Molecular Dynamics of DNA–Protein Conjugates on Electrified Surfaces: Solutions to the Drift-Diffusion Equation

A. Langer,†‡ W. Kaiser,†‡ M. Svejda, † P. Schwertler, † and U. Rant‡*,†‡

†Walter Schottky Institute and Chemistry Department, Technische Universität München, 85748 Garching, Germany
‡Dynamic Biosensors GmbH, 82152 Munich, Germany

Supporting Information

ABSTRACT: Self-assembled monolayers of charged polymers are an integral component of many state-of-the-art nanobiosensors. Electrical interactions between charged surfaces and charged biomolecules, adopting the roles of linkers or capture molecules, are not only crucial to the sensor performance but may also be exploited for novel sensing concepts based on electrically actuated interfaces. Here we introduce an analytical model describing the behavior of double-stranded DNA and proteins tethered to externally biased microelectrodes. Continuum electrostatic Poisson–Boltzmann models and the drift-diffusion (Smoluchowski) equation are used to calculate the steady state as well as the dynamic behavior of oligonucleotide rods in DC and AC electric fields. The model predicts the oligonucleotide orientation on the surface and calculates how the increased hydrodynamic drag caused by a protein bound to the DNA’s distal end affects the molecular dynamics of the DNA–protein complex. The results of the model are compared to experiments with electrically switchable DNA layers, and very good agreement between theory and experiment is found. The hydrodynamic diameter of the bound protein can be analyzed from experimental data of the slowed motion of the DNA–protein conjugate with angstrom precision.

INTRODUCTION

Functional surfaces are a key component for various microarray and biosensing techniques. A common feature of these “smart surfaces” is that their interfacial properties can be controlled and even dynamically altered by choice of solvent, temperature, pH, or electric fields. Many schemes involve the immobilization of charged polymers (in particular DNA), and their performance crucially depends on the orientation of the polymer with respect to the surface, which in turn is affected strongly by electrostatics, ligand-induced conformational transitions, or surface heterogeneity. Although much work has been done theoretically and especially on the single-molecule level, the molecular conformation of surface-immobilized oligonucleotides still lacks a complete understanding. Conformational changes in aqueous solution that are driven by Brownian motion or electrically induced occur on time scales that are too fast to be resolved with standard AFM or single-molecule fluorescence techniques. In addition to the dynamic hinge motion, elastic bending of oligonucleotides may also play a significant role. In particular, it has become apparent that although electrical forces between DNA and surfaces seem to be predominant at first glance, thermal motion on these nanoscales cannot be ignored.

Because of their unique mechanical and electrical properties, short double-stranded DNA molecules that are tethered to a gold microelectrode at one end show characteristic conformational changes under externally applied alternating electric potentials. The intrinsically negatively charged molecules are repelled from and attracted to the surface at negative and positive potentials, respectively. A recently developed time-resolved measurement technique revealed that conformational switching occurs on a microsecond time-scale. Tracing the upward and downward motion of the DNA, it was found that the attachment of a protein to the surface-distal end of the DNA slows down the switching dynamics. Previous coarse grain simulations indicated a qualitative correlation between protein size and slowed molecular dynamics of DNA–protein conjugates, but it was not possible to quantitatively describe experimental data.

Here we present an analytical model that describes the dynamic conformation of surface-grafted oligonucleotides on externally biased microelectrodes. Using continuum electrostatic Poisson–Boltzmann models, the free energy of a DNA cylinder end-grafted to a charged surface is calculated. After discussing the equilibrium case, the molecular dynamics of DNA in alternating electric fields are treated, making use of similar theoretical models for diffusion constrained on a spherical surface that have been developed for cyclic voltammetry measurements. The Smoluchowski equation for time-dependent parameters is solved for the appropriate boundary conditions and considering the entropic loss of conformations due to the presence of a surface. Finally, theoretical results from the model are compared to fluorescence experimental data with angstrom precision.

Received: October 28, 2013
Revised: December 30, 2013
experiments: the theory agrees very well with experimental data and allows us to determine the effective hydrodynamic Stokes radius of proteins attached to the DNA.

**FREE ENERGY OF A DNA CYLINDER TETHERED TO A CHARGED SURFACE**

**Entropy.** Double-stranded (ds) DNA is a relatively stiff polymer with a persistence length of ~50 nm (in 0.1 M aqueous NaCl). Thus, short dsDNA can be modeled as a charged cylinder of length $L = nb$ and diameter $2R$ (Figure 1),

where $n$ denotes the number of base pairs, $b$ the base pair spacing (0.34 nm), and $R$ the hydrodynamic radius of B-DNA (1.3 nm). The orientation of an end-tethered DNA cylinder is characterized by the angle $\alpha$ between the DNA and the surface plane (out-of-plane angle). However, because the DNA molecule can freely rotate around the surface normal through its anchor point (azimuthal rotation), the probability that the DNA adopts a certain out-of-plane angle $\alpha$ depends on $\alpha$. The number of accessible microstates (density of azimuthal angles) decreases with $\cos \alpha$ and becomes minimal for $\alpha = 90^\circ$, where only one microstate exists. Thus, the orientation entropy of the DNA cylinder depends on $\alpha$

$$S(\alpha) = k_B \ln \Omega(\alpha) = k_B \ln(2\pi n \cos \alpha) \quad (1)$$

in accordance with the Boltzmann definition, where $k_B$ is the Boltzmann constant, $\Omega(\alpha)$ the angle-dependent number of microstates the DNA can adopt for a given macrostate $\alpha$, and $N$ a density of states. Because $dS(\alpha)/d\alpha < 0$ for all values of $0 < \alpha < 90^\circ$, a surface-tethered DNA molecule is always subjected to an entropic force which attempts to tilt the molecule toward the surface.

