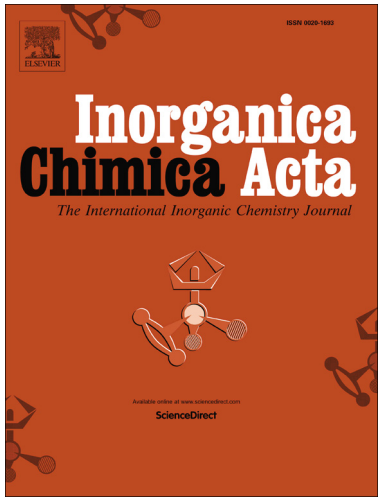

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A new rhodamine B-based 'off-on' colorimetric chemosensor for Pd^{2+} and its imaging in living cells

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ABSTRACT

A colorimetric rhodamine-based fluorescent probe **RBPTA** has been designed to detect Pd^{2+} under mild condition (pH>4) in aquenous solution and living HeLa cells. The probe displays high sensitivity (DL 4.2 nM), selectivity and quick response (20 min) toward Pd^{2+}, driven by a PET process.

Keywords: Fluorescent probe; Rhodamine derivative; Palladium; cell imaging

1. Introduction

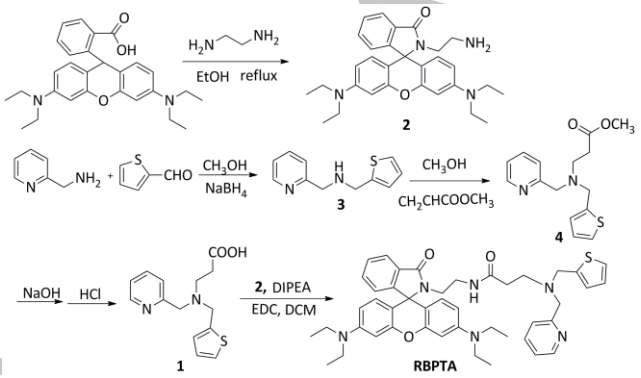
Palladium, one of the platinum group elements, has been widely used in various fields, such as catalytic converters, fuel cells, electronics, medical instruments and jewelry [1-5]. Frequent use of palladium species has resulted in a high level of residual palladium ions in water and soil [6]. Palladium may coordinate with thiols-containing amino acids, DNA, RNA, proteins, vitamin B_6 and further disturb several cellular processes. Excess palladium accumulation would pose a great threat to human health as well [7-9]. Because of the hazardous effects of palladium, European Agency for the Evaluation of Medicinal Products had set the threshold limit of palladium found in drugs as 5-10 ppm, and suggested dietary intake of palladium less than 1.5-15 μg per person per day [10, 11].

Some conventional methods and techniques, atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), solid phase micro extraction-high performance liquid chromatography (SPME-HPLC), and X-ray fluorescence (XRF), have been used to detect palladium [12-16]. Nevertheless, all these approaches require sophisticated and expensive instruments, complicated sample preparation, rigorous experimental conditions, as well as specialized and experienced operators. In contrast, fluorometric determination could avoid the shortcoming of these convention methods in the term of trace analysis and detection, meanwhile maintains the high selectivity and accuracy [18]. Owning to the nature of

fluorometric detection, for instance simple operation, fast response, in situ analysis, low cost, even naked-eye observation without instruments[19, 20], quite a number of efforts have been done to develop fluorescent probes for palladium.

A series of fluorescent probes for palladium detection have been synthesized based on various fluorophores, oxime derivatives [21], rhodamine dyes [22-24], triazole [25], cyanines [26], boron dipyrroethane dyes [27], benzothiazole [28], chromen [29], Naphtalimide [30]. yet companying with some defects in their nature, for example the changes of fluorescence signature can't be observed by eyes, high detection limit, long response time and harsh pH condition [31-35].

Rhodamine fluorophore has a special photoluminescent property, its spirolactam form (no fluorescence) ring-open form (fluorescence) would be triggered by a specific analyte [36-37]. On this basis, we developed a new rhodamine based fluorescence **RBPTA** from pyridine and thiophene moiety with remarkable nature of colorimetric, naked-eye, 'off-on' sensing system for the detection of Pd^{2+} (Scheme 1). To our delight, **RBPTA** exhibited a desired high selectivity and sensitivity for the detection of Pd^{2+}. The sensing process of **RBPTA** for palladium is based on induced opening of spirolactam ring with a brilliant pink colortion and fluorescence emitted (Scheme 2).



