Supporting Information for

A Fluorescence Turn-On Sensor for the Detection of Palladium Ions that Operates Through *In-Situ* Generation of Palladium Nanoparticles

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Experimentals

General methods, materials, instrumentation and measurements

Iodo-BODIPY **1** and H-BODIPY **2** were prepared according to literature procedures.[1] Aqueous solutions were freshly prepared with deionized water from a water purification system (Human Corp. Korea). All metal ions were used as chloride salts except for $AgNO_3$. UV-Vis absorption spectra were obtained on a Shimadzu UV-2501 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer at 25 °C using 10 mm quartz cuvettes with a path length of 1 cm. Fluorescence quantum yields were determined by standard methods, using rhodamine 6G (Φ_F = 0.95 in EtOH)[2] for iodo-BODIPY **1** and fluorescein (Φ_F = 0.95 in 0.1 M NaOH)[3] for H-BODIPY **2** as standards, respectively. Fluorometric assays with various metal analytes were measured by monitoring changes in fluorescence intensity using a Synergy Mx Microplate Reader (BioTek, USA).

1. Studies on Formation of PdNPs and TEM Images of PdNPs

(a) Time-dependent absorption spectra of Pd^{2+} in ethanol-water (1:4, v/v) at 25 ^oC

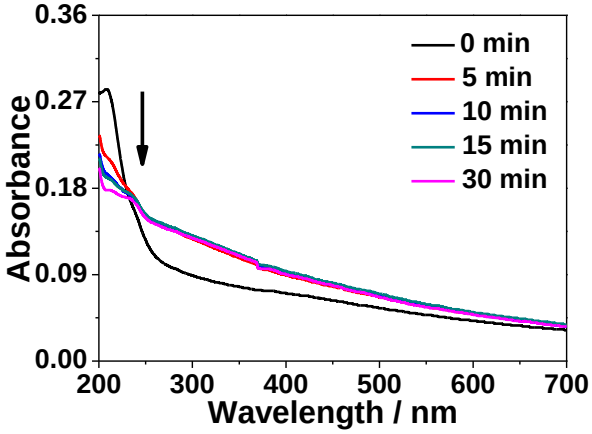


Figure S1. Time-dependent UV-Vis absorption spectra of $PdCl_2$ (50 μM) in ethanol-water (1:4, v/v) at 25 ^oC.

(b) Determination of conversion yield of Pd^{2+} in ethanol-water (1:4, v/v) to PdNPs at 25 ^{o}C

In order to determine the correlation of absorbance and concentration of $PdCl_2$, UV-Vis absorption spectra of $PdCl_2$ at various concentrations in water was measured. The extinction coefficient of absorbance at 208 nm and concentration of $PdCl_2$ was calculated from the slope of a plot of the absorbance at 208 nm versus concentration of $PdCl_2$ in water. The calculated coefficient was used in determining the conversion yield of $PdCl_2$ in ethanol-water (1:4, v/v) to PdNPs. For example, the conversion yield was determined by measuring absorbance at 208 nm of supernatant after centrifugation of the suspended PdNPs that are readily generated by mixing $PdCl_2$ in aqueous ethanol, and comparing it with an absorbance vs. concentration calibration curve (λ_{abs} = 208 nm) for $PdCl_2$ in water. $PdCl_2$ (50 μM) was converted to PdNPs in aqueous ethanol in *ca.* 90% yield.

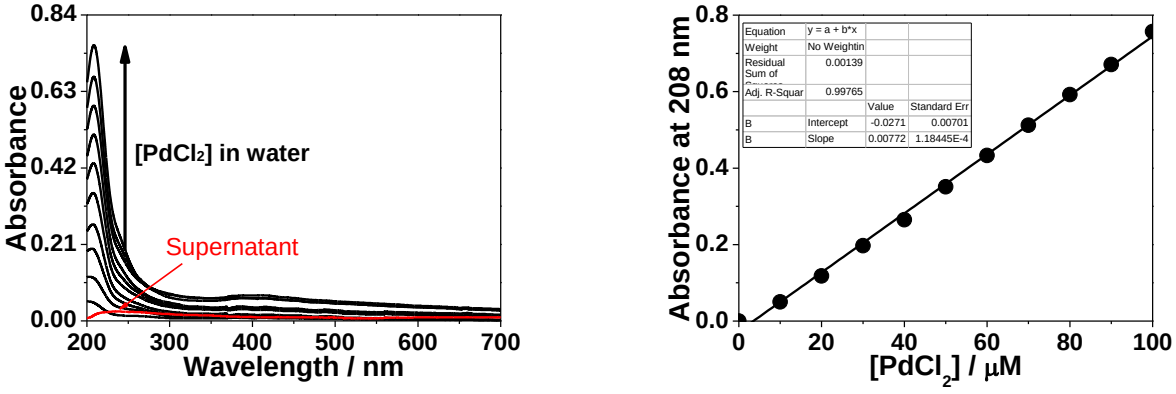


Figure S2. (A) UV-Vis absorption spectra of $PdCl_2$ (10-100 μM) in water at 25 ^{o}C. (B) A plot of absorbance at 208 nm vs [$PdCl_2$] in water at 25 ^{o}C. UV-Vis absorption spectra (red line) of the supernatant after centrifugation of the suspended PdNPs that were obtained 30 min after mixing $PdCl_2$ (50 μM) in aqueous ethanol, were shown in Figure S2(A).

