Photoactivated ratiometric copper (II) ion sensing with semiconducting polymer dots

Pei-Jing Wu, [‡] *Jia-Lin Chen,* [‡] *Chuan-Pin Chen, and Yang-Hsiang Chan**

Department of Chemistry, National Sun Yat-sen University, 70 Lien Hai Road,
Kaohsiung, Taiwan 80424

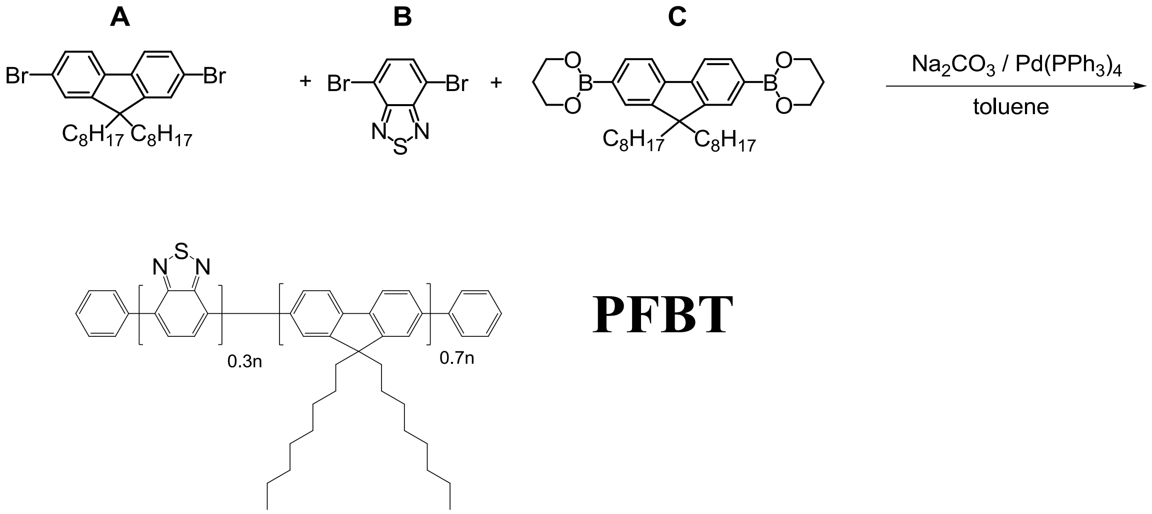
E-mail: yhchan@mail.nsysu.edu.tw

Supporting Information

Materials. The following inorganic salts were purchased from Sigma-Aldrich and used as received: CO-520, PPE, 2,7-dibromofluorene, sodium hydroxide, 9,9-Dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester, 4,7-Dibromo-2,1,3-benzothiadiazole, tetrahydrofuran (THF; anhydrous, ≥ 99.9%, inhibitor-free), toluene, tetrabutylammonium bromide (Bu_4NBr), phenylboronic acid, bromobenzene, methanol, tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$), dichloromethane (CH_2Cl_2), 4,7-Dibromobenzo[c]-1,2,5-thiadiazole, 9,9-Dihexylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester, sodium carbonate, copper(II) sulfate, potassium chloride, sodium chloride, nickel(II) sulfate, iron(II) sulfate, magnesium(II) sulfate, cobalt(II) sulfate, calcium sulfate, zinc sulfate, and lead sulfate. High purity water (18.2 MΩ•cm) was used throughout the experiment.

Synthesis of PFBT.

Scheme S1.

