*Supporting Information for*

# Development of a FRET-based ratiometric fluorescent probe to monitor the changes of palladium(II) in aqueous solution and living cells

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## Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

## Method of spectral measurements

Unless otherwise noted, all the measurements were made according to the following procedure. A stock solution (1.0 mM) of **CR-Pd** was prepared by dissolving the requisite amount of it in DMF. In a 10 mL tube the test solution of compounds **CR-Pd** was prepared by placing 0.1 mL of stock solution, 4.9 mL of EtOH and 5 mL of 0.1 M PBS buffer (different pH). After adjusting the final volume to 10 mL with distilled-deionized water, standing at room temperature 3 min, 3 mL portion of it was transferred to a 1 cm quartz cell to measure absorbance or fluorescence. The stock solutions of metal ions for selectivity experiments were prepared respectively by dissolving $Mg^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Cr^{2+}$, $S_2O_3^{2-}$, $Ag^{+}$, $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $NH_4^{+}$, $Al^{3+}$, Cys , $Co^{2+}$, $HSO_3^{-}$, blank , $Pd^{2+}$ in twice-distilled water. The slight pH variations of the solutions were achieved by adding the minimum volumes of NaOH (0.1 M) or HCl (0.2 M).

## Culture and preparation of HeLa and A549 cells

HeLa cells and A549 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10 % FBS (fetal bovine serum) in an atmosphere of 5 % $CO_2$ and 95 % air at 37 °C. Before the experiments, seed the HeLa or A549 cells in 35 mm glass-bottomed dishes at a density of $2\times10^5$ cells per dish in 2 mL of culture medium and incubate them inside an incubator containing 5 % $CO_2$ and 95 % air at 37 °C. Incubating the cells for 24 h. Cells will attach to the glass surface during this time.

## Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 24-well tissue culture plate in the presence of 500 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37 °C and 5 % $CO_2$ atmosphere for overnight and then incubated for 24 h in the presence of **CR-Pd** at different concentrations (0 μM, 5 μM, 10 μM, 20 μM, 30 μM, 50 μM). Then cells were washed with PBS buffer and 500 μL supplemented DMEM medium was added. Subsequently, 50 μL MTT (5 mg/ mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 500 μL sodium dodecyl sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 570 nm using a microplate reader. The cell viability was determined by assuming 100 % cell viability for cells without **CR-Pd**.

## Imaging of $Pd^{2+}$ in living cells

### *1. Ratiometric imaging of exogenous $Pd^{2+}$ in HeLa cells*

Before the experiments, the HeLa cells were seeded on two 35 mm glass-bottomed dishes and allowed to adhere for 24 h. the cells were washed with PBS (pH = 7.4) buffer three times. Subsequently, the first group was incubating with probe **CR-Pd** (10 μM) (containing 0.1 % DMSO as a cosolvent) for 30 min at 37 ˚C, the HeLa cells were rinsed with PBS three times. The second group was incubated with probe **CR-Pd** (10 μM) (containing 0.1% DMSO as a cosolvent) for 30 min at 37 ˚C, the HeLa cells were rinsed with PBS three times and the cells were incubated with $Pd(AcO)_2$ (30 μM) for 30 min at 37 ˚C, and then washed with PBS three

times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

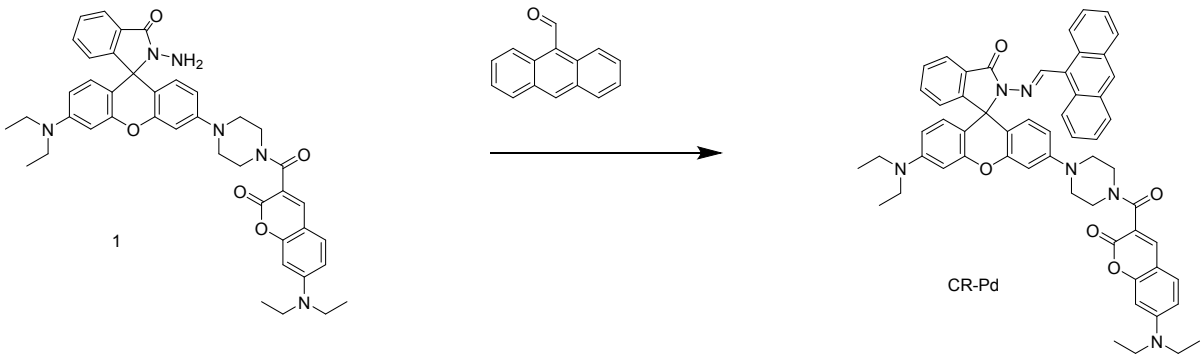
***2. Ratiometric imaging of exogenous $Pd^{2+}$ in A549 cells***

