Supporting Information

Cellular uptake and binding of guanidine-modofied phthalocyanines to *KRAS*/*HRAS* G-quadruplexes

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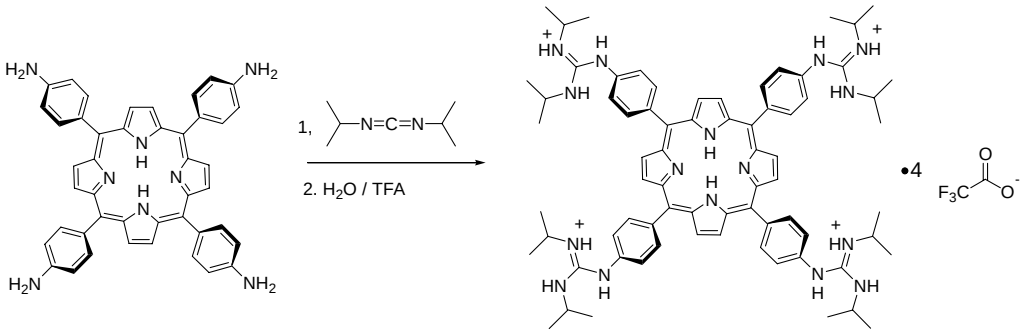
Materials and Methods

Compounds 1 – 4

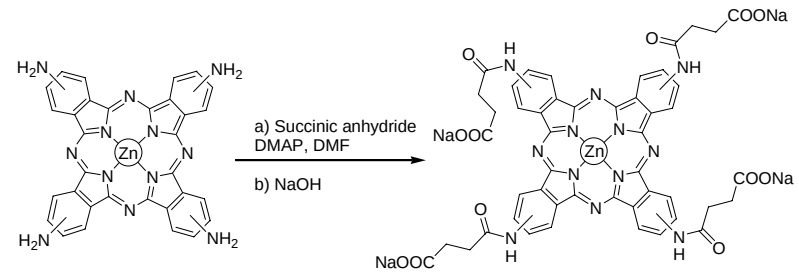
The synthesis and characterization of tetrakis-(diisopropyl-guanidine) phthalocyanine "DIGP" (**1**), and its Zn-containing derivative "Zn-DIGP" (**2**) have been reported (reference 13 in the main manuscript). Compounds (**3**) and (**4**) were synthesized at the University of Zürich according to the following procedures:



5,10,15,20-tetrakis (4-aminophenyl)-21H,23H-porphine

5,10,15,20-tetrakis (diisopropyl-guanidine)-21H,23H-porphine . (TFA)4 salt (**3**)

5,10,15,20-tetrakis(diisopropyl-guanidine)-21H,23H-porphine . $(TFA)_4$ salt (DIGPor, 3): The starting material 5,10,15,20-tetrakis (4-aminophenyl)-21H,23H-porphine was obtained from TCI Europe and 35 mg (52 μmoles) was combined with pyridine (4 mL), pyridine-HCl (2 g), and diisopropylcarbodiimide (500 μL, 3.3 mmoles, 59 equiv) and stirred under N_2 at 110 ºC for 18 h. The reaction was removed from the heat, and 15 mL of H_2O was used to transfer the hot mixture into a polypropylene centrifuge tube. TFA (1 mL) was added, mixed, and the resulting precipitate was collected by centrifugation at 6'500 r.p.m. The precipitate was suspended into water (2 mL), sonicated, and TFA (40 μL) added. The resulting precipitate was collected by centrifugation at 6'500 r.p.m. This was repeated a total of three times. The resulting precipitate was dissolved into 1.2 mL of 1:1 acetonitrile / water and lyophilised to yield 55 mg (65 %) of a red powder. 1H-NMR (400 MHz, d_6-DMSO / d_4-methanol, 10 : 1 mixture) δ 8.89 (br s, 8H), 8.15 (d, J = 8.4 Hz, 8H), 7.55 (d, J = 8.4 Hz, 8H), 4.05 (m, J = 6.5 Hz, 8H), 1.26 (d, J = 6.5 Hz, 48H), ESI MS (m/z): [M+H]+ calcd for $C_{72}H_{91}N_{16}$, 1180; found 1180.



