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The effect of DNA probe distribution on the reliability of label-free biosensors

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ABSTRACT

As the design of label-free DNA biosensors matures, and their sizes reduced to enhance their sensitivity, not much has been researched about the variations in the received signal with the positioning of the probes on the sensitive surface. We approach this issue computationally in this paper. By adopting the finite-element model on a three-dimensional biological field-effect transistor (BioFET) slice, and running Monte-Carlo simulations on the positions of the DNA molecules, we extract the expected variations in the signal. Then, we show that signal-to-noise (SNR) ratio can be low enough to hinder the functionality of the device, placing a limitation on how low the sensitivity of a sensor of a certain size can be.

1. Introduction

The need for label-free biological sensors has been spurring a lot of research on different techniques to achieve this, using conventional semiconductors [1–3], MEMS devices [4–5], nanostructures [6–8], or microelectrodes [9]. It is an essential step in the ultimate quest for realizing portable, microfabricated lab-on-chip devices, as this will eliminate the need for expensive and bulky optical detection techniques, as well as costly stains and reagents that would also need to be administered in micro-scale, complicating the design of the microfluidic devices on the chip. The ultimate goal of a biosensor is to achieve the highest possible sensitivity (down to a single molecule), while not compromising selectivity (insensitivity to foreign species). Several challenges lie ahead of such attempts, including the miniaturization of electrodes [10], controlled growth of optimized nanostructures to build the sensor, and controlled immobilization of the probe molecules on the sensitive surface.

The way to achieve the ultimate control over sensitivity is by miniaturizing the dimensions of the active region in the sensor to allow the smallest number of probe molecules to modulate electrical properties. However, there is a downfall to this continuous miniaturization: The loss of the ensemble effect and the introduction of statistical bias. Whereas a macroscopic ion-sensitive electrode might be insensitive to the binding locations of the sensed molecules (such as DNA or proteins), a microscopic version's response characteristics can be very dependent on the exact geometry of the sensor and the positions of the probes, as well as the locations of pairing with targets. An example of this dependence was illustrated in [11] on nanowire DNA biosensors, wherein the spatial variations of the doping density of the semiconductor can cause severe variations in the sensed signal. This variation can be seen as a source of noise, especially in microarray sensors, since the response of each cell may differ considerably from the other cells.

In the case of DNA biosensors, even when still larger than nanostructures, the variations in the locations of the probe molecules can also introduce ambiguity in the sensed signal. This effect has not been previously addressed. In this paper, through mean-field simulations, we provide evidence for the existence of such variations in DNA sensors, and we give numerically calculated estimates for the variation, and the expected SNR of the device with different probe densities.

2. Aggregation of DNA

Immobilized DNA molecules on surfaces are known to form aggregates, rather than distribute over the surface by means of thermodynamic diffusive forces [12]. This phenomenon is, to a great extent, absent in surfaces with high DNA coverage ratio. However, high surface coverage can severely hinder the hybridization efficiency [13]. DNA probes must have the full ability of thermodynamic motion (with the exception of the tethered end) to achieve the maximum hybridization efficiency. The aggregation of DNA probes into clustered areas at the sensor's surface is a result of

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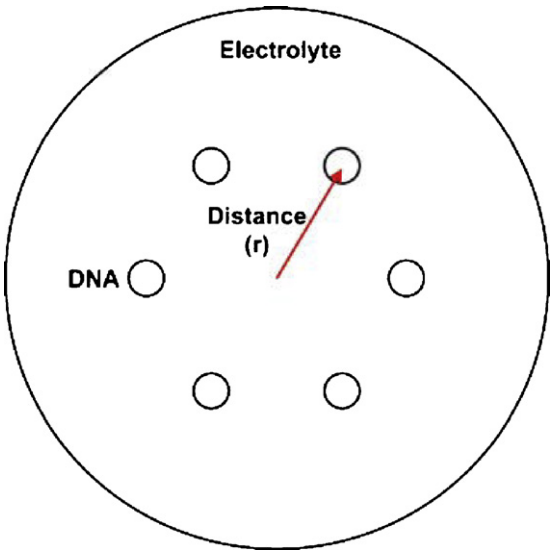

Fig. 1. Top view of the simulation domain of six DNA molecules in an electrolyte medium.

energetic considerations, of which counter-ions and their bulk concentrations play an important rule.

