Supporting Information

Impact of a Snap-Back Loop on Stability and Ligand Binding to a Parallel G-Quadruplex

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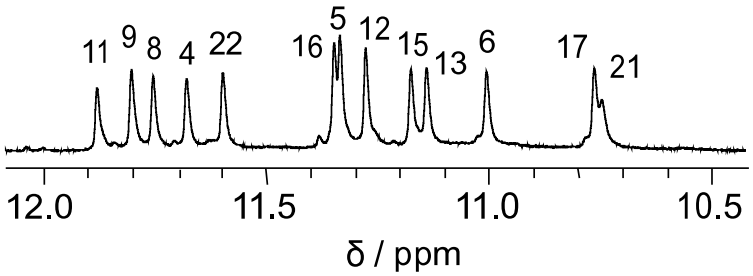

Figure S1. Imino proton spectral region of ***Pu22T*** (0.5 mM) at 25 °C with signal assignments.

NMR spectral analysis. The number of imino signals indicate the formation of a single ***Pu22T*** quadruplex species with three tetrads (Figure S1). Consecutive runs of intranucleotide and sequential H8-H1' and H8-H2'/H2" NOE connectivities identify residues T1 to G6 and G15 to G21 (Figure S2, top). A strong H8-H1' NOE crosspeak at 7.71/6.37 ppm points to a *syn*-conformation and was assigned to G22 based on the close similarity with spectral regions of *Pu24* variants reported by Phan *et al.*.[1] This guanosine is also linked through sequential connectivities to the G9-G8 tract, further establishing the proposed snap-back loop structure. A fourth G-tract interrupted by two propeller loops must run from G11 to G13. Single-residue propeller loops give rise to a thymidine H6 at 7.92 ppm and two additional isochronic T H6 at 7.86 ppm with no sequential NOE contacts. Tetrad alignments are further assessed using H8-imino NOE correlations within a tetrad plane, but weak inter-tetrad contacts can also be observed (Figure S2, bottom). Supported by additional $^{1}H-^{13}C$ HMBC experiments with correlations between H8 and H1 protons (data not shown), the three tetrads (4-8-11-15), (5-9-12-16), and (6-22-13-17) can be identified.

More structural details of the fold-back structure are revealed by connectivities of residue A20 located within the four-nucleotide snap-back loop. In addition to sequential contacts with A19 and G21, H8 of this residue has crosspeaks to G6, G22, and G21 imino protons. Although weak, observation of a G21 imino resonance suggests its participation in a G18·A20·G21 base triad capping the 3'-tetrad as previously described for a modified *Pu24* quadruplex.[1] Stacking of A20 on the 3'-outer tetrad was further supported by contacts of A20 H1' to G22 H1 and A20 H2 to several sugar protons of G6. On the other hand, stacking of the 5'-overhang residue A3 on the 5'-outer tetrad is suggested by contacts of A3 H2 to G4 and G15 imino protons.

