Electronic Supplementary Material (ESI) for Chemical Communications

Ni^{2+} and Cu^{2+} complexes of phenanthroline-based ligand bind to G-quadruplex at non-overlapping sites.

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Supporting Information

Material and Methods

Synthetic oligonucleotides.

Deoxyoligonucleotides used for fluorescence melting studies were the human telomeric sequence HTS (AGGGTTAGGGTTAGGGTTAGGGT, labeled with Dabcyl at 5' end and Fluorescin at 3' end) and ds-random (Fluorescin 5' labelled GTGAGATACCGACAGAAG annealed with its complementary strand labeled with Dabcyl at 3' end). They were synthesized and HPLC purified by Oswel Research Products Ltd (Southampton, UK).

DNA oligonucleotide used for CD and EMSA assays was Tel22: AGGGTTAGGGTTAGGGTTAGGG. It was synthesized by Eurogentec (Belgium) and HPLC purified.

DNA concentrations were determined spectroscopically. An annealing protocol (heating to 95 °C for 10 min and slow cooling up to room temperature o.n.) was applied to all DNA solution before use.

Ligand, metal ions and metal complexes solutions.

The synthesis and characterization of P120 has been described (Krapcho, A. P.; Sparapani, S.: Boxer, M. *Tetrahedron Lett.*, **2007,** *48*, 5593-5593).

Stock solutions (4 mM) of P120 were prepared in DMSO. They were diluted to the appropriate concentration in the working buffer prior to use.

$CuSO_4$ (1 M) and $NiCl_2$ (0.8 M) were dissolved in deionized water and metal ion concentrations were determined by ICP (Optima 3000 DV Perkin Elmer).

Metal complexes were prepared by mixing the required amount of ligand and metal ion stock solution. Solutions prepared in water/methanol (10%-90%) and contained 100 µM ligand and different stoichiometric amounts of metal ions were further analyzed by mass spectrometry using a Mariner^{TM} mass spectrometer, Applied Biosystems (Foster City, CA). A Harvard model 11 syringe pump (Holliston, MA) set at flow rate of 20 µl/min was used to infuse the sample solutions. The ESI source was operated in positive ion mode with an electrospray voltage of 4.5 kV. Spectra were acquired over the *m/z* range 100-4000 by summing 100 scans.

P120 (m/z) 290.0750 (L; 100%)
P120 + 1 eq $CuSO_4$ (m/z) 290.0750 (L; 25%), 215.0174 (L-Cu-DMSO; 43%), 397.008 (L-Cu-2DMSO; 32%)
P120 + 2 eq $CuSO_4$ (m/z) 290.0750 (L; 6%), 215.0174 (L-Cu-DMSO; 18%), 397.008 (L-Cu-2DMSO; 44%), 641.0722 (2L-Cu; 26), 320.5452 (2L-Cu; 6%)
P120 + 1 eq $NiCl_2$ (m/z) 212.5063 (L-Ni-DMSO; 100%)
P120 + 2 eq $NiCl_2$ (m/z) 212.5063 (L-Ni-DMSO; 35%)), 318.0317 (2L-Ni; 65%)

UV Titrations.

Spectrophotometric titrations were performed at 25°C in 10 mM Tris-HCl, 50 mM KCl, pH 7.5, with a Perkin-Elmer Lambda 20 equipped with Haake C25P thermostat. Complex formation was followed recording the ligand spectra upon incremental additions of metal ion solutions in the presence/absence of proper Tel22 concentrations. When required the spectra were recorded at increasing temperature.

Fluorescence melting studies.

Melting experiments were performed on HTS and ds-random sequences in a Roche LightCycler, using an excitation source at 488 nm and recording the fluorescence emission at 520 nm. Mixtures (20 μL) contained 0.25 μM of target DNA and variable concentrations of tested derivatives in 50 mM potassium buffer (10 mM LiOH; 50 mM KCl pH 7.4 with H_3PO_4). Temperature was slowly increased (0.2 °C/min) up to 90 °C and again lowered at the same rate to 30°C. This cycle was repeated at least twice for each solution. T_m values were determined from the first derivatives of the melting profiles using the Roche LightCycler software. Each curve was repeated at least three times and errors were ± 0.4°C.

Circular dichroism measurements.

Circular dichroism spectra from 230 to 450 nm were recorded using 10 mm path length cells on a Jasco J 810 spectropolarimeter equipped with a NESLAB temperature controller in 10 mM Tris-HCl, 50 mM KCl pH 7.4. Before data acquisition, Tel22 solutions (4 μM) were heated at 95°C for 5 min and left to cool at room temperature o.n.. The reported spectrum of each sample represents the average of 3 scans recorded with 1-nm step resolution. Observed ellipticities were converted to mean residue ellipticity [θ] = deg x cm^2 x $dmol^{-1}$ (Mol. Ellip.).

