† Electronic Supplementary Information (ESI)

A smart NIR fluorescent probe for the highly selective detection of palladium

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equiv) , $Ru_3(CO)_{12}$ (3 equiv) and the excess of representative metal ions (10 equiv) in ET-PBS (9:1, v/v) solution.

Fig. S8 UV-vis (A) and fluorescence spectral changes (B) of **MB-APC** (10 μM) in the presence of $Pd(PPh_3)_4$ (3 equiv) in EtOH-PBS (9:1, v/v), with or without EDTA (3 equiv).

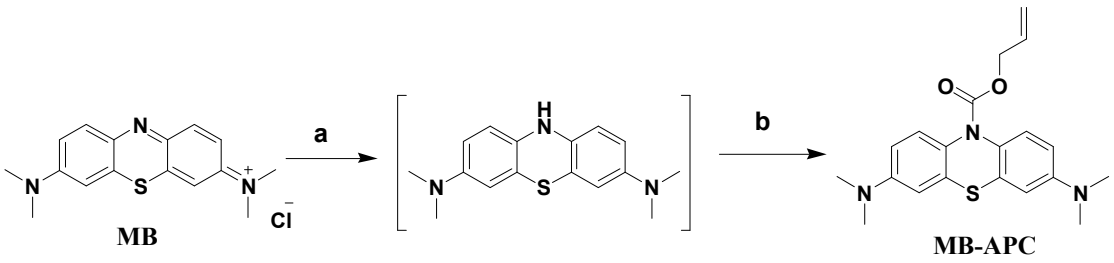
Fig. S9 MTT assay of HeLa cells incubated with **MB-APC** (0-100 μM) for 48 h.

Materials and measurements

All spectroscopic measurements were performed in EtOH-PBS solution (v/v=9:1). For the spectrometer measurements, a quartz cuvette with 10 mm path length was used to hold each sample. The UV-vis spectra were measured using a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were acquired using an FL-4500 fluorescence spectrophotometer (Hitachi, Japan). Excitation wavelength was 650 nm and fluorescence was detected in a wavelength range between 650 nm and 800 nm. Unless otherwise noted, the spectra were measured in EtOH-PBS (v/v=9:1) solution recorded at 30 min intervals at room temperature.

Synthesis and characterization of MB-APC

Methylene blue (MB, 748 mg, 2.0 mmol), sodium bicarbonate (600 mg, 7.1 mmol) and sodium dithionate (1.00 g, 5.7 mmol) were dissolved in deionized water (10 ml) and toluene (40 ml). The mixture was stirred at 50 °C for 3 h, then cooled to room temperature and the toluene phase containing leuco-MB was separated. Potassium carbonate (600 mg, 7.1 mmol) and Allyl chloroformate (480 μL, 4.0 mmol) were added to the solution in an ice bath. 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 **MB-APC** (0.147 g, 19.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 2.8 Hz, 2H), 6.66 (d, J = 2.8 Hz, 1H), 6.64 (d, J = 2.8 Hz, 1H), 6.04 – 5.90 (m, 1H), 5.34 - 5.27 (m, 1H), 5.25 - 5.19 (m, 1H), 4.71 (d, J = 4.3 Hz, 2H), 2.95 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.31, 148.82, 132.81, 132.56, 128.34, 126.98, 117.43, 111.10, 110.39, 66.64, 40.71. HRMS (ESI): calcd for $(M+H)^+$ ($C_{20}H_{24}O_2N_3S^+$) 370.1584, found 370.1587.



Reagents and conditions: (a) Sodium dithionate, Sodium bicarbonate, H_2O/Toluene, 50 ^{o}C, 3 h; (b) Allyl chloroformate, Toluene, room temperature, overnight.

Scheme S1. Synthetic route of **MB-APC**

General spectral Measurements

The stock solutions of **MB-APC** (10 mM) were prepared by dissolving the required amount in dimethyl sulfoxide. The stock solution of Pd^0 (2 mM) was prepared in dimethyl sulfoxide. The stock solution of phosphate buffer saline (PBS, 10 mM, pH7.4) were prepared in required amount monopotassium phosphate, hydrogen phosphate sodium, potassium chloride and sodium chloride dissolved in water. $Hg(NO_3)_2$ ion (100 mM) was dissolved in methanol. $Ru_3(CO)_{12}$ (2 mM) was dissolved in dimethyl sulfoxide. Metal ion (Ca^{2+}, K^+, Mg^{2+}, Na^+, Cd^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Ag^+, Ba^{2+}, Pt^{2+}, Cd^{2+}, Al^{3+}, Cr^{3+}) stock solutions (100 mM) were obtained by diluting the standard solutions of the corresponding nitrate salt, respectively. The stock solution of EDTA (1 mM) was dissolved in water at 60 °C.

