Surface Plasmon Resonance study of the interaction of N-methyl mesoporphyrin IX with G-quadruplex DNA

M. Perenon, H. Bonnet, T. Lavergne, J. Dejeu*, E. Defrancq

Univ. Grenoble Alpes, CNRS, DCM UMR-5250, F-38000 Grenoble, France

Corresponding author: Jérôme Dejeu, + 33 4 56 52 08 13, jerome.dejeu@univ-grenoble-alpes.fr

Synthesis of CEB25-L111(T) G4 parallel conjugate 2

General details

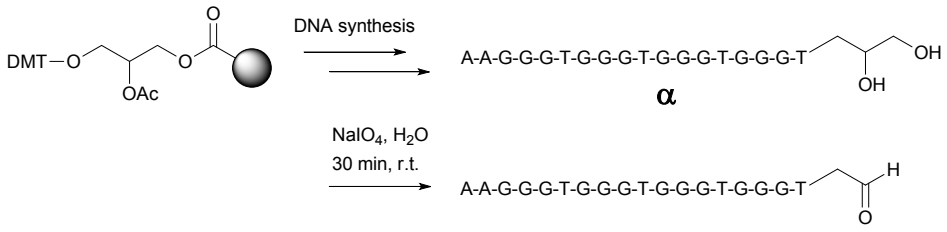
The oligonucleotide α was prepared using β-cyanoethylphosphoramidite chemistry on a 3400 DNA synthesizer at 1 µmol scale.

The RP-HPLC purifications were performed on a Gilson system with Nucleosil C-18 colunm (Macherey-Nagel, 100 Å, 250 x 10 mm, 7 µm) with UV-monitoring at 260 nm and 280 nm using 4 mL/min flow linear gradient. A stepwise gradient of 0-30% B in 20 min then from 30 to 100% B in 10 min was applied.

Desalting of oligonucleotides was performed by SEC on NAP 25 cartridge using manufacturer's protocol.

Quantification of oligonucleotides was performed at 260 nm using Nanodrop apparatus (molar extinction Ɛ260nm was estimated according to the nearest neighbor model).

Oligonucleotide α



Scheme SI-1 : oligonucleotide α

Oligonucleotide α [$^{5'}$AAGGGTGGGTGGGTGGGT$^{3'}$-diol] was obtained from automated synthesis on a 3'-glyceryl CPG resin at 1 µmol scale using a 3400 DNA synthesizer from Applied Biosystems. After synthesis, cyanoethyl protecting groups were removed using 20% piperidine in acetonitrile. Cleavage from the resin and deprotection was performed in 28% NH_4OH for 16h at 55°C. The product was purified on RP-HPLC and analyzed by HPLC and ESI. ε_{260nm} = 182700$M^{-1}.cm^{-1}$. ESI-MS (-) m/z calcd for $C_{183}H_{228}N_{78}O_{113}P_{18}$: 5882.9, m/z found: 5882.7 $[M-H]^-$. 300 nmoles were readily oxidized as sodium metaperiodate (20 eq; 6 µmol; 1.3 mg) was added to a solution of oligonucleotide (1 eq; 300 nmol) in water (400 µL). The reaction was stirred for 1h at room temperature in dark conditions. The product was then desalted on NAP 25 and the fractions were collected to obtain the crude product (UV-monitored at 260 nm). The oxidation was considered quantitative and the crude oligonucleotide was used in the next step without further purification.