tetraamino-zinc-phthalocyanine

tetrakis(succinamic acid)-zinc-phthalocyanine • Na_4 salt (**4**)

Tetrakis(succinamic acid)-zinc-phthalocyanine . Na_4 salt (4): At room temperature and N_2 atmosphere, succinic anhydride (196 mg, 1.96 mmol) and dimethylaminopyridine (138 mg, 0.3 mmol) was added, at room temperature, to a solution of tetraamino-zinc-phthalocyanine (50 mg, 0.078 mmol) in DMF (6 mL). After 7 days, the reaction mixture was diluted with EtOAc (66 mL) and the resulting precipitate was collected by centrifugation. The dark green precipitate was washed repeatedly with H_2O. The precipitate was dissolved in TFA (4 mL) and then mixed water (20 mL) and centrifuged. The resulting ppt was dissolved in 1 N NaOH (50 mL), precipitated with MeOH (100 mL) and dried to afford **4** (76 mg, 86%) as a dark green solid. 1H-NMR (300 MHz, d_6-DMSO) δ 8.93 (br. s, 4H), 8.13 (br. m, 4H), 8.01 (br. s, 4H), 6.66 (br. m, 4H), 2.92 (br. s, 8H), 2.81 (br. s, 8H). MALDI TOF MS (*m*/*z*): $[M+H]^+$ calcd for $C_{48}H_{36}N_{12}O_{12}Zn$, 1037.19; found 1037.2. UV-Vis (DMSO) λmax (nm) and ε ($cm^{-1}M^{-1}$): 360 (6.1 x 10^4), 630 (2.57 x 10^4), and 690 (1.32 x 10^5).

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. Dual-labeled F-28R-T (5'end with FAM, 3'end with TAMRA) was HPLC purified. 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.

Circular Dichroism

CD spectra were obtained using a JASCO J-600 spectro-polarimeter equipped with a thermostatted cell holder. The oligonucleotides used for the CD experiments were at a concentration of 3 μM, in 50 mM Tris-HCl, pH 7.4 and 25 mM KCl (or 100 mM NaCl when duplex DNA was used). Spectra were recorded in 0.5 cm quartz cuvette. A thermometer placed in the cuvette holder allowed a precise measurement of the sample temperature. The spectra were calculated with J-700 Standard Analysis software (Japan Spectroscopic Co., Ltd) and are reported as ellipticity (mdeg) versus wavelength (nm). Each spectrum was recorded three times, smoothed and subtracted from the baseline.

FRET spectroscopy

Fluorescence measurements were carried out with a Microplate Spectrofluorometer System (Molecular Devices) using a 96-well black plate, in which each well contained 50 μl of 200 nM dual-labelled Fam-GGGAGGGAGGGAAGGAGGGAGGGAGGGA-Tamra in 50 mM Tris-HCl, pH 7.4 and increasing amounts of KCl. In the presence of KCl, DNA assumes a folded quadruplex conformation and FRET is expected between the 5' and 3' fluorophores.

The interaction of the G-quadruplex with the phthalocyanines can be studied by following the quenching of FAM emission at 584 nm by the added GPcs, as they absorb at 584 nm (see spectrum of DIGP reported below). For the fluorescence quenching data we obtained the fraction of bound GPc at increasing ligand concentrations. Plotting the fraction of bound GPc (moles of bound GPc divided by total number of DNA moles) against the free ligand concentrations we obtained experimental points that were best-fitted to a standard binding equation $y=B_{max}\ L/(K_D+L)$ by using SigmaPlot 10.0.1.

