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Dynamic Electrical Switching of DNA Layers on a Metal Surface

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ABSTRACT

We report on the dynamic control over the orientation of short oligonucleotide strands which are tethered to gold surfaces in electrolyte solution. By applying alternating electrical bias potentials to the supporting electrodes we are able to induce a switching of the layer conformation between a "lying" and a "standing" state, simultaneously monitored in a contactless mode by fluorescence techniques. We demonstrate that our electrooptical experiments allow for an in-depth investigation of the intriguing molecular dynamics of DNA at surfaces and, moreover, how the dynamic response of these switchable biomolecular layers opens new prospects in label-free biosensing.

Monolayers of DNA immobilized on solid substrates are of considerable importance not only for applications as DNA-based sensors and microarrays[1−4] but also for investigations of the complex behavior of polyelectrolyte molecules at interfaces.[5,6] Active manipulation of DNA on surfaces[7−11] may significantly enhance the functionality of these grafted layers.

The polarization of a solid/liquid interface involves accumulation of ions in solution adjacent to the surface to compensate for the induced charge on the metal surface. Placing a charged macromolecule within this environment by grafting it to the surface at one end allows for an efficient electrical manipulation at low electrode potentials, taking advantage of the high electric field strength within the interface region. A particularly appealing yet undisclosed aspect is to investigate the dynamics of the surface-tethered macromolecule as it is repelled from or attracted to the surface upon reversing the charge of the metal. Moreover, given that such dynamic properties should susceptibly depend on the intrinsic molecular characteristics (i.e., molecular weight, size, charge, etc.) functional probe layers of this kind would directly meet potential applications in biosensing (e.g., the label-free detection of specific affinity binding reactions).

In this contribution, we introduce a biological nano-electro-mechanical-optical system (NEMOS); that is, we electrically induce a rotation of oligonucleotides tethered to gold surfaces while probing the orientation of the nanometer-sized molecules using optical techniques. We present studies on 24-mer oligonucleotides in their single stranded (ss) as well as double stranded (ds) conformation; for fluorescence detection, the DNAs were dye labeled (Cy3) at their 3′ end, while the opposing 5′ end was modified with a thiol linker to chemically graft the strands to gold surfaces. DNA was obtained from IBA GmbH in Goettingen, Germany, and the sequence of the 24-mer single stranded (ss) oligonucleotides was 5′ HS-$(CH_2)_6$-TAG TCG GAA GCA TCG AAG GCT GAT-Cy3 3′. Details of the DNA immobilization on Au surfaces are described elsewhere.[12] Briefly, the surface coverage of DNA-molecules was adjusted by varying the following parameters during the self-assembly process from aqueous solution: assembly time (5 s − 2h), DNA concentration (0.05−10 μM), salt concentration (3−1000 mM), and salt valence (NaCl and $MgCl_2$ for monovalent and divalent cations, respectively). The influence of the assembly time and the DNA concentration can be described fairly well in terms of (diffusion limited) Langmuir adsorption theory. The salinity of the aqueous electrolyte solution bears a particular importance since higher salt concentrations (as well as salt valence) facilitate an enhanced screening of the DNA's intrinsic negative charge. This has a pronounced effect on the layer packing density which is influenced by the mutual electrostatic repulsion of neighboring DNA strands. Therefore, layers of dilute surface coverage, as eligible for the distinct observation of "DNA-switching", were prepared at low concentrations of monovalent salt (<50 mM). Following DNA immobilization, mercaptohexanol (MCH) was coadsorbed to prevent nonspecific DNA−Au interactions by formation of a mixed DNA/MCH monolayer.[13] Upon requirement, hybridization of ss-DNA was carried out by exposing the surface-grafted layers to a large excess of

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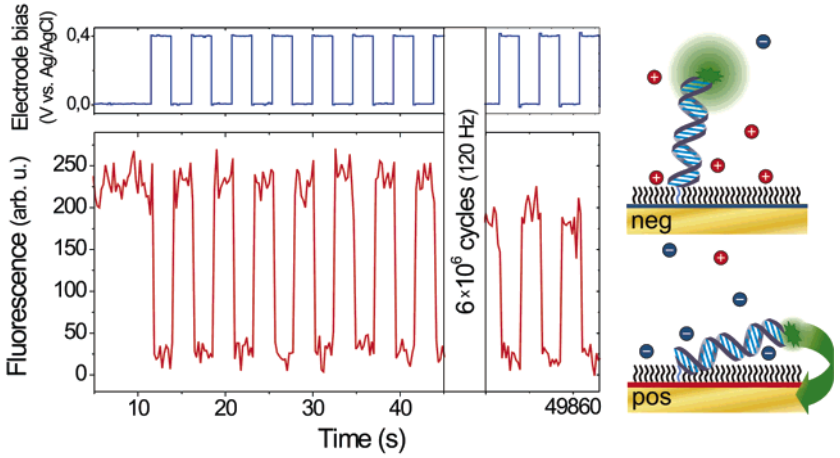

