Supplementary Information

Diazobenzene-containing conjugated polymers as dark quenchers

JiataoWu,[a,b] Ying Tan,[b] YonghuaXie,[b,c] Yi Wu,[a,b] RuiZhao,[b] YuyangJiang[b,c*] and ChunyanTan[b,*]

[a]The Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education)Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China;

[b]The Ministry-Province Jointly Constructed Base for State Key Lab-Shenzhen Key Laboratory of Chemical Biology, the Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, P. R. China;

[c]Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Beijing 100084, P. R. China

RECEIVED DATE (automatically inserted by publisher); Email: tancy@sz.tsinghua.edu.cn

Experimental

Materials. 1,2-bis(4-iodophenyl)diazene, 1-(4-iodophenyl)-2-phenyldiazene, 1,2-bis (4-iodophenyl) hydrazine and 1,4-diiodo-2,5-hydroquinone were synthesized according to literature reported procedures.[1-3] 7-amino-4-methylcoumarin (AMC), coumarin 314, coumarin 6, rhodamine 6G were purchased from Aladdin (Shanghai, China). Rhodamine 123 was purchased from Aldrich (USA). Fish sperm native DNA and acridine orange hemi (zinc chloride) salt were purchased from Sangon Biotech (Shanghai, China). The water used in all experiments was prepared in a SG water purification system and displayed a resistivity of ≥ 18.2 MΩcm^{-1}.

Azo-PPE-$CO_2C_{12}H_{25}$. A solution of didodecyl 2,2'-(2,5-diethynyl-1,4-phenylene) bis(oxy)diacetate (306 mg, 0.5mmol) and 1,2-bis(4-iodophenyl) diazene (217 mg, 0.5mmol) in 20 mL of dry THF/Et_3N (v/v = 2/1) fitted with a condenser were degassed with argon for 5 minutes. Then 17.4 mg of $Pd(PPh_3)_4$ (15 μmol) and 8 mg of CuI (15 μmol) were added under argon. The reaction mixture was stirred at 60 ºC for 20 hr. The obtained reaction solution was poured into 300 mL of methanol, and the precipitation were further purified by two repeated cycles of dissolution in THF and precipitation into methanol and light yellow fiber solid was collected and dried (265 mg, 67%): ^{1}H NMR (400 MHz, $CDCl_3$-d_6) 7.93 (br, s, 4H), 7.71 (br, s, 4H), 7.04 (br, d, 2H), 4.76 (br, t, 4H), 4.25 (br, t, 4H), 1.68 (br, d, 4H), 1.23 (br, d, 36H), 0.68 (br, t, 6H); ^{13}C NMR (100 MHz, $CDCl_3d_6$) 168.56, 153.46, 152.04, 138.43, 132.59, 124.53, 123.08, 118.24,67.09, 65.63, 31.90, 29.68, 29.62, 29.58, 29.50, 29.32, 29.23, 28.59, 25.84, 22.66, 14.07. Mn: 2.41×10^4, Mw: 5.81×10^3, PDI: 2.412.

Phz-PPE-$CO_2C_{12}H_{25}$. A solution of didodecyl 2,2'-(2,5-diethynyl-1,4-phenylene)bis(oxy) diacetate (121 mg, 0.2mmol) and 1,2-bis(4-iodophenyl)hydrazine (87.2 mg, 0.2mmol) in 20 mL of dry THF/Et_3N (v/v = 3/1) and 0.5 ml hydrazine monohydrate fitted with a condenser were degassed with argon for 5 minutes. Then 12 mg of $Pd(PPh_3)_4$ (10 μmol) and 5.3 mg of CuI (10μmol) were added under argon. The reaction mixture was stirred at 60 ºC for 48 hr. The obtained reaction solution was poured into 300 mL of methanol, and the precipitation were further purified by two repeated cycles of dissolution in THF and precipitation into methanol and light yellow fiber solid was collected and dried (82 mg, 56%): ^{1}H NMR (400 MHz,

$CDCl_3$-d_6) 7.93 (br, s, 4H), 7.71 (br, s, 4H), 7.52 (br, s, 2H), 7.04 (br, d, 2H), 4.76 (br, t, 4H), 4.25 (br, t, 4H), 1.67 (br, d, 4H), 1.23 (br, d, 36H), 0.86 (br, t, 6H).

