

† 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)₃Cl(glycinate)] was purchased from Selleck Co., Ltd. ¹H and ¹³C NMR spectra were recorded in CDCl₃ 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**, Na₂PdCl₄ and Pd(PPh₃)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 (H₂O₂, NaClO, NaHSO₃, Na₂SO₃, Hcy and GSH) were prepared in distilled water. The stock solutions of relevant analytes (KO₂ and Cys) were prepared in DMSO. The stock solution of H₂S was prepared in Na₂S 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 Na₂PdCl₄ to HeLa and 293T cells. Cells were seeded into a 96-well cell-culture plate. Various concentrations of **NF-APC**, CORM-3 and Na₂PdCl₄ were added to the wells. The cells were incubated at 37 °C under 5% CO₂ 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% CO₂ for 4 h. The MTT

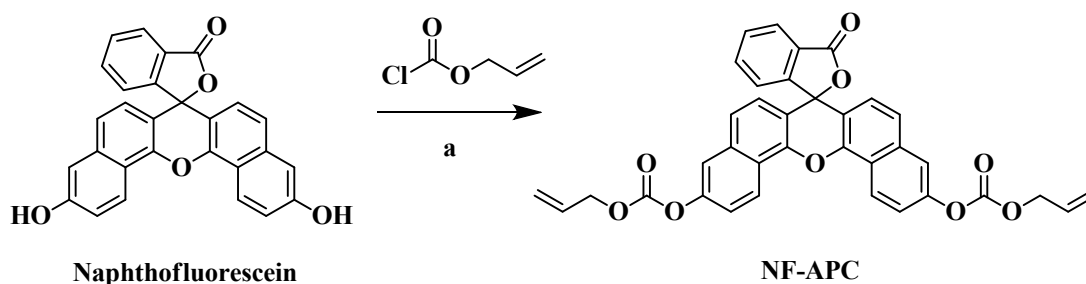
solution was removed and the yellow precipitate (formazan) observed in the plates was dissolved in 100 μ L DMSO. Microplate reader was used to measure the absorbance at 570 nm for each well. The viability of cells was calculated according to the following equation: Cell viability = $A_{570(\text{sample})}/A_{570(\text{control})}$

General procedure of cell imaging

HeLa cells were grown in DMEM media containing 10% fetal bovine serum, 100 U/mL penicillin and 100 μ g/mL streptomycin at 37 °C in 5% CO₂ atmosphere. The cells were seeded on a Ø 30 mm glass-bottomed dish at the density of 1×10^5 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 μ M) for 30 min at 37 °C and washed three times with PBS, then treated with Na₂PdCl₄ (20 μ M), Hoechst 33342 (10 μ M) and NF-APC (10 μ 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 \times objective lens. The excitation wavelengths were 405 nm for Hoechst 33342 and 633 nm for NF-APC, respectively.

Synthesis and Characterization

Scheme S1. Synthesis of NF-APC*



* Reagents and conditions: (a) THF, Et₃N, 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 CH₂Cl₂ 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. ¹H NMR (400 MHz, CDCl₃) δ 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 (ddd, J = 10.4, 2.3, 1.1 Hz, 2H), 4.83 (dt, J = 5.9, 1.3 Hz, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 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)⁺ (C₃₆H₂₅O₉)⁺ 601.1493, found 601.1481.

^1H NMR, ^{13}C NMR, MS, HRMS and HPLC spectra of compound NF-APC.