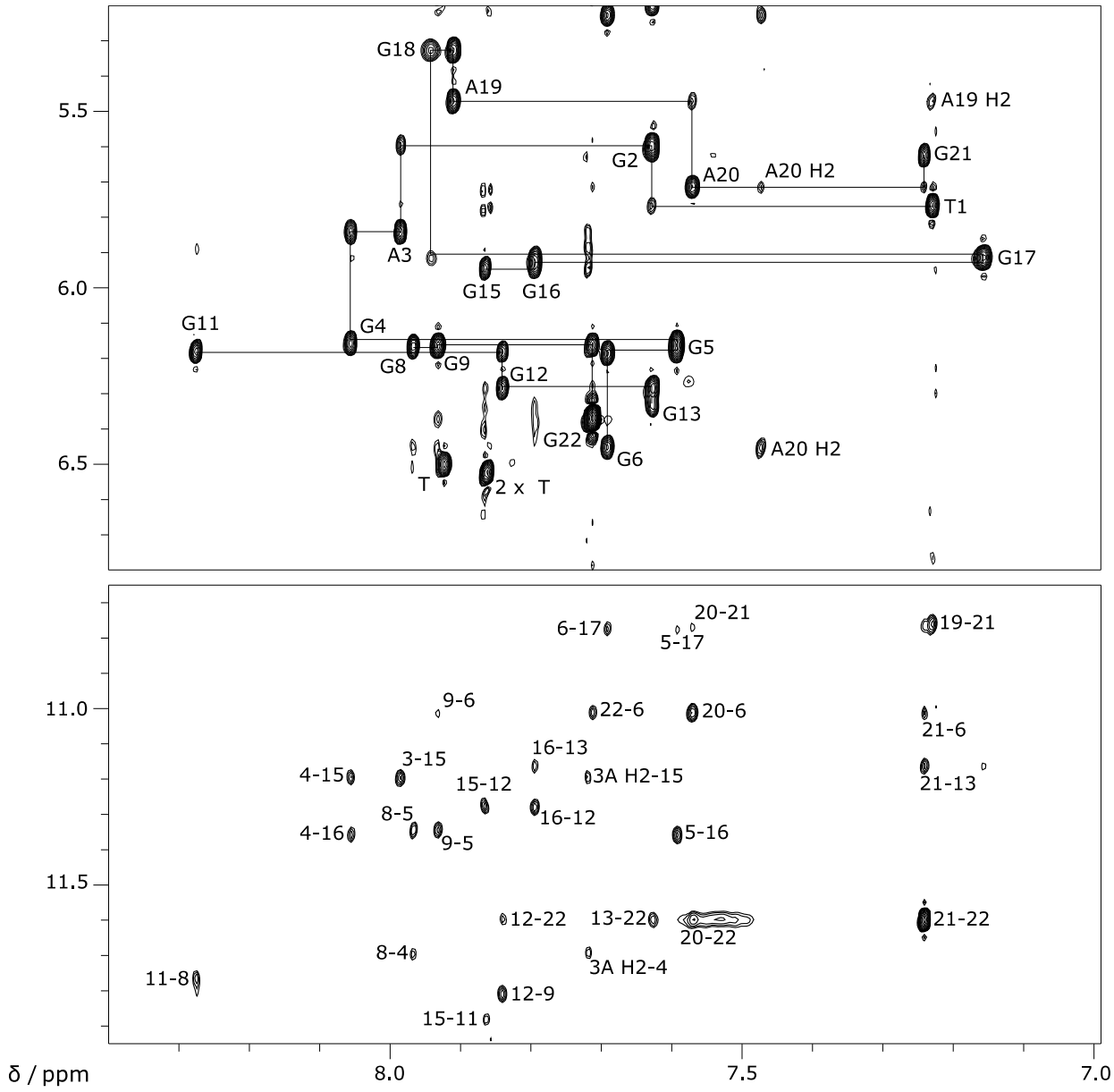

Figure S2. H8/H2/H6(ω_2)-H1'(ω_1) (top) and H8/H2/H6(ω_2)-imino(ω_1) (bottom) 2D NOE spectral regions of ***Pu22T*** (0.5 mM) acquired at 25 °C with a mixing time of 300 ms. Continuous H8-H1' NOE connectivity walks are shown. First and second numbers in the bottom spectral region refer to H8/H2 and imino protons, respectively.

CD experiments. Spectra were acquired with a Jasco J-810 spectropolarimeter equipped with a thermoelectrically controlled cell holder. Measurements were performed with 1 cm quartz cuvettes at 20 ^{o}C on 5 μM quadruplex in a buffer (pH 7) of either 10 mM potassium phosphate or 20 mM potassium phosphate with 100 mM KCl. Spectra were recorded with a bandwidth of 1 nm, a response time of 1-2 s, and a scanning speed of 50 nm/min and finally blank-corrected.

