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SUPPLEMENTARY INFORMATION

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Routing of individual polymers in designed patterns

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Materials and Methods

Reagents and equipment

All chemicals were purchased from Sigma-Aldrich and used without further purification unless otherwise stated. HPLC grade solvents were used and anhydrous solvents were purchased in Sure/Seal bottles with inert atmospheres or dried using an MBRAUN MB SPS-800 Solvent Purification System. Reactions were carried out under argon atmosphere unless otherwise stated (argon gas was dried over phosphorus pentoxide). Organic reactions were monitored by thin-layer chromatography (TLC) whenever possible and flash chromatography was carried out on Silica gel 60 (230 -400 mesh). NMR spectra were recorded on a Varian Gemini System connected to a 400 MHz Oxford magnet or on a Bruker Avance III HD system connected to a 400 MHz Bruker Ascend magnet. Chemical shifts are reported in ppm and corrected according to the solvent residual peak[31]. Mass spectra of small molecules were obtained on a Bruker Maxis Impact (ESI-TOF) using a Dionex Ultimate 3000 RS (HPLC) as interface. Melting points were obtained on a BÜCHI Melting Point B-540 system. Water used for DNA experiments was purified on a Milli-Q Biocel system by Millipore. DNA oligonucleotides used as staple strands in DNA origami structures were desalted by the manufacturer. DNA origami scaffold (M13mp18) was purchased from New England BioLabs. DNA oligonucleotides were purchased from Sigma-Aldrich or synthesized in house on a BioAutomation MerMade-12 automated oligonucleotide synthesizer. Preloaded standard 1000Å CPG columns were purchased from BioAutomation in the US and used for the synthesis of unmodified DNA oligos. Standard chemicals used in the automated DNA synthesis and preloaded 3000Å CPG were purchased from Link Technologies Ltd in Scotland: Deblock mix (3% trichloroacetic acid in dichloromethane), ETT activator (0.25 M 5-ethylthio-1H-tetrazole in anhydrous acetonitrile), cap mix A (tetrahydrofuran/lutidine/acetic anhydride), cap mix B (10% methylimidazole in THF), oxidizer (0.02 M Iodine in tetrahydrofuran/pyridine/water). Acetonitrile was purchased as DNA synthesis grade from Sigma Aldrich and stored over molecular sieves purchased from BioAutomation in the US. Dichloromethane was purchased as HPLC grade and

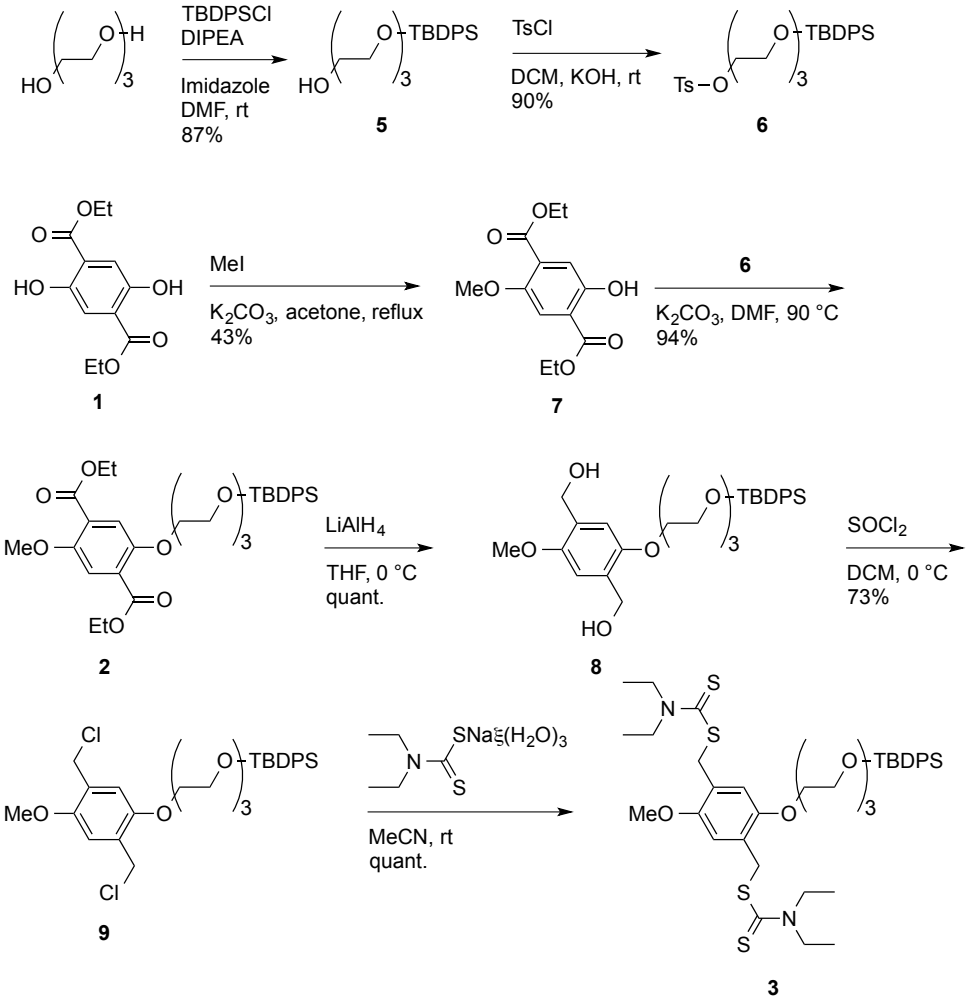
stored over molecular sieves purchased from VWR. Synthesized oligonucleotides were cleaved from the solid-support either using 33% ammonium hydroxide solution or AMA (ammonia hydroxide 30-33% and aq. methylamine 40% v/v 1:1). Oligonucleotides were HPLC purified on an Agilent 1100 or 1200 Series HPLC systems equipped with a Phenomenex Clarity 3u Oligo-RP, 50 mm x 4.6 mm column running a gradient of MeCN in TEAA (100 mM, pH 7) buffer. All DNA concentrations were determined using a Nanodrop ND -1000 spectrophotometer. Mass spectra of oligonucleotides were either obtained on a Bruker Daltonics Autoflex Speed MALDI –TOF MS spectrometer using AnchorChip target plates, or on a Shimadzu LCMS-2020EV connected to a Shimadzu Prominence RP-UPLC system equipped with a Phenomenex Gemini 3u C18, 100 mm x 4.6 mm column and running a gradient of MeOH in 1,1,1,3,3,3-hexafluoroisopropanol/triethylamine buffer (HFIP, 100 mM / TEA, 8 mM). The matrix used for MALDI –TOF MS was 90% 3-hydroxypicolinic acid (50 mg/mL) in water/MeCN 1:1 and 10% diammonium citrate (50 mg/mL) in water. Gel permeation chromatography (GPC) was performed on a system comprising an LC-20AD Shimadzu HPLC pump, a Shimadzu RID-10A refractive index detector and a DAWN HELEOS 8 LS detector. For polymers soluble in organic solvents, the system was equipped with an Mz-Gel SDplus Linear column with 5 µm particles length of 300 mm and an internal diameter of 8 mm from MZ-Analysentechnik providing an effective molecular weight range of 1,000-1,000,000. The eluent was DMF with 10 mM LiBr at 30 °C (flow rate: 1 mL min^{-1}). For polymers soluble in water, the system was equipped with a HEMA-Bio Linear column with 10 µm particles, a length of 300 mm and an internal diameter of 8 mm from MZ-Analysentechnik providing an effective molecular weight range of 1,000−1,000,000. The eluent is 0.1 µm filtered PBS buffer (0.01 M phosphate, NaCl 0.138 M, KCl 0.027 M) with 300 ppm sodium azide to inhibit bacterial growth. XPS data acquisition was performed using a Kratos Axis UltraDLD instrument (Kratos Analytical Ltd., Telford, UK) equipped with a monochromated Alkα x-ray source (hv = 1486.6 electron volts, eV) operated at 15 kV and 10 mA (150 W). A hybrid lens mode was employed during analysis with an analysis area of approximately 700 μm × 300 μm. The XPS spectra for all samples were taken

at a photoemission angle of 0°, which has a probe depth of around 10 nm. Survey spectra (Binding Energy (B.E.) of 0 - 1100 eV with pass energy of 160 eV) were used for element identification and quantification. The acquired data were converted to VAMAS format and analysed using CASAXPS software. Absorbance spectra and DNA melting profiles were recorded at a Varian Cary 100 Bio UV-visible spectrophotometer using a Hellma Quartz Suprasil 4 mm x 10 mm, 1400 µL cuvette. For some of the absorption measurements we used a Cary 60 instrument from Agilent Technologies. Fluorescence measurements were recorded at a Horiba JobinYvon FluoroMax-3 instrument using a Hellma Quartz Suprasil 3 mm x 3 mm, 60 µL cuvette. DNA origami structures were annealed on an Eppendorf Mastercycler Personal thermal cycler.

For the 3D PAINT superresolution imaging, the non-modified DNA oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Fluorescently modified DNA oligonucleotides were purchased from Biosynthesis (Lewisville, TX). Streptavidin was purchased from Invitrogen (S-888, Carlsbad, CA). Bovine serum albumin (BSA), and BSA-Biotin was obtained from Sigma Aldrich (A8549, St. Louis, MO). Glass slides and coverslips were purchased from VWR (Radnor, PA). Trolox, PCA (protocatechuic acid), and PCD (protocatechuate 3,4-dioxygenase) were purchased from Sigma Aldrich (238813-1G, 03930590-50MG, P8279-25UN, St. Louis, MO).

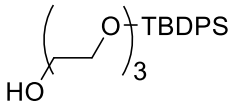
Synthesis of monomer 3 used for polymerization via the dithiocarbamate precursor route

An overview of the synthesis of the monomer shown in Supplementary Fig. 1.



Supplementary Fig. 1. Detailed scheme of the synthesis of the monomer (**3**) used for the formation of poly(APPV-TBDPS) via the dithiocarbamate precursor route. The monomer was synthesized in 7 steps from commercially available triethylene glycol and 2,5-dihydroxy-terephthalic acid diethyl ester.

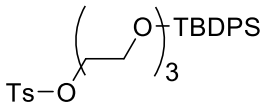
<u>Synthesis of 2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecan-12-ol (**5**)[46-48]</u>



To a flask were added triethylene glycol (24.3 mL, 182 mmol), TBDPSCl (9.46 mL, 36.4 mmol), imidazole (248 mg, 3.64 mmol), anhydrous DMF (50 mL) and anhydrous DIPEA (7.12 mL, 40

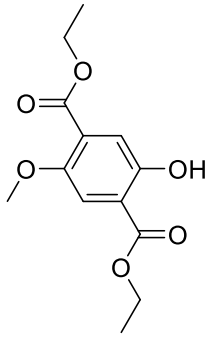
mmol). After stirring at rt for 3 hours, the solution was diluted with EtOAc (200 mL) and washed with H_2O (2 x 80 mL) and sat. aq. NaCl (2 x 80 mL). The organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. Flash chromatography on silica (Et_2O) afforded **(5)** as a clear oil (12.33 g, 87%). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.76 - 7.73 (m, 4H), 7.46 - 7.41 (m, 6H), 3.83 (dd, *J* = 4.6, 5.6 Hz, 2H), 3.64 - 3.60 (m, 8H), 3.54 - 3.49 (m, 3H), 1.05 (s, 9H).^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 135.5, 133.6, 129.7, 127.7, 72.7, 72.2, 70.5, 70.3, 63.5, 61.1, 26.3, 18.9.HRMS (ESI): [M+K]; Calc.: 427.1701, found: 427.1703.

Synthesis of 2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecan-12-yl 4-methylbenzenesulfonate (**6**)



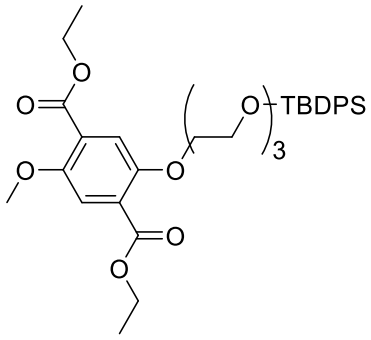
To a flask were added (**5**) (9.10 g, 23.42 mmol), TsCl (4.46 g, 23.42 mmol) and anhydrous DCM (230 mL). To this solution was added finely powdered KOH (10.51 g, 187.35 mmol) in small portions. The solution was stirred at rt for 1 hour before the mixture was diluted with H_2O (100 mL) and the aqueous layer extracted with DCM (3 x 100 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo*. Flash chromatography on silica (pentane/Et_2O 3:1 to 2:3) afforded (**6**)as a clear oil (11.32 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.70 (dd, *J* = 1.6, 7.9 Hz, 4H), 7.45 - 7.36 (m, 6H), 7.32 (d, *J* = 8.0 Hz, 2H), 4.16 (dd, *J* = 4.3, 5.4 Hz, 2H), 3.82 (dd, *J* = 5.0, 5.5 Hz, 2H), 3.69 (dd, *J* = 4.5, 5.2 Hz, 2H), 3.60 - 3.55 (m, 6H), 2.42 (s, 3H), 1.07 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 144.8, 135.6, 133.7, 133.0, 129.8, 129.6, 128.0, 127.7, 72.5, 70.8, 70.7, 69.3, 68.7, 63.4, 26.8, 21.6, 19.2.HRMS (ESI): [M+Na]; Calc.: 565.2051 found: 565.2051.

Synthesis of diethyl 2-hydroxy-5-methoxyterephthalate (**7**)



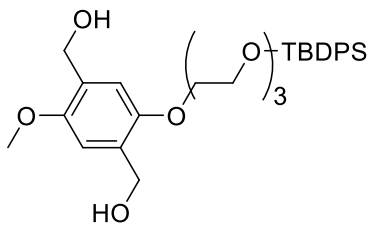
To 2,5-dihydroxy-terephthalic acid diethyl ester (10.00 g, 39.33 mmol) were added acetone (130 mL) and anhydrous K_2CO_3 (5.44 g, 39.33 mmol). The mixture was heated to reflux and then iodomethane (2.69 mL, 43.27 mmol) was added. The solution was refluxed for 24 hours before the solvent was removed *in vacuo*. The residue was dissolved in DCM (120 mL), H_2O (60 mL) and sat. aq. NH_4Cl (60 mL). The layers were separated and the aqueous phase extracted with DCM (2 x 100 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo*. Flash chromatography on silica (pentane/Et_2O 4:1 to 2:1) afforded (**7**) as a slightly yellow solid (4.55 g, 43%).^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 10.38 (s, 1H), 7.48 (s, 1H), 7.17 (s, 1H), 4.47 (q, *J* = 7.1 Hz, 2H), 4.32 (q, *J* = 7.1 Hz, 2H), 3.83 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.34 (t, *J* = 7.1 Hz, 3H).^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 169.1, 164.8, 155.0, 150.3, 129.4, 118.8, 114.5, 112.5, 61.9, 60.9, 56.2, 13.6, 13.5.HRMS (ESI): [M+Na]; Calc.: 291.0839, found: 291.0840.M_P (uncorr.) 52.5 - 53.8 °C.

<u>Synthesis of diethyl 2-((2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecan-12-yl)oxy)-5-methoxyterephthalate (**2**)</u>



To a flask were added (**7**) (8.75 g, 16.12 mmol), (**6**) (4.54 g, 16.93 mmol) and anhydrous DMF (65 mL). The mixture was heated to 90 °C before K_2CO_3 (4.46 g, 32.24 mmol) was added. The mixture became yellow and was stirred for 3 hours at 90 °C. After cooling to rt the mixture was diluted with EtOAc (150 mL) and the organic layer was washed with H_2O (2 x 100 mL) and sat. aq. NaCl (2 x 50 mL). The organic layer was dried over Na_2SO_4 and concentrated*in vacuo*. Flash chromatography on silica (pentane/Et_2O 2:1 to Et_2O) afforded (**2**) as a clear oil (9.72 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 - 7.67 (m, 4H), 7.41 - 7.34 (m, 8H), 4.35 (tq, *J* = 0.9, 7.0 Hz, 4H), 4.16 (t, *J* = 5.0 Hz, 2H), 3.87 - 3.85 (m, 5H), 3.82 (t, *J* = 5.3 Hz, 2H), 3.72 – 3.65 (m, 4H), 3.61 (t, *J* = 5.3 Hz, 2H), 1.36 (dt, *J* = 0.8, 7.1 Hz, 6H), 1.05 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 165.3, 152.8, 151.5, 135.6, 133.7, 129.6, 127.6, 125.3, 124.3, 117.9, 115.2, 72.5, 71.0, 70.8, 70.0, 69.7, 63.4, 61.3, 61.3, 56.7, 26.8, 19.2, 14.3 (2H).HRMS (ESI): [M+K]; Calc.: 677.2543, found: 677.2541.

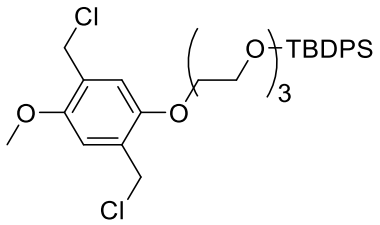
Synthesis of (2-((2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecan-12-yl)oxy)-5-methoxy-1,4-phenylene)dimethanol (**8**)



To a flask were added (**2**) (9.72 g, 15.22 mmol) and anhydrous THF (150 mL). The solution was cooled to 0 °C and $LiAlH_4$ (2.31 g, 60.86 mmol) was slowly added. The reaction was stirred for 10 minutes before quenched with a mixture of H_2O and THF 1:2. The mixture was diluted with H_2O (250 mL) and sat. aq. NaCl (100 mL). The aqueous layer was extracted with DCM (3 x 200 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo*. Flash chromatography on silica (Et_2O to Et_2O/acetone 10:2) afforded (**8**) as a clear oil (8.40 g, 99%).^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.76 - 7.73 (m, 4H), 7.45 - 7.40 (m, 6H), 7.09 (s, 1H), 7.01 (s, 1H),

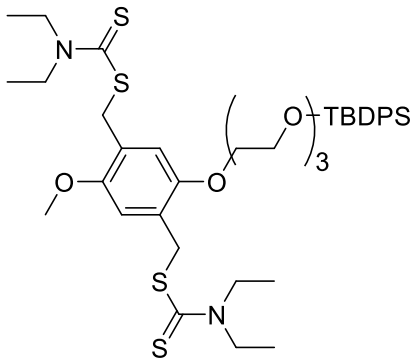
4.63 (t, *J* = 6.0 Hz, 4H), 4.11 (dd, *J* = 4.0, 5.5 Hz, 2H), 3.96 (q, *J* = 6.0 Hz, 2H), 3.84 (dd, *J* = 4.8, 5.4 Hz, 2H), 3.81 - 3.78 (m, 2H), 3.78 (s, 3H), 3.67 - 3.61 (m, 6H), 1.05 (s, 9H).^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 150.7, 150.0, 135.5, 133.7, 130.2, 129.7, 129.6, 127.7, 112.3, 110.2, 72.2, 70.6, 70.5, 69.6, 68.7, 63.5, 59.6, 59.0, 55.2, 26.3, 8.9.HRMS (ESI): [M+Na]; Calc.: 577.2592, found: 577.2593.

Synthesis of 12-(2,5-bis(chloromethyl)-4-methoxyphenoxy)-2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecane (**9**)



To a flask was added anhydrous DCM (400 mL), which was cooled to 0 °C before $SOCl_2$ (7.37 mL, 101.6 mmol) was added. To this mixture was added a solution of (**8**) (9.40 g, 16.94 mmol) in anhydrous DCM (25 mL). The reaction was stirred at 0 °C for 2 hours and then diluted with H_2O (200 mL) and sat. aq. NaCl (200 mL). The aqueous layer was extracted with DCM (2 x 200 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo*. Flash chromatography on silica (pentane/Et_2O 3:1 to 3:2) afforded (**9**) as a clear oil (7.36 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 - 7.67 (m, 4H), 7.43 - 7.35 (m, 6H), 6.94 (s, 1H), 6.91 (s, 1H), 4.64 (s, 2H), 4.60 (s, 2H), 4.14 (dd, *J* = 4.7, 5.3 Hz, 2H), 3.86 - 3.81 (m, 7H), 3.72 - 3.66 (m, 4H), 3.62 (t, *J* = 5.4 Hz, 2H), 1.05 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 151.5, 150.4, 135.6, 133.7, 129.6, 127.6 (2C), 126.9, 115.4, 113.1, 72.5, 71.0, 70.9, 69.8, 69.3, 63.5, 56.2, 41.3, 41.2, 26.9, 19.2.HRMS (ESI): [M+Na]; Calc.: 613.1914, found: 613.1924.

