Supporting Information for

Modulation in Charge Transfer Characteristics of Flexible Fluorescent Bis-Benzimidazole Probes: Independent Sensing Mechanisms for Hg^{2+} and F^{-}

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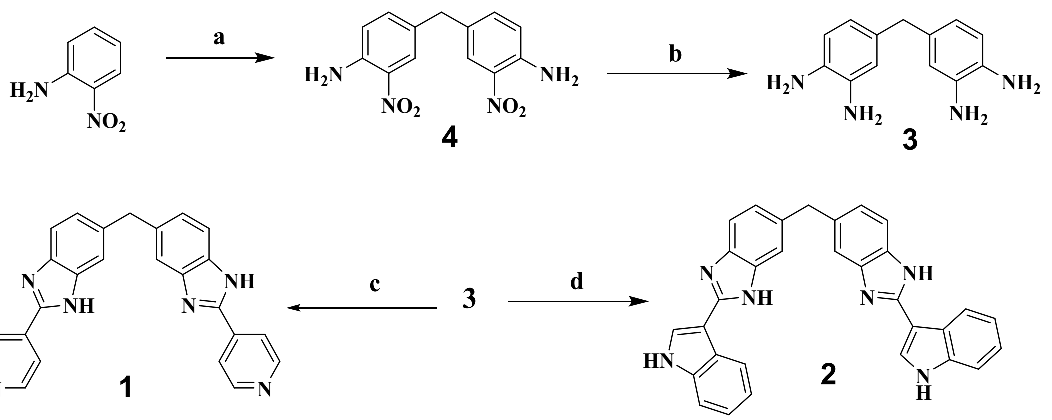
Synthesis and Characterization



Reaction conditions. (a) HCHO, HCl, reflux, 5 h (b) Zn powder, NaOH, reflux. (c) 4-Pyridinecarboxaldehyde, $Na_2S_2O_5$, reflux. (d) indole-3-carboxaldehyde, $Na_2S_2O_5$, reflux

Di(4-amino-3-nitrophenyl)methane (4). 2 g (14.49 mmol, 1 eq.) of 2-Nitroaniline and 218 mg (7.27 mmol, 0.5 eq.) of p-Formaldehyde were heated in 25 % HCl (32ml) for 5 h. The orange-yellow solution was then poured into water (100ml) and neutralized with 25 % aqueous ammonia (pH 10). The precipitate was filtered and washed with water and dried under vacuum to give a yellow-orange solid. **^{1}H-NMR ($CDCl_3$, 400 MHz):** ppm: 7.93 (s, 1H); 7.16 (d, 1H,); 6.75 (d, 1H,); 3.81 (s, 1H). **HRMS:** m/z = 289.0939[M+H]$^+$, (Calcd. : 289.10)

Di(3,4-diaminophenyl)methane (3). Compound 1 (500 mg, 1.73mmol, 1 eq.) was mixed with 1.15 g of Zn powder and refluxed in ethanol under N_2. To this 1 ml of 0.1M NaOH solution was added drop wise under vigorous stirring. After 1h the orange solution turned to pale yellow, the refluxing the reaction mixture was continued for another 1h and then was filtered. After that solvent was evaporated in vacuum and poured into water. The solid was filtered and washed thoroughly until neutral pH and dried under vacuum. Due to instability of the tetra-amine, it is used directly without further purification.

Compound 1. 4-pyridinecarbaxaldehyde (300 mg, 2.8 mmol, 2 eq.) was dissolved in 20 ml of ethanol and to it 264 mg $Na_2S_2O_5$, dissolved in water, was added, and stirred for 15 minute and to this 320 mg (1.4mmol, 1 eq.) of compound **3** was added and refluxed for 24 h. The pure product (~62 %) was isolated by neutral alumina column chromatography with 2.5-3.5 % MeOH/$CHCl_3$ as eluent. **^{1}H-NMR (DMSO-*d*$_6$, 400 MHz):** δ ppm: 13.136, 13.081 (broad, 2H, NH); 8.728 (d, *J* = 5 Hz, 4H); 8.044 (t, 4H); 7.629 (t, 2H); 7.491 (dd, *J* = 7.76, 4.2 Hz, 1H); 7.418 (s, 1H); 7.187 (dd, *J* = 8.7, 4.28 Hz, 2H); 4.206 (t, 2H). **^{13}C-NMR (DMSO-*d*$_6$, 400 MHz):** δ ppm: 150.8, 149.1, 144.4, 142.6, 137.6, 135.7, 133.9, 125.3, 124.2, 119.6, 111.8, 41.8. **HRMS:** m/z= 403.1669 [M+H]$^+$ (Calcd. : 403.1671)

