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A Readily Available Fluorescent Probe for Carbon Monoxide Imaging in Living Cells

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ABSTRACT:

Carbon monoxide (CO) is an important gasotransmitter in living systems and its fluorescent detection is of particular interest. However, fluorescent detection of CO in living cells is still challenging due to lack of effective probes. In this paper, a readily available fluorescein-based fluorescent probe was developed for rapid detection of CO. This probe can be used to detect CO in almost wholly aqueous solution under mild conditions, and shows high selectivity and sensitivity for CO with colorimetric and remarkable fluorescent turn-on signal changes. The detection limit of this probe for CO is as low as 37 nM with a linear range of 0-30 μM. More importantly, this probe (1 μM dose) can be conveniently used for fluorescent imaging CO in living cells.

INTRODUCTION

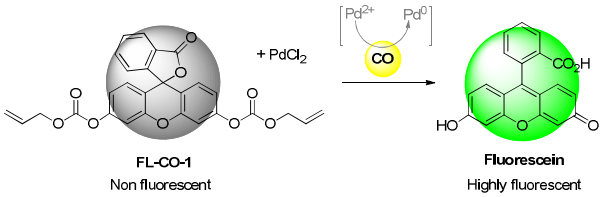
Although carbon monoxide (CO) is well known for its high toxicity and lethal effects in mammals,[1] this cannot change the fact that CO can be endogenously produced during the haem catabolism in the human body and other biological systems by heme oxygenase (HO) enzymes.[2] More importantly, it is now evident that endogenously produced CO is an important cell signaling molecule that involved in a vast number of physiological and pathological processes.[3] In addition, studies have shown that CO can be used as a potential therapeutic agent because of its reported antihypertensive, anti-inflammatory and cell-protective effects.[4] Accordingly, devising new ways to detect and sense this gas molecule in living systems is of great significance.[5-9]

Traditional methods for CO detection, including gas chromatography[10], chromogenic detection[11-14], and electrochemical assays[15], typically result in sample destruction and/or are limited to detect CO in living systems. Recently, fluorescence sensing and imaging with fluorescent probes has emerged as one of the most powerful techniques to detect biologically important molecules in living systems. However, development of fluorescent probes for monitoring CO at the cellular level still remains challenging.[16-21] Only until very recently, a significant breakthrough came from the seminal work of He et al.[22] and Chang et al.[23], who independently reported two different types of fluorescent CO probes for the first time of imaging of CO in living cells.[7] After that, several more probes have been developed and applied for cell-imaging of CO.[24-27] However, these probes are either complicated or difficult to

synthesize[22-25], or need to be excited by UV light[25], or need to use a high concentration of organic solvent[27]. Therefore, development of new fluorescent probes with improved properties and capable of tracking CO in living cells is highly desired.

Herein, we report a novel fluorescent probe system for visual and fluorescent detection of CO. This probe system uses an allyl chloroformate functionalized fluorescein (**FL-CO-1**) as the CO signaling molecule and $PdCl_2$ as an additive to trap CO (Scheme 1). We choose the fluorescein as a signaling unit because it is a well-known fluorophore with excellent photophysical properties such as high absorption coefficient, high fluorescence quantum yield (Φ = 0.92, 0.1 M NaOH), high photostability, and good water solubility.[28-31] In addition, fluorescein is commercially available and cheap. The strategy of this probe system relies on the in situ conversion of Pd^{2+} to Pd^0 by CO[25] and the well-known Pd^0-mediated Tsuji–Trost reaction to remove the allyl group[32-33]. Importantly, our studies showed that this probe system has the following merits: (1) it is readily available and can be used in almost wholly water solution; (2) it exhibits near-zero background fluorescence, but shows a rapid, colorimetric and remarkable fluorescent turn-on response for CO, which allows a convenient visual and sensitive fluorescent detection for CO; (3) it shows high selectivity and sensitivity for CO with a detection limit as low as 37 nM and it can be used in a low dosage (sub-micromolar level); (4) it can be conveniently used to image both exogenous and heme stimulation produced CO in living cells. As far as we know, this is the first time of showing a fluorescent probe capable of imaging heme stimulation produced CO in living cells.