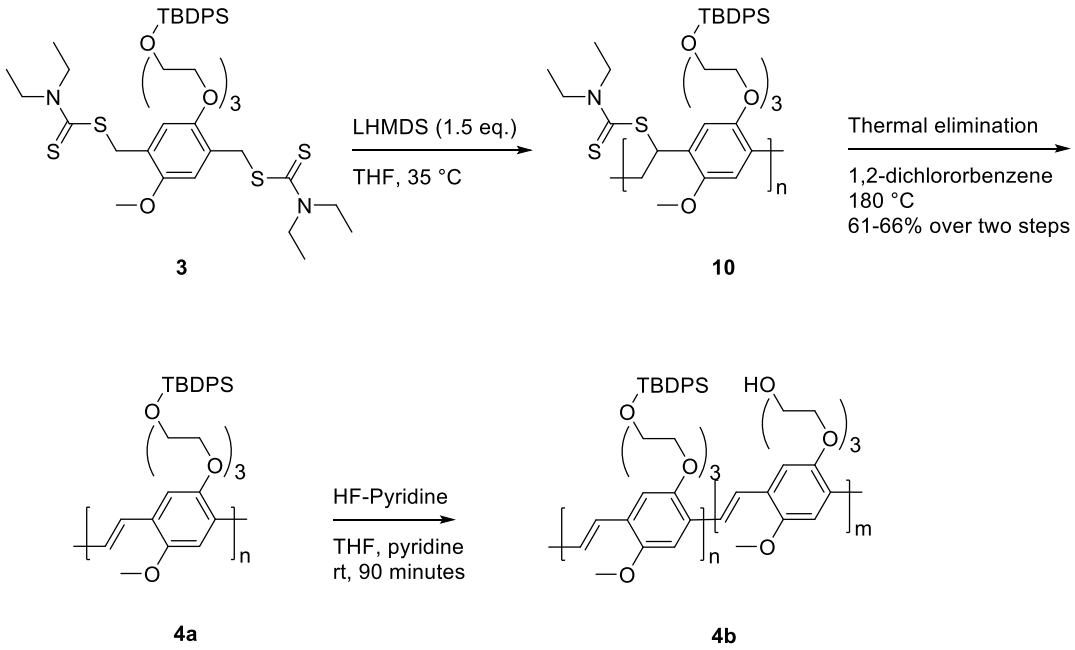
Synthesis of (2-((2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecan-12-yl)oxy)-5-methoxy-1,4-phenylene)bis(methylene) bis(diethylcarbamodithioate) (**3**)



To a flask were added (**9**) (7.36 g, 12.44 mmol), anhydrous MeCN (125 mL) and sodium diethyl-dithiocarbamatetrihydrate (16.82 g, 74.64 mmol). The solution was stirred at rt for 1 hour and then diluted with H_2O (100 mL) and sat. aq. NaCl (100 mL). The aqueous layer was extracted with DCM (2 x 300 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo*. Flash chromatography on silica (pentane/EtOAc 19:1 to 3:1) afforded (**3**) as a clear oil (10.10 g, 99%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 - 7.66 (m, 4H), 7.43 - 7.34 (m, 6H), 7.04 (s, 1H), 7.03 (s, 1H), 4.59 (s, 2H), 4.53 (s, 2H), 4.10 (dd, *J* = 4.9, 5.2 Hz, 2H), 4.03 (q, *J* = 7.0 Hz, 4H), 3.82 (q, *J* = 5.1 Hz, 4H), 3.79 (s, 3H), 3.71 - 3.64 (m, 8H), 3.61 (t, *J* = 5.4 Hz, 2H), 1.28 - 1.20 (m, 12H), 1.05 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 196.1, 196.0, 151.8, 150.7, 135.7, 133.8, 129.7, 127.7, 125.6, 124.8, 115.6, 113.9, 72.6, 71.1, 71.0, 70.0, 69.1, 63.6, 56.3, 49.5, 46.7, 36.8, 36.8, 27.0, 19.3, 12.6, 11.8.HRMS (ESI): [M+K]; Calc.: 855.2786, found: 855.2793.

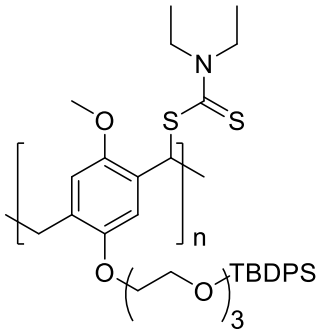
Synthesis of APPV polymers

An overview of the APPV synthesis is shown in Supplementary Fig. 2.



Supplementary Fig. S2. The monomer (**3**) is deprotonated using LHMDS in THF and then it polymerizes into the prepolymer (**10**). This prepolymer is converted into poly(APPV-TBDPS) (**4a**) via thermal elimination at 180 °C in 1,2-dichlorobenzene. To prepare the APPV-TBDPS polymer for DNA functionalization, a fraction of the TBDPS ethers is removed using HF-pyridine to afford the partially deprotected random-copolymer; poly[(APPV-TBDPS)-*ran*-(APPV-OH)] (**4b**).

Procedure for polymerization via the dithiocarbamate precursor route to afford the prepolymer (**10**)

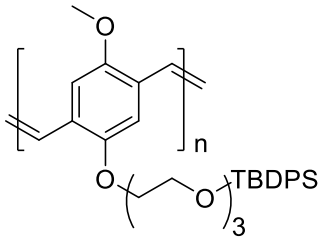


The method is based on a report by J. Vandenbergh *et al*[49]. Monomer (**3**) was added to a flask that had been cleaned with methanol and thoroughly flame dried. The flask was evacuated for 2 hours before it was refilled with argon. Anhydrous THF (inhibitor free, volume specified in Supplementary Table 1) was added and the monomer was allowed to dissolve for 30 minutes, after which the mixture was degassed with argon for 20 minutes. The solution was heated to 35 °C, and then LHMDS (1.0 M in THF, 1.5 eq., volume specified in Supplementary Table 1) was added in one portion. The solution was stirred under argon flow for 60 min. The mixture was diluted with 2-methyltetrahydrofuran (100 mL) and washed with H_2O (2 x 100 mL). The organic layer was separated and the solvent removed *in vacuo* to afford a yellow foam. The crude prepolymer **(10)** was dried *in vacuo* for 16 hours before it was subjected to the following thermal elimination without further purification or characterization.

Supplementary Table 1. Final concentration of monomer and amount of monomer, solvent, and base added.

Final concentration	Monomer (**3**)	THF	LHMDS (1.0 M in THF)
0.08 M	966 mg	13.0 mL	1.77 mL
0.10 M	839 mg	8.5 mL	1.54 mL
0.15 M	711 mg	4.5 mL	1.30 mL
0.20 M	724 mg	3.0 mL	1.33 mL

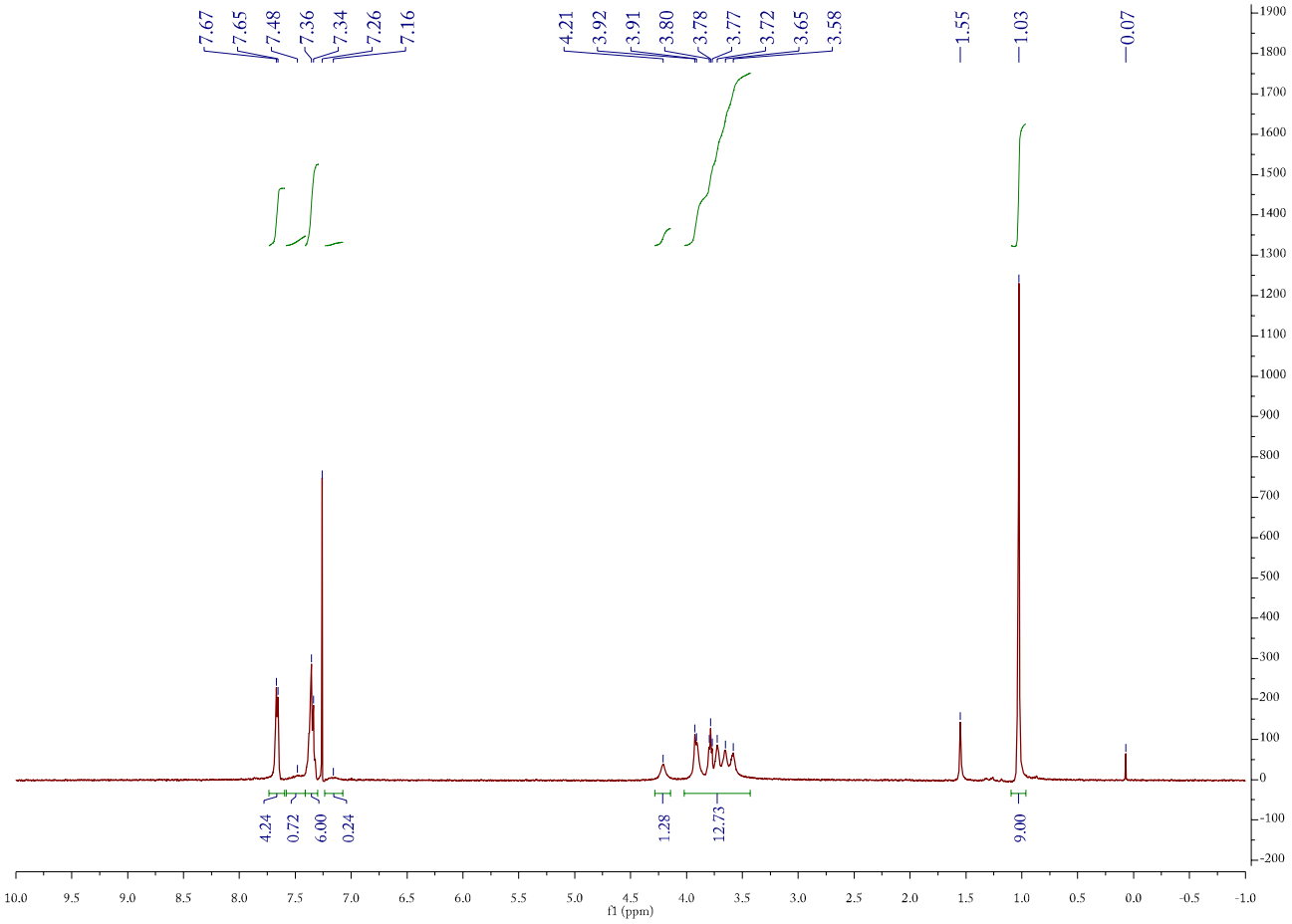
Procedure for thermal elimination of the TBDPS-prepolymer to afford the APPV-TBDPS polymer **(4a)**



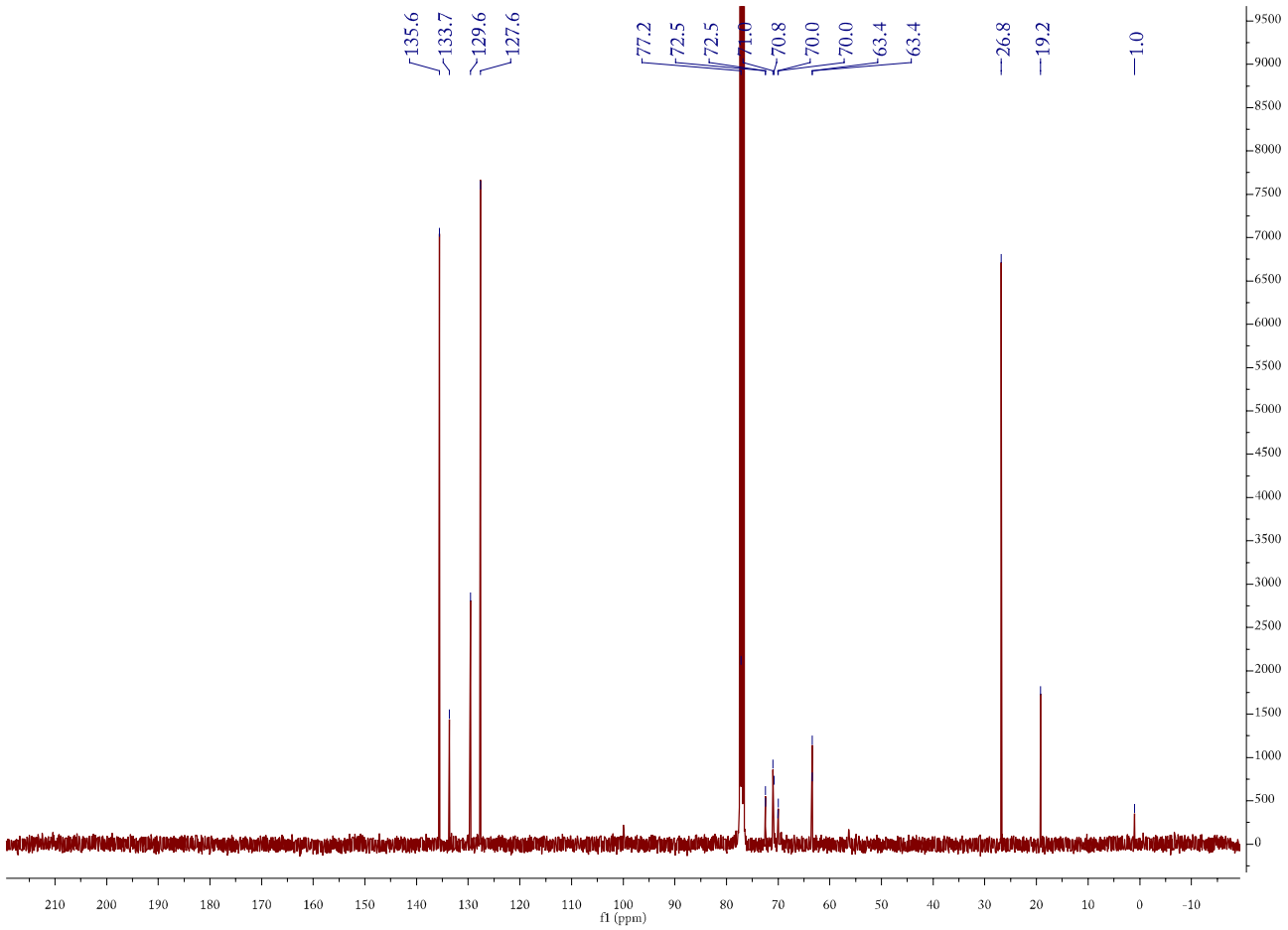
The method is based on a previous report by J. Vandenbergh *et al.*[35]. To a thoroughly flame-dried and argon filled three necked flask was added a solution of the prepolymer (**10**) in 1,2-dichlorobenzene. Dissolvation and transfer of the prepolymer were performed under argon atmosphere and gentle heating to improve polymer solubility. Additional 1,2-dichlorobenzene was added until a volume of around 300 mL. It was important to keep the concentration of prepolymer low (below 3 mg per mL solvent) due to gelation issues in the reaction. The solution was degassed with argon for 1 hour before the mixture was refluxed for 3 hours at 180 °C in complete darkness using a heating mantle. The now orange solution was allowed to cool to rt, after which the solvent was removed *in vacuo* until approximately 10 mL remained (the temperature was set to 70 °C and the rotary evaporator was connected to a Schlenk line to achieve lower pressure). After the elimination reaction, light was avoided as much as possible. The resulting dark orange polymer residue was precipitated in MeOH (600 mL) directly from 1,2-dichlorobenzene to afford a dark red polymer. The polymer was dried *in vacuo* for 36 hours to yield the desired product (**4a**) as a red solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (br d, *J* = 5.9, 4H), 7.46 (br s, 1H), 7.40 - 7.32 (br m, 6H), 7.16 (br s, 1H), 4.21 (br s, 2H), 3.92 (br s, 3H), 3.78 - 3.58 (m, 10H), 1.02 (s, 9H).^{13}C-NMR (100 MHz, $CDCl_3$) δ 135.6, 133.7, 129.6, 127.6, 77.2, 72.5, 71.0, 70.8, 70.0, 63.4, 26.8, 19.2.
The observed broadening of the ^{1}H NMR signals and missing backbone signals in the ^{13}C NMR analysis, likely arise from inhomogeneous broadening due to for example aromatic ring stacking, aggregation, and inhomogeneity in the backbone.

Supplementary Table 2. Amount of prepolymer (**10**) added (estimated with 100% yield from the polymerization reaction), solvent, and yield over two steps.

Final concentration	Prepolymer (**10**)	Yield (**4a**) (two steps)
poly(APPV-TBDPS) 0.08 M	789.6 mg	397 mg, 65 %
poly(APPV-TBDPS) 0.10 M	685.8 mg	349 mg, 66 %
poly(APPV-TBDPS) 0.15 M	581.1 mg	275 mg, 61 %
poly(APPV-TBDPS) 0.20 M	591.8 mg	284 mg, 62 %

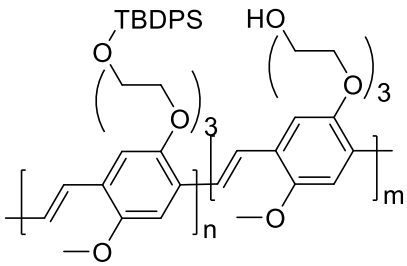


Supplementary Fig. 3. ^{1}H NMR of poly(APPV-TBDPS) (**4a**) in $CDCl_3$.



Supplementary Fig. 4. ^{13}C-NMR of poly(APPV-TBDPS) (**4a**) in $CDCl_3$.

Procedure for partial removal of TBDPS ethers from poly(APPV-TBDPS) to provide the random copolymer poly[(APPV-TBDPS)-*ran*-(APPV-OH)] **(4b)**.



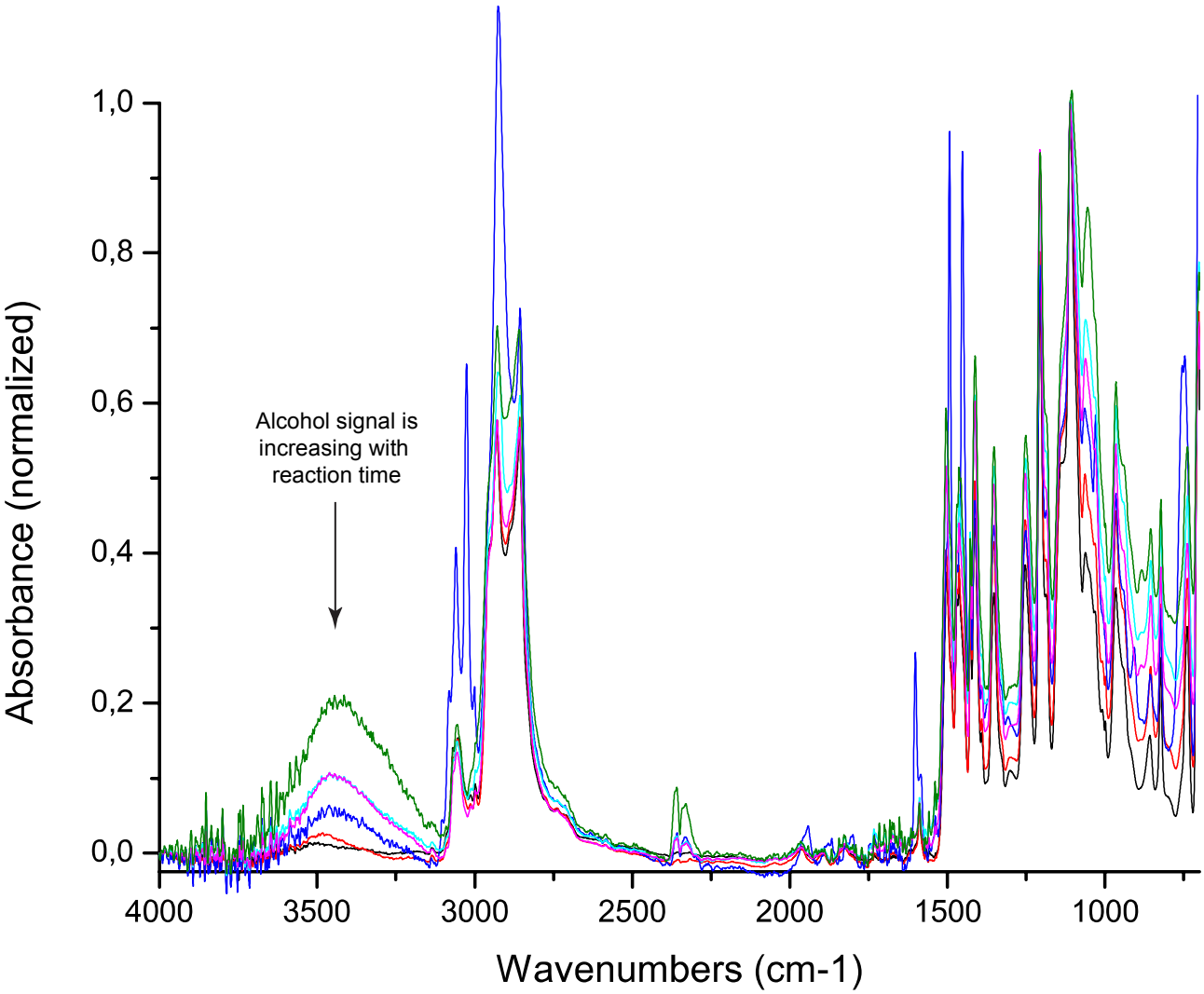
To the APPV-TBDPS polymer (**4a**) (75 mg, 145 μmol TBDPS groups) in a 50 mL Corning centrifuge tube (polypropylene tube) was added THF (22 mL, polymer concentration of 3.4 mg/mL)

and the polymer was allowed to dissolve for 48 hours. A mixture of HF-Pyridine 70%, THF and pyridine (3.1 mL, v/v 1:8:2, approx. 11 mmol HF, 75 eq.) was added, and the mixture was stirred at rt for 90 minutes before it was directly precipitated in MeOH (600 mL) to obtain (**4b**) as a red solid. The polymer was dried *in vacuo* for 16 hours before further use.

