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Identification of Single-Strand DNA by in situ Scanning Tunneling Microscopy

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In this report we show that bound ruthenium-hexammine(II)/(III) (RuHex) can be used to identify DNA strands on a Au(111)-surface by in situ electrochemical scanning tunneling microscopy (in situ STM) at the single-molecule level.

The molecular orientation and accessibility of oligonucleotides attached to a surface are crucial in DNA biosensor function. Electrochemical detection of immobilization and hybridization of DNA-based redox probes is a facile and inexpensive alternative to optical or radioactive techniques. The RuHex redox couple has been broadly used to probe quantitatively monolayer coverages of oligonucleotides.[1−3] RuHex binds to the backbone of DNA, and the reversible transition between the two oxidation states can be detected by cyclic voltammetry or chronocoulometry.

Previous work from our group has shown that monolayers of single-strand oligonucleotides self-assembled on Au(111) at the open circuit potential are disordered and individual oligonucleotide strands almost indistinguishable. As the Au(111)-electrode potential is shifted to a strongly negative value the strands form highly ordered dense monolayers,[4] for which the lattice structure can be determined. A recent theoretical study based on Monte Carlo simulations of the surface grafting of rodlike polyelectrolyte brushes indicates that a new nematic phase is formed at high grafting densities. This resembles the observed ordered thiol-modified oligonucelotide adlayers and provides a rationale for both the upright orientation and high surface density.[5] The high coverage by single-strand (ss) oligonucleotide molecules is, however, prohibitive for hybridization with the complementary strand and even stimulates dehybridization of adsorbed preformed double-strand molecules.[6] A mixed monolayer of the ss-oligonucleotide and a diluent such as mercaptohexanol (MCH) provides, however, ample space for hybridization.[2,3,7] MCH also prevents the oligonucleotide from nonspecific adsorption to the gold-surface. The number of oligonucleotide molecules in the mixed monolayer can, finally, be controlled via the adsorption time, ionic strength of the buffer, and the structure of the linker.[2,8,9]

In this report we have used a sequence of 13 bases with a mercaptohexyl linker (HS−$(CH_2)_6$-5′-CGC ATT ATT ACG C, **HS-13mix**). The mixed-sequence strand adsorbed on Au(111) shows broadly similar electrochemical and in situ STM patterns as ss-oligonucleotides consisting of 10 adenine bases and a mercaptohexyl linker, HS-10A, studied previously.[10] Figure 1 shows an in situ STM image of a HS-13mix monolayer on Au(111) in 10 mM Tris-(hydroxymethyl)aminomethane (Tris) + 50 mM NaCl buffer, pH 7.6. The self-assembled monolayer (SAM) of the oligonucleotide was disordered, but by stepping the potential to −0.61 V (SCE) an ordered, densely packed monolayer was formed.

A SAM of MCH in 10 mM Tris + 50 mM NaCl (pH 7.6) was investigated as a reference (Figure 2). MCH also forms a highly ordered SAM, as expected for a thiolated alkane chain.[11] A mixed monolayer was prepared by adsorbing HS-13mix on a freshly annealed Au(111) electrode for 1 h. This was followed by thorough rinsing with buffer (10 mM Tris + 50 mM NaCl, pH 7.6) and then by adsorption of MCH for 1 h.[1−3] In situ STM of the mixed monolayer at −0.26 V (SCE) showed a disordered structure, Figure 3. All in situ STM images of both pure and mixed monolayers show pits, which are characteristic for thiol-bound adlayers. Pits are caused by missing gold atoms beneath the thiol adlayer, i.e. thiols are adsorbed in the dark patches just a gold atom layer deeper.[12]

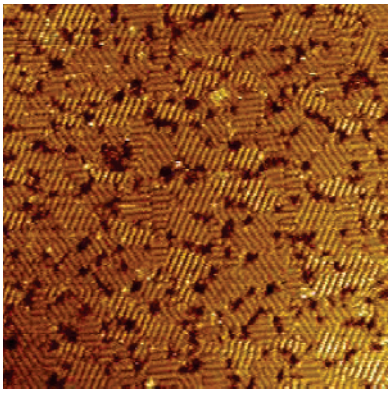

Figure 1. 60 nm × 60 nm in situ STM image of HS-13mix in 10 mM Tris + 50 mM NaCl (pH 7.6). I_t = 0.35 nA, V_{bias} = −0.08V, E_{sample} = −0.21 V (SCE).