(c) Transmission electron microscopy (TEM) analysis

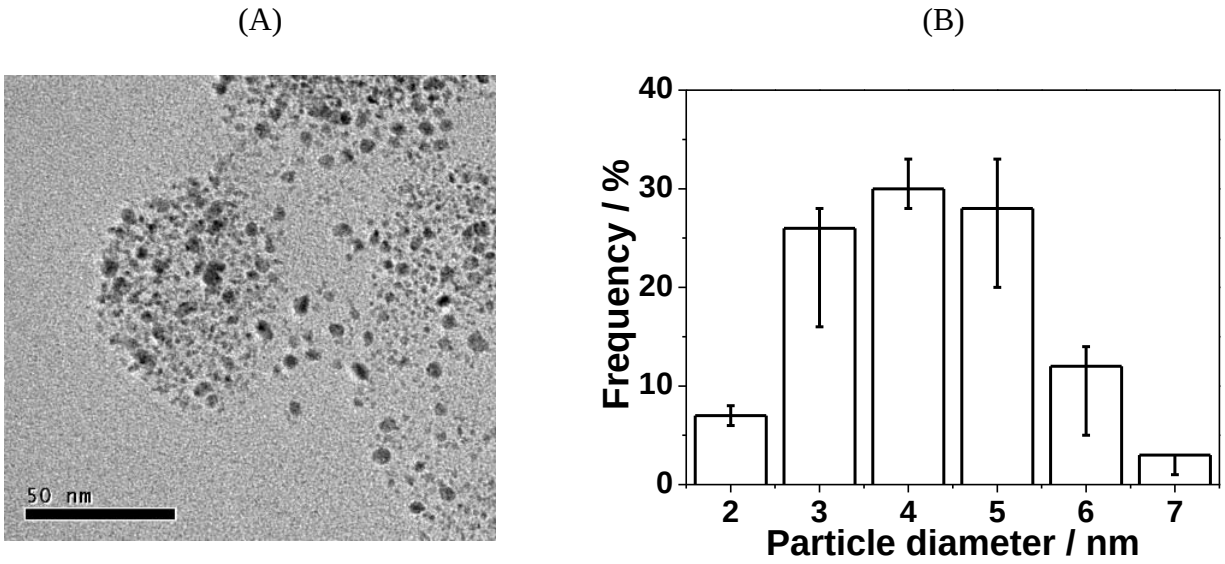


Figure S3. TEM image (A) and size distribution (B) of *in-situ* generated PdNPs from a solution of $PdCl_2$ (50 μM) in ethanol-water (1:4, v/v) at 25 ^oC. Average diameter of PdNPs is 4.2 ± 0.2 nm.

2. Photophysical Properties of iodo-BODIPY and H-BODIPY

Table S1. Photophysical properties of iodo-BODIPY **1** and H-BODIPY **2**[a]

Compounds	$\lambda_{abs.\ max}$, nm	ε, $M^{-1}cm^{-1}$	$\lambda_{em.\ max}$, nm[b]	Φ_{FL}[c]
iodo-BODIPY **1**	535	51964	555	0.017
H-BODIPY **2**	501	90298	512	0.55

[a]Data were obtained in ethanol-water (1:4, v/v) at 25 ^oC. [b]Excited at 465 nm [c]Quantum yields vs. rhodamine 6G in EtOH (Φ_F = 0.95) for iodo-BODIPY **1**[2] and fluorescein in 0.1 N NaOH (Φ_F = 0.95) for H-BODIPY **2**.[3]

3. Fluorescence Turn-on Response

Preparation of stock solution and general procedure: $PdCl_2$ dissolved in deionized water (0.5 mM, 20 µL) was mixed with a mixture solution of ethanol and water (1:7, v/v, 160 µL). To the solution was added iodo-BODIPY **1** (0.05 mM in EtOH, 20 µL) to have final concentration of 5 µM iodo-BODIPY **1** and 50 µM $PdCl_2$ in ethanol and water (1:4, v/v), respectively. The reactions were monitored at 25 ^{o}C for 30 minutes. The fluorescence signal for each well was measured at 510 nm (λ_{ex} = 465 nm).

(a) Fluorescence turn-on response of 1 in the presence of $PdCl_2$ in ethanol-water

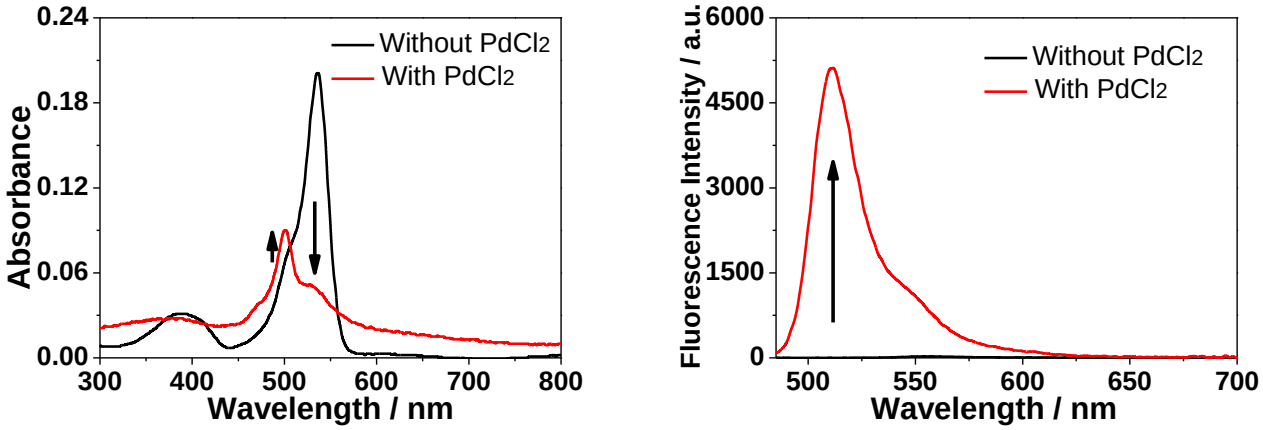


Figure S4. (left) Absortion and (right) fluorescence emission spectra of iodo-BODIPY **1** (5 µM) without (black) and with (red) addition of Pd^{2+} (50 µM) in ethanol-water (1:4, v/v) at 25 ^{o}C. The spectra were acquired 30 min after the addition of **1** to the solution of Pd^{2+} in ethanol-water (1:4, v/v). Excited at 465 nm.

