† Electronic Supplementary Information (ESI)

Development of a colorimetric and NIR fluorescent dual probe for carbon monoxide

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Materials and General Information

All solvents and reagents (analytical grade) were obtained commercially and used as received unless otherwise mentioned. Column and layer chromatographic silica gel were purchased from Qingdao Haiyang Chemical Co., Ltd. Flash column chromatography was performed with silica gel (200-300 mesh). CORM-3 [Ru(CO)$_3$Cl(glycinate)] was purchased from Selleck Co., Ltd. $^1$H and $^{13}$C NMR spectra were recorded in CDCl$_3$ with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively, using TMS as the internal standard. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI-ACPI mass selective detector, and high-resolution mass spectra (HRMS) were recorded on a Shimadzu LCMS-IT-TOF. HPLC was recorded on Agilent1200 with C18 column (5μ, 250×4.6mm) in the gradient method using methanol and water eluent. All chemicals were purchased from commercial sources unless otherwise specified. All the solvents were of analytical reagent grade and were used without further purification.

The UV-vis absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan), using quartz cell of 10.0 mm pathlength. Fluorescence measurements were performed on an FL-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with quartz cell of 10.0 mm pathlength. Unless otherwise noted, the spectra were measured in PBS buffer solution after the mixtures were equilibrated at room temperature. The cells were imaged using a LSM 710 laser scanning confocal microscope (Carl Zeiss, Germany).

General procedure of spectral Measurements

The stock solutions of NF-APC, Na$_2$PdCl$_4$ and Pd(PPh$_3$)$_4$ (10 mM) were prepared by dissolving the required amount in DMSO. CORM-3 stock solutions (10 mM) were prepared in DMSO according to the instructions of the provider. The fresh CORM-3 solution used as CO source was prepared by diluting CORM-3 stock solutions with distilled water. The stock solutions of relevant analytes (H$_2$O$_2$, NaClO, NaHSO$_3$, Na$_2$SO$_3$, Hcy and GSH) were prepared in distilled water. The stock solutions of relevant analytes (KO$_2$ and Cys) were prepared in DMSO. The stock solution of H$_2$S was prepared in Na$_2$S solution. NO solution was prepared by dissolving sodium nitroprussiate dehydrate in distilled water.

Absorption and fluorescence titrations were performed by adding samples into a quartz cell (10.0 mm pathlength). The fluorescence intensity was measured at the excitation wavelength of 620 nm. The excitation and emission slit width were both 10 nm.

General procedure of MTT assay

The MTT assay was used to measure the cytotoxicity of NF-APC, CORM-3 and Na$_2$PdCl$_4$ to HeLa and 293T cells. Cells were seeded into a 96-well cell-culture plate. Various concentrations of NF-APC, CORM-3 and Na$_2$PdCl$_4$ were added to the wells. The cells were incubated at 37 °C under 5% CO$_2$ for 48 h. An aliquot of 10 μL MTT (5 mg mL$^{-1}$) was added to each well and incubated at 37 °C under 5% CO$_2$ for 4 h. The MTT
solution was removed and the yellow precipitate (formazan) observed in the plates was dissolved in 100 \( \mu L \) DMSO. Microplate reader was used to measure the absorbance at 570 nm for each well. The viability of cells was calculated according to the following equation: 

\[
\text{Cell viability} = \frac{A_{570\text{(sample)}}}{A_{570\text{(control)}}}
\]

**General procedure of cell imaging**

HeLa cells were grown in DMEM media containing 10% fetal bovine serum, 100 U/mL penicillin and 100 \( \mu g/mL \) streptomycin at 37 °C in 5% CO\(_2\) atmosphere. The cells were seeded on a Ø 30 mm glass-bottomed dish at the density of 1\( \times 10^5 \) cells in a culture medium and incubated overnight for living cell imaging by confocal laser scanning microscopy (CLSM). The HeLa cells were incubated with CORM-3 (50 \( \mu M \)) for 30 min at 37 °C and washed three times with PBS, then treated with Na\(_2\)PdCl\(_4\) (20 \( \mu M \)), Hochest 33342 (10 \( \mu M \)) and NF-APC (10 \( \mu M \)), incubated for 30 min at 37 °C and washed three times with PBS before imaging by CLSM. The cells were imaged with a 40× objective lens. The excitation wavelengths were 405 nm for Hochest 33342 and 633 nm for NF-APC, respectively.

**Synthesis and Characterization**

**Scheme S1. Synthesis of NF-APC**

![Chemical structure](image)

* Reagents and conditions: (a) THF, Et\(_3\)N, RT, overnight.

