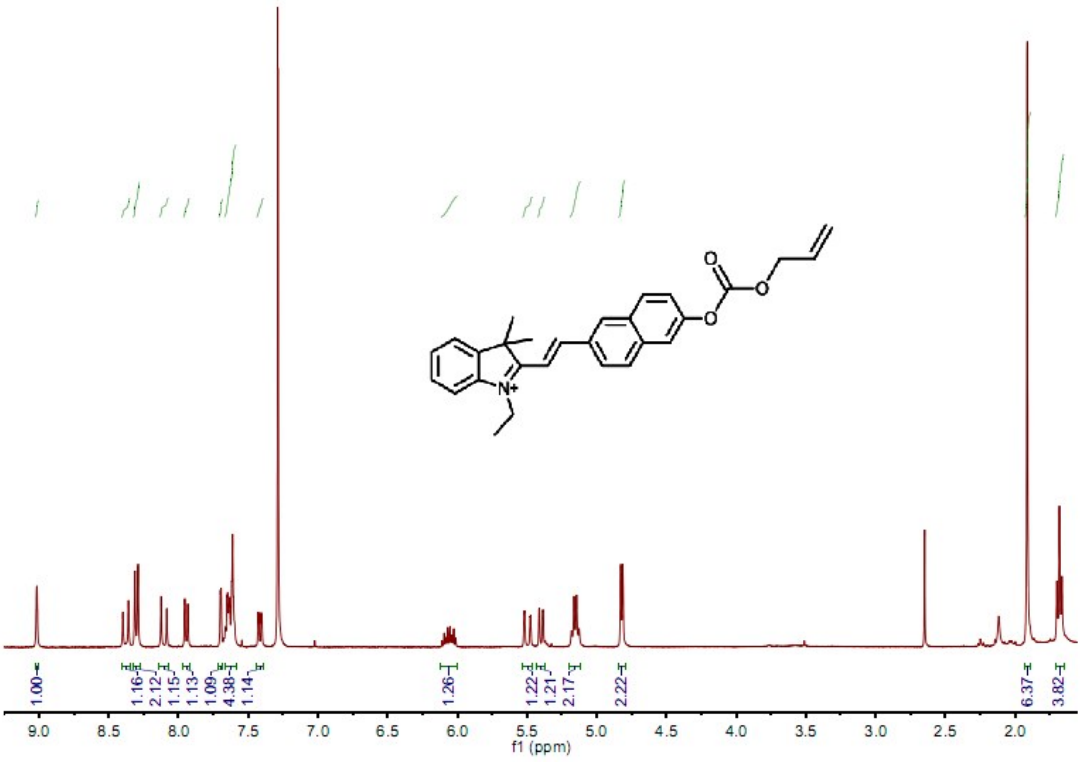


Fig. S7. [1]HNMR spectrum of **Hcy-CO** in $CDCl_3$.

