Supplementary Information

1.Materials and methods

1.1 Materials

The anticancer peptide Lasioglossin-III (LL-III) with sequence VNWKKILGKIIKVVK-NH$_2$ (M.W. of 1764.19 g mol^{-1}) and net charge +6 was purchased as a dry powder by Primm SRL (Milan, Italy) with a HPLC purity > 95%. The stock solutions were prepared by dissolving the powder in the same buffers used for the DNA sequences. Its concentration was spectrophotometrically determined by using the extinction coefficient of $\varepsilon(280)$ = 5690 M^{-1} cm^{-1}. Unlabeled and 5'-labeled with carboxyfluorescein (FAM) or Texas-Red (Tx-Red) DNA oligonucleotides were purchased as a HPLC grade dry powder from Merck (Darmstadt, Germany) or Eurogentec (Seraing, Belgium) and resuspended in an appropriate volume of H$_2$O. The concentration of the stock solutions was determined with UV-Vis spectroscopy by recording the absorbance at 260 nm and using the theoretical extinction coefficient determined with the nearest-neighbor model.[1] The samples were then diluted at a micromolar concentration (about 10 μM) in the desired buffer, heated at 95 °C for 5 min, left to slowly cool at room temperature and then stored for a night at 4 °C before use. The chosen buffers were: (i) 10 mM potassium phosphate (KP) + 70 mM potassium chloride (KCl) + 0.2 mM EDTA (buffer K$^+$) for mixed-type Tel-23, Mut_Tel-23, cMyc and cKIT1; (ii) 10 mM sodium phosphate (NaP) + 70 mM sodium chloride (NaCl) + 0.2 mM EDTA (buffer Na$^+$) for the antiparallel Tel-23 and 12CG duplex; (iii) 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) + 70 mM lithium chloride (LiCl) + 0.2 mM EDTA (buffer Li$^+$) for the disordered single-stranded Tel-23 and Mut_Tel-23. The pH was adjusted to 7.0 for every buffer composition.

Oligonucleotide	Sequence	Label	Topologies	Buffer ion
Tel-23	TAG GGT TAG GGT TAG GGT TAG GG	FAM	Mixed-Type Antiparallel Disordered	K$^+$ Na$^+$ Li$^+$
Mut_Tel-23	TAT GGT TAG TGT TAG TGT TAG GT	FAM	Disordered	K$^+$ Li$^+$
cMyc	TGA GGG TGG GTA GGG TGG GTA A	Tx-Red	Parallel	K$^+$
cKIT1	AGG GAG GGC GCT GGG AGG AGG G	Tx-Red	Parallel	K$^+$
12CG	CGC GTT AAC GCG	Tx-Red	B-DNA Duplex	Na$^+$ K$^+$

1.2 Fluorescence Spectroscopy

All the experiments were performed on a Fluoromax-4 spectrofluorometer from Horiba Scientific (Edison, USA) at a fixed temperature of 20 °C and in a 1 cm path-length quartz cuvette.

1.2.1 Fluorescence anisotropy assays

To determine the dissociation constant (K_D) for each DNA-peptide complex, a fluorescence anisotropy assay was performed by adapting a previously reported methodology.[2] Briefly, a

concentrated peptide solution was titrated into a sample containing a fixed concentration of the label oligonucleotide (50 nM). Tx-Red and FAM-labeled DNA were excited at 589 and 492 nm and their anisotropy collected at 615 and 518 nm, respectively. The excitation and emission monochromator slits were adjusted to obtain an initial S_{VV} signal (where the subscripts indicate the vertical orientation of both the excitation and emission polarizer) between $1·10^6$ and $2·10^6$ CPS as suggested by the manufacturer. The anisotropy of each sample was recorded at least 7 times to assure that equilibrium was reached, and the results were averaged. During the experiments, a total intensity variation was observed, revealing that the free and bound states were characterized by a different quantum yield. For this reason, the fraction of bound oligonucleotide was calculated by means of the following equation:[3]

$$f_b = \frac{r_i - r_f}{(r_i - r_f) + \frac{I_b}{I_f}(r_b - r_i)}$$

Where f_b is the oligonucleotide bound fraction, r_i is the anisotropy recorded at the i-th point of titration, r_f is the anisotropy in the absence of the peptide, r_b is the anisotropy value at saturation, I_b is the total emission intensity at saturation, and I_f is the total emission intensity in the absence of the peptide. The binding isotherms were obtained by plotting f_b as a function of peptide concentration. The K_D values were obtained by fitting the binding curves to the following equation:

$$f_b = \frac{1}{[DNA]}\left(-\frac{\sqrt{(1 + [DNA]\cdot K_D^{-1} + X\cdot K_D^{-1})^2 - 4K_D^{-2}\cdot [DNA]\cdot X} - (1 + [DNA]\cdot K_D^{-1} + X\cdot K_D^{-1})}{2K_D^{-1}}\right)$$

where [DNA] is the fixed DNA concentration and X is the total peptide concentration. The reported K_D values are the average of at least three independent measurements.

1.2.2 Fluorescence quenching

Fluorescence quenching experiments were performed to estimate the degree of exposition of LL-III Trp to the solvent by titrating samples containing the peptide alone or the peptide-DNA complex with acrylamide up to 0.055 M. The LL-III concentration was 2 µM and the DNA concentration was in the 4-6 µM range. The excitation wavelength was set to 295 nm and the emission collected between 310 and 550 nm. Each spectrum was recorded not less than three times to assure that at least two spectra were superimposable, indicating that the system was at equilibrium. The excitation and the emission monochromator slits were adjusted to obtain a starting signal intensity > $8·10^5$ CPS. To determine the collisional quenching constant of Stern-Volmer (K_{SV}),[3] the fluorescence intensity was corrected for the DNA's inner filter effect by recording the absorbance at 295 nm at the same concentration in a separate UV-vis spectroscopy experiment. The ratio I_0/I with I_0 indicating the corrected LL-III fluorescence intensity and I indicating the corrected intensity at a given concentration of acrylamide was calculated at 356 nm and reported as a function of acrylamide. The K_{SV} was determined by fitting the experimental data with a least squares regression line. The results reported are the average of at least three independent measurements.

1.3 Circular Dichroism (CD)

Circular dichroism spectra in the range 210-340 nm were recorded on a Jasco 1500 spectropolarimeter from Jasco Corporation (Tokyo, Japan) at a fixed temperature of 20 °C and in a 1 cm path-length quartz cuvette. The experimental parameters were set as follows: scan rate: 50 nm/min; response time: 2 s; data pitch: 0.5 nm; bandwidth: 4 nm; accumulations: 5. For the samples containing Tel-23 and cMyc, the concentration was 3 μM, while for cKIT1 1 μM. For each sample, the spectra of DNA only and in the presence of the peptide (at 1:1 molar ratio) were recorded and elaborated after subtracting the corresponding blank. The spectrum of the bound peptide was obtained by subtracting the DNA spectrum from that of the complex. For comparison, the spectrum of LL-III in neat buffer alone was also recorded. Peptide spectra were normalized and reported in mean residue ellipticity, $[\theta]_{MRW}$, and measured in deg cm^2 dmol^{-1}. The helix fraction, f_H, was calculated according to the relation:

$$f_{\mathrm{H}} = \frac{(\theta - \theta_{\mathrm{C}})}{\theta_{\mathrm{H}} - \theta_{\mathrm{C}}}$$

where θ is the experimental molar ellipticity at 222 nm, θ_C is the theoretical molar ellipticity for a 100% random coil calculated as $2200-53T$ and θ_H is the theoretical molar ellipticity for a 100% alpha-helix calculated as $-40000+250T(1-3/n)$ with n, the number of amino acids and T, the temperature (20 °C) expressed in Celsius degree. [4] Moreover, CD experiments were performed to verify that the Mut_Tel-23 sequence did not form a G4 structure. The oligonucleotide spectra were recorded with the aforementioned parameters at a concentration of 3 μM in K$^+$ buffer, Li$^+$ buffer and water.

