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Polarizability of DNA Block Copolymer Nanoparticles Observed by Electrostatic Force Microscopy

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In this study, DNA block copolymer (DBC) micelles with a polystyrene (PS) core and a single-stranded (ss) DNA shell were doped with ferrocene (Fc) molecules. Tapping mode atomic force microscopy (AFM) was used to study the morphology of the doped and undoped block copolymer aggregates. We show that introducing Fc molecules into the hydrophobic core does not affect the structural properties such as shape or size. In contrast, doping with Fc significantly changes the micelles’ electrical properties, namely their polarizability. Electrostatic force microscopy (EFM) measurements reveal that the undoped micelles show no significant polarization signal, while the Fc-doped aggregates exhibit strongly enhanced polarizability. Furthermore, the nucleic acid moieties were utilized in combination with complementary ssDNA strands to assemble single particles into linear arrays of DBC nanoobjects. The ability to tune the electrostatic properties of the polymer core and the presence of nucleic acids might open the way for using these bioorganic nanoparticles as building blocks for nanoelectronic or biosensing devices.

Introduction

Since the introduction of oligonucleotide-functionalized gold nanoparticles (DNA–Au NPs) in 1996, numerous studies and potential applications of these materials have been demonstrated. Among them are the detection of nucleic acids, proteins, metal ions, and small molecules and the delivery of gene silencing agents and drugs [1–5].

An alternative route to form biohybrid nanoparticles is based on supramolecular chemistry. It was previously demonstrated that amphiphilic block copolymers comprised of organic polymers and oligonucleotides form micelles in aqueous solution due to microphase separation. These DNA block copolymer (DBC) aggregates are composed of a core containing the hydrophobic polymer segments and a corona of single-stranded (ss) DNA [6–13]. It is even possible to transform the shape of these self-assembled structures from spheres to rods by hybridization, providing further
degrees of structural control over the nanoparticles.\textsuperscript{[14]} The self-assembled structures of DBC aggregates have been studied by different techniques including atomic force microscopy (AFM), fluorescence correlation spectroscopy, and dynamic light scattering.\textsuperscript{[7,12,13]} However, little is known about the electrical features of these bioorganic nanoobjects and how they can be manipulated. This issue is critical for exploiting such conjugates for sensing applications. Since most organic polymers that are coupled to DNA in these materials are non-conductive, it is assumed that the electrical transport of such materials is rather poor. However, modification of the micellar core through the non-covalent introduction of hydrophobic moieties offers a promising opportunity for improving the electrical properties of DBC micelles.

Herein, we aim to address this issue by doping the polymeric core of the micelles with a metallorganic moiety without significantly affecting the structural features of the self-assembled nanoparticles. The effect of doping on the micelles’ polarizability was studied using AFM and EFM to explore the structural and electrical properties (polarizability) of the bioorganic hybrid structures.\textsuperscript{[15–20]} Furthermore, doped DBC aggregates were transformed into superstructures by hybridization with template nucleic acid strands.

Experimental Part

**AFM Tapping Mode**

We used an AFM system and the WSxM software designed by Nanotec Electronica (Madrid, Spain) to obtain and analyze the data.\textsuperscript{[21]} Soft, non-conductive, rectangular, commercial SiN\textsubscript{4} cantilevers (NSG 10, NT MDT Co., LTD.) with spring constants of 5.5–22.5 N·m\textsuperscript{-1} and resonance frequencies from 190 to 325 kHz were employed.

**EFM Mode**

For the EFM measurements, we utilized platinum-coated silicon tips with spring constants of 0.5–2.2 N·m\textsuperscript{-1} and resonance frequencies from 90 to 116 kHz. The tip was lifted 25 nm above the set point height at zero voltage. EFM measurements were performed using the two-pass technique. In the first pass, a topography image was acquired. During the second pass, a conductive AFM cantilever was piezo-driven at resonant frequency and grounded or biased by DC voltage. The capacitive tip-sample electric force leads to a resonance frequency shift and accordingly a decrease in the amplitude and a change in the phase of the cantilever oscillations. Amplitude and phase deviations could both be measured and thus the electric potential distribution over the sample surface could be imaged.\textsuperscript{[15–18]}

Sample preparation for AFM and EFM measurements: A 10 μL droplet of the sample solution containing the DBC at a concentration of 10 nM was deposited on a mica surface for 5 min. After that, the surface was gently rinsed with triple distilled water and dried with nitrogen gas.

**Results and Discussion**

The synthesis of the polymeric bioorganic hybrid structure DNA\textsuperscript{-}block (b)\textsuperscript{-}PS has been described previously.\textsuperscript{[22]} The amphiphilic block copolymer used in this study consisted of an amino-terminated polystyrene (PS) polymer of...
MW = 5 000 g·mol⁻¹ and a 22 mer ODN (MW = 6 670 g·mol⁻¹, sequence: 5'-CCTCGCTCTGCTAATCCTGTTA-3') that were covalently connected by a linker system as shown in Scheme 1a. The micelles were prepared by solvation of the DNA diblock copolymer in dimethylformamide (DMF) as cosolvent and subsequent dialysis against water.[6,22,23] Scheme 1b exhibits a schematic representation of a DNA-b-PS micelle. For doping with Fc, a procedure similar to that of loading a hydrophobic drug into the micelle core was followed.[24] A schematic drawing of a Fc-doped micelle is shown in Scheme 1c. In order to verify the incorporation of Fc in the micelles, energy dispersive microanalysis measurements (EDS) were performed using a high resolution transmission electron microscope (HRTEM). The samples were prepared by depositing a small amount of micelle solution on top of a carbon-coated TEM grid. The analysis presented in Figure 1 proved that ferrocene was present within the DBC micelles. Control experiments with undoped micelles did not show any presence of iron, as it can be seen in Figure S1 (Supporting Information). The source of the other elements found in the analysis was from the buffer solution and the HRTEM grid.

