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Electronical supporting information for

Responsive DNA G-quadruplex micelles

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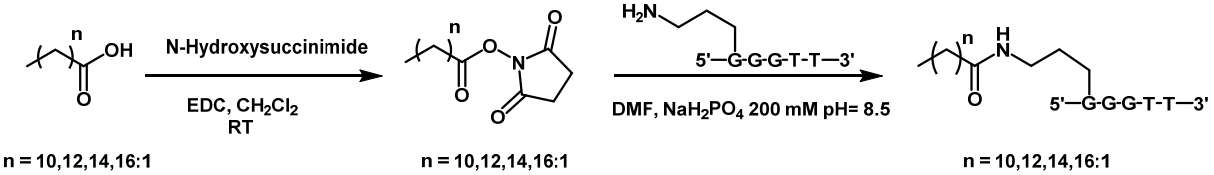
General remarks:

Chemicals were purchased from Sigma Aldrich or Acros and used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 400 MHz in $CDCl_3$. Chemical shifts (δ) are denoted in ppm using residual solvent peaks as internal standard. Syntetic oligonucelotides were purchased from Biotex Berlin-Buch GmbH. Oligonucleotide concentrations were calculated using Nanodrop 2000 (Thermo Fisher Scientific). Extinction coefficients of the oligonucleotides (ε_{260}) were calculated by Oligo Analyzer 3.1 from IDT (Integrated DNA Technologies). Reversed-phase HPLC (RP-HPLC) purifications were performed on a Shimadzu LC-10AD VP using a Xbridge Prep C8 column (10 x 150 mm, particle size 5 μm) from Waters Corporation. 0.1 mM triethylammonium acetate (TEAA) at pH=7.0 (solvent A) and acetonitrile (solvent B) were used as the mobile phase at a flow rate of 1 mL/min. Gradient: 5% B for 5 min, linear gradient to 90% B in 5 min, to 100% B in 10 min, isocratic for 5 min. Re-equilibration of the column at 5% B for 5 min. The column was heated to 65˚C. UPLC-MS on the conjugates was performed on an Acquity TOF Detector (ESI TOF- MS) coupled to Waters Acquity Ultra Performance LC using a Acquity BEH C4 (1.7 µm 2.1 x 150 mm). 15 mM TEAA at pH=7.0 (solvent A) and methanol (solvent B) were used as the mobile phase at a flow rate of 0.2 mL/min. Gradient: 95% A for 5 min, linear gradient to 5% A in 5 min. Re-equilibration of the column at 95% A for 4 min. The column was heated to 60˚C. The ESI ion source was operated in negative mode and mass spectra was collected between 200 and 3000 *m/z*. UPLC-MS chromatograms were analyzed with MassLynx V4.1. Circular dichroism (CD) and UV-visible spectra were recorded on Jasco J-815 Spectropolarimeter and Jasco V-660 Spectrophotometer, respectively. Fluorescence was recorded on a Jasco FP-8300 Spectrofluorometer.

Scheme S1. Synthesis of the alkyl-functionalized oligonucleotides



General procedure for the synthesis of the N-hydroxysuccinimidic esters

The carboxylic acid (3 mmol, 1 eq) and N-hydroxysuccinimide (3.3 mmol, 1.1 eq.) were dissolved in 20 mL dichloromethane under N_2 atmosphere. Thus EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) (3.3 mmol, 1.1 eq) was added to the mixture in two portions. The reaction was stirred overnight. The mixture was extracted with H_2O (2 x 30 mL) and then washed with brine (2 x 30 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residual solid was recrystallized from EtOH. The obtained white crystals were dried overnight under vacuo (y=50-80%). The $C_{18:1}$ ester was purified by flash column chromatography using dichloromethane as eluent (y=50%).

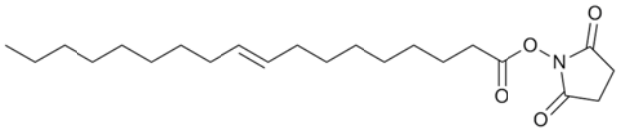
C12 (n=10). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J=6.8 Hz, 3H), 1.26-1.32 (m, 14H), 1.37-1.44 (m,2H), 1.74 (m, 2H), 2.6 (t, J = 7.5 Hz, 2H), 2.8 (s, 4H).

C14 (n=12). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.26-1.32 (m, 18H), 1.37-1.44 (m,2H), 1.74 (m, 2H), 2.6 (t, J = 7.5 Hz, 2H), 2.8 (s, 4H).

C16 (n=14). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.26-1.32 (m, 22H), 1.37-1.44 (m,2H), 1.74 (m, 2H), 2.6 (t, J = 7.5 Hz, 2H), 2.8 (s, 4H).

C18:1 (n=16:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.22 – 1.44 (m, 20 H), 1.74 (m, 2H), 1.97 – 2.06 (m, 4H), 2.60 (t, J = 7.5 Hz, 2H), 2.83 (d, J = 3.3 Hz, 4H), 5.29 – 5.40 (m, 2H).

Figure S1. Structure of $C_{18:1}$–NHS ester

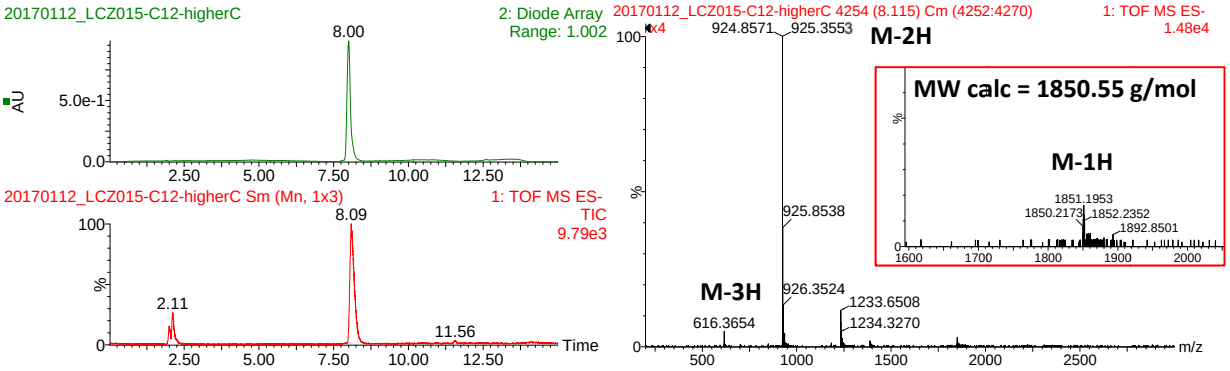


General procedure for the synthesis of the DNA-lipids conjugates

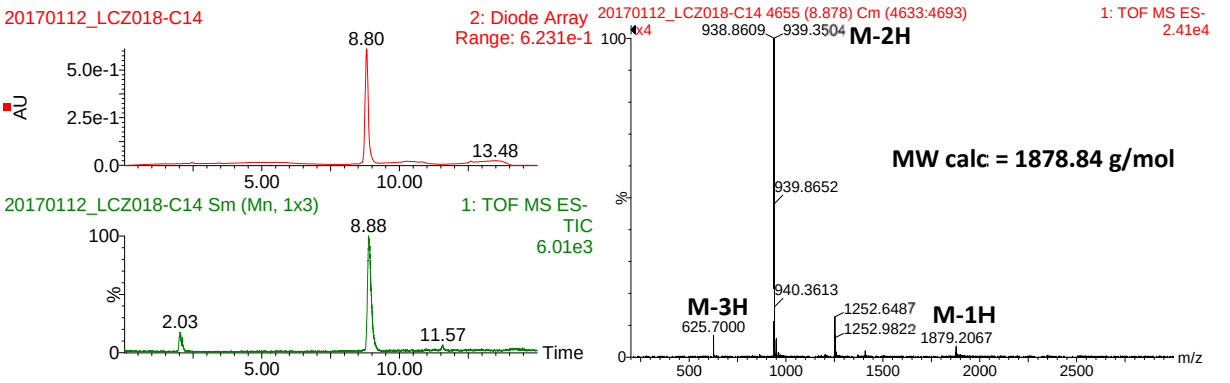
The amino-modified oligonucleotide was solubilized in 350 µL of 200 mM NaH_2PO_4 pH=8.5 (350 µM) and 250 µL of the alkyl N-hydroxysuccinimyl-ester in DMF (20 mg/mL) were added. The reaction was stirred overnight at 37˚C or 70 ˚C when precipitation of the alkyl NHS-ester occurred. The functionalized DNA was purified by size exclusion chromatography (NAP-10, GE Healtcare) using triethylamine acetate (TEAA) buffer 50 mM pH=7.2. The lyophilized conjugates were subsequently purified by RP-HPLC and analyzed by UPLC-MS (TOF).

Figure S2. UPLC-MS chromatograms and ESI(-) mass traces of DNA-lipid conjugates.

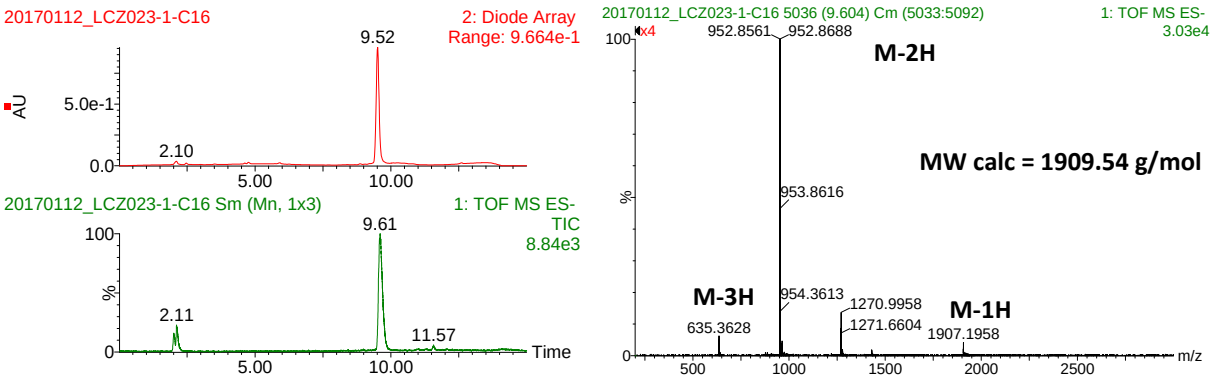
C_{12}-GGGTT



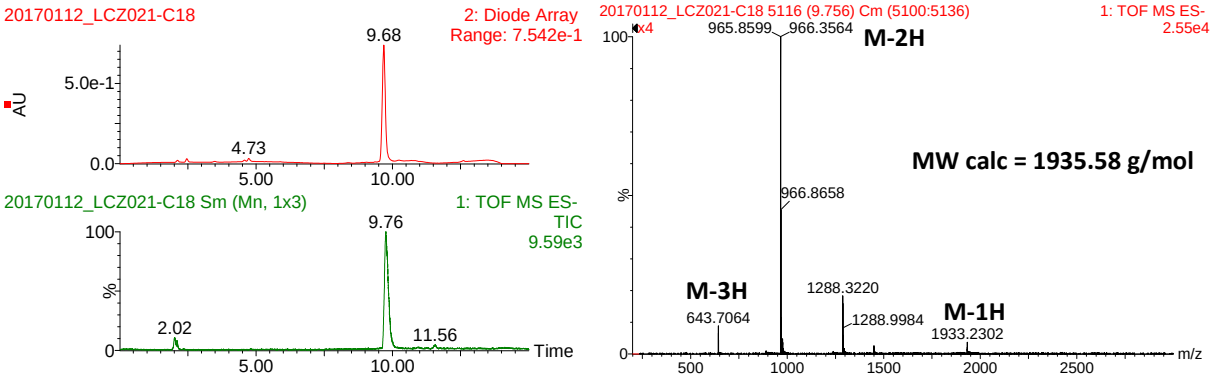
C_{14}-GGGTT



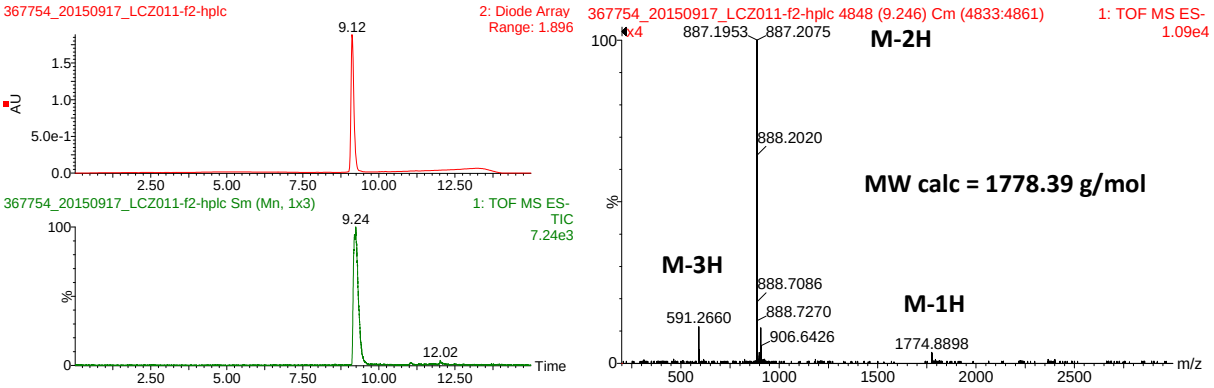
C_{16}-GGGTT



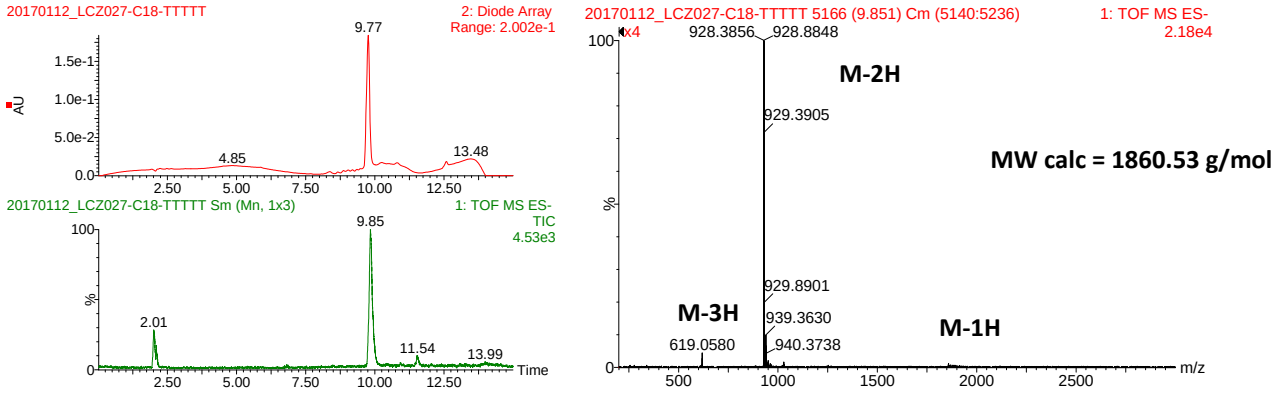
$C_{18:1}$-GGGTT



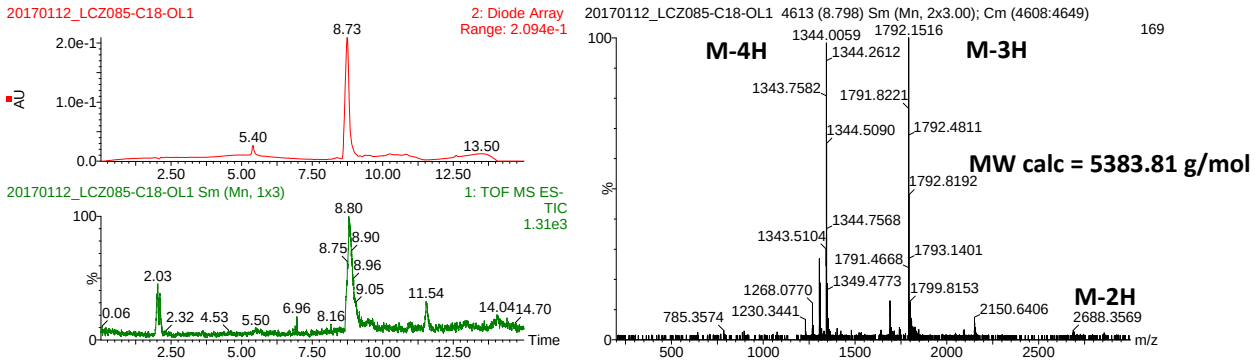
C_{12}-TTTTT



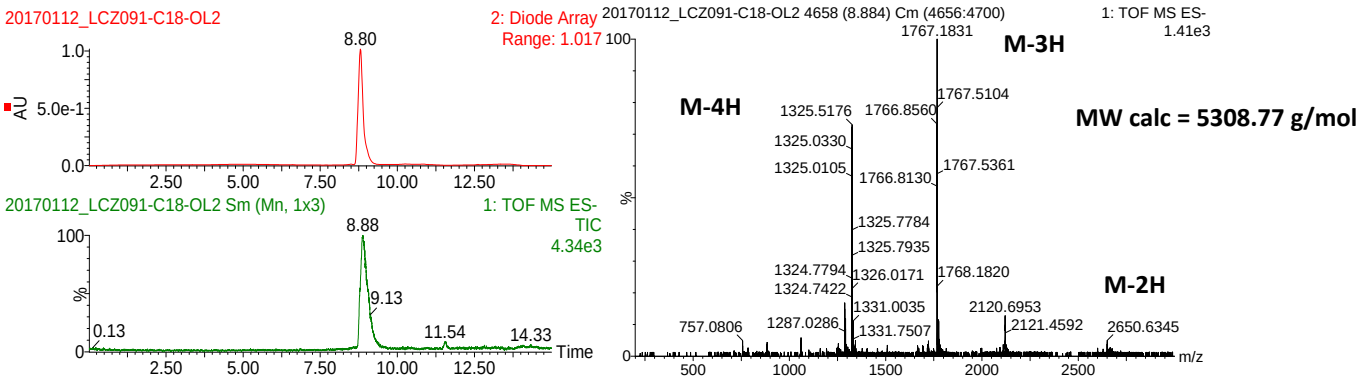
$C_{18:1}$-TTTTT



$C_{18:1}$-GGGTTTAAGTGTAGTT ($C_{18:1}$-OL1)



$C_{18:1}$-TTTTTTAAGTGTAGTT ($C_{18:1}$-OL2)



Annealing procedure for the G-4 formation

The purified DNA-lipids were solubilized in 30 mM Tris-HCl, 80 mM KCl, pH=7.2and then heated to 90˚C for 15 min. The solution was slowly cooled to room temperature and then stored at 4˚C overnight.

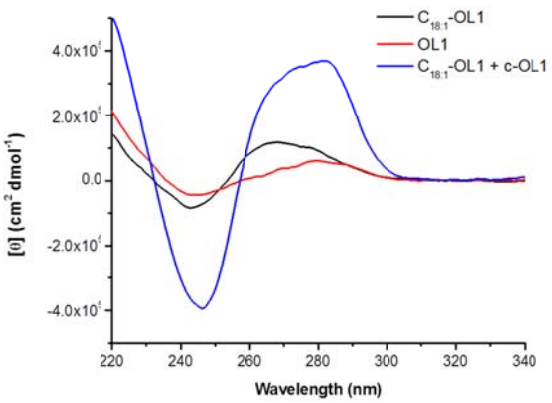
General procedure for CD measurements

The CD measurements were performed after annealing of the samples. The CD signal was measured in the range between 220 nm and 350 nm in continuous mode. The measured CD spectra were corrected for the concentration of the samples using the following equation:

$$[\theta] = \frac{100 \times \theta}{C \times l} \quad [cm^2 dmol^{-1}]$$

Where θ is the ellipticity in degrees, C is the concentration in M and l is the pathlength in cm.

Figure S3. CD spectra of $C_{18:1}$-OL1 (black line) compared to the oligonucleotide OL1 before conjugation (red line) and after addition of the complementary strand c-OL1. All the samples were dissolved in Tris-HCl 30 mM KCl 80 mM pH=7.2. C= 5 µM.



Critical micellar concentration (CMC) determination.

A 2.5 mM Nile Red stock solution was made in ethanol and diluted 1000-fold in the DNA-lipid sample in30 mM Tris-HCl, 80 mM KCl, pH=7.2. The samples were annealead before fluorescence measurements.

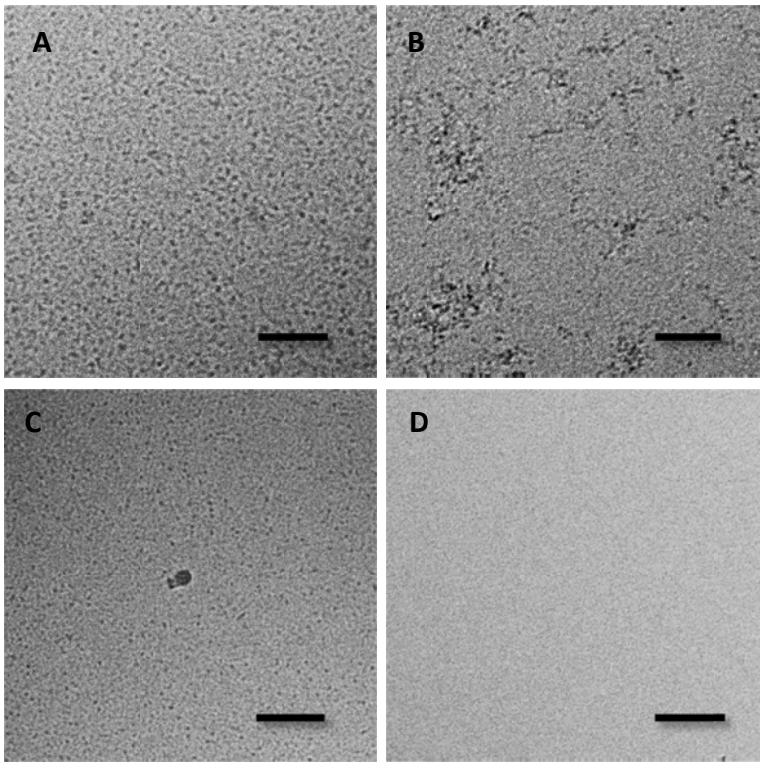
Nile Red fluorescence was measured at different concentrations of the samples using excitation at 550 nm. Fluorescent emission was measured from 600 nm to 700 nm at 1 nm intervals. The Nile Red emission maximum (λ_{max}) was calculated using a log-normal fit.[1]

Cryo-transmission electron microscopy (cryo-TEM)

The samples containing the DNA-lipids were annealed as described above and analyzed by cryo-TEM at a concentration of 400 µM.

A 3µl solution was put on a Quantifoil (3.5/1) holy carbon coated grid. The grid was blotted and vitrified in ethane in a Vitrobot (FEI, Eindhoven, The Netherlands). The grids were observed in a Philips CM120, operating at 120 keV using a Gatan (model626) cryo-stage. Images were recorded with a slow scan CCD camera under low-dose conditions.

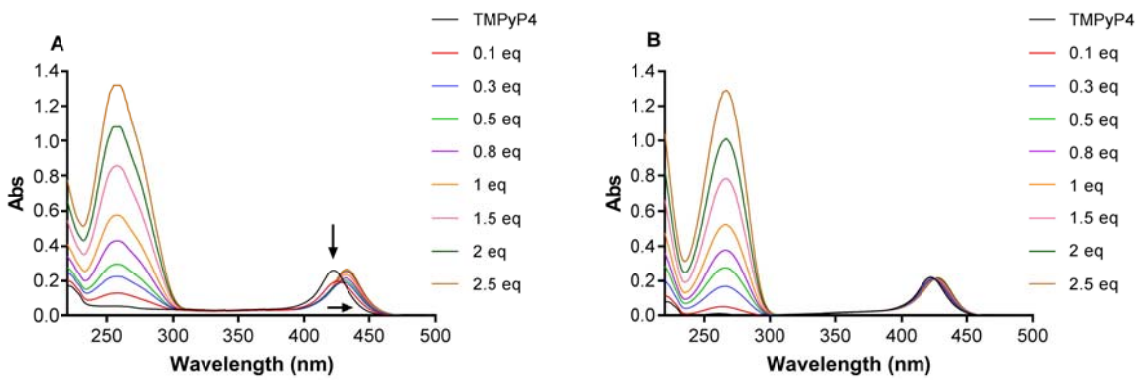
Figure S4. Cryo-TEM images of A) C_{12}-GGGTT, B) C_{16}-GGGTT, C) $C_{18:1}$-GGGTT and D) C_{12}-TTTTT . Scale bar represents 50 nm.



Binding of G-4 micelles to TMPyP4

The binding of the micelles with the cationic porphyrin *meso*-5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)porphine (TMPyP4) was assessed by UV-Vis titrations. To a stock solution of TMPyP4, increasing concentrations of lipid conjugate, previously annealed, were added. Upon addition of the G-4 micelles, the intensity of the Soret band of the porphyrin (422 nm) decreased. This, together with the observed bathochromic shift are clear indications for the interaction of G4 with the porphyrin, as previously reported [2–4]. Conversely, no change in the Soret band was detected upon titrations with DNA-conjugates unable to assemble into a G-4 structure.

Figure S5. Uv-Vis absorption spectra of a solution of TMPyP4 upon titration with increasing amounts of A) C_{12}-GGGTT and B) C_{12}-TTTTT.



Native polyacrylamide electrophoresis (PAGE)

Electrophoresis experiments were performed according to standard procedures with 10% polyacrylamide gels (acrylamide-bis acrylamide 19:1, 40% w/v). 1 mM KCl was added to both the gel and the running buffer (1x TBE). The samples were prepared in 30 mM Tris-HCl, 80 mM KCl, pH=7.2 (C=15 µM) and annealed beforehand. The gel was run at 80V for 1.5h at 4˚C and stained with Stains-All (Sigma Aldrich).

Förster resonance energy transfer (FRET) experiment procedure.

The two dyes 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) were suspendend in ethanol at 2 mg/ml. The DNA-lipid conjugates were also dissolved in ethanol and 5 wt% of each dye was added to the samples. The ethanol was evaporated in a vacuum concentrator, resulting in a thin lipid film. The film was rehydrated with 25 mM $NaHPO_4$, 50 mM KCl pH=7.2 to reach a final concentration of 1 mg/mL. The mixture was heated to 65 ˚C for 15 mins and then cooled slowly at room temperature. The sample was centrifugated in tube filters with a pore size of 0.45 µm to remove aggregates and encapsulation of the dyes was confirmed by UV-Vis (Fig. S3). For fluorescence experiments the samples were diluted in 90% bovine serum albumin (BSA) in PBS (50 mg/ml in 15 mM $NaHPO_4$, 150 mM NaCl). The concentration of the DNA-lipids was 18 µM. The fluorescence was measured in a quartz cuvette with 1.0 cm path length. The samples were excited at 450 nm and emission was measured from 465 nm to 700 nm for 5 hours at 37˚C. The complementary oligonucleotide (1 eq) was added after 60 mins and mixed by pipetting up and down. FRET efficiency was measured by calculating the FRET ratio ($I_{575}/(I_{575} + I_{510})$) at each time point to monitor micelle stability.

The same procedure was followed for the preparation of the ATP-responsive micelles ($C_{18:1}$-OL4) but using pH=8.5,due to binding of BSA with ATP at lower pH.[5,6] Addition of the aptamer (1.2 eq) or aptamer and ATP (5 mM) was done after 80 min.

Table S1. Sequences of the used oligonucleotides in the FRET experiment.

Name	Sequence (5'-3')
OL1	$C_{18:1}$-GGGTTTAAGTGTAGTT
OL2	$C_{18:1}$-TTTTTTAAGTGTAGTT
c-OL1	AACTACACTTAAACCC
c-OL2	AACTACACTTAAAAAA
OL3	GACATGTCTGACCTTG
OL4	GGGTTCACCTGGA
APT	C**ACCTGGGGGAGTATTGCGGAGGAAGGT**TCCAGGTGAACCC

Blue: ATP-binding domain; **Red:** responsive domain.

Figure S6. Fluorescence emission spectra of FRET pairs (DiO and DiI) encapsulated in the $C_{18:1}$-OL1 micelles. λ ex=450 nm, T=37°C. (a) $C_{18:1}$-OL1 micelles; (b) $C_{18:1}$-OL1 micelles upon addition of the complementary strand. Addition was done after 60 min and coincides with a decrease of the band at 575 nm and an increase of the band at 510 nm. (c) $C_{18:1}$-OL2 did not show any energy transfer between the two dyes after 300 mins. (d) UV-Vis measurements to confirm the encapsulation of the dyes into the micelles. Samples were diluted in 10 mM Tris-HCl, 80 mM KCl, 5 mM $MgCl_2$ pH=7.2. For comparison also micelles with only DiO (black line) or DiI (blue line) were prepared with the same procedure.

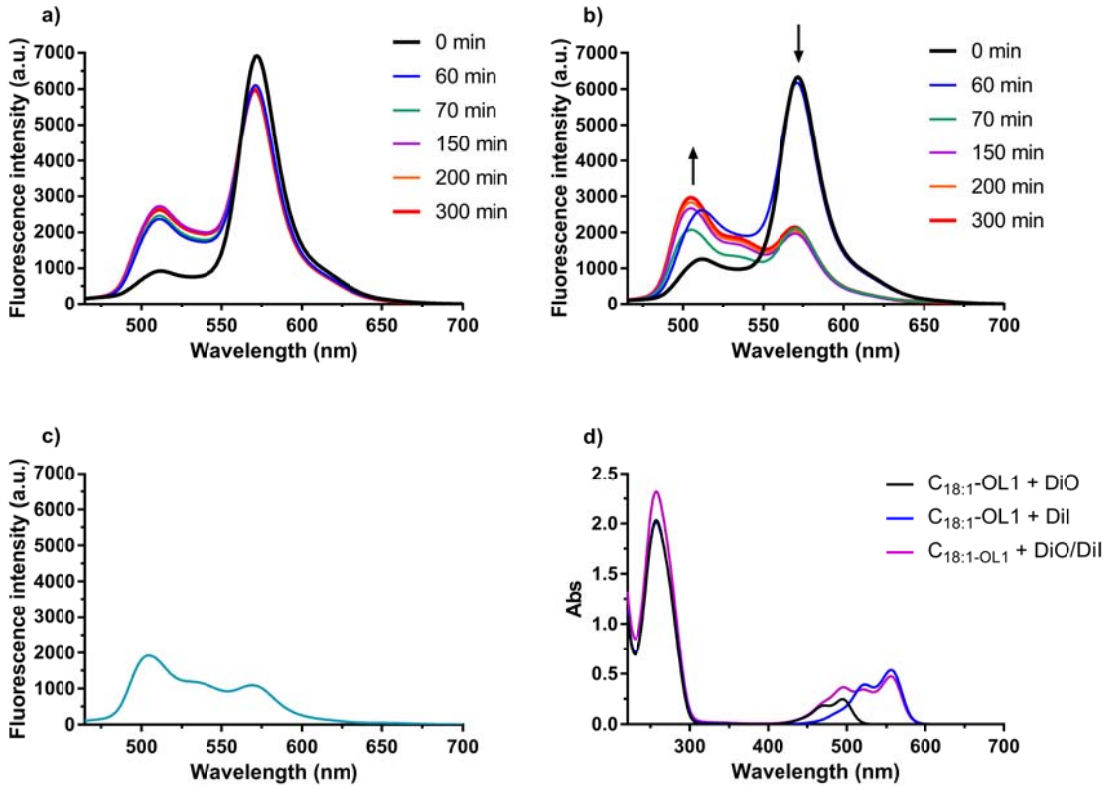


Figure S7. Fluorescence emission spectra of DiO (5 wt%) encapsulated in the DNA-lipids micelles over 5h at 37˚C. λ_{ex}=450 nm C=8 μM. (a) $C_{18:1}$-OL1; (b) $C_{18:1}$-OL1 upon addition of 1 equivalent complementary oligonucleotide c-OL1. After incubation with c-OL1 the dye is released and fluorescence emission at 510 nm decreased.

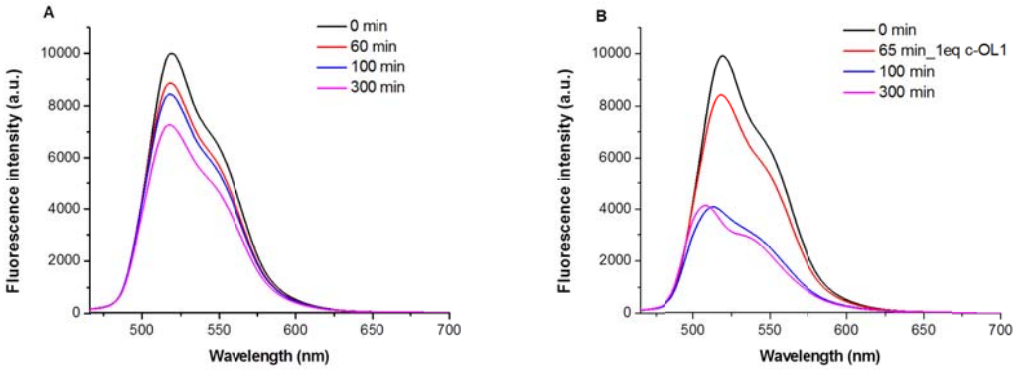


Figure S8. Fluorescence emission spectra of DiO (5 wt%) encapsulated in the DNA-lipids micelles over 5h at 37˚C. λ_{ex}=450 nm C=18 μM. (a) $C_{18:1}$-OL1; (b) $C_{18:1}$-OL1 upon addition of 1 equivalent complementary oligonucleotide c-OL1. Emission at 575 nm is measured due to absorbance of DiI at 450 nm, but the fluorescence intensity is significantly lower when compared to the system with both dyes encapsulated. Also in this case addition of the c-OL1 determines a small decrease in the emission.

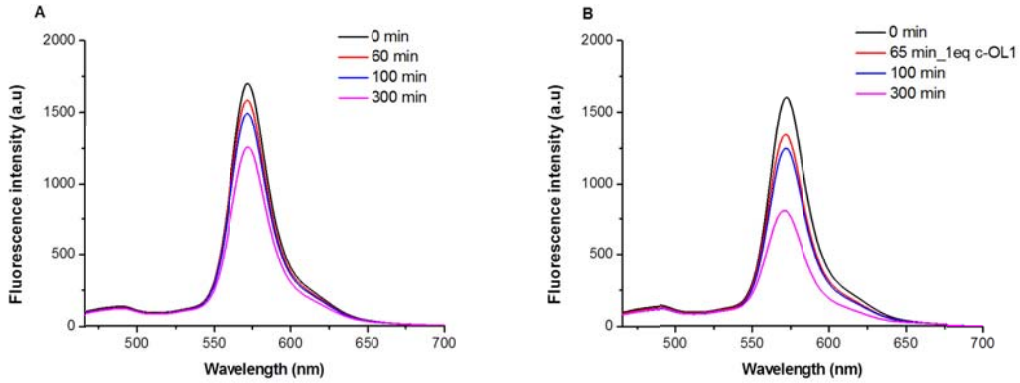


Figure S9. Normalized FRET measured over 5h for the ATP-responsive micelles $C_{18:1}$-OL4 (blue). Upon addition of 1.2 eq of aptamer (APT) the micelles are slowly destabilized (black). In the presence of both APT and ATP (5 mM) the FRET ratio decreases abruptly, indicating the release of the dyes from the micelles (purple). The arrow indicates the time point at which the addition was done. No destabilization was observed when ATP (5 mM) was added to the micelles in the absence of the aptamer (orange).

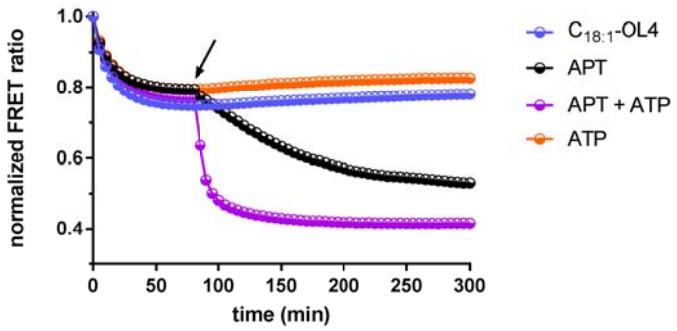
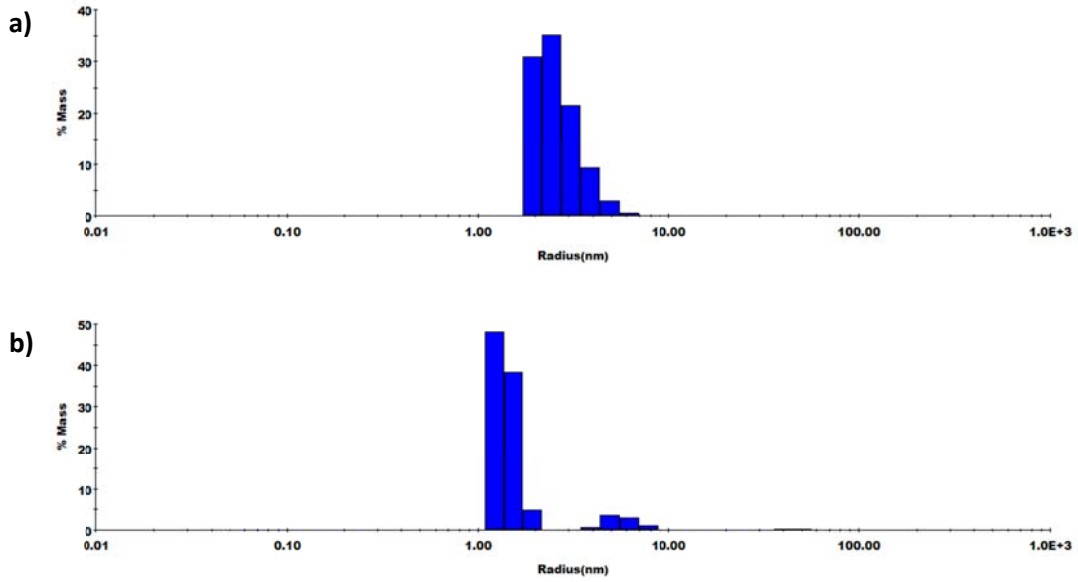


Figure S10. Dynamic light scattering (DLS) measurements for (a) $C_{18:1}$-GGGTT and (b) C_{16}-GGGTT.



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