Scheme 1. Synthesis of **RBPTA**

2. Experimental

2.1 Materials and methods

2-Aminomethylpyridin, 2-Thenaldehyde, methyl acrylate, sodium borohydride, Rhodamine B, ethanediamine, EDC·HCl and DIPEA were purchased from Energy Chemical, metal ions $AgNO_3$, $CdCl_2$, Cs_2CO_3, CuCl, $FeCl_3$, $SnCl_2{\cdot}2H_2O$, $HgCl_2$, $CaCl_2$, $FeCl_2{\cdot}4H_2O$, $NiCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$,$MgCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H2O$, $ZnCl_2$, $Pb(NO_3)_2$, $PdCl_2$, NaCl, $AlCl_3$,$MnCl_2$ were purchased from Sinopharm Chemical Chemical Co., Ltd., and reagents of analytical reagent grade. All solvernts purchased from Shanghai Wohua Chemical Co., Ltd., and double-distilled water was used in all experiments. All reagents and organic solvents used were used without further purification. Thin-layer chromatography was performed on a HAIYANG silica gel F254 Plate, and Column chromatography was performed on HAIYANG silica gel (type: 200-300 mesh, 300-400 mesh).

^{1}H NMR and ^{13}C NMR spectra were collected on Bruker-Avance DPX 400MHz, the chemical shifts in parts-per-million (ppm) for NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference. Mass spectra were obtained from mass spectrometer (Agilent-6110). Fluorescent spectra were measured on Spectofluorometer FS5. UV-vis spectra were obtained on U3010-vis spectrophotometer. The pH levels were measured using FE20.

2.2 Sythesis

2.2.1. Compound 3: 1-(pyridin-2-yl)-N-(thiophen-2-ylmetheyl) methanamine:

2-Aminomethylpyridin (1 g, 9.25 mmol) was added to a solution of 2-Thenaldehyde (1.036 g, 9.25 mmol) in methanol (15 ml) with nitrogen atmosphere. The mixture was stirred at room temperature overnight, before sodium borohydride (1.05 g, 27.75 mmol) was slowly added to the mixture at 0 ℃, and the mixture was stirred for 8 hours. The reaction mixture was quenched by water, after methanol was evaporated under reduced pressure, then extracted with dichloromethane (20 mL×3). The organic phases were combined and then washed with saturated brine, dried with Na_2SO_4, then filtered. Removal of the solvents under reduced pressure gave a crude product, which was purified by silica gel chromatography (eluent: EA:PE 1:5) to produce a yellow oily liquid (1.06g, 56%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 7.67 (td, J = 7.6, 1.8 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.27 – 7.21 (m, 1H), 7.19 (ddd, J = 7.5, 4.9, 1.1 Hz, 1H), 6.97 (d, J = 3.4 Hz, 2H), 4.07 (s, 2H), 3.98 (s, 2H), 2.44 (s, 1H). ESI-MS: calcd. for $[M+H]^+$, 205.07; found, 205.0.

2.2.2. Compound 4: Methyl-3-((pyridine-2-ylmethyl) (thiophen-2-ylmethyl) amino) propanoate:

A mixture of compound 3 (0.82 g, 4 mmol) and methyl acrylate (0.69 g, 8 mmol) in dry methanol (10 mL) was refluxed for 5 hours, then methyl acrylate and methanol was evaporated under reduced pressure and the crude compound was purified by silica gel chromatography (eluent: EA:PE 1:5) to produce a yellow oily liquid (0.86 g, 74 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (ddd, J = 4.9, 1.9, 1.0 Hz, 1H), 7.70 (td, J = 7.6, 1.8 Hz, 1H), 7.57 (dt, J = 7.9, 1.1 Hz, 1H), 7.27 – 7.21 (m, 1H), 7.18 (ddd, J = 7.5, 4.9, 1.3 Hz, 1H), 6.98 – 6.91 (m, 2H), 3.90 (s, 2H), 3.83 (s, 2H), 3.67 (s, 3H), 2.92 (t, J = 7.2 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H). ESI-MS: calcd. for $[M+H]^+$, 291.11; found,290.9.

2.2.3. Compound 1: 3-((pyridine-2-ylmethyl)(thiophen-2-ylmethyl)amino)-propanoic acid

A solution of compound 4 (1 g, 3.5 mmol) in 10mL methanol was added NaOH (0.42 g, 10.5 mmol), then the mixture was refluxed for 3 hours, then the reaction mixture was cooled to 0 ℃, and neutralized with dilute HCl, the solvent was evaporated, the residue was purified silica gel chromatography (eluent: MeOH:DCM 1:50) to produce a light

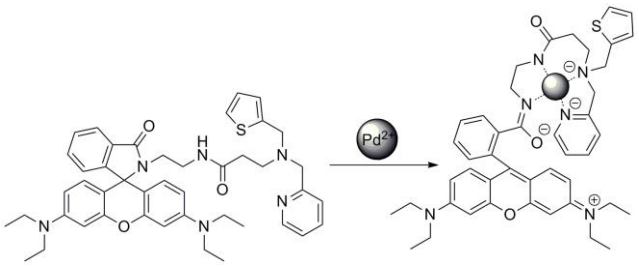
yellow powder (0.54 g, 56 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.46 (s, 1H), 8.58 (d, J = 5.0 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.24 (dd, J = 11.1, 6.4 Hz, 2H), 6.97 (p, J = 3.4 Hz, 2H), 3.99 (s, 2H), 3.88 (s, 2H), 2.96 (t, J = 6.4 Hz, 2H), 2.58 (t, J = 6.4 Hz, 2H). ESI-MS: calcd. for $[M+H]^+$ 277.35; found, 276.2.

