DNA Multiblock Copolymers

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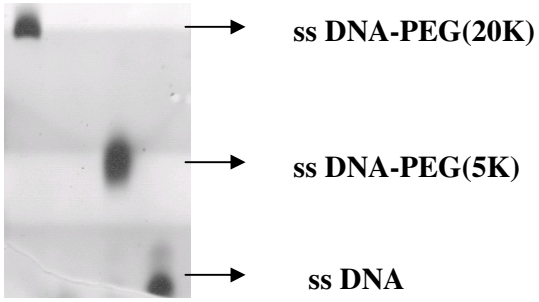
I. General Considerations

Unless otherwise stated, materials were obtained from commercial suppliers and were used without further purification. The poly(ethyleneglycol) (PEG), N-diisopropyl-2-cyanoethyl-chlorophosphoramidite, diisopropylethylamine were purchased from Aldrich. Succinimide activated carboxy-terminated PEGs were obtained from Nektar (USA). The dimethoxytrityl (DMTr) protected phosphoramadites were purchased from Link Technologies (UK) or SAFC (Germany). ss DNA-PEG-ss DNA triblock copolymers were synthesized using AKTA Oligopilot DNA synthesizer (Amersham Biosciences, Sweden). Tetramethylenesilane and triphenylphosphine were used as the references for the ^{1}H NMR and ^{31}P NMR spectra, respectively. The spectra were recorded on Bruker AMX 250 (250MHz) or DRX 500 (500 MHz) spectrometer. Molecular weights were determined using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) using 3-hydroxypicolinic acid as the matrix. The spectra were recorded on a Bruker MALDI-TOF (Reflex-TOF) mass spectrometer. HPLC analysis and purifications were performed on an Äkta Purifier (Amersham Biosciences, Sweden) using a C-18 column with UV detection at 260 nm. In all experiments, MilliQ standard water (Millipore Inc., USA) with a typical resistivity of 18.2 MΩ/cm was used. Oligonucleotides were quantified spectrophotometrically at a wavelength of 260 nm (SpectraMax M2, Molecular Devices, USA) and by denaturing polyacrylamide gel electrophoresis (PAGE) followed by staining with ethidium bromide and UV transillumination. The densiometric quantification was done using GelPro programme distributed from Intas GmbH (Germany).

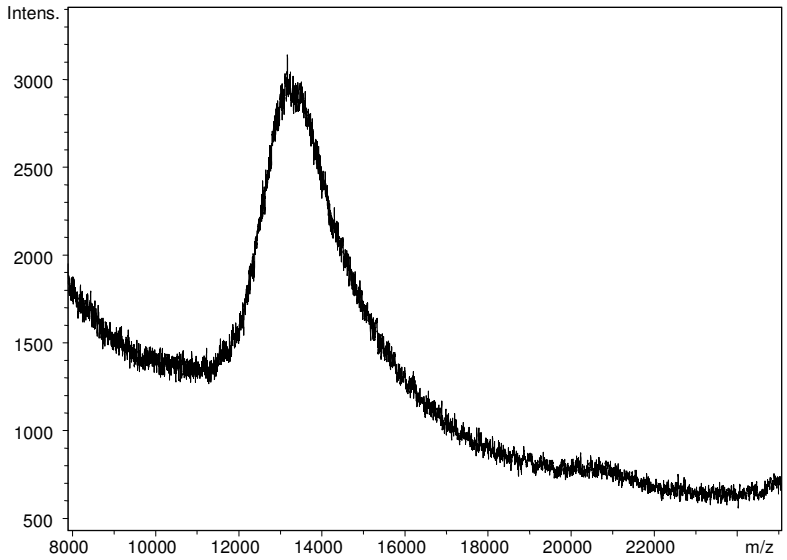
II. Synthesis of DNA-PEG Diblock Copolymers

The synthesis of DNA-PEG diblock copolymers was carried out by mixing 5'-amino-modified oligonucleotide (5'-TAACAGGATTAGCAGAGCGAGG-3', 22mer, Mw = 6950 g/mol) (1 µmol) with succinimide activated carboxy-terminated PEG (Mn = 5000 or 20000 g/mol) (5 µmol) in 500 µl 0.1 M sodiumtetraborate buffer (pH 8). The mixture was allowed to react for 2 days at room temperature. The block copolymer products were purified using 8 % denaturing PAGE. After excision of the bands, they were dialyzed against water for 24 hours. Subsequently, the DNA block copolymers were lyophilized yielding 0.6 µmol (60 %) ss DNA-*b*-PEG(5K) and 0.5 µmol (50 %) ss DNA-*b*-PEG(20K), respectively. Characterization of the products was carried out by PAGE, HPLC and MALDI-TOF mass spectrometry.