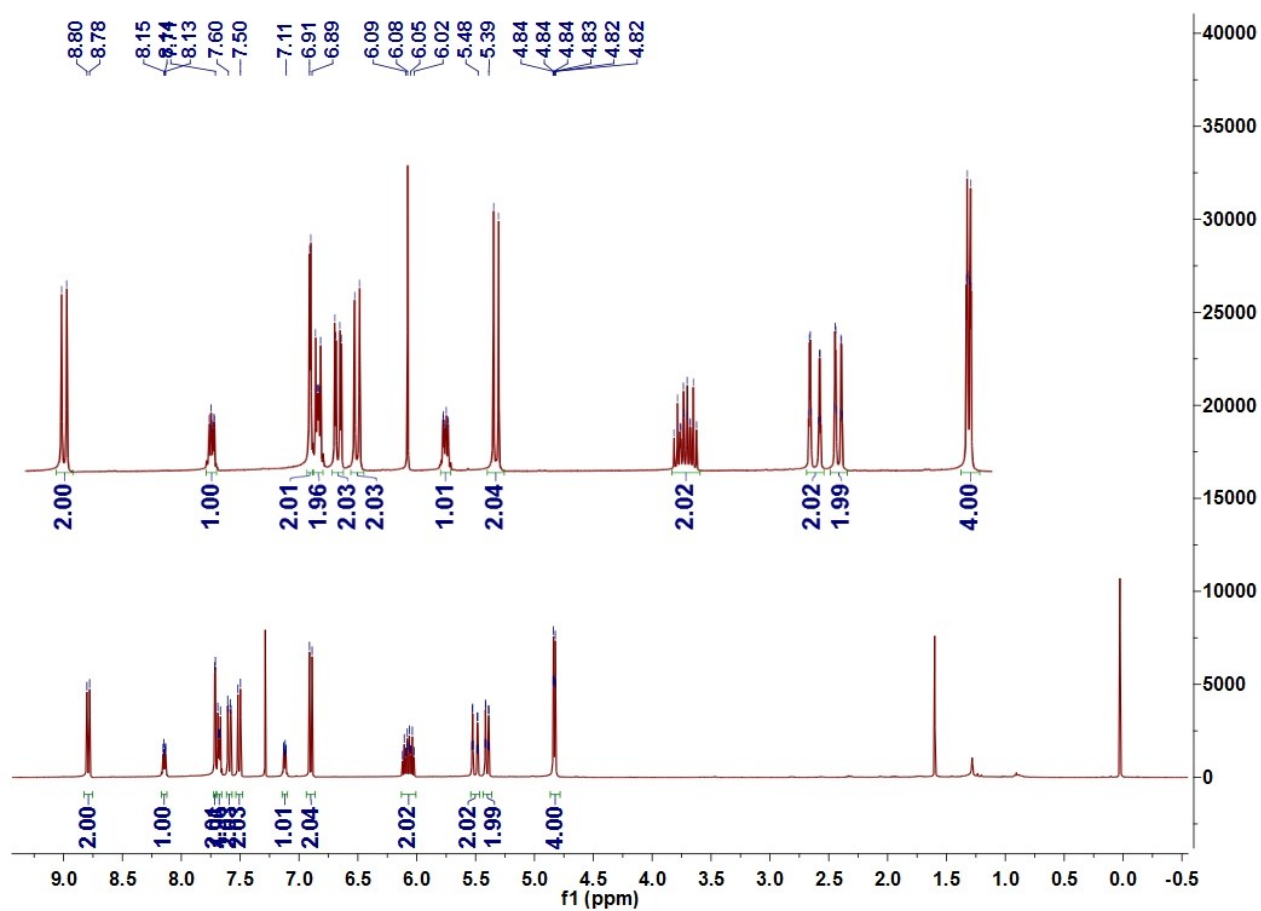


Fig. S1 ^1H NMR spectrum of NF-APC

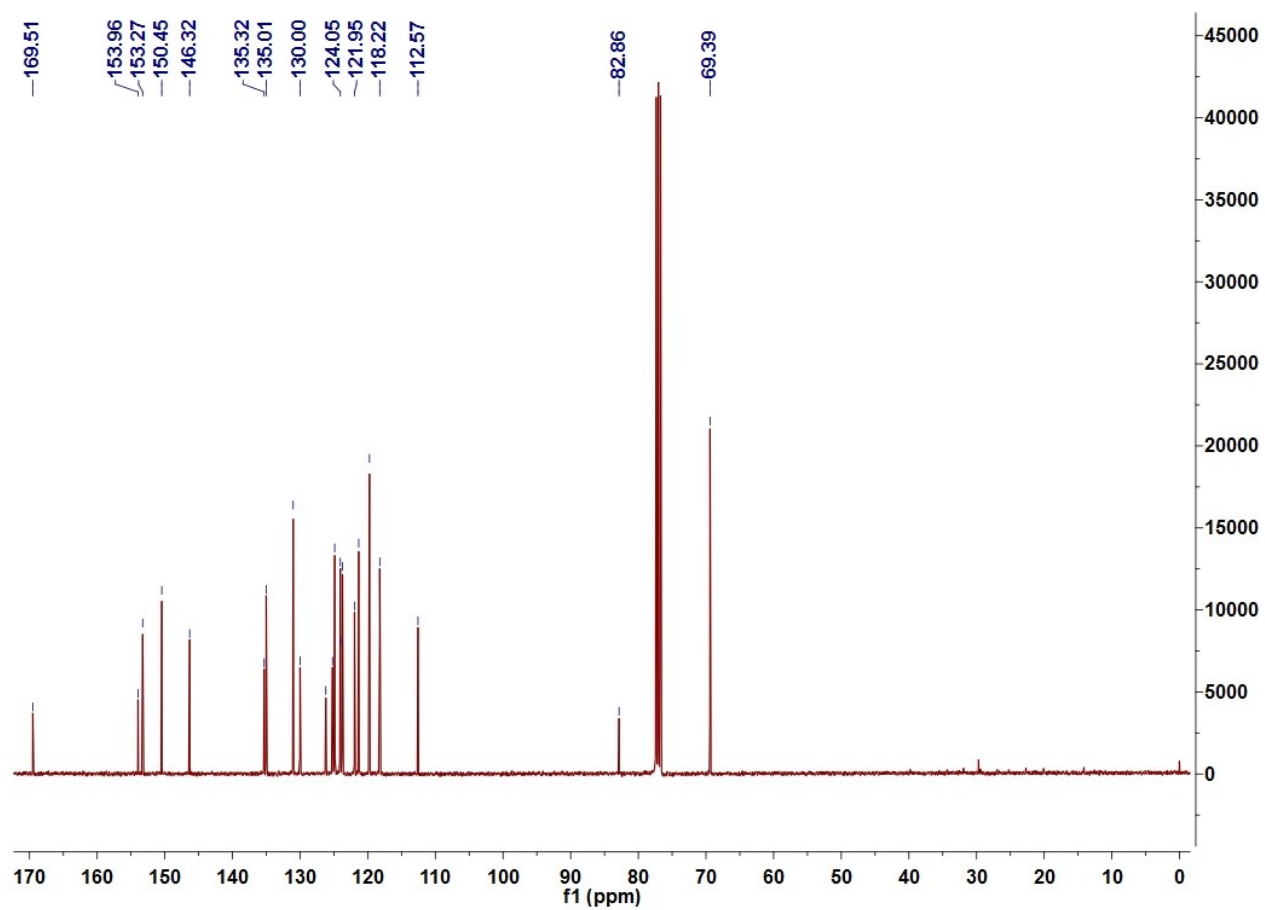


Fig. S2 ^{13}C NMR spectrum of NF-APC

