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Supporting Information to:

Organization of proteins at the nanoscale via block copolymer lithography and non-covalent bioconjugation

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Materials

The hydroxyl-functionalized OH-PEG_{136}-*b*-PS_{182} was obtained from Polymer Source. (+)-Biotin-4-nitrophenyl ester (≥ 98 %) was obtained from Fluka and used as received. Triethylamine (99 %) was purchased from Acros. Streptavidin was obtained from Sigma Aldrich. Toluene and dichloromethane (Baker) were distilled prior to use and stored over molecular sieves. Ultrapure water was purified by using a WaterPro PS polisher (Labconco, Kansas City, MO) set to 18.2 MΩ cm^{-1}. Syntheses were carried out under an argon atmosphere.

Synthesis and methods

*Block copolymer biotinylation (**2**)*

Hydroxyl-capped poly(ethylene glycol)-*block*-polystyrene (OH-PEG_{136}-*b*- PS_{182}, **1**, 25000 g mol^{-1}, 1 g, 4 × 10^{-2} mmol) was dried by dissolution in toluene and subsequent azeotropic distillation. This procedure was repeated three times. The dried polymer was dissolved in dry dichloromethane (10 mL) and the solution was cooled to 0 °C, after which triethylamine (0.0476 g, 4 × 10^{-4} mmol) was added. (+)-Biotin-4-nitrophenyl ester (0.146 mg, 4 × 10^{-4} mmol) in dry dichloromethane was then added dropwise to the polymer solution. After complete addition, the reaction mixture was allowed to warm to room temperature and was stirred for 4 days. Subsequently, the mixture was concentrated in vacuo and the crude product was redissolved in dichloromethane. After washings with aqueous saturated $NaHCO_3$ solution and water, the organic layer was dried over anhydrous Na_2SO_4, filtrated, and evaporated to dryness. The crude product was further purified by column chromatography using silica, CH_2Cl_2/MeOH 99:1 v/v yielding the polymer as a white solid (80% functionalization).

^{1}H-NMR (300 MHz, $CDCl_3$, 22 °C, TMS): δ = 6.9-6.2 (br, CH_2CH(*Ph*)-), 5.90 (br, 1H; biotin-CHCHN*H*), 5.09 (br, 1H; BiotinCH_2CH*NH*), 4.38 (br, 1H; biotin-CH_2*CH*NH), 4.21 (br, 1H; biotin-CH*CH*NH), 5.1 (br, 1H; NH), 3.6 (br, -

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O(CH_2CH_2O)$_n$$CH_2CH_2$O(C=O)-), 3.4-3.2 (br, -$CH_2CH$(Ph)(C=O)-), 2.7-2.4 (br, -SCH_2CH-), 2.2-0.8 (br, CH_3-CH_2CH(CH_3)(CH_2CH(Ph)$_n$(C=O)-, -NH(C=O)$CH_2CH_2CH_2CH_2CH$Bt, $CH_3CH_2CH(CH_3)(CH_2CH(Ph)_n(C=O)$-).
SEC/$CHCl_3$: M_n = 25000 g mol^{-1}, *PDI* = 1.03

Preparation of polymer solutions in toluene
Polymer solutions in toluene were prepared by adding toluene to a known amount of PS-*b*-PEG until the concentration of the solution was 1 wt%. Subsequently, the solution was shaken for 3 hours to completely dissolve the polymer and the solution was kept at 4 ^{o}C.

Binding of streptavidin to polymer films
The silicon wafers coated with block copolymer films were dipped in a buffered solution of SAv (phosphate buffer 20 mM, pH 7.2) at a known concentration comprised between 20 and 100 mg mL^{-1} for a precise period of time. Subsequently, the wafers were extensively washed with fresh buffer solution and ultrapure water. The wafers were finally dried in a stream of nitrogen.

Characterization

Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker DPX300 spectrometer using tetramethylsilane as an internal standard.

Size-Exclusion Chromatography
Molecular weight distributions were measured with a Shimadzu Prominence apparatus equipped with a guard column and a PL gel 5 µm mixed-D column, 300 × 7.5 mm (Polymer Laboratories) with differential refractive index detection using either $CHCl_3$ as an eluent (1 mL min^{-1} at 35°C). Polystyrene standards in the range of 580 to 377400 g mol^{-1} were used for calibration.