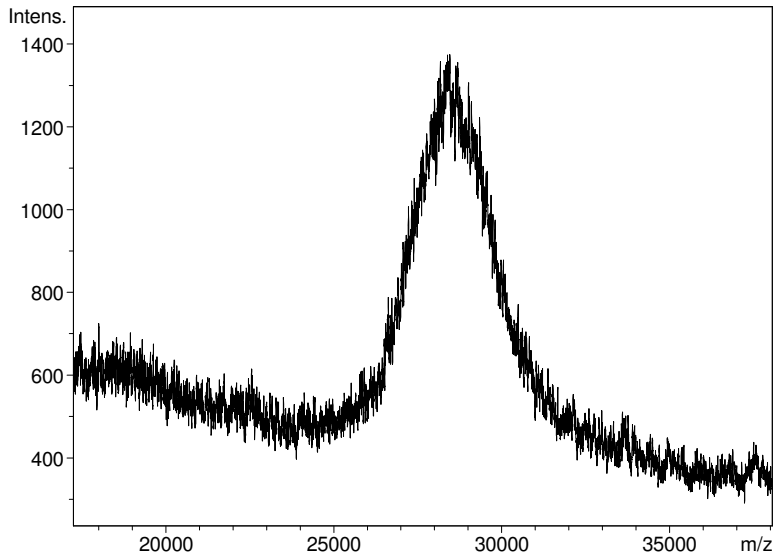


Supporting Figure 1: PAGE analysis of purified DNA diblock copolymers.

A



B

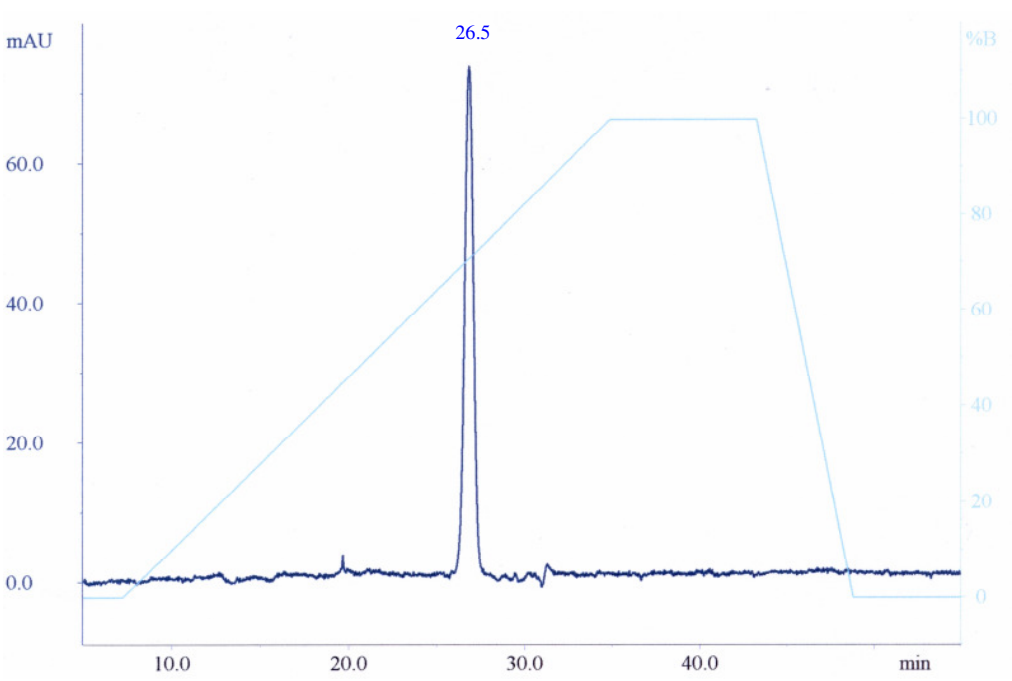


Supporting Figure 2: The MALDI-TOF spectrum of **(A)** ss DNA-PEG(5K) (found: 13200 g/mol, calculated: 13100 g/mol) and **(B)** ss DNA-PEG(20K) (found: 28300 g/mol, calculated: 28100 g/mol).