1.4 Isothermal Titration Calorimetry (ITC)

ITC experiments were performed at 20 °C by means of a Nano ITC-III from TA Instruments (New Castle, DE, USA). The standard enthalpy of binding ΔH_b° for the complex formation between LL-III and Tel-23 K$^+$, Tel-23 Na$^+$, cMyc and cKIT1 was determined by titrating a 20 (or 10 for cMyc) μM DNA solution, placed in the calorimeter vessel, with a 200 (or 100 for cMyc) μM peptide solution (placed in the syringe). The stirrer was set at 250 rpm, the time between each injection was set at 400 s and 10 injections of 10 μL were performed for each experiment. The contribution of the LL-III heat of dilution was determined in separate experiments by injecting the peptide solution into the buffer solution, and then subtracted from the heats of binding. Each raw calorimetric peak was integrated and normalized to the moles of complex formed at the corresponding injection. The concentration of the complex at each injection was determined by means of the K_D obtained in the fluorescence anisotropy assays. The standard Gibbs free energies of binding ΔG_b° were determined from K_D according to $\Delta G_b^\circ = -RT\ln(K_D^{-1})$ and the standard entropies of binding ΔS_b° from the Gibbs relation $\Delta G_b^\circ = \Delta H_b^\circ - T\Delta S_b^\circ$. The results reported are the average of at least three independent measurements.

