DNA on Gold Surfaces

Dynamics of Ordered-Domain Formation of DNA fragments on Au(111) with Molecular Resolution**

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Molecular alignment or recognition between reacting biological molecules and macromolecules such as protein–protein and DNA–protein interactions, or DNA hybridization with electrostatic interactions as broadly controlling factors is

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[**] Financial support from the Danish Technical Science Research Council is acknowledged. We also wish to acknowledge discussions with Dr. Richard J. Nichols, University of Liverpool and Rodolphe Marie, The Microelectronics Center, Technical University of Denmark.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/ange.200352146

crucial both in natural biological processes and in biotechnological applications. This applies for example, to DNA-based biosensors, in which subtle control of probe immobilization is required to ensure suitable adsorbate oligonucleotide orientation and accessibility of complementary strands or other substrate molecules.[1] Electric fields caused by an electrochemical electrode in contact with the natural aqueous buffer media here offer a highly convenient tool.

However, efforts in DNA-based biosensor technology towards the nanoscale and single-molecule levels require: first that single-crystal, atomically planar electrode surfaces be the environment for biomolecular function. Second, chemical linking and some degree of supramolecular order must prevail, otherwise adsorbate structures are too conformationally labile for functional biomolecules to be imaged.[2,3] We have previously exploited the high sensitivity of single-crystal electrochemistry and the high resolution of scanning tunneling microscopy directly in aqueous buffer (in situ STM) to study assembled monolayers of several proteins.[2–6] These combined techniques disclosed dense monolayers of the proteins, which could be structurally mapped to single-molecule resolution in their full functional state. Herein, we report that single-crystal electrochemistry and in situ STM are introduced to characterize surface-bound thiol-modified short oligonucleotides. We have focused on single-strand oligonucleotides with ten adenine nucleobases (HS-10A) and with a single adenine base (HS-A) to which a hexamethylene thiol linker is covalently attached at the 5′-end. The oligonucleotide strand is short enough that details about interfacial structural and dynamic behavior can be obtained compared with the complex macromolecular structure of longer DNA-fragments. The strand is, however, long enough to offer insight into oligonucleotide collective properties and novel structural features compared with single-nucleotide bases.

The oligo- or mononucleotide was first adsorbed at open circuit potential by immersing the electrodes into the appropriate solution. Figure 1 A shows cyclic voltammograms (CV) of HS-10A and HS-A in the potential range from $+0.6$ to -0.9 V. All potentials are referenced to saturated calomel electrode (SCE). HS-10A (a) and HS-A (b) show a dominant peak at -0.671 V and -0.675 V, respectively. The latter peak has a shoulder at the negative side of the peak potential, which is also sometimes observed for HS-10A. With reference to the in situ STM data (see below) the shoulder can be assigned to the coexistence of ordered adsorbate domains separated by regions of disordered adsorption of variable abundance. We have also recently observed such a pattern for *N*-phenyl-mercaptoacetamide disulfide on Au(111).[4] Figure 1 B shows three consecutive scans of HS-10A. The -0.671 V peak has almost disappeared in the second and completely in the third scan. The data in Figure 1 point unambiguously to the strong -0.671 V peak being caused by reductive desorption of HS-10A, that is, a one-electron reduction of the Au–S bond and release of the adsorbed molecules.[7a] The formation of the Au–S bond is confirmed by X-ray photoelectron spectroscopy (XPS; see Supporting Information). The coverage of the thiol-linked oligonucleotide can be determined from the Faradaic charge associated