Scheme 1. Detection of CO by a mixture of **FL-CO-1** and $PdCl_2$.



EXPERIMENTAL SECTION

Materials and Instrumentation. All reagents and chemicals were purchased from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer, and resonances (δ) are given in parts per million relative to tetramethylsilane (TMS). Coupling constants (J values) are reported in hertz. High-resolution mass spectrometry (HR-MS) spectra were obtained on a Bruker microTOF-Q instrument. UV-Vis and fluorescence spectra were recorded on an Agilent Cary-100 UV-Vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. HPLC spectra were recorded on an Agilent 1100 high performance liquid chromatography. Cell imaging was performed in an inverted fluorescence microscopy with a 20× objective lens.

Synthesis of the Probe. To a solution of fluorescein (332 mg, 1.0 mmol) in dry THF (10 mL) was added Et_3N (310 mg, 425 μL) under the atmosphere of nitrogen. After stirring for 10 min, the resulting red solution was cooled in an ice-bath, and then allyl chloroformate (1.04 g, 4 mmol) was slowly added over 10 min. During this time, the solution turned into yellow and the mixture was then stirred over night at room temperature. After filtration, the filtrate was concentrated under reduced pressure. The

crude product was purified by column chromatography (petroleum ether:ethyl acetate = 10:1, v/v) to afford two white solid products **FL-CO-1** and **FL-CO-2**.

FL-CO-1: 210 mg (yield 42%). Mp: 101-102.4 °C. TLC (silica plate): R_f~0.63 (petroleum ether:ethyl acetate 3:1, v/v). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, J = 5.9 Hz, 1H), 7.70 (t, J = 5.8 Hz, 1H), 7.65 (t, J = 5.9 Hz, 1H), 7.22 (s, 2H), 7.18 (d, J = 6.0 Hz, 1H), 6.94 (d, J = 6.8 Hz, 2H), 6.86 (d, J = 6.9 Hz, 2H), 6.00 (m, 2H), 5.45 (d, J = 13.7 Hz, 2H), 5.35 (d, J = 8.3 Hz, 2H), 4.76 (d, J = 4.4 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.29, 152.82, 152.78, 152.34, 151.51, 135.50, 130.79, 130.12, 129.12, 126.03, 125.29, 123.87, 120.03, 117.16, 116.53, 110.03, 81.29, 69.55. HR-MS (ESI): calcd for $C_{28}H_{21}O_9^+$ ($M + H^+$): 501.1180; found 501.1184. Elemental analysis calcd. (%) for $C_{28}H_{20}O_9$: C 67.20, H 4.03; found C 67.14, H 3.96.

FL-CO-2: 100 mg (yield 24%). Mp: >240 °C (decomposes). TLC (silica plate): R_f~0.40 (petroleum ether:ethyl acetate 3:1, v/v). ^{1}H NMR (500 MHz, d_6-DMSO) δ 10.25 (s, 1H), 8.04 (d, J = 6.1 Hz, 1H), 7.81 (t, J = 5.9 Hz, 1H), 7.74 (t, J = 5.9 Hz, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.34 (d, J = 6.1 Hz, 1H), 7.04 (dd, J = 6.9 and 1.6 Hz, 1H), 6.87 (d, J = 7.0 Hz, 1H), 6.76 (s, 1H), 6.63 (s, 2H), 6.00 (m, 1H), 5.42 (d, J = 13.8 Hz, 1H), 5.31 (d, J = 8.3 Hz, 1H), 4.75 (d, J = 4.3 Hz, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 169.01, 160.23, 152.76, 152.37, 152.01, 151.65, 136.26, 132.04, 130.79, 129.70, 129.61, 126.28, 125.30, 124.53, 119.53, 117.97, 117.45, 113.62, 110.41, 109.55, 102.77, 82.38, 69.50, 65.39. HR-MS (ESI): calcd for $C_{24}H_{17}O_7^+$ ($M + H^+$): 417.0969; found 417.0972. Elemental analysis calcd. (%) for $C_{24}H_{16}O_7$: C 69.23, H 3.87; found C 69.40, H 3.93.