The binding data obtained from polymerase stop assays were analysed according to Scatchard equation $r/[L]=n\ K_A - rK_A$ where r is the ratio of the moles of bound ligand divided by the total available binding sites, [L] is the concentration of free ligand, and *n* is the number of binding sites per DNA molecule.

Table S_1: Apparent dissociation constants of GPcs **1** and **2** binding to quadruplex DNA[a]

	KCl, mM	K_D (**1**), M	K_D (**2**), M
m*KRAS*	100	8.6 (±2,1) x 10^{-7}	8.9(±1.7) x 10^{-7}
PTHR1	100	5.8 (±1.3) x 10^{-7}	3.2(±0.8) x 10^{-7}
HRAS-1	100	3.7 (±3.4) x 10^{-7}	-

[a] Data obtained using FRET constructs in 50 mM Tris-HCl, pH 7.4, 100 mM KCl. Higher apparent affinities were observed in 50 mM KCl (Table S_1).

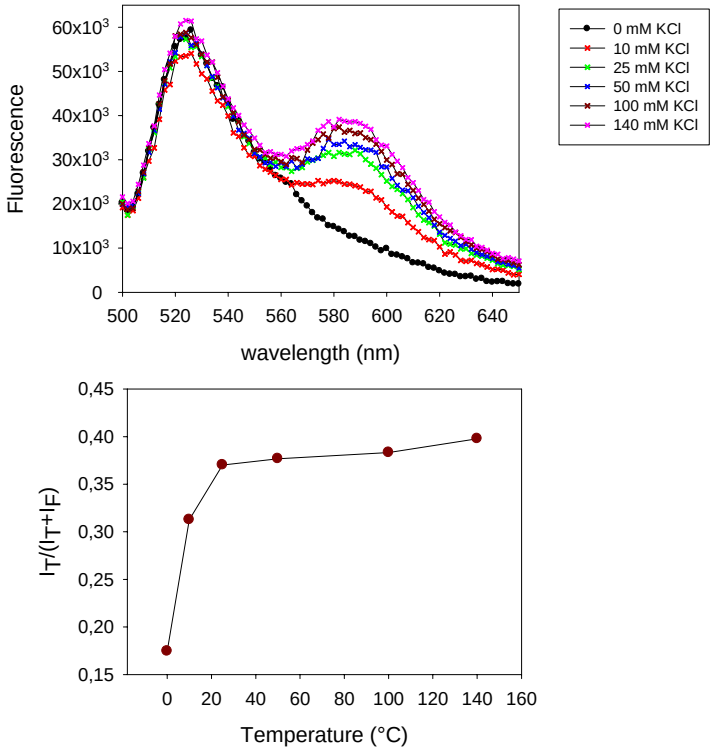


Figure S_1: Folding of murine GA-element followed by FRET as a function of KCl concentration. At 25 mM, the sequence is folded into its characteristic quadruplex structures. GA-element has been doubled labelled: 5'Fam-GGGAGGGAGGGAAGGAGGGAGGGAGGGA-tamra

Figure S_2. Taq polymerase stop assay of the DNA template containing the murine KRAS GA-element in the absence (lane 1) and presence of 0.5, 1 and 2.5 μM phthalocyanine **1** (lanes 2-4) or **2** (lanes 5-7). Buffer: 50 mM Tris-HCl, pH 7.4, 25 mM LiCl. Reaction conditions: template 50 nM, primer 50 nM, 1 h reaction at 37°C. Reaction products run in 12% PAGE in TBE-urea.

