Electronic Supplementary Information

A New Solvatochromic Fluorophore for Exploring Nonpolar Environments Created by Biopolymers

Abulfazl Fakhari M. and Steven E. Rokita

Contribution from the Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742

General methods. NMR spectra were collected on a Bruker AM500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer and referenced to residual protons in the solvents. Chemical shifts (δ) and coupling constants (*J*) are reported in parts per million (ppm) and Hertz (Hz), respectively. All UV/Vis measurements were obtained with a Hewlett Packard 8453 UV/Vis spectrophotometer, and the fluorescence spectra were collected with a Hitachi F-4500 spectrofluorometer. Extinction coefficients of **2** were measured by serial addition of **2** in MeCN to a cuvette containing the solvent of interest. Absorbance measurements between 0.1 and 1.0 AU were recorded and normalized to the final volume after sequential additions. The average of three independent measurements of $\lambda_{max.abs}$ at each concentration was plotted, and linear best fit of absorbance versus concentration of **2** provided the extinction coefficient.

General materials. All chemicals and reagents of the highest commercial grade were used without further purification unless otherwise noted. Solvents with an assay purity of 99.9% were purchased from Fisher Scientific and used without further purification. All aqueous solutions were prepared with water purified to a resistivity of 17.8-18.0 MΩ.cm. Oligodeoxynucleotides were purchased from IDT (Coralville, IA).

1-Bromo-5-cyanonaphthalene (1). A mixture of 1-cyanonaphthalene (3.1 g, 20 mmol) and $FeCl_3$ (23 mg, 0.14 mmol) was heated to 90 °C in a round bottom flask and stirred for ca. 5 min before dropwise addition of bromine (1.0 ml, 20 mmol). The reaction slurry was stirred at 90 °C for 3 h and then cooled to room temperature. The resulting solidified mixture was recrystallized from ethanol to give **1** as orange needle-shaped crystals in 60% yield (2.8 g, 12 mmol). ^{1}H NMR (d_6-DMSO) δ 8.43 (d, *J* = 8 Hz, 1H), 8.26 (d, *J* = 7 Hz, 1H), 8.1 (d, *J* = 8 Hz, 1H), 8.1 (d, *J* = 7 Hz, 1H), 7.8 (t, *J* = 8 Hz, 1H), 7.7 (t, *J* = 8 Hz, 1H). ^{13}C NMR (d_6-DMSO) 135.5, 134.0, 133.3, 133.1, 131.9, 131.0, 128.6, 125.8, 124.1, 118.3, 110.9. Melting point 146-148 °C (reported 147 °C).[1]

1-Cyano-5-methylaminonaphthalene (2). A mixture of ±-BINAP (53 mg, 0.085 mmol) and anhydrous toluene (25 ml) was sealed in a round-bottom flask with a magnetic stir bar. The mixture was heated to 80 °C until the ±-BINAP dissolved. After cooling the solution to ca. 40 °C, palladium (II) acetate (21 mg, 0.086 mmol) was added. This generated a dark brown solution that was stirred for ca. 10 min at room temperature before consecutive addition of **1** (200 mg, 0.86 mmol) and cesium carbonate (363 mg, 1.11 mmol). The mixture was stirred for an additional 20 min at reflux followed by addition of methylamine (2 M solution in THF, 1.0 ml, 2.0 mmol). The reaction vessel was finally sealed and stirred at 80 °C overnight. The resulting reaction mixture was filtered through a plug of Celite 545®, and the filtrate was concentrated to dryness. The crude product was purified by silica gel flash chromatography (petroleum ether-ethyl acetate 6:1 to 1:1) providing the desired product as a yellow solid in 69% yield (110 mg, 0.59 mmol). ^{1}H NMR (CD_3CN) δ 8.19 (d, *J* = 8 Hz, 1H), 7.93 (d, *J* = 8, 1 Hz, 1H), 7.64 – 7.36 (m, 3H), 6.66 (dd, *J* = 8 Hz, 1H), 5.37 (s, 1H), 2.94 (s, *J* = 5 Hz, 3H). ^{13}C NMR (CD_2Cl_2) δ 146.0, 134.1, 133.0, 130.0, 125.9, 123.9, 123.6, 118.8, 114.0, 111.0, 105.6, 31.3. IR (solid phase) 3405, 2894, 2219, 1582, 1532, 1491, 1408, 1293, 1163, 767 cm^{-1}. HRMS calcd for $C_{12}H_{10}N_2$ 183.0878, found 183.0917 ($[M+H]^+$); mp 150-152 °C.

