Supporting Information

A dual responsive fluorescent probe based on a BODIPY/Pyridine conjugate for reversible detection of Au^{3+} and Hydronium ions

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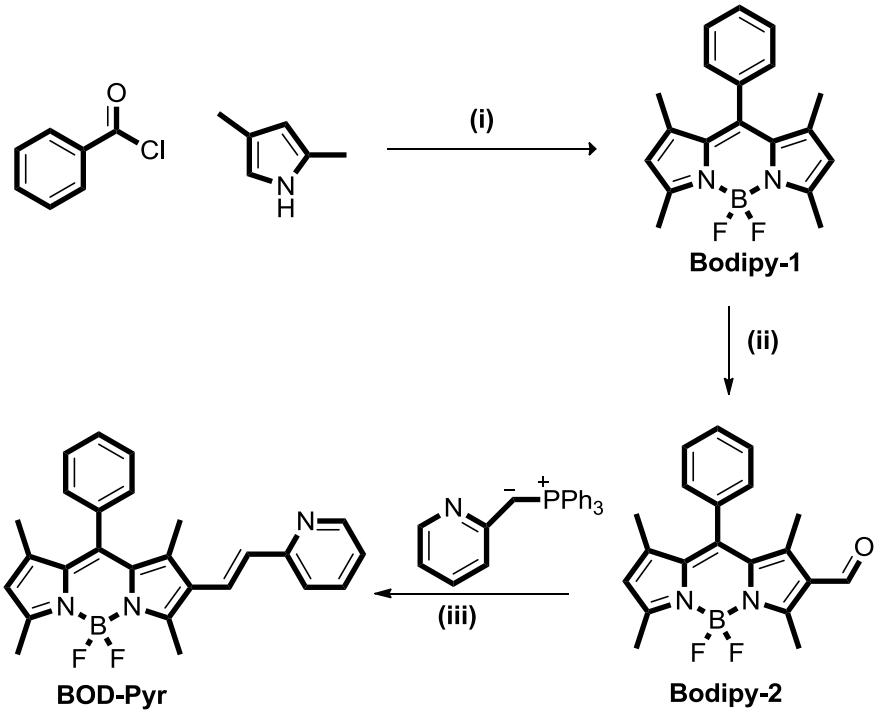
1. General methods

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. Bruker MALDI-TOF-TOF Mass Spectrometer was used for mass spectrometry analysis. 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 500 nm, the emission spectra were integrated over the range 520 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.

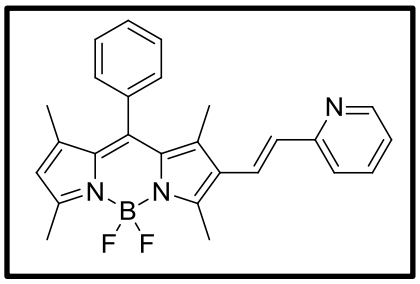
2. Synthesis of probe molecule

The synthesis pathway for **BOD-Pyr** was shown in Scheme 1. **Bodipy-1** and **Bodipy-2** were synthesized by using literature procedure [1]. **Bodipy-2** was synthesized from **Bodipy-1** by using well known Vilsmeier Haack's formylation reaction. The obtained molecule was converted to **BOD-Pyr** by using Wittig reagent[2] and triethylamine base in dioxane.



Scheme 1: Synthesis pathway of **BOD-Pyr. (i)** DCM, RT, overnight, **(ii)** $POCl_3$, DMF, 0^oC, then DCE, 60^oC, overnight, **(iii)** Et_3N, Dioxane, RT, overnight.

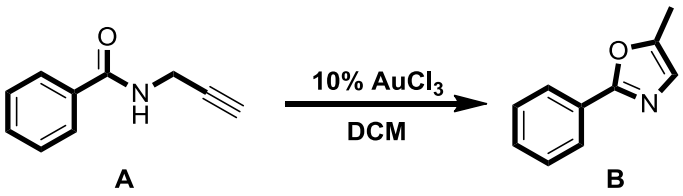
Synthesis of BOD-Pyr



To a solution of **Bodipy-2** (100 mg, 0.285 mmol) in dioxane (10 mL) was added triphenyl(2-pyridylmethyl)phosphonium chloride hydrochloride (389 mg, 0.896 mmol). Then, 250 µl of triethyl amine was added drop by drop and the resultant solution was stirred at room temperature for overnight. After reaction completed, the solution was concentrated in vacuum and extracted three times with dichloromethane. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane / ethyl acetate (8/1)) to afford **BOD-Pyr** as green solid (68.1mg, 56% yield). Mp: 267-269 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.56 (d, J= 4.0 Hz, 1H), 7.62 (dt, J= 8.0, 1.6 Hz 1H), 7.52-7.50 (m, 3H), 7.46 (s, 1H), 7.32-7.29 (m, 2H), 7.28 (s, 1H), 7.12-7.09 (m, 1H), 6.72 (d, J= 16.0 Hz, 1H), 6.01 (s,

1H), 2.76 (s, 3H), 2.58 (s, 3H), 1.51 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.3, 155.9, 154.9, 149.6, 143.8, 141.8, 139.3, 136.5, 135.0, 132.0, 131.0, 129.5, 129.2, 129.1, 128.1, 127.8, 123.9, 121.8, 121.8, 14.7, 14.5, 14.1, 12.9. Calcd. for $C_{26}H_{24}BF_2N_3$: 427.203 $[M]^+$, Found: 428.244 $[M+H]^+$.

Synthesis of compound B[3]



Scheme 2: Synthesis of compound B by gold-catalyzed cyclization of propargylic amide

3. Effect of solvent

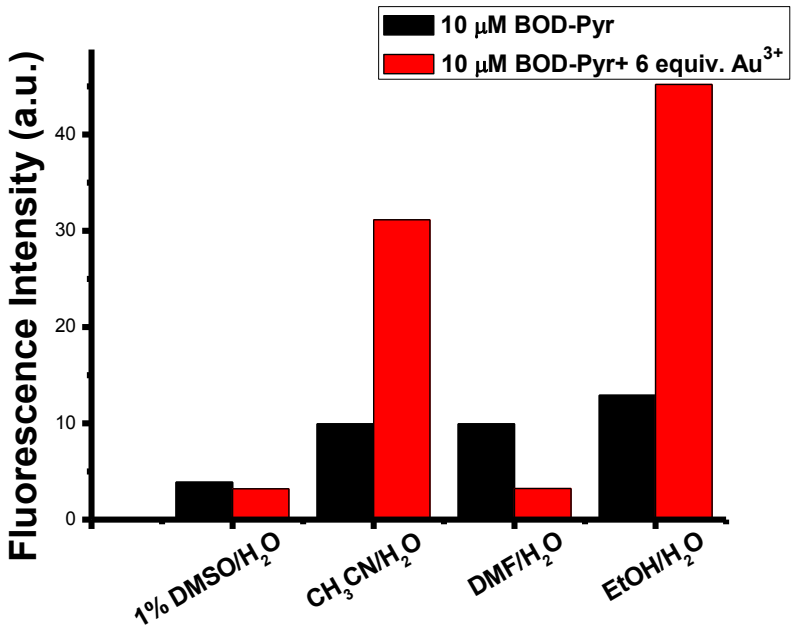


Figure S1: Effect of solvent on the interaction of **BOD-Pyr** (10.0μM) with Au^{3+} (60 μM, 6 equiv.) in various solvent combinations ((Solvent/H_2O) v/v, 1:1) (λ_{ex}: 500 nm, at 25 ^oC).