Transmission Electron Microscopy
Samples for TEM were prepared by depositing small amounts of aqueous dispersions onto a carbon-coated copper grid. After two minutes, the excess water was drained off and the grids were further dried under vacuum. TEM images were recorded using a *JEOL JEM 1010* microscope (60 kV) equipped with a CCD camera.

AFM
Silicon wafers (4 inches, resistivity: 1 ohm/cm, 500 µm thickness, p/boron) were cleaned by O_3/UV irradiation. Samples were prepared in the following way; a silicon wafer was covered with polymer solution and protected with a beaker flask. If necessary cotton impregnated with 10 mL of solvent (water and/or toluene) was placed inside this chamber (2.84 × 10^{-3} m^3). After x seconds, the spin-coating process was started (3000 rpm for 40 seconds). After a color change was observed the beaker glass was removed. AFM images were taken in both height and phase contrast using a Dimension 3100 instrument in tapping mode. Etched silicon tips were used with spring constants between 40 and 60 N m^{-1} (as specified by the manufacturer).

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Calculations

Approximative block volumic fractions of the block copolymers

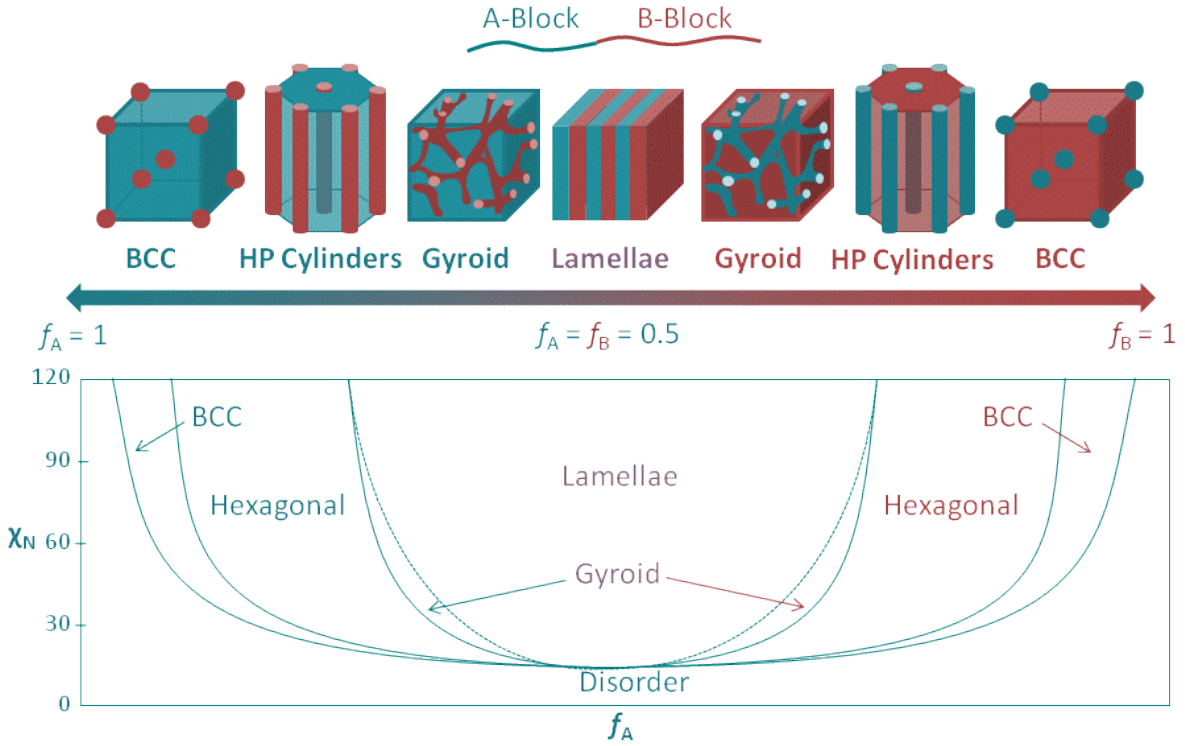

Fig. S1 (Top) Variety of constant-radius geometries observed as a function of relative lengths of the two blocks. (Bottom) Predicted phase diagram of diblock copolymers self-assembly according to self-consistent mean field theory. Adapted from the literature.[1]

According to Madsen and Bates,1 the morphology of block copolymers in bulk depends on the Flory-Huggins parameter (χ_N), which depends on the incompatibility of the two blocks, the overall molecular weight, and the relative volumic fractions of both blocks ($\boldsymbol{f}_A = 1 - \boldsymbol{f}_B$).

