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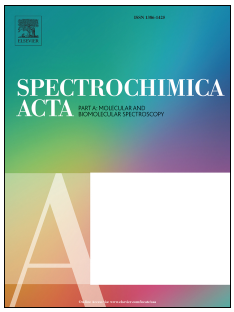

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PII: S1386-1425(19)31047-9

DOI: https://doi.org/10.1016/j.saa.2019.117657

Reference: SAA 117657

To appear in: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*

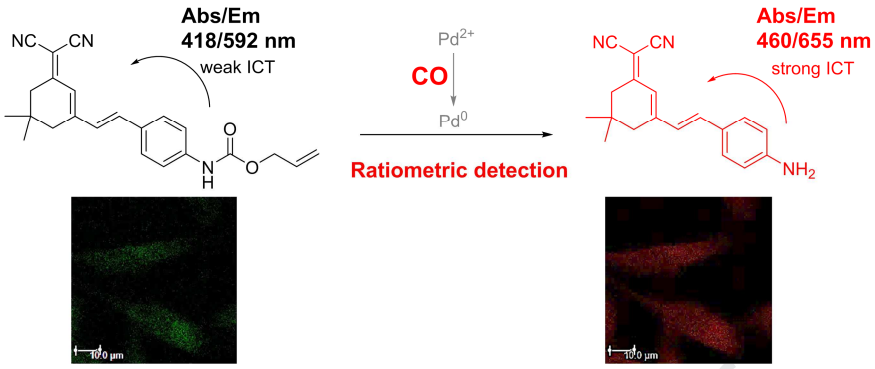
Received Date: 12 June 2019

Revised Date: 12 October 2019

Accepted Date: 14 October 2019

Please cite this article as: E. Zhou, S. Gong, J. Hong, G. Feng, Development of a new ratiometric probe with near-infrared fluorescence and a large Stokes shift for detection of gasotransmitter CO in living cells, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2019), doi: https://doi.org/10.1016/j.saa.2019.117657.

Graphical Abstract



Development of a new ratiometric probe with near-infrared fluorescence and a large Stokes shift for detection of gasotransmitter CO in living cells

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Abstract: A near-infrared (NIR) ratiometric fluorescent probe, **NIR-Ratio-CO**, was developed for rapid detection of carbon monoxide (CO) in both solution and living cells through the strategy of Pd^0-mediated Tsuji-Trost reaction. This probe shows a rapid, highly specific and sensitive detection process for CO, accompanied by colorimetric and distinct ratiometric fluorescence changes at 655 and 592 nm with a large Stokes shift up to 195 nm. The detection limit for CO was measured to be about 61 nM by the fluorescence method. In addition, this probe was successfully applied for ratiometric imaging of both exogenous and endogenous CO in living cells, indicating that it can be used as a novel tool for ratiometric fluorescent detection of CO in living systems.

Keywords: near-infrared; ratiometric; fluorescent probe; carbon monoxide; imaging

1. Introduction

Carbon monoxide (CO), a highly toxic gas molecule, has become recognized as an important biological gasotransmitter in living systems [1]. CO can be endogenously generated during the haem catabolism in our human body and other biological systems by haem oxygenase (HO) enzymes, and plays a significant regulatory role in various physiological and pathological processes [2]. Abnormal metabolism of CO has been involved in a numerous of diseases, such as hypertension, inflammation, Alzheimer's disease, and heart failure, however, proper amount of CO was found to be a potential therapeutic agent for therapy of many diseases because of its cytoprotective effects [3,4]. Despite of these discoveries, many precise cellular functions of CO remain unclear, which may largely due to the lack of a reliable method to effectively detect this small gas molecule in living systems [5]. To disclose more details of the biological CO, the development of convenient and reliable methods for real-time detection of CO in living systems is therefore important and urgent [6-8].

Although several methods have been developed to detect CO, such as gas chromatography [9], chromogenic detection [10,11], and electrochemical analysis [12], these methods are limited to detect CO in living systems due to their poor bio-compatibility. Recently, small molecular fluorescent probes has been proved particularly useful for detection and imaging in living systems due to their many advantages such as high sensitivity, great convenience, noninvasive manner, and

