Supporting Information

Naphthalimide-based fluorescent 'turn off' probes for palladium ion: structure-activity relationships

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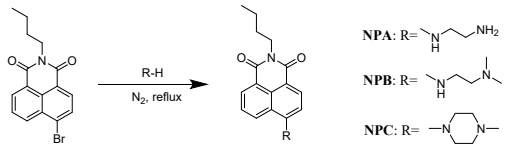
1. Materials and instruments

Unless otherwise specified, all reagents and chemicals used in this study were purchased from commercial companies. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Bruker Avance 400 MHz or 600 MHz spectrometer with tetramethylsilane (TMS) as the internal standard substance. Mass spectra were recorded with a Waters Xevo G2XS Tof mass spectrometer. The absorption and emission spectra were respectively measured with an Evolution 220 and a Lumina spectrophotometer from Thermo Fisher Scientific. The confocal fluorescence image was carried out with an A1R laser scanning confocal microscope from Nikon.

2. Cell culture and fluorescence imaging

The resuscitated and passaged L929 cells and Hela cells were incubated with DMEM medium containing 10% fetal bovine serum in the incubator (37 ℃, 5% CO_2) for 24 h. Subsequently, the L929 cells and Hela cells were transferred to confocal culture dishes and incubated in the incubator (37 ℃, 5% CO_2) for another 24 h. The experiments were divided into three groups: 1) in the control group, the cells were incubated with serum-free medium containing 5 μM probe for 20 min; 2) in the test group, the cells were pre-incubated with serum-free medium containing 10 μM $PdCl_2$ for 30 min, followed by incubated with serum-free medium containing 5 μM probe for 20 min; 3) in the validation group, to verify the targeting ability of probe **NPE** and **NPF** toward cancer cells through receptor-mediated endocytosis, Hela cells were respectively preincubated with 1 mmol/L biotin or 100 μmol/L celecoxib for 30 min to compete with biotin or indomethacin receptors, and then incubated with serum-free medium containing 5 μM probe for 20 min; while L929 cell were pretreated with 1 μg/mL LPS for 12 h to induce inflammation followed by incubated with serum-free medium containing 5 μM probe for 20 min. After washed with PBS for three times, the cells were treated with 1 mL of culture medium for confocal experiments. In the confocal experiments, the fluorescence of blue channel (425-475 nm) and green channel (500-550 nm) were recorded under excited with a 405 nm/488 nm laser, respectively.

3. Synthesis of probes NPA-NPF



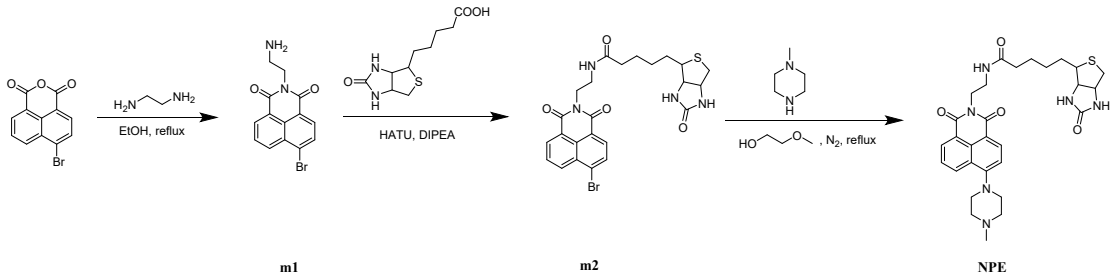
Scheme S1 Synthesis routes of **NPA, NPB** and **NPC**

NPA-NPC

N-butyl-4-bromo-1,8-naphthalimide (331 mg, 1 mmol) and ethylenediamine (1.2 mL) were dissolved in 20 mL of ethylene glycol monomethyl ether and refluxed for 6 hours under nitrogen protection. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (DCM: MeOH = 50:4) to give yellow powder **NPA** (192 mg, 62%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.57 (d, *J* = 7.3 Hz, 1H), 8.44 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 6.20 (t, *J* = 5.0 Hz, 1H), 4.20-4.12 (m, 2H), 3.42 (q, *J* = 5.3 Hz, 2H), 3.18 (t, *J* = 5.7 Hz, 2H), 1.70 (q, *J* = 8.0 Hz, 2H), 1.61 (s, 2H), 1.44 (h, *J* = 7.4 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (151 MHz, $CDCl_3$) δ (ppm): 164.76, 164.23, 149.67, 134.46, 131.10, 129.82, 126.23, 124.68, 123.13, 120.48, 110.36, 104.41, 44.90, 40.17, 39.99, 30.33, 29.70, 29.32, 20.45, 13.89. HR-MS m/z calcd for $C_{18}H_{21}N_3O_2$: $[M+H]^+$

312.1712; found 312.1701. Compounds **NPB** and **NPC** were obtained by the same procedure with N,N-dimethylethylenediamine or methylpiperazine instead of ethylenediamine. **NPB** (151 mg, 43%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.60-8.55 (m, 1H), 8.45 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.33 (t, *J* = 4.3 Hz, 1H), 4.16 (dd, *J* = 8.6, 6.5 Hz, 2H), 3.43-3.36 (m, 2H), 2.75 (t, *J* = 5.8 Hz, 2H), 2.35 (s, 6H), 1.75-1.66 (m, 2H), 1.45 (q, *J* = 7.5 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C-NMR (151 MHz, $CDCl_3$) δ (ppm): 164.79, 164.25, 149.63, 134.52, 131.10, 129.80, 126.46, 124.64, 123.07, 120.43, 110.21, 104.36, 56.94, 45.01, 40.14, 39.98, 30.34, 20.45, 13.90. HR-MS m/z calcd for $C_{20}H_{26}N_3O_2$: $[M+H]^+$ 340.2025; found 340.2014. **NPC** (172 mg, 51%): ^{1}H-NMR (600 MHz, DMSO-d_6) δ (ppm): 8.46 (dd, *J* = 7.3, 1.3 Hz, 1H), 8.42 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 7.80 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 4.05-3.99 (m, 2H), 3.23 (t, *J* = 4.9 Hz, 4H), 2.64 (t, *J* = 4.2 Hz, 4H), 2.31 (s, 3H), 1.63-1.56 (m, 2H), 1.34 (h, *J* = 7.4 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (151 MHz, DMSO-d_6) δ (ppm): 163.99, 163.46, 156.10, 132.63, 131.07, 130.92, 129.55, 126.45, 125.74, 123.01, 115.95, 115.51, 55.10, 52.98, 46.21, 30.18, 20.27, 14.20. HR-MS m/z calcd for $C_{21}H_{26}N_3O_2$: $[M+H]^+$ 352.2025; found 352.2017.

