Electronic Supplementary Information for Dalton Transactions

Interactions of Ruthenium Coordination Cubes with DNA

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Electronic Supplementary Information

1. Stability Experiments

UV-visible absorption spectra were recorded on a Perkin Elmer spectrophotometer model Lambda 25.

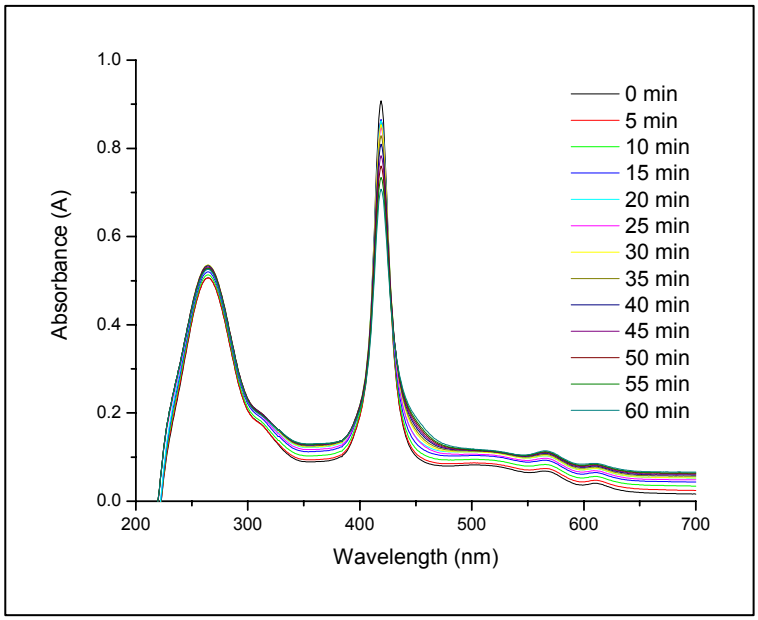

Figure 1S. UV-visible spectra of a 2.5 μM solution of cube [**2**][CF_3SO_3]$_8$ in HBS-EP buffer supplemented with 0.2 M KCl. The spectra were recorded over a 1 h period.

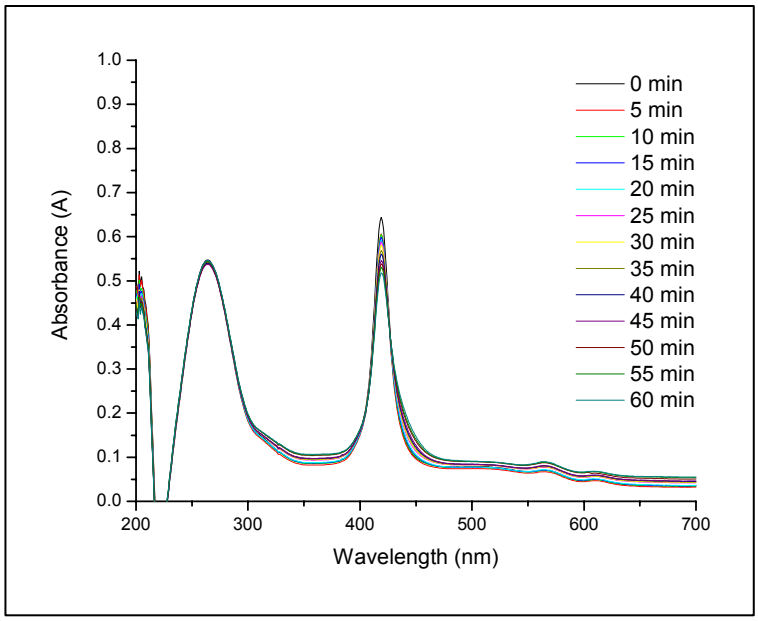

Figure 2S. UV-visible spectra of a 2.5 μM solution of cube [**2**][CF_3SO_3]$_8$ + 0.25 μM solution of *Htelo* DNA, HBS-EP buffer supplemented with 0.2 M KCl. The spectra were recorded over a 1 h period.

2. DNA binding studies

2.1 Stock solution preparation

Complexes **1** and **2** were dissolved in acetone to give 10 mM stock solutions. Complexes **3** and **4** were dissolved in acetone to give 1 mM stock solutions. Thiazole Orange (TO, Sigma-Aldrich) was prepared as a 10 mM stock solution in DMSO. These stock solutions were freshly prepared prior to use and were further diluted to final concentrations using 60 mM potassium cacodylate buffer (pH 7.4) (NOTE: the potassium cacodylate buffer also contains 50 mM KCl).

2.2 Fluorescence Intercalator Displacement (FID) assay

The FID assays were performed on a Varian Cary Eclipse Spectrometer following a reported procedure.[1] The oligonucleotides 22 AG human telomeric DNA, 5'-AGGG TTA GGG TTA GGG TTA GGG-3'; 17bp duplex-DNA: [5'-CCAGTTCGTAGTAACCC-3']/[5'-GGGTTACTACGAACTGG- 3']; and 21bp c-myc: 5'-GGG-GAG-GGT-GGG-GAG-GGT-GGG-3' were purchased from Eurogentec and used without any further purification. The corresponding

oligonucleotides were dissolved in MilliQ water to yield 20 μM stock solutions. These were diluted to yield 500 nM solutions using a 60 mM potassium cacodylate buffer (pH 7.4) and annealed to 90^{o}C for 5 min and then allowed to cool at room temperature overnight.