MS Spectrum

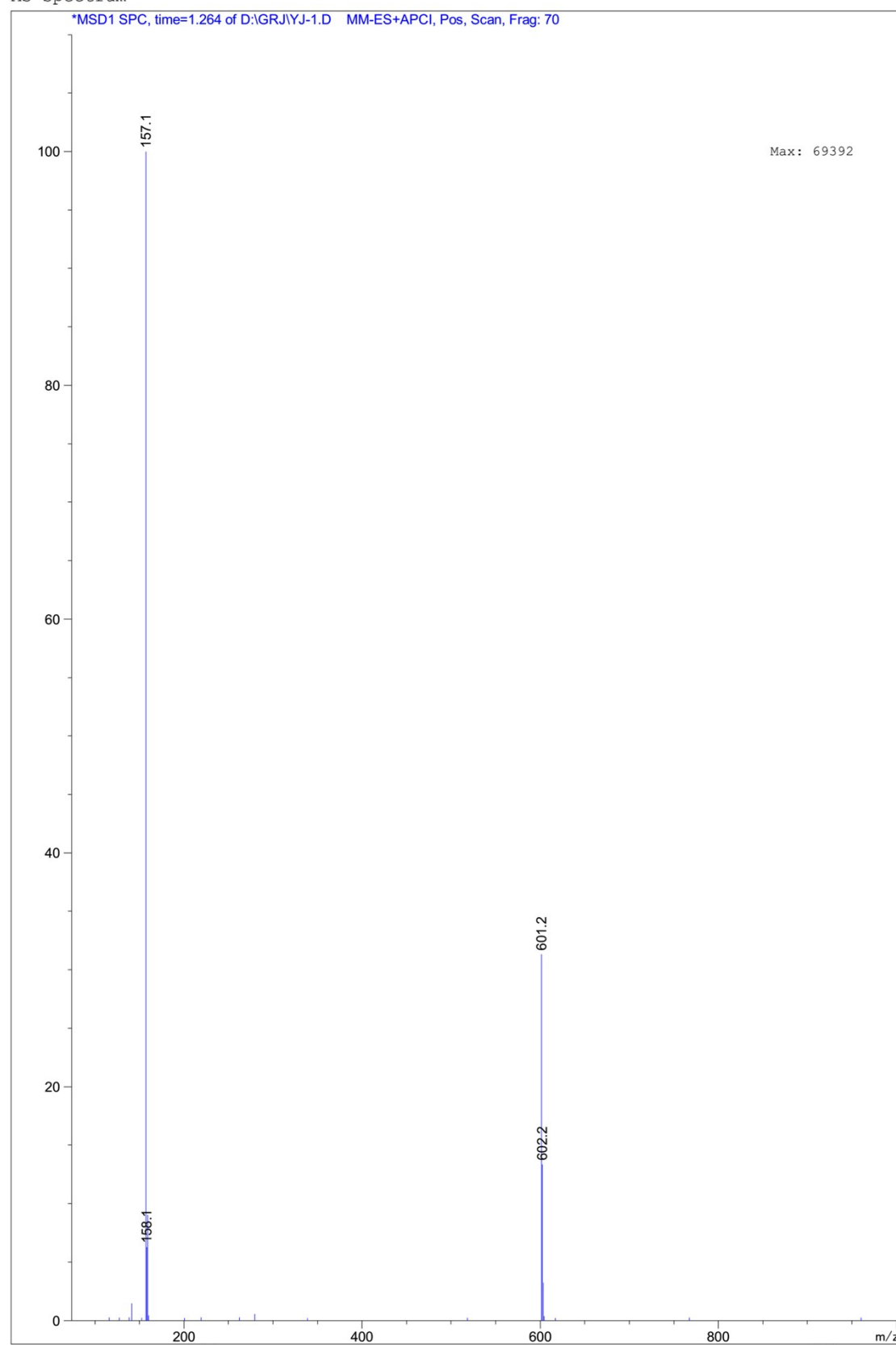
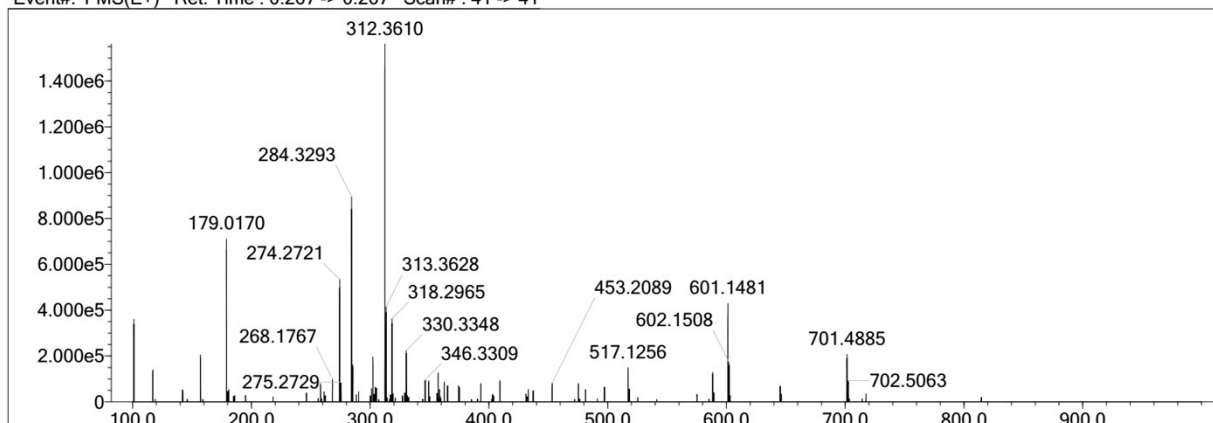
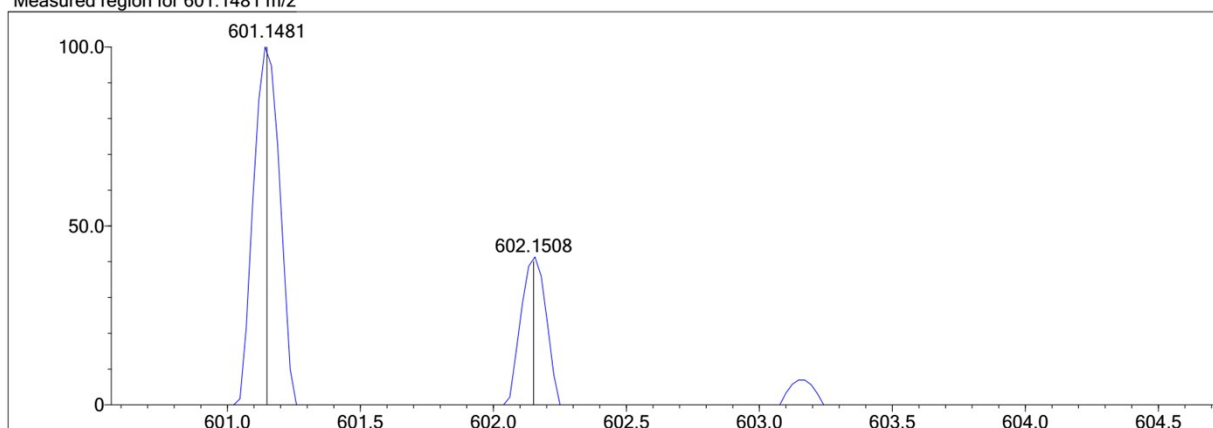


