Supporting information

For

An RNA hairpin to G-quadruplex conformational transition

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Experimental procedures:

CD spectroscopy

Circular dichroism experiments were conducted on a Chirascan spectropolarimeter using a quartz cuvette with an optical path length of 1 mm. Samples were prepared by diluting the oligonucleotides in the appropriate buffer, by heating them at 95 °C and then equilibrating at 4 °C for at least 12 h. Scans were performed over the range of 200-320 nm at 20 °C. Each trace is the result of the average of three scans taken with a step size of 1 nm, a time per point of 1 s and a bandwidth of 1 nm. A blank sample containing only buffer was treated in the same manner and subtracted from the collected data. The data were finally zero-corrected at 320nm.

UV spectroscopy

UV melting curves were collected using a Varian Cary 400 Scan UV-visible spectrophotometer by following the absorbance at 260, 280 or 295 nm.
Samples were transferred to a 1cm path-length quartz cuvette, covered with a layer of mineral oil, placed in the spectrophotometer and equilibrated at 5 °C for 10 minutes. Samples were then heated to 95 °C and cooled to 5 °C at a rate of 1 °C/min, with data collection every 1 °C during both melting and cooling. T_m values were obtained from the minimal of the first derivative of the melting curve. Thermal differential spectra were obtained by subtracting the UV spectra collected at 25 °C from the one collected at 80 °C of the oligonucleotide solutions.

^{1}H NMR spectroscopy

NMR spectra were recorded at 298 K using a 500 MHz Bruker Avance TCI spectrometer equipped with a cryogenic TCI ATM probe. Water suppression was achieved using excitation sculpting. The oligonucleotides were annealed at 100 μM in a 10mM PBS buffer (pH 7.0) by heating at 95 °C for 10 min. $MgCl_2$ and KCl were then added directly to the warm solution. The samples were then slowly cooled to room temperature and equilibrated at 4 °C for at least 24h.

Titration experiments with an increasing concentration of cations were performed by adding increasing amount of a 1 M solution of KCl or a 1 mM solution of $MgCl_2$.

Titration experiments with the small molecule **1** were performed by adding increasing amount of a 10 mM solution of the TAP derivative.

During titration experiments several ^{1}H NMR spectra were acquired after the addition of the cations or molecule in order to check that the thermodynamic equilibrium was reached.