Before the experiments, the A549 cells were seeded on two 35 mm glass-bottomed dishes and allowed to adhere for 24 h. the cells were washed with PBS (pH = 7.4) buffer three times. Subsequently, the first group was incubating with probe **CR-Pd** (10 μM) (containing 0.1 % DMSO as a cosolvent) for 30 min at 37 ˚C, the A549 cells were rinsed with PBS three times. The second group was incubating with probe **CR-Pd** (10 μM) (containing 0.1 % DMSO as a cosolvent) for 30 min at 37 ˚C, the A549 cells were rinsed with PBS three times and the cells were incubated with $Pd(AcO)_2$ (30 μM) for 30 min at 37 ˚C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

***3. Colocation experiment in HeLa cells***

HeLa cells were seeded on two 35 mm glass-bottomed dishes and allowed to adhere for 24 h. the cells were washed with PBS (pH = 7.4) buffer three times. Subsequently, the cells incubating with probe **CR-Pd** (10 μM) (containing 0.1 % DMSO as a cosolvent) and 25 nM MitoTracker Deep Red for 30 min at 37 ˚C, the cells were rinsed with PBS three times and the cells were incubated with $Pd(AcO)_2$ (10 μM) for 20 min at 37 ˚C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

## Synthesis

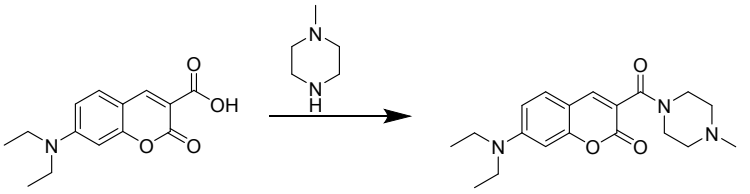



**Scheme 1** Synthesis of the probe ***CR-Pd***

**Compounds 1 were synthesized according to the reported method [1,2,3]**

***Synthesis of compound CR-Pd***

Compound **1** (70 mg, 0.1 mmol, 1 eq) and anthracene-9-carbaldehyde (21 mg, 0.1 mmol, 1 eq) were dissolved in 3 mL of anhydrous ethanol was heated to reflux for 24 h, and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (MeOH / $CH_2Cl_2$ = 1: 40, v/v) to afford a yellow solid as compound **CR-Pd** (56 mg, yield: 63 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.88 (s, 1H), 8.65 (s, 1H), 8.08 (d, *J* = 8.5 Hz, 2H), 8.03 – 7.98 (m, 2H), 7.93 (d, *J* = 8.9 Hz, 2H), 7.73 – 7.62 (m, 2H), 7.50 (q, *J* = 8.8, 8.1 Hz, 3H), 7.39 (t, *J* = 7.9 Hz, 2H), 7.21 (d, *J* = 7.3 Hz, 1H), 6.80 (s, 1H), 6.74 (d, *J* = 9.9 Hz, 3H), 6.64 (d, *J* = 9.5 Hz, 1H), 6.56 (d, *J* = 2.3 Hz, 1H), 6.46 (d, *J* = 7.0 Hz, 2H), 3.69 (s, 2H), 3.45 (d, *J* = 7.2 Hz, 6H), 3.24 (d, *J* = 20.7 Hz, 4H), 1.40 (s, 1H), 1.24 (s, 1H), 1.10 (dt, *J* = 16.5, 6.9 Hz, 12H), 0.84 (dd, *J* = 9.8, 6.3 Hz, 2H); $^{13}$C NMR (101 MHz, DMSO) δ 164.58, 164.37, 158.91, 157.09, 152.91, 152.71, 152.11, 151.75, 151.49, 149.18, 147.65, 144.36, 134.58, 131.22, 130.60, 129.88, 129.52, 129.30, 128.22, 128.09, 127.34, 126.10, 125.93, 124.85, 124.33, 123.73, 116.23, 112.40, 109.90, 109.01, 107.59, 105.50, 102.46, 97.94, 96.77, 65.70, 44.64, 44.18, 26.81, 12.85, 12.74; HRMS (EI) *m/z* calculated for $C_{57}H_{52}N_6O_5$: 900.3999 Found: 901.4072 (M+H).