(b) Time-dependent fluorescence turn-on response of 1 in the presence of different concentrations of Pd^{2+} in ethanol-water

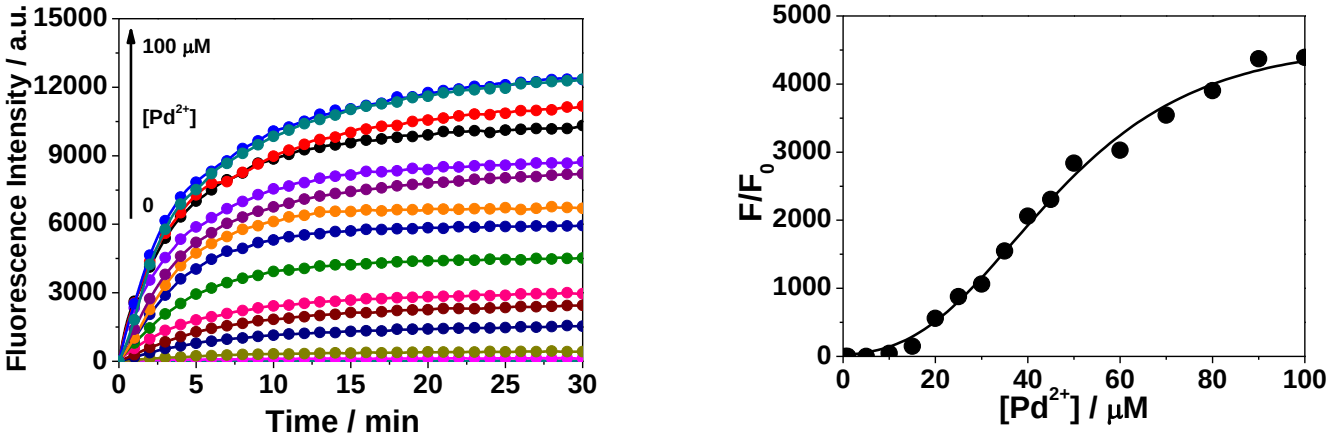


Figure S5. (left) Time-dependent fluorescence intensity at 510 nm of **1** (5 µM) in the presence of different concentrations of Pd^{2+} in ethanol–water (1:4, v/v) at 25 ^{o}C. [Pd^{2+}] = 0, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 µM. Excited at 465 nm. The values were obtained every 2 min (0 – 30 min). (right) A plot of the relative fluorescence intensity at 510 nm as a function of [Pd^{2+}]. Incubation time = 30 min. F_0 and F correspond to the fluorescence intensity of iodo-BODIPY **1** in the absence and the presence of $PdCl_2$ in ethanol-water (1:4, v/v), respectively.

4. Identification of Fluorescent Reaction Product

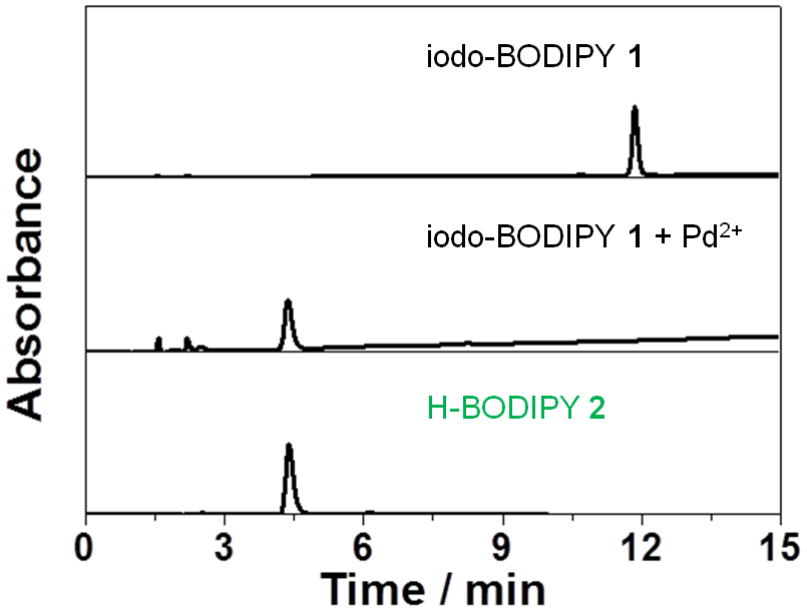


Figure S6. HPLC chromatograms of **1** before (top); after addition of Pd^{2+} (50 μM) in ethanol–water (1:4, v/v) at 25 °C (middle); **2** only (bottom).The samples were analyzed by using HPLC-MS with a linear gradient elution (eluent A/B =20/80, A: deionized water, B: Methanol with 5 mM ammonium formate, flow rate 0.3 mL/min, UV: 500 nm). [**1**] = [**2**] = 5 μM, [Pd^{2+}] = 50 μM.

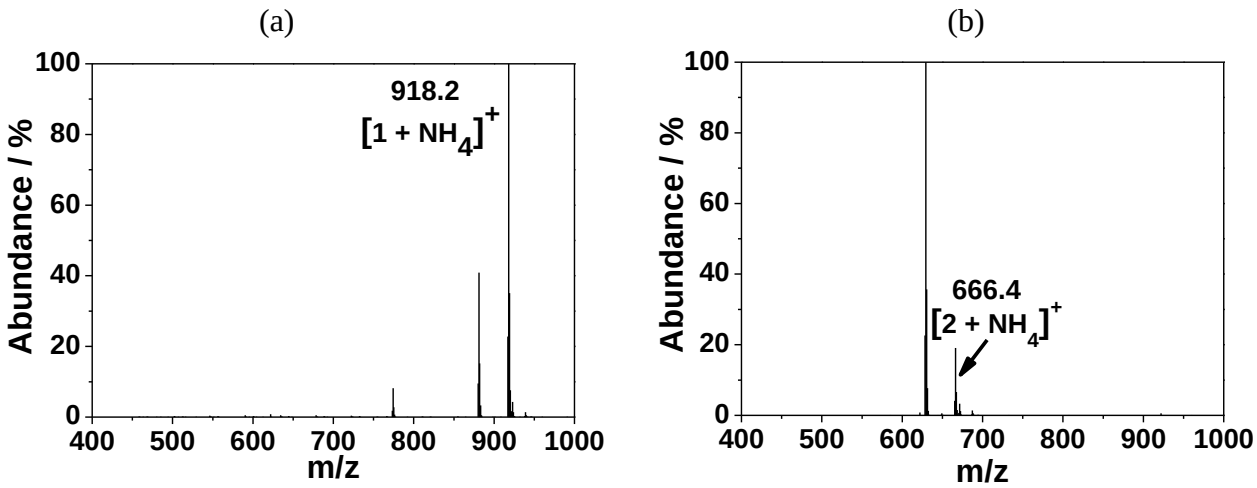


Figure S7. ESI-MS spectra of the peak of retention time at 12.0 min (a) and 4.5 min (b). The MW of the material eluting with a retention time of 12.0 min is 918.2, which corresponds to $[M+NH_4]^+$ for iodo-BODIPY **1** and the MW of the substance with a retention time of 4.5 min is 666.4, which corresponds to $[M+NH_4]^+$ for **2**.

