Supplementary Material (ESI) for Chemical Communications

Supporting information

Primer extension reactions as tool to uncover folding motifs within complex G-rich sequences: analysis of the human *KRAS* NHE†

Manikandan Paramasivam,[¶] Susanna Cogoi and Luigi E. Xodo[*]
Department of Medical and Biological Science, University of Udine, 33100 Udine, Italy; Fax: +39 0432 494301; Tel:+39 0432 494395; E-mail: luigi.xodo@uniud.it;

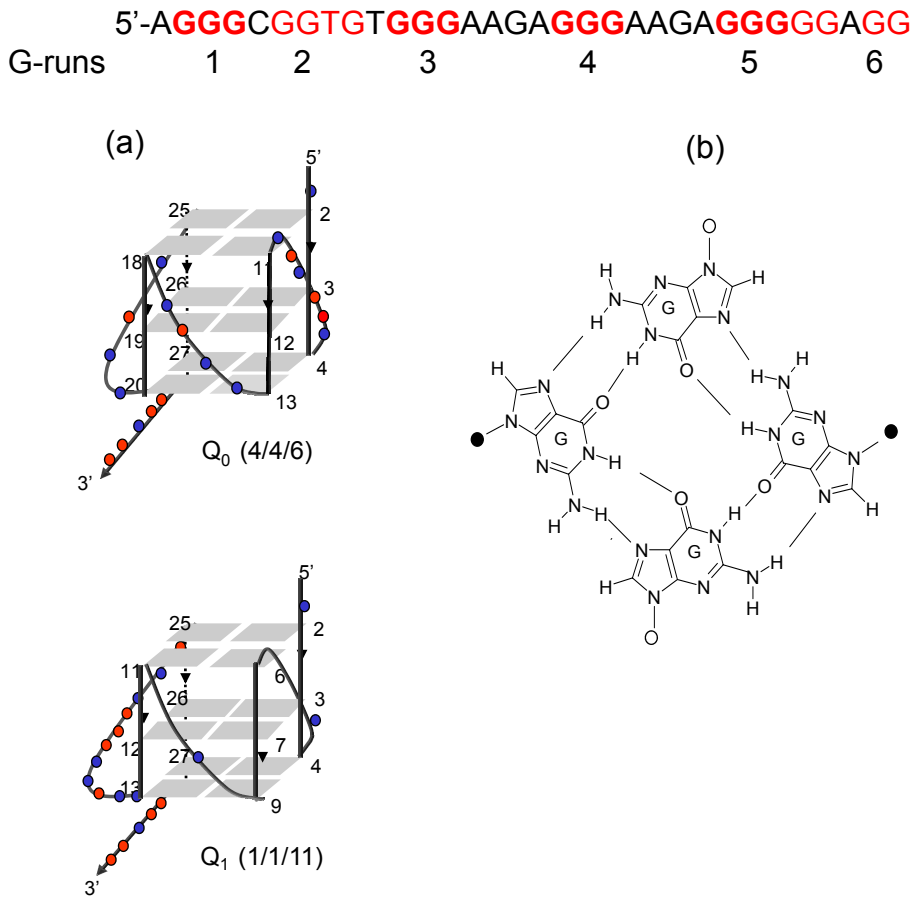


Fig. S1: (a) **Q_1** (1/1/11) shows the putative quadruplex structure assumed by the G-runs 1-2-3-5, inferred by DMS footprinting experiments. [2b] The expected 4/4/6 conformation that should be formed by the G-runs 1-3-4-5 (**Q_0**) is not supported by DMS footprintings. [2b]