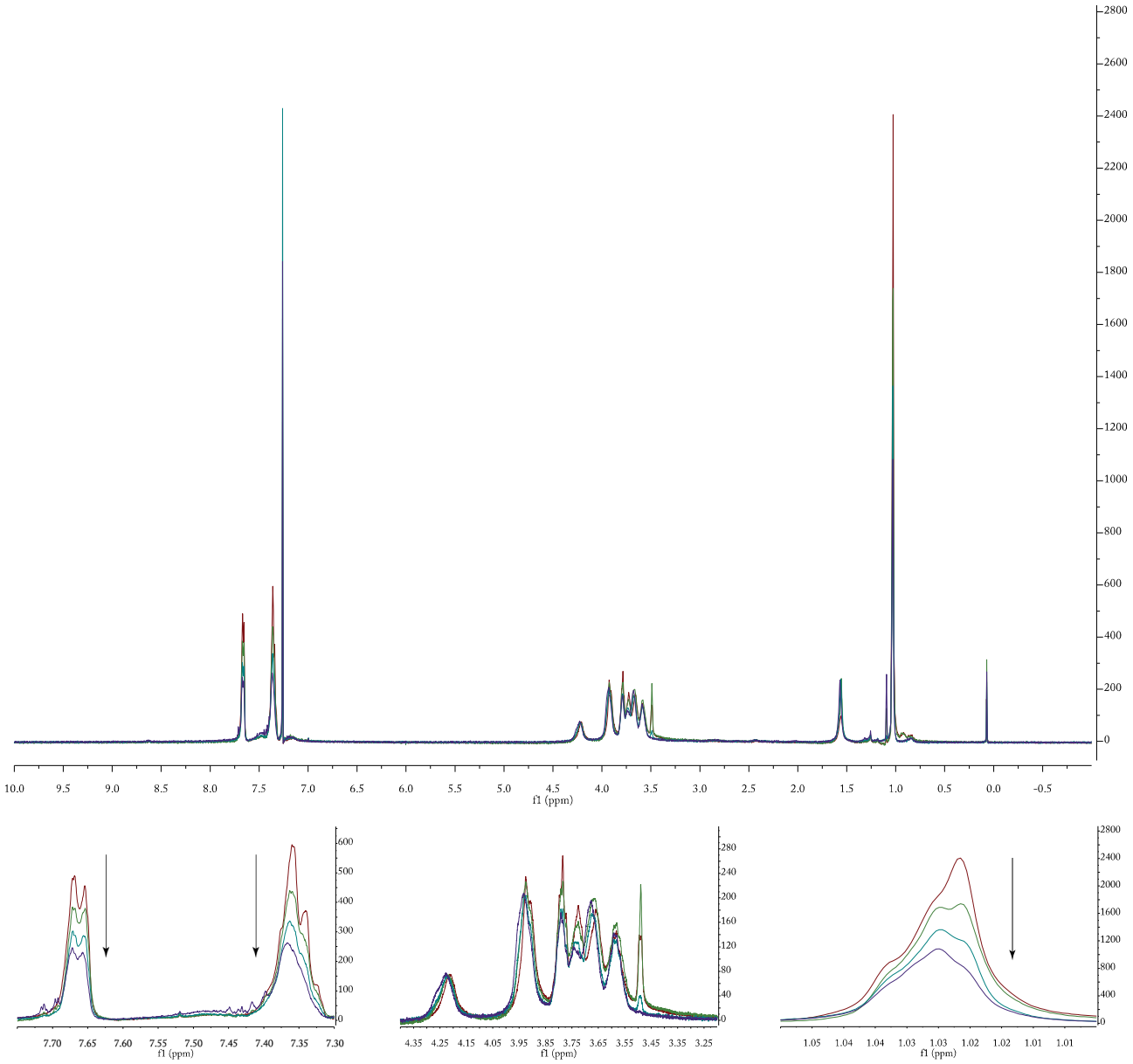
As an example, the naming of the resulting copolymer poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M was performed in correspondence to the starting material poly(APPV-TBDPS) 0.20 M.

As will be mentioned later, poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M was used for the synthesis of all poly(APPV-DNA) used.

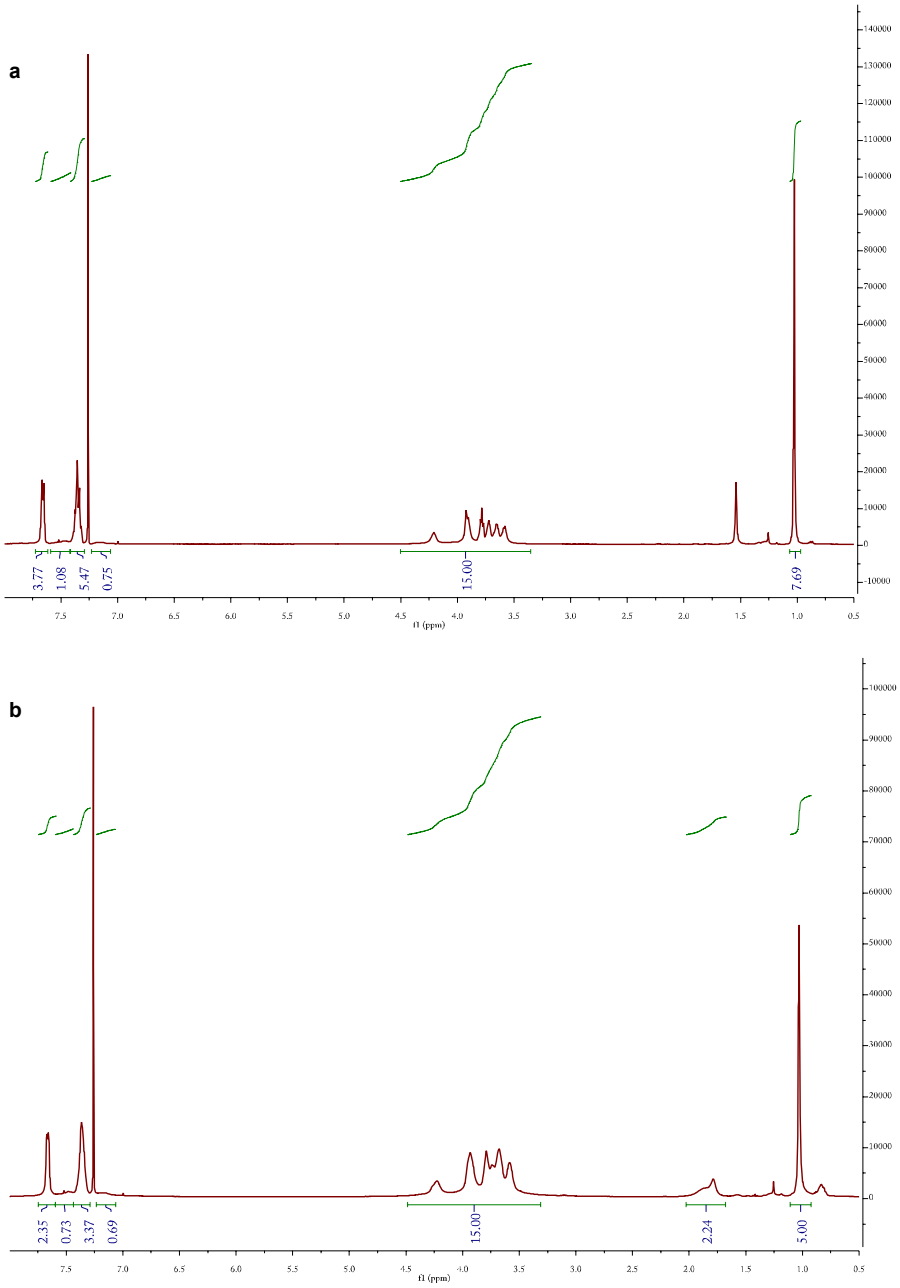
The experiment was performed in plastic equipment, and HF solutions were handled with plastic pipettes to avoid corrosion and loss of active fluoride due to reaction with glassware.
For the optimization of the partial deprotection reaction initial experiments were performed on low molecular weight polymers from the 0.08 M APPV-TBDPS polymerization. The partial deprotection could easily be observed by both ATR-IR and ^{1}H NMR, where the presence of an alcohol and the loss of the TBDPS ether were observed respectively. For the higher molecular weight (APPV-TBDPS) polymers, 0.15 M and 0.20 M polymerizations, the polymers had an increased tendency to aggregate upon removal of the protection groups.



Supplementary Fig. 5. ATR-FTIR data of poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.08 M (**4b**). Poly(APPV-TBDPS) 0.08 M (**4a**) was deprotected with HF using the procedure given above, and the reaction was quenched by precipitation in MeOH at different reaction times 0 (black), 30 (red), 60 (blue), 90 (cyan), 120 (magenta), and 150 (green) minutes. The resulting polymers were washed with MeOH and dried *in vacuo* for 16 hours before analysis (the arrow indicates the formation of alcohol groups during the reaction). The spectra have been normalized at 1111 cm^{-1}.



<u>Supplementary</u> Fig. 6. ^{1}H NMR spectra of poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.08 M (**4b**). Poly(APPV-TBDPS) 0.08 M (**4a**) was deprotected with HF using the procedure given above, and the reaction was quenched by precipitation in MeOH at different reaction times of 0 (red), 30 (green), 60 (blue), and 90 (purple) minutes. The resulting polymers were washed with MeOH and dried *in vacuo* for 16 hours before analysis in $CDCl_3$. The arrows indicate increasing reaction times. The removal of the silyl ether is evident by the decrease in the integral (indicated by arrows) relative to the ethers between 4.50 and 3.00 ppm. For reaction times of 120 and 150 minutes liquid state NMR was not possible.



Supplementary Fig. 7. a) ^{1}H NMR of poly(APPV-TBDPS) 0.20 M (**4a**) in $CDCl_3$. b) ^{1}H-NMR of poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M (**4b**) in $CDCl_3$. Based on the integral from the *tert*-butyl groups on the TBDPS moiety, it was estimated that 35% of the protective groups were removed. The analyzed poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M sample was prepared by treating poly(APPV-TBDPS) 0.20 M with HF for 90 minutes using the procedure given above. The viscosity of this sample was very high and aggregation was observed. To ease handling and avoid

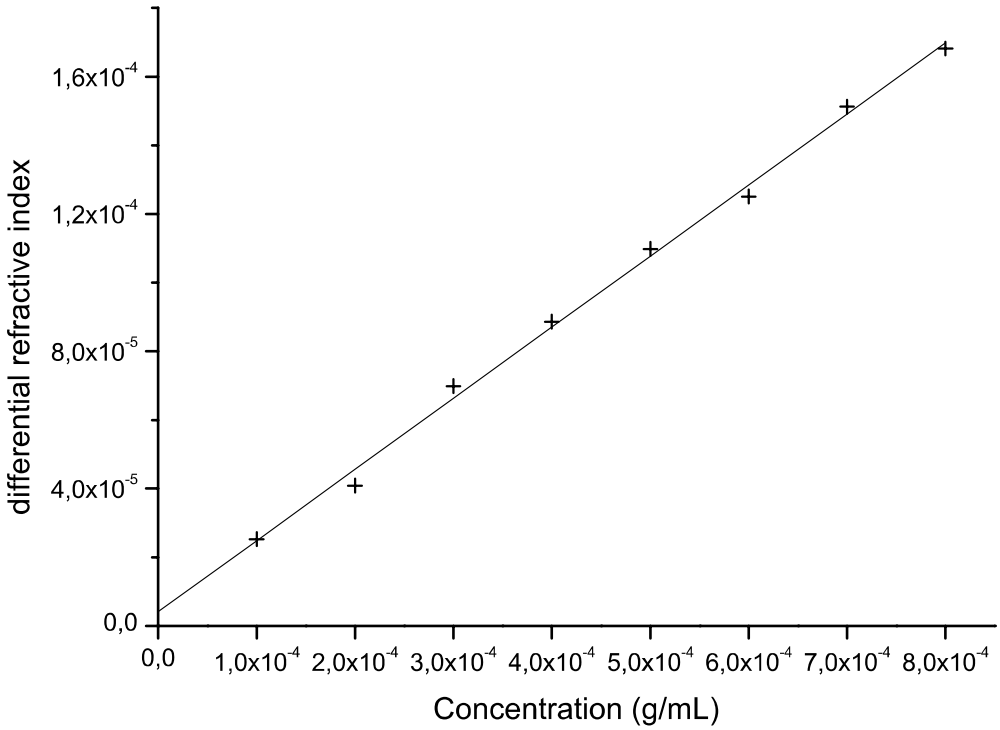
precipitation, a degree of removal of TBDPS below 35% was generally persued. During deprotection some variation in the required reaction time was observed, probably due to the moisture sensitivity of the HF-pyridine mixture.

Mass analysis of the APPV-TBDPS polymer by GPC

The APPV-TBDPS polymers were analyzed by GPC (gel permeation chromatography) at 30 °C using a mobile phase of DMF. Prior to analysis, polymers were allowed to dissolve for a minimum of 4 hours at 80 °C to obtain a well dispersed solution.

The specific refractive index increment (dn/dc) for poly(APPV-TBDPS) in DMF at 30 °C was estimated to 0.2070 ± 0.0048 mL/g using the refractive index of various concentrations of poly(APPV-TBDPS) 0.08 M. The data is shown below in Supplementary Fig. 8.

The mass distributions of poly(APPV-TBDPS) polymerized at different monomer concentrations are summarized in Supplementary Table 3.

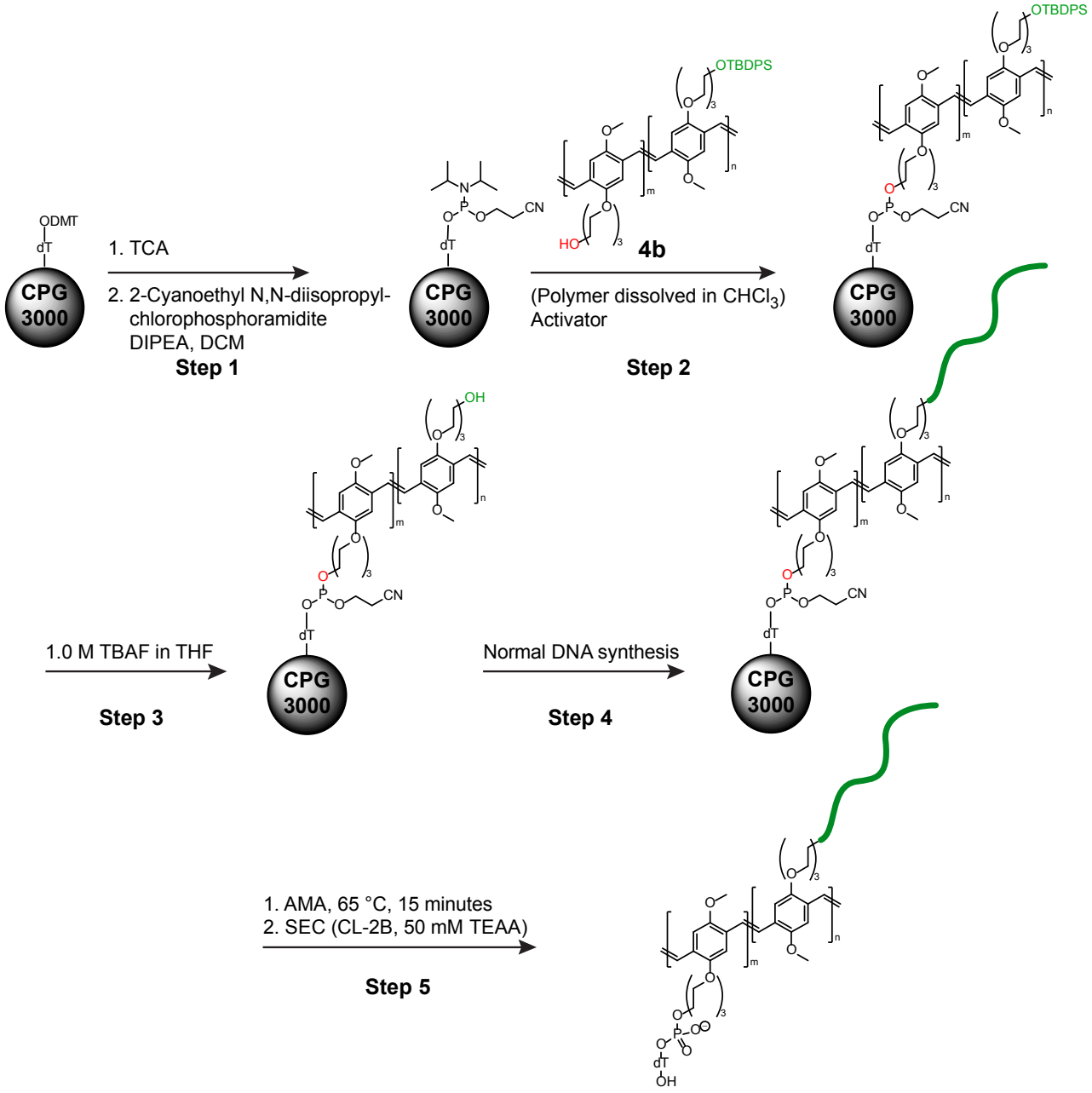


Supplementary Fig. 8. Determination of dn/dc of poly(APPV-TBDPS) in DMF using refractive index. The (+) represents the data and the (–) represents the fit.

Supplementary Table 3. GPC analysis of fully intact poly(APPV-TBDPS) (0.08 M, 0.1 M, 0.15 M, and 0.2 M).

Sample	**Mn** (kDa)	**Mw** (kDa)	**PDI** (Mw/Mn)	**Rz rms** (nm)	**dn/dc** (mL/g)
poly(APPV-TBDPS) 0.08 M	48.8 (±1.8%)	69.7 (±0.5%)	1.440 (±1.8%)	13.3 (±8.8%)	0.2070
poly(APPV-TBDPS) 0.10 M	51.4 (±3.0%)	71.1 (±0.9%)	1.385 (±3.2%)	16.3 (±10.3%)	0.2070
poly(APPV-TBDPS) 0.15 M	59.1 (±2.1%)	90.4 (±0.4%)	1.530 (±2.1%)	17.9 (±3.4%)	0.2070
poly(APPV-TBDPS) 0.20 M	66.9 (±1.6%)	100.4 (±0.4%)	1.501 (±1.6%)	19.2 (±2.9%)	0.2070

Automated DNA synthesis on the APPV-polymer.



Supplementary Fig. 9. Synthetic scheme for the preparation of the APPV-DNA polymer. Step 1) CPG 3000 preloaded with thymidine is deprotected and the resulting alcohol group is converted into a phosphoramidite using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and DIPEA in DCM. Step 2) The phosphoramidite modified CPG allows for immobilization of poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M (**4b**) when added simultaneously with activator. Step 3) After

immobilization, polymer **4b** is deprotected with TBAF in THF to further expose the alcohol groups for DNA synthesis. Step 4) Using normal DNA synthesis, the alcohol groups on the APPV polymer can be functionalized with DNA, synthesized one nucleotide at a time (green strand). Step 5) After DNA synthesis, the APPV-DNA polymer is cleaved from the CPG 3000 and the protective groups removed using ultrafast deprotection conditions (AMA, 65 °C, 15 minutes) and the resulting poly(APPV-DNA) immediately purified using SEC CL-2B material and a mobile phase of TEAA (50 mM, pH 7).