UV-vis and fluorescence spectra titration experiments were performed by addition of small aliquots of Pd^0 stock solution into the **MB-APC** concentration of 10 μM in the EtOH-PBS (v/v=9:1) solution. The absorption was measured covered the wavelength range of 550-750 nm and the fluorescence intensity was measured at the excitation wavelength of 650 nm with the emission covered over the wavelength range of 650-800 nm.

General procedure of MTT assay

The MTT assay was used to measure the cytotoxicity of **MB-APC** to HeLa cells. Cells were seeded into a 96-well cell-culture plate. Various concentrations of NF-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 cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin, 100 mg/mL streptomycin and 10% heat inactivated fetal bovin

serum. The cells were seeded on a Ø 30mm glass-bottomed dish at the density of 1×10^5 cells in a culture medium and incubated overnight for live-cell imaging by confocal laser scanning microscopy (CLSM). The HeLa cells were treated with 10 μM of Hochest 33342 and **MB-APC**, 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 (30 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 633 nm for **MB-APC**, respectively.

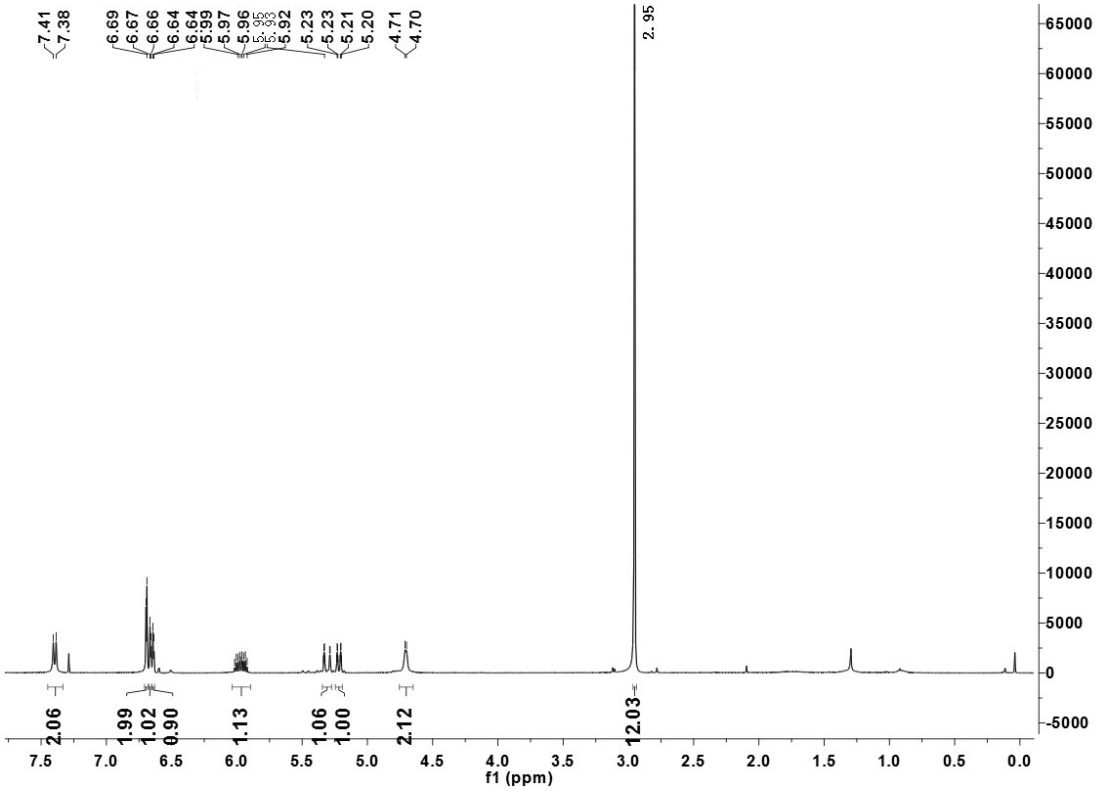


Fig. S1. ^{1}H NMR spectrum of **MB-APC** in

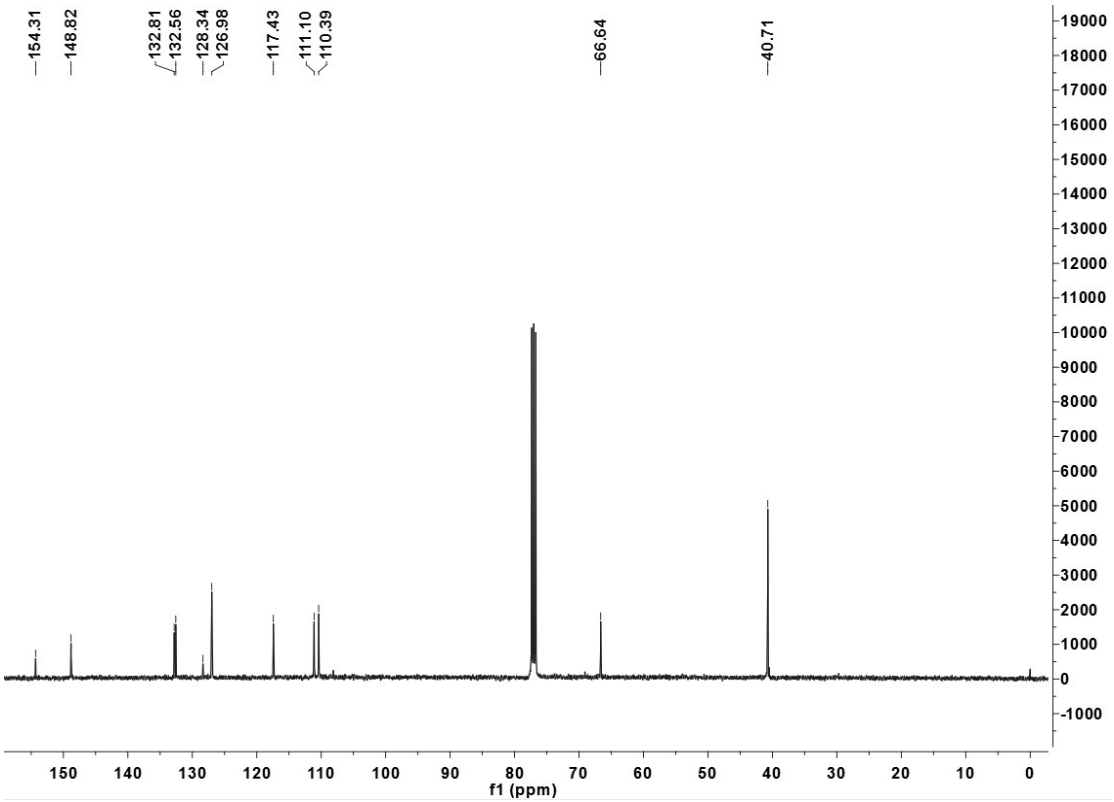


Fig. S2. ^{13}C NMR spectrum of **MB-APC**

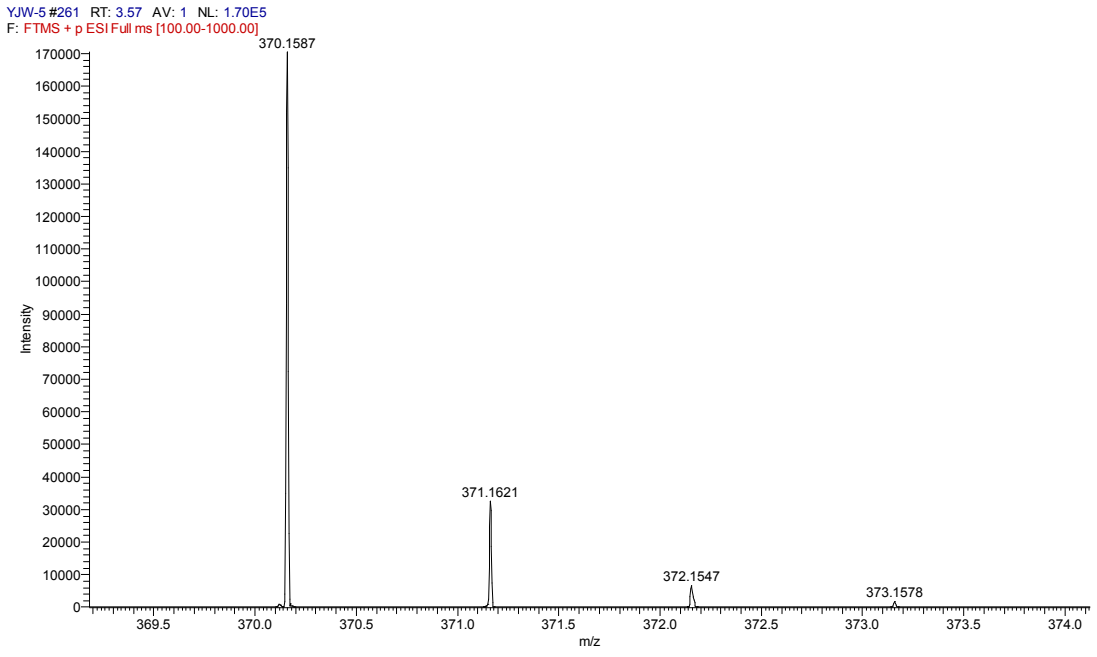


Fig. S3. HRMS spectrum of **MB-APC**

A B

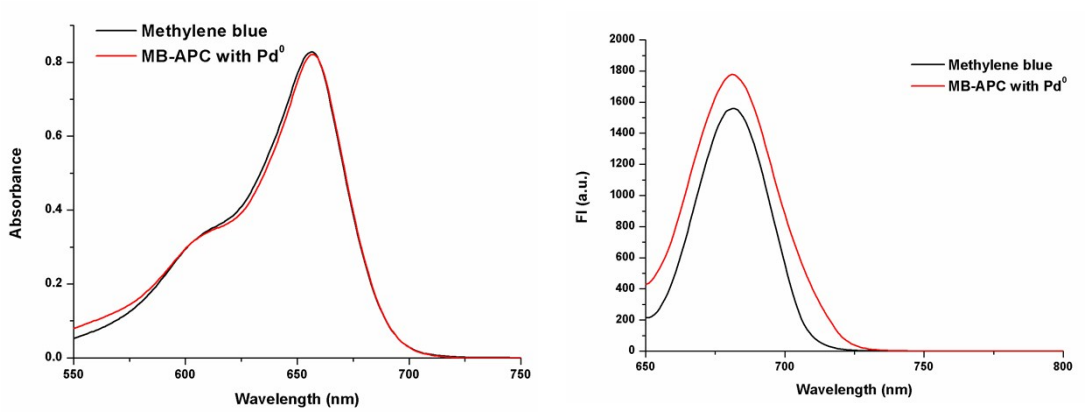


Fig. S4 UV-vis (A) and fluorescence spectra (B) of **Methylene blue** (10 μM) from commercial source and **MB-APC** (10 μM) with $Pd(PPh_3)_4$ (30 μM) in EtOH-PBS (9:1, v/v).