5′-GGGAGGGAGGGAAGGAGGGAGGGAGGGA-3′

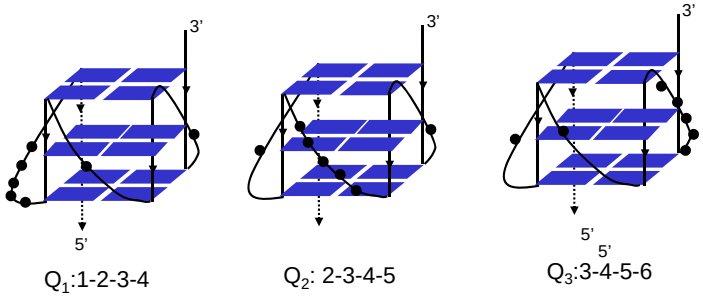


Figure S_3: Putative structure of the G4-DNA formed by the murine GA-element, as determined by DMS-footoprinting (Nucleic Acids Res, 2006, 34, 2536).

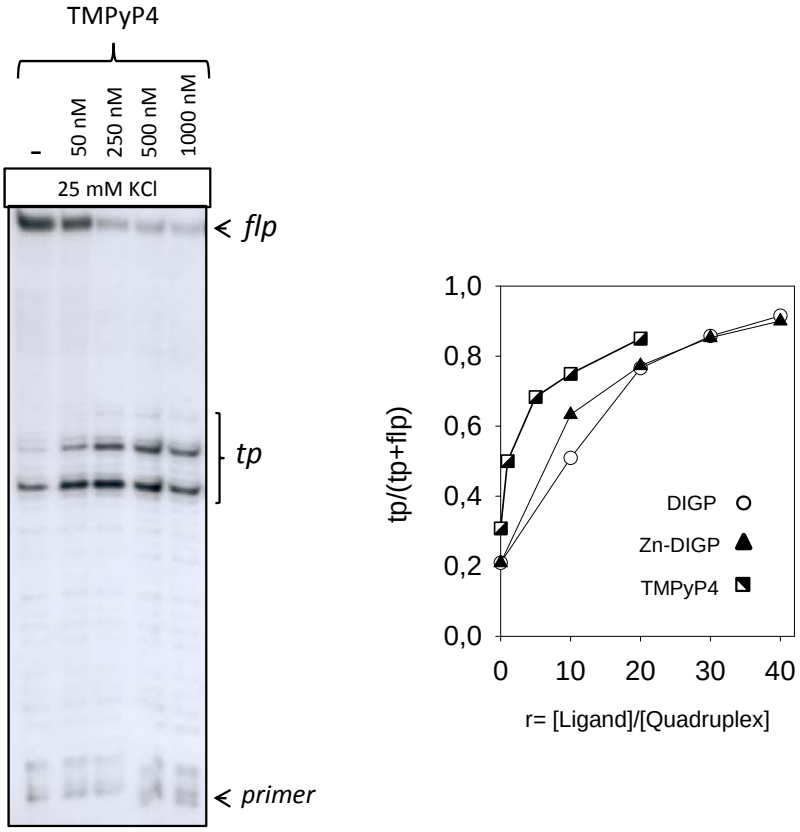


Figure S$_4$: (Left) Taq polymerase stop assay of the DNA template containing the murine KRAS GA-element in the absence (lane 1) and presence of 50, 250, 500 and 1000 nM TMPyP4. Buffer: 50 mM Tris-HCl, pH 7.4, 25 mM KCl. Reaction conditions: template 50 nM, primer 50 nM, 1 h reaction at 37°C. Reaction products run in 12% PAGE in TBE-urea. (Right) Plots showing the fraction of truncated products as a function of r for DIGP, Zn-DIGP and TMPyP4.

