Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supporting Information

Introducing Structural Flexibility Into Porphyrin-DNA Zipper Arrays

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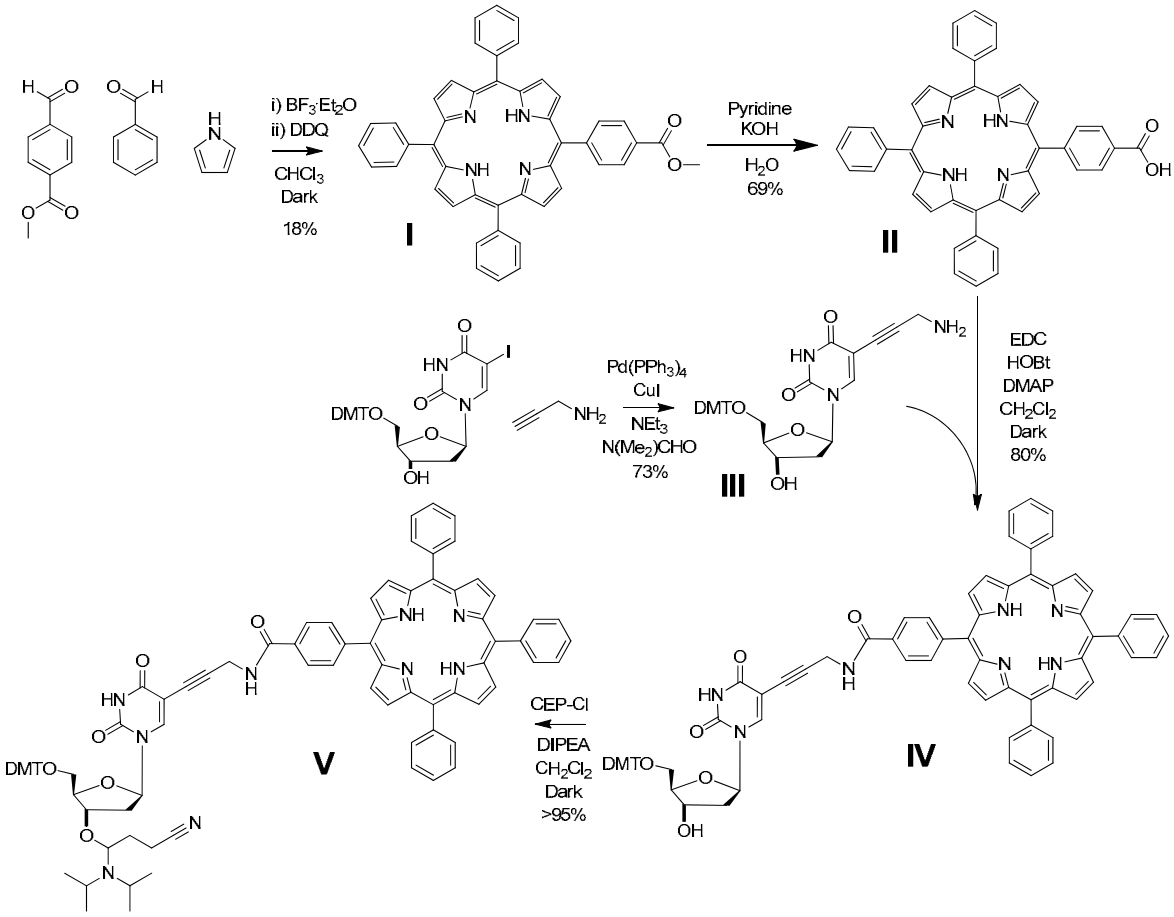
General

Reagents and solvents were purchased from Fisher, Sigma Aldrich, Fluka and Alfa and were used as received. Solid supported DNA synthesis reagents were purchased from Proligo, DNA purification columns were puchased from Glen Research and Berry and Associates, desalting columns was purchased from GE Healthcare. For flash chromatography, silica gel 60 (Merck and Fisher), silica gel H (Fluka) and basic aluminium oxide (Acros) were used. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 instrument (400 and 100 MHz respectively). Chemical shift data are given as δ in units of parts per million (ppm) relative to residual undeuterated solvent. MALDI-ToF-MS were recorded on a ThermoBioAnalysis Dynamo using a *p*-nitroaniline matrix and referenced against TPP (MW 614.25) and 2,8,12,18-tetrahexyl-3,7,13,17-tetramethyl-5,15-di(*p*-(3-hydroxy-3-methyl but-2-ynyl)phenyl porphyrin (MW 1082.88). Low resolution and high resolution electrospray MS were conducted on a Walters ZMD and an LTQ Orbitrap XL respectively. DNA Melting profiles were recorded using a Varian Cary 300 Biospectrophotometer and are an average of at least two denaturing-annealing cycles. The absorptions of oligonucleotide solutions were measured at 260 nm in a quartz cuvette with a path length of 1 cm. Fluorescence spectroscopy was conducted on a Varian Eclipse spectrometer in quartz cells with path length of 1 cm, fluorescence melting samples were excited at 420 nm and monitored at 655 nm. pH values were determined using an Accumet AB10 basic pH meter. CD spectroscopy was performed using an Applied Photophysics Chirascan Circular Dichroism Spectrometer (150 W Xe arc).

Acetylene linked building block (**1**) was synthesized as per previously published methods.[1]



Scheme S1. Synthesis route to amide monomer **V**

5-(*p*-methyl benzoate)-10,15,20-triphenyl porphyrin (I)

Pyrrole (2.52 ml, 6.0 eq, 36 mmol), benzaldehyde (3.64 ml, 6.0 eq, 36 mmol) and methyl-*p*-formylbenzoate (985.0 mg, 1.0 eq, 6 mmol) were dissolved in chloroform (500 ml), purged with N_2 (1 hour) in the dark before boron trifluoride etherate (0.69 ml, 0.9 eq, 5.4 mmol) was added and the reaction allowed to stir at room temperature. After one hour DDQ (8.14 g, 6.0 eq, 36 mmol) was added and stirred for 16 hours. Reaction mixture was concentrated *in vacuo* before purification thrice by column chromatography (first column - silica/alumina, eluent – DCM; second and third columns - silica, eluent – toluene) to yield 729.3 mg (18 %) of a dark purple crystalline solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm -2.57 (s, 2 H), 4.19 (s, 3 H), 7.78 - 7.88 (m, 9 H), 8.30 - 8.37 (m, 6 H), 8.43 (d, *J*=8.16 Hz, 2 H), 8.55 (d, *J*=8.16 Hz, 2 H), 8.94 (d, *J*=4.77 Hz, 2 H), 9.00 (s, 4 H), 9.00 (d, *J*=4.77 Hz, 2 H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$): δ ppm 52.38 (CH_3), 118.54 (C), 120.40 (C), 120.60 (C), 126.69 (CH), 127.75 (CH), 127.90 (CH), 129.55 (C), 130.20 - 132.50 (m, C), 134.52 (CH), 134.57 (CH), 142.03 (C), 142.05 (C), 147.05 (C), 167.30 (C). **MALDI-ToF** ($C_{46}H_{32}N_4O_2$): *m/z* calcd. 672.77; found 673.76 $[M+H]^+$.

