Supporting information

Syntheses, DNA interactions, and photodynamic antimicrobial properties of near-infrared indolium carbocyanine dyes

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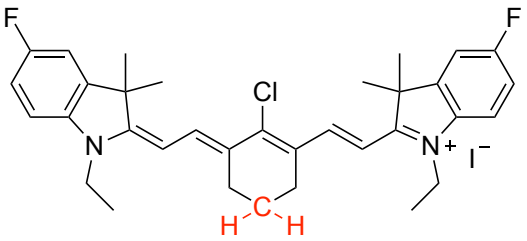
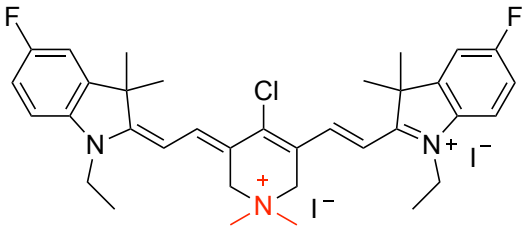


9 *(charge +1, QAC 0)*



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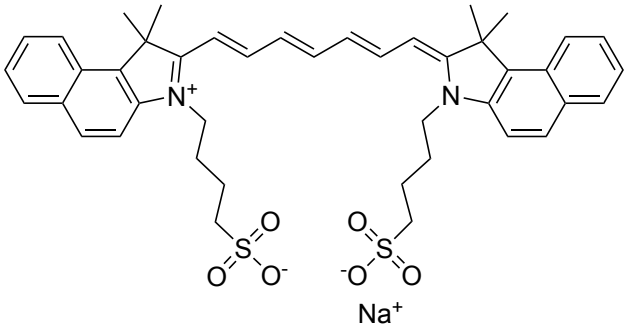
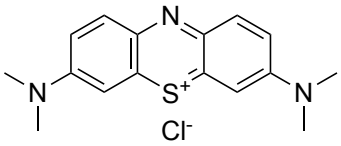


11
indocyanine green (ICG; *net charge -1, QAC 0)*



12
methylene blue (MB; *net charge +1, QAC 0)*

Fig. S1 Chemical structures of the dyes employed in this study. Abbreviations: charge = net charge at pH 7.0; QAC = quaternary ammonium content, excluding quaternary amines by resonance.

Synthetic Procedures: Precursor Compounds **1** through **8** in Scheme 1

5-fluoro-2,3,3-trimethyl-3H-indole (2). Known compound **2** was prepared according to a reported procedure.[1, 2] A solution of commercially available 4-fluorophenylhydrazine (**1**) 15 g (92.3 mmol) and 3-methyl-2-butanone (14.8 mL, 138.4 mmol) in acetic acid (200 mL) was reflux overnight. The solution was then cooled to room temperature and filtered. The solvent was removed under reduced pressure, and the remaining dark-brown residue was dissolved in DCM. The resulting solution was extracted cautiously with $NaHCO_3$ solution (2 times), then washed with water and brine, dried with $MgSO_4$, and concentrated under vacuum to obtain product **2**. Yield: 76 % (16.0 g).

1-ethyl-5-fluoro-2,3,3-trimethyl-3H-indol-1-ium iodide (3).[3] Iodoethane was added to 5-fluoro-2,3,3-trimethyl-3*H*-indole (**2**) in acetonitrile under reflux conditions. The reaction mixture was stirred for 18 h at 85 °C to afford product as a precipitate (**3**). Then, the precipitate was filtered under vacuum and washed with ether to yield the product.

1,1-dimethyl-4-oxopiperidin-1-ium iodide (6). Known compound **6** was prepared according to the reported procedure.[2, 7] Compound **5** was stirred in acetone and excess iodomethane was added. The mixture was stirred at rt for 8 h, while monitoring the reaction progress by TLC. After the reaction was completed, the mixture was filtered, and the white precipitate was washed with acetone and ether. The product **6** was dried under vacuum and used directly in next step without further purification.

(E)-2-chloro-3-(hydroxymethylene)cyclohex-1-ene-1-carbaldehyde (7). Known compound **7** was prepared according to a reported procedure.[4, 5] $POCl_3$ (9 mL, 96 mmol) in DCM (1 mL) was added dropwise to a chilled solution of 1:1 DMF:DCM (10 mL each) in an ice bath. The mixture was stirred for 30 min, then commercially available cyclohexanone (**4**) (4.45 mL, 43 mmol) was added. The resulting solution was refluxed for 3 h at 80 °C, cooled, and poured into ice cold H_2O. The mixture was kept overnight in a 4 °C refrigerator to obtain product **7**. Yield: 70 %.

(E)-4-chloro-5-formyl-3-(hydroxymethylene)-1,1-dimethyl-1,2,3,6-tetrahydropyridin-1-ium (8). Known compound **8** was prepared according to a reported method as follows.[2, 6] DMF was cooled down (9.0 mL, 0.12 mol) to 0 °C and $POCl_3$ was slowly added (5.5 mL, 0.06 mol) to the reaction mixture, which was stirred at 0 °C for 30 min. Then, compound **6** (5.00 g) was transferred to the reaction and the resulting the solution was heated at 80 °C for 3 h. The mixture was cooled down, then aqueous hydrochloric acid (20 % *v/v*, 30.0 mL) was added dropwise and stirred at room temperature for 1 h. The solution was sonicated for 20 min and left to stand in freezer overnight (-20 °C). The precipitated product was subsequently filtered to afford **8**. Yield: 25 %.