Optical Studies. Stock solutions of **FL-CO-1** (1-2 mM), $PdCl_2$ (2-10 mM) and CORM-3 (10 mM) were separately prepared in HPLC grade DMSO and used freshly. Stock solutions (5-10 mM) of the analytes including NaF, NaCl, NaBr, NaI, NaN_3, Na_2SO_4, NaAcO, $NaHSO_3$, $NaHCO_3$, NaHS, KCN, n-butylamine, diethylamine, Cys, Hcy, GSH, Ala, Leu, Trp, Gly, Ile, and Lys were prepared in ultrapure water. ROS/RNS species such as ClO^-, H_2O_2, NO_2^-, NO, $ROO^\bullet$, $^tBuOO^\bullet$ and $^\bullet OH$ were prepared according our published procedure and used freshly.[34-35] For a typical optical study, a 3.0 mL solution containing **FL-CO-1** (5 μM) and $PdCl_2$ (5 μM) in PBS buffer (10 mM, pH 7.4, with 0.5% DMSO, v/v) was prepared in a quartz cuvette. The UV-Vis or fluorescent spectra were then recorded after addition of an analyte of interest at 37 °C (controlled by a temperature controller).

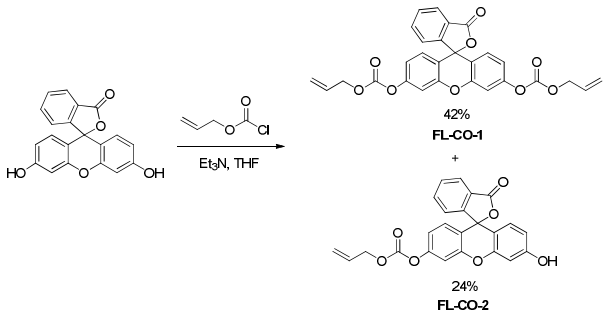
Imaging of CO in living cells. A549 human lung carcinoma cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100 μg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 °C, and then were seeded in a 24-well culture plate for one night before cell imaging experiments. In the experiment of cell imaging, as controls, living cells were incubated with **FL-CO-1** (1 μM) and a mixture of **FL-CO-1** (1 μM) and $PdCl_2$ (1 μM) at 37 °C for 30 min, respectively, and they were imaged after washing with PBS for three times. For imaging of exogenous CO, A549 cells were pre-treated with CORM-3 (1, 5, and 10 μM, respectively) for 30 min at 37 °C, and then were incubated with a mixture of **FL-CO-1** (1 μM) and $PdCl_2$ (1 μM) for 30 min at 37 °C. The imaging of CO was then carried out after washing the cells

with PBS buffer. For imaging of heme stimulation produced CO, the procedure is almost the same to that of imaging exogenous CO, but using heme (100 μM) instead of CORM-3 in the cell pre-treatment.

RESULTS AND DISCUSSION

Probe Synthesis. The probe **FL-CO-1** was prepared from the commercially available fluorescein and allyl chloroformate in dry THF under basic conditions (Scheme 2). The synthetic procedure is simple and the products can be isolated and purified by column chromatography to afford **FL-CO-1** (42%) as the major product and **FL-CO-2** (24%) as the minor product. The structure of these two products was proved by NMR, Mass and elemental analyses. Synthetic details and product characterizations can be found in the experimental section and the Supporting Information.