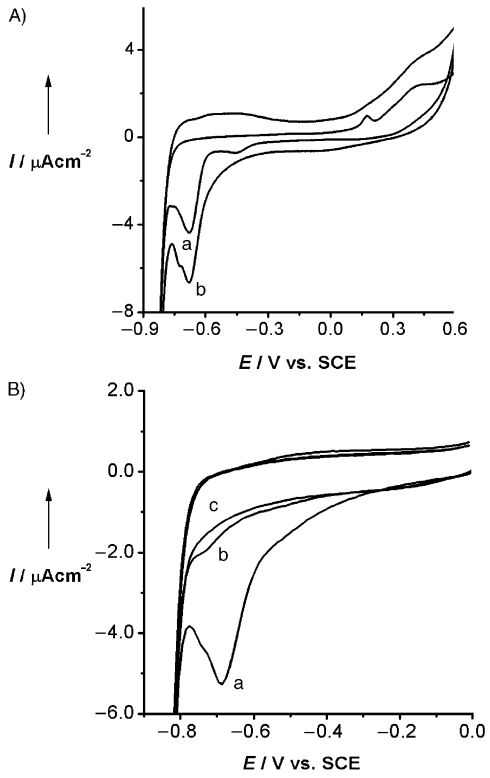


Figure 1. Cyclic voltammetry of reductive desorption in 0.1 M phosphate, pH 6.9. Scan rate 10 $mV s^{-1}$. (A) CV of HS-10A (a) and HS-A (b) adsorbed on Au(111). (B) First scan (a), second scan (b), and the third scan (c) of HS-10A reflect the desorption of the molecules from the surface.

with the voltammetric peak. Peak integration gives 27 ± 5 and 28 ± 4 $\mu C cm^{-2}$ for HS-10A and HS-A, respectively.

The reductive-current peak of organic-thiol layers has two components. The dominant component stems from interfacial charge transfer from the electrode to chemisorbed thiolate, but there is also a capacitive component caused by the formation of the aqueous double layer on the uncoated gold surface after thiol desorption.[7b,c] The non-Faradaic contribution can be estimated to be 1.9 $\mu C cm^{-2}$ for HS-10A by interfacial capacitance data (see Supporting Information). Hence, the resulting coverage is 260 ± 60 $pmol cm^{-2}$. The coverage is significantly higher than values reported by Tarlov and co-workers for longer single- and double-strand (25 bases or base pairs), who found coverages in the range 10–90 $pmol cm^{-2}$, and by Demers et al., who found values of ≈ 20 $pmol cm^{-2}$ for 12-base oligonucleotides.[1,8,9] The coverage is, however, determined by a variety of different parameters such as adsorption time, ionic strength, oligonucleotide length and base sequence, and electrode surface morphology. The research groups of Tarlov and Demers used Au-film substrates treated by "piranha solution", which is known to give a surface morphology quite different from the atomically planar Au(111) surface used in this work. A thiol-modified single-stranded oligonucleotide with 25 bases on Au(111)-surface gives similar coverages as HS-10A (unpublished results). The corresponding double-stranded oligonucleotide also provides a high coverage, which is in accordance with the coverage reported by Kelly et al.[10] We have focused

on the oligonucleotide adsorption features on a well-defined Au(111) electrode surface and the effect of the electrode polarization on the adsorbate structural organization.

A simple estimate shows that densely packed oligonucleotides in an upright or tilted position, based on the assumption that a cylindrical geometry with a diameter of 10 Å gives a coverage very close to 210 pmol cm^{-2}. Recumbent or flat-lying oligonucleotides give significantly lower coverages, that is, between 40 and 80 pmol cm^{-2}. In an upright/tilted position the coverage would, moreover, be little affected by the number of adenines. The almost identical charges determined from reductive desorption of the gold–sulfur bond for HS-A and HS-10A therefore substantiate an upright or tilted position of the adsorbates, bound to the surface solely by the thiol-linker group.

In situ STM images show the electronic structure and electronic conductivity of the adsorbates.[5,11] The properties are also controlled by the molecular-adsorbate conformation. Packing constraints in the adlayer can, for example, impose molecular conformations with poor electronic conductivity unfavorable for in situ STM imaging.[12] Reliable imaging also requires that environmental conditions for the formation of monolayers of some lateral order are defined. Spatial constraint of the conformationally labile adsorbate molecules packed into dense, closely spaced layers or domains with long-range order by collective lateral interactions is hence essential for robust high-resolution imaging.