5-(*p*-benzoic acid)-10,15,20-triphenyl porphyrin (II)

5-(*p*-methyl benzoate)-10,15,20-triphenyl porphyrin (729.3 mg, 1.09 mmol, 1.0 eq) and potassium hydroxide (2.96 g, 52.7 mmol, 50 eq) were dissolved in pyridine (15 ml) and water (2 ml) under N_2 and heated to 40^{o}C for 20 hours. The reaction mixture was poured into brine (150 ml) and DCM (300 ml), 2M hydrochloric acid (6 ml) was added. The organic phase was washed twice with brine (2 X 100 ml) before drying over $MgSO_4$. Pyridine was removed through co-evaporation with toluene and chloroform. The crude product was filtered twice through Celite 545 and eluted with chloroform before crystallisation from toluene to give 489.9 mg (69 %) of a purple solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm -2.82 (br. s., 2 H), 7.62 - 7.79 (m, 9 H), 8.10 - 8.21 (m, 6 H), 8.27 (d, *J*=8.03 Hz, 2 H), 8.41 (d, *J*=8.03 Hz, 2 H), 8.82 (br. s., 8 H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$): δ ppm 118.68 (C), 120.21 (C), 120.37 (C), 126.58 (CH), 127.65 (CH), 127.97 (CH), 130.67 (C), 134.40 (CH), 141.88 (C), 146.48 (C), 180.71 (C). **MALDI-ToF** ($C_{45}H_{30}N_4O_2$): *m/z* calcd. 658.75; found 659.70 $[M+H]^+$.

5'-(4'',4'''-dimethoxytrityl)-5-propargylamine-dU (III)

5'-(4'',4'''-dimethoxytrityl)-5-iodo-dU (492.0 mg, 0.75 mmol, 1.00 eq) and triethylamine (0.73 ml, 5.25 mmol, 7.00 eq) were dissolved in anhydrous DMF (5 ml) in the dark and purged with N_2 for 30 mins. Propargylamine (103 µL, 1.50 mmol, 2.00 eq) and copper (I) iodide (35.7 mg, 0.188 mmol, 0.25 eq) were added and purged for 20 minutes prior to the addition of palladium *tetrakis*(triphenyl phosphine) (86.5 mg, 75.0 µmol, 0.10 eq), the reaction was stirred for 4.5 hours. The reaction mixture was poured into EDTA solution (aq, 5 % w/v, pH 9.0, 100 ml), partitioned with chloroform (100 ml), the aqueous phase was re-extracted with chloroform (100 ml). The organic phases were combined, washed with EDTA solution (aq, 5 % w/v, pH 9.0, 100 ml) and dried over $MgSO_4$. Column chromatography (silica neutralised with NEt_3, eluent – 5 % MeOH in DCM) gave the product as a golden foam, 327.1 mg (73 %). **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 2.33 (d, *J*=6.78 Hz, 1 H), 2.54 (d, *J*=6.65 Hz, 1 H), 3.22 (br. s., 1 H), 3.31 (d, *J*=7.91 Hz, 1 H), 3.44 (d, *J*=9.29 Hz, 1 H), 3.78 (s, 6 H), 4.13 (br. s., 1 H), 4.55 (br. s., 1 H), 6.35 (dd, *J*=6.27, 5.90 Hz, 1 H), 6.87 (d, *J*=8.53 Hz, 4 H), 7.22 (t, *J*=7.22 Hz, 1 H), 7.31 (dd, *J*=7.78, 7.53 Hz, 2 H), 7.37 (d, *J*=7.28 Hz, 4 H), 7.47 (d, *J*=7.65 Hz, 2 H), 8.19 (s, 1 H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$): δ ppm 31.54 (CH_2), 41.49 (CH_2), 55.18 (CH_3), 63.54 (CH_2), 71.61 (CH), 73.35 (C), 85.65 (CH), 86.56 (CH), 86.78 (C), 94.12 (C), 99.90 (C), 113.24 (CH), 126.79 (CH), 127.83 (CH), 127.94 (CH), 129.90 (CH), 129.96 (CH), 135.43 (C), 135.57 (C), 142.60 (CH), 144.61 (C), 149.50 (C), 158.46 (C), 162.29 (C). **ESI(pos) (**$C_{33}H_{33}N_3O_7$): *m/z* calcd. 583.63, found 584.30 $[M+H]^+$.

***N*-(5'(4'',4'''-dimethoxytrityl)-5-propargyl-dU)-5'''',10'''',15''''-triphenyl-20''''-(*p*-benzamide)-porphyrin (IV)**

5,10,15-triphenyl-20-*p*-benzoic acid porphyrin (105.0 mg, 0.16 mmol, 1.00 eq), 5'-(4'',4'''-dimethoxytrityl)-5-propargylamine-dU (121.0 mg, 0.21 mmol, 1.3 eq), EDC (56 µL, 0.32 mmol, 2.0 eq), HOBT (24.4 mg, 0.16 mmol, 1.0 eq) and DMAP (38.9 mg, 0.32 mmol, 2.0 eq) were dissolved in anhydrous DCM (5 ml) and stirred in the dark under N_2 for 5 ½ hours. The reaction mixture was washed with brine (25 ml) and dried over Na_2SO_4. The product was purifed by column chromatography (silica neutralised with NEt_3, eluent – 0.5 % → 2.5 % MeOH in DCM)to yield156.7 mg of a purple solid (80 %). **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm -2.75 (s, 2 H), 2.27 - 2.39 (m, 1 H), 2.47 - 2.59 (m, 1 H), 3.35 (dd, *J*=10.42, 2.76 Hz, 1 H), 3.41 (dd, *J*=10.67, 2.13 Hz, 1 H), 3.68 (s, 6 H), 4.09 (d, *J*=2.26 Hz, 1 H), 4.30 (dd, *J*=17.82, 4.52 Hz, 1 H), 4.37 (dd, *J*=17.94, 4.89 Hz, 1 H), 4.51 - 4.59 (m, 1 H), 6.34 (t, *J*=6.53 Hz, 1 H), 6.70 (t, *J*=4.52 Hz, 1 H), 6.82 (dd, *J*=8.78, 2.01 Hz, 4 H), 7.18 (t, *J*=7.28 Hz, 1 H), 7.28 (t, *J*=7.65 Hz, 2 H), 7.36 (d, *J*=8.16 Hz, 4 H), 7.45 (d, *J*=7.65 Hz, 2 H), 7.67 - 7.85 (m, 9 H), 7.96 (d, *J*=7.91 Hz, 2 H) 8.20 (d, *J*=7.40 Hz, 6 H), 8.22 (d, *J*=7.91 Hz, 2 H), 8.27 (s, 1 H), 8.77 (d, *J*=4.77 Hz, 2 H), 8.85 (d, *J*=4.77 Hz, 2 H), 8.86 (s, 4 H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$): δ ppm 30.86 (CH_2), 41.65 (CH_2), 55.21 (CH_3), 63.54 (CH_2), 72.21 (CH), 74.58 (C), 85.90 (CH), 86.69 (CH), 87.12 (C), 89.50 (C), 99.47 (C), 113.40 (CH), 118.65 (C), 120.33 (C), 120.51 (C), 125.48 (CH), 126.67 (CH), 127.01 (CH), 127.74 (CH), 127.87 (CH), 128.10 (CH), 129.96 (CH), 130.49 - 132.35 (m, CH), 132.95 (C), 134.48 (CH), 134.51 (C), 135.50 (C), 142.20 (C), 143.41 (CH), 144.54 (C), 145.60 (C), 149.21 (C), 158.61 (C), 162.08 (C), 166.93 (C). **HR-ESI(pos)** ($C_{78}H_{61}N_7O_8$): *m/z* calcd.1224.4582; found 1224.4663 $[M]^+$.

