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A 4-hydroxynaphthalimide-derived ratiometric fluorescent chemodosimeter for imaging palladium in living cells†

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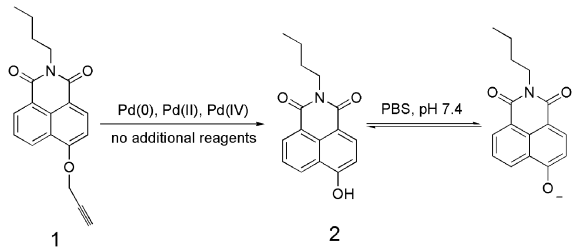
A highly selective ratiometric fluorescent chemodosimeter derived from 4-hydroxynaphthalimide was designed and synthesized to image palladium species in living cells by virtue of a palladium-catalyzed depropargylation reaction, and it could monitor three typical palladium species (0, + 2 and + 4) without additional reagents.

Fluorescent indicators for the imaging of poisonous species in living matrices are of increasingly significant importance for biological toxicity research.[1] Recently, great attention has been paid to the determination of palladium (Pd) species owing to serious environmental and health problems resulting from its wide use in various materials, such as dental crowns, fuel cells, jewelry, and especially catalysts in synthesizing various drugs.[2] Despite advances in the development of highly selective and sensitive fluorescent indicators for palladium,[3] there are still some limitations in the quantitative detection and bioimaging. For example, almost all of these available systems recognize palladium only by the changes in fluorescence intensity, and are prone to be disturbed in the quantitative detection by many factors, such as variabilities in excitation and emission efficiency, sample environments, and probe distribution.[4] Additionally, most of the reported fluorescent indicators for palladium are based on the fluorescein and rhodamine fluorophores possessing small Stokes shifts, which have potential difficulties in the quantitative determination and bioimaging because of the excitation interference.[5] A possible solution to the above-mentioned problems is to utilize a ratiometric indicator that not only exhibits a larger Stokes shift, but can eliminate most or all ambiguities by self-calibration of two emission bands.[6] Furthermore, so far, few reports on the imaging of palladium in living systems have been published.[3n] Thus, novel cell-permeable ratiometric fluorescent indicators for palladium species become our target.



Scheme 1 Recognition mechanism of **1** toward palladium.

Very recently, we reported some ratiometric fluorescent indicators based on the internal charge transfer (ICT) mechanism and their bioimaging applications.[7] In connection with our continuing research, we herein describe the design and synthesis of a cell-permeable ratiometric fluorescent chemodosimeter **1** (Scheme 1, **1**) for the palladium employed ICT mechanism.

1,8-Naphthalimide with an electron donor and an acceptor group is characteristic of an ICT fluorophore, and its derivatives including 4-amino-1,8-naphthalimide, 3-amino-1,8-naphthalimide, 4,5-diamino-1,8-naphthalimide, and 3,4-diamino-1,8-naphthalimide, have been frequently used in ratiometric fluoroionophores.[8] However, up to now, to the best of our knowledge, no reports on fluorescent indicators derived from 4-hydroxy-1,8-naphthalimide (Scheme 1, **2**) have been published. To exploit its application in ratiometric fluorescent indicators, we investigated the photophysical and chemical properties of **2**. Gratifyingly, we found that compound **2** in the alkalescent aqueous solution has longer wavelength fluorescence (green fluorescence) than 4-alkoxy derivatives owing to the stronger electron-donor ability of oxygen anions. Additionally, chemodosimeters have been used to detect an analyte through a highly selective chemical reaction between the dosimeter molecule and the target analyte, and they provide an ideal way to design off–on fluorescent indicators for the quenching of the heavy metal ions.[9] So, the aim of our work was to find a specific reaction for palladium species. Pal and co-workers demonstrated in detail the deprotection of aryl propargyl ethers/amines in the presence of a palladium catalyst.[10] This strategy inspired us to construct a colorimetric and ratiometric fluorescent chemodosimeter **1**. We expected that palladium

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would react with **1** and further lead to the cleavage of propargyl ethers, and as a result, restore the longer wavelength fluorescence of **2**. The recognition mechanism of **1** toward palladium is shown in Scheme 1. Chemodosimeter **1** was easily synthesized from propargyl hydroxide and *N*-butyl-4-bromo-1,8-naphthalimide, which is prepared from 4-bromo-1,8-naphthalic anhydride and *n*-butylamine. Detailed procedures and characterizations were described in the ESI.†