Fig. S3 MS spectrum of NF-APC

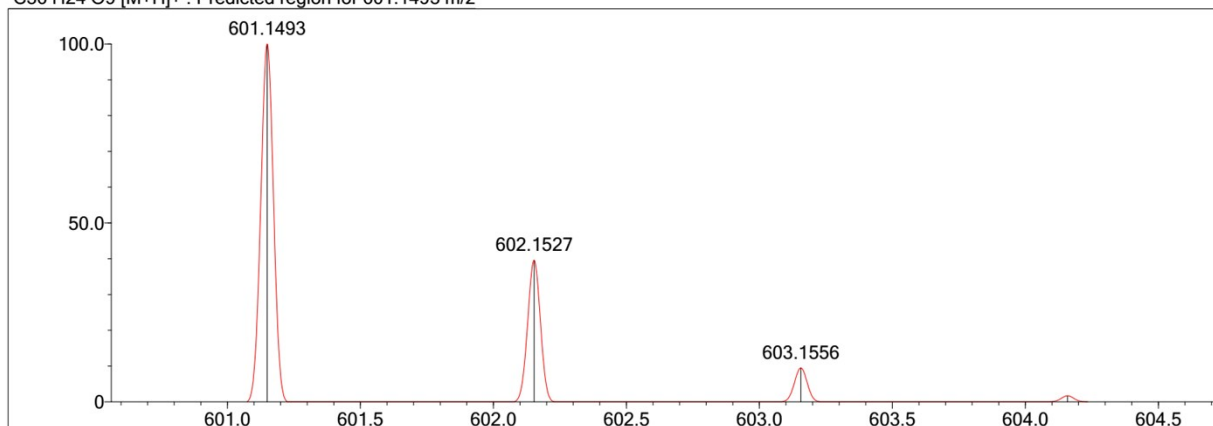
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Measured region for 601.1481 m/z

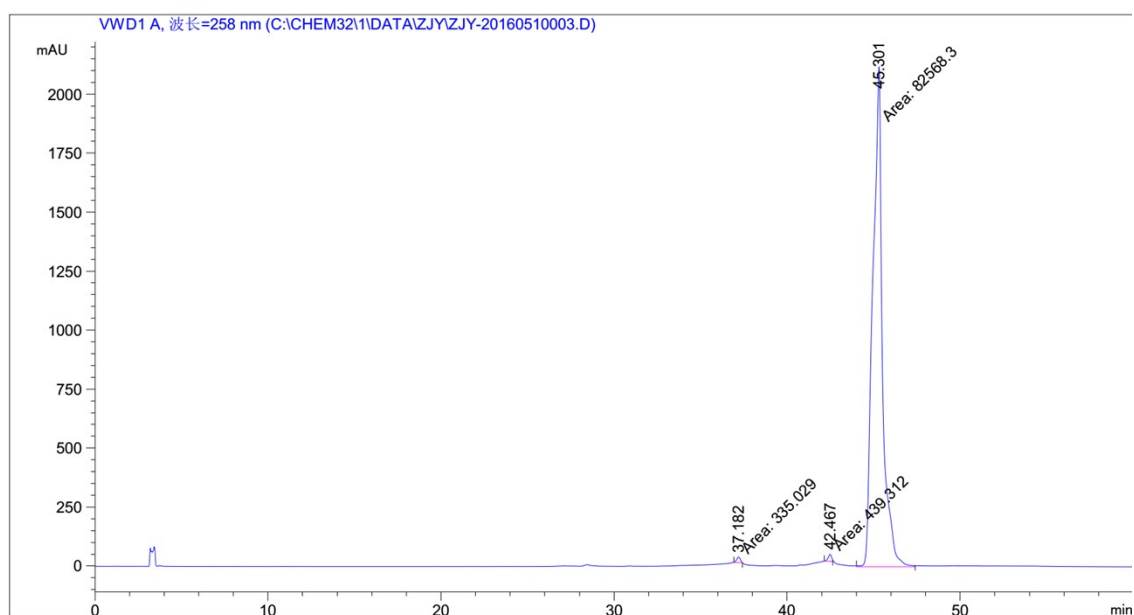


C36 H24 O9 [M+H]⁺ : Predicted region for 601.1493 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	93.12	C36 H24 O9	[M+H] ⁺	601.1481	601.1493	-1.2	-2.00	95.50	25.0

Fig. S4 HRMS spectrum of NF-APC



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

Signal 1: VWD1 A, 波长=258 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	37.182	MM	0.2385	335.02948	23.41117	0.4020
2	42.467	MM	0.2385	439.31219	30.70056	0.5271
3	45.301	MM	0.6490	8.25683e4	2120.44849	99.0709

