Supplementary Information

Selective binding of c-MYC G-quadruplex caged in a dsDNA by a hemopeptide

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Material and Methods.

Tris(hydroxymethyl)aminomethane (tris), 2-(N-morpholino)ethanesulfonic acid (MES), cacodylic acid, lithium hydroxide, potassium chloride, sodium chloride, microperoxidase-11, hemin, 30% hydrogen peroxide solution and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{2-}$) were purchased from Sigma-Aldrich. Ultrapure water from NANOpure Diamond (Barnstead) source was used in all the experiments.

Microperoxidase-11 (MP-11) was dissolved in an aqueous solution to form a stock solution. The concentration of MP-11 stock solution was determined using the pyridine hemochromagen assay.[1] Acetylated MP-11 (AcMP-11) was prepared according to a published procedure and mass spectrometry was used to verify the mass of the resulting metallopeptide.[2] AcMP-11 was dissolved in water and the concentration was determined spectroscopically using the UV-Vis absorbance intensity at 397 nm (ε_{397} = 147167 cm^{-1} M^{-1}).[3] Hemin stock solutions were prepared by dissolving hemin in DMSO and the concentration was determined using the pyridine hemochromagen assay. All oligonucleotides were purchased from Integrated DNA Technologies and were dissolved in ultrapure water with a final concentration of 500 μM.

The list of oligonucleotides used in this study includes the flowing list:

Oligomer	Topology	Sequence[a]
Htel	Antiparallel	AGGGTTAGGGTTAGGGTTAGGG
532	Hybrid	GGGTTTTTGGGTTGGGTTTGGG
c-KIT	Parallel	CGGGCGGGCGCGAGGGAGGGG
c-MYC	Parallel	AGGGTGGGTAGGGTGGG
KRAS	Parallel	AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG
dsDNA		GACGTGTGGACTGTGA
$dsDNA_{comp}$		TCACAGTCCACACGTC
ssDNA:Htel		GCGGTTCTGAACTCGATAT AGGGTTAGGGTTAGGGTTAGGG ATTAGTGCTAGCTACGCG
$ssDNA:Htel_{comp}$		CGCGTAGCTAGCACTAAT TCCCTAACCCTAACCCTAACCC ATATCGAGTTCAGAACCGC
ssDNA:532		GCGGTTCTGAACTCGATAT GGGTTTTTGGGTTGGGTTTGGG ATTAGTGCTAGCTACGCG
$ssDNA532_{comp}$		CGCGTAGCTAGCACTAAT CCCAAACCCAACCCAAAAACCC ATATCGAGTTCAGAACCGC
ssDNA:c-KIT		GCGGTTCTGAACTCGATAT CGGGCGGGCGCGAGGGAGGGG ATTAGTGCTAGCTACGCG
$ssDNA:c\text{-}KIT_{comp}$		CGCGTAGCTAGCACTAAT CCCCTCCCTCGCGCCCGCCCG ATATCGAGTTCAGAACCGC
ssDNA:KRAS		GCGGTTCTGAACTCGATAT AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG ATTAGTGCTAGCTACGCG
$ssDNA:KRAS_{comp}$		CGCGTAGCTAGCACTAAT CCTCCCCCTCTTCCCTCTTCCCACACCGCCC TATATCGAGTTCAGAACCGC

ssDNA:c-MYC		GCGGTTCTGAACTCGATAT TGAGGGTGGGGAGGGTGGGGAA ATTAGTGCTAGCTACGCG
ssDNA:c-MYC$_{comp}$		CGCGTAGCTAGCACTAAT TTCCCCACCCTCCCCACCCTCA ATATCGAGTTCAGAACCGC
ssDNA:^{G17A}c-MYC		GCGGTTCTGAACTCGATAT TGAGGGTGGGGAG**A**GTGGGGAA ATTAGTGCTAGCTACGCG
ssDNA:^{G17A}c-MYC$_{comp}$		CGCGTAGCTAGCACTAAT TTCCCCAC**T**CTCCCCACCCTCA ATATCGAGTTCAGAACCGC
ssDNA:^{G17A}c-MYC +5		GCGGTTCTGTTCTGAACTCGATA TTGAGGGTGGGGAGGGTGGGGAA ATTAGTGCTAGCTACGCG
ssDNA:c-MYC$_{comp}$ +5		CGCGTAGCTAGCACTAAT TTCCCCACCCTCCCCACCCTCA ATATCGAGTTCAGAACAGAACCGC
ssDNA:^{G17A}c-MYC +10		GCGGTTCTGTTCTGAACTCGATAT TGAGGGTGGGGAGGGTGGGGAA ATTAGTGCTAGCTATGTGACGCG
ssDNA:c-MYC$_{comp}$ +10		CGCGTCACATAGCTAGCACTAAT TTCCCCACCCTCCCCACCCTCA ATATCGAGTTCAGAACAGAACCGC
ssDNA:^{G17A}c-MYC +20		GCGGTTCTGTGGACTTCTGAACTCGATAT TGAGGGTGGGGAGGGTGGGGAA ATTAGTGCTAGCTATGTGAGCAGTCGCG
ssDNA:c-MYC$_{comp}$ +20		CGCGACTGCTCACATAGCTAGCACTAAT TTCCCCACCCTCCCCACCCTCA ATATCGAGTTCAGAAGTCCACAGAACCGC
dsDNA59		GCGGTTCTGAACTCGATATGCAGTGTGGACTGTGACTTCTGATTAGTGCTA GCTACGCG
dsDNA59$_{comp}$		CGCGTAGCTAGCACTAATCAGAAGTCACAGTCCACACTGCATATCGAGTTC AGAACCGC

[a]Mutated nucleotides at position 17 in the c-MYC GQ sequence of the ssDNA:^{G17A}c-MYC and ssDNA:^{G17A}c-MYC$_{comp}$ are in bold. Added nucleotides to the ssDNA:c-MYC and ssDNA:c-MYC$_{comp}$ sequences are underlined.

