Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

A BODIPY-based reactive probe for the detection of Au(III) species and its application to cell imaging

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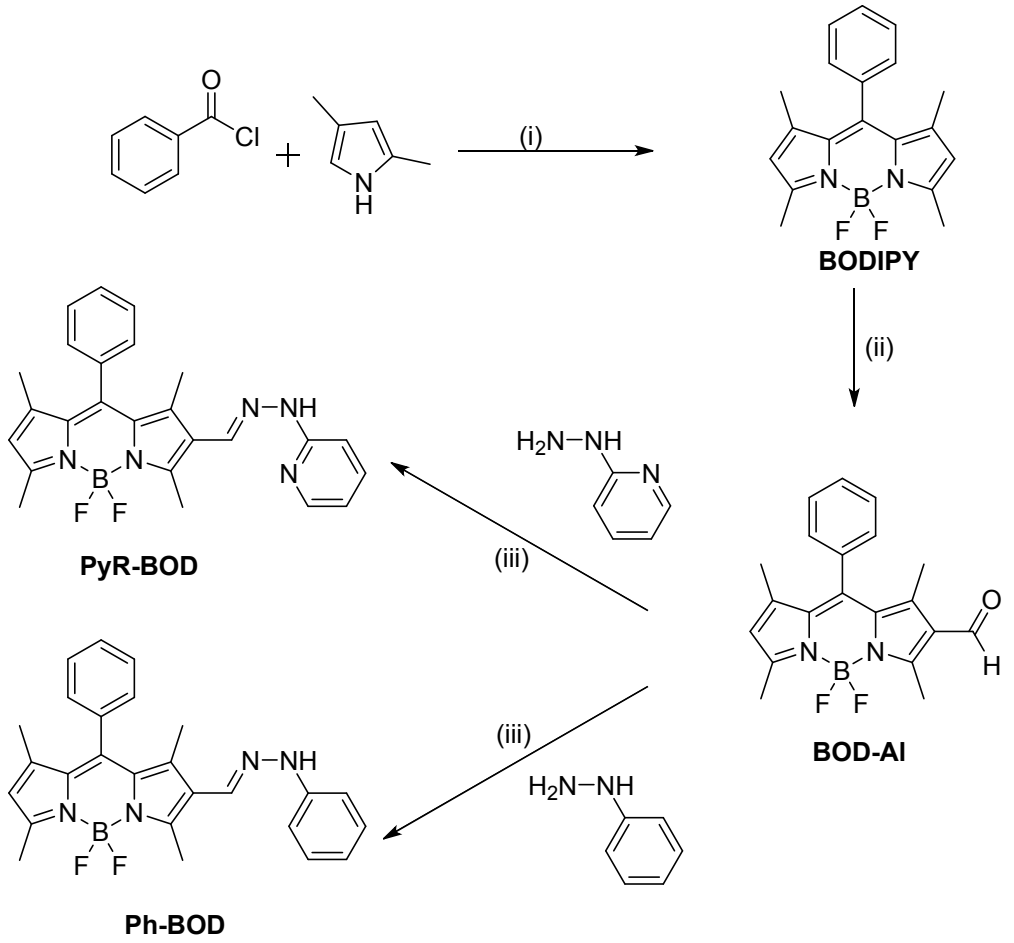
1. General methods

All reagents were purchased from commercial suppliers (Aldrich and Merck) and they were used without further purification. ^{1}H NMR and ^{13}C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. HRMS data were acquired on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence measurements were performed by using Varian Cary Eclipse Fluorescence spectrophotometer. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 460 nm, the emission spectra were integrated over the range 480 nm to 750 nm. The slit width was 5 nm for both excitation and emission. Melting points were determined by using an Electrothermal Melting Point Apparatus 9200. The pH was recorded by HI-8014 instrument (HANNA). All measurements were conducted at least in triplicate.

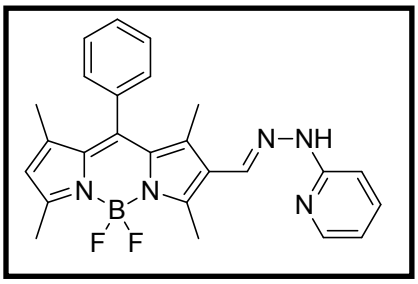
2. Synthesis of probe molecules

The synthesis pathway for **PyR-BOD** and **Ph-BOD** was shown in Scheme 1. **BODIPY** and **BOD-Al** were synthesized by using literature procedure.[1] **BOD-Al** was synthesized from **BODIPY** by using well known Vismeier Haack's formylation reaction. The obtained molecule was converted to **PyR-BOD** and **Ph-BOD** by using appropriate hydrazines[2] and catalytic amount of acetic acid in ethanol in a short reaction times.[3]



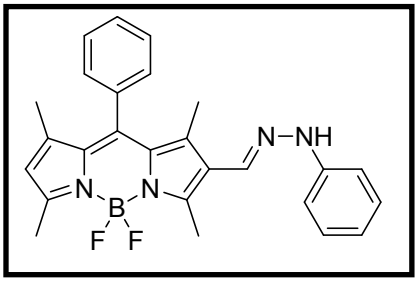
Scheme 1: Synthesis pathway of **PyR-BOD** and **Ph-BOD. (i)** DCM, RT, overnight, then Et_3N and BF_3OEt_2 **(ii)** $POCl_3$, DMF, 0°C, then DCE, 60°C, overnight, **(iii)** Hydrazine derivative, ethanol, CH_3COOH, reflux.