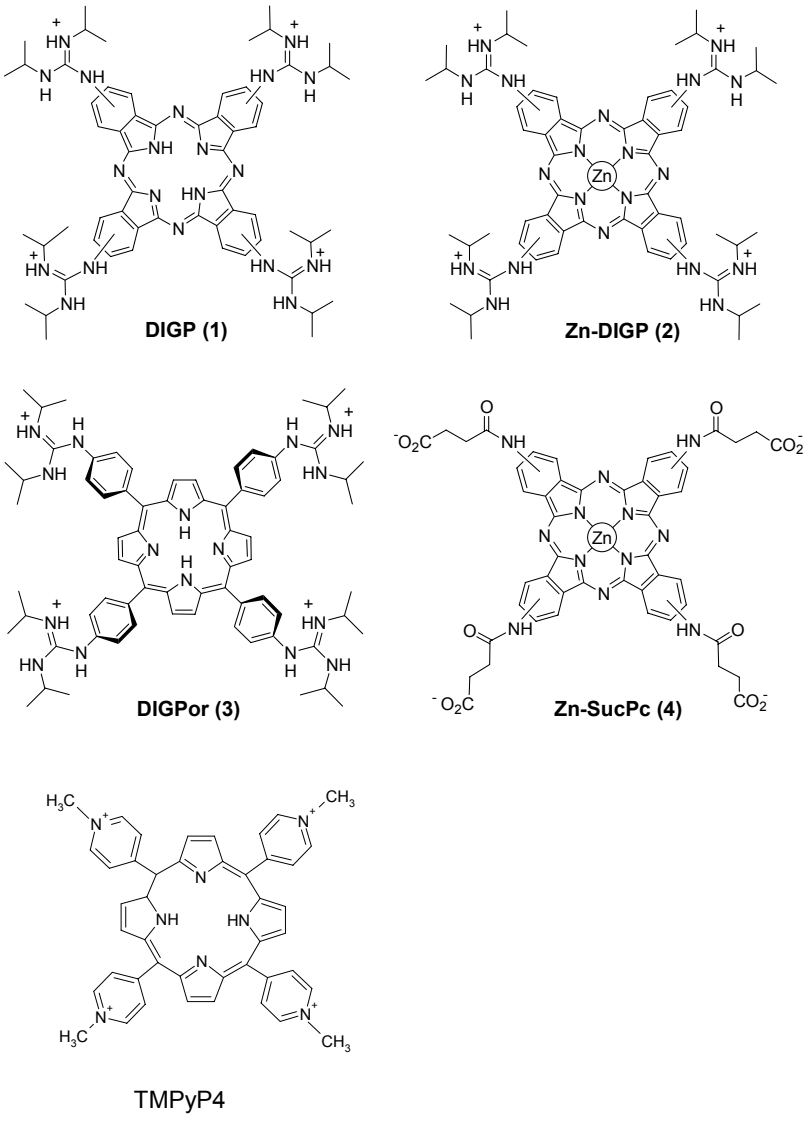


Fig. S2: Structures of the guanidine phthalocyanines DIGP, Zn-DIGP and porphyrin TMPyP4 used as quadruplex stabilizers. Compounds DIGPor and Zn-Suc Pc that do not bind to quadruplex DNA have been used as control.

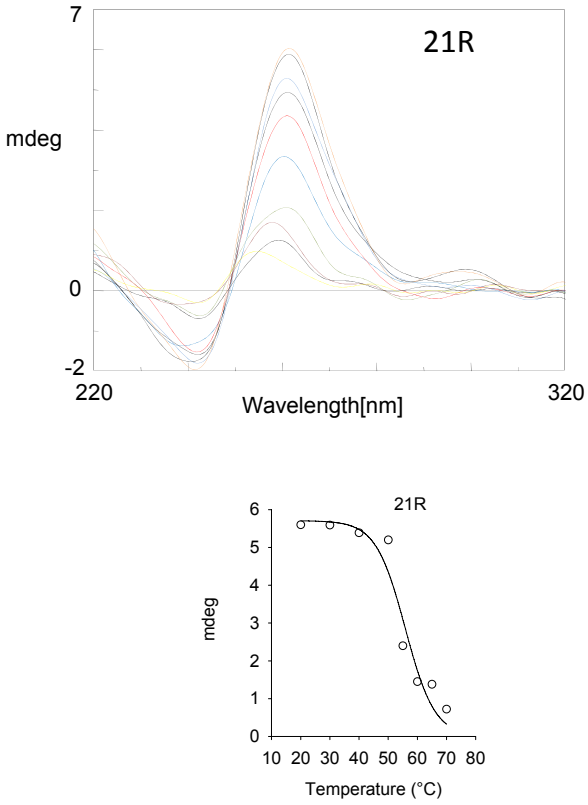


Fig. S3: CD of **21R** in 50 mM Tris-HCl pH 7.4, 100 mM KCl as a function of temperature from 20 to 70 °C. Ellipticity at 260 nm, measured while heating the sample, has been plotted as a function of temperature. The cooling curve gave a T_M of 56 °C.