Model compound. A solution of didodecyl 2,2'-(2,5-diethynyl-1,4-phenylene)bis(oxy) diacetate (153 mg, 0.25 mmol) (2) and 1-(4-iodophenyl)-2-phenyldiazene (154 mg, 0.5 mmol) in 20 mL of dry THF/Et_3N (v/v = 2/1) fitted with a condenser were degassed with argon for 5 minutes. Then 17.4 mg of $Pd(PPh_3)_4$ (15 μmol) and 8 mg of CuI (15 μmol) were added under argon. The reaction mixture was stirred at 60 ºC for 20 hr. The reaction solvent was removed *in vacuo*. Then the remainders were partitioned between water and CH_2Cl_2. The organic layer was washed with 1M HCl, water, and brine. The organic solution was removed and then dissolved in a minimum of CH_2Cl_2 and loaded on the top of silica column, eluted with CH_2Cl_2 /petroleum ether(v/v = 1/5) and a orange solid was obtained (126 mg, 52%):^{1}H NMR (400 MHz, $CDCl_3$-d_6) 7.93 (d, 8H), 7.71 (d, 4H), 7.52 (m, 6H), 7.04 (s, 2H), 4.75 (s, 4H), 4.24 (t, 4H), 1.67(m, 4H), 1.22 (m, 36H), 0.85 (t, 6H); ^{13}C NMR(100 MHz, $CDCl_3$-d_6) 168.57, 153.47, 152.73, 152.03, 132.55, 131.25, 129.11, 125.70, 122.97, 122.96, 118.29, 114.79, 95.80, 87.50, 67.12, 65.62, 31.90, 29.63, 29.62, 29.58, 29.50, 29.32, 29.23, 28.60, 25.84, 22.65, 14.06.

Azo-PPE-CO_2H. To a solution of Azo-PPE-CO_2R (198 mg, 0.25 mmol) in 30 mL of dioxane/THF (v/v = 5/1) was added 1.5 mL of 1 M $(n\text{-}Bu)_4NOH$ in methanol, and stirred at room temperature for 24 hr. During the course of the hydrolysis reaction, 2 mL of water was systematically added in order to keep the solution clear. Then a solution of 0.20 g of $NaIO_4$ in 3 mL of water was added to the hydrolyzed polymer solution, and the resulting mixture was poured into 400 mL of cold acetone, resulting in the precipitation of azo-PPE-CO_2R as orange powders. The polymer was then dissolved in 50 mL of deionized water (several drops of 1 M NaOH solution were added) and was purified by dialysis against deionized water using a regenerated cellulose membrane (7 kD molecular weight cut-off). After dialysis, the solution was stored as the stock solution in the refrigerator.

Instrumentation. Fluorescence quenching study was performed on a SPEX Flurolog 3-TCSPC instrument with 1 cm path length cuvette, and absorption spectra were recorded on a Beckman DU 800 spectrophotometer. NMR spectra were recorded on Bruker 400 MHz spectrometers, and chemical shifts were reported in ppm using TMS as internal reference.

Quenching experiments. The concentration of stock azo-PPE-CO_2R stock solution concentration was 1.1 mM and azo-PPE-CO_2H was 0.62 mM (all concentrations are provided as polymer repeat unit concentration, [PRU]). The stock concentration of the dyes: AMC, coumarin 314, coumarin 6, rhodamine 123, rhodamine 6G were 2 mM in DMSO, model compound and DABCYL were 1 mM in $CHCl_3$. The stock solution was diluted as needed to prepare solutions used for spectroscopic experiments. Final concentrations of the diluted azo-PPE-CO_2R and azo-PPE-CO_2H solutions were determined based on the polymers's extinction coefficient $\varepsilon = 5.4 \times 10^5$ $M^{-1}cm^{-1}$ and 1.0×10^5 $M^{-1}cm^{-1}$, respectively. For a typical quenching experiment, 2 mL of a 2 µM dye solution in $CHCl_3/CH_3CN$ (v/v = 1/1) was placed in a 1 cm quartz fluorescence cell. The UV-visible absorption and fluorescence spectra were recorded at roomtemperature. Then absorption and/or fluorescence spectra were repeatedly acquired after the addition of microliter aliquots of a concentrated quencher by use of 10 µL and 100 µL syringe.