Synthesis of PyR-BOD



To a mixture of **BOD-Al** (35.2 mg, 0,1 mmol) and 2-hydrazinylpyridine[2] (12 mg, 0,11mmol) in 4 ml ethanol and a drop of glacial acetic acid was added. The resulting solution was refluxed for two hours. After cooling room temperature, the solvent was removed under reduced pressure. The resultant residue was purified by column chromatography (2:1 (Hexane:Ethyl acetate)) to afford **PyR-BOD** as purple solid (34 mg, 76 % yield). Mp: 274-276 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.33 (s, 1H), 8.10 (d, J = 4.0 Hz, 1H), 7.71 (s, 1H), 7.58-7.50 (m, 3H), 7.31-7.29 (m, 2H), 7.14 (d, J = 8.0 Hz, 1H), 6.72 (t, J = 6.4 Hz, 1H), 6.03 (s, 1H), 2.82 (s, 3H), 2.59 (s, 3H), 1.68 (bs, 1H), 1.54 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.1, 156.6, 154.8, 147.5, 144.3, 142.0, 138.8, 138.2, 134.9, 133.5, 132.2, 130.7, 129.2, 129.1, 128.0, 124.3, 122.0, 115.4, 107.2, 14.7, 14.5, 14.2, 12.3. MS (TOF-ESI): m/z: Calcd: 441,20564 $[M-H]^-$, Found: 441,20585 $[M-H]^-$, Δ=-0.48 ppm

Synthesis of Ph-BOD



The sensor molecule, **Ph-BOD**, was synthesized by using above procedure as purple solid (31 mg, 70%yield). Mp: 247-249 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.64 (s, 1H), 7,50-7.48 (m, 4H), 7.26-7.20 (m, 4H), 6.95 (s, 2H), 6.81 (s, 1H), 6.01 (s, 1H), 2.81 (s, 3H), 2.58 (s, 3H), 1.52 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 155.0, 143.5, 141.6, 138.2, 134.7, 131.4, 129.4, 129.3, 129.2, 129.0, 128.9, 127.9, 127.5, 125.0, 121.5, 119.2, 112.1, 14.5, 14.3, 14.1, 12.2.

3. Determination of detection limit

The detection limit was calculated based on the fluorescence titration [1]. To determine the detection limit, the emission intensity of **PyR-BOD** (10.0 μM) without Au^{3+} was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Au^{3+} concentration could be obtained in the 0,025 – 0,8μM (R = 0.9924). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 44 nM.

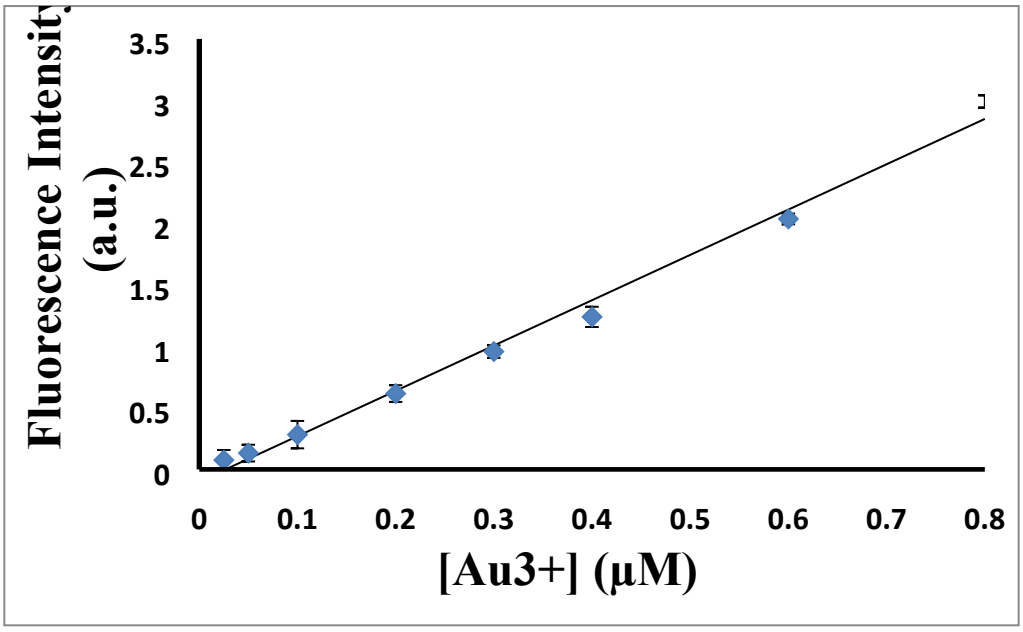


Figure S1: Fluorescence changes of **PyR-BOD** (10.0 μM) upon addition of Au^{3+} (0,025 to 0,8 μM, 0. 025 to 0.08 equiv.) in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 460 nm, λ_{em}= 512 nm at 25 ^{o}C).

4. Cell Imaging

A549 Human Lung Adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5 % CO_2 at 37 ^oC. The cells were plated on 12mm cover glasses in 6-well plate and allowed to grow for 24h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated **PyR-BOD** (5 μM) for 20 min at 37 ^oC then washed with PBS three times. After incubating with Au^{3+} (10 μM) for 20 min at 37 ^oC, cells were rinsed with PBS three times, and DAPI for 10 min at 37^oC then washed with PBS three times. Then, the fluorescence images were acquired through an Olympus IX71 fluorescence microscope.

5. References

1. (a) Emrullahoğlu, M.; Üçüncü, M.; Karakus, E. *Chem. Commun.*, **2013**, 49, 7836. (b) Isik, M.; Ozdemir, T.; Turan, I. S.; Kolemen, S.; Akkaya, E. U. *Org. Lett.* **2013**, 15, 216.
2. Padalkar, V. S.; Patil, V. S.; Phatangare, K. R.; Umape, P. G., Sekar, N. *Synth. Commun.* **2011**, 41, 925.
3. Chaur, M. N.; Collado, D.; Lehn, J. M. *Chem. Eur. J.* **2011**, 17, 248.

