Electronic supplementary information

Single photon DNA photocleavage at 830 nm by quinoline dicarbocyanine dyes

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Contents:

A. Experimental Section. …… p.S2

B. Supporting Figures/Table. …… p.S8

C. References. …… p.S29

A. Experimental Section:

General

Deionized distilled water was used for buffer and DNA sample preparation. PUC19 plasmid DNA was cloned in XL-1 blue *E. coli* competent cells (Stratagene) according to standard laboratory protocols[1] and was purified using a QIAfilter Plasmid Mega Kit (Qiagen™, Cat. No. 12263). Sonicated calf thymus (CT) DNA was obtained from Invitrogen (Cat. No. 15633-019; 10 mg/mL, average size ≤ 2000 bp). Sodium phosphate monobasic and sodium phosphate dibasic came from Thermo Fisher Scientific. Deuterium oxide (99.9%) was supplied by Cambridge Isotope Laboratories. All other chemicals, including sodium azide (≥99.99%), sodium benzoate (99%), and dimethyl sulfoxide (DMSO, ≥99.99%) were from Sigma-Aldrich and were used as-is without further purification.

A PerkinElmer Lambda 35 spectrophotometer or a Shimadzu UV-2401 PC spectrophotometer and a PerkinElmer LS55 fluorescence spectrophotometer were respectively used to record UV-visible absorption spectra and fluorescence emission spectra. Circular dichroism (CD) and induced circular dichroism (ICD) spectra were acquired with a Jasco J-810 or a Jasco J-1500 CD spectropolarimeter. At wavelengths from 700 nm to 1000 nm, the Jasco J-1500 spectropolarimeter was fitted with a Jasco EXPM-531 NIR extender to enhance the sensitivity of the CD signal. NMR spectra were recorded on a Bruker Advance 400 MHz NMR spectrometer. High-resolution mass spectra (HRMS) were obtained from the Georgia State University Mass Spectrometry Facility using a Waters Q-TOF micro (ESI-Q-TOF) mass spectrometer. All melting points were recorded on a Mel-Temp Electrothermal apparatus and are uncorrected. Pre-coated Silica GEL 60 F-254TLC plates were from Merck KGaA (Darmstadt, Germany).

Synthetic procedures

*4-methylquinolinium iodide **(1)***: Quinolinium salt **1** (Scheme S1) was obtained by the reaction of 4-methylquinoline (1 equiv) with iodomethane (4 equiv) in anhydrous acetonitrile refluxed at 90 °C for 72 h. Thin layer chromatography (TLC) was used to monitor the progress of the reaction eluting with a 4:1 mixture of DCM:hexanes. Upon completion, the reaction mixture was allowed to cool to room temperature and diethyl ether was added to precipitate the iodide salt. The solid was collected by vacuum filtration and washed with diethyl ether (3x25 mL). The salt was used without further purification in subsequent reactions.

General procedure for the synthesis of polymethine precursors

Polymethine precursor **3** (Scheme S1) was purchased from Sigma Aldrich and used as-is. Precursor **2** was prepared as described previously.[2] Briefly, mucochloric acid (1 equiv) was dissolved in ethanol. A solution of aniline (2 equiv) was added dropwise over 10 min, and the resulting mixture was stirred and heated to 40 °C until the evolution of CO_2 (g) was observed to cease. The mixture was then cooled in an ice bath and diethyl ether was slowly added to induce precipitation of the reaction product. The resulting solid was collected by vacuum filtration, washed with diethyl ether (3x25 mL), and used without any additional purification.

General procedure for the synthesis of dyes 4 and 5

The final dyes **4**[3] and **5**[4] (Scheme S1) are reported in the literature; however, the publications fail to provide any synthetic methods or characterization of the compounds or provide only superficial characterization (UV-visible spectra). Due to this, we are reporting these compounds with the characterization included. For dye **5,** solubility in deuterated solvents proved too poor to obtain sufficient concentrations of the compound for ^{13}C NMR spectroscopy.

Salt **1** (2 equiv) and the corresponding polymethine precursors **2** or **3** (1 equiv) were dissolved in acetic anhydride. Trimethylamine (TEA) (0.1 mL) was added and the reaction mixture was stirred and heated to 75 °C. Reaction progress was monitored by UV-visible spectrophotometry. Upon completion of the reaction, diethyl ether was added to precipitate the final dyes **4** and **5**, which were collected by vacuum filtration. The dyes were purified by recrystallization from methanol/diethyl ether.

*1-methyl-4-((1E,3E)-5-((Z)-1-methylquinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-ium iodide **(4)***: MP 239 °C (Dec); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 3.97 (s, 6 H) 6.65 (t, *J* = 12.25 Hz, 1 H) 6.99 (d, *J* = 13.39 Hz, 2 H) 7.32 (d, *J* = 7.33 Hz, 2 H) 7.59 (t, *J* = 7.07 Hz, 2 H) 7.78 - 7.89 (m, 4 H) 7.95 (t, *J* = 13.01 Hz, 2 H) 8.09 (d, *J* = 7.07 Hz, 2 H) 8.42 (d, *J* = 8.34 Hz, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 41.47, 107.80, 110.48, 117.22, 118.93, 124.18, 124.74, 125.90, 128.62, 132.46, 138.86, 141.36, 145.80, 146.89; HRMS (TOF MS ESI+): calc'd for $C_{25}H_{23}N_2^+$: m/z 351.1856 ($[M]^+$), Found: m/z 351.0218 $[M]^+$

*4-((1E,3Z)-3-chloro-5-((Z)-1-methylquinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-methylquinolin-1-ium iodide **(5)***: MP >260 °C; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 4.05 (s, 6 H) 6.94 (d, *J* = 13.14 Hz, 2 H) 7.41 (d, *J* = 7.33 Hz, 2 H) 7.67 (t, *J* = 5.81 Hz, 2 H) 7.86 - 7.97 (m, 4 H) 8.14 (d, *J* = 12.88 Hz, 2 H) 8.30 (d, *J* = 7.07 Hz, 2 H) 8.41 (d, *J* = 8.34 Hz, 2 H); HRMS (TOF MS ESI+): calc'd for $C_{25}H_{22}ClN_2^+$: m/z 385.1466 ($[M]^+$), Found: m/z 385.1964 $[M]^+$

Cyanine dyes **4** and **5** were stored in a -4 °C freezer as 2.5 mM stock solutions in DMSO.

UV-visible absorption spectrophotometry, dye stability and DNA interactions

The absorbance of cyanine dyes **4** and **5** was measured at 22 °C with a UV-visible spectrophotometer. Cuvettes contained 10 μM of dye in DMSO or 10 μM of dye in 10 mM of sodium phosphate pH 7.0 buffer without and with 150 μM bp CT DNA. Absorption spectra were recorded at 5 min time intervals from 0 min up to 25 or 30 min.

In DNA titration experiments, small volumes of an aqueous solution of 15,111 μM bp CT DNA were sequentially added to samples containing 20 μM of cyanine dye in 10 mM sodium phosphate buffer pH 7.0 (500 μL initial volume). All absorption spectra were corrected for sample dilution. Final concentrations of CT DNA in each sample ranged from 0 μM bp up to 2684 μM bp.