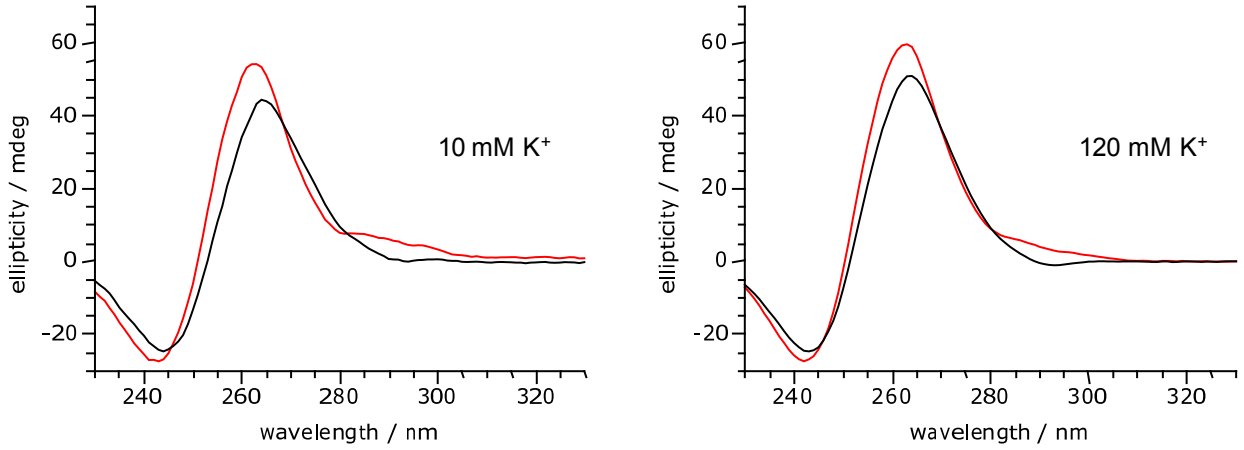

Figure S3. Circular dichroism spectra of the ***MYC*** (black) and ***Pu22T*** (red) quadruplex in the presence of 10 mM K^+ (left) and 120 mM K^+ (right).

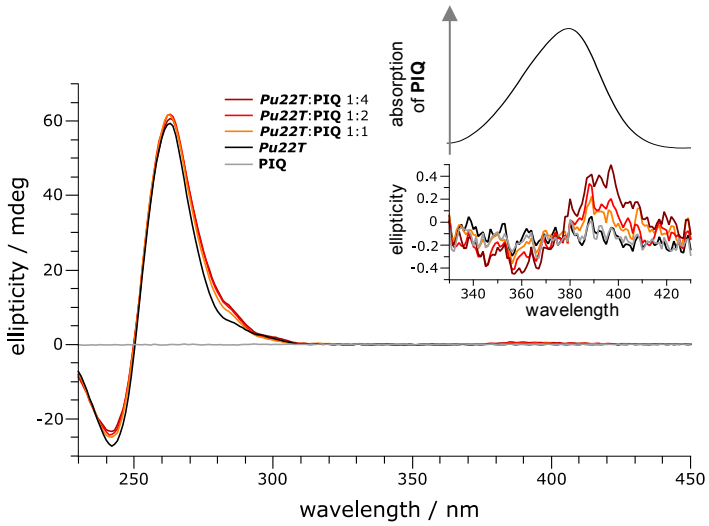

Figure S4. Circular dichroism spectra of free **PIQ** and of ***Pu22T*** (5 μM) in the presence of increasing amounts of the ligand at 20 °C in 20 mM KP_i buffer, 100 mM KCl, pH 7. The inset shows induced Cotton effects together with the ligand UV-vis absorption on top.