Totals : 8.33426e4 2174.56022

Fig. S5 HPLC spectrum of NF-APC

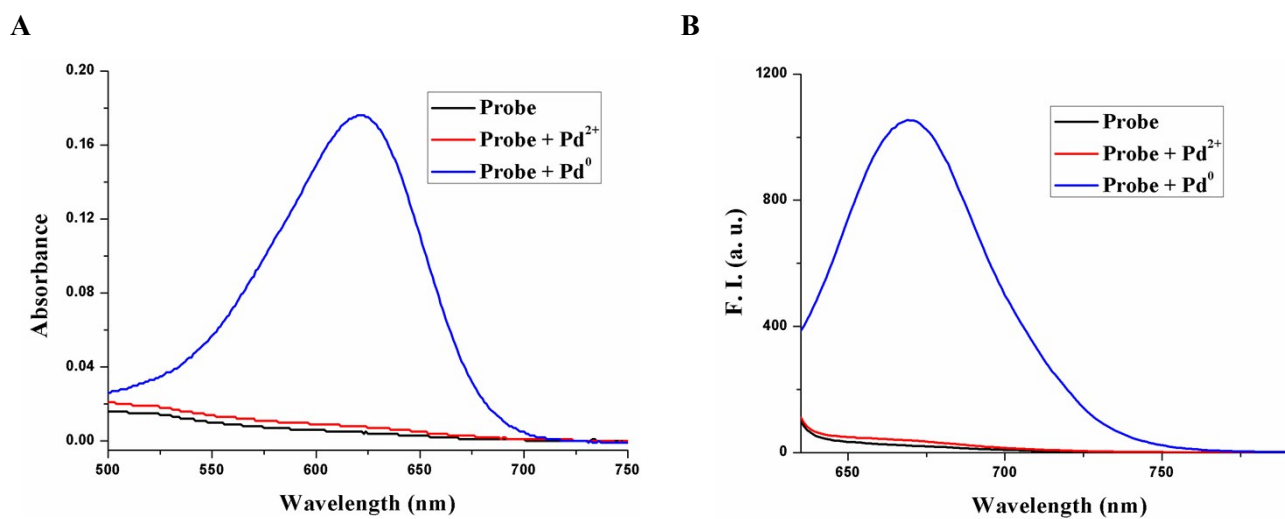


Fig. S6 UV-vis (A) and fluorescence spectra (B) of **NF-APC** (10 μ M) alone, and in the presence of a Pd^{2+} source (Na_2PdCl_4 , 60 μ M) or a Pd^0 source ($\text{Pd}(\text{PPh}_3)_4$, 60 μ M), respectively. Conditions: DMSO-PBS buffer (4:6, v/v), pH 7.4, λ_{ex} = 620 nm. The spectra were recorded after incubation for 45 min.

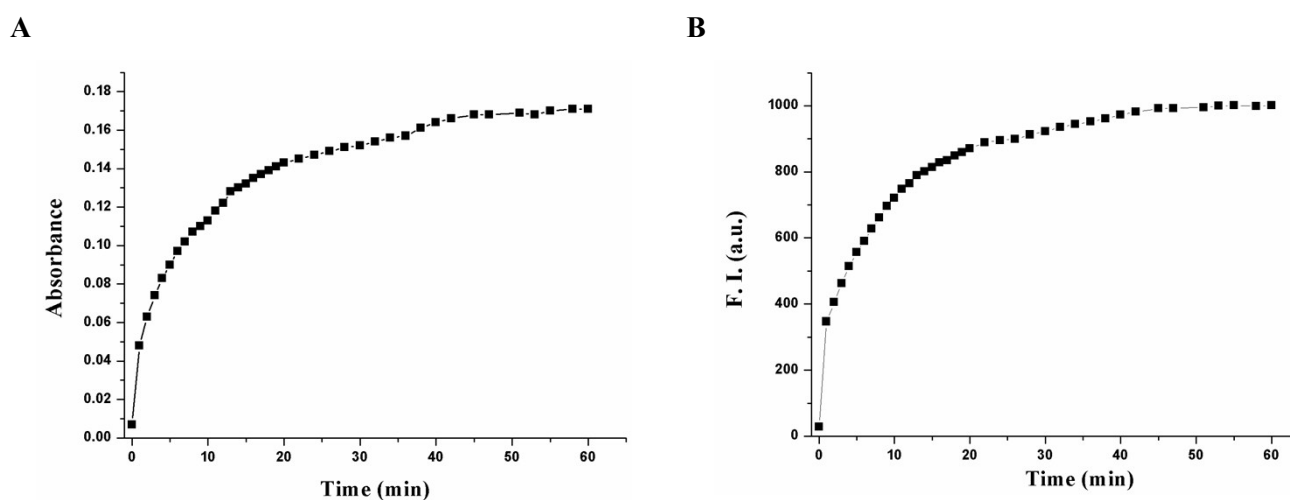


Fig. S7 Changes of absorbance at 620 nm (A) and emission intensity at 670 nm (B) of **NF-APC** against time. Conditions: $[\text{NF-APC}] = 10 \mu\text{M}$; $[\text{Na}_2\text{PdCl}_4] = 60 \mu\text{M}$; $[\text{CORM-3}] = 100 \mu\text{M}$; DMSO-PBS buffer (4:6, v/v), pH 7.4.

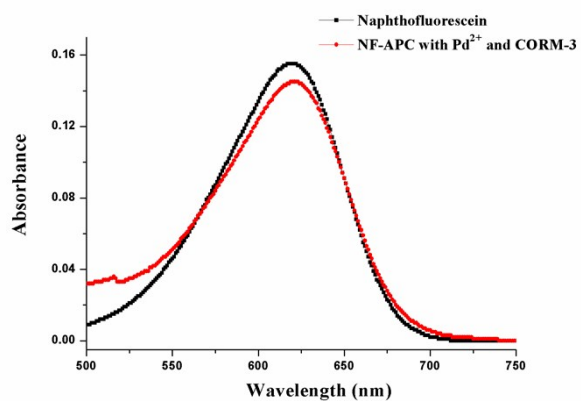
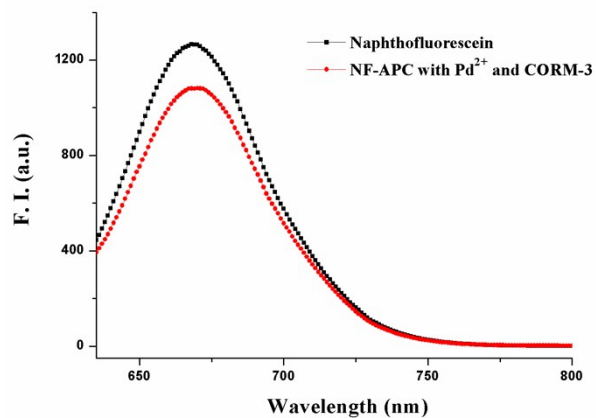
A**B**

Fig. S8 UV-vis (A) and fluorescence spectra (B) of Naphthofluorescein (10 μ M) from commercial source and **NF-APC** (10 μ M) with Na₂PdCl₄ (60 μ M) and CORM-3 (100 μ M). Conditions: DMSO-PBS buffer (4:6, v/v), pH 7.4, λ_{ex} = 620 nm.

