Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

The Donor-Acceptor Complexes of Quantum Dots and Ionic Perylene Diimides for Ratiometric Detection of Double-Stranded DNA

Gyan H. Aryal,[a] Liming Huang,[a*] and Kenneth W. Hunter [a*]

Department of Microbiology and Immunology, School of Medicine, University of Nevada, Reno, NV 89557, USA

Experimental

Unless otherwise stated, all solvents and chemicals were purchased from Sigma Aldrich and used without further purification. ^{1}H- and ^{13}C-NMR spectra were recorded on either a Varian 400 or 500 MHz spectrometer in $CDCl_3$, DMSO-d_6, CF_3COOD, D_2O, or a mixture of D_2O and DMSO-d_6. Mass spectra were recorded with Waters Micromass XQ detector using ESI^-. MALDI-TOF spectra were recorded on a Bruker Autoflex3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS). Quantum dots were purchased from Ocean Nano tech (San Diego, CA, USA). The oligonucleotides used in the studies were custom synthesized by Integrated DNA Technologies, Inc. (IDT).

UV-vis spectra were recorded with a dual-beam Perkin Elmer Lambda 950 Spectrophotometer using UV-WIN Lab version 5.1.5 software. Fluorescence spectra were acquired using a Jobin-Yvon Horiba Fluorolog 3-222 Fluorescence Spectrophometer. 1-cm or 0.5-cm quartz cuvettes were used for both UV-vis and fluorescence studies.

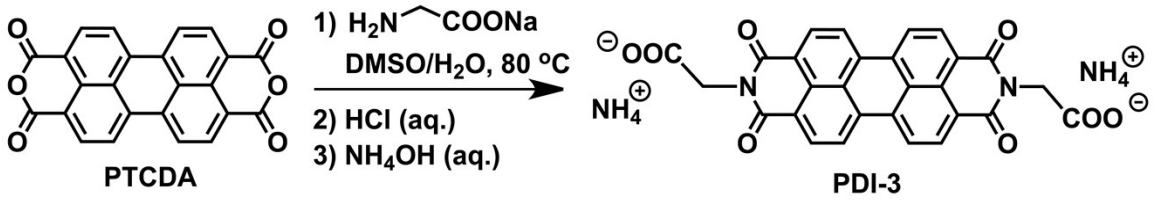
Synthesis of PDI-1

A mixture of 3, 4, 9, 10-perylenetetracarboxylic dianhydride (PTCDA) (0.50 g, 1.27 mmol) and *N, N*-dimethyl ethylene diamine (0.45 g, 5.09 mmol) were added into 10 mL of DMF in a round-bottom flask. The mixture was stirred at 80 ^{o}C overnight under nitrogen. The hot reaction mixture was cooled down to r.t. and precipitated out using excess acetone. The resulting precipitate was collected by centrifugation and washed with acetone. The solid was dried under vacuum at 110 ^{o}C to yield a dark red product **PDI-1** (0.66 g, 97%). ^{1}H-NMR (Varian 400 MHz, CF_3COOD): δ 8.88 (d, *J* = 8.4 Hz, 4H), 8.82 (d, *J* = 8 Hz, 4H), 4.78 (t, *J* = 4.8 Hz, 4H), 3.75 (t, *J* = 6 Hz, 4H), 3.19 (s, 12H); ^{13}C-NMR (100 MHz, CF_3COOD) δ 168.5, 138.9, 136.1, 132.1, 129.1, 127.2, 124.5, 61.0, 46.7, 39.3; MALDI-TOF: m/z = 533.57 $[M+H]^+$ (calc'd. 533.59 for $C_{32}H_{29}N_4O_4^+$).

Synthesis of PDI-2

In a round-bottom flask, compound **PDI-1** (100 mg, 0.19 mmol) and ethylbromide (0.5 mL) were added to toluene (5 mL). The resulting mixture was stirred overnight under nitrogen at 110 ^{o}C. The reaction mixture was precipitated out with ethyl acetate. The solid suspended in ethyl acetate was collected by centrifugation. The resulting product was dissolved in water and filtered using a fine porosity fritted glass filter. The filtrate was collected and the water was removed using rotary evaporation. A solid was collected and dried under vacuum at 110 ^{o}C to yield the product **PDI-2** (90 mg, 0.16 mmol, 62%). ^{1}H-NMR (Varian 400 MHz, CF_3COOD): δ 8.87 (d, *J* = 8.5 Hz, 4H), 8.82 (d, *J* = 8 Hz, 4H), 4.85 (t, *J* = 7.5 Hz, 4H), 3.85 (t, *J* = 8 Hz, 4H), 3.68-3.63 (q, *J* =7 Hz, 4H); 3.33 (s, 12H); 1.57 (t, *J* = 7 Hz, 6H); ^{13}C-NMR (100 MHz, CF_3 COOD) δ 167.8, 138.5, 135.6, 131.7, 128.6, 126.9, 124.2, 63.9, 62.3, 53.1, 37.1, 9.6;. MALDI-TOF: m/z = 590.73 $[M]^{2+}$ (calc'd. 590.71 for $C_{36}H_{38}N_4O_4^{2+}$).

