Electronic Supplementary Material (ESI) for ChemComm.

# Supporting Information

# Cascade Sensing of Gold and Thiol with Imidazole-bearing Functional Porphyrins

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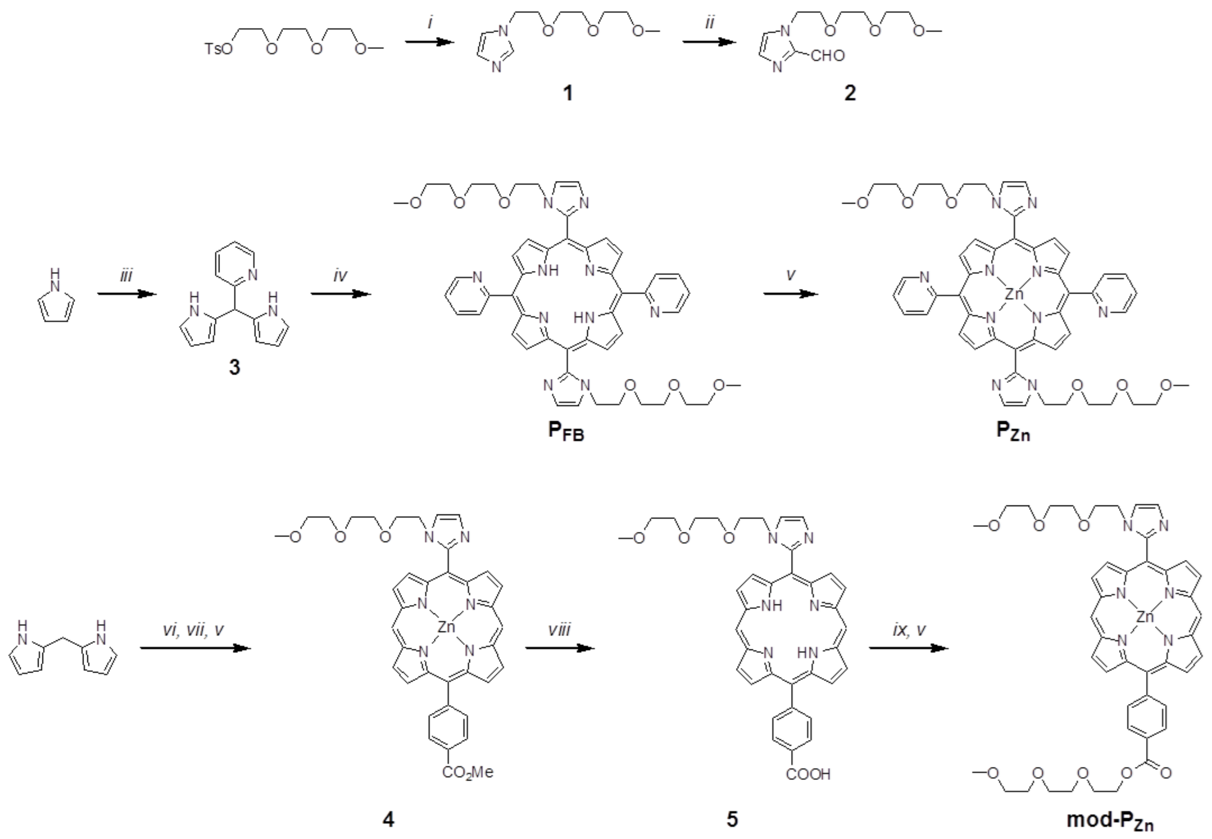
## Experimental Details

**Materials and measurements:** All commercially available reagents were reagent grade and used without further purification. For metal selectivity experiments and titration experiments, the $Cu^{2+}$, $Hg^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Ag^{+}$ solutions were prepared from the acetate salts. The solutions of $Fe^{3+}$, $Fe^{2+}$ were prepared from chlorate salts. The $Mg^{2+}$, $Cd^{2+}$, $Ni^{2+}$ solutions were prepared from nitrate salts. The solution of $Co^{2+}$, $Ru^{3+}$ and $Pt^{2+}$ were prepared from chloride salts and $Au^{3+}$ was prepared from $HAuCl_4$. For *in vitro* fluorescence imaging, $Au^{3+}$ solution was prepared from $AuCl_3$. All experiments were carried out with phosphate buffered solution (PBS, pH 7.4, 10 mM). UV/Vis absorption and fluorescence emission spectra were recorded on a JASCO model V-660 and FP-6300 spectrophotometer, respectively. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer at 25 ºC. MALDI-TOF-MS analysis was carried out using a Bruker model LRF20 using dithranol as a matrix. Visual observation of fluorescence emission was carried out under UV-light (Sankyo FL10BLB) illumination.

