† Electronic Supplementary Information (ESI)

CV-APC, a colorimetric and red-emitting fluorescent dual probe for the highly sensitive detection of palladium

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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). ^{1}H and ^{13}C NMR spectra were recorded in DMSO-*d6* with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively, using TMS as the internal standard. High-resolution mass spectra (HRMS) were recorded on a Shimadzu LCMS-IT-TOF. 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 CH_3CN-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 **CV-APC** and $Pd(PPh_3)_4$ (10 mM) were prepared by dissolving the required amount in DMSO. $Hg(NO_3)_2$ (10 mM) was dissolved in methanol. Metal ion (Fe^{2+}, Hg^{2+}, Ag^{+}, Ni^{+}, K^{+}, Mn^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, Na^{+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Pb^{2+}) stock solutions (10 mM) were obtained by diluting the standard solutions of the corresponding nitrate salt, respectively.

UV-vis and fluorescence spectra titration experiments were performed by addition of small aliquots of Pd^{0} stock solution into the **CV-APC** solution. The fluorescence intensity was measured at the excitation wavelength of 588 nm. The excitation and emission slit width were both 10 nm.

General procedure of MTT assay

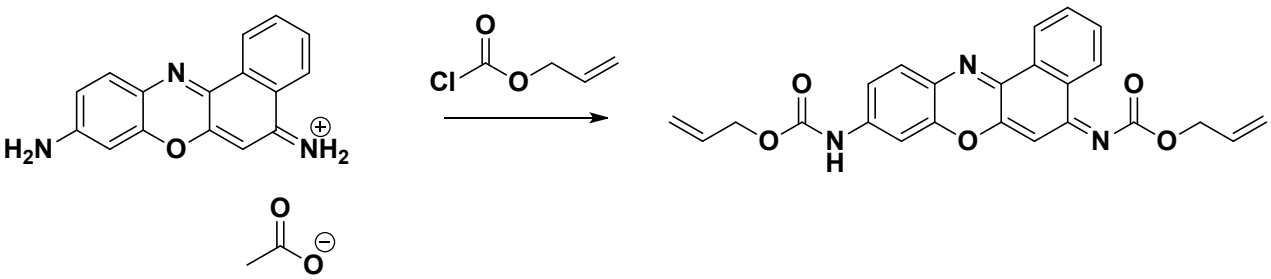
The MTT assay was used to measure the cytotoxicity of **CV-APC** to HeLa cells. Cells were seeded into a 96-well cell-culture plate. Various concentrations of **CV-APC** were added to the wells. The cells were incubated at 37 °C under 5% CO_2 for 48 h. 10 μL MTT (5 mg mL-1) was added to each well and incubated at 37 °C under 5% CO_2 for 4 h. Remove the MTT solution and yellow precipitates (formazan) observed in plates were dissolved in 100 μL 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 = $A570_{(sample)}/A570_{(control)}$

General procedure of cell imaging

HeLa cells were grown in DMEM media containing 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/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 1×10^5 cells in a culture medium and incubated overnight for living cell imaging by confocal laser scanning microscopy (CLSM). The HeLa cells were incubated with 10 μM of Hochest 33342 and **CV-APC**, which are prepared by diluting 2.0 μL of stock solution (10 mM in DMSO) with 2 mL of PBS solution and incubated for 30 min at 37 °C and washed with three times with PBS before imaging by CLSM. And the cells were subsequently incubated with Pd^0 (10 μM) 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 561 nm for **CV-APC**, respectively.

Synthesis and Characterization

Scheme S1. Synthesis of **CV-APC**[*]



[*] Reagents and conditions: DCM, Et_3N, RT, overnight.