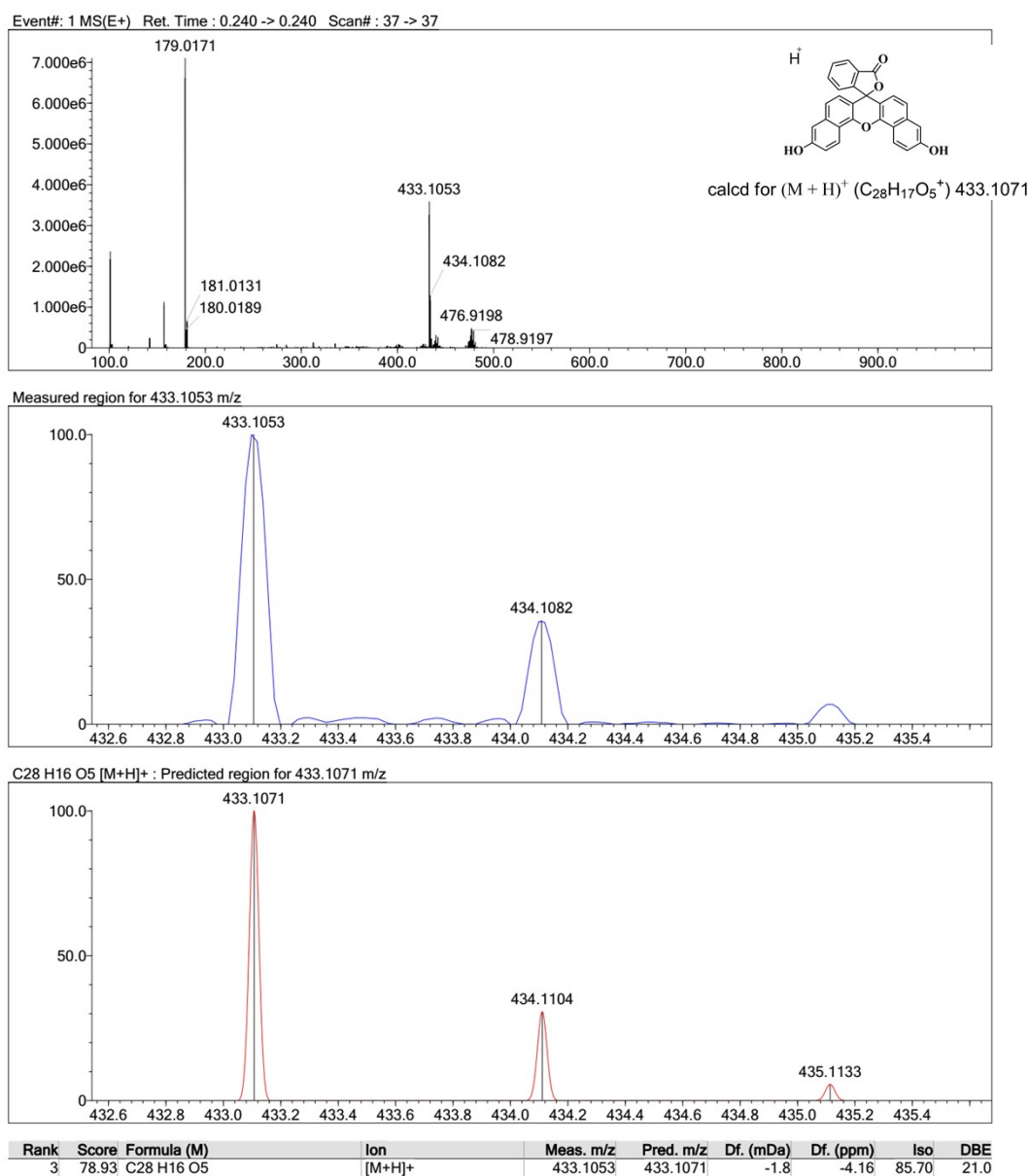


Fig. S9 HRMS spectrum of the reaction product of **NF-APC** incubated with Na₂PdCl₄ and CORM-3 for 45 min in 25 °C. Conditions: [NF-APC] = 10 μM; [Na₂PdCl₄] = 60 μM; [CORM-3] = 100 μM; DMSO-PBS buffer (4:6, v/v), pH 7.4.