***In vitro* fluorescence imaging:** HeLa cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in a humidified atmosphere containing 5% $CO_2$ at 37°C. First, 160 μM of $\mathbf{P_{Zn}}$ in 1% acetonitrile/Hank's buffered salt solution (HBSS) was treated to HeLa cells for 30 min and washed with HBSS by three times to remove unassociated $\mathbf{P_{Zn}}$ to get blank cells. Second, in order to prepare cysteine enriched cells, HeLa cells were incubated with cysteine (2 mM) for 24 hrs and washed with HBSS. Then, $\mathbf{P_{Zn}}{\cdot}Au^{3+}$ was treated in a similar manner with above, where 160 μM of $\mathbf{P_{Zn}}$ in 1%

acetonitrile/HBSS was mixed with 1.0 equivalent of $Au^{3+}$. The *in vitro* imaging was carried out using a fluorescence microscope.

## Synthesis



**Scheme S1**. Synthetic procedures of imidazole-bearing porphyrins. Reagents and conditions; i) NaH, imidazole, THF, reflux, 12 h, ii) n-BuLi, DMF, THF, -78 °C, 12 h, iii) pyrrole, TFA, 30 min, iv) **2**, propionic acid, reflux, 12 h, v) $Zn(OAc)_2 \cdot 2H_2O$, $CH_2Cl_2$/MeOH, 2 h, vi) **2**, methyl 4-formyl benzoate, $CH_2Cl_2$, TFA, 25 °C, 12 h, vii) Chloranil, $CH_2Cl_2$, 25 °C, 3 h, viii) 3.0 M NaOH(aq), THF/$H_2O$, reflux, 12 h, ix) DCC, DMAP, tri(ethylene glycol) monomethyl ether, $CH_2Cl_2$, 25 °C, 12 h.

**1**: To a suspension of NaH (1.90 g, 44.1 mmol) in dry THF (200 mL), imidazole (2.00 g, 29.4 mmol) was added and stirred for 1 h at 0 ºC. After then, [2-[2-(2-methoxyethoxy)ethoxy]ethoxy]p-toluenesulfonate (14.0 g, 44.1 mmol) was added and refluxed for 12 h. The reaction mixture was cooled to 0 ºC, and excess NaH was quenched by the addition of small amount of water. The reaction mixture was poured into water and extracted with $CH_2Cl_2$. The combined organic layer was evaporated to dryness. And the mixture was purified with silica column chromatography as an eluent of

EtOAc/$CH_2Cl_2$/MeOH (5:5:1, v/v) to give **3** (5.5 g, 87%) as colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.53 (s, 1 H), 7.03 (s, 1 H), 6.99 (s, 1 H), 4.10 (t, 2 H), 3.74 (t, 2 H), 3.62 (m, 8 H), 3.38 (s, 3 H).

**2**: To a solution of **1** (19.0 g, 88.7 mmol) in dry THF (200 mL), *n*-butyl lithium (2.5 M in $CH_2Cl_2$, 45.0 mL, 115 mmol) was slowly added under $N_2$ for 1 h at -78 ºC. After then, dry DMF (10.0 mL, 133 mmol) was added and stirred for 3 h. Small amount of water was added to quench excess *n*-butyl lithum. The mixture was poured into water and extracted with $CH_2Cl_2$. The combined organic layer was evaporated to dryness and purified by silica column chromatography as an eluent of EtOAc/$CH_2Cl_2$/MeOH (5:5:1, v/v) to give **2** (11.0 g, 51%) as colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.80 (s, 1 H), 7.35 (s, 1 H), 7.27 (s, 1 H), 4.60 (t, 2 H), 3.76 (t, 2 H), 3.58 (m, 8 H), 3.51 (s, 3 H).

**3**: To a solution of 2-pyridinecarboxaldehyde (19.0 mL, 200 mmol) in pyrrole (200mL, 2800 mmol), trifluoroacetic acid (1.0 mL) was added and stirred for 30 min at 25 °C. After then, triethylamine (1.0 mL) was added to neutralize trifluoroacetic acid. The reaction mixture was poured into $CH_2Cl_2$ (400 mL). The combined organic layer was washed with water (100 mL) for three times and evaporated to dryness. The residue was purified by silica column chromatography with EtOAc/*n*-hexane (3:7, v/v). The second fraction was collected and recrystallized in $CH_2Cl_2$ and *n*-hexane to give **3** (12.3 g, 28%) as a white crystalline solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.88 (broad s, 1 H), 8.61 (m, 1 H), 7.68 (m, 1 H), 7.30 (m, 1 H), 7.20 (m, 1 H), 6.72 (m, 2 H), 6.13 (q, 2 H), 5.94 (m, 2 H), 5.50 (s, 1 H).

