Electronic Supplementary Material (ESI) for Analyst

Supporting Information for

Highly Selective Fluorescence Turn-on Sensing of Gold Ions by Nanoparticle Generation/C-I Bond Cleavage Sequence

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Table of Contents:

Experimentals

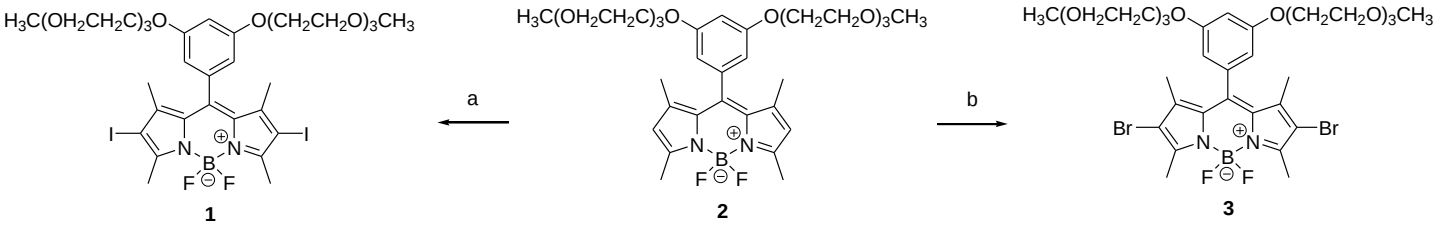
Materials

All reagents were of the highest commercial quality and used as received without further purification. All solvents were spectral grade unless otherwise noted. Anhydrous CH_2Cl_2 and DMF and THF were obtained as a sure-seal bottle from Aldrich Co. Inc. (Milwaukee, WI). Silica gel (40 μm) was obtained from Merck Inc. Aqueous solutions were freshly prepared with deionized water from a water purification system (Human Corp. Korea). $AuCl_3$ and Rose Bengal were purchased from Aldrich Co. Inc. (Milwaukee, WI). All metal ions were used as chloride salts except for $AgNO_3$.

General methods, instrumentation and measurements

Synthetic manipulations that required an inert atmosphere (where noted) were carried out under argon using standard Schlenk techniques. NMR (^{1}H and ^{13}C) spectra were recorded on Bruker Advance 500 MHz spectrometers. The chemical shift data for each signal are given in units of δ (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0, and referenced to the residual solvent resonances. Splitting patterns are denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). High-resolution electrospray ionization (ESI) mass spectra were obtained at national center for inter-university research facilities. 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. Fluorometric assays with various metal analytes were measured by monitoring changes in fluorescence intensity using a Synergy Mx Microplate Reader (BioTek, USA). 3,5-Bis(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)benzaldehyde was synthesized according to the literature procedure.[1]

1. Synthesis



Scheme S1. Synthetic scheme of compounds **1-3**, Reagents and conditions a) I_2, iodic acid, EtOH/H_2O, 25 ^{o}C to 60 ^{o}C, 85%; (b) NBS, CH_2Cl_2, 12 hours, r.t., 82%.

8-[3,5-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (2). To a stirred mixture of 2,4-dimethylpyrrole (100 µL, 1.02 mmol) and 3,5-bis(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)benzaldehyde[1](200 mg, 0.465 mmol) in dry THF (50 mL) was added 2 drops of TFA. After the mixture was stirred at room temperature for 6 hours under argon atmosphere, a solution of 160 mg (0.7 mmol) DDQ (tetrachloro-1,4-benzoquinone) in CH_2Cl_2 (30 mL) was added to the reaction solution. The solution was stirred at room temperature for 6 hours. To the mixture were added triethylamine (870 µL, 7 mmol) and $BF_3{\cdot}OEt_2$ (1 mL, 7 mmol) and the reaction mixture was stirred for overnight at room temperature. The mixture solution was evaporated and crude product was dissolved with CH_2Cl_2 and washed with water (3 x 100 mL). The separated organic layers were dried with $MgSO_4$, filtered, and evaporated under vacuum to yield a black crude compound. The crude product was purified by column chromatography on silica gel using progressively more polar 5:1 to

1:1 hexanes : ethyl acetate as the mobile phase to afford **2** as a red solid (120 mg, 40%).; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.58 (s, 1H), 6.45 (d, 2H, J = 2.5 Hz), 5.98 (s, 2H), 4.09 (m, 4H), 3.84 (m, 4H), 3.73 (m, 4H), 3.66 (m, 8H), 3.54 (m, 4H), 3.37 (s, 6H), 2.54 (s, 6H), 1.53 (s, 6H).; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 160.7, 155.4, 143.1, 141.2, 136.4, 131.0, 121.1, 106.6, 102.4, 71.8, 70.8, 70.6, 70.5, 69.6, 67.7, 59.0, 14.5, 14.3.; HR-MS (ESI): calcd. for $C_{33}H_{47}BF_2N_2O_8$ $[M + Na]^+$ 671.3286, found 671.3282.

8-[3,5-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl]-2,6-bis(iodo)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (1). 8-[3,5-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene **2** (250 mg, 0.385 mmol) and iodine (240 mg, 0.96 mmol) were dissolved in 20 mL of EtOH. To the reaction solution was slowly added a solution of iodic acid (135 mg, 0.77 mmol) in water (0.5 mL) at room temperature and stirred for 20 min. The reaction solution was gradually heated to 60 ^{o}C. After stirring for 1 hour at the temperature, the reaction mixture was evaporated under reduced pressure, diluted with methylene chloride, and washed with $Na_2S_2O_4$ (3 x 100 mL). The combined organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using progressively more polar 10:1 to 5:1 hexanes : ethyl acetate as the mobile phase to afford **1** as a red solid (280 mg, 85%).; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.63 (s, 1H), 6.42 (d, 2H, J = 2.0 Hz), 4.1 (m, 4H), 3.85 (m, 4H), 3.73 (m, 4H), 3.67 (m, 8H), 3.55 (m, 4H), 3.38 (s, 6H), 2.64 (s, 6H), 1.55 (s, 6H).; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 161.0, 156.7, 145.3, 140.9, 136.1, 130.9, 106.4, 102.7, 85.5, 71.9, 70.8, 70.6, 70.5, 69.6, 67.8, 59.0, 16.9, 16.0.; HR-MS (ESI): calcd. for $C_{33}H_{45}BF_2I_2N_2O_8$ $[M + Na]^+$ 923.1219, found 923.1210.

8-[3,5-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl]-2,6-bis(bromo)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (3). To 8-[3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene **2** (100 mg, 0.15 mmol) dissolved in 15 mL of dry CH_2Cl_2 was added a solution of 60 mg (0.33 mmol) N-bromosuccinimide (NBS) in dry CH_2Cl_2 (5 mL) at room temperature. After stirring for 12 hours, the reaction mixture was evaporated under reduced pressure, the crude product was purified by column chromatography on silica gel using progressively more polar 5:1 to 2:1 hexanes : ethyl acetate as the mobile phase to afford Br-BODIPY **3** as a red solid (100 mg, 82%).; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.63 (s, 1H), 6.42 (d, 2H, J = 2.0 Hz), 4.1 (m, 4H), 3.85 (m, 4H), 3.72 (m, 4H), 3.67 (m, 8H), 3.55 (m, 4H), 3.38 (s, 6H), 2.60 (s, 6H), 1.53 (s, 6H).; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 161.0, 153.9, 140.6, 135.8, 130.0, 111.6, 106.5, 102.7, 71.9, 70.8, 70.6, 70.5, 69.6, 67.8, 59.0, 14.1, 13.7.; HR-MS (ESI): calcd. for $C_{33}H_{47}BF_2N_2O_8$ $[M + Na]^+$ 827.1496, found 827.1899.

2. Studies on formation of AuNPs

General procedure: For general formation of AuNPs from $AuCl_3$ and HEPES buffer, $AuCl_3$ solutions at different concentrations (0 ~ 5 mM) were prepared by dissolving in deionized water, and each $AuCl_3$ solution (20 µL) was mixed with HEPES buffer (50 mM, 180 µL, pH 7.0) to be a final concentration of 0 ~ 500 µM $AuCl_3$ in HEPES buffer.