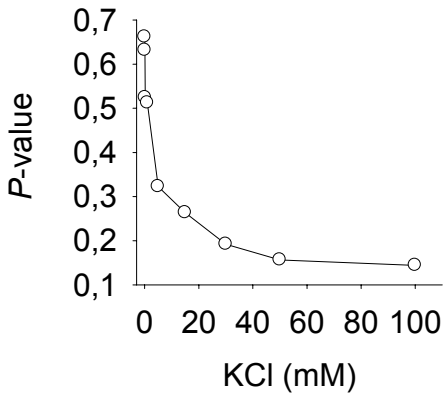
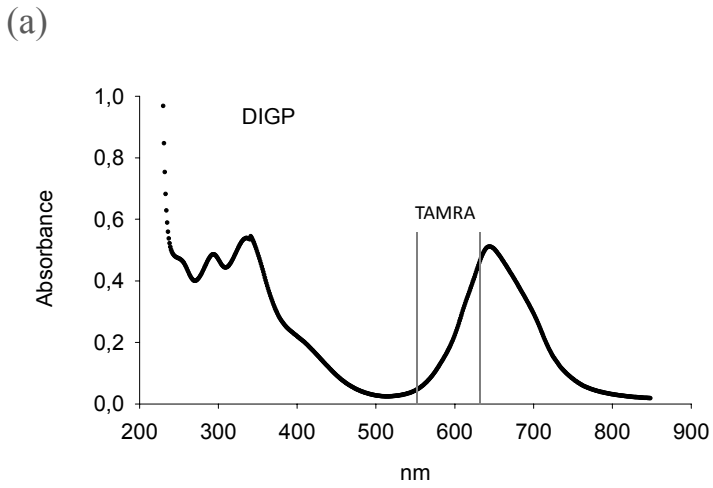


Fig. S4: P-value, $(I_D/(I_D+I_A)$ where I_D and I_A are the fluorescence intensities of donor (FAM) and acceptor (TAMRA), relative of quadruplex F-21R-T.