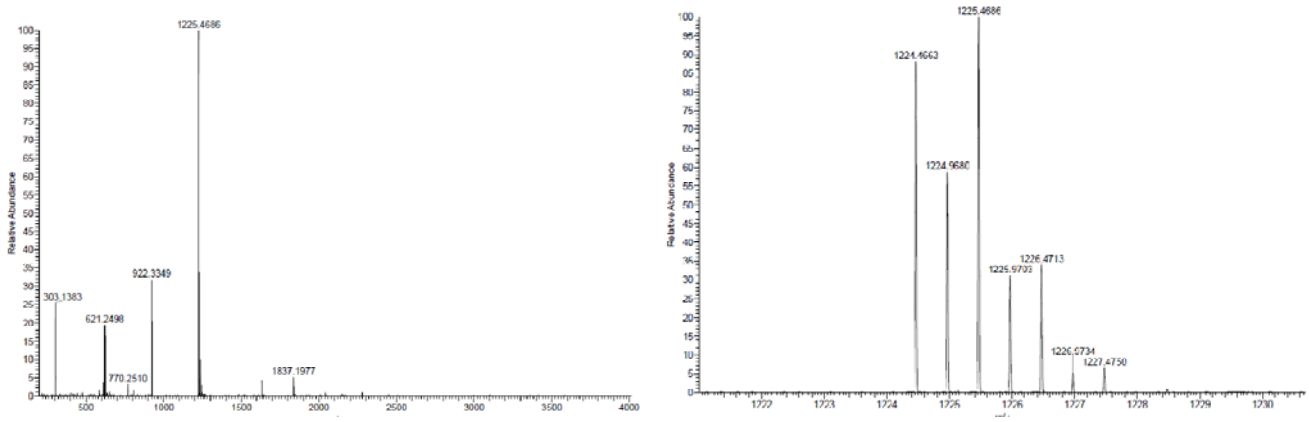


Figure S1. Overview and expansion of high resolution mass spectrometry of compound **IV**

***N*-(5'(4'',4'''-dimethoxytrityl)-5-propargyl-dU)-5'''',10'''',15''''-triphenyl-20''''-(*p*-benzamide)-porphyrin phosphor-amidite (V)**

N-(5'(4'',4'''-dimethoxytrityl)-5-propargyl-dU)-5'''',10'''',15''''-triphenyl-20''''-(*p*-benzamide)-porphyrin (120.0 mg, 98.0 µmol, 1.0 eq) was dissolved in anhydrous DCM (2 ml) and purged with N_2 in the dark prior to the addition of DIPEA (68.3 µL, 0.39 mmol, 4.0 eq), this was purged with N_2 for 5 minutes and 2-cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine (69.9 µL, 0.29 mmol, 3.0 eq) was added. Reaction reached completion after 2.5 hours, the product was precipitated with hexanes (25 ml) and cooled (-18 ^{o}C) for 20 minutes. Hexanes were removed and the precipitated product washed with an additional portion of hexanes (10 ml), the crude product was dried *in vacuo* for 2 hours prior to use in DNA synthesis. 120.5 mg crude product obtained. Due to the very high oxidative sensitivity of the compound no characterisation bar TLC was performed. The crude product was used immediately for DNA synthesis. $\mathbf{R_f}$ (10:1 DCM:MeOH): 0.46.

DNA synthesis

3' to 5' solid phase DNA synthesis was conducted on an Expedite synthesiser using standard reagents, the coupling times for modified nucleobases was increased to 5 minutes (20 – 30 mM solution in DCM-CH_3CN 1:1). Modified sequences were terminated with the addition of a fluorous-DMT tagged nucleobase for purification by fluorous affinity columns (Fluoro-Pak), standard DNA sequences were purified by Glen-Pak columns.

UV-Vis and fluorescence spectra

UV-Vis and fluorescence spectra were conducted on samples of equal absorbance at 420 nm (0.48 ± 0.02 a.u.) in 1 mL of phosphate buffer (100 mM sodium phosphate, 100 mM sodium chloride, 1 mM di-sodium EDTA, pH 7).

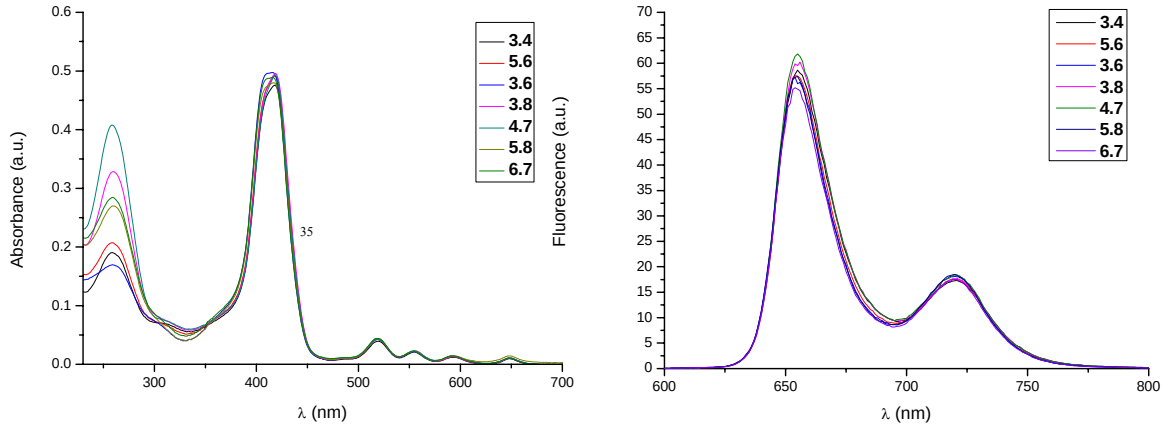
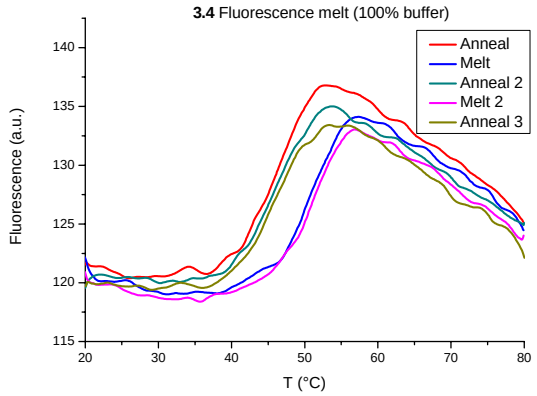
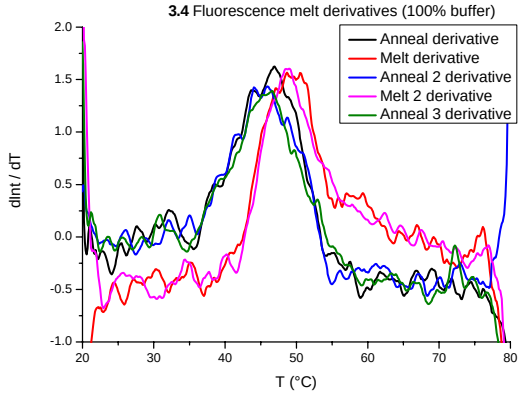


