Supplementary Information (ESI)

Enhanced binding of 5,10,15,20 (N-Propyl-4-Pyridyl) Porphyrine (TPrPyP4): a comparative study with TMPyP4

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Materials and Methods

Oligonucleotides and porphyrins

The oligonucleotides were obtained from Microsynth (Switzerland). They were purified by PAGE using a denaturing 20% gel (acrylamide: bisacrylamide, 19:1) in TBE, 7 M urea, 55°C. The bands were excised from the gel and eluted in water. The DNA solutions were filtered (Ultrafree-DA, Millipore) and precipitated. The concentration of each DNA was determined from the absorbance at 260 nm in milli Q water, using as extinction coefficients 7500, 8500, 15000 and 12500 $M^{-1}cm^{-1}$ for C, T, A and G, respectively. Porphyrins TMPyP2 (P2), TMPyP3 (P3) were purchased from Porphyrin Systems (Lübeck, Germany), TMPyP4 (P4) from Sigma (Milan, Italy).

Polymerase stop assay

Single-stranded DNA fragments with a number of nt between 79-82, containing in the middle a quadruplex forming G-rich element from the murine *KRAS* or human *HRAS* promoters, were used as templates in the Taq polymerase primer-extension reactions. The DNA sequences have been purified by PAGE under denaturing conditions. The template (25 nM) was mixed with the ^{32}P-labelled primer (25 nM), in the presence or absence of porphyrins (P2, P3, P4, PP4 or 4) or phthalocyanines (**1-4**), in 25 mM KCl, Taq buffer 1X and incubated overnight at 37°C. The primer extension reactions have been carried out for 1h, by adding 10 mM DTT, 100 mM dATP, dGTP, dTTP, dCTP and 3.75U of Taq polymerase (Euro Taq, Euroclone, Milan). The reactions were stopped by adding an equal volume of stop buffer (95% formamide, 10mM EDTA, 10mM NaOH, 0.1% xylene cyanol, 0.1% bromophenol blue). The products were separated on a 12% polyacrylamide sequencing gel prepared in TBE1X, 8 M urea. The gel was dried and exposed to autoradiography. Standard dideoxy sequencing reactions were performed to detect the exact positions in which DNA polymerase was arrested.

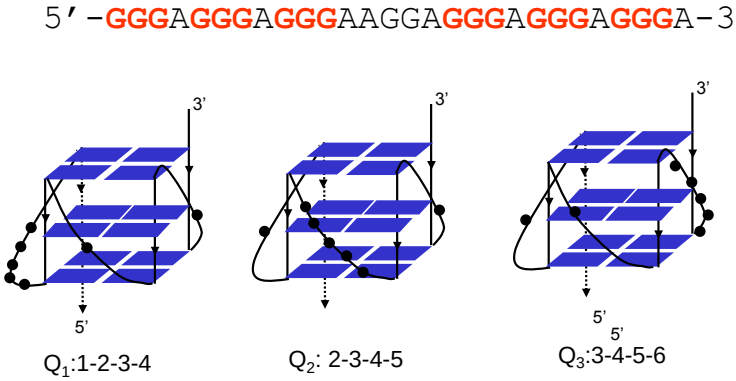


Figure S_1: Putative structure of the G4-DNA formed by the murine GA-element, as determined by DMS-footoprinting (Nucleic Acids Res, 2006, 34, 2536).