We firstly assessed the spectroscopic properties of chemodosimeter **1** under simulated physiological conditions (20 mM phosphate buffer saline (PBS), pH 7.4). In the absence of palladium species, the chemodosimeter solution exhibits one major absorption peak at 364 nm and fluorescence emission peak at 480 nm. Exhilaratingly, under the above mild conditions, the additions of three typical palladium species (0, + 2 and + 4) resulted in the remarkable absorption and fluorescence changes of chemodosimeter **1** without extra reagents, respectively. To disclose the sensing mechanism of **1** toward palladium species, the reaction of **1** with palladium species was conducted under the same conditions as described above. The reaction products were subjected to electrospray ionization mass spectral analysis. The peak at *m*/*z* 268.09794 $[M–H]^-$ corresponding to compound **2** was observed (Fig. S1, ESI†). Additionally, the green fluorescent reaction product was obtained and characterized to be compound **2** by ^{1}H NMR. So, this recognition is implemented by the depropargylation reaction of chemodosimeter **1** (Scheme 1). We then attempted to clarify the process of depropargylation reaction by investigating the kinetic profiles of the recognition. The result reveals that chemodosimeter **1** responds to palladium species in the following order: Pd(IV) > Pd(II) > Pd(0) (Fig. S3, ESI†). Thus, combined with the previously reported conclusions,[3n,10,11] the depropargylation reaction can occur *via* directly Pd(IV)/Pd(II)-catalyzed hydration intermediates or allenylpalladium resulted from the oxidative addition of Pd(0) without additional reagents.

$PdCl_2$ was selected as the representative palladium species in the following experiments for it is the most toxic among them. When $PdCl_2$ was added gradually to the solution of **1**, the maximum absorption peak showed an 89 nm red shift with an isosbestic point at 391 nm (Fig. S5, ESI†). In the fluorescence emission spectrum, upon addition of $PdCl_2$ (final concentration: 50 μM), the maximum emission peak undergoes a red shift to 553 nm, and the ratio of fluorescence intensities (F_{553}/F_{480}) changes from 0.32 to 3.31 (R = 10.3-fold) (Fig. 1a). In addition, a well-defined isoemission point at 516 nm is also observed (Fig. 1a). More importantly, there was a good linearity between the fluorescence intensity ratio, R (F_{553}/F_{480}), and the concentrations of $PdCl_2$ in the range of 0 to 7 μM with a detection limit of 0.07 μM (Fig. 1b), which allowed the determination of $PdCl_2$ by a ratiometric fluorescence method.

The selectivity of chemodosimeter **1** toward different metal ions was investigated. Under the same conditions, nearly no fluorescence intensity changes were observed in the presence of K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Ag^+, Hg^{2+}, and Au^{3+} (Fig. 2). Furthermore, the effects of interference of the above-mentioned metal ions on monitoring $PdCl_2$ were also studied (Fig. S6, ESI†). These results demonstrated that chemodosimeter **1** possesses high selectivity toward palladium species when present with other metal ions.