In other words, if the block copolymer is long enough and the incompatibility between the two blocks strong enough, nanostructuration is obtained.

Previous works testify that a molecular weight of 25000 g mol^{-1} for a PEG$_n$-*b*-PS$_m$ copolymer is sufficient to obtain nanostructuration.

The composition of the copolymer used in this study is n = 136 and m = 182. By calculating the block volumic fractions, we can predict the type of morphology which will be obtained.

$$\boldsymbol{f}_A = (\boldsymbol{V}_{PEG})/(\boldsymbol{V}_{PEG} + \boldsymbol{V}_{PS})$$
$$= (\boldsymbol{M}_{PEG}.\boldsymbol{\rho}_{PEG}) / (\boldsymbol{M}_{PEG}.\boldsymbol{\rho}_{PEG} + \boldsymbol{M}_{PS}.\boldsymbol{\rho}_{PS})$$
$$= (136 \times 44 \times 1.23) / (136 \times 44 \times 1.23 + 182 \times 104 \times 1.11)$$
$$= 0.26$$

with $\boldsymbol{V}$ being the volume of the considered block, $\boldsymbol{M}$ its molecular weight, and $\boldsymbol{\rho}$ its density.

The expected structure is thus a hexagonally packed phase of PEG in a PS continuous phase.

[1] M. W. Matsen, F. S. Bates, *Macromolecules*, 1996, **29**, 1091.

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Additional data

Table S1 SAv binding experiments with films made of biotin-functionalized BCP **2**

Dipping time / min	SAv concentration / mg mL^{-1}	Additive	Results
5, 20, 30	100	-	SAv clusters
5, 10	50	-	SAv clusters
5, 10, 30	20	-	SAv clusters
5	20	100 mM NaCl	Film removal

Table S2 SAv binding experiments with films made from mixtures of non-functionalized (**1**) and biotin-functionalized (**2**) BCPs[a]

[**2**]:[**1**]	Toluene/Water v/v	Type of cylinders	Dipping time / min	SAv on the surface
1:0	-	Perpendicular	5, 60	Clusters
4:1	-	Perpendicular/Parallel	10, 30	Clusters
2:1	-	Perpendicular/Parallel	10, 20, 30	Clusters
1:1	4:1	Perpendicular/Parallel	10, 30	Clusters
1:2	4:1	Perpendicular/Parallel	5, 20, 30, 60	Clusters
1:4	4:1	Perpendicular/Parallel	10, 60	Clusters
5:95	4:1	Perpendicular	5,[b] 60, 165	Single proteins
1:99	4:1	Perpendicular	10,[b] 90	Single proteins
0:1	4:1	Perpendicular	10, 60, 90	No protein at all

[a] The substrates were dipped in a 20 mg mL^{-1} solution of SAv (phosphate buffer 20 mM, pH 7.2).

[b] The dipping time was too short as no protein could be observed on the surface.

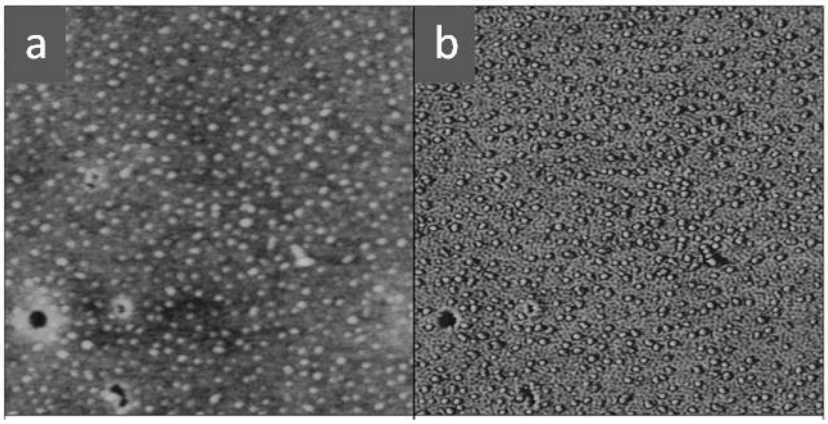

Fig. S2 Tapping-mode atomic force microscopy images of a thin films of **1** and **2** (1:1) consisting of a mixture of PEG perpendicular and parallel cylinders after dipping 10 min in a 20 mg mL^{-1} SAv solution (2 × 2 µm). Left: height image. Right: phase image.