Supporting Information

Unravelling the cellular emission fingerprint of the benchmark G-quadruplex-interactive compound Phen-DC_3

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Experimental Section

General

Solvents, reagents, chemicals, and biological templates were purchased from commercial suppliers (Sigma-Aldrich and Eurofins Genomics) and used without further modifications, unless otherwise stated. G4 formation of *c-MYC* Pu24T (5'-TGAGGGTGGTGAGGGTGGGGAAGG-3'), Tel-22 (5'-AGGGTTAGGGTTAGGGTTAGGG-3') and $4G_3U_3$ (5'-GGGUUUGGGUUUGGGUUUGGG-3') was induced by heating the oligonucleotide at 95 °C for 5 min in the presence of 100 mM KCl and then slowly allowed to reach room temperature overnight. The exact oligonucleotide concentration was determined by UV/Vis spectroscopy using the molar extinction coefficient (ε_{260}) calculated by using oligo analyzer on the IDT web site ($\varepsilon_{c\text{-}MYC\ Pu24T}$ = 248200 M^{-1} cm^{-1}; $\varepsilon_{Tel\text{-}22}$ = 228500 M^{-1} cm^{-1}; ε_{4G3U3} = 185600 M^{-1} cm^{-1}). The stock solution of Phen-DC_3 (Article number: SML2298, Sigma-Aldrich) was prepared in DMSO at a final concentration of 10 mM. The final concentration of DMSO in all the assays reached the maximum value of 2 % (v/v).

Absorption and steady-state emission spectra

UV/Vis absorption spectra were recorded on T90+ UV/Vis spectrometer (PG instruments Ltd). Fluorescence spectra were recorded on Jasco FP-6500 spectrofluorometer equipped with the Jasco Peltier-type temperature controller (ETC2736). The slits width of both monochromators was 5 nm. Appropriate references were subtracted from both absorption and emission spectra of the Phen-DC_3-G4 systems in order to resolve only the contribution of the interactions.

LOD calculation

Limit of detection (LOD) was calculated by plotting the changes in the absorption at 405 nm as a function of Tel-22 concentration. LOD was calculated according to the following equation:

$$LOD = \frac{s_b \times k}{m} \qquad (Eq.S1)$$

where s_b is the standard deviation calculated out of 20 independent measurements on blank solution, *k* is 3, according to IUPAC recommendations and *m* is the slope obtained from the linear fitting optical density$_{(OD\ at\ 405\ nm)}$ *vs.* [Tel-22].

Electronic circular dichroism spectra

Electronic circular dichroism spectra were measured with a Jasco J-720 spectropolarimeter equipped with the JascoPeltier-type temperature controller (PTC-423L) and are presented as a sum of 6 accumulations. Before use, the optical chamber of the CD spectrometer was deoxygenated with dry nitrogen and was held under nitrogen atmosphere during the measurements. Appropriate references were subtracted from the obtained ECD spectra. Quartz cuvettes with a light path of 10 • 4 mm were used.

Taq-DNA polymerase stop assay

The DNA polymerase stop assay was performed as previously described.[S1] Briefly, reactions containing 40 nM template DNA (*c-MYC* Pu24T or non-G4) annealed to a TET-labeled primer was incubated with Phen-DC_3 (0.008, 0.02, 0.05, 0,13, 0.32, 0.8, 2 and 5 µM) in the presence of 50 mM KCl. Control reactions contained 2% DMSO instead of compound. 0.625 U/uL of Taq-DNA polymerase was used in each reaction, and incubated for 30 minutes at 50 °C. Samples were run on 10% denatured PAGE.

For the kinetic experiments, *c-MYC* Pu24T template was incubated with 0.5 μM Phen-DC_3 and the reactions were monitored over time (0, 1, 5, 10, 20, 30, 40 50 and 60 min). Control reactions contained 2% DMSO instead of Phen-DC_3. Gels were visualized by Typhoon 9400 scanner and quantified by ImageQuant TL software. Fluorescence signal was normalized relatively to DMSO reaction. The last band was assigned to the full-length product. The following oligonucleotide sequences were used:

Primer 5´-3´:

TET-TGAAAACATTATTAATGGCGTCGAGCGTCCG

Template *c-MYC* Pu24T 5´-3´:

ATATATATATTGAGGGTGGTGAGGGTGGGGAAGGATATATATATCGGACGCTCGACG
CCATTAATAATGTTTTCA

Template NonG4 5´-3´:

