

Supplementary Information

to the manuscript:

DNA binding mode analysis of a core-extended naphthalene diimide as a conformation-sensitive fluorescent probe of G-quadruplex structures

Chiara Platella ¹, Rosa Gaglione ¹, Ettore Napolitano ¹, Angela Arciello ¹, Valentina Pirota ², Filippo Doria ², Domenica Musumeci ^{1,3}, Daniela Montesarchio ^{1,*}

¹*Department of Chemical Sciences, University of Naples Federico II, via Cintia 21, 80126 Naples, Italy*

³*Department of Chemistry, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy*

³*Institute of Biostructures and Bioimaging (IBB) of the National Research Council (CNR), Napoli*

* Correspondence: daniela.montesarchio@unina.it

Present Address: Daniela Montesarchio, Department of Chemical Sciences, University of Naples Federico II, via Cintia 21, I-80126 Naples, Italy

Tel: +39 081 674126; Fax: +39 081 674313

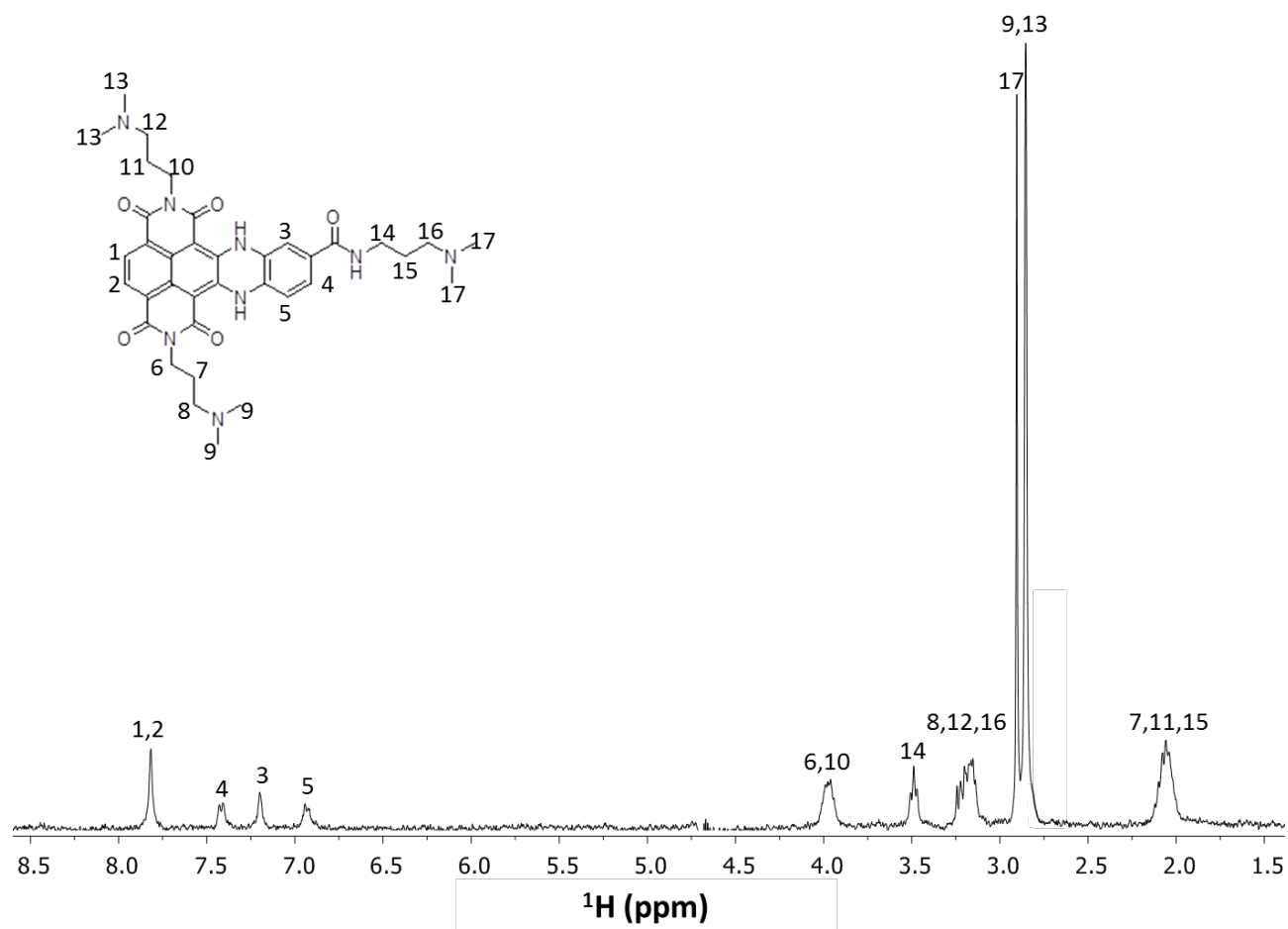


Figure S1. ¹H NMR spectrum of **c_{ex}-NDI** and related chemical structure and atom numbering used in the ¹H NMR assignments.