Deglycosylation of an uracil-containing oligonucleotide and generation of duplex DNA containing abasic sites. An oligonucleotide strand in which Z = uridine (2 nmol) was combined with uracil-DNA deglycosylase (1 unit, New England Biolabs) in 20 μL of Tris-HCl (10 mM, pH 7.4), KCl (50 mM), dithiotreitol (1 mM), and EDTA (1mM) and incubated at 37° C for 3 h. This solution was then extracted with phenol/chloroform and dried under reduced pressure. The resulting DNA was resuspended in an approximately equal volume of water (20 μL) and alternatively annealed to the appropriate complementary strand to form the duplexes in Scheme 2. The desired deglycosylated product was confirmed by MALDI mass spectroscopy (exp. m/z 5381.8 ± 0.9, calc. for $C_{171}H_{219}N_{62}O_{107}P_{17}$, m/z 5381.4) and no remaining starting material was observed (calc for $C_{175}H_{221}N_{64}O_{108}P_{17}$, m/z 5475.6).

Fluorescence quantum yields. Fluorescence quantum yields (Φ) were determined under aerobic conditions using six different pairs of optically matched solutions containing **2** (indicated solvents) and anthracene (ethanol, $\Phi_{anthr} = 0.27$), respectively.[2] Excitation at 346 nm was chosen since the absorption spectrum of **2** is relatively independent of solvent at this wavelength. Excitation and emission slits were set at 5 nm unless otherwise noted, and the UV absorption of each solution at 346 nm was held below 0.2 AU. Fluorescence spectra of the matched solutions were integrated after background correction and plotted against their A_{346} values. The linear fit of these data ($Grad_{EtOH}$ for anthracene in ethanol and $Grad_X$ for **2** in each x solvent) was generated by Origin (6.0) using eq. S1 for which η is the refractive index of the individual solvents. An example of the data is illustrated in Figure S2 for **2** in DMSO.

$$\Phi_{\mathbf{2}} = \Phi_{anthr}\,(Grad_X\,(\mathbf{2}) / Grad_{EtOH}\,(anthracene))(\eta_X / \eta_{EtOH}) \qquad \textbf{eq. S1}$$

E_T(30) value of aqueous buffer. Reichardt's dye (ca. 1 mg) was added to sodium phosphate (10 mM) pH 7 and 100 mM NaCl in a quartz cell (1 cm path length), and the $\lambda_{max.abs}$ value was determined manually and by the "peak-find" function of the spectrophotometer. Three independent measurements were averaged and used to determine the E_T(30) value using equation S2.[3] All measurements were performed under ambient conditions.

$$E_T(30) = 28591\ \text{kcal} / \lambda_{max.abs}\,(\text{nm}) \qquad \textbf{eq. S2}$$

Binding affinity of 2 for duplex DNA. Changes in fluorescence emission intensity and $\lambda_{max.em}$ resulting from parallel titration of each DNA sample into **2** (10 μM) were independently converted to (ΔX). $[DNA]_{free}$ was calculated from the difference of $[DNA]_{total}$ and $[DNA]_{bound}$ for which $[DNA]_{bound}$ is $(\Delta X / \Delta X_{max})[\mathbf{2}]_{total}$. K_d is related to $\Delta X / \Delta X_{max}$ as shown in eq. S3 and calculated as the concentration of **2** at $\Delta X / \Delta X_{max} = 0.5$ by non-linear best fit (Origin 6.0) of $\Delta X / \Delta X_{max}$ versus $[DNA]_{free}$ for a 1:1 binding isotherm.[4]

$$\Delta X / \Delta X_{max} = ([DNA]/K_d) / (1+[DNA]/K_d) \qquad \textbf{eq. S3}$$

References

1. M. Novi, G. Garbarino, G. Petrillo and C. Dellerba, *Tetrahedron*, 1990, **46**, 2205-2212.
2. W. H. Melhuish, *J. Phys. Chem.*, 1961, **65**, 229-235.
3. J. Catalan, J. L. G. de Paz and C. Reichardt, *J. Phys. Chem. A*, 2010, **114**, 6226-6234.
4. K. E. van Holde, in *Physical Biochemistry*, Prentice-Hall, INC. Englewood Cliffs, NJ, 2 edn., 1985, pp. 51-92.