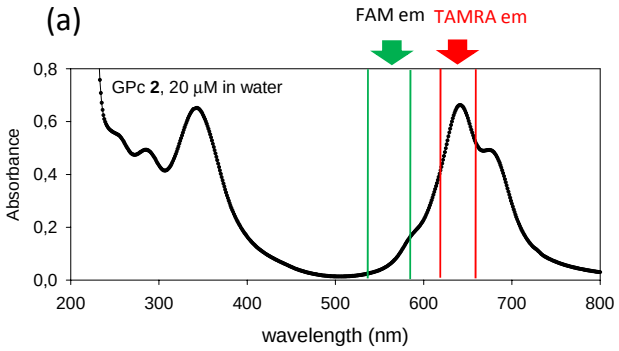


Figure S$_5$: (a) Absorption spectrum of phthalocyanine **2** in water;

Human parathyroid hormone PTH/PTH-related peptide receptor (PTHR1) gene

FAM-CCCGGGAGGGCGCCGGGGGAGGGAAGA-TAMRA

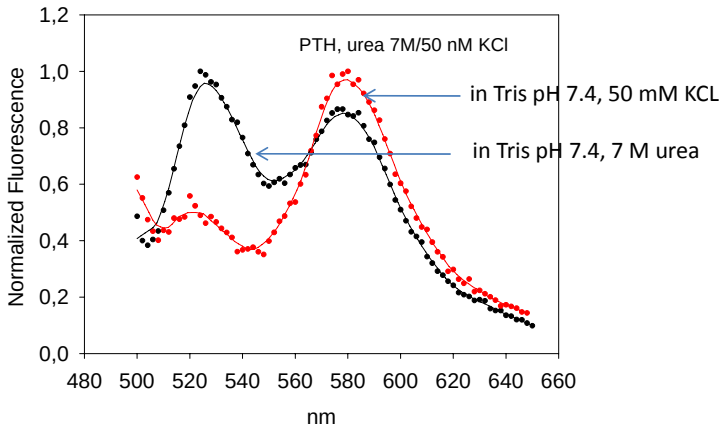


Figure S_6: Sequence of the human parathyroid hormone PTH/PTH-related peptide receptor (PTHR1) gene, forming a parallel G-quadruplex. The figure shows that the PTHR1 sequence folds in KCl, even in the presence of 7 M urea. In 50 mM Tris-HCl, pH 7.4, 50 mM KCl, the energy transfer $P=I_T/(I_T+I_F)$ is 0.66, indicating the folding of the sequence into a G-quadruplex with a T_M=67°C. As expected, in CsCl or LiCl this transition is not observed.