HPLC:

For the HPLC analysis the gradient was held constant for 7 min. at 0% **B** and then increased to 100% **B** in 42.5 min. with a flow rate of 1.0 ml/min. Eluent **A** was 0.1 M Triethylamine ammonium acetate (TEAAc pH: 7.0) and eluent **B** was 0.1 M TEAAc/ACN (20:80) mixture.

A



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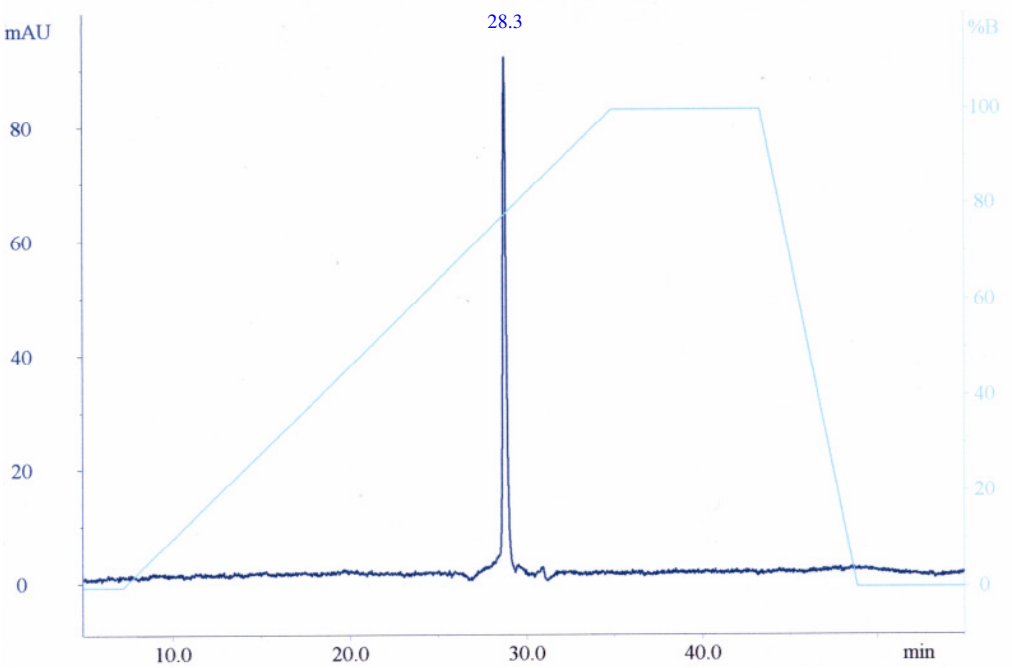
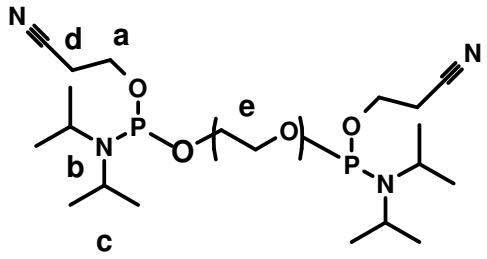


Supporting Figure 3. The HPLC elugrams represent **(A)** ss DNA-PEG(5K) and **(B)** ss DNA-PEG(20K). At 26.5 min. and 28.3 min. ss DNA-PEG(5K) and ss DNA-PEG(20K) elute, respectively.

III. Synthesis of Phosphoramidite Functionalized Polymer



Poly(ethyleneglycol) with a molecular weight of 1.000 g/mol (1.0 mmoles, 1.0 g) was dissolved in dry THF and reacted with N-diisopropyl-2-cyanoethyl-chlorophosphoramidite (2.10 mmol, 500 mg) in the presence of diisopropylethylamine at room temperature under argon atmosphere for 2h. It was then filtered and then the filtrate was dried under high vacuum. (Yield: 99%)