Synthesis of PDI-3



A mixture of 3, 4, 9, 10-perylenetetracarboxylic dianhydride **1** (0.25 g, 0.64 mmol) and glycine sodium salt hydrate (0.25 g, 2.56 mmol) were suspended in 10 mL of DMSO/H_2O (3:1). The mixture was stirred at 80 °C overnight under nitrogen. The hot reaction mixture was allowed to cool and precipitated out using excess acetone. The resulting precipitate was collected by centrifugation and washed with acetone. The solid was dissolved in 10 mL of distilled water and precipitated out by adding 1 mL of aq. HCl (1 M). A solid was collected by centrifugation and washed with excess water to remove the glycine residue. Then the solid was dissolved in aq. NH_4OH solution (5%) and precipitated with excess acetone. A red solid was washed with ethanol and collected using filtration. The solid was dried under vacuum at 80°C to yield the product **PDI-3** (0.28 g, 80%). ^{1}H-NMR (Varian 400 MHz, D_2O/DMSO-d_6 (1;1), 70 °C): δ 8.34 (s, 4H), 8.23 (s, 4H), 4.46 (s, 4H). MS (ESI$^-$, m/z) Calcd for [$C_{28}H_{14}N_2O_8$]: 506.40; Found: = 506.24.

^{1}H- and ^{13}C-NMR of PDI-1, PDI-2, and PDI-3

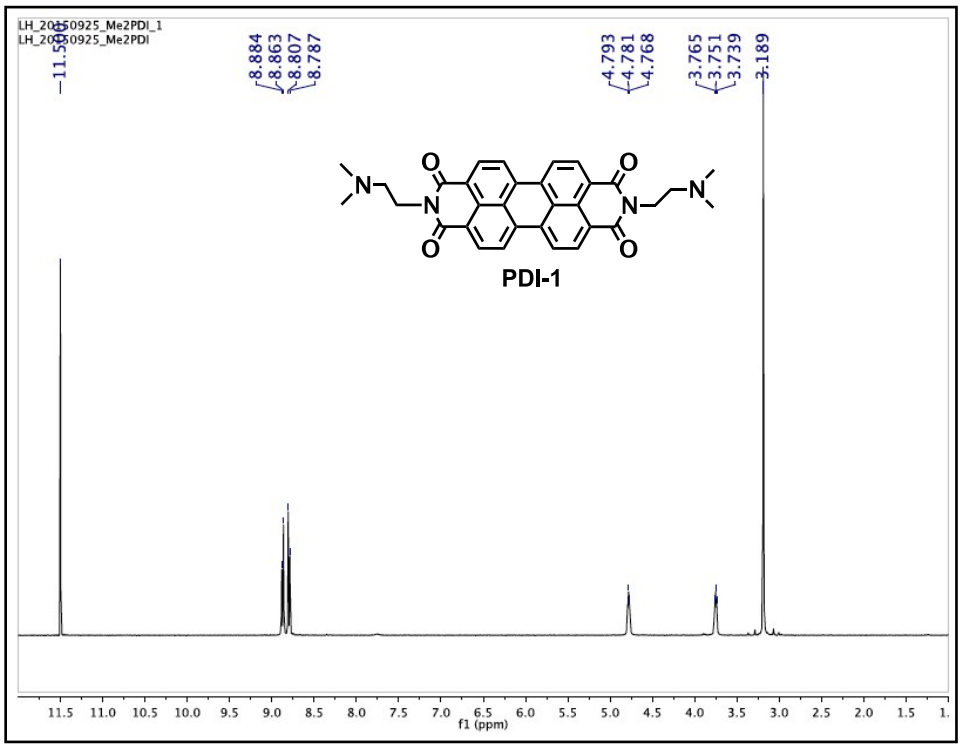


Figure S1. ^{1}H-NMR of **PDI-1** in CF_3COOD.