**Electric Interactions.** For treating the electrical interactions between the charged surface and the DNA, we make the following approximations: the negative charge of the DNA ($-2e$ per base pair) is considered to be homogeneously distributed along the cylinder axis. This intrinsic charge is partly compensated by counterion condensation, which leads to an effective line charge density of $\rho = -2e/(2b)$, with the Manning parameter $\xi = 4.2$ for monovalent salt. The electric surface potential, which is externally applied to the metal electrode, is screened by counterions in solution. The screening effect causes the effective surface potential $\phi_{\text{eff}}$ to decay exponentially with increasing distance from the surface (Debye–Hückel approximation for low voltages <50 mV and monovalent electrolyte). Using a spherical coordinate system with its origin in the DNA’s anchor point, $\phi(\alpha, \alpha)$ can be written as $\phi(\alpha, \alpha) = \phi_{\text{eff}} \cdot e^{-\kappa^2} \cdot \cos \alpha$, where $\kappa$ is the inverse Debye length which depends on the ionic strength of the solution. For typical electrolyte solutions containing 0.01 M, 0.1 M, or 1 M ($M = \text{mol/L}$) monovalent salt, $\kappa^{-1}$ is 3, 1, or 0.3 nm, respectively. Hence, the potential is strongly localized within the first few nanometers from the surface and drops quickly on the length scale of oligonucleotides. At large distances ($\gg \kappa^{-1}$) from the surface, Brownian motion prevails over electric interactions and dominates the system behavior.

Figure 1. Lollipop model for DNA–protein complexes on an electrified surface. The DNA is modeled as a charged cylinder of length $L$ and diameter $2R$. The negative DNA charge is approximated as a homogeneous charge distribution along the cylinder axis with an effective line charge density $\rho$. The scheme also accounts for a linker between the DNA and the Au surface and a surface passivation layer of equal height which reflects the most common experimental conditions where C6-linkers and mercaptohexanol self-assembled monolayers (SAM) are used. The applied external potential mainly drops across the dielectric SAM so that a strongly reduced effective line charge density $\rho_{\text{eff}}$ adjusts at the SAM/electrolyte interface (see Figure 7 and the Supporting Information for details). Because of the rotational symmetry, in-plane rotations are degenerate in energy and the DNA orientation is characterized by only the out-of-plane angle $\alpha$ between the cylinder and the surface plane. A rotation around the cylinder axis is not allowed for steric reasons because of the asymmetric connection of only one DNA strand to the surface. Inspired by experiments, where capture molecules for proteins are attached at the DNA’s distal end, a bound protein is assumed to be located on top of the DNA helix. The protein is modeled as a sphere of diameter $D$ and charge $Q$.

Figure 2. Free energy and orientation probability density distribution. (A, B) Gibbs free-energy differences $\Delta G(\alpha)$ in units of $k_B T$ and (C, D) probability density distribution $p(\alpha)$ for a positively (attractive) and a negatively charged (repulsive) surface ($\phi_{\text{eff}} = \pm 50 \text{ mV}$) and three different DNA lengths ($n = 24, 48, 72 \text{ bp}$). The gray dashed line in (C) shows the probability density distribution $p(\alpha) = \cos \alpha$ for an uncharged surface. Note the different scales in (C) and (D). $T = 298 \text{ K}$, $\kappa^{-1} = 1 \text{ nm}$ (100 mM monovalent salt).
The energy of the negatively charged DNA cylinder in the exponentially decaying potential, $U(\alpha)$, depends on the out-of-plane angle and can be calculated by integration over the homogeneous line charge density along the cylinder axis.

$$
U(\alpha) = \int_0^L \phi \, dq = \phi_0 \rho \int_0^L \exp[-\kappa(\sin \alpha + R \cos \alpha)] \, dr
= -\frac{2\rho_0}{\xi k_b} \frac{1 - e^{-\xi L \sin \alpha}}{e^{\xi R \cos \alpha} \sin \alpha}
$$

(2)

**Free Energy.** From eq 1 and 2 we can derive the corresponding Gibbs free energy $G(\alpha)$ of a particular DNA orientation as

$$
G(\alpha) = U(\alpha) - TS(\alpha) = \frac{2\rho_0}{\xi k_b} \frac{1 - e^{-\xi L \sin \alpha}}{e^{\xi R \cos \alpha} \sin \alpha}
- k_b T \ln(2\pi e \alpha) \cos \alpha
$$

(3)

where $T$ denotes the temperature.

**DNA ORIENTATION IN EQUILIBRIUM**

In equilibrium, the probability density for a certain orientation of the DNA cylinder is given by the Boltzmann distribution

$$
p(\alpha) = \frac{1}{Z'} \exp\left(-\frac{G(\alpha)}{k_b T}\right)
= \frac{1}{Z} \exp\left(\frac{2\rho_0}{\xi k_b k_b T} \frac{1 - e^{-\xi L \sin \alpha}}{e^{\xi R \cos \alpha} \sin \alpha} \cos \alpha\right)
$$

(4)

where the normalization constants $Z'$ and $Z$ are defined by

$$
\int_0^{2\pi} p(\alpha) \, d\alpha = 1.
$$

Figure 2 shows plots of the Gibbs free-energy differences $\Delta G(\alpha) = G(\alpha) - G(\alpha = 0)$ and the corresponding DNA orientation probability density $p(\alpha)$ as a function of the DNA angle $\alpha$. Irrespective of the applied potential, $\Delta G(\alpha)$ features a maximum for $\alpha \to 90^\circ$ because the gradual reduction of accessible microstates results in an overwhelming entropic penalty.

