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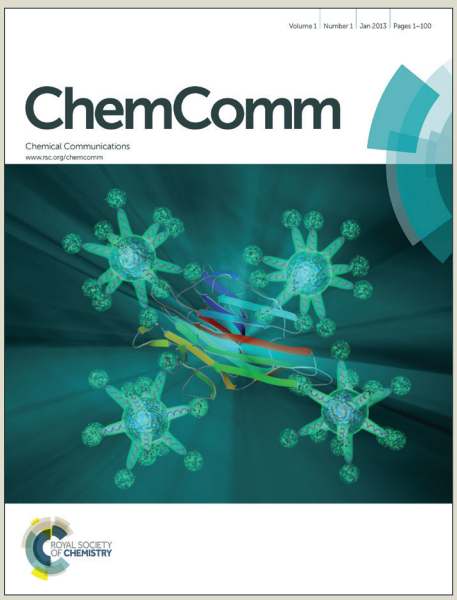

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A naked-eye and ratiometric near-infrared probe for palladium via modulating π-conjugated system of cyanines

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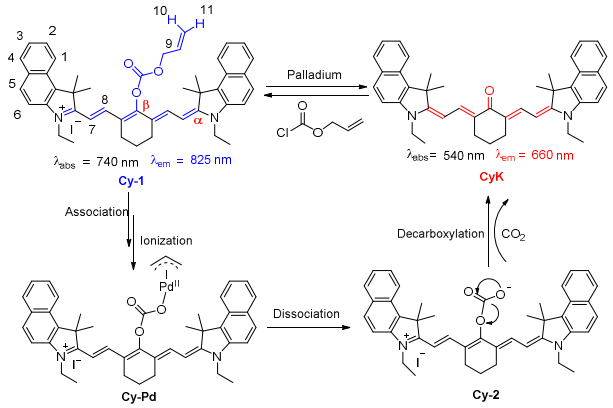
A ratiometric and colorimetric cyanine-based palladium sensor with an excellent selectivity and sensitivity has been designed. Notably, the modulation in the conjugation π-electrons of cyanine dyes can result in ratiometrically fluorescent change with a large stokes shift (270 nm), especially for realizing palladium detection in aqueous samples by indicator paper and in living cells by ratiometric mode. The limit of detection is as low as 0.3 ppb.

Rational design of visual and fluorescent probes for poisonous and biologically important species in the environment and living systems has become an active research field.[1] In particular, consideration attention has been paid to develop Pd-selective fluorescent probes because a serious health hazard would be caused by the formation of palladium complexes with some biomacromolecules such as thiol-containing amino acids, proteins, DNA and RNA.[2-3] The current palladium contamination are mainly coming from two sources:[4] one is the application of palladium in catalytic converters of automobiles that release palladium to the environment, and the other is the residual palladium of synthetic intermediates in active pharmaceutical ingredients (APIs).[4e] Despite some advances in sensing palladium species,[5] the currently methods such as atomic absorption spectroscopy (AAS), X-ray fluorescence, plasma emission methods (e.g., ICP-MS and ICP-AES) are still a challenge for the rational design of palladium probes, especially for meeting the quantitative criteria of both imaging in living system and visually detecting its residual in the environment.

Optical detection approaches have great advantages in the development of highly sensitive and relatively simple analysis protocols.[6] Especially, owing to the unique advantages of the colorimetric and fluorimetric analytical approach at low cost, simply pretreatment and naked-eye mode in a high-throughput fashion, many groups focus on colorimetric and fluorimetric probes for palladium.[7] However, most palladium sensors are generally off-on signal output changes in fluorescence intensity, in which quantitative detection could be significantly influenced by environmental effects, along with a decrease in signal fidelity.[8] Although several ratiometric fluorescent palladium probes has been exploited, their short emission wavelength is limited to their application in vivo bioimaging.[7] Furthermore, given the low residue threshold of palladium in drugs (ca. 5–10 ppm),[9] the typical metal binding motif is often difficult to achieve high selectivity and sensitivity for palladium.[10] Thus, the reaction-based fluorescence sensing system is a priority strategy in designing fluorescent Pd-selective sensors.