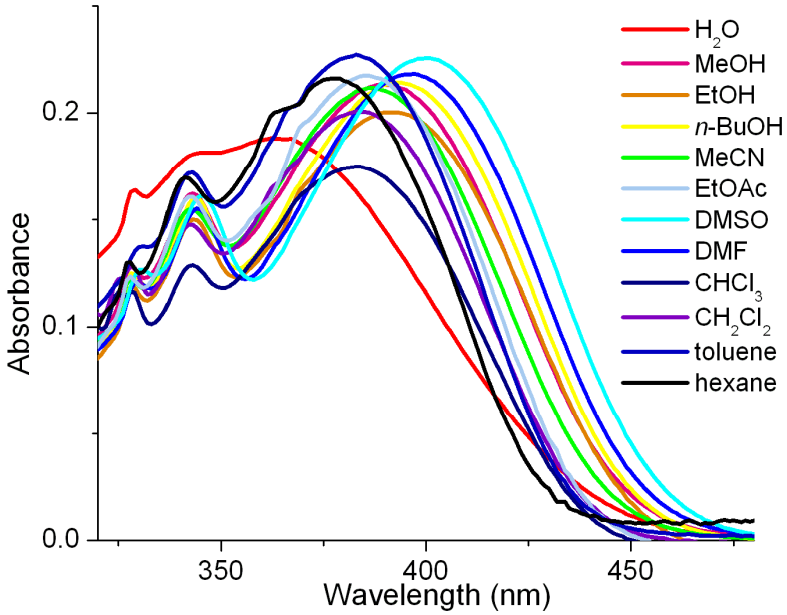

Figure S1. Absorption spectra of 2 in solvents of varying polarity. Solutions of **2** (50 μM) were prepared with the indicated solvents and their spectra were recorded under ambient conditions.

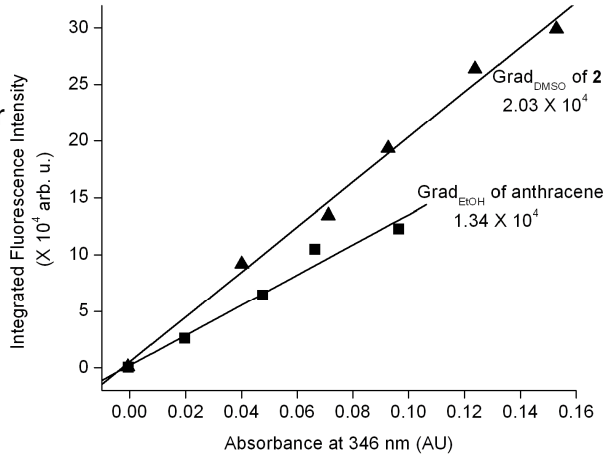

Figure S2. Integrated fluorescence emission versus sample absorbance at 346 nm. The linear best fits for anthracene in ethanol (■) and **2** in DMSO (▲) are proportional to their fluorescence quantum yields. Conversion to an absolute quantum yield is achieved through eq S1.

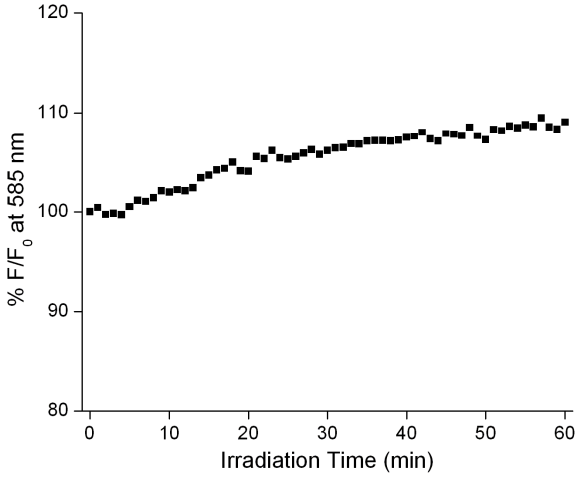

Figure S3. Photochemical stability of 2. A solution of **2** (1 μM) in sodium phosphate (10 mM) pH 7 and 100 mM NaCl was irradiated continuously at 346 nm for 1 h by the spectrofluorometer (excitation slit: 5 nm; emission slit: 10 nm). Fluorescence emission at 585 nm was recorded in 1 sec intervals and averaged per minute. F_0 and F denote fluorescence intensities of **2** initially and after the indicated time of irradiation time, respectively.