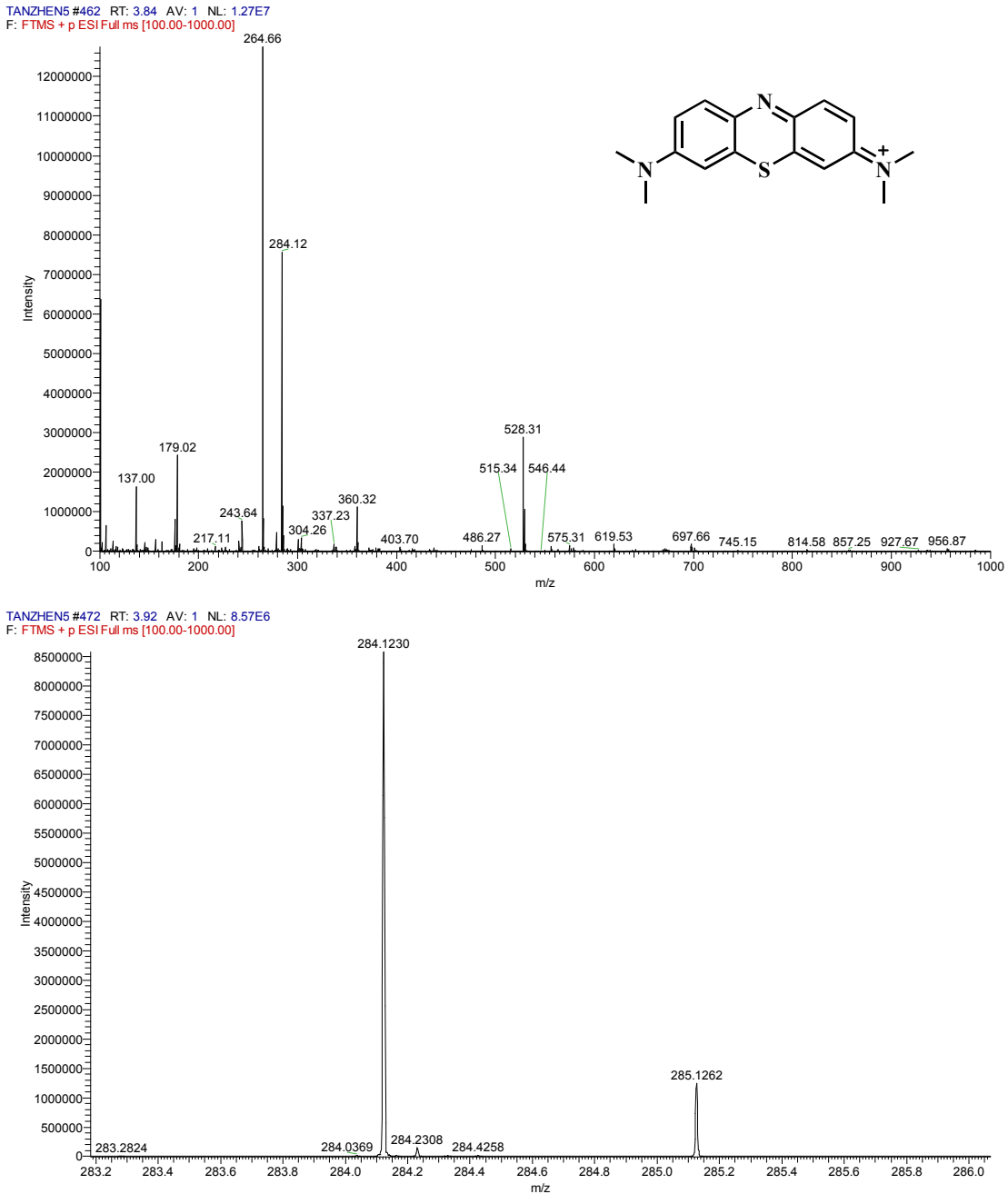


Fig. S5 HRMS spectrum of the reaction product of **MB-APC** (10 μM) in the presence of $Pd(PPh_3)_4$ (3 equiv) in EtOH-PBS (9:1, v/v) solution. HRMS (ESI): calcd for M^+ ($C_{16}H_{18}N_3S^+$) 284.1216, found 284.1230.