UV-vis titrations. Spectra were acquired with a Jasco V-650 spectrophotometer equipped with a Peltier temperature control unit. Measurements were performed in 20 mM potassium phosphate buffer, 100 mM KCl, pH 7, by titrating aliquots of a G4 solution to 15 μM ligand in 1 cm quartz cuvettes. Between measurements, the sample was equilibrated for 5 min under stirring. Spectra were recorded between 300-600 nm with a bandwidth of 1 nm, a scanning speed of 100 nm/min, and three accumulations. Before data analysis, the spectra were blank-corrected and dilution effects were eliminated. The absorption at 376 nm was normalized, plotted over the quadruplex-to-ligand molar ratio and the resulting isotherm fitted based on a model with equivalent and independent binding sites.[2,3]

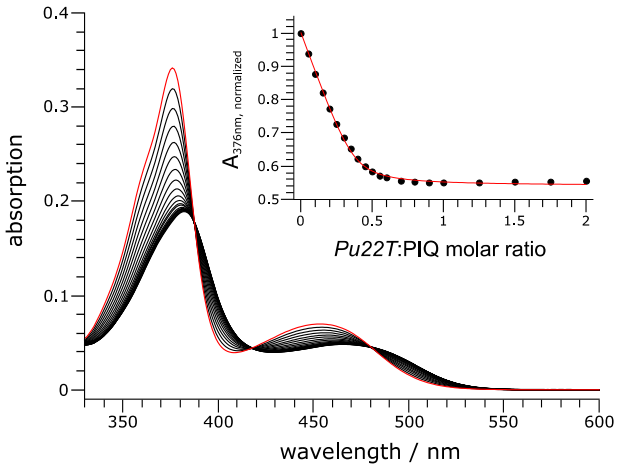

Figure S5. Exemplary UV-vis titration of **PIQ** with the ***Pu22T*** quadruplex in the presence of 120 mM K^+ at 40 °C. The initial free ligand spectrum is shown in red; normalized absorbances at 376 nm as a function of quadruplex-to-ligand molar ratios and fitted isotherm are shown as inset.

Table S1. Binding constants K_a and number of bound ligands N from UV-vis titrations at 40 °C.[a]

	MYC	***Pu22T***
K_a (M^{-1})	$(0.9 \pm 0.1)\cdot 10^6$	$(1.6 \pm 0.6)\cdot 10^6$
N	4.2 ± 0.1	2.6 ± 0.1

[a]Averages with standard deviations from three independent measurements in the presence of 120 mM K^+.

Table S2. Melting temperature of ^{FRET}***G4*** (0.2 μM) in the presence and absence of **PIQ** and of competitor quadruplex $G4_{comp}$.[a]

$G4_{comp}$	**PIQ**	T_m / ℃
0	0	40.0 ± 0.6
0	2 eq	80.7 ± 0.6
1 eq ***MYC***	2 eq	55.0 ± 0.5
1 eq ***Pu22T***	2 eq	70.8 ± 2.7

[a]Averages with standard deviations from three independent measurements in 10 mM KP_i buffer.