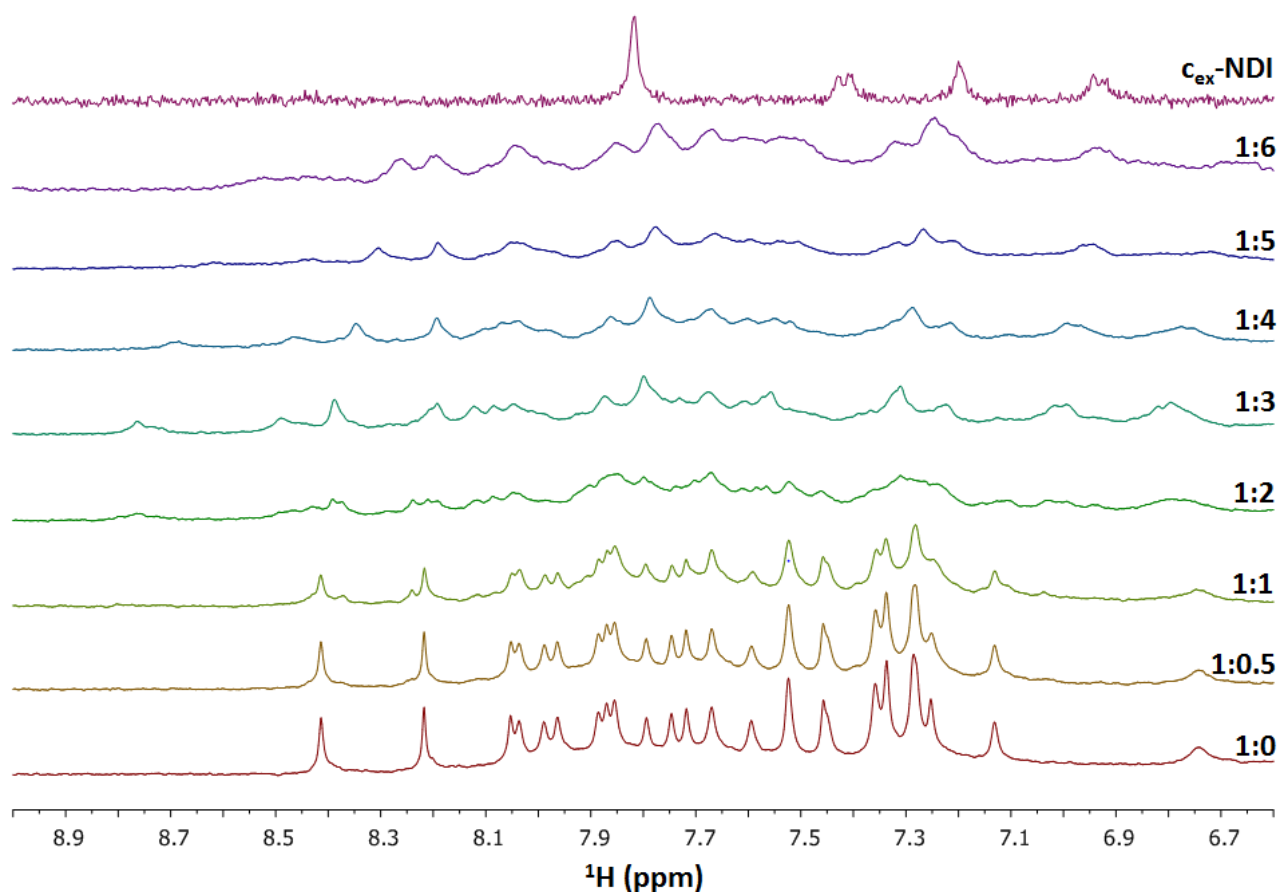


Figure S2. Aromatic proton regions of the ^1H NMR spectra of m-tel24 G-quadruplex upon titration with c_{ex} -NDI (from 0.5 to 6 equivalents). On top, NMR spectrum of free c_{ex} -NDI (0.2 mM) is reported. DNA/ c_{ex} -NDI ratios are shown on the right of the corresponding spectrum.

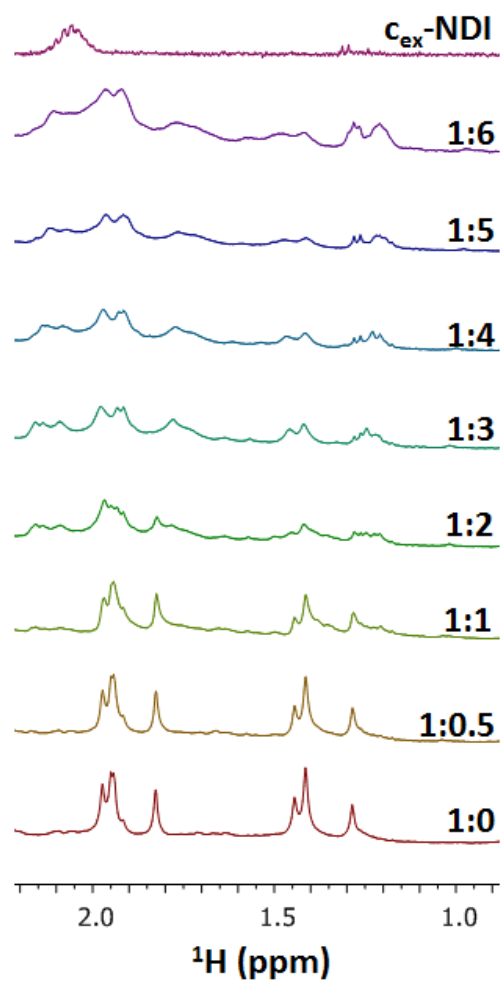


Figure S3. Methyl proton regions of the ^1H NMR spectra of m-tel24 G-quadruplex upon titration with $\text{c}_{\text{ex}}\text{-NDI}$ (from 0.5 to 6 equivalents). On top, NMR spectrum of free $\text{c}_{\text{ex}}\text{-NDI}$ (0.2 mM) is reported. DNA/ $\text{c}_{\text{ex}}\text{-NDI}$ ratios are shown on the right of the corresponding spectrum.