Scheme 1 Sensing mechanism of **Cy-1** based on palladium catalyzed Tsuji-Trost Allylic Reaction

With these minds in hand, the ratiometric near-infrared (NIR) fluorescent palladium probe are designed for meeting the criteria of both imaging in vivo and quantitative visual detection for palladium, which features high sensitivity with low background. Our strategy is relied on modulating the π-conjugated systems of cyanines to construct the ratiometric sensor in the presence of palladium. A novel cyanine derivative **CyK** as a scaffold is chosen on basis of the following considerations[11]: its polymethine π-electron system between two nitrogen atoms could be rationally modulated by

introducing the masking group on the meso-oxygen atom of cyanine; both NIR emitting and large extinction coefficient would be greatly preferable to in vivo bioimaging and constructing naked-eye sensor. Specifically, for **Cy-1**, allyl chloroformate as a functional group to trap palladium was introduced to implement the change in π-electron system of **CyK**, while removal of the masking group could restore the polymethine π-electron system of parent cyanine (Scheme 1). Consequently, a large wavelength shift can take place in both absorption and emission spectra with a low background, making the probe high sensitivity for palladium.

Cy-1 was prepared from the reaction of **CyK** and allyl chloroformate in a satisfactory yield of about 60%. Their chemical structures were well characterized by ^{1}H, ^{13}C NMR, and high resolution mass spectra (HRMS) as described in the Experimental Section. Obviously, the absorption peak of **CyK** was located at 545 nm because the typical polymethine π-electron systems of cyanine between two nitrogen atoms was disrupted and shorten in its keto structure (Fig. S1†).[11] In contrast, **Cy-1** exhibited a very intense band at 810 nm (ε = 1.2 × 10^5 M^{-1} cm^{-1}) with a large red-shift by about 265 nm. This large red-shift in the absorption spectra could be attributed to the masking group of the allyl chloroformate, which can recover and extends the conjugated π-electron system like the traditional cyanine (Scheme 1).

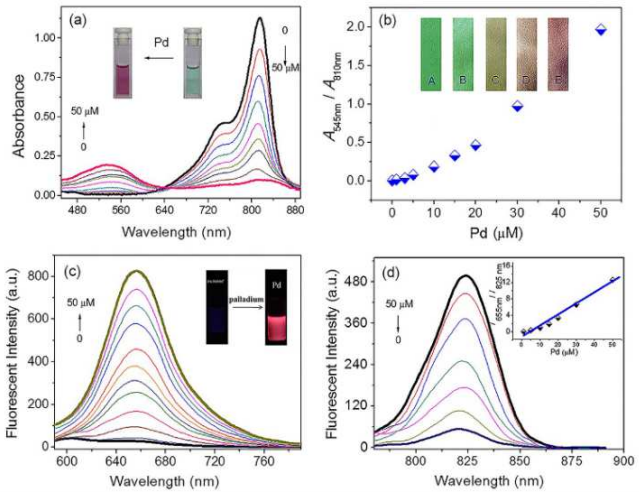


Fig. 1 Spectra properties of **Cy-1** (10.0 μM) upon titration of palladium ($Pd(PPh_3)_4$, 0-50 μM) in a mixed solution of CH_3CN-PBS (v/v = 1/3, pH = 7.4, 0.01 M): (a) Absorption spectra, (b) the ratio (A_{545} nm/A_{810} nm) of absorbance intensity as a function of Pd concentration; (c) 8mission spectra, λ_{ex} = 545 nm; (d) emission spectra, λ_{ex} = 740 nm; Each spectrum was recorded at 20 min. Inset: color changes (a) and fluorescence imaging (c) of **Cy-1** with palladium; (b) visible color changes of **Cy-1** by indicator paper dipping into palladium solution (from left to right: **Cy-1**, 5, 10, 20, 50 μM); (d) the ratio (I_{655} nm/I_{825} nm) of fluorescence intensity as a function of palladium concentration.