(a) Time-dependent absorption spectra of Au(III) in various aqueous media

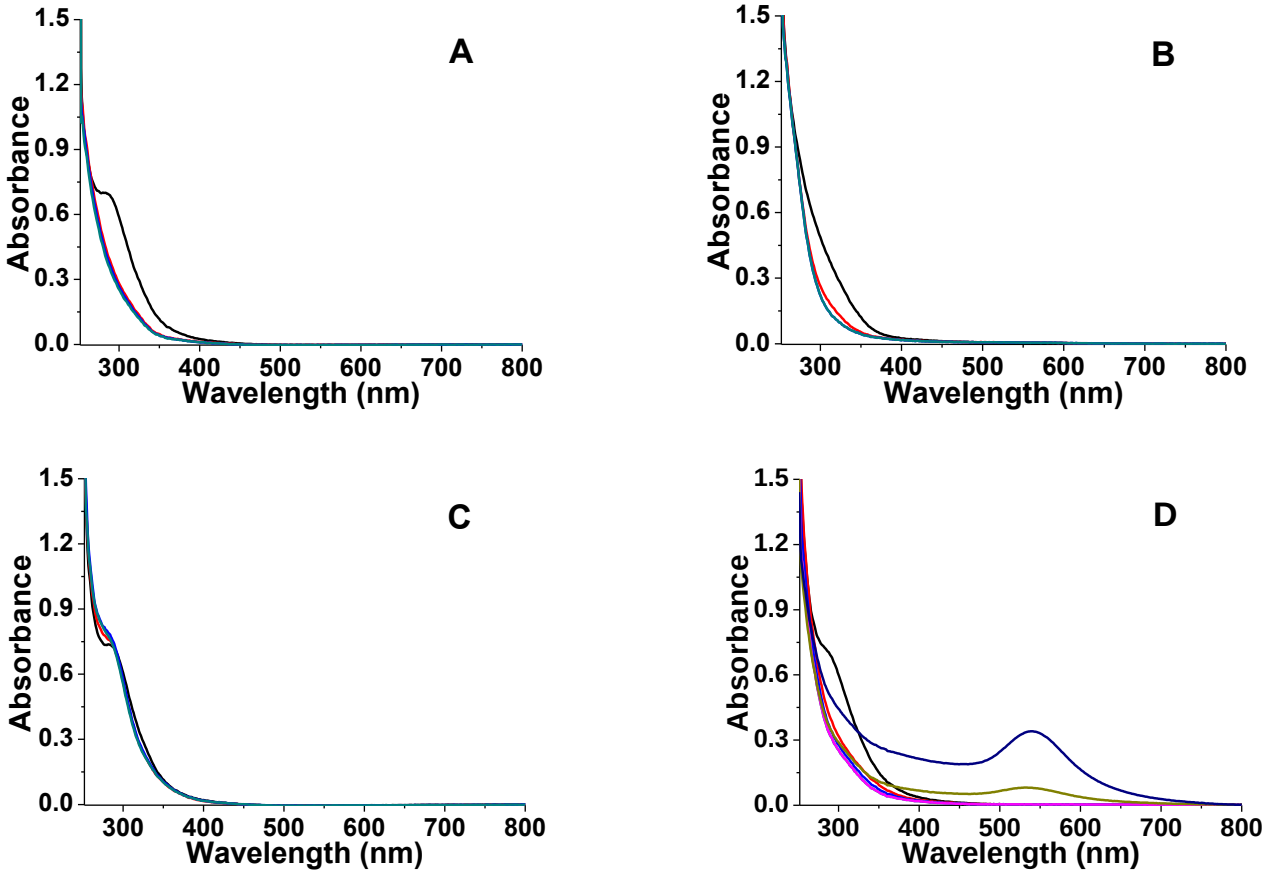


Figure S1. Time-dependent UV-Vis absorption spectra of $AuCl_3$ (300 µM) in various aqueous media: (A) PBS (50 mM, pH 7.0), (B) Tris buffer (50 mM, pH 7.0), (C) water, and (D) HEPES buffer (50 mM, pH 7.0) at 25 ^{o}C. The spectra were obtained every 10 min (0 – 30 min) for A, B, C and every 5 min (0 – 30 min) for D.

(b) Time-dependent formation of AuNPs by varying Au(III) concentrations in HEPES buffer (50 mM and 100 mM)

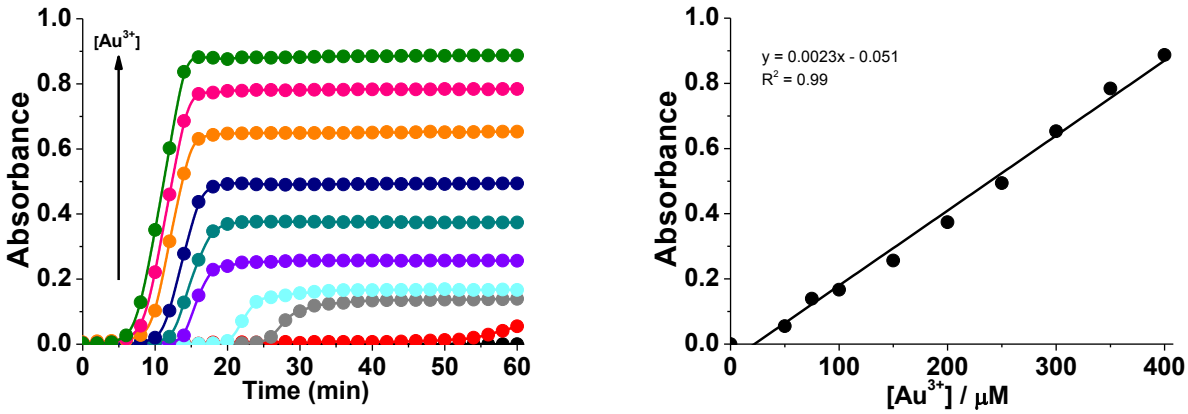


Figure S2. (Left) Time-dependent absorbance (λ = 550 nm) of AuNP solution formed in the presence of different concentrations of $AuCl_3$ ([bottom] 0, 50, 75, 100, 150, 200, 250, 300, 350, 400 µM [top]) in HEPES buffer (50 mM, pH 7.0, 25 °C). (Right) Absorbance at λ = 550 nm as a function of Au(III) concentration. Incubation time = 60 min. Data points are averages of three independent measurements.

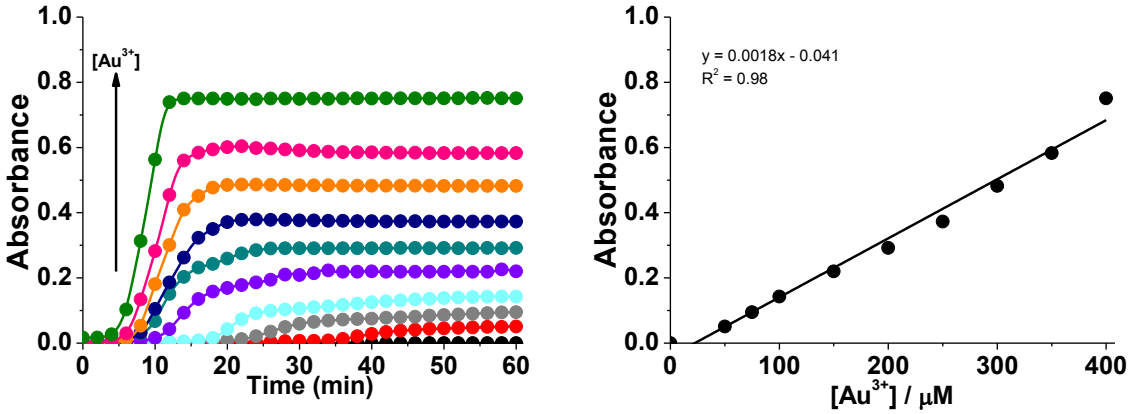


Figure S3. (Left) Time-dependent absorbance (λ = 550 nm) of AuNP solution formed in the presence of different concentrations of $AuCl_3$ ([bottom] 0, 50, 75, 100, 150, 200, 250, 300, 350, 400 µM [top]) in HEPES buffer (100 mM, pH 7.0, 25°C). (Right) Absorbance at λ = 550 nm as a function of Au(III) concentration. Incubation time = 60 min. Data points are averages of three independent measurements.