An increasing concentration of the metal complex (ligand) (from 0.125 μM to 2.5 μM which correspond to 0.5 to 10 equivalent) was added onto a mixture of pre-folded quadruplex DNA (0.25 μM of either *Htelo* or *c-myc*) or duplex DNA (17bp, 0.25 μM) and thiazole orange, TO (0.50 μM), in a 60 mM potassium cacodylate (pH 7.4). After 3 minutes equilibration time, the corresponding emission spectrum (between 510 and 750 nm) with an excitation wavelength of 501 nm was recorded. The fluorescence area (FA, 510-750 nm) of each spectrum obtained was converted to percentage of displacement in which % TO displacement = 100 – [(fluorescence area upon ligand addition /fluorescence area of standard) × 100], where fluorescence area of standard being fluorescence area before addition of ligand. The FA was plotted against the concentration of added ligand, from which the ability of ligand to induce TO displacement is expressed as DC_{50} ($^{Htelo}DC_{50}$ for *Htelo* DNA, $^{ds}DC_{50}$ for duplex DNA and $^{c\text{-}myc}DC_{50}$ for *c-myc* DNA) value which is the required concentration to displace 50% of TO from the DNA (either *Htelo*, *c-myc* or duplex DNA). The selectivities for *Htelo*- and *c-myc*-DNA over duplex-DNA are expressed by $^{ds}DC_{50}$ /$^{Htelo}DC_{50}$ value and $^{ds}DC_{50}$ /$^{cmyc}DC_{50}$ value, respectively.

2.3 Surface Plasmon Resonance (SPR) assay

SPR measurements were conducted on a four-channel BIACore 3000 optical biosensor instrument adopting published procedures.[2,3] All experiments were performed on streptavidin-coated sensorchips (SA) purchased from GE Healthcare UK Ltd. The biotinylated oligonucleotides 5'-biotin-AGGGTTAGGGTTAGGGTTAGGG 22 bases human telomeric DNA, 5'-biotin-TTCGCGCGCGTTTTCGCGCGCG 22 bases CG-rich duplex and 5'-biotin-ATG-CAT-GCG-GGG-AGG-GTG-GGG-AGG-GTG-GGG-AAG-GTG-GGG 39 bases *c-myc* quadruplex were purchased from Eurogentec and used without any further purification. HBS-EP buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA,0.005% v/v surfactant P20) was obtained from BIACore and supplemented with degassed 0.2 M KCl solution and filtered beforehand.

2.3.1 Immobilization of biotinylated DNA probes.

The corresponding biotinylated oligonucleotides were dissolved in MilliQ water to yield 20 μM stock solutions. They were further diluted to 10 nM in HBS-EP buffer supplemented with 0.2 M KCl solution. The 10 nM oligonucleotides were then annealed by heating them at 95 ^{o}C for 5 minutes and leave to cool down slowly at room temperature overnight prior to chip loading. The sensorchip was initially conditioned with injections of 1M NaCl in 50 mM NaOH followed by extensive washing with buffer. Manual injections of the labeled-oligonucleotides were performed at a flow rate of 2 μL/min for 7 minutes to reach sufficient RU level (~600RU). One of the flow cells was left blank as a reference.

2.3.2 Binding studies

DNA binding experiments were carried out in running buffer by multiple injections of a range of compound concentrations (analytes) simultaneously over the immobilized DNA (ligands) and the blank reference on the four channels (flow cells) at a flow rate of 20 μL/min for 5 minutes. Compounds' solutions were prepared in 9 mm glass vials with pierce able crimp caps (Biacore Inc.) by serial dilutions from stock solution. Between injections, the sensor surface was regenerated with injection of buffer. The reference sensorgrams were subtracted from the series of sensorgrams obtained at different compound concentrations to give true binding response, response unit, RU versus time. Double referencing was also done by subtracting the injection of running buffer alone from the data.

The observed response in the steady-state region (R_{eq}) is proportional to the amount of bound compound and was determined by linear averaging over a 50 s time span by using BIAevaluation 4.0.1 software. The values were then converted to r, which represents the moles of bound compound per mole of DNA using equation 1.

$$r = R_{eq} / R_{max} \quad (1)$$

The theoretical maximum response proportional to the amount of immobilized ligand, R_{max} was calculated using equation 2 as previously described.[3,4]

$$R_{max} = R_L \times S_m [(\text{Analyte molecular weight})/(\text{ Ligand molecular weight })] \quad (2)$$

Where R_L is the immobilization level and S_m is the stoichiometric ratio. The number and type of binding sites was estimated using Scatchard plots derived from r/concentration versus plots of r. r values were then plotted against concentrations of compound to generate a hyperbolic binding curve. The binding constant at equilibrium, K_A was obtained by fitting the binding curve according to a steady-state affinity model determined from the Scatchard plots.[2,3] The results of compound-quadruplex binding experiment were fitted to a non-equivalent two-site binding model (equation 3):

$$r = (K_1C_{free}+2K_1K_2C^2_{free})/(1+K_1C_{free}+K_1K_2C^2_{free}) \quad (3)$$

In this model, K_1 and K_2 values are the binding constants, C_{free} is the concentration of the compound in equilibrium with the complex and is fixed by the concentration in the flow solution.