^{31}P NMR (200 MHz, THF): 144.1, 144.7 ppm

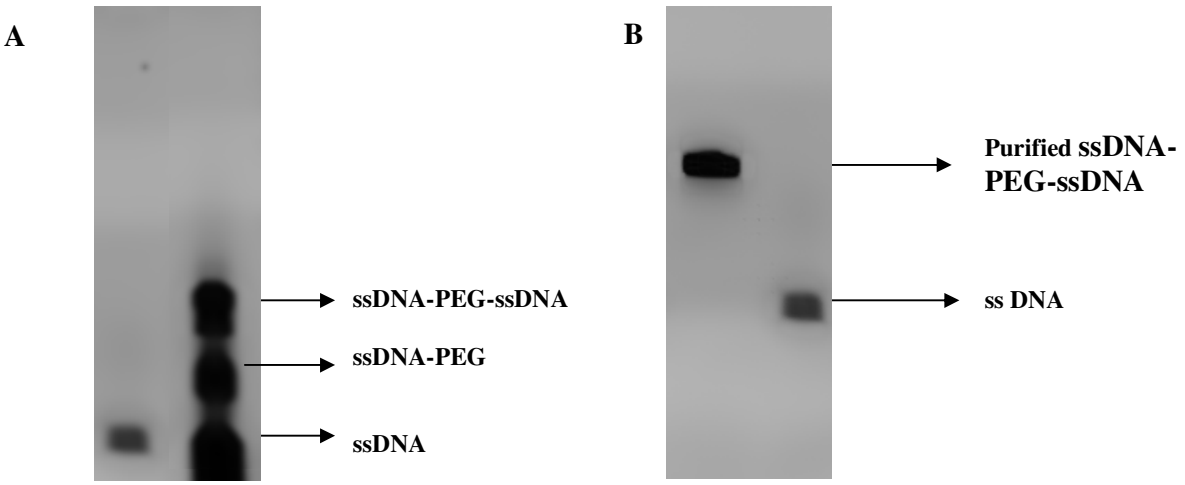
^{1}H-NMR (500 MHz, $CDCl_3$): 1.10 (d, 24H, **c**), 2.65 (t, 2H, **d**), 3.04 (m, 2H, **b**), 3.60-3.86 (b, 105H, **e**), 3.94 (t, 2H, **a**),