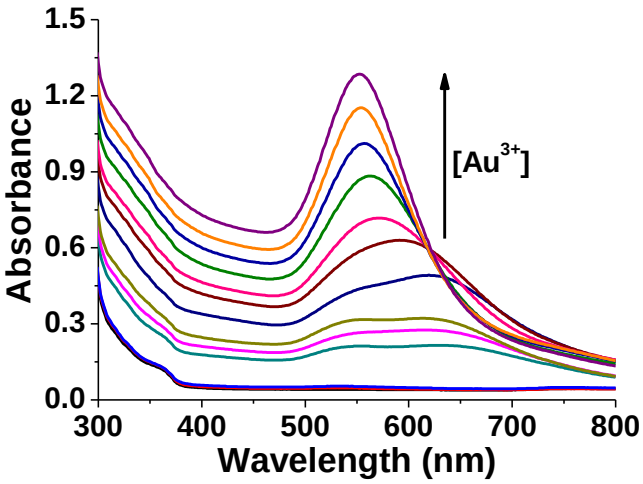


Figure S4. UV-Vis absorption spectra of HEPES buffer (50 mM, pH 7.0, 25 °C) treated with different concentrations of Au(III) (0–500 μM). The spectra were obtained 30 min after the reaction between HEPES and Au(III).

(c) Absorption spectra of Au(III) in HEPES buffer (50 mM) at different pHs

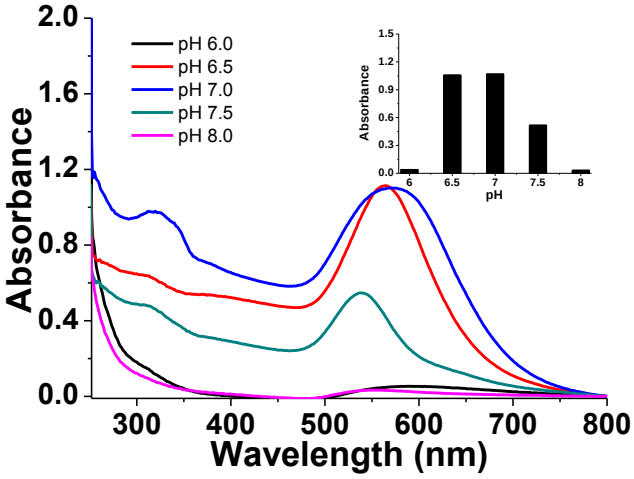


Figure S5. UV-Vis absorption spectra of Au(III) (300 μM) in HEPES buffer (50 mM, 25 °C) at different pHs. The spectra were obtained 35 min after the reaction between HEPES and Au(III).

(d) Investigation of formation of nanoparticles using various metal ions in HEPES buffer

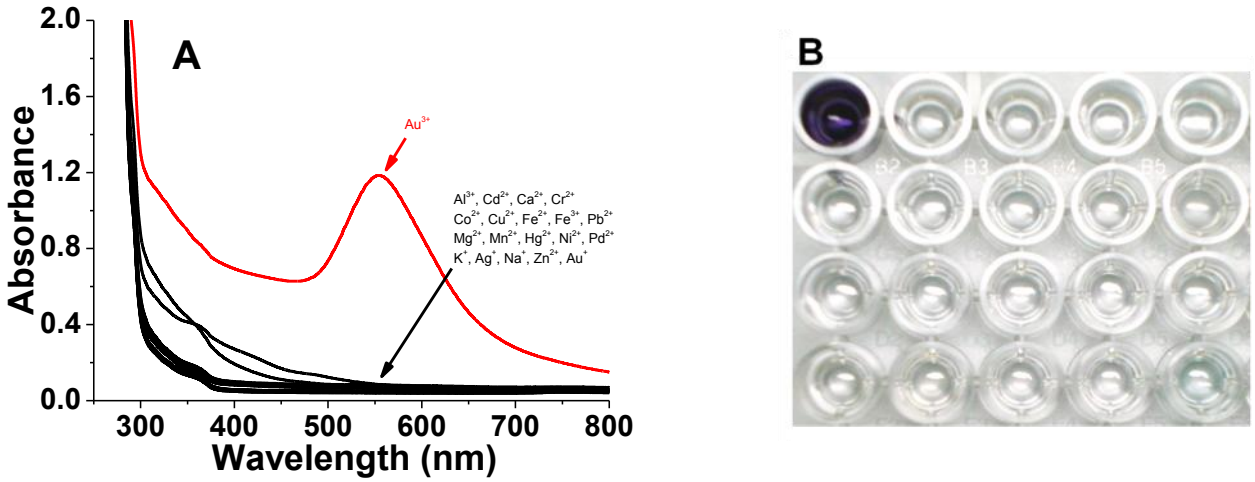


Figure S6. (A) UV-Vis absorption spectra of various metals (300 μM for all metals) in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C). The spectra were obtained at 40 min after addition of each metal ion in HEPES buffer. (B) Photographs of various metals (300 μM) in HEPES buffer (50 mM, pH 7.0) under ambient light. First row (left to right):Au^{3+}, Al^{3+}, Cd^{2+}, Ca^{2+}, Cr^{2+}; Second row (left to right): Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}; Third row (left to right): Mg^{2+}, Mn^{2+}, Hg^{2+}, Ni^{2+}, Pd^{2+}; Fourth row (left to right): K^{+}, Ag^{+}, Na^{+}, Zn^{2+}, Au^{+}.

(e) Transmission electron microscopy (TEM) analysis of AuNPs generated from a solution of $AuCl_3$ in HEPES buffer.

In order to confirm the formation of AuNPs by mixing Au(III) in HEPES buffer, transmission electron microscopy (TEM) analysis was performed. TEM images were obtained from products, which were recovered 30 min after mixing different concentration of $AuCl_3$ (150 and 300 μM, respectively) with HEPES buffer (50 mM, pH 7.0) and $AuCl_3$ (300 μM) with HEPES buffer (100 mM, pH 7.0).

TEM images of AuNPs obtained from 100 mM HEPES buffer showed inhomogenous branched particles with different number of tips, Even though fluorescence enhancement was greater with AuNPs obtained from higher concentrations of HEPES, we performed all experiments with 50 mM HEPES buffer to exclude size effect of AuNPs on fluorescence enhancement.

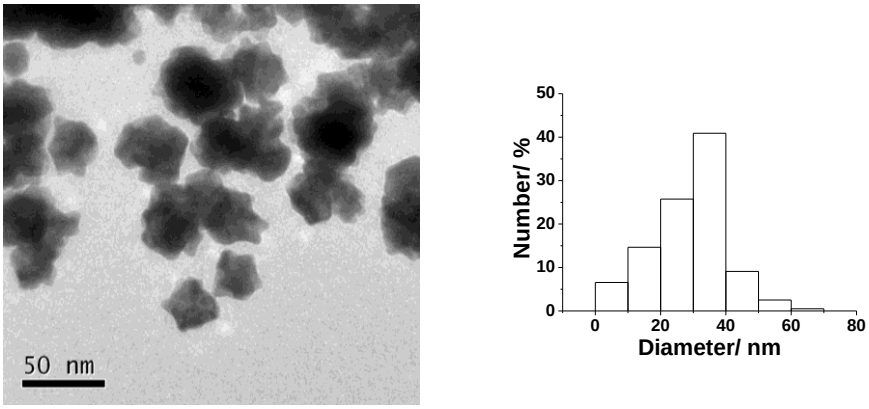


Figure S7. TEM image (left) and size distribution (right) of AuNPs *in situ* generated from a solution of $AuCl_3$ (150 μM) in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C).

