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Qualitative Mapping of Structurally Different Dipeptide Nanotubes

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ABSTRACT

Biological self-assembled structures are receiving increasing focus within micro- and nanotechnology, for example, as sensing devices, due to the fact that they are cheap to produce and easy to functionalize. Therefore, methods for the characterization of these structures are much needed. In this paper, electrostatic force microscopy (EFM) was used to distinguish between hollow nanotubes formed by self-assembly by a simple aromatic dipeptide, L-phenylalanine, silver-filled peptide-based nanotubes, and silver wires placed on prefabricated SiO_2 surfaces with a backgate. The investigation shows that it is possible to distinguish between these three types of structures using this method. Further, an agreement between the detected signal and the structure of the hollow peptide was demonstrated; however only qualitative agreement with the mathematical expressing of the tubes is shown.

In recent years a lot of research activities have been channeled into looking for suitable biological materials for micro- and nanodevices.[1,2] A promising material is the aromatic dipeptide, L-phenylalanine, as it has been shown to be biocompatible as well as easily functionalized.[3] These abilities of the peptides give rise to interesting application possibilities, as it has been shown, that they can self-assemble into tubelike structures[4−7] with a very small diameter. It has been proven that their hollow structures can be directionally grown, which overcomes one of the barriers which most other biological materials have, when integration with microdevices is wanted.[8] Further, they are resilient to different microfabrication techniques, which make them candidates for use in microfabrication.[9,10] Suggestions for applications range from improved sensing to electronic circuits.[11,12] It has also been shown that these structures can be used, for example, as a mold for creating nanoscale silver wires[13] or as biosensors[14,15] by surface functionalization.[3] So far most investigations with scanning electron microscopy (SEM) or transmission electron microscopy (TEM) have been focusing on determining the physical properties of these tubes,[13] but even so very few scanning probe microscopy investigations of the tubes can be found in the literature. The scanning probe microscopy method known as electrostatic force microscopy (EFM) provides a number of possibilities for the investigation of different electrical properties of nanoscale structures. EFM has in the previous years been used to estimate the conductivity of single-walled carbon nanotubes and DNA,[16] to distinguish between conductive and insulating polymer nanofibers,[17] measure trapped surface charges,[18] and to estimate the dielectric constant of insulating polymer nanofibers[19] and chromosomes.[20] In this report, investigations of the ability of the EFM phase mode[16] method to distinguish between different types of β-amyloid polypeptide fibers and silver wires will be presented. As such the EFM method appears to offer a simple way of looking at the composition of samples in the 100 nm range. We show that the method can be used to distinguish between hollow polypeptide tubes, silver-filled polypeptide tubes, and silver wires. We further demonstrate that the method can detect the structure of polypeptide tube.

Substrates for the experiments were fabricated by the use of 4 in. 350 μm thick heavily p-doped silicon wafers. A 100 nm thick silicon oxide layer was grown on the substrates, the oxide on the back was removed by HF, and a 20 nm layer of titanium was evaporated on the backside followed by a 500 nm layer of gold.

For the EFM experiments, three different types of samples were used. The peptide solution was prepared by dissolving the lyophilized form of the diphenylalanine (Bachem (cat. no. G-2925, Germany) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Fluka Cat. No. 52517) at a final concentration of 100 mg/mL. Peptide stock solution was diluted in distilled water to a final concentration of 2 mg/mL. For the casting of silver nanowires inside the peptide nanotubes, ionic silver

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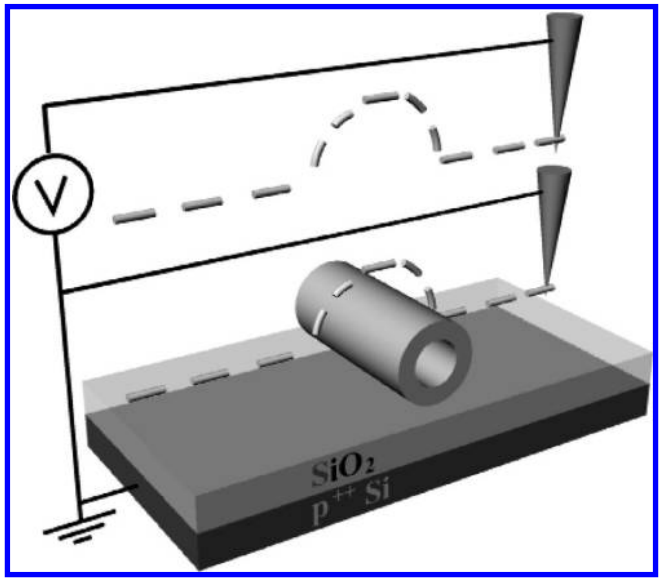