Whereas, for duplex DNA binding, independent multi-equivalent binding model was employed to fit the binding curve (equation 4):

$$r = R_{eq} / R_{max} = nKC / (1 + KC) \quad (4)$$

Where K is the binding constant, n is the number of binding sites per oligonucleotide molecule, and C is the free analyte concentration.

2.4 Plots from Fluorescence Intercalator Displacement (FID) assay

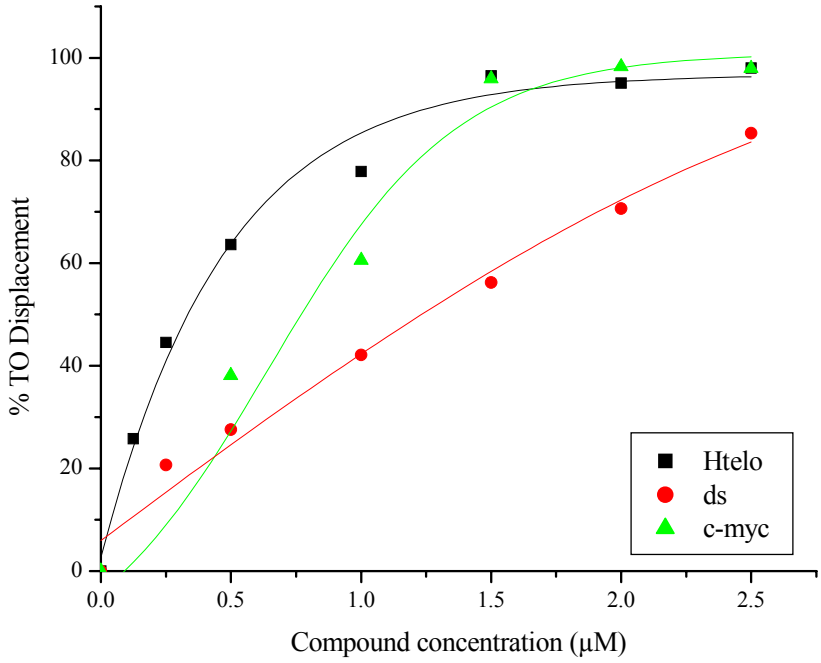

Figure S3. Graphical representation of TO displacement from human telomeric (*Htelo*) quadruplex-, duplex- (*ds*) and *c-myc* quadruplex-DNA upon increasing concentration of complex **2** from 0.125 to 2.5 μM.

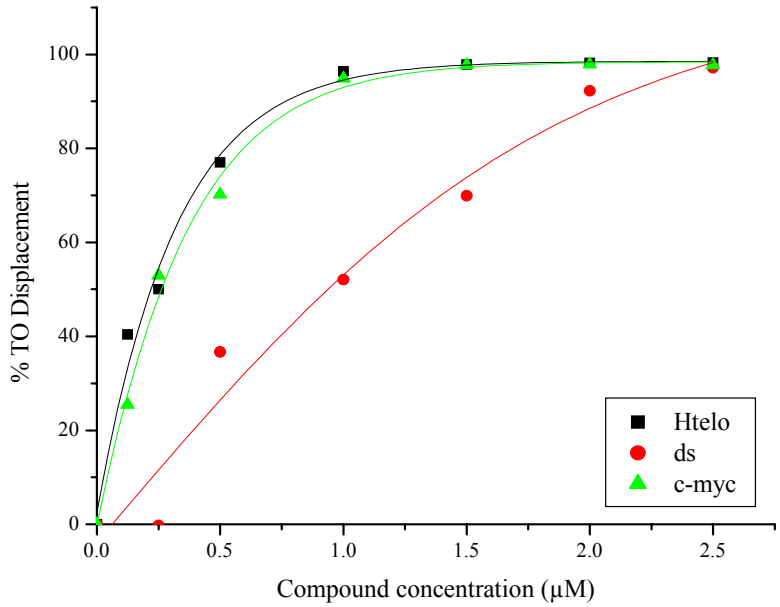

Figure S4. Graphical representation of TO displacement from human telomeric (*Htelo*) quadruplex-, duplex- (*ds*) and *c-myc* quadruplex-DNA upon increasing concentration of complex **3** from 0.125 to 2.5 μM.