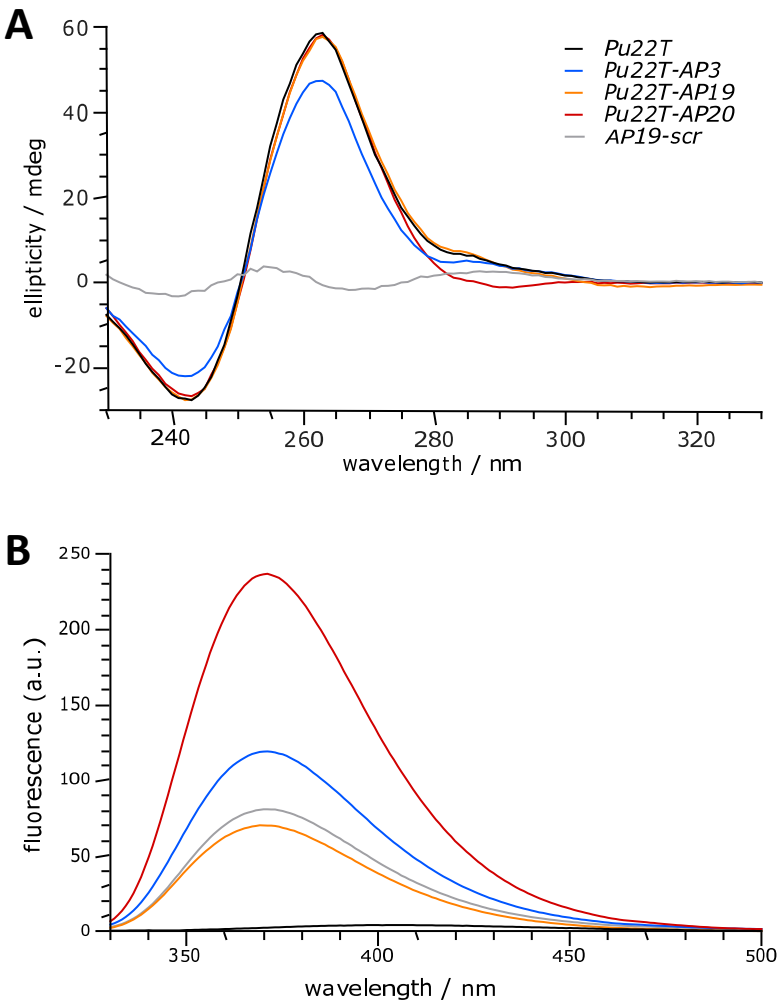

Figure S6. CD spectra (A) and fluorescence emission spectra (B) of ***Pu22T*** and analogs with a 2-aminopurine substitution at position 3, 19, or 20. An exemplary ***AP19-scr*** sequence with single G-to-T substitutions in three of its G-tracts to block folding into a distinct secondary structure derives from ***Pu22T-AP19*** and serves as a control. Note that negligible fluorescence is observed for unsubstituted ***Pu22T***. Spectra were acquired with 5 µM DNA in the presence of 10 mM K^+ at 20 °C (A) and with 5 µM DNA in the presence of 120 mM K^+ at 25 °C (B).

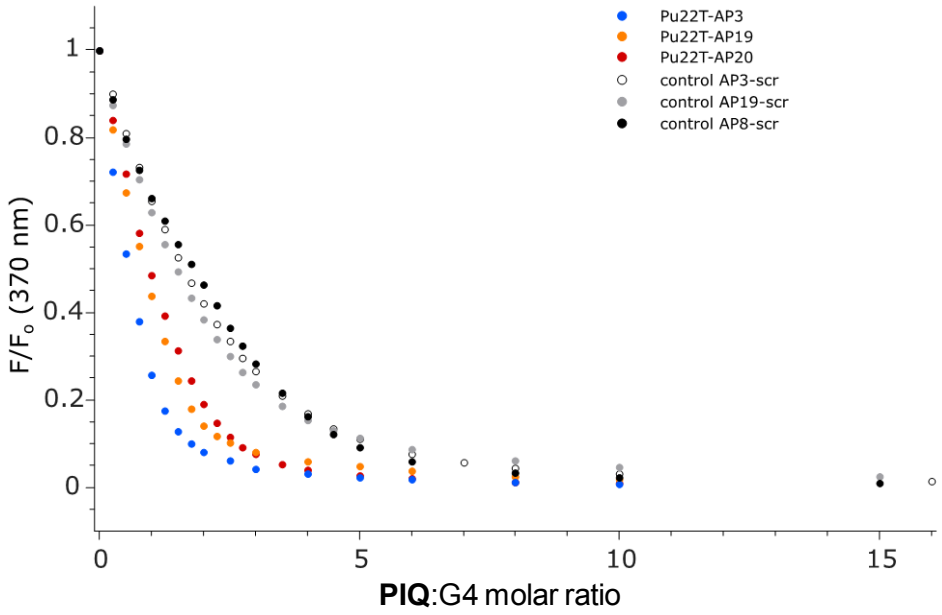

Figure S7. Normalized fluorescence titration data of 2-aminopurine substituted ***Pu22T*** analogs and of three unfolded control sequences. The significant exponential fluorescence decay when titrating the **PIQ** ligand to the latter sequences can be attributed to inner filter effects. Experiments were performed with 5 μM DNA in the presence of 120 mM K^+ at 25 °C.

