Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2014

Coumarin dye-embedded semiconducting polymer dots for ratiometric sensing of fluoride ions in aqueous solution and bioimaging in cells

*Ya-Chi Huang, Chuan-Pin Chen, Pei-Jing Wu, Shih-Yu Kuo, and Yang-Hsiang Chan**

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

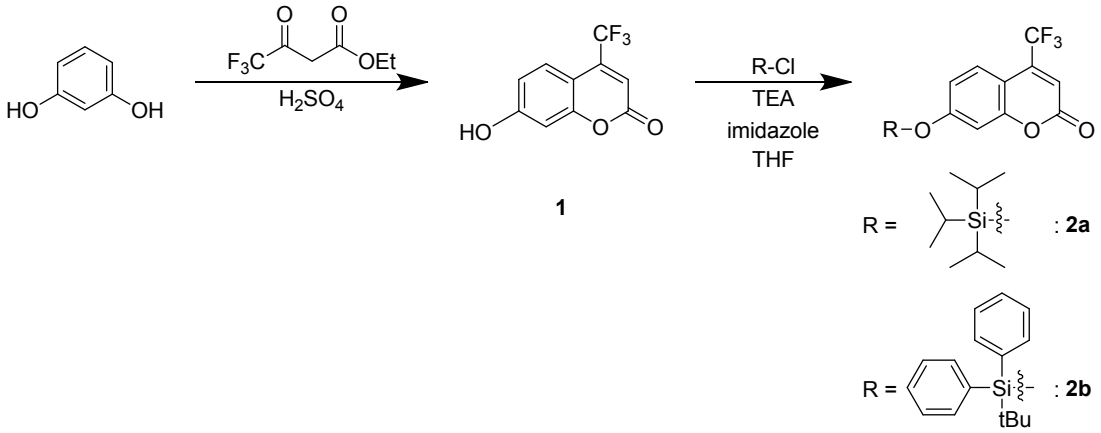
E-mail: yhchan@mail.nsysu.edu.tw

Supporting Information

Materials. The following inorganic salts were purchased from Sigma-Aldrich and used as received: resorcinol, PPE, ethyl 4,4,4-trifluoroacetoacetate, sulfuric acid, triethylamine, imidazole, chlorotriisopropylsilane, tetrahydrofuran (THF; anhydrous, ≥ 99.9%, inhibitor-free), tert-butylchlorodiphenylsilane, methanol, dichloromethane (CH_2Cl_2), hexane, and all salts. High purity water (18.2 MΩ•cm) was used throughout the experiment.

Synthesis of Coumarin Derivatives.

Scheme S1.