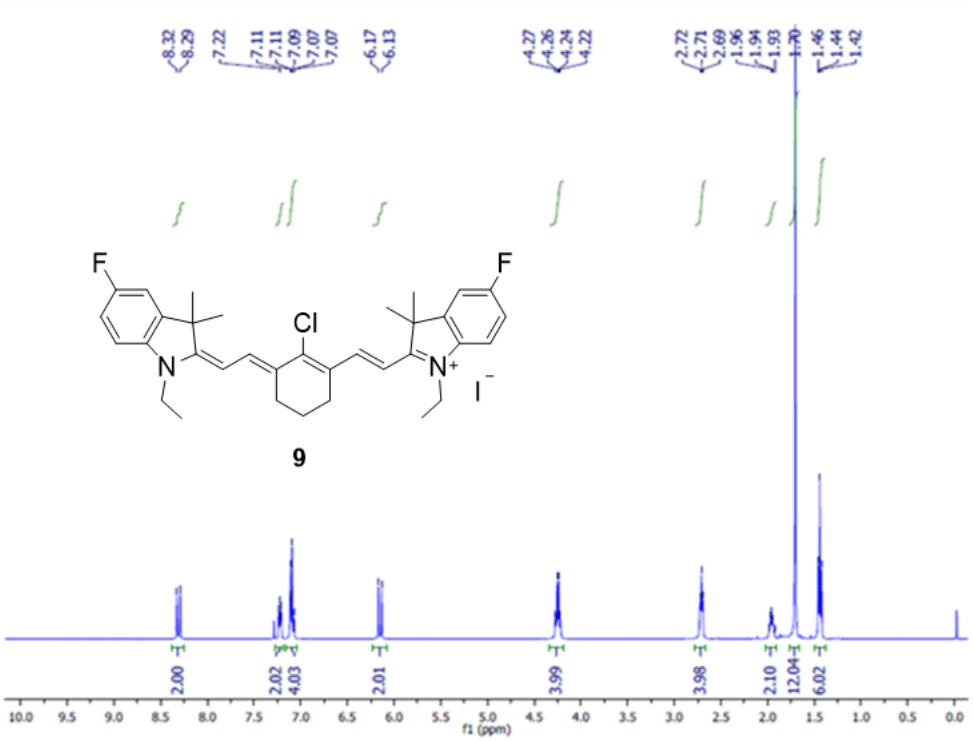


Fig S2 ^{1}H NMR spectrum of dye **9** (400 MHz, $CDCl_3$)**: δ** 8.31 ppm (d, *J* = 14.1 Hz, 2H), 7.22 (dd, *J* = 9.5, 3.7 Hz, 2H), 7.09 (t, *J* = 7.6 Hz, 4H), 6.14 (d, *J* = 14.1 Hz, 2H), 4.24 (q, *J* = 7.1 Hz, 4H), 2.70 (t, *J* = 6.0 Hz, 4H), 2.01 – 1.90 (m, 2H), 1.70 (s, 12H), 1.44 (t, *J* = 7.2 Hz, 6H).

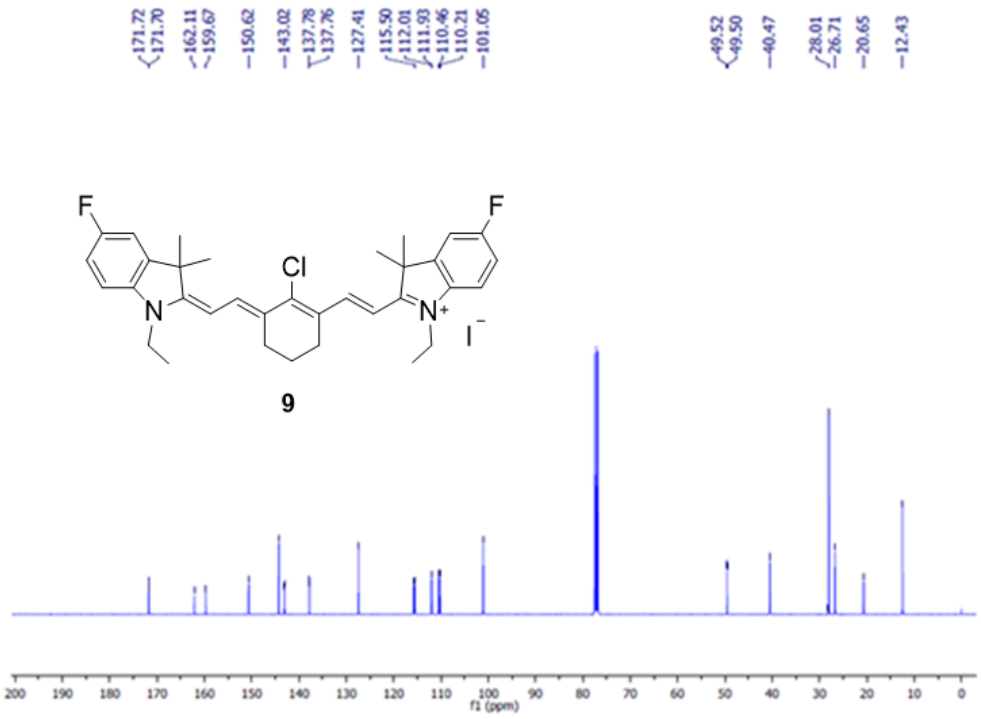


Fig S3 ^{13}C NMR spectrum of dye **9** (101 MHz, $CDCl_3$)**: δ** ppm 171.72, 171.70, 162.11, 159.67, 150.62, 144.26, 143.10, 143.02, 137.78, 137.76, 127.41, 115.74, 115.50, 112.01, 111.93, 110.46, 110.21, 101.05, 49.52, 49.50, 40.47, 28.01, 26.71, 20.65, 12.43.