# Supplementary Figures and Tables
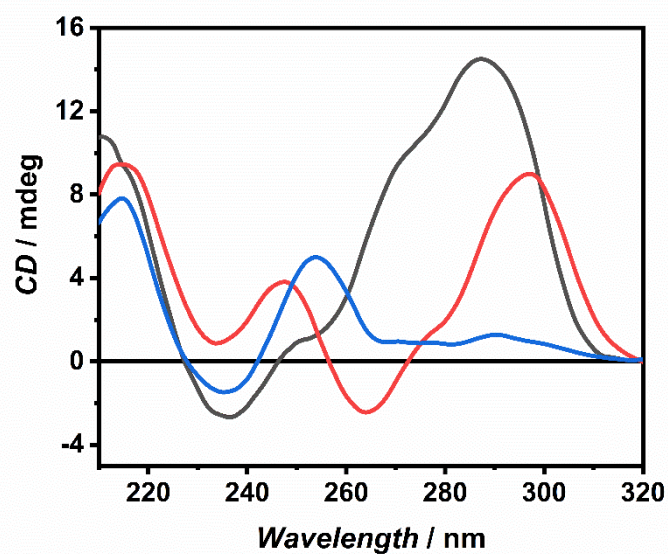
Figure S1. CD spectra of Tel-23 in K^+ buffer (black curve), Na^+ buffer (red curve) and Li^+ buffer (blue curve). The spectra were recorded at 20 °C and at 3 µM strand concentration.
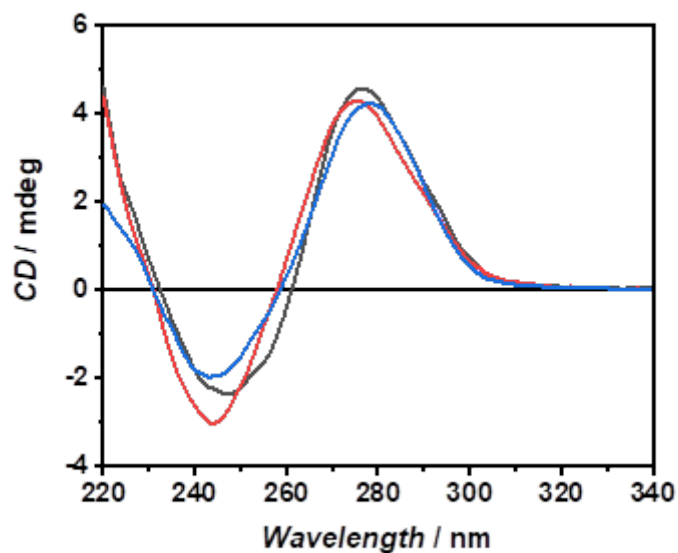
Figure S2. CD spectra of Mut_Tel-23 in K^+ buffer (black curve), Li^+ buffer (red curve) and water (blue curve). The spectra were recorded at 20 °C and at 3 µM strand concentration. The obtained spectrum in K^+ does not correspond to any known GQ spectrum. Further, as expected for a DNA sequence that does not form a GQ, CD spectrum of Mut_Tel-23 is similar in pure water, in Li^+ and in K^+ buffer.
