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Communication

A unique two-photon fluorescent probe based on ICT mechanism for imaging palladium in living cells and mice

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ABSTRACT

Palladium(0) as one of the vital transition metals, is employed in numerous industries, such as drug synthesis, aerospace high-tech field and automobile industry. When the Pd(0) enter into the body, it will bind with thiol-containing amino acids, DNA, RNA, and other biomolecules damaging to human health. Thus, developing a novel tool for monitoring and imaging of Pd(0) *in vivo* is very urgent. In the work, based on a intramolecular charge transfer (ICT) mechanism a two-photon fluorescent probe NIPd had been designed and synthesized for the recognition Pd(0). *In vitro* experiments data displayed that probe NIPd exhibited a 13-fold fluorescent increase for Pd(0) in 30 min in the aqueous solution with a detection limit of 16 nmol/L. It also showed the outstanding selectivity and antijamming performance. More importantly, NIPd could be served as a two-photon fluorescent probe for real-time monitoring Pd(0) in living cells and mice.

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Palladium(0) is a transition metal element and has a wide range of applications in organic catalysis, pharmaceuticals, fuel cells and some fine chemicals [1–5]. Many substances containing palladium can bind tightly with thiol-containing biomolecules, such as proteins, DNA and RNA, causing some biological signals to be disordered and affecting people's health and even causing major diseases [6–8]. Precisely, the Pd(0) limit in pharmaceutics is 5−10 ppm and the suggested nutritional intake for Pd(0) is no more than 0.03 μg/mL from drinking water and < 2 μg/person from food. The uptake of a person with a dental prosthesis containing palladium might reach up to 15 μg per day [9]. Therefore, developing fast, reliable, simple and practical methods to detect Pd(0) are of great importance [10].

Up to now, there are several methods to detect Pd(0) *in vitro* or *in vivo* including atomic absorption spectrometry [11], solid phase microextraction-high performance liquid chromatography [12], inductively coupled plasma mass spectroscopy and fluorescence-based assays [13,14]. Among them, fluorescent probes are very attractive due to their simple operation, high sensitivity, selectivity, and non-invasiveness and the capability for real-time detection in living systems [15–20]. Compared to one-photon technology, two-photon imaging has incomparable advantages, such as near-infrared excitation, high-depth imaging, tissue spontaneous fluorescence interference avoiding [21–25]. Thus, based on the above considerations, it encourages us to design a new two-photon fluorescent probe for monitoring Pd(0) in living cells and mice.

Herein, we reported a new fluorescent probe NIPd (Scheme 1) based intramolecular charge transfer (ICT) mechanism for the detection of palladium(0). The molecular framework of NIPd was designed based on a naphthalimide fluorophore owing to possessing good two-photon absorption cross section, biocompatibility, and structure easily modified [17,26,27]. When the phenol allyl ester structure of NIPd encounters Pd(0), a hydrolysis reaction occurs to form a phenol, and recovering ICT effect and emitting strong fluorescence signal. Due to the D-π-A structure, NIOH could be also applied to two-photon imaging for Pd(0) in living MCF-7 cells. In addition, probe NIPd

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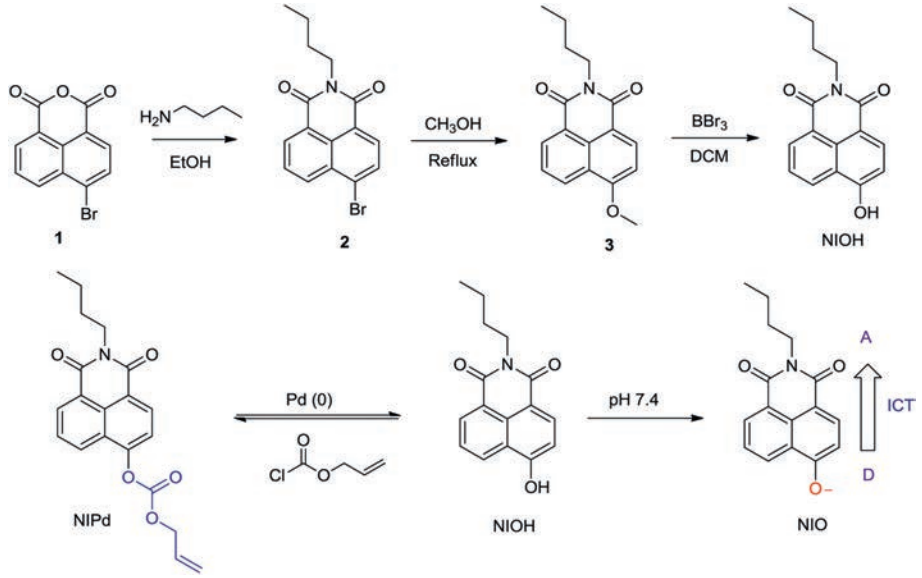

Scheme 1. Synthesis route and recognition mechanism of probe NIPd.

had been successfully used for the recognition of palladium(0) in BALB/c mice.