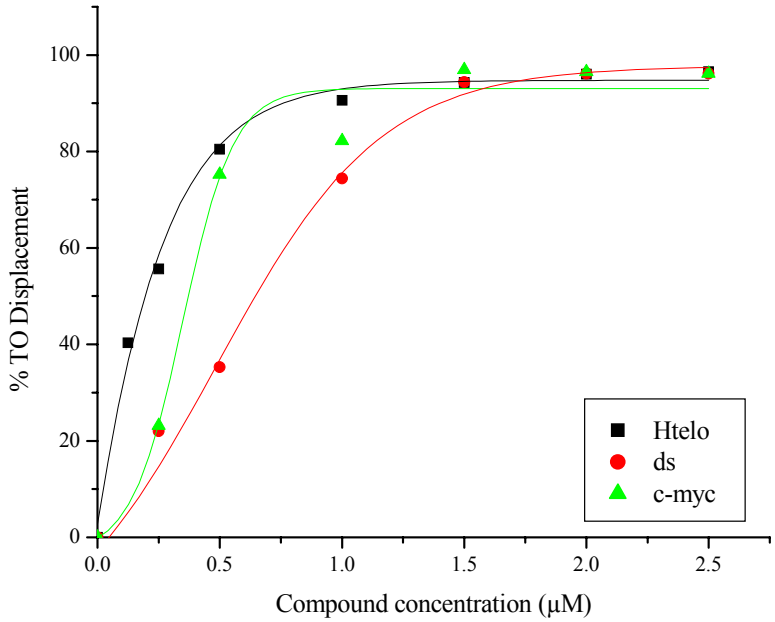

Figure S5. Graphical representation of TO displacement from human telomeric (*Htelo*) quadruplex-, duplex- (*ds*) and *c-myc* quadruplex-DNA upon increasing concentration of complex **4** from 0.125 to 2.5 μM.

2.5 Concentration-dependant sensorgrams from SPR

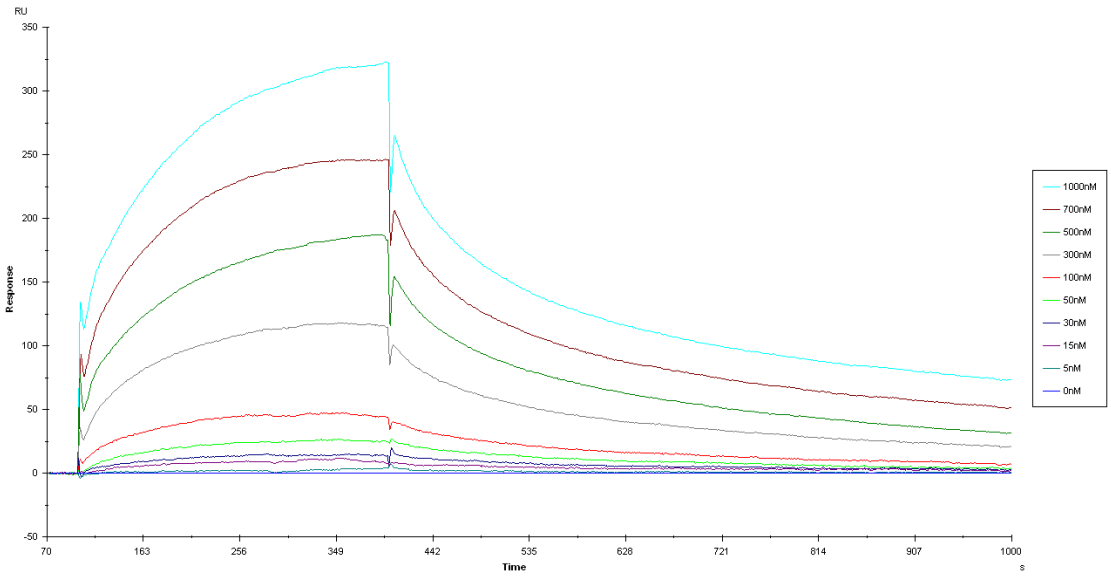

Figure S6. Sensorgrams overlay obtained for complex **1** (from 5-1000 nM) binding to human telomeric (*Htelo*) quadruplex DNA.

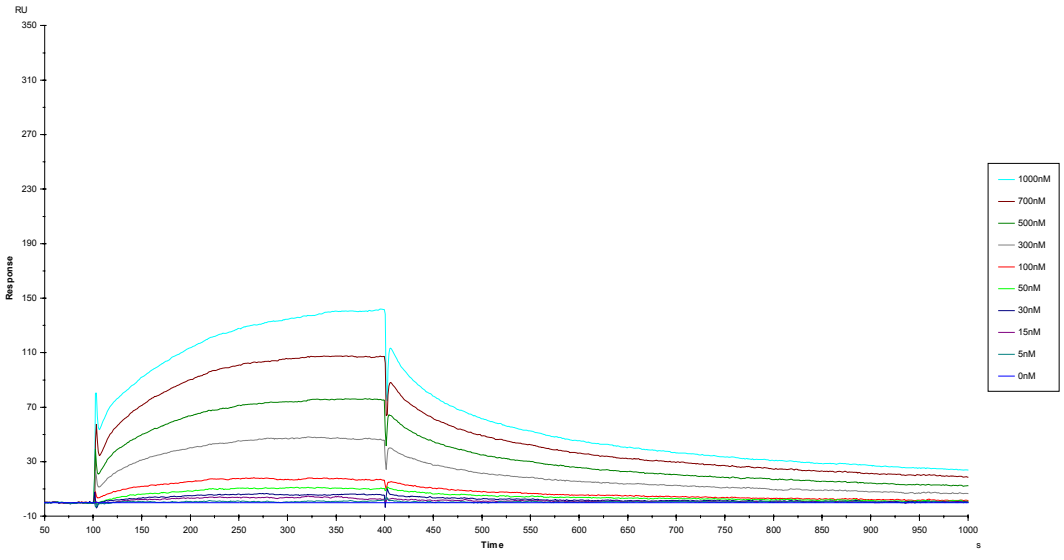

Figure S7. Sensorgrams overlay obtained for complex **1** (from 5-1000 nM) binding to CG-rich (*ds*) duplex DNA.

