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Supporting Information

for

Photo-induced spin switching in a modified anthraquinone modulated by DNA binding

by

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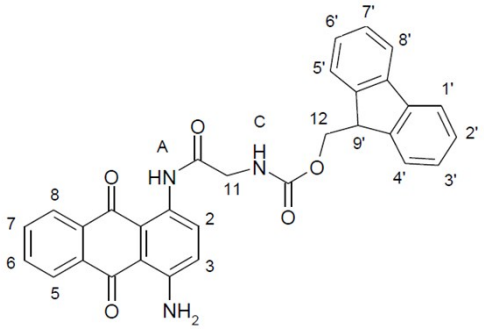
S1. Synthesis & Characterization

Commercially available chemicals were purchased from Aldrich and used as received, unless otherwise stated. ^{1}H NMR spectra were recorded on a Bruker AMX III 300 MHz spectrometer. All spectra were recorded at room temperature; the solvent for each spectrum is given in parentheses. Chemical shifts are reported in ppm and are relative to TMS internally referenced to the residual solvent peak. Datasets were edited with Bruker TopSpin suite. The multiplicity of signals is reported as singlet(s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination of any of these. High-resolution mass spectra were recorded on a ESI–TOF Mariner from Perseptive Biosystem (Stratford, Texas, USA), using electrospray (ES) ionization. The purity profile of the compounds was assayed by HPLC using a Varian Pro-Star system equipped with a Biorad 1706 UV–VIS detector (260 nm) and an Agilent C-18 column (5 µm, 4.6 × 250 mm). An appropriate ratio of water (A) and acetonitrile (B) was used as mobile phase with an overall flow rate of 1 mL min−1; the general method for the analyses is reported here: 0 min (95% A–5% B), 5 min (95% A–5% B), 35 min (5% A–95% B), 38 min (5% A–95% B), and 40 min (95% A–5% B). The purity of all compounds was ≥ 97%, unless otherwise stated.

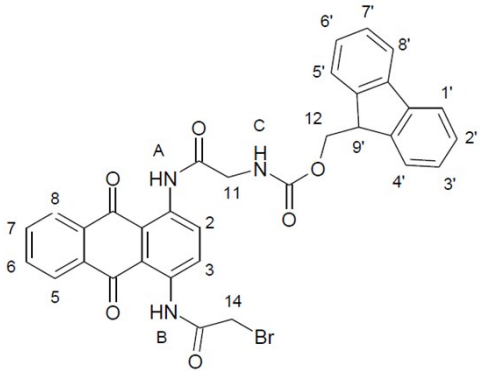
The color of **1** and **2** as a dry powder is orange, slightly lighter for **2** than for **1**.

Synthesis of 1-(N-FMOC- aminoacetamido)-4-aminoanthracene-9,10-dione



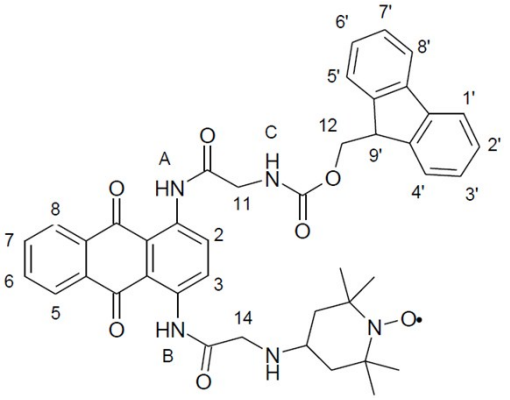
984 mg of DCC was dissolved in 50 mL of freshly distilled THF. 1.27 g of N-FMOC-glycine and 1 g of 1,4-diaminoanthraquinone were added to the solution. The mixture was stirred at room temperature for 18 h giving a precipitate which was filtered. The solid was extracted with THF/water. The organic solvent was evaporated giving 1.5 g of 1-(FMOC- aminoacetamido)-4-aminoanthracene-9,10-dione as a purple solid. Yield 68%. ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 12.84 (s, 1H; HA), 8.82 (d, 1H, *J* = 9.6 Hz; H2), 8.61 (s, 2H; HB), 8.25 (m, 1H; H7), 8.17 (d, 1H, *J* = 9.6 Hz; H3), 7.94-7.79 (m, 5H; H6,1',4',5',8'), 7.65 (m, 1H; HC), 7.54-7.30 (m, 6H; H5,8,2',3',6',7'), 6.54 (s, 2H; HB), 4.43-4.35 (m, 3H; H12,9'), 3.85 (d, 2H, J = 5.6 HZ; H11). HRMS (ESI): calculated for $C_{31}H_{24}N_3O_5$ ($M+H^+$) 518.1710, found 518.1835.