To determine complex stoichiometry by Job plot, different amounts of metal ions complexes and Tel22 solutions were mixed to reach a final constant total concentration (80 μM). The relative variation of the molar ellipticities recorded at 358 nm or 290 nm were reported as a function of the molar fraction defined as χ = [$(P120)_2$Me(II)]/([$(P120)_2$Me(II)]+[Tel22]) where Me(II) was Ni(II) or Cu(II).

Electrophoretic mobility shift assay (EMSA).

Single stranded Tel22 was 5'-labeled with ^{32}P and T4 polynucleotide kinase, by incubating the reaction mixture at 37 °C for 30 min. The kinase activity was inactivated by heating the reaction mixture at 85°C for 5 min, followed by two phenol extractions.

A mixture of purified labelled and unlabelled oligonucleotides (total final concentration 1 μM folded in 10 mM Tris-HCl, 50 mM KCl, pH 8.0) was added of increasing concentrations of tested ligand. After incubation (ranging from 30 min up to o.n. at r.t.) the samples were analyzed by native

16% PAGE in TBE 0.5X containing 20 mM KCl in the running buffer. Gels were dried and resolved bands were visualized and quantified on a PhosphorImager (Amersham).

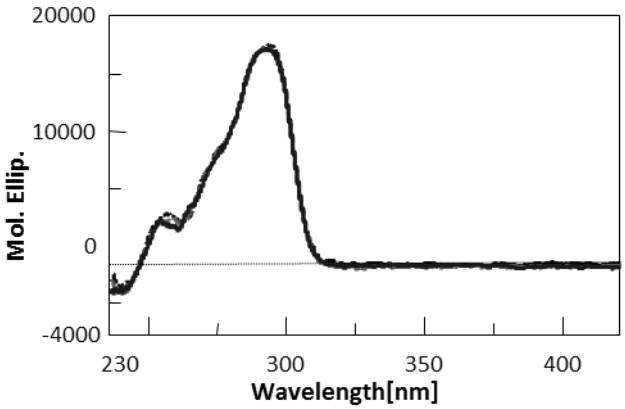

Figure S1. CD spectra of 4 μM Tel22 (solid line) upon addition of 16 μM Ni^{2+} (dotted line) or 16 μM P120 (dashed line) in 10 mM Tris, 50 mM KCl, pH 7.4.

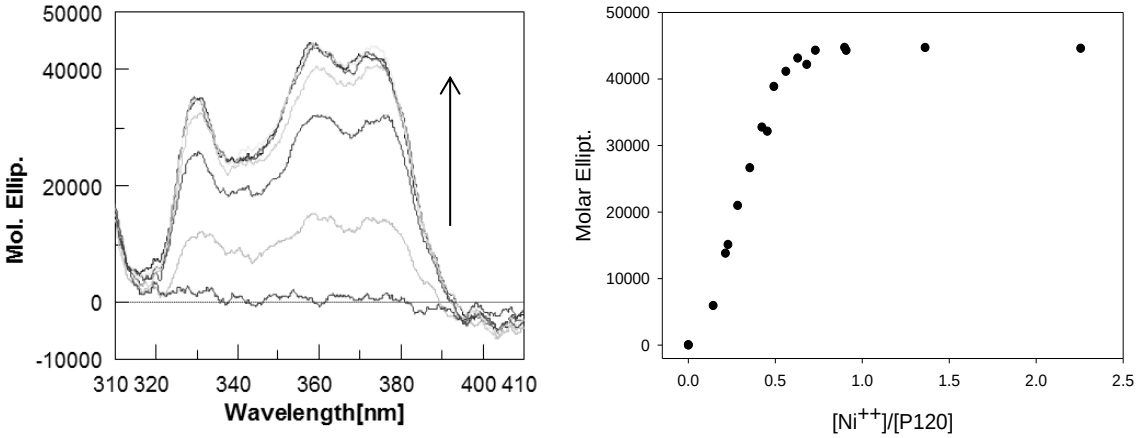

Figure S2. CD spectra of 6 μM P120 in the presence of 4 μM Tel22 upon addition of increasing concentrations of Ni^{2+} (PANEL A) and corresponding variation of the induced signal recorded at 358 nm (PANEL B) determined in 10 mM Tris, 50 mM KCl, pH 7.4. The $[Ni^{++}]/[P120]$ ratio refers to the total metal ion vs total ligand concentrations. Molar ellipticities were calculated taking into account the molar concentration of P120. Since experimental results support that the optical active species is represented by $(P120)_2Ni(II)$, the ellipticity referred to this complex should be doubled.