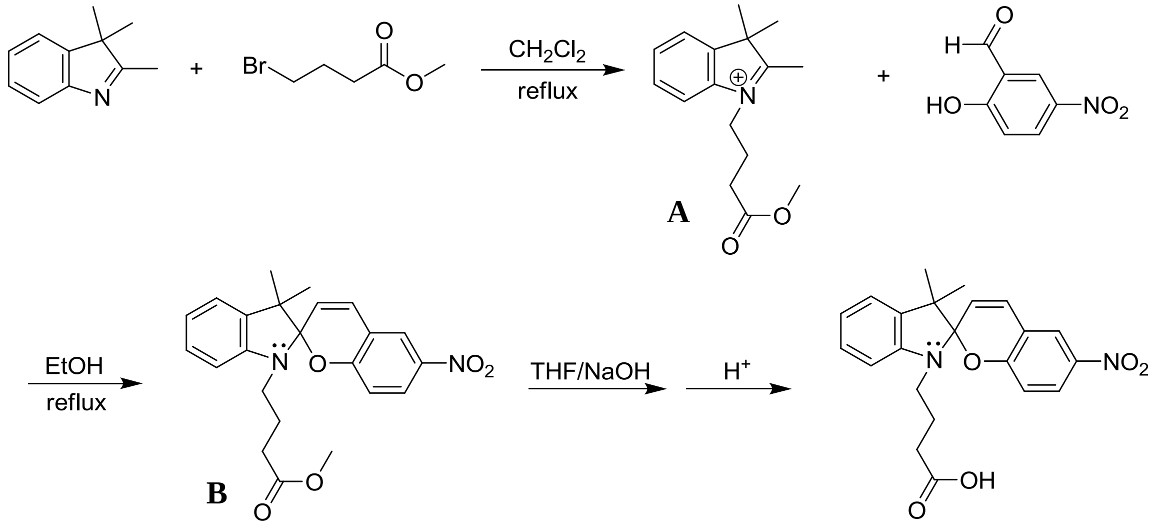


PFBT

PFBT Polymer: Polymers were synthesized by copolymerization of Monomers **A**, **B**, and **C** by Suzuki coupling. In a 100 mL flask, monomer **A** (0.4 mmol), **B** (0.6 mmol), and **C** (1 mmol) were dissolved in toluene (20 mL), and then Bu_4NBr (0.04 mmol, 12.5 mg) and Na_2CO_3 (2M, 12 mL) was added. The mixture was degassed and refilled with N_2 (repeated 4 times) before and after addition of $Pd(PPh_3)_4$ (0.035 mmol, 55 mg). The reactants were stirred at 90°C for 40 hours and phenylboronic acid (100 mg) dissolved in THF (1 mL) was added. After two hours, bromobenzene (1 mL) was added and further stirred for 3 hours. The mixture was poured into methanol (250 mL). The precipitate was filtered, washed with methanol, water, and acetone to remove monomers, small oligomers, and inorganic salts. The crude product was dissolved in CH_2Cl_2 (15 mL), filtered through 0.2μm membrane, and re-precipitated in methanol (150 mL). The powder was then stirred in acetone (200 mL) for 4 hours and then collected by filtration, and dried in vacuum. Yield: 402 mg (74%). M_n: 23290, M_w: 79396, PDI: 3.40.

Synthesis of Spiropyran Functionalized with Carboxyl Group.

Scheme S2.



Spiropyran Functionalized with Carboxyl Groups: A solution of 2,3,3-trimethylindolenine (7.72 g) and methyl 4-bromobutyrate (8.70 g) in 20 mL of acetonitrile was refluxed for 24 h. After reaction, the solvent was evaporated, and the residue was washed with copious diethyl ether and recrystallized from a mixture of diethyl ether and methanol (15:1) to give 11.7 g (72% yield) of the pink solid as the quaternary ammonium salt **A**. A solution of A (11.7 g) in absolute ethanol (200 mL) was heated to 70 ^{o}C and another solution of 5-nitro-salicylaldehyde (5.2 g) in 30 mL of ethanol was added slowly with vigorous stirring. The reaction mixture was heated at reflux for 12 h and then cooled to -20 ^{o}C for 1h. The residue was filtered and washed thoroughly with cold ethanol to obtain the crude product **B**. The crude product was then suspended in $CHCl_3$ and extracted with water. The organic layer was separated, dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield 4.3 g of **B**. 2 g of Compound **B** was dissolved in 40 mL of THF and then 10 mL of 10% NaOH was added. The reaction mixture was stirred in the dark under nitrogen atmosphere for 40 h. After that, the reaction mixture was added 100 mL of 10% citric acid and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 10% sodium bisulfide, brine, and dried over $MgSO_4$. Removal of the solvent under reduced pressure afforded 1.3 g of spiropyran functionalized with carboxyl groups. Figure S1 shows the ^{1}H NMR (δ, $CDCl_3$) data.