Synthesis of 1-(FMOC- aminoacetamido)-4-(bromoacetamido)anthracene-9,10-dione



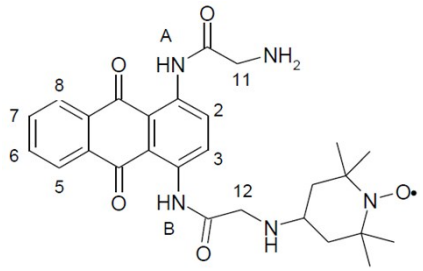
600 mg of 1-(aminoacetamido)-4-aminoanthracene-9,10-dione was suspended in 600 mL of dry toluene. 4.35 mmol of pyridine and 380 µl of bromoacetylbromide were added to the mixture which was heated to reflux for 12 h. The orange mixture was cooled and the solvent was reduced to a small volume (20 mL). 150 mL of ethanol were added to the suspension and the mixture was cooled to -20°C, promoting the formation of a crystalline solid. This orange solid was filtered and washed with water and ethanol to give 620 mg of pure 1-(FMOC- aminoacetamido)-4-(bromoacetamido)anthracene-9,10-dione. Yield 84%. ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 12.77 (s, 1H; HA), 12.56 (s, 1H; HB), 9.04 (d, 1H, *J* = 9.5 Hz; H2), 8.86 (d, 1H, *J* = 9.5 Hz; H3), 8.27 (d, 1H, *J* = 7.4 Hz; H8), 8.15 (d, 1H; = 7.4 Hz; H5), 7.94-7.80 (m, 5H; H6,1',4',5',8'), 7.70 (m, 1H; HC), 7.54-7.20 (m, 5H; H7,2',3',6',7'), 4.43-4.35 (m, 3H; H12,9'), 3.85 (m, 4H; H11,14). HRMS (ESI): calculated for $C_{33}H_{25}N_3O_6$ ($M+H^+$) 638.0921, 640.0907, found 638.0968, 640.0910.

Synthesis of 1-(FMOC- aminoacetamido)-4-(2'-TEMPOaminoacetamido)anthracene-9,10-dione



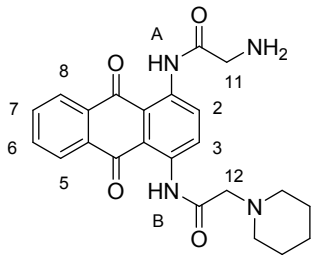
9.43 µl of pyridine and 33 mg of 4-Amino-TEMPO (4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl) were added to a solution of 50 mg of 1-(FMOC- aminoacetamido)-4-(bromoacetamido)anthracene-9,10-dione in 10 mL of amine-free DMF. The mixture was heated to 70 °C for 6 h (the reaction was monitored by TLC with EtOAc as eluent). The solvent was subsequently evaporated and the orange solid obtained was suspended in EtOH, filtered and washed again with H_2O and EtOH to obtain 35 mg of 1-(FMOC- aminoacetamido)-4-(2'-TEMPOaminoacetamido)anthracene-9,10-dione. ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 12.60 (bs, 2H; HA,B), 9.10 (bs, 1H; H2), 8.90 (bs, 1H; H3), 8.20 (bs, 2H; H5,8), 7.90-7.70 (m, 5H; H6,1',4',5',8'), 7.50-7.10 (m, 5H; H7,2',3',6',7'), 4.40-4.25 (m, 3H; H12,9'), 3.80 (m, 4H; H11,14). HRMS (ESI): calculated for $C_{42}H_{43}N_5O_7$ ($M+H^+$) 729.3157, found 729.3197.