The fluorescent probe NIPd was prepared as outlined in Scheme 1. The chemical structure of NIPd was fully characterized by ^{1}H NMR and ^{13}C NMR spectral and mass spectrum analytical data (Figs. S10–S12 in Supporting information).

With probe NIPd in hand, the basic spectral response of the probe in solution was studied. Below the concentration of 80 μmol/L, the absorption intensity has a good linear relationship with the probe concentration (Fig. S1 in Supporting information). The probe had good water solubility, which was in favor of entering into the living cells. In order to obtain better results, we measured the fluorescence emission spectra of NIPd in different ratios of PBS: DMSO system. It was found that when the ratio of PBS: DMSO was 8:2, the emission was the strongest, and this was used as subsequent tests condition (Fig. S2 in Supporting information). Then we measured the excitation and emission spectra at a PBS: DMSO ratio of 8:2, with a maximum excitation wavelength of 450 nm and a maximum emission wavelength of 550 nm (Fig. S3 in Supporting information). Next, as seen in Fig. S5 (Supporting information), the maximum absorption of probe is at 350 nm, and the maximum absorption peak appears at 450 nm only when it encountered palladium(0). Under the naked eye, the color of the probe solution changes from colorless and transparent to light yellow. When NIPd (10 μmol/L) was treated with increasing concentration of $Pd(PPh_3)_4$ (0–18 μmol/L). Fluorescence emission intensity at 560 nm enhanced from 25 to 330 (about 13-fold shown in Fig. 1a). Besides, the fluorescence intensity at 560 nm of NIPd has a good linear correlation with the concentrations of $Pd(PPh_3)_4$ (R^2 = 0.985). The detection limit of probe NIPd was determined to be 16 nmol/L in aqueous solution for Pd(0) using the $3\delta/k$ method (Fig. 1b and Fig. S4 in Supporting information). We further tested the selectivity of NIP for other metal cations in the test system. When different metal cations were added to the NIPd solution, as expected, NIPd only responded to Pd(0) (Fig. 1c). However, other ions (including Na^+; K^+; Pb^{2+}; Ni^{2+}; Mn^{2+}; NH_4^+; Br^-; F^-; CH_3COO^-; HCO_3^-; S^{2-}; HPO_4^-; I^-; CO_3^{2-}; NO_3^-; Cl^-; SO_4^{2-}; Co^{2+}) and biomolecules (AA; GSH) had no obvious fluorescence enhancement due to the highly specific Pd-triggered cleavage process (Fig. 1d). As seen in Fig. S6 (Supporting information), probe NIP was unaffected by the presence of other ions. We obtain the high resolution mass spectrum (Fig. S7 in Supporting information) of NIO, the final product of NIPd reacted with Pd(0), which proves the mechanism in Scheme 1.

Since the ester group undergoes hydrolysis under both acid and base conditions, we have examined the fluorescence stability in the range of pH 3.95–10.01 before and after the reaction of NIPd with Pd(0). As shown in Fig. 2a, when NIPd is present, pH has little effect on the fluorescence intensity of Pd(0), while after reacted with Pd(0), it is hydrolyzed when pH is less than 7, and in the range of 7–10 is stable, indicating that Pd(0) is stable and applicable under physiological conditions. We further evaluated the cytotoxicity of NIPd by MTT assay. MCF-7 cells were incubated with different concentrations of NIPd (1–50 μmol/L) for 12 h. As shown in Fig. 2b, the cell viability was higher than 90 %, indicating that toxicity of NIPd is low intracellularly and NIPd has the potential to detect Pd(0) *in vivo*.