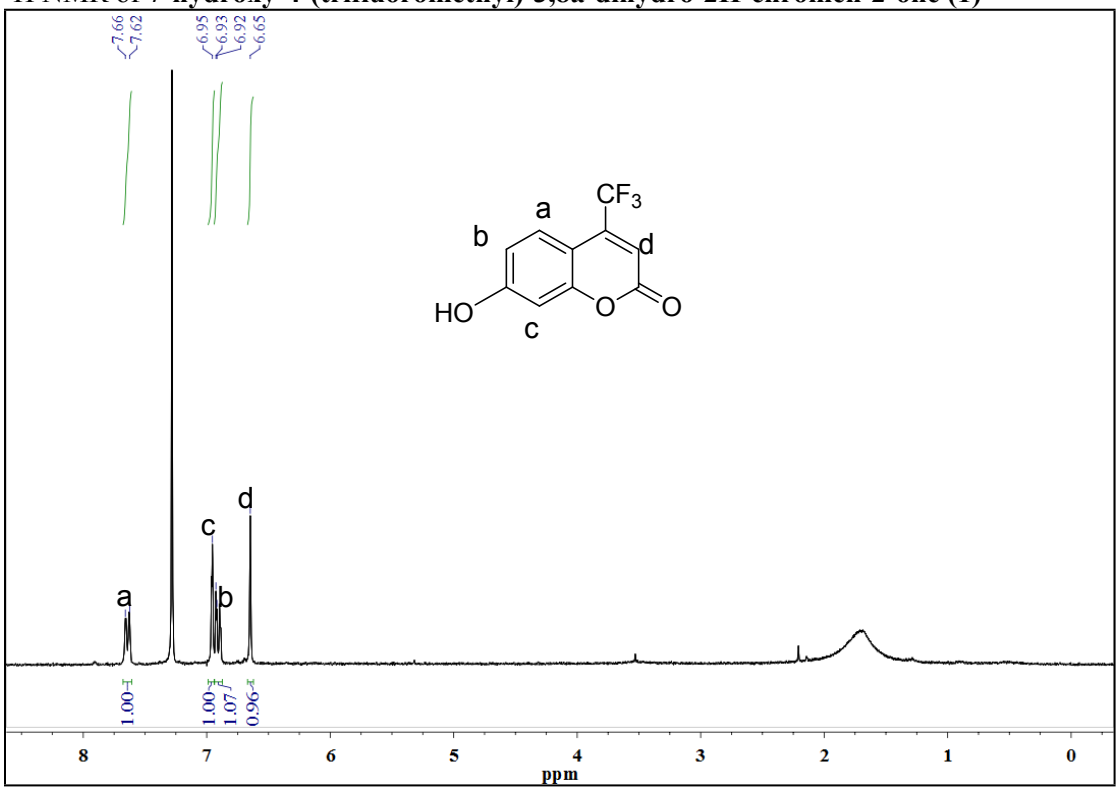
Synthesis of 7-hydroxy-4-(trifluoromethyl)-3,8a-dihydro-2H-chromen-2-one (1). Compound **1** was synthesized according to a modified reported procedure.[1] 2 g (18.2 mmol) of resorcinol in 7 ml of concentrated sulfuric acid in a round-bottom single-neck flask was cooled to 0 °C with stirring for 30 min. After that, 4 ml (27.4 mmol) of ethyl 4,4,4-trifluoroacetoacetate and 10 ml of concentrated sulfuric acid was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for another 12 h. After that, the reaction mixture was poured into ice water and the precipitate was filtered. The solid product was washed with methanol and water several times to give 3 g (70%) of **1** as pink solid. ^{1}H NMR (δ, $CDCl_3$): 7.62-7.66 (d, 1H, J = 12Hz), 6.95 (s, 1H), 6.92-6.93 (d, 1H, J = 1.5Hz), 6.65 (s, 1H).

Synthesis of 4-(trifluoromethyl)-7-((triisopropylsilyl)oxy)-2H-chromen-2-one (2a). 0.5 g (2.2 mmol) of **1**, 0.5 ml (3.2 mmol) of triethylamine, and 1 mg of imidazole was dissolved in 10 ml of THF in a round-bottom single-neck flask and then 0.51 ml (2.4 mmol) of chlorotriisopropylsilane was added dropwise. After being stirred at room