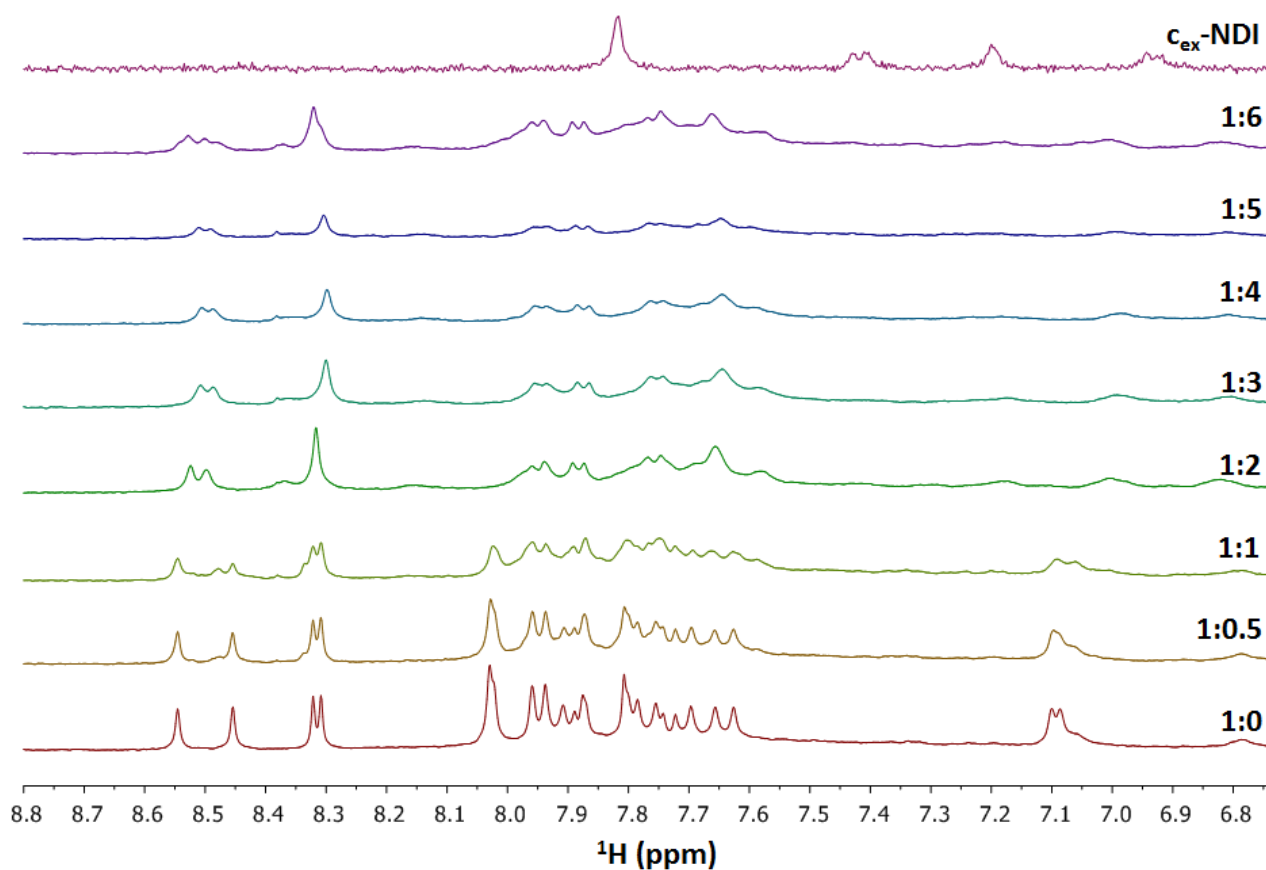


Figure S4. Aromatic proton regions of the ^1H NMR spectra of M2 G-quadruplex upon titration with $c_{\text{ex}}\text{-NDI}$ (from 0.5 to 6 equivalents). On top, NMR spectrum of free $c_{\text{ex}}\text{-NDI}$ (0.2 mM) is reported. DNA/ $c_{\text{ex}}\text{-NDI}$ ratios are shown on the right of the corresponding spectrum.

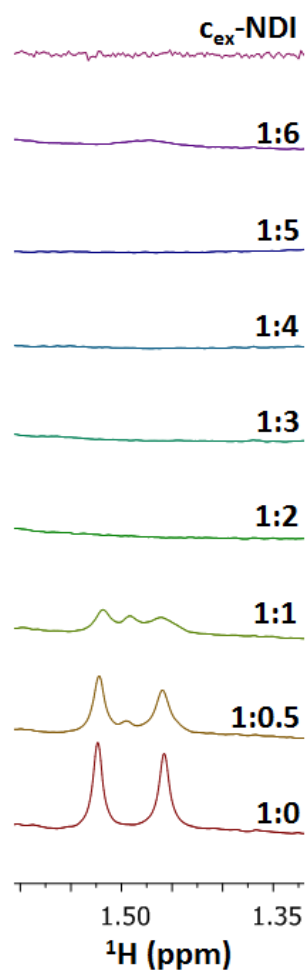


Figure S5. Methyl proton regions of the ^1H NMR spectra of M2 G-quadruplex upon titration with $c_{\text{ex}}\text{-NDI}$ (from 0.5 to 6 equivalents). On top, NMR spectrum of free $c_{\text{ex}}\text{-NDI}$ (0.2 mM) is reported. DNA/ $c_{\text{ex}}\text{-NDI}$ ratios are shown on the right of the corresponding spectrum.