Sample preparation for UV-Vis measurements.

Titrations of either MP-11 or AcMP-11 with oligonucleotides. A buffer solution containing either 10 mM tris buffer, pH = 7.5, or MES buffer, pH = 5.5 containing 50 mM KCl and 2 mM $MgCl_2$ was added with the appropriate metallopeptide with a final concentration of 5 µM. Additionally, stock solutions of the various DNA sequences at the same buffer solutions were prepared with a concentration of 100 µM. The solutions were heated to 95 ^{o}C for 5 minutes and then allowed to slowly cool down to room temperature. Next, either MP-11 or AcMP-11 were titrated with the DNA stock solution with increments of 0.5 µM at each addition, with a 2-minute incubation period.

Peroxidase inhibition assays. A buffer solution of 10 mM tris, pH = 7.5 containing 50 mM KCl and variable concentrations of c-MYC was first heated to 95 ^{o}C for 5 minutes and then allowed to slowly cool down to room temperature. Subsequently, the solution was added with 0.1 µM MP-11 and incubated for 30 minutes. Upon addition of $ABTS^{2-}$ and H_2O_2 with final concentrations of 500 µM and 300 µM, respectively, the absorbance at 414 nm was recorded in a time-dependent manner to monitor the MP-11-catalyzed oxidation of the $ABTS^{2-}$ substrate until saturation of the signal was reached.

Sample preparation for Circular dichroism spectroscopy measurements.

Melting curve measurement. Sample solutions containing buffer solutions of 10 mM Lithium cacodylate, pH = 7.4 containing 10 mM KCl and 5 μM of GQ oligonucleotides were prepared. The samples were heated to 95 ^{o}C for 5 minutes and then allowed to slowly cool down to room temperature in order to obtain the properly folded and thermodynamically stable GQ structures. Next, MP-11 was added to the solution with a final concentration of 5 μM and incubated for 30 minutes. Samples were heated at a 5 ^{o}C / min rate and allowed to equilibrate for 2 minutes at each temperature prior to the measurement.

MP-11 binding to DNA sequences. Sample solutions containing buffer solutions of 10 mM tris buffer, pH = 7.5 containing 50 mM KCl and 5 μM of GQ oligonucleotides were prepared. Alternatively, in the case of c-MYC folding in the presence of Na^+ ions, a sample was also prepared in a 10 mM tris buffer solution, pH = 7.5 containing 100 mM NaCl. In the case of dsDNA, the final concentration of the double-stranded oligonucleotide was 5 μM. The samples were, then, heated to 95 ^{o}C for 5 minutes and then allowed to slowly cool down to room temperature. Next, MP-11 was added to the solution with a final concentration of 5 μM and incubated for 30 minutes prior to measurement.

Data acquisition and analysis.

UV-Vis spectroscopy. The UV-Vis absorbance spectra of the complexes were measured using the Evolution ONE Plus UV-VIS spectrophotometer (Thermo Fisher) where the sample was placed in a quartz cuvette with a 1 cm path length. All measurements were performed at 25 ^{o}C.

For monitoring the peroxidase activity, the absorption at 414 nm was measured every 10 s to ensure accuracy in the calculation of the initial rate, R0. R0 was calculated by deriving the slope of the initial reaction and employing the extinction coefficient of $ABTS^{2-}$ at 414 nm (ε_{414} = 31,100 cm^{-1} M^{-1}).[4]

The dissociation constants, K_d, were determined at pH = 5.5 where a clear transition was observed from non-specific MP-11-bound GQs to specific stacking of the hemin moiety on the external G-tetrad of the GQs. The spectroscopic data was fitted to the following equation [51]:

$$[DNA_0] = K_d(A–A_0)/(A_\infty–A) + [P_0](A– A_0)/(A_\infty– A_0).$$

A clear bimolecular conversion of the MP-11 into an MP-11/GQ complex was observed only at pH = 5.5 and certain sequences. Since the spectral changes included an initial drop in optical density prior to an increase, we defined A_0 as the absorbance at 403 nm at the minimal intensity; A_∞ corresponds to MP-11 absorbances of the Soret band at 403 nm at DNA concentrations where MP-11-binding is saturated; DNA_0 corresponds to the concentration of oligonucleotide; and P_0 corresponds to the fixed concentration of MP-11.

CD spectroscopy. The CD signal of the oligonucleotides was recorded using a Chirascan CD spectrometer (Applied Photophysics) and the sample was placed in a quartz cuvette with 1 cm path length. All measurements were performed at 25 ^{o}C unless stated otherwise. In melting temperature experiments we heated the samples from 20 ^{o}C to 95 ^{o}C in 5 ^{o}C increments and allowed the system to equilibrate at each point before measuring.