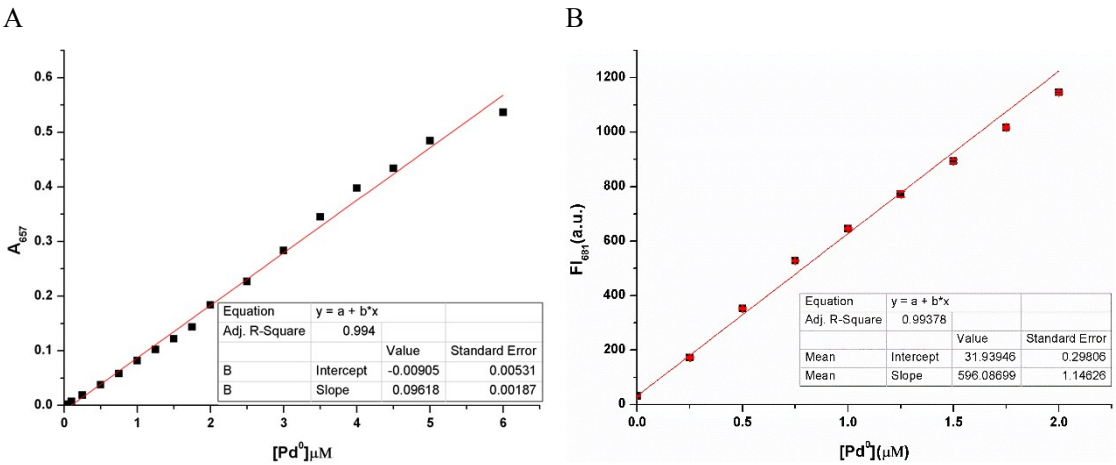


Fig. S6 Absorption (A) at 657 nm and emission intensity (B) at 681 nm of **MB-APC** (10 μM) as a function of the concentration of $Pd(PPh_3)_4$ in EtOH-PBS (9:1, v/v).

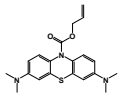
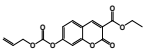
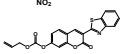
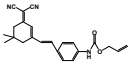
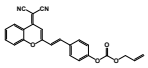
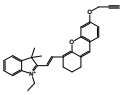
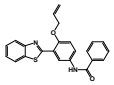
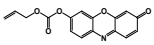
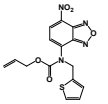
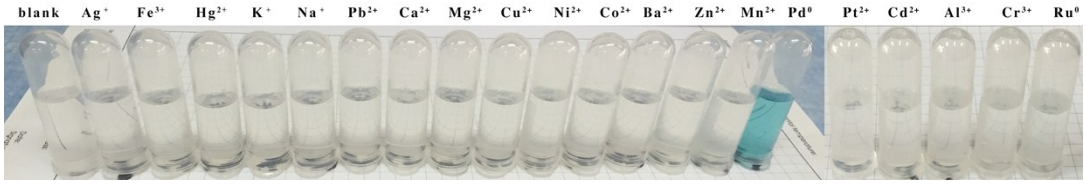
Name	Structure	λ_{ex} (nm)	λ_{em} (nm)	Detection limit	Intensity enhancement (fold)
MB-APC		650	681	5.7 nM	55
PC[1]		400	450	0.34 nM	-
PdL1[2]		465	549	15 nM	-
Cou-1[3]		462	498	17.4 nM	-
Probe 1[4]		472	643	24.2 nM	85
DCM-1[5]		560	700	52 nM	10
NIR-Pd[6]		690	714	340 nM	43
OHBT[7]		350	550	1 μM	-
Res-1[8]		570	590	2.1 nM	-
NBDTC[9]		480	526	1.13 nM	50

Table 1 Comparison of **MB-PAC** and reported Pd^0 probes from 2013 to 2016

A



B

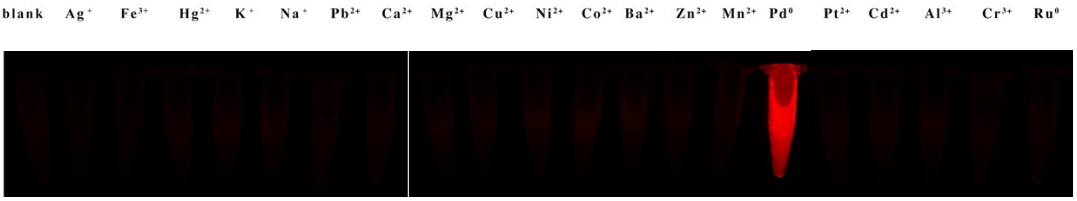


Fig. S7. Color (A) and emission (B) changes of **MB-APC** (10 μM)in the presence of $Pd(PPh_3)_4$ (3 equiv) , $Ru_3(CO)_{12}$ (3 equiv) and the excess of representative metal ions (10 equiv) in EtOH-PBS (9:1, v/v) solution.