6. **Effect of water ratio**

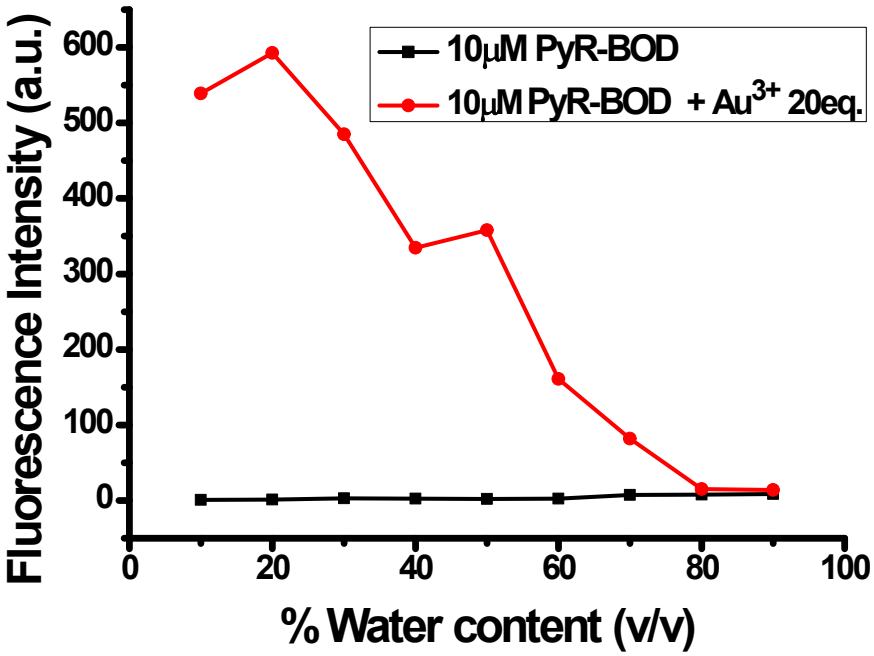


Figure S2: Effect of fraction of water on the interaction of **PyR-BOD** (10 μM) with Au^{3+} (200 μM, 20 equiv.) in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 460 nm, λ_{em}= 512 nm at 25 ^{o}C).

7. Effect of pH

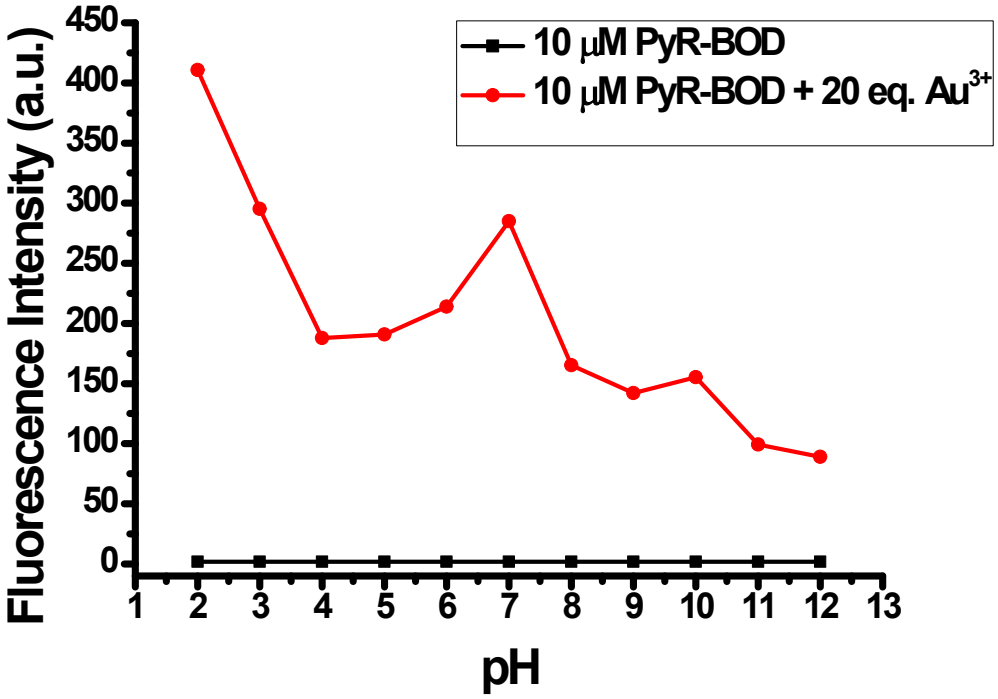


Figure S3: Effect of fraction of pH on the interaction of **PyR-BOD** (10 μM) with Au^{3+} (200 μM, 20 equiv.) in 0.1M potassium phosphate buffer, pH/EtOH (v/v, 1:1) (λ_{ex}: 460 nm, λ_{em}= 512 nm at 25 °C).