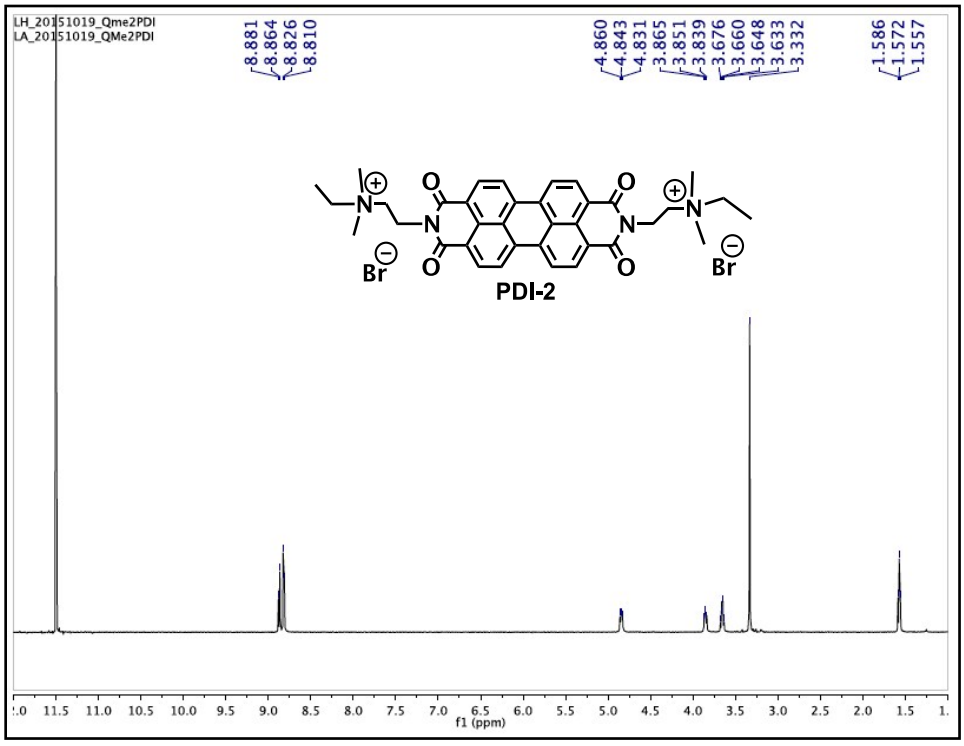


Figure S2. ^{1}H-NMR of **PDI-2** in CF_3COOD.

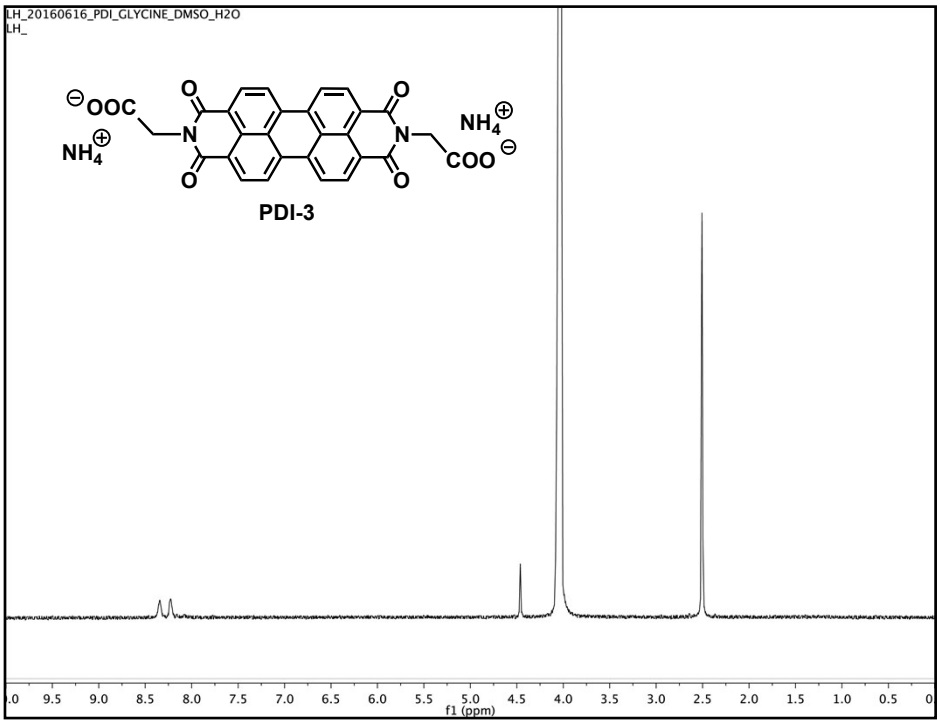


Figure S3. ^{1}H-NMR of **PDI-3** in D_2O/DMSO-d_6 (1:1) at 70 ^{o}C. The sample was heated to 70 ^{o}C due to the poor solubility and strong aggregation of **PDI-3** in D_2O/DMSO-d_6**.**