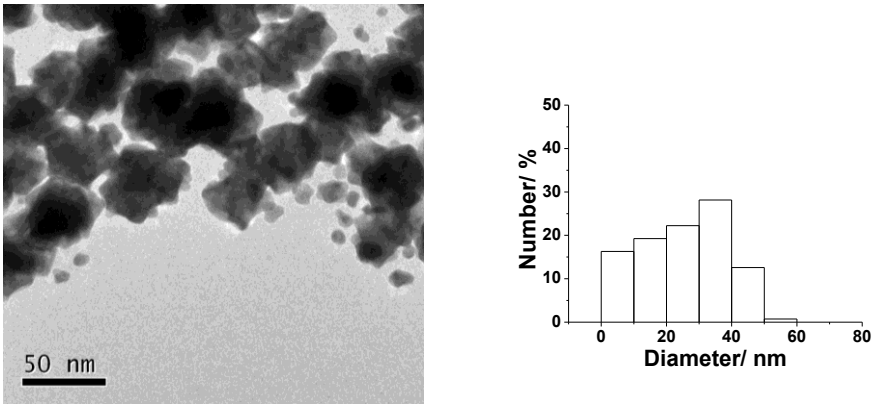


Figure S8. TEM image (left) and size distribution (right) of AuNPs *in situ* generated from a solution of $AuCl_3$ (300 μM) in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C).

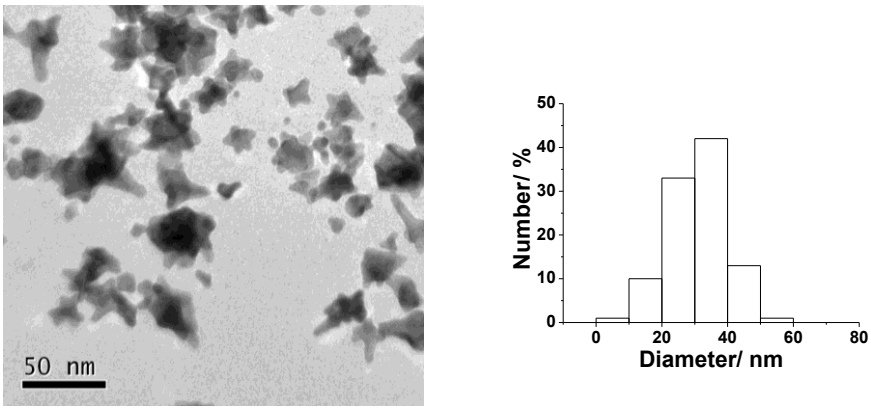


Figure S9. TEM image (left) and size distribution (right) of AuNPs *in situ* generated from a solution of $AuCl_3$ (300 μM) in HEPES buffer (100 mM, pH 7.0, 25 ^{o}C).

3. Photophysical properties of I-BODIPY 1 and H-BODIPY 2

Table S1. Photophysical properties of I-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]
1	538	58 000	558	0.005
2	498	72 000	512	0.56

[a]Data were obtained in 10 mM HEPES buffer (pH 7.0) containing 5% EtOH as a cosolvent. [b]Excited at 465 nm [c]Quantum yields vs. rhodamine 6G in EtOH (Φ_F = 0.95) for **1** and fluorescein in 0.1 N NaOH (Φ_F = 0.95) for **2**.[2]

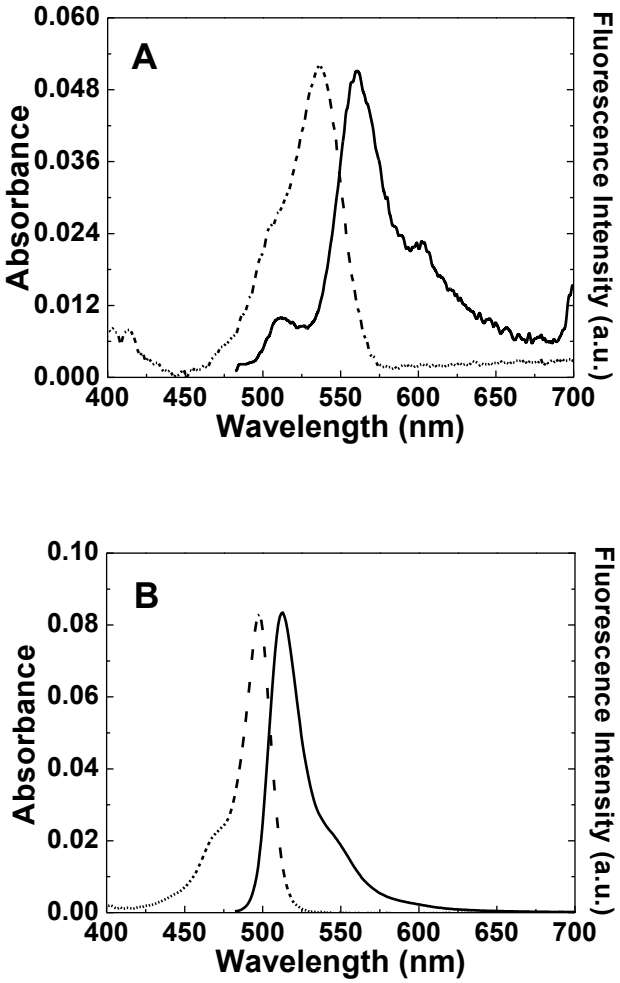


Figure S10. Absorption (dashed lines) and emission spectra (solid lines) of probe **1** (A) and **2** (B) in HEPES buffer (50 mM, pH = 7.0, 25 ^{o}C) containing 5% EtOH as a cosolvent. Excited at 465 nm. [**1**] = [**2**] = 1 μM.

4. Fluorescence *turn-on* response of I-BODIPY 1

Preparation of stock solution and general procedure: A stock solution of I-BODPIY **1** (0.1 mM in EtOH, 100 µL) was diluted with a mixture solution of ethanol and water (4: 5, v/v, 900 µL) to give a final concentration of 0.01 mM I-BODPIY **1**. $AuCl_3$ dissolved in deionized water was mixed with HEPES buffer (50 mM, pH 7.0, 180 µL) to be a final concentration of 300 µM $AuCl_3$ in HEPES buffer. The solution was stirred for 30 min to make sure the complete formation of AuNPs. To the solution was added I-BODIPY **1** (0.01 mM in EtOH/water (1:1, v/v), 20 µL) to have final concentration of 1 µM. The reactions were monitored at 25 ^{o}C for 60 minutes. The fluorescence signal for each well was measured at 510 nm (λ_{ex} = 465 nm).

The fluorescence enhancement was affected by the reaction conditions such as the concentration of HEPES buffer and pH value. For example, when pH was adjusted to 6, less signal to noise ratio (S/N) was observed and higher concentrations of HEPES buffer resulted in higher S/N.

*(a) Fluorescence turn-on response of I-BODIPY **1** by citrate-capped AuNPs*

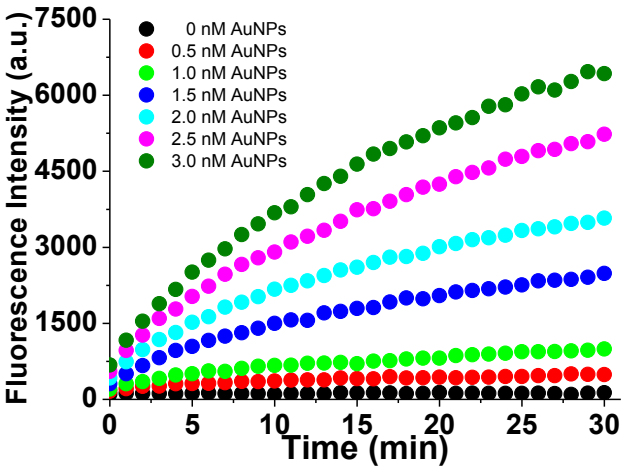


Figure S11. Time-dependent fluorescence turn-on response of **1** (1 µM) upon incubation with various concentrations of citrate-capped AuNPs solution (water, 25 ^{o}C) containing 5% EtOH as a cosolvent. Excited at 465 nm. Fluorescence intensity was measured at 510 nm. The spectra were obtained every 1 min (0 – 30 min).