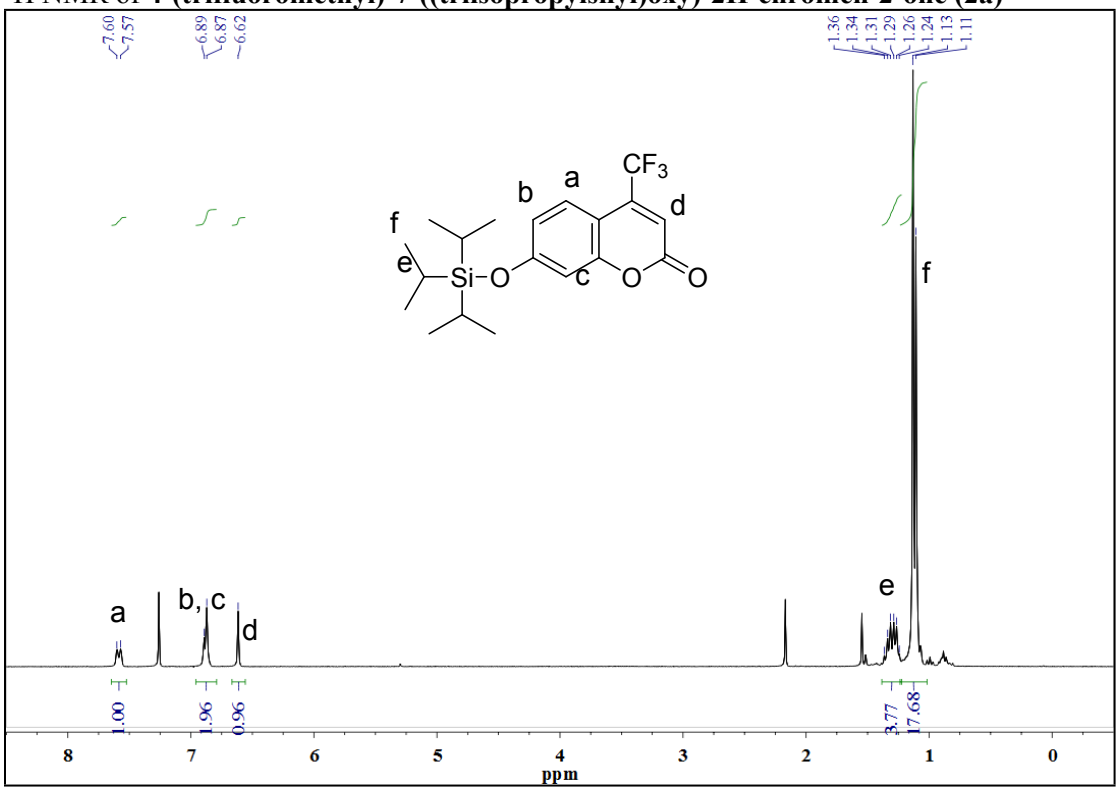
temperature for 6 h under nitrogen, the reaction mixture was poured into water, diluted with CH_2Cl_2, and extracted with brine for 3 times. The organic extract was separated, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified on a silica gel column by eluting with CH_2Cl_2/hexane (30:100, v/v) to yield 0.55 g (66%) of **2a** as light yellow oil. MALDI MS: *m/z* 387.1 (M^+). ^{1}H NMR (δ, $CDCl_3$): 7.57-7.60 (d, 1H, *J* = 9Hz), 6.87-6.89 (m, 2H), 6.62 (s, 1H), 1.24-1.36 (m, 3H), 1.11-1.13 (d, 18H, *J* = 6Hz). Anal. Calcd for $C_{19}H_{25}F_3O_3Si$: C, 59.05; H, 6.52. Found: C, 59.31; H, 6.43.

Synthesis of 7-((tert-butyldiphenylsilyl)oxy)-4-(trifluoromethyl)-3,8a-dihydro-2H-chromen-2-one (2b). 0.5 g (2.2 mmol) of **1**, 0.5 ml (3.2 mmol) of triethylamine, and 1 mg of imidazole was dissolved in 10 ml of THF in a round-bottom single-neck flask and then 0.62 ml (2.4 mmol) of tert-butylchlorodiphenylsilane was added dropwise. After being stirred at room temperature for 6 h under nitrogen, the reaction mixture was poured into water, diluted with CH_2Cl_2, and extracted with brine for 3 times. The organic extract was separated, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified on a silica gel column by eluting with CH_2Cl_2/hexane (20-30:100 by vol.). The oil-like product was further recrystallized from hexane to yield 0.61 g (60%) of **2b** as white solid. MALDI MS: *m/z* 469.1 (M^+). ^{1}H NMR (δ, $CDCl_3$): 7.69-7.71 (d, 4H, *J* = 6Hz), 7.37-7.48 (m, 7H), 6.77-6.81 (m, 1H), 6.72-6.73 (d, 1H, *J* = 6Hz), 6.57 (s, 1H), 1.12 (s, 9H). Anal. Calcd for $C_{26}H_{23}F_3O_3Si$: C, 66.65; H, 4.95. Found: C, 67.12; H, 4.82.