Scheme 2. The synthesis of **FL-CO-1** and **FL-CO-2**



Colorimetric and Fluorescent Detection of CO. To our delight, **FL-CO-1** exhibits good solubility in almost pure aqueous solution, as we can easily make clear solutions of **FL-CO-1** at concentrations of 1-10 μM in PBS buffer (10 mM, pH 7.4) with a very small amount of DMSO (<1%, v/v). Thus, in this work, almost pure PBS buffer (containing only 0.5% DMSO) was used as solvent to investigate the sensing

properties of **FL-CO-1**. As expected, **FL-CO-1** is non fluorescent (due to the protection of two hydroxyl groups of fluorescein) and not responsive to Pd^{2+} ($PdCl_2$ was used). However, **FL-CO-1** showed significant fluorescence turn-on responses for Pd^0 upon addition of $Pd(PPh_3)_4$ (Figure S1, Supporting Information). This selective response of **FL-CO-1** for Pd^0 over Pd^{2+} is consistent with that of recently reported allyl chloroformate functionalized probes for Pd^0.[36-37] Since Pd^{2+} can be readily reduced by CO to generate Pd^0 in-situ,[25-27] **FL-CO-1** was expected to be a potential probe for detection of CO.

The response of **FL-CO-1** for CO was then tested in the presence of $PdCl_2$. Commercially available $[Ru(CO)_3Cl(glycinate)]$ (CORM-3), a water soluble CO-releasing molecule, was used in these studies as an easy-to-handle CO source.[22-27] As shown in Figure 1a, upon addition of 50 μM of CORM-3, the probe (**FL-CO-1** + $PdCl_2$, 5 μM each) in PBS buffer (10 mM, pH 7.4, with 0.5% DMSO, v/v) showed significant time-dependent fluorescent turn-on changes in the fluorescence spectra. Remarkably, the fluorescence intensity of the probe solution at 520 nm increased over 100-fold within 15 min and as a result, the solution started to emit strong yellow-green fluorescence (inserted in Figure1a). Fluorescent kinetics of the probe (**FL-CO-1** + $PdCl_2$) upon addition of CO showed that the fluorescence intensity at 520 nm was gradually increased, which is in sharp contrast to that of **FL-CO-1** itself against time and the response of **FL-CO-1** to CO in the absence of $PdCl_2$ (Figure 1b). Meanwhile, in the UV-Vis spectra, a peak at 490 nm showed a significant increase, which accompanied by distinct color changes from colorless to bright yellow (Figure

S2, Supporting Information). Clearly, these results indicate that the probe (**FL-CO-1** + $PdCl_2$) can be used as a visual and fluorescent turn-on probe for rapid detection of CO with remarkable fluorescence enhancement in aqueous solution under mild conditions. Although **FL-CO-2** also showed some potential for fluorescent detection of CO, it was found that **FL-CO-2** showed less fluorescence enhancement for CO (~20-fold at 15 min) due to its more background fluorescence and much less stability in comparison to **FL-CO-1** (Figure S3, Supporting Information). Therefore, **FL-CO-2** was not further studied and we concentrated on the sensing properties of **FL-CO-1** in the following studies.