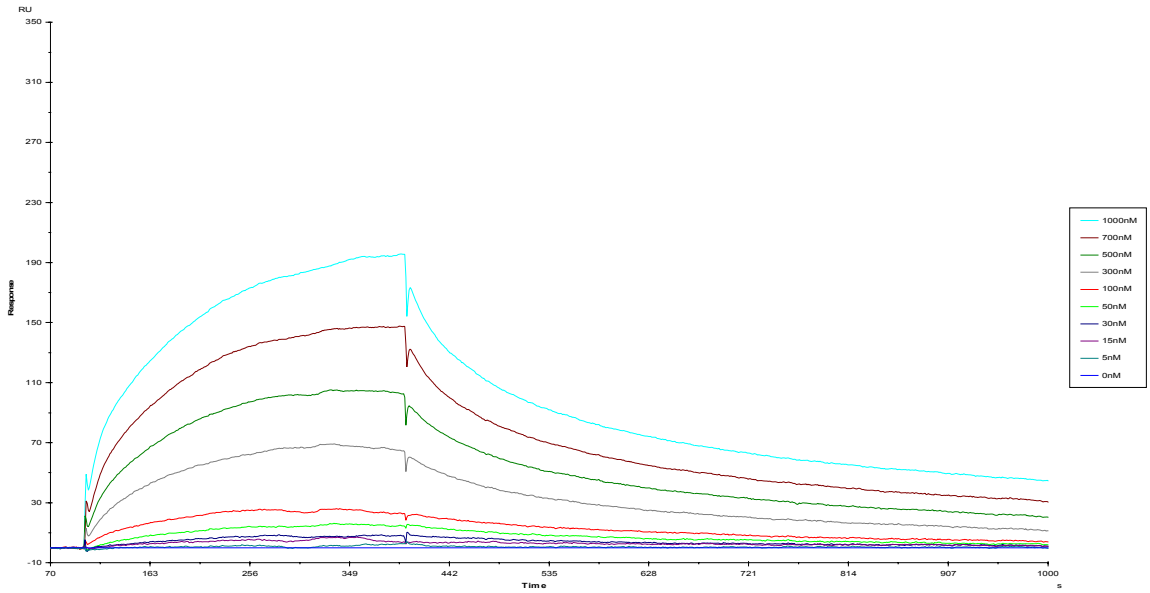

Figure S8. Sensorgrams overlay obtained for complex **1** (from 5-1000 nM) binding to *c-myc* quadruplex DNA.

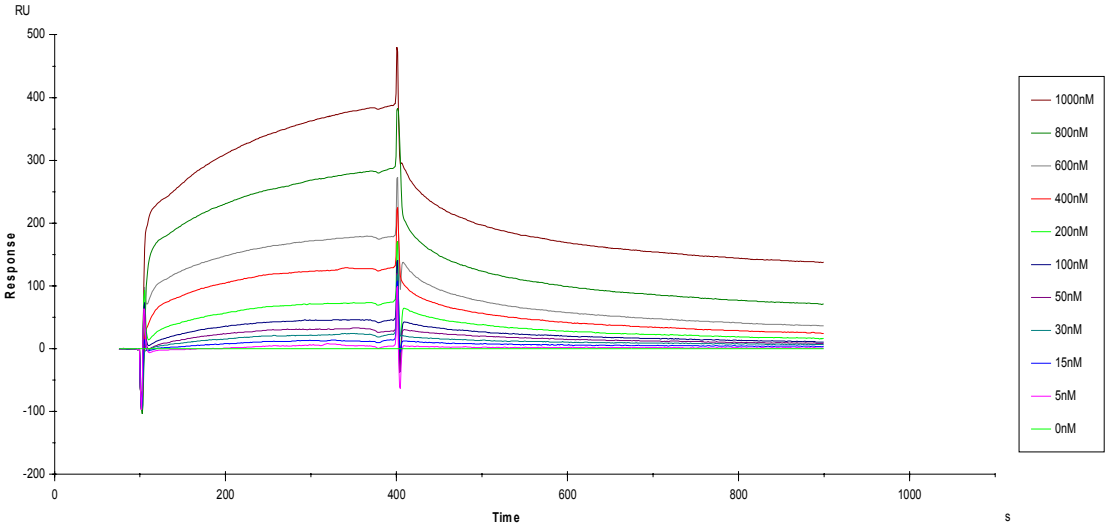

Figure S9. Sensorgrams overlay obtained for complex **3** (from 5-1000 nM) binding to human telomeric (*Htelo*) quadruplex DNA.