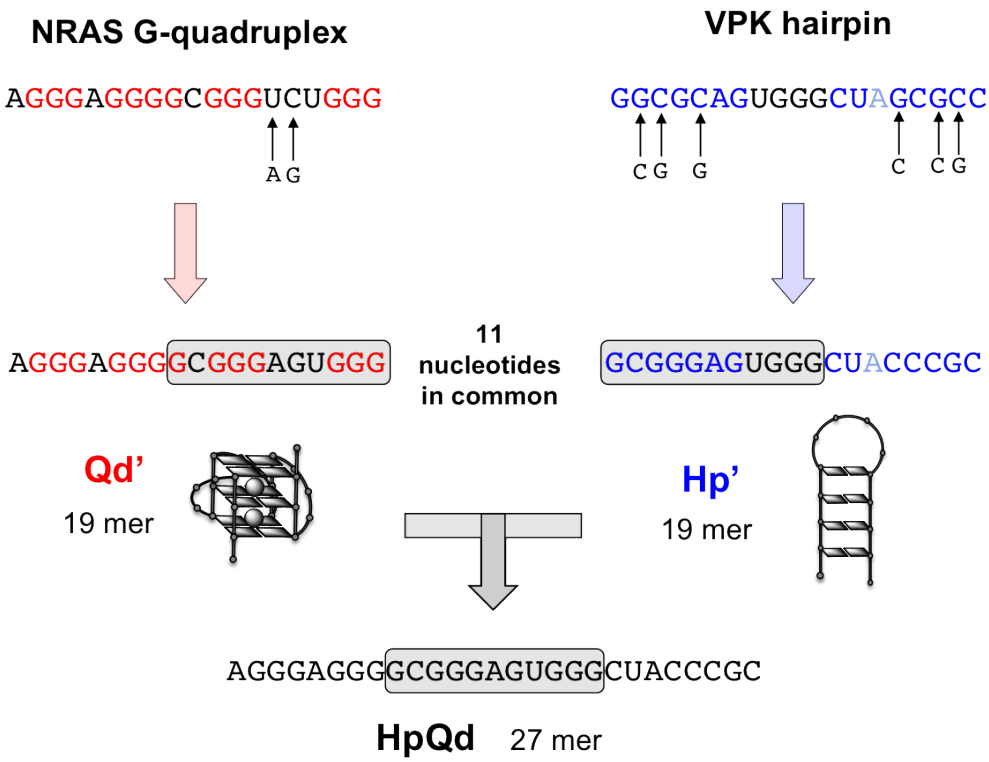


Figure S1. Rational design of the **HpQd** sequence.

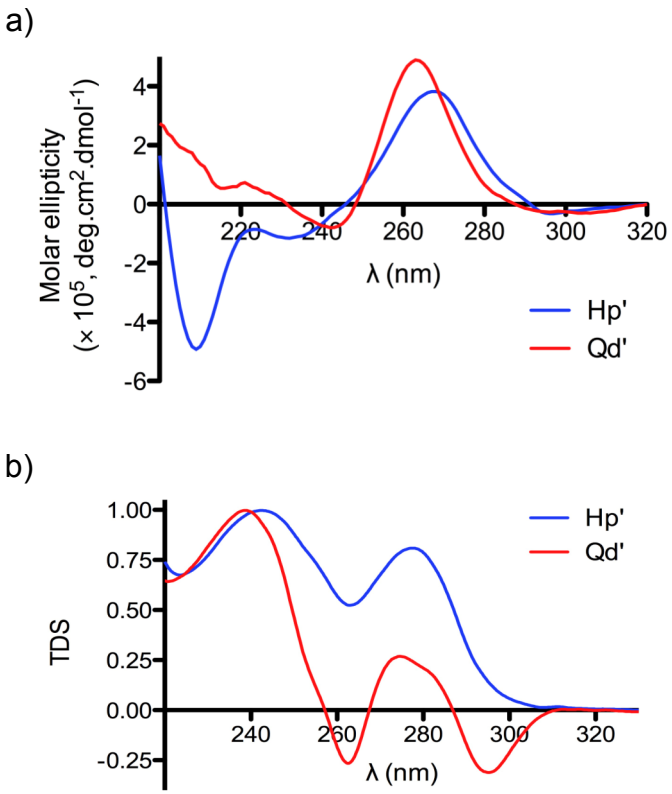


Figure S2. a) CD and b) TDS profiles of **Hp'** and **Qd**' in sodium cacodylate (pH 7.0), 10 mM KCl and 100 μM $MgCl_2$.