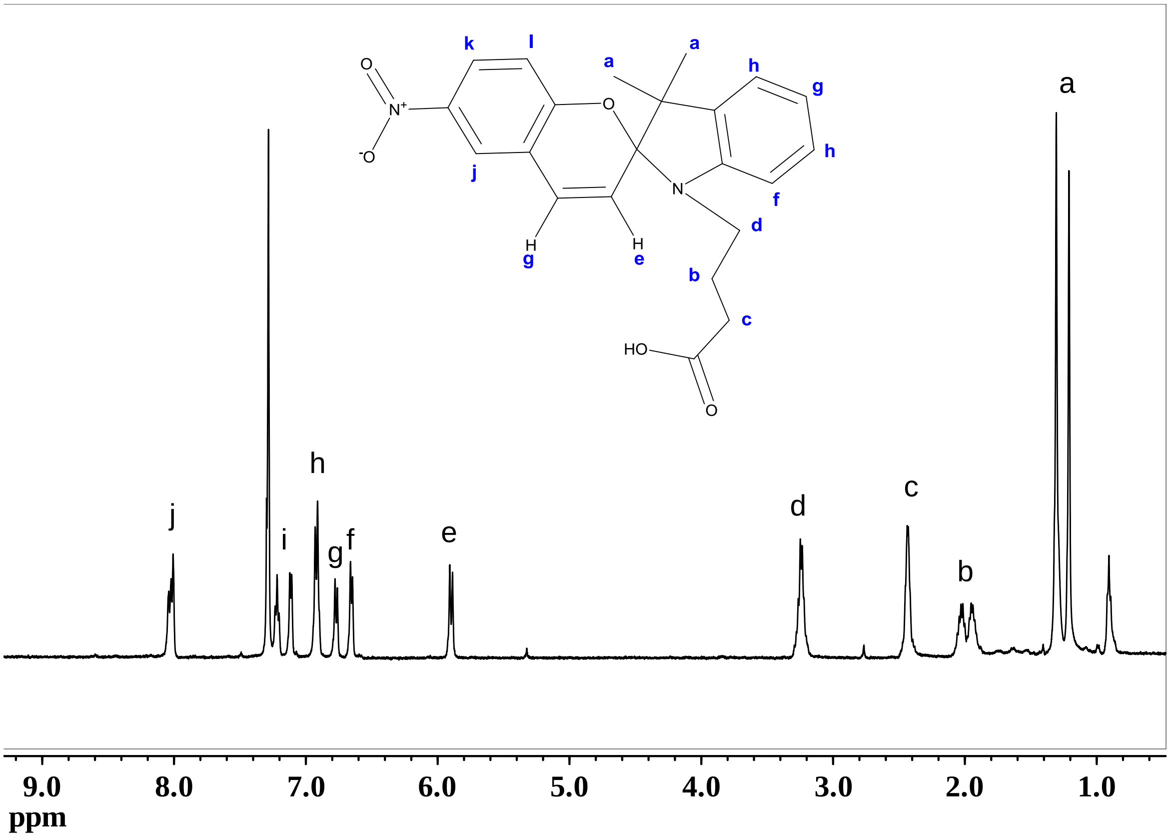


Figure S1. ^{1}H NMR of carboxyl-functionalized spiropyran. ^{1}H NMR(δ, $CDCl_3$): 1.21 (s, 6H), 1.30 (s, 6H), 1.88-2.08 (m, 2H), 2.40-2.45 (t, *J*=9.0 Hz, 2H), 3.21-3.27 (t, *J*=6.0 Hz, 2H), 5.88-5.91 (m, 1H), 6.64-6.64 (m, 1H), 6.75-6.78 (m, 2H), 6.88-6.93 (t, *J*=9.0 Hz, 2H), 7.19-7.24 (t, *J*=6.0 Hz,1H), 8.00-8.02 (m, 1H)

<u>Preparation, Characterization, and Application of Spiopyran-Functionalized Pdots for Cu^{2+} Measurements.</u> First, 10 mg of CO-520 dissolved, 17 mg of carboxyl-functionalized spiropyran, 5 mg of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and 1 mg of 4-Dimethylaminopyridine (DMAP) was dissolved in 10 mL of anhydrous dimethylformamide (DMF) in a vial. The mixture was stirred in the dark at room temperature for 12 h and then stored at 4 ^{o}C until further usage. After that, 30 μL of SP-conjugated CO-520 and 200 μL of PPE or PFBT (1 mg/mL in THF) were added into 5 mL of THF. This mixture was then quickly injected into 10 mL of water under vigorous sonication. THF was then removed by purging with nitrogen on a 96 ^{o}C hotplate for 30 min. The resulting Pdot solution was first filtered through 0.2 μL cellulose acetate membrane filter to remove any aggregates formed during preparation and then purified by gel filtration using Sephacryl HR-300 gel media.

The particle size was determined by DLS and TEM to be 11 nm in diameter for PPE Pdots and 28 nm in diameter for PFBT Pdots. TEM images of the synthesized Pdots were acquired using a JEOL 2010 transmission electron microscope at an acceleration voltage of 200 kV. For TEM, a drop of Pdot aqueous solution was placed onto a carbon-coated grid and allowed to evaporate at room temperature. The absorption spectra of Pdots were measured using UV-visible spectroscopy (Spectra System UV2000 HR, Thermo Separation Products). For the fluorescence measurements, various concentrations of copper ions were introduced into the Pdot solutions and the fluorescence spectra were collected using a Hitachi F-7000 fluorometer (Hitachi, Tokyo, Japan) under 405 nm excitation. We didn't observe any photobleaching of PPE or PFBT Pdots under our experimental conditions.