**Compound 2** **Control compound 3**

***Synthesis of Control compound 3***

Compound **2** (261 mg, 1 mmol, 1 eq) is dissolved in 3 mL DMF, add HOBT (1-Hydroxybenzotriazole) (67.5 mg, 0.5 mmol, 0.5 eq), EDCI (1-(3-Dimethylaminopropyl) -3-ethylcarbodiimide hydrochloride) (573 mg, 3 mmol, 3 eq) and stir for 10 minutes, then add n-methypiperazine (100 mg, 1 mmol, 1 eq), stirring for 10 minutes, and add 2 drops of DIEA (Ethyldiisopropylamine), stirring at room temperature for 2 hours. Pour the solution into ethyl acetate for extraction and wash with saturated sodium chloride, Sodium sulfate drying and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (MeOH / $CH_2Cl_2$ = 1: 50, V/V) to afford a yellow solid as compound **3**. $^1$H NMR (400 MHz, DMSO) δ 7.96 (s, 1H), 7.49 (d, *J* = 8.9 Hz, 1H), 6.74 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.55 (d, *J* = 2.1 Hz, 1H), 3.57 (s, 2H), 3.45 (q, *J* = 6.9 Hz, 4H), 2.30 (d, *J* = 18.6 Hz, 4H), 2.19 (s, 3H), 1.92 (s, 2H), 1.13 (t, *J* = 7.0 Hz, 6H).

**References:**

[1] Yan-Ru Zhang, Xin-Peng Chen, Jing-Shao, Jia-Yi Zhang, Qiong Yuan, Jun-Ying Miao, and Bao-Xiang Zhao, *Chem. Commun.*, 2014, **50**, 14241-14244.

[2] Peng-Zhong Chen, Yu-Xiang Weng, Li-Ya Niu, Yu-Zhe Chen, Li-Zhu Wu, Chen-Ho Tung, and Qing-Zheng Yang, *Angew. Chem. Int. Ed.*, 2016, **55**, 2759 –2763.

[3] Mingguang Ren, Jing Nie, Beibei Deng, Kai Zhou, Jian-Yong Wang and Weiying Lin *New J. Chem.*, 2017, **41**, 5259-5262.