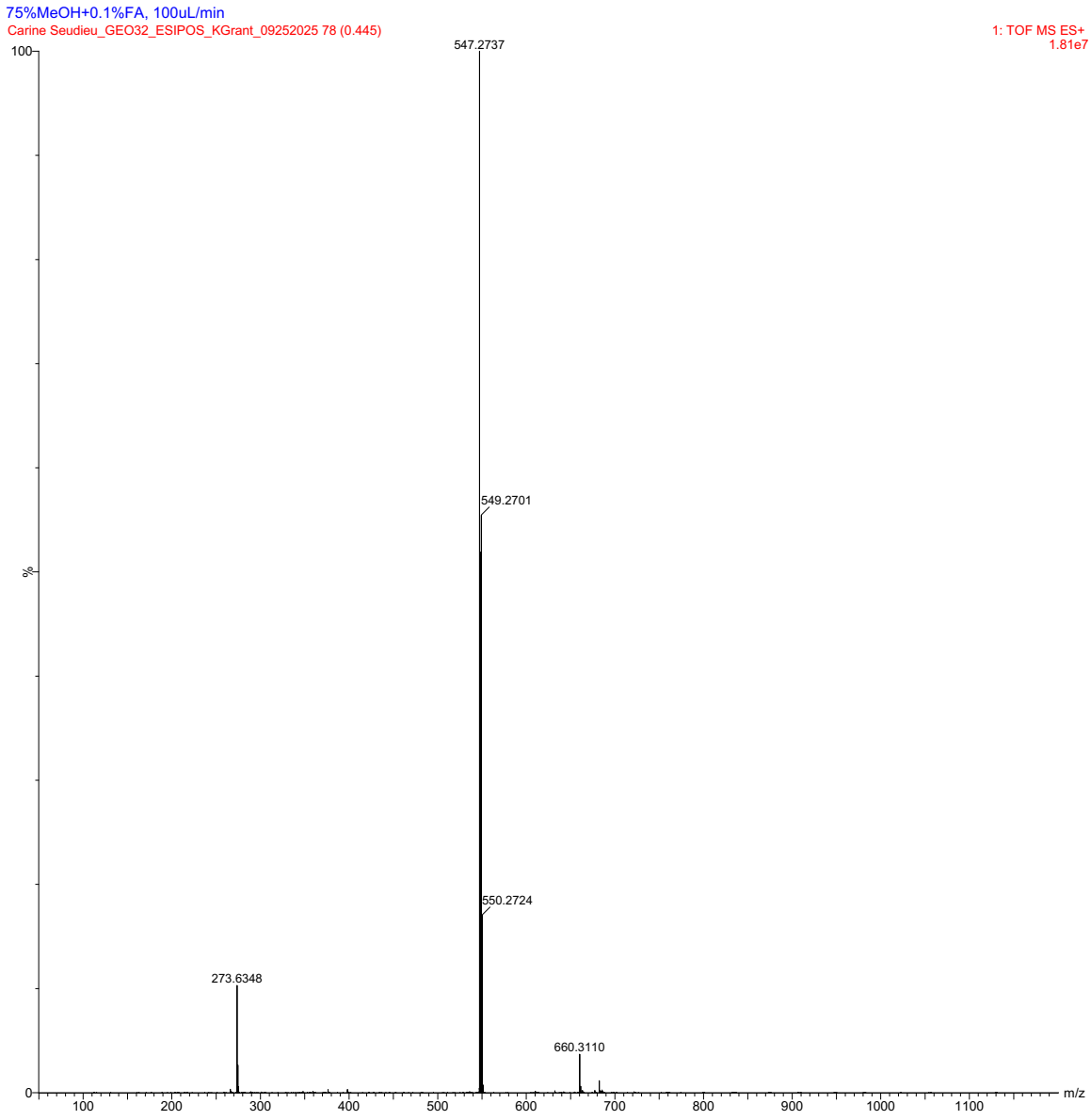


Fig S4 High Resolution ESI mass spectrum of dye **9** (positive mode) calculated for $C_{34}H_{38}ClF_2N_2^+$: *m/z* 547. 2692, found *m/z* 547.2737.

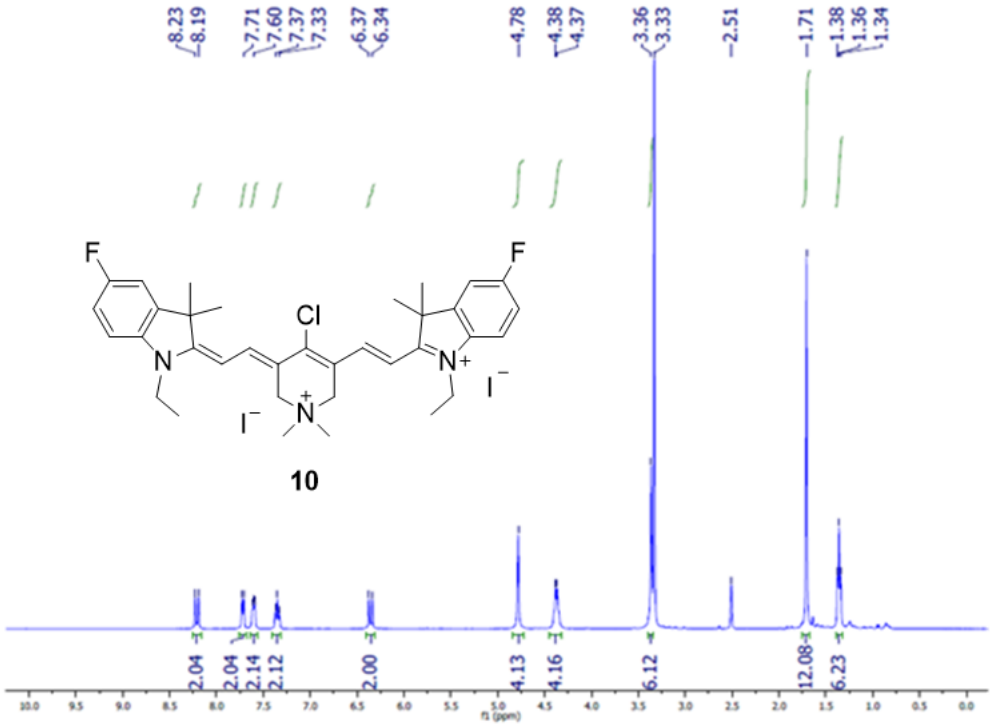


Fig S5 ^{1}H NMR spectrum of dye **10** (400 MHz, DMSO-d_6)**: δ** 8.21 ppm (d, J = 14.6 Hz, 2H), 7.72 (d, J = 8.1 Hz, 2H), 7.64 – 7.56 (m, 2H), 7.35 (t, J = 8.9 Hz, 2H), 6.36 (d, J = 14.7 Hz, 2H), 4.78 (s, 4H), 4.37 (d, J = 6.9 Hz, 4H), 3.36 (s, 6H), 1.71 (s, 12H), 1.36 (t, J = 6.8 Hz, 6H).