5. Control Experiments

(a) Fluorescence turn-on response of 1 in the presence of $PdCl_2$ in various aqueous media

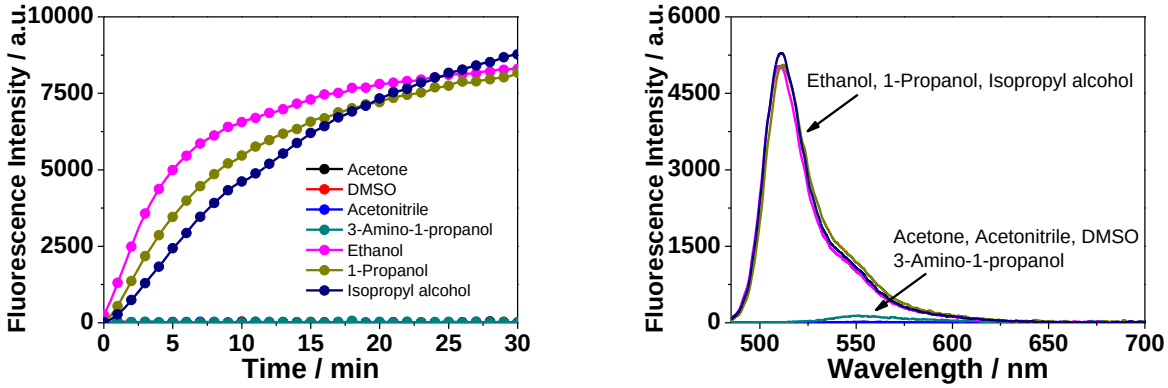


Figure S8. (left) Time-dependent fluorescence intensity at 510 nm of **1** upon addition of Pd^{2+} in water containing different organic solvents (20%) as a cosolvent at 25 °C. (right) Fluorescence emission spectra of iodo-BODIPY **1** upon addition of Pd^{2+} in water containing different organic solvents (20%) as a cosolvent at 25 °C. The spectra were acquired 30 min after the addition of **1** to the solution of Pd^{2+} in aqueous media. Excited at 465 nm. [**1**] = 5 μM, [$PdCl_2$] = 50 μM

(b) Effect of reducing agent ($NaBH_4$) on the iodo-BODIPY 1

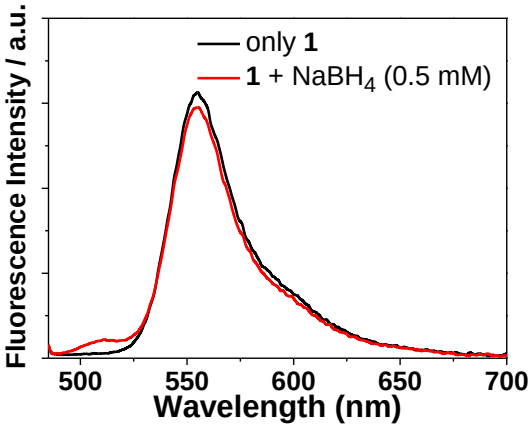


Figure S9. Fluorescence emission spectra of iodo-BODIPY **1** upon addition of $NaBH_4$ (500 μM) in ethanol–water (1:4, v/v) at 25 °C. Excited at 465 nm.

(c) Stability of deiodinated product, H-BODIPY 2 in the presence of Pd^{2+}

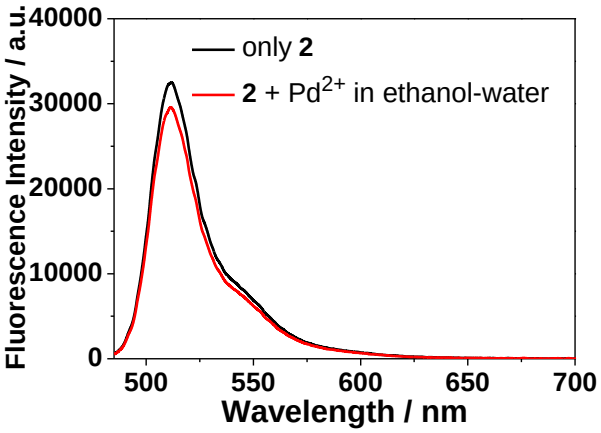


Figure S10. Fluorescence emission spectra of H-BODIPY **2** (5 μM) upon addition of Pd^{2+} (50 μM) in ethanol–water (1:4, v/v) at 25 ^{o}C. The spectra were obtained 30 min after the addition of Pd^{2+} to the solution of H-BODIPY **2**. Excited at 465 nm.

(d) Photostability of iodo-BODIPY 1

We have investigated photostability of **1** in ethanol–water (1:4, v/v) at 25 ^{o}C .The photooxidation studies were performed by continuous visible light irradiation using a 150 W steady-state Xe lamp as the irradiation source under aerobic conditions. The photostability was quantified by monitoring the change of fluorescence intensity of **1** after photoirradiation for 2.5 hours.