Fluorescence quenching and recovery of acridine orange by azo-PPE-CO_2H and fish sperm native DNA. The concentration of stock azo-PPE-CO_2H was 0.62 mM (all concentrations are provided as polymer repeat unit concentration, [PRU]). The concentrations of acridine orange stock solution and fish sperm native DNA were 50 µM and 5 mg/ml in water, respectively. The quenching experiment was carried out in 25 nM acridine orange solution by titrations of azo-PPE-CO_2H until 7.7 µM. The fluorescence recovery was then measured by titrations of fish sperm native DNA until its concentration reached 15 µg/ml.

Results

1. ^{1}H NMR characterization of the model compound azo-PE, azo-PPE-CO_2R, and phz-PPE-CO_2R.

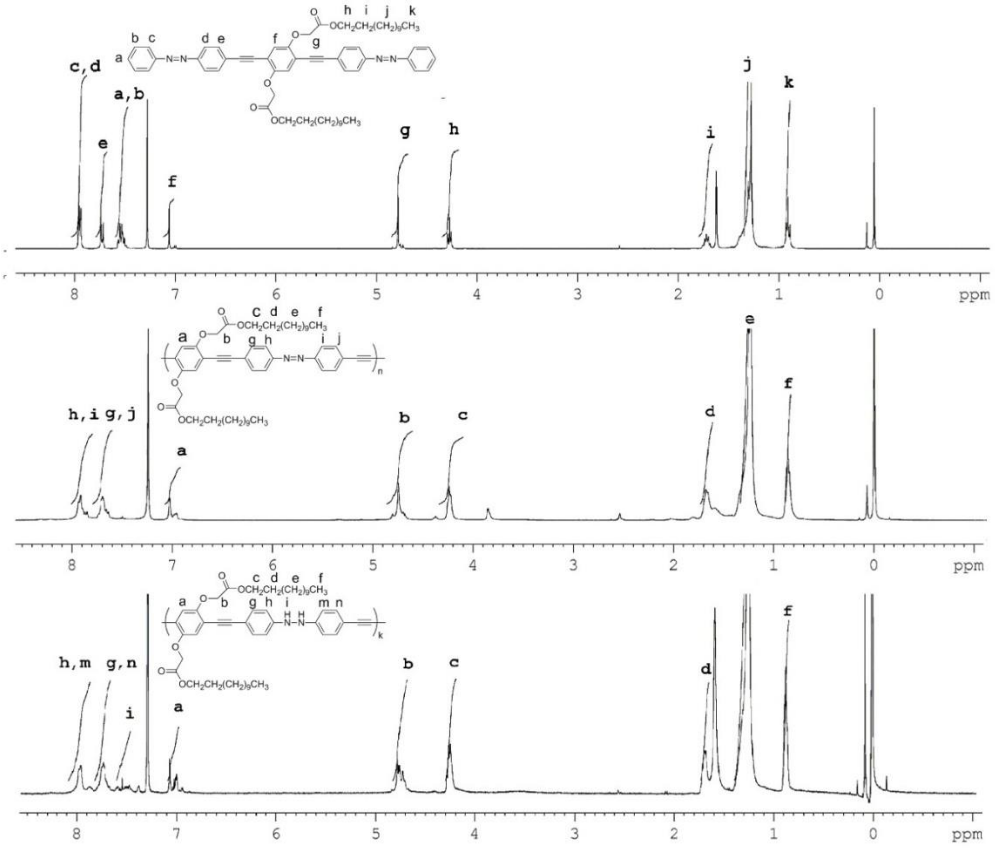

Figure S1. ^{1}H NMR characterization of the model compound, azo-PPE-CO_2R and Phz-PPE-CO_2R.