DNA photocleavage

Individual DNA cleavage reactions containing 5 μM to 50 μM concentrations of cyanine dye **4** or **5**, 38 μM bp of pUC19 plasmid, and 10 mM of sodium phosphate pH 7.0 were prepared in a total volume of 40 μL. In order maintain reaction temperature at 10 °C, 22 °C, or 37 °C, the samples were placed in a thermometer-fitted metal block that was either heated, kept at room temperature, or immersed in an ice bath. While monitoring temperature with the thermometer, the samples were either kept in the dark or were irradiated at time intervals ranging from 5 min to 120 min using a light emitting diode (LED) laser (LaserLand) with a peak emission wavelength of either 532 nm (1.0 W/cm^2), 808 nm (2.8 W/cm^2), or 830 nm (2.8 W/cm^2). At the end of the

irradiation time interval, a total of 3 μL of electrophoresis loading buffer containing 15.0% (*w/v*) ficoll and 0.025% (*w/v*) bromophenol blue) was added to each reaction and 20 μL of the resulting solution were added to one of the wells of a 1.5% agarose gel stained with ethidium bromide (0.5 μg/mL, final concentration). Completely loaded gels were then electrophoresed for ~60 min at 105 V in a Bio-Rad Laboratories gel box using 1 × tris-acetate-EDTA (TAE) containing 0.5 μg/mL ethidium bromide as the running buffer. Electrophoresed gels were visualized at 302 nm with a VWR Scientific LM-20E transilluminator and then photographed with a UVP PhotoDoc-It™ Imaging System. For quantitating the gels, ImageQuant version 5.2 software was employed. The DNA photocleavage yields were then calculated using the formula:

Percent Photocleavage = [(Linear + Nicked DNA)/(Linear + Nicked + Supercoiled DNA)] × 100.

Circular dichroism

Individual samples for CD analysis consisted of 10 mM sodium phosphate buffer pH 7.0 with 10 μM of dye and 120 μM bp of CT-DNA present alone and in combination (total volume of 2000 μL). Spectra were collected from 900 to 200 nm in 3 mL (1.0 cm) quartz cuvettes (Starna) using the following instrument settings: scan speed, 100 nm/min; response time, 2 s; bandwidth, 0.5 nm; sensitivity, 100 millidegrees. Final spectra were averaged over 12 acquisitions.

Extended, near-infrared circular dichroism spectra were recorded from 1000 nm to 600 nm for samples containing 10 mM sodium phosphate buffer pH 7.0, 25 μM of dye, and 990 μM bp of CT DNA (2000 μL total volume). The scan speed was set at 100 nm/min, the response time was 2 s, and the bandwidth and sensitivity were 0.5 nm and 200 millidegrees, respectively. Final spectra were averaged over 12 acquisitions.

Fluorescence emission spectra

Solutions containing 10 mM sodium phosphate buffer pH 7.0 and 10 μM of cyanine dye in the absence and presence of either 100 μM bp or 990 μM bp CT DNA were transferred to 3.0 mL Starna quartz cuvettes (2000 μL, total volume). The samples were excited at 550 nm and 800 nm and emission spectra were respectively recorded from 555 nm to 900 nm and from 805 nm to 900 nm (22 °C).

Reagent induced changes in DNA photocleavage

In an argon-purged glove box, 40 μL photocleavage reactions containing 10 mM sodium phosphate buffer pH 7.0, 20 μM of cyanine dye, and 38 μM bp of pUC19 plasmid were prepared from solutions bubbled with argon and then irradiated at 830 nm for 30 min. The procedure was repeated using aerated solutions in a glove box purged with air.

A second set of reactions containing 10 mM of sodium phosphate buffer pH 7.0, 38 μM bp pUC19 plasmid DNA and 20 μM of dye were prepared in the presence and absence of either 100 mM of the singlet oxygen scavenger sodium azide, 100 mM of the hydroxyl radical scavenger sodium benzoate, or 70% D_2O *(v/v)*. The reactions were aerobically irradiated on the bench top for 30 min (830 nm).

After the irradiation, the above DNA reactions were electrophoresed on 1.5% non-denaturing agarose gels, visualized, and quantitated as just described. The percent change in DNA photocleavage was then calculated using the following formula, where the additive was either argon, sodium azide, sodium benzoate, or D_2O:

Percent Change in Cleavage = [(% Total of Linear and Nicked DNA $_{\text{with additive}}$ – % Total of Linear and Nicked DNA $_{\text{without additive}}$)/(% Total of Linear and Nicked DNA $_{\text{without additive}}$)] x 100.

ROS detection using HPF

Solutions containing 10 mM sodium phosphate buffer pH 7.0, and 3 μM of hydroxyphenyl fluorescein (HPF) in the presence and absence of 20 μM of **5** were prepared. In a parallel reaction, a total 100 mM of sodium benzoate was used as a hydroxyl radical scavenging reagent. Samples were then kept in the dark or irradiated with an 830 nm LED laser (2.8 W/cm^2) for 30 min. To generate hydroxyl radicals, aqueous solutions containing 10 μM H_2O_2, 10 μM ammonium iron(II) sulfate, 3 μM HPF, and 10 mM sodium phosphate buffer pH 7.0 in the presence and absence of 100 mM of sodium benzoate were equilibrated in the dark for a few minutes (22 °C).[5] Fluorescence emission spectra were immediately recorded using a PerkinElmer LS55 spectrofluorometer (λ_{ex} = 490 nm).

ROS detection using SOSG

Reactions containing 10 mM sodium phosphate buffer pH 7.0 and 0.75 μM of Singlet Oxygen Sensor Green® (SOSG) in the presence and absence of 20 μM of **5** were prepared. Samples were then kept in the dark or irradiated with an 830 nm LED laser (2.8 W/cm^2) for 30 min. As a positive control for hydroxyl radical detection, an aqueous solution containing 10 μM H_2O_2, 10 μM ammonium iron(II) sulfate, 750 nM of SOSG, and 10 mM sodium phosphate buffer pH 7.0 was equilibrated in the dark for a few minutes (22 °C).[5] To generate singlet oxygen, solutions containing 1 μM of methylene blue and 10 mM sodium phosphate buffer pH 7.0 were kept in the dark or irradiated with a 638 nm LED laser (2.8 W/cm^2, LaserLand) for 2 s. Fluorescence emission spectra were immediately recorded with a PerkinElmer LS55 spectrofluorometer (λ_{ex} = 480 nm).

Cell culture

ES2 human clear cell ovarian carcinoma cell line was obtained from ATCC (Manassas, VA). All cancer cells were cultured in DMEM medium (Sigma, St. Louis, MO) with 10% fetal bovine serum (VWR, Visalia, CA) and 1.2 mL/100 mL penicillin−streptomycin (Sigma, St. Louis, MO). All cells were grown in a humidified atmosphere of 5% CO_2 (v/v) in air at 37 °C.[6]

Cellular uptake and fluorescence imaging

ES2 cells were plated in 96-well plates at a density of 10 × 10^3 cells/well and cultured for 24 h. After that cells were incubated with the dye **5** (10 μg/mL) dissolved in DMEM (10% fetal bovine serum) for 24 h. To visualize the subcellular distribution of the dye, nuclei of ES2 cells were stained with Hoechst 33342. Before imaging, cells were washed with DPBS. Images were collected with an BZ-X710 Keyence microscope using DAPI filter and Cy® 7 filter cubes.[7]

ROS measurements

Intracellular ROS levels were evaluated with the 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) assay according the previously described procedure.[8] Briefly, ES2 cells were seeded in 96-well plates at a density of 10×10^3 cells/well and cultured for 24 h. Subsequently, cells were incubated with the dye **5** dissolved in cell culture medium (1.0 μg/mL) for 24 h. Then, the cells were rinsed with DPBS and 100 μL of 10 μM DCFH-DA was added under dark conditions and incubated for 30 min prior to light treatment. The test samples were exposed to a 808 nm laser diode light for 5 min (0.3 W/cm^2). Non-treated cells, cells incubated with the same concentration of the dye **5** under dark conditions, and cells exposed to a 808 nm laser diode for 5 min were used as controls. Fluorescence was measured using a multiwell plate reader with 485 nm excitation and 528 nm emission filters.

