Electronic Supplementary Information (ESI)

Fluorescent "Light-Up" Bioprobes Based on Tetraphenylethylene Derivatives with Aggregation-Induced Emission Characteristics

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Materials and Instrumentations

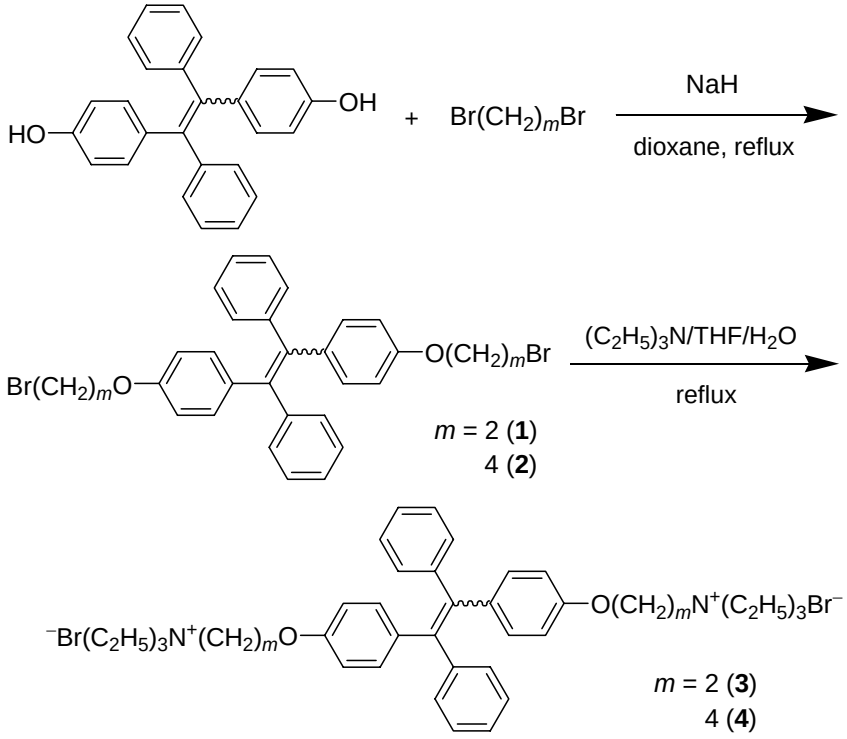
1,4-Dibromobutane, 1,2-dibromoethane, sodium hydride (60% dispersion in mineral oil), and triethylamine were purchased from Aldrich and used as received without further purification. Solvents were purified by standard distillation procedures. Bovine serum albumin (BSA; >98%, fraction V) and calf thymus (ct) DNA (highly polymerized sodium salt) were purchased from Sigma.

^{1}H and ^{13}C NMR spectra were measured on a Bruker ARX 300 or Varian 300 spectrometer using deuterated chloroform as solvent. Tetramethylsilane (TMS) was used as internal reference for the NMR analyses. Mass spectra were recorded on a triple quadrupole mass spectrometer (Finnigan TSQ7000). UV-vis absorption spectra were recorded on a Milton Roy Spectronic 3000 Array spectrophotometer and photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 50B spectrofluorometer with Xenon discharge lamp excitation.

Synthesis and Characterization

1,2-Bis(4-hydroxyphenyl)-1,2-diphenylethene was prepared following the experimental procedures described in the literature.[1] Tetraphenylethene derivatives **1**–**4** were prepared according to the synthetic routes given in Scheme S1. All the reactions were carried out in an inert atmosphere.

Scheme S1



1,2-Bis[4-(2-bromoethoxy)phenyl]-1,2-diphenylethene (1). To a mixture of sodium hydride (84 mg) and 1,2-bis(4-hydroxyphenyl)-1,2-diphenylethene (0.50 g) in dry dioxane (20 mL), 1,2-dibromo-ethane (1.50 g) was added at room temperature. The mixture was heated to reflux and stirred for 24 h. After filtration and concentration, the product was isolated and purified by silica gel chromatography using chloroform/hexane (1:1 v/v) as elute. The product (**1**) was obtained in 32% yield. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.10–7.02 (m, 10H), 6.95–6.92 (m, 4H), 6.65–6.59 (m, 4H), 4.15–4.11 (m, 4H), 3.55–3.49 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 156.6, 144.1, 139.8, 137.2, 132.7, 131.5, 127.8, 126.4, 114.0, 67.8, 29.3. MS (TOF), *m/e*: 578.03 ($[M]^+$, calcd. 578.03).