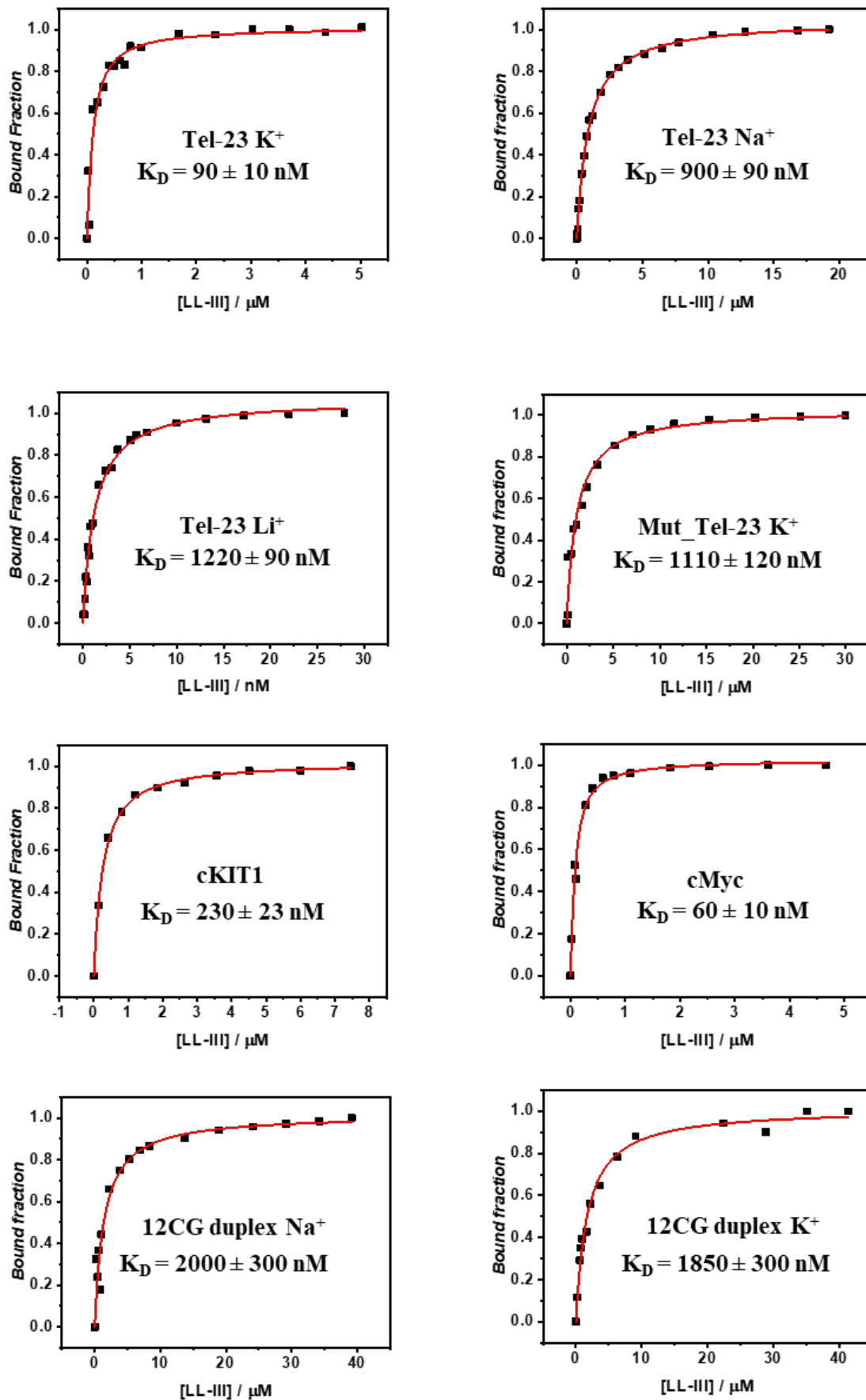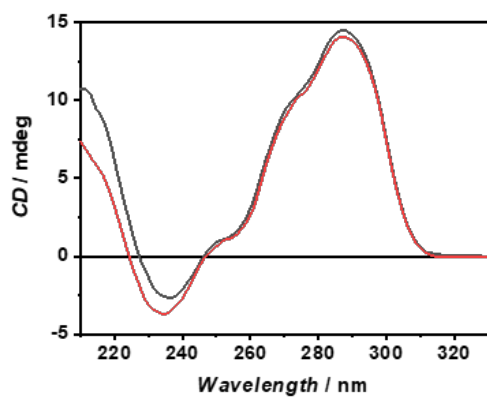
Figure S3. Binding curves for the complexes formed by LL-III and the indicated oligonucleotides. The experimental data are reported as black squares. The red curves represent the best fit of the experimental points.
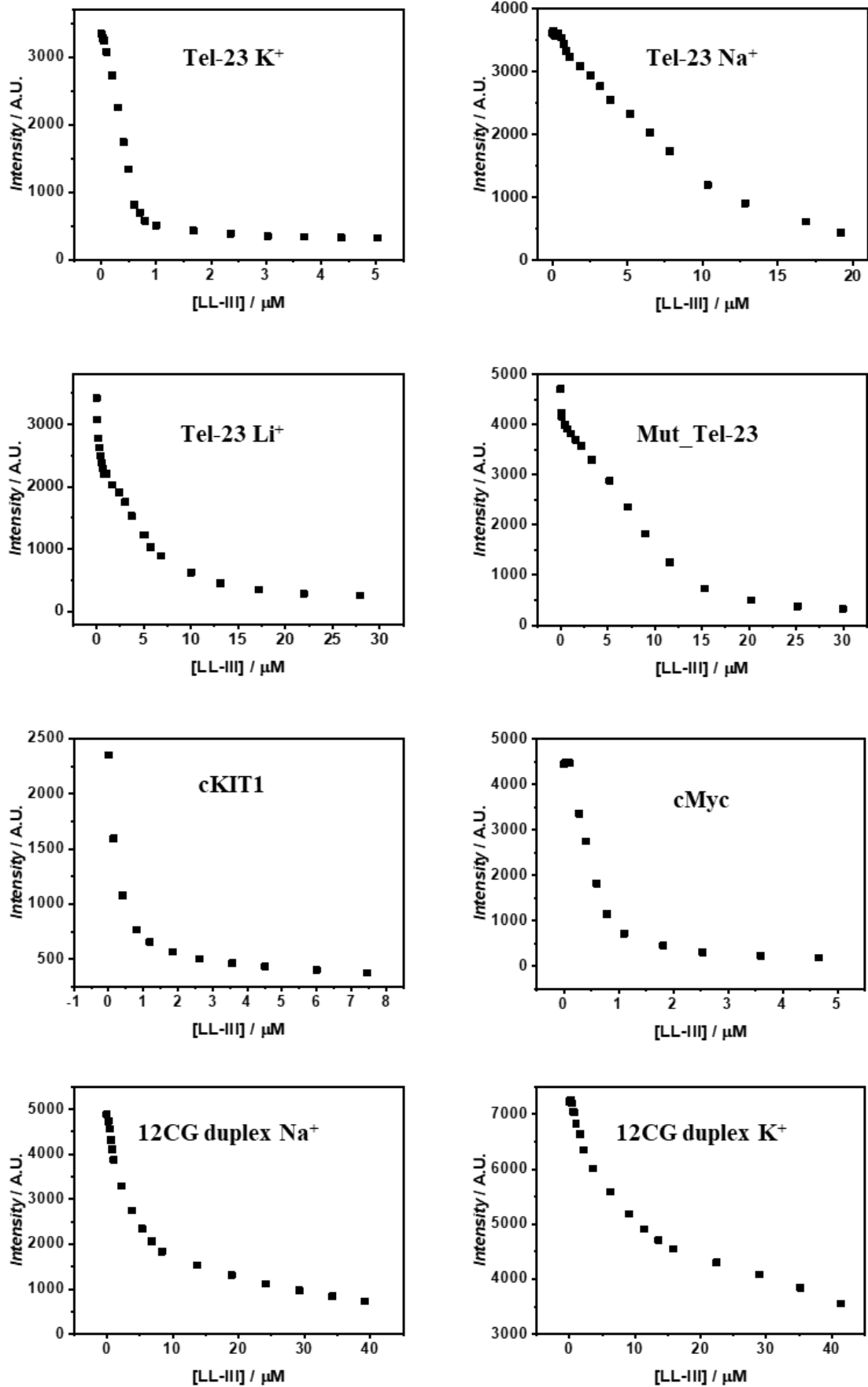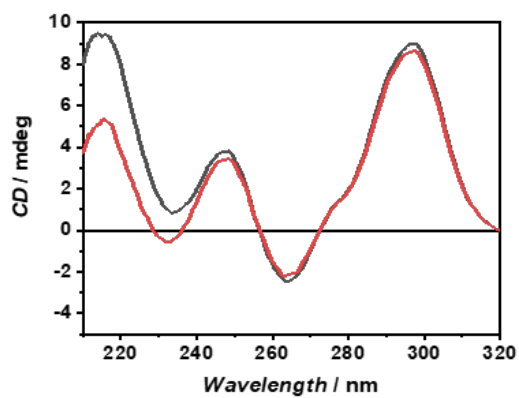
Figure S4. Total intensity curves recorded in the binding experiments of LL-III with the indicated oligonucleotides.
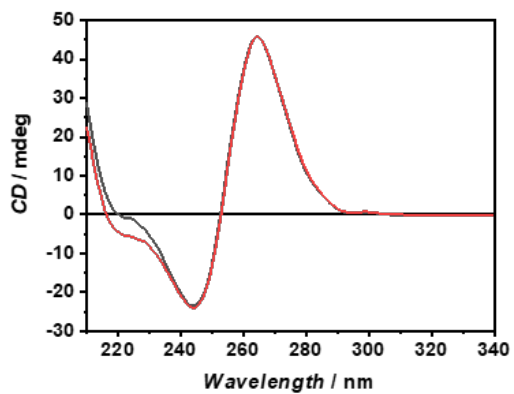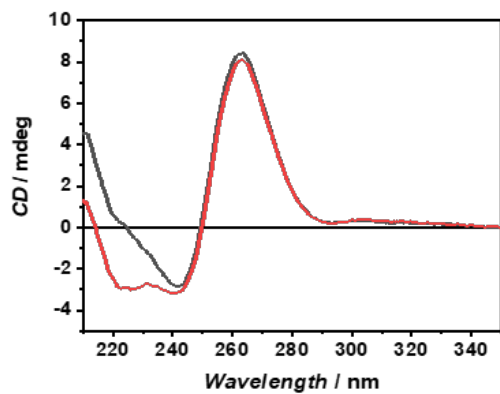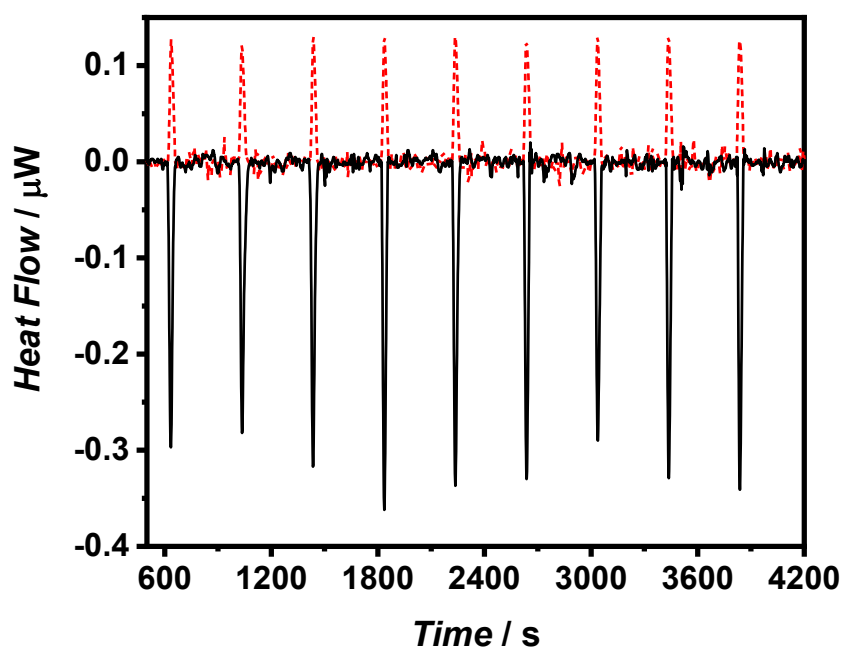
Figure S5. CD spectra of the studied DNA quadruplexes in the absence (black curve) and in the presence of the peptide at 1:1 molar ratio (red