Evaluation of phototherapeutic effect

ES2 cells were plated in 96-well plates at a density of 10×10^3 cells/well and cultured for 24 h. After that cells were incubated in the dark with the dye **5** (10 μg/mL) dissolved in DMEM (10% fetal bovine serum) for 24 h. The dye-containing medium was then removed and the cells were rinsed with warm DPBS before fresh medium was added. Subsequently, cells were exposed to a 808 nm laser diode light for 10 min (0.3 W/cm^2). After treatment, cells were cultured for 24 h in growth medium prior to viability measurements with Calcein AM as previously described.[9] Non-treated cells, cells incubated with the same concentration of the dye **5** under dark conditions, and cells exposed to a 808 nm laser diode for 5 min were used as controls.

B. Supporting Scheme, Figures and Table:

Scheme S1. Synthesis of cyanine dyes **4** and **5** …… S8

Fig. S1. ^{1}H NMR spectrum of dye **4** …… S9

Fig. S2. ^{13}C NMR spectrum of dye **4** …….. S10

Fig. S3. HRMS of dye **4** ……... S10

Fig. S4. ^{1}H NMR spectrum of dye **5** …….. S11

Fig. S5. HRMS of dye **5** …….. S12

Fig. S6. UV-visible spectra vs. time: dyes **4** and **5** in DMSO……..…… S13

Fig. S7. UV-visible spectra vs. time: dyes **4** and **5** in aqueous buffer……..…. S14

Fig. S8. DNA photocleavage at 808 nm and 830 nm: dyes **4** and **5** at 22 °C …….. S15

Fig. S9. Dark cleavage: dyes **4** and **5** at 10 °C, 22 °C, and 37 °C ……..… S16

Fig. S10. DNA photocleavage at 830 nm vs. dye concentration: dye **5** at 22 °C …….. S17

Fig. S11. DNA photocleavage at 830 nm vs. time: dye **5** at 10 °C …….. S18

Fig. S12. UV-visible absorption DNA titration spectra: dye **5** ……..... S19

Fig. S13. Near-infrared circular dichroism spectra vs. absorbance: dye **5** ……..… S20

Fig. S14. Fluorescence spectra vs. absorbance: dye **5** ……..……..… S21

Fig. S15. DNA photocleavage at 532 nm: dye **5** at 10 °C ……..... S22

Fig. S16. DNA photocleavage at 830 nm: dye **5**, air vs. argon…….. S23

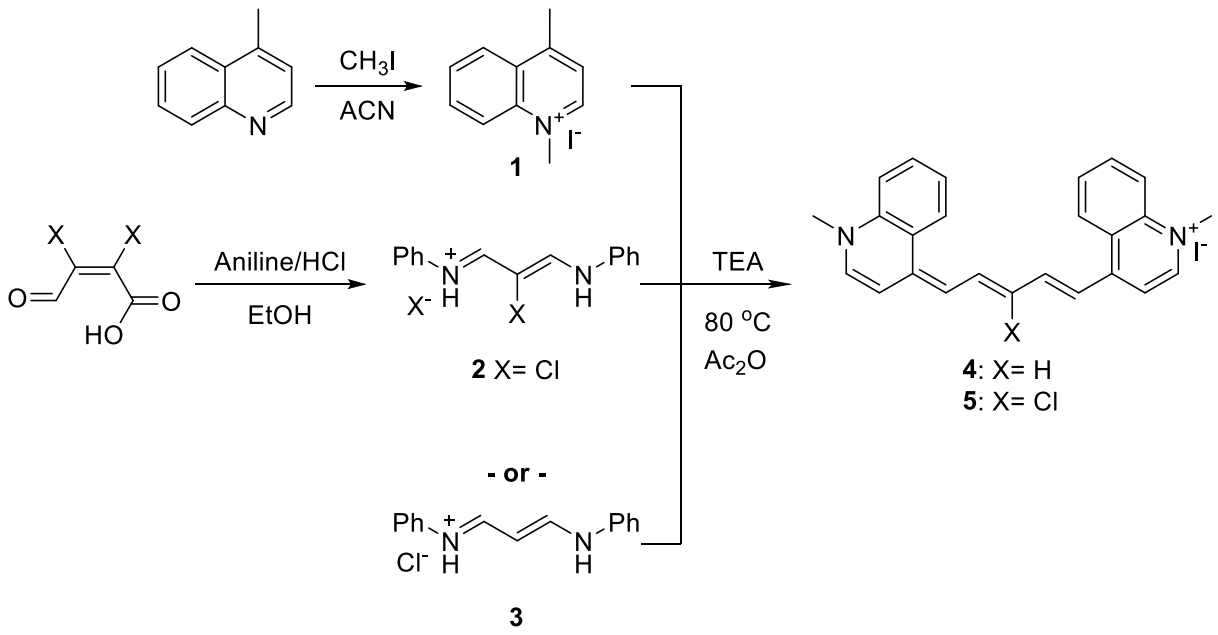
Fig. S17. DNA photocleavage st 830 nm: dye **5**, no additive vs. scavenger ……..... S24

Fig. S18. DNA photocleavage at 830 nm: dye **5**, H_2O vs. D_2O ……..…… S25

Fig. S19. Fluorometric detection of ROS: Fenton reagent, methylene blue ……..… S26

Fig. S20. Fluorometric detection of dye **5**-sensitized ROS in cancer cells……..… S27

Table S1. Inhibition of dye **5**-sensitized photocleavage by chemical additives …….. S28