Figure 2 shows two representative in situ STM images of HS-10A adsorbed at open circuit potential on a Au(111)-electrode surface in contact with aqueous buffer. The open-circuit potential was measured to be 0.19 V. Extensive oligonucleotide adsorption is clearly apparent but with little or no long-range structural order at the sample potential −0.21 V (Figure 2A). Clearly ordered domains, however, appear when the sample potential for the same sample is lowered in situ to −0.61 V, Figure 2B. The sample electrochemical potential is thus a crucial determining factor in the adsorption dynamics and the resulting supramolecular two-dimensional HS-10A adsorbate organization on the Au(111)-surface.

Figure 3 shows high-resolution in situ STM images of other features of HS-10A domains adsorbed under potential control at −0.61 V and recorded at −0.21 V, still significantly lower than the open circuit potential. The character of the observed domains is unchanged in the potential range from −0.61 to −0.21 V but a better quality image is achieved at −0.21 V. A further increase to slightly positive potentials results in the slow disappearance of the domains leaving the adlayer in an entirely disordered state but with the same high overall coverage as in the ordered domains. When the potential was set back to −0.61 V, an ordered structure was reconstituted, thus demonstrating the reversibility of the potential-induced domain formation. Figure 3A shows long-range adsorbate order and pits typical for adsorbed organic-thiolate layers on Au(111) surfaces located at the domain boundary regions. The domains are oriented with an angle of almost 60° to each other and follow the triangular Au(111) structure, which is clearly seen underneath the adsorbate layer. The high resolution in Figure 3B displays bright spots organized in rows with lower contrast in between. The

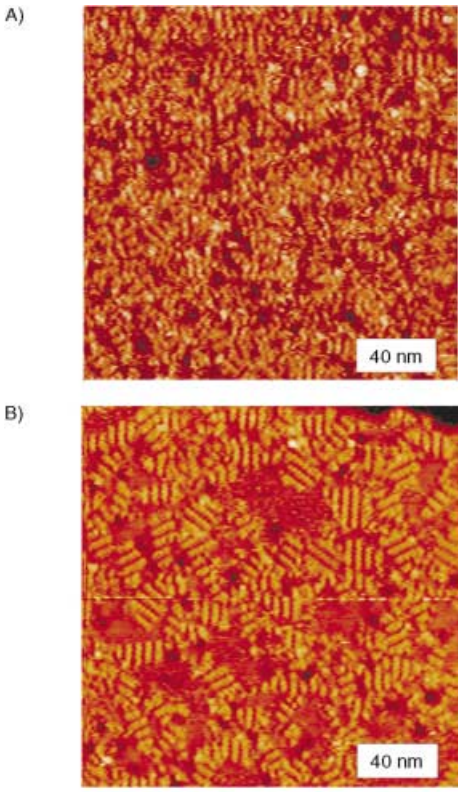


Figure 2. In situ STM images of HS-10A in 0.01 M phosphate buffer, pH 7.1, recorded in the constant-current mode, tunneling current 0.5 nA and bias voltage −0.15 V. The oligonucleotide was adsorbed under open circuit potential and scanned at the sample potential −0.21 V versus SCE. (A) By decreasing the sample potential to −0.61 V versus SCE (B) domain formation, displayed by areas with ordered structure, was observed.

distance between the spots along a row is about 5 Å and the spacing between the spots in parallel rows is 11 Å. This is clearly reflected by the height profiles along and orthogonal to the rows (Figure 3B insets). The rows form a $(\sqrt{3}\times 4)$R30° surface lattice, with a total coverage of 288 pmol cm^{-2}.[13] This is close to the coverage of 260 ± 60 pmol cm^{-2} determined by CV, especially in view that the coverage calculated from the surface lattice represents only the ordered structure inside the domains (50 nm^2 compared to the electrode surface 0.2 cm^2) and not the inter-domain region, pits, steps, and disordered areas. The close values of the coverage obtained by CV and in situ STM thus point to the overall highly uniform character of the electrode surface. The highly ordered-surface lattice of HS-10A was not observed for thiol-free 10-A oligonucleotide adsorption under electrochemical potential control. This emphasizes the fundamentally different adsorption modes for thiolated and thiol-free 10-A oligonucleotide forms.