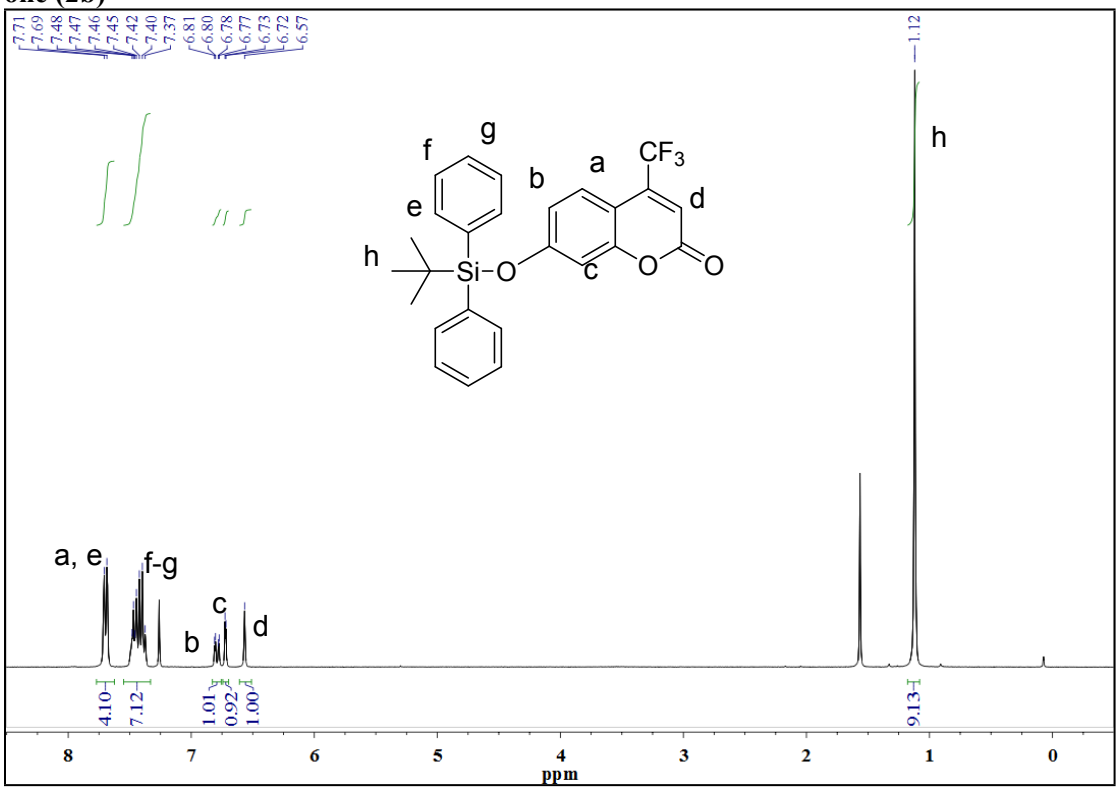
^{1}H NMR of **7-hydroxy-4-(trifluoromethyl)-3,8a-dihydro-2H-chromen-2-one (1)**



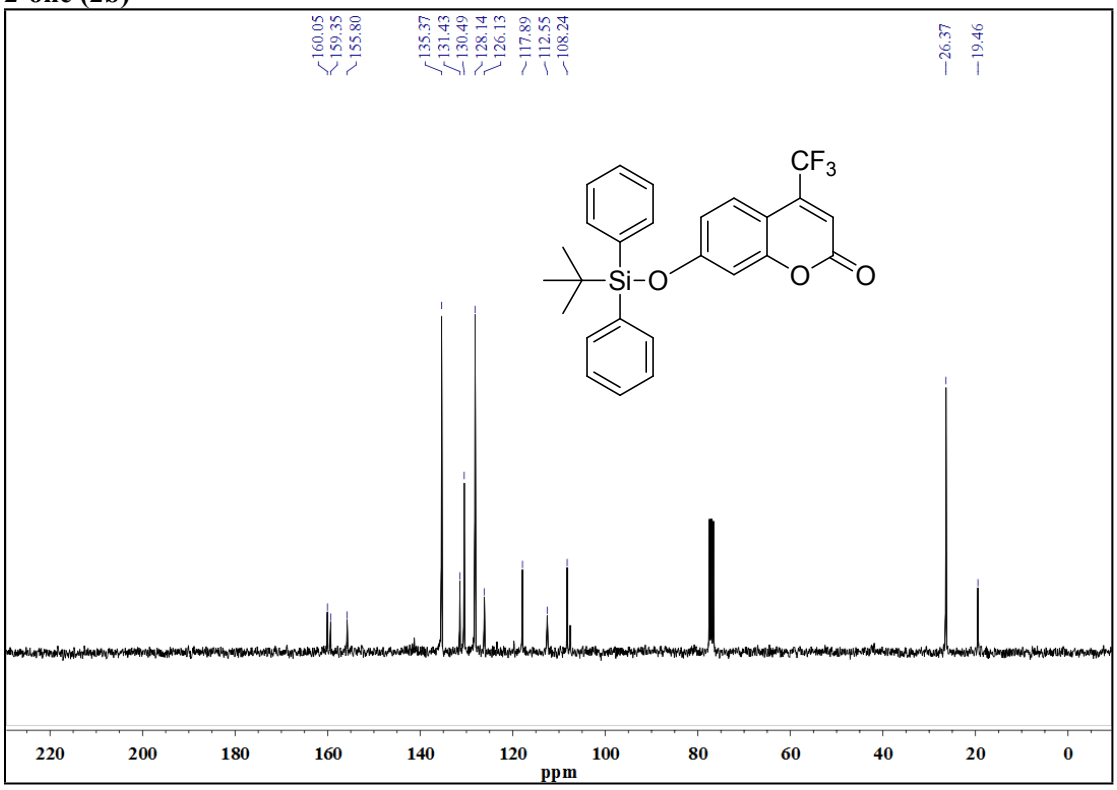
^{1}H NMR of **4-(trifluoromethyl)-7-((triisopropylsilyl)oxy)-2H-chromen-2-one (2a)**