*Scheme S1: Synthesis of cyanine dyes **4** and **5**.*

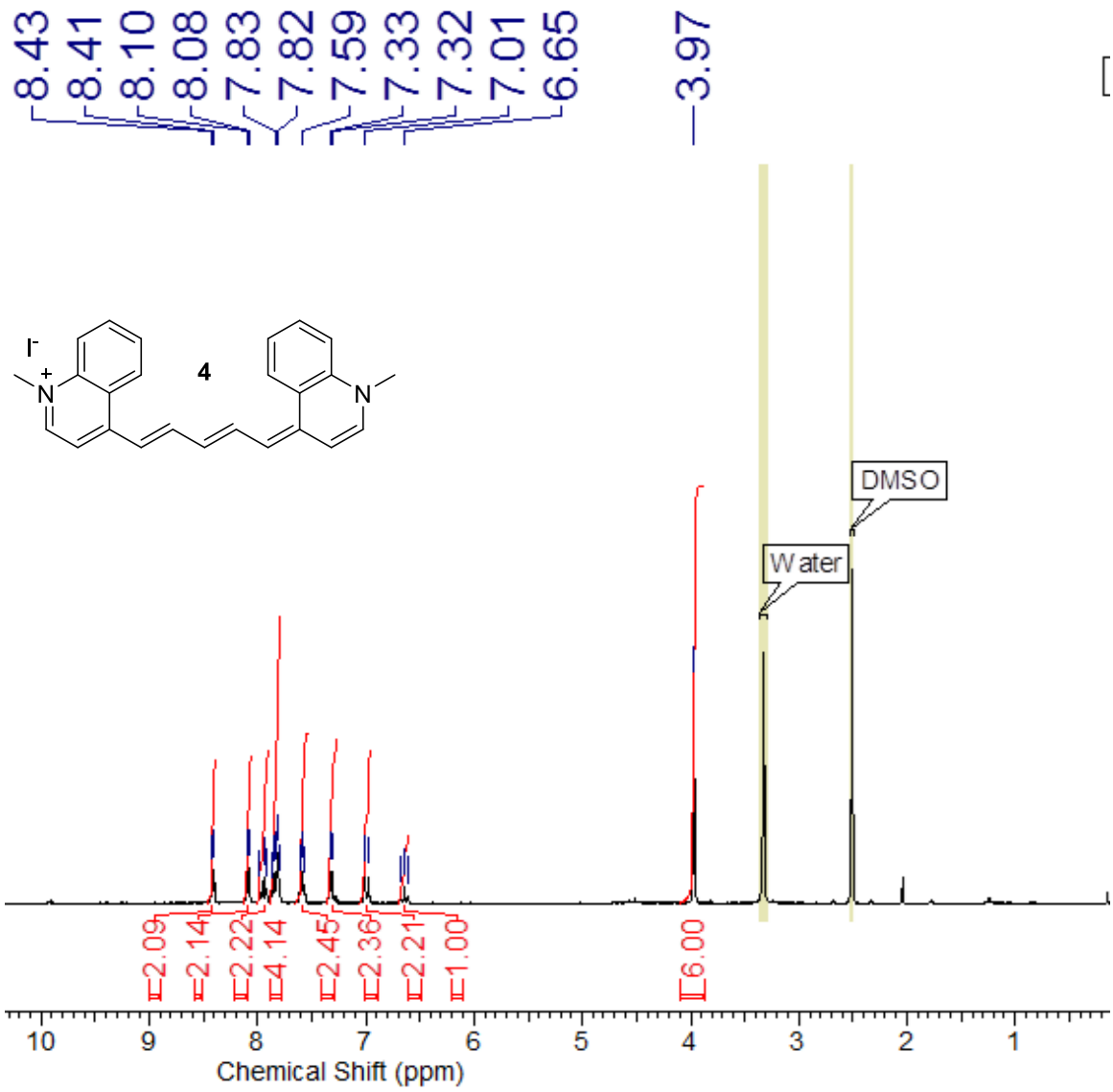


Fig. S1 ^{1}H NMR spectrum of dye **4**.

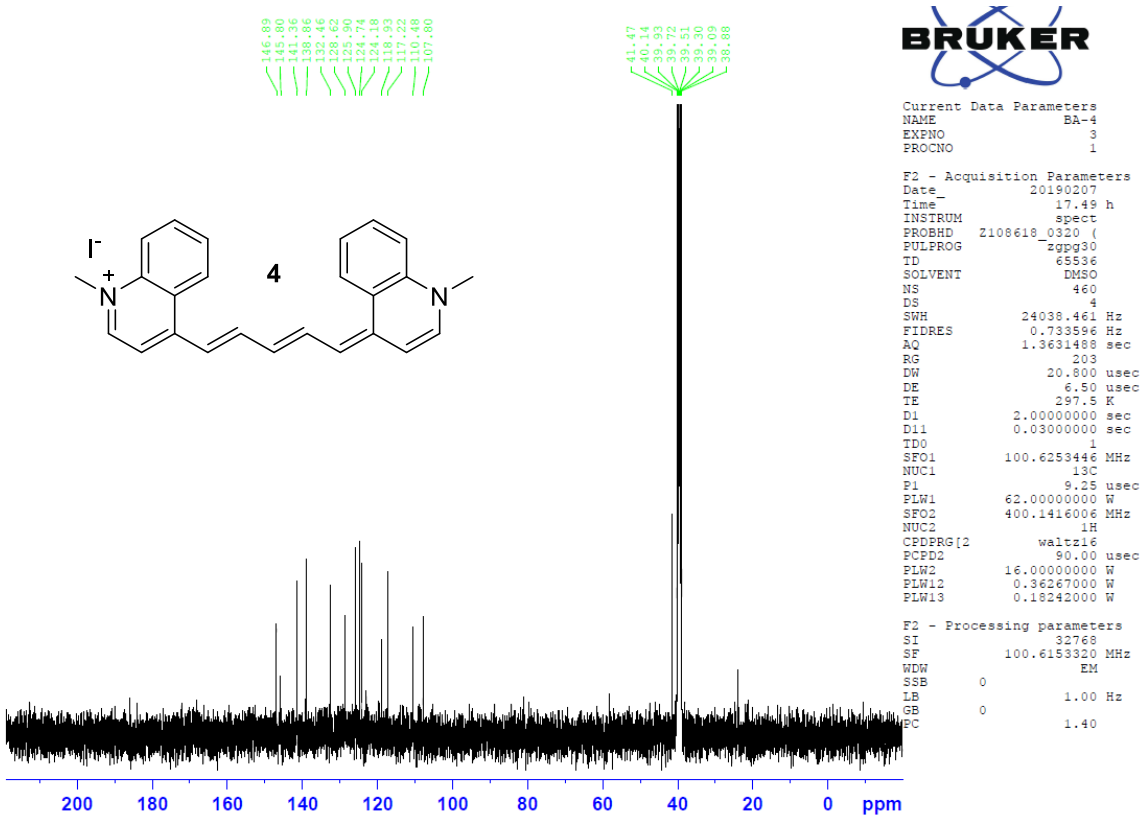


Fig. S2 ^{13}C NMR spectrum of dye **4**.

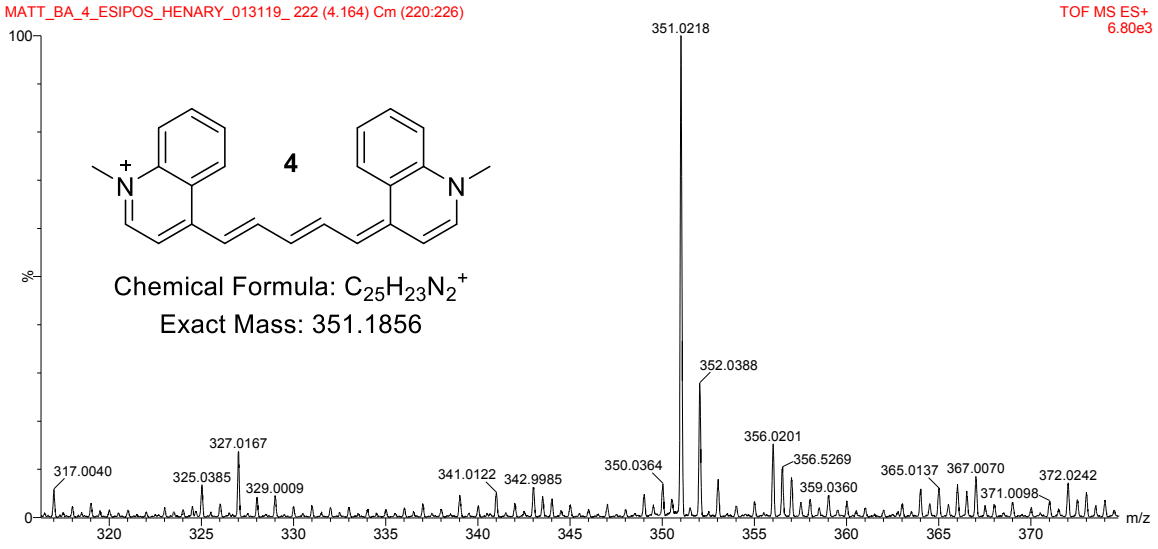


Fig. S3 HRMS of dye **4**.