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[1] S. Watanaba, A. Kobayashi, M. A. Kakimoto and Y. Imai, *J. Polym. Sci., Part A: Polym. Chem.* 1994, **32**, 909.

1,2-Bis[4-(4-bromobutoxy)-phenyl]-1,2-diphenylethene (2). To a mixture of sodium hydride (30 mg) and 1,2-bis(4-hydroxyphenyl)-1,2-diphenylethene (0.11 g) in dry dioxane (20 mL), 1,2-dibromo-butane (0.33 g) was added at room temperature. The mixture was heated to reflux and stirred for 24 h. After filtration and concentration, the product was isolated and purified by silica gel chromatography using chloroform/hexane (1:1 v/v) as elute. The product (**2**) was obtained in 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.09–7.00 (m, 10H), 6.97–6.87 (m, 4H), 6.61–6.54 (m, 4H), 3.90–3.84 (m, 4H), 3.45–3.40 (m, 4H), 2.00–1.97(m, 4H), 1.88–1.84 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 157.9, 144.9, 140.3, 137.2, 133.2, 132.1, 128.3, 126.9, 114.2, 67.3, 34.2, 30.2, 28.6. MS (TOF), *m/e*: 634.09 ($[M]^+$, calcd. 634.09).

1,2-Bis{4-[2-(*N*,*N*,*N*-triethylammonium)ethoxy]phenyl}-1,2-diphenylethene dibromide (3). A 250 mL flask with a magnetic spin bar was charged with **1** (100 mg) dissolved in 100 mL of THF. To this solution was added triethylamine (5 mL). The mixture was heated to reflux and stirred for 3 days. During this period, 10 mL of water was added at several intervals. THF and extra triethylamine were evaporated. The water solution was washed by chloroform three times. After solvent evaporation, the residue was washed with chloroform and acetone and then dried overnight in vacuo at 50 °C. The product (**3**) was isolated in 56% yield. ^{1}H NMR (300 MHz, *d*-DMSO), δ (ppm): 7.22–7.18 (m, 6H), 7.07–6.95 (m, 8H), 6.90–6.83 (m, 4H), 3.90–3.84 (m, 4H), 4.40 (br, 4H), 3.31–3.12 (m, 12H), 1.37–1.25 (m, 18H). ^{13}C NMR (75 MHz, *d*-DMSO), δ (ppm): 156.3, 144.1, 139.9, 137.0, 132.5, 131.2, 128.4, 127.0, 114.2, 61.4, 60.4, 53.4, 7.8. MS (TOF), *m/e*: 699.36 ($[M - Br]^+$, calcd. 699.35).

1,2-Bis{4-[4-(*N*,*N*,*N*-triethylammonium)butoxy]phenyl}-1,2-diphenylethene dibromide (4). A 250 mL flask with a magnetic spin bar was charged with **2** (100 mg) dissolved in 100 mL of THF. To this solution was added triethylamine (5 mL). The mixture was heated to reflux and stirred for 3 days. During this period, 10 mL of water was added at several intervals. THF and extra triethylamine were evaporated. The water solution was washed by chloroform three times. After solvent evaporation, the residue was washed with chloroform and acetone and then dried overnight in vacuo at 50 °C. The product (**4**) was isolated in 68% yield. ^{1}H NMR (300 MHz, *d*-DMSO), δ (ppm): 7.25–7.19 (m, 6H),

7.07–6.92 (m, 8H), 6.83–6.77 (m, 4H), 4.04–4.02 (m, 4H), 3.36–3.29 (m, 16H), 1.86–1.81(m, 8H), 1.34–1.11 (m, 18H). ^{13}C NMR (75 MHz, *d*-DMSO), δ (ppm): 156.9, 143.8, 139.3, 135.7, 132.0, 130.7, 127.9, 126.4, 113.7, 66.5, 55.6, 52.0, 25.5, 18.0, 7.2. MS (TOF), *m/e*: 789.50 ($[M{\cdot}2H_2O - HBr]^+$, calcd. 789.44).