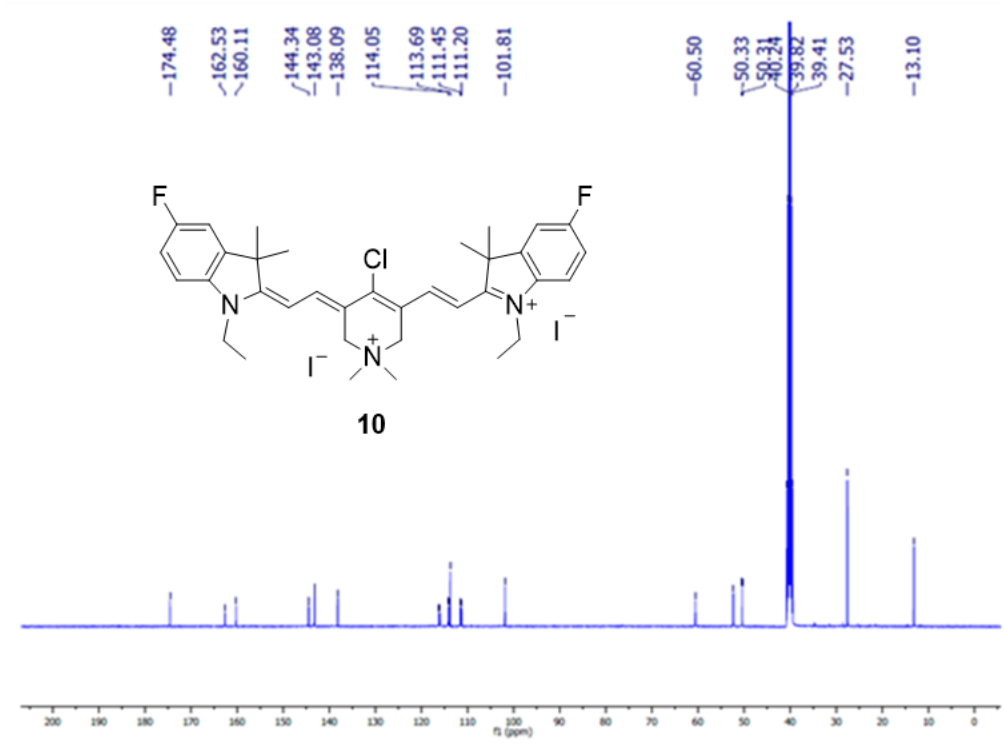


Fig S6 ^{13}C NMR spectrum of dye **10** (100 MHz, DMSO-d_6)**: δ** ppm 174.48, 162.53, 160.11, 144.43, 144.34, 143.12, 143.08, 138.09, 116.13, 115.88, 114.05, 113.96, 113.69, 111.45, 111.20, 101.81, 60.50, 52.25, 50.33, 50.31, 27.53, 13.10.

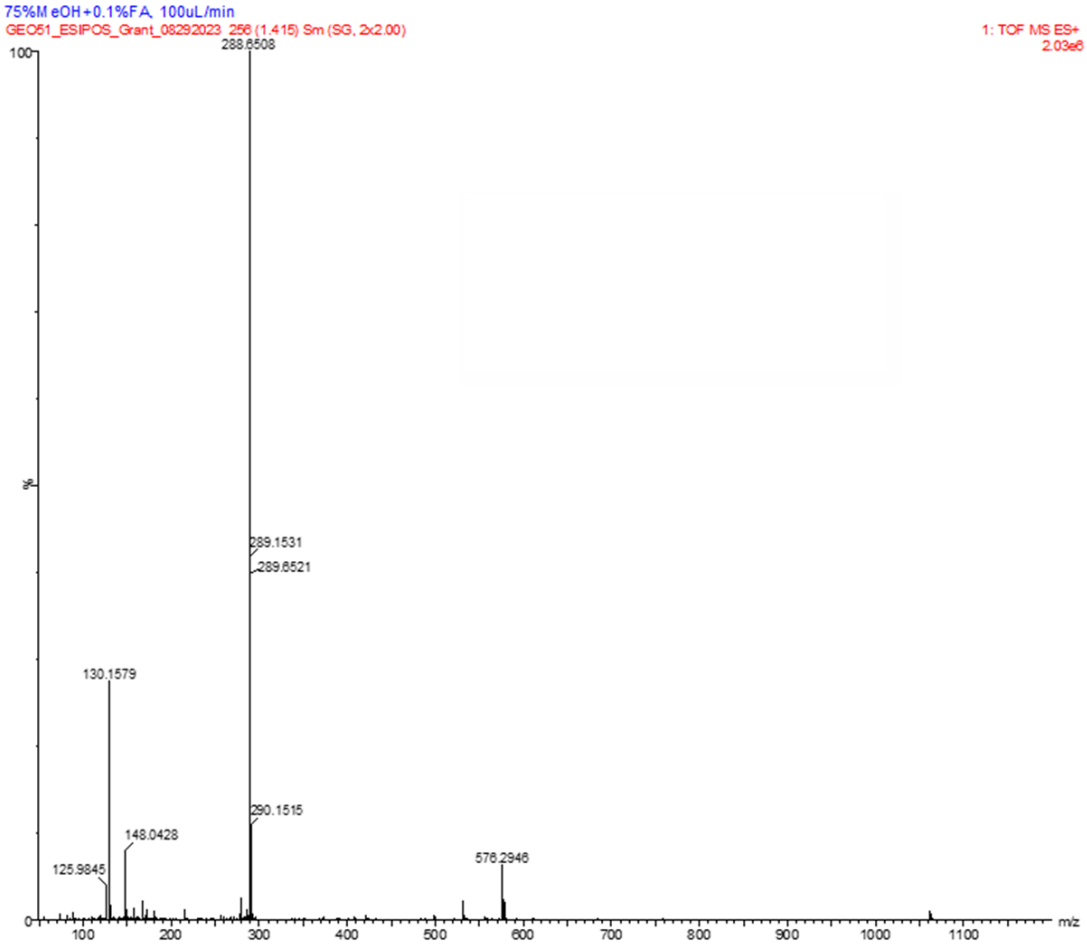


Fig S7 High Resolution ESI mass spectrum of dye **10** (positive mode) calculated for $C_{35}H_{42}ClF_2N_3^{2+}$: *m/z* 288.65, found *m/z* 288.6508.