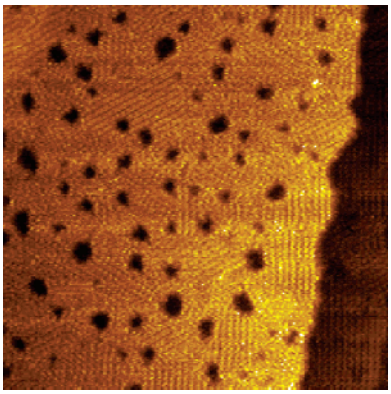

Figure 2. 60 nm × 60 nm in situ STM image of MCH in 10 mM Tris + 50 mM NaCl, pH 7.6. I_t = 0.4 nA, V_{bias} = 0.02 V, E_{sample} = −0.21 V (SCE).

A potential shift to −0.61 V (SCE), where oligonucleotide domains normally appear showed no formation of an ordered monolayer.

The absence of domains suggests that MCH and oligonucleotides are completely mixed in the monolayer, since areas containing solely MCH or oligonucleotide would give ordered structures. The oligonucleotide cannot be distinguished from MCH in the in situ

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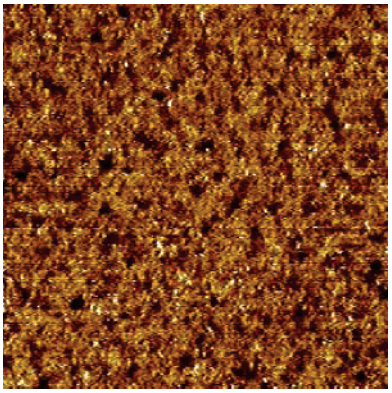

Figure 3. 60 nm × 60 nm in situ STM image of mixed monolayer of HS-13mix/MCH with 10 mM Tris + 50 mM NaCl (pH 7.6) as electrolyte. STM: $I_t = 0.1$ nA, $V_{bias} = -0.05$ V, $E_{sample} = -0.26$ V (SCE).

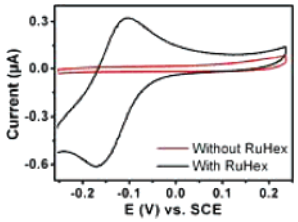

Figure 4. Cyclic voltammograms recorded before and after addition of RuHex. First scans. Scan rate 50 mV/s.

STM image (Figure 3) consistent with the results for a disordered layer of oligonucleotides.[4] The voltammogram in Figure 4 recorded directly in the STM cell reflects the featureless properties of the adlayer.

Addition of RuHex to the STM cell gives strong, well-defined cathodic and anodic peaks in the cyclic voltammogram (Figure 4). The RuHex concentration in the cell was 0.4 mM, and because of the high concentration the peak positions correspond to solution electrochemistry of RuHex and not to the adsorbed RuHex.[13]. In situ STM showed further that a large number of randomly distributed bright spots started to appear after about 10 min (Figure 5). Addition of RuHex to a pure MCH monolayer gave no change in the in situ STM images and poor voltammetry with strongly distorted signals.