*(b) Fluorescence turn-on response of I-BODIPY **1** in the presence of Au(III) in various aqueous media*

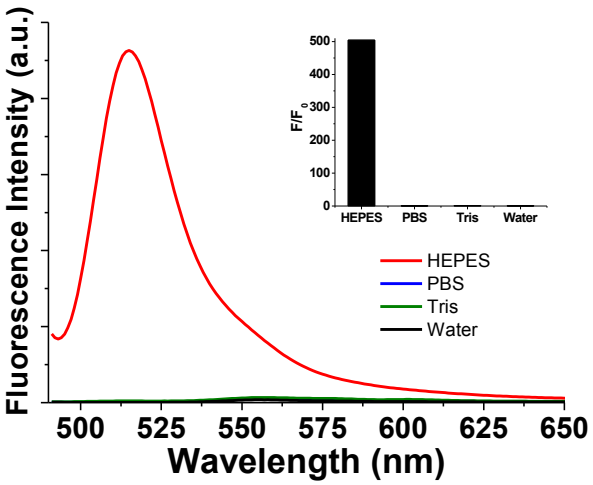


Figure S12. Fluorescence emission spectra of probe **1** (1 μM) in the presence of $AuCl_3$ (300 μM) at various aqueous media: HEPES (red), PBS (blue), and Tris (green) buffers (50 mM, pH 7.0, 25 °C) and water (black) containing 5% EtOH. Excited at 465 nm. The spectra were obtained 30 min after the addition of probe **1** to AuNPs obtained from Au(III) treated with HEPES buffer solution (50 mM, pH 7.0). Inset shows the relative emission intensity (F/F_0) at 510 nm depending on buffer systems.

*(c) Fluorescence turn-on response of I-BODIPY **1** in the presence of Au(III) in HEPEs buffer solutions at various concentrations*

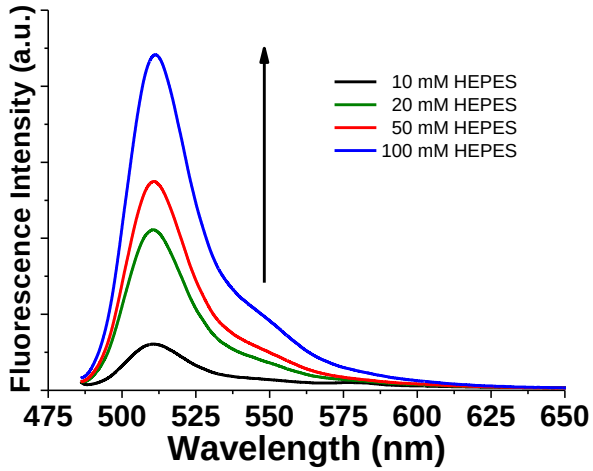


Figure S13. Fluorescence emission spectra of probe **1** (1 μM) in the presence of $AuCl_3$ (300 μM) at various concentrations of HPEPS buffer (10 – 100 mM, 25 ^{o}C, pH 7.0) containing 5% ethanol. Excited at 465 nm. The spectra were obtained 30 min after the addition of probe **1** to AuNPs obtained from Au(III) treated with HEPES buffer solution.

*(d) pH effect of assay solution on fluorescence turn-on response of I-BODIPY **1***

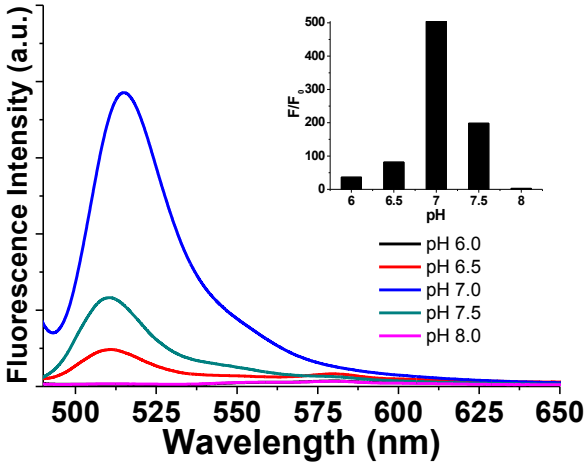


Figure S14. Fluorescence emission spectra of probe **1** (1 μM) in the presence of $AuCl_3$ (300 μM) in HEPES buffer (50 mM, 25 ^{o}C, 5% ethanol) at various pH conditions. Excited at 465 nm. The spectra were obtained 30 min after the addition of probe **1** to AuNPs obtained from Au(III) treated with HEPES buffer solution. Inset shows the relative emission intensity (F/F_0) at 510 nm depending on the pH of assay solution.

*(e) Time-dependent fluorescence turn-on response of I-BODIPY **1** in the presence of different concentrations of Au(III) in HEPEs buffer*

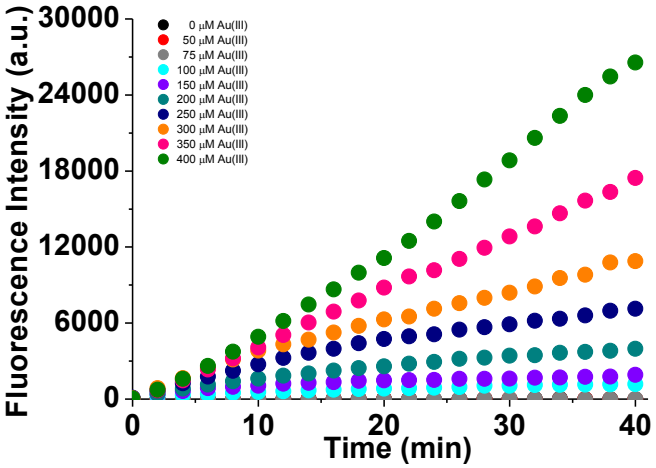


Figure S15. Time-dependent fluorescence intensity at 510 nm of **1** (1 μM) upon incubation with various concentrations of $AuCl_3$ (0 – 400 μM) in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C) containing 5% EtOH as a cosolvent. Excited at 465 nm. The values were obtained every 2 min (0 – 40 min).

(f) Kinetic studies of the conversion of I-BODIPY to H-BODIPY

Standard fluorescence curve: In order to determine the correlation of fluorescence intensity and concentration of 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 probe **1** by AuNPs.

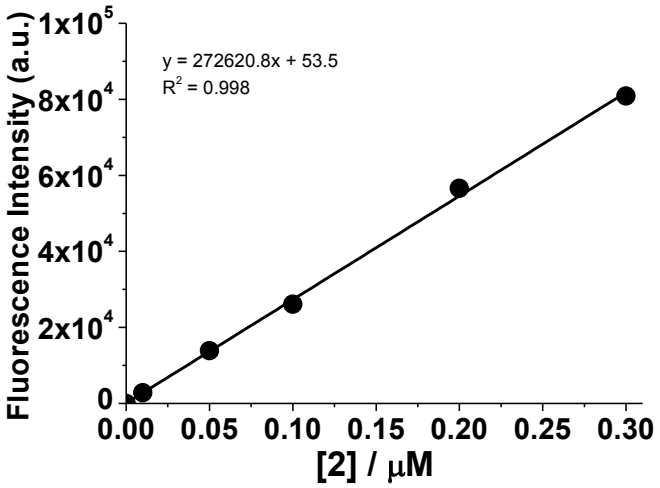


Figure S16. Standard fluorescence curve of compound **2** in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C) containing 5% EtOH as a cosolvent. Fluorescence intensity at 510 nm was measured.

Determination of kinetic constant: To determine kinetic constant, probe **1** (1 μM) was added to the solution of $AuCl_3$ (500 μM) in 50 mM HEPES (pH 7.0) containing 5% ethanol as a cosolvent.