NPE



Scheme S2 Synthesis routes of **NPE**

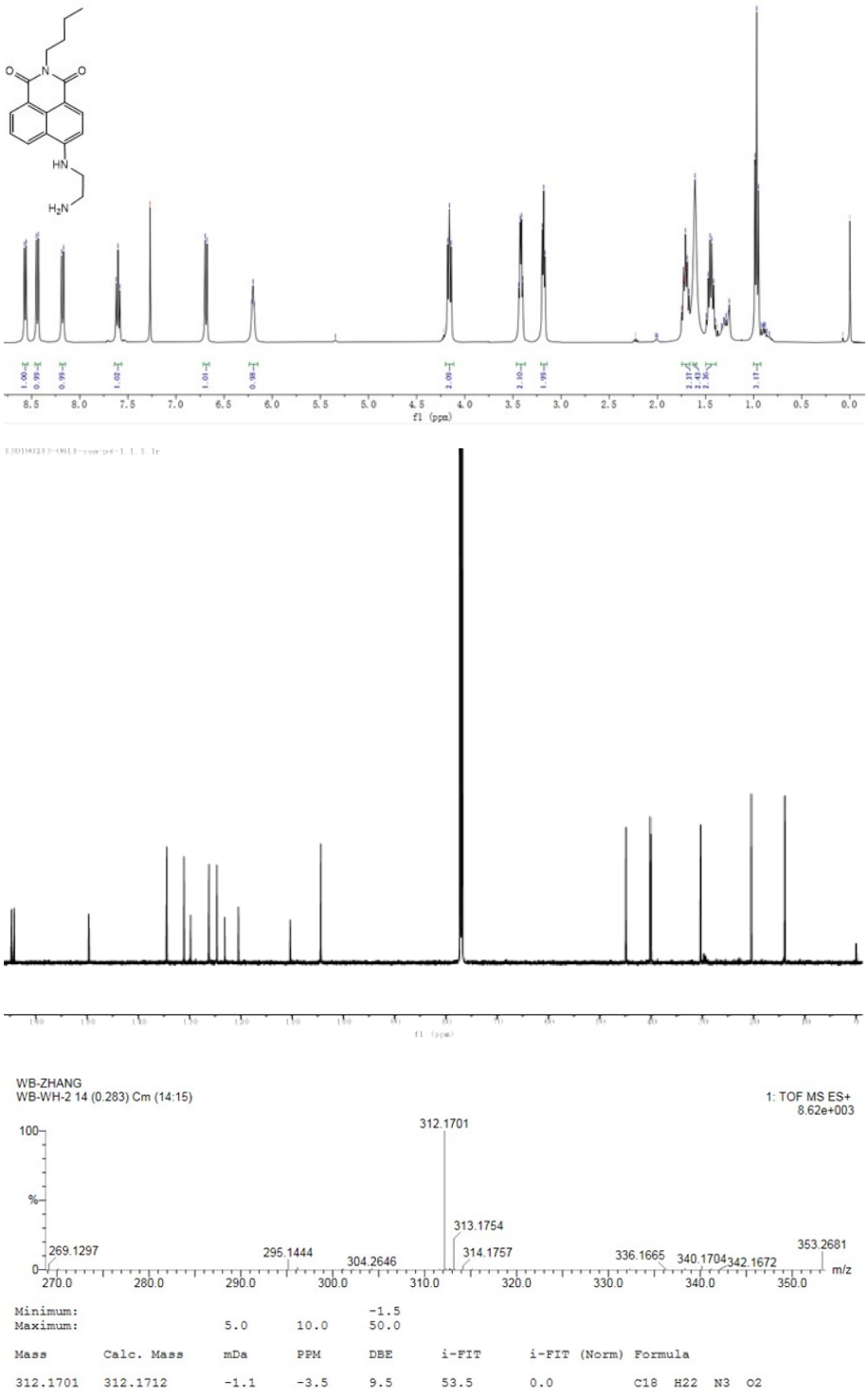
m1: 4-Bromo-1,8-naphthalic anhydride (2.77 g, 10.0 mmol) and ethylenediamine (1 g, 15.1 mmol) in anhydrous ethanol (100 mL) were refluxed for 6 hours under nitrogen protection. After filtering to remove the solid, the resulting solvent was distilled under reduced pressure. The obtained solid was recrystallized with ethanol to give a yellow powder **m1** (870 mg, 27%).

m2: Under room temperature, biotin (241.0 mg, 0.99 mmol) and HATU (294.0 mg, 0.77 mmol) in N,N-dimethylformamide (5 mL) were stirred for 1 hour to activate the carboxyl group. Then, DIPEA (0.56 mL) and compound **m1** (207 mg, 0.65 mmol) were added to the above solution. The resulting solution was stirred for another 16 hours and then added dropwise into water (200 mL). The solid was filtered under vacuum to give **m2** (326.5 mg, 60%).

NPE: Compound **m2** (326 mg, 0.60 mmol) and methylpiperazine (1.2 mL) in ethylene glycol monomethyl ether (20 mL) were refluxed under nitrogen protection for 16 hours. After cooling to room temperature, the solvent was removed by rotary evaporation. The resulting solid was purified by silica gel column chromatography (DCM: MeOH = 50:4) to yield a yellow solid **NPE** (102 mg, 30%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.51-8.37 (m, 3H), 7.89 (t, *J* = 6.1 Hz, 1H), 7.83 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 6.40 (s, 1H), 6.36 (s, 1H), 4.32-4.26 (m, 1H), 4.12 (t, *J* = 6.0 Hz, 2H), 4.06 (t, *J* = 5.9 Hz, 1H), 3.25 (s, 4H), 3.03-2.98 (m, 1H), 2.82 (ddd, *J* = 12.4, 9.9, 5.1 Hz, 2H), 2.66 (s, 4H), 2.32 (s, 3H), 1.94 (t, *J* = 7.5 Hz, 2H), 1.51 (dd, *J* = 12.6, 5.9 Hz, 2H), 1.36 (dt, *J* = 15.7, 7.8 Hz, 4H), 1.24 (s, 2H). ^{13}C-NMR (151 MHz, DMSO-d_6) δ (ppm): 174.93,

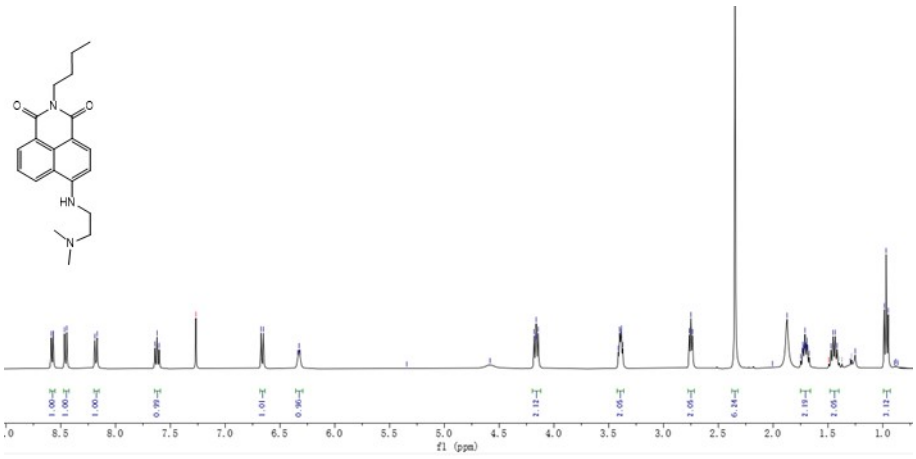
172.69, 164.27, 163.74, 163.17 (d, J = 3.4 Hz), 156.01, 132.54, 130.99, 130.82, 129.75, 126.47, 125.79, 123.30, 116.29, 115.51, 61.40, 59.65, 55.81, 55.09, 52.98, 46.19, 37.07-36.49 (m), 35.69 (d, J = 3.3 Hz), 28.52 (d, J = 3.9 Hz), 28.42 (d, J = 3.6 Hz), 25.63, 25.01. HR-MS m/z calcd for $C_{29}H_{37}N_6O_4S$: $[M+H]^+$ 565.2597; found 565.2600.

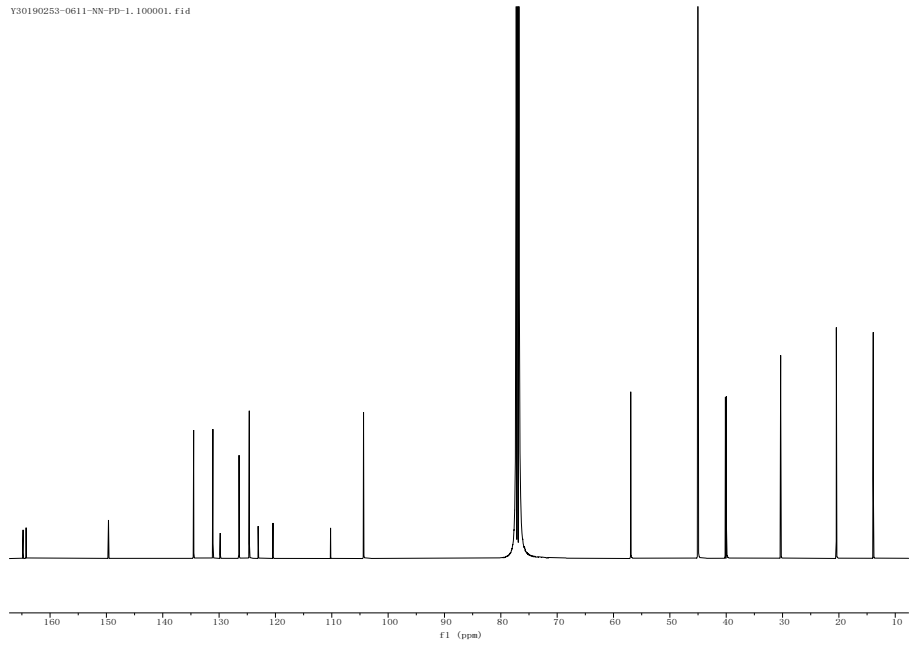
NPD and **NPF** were synthesized according to the literature [1].

