Supporting Information

DNA structure-specific sensitization of a metalloporphyrin leads to an efficient *in vitro* quadruplex detection molecular tool

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Part 1. Chemistry

General procedure. The handling of all air/water sensitive materials was carried out using standard techniques. Unless specified otherwise all other solvents were used as commercially supplied. ^{1}H NMR spectra were recorded at room temperature on a Bruker Avance II 300; chemical shifts are expressed in ppm relative to MeOD-*d4* (3.31 ppm). UV-Vis spectra were recorded in solutions using a Varian Cary 50 spectrophotometer (1 cm path length quartz cell). Accurate mass measurements (HR-MS) were carried out using a LTQ Orbitrap XL (THERMO) mass spectrometer. All measurements were made at the "*Welience, Pôle Chimie Moléculaire de l'Université de Bourgogne* (*WPCM*)".

Synthesis of Pd.TEGPy. TEGPy (40.0 mg, 26.2 µmol, obtained according to the procedure described in A. Laguerre *et al.*, *Org. Biomol. Chem.*, 2015, **13**, 7034) and $PdCl_2$ (5.60 mg, 31.6 µmol) were added into 2.0 mL deionized water. The resultant mixture was heated under refluxed until the absorbance peak at 426 nm (Soret band of TEGPy in MeOH) blue-shifted to 418 nm (Soret band of Pd.TEGPy in MeOH) without any change afterwards. The mixture was evaporated and purified by size-exclusion chromatography (Bio-rad Bio-Beads S-X1, DMF). The resulting product was passed through a Dowex 1x8 100 mesh ion-exchange column (H_2O). The title compound was isolated as a red solid in 96% yield (36.6 mg, 25.1 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 9.39 (d, J = 6.3 Hz, 8H, C_5H_4N), 9.07 (s, 8H, pyrrole-H), 8.87 (d, J = 6.3 Hz, 8H, C_5H_4N), 5.12 (m, 8H, CH_2), 4.24 (m, 8H, CH_2), 3.80 (m, 8H, CH_2), 3.67 (m, 8H, CH_2), 3.61 (m, 8H, CH_2), 3.45 (m, 8H, CH_2), 3.11 (s, 12H, OCH_3). HRMS (ESI): calcd for $[C_{68}H_{86}N_8O_{12}Pd]^{3+}$, m/z = 436.84164, found: 436.84114 ($[M-4Cl]^{3+}$). UV−Vis (MeOH): λ_{max} [nm] (ε $M^{-1}cm^{-1}$): 418 (52290) 526 (6310), 558 (2770).