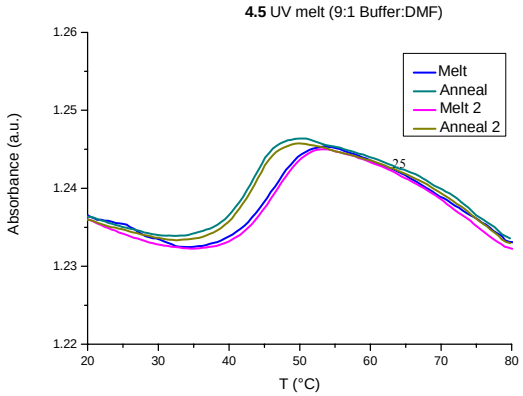
Figure S2. UV-Vis (left) and fluorescence (right) spectra of porphyrin modified DNA.

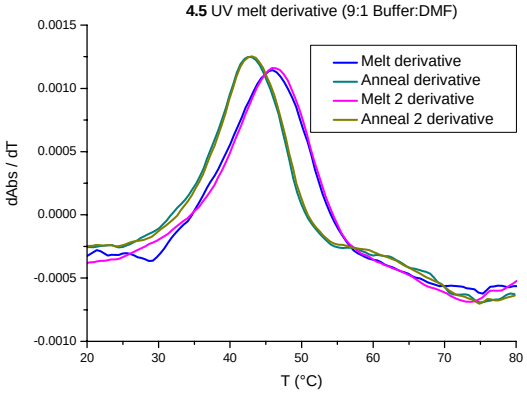
Thermal denaturing

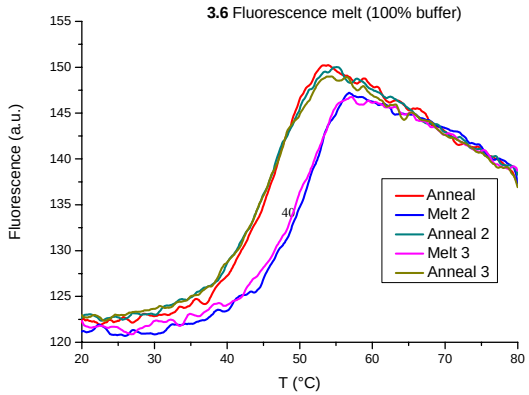
Thermal denaturing of DNA samples for UV-Vis and fluorescence melting were conducted at a duplex concentration of 1 µM in 1 mL of phosphate buffer (100 mM sodium phosphate, 100 mM sodium chloride, 1 mM di-sodium EDTA, pH 7). Melting and annealing rates were set to 1 °C min^{-1}.

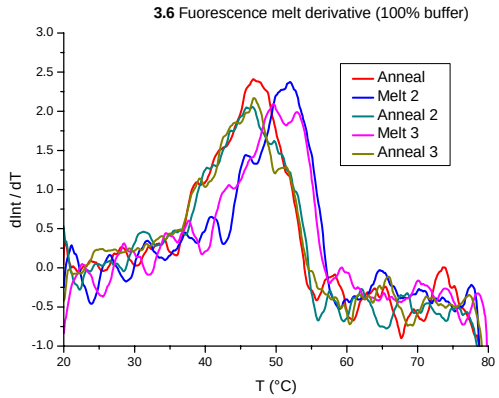


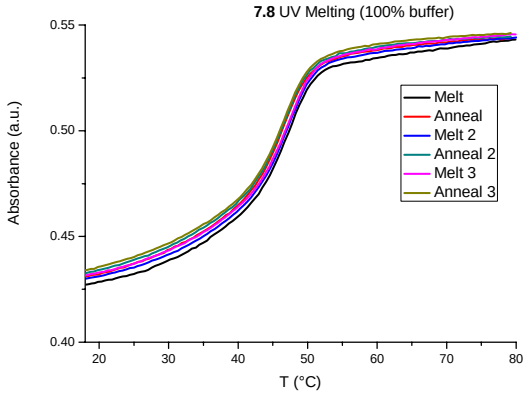


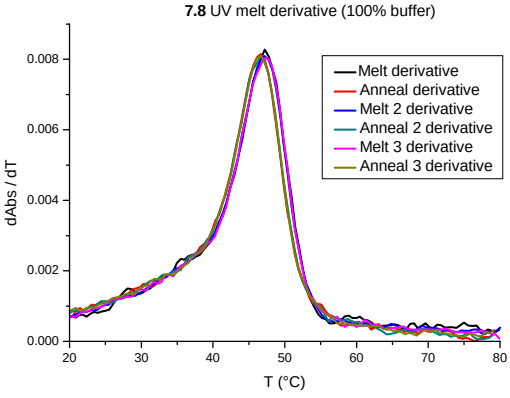












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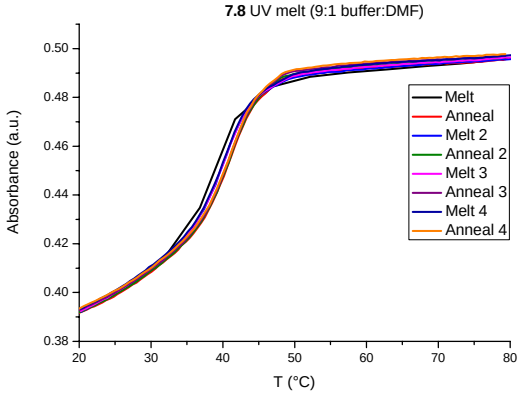
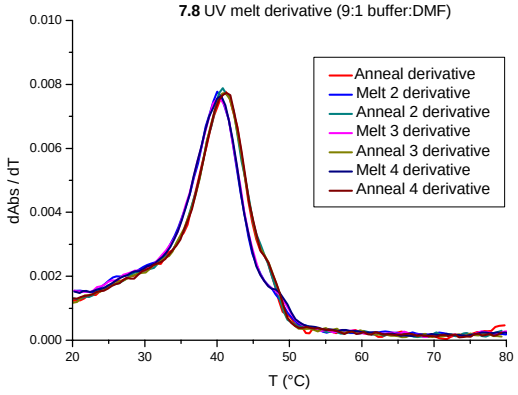