**P$_{\mathbf{FB}}$**: The mixture solution of **2** (3.31 g, 13.6 mmol) and **3** (3.05 g, 13.8 mmol) in propionic acid (200 mL) was refluxed for 12 h. The reaction mixture was evaporated and extracted with water and $CH_2Cl_2$. The combined organic layer was evaporated and silica column chromatographed with an eluent of EtOAc/$CH_2Cl_2$/MeOH (5:5:1, v/v). The first red-colored fraction was collected and freeze-dried to give **P$_{FB}$** (1.3 g, 11%) as purple powder. MALDI-TOF-MS: *m/z* calcd. for $C_{50}H_{52}N_{10}O_6$: 889.41 [M + H]$^+$; found: 889.86. $^1$H NMR (400 MHz, CDCl3): $\delta$ 9.16 (s, 2 H), 8.91 (d, 4 H), 8.83 (d, 4 H), 8.23 (d, 2 H), 8.14 (d, 2 H), 7.77-7.70 (m, 6 H), 3.91 (t, 2 H), 3.82 (t, 2 H), 3.58 (m, 8 H), 3.51 (s, 3 H), -2.88 (s, 2 H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 160.80, 150.00, 149.89, 148.61, 136.69, 136.60, 133.07, 131.34, 131.28, 129.16, 124.34, 122.60, 121.10, 109.54, 109.47, 72.57, 72.51, 72.10, 70.92, 70.89, 70.84, 70.80, 58.97, 58.93, 48.31, 41.31.

**P$_{Zn}$**: To a solution of **P$_{FB}$** (1.00 g, 1.12 mmol) in $CH_2Cl_2$ (30.0 mL), zinc acetate dehydrate (2.50 g, 11.2 mmol) and methanol (10.0 mL) was added and stirred for 2 h. The reaction mixture was washed with water and aqueous KOH (0.1 M). The combined organic layer was dried over $MgSO_4$ and freeze-dried to give **P$_{Zn}$** (1.0 g, 93%) as greenish purple powder. MALDI-TOF-MS: *m/z* calcd. for $C_{50}H_{50}N_{10}O_6Zn$: 951.32 [M + H]$^+$; found: 951.49.

**4**: To a mixture solution of methyl 4-formylbenzoate (1.12 g, 6.64 mmol), **2** (1.66 g, 6.64 mmol), and dipyrromethane (2.00 g, 13.7 mmol) in $CH_2Cl_2$ solution (800 mL), trifluoroacetic acid (1.00 mL) was added and stirred for 12 h. After then, triethylamine (1.00 mL) and chloranil (5.05 g, 20.52 mmol) was added and stirred for 6 h. Zinc acetate dihydrate (4.51 g, 20.5 mmol) and methanol (50.0 mL) was added and further stirred for 2 h. The reaction mixture was evaporated and purified with silica column chromatography as an eluent of $CH_2Cl_2$/MeOH (93:7, v/v). The product was recrystallized with $CH_2Cl_2$ and *n*-hexane to give **4** (450 mg, 10%) as purple crystalline solid. MALDI-TOF-MS: *m/z* calcd. for $C_{38}H_{34}N_6O_5Zn$: 719.19 [M + H]$^+$; found: 719.85. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.20 (s, 2 H), 9.48 (d, 2 H), 9.16 (d, 2 H), 8.86 (d, 1 H), 8.80 (d, 2 H), 8.66 (d, 1 H), 8.50 (d, 1 H), 8.28 (d, 1 H), 5.71 (s, 1 H), 5.55 (d, 2H), 4.20 (s, 3 H), 3.04, 2.93, 2.80, 2.49, 1.96, and 1.75 (m, 15 H), 1.86 (s, 1 H).

**5**: **4** (400 mg, 0.555 mmol) was dissolved in THF (20.0 mL) and aqueous NaOH (3.0 M, 20.0 mL) was added and refluxed for 12 h. After then, the reaction mixture was neutralized with conc. HCl and poured into $CH_2Cl_2$ (300 mL). The organic layer was washed with water (100 mL) for three times. The combined organic layer was evaporated to dryness and the residue was recrystallized with $CH_2Cl_2$ and *n*-hexane to give **5** (290 mg, 81%) as purple crystalline solid. MALDI-TOF-MS: *m/z* calcd. for $C_{37}H_{34}N_6O_5$: 643.26 $[M + H]^+$; found: 643.35. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.80 (s, 2 H), 9.83 (d, 2 H), 9.74 (d, 2 H), 9.10 (m, 4 H), 8.50-8.37 (m, 6 H), 4.34, 3.53, 3.10-3.03, 2.95-2.93 (m, 15 H), -3.30 (d, 2 H).

**mod-P$_{\mathbf{Zn}}$**: **5** (250 mg, 0.389 mmol), *N*,*N*'-dicyclohexylcarbodiimide (120 mg, 0.583 mmol), 4-dimethylaminopyridine (4.8 mg, 0.0359 mmol), and tri(ethylene glycol) monomethyl ether (640 mg, 3.89 mmol) was dissolved in $CH_2Cl_2$ (100 mL) and stirred for 12 h. Zinc acetate dihydrate (2.561 g, 853.9 mmol) and methanol (10 mL) was added and further stirred for 2 h. The reaction mixture was washed with water (100 mL) for three times. The combined organic layer was evaporated and purified with silica column chromatography as an eluent of $CH_2Cl_2$/MeOH (93:7, v/v). The first fraction was collected and evaporated to give **6$_{\mathbf{Zn}}$** (280 mg, 84%) as purple solid. MALDI-TOF-MS: *m/z* calcd. for $C_{44}H_{46}N_6O_8Zn$: 851.27 $[M + H]^+$; found: 852.16. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 10.20 (s, 2 H), 9.48 (d, 2 H), 9.16 (d, 2 H), 8.84 (d, 1 H), 8.80 (d, 2 H), 8.68 (d, 1 H), 8.50 (d, 1 H), 8.28 (d, 1 H), 5.71 (s, 1 H), 5.55 (d, 1 H), 4.76, 4.07, 3.91, 3.64, and 3.44 (m, 15 H), 3.04, 2.93, 2.80, 2.49, 1.96, and 1.75 (m, 15 H), 1.86 (s, 1 H).