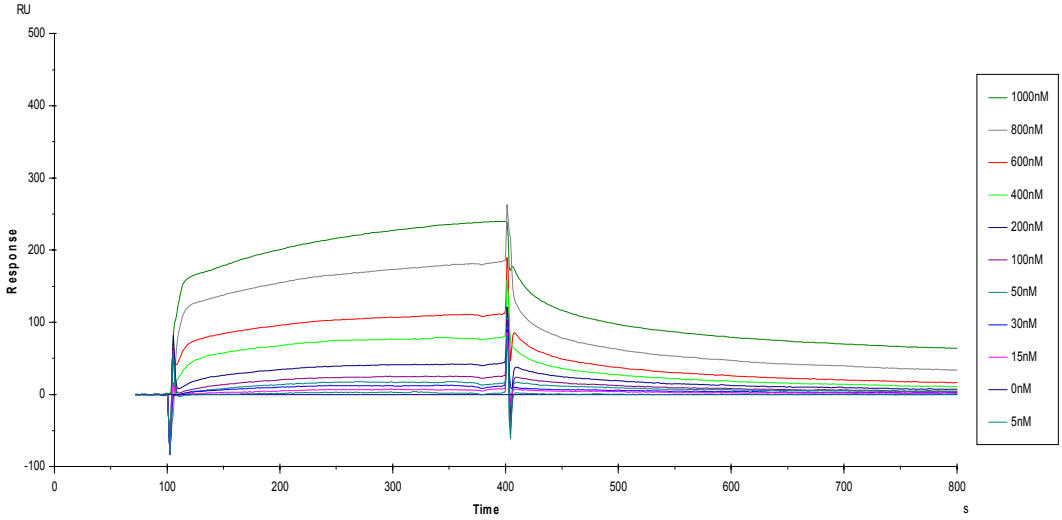

Figure S10. Sensorgrams overlay obtained for complex **3** (from 5-1000 nM) binding to CG-rich (*ds*) duplex DNA.

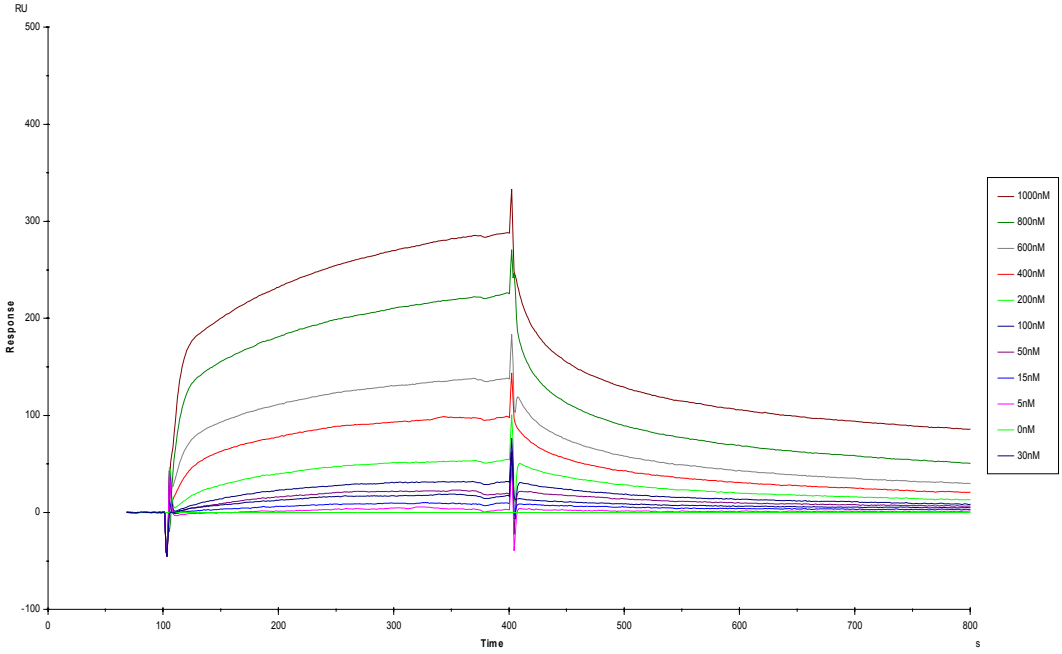

Figure S11. Sensorgrams overlay obtained for complex **3** (from 5-1000 nM) binding to *c-myc* quadruplex DNA.

2.6 SPR curve fitting

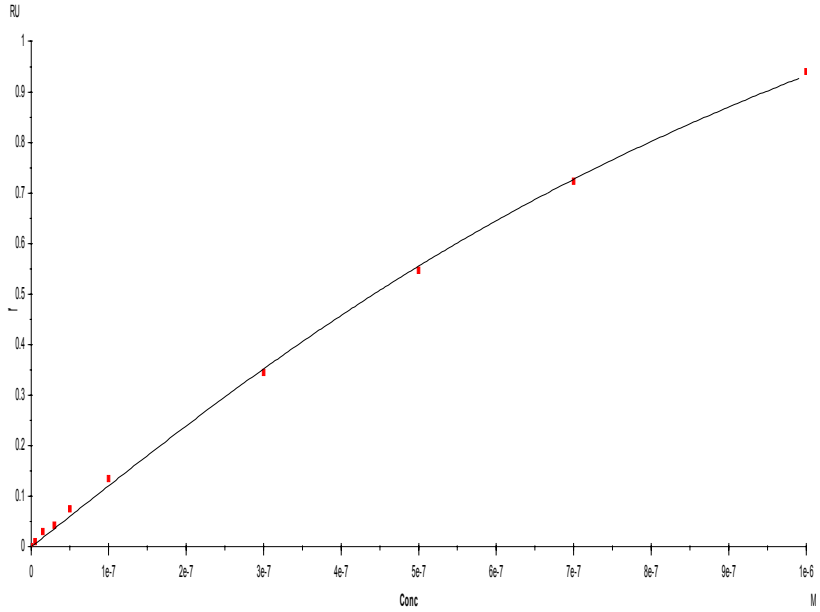

Figure S12. Binding curve of complex **1** to human telomeric DNA fitted with a two-non-equivalent binding site model obtained using BIAeval software (BIAcore).