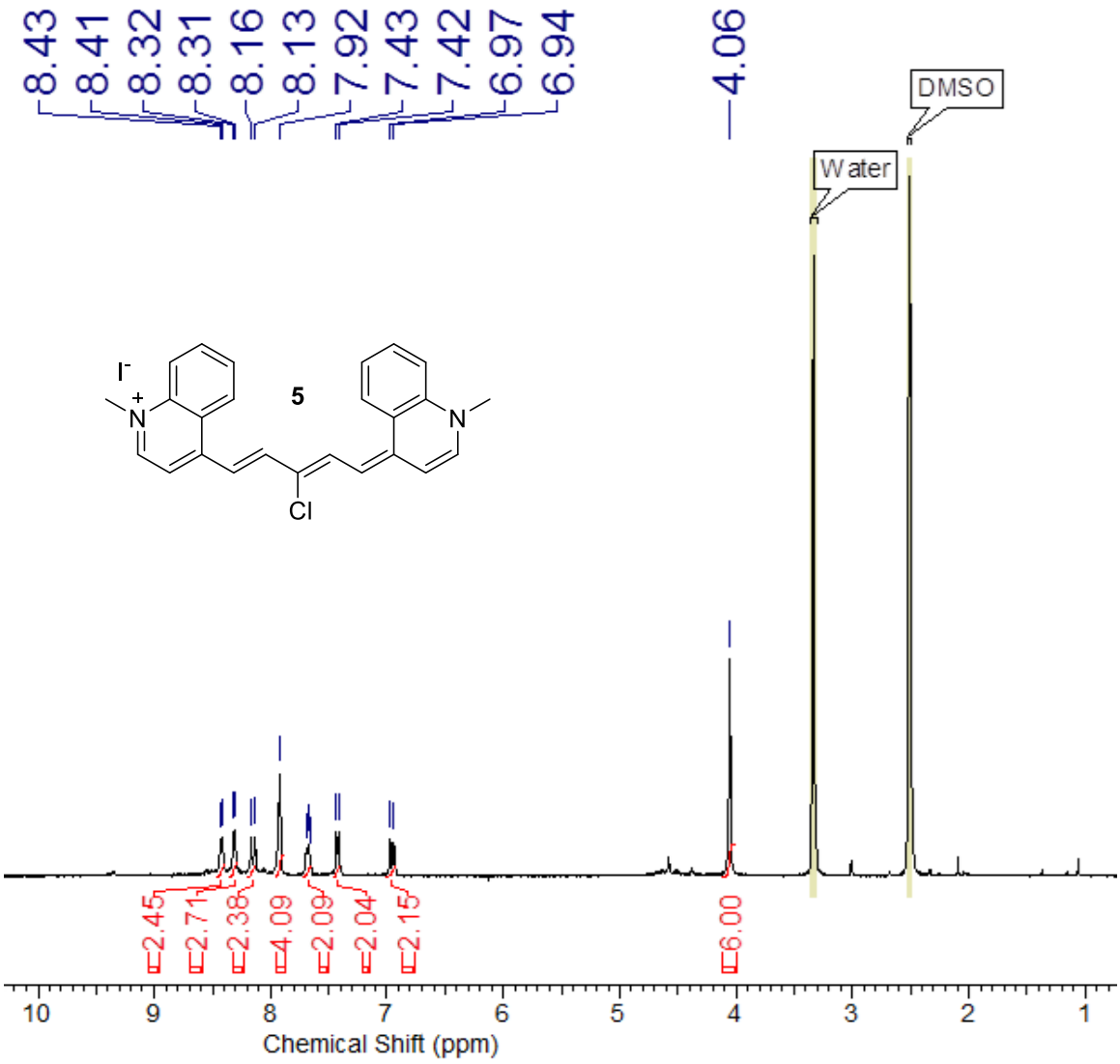


Fig. S4 ^{1}H NMR spectrum of dye **5**.

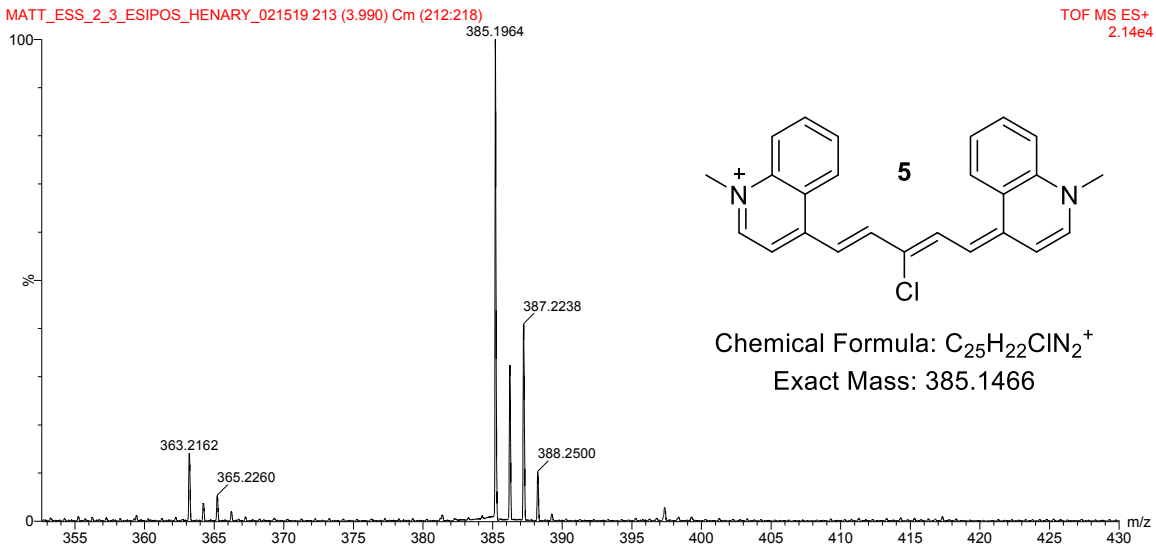


Fig. S5 HRMS of dye **5**.

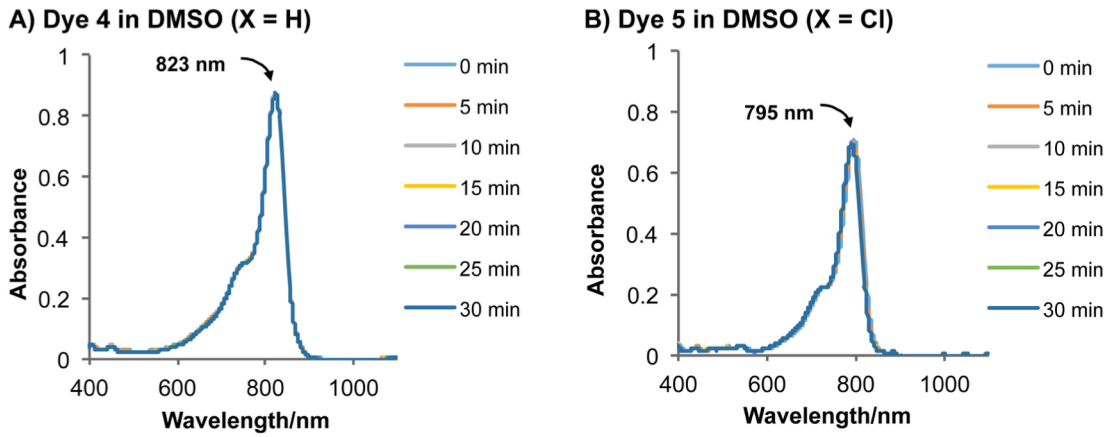


Fig. S6 UV-visible spectra recorded as a function of time for 10 μM of dyes **4** and **5** in DMSO (22 °C).