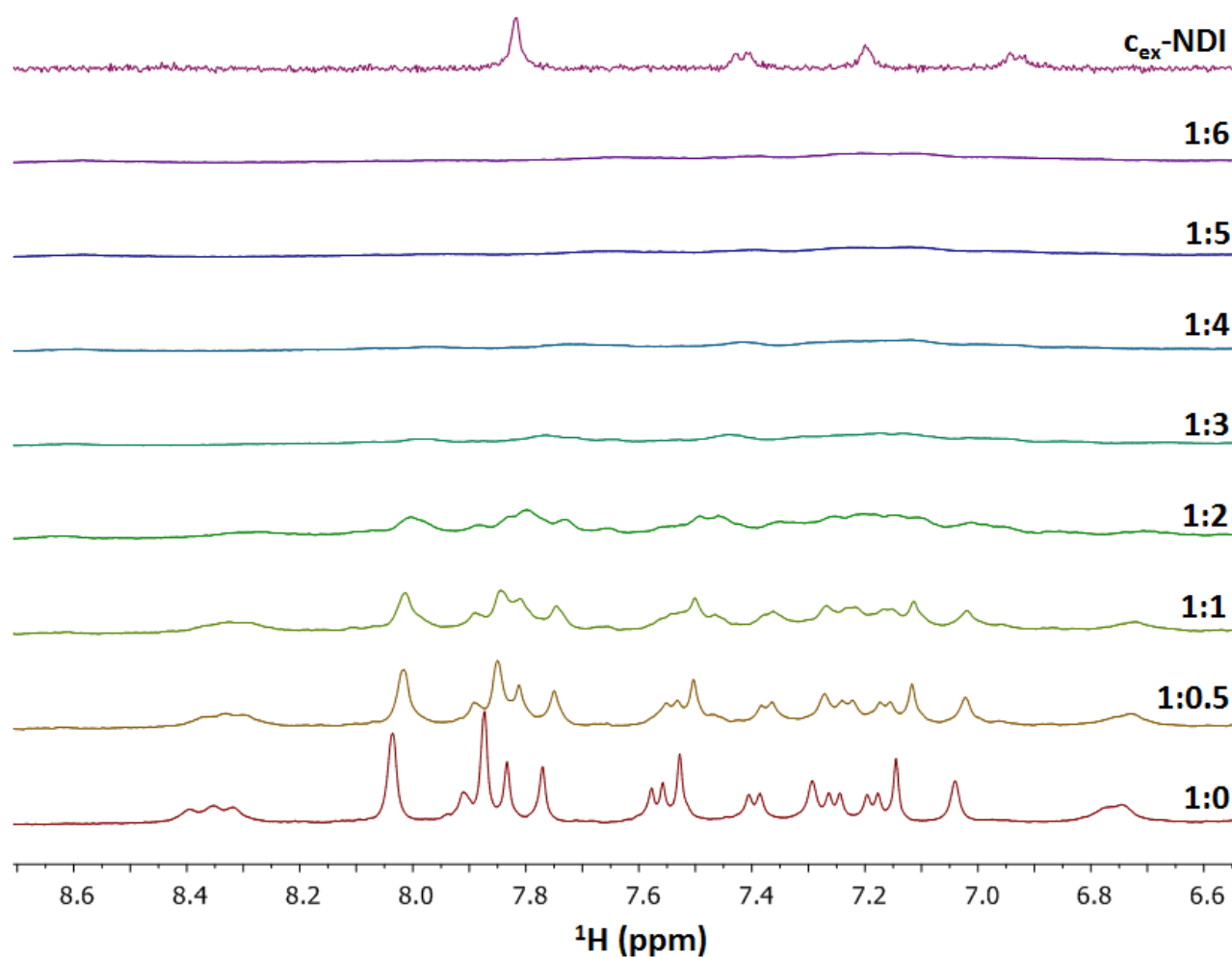


Figure S6. Aromatic proton regions of the ^1H NMR spectra of ds12 duplex upon titration with $\text{c}_{\text{ex}}\text{-NDI}$ (from 0.5 to 6 equivalents). On top, NMR spectrum of free $\text{c}_{\text{ex}}\text{-NDI}$ (0.2 mM) is reported. DNA/ $\text{c}_{\text{ex}}\text{-NDI}$ ratios are shown on the right of the corresponding spectrum.

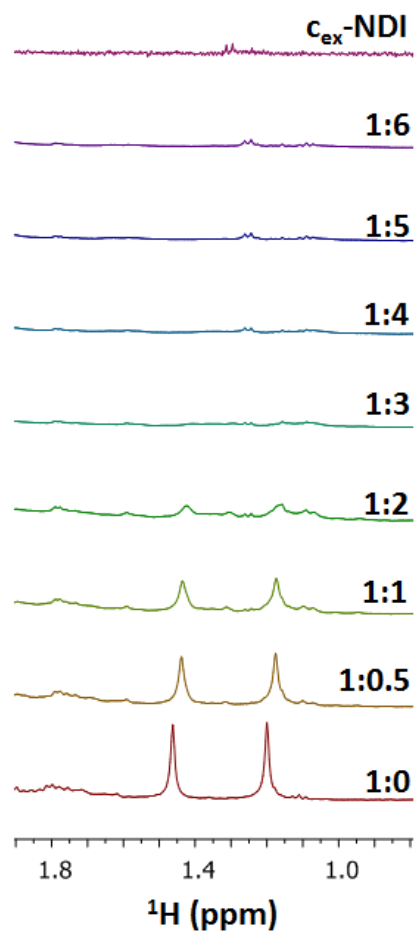


Figure S7. Methyl proton regions of the ^1H NMR spectra of ds12 duplex upon titration with $\text{c}_{\text{ex}}\text{-NDI}$ (from 0.5 to 6 equivalents). On top, NMR spectrum of free $\text{c}_{\text{ex}}\text{-NDI}$ (0.2 mM) is reported. DNA/ $\text{c}_{\text{ex}}\text{-NDI}$ ratios are shown on the right of the corresponding spectrum.

