Direct visualization of quadruplex/ligand interactions *via* high-speed atomic force microscopy

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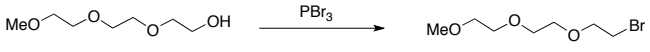
-- Supporting Information --

Part 1. Chemistry.

General procedures. The handling of all air/water sensitive materials was carried out using standard techniques. DMF was dried over activated molecular sieves under Ar. Unless specified otherwise all other solvents were used as commercially supplied. Where mixtures of solvents were used, ratios are reported by volume. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance II 300 or 500 MHz; chemical shifts are expressed in ppm relative to chloroform (7.26 ppm) or Methanol-d_4 (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 Bruker microTOF-QTM ESI-TOF mass spectrometer. HPLC was carried out with a Thermo Ultimate 3000 spectrometer. All measurements were made at the "*Welience, Pôle Chimie Moléculaire de l'Université de Bourgogne* (*WPCM*)".



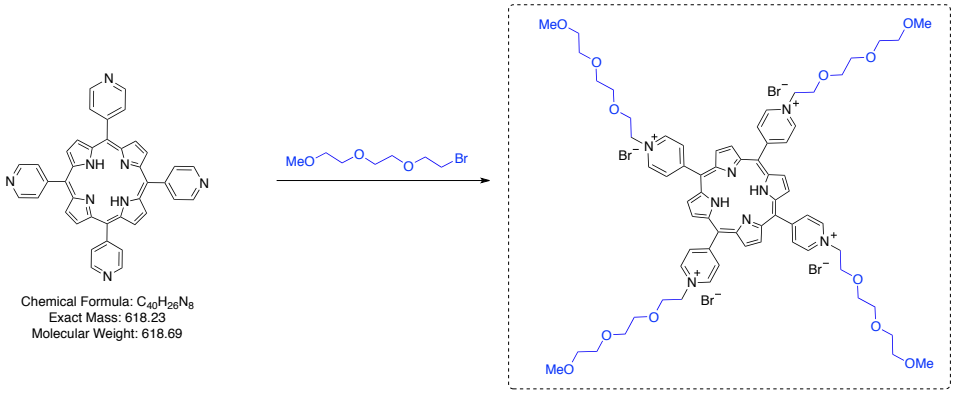
Chemical Formula: $C_7H_{16}O_4$
Exact Mass: 164.10
Molecular Weight: 164.20

Chemical Formula: $C_7H_{15}BrO_3$
Exact Mass: 226.02
Molecular Weight: 227.10

Step 1 : This reaction was carried out by a modification of the procedure reported by Cappelli et al. (see A. Cappelli, S. Galeazzi, M. Anzini, S. Vomero, "*Novel polybenzofulvene derivatives, synthesis and uses thereof*", WO2008037604, publication date: 2008-04-03) 2-(2-(2-methoxyethoxy)ethoxy)ethanol (20.5 g, 0.125 mol) was dissolved in ethyl ether (100 mL)

under N_2. Cooled it to 0 °C, then PBr_3 (6.00 mL, 63.2 mmol) was added dropwise into the solution. The reaction mixture was stirred for 15 min at this temperature. After that, methanol (7.5 mL) was added slowly and stirred for another 30 min. After the completion, the solution was diluted with water (25 mL) and washed thoroughly with saturated $NaHCO_3$, brine and dried over $MgSO_4$. Evaporated the solvent and dried under vacuum to obtain the light yellow liquid. **Br-PEG**: liquid, 5.55 g, yield: 20%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (t, 2H, J = 6.3 Hz), 3.65-3.56 (m, 6H), 3.52-3.46 (m, 2H), 3.40 (t, 2H, J = 6.3 Hz), 3.31 (s, 3H).

Step 2: The commercially availbale Meso-tetrakis-(4-pyridyl) porphine (TPyP) (100 mg, 0.162 mmol) and Br-PEG (1.00 g, 4.40 mmol) were mixed in DMF (6 mL) under Ar. The reaction mixture was stirred at 100 °C in the dark. The reaction was monitored by MALDI/TOF mass spectrometry. After the completion, removed the solvent under vacuum and recrystallized with water/acetone (1:2) to obtain a solid that is washed with CH_2Cl_2 and acetone.



Chemical Formula: $C_{40}H_{26}N_8$
Exact Mass: 618.23
Molecular Weight: 618.69

Chemical Formula: $C_{68}H_{86}Br_4N_8O_{12}$
Exact Mass: 1522.31
Molecular Weight: 1527.07

TEGPy: violet sticky solid, 200 mg, yield: 81%; ^{1}H NMR (300 MHz, Methanol-d_4) δ 9.51 (d, 8H, J = 6.6 Hz, C_5H_4N), 9.24 (se, 8H, pyrrole-H), 9.01 (d, 8H, J = 6.6 Hz, C_5H_4N), 5.23 (m, 8H, CH_2), 4.36 (m, 8H, CH_2), 3.91 (m, 8H, CH_2), 3.78 (m, 8H, CH_2), 3.71 (m, 8H, CH_2), 3.56 (m, 8H, CH_2), 3.21 (s, 12H, OCH_3); ^{13}C NMR (126 MHz, Methanol-d_4) δ 159.6, 146.4, 145.6, 145.4, 134.0, 117.2, 73.0, 71.6 (2C), 71.3, 70.3, 62.8, 59.0. ESI-HR-MS: calcd for $[C_{68}H_{86}N_8O_{12}]^{4+}$, m/z = 301.65858, found: 301.65807 ($[M-4Br]^{4+}$); calcd for $[C_{68}H_{85}N_8O_{12}]^{3+}$, m/z = 401.87568, found: 401.87420 ($[M-4Br-H]^{3+}$). UV–vis (water): λ_{max} [nm] (ε $M^{-1}cm^{-1}$) = 420 (105000), 518 (8000), 555 (4300), 585(5200), 645 (2900).