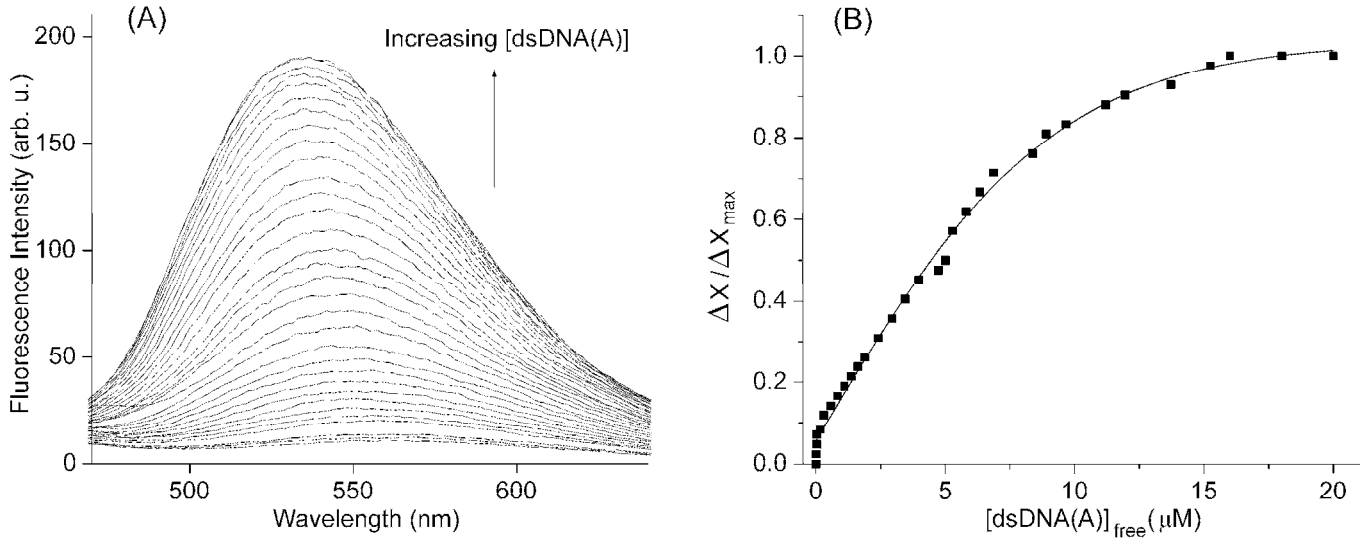

Figure S4. Titration of 2 with an oligonucleotide duplex containing an abasic site counter to an adenine residue, dsDNA(A). (A) The fluorescence emission (excitation: 346 nm) was monitored for a solution of **2** (10 μM) in sodium phosphate (10 mM) pH 7 and 100 mM NaCl after sequential addition dsDNA(A). The titration was stirred for 3 min under ambient conditions after each addition of DNA before the fluorescence spectrum was acquired. Sample volumes remained within 10% of their initial value. (B) Fractional saturation of binding was monitored as a function of the relative hypsochromic shift of $\lambda_{max.em}$ as a function of [dsDNA(A)] and fit to eq. S3 (Origin 6.0) to provide a K_d of 5 μM (R^2= 0.996, σ = 0.023).