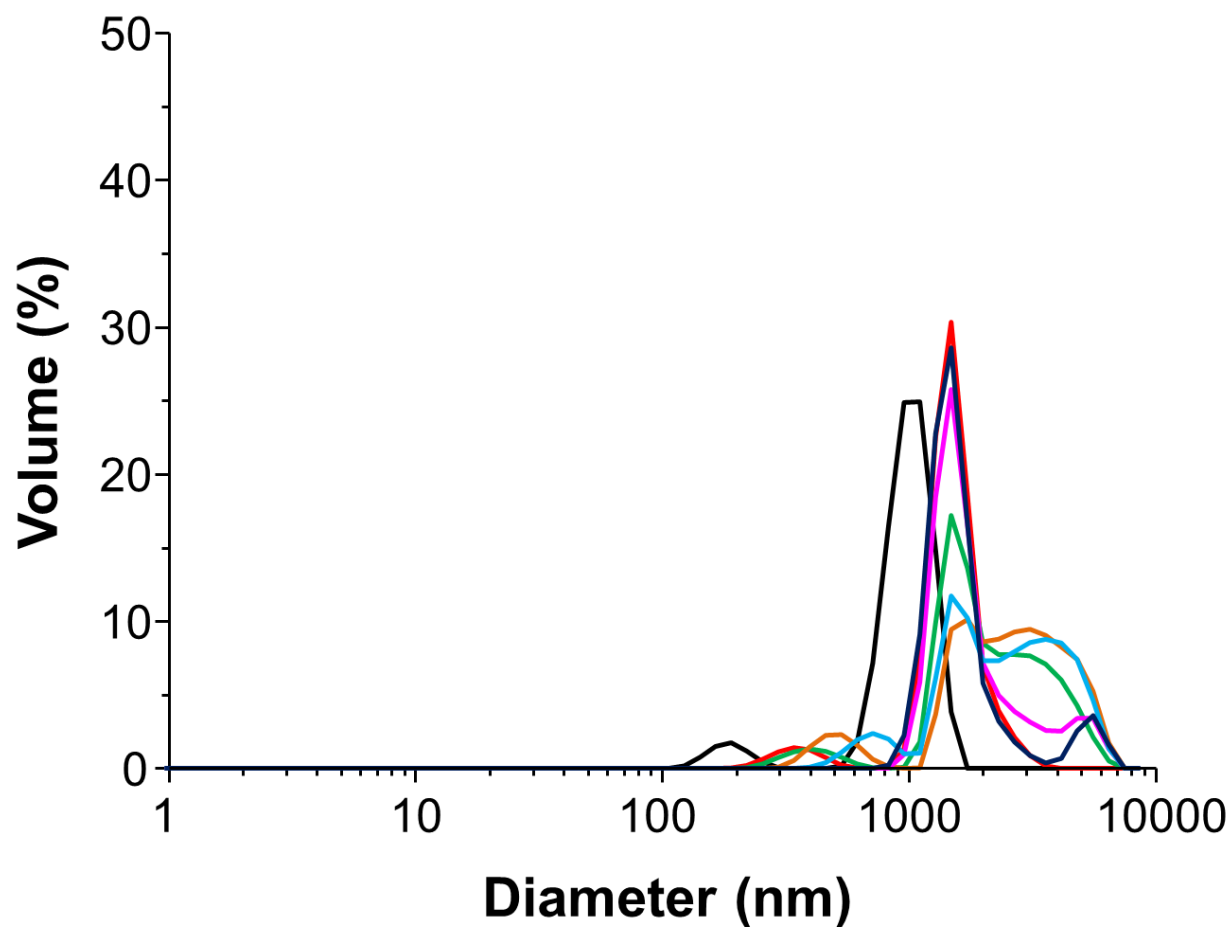


Figure S8. Volume-based particle size distribution for different amounts of c_{ex} -NDI corresponding to 0.5 (black line), 1 (red line), 2 (blue line), 3 (fuchsia line), 4 (green line), 5 (cyan line) and 6 (orange line) ligand equivalents with respect to DNA in the DLS titration experiments.