4. Effect of water ratio

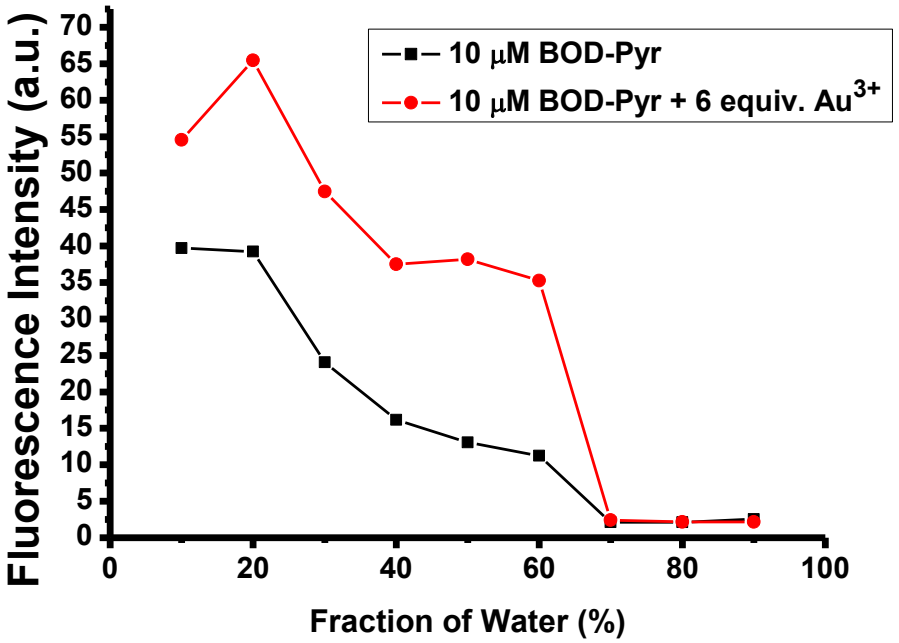


Figure S2: Effect of fraction of water on the interaction of **BOD-Pyr** (10.0μM) with Au^{3+} (60 μM, 6 equiv.) in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 500 nm, λ_{em}= 577 nm at 25 ^{o}C).

5. Effect of pH

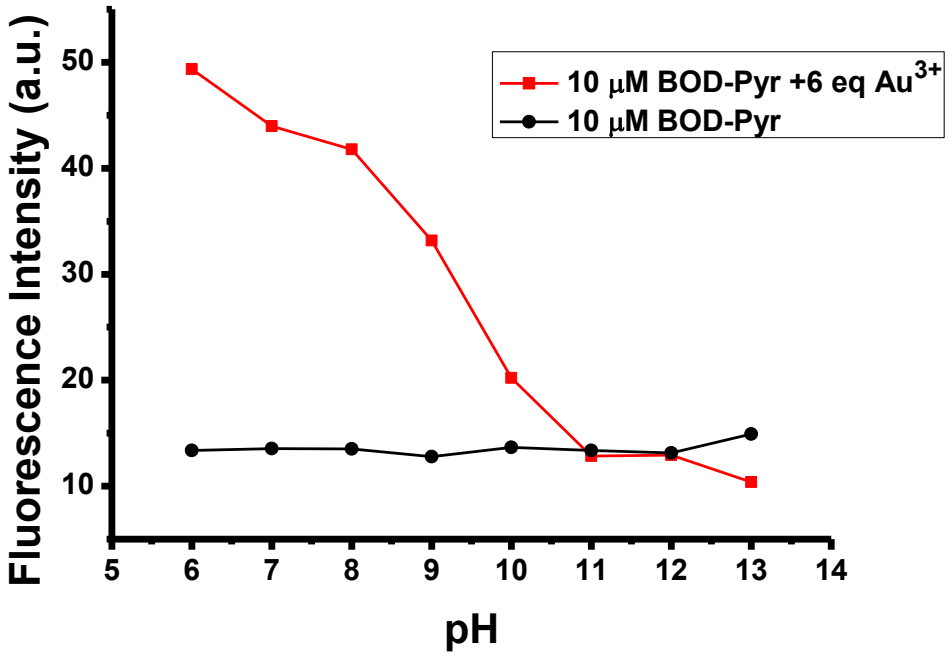


Figure S3: Effect of fraction of pH on the interaction of **BOD-Pyr** (10.0μM) with Au^{3+} (60 μM, 6 equiv.) in 0.1M potassium phosphate buffer/EtOH (v/v, 1:1) (λ_{ex}: 500 nm, λ_{em}= 577 nm at 25 ^{o}C).

6. Reaction – Time Profile of BOD-Pyr with Au^{3+}

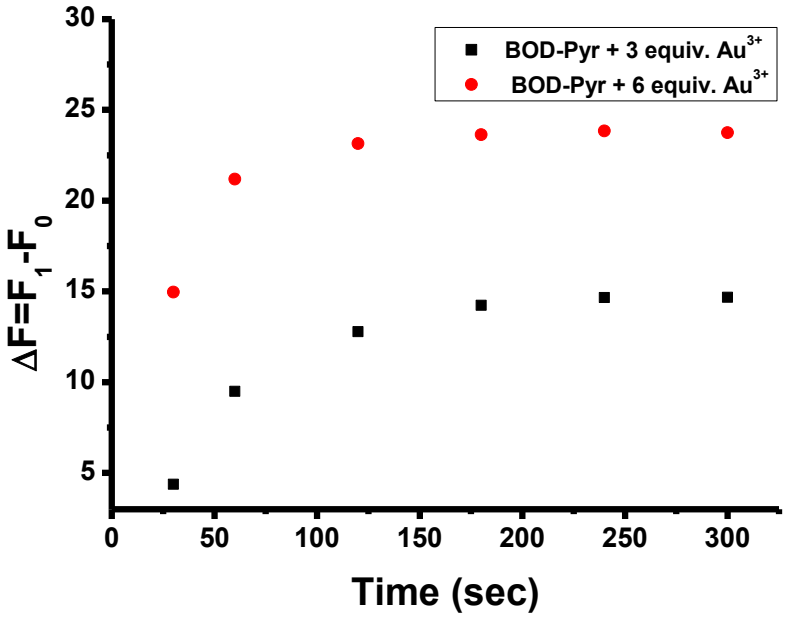


Figure S4: Reaction time profiles of **BOD-Pyr** (10.0 µM) in the presence of Au^{3+} [30 (■) and 60 (●) µM.]. The fluorescence intensities at 577 nm were continuously monitored at time intervals in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (λ_{ex}: 500 nm, λ_{em}= 577 nm at 25 ^{o}C).

7. Absorption and emission spectra of BOD-Pyr

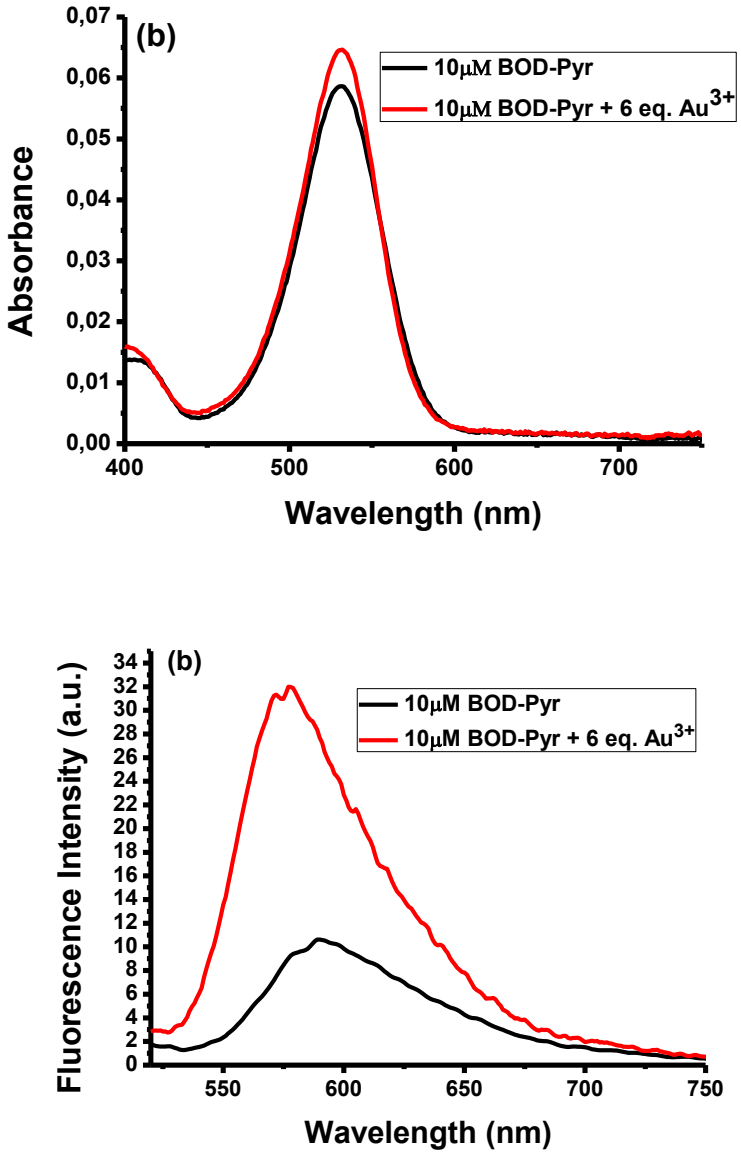


Figure S5: **(a)** Absorbance and **(b)** fluorescence spectra of **BOD-Pyr** (10μM) in the absence and presence of 6 equiv. (60μM) of Au^{3+} in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1).