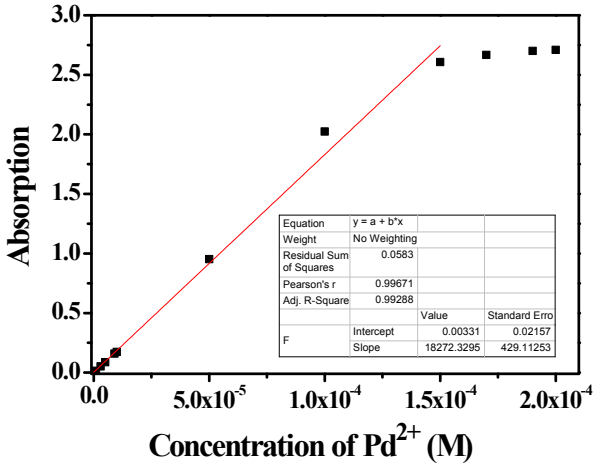



**Figure S1** The linear relationship between absorption intensity and probe **CR-Pd** concentration.

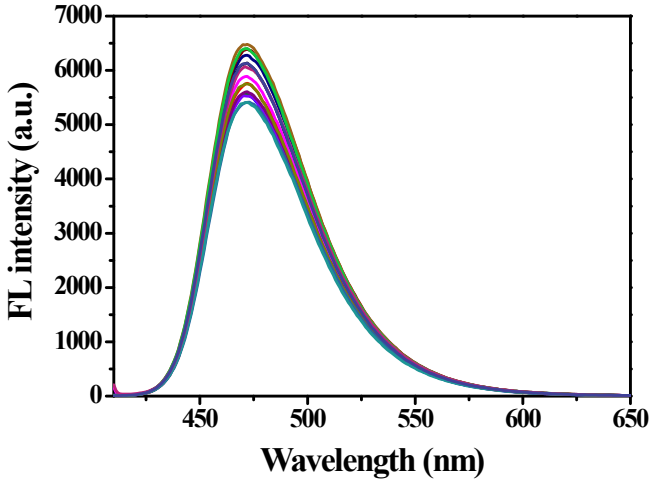



**Figure S2** The Fluorescence intensity titration profiles of Control Compound **3** (10 μM) in the presence of $Pd^{2+}$ (0-150 μM) in PBS buffer containing 50% EtOH as a cosolvent ($\lambda_{ex}$= 400 nm).

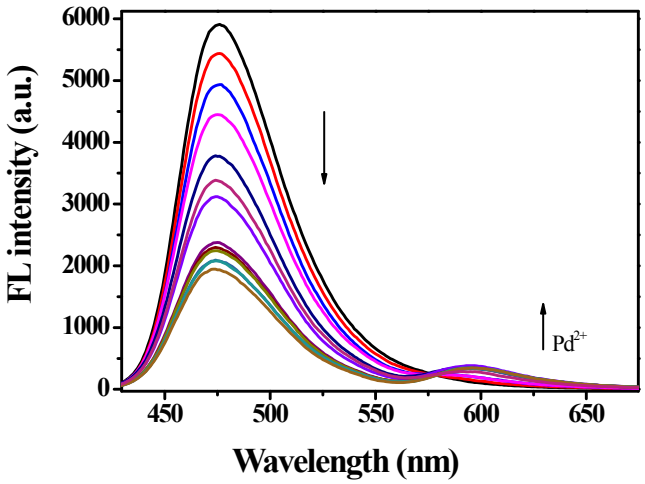



**Figure S3** The Fluorescence intensity titration profiles of **CR-Pd** (10 μM) in the presence of $Pd^{2+}$ in PBS buffe containing 50 % EtOH as a cosolvent excitation at 400 nm.

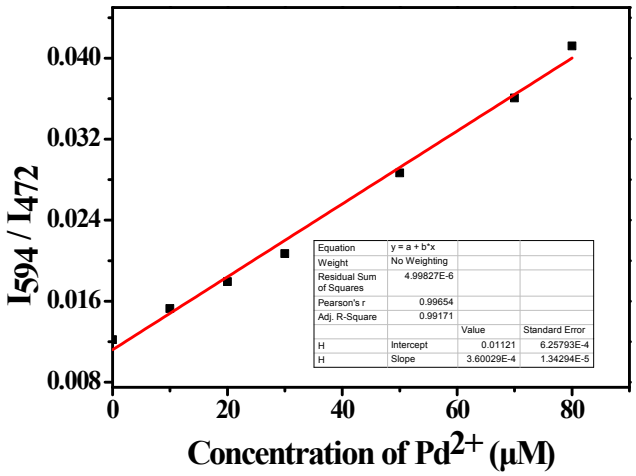



**Figure S4** The linear relationship between the fluorescence intensity ratio ($I_{594}/I_{472}$) and the concentration of $Pd^{2+}$ ($\lambda_{ex}$ = 400 nm).