GAGACCATTCAAAAGGATAATGTTTGTCATTTAGTATATGCCCCTGCTCGTCTTCCCTT
CTCCGGACGCTCGACGCCATTAATAATGTTTTCA

Fluorescence microscopy

Human cervical carcinoma (HeLa) cells and human embryonic kidney (HEK 293) cells were cultured at 37 °C in 5% CO_2 in DMEM high glucose medium (Gibco) supplemented by 10% fetal bovine serum and penicillin-streptomycin. HeLa cells were fixed for 10 min either with 2% paraformaldehyde (PFA) at room temperature or cold methanol (MeOH) at -20 °C, and permeabilized with PBST (phosphate buffer saline supplemented by 0.1% TritonX-100). HEK 293 cells were fixed for 10 min with 2% paraformaldehyde (PFA) at room temperature and permeabilized with PBST. HeLa and HEK 293 fixed cells were treated with Phen-DC_3 (20 μM) for 30 min at room temperature. Then, Phen-DC_3 solution was removed from the

glass-bottomed microwell dishes (MaTek Corp.) and washed two times with 1X PBS (phosphate buffer saline). For RNA degradation controls, 0.1 mg/ml RNase A (Thermo Fisher Scientific) was used and the cells were pre-treated with RNase A for 2 hours at 37 °C prior the treatment with Phen-DC_3. For DNA degradation controls, 50 U/µL DNase I (Thermo Fisher Scientific) was used and samples were pre-treated for 2 hours at 37 °C, and then treated with Phen-DC_3. To confirm the absence of fluorescence from the unbound Phen-DC_3 state, MeOH-fixed HeLa cells were also pre-treated simultaneously with both RNase A (0.1 mg/ml) and DNase I (50 U/µL) for 2 hours at 37 °C prior the treatment with Phen-DC_3. Competition assays were performed by either using Phen-DC_3-stained cells (20 µM) and different concentrations of BRACO-19 (10 and 20 µM), or TmPyP4-stained cells (10 µM) and different concentrations of Phen-DC_3 (10 and 20 µM) upon 30 min incubation at room temperature. Images were acquired by confocal microscope Leica SP8 FALCON (FAst Life time CONtrast) using HC PL APO 63x/1.20 Water motCORR CS2 objective. Maximum intensity projection of Z-stack images was used for data presentation to preserve raw fluorescence signal. Quantitative data analysis was performed by manually selecting the area of the cellular compartments and measuring the mean gray value. All data were processed using ImageJ software available at https://imagej.nih.gov/ij/.