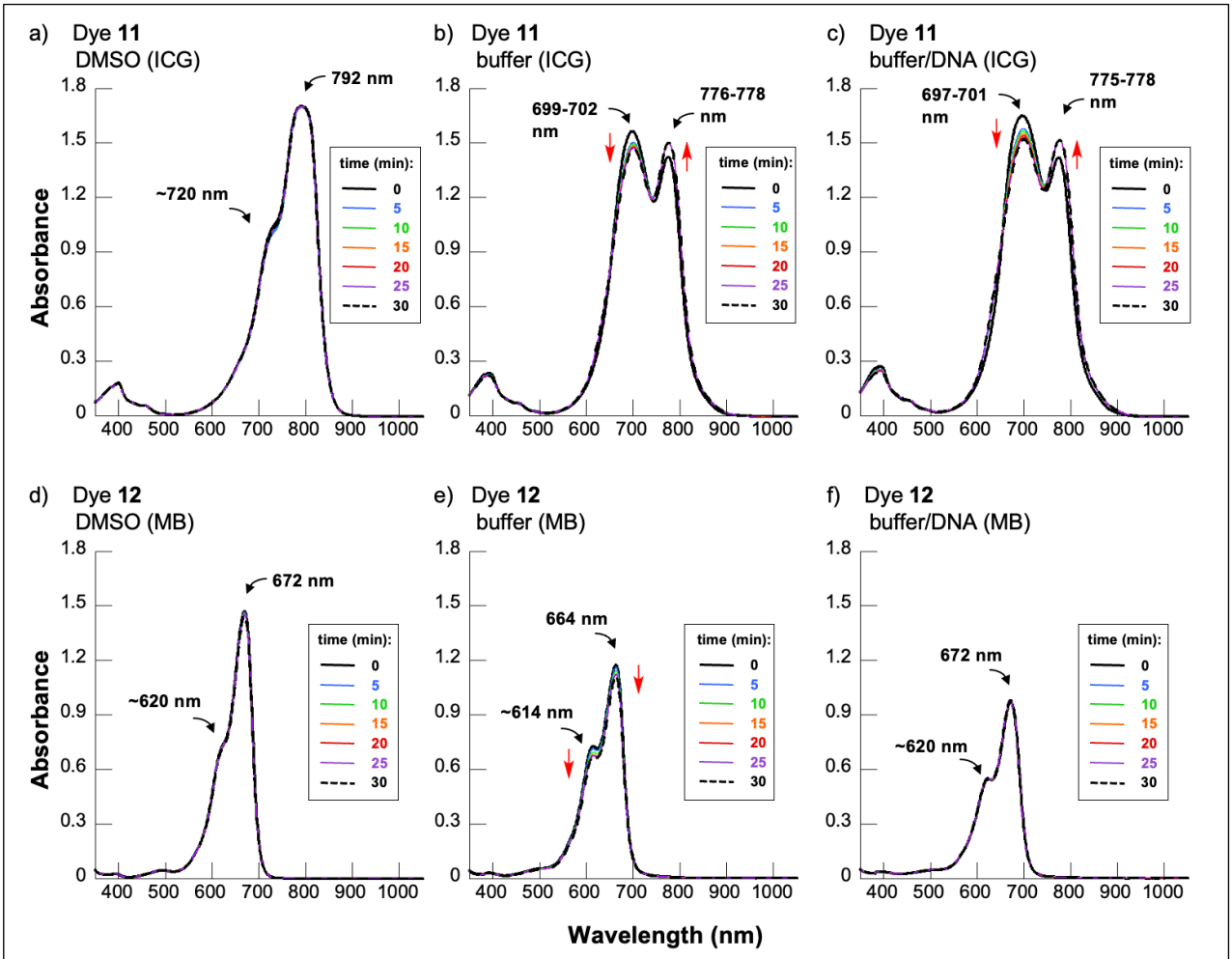


Fig. S8 UV-visible spectra recorded over 30 min for 10 μM of indocyanine green (ICG, **11**) and methylene blue (MB, **12**) in: a), d) neat DMSO; b), e) 10 mM sodium phosphate buffer pH 7.0; c), f) 10 mM sodium phosphate buffer pH 7.0 and 150 μM bp calf thymus (CT) DNA (22 °C).