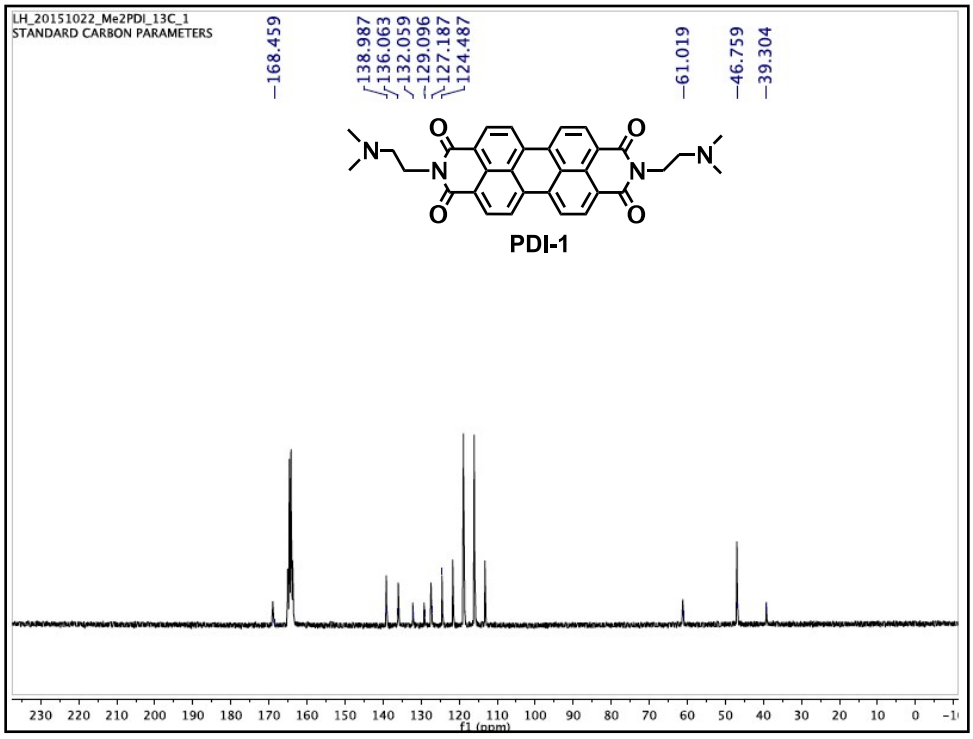


Figure S4. ^{13}C-NMR of **PDI-1** in CF_3COOD.