^{1}H NMR of **7-((tert-butyldiphenylsilyl)oxy)-4-(trifluoromethyl)-3,8a-dihydro-2H-chromen-2-one (2b)**



^{13}C NMR of **7-((tert-butyldiphenylsilyl)oxy)-4-(trifluoromethyl)-3,8a-dihydro-2H-chromen-2-one (2b)**



Preparation of PDA-enclosed Coumarin-doped Pdots for F^- Measurements.

First, 50 μL of PPE (1 mg/mL in THF) and 2 μL of coumarin (4 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 ^oC hotplate for one hour to obtain coumarin-embedded PPE Pdots. The resulting Pdot solution was then filtered through 0.2 μL cellulose acetate membrane filter to remove any aggregates formed during preparation. After that, 200 μL of 10,12-pentacosadiynoic acid solution (2 mg/mL in THF) was added dropwise into 4 mL of coumarin-embedded PPE Pdot solution under stirring. The mixture was further stirred for 15 min and then THF was removed at 67 ^oC for 25 min. The solution was then cooled at 4 ^oC for 2h. The coated diacetylenes were then polymerized upon exposure to a 254-nm UV lamp for 15 min under nitrogen atmosphere. The solution was further centrifuged (7000 rpm, 5 mins) to remove large aggregates. 2 mL of the supernatant solution was transferred to a centrifugal filtration device (Amicon® Ultra-4, MWCO: 100 kDa), and then centrifuged (2000 rpm, 1 min) to separate free coumarin dyes or any empty PDA nanoparticles (either micelles or vesicles) formed during the processes of encapsulation. Considering a 9 nm coumarin-embedded PPE Pdot whose density is assumed to be 1 g/cm^3 and the molecular weight was determined to be ~14900 by gel permeation chromatography, the number of PPE polymer is ~123 inside each individual Pdot and each Pdot contains ~610 coumarin dyes. Because the coumarin-embedded PPE Pdots were prepared by use of coprecipitation method, we believe coumarin dyes should distribute evenly over the Pdot nanosphere.

Characterization of PDA-enclosed Coumarin-embedded Pdots. The average particle size was determined by dynamic light scattering and transmission electron microscopy (TEM) to be ~17 nm in diameter for PDA-enclosed coumarin-doped PPE Pdots. TEM images of the synthesized Pdots were acquired using a JEOL 2100 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, the fluorescence spectra were collected using a Hitachi F-7000 fluorometer (Hitachi, Tokyo, Japan) under 405 nm excitation. Absolute fluorescence quantum yield of Pdots was determined by using an integrating sphere unit of Hitachi F-7000 fluorescence spectrophotometer. Due to extensive spectral overlap between the emission of PPE and coumarin in the range of 430 to 600 nm, we could only determine the overall quantum yield. The quantum yield of coumarin-embedded PPE Pdots was 6.1% in the absence of fluoride, and increased to 6.6% and 11.8% after the addition of 90 μM and 520 μM of NaF. The extinction coefficient of TBDPSCA-embedded PPE Pdots at 405 nm was determined to be 5.61×10^7 M^{-1} cm^{-1}. According to the previously reported literature,[2] the enhanced fluorescence of protected coumarin dyes in water is attributed to the intramolecular charge transfer events via stronger Si-O bond polarization upon the interaction with water molecules, rather than actual desilylation (deprotection) by fluoride anions.