Next, we performed a one-photon (OP) and two-photon (TP) confocal experiment to detect Pd(0) in the living MCF-7 cells using probe NIPd (Figs. 3 and 4). No matter in OP or TP conditions, NIPd gave out no fluorescence before Pd(0) was added (Figs. 3b and d). However, after Pd(0) (37.5 μmol/L) was added for 45 min, NIPd was combined with Pd(0), emitting obvious fluorescence signal by OP and TP confocal microscopy (Figs. 3g and i). The results showed that NIPd could penetrate the cell membrane and was evenly dispersed in the cytoplasm. Thus NIPd can detect Pd(0) in living cells. The two-photon stability of probe determines the image quality. Subsequently, the cell morphology of living MCF-7 cells did not change significantly under continuous two-photon laser irradiating (Figs. 4a–c). In addition, fluorescent intensities of probe NIPd basically remained unchanged, clearly showing that probe NIPd had good light stability under two-photon microscope.

Finally, in order to verify probe NIPd could be employed to detect Pd(0) *in vivo*, so, mice imaging experiments were carried out. As shown in Fig. 5, subcutaneous injection of PBS buffer showed almost no fluorescence (Fig. 5a). Next, only injected with NIPd (100 μmol/L, 45 min), NIPd gave out weak fluorescence signal (Fig. 5b). After Pd(0) (100 μmol/L, 45 min) was injected, strong fluorescence emitted (Fig. 5c). Thus, *in vivo* experiments showed that NIPd can emit strong fluorescence in response to Pd(0) *in vivo*.

Above all, probe NIPd can specifically detect Pd(0) in complex systems. NIPd can detect Pd(0) in live cells under one and two

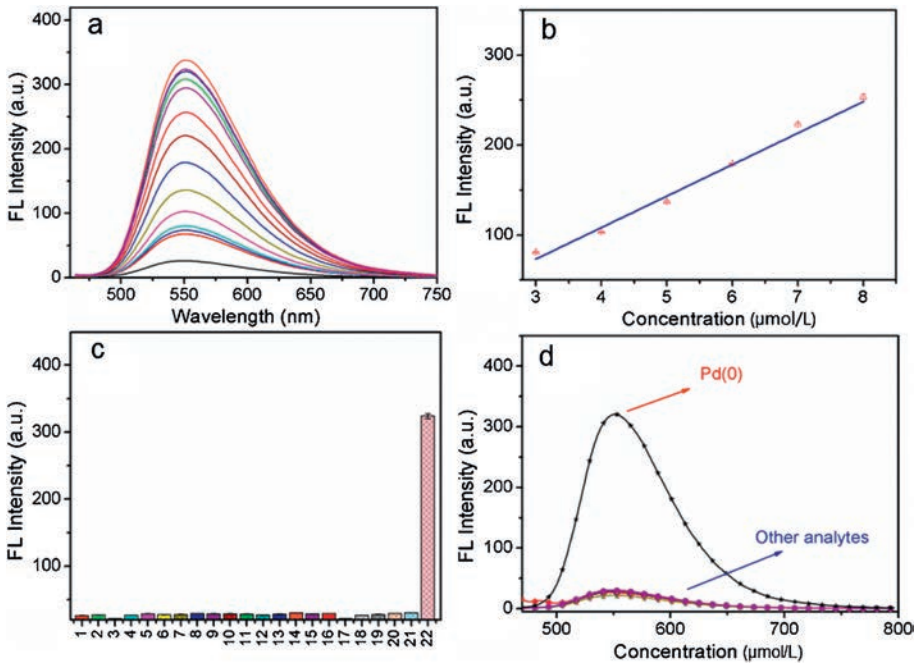

Fig. 1. (a) Fluorescence spectra of NIPd (10 μmol/L) in the presence of increasing concentrations of $Pd(PPh_3)_4$ (0–18 μmol/L) in DMSO/PBS buffer (pH 7.4, 2:8, v/v) with excitation at 450 nm. (b) Linear relationship between fluorescence intensity of NIPd at 550 nm and $Pd(PPh_3)_4$ concentrations. (c) Fluorescence intensity and (d) spectra of NIPd (10 μmol/L) with excitation at 450 nm in the presence of different metal species (100 μmol/L). Other analytes, from 1 to 22 in turn: 1) blank; 2) Na^+; 3) K^+; 4) Pb^{2+}; 5) Ni^{2+}; 6) Mn^{2+}; 7) NH_4^+; 8) Br^-; 9) F^-; 10) CH_3COO^-; 11) HCO_3^-; 12) S^{2-}; 13) HPO_4^-; 14) AA; 15) GSH; 16) I^-; 17) CO_3^{2-}; 18) NO_3^-; 19) Cl^-; 20) SO_4^{2-}; 21) Co^{2+}; 22) Pd(0).