8. Fluorescence titration of BOD-Pyr with Au(III) in EtOH/Water

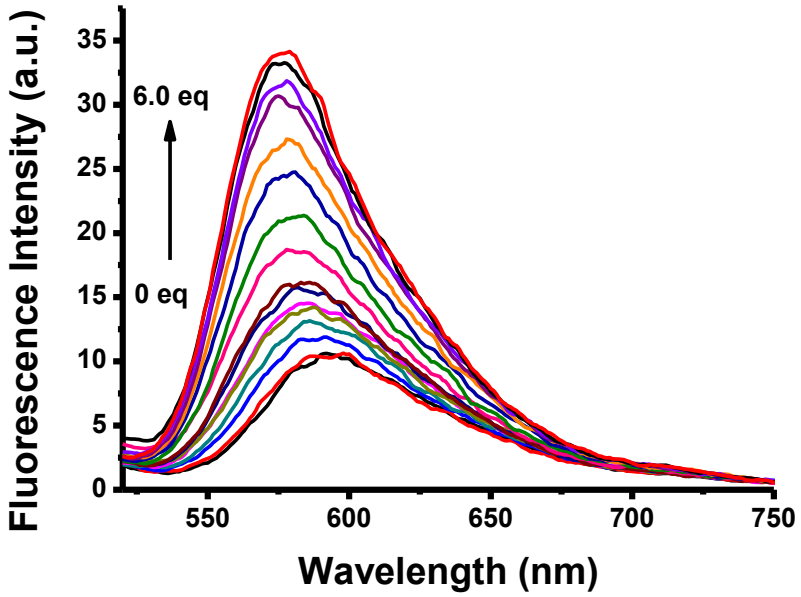


Figure S6: Fluorescence spectra of **BOD-Pyr** (10µM) in the presence of increasing concentrations of Au^{3+} (0-60µM, 0-6 equiv.) in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (λ_{exc}=500 nm at 25^{o}C).

9. Job's plot analysis of BOD-Pyr with Au^{3+}

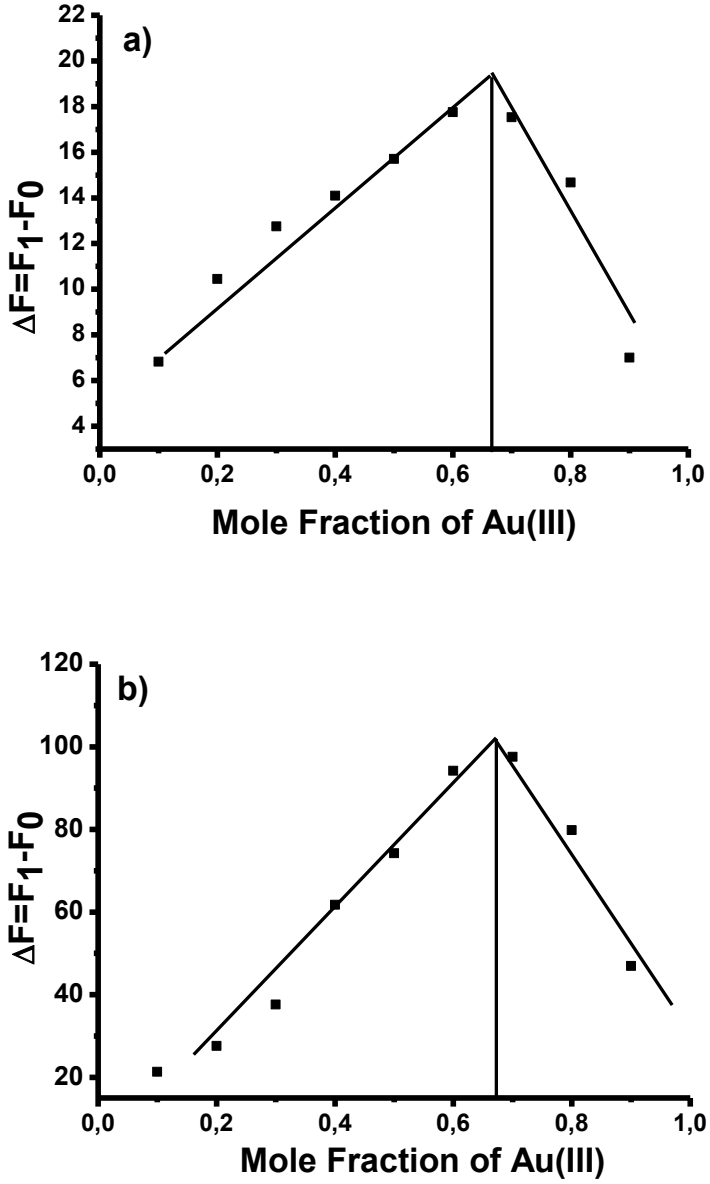


Figure S7: The Job's plot analysis between **BOD-Pyr** and Au^{3+} (a) in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (b) in ethanol. The total concentration of **BOD-Pyr** and Au^{3+} was kept constant at 20 µM (λ_{exc}=500 nm, λ_{em}= 577 nm at 25 ^{o}C).

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

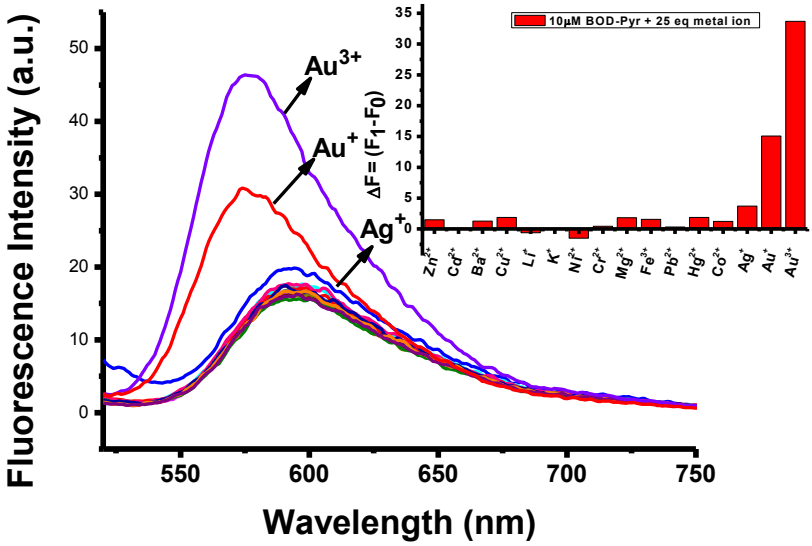


Figure S8: Fluorescence intensities of **BOD-Pyr** (10.0μM), **BOD-Pyr** (10.0μM) + Au^{3+} (60 μM, 6 equiv.), **BOD-Pyr** (10.0μM) + other ions (250μM, 25 equiv.) in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 500 nm, at 25 ^{o}C). Inset: Bar graph notation.

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

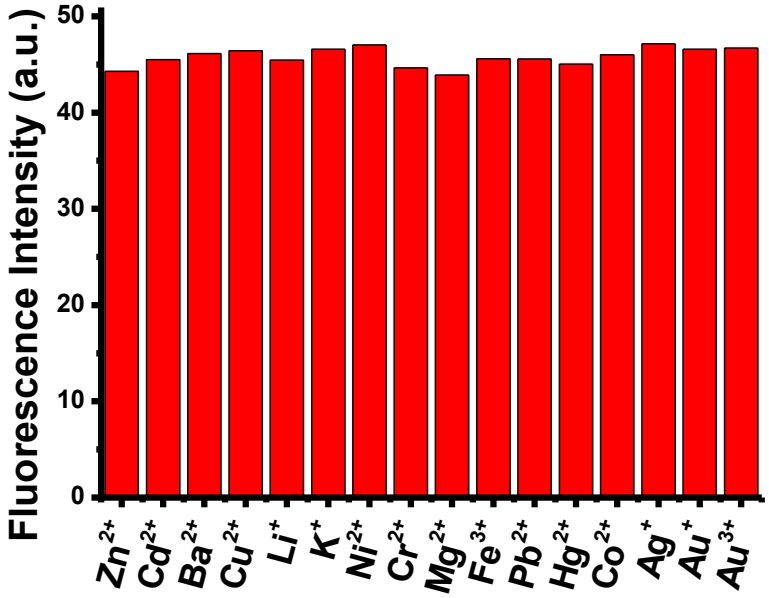


Figure S9: Fluorescence intensities of **BOD-Pyr** (10μM) in the presence of Au^{3+} (6 equiv.) and 10 equiv. of other metal ions in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:1) (λ_{exc}=500 nm, λ_{em}= 577 nm at 25 ^{o}C).