General procedure for immobilization of poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M (**4b**) on CPG 3000 and subsequent oligonucleotide synthesis directly on the immobilized polymers

The synthesis of DNA coated APPV polymer, poly(APPV-DNA) was solely performed on a MerMade 12 oligonucleotide synthesizer from Bioautomation using a fully automated procedure. This equipment is based on small columns loaded with CPG3000, and the different reagents are added to the column and eluted from the CPG using reduced pressure. Two amidite positions on the synthesizer, one containing a solution of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (200 µL) in anhydrous DCM (5 mL) and the other containing anhydrous DIPEA, were modified in such a way, that the two solutions were injected simultaneously to the synthesis column. This approach evades premixing of the chemicals prior to synthesis and allows for prolonged storage of the chemicals under argon atmosphere on the automated synthesizer. To functionalize the 3000Å CPG (preloaded with 200 nmol thymidine) as a phosphoramidite, the solid support was subjected to the following steps; 2 x deblock, 2 x MeCN wash, 3 x coupling steps with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in DCM added together with DIPEA, and then 5 x MeCN wash. The poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M **(4b)** (7 mg) was dissolved in anhydrous $CHCl_3$ (4 mL) and added to the column together with activator (0.25 M 5-ethylthio-1H-tetrazole in anhydrous acetonitrile). The following steps were performed in the immobilization of the polymer to the solid support; 1 x coupling steps with the poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M in

$CHCl_3$ added together with activator, 10 x DCM wash, 4 x MeCN wash. To remove the majority of remaining silyl ethers on the polymer, TBAF in THF (1 M) was added to the column without any additives. The following steps were used for this procedure; 2 x TBAF treatments, 4 x MeCN wash, 2 x DMF wash, 2 x TBAF treatments, 4 x MeCN wash, 2 x DMF wash, 2 x TBAF treatments, 2 x MeCN wash. The free alcohol groups on the immobilized polymer were used as handles for the DNA coating. The following steps in the automated synthesis were used for synthesis of each nucleotide on the polymer; 2 x deblock, 2 x MeCN wash, 2 x coupling of nucleoside phosphoramidite, 4 x MeCN wash, 2 x DMF wash, 4 MeCN wash, 2 x coupling of nucleoside phosphoramidite, 4 x MeCN wash, 2 x DMF wash, 4 MeCN wash, 2 x coupling of nucleoside phosphoramidite, 1 x MeCN wash, 1 x capping, 1 x oxidation, 2 x MeCN wash.
Deblock, capping, and oxidation steps were omitted before the first nucleotide was applied to the polymer since the OH groups on the polymer are already deprotected.
The following reaction times per addition were used in the reactions (number of vacuum pulses, wait time between vacuum pulses, and amount added): Nucleoside phosphoramidite (4 x 15 seconds, 70 µL amidite, 85 µL activator), phosphoramidite formation on CPG (8 x 75 seconds, 125 µL 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in $CHCl_3$, 125 µL DIPEA), polymer immobilization (8 x 75 seconds, 125 µL polymer, 125 µL activator), TBAF cleavage (8 x 75 seconds, 250 µL 1.0 M TBAF in THF), MeCN wash (1 x 10 seconds, 250 µL MeCN), DCM wash (1 x 60 seconds, 200 µL DCM), DMF wash (8 x 75 seconds, 250 µL DMF), deblock mix (4 x 10 seconds, 150 µL deblock mix), capping (5 x 8 seconds, 75 µL cap mix A, 75 µL cap mix B), and oxidizer (3 x 15 seconds, 150 µL oxidizer).

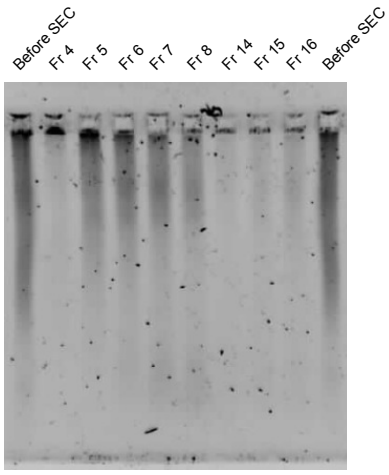
Procedure for purification of poly(APPV-DNA)

The PPV-DNA was cleaved from the CPG by ultrafast deprotection conditions using AMA (ammonia hydroxide 30-33% and aq. methylamine 40% v/v 1:1) at 65 °C for 15 minutes. The

mixture was cooled and loaded onto a size exclusion column (PD-10 columns packed with Sepharose CL-2B material) and eluted with TEAA (50 mM, pH 7) buffer. Fractions containing poly(APPV-DNA) were lyophilized, dissolved in water, and again lyophilized before stored at -20 °C. Care was taken to avoid light during the purification and storage. Before use, the polymers were dissolved in water. Early fractions contain high molecular weight poly(APPV-DNA) while later fractions contain small molecules such as DNA strand, ammonia hydroxide, methyl amine, and protective groups. The polymers were analysed using GPC and the data is shown in Supplementary Table 4.

The poly(APPV-DNA) samples were named according to the fraction number from the purification, e.g. poly(APPV-DNA) fr. 3. The earliest fraction containing poly(APPV-DNA) was fraction 3. For the AFM imaging mentioned later, poly(APPV-DNA) fr. 3 was used, as this fraction contains polymers of highest molecular weight.

Several attempts were made to improve the quality and decrease the polydispersity of the APPV-DNA polymers. Prior to the DNA synthesis, attempts were made to purify the APPV-TBDPS polymers on Biorad SX-1 material using THF as the eluent. However, no significant improvements of the lengths or PDIs were observed by AFM of the resulting APPV-DNA polymers.
In order to separate high molecular weight poly(APPV-DNA) from low molecular weight ones, gel electrophoresis using a 0.4% agarose gel was attempted. Samples taken before and after SEC purification on CL-2B material were analyzed and the resulting gel is shown in Supplementary Fig. 10. It is observed that the APPV-DNA polymers in early fractions tend to remain in the wells, while the lower molecular ones migrate in the gel.



Supplementary Fig. 10. Gel electrophoresis (0.4% agarose, 50 V, 2 hours, SYBR® Gold stain) of poly(APPV-DNA) before and after SEC purification. Lane 1 and 10: Before SEC purification. Lane 2 to 9: Selected fractions from SEC purification using CL-2B as solid phase.

Dialysis of the APPV-DNA polymers using Biotech CE tubing MWCO 1000 kDa proved unsuccessful since the polymers either could not penetrate the pores in the membrane material or simply adhered to the membrane due to potential presence of cellulose nitrate in the membrane composition.

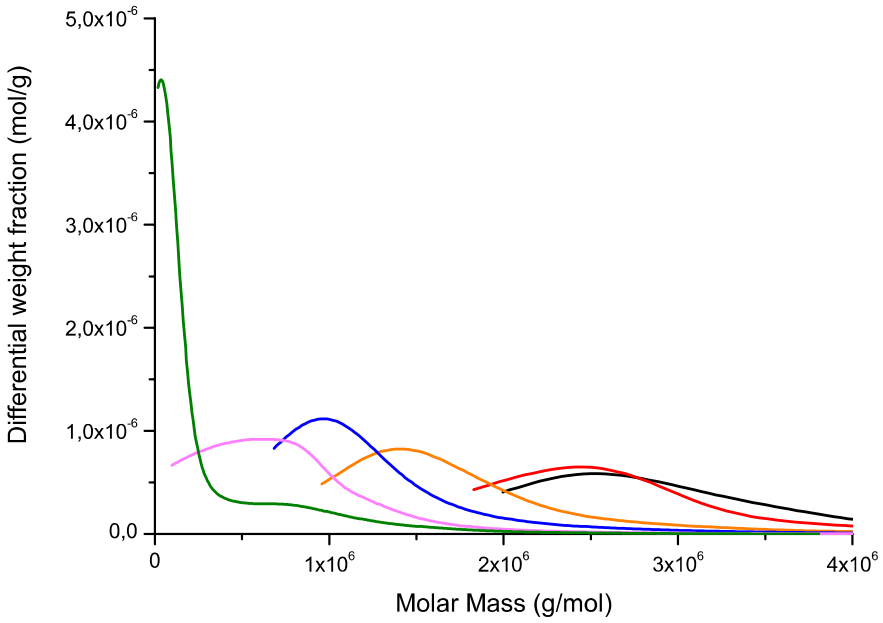
Mass analysis of the APPV-DNA polymer by GPC

The mass analysis of poly(APPV-DNA) was performed in PBS buffer using a dn/dc value of 0.1850 mL/g. The different fractions from the SEC purification were lyophilized, dissolved in water and lyophilized. The dry polymers were dissolved in PBS buffer before analysis.

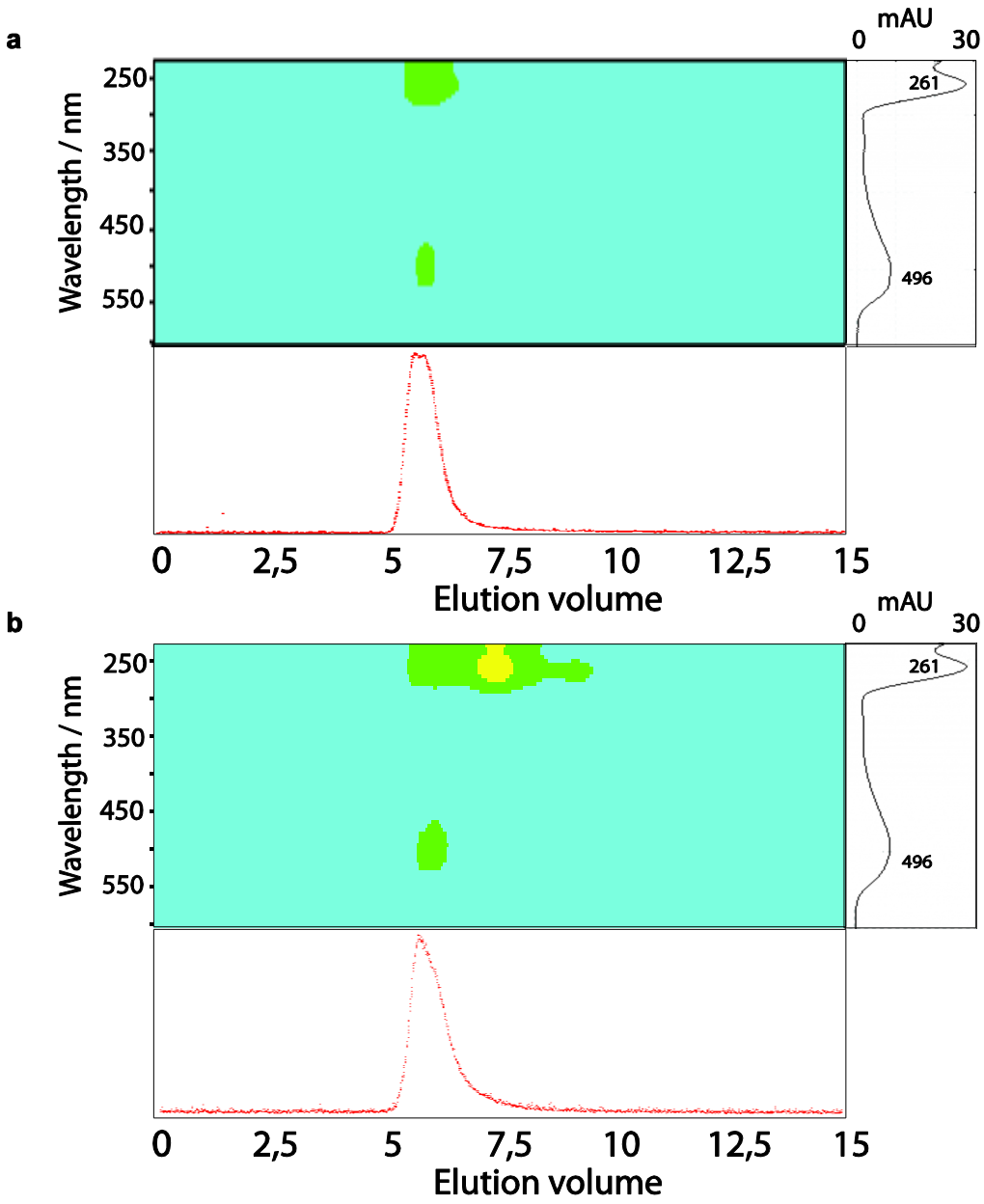
Supplementary Table 4. GPC-MALS analysis of poly(APPV-DNA) resulting from DNA grafting on poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M. The analyses were performed in PBS buffer and on the different fractions after SEC purification. Due to the high molecular weight of the APPV-DNA polymers and the void volume of the column, no significant separation of the polymers were

observed, and the M_n values are therefore calculated to be close to the M_w values. This causes an artificially low PDI. The exceptions to this are fraction 7 and 8, containing large amounts of free DNA, probably arising from oligonucleotide synthesis on alcohol groups on the CPG 3000 which have not been functionalized with the APPV polymer.

Sample	Mn[a] (kDa)	Mw (kDa)	PDI[a] (Mw/Mn)	Rz rms (nm)	dn/dc (mL/g)
poly(APPV-DNA) fr. 3	2908.0 (±0.4%)	3344.5 (±0.6%)	1.150 (±0.7%)	81.7 (±0.6%)	0.1850
poly(APPV-DNA) fr. 4	2663.1 (±0.5%)	3240.1 (±0.9%)	1.217 (±1.0%)	88.7 (±1.0%)	0.1850
poly(APPV-DNA) fr. 5	1540.8 (±0.6%)	1906.2 (±0.6%)	1.237 (±0.8%)	71.8 (±0.7%)	0.1850
poly(APPV-DNA) fr. 6	1046.1 (±0.7%)	1291.2 (±0.6%)	1.234 (±1.0%)	62.7 (±0.7%)	0.1850
poly(APPV-DNA) fr. 7	347.6 (±4.6%)	724.8 (±1.0%)	2.085 (±4.7%)	49.7 (±1.7%)	0.1850
poly(APPV-DNA) fr. 8	54.9 (±6.8%)	343.9 (±1.1%)	6.260 (±6.9%)	46.1 (±2.1%)	0.1850



Supplementary Fig. 11. The distribution analysis of APPV-DNA polymers after SEC purification. Black: poly(APPV-DNA) fr. 3, red: poly(APPV-DNA) fr. 4, orange: poly(APPV-DNA) fr. 5, blue: poly(APPV-DNA) fr. 6, pink: poly(APPV-DNA) fr. 7, green: poly(APPV-DNA) fr. 8. Fraction 7 and 8 are dominated by small molecular weight molecules, probably DNA strands synthesized on non-functionalized hydroxyl groups on the CPG 3000 material.



Supplementary Fig. 12. Elution and UV-vis profiles of GPC analyzed poly(APPV-DNA). a) poly(APPV-DNA) fr. 3 showing the polymer harbours UV from both DNA (261 nm) and the polymer backbone (496 nm). b) poly(APPV-DNA) fr. 8 shows free DNA oligomers clearly visible at a larger elution volume than the polymer. These DNA oligomers are not bound to the polymer as they do not co-elude.

Supplementary Table 5. Estimation of average theoretical molecular length of poly(APPV-DNA). The calculations are based on a repeating unit weight of 2999.8 Da for poly(APPV-DNA). The calculations assume a complete functionalization of each repeating unit with a 9mer DNA strand (5'-TCA TCT AAC, MW 2657.8 g/mol). The repeating unit length of 0.665 nm for APPV was estimated using ChemBio3D Ultra 13.0 on a minimized structure. For DNA functionalization, poly(APPV-TBDPS) 0.20 M was used. It should be noted that the lengths of the APPV-DNA polymers in fractions 3 and 4 appears shorter when characterized by AFM (See Supplementary Fig. 17).

Sample	Mn (kDa)	Mw (kDa)	Repeating unit (Da)	Repeating unit length (nm)	Average DP (Mn)	Average DP (Mw)	Average length in nm (Mn)	Average length in nm (Mw)
poly(APPV-DNA) fr. 3	2908.0	3344.5	2999.8	0.665	969	1115	644.6	741.4
poly(APPV-DNA) fr. 4	2663.1	3240.1	2999.8	0.665	888	1080	590.4	718.3
poly(APPV-DNA) fr. 5	1540.8	1906.2	2999.8	0.665	514	635	341.6	422.6
poly(APPV-DNA) fr. 6	1046.1	1291.2	2999.8	0.665	349	430	231.9	286.2
poly(APPV-DNA) fr. 7	347.6	724.8	2999.8	0.665	116	242	77.1	160.7
poly(APPV-DNA) fr. 8	54.9	343.9	2999.8	0.665	18	115	12.2	76.2

XPS elemental analysis of the APPV-DNA polymer

XPS (X-ray photoelectron spectroscopy) was performed on the different polymer samples to estimate the degree of DNA functionalization of the polymers. The polymer samples were coated on a titanium surface by evaporation of the solvent under ambient conditions.

The data are associated with some degree of uncertainty and are mainly suitable for qualitative indications. As an example, the amount of silicon in poly(APPV-TBDPS) appears to be higher after the partial deprotection, poly[(APPV-TBDPS)-*ran*-(APPV-OH)]. However, the data for poly(APPV-TBDPS) agrees well with the theoretical values, poly(APPV-TBDPS) theoretical. Theoretical values are calculated based on a complete functionalization of each repeating unit of the APPV with the corresponding structure (TBDPS, DNA, or dT). Based on the ratios of P/C and N/C in the

poly(APPV-DNA) sample compared to the calculated value, the DNA coverage of the polymer is roughly estimated to 63%.

Supplementary Table 6. XPS analysis and theoretical values of selected functionalized APPV polymers. The standard deviations (SD) were calculated from samples analyzed in triplicate. The poly(dT-APPV) resembles the situation where all alcohol groups from the APPV polymer have been attached to the 5' alcohol group on the dT preloaded CPG 3000 material. ND – not detected. The unexpectedly high silicon content in poly[(APPV-TBDPS)-*ran*-(APPV-OH)] may be due to a contamination.

Sample	C	O	P	N	Si	Ti	S	Na	F
poly(APPV-TBDPS)	83.85	13.22	ND	ND	2.19	0.46	0.28	ND	ND
SD	3.28	2.70			0.36	0.40	0.17		
poly[(APPV-TBDPS)-*ran*-(APPV-OH)]	83.80	12.79	ND	ND	3.25	0.16	ND	ND	ND
SD	0.10	0.25			0.22	0.14			
poly(APPV-DNA)	57.44	25.93	2.90	11.82	0.60	0.28	0.05	0.36	0.62
SD	2.79	2.49	0.19	0.71	0.55	0.48	0.09	0.11	0.58
poly(APPV-TBDPS) theoretical	83.80	13.50	0	0	2.70	0	0	0	0
poly(APPV-DNA) theoretical	51.00	29.50	4.50	15.00	0	0	0	0	0
poly(APPV-dT) theoretical	62.50	30.00	2.50	5.00	0	0	0	0	0

Yield determination of oligonucleotide synthesis on the poly(APPV) backbone

The yields of the synthesis of oligonucleotide brushes on the poly(APPV) backbone are determined based on the amount of single stranded DNA attached to the polymer as measured by UV-absorbance (260 nm). The synthesis is performed on 200 nmol CPG columns and the product is isolated by size exclusion chromatography (SEC). Typically poly(APPV-DNA) is isolated in 1 mL fractions during SEC and the product typically elutes in fraction 3 to 8. Single stranded DNA also elutes in fractions 7 and 8, and therefore the isolated yield of poly(APPV-DNA) is based on the added amounts from fraction 3 to 6.

Supplementary Table 7. Calculated amounts of single stranded DNA in poly(APPV-DNA) from seven different synthesis batches. The results are based on UV (260 nm) measurements and extinction coefficients determined using IDT oligoanalyzer software.

Synthesis number	Isolated yield (nmol ssDNA in poly(APPV-DNA)
1	58.2
2	112
3	102
4	9.7
5	15.1
6	10.1
7	15.9

It is observed that the yields vary from around 10 nmol to above 100 nmol of ssDNA in the poly(APPV-DNA). Although varying, the yields are comparable to the yields obtained from standard solid phase oligonucleotide synthesis on 200 nmol solid supports. It should also be noted that the amount of poly(APPV-DNA) required for one DNA-origami experiment is typically in the range from 20 pmol to 1 nmol dependent on the kind of experiment. Therefore, the amount of isolated poly(APPV-DNA) from one synthesis column is sufficient for several experiments.