The contributions from electric interactions become significant for small angles. In the case of a negative applied potential, $\Delta G$ features another maximum for $\alpha \to 0^\circ$ due to electrostatic repulsion. Conversely, applying a positive potential produces a minimum in $\Delta G(\alpha \to 0^\circ)$ due to electrostatic attraction. The resulting distributions of the DNA orientation probability are very different for attractive and repulsive potentials, however. The DNA is repelled from a negatively charged surface, $p(\alpha \to 0^\circ) \to 0$, but at the same time entropy forbs a perfectly vertical orientation, $p(\alpha \to 90^\circ) \to 0$. As a consequence, a broad, asymmetric bell-shaped distribution develops (Figure 2C). Notably, the DNA length does not affect the distribution substantially for repulsive potentials. On the other hand, when attracted to the surface by positive potentials, the DNA lies down efficiently and the orientation distribution of long oligonucleotides (48 and 72 bp) is very narrow and almost parallel to the surface ($0^\circ < \alpha < \sim 1^\circ$). For short 24 bp DNA the distribution spreads out from the surface, which is an entropic effect. While the strength of electrostatic attraction scales with the DNA length (charge), Brownian agitation does not. Thus, short DNA strands, which are not held as tightly by the surface-proximal field, are more prone to “flap away” from the surface.

The influences of the magnitude of the applied electrode potential and screening of the electrolyte solution are depicted in Figure 3. In solutions of low ($\kappa^{-1} = 3$ nm) and moderate ($\kappa^{-1} = 1$ nm) screening, positive potentials of merely a few 10 mV suffice to efficiently hold the DNA in a near-horizontal orientation. For negative potentials, the repulsion efficiency strongly depends on the screening length of the electrolyte: under conditions of low screening the center of the DNA angle distributions reaches $\sim 60^\circ$, whereas at moderate screening it stays below $40^\circ$. Under conditions of strong screening ($\kappa^{-1} = 0.3$ nm), electrostatic interactions between the DNA and the surface are almost completely suppressed and the DNA orientation is hardly affected by the charging state of the surface. These results are in good agreement with previous experimental observations.18,23,37

The average DNA angle $\overline{\alpha}$ can be calculated from the angular distribution function $p(\alpha)$:

$$
\overline{\alpha} = \int_0^{\pi/2} \alpha p(\alpha) \, d\alpha
$$

(5)

For an uncharged surface, $\overline{\alpha} = 32.7^\circ$ and does not depend on the DNA length. Experimentally, tilt angles of oligonucleotides have been determined by neutron reflectivity28 ($\overline{\alpha} \approx 60^\circ$ for $n = 25$ bp) and fluorescence self-interference39 ($\overline{\alpha} \approx 50^\circ$ for $n = 50$ bp and $\overline{\alpha} \approx 40^\circ$ for $n = 21$ bp). The reported values are higher than the theoretical prediction, which is most likely due to the relatively high surface densities in these studies. Steric interactions in densely packed layers result in more vertical DNA orientations; moreover, even if external potentials are not applied intentionally, surface charges of capacitive or chemical origin which can lead to repulsion are almost always present.

Figure 4 illustrates that the DNA must be of adequate length in order to allow for an efficient manipulation of the DNA.

---

**Figure 3.** Influence of the effective potential and electrolyte screening on the DNA orientation. Probability density distributions $p(\alpha)$ are plotted for 48 bp DNA for Debye lengths of (A) $\kappa^{-1} = 3$ nm, (B) $\kappa^{-1} = 1$ nm, and (C) $\kappa^{-1} = 0.3$ nm, corresponding to concentrations of monovalent salt of approximately 0.01, 0.1, and 1 M, respectively. $T = 298$ K. In the white regions, $p(\alpha) > 2$. 

---

The Journal of Physical Chemistry B
orientation. Very short DNAs like 12 bp oligonucleotides do not possess enough charge to generate electrical interactions that are strong enough to prevail over Brownian agitation; thus, short DNAs do not align well in the applied electric field. The alignment efficiency improves with increasing DNA lengths, in particular when the DNA is attracted by a positively charged surface. In this case, almost all charges along the lying DNA strand reside within the screening layer and may cooperatively contribute to a strong electrical attraction, holding long strands tightly close to the surface. At repulsive surface potentials, only the surface-proximal base-pairs of the standing DNA strongly interact with the surface field. Hence, a minimal length of ~30 bp seems sufficient to produce maximal repulsion efficiency.

Molecular Dynamics of a DNA Cylinder in Alternating Electric Fields

After having established steady-state solutions of the DNA orientation probability \( p(\alpha) \) for constant electrode potential above, we will now calculate the temporal evolution of \( p(\alpha, t) \) when alternating potentials are applied to the electrode.