12. Determination of detection limit

In Water/EtOH (1/1, pH = 7.0)

The detection limit was calculated based on the fluorescence titration [1]. To determine the detection limit, the emission intensity of **BOD-Pyr** (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 2 – 7 μM (R = 0.9828). 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 4.0 μM.

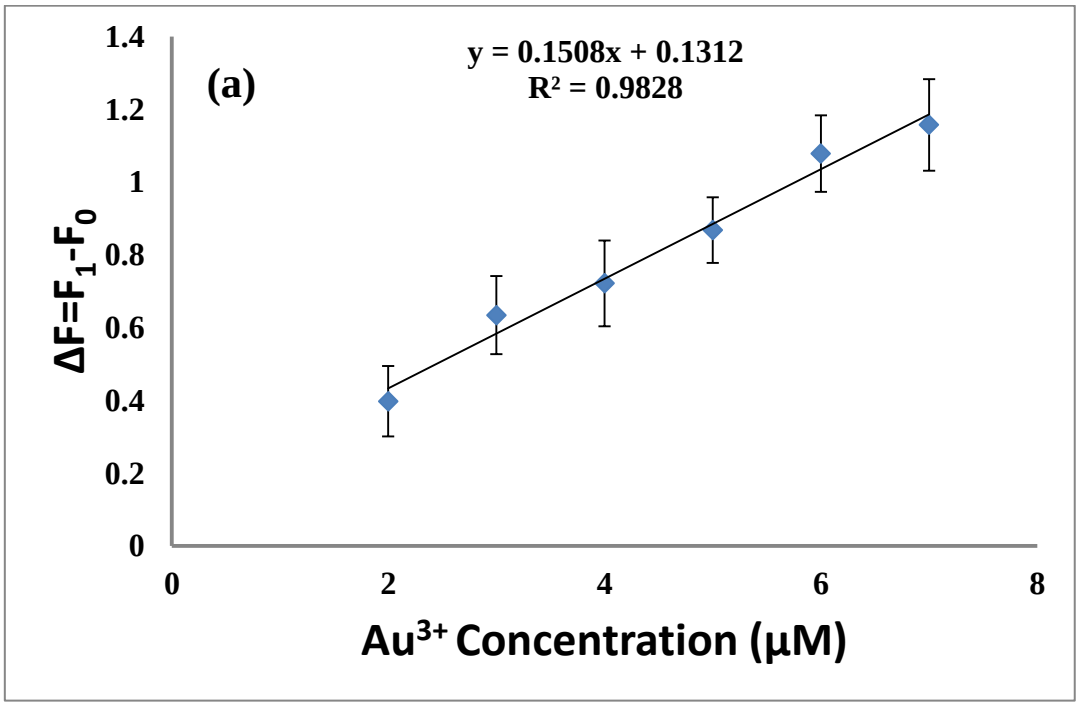


Figure S10: Fluorescence changes of **BOD-Pyr** (10.0 μM) upon addition of Au^{3+} (2 to 7 μM, 0. 2 to 0.7 equiv.) in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 500 nm, at 25 ^{o}C).

In Dichloroethane

Under the present conditions, a good linear relationship between the fluorescence intensity and Au^{3+} concentration could be obtained in the 0.05 – 1.0 μM (R = 0.9954). The detection limit was measured to be 63 nM.

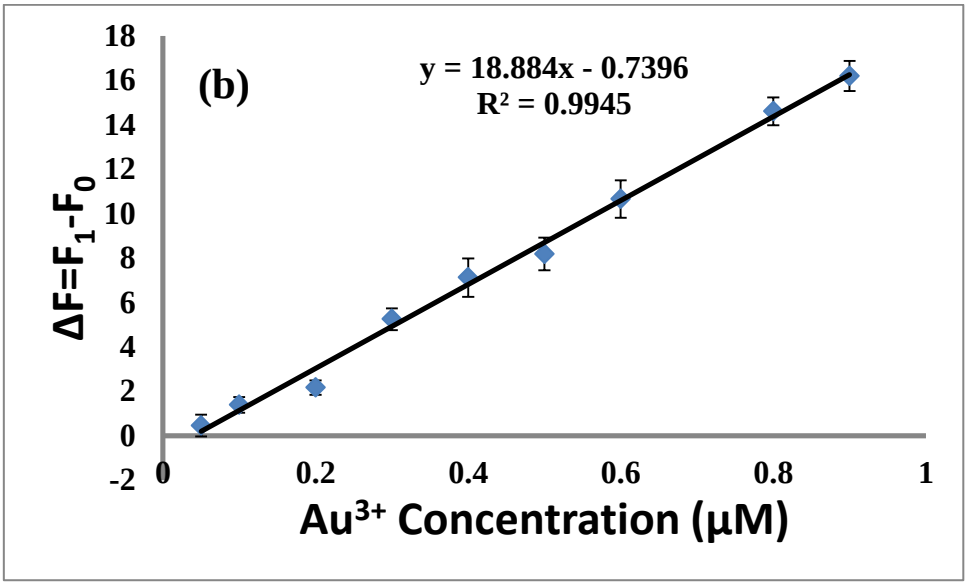


Figure S11: Fluorescence changes of **BOD-Pyr** (10.0 μM) upon addition of Au^{3+} (0. 05 to 1.0 μM, 0.005 to 0.1 equiv.) in dichloroethane (λ_{ex}: 500 nm, at 25 ^{o}C).

13. Reversibility of Au^{3+} sensing event

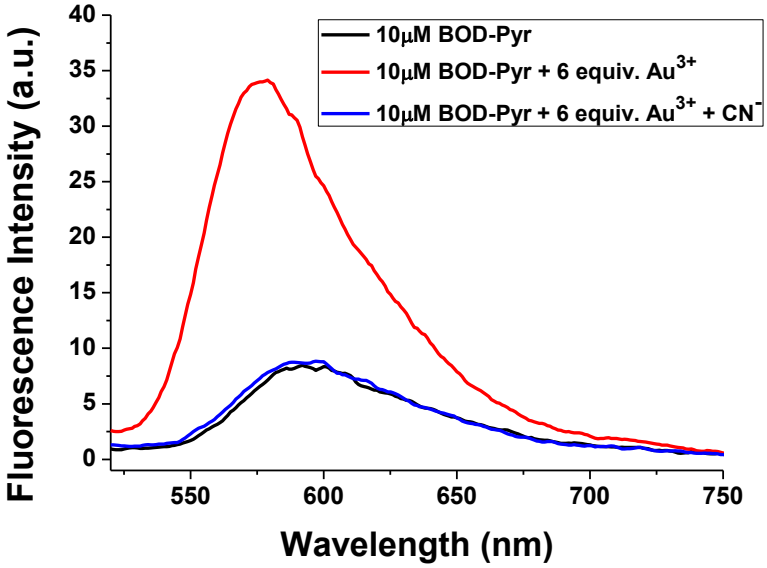


Figure S12: Fluorescence intensities of **BOD-Pyr** (10.0μM) (black), **BOD-Pyr** (10.0μM) + Au^{3+} (60 μM, 6 equiv.) (red), **BOD-Pyr** (10.0μM) + Au^{3+} (60 μM, 6 equiv.) + excess amount of CN^- (blue), in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 500 nm, at 25 ^oC).

14. Determination of the association constant and stoichiometry

The association constant of [Au^{3+}] was determined by using fluorescence titration data with the help of following equation;[4]

$$\ln[(F - F_0) / (F_{max} - F_0)] = n \ln[Au^{3+}] + n \ln(K_{asscn}) \quad \text{.....(1)}$$

where n is the number of gold ions associating with each molecule of **BOD-Pyr**, K_{assoc} is the association constant, F_0 is the fluorescence of the free probe, F_{max} is the fluorescence intensity at saturation point, and F is the fluorescence of probe obtained with Au^{3+} addition.