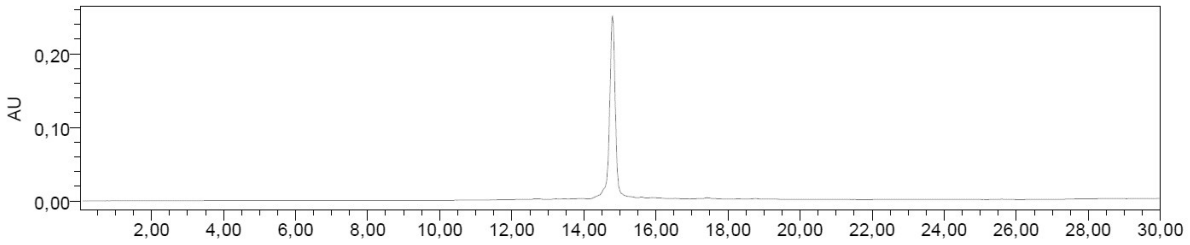


Figure SI-1: RP-HPLC chromatogram of oligonucleotide α

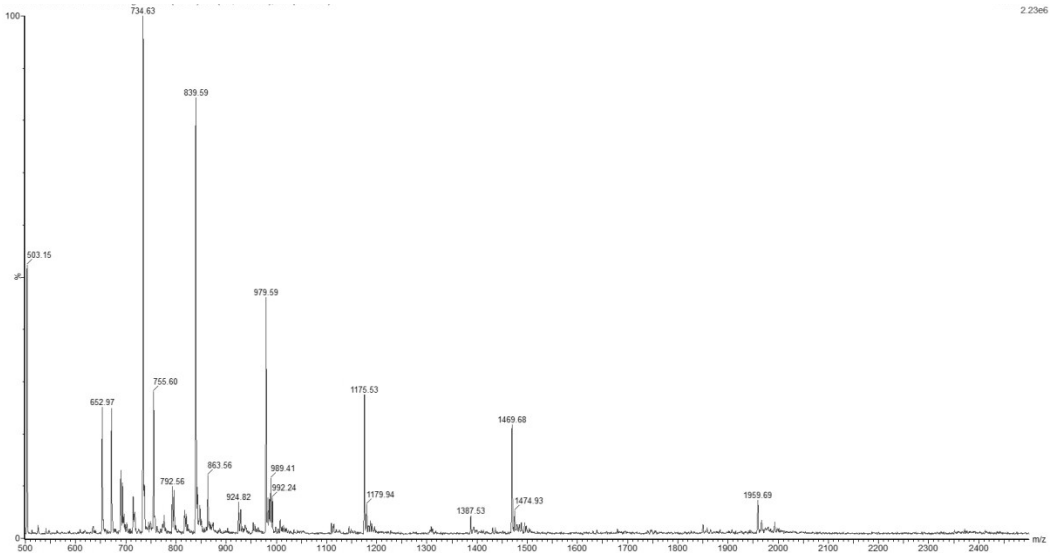
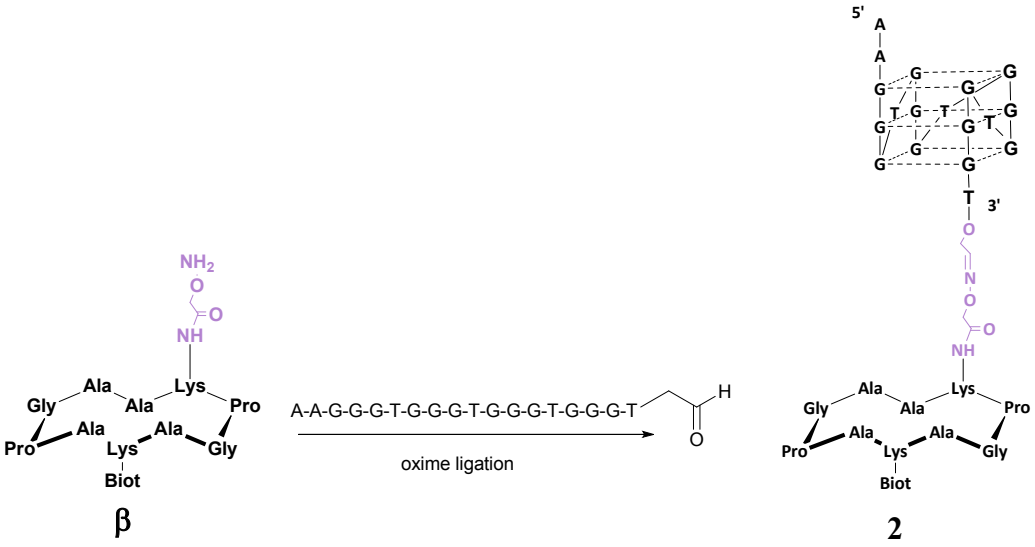


Figure SI-2: ESI mass spectrum of oligonucleotide **α**

Conjugate 2



3’ aldehyde oligonucleotide (1 eq, 300 nmoles) was dissolved in 0.4 M ammonium acetate buffer (pH 4.5, concentration 10^{-3} M) and previously reported aminooxy peptide **β** (2 eq, 600 nmoles) was added. The solution was stirred at 55°C for 45 min. The crude was purified using RP-HPLC conjugate **2** was desalted by SEC and freeze dried. Quantification was performed

by UV-spectrometry (180 nmoles, yield 60%), ε_{260}nm = 182700 $M^{-1}.cm^{-1}$. MALDI-MS (-) m/z calcd for $C_{232}H_{303}N_{93}O_{125}P_{18}S$: 6980.5, m/z found: 6979.7 $[M-H]^-$.