Synthesis of (5-Allyloxycarbonylimino-5H-benzo[a]phenoxazin-9-yl)-carbamic acid allyl ester (CV-APC) Allyl chloroformate (0.38 g, 3.0 mmol) was added to a solution of cresyl violet acetate (0.321 g, 1 mmol) and triethylamine (0.30 g, 3.0 mmol) in 8 mL DCM. The reaction mixture was stirred overnight at room temperature, and water and CH_2Cl_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 **CV-APC** (0.133 g, 31%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.32 (s, 1H), 8.55 (d, *J* = 7.7 Hz, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 7.84 - 7.74 (m, 2H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.56 (s, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 6.44 (s, 1H), 6.11 - 5.93 (m, 2H), 5.39 (dd, *J* = 16.5, 12.2 Hz, 2H), 5.28 (dd, *J* = 15.4, 10.7 Hz, 2H), 4.75 (d, *J* = 5.4 Hz, 2H), 4.64 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 163.27, 159.25, 153.50, 149.05, 144.90, 142.90, 133.25, 133.16, 132.22, 131.94, 131.42, 131.26, 130.37, 128.73, 125.27, 124.67, 119.02, 118.49, 115.78, 104.35, 100.54, 67.02, 65.69. HRMS (ESI): calcd for $(M+H)^+$ ($C_{36}H_{25}O_9^+$) 430.1397, found 430.1403.