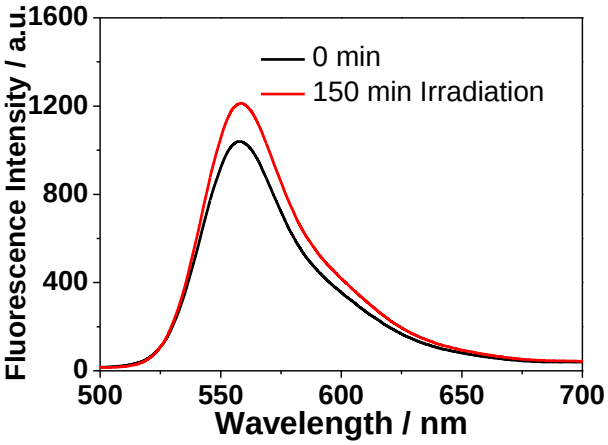


Figure S11. Fluorescence emission spectra of iodo-BODIPY **1** (5 μM) in ethanol–water (1:4, v/v) at 25 ^{o}C before and after photoirradiation (λ_{ex} =465 nm) for 2.5 h.

6. Selectivity Studies

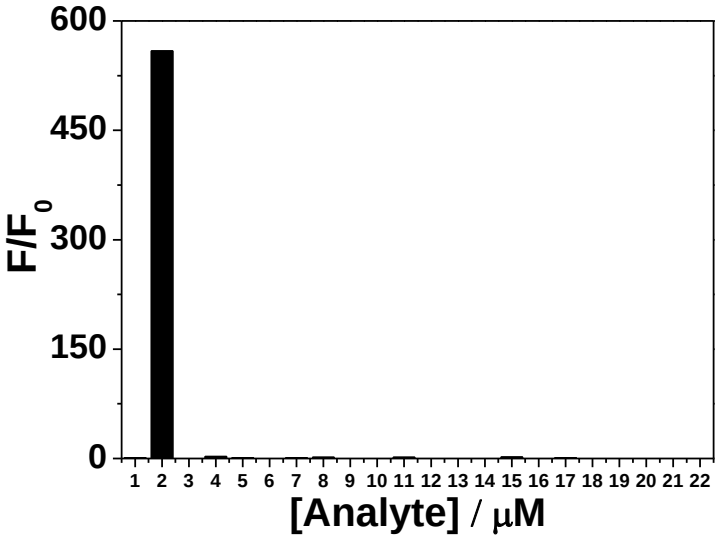


Figure S12. Relative fluorescence intensities of iodo-BODIPY **1** toward various metal ions (as their chloride salts except for $AgNO_3$) in ethanol–water (1:4, v/v), measured 30 minutes after addition of each metal ion at 25 °C. 1, iodo-BODIPY **1** only; 2, Pd^{2+}; 3, Al^{3+}; 4, Ca^{2+}; 5, Cd^{2+}; 6, Cu^{2+}; 7, Co^{2+}; 8, Cr^{2+}; 9, Fe^{2+}; 10, Fe^{3+}; 11, Pb^{2+}; 12, Zn^{2+}; 13, Mg^{2+}; 14, Hg^{2+}; 15, Ni^{2+}; 16, Mn^{2+}; 17, K^{+}; 18, Ag^{+}; 19, Na^{+}; 20, Au^{+}; 21, Au^{3+}; 22, Pt^{2+} [**1**] = 5 μM, [metal ion] = 20 μM for Pd^{2+} and 50 μM for all other metal ions. Excited at 465 nm.

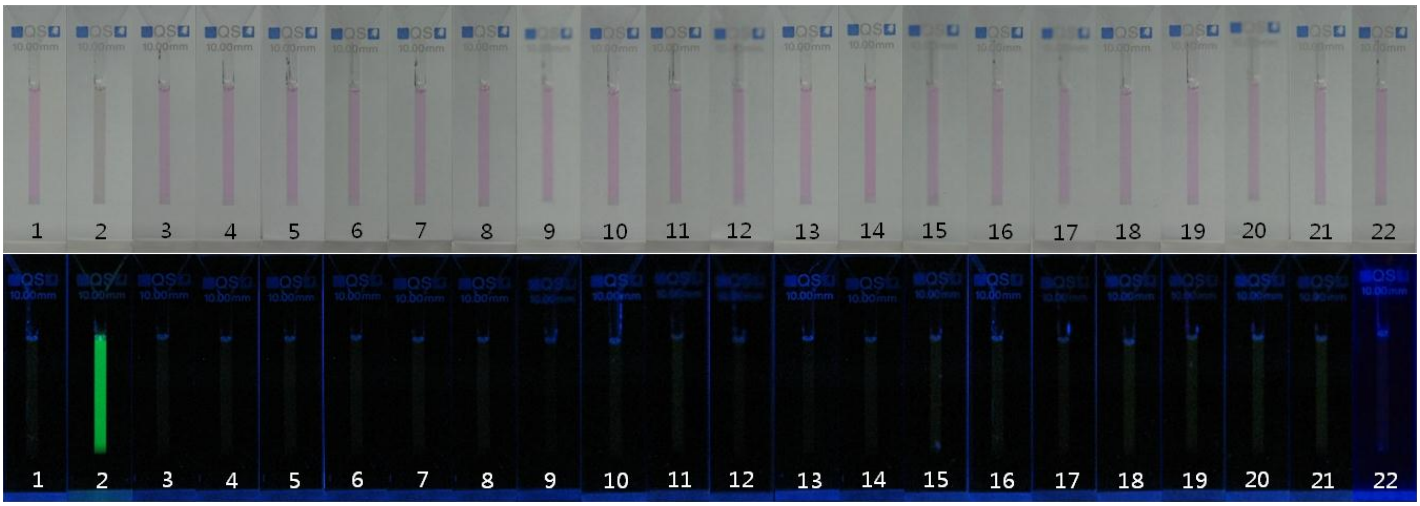


Figure S13. Photographs of probe **1** upon addition of various metal ions under (top) ambient light and (bottom) UV irradiation (365 nm). 1, iodo-BODIPY **1** only; 2, Pd^{2}+; 3, Al^{3+}; 4, Ca^{2+}; 5, Cd^{2+}; 6, Cu^{2+}; 7, Co^{2+}; 8, Cr^{2+}; 9, Fe^{2+}; 10, Fe^{3+}; 11, Pb^{2+}; 12, Zn^{2+}; 13, Mg^{2+}; 14, Hg^{2+}; 15, Ni^{2+}; 16, Mn^{2+}; 17, K^{+}; 18, Ag^{+}; 19, Na^{+}; 20, Au^{+}; 21, Au^{3+}; 22, Pt^{2+} [**1**] = 5 μM, [metal ion] = 20 μM for Pd^{2+} and 50 μM for all other metal ions.