8. Absorption and emission spectra of PyR-BOD

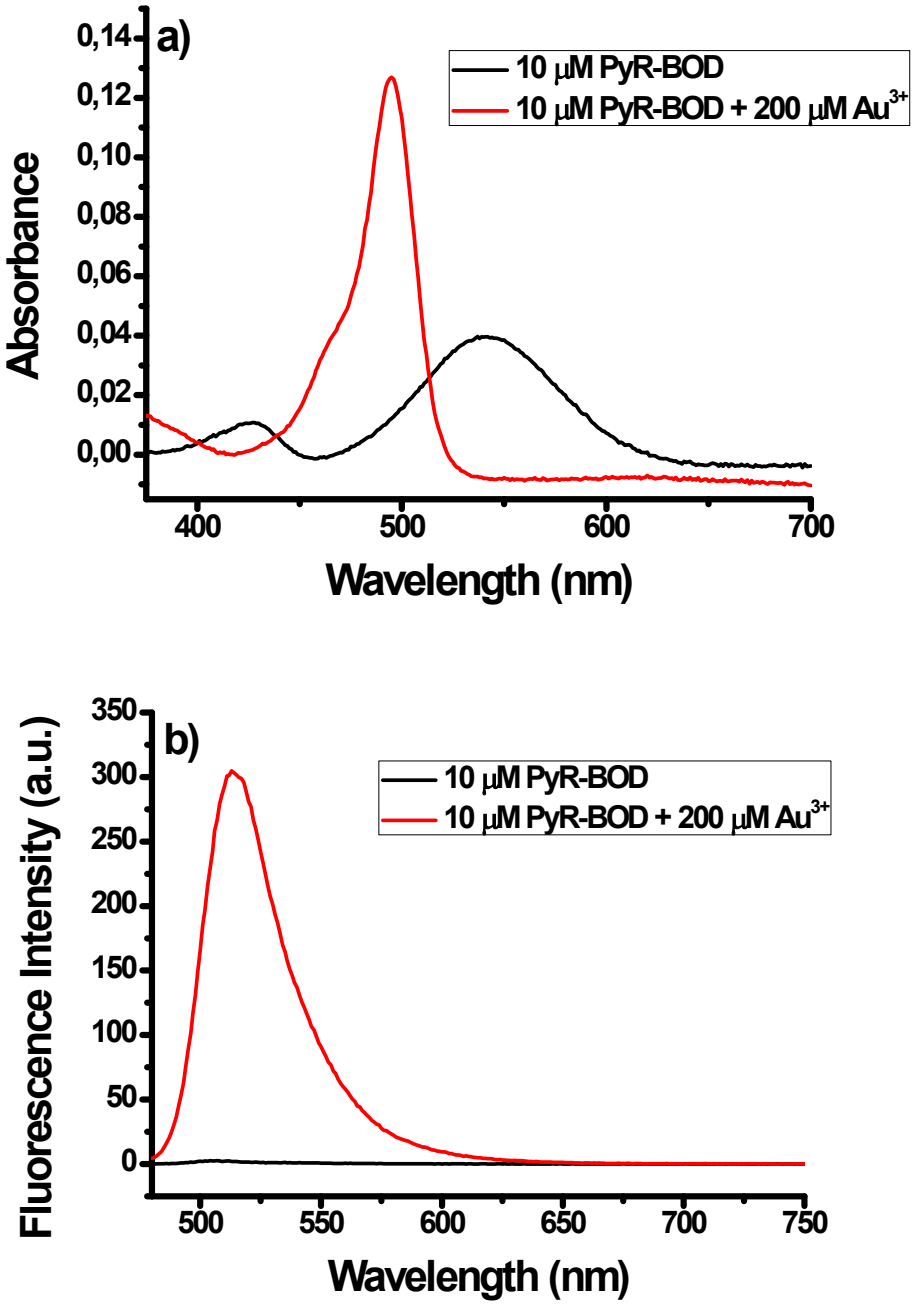


Figure S4: **(a)** Absorbance and **(b)** fluorescence spectra of **PyR-BOD** (10 μM) in the absence and presence of 20 equiv. (200 μM) of Au^{3+} in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (λ_{ex}: 460 nm at 25 °C).

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9. Titration of PyR-BOD with Au(III)

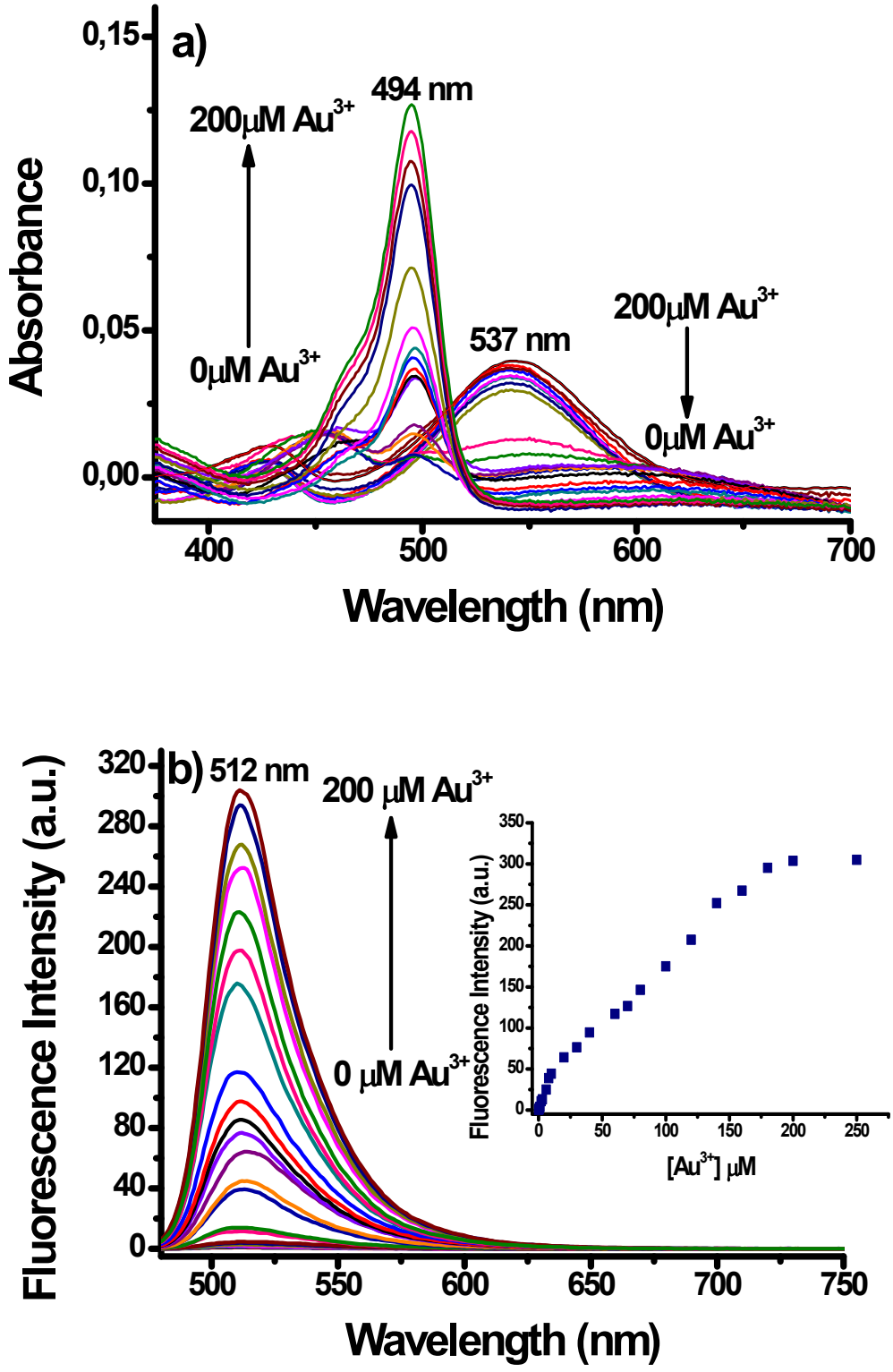


Figure S5: **(a)** Absorbance and **(b)** fluorescence spectra of **PyR-BOD** (10 μM) in the presence of increasing amount of Au^{3+} (0-200 μM) 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1).Inset: Calibration curve. (λ_{ex}: 460 nm at 25 °C).