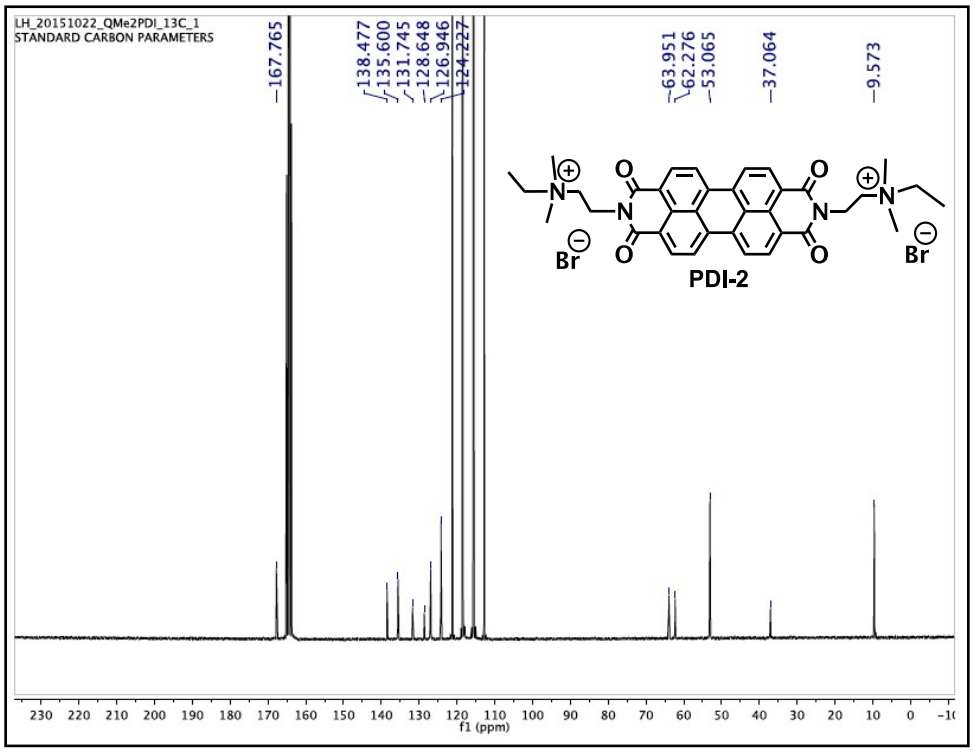


Figure S5. ^{13}C-NMR of **PDI-2** in CF_3COOD.

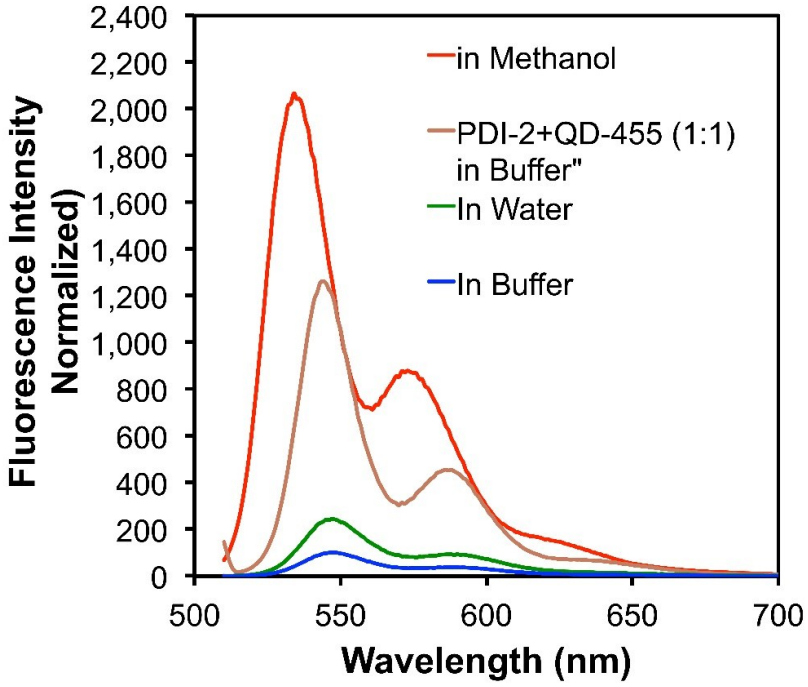


Figure S6. Fluorescence emission spectra of **PDI-2** ($5x10^{-6}$ M) in methanol, water, Tris-HCl buffer (pH 7.4), and in Tris-HCl buffer (pH 7.4) with 1.0 equivalent QD-455. The emission spectra were collected when the samples were excited at 490 nm.