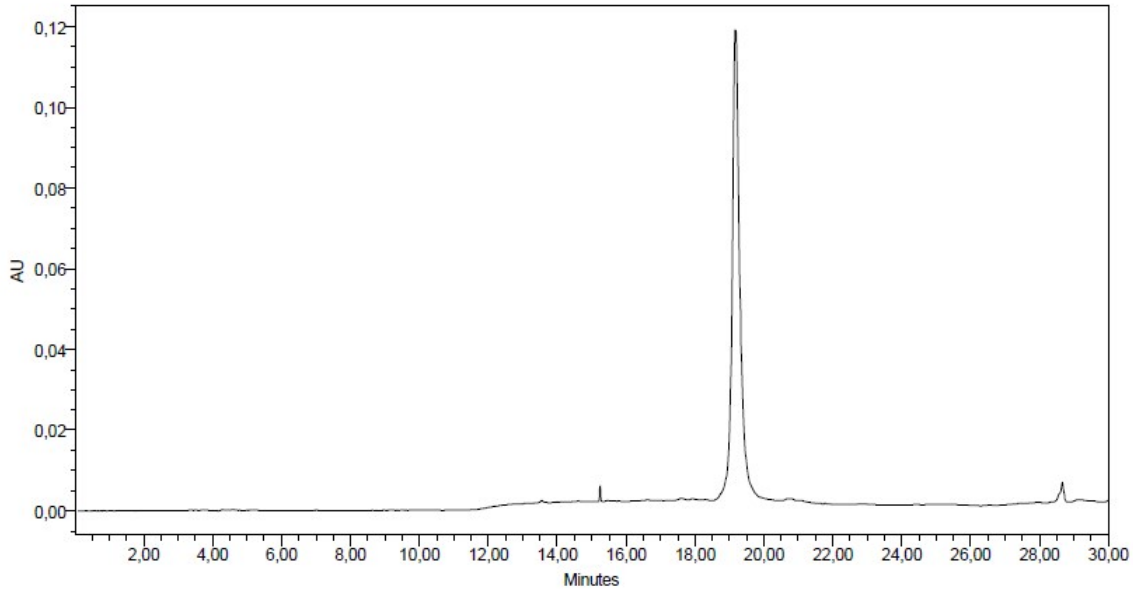


Figure SI-3: RP-HPLC chromatogram of oligonucleotide **2**

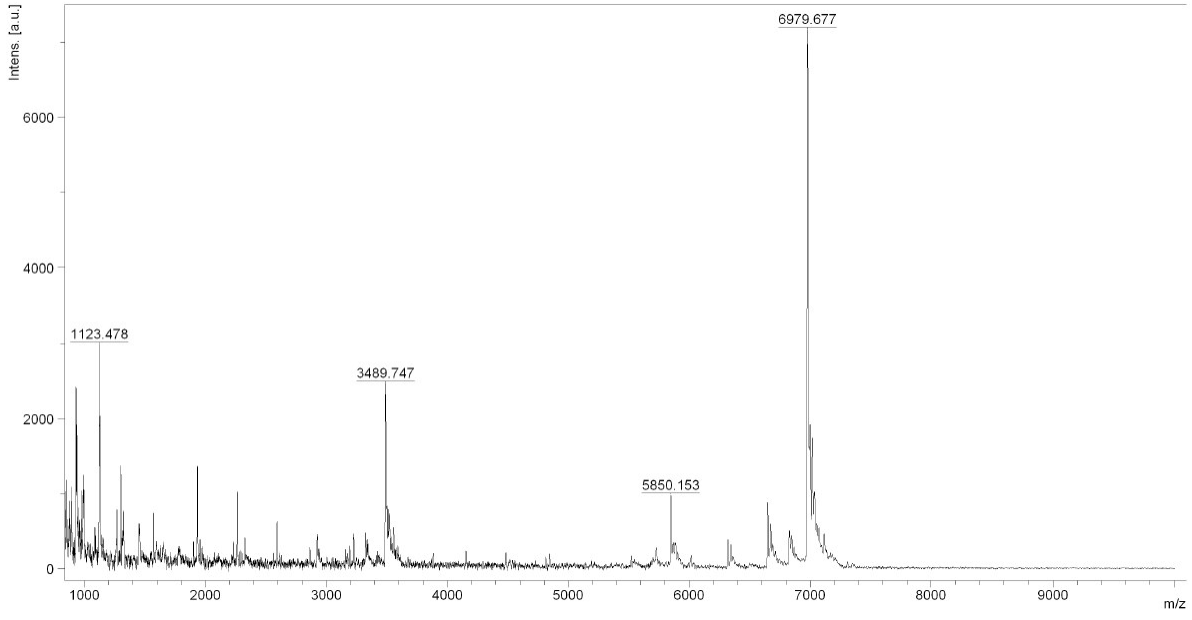


Figure SI-4: MALDI mass spectrum of oligonucleotide **2**

NMM

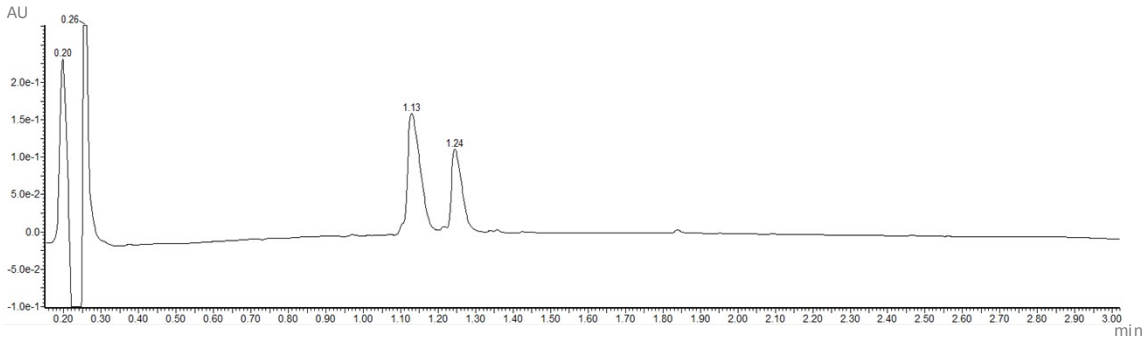


Figure SI-5: UPLC chromatogram of NMM in RB buffer