10. The Fluorescence responses of PyR-BOD with Au^{3+} and other ions

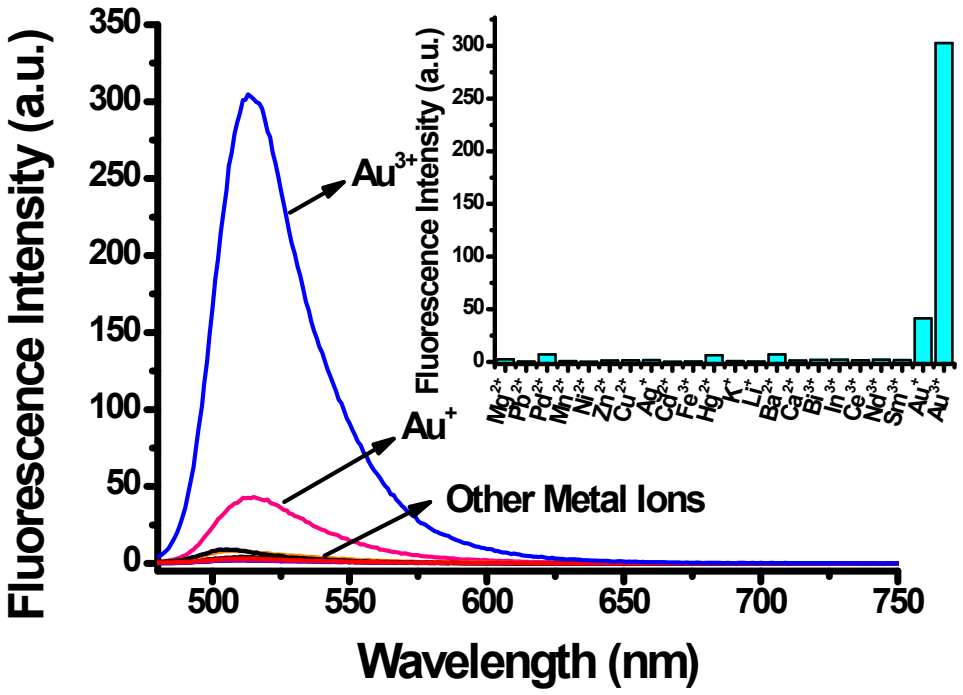


Figure S6: Fluorescence intensities of **PyR-BOD** (10 μM), **PyR-BOD** (10 μM) + Au^{3+} (200 μM, 20 equiv.), **PyR-BOD** (10 μM) + other metal ions (200 μM, 20 equiv.) in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 460 nm, at 25 °C). Inset: Bar graph notation.

11. The Fluorescence responses of PyR-BOD in the presence of Au^{3+} and other metal ions

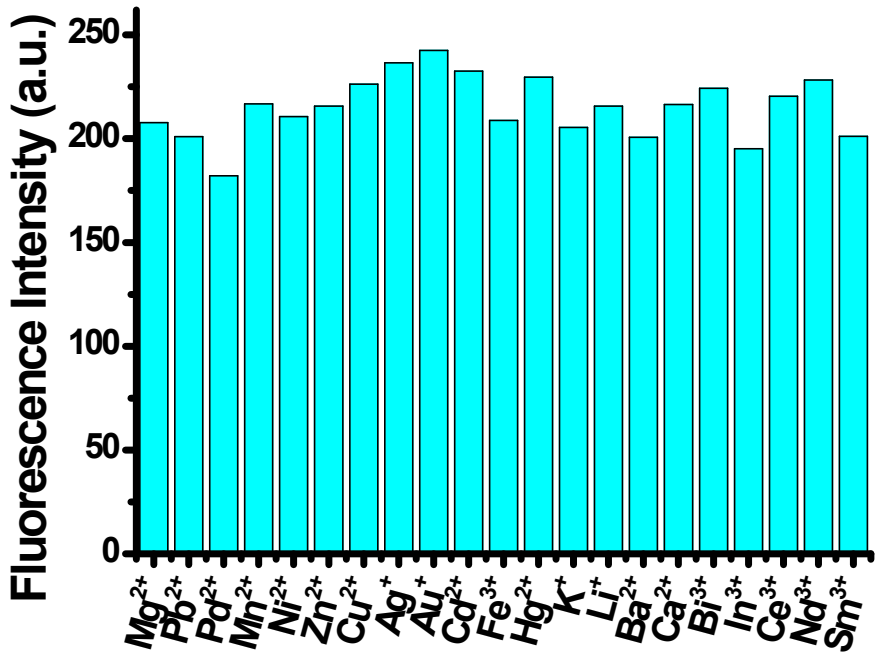
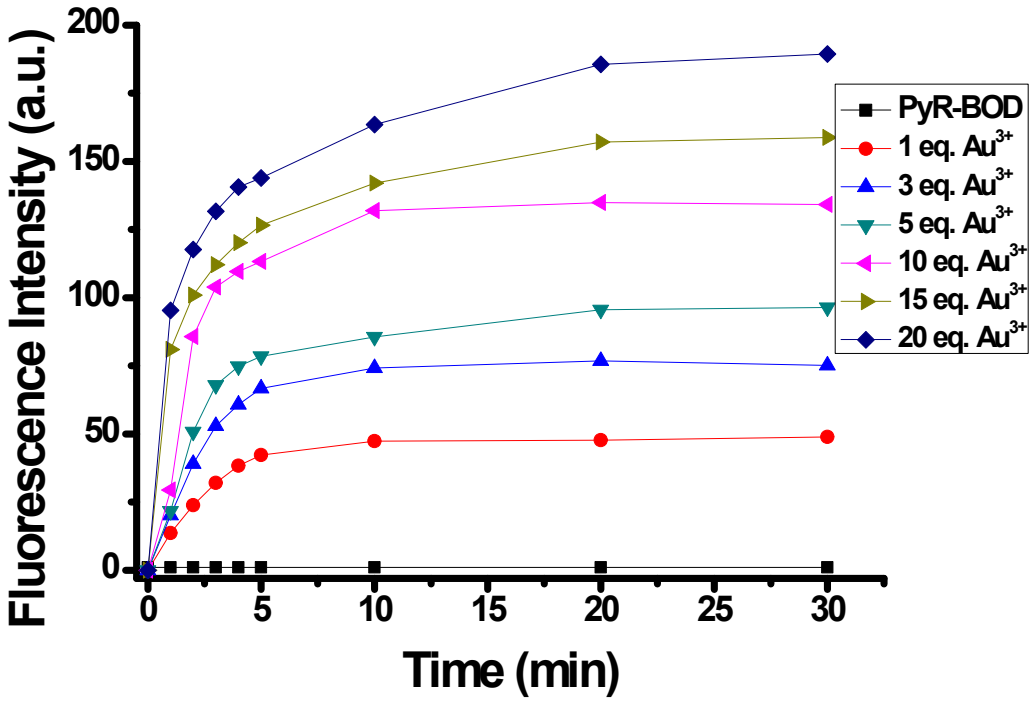