Compound 2. 300 mg (0.62 mmol, 2 eq.) of indole-3-carbaxaldehyde was dissolved in 20 ml of ethanol and to it 264 mg $Na_2S_2O_5$, dissolved in water, was added and stirred for 15 minute and to this 70 mg

(0.31 mmol, 1 eq.) of compound **3** was added and refluxed for 24 h. The pure product (~70 %) was isolated by neutral alumina column chromatography with 2.5 % MeOH/$CHCl_3$ as eluent.

^{1}H-NMR (DMSO-*d*$_6$, 400 MHz): δ ppm: 12.24 (broad, 2H, NH); 11.55 (s, 2H, NH); 8.47 (d, *J* = 7.2 Hz, 2H); 8.07 (d, *J* = 2 Hz, 2H); 7.47 (d, *J* = 6.8 Hz, 4H); 7.17 (m, 4H); 7.06 (dd, *J* = 1.2, 8, 2H); 4.157 (s, 2H), **^{13}C-NMR (DMSO-*d*$_6$, 400 MHz):** δ ppm: 147.8, 136.9, 135.7, 126.3, 125.6, 122.8, 122.6, 121.8, 120.6, 112.3, 107.2, 79.6, 41.9, **HRMS:** m/z= 479.1987 $[M+H]^+$ (Calcd. : 479.1984)

Experimental Details

Materials and Methods. All necessary chemicals (precursors, starting materials, reagents, and solvents) were purchased from best-known suppliers and used as received. FT-IR spectra were recorded on a PerkinElmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-400 Advance NMR spectrometer. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane. Mass spectra were recorded on a Micromass Q-TOF Micro TM spectrometer.

Sampling Procedure of Sensing. The sensing studies with metal ions (added in the forms of nitrate and perchlorate salts in water and acetonitrile medium respectively) was carried out by adding 10 μL DMSO solution of **1** and **2** from stock (1×10^{-3} M) in acetonitrile or water medium to make the final volume of 1 mL (conc. = 1×10^{-5} M) followed by addition of DMSO or water solutions of metal ions respectively. Similarly for anion sensing studies, we have involved anions in the forms of either potassium (in water medium) or tertbutyl ammonium salts (in acetonitrile medium). In all cases, the final concentration of DMSO in the solution did not exceed 1%.

UV−Vis and Fluorescence Spectroscopy. The UV−vis and fluorescence spectroscopy were recorded on a Shimadzu model 2100 spectrometer and Cary Eclipse spectrofluorimeter respectively. The slit-width for the fluorescence experiment was kept at 5 nm (excitation) and 5 nm (emission) and the excitation wavelength was set at 320 nm.

Dynamic Light Scattering Studies (DLS). DLS measurements were done using a Malvern Zetasizer NanoZS particle sizer (Malvern Instruments Inc., MA) instrument. Samples (**1** with and without Hg^{2+}) were prepared and examined under dust free conditions. Reported mean hydrodynamic diameters were obtained from Gaussian analysis of the intensity weighted particle size distributions.

Detection limit determination. The method used for the calculation of the detection limit is known as the blank variation method. In this method, the calibration curve was prepared by fluorescence titration of **1** (10 μM) with Hg^{2+} and F^{-} ions in CH_3CN medium. The fluorescence signals of the compound without the added Hg^{2+} or F^{-} were considered as blank reading. The standard deviation value was calculated from the blank readings and fluorescence titration data. Using this standard deviation value, we calculated limit of decision by this following equation.