real-time detection ability with high temporal-spatial resolution [13]. In 2012, Chang's group reported a small molecular fluorescent probe based on an organic palladium complex, which realized CO detection in living cells for the first time [14]. Soon after, many other fluorescent probes for detection of CO in living cells were reported [15-35]. Among them, the Pd^0-mediated Tsuji–Trost reaction has been proved to be a simple but very attractive strategy to develop probes for detecting CO in living systems [22-36]. However, most of the reported CO probes have some shortcomings. For example, almost all of the reported CO probes are single emissive (intensity-based), which may be interfered from a variety of factors, such as the concentration of probe, temperature, pH, and instrument efficiency etc. [26,36]. In comparison, ratiometric fluorescent probes can effectively overcome these problems by self-calibration at two emission bands, which greatly increases the reliability and accuracy of detection [37]. Besides, most of the reported CO probes showed fluorescence changes in the visible region (<650 nm) and small Stokes shift (<50 nm), which also limited their biological applications due to the potential strong interference from the background fluorescence of biomolecules and the scattering light from the excitation [38,39]. In view of these shortcomings, development of ratiometric fluorescent CO probes with enhanced near-infrared (NIR) fluorescence and a large Stokes shift are urgently needed.

Herein, a NIR ratiometric fluorescent probe (**NIR-Ratio-CO**, Scheme 1) was designed for detection of CO both in solution and living cells. Probe **NIR-Ratio-CO** uses **DCI-NH2** as the ratiometric fluorescent signaling unit due to its well-known

easy-to-get property with efficient intramolecular charge transfer (ICT) process, desirable NIR fluorescence properties with a remarkable large Stokes shift (~200 nm), and excellent biocompatibility to living systems [40,41]. An allyl carbamate moiety was chosen as the reactive unit because it not only offered an efficient Tsuji–Trost reaction site [22,26] but also could effectively modulate the electron push-pull system and alter the photophysical property of **DCI-NH2**. Due to the electronic-withdrawing property of allyl carbamate, the probe shows weak ICT and has an emission at 592 nm. However, after the addition of CO in the presence of Pd^{2+}, the allyl carbamate is cleaved, resulting in the generation of **DCI-NH2** with stronger ICT and a red-shifted emission at 655 nm (Scheme 1). As a result, by self-calibration at two emission bands, **NIR-Ratio-CO** could provide an efficient ratiometric NIR fluorescent probe for CO with desirably enhanced fluorescence in the NIR region. Moreover, we also showed that this probe exhibited excellent sensing performance for CO and could be used to detect both exogenous and endogenous in living cells. To the best of our knowledge, **NIR-Ratio-CO** is the first ratiometric fluorescent probe reported to date with enhanced NIR fluorescence and a large Stokes shift for CO detection in living cells (Table S1 in the supplementary data).

[**Scheme 1**]

2. Materials and methods

2.1. Materials and instrumentation

Unless otherwise stated, all the chemicals involved in this work were purchased from commercial suppliers and used without further purification. CORM-3, a

commercially available CO-releasing molecule was used as the safe and easy-to-handle CO source in all the experiments according to the literature [14,15,23,42]. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 or 600 spectrometer. Data of high-resolution mass spectrometry (HR-MS) were obtained with an LC/Q-TOF MS spectrometer (Agilent). The UV-vis and fluorescent spectra were recorded on an Agilent Cary-100 UV-vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. The pH was measured by a PB-10 digital pH-meter (Sartorius). Cell imaging was performed on a Leica TCS SP8 confocal fluorescence microscope.

2.2. Synthesis

DCI-NH2 was synthesized from isophorone (**ISO**) as a deep purple solid according to the literature method [41].

Synthesis of NIR-Ratio-CO. To a solution of **DCI-NH2** (289 mg, 1 mmol) was added allylchloroformate (0.5 ml, 5 mmol) in dry pyridine (8 mL) under the atmosphere of nitrogen at 0 °C. The resulting mixture was stirred at room temperature for 4 h. Then, the reaction was quenched with diluted HCl and extracted with ethyl acetate (20 mL × 3). The combined organic layers were dried, filtered and concentrated. The crude product was purified by column chromatography (eluent: petroleum ether/ethyl acetate = 10/1, v/v) to afford **NIR-Ratio-CO** as a red powder (112 mg, yield 30 %). M.p.185-186 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.01 (d, *J* = 16.0 Hz, 1H), 6.91 (s, 1H), 6.90 (d, *J* = 15.5 Hz, 1H), 6.81 (s, 2H), 5.97 (ddt, *J* = 16.6, 11.0, 5.8 Hz, 1H), 5.38 (d, *J* = 16.9 Hz,

1H), 5.28 (d, *J* = 10.5 Hz, 1H), 4.69 (d, *J* = 5.6 Hz, 2H), 2.59 (s, 2H), 2.46 (s, 2H), 1.08 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.24, 154.03, 152.82, 139.11, 136.44, 132.10, 130.70, 128.52, 127.81, 123.05, 118.61, 118.45, 113.56, 112.79, 103.81, 78.03, 66.02, 42.92, 39.11, 31.95, 27.94. MS (EI): m/z found 373.1 (M^+). HR-MS (ESI) calcd. for $C_{23}H_{23}N_3O_2Na^+$ ($M + Na^+$) 396.1682, found 396.1685.