Electronic circular dichroism spectra of DNA and RNA G4s

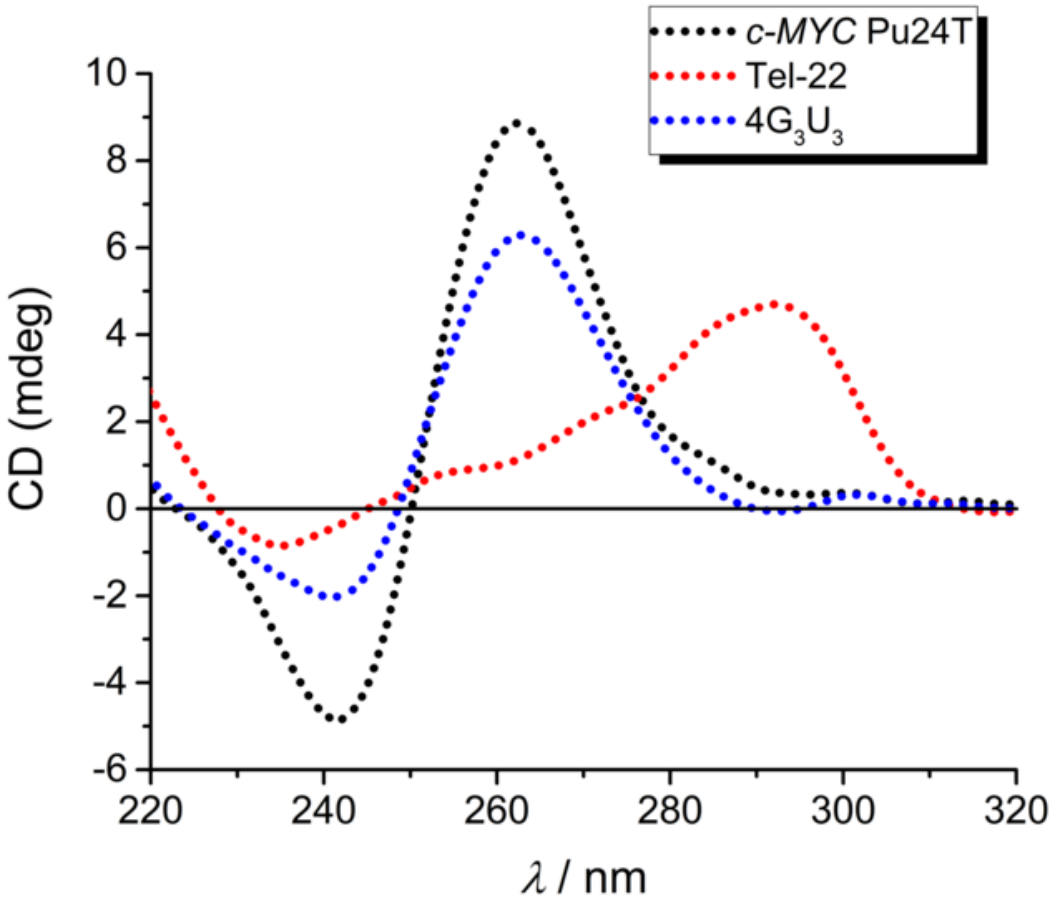

Figure S1. Characteristic ECD spectral signatures of the G4s structures used in this study (c_{Tris} = 50 mM, c_{KCl} = 100 mM, c_{G4} 2μM).

Electronic absorption spectra of Phen-DC_3 and Tel-22

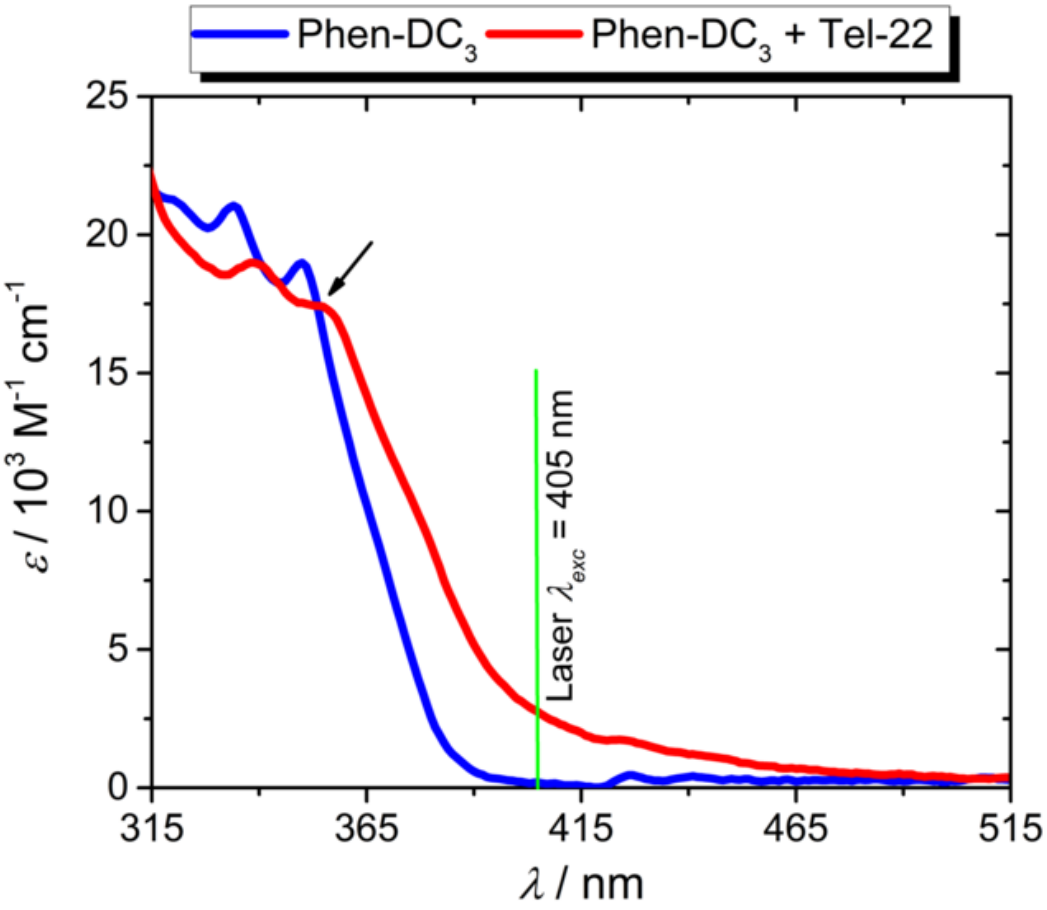

Figure S2. Absorption spectra of Phen-DC_3 in the absence (blue line) and presence (red line) of an equimolar concentration of Tel-22 (c_{Tris} = 50 mM, c_{KCl} = 100 mM, $c_{Phen\text{-}DC3}$ = 5.0 μM and $c_{Tel\text{-}22}$ = 5.0 μM).