In Water/EtOH (1/1, pH=7.0)

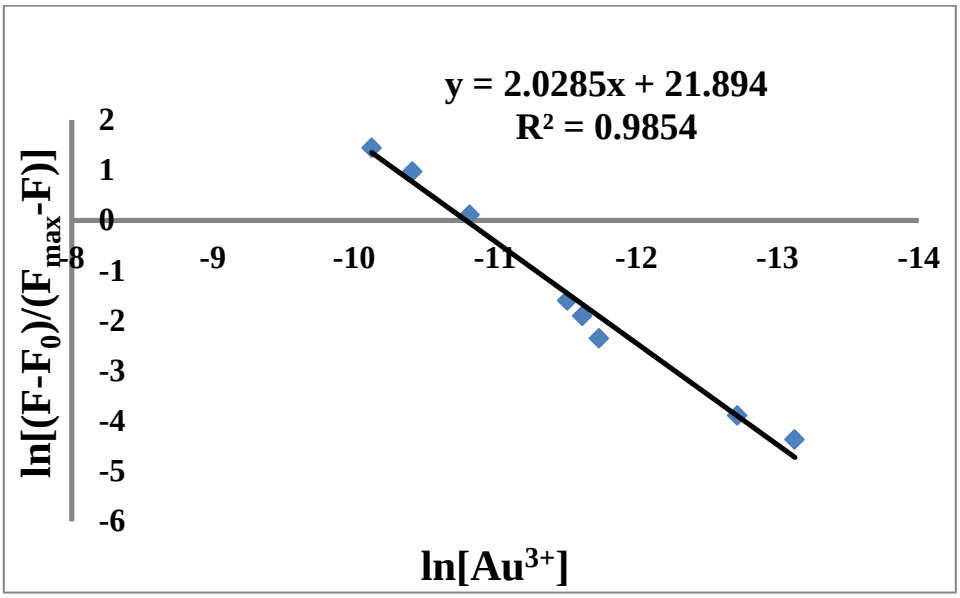


Figure S13: Plot of $\ln[(F-F_0)/(F_{max}-F)]$ against $\ln[Au^{3+}]$; the stoichimetry of **BOD-Pyr** Au^{3+} association, obtained directly from the slope, is 2.03 ≅ 2. Following equation 1, the intercept gave an association constant of **BOD-Pyr** as 4.9 x 10^4 M^{-2} (in 0.1M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:1) (λ_{ex}: 500 nm, at 25 ^oC).

In Dichloroethane

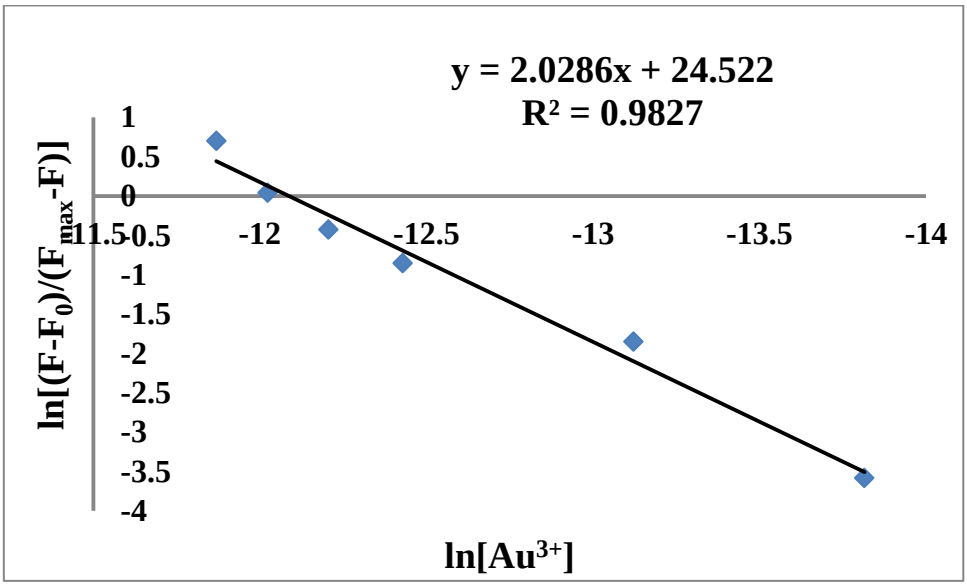


Figure S14: Plot of $\ln[(F-F_0)/(F_{max}-F)]$ against $\ln[Au^{3+}]$; the stoichimetry of **BOD-Pyr** Au^{3+} association, obtained directly from the slope, is 2.03 ≅ 2. Following equation 1, the intercept gave an association constant of **BOD-Pyr** as 1.8×10^5 M^{-2} (in dichloroethane).

15. ^{1}H NMR titration study of BOD-Pyr with Au^{3+}

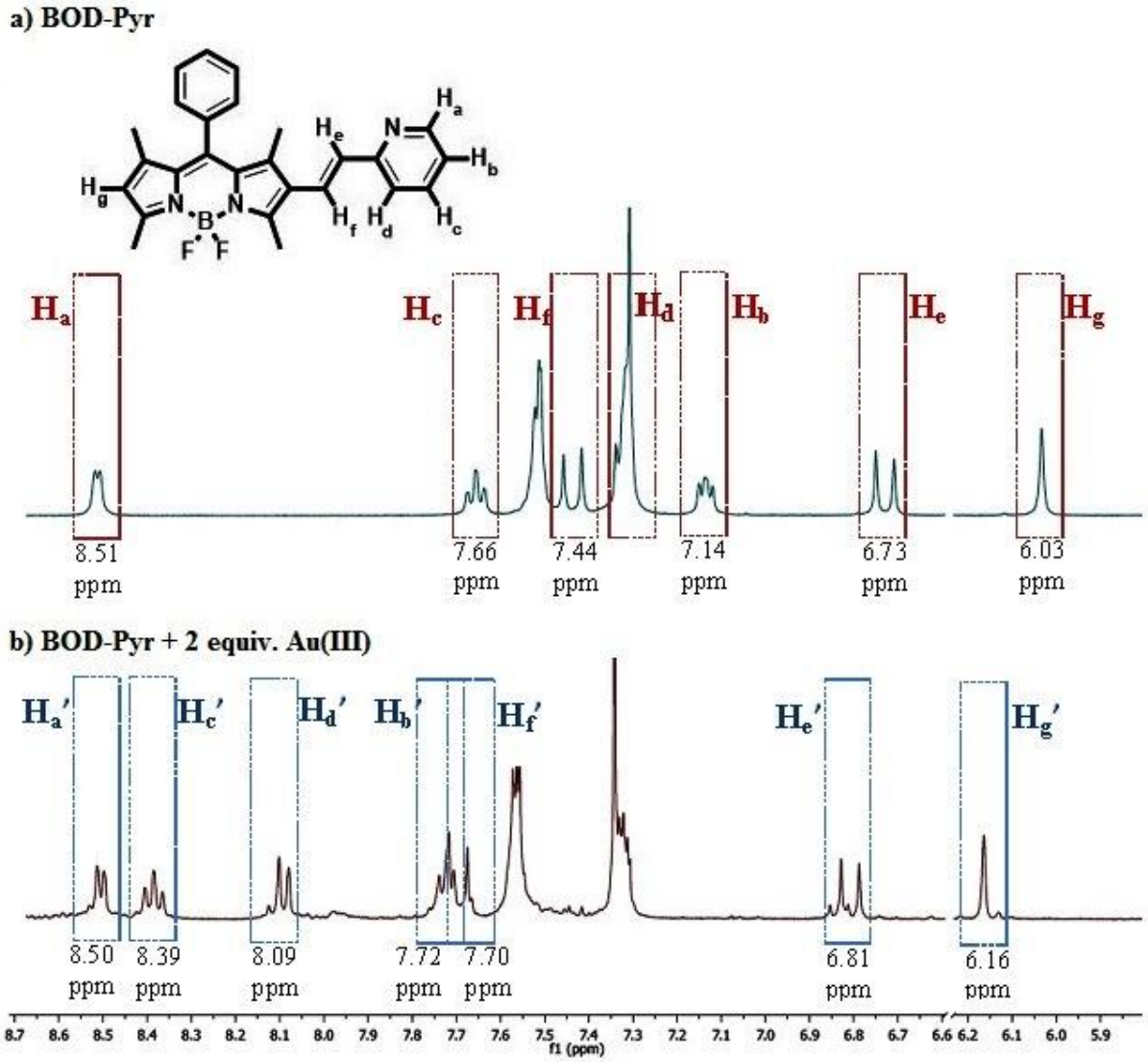


Figure S15: (a) ^{1}H-NMR of **BOD-Pyr** in chloroform-d1 (a drop of methanol-d4). **(b)** ^{1}H-NMR of **BOD-Pyr** + Au^{3+} (2 equiv.) in chloroform-d1 (a drop of methanol-d4).