Smoluchowski (Drift-Diffusion) Equation. In a system that is neither subjected to entropic constraints nor to external forces (constant free energy), Brownian motion leads to diffusion which can be described by Fick’s law

\[
j(\alpha, t) = -D_1 \frac{\partial p(\alpha, t)}{\partial \alpha}
\]

Here, \( j(\alpha, t) \) is the one-dimensional rotational probability flux of DNA molecules along the \( \alpha \)-direction and \( D_1 \) denotes the DNA’s rotational diffusion coefficient which can be calculated by the formula of Tirado and Torre. Together with the continuity equation

\[
\frac{\partial p(\alpha, t)}{\partial t} = - \frac{\partial j(\alpha, t)}{\partial \alpha}
\]

we obtain the one-dimensional diffusion equation

\[
\frac{\partial p(\alpha, t)}{\partial t} = D_1 \frac{\partial^2 p(\alpha, t)}{\partial \alpha^2}
\]

In the case at hand, the entropy of the DNA cylinder depends on its orientation, \( S = S(\alpha) \), and the DNA is subjected to an electrical potential \( \phi = \phi(\alpha, t) \) that varies with time; hence, \( G = G(\alpha, t) \). This gives rise to a torque \( -\partial G(\alpha)/\partial \alpha \) that acts on the DNA and results in a drift term which must be added to eq 6

\[
j(\alpha, t) = -D_1 \frac{\partial p(\alpha, t)}{\partial \alpha} - \frac{1}{\zeta} \frac{\partial G(\alpha)}{\partial \alpha} p(\alpha, t)
\]

\( \zeta \) denotes the rotational friction coefficient of the DNA. Using the Einstein relation \( D_1 \zeta = k_B T \), we obtain the drift-diffusion Smoluchowski equation in one rotational dimension,

\[
\frac{\partial p(\alpha, t)}{\partial t} = D_1 \left[ \frac{\partial^2 p(\alpha, t)}{\partial \alpha^2} + \frac{1}{k_B T} \frac{\partial}{\partial \alpha} \left( \frac{\partial G(\alpha, t)}{\partial \alpha} p(\alpha, t) \right) \right]
\]

Note that because viscous drag dominates in a low Reynolds number regime, the motion of DNA molecules in solution is highly overdamped and we can neglect inertia terms. If no external potential is applied to the electrode, i.e., \( \phi_{\text{eff}} = 0 \), eq 10 simplifies to

\[
\frac{\partial p(\alpha, t)}{\partial t} = D_1 \left[ \frac{\partial^2 p(\alpha, t)}{\partial \alpha^2} + \frac{\partial}{\partial \alpha} \left( \tan \alpha \cdot p(\alpha, t) \right) \right]
\]

with the steady-state solution \( p(\alpha, t \to \infty) = \cos \alpha \), in accordance with eq 4 and in analogy to the entropically driven diffusion of particles. What are solutions to the Smoluchowski equation if the externally applied potential changes over time? Let us first assume an instantaneous potential step with

\[
\phi_{\text{eff}}(t) = \begin{cases} \phi_1, & t < 0 \\ \phi_2, & t \geq 0 \end{cases}
\]

(the experimentally more realistic situation of a finite electrode charging time will be considered later). To obtain \( p(\alpha, t) \) we need to solve eq 10 with a time-dependent free energy \( G(\alpha, t) \) subject to the Neumann boundary conditions

\[
j(\alpha, t) = -D_1 \left( \frac{\partial}{\partial \alpha} + \frac{1}{k_B T} \frac{\partial G(\alpha)}{\partial \alpha} \right) p(\alpha, t) = 0
\]

at \( \alpha = 0 \) and \( \alpha = \frac{\pi}{2} \)

The initial probability density distribution \( p_0(\alpha, 0) \) can be obtained from eq 4, using \( \phi_{\text{eff}} = \phi_1 \). Then, the time-dependent solutions of the probability distribution can be calculated numerically, e.g., with the Python FiPy library for partial differential equations (c.f. Materials and Methods).

First we consider the upward motion: assuming that the electrode potential has been attractive (\( \phi_{\text{eff}} = 50 \text{ mV} \)) for a sufficient amount of time, the DNA orientation \( p \) has equilibrated in the narrowly distributed lying state as shown in Figure 2D. At \( t = 0 \mu s \), the potential is switched to \( \phi_{\text{eff}} = -50 \text{ mV} \), and the DNA is suddenly repelled from the surface. Figure 5 depicts exemplary solutions to eq 10 for 48 bp DNA. In the beginning, \( p(\alpha, t) \) starts as a relatively sharp peak, but the distribution broadens due to diffusion as the DNA molecules rotate away from the surface and emerge from the thin screening layer that mediates strong electric interactions. A new steady state featuring a broad standing distribution is reached after approximately 0.5 \( \mu s \). In contrast to the upward motion, which is of a collective nature, the downward transition features an interesting, noncollective behavior: Figure 5B shows that two DNA subensembles exist at the same time before the system relaxes to the energetically preferred state of lying DNA orientations. While a fraction of the DNA ensemble quickly accumulates in the lying orientation, another substantial
fraction remains in the standing orientation for more than 0.5 μs after switching from repulsive to attractive potentials. This behavior can be rationalized by the following argument: the DNA first needs to diffuse from large to small angles in order to dive into the short-ranged electric field region, where it gets “pulled down” efficiently. This is in agreement with the stochastic interpretation of coarse-grained molecular dynamics simulations.25

As expected, the DNA length affects the upward and downward switching dynamics, cf. Figure 5C,D. Long DNA cylinders move slower than shorter ones, as the hydrodynamic drag increases with DNA length and, conversely, the rotational diffusion coefficient $D_r$ decreases (cf. Table 1). Switching times vary from approximately 0.2 to 3 μs when the DNA is extended from 12 to 72 bp, respectively.