Electronic absorption spectra of Phen-DC_3 and $4G_3U_3$

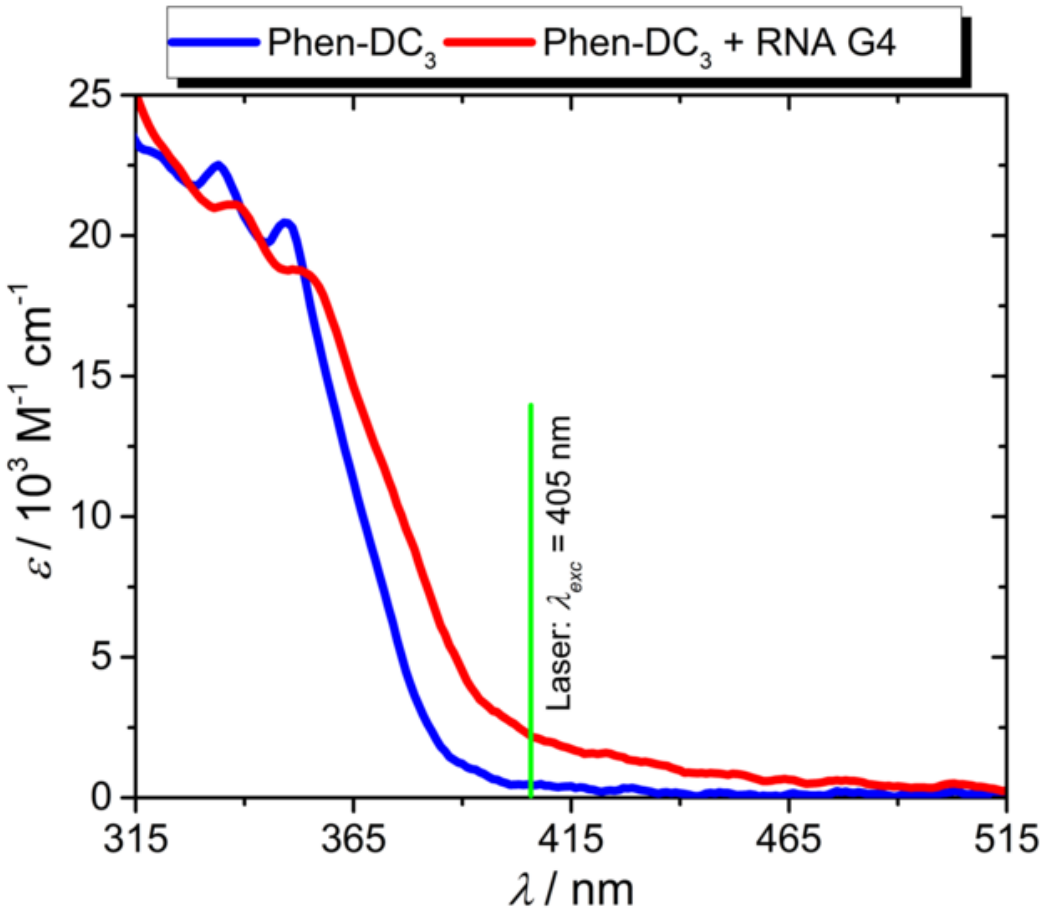

Figure S3. Absorption spectra of Phen-DC_3 in the absence (blue line) and presence (red line) of an equimolar concentration of RNA G4 $4G_3U_3$ (c_{Tris} = 50 mM, c_{KCl} = 100 mM, $c_{Phen\text{-}DC3}$ = 5.0 μM and c_{4G3U3} = 5.0 μM).