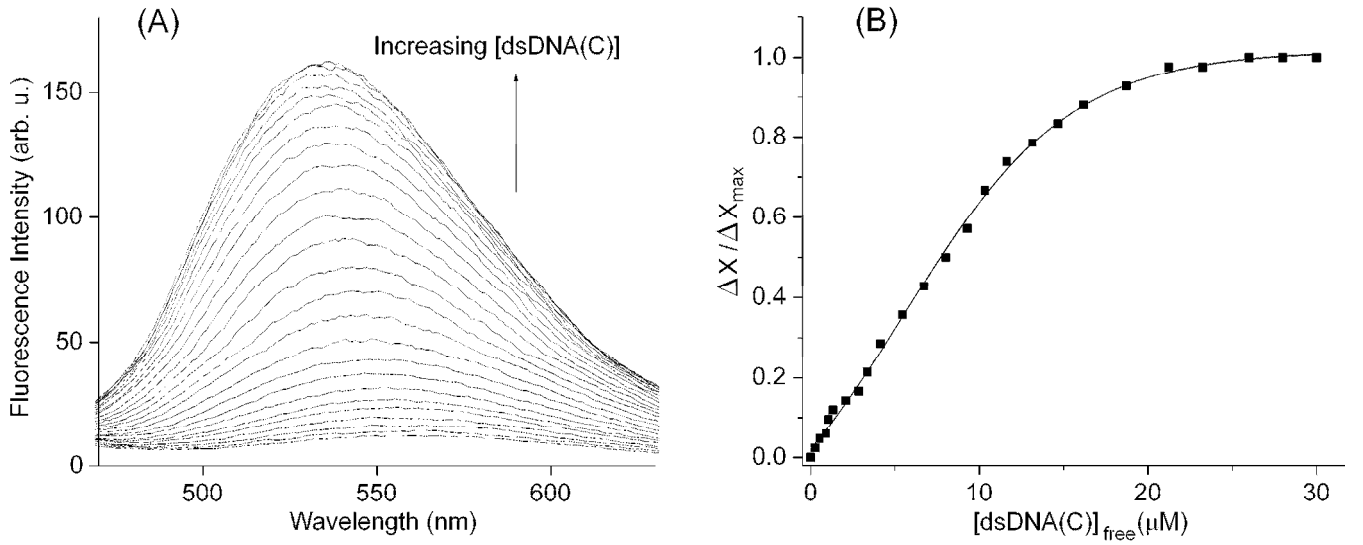

Figure S5. Titration of 2 with an oligonucleotide duplex containing an abasic site counter to an cytosine residue, dsDNA(C). (A) The fluorescence emission (excitation: 346 nm) was monitored for a solution of **2** (10 μM) in sodium phosphate (10 mM) pH 7 and 100 mM NaCl after sequential addition dsDNA(C). The titration was stirred for 3 min under ambient conditions after each addition of DNA before the fluorescence spectrum was acquired. Sample volumes remained within 10% of their initial value. (B) Fractional saturation of binding was monitored as a function of the relative hypsochromic shift of $\lambda_{max.em}$ as a function of [dsDNA(A)] and fit to eq. S3 (Origin 6.0) to provide a K_d of 8 μM (R^2=0.999, σ = 0.014).