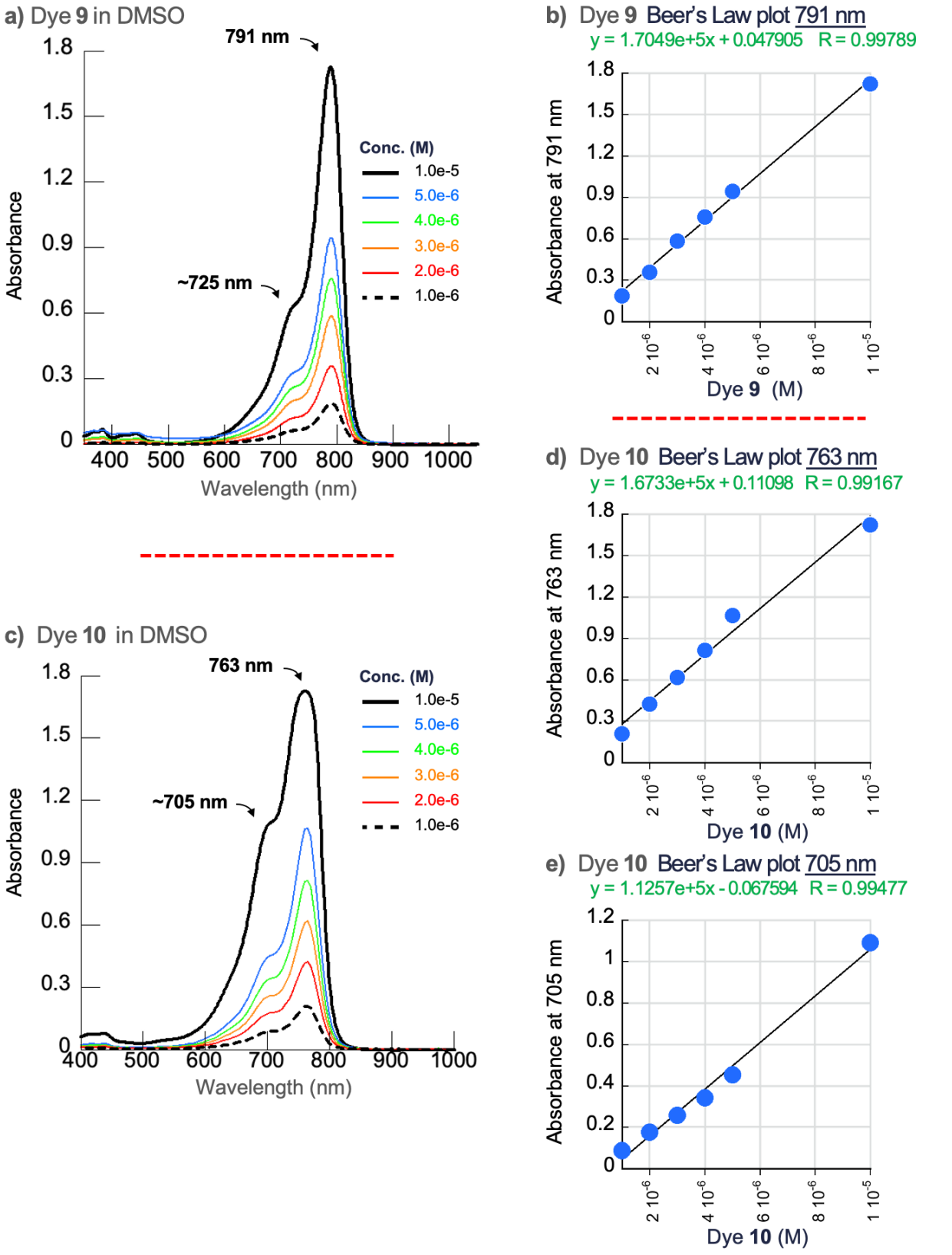


Fig. S9 UV-visible absorption spectra in neat DMSO and corresponding absorbance values plotted as a function increasing 1.0×10^{-6} to 1.0×10^{-5} M dye concentrations for: **a, b)** dye **9** and **c, d, e)** dye **10**. Linear regression analyses (right) were used to fit the λ_{max} absorbance values to the best straight-line y = mx + b, where the slope m represents dye molar extinction coefficient ($\varepsilon_{\lambda max}$ = $M^{-1}cm^{-1}$) in DMSO.

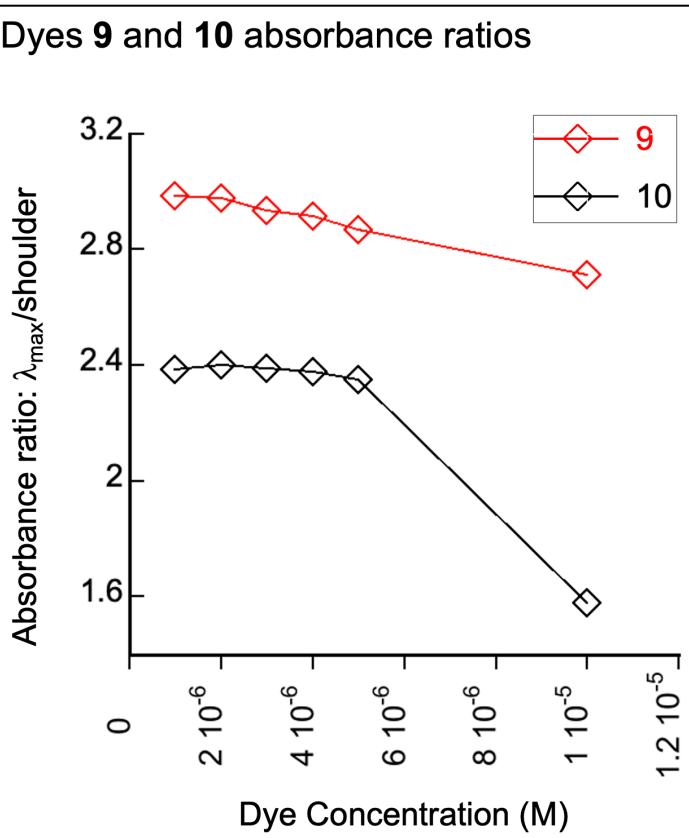


Fig. S10 Absorbance ratios in neat DMSO: (absorption at λ_{max})/(maximum absorption at the hypsochromic shoulder for dyes **9** *(791 nm/725 nm)* and **10** *(763 nm/705 nm)*. Corresponding spectra are shown in Fig. S9.