Table S3. Binding constants K_a and stoichiometries N for the association between 2-aminopurine analogs of ***Pu22T*** and **PIQ** as determined from fluorescence titration data after correction for inner filter effects.[a]

	Pu22T-AP3	***Pu22T-AP19***	***Pu22T-AP20***
K_a (M^{-1})	$(1.0 \pm 0.3)\cdot 10^6$	$(3.8 \pm 0.5)\cdot 10^5$	$(3.1 \pm 0.3)\cdot 10^5$
N	0.9 ± 0.1	2.0 ± 0.2	2.3 ± 0.1

[a]Average values with standard deviations from three independent experiments in the presence of 120 mM K^+ at 25 °C.

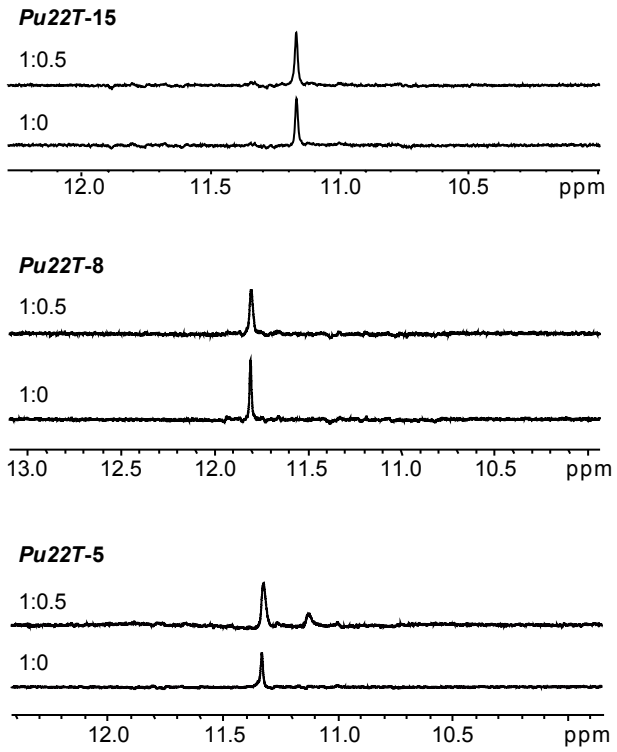

Figure S8. ^{15}N filtered 1D ^{1}H-^{15}N HMQC imino proton spectral region of ***Pu22T*** in the absence and presence of 0.5 eq **PIQ** acquired at 25 °C with a site-specifically ^{15}N-enriched (10%) G5, G8, or G15 residue.