## Additional UV/Vis and fluorescence spectra, and further characterization data

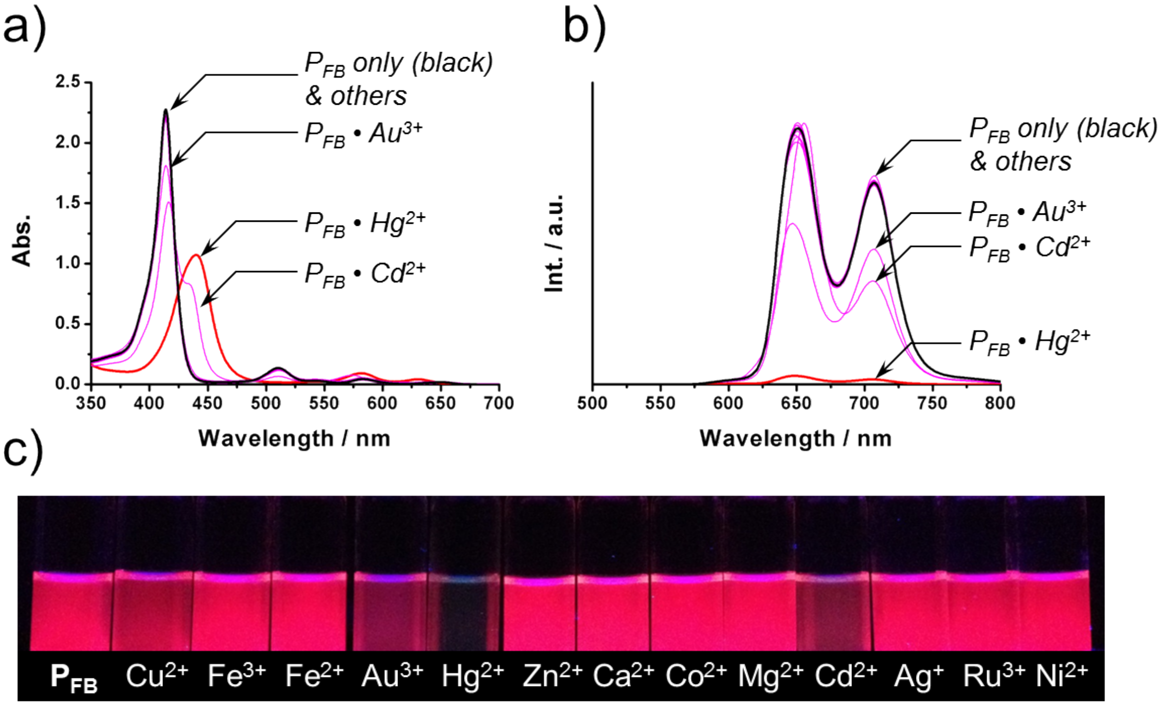


**Figure S1.** Optical responses of $\mathbf{P_{FB}}$ (10 μM) by the addition of various metal ions (2 eq.) in 50% MeCN/$H_2O$. (a) UV/Vis absorption spectra, (b) fluorescence emission spectra ($\lambda_{ex}$ = 413 nm), and (c) visible fluorescence emission under UV-A light illumination are shown.