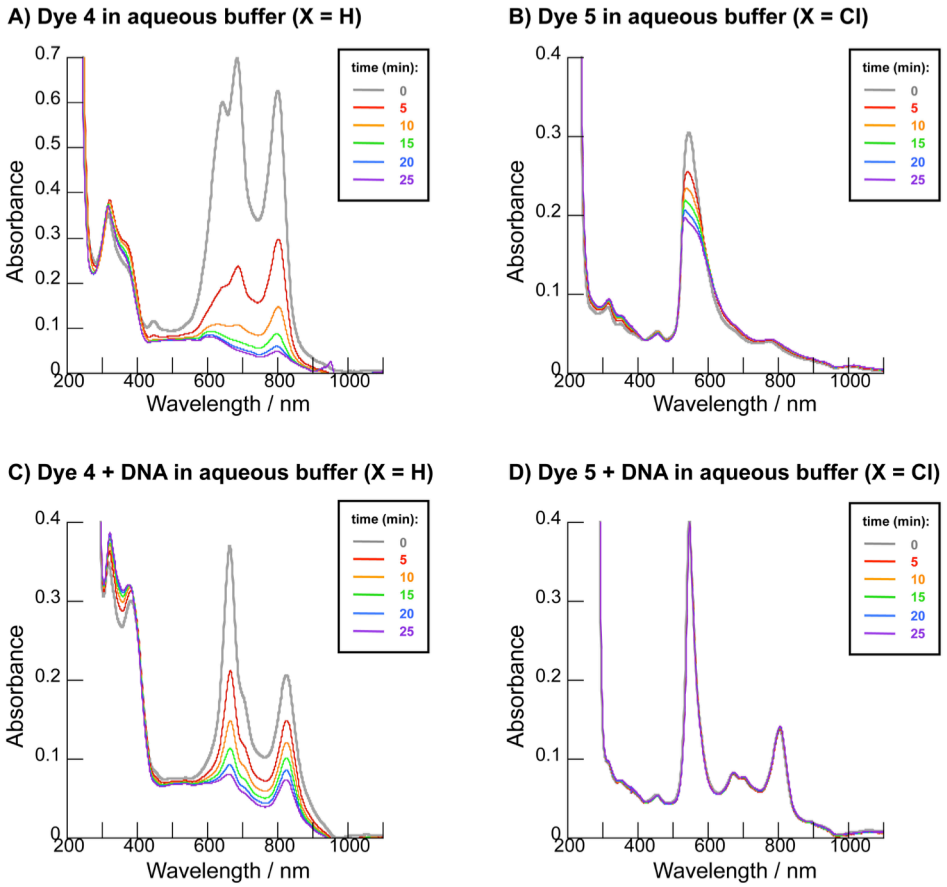


Fig. S7 UV-visible spectra recorded as a function of time for 10 μM of dyes **4** and **5** in the absence and presence of 150 μM bp CT DNA (10 mM sodium phosphate buffer pH 7.0; 22 °C).

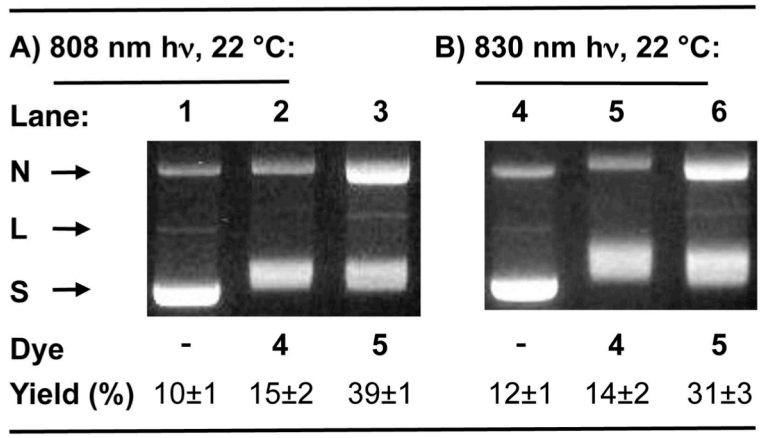


Fig. S8 Agarose gels showing cyanine dye-sensitized photocleavage of pUC19 plasmid DNA with (**A**) 808 nm and (**B**) 830 nm LED lamps (2.8 W/cm^2; 30 min hν at 22 °C). Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 38 μM bp DNA in the absence and presence of 20 μM of dye. Yields and standard deviation were obtained over 3 trials. Abbreviations: **L** = linear; **N** = nicked; **S** = supercoiled).

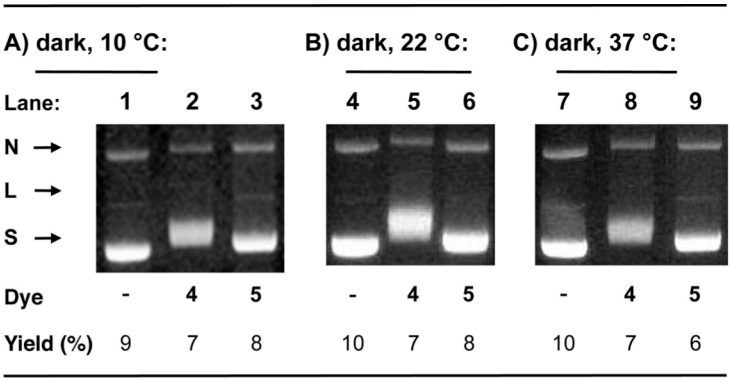


Fig. S9 Agarose gels showing controls in which cyanine dyes **4** and **5** were equilibrated with pUC19 plasmid DNA in the dark at temperatures ranging from 10 °C to 37 °C (30 min no hν). The reactions contained 10 mM sodium phosphate buffer pH 7.0 and 38 μM bp DNA in the absence and presence of 20 μM of dye. Abbreviations: **L** = linear; **N** = nicked; **S** = supercoiled).

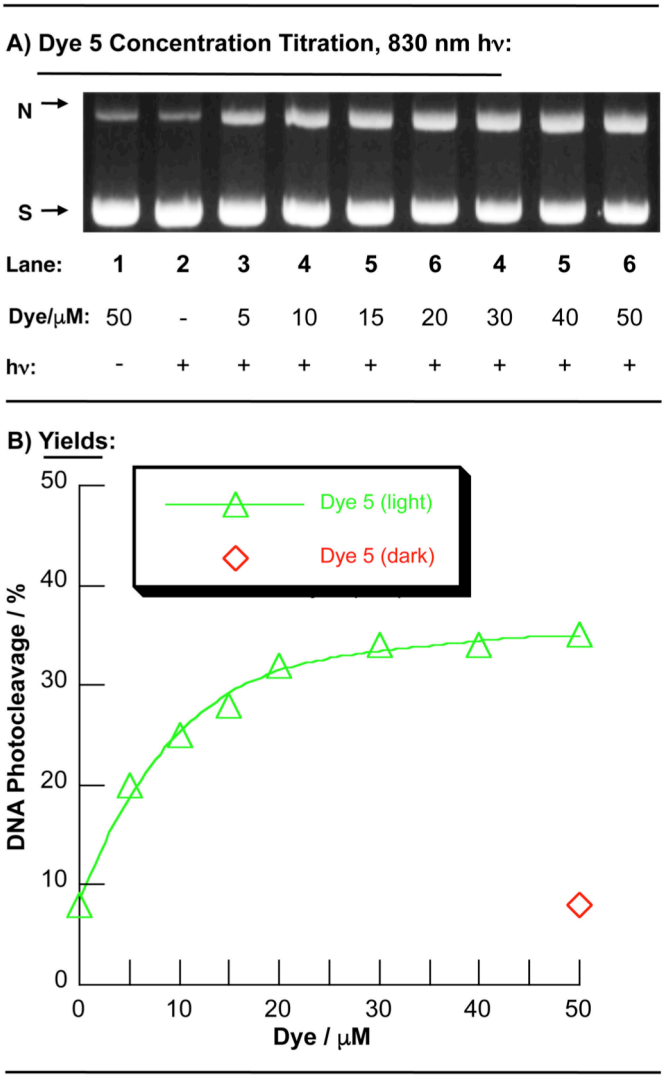


Fig. S10 Agarose gel (**A**) and corresponding yields (**B**) showing photocleavage of 38 μM bp pUC19 DNA by 0 to 50 μM of dye **5** (10 mM sodium phosphate buffer pH 7.0). With the exception of the dark controls in lane 1, reactions were irradiated with a 830 nm LED lamp (2.8 W/cm^2) for 30 min at 22 °C. Abbreviations: **N** = nicked; **S** = supercoiled.