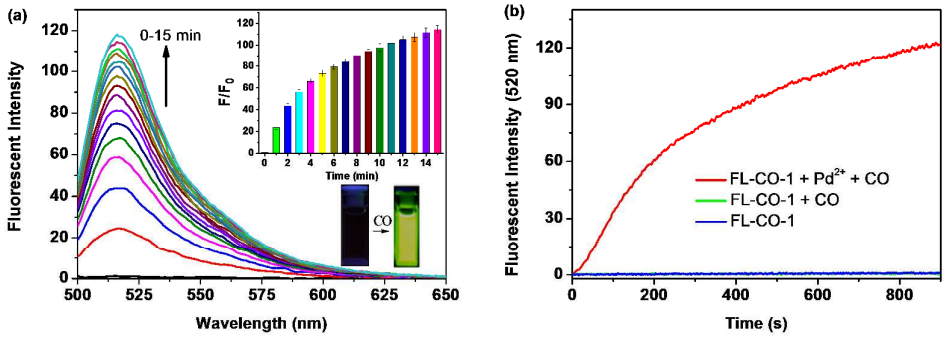


Figure 1. (a) Fluorescence spectra changes of the probe (5 μM **FL-CO-1** + 5 μM $PdCl_2$) upon addition of CORM-3 (50 μM) in PBS buffer (10 mM, pH 7.4, with 0.5% DMSO, v/v) at 37 °C. The spectra were recorded per min. Insert: fluorescent intensity ratio (F/F_0) changes at 520 nm as a function of time (the data were reported as the mean ± standard deviation of triplicate experiments). An emission color change under a light of 365 nm was also inserted. (b) Fluorescent stability of **FL-CO-1** (5 μM) against time and fluorescent kinetics of **FL-CO-1** (5 μM) for CORM-3 (50 μM) in the

absence and presence of $PdCl_2$. The data were recorded at 520 nm per second. For all spectra measurement, λ_{ex} = 490 nm, slit width: $d_{ex} = d_{em}$ = 2.5 nm.

To find out the best amount of $PdCl_2$ for CO detection, different concentration of $PdCl_2$ were added to the probe solution. As shown in Figure S4a (Supporting Information), although increasing the amount of $PdCl_2$ was found more favorable for fluorescence signal changes, fluorescence enhancement becomes slower after addition of one equivalent of $PdCl_2$. Thus, a 1:1 ratio of **FL-CO-1** and $PdCl_2$ was used in the following studies because this ratio is good enough for detection of CO with a remarkable fluorescent readout. The pH effect was also investigated (Figure S4b, Supporting Information). It was found that the probe system itself was stable at pH values of 2–10, whereas significant fluorescence enhancements can be observed at pH 6–10 in the presence of CO. This indicates that this probe system can be used over a wide pH range including a physiological relevant pH, which is highly favorable for its biological applications.

To understand the sensing mechanism of the probe system (**FL-CO-1** + $PdCl_2$) for CO, Mass spectrometry, TLC and HPLC analyses of the reaction mixture were performed (Figure S5-7, Supporting Information). Mass analysis of the reaction mixture at 5 min showed that the reaction produced **FL-CO-2** and fluorescein (Figure S5, Supporting Information). The results of TLC and HPLC analyses at different reaction times indicated that the reaction initially generated **FL-CO-2** as an intermediate, but eventually transformed to fluorescein as the final product (Figure S6-7, Supporting Information). Combing these analyses and the optical changes

shown above, the sensing mechanism of the probe system for CO is most likely the process as proposed in Scheme S1 (Supporting Information). In this process, Pd^{2+} was first reduced by CO to generate Pd^0, which subsequently mediated the Tsuji–Trost reaction to release **FL-CO-2** (intermediate) and the highly fluorescent fluorescein (final product), thus producing the observed optical changes.

The Selectivity. The selectivity of this new CO-detection system (**FL-CO-1** + $PdCl_2$) was then investigated. Various analytes including common anions such as F^-, Cl^-, Br^-, I^-, N_3^-, SO_4^{2-}, AcO^-, HCO_3^-, HSO_3^-, HS^-, and CN^-, primary and secondary amines such as n-butylamine and diethylamine, amino acids such as Ala, Leu, Trp, Gly, Ile, and Lys, biothiols such as Cys, Hcy, and GSH, reactive oxygen/nitrogen species (ROS/RNS) such as ClO^-, H_2O_2, NO_2^-, NO, $ROO^{\bullet}$, $^tBuOO^{\bullet}$ and $^{\bullet}OH$, as well as CORM-3 were investigated in parallel under the same test conditions using probe **FL-CO-1** and **FL-CO-1** in the presence of $PdCl_2$ as blank. As shown in Figure 2, among these analytes, only addition of CO induced the probe solution a significant fluorescence enhancement, while the other analytes showed almost no effect. These results clearly indicate that this probe system has a high selectivity for CO. Moreover, this highly selective response for CO can be conveniently observed by the naked eye (Figure S8, Supporting Information).