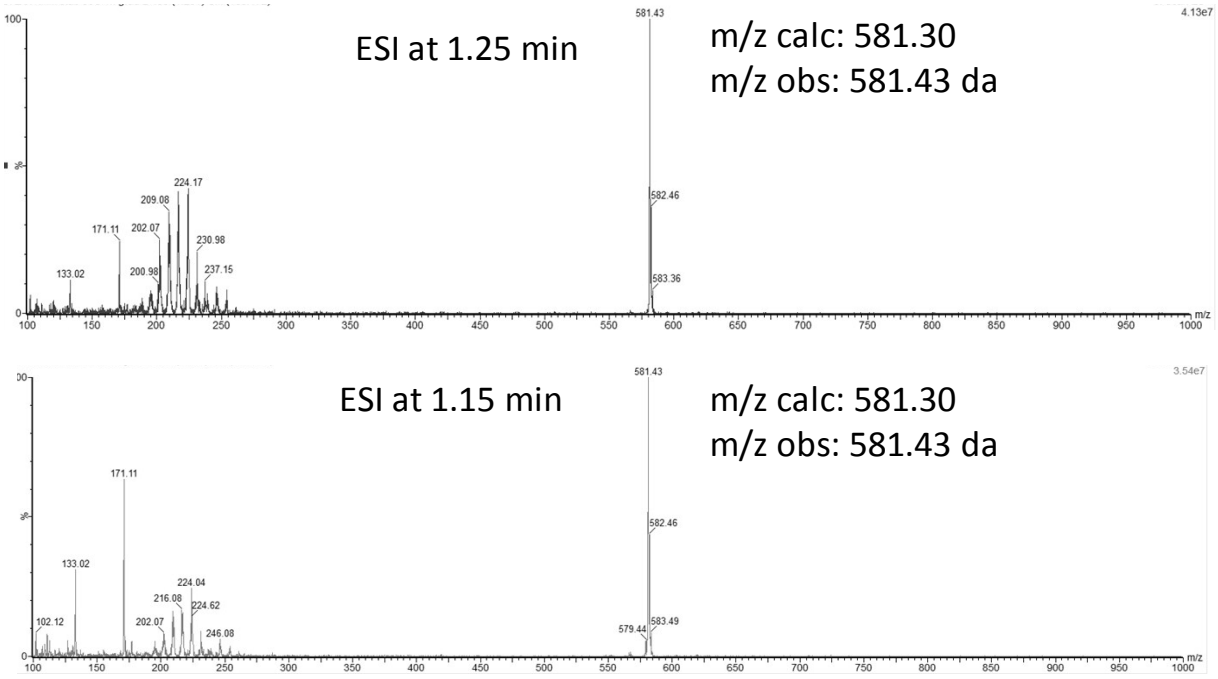


Figure SI-6: ESI mass spectrum of NMM corresponding to the UPLC-MS peaks at 1.15 min (bottom) and 1.25 min (top)

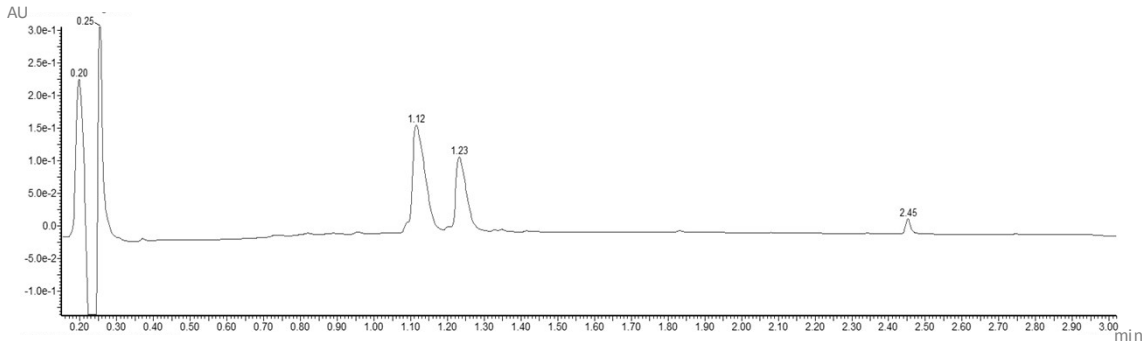


Figure SI-7: UPLC chromatogram of NMM after incubation in RB buffer at 95°C for 1h