$L_C = t_C \times s \times (1 + 1/N)^{1/2}$……………….....………………………………….. (1)

where, N = the number of blank replicates taken; the value of tc for 10 blank readings is 1.833; and s = the standard deviation value. The detection limit (L_D) was calculated as the double of the decision limit obtained,

$L_D = 2\ L_c$ ……………………………………………………………….....…. (2)

^{1}H NMR Titration Studies. ^{1}H NMR titration studies with probe **1** was performed upon dissolving **1** (5 mM) in $DMSOd_6$ medium. To that Hg^{2+} and F- were added independently gradually added (- 1.2 equiv.) and the spectra were recorded using identical parameters. The chemical shifts have been represented as 'δ ppm'.

Scanning Electron Microscopy: Solution of **1** (concentration 10 μM) in water medium with and without Hg^{2+} were drop cast over double-sided tapes attached onto the brass stubs and air-dried for 48 h. The samples were then coated with gold vapor and analysed on a Quanta 200 SEM operated at 15 kV.

Stoichiometry determination by Job plot: The Job plot is a method of continuous variation for determining the stoichiometry of interaction between the two species. The total molar concentration of the two binding species (here, **1** and Hg^{2+} and/or F^{-} ions) was kept constant (1×10^{-4} M) and the mole fraction was varied. Further the change in absorbance was plotted against the mole-fraction. The maxima or minima thus obtained gave the stoichiometry of interaction. In all cases, we have plotted ΔA*[analyte] vs [**1**] / {[analyte] + [**1**]}. Where, $\Delta A = A - A_0$, A = absorbance of probe molecule after addition of analyte at specific wavelengths and A_0 = absorbance of probe molecule without the analyte. [**1**] / {[analyte] + [**1**]} is the mole fraction of probe molecule in the mixture, analyte = Hg^{2+} and F^{-} etc.

Additional Spectral Data

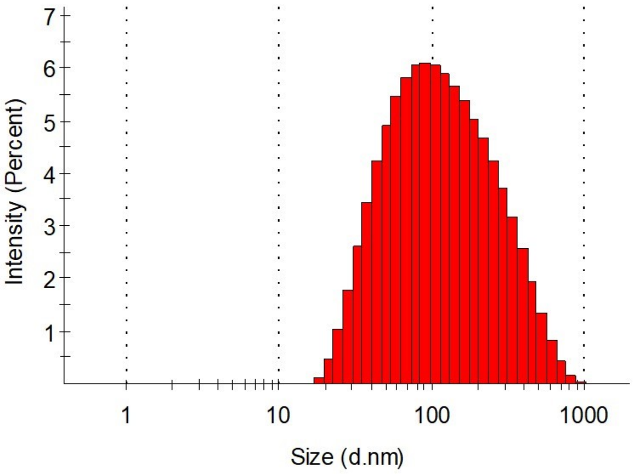


Figure S1. Dynamic light scattering experiment of compound **1** (10 μM) in water medium.

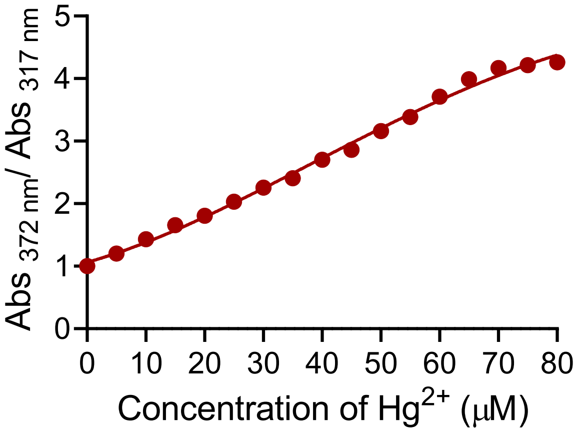


Figure S2. Ratiometric Changes in the absorbance of compound **1** (10 μM) upon addition of Hg^{2+} (80 μM) in CH_3CN medium.