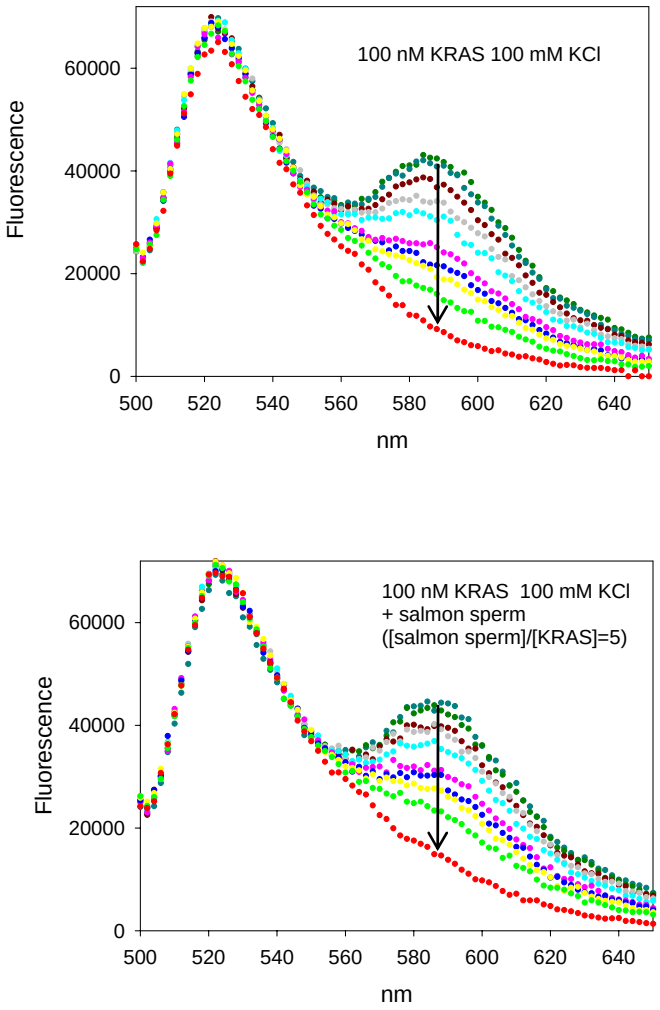


Figure S$_7$: Titration of 100 nM F-28-T in 50 mM Tris-HCl, pH 7.4, 100 mM KCl with increasing amounts of DIGP **1** in the absence (top) and presence (bottom) of salmon sperm DNA ([ssDNA]/[F-28R-T]=5).

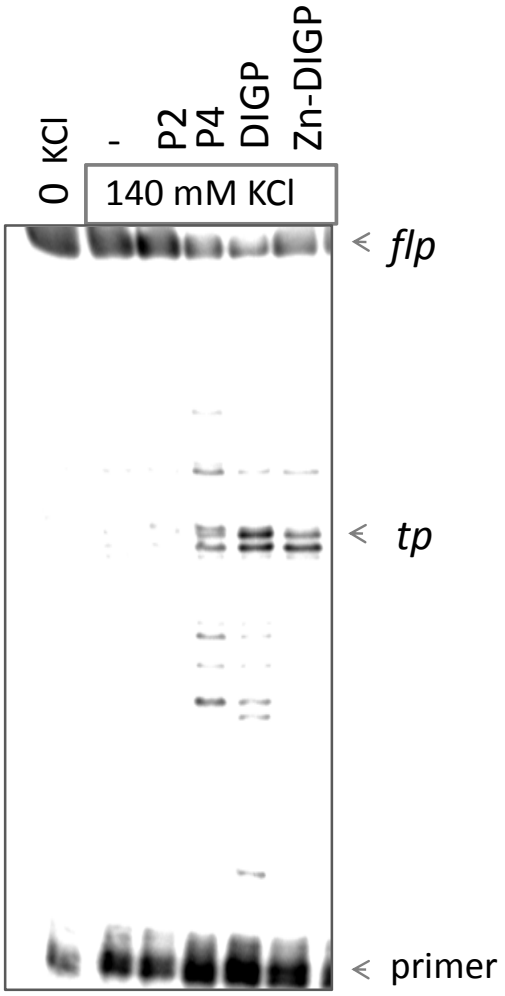
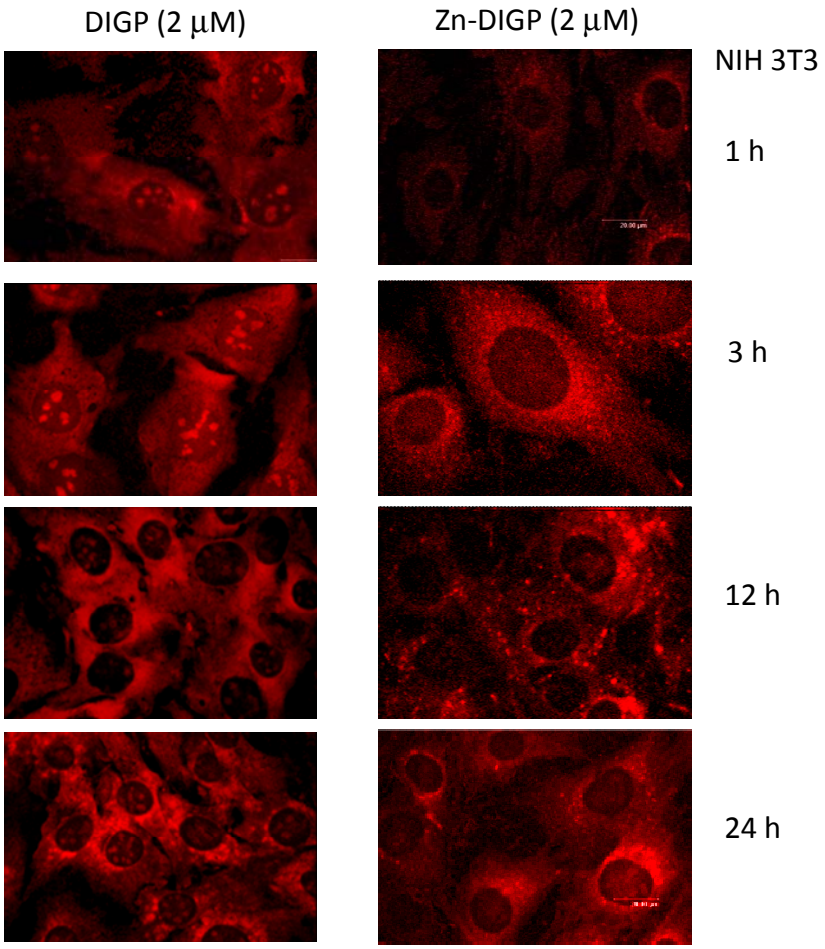


