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Supporting Information

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Controlling the Size of Nanoparticles by an Enzymatic Reaction

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I. Material Preparation

The preparation of ss DNA-*b*-PPO diblock copolymers, and the formation of micelles were carried out as described previously.^[1] Oligonucleotides were quantified spectrophotometrically at a wavelength of 260 nm.

General Hybridization Procedure for Fluorescent Labelling

The hybridization was carried out by dissolving ss DNA-*b*-PPO diblock copolymer and the complementary strand with the fluorophore (Alexa488, Invitrogen, USA) attached at the 5' end 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 Mg²⁺ (60 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 Biometra polymerase chain reaction (PCR) thermocycler (Biometra GmbH, Germany). The final concentration of DNA was between 5-8 µM.

Enzymatic Reaction

The enzymatic reactions were carried out by mixing reaction buffer, 1 nmol of ss DNA-*b*-PPO block copolymers, 150 nmol of dTTP and 40-60 units of TdT. This mixture was incubated for different times at 37°C in a thermoshaker. The reactions were completed by placing the samples in the freezer at -20°C.

Material Preparation for FCS Experiments

ss DNA-*b*-PPO: ss DNA-*b*-PPO micelles were hybridized with the complementary sequence which was functionalized with Alexa488 (Invitrogen, USA) at the 5' end. The ratio of ss DNA-*b*-PPO to ODN carrying the dye was adjusted to be 1 % so that the predominant form of DNA within the corona remains single stranded.

II. FCS Measurements

FCS was performed on a custom-built confocal microscope based on an Olympus IX71. An Argon ion laser (Spectra Physics) was used to excite the micelle solutions at 488 nm with 20 μ W or 50 μ W, respectively. In epi-fluorescence configuration, diffraction-limited excitation and fluorescence collection was achieved through a water immersion objective (UPlanSapo 60xW, 1.2 n.a., Olympus, Hamburg). A 50 μ m pinhole blocked the out-of-focus fluorescence. Fluorescence in the spectral range between 500 and 570 nm was separated from scattered light by an interference filter (HQ 532/70, AHF, Tübingen) and split in two channels by a polarizing beam splitter. The signals of the two single photon counting avalanche photodiodes (SPCM AQR-14, Perkin-Elmer) were fed into the autocorrelator card (ALV-5000/E, ALV, Langen) in cross-correlation configuration and in parallel to a set of synchronized, fast counter cards (SPC-152, Becker&Hickl, Berlin) for software-based autocorrelation.^[2] A diluted Rhodamine-110 solution in pure water was used as the reference to yield the optical parameters of the confocal detection volume.

The addition of the nucleotides by TdT to the single stranded DNA of the preformed DNA-*b*-PPO micelles was monitored by fluorescence correlation spectroscopy, FCS. To maintain the micelles during the FCS measurements, the concentrations were kept well above the critical micelle concentration resulting in 50 to 100 fluorescently labelled micelles in the diffraction-limited confocal detection volume.

For spherical micelles the diffusion time, τ_D , can be directly related to the hydrodynamic radius, r_H (equation 1):

$$r_H = kT (4 \tau_D) / 6\pi \eta \omega^2 \quad (1)$$

where k is the Boltzmann constant, T is the temperature, η is the viscosity of the solution, and ω is the approximated axial $1/e^2$ radius of the confocal volume.

Fitting the autocorrelation function of rhodamine 110 in water yielded the axial radius $\omega = 220$ nm of confocal volume using a diffusion coefficient $D = 2.8 \cdot 10^{-6} \text{ cm}^2/\text{s}$.^[3] The corresponding effective hydrodynamic radius of 0.77 nm for rhodamine 110 is similar as reported previously.^[4] Accordingly, the diffusion times of the micelles were used to calculate the respective radii. The diameter increased from 9.9 nm for a TdT reaction time of 15 minutes up to 23 nm after 16 hours reaction time. The kinetics of micelle growth could be described by a logarithmic time dependence with an initial growth rate of about $1.6 \pm 0.1 \text{ nm / h}$.

III. SFM Visualization

Five microliters of the solution after enzymatic reaction were diluted to 20 μl with imaging buffer (10 mM Tris PH 7.4, 1 mM NiCl_2) and then deposited onto cleaved mica (Plano GmbH, Germany). After 5 min incubation the samples were rinsed with 200 μl of imaging buffer. 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 μm long, 17 μm wide, 0.6 μm 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 μm . 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 μm and a z-extension of 2.5 μm . The scanner was calibrated by imaging a rectangular grid of 1 $\mu\text{m} \times 1 \mu\text{m}$ mesh size.

In liquids, we selected a cantilever excitation frequency between 8 – 10 kHz for imaging. SFM images (512×512 pixels) were recorded at a scan rate of 1 Hz. Images were processed by first

order flattening to remove a background slope. The maximum height of individual micelles was calculated by means of local roughness analysis. Height values determined from > 440 micelles after each time period are plotted together in the histograms. The images and the corresponding histograms are shown below.

Supporting Figure 1. *SFM topography and corresponding histograms of the TdT reaction products after various time periods.*

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