Biophysical study of the interaction NMM/G4

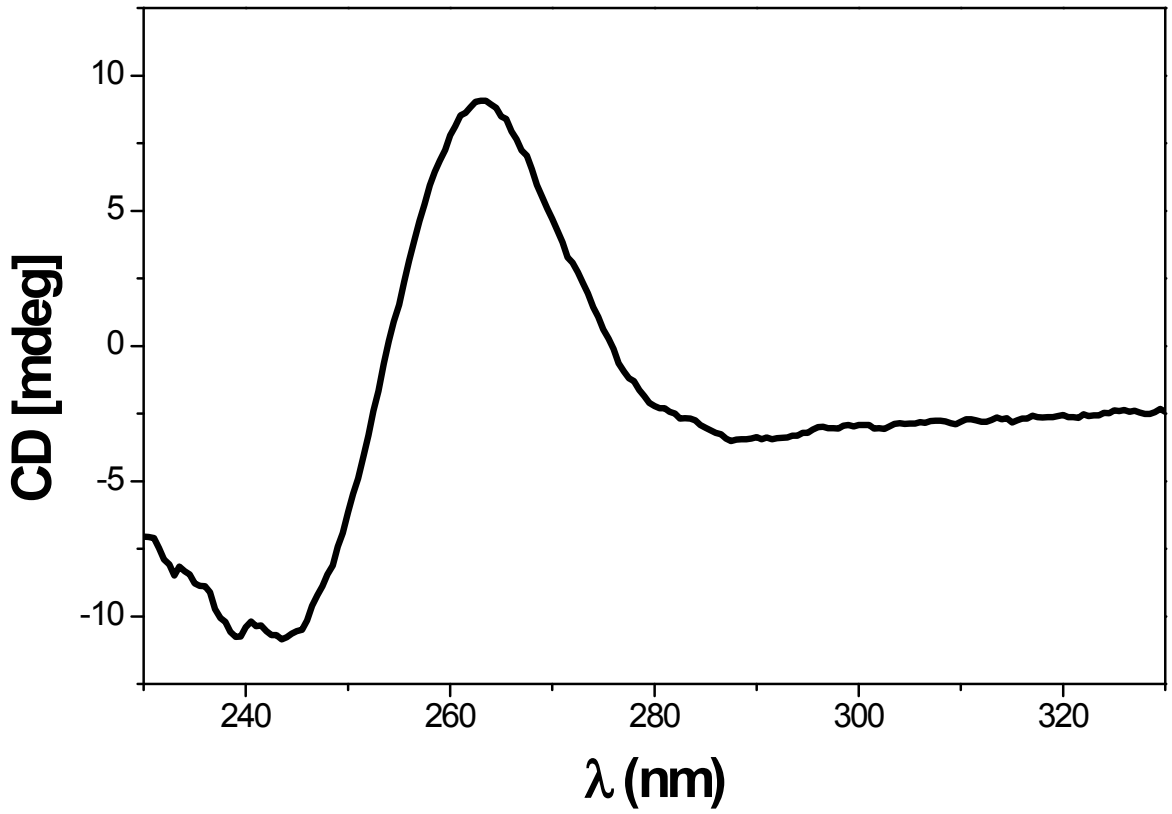


Figure SI-8: CD spectra of conjugate **2** before addition of NMM

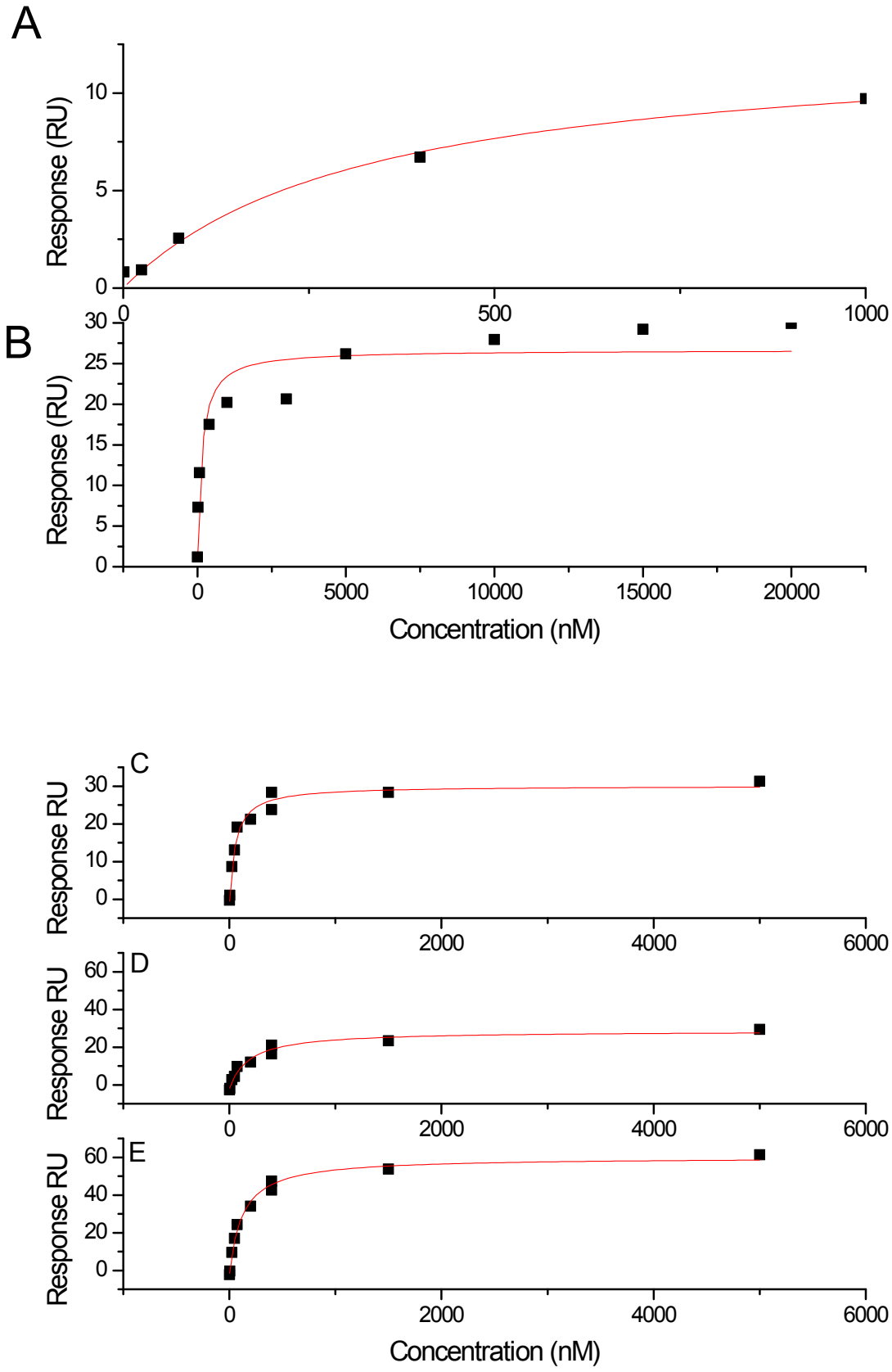


Figure SI-9: Adsorption isotherm (square) and fitting curve (line) using a 1:1 Langmuir interaction model: A) system **1**, B) system **2**, C) system **3**, D) system **4** and E) system **5**.