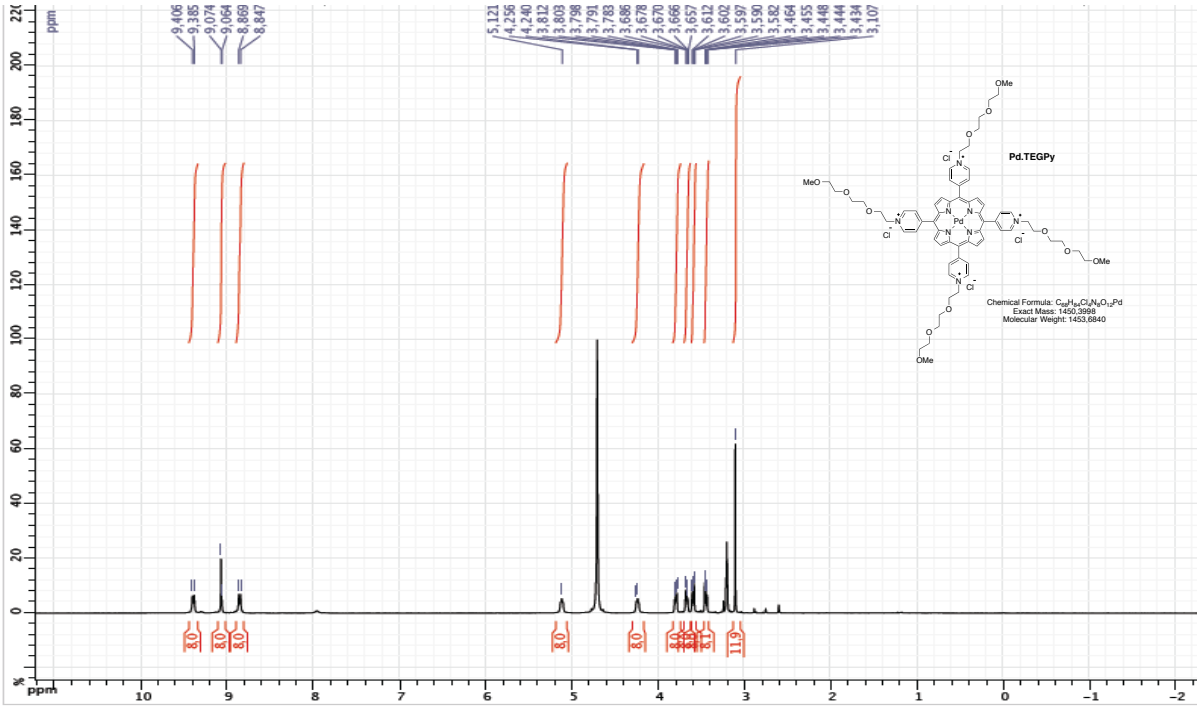


Figure S1. ^{1}H NMR spectrum of **Pd.TEGPy** (MeOD, 298K).

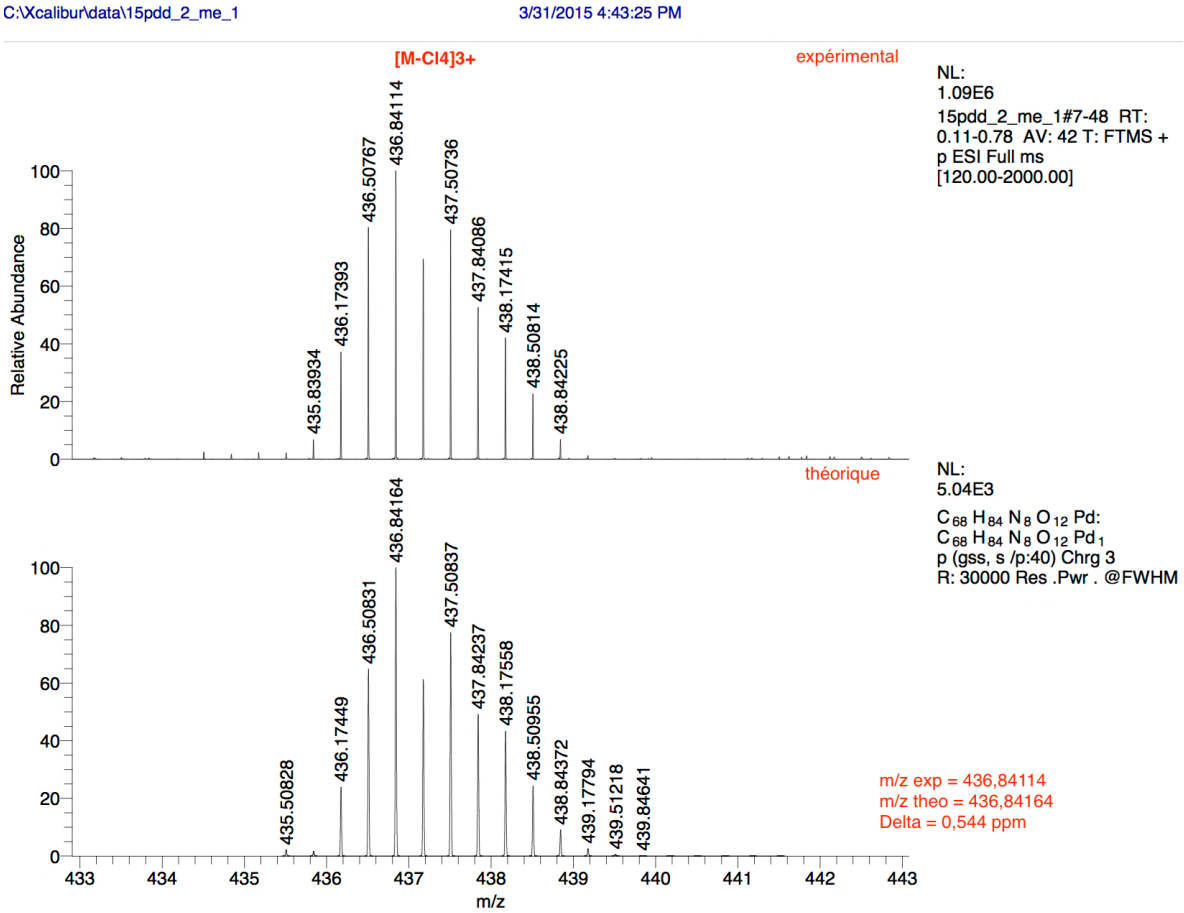


Figure S2. HRMS (ESI) mass spectrum of **Pd.TEGPy**.