UV and PL Spectra

Stock solutions of **1** and **2** were 1.0×10^{-3} M in acetonitrile. Sample mixtures for measuring the UV and PL spectra were prepared by adding 1 mL of a stock solution to 99 mL of acetonitrile or water under vigorous stirring at room temperature. The mixtures were stirred for half an hour prior to taking their spectra. The relative fluorescence quantum yields (Φ_F) were determined by the standard method using 1.0×10^{-5} M quinine sulfate in 0.1N H_2SO_4 solution as reference. The refractive indices of the solvents were taken into account in the measurements.

BSA was dissolved in a pH 7.0 phosphate buffer solution (1.0 mg/mL). DNA was dissolved in deionized water (1.0 mg/mL) and filtered through a 0.45 μm filter. The actual concentration (in nucleic base) was determined by UV photometry using the extinction coefficient ε_{260}= 6600 M^{-1} cm^{-1}.

Stock solutions of **3** and **4** were 2.5×10^{-4} M in water. Fluorescence titration was carried out by sequentially adding 100 μL aliquots of DNA or BSA solution to a 100 μL stock solution of **3** or **4**, followed by adding an aqueous phosphate buffer (10 mM, pH 7) to acquire a 10.00 ml solution. The mixtures were stirred for half an hour prior to taking their spectra.

Temperature Effect on NMR Spectra

According to previous studies of rotation-induced conformational changes in many molecular systems, fast conformational exchanges caused by fast intramolecular rotations upon single-bond axes give sharp resonance peaks, whereas slower exchanges due to slowed rotations at lower temperatures broaden the resonance peaks.[2]

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[2] (a) J. Sandstrom, *Dynamic NMR Spectroscopy*, Academic Press, London, UK, 1982. (b) P. U. Biedermann, J. J. Stezowski and I. Agranat, *Eur. J. Org. Chem*. 2001, **1**, 15. (c) R. E. Carter, B. Nilsson and K. Olsson, *J. Am. Chem. Soc*. 1975, **97**, 6155. (d) K. Mislow, *Acc. Chem. Res*. 1976, **9**, 26.

The dichloromethane solution of **1** exhibits sharp NMR peaks at room temperature, which are clearly broadened when the temperature is decreased (Figure S7). The solvent (dichloromethane) should be in the liquid state and experience little viscosity change in the whole measured temperature range (i.e., from 25 to –53 °C). Dye **1** should remain molecularly dissolved at the temperatures and the effects of aggregate formation and viscosity change should be marginally small. The plot of Ln δ_{fwhm} (full width at half maximum) versus $1/T$ is a linear line (Figure S8), suggesting that the band shape broadening follows a single mechanism. The restriction of intramolecular rotations is thus believed to be the predominant factor for the band shape broadening.[3]

Table S1 Absorption and emission characteristics of tetraphenylethene (TPE) derivatives **1** and **2** in the solution[a] and aggregate[b] states

TPE	λ_{ab}, nm[c]		λ_{em}, nm[d]	
	solution	aggregate	solution (Φ_F, %)	aggregate (Φ_F, %)
1	310	325	394 (0.49)	476 (19.67)
2	312	328	390 (0.28)	478 (16.32)

[a] In acetonitrile solutions (10 μM). [b] In acetonitrile/water (1:99 by volume) mixtures (10 μM). [c] Absorption maximum. [d] Emission maximum [with quantum yield (Φ_F, %) given in the parentheses, relative to 10^{-5} M quinine sulfate in 0.1 N H_2SO_4 solution]; excitation wavelength: 350 nm.

[3] (a) R. A. Alberty and R. J. Silbey, *Physical Chemistry*, Wiley, New York, 1992. (b) L. Goodman, V. Pophristic and F. Weinhold, *Acc. Chem.Res*. 1999, **32**, 983. (c) K. B. Wiberg, *Acc. Chem. Res*. 1999, **32**, 922. (d) J. W. Chen, C. C. W. Law, J. W. Y. Lam, Y. P. Dong, S. M. F. Lo, I. D. Williams, D. B. Zhu and B. Z. Tang, *Chem. Mater.*, 2003, **15**, 1535.