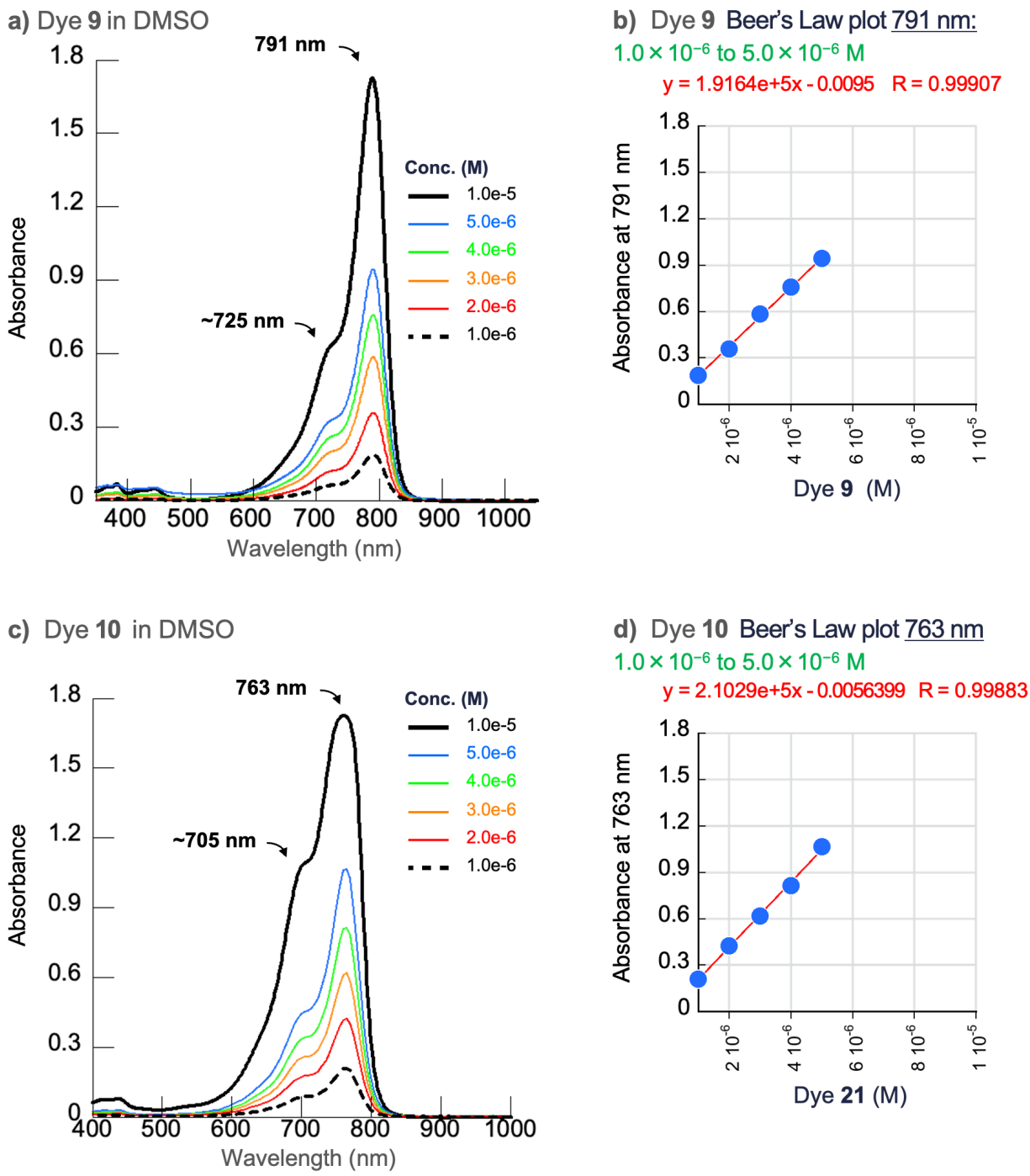


Fig. S11 UV-visible absorption spectra in neat DMSO and corresponding absorbance values plotted as a function increasing 1.0×10^{-6} to 5.0×10^{-6} M dye concentrations for: **a, b)** dye **9** and **c, d)** dye **10**. Linear regression analyses (right) were used to fit the λ_{max} absorbance values to the best straight-line y = mx + b, where the slope m represents dye molar extinction coefficient ($\varepsilon_{\lambda max}$ = $M^{-1}cm^{-1}$) in DMSO.

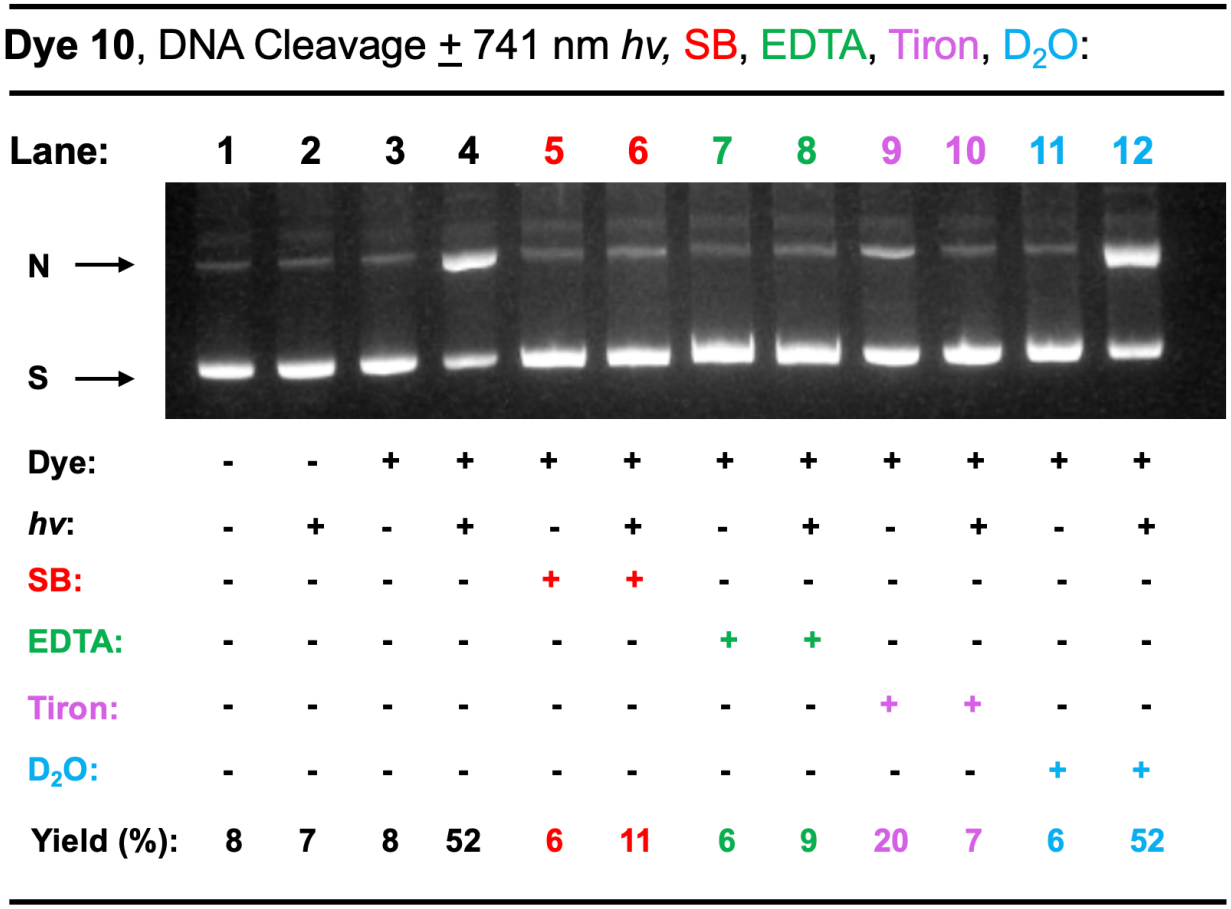


Fig. S12 Representative trial: agarose gel stained with ethidium bromide showing pUC19 plasmid photocleavage by dye **10**. Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 38 μM bp DNA in the absence and presence of 30 μM of **10** and a chemical agent, either: 100 mM of sodium benzoate (SB); 100 mM ethylenediaminetetraacetic acid (ETDA), 10 mM Tiron, or 79 % *(v/v)* of deuterium oxide (D_2O). Each reaction was irradiated for 30 min with 741 nm LED lamp, 0.3 W/cm^2 or kept in dark (22 °C). (In Table 1 of the main manuscript, photocleavage inhibition percentages for each chemical additive are calculated as the average of three independent trials, with standard deviations representing the error.) Abbreviations: N, nicked; S, supercoiled.