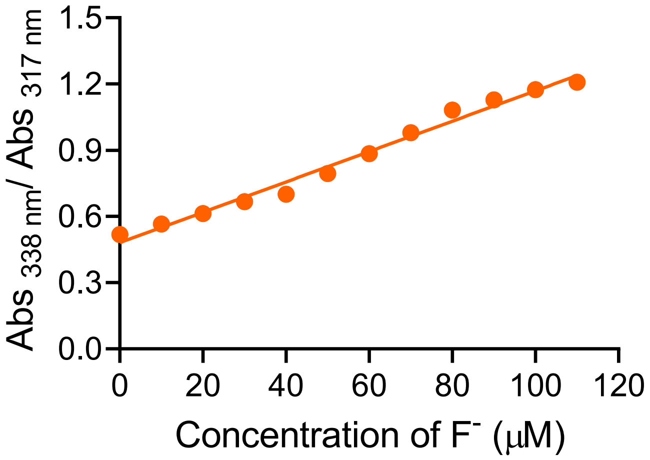


Figure S3. Ratiometric Changes in the absorbance of compound **1** (10 μM) upon addition of F^- (110 μM) in CH_3CN medium.

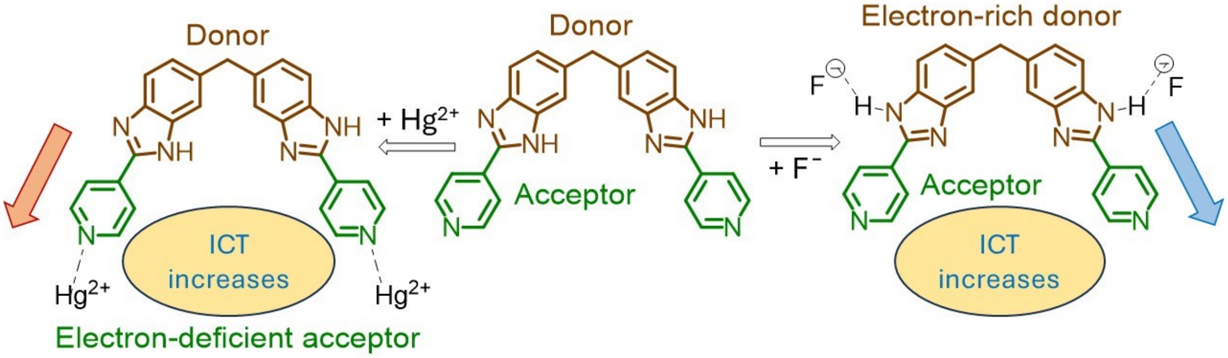


Figure S4. Schematic diagram shows interaction of compound **1** with Hg^{2+} and F^- ion.

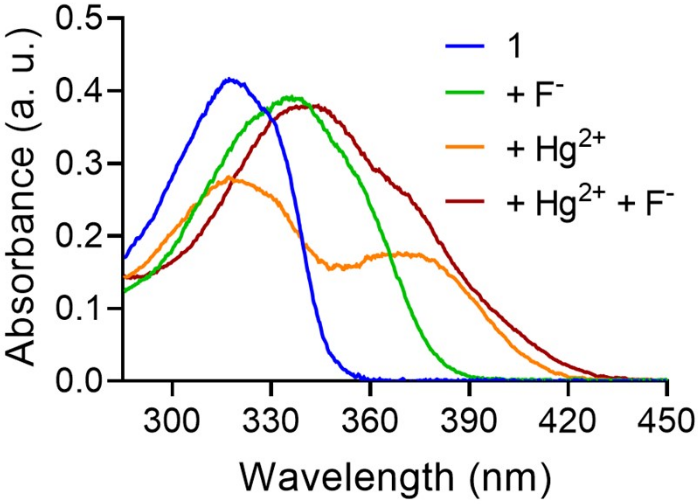


Figure S5. Fluorescence spectra of compound **1** (10 μM, λex = 320 nm) with Hg^{2+} (80 μM) and F^- (110 μM) when added individually and as a 1:1 mixture.