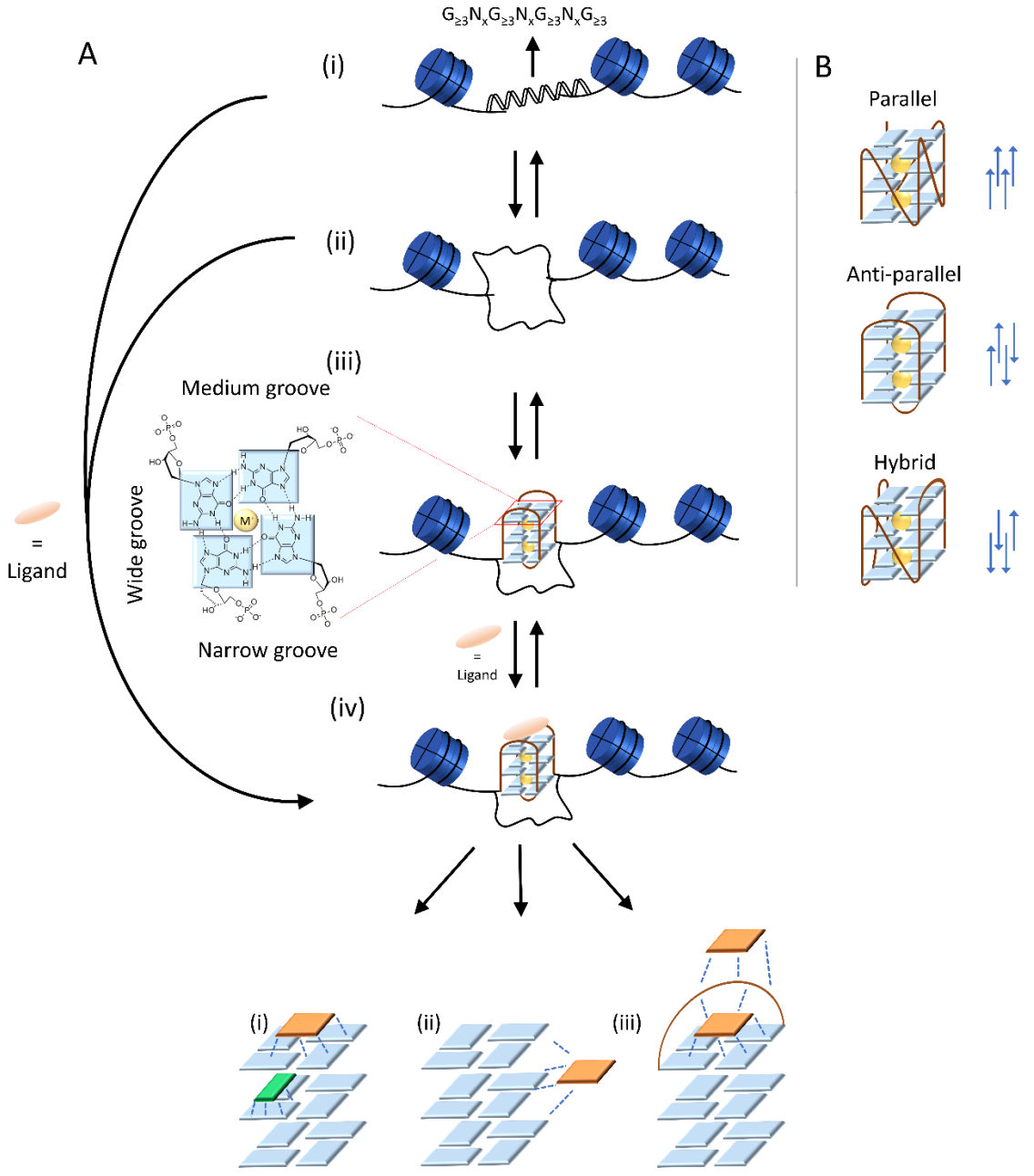

Fig. S1 (A) Dynamic equilibria of G-quadruplex-containing DNA sequences in the presence of a binding ligand. An Interconversion between the different secondary structures of the DNA a presented, with various binding modes of ligands towards the GQ: (i) π-stacking either via the external tetrad or intercalation between the G-quartets. (ii) Interactions with the grooves of the G-quadruplex, and (iii) interactions with flanking loops and nucleotides. (B) The different topologies of G-quadruplex.