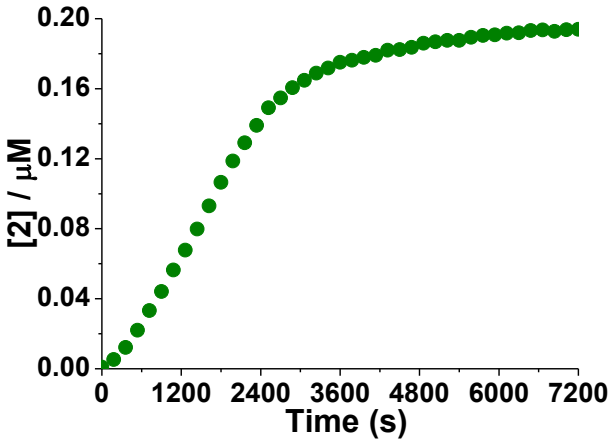


Figure S17. Kinetics for the fluorescence response of probe **1** with $AuCl_3$ (500 μM) in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C)

5. Identification of fluorescent reaction product

(a) LC-MS analysis

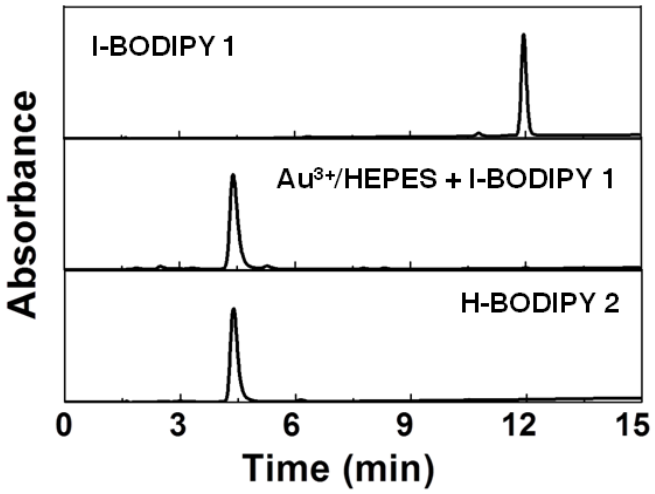


Figure S18. HPLC chromatograms of I-BODIPY **1** before (top); after addition of AuNPs generated *in situ* from a solution of $AuCl_3$ (300 μM) in HEPES buffer (50 mM, pH 7.0, 5% ethanol) at 25 ^{o}C (middle); H-BODIPY **2** only (bottom). The samples were analyzed by LC-MS with a linear gradient elution (from 0 to 80% B, A: 5 mM ammonium formate buffer, B: methanol, flow rate 0.3 $mLmin^{-1}$, UV: 460 nm and 520 nm). The MW of the retention time at 12.0 min is 918.2, which corresponds to $[M+NH_4]^+$ for I-BODIPY **1** and MW of the retention time at 4.5 min is 666.4, which corresponds to $[M+NH_4]^+$ for compound H-BODIPY **2**.

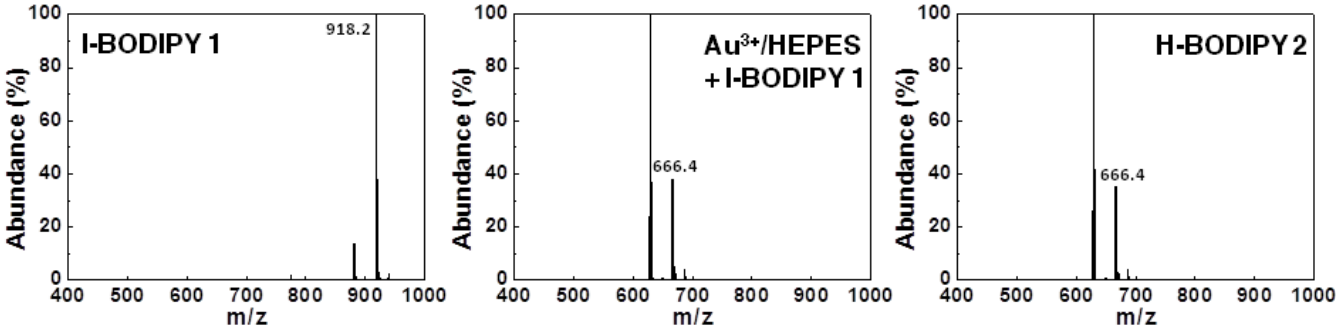


Figure S19. ESI-MS spectra of I-BODIPY **1** (a) before and (b) after addition of AuNPs generated *in situ* from a solution of $AuCl_3$ (300 μM) in HEPES buffer (50 mM, pH 7.0, 5% ethanol)at 25 ^{o}C, (c) H-BODIPY **2**. The MW of the retention time at 12.0 min is 918.2, which corresponds to $[M+NH_4]^+$ for I-BODIPY **1** and MW of the retention time at 4.5 min is 666.4, which corresponds to $[M+NH_4]^+$ for compound H-BODIPY **2**.

(b) ^{1}H-NMR analysis

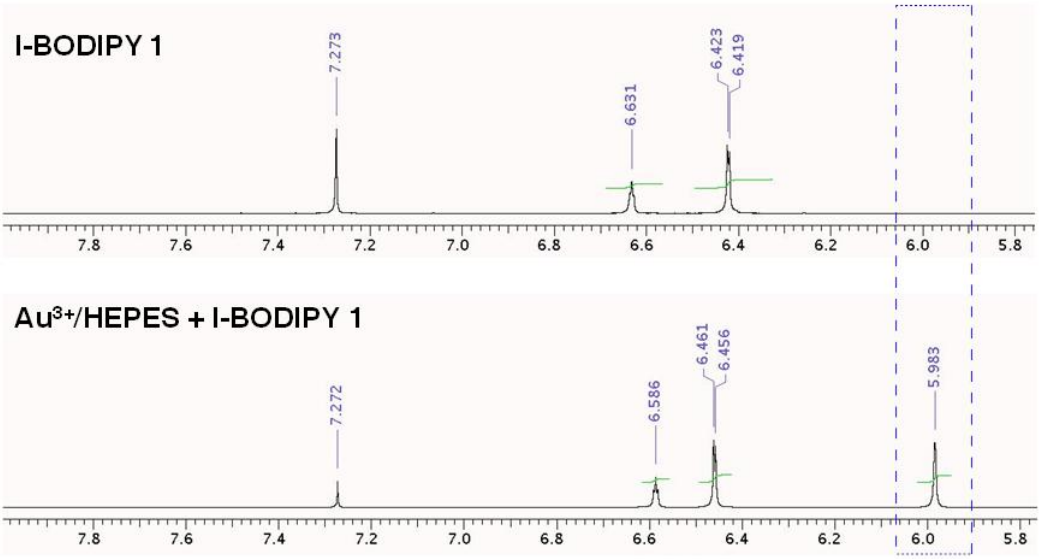


Figure S20. Partial ^{1}H-NMR ($CDCl_3$) spectra of (top) I-BODIPY **1** only and (bottom) the isolated reaction product, obtained upon addition of I-BODIPY **1** to the solution of $AuCl_3$ in HEPES buffer (50 mM, pH 7.0, 5% ethanol).

(c)TLC analysis

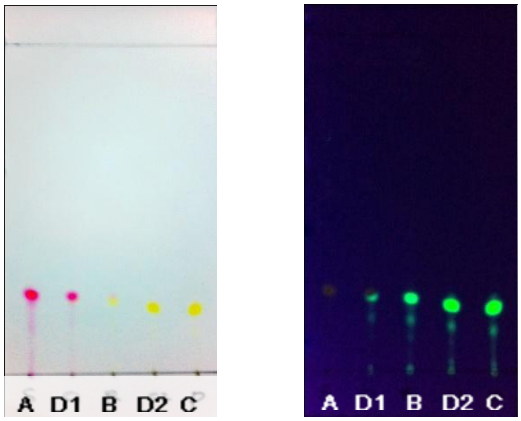


Figure S21. UV-Vis (left) and fluorescence (right) thin layer chromatograms of probe **1** upon addition of $AuCl_3$ (300 μM) in HEPES buffer (50 mM, pH 7.0). (A) **1**, (B) reaction mixture **R,** (C) **2**, (D1) **1** + **R**, and (D2) **2** + **R**; R_f: 0.25 for **1**, 0.2 for **2** (MC/MeOH, 40:1, v/v).