16. Fluorescence titration of BOD-Pyr with Au(III) in dichloroethane

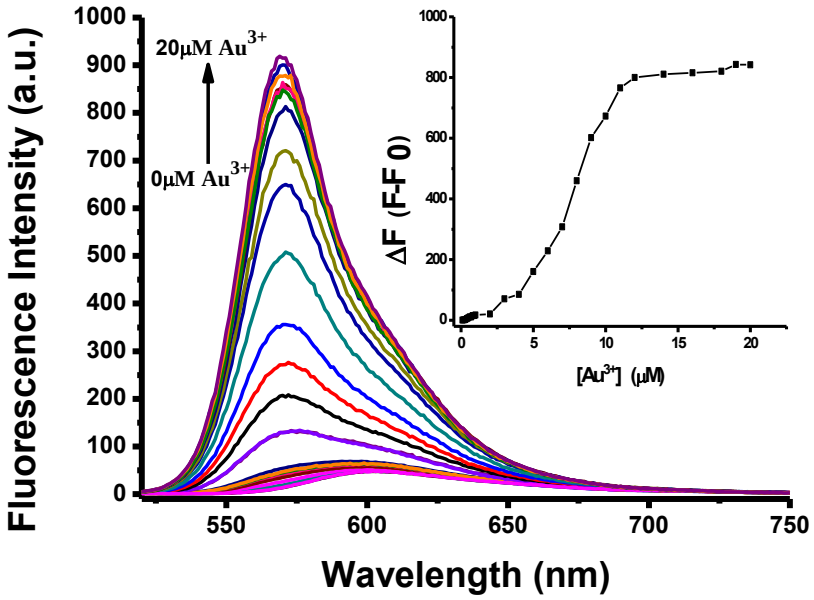


Figure S16: Fluorescence spectra of **BOD-Pyr** (10μM) in the presence of increasing concentrations of Au^{3+} (0-20μM, 0-2 equiv.) in dichloroethane (λ_{exc}=500 nm, λ_{em}= 570 nm at 25 ^{o}C). Inset: Calibration curve.

17. Quantitative detection of residual Au^{3+} content in compound B purified by silica gel chromatography

Detection of residual Au^{3+} content in compound B using BOD-Pyr

A sample of compound B (2.0 mg) was weighed and dissolved in 2.0 mL of in 0.1 M phosphate buffer/EtOH. Stock solution of **BOD-Pyr** solution was prepared (1.0×10^{-3} M) and 20 µL added into the solution containing compound B. The resulting solution was shaken at room temperature before recording the fluorescence spectra. Based on the standard calibration curve, the content of residual gold ions in compound B was determined as 1.8×10^{-8} mol mg^{-1}

Detection of residual Au^{3+} content in compound B using inductively-coupled plasma mass spectroscope (ICP-MS)

A sample of compound B (2.0 mg) prepared in 2.0 mL aqua regia (HNO_3: HCl, 1:3 v/v) and incubated 30 min. Then, degradation process was applied with Cem Mars X microwave instrument. The solution was completed to 4.0 mL with deionized water. The resulting solution was subjected to ICP-MS analysis. A standard calibration curve was acquired with the known concentration of Au^{3+} solutions. The measurement was conducted in triplet. The residual gold ions in compound B were measured as 1.27×10^{-8} mol mg^{-1}.

18. Sensitivity in various organic solvents

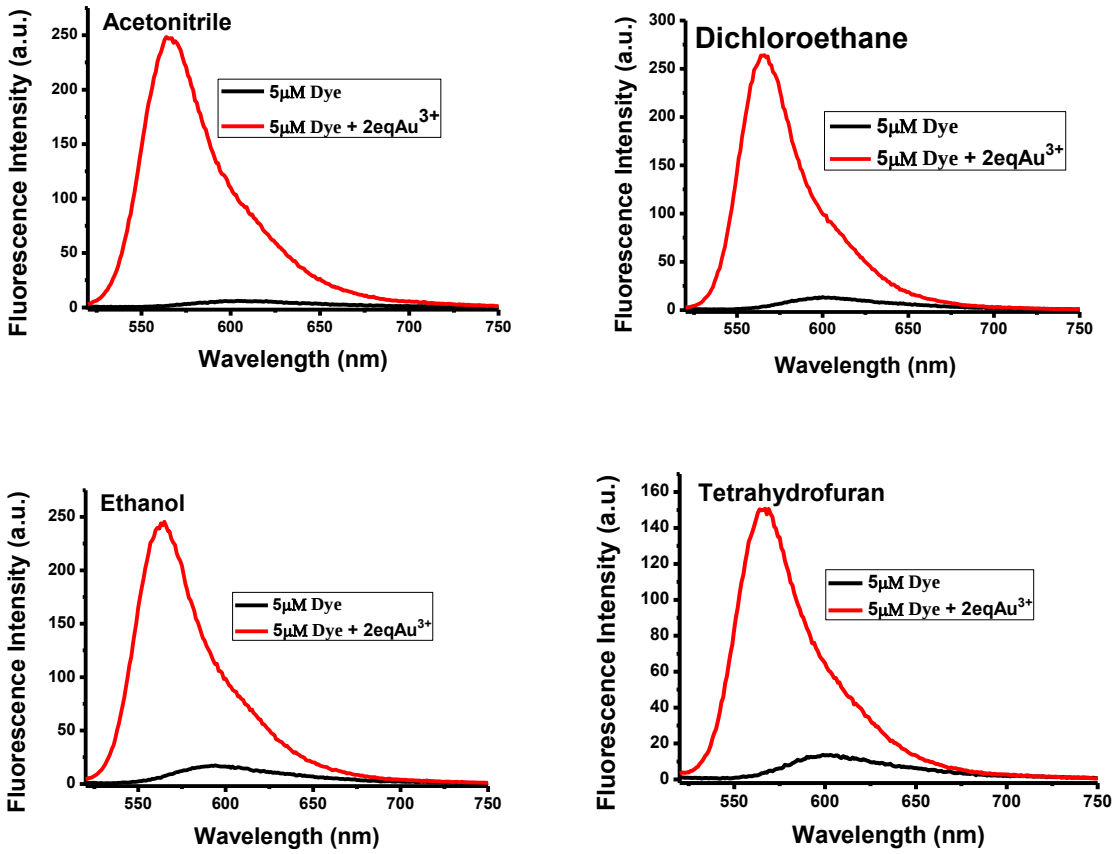


Figure S17: Fluorescence intensities of **BOD-Pyr** (10.0μM) (black), **BOD-Pyr** (5.0μM) + Au^{3+} (10 μM, 2 equiv.) (red) in various organic solvents. (λ_{ex}: 500 nm, at 25 ^{o}C).

19. Determination of quantum yields

Fluorescence quantum yields of **BOD-Pyr** were determined by using optically matching solutions of Rhodamine 6G (Φ_F=0.95 in water) as a standard [6]. The quantum yield was calculated according to the equation;

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

Solvent	**Ɛ (only dye) $M^{-1}xcm^{-1}$**	**Quantum yield (only dye)**	**Ɛ (dye + Au^{3+}) $M^{-1}xcm^{-1}$**	**Quantum yield (dye + Au^{3+})**
Acetonitrile	21063	1,7	37901	48,1
Dichloroethane	18325	3,8	44112	76,9
Ethanol	21409	5,3	37333	33,2
Tetrahydrofuran	17166	4,1	38947	24,3

Table S1: Quantum yields in different organic solvents.

