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Supporting Information

Lipid-DNAs as Solubilizers of *m*THPC

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Supporting Information

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Materials and methods

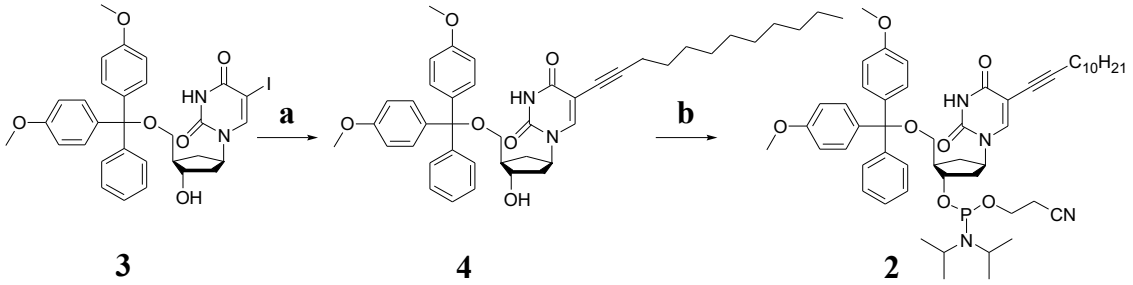
All chemicals and reagents were purchased from commercial suppliers and were used without further purification, unless otherwise noted. In all experiments, MilliQ standard water (Millipore Inc., USA) with a typical resistivity of 18.2 MΩ/cm was used. The 1-dodecyne, copper(I) iodide, tetrakis(triphenylphosiphine)palladium(0) and diisopropylamine were purchased from Sigma-Aldrich and used as received. *m*-THPC was provided kindly by Professor Mathias O. Senge (School of Chemistry, SFI Tetrapyrrole Laboratory, Trinity College Dublin, Dublin 2, Ireland). Other special chemicals acquired from different chemical sources were 5'-DMT-5-iodo deoxy uridine (Chemgenes). All lipid-DNAs were synthesized using standard automated solid-phase phosphoramidite coupling methods on an ÄKTA oligopilot plus (GE Healthcare) DNA synthesizer. All solvents and reagents for DNA synthesis were purchased from Novabiochem (Merck, UK) and SAFC (Sigma-Aldrich, Netherlands). Solid supports (Primer SupportTM, 200 μM/g) from GE Healthcare were used for the synthesis of DNA. Lipid-DNAs were purified by reversed-phase high performance liquid chromatography (RP-HPLC) using a C15 RESOURCE RPCTM 1 ml reverse phase column (GE Healthcare) through custom gradients using elution buffers (A: 100 mM triethylammonium acetate (TEAAc) and 2.5% acetonitrile (ACN), B: 100 mM TEAAc and 65% ACN). Afterwards the lipid-DNAs were characterized by MALDI-TOF mass spectrometry using a 3-hydroxypicolinic acid matrix. Spectra were recorded on an ABI

Voyager DE-PRO MALDI-TOF (delayed extraction reflector) Biospectrometry Workstation mass spectrometer. The concentrations of the DNA were determined on a Jasco V-630 spectrophotometer (Jasco Benelux B.V., Netherlands) using 1 cm light-path quartz cuvette. Pristine DNAs were purchased from Biomers.net at HPLC purification grade. Absorption spectra were recorded on a Specord S 600 (Analytic Jena) spectrometer. A 0.1 x 1 cm cuvette was used for solubilizer-screening experiments and 1 x 1 cm cuvette for other measurements. The loading concentrations of *m*THPC were determined by RP-HPLC using Xterra Prep MS C18, 10 μm, 7.8 x 150 mm column (Waters) through custom gradients using elution buffers (A: H_2O (0.1% TFA), B: ACN (0.1% TFA)) at a wavelength of 417 nm. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorometer (Varian Nederland B.V.) at room temperature, an excitation wavelength of 417 nm was used for *m*THPC-loaded samples.

Synthesis and characterization of lipid-DNAs

The modified 5-(dodec-1-ynyl)uracil phosphoramidite **2** was synthesized in two steps as previously reported starting from **1** (Scheme S1).[1] Subsequently, the modified uracil phosphoramidite was dissolved in ACN to adjust the concentration to 0.15 M and directly connected to the DNA synthesizer. All oligonucleotides were synthesized on a 50 μmol scale on an ÄKTA oligopilot plus (GE Healthcare) DNA synthesizer using standard β-cyanoethylphosphoramidite coupling chemistry. Deprotection and cleavage from the PS support was carried out by incubation in concentrated aqueous ammonium hydroxide

solution at 55 °C for 5 h. Following this step, the oligonucleotides were purified by using RP-HPLC, using a C15 RESOURCE RPC^{TM} 1 mL reversed phase column (GE Healthcare) through custom gradient elution (A: 100 mM TEAAc and 2.5% ACN, B: 100 mM TEAAc and 65% ACN). Fractions were desalted using centrifugal dialysis membranes (MWCO 3000, Sartorius Stedim) or a HiTrap Desalting column (GE Healthcare). DNA concentrations were determined by UV absorbance using their respective extinction coefficients. The purity was characterized by RP-HPLC (Figure S1) on a Shimadzu VP series HPLC system with PDA detector using a C4 Jupiter 5 µm 300 Å 1 mL reversed phase column (Phenomenex) through custom gradient elution (A: 100 mM TEAAc and 5% ACN, B: Methanol). Finally, the identity of the oligonucleotides was confirmed by MALDI-TOF mass spectrometry (Figure S2).



Scheme S1. Synthetic scheme of 5-(dodec-1-ynyl) uracil phosphoramidite. a) 1-dodecyne, $Pd(PPh_3)_4$, CuI, DMF/(iPr)$_2$N (v/v = 1/1), room temperature for 18 h, yield 70 %; b) N-diisopropyl-2-cyanoethyl-chlorophosphoramidte, (iPr)$_2$EtN, CH_2Cl_2, room temperature for 3 h, yield 80 %.

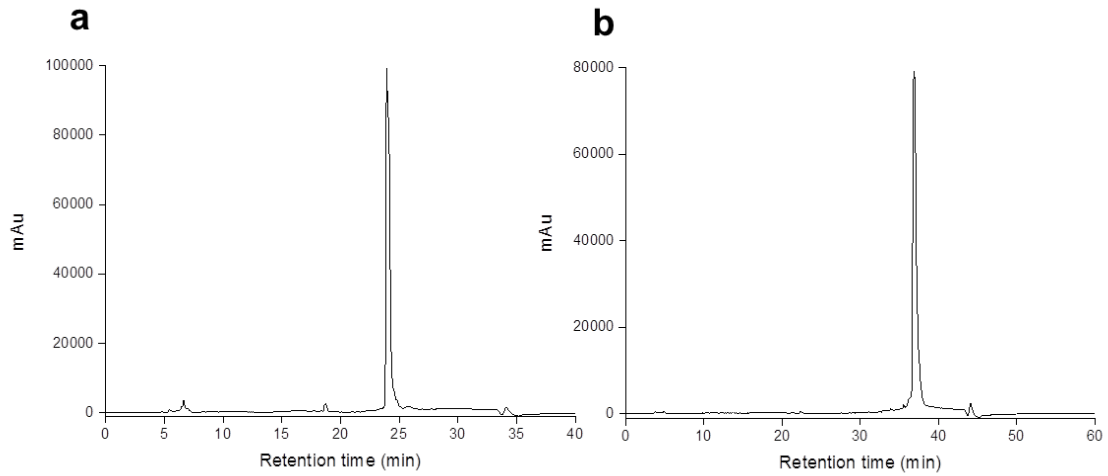


Figure S1. Purity analysis of a) **<u>UU</u>11mer** and b) **<u>UUUUUU</u>12mer** characterized with RP-HPLC, while monitoring at 260 nm.

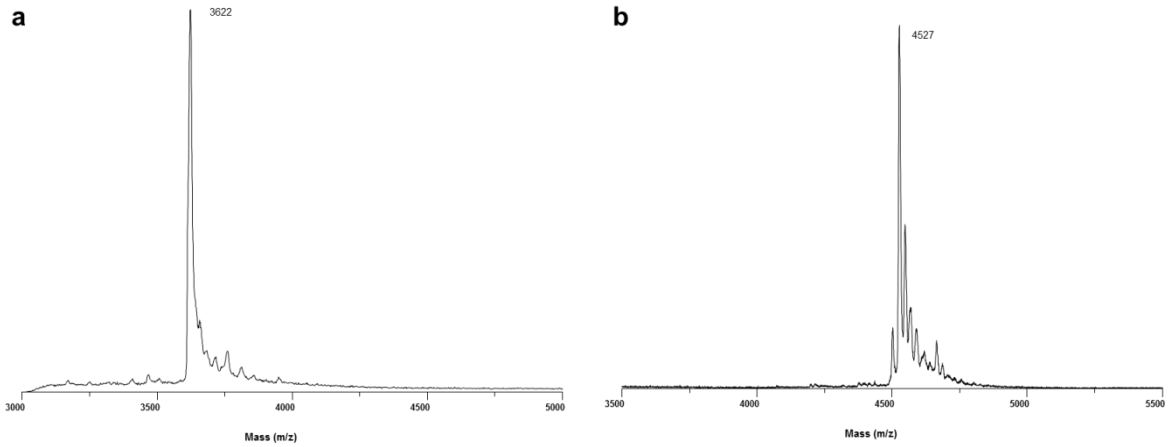


Figure S2. Characterization of amphiphilic oligonucleotides by MALDI-TOF mass spectrometry. Spectrum of (a) **<u>UU</u>11mer** (calc. 3629 g/mol, found 3622 g/mol) and (b) **<u>UUUUUU</u>12mer** (calc. 4534 g/mol, found 4527 g/mol).

Pretreatment of DNA aqueous solutions

Single-stranded DNAs (**11mer**, **c11mer**, **12mer**, **UU11mer**, **UUUUUU12mer**, 50 μM), **dsUU11mer** (**UU11mer** 50 μM, **c11mer** 50 μM, $MgCl_2$ 10 mM) and **ds11mer** (**11mer** 50 μM, **c11mer** 50 μM, $MgCl_2$ 10 mM) were thermally cycled (90 °C, 30 min; –1 °C/2 min until room temperature) by using a polymerase chain reaction (PCR) thermocycler (Biorad, USA) before use.

Critical micelle concentration (CMC) determination

For CMC determination, firstly 10 pmol of 1,6-diphenyl-1,3,5-hexatriene (DPH) was loaded in Eppendorf DNA low-binding tubes using a solution in acetone (1 μM). The solvent was allowed to evaporate at room temperature for 5 h after which DNA amphiphile solution (100 μL) was added. The oligonucleotides were prepared at concentrations ranging from 0.0025 to 1 g/L in 1x TAE buffer (10 mM Tris Acetate, 0.2 mM EDTA, 20 mM NaCl, 12 mM $MgCl_2$, pH 8.0) and thermally cycled before use. After addition to the DPH-containing tubes, the solutions were shaken at 37 °C overnight. Subsequently, fluorescence spectra (375–500 nm) were recorded on a Varian Cary Eclipse fluorometer (Varian Nederland B.V.) at room temperature using an excitation wavelength of 350 nm. For the CMC analysis, the intensity at 425 nm (maximum) was plotted against the logarithm of the lipid-DNA concentration (Figure S3). The CMCs were determined to be 29 and 24 μM for **UU11mer** and **UUUUUU12mer**, respectively.

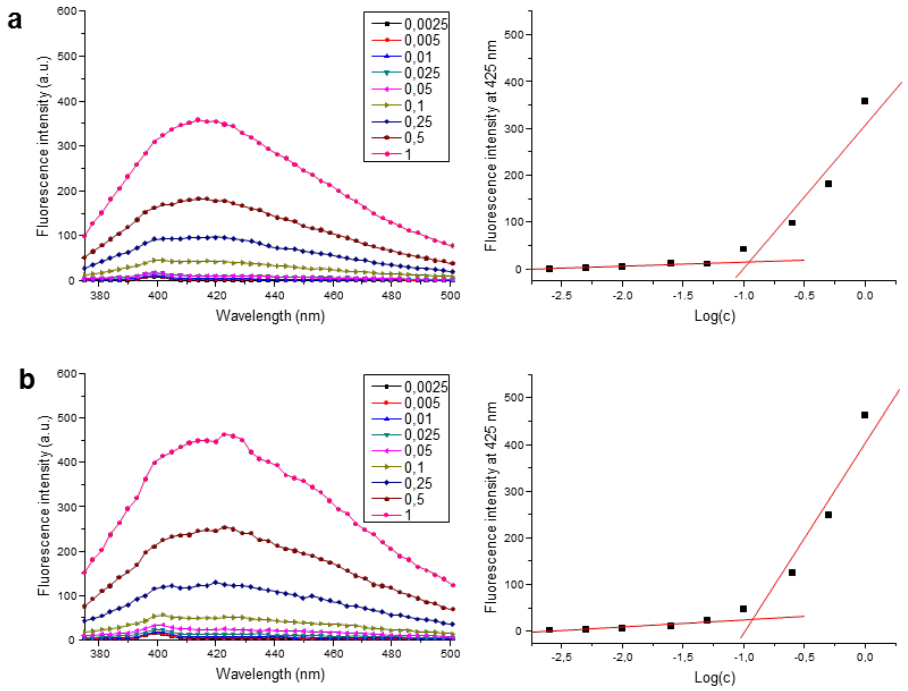


Figure S3. Determination of critical micelle concentrations (CMCs) of the amphiphilic oligonucleotides. Fluorescence spectra of micelle-incorporated 1,6-diphenyl-1,3,5-hexatriene (DPH) at different concentrations (g/L) (left) and intensity at 425 nm (maximum) plotted against the logarithm of the concentration (right) for a) **UU11mer** and b) **UUUUUU12mer**.

Cryogenic electron microscopy (Cryo-EM)

Cyro-EM was performed according to a standard procedure. A sample suspension (3 µL) was placed on a glow-discharged holy carbon coated grid (Quantifiol 3.5/1) blotted and vitrified in a Vitrobot (FEI). Samples were observed in a Gatan 626 cryo-stage in a Philips CM120 operating at 120 keV or in a FEI Tecnai T20 operating at 200 keV. Images were recorded under low-dose conditions on a slow-scan CCD camera.

Table S1. Diameters of lipid-DNA micelles before and after *m*THPC loading obtained through cryo-EM.

Sample	**UU11mer**	**UU11mer**	**dsUU11mer**	**dsUU11mer**	**UUUUUU12mer**	**UUUUUU12mer**
	(–)	(+)	(–)	(+)	(–)	(+)
Diameter (nm)	9.8 ± 1.0	11.1 ± 1.7	9.9 ± 2.0	11.4 ± 1.6	8.2 ± 1.8	9.0 ± 2.0

(–) Without *m*THPC; (+) Loaded with *m*THPC.

Dynamic Light Scattering (DLS)

The measurements used a 3D DLS spectrometer (LS Instruments, Fribourg, Switzerland) equipped with a 25mW HeNe laser (JDS uniphase) operating at λ=632.8 nm, a two channel multiple tau correlator (1088 channels in autocorrelation), a variable-angle detection system, and a temperature-controlled index matching vat (LS Instruments). The scattering spectrum was measured using two single mode fibre detections and two high sensitivity APD detectors (Perkin Elmer, model SPCM-AQR-13-FC).

Fluctuations in the scattered intensity with time $I(q,t)$ (also called count rate), measured at a given scattering angle θ or equivalently at a given scattering wave vector $q=(4\pi n/\lambda)\sin(\theta/2)$, are directly reflecting the so-called Brownian motion of the scattering particles (refractive index n=1.33 at 20 °C). In dynamic light scattering (DLS), the fluctuation pattern is translated into the normalized time autocorrelation function of the

scattered intensity, $g^{(2)}(q,t)=\frac{\langle I(q,0)I(q,t)\rangle}{\langle I(q,0)\rangle^2}$. For a diffusive process, with a characteristic time inversely proportioned to q^2, $g^{(2)}(q,t)\sim\exp(-2Dq^2t)$, with D the mutual diffusion coefficient. The Stokes-Einstein relation allows one to determine the hydrodynamic radius R_h of the scattered objects; $R_h=kT/6\pi\eta D$, if the temperature T and solvent viscosity η are known (here η=1.002 cP at 20 °C for water). The size distribution was determined using the CONTIN algorithm based on the inverse Laplace transform of the correlation function.

Setup for singlet oxygen ($^1O_2$) generation experiments

A 1 x 1 cm cuvette was used for the measurements. Near–infrared emission spectra were recorded using an Andor iDus InGaAs detector coupled with a Shamrock 163 spectrograph with excitation using a 4 mW 405 nm diode laser (Thorlabs LDM 405). Tris(bipyridine)ruthenium(II) chloride ($Ru(bpy)_3Cl_2$) was used as reference sensitizer.

Preparation of samples for solubilizer-screening experiment

First, *m*THPC (340 μg, 0.5 μmol) in ethanol (1 mg/mL) was loaded into a vial and the solvent was removed under vacumn at 30 °C for 3 h. The pretreated DNA aqueous solution (500 μL) was added directly to *m*THPC, and the mixture was stirred (1000 r/min) at 37 °C for 12 h. After centrifugation (10000 r/min) for 15 min, the supernatant loaded with *m*THPC was obtained without touching the pellet. Aluminum foil was used to cover

the samples to avoid photochemical degradation of *m*THPC throughout the experiments.

Preparation of maximum *m*THPC-loaded lipid-DNA micelles

*m*THPC (680 μg, 1 μmol) in ethanol (1 mg/mL) was mixed with pretreated aqueous DNA solution (1000 μL) and stirred (1000 r/min) at room temperature for 1 h. After that, the mixture was lyophilized, and H_2O (1000 μL) was added. The mixture was stirred (1000 r/min) at room temperature for 1 h. After centrifugation (10000 r/min) for 15 min, the supernatant loaded with *m*THPC was obtained without touching the pellet. Aluminum foil was used to cover the samples to avoid photochemical degradation of *m*THPC throughout the experiments.

Preparation of samples for $^1O_2$-generation experiment

*m*THPC 680 μg (1 μmol) in ethanol (1 mg/mL) was loaded into a vial, the solvent was removed under vacuum at 30 °C for 5 h. In the meantime, the aqueous solution of **UU11mer** (50 μM in D_2O, 4000 μL) and **ds<u>UU</u>11mer** (**<u>UU</u>11mer** 50 μM, **c11mer** 50 μM, $MgCl_2$ 10 mM in D_2O, 4000 μL) was prepared and thermally cycled as described above. After that, the solutions obtained were added directly to *m*THPC, and the mixtures were stirred (1000 r/min) at room temperature for 6 h. After centrifugation (10000 r/min) for 15 min, the supernatants were obtained without touching the pellet. Aluminum foil was used to cover the sample to avoid photochemical degradation of *m*THPC throughout the experiments.

Determination of *m*THPC loading concentrations and loading capacities

To determine the loading capacities, the isolated supernatant (400 μL) was lyophilized, followed by the addition of cold ethanol (400 μL) to extract *m*THPC. After centrifugation (10000 r/min) for 15 min, the supernatant was removed to determine the drug loading concentration and loading capacity through RP-HPLC measurement. Loading capacities were calculated by using equation S1:

$$\%Loading\ capacity = \frac{\text{Weight of } m\text{THPC loaded}}{\text{Weight of DNA}} \times 100\% \quad \text{(S1)}$$

Preparation of the stock solution of *m*THPC (2 mM) loaded UU11mer micelles

UU11mer (1.7 mM, 4 mL) in MiliQ H_2O was thermally cycled (90 °C, 30 min; –1 °C/2 min until RT) by using a polymerase chain reaction (PCR) thermocycler (Biorad, USA) before use. Then it was diluted to 800 μM (8.5 mL) with MiliQ H_2O. *m*THPC (6.8 mg) was dissolved in ethanol (6.8 mL), and it was mixed with pretreated aqueous DNA solution (8.5 mL) and stirred (1000 r/min) at room temperature for 1 h. After that, the mixture was lyophilized, and H_2O (2 mL) was added. The mixture was stirred (1000 r/min) at room temperature for 1 h. After centrifugation (10000 r/min) for 15 min, the solution A was obtained. Aluminum foil was used to cover the samples to avoid photochemical degradation of *m*THPC throughout the experiments.

To determine the loading concentration of solution A, the isolated supernatant (20 μL) was lyophilized, followed by the addition of cold ethanol (1360 μL) to extract *m*THPC.

After centrifugation (10000 r/min) for 15 min, the supernatant was removed and diluted five times for RP-HPLC measurement to determine and calculate the drug loading concentration of solution A. The drug loading concentration of solution A was found to be 7045 μM. Finally, solution A was diluted with MiliQ water to obtain the stock solution.

***In vitro* phototoxcity of *m*THPC loaded UU11mer**

Human epidermoid carcinoma A253, human epithelial carcinoma A431, human oral adenosquamous carcinoma CAL27, murine hematopoiesis monocytic macrophages J774A.1, murine fibroblasts L929 and human colorectal adenocarcinoma HT29 cells were grown in Dulbecco's modified eagle medium (DMEM) with 10% heat inactivated FCS, 1% penicillin (10000 IU) and streptomycin (10000 μg/mL). A stock solution (2 mM) of *m*THPC loaded **UU11mer** micelles was prepared at 4 °C in MiliQ water and kept in the dark. DMEM (without phenol red) with 10 % FCS was used for further dilution to reach concentration 2 or 10 μM of *m*THPC, respectively. As a control for cytotoxicity, 2 concentrations (8 and 80 μM) were prepared from the 800 μM stock solution of **UU11mer** (empty) at 4 °C in DMEM (without phenol red) with 10 % FCS and kept in the dark. As a positive control a stock solution (2 mM) of the pure *m*THPC was prepared at 4 °C in ethanol and kept in the dark. DMEM (without phenol red) with 10 % FCS was used for further dilution to reach concentration 2 or 10 μM of *m*THPC, respectively. In microplates 2×10^4 cells per well of all 6 cell lines were seeded in fresh medium (DMEM without phenol red) containing 10 % FCS with 2 or 10 μM of *m*THPC loaded **UU11mer** micelles

or the described two controls and incubated for 24 h. After exchange of medium (to remove any *m*THPC loaded **UU11mer** or empty **UU11mer** or free *m*THPC not taken up by the cells), the photosensitization was performed at RT with a laser (biolitec AG, Jena) at 652 nm at a dose rate of app. 50 J/cm^2. The cell viability of the samples was measured with a Tecan InfiniTE *200* microplate reader, at a wavelength of 490 nm, assessed using the XTT assay[2] and the absorbance. A wavelength of 630 to 690 nm was used to measure the reference absorbance (for measuring the non-specific readings).

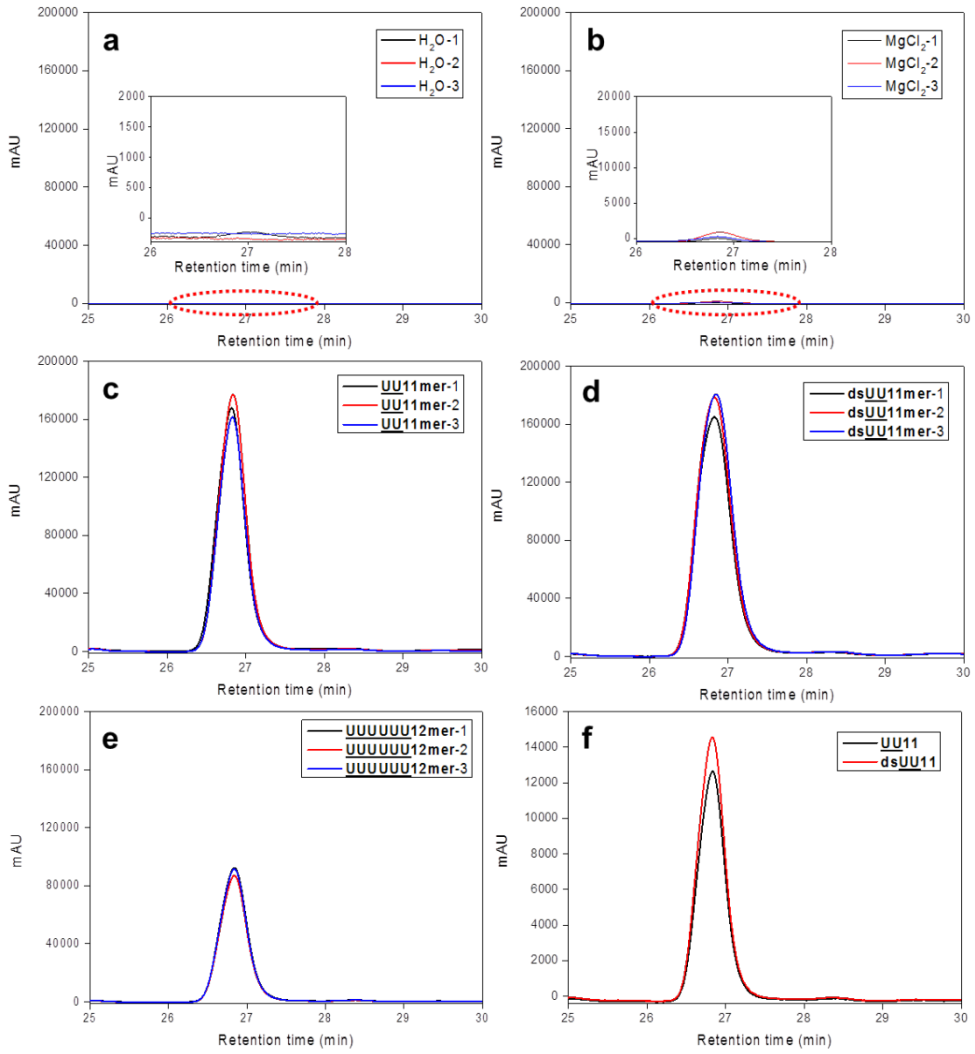


Figure S4. RP-HPLC spectra of a) H_2O; b) $MgCl_2$ (10 mM) aqueous solution; c) **UU11mer** (50 μM); d) **dsUU11mer** (50 μM); e) **UUUUUU12mer** (50 μM) *m*THPC-loaded samples for maximum loading capacity determination and f) *m*THPC-loaded **UU11mer** (50 μM) and **dsUU11mer** (50 μM) micelles in D_2O for $^1O_2$-generation. Spectra were recorded at a wavelength of 417 nm.

Table S2. Concentrations and loading capacities of *m*THPC-loaded samples determined by RP-HPLC.

Samples	Integral	Concentration (μM)	Average concentration (μM)	Loading capacity (%)	*m*THPC/carrier ratio
H_2O-1	0	0	0	0	-
H_2O-1	0	0			
H_2O-1	0	0			
$MgCl_2$-1	15454	0.2	0.3 ± 0.1	-	-
$MgCl_2$-2	34958	0.4			
$MgCl_2$-3	16779	0.2			
UU11mer-1	4235026	31.1	31.1 ± 1.6	11.7 ± 0.6	1 : 1.61
UU11mer-2	4438431	32.6			
UU11mer-3	4015920	29.5			
dsUU11mer-1	5162632	37.9	40.0 ± 2.1	7.8 ± 0.4	1 : 1.25
dsUU11mer-2	5543864	40.7			
dsUU11mer-3	5639095	41.4			
UUUUUU12mer-1	2316226	17.1	16.7 ± 0.6	5.1 ± 0.2	1 : 2.99
UUUUUU12mer-2	2183281	16.1			
UUUUUU12mer-3	2286009	16.8			

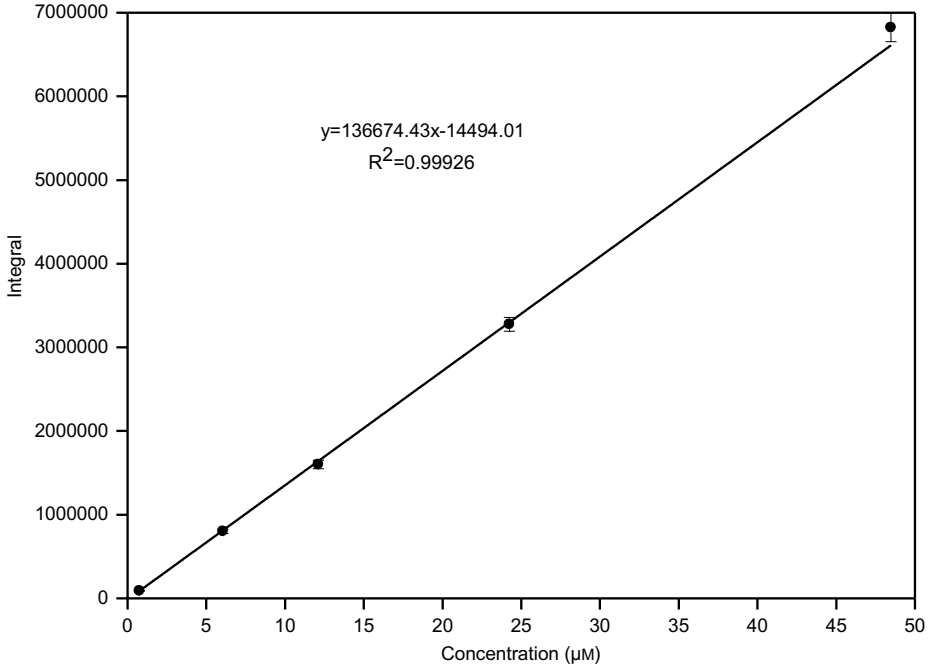


Figure S5. Calibration curve of *m*THPC obtained through RP-HPLC in ethanol, while monitoring at 417 nm.

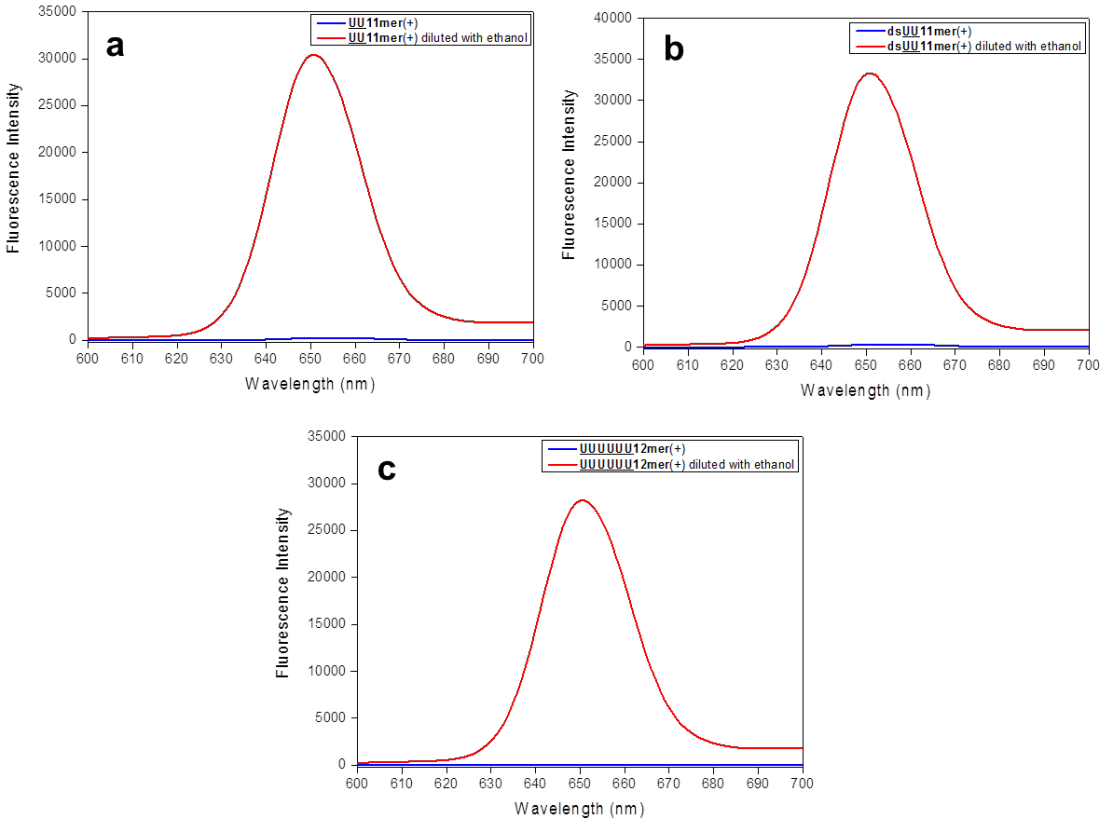


Figure S6. Fluorescence-emission spectra of *m*THPC-loaded lipid-DNA aqueous solutions (blue line) and dilutions with 1 equivalent volume of EtOH (red line) at an excitation wavelength of 417 nm. a) **UU11mer (**50 μM); b) **dsUU11mer (**50 μM); c) **UUUUUU12mer (**50 μM).

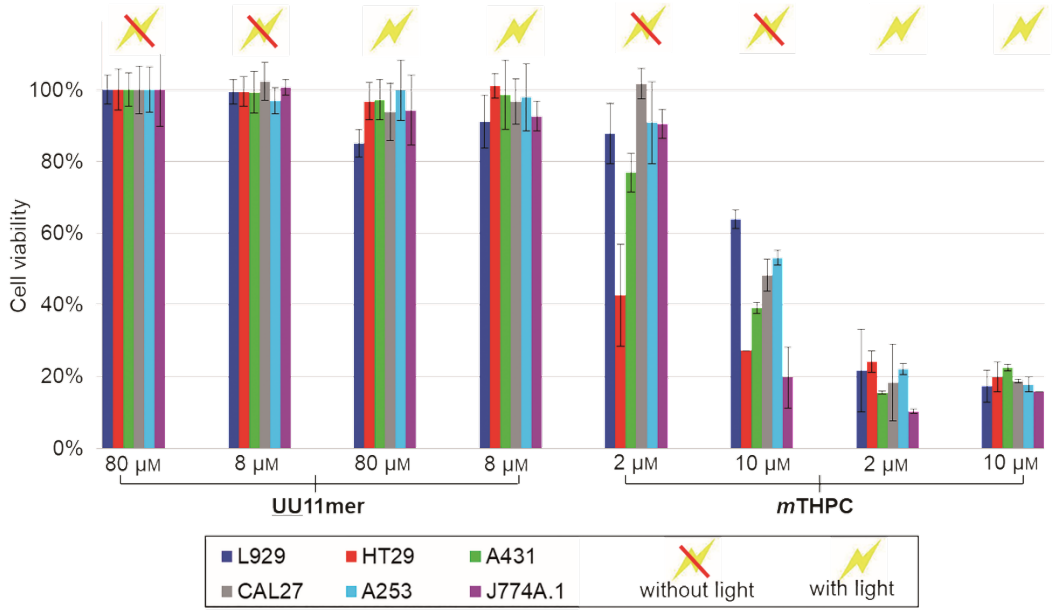


Figure S7. Phototoxicity and dark toxicity of **UU11mer** (8 and 80 µM) aqueous solutions and pure *m*THPC (2 and 10 µM) in ethanol. They were tested in six different cell lines (A431, HT29, L929, J744A.1, CAL27 and A253). The controls were incubated with the cells for 24 h. After exchange of medium, the photosensitization was performed at RT with a laser at 652 nm at a dose rate of app. 50 J/cm^2. The cell viability was measured with a Tecan InfiniTE *200* microplate reader, at a wavelength of 490 nm. A wavelength of 630 to 690 nm was used to measure the reference absorbance.

Table S3. Concentrations of the samples used for $^1O_2$-generation experiments measured in D_2O determined by RP-HPLC.

Samples	Integral	Concentration (µM)
UU11mer	319170	2.4
dsUU11mer	366328	2.8

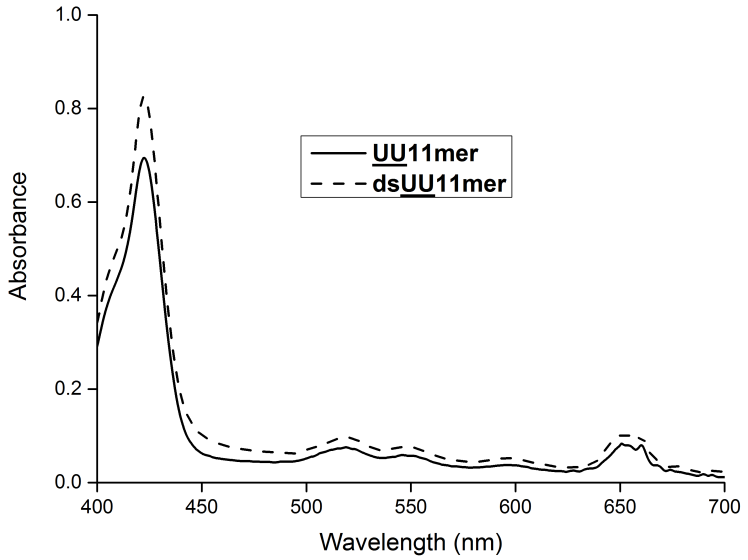


Figure S7. Absorption spectra of the samples for $^1O_2$-generation experiment measured in D_2O.

References

[1] M. Kwak, I. J. Minten, D. M. Anaya, A. J. Musser, M. Brasch, R. J. M. Nolte, K. Müllen, J. J. L. M. Cornelissen and A. Herrmann, A. *J. Am. Chem. Soc.*, 2010, **132**, 7834–7835.

[2] M. V. Berridge, P. M. Herst and A. S. Tan, A. *Biotechnol. Ann. Rev.*, 2005, **11**, 127–152.