Cell Culture and Labeling. The cervical cancer cell line HeLa was ordered from Food Industry Research and Development Institute (Taiwan). 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 (5000 units/mL penicillin G, 50 μg/mL streptomycin sulfate in 0.85% NaCl) at 37 °C with 5% CO_2 humidified atmosphere. The cells were pre-cultured in a T-25 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 0.8 mL trypsin-ethylenediaminetetraacetic (EDTA) solution (0.25 w/v % trypsin, 0.25 g/L EDTA) at 37°C for 3 min. The cell suspension solution was then centrifuged at 1000 rpm for 5 min to precipitate the cells to the bottom of the tube. After taking out the upper media, the cells was rinsed and re-suspended in 5 mL of culture media. Approximately tens of thousands 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 non-specifically bound Pdots on the cell surface.

Cell Imaging. The fluorescence spectra of Pdot-tagged cells were acquired with a fluorescence confocal microscope (Nikon D-Eclipse C1) under ambient conditions (24 ± 2 ^{o}C). The confocal fluorescence images were collected using a diode laser at 488 nm (~15 mW) as the excitation source and an integration time of 1.6 μs/pixel. A CF1 Plan Fluor 40x (N. A. 0.75, W.D. 0.66 mm) objective was utilized for imaging and spectral data acquisition; the laser was focused to a spot size of ~7 μm^2. The blue fluorescence was collected by filtering through a 450/35 band-pass, while the green fluorescence was collected by integrating the spectral region from 500 to 530 nm (λ_{ex} = 408 nm).

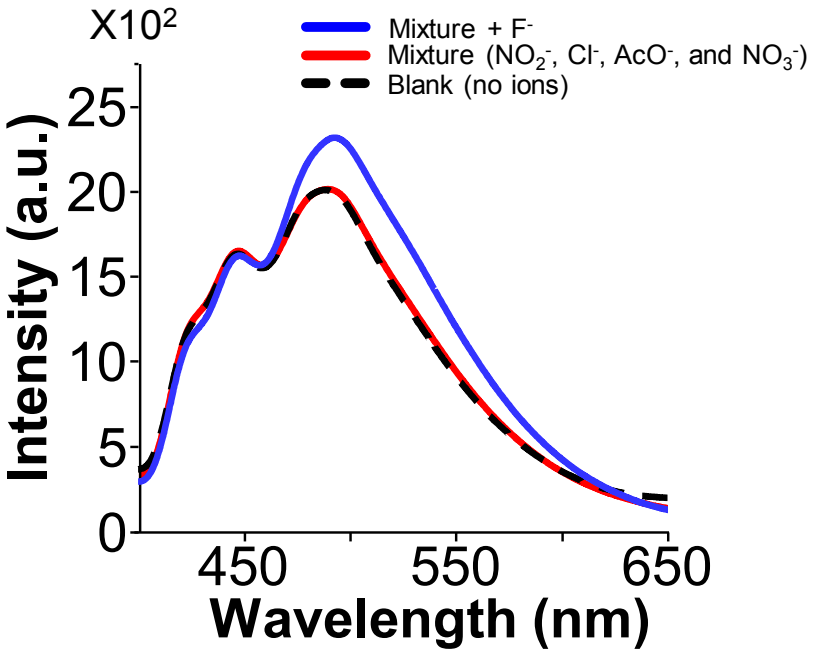


Figure S1. Interference effect of mixed anions on the emission spectra of solutions containing PDA-enclosed PPE/TBDPSCA Pdots. Fluorescence of PDA-enclosed TBDPSCA-doped PPE Pdots in pure water (black line), and in solution containing 90 μM of various anions before (red line) and after (blue line) the addition 90 μM of fluoride ions.

Calculation of Förster Radius and FRET Efficiency for PPE-Coumarin Pair.

To obtain the value of R_o, we had to calculate J first from the following equation:

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

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 coumarin as the acceptor at λ.

Figure S2 shows the degree of spectral overlap between the PPE emission and the coumarin absorption. Based on this information, we calculated that J was ~5.5×10^{14} $M^{-1}cm^{-1}nm^4$ using equation S1.