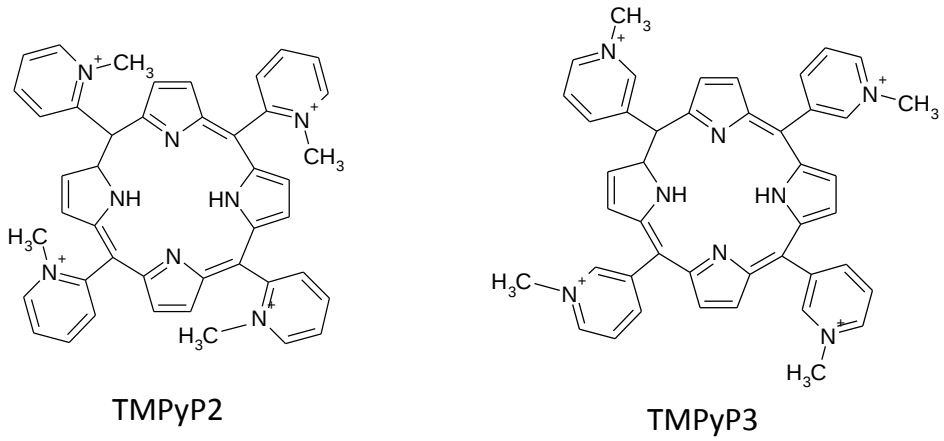
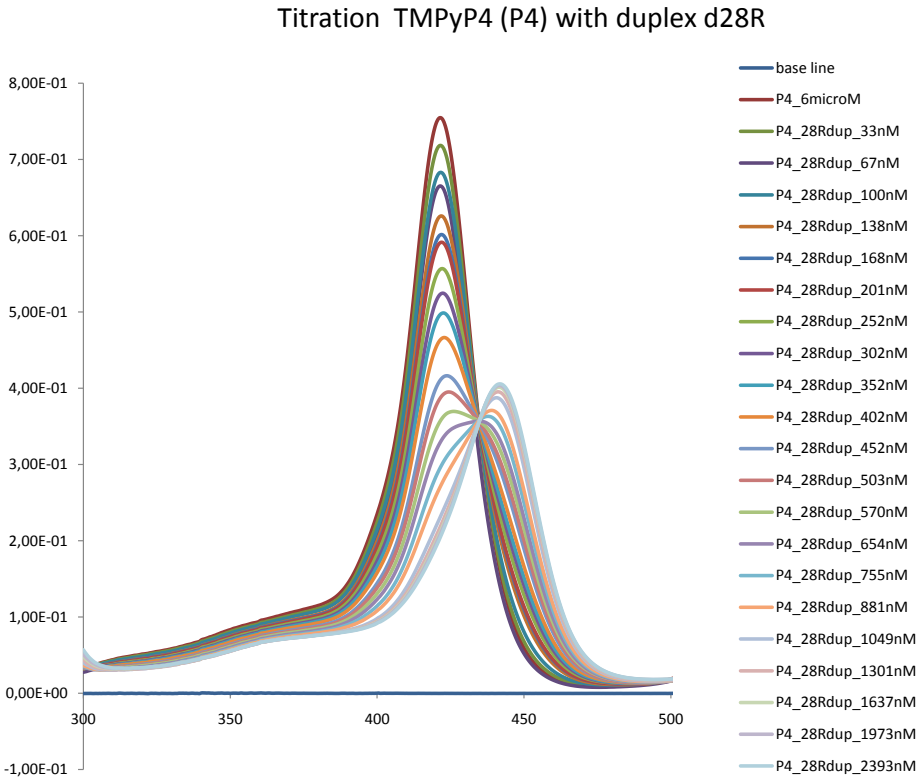


Figure S_2: Structures of pentaphyrins TMPyP2 (P2) and TMPyP3 (P3).