Figure 1. Electrically induced, persistent switching of a DNA layer (double stranded, 24-mer) on a Au surface monitored by optical measurements. Left: The fluorescence intensity observed from the dye-labeled DNA layer alternates upon periodically reversing the electrode charge. Right: Negatively biased electrodes repel the likewise charged DNA strands, bright fluorescence is emitted from the dye attached to the DNA's top end. Positive surface charge attracts the strands and due to the close proximity to the metal efficient energy transfer from the excited dye to the Au results in a substantial quenching of fluorescence. Note that the layers maintain their functionality over millions of cycles (>13.8 h) showing outstanding persistency. No indications for desorption of molecules have been found. Salt in solution: [Tris] = 10 mM. Molecule surface density: 5×10^{15} m^{-2}.

complementary DNA in solution ([Tris] = 10 mM, [NaCl] = 1 M, pH = 7.3) for 1.5 h. Afterward, the electrodes were thoroughly rinsed with buffer solution ([Tris] = 10 mM, pH = 7.3, [NaCl] = 50 mM]). DNA coverage was quantified routinely using electrochemical methods as introduced by Steel et al.[14] MCH adsorption was performed routinely prior to electrochemical measurements.

After preparation, the samples were installed in an uncapped liquid cell (continuously purged with Argon gas) which allowed for optical as well as electrochemical measurements. Monovalent salt in the buffered solution (pH = 7.3) was Tris (up to 10 mM) and additional NaCl, when required. For electrochemical experiments, a potentiostat was utilized to control the potential of the Au work electrodes with respect to a Ag/AgCl reference electrode using a Pt wire counter electrode.

Fluorescence measurements were conducted by positioning optical fibers atop the electrode.[15] An Ar^+−laser (λ = 514 nm) was used for optical excitation. For detection, the light was coupled into a spectrometer (set to the Cy3-peak-emission wavelength, 560 nm) equipped with a cooled photomultiplier. Frequency response analysis was performed using a lock-in amplifier to control the potentiostat and enable phase-sensitive detection of the optical signal. Reference measurements of unmodified Au surfaces were used for background correction.

Figure 1 depicts the electrically induced and, simultaneously, optically detected switching of DNA orientations on a gold surface. Here, we observe the fluorescence intensity emitted from the dye-labeled DNA-layer while alternating the bias potential applied to the supporting Au electrodes with respect to the point of zero charge (~0.2 V vs Ag/AgCl reference). Depending on the charging state of the surface, the intrinsically negatively charged DNA is either driven away from, or pulled toward the electrode and hence adopts an upright or considerable tilted orientation for negative and positive bias, respectively.[8] Optical probing of the DNA orientation takes advantage of nonradiative energy transfer from the excited dye molecule (attached to the DNA's top end) to the metal.[16,17] Since this process imposes a nonlinear distance-dependence on the dye's quantum yield (QY), the observable fluorescence intensity (F) not only varies with the number of molecules per area (σ) but depends significantly on their orientation relative to the surface. To a good approximation, it can be written as

$$F \propto \sigma QY(z) \propto \sigma \cdot z^3 = \sigma(l_{\mathrm{DNA}} \sin \theta)^3 \qquad (1)$$

where z, l_{DNA}, and θ, are the dye−metal distance, DNA effective length, and its angle to the surface-plane, respectively. As a consequence, bright fluorescence can be observed for upright strand orientations, whereas the emission from tilted oligonucleotides is substantially quenched (the fluorescence intensities deduced from Figure 1 for negative and positive bias, i.e., $F^- \approx 10F^+$, correspond to orientations of $\theta^- \approx 90°$ and $\theta^+ \approx 25°$, respectively).

Most remarkably, the switching of DNA orientations shows outstanding persistency and stability owing to a suitable, gentle choice of electrochemical potentials that practically prevents desorption of molecules from the surface as well as degradation of the dye. The slight reduction in signal amplitude after nearly 14 h of continuous switching and simultaneous illumination with laser light depicted in Figure 1 can be attributed to photobleaching (moving the illumination spot on the electrode restores the initial fluorescence). Noteworthy, the strong energy transfer to the metal substrate has a beneficial effect on the photostability of the dye, i.e., as a result of the reduced average excited-state lifetime, photobleaching reactions are slowed.