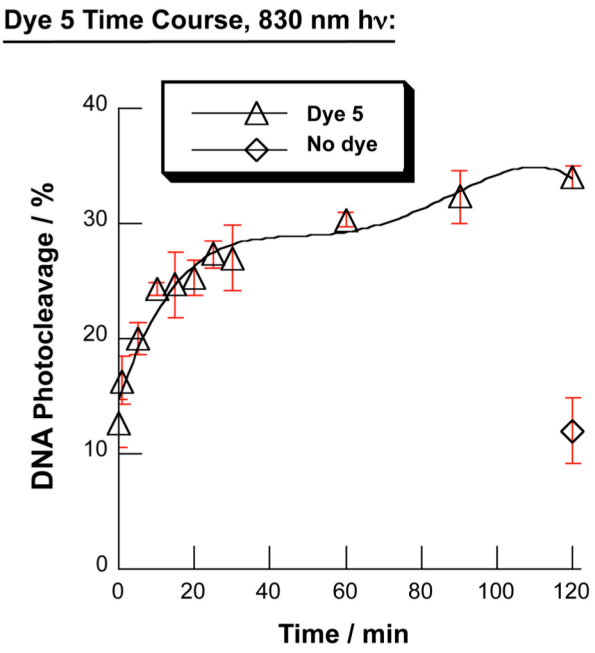


Fig. S11 Photocleavage of 38 μM bp pUC19 DNA by 20 μM of dye **5** (10 mM sodium phosphate buffer pH 7.0). Reactions were irradiated with a 830 nm LED lamp for 0, 1, 5, 10, 15, 20, 25, 30, 60, 90, and 120 min time intervals at 10 °C (2.8 W/cm^2). Data points are averaged over three trials. Error bars represent standard deviation. Abbreviations: **N** = nicked; **S** = supercoiled.

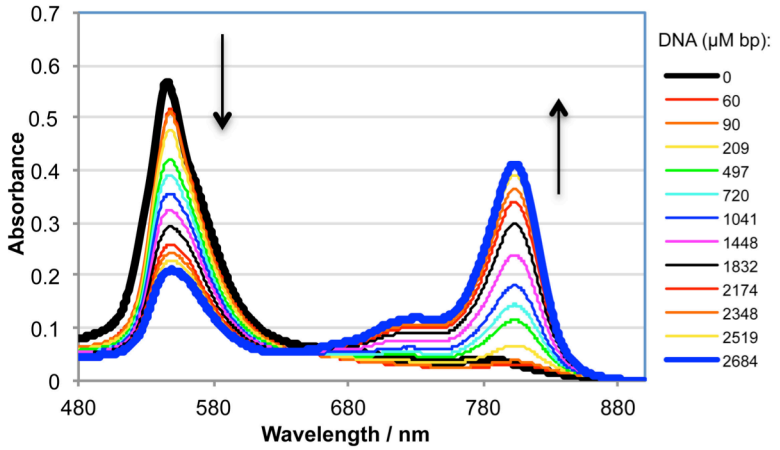


Fig. S12 Representative UV-visible absorption titration spectra of 20 μM of cyanine dye **5** in the absence and presence of increasing concentrations of CT DNA (10 mM sodium phosphate buffer, pH 7.0, 22 °C). All absorption spectra were corrected for sample dilution.

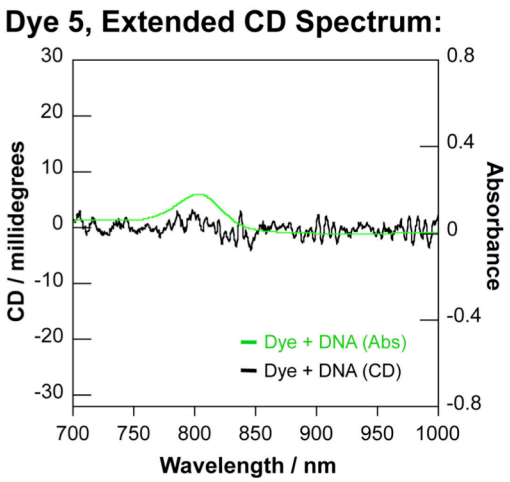


Fig. S13 Double y-axis plots superimposing the extended, near-infrared circular dichroism (CD) and UV-visible absorption (Abs) spectra of dye **5** (22 °C). Samples contained 10 mM sodium phosphate buffer pH 7.0, 20 μM (Abs) or 25 μM (CD) of dye and 990 μM bp of CT DNA.

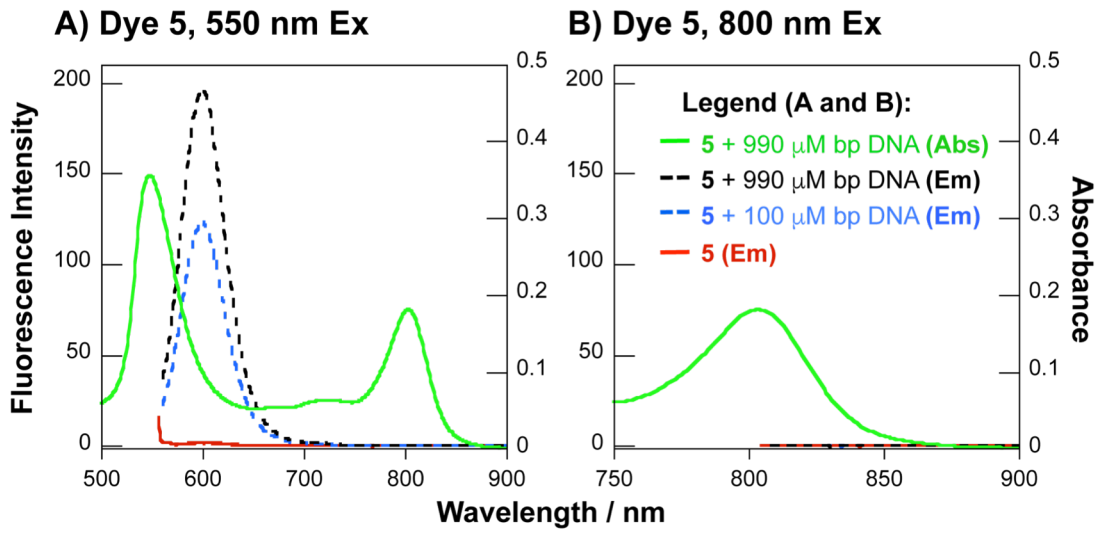


Fig. S14 Double y-axis plots superimposing the fluorescence emission (Em) and UV-visible absorption (Abs) spectra of dye **5** (22 °C). Samples contained 10 mM sodium phosphate buffer pH 7.0, 10 μM (Em) or 20 μM (Abs) of dye and/or 100 μM to 990 μM bp of CT DNA. The emission spectra were recorded at excitation wavelengths (Ex) of 550 nm (**A**) and 800 nm (**B**).

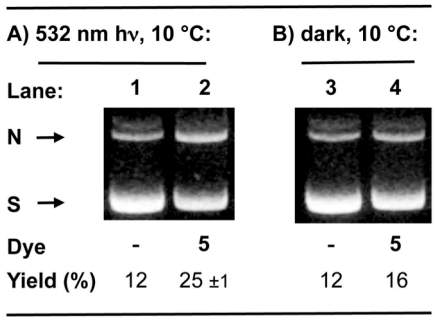


Fig. S15 Agarose gels showing cyanine dye-sensitized photocleavage of pUC19 plasmid DNA (**A**) after 30 min of irradiation with a 532 nm LED laser (1.0 W/cm^2) and (**B**) after a 30 min incubation period in the dark (10 °C). Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 38 μM bp DNA in the absence and presence of 20 μM of dye **5**. Abbreviations: **N** = nicked; **S** = supercoiled).

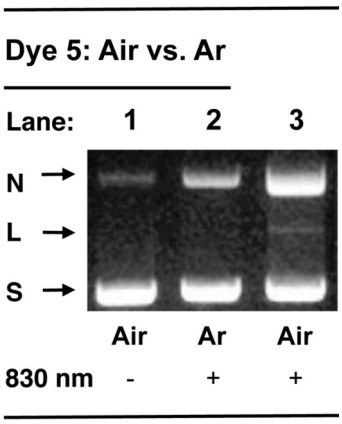


Fig. S16 Agarose gel showing cyanine dye-sensitized photocleavage of pUC19 plasmid DNA under aerobic and anaerobic conditions. Reactions containing 10 mM sodium phosphate buffer pH 7.0, 20 μM of dye **5**, and 38 μM bp DNA were purged in a glove box with air or argon and then either irradiated with a 830 nm LED lamp (2.8 W/cm^2) or kept in the dark in the purged glove box (30 min, 22 °C). Abbreviations: **L** = linear; **N** = nicked; **S** = supercoiled.