curve). (A) Tel-23 in K$^+$-buffer, (B) Tel-23 in Na$^+$-buffer, (C) cMyc in K$^+$-buffer, and (D) cKIT1 in K$^+$-buffer All spectra were recorded at 20 °C.
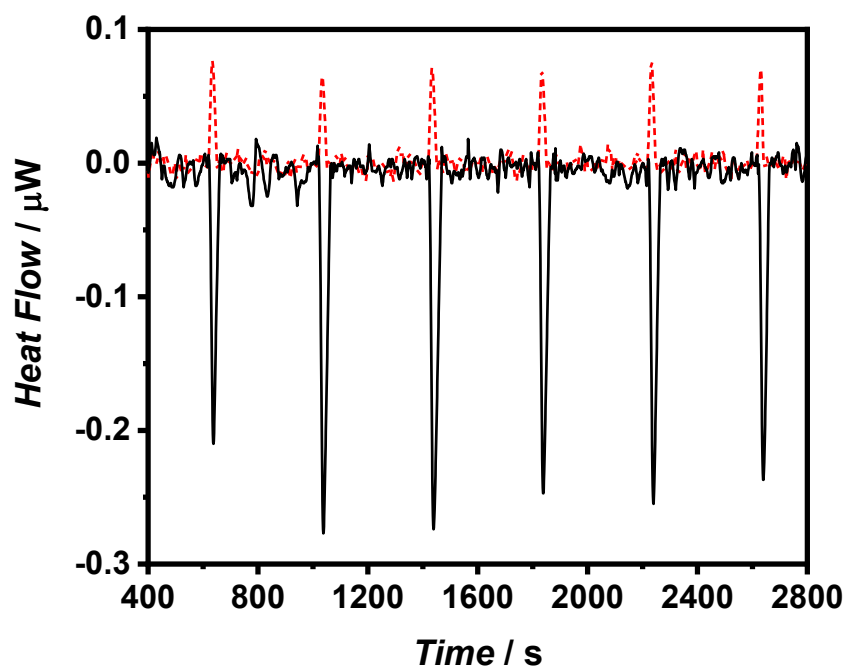
Figure S6. ITC trace obtained from a titration of 20 µM Tel-23 in K⁺ buffer (black curve) with 200 µM LL-III solution. The ITC trace obtained for LL-III dilution into buffer is reported as a red dashed line. The experiments were performed at 20 °C.
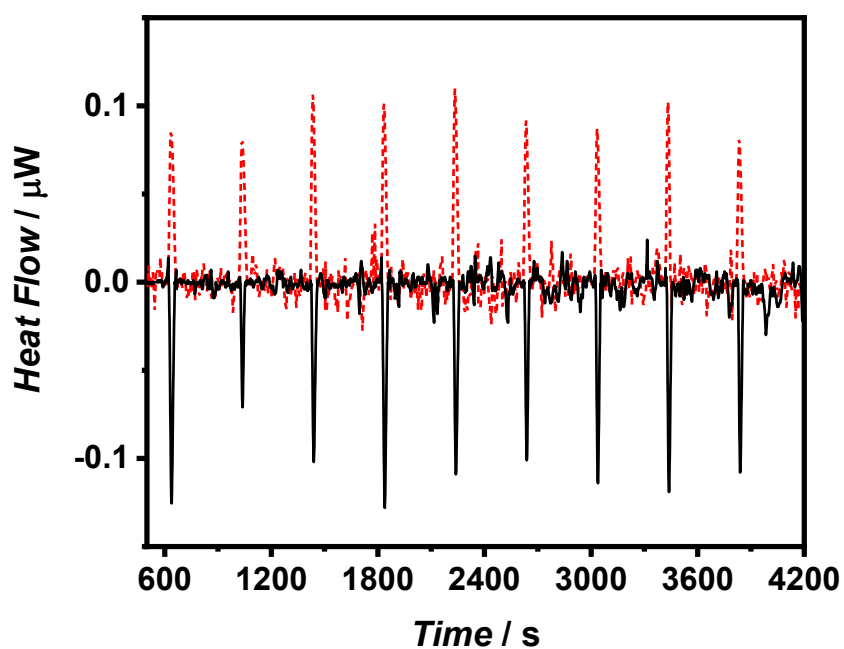
Figure S7. ITC trace obtained from a titration of 20 µM Tel-23 in Na⁺ buffer (black curve) with 200 µM LL-III solution. The ITC trace obtained for LL-III dilution into buffer is reported as a red dashed line. The experiments were performed at 20 °C.