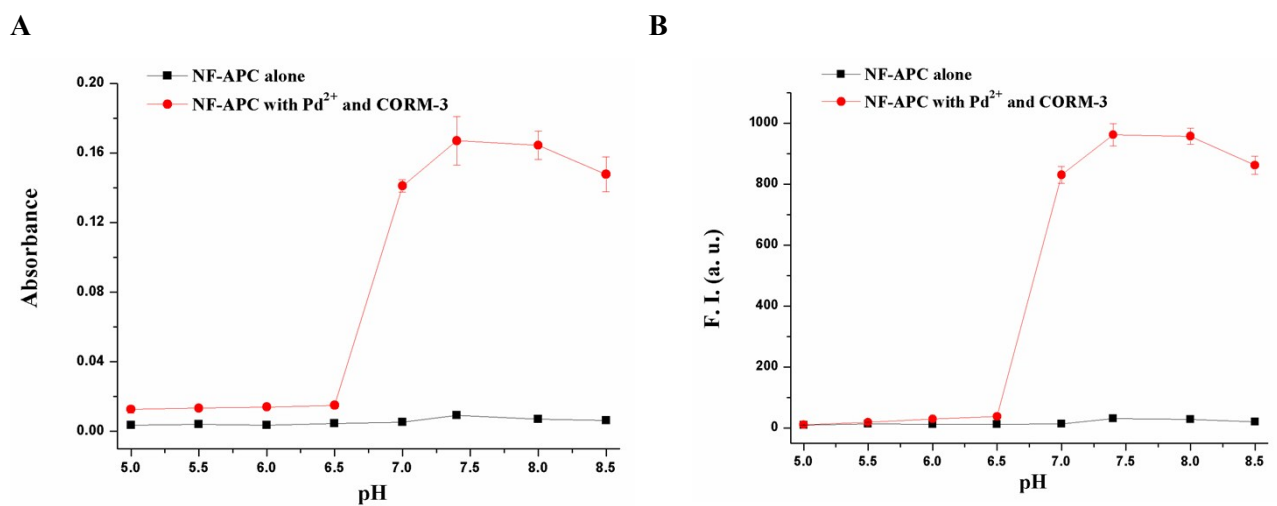


Fig. S10 The effect of pH on absorbance at 620 nm (A) and emission intensity at 670 nm (B) of NF-APC alone or with Na_2PdCl_4 (60 μM) and CORM-3 (100 μM). Conditions: DMSO-PBS buffer (4:6, v/v), pH 7.4, λ_{ex} = 620 nm. The spectra were recorded after incubation for 45 min.

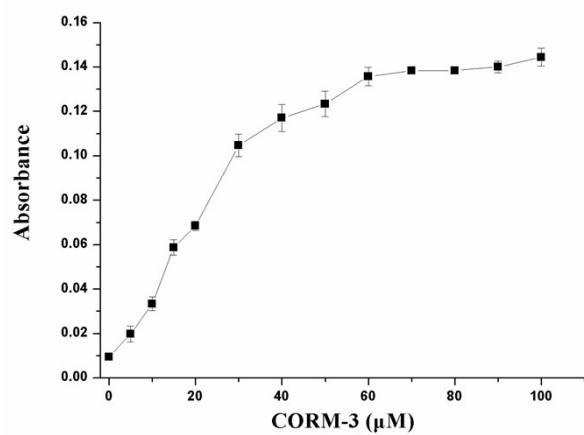
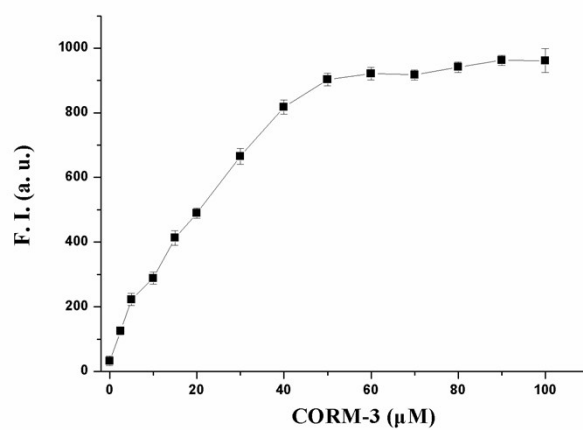
A**B**

Fig. S11 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_2PdCl_4] = 60 μM; DMSO-PBS buffer (4:6, v/v), pH 7.4, λ_{ex} = 620 nm.

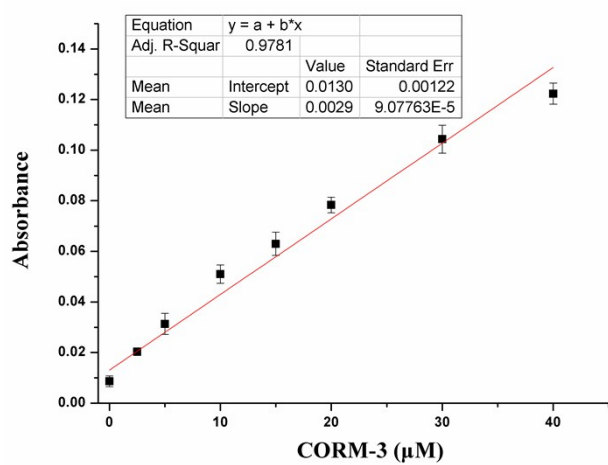
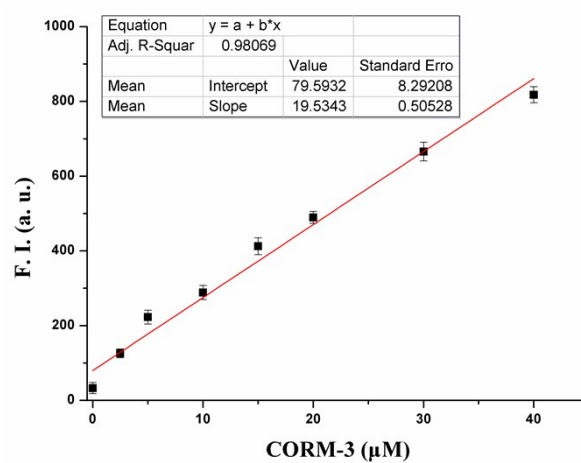
A**B**

Fig. S12 Absorbance at 620 nm (A) and emission intensity at 670 nm (B) of **NF-APC** (10 μ M) as a function of the concentration of CORM-3 in DMSO-PBS buffer (4:6, v/v) at 25 °C.