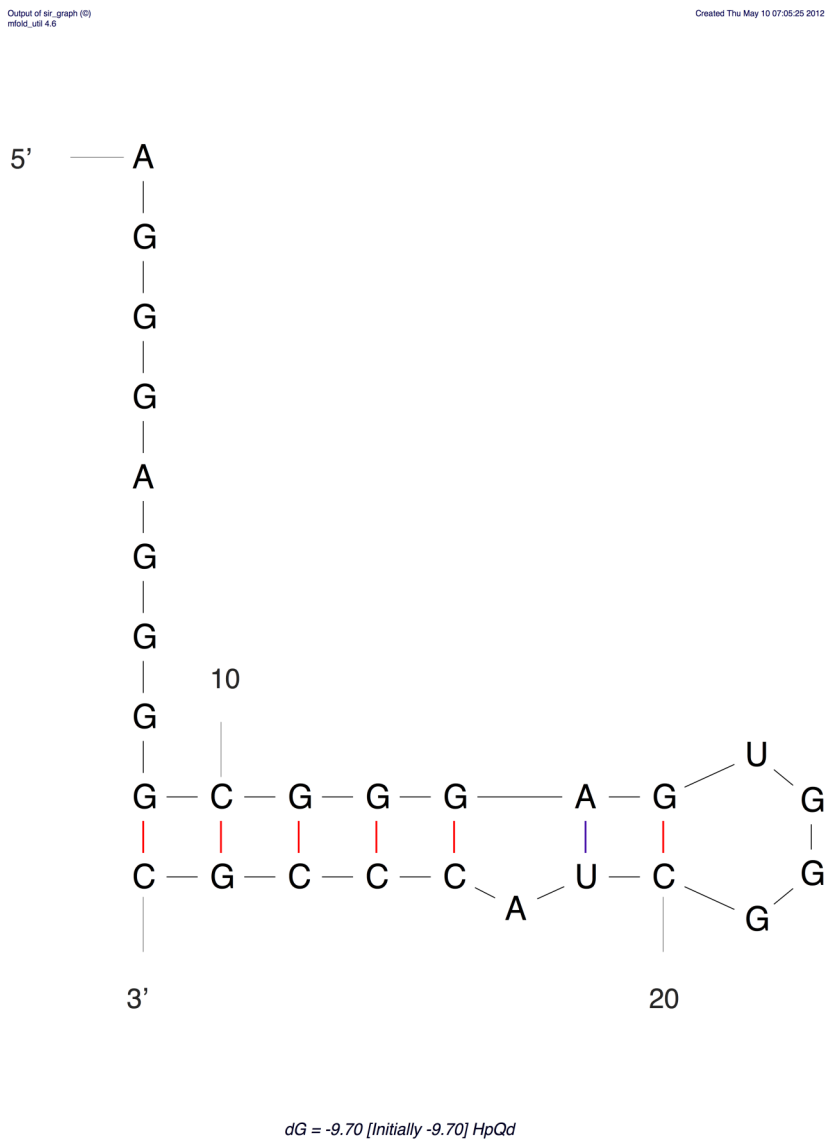


Figure S3. Most stable predicted structure of **HpQd** sequence using MFold RNA folding software (http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form).

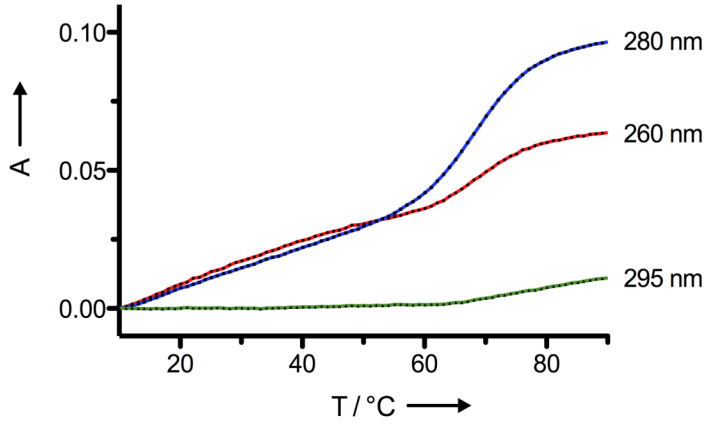


Figure S4. Thermal denaturation studies of **HpQd** in sodium cacodylate (pH 7.0), 1 mM KCl recorded at 280, 260 and 295 nm.