The Fc containing and undoped DNA-b-PS micelles were characterized by AFM topography images in tapping mode. These measurements revealed singly separated uniform particles with heights of 5–9 nm. The average height of the doped and undoped micelles was found to be 6.5 ± 0.4 and 6.2 ± 0.5 nm, respectively. The results clearly demonstrated that doping with Fc did not affect the shape or the size of the micelles (see Figure S2 for details). Our AFM tapping mode topography measurements are consistent with results reported on similar micelle systems adsorbed on an
aminopropyltrimethoxysilane-functionalized mica surface. Li et al.\[9\] reported spherically shaped particles with diameters of 8–30 nm. The discrepancy in height is likely due to different surfaces or different structural parameters of the constituent block copolymers.

The EFM measurements on the undoped micelles showed no signal at positive (+5 V), zero, or negative (−5 V) bias voltages. In contrast, the EFM measurements of the Fc-doped micelles shown in Figure 2a exhibit a markedly different behavior. A strong pattern of negative phase shift signal at the position of the doped micelles was detected when bias voltages of +5 V (Figure 2b) and −5 V (Figure 2d) were applied to the tip, while no EFM signal was measured at 0 V (Figure 2c). The line profiles in Figure 2e represent the magnitude of the phase shift signal at the position of the doped micelles at the different bias voltages. This behavior is indicative of strongly enhanced polarizability.

Control EFM measurements on ssDNA with the same sequence composition as in the micellar corona and adsorbed onto a mica surface showed no EFM signal, as seen in Figure S3 (Supporting Information). From these measurements, it was concluded that, the Fc incorporated into the core of the micelles is responsible for the different polarization behaviors of doped and non-doped particles. In order to validate our results, we applied the same technique to multi-walled carbon nanotubes (MWCNTs), strongly polarizable objects similar in height to the micelles presented herein (Figure S4, Supporting Information). A strong pattern of negative phase shift signal was clearly observed at the position of the CNTs when bias voltage was applied to the tip, while no EFM signal was measured at 0 V. The results for the polarizability of CNTs are consistent with the results reported by other groups, supporting the reliability and accuracy of our EFM measurements.\[15,16\]

After exploiting the hydrophobic core of the aggregates for incorporation of polarizable molecules, the nucleic acid shell of the DBC particles was utilized for the formation of superstructures. Therefore, the micelles were hybridized with ss 42 mer ODN templates and 20 mer bridging ODNs (Figure 1d). The central part of 22 mer template sequence was selected to be complementary to the DNA present in the corona of the micelles, while the two 10 mer overhangs at the both ends were used to hybridize with seven 20 mer connector strands. In this way, strings of Fc-doped DBC particles were generated. For the exact sequence composition see Supporting Information (Table S1). Figure 3a shows
an AFM topography image of DNA-b-PS linear particle strings adsorbed on a mica surface. The line profile in Figure 3b illustrates the dimensions of one particle string (black) relative to a single DBC micelles (grey). The EFM measurements of the Fc-doped particle strings are shown in Figure 3c–e. A strong pattern of negative phase shift signal at the position of the doped rods was observed when bias voltages of +5 V (Figure 3c) and –5 V (Figure 3e) were applied to the tip, while no EFM signal was measured at 0 V (Figure 3d). The line profiles in Figure 3f represent the magnitude of the phase shift signal at the position of the doped particle strings at the different bias voltages. It should be stated that the design of DNA-b-PS micelles aligned into strings presented here was different from our previous work, which also aimed at rod-like particle formation.[6] While spherical DBC aggregates with poly(propylene oxide) as hydrophobic component disaggregate upon rod formation, the PS-containing particles presented here with a higher glass transition temperature remain largely unchanged when assembling into linear arrangements with template and connector DNA.

Incorporation of polarizable species into PS-containing block copolymer matrices and their effects on the properties of thin films have already been investigated.[25–27] In these studies as well, successful confinement could be clearly confirmed through polarizability measurements using EFM. However, these systems are focused on the dispersion and organization of large nanoparticles within a film. By contrast, in the system reported here significant polarizability is introduced through the incorporation of small guest molecules into individual well-defined nanoscale objects that can be further transformed into aggregates of higher structural order; this is thus a notable departure from previous work.

**Conclusion**

Advanced AFM techniques were applied to characterize the morphological and electrical properties of DBC micelles. The polarizability of these bioorganic nanoobjects can be easily altered by doping the hydrophobic core with highly polarizable molecules such as ferrocene. Doping does not alter the size or shape of the micelles but significantly changes their electrical properties. Single-doped particles could be aligned into strings of micelles employing the self-recognition properties of the DNA corona in combination with template and connector strands. These findings open the way for exploring the possibility of using these nanoobjects in nanoelectronic and sensing applications, as the dopant could be used as an electrical tag.

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