In addition to an appropriate choice of electrochemical parameters, considerate tuning of monolayer properties is

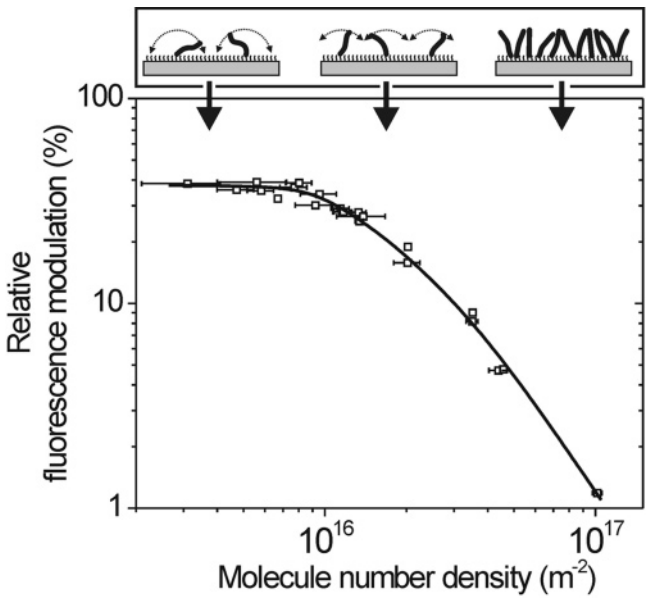

Figure 2. Influence of the packing density of molecules within layers of 24-mer single stranded DNA on the observable fluorescence modulation ($\Delta F/F_{avg}$). The top inset illustrates how steric interactions between adjacent molecules constrain the attainable free gyration of individual strands. The solid line is a guide to the eye; salt in solution: 60 mM; $E_{DC} = 0.2$ V vs Ag/AgCl, $E_{AC} = 0.2$ V, 0.2 Hz square wave.

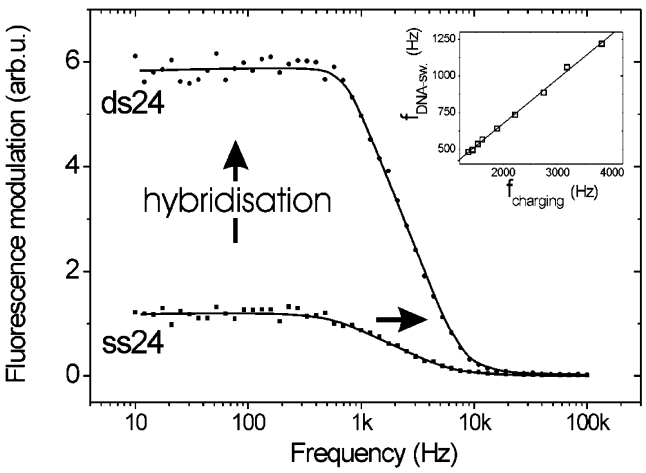

Figure 3. Response of the fluorescence modulation amplitude as a function of the frequency of the driving electrical AC potentials. Upon hybridization, the double stranded 24-mer DNA layer (red circles) shows substantially enhanced switching compared to the single stranded conformation (black squares), accompanied by a shift of its cutoff frequency to higher values. Salt in solution: [Tris] = 10 mM, [NaCl] = 50 mM; electrode bias: $E_{DC} = 0.2$ V vs Ag/AgCl, $E_{AC} = 0.2$ V rms, sine wave. The lines are a guide to the eye. Inset: Correlation between the cutoff frequencies of the DNA switching and the electrochemical charging current (values determined from evaluating the frequency at which the measured phase has shifted 45° from its low-frequency value). Data points are for solution salt concentrations of 3, 5, 7, 10, 15, 25, 40, 60, 100 mM, from left to right, respectively. Molecule surface density: 3×10^{15} m^{-2}.

essential to realize an efficient switching behavior of the tethered oligonucleotides. Figure 2 shows that the mobility of individual strands is strongly affected by the monolayer packing density. For high packing densities ($\approx 10^{17}$ molecules/m^2), steric interactions prevent free gyrations of the strands about their anchoring points and the DNA molecules are forced to take an upright orientation on the surface.[12] Decreasing the coverage density leads to an increase in the rotational mobility of the strands (and hence the maximal achievable switching amplitudes) until eventually mutual collisions do not occur anymore. As expected, this regime of low surface coverage is characterized by a saturation of the switching amplitude (cf. Figure 2). Thus, by adjusting the monolayer surface coverage carefully, it is possible to access cooperative layer behavior as well as to study the dynamics of (an ensemble of) isolated DNA strands.