2. Infrared absorption spectrum of azo-PPE-CO_2R and phz-PPE-CO_2R.

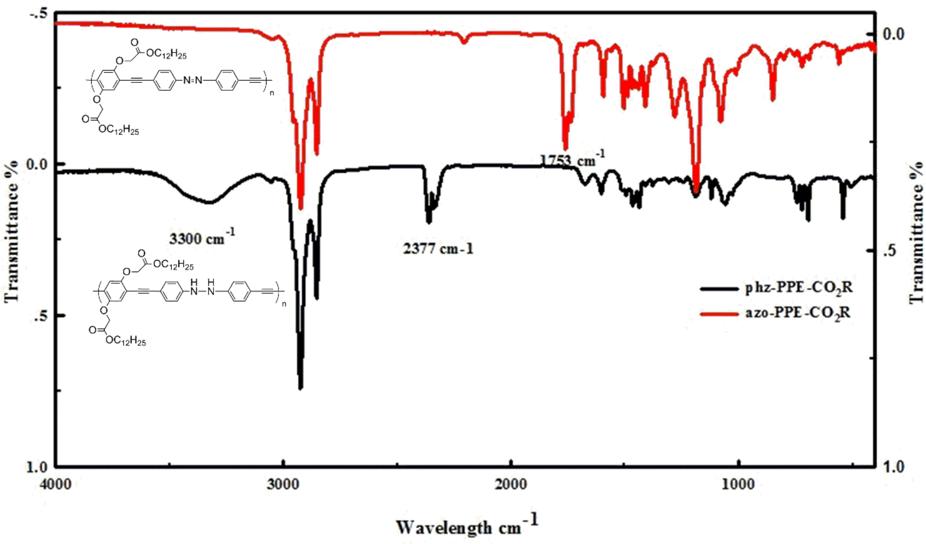

Figure S2. Infrared absorption spectra of azo-PPE-CO_2R and phz-PPE-CO_2R.

3. Photophysical characterization of the polymers.

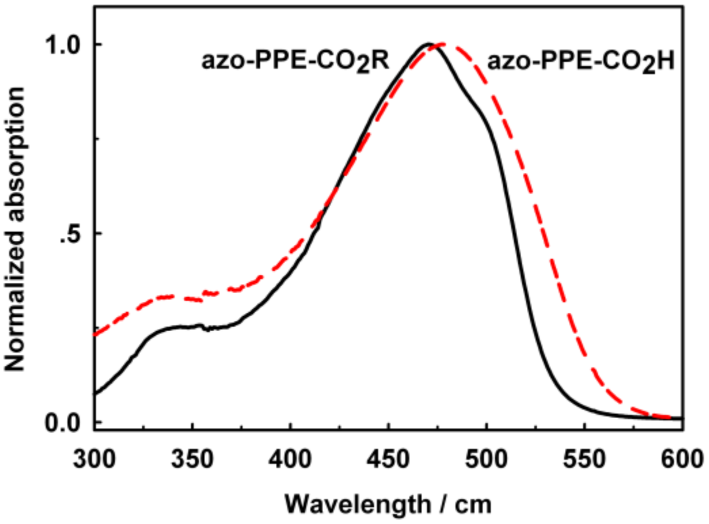

Figure S3. Absorption spectra of azo-PPE-CO_2R (—) in acetonitrile and azo-PPE-CO_2H (– –) in aqueous solution..