6. Selectivity Studies of fluorometric assays with I-BODIPY 1

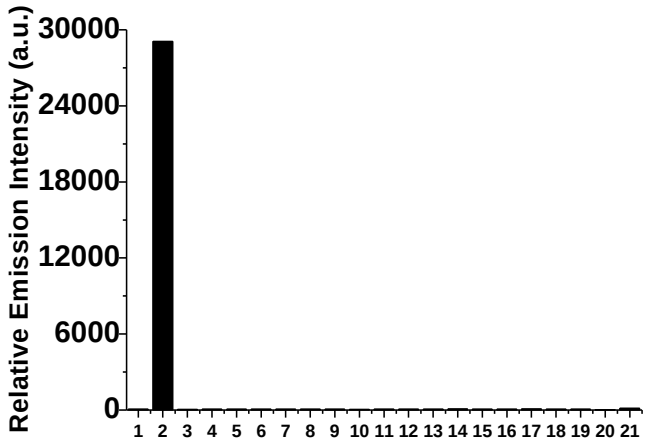


Figure S22. Relative emission intensity at 510 nm of probe **1** (1 μM) with different metal ions (300 μM for each metal ion) in HEPES buffer (50 mM, pH 7.0, 25 ^{o}C). From 1 to 21: None, Au^{3+}, Al^{3+}, Cd^{2+}, Ca^{2+}, Cr^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}, Ni^{2+}, Pd^{2+}, K^{+}, Ag^{+}, Na^{+}, Zn^{2+}, and Au^{+} (all were chloride salts except for $AgNO_3$). The values are averages of three independent measurements.

7. Fluorescence *turn-on* response of Rose Bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein)

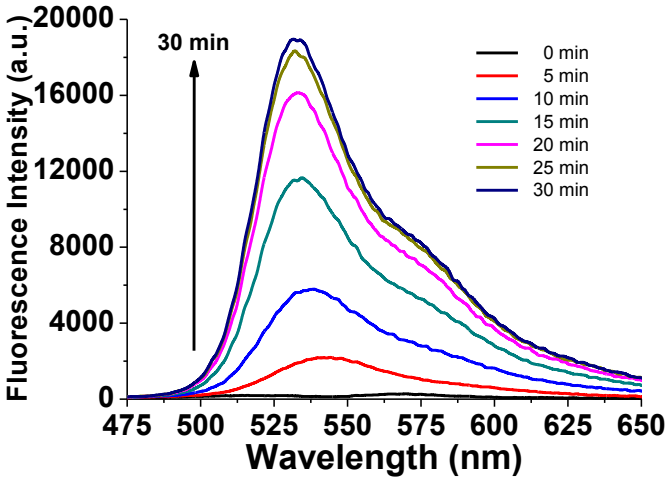
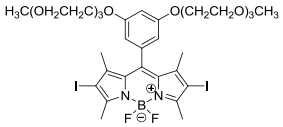
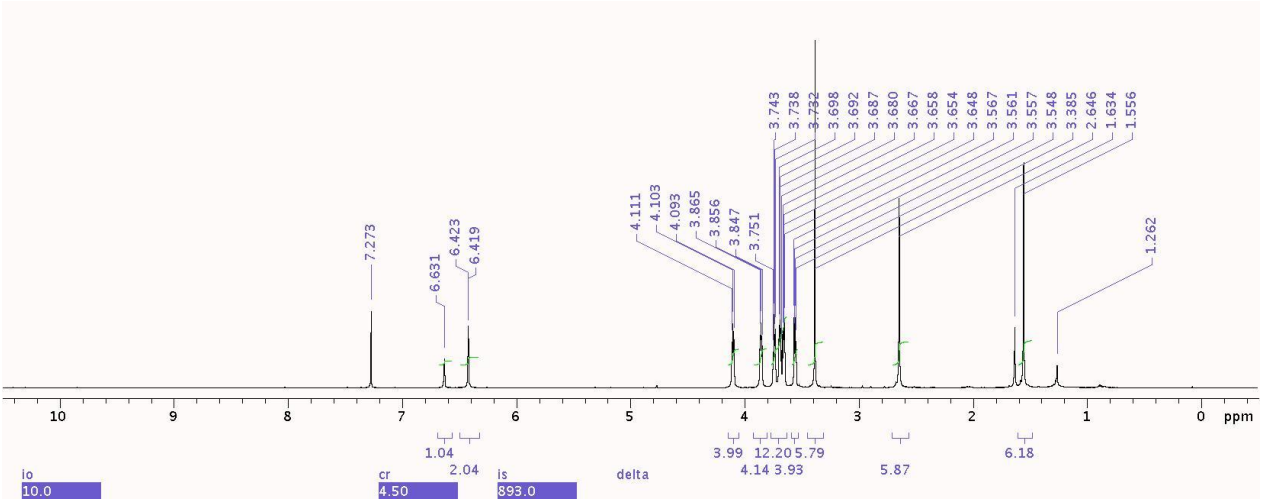


Figure S23. Changes in fluorescence spectra of Rose Bengal (1 μM) upon addition of $AuCl_3$ (300 μM) in HEPES buffer (50 mM, pH 7.0, 5% ethanol, 25°C) for different time periods (0 – 30 min). Excited at 450 nm ($\lambda_{em,\ max}$ = 535 nm).

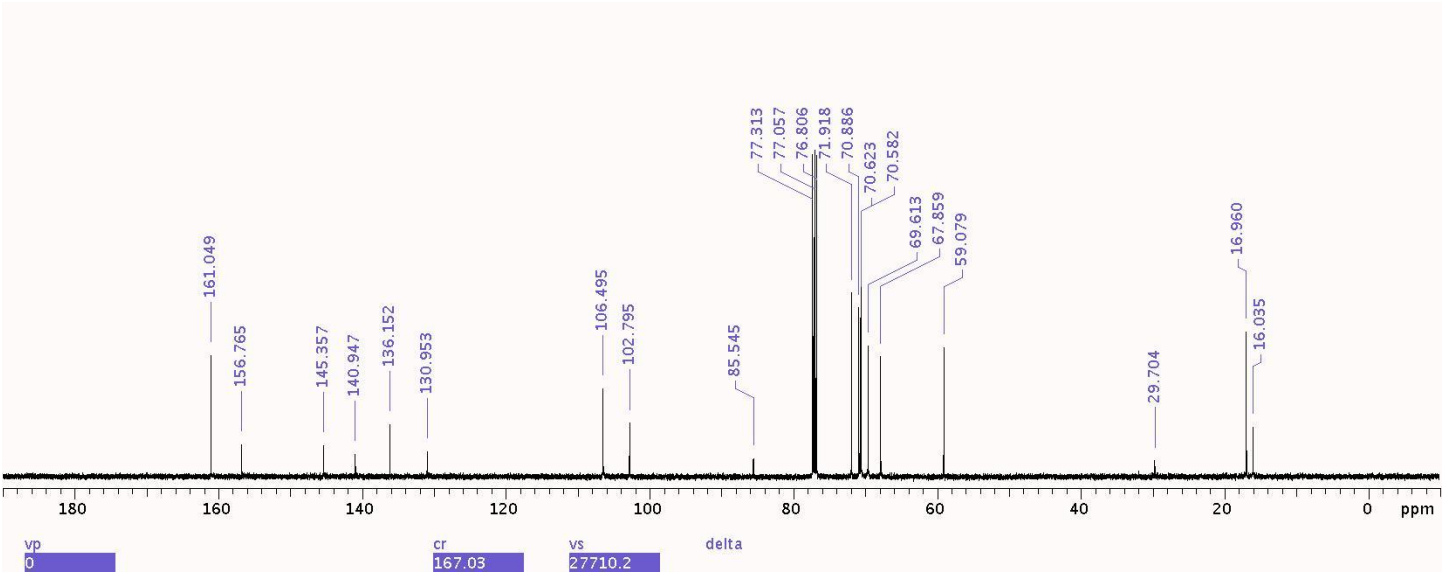
8. ^{1}H-NMR and ^{13}C-NMR Spectra

^{1}H-NMR Spectrum of **1** in $CDCl_3$ (500 MHz):