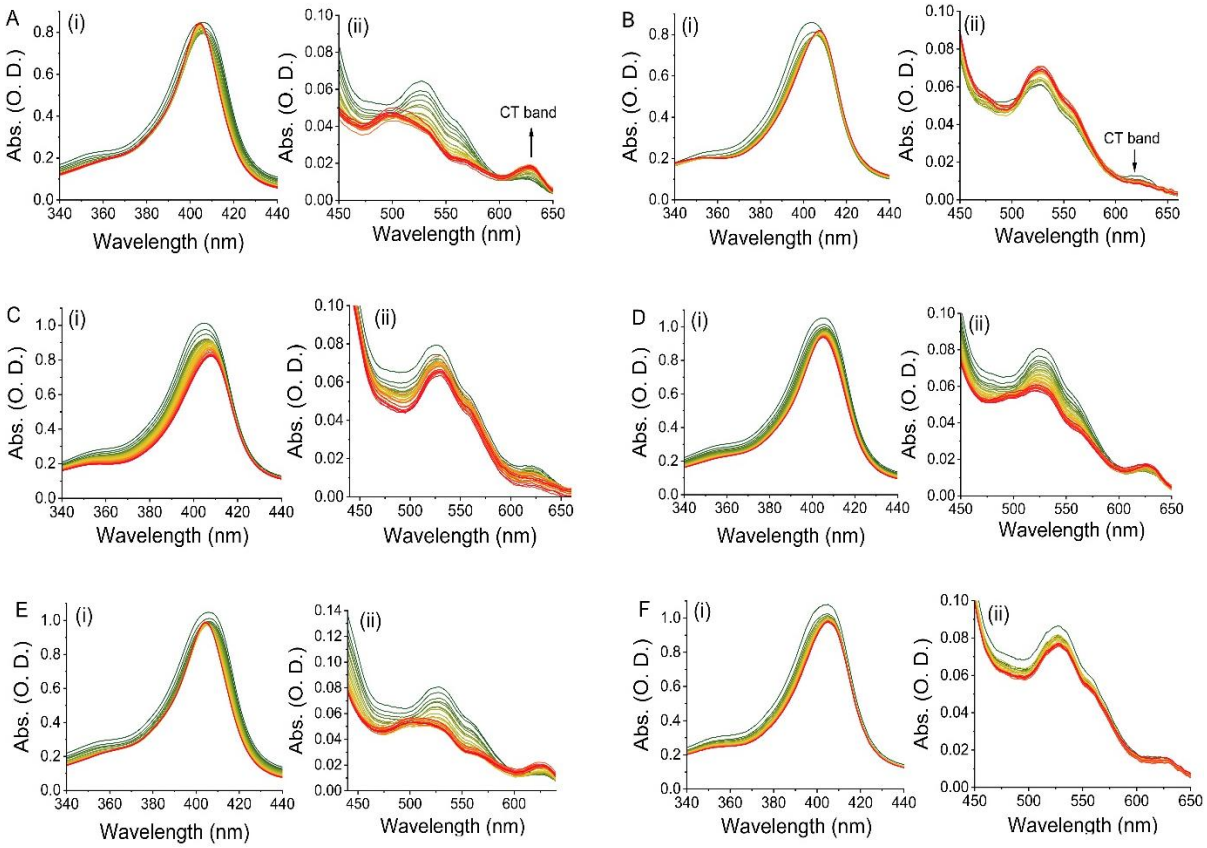

Fig. S2. Titration curves corresponding to the UV-Vis absorbance spectra of MP-11 upon interaction with various DNA sequences with both (i) the Soret band and (ii) Q bands: (A) c-MYC, (B) dsDNA, (C) Htel, (D) c-KIT, (E) 532 and (F) KRAS. All measurements were performed at 10 mM tris buffer solution, pH = 7.5 containing 50 mM KCl at 25 ^{o}C.

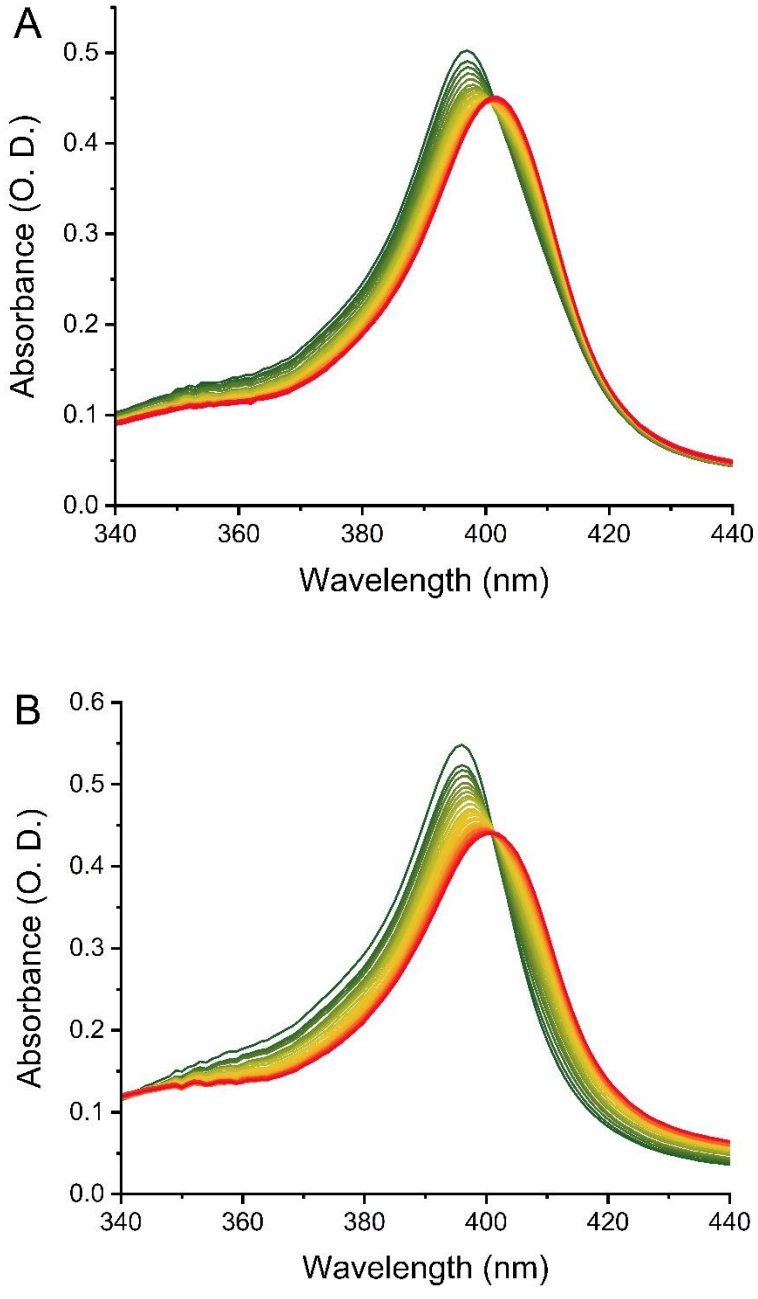

Fig. S3. AcMP-11 Titration curves corresponding to the UV-Vis absorbance spectra of acetylated MP-11 upon interaction with either: (A) c-MYC or (B) dsDNA sequence. All measurements were performed at 10 mM tris buffer solution, pH = 7.5 containing 50 mM KCl at 25 ^{o}C.