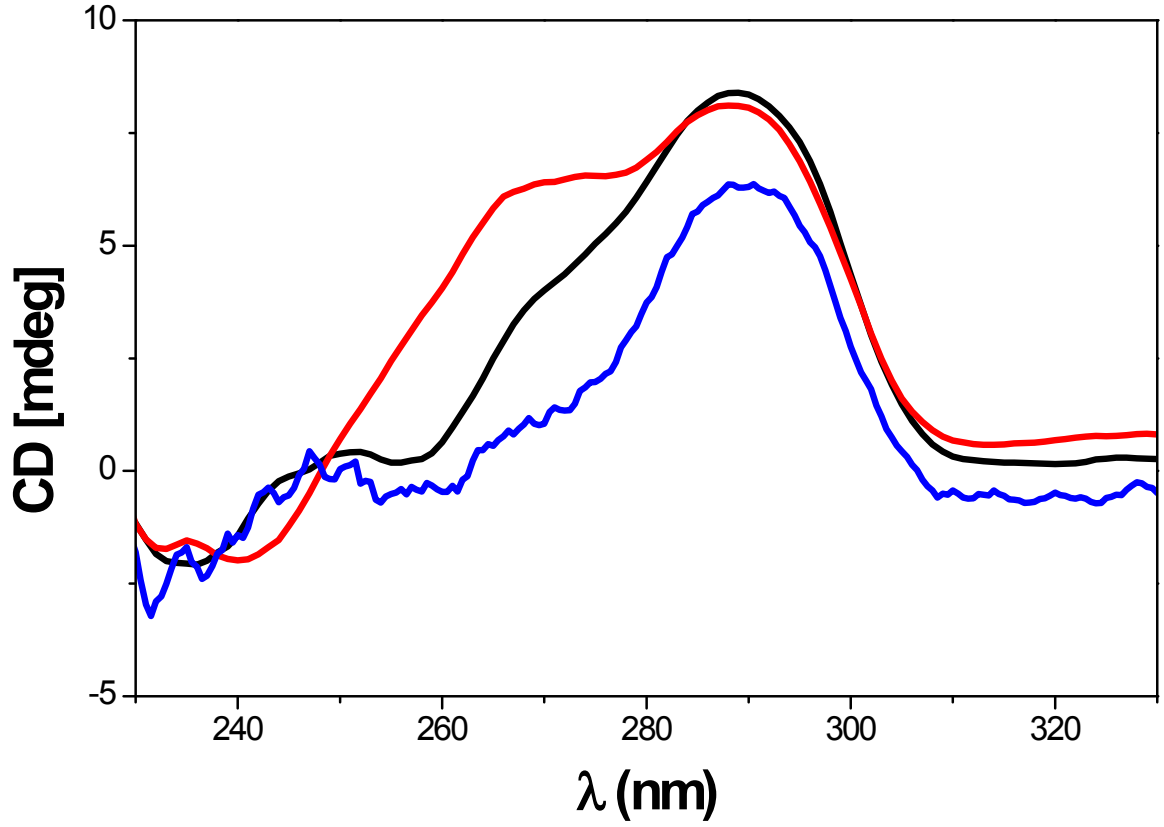


Figure SI-10: CD spectra of hybrid topology :system **6** (blue), system **7** (wtTel26, red), system **8** (Tel 23, blanck) alone before NMM adding.