To examine the response of **Cy-1** to palladium, the probe was carried out in a mixture solution of PBS-CH_3CN (3-1, pH = 7.4, 0.01 M). As expected, **Cy-1** displayed distinct color changes from light blue to red (inset of Fig. 1a) when incubated with $Pd(PPh_3)_4$. Specifically, upon titration of palladium to **Cy-1**, the absorption peak at 810 nm decreased sharply and a new band at 545 nm appeared with an isosbestic point at 640 nm (Fig. 1a), displaying extremely large blue shift by about 265 nm. The isosbestic point at 640 nm clearly revealed that only two species coexisted. In this analysis, the absorbance at the two wavelengths almost reached equilibrium at about 20 min, and the absorbance ratio A_{545} nm/A_{810} nm was found to almost increase linearly as a function of palladium concentration (Fig. 1b). Obviously, **Cy-1** serves as an excellent "naked-eye" colorimetric probe for quantitative determinations of palladium concentrations.

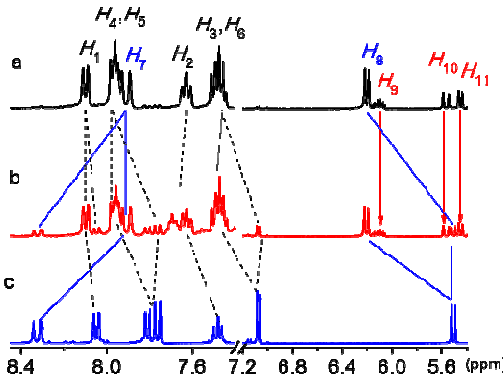


Fig. 2 Partial ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of (a) only **Cy-1**, (b) **Cy-1** with 1 equiv of $Pd(PPh_3)_4$ and (c) **CyK**.

The removal of the trigger by palladium should recover the conjugation π-electrons of **Cy-1**, resulting in a ratiometric signal from two wavelengths in emission spectra. Actually, a large hypsochromic shift in the emission spectra of **Cy-1** was observed in the presence of palladium. Upon titration of 50 μM palladium, the NIR emission band of **Cy-1** at 825 nm (excitation at 740 nm) decreased (Fig. 1c), and a concomitant sharp increase took place in an emission peak at 655 nm (excitation at 545 nm, Fig. 1d). Here the characteristic emission band of **Cy-1** underwent a large 170 nm hypsochromic shift upon specific reaction with palladium. The new turn-on fluorescence at 655 nm could be attributed to the trigger removing and generation of **CyK**. Furthermore, the ratiometric mode of **Cy-1** was built for more accurate and quantitative measurements for palladium. The fluorescence ratio (I_{655} nm/I_{825} nm) was measured, along with an increased linear in the concentration range from 0-50 μM palladium (inset of Fig. 1d). Moreover, another ratiometric mode of the fluorescence ratio (I_{560} nm/I_{745} nm) by using 825 nm as a monitor wavelength was also established (Fig. S2†) when the palladium concentration was increased from 0 to 50 μM. All these results indicate the fluorescence ratio of **Cy-1** can be employed to measure palladium concentrations directly.

The time-dependent changes in the emission spectra of **Cy-1** (10.0 μM) monitored at 655 nm was also investigated (Fig. S3†). By examining the kinetic data for palladium, it is apparent that no more than 20 minutes it would take to reach reaction equilibrium, which could practicably make the detection for the existence of palladium in a short time by using

Cy-1. Furthermore, the detection limit was calculated to be 0.3 ppb (Fig. S4†), which is lower than the palladium content in persons found from samples of morning saliva (10.6 ±7.4 μg L^{-1}).[12]