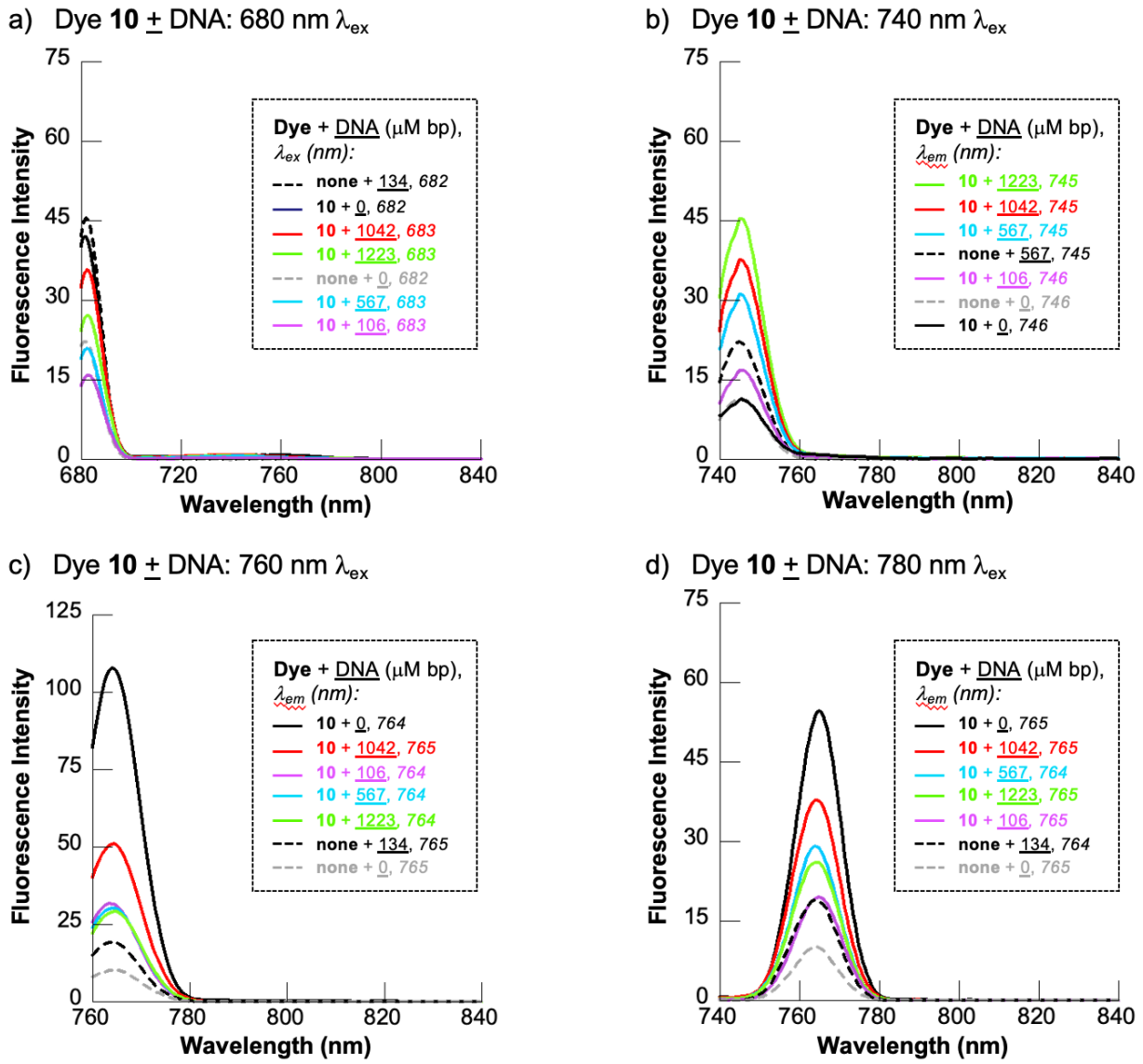


Fig. S13 Fluorescence emission spectra recorded at excitation wavelengths (λ_{ex}) of 680, 740, 760, and 780 nm in the absence and presence of 10 μM of dye **10** and 106 up to 1312 μM bp CT DNA (10 mM sodium phosphate buffer pH 7.0, 22 °C). Abbreviation: λ_{em} = wavelength of maximum emission.

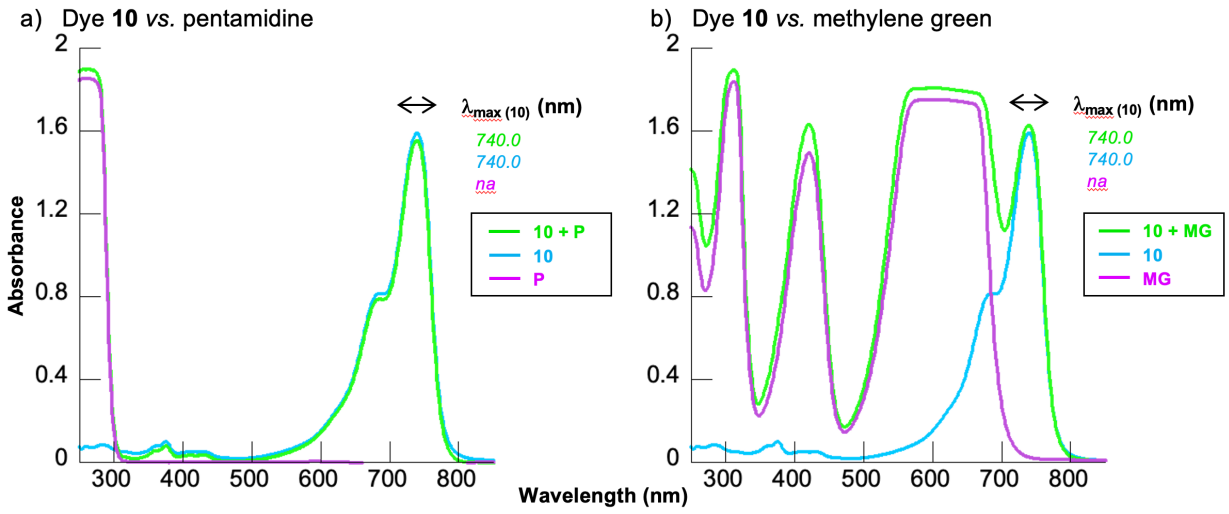


Fig. S14 The UV-visible absorption spectra were recorded for solutions containing 10 mM sodium phosphate buffer pH 7.0 with: (**a**) 10 µM of dye **10**, and/or 250 µM of pentamidine (P); (**b**) 10 µM of dye **10**, and/or 250 µM of methyl green (MG).

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