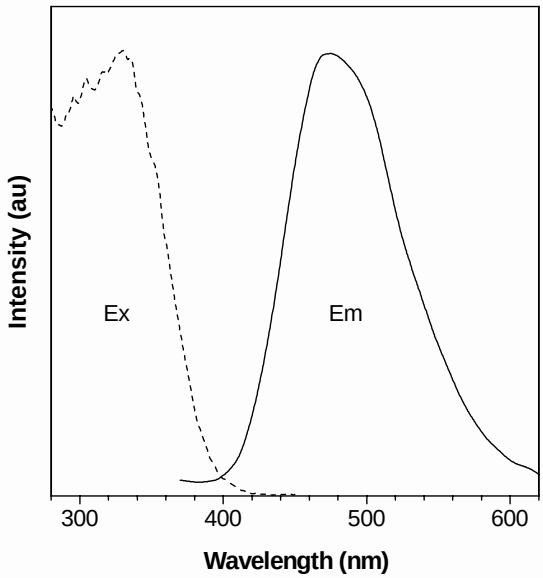

Figure S1. Excitation (Ex) and emission (Em) spectra of **1** in an acetonitrile/water (1:99 by volume) mixture.

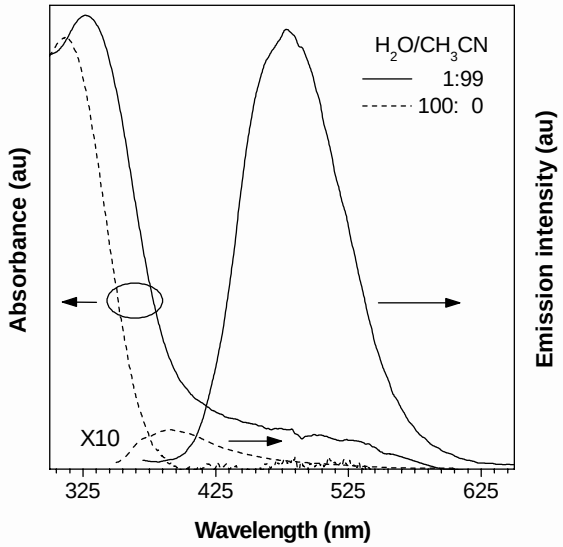

Figure S2. Absorption and emission spectra of **2** (10 μM) in acetonitrile and acetonitrile/water mixture.

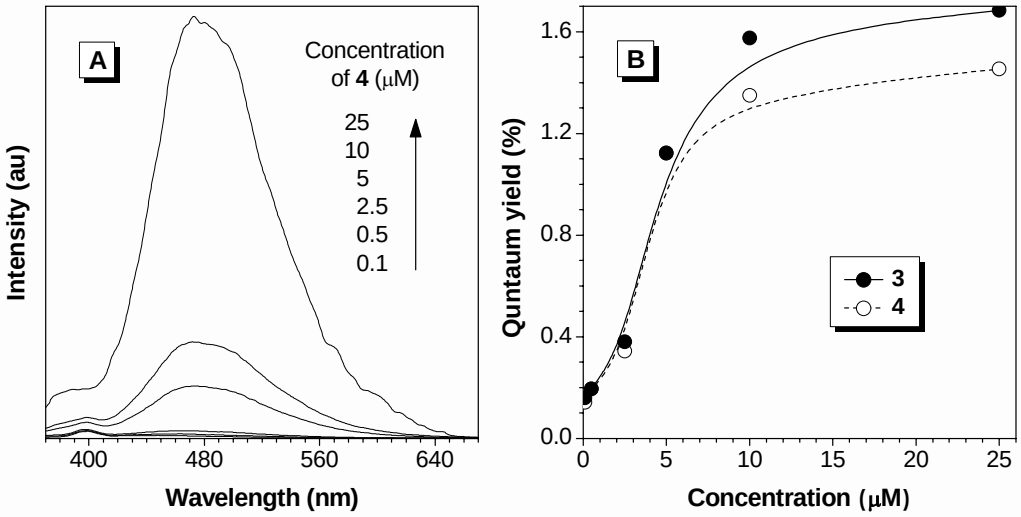

Figure S3. (A) Emission spectra of **4** in aqueous phosphate buffer (pH = 7.0) at different concentrations. (B) Effects of solution concentrations on quantum yields of the buffer solutions of **3** and **4**.