Finally, the percentage yield of oligonucleotide synthesis on the poly(APPV) can be roughly estimated based on the amount of injected polymer, and the DNA-coverage of the resulting product. These estimations give yields of 3.1% to 35%, and the details of the calculations can be found below.

Determining the percentage yield of the oligonucleotide synthesis on the poly(APPV) polymer is not straightforward, and in general, only rough estimates of the percentage yields can be made. This is due to the fact that the structure of poly(APPV-DNA) cannot be characterized at the atomic level. Moreover, the shortest fractions of the synthesized DNA-APPV cannot be completely separated from single stranded DNA synthesized along with the poly(APPV-DNA) synthesis. Typically, the poly(APPV-DNA) is purified by gravity size exclusion chromatography where fractions of approximately 1 mL are collected. Fractions 3 to 6 contain poly(APPV-DNA) isolated from single stranded DNA. We here give a representative example of the determination of the percentage yield based on the amount of poly(APPV-DNA) isolated in fraction fraction 3-6 during purification. The absorbance at 260 nm is used as a measure of the amount of isolated DNA-APPV. Moreover, the DNA-coverage of 63% as determined by XPS has to be taken into account when calculating the yields. It shall be noted that we have no means of estimating the number of attachment sites to the solid support. In these calculations, we therefore do not take attachment sites into account.

For the synthesis of one batch of DNA-APPV, 125 µL of partly deprotected poly(TBDPS-APPV) (1.75 mg/mL) is added to the synthesis column.

$$0.125\ mL \cdot 1.75\ \frac{mg}{mL} = 0.219\ mg$$

During the partial deprotection of the poly(TBDPS-APPV) approximately 35% of the protection groups are removed. The protecting group makes up 46% of the mass of the repeating unit. From this we can calculate the percentage of mass lost during the partial deprotection:

$$mass\ loss(\%) = 0.35 \cdot 0.46 = 0.16 = 16\%$$

The M_N of the poly(TBDPS-APPV) batch was 66.9 kDa. The amount of added polymer can now be calculated:

$$66.9\ kDa - (66.9\ kDa\ \cdot 16\%) = 56.2\ kDa$$

$$n_{poly(APPV)} = \frac{0.219\ mg}{149.0\ kDa} = 3.90\ nmol$$

From these figures the maximum theoretical amount of single stranded DNA synthesized on the polymers based on a 63% coverage can be calculated.

The degree of polymerization for the described batch was 129 (66.9 kDa/518.73 Da). With a 63% coverage of DNA this corresponds to an average of 81 molecules of single stranded DNA grafted to each poly(APPV) backbone. The maximal amount of single stranded DNA synthesized on poly(APPV) can therefore be determined:

$$n_{max,DNA} = 3.90\ nmol \cdot 81 = 316\ nmol$$

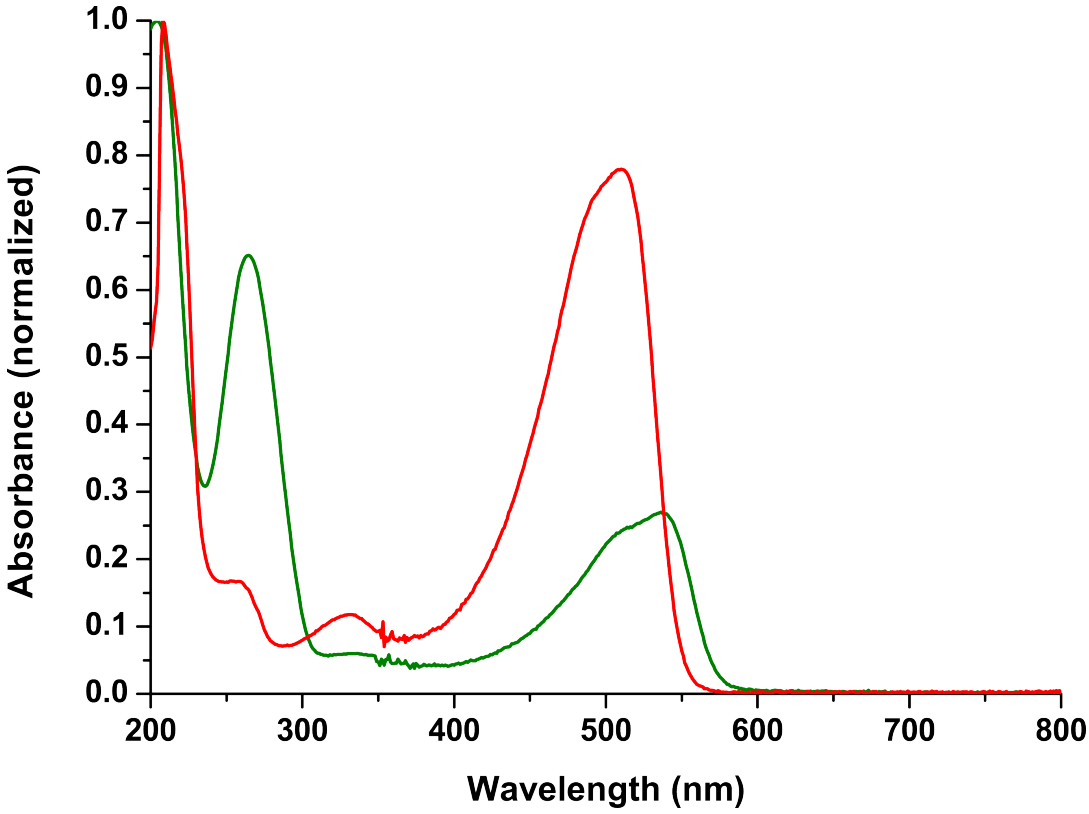
Based on these calculations, the estimated percentage yield of the DNA oligonucleotide synthesis varies from 3.1% to 35%:

$$\frac{9.7\ nmol}{316\ nmol} \cdot 100\% = 3.1\% \quad \frac{112\ nmol}{316\ nmol} = 35\%$$

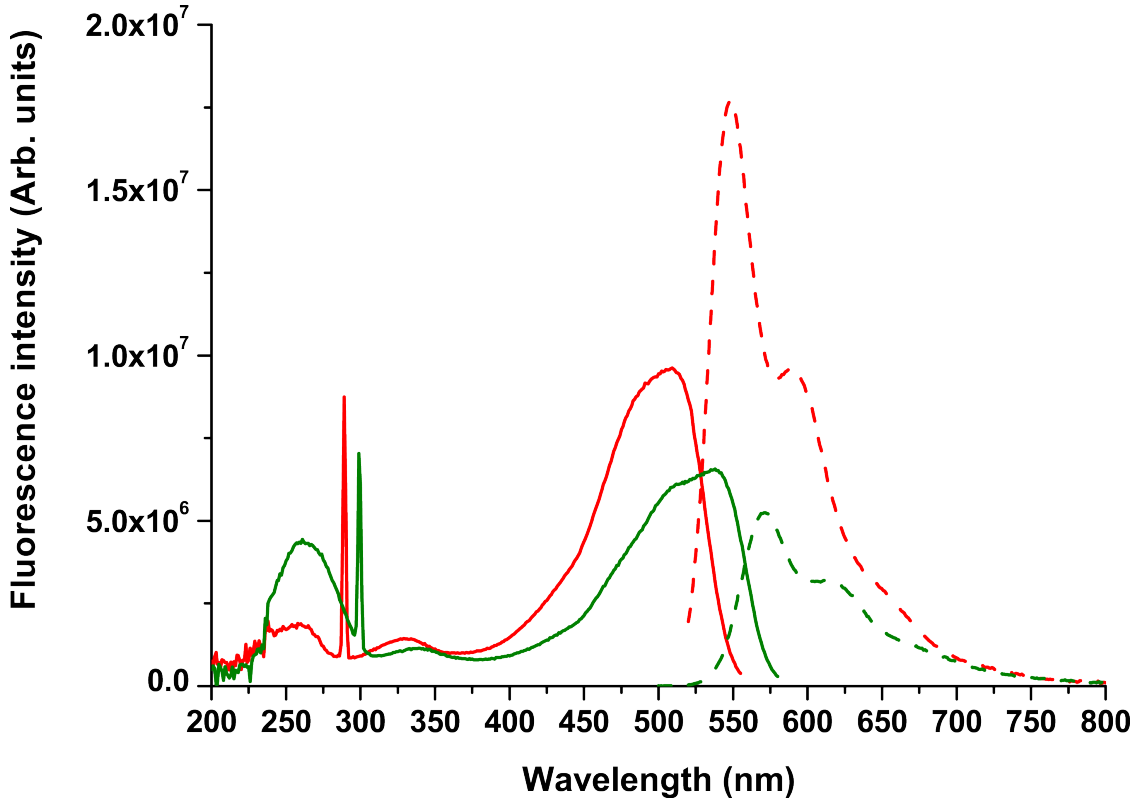
Optical properties of poly(APPV-DNA)

The optical properties of the material have been investigated in terms of UV-Vis absorption and fluorescence. Moreover, the possibility to turn on fluorescence of small-molecule fluorophores by Förster Resonance Energy Transfer (FRET) from the polymer to acceptor fluorophores on DNA-origami, has been investigated.

UV-Vis absorption and fluorescent properties of poly(APPV-TBDPS) and poly(APPV-DNA)



Supplementary Fig. 13. UV-vis absorption spectra of poly(APPV-TBDPS) in THF, red (---) and poly(APPV-DNA) in water, green (---). The light source is shifted at 350 nm between UV and visible light.



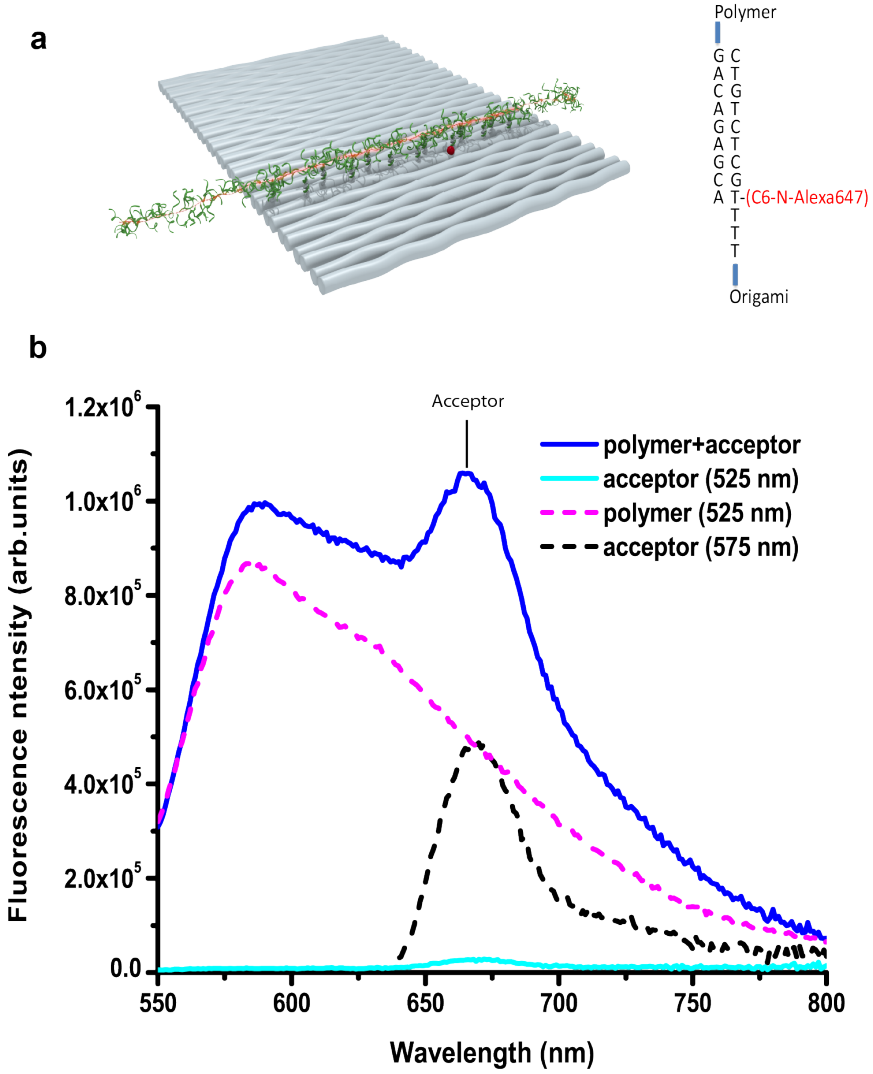
Supplementary Fig. 14. Fluorescence spectra of poly(APPV-TBDPS) in THF and poly(APPV-DNA) in water.

Solid red (–––): Excitation spectrum of poly(APPV-TBDPS) with a fixed emission wavelength at 580 nm. Dashed red (- - -): Emission spectrum of poly(APPV-TBDPS) with a fixed excitation wavelength at 500 nm. Solid green (–––): Excitation spectrum of poly(APPV-DNA) with a fixed emission wavelength at 600 nm. Dashed green (- - -): Emission spectrum of poly(APPV-DNA) with a fixed excitation wavelength at 475 nm. Front entrance and exit slits were set at 2 nm. The poly(APPV-DNA) shows a significantly higher absorption in the region around 260 nm than the poly(APPV-TBDPS). The peaks at 290 and 300 nm are artefacts arising at half the detected emission wavelength.

FRET from the PPV polymer to a fluorophore on DNA origami

Fluorescence of the polymer is also observed after specific immobilization on DNA origami as shown in Supplementary Fig. 15a. We demonstrate how the positioning of the polymer on origami

can be used to obtain Förster Resonance Energy Transfer ((FRET) between the polymer and a single acceptor co-localized on the origami. An Alexa647 dye was conjugated to one of the polymer-binding extended staple strands on the DNA origami. Thus specific polymer binding onto the DNA-origami board is expected to occur in close proximity to the Alexa647 acceptor position. Before addition of poly(APPV-DNA) to the fluorophore-labelled DNA origami sample, very low acceptor emission was observed upon excitation at 525 nm (Supplementary Fig. 15b). After addition of the polymer to the sample, however, a ~20x signal enhancement of acceptor fluorescence was observed arising from FRET from the polymer to acceptor fluorophores on DNA-origami. Fluorescence from a control measurement with no acceptor fluorophore incorporated to the DNA origami, shows only signal arising from polymer fluorescence (Supplementary Fig. 15b).



Supplementary Fig. 15. FRET between the polymer and a single fluorophore. a) Illustration of the set-up for precise positioning of poly(APPV-DNA) and Alexa647 (red sphere). The poly(APPV-DNA) is aligned on rectangular DNA-origami with an Alexa647 dye attached to one of the polymer-binding extended staple strands. b) Fluorescence spectra for: Alexa647 and poly(APPV-DNA) immobilized on the origami (blue curve), poly(APPV-DNA) on DNA-origami without acceptor fluorophore (purple dashed curve), Alexa647 labelled DNA-origami without poly(APPV-DNA) (cyan curve), and fluorophore excited at 575 nm (black dashed curve). Apart from the latter example the samples were excited at 525 nm and the fluorescence measured from 550 nm to 800 nm. Background spectra were subtracted.

Experimental description of fluorescence measurements

For FRET experiments poly(APPV-DNA) with a 9mer ssDNA complementary to the extended staple strands on DNA-origami was used. The applied sequence was:

5'-ACG AGA CAG-APPV

DNA-origami was folded at a 0.20 pmol scale to a final volume of 20 µL TAE(x1) buffer with 12.5 mM $MgCl_2$. Alexa647 was incorporated by labelling of an amino modified extended staple strand with the following sequence:

5'-**CTGTCTCGT(c6-N-Alexa647)-**TTT-CCAGGCGCGAGGACAGATGAACGGGTAGAAAA

The part of the sequence denoted in bold is the polymer binding part of the sequence.

Before measurements the samples were diluted to 80 µL buffer. In general, fluorescence spectra were obtained using 80 µL samples. A spectrum of the origami without polymer was obtained, and then the polymer was added. Polymer addition was carried out from a concentrated solution of poly(APPV-DNA) in milliQ water and the resulting dispersion was mixed thoroughly by pipette. Typically, 1 uL of a 50 µM polymer solution based on the concentration of ssDNA on poly(APPV-DNA) was added. The samples were excited at 525 nm, and the emission was measured from 550 nm to 800 nm. Background spectra were obtained by exciting a DNA-origami sample without any dyes at 525 nm and measuring the emission from 550 nm to 800 nm. To obtain a spectrum of the poly(APPV-DNA) on origami without any fluorophores, a separate DNA-origami sample was prepared. The spectrum was obtained using the same protocol as described above. All measurements were performed at 25 °C.

Fluorescense quantum yield of poly(APPV-DNA)

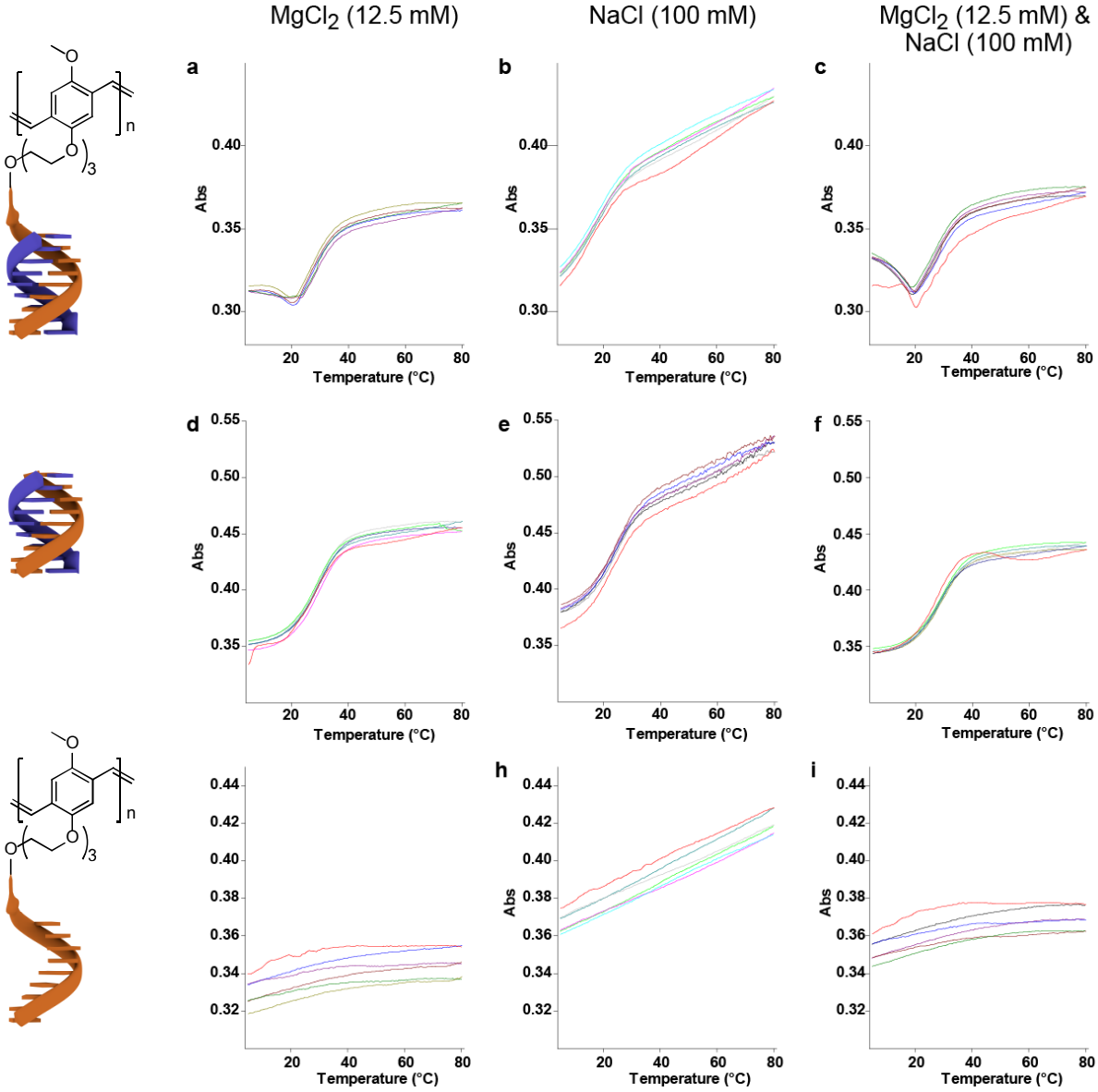
The polymer fluorescence quantum yield Φ_{PPV} was determined using absorption and fluorescence measurements of both sample and a reference using:

$$\Phi_{\mathrm{PPV}} = \Phi_{ref} \frac{I_{PPV} \cdot Abs_{Ref}}{I_{ref} \cdot Abs_{PPV}} \frac{n_{PPV}^2}{n_{ref}^2}$$

where the subscripts PPV and ref stand for the PPV sample and the reference standard. The parameter *Abs* is the absorbance at the fluorescence excitation wavelength and *I* is the integrated area under the fluorescence spectrum. The square of the refractive index, *n*, is employed to make the correction due to the difference in media of the standard and the polymer sample. Sulforhodamine 101 (Reference dye sample kit, Life technologies) in ethanol was used as a reference (with fluorescence quantum yield Φ_{ref} = 1).[50] A quartz cuvette with 1 cm path length was used for both absorption and fluorescence measurements. The absorbance of both sample and reference was kept below 0.05. We estimated an uncertainty of ~20% on our quantum yield measurements by further using 5-carboxytetramethylrhodamine (CMTR, Reference dye sample kit, life technologies) in methanol as a reference (Φ_{CTMR}=0.68).[51] The polymer sample was prepared by dilution of the stock concentration in 20 mM Tris-acetate-EDTA buffer with pH 8.3. Prior to measurements, this sample was heated to 95 degrees for 5 minutes and left to cool down slowly to room temperature overnight.