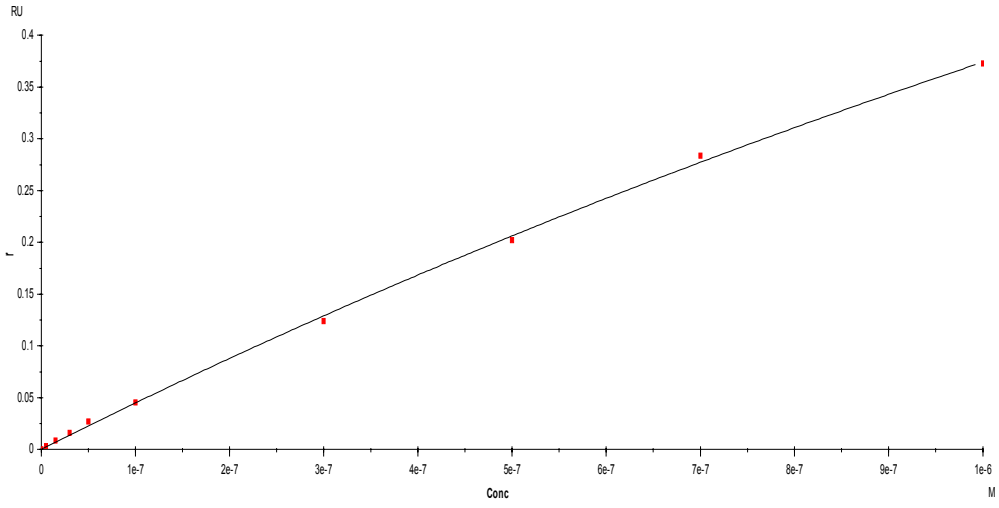

Figure S13. Binding curve of complex **1** to CG-rich duplex DNA fitted with two-equivalent binding site model obtained using BIAeval software (BIAcore).

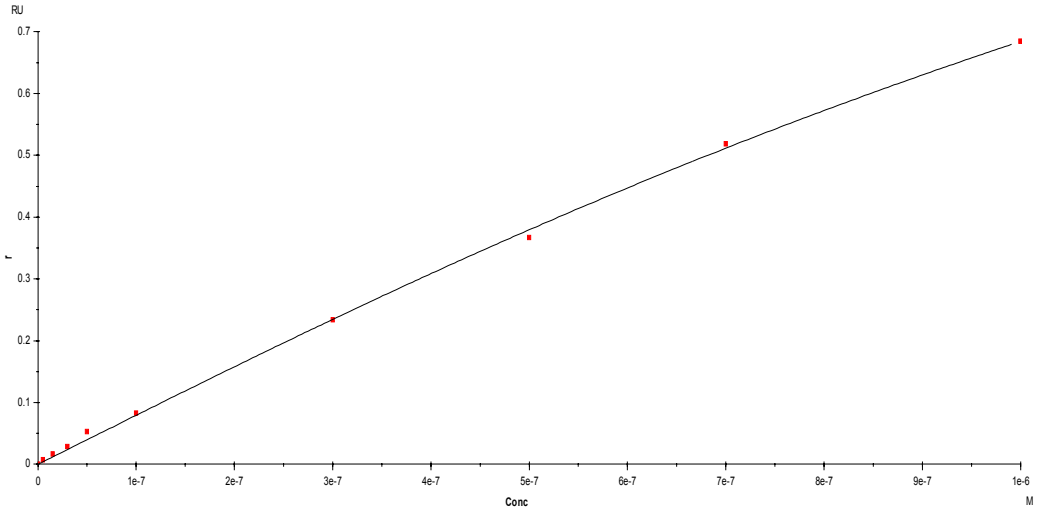

Figure S14. Binding curve of complex **1** to *c-myc* DNA fitted with two-non-equivalent binding site model obtained using BIAeval software (BIACore).

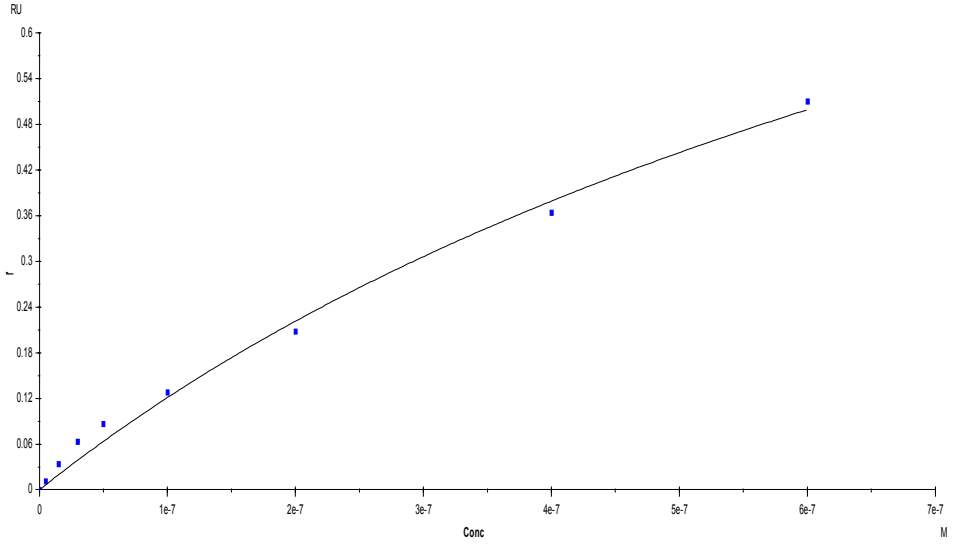

Figure S15. Binding curve of complex **3** to human telomeric DNA fitted with a two-non-equivalent binding site model obtained using BIAeval software (BIAcore).