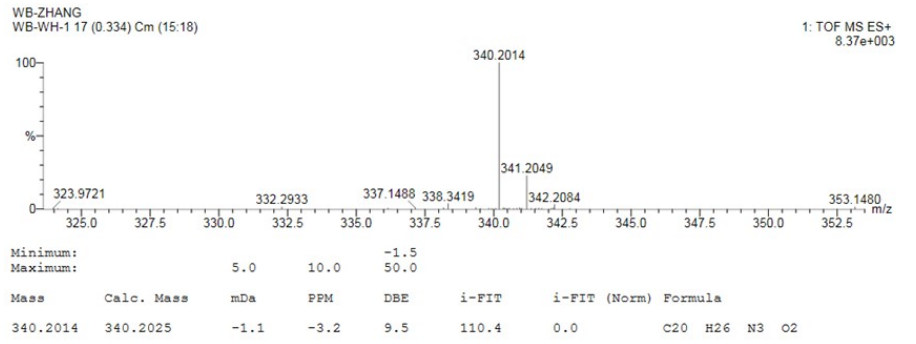


Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
312.1701	312.1712	-1.1	-3.5	9.5	53.5	0.0	C18 H22 N3 O2

Fig. S1 ^{1}H-NMR, ^{13}C-NMR and HR-MS of **NPA**

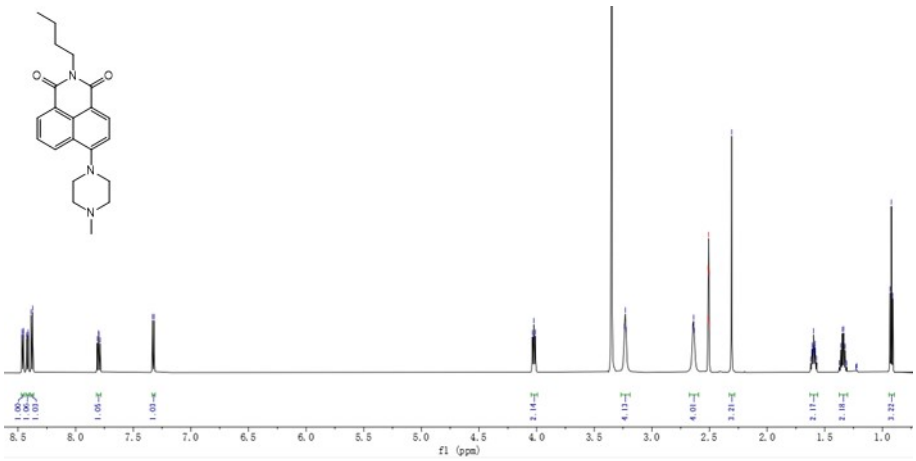


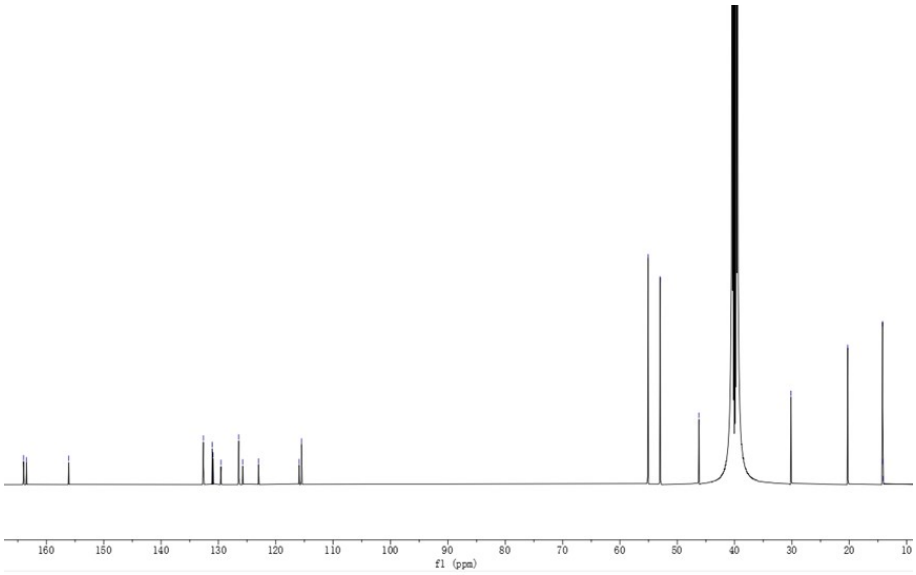


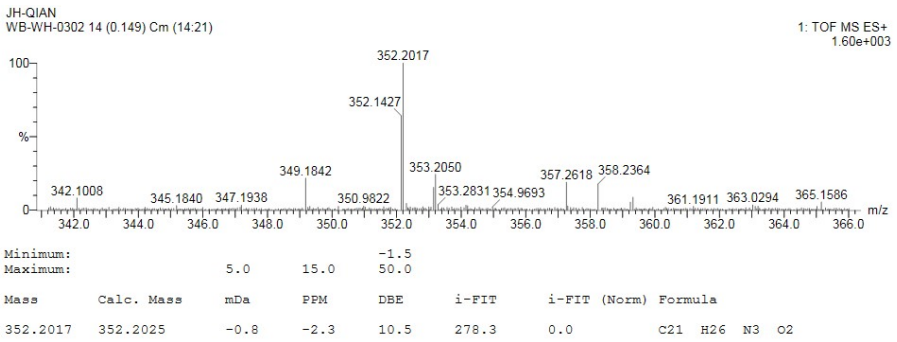


Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
340.2014	340.2025	-1.1	-3.2	9.5	110.4	0.0	C20 H26 N3 O2

Fig. S2 ^{1}H-NMR, ^{13}C-NMR and HR-MS of **NPB**

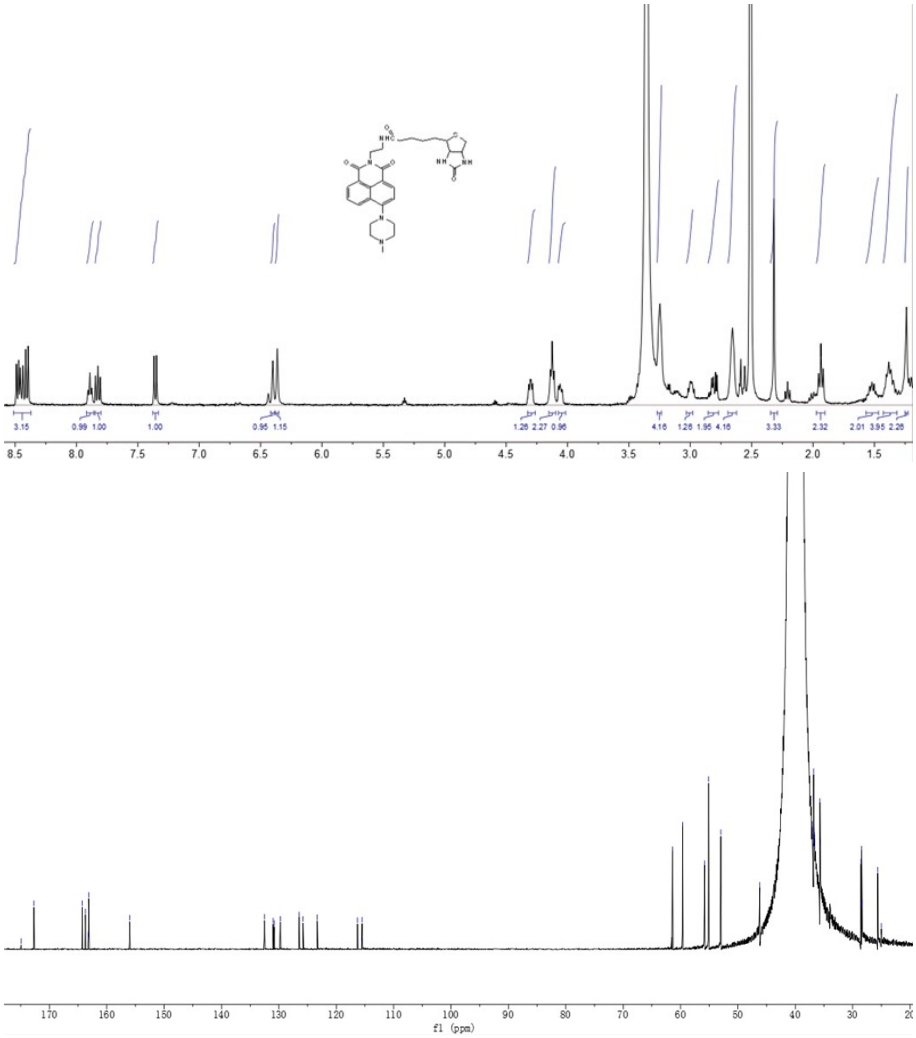


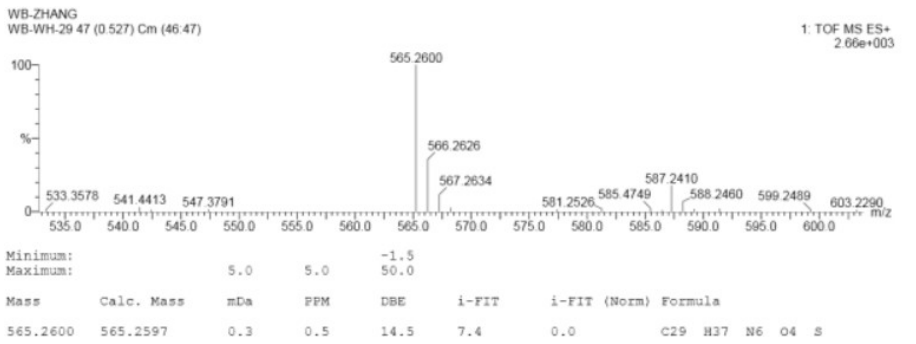




Minimum:				-1.5			
Maximum:		5.0	15.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
352.2017	352.2025	-0.8	-2.3	10.5	278.3	0.0	C21 H26 N3 O2