2.2.4. Compound 2 2-(2-aminoethyl)-3', 6'-bis (diethylamino) spiro [isoindoline- 1,9'-xant-hen]-3-one

A solution of Rhodamine B (2 g, 4.499 mmol) in ethyl alcohol (30 mL) was heated for 20 minutes, then ethanediamine (3.0 ml, 44.98 mmol), the mixture was refluxed for 12 hours. The mixture was evaporated in reduced pressure, the distilled water was added to the residue, and the mixture was extracted with dichloromethane (50 mL×3), then the organic phase was combined, washed with saturated brine, and dried with Na_2SO_4, then filtered, Removal of the solvents under reduced pressure gave a crude product, which was purified by silica gel chromatography (eluent: MeOH:DCM 1:50) to produce a light yellow powder (1.81 g, 83 %).^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 – 7.87 (m, 1H), 7.51 – 7.44 (m, 2H), 7.16 – 7.07 (m, 1H), 6.45 (d, J = 8.8 Hz, 2H), 6.39 (d, J = 2.6 Hz, 2H), 6.29 (dd, J = 8.9, 2.6 Hz, 2H), 3.35 (q, J = 7.1 Hz, 8H), 3.21 (t, J = 6.6 Hz, 2H), 2.45 (t, J = 6.6 Hz, 2H), 1.65 (s, 2H), 1.18 (t, J = 7.0 Hz, 12H). ESI-MS: calcd. for $[M+H]^+$ 485.28; found, 485.3.

2.2.5. Compound RBPTA

Compound 1 (0.5g, 1.81mmol), EDC (0.38g, 2mmol), DIPEA (0.78g, 6mmol) were stirred in dichloromethane for 0.5 hour. Compound 2 (0.88g, 1.8 1mmol) was added the mixture was stirred in room temperature for 8 hours. The mixture was evaporated under reduced pressure, the residue was dissolve by dichloromethane, the solution was washed by diluted citric acid, saturated sodium bicarbonate, and saturated brine, the mixture was dried with Na_2SO_4, then filtered, Removal of the solvents under reduced pressure gave a crude product, which was purified by silica gel chromatography (eluent: MeOH:DCM 1:50) to produce a pink powder (1.05g, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.49 (dd, J = 5.0, 1.7 Hz, 1H), 7.96 – 7.88 (m, 1H), 7.62 (td, J = 7.7, 1.8 Hz, 1H), 7.47 (tt, J = 7.5, 3.4 Hz, 3H), 7.39 (t, J = 4.8 Hz, 1H), 7.18 (dd, J = 5.0, 1.3 Hz, 1H), 7.16 – 7.05 (m, 2H), 6.97 – 6.87 (m, 2H), 6.48 – 6.35 (m, 4H), 6.26 (dd, J = 8.9, 2.6 Hz, 2H), 3.88 (s, 2H), 3.80 (s, 2H), 3.33 (p, J = 6.7 Hz, 10H), 3.06 (q, J = 5.5 Hz, 2H), 2.84 (t, J = 6.9 Hz, 2H), 2.37 (t, J = 6.8 Hz, 2H), 1.17 (t, J = 7.0 Hz, 12H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.52 , 169.47 , 159.22 , 153.87 , 153.29 , 148.96 , 148.86 , 141.85 , 136.50 , 132.63 , 130.61 , 128.52 , 128.08 , 126.46 , 126.17 , 124.99 , 123.88 , 122.97 , 122.79 , 122.02 , 108.20 , 105.03 , 97.83 , 65.46 , 59.33 , 52.45 , 49.66 , 44.33 , 40.02 , 39.81 , 34.22 , 12.63 . ESI-MS: calcd. for $[M+H]^+$ 743.37; found, 743.3 Elemental analysis (%): calcd. for RBPTA C 71.13, N 11.31, H 6.78; found, C 71.21, N 11.45, H 6.79.



Scheme 2. **RBPTA** and Pd^{2+} sensing process

2.3. Stock solution

Ionic stock solution(10^{-3} M) of chloride salts of Cd^{2+}, Cu^{+}, Fe^{3+}, Sn^{2+}, Hg^{2+}, Ca^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, Co^{2+}, K^{+}, Zn^{2+}, Pb^{2+}, Na^{+}, Al^{3+}, Mn^{2+}, Pd^{2+}, Pt^{2+} and Cs_2CO_3, $AgNO_3$, $Ba(NO_3)_2$ were prepared in EtOH/H_2O (3:2, v/v, pH 7.4, HEPES buffer, 0.5 mM). And probe's stock solution (1 mM) of RBPTA was prepared in EtOH/H_2O (3:2, v/v, pH 7.4, HEPES buffer 0.5 mM).