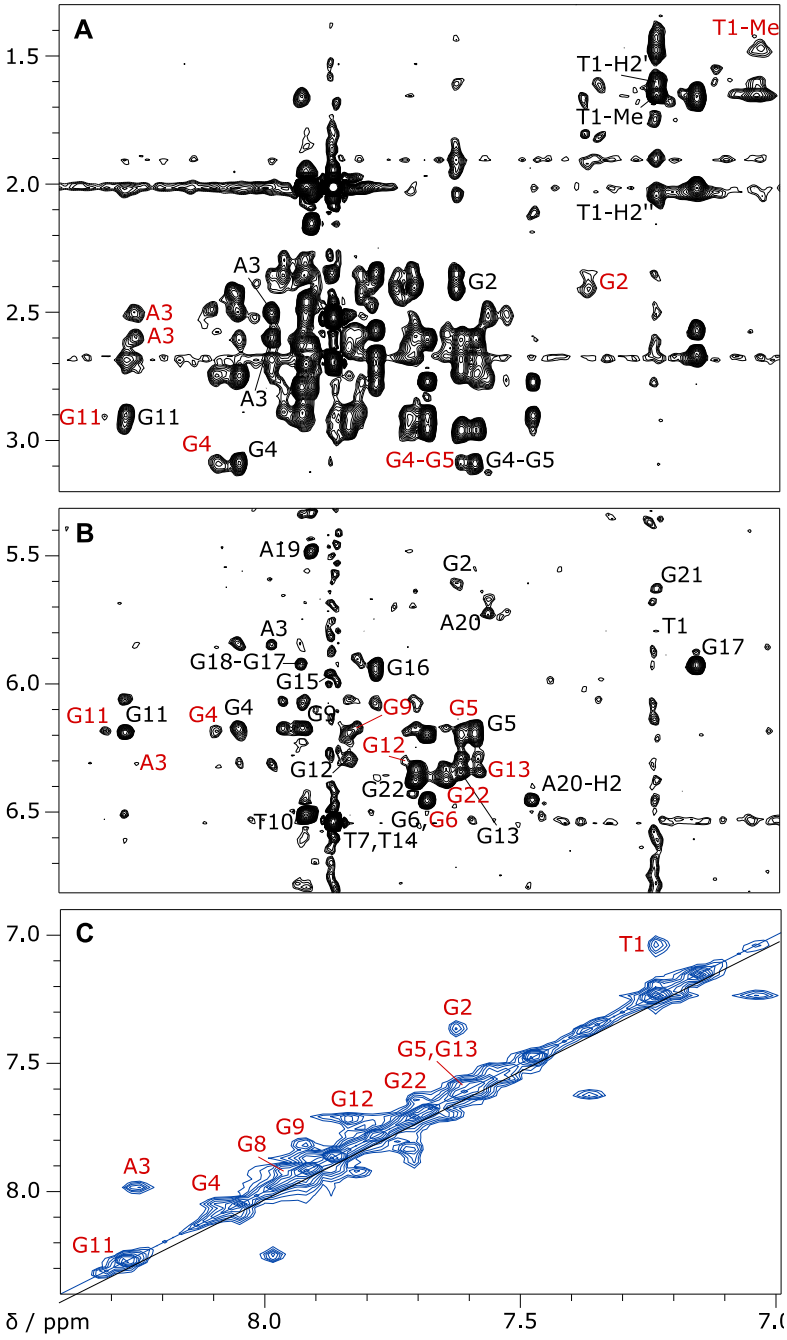

Figure S9. 2D NOE (mixing time 500 ms) H8/H6(ω_2)-H2'/H2''/Me(ω_1) (A) and H8/H6(ω_2)-H1'(ω_1) (B) spectral region as well as an EASY ROESY (mixing time 80 ms) H8/H6-H8/H6 spectral region (C) of a ***Pu22T*** – **PIQ** mixture (molar ratio 1:0.5) at 25 °C. Assignments for the free quadruplex and the ligand bound quadruplex as well as exchange peaks are given in black and red, respectively.

NMR spectral analysis. The G4 H8 resonance in the complex was assigned through its ROESY exchange peak (Figure S9C). Although G5 H8 overlaps with G13 H8 in the free quadruplex and prevents an unambiguous assignment through ROESY exchange crosspeaks in the complex, sequential H8-H2' NOE connectivities between G4 and G5 identifies G5 resonances (Figure S9A). Following H8-H1' NOE connectivities, no change in chemical shift is observed for G6 H8 (Figure S9B). H8 resonances of G11, G12, and G13 were likewise assigned through H8-H1' continuous NOE walks and the identification for G12 and G13 was further supported by ROESY exchange peaks. These also allowed the assignment of G8 H8, G9 H8, and G22 H8 despite signal overlap of G8 H8 and G9 H8 in the complex and free quadruplex, respectively.

