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# Generation of Multiblock Copolymers by PCR:

## Synthesis, Visualization and Nanomechanical Properties

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## I. General Considerations

Unless otherwise stated, materials were obtained from commercial suppliers and were used without further purification. Poly(ethyleneglycol) (PEG), N-diisopropyl-2-cyanoethyl-chlorophosphoramidite, diisopropylethylamine were purchased from Aldrich. Carboxy-terminated PEGs were obtained from Nektar (USA). The dimethoxytrityl (DMTr) protected phosphoramidites 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  $^1\text{H}$  NMR and  $^{31}\text{P}$  NMR spectra, respectively. The spectra were recorded on Bruker AMX 250 (250MHz) or DRX 500 (500 MHz) spectrometers. Molecular weights were determined using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) employing 3-hydroxypicolinic acid as the matrix. The spectra were recorded on a Bruker MALDI-TOF (Reflex-TOF) mass spectrometer. In all experiments, MilliQ standard water (Millipore Inc., USA) with a typical resistivity of 18.2 M $\Omega$ /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 densitometric quantification was done using GelPro programme distributed from Intas GmbH (Germany). The PCR reactions were carried out in a MyCycler™ Thermal Cycler from Bio-RAD (USA). The PCR products were analyzed by agarose gel electrophoresis using ethidium bromide staining and transillumination.

## II. Synthesis of ds DNA tri- and pentablock copolymers

### The Sequences

Id.	DNA sequence 5'-3'-direction
<b>ssTB1</b>	CCTCGCTCTGCTAATCCTGTTA
<b>ssDB1</b>	TCAGTTCGGTGTAGGTC
<b>ssDB2</b>	TCCGCCTTTCTCCCT TC
<b>ssDB3</b>	GCTCACTCAAAGGCGGTAAT

### Synthesis of ds DNA triblock copolymers

Double stranded (ds) DNA triblock copolymers were synthesized by a modified PCR procedure employing the DNA triblock copolymer (ssTB1) and a conventional oligonucleotide as primers. A total of 200 µl PCR reaction mixture containing 0.5 mM dNTPs, 1 U Taq DNA polymerase, 50 pg plasmid DNA pBR322, 1 µM forward and backward primers, PCR buffer (100 mM Tris-HCl, 500 mM KCl and 0.8% Nonidet P40), and 2-2.5 mM of magnesium chloride were subjected to thermal cycling (4 min at 95 °C and then 30 cycles of 30 sec at 95 °C for denaturation, 4 min at 55-59 °C for annealing and 30 sec-2 min at 72 °C for extension) in a thermocycler. The PCR amplified products were purified by QIAquick Gel Extraction Kit from Qiagen GmbH (Germany) using deionized water for eluting the amplicons. The PCR products were characterized by agarose gel electrophoresis.

### Synthesis of ds DNA pentablock copolymers

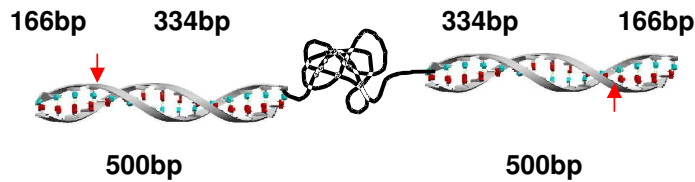
Double stranded DNA pentablock copolymers were synthesized by modified PCR protocols employing polymer functionalized primers (backward primer: ssTB1; forward primers: ssDB1, ssDB2 and ssDB3). PCR was carried out in 200 µl PCR reaction mixture containing 0.5 mM dNTPs, 4 U Taq DNA

polymerase, 200 pg plasmid DNA pBR322, 1  $\mu$ M forward and backward primers, PCR buffer (100 mM Tris-HCl (pH 8.8 at 25 °C), 500 mM KCl and 0.8% Nonidet P40), and magnesium chloride (2-3 mM). The PCR conditions were as follows: 95 °C, 4 min; (95 °C, 30 sec; 59 °C, 4 min; 72 °C, 1 min) / 30 cycles; 72 °C, 7 min. The amplified products were purified by electroelution into dialysis bags. The ds DNA pentablock copolymers were characterized by agarose gel electrophoresis.