The length of possible flat-lying oligonucleotides on the surface would affect the formation of the domains. The HS-A nucleotide adlayer was, therefore, also mapped by in situ STM. Potential-controlled adsorption of HS-A showed clearly ordered domains with the same $(\sqrt{3}\times 4)$R30° surface lattice as HS-10A (see Supporting Information). The same surface lattice for the thiolated mono- and oligonucleotide

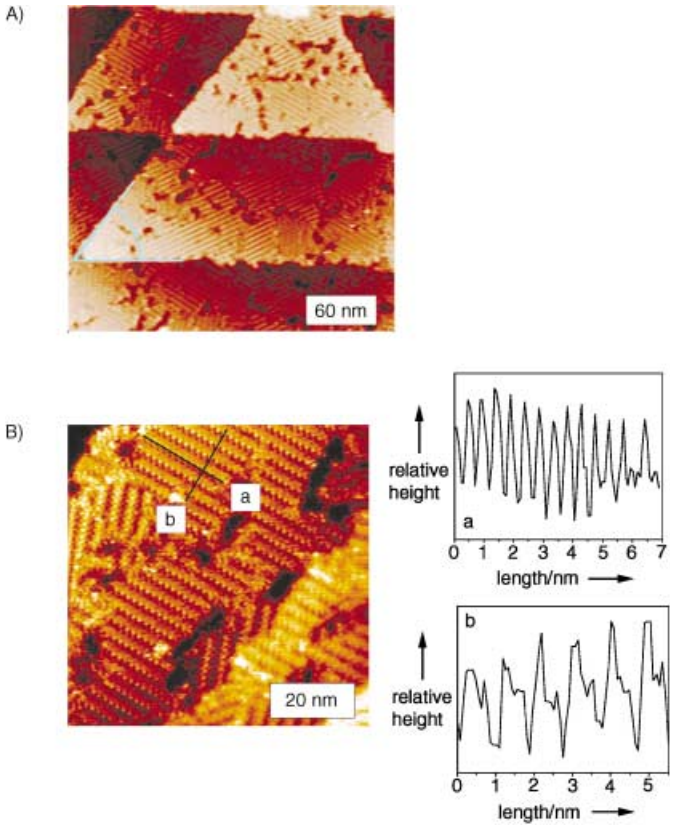
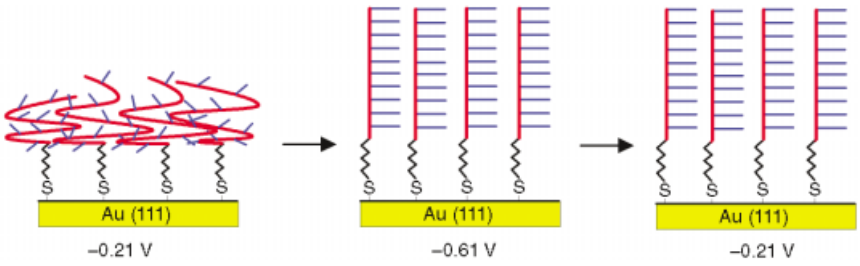


Figure 3. In situ STM images of HS-10A in 0.01 M phosphate buffer, pH 7.1, obtained in constant-current mode. The HS-10A layer was assembled at −0.61 V and scanned at −0.21 V sample potential versus SCE. The bias voltage was −0.15 V. A) The oligonucleotide adlayer follows the triangular Au(111) structure underneath (angle is indicated in blue), scan area 60×60 nm^2, tunneling current 0.3 nA. B) The bright spots represent single oligonucleotide molecular units, scan area 20×20 nm^2, tunneling current 0.8 nA. Relative height profiles along the lines a and b indicated in the image.[14]

can best be explained by perpendicular or tilted adsorption of both adsorbates, which are attached to the electrode surface solely through the thiol linker. The bright spots are likely to represent thiolate adsorbed on three-fold hollow sites, that is, in the "hole" between three adjacent surface Au-atoms. These holes are better conducting sites for STM for adsorbed thiolate than bridge states, that is, bridge sites between a pair of neighboring Au-atoms. The weaker contrasts represent the nucleotide part but the similarity between HS-10A and HS-A could imply that only residues close to the thiolate group are monitored.