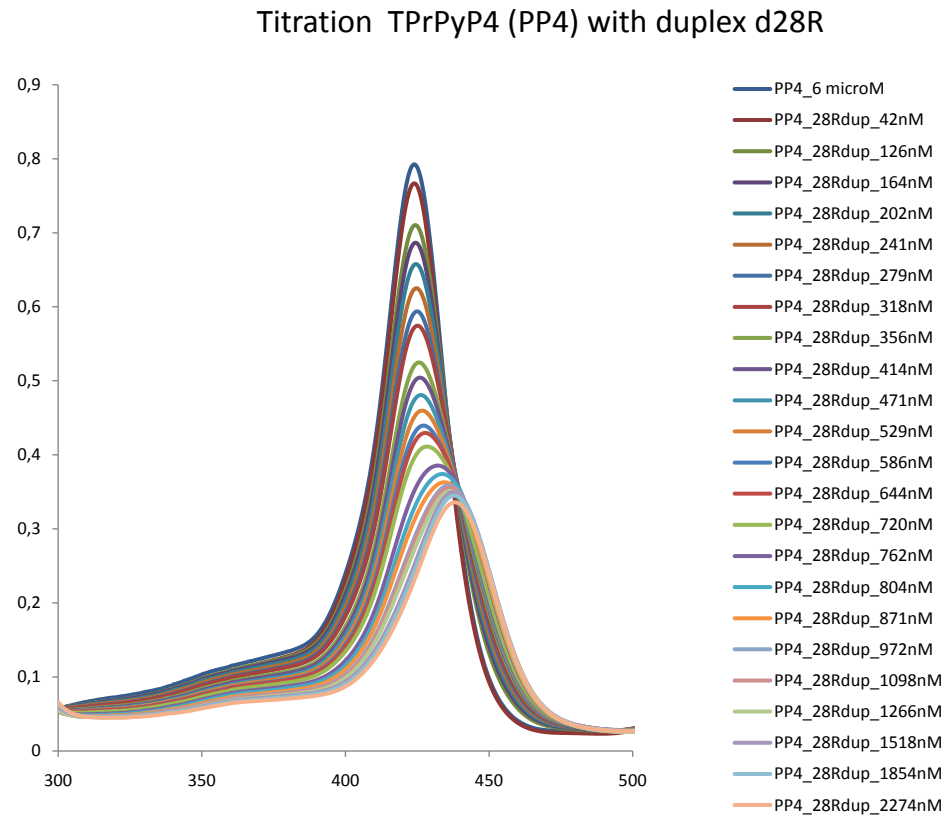


Figure S$_3$: UV-vis titrations of porphyrins 6 μM TMPyP4 and TPrPyP4 with duplex d28R [5'-GGGAGGGAGGGAAGGAGGGAGGGAGGGA-3'CCCTCCCTCCCTTCCTCCCTCCCTCCCT] in 50 mMTris-HCl, pH 7.4, 100 mM NaCl**.**