To insight the mechanism of **Cy-1** for sensing palladium, 1H NMR titration of the probe with $Pd(PPh_3)_4$ in $CDCl_3$ was firstly carried out in Fig. 2. Obviously, the chemical shifts of the alkene-H (H_7 and H_8) in polymethine chain of **Cy-1** were significantly field-shifted in the presence of palladium. Specifically, the significant chemical shifts of H_7 and H_8 were observed from 6.19 and 7.91 ppm to 5.51 and 8.40 ppm, respectively (Fig. 2 and Fig. S5†). These changes in chemical shifts could be attributed to the electronic redistribution of π-electron system, which shorten the polymethine π-conjugation system to enlarge the two neighbor alkene-H differentials in chemical shifts (Table S1†). For other three alkene-H (H9, H10 and H11) peaks in allyl chloroformate unit of **Cy-1**, they were all disappeared (Fig. 2) when **Cy-1** reacted with palladium completely. Furthermore, the ^{13}C NMR signal of Cα is also greatly shielded from 172.8 to ca. 163.7 ppm (Fig. S6†).[13] Actually, at room temperature, the anticipated compound **CyK** can be easily obtained via the addition of $Pd(PPh_3)_4$ to a solution of **Cy-1** with high yield (ESI†). Thus, all these results provide solid evidences that the trigger moiety of allyl chloroformate unit in **Cy-1** is removed in the presence of palladium.

As shown in Scheme 1, it is expected that the trigger moiety of allyl chloroformate unit is initially conjugated with palladium and ionized to form **Cy-Pd**, then dissociated and decarboxylated to transform into **CyK**. In the Tsuji-Trost reaction, Pd(0) is capable of catalyzing the allylic oxidative insertion to cleave the allylic C-O bond of allylic ethers. In our case, high-resolution ESI-MS spectra of **Cy-1** were also employed for monitoring this sensing process for palladium (Fig. S7†). In practice, the peak of π-allylpalladium(II) complex **Cy-Pd** as Pd(0) reacted with the allyl carbamate group of **Cy-1** was observed at *m/z* 779.3. Correspondingly, the dissociative compound peak of **Cy-Pd** was also found at *m/z* 637.3 corresponding to **Cy-2**, and the final product peak of **CyK** that decarboxylated of **Cy-2** was found at *m/z* 593.4 (Fig. S7†). These results confirm our proposed schematic mechanism (Scheme 1).

The metal-specificity of **Cy-1** toward different metal ions such as Pb^{2+}, Pt^{2+}, Cr^{3+}, Fe^{3+}, Co^{2+}, Cd^{2+}, Ni^{2+}, was investigated in Fig. 3. It was found that only the addition of palladium made such an obvious signal changes in the absorption and emission spectra of **Cy-1** in the test system. Specifically, the fluorescence intensity ratio of I_{655} nm/I_{825} nm displayed an enhancement at about 130 times in the presence of palladium species with the color changes from green to red, which can be easily observed by naked eye. Clearly, the results indicated that palladium could be detected through both ratiometric fluorescence and colorimetric methods by **Cy-1**. Moreover, our results also verify the generality of **Cy-1** as a Pd sensor, which exhibits the same sensitivity with both Pd(0) and Pd(II) under reducing conditions (Fig. S8†).[7b,7e,8h]

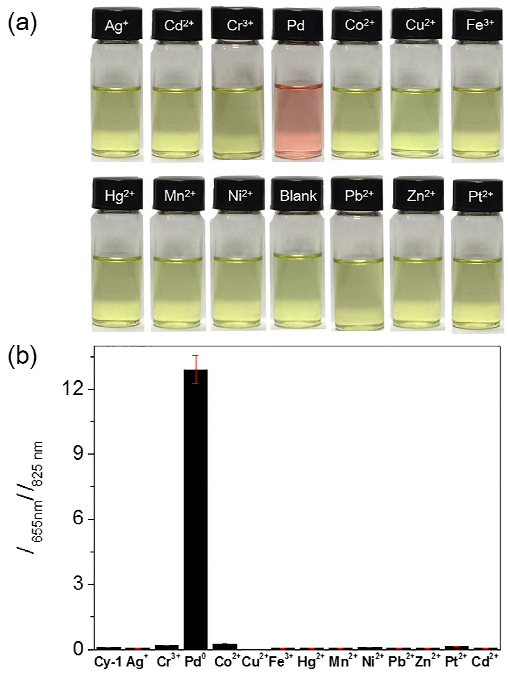


Fig. 3 (a) Color changes and (b) fluorescence ratio responses (I_{655} nm/I_{825} nm) of **Cy-1** (10 μM) toward various metal ions (50 μM), λ_{ex} = 545 nm, in a mixture solution of CH_3CN-PBS (v/v = 1/3, pH = 7.4, 0.01 M). Each spectrum was recorded at 20 min after addition of palladium and each spectrum was repeated for three times.