Figure S7: Fluorescence intensities of **PyR-BOD** (10 μM) in the presence of Au^{3+} (200 μM, 20 equiv.) and 20 equiv. of other metal ions in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (λ_{exc}=460 nm, λ_{em}= 512 nm at 25 ^{o}C)

12. Reaction – Time Profile of PyR-BOD with Au3+



13.

Figure S8: Reaction time profiles of **PyR-BOD** (10 μM) in the absence (■) or presence of Au^{3+} [10 (●), 30(▲), 50(▼), 100 (◄), 150(►), 200(♦) μM.]. The fluorescence intensities at 512 nm were continuously monitored at time intervals in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (λ_{exc}=460 nm, λ_{em}= 512 nm at 25 °C).

14. Determination of Stability of PyR-BOD and Ph-BOD in Reaction Conditions over Time

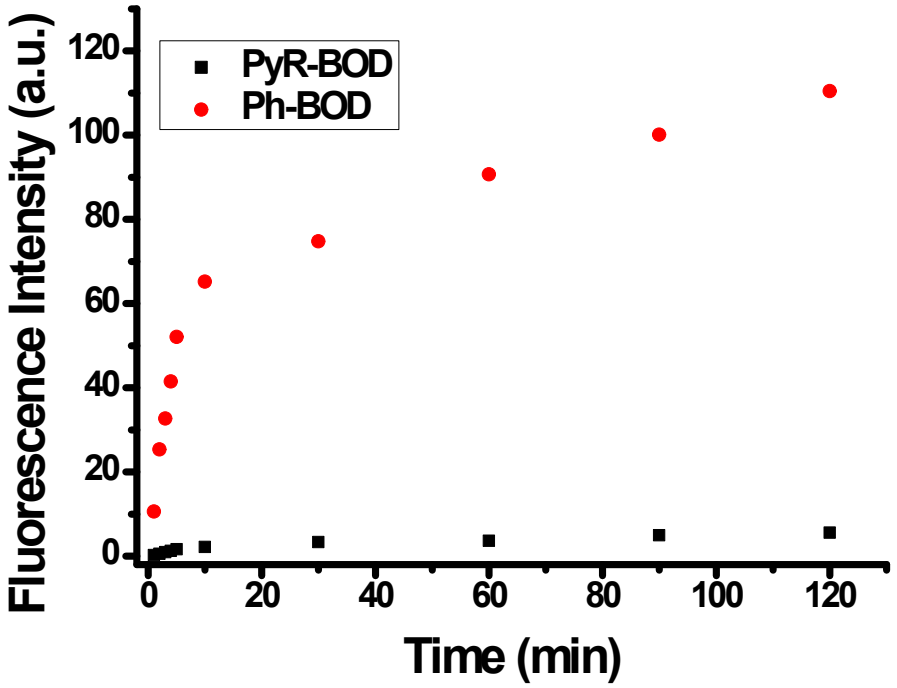
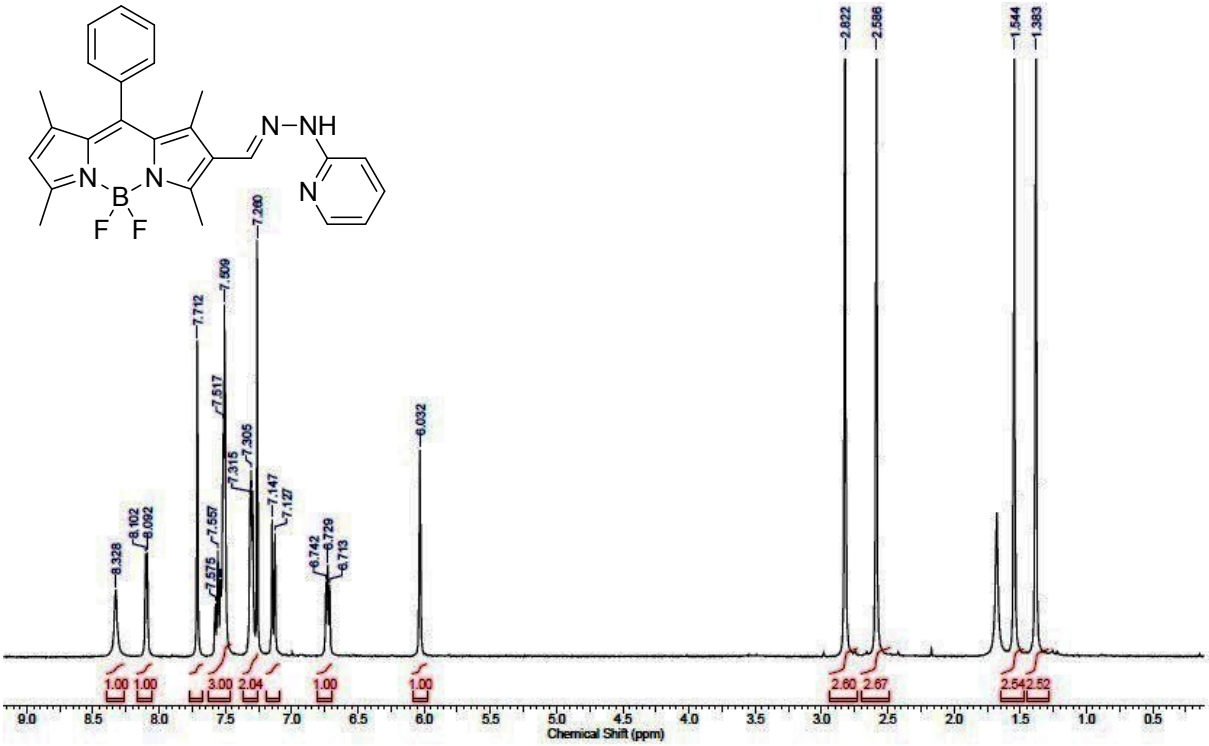
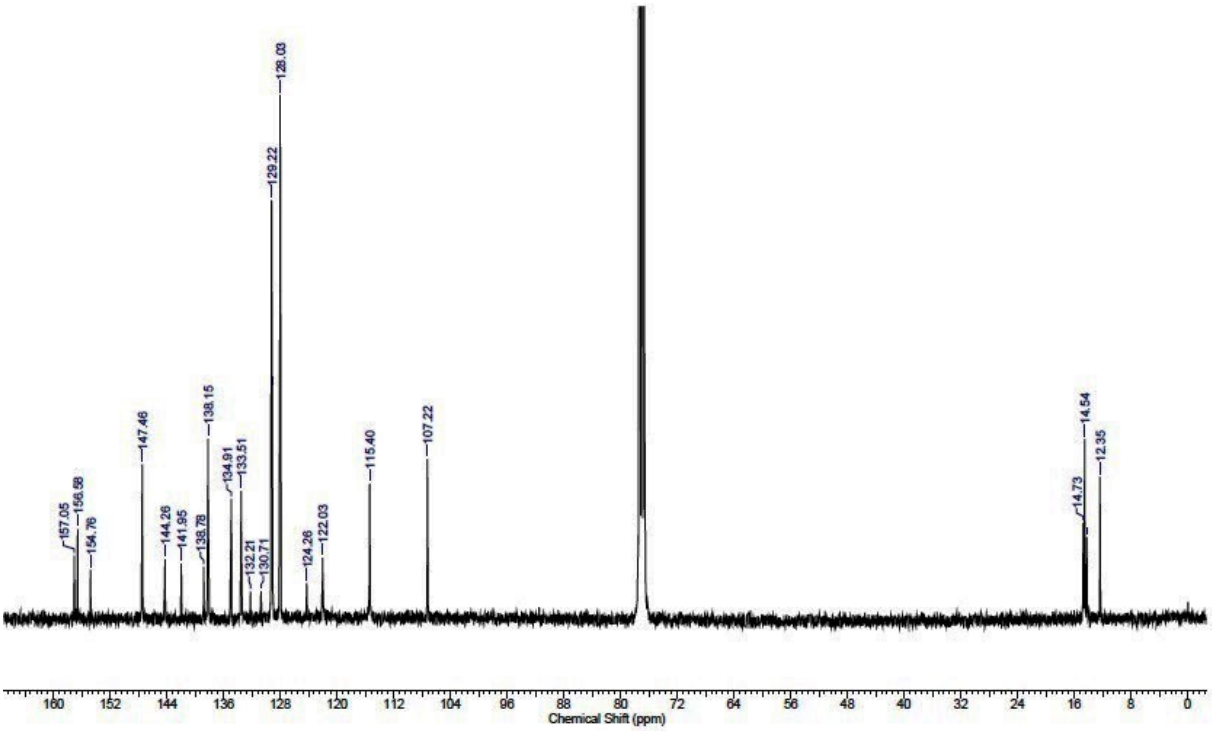


Figure S9: Fluorescence responses of **PyR-BOD** (10 μM) **and Ph-BOD** (10 μM) in reaction conditions (0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) without addititon of Au^{3+}. (λ_{exc}=460 nm, λ_{em}= 512 nm at 25 ^{o}C).