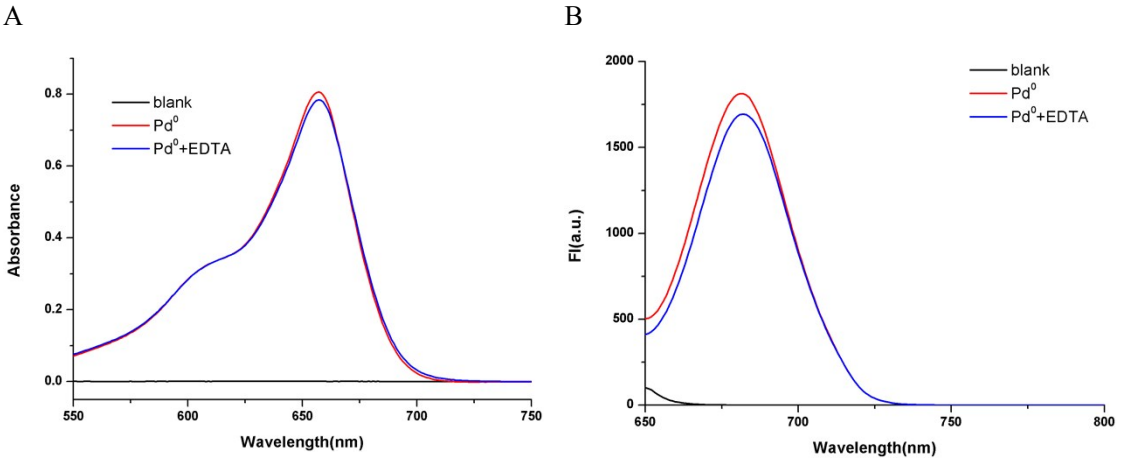


Fig. S8 UV-vis (A) and fluorescence spectral changes (B) of **MB-APC** (10 μM) in the presence of $Pd(PPh_3)_4$ (3 equiv) in EtOH-PBS (9:1, v/v), with or without EDTA (3 equiv).

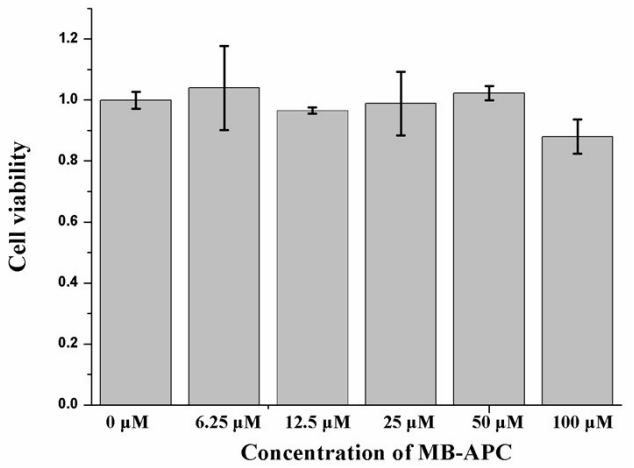


Fig. S9 MTT assay of HeLa cells incubated with **MB-APC** (0-100 μM) for 48 h.

References

1. Y. Liu, K. Xiang, M. Guo, B. Tian and J. Zhang, *Tetrahedron Lett.*, 2016, 57, 1451-1455.
2. Z. Ma, X. Wang, Y. Sun, J. Liu, Y. Tong and Z. Liu, *J. Fluoresc.*, 2016, 26, 1917-1921.
3. K. Wang, G. Lai, Z. Li, M. Liu, Y. Shen and C. Wang, *Tetrahedron*, 2015, 71, 7874-7878.
4. K. Xiang, Y. Liu, C. Li, B. Tian and J. Zhang, *RSC Adv.*, 2015, 5, 52516-52521.
5. P. Wang, J. Xia and Y. Gu, *Tetrahedron Lett.*, 2015, 56, 6491-6494.
6. H. Chen, W. Lin and L. Yuan, *Org. Biomol. Chem.*, 2013, 11, 1938-1941.
7. L. Cui, W. Zhu, Y. Xu and X. Qian, *Anal. Chim. Acta*, 2013, 786, 139-145.
8. J.-w. Yan, X.-l. Wang, Q.-f. Tan, P.-f. Yao, J.-h. Tan and L. Zhang, *Analyst*, 2016, 141, 2376-2379.
9. Z.-Y. Xu, J. Li, S. Guan, L. Zhang and C.-Z. Dong, *Spectrochim. Acta, Part A*, 2015, 148, 7-11.