7.8 UV melt derivative (9:1 buffer:DMF)



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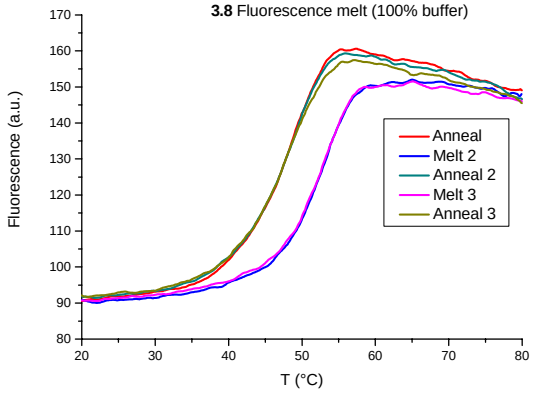

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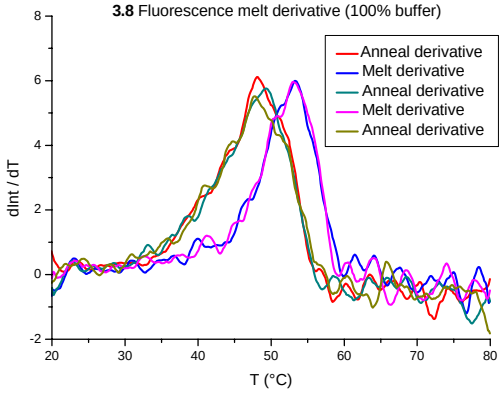

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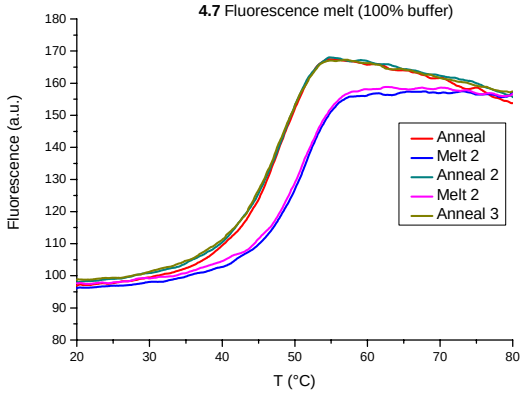

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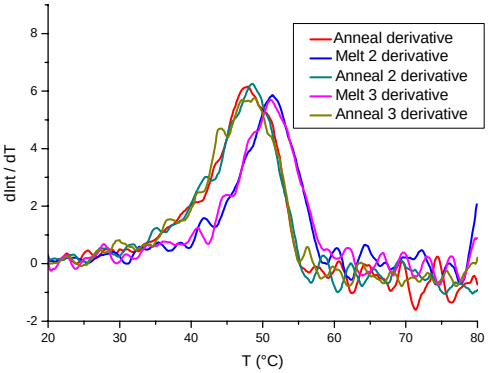
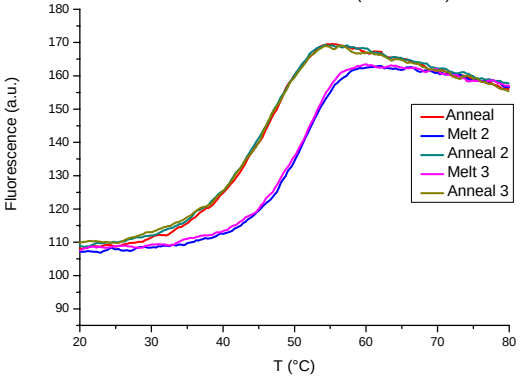


5.8 Fluorescence melt (100% buffer)



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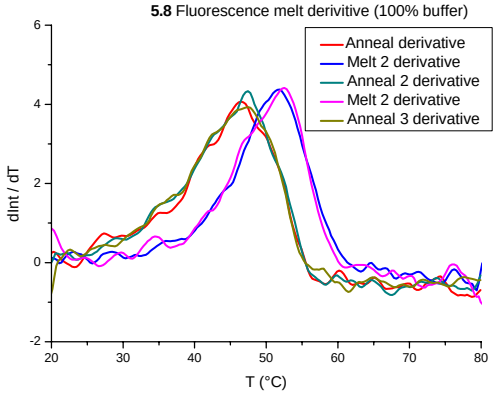

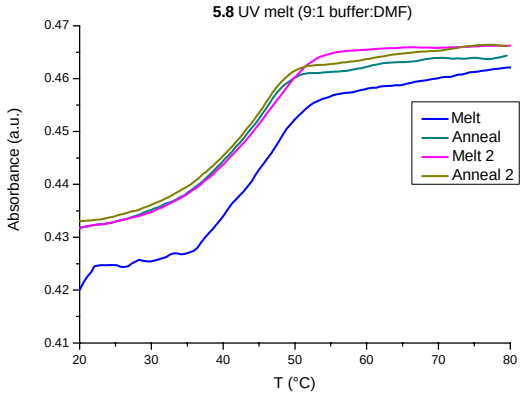
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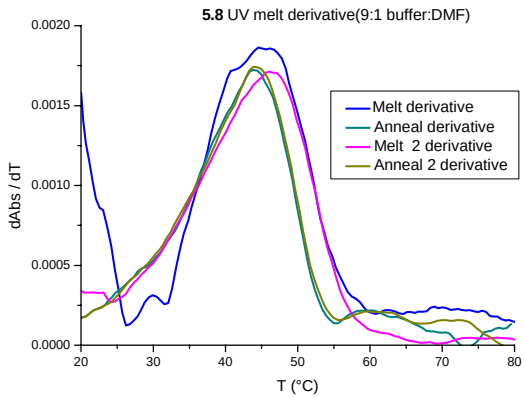
Absorbance (a.u.)
T (°C)
Melt
Anneal
Melt 2
Anneal 2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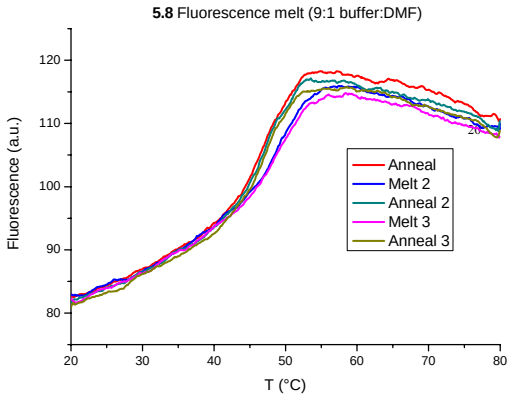
dAbs / dT
T (°C)
Melt derivative
Anneal derivative
Melt 2 derivative
Anneal 2 derivative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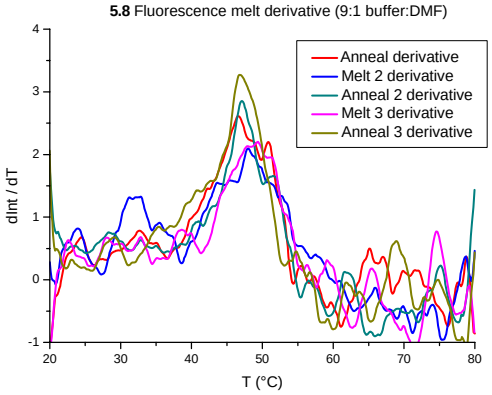
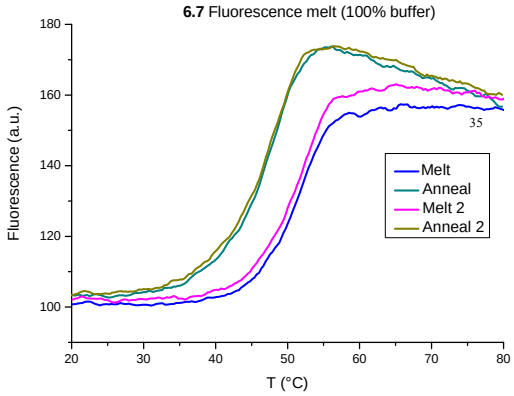
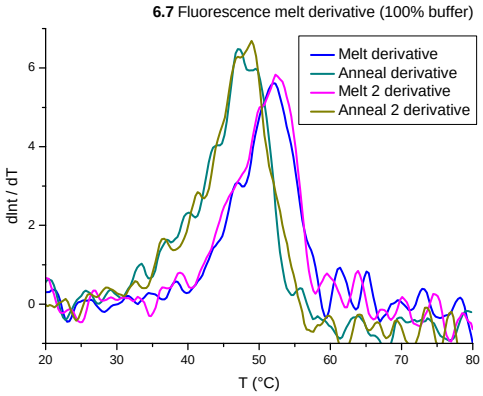
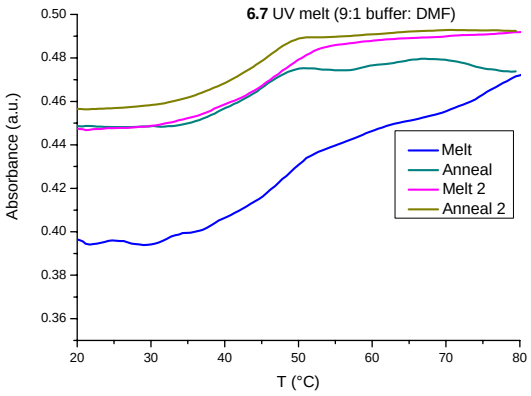
Fluorescence (a.u.)
T (°C)
Anneal
Melt 2
Anneal 2
Melt 3
Anneal 3