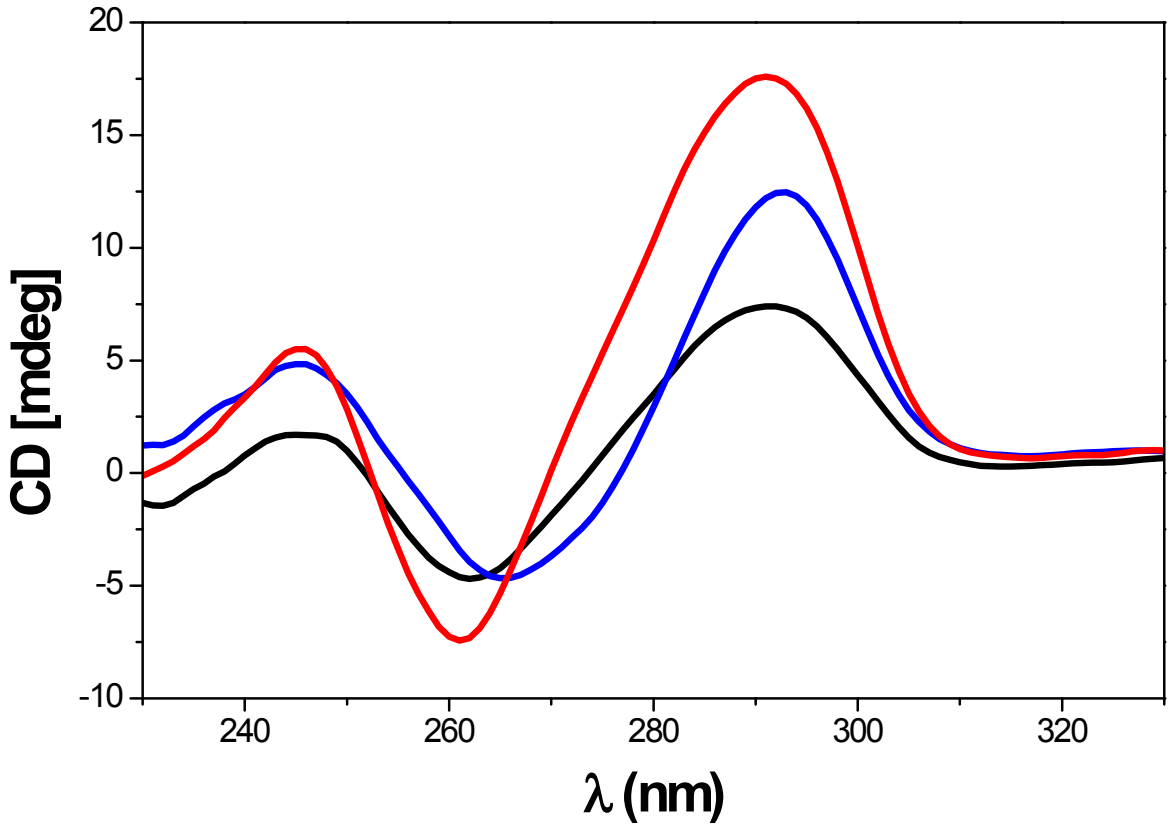


Figure SI-11: CD spectra of antiparallel topology : constrained system **9 (red)**, system **10** (22 CTA sequence, black) and system **11** (TBA, blue) alone before NMM adding.

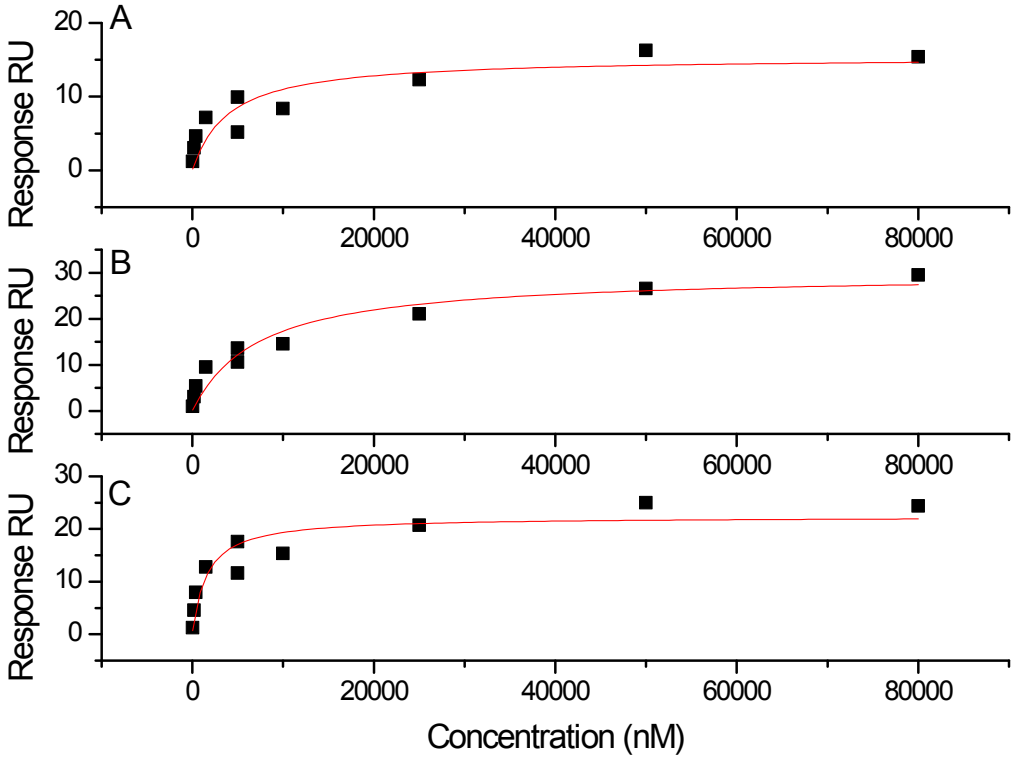


Figure SI-12: Adsorption isotherm (square) and fitting curve (line) using a 1:1 Langmuir interaction model for the G4 hybrid conformation: A) system **6**, B) system **7**, C) system **8**.