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

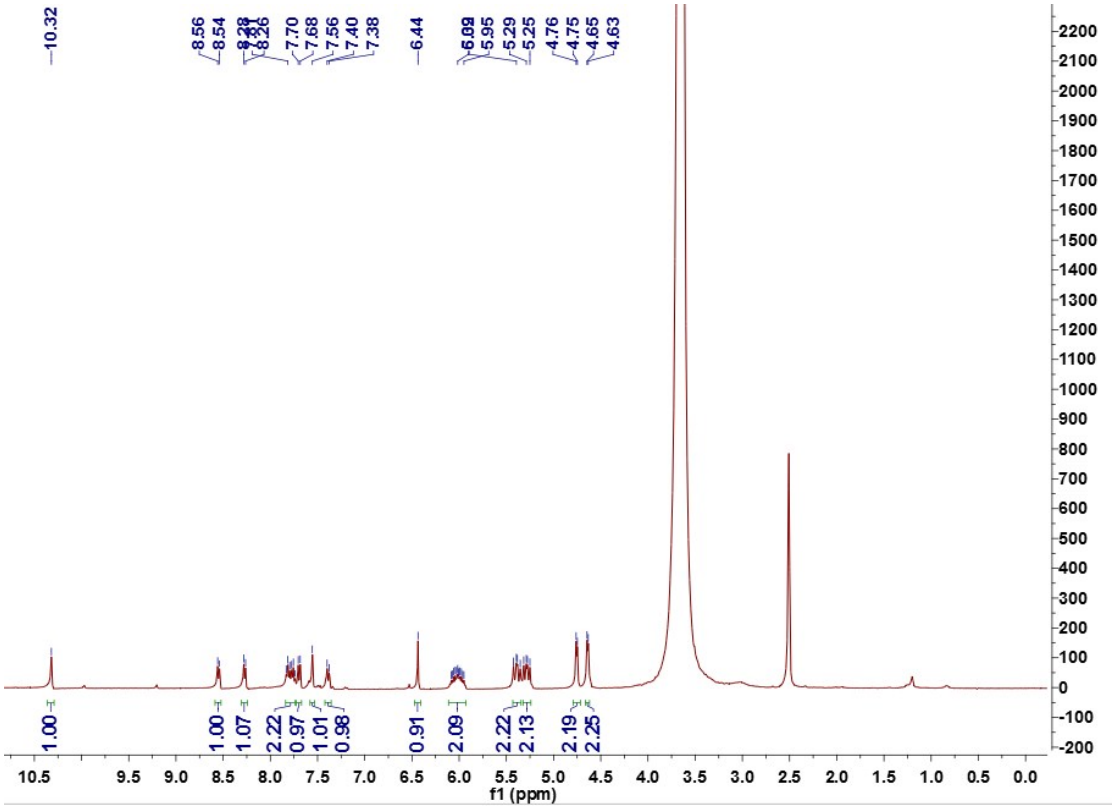


Fig. S1 ^{1}H NMR spectrum of **CV-APC**

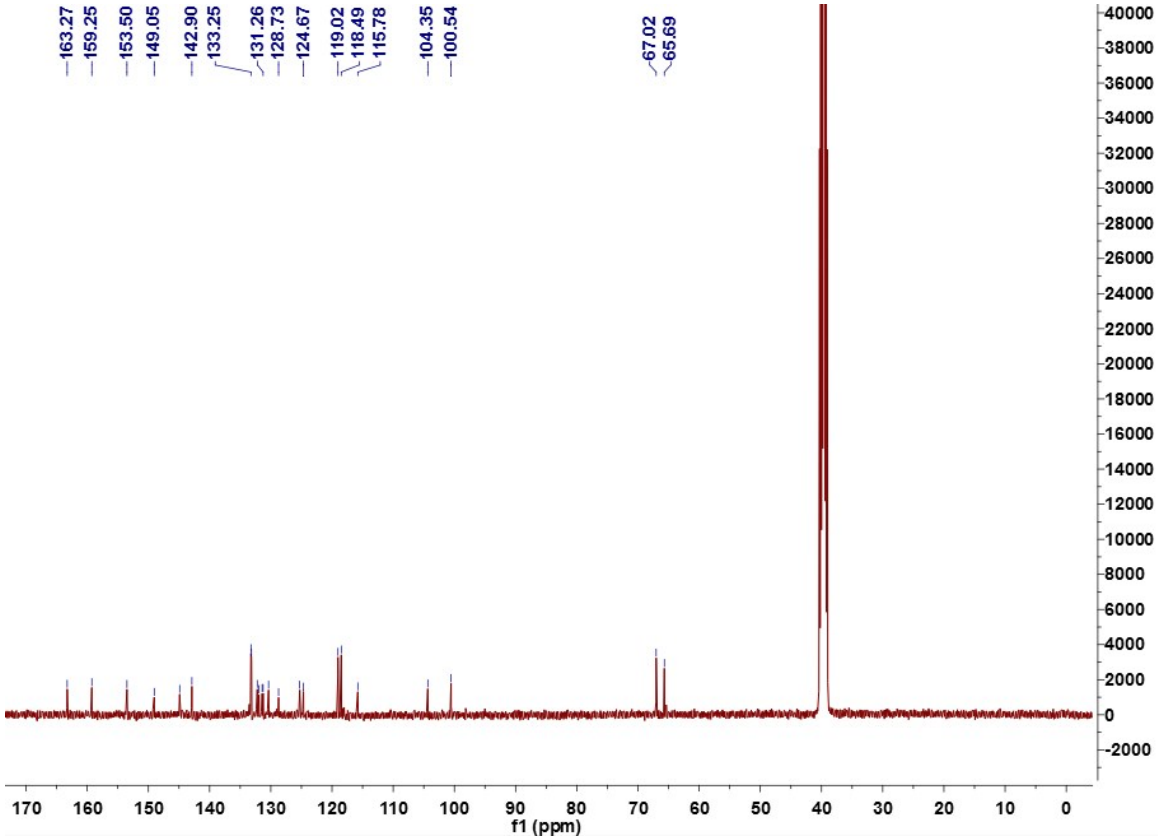


Fig. S2 ^{13}C NMR spectrum of **CV-APC**

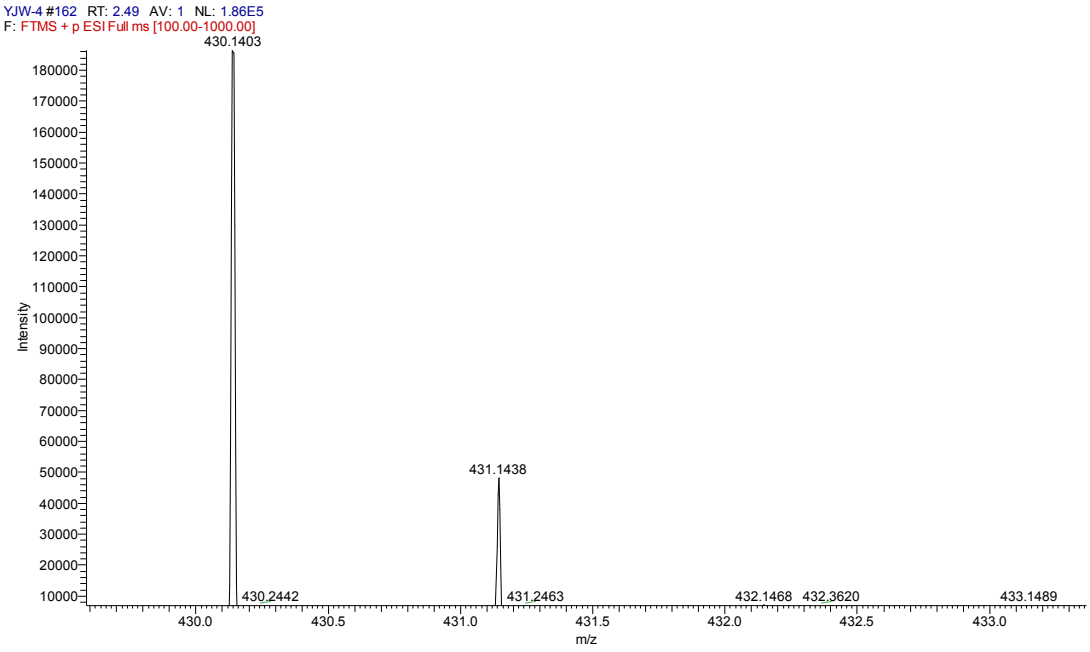


Fig. S3. HRMS spectrum of **CV-APC**

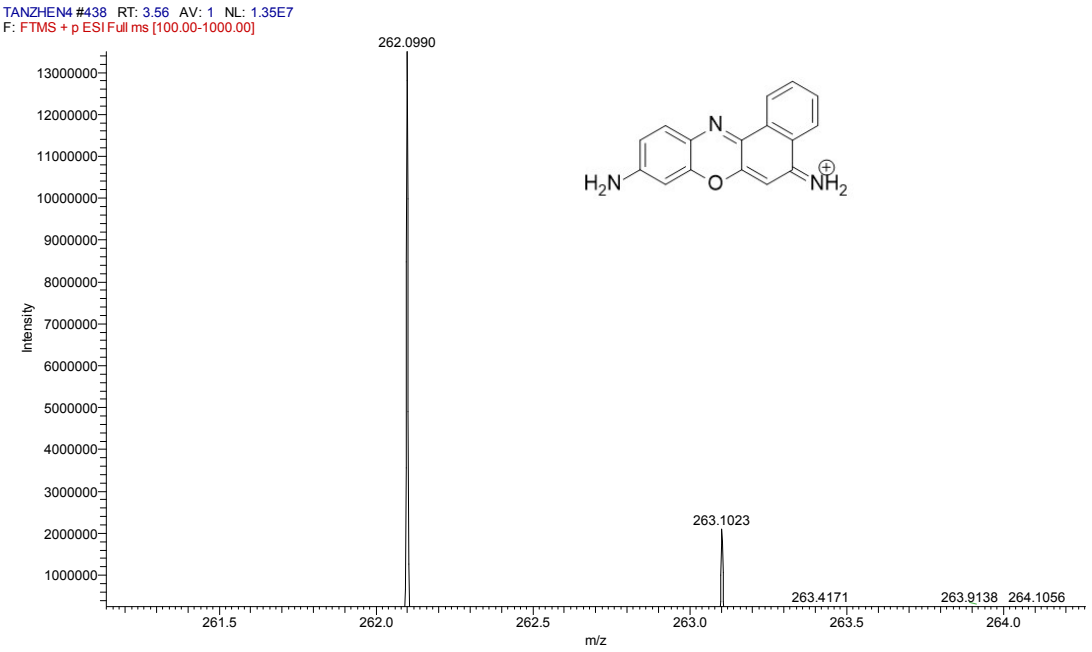


