Supporting Information for

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

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1. Materials and general methods

All chemicals used in this paper were commercial products of analytical grade. $^1$H-NMR and $^{13}$C-NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in CDCl₃, TMS as internal standard). High-resolution mass data were measured with fourier transform ion cyclotron resonance mass spectrometer (APEX IV). Absorption spectra were recorded on UV-3101PC spectrophotometer. Fluorescence emission and excitation spectra were measured on Perkin-Elmer Model LS-55. All pH measurements were made with a Sartorius basic pH-meter PB-10.

Parent stock solutions (10 mM) of the perchlorate salts of K⁺, Ca²⁺, Mg²⁺, Al³⁺, Zn²⁺, Fe²⁺, Cu²⁺, Ni²⁺, Co²⁺, Cd²⁺, Pb²⁺, Ag⁺, Hg²⁺ and the chloride salt of Au³⁺ were prepared in ultra-pure water. Parent stock solutions of PdCl₂ (5 mM) and (NH₄)₂PdCl₆ (1 mM) were prepared in the mixture of brine and methanol (3:1, v/v). Parent stock solutions of chemodosimeter 1 (1 mM) and Pd(PPh₃)₄ (1 mM) also were prepared in DMSO. Test solutions were prepared by placing 50 µL of the chemodosimeter stock solution into a test tube, adding an appropriate aliquot of each ion stock solution, and then diluting the solution to 10 mL with PBS (20 mM, pH = 7.4). The fluorescence spectra were obtained after various analytes addition at 35 °C for 2 h (other than time-dependent experiments) by excitation at 410 nm. The excitation and emission slit widths were 10 nm and 15 nm, respectively.

2. Synthesis of chemodosimeter 1

Scheme S1 Synthesis of chemodosimeter 1
**N-butyl-4-bromo-1,8-naphthalimide**

N-butyl-4-bromo-1,8-naphthalimide was synthesized according to the method described by Qian et al. with some modifications. The condensation of 4-bromo-1,8-naphthalic anhydride (10 g, 36 mmol) and \(n\)-butylamine (2.9 g, 40 mmol) was carried out in toluene (200 mL) at refluxing under nitrogen atmosphere for 24 h. After removal of toluene, the residues were purified by silica gel column chromatography using dichloromethane as eluent to afford 9.9 g (83%) pure product. m.p.: 104.1- 105.2 °C.

**Chemodosimeter 1**

A mixture of \(N\)-butyl-4-bromo-1,8-naphthalimide (1.5 g, 4.5 mmol) and propargyl hydroxide (1.1 g, 20 mmol) in dimethylsulfoxide (DMSO) (15 mL) was heated at 120 °C for 2 h, then the reaction mixture was cooled to room temperature and diluted with water to afford a crude product after filtration. The crude product was purified by silica gel column chromatography using chloroform as eluent to afford 1.2 g (85%) chemodosimeter 1. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) (\(\times 10^{-6}\)): 0.97(t, \(J = 7.4\) Hz, 3H), 1.41-1.47(m, 2H), 1.67-1.74(m, 2H), 2.63(s, 1H), 4.16(t, \(J = 7.6\) Hz, 2H), 5.02(s, 2H), 7.18(d, \(J = 8.2\) Hz, 1H), 7.71(d, \(J = 7.8\) Hz, 1H), 8.54-8.61(m, 3H). \(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (\(\times 10^{-6}\)): 45.41, 56.57, 111.18, 112.33, 121.08, 122.06, 122.95, 123.08, 123.59, 130.25, 136.54, 143.06, 143.61, 145.17, 149.13, 149.15, 150.40, 152.96, 157.89. HRMS (ESI positive) calcd for C\(_{19}\)H\(_{17}\)NO\(_3\) [M+H]\(^+\) 308.12812, found 308.12763.
3. The HRMS data of reaction product of 1 with PdCl₂

![Figure S1](image1.png)

**Fig. S1** HRMS of reaction product of chemodosimeter 1 with PdCl₂

4. Absorption responses of 1 toward Pd(IV), Pd(II), Pd(0)

![Figure S2](image2.png)

**Fig. S2** Absorption responses of 1 toward Pd(IV), Pd(II), Pd(0) in PBS (20 mM, pH 7.4) solution.
5. The kinetic profiles of the recognition of three palladium species

![Fig. S3](image)

Fig. S3 Time-dependent fluorescence changes of 1 (5 µM) in the presence of three palladium species (50 µM) in PBS (20 mM, pH 7.4) solution at 25 °C: (a) Pd(IV), (b) Pd(II), (c) Pd(0), and (d) time-dependent changes of fluorescence intensity ratio $F_{553}/F_{480}$.