2.3. Optical studies

Unless otherwise stated, all the optical tests were performed in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v/v) at 37 °C with 5 μM of **NIR-Ratio-CO** in the presence of 10 μM of $PdCl_2$. Standard quartz cuvettes with a 10 mm lightpath were used for the UV-vis and fluorescent spectral measurements. λ_{ex} = 440 nm and slit width $d_{ex} = d_{em}$ = 10 nm were set for all fluorescence measurements. The fluorescence quantum yields were determined in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v/v) using Rhodamine B (Φ = 0.89 in ethanol) as standard.

2.4. Imaging of CO in living cells

HeLa cells were cultured in DMEM medium supplemented with FBS (fetal bovine serum, 10%) at 37 °C in a 5% CO_2 and 95% air incubator. In the cell imaging experiments, a mixture of probe **NIR-Ratio-CO** (5 μM) and $PdCl_2$ (10 μM) in PBS buffer with 0.5% of DMSO was used for the imaging. As the control, cells were imaged after incubation with a mixture of probe **NIR-Ratio-CO** (5 μM) and $PdCl_2$ (10 μM) at 37 °C for 30 minutes. For imaging of exogenous CO, cells were incubated with CORM-3 (10, 20 and 30 μM, respectively) for 30 min, and then with a mixture of probe **NIR-Ratio-CO** (5 μM) and $PdCl_2$ (10 μM) for another 30 min. For imaging

of endogenous CO, cells were incubated with heme (100 μM) for 1, 4 and 8 h at 37 °C, respectively, and then with a mixture of probe **NIR-Ratio-CO** (5 μM) and $PdCl_2$ (10 μM) for another 30 min. Imaging was taken on a confocal fluorescence microscope after washing the cells three times with PBS buffer solution. The fluorescence of each channel (red or green) was analyzed with three different cell images, and then the average fluorescence intensity of each channel was obtained. Finally, the F_{red}/F_{green} ratio was calculated.

3. Results and discussion

3.1. Probe synthesis

Probe **NIR-Ratio-CO** was successfully synthesized according to the route as shown in Scheme 2. **DCI-NH2** was prepared from commercially available isophorone (**ISO**) according to the literature method [41]. Structural identification of **NIR-Ratio-CO** was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectroscopy, which can be found in the supplementary data.

[**Scheme 2**]

3.2. Ratiometric fluorescent detection of CO

With **NIR-Ratio-CO** in hand, we tested its sensing properties for CO in the presence of $PbCl_2$ by the absorption and fluorescent spectral changes in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v/v) at 37 °C. As shown in Figure 1, in the absence of CO, the probe solution (5 μM **NIR-Ratio-CO** + 10 μM Pd^{2+}) showed a major absorption peak at 418 nm and a marked fluorescence at 592 nm (Φ = 0.02). Upon addition of CORM-3 (100 μM), the absorption peak at 418 nm decreased

gradually and simultaneously a new absorption peak at 460 nm appeared, accompanied by an apparent color change from light yellow to pink. Meanwhile, a marked decrease of fluorescence intensity at 592 nm and concurrently a dramatic enhancement of fluorescence intensity at 655 nm with an obvious red-shift (63 nm) of emission and a marked emission color change from orange to red can be observed, as shown in Figure 1b. Furthermore, from the reaction kinetic studies as shown in Figure S1, upon addition of CORM-3, the fluorescence intensity of the probe solution at 655 nm increased rapidly and reached saturation at about 15 min, indicating it showed a rapid detection process. In addition, the fluorescence intensity ratio at 655 and 592 nm (I_{655}/I_{592}) increased from 0.42 to 7.5 after incubation with CORM-3 for 15 min, which corresponds to a ~18 fold enhancement, indicating the probe system shows a fairly good ratiometric fluorescence response for CO. In contrast, **NIR-Ratio-CO** shows negligible response to $PbCl_2$ and does not respond to CO in the absence of $PbCl_2$ (Figure S2). All these results indicate that **NIR-Ratio-CO** can be used as a potential NIR ratiometric fluorescent probe for rapid detection of CO in the presence of $PbCl_2$. Notably, the fluorescence shows significant enhancement in the far-red to NIR region, which is highly favorable for the biological applications of **NIR-Ratio-CO**.