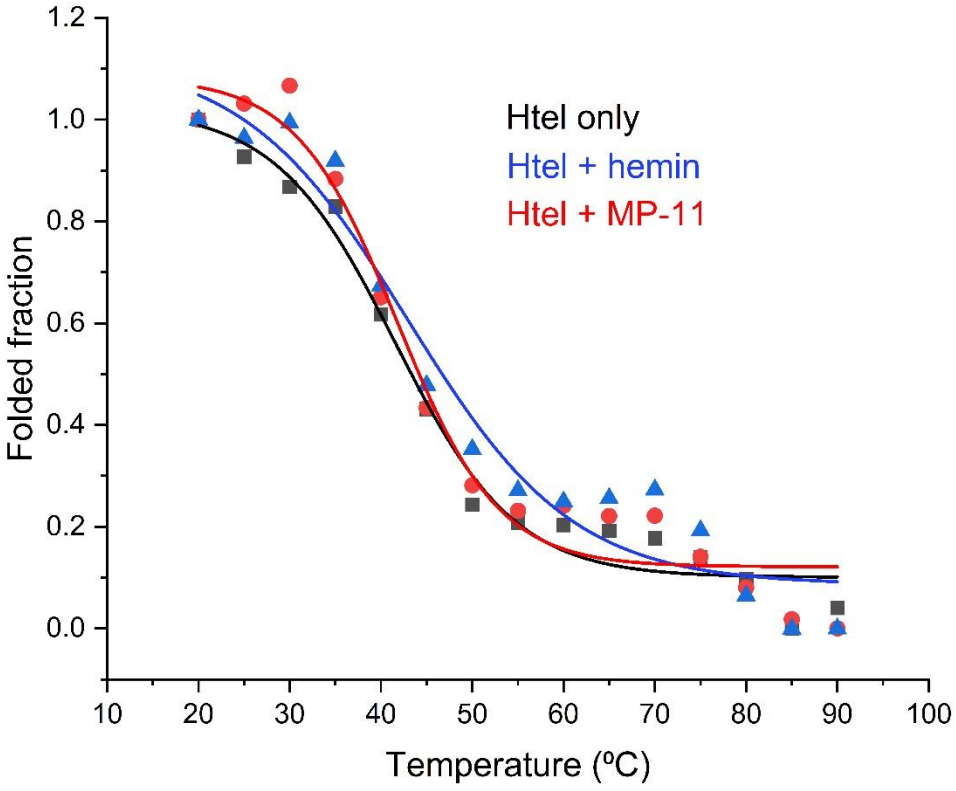

Fig. S4. Normalized CD melting curves corresponding to the Influence of hemin or MP-11 on the stability of telomeric G-quadruplex oligonucleotides. All measurements were performed at 10 mM Li cacodylate buffer solution, pH = 7.5 containing 1 mM KCl, at 25 ^{o}C.

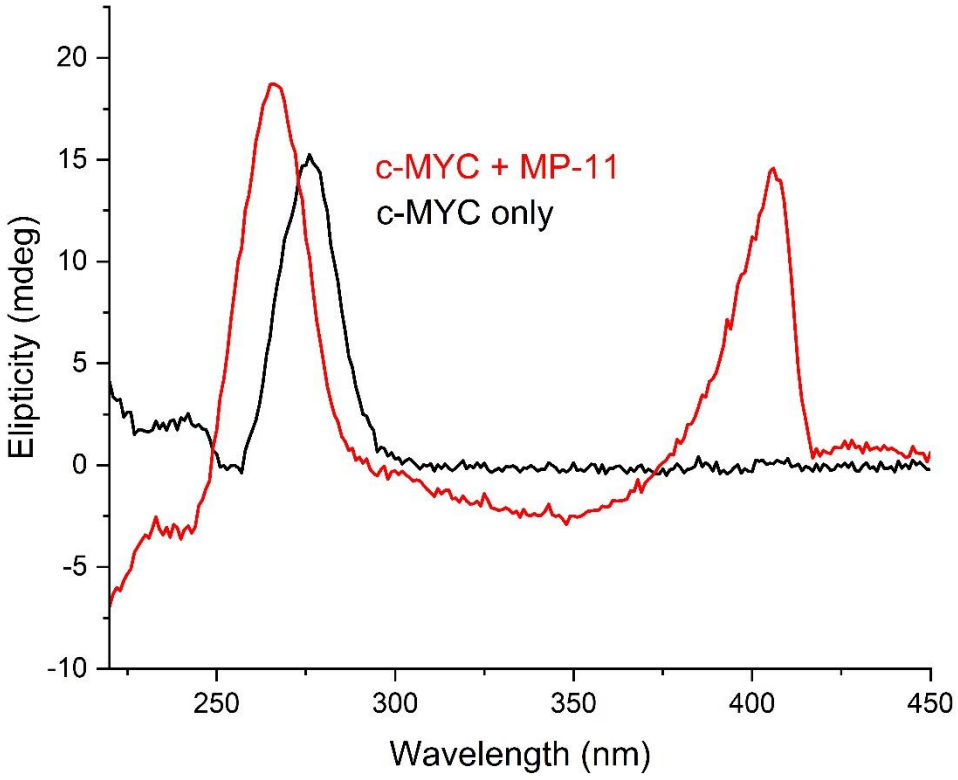

Fig. S5. CD spectra of Na^+-induced GQ structure corresponding to the c-MYC oligonucleotide folded either into an antiparallel topology (in the absence of MP-11, black curve) or into parallel topology (upon addition of MP-11, red curve). All measurements were performed at 10 mM tris buffer solution, pH = 7.5 containing 100 mM NaCl at 25 ^{o}C.