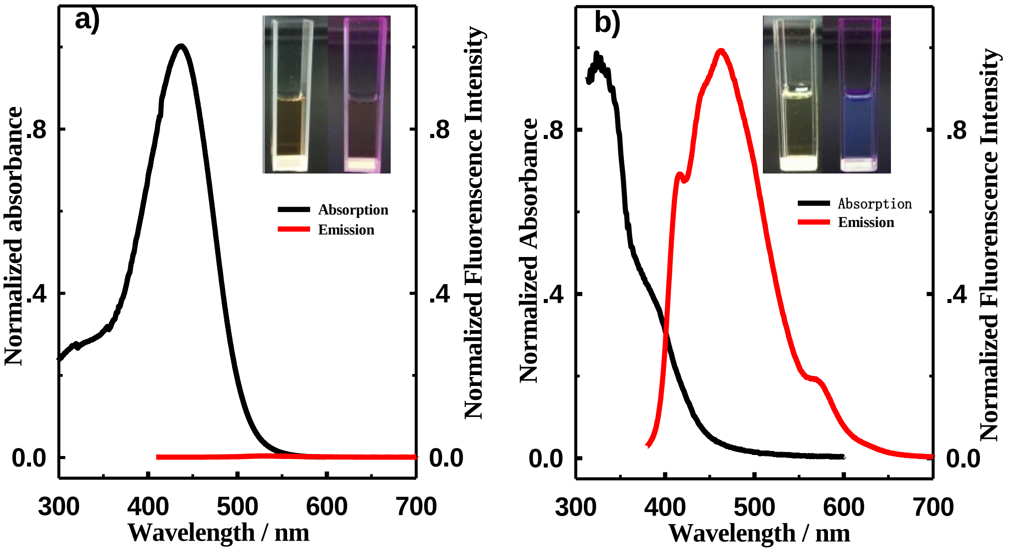

Figure S4. Absorption (black) and emission spectra (red) of azo-PPE-CO_2R (left) and phz-PPE-CO_2R (right). Insets show the pictures of corresponding samples under the normal light and UV irradiation at 360 nm.

4. Spectral overlap between the normalized absorption of model compound and dabcyl and the emission of some fluorescent dyes.

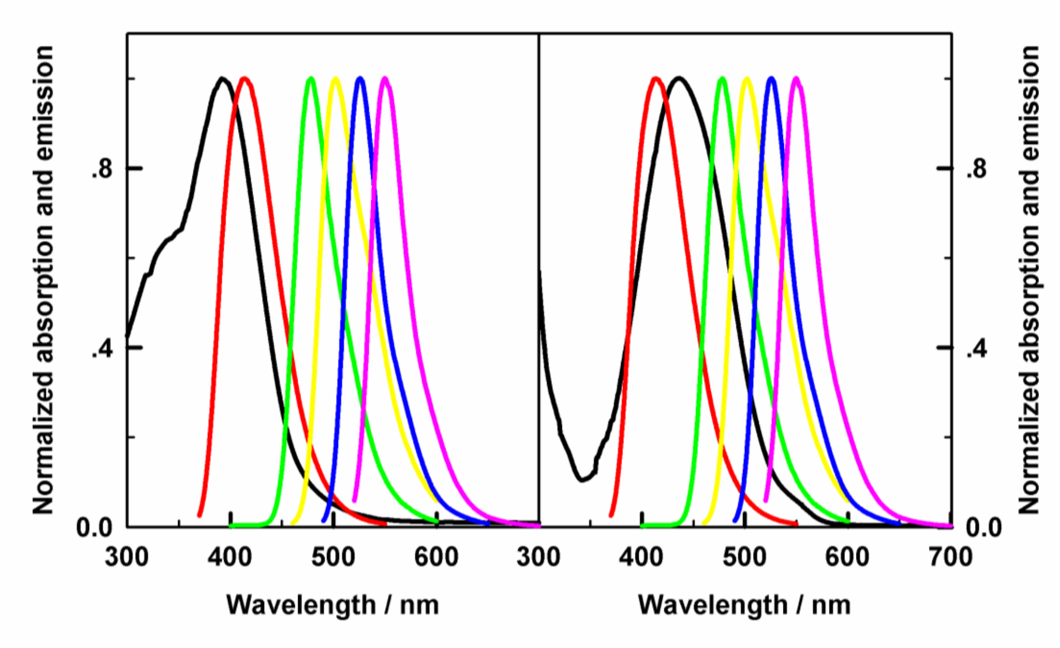

Figure S5. Spectral overlap between the normalized absorption of model compound (left, black), dabcyl (right, black) and the emission of fluorescent dyes including AMC (red), coumarin 314 (green), coumarin 6 (yellow), rhodamine 123 (blue) and rhodamine 6G (purple), respectively.