7. Kinetic Studies

Standard fluorescence curve: In order to determine the correlation of fluorescence intensity and concentration of dehalogenation product **2**, fluorescence intensities of various concentrations of **2** at 510 nm were measured. The extinction coefficient of fluorescence intensity and concentration of **2** was calculated from the slope of a plot of the fluorescence intensity versus concentration of **2**. The calculated coefficient was used in determining kinetic constants of the conversion of iodo-BODIPY **1** to H-BODIPY **2** by Pd^{2+} in ethanol-water (1:4, v/v) at 25 ^{o}C.

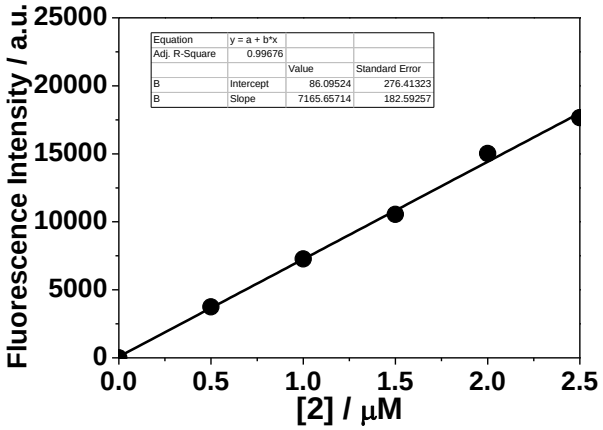


Figure S14. Standard fluorescence curve of H-BODIPY **2** in ethanol-water (1:4, v/v) at 25 ^{o}C. Fluorescence intensity at 510 nm was measured. Excited at 465 nm.

Determination of kinetic constant: To determine kinetic constant, iodo-BODIPY **1** (5 μM) was added to the solution of Pd^{2+} (50 μM) in ethanol-water (1:4, v/v) at 25 ^{o}C.

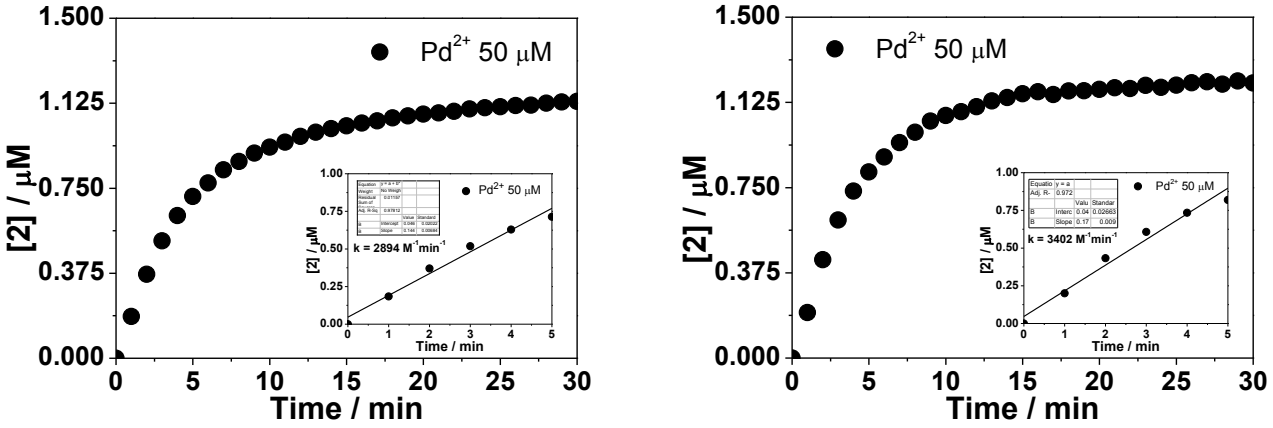


Figure S15. Kinetics for the fluorescence response of iodo-BODIPY **1** (5 μM) with Pd^{2+} (50 μM) in ethanol-water (1:4, v/v) at 25 ^{o}C. Fluorescence intensity was measured at 510 nm. Excited at 465 nm.