6. Determination of reaction time in sensing PdCl$_2$

According to the kinetic profiles of the recognition of three palladium species, the Pd(II)/Pd(0)-catalyzed depropargylation reaction velocity is very slow at 25 °C. To improve this reaction, enhancing the reaction temperature is a preference. Considering the application to the medicament analysis and bioimaging, 35 °C was selected for the recognition of PdCl$_2$.

![Fig. S4](image)

Fig. S4 (a) Time-dependent fluorescence change of 1 (5 µM) in the presence of PdCl$_2$ (50 µM) in PBS (20 mM, pH 7.4) solution at 35 °C. (b) time-dependent changes of fluorescence intensity ratio $F_{553}/F_{480}$. 

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7. Absorption responses of 1 toward PdCl$_2$

![Absorption responses of 1](image)

*Fig. S5* Absorption responses of 1 (5 µM) toward different concentrations of PdCl$_2$ in PBS (20 mM, pH 7.4) solution. Each spectrum was acquired 2 h after PdCl$_2$ addition at 35 °C.

8. The effects of interference of common metal ions on monitoring PdCl$_2$

![Fluorescence responses of 1](image)

*Fig. S6* Fluorescence responses of 1 toward PdCl$_2$ (50 µM) in the absence and presence of other analytes (50 µM). Bars represent fluorescence intensity ratio $F_{553}/F_{480}$.

9. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of chemodosimeter 1 was measured by five times and the standard deviation of blank measurement was achieved. To gain the slop, the ratio of the fluorescence intensity at 553 nm to the fluorescence intensity at 480 nm ($F_{553}/F_{480}$) was plotted as a concentration of Pd$^{2+}$. So the detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where $\sigma$ is the standard deviation of blank measurement, k is the slop between the
fluorescence intensity ratio versus Pd\textsuperscript{2+} concentration.

10. Cell culture and imaging

RAW 264.7 macrophage cells (gifted from the center of cells, Peking Union Medical College) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 U/mL of penicillin and 100 μg/mL streptomycin at 37 °C under a humidified atmosphere containing 5% CO\textsubscript{2}. RAW 264.7 macrophage cells were seeded in a 6-well plate at a density of 104 cells per well in culture media. After 24 h, they were incubated with 5 μM chemodosimeter 1 in culture media for 15 min at 37 °C, and washed with phosphate buffered saline (PBS) three times. Then the fluorescence imaging of cells was carried out. Next, 40 μM PdCl\textsubscript{2} was added to the above cells for another 10 min, and the fluorescence imaging of cells was implemented. To further confirm the distinct changes of ratiometric fluorescence responses resulted from PdCl\textsubscript{2}, additional 20 μM PdCl\textsubscript{2} was added to the above cells for another 10 min, again. The fluorescence imaging of RAW264.7 macrophage cells was observed under Nikon Ci Si confocal and multi-photo system (confocal excitation: a diode laser at 408 nm). The blue channel was set at 460 ± 30 nm, and the green channel was set at 560 ± 30 nm.

![Fig. S7 Confocal fluorescence images of live RAW 264.7 macrophage cells with the additions of different concentration PdCl\textsubscript{2}: (a) 0 μM, (b) 40 μM, (c) 60 μM.](image)

11. Cytotoxicity assays

HeLa cells were cultured in the iscove’s modified dulbecco’s medium (IMDM)
supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO₂ and 95% air at 37°C. The cells were seeded into 96-well plates, then 5 and 10 µM (final concentration) chemodosimeter 1 (99% IMDM and 1% DMSO) were added respectively (n = 6). Next, the cells were incubated at 37°C in an atmosphere of 5% CO₂ and 95% air for 1 h. Untreated assay with IMDM (n = 6) was also conducted under the same conditions.

Figure S8. Cytotoxicity assays of chemodosimeter 1 at different concentrations for HeLa cells

12. The characterization data of chemodosimeter 1

{H-NMR of 1}
\(^{13}\)C-NMR of 1

HRMS of 1

Fig. S9 The characterization data of chemodosimeter 1

13. Reference