Figure 1. The principle of the EFM phase mode method. First a topography line scan is made, then the tip is lifted and another line scan is made with a potential difference between the AFM tip and the backgate.

was reduced to metallic silver inside the nanotube. The peptide mold was then removed by enzyme degradation using Proteinase K (Sigma-Aldrich cat. no. P6556) following the procedure by Reches et al.[13,21] The solutions with the peptide and silver wires were then added onto the fabricated substrates.

The EFM phase mode method has been well documented in previous reports.[16−19] The working principle of EFM is illustrated in Figure 1 and can be outlined as follows: First a line scan acquires the topography in tapping mode with no bias applied between the tip and the doped substrate. Then the tip is raised a few tens of nanometers above the sample, a potential is applied (as sketched in Figure 1), and the tip retraces the topography of the previous scan at a constant height over the sample. During the second scan, the phase of the oscillation, ϕ, of the cantilever is recorded. Since the tip is raised some tens of nanometers and due to the potential difference between the tip and the substrate it can be assumed that the only force acting on the cantilever is an electrostatic force, F, caused by the applied potential. According to Staii et al.[19] and Jespersen et al.,[18] the phase is proportional to the derivative of the force acting on the cantilever or

$$\phi \approx -\frac{Q}{k}\frac{\partial F}{\partial z} \tag{1}$$

where Q is the quality factor, k the spring constant of the cantilever, and z the distance between the tip and the doped substrate. Using electrostatics, the derivative of the force can be written as[6]

$$\frac{\partial F}{\partial z} \approx \frac{1}{2}\frac{\partial^2 C}{\partial z^2}V^2 \tag{2}$$

where C is the capacitance between the tip and the substrate and V is the potential difference between the tip and the substrate. From eq 2, it can be seen that changes in the phase can only be caused by changes in the capacitance; so in this case by changes are caused in the material between the tip and the cantilever. Therefore changes in the phase can be written as

$$\Delta\phi \approx \frac{Q}{2k}\left(\frac{\partial^2 C_1}{\partial^2 z} - \frac{\partial^2 C_2}{\partial^2 z}\right)V^2 \tag{3}$$

where C_1 is the capacitance between the tip and the substrate without a sample inserted and C_2 is the capacitance between tip and substrate with the sample introduced.

It is also necessary to take into consideration that the interaction of the capacitance coupling is not the only force acting on the cantilever tip. The substrate also interacts with the cone of the tip and the cantilever beam. However, theoretical considerations by Colchero et al.[22] indicate that for the purpose of this work these interactions with the substrate as a function of varying height can be neglected as the interacting is mostly constant. Further the calculations indicate that for optimal readout, changes in height (the height the tip is raise during the second scan) should be in the range of 10 to 50 nm. Taking this into account and modeling the tip substrate interaction as a plate capacitor (Jespersen et al.),[18] where the shape of the cantilever tip is assumed to be a flat disk with radius r_{tip}, the change in the lift phase can be expressed by[18,19]

$$\Delta\phi \approx \frac{Q\pi {r_{\mathrm{tip}}}^2 \varepsilon_0}{k}\left(\frac{1}{(x + t/\varepsilon_{\mathrm{SiO_2}})^3} - \frac{1}{x + t/\varepsilon_{\mathrm{SiO_2}} + h/\varepsilon_{\mathrm{P}})^3}\right)V^2 \tag{4}$$

where r_{tip} is the radius of the tip, ε_0 is the vacuum permittivity, x is the lift height, t the height of the oxide layer, $\varepsilon_{\mathrm{SiO_2}}$ is the permittivity of the oxide, h the height of the sample, and ε_{P} is the permittivity of the sample in question.