[**Figure 1**]

3.3. The selectivity

To evaluate the selectivity of **NIR-Ratio-CO** toward CO, we tested its responses to various biologically relevant analytes. As shown in Figure 2, upon addition of various analytes including biothiols and amino acids ((cysteine (Cys), homocysteine

(Hcy), glutathione (GSH), hydrogen sulfide (H_2S, NaHS was used as the source), methionine (Met), threonine (Thr), arginine (Arg), tyrosine (Tyr), alanine (Ala), aspartic (Asp), lysine (Lys), pyroglutamic acid (Pyr), phenylalanine (Phe), glutamic acid (Glu), isoleucine (Ile), histidine (His), glycine (Gly), valine (Val), leucine (Leu), tryptophan (Trp), serine (Ser)), anions (F^-, Cl^-, Br^-, I^-, NO_3^-, CO_3^{2-}, HCO_3^-, HSO_4^-, SO_3^{2-}, $S_2O_3^{2-}$, HSO_3^-, $S_2O_7^{2-}$, $S_2O_8^{2-}$, SCN^-, Ac^-, N_3^-, and $C_2O_4^{2-}$, all with sodium salts), and reactive oxygen/nitrogen species (ROS/RNS) [43] such as ClO^-, H_2O_2, NO_2^-, NO, HNO, $ONOO^-$, $ROO^\bullet$, $^tBuOO^\bullet$ and $O_2^{\bullet-}$ to the probe solution (5 μM **NIR-Ratio-CO** + 10 μM Pd^{2+}) in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v/v) at 37 °C, only negligible changes at 592 nm in the fluorescence spectra were observed. These results are clearly different to the ratiometric fluorescent response of the probe solution for CO (CORM-3), indicating **NIR-Ratio-CO** has high selectivity for CO.

[**Figure 2**]

3.4. The sensitivity

Fluorescent titration method was then used to investigate the sensitivity of the probe for CO. As shown in Figure 3, with the increase of the concentration of CORM-3 to the test solution of **NIR-Ratio-CO** (5 μM with 10 μM of Pd^{2+}), the emission at 592 nm gradually decreased, while the emission at 655 nm increased dramatically. The ratios of fluorescence intensity at 655 and 592 nm (I_{655}/I_{592}) showed a dose-dependent response to CORM-3 and a saturation was observed after about 50 μM of CORM-3 was added (Figure 3a, inset). Importantly, the ratio I_{655}/I_{592} showed a linear increase to the concentration of CORM-3 from 0 to 10 μM (Figure 3b). Since 1

mole CORM-3 can release 1 mole CO in aqueous solution [42], the detection limit of probe **NIR-Ratio-CO** for CO was thus determined to be about 61 nM based on the 3σ/k method (S/N = 3). These results indicate that **NIR-Ratio-CO** can be used as a ratiometric fluorescent probe for quantitative analysis of CO with high sensitivity.

[**Figure 3**]

3.5. The sensing mechanism

We compared the spectra of the probe solution after addition of CORM-3 with those of **DCI-NH2** and found that they are in good agreement (Figure S3). This suggests that the formation of **DCI-NH2** is responsible for the optical changes. Recently, several Tsuji–Trost reaction-based fluorescent turn-on probes for CO have been reported and their sensing mechanism were well studied [22-36]. Based on these reports, the reaction mechanism of **NIR-Ratio-CO** for sensing CO in the presence of Pd^{2+} is proposed in Scheme S1. To confirm this sensing mechanism, the reaction mixture of probe **NIR-Ratio-CO** system with CORM-3 was analyzed by thin-layer chromatography (TLC) and high-resolution mass spectrometry. As shown in Figures S4-5, the red fluorescent product in TLC plate and the peak with a mass of 288.1487 in the mass spectrum clearly showed that **DCI-NH2** was formed.

3.6. The effect of pH

The pH effects of the probe for NIR fluorescent detecting CO was also investigated. As presented in Figure S6a, the **NIR-Ratio-CO** solution in the presence of Pd^{2+} shows very weak fluorescence at 655 nm and the fluorescence is not pH sensitive. However, upon addition of CORM-3, the NIR fluorescence of the probe solution (5

μM **NIR-Ratio-CO** + 10 μM Pd^{2+}) showed significant enhancement over a wide range of pH, and the best pH was found around the physiological pH (~7.0). Similar results can be obtained from the change of fluorescence intensity ratio (I_{655}/I_{592}) under different pH (Figure S6b). This clearly indicates that **NIR-Ratio-CO** has great potential for working in living systems.