5.8 Fluorescence melt derivative (9:1 buffer:DMF)
dInt / dT
T (°C)
Anneal derivative
Melt 2 derivative
Anneal 2 derivative
Melt 3 derivative
Anneal 3 derivative


6.7 Fluorescence melt (100% buffer)
Fluorescence (a.u.)
T (°C)
Melt
Anneal
Melt 2
Anneal 2


6.7 Fluorescence melt derivative (100% buffer)
dInt / dT
T (°C)
Melt derivative
Anneal derivative
Melt 2 derivative
Anneal 2 derivative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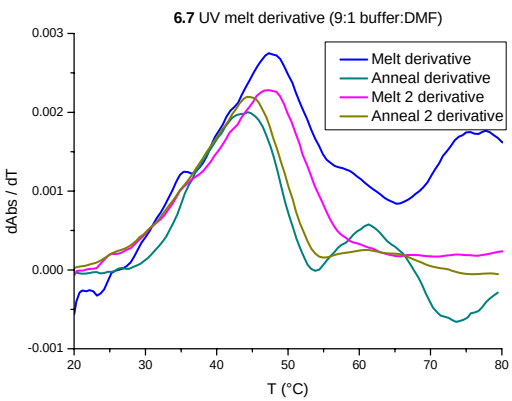
Absorbance (a.u.)
T (°C)
Melt
Anneal
Melt 2
Anneal 2


6.7 UV melt derivative (9:1 buffer:DMF)
dAbs / dT
T (°C)
Melt derivative
Anneal derivative
Melt 2 derivative
Anneal 2 derivative

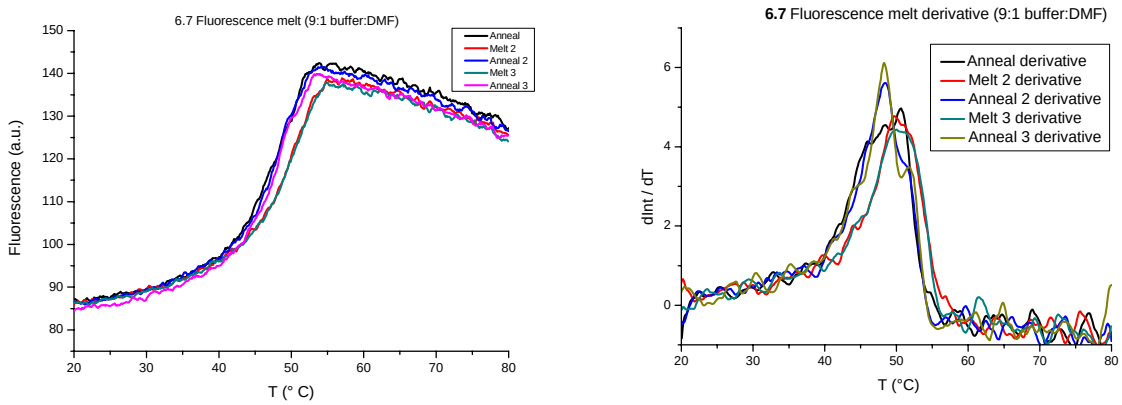


Figure S3. Thermal denaturing traces and first derivatives of porphyrin modified DNA.

Molecular modelling

Molecular modeling of **3.4**, **5.6** and **3.6** was carried out with Schrodinger's MacroModel program using an AMBER* forcefield, the 'sticky ends' were omitted from the calculations for clarity. Calculations were carried out using water solvent and energy minimisation conducted until the gradient of the potential energy reached an order of magnitude of 10^{-2}, typically around 15,000 iterations. Starting structures for all models were identical and idealised B type DNA helices.