The distorted electrochemical signals recorded for the MCH layer possibly originate from RuHex trapped in pits or on domain boundaries, where the interfaces are different compared to the one on top of the ordered domains. We therefore conclude that RuHex is only bound to the oligonucleotides in the mixed monolayer. This accords with previous reports.[1−3]

The significant molecular-size in situ STM contrast changes on RuHex attachment to the oligonucleotides raises issues regarding the in situ STM tunneling mechanisms. Barton and co-workers have recently shown that the charge transfer in DNA in electrochemical environment occurs through the bases pairs and not the sugar−phosphate backbone.[14] However, the low-lying RuHex redox level is likely to open new, multistep electron-transfer hopping channels in the tunneling gap via bound RuHex units.[15] Together with some of RuHex units bound to each HS-13mix molecule such a mechanism would be extremely efficient compared with tunneling

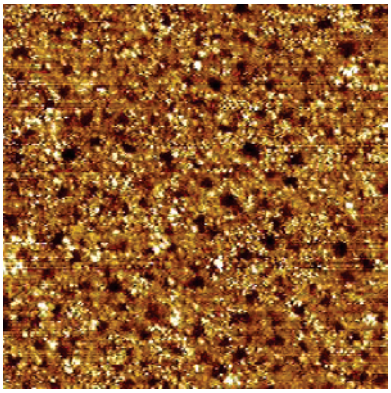

Figure 5. 60 nm × 60 nm in situ STM image of mixed monolayer of HS-13mix/MCH after addition of RuHex. 10 mM Tris + 50 mM NaCl (pH 7.6) as electrolyte. RuHex concentration in the cell was 0.4 mM. STM: $I_t = 0.1$ nA, $V_{bias} = -0.05$ V, $E_{sample} = -0.26$ V (SCE).

through bare ss-oligonucleotides. The bright spots can therefore reasonably be assigned to single oligonucleotide molecules with RuHex bound to the backbone. It is notable that RuHex binding induces strong conductivity in the ss-oligonucleotide, commonly assigned much lower conductivity than the double-strand.[16] The RuHex-based hopping channel and possibly RuHex-induced structural rigidity of the oligonucleotide can account for this.

The results show that ss-oligonucleotide molecules on a Au(111)-surface can be identified to molecular resolution by in situ STM using RuHex as a marker. This offers interesting perspectives for recording hybridization at the single-molecule level by in situ STM.

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References

(1) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *70*, 4670.
(2) Rant, U.; Arinaga, K.; Fujita, S.; Yokoyama, N.; Abstreiter, G.; Tornow, M. *Nano Lett.* **2004**, *4*, 2441.
(3) Wong, E. L. S.; Gooding, J. J. *Anal. Chem.* **2003**, *75*, 3845.
(4) Wackerbarth, H.; Grubb, M.; Zhang, J.; Hansen, A. G.; Ulstrup, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 198.
(5) Fazli, H.; Golestanian, R.; Hansen, P. L.; Kolahchi, M. R. *Europhys. Lett.* **2006**, *73*, 429.
(6) Wackerbarth, H.; Grubb, M.; Wengel, J.; Chorkendorff, I.; Ulstrup, J. *Surf. Sci.* **2006**, *600*, 122.
(7) Peterson, A. W.; Heaton, R. J.; Georgiadis, R. M. *Nucleic Acids Res.* **2001**, *29*, 5163.
(8) Herne, T. M.; Tarlov, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 8916.
(9) Wackerbarth, H.; Marie, R.; Grubb, M.; Zhang, J.; Hansen, A. G.; Chorkendorff, I.; Christensen, C. B. V.; Boisen, A.; Ulstrup, J. *J. Solid Sate. Electrochem.* **2004**, *8*, 474.
(10) The sequence of HS-10A is HS−$(CH_2)_6$-5′-AAA AAA AAA A.
(11) Poirer, G. F. *Chem. Rev.* **1997**, *97*, 1117.
(12) Zhang, J.; Chi, Q.; Ulstrup, J. *Langmuir* **2006**. In press.
(13) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Bioconjugate Chem.* **1999**, *10*, 419.
(14) Liu, T.; Barton, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 10160.
(15) Kuznetsov, A. M. and Ulstrup, J. *J. Phys. Chem. A* **2000**, *104*, 11531.
(16) Schuster, G. B., Ed. *Top. Curr. Chem.* **2004**, *236*−*237*.

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