Figure S$_8$. Polymerase stop assays of 50 nM 82-mer template containing the *hras*-1 sequence forming an antiparallel G-quadruplex incubated with primer and GPc at r=10. Primer extension with Taq polymerase was carried out for 1 h at 37°C. P2=TMPyP2, P4= TMPyP4; flp= full length product, tp= truncated products.


DIGP (2 μM)
Zn-DIGP (2 μM)
NIH 3T3
1 h
3 h
12 h
24 h

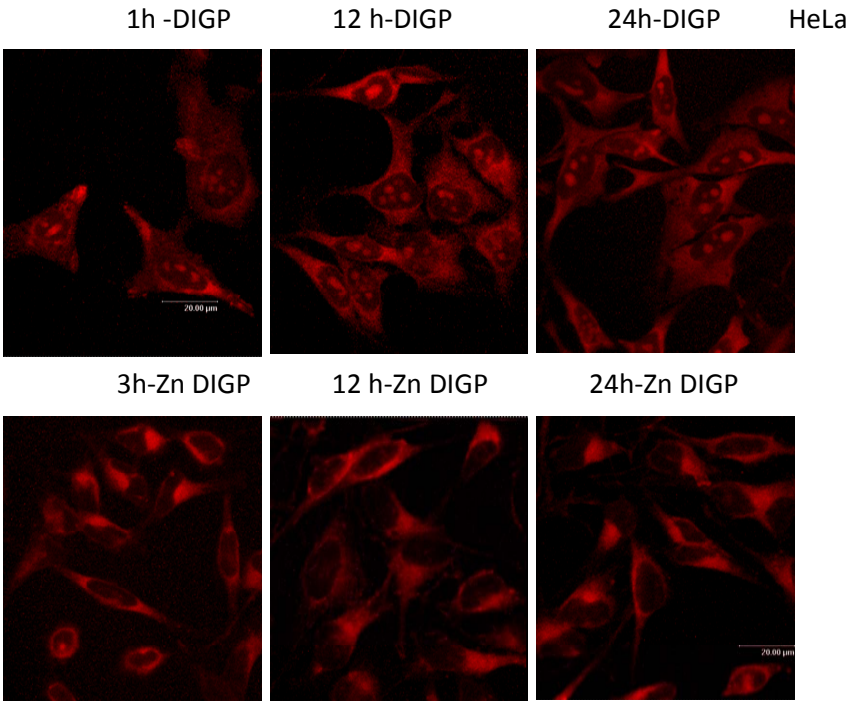


Figure S$_9$. NIH 3T3 (top) and HeLa (bottom) cells treated for 1, 3, 12 and 24 h with 2 μM DIGP and Zn-DIGP, fixed on glass slides, and analysed by confocal microscopy. Excitation= 633 nm, emission= 680-800 nm.