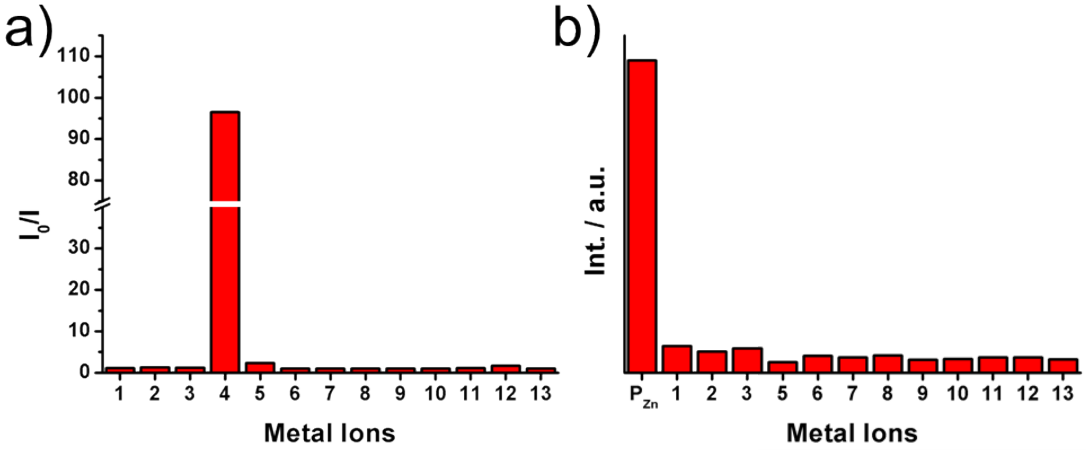


**Figure S2**. Metal selectivity and metal competition experiments of $\mathbf{P_{Zn}}$ (10 μM) in 50% MeCN/$H_2O$. (a) Relative fluorescence intensity ratio ($\lambda_{ex}$ = 425 nm) in the presence of various metal ions (2 eq.), and (b) intensity of system [$\mathbf{P_{Zn}}$·$M^+$] + $Au^{3+}$. Metal ions 1: $Cu^{2+}$, 2: $Fe^{3+}$, 3: $Fe^{2+}$, 4: $Au^{3+}$, 5: $Hg^{2+}$, 6: $Zn^{2+}$, 7: $Ca^{2+}$, 8: $Co^{2+}$, 9: $Mg^{2+}$, 10: $Cd^{2+}$, 11: $Ag^+$, 12: $Ru^{3+}$, 13: $Ni^{2+}$.