After having introduced the basic features of the manipulation of oligonucleotide orientations on metal surfaces, the mechanism of the electrically induced switching will be elucidated in more detail in the next paragraphs by discussing frequency response data and electrostatic screening effects. In this respect, it is essential to consider the ion- and potential-distribution in solution at the interface with the biased metal surface. In general, charging an electrode surface that is in contact with electrolyte solution induces a redistribution of the dissolved ions in solution, the so-called "double layer" (DL),[18] which eventually screens the electrode-charge (here, the DNA strands are predominantly located in the diffuse part of the DL, described by the Gouy−Chapman, GC, model). The ionic double layer is associated with an interfacial capacitance, which, upon a change of electrode bias, is charged by transport of ions from solution toward the interface. It will become evident from the following discussion that the formation of the ionic DL significantly influences and determines the DNA switching.

To investigate the dynamic response of the switchable DNA layers, we performed frequency sweeps of the driving AC potentials applied to the supporting Au electrodes (cf. Figure 3). For frequencies up to the kHz regime, we observe constant fluorescence modulation amplitudes, followed by a transition region of decreasing switching efficiency. At high frequencies ($> \approx 10$ kHz), the DNA layer ceases to follow the electrical excitation; a comparison with measurements of the absolute fluorescence intensity indicates that the DNA strands take an average position between a lying and a standing orientation on the surface.

The appearance of a distinct decline in amplitude in the frequency traces of the DNA switching is intrinsically related to the polarization (-time) of the liquid interface. This becomes apparent when comparing the cutoff frequencies of the optically determined DNA switching with those from measurements of the electrochemical current. By gradually increasing the salt concentration in solution (i.e., changing the solution conductance and hence the response time of the electrochemical cell) it is possible to influence the (cell specific) double layer charging time. The inset of Figure 3 illustrates that, first, the dynamics of the DNA switching are virtually perfectly correlated to the formation of the ionic double layer, and, second, that the induced rotation of the DNA strands is lagging behind the DL formation. Therefore, we conclude that the accumulation of ions in the surface region is required to manipulate the DNA strands, which is

comprehensible when considering the enormous electric field strength (and gradient) present within the DL: e.g., the Gouy−Chapman model (adopting a salt concentration of 60 mM and a surface potential of 0.2 V) predicts an electric field strength of 160 kV/cm 20 Å from the surface, whereas its magnitude has decreased to 0.5 kV/cm at 92 Å, which corresponds to the DNA's top end when "standing". Accordingly, the electrostatic energy of a ds-24mer-DNA[19] oriented at, for example, 45° with respect to the surface placed within the GC-layer is (merely) 5 times the thermal energy k_BT (k_B being the Boltzmann constant). Hence it is evident that the strong field within the GC layer is essential to provide the interaction strength required to induce a distinct conformational state. At frequencies too high for the DL to accumulate, electrostatic interactions with the surface are negligible and the DNA's orientation is governed by thermal fluctuations. As a consequence, a manipulation of the layer conformation is not feasible.

Besides the fundamental frequency response, Figure 3 contains another key result of the presented work, namely a comparison of layers of single and double stranded DNA, respectively, showing a remarkable difference in the observed switching behavior. The modulation amplitude exhibits a substantial increase upon hybridization (factor 4.8 at low frequencies), accompanied by a positive shift of the cutoff frequency (500 to 700 Hz). Obviously, the layer of ds-DNA can be switched more effectively and faster than the ss-DNA layer. We note that the higher cutoff frequency is not caused by a change in the double layer charging time, which stayed constant upon hybridization. Thus, it reflects the different molecular properties of the DNA in its single and double stranded conformation, respectively.

We assign the change in the dynamics of ss- and ds-DNA to their distinct flexibilities, as well as to the effective charges of the molecules; both of which are challenging issues to be addressed in the nonuniform environment of a polarized interface. However, in a straightforward view, electrostatic interactions are limited to the fraction of the charged backbone within the very proximity to the surface, due to efficient screening of electrical fields in electrolytes of even moderate salinity (the Debye screening length is approximately 12 Å for 60 mM monovalent salt). As a consequence, one can anticipate a significantly different behavior for the stiff ds-DNA (mechanical persistence length ≈500 Å,[20] cf. the molecular length of ds-24 oligonucleotides is 82 Å), compared to the flexible single stranded form (persistence length <20 Å),[21] for which the upper, unaffected part will be dangling about its lower, electrically stabilized part.

