Supporting Information

Ratiometric Temperature Sensing with Semiconducting Polymer Dots

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Synthesis of Polystyrene-Rhodamine B (PS-RhB).

For carrying out the NH_2-isothiocyanate reaction depicted in Figure 1A, 200-μL of 10-mg/mL RhB-isothiocyanate in DMF (anhydrous) and 1 mg $NaHCO_3$ were added into 2 mL of 1-mg/mL amine-terminated polystyrene polymer (PS-NH_2, MW 1000, polydispersity 1.1) (in DMF) in a 10-mL round-bottom flask. The mixture was gently stirred overnight under N_2. DMF was removed by rotary evaporation at 75 °C. The resulting red solid was then dissolved in 1-mL THF (anhydrous). $NaHCO_3$ was filtered off with a 200-nm membrane filter because it did not dissolve in THF. The resultant PS-RhB in THF was then blended with the semiconducting polymer to make Pdot-RhB.

Preparation of Pdots.

The preparation of Pdots has been described previously[1, 2]. Briefly, 10 mg of the semiconducting polymer—either poly[{9,9-dioctyl-2,7-divinylene-fluorenylene}-alt-co--{2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene}] (PFPV, MW 220,000, polydispersity 3.1) or poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-(1,4-benzo-{2,1',3}-thiadiazole)] (PFBT, MW 150,000, polydispersity 3.0); both from ADS Dyes, Inc. (Quebec, Canada) —was dissolved in 10-mL THF by stirring overnight under inert atmosphere. The solution was then filtered through a 0.7-μm glass fiber filter to remove any insoluble material. Pdot-RhB was prepared by first mixing 200-μL of 1-mg/mL PFPV or PFBT (in THF) with 10-μL of 2-mg/mL PS-RhB that had been dissolved in 5-mL THF. The mixture was injected into10 mL of MilliQ water under sonication. The THF was removed by nitrogen stripping, and the solution was concentrated by continuous nitrogen stripping to 10 mL on a 90 ºC

hotplate, followed by filtration through a 0.2-μm filter. Free RhB was removed by passing the solution through a Bio-Rad Econo-Pac® 10DG size exclusion column (Hercules, CA, USA).

Characterization of Pdots.

TEM experiments were performed on a FEI Tecnai F20 transmission electron microscope. DLS experiments were conducted using a Malvern Zetasizer NanoZS. UV-Vis absorption spectra were recorded with a DU 720 spectrophotometer. Fluorescence spectra were collected with a Fluorolog-3 fluorometer. The temperature-dependent fluorescence of Pdots was measured in a Fluorolog-3 fluorometer coupled with a heating/cooling system. The absolute temperature of Pdot solutions was measured by digital thermometer (TM902C) where the temperature probe was inserted into the solution. The solution was stirred gently to yield homogeneous cooling and heating during the experiment.

Cell Culture and Labeling.

The cervical cancer cell line HeLa was ordered from American Type Culture Collection (ATCC, Manassas, VA, USA). Primary cultured HeLa cells were grown in Dulbecco's Modified Eagle Medium (cat. no. 11885, Invitrogen) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin solution at 37 ºC with 5% CO_2 humidified atmosphere. The cells were cultured in a T-75 flask and allowed to grow for 5-7 days prior to experiments until ~80% confluence was reached. To prepare cell suspensions, the adherent cancer cells were quickly rinsed with media and then incubated

in 5 mL of trypsin-ethylenediaminetetraacetic (EDTA) solution (0.25 w/v % trypsin, 2.5 g/L EDTA) at 37 ºC for 5 min. The cell suspension solution was then centrifuged at 4500 rpm for 10 min to precipitate the cells in the bottom of the tube. After taking out the supernatant, the cells were rinsed and resuspended in 5 mL of culture media. Approximately tens of thousands of HeLa cells were split onto a glass-bottomed culture dish and allowed to grow for 12 h before Pdot tagging. Prior to fluorescence imaging, the cells were rinsed with PBS buffer to remove any nonspecifically attached Pdots on the cell surface.

Cell Imaging.

The fluorescence images of PFBT-RhB-tagged cells were acquired with a fluorescence confocal microscope (Zeiss LSM 510). The temperature was controlled by a heating stage coupled to the microscope. The temperature of the cell solution was determined by the digital thermocouple (TM902C), whose probe was dipped into the cell solution. The confocal fluorescence images were collected using a diode laser at 458 nm as the excitation source with an integration time of 1.6 μs/pixel. A Carl Zeiss 63X ("C-Apochromat" 63X/1.2 W Corr) objective was utilized for imaging and spectral data acquisition; the laser was focused to a spot size of ~5 μm, and the laser power in the sample stage was about 3 uW.

Reference

1. Wu, C.; Jin, Y.; Schneider, T.; Burnham, D. R.; Smith, P. B.; Chiu, D. T., *Angew. Chem., Int. Ed.* **2010,** 49, 9436-9440.
2. Wu, C.; Schneider, T.; Zeigler, M.; Yu, J.; Schiro, P. G.; Burnham, D. R.; McNeill, J. D.; Chiu, D. T., *J Am.Chem. Soc.* **2010,** 132, 15410-15417.