DNA melting temperatures of the poly(APPV-DNA) DNA sequences

The hybridization properties of the attached DNA on the polymer was investigated by measuring the melting profiles of the APPV-DNA polymers with complementary ssDNA oligonucleotides, ODN1 together with ODN1*, and APPV-DNA polymers without any complementary DNA oligoes. ODN1 denotes the 9mer DNA sequence coated on the APPV-DNA polymer used in the majority of this study, including origami experiments. ODN1* is complementary to ODN1. The melting profiles are shown in Supplementary Fig. 16. The experiments were carried out in the presence of $MgCl_2$, NaCl, or both. In some cases, precipitation was observed when $MgCl_2$ was present, but precipitation was never observed at isothermic conditions over a time period of 12 hours. Melting points for hybridized strands are given in Supplementary Table 8.



Supplementary Fig. 16. Melting temperature determination of poly(APPV-DNA) with complementary ssDNA in TAE buffer (1x). All samples were monitored at an absorbance wavelength of 260 nm. $MgCl_2$ and NaCl concentrations were 12.5 mM and 100 mM, respectively. ODN1 and ODN1* are depicted as blue and orange respectively. a) poly(APPV-DNA) ODN1 fr. 5, ssDNA ODN1*, and $MgCl_2$. b) poly(APPV-DNA) ODN1 fr. 5, ssDNA ODN1*, and NaCl. c) poly(APPV-DNA) ODN1 fr. 5, ssDNA ODN1*, $MgCl_2$, and NaCl. d) ssDNA ODN1, ssDNA ODN1*, and $MgCl_2$. e) ssDNA ODN1, ssDNA ODN1*, and NaCl. f) ssDNA ODN1, ssDNA ODN1*, $MgCl_2$

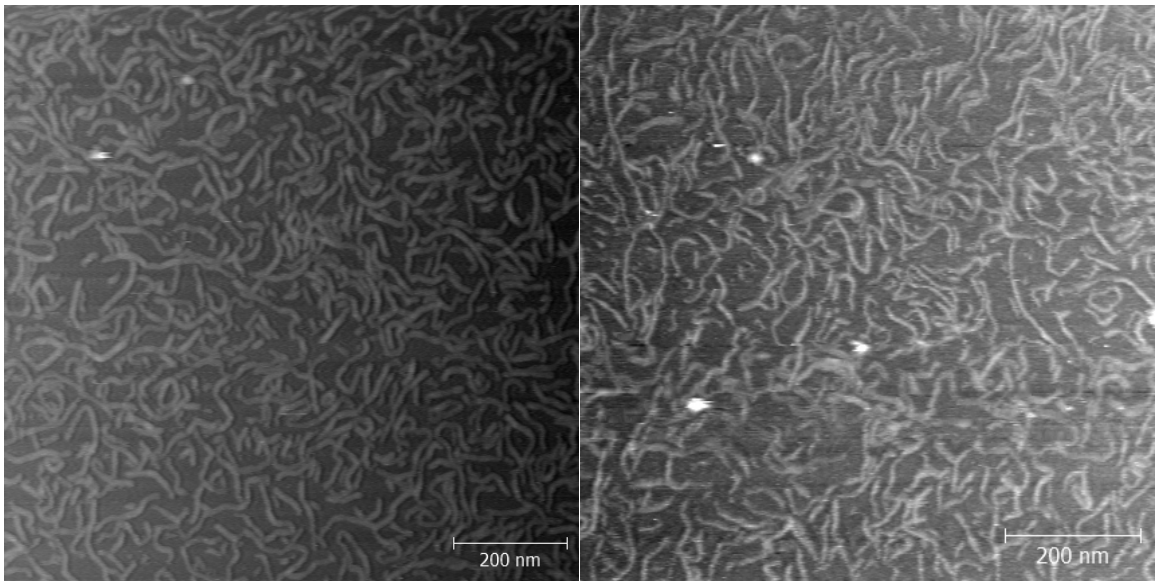
and NaCl. g) poly(APPV-DNA) ODN1 fr. 4 and $MgCl_2$. h) poly(APPV-DNA) ODN1 fr. 4 and NaCl. i) poly(APPV-DNA) ODN1 fr. 4, $MgCl_2$,and NaCl.

Supplementary Table 8. Summarized melting temperatures (degrees Celsius) between poly(APPV-DNA) ODN1 & ODN1* and between ODN1 & ODN1*. The listed melting temperatures are average values extracted from six temperature ramps. $MgCl_2$ and NaCl concentrations were 12.5 mM and 100 mM.

	$MgCl_2$	NaCl	$MgCl_2$ & NaCl
poly(APPV-DNA) ODN1 & ODN1*	27.2	17.6	27.2
ODN1 & ODN1*	29.3	24.0	28.3

AFM imaging of single polymers

APPV-DNA polymers were analysed by AFM revealing isolated polymers with only a small amount of aggregation observed. The analysed APPV-DNA polymers were obtained from DNA grafting on poly[(APPV-TBDPS)-*ran*-(APPV-OH)] 0.20 M. After SEC purification, the first fraction containing APPV-DNA polymers, named poly(APPV-DNA) fr. 3, was used for the AFM imaging. The APPV-DNA polymers were adsorbed to a freshly cleaved mica surface for 2 min and imaged in TAE buffer (1x) with $MgCl_2$ (12.5 mM) and $NiCl_2$ (1 mM).



Supplementary Fig. 17. AFM images of poly(APPV-DNA) fr. 3 on a mica surface. The polymers observed by AFM are up to more than 200 nm long which is significantly shorter than the theoretical average lengths of 640-740 nm derived from GPC (Supplementary Table 5). The longer polymers may be lost during handling as it is observed that the polymers stick to plastic tubes and pipettes and the longer polymers may also be mechanically cleaved during handling and imaging.

Scanning probe microscopy measurements of nanomechanical and conductivity properties of the APPV-DNA polymer

Quantitative nanomechanical microscopy

The quantitative nanomechanical mapping mode was operated under ambient conditions, which allowed control of the applied force during the measurement. Commercial silicon tips with a nominal spring constant of 20 N/m were used in the experiment, and the substrate used in AFM experiments is silicon. The following formula derived for a spherical tip indenting a semi-infinite planar sample was used to estimate a local reduced elastic modulus.

$$F_{interaction} = \left(\frac{4}{3}\right) E^{*}\sqrt{R}(d - d_0)^{\frac{3}{2}} + F_{adh} \qquad (1)$$

where $F_{interaction}$ is the tip-sample force, E^* the reduced elastic modulus of the tip and the sample, R the tip radius, d_0 the surface rest position, $d - d_0$ the depth of indentation and F_{adh} the constant adhesion force during the contact. The topography, elastic modulus and force maps were processed using SPIP™ software.

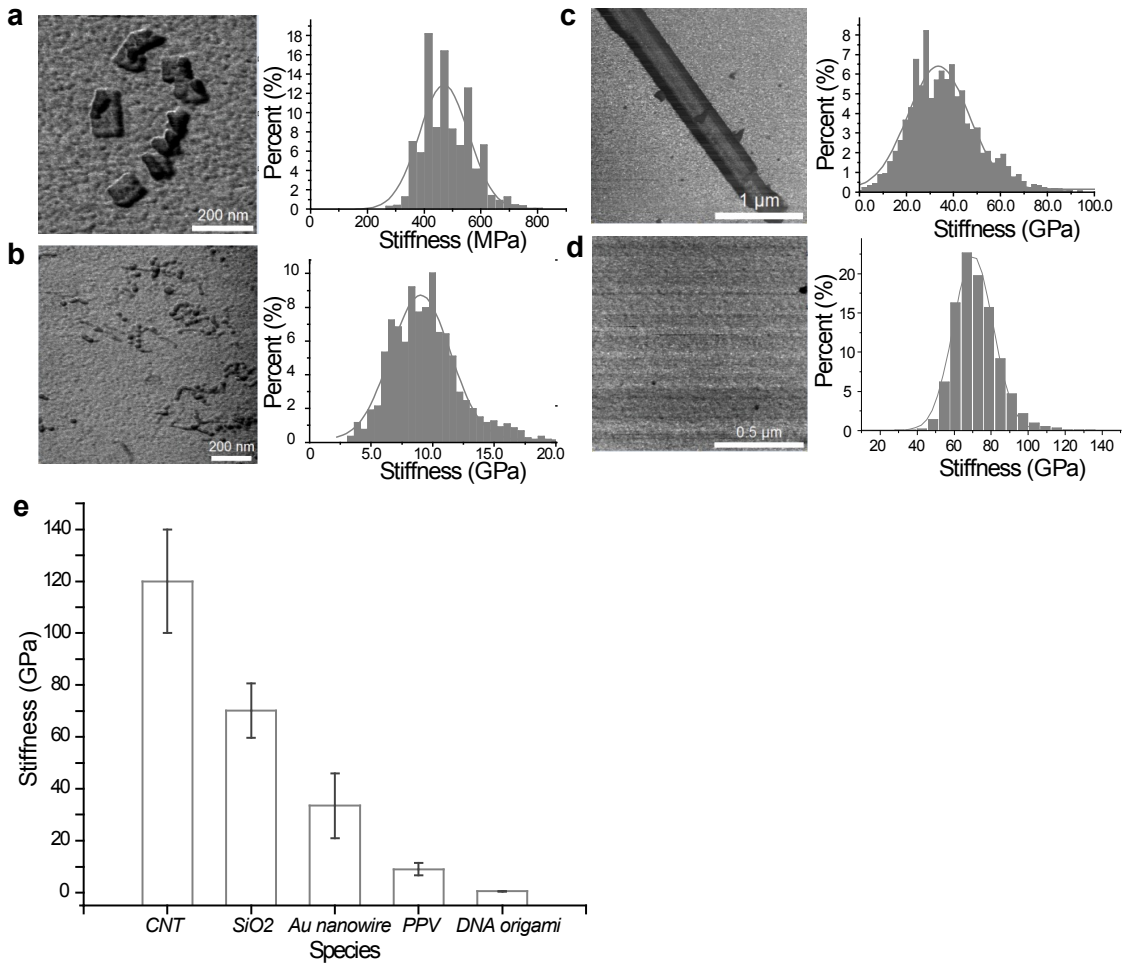
Surface Potential measurements with Kelvin probe force microscopy

The surface potential measurements were performed on an atomic force microscope (Multimode VIII, Bruker) with a Co/Cr-coated tip (MESP) using a two-pass scan technique. Topography information was acquired using a tapping mode on the first pass. During the second pass, the probe was lifted at 15nm with respect to the sample topography, while an oscillating voltage was applied to generate the vibration of the probe. The feedback controller monitored the vibrating amplitude of probe and recorded the potential difference to construct the surface potential image. The result of the well-known mathematical treatment is that the force felt by the vibrating capacitor is proportional to the difference in voltage between the probe and the substrate

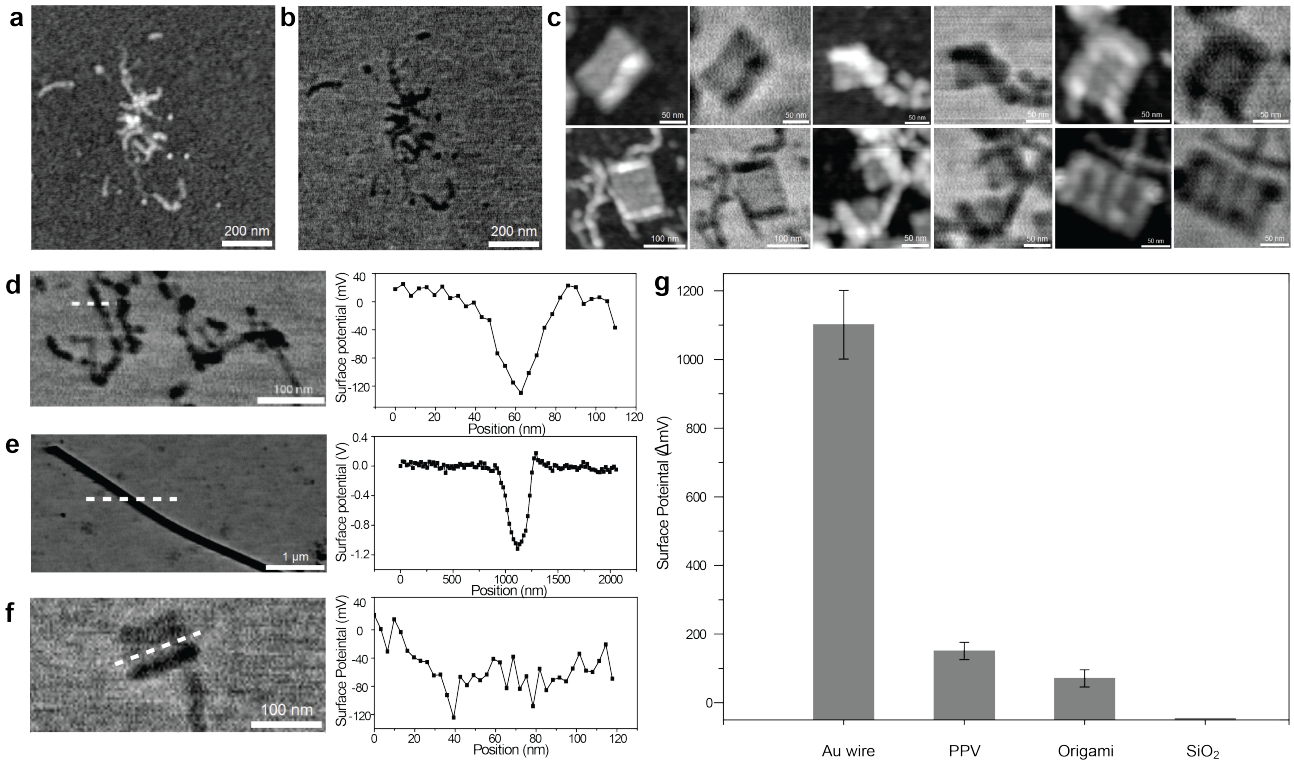
$$\mathrm{F_{cap}} \approx \mathrm{F_{cap}}(\omega) = -\frac{\mathrm{dy}}{\mathrm{dx}}\Delta \mathrm{V_{Dc}}\Delta \mathrm{V_{Ac}} \sin \omega \mathrm{t} \qquad (2)$$

where C is capacitance, Z is separation, VDC and VAC are the DC and AC voltage differences between the probe and substrate, and ω is the resonant frequency of the Kelvin probe. The applied DC voltage is adjusted until the magnitude of the oscillating deflection of the probe is minimized. This is the voltage where the capacitor plates are applying a minimum force on one another and corresponds to the voltage equal to the difference in work function between the two

plates. It is instructive to note that the force is proportional to the magnitude of the AC voltage, providing a simple way to increase the sensitivity of the KPFM.



Supplementary Fig. 18. The stiffness of different species measured by quantitative nanomechanical microscopy. The stiffness map and histogram of, a) DNA origami, b) poly(APPV-DNA) fr. 3, c) Au nanowire, and d) and SiO_2. e) The summary of stiffness of different species.



Supplementary Fig. 19. The surface potential measurement of different species. a) The topography map of poly(APPV-DNA) fr. 3. b) The surface potential map of poly(APPV-DNA) fr. 3. c) Topography and surface potential maps of poly(APPV-DNA) fr. 3 on DNA origami. The surface potential and line profiles of d) poly(APPV-DNA) fr. 3, e) gold nanowire, and f) DNA origami. g) The summary of surface potential of different species.

DNA origami

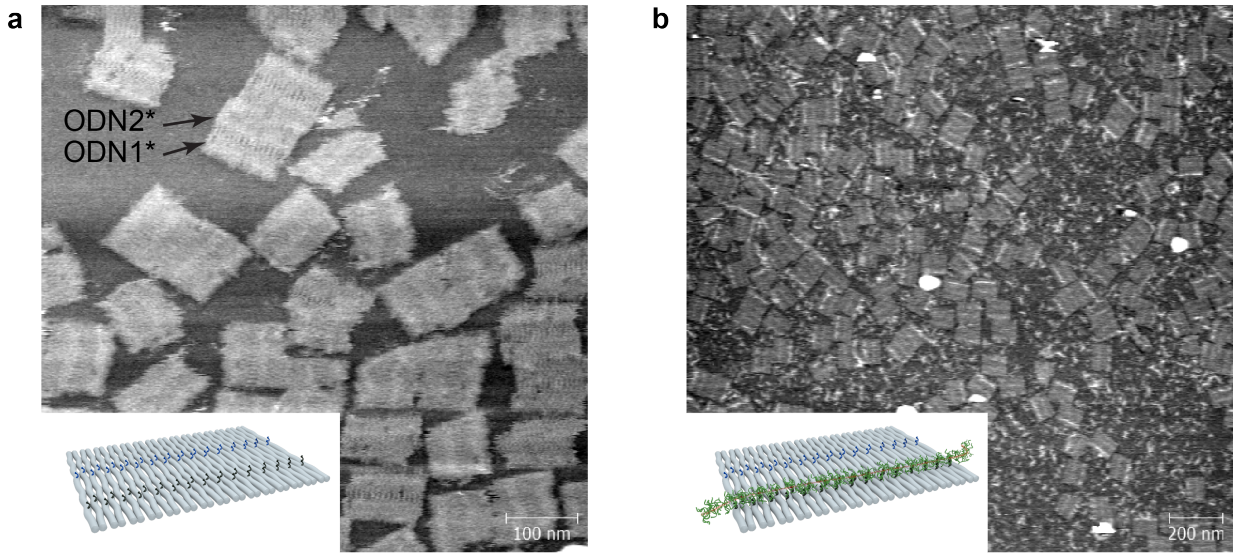
Origami annealing protocol

Rectangular DNA origami was formed according to a modified version of Rothemund's original protocol (*18*). The M13mp18 scaffold (10 nM) was mixed with unpurified staple strands (100 nM) in TAE buffer (1x) with $MgCl_2$ (12.5 mM). The mixture was annealed on an Eppendorf Mastercycler Personal machine (Ramp: 80 to 55 °C over 10 min. and 55 to 4 °C over 80 min.). After annealing excess staples were removed using Amicon centrifugal filter units (100,000 MWCO, 6000 g, 10 min.) and washed twice with TAE-Mg^{2+}-buffer (1x, 12.5 mM).
A detailed illustration and a list of the DNA oligo sequences for the self-assembly of the different DNA origami structures are listed at the end of this document.