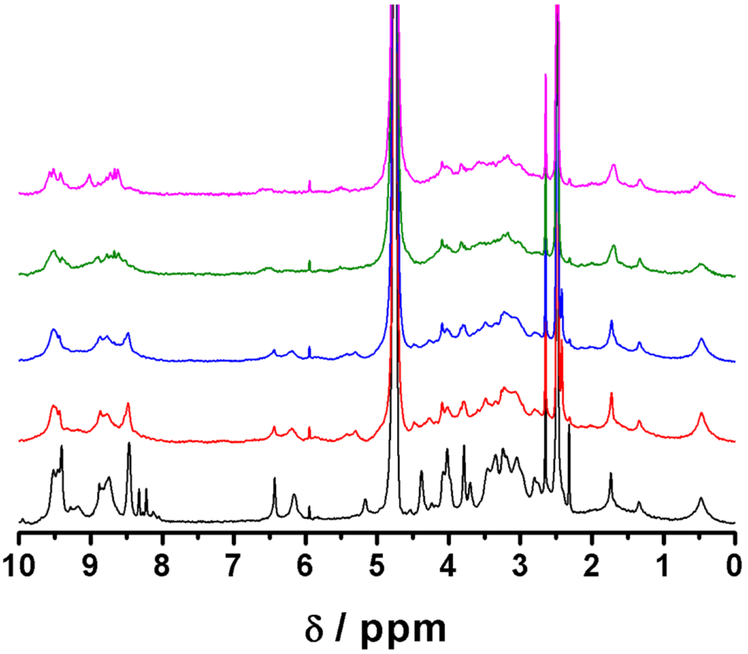


**Figure S3.** $^1$H-NMR titration spectra of $\mathbf{P_{Zn}}$ (5 mM) in addition with $HAuCl_4$ in $CD_3CN$.

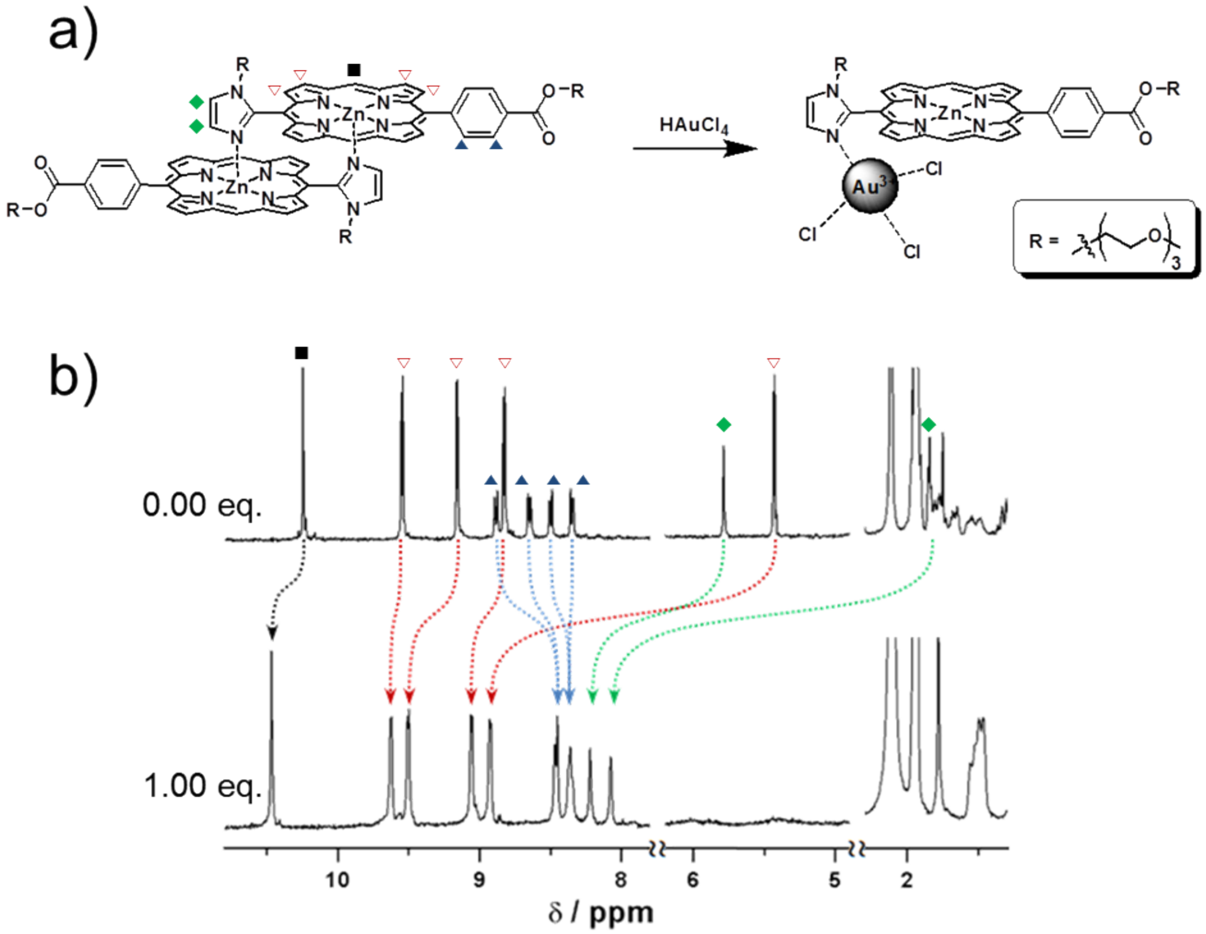


**Figure S4.** (a) Plausible binding mechanism of **mod-P$_{Zn}$** upon addition of Au$^{3+}$ and (b) $^1$H-NMR spectra of **mod-P$_{Zn}$** (5 mM) in $CD_3CN$ with/without Au$^{3+}$ are shown.