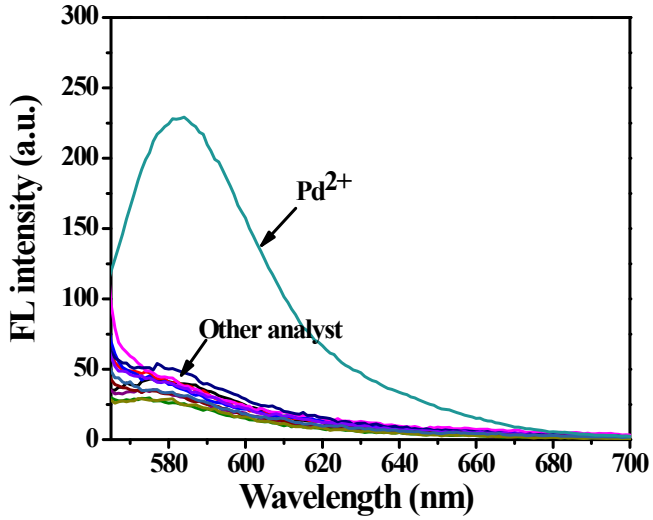



**Figure S5** The fluorescence spectra changes of probe **CR-Pd** (10 μM) in the presence of various analytes (50 μM) in PBS buffer containing 50 % EtOH as a cosolvent ($\lambda_{ex}$=550 nm)

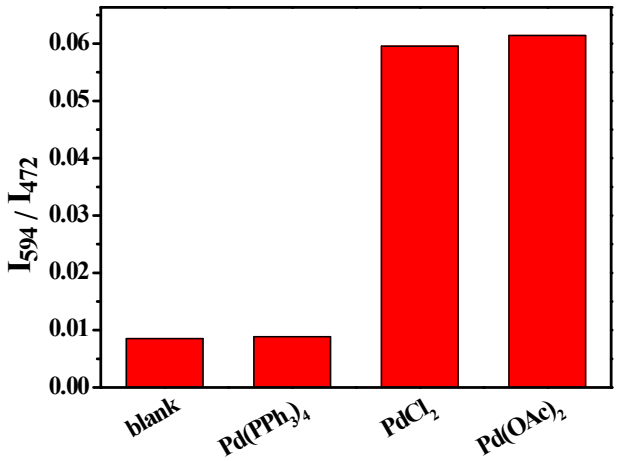



**Figure S6** Fluorescence intensity ratio ($I_{594}$ / $I_{472}$) of probe **CR-Pd** (10 μM) was added to a different palladium solution (100 μM) in PBS buffer containing 50 % EtOH as a cosolvent ($\lambda_{ex}$ = 400 nm).

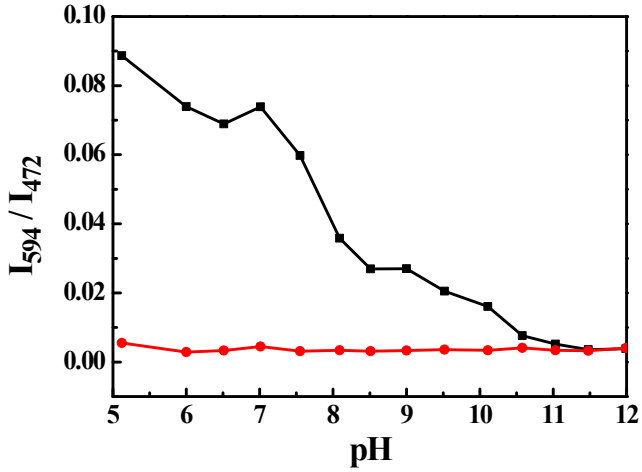



**Figure S7** The fluorescence intensity ratio ($I_{594}$ / $I_{472}$) of the probe **CR-Pd** (10 μM) before and after addition of $Pd(AcO)_2$ (100 μM ) in PBS buffer with different pH values, containing 50 % EtOH as a cosolvent ($\lambda_{ex}$ = 400 nm).