*Synthesis of 1-(aminoacetamido)-4-(2'-TEMPOaminoacetamido)anthracene-9,10-dione (Compound **1**)*



10 mg of 1-(FMOC- aminoacetamido)-4-(TEMPOaminoacetamido)anthracene-9,10-dione was dissolved in 500 µl of a 10 % piperidine solution in DMF. The solution was stirred at room temperature for 30 minutes (the reaction was followed by TLC using THF as eluent). The solvent was evaporated and the crude solid was suspended in cold Et_2O (7 mL). The suspension was centrifuged and the solid was collected, obtaining 6 mg of 1-(aminoacetamido)-4-(2'-TEMPOaminoacetamido) anthracene-9,10-dione. Yield 85%. ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 12.50 (bs, 2H; HA,B), 9.05 (bs, 1H; H2), 8.80 (bs, 1H; H3), 8.15 (bs, 2H; H5,8), 7.80 (m, 1H; H6), 7.45 (m, 1H; H7), 3.90-3.60 (m, 4H; H11,12). ^{13}C-NMR ($CDCl_3$, 75MHz) δ (ppm): 181.1, 169.8, 169.2, 141.1, 136.1, 130.9, 129.8, 129.2, 121.3, 118.0, 55.1, 47.2. HRMS (ESI): calculated for $C_{27}H_{33}N_5O_5$ ($M+H^+$) 507.3476, found 507.3604.

*Synthesis of 1-(aminoacetamido)-4-(piperidinoaminoacetamido)anthracene-9,10-dione (Compound **2**)*



Compound **2** was prepared according to the procedure described above from 1-(FMOC-aminoacetamido)-4-(bromoacetamido)anthracene-9,10-dione using piperidine instead of 4-Amino-TEMPO (4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl). Yield 76%. ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 12.48 (bs, 2H; HA,B), 9.11 (bs, 1H; H2), 8.83 (bs, 1H; H3), 8.15 (bs, 2H; H5,8), 7.87 (m, 1H; H6), 7.38 (m, 1H; H7), 3.90-3.60 (m, 4H; H11,12), 2.20 (m, 4H, NCH_2), 1.51 (m, 6H, CH_2). ^{13}C-NMR ($CDCl_3$, 75MHz) δ (ppm): 182.1, 170.2, 169.2, 138.7, 135.2, 131.1, 130.8, 129.2, 125.4, 118.1, 59.1, 52.2, 42.1, 27.3, 22.1. HRMS (ESI): calculated for $C_{23}H_{25}N_4O_4$ ($M+H^+$) 421.1870, found 421.1875.

HPLC of 1 and 2 following EPR experiments

In Figure S1, we report the HPLC profiles of 1 and 2 recorded after the EPR experiments in toluene solution. The Figure shows that both compounds are pure and photodegradation is absent. The conditions of the elution are as follows:

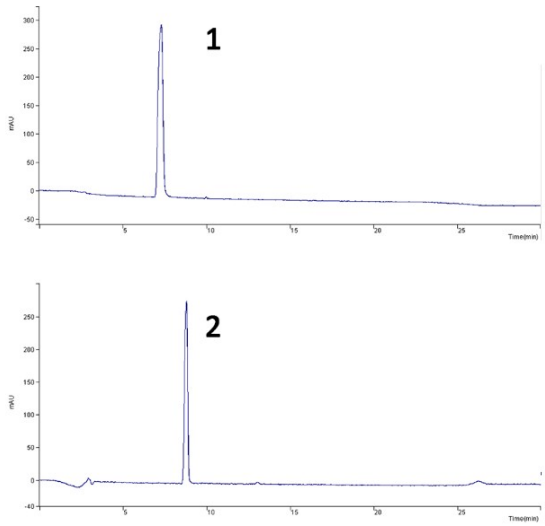


Figure S1 HPLC elution profiles of **1** and **2** from toluene solution taken from the EPR tube after the experiment.