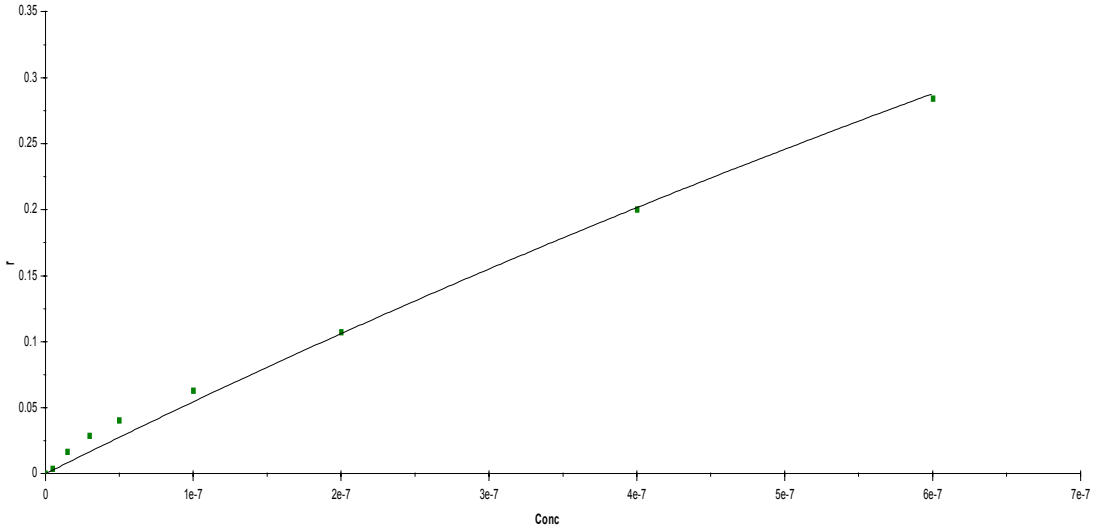

Figure S16. Binding curve of complex **3** to CG-rich duplex DNA fitted with two-equivalent binding site model obtained using BIAeval software (BIAcore).

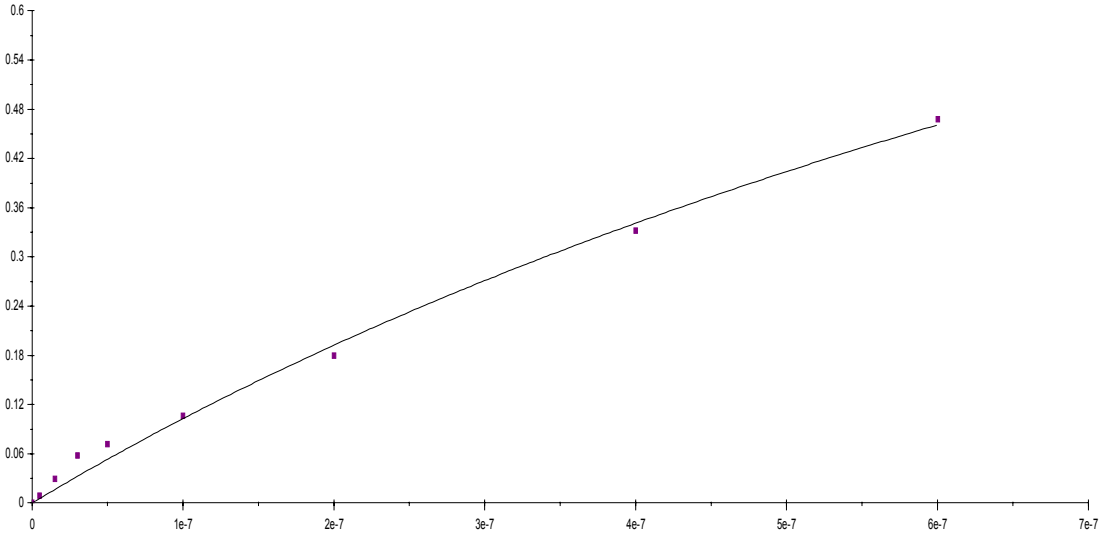

Figure S17. Binding curve of complex **3** to *c-myc* DNA fitted with two-non-equivalent binding site model obtained using BIAeval software (BIACore).

1 D. Monchaud, C. Allain, M.-P. Teulade-Fichou, *Bioorg. Med. Chem. Lett.* 2006, **16**, 4842.
2 I. M. Dixon, F. Lopez, J.-P. Estève, A. M. Tejera, M. A. Blasco, G. Pratviel, B. Meunier, *ChemBioChem* 2005, **6**, 123.
3 J. E. Redman, *Methods* 2007, **31**, 302.