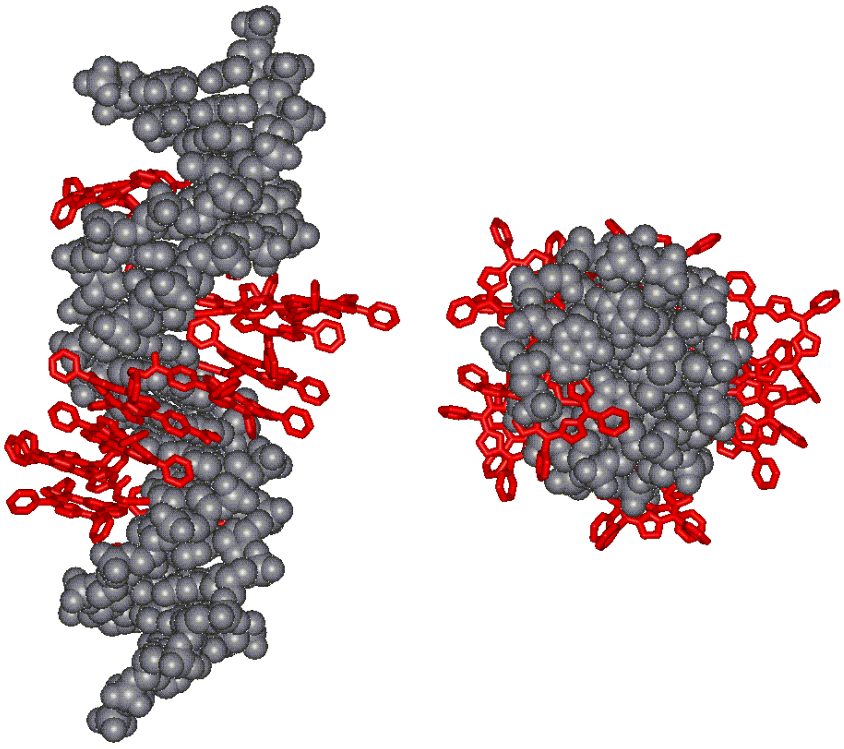


Figure S4. Duplex **3.4,** side on and top down views

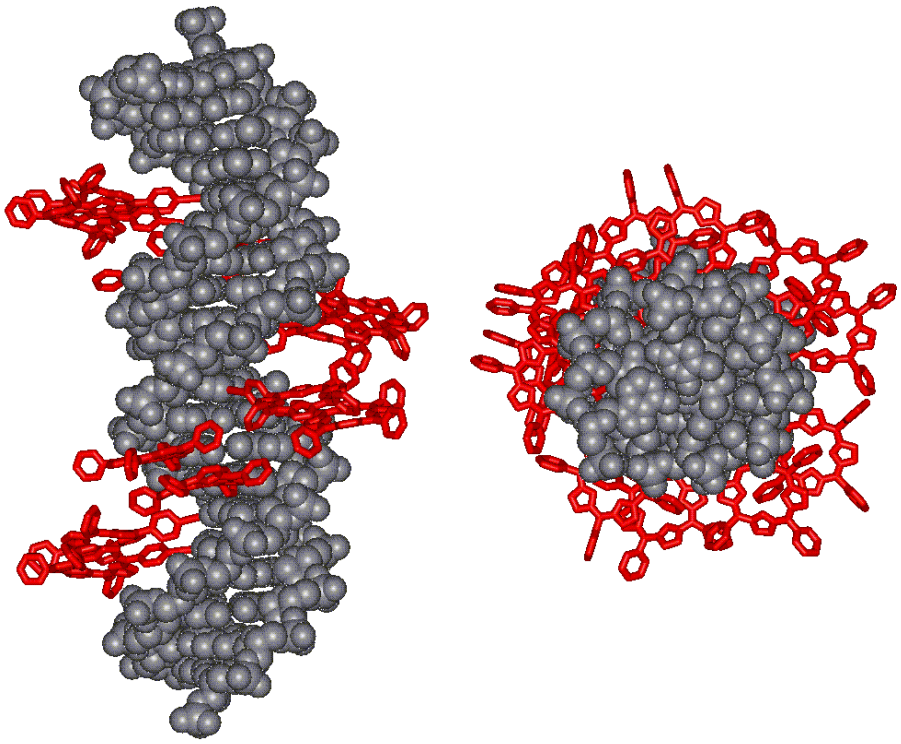


Figure S5. Duplex **5.6**, side on and top down views

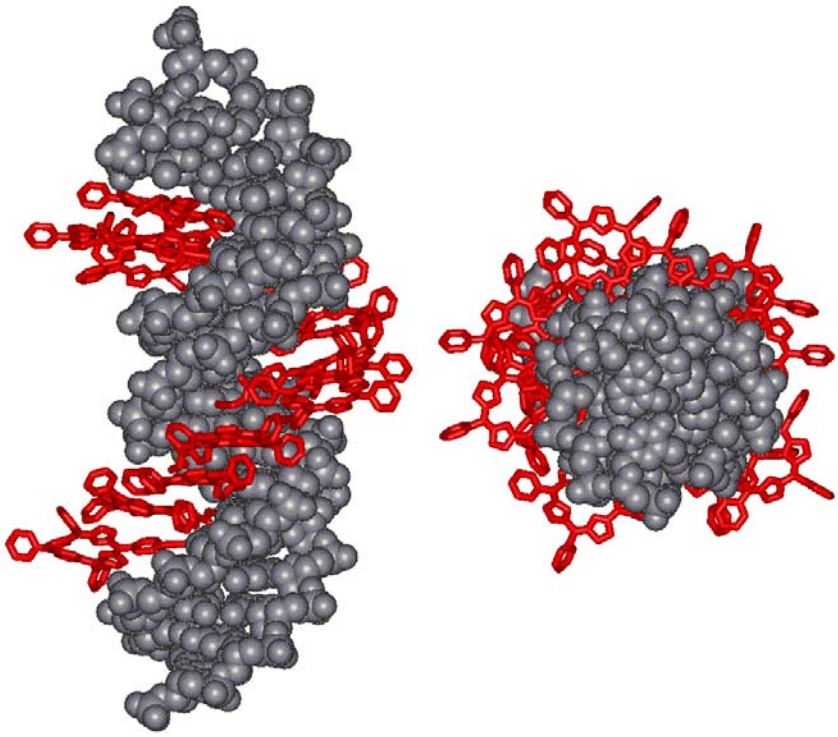


Figure S6. Duplex **3.6**, side on and top down views

Circular Dichroism (CD) spectroscopy

Circular dichroism was conducted with a 1 nm step size, 1 nm bandwidth and integration time of 4 seconds per point with sample concentrations of 1.2 ± 0.1 μM in 400 μL of phosphate buffer (100 mM sodium phosphate, 100 mM sodium chloride, 1 mM disodium EDTA, pH 7).