### **Purification of ds DNA Pentablock Copolymers**

The purification was done according to the literature<sup>1</sup> with minor modifications. The procedure is detailed below. After the PCR reaction, the reaction mixture was run in a 1.5% agarose gel. By using a sharp scalpel a small slice of agarose gel containing the band of pentablock copolymers was cut out, and placed on a square of Parafilm wetted with 0.25x TBE. One end of a piece of dialysis tubing was sealed with a secure knot. The dialysis bag was filled with 0.25x TBE and the gel slice was transferred into the buffer-filled bag. The bag was immersed in a shallow layer of 0.25x TBE in a horizontal electrophoresis tank. The gel fragments should be maintained parallel to the electrodes. Electric current through the bag (7.5 V/cm) was passed for 45-60 minutes. By using a long-wavelength UV lamp the movement of the DNA fragment out of the gel slice was monitored. The polarity of the current was reversed for 20 seconds to release the DNA from the wall of the bag. After turning off the electric current the bag was recovered from the electrophoresis chamber. After the reverse electrophoresis, the buffer surrounding the gel slice was transferred to a plastic tube. The gel slice was removed from the bag and stained. It was examined by UV illumination to confirm that the entire pentablock copolymer has eluted. The product was then desalted by Microspin G25 Columns (Amersham Biosciences, Sweden).

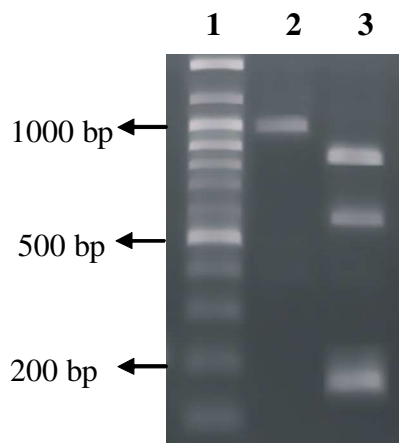
## Restriction endonuclease analysis of ds DNA tri- and pentablock copolymers



**Supporting Figure 1.** *The graphical representation of restriction endonuclease analysis of ds DNA triblock copolymer.*

Several DNA tri- and pentablock copolymers have been analyzed by a sequence specific restriction endonuclease. As an example, the restriction analysis of the triblock copolymer ds DNA(500bp)-*b*-PEG(1K)-*b*-DNA(500 bp) is described below.

The DNA triblock copolymer was digested by the enzyme AasI (DrdI) at 37 °C for 15 h in the reaction buffer containing 10 mM Tris-HCl, 10 mM MgCl<sub>2</sub> and 0.1 mg/ml BSA. The product was analyzed by agarose gel electrophoresis (**Supporting Figure 2**). Lanes 1 and 2 show the DNA ladder and the triblock copolymer, respectively. The restriction resulted in three different products which are shown in Lane 3. The band with the lowest electrophoretic mobility represents the triblock hybrid digested once. Other restriction products are the copolymer digested twice with an intermediate mobility and the nucleic acid segment of 166 bp with the highest mobility.



**Supporting Figure 2.** Gel analysis of the restriction of a DNA triblock copolymer. Lane 1: DNA Ladder (10000-100 bp). Lane 2: ds DNA triblock copolymer with 500 bp nucleic acid segments. Lane 3: The endonuclease restriction products of the DNA triblock copolymer.

### III. SFM Measurements of DNA Block Copolymers in Buffer

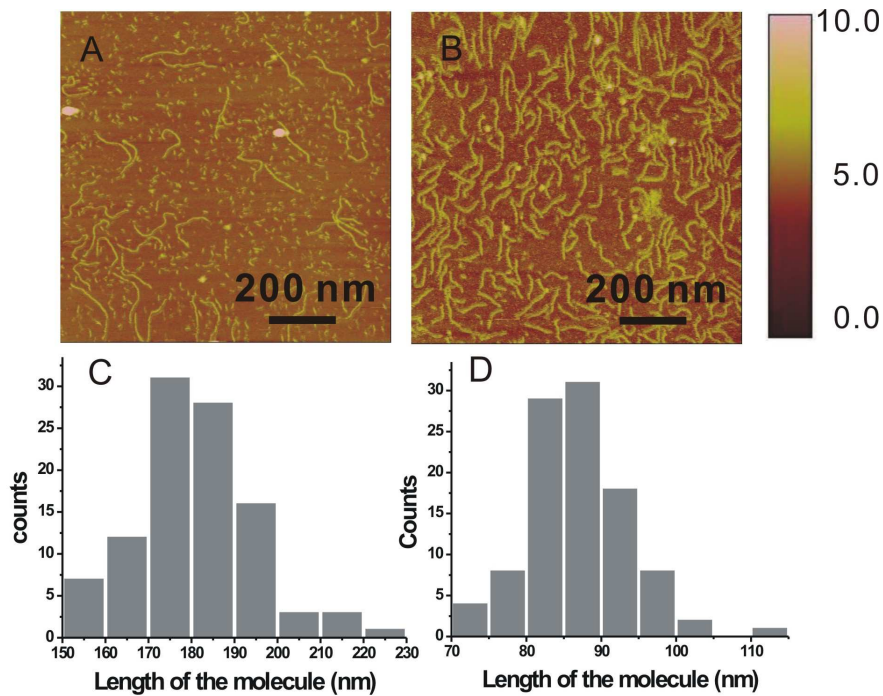
Twenty microliters of a 10  $\mu\text{g/ml}$  DNA-*b*-PEG-*b*-DNA solution in buffer (10 mM Tris PH 7.4, 1 mM  $\text{NiCl}_2$ ) were deposited onto freshly cleaved mica (Plano GmbH, Germany). After 5 min incubation the samples were rinsed with 200  $\mu\text{l}$  of buffer solution. The mica sheet was then mounted in the SFM keeping the surface always covered by buffer solution. All images were recorded using a commercial SFM (Multimode, Nanoscope IIIa, Veeco Instruments, California USA) in soft tapping mode in liquid. Oxide-sharpened silicon nitride cantilevers (NP-S, Veeco Instruments, California; 115  $\mu\text{m}$  long, 17  $\mu\text{m}$  wide, 0.6  $\mu\text{m}$  thick) with an integrated tip (a spring constant of 0.32 N/m and a resonance frequency of 56 kHz in air) were used. The height of the tip was 2.5 to 3.5  $\mu\text{m}$ . The tip radius was confirmed by scanning electron microscopy after having performed the SFM measurements. We found tip radii of curvatures < 20 nm in all cases. A piezoelectric E-scanner (Veeco Instruments, California) was used, which supplies a maximum x-, y-scan of 12.5  $\mu\text{m}$  and a z-extension of 2.5  $\mu\text{m}$ . The scanner was calibrated by imaging a rectangular grid of 1  $\mu\text{m}$  \* 1  $\mu\text{m}$  mesh size.