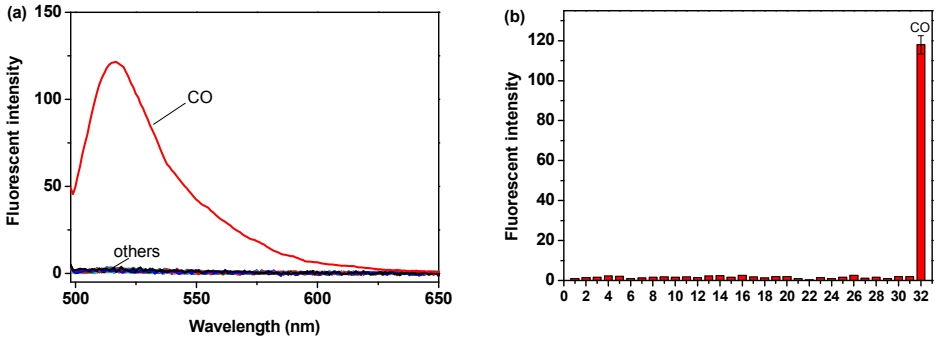


Figure 2. (a) Fluorescent spectra and (b) fluorescent intensity responses of the probe system (**FL-CO-1** + $PdCl_2$, 5 μM each) at 520 nm for various analytes (100 μM, except 50 μM of CORM-3). Numbers 1-29 in (b) represent: 1. **FL-CO-1**, 2. **FL-CO-1** + Pd^{2+}, 3. F^-, 4. Cl^-, 5. Br^-, 6. I^-, 7. N_3^-, 8. SO_4^{2-}, 9. AcO^-, 10. HS^-, 11. HSO_3^-, 12. HCO_3^-, 13. Cys, 14. Hcy, 15. GSH, 16. ClO^-, 17. CN^-, 18. n-butylamine, 19. diethylamine, 20. Ala, 21. Leu, 22. Trp, 23. Gly, 24.Ile, 25. Lys, 26. H_2O_2, 27. NO, 28. NO_2^-, 29. $ROO^{\bullet}$, 30. ^{t}BuOO$^{\bullet}$, 31. $^{\bullet}$OH, 32. CO. All spectra were monitored 15 min after mixing in PBS buffer (10 mM, pH 7.4) at 37 °C. λ_{ex} = 490 nm, d_{ex} = d_{em} = 2.5 nm.

The Sensitivity. Figure 3 shows the concentration-dependent fluorescence spectra changes of the probe system (**FL-CO-1** + $PdCl_2$) for CO. One can see that, upon progressive increasing of the concentration of CORM-3, the probe solution showed a progressive increase in fluorescence intensity at 520 nm until gradually close to saturation (Figure 3a). In addition, a satisfactory linear relationship between the fluorescence intensity changes at 520 nm and the CO concentrations in the range of 0-30 μM can be observed with a correlation coefficient as high as 0.99856 (Figure 3b). The detection limit of the probe system for CO was determined to be about 37 nM

(based on signal-to-noise ratio S/N = 3), indicating that the probe system is highly sensitive for CO.