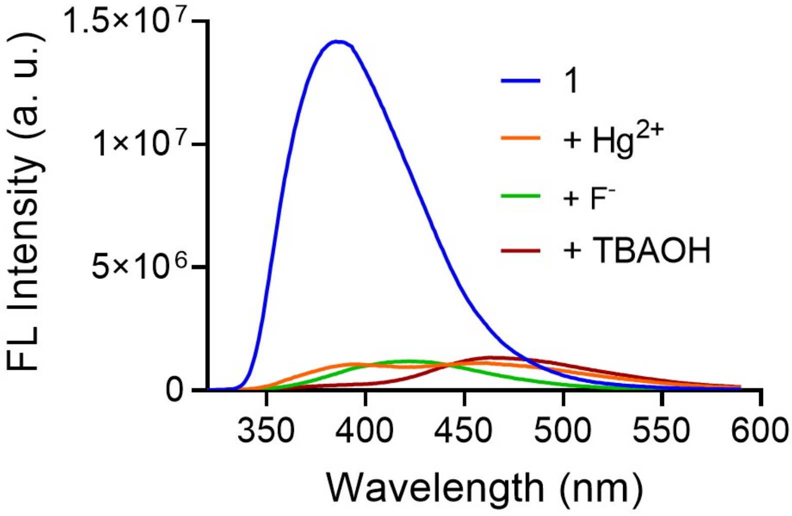


Figure S6. Fluorescence spectra of compound **1** (10 µM, λex = 320 nm) with Hg^{2+} (80 µM), F^{-} (110 µM) and TBAOH (110 µM) in acetonitrile medium.

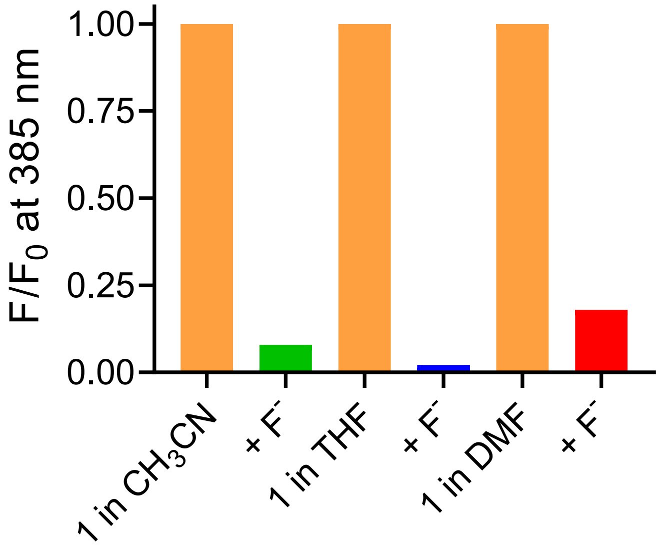


Figure S7. Changes in fluorescence intensity of compound **1** (10 µM, λex = 320 nm) upon addition of F^{-} (110 µM) ion in different organic medium.

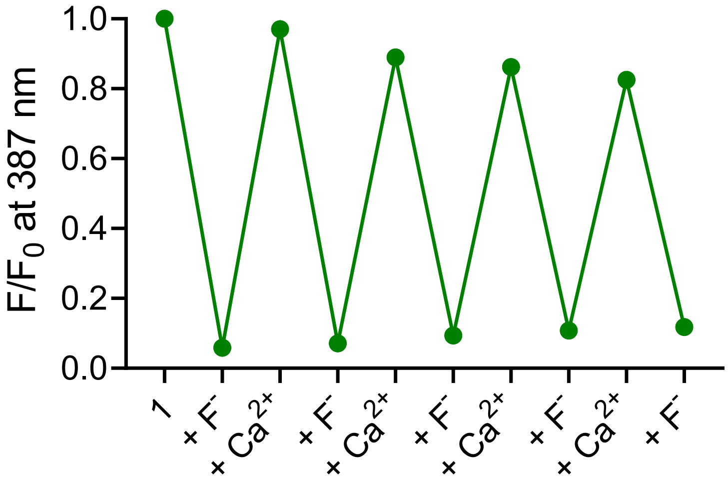


Figure S8. Changes in fluorescence intensity of **1** upon sequential addiions of Ca^{2+} (80 µM) and F^- (110 µM) ion in CH_3CN medium.