For the determination of Cu^{2+} in simulated biological backgrounds, the SP-functionalized PPE Pdots (0.2 μg/mL) and SP-functionalized PFBT Pdots (0.2 μg/mL) were added into the diluted (1:10 v/v with high purity water) EMDM (D5921, Sigma) solutions, which contained various inorganic salts, amino acids, vitamins, and other supplementary components.[1] After that, solutions containing 20 μM of Cu^{2+} was prepared by adding $CuSO_4$ into EMDM solutions for the subsequent fluorescence measurements. The concentration of Cu^{2+} was then determined from the fluorescence ratio of I_{535nm}/I_{466nm} as described in the main text.

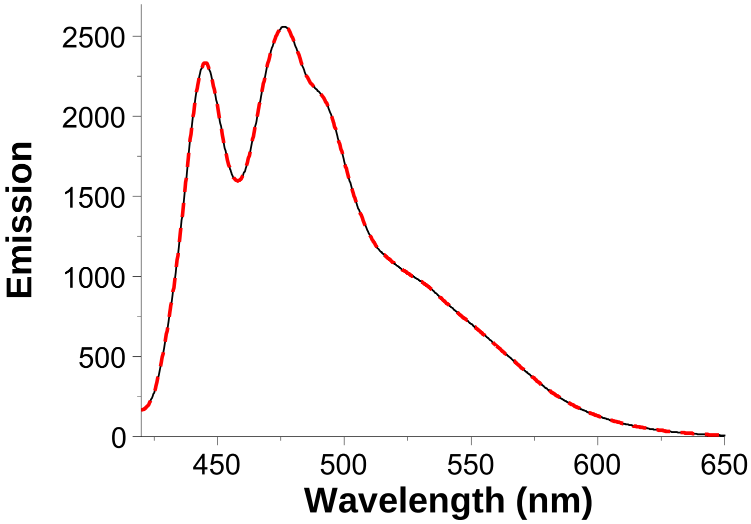


Figure S1. Effect of Cu^{2+} on the emission intensity of SP-PPE/SP-PFBT Pdot mixture solution without exposure to UV light. Fluorescence of SP-PPE/SP-PFBT Pdot mixture solution before (solid black line) and after (dashed red line) the addition of 20 μM of Cu^{2+} without UV irradiation.

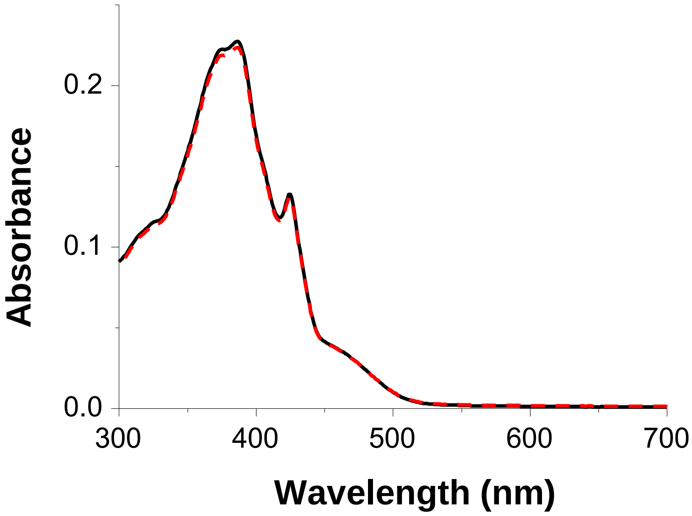


Figure S2. The absorption spectra of SP-PPE/SP-PFBT Pdot mixture solution before (solid black line) and after (dashed red line) the addition of Cu^{2+} followed by UV irradiation. Note the slight decrease in the absorption intensity after Cu^{2+} addition, which we attribute to dilution of the solution when we added the Cu^{2+} solution.