We demonstrate the favourable clustering of the DNA probes by examining a two-dimensional top-view mean-field finite-element model of the DNA probes (treated as vertical cylinders in this paper). Shown in Fig. 1, we study the total electrostatic energy of the system (DNA + counter-ions) as the mutual distance between the six immobilized DNA strands is reduced. This simulation will give the mean-field energy, within the Poisson–Boltzmann approximation. The simulation does not include energy wells stemming from chemical reactions, such as those responsible for the immobilization in the first place. Such chemical reactions are assumed to be isotropic within the surface and not significant when dealing with variations in energy with DNA clustering, even though the absolute values of the chemical forces can be much larger than the electrostatic energy of the system.

The charges on the DNA backbone are averaged over the DNA molecule, and the volume occupied by the DNA molecule is assumed impermeable to ions. This of course neglects the possible associations in the major and minor grooves of the DNA molecules, but such an approximation is common in the study of biosensors [14,15]. The solution ionic population is studied in the framework of the Poisson–Boltzmann model for a z–z electrolyte:

$$\nabla^2 V = \frac{2qn_0}{\varepsilon} \sinh(z\beta V) \tag{1}$$

where V is the electrostatic potential (V), q is the electronic charge (C), ε is the permittivity of the solution medium (F/cm), n_0 is the bulk salt concentration (cm^{-3}), z is the valence of the ions, and β is the inverse thermal voltage (V^{-1}). Eq. (1) models the aggregation of the salt around the DNA molecule (counter-ion condensation). The DNA cylinders themselves are considered charged circles (top view) and are placed at hexagonal equidistant points. For each distance, the charge and potential distribution is calculated using the finite-element method. The total electrostatic energy is calculated using the following equation:

$$E = \frac{1}{2}\int_{\Omega} \rho V \, dr \tag{2}$$

where ρ is the charge density. In the solution, this is given by the electrolyte charge density:

$$\rho = -2qn_0 \sinh(z\beta V) \tag{3}$$

The electrostatic energy is not the only factor that affects the charge separation. It is the total free energy of the system that determines the most energetically favourable distancing. The Poisson–Boltzmann model ensures maximum entropy for the ionic distribution within minimum energy constraints. However, it does not give the amount of this entropy. An estimate of the entropy of the ions, which is valid at infinite dilution, and which is often used, is given by:

$$S = k\int_{\Omega} (n \ln n - n)\, dr \tag{4}$$

where k is Boltzmann's constant, and n is the volumetric density of the ionic species. Eq. (4) is correct to a constant value. The constant is of no significance here as we are seeking the density of DNA at minimum free energy $G = (E - TS)$, which is not influenced by the value of this constant.

Fig. 2A shows the simulated electrostatic energy for six DNA probes as a function of their mutual distances, at different bulk electrolyte concentrations. As is shown, a quite steep electrostatic minimum energy exists (at least 100 meV) and the distance at which this occurs is independent on the electrolyte concentration. This minimum energy distance is around 4 nm, which results in an average DNA packing fill factor less than 50%. However, when the complete free energy is calculated, including entropy, then the equilibrium separation is much more sensitive to the bulk electrolyte density, as evident from Fig. 2B. At very low electrolyte concentration, it appears that the DNA molecules are less likely to aggregate, due to the absence of an energy minimum (to within 60 nm inter-distance). However, as the bulk electrolyte concentration is increased, the minimum energy location appears to favour aggregation of DNA molecules. During hybridization and immobilization, the electrolyte concentration is kept high, and the aggregation is expected to allow the formation of clustered DNA regions. Furthermore, the depth of the energy well seems to be in the range of 50 meV, and biological temperatures can therefore allow considerable fluctuations in the DNA density from the value at the minimum energy. The strong dependence of the equilibrium distances on the entropy and on the electrolyte concentration indicates that the immobilization step during the biosensor development must be done in an extremely stable environment with controlled salt and temperature.

Although the DNA probes have been shown to have a tendency to immobilize close to each other, it is worth mentioning that the wobbly nature of oligonucleotides can increase their steric hindrance radius [16]. It is plausible, therefore, that the DNA molecules might space out more evenly under steric volume exclusion effects. However, this aggregation of molecules can also happen during hybridization. Firstly, for denser probe layers, the conformation change (from flailing probes to stiff rods) will allow more interaction between neighbouring probes and targets. Secondly, the energetics of the reaction will be reduced in the vicinity of rod-shaped hybridized DNA molecules, as shown in the simulations above, due to counter-ion sharing. Thus, even with steric interactions, hybridized DNA chips will also exhibit non-uniformity in the coverage, and this will be particularly important for high-sensitivity detection, especially in the absence of PCR pre-amplification.

3. Monte Carlo simulation of BioFET signal

The apparent sensitivity of micro- and nanoscale sensors to the geometry and positions of the probe and target molecules is a motivation for the study of this variation by simulating the response of the entire sensor. To accomplish this, we have combined finite-element analysis of the BioFET with Monte-Carlo