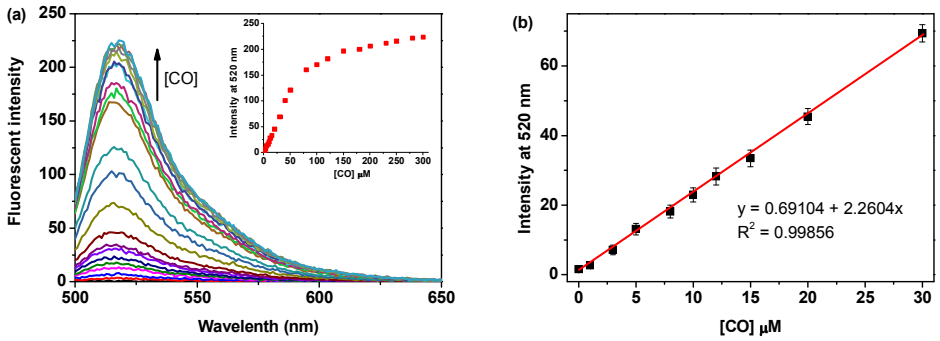


Figure 3. (a) Fluorescent spectra changes of the probe system (**FL-CO-1** + $PdCl_2$, 5 μM each) upon addition of different concentrations of CORM-3 in PBS buffer (10 mM, pH 7.4, with 0.5% DMSO, v/v) at 37 °C. Insert: fluorescence intensity changes of the probe system at 520 nm as a function of the concentrations of CORM-3. Each data was obtained 15 min after mixing. λ_{ex} = 490 nm, slit width: d_{ex} = d_{em} = 2.5 nm. (b) A linear relationship of fluorescence intensity changes of the probe system at 520 nm against the concentration of CORM-3 from 0 to 30 μM. The data were reported as the mean ± standard deviation of triplicate experiments and fitted (red line) by the equation: y = 0.69104 + 2.2604×[CO] with R^2 = 0.99856.

Considering the potential toxicity of palladium ions, it should be more preferable if we can use the probe in a lower dose. Thus, the dose of the probe was gradually decreased to see whether fluorescent detection of CO can be still achieved. As shown in Figure S9 (Supporting Information), upon reducing the probe dose from 1 μM to 0.05 μM, significant fluorescent enhancements were all observed after addition of 10 equiv. of CO to the probe solution. Notably, even the dose of the probe system was

reduced to as low as 0.05 μM, addition of 0.5 μM of CORM-3 (~0.014 ppm CO) can still induce remarkable fluorescence enhancement within 15 min, indicating that the probe system can be used in a very low dosage to detect CO efficiently, which should be very useful for its practical applications.

Imaging of CO in Living Cells. Based on the inspiring results shown above, we investigated the potential applications of **FL-CO-1** for imaging of CO in living cells. The cytotoxicity of **FL-CO-1** and **FL-CO-1** + $PdCl_2$ was firstly explored by MTT assays, and the results indicate that both **FL-CO-1** (up to 50 μM) and the **FL-CO-1** + $PdCl_2$ system showed very low cytotoxicity to living cells (Figure S10, Supporting Information). Even so, the high sensitivity of the probe inspired us to use a low dose probe for fluorescent imaging of CO in living cells. As shown in Figure 4, when A549 human lung carcinoma cells were incubated with **FL-CO-1** (1 μM) or the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each) for 30 min, no fluorescence was observed (A1 and B1). However, when the cell culture was pre-incubated CORM-3, then incubated with the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each), a dose-dependent intracellular fluorescence was observed (C1-E1). It was reported that purging CO gas into water (at 1 atm) can give a CO stock solution with an estimated concentration of 1 mM.[22] Such a CO stock solution was made (by bubbling CO gas gently into water for 60 min) as another CO source instead of CORM-3. As shown in Figure S11 (Supporting Information), preliminary experiments showed that addition of this solution with a final CO concentration of ~50 μM to the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each) resulted in significant increase in fluorescence, indicating the probe system is indeed

effective for CO detection. In addition, when the cell culture was pre-incubated with this CO gas made solution, and then incubated with the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each), a dose-dependent intracellular fluorescence can be also observed (Figure S11, Supporting Information). All these results clearly indicate that **FL-CO-1** can be used as an effective probe candidate for monitoring changes in intracellular CO.