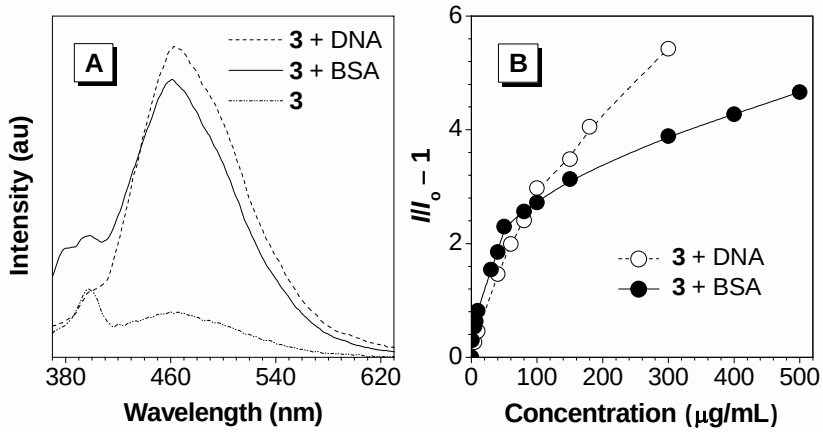

Figure S4. (A) Emission spectra of **3** (2.5 μM) in an aqueous phosphate buffer (pH = 7) and in the buffers containing 300 μg/mL DNA and 500 μg/mL BSA. (B) Plots of fluorescence intensities of buffer solutions of **3** at 462 nm versus concentrations of DNA and BSA.

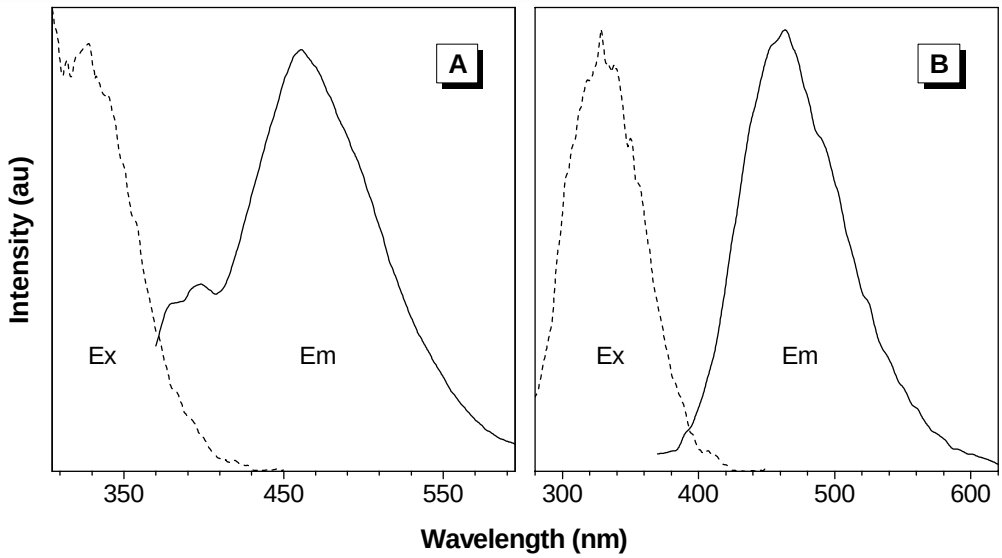

Figure S5. Excitation (Ex) and emission (Em) spectra of buffer solutions (2.5 μM) of (A) **3** in the presence of 500 μg/mL of BSA and (B) **4** (2.5 μM) in the presence of 300 μg/mL of ct DNA.

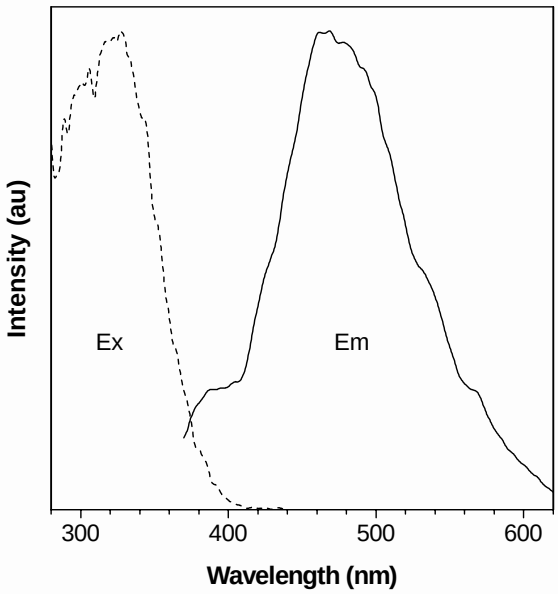

Figure S6. Excitation (Ex) and emission (Em) spectra of **4** (2.5 μM) in a glycerol/water mixture (99:1 by volume) at 25 °C.