2.4. General method of UV-vis and fluorescence

The UV-vis and fluorescent methods were carried out to evaluate the nature of RBPTA. Test samples were prepared by free stock solution (EtOH/H_2O 3:2 v/v, pH 7.4, HEPES buffer, 0.1 mM), and the UV-vis absorption spectra fluorescent spectra were measure in 25 ℃.And in fluorescence measurement fluorescence intensity at λ_{ex} = 566 nm, λ_{em} = 584 nm.

2.5. Cells studies of RBPTA

2.5.1. Cytotoxicity of RBPTA

RBPTA was dissolved in DMSO as a stock solution, then **RBPTA** concentrations in cell-culture medium were controlled from 0 to 15 μM, then HeLa cells were incubated with the solutions above. Last MTT assay was executed to evaluate the cytotoxicity of RBPTA.

2.5.2. fluorescent imaging in living cells

Control group: HeLa cells was incubated with **RBPTA** (10 μM). After 30 min, cell-culture medium was removed, and fresh medium was added. Then fluorescence imaging experiment was taken (λ_{ex}=566 nm, λ_{em}=586 nm).

Experimental group: HeLa cells was incubated with **RBPTA** (10 μM) for 30 min, then with $PdCl_2$ (10 μM) for 30 min. Cell-culture medium was removed, and fresh medium was added. Last fluorescence imaging experiment was taken (λ_{ex}=566 nm, λ_{em}=586 nm).

2.6. Preparation of paper test

Filter papers was dipped in the stock solution of **RBPTA** (1 mM), then test papers were dried in air to measure different concentrations of Pd^{2+}.

3. Results and discussion

3.1. pH response of RBPTA

The pH response of **RBPTA** in EtOH/H_2O (3:2, v/v, pH 7.4, HE-PES buffer, 0.5 mM) solution, acid base titration was taken (pH=1.00, 2.06, 3.03, 4.08, 5.14, 6.03, 7.0 6, 8.06, 8.9 2, 10.05, 11.04, 12.03), and the fluorescent intensities of **RBPTA** at 566 nm were recorded (Figure 1). It showed that **RBPTA** did not emit fluorescence (λ_{ex}=566 nm) at pH>5, it means that the spirolactam of **RBPTA** would be kept in pH 5-12. And the fluorescence intensity (λ_{ex}=566 nm) tended to be stable at pH<3, for the ring-opening of the spirolactam. These results suggested that **RBPTA** recognized palladium with pretty low background fluorescence at pH from 5 to 12. Herein, further UV-vis and fluorescence studies were carried out in EtOH /H_2O (3:2, v/v, PH 7.40, HEPES buffer, 0.5 mM) solution.

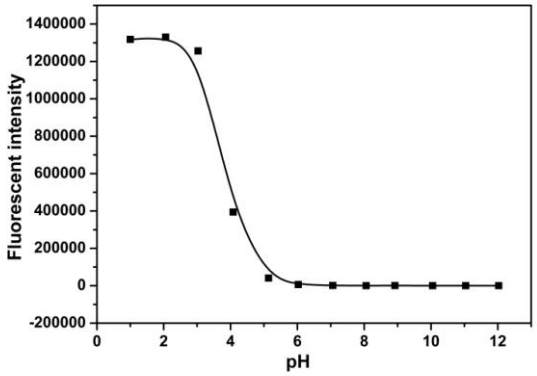


Figure 1 The fluorescent intensity of **RBPTA** (10 μM) show effect of ph in EtOH/H_2O (3:2 V/ V) at 25 ℃. λ_{ex}=566 nm, λ_{em}=586 nm.

3.2. Response time

Response time of probe of **RBPTA** was investigated by fluorescence method (Figure 2). Test sample was prepared by placing 10 μM **RBPTA** and 5 equiv of Pd^{2+} in 3 mL free stock solution (EtOH/H_2O 3:2 v/v, pH 7.4, HEPES buffer, 0.1mM), and the sample was tested immediately, until the fluorescence intensity of sample reached the maximum value(0-28 min).

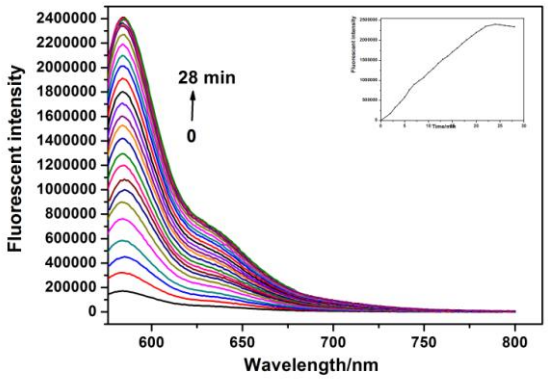


Figure 2. Fluorescence intensity of **RBPTA** ((10 μM)) with Pd^{2+} (5 eq) over a period of 28 min, λ_{ex}= 566 nm. Inset: Plot of the fluorescence intensities at 584 nm over a period of 28 min.

3.3. Job's plot of RBPTA and Pd^{2+}

The solutions of Pd^{2+} and **RBPTA** were prepared with a total concentration of 50 μM, and the mole fraction of Pd^{2+} to **RBPTA** from ranged from 0 to 1. A job's plot (Figure. 3) was generated to revealed that **RBPTA** and palladium complex is 1:1 stoichiometry.

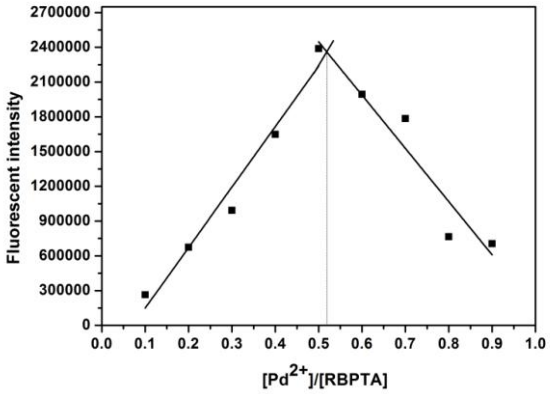


Figure 3. Job's plot of RBPTA complexing with Pd^{2+}, λ_{ex}=566 nm, λ_{em}=586 nm.

3.4. Fluorescence titration

The cation binding properties of **RBPTA** are studied by using the chloride salts of Cd^{2+}, Cs^{3+}, Cu^{+}, Fe^{3+}, Sn^{2+}, Hg^{2+}, Ca^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, Co^{2+}, K^{+}, Zn^{2+}, Pb^{2+}, Na^{+}, Al^{3+}, Mn^{2+}, Pd^{2+}, Pt^{2+} and $AgNO_3$, $Ba(NO_3)_2$ in $EtOH/H_2O$ (3:2, v/v, pH 7.4, HEPES buffer, 0.5 mM) solution. Upon addition of cations to a colorless solution of **RBPTA** (0.2 equiv. 10 μM), only Pd^{2+} could urge the mixture to emit fluorescence and become pink (Figure 4a), whereas others cannot. It suggested that **RBPTA** can function as a highly selective chemosensor for Pd^{2+}. Then, the fluorescence titration experiment (Figure 4b) was taken to further demonstrated high sensitivity of **RBPTA** to detect Pd^{2+}.

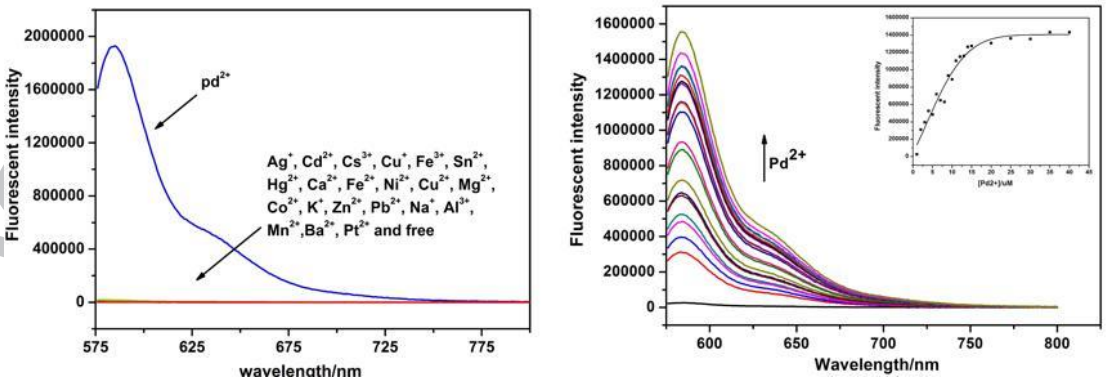


Figure 4. a) **RBPTA** (10 μM) and cations (50 μM) in $EtOH/H_2O$ (3:2, v/v, pH 7.4, HEPES buffer, 0.5 nM) solution, after 4 hours' standing at 25 ℃. The excitation and emission wavelengths were 566 nm and 584 nm, respectively. b) fluorescence spectra of addition of different amounts of Pd^{2+} in $EtOH/H_2O$ (3:2, v/v, pH 7.4, HEPES buffer, 0.5 nM) solution, after 4 hours' standing at 25 ℃. λ_{ex}=566 nm, λ_{em}=586 nm.

3.5. Binding association constant

Moreover, association constant value of Pd^{2+} with **RBPTA** (Figure 5), based on fluorescence titration, has been calculated by emission intensity date following the modified Benesi-Hildenbrand equation, $F - F_0 = \Delta F = [Pd^{2+}](F_{max} - F_0)/(1/Ka + [Pd^{2+}])$. Here F is fluorescence intensity at λ_{ex} = 566 nm, λ_{em} = 584nm, F_0 is fluorescence intensity of **RBPTA** at λ_{ex} = 566 nm, λ_{em} = 584 nm, Fmax is saturated fluorescence intensity at λ_{ex} = 566 nm, λ_{em} = 584nm at λ_{ex} = 566 nm, λ_{em} = 584nm of **RBPTA**-Pd complex, and Ka is association constant. Ka was evaluated to be 4.88×10^4 M^{-1}.

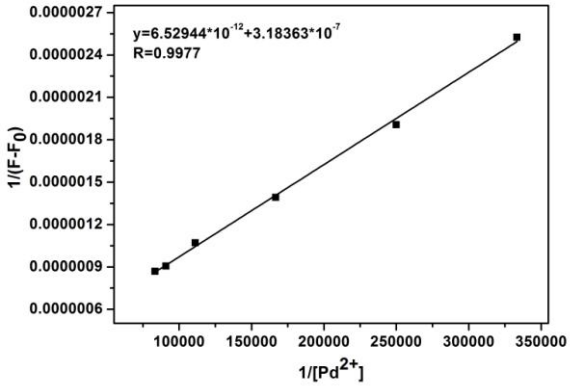


Figure 5. Fluorescence intensity of **RBPTA** (10 μM) at 584 nm upon addition of Pd^{2+} (3 μM, 4 μM, 7 μM, 9 μM, 11 μM, 12 μM).

3.6. Detection limit

The detection limit of **RBPTA**, based on fluorescence titration as well, was calculated by the equation: Detection limit = 3SD/S. The fluorescence emission spectrum of RBPTA was measured 10 times, then the standard deviation (SD) was calculated (supporting materials). Meanwhile, the slop (S) was obtained by plotted the fluorescence intensity at 584 nm with various concentrations of Pd^{2+} (Figure 6). Thus the detection limit was evaluated to be 4.22 nM.

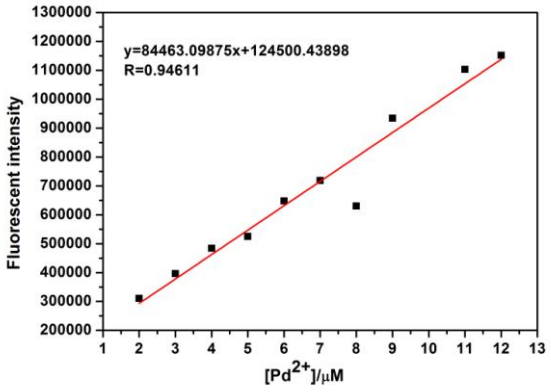


Figure 6. Fluorescence intensity of **RBPTA** ((10 μM)) at 584 nm with different concentration of Pd^{2+}.

3.7. Competition expriments

Competition experiments, to further explore the selectivity of **RBPTA** for palladium, were performed in the presence of Pd^{2+} (5 equiv, 50μM) mixed with others cations (5

equiv, 50 μM): Ag^{+}, Cd^{2+}, Cs^{3+}, Cu^{+}, Fe^{3+}, Sn^{2+}, Hg^{2+}, Ca^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, Co^{2+}, K^{+}, Zn^{2+}, Pb^{2+}, Na^{+}, Al^{3+}, Mn^{2+}, $Ba^{2+}$and Pt^{2+}, respectively, bound to **RBPTA** (1 equiv. 10 μM), the fluorescence studied (Figure 7) showed those free cations would have little interference the sensing of palladium by **RBPTA**.

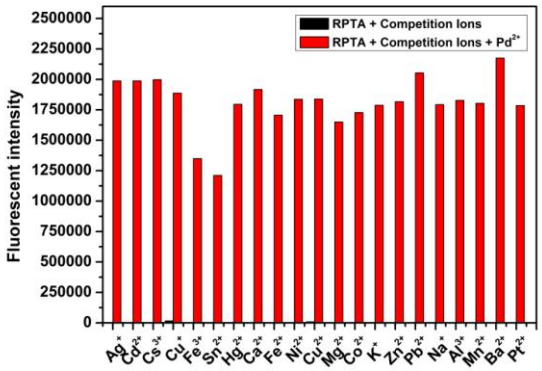


Figure 7. Pd^{2+} (5 equiv, 50 μM) ,free cations (5 equiv, 50 μM), and **RBPTA** (1 equiv 10 μM). after 4 hours' standing at 25 ℃. λ_{ex}=566 nm, λ_{em}=586 nm.

3.8. MS spectra study

The MS spectra Figure 8 shown that the peak appeared at m/z 743.3 before complexing it is corresponds to [**RBPTA** + H]$^{+}$, while the peak appeared at 849.3 m/z after complexing, it assigned to the complex [**RBPTA**$^{2-}$ + Pd^{2+} + H^{+}]$^{+}$. It further confirms that the coordination mode of **RBPTA** and palladium is 1:1 stoichiometry.

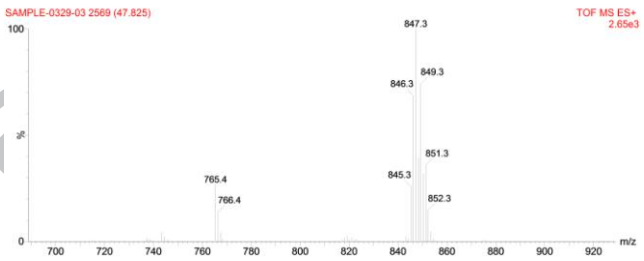


Figure 8. The MS spectrum of RBPTA-Pd^{2+} complex

3.10. Density functional theory (DFT) calculation

The spatial distributions and obital energies of the HOMO and LUMO of RBPTA and **RBTPA-**Pd were generated using DFT calaulations with the B3LYP method using 6-31+G (d, p) as the basis set(Figure 10). It shown that the energy gaps between the HOMO and LUMO in the **RBPTA** and **RBTPA-**Pd were calculated to be 378.3 kJ/mol and 200.3 kJ/mol, respectively, the results exhibited that the binding of palladium to **RBPTA** lowered the HOMO-LUMO energy gap of the complex and stabilized the system

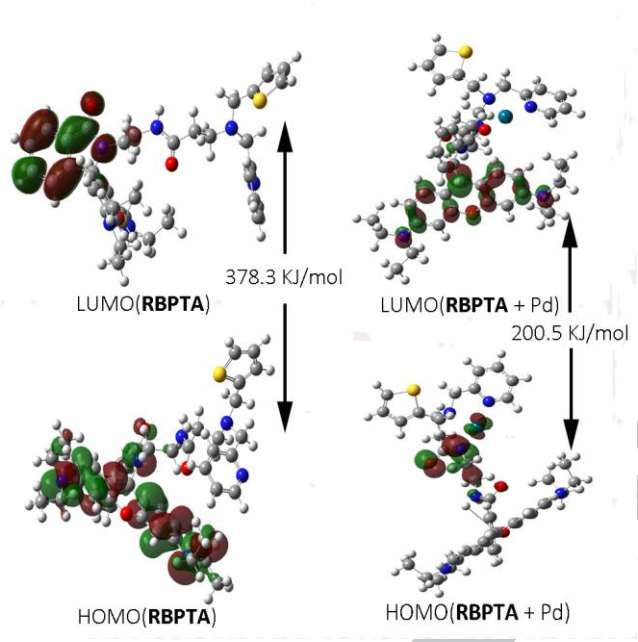


Figure 9. HOMO and LUMO orbitals of **RBPTA** and the **RBPTA-Pd** complex.

3.11. Cell studies

Moreover, cytotoxicity assays were taken to evaluate the cytotoxicity of **RBPTA**. An MTT assay was taken with different **RBPTA** concentrations from 0 to 15 μM in HeLa cells. As shown in Figure 10, the probe showed no toxicity to HeLa cells, the results suggested that the probe could be used to detect Pd^{2+} in biological samples.

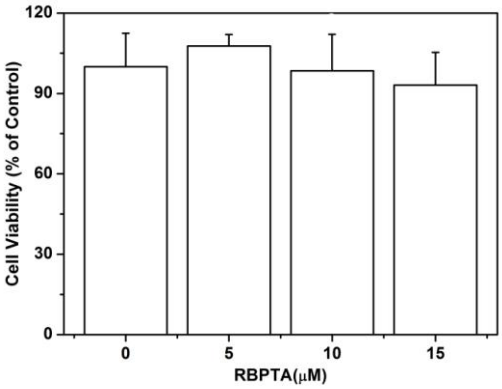


Figure 10 Cytotoxicity of **RBPTA** in HeLa

Encouraged by the promising properties of **RBPTA**, the imaging applications in living cells was investigated to search whether **RBPTA** could realize the detection of Pd^{2+} in living cells. As shown in Figure 11, HeLa cells which were incubated with **RBPTA** (10 μM) showed no fluorescence (Figure 11c), while it display strong green fluorescence after being treated with $PdCl_2$ (Figure 11g).The result indicate that **RBPTA** is capable of fluorescence imaging of palladium in living cells.

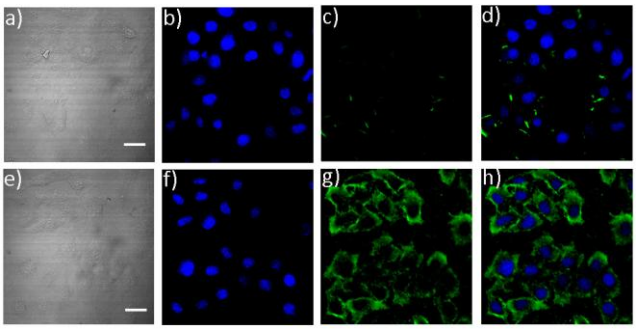


Figure 11. Bright-field and fluorescence image of HeLa cells: (a-d)HeLa cells only incubated with the probe (10 μM) for 0.5 h in 37℃: (a) bright-field image; (b) fluorescence image from blue channel; (c) fluorescence image from green channel; (d) merge image of (b) and (c); further treated with $PdCl_2$ for 0.5 h in 37 ℃: (e) bright-field image; (f) fluorescence image from blue channel; (g) fluoresceence image from green channel; (h) merge image of (f) and (g). scale bar 2 μm.

3.12. Test papers

What is more, practical application, test papers, was conducted. Filter paper was dipped in stock solution with **RBPTA** (1 mM), then was exposed to different concentration of Pd^{2+}. Last test paper was observed under 356 nm UV light (Figure12). It shown that the **RBPTA** can be made as a test paper to ration detect for Pd^{2+}.

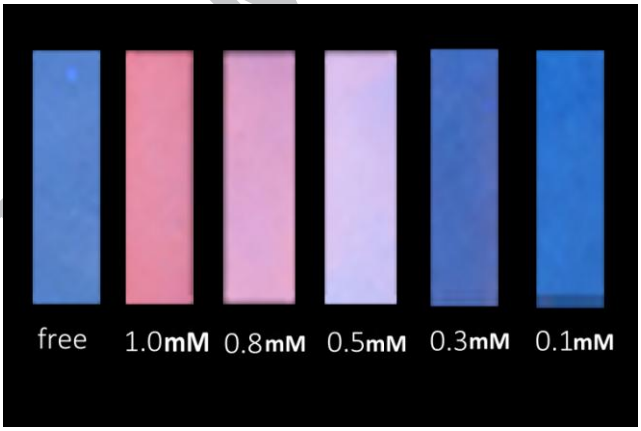
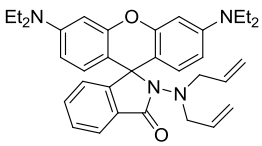
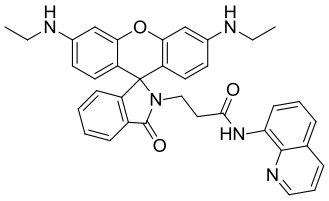
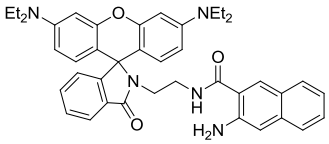
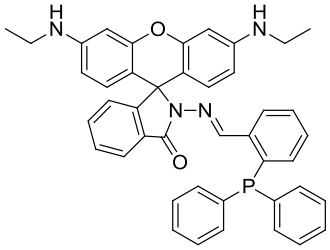
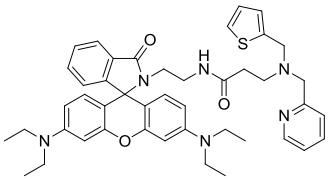


Figure 12. Test paper, treated with different concentrations of Pd^{2+}, taken under 356 nm UV light.

3. 13. Comparison of Pd^{2+} probes

Table 1 comparison of Pd^{2+} probes

Probe	Chemical structure	E_x/E_m	Detection limit/mol·L^{-1}	Solvent system	Application
Ref. 18		530/580	1.85×10^{-7}	Ethanol/Water (1:1)	Test paper

Ref. 4		550/562	Not mentioned	Ethanol/Water (1:1)	Not mentioned
Ref. 29		420/590	Not mentioned	Ethanol/Water (1:1)	Mice imaging
Ref. 34		505/552	5.0×10^{-9}	Ethanol/Water (1:1)	Test paper
This work		566/586	4.2×10^{-9}	Ethanol/Water (3:2)	Cells imaging Test paper

4. Conclusion

In conclusion, a new rhodamine fluorescent probe for palladium species with high sensitivity and selectivity and quick response was developed. Additionally, **RBPTA** can be applied to detect Pd^{2+} in both water sample, and living cells. **RBPTA** could be made as test paper to detect Pd^{2+} with quantitation as well.

Acknowledgements

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Some of the important highlights of this work are following.

1. **RBPTA**is used as a chemosensor for selective and quantitative detection of palladium with a quite low limitation as 4.22 nM.
2. **RBPTA**is highly selective to palladium without interference from other cations.
3. **RBPTA**detects palladium under mild condition (pH>4) in aquenous solution.
4. **RBPTA**displays quick response (20 min)toward palladium.
5. **RBPTA**can be utilized as a fluorescent probe for the detection of palladium in living cells (HeLa cells).
6. **RBPTA** can be made as a test paper to ration detect for palladium.