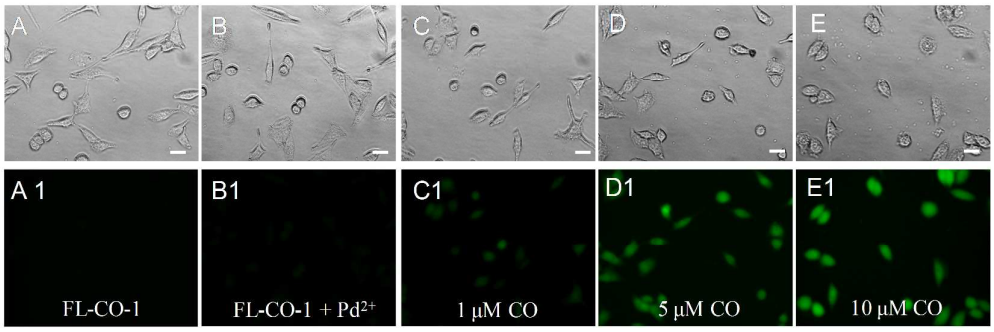


Figure 4. Fluorescent imaging of CO in A549 cells by the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each). Top row A-E: bright field images. Bottom row A1-E1: fluorescent images of A-E, respectively, with excitation wavelength at 450-480 nm. A and A1: The cells were incubated with **FL-CO-1** (1 μM) for 30 min. B and B1: The cells were incubated with the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each) for 30 min. C and C1, D and D1, and E and E1: cells were pre-incubated with 1, 5 and 10 μM of CORM-3 for 30 min, then with the probe system for 30 min, respectively. Scale bar = 20 μm.

The above results also hint that the concentration of endogenous CO is very low. Considering that CO is naturally produced by the action of heme oxygenase (HO) on the heme from hemoglobin breakdown,[2] and it was reported that the heme oxygenase activity can be increased by heme treatment and heme can contribute to produce more

HO-derived CO,[38] heme was added to living cells to stimulate more CO in living cells. As shown in Figure 5, when the cells were pre-incubated with heme (100 μM) for 0.5 h prior to incubation with the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each), we observed obvious green fluorescence inside the cells (F1), which is clearly different to that of cells incubated only with the probe system (B1 in Figure 4). In addition, the fluorescence insider the cells showed time dependent enhancement (F1 to H1), indicating more CO was produced over time. These results showed that the probe system has great potential to image heme stimulation produced CO in living cells.

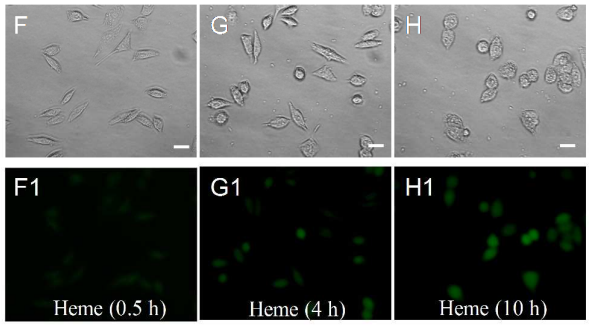


Figure 5. Fluorescent imaging of CO produced via heme stimulation in A549 cells using the probe system (**FL-CO-1** + $PdCl_2$). Top row F-H and bottom row F1-H1 are bright field images and the corresponding fluorescent images, respectively, with excitation wavelength at 450-480 nm. The cells were pre-incubated with 100 μM of heme for 0.5 h (F and F1), 4 h (G and G1) and 10 h (H and H1), then with the probe system (**FL-CO-1** + $PdCl_2$, 1 μM each) for 30 min, respectively. Scale bar = 20 μm.

CONCLUSION

In summary, we developed a new fluorescent probe system for CO. This probe system is readily available and able to detect CO in almost wholly water solution under mild conditions. More importantly, this probe system shows high selectivity

and sensitivity for CO with rapid and distinct colorimetric and fluorescent turn-on responses, and can be used in a very low dosage to image CO in living cells. Based on these excellent sensing properties, this probe system can be appreciated as an invaluable research tool candidate for detection of CO in living systems.

ASSOCIATED CONTENT

Supporting Information.

Structure characterizations for the probe, data for investigation of the sensing mechanism, and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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