3.7. Bioimaging applications

An evaluation of the ability of probe **NIR-Ratio-CO** to image CO in living cells was carried out with a fluorescence confocal microscope. A standard 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was first conducted with cells treated with di□erent concentrations of probe **NIR-Ratio-CO** (10, 20, 30, 40, and 50 μM). As shown in Figure S7, even after incubation with 50 μM probe, more than 80% of the cells still survive either in the absent or in the presence of two equiv. of Pd^{2+}, indicating that the probe shows low cytotoxicity. In the bioimaging experiments, the cells were exposed to 0, 10, 20, and 30 μM of CORM-3 for 30 min, washed with PBS, and then stained with a 5 μM probe **NIR-Ratio-CO** solution (with 10 μM $PdCl_2$) for 30 min before confocal microscope images were taken (Figure 4). One can see that, the cells incubated with only the probe emitted fluorescence in the green channel but hardly any fluorescence in the red channel. However, with exposure to an incremental amount of CORM-3, the fluorescence emission decreased gradually in the green channel, accompanied by an increase of fluorescence in the red channel. Correspondingly, the ratiometric images showed that the F_{red}/F_{green} value increased from 0.12 to 4.31. These results indicate that probe

NIR-Ratio-CO is capable of ratiometric detection of exogenous CO in living cells.

[**Figure 4**]

The capability of probe **NIR-Ratio-CO** to image endogenous CO in living cells was also investigated. It is known that endogenous CO is generated from the enzymatic degradation of heme catalyzed by heme oxygenases [2,44]. In this work, heme was used to stimulate endogenous CO generation [23, 29, 32]. As shown in Figure 5, when HeLa cells were pre-incubated with 100 μM heme and then indicated with the probe system, the fluorescence in the green channel decreased gradually over the incubation time of heme from 0 h to 8 h (images E1, F1 ,G1, and H1), meanwhile, the fluorescence in the red channel showed an obvious increase over time. Correspondingly, with the increase of heme-stimulation time, the ratiometric images showed the F_{red}/F_{green} value increased from 0.32 to 2.98, indicating the intracellular CO level was gradually increased. This result showed that **NIR-Ratio-CO** can be used for ratiometric imaging of endogenous CO produced by heme-stimulation in living cells.

[**Figure 5**]

Conclusions

In summary, a NIR ratiometric fluorescent probe **NIR-Ratio-CO** was successfully developed for the detection of CO in living cells based on the Pd-triggered Tsuji-Trost reaction. The probe system shows rapid, colorimetric and distinct ratiometric fluorescence responses at 655/592 nm for CO in aqueous solution with desirable properties including high selectivity, good sensitivity and a remarkable large Stokes

shift. In addition, **NIR-Ratio-CO** was proved to be capable of ratiometric fluorescent imaging of both exogenous and endogenous CO in living cells. Taken together, this work provided a valuable ratiometric NIR fluorescent probe for rapid detection of CO in living systems.

Acknowledgment

Financial support from the National Natural Science Foundation of China (21672080 and 21472066) and the Fundamental Research Funds for the Central Universities (CCNU19TS007) is gratefully acknowledged.

Appendix

Structure characterizations for the probe, data for study the sensing mechanism, and other data, please refer to supplementary data.

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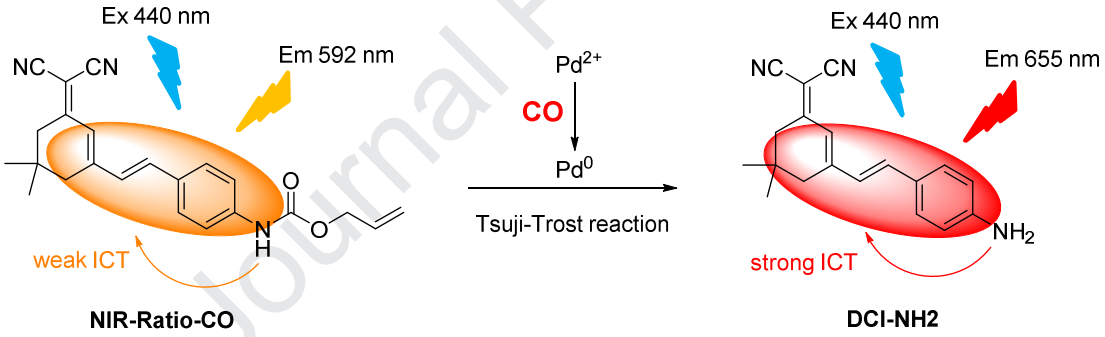
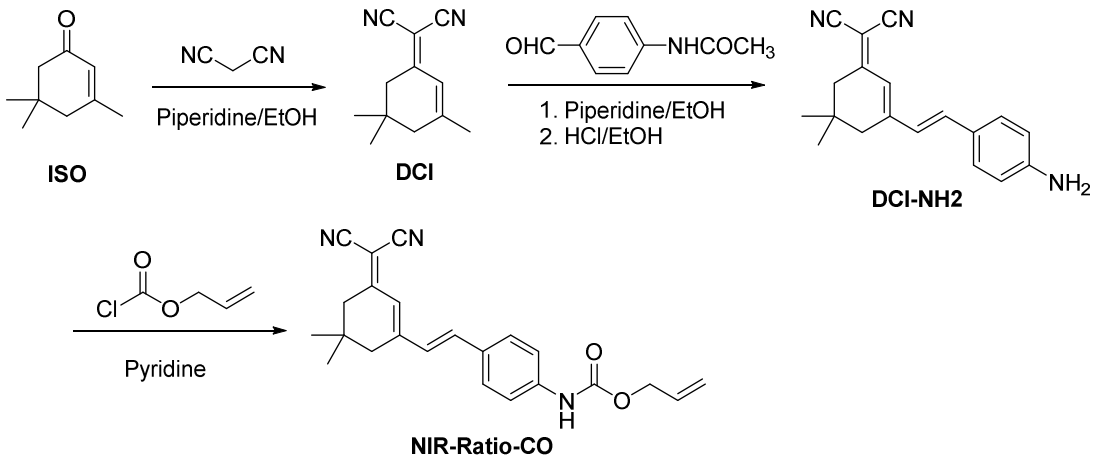