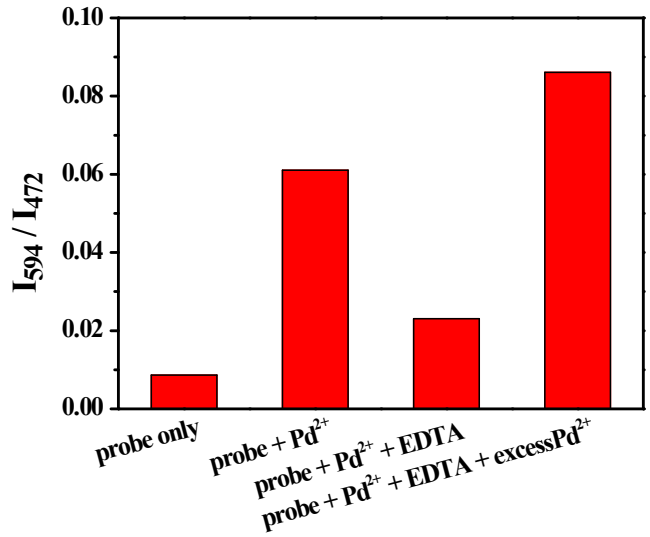



**Figure S8** The fluorescence intensity ratio ($I_{594}/I_{472}$) of the probe **CR-Pd** (10 μM) in PBS buffer containing 50 % EtOH as a cosolvent. Separately, blank, adding $Pd(AcO)_2$ (100 μM ), adding $Pd(AcO)_2$ an then add EDTA, adding $Pd(AcO)_2$ and EDTA, then add excess $Pd(AcO)_2$.

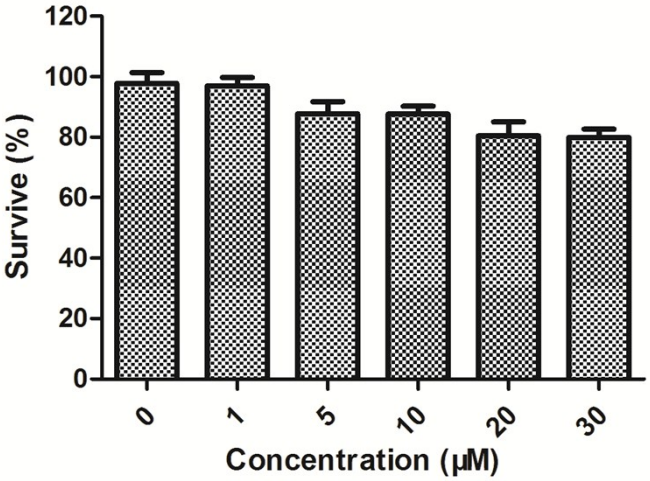



**Figure S9** Cytotoxicity assays of **CR-Pd** at different concentrations for HeLa cells.

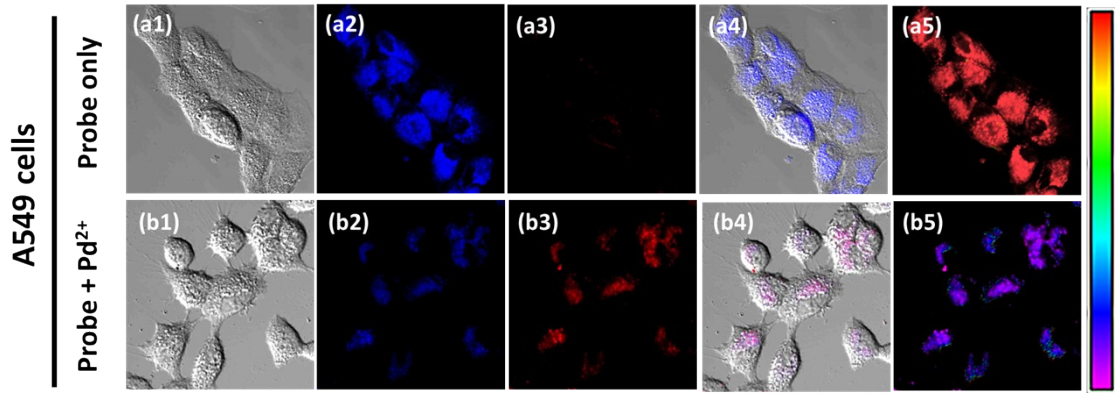



**Figure S10** Ratiometric fluorescence imaging of $Pd^{2+}$ in A549 cells using the probe **CR-Pd**: (a1-5) brightfield and fluorescence images of the A549 cells only incubated with the probe **CR-Pd** (10 μM) for 30 min; (b1-5) brightfield and fluorescence images of the A549cells incubated with the probe **CR-Pd** (10 μM) for 30 min, and then with 30 μM $Pd(OAc)_2$ for 30 min.