Part 2. Dynamic light scattering.

a) DLS measurements performed on a Malvern apparatus with 100µM of TMPyP4 in water:

Sample Details

Sample Name:	TMPyP4 100microM 3	**SOP Name:**	mansettings.nano
File Name:	GATTACA.dts	**Dispersant Name:**	Water
Record Number:	47	**Dispersant RI:**	1,330
Measurement Date and Time:	jeudi 21 mai 2015 15:20:18	**Viscosity (cP):**	0,8872

System

Temperature (°C):	25,0	**Duration (s):**	10
Count Rate (kcps):	338,0	**Duration Used (s):**	60
Derived Count Rate (kcps):	7681,1	**Measurement Position (mm):**	4,65
Cell Description:	Low volume glass cuvette (45...	**Attenuator:**	8

Results

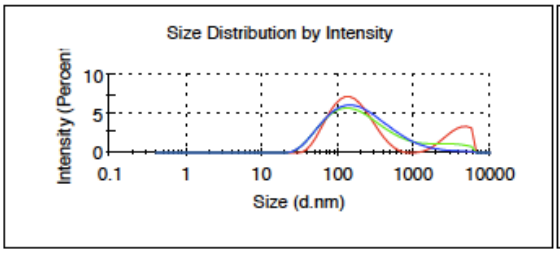
Z-Average (d.nm):	130,5		**Size (d.n...**	**% by Intensity**	**% by Volume**
PdI:	0,449	**Peak 1:**	336,1	100,0	66,8
Intercept:	0,009	**Peak 2:**	0,000	0,0	24,5
CPF:	0,00	**Peak 3:**	0,000	0,0	8,7
Result quality	Refer to quality report				

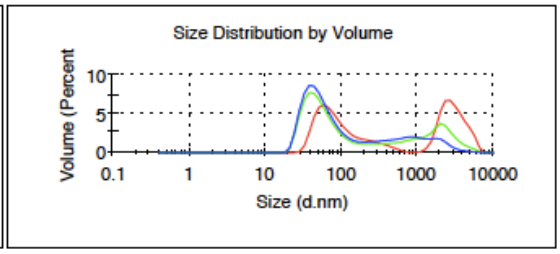
Cumulants Analysis Parameters

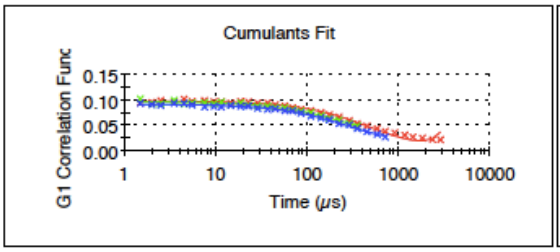
First Point:	3
Cut-off:	0,100

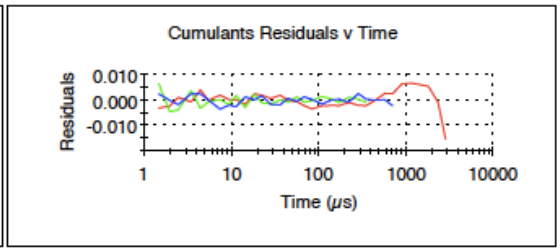
Multimodal Analysis Parameters

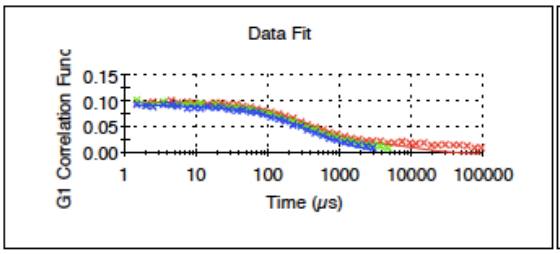
FirstPoint:	3	**Lower Size (d.nm):**	0,6000
		Upper Size (d.nm):	6000

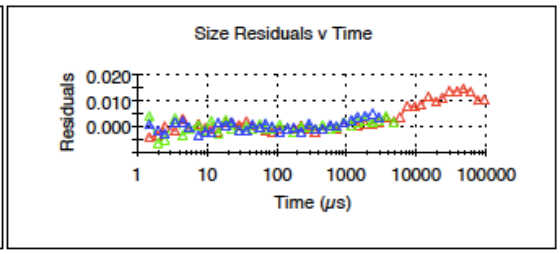












b) DLS measurements performed with 100µM of TEGPy in water:

Sample Details

Sample Name:	Base libre 100microM 3	**SOP Name:**	mansettings.nano
File Name:	GATTACA.dts	**Dispersant Name:**	Water
Record Number:	44	**Dispersant RI:**	1,330
Measurement Date and Time:	jeudi 21 mai 2015 15:13:11	**Viscosity (cP):**	0,8872

System

Temperature (°C):	25,0	**Duration (s):**	10
Count Rate (kcps):	263,2	**Duration Used (s):**	70
Derived Count Rate (kcps):	20864,8	**Measurement Position (mm):**	4,65
Cell Description:	Low volume glass cuvette (45...	**Attenuator:**	7

Results

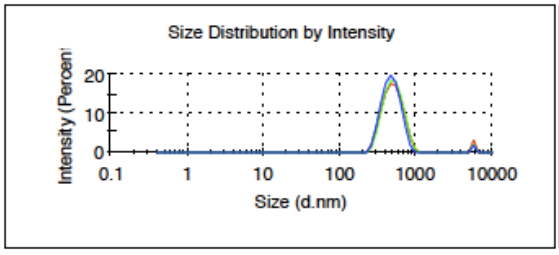
Z-Average (d.nm):	670,2		**Size (d.n...**	**% by Intensity**	**% by Volume**
PdI:	0,477	**Peak 1:**	476,3	98,2	97,6
Intercept:	0,338	**Peak 2:**	5560	1,8	2,4
CPF:	0,00	**Peak 3:**	0,000	0,0	0,0
Result quality	Refer to quality report				

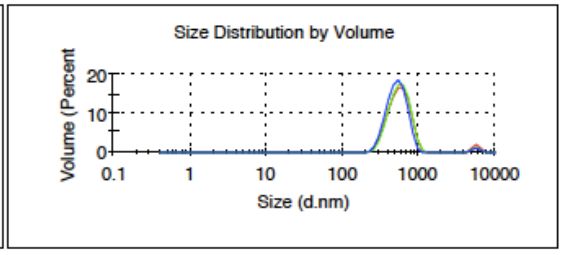
Cumulants Analysis Parameters

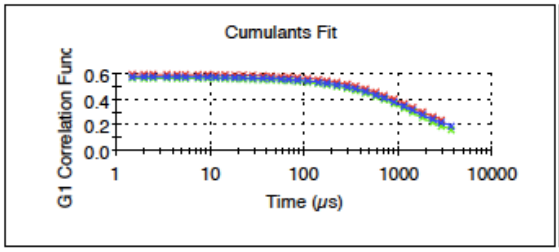
First Point:	3
Cut-off:	0,100

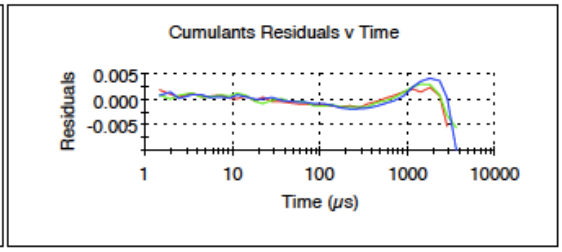
Multimodal Analysis Parameters

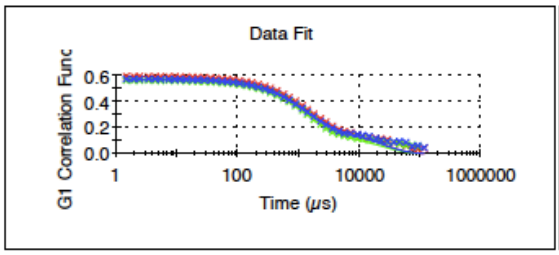
FirstPoint:	3	**Lower Size (d.nm):**	0,6000
		Upper Size (d.nm):	6000

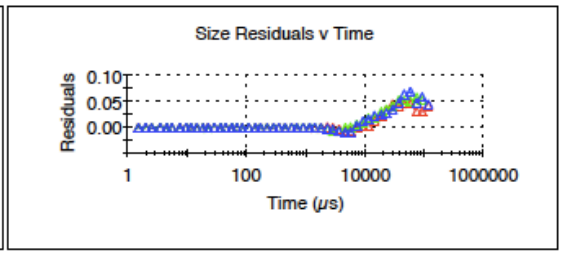












c) DLS measurements performed with 100µM of Pd.TEGPy in water:

Sample Details

Sample Name:	TEGPy Palladium 100microM 3	**SOP Name:**	mansettings.nano
File Name:	GATTACA.dts	**Dispersant Name:**	Water
Record Number:	66	**Dispersant RI:**	1,330
Measurement Date and Time:	jeudi 21 mai 2015 16:14:26	**Viscosity (cP):**	0,8872

System

Temperature (°C):	25,0	**Duration (s):**	10
Count Rate (kcps):	270,4	**Duration Used (s):**	60
Derived Count Rate (kcps):	2436,3	**Measurement Position (mm):**	4,65
Cell Description:	Low volume glass cuvette (45...	**Attenuator:**	9

Results

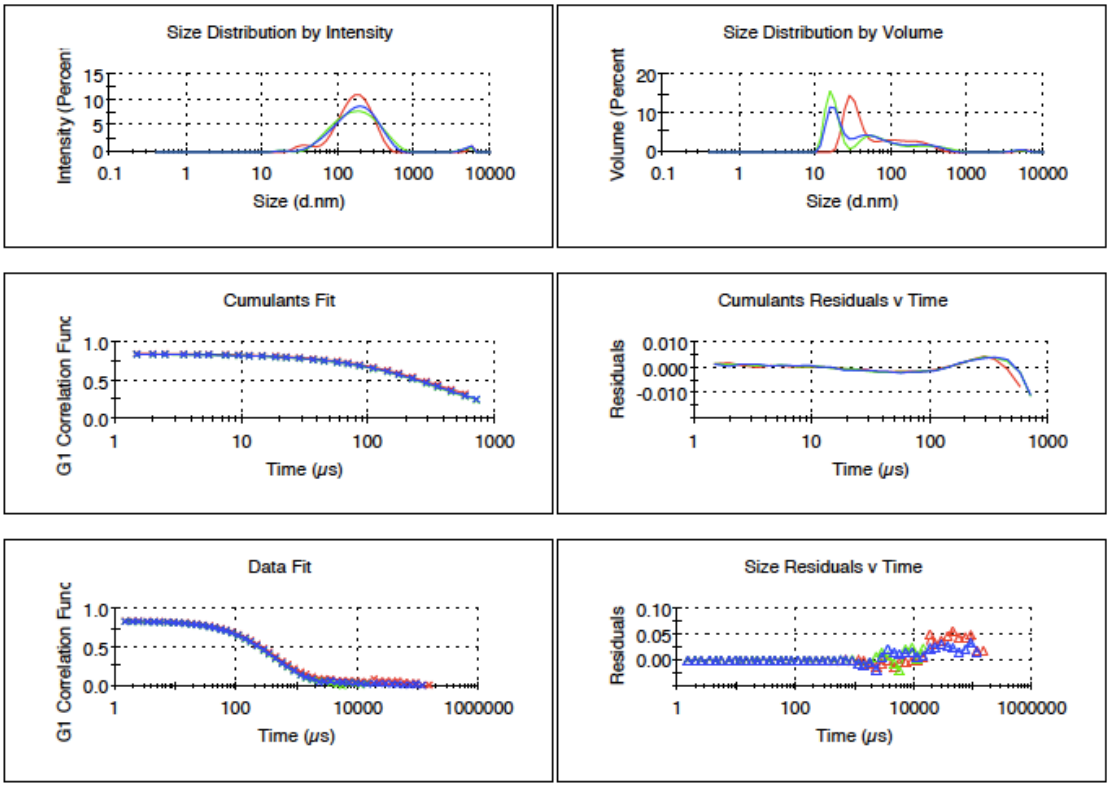
Z-Average (d.nm):	145,1		**Size (d.n...**	**% by Intensity**	**% by Volume**
PdI:	0,374	**Peak 1:**	195,4	96,4	44,9
Intercept:	0,706	**Peak 2:**	4976	2,6	39,2
CPF:	0,00	**Peak 3:**	19,44	0,9	14,0
Result quality	Good				

Cumulants Analysis Parameters

First Point:	3
Cut-off:	0,100

Multimodal Analysis Parameters

FirstPoint:	3	**Lower Size (d.nm):**	0,6000
		Upper Size (d.nm):	6000



Part 3. Circular dichroism spectroscopy.

CD spectra were recorded on a JASCO J-815 spectropolarimeter in a 10mm path-length quartz semi-micro cuvette (Starna). CD spectra were recorded over a range of 500-600nm (bandwidth =

3nm, 1nm pitch, 1s response, scan speed = 500nm.mn^{-1}, averaged over 3 scans, zeroed at 600nm) with 100μM of TEGPy, Pd.TEGPy and TMPyP4 in water. Final data were analyzed with OriginPro®8 (OriginLab Corp.).

Part 4. Oligonucleotides.

Preparation of stock solutions. The lyophilized DNA/RNA strands (purchased from Eurogentec, Seraing, Belgium) were firstly diluted in deionized water (18.2 MΩ.cm resistivity) at 500μM for monomolecular (*i.e.*, F21T, F-DS-T, L-TERRA, 22AG, c-myc, c-kit,) and bimolecular structures (*i.e.*, ds17 and ds26) or 1000μM for tetramolecular structures, (*i.e.*, TG5T). All DNA structures were prepared in a Caco.K buffer, comprised of 10mM lithium cacodylate buffer (pH 7.2) plus 10mM KCl/90mM LiCl. Monomolecular structures were prepared by mixing 40μL of the constitutive strand (500μM) with 8μL of a lithium cacodylate buffer solution (100mM, pH 7.2), plus 8μL of a KCl/LiCl solution (100mM/900mM) and 24μL of water. Bimolecular structures were prepared by mixing 40μL of each constitutive strand (500μM) with 16μL of a lithium cacodylate buffer solution (100mM, pH 7.2), plus 16μL of a KCl/LiCl solution (100mM/900mM) and 48μL of water. Tetramolecular structures were prepared by mixing 20μL of each constitutive strand (1000μM) with 32μL of a lithium cacodylate buffer solution (100mM, pH 7.2), plus 32μL of a KCl/LiCl solution (100mM/900mM) and 96μL of water. The final concentrations were theoretically 250, 125 and 83.3μM respectively for mono-, bi- and tetra-molecular DNA structures, respectively.

Name	**Sequence**
F21T	FAM-d[$^{5'}$GGGTTAGGGTTAGGGTTAGGG$^{3'}$]-TAMRA
F-DS-T	FAM-d[$^{5'}$TATAGCTATATTTTTTTATAGCTATA$^{3'}$]-TAMRA
L-TERRA	FAM-r[$^{5'}$GGGUUAGGGUUAGGGUUAGGG$^{3'}$]-TAMRA
22AG	d[$^{5'}$AGGGTTAGGGTTAGGGTTAGGG$^{3'}$]
c-myc	d[$^{5'}$GAGGGTGGGGAGGGTGGGGAAG$^{3'}$]
c-kit	d[$^{5'}$CGGGCGGGCGCGAGGGAGGGG$^{3'}$]
TG5T	*strands 1-4:* d[$^{5'}$TGGGGGT$^{3'}$]
ds17	*strand 1*: d[$^{5'}$CCAGTTCGTAGTAACCC$^{3'}$] *strand 2*: d[$^{5'}$GGGTTACTACGAACTGG$^{3'}$]
ds26	*strand 1-2*: d[$^{5'}$CAATCGGATCGAATTCGATCCGATTG$^{3'}$]

The actual concentration of each DNA was determined through a dilution to 1μM theoretical concentration (expressed in motif concentration) for monomolecular structures (*i.e.*, 4μL in 996μL water), to 1μM for bimolecular structures (i.e., 8μL in 992μL water) and to 0.2μM for tetramolecular structure (i.e., 2.4μL in 997.6μL water) through UV spectral analysis at 260nm (after 5min at 90°C) with the following molar extinction coefficient values: 268300 (F21T), 258900 (F-ds-T), 276700 (L-TERRA), 228500 (22AG), 232000 (c-myc), 205600 (c-kit), 271600 (TG5T), 328300 (ds17) and 506400 M^{-1}.cm^{-1} (ds26).

Folding of higher-order structures. The higher-order DNA/RNA structures were folded according to two procedures: (a) for the monomolecular architectures, solutions were heated (90°C, 5 min), cooled on ice (7hrs) and then stored at least overnight (4°C); (b) for the folding of all other

structures, the solutions were heated (90°C, 5min), gradually cooled (65, 60, 55, 50, 40 and 30°C (60mn/step), 25°C (2hr)) and then stored at least overnight (4°C).

Part 5. FRET-melting experiments.

Experiments were performed in a 96-well format using a Mx3005P qPCR machine (Agilent) equipped with a FAM filter (λ_{ex} = 492nm; λ_{em} = 516nm) in 100µL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 99mM LiCl/1mM KCl for L-TERRA and + 90mM LiCl/10mM KCl for F21T and F-DS-T, with 0.2µM of labeled oligonucleotide and 5 equiv. of ligands (TMPyP4, TEGPy and Pd.TEGPy). Competitive experiments were carried out with labeled oligonucleotide (0.2µM), 5 equiv. ligands (TMPyP4, TEGPy and Pd.TEGPy) and increasing amounts (0, 15 and 50 equiv.) of the unlabeled competitor ds26. After a first equilibration step (25°C, 30s), a stepwise increase of 1°C every 30s for 65 cycles to reach 90°C was performed, and measurements were made after each cycle. Final data were analyzed with Excel (Microsoft Corp.) and OriginPro®8 (OriginLab Corp.). The emission of FAM was normalized (0 to 1), and $T_{1/2}$ was defined as the temperature for which the normalized emission is 0.5; $\Delta T_{1/2}$ values are means of 3 experiments.

Part 6. Fluorescence and UV-Vis investigations.

UV-Vis spectra were recorded on a JASCO V630Bio and fluorescence spectra on a JASCO FP8500 in a 10mm path-length quartz semi-micro cuvette (Starna). UV-Vis experiments were carried out in 1mL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 90mM LiCl/10mM KCl, with ligands (TMPyP4, TEGPy and Pd.TEGPy, 2µM) alone and in presence of TG5T (up to 20µM).

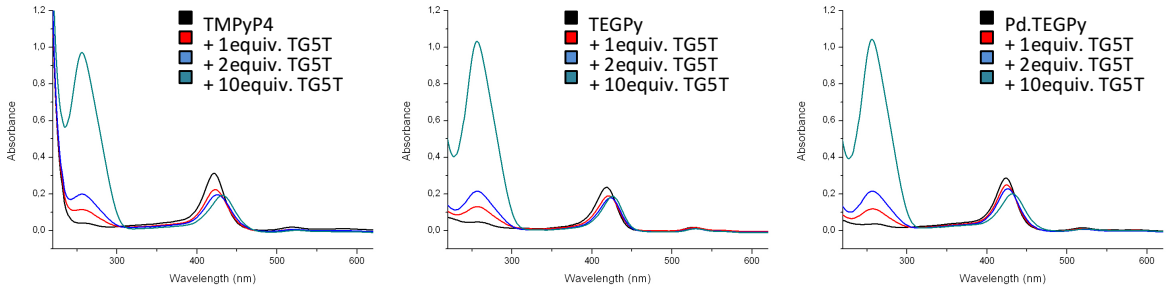


Figure S3. UV-Vis titrations of TMPyP4, TEGPy and Pd.TEGPy with increasing amounts (1, 2 and 10 equiv.) of TG5T.

Fluorescence experiments were carried out in 1mL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 90mM LiCl/10mM KCl, with ligands (TMPyP4, TEGPy and Pd.TEGPy, 2µM) alone and in presence of DNA oligonucleotides (up to 20µM) being either duplexes (ds17, ds26) or quadruplexes (22AG, myc, kit and TG5T). Spectra (λ_{ex} = 256nm, λ_{em} = 425-625nm, Ex and Em slits = 5nm, 1nm pitch, 1s response, scan speed = 500nm.mn^{-1}) were recorded 5mn (at 25°C) after the addition of the oligonucleotides.

DNA-sensitizing experiments were carried out in 1mL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 90mM LiCl/10mM KCl, ligands (TMPyP4, TEGPy and Pd.TEGPy, 2µM) in presence of oligonucleotides (TG5T or ds26, 20µM). Spectra were recorded at 15 different λ_{ex} between 226 and 306nm (Ex and Em slits = 5nm, 1nm pitch, 1s response, scan speed = 500nm.mn^{-1}) at 25°C.

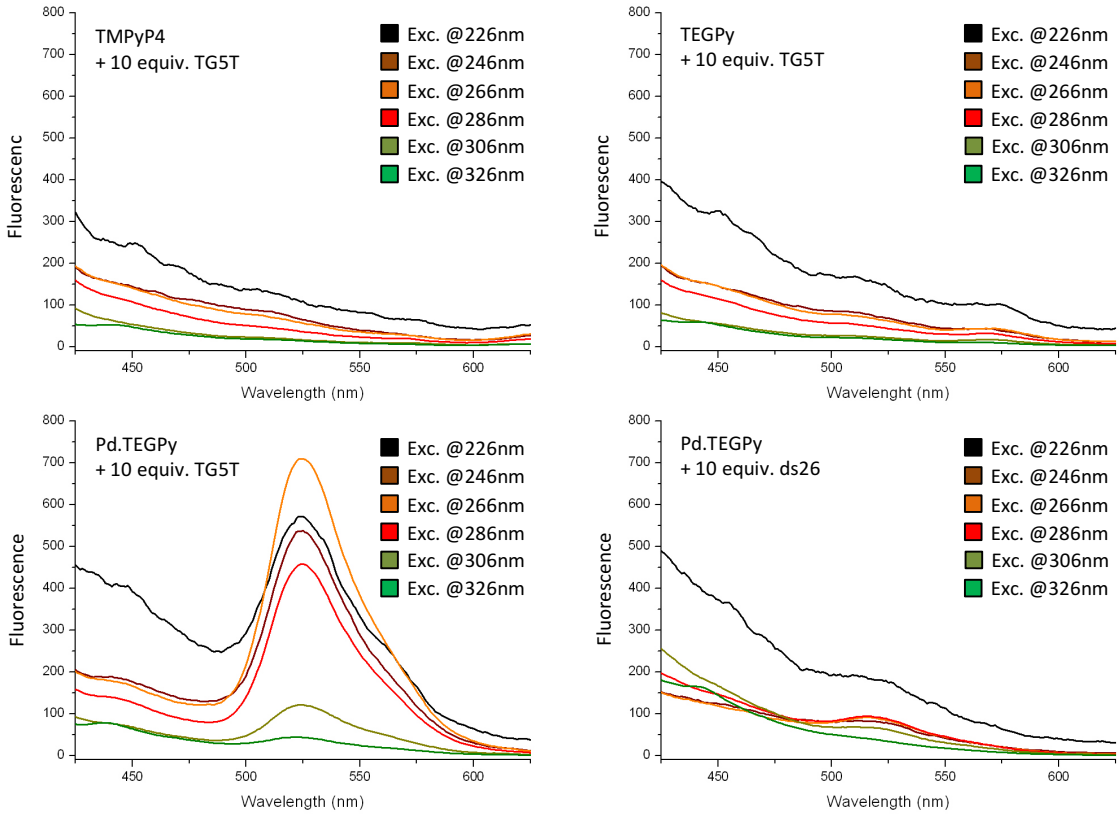


Figure S4. DNA-mediated fluorescence experiments carried out with TMPyP4, TEGPy and Pd.TEGPy and 10 equiv. of TG5T or ds26 at various λ_{ex} between 226 and 306nm.

Control experiment for the screening effect described in Figure 6B of the main manuscript: experiments were carried out in 1mL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 90mM LiCl/10mM KCl, with Pd.TEGPy (2µM) in presence of of an excess of 22AG (up to 20µM). Spectra (λ_{ex} = 256nm, λ_{em} = 518nm, Ex and Em slits = 5nm, 1nm pitch, 1s response, scan speed = 500nm.mn^{-1}) were recorded 5mn (at 25°C) after the addition of every 2µM of 22AG.

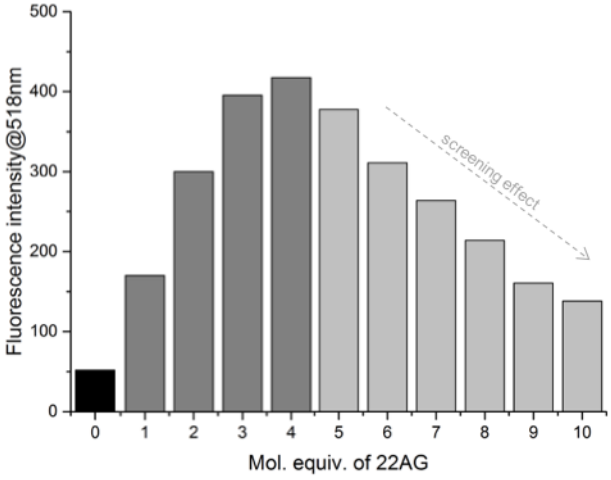


Figure S5. Figure S5. Fluorescence titrations (λ_{ex} = 256nm) of 2µM Pd.TEGPy upon addition of increasing amounts of 22AG (0 to 20µM, 2µM-step), carried out in lithium cacodylate buffer (pH 7.2) plus 10mM KCl/90mM LiCl.