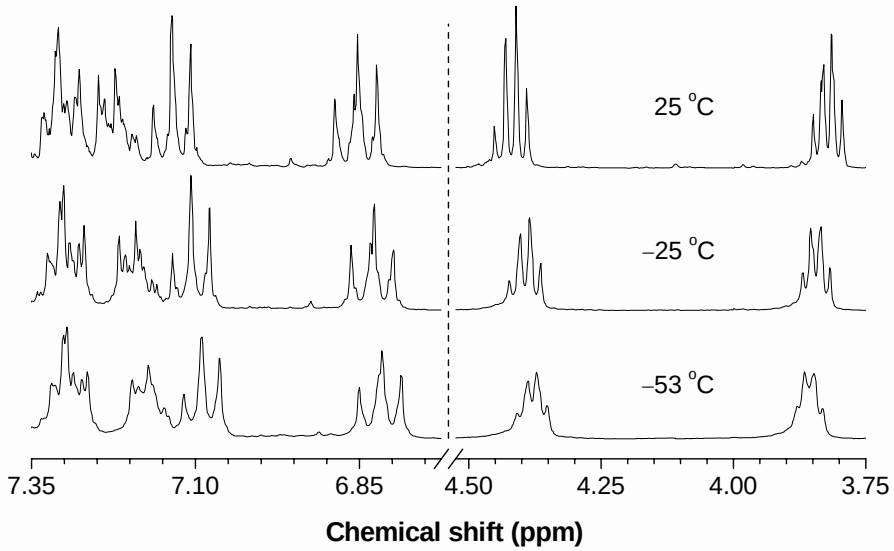

Figure S7. ^{1}H NMR spectra of **1** in dichloromethane-d_2 at different temperatures.

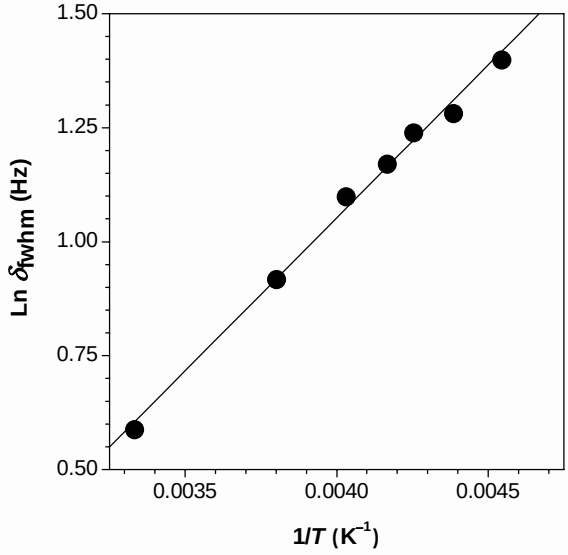

Figure S8. Effect of temperature (T) on the full width at half-maximum of resonance peak (δ_{fwhm}) of phenyl protons of **1** at δ~6.8 in dichloromethane-d_2.

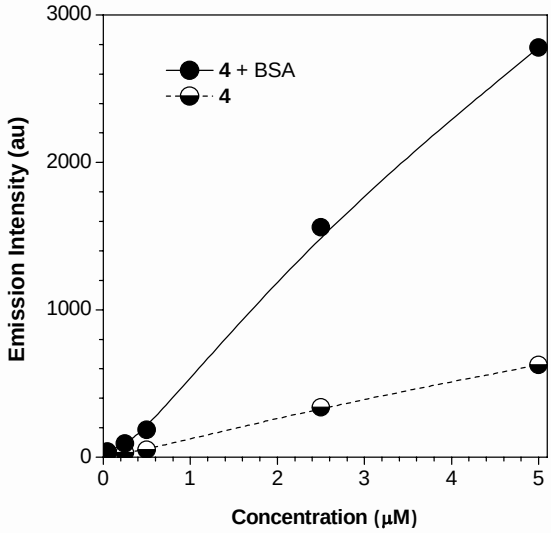

Figure S9. Effect of concentration on the emission intensity of aqueous phosphate buffer solution (pH = 7.0) of **4** at 463 nm in the absence or presence of BSA (100 μg/mL).