Scheme 1. NIR ratiometric fluorescent probe **NIR-Ratio-CO** for detection of CO.



Scheme 2. Synthesis route for **NIR-Ratio-CO**

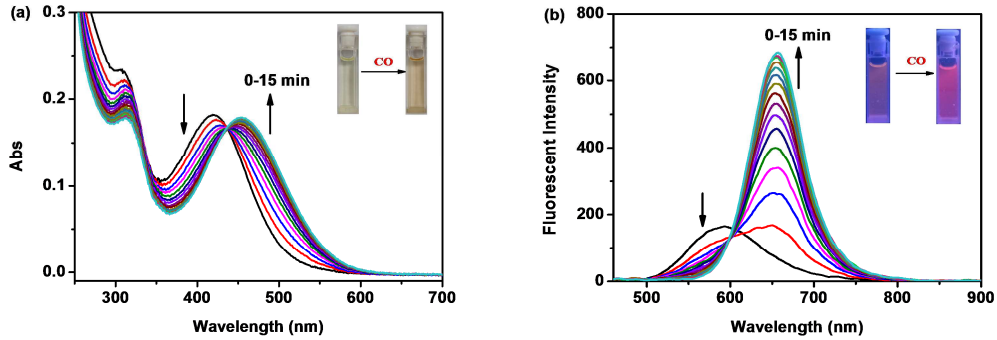


Figure 1. (a) UV-vis and (b) fluorescence spectral changes of the probe system (5 μM **NIR-Ratio-CO** + 10 μM $PdCl_2$) upon addition of CORM-3 (100 μM) in PBS buffer (10 mM, pH 7.4, 20% DMSO, v/v) at 37 □. For fluorescence measurement, λ_{ex} = 440 nm, slit width: d_{ex} = d_{em} = 10 nm. Inset: color changes and emission color changes under a portable 365 nm light.