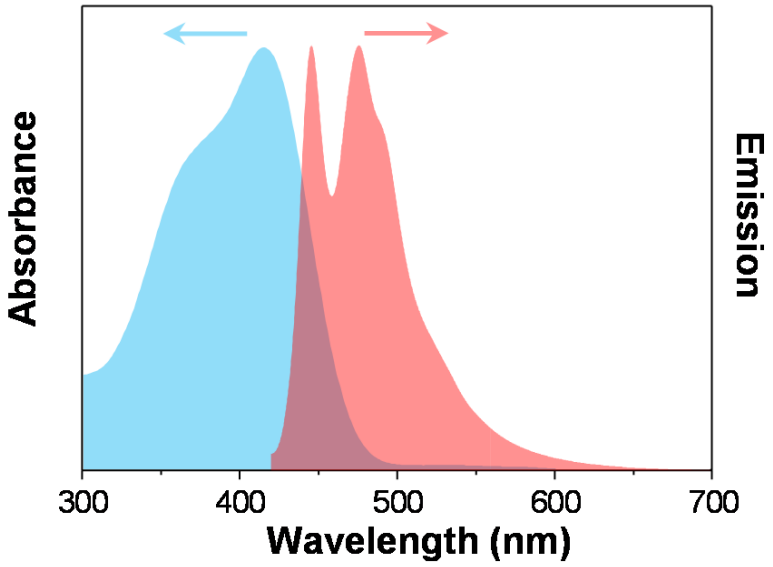


Figure S2. Illustration of spectral overlap between the emission of donor (i.e., PPE) and the absorption of acceptor (i.e., coumarin).

The FRET efficiency (E) is given by:

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

where r is the distance between the donor and the acceptor which, in our case, is the distance between PPE and coumarin. R_o is the Förster-like radius that represents a specific separation distance where the energy transfer efficiency is reduced to 50% of the maximal value. To calculate the FRET efficiency by use of eq S2, we have to calculate the distance (r) between the donor and the acceptor first. If we consider a 17 nm coumarin-encapsulated PPE Pdots whose density assumed to be 1 g/cm^3, the number of PPE polymers is ~123 inside each individual Pdot, and each Pdot contains ~610 coumarin dyes (*vide supra*). Consequently, the average distance between each two neighboring PPE polymers is 3.4 nm, and the average distance (r) between each polymer-coumarin pair is $\sqrt[3]{(1.7^3/5)}$ = 1.0 nm. The FRET efficiency (E) can then be calculated from eq S2: $E = [1 + (1.0/2.9)^6]^{-1} > 99\%$ if all of the embedded coumarin dyes were

deprotected by F^-. Experimentally (6 mM of fluoride ions as an example), we observed a reduced FRET efficiency of 61% from the steady-state quenching, and 58% (from 0.26 ns to 0.11 ns) from lifetime measurements (Figure S4). We think it might be attributed to the uneven distribution of coumarin dyes inside Pdots or the hydrophobic core of Pdot matrix that might prevent the fluoride ions from reaching the interior of the Pdots. Additionally, we can roughly calculate the number of deprotected coumarins from the FRET efficiency. For example, the experimental FRET efficiency is 58-61% at 6 mM of fluoride ions, which in turn indicates that the average distance (r) between each polymer-dye pair is 2.7 nm. In this case, the number of deprotected coumarins is ~152.

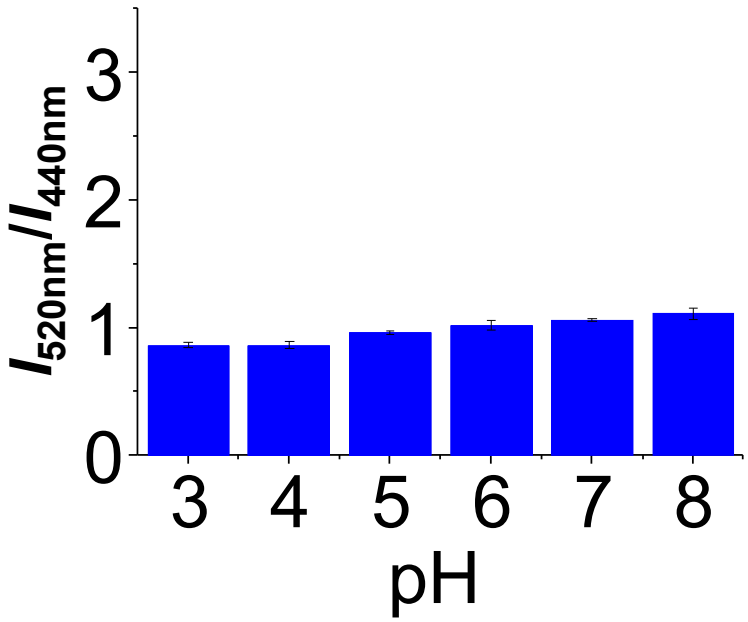


Figure S3. Fluorescence intensity ratios (I_{520nm}/I_{440nm}) of PDA-coated PPE/TBDPSCA Pdots at different pH, ranging from 3 to 8.