**Synthesis of diallyl (3'-oxo-3'H-spiro[dibenzo[c,h]xanthene-7,1'-isobenzofuran]-3,11-diyl) dicarbonate (NF-APC).** Allyl chloroformate (0.144g, 1.2 mmol) was added to a solution of naphthofluorescein (0.172 g, 0.4 mmol) and triethylamine (0.120 g, 1.2 mmol) in 8 mL THF. The reaction mixture was stirred overnight at room temperature, and water and CH\(_2\)Cl\(_2\) were added to the resulting solution. The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography to afford NF-APC (0.073 g, 30.2%) as a white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta 8.79 \) (d, \( J = 9.1 \) Hz, 2H), 8.17-8.12 (m, 1H), 7.71 (d, \( J = 2.3 \) Hz, 2H), 7.70-7.66 (m, 2H), 7.59 (dd, \( J = 9.1, 2.3 \) Hz, 2H), 7.51 (d, \( J = 8.7 \) Hz, 2H), 7.14-7.10 (m, 1H), 6.90 (d, \( J = 8.7 \) Hz, 2H), 6.13-6.01 (m, 2H), 5.51 (ddd, \( J = 17.2, 2.8, 1.4 \) Hz, 2H), 5.40 (ddt, \( J = 10.4, 2.3, 1.1 \) Hz, 2H), 4.83 (dt, \( J = 5.9, 1.3 \) Hz, 4H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta 169.51, 153.96, 153.27, 150.45, 146.32, 135.32, 135.01, 131.03, 130.00, 126.20, 125.21, 124.88, 124.05, 124.02, 123.73, 121.95, 121.33, 119.74, 118.22, 112.57, 82.86, 69.39. ESI-MS m/z: 601.2 [M+H]\(^+\). Purity: 99.06% by HPLC. HRMS (ESI): calcd for (M+H)\(^+\) (C\(_{36}\)H\(_{25}\)O\(_9\)) 601.1493, found 601.1481.
$^{1}H$ NMR, $^{13}C$ NMR, MS, HRMS and HPLC spectra of compound NF-APC.

Fig. S1 $^{1}H$ NMR spectrum of NF-APC
Fig. S2 $^{13}$C NMR spectrum of NF-APC
Fig. S3 MS spectrum of NF-APC
Fig. S4 HRMS spectrum of NF-APC
HPLC spectrum of NF-APC

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**Area Percent Report**

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Sorted By : Signal
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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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Totals : 8.33426e4 2174.56022

**Fig. S5** HPLC spectrum of NF-APC
Fig. S6 UV-vis (A) and fluorescence spectra (B) of NF-APC (10 μM) alone, and in the presence of a Pd²⁺ source (Na₂PdCl₄, 60 μM) or a Pd⁰ source (Pd(PPh₃)₄, 60 μM), respectively. Conditions: DMSO-PBS buffer (4:6, v/v), pH 7.4, λₑx = 620 nm. The spectra were recorded after incubation for 45 min.

Fig. S7 Changes of absorbance at 620 nm (A) and emission intensity at 670 nm (B) of NF-APC against time. Conditions: [NF-APC] = 10 μM; [Na₂PdCl₄] = 60 μM; [CORM-3] = 100 μM; DMSO-PBS buffer (4:6, v/v), pH 7.4.
Fig. S8 UV-vis (A) and fluorescence spectra (B) of Naphthofluorescein (10 μM) from commercial source and NF-APC (10 μM) with Na₂PdCl₄ (60 μM) and CORM-3 (100 μM). Conditions: DMSO-PBS buffer (4:6, v/v), pH 7.4, λ<sub>ex</sub> = 620 nm.
Fig. S9 HRMS spectrum of the reaction product of NF-APC incubated with Na$_2$PdCl$_4$ and CORM-3 for 45 min in 25 °C. Conditions: [NF-APC] = 10 μM; [Na$_2$PdCl$_4$] = 60 μM; [CORM-3] = 100 μM; DMSO-PBS buffer (4:6, v/v), pH 7.4.
Fig. S10 The effect of pH on absorbance at 620 nm (A) and emission intensity at 670 nm (B) of NF-APC alone or with Na$_2$PdCl$_4$ (60 μM) and CORM-3 (100 μM). Conditions: DMSO-PBS buffer (4:6, v/v), pH 7.4, $\lambda_{ex} = 620$ nm. The spectra were recorded after incubation for 45 min.
Changes of absorbance at 620 nm (A) and emission intensity at 670 nm (B) of NF-APC against concentration of CORM-3. Conditions: [NF-APC] = 10 μM; [Na$_2$PdCl$_4$] = 60 μM; DMSO-PBS buffer (4:6, v/v), pH 7.4, $\lambda_{ex} = 620$ nm.
Fig. S12 Absorbance at 620 nm (A) and emission intensity at 670 nm (B) of NF-APC (10 μM) as a function of the concentration of CORM-3 in DMSO-PBS buffer (4:6, v/v) at 25 °C.
Fig. S13 The photostability study of NF-APC. Emission intensity at 670 nm of NF-APC with (red) or without (black) CO was measured by continuous irradiation with a Xe lamp at 10 nm slit width at excitation wavelength 620 nm. Conditions: [NF-APC] = 10 μM; [Na2PdCl4] = 60 μM; DMSO-PBS buffer (4:6, v/v), pH 7.4, λex = 620 nm.
Fig. S14 MTT assay of HeLa (A, C, E and G) and 293T (B, D, F and H) cells incubated with NF-APC (0-100 μM), CORM-3 (0-200 μM), Na₂PdCl₄ (0-200 μM) and the mixture of NF-APC (10-20 μM) and Na₂PdCl₄ (20-120 μM) for 48 h,
respectively.