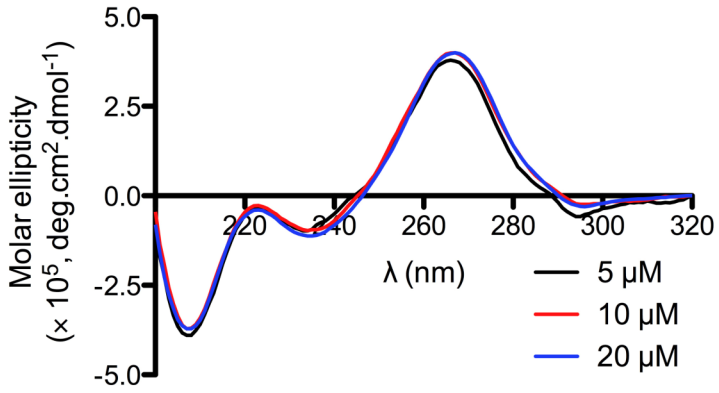
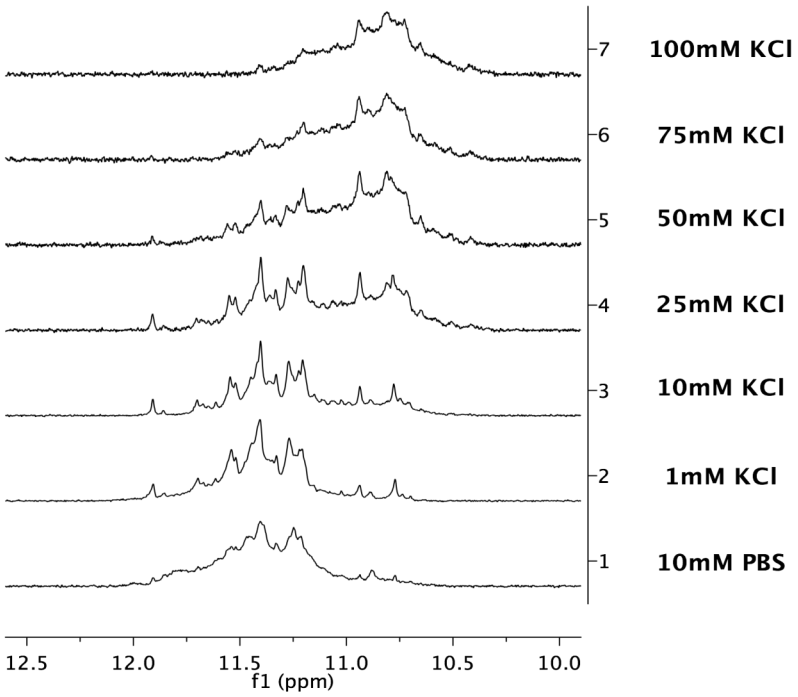


Figure S5. CD profile of **HpQd** in sodium cacodylate (pH 7.0) at different concentrations. The spectra were found to overlay indicating that the **HpQd** conformation is independent of **HpQd** concentration.

a)



b)

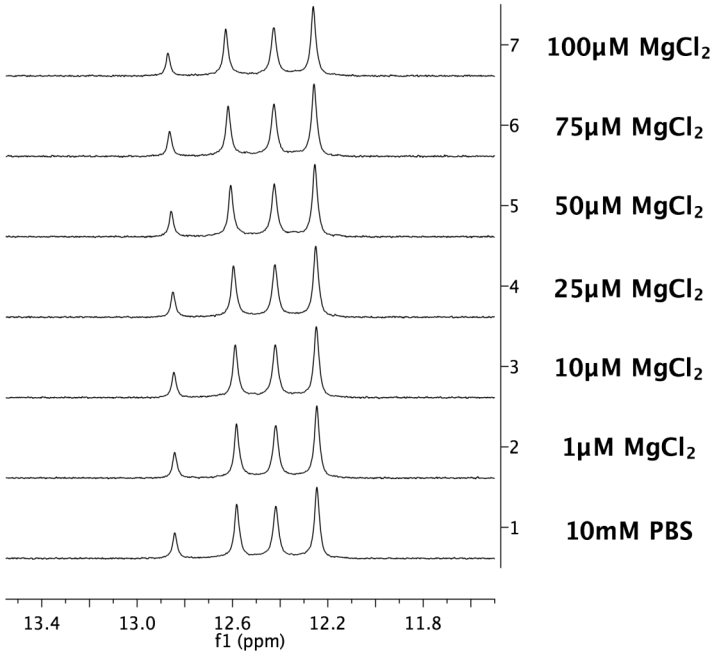


Figure S6. a) ^{1}H NMR titration of **Qd'** with an increasing amount of KCl starting from 10 mM PBS. b) ^{1}H NMR titration of **Hp'** with an increasing amount of $MgCl_2$ starting from 10 mM PBS.