(a) Several factors to improve kinetics: amount of ethanol in water

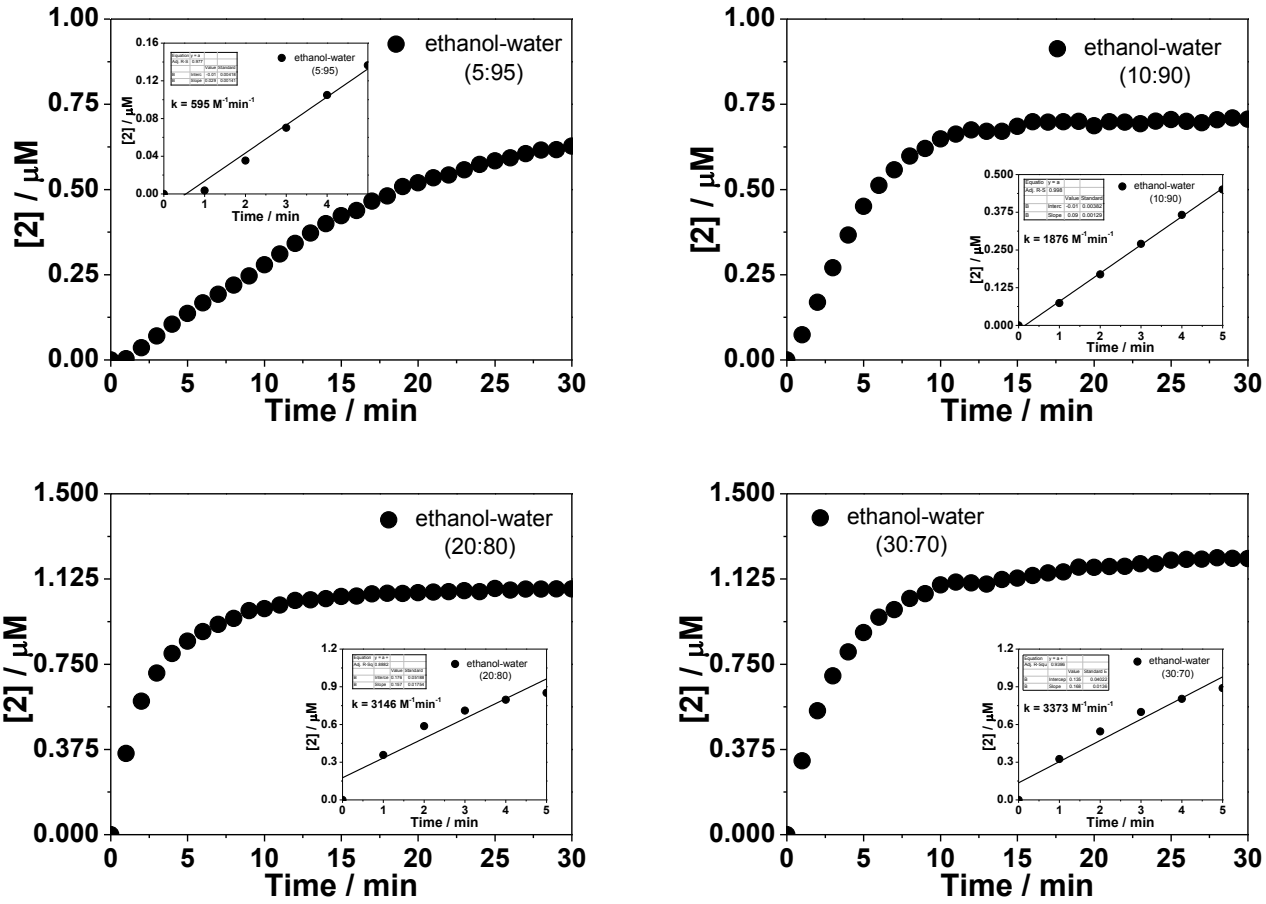


Figure S16. Kinetics for the fluorescence response of iodo-BODIPY **1** (5 μM) with Pd^{2+} (50 μM) in water containing different % ethanol (5-30%) as a cosolvent at 25 ^{o}C. Fluorescence intensity was measured at 510 nm. Excited at 465 nm.

(b) Several factors to improve kinetics: temperature

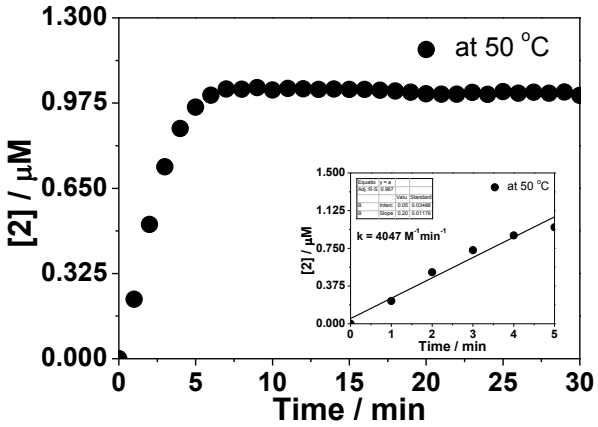


Figure S17. Kinetics for the fluorescence response of iodo-BODIPY **1** (5 μM) with Pd^{2+} (50 μM) in ethanol-water (1:4, v/v) at 50 ^{o}C. Fluorescence intensity was measured at 510 nm. Excited at 465 nm.

(c) Several factors to improve kinetics: additional reducing agent, $NaBH_4$

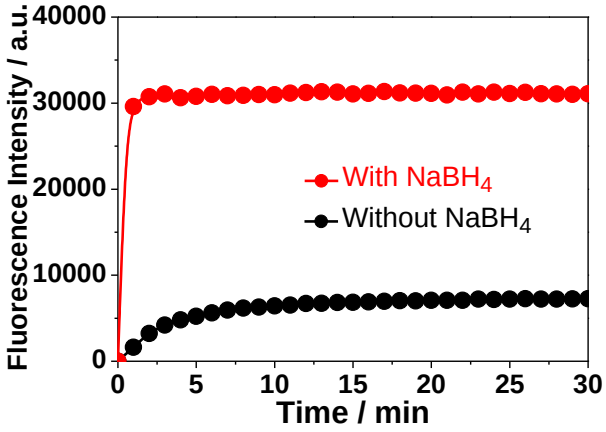


Figure S18. Time-dependent fluorescence intensity at 510 nm of **1** (5 μM) upon addition of Pd^{2+} (50 μM) in the presence of $NaBH_4$ (0.5 mM) in ethanol–water (1:4, v/v) at 25 ^{o}C. Excited at 465 nm.

8. Determination of Detection Limit

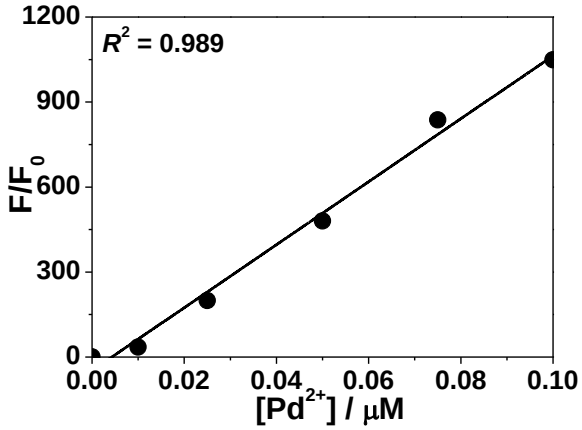


Figure S19. A plot of relative fluorescence intensity at 510 nm of iodo-BODIPY **1** (5 μM) as a function of $[Pd^{2+}]$. Fluorescence spectra of iodo-BODIPY **1** (5 μM) were measured 30 min after addition of Pd^{2+} in ethanol–water (1:4, v/v, 0.5 mM $NaBH_4$) at 25 ^{o}C. Excited at 465 nm. F_0 and F correspond to the fluorescence intensity of iodo-BODIPY **1** in the absence and the presence of $PdCl_2$ in ethanol-water (1:4, v/v, 0.5 mM $NaBH_4$), respectively. $[Pd^{2+}]$ = 0-0.1 μM