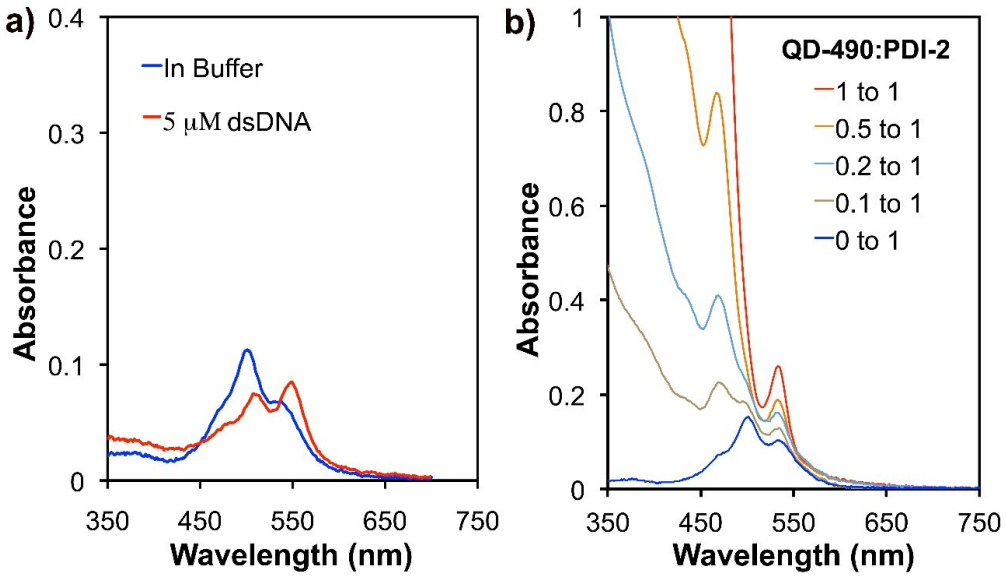


Figure S7. a) UV-vis spectra of **PDI-2** ($5x10^{-6}$ M) in Tris-HCl buffer and in the absence or presence of 5 μM dsDNA S1:S2; b) UV-vis spectra of **PDI-2** ($5x10^{-6}$ M) in the presence of 0, 0.1, 0.2, 0.5, and 1.0 equivalent of QD-490.

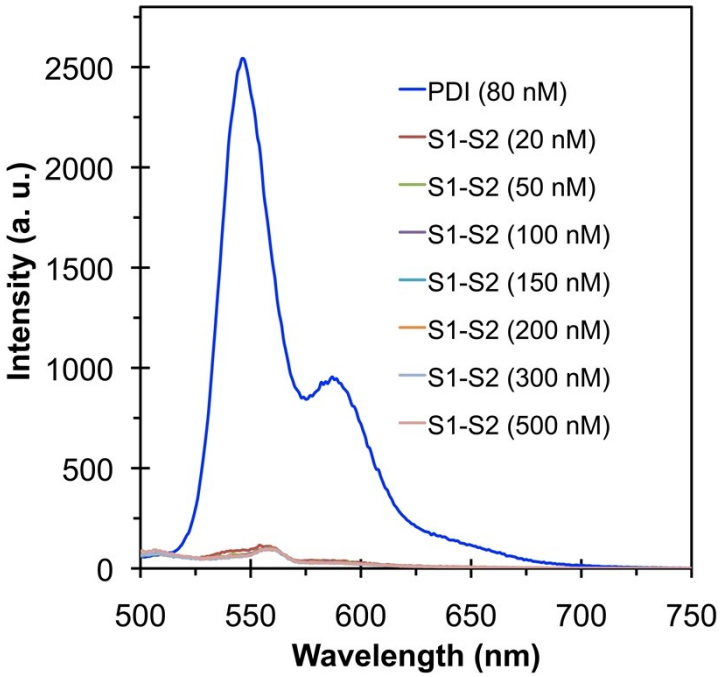


Figure S8. a) Fluorescence spectra of **PDI-2** (80 nM) in Tris-HCl buffer (pH 7.4) in the absence or presence of 20 to 500 nM of dsDNA S1:S2. The emission spectrum was collected when the sample was excited at 490 nm.