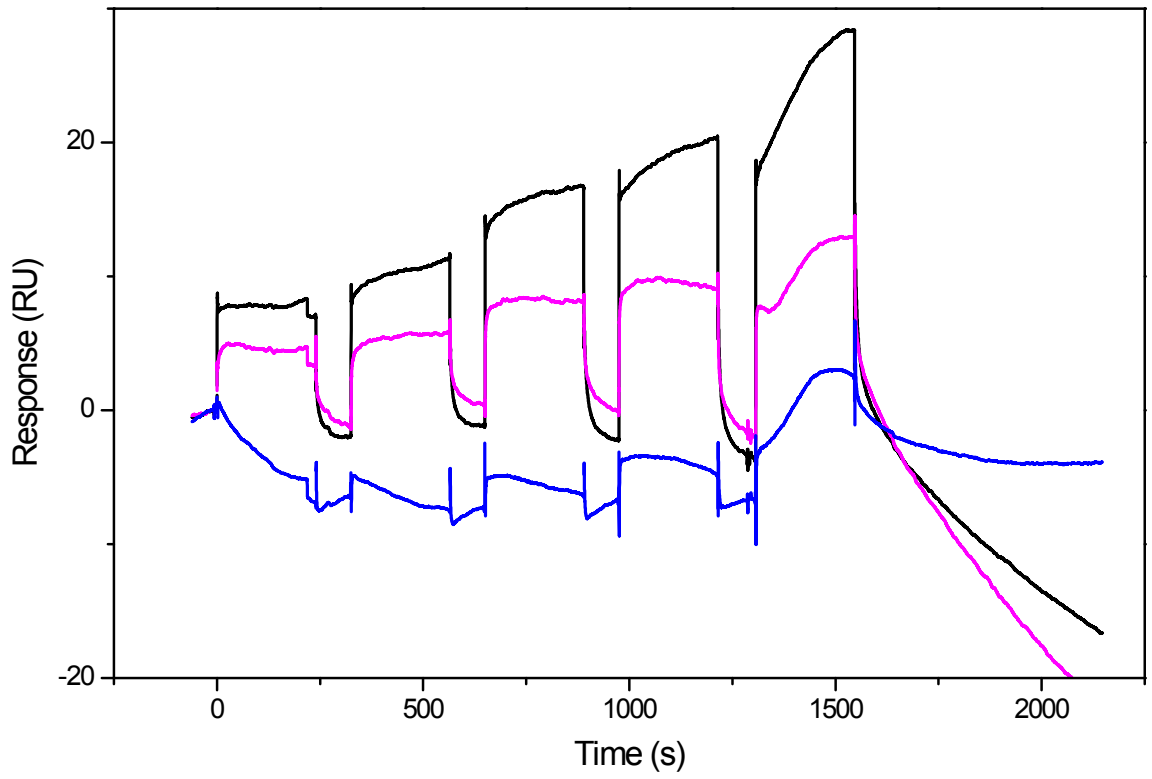


Figure SI-13: Single Cycle Kinetic titration analysis realized for the NMM interaction with antiparallel G4 conformation: system **9** (blue), system **10** (22 CTA, pink) and system **11** (TBA, black). The interaction of NMM with different DNA structures was tested at concentrations of 5, 10, 25, 50 and 80 µM. Sensorgrams corresponded to double subtracted data (blank and reference subtraction).

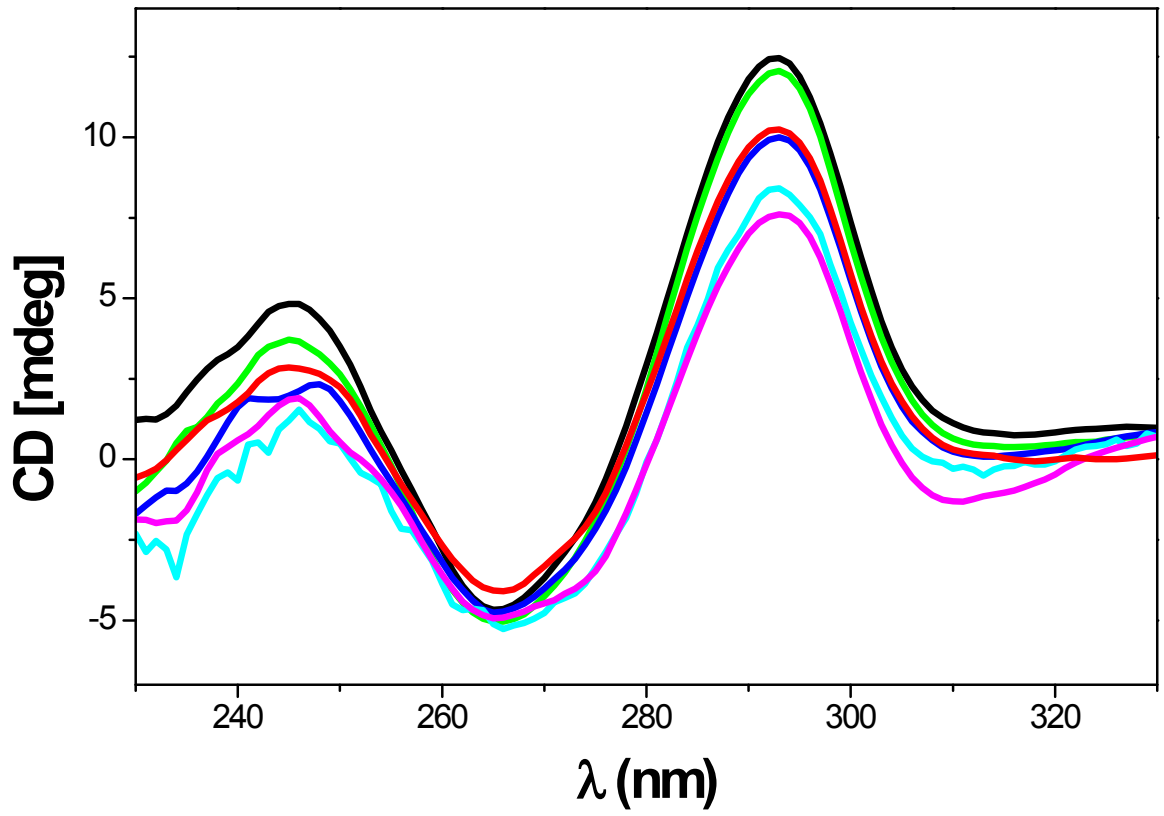


Figure SI-14: CD spectra analysis of antiparallel G-quadruplex topology of system **11** (TBA) upon addition of NMM (1:10 ratio) and increased incubation time: G4 without NMM (black), G4 with NMM after 1 min (green), 4H (blue), 24H (cyan), 48H (purple) and control G4 annealing with the NMM (1:10 ratio) (red).