From the discussion so far, it has become apparent that the orientation of the tethered oligonucleotides on the surface is strongly influenced by interactions with the ionic double layer. Its extension can be adjusted by varying the concentration of salt in solution (the Debye screening length is inversely proportional to the square root of the monovalent salt concentration). By this it is possible to tune the magnitude of the electrostatic torque acting on the DNA strand, which leads to a dependency of the modulation amplitude on the solution Debye length, as depicted in Figure 4. For salt concentrations <10 mM and frequencies up to the kHz regime, we find constant, maximal switching amplitudes, denoted by a plateau in that region. Upon increasing the concentration of NaCl in solution when switching at low or moderate frequencies (e.g. 100 Hz), the modulation amplitude starts to decline quickly as the Debye length decreases below approximately 20 Å, which corresponds to 1/4 of the DNA's length. For high salt concentrations (as well as for high frequencies), the modulation of DNA orientations is almost completely suppressed.

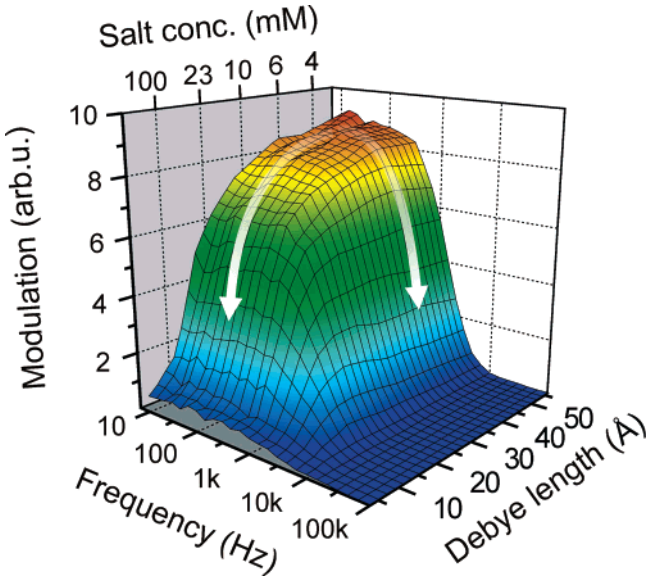

Figure 4. Fluorescence modulation amplitude vs Debye screening length of the electrolyte solution and frequency of the driving AC bias that is applied to the electrodes that support the ds-24-mer DNA layer. In the plateau region of maximal switching amplitude, electrostatic interactions between the strands and the surface prevail over thermodynamically governed, statistical gyrations. Electrode bias: $E_{DC} = 0.2$ V, $E_{AC} = 0.2$ V rms, sine wave. Molecule surface density: 3×10^{15} m^{-2}.

The results of Figure 4 illustrate how the efficiency of DNA manipulation is determined by the balance between statistical gyrations of the strands due to thermal agitation and the total strength of electrostatic interactions. In the case of highly concentrated salt solutions, the electrostatic energy of the DNA strands within the quickly decaying potential is too small to compete with k_BT and so Brownian motion dominates. On the other hand, at low salt concentrations the DL is far-reaching, so that it interacts with many charged sites on the DNA's backbone. The associated energy eventually exceeds k_BT and manipulation of the strands becomes feasible. Once the electrostatic energy is sufficiently dominating over k_BT, the strands will be pushed to a nearly fully upright orientation for negative electrode potentials. Therefore, a further increase in electrostatic repulsion (i.e., diminished salt concentration) cannot lead to an additional increase in switching amplitude and saturation is observed.

In conclusion, we have been introducing the electrically controlled, dynamic, and persistent switching of conformations of oligonucleotide layers which are tethered to Au surfaces in electrolyte solutions. The method proves to be applicable to address the complex behavior and interactions

of (bio-) polyelectrolytes within the polarized region at liquid/metal interfaces; moreover, by tuning the number density of molecules on the surface, properties of the cooperative layer as well as of ensembles of individual, noninteracting molecules can be examined in a straightforward manner.

Investigations of the molecular dynamics show a strong correlation of the DNA kinetics with ions dissolved in solution. At the same time, the remarkable difference in switching behavior for single and double stranded DNA, respectively, indicates the sensitivity of the technique to investigate intrinsic properties of the involved molecules. Hence, this suggests the controlled switching of functional polyelectrolyte layers on solid substrates as a novel, label-free, and, due to its versatility, outstanding method to be employed for biosensing purposes. In principle, its applicability is not limited to DNA sensing but includes all kinds of (bio-) molecules, that, upon specifically binding to the grafted probe layer, would alter its switching dynamics (e.g., by means of the target molecules' charge, size, hydrodynamic properties).

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Supporting Information Available: Reference measurements confirming presented "switching" interpretation. This material is available free of charge via the Internet at http://pubs.acs.org.

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