A total of 22 polypeptide hollow tubes were scanned and their topography height was in the range of 50 to 220 nm. A typical topography scan and a lift phase scan of a single hollow peptide nanotube are shown in Figure 2A,B, respectively, while line profiles outlined in these figures are plotted in Figure 2C. A dip in the phase line profile is observed in the center of the peptide nanotubes. From eq 2, the dip in the phase can be explained by a change in the capacitance between the tip and substrate, which in turn from eq 4 indicates a change in the dielectric properties of the tube. Such a change can be attributed to the presence of hollow tubes, as shown in Figure 2D, since in that case the permittivity of the tubes would decrease in the middle, where air is present. This conclusion is supported also by the work of Reches et al.[13] and Song et al.,[23] which indeed report that the tubes are hollow. In order to estimate the dielectric constant of the fibers eq 4 was used for the hollow peptide fiber data, mimicking the work done by Staii et al.[19] with poly(ethylene oxide) nanofibers. Due to the peptide fibers hollow structure the maximum change in the lift phase was used for fibers (see Figure 2D) of various heights. The estimated dielectric constants seem to depend on the height of the peptide tubes and are in the range around 2 to 12. This suggests that eq 4 is not suitable for describing the dielectric properties of these peptide tubes. An explanation for this could be that the wall of the peptide tube is porous, as suggested by theoretical studies done by Song et al.[23] All

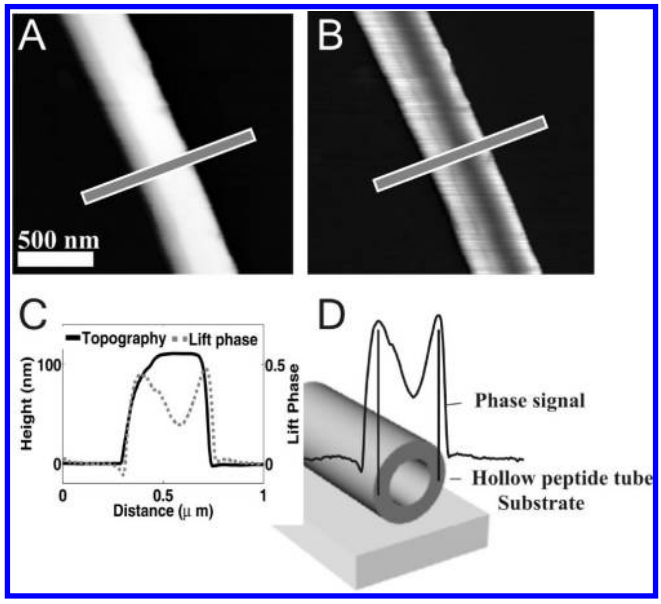

Figure 2. (A) Topography image of a peptide tube; (B) the lift phase image of the same peptide tube; (C) the line profile indicated by the gray bars in A and B plotted together; (D) schematic cross-sectional drawing of the expected hollow structure of the peptide as interpreted by the lift phase signal.

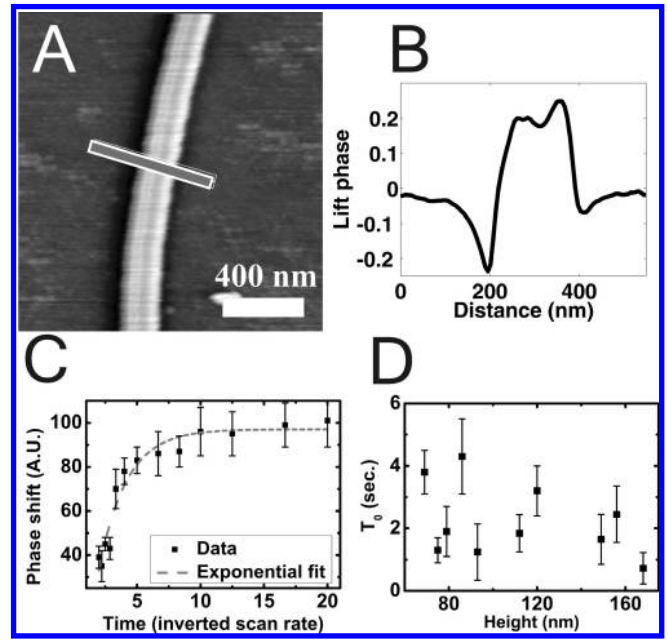

Figure 3. (A) Lift phase image of a silver-filled peptide; (B) the line profile indicated by the gray bar in panel A; (C) change in the lift phase as a function of the inverted scan rate for a silver-filled peptide tube; (D) the time constant of the exponential of different tubes as a function of their height.