Supplementary Figures

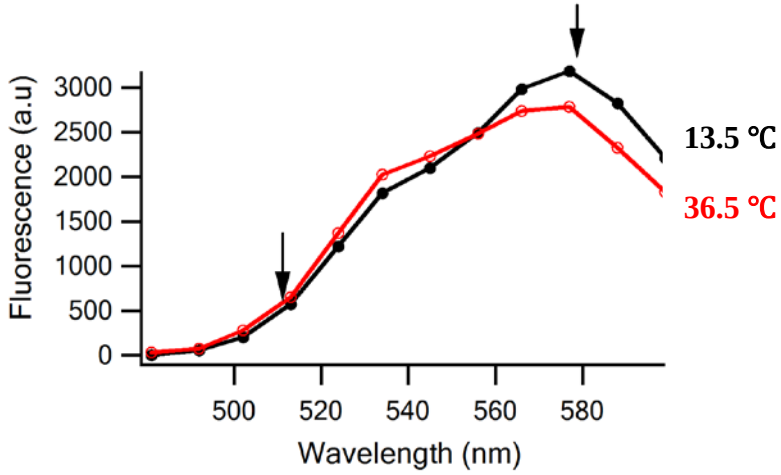

Figure S1. Single cell "spectra" (of the cell pointed to with an arrow in Figure 3) at 13.5 ℃ (Black) and 36.5 ℃ (Red). The ratio of the average fluorescence intensity of 571-582nm to 507-518nm is 5.54 and 4.26, which correspond to a temperature of 13.5 ℃ and 35.6 ℃, respectively.

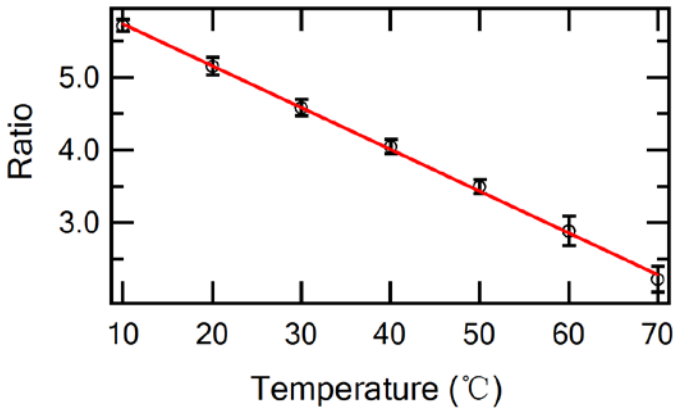

Figure S2. Plot of the ratios of the average fluorescence intensities of 507-518nm to 571-582nm as a function of temperature for PFBT-RhB. The intensity values and ratios used in this figure were derived from the spectra shown in Figure 2C. The red line shows the linear fit, from which we calculated the cell temperatures.

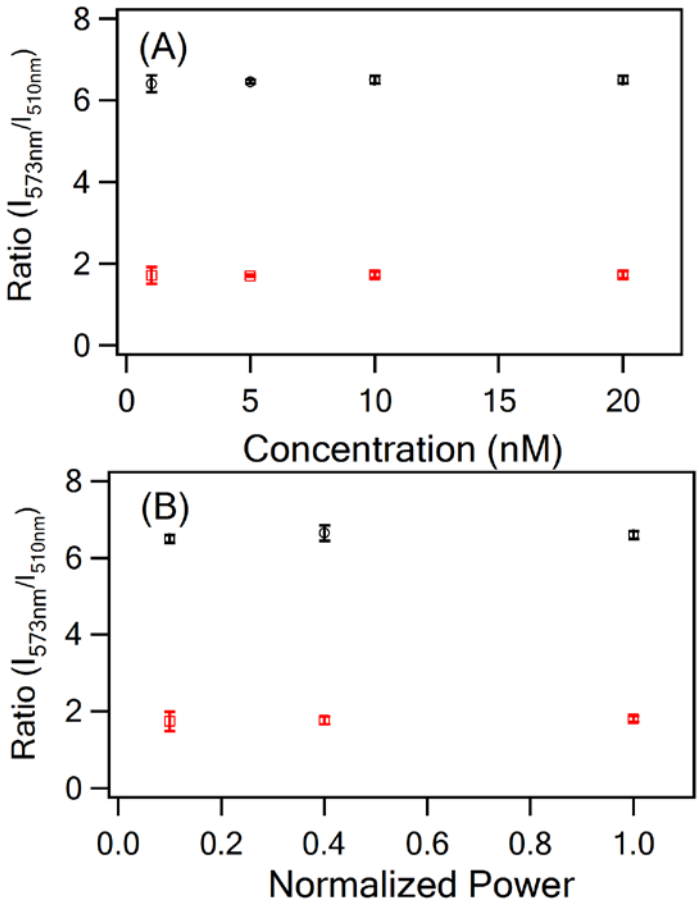

Figure S3. Plot of the intensity ratios (I_{573nm}/I_{510nm}) for PFBT-RhB (black circle) and PFPV-RhB (red square) Pdots as a function of: (A) different concentrations of Pdots (from 1nM to 20 nM), and (B) different normalized excitation powers. All measurements were made at room temperature. The excitation power was varied by placing a neutral density filter into the excitation path of the fluorometer; 1.0 is the power output from the 450W Xenon lamp without any neutral density filter present in the beam path.