Fig. S3 ^{1}H-NMR, ^{13}C-NMR and HR-MS of **NPC**

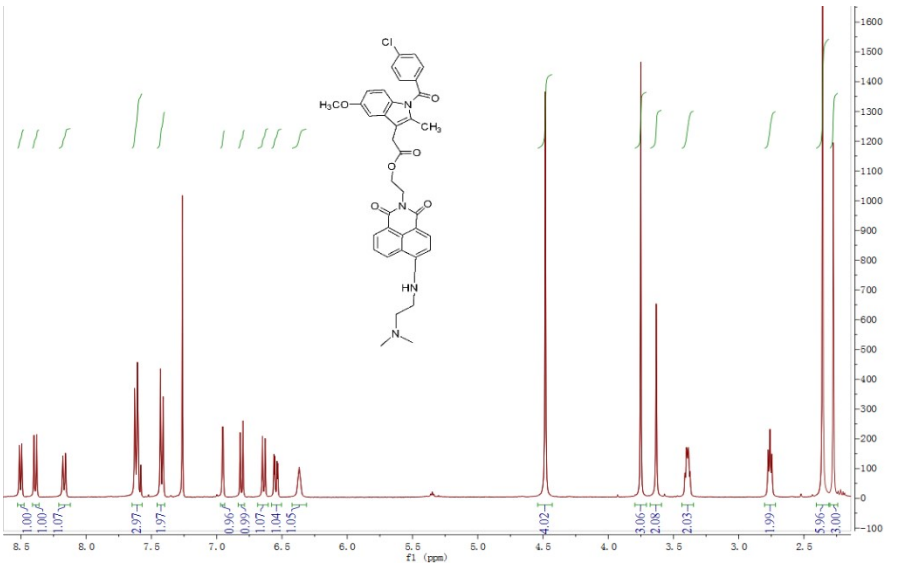


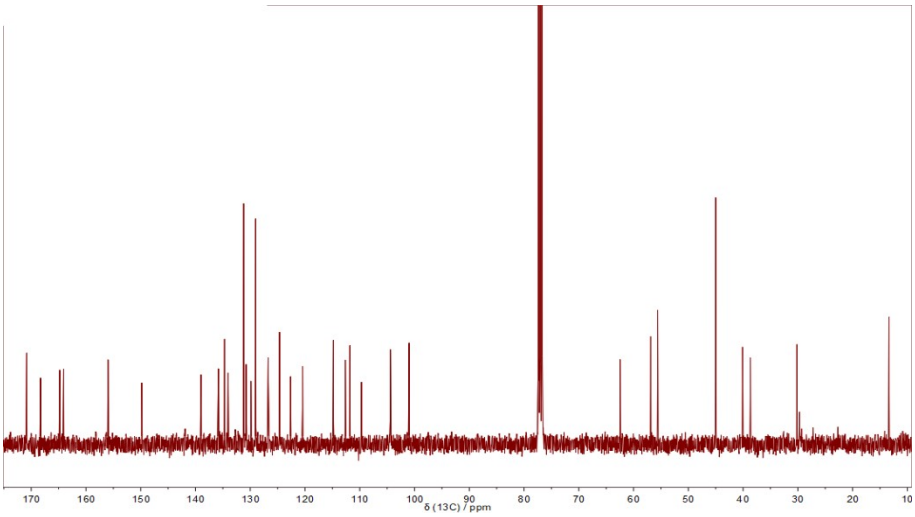


Minimum: -1.5
Maximum: 5.0 5.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
565.2600	565.2597	0.3	0.5	14.5	7.4	0.0	C29 H37 N6 O4 S

Fig. S4 ^{1}H-NMR, ^{13}C-NMR and HR-MS of **NPE**





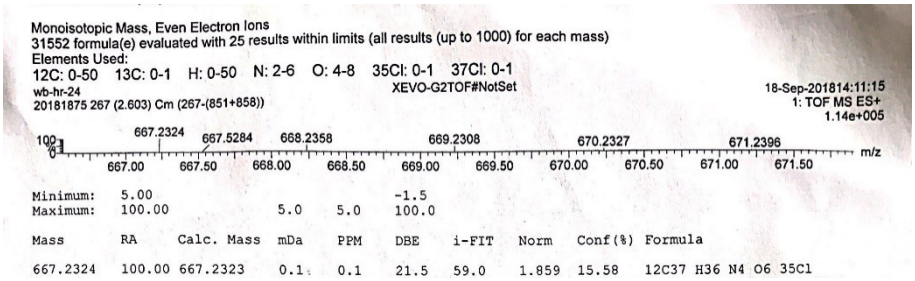


Fig. S5 ^{1}H-NMR, ^{13}C-NMR and HR-MS of **NPF**

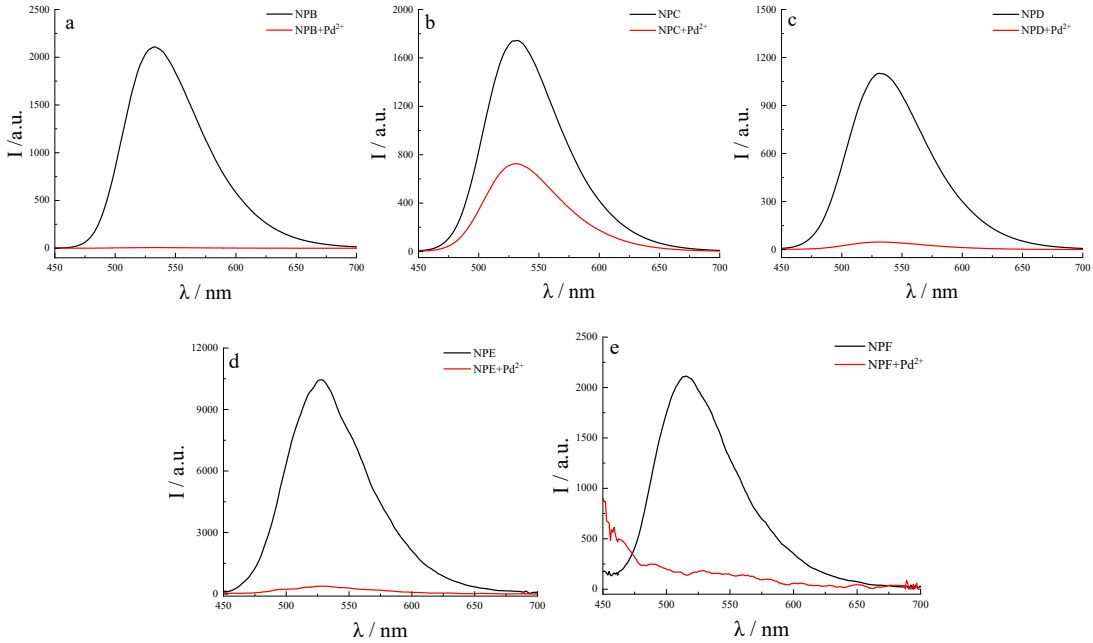


Fig. S6 The emission spectra of the probes with (red line) and without (black line) Pd^{2+} in PBS (20 mM, pH 7.0). [probe] = 10 μM, [Pd^{2+}] = 200 μM, λ_{ex} = 425 nm for **NPB** and **NPF**, λ_{ex} = 382 nm for **NPC**, **NPD** and **NPE**.