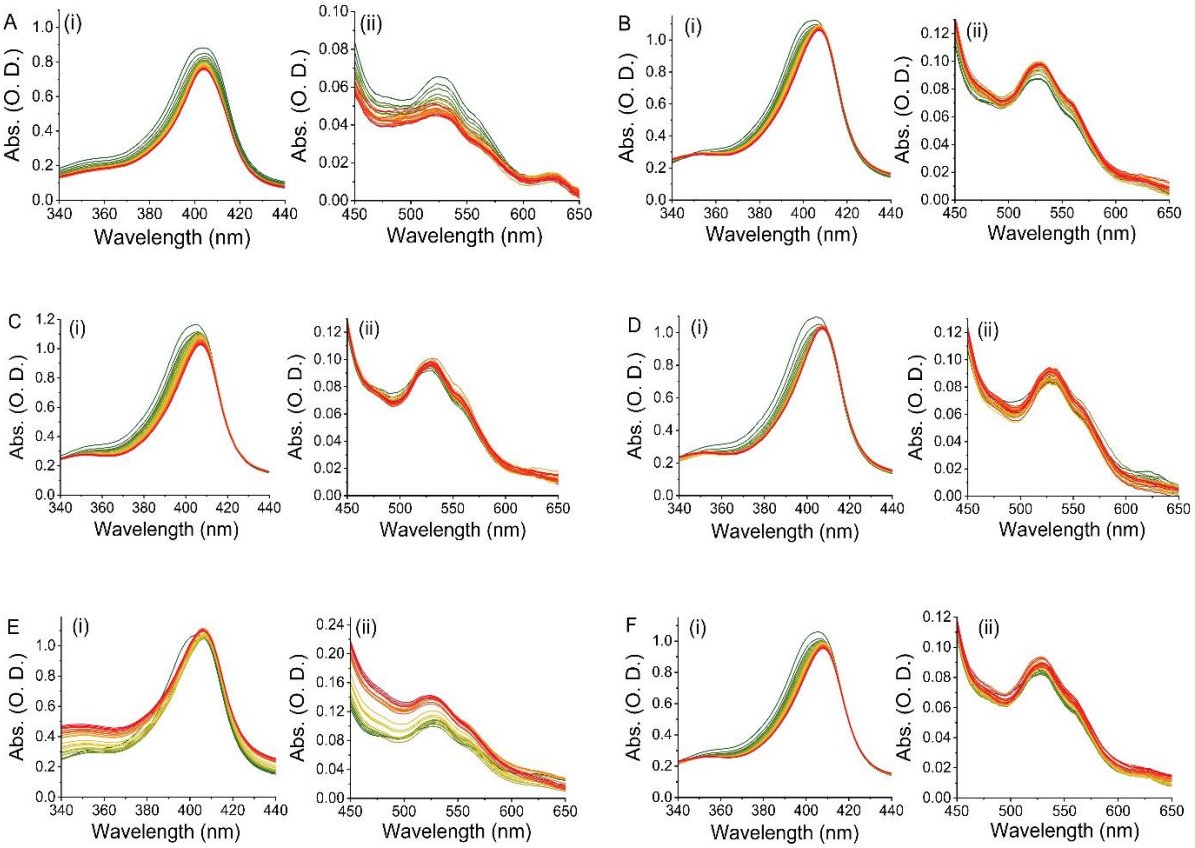

Fig. S6. Titration curves corresponding to the UV-Vis absorbance spectra of MP-11 upon interaction with various dsDNA:GQ sequences displaying both (i) the Soret band and (ii) Q bands. The GQ embedded GQ sequences correspond to (A) c-MYC, (B) dsDNA59, (C) Htel, (D) c-KIT, (E) 532 and (F) KRAS. All measurements were performed at 10 mM tris buffer solution, pH = 7.5 containing 50 mM KCl and 2 mM $MgCl_2$ at 25 ^{o}C.