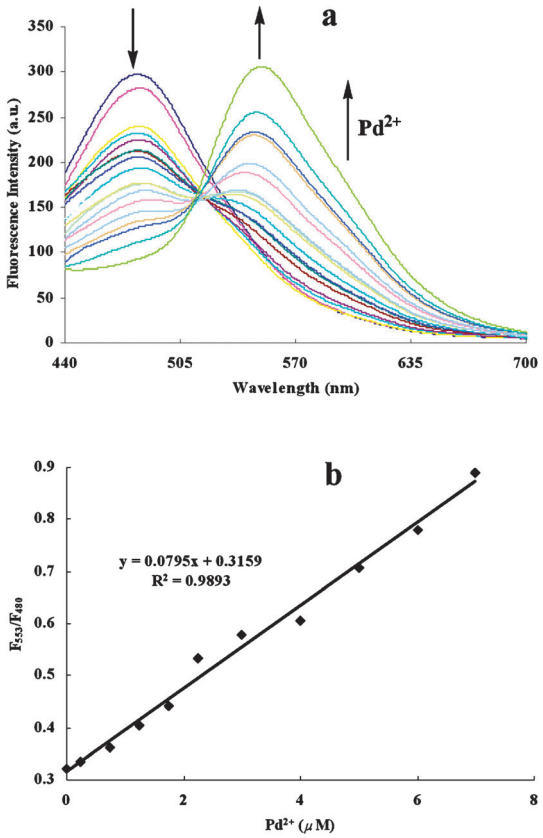

Fig. 1 Fluorescence responses of **1** (5 μM) toward different concentrations of $PdCl_2$ in PBS (20 mM, pH 7.4) solution. (a) Fluorescence spectra of **1** in the presence of increasing concentrations of $PdCl_2$ (final concentration: 0, 0.25, 0.75, 1.25, 1.75, 2.25, 3, 4, 5, 6, 7, 8, 10, 14, 18, 25, 30, 40, 50 μM); (b) fluorescence intensity ratio (F_{553}/F_{480}) of **1** *versus* increasing concentrations of $PdCl_2$ (final concentration: 0, 0.25, 0.75, 1.25, 1.75, 2.25, 3, 4, 5, 6, 7 μM). Each spectrum was acquired 2 h after $PdCl_2$ addition at 35 °C.

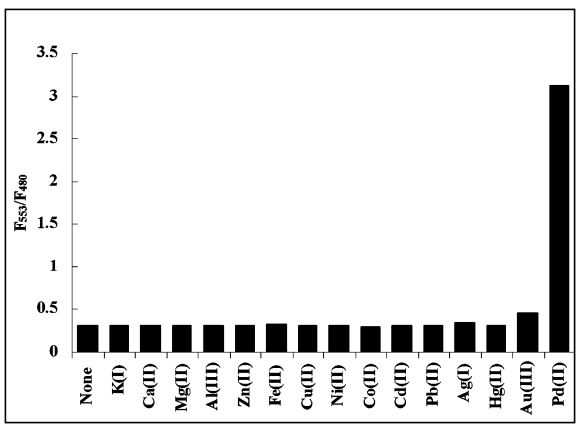

Fig. 2 Fluorescence responses of **1** (5 μM) toward common metal ions (50 μM). Bars represent the fluorescence intensity ratio F_{553}/F_{480}. Each spectrum was acquired 2 h after metal ions addition at 35 °C.

To further demonstrate the ability of chemodosimeter **1** to image palladium species in living systems, we carried out experiments in live RAW 264.7 macrophage cells. The cells incubated with chemodosimeter **1** (5 μM) for 15 min showed an intense intracellular fluorescence (Fig. 3a–c). The result

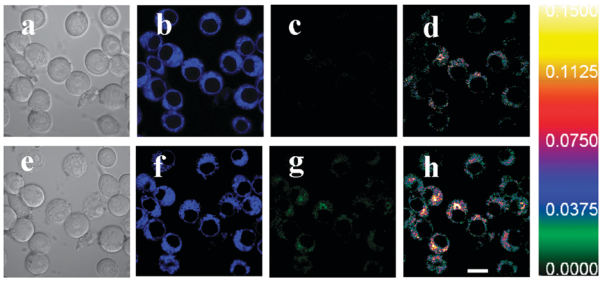

Fig. 3 Confocal fluorescence images of live RAW 264.7 macrophage cells. The cells were incubated with chemodosimeter **1** (5 μM) for 15 min: (a) bright-field transmission image, (b) blue channel, (c) green channel, and (d) ratio image generated from (c) and (b); the above cells after addition of $PdCl_2$ (40 μM) for another 10 min: (e) bright-field transmission image, (f) blue channel, (g) green channel, and (h) ratio image generated from (g) and (f). Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO_2. Scale bar = 20 μm.

reveals that chemodosimeter **1** can penetrate the cell membrane. And then, 40 μM $PdCl_2$ was added to the above cells for another 10 min (Fig. 3e–g). As expected, distinct changes of ratiometric fluorescence responses in living cells were observed (Fig. 3d and h). These results showed that chemodosimeter **1** can be used for the ratiometric fluorescence imaging of $PdCl_2$ in living cells. Moreover, to evaluate cytotoxicity of chemodosimeter **1**, we performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in HeLa cells with 5 and 10 μM chemodosimeter **1** for 1 h, respectively. The result clearly showed that chemodosimeter **1** was of low toxicity or non-toxic to cultured cells under the experimental conditions at the concentration of 5 μM for 25 min (Fig. S8, ESI†).

In conclusion, we have developed a simple but highly selective fluorescent chemodosimeter **1** for the ratiometric determination of three typical palladium species (0, + 2 and + 4) without additional reagents. Additionally, chemodosimeter **1** can monitor $PdCl_2$ in live RAW 264.7 macrophage cells by ratiometric fluorescence imaging. More importantly, we provide a novel cell-permeable fluorophore containing the ICT structure for designing ratiometric fluorescent chemodosimeters for other target analytes. And we anticipate that the platform could become a popular moiety in the future, just as aminonaphthalimide derivatives have done.

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