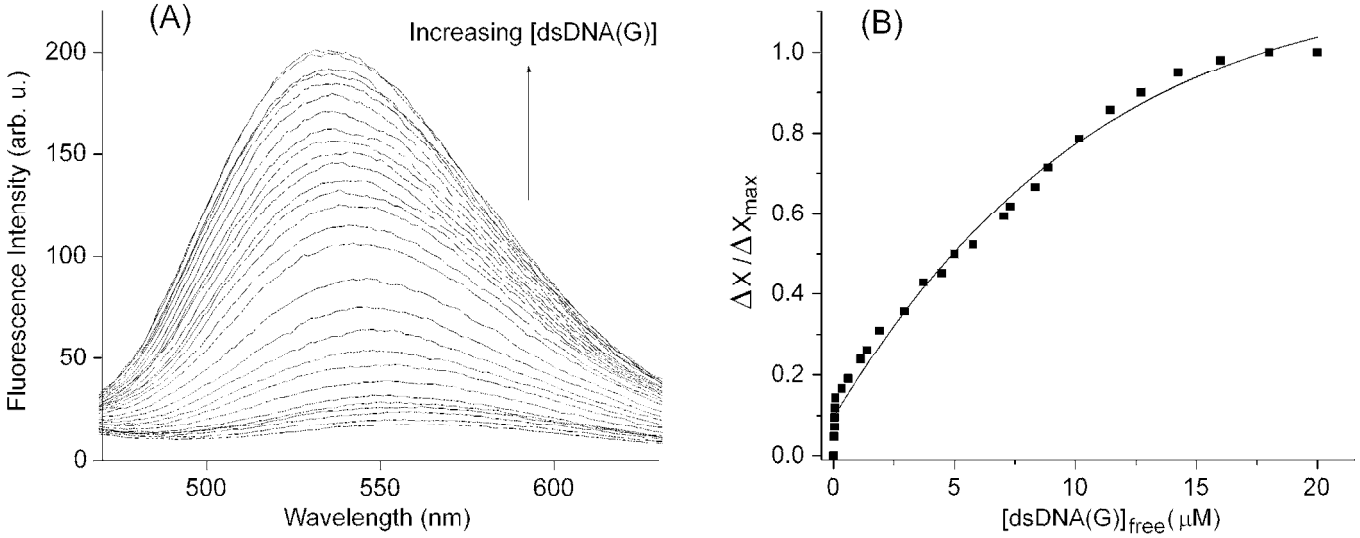

Figure S6. Titration of 2 with an oligonucleotide duplex containing an abasic site counter to an guanine residue, dsDNA(G). (A) The fluorescence emission (excitation: 346 nm) was monitored for a solution of **2** (10 μM) in sodium phosphate (10 mM) pH 7 and 100 mM NaCl after sequential addition dsDNA(G). The titration was stirred for 3 min under ambient conditions after each addition of DNA before the fluorescence spectrum was acquired. Sample volumes remained within 10% of their initial value. (B) Fractional saturation of binding was monitored as a function of the relative hypsochromic shift of $\lambda_{max.em}$ as a function of [dsDNA(A)] and fit to eq. S3 (Origin 6.0) to provide a K_d of 5 μM (R^2=0.989, σ = 0.036).

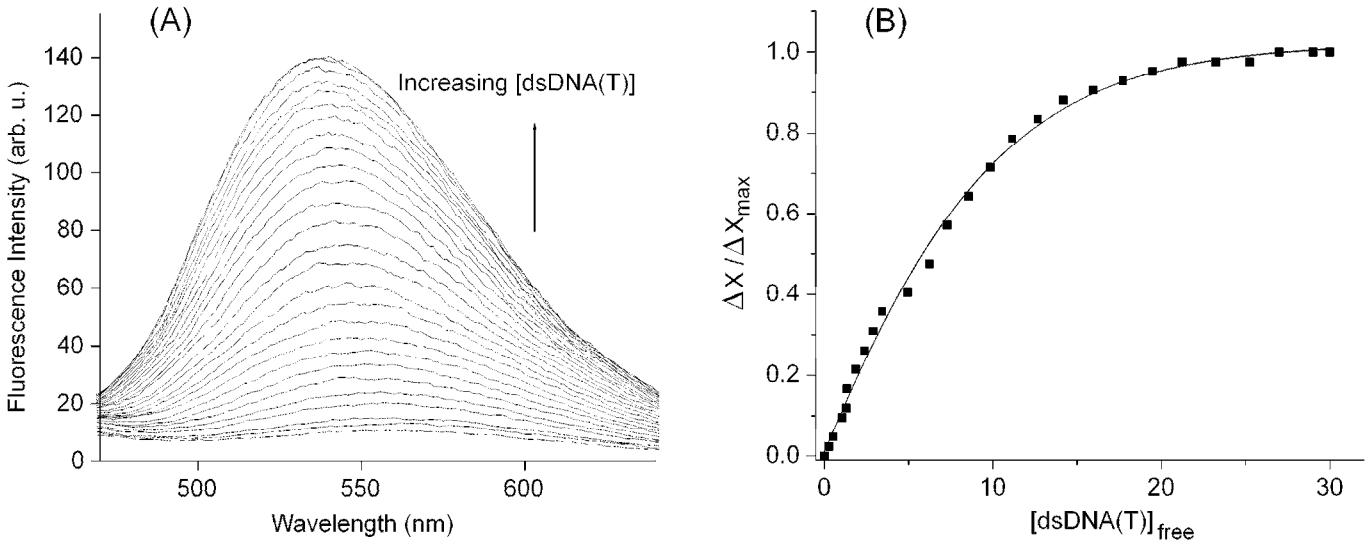

Figure S7. Titration of 2 with an oligonucleotide duplex containing an abasic site counter to an thymine residue, dsDNA(T). (A) The fluorescence emission (excitation: 346 nm) was monitored for a solution of **2** (10 μM) in sodium phosphate (10 mM) pH 7 and 100 mM NaCl after sequential addition dsDNA(T). The titration was stirred for 3 min under ambient conditions after each addition of DNA before the fluorescence spectrum was acquired. Sample volumes remained within 10% of their initial value. (B) Fractional saturation of binding was monitored as a function of the relative hypsochromic shift of $\lambda_{max.em}$ as a function of [dsDNA(A)] and fit to eq. S3 (Origin 6.0) to provide a K_d of 7 μM (R^2=0.997, σ = 0.019).