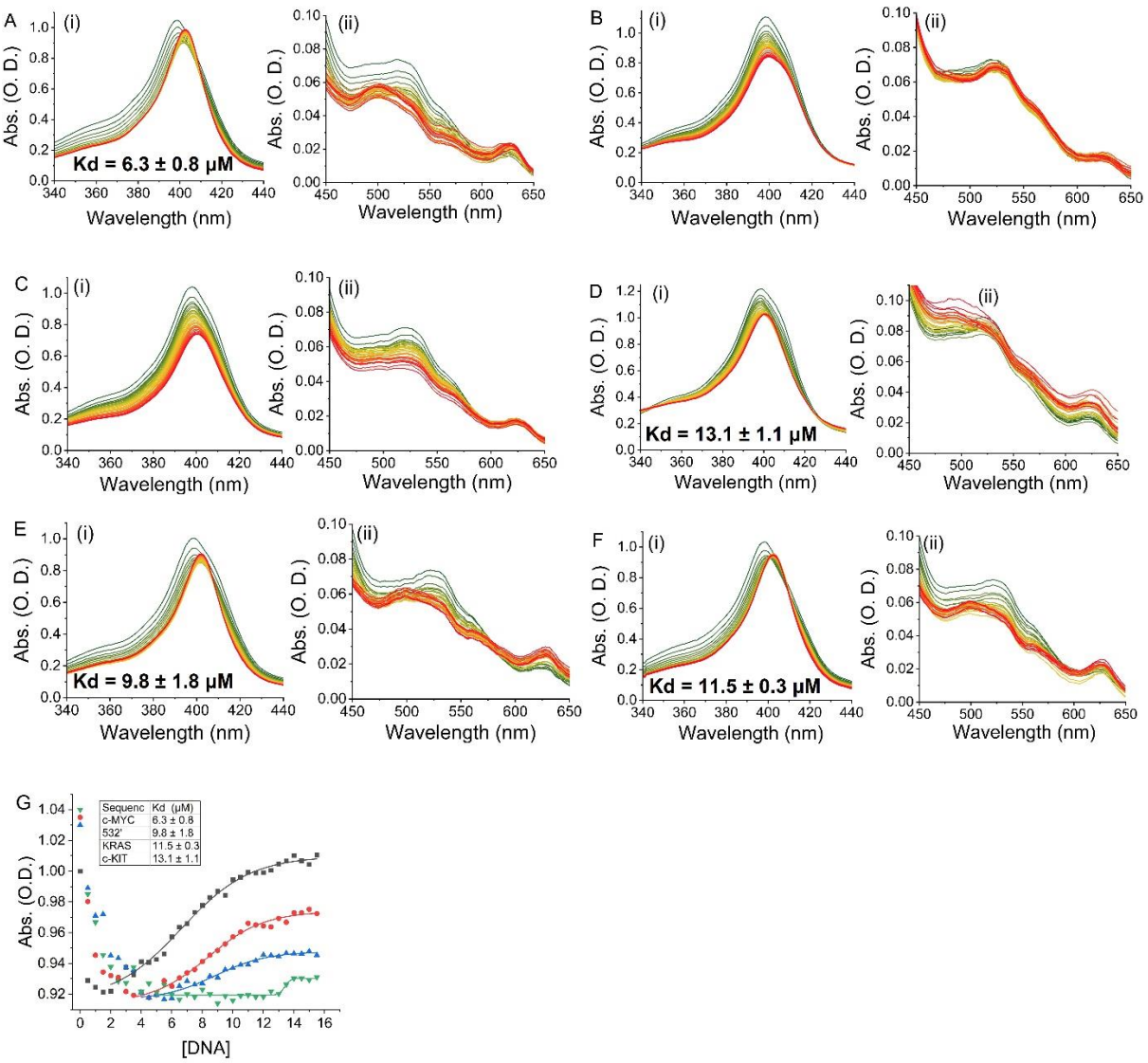

Fig. S7. Titration curves corresponding to the UV-Vis absorbance spectra of MP-11 upon interaction with different GQ sequences with both (i) the Soret band and (ii) Q bands: (A) c-MYC, (B) dsDNA, (C) Htel, (D) c-KIT, (E) 532 and (F) KRAS. All measurements were performed at 10 mM MES buffer solution, pH = 5.5 containing 50 mM KCl at 25 ^{o}C. The apparent dissociation constants for the MP-11/GQ complex formation are listed in Figures A, D, E, and F where the GQ sequences revealed clear isosbestic point implying a specific binding mode with the ligand. G. Titration curves of MP-11 at pH = 5.5 in the presence of various GQ sequences corresponding to the absorbance changes at 403 nm as a function of an increasing concentration of MP-11: c-MYC GQ (black curve), 532 (red curve), KRAS (blue curve) and c-KIT (green curve). Inset: the apparent dissociation constants for the MP-11/GQ complex formation.