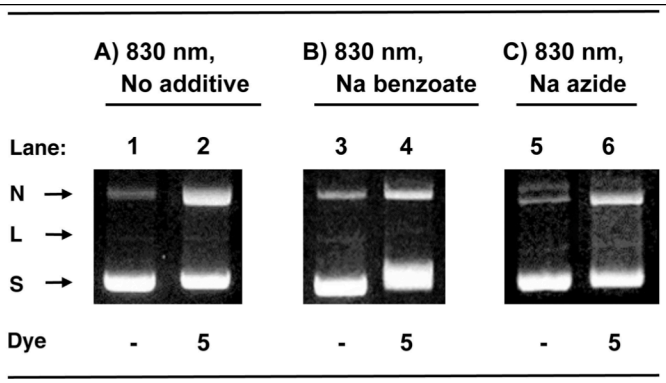


Fig. S17 Agarose gels comparing levels of cyanine dye-sensitized photocleavage of pUC19 plasmid DNA generated in the absence (**A**) and presence of the ROS scavenging agents sodium benzoate (**B**), and sodium azide (**C**) (830 nm hν for 30 min at 22 °C). All reactions contained 10 mM sodium phosphate buffer pH 7.0, 20 μM of dye **5**, and 38 μM bp DNA. Abbreviations: **L** = linear; **N** = nicked; **S** = supercoiled.

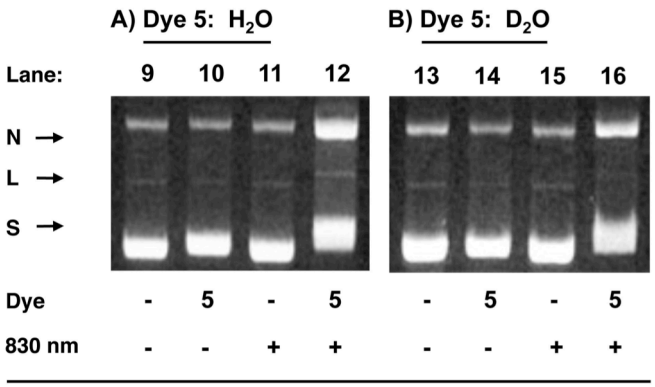


Fig. S18 Agarose gels showing cyanine dye-sensitized photocleavage of pUC19 plasmid DNA in 100% H_2O *(v/v)* **(A)** vs. 70% D_2O *(v/v)* **(B)**. The reactions, which contained 10 mM sodium phosphate buffer pH 7.0 and 38 μM bp DNA in the absence and presence of 20 μM of dye **5**, were either kept in the dark or irradiated with a 830 nm LED lamp (2.8 W/cm^2, 30 min hν at 22 °C). Abbreviations: **L** = linear; **N** = nicked; **S** = supercoiled.

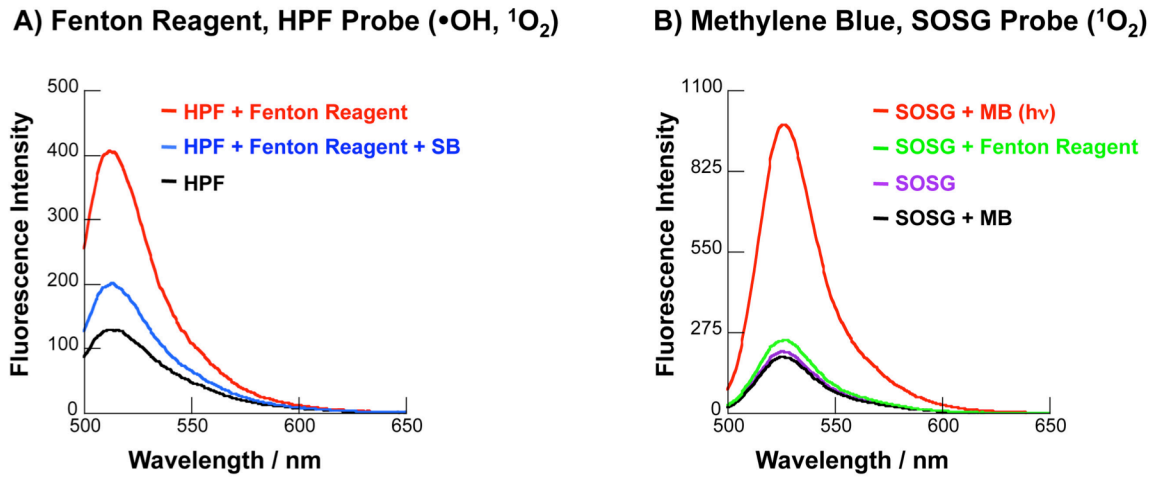
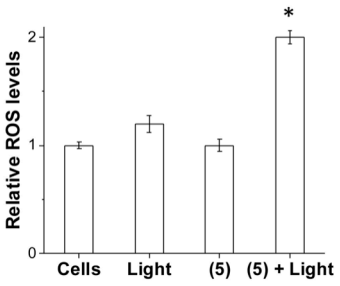


Fig. S19 Fluorescence spectra recorded at 22 ºC of: (**A**) 3 µM hydroxyphenyl fluorescein (HPF) in the absence (black line) and presence of either 10 µM ammonium iron(II) sulphate/10 µM H_2O_2 (red line) or 10 µM ammonium iron(II) sulphate/10 µM H_2O_2 and 100 mM sodium benzoate (SB; blue line); (**B**) 0.75 µM Singlet Oxygen Sensor Green® (SOSG) in the absence (purple line) and presence of either 10 µM ammonium iron(II) sulphate/10 µM H_2O_2 (green line), 1 µM methylene blue (black line), or 1 µM methylene blue irradiated for 2 s with a 638 nm LED laser (2.8 W/cm^2, Laserland; red line). All samples contained 10 mM sodium phosphate buffer pH 7.0.



	Relative ROS level	STDV
Cells	1	0.03
Light	1.2	0.08
(**5**)	1	0.06
(**5**) + Light	2	0.06

Fig. S20 Relative intracellular ROS levels detected by DCFH-DA in ES2 cancer cells after the following treatments: **Cells**- no treatment; **Light-**cells exposed to a 808 nm laser (0.3 W/cm^2) for 5 min; **(5) –** cells incubated with dye **5** (1μg/mL) for 24 h under dark conditions; **(5) + Light -** cells incubated with dye **5** (1μg/mL) for 24 h and exposed to a 808 nm laser (0.3 W/cm^2) for 5 min. ROS level of non-treated cells was set to 1. *p < 0.05 when compared with non-treated cells.

Table S1 Inhibition of dye **5**-sensitized DNA photocleavage induced by chemical additives

Reagents added	Target ROS	Photocleavage inhibition (%)
Argon	$^1O_2$ & •OH	75 ± 3
Na benzoate	•OH	40 ± 2
Na azide	$^1O_2$ > •OH	24 ± 2
D_2O	$^1O_2$	12 ± 1

[a] Reactions consisting of 38 μM bp of pUC19 plasmid DNA and 20 μM of **5** with and without 100 mM of scavenger (sodium benzoate, sodium azide) or 70% D_2O *(v/v)* were irradiated for 30 min with a 830 nm, 2.8 W/cm^2 LED laser (10 mM sodium phosphate buffer pH 7.0; Figs. S16 - S18). Data were averaged over three trials with error reported as standard deviation.

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