![Figure 5](image)

**Figure 5.** Dynamic response of the DNA orientation upon instantaneous reversal of the electrode potential. (A) Calculated probability density distribution $p(\alpha, t)$ for instantaneous potential steps at $t = 0$ μs: (A) from $\phi_1 = 50$ mV to $\phi_2 = -50$ mV and (B) from $\phi_1 = -50$ mV to $\phi_2 = 50$ mV ($n = 48$ bp). (C, D) Average angles $\bar{\alpha}(t)$ for the upward and downward motions for DNA of different lengths (in base-pairs) for the same potential steps as in (A) and (B). $\kappa^{-1} = 1$ nm, $\eta = 0.89$ mPas ($T = 298$ K). In the white regions, $p(\alpha, t) > 2$.

Table 1. Calculated Rotational Diffusion Coefficients $D_r$ $(\mu s^{-1})$ for Different Protein Diameters $D$ and DNA Lengths

<table>
<thead>
<tr>
<th>$D$ (nm)</th>
<th>$n = 24$ bp</th>
<th>$n = 48$ bp</th>
<th>$n = 72$ bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>bare DNA</td>
<td>2.7</td>
<td>0.49</td>
<td>0.17</td>
</tr>
<tr>
<td>1</td>
<td>1.9</td>
<td>0.38</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>0.31</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>0.25</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>0.79</td>
<td>0.21</td>
<td>0.087</td>
</tr>
<tr>
<td>5</td>
<td>0.62</td>
<td>0.17</td>
<td>0.075</td>
</tr>
</tbody>
</table>

Calculated from Tirado40 and eq 14. $\eta = 0.89$ mPas ($T = 298$ K).

## DYNAMICS OF A DNA–PROTEIN “LOLLIPOP” COMPLEX

Finally, we will expand the model by attaching a sphere of arbitrary size and charge to the distal end of the DNA cylinder (cf. Figure 1). The resulting shape resembles a lollipop, with the sphere representing a globular protein of diameter $D$ and net charge $Q = me$. It corresponds to the experimental situation where a protein binds to a dedicated capture molecule which is attached to the upper DNA end.24,43,44 This modification requires three adjustments to the model, two of which are related to the sphere’s size and one results from its charge.

First, the attached sphere contributes to a higher total rotational friction; hence, $D_r$ is reduced. Using the expression derived by Happel and Brenner,41 the new value of $D_r$ can be calculated as

$$D_r = \left(\frac{n_0 D^3 + 3n_0 D(D/2 + L)^2}{k_B T} + D_r^{DNA}\right)^{-1}$$

(14)

where $D_r^{DNA}$ is the rotational diffusion coefficient of the bare DNA. Table 1 lists rotational diffusion coefficients $D_r$ calculated with eq 14 for different values of $D$ and $L = nb$.

Second, the protein sphere prohibits the DNA from lying down completely on the surface if its diameter is larger than the DNA diameter. This steric restriction can be accounted for by adding a potential barrier term $B(\alpha)$ to the free energy:

$$B(\alpha) = \begin{cases} 0, & \alpha \geq \alpha_c \\ \infty, & \alpha < \alpha_c \end{cases}$$

(15)

where $\alpha_c \approx \sin^{-1}\left(\frac{(D/2 - R)/(L + D/2)}{2}\right)$ for $D \geq 2R$ and $\alpha_c = 0$ for $D < 2R$.

Third, the charge of the protein sphere gives rise to an additional electrical interaction term

$$U_{sphere}(\alpha) = me\Phi_{eff} \exp\left(-k\left[(L + D/2) \sin \alpha + R \cos \alpha\right]\right)$$

(16)

which must be added to the free energy. For simplicity, eq 16 assumes that the effective protein charge $m$ is concentrated in the sphere center and that the sphere’s dielectric properties are the same as those for the surrounding electrolyte. A positively charged protein ($m > 0$) neutralizes some of the negative DNA charges and reduces the strength of the electric attraction or repulsion, while a negatively charged protein ($m < 0$) has an opposite effect. It is enlightening to estimate representative $m$-values from $\zeta$-potential values $\phi_\zeta$ which are typically measured for real proteins. The net charge $m$ of a spherical protein is related to its $\zeta$-potential via

$$m = \frac{\pi \varepsilon_0 \varepsilon_\infty D(2 + \kappa D) \phi_\zeta}{\varepsilon}$$

(17)

with the vacuum permittivity $\varepsilon_0$ and the permittivity of water $\varepsilon_\infty ($~80). Table 2 summarizes protein net charges $m$ calculated with eq 17 for different values of $\phi_\zeta$.