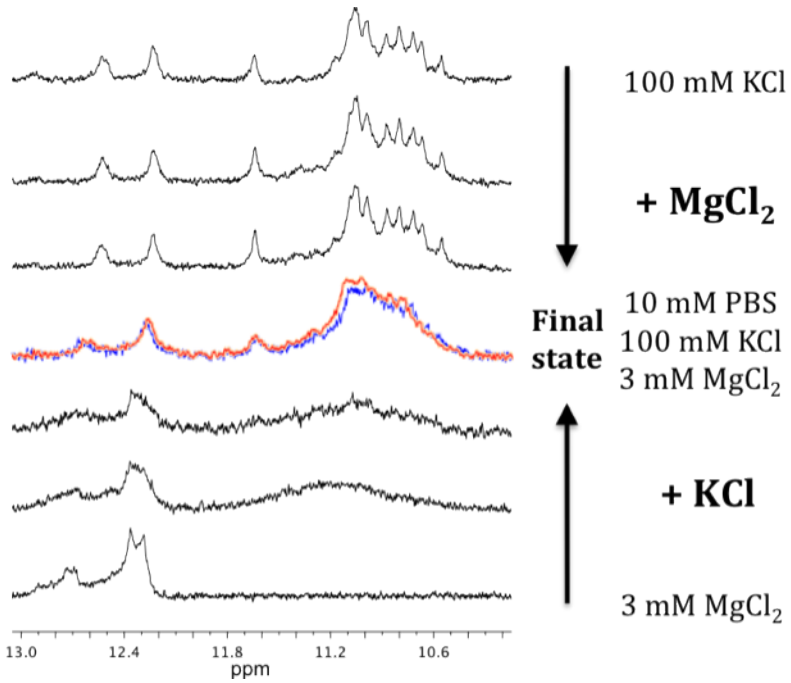
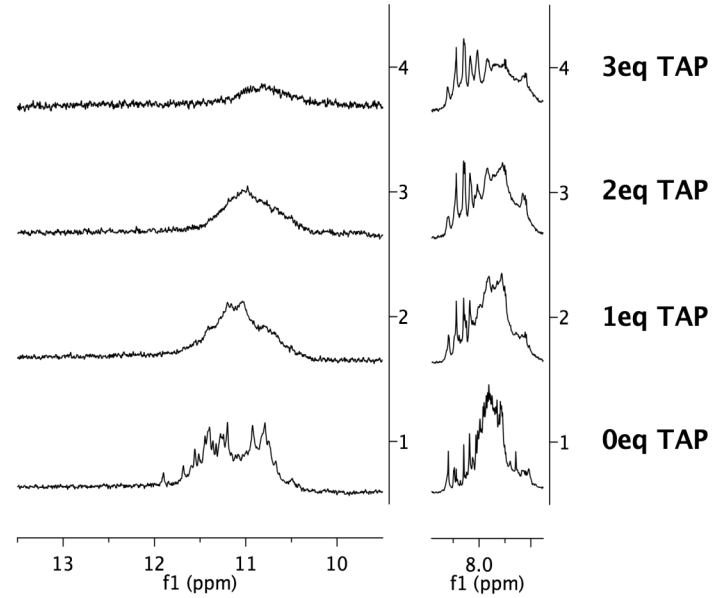


Figure S7. Summary of 1D ^{1}H NMR titration experiments of **HpQd** with an increasing amount of either KCl or $MgCl_2$ starting from two different initial states (presence of KCl or $MgCl_2$). These experiments demonstrate that **HpQd** preferentially folds into a G-quadruplex conformation at near physiological condition independently of its initial state. The blue and red spectra were obtained while starting the titration with either the presence of KCl or $MgCl_2$, respectively. These two spectra were found to overlay showing that the conformations of **HpQd** at the end of both titrations are identical.

a)



b)

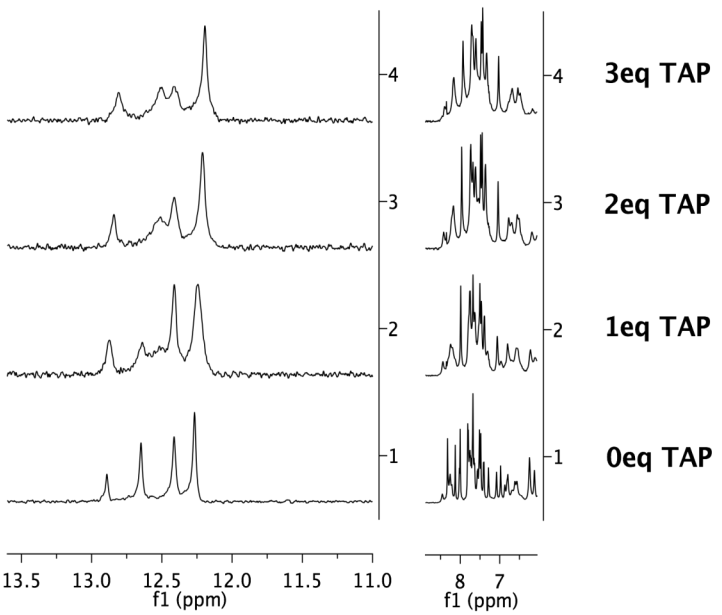


Figure S8 – ^{1}H NMR titration of a) **Qd'** and b) **Hp'** with an increasing amount of the triarylpyridine derivative **1** (TAP) in 10 mM PBS pH 7.0, 10mM KCl and 200μM $MgCl_2$.