20. Absorbance and fluorescence spectra of BOD-Pyr at various pH values

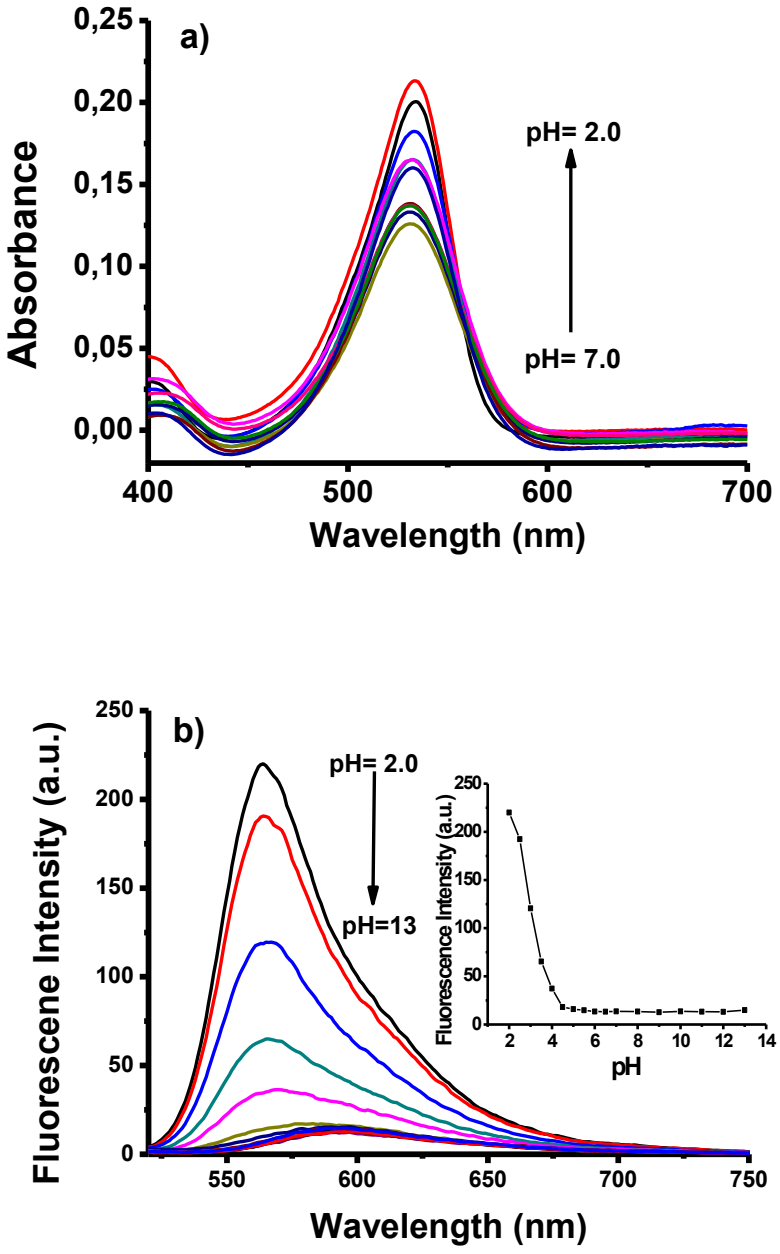


Figure S18: (a) Absorbance and **(b)** fluorescence spectra of **BOD-Pyr** (10 µM) in 0.1 M phosphate buffer/EtOH (v/v, 1:1) at various pH values (2.0-13.0) (λ_{exc}=500 nm, 25 ^{o}C). Inset: The variation of fluorescence intensity at 564 nm of **BOD-Pyr** with pH (2.0-13.0).

21. Reversibility of pH Sensing

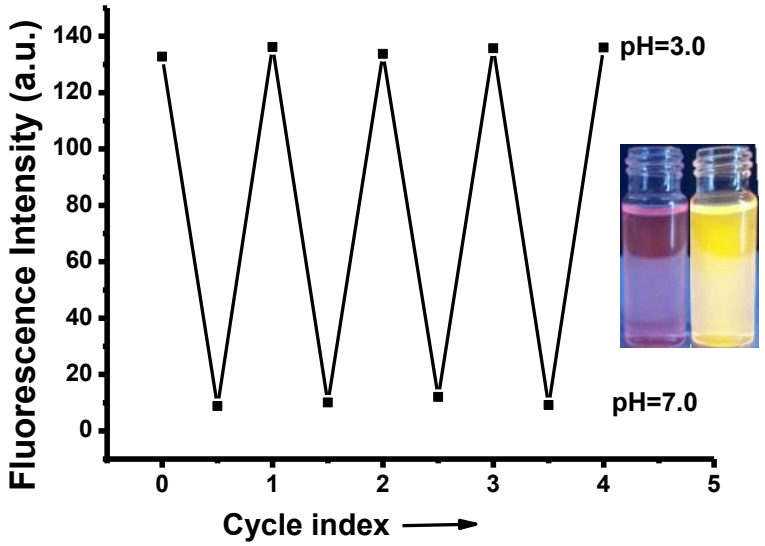


Figure S19: pH reversibility study of **BOD-Pyr** (10μM) between pH 7.0 and 3.0 in 0.1 M phosphate buffer/EtOH (v/v, 1:1) (λ_{exc}=500 nm, λ_{em}=564 nm, 25 ^{o}C). Inset: Fluorescence photographs of **BOD-Pyr** at pH 7.0 (left) and 3.0 (right) under illumination with 365 nm light.

22. Determination of pKa of BOD-Pyr

The acidity constant pK_a of **BOD-Pyr** was calculated by using fluorescence titration method in water:ethanol (1:1, v/v) solvent system at various pHs. The linear response was obtained within the range of pH from 2.5 to 6.0 and with the help of a Henderson–Hasselbalch type equation [5];

$$\log \frac{(F_{max} - F)}{(F - F_{min})} = \text{pH} - \text{pK}_a \quad \text{---------- Eq 2}$$

where, F_{min} is the fluorescence of the free probe, F_{max} is the fluorescence intensity at saturation point, and F is the fluorescence of probe obtained at different pHs, pK_a value was determined as 3.06 ± (0.14) for **BOD-Pyr**.