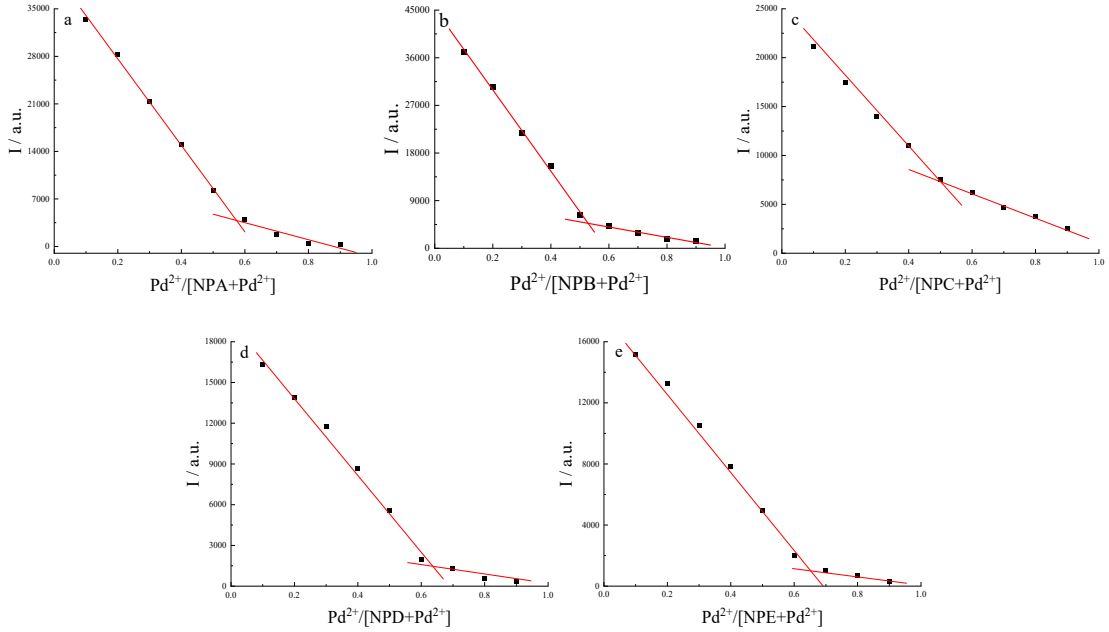


Fig. S7 The Job's plots of **NPA-NPE** and Pd^{2+} in PBS (20 mM, pH 7.0), [probe]+[Pd^{2+}] = 15.0 μM.

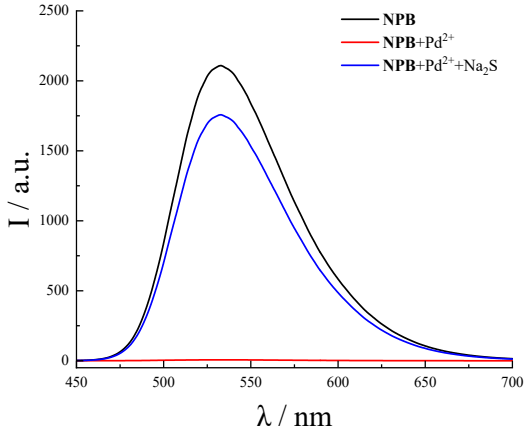


Fig. S8 Fluorescence spectra of **NPB** after addition of Pd^{2+} and further addition of Na_2S in PBS solution (20 mM, pH 7.0).

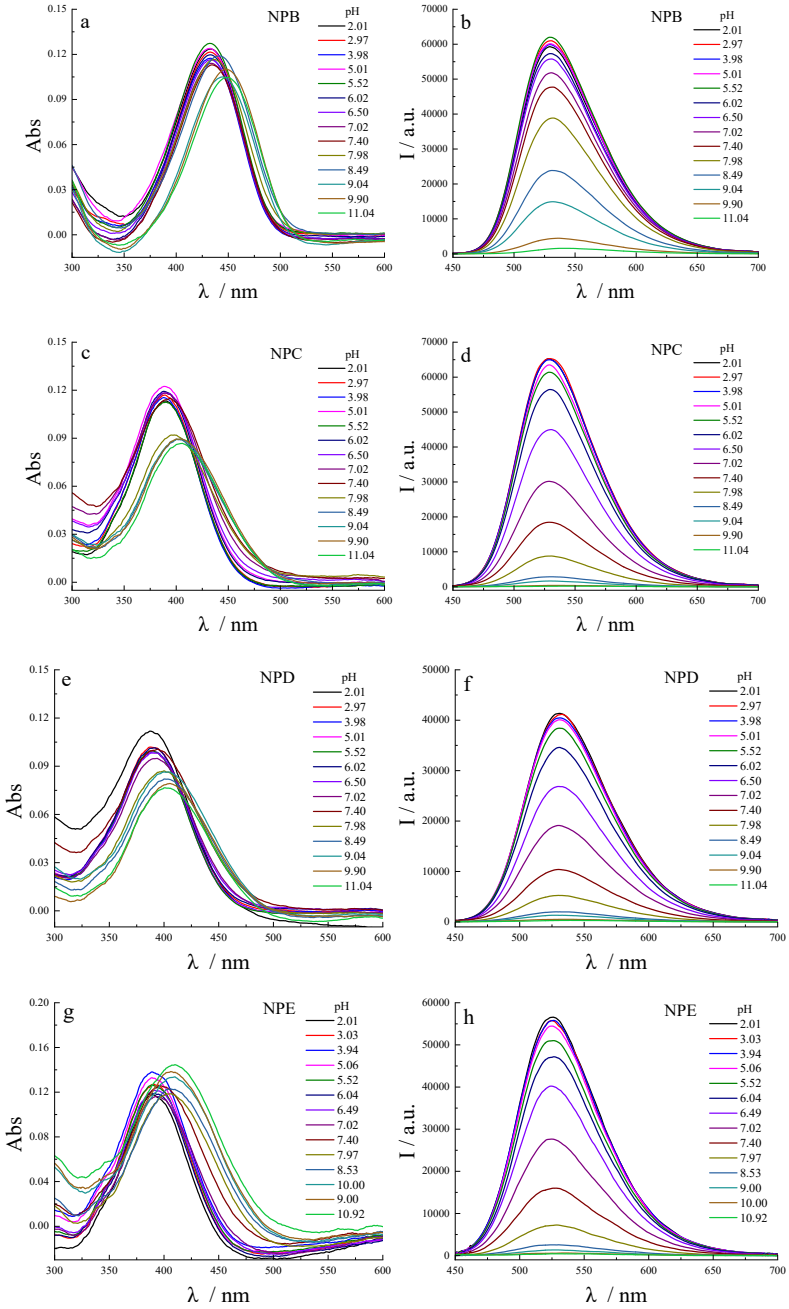


Fig. S9 The absorption (a, c, e, g) and emission (b, d, f, h) spectra of **NPB/NPC/NPD/NPE** under different pH conditions. [probe] = 10 μM, λ_{ex} = 425 nm for **NPB**, and λ_{ex} = 382 nm for **NPC**, **NPD** and **NPE**.