Figure S8. ITC trace obtained from a titration of 10 µM cMyc in K$^+$ buffer (black curve) with 100 µM LL-III solution. The ITC trace obtained for LL-III dilution into buffer is reported as a red dashed line. The experiments were performed at 20 °C.
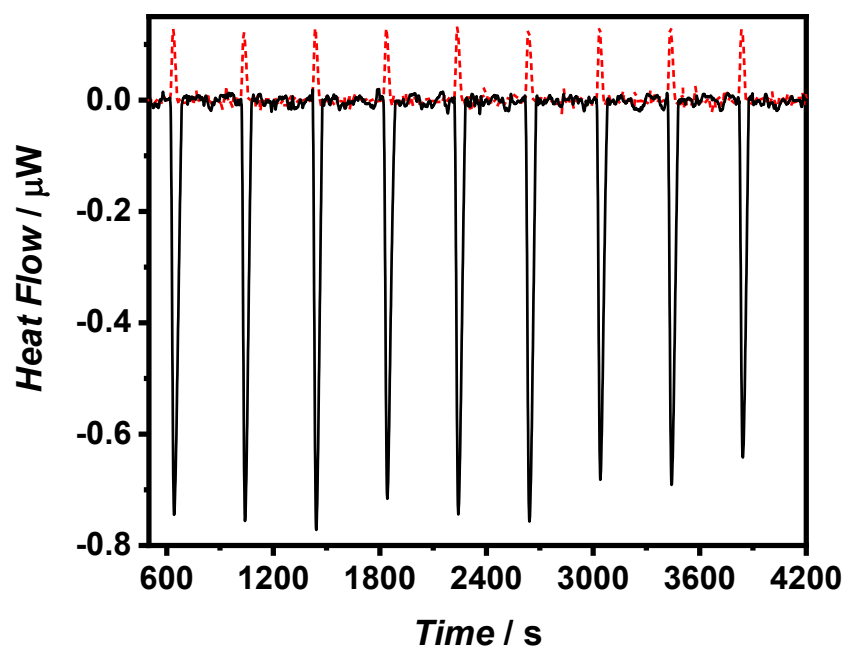