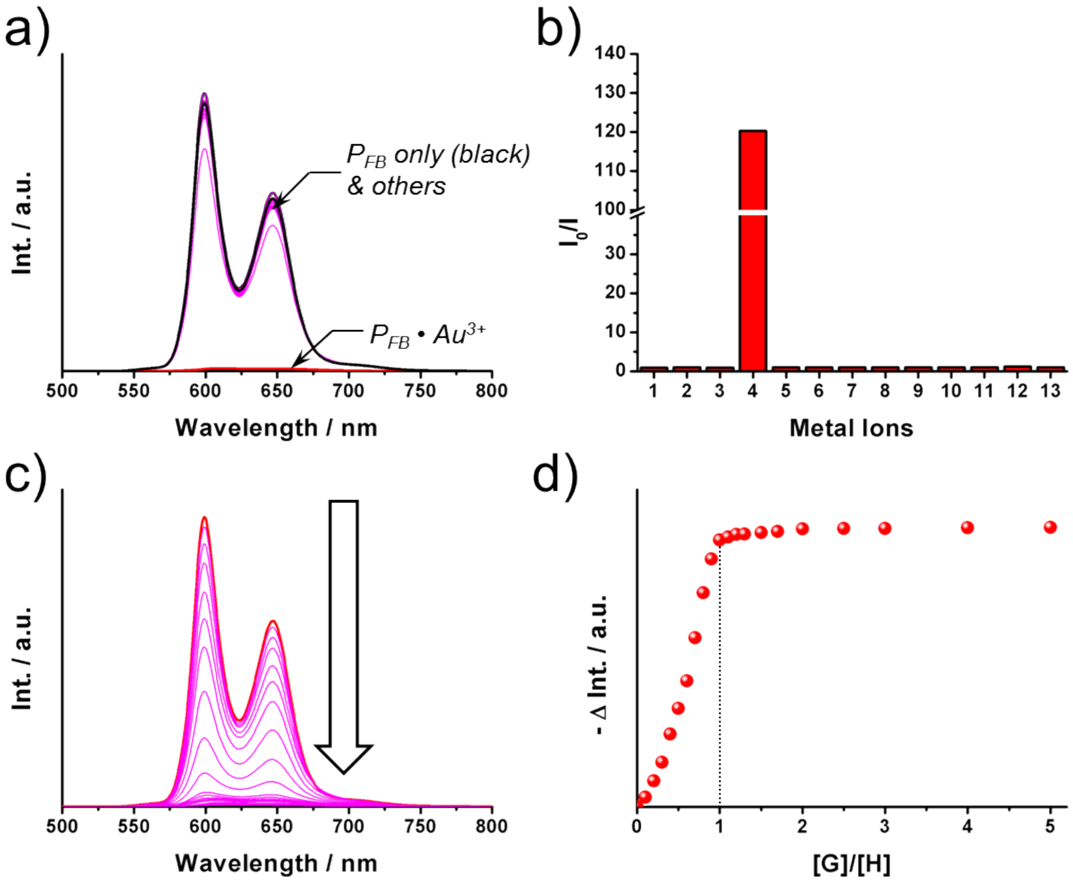


**Figure S5.** Optical responses of **mod-P$_{\mathbf{Zn}}$** (10 μM) in the presence of various metal ions (two eq.) and titration with $Au^{3+}$ ion in 50% MeCN/$H_2O$. (a) Fluorescence emission spectra ($\lambda_{ex}$ = 420 nm), (b) relative fluorescence intensity, (c) change in fluorescence emission upon $Au^{3+}$ addition, and (d) spectroscopic titration curve ($\lambda_{em}$ = 600 nm) are shown. Metal ions; 1: $Cu^{2+}$, 2: $Fe^{3+}$, 3: $Fe^{2+}$, 4: $Au^{3+}$, 5: $Hg^{2+}$, 6: $Zn^{2+}$, 7: $Ca^{2+}$, 8: $Co^{2+}$, 9: $Mg^{2+}$, 10: $Cd^{2+}$, 11: $Ag^{+}$, 12: $Ru^{3+}$, 13: $Ni^{2+}$.

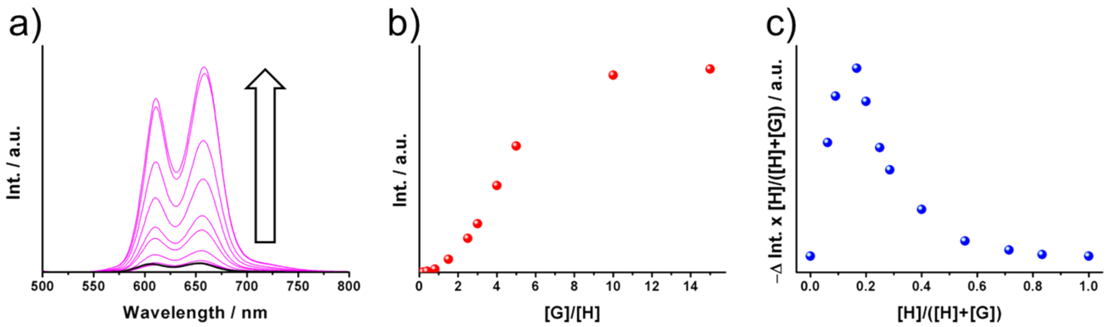


**Figure S6.** Optical response of $\mathbf{P_{Zn}}\cdot Au^{3+}$ (10 μM) upon addition of cysteine in 50% MeCN/$H_2O$. (a) Fluorescence emission spectra ($\lambda_{ex}$=425 nm), (b) spectroscopic titration curve, and (c) Job's plot ($\lambda_{em}$ = 660 nm).