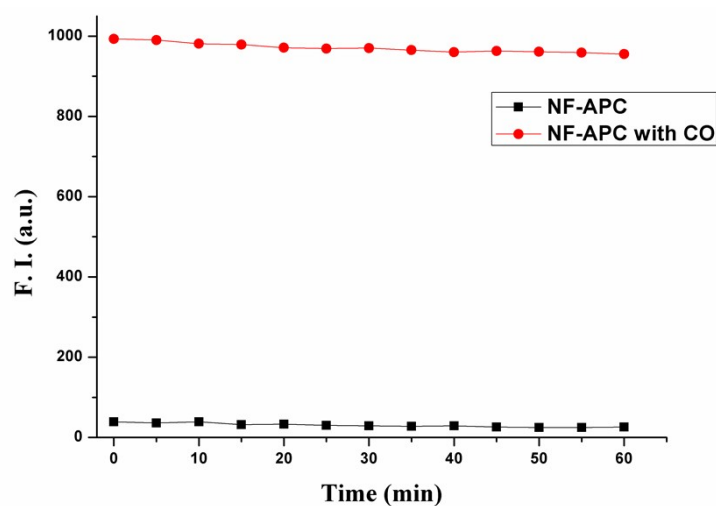


Fig. S13 The photostability study of **NF-APC**. Emission intensity at 670 nm of **NF-APC** with (red) or without (black) CO was measured by continuous irradiation with a Xe lamp at 10 nm slit width at excitation wavelength 620 nm. Conditions: **[NF-APC]** = 10 μ M; **[Na₂PdCl₄]** = 60 μ M; DMSO-PBS buffer (4:6, v/v), pH 7.4, λ_{ex} = 620 nm.

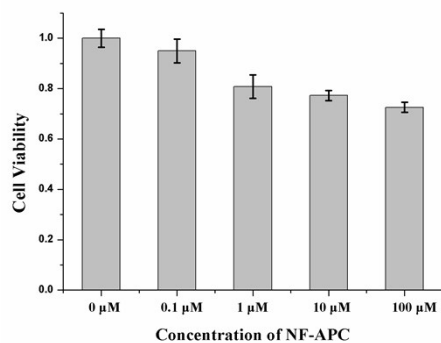
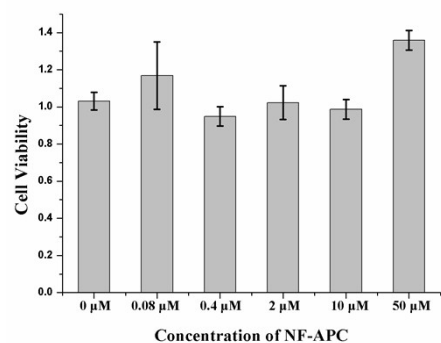
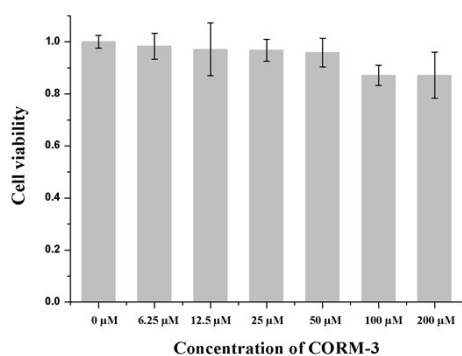
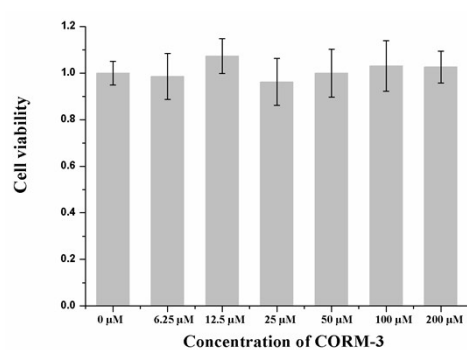
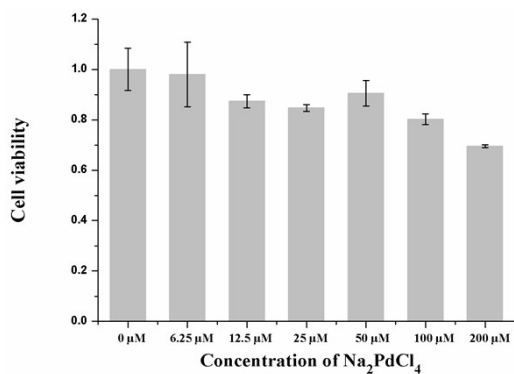
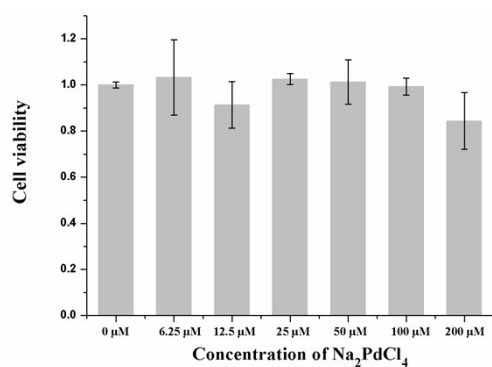
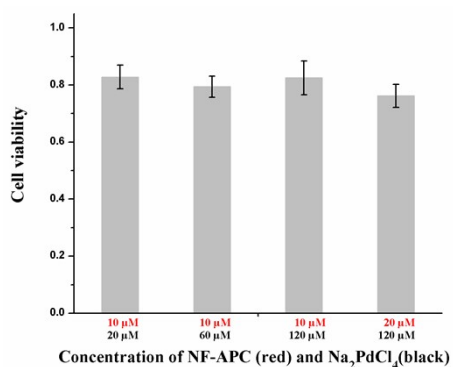
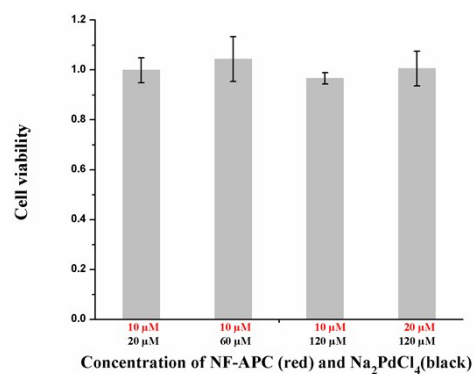
A**B****C****D****E****F****G****H**

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.