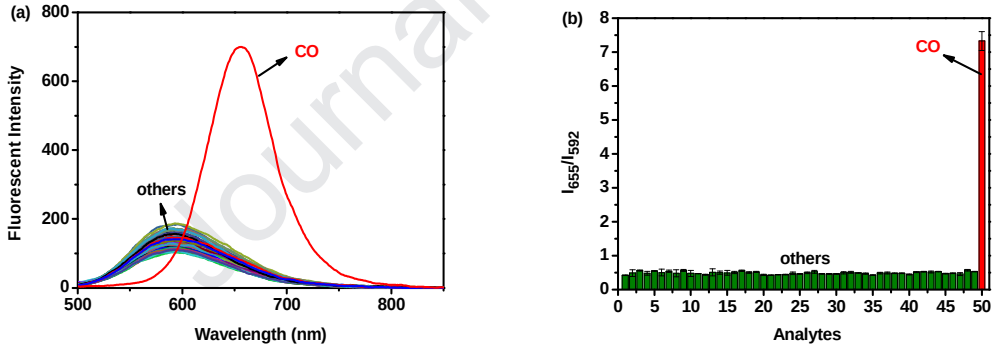


Figure 2. (a) Fluorescent spectra and (b) fluorescent intensity ratio (I_{655}/I_{592}) responses of the probe system (5 μM **NIR-Ratio-CO** + 10 μM $PdCl_2$) for various analytes (100 μM each, except 1 mM of GSH) including: (1) **NIR-Ratio-CO** itself, (2) **NIR-Ratio-CO** + Pd^{2+}, (3) F^-, (4) Cl^-, (5) Br^-, (6) I^-, (7) NO_3^-, (8) CO_3^{2-}, (9) HCO_3^-, (10) HSO_4^-, (11) SO_3^{2-}, (12) $S_2O_3^{2-}$, (13) HSO_3^-, (14) $S_2O_7^{2-}$, (15) $S_2O_8^{2-}$, (16) SCN^-, (17) Ac^-, (18) N_3^-, (19) $C_2O_4^{2-}$, (20) Cys, (21) Hcy, (22) GSH, (23) H_2S, (24) Met, (25) Thr, (26) Arg, (27) Tyr, (28) Ala, (29) Asp, (30) Lys, (31) Pyr, (32) Phe, (33) Glu, (34) Ile, (35) His, (36) Gly, (37) Val, (38) Leu, (39) Trp, (40) Ser, (41) H_2O_2, (42) ClO^-, (43) $NaNO_2$, (44) $^tBuOO^{\bullet}$, (45) $ROO^{\bullet}$, (46) HNO, (47) NO, (48) $O_2^{\bullet-}$, (49)

$ONOO^-$, (50) CO (CORM-3). All spectra were obtained 15 min after mixing in PBS buffer (10 mM, pH 7.4, 20 % DMSO, v/v) at 37 □, λ_{ex} = 440 nm, d_{ex} = d_{em} = 10 nm.

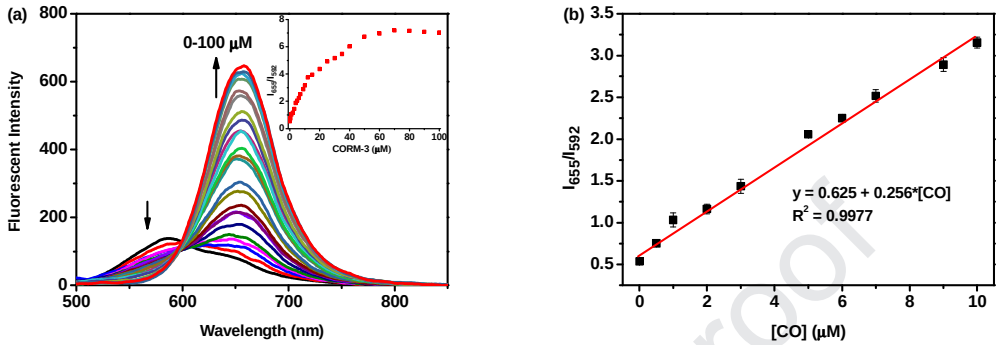


Figure 3. (a) Fluorescent spectral changes of the probe system (5 μM **NIR-Ratio-CO** + 10 μM $PdCl_2$) upon addition of different concentrations of CORM-3. Each spectrum was obtained 15 min after mixing. Inset: the saturation curve of the fluorescence intensity ratio changes. (b) Linear relationship (I = 0.625 + 0.256 × [CORM-3], R^2 = 0.9977) of the fluorescence intensity ratios (I_{655}/I_{592}) to CO concentrations from 0 to 10 μM. All data were obtained in PBS buffer (10 mM, pH = 7.4, with 20% DMSO, v/v) at 37 □. λ_{ex} = 440 nm, d_{ex} = d_{em} = 10 nm.