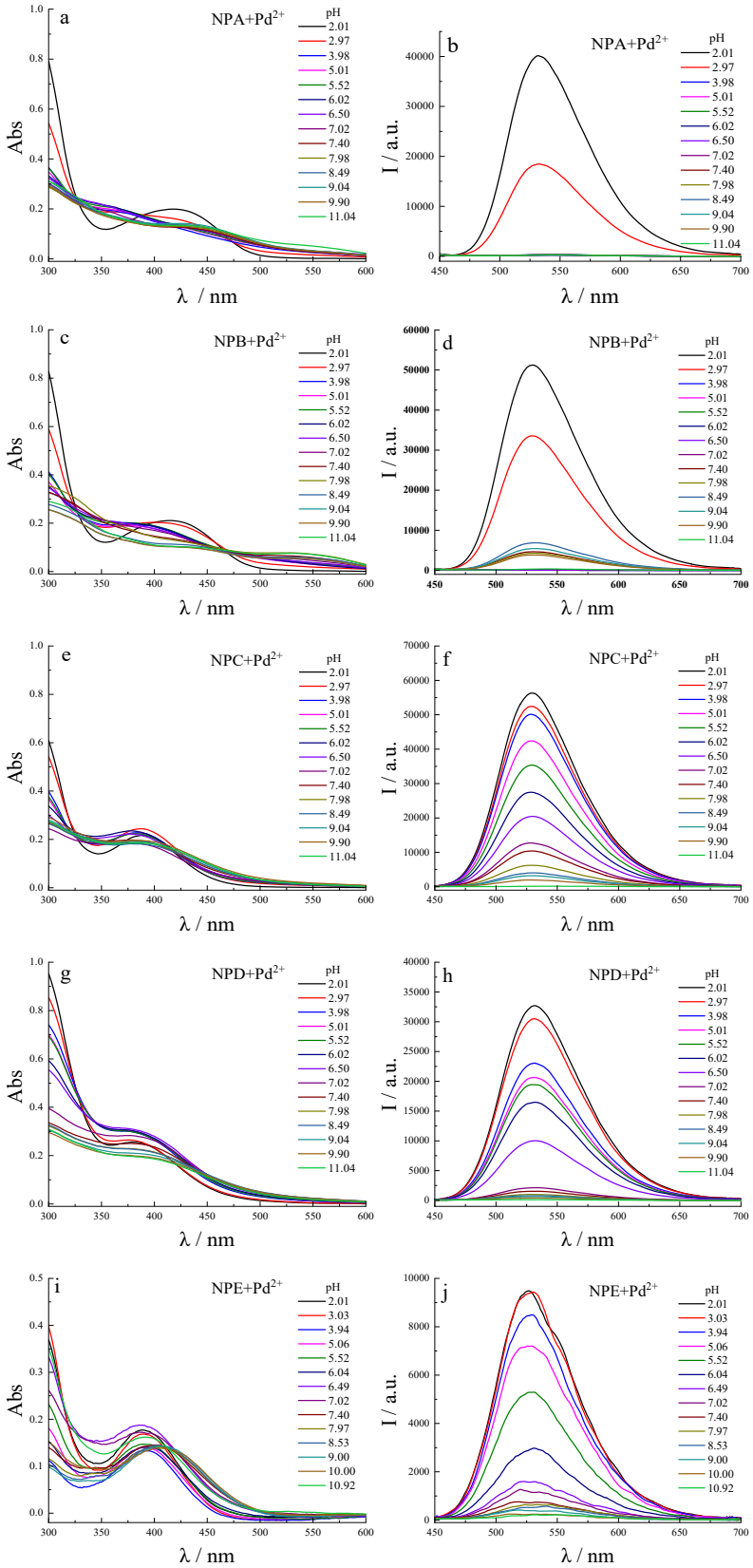


Fig. S10 The absorption (a, c, e, g, i) and emission (b, d, f, h, j) spectra of probe-Pd^{2+} mixed system under different pH conditions. [probe] = 10 μM, [Pd^{2+}] = 200 μM, λ_{ex} = 425 nm for **NPA** and **NPB**, and λ_{ex} = 382 nm for **NPC**, **NPD** and **NPE**.

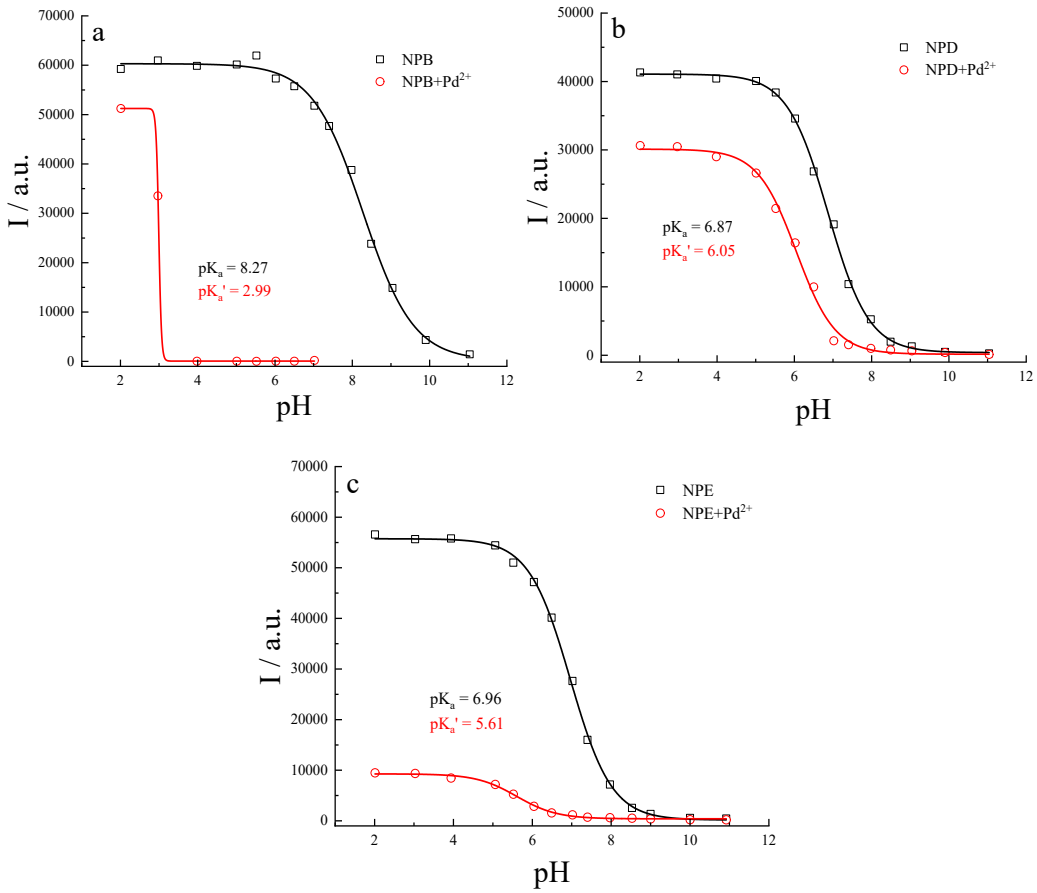


Fig. S11 The plots of fluorescent intensities of **NPB** (a), **NPD** (b) and **NPE** (c) without (black line) and with Pd^{2+} (red line) as a function of pH. [**NPB**] = [**NPD**] = [**NPE**] = 10 μM, [Pd^{2+}] = 200 μM, λ_{ex} = 425 nm for **NPB**, λ_{ex} = 382 nm for **NPD** and **NPE**.

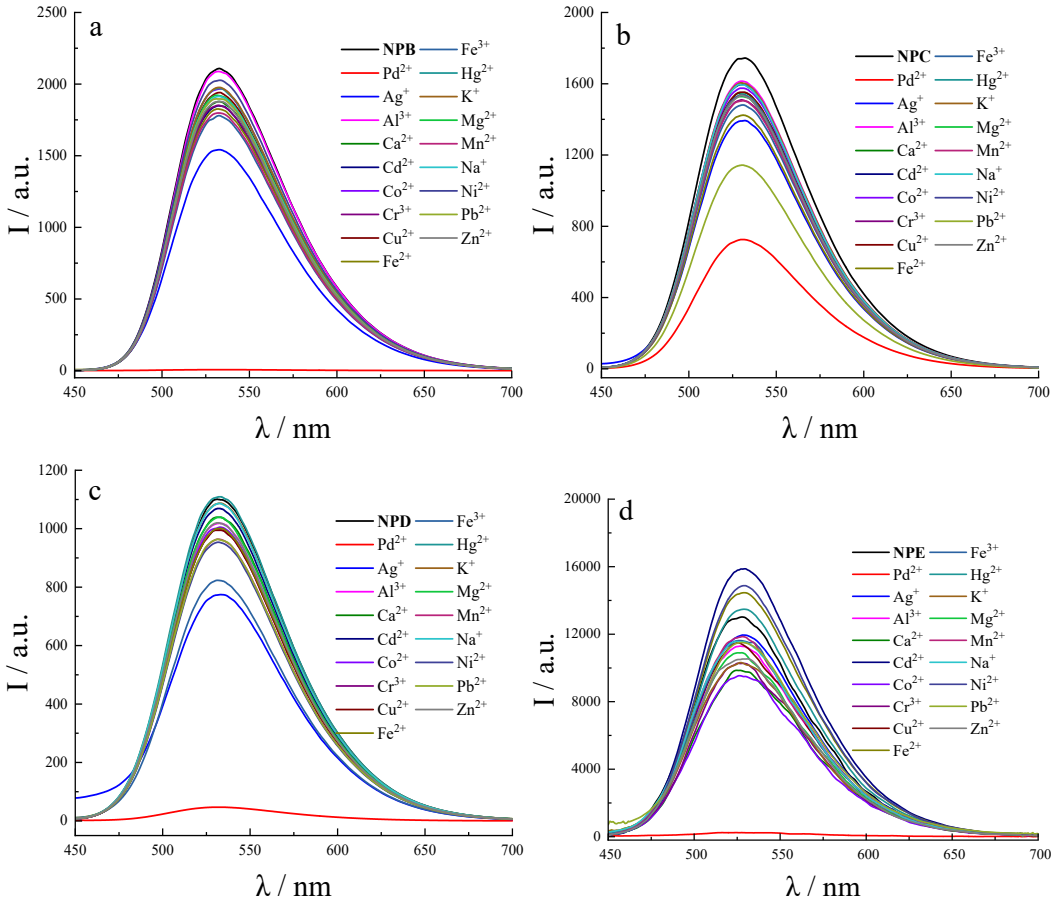


Fig. S12 Effects of metal ions on the emission spectra of **NPB**-**NPE**. [probe] = 10 μM, [metal ion] = 200 μM, λ_{ex} = 425 nm for **NPB**, and λ_{ex} = 382 nm for **NPC**, **NPD** and **NPE**.