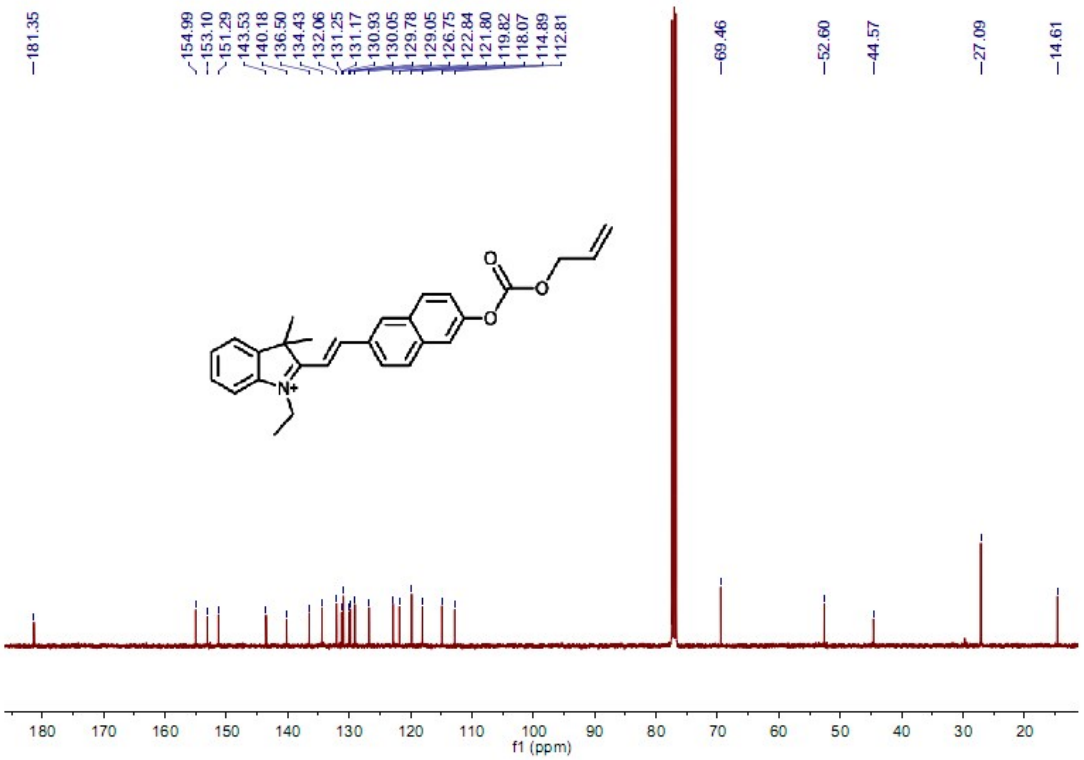


Fig. S8. ^{13}CNMR spectrum of **Hcy-CO** in $CDCl_3$.

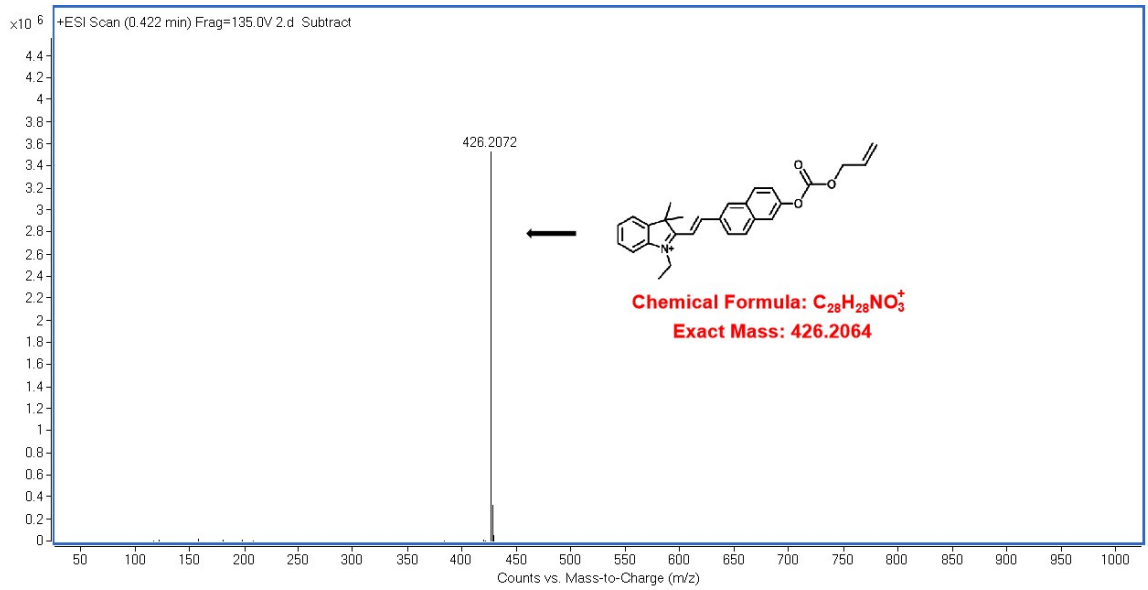


Fig. S9. The HRMS spectrum of **Hcy-CO**.

References

1. G. Liu, D. Liu, X. Han, X. Sheng, Z. Xu, S. H. Liu, L. Zeng and J. Yin, *Talanta*, 2017, **170**, 406-412.
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3. D. Verga, M. Nadai, F. Doria, C. Percivalle, M.D. Antonio, M. Palumbo, et al., *J. Am. Chem. Soc*. 2010, **132**, 14625–14637.