Viscosity-dependent absorption spectra of Phen-DC_3

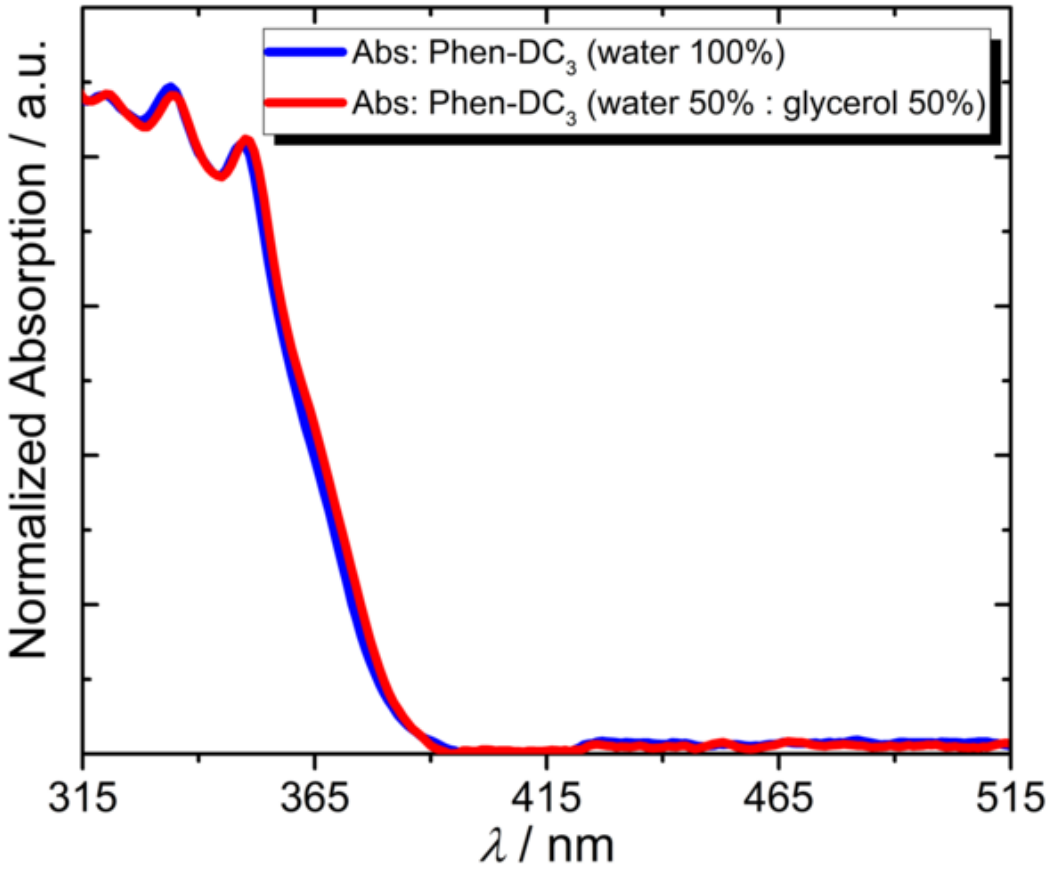

Figure S4. Absorption spectra of Phen-DC_3 in pure water (blue line) or in a glycerol:water (50/50 %) mixture (red line) ($c_{Phen\text{-}DC3}$ = 5.0 µM).