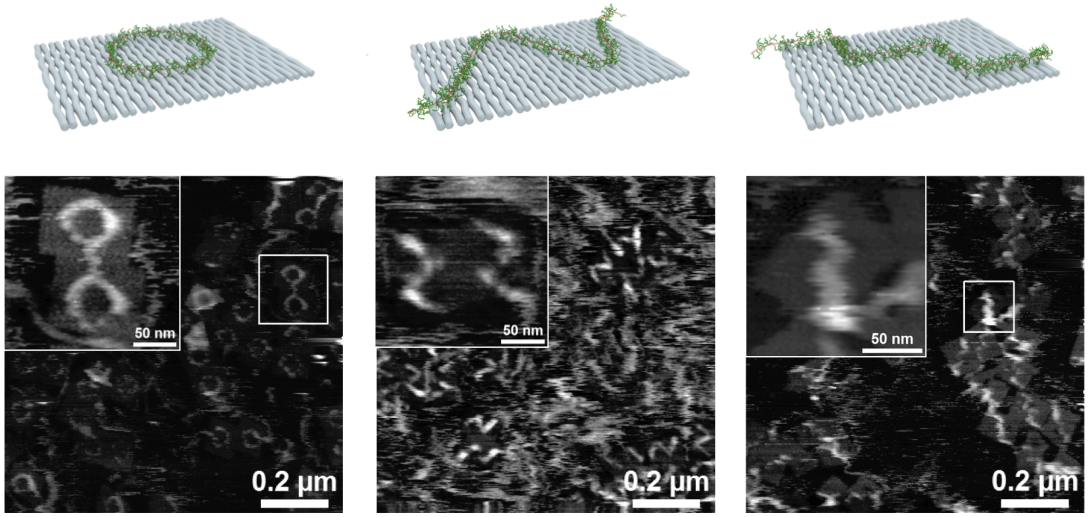
General procedure for AFM Scanning of DNA Origami

The APPV-DNA polymer binding to the DNA origami was visualized by atomic force microscope (AFM) (Multimode VIII, Bruker). The purified DNA origami solution (2 µL) was deposited onto a freshly-cleaved mica surface, immediately followed by addition of diluted polymer solution (10 µl). After incubation (0.5-5 min.), sufficient TAE buffer (1x) with $MgCl_2$ (12.5 mM) was added for the AFM scanning. For some experiments the origami was allowed to immobilize first for 1 min. followed by addition of the APPV-DNA polymer (Fig. 3c and Supplementary Fig. 21).

Self-assembly of poly(APPV-DNA) on DNA origami



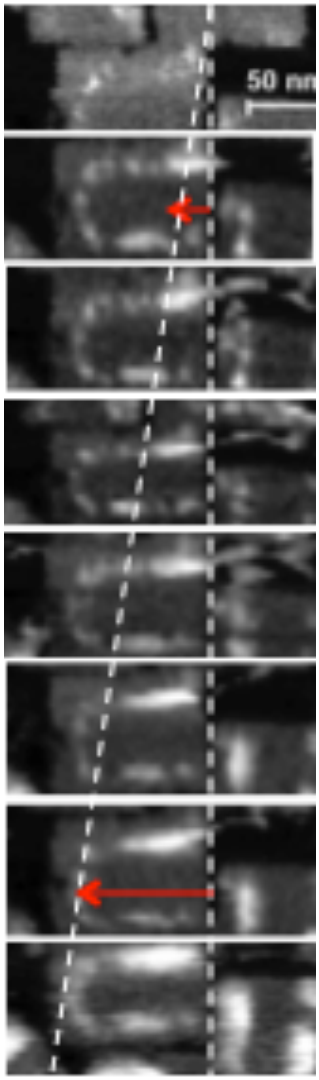
Supplementary Fig. 20. AFM images of poly(APPV-DNA) fr. 3 positioned on 2D DNA origami rectangles showing full control of the positioning of poly(APPV-DNA) on DNA origami depending on DNA sequence. a) Plain origami with two linear lanes of extended staple strand sequences: ODN1* (Black in inset) and ODN2 (Blue in inset). The lane closest to the origami edge is complementary to poly(APPV-DNA)-ODN1, while he lane closer to the middle is not complementary to poly(APPV-DNA)-ODN1. Faint lines of the ssDNA extensions are observed. b) Poly(APPV-DNA) ODN1 is hybridized to the complementary lane closest to the edge and lines of polymer are only observed on the line closest to the edge.



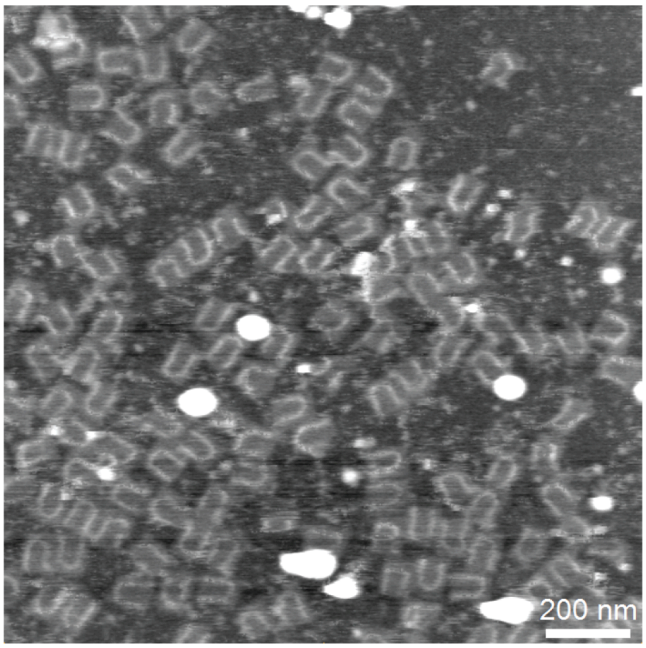
Supplementary Fig. 21. Illustrations of patterns and AFM images of poly(APPV-DNA) fr. 3 positioned on 2D DNA origami rectangles in circular, wave and staircase line pattern.

High speed AFM imaging

The assembly process of the APPV-DNA polymer binding to the DNA origami was monitored via high speed AFM (Fast-Scan, Bruker). The purified DNA origami solution of 2 µL was deposited onto a freshly-cleaved mica surface, and after incubation (around 30 seconds), 100 µL TAE buffer (1x) with $MgCl_2$ (12.5 mM) was added. Next, 2 µL poly(APPV-DNA) fr. 3 solution (30 µM ssDNA on the polymer) was carefully placed on the top of a fast-scan tip, which was then rapidly lowered onto the mica surface. Images where then captured sequentially.



Supplementary Fig. 22. Direct visualization of the dynamic assembly process for the poly(APPV-DNA) fr. 3 on the DNA origami. The AFM images were recorded in up to ~21 second per image and a wait-time of around 1-3 minutes between selected images. As shown by the red arrows, it's clear to see a polymer was initially attaching on the edge of the DNA origami. Then, the polymer was gradually zippering up on the DNA origami along the extended staple strand path.



Supplementary Fig. 23. Immobilization of poly(APPV-DNA) fr. 3 on DNA origami containing the U-shaped pattern.

3D DNA-PAINT super-resolution imaging of the APPV-DNA polymer

Optical setup

Fluorescence imaging was carried out on an inverted Nikon Eclipse Ti microscope (Nikon Instruments, Melville, NY) with the Perfect Focus System, applying an objective-type TIRF configuration using a Nikon TIRF illuminator with an oil-immersion objective (CFI Apo TIRF 100×, NA 1.49, Oil). A total magnification of 100× was used, yielding a pixel-size of 160 nm. A 561 nm laser (200 mW nominal, Coherent Sapphire) was used for excitation. The laser beam was passed through a cleanup filter (ZET561/10, Chroma Technology, Bellows Falls, VT) and coupled into the microscope objective using a beam splitter (ZT561rdc, Chroma Technology). Fluorescence light was spectrally filtered with an emission filter (ET600/50m, Chroma Technology) and imaged on an EMCCD camera (iXon X3 DU-897, Andor Technologies, North Ireland).

Super-resolution DNA-PAINT imaging

For sample preparation, a piece of coverslip (No. 1.5, 18 mm × 18 mm, ≈0.17 mm thick) and a glass slide (3" × 1", 1 mm thick) were sandwiched together by two strips of double-sided tape to form a flow chamber with inner volume of ≈5 µl. First, 20 µl of biotin-labeled bovine serum albumin (1 mg/ml, dissolved in buffer A) was flown into the chamber and incubated for 2 min. The chamber was then washed using 40 µl of buffer A. 20 µl of streptavidin (0.5 mg/ml, dissolved in buffer A) was then flown through the chamber and allowed to bind for 2 min. After washing with 40 µl of buffer A and subsequently with 40 µl of buffer B. To increase the density of structures on the surface, the chamber was incubated with the undiluted biotin-labeled DNA structures three times and incubated for 5 min each time. The chamber was washed using 20 µl of buffer B.

Direct imaging of DNA Origami structures. The final imaging buffer solution contained 2 nM Cy3b-labeled imager strand in buffer B with Trolox, PCA and PCD. The chamber was sealed with epoxy before imaging. Imaging was performed under the following conditions: The CCD readout bandwidth was set to 1 MHz at 16 bit and 5.1 pre-amp gain. No EM gain was used. 15000 frames were acquired with an integration time of 300 ms. The Nikon N-STROM package, including a cylindrical astigmatism lens, was used to acquire 3D images.

DNA-PAINT of polymer on DNA origami structures. To the chamber containing biotinylated DNA nanostructures the purified polymer was added at a 1:40 dilution and incubated for 10 min. The chamber was washed with 20 µl of buffer B and the imaging buffer solution was added (1 nM Cy3b-labeled imager strand in buffer B with Trolox, PCA and PCD). The chamber was sealed with epoxy before imaging. Imaging conditions as described in the paragraph above.

Super-resolution reconstruction was performed using the N-STORM package and a custom MATLAB software. EMAN2[52] was used for EM-type class averaging of DNA-PAINT super-resolution images. DNA-PAINT localization data was binned with a bin size of 2.66 nm/px and imported in EM2 for subsequent classification, alignment, and averaging. Distance measurements using intensity line profiles on class-averaged datasets was performed with ImageJ, LabVIEW, and

Origin.

3D Visualization and helix fitting software

An x-y-projection of the helix is used to fit an ellipse to the localizations with a least square algorithm described by Fitzgibbon *et al*[53]. The orientation and radius of the ellipse determines the amplitude of a sinusoidal function that is fitted when projecting the point cloud sidewise in the YZ-Plane with a nonlinear least square model. Accordingly, a projection in the XZ-Plane is fitted with a sinusoidal function with phase shift of π/2 (hence a cosinusoidal function). The height of the helix is calculated as the difference of the mean values of the minimum and maximum z position of the sinusoidal functions with a length of 2π. The fit values of the sinusoidal and cosinusoidal function determine the helix as it may be described in Cartesian Coordinates as [X,Y,Z] = [a*cos(t),b*sin(t),t]

DNA-PAINT sequences.

Docking strand on DNA Origami: 5' – ATCCATTAT – staple

Imager strand sequence for polymer imaging: 5' – GATCCATTAT – Cy3b

Imager strand sequence for guide strand (origami) imaging: Cy3b – ATAATGGATC – 3'

Polymer sequence for Poly(APPV-DNA-)-PAINT experiments: 5' TTAGGTAAAG-polymer (10 nt)-ATAATGGAT-Polymer

Trolox, PCA and PCD preparation.

Trolox was prepared as 100x stock solution with 100mg Trolox, 430 µl 100% methanol, 3.2 ml H2O, 345 µl 1 N NaOH.

PCA was prepared as 40x stock solution with 154 mg PCA in 10 ml H_2O. NaOH was used to adjust

pH = 9.0.

PCD was prepared as 100x stock solution with 9.3 mg PCD in 13.3 ml buffer (50% glycerol in 50 mM KCl, 1 mM EDTA and 100 mM Tris-HCl pH 8).

Stocks were stored at -20C. To prepare imaging buffers, stock solutions were brought to 1x by dilution in buffer B.

Buffers.

Buffer A (10 mM Tris-HCl, 100 mM NaCl, 0.05 % Tween-20, pH 7.5).

Buffer B (5 mM Tris-HCl, 10 mM MgCl2, 1 mM EDTA, 0.05 % Tween-20, pH 8).

DNA sequences and DNA origami design

DNA-polymer sequences:

poly(APPV-DNA)-ODN1: 5’ TCATCTAAC-polymer

ODN1*: 5’ GTTAGATGA 3’

ODN2: 5’ TTATGCACC 3’

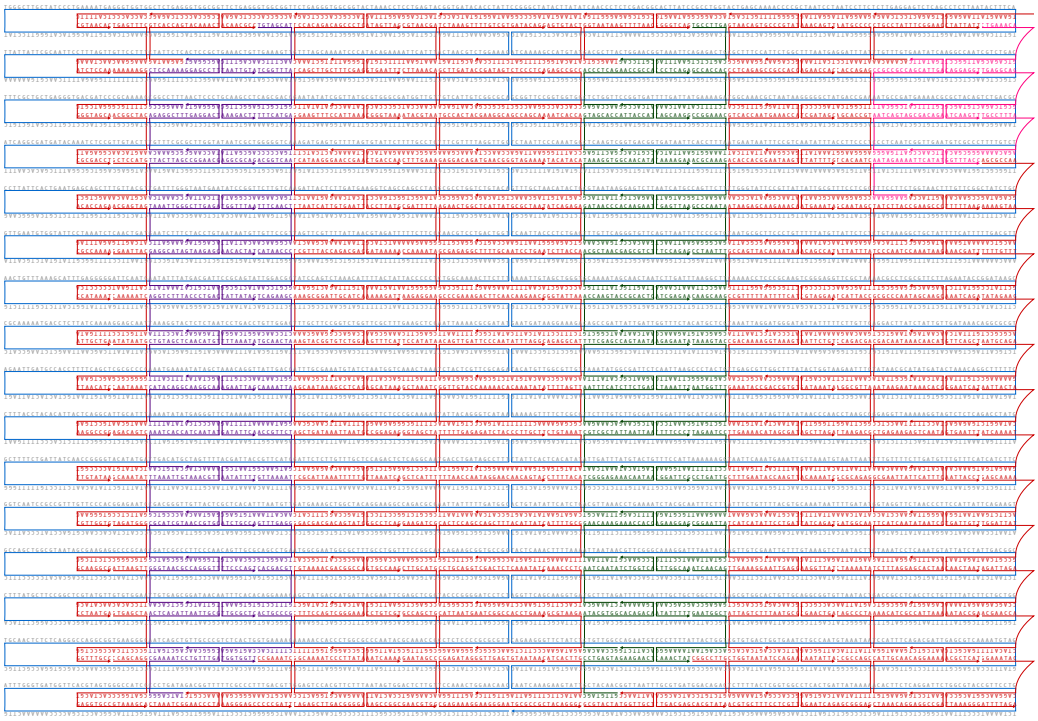
ODN3: 5’ CTGTCTCGT 3’

Poly(APPV-DNA)-PAINT: 5'-ATAATGGAT-Polymer

Poly(APPV-DNA)-FRET: 5’-ACGAGACAG-Polymer

The poly(APPV-DNA)-ODN1 was used for all experiments except the DNA PAINT on a 3D nanostructure and the FRET on DNA-origami experiments. ODN1*, ODN2 and ODN3 are used as 5’ staple strand extensions for 2D DNA origami experiments. A 3T spacer is incorporated between the origami and the extension. Poly(APPV-DNA)-ODN1 binds to ODN1* and ODN2 serves as a sequence specificity reference. Poly(APPV-DNA)-FRET binds to ODN3.

Origami with two linear paths:



Purple line sequences:

GTTAGATGATTTCCAAATCATTACTTAGCCGGAACGTACCAAGC

GTTAGATGATTTTTTCATTTCTGTAGCTCAACATGTTTAGAGAG

GTTAGATGATTTGCGAAACAAGAGGCTTTGAGGACTAGGGAGTT

GTTAGATGATTTCAAAATTAGGATAAAAATTTTTAGGATATTCA

GTTAGATGATTTGTTTGAGGTCAGGCTGCGCAACTGTTCCCAGT

GTTAGATGATTTTTTCAACTACGGAACAACATTATTAACACTAT

GTTAGATGATTTCACGACGTGTTTCCTGTGTGAAATTTGCGCTC

GTTAGATGATTTTGTAGCATAACTTTCAACAGTTTCTAATTGTA

GTTAGATGATTTCATAACCCGCGTCCAATACTGCGGTATTATAG

GTTAGATGATTTTGCAACTAGGTCAATAACCTGTTTAGAATTAG

GTTAGATGATTTAAAGGCCGCTCCAAAAGGAGCCTTAGCGGAGT

GTTAGATGATTTTTTCATGATGACCCCCAGCGATTAAGGCGCAG

GTTAGATGATTTGGCGATCGCGCATCGTAACCGTGCGAGTAACA

GTTAGATGATTTACCCGTCGTTAAATTGTAAACGTTAAAACTAG

GTTAGATGATTTTATATTTTCATACAGGCAAGGCAAAGCTATAT

GTTAGATGATTTAGCTGATTACTCACATTAATTGCGTGTTATCC

GTTAGATGATTTTACCTTTAAGGTCTTTACCCTGACAATCGTCA

GTTAGATGATTTACGGTCAATGACAAGAACCGGATATGGTTTAA

GTTAGATGATTTGTTAAAATAACATTAAATGTGAGCATCTGCCA

GTTAGATGATTTAAAGATTCTAAATTGGGCTTGAGATTCATTAC

GTTAGATGATTTTCAGAAGCCTCCAACAGGTCAGGATTTAAATA

GTTAGATGATTTACCGTTCTGATGAACGGTAATCGTAATATTTT

GTTAGATGATTTACTGCCCGCTTTTCACCAGTGAGATGGTGGTT

GTTAGATGATTTTATCAGGGCGAAAATCCTGTTTGACGGGCAAC

GTTAGATGATTTTCGGTTTAGGTCGCTGAGGCTTGCAAAGACTT

GTTAGATGATTTGCTCACAAGGGTAACGCCAGGGTTTTGGGAAG

GTTAGATGATTTCATGTCAAAAATCACCATCAATATAACCCTCA

GTTAGATGATTTTAAATATTGAGGCATAGTAAGAGCACAGGTAG

Green line sequences:

TTATGCACCTTTATCAACAGGAGAGCCAGCAGCAAAATATTTTT

TTATGCACCTTTCCTGATTGAAAGAAATTGCGTAGAAGAAGGAG

TTATGCACCTTTGAATGGCTACCAGTAATAAAAGGGCAAACTAT

TTATGCACCTTTTGCCTTGACAGTCTCTGAATTTACCCCTCAGA

TTATGCACCTTTCCTAATTTAAGCCTTAAATCAAGAATCGAGAA

TTATGCACCTTTTAAAGTACCAGTAGGGCTTAATTGCTAAATTT

TTATGCACCTTTTTTAACGTTCGGGAGAAACAATAACAGTACAT

TTATGCACCTTTTGAACAAAGATAACCCACAAGAATAAGACTCC

TTATGCACCTTTCATATTTATTTCGAGCCAGTAATAAATCAATA

TTATGCACCTTTTAGAATCCCCTTTTTTAATGGAAACGGATTCG

TTATGCACCTTTAAATCAATCGTCGCTATTAATTAAATCGCAAG

TTATGCACCTTTCGGAATTACGTATTAAATCCTTTGGTTGGCAA

TTATGCACCTTTAATGGTTTTGCTGATGCAAATCCATTTTCCCT

TTATGCACCTTTGCCACCACTCTTTTCATAATCAAATAGCAAGG

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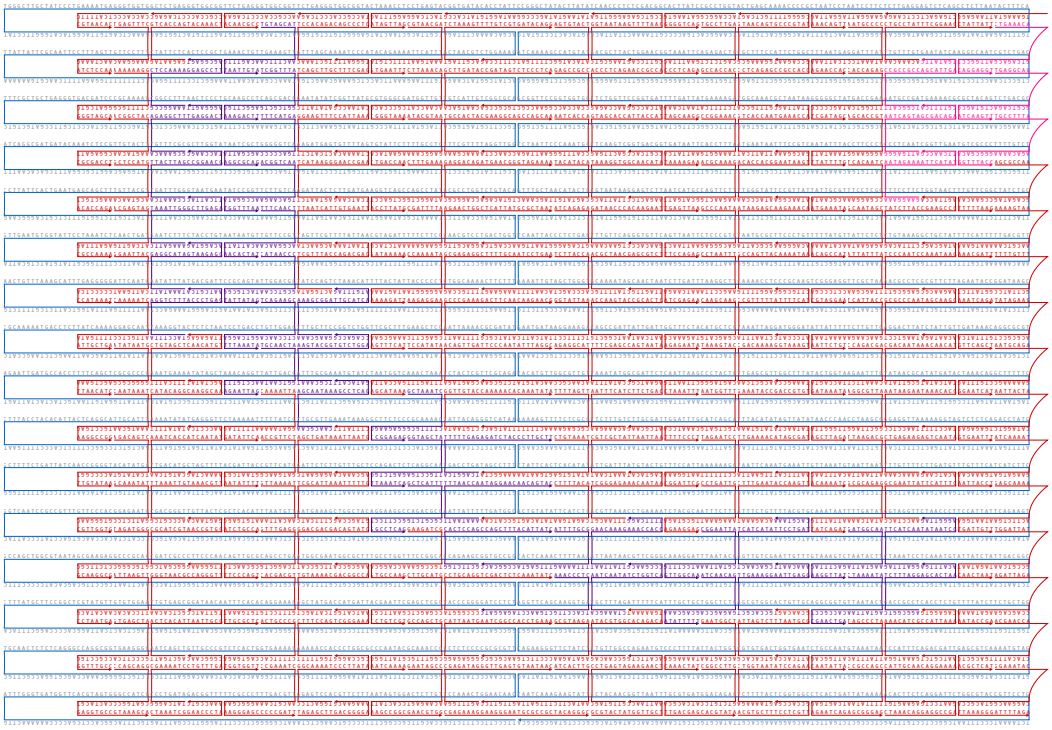
Origami with 90 degree curve:



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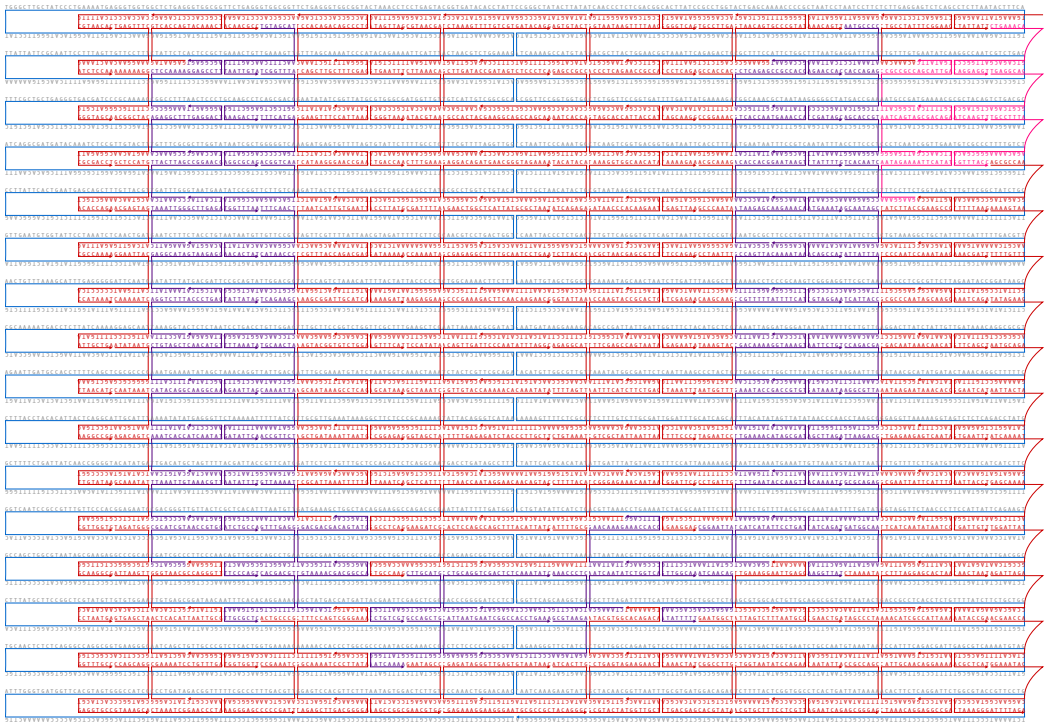
Origami with 180 degree curve:



Purple 180 degree curve sequences:

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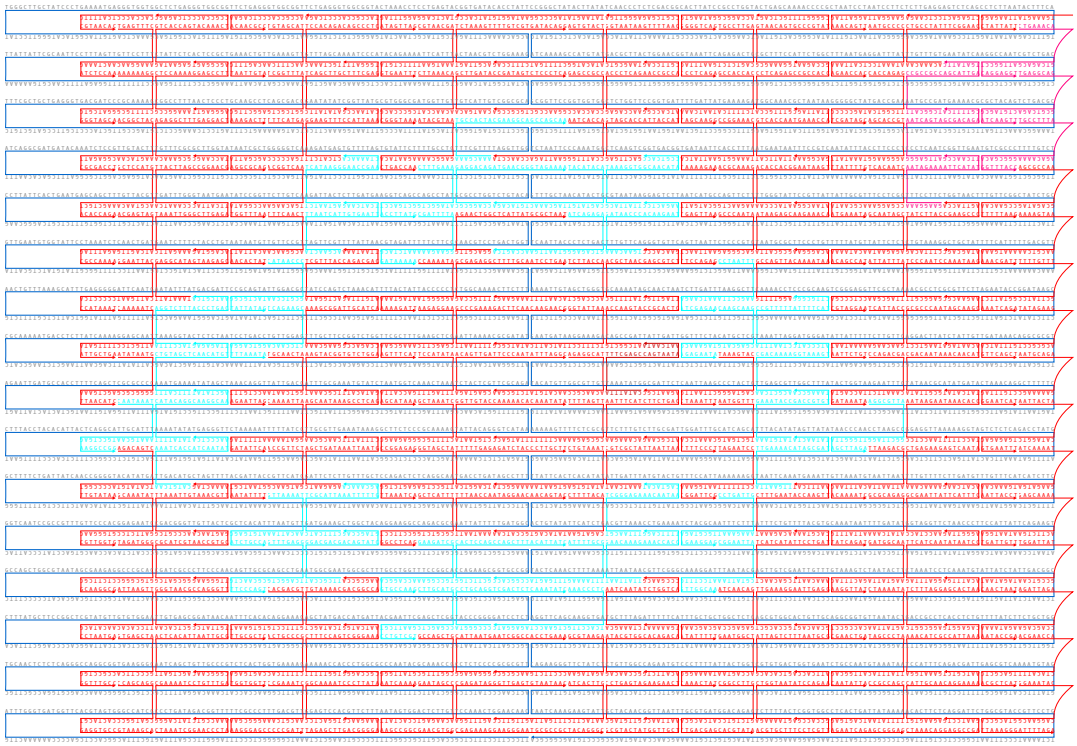

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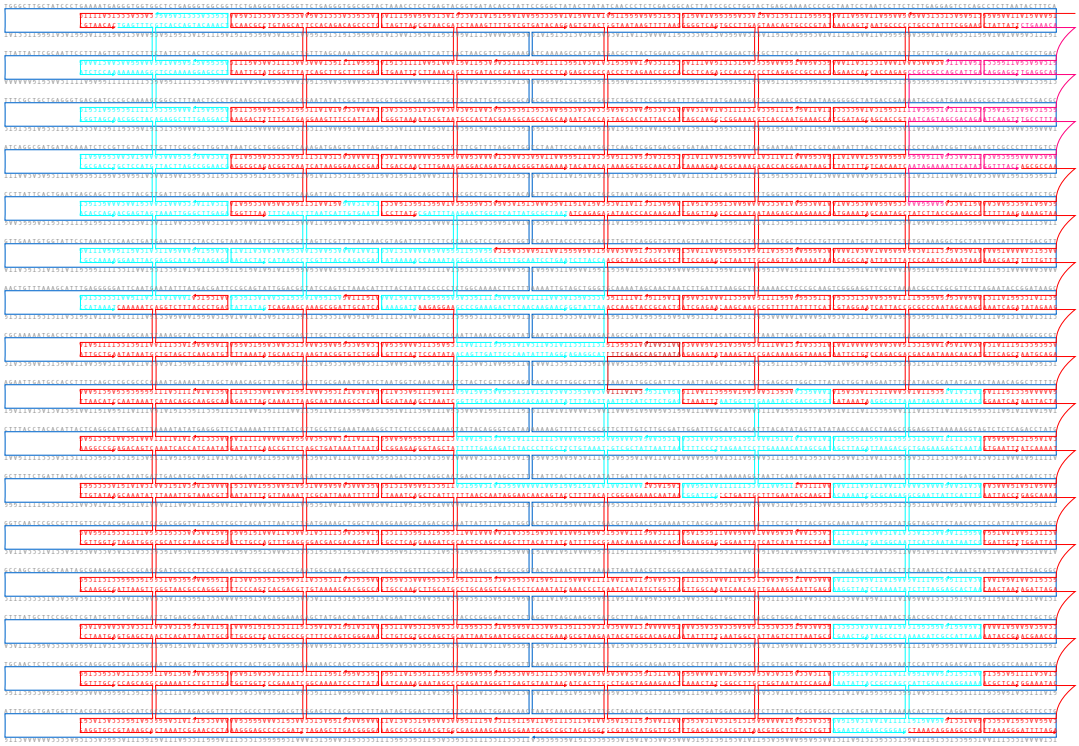
Origami with staircase pattern:



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Turquoise zigzag pattern sequences:

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TGGACTCCGGCAAAATCCCTTATACGCCAGGG

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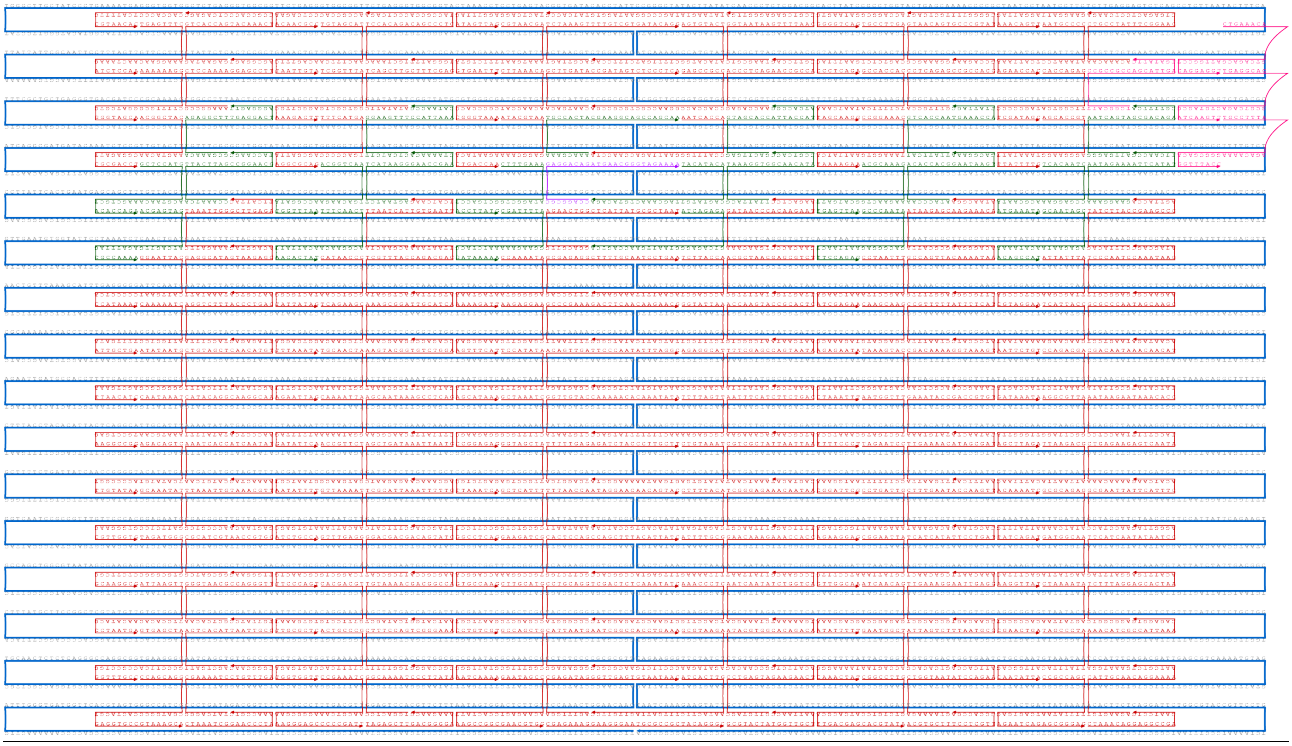
GATGGCAAAAGTATTAGACTTTACAAGGTTAT

Origami for FRET experiment with polymer line and one Alexa647 fluorophore:



Polymer binding extended staple strands (Green in figure):

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CTGTCTCGTTTTCTCATCTTGGAAGTTTCCATTAAACATAACCG

CTGTCTCGTTTTAAACGAAATGCCACTACGAAGGCAGCCAGCAA

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CTGTCTCGTTTTTTATTCATGTCACCAATGAAACCATTATTAGC

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CTGTCTCGTTTTGCAATAGCAGAGAATAACATAAAAACAGCCAT

Index sequences:

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TGAGGCAGTTTTGCGTCAGACTGTAGCGATCAAGTT

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TGCCTTTATTTTAGACAAAAGGGCGACAGGTTTACC

Acceptor strand:

CTGTCTCGT(c6-N-Alexa647)TTTCCAGGCGCGAGGACAGATGAACGGGTAGAAAA

Additional sequences:

CACCACCCTCATTTTCCGTAACAC

ACCGCCACCCTCAGAAACAACGCC

TACTCAGGAGGTTTAGATAGTTAG

TATAAGTATAGCCCGGAATAGGTGTATCACCG

ACCAGGCGGATAAGTGGGGGTCAG

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GGAAAGCGGTAACAGTGCCCGTATCGGGGTTTTGCTCAGT

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AAAAAAGGCTTTTGCGGGATCGTCGGGTAGCA

TCGGTTTAGGTCGCTGAGGCTTGCAAAGACTT

CTTAAACAACAACCATCGCCCACGCGGGTAAA

GAGCCGCCCCACCACCGGAACCGCTGCGCCGA

GCCACCACTCTTTTCATAATCAAATAGCAAGG

CACCAGAGTTCGGTCATAGCCCCCTCGATAGC

AAAGGCCGCTCCAAAAGGAGCCTTAGCGGAGT

ATATATTCTCAGCTTGCTTTCGAGTGGGATTT

CAATGACAGCTTGATACCGATAGTCTCCCTCA

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GTTTGCCACCTCAGAGCCGCCACCGCCAGAAT

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TTTCATGATGACCCCCAGCGATTAAGGCGCAG

ATACGTAAGAGGCAAAAGAATACACTGACCAA

AATCACCACCATTTGGGAATTAGACCAACCTA

CCGGAAACTAAAGGTGAATTATCATAAAAGAA

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GAAGATCGTGCCGGAAACCAGGCAGTGCCAAG

ATTTTGCGTTTAAAAGTTTGAGTACCGGCACC

CGGAATTACGTATTAAATCCTTTGGTTGGCAA

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CACGACGTGTTTCCTGTGTGAAATTTGCGCTC

CTTGCATGCCGAGCTCGAATTCGTCCTGTCGT

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GCGTAAGAAGATAGAACCCTTCTGAACGCGCG

GAATGGCTACCAGTAATAAAAGGGCAAACTAT

TAGCCCTATTATTTACATTGGCAGCAATATTA

AGCTGATTACTCACATTAATTGCGTGTTATCC

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GGGAGAGGCATTAATGAATCGGCCACCTGAAA

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GTCACACGATTAGTCTTTAATGCGGCAACAGT

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CCAGCAGGCGATGGCCCACTACGTGAGGTGCCGTAAAGCA

CCGAAATCAACGTCAAAGGGCGAAAAGGGAGCCCCCGATT

GAATAGCCACAAGAGTCCACTATTAAGCCGGCGAACGTGG

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TGGACTCCGGCAAAATCCCTTATACGCCAGGG

AGTTTGGACGAGATAGGGTTGAGTGTAATAAC

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GTAAAAGACTGGTAATATCCAGAAATTCACCA

AGAAGTGTCATTGCAACAGGAAAAAATCGTCT

CTAAATCGGAACCCTAAAACCGTC

TAGAGCTTGACGGGGAAAAGAACG

CGAGAAAGGAAGGGAATGCGCCGCTACAGGGC

GCGTACTATGGTTGCTAATTAACC

ACGTGCTTTCCTCGTTGCCACCGA

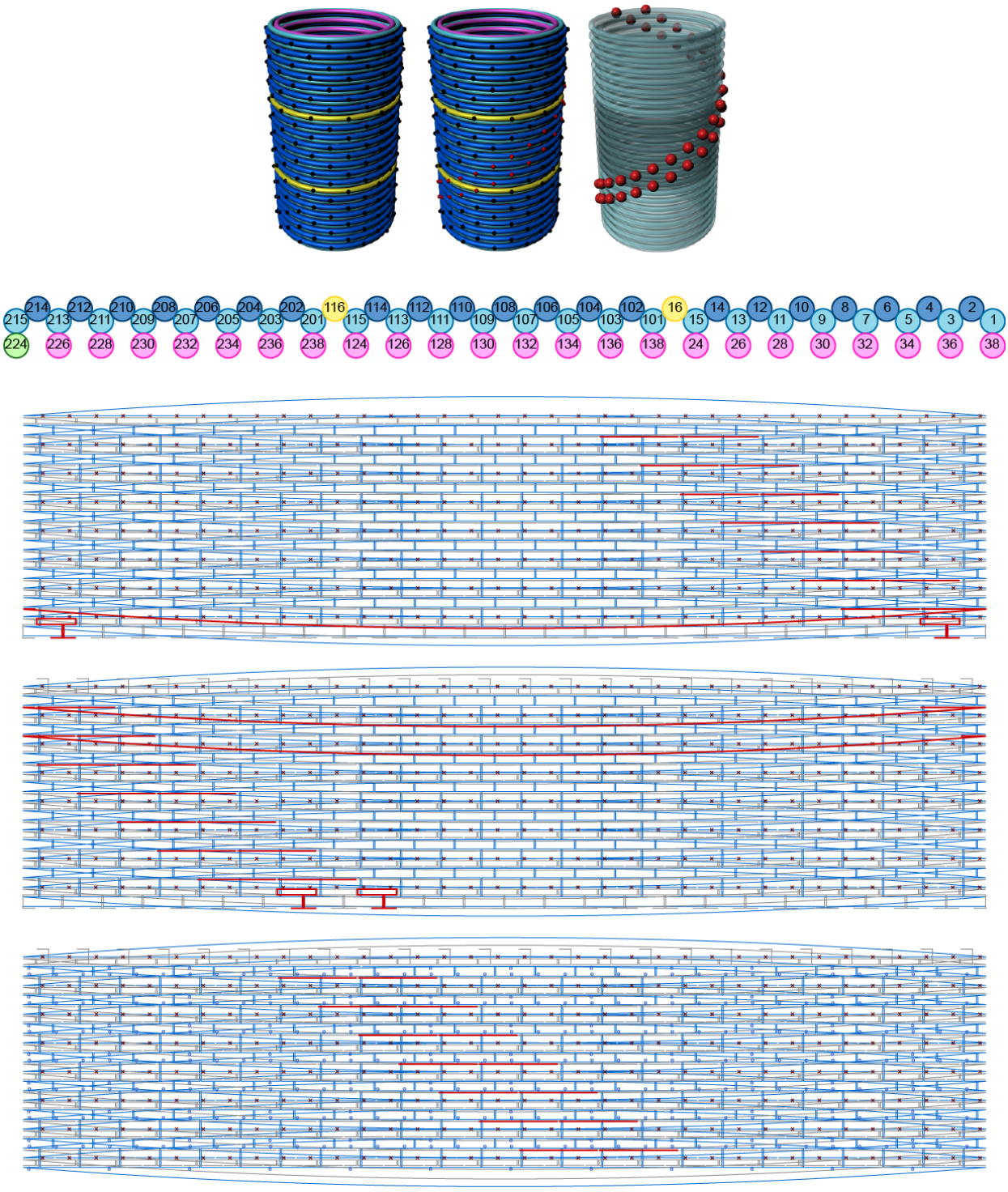
CTAAACAGGAGGCCGAGAATCCTG

3D DNA origami

3D Origami annealing and assembly protocol

Monomers of the three-dimensional DNA origami cylinder were annealed following the method from Douglas et al[38].. The 8064-nt scaffold (10 nM) was mixed with unpurified staple strands (100 nM) in 5 mM Tris, 1 mM EDTA, pH 8.0 with $MgCl_2$ (10 mM), and annealed (Ramp: 80 to 65 °C over 15 min. and 65 to 20 °C over 18 hours.). After annealing, monomers were purified by agarose gel electrophoresis. Trimers were assembled by combining monomers in equimolar ratio in TAE buffer (1x) with $MgCl_2$ (20 mM), and annealing from 40 to 20 °C over 12 hours.

3D DNA Origami cylinder with helical path:



Supplementary Fig. 24. Schematic illustration of the cylindrical DNA origami structure with the

connector strands between the individual origami rings shown in yellow and the handle strands for DNA-PAINT in red. The design and sequence routing of the three cylinders is shown in the lower four panels. The details of the design and the sequences will be published separately.

Legends for supplementary videos

Supplementary Movie 1 Origami. Animated 3D DNA-PAINT super-resolution images of a single DNA structure (color indicates height). Localizations are shown along with a helical fit (red line).

Supplementary Movie 2 Polymer. Animated 3D DNA-PAINT super-resolution images of a single polymer bound to a DNA structure (color indicates height). Localizations are shown along with a helical fit (red line).

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