Silicon-on-insulator based thin film resistors for quantitative biosensing applications

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Field-effect based semiconductor devices for the label-free detection of molecular interactions represent a promising development for biosensor applications. Recently, several such devices have been presented for the direct electrical detection of nucleic acids and proteins. However, a detailed and quantitative understanding of experimental observations is still elusive in most cases. Here we employ a recently introduced Silicon-on-Insulator (SOI) based field-effect sensor for the label-free detection of molecules by their intrinsic charge. We present a theoretical description for the quantitative analysis of the sensor response. A capacitor model was developed which accounts for dielectric effects as well as for Debye screening by mobile ions within the layers of molecules bound to the surface. We successfully applied the model to the detection of charged peptides and multilayers at the functionalized sensor surfaces. The electrical detection of the adsorption of bovine serum albumin (BSA) to the sensor surface is demonstrated and can be explained in terms of a dipolar orientation of the bound molecules.

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1 Introduction

Advances in the development of biofunctional semiconductor systems allow for the label-free electrical detection of molecules by their intrinsic charge. Recently, several field-effect-based semiconductor devices for the detection of nucleic acids [1–3] and proteins [4–6] have been presented. Currently different substrate materials are studied for their potential in biosensing applications. For example, group III-nitrides have been shown to be nontoxic and stable under physiological conditions [7] operating at a high signal-to-noise ratio [8]. The successful electrochemical passivation of GaAs/AlGaAs heterostructures has been demonstrated allowing stable measurements at a high sensitivity [9]. Moreover, diamond exhibits a good biocompatibility as well as a high stability and has been used for the fabrication of biosensors [5, 10]. Apart from the direct detection by field effect devices another promising strategy is the application of electrical fields for the active manipulation and detection of DNA hybridizations, which might be useful for actively controlled biosensing devices [11, 12]. For all biosensing field effect devices, sensor signals strongly depend on screening effects in the electrolyte solution and on the charge distribution within the biofunctional layers. For a quantitative understanding of the detection mechanisms and the experimental results, studies with well defined model systems are necessary. Here we use a recently introduced Silicon-on-Insulator (SOI) based field-effect sensor [13] for the investigation of such model systems comprising small peptides, charged multilayers and proteins. We present a theoretical description based on a capacitor model and a linearized Debye Hückel theory for the quantitative analysis of the differently functionalized devices.

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2 Capacitor model

Field effect devices allow the determination of the surface potential $\psi_s$ at the sensor/electrolyte interface. Surface potential variations measured by such a device are strongly dependent on screening effects inside the adjacent phase. In the case of a SOI based sensing device, the sheet resistance is sensitive to variations of $\psi_s$. A calibration measurement using a Ag/AgCl reference electrode is used to relate a certain change in sheet resistance $\Delta R$ to a corresponding change in potential $\Delta \psi_s$ [14]. Relating a sensor signal quantitatively to the binding of charged molecules to the sensor surface can be achieved by modelling the change in surface potential $\Delta \psi_s$ with an equivalent circuit, incorporating screening effects in the electrolyte solution.

The sensor/electrolyte structure can be illustrated by a series of capacitors as depicted in Fig. 1a. In this simple one-dimensional capacitor model the system is described by the capacitance $C_s$ of the device, the capacitance $C_p$ of the layers adsorbed to the sensor surface and the capacitance of the double layer $C_d$. $C_s$ results as a combination of the semiconductor capacitance, the oxide capacitance and the interface state capacitance. Figure 1b gives a more detailed picture of the layers and charge distributions used in modelling a sensor/electrolyte structure functionalized with one layer of charged molecules. $\sigma_i$ is the semiconductor surface charge, $\sigma_{ox}$ the oxide surface charge. Adsorbed layers are described by the volume charge density $\rho$, the dielectric constant $\varepsilon$ and the screening length $\kappa^{-1}$. The double layer is described by the surface charge $\sigma_{dl}$ at the distance of the screening length $\kappa_{dl}$ in the electrolyte with the dielectric constant for water $\varepsilon_w$. Correspondingly, a system functionlized by several layers can be described as a series of $N$ layers with the volume charge distribution $\rho(x)$. For small potentials ($\psi < 2kT$) [15] the linearized Debye–Hückel equation $d^2/\text{d}x^2 \psi(x) - \kappa^2 \psi(x) = -1/\varepsilon \rho(x)$ must be solved for each of the layers of charged molecules containing mobile ions. We can describe the potential within the electrolyte by a plate capacitor with the Debye capacitance per area $C_{Dw} = \varepsilon_w \varepsilon_0 \kappa_{Dw}$. The potential difference between the semiconductor and the bulk electrolyte is $U_{tot}$ and is set by the reference electrode. Applying the appropriate boundary conditions for the particular system, we can calculate the potential at the silicon oxide/electrolyte interface $\psi_s$ which determines the sheet resistance of the device. This is shown for a multilayered system in [16].