Polarity-dependent absorption spectra of Phen-DC_3

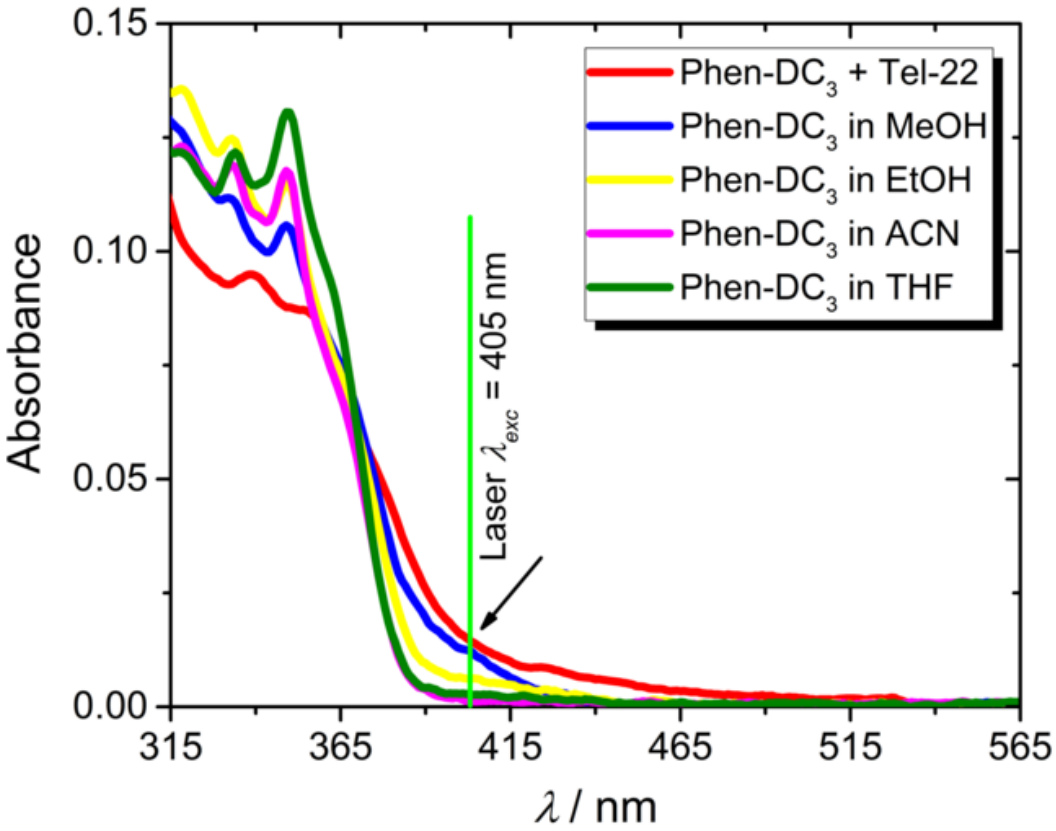

Figure S5. Absorption spectra of Phen-DC_3 in organic solvents having different polarities. The long-wavelength tail present in the Phen-DC_3-Tel-22 complex is similar to that observed for Phen-DC_3 alone in MeOH. ($c_{Phen\text{-}DC3}$ = 5.0 μM).

Emission spectra of Phen-DC_3 in the presence of DNA and RNA G4s

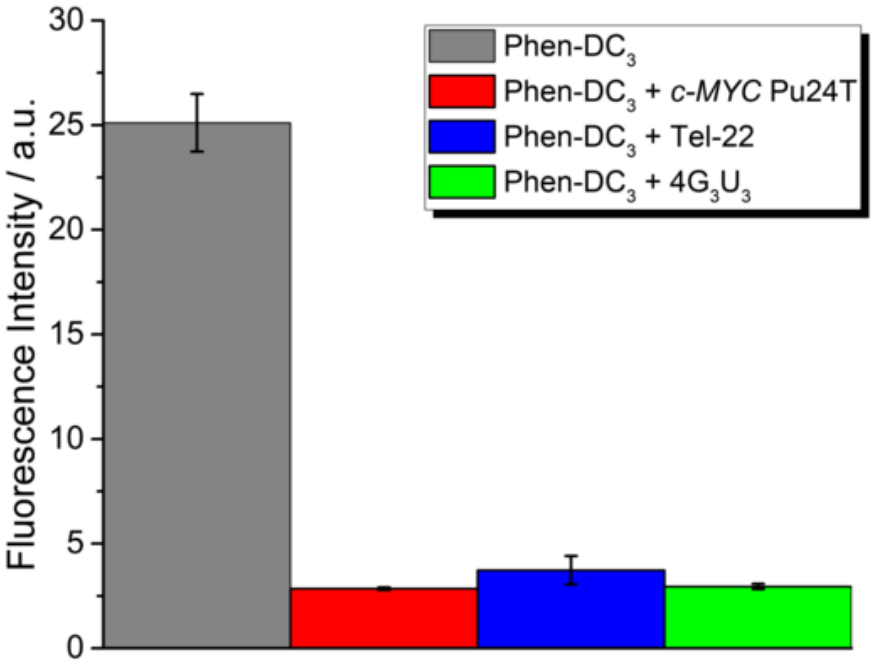

Figure S6. Changes in the Phen-DC_3 fluorescence intensity in the presence of DNA and RNA G4s. (c_{Tris} = 50 mM, c_{KCl} = 100 mM, $c_{Phen\text{-}DC3}$ = 2.5 μM, $c_{c\text{-}MYC\ Pu24T}$ = 2.5 μM, $c_{Tel\text{-}22}$ = 2.5 μM and c_{4G3U3} = 2.5 μM, λ_{exc} = 350 nm).

Limit of detection (LOD)