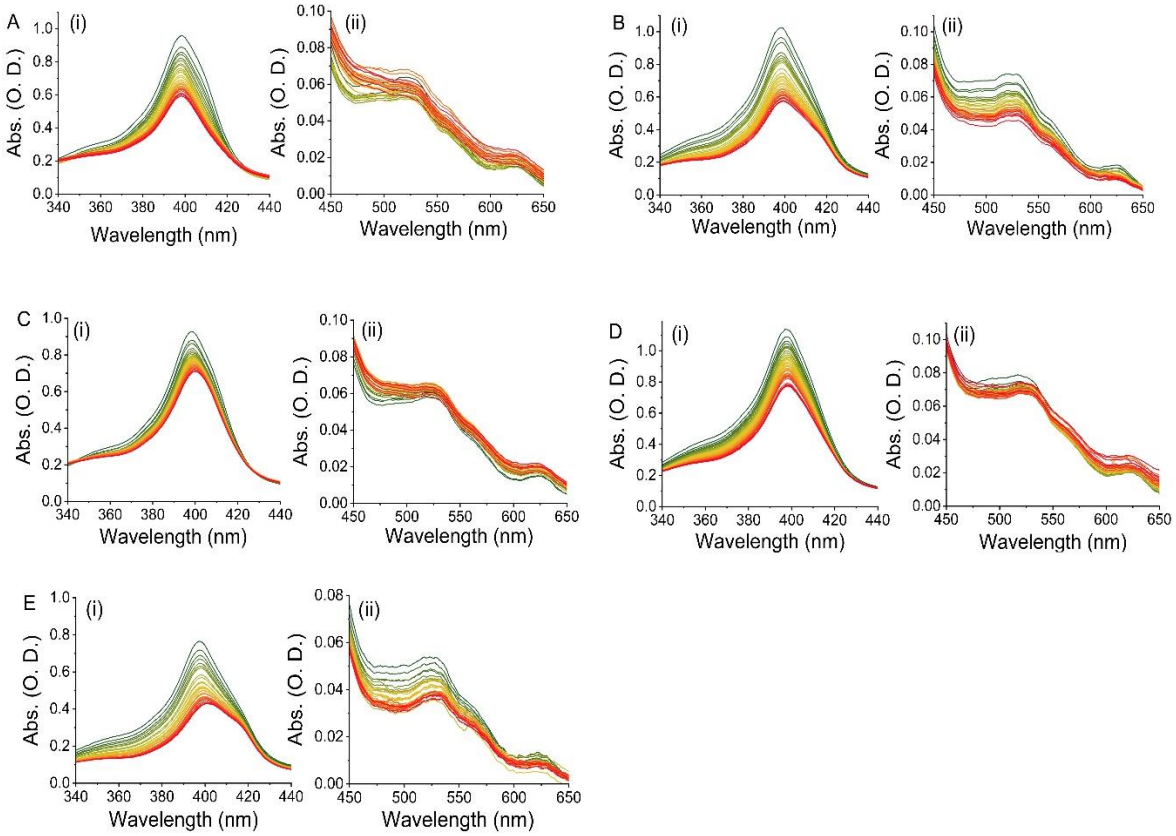

Fig. S8. Titration curves corresponding to the UV-Vis absorbance spectra of MP-11 upon interaction with different dsDNA:GQ sequences with both (i) the Soret band and (ii) Q bands: (A) dsDNA59, (B) c-KIT, (C) KRAS, (D) 532 and (e) htel oligonucleotide sequences. All measurements were performed at 10 mM MES buffer solution, pH = 5.5 containing 50 mM KCl and 2 mM $MgCl_2$ at 25 ^{o}C.

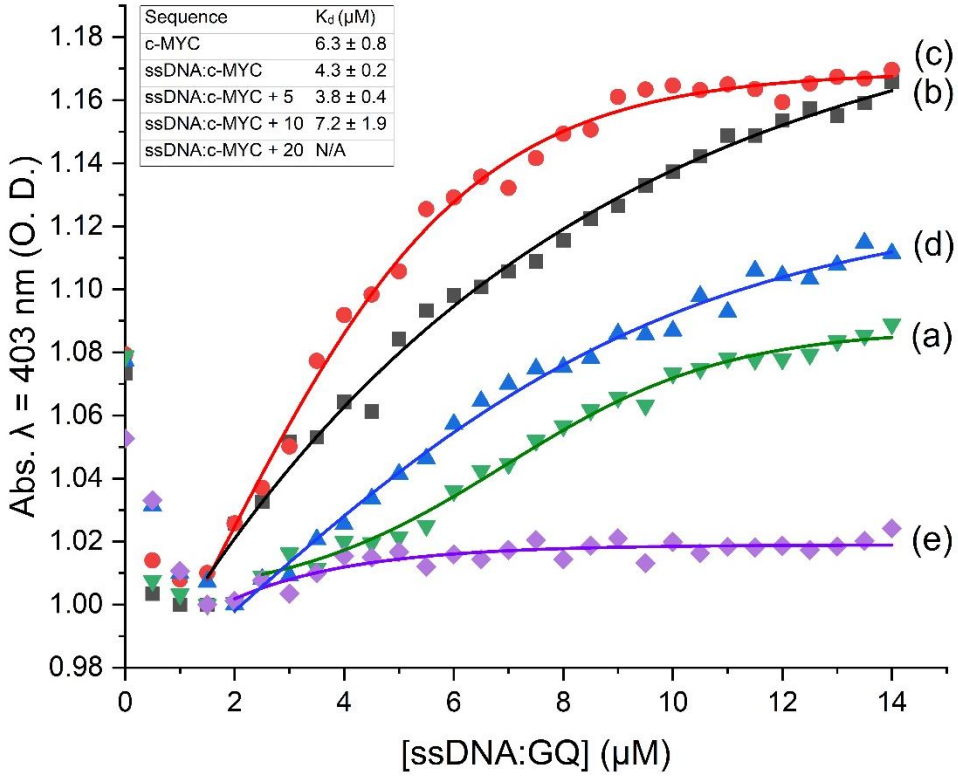

Fig. S9. Titration curves of MP-11 at pH = 5.5 in the presence of ssDNA:c-MYC corresponding to the absorbance changes at 403 nm as a function of an increasing number of flanking nucleotides: (a) c-MYC GQ, (b) ssDNA:c-MYC, (c) ssDNA:c-MYC+5, (d) ssDNA:c-MYC+10 and (e) ssDNA:c-MYC+20. Inset: the apparent dissociation constants for the MP-11/ssDNA:GQ complex formation.

Supplementary References

1. I. Barr and F. Guo, *Bio Protoc.*, 2015, **5**, e1594.
2. H. M. Marques and C. B. Perry, *J. Inorg. Biochem.*, 1999, **75**, 281-291.
3. N. J. O'Reilly and E. Magner, *Phys. Chem. Chem. Phys.*, 2011, **13**, 5304-5313.
4. N. J. Miller, J. Sampson, L. P. Candeias, P. M. Bramley and C. A. Rice-Evans CA, *FEBS Lett.*, 1996, **384**, 240–242.