S2. TR-EPR surfaces

For convenience, we report in Figure S2 the TR-EPR surfaces of **1** in toluene (left) and buffered solution containing DNA (right). The letters highlight positions on the surfaces referred to in the text.

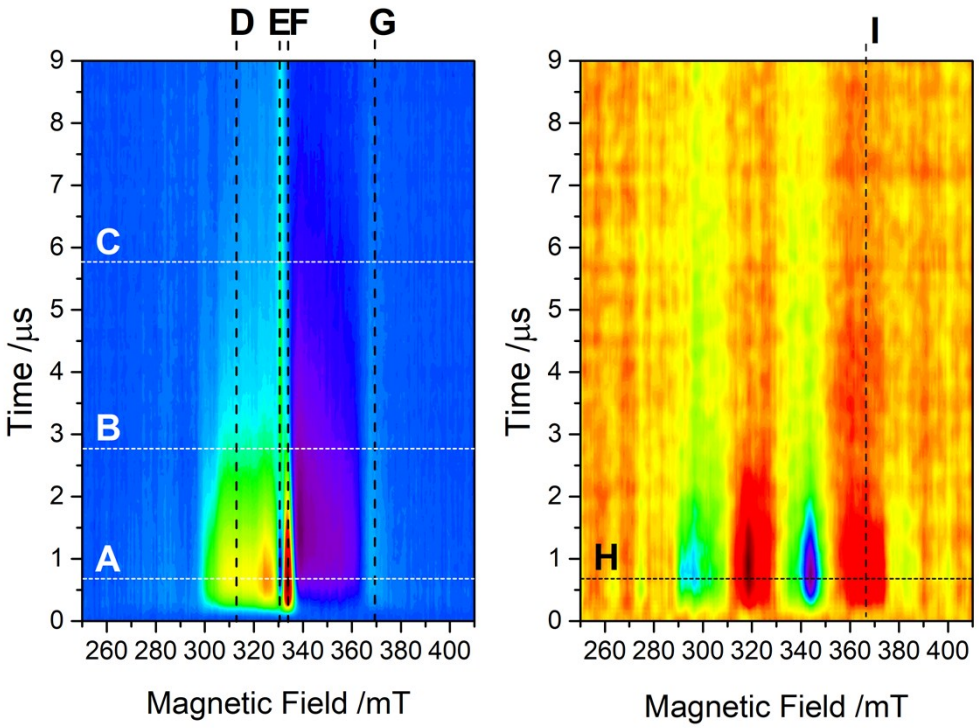


Figure S2 TR-EPR surfaces of **1** in frozen solution of toluene, left, and buffered solution of DNA, right. T = 130 K. The colors in the rainbow palette represent the intensity of the signal from emission (purple) to enhanced absorption (red), the zero-intensity level depends on the plot and can be determined from the low and high field regions.

S3. Time evolution of the central region of antraquinone 1

In Figure S3, we report the TR-EPR spectra of the central region of **1** in toluene at 130 K. In the figure, the spectra are shown with the simulations of the D_1 and D_0 states that form the main contribution to the spectral lineshape. As mentioned above, the broad shoulders in these spectra are of uncertain attribution. One possible explanation is that these featureless signals arise from a small pool of molecules in which the conformation of the nitroxide side chain is at an intermediate distance from the anthraquinonic core leading to a weakly coupled state.