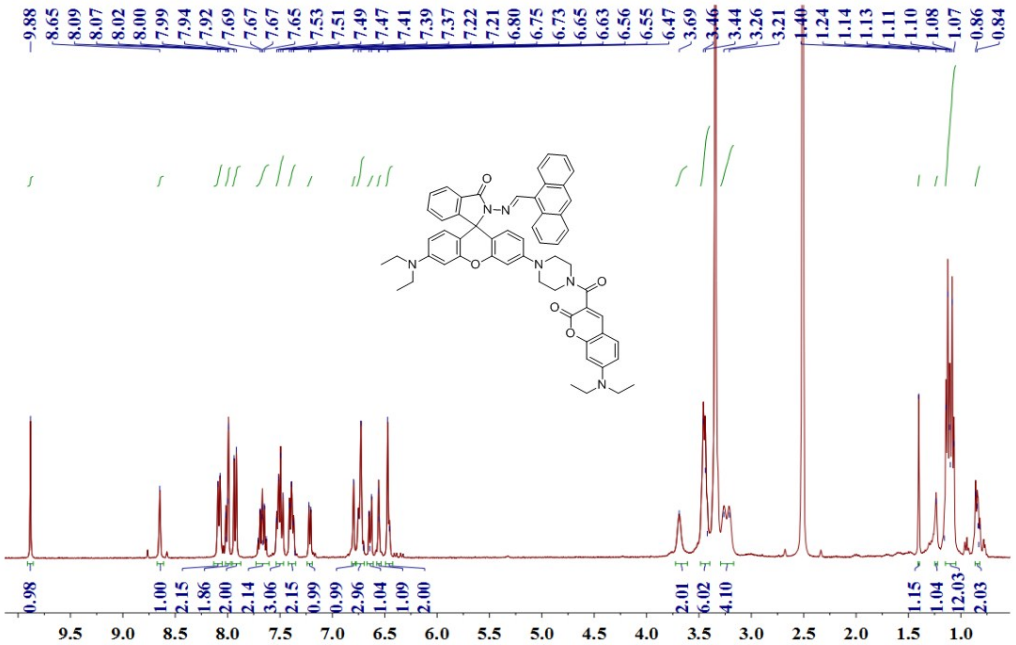


**Figure S11** $^{1}$H-NMR (DMSO-$d_6$) spectrum of compound **CR-Pd.**

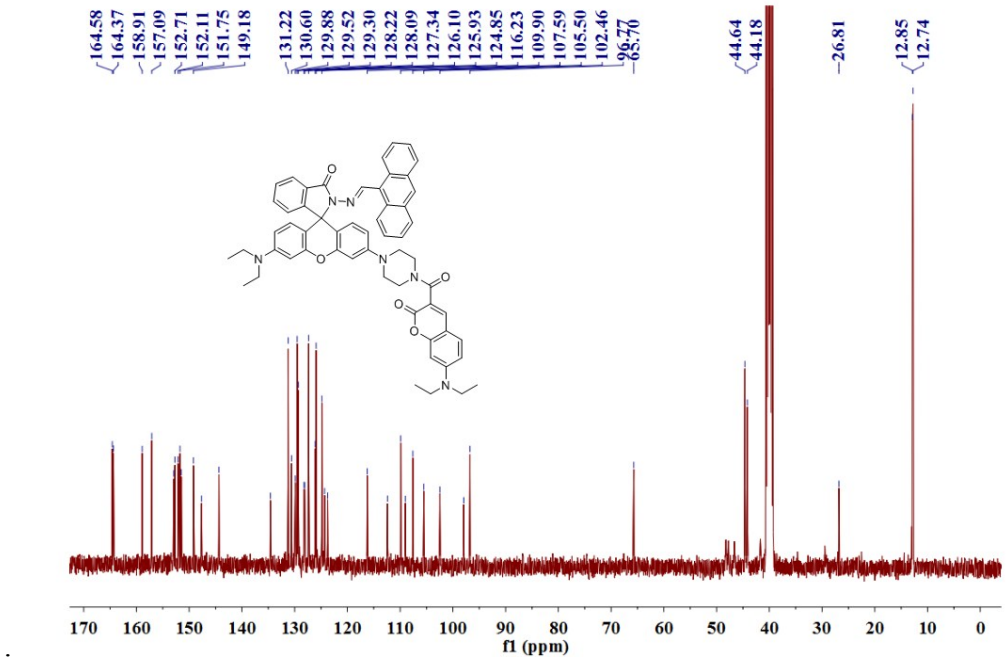


**Figure S12** $^{13}$C-NMR (DMSO-$d_6$) spectrum of compound **CR-Pd**.