Fig. S4 HRMS spectrum of the reaction product of **CV-APC** (10 μM) in the presence of $Pd(PPh_3)_4$ (3 equiv) in CH_3CN -PBS (9:1, v/v) solution. HRMS (ESI): calcd for M^+ ($C_{16}H_{18}N_3S^+$) 262.0975, found 262.0990.

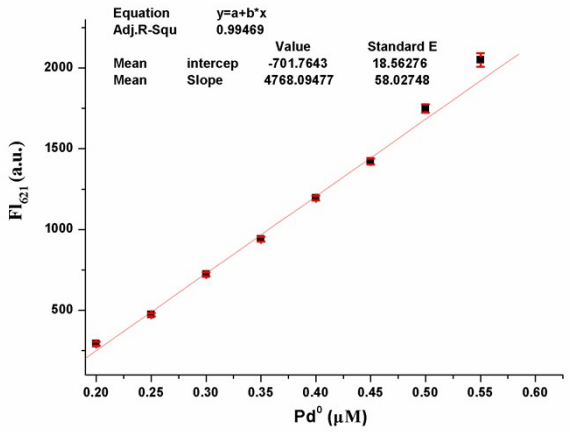


Fig. S5 Fluorescence intensity at 621 nm of **CV-APC** (0.5 μM) as a function of the concentration of Pd^0 in CH_3CN-PBS (1:1, v/v), excited at 588 nm.