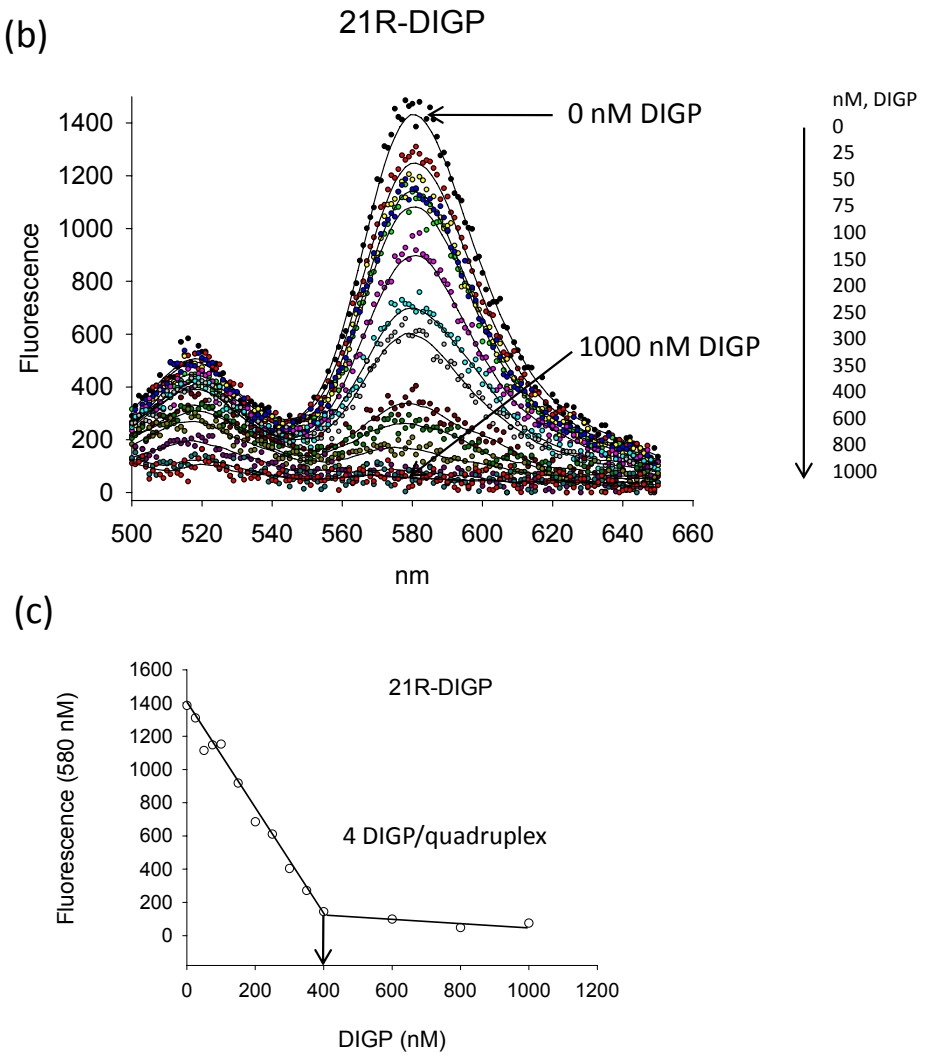


Fig. S5: (a) UV-Vis spectrum of 20 μM DIGP in water; (b, top panel) Titration of 100 nM quadruplex **21R** tagged with FAM and TAMRA in 50 mM Tris-HCl, pH 7.4, 50 mM KCl with 0,

25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 600, 800, 1000 nM DIGP (from top to bottom). The addition of DIGP results in selective quenching of TAMRA emission, as DIGP absorbs in the region of TAMRA emission; Bottom panel shows the fluorescence emitted at 580 nm by quadruplex **21R** as a function of DIGP concentration. The binding stoichiometry estimated is 4 DIGP *per* quadruplex.

5′FAM-AGGGCGGTGTGGGAAGAGGGA-TAMRA 21R, T_M=56 °C
5′FAM-ATGGCGGTGTTGGAAGAGGTA-TAMRA 21Rm1, T_M=30 °C
5′FAM-AGGGCGG-GTGGGAAGAGGGA-TAMRA 21Rm2, T_M=80 °C

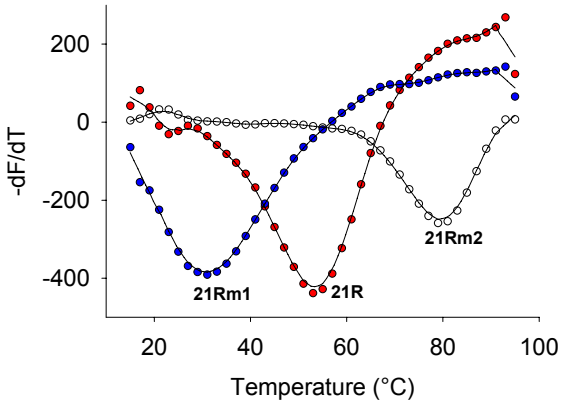


Fig. S6: FRET-melting in 50 mM Tris-HCl, pH 7.4, 100 mM KCl of **21R** and mutant sequences **21Rm1** and **21Rm2**.

Fig. S7: Figure 5b in the paper shows an increase in fluorescein (FAM) emission with added UP1 that is not accompanied by a decrease in rhodamine (TAMRA) emission. This may be related to change in quantum yield of rhodamine due to deprotonation of protein-bound dyes that are not ionized when dispersed free of protein in the pH 7.4 buffer.