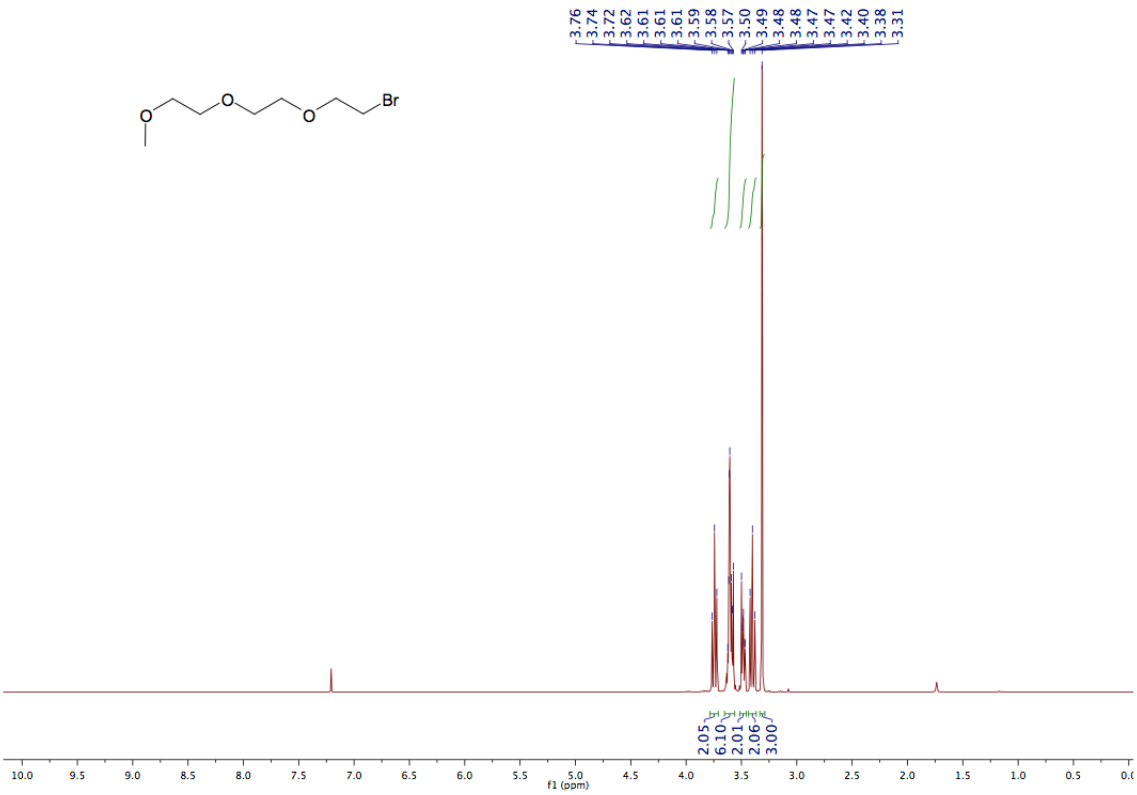


Figure S1. ^{1}H NMR spectrum of **Br-PEG** ($CDCl_3$, 298K)

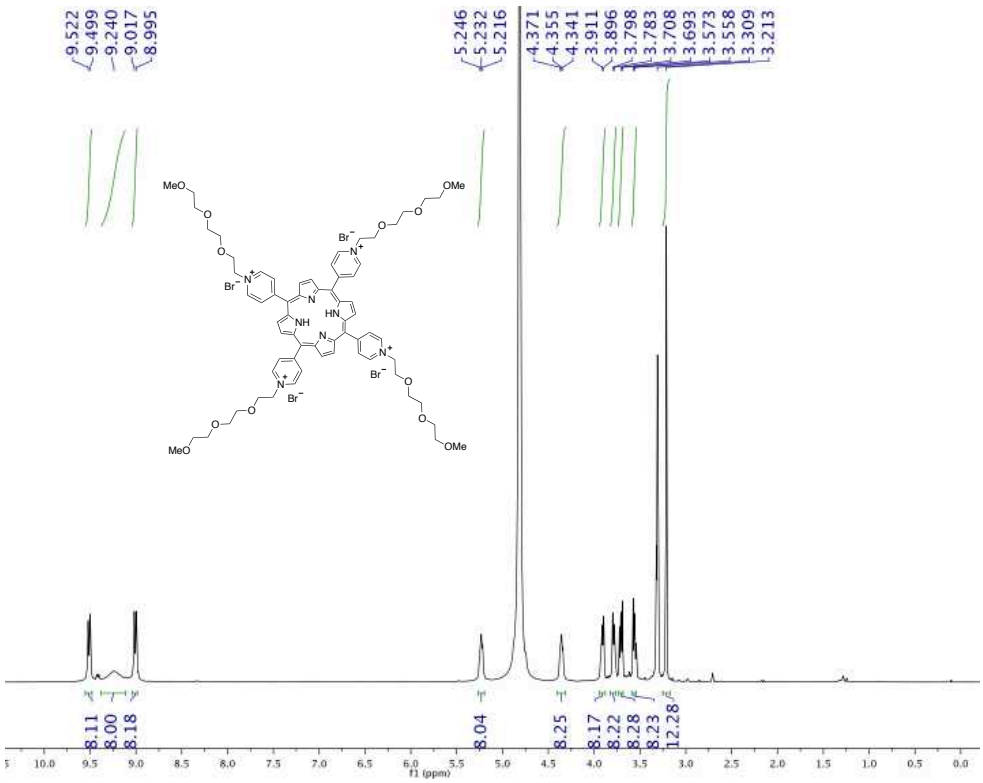


Figure S2. ^{1}H NMR spectrum of **TEGPy** (Methanol-d_4, 298K)

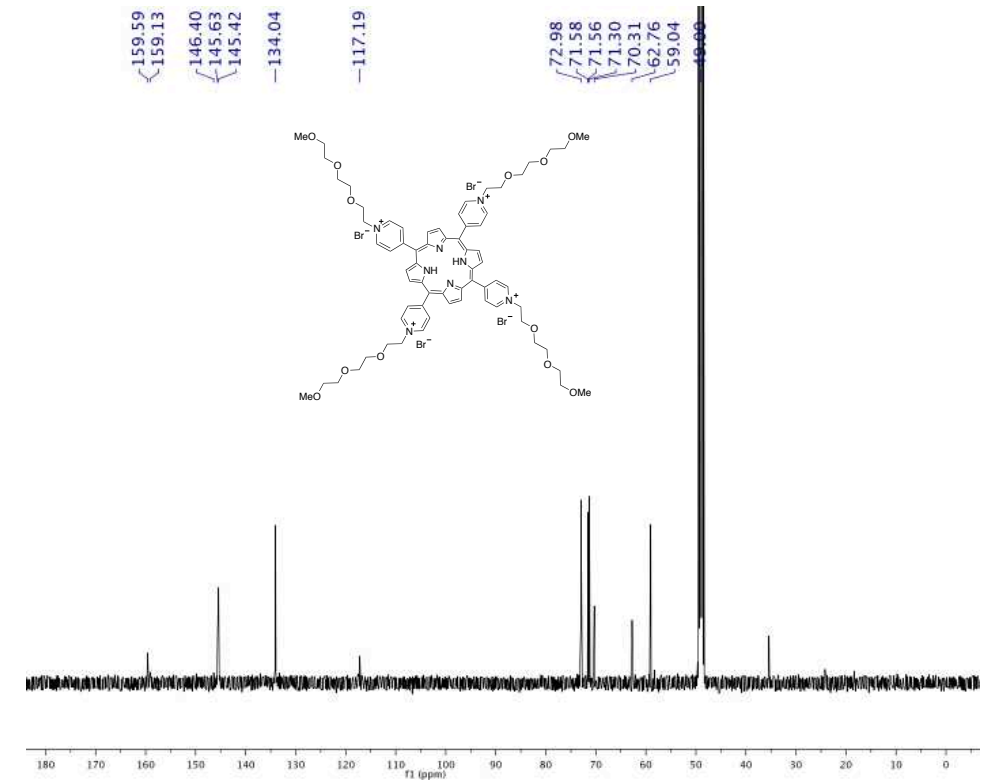
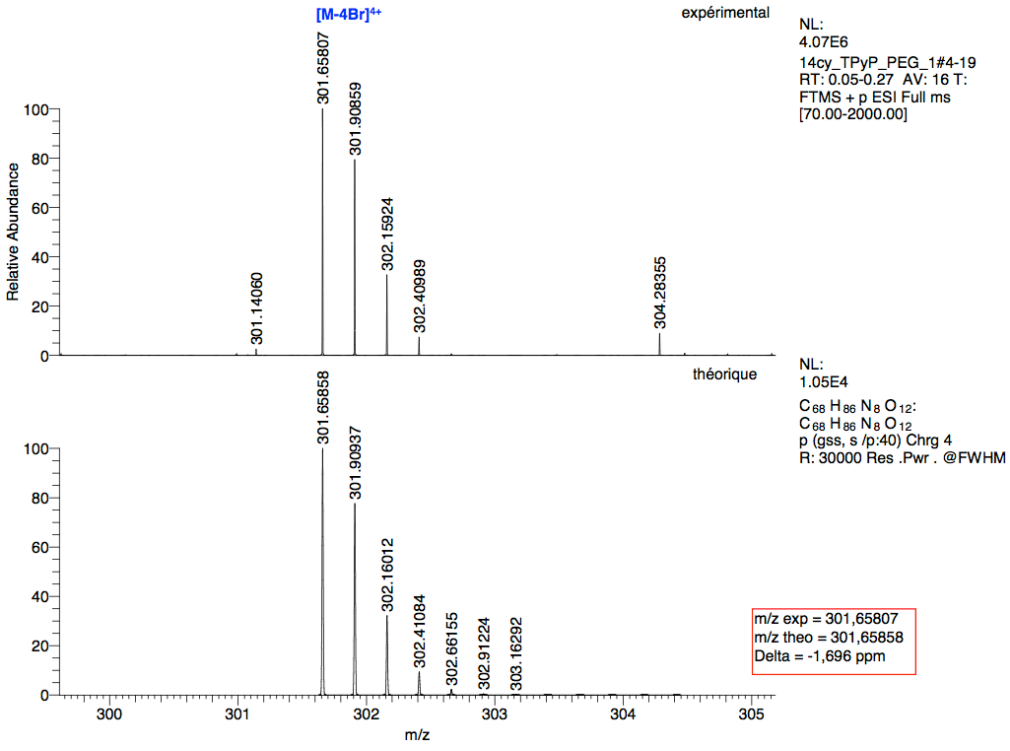


Figure S3. ^{13}C NMR spectrum of **TEGPy** (Methanol-d_4, 298K)



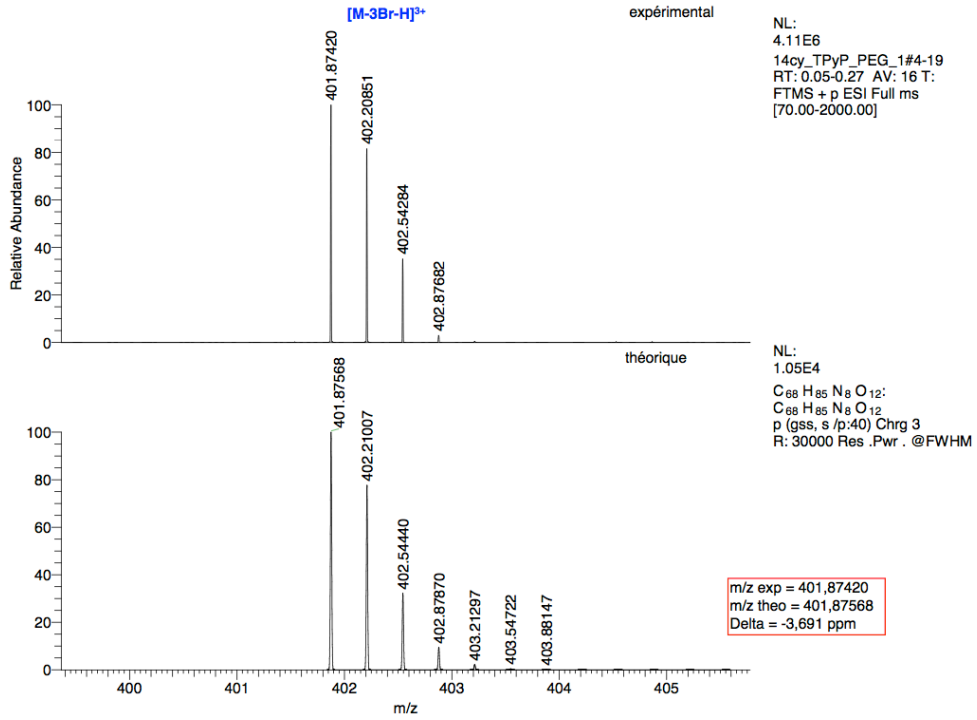


Figure S4. HR-MS (ESI) mass spectra of **TEGPy**.

Part 2. Oligonucleotides

The lyophilized DNA/RNA strands (purchased from Eurogentec) were firstly diluted in deionized water (18.2 MΩ.cm resistivity) at 500µM (for monomolecular (F21T, F-Myc-T, F-kit-T, F-duplex-T, L-TERRA, L-TRF2, L-VEGF, 22AG) and bimolecular structures (ds26)) or 1000µM (for tetramolecular structures (TG5T)). All DNA/RNA 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. 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), 285300 (F-myc-T), 258900 (F-kit-T), 258900 (F-duplex-T), 276700 (L-TERRA), 231000 (L-TRF2), 242400 (L-VEGF), 228500 (22AG), 271600 (TG5T) and 506400 $M^{-1}.cm^{-1}$ (ds26). 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 3. UV-Vis investigations.

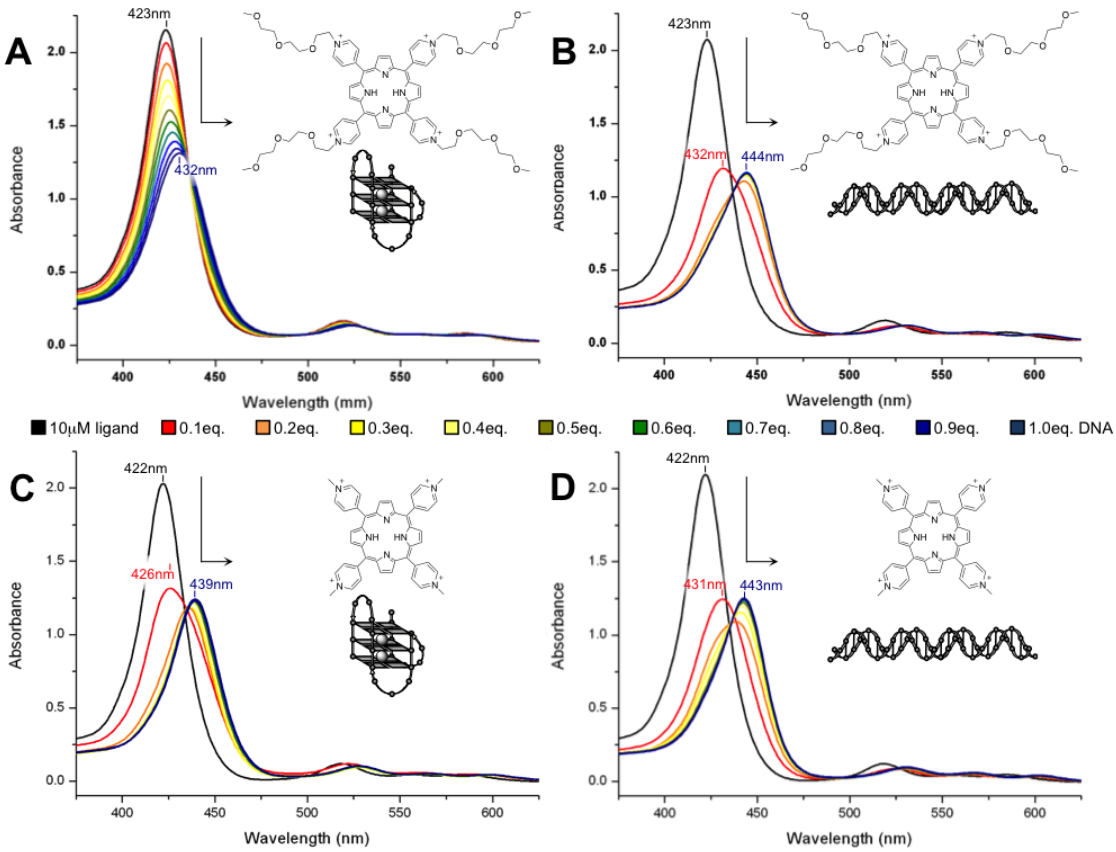


Figure S5. Quantification of the DNA-interacting properties of TEGPy (A,B) or $TMPyP_4$ (C,D), upon addition of increasing concentrations of 22AG (A,C) or ds26 (B,D).

Spectra were recorded on a JASCO V630Bio spectrophotometer in a 10mm path-length quartz semi-micro cuvette (Starna). UV-Vis experiments were carried out with ligand alone, either TEGPy (A,B) or TMPyP4 (C,D) (10µM, 10mM in H_2O, 1.0µL) in 1mL (final volume) of water and in presence of increasing amounts (from 0 to 1equiv., 0.1 equiv./step) of DNA, being either 22AG (A,C) or ds26 (B,D).

Part 4. FRET-melting experiments.

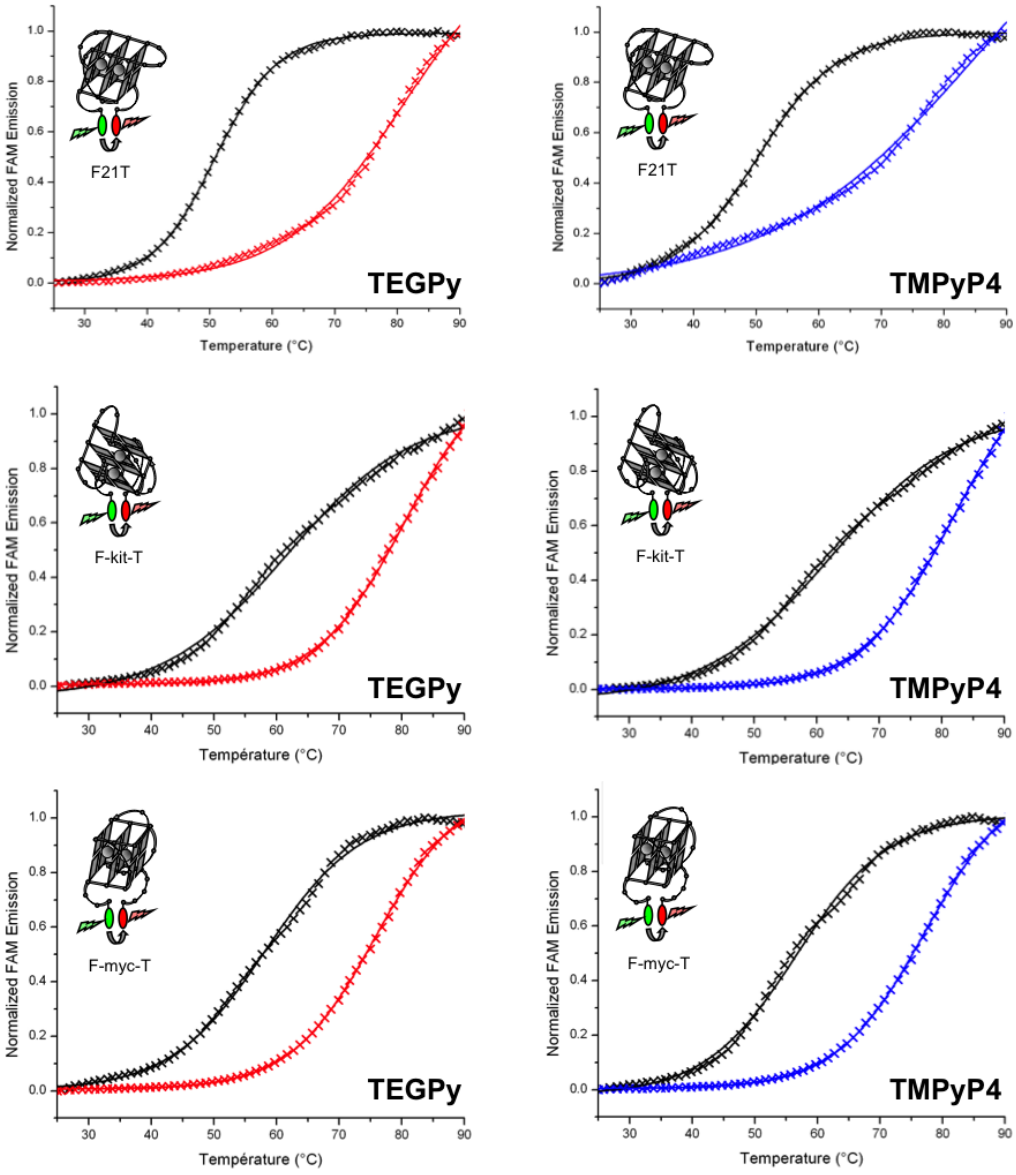


Figure S6. Evaluation of the quadruplex-DNA affinity of both TEGPy (left) and TMPyP4 (right) *via* experiments carried out with F21T, F-kit-T or F-myc-T (0.2µM) in presence of 1µM ligand (5 molar equivalents).

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).

Experiments (Figures S6 and S7) were carried out in 100µL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 99mM LiCl/1mM KCl (except for F21T: 90mM LiCl/10mM KCl), with 0.2µM of labeled DNA (Figure S6, F21T (28µM, 0.7µL), F-kit-T (31µM, 0.6µL) and F-myc-T (28µM, 0.7µL)) or RNA oligonucleotides (Figure S7, L-TERRA (18µM, 1.1µL), L-VEGF (21µM, 0.9µL) and L-TRF2 (23µM, 0.8µL)) in absence (black curves) or in presence of 1.0µM (5equiv.) of TEGPy (red curves) or TMPyP4 (blue curves).

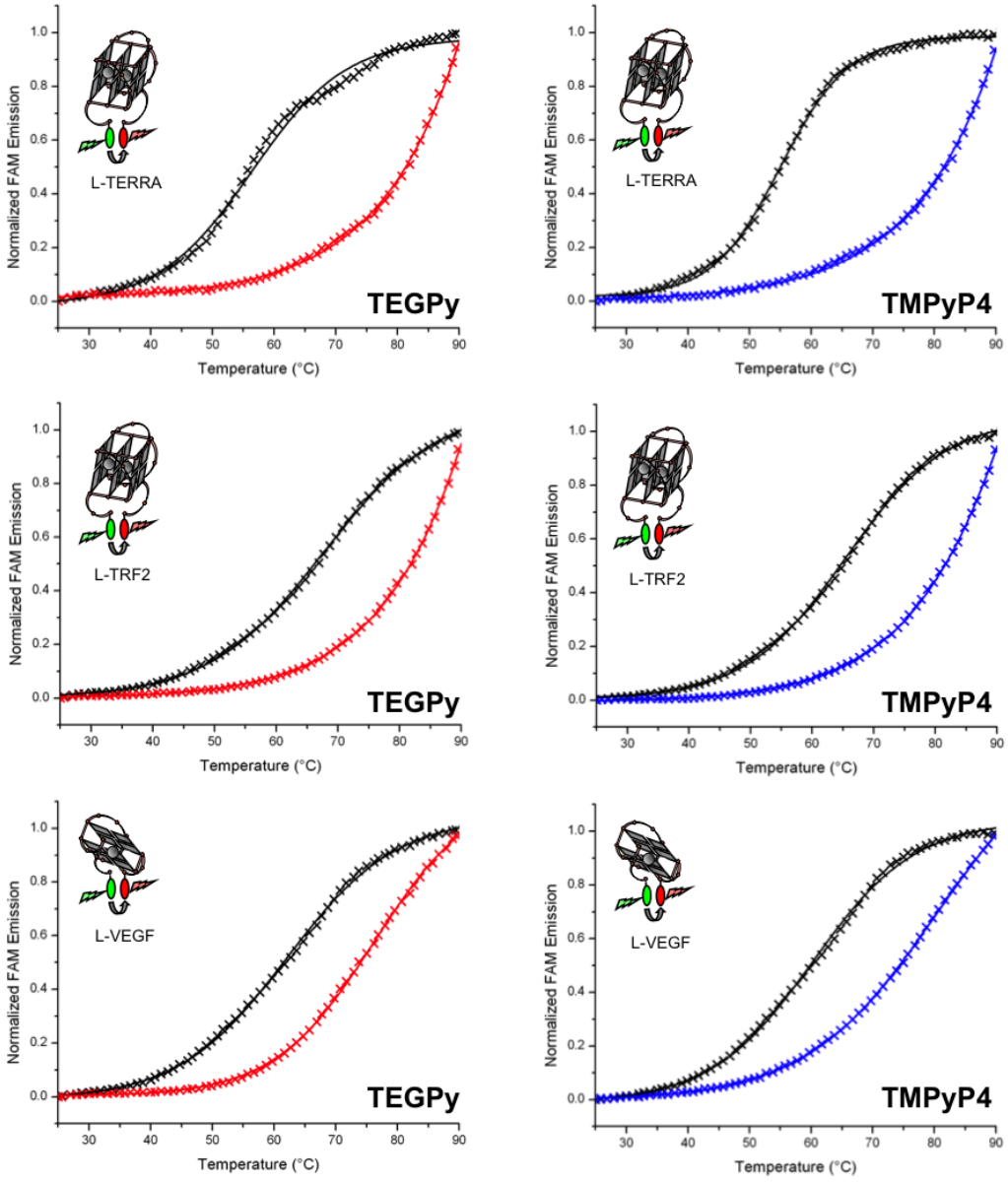


Figure S7. Evaluation of the quadruplex-RNA affinity of both TEGPy (left) and TMPyP4 (right) *via* experiments carried out with L-TERRA, L-TRF2 or L-VEGF (0.2µM) in presence of 1µM ligand (5 molar equivalents).

Experiments (Figure S8, upper panel) were carried out in 100µL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 90mM LiCl/10mM KCl, with 0.2µM of labeled F-duplex-T (36µM, 0.5µL) in presence of 1.0µM (5equiv.) of TEGPy (100µM in H_2O). Competitive experiments (Figure S8, lower panel) were carried out in 100µL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 90mM LiCl/10mM KCl (F21T) with labeled DNA F21T (0.2µM, 28µM, 0.7µL) in presence of either TEGPy or TMPyP4 (1.0µM) and increasing amounts (0, 15 and 50equiv.) of the unlabeled competitor ds26 (205µM, 1.4 and 4.9µL).

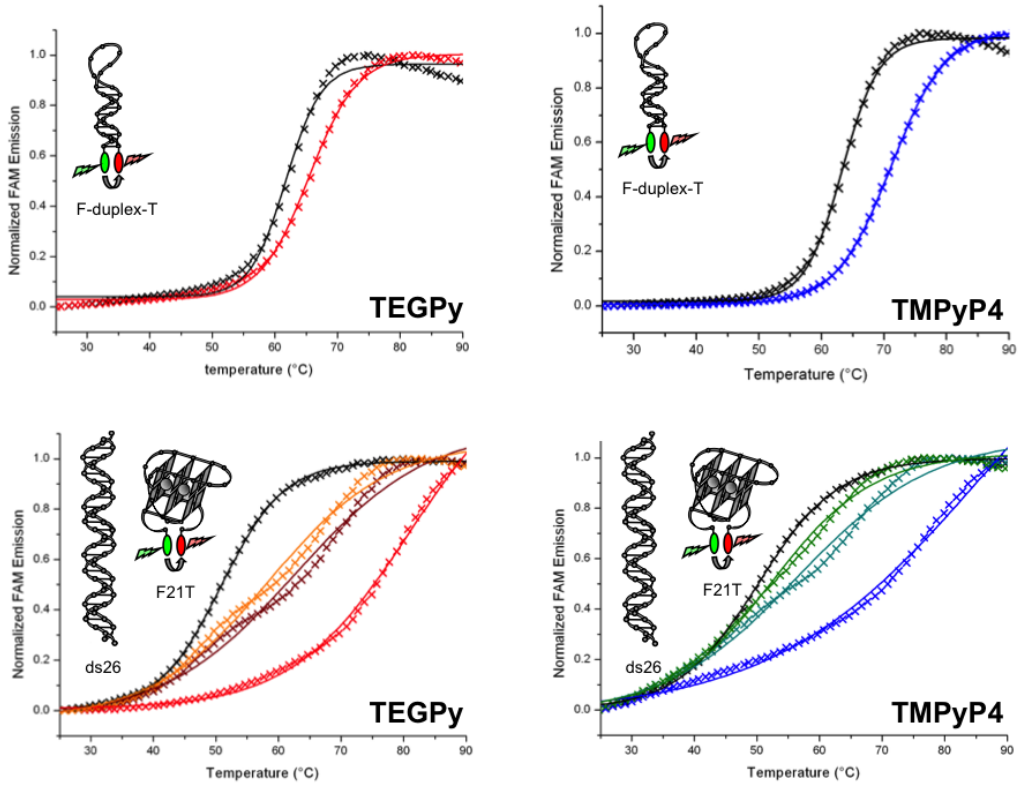


Figure S8. Evaluation of the quadruplex affinity/selectivity of both TEGPy (left) and TMPyP4 (right) *via* experiments carried out with F-duplex-T (0.2µM, upper panel) or competitive FRET-melting experiments performed with F21T (0.2µM, lower panel), 1µM ligand and increasing concentrations of duplex-DNA competitor (ds26, 0, 3 and 10µM).

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 2 to 4 experiments.

Part 5. 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 350-700nm (bandwidth = 1nm, 1nm pitch, 1s response, scan speed = 200nm.mn^{-1}, averaged over 30 scans) with TEGPy (10µM, 10mM in H_2O, 1.0µL) or TMPyP4 (10µM, 10mM in H_2O:DMSO 10:1, 1.0µL) in 1mL (final volume) of H_2O.

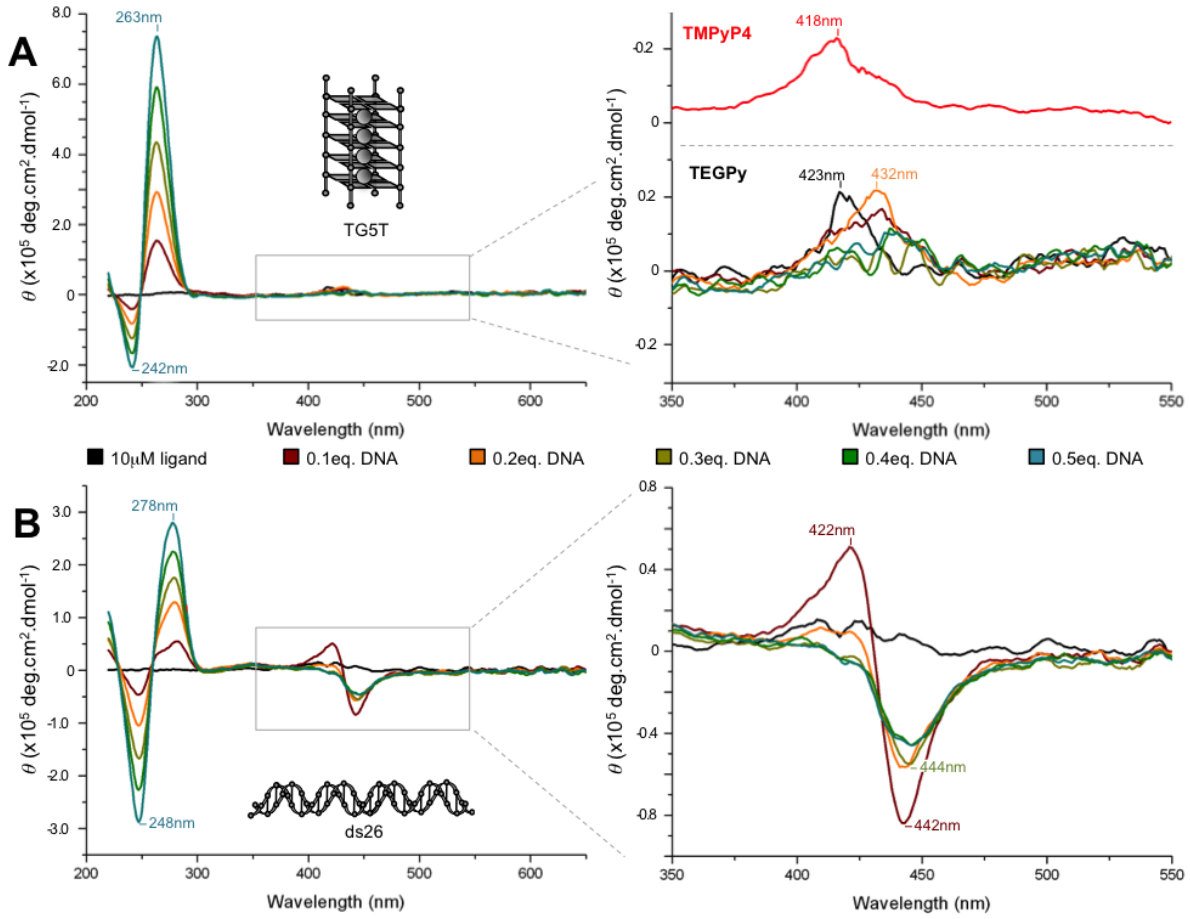


Figure S9. Insights into the DNA-binding mode of TEGPy *via* CD titrations carried out with increasing concentrations of TG5T (A) or ds26 (B) in water. Right panel: focus on the region corresponding to the absorbance Soret band of TEGPy (350-550nm); upper panel, red line: the signal obtained when TMPyP4 is studied in similar conditions (10µM in water).

Spectra were firstly recorded with TEGPy or TMPyP4 alone (black and red curves, respectively, Figure S9). Afterwards, DNA titrations were performed over a range of 200-700nm (bandwidth = 1nm, 1nm pitch, 1s response, scan speed = 500nm.mn^{-1}, averaged over 3 scans) with TEGPy (up to 10µM, 10mM in H_2O, 1.0µL) in 1mL (final volume) of 10mM

lithium cacodylate buffer (pH 7.2) plus 10mM KCl/90mM LiCl and increasing amounts of either quadruplex (TG5T, up to 0.5equiv.) or duplex (ds26, up to 0.5equiv.).

Part 6. AFM and HS-AFM studies.

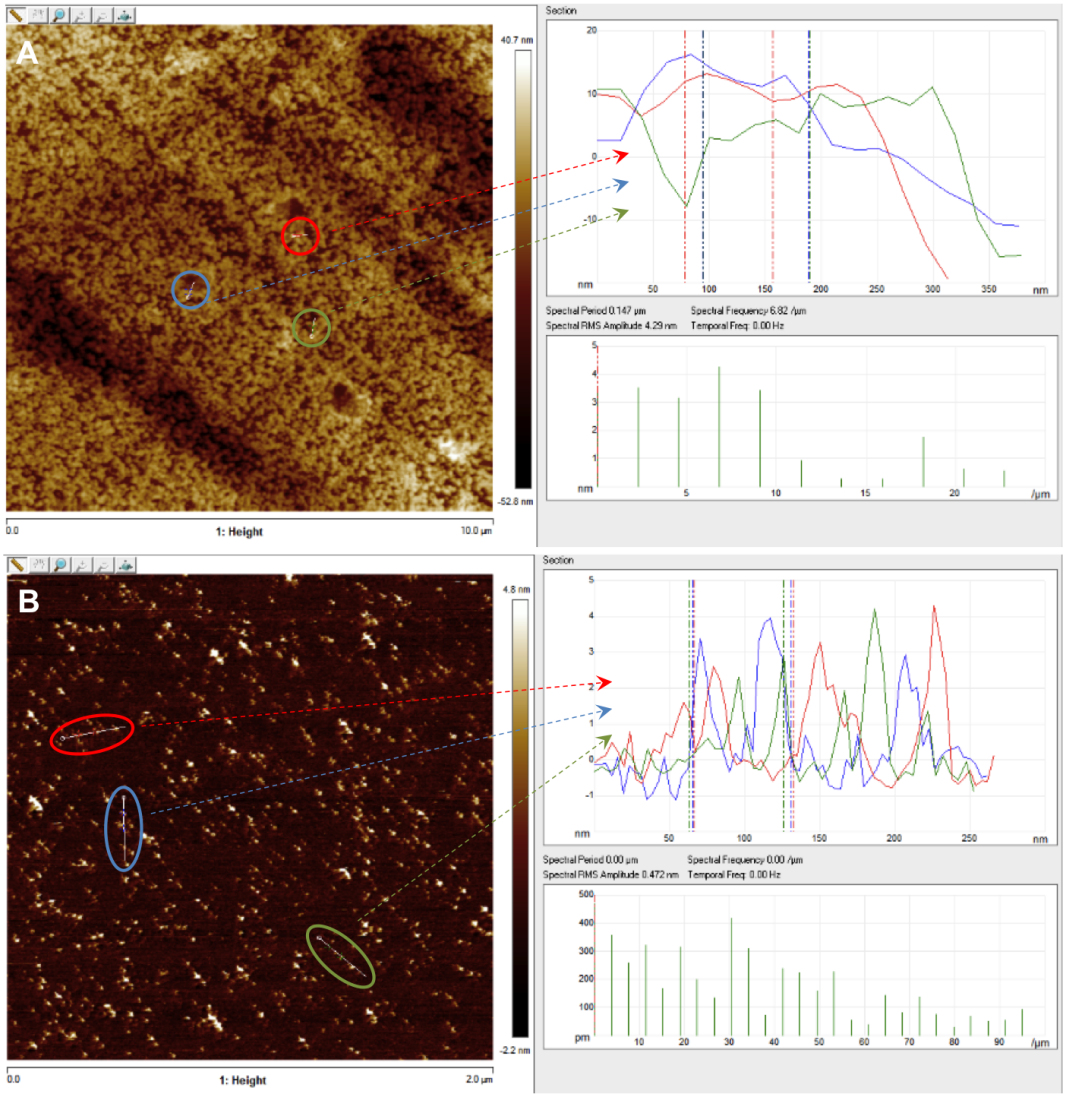


Figure S10. Quantification of the AFM images corresponding to experiments carried out with TEGPy (A) and TMPyP4 (B). The heights of the objects were calculated by a random selection of three ~300-nm sections

Static atomic force microscopy (AFM) images have been recorded in Peak Force mode on a Multimode 8 BRUKER microscope with 15µm scanner range. Selected ScanAsyst-HR probes of spring constant 0.3-0.4 N/m were used. 2µL of TEGPy or TMPyP4 (10µM in H_2O for TEGPy 1%DMSO in H_2O for TMPyP4) are deposited on a freshly cleaved 1.5mm diameter mica disk (RIBM Co., Japan). After 10min of adsorption on the surface and drying step, 150µL of H_2O are added and the images were collected at 25°C. The average height of the

basic block of the TEGPy was determined to be between 20 and 40nm (Figure S10A) while that of TMPyP4 between 2 and 4nm (Figure S10B). Experiments were carried out at least two independent times, that is, on separate days with different mica disks, porphyrin solutions and cantilevers. The influence of the nature of the stock solutions has also been verified: as seen in the Figure S11, ~15-nm aggregates were obtained when TEGPy is first diluted in pyridine (and not H_2O), following the aforementioned drying/re-solubilizing protocol.

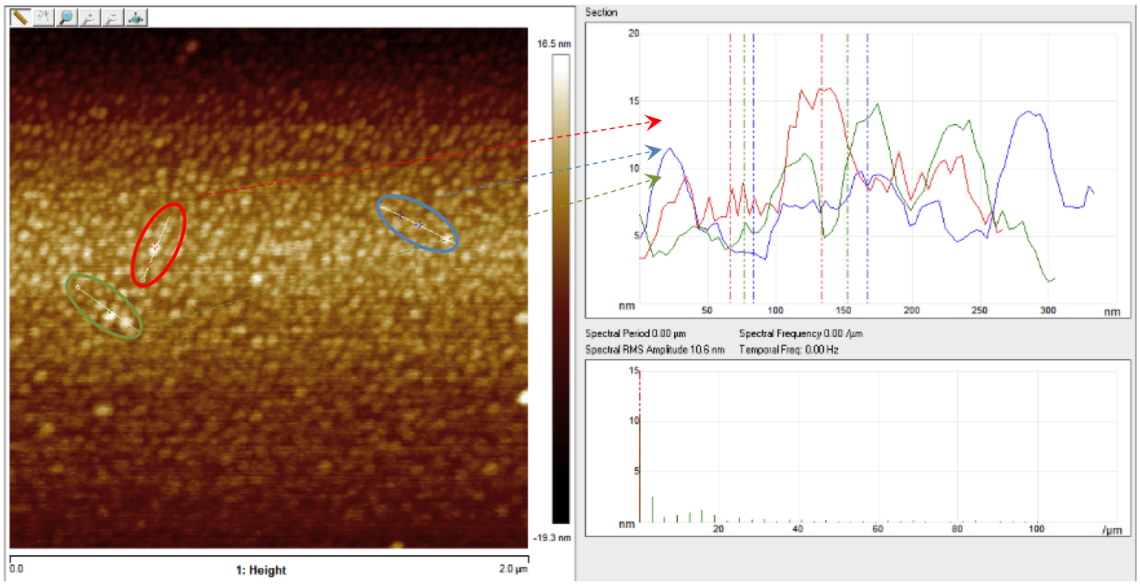


Figure S11. Quantification of the AFM images corresponding to experiments carried out with TEGPy first diluted in pyridine. The heights of the objects were calculated by a random selection of three ~300-nm sections

High-speed atomic force microscopy (HS-AFM) measurements were carried as follows: 2µL of TEGPy solution (10µM in H_2O) is deposited on a freshly cleaved 1.5mm diameter mica disk (RIBM Co., Japan); after 10min of adsorption on the surface, 150µL of water are added and the images were collected at 25°C using EBD tip (3µm long) deposited on small cantilevers (Nanoworld USC, f = 1.2MHz, k = 0.1-0.15N.m^{-1} with an oscillation amplitude limited to 0.5nm). During the scanning process, 8.8µL of 180µM water solutions of either TG5T or ds26 were added (for obtaining a final 10µM DNA concentration), and images were collected over a 10-min period. For a dynamic view of the process, see the two corresponding movies available as supporting material free of charge *via* the internet at http://pubs.rsc.org/en/journals/journalissues/ob. Experiments were conducted at least two independent times (*i.e.*, with different mica disks, TEGPy and DNA solutions). All HS-AFM images are in 400x100nm^2 size and recorded with the scan rate of 1-4 frame.s^{-1}.