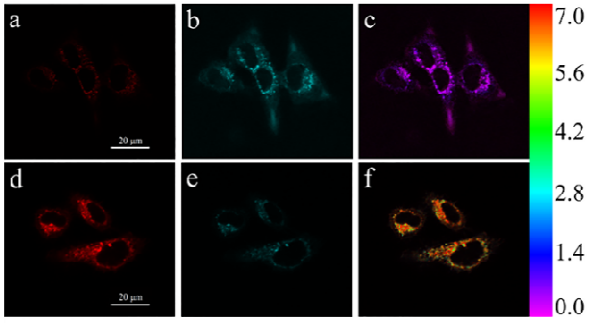


Fig. 4 Confocal fluorescence images of HeLa cells incubated with **Cy-1** (a) and (b) for 25 min; then treated with palladium for 25 min (d) and (e). (a) and (d) λ_{ex} = 540 nm, collected in optical windows between at 600-700 nm (red channel); (b) and (e) λ_{ex} = 633 nm, collected in optical windows between at 700-750 nm (cyan channel); (c) and (f) Pseudocolored ratiometric ratio was collected in two channels (Fred/Fcyan). Note: the ratiometric images were obtained by the image analysis software, Image Pro-plus 6.0 (Scale bar = 20 μM).

In order to realize the value of **Cy-1** for sensing palladium as a straightforward detection manner, the fast qualitative indicator paper for palladium was also demonstrated in real water samples. The visible color change of **Cy-1** by indicator paper was observed upon dipping into different concentration of palladium solution (Fig. 1b). To further demonstrate the ability of **Cy-1** to image palladium species in living systems, Hela cells were chosen for its in vivo application. As shown in Fig. 4, after incubating the Hela cells with sensor **Cy-1** for 25 min, there was a weak fluorescence at 600-700 nm (red

channel), while a strong NIR fluorescence was obtained at 700-750 nm (cyan channel, Fig. 4a and Fig. 4b), indicating that **Cy-1** had good cell-permeable. As expected, after incubated with palladium for another 25 min, the distinct enhanced fluorescence changes in red channel were observed, and a containment decrease in fluorescence intensity took place in cyan channel (Fig. 4d and Fig. 4e). The ratiometric fluorescence imaging of living Hela cells before (Fig. 4c) and after (Fig. 4f) the treatment of **Cy-1** with palladium were obtained. The observations indicate that **Cy-1** is both cell-permeable and capable of bioimaging palladium in a ratiometric manner.

In conclusion, we presented a new naked-eye and ratiometric palladium sensor **Cy-1** based on the specific palladium catalyzed Tsuji-Trost allylic reaction. The efficient modulation in the π-conjugation electrons of the cyanine dye can result in a distinct ratiometric change in fluorescence mode. **Cy-1** can be potentially exploited by bioimaging in vivo and by naked-eye as indicator paper in sensing palladium, along with several meritorious features such as high sensitivity, short response time, low detection limit (0.3 ppb), a large spectral shift with low fluorescence background. This strategy provides a new opportunity for biomedical researchers to explore ratiometric cyanine-based NIR probes.

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Notes and references

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†*Electronic Supplementary Information (ESI) available: Reagents and instruments, synthesis procedures, additional spectroscopic data, ^{1}H, ^{13}C NMR, and FRMS. See DOI:10.1039/c000000x/*

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Graphics for Contents

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The modulation in the conjugation π-electrons of cyanine dyes can result in ratiometrically fluorescent change with a large stokes shift (270 nm), especially for realizing palladium detection in aqueous samples by indicator paper and in living cells by ratiometric mode.