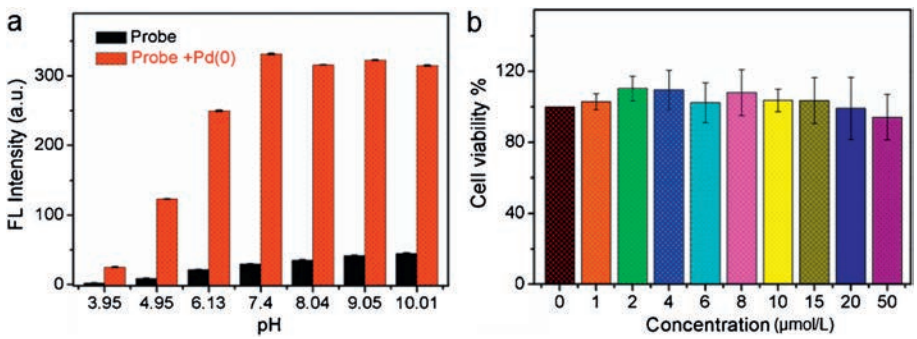

Fig. 2. (a) Fluorescence stability of NIPd (10 μmol/L) and NIPd with Pd(0) in DMSO-PBS buffer (pH 7.4, 8:2, v/v) buffer within the range of pH 3.95–10.01. (b) Cell viability of probe NIPd in MCF-7 cells for 12 h.

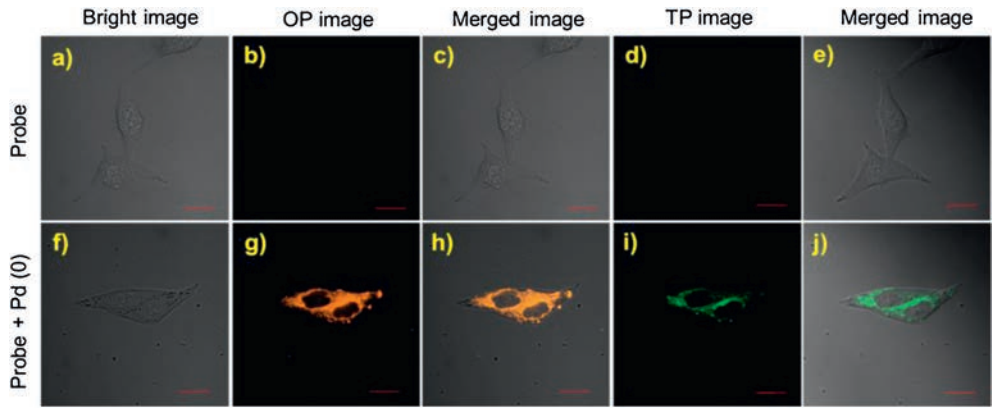

Fig. 3. Recognition of Pd(0) by probe NIPd in living MCF-7 cells by one-photon (OP) and two-photon (TP) confocal microscopy. (a–e) Only probe NIPd (10 μmol/L, 30 min). (f–j) Probe (10 μmol/L, 30 min) with Pd(0) (37.5 μmol/L, 45 min). OP model: λ_{ex} = 488 nm; λ_{em} = 520 - 560 nm. TP model: λ_{ex} = 810 nm; λ_{ex} = 520 - 560 nm. Scale bar =20 μm.

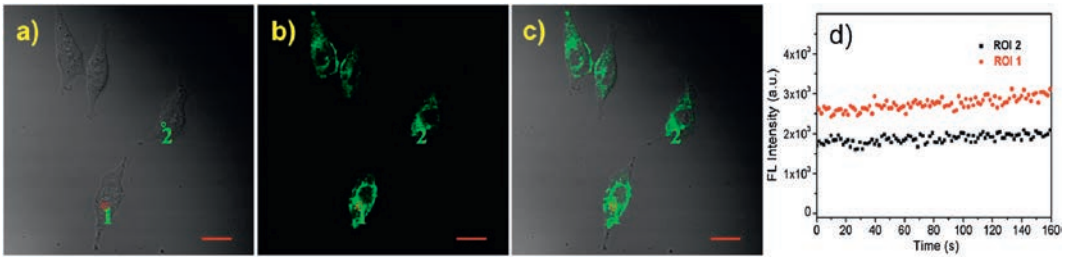

Fig. 4. Two-photon optical stability experiment in living MCF-7 cells. Probe (10 μmol/L, 30 min) with Pd(0) (37.5 μmol/L, 45 min). λ_{ex} =810 nm; λ_{em} = 520−560 nm. Scale bar =20 μm.