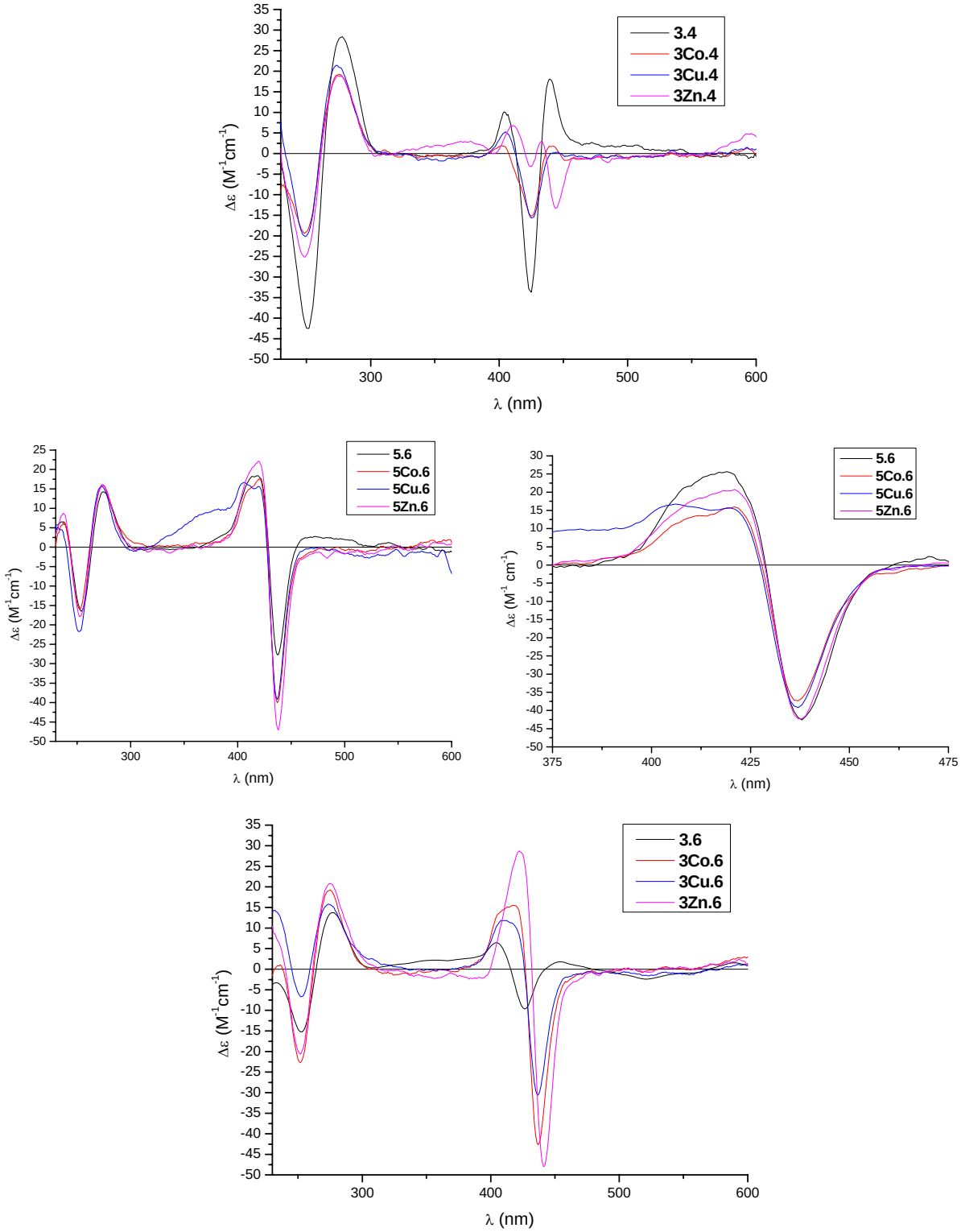


Figure S7. Circular dichroism of porphyrin modified DNA including expansion of **5M.6**

General method for metallation of porphyrin modified DNA

Single stranded porphyrin DNA (5 nmoles) was mixed with a metal acetate (6 μmoles) in water (100 μL), this was degassed with nitrogen before heating to 85 °C for 5 minutes. The sample was cooled to room temperature before the addition of an EDTA solution (pH 8, 0.5 M, 600 μmoles, 120 μL). The metallated DNA was loaded onto a conditioned Glen Pak column, washed with TEAA (0.1 M, 3 mL) before eluting with MeCN:H_2O (50:50 2 mL). Typically yielding 50-60% metallated porphyrin DNA.

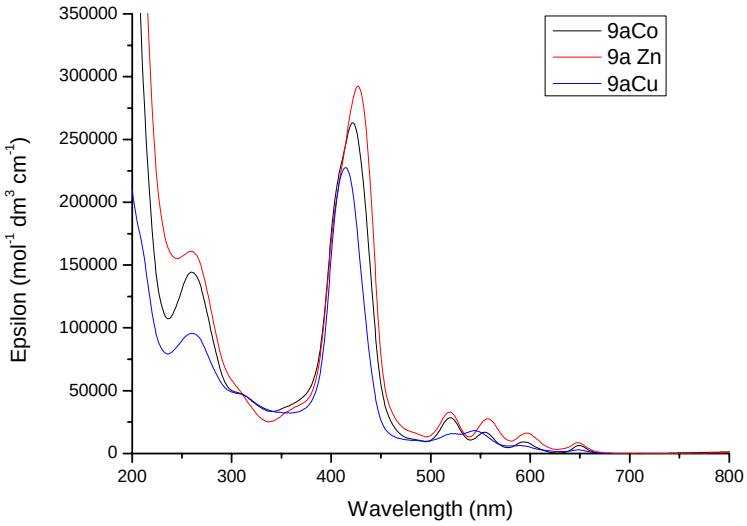


Figure S8. Example UV-vis spectra of metallated porphyrin DNA.

SAXS measurements of 3.4

The structure of the DNA construct in solution was studies at high concentration (~50 uM) by small angle x-ray scattering. The DNA was resuspended in buffer, centrifuged, and the solution mounted in a capillary. SAXS was recorded on an Anton Parr SAXess instrument with a slit geometry source with 1.54 Å x-ray radiation. Data collection was 12 hours for the sample and 12 hours for the buffer background. The background buffer scattering was subtracted from the sample scattering, the resulting SAXS pattern rebinned and then desmeared using the method of *Lake*[2] as implemented by Anton Parr in the program LAKE with the known slit size and dimensions of the instrument. The data was fitted from = 0.14 nm^{-1} to 1.02 nm^{-1} using the Small Angle Scattering Analysis Package from NIST in Igor Pro.[2] A variety of elongated models including cylinders, parallelepipeds, elliptical cylinders and two dimensional ellipsoids fitted the data and gave broadly similar parameters. The simplest model with a good fit was a cylinder (four free parameters) with a radius of 3.9 nm and a length of 13 nm giving a chi^2 of 4.23. Other models with more free parameters gave chi^2 between 3.5 and 4. The figure left shows SAXS data recorded on a solution of porphyrin modified DNA at approximately 50 μM. Error bars are the square root of the number of counts and the fit is to a simple rigid cylinder with a radius of 3.9 nm and a length of 13 nm.

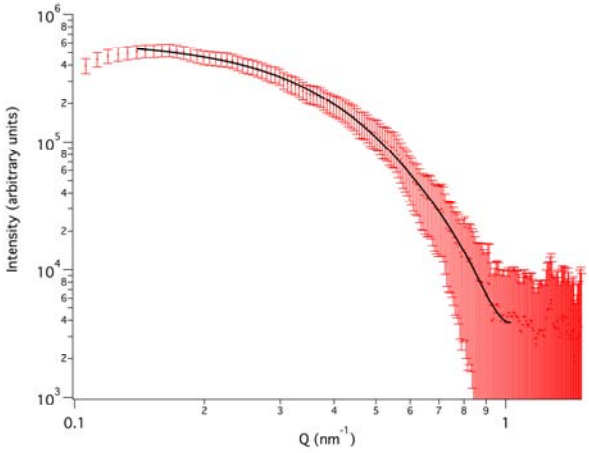


Figure S9. SAXS data of duplex **3.4**

References

1. L. A. Fendt, I. Bouamaied, S. Thöni, N. Amiot and E. Stulz, *J. Am. Chem. Soc.*, 2007, **129**, 15319-15329.
2. J. Lake, *Acta Crystallographica*, 1967, **23**, 191-194.
3. S. R. Kline, *J. Appl. Cryst.*, 2006, **39**, 895–900.