5. Steady-state fluorescence quenching study.

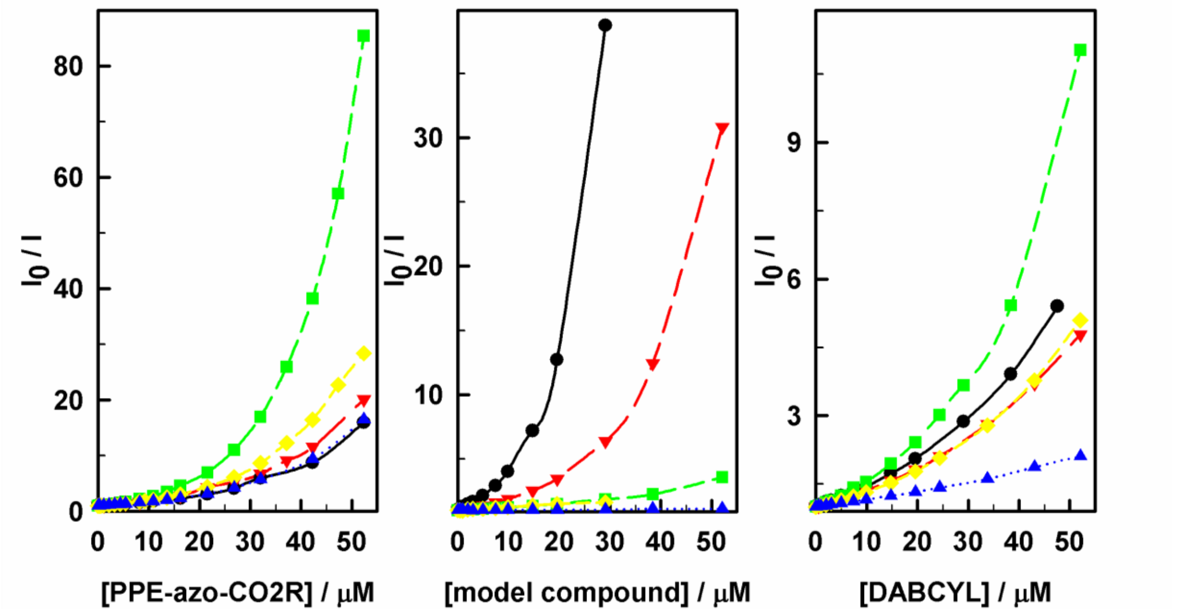

Figure S6. Stern-Volmer plots of quenching of AMC (black), coumarin 314 (red), coumarin 6 (green), rhodamine 123 (yellow), rhodamine 6G (blue) by azo-PPE-CO_2R, model compound and Dabcyl.

6. FRET parameters calculation

The Förster radius, R_0, which is the distance between the donor and the acceptor when the energy transfer efficiency is 50%, is calculated by the equation:

$R_0 = 0.211 \times [\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6}$,

where κ^2 is the dipole orientation factor (range 0 to 4, $\kappa^2 = 2/3$ for randomly oriented donors and acceptors), Q_D is the fluorescence quantum yield of the donor in the absence of the acceptor, n is the refractive index of the solvent, $J(\lambda)$ is the spectral overlap integral.

Table S1. FRET parameters Calculation of the quenchers and dyes.

Model compound

	Q_D	$J(\lambda)$	R_0 (Å)	K_{SV} (M^{-1})
AMC	0.66	9.57×10^{14}	46.16	2.15×10^{5}
Coumarin 314	0.68	1.69×10^{14}	34.76	1.99×10^{5}
Coumarin 6	0.79	7.74×10^{13}	31.28	4.39×10^{4}
Rhodaime 123	0.9	4.33×10^{13}	29.02	1.11×10^{4}
Rhodamine 6G	0.95	2.17×10^{13}	26.09	4.52×10^{3}