Taken together the voltammetric data and the in situ STM images point unambiguously to a densely packed adlayer for thiolated mono- and oligonucleotides, linked to the gold surface solely through the thiol group. The organization of the oligonucleotides can be illustrated by the Scheme 1. Capacitance data suggest that the potential of zero charge of the Au(111)-electrode in 0.1M phosphate solution is about 0.3 V (see Supporting Information). Oligonucleotide adsorption either at the open-circuit potential or at controlled, more negative potentials is thus at a negatively charged electrode surface. Specific adsorption by thiolate linking is then likely to be favored over nonspecific adsorption because of electrostatic repulsion of the negatively charged oligonucleotide backbone from the electrode surface. Densely packed monolayers of HS-10A or HS-A with the 10-A backbone oriented towards the solution, which are not in direct contact with the Au(111)-surface are therefore expected, but the solvent-exposed backbone would still be disordered in random conformations close to the potential of zero charge. The electrochemical potential shifts to more negative values, thus increasing the electric field in the interfacial region. This would, first, push the oligonucleotide backbone further into the solution, and second, induce conversion from the coiled conformation towards an extended, more upright conformation. A potential-dependent orientation of thiol-modified double-stranded oligonucleotides has also been shown by electrochemical-potential-controlled atomic force microscopy.[15] In either case the intermolecular electrostatic repulsion between the strongly charged oligonucleotide anions would be screened by a network of counter ions, which can even be expected to stabilize the domain formation, analogous to cation-induced DNA aggregation (see below).[16] The strong collective intermolecular interaction would also induce Au–S bond breaking and reformation during the domain-ordering process. This would most likely involve Au–S bond labilization and some degree of adsorbate surface diffusion. The energies of the electrostatic backbone repulsion from the surface and the surface diffusion are comparable and would roughly cancel out each other.

Several other effects would reinforce this conversion. One is exposure of the negatively charged backbone phosphate groups, with favored solvation compared to the coiled conformation. Another effect would be stabilization of the extended orientation by hydrophobic stacking or hydrogen bonding between the adenines. Both thermodynamics and kinetics of the folding of a single-strand polyadenine with 16 adenines and short complementary sequences at each end into a roughly circular molecular beacon was also recently found to show molecular rigidity originating from base stacking. The stacking energy corresponding to ten adenine



Scheme 1. Orientation of HS-10A oligonucleotide adsorbed in ordered domains on the Au(111)-electrode. Repulsion between the phosphate backbone and the highly negatively polarized surface forces the oligonucleotide into an extended position. Hydrophobic base stacking and lateral hydrogen bonding between neighboring units stabilize the adsorption mode of the extented HS-10A.

bases comes close to the surface-diffusion activation energy and therefore contributes significantly to the ordering process. Similar folding of polythymine showed no rigidity.[17]

A mechanistic view of the disorder–order transition therefore emerges where the thiol-modified oligonucleotides are first adsorbed specifically at high surface coverage but in random conformations on the solution side. Stacking, hydrogen-bond formation, and other interactions favorable for ordered-domain formation are kinetically hindered in this state. Subsequent backbone repulsion at large negative potentials ushers the adsorbed oligonucleotides into extended conformations in which stacking and other lateral interactions are much less hindered, thus leading to facile domain formation. Electrostatic triggering by the electrode-surface charge is thus a key feature in maintaining the ordered-surface adsorbate domains.

The results reported are the first case for the formation of domains of specifically adsorbed thiol-modified oligonucleotides with long-range order mapped to single-molecule or higher resolution. The basis for this novel observation is the use of high-quality single-crystal electrode surfaces and electrochemical-potential control of the adsorption process. Previous reports have mostly been based on polycrystalline electrodes or etched gold films[1,10,18] giving rough surface morphologies that are hard to reproduce.[19] A study based on annealed commercial Au-films disclosed a dense monolayer but not domain formation nor single-molecule resolution.[15] These results add to evolving high-resolution DNA-related science and biotechnology towards the nanoscale and single-molecule levels.[20–22] Single-molecule, *ss*- and *ds*-oligonucleotide molecular electronic-conductivity mechanisms is immediately one such area. Two-dimensional or columnar DNA-based molecular aggregation induced by transition-metal complexes, presently in intense focus, is another area in which single-molecule resolution by the comprehensive approach suggested could be within reach.[16] Systematic extension to longer and variable-base composition would finally offer novel nanoscale biotechnological importance to single-molecule hybridization of immobilized DNA-based molecules. This approach requires, however, that studies of the specific adsorption mode and the long-range domain-order phenomenon observed for the simple repetitive 10A sequence be extended to variable-length and base-sequence oligonucleotides. The thiol-modified 25-base pair oligonucleotide from the breast-cancer-susceptibility gene (BRCA1) has been shown by single-crystal voltammetry and XPS to display the same high-coverage binding mode as the shorter HS-A and HS-10A oligonucleotides.[23] In situ STM of this sequence is, however, not presently available.

Experimental Section

Hexanesulfonyl mono- and oligonucleotides modified at the 5′ end were obtained from TAG Copenhagen. The sample quality was checked by matrix-assisted laser desorption ionization time-of flight (MALDI-TOF) mass spectroscopy. K_2HPO_4, and KH_2PO_4 were of "superpure" quality. Millipore water (Milli-Q-Housing) was used throughout.

Single-crystal gold electrodes for electrochemistry were prepared as bead electrodes by the method of Clavilier and Hamelin.[24a,b] The hanging meniscus method was used in voltammetric measurements. Cyclic voltammograms were recorded by using an Autolab system (Eco Chemie, The Netherlands). A coiled bright platinum wire and a saturated calomel electrode were used as counter and reference electrode, respectively. The medium was 0.1M K_2HPO_4/KH_2PO_4 buffer, pH 6.9. The solutions were deoxygenated by purging the solutions with purified argon prior to use and an argon atmosphere was maintained above the solutions during experimental recordings.

In situ STM was recorded by a PicoSPM instrument (Molecular Imaging Co., USA) with a bipotentiostat for independent control of substrate and tip potential, and of the in-house built three-electrode KEL-F cells. The constant-current mode was used. The substrate was a Au(111)-disc prepared and checked as previously reported.[25] Reference and counter electrodes were platinum wires. 0.01M phosphate (pH about 7.0) was supporting electrolyte. Tungsten tips were prepared and coated as previously.[25] The disc or bead electrodes were immersed in buffer solution, 0.01M K_2HPO_4/KH_2PO_4, pH 6.9, containing 1 μM oligonucleotide, and soaked for three to five hours at room temperature. After completion of the surface immobilization, the samples were thoroughly rinsed with Millipore water. Glassware and other utensils were cleaned as previously reported.[6]

Received: June 16, 2003
Revised: August 29, 2003 [Z52146]

Keywords: adsorption · electrochemistry · oligonucleotides · scanning probe microscopy · self-assembly

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[13] The $(\sqrt{3}\times 4)$R30° notion implies that the adsorbate molecules are organised in a rectangular unit cell in which each molecule is separated by four Au atoms along the atomic rows of the face-centered cubic Au(111)-lattice (4×2.885 Å $= 11.54$ Å), and by $\sqrt{3}$ times a Au-atom diameter perpendicular to this direction.
[14] The height profile is shown as relative apparent heights along and perpendicular to the atomic rows of the Au(111)-surface.

The absolute heights can only be obtained subject to details of the interfacial electronic tunneling mechanisms.
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