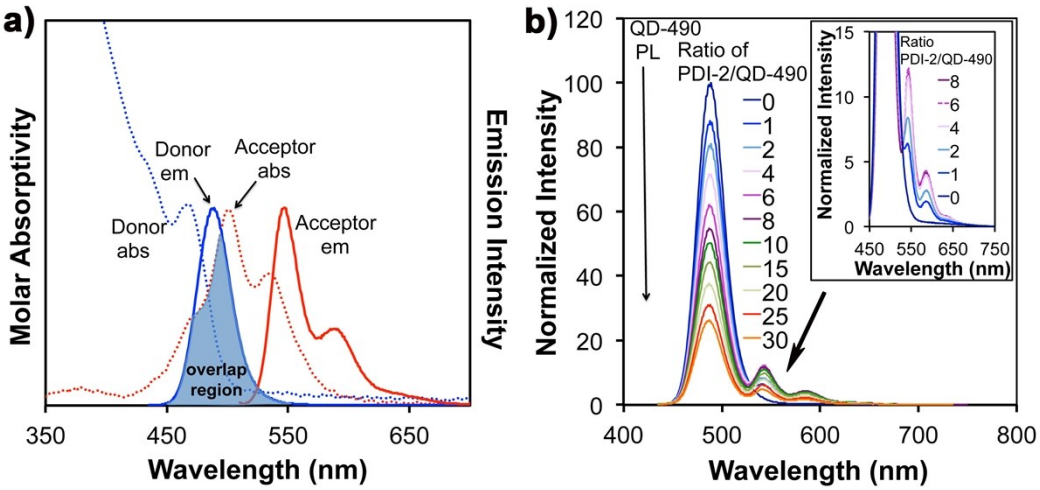


Figure S9. a) FRET diagram. UV-vis molar absorptivity (dashed lines) of QD-490 ($1x10^{-6}$ M) and **PDI-2** ($5x10^{-6}$ M). The absorptivity of **PDI-2** was multiplied by 8. Emission spectra (solid lines, normalized) of QD-490 ($2x10^{-8}$ M) and **PDI-2** ($1.0x10^{-6}$ M) in Tris-HCl buffer (pH 7.4) are shown; b) Emission spectra of QD-490 ($2.0x10^{-8}$ M) in the absence and presence of **PDI-2** with a molar ratio from 0 to 30. Inset: FRET fluorescence emission in the range from 450 to 750 nm. The emission spectra were collected when the samples were excited at 405 nm.

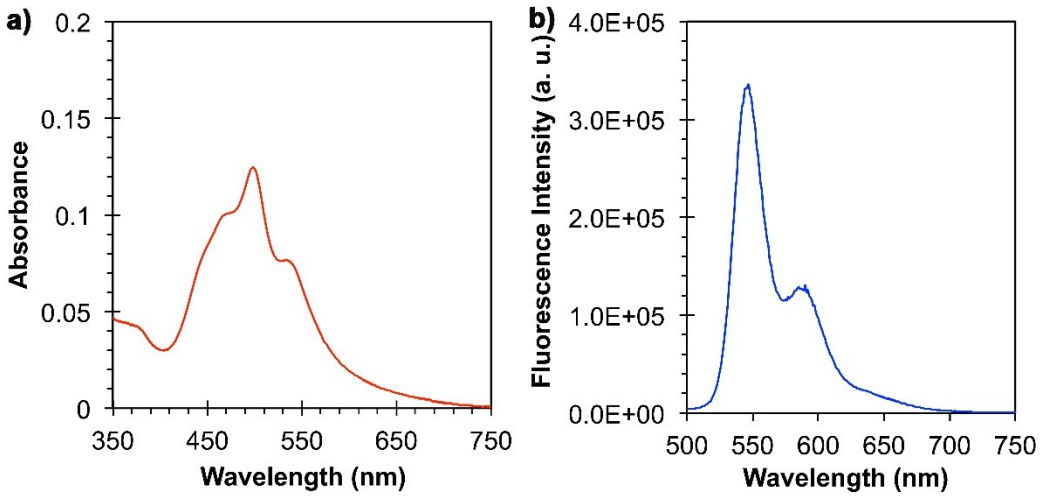


Figure S10. a) UV-vis spectra of **PDI-3** (1.0×10^{-5} M) in Tris-HCl buffer (pH 7.4); b) Fluorescence spectrum of **PDI-3** (1.0×10^{-6} M) in Tris-HCl buffer (pH 7.4). The emission spectrum was collected when the sample was excited at 490 nm.

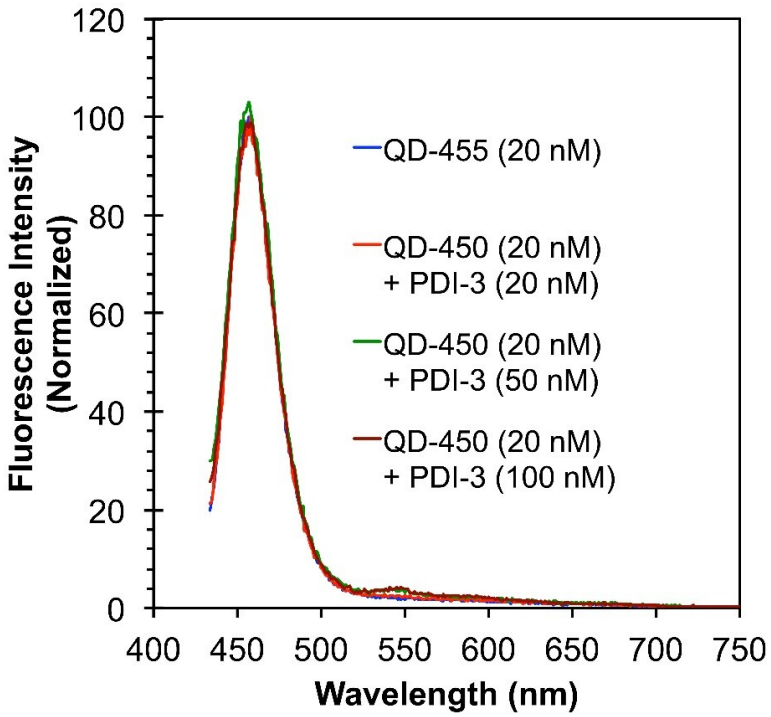


Figure S11. Fluorescence spectra of QD-455 (20 nM in Tris-HCl buffer) in the absence or presence of 20, 50, and 100 nM of **PDI-3**. The emission spectra were collected when the samples were excited at 405 nm.