Incorporating eq 15 and eq 16 into eq 3 yields the free energy of a DNA–protein lollipop:

$$G(\alpha) = -\frac{2e\phi_{eff}}{\bar{\zeta}k_B} \frac{1 - e^{-\bar{\zeta}k_B}}{\bar{\zeta}k_B} - k_B T \ln(2\pi N \cos \alpha)$$

$$+ U_{sphere}(\alpha) + B(\alpha)$$

(18)
Table 2. Calculated Net Protein Charges $m$

<table>
<thead>
<tr>
<th>$\phi_c$ (mV)</th>
<th>$D = 2$ nm</th>
<th>$D = 4$ nm</th>
<th>$D = 6$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.6</td>
<td>1.7</td>
<td>3.4</td>
</tr>
<tr>
<td>10</td>
<td>1.1</td>
<td>3.4</td>
<td>6.7</td>
</tr>
<tr>
<td>20</td>
<td>2.2</td>
<td>6.7</td>
<td>13.4</td>
</tr>
<tr>
<td>40</td>
<td>4.5</td>
<td>13.4</td>
<td>27.9</td>
</tr>
</tbody>
</table>

Calculated from eq 17, $\kappa^{-1} = 1$ nm.

Figure 6 shows exemplary solutions of the Smoluchowski equation with the Gibbs energy from eq 18 for a 48 bp DNA molecule with an uncharged protein ($D = 3$ nm, $m = 0$) attached to its upper end. In comparison to Figure S, the DNA–protein complex lags behind bare DNA because of its higher friction. Figure 6C,D depicts the time evolution of the average angle $\alpha$ for differently sized protein spheres ($D = 0, 1, 2, 3, 4, 5$ nm, $m = 0$). Evidently, the switching dynamics slow down with increasing protein diameter. Note also that because of steric collisions with the surface, $\alpha$ is shifted to higher starting values for proteins with $D > 2R$ (i.e., $\alpha_c > 0$). Figure 6E,F shows the effect of a nonzero protein charge on the time evolution of $\alpha$. Whereas a negative protein charge ($m = -10$) simply amplifies the DNA’s negative charge and has almost no influence on the DNA motion, a positive protein charge ($m = 10$) counteracts the DNA and repulsively interacts with a positively charged surface. As the protein is pushed away from a positively charged surface, the DNA–protein complex does not perfectly lie on the surface (the initial value of $\alpha$ is increased). It is also remarkable that the effect of a positive protein charge is more pronounced for the downward switching than for the upward switching, for which the dynamics remain almost unaffected.

### COMPARISON WITH EXPERIMENTS

The steady state and the dynamic evolution of the orientation probability distribution can be calculated with eqs 4 and 10, but $p(\alpha, t)$ cannot be directly measured. Instead, we use a fluorescence energy transfer technique to analyze the extension of a DNA layer from an electrode surface as function of time and applied potential. The experimentally investigated DNA layers comprise approximately 1 million strands, which are self-assembled on a gold microelectrode ($\phi = 120 \mu$m). Special care is taken to keep the DNA surface density very low ($\sim10^{10}$ strands/cm$^2$) so that steric interactions between proximal molecules are avoided and the layer can be regarded as an ensemble of single molecules. For assessing the DNA orientation in real-time, the surface-distal DNA ends are modified with cyanine dyes (Cy3). The intensity of the fluorescence emission depends on the dye’s distance to the gold surface because a resonant energy transfer to the gold strongly quenches the fluorescence when the dye (i.e., the upper DNA end) approaches the surface. In previous work we calculated that the emitted fluorescence $f(\alpha)$ of a Cy3 dye above a gold surface can be approximated by the simple analytical function

$$f(\alpha) = 0.45[1 - \exp(-0.042h(\alpha))]^{3.1}$$

where $h(\alpha) = L \sin \alpha + R \cos \alpha + d$ is the vertical distance of the dye to the surface and $d \approx 1$ nm is the height of the MCH spacer layer. With this function, the calculated probability distribution $p(\alpha, t)$ can be converted to a measurable fluorescence signal $F(t)$ via

$$F(t) = \int_0^{\pi/2} f(\alpha) \cdot p(\alpha, t) \, d\alpha$$

For a comparison of theoretical and experimental data, we evaluate normalized fluorescence intensities $F_{\text{norm}}$ instead of absolute values. To this end, the equilibrated fluorescence levels at attractive potential ($F_\alpha$) and repulsive potential ($F_r$) are measured for $t \to \infty$. $F_{\text{norm}}$ is then calculated by subtracting the minimal intensity ($F_r$) from the fluorescence trace and dividing by the modulation amplitude: $F_{\text{norm}}(t) = (F(t) - F_r)/(F\alpha - F_r)$. This procedure consistently yields values between 0 and 1 and allows us to compare measurements on different electrodes, irrespective of their individual background intensities. We note, however, that the underlying assumption is that all DNA molecules may unrestrictedly react to the applied potential. The presence of high-density clusters in real experiments, for instance, where the motion of individual strands is hampered by neighboring DNAs, would introduce inaccuracies in the comparison of experimental data and theoretical calculations. However, the good agreement between experiment and theory
strongly suggests that this assumption is adequately justified here.

Equation 4 predicts that effective surface potentials of the order of a few tens of millivolts should be sufficient to manipulate the DNA conformation efficiently, but experimentally much higher potentials need to be applied in order to switch the DNA from a standing to a lying orientation and vice versa. This is due to the used mercaptohexanol layer which is needed as both spacer and surface passivation. Because of its dielectric properties it also acts as a serial capacitance, and in fact, the externally applied potential \( \phi_{\text{electrode}} \) mainly drops across this layer. This effect can be translated into a parameter \( \gamma \), which accounts for the dielectric voltage drop. The effective potential at the MCH/electrolyte interface then reads

\[
\phi_{\text{eff}} = \gamma \phi_{\text{electrode}}
\]

(21)

To test the validity of the theoretical predictions for the steady state, we measured the fluorescence emission of a Cy3-labeled 48 bp DNA layer as a function of the applied electrode potential. Figure 7 shows that the Boltzmann distribution accurately reproduces the experimental results. The DNA strands stand on the surface for negative potentials (high fluorescence) and lay on the surface for positive potentials (low fluorescence). A best fit for the dielectric parameter \( \gamma \approx 0.075 \pm 0.005 \) is identified as the best fit. In order to account for the electrochemical potential difference between the gold work and ITO counter electrodes, the theoretical curve has been offset by 230 mV. Electrolyte: 50 mM NaCl, 10 mM Tris buffer, pH 7.4, \( \kappa^{-1} = 1.3 \) nm.