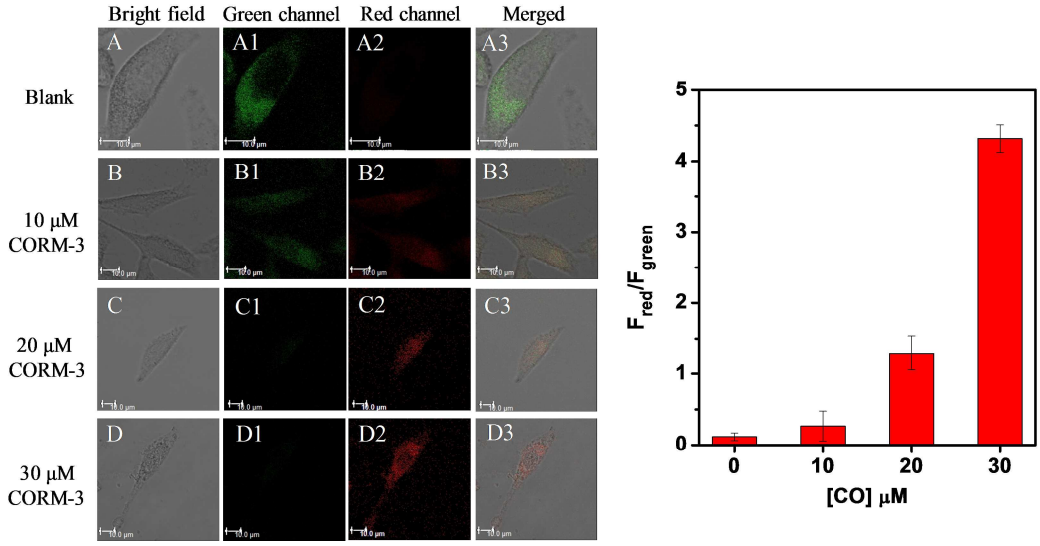


Figure 4. Confocal fluorescent imaging of CO in HeLa cells by the probe system (5 μM **NIR-Ratio-CO** + 10 μM $PdCl_2$). A, A1, A2 and A3: the cells were incubated with

the probe system for 30 min. B, B1, B2 and B3, C, C1, C2 and C3, and D, D1, D2 and D3: the cells were pre-incubated with 10, 20 and 30 μM of CORM-3 for 30 min, then with the probe system for 30 min, respectively. Scale bar = 10 μm. Green channel: λ_{ex} = 458 nm, λ_{em} was collected at 549-620 nm. Red channel: λ_{ex} = 458 nm, λ_{em} was collected at 620-721 nm. A histogram on the right shows the quantitative information of fluorescence ratio of the red channel and the green channel (F_{red}/F_{green}) upon incubation of different concentrations of CO.

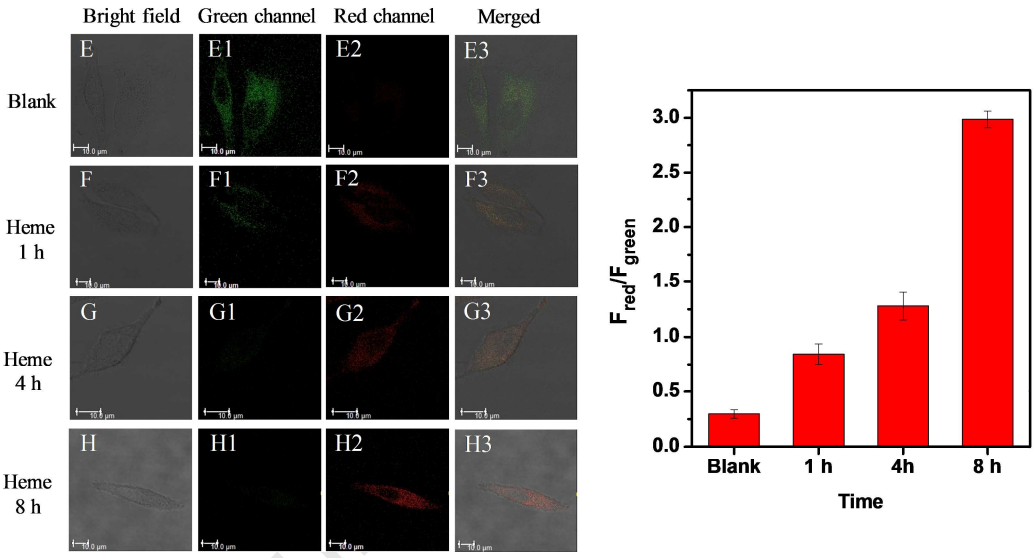


Figure 5. Confocal imaging of endogenous CO via heme stimulation in HeLa cells with the probe **NIR-Ratio-CO** system (5 μM probe + 10 μM $PdCl_2$). E-H: Bright field images. E1-H1: Green channel (λ_{ex} = 458 nm, λ_{em} = 549-620 nm). E2-H2: Red channel (λ_{ex} = 458 nm, λ_{em} = 620-721 nm). E3-H3: Merged images of green and red channel. The cells were pre-incubated with 100 μM heme for 1 h (F, F1, F2 and F3), 4 h (G, G1, G2 and G3), and 8 h (H, H1, H2 and H3), and then incubated with the probe system for 30 min, respectively. Scale bar = 10 μm. A histogram on the right shows the quantitative information of fluorescence ratio of the red channel and the green channel (F_{red}/F_{green}) upon incubation of heme at different times.

Highlights

1. A NIR ratiometric fluorescent probe for detection of CO was reported.
2. This probe shows enhanced fluorescence in the NIR region with a large Stokes shift.
3. This probe shows high selectivity and sensitivity for CO with a rapid response.
4. Imaging both exogenous and endogenous CO in living cells was successfully applied.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: