Electronic Supplementary Material (ESI) for RSC Advances.

Assembly and Functionalization of Supramolecular Polymers from DNA-Conjugated Squaraine Oligomers

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GENERAL INFORMATION

Chemical reactions were monitored by TLC (Silica gel, Macherey-Nagel).

^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR spectra were measured on a *Bruker avance 300* spectrometer, in DMSO-d_6 or $CDCl_3$-d; setting the solvent residual peak as reference.

Mass spectrometry was performed with an *Applied Biosystems* MDS-Sciex QTRAP LC/MS/MS system and LTQ Orbitrap XL system.

Absorption spectra were recorded in 1 cm quartz cells at 20°C (if not otherwise stated) on *Varian Cary-100 Bio*-UV/VIS spectrophotometer equipped with a *Varian Cary*-block temperature controller.

CD spectra were recorded on a *JASCO J-715* spectropolarimeter using quartz cuvettes with an optical path of 1 cm.

Atomic force microscopy (AFM) measurements were conducted on a *Nanosurf FlexAFM* using tapping mode AFM probes (Budget Sensors). Samples were deposited on a (3-aminopropyl)triethoxysilane (APTES)-modified mica that was prepared according to published procedures.[1] Unmodified mica was purchased from PLANO GmbH. The following procedure for sample deposition was applied: 30 µL of sample were transferred onto the modified mica, after 3 min the solution was removed, the surface was rinsed (3 times) with 0.5 mL Milli-Q water and dried with a stream of argon gas.

Transmission electron microscopy (TEM) was performed on *ANA FEI Tecnai Spirit* Transmission electron microscope provided by Microscopy Imaging Center (MIC), University of Bern, Switzerland. Samples were deposited on the carbon-coated copper grids that were purchased from Agar Scientific. The procedure of deposition: 5 µL of sample were transferred onto the grid, after 5 min the solution was removed, the grid was rinsed (3 times) with 5 µL Milli-Q water, 5 µL of aqueous uranyl acetate were added to the sample and after 5 min the solution was removed.

Scanning electron microscope (SEM) images were obtained on *Zeiss Gemini 450* microscope (MIC, University of Bern). Samples were deposited on APTES-modified mica (same deposition procedure as in AFM experiments) or silicon nitride discs with hydrophilic coating (Ted Pella Inc., USA) (sample deposition: see TEM measurements, but without the addition of uranyl acetate).

Reversed-phase HPLC purification and analysis of the modified oligonucleotides was carried out on a *LC-20AT / SPD-M20A* system (Shimadzu, Kyoto, Japan).

Reagents and solvents for the synthesis of the squaraine phosphoramidite were purchased from commercial suppliers: Acros Organics, Fluka, and Sigma Aldrich.

UltraMILD CE phosphoramidites, the reagents and solvents required for the synthesis of the DNA-functionalized squaraine oligomers **Sq1**, **Sq3**, and **Sq6** were purchased from Glen Research and Sigma Aldrich. Oligonucleotide-synthesis of **Sq1**, **Sq3**, **Sq6** and 3-amino-modified oligomer **T6** was performed by an automated oligonucleotide synthesis on a *394-DNA/RNA synthesizer* (Applied Biosystems), based on phosphoramidite chemistry. Unmodified oligonucleotides **NM** and **cNM** as well as 5-amino-modified oligonucleotides **ON1** and **ON2** were commercially obtained from Microsynth (Balgach, Switzerland).

Table S1. The list of the oligonucleotides used in this work.

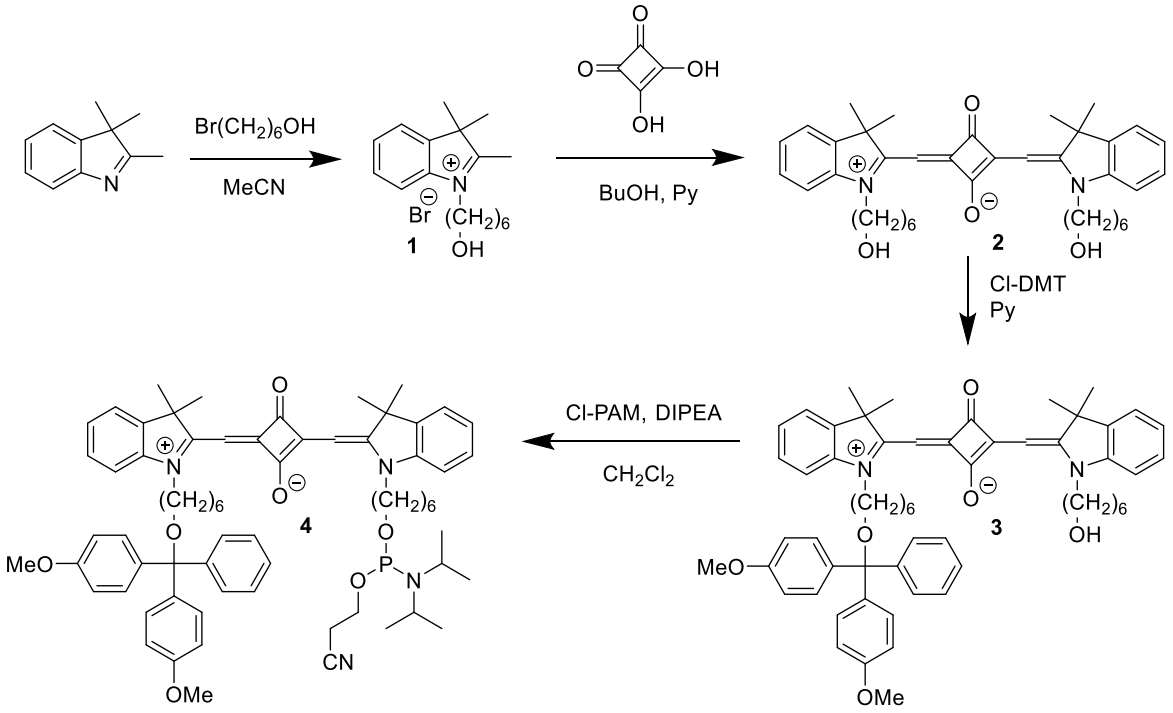
ID:	Sequence
Sq1	3'-GAAGGCACTC-Sq
Sq3	3'-GAAGGCACTC-Sq-Sq-Sq
Sq6	3'-GAAGGCACTC-Sq-Sq-Sq-Sq-Sq-Sq
T6	NH_2-TTTTTT-5'
ON1	3'-GAGTGCCTTCTTTTTTTTTT-NH_2
ON2	3'-CCTGAAGTTATTTTTTTTTT-NH_2
T6-SS	Thioctic acid-NH-TTTTTT-5'
ON1-SS	3'-GAGTGCCTTCTTTTTTTTTT-NH-Thioctic acid
ON2-SS	3'-CCTGAAGTTATTTTTTTTTT-NH-Thioctic acid
NM	3'-GAAGGCACTC-5'
cNM	3'-GAGTGCCTTC-5'

Concentrations of the oligonucleotides were determined based on the molar absorptivities. For the calculation of the molar absorptivities, values ε_{260}(Sq) = 11,000 M^{-1} cm^{-1},[2] ε_{260}(A) = 15,060 M^{-1} cm^{-1}, ε_{260}(T) = 8,560 M^{-1} cm^{-1}, ε_{260}(G) = 12,180 M^{-1} cm^{-1}, and ε_{260}(C) = 7,100 M^{-1} cm^{-1} were applied.[3]

The preparation of the phosphate buffer (PB) (0.1 M, pH = 7.0) was adapted from the literature:[4] a mixture of (A) 60 mL $Na_2HPO_4 \cdot 2H_2O$ (17.8 g/L) and (B) 40 mL KH_2PO_4 (13.61 g/L). The use of either KH_2PO_4 or NaH_2PO_4 in the buffer solution did not alter the results (e.g. the self-assembly of the oligomers into supramolecular polymers).

SYNTHESIS OF THE SQUARAINE PHOSPHORAMIDITE

The synthesis of the squaraine phosphoramidite was adapted from published procedures,[2] with small changes to the original protocols (see below). The synthesis consists of four steps:



Synthesis of compound (1)

A mixture of 2,3,3-trimethylindolenine (4 g, 25.1 mmol) and 6-bromo-1-hexanol (5.46 g, 30.1 mmol) in acetonitrile (30 mL) was refluxed under argon for 30 hours. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel 60, 0-30% MeOH in CH_2Cl_2), to give the product 1-(6-hydroxyhexyl)-2,3,3-trimethyl-3*H*-indolium bromide (**1**) (R_f = 0.67; Silica gel 60, 15% MeOH in CH_2Cl_2), 3.64 g (43%).
λ_{max} (Abs) of (**1**) = 279 nm (in MeOH).
^{1}H-NMR (300 MHz, DMSO, ppm): δ 8.03-7.94 (1H, m, arom H), 7.89-7.81 (1H, m, arom H), 7.66-7.59 (2H, m, arom H), 4.46 (2H, t, 7.72 Hz, NCH_2), 3.37 (2H, t, 5.93 Hz, $C\underline{H}_2OH$), 2.86 (3H, s, 2-CH_3), 1.91-1.72 (2H, m, CH_2), 1.54 (6H, s, [3-CH_3]$_2$), 1.49-1.29 (6H, m, CH_2).
^{13}C-NMR (75 MHz, DMSO, ppm): δ 196.41, 141.87, 141.04, 129.37, 128.93, 123.52, 115.48, 60.43, 54.14, 47.58, 40.35, 40.08, 39.80, 39.52, 39.24, 38.96, 38.69, 32.17, 27.25, 25.74, 25.10, 22.01, 14.06.
ESI-MS (pos., MeOH): mass calc. for $C_{17}H_{26}NO^+$ = 260.2009; mass found = 260.2002.

Synthesis of compound (2)

Compound **1** (950 mg, 2.79 mmol) was dissolved in pyridine (10 mL). Then 3,4-dihydroxy-3-cyclobutene-1,2-dione (150 mg, 1.32 mmol) and 1-butanol (10 mL) were added. The mixture was stirred at 110 °C for 5 hours. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel 60, 0–8% MeOH in CH_2Cl_2) to give the diol (**2**) as a blue powder (R_f = 0.23; silica gel 60, 5% MeOH in CH_2Cl_2), 340 mg (43%).
^{1}H-NMR (300 MHz, DMSO, ppm): δ 7.51 (2H, d, 7.35 Hz, arom H), 7.40-7.25 (4H, m, arom H), 7.16 (2H, t, *J* = 6.87 Hz, arom H), 5.80 (2H, s, CH), 4.36 (2H, t, *J* = 5.18 Hz, OH), 4.07 (4H, t, *J* = 6.5 Hz, NCH_2), 3.38 (4H, t, *J* = 5.56 Hz, $C\underline{H}_2OH$), 1.81-1.55 (4H, m, CH_2), 1.68 (12H, s, 3-CH_3), 1.50-1.26 (12H, m, CH_2).
^{13}C-NMR (75 MHz, $CDCl_3$, ppm): δ 178.63, 170.27, 142.42, 142.29, 127.89, 123.85, 122.41, 109.45, 86.66, 62.56, 49.37, 43.19, 32.50, 27.10, 26.25, 26.21, 24.93.
ESI-MS (pos., MeOH): mass calc. for $C_{38}H_{48}N_2O_4$ = 596.3614; mass found = 596.3601.

Synthesis of compound (3)

4,4'-Dimethoxytrityl chloride (320 mg, 0.947 mmol) was added to a solution of **2** (565 mg, 0.947 mmol) in dry pyridine (12 mL). The mixture was stirred overnight at room temperature and under argon. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel 60, 0–4% MeOH in CH_2Cl_2/EtOAc/NEt_3, 49/49/2, v/v/v), to yield the mono(dimethoxytrityl)-protected derivative **3** (R_f = 0.51; silica gel 60, 5% MeOH in CH_2Cl_2/EtOAc/NEt_3, 49/49/2, v/v/v), 390 mg (37%).

^{1}H-NMR (300 MHz, DMSO, ppm): δ 7.51 (2H, d, *J* = 7.53 Hz, arom H), 7.41-7.09 (15H, m, arom H), 6.86 (4H, d, *J* = 9.04 Hz arom H), 5.80 (1H, s, CH), 5.79 (1H, s, CH), 4.35 (1H, t, *J* = 5.18 Hz, OH), 4.16-3.93 (4H, m, NCH_2), 3.71 (6H, s, OCH_3), 3.42-3.32 (2H, m, $C\underline{H}_2OH$), 2.93 (2H, t, *J* = 6.21 Hz, CH_2DMT), 1.80-1.59 (4H, m, CH_2), 1.67 (6H, s, 3-CH_3), 1.66 (6H, s, 3-CH_3), 1.58-1.47 (2H, m, CH_2), 1.47-1.25 (10H, m, CH_2).

^{13}C-NMR (75 MHz, $CDCl_3$, ppm): δ 179.22, 179.11, 170.23, 170.15, 158.40, 145.45, 142.55, 142.46, 142.33, 136.78, 130.10, 128.30, 127.88, 127.80, 126.68, 123.81, 122.42, 117.57, 113.09, 109.47, 109.40, 86.73, 86.62, 85.79, 77.58, 77.16, 76.74, 63.30, 62.64, 55.30, 49.41, 49.36, 45.91, 43.79, 43.14, 32.51, 30.07, 27.15, 27.13, 26.28, 26.24, 26.15, 24.88.

ESI-MS (pos., CH_2Cl_2): mass calc. for $C_{59}H_{66}N_2O_6$ = 898.4921; mass found = 898.4888.

Synthesis of compound (4)

2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (145 µL, 0.651 mmol) was added under argon to a solution of compound **3** (390 mg, 0.434 mmol) and diisopropylethylamine (226 µL, 1.3 mmol) in anhydrous dichloromethane (8 mL). The mixture was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by column chromatography (silica gel 60, 50-100% EtOAc in hexane containing 1% of NEt_3). Note: use only freshly distilled NEt_3; and anhydrous EtOAc and hexane. The fractions containing the product were combined and concentrated to a volume of about 2 mL. Cold (-28 °C) hexane (100 mL) was added and the solution was kept at -28 °C for 2 hours. A blue precipitate was formed, the material was filtered-off and the product was dried under high-vacuum to yield phosphoramidite **4** (R_f = 0.76; silica gel 60, 5% MeOH in CH_2Cl_2/EtOAc/NEt_3, 49/49/2, v/v/v), 215 mg (45%).

^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 7.46-7.07 (15H, m, arom H), 6.96 (2H, t, *J* = 7.82 Hz, arom H), 6.82 (4H, d, *J* = 8.85 Hz, arom H), 5.94 (2H, s, CH), 4.09-3.89 (4H, m, NCH_2), 3.89-3.75 (2H, m, $C\underline{H}CH_3$), 3.77 (6H, s, OCH_3), 3.70-3.50 (4H, m, CH_2OP), 3.03 (2H, t, *J* = 6.5 HZ, CH_2ODMT), 2.62 (2H, t, *J* = 6.5 Hz, CH_2CN), 1.88-1.71 (4H, m, CH_2), 1.78 (12H, s, 3-CH_3), 1.68-1.55 (4H, m, CH_2), 1.51-1.37 (8H, m, CH_2), 1.22-1.10 (12H, m, $CHC\underline{H}_3$).

^{13}C-NMR (75 MHz, $CDCl_3$, ppm): δ 179.59, 179.49, 170.10, 170.05, 158.37, 145.43, 142.54, 142.28, 136.75, 130.08, 128.27, 127.84, 127.78, 126.65, 123.75, 122.38, 117.79, 113.06, 109.42, 86.61, 85.77, 77.58, 77.16, 76.74, 63.57, 63.34, 63.27, 58.52, 58.27, 55.27, 49.36, 43.76, 43.71, 43.15, 42.98, 31.18, 31.08, 30.04, 27.16, 27.12, 26.90, 26.25, 25.87, 24.78, 24.73, 24.69, 24.64, 20.50, 20.41.

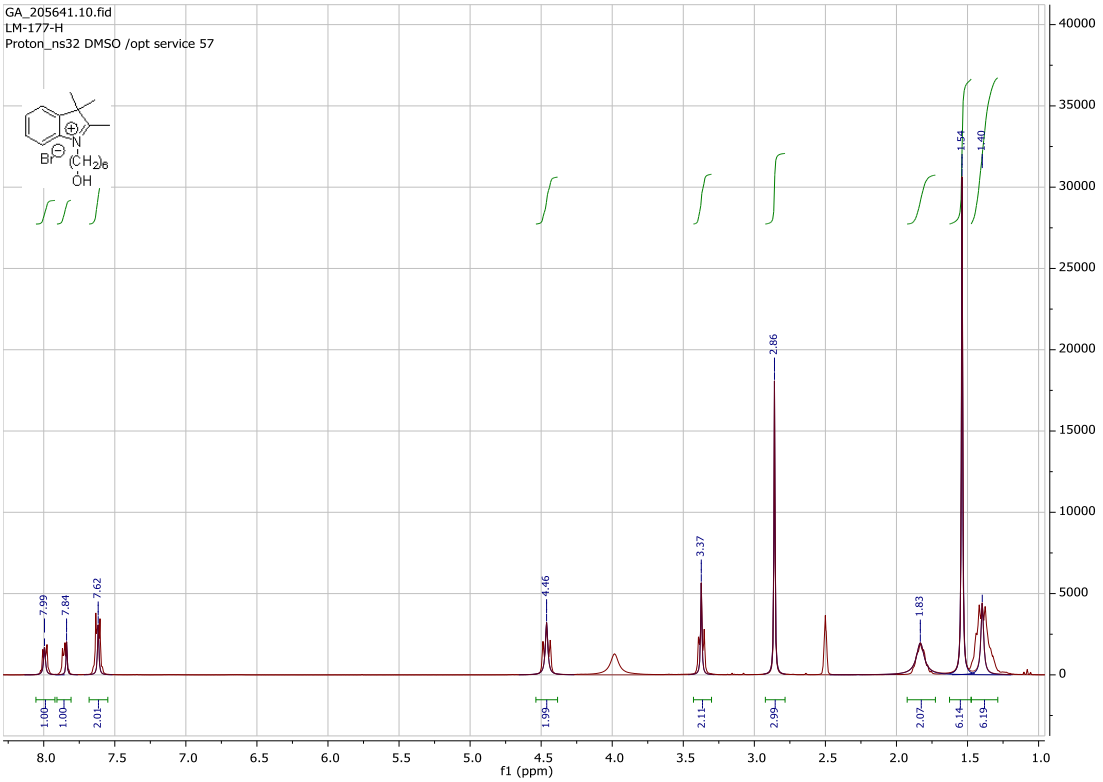
^{31}P-NMR (121 MHz, $CDCl_3$): δ = 147.25.

ESI-MS (pos., CH_2Cl_2): mass calc. for $C_{68}H_{83}N_4O_7P$ = 1098.5999; mass found = 1098.5958.

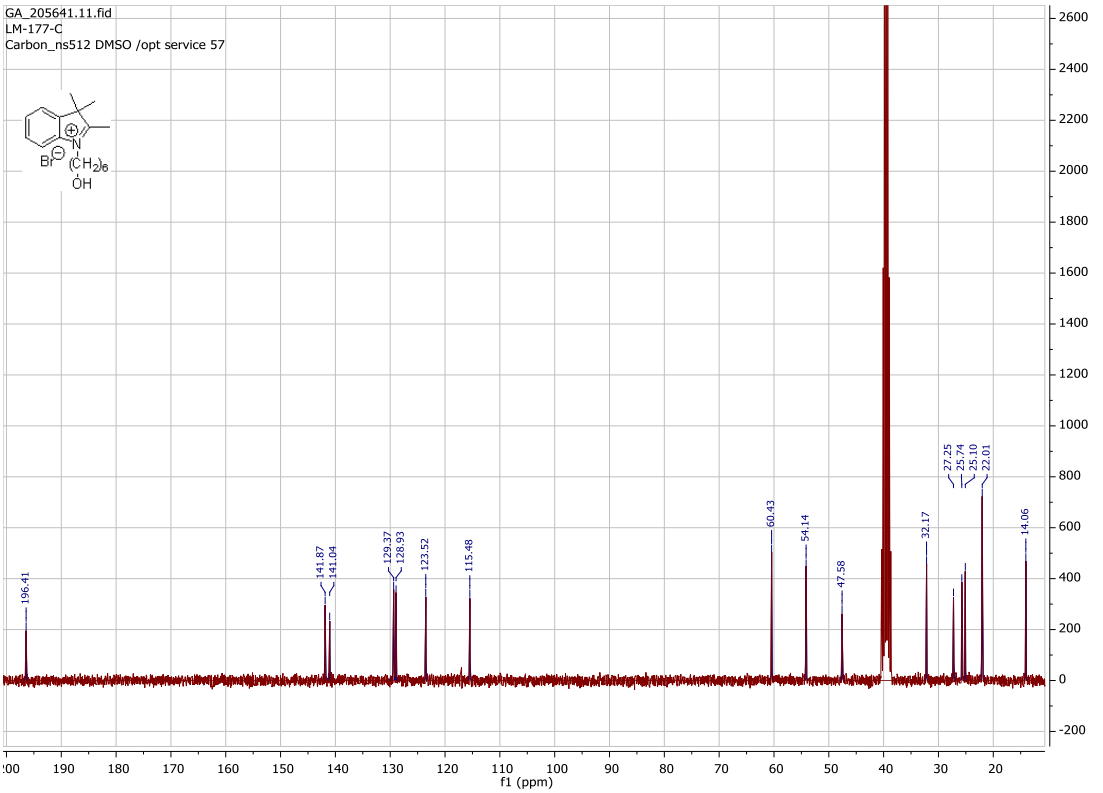
NMR DATA

Compound (1)

^{1}H-NMR:



^{13}C-NMR:



Compound (2)

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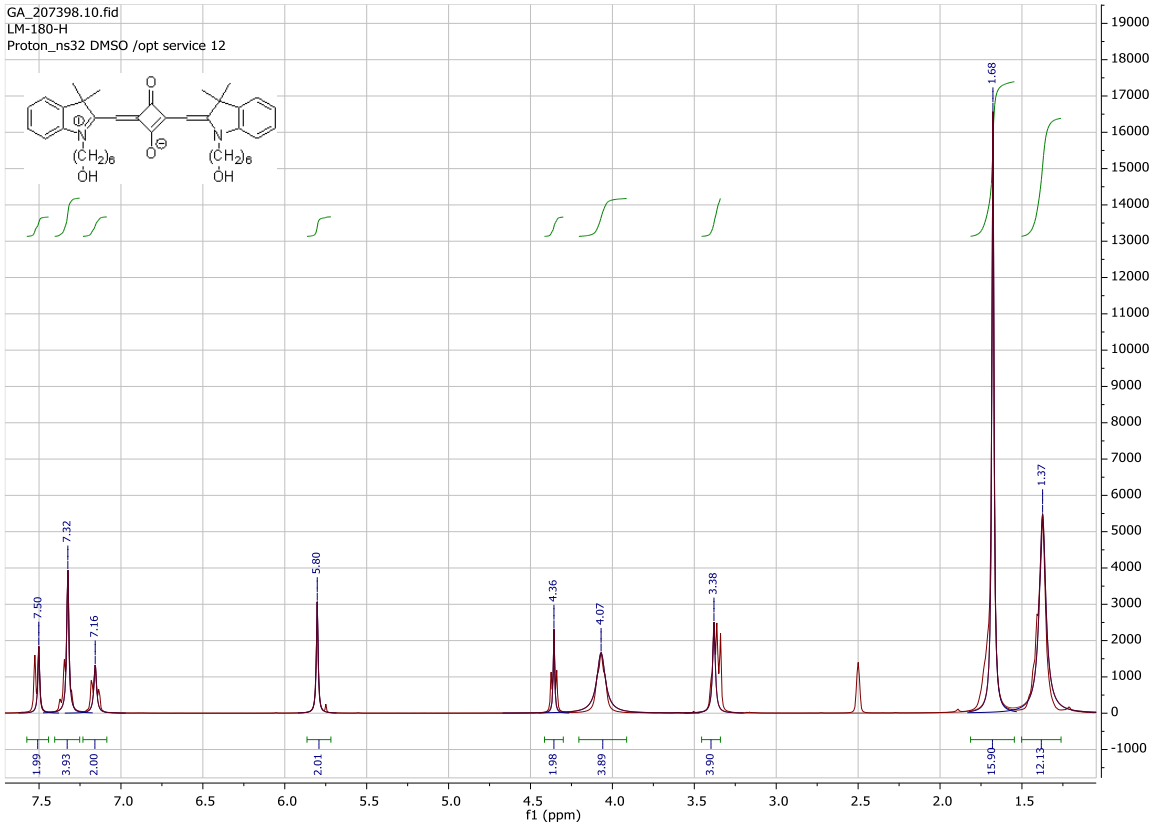

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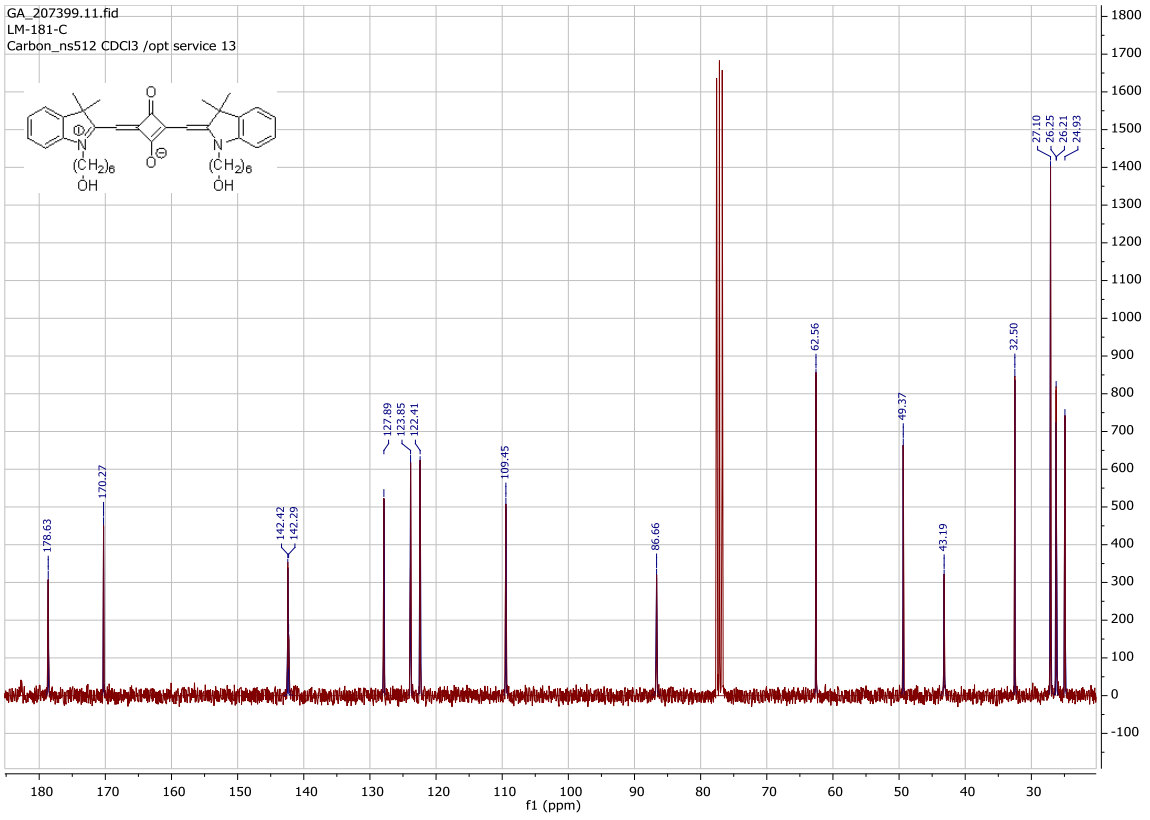

Compound (3)

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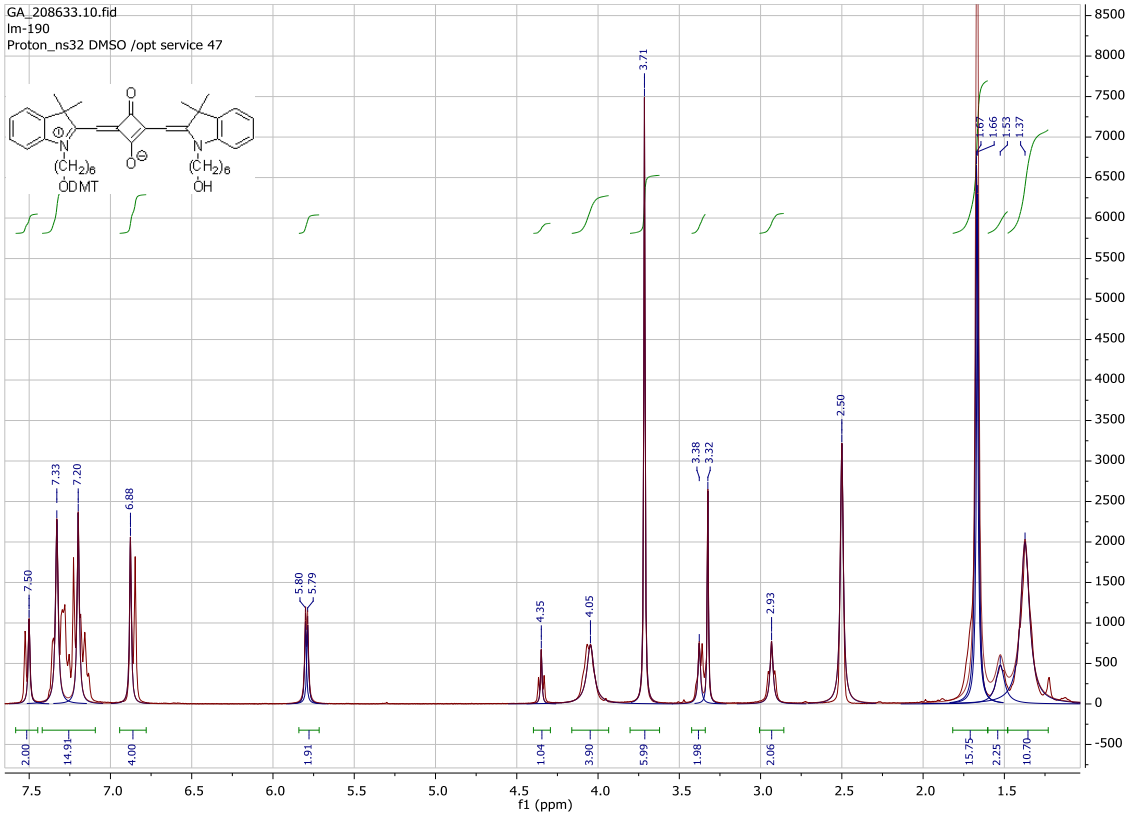
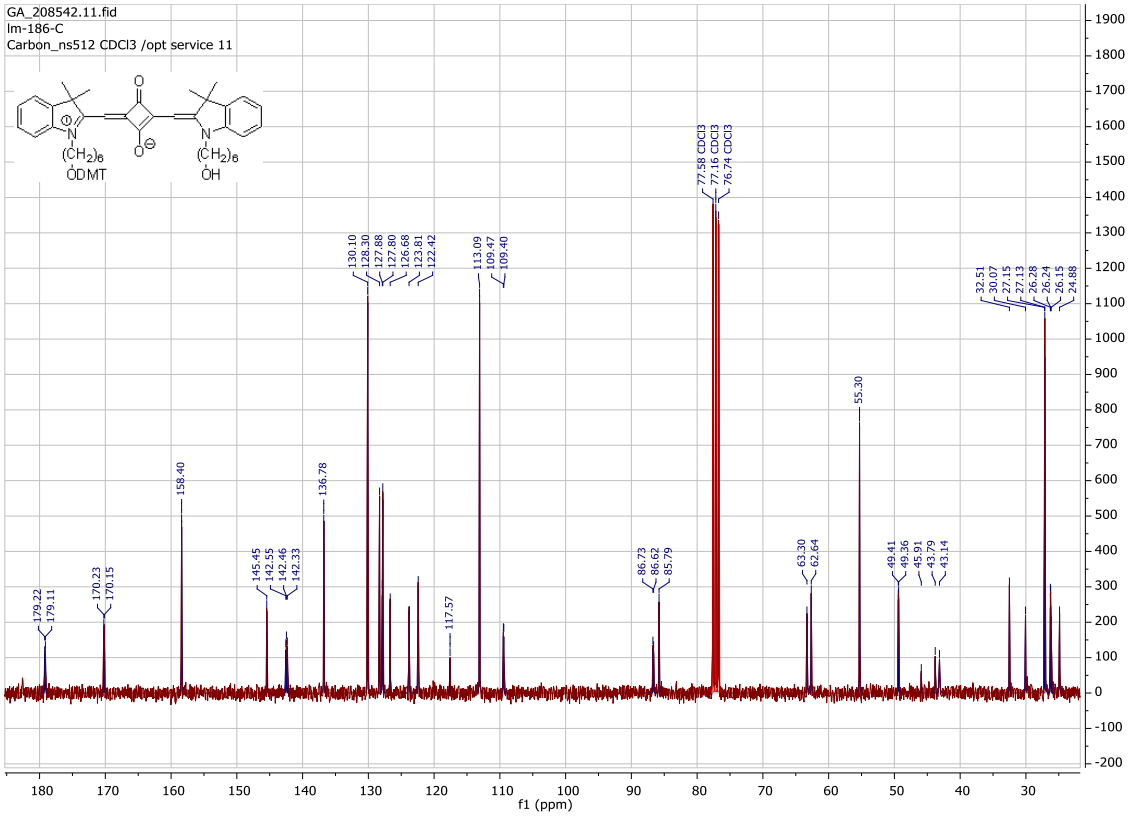


^{13}C-NMR:



Compound (4)

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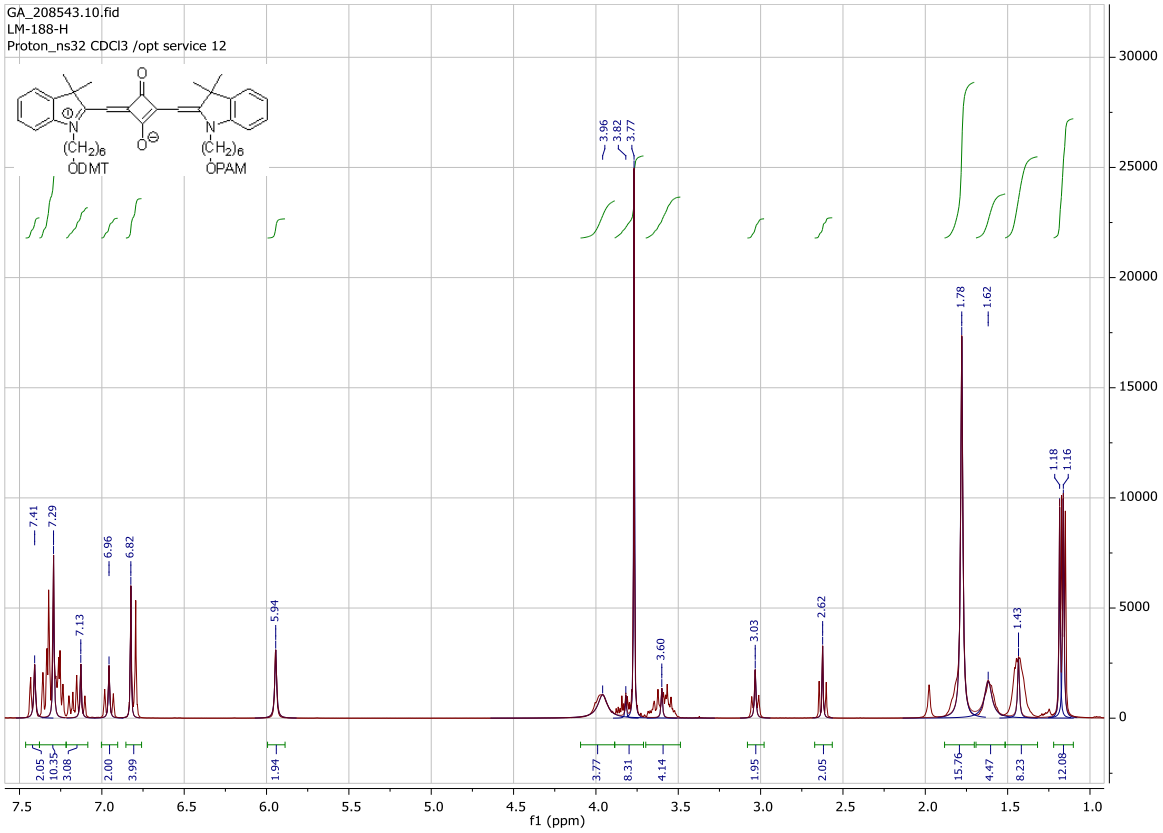

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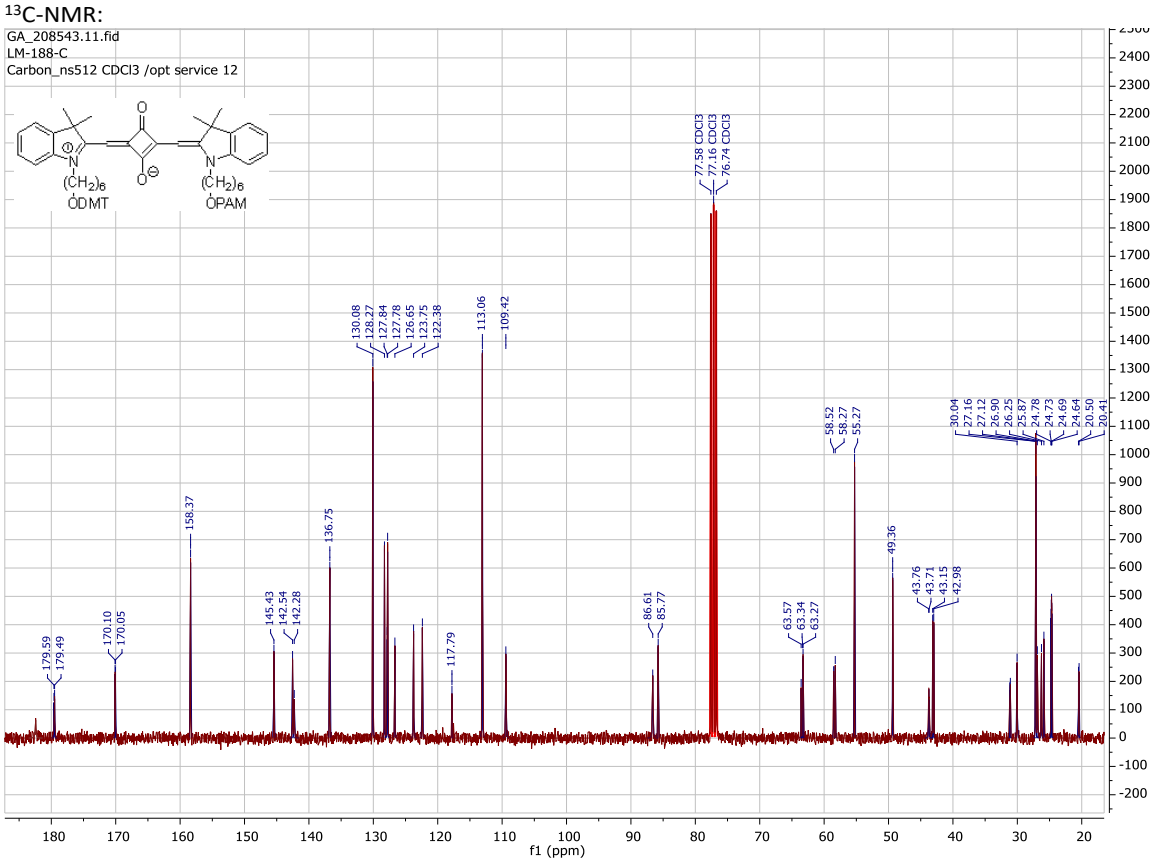
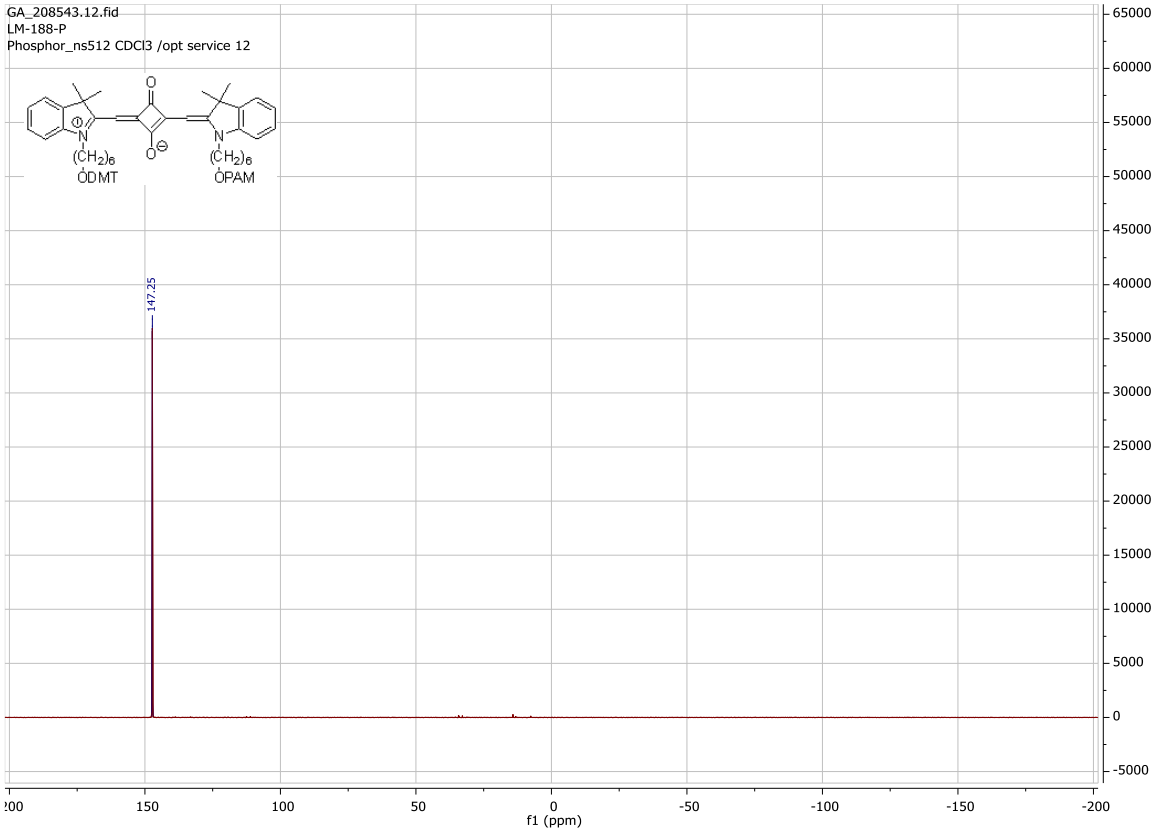


^{31}P-NMR: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60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
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5000
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100
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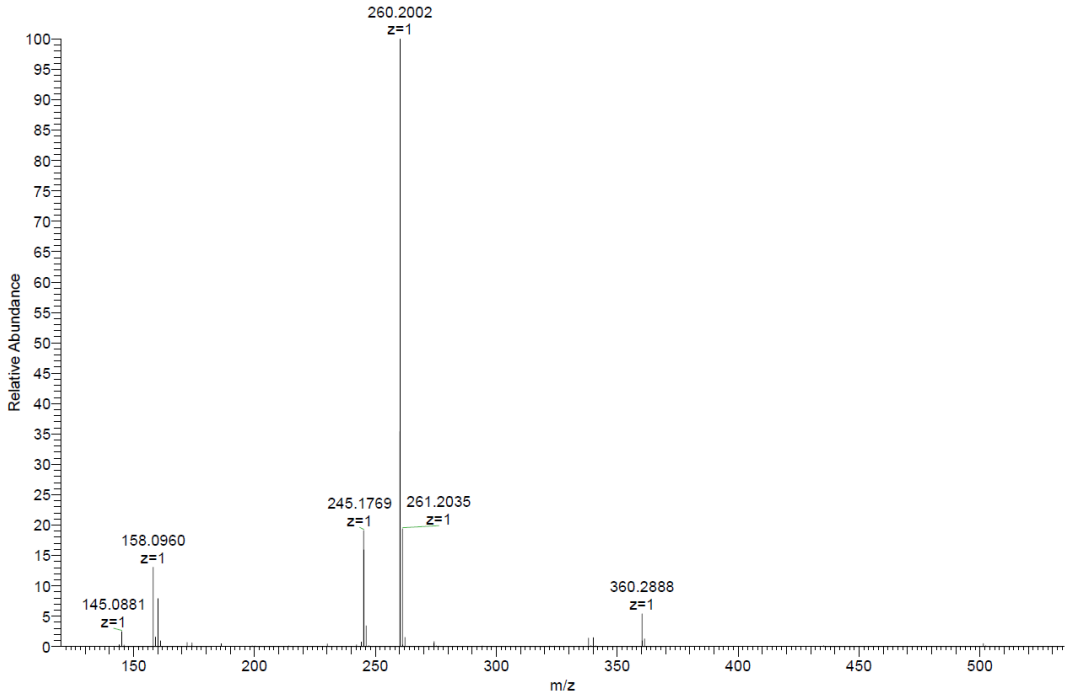
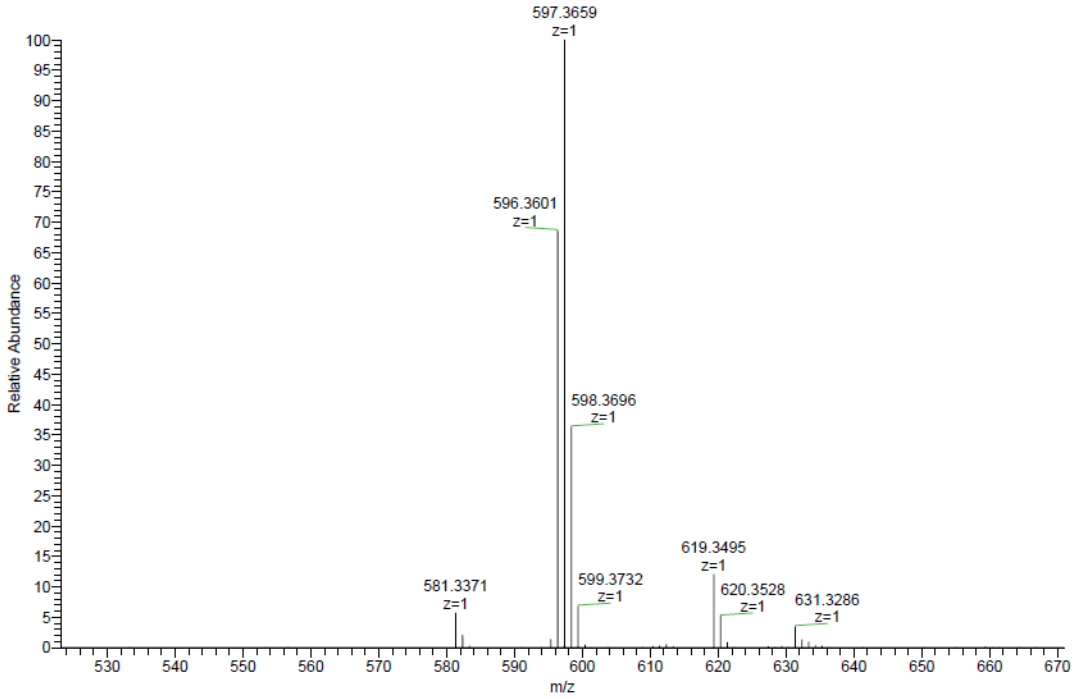
0
-50
-100
-150
-200
f1 (ppm)

MS ANALYSIS

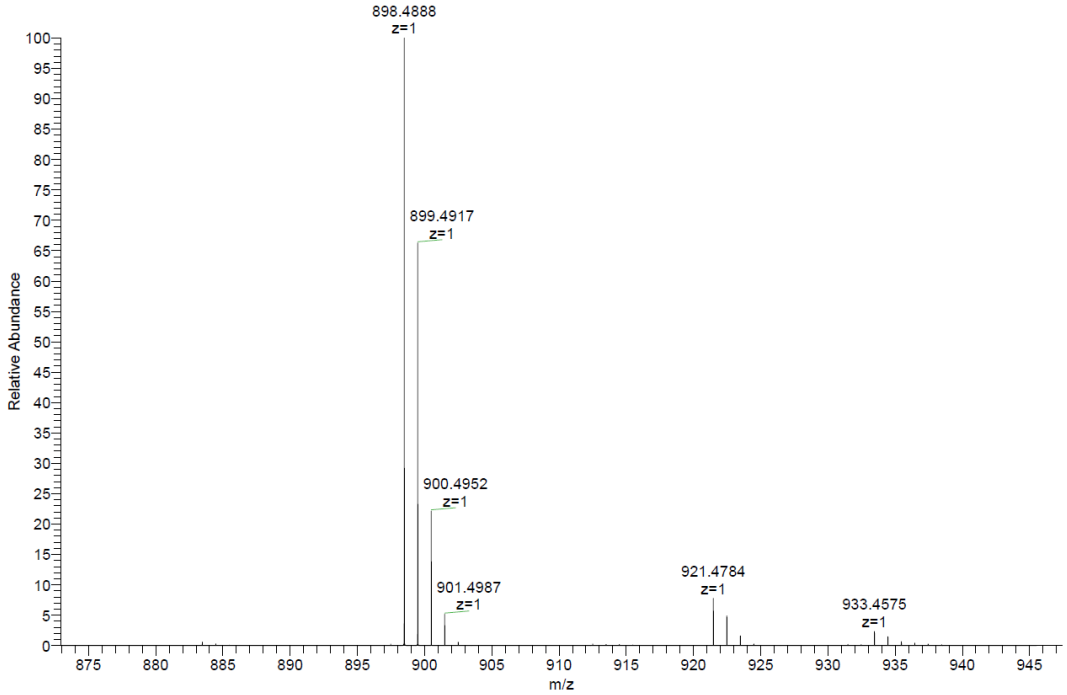
Compound (1)



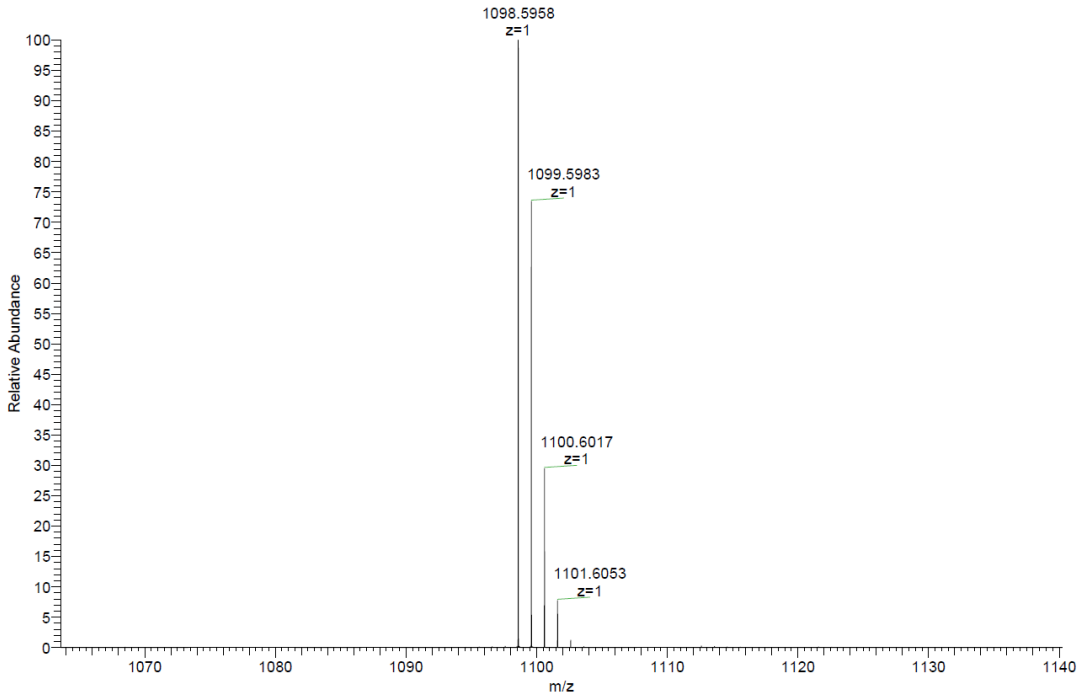
Compound (2)



Compound (3)



Compound (4)



SOLID PHASE SYNTHESIS OF THE SQUARAINE OLIGOMERS

Synthesis of the oligomers **Sq1**, **Sq3**, and **Sq6** were performed *via* an automated oligonucleotide synthesis on a 394-DNA/RNA synthesizer (*Applied Biosystems*). The UltraMILD procedure (GlenResearch) was applied, using the corresponding phosphoramidites, solid-supports and reagents (Cap A). Coupling time of the squaraine building block was increased to 3 minutes. Cleavage from the solid support and removal of the protecting groups was carried out by a treatment with 50 mM potassium carbonate solution in methanol at room temperature for 4 hours. The oligomers were purified by RP-HPLC: using a C4 polymer column, 250 mm × 4.6 mm, Supelco; Mobile phase: Solvent A = triethylammonium acetate (TEAA) buffer (0.1 M, pH 7.0), solvent B = MeCN, gradient of 40% to 100% solvent B within 15 min; flow rate of 0.5 mL/min; Temp. of the column oven set to 40 °C.

Table S2. ID, sequence, chemical formula, calculated and found mass (from the MS analysis) of **Sq1**, **Sq3**, and **Sq6**. The obtained amount of oligonucleotides, after the purification are given on the right.

ID:	Sequence:	Chemical formula:	calc. mass:	found mass:	Yield [nmol]:
Sq1	3`-GAAGGCACTC-Sq	$C_{135}H_{169}N_{43}O_{62}P_{10}$	3693.8770	3693.8836	160
Sq3	3`-GAAGGCACTC-$(Sq)_3$	$C_{211}H_{263}N_{47}O_{74}P_{12}$	5010.5113	5010.5172	113
Sq6	3`-GAAGGCACTC-$(Sq)_6$	$C_{325}H_{404}N_{53}O_{92}P_{15}$	6985.4629	6985.4792	116

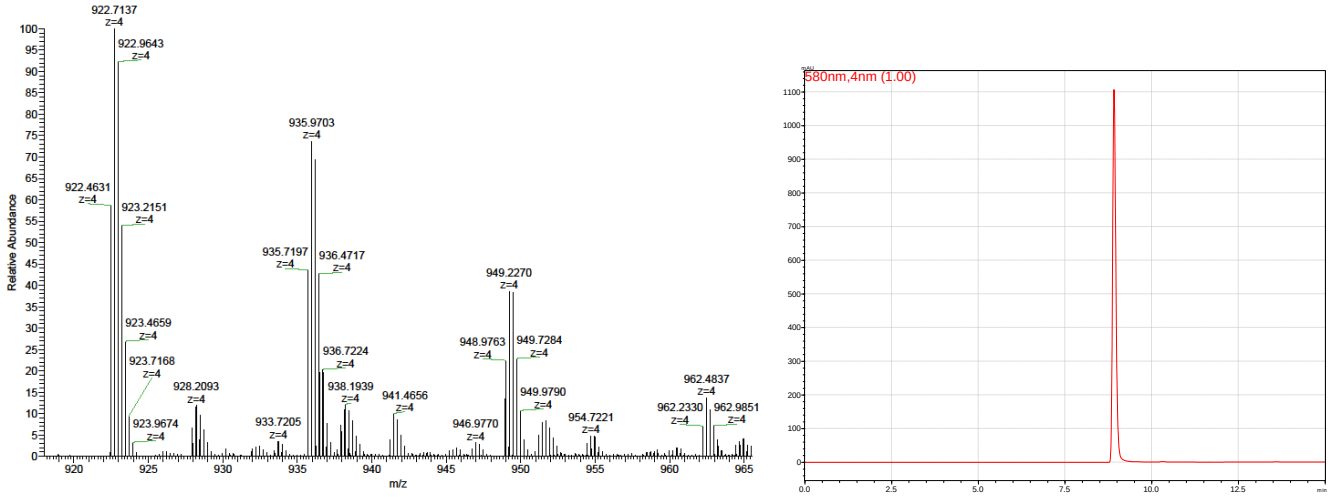


Figure S1. Left: ESI-MS spectrum of oligomer **Sq1** (negative mode, MeOH). Right: HPLC analysis of oligomer **Sq1**.

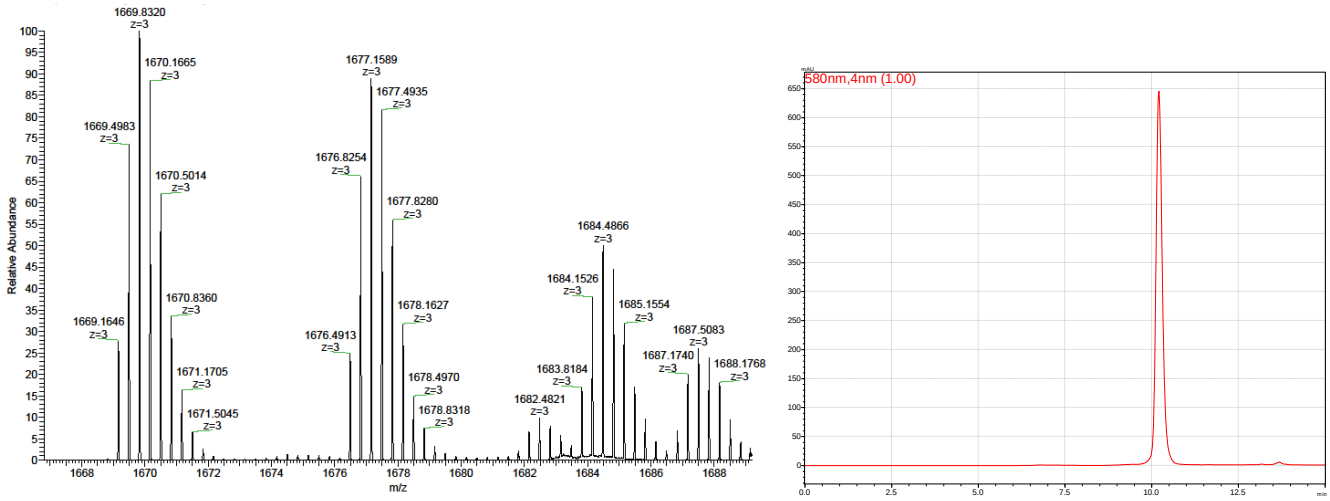


Figure S2. Left: ESI-MS spectrum of oligomer **Sq3** (negative mode, MeOH). Right: HPLC analysis of oligomer **Sq3**.

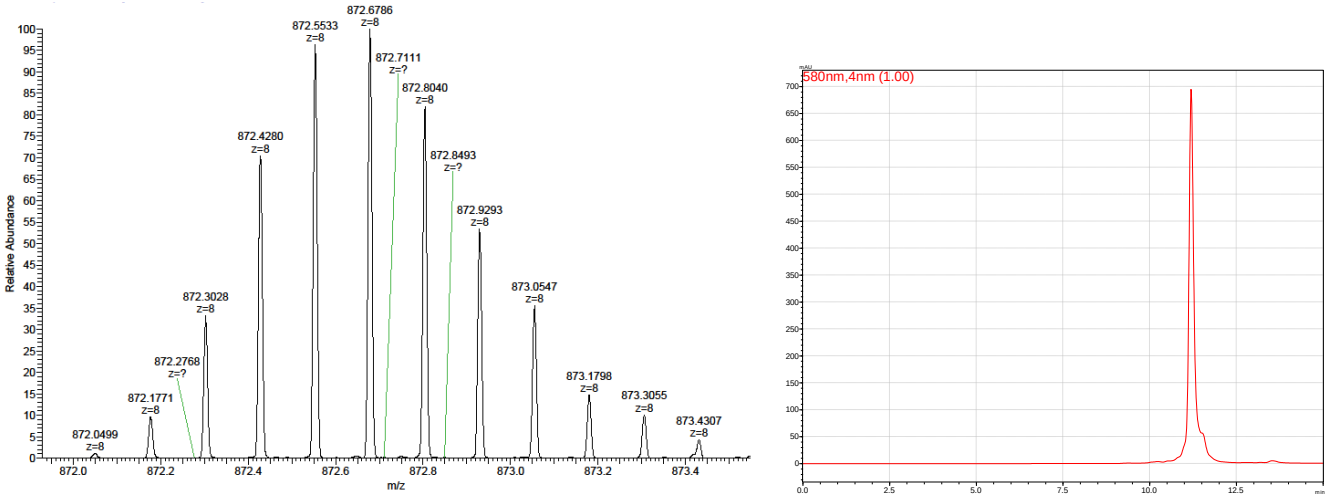


Figure S3. Left: ESI-MS spectrum of oligomer **Sq6** (negative mode, MeOH). Right: HPLC analysis of oligomer **Sq6**.

SPECTRO- AND MICROSCOPIC PROPERTIES OF OLIGOMERS

Oligomer Sq1 (Figure S4)

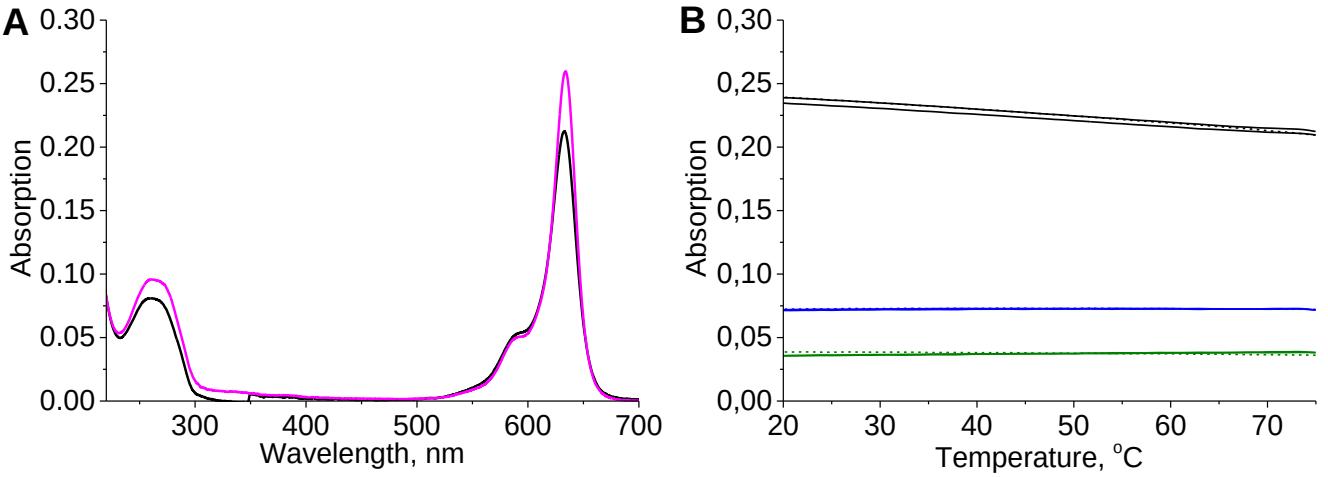


Figure S4. A: Absorption spectra of **Sq1** in aqueous ethanol (black) and ethanol (magenta). B: Temperature-dependent UV-Vis absorption spectrum of **Sq1** monitored at 560 nm (olive), 587 nm (blue), and 633 nm (black). The solid lines represent the cooling cycles, whereas the dashed lines correspond to the heating cycle. Conditions: 1 µM **Sq1**; 15% EtOH; 100 mM NaCl; 10 mM PB, pH=7.

Oligomer Sq3 (Figure S5)

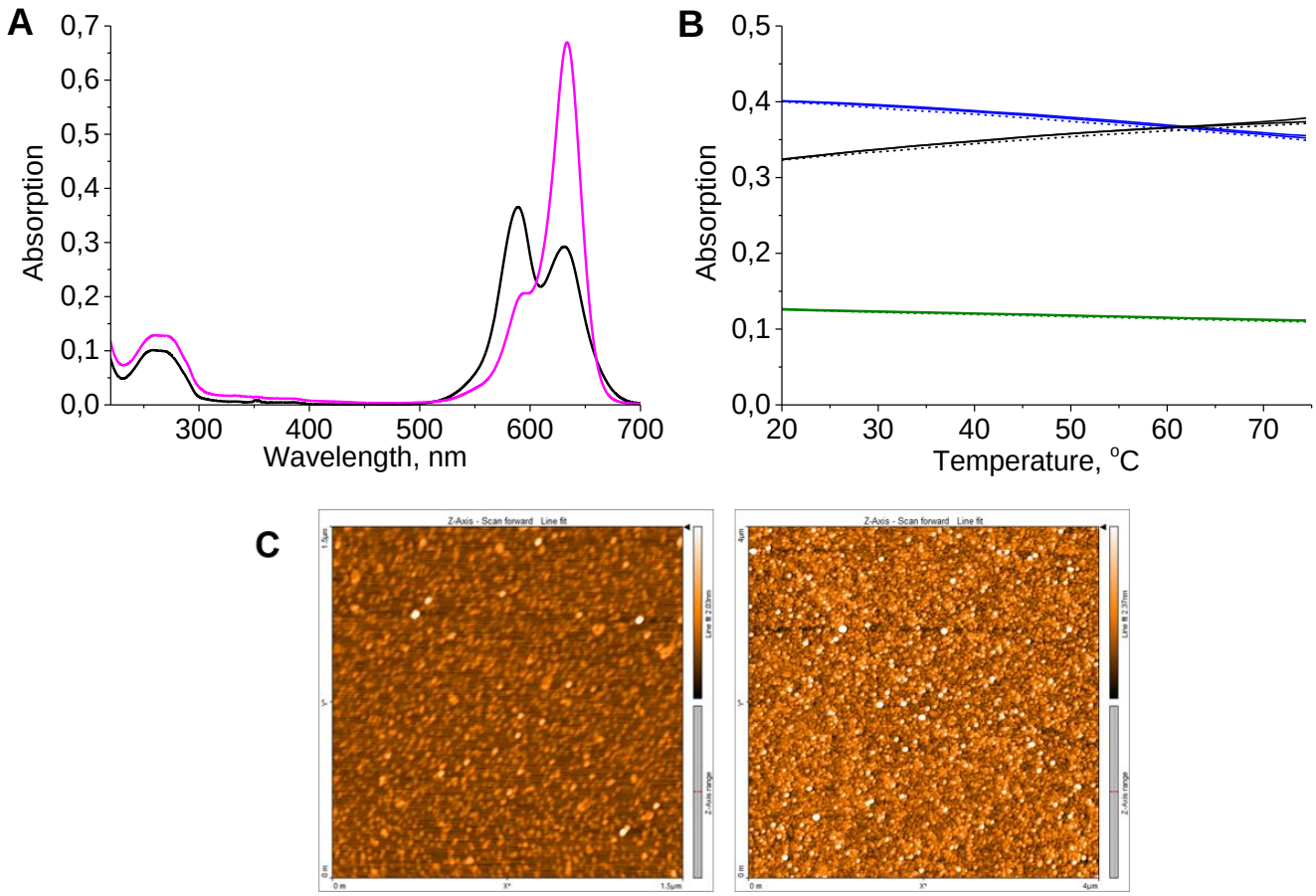


Figure S5. A: Absorption spectra of **Sq3** in aqueous ethanol (black) and ethanol (magenta). B: Temperature-dependent UV-Vis absorption spectra of **Sq3** monitored at 560 nm (olive), 587 nm (blue) and 633 nm (black). Solid lines: cooling cycles; dashed lines: heating cycle. C: AFM images of **Sq3** aggregates. Conditions: 1 µM **Sq3**; 15% EtOH; 100 mM NaCl; 10 mM PB, pH=7.

Oligomer Sq6 (Figure S6)

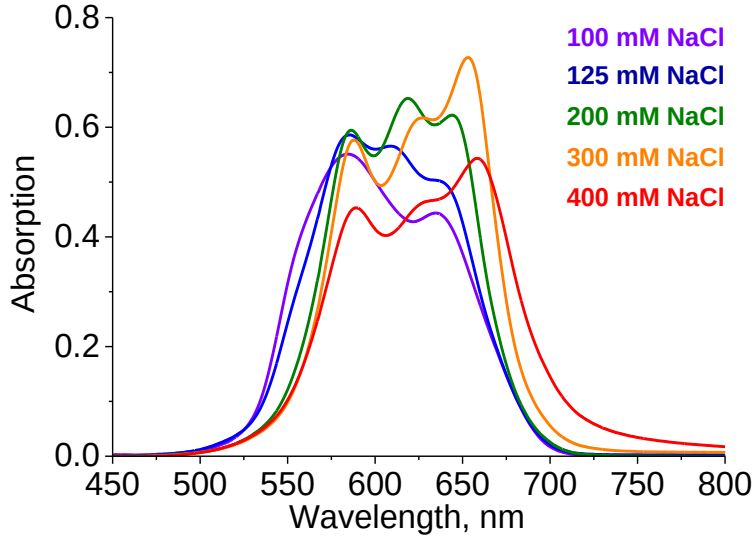


Figure S6. The absorption spectra of oligomer **Sq6** recorded for different NaCl concentrations; conditions: 1 µM **Sq6**; 15% EtOH; 10 mM PB (pH=7); for NaCl see legend on the right.

CHARACTERIZATION OF SQ6-SPS

Reversibility of Sq6-SPs formation (Figure S7)

The sample (containing 1 µM of **Sq6**; 15% EtOH; 300 mM NaCl and 10 mM of PB, pH = 7) was cooled and heated (temperature range of 20 °C – 75 °C) with a rate of 0.5 °C/min. Cooling and heating cycles were repeated 10 times for the same sample:

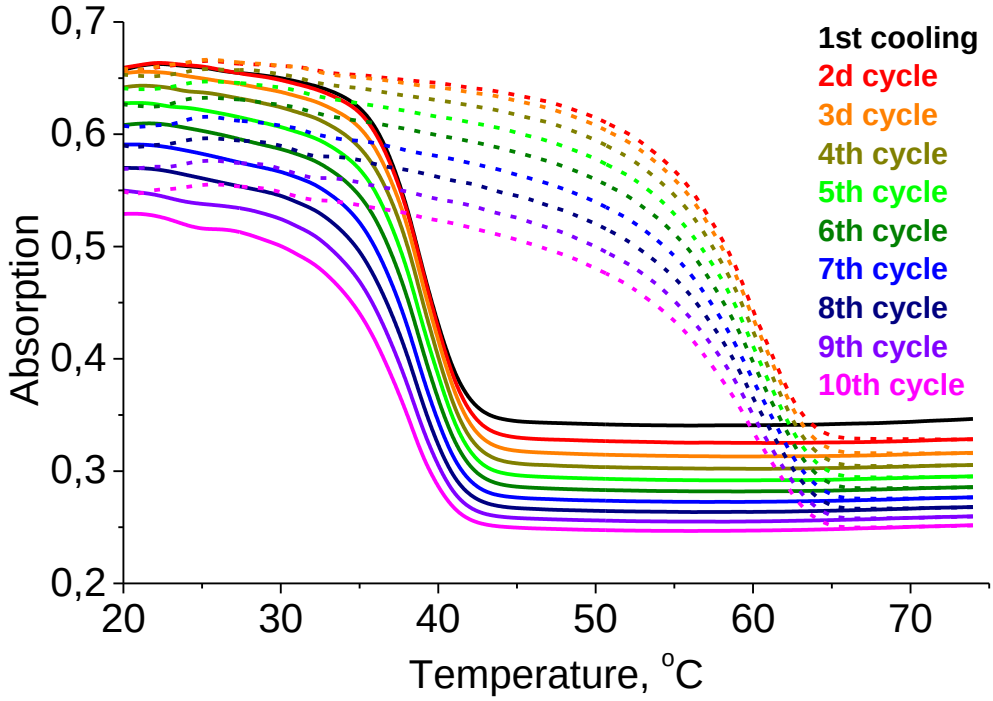


Figure S7. Temperature-dependent UV-Vis absorption spectra of **Sq6** monitored at 660 nm. Solid lines: cooling cycles; dashed lines: heating cycles. Conditions: see above.

SEM visualization (Figure S8 and S9)

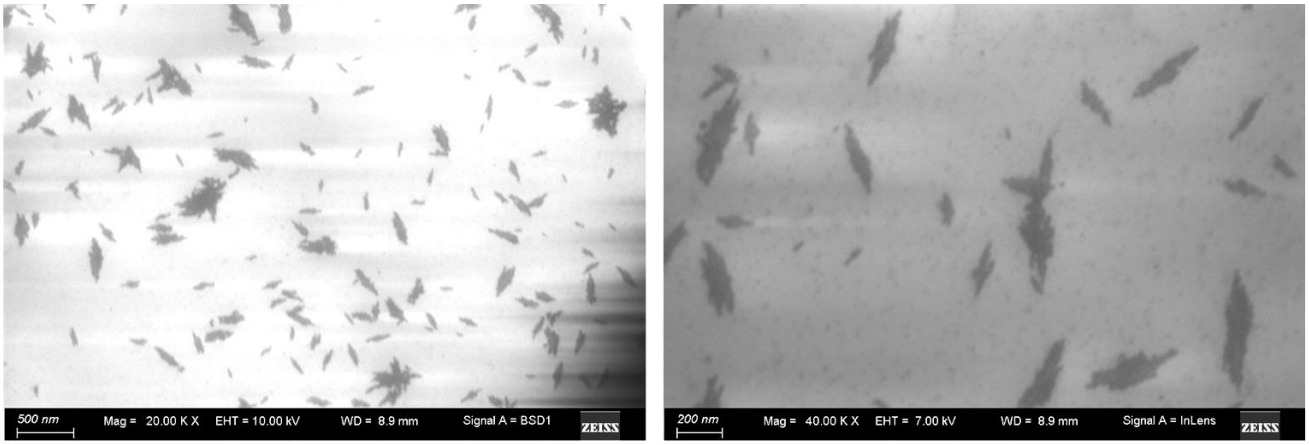


Figure S8. SEM images of **Sq6**-SPs deposited on APTES-modified mica. For sample preparation, see "*General Information*".

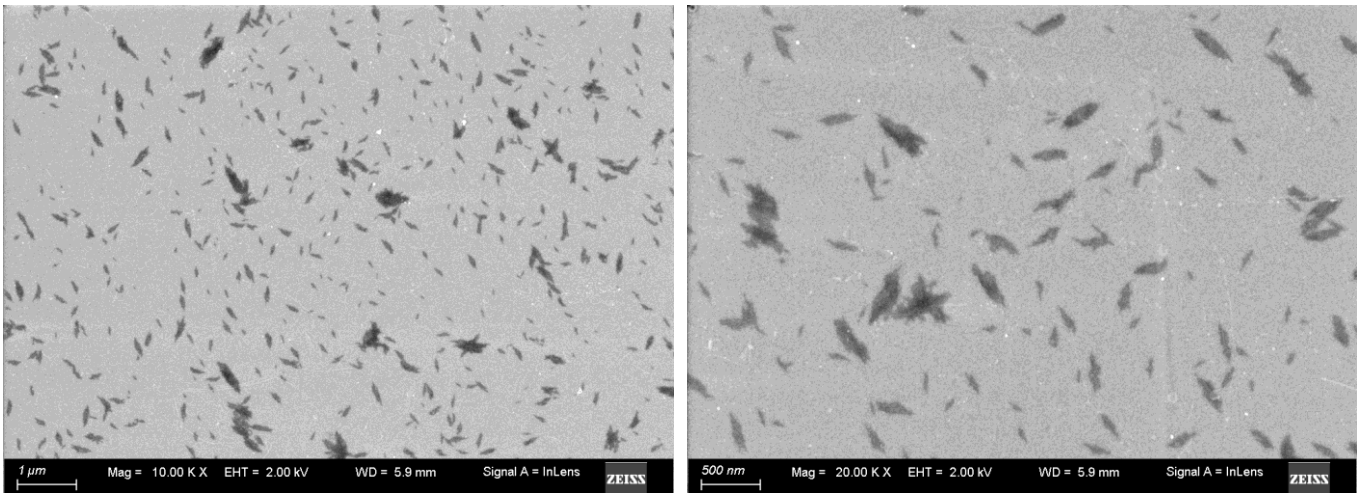


Figure S9. SEM images of **Sq6**-SPs deposited on silicon nitride discs with hydrophilic coating. For sample preparation, see "*General Information*".

Statistical analysis of the dimensions of the SPs

The statistical analysis was performed based on AFM images.

The SPs were prepared from oligomer **Sq6** (1 µM **Sq6**; 15% EtOH; 300 mM NaCl; 10 mM PB), the sample was cooled from 75 °C to 20 °C with a rate of 0.5 °C/min prior to the measurement.

For the sample deposition, see "*General Information*".

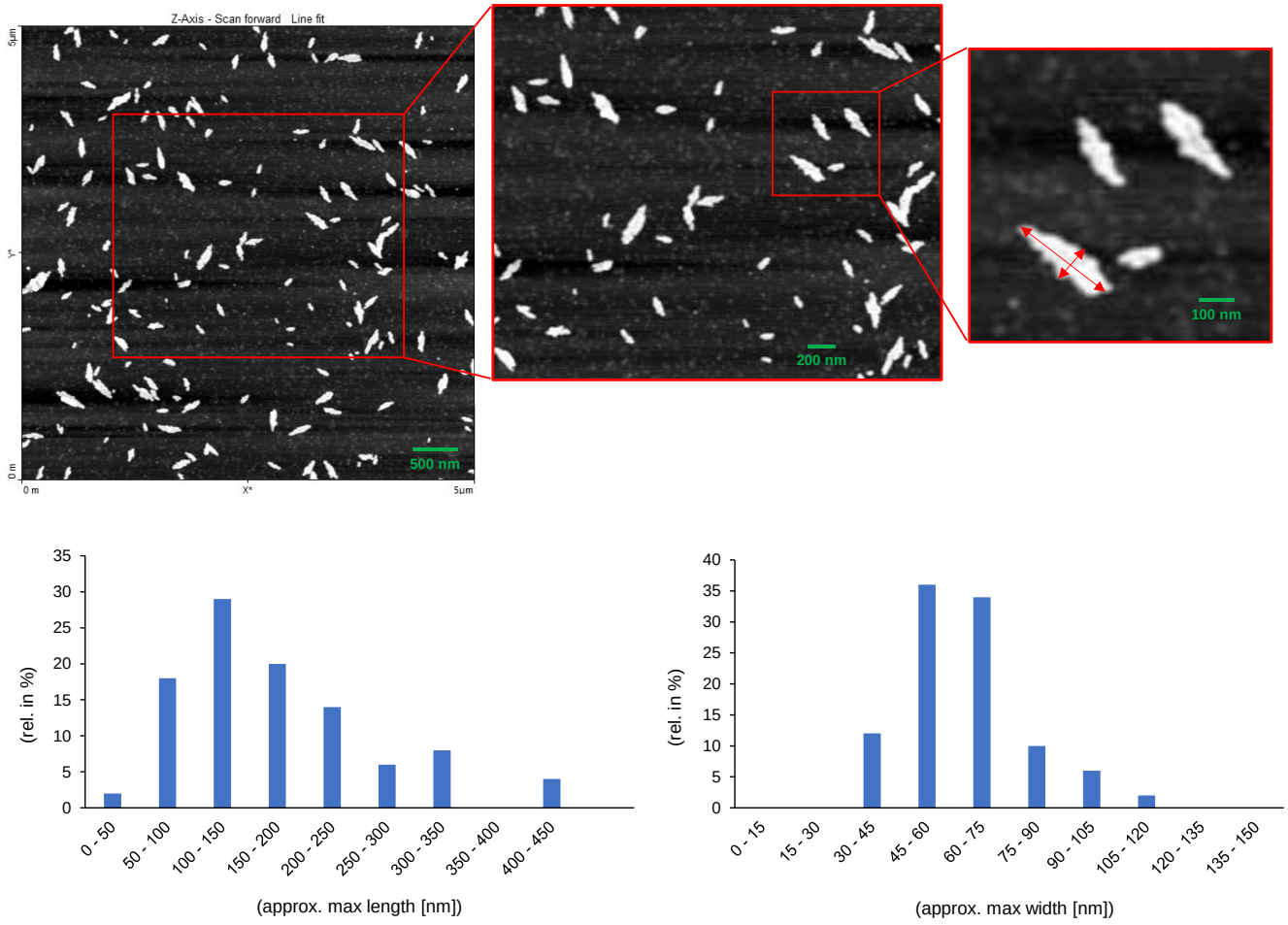


Figure S10. Determination of the length and width of **Sq6**-SPs based on AFM images. The pictures were analyzed using Fiji.[5]

Preliminary model of Sq6 and ssDNA availability on the surface of the SP

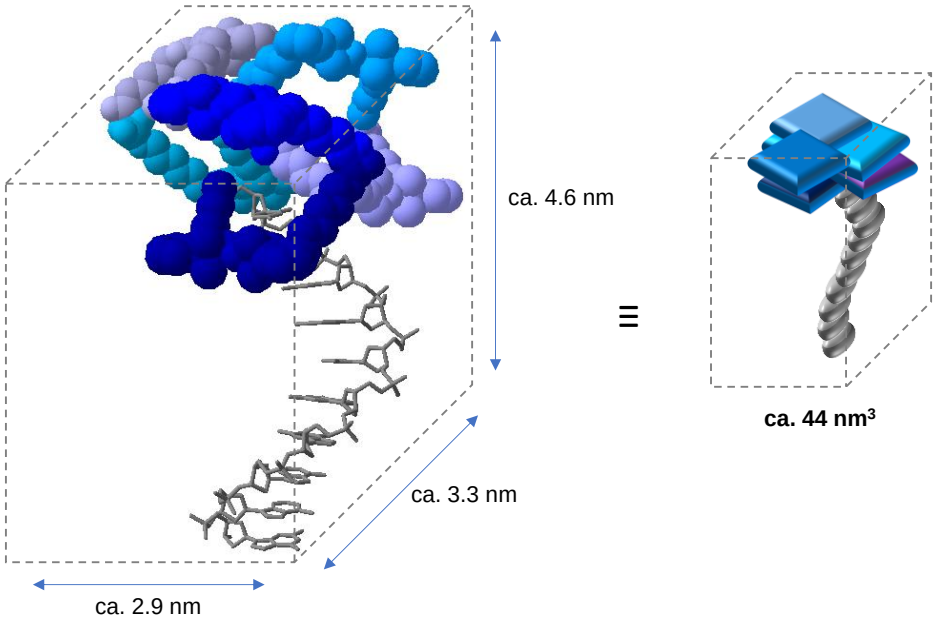
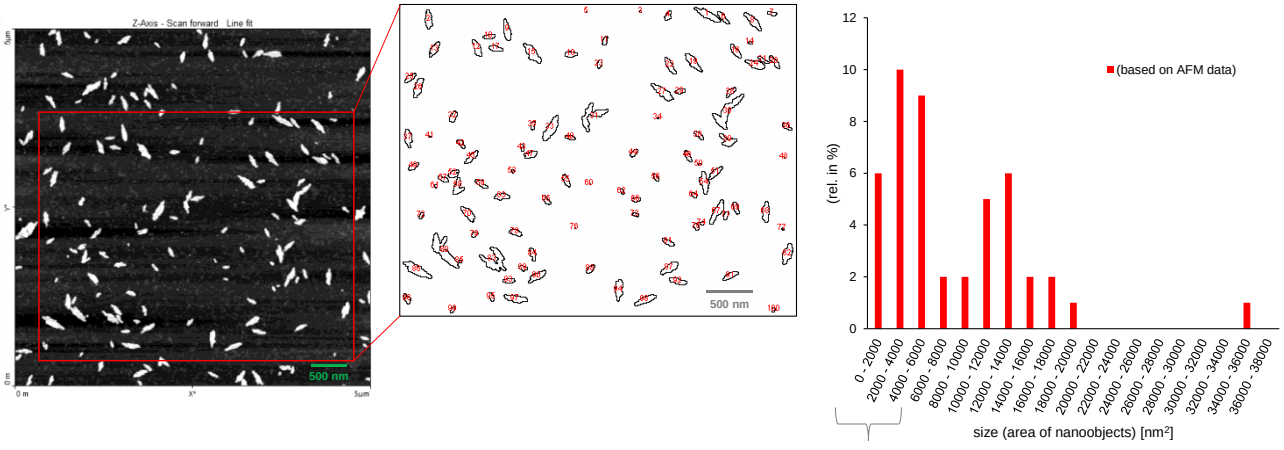


Figure S11. A hypothetical model of the **Sq6** oligomer. Based on UV/Vis measurements, we assume an "oblique" but regular arrangement of the chromophores (squaraines, colored). Since the interactions of the oligomers in the supramolecular polymers is presumably dominated by intermolecular squaraine-squaraine interactions, the DNA strand (gray) is predicted to be (at least to a certain extent) accessible for complementary strand hybridization. The structures were created using *ChemOffice* and *DeepView/Swiss-PdbViewer*.

The data the model provides were then taken into account to give a raw estimation on the number of oligomers present in 1 SP (see below).



avg. size of about 4000 nm²

(avg. size) x (avg. height = 4 nm) = 16000 nm³ → avg. volume of SP

→ (avg. volume of SP) / (avg. volume per oligomer*) = ca. **363 oligomers**

*(avg. volume per oligomer = 44 nm³)

Figure S12. Based on the approx. volume of 1 oligomer, an average SP consists of about 360 **Sq6** oligomers. Picture analysis was carried out using Fiji.[5]

FUNCTIONALIZATION OF THE SUPRAMOLECULAR POLYMERS WITH GOLD NANOPARTICLES

Preparation of the gold nanoparticles (AuNPs)

The gold nanoparticles, with an average diameter of 5 nm, were prepared according to literature.[6,7] Since $HAuCl_4$ is corrosive, a glass spatula was used to avoid any contact with metal. All glassware and magnetic stir bar used in the preparation were thoroughly cleaned with aqua regia ($HCl:HNO_3$, 3:1, v:v), rinsed with distilled water and dried in the oven prior to the use.[8] The solutions of 4% $HAuCl_4$, 0.2 M K_2CO_3 and $NaBH_4$ were filtered through 0.2 µm syringe filters prior to the application.

In short: A 4% aqueous solution of $HAuCl_4$ (375 µL) was combined with a 0.2 M K_2CO_3 solution (0.5 mL) and added to H_20 (100 mL) at 4 °C. Under vigorous stirring, 5 x 1 mL of a freshly prepared $NaBH_4$ solution (0.5 mg/mL; in water) were added to the $HAuCl_4/K_2CO_3$ solution. The mixture was stirred for 5 min at 4 °C. The solution was centrifuged at 12'000 rpm for 10 min and the supernatant was collected. Bis(*p*-sulfonatophenyl)phenylphosphine (BSPP) (20 mg) was added to the solution and the mixture was shaken overnight at room temperature. Under stirring, NaCl (solid) was added till the color of the solution changed from deep burgundy to light purple. The mixture was centrifuged at 3'000 rpm for 30 min and the supernatant was discarded. The AuNPs were resuspended in a solution of BSPP (1 mL; conc.: 250 mg/L) and MeOH (1 mL) was added. The mixture was again centrifuged, the supernatant was discarded and the AuNPs were finally resuspended in a solution of BSPP (1 mL; conc.: 250 mg/L).

The concentration of the AuNPs was determined as follows:

The avg. diameter of the AuNPs was obtained from transmission electron microscopy image via automatically generated image analysis by the Fiji open-source platform.[5]

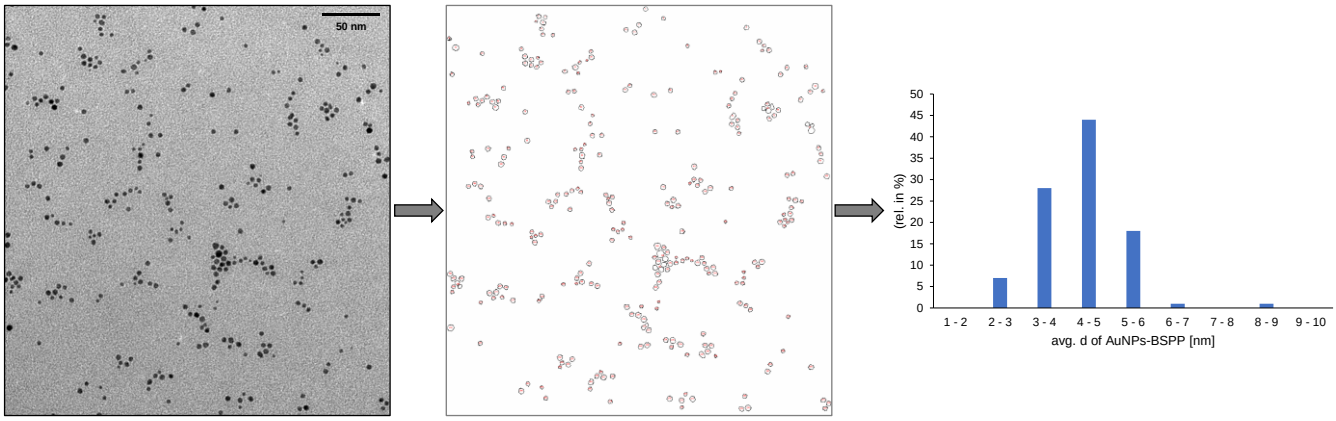


Figure S13. TEM image of BSPP stabilized AuNPs (left), the Fiji particle analysis output (center) and the histogram (right) showing a maximum number of particles centered at around a diameter of 5 nm.

An average diameter of 5 nm, based on published data,[8] is associated with a molar absorptivity of approx. $1x10^7$ $M^{-1}cm^{-1}$.

Next, the absorbance at 520 nm was measured with UV/Vis absorption spectroscopy (the sample was measured in a cuvette with an optical path length of 0.1 cm).

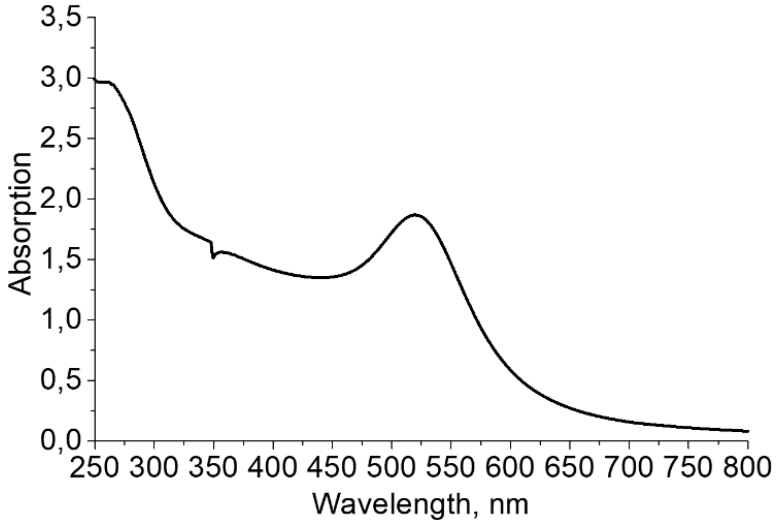
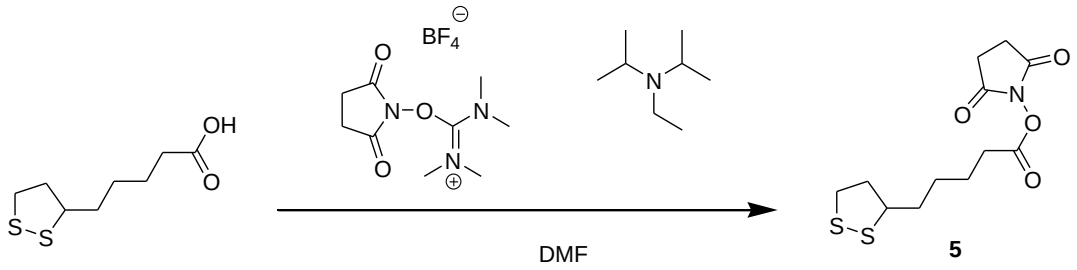


Figure S14. UV/Vis spectrum of BSPP stabilized AuNPs.

The concentration was then calculated according to *Beer–Lambert*: c = (1.84) / (1x10^{7} M^{-1}cm^{-1} x 0.1 cm) = 1.8x10^{-6} M

Synthesis of the oligonucleotides linked to thioctic acid.

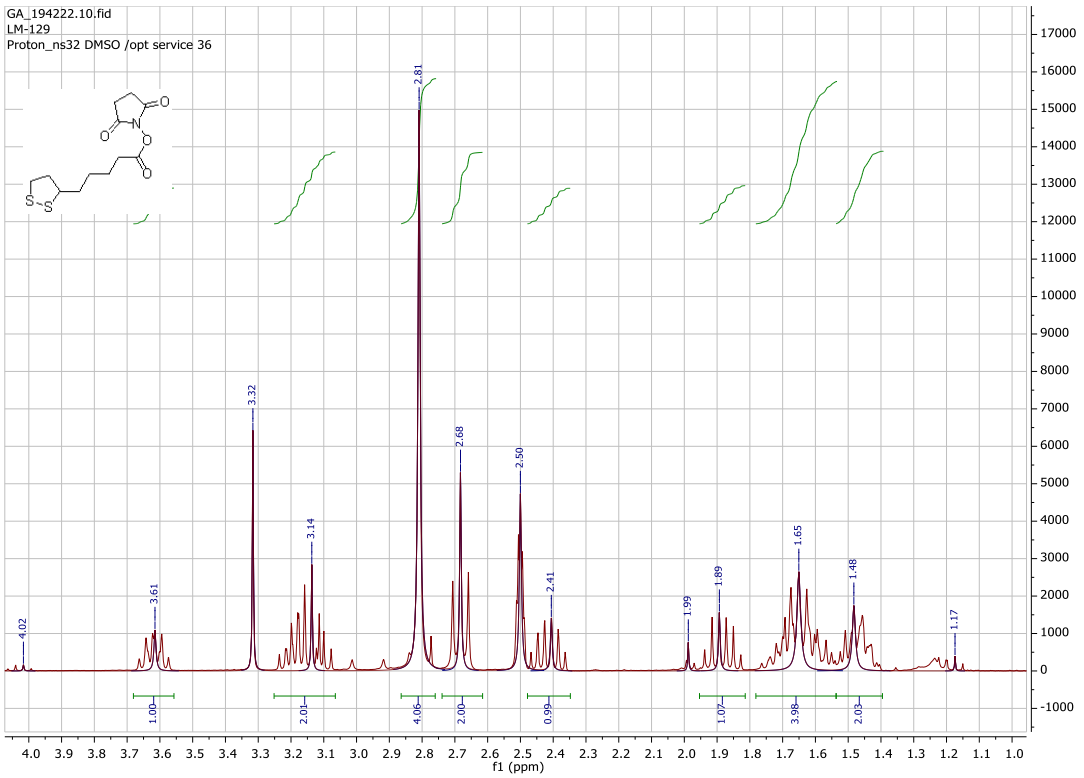
Synthesis of the N-hydroxysuccinimide ester (NHS-ester) of thioctic acid **5**, according to reported procedures.[6,9]



DIPEA (0.42 mL, 2.42 mmol) and *N*,*N*,*N*',*N*'-tetramethyl-O-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU, 290 mg, 0.969 mmol) were added to a solution of thioctic acid [(±)-α-lipoic acid; 100 mg, 0.485 mmol] in dry DMF (1.5 mL). The mixture was stirred under argon at room temperature for 1 h. Diethyl ether (40 mL) was added and the mixture was kept at -30 °C for 16 hours. The liquid containing the crude product was decanted and the solvents were evaporated under reduced pressure. The material was purified by column chromatography (on SiO_2, using EtOAc) to afford the product (100 mg, 68%).

^{1}H-NMR (300 MHz, DMSO, ppm): δ 3.68-3.56 (1H, m, CH_2), 3.25-3.06 (2H, m, CH, CH_2), 2.81 (4H, s, NHS), 2.68 (2H, t, J = 7.16 Hz, C$\underline{H_2}$COONHS), 2.48-2.35 (1H, m, CH_2), 1.96-1.79 (1H, m, CH_2), 1.79-1.53 (4H, m, CH_2), 1.54-1.38 (2H, m, CH_2).

ESI-MS (pos. mode, sample in MeCN): calc. mass for $C_{12}H_{17}NO_4S_2$ = 303.0599; mass found = 303.0596.



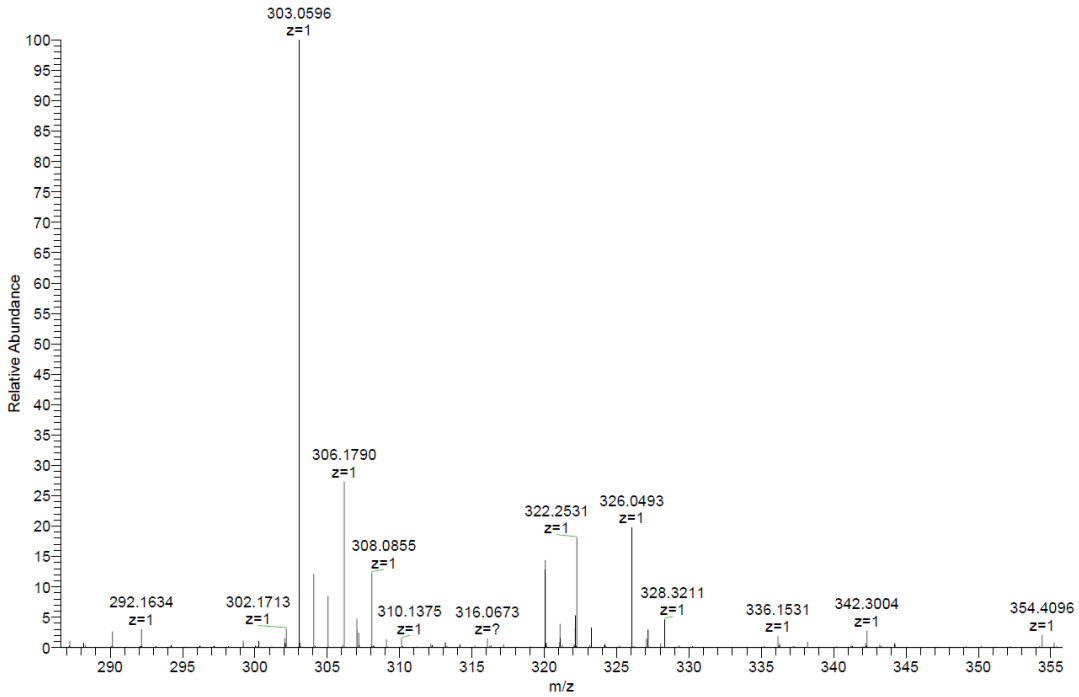
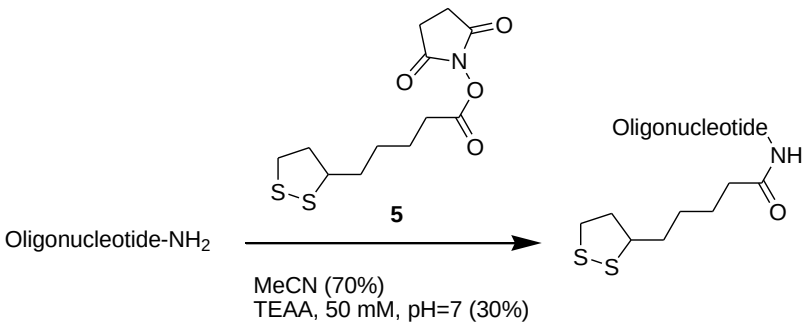


Figure S15. Top: ^{1}H-NMR spectrum. Bottom: ESI-MS spectrum of the NHS-ester of thioctic acid **5**.

Synthesis of the thioctic acid-modified oligonucleotides:[6,9]



The amino-modified oligonucleotides **T6**, **ON1**, and **ON2** were dissolved in 500 µL of a mixture of MeCN (70%) and TEAA (50 mM, pH = 7) (30%). The NHS-ester of thioctic acid **5** (see above) was added in a 200 equimolar excess.[6] The reaction was carried out in a lab-shaker at 25 °C overnight. To the reaction mixture TEAA (2 mL, 0.1 M, pH = 7) was added. The solution was filtered *via* a 0.45 µm disposable syringe filter and the crude product was purified by RP-HPLC: Clarity® 5 µm Oligo-RP column, 250 mm × 4.6 mm, Phenomenex®; gradient of 0% to 100% solvent B within 20 min; Temp. of column oven at 40 °C; flow rate = 1 mL/min; solvents, see chapter "SOLID PHASE SYNTHESIS OF THE SQUARAINE OLIGOMERS". Overall yields of 41-66% were achieved.

Table S3. ID, sequence, chemical formula, calculated and found mass (MS analysis) of the thioctic acid-modified oligonucleotides.

ID:	Sequence:	Chemical formula:	mass calc.:	mass found:
T6-SS	Thioctic acid-NH-TTTTTT-5`	$C_{75}H_{107}N_{13}O_{45}P_6S_2$	2159.4351	2159.4396
ON1-SS	3`-GAGTGCCTTCTTTTTTTTTT-NH-Thioctic acid	$C_{211}H_{280}N_{56}O_{134}P_{20}S_2$	6425.1012	6425.1040
ON2-SS	3'-CCTGAAGTTATTTTTTTTTT-NH-Thioctic acid	$C_{212}H_{280}N_{58}O_{132}P_{20}S_2$	6433.1175	6433.1200

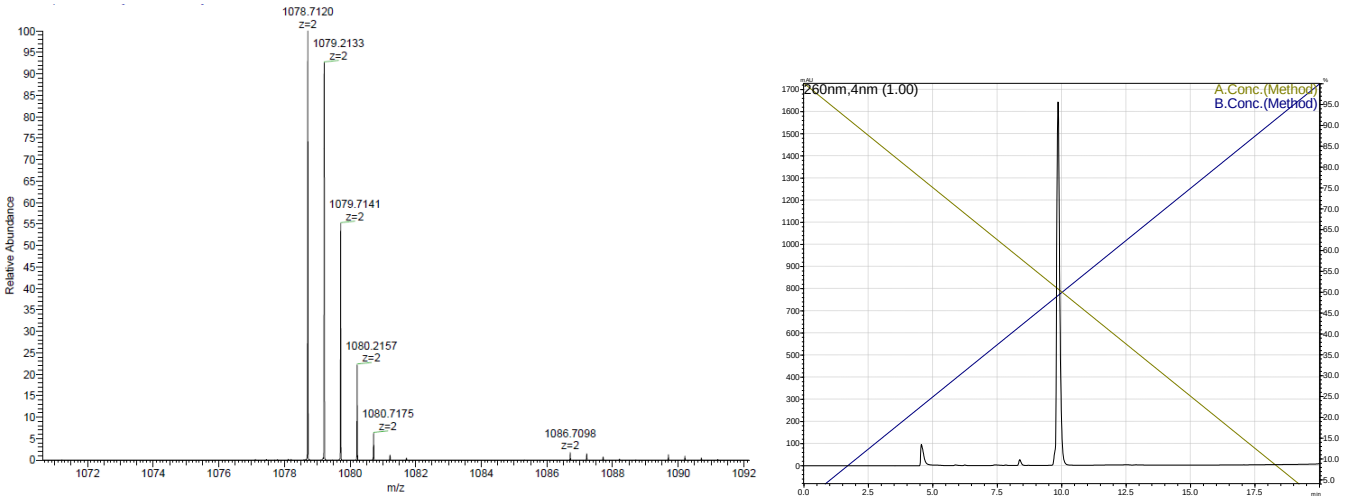


Figure S16. Left: ESI-MS analysis (negative ion mode, sample in MeOH). Right: HPLC analysis of **T6-SS**.

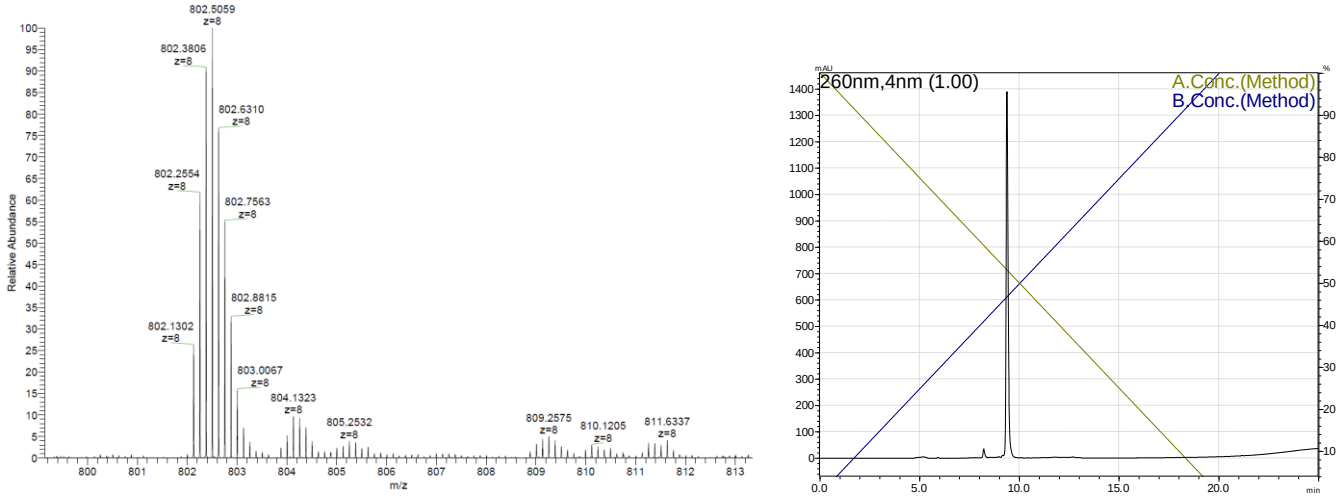


Figure S17. Left: ESI-MS analysis (negative ion mode, sample in MeOH). Right: HPLC analysis of **ON1-SS**.

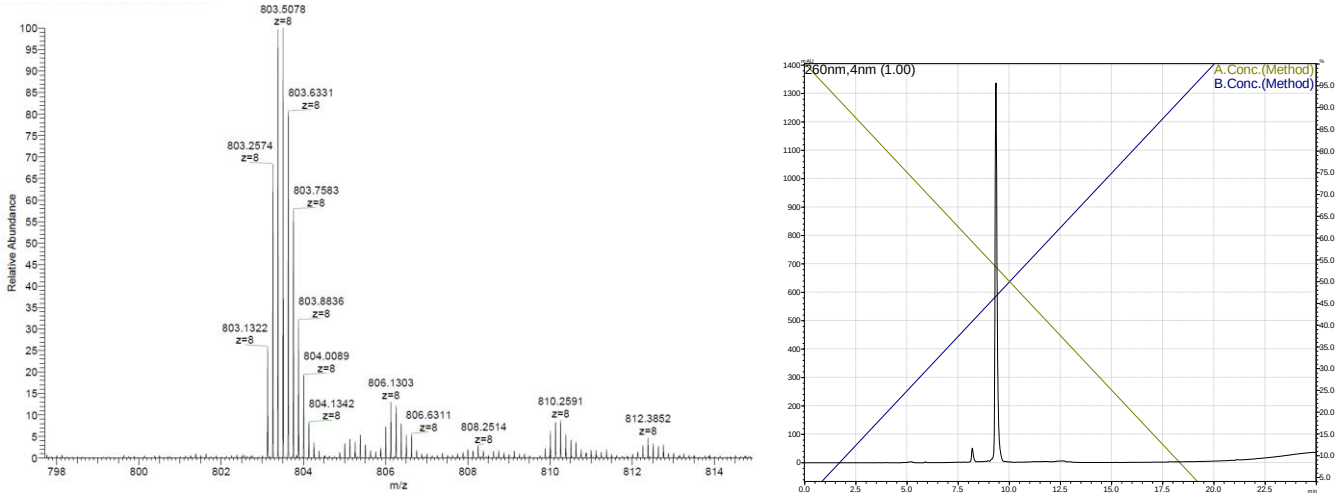


Figure S18. Left: ESI-MS analysis (negative ion mode, sample in MeOH). Right: HPLC analysis of **ON2-SS**.

Functionalization of the SPs with AuNPs

The general procedure to prepare the AuNP-modified oligonucleotides was as follows:[6,7] AuNPs (0.3 nmol) and the oligonucleotide (containing thioctic acid) (0.6 nmol) were incubated overnight at room temperature in 0.5 × TBE buffer containing 50 mM NaCl. Afterwards, oligo **T6-SS** (30 nmol) was added and the mixture was incubated for an additional 12 h. Then, the sample was centrifuged (12'000 rpm, 35 min), the supernatant was removed and the remaining precipitate was diluted with Milli-Q water (this process was repeated twice).

Next, the SPs were prepared from oligonucleotide **Sq6** (1 µM **Sq6;** 15% EtOH; 300 mM NaCl; 10 mM PB), the sample was heated to 75 °C and then cooled to 20 °C (with a rate of 0.5 °C/min).

Finally, the **Sq6**-SPs (1 eq.) and the AuNP-modified samples (0.03 eq.) were combined and kept in the lab-shaker (700 rpm) for 16 hours at room temperature.

TEM images of ON1-AuNP

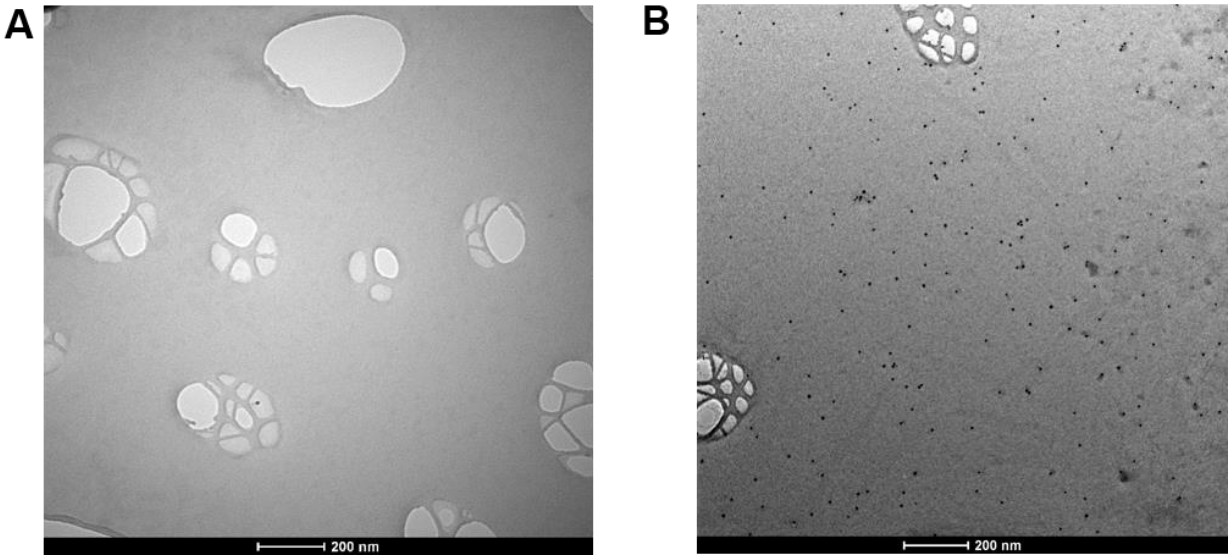
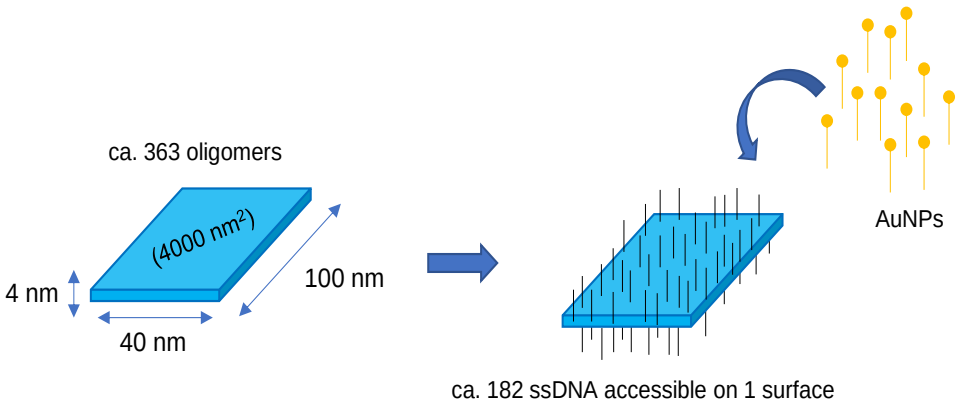


Figure S19. TEM images obtained after deposition of **ON1-AuNPs** suspended in water (A) and water containing 15% EtOH; 300 mM NaCl; 10 mM PB, pH=7 (B). For the handling and deposition procedure of the samples, see *General information*, *TEM*.

Calculation of surface coverage of the SPs with AuNPs

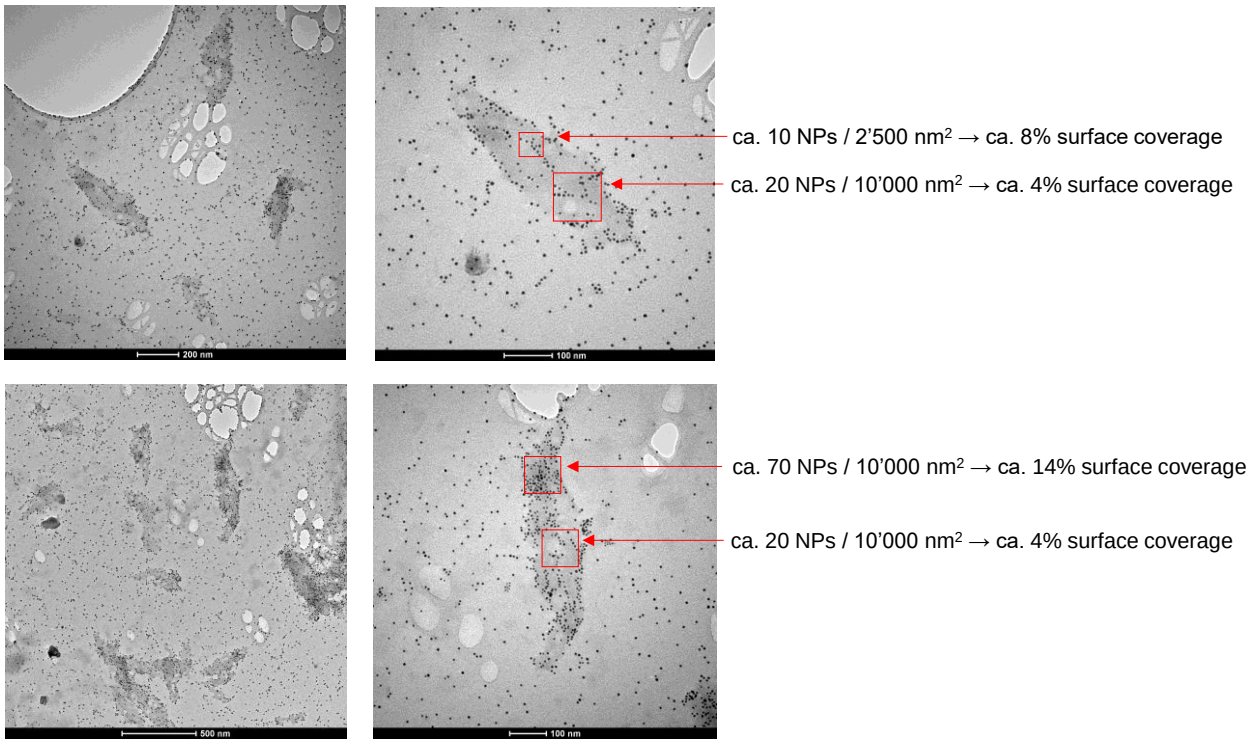
With the average number of oligomers present in 1 SP (about 360 oligomers, calculations see above), it is also possible to calculate the max. (possible) density of ssDNA (the DNA nucleotides of **Sq6**) on a SP:



→ max. *theoretical* surface coverage with AuNPs: 182 NPs per 4000 nm^2

→ max. surface coverage *measured*, based on TEM images: 70 NPs per 10000 nm^2 → **15%**

Figure S20. Assuming there is an equal distribution of the ssDNAs on the surface(s) and the DNA part is always completely accessible (which is most certainly not the case, due to structural irregularities and errors in creating the model), the theoretical surface coverage with AuNPs carrying complementary strands can be calculated. The actual value that was measured with TEM is also given (TEM images see below).



r_{NP} = 2.5 nm

$A_{NP} = \pi \times r_{NP}^2$ = 19.6 nm²

Figure S21. Approximation of the surface coverage based on TEM images. Sample: oligomers **ON1-AuNP*Sq6** (preparation and conditions, see above); r_{NP}: average nanoparticle radius; A_{NP}: projected area of nanoparticles.

REFERENCES

1 Y. L. Lyubchenko and L. S. Shlyakhtenko, *Methods*, 2009, **47**, 206–213.

2 L. I. Markova, V. L. Malinovskii, L. D. Patsenker and R. Häner, *Org. Biomol. Chem.*, 2012, **10**, 8944–8947.

3 M. J. Cavaluzzi and P. N. Borer, *Nucleic Acids Res.*, 2004, **32**, e13–e13.

4 R. M. C. Dawson, D. C. Elliott, W. H. Elliott and K. M. Jones, *Data for Biochemical Research*, Oxford Science Publications, OUP, Oxford, Third Edition., 1986.

5 J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat. Methods*, 2012, **9**, 676–682.

6 T. Zhang, P. Chen, Y. Sun, Y. Xing, Y. Yang, Y. Dong, L. Xu, Z. Yang and D. Liu, *ChemComm*, 2011, **47**, 5774–5776.

7 C. J. Loweth, W. B. Caldwell, X. Peng, A. P. Alivisatos and P. G. Schultz, *Angew. Chem. Int. Ed.*, 1999, **38**, 1808–1812.

8 W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal Chem.*, 2007, **79**, 4215–4221.

9 J. Sharma, R. Chhabra, C. S. Andersen, K. v. Gothelf, H. Yan and Y. Liu, *J. Am. Chem. Soc.*, 2008, **130**, 7820–7821.