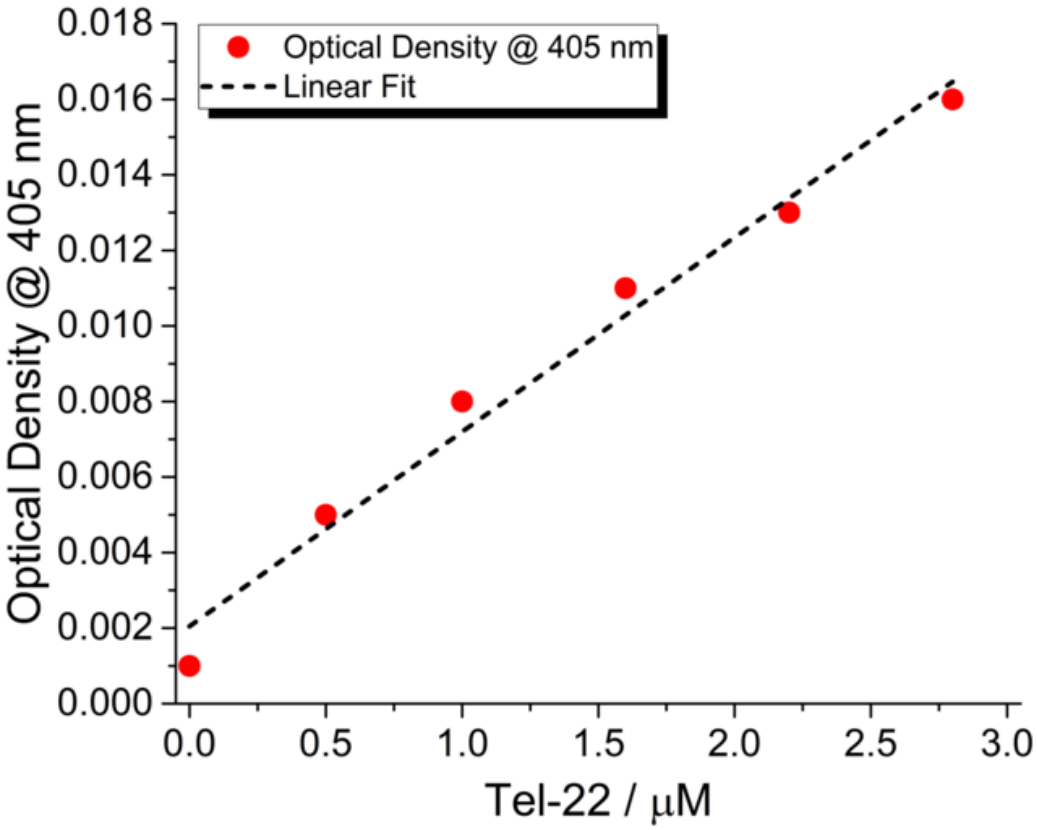

Figure S7. Calculated limit of detection (LOD) for Phen-DC_3-Tel-22 system. The changes in the optical density at 405 nm were plotted as a function of Tel-22 concentration.