^{13}C-NMR (125MHz, $CDCl_3$): 20.2, 23.6, 23.9, 44.3, 59.1, 65.1, 65.6, 116.6, 117.5

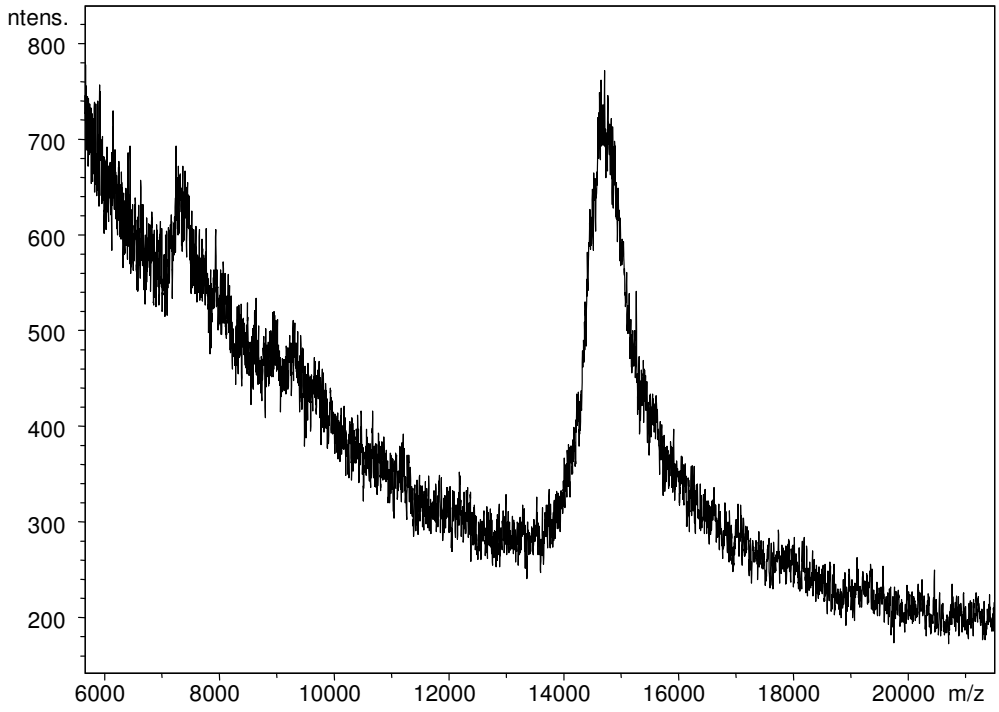
IV. Synthesis of DNA ssDNA-PEG-ssDNA

5'-CCTCGCTCTGCTAATCCTGTTA-3' (22mer, Mw = 6780 g/mol) was synthesized in 120 micromole scale using a standard phosphoramidite DNA synthesis protocol.[1] Phosphoramidite functionalized polymer was dissolved in dry dichloromethane and attached to the 5' ends of the sequence by an optimized coupling procedure.[2] After that, **ss DNA-PEG-ss DNA** was liberated from the solid support using concentrated ammonia for 16 h accompanied by deprotection of the bases. The solid support was removed by filtering and was then washed with an ethanol/water mixture. After evaporation of the solvent the conjugate was purified by denaturing PAGE, filtered and desalted. Finally, the product was analyzed by PAGE followed by ethidium bromide staining and MALDI-TOF mass spectrometry. (Yield: 54 %)

MALDI-TOF MS: 15100 m/z



Supporting Figure 4. PAGE analysis of **(A)** crude reaction mixture and **(B)** the purified ss DNA-PEG-ss DNA.



Supporting Figure 5: MALDI-TOF mass spectrum of ss DNA-PEG-ss DNA (found: 15100 g/mol, calculated: 15100 g/mol).

V. Synthesis of Multiblock Architectures

General Hybridization Procedure

The hybridization was carried out by dissolving the desired stoichiometric quantities of ss entities in in TAE buffer (20 mM tris(hydroxymethyl)aminomethane-HCl, pH 8.0; 10 mM acetic acid, 0,5 mM EDTA) containing Na^+ (100 mM) and Mg^{2+} (20 mM). The mixture was heated to 95°C and was slowly cooled to room temperature over the course of 3 days (1 degree per hour) by using a polymerase chain reaction thermocycler (Biometra GmbH, Germany). The final concentration of DNA was between 2-5 μM.

DNA Sequences:

ss DNA-PEG-ss DNA: 5'- CCTCGCTCTGCTAATCCTGTTA-3'

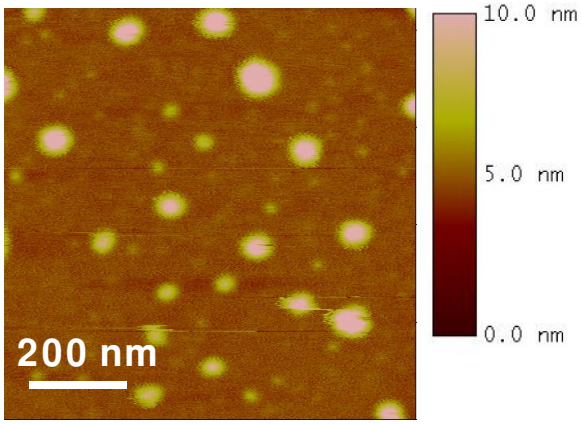
Complementary: 5'- TAACAGGATTAGCAGAGCGAGG -3'

VI. Scanning Force Microscopy (SFM) Measurements of DNA Triblock Copolymer Micelles

20 microliters of a 0.2 nM DNA-triblockcopolymer solution in dichloromethane were deposited onto silicon wafer (Si-Mat-Silicon Materials, Landsberg am Lech, Germany). After evaporation of the solvent, the sample was mounted in the SFM.

The images were recorded using a commercial SFM (Multimode, Nanoscope IIIa, Veeco Instruments, California, USA) in tapping mode in air with an E-scanner. Silicon cantilevers (OMCLAC 160 TS-W2, Olympus, Japan; 160 µm long, 50 µm wide, 4.6 µm thick) with resonance frequencies of ~300 kHz were used. The height of the tip was 7-15 µm, and the tip radius of curvature was < 10 nm.

SFM images (512×512 pixels) were recorded at a scan rate of 1 Hz. The raw data has been modified by applying the second order "flatten" filter. The maximum height of individual micelles was calculated by means of local roughness analysis. The maximum height of the micelles varied from 3 nm to 11 nm, and the diameter of the micelles varied from 15 nm to 77 nm.



Supporting Figure 5: SFM topographical image of DNA-triblockcopolymer PEG(20K)-DNA(22bp)-PEG(20K). The height is indicated with a color scale bar on the right. The z-scale in this image is 10 nm.

References:

1. M. H. Caruthers, *Accounts Chem. Res.*, 1991, **24**, 278-284.
2. F. E. Alemdaroglu, K. Ding, R. Berger and A. Herrmann, *Angew. Chem. Int. Ed.*, 2006, **45**, 4206-4210.