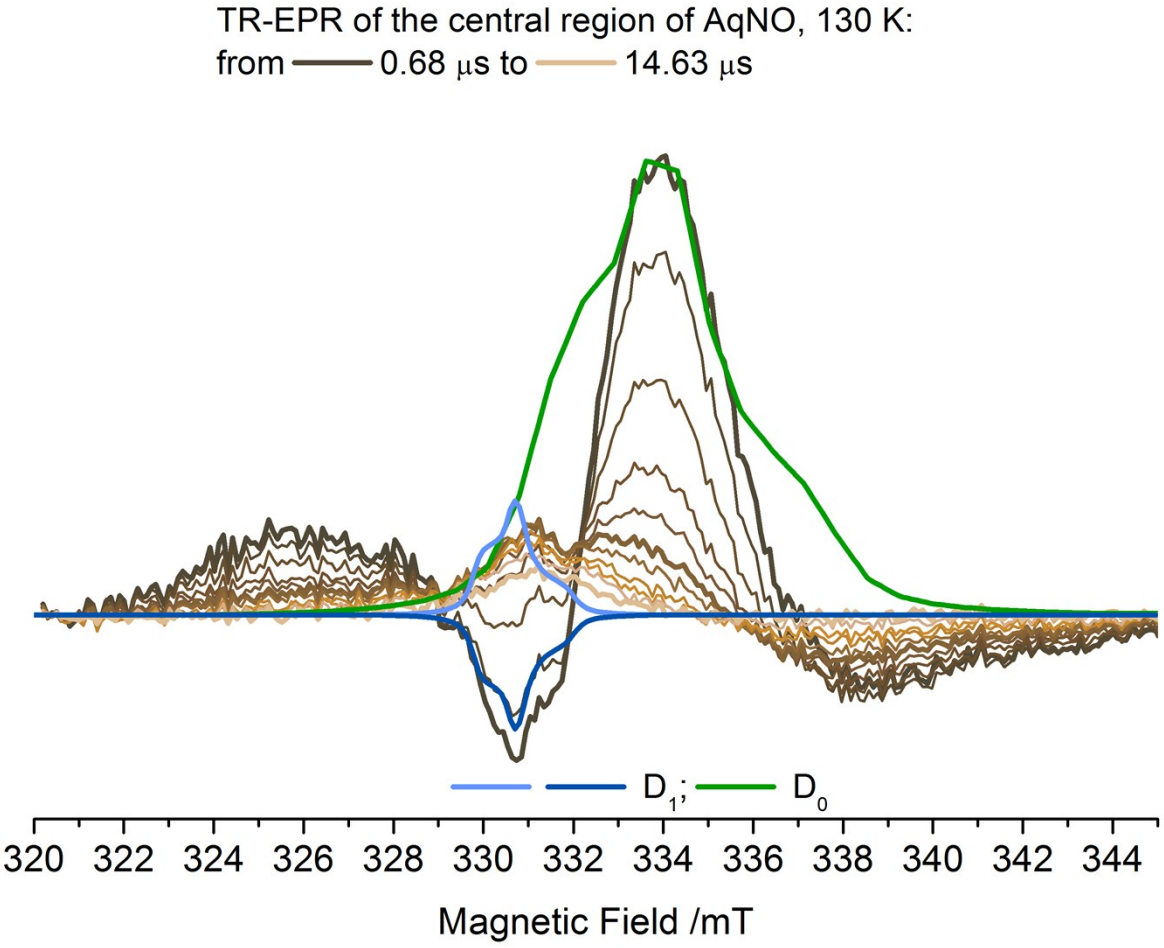


Figure S3 TR-EPR of the central region of **1** in frozen solution of toluene/polystyrene, T = 130 K. In blue, the simulation of the D_1 state polarized in emission (dark blue) and enhanced absorption (light blue); in green, the simulation of the D_0 state polarized in enhanced absorption.

S4. Time evolution profiles at different magnetic field values

In Figure S4, we show the fitting of the time evolution traces of **1** (left) and **2** (right) in frozen toluene solution and buffered frozen solution containing DNA. For **1**, the letters refer to the DAF shown on the surfaces shown in Figure S2, above. The decay curves represent all possible routes of triplet depopulation, but are mainly dominated by spin lattice relaxation processes with loss of the polarization and return to a Boltzmann distribution.