In liquids, we selected a driving frequency between 8 – 10 kHz for imaging. SFM images (512  $\times$  512 pixels) were recorded at a scan rate of 1 Hz. Images were processed by flattening to remove the

background slope. Contour lengths measured from 100 molecules were plotted together in the histograms.

### Control experiments

The DNA segments having 500 and 225 bp have been prepared by PCR and measured. A mean length of  $180.1 \pm 11.1$  nm was measured for the 500-bp fragments, yielding a rise of  $0.36 \pm 0.02$  nm per bp, and a mean length of  $86.3 \pm 5.7$  nm was measured for the 225-bp fragments, yielding a rise of  $0.38 \pm 0.03$  nm. The height of the molecules is  $\sim 2$  nm. The width is 6~8 nm.



**Supporting Figure 3.** *Structural properties of 500 bp-DNA (A, C) and 225 bp-DNA (B, D) fragments investigated by Scanning Force Microscopy. (A, B) Tapping mode SFM topographical images in buffer. The height is indicated with a color scale bar on the right. The z-scale of the images is 10 nm. (C, D) Histograms of contour length distribution.*



## IV Manipulation of Block Copolymers by SFM

For pre-coating the surface of highly oriented pyrolytic graphite (HOPG), a droplet (ca. 10  $\mu$ l) of  $C_{12}H_{25}NH_2$  (dodecylamine) chloroform solution (0.3 g/L) was spin coated onto HOPG at spinning rate of 40 rps. The amphiphile pre-coated surface was annealed afterwards at 40°C for 20 min in order to evaporate the solvent remaining on the surface. 10  $\mu$ l of a 5  $\mu$ g/ml DNA (500 bp) *-b*-PEG-*b*-DNA (500 bp) solution in distilled water were deposited onto the precoated HOPG surface for 10 sec and spun off subsequently. SFM images were recorded before and after manipulation using a MultiMode scanning probe microscope (Digital Instruments, Inc., Santa Barbara, CA, USA) operated in tapping-mode. Height and phase images were recorded with a scan rate of 2-4 lines/s and a resolution of 512\*512 pixels. Olympus etched silicon cantilevers were used with a typical resonance frequency in the range of 200-400 kHz and a spring constant around 42 N/m. All samples were investigated at room temperature open to the air. The contour length of single polymer molecule was determined by a home made software.<sup>2</sup>

For dragging the molecules across the surface (lateral manipulation), a commercial SFM lithography program “Litho” (from Digital Instruments) based on the Multimode head and the Nanoscope III controller was used. For the purpose of manipulation, the SFM can be gently switched from tapping mode to contact mode at a predefined point while the tip is passing along the predefined trace. From the tip-molecule contact point, the interaction between SFM tip and sample is enhanced, and thus can be used to drag a molecule across the surface.<sup>3</sup>

For blowing circular topologies, a droplet of chloroform liquid was additionally spin-coated at 40 rps onto the triblock molecules deposited on the HOPG surface for 20 sec.<sup>4</sup> Then the sample was immediately scanned by SFM in tapping mode with scan rate of 4-5 lines/s. Height and phase images were recorded while the blowing manipulation was performed.

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