9. Fluorescence Turn-on Response of 1 with Various Palladium Species

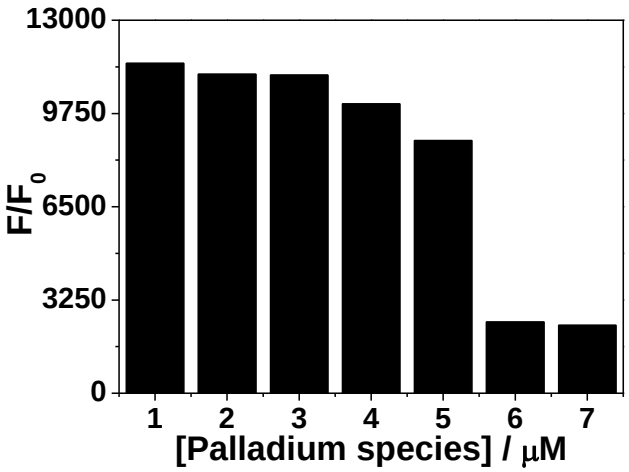
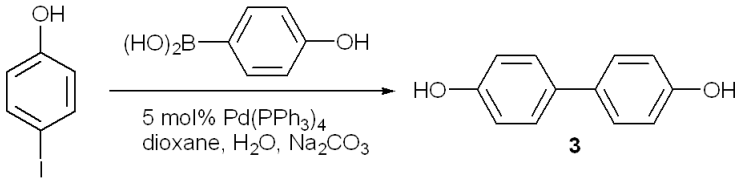


Figure S20. Relative fluorescence intensities at 510 nm of iodo-BODIPY **1** (5 μM) toward various different palladium species (50 μM) in ethanol–H_2O (1:4, v/v, 0.5 mM $NaBH_4$), measured 30 minutes after addition of each analyte at 25 °C. 1, $Pd(NO_3)_2$; 2, $Pd(OAc)_2$; 3, $PdCl_2$; 4, Na_2PdCl_4; 5, $Pd(PPh_3)_4$; 6, $PdCl_2(PPh_3)_2$; 7, $PdCl_2(dppf)_2$. Excited at 465 nm.

10. Determination of Palladium Contents in Chemical Products

Biphenyl derivative **3** was synthesized by a Suzuki-Miyaura cross coupling reaction of an arylboronate (210 mg, 1.10 mmol) and an aryl halide (300 mg, 1.36 mmol) in the presence of $Pd(PPh_3)_4$ (81 mg, 0.07 mmol) and purified by using column chromatography (Scheme S1). Standard curve was obtained by measuring fluorescence intensity at 510 nm of iodo-BODIPY **1** (5 μM) 30 min after the addition of $Pd(PPh_3)_4$ at various concentrations (1-50 μM) in ethanol–H_2O (1:4, v/v, 0.5 mM $NaBH_4$) at 25 °C. For the determination of amount of Pd in the synthesized **3**, biphenyl derivative **3** (1.9 mg) was dissolved in ethanol (1 mL). An aliquot of this solution (20 μL) was mixed with a solution of $NaBH_4$ in water (160 μL, final [$NaBH_4$] = 0.5 mM). To the solution was added a solution of iodo-BODIPY **1** in EtOH (20μL, [**1**] = 0.05 mM) to result in an assay solution nominally 5 μM in **1**. The reactions were monitored at 25 °C for 30 minutes in a microplate reader, during which the fluorescence signal for each well was measured at 510 nm (λ_{ex} = 465 nm). Comparison of the fluorescence signals from analysis of **3** (38 μg), obtained using sensing system comprised of iodo-BODIPY **1** (5 μM) in ethanol–H_2O (1:4, v/v, 0.5 mM $NaBH_4$,) at 25 °C, with those from a standard curve show that that the purified biphenyl derivative **3** (38 μg) contains 2 ± 0.1 ppm of Pd.



Scheme S1. Synthesis of compound **3**.

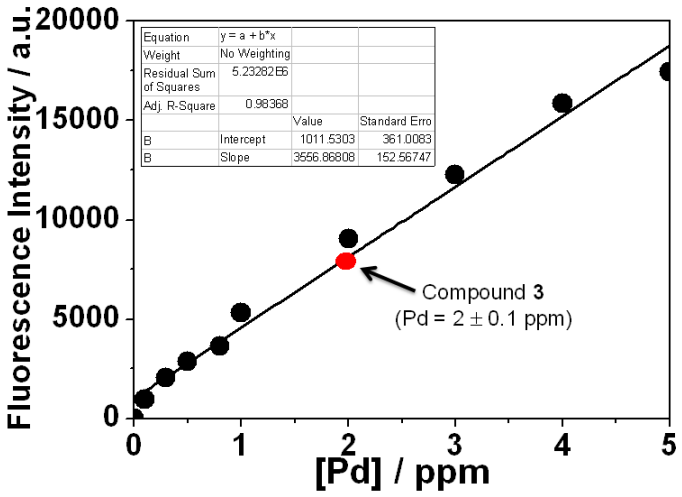


Figure S21. Determination of residual palladium contents in biphenyl derivative **3** (38 μg). Comparison of the fluorescence signals from analysis of **3** (red circle), obtained using sensing system comprised of iodo-BODIPY **1** (5 μM) in ethanol–H_2O (1:4, v/v, 0.5 mM $NaBH_4$, 25 °C), with those from a standard curve (black circle). Excited at 465 nm. The measured [Pd] in **3** was determined from the average of 4 independent measurements to be 2 ± 0.1 ppm

11. References

1 J. Park, S. Choi, T.-I. Kim and Y. Kim, *Analyst*, 2012, 137, 4411.
2 R. F. Kubin and A. N. Fletcher, *J. Luminescence*, 1982, **27**, 455.
3 A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, **108**, 1067.