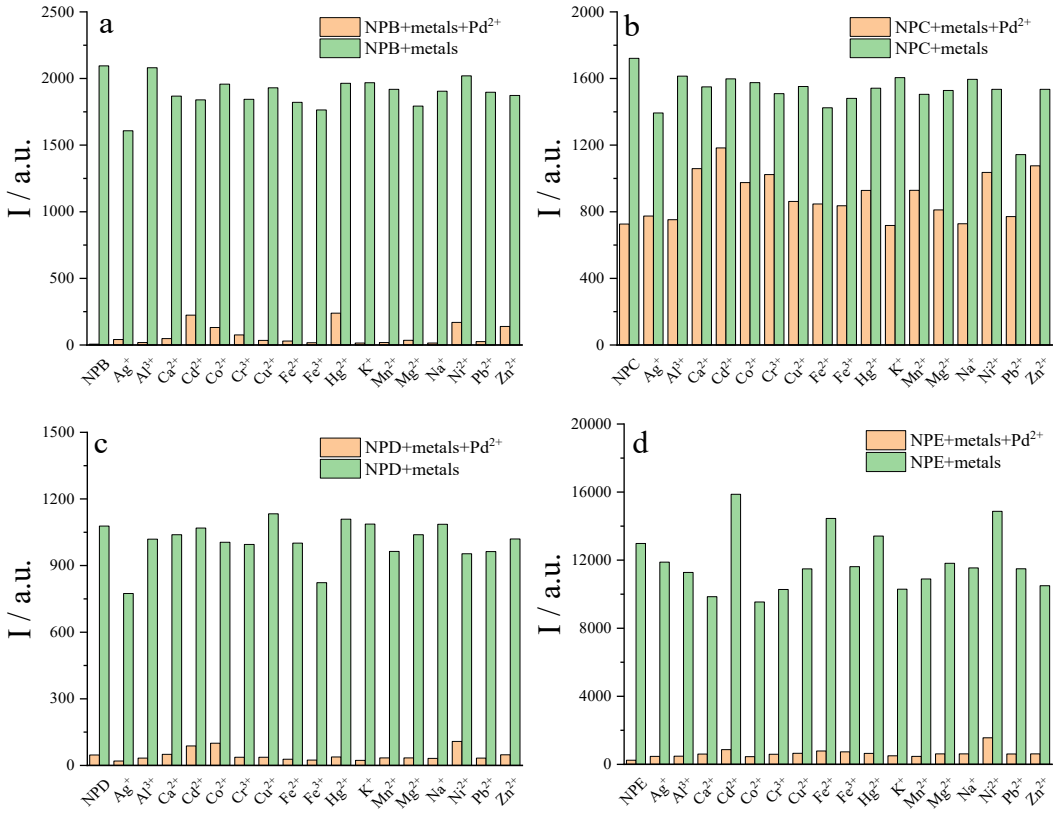


Fig. S13 Effects of metal ions on the fluorescence intensities of **NPB-NPE** in the absence (green) and presence (orange) of Pd^{2+}

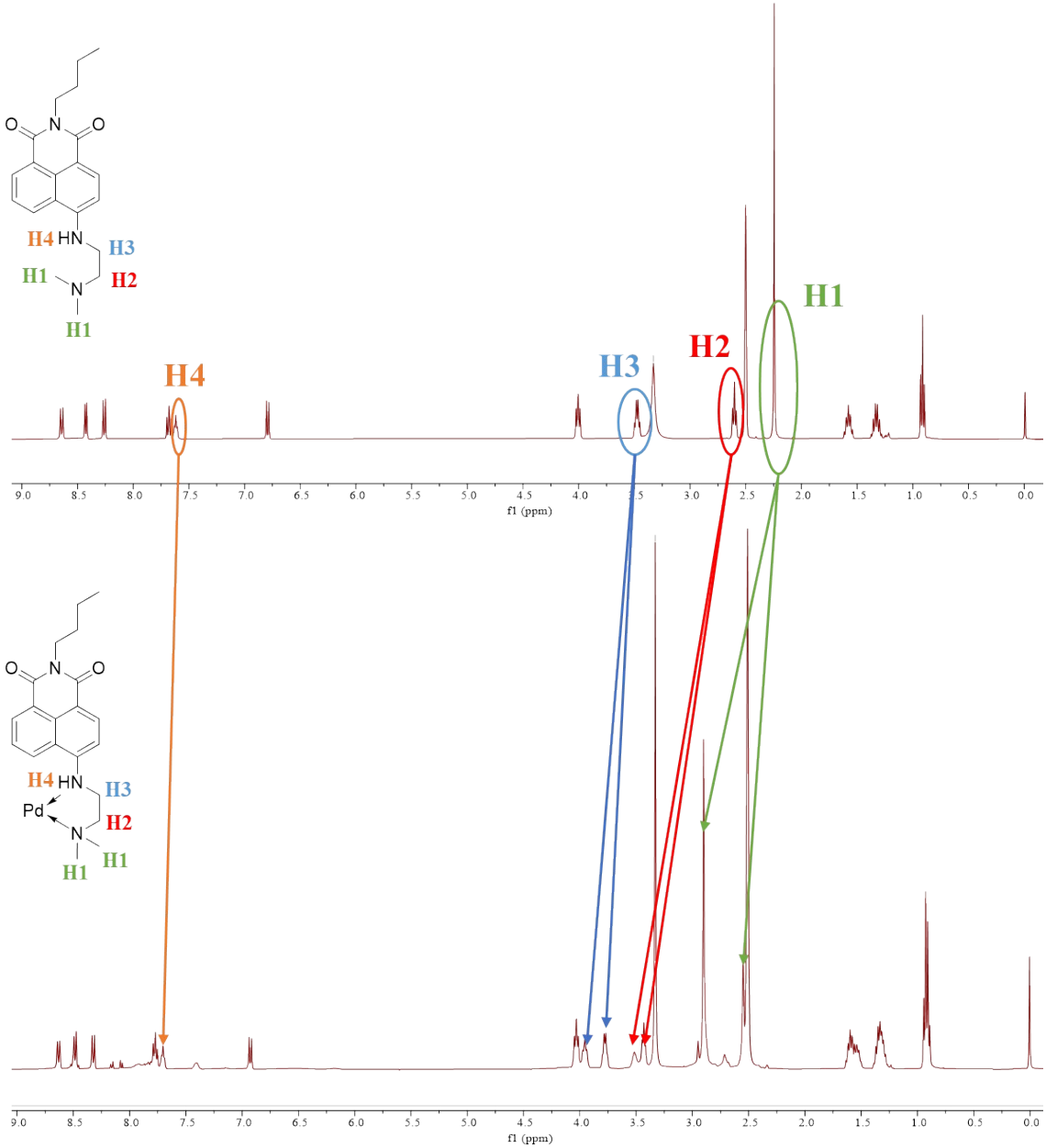


Fig. S14 Partial ^{1}H NMR spectra of **NPB** and **NPB** after reaction with $PdCl_2$

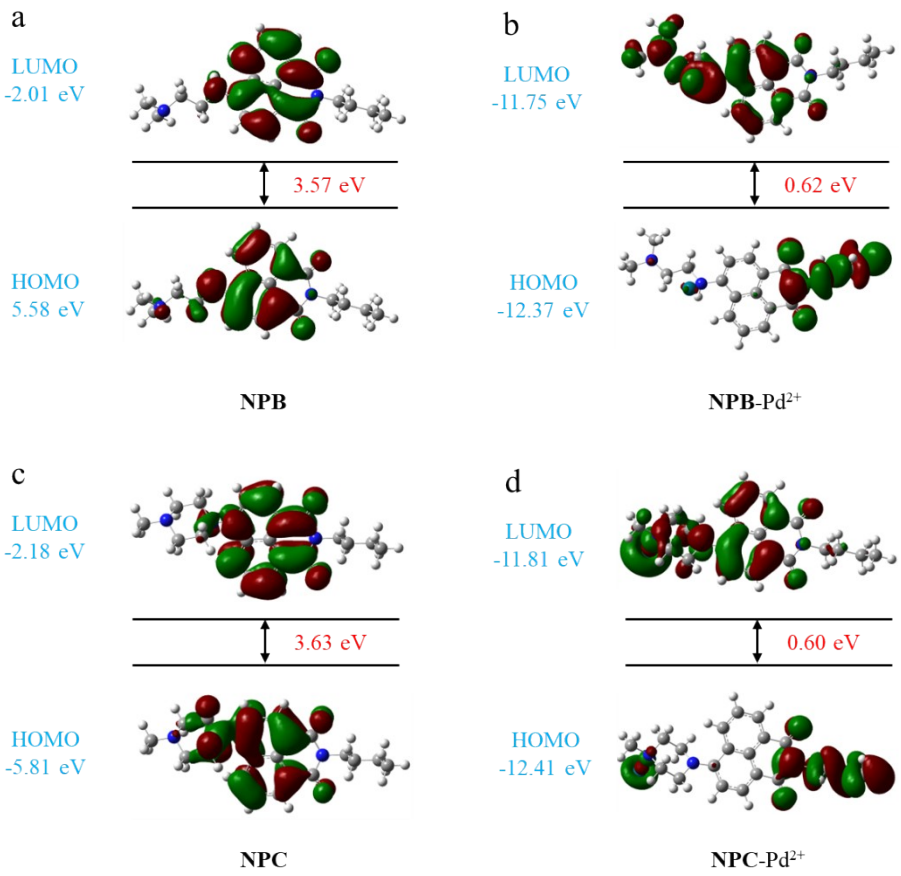


Fig. S15 Frontier molecular orbital of probe **NPB/NPC** in the absence and presence of Pd^{2+}. LANL2DZ basis group for Pd(II) and 6-31G(d, p) for other atoms.