Figure S9. ITC trace obtained from a titration of 20 µM cKIT1 in K$^+$ buffer (black curve) with 200 µM LL-III solution. The ITC trace obtained for LL-III dilution into buffer is reported as a red dashed line. The experiments were performed at 20 °C.

	K_{D} / nM	f_{H}	ΔH_b^0 / kJ mol^{-1}	ΔG_b^0 / kJ mol^{-1}	$-T\Delta S_b^0$ / kJ mol^{-1}	K_{SV} / M^{-1}
LL-III	\	0.15	\	\	\	17.8 ± 0.3
+ Tel-23 K$^+$	90 ± 10	0.17	5 ± 1	-39.5 ± 0.3	-44 ± 1	13.7 ± 0.5
+ Tel-23 Na$^+$	900 ± 90	0.17	2.3 ± 0.4	-33.9 ± 0.4	-36.2 ± 0.8	11.5 ± 0.6
+ cMyc	60 ± 10	0.26	5.3 ± 0.7	-40.5 ± 0.4	-46.4 ± 0.8	8.2 ± 0.3
+ cKIT1	230 ± 23	0.49	15 ± 1	-37.2 ± 0.4	-52 ± 1	9.9 ± 0.4

Table S1. Summary of the parameters determined for the G-quadruplexes investigated in this study: dissociation constants (K_{D}), LL-III helical fraction (f_{H}), standard enthalpies of binding (ΔH_b^0), standard Gibbs free energies of binding (ΔG_b^0), the standard entropic terms of binding ($-T\Delta S_b^0$) and the Stern-Volmer constants (K_{SV}). The thermodynamic parameters are reported at 20 °C.

Peptide	Sequence	Length	Charge	K_{D} / nM	Ref.
LL-III	VNWKKILGKIIKVVK-NH$_2$	15	+6	60 (cMyc)	This work
DM039	PGHLKGREIGLWYAKKQGQKNK	22	+5	112 (cMyc)	[2]
LL-37	LLGDFFRKSKEKIGKEFKRIVQ RIKDFLRNLVPRTES	37	+6	40000 (cMyc)	[5]
Cyclic RHAU23	**G**LHPGHLKGREIGMWYAKKQ GQKNK**N**	26	+5	129 (parallel G4 T95-2T)	[6]
CIRBP peptide 12	RGGSAGGRGFFRGGRGRGRGF SRGG	25	+7	5200 (cMyc)	[7]

Table S2. Examples of G4-binding peptides reported in the literature. The charges are those calculated assuming a pH of 7.

References

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