Confocal fluorescence of MeOH-fixed HeLa cells

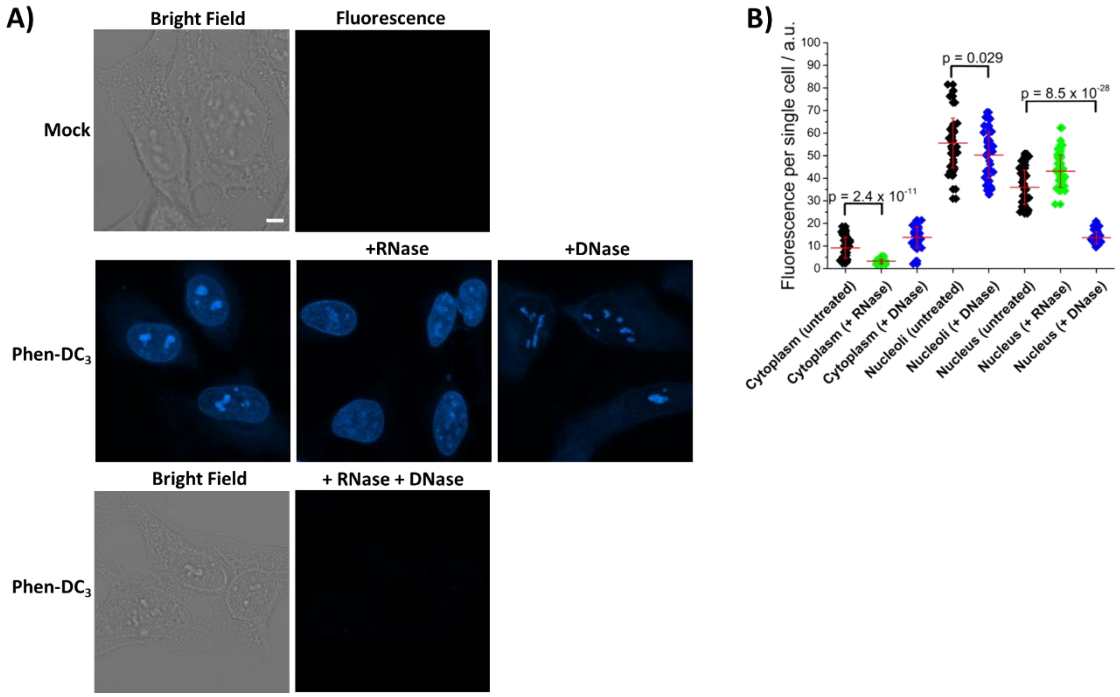

Figure S8. A) Confocal fluorescence images of MeOH-fixed HeLa cells either unstained or stained with Phen-DC_3 (20 μM). The bright field (BF) image acquired for unstained MeOH-fixed HeLa cells and the associated background level is also shown. No autofluorescence from unstained cells was observed. Where denoted the fixed cells were pre-treated with either RNase or DNase prior to Phen-DC_3-treatment. To confirm the absence of background signal from the unbound Phen-DC_3 state, fixed cells were pre-treated with both RNase and DNase. No fluorescence signal was observed in this condition supporting the fact that the only fluorescence species is the Phen-DC_3 bound state. The associated BF image is also shown for sake of clarity. **B)** Quantification of fluorescence signal per single cell. Data represent populations of individual cells for each condition of the final experiment: untreated (N = 40 cells), RNase treated (N = 40 cells), DNase treated (N = 40 cells). Means ± SD are indicated. Analysis of the data was performed using two-sample t tests and p values are indicated. Scale bar = 5 μm. Diode 405 nm laser was used and the fluorescence emission was recorded in the range λ_{em}: 430-630 nm.

Confocal fluorescence of PFA-fixed HEK 293 cells

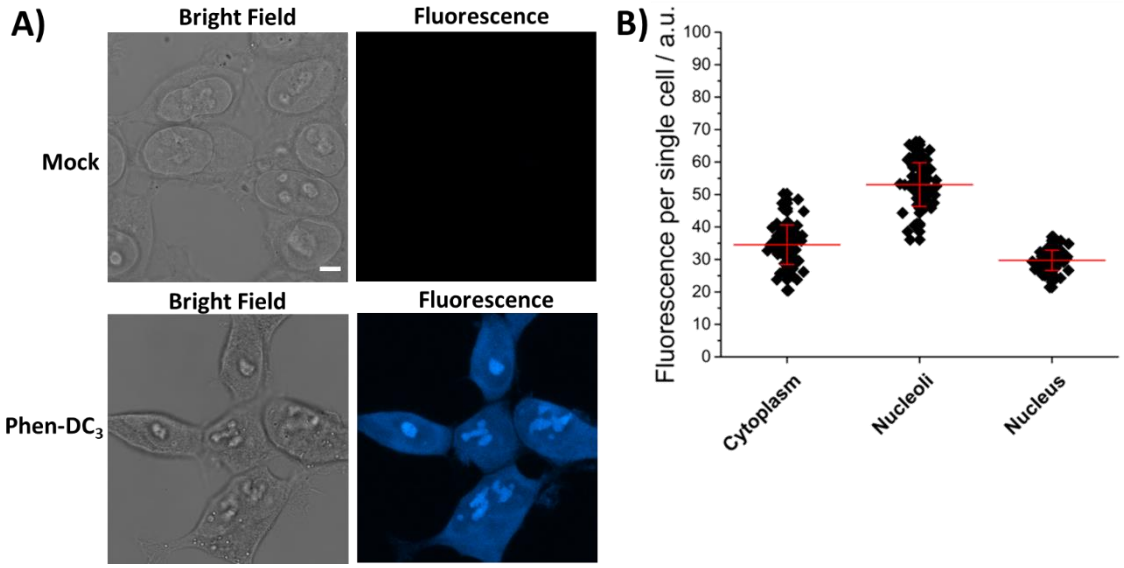

Figure S9. A) Confocal fluorescence images of PFA-fixed HEK 293 cells either unstained or stained with Phen-DC_3 (20 µM). The bright field (BF) image acquired for unstained PFA-fixed HEK 293 cells and the associated background level is also shown. No autofluorescence from unstained cells was observed. **B)** Quantification of fluorescence signal per single cell. Data represent populations of individual cells for each condition of the final experiment: Phen-DC_3 treated cells (N = 60 cells). Means ± SD are indicated. Scale bar = 5 µm. Diode 405 nm laser was used and the fluorescence emission was recorded in the range λ_{em}: 430-630 nm.

References

S1. J. Jamroskovic, M. Livendahl, J. Eriksson, E. Chorell and N. Sabouri, *Chem. Eur. J.*, 2016, **22**, 18932–18943.