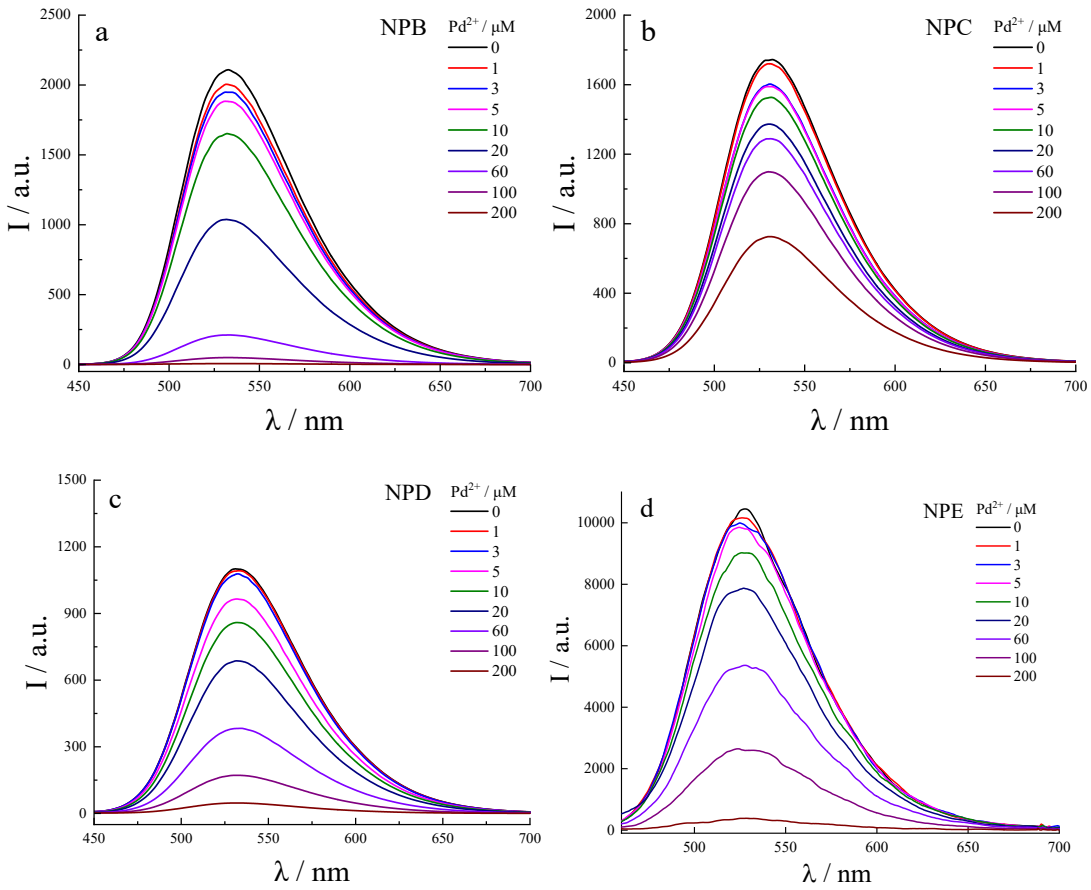


Fig. S16 Pd^{2+} effect on the emission spectra of **NPB-NPE**. [probe] = 10 μM, [Pd^{2+}] = 0-200 μM, λ_{ex} = 425 nm for **NPB**, and λ_{ex} = 382 nm for **NPC**, **NPD** and **NPE**.

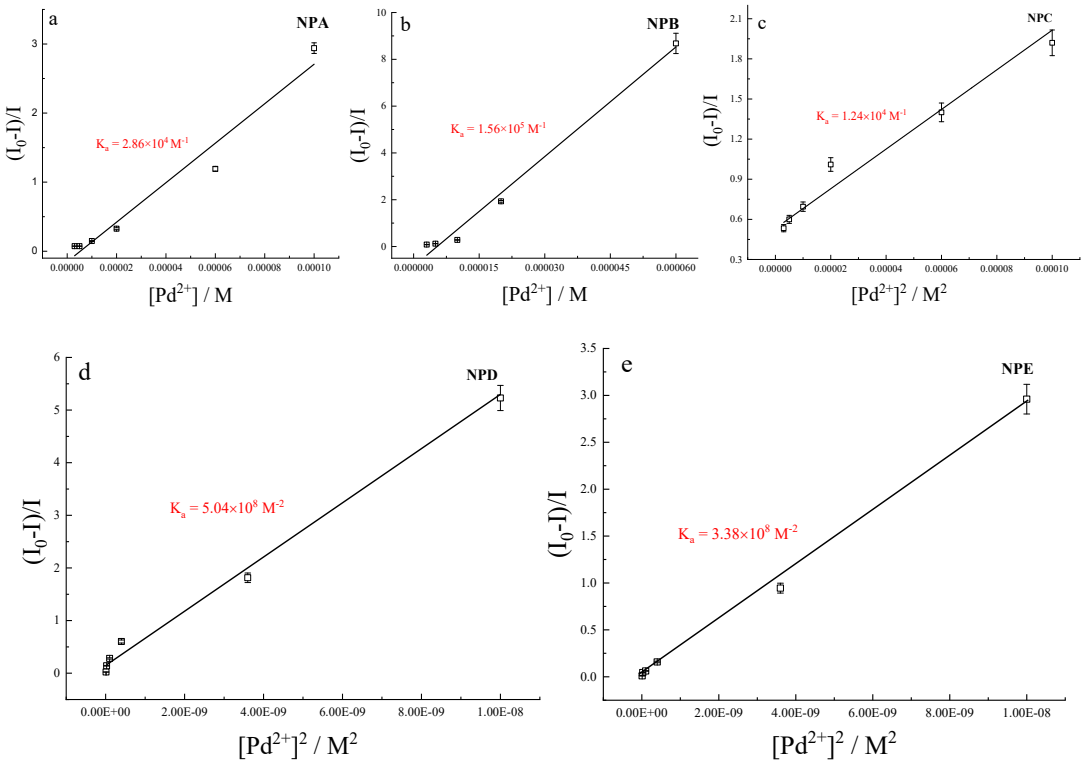


Fig. S17 Evaluation of binding constants of Pd^{2+} to **NPA-NPE**

Table S1 Comparation of the detection limits of Pd^{2+} with some reported probes

Probes	Detection limit	Linear range	Ref.
PPC	0.90 μM	0-50 μM	[2]
Cy202	0.52 μM	0-100 μM	[3]
3c	0.45 μM	0-25 μM	[4]
PPIX-L2	0.38 μM	0-4 μM	[5]
IMQU-8	0.0025 μM	0-2 μM	[6]
BTANC	0.0225 μM	0-9 μM	[7]
L_1	0.041 μM	0.25-0.5 μM	[8]
QA	0.485 μM	0-6 μM	[9]
PS-1	0.26 μM	0-5 μM	[10]
1	0.055 μM	0-6 μM	[11]
Bis-TPI	0.92 μM	0-1 μM	[12]
QB-3	0.089 μM	0-5 μM	[13]
NPA	3.30 μM	1-20 μM	This work
NPB	0.23 μM	1-20 μM	This work
NPC	9.48 μM	1-20 μM	This work
NPD	0.57 μM	1-20 μM	This work
NPE	4.06 μM	1-20 μM	This work

Table S2 Comparation analysis of Pd^{2+} in different samples

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%, n = 3)
River water	3	3.61	120	5.67
	5	6.15	123	5.50
	8	7.93	99	2.61
Tap water	3	2.79	93	5.64
	5	4.47	89	3.94
	8	7.52	94	1.78
Drinking water	3	2.72	91	5.87
	5	4.25	85	5.09
	8	8.09	101	0.61

Before detection, the water samples were filtered through 0.22 μm membrane filters.

References

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