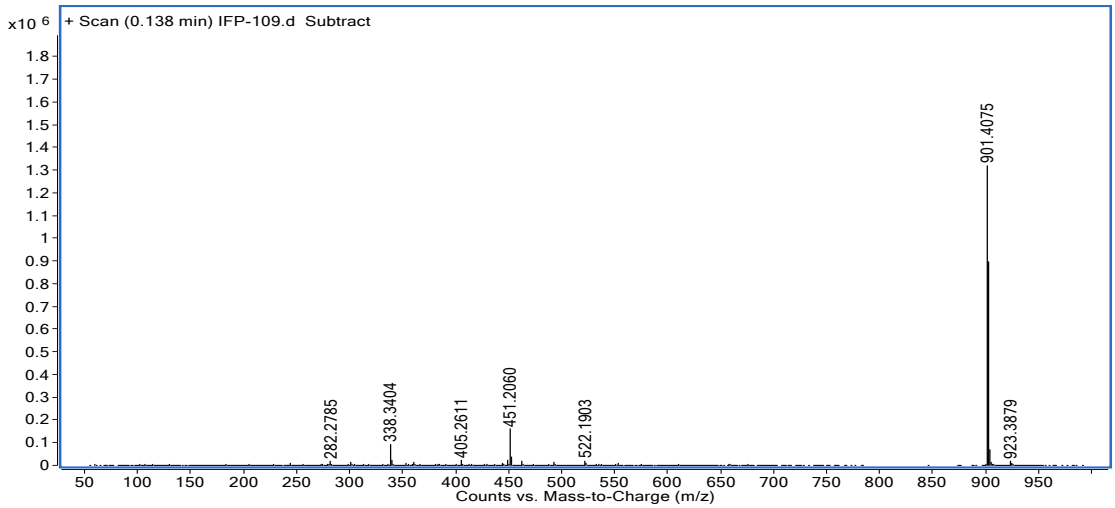



**Figure S13** HRMS (ESI) spectrum of **CR-Pd.**

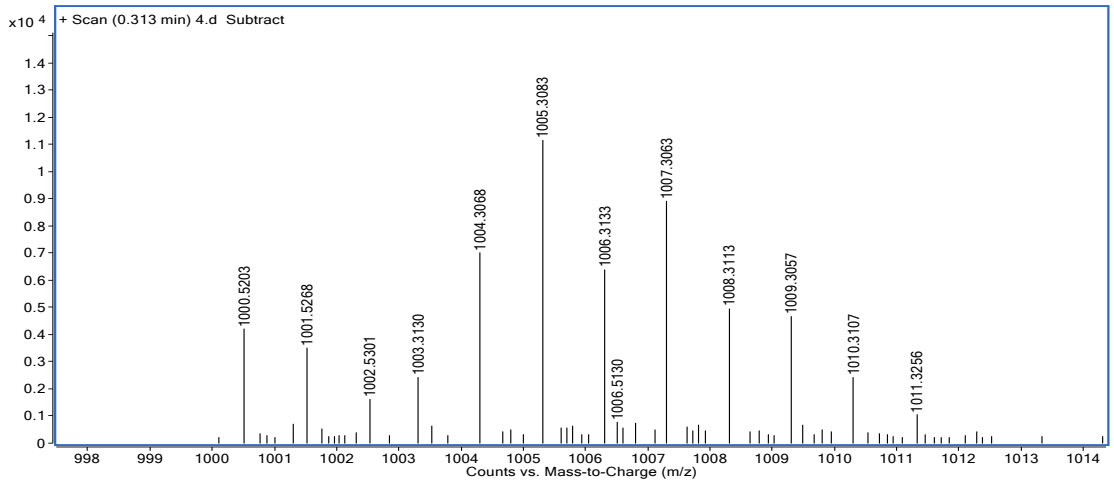



**Figure S14** HRMS (ESI) spectrum of product of **CR-Pd** reacted with $Pd(AcO)_2$ .