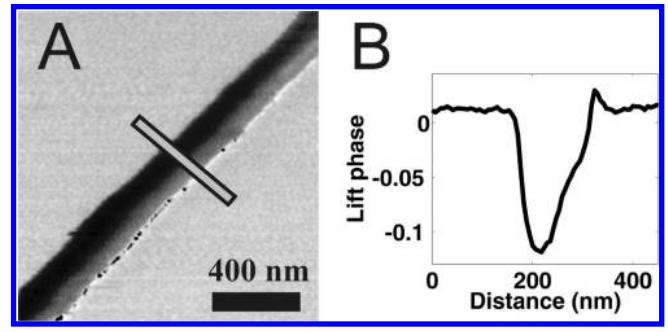

Figure 4. (A) Lift phase image of a silver wire; (B) the line profile indicated by the gray bar in B.

the scanned fibers down to around 60 nm in height showed the same change in shape as shown in Figure 2B. For fibers with a smaller diameter, the dip began to be unobservable, most likely due to the dimensions of the AFM tip, as the radius is around 25 nm. The phase amplitude varied from around 0.2 to 0.65 with respect to the substrate. Various comparisons were made between the different measured values for the phase amplitude and fiber dimensions, but no clear trends were identified. This is properly due to the fact that the self-assembling process produces structures with different wall thicknesses.

A total of 34 silver-filled peptide tubes were scanned and their topography height was in the range of 70 to 170 nm. The lift phase for a silver-filled peptide tube is shown in Figure 3A, while Figure 3B shows the line profile illustrated by the gray line in Figure 3A. The phase shift for the silver-filled peptide tube resembles the signal which Staii et al.[19] measured for conducting Pan.HCSA/PEO nanofibers using the EFM method, indication that the silver-filled peptide tubes have similar electrical properties. The signal shows a negative−positive phase shift response. All the scanned silver-filled tubes showed this characteristic negative−positive phase response with a variation in the amplitude in the range around 0.1 to 0.48 for the positive peak. Staii et al.[19] explanation for this behavior is the existence of an additional attractive force, which interacts between the tip and the silver-filled tube, as the tip approaches the tube. Another cause for this negative−positive phase response might be the structure of the tube itself, since the wall of the tube could cause the negative part while the silver in the middle could cause the positive part. In order to investigate this effect further, we have plotted the amplitude of the negative part of the phase signal as a function of the inverted scan rate (shown in Figure 3C). These investigations were carried out on 10 of the 34 silver-filled peptide tubes. As the response indicated, an exponential curve an exponential function has been fitted to the data. The time constant of the exponential for the different curves has been plotted as a function of their height in Figure 3D. Figure 3C therefore suggests that the initial dip in the phase is due to the insulating-conducting structure of the tube. As the AFM tip approaches the silver-filled peptide, a capacitor is formed by the AFM tip and the silver inside the tube, with the wall of the tube acting as the dielectric. Due to the applied voltage (the AFM tip on the one side and a potential on the silver due to the capacitor formed by the backgate and the silver) this capacitor is charging while the AFM tip scans the peptide with the dip size depending on the scan rate.

Twelve silver wires were scanned during the experiments with a topography height in the range of 30 to 80 nm. The lift phase for a pure silver wire is shown in Figure 4A, while Figure 4B shows the line profile illustrated by the gray line in Figure 4A. Figure 4B shows the lift phase of the silver wire fabricated from the peptide as described by Reches et

al.[13] The phase shift follows relatively well the topography, as expected for a solid and conducting material.[19] As the silver wires are made from the peptide shell, their topography height tends to be smaller compared to the peptide structures. The typical phase signals for the silver wires are of the same amplitude as the peptide tubes making the ratio between the height of the sample and the phase shift a possible way to distinguish between the two types of samples. This seems to hold with the theory of eqs 3 and 4 since silver has a high dielectric constant while the peptide is an insulating material.

In conclusion, we have used the EFM method to distinguish between three different types of structures (hollow and silver-filled peptides, and silver wires) fabricated using a simple aromatic dipeptide, L-phenylalanine. We have shown that it is possible to detect the geometric structure of hollow nanotubes using EFM. Further the dip in lift phase for the hollow tubes can be qualitatively explained by previously reported theoretical considerations.[18,19] Also investigations of the signal from the silver-filled tubes show an exponential behavior with scan rate, which may be explained by charging of a capacitor formed between the AFM tip and the silver core of the peptide tube with the peptide wall acting as the dielectric. Therefore this method seems to be a promising tool for the characterization of self-assembled microdevices using peptides nanotubes.

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