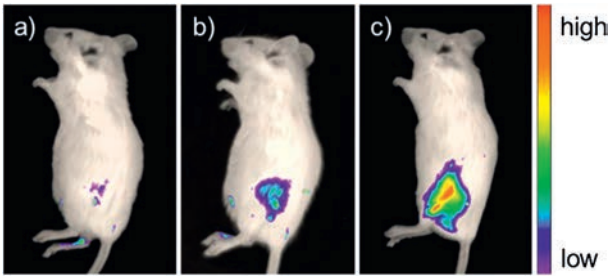

Fig. 5. Detection of Pd(0) in the Balb/c mice using probe NIPd. (a) Subcutaneous injection with PBS; (b) Subcutaneous injection with NIPd (100 μmol/L); (c) Subcutaneous injection with NIPd (100 μmol/L) and Pd(0) (100 μmol/L). Incubation time: 45 min.

photon microscopes, and can even be used to detect Pd(0) *in vivo*, which has a good practicality application potential.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.01.006.

References

[1] J.Y. Lee, B. Raju, B.N. Kumar, et al., Sep. Purif. Technol. 73 (2010) 213–218.
[2] H. Doucet, J.C. Hierso, Curr. Opin. Drug. Disc. 10 (2007) 672–690.
[3] J.C. Wataha, C.T. Hanks, J. Oral. Rehabil. 23 (1996) 309–320.
[4] J.C. Wataha, K. Shor, Expert. Rev. Med. Devic. 7 (2010) 489–501.
[5] A.C. Bosch, B. O'Neill, G.O. Sigge, et al., J. Sci. Food. Agr. 96 (2016) 32–48.
[6] B.A. Lesniewska, B. Godlewska-Zylkiewicz, B. Bocca, et al., Sci. Total. Environ. 321 (2004) 93–104.
[7] Z. Wu, Z. Zhen, J.H. Jiang, et al., J. Am. Chem. Soc. 131 (2009) 12325–12332.
[8] J. Kielhorn, C. Melber, D. Keller, et al., Int. J. Hyg. Envir. Heal. 205 (2002) 417–432.
[9] M. Zischka, P. Schramel, H. Muntau, et al., Trac-Trend. Anal. Chem. 21 (2002) 851–868.
[10] K. Wang, G.Q. Lai, Z.F. Li, et al., Tetrahedron 71 (2015) 7874–7878.
[11] C. Locatelli, D. Melucci, G. Torsi, Anal. Bioanal. Chem. 382 (2005) 1567–1573.
[12] V. Kaur, J.S. Aulakh, A.K. Malik, Anal. Chim. Acta 603 (2007) 44–50.
[13] P.P. Petrova, S.V. Velichkov, I.P. Havezov, et al., Bulg. Chem. Commun. 41 (2009) 65–71.
[14] B. Dimitrova-Koleva, K. Benkhedda, E. Ivanova, et al., Talanta 71 (2007) 44–50.
[15] W.Y. Feng, L.Y. Bai, S.W. Jia, et al., Sensor. Actuat. B -Chem. 260 (2018) 554–562.
[16] P. Wang, J.X. Xia, Y.Q. Gu, Tetrahedron. Lett. 56 (2015) 6491–6494.
[17] P.C. Zhou, J. Yao, G.D. Hu, et al., ACS Chem. Biol. 11 (2016) 1098–1105.
[18] L. Yu, Y.M. Qiao, L.X. Miao, et al., Chin. Chem. Lett. 29 (2018) 1545–1559.
[19] X.J. Peng, Adv. Mater. Res. 441 (2012) 783.
[20] C.Y. Chen, L.F. Zhou, F.L. Liu, et al., J. Hazard. Mater. 386 (2019) 121943.
[21] L.C. Li, R.F. Guan, M.M. Guo, et al., Sensor. Actuat. B -Chem. 254 (2018) 949–955.
[22] W.F. Luo, W.S. Liu, J. Mater. Chem. B 4 (2016) 3911–3915.
[23] H.J. Cui, H. Chen, Y. Pan, et al., Sensor. Actuat. B -Chem. 219 (2015) 232–237.
[24] J.Y. Wang, Z.R. Liu, M. Ren, et al., J. Photoch. Photobio. A 317 (2016) 108–114.
[25] H.L. Li, J.L. Fan, X.J. Peng, Chem. Soc. Rev. 42 (2013) 7943–7962.
[26] X.H. Cao, T.T. Zhang, A.P. Gao, et al., Org. Biomol. Chem. 12 (2014) 6399–6405.
[27] Z. Chen, Y.F. Xu, X.H. Qian, Chin. Chem. Lett. 29 (2018) 1741–1756.