Note that the capacitor model is a first order approximation, which does not include interfacial ion condensation and changes of the surface pH caused by the binding of charged molecules. These effects could alter the measured surface potential changes. However, incorporating site-binding theory, a linear relation between the surface charge and the surface potential can be found numerically in the measurement regime, where the coefficient depends on detailed knowledge of the number of available surface sites. As here we are interested mainly in screening effects and small potential variations, such effects can safely be neglected.
3 Materials and methods

SOI-based thin film resistor: The sensor chips were fabricated from commercially available silicon-on-insulator (SOI) wafers (ELTRAN, Canon) using standard lithographic methods and wet chemical etching as described in detail elsewhere [13]. The top silicon layer of these wafers was 30 nm thick and slightly doped with boron ($10^{16}$ cm$^{-3}$). Metal contacts were deposited in an electron beam evaporation chamber (20 nm Ti, 300 nm Au). The chips were glued into a chip carrier and the contacts were Au-wire bonded to the carrier. Afterwards, the chips were encapsulated with a silicone rubber glue to insulate the contacts from the electrolyte solution. A flow chamber was mounted on top of the sensor and a Ag/AgCl reference electrode was used to control the potential of the electrolyte solution and for calibration. The sheet resistance of the device was measured as described elsewhere [14].

Production of NTA-lipid surfaces: The bare surface sensors were passivated by the covalent binding of ODTMS (octadecyltrimethoxysilane) to the oxide surface, resulting in a hydrophobic surface. The desired amounts of lipids in chloroform solution were mixed in a glass flask to yield a total lipid concentration of 1 mg/ml. Afterwards, the solvent was evaporated under nitrogen, and the glass was stored under vacuum overnight. The dried lipids were dissolved in pure ethanol and injected into the flow chamber. The spontaneous formation of the lipid monolayer was initiated by rinsing the chamber with 10 mM PBS buffer (pH 7.5 with varying KCl concentrations) at a flow rate of 10 µl/s. A peristaltic pump (ISMATECH, Germany) applied this flow for 10 s, followed by a rest time of 90 s. After approximately 2 h, the chamber was rinsed with buffer to remove all residual lipids and ethanol. A mixture of DMPC and cholesterol was used as the matrix lipid, and 5% DOGS-NTA (1,2-dioleoyl-sn-glycero-3-[N(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl) was added. For the Ni$^{2+}$ buffer an additional 1 mM NiCl$_2$ was added to the PBS buffer. The EDTA buffer (10 mM PBS, pH 7.5) contained 90 mM KCl and 50 mM EDTA. Both Ni$^{2+}$ and EDTA containing buffers were equilibrated prior to the binding experiments: The buffers were titrated with KCl until the sensor’s response to the buffer exchange between Ni$^{2+}$ and EDTA buffer was no longer detectable.

Deposition of PEMs: PSS (MW 70,000) and PAH (MW 60,000) were purchased from Sigma–Aldrich. 5 mg/ml polyelectrolyte solutions were prepared by direct dissolution in 10 mM Tris buffer at pH 7.5 containing 50 and 500 mM NaCl, respectively. PAH and PSS solutions were injected twice into the flow chamber to insure full coverage of the sensor surface, starting with the positively charged PAH. After obtaining a stable sensor signal, the chamber was rinsed twice with buffer of the same salt concentration as the polyelectrolyte solutions. As soon as a stable signal was obtained, the next polyelectrolyte solution was injected and the procedure was repeated up to 20 times. The sheet resistance of the thin film resistor was monitored continuously during the multilayer deposition.

Adsorption of BSA: BSA (minimum 98%) was purchased from Sigma-Aldrich. Solutions of various BSA concentrations were prepared by direct dissolution in 10 mM Tris buffer at pH 7.5 containing 50 mM NaCl. The BSA solutions were injected into the flow chamber starting with the lowest concentration. When a stable signal was obtained, the procedure was repeated with a higher BSA concentration.

4 Detection of charged peptides

First we examine a system consisting of only one layer of charged molecules. Here, we functionalize the SOI sensor surface by biomimetic lipid membranes. Therefore, the native oxide surface was passivated by covalent coupling of a silane layer. Subsequently, the sensor device was covered with a lipid monolayer by the solvent exchange method. The incorporation of metal-chelating lipids (1,2-dioleoyl-sn-glycero-3-[N(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl – DOGS-NTA) into the lipid layer allows the binding of polyhistidine tagged peptides if Ni$^{2+}$ ions are bound to the NTA headgroups [6]. The unbinding of a polyhistidine peptide can be achieved by the addition of a chelating agent for Ni$^{2+}$, such as ethylenediaminetetraacetic acid (EDTA) as it is shown in Fig. 2a. The binding of polyhistidine
Fig. 2 (online colour at: www.pss-a.com) NTA lipids incorporated into a lipid monolayer allow the specific binding of polyhistidine tagged peptides to the sensor surface (a). Binding only occurs if Ni$^{2+}$ ions are bound to the NTA headgroups. The Ni$^{2+}$ ions can be removed by the chelating agent ethylenediaminetetraacetic acid (EDTA). An increasing number of charged aspartates results in a bigger change of the surface potential $\Delta \psi_s$ caused by the peptide binding which is shown in (b) for two different salt concentrations.

tagged peptides with different numbers of charged residues (aspartates), varying from a single charged residue up to eight charged residues (His$_6$Asp$_1$ to His$_6$Asp$_8$), could be measured in terms of a change in sheet resistance. A solution containing 7 µM of peptide was applied to the sensor device. Upon binding of the peptides, a shift of the surface potential is observed, which is shown in Fig. 2b for peptides with a different number of charged residues. The sensitivity of the sensor allows the discrimination between peptides with one or two charged residues. Peptides with higher charges result in an increased sensor response. However, the signal increase for each additional charged amino acid decreases with increasing number of amino acids (Fig. 2b).

In a first approximation, the system can be described as a single charged layer with the surface charge $\sigma$ at a distance $d$ from the sensor surface. The measured signal caused by the adsorption of such a layer is represented by the change in sheet resistance $\Delta R$ corresponding to a change in surface potