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Naphthylamine–Rhodamine-Based Ratiometric Fluorescent Probe for the Determination of Pd^{2+} Ions

Shiguo Sun,* Bo Qiao, Na Jiang, Jitao Wang, Si Zhang, and Xiaojun Peng*

State Key Laboratory of Fine Chemicals, Dalian University of Technology, E224 West Campus, No. 2, Linggong Road, Ganjingzi District, 116024 Dalian, China

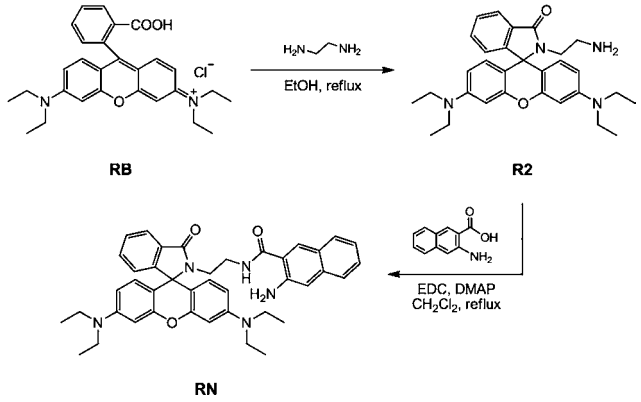
Supporting Information

ABSTRACT: A naphthylamine–rhodamine hybrid ratiometric and colorimetric fluorescent probe (**RN**) was designed and synthesized. **RN** can identify Pd^{2+} ions with high selectivity and sensitivity. Furthermore, the probe can be used to monitor Pd^{2+} ions in live mice by fluorescence imaging.

Palladium, one of the platinum-group elements, is hazardous to humans because it can cause severe primary skin and eye irritations.[1] Palladium can coordinate with DNA, thiol-containing amino acids, proteins, and vitamin B6 and disturb several cellular processes.[2] However, palladium is ideally suited for catalysts (chemical synthesis catalysts[3] and automobile exhaust catalysts[4]), dental materials, electrical equipments, fuel cells, and jewelry. The extensive use of palladium increases the risks associated with health hazards;[5] therefore, methods for highly selective and sensitive detection of palladium are necessary. Traditional analytical methods (atomic absorption spectrometry, plasma emission spectroscopy, solid-phase microextraction–high-performance liquid chromatography, and X-ray fluorescence) can quickly detect palladium with high sensitivity but need expensive instrumentation and highly skilled individuals.[6] Fluorescence methods, however, can avoid these shortcomings while maintaining the efficiency and accuracy of the traditional methods and therefore have been exploited by researchers.[7]

Pd^{2+} ion is well-known for its fluorescence-quenching abilities. Qian and co-workers[8] designed and synthesized a naphthalimide derivative to detect Pd^{2+} ions. On selective complexation with Pd^{2+}, the fluorescence of naphthalimide probe was quenched. Practically, for small changes in fluorescence, monitoring an off–on fluorescence signal is more reliable than an on–off signal (as in the case of naphthalimide derivatives). The rhodamine platform has been widely exploited to construct off–on fluorescence probes for the identification of Pd^{2+} ions.[9] However, these on–off and off–on probes are significantly influenced by the excitation power and detector sensitivity, while some of the synthesized ratiometric probes are less sensitive to these factors.[10] In this study, naphthylamine and rhodamine were conjugated to synthesize a ratiometric fluorescent probe (**RN**, Scheme 1) to identify Pd^{2+} ions. This probe was based on coordination reaction. When complexed with Pd^{2+}, the aquamarine blue fluorescence of naphthylamine was quenched; simultaneously, the spirolactam ring of rhodamine was opened and accompanied by the appearance of red fluorescence. The probe has high selectivity for Pd^{2+} among the metal ions (including the platinum-group metal ions).

Scheme 1. Structure and Synthesis of RN



The synthetic scheme for the synthesis of **RN** is shown in Scheme 1. The intermediate **R2** was synthesized by a reported synthetic procedure using rhodamine B and ethylenediamine as reactants.[11] Reaction of **R2** with 3-amino-2-naphthoic acid afforded the probe **RN**. The structure of **RN** was characterized by ^{1}H NMR, ^{13}C NMR, and HRMS.

Both in solid state and in solution, **RN** emits aquamarine blue fluorescence (characteristic of the naphthylamine moiety). The absence of red fluorescence in the emission spectra indicated a spirolactam ring-closed form of the rhodamine moiety in the metal-free solution of the probe. The spirolactam ring of the rhodamine moiety is susceptible to changes in the

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pH; pH promotes ring-opening in the rhodamine and leads to emission of red fluorescence. Therefore, the fluorescence properties of **RN** were monitored by measuring the fluorescence intensity ratio ($I_{590\ nm}/I_{490\ nm}$) over a range of pH values (2−12, Figure 1). It can be clearly seen that the

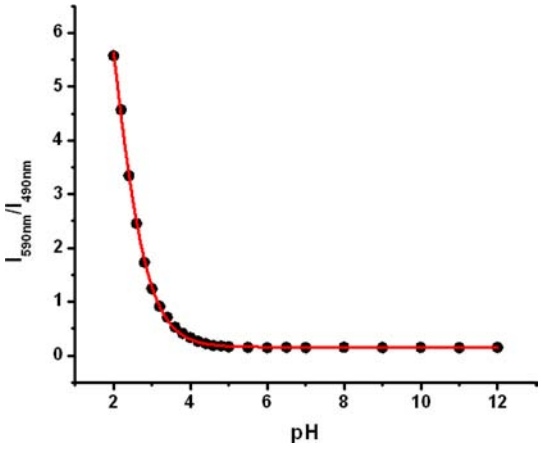


Figure 1. pH-dependent variation in fluorescence intensity ratio ($I_{590\ nm}/I_{490\ nm}$) of **RN** (10 μM) in EtOH/H_2O (1:1, v/v), λ_{ex} = 420 nm.

fluorescence intensity ratio ($I_{590\ nm}/I_{490\ nm}$) of the probe is nearly constant in the 4−12 pH range, and the pK_a value of **RN** is 1.97 ± 0.06. Therefore, the near-neutral EtOH/H_2O (1:1, v/v) system was used in the subsequent assays.

The equilibration time for the complexation was evaluated between **RN** and Pd^{2+} ion (Figure S1, Supporting Information). After the addition of Pd^{2+} ions, the fluorescent intensity ratio ($I_{590\ nm}/I_{490\ nm}$) quickly increased for the first 5 min and reached a maximum in 10 min. Therefore, subsequent fluorescent measurements were recorded after a 10 min incubation period.

The change in fluorescence spectra of **RN** upon titration with $PdCl_2$ is displayed in Figure 2. In the absence of palladium, **RN** (10 μM) in the EtOH/H_2O (1:1, v/v) displayed an emission peak with a maxima at 490 nm. With continuous addition of

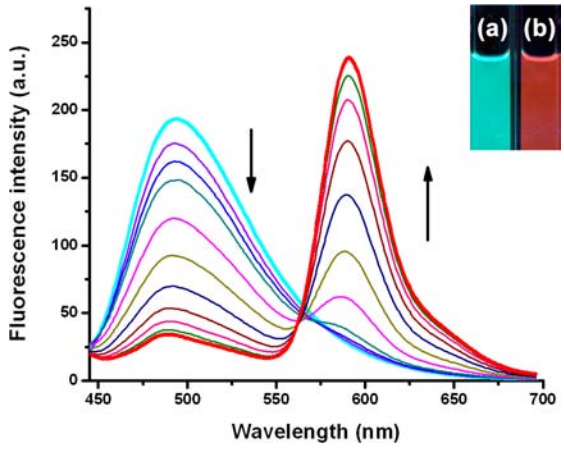


Figure 2. Fluorescence spectra of **RN** (10 μM) upon titration with $PdCl_2$ (0−10 μM) in EtOH/H_2O (1:1, v/v) at room temperature. All spectra were recorded 10 min after the addition of Pd^{2+} ions. λ_{ex} = 420 nm. Inset: Images showing the change in fluorescence of **RN** (a) before and (b) after the addition of $PdCl_2$.

Pd^{2+} ions, the fluorescence intensity of the peak at 490 nm gradually decreased, while a new emission peak with the maxima at 590 nm appeared and then increased gradually. The fluorescence intensity ratios ($I_{590\ nm}/I_{490\ nm}$) of **RN** exhibited a linear relationship in the concentration range from 0 μM to 2 μM Pd^{2+} ions (Figure S2, Supporting Information). The limit of detection for Pd^{2+} using the probe **RN** was 45.9 nM.[12] For all concentrations of Pd^{2+} ions above 10 μM, the fluorescence spectra exhibited no significant changes, and the intensity of absorption at 569 nm also reached saturation (Figure S3, Supporting Information). The changes in emission spectra occur up to a 1:1 [Pd^{2+}]/[**RN**] ratio, indicating the formation of a 1:1 complex. This ratio between the metal and **RN** was confirmed by the Job plot (Figure 3). Mass spectrometric

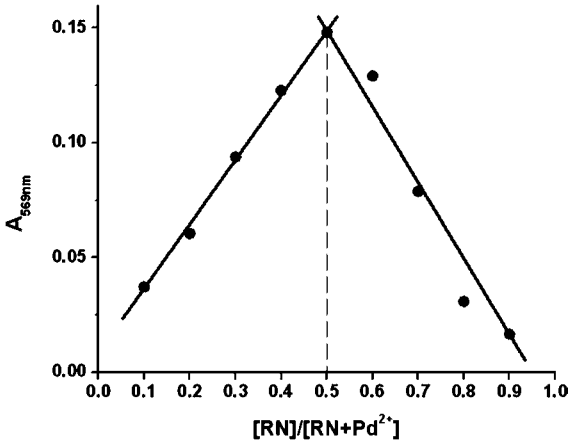


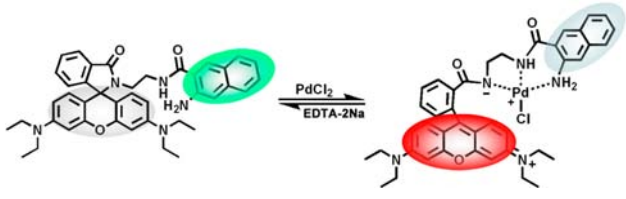
Figure 3. Job plot for the complexation of Pd^{2+} ion with **RN** determined by UV−vis method (at 569 nm). Total concentration of **RN** and Pd^{2+} ions is 20 μM.

analysis (Figure S4, Supporting Information) provided further support for the formation of the 1:1 complex; m/z_{obsd} = 794.25, m/z_{calcd} for [**RN** + Pd^{2+} + Cl^-]$^+$ = 794.21.

The **RN**/Pd^{2+} complex was titrated with EDTA−2Na to determine the nature of the binding (Figure S5, Supporting Information). With the increase in the concentration of EDTA−2Na, the fluorescence intensity at 590 nm gradually decreased, while the intensity of the peak with the maxima at 490 nm increased. Excess EDTA−2Na completely quenched the fluorescence at 590 nm. This phenomenon likely resulted from the removal of Pd^{2+} ion from **RN**, leading to the reconstitution of the spirolactam ring in the rhodamine moiety and hence the loss of fluorescence at 590 nm. From these observations, the mechanism can be proposed in Scheme 2 for the detection of Pd^{2+} ions by **RN**.

Next, the selectivity of **RN** was evaluated. Common metal ions (Na^+, K^+, Ag^+, Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+}, Fe^{3+},

Scheme 2. Proposed Mechanism for the Identification of Pd^{2+} Ion by RN



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Fe^{2+}, Co^{3+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Al^{3+}, Mg^{2+}, and La^{3+}) and the platinum-group metal ions (Ru^{3+}, Rh^{3+}, and Pt^{2+}) were tested for their ability to influence the fluorescence behavior of **RN** in these experiments. Only Pd^{2+} ions induced a large increase in fluorescence intensity ratio of **RN**, while the increase due to other metal ions was barely above the background (Figure 4).

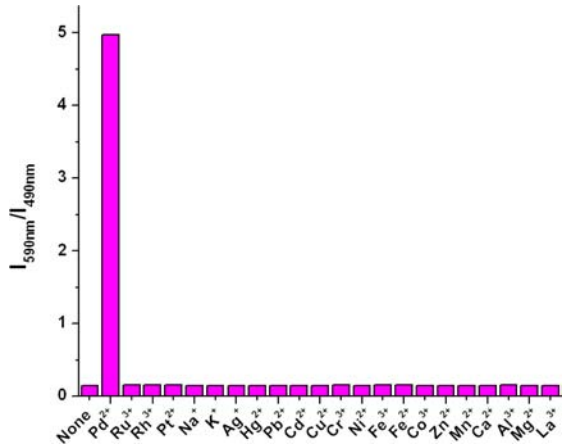

Figure 4. Fluorescence intensity ratio ($I_{590\ nm}/I_{490\ nm}$) of **RN** (10 μM) in the presence of different metal ions (10 μM for Pd^{2+} ions and 20 μM for other metals ions) in EtOH/H_2O (1:1, v/v). λ_{ex} = 420 nm.

These observations demonstrate that the probe **RN** can specifically recognize Pd^{2+} ions with high selectivity. Interference experiments to study the effects of other ions in the identification of Pd^{2+} ions was also performed. None of metal ions caused any significant quenching of the fluorescence resulting from the complexation of **RN** with Pd^{2+} ion (Figure S7, Supporting Information).

The ability of **RN** to determine in vivo Pd^{2+} ion concentration was evaluated by fluorescent imaging. First, an EtOH/H_2O/DMSO (2:2:1, v/v) solution (100 μL) containing **RN** (200 μM) was introduced by intraperitoneal injection into shaved living mice; no fluorescence signal was collected (Figure 5, a). Then an EtOH/H_2O (1:1, v/v) solution (100 μL) containing $PdCl_2$ (100 μM) was subcutaneously injected, and it was clear that a fluorescence signal was received (Figure 5, b). The same results were achieved when the $PdCl_2$ concentrations were 200 μM (Figure 5, c) and 500 μM (Figure 5, d), and the fluorescence intensity increased with an increase of $PdCl_2$ concentration. These results showed that probe **RN** can be used as a Pd^{2+}-selective probe for in vivo fluorescence imaging.

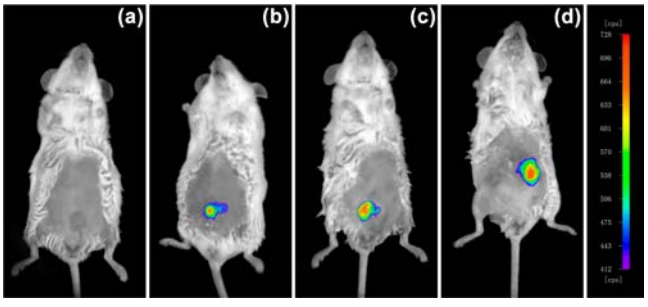

Figure 5. Fluorescent images of living mice. Subcutaneous injection of the solution of **RN** (200 μM, a), subcutaneous injection of the solution of **RN** (200 μM), and then subcutaneous injection of the solution of $PdCl_2$ (b, 100 μM; c, 200 μM; d, 500 μM). Images were taken 20 min after the subcutaneous injection of $PdCl_2$, with the excitation filter at 480 nm and the emission filter set at 600 ± 20 nm.

In conclusion, an **RN** probe for highly selective detection of Pd^{2+} ions was developed. The application of this probe for in vivo imaging was demonstrated. Unlike previously reported Pd-catalyzed reaction-based ratiometric probes, the change in the optical character of the probe **RN** is induced by complexation with Pd^{2+} ion, thereby providing a reversible ratiometric probe for the identification of Pd^{2+}.

■ ASSOCIATED CONTENT

Supporting Information

Reagents and instruments, synthesis procedures, additional spectroscopic data, ^1H NMR, ^{13}C NMR, and MS. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: shiguo@dlut.edu.cn.
*E-mail: pengxj@dlut.edu.cn.

Notes

The authors declare no competing financial interest.

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