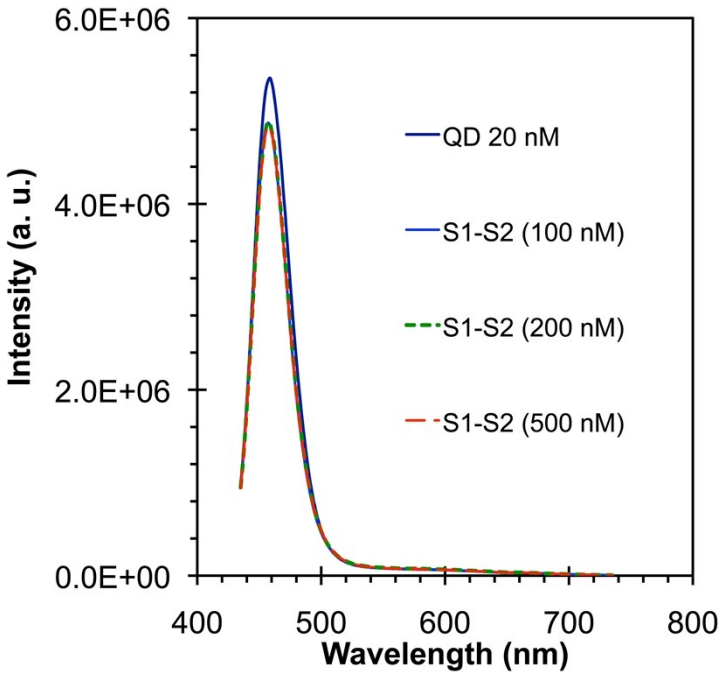


Figure S12. Fluorescence spectra of QD-455 (20 nM in Tris-HCl buffer) in the absence or presence of 100, 200, and 500 nM of dsDNA S1:S2. The emission spectra were collected when the samples were excited at 405 nm.

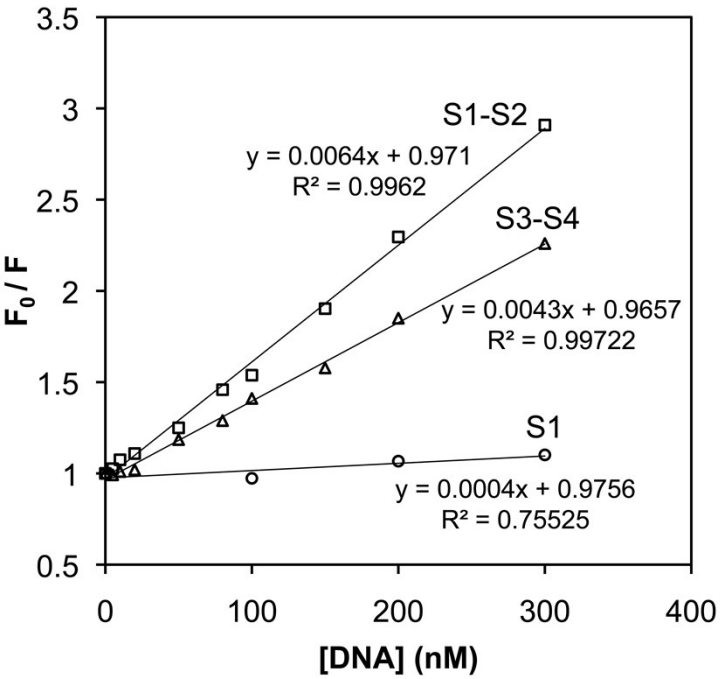


Figure S13. Stern-Volmer plots for the DNA quenching of FRET emission (at 545 nm) of **PDI-2**. K_{SV} quenching constant was calculated according to the equation $F_0/F=1+K_{SV}[DNA]$ (In the equation K_{SV} is referred to as the Stern–Volmer constant, and F_0 and F is the FRET emission intensity of **PDI-2** in the absence and presence of DNA, respectively.)