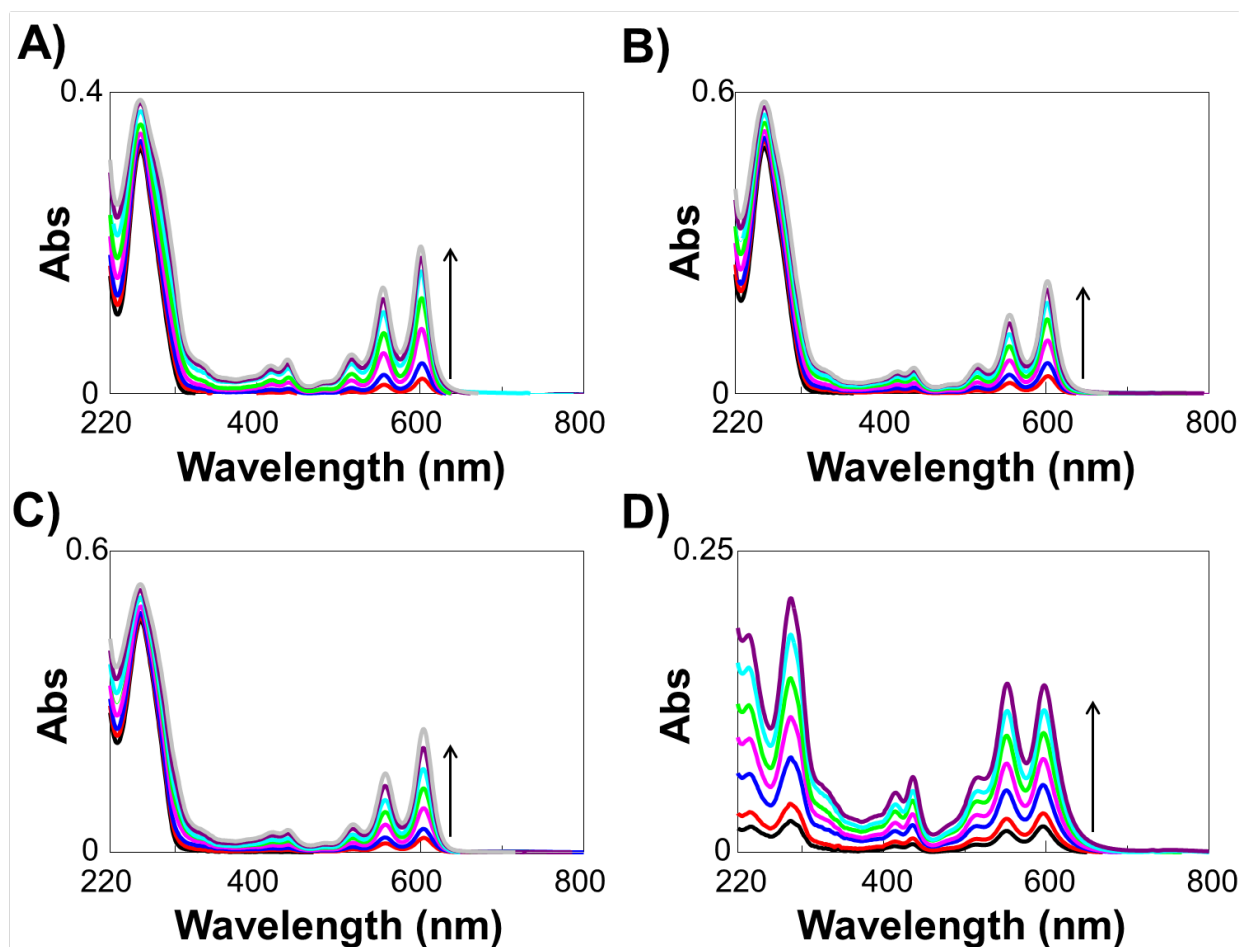


Figure S9. UV-vis spectra in 100 mM KCl and 20 mM potassium phosphate buffer (pH 7.0) of 2 μ M solutions of A) m-tel24 G-quadruplex, B) M2 G-quadruplex and C) ds12 duplex in the absence or presence of increasing amounts (up to 6 molar equivalents) of c_{ex} -NDI and D) free c_{ex} -NDI at different concentration (from 1 up to 12 μ M). The arrows indicate UV-vis band intensity variation on increasing c_{ex} -NDI concentration.

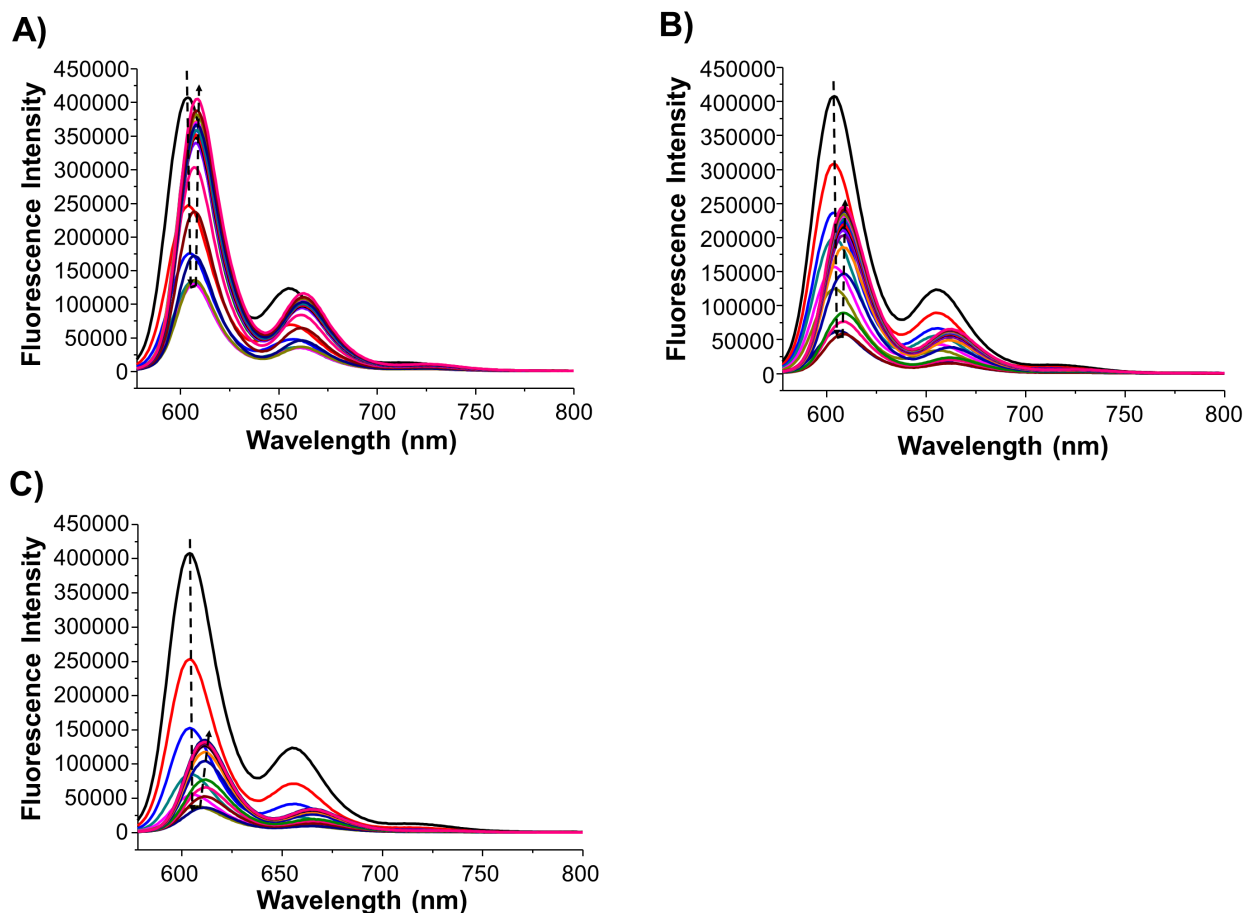


Figure S10. Fluorescence titration experiments of c_{ex} -NDI with A) m-tel24 G-quadruplex, B) M2 G-quadruplex and C) ds12 duplex. The arrows indicate fluorescence band intensity variation on increasing DNA concentration.

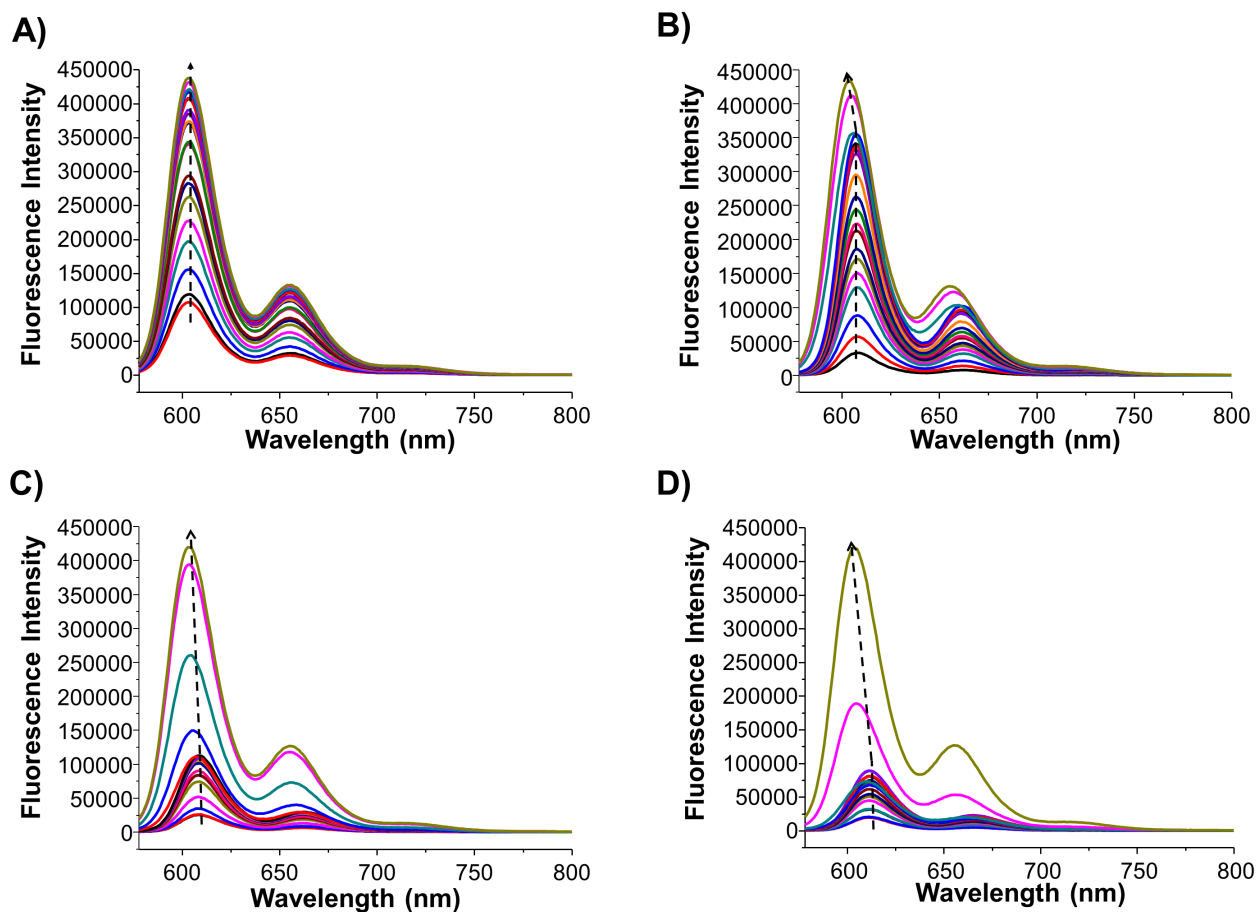


Figure S11. Fluorescence spectra of c_{ex} -NDI in the A) absence and presence of B) m-tel24 G-quadruplex, C) M2 G-quadruplex and D) ds12 duplex recorded for Job plot analysis. The arrows indicate fluorescence band intensity variation on increasing c_{ex} -NDI concentration.

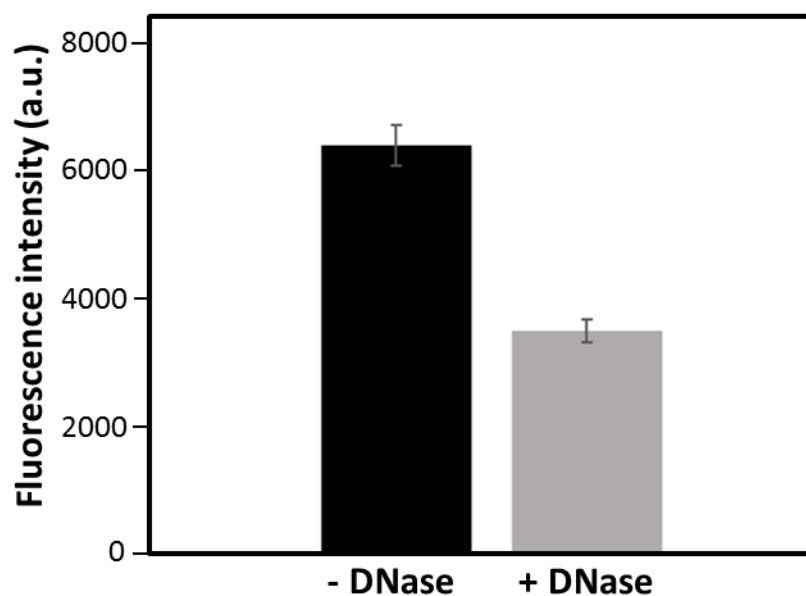


Figure S12. Fluorescence analysis of MCF7 cells incubated with 1.25 μM **c_{ex}-NDI** with or without a subsequent treatment with DNase. Quantification of the fluorescence signal intensity was performed with Zen Blue software on at least 6 different cell acquisitions.

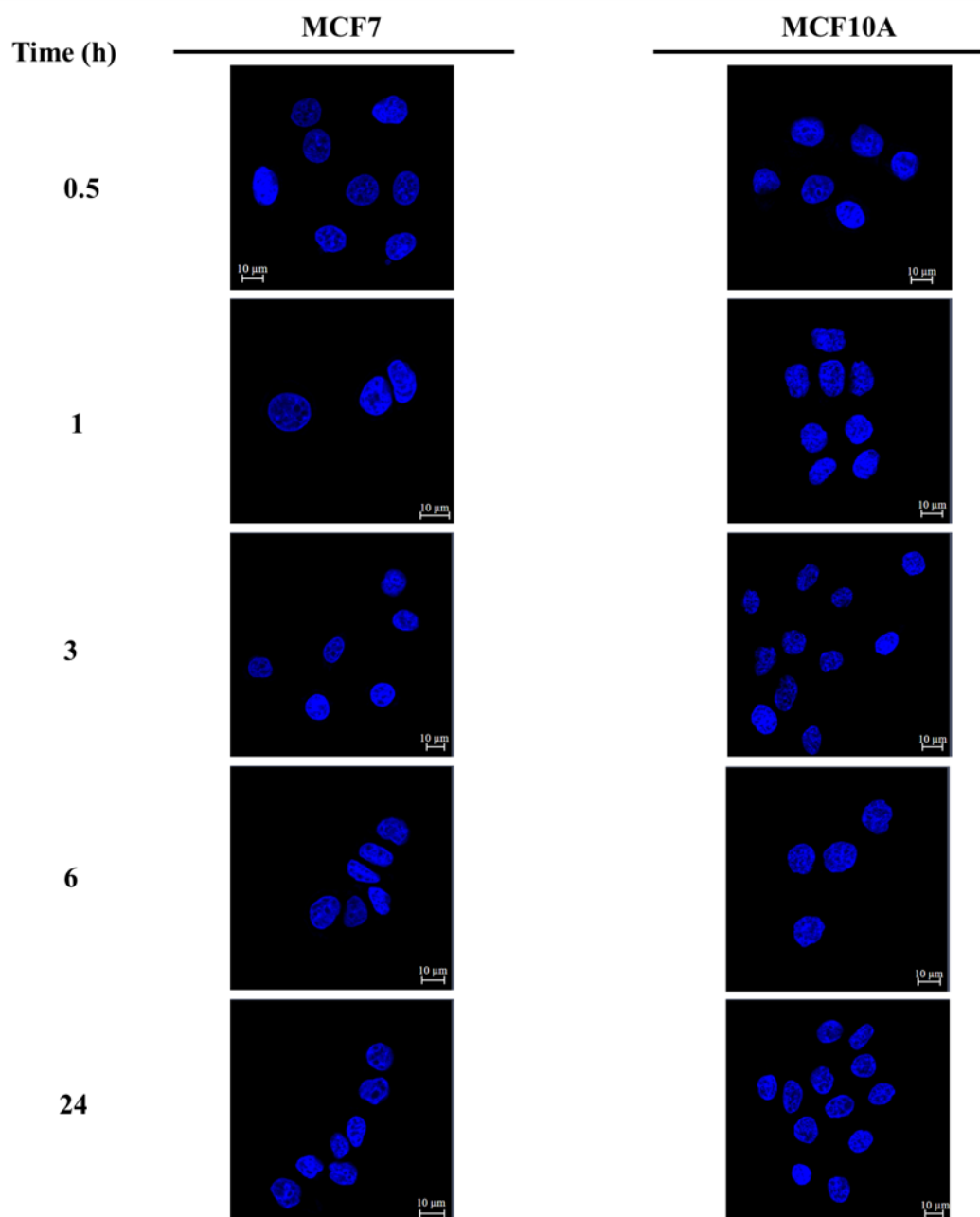


Figure S13. Confocal microscopy analysis of MCF7 and MCF10A cells. Cells were cultured on glass coverslips in 24-well plates, grown to semi-confluency and analysed after 0.5 - 1 - 3 - 6 - 24 h. Cell nuclei were stained by incubating the cells with 0.001 mg/mL Hoechst in PBS 1X for 20 min in the dark. After fixing the cells with 4% paraformaldehyde, they were analysed by CLSM by using a 63x oil immersion objective. Scale bars correspond to 10 μ m.