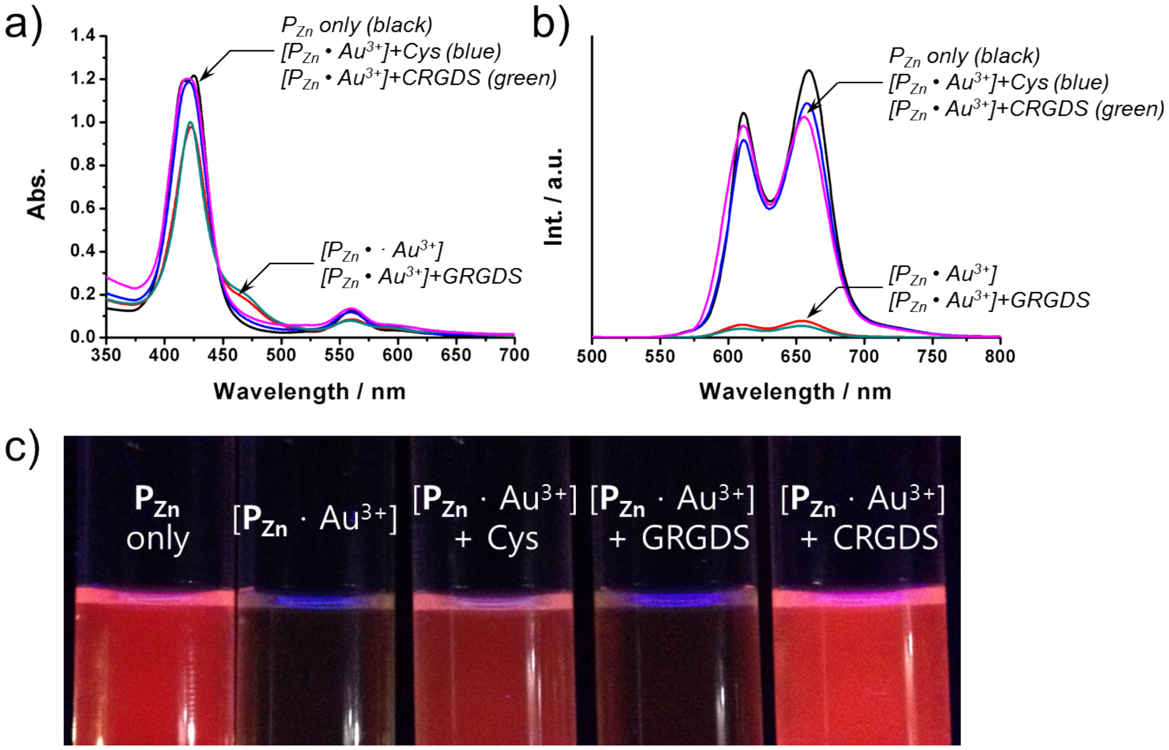


**Figure S7**. Optical response of $\mathbf{P_{Zn}}\cdot Au^{3+}$ (10 μM) upon addition of cysteine, GRGDS, CRGDS (4 eq.) in 50% MeCN/$H_2O$. (a) UV-Vis absorption spectra, (b) fluorescence emission spectra ($\lambda_{ex}$ = 425 nm), and (c) visible fluorescence emission ($\lambda_{ex}$ = 365 nm) are shown.

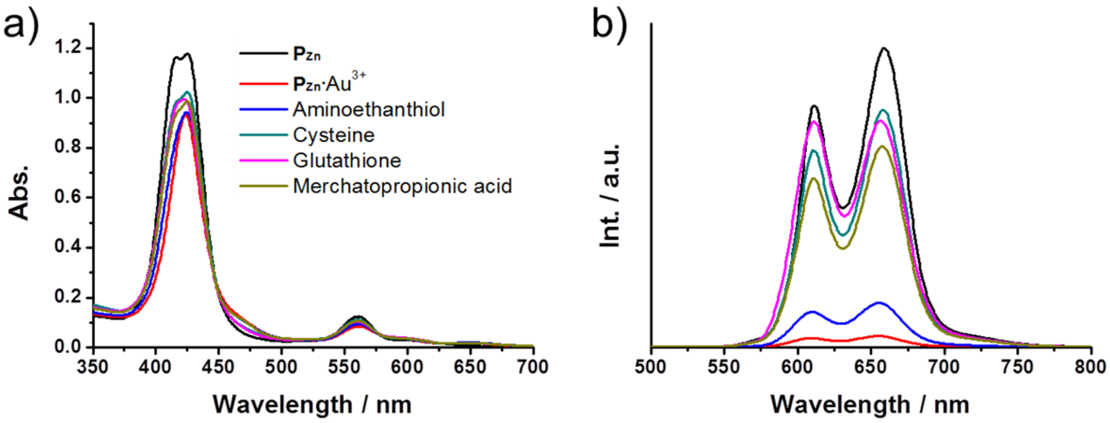


**Figure S8.** Optical response of $\mathbf{P_{Zn}}\cdot Au^{3+}$ (10 μM) upon addition of various thiol-containing species (4 eq.) in 50% MeCN/$H_2O$. (a) UV-Vis absorption spectra, (b) fluorescence emission spectra ($\lambda_{ex}$ = 425 nm).

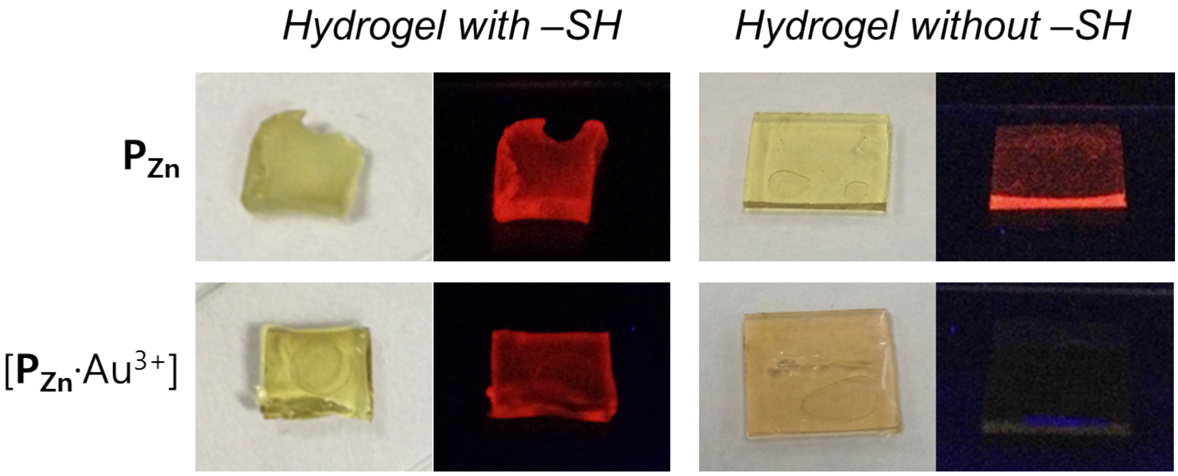


**Figure S9**. Treatment of $\mathbf{P_{Zn}}$ and [$\mathbf{P_{Zn}}\cdot Au^{3+}$] to thiol-containing and thiol-free hydrogels. Each dried hydrogel was swollen in 20 μM of $\mathbf{P_{Zn}}$ or [$\mathbf{P_{Zn}}\cdot Au^{3+}$] containing MeCN/$H_2O$ (50 v/v).