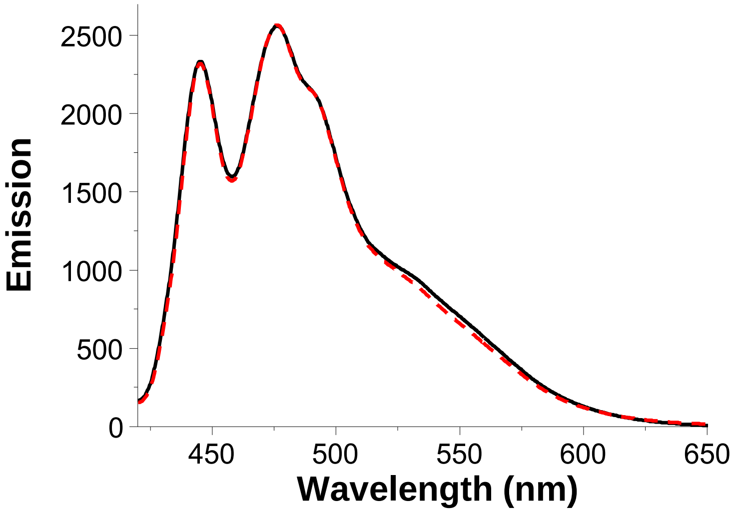


Figure S3. Effect of UV irradiation on the emission intensity of SP-PPE/SP-PFBT Pdot mixture solution in the absence of Cu^{2+}. Fluorescence of SP-PPE/SP-PFBT Pdot mixture solution before (solid black line) and after (dashed red line) 30 s of UV irradiation (λ = 365 nm, power = 3.5 μW).

<u>Calculation of Förster Radius for PPE-PFBT Pair and FRET Efficiency.</u> To obtain the value of R_o, we had to calculate J first from the following equation:

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \qquad \text{(in M}^{-1}\text{cm}^{-1}\text{nm}^4\text{)} \qquad \text{(S1)}$$

where $F_D(\lambda)$ is the dimensionless emission intensity, λ is the wavelength in units of nm, $\varepsilon_A(\lambda)$ is the molar absorption coefficient of PFBT as the acceptor at λ.

Figure S4 shows the degree of spectral overlap between the PPE emission and the PFBT absorption. Based on this information, we calculated that J was ~2.0×10^{18} $M^{-1}cm^{-1}nm^4$ using equation S1. FRET efficiency (E) is given by:

$$E = \left[1 + \left(\frac{r}{R_o}\right)^6\right]^{-1} \qquad (S2)$$

where *r* is the distance between the donor and the acceptor which, in our case, is the distance between PPE and PFBT. To simplify the conditions, we assume that there is only one PFBT Pdot (acceptor) in close proximity to each PPE Pdot (donor) so the average distance (*r*) from core to core between each PPE-PFBT pair is 11/2 + 28/2 + 5x0.35 nm (per polyoxyethylene unit) + 4x0.14 nm (per C-C single bond) = 21.8 nm. The FRET efficiency (*E*) can then be calculated from equation S2: $E = [1+(21.8/12.8)^6]^{-1}$ = 4%. Experimentally we observed a better FRET efficiency of 4-20% from the steady-state quenching at different Cu^{2+} concentrations. It could be attributed to the fact that multiple acceptors could bind to a single donor at higher Cu^{2+} concentrations and/or the high flexibility of polyoxyethylene that could bring donor-acceptor pairs in more close proximity.

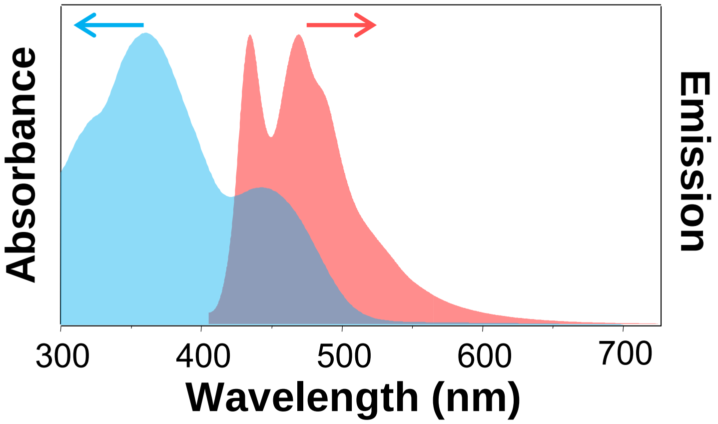


Figure S4. Illustration of spectral overlap between the emission of donor (i.e., PPE) and the absorption of acceptor (i.e., PFBT).

References

(1) http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Formulation/d5921for.Par.0001. File.tmp/d5921for.pdf

Acknowledgements

We would like to thank the National Science Council (NSC 101-2113-M-110-015-MY2) and National Sun Yat-sen University. We also thank Prof. Wei-Lung Tseng for UV/FL/DSL measurements and Prof. Chao-Ming Chiang for chemical support.