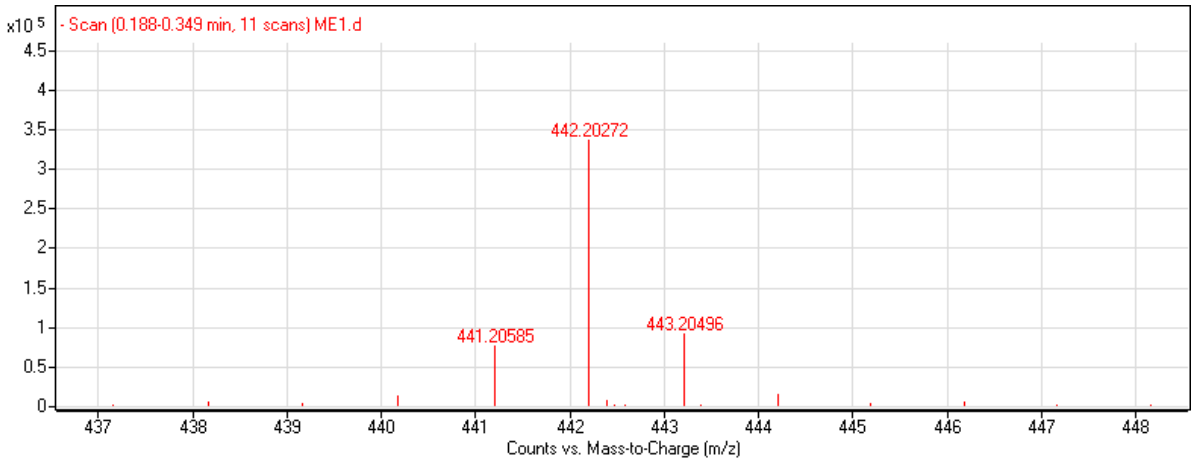
15. ^{1}H NMR of PyR-BOD



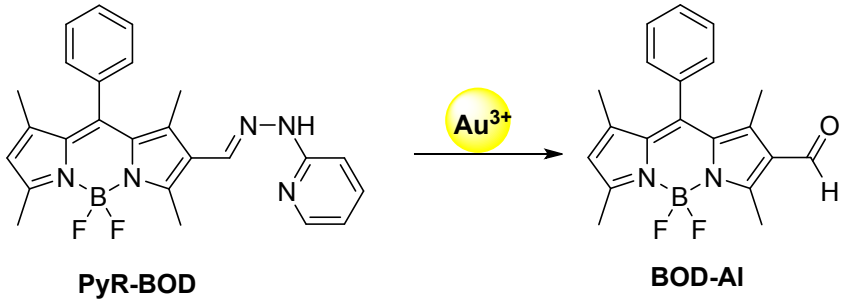
16. ^{13}C NMR of PyR-BOD



17. HRMS Spectrum of PyR-BOD



18. TLC Image of the Hydrolysis Reaction of PyR-BOD Mediated by Au(III) Ions



PyR-BOD **BOD-Al**

a)

b)

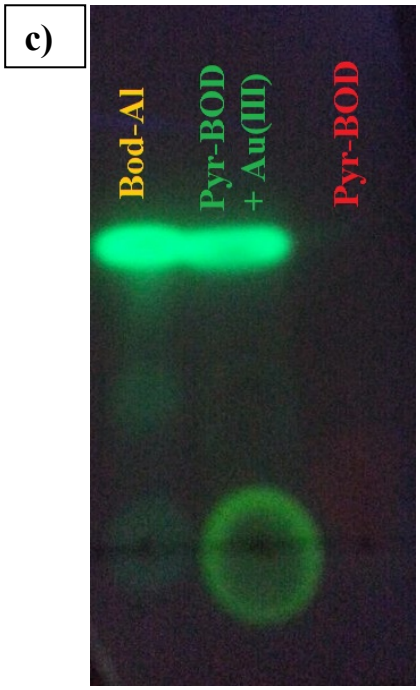
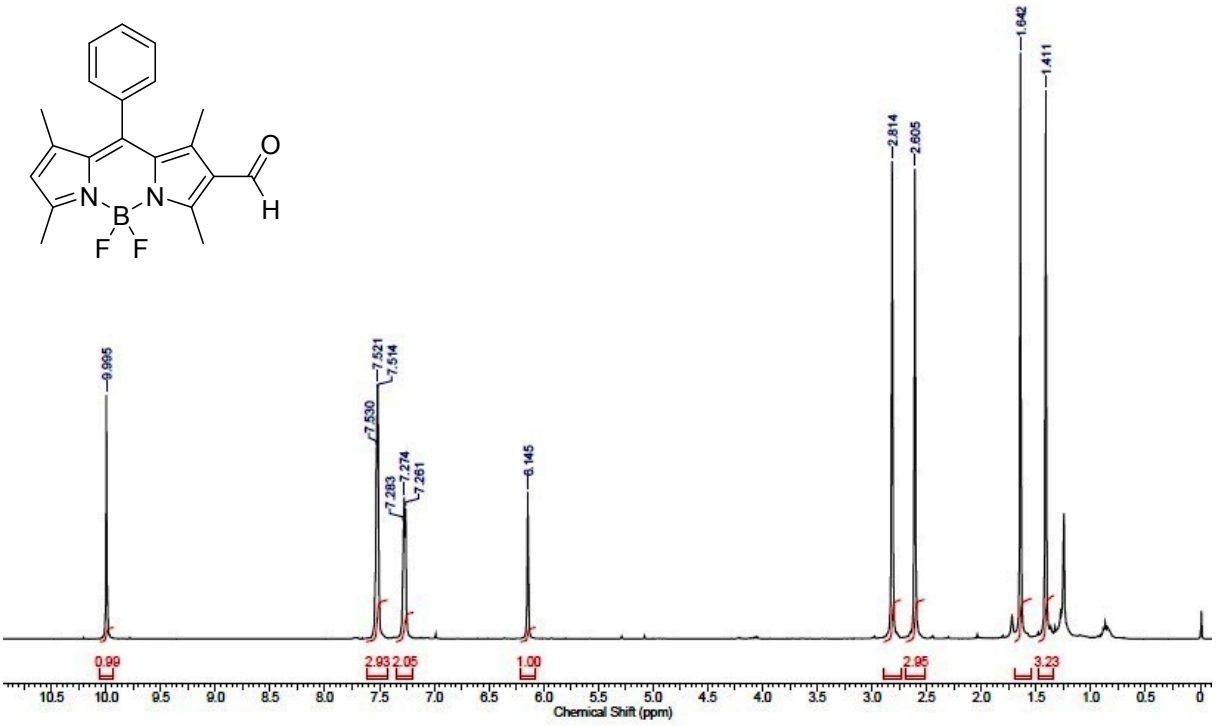


Figure S10: (a) Day light photograph image of **PyR-BOD** + Au(III) (left), **PyR-BOD** (right), **(b)** fluoresccence image of **PyR-BOD** + Au(III) (left), **PyR-BOD** (right), **(c)** TLC image of the hydrolysis reaction of **PyR-BOD** mediated with Au(III) ion (4/1, v/v, hexane/ethylacetate).

19.^{1}H NMR of BOD-Al



20.^{13}C NMR of BOD-Al