The different decay curves for **1** in toluene are taken at field values where one of the different species detected in the experiment dominates: the main state at field position **D** is the excited quartet Q_1; at position **E** the two states that contribute with comparable intensity are the excited doublet D_1 and the central transition of the quartet state Q_1; at position **F** the polarized doublet D_0 is the main contributing state; the triplet state T_1 dominates at **G** position. The decay curves for **1** and for **2** in the buffered solution containing DNA are taken at approximately the field position corresponding to the field position **G** in toluene for comparison.

The fittings of the decaying region of the time traces are single exponential fittings for curves (D), (F), (G), and (I) while curve (E) is fitted by a double exponential. The fitting starts immediately following the end of the rise time of the TR-EPR signal, approximately at 0.9 μs. The values of the time constants of the exponentials are: **1** in toluene: (D) 1.77±0.01 μs; (E) rising, 0.97±0.01 μs and decaying, 8.5±0.1 μs; (F) 1.06±0.005 μs; (G) 5.2±0.3 μs; **2** in buffered DNA solution: (I) 2.0±0.2 μs; **2** in toluene: 3.8±0.1 μs; **1** in buffered DNA solution: 2.3±0.1 μs. We must note that in DNA the triplet state decay for both **1** and **2** is significantly faster than in toluene frozen solution.