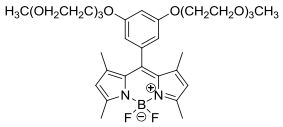


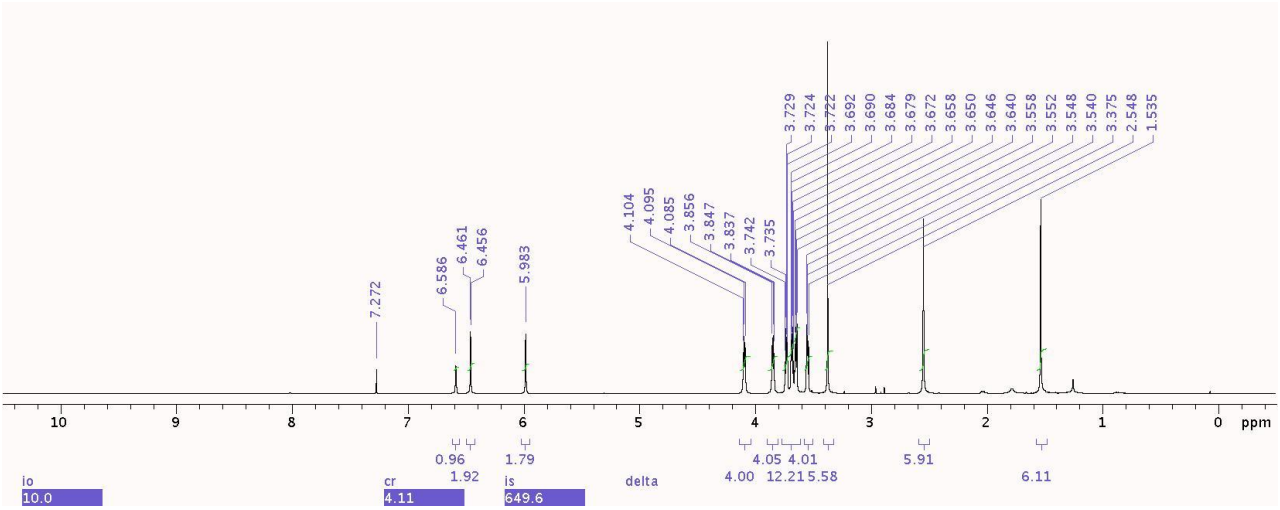


^{13}C-NMR Spectrum of **1** in $CDCl_3$ (125 MHz):

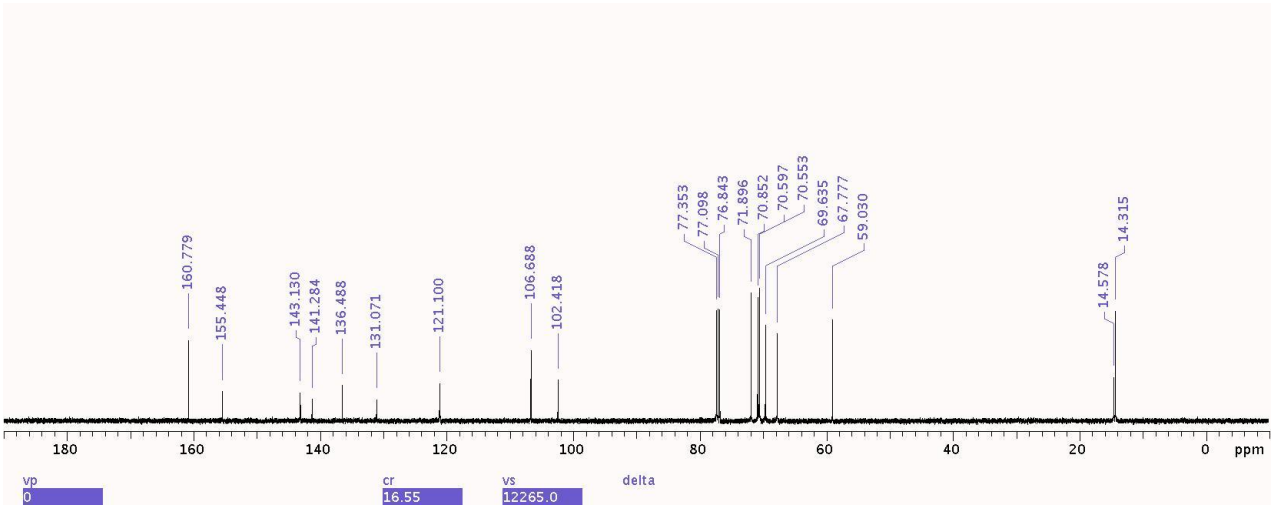


^{1}H-NMR Spectrum of **2** in $CDCl_3$ (500 MHz):

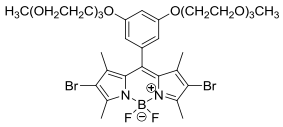


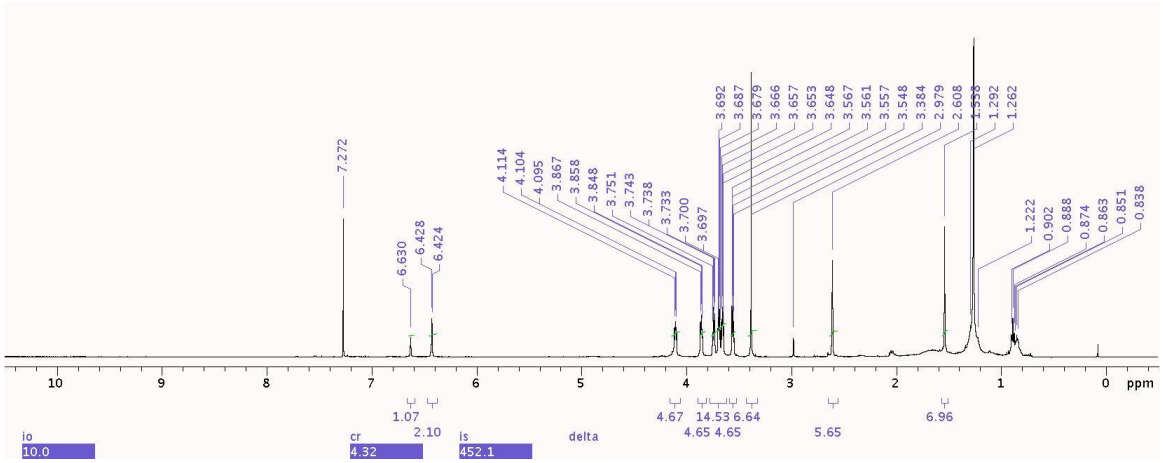


^{13}C-NMR Spectrum of **2** in $CDCl_3$ (125 MHz):

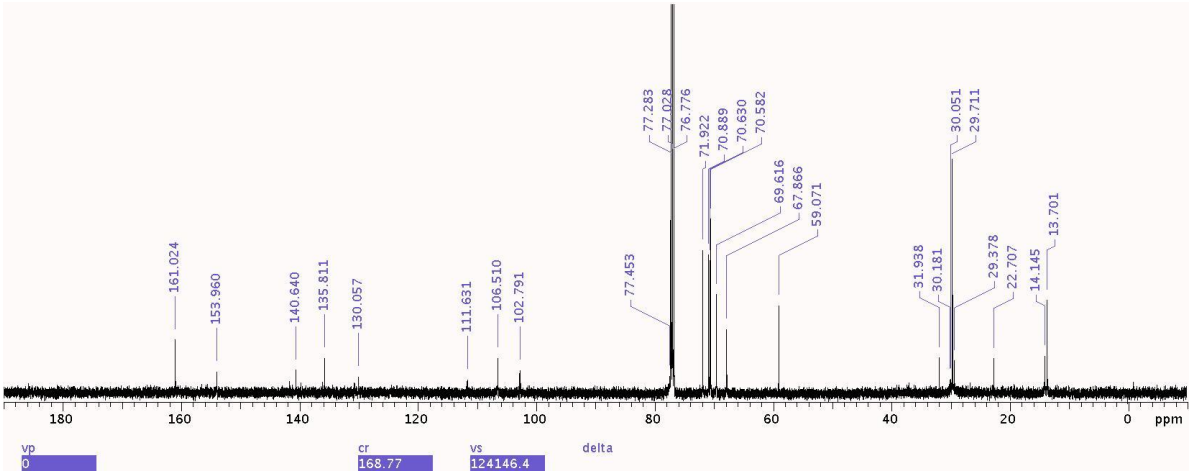


^{1}H-NMR Spectrum of **3** in $CDCl_3$ (500 MHz):





^{13}C-NMR Spectrum of **3** in $CDCl_3$ (125 MHz):



9. References

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2 A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, **108**, 1067.