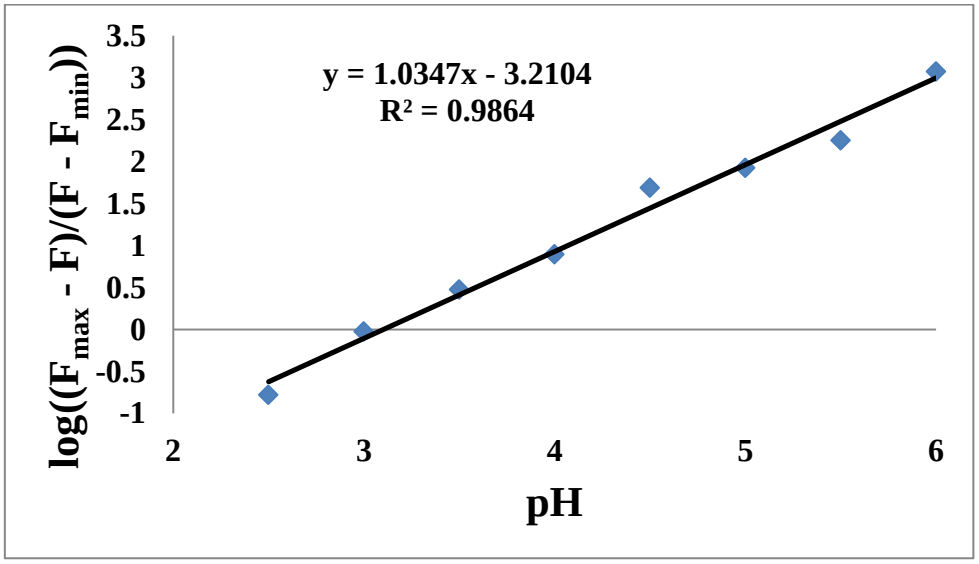
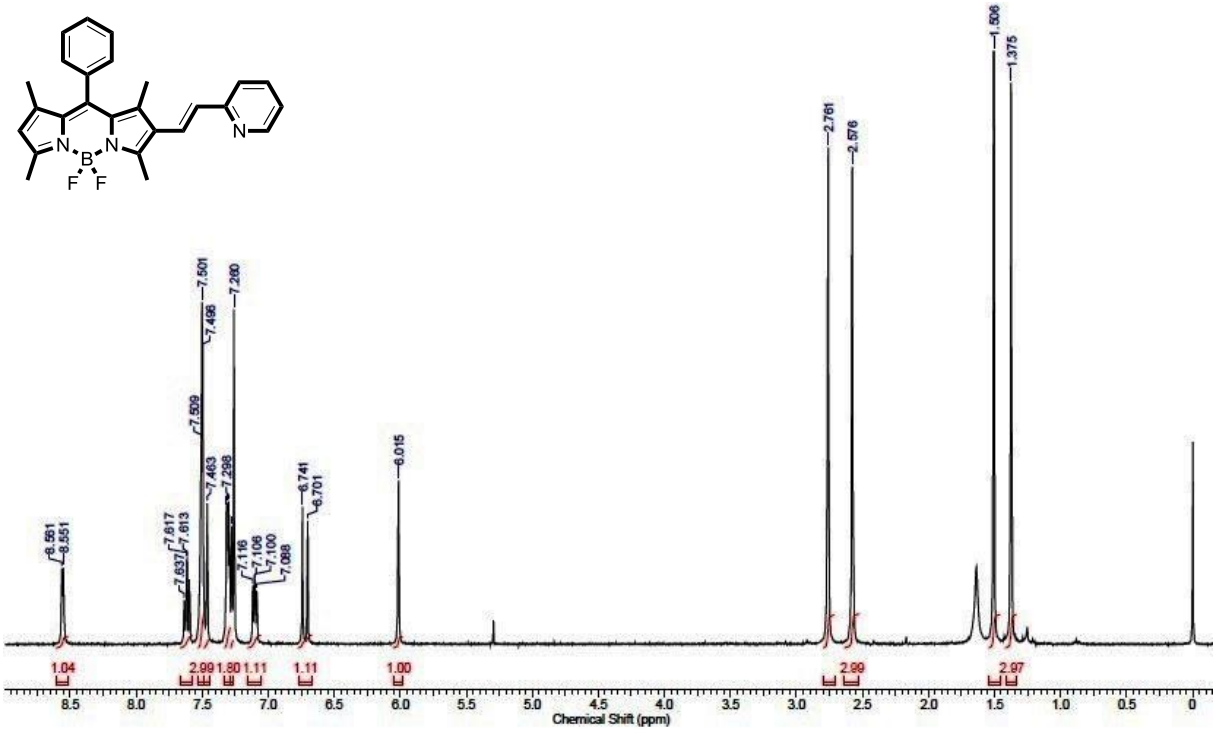
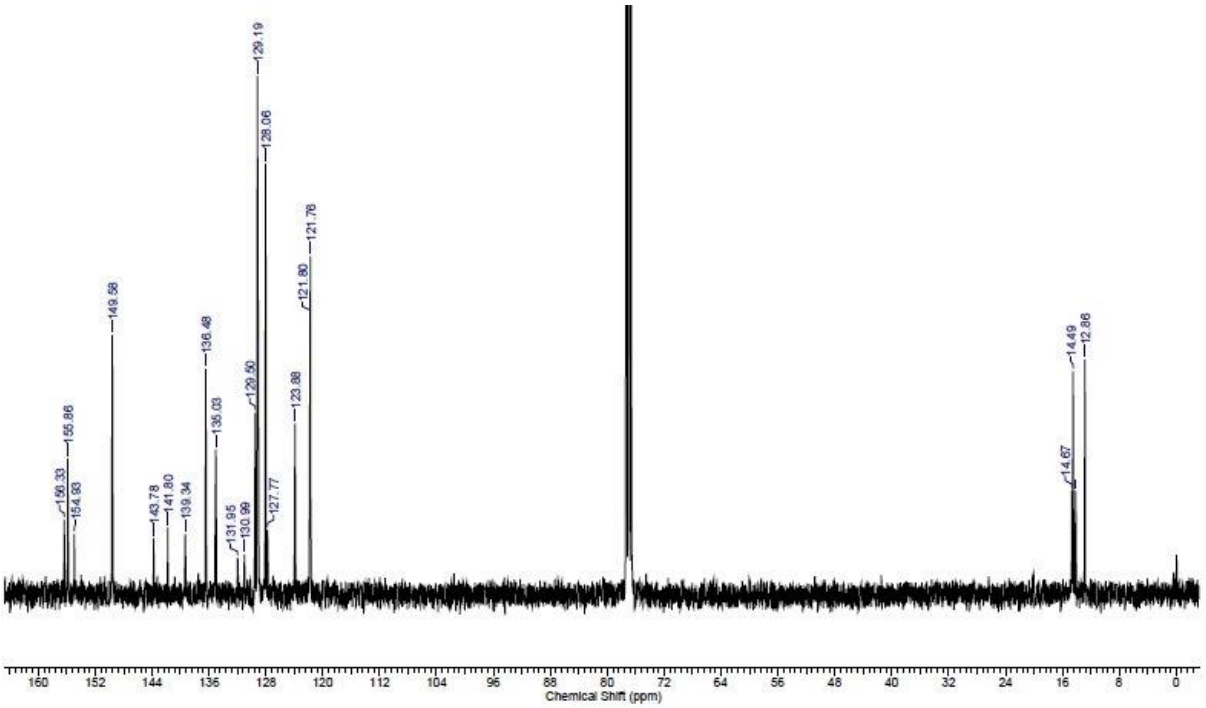


Figure S20: Plot of $\log((F_{max} - F)/(F - F_{min}))$ as a function of pH for determination of pK_a of **BOD-Pyr** (λ_{ex}: 500 nm, at 25 ^{o}C).

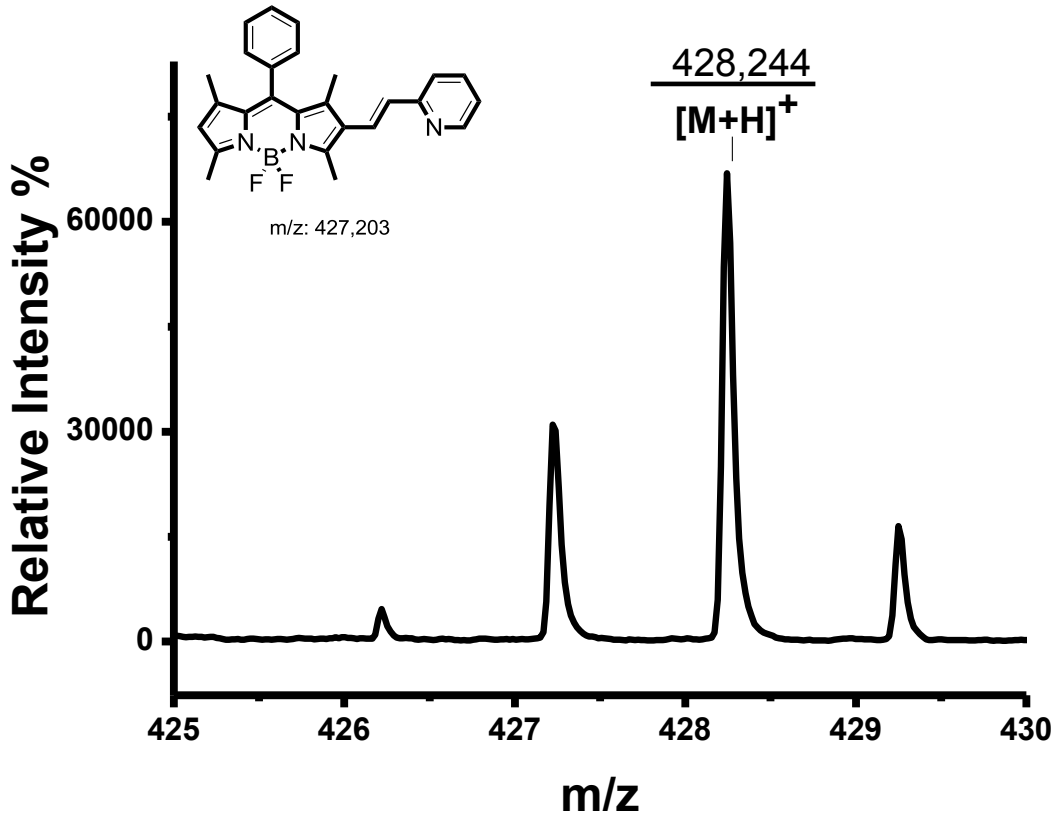
23. ^{1}H NMR of BOD-Pyr



24. ^{13}C NMR of BOD-Pyr



25. MALDI-TOF-TOF MS of BOD-Pyr



26. References

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