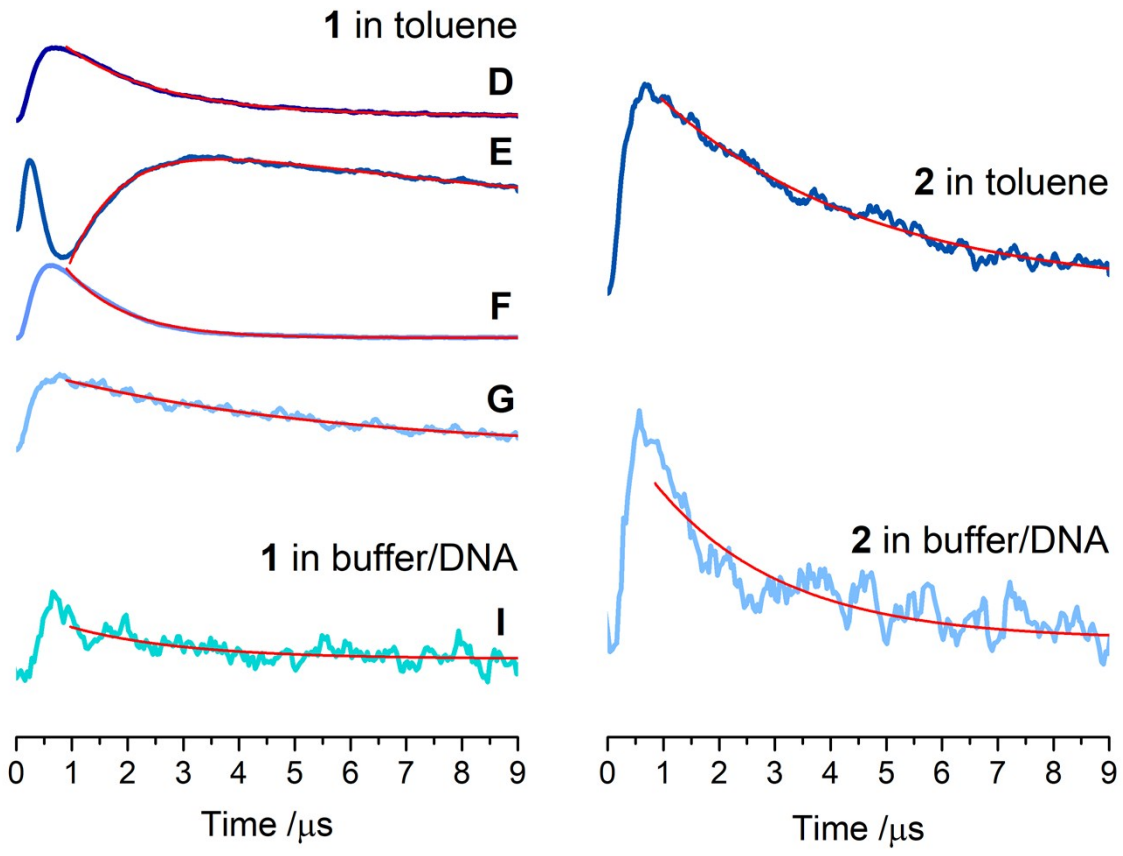


Figure S4 Left, time evolution profiles (shades of blue) and fittings (red) for **1** in toluene (D-G) and in buffered DNA solution (I) at 130 K. Field values: D) 312.9 mT; E) 330.5 mT; F) 334.0 mT; G) 369.3 mT; I) 366.5 G. Right, time evolution profiles and fittings for **2** in toluene and in buffered DNA solution at 130 K. Field values: 369.4 mT and 366.5 mT, respectively.

S5. Modelling of anthraquinone 1

In Figure S5, the minimized conformations of anthraquinone **1** in vacuum and intercalated in a duplex DNA sequence are compared. The image shows the same models as in Figure 6 in the main text from a different viewing angle. As highlighted in Figure S5, the side chain of compound **1** is pointing towards the anthraquinone scaffold in the unbound, minimized model. When intercalation occurs, the compound binds the DNA showing the typical anthraquinone-duplex interaction motif [*Belinda S. Parker, Trevor Buley, Ben J. Evison, Suzanne M. Cutts, Greg M. Neumann, Magdy N. Iskander, and Don R. Phillips. A Molecular Understanding of Mitoxantrone-DNA Adduct Formation. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 18, 18814–18823, 2004*], constraining the side chain outward and pointing towards the solvent. Molecular graphics were obtained with the UCSF Chimera package (Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, supported by NIGMS P41-GM103311) [*Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera-a visualization system for exploratory research and analysis. J Comput Chem. 2004 Oct; 25(13):1605-12*].

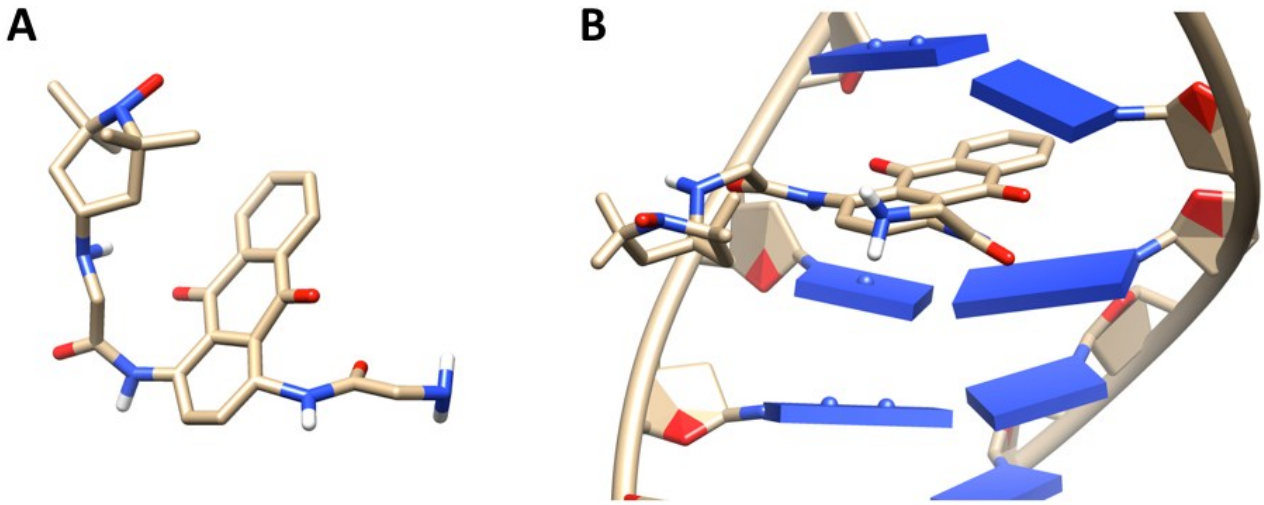


Figure S5 Minimized molecular models of unbound (A) and intercalated (B) compound **1**.