Dabcyl

	Q_D	$J(\lambda)$	R_0 (Å)	K_{SV} (M^{-1})
AMC	0.66	4.52×10^{14}	40.74	4.44×10^{4}
Coumarin 314	0.68	6.03×10^{14}	42.96	4.75×10^{4}
Coumarin 6	0.79	5.62×10^{14}	43.52	5.82×10^{4}
Rhodaime 123	0.9	5.11×10^{14}	43.78	3.14×10^{4}
Rhodamine 6G	0.95	3.85×10^{14}	42.14	1.60×10^{4}

Azo-PPE-CO_2R

	Q_D	$J(\lambda)$	R_0 (Å)	K_{SV} (M^{-1})
AMC	0.66	1.19×10^{15}	47.87	7.19×10^{4}
Coumarin 314	0.68	1.96×10^{15}	52.26	8.74×10^{4}
Coumarin 6	0.79	1.37×10^{15}	50.48	1.27×10^{5}
Rhodaime 123	0.9	7.17×10^{14}	46.32	9.97×10^{4}
Rhodamine 6G	0.95	1.78×10^{14}	37.06	6.97×10^{4}

7. Absorption and fluorescence emission quenching spectra of rhodamine 6G by azo-PPE-CO_2H in methanol

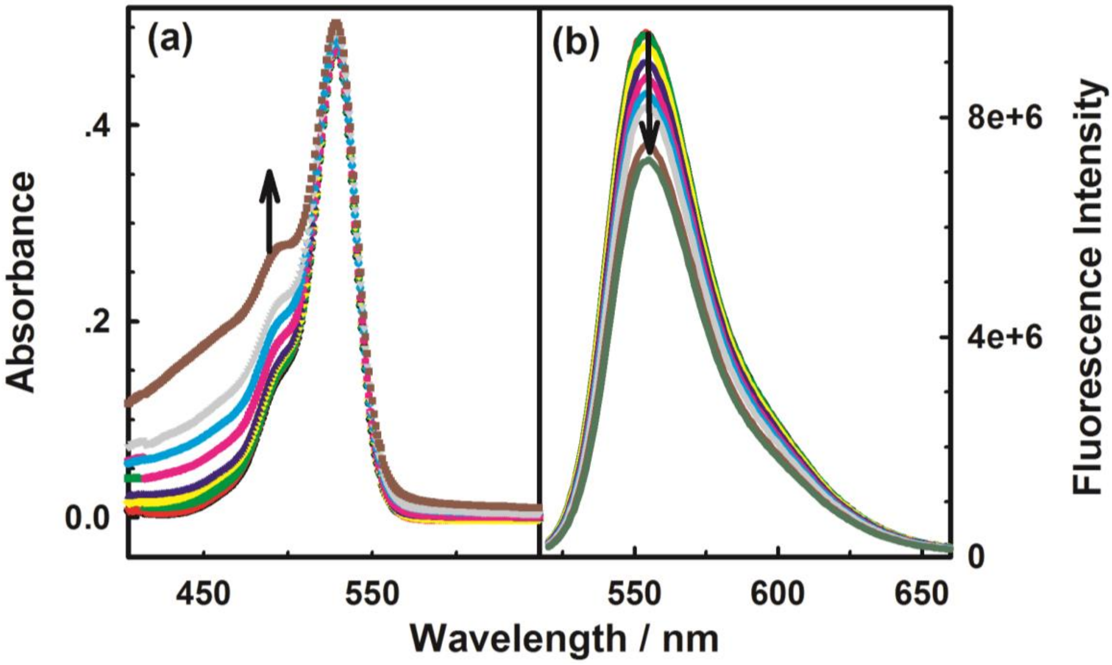

Figure S7. Absorption and fluorescence emission quenching spectra of rhodamine 6G by azo-PPE-CO_2H in methanol (a and b).

References:

(1) Funston, A. M.; Silverman, E. E.; Miller, J. R.; Schanze, K. S. *The Journal of Physical Chemistry B* **2004**, *108*, 1544.

(2) Prasad, H.; Gowda, S.; Channe Gowda, D. *Synthetic communications* **2004**, *34*, 1.

(3) Traber, B.; Wolff, J. J.; Rominger, F.; Oeser, T.; Gleiter, R.; Goebel, M.; Wortmann, R. *Chemistry-A European Journal* **2004**, *10*, 1227.