Due to the absence of clearly observable H8-H1' NOE contacts, assignment of the remaining H8 ROESY exchange peaks made use of NOE contacts to H2'/H2" and T methyl protons. In some cases, identification of NOE crosspeaks within the complex were also facilitated by corresponding spectra acquired on a 1:1 ligand to quadruplex mixture, associated with elevated and reduced crosspeak intensities for the ligand-bound and free G4, respectively.

Table S4. ^{1}H chemical shifts of ***Pu22T*** without and with ligand **PIQ** (1:0.5 molar ratio).[a]

	Pu22T					***Pu22T*** **– PIQ**		
residue	H1	H8/H6	H2/H5/Me	H1'	H2'/H2''	H1	H8/H6	H1'
T1	--	7.23	1.65	5.77	1.60/2.05	--	7.04	n.d.
G2	n.d.	7.63	--	5.60	2.36/2.42	n.d.	7.36	n.d.
A3	--	7.98	7.72	5.84	2.49/2.60	n.d.	8.25	6.30
G4	11.69	8.06	--	6.16	2.76/3.09	11.56	8.09	6.17
G5	11.35	7.59	--	6.18	2.61/2.96	11.15	7.61	6.19
G6	11.01	7.69	--	6.45	2.78/2.91	10.92	7.68	6.44
T7	--	n.d.	n.d.	n.d.	n.d.	--	n.d.	n.d.
G8	11.76	7.97	--	6.17	2.36/2.90	n.d.	7.92	n.d.
G9	11.81	7.93	--	6.16	2.61/2.92	11.53	7.82	6.16
T10	--	7.92	2.02	6.50	2.46/2.70	--	n.d.	n.d.
G11	11.88	8.27	--	6.18	2.90/2.95	11.24	8.31	6.17
G12	11.28	7.84	--	6.28	2.65/2.97	11.13	7.72	6.28
G13	11.16	7.63	--	6.33	2.68/2.74	11.08	7.58	6.33
T14	--	n.d.	n.d.	n.d.	n.d.	--	n.d.	n.d.
G15	11.19	7.86	--	5.94	2.72/2.72	n.d.	n.d.	n.d.
G16	11.36	7.79	--	5.92	2.57/2.67	11.14	n.d.	n.d.
G17	10.77	7.16	--	5.91	1.68/2.02	10.72	n.d.	n.d.
G18	n.d.	7.94	--	5.33	2.64/2.80	n.d.	n.d.	n.d.
A19	--	7.91	7.23	5.47	2.04/2.16	--	n.d.	n.d.
A20	--	7.57	7.47	5.71	2.53/2.78	--	7.52	5.66
G21	10.76	7.24	--	5.62	1.76/2.64	n.d.	n.d.	n.d.
G22	11.60	7.71	--	6.37	2.66/3.38	11.48	7.64	6.32

[a]At 25 °C in 10 mM KP_i, pH 7; n.d.: not determined.

References

(1) Phan, A. T.; Kuryavyi, V.; Gaw, H. Y.; Patel, D. J. Small-molecule interaction with a five-guanine-tract G-quadruplex structure from the human *MYC* promoter. *Nat. Chem. Biol.* **2005**, *1*, 167–173.

(2) Stootman, F. H.; Fisher, D. M.; Rodger, A.; Aldrich-Wright, J. R. Improved curve fitting procedures to determine equilibrium binding constants. *Analyst* **2006**, *131*, 1145–1151.

(3) Jäger, K.; Bats, J. W.; Ihmels, H.; Granzhan, A.; Uebach, S.; Patrick, B. O. Polycyclic azoniahetarenes: Assessing the binding parameters of complexes between unsubstituted ligands and G-quadruplex DNA. *Chem. - A Eur. J.* **2012**, *18*, 10903–10915.