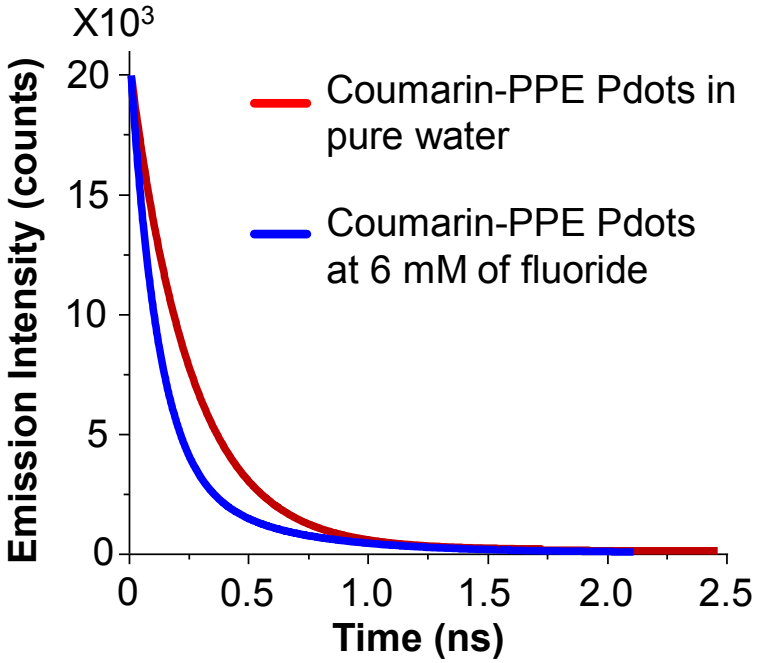


Figure S4. Time-resolved fluorescence decay of coumarin-embedded PPE Pdots in pure water (0.26 ns, red line) and in the presence of 6 mM fluoride ions (0.11 ns, blue line).

Table S1. Original composition of RPMI-1640[3]

Component	mg/L
Inorganic Salts	
$Ca(NO_3)_2 \cdot 4H_2O$	100.0
$MgSO_4$	48.81
KCl	400.0
$NaHCO_3$	2000.0
NaCl	6000.0
NaH_2PO_4	800.0
Amino Acids	
L-Arginine	200.0
L-Asparagine	50.0
L-Aspartic Acid	20.0
L-Cysteine • 2HCl	65.0
Glycine	10.0
L-Glutamic Acid	20.0
L-Glutamine	300.0
L-Histidine	15.0
L-Hydroxyproline	20.0
L-Isoleucine	50.0
L-Leucine	50.0
L-Lysine • HCl	40.0
L-Methionine	15.0
L-Phenylalanine	15.0
L-Proline	20.0
L-Serine	30.0
L-Threonine	20.0
L-Tryptophan	5.0
L-Tyrosine • 2Na • $2H_2O$	29.0
L-Valine	20.0
Vitamins	
Biotin	0.2
Choline Chloride	3.0
D-Calcium pantothenate	0.25
Folic Acid	1.0
i-Inositol	35.0
Niacinamide	1.0
Para-Aminobenzoic Acid	1.0
Pyridoxine • HCl	1.0
Riboflavin	0.2
Thiamine • HCl	1.0
Vitamin B12	0.0050
Other	
D-Glucose (Dextrose)	2000.0
Glutathione (reduced)	1.0
Phenol Red • Na	5.0

References.

1. W. Lin, L. Long, J. Feng, B. Wang, C. Guo, *Eur. J. Org. Chem.*, 2007, 4301-4304
2. S. Y. Kim, J. Park, M. Koh, S. B. Park, J.-I. Hong, *Chem. Commun.*, 2009, 4735-4737
3. http://www.lifetechnologies.com/tw/zt/home/technical-resources/media-formulation.114.html

Acknowledgements. We would like to thank the Ministry of Science and Technology (101-2113-M-110-015-MY2), NSYSU-KMU Joint Research Project (103-I 004), and National Sun Yat-sen University. We also thank Prof. Wei-Lung Tseng for UV/DSL measurements and Prof. Chao-Ming Chiang for chemical support.