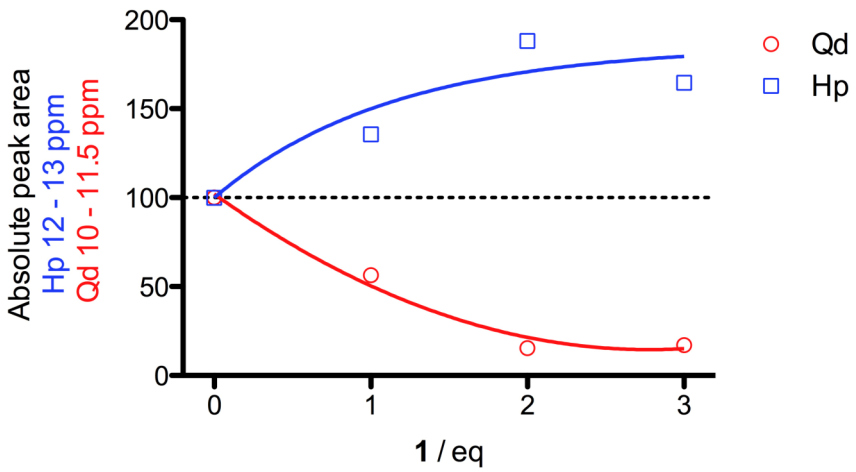
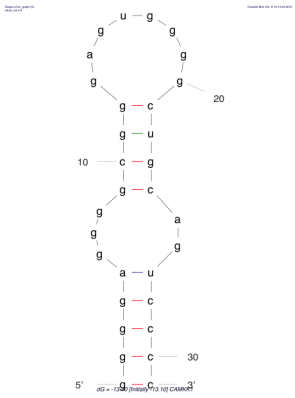


Figure S9 – Change of the absolute area of imino envelopes relative to **Hp** (12 – 13 ppm) in blue and to **Qd** (10 – 11.5 ppm) in red in the ^{1}H NMR spectra of **HpQd** while titrated by an increasing amount of **1**. The NMR spectra are depicted in **Figure 5** in the manuscript.

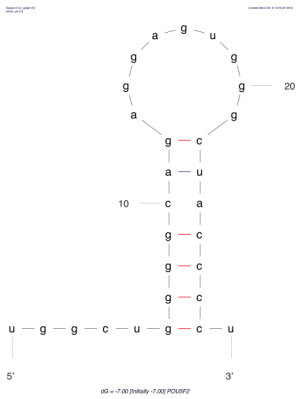
Calcium/calmodulin-dependent protein kinase 1
CAMKK1

mRNA : ggggaggggcgggauggggcugcaguccccc



POU domain class 5, transcription factor 2
POU5F2

mRNA : uggcuggggcagaggaguggggcuacccccu



Guanine nucleotide-binding protein G(olf) subunit alpha
GNAL

mRNA : cggaggggcgggaggggcugcacccgg

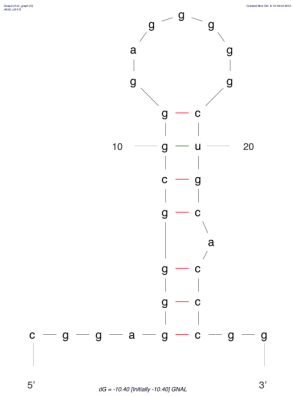


Figure S10 – The **HpQd** sequence was not found in the human transcriptome. Nevertheless G-rich sequences presenting some homology with **HpQd** were found. The nucleotides in red are identical to the **HpQd** sequence. MFold-predicted structures are presented to show the ability of these sequences to fold into hairpin structures. These G-rich sequences also possess the ability to fold into G-quadruplexes (underlined nucleotides).