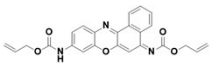
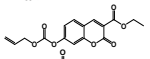
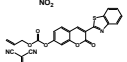
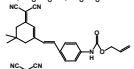
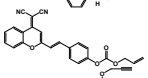
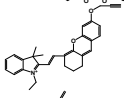
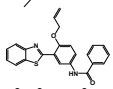
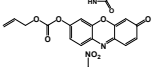
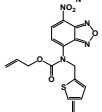
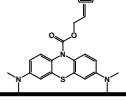
Name	Structure	λ_{ex} (nm)	λ_{em} (nm)	Detection limit
CV-APC		588	621	0.78 nM
PC[1]		400	450	0.34 nM
PdL1[2]		465	549	15 nM
Cou-1[3]		462	498	17.4 nM
Probe 1[4]		472	643	24.2 nM
DCM-1[5]		560	700	52 nM
NIR-Pd[6]		690	714	340 nM
OHBT[7]		350	550	1 μM
Res-1[8]		570	590	2.1 nM
NBDTC[9]		480	526	1.13 nM
MB-APC[10]		650	681	5.7 nM

Table S1 Comparison of **CV-PAC** and reported Pd^0 probes from 2013 to 2017

A **B**

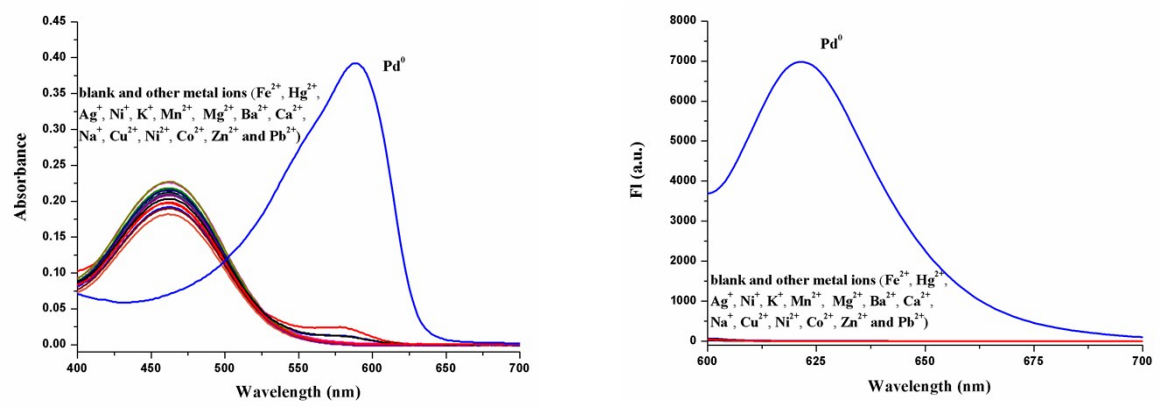


Fig. S6 Absorbance at 588 nm (A) and fluorescence intensity at 621 nm (B) of **CV-APC** (10 μM and 0.5 μM, respectively) in the presence of $Pd(PPh_3)_4$ (3 equiv) and the excess of representative metal ions (10 equiv) in CH_3CN-PBS (1:1, v/v) solutions. The spectra were recorded after incubation for 80 min.

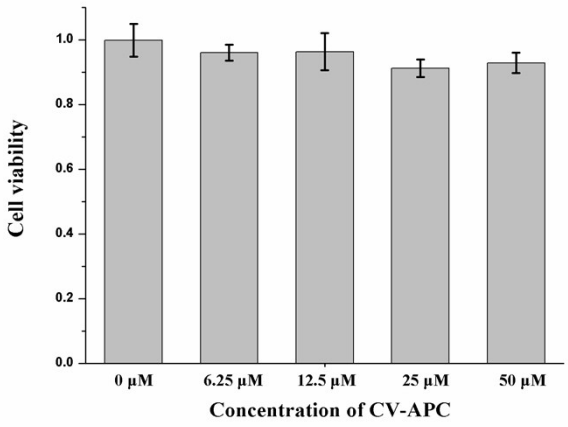


Fig. S7 MTT assay of HeLa cells incubated with **CV-APC** (0-50 μM) for 48 h.

References

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