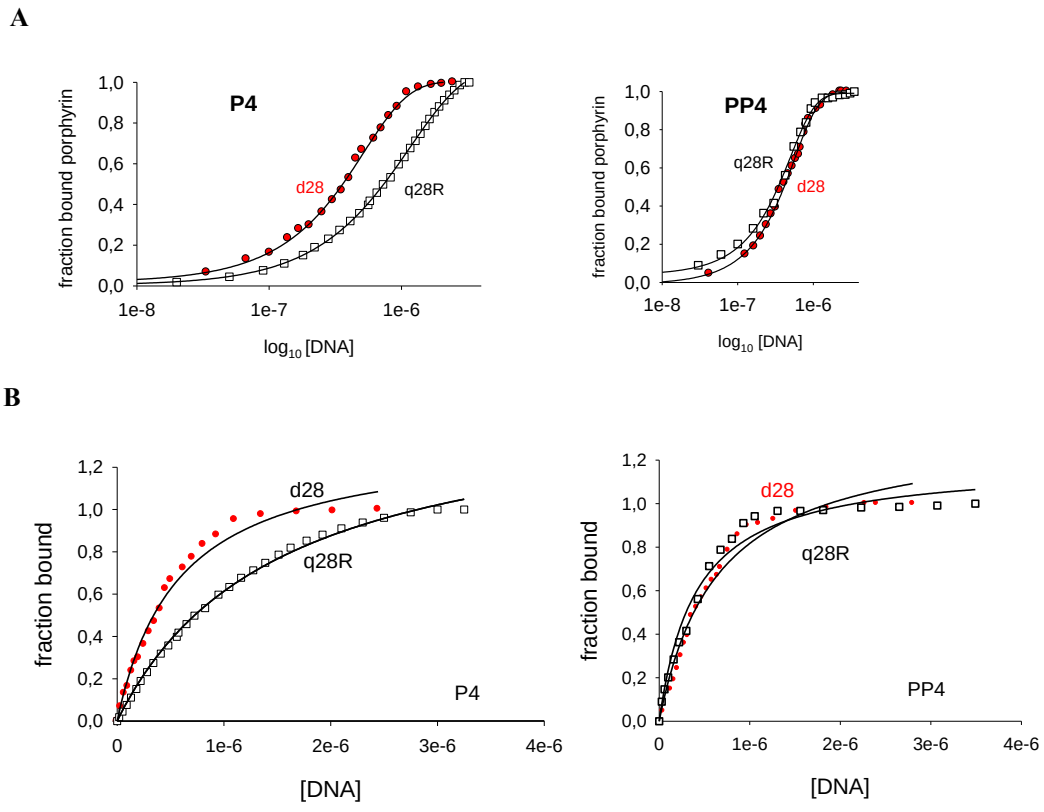


Figure S_4: (A) Binding curves for P4 and PP4 on a logarithm scale showing that P4 has more affinity for duplex d28 than quadruplex q28R, whereas PP4 shows similar affinity for duplex and quadruplex; (B) Binding curve on a linear scale and best fitted to a 1:1 binding model. P4-d28: K_d=5.7x10^{-7}±5.7x10^{-7}; P4-q28R: K_d=1.5x10^{-6}±5.0x10^{-8}; PP4-d28: K_d=4.1x10^{-7}±4.5x10^{-7}; PP4-q28R: K_d=6.4x10^{-7}±6.6x10^{-8}.

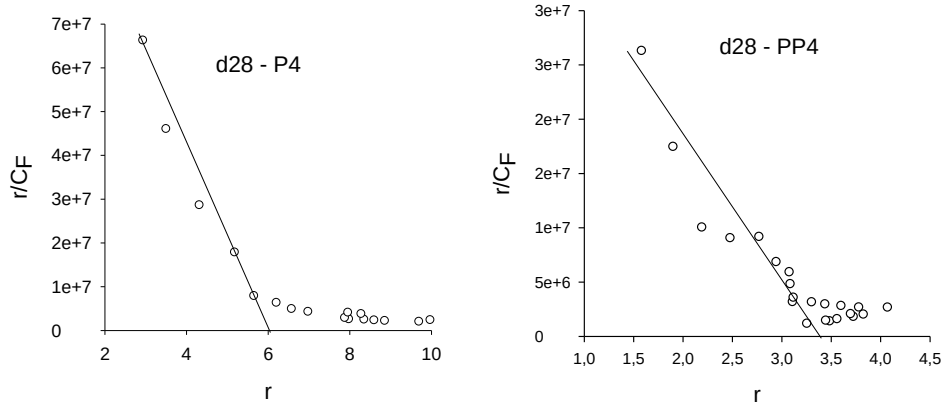
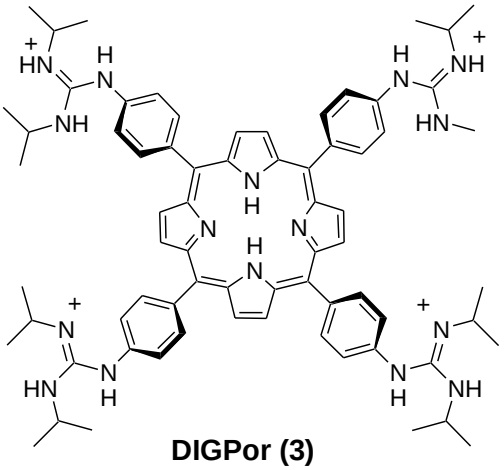
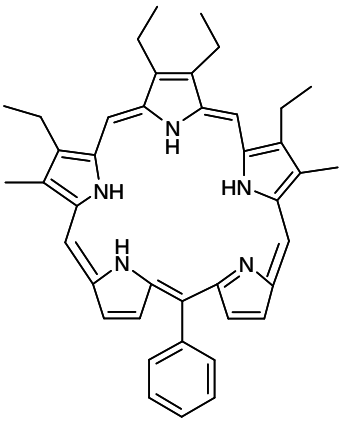


Figure S_5: Typical Scatchard plots for duplex d28R and porphyrins P4 and PP4. Titrations performed in 50 mM Tris-HCl pH 7.4, 100 mM KCl, quartz cuvette, 0.5 cm pathlength, 28R concentration 6 μM.



DIGPor (3)



Pentaphyrine

Figure S_6: (Up) Porphyrine (DIGPor) used in this study. [P Alzeer, N Roth, NW Luedtke. ChemComm 2009, 15, 1970; A Membrino, M Paramasivam, S Cogoi, J Alzeer, NW Luedtke, LE Xodo 2010, ChemComm 46, 625] ; (Down) Pentaphyrins used in this study [C Comuzzi, S Cogoi, M Overhand, GA Van der Marel, HS Overkleeft, LE Xodo. J Med Chem. 2006, 49, 196.