![Figure 7. Steady-state voltage response: theory versus experiment. The fluorescence emission of a Cy3-labeled 48 bp DNA layer was measured and calculated (eqs 4, 19, and 20) as a function of the applied potential. A dielectric parameter \( \gamma \approx 0.075 \pm 0.005 \) is identified as the best fit. In order to account for the electrochemical potential difference between the gold work and ITO counter electrodes, the theoretical curve has been offset by 230 mV. Electrolyte: 50 mM NaCl, 10 mM Tris buffer, pH 7.4, \( \kappa^{-1} = 1.3 \) nm.](image)

We measured the switching dynamics of DNA layers using a setup for time-correlated single photon counting, which has been described in detail previously. In brief, the setup records the arrival time of individual fluorescence photons emitted by the Cy3-labeled DNA layer with respect to the edge of the applied voltage pulse. The DNA oscillation is driven by a square wave potential at 10 kHz while histograms of the fluorescence change during the DNAs’ upward and downward motions are collected.

Figure 8 compares time-resolved experimental and theoretical data for DNA strands ranging in length from 24 to 72 bp. The model describes the motion of oligonucleotides up to \( n \leq 54 \) bp almost perfectly. The experimental fluorescence trace progressively increases before reaching its maximal steepness, which results from the gradual electrode charging process (compare the difference to the abrupt onset that was calculated for a hypothetical instantaneous charging in Figure 4). A charging time of the microelectrode of \( \tau = 1.6 \) \( \mu \)s was measured with an oscilloscope. During the discharging and recharging process, the DNA strands have time to start diffusing away from the electrode before the repulsive field takes effect and additionally pushes the strands upward. As the DNA gradually stands up, more and more DNA charges leave the region of strong electrical repulsion. Consequently, the fluorescence change slows until finally diffusion leads to the steady-state equilibrium distribution.

For long DNA \( (n \geq 60) \), however, the theory diverges from the experiment. We attribute this to the flexibility of the DNA helix and the influence of sequence-dependent curvature. Bending of a polymer becomes significant long before the contour length matches the persistence length (50 nm), and accordingly, a 72 bp DNA strand (24 nm) cannot be regarded as a rigid DNA cylinder anymore. Aspects of this, including the entropic contributions, have also been dealt with by Ambia-Garrido et al. The high shearing forces induced by the strongly repulsive field gradient may lead to additional DNA bending and hence, the fluorescence response lags behind the theoretical prediction.

**Determination of a Protein’s Hydrodynamic Diameter.** Inspired by experiments with proteins binding to the distal end of the DNA, one of the initial goals for the development of
the lollipop model has been the analysis of the protein size from the upward switching curve of a DNA−protein complex. Indeed, using eqs 14 and 18, the hydrodynamic protein diameter $D$ together with the protein charge $m$ are the essential free parameters to be determined. In Figure 9 we use the lollipop model to fit time-resolved experimental data for layers of 48 bp DNA before and after binding to proteins with hydrodynamic diameters ranging from approximately 2 to 7 nm. Proteins were attached to the distal DNA end using different approaches: His-tagged proteins G and L as well as ubiquitin (Ub), interferon-α (INF α), chloramphenicol acetyl transferase (CAT), and protein kinase ERK2 were captured via layers of trisNTA-modified DNA;44 dihydrofolate reductase (DHFR) was bound via methotrexate-modified DNA; and antibiotin Fab fragment was bound via biotin-modified DNA. Cytochrome-C (Cyt-C), carbonic anhydrase (CA), and protein A were covalently attached to the DNA.24 Because of the increased rotational friction of the DNA−protein complexes, the time-resolved upward switching curves lag behind the curves for bare DNA. As expected, large proteins yield large shifts in the rising curves and high values of the parameter $D$ are obtained from a fit analysis. Despite the fact that the molecular weight tends to scale with the hydrodynamic diameter, tertiary protein structures can be very different, so that proteins of almost identical molecular weight may show very different hydrodynamic diameters (e.g., CA and CAT). When the protein diameter is significantly larger than the DNA diameter (2.6 nm), steric interactions with the surface can be observed (Figure 9C,D): Initially, the DNA−protein complexes rise almost as fast as the bare DNA ($t < 1 \mu$s) because of the higher starting angle $\alpha$, which reduces the initial electrostatic attraction (c.f. Figure 6C). Shortly after, however, the motion slows down because of the much higher friction, and the rising curve flattens. Figure 9E summarizes fit results from 11 different proteins and shows an excellent agreement between diameters determined from the switching dynamics and from dynamic light-scattering measurements and literature X-ray diffraction data. On average, the evaluated diameters deviate by less than 0.3 nm from the calibration line.

The lollipop model predicts that the protein charge $m$ has only little effect on the upward DNA switching (cf. Figure 6E). Indeed, all fit curves in Figure 9 were calculated by setting $m = 0$. Even if a protein carries an effective charge, it is quickly lifted out of the region of strong electric interactions by the DNA lever because the electric field decays rapidly above the surface (on the length scale of the Debye length $\kappa^{-1} \sim 1$ nm). Consequently, most of the upward motion is predominantly governed by the electric charges of the DNA. Hence, instead of determining $m$ from time-resolved data, the analysis of steady-
state voltage response measurements (cf. Figure 7) seems to be more promising for gauging the electrical properties of a protein (i.e., a pI value). This shall be investigated in more detail in the future.

CONCLUSION

The presented analytical model describes the steady-state and dynamic behavior of short double-stranded DNA molecules that are tethered to an electrically charged surface. Focusing on the interplay between thermal energy and electrostatic interaction, we could show how the increased hydrodynamic drag caused by a protein bound to the DNA’s distal end affects the molecular dynamics and how the size of the protein can be analyzed from the slowed motion. Furthermore, the model suggests that the protein charge plays a minor role for the molecular dynamics, but rather affects the near-horizontal orientations in the steady-state. Experimental data from time-resolved fluorescence measurements were compared to the theory and found to be in good agreement. Hydrodynamic diameters of different proteins could be determined with an accuracy of 0.3 nm.

Limitations of the lollipop model become apparent when dealing with long oligonucleotides (>60 bp or >20 nm) for which the approximation of a rigid DNA rod is no longer valid. At the same time, the DNA length limits the size of proteins that can be quantitatively evaluated: because of steric restrictions, an effective DNA switching can be achieved only if the protein diameter is significantly smaller than the length of the DNA lever that actuates the protein. The quantitative sizing of very large proteins (D > 10 nm) requires long, mechanically reinforced levers which could be realized by specifically designed DNA nanostructures. In addition to the quantitation of effective protein diameters and the evaluation of DNA lengths, the described analytical principle also enables the detection of changes in protein conformation, given that they alter the protein’s hydrodynamic friction.

This paves the way for a new class of chip-based biosensors which enable the detection of biomolecules and the characterization of their biophysical parameters in the same measurement.

MATERIALS AND METHODS

DNA was obtained from Metabion GmbH, Germany. The sequences of the used oligonucleotide were selected with the intention to minimize the occurrence of complicating secondary structures: 5′ HS C6 TAG TCG TGA GCA CAT GGA CCT GGC Cy3 3′ (24 bp), 5′ HS C6 TAG TCG TGA GCA CAT GGA CCT GGC Cy3 3′ (36 bp), 5′ HS C6 TAG TCG TAA GCT GAT GCT GAT TAG TCG GAA GCA TCG AAT GCT GAT Cy3 3′ (48 bp), 5′ HS C6 TAG TCG TGA GCA CAT GGA CCT GAT TAG TCG TAA GCT GAT GCT GAT TAG GGC Cy3 3′ (54 bp), 5′ HS C6 TAG TCG TGA GCA CAT GGA CCT GAT TAG TCG TAA GCT GAT GCT GAT TAG TCG GAA GCA TCG AAT GCT GAT Cy3 3′ (60 bp), 5′ HS C6 TAG TCG TGA GCA CAT GGA CCT GAT TAG TCG TAA GCT GAT GCT GAT TAG GGC Cy3 3′ (72 bp). DNA probes were end-rafted onto the gold electrode with a picoliter dispensing system (10 mM Tris, pH 7.3, 200 mM NaCl, 1 μM ssDNA), followed by passivation of the Au electrode through coadsorption of 1 mM mercaptohexanol for 1 h and hybridization with complementary DNA (50 nM) on the surface. All measurements of the fluorescence response were performed in 10 mM Tris buffer pH 7.3, 50 mM NaCl (κ−1 = 1.3 nm) at T = 298 K (η = 0.89 mPas). The preparation of Au electrodes and the modification of DNA with capture molecules and protein has been described in detail elsewhere.24 Numerical solutions to the Smoluchowski equation eq 10, i.e., the time-evolution of the probability distribution, were calculated using the FiPy library, a partial differential equation (PDE) solver written in Python that uses a standard finite volume approach for numerically solving PDEs. FiPy supports terms for convection, transient diffusion, and standard sources. With this approach, elliptic, hyperbolic, and parabolic PDEs (or combinations thereof) can be solved. The angle of the DNA was discretized into steps of 0.01 radians and the time-resolution into steps of 0.01 μs. These values proved to provide accurate results while still maintaining a good execution time (around 5 s per parameter configuration on a normal desktop computer). As boundary conditions, a fixed flux value of zero at both borders of the angle (0 and π/2) was defined.

ASSOCIATED CONTENT

Supporting Information

Information concerning the rotational diffusion of end-tethered rods and the potential drop across the SAM at the electrolyte–metal interface. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: rant@dynamic-biosensors.com.

Notes

The authors declare the following competing financial interest(s): UR and WK are cofounders of Dynamic Biosensors GmbH, a company which commercializes electrically switchable DNA layers for analytical purposes. AL is financially supported by DB.

ACKNOWLEDGMENTS

Financial support for this work is gratefully acknowledged from BMBF via Go-Bio and the Excellence Initiative via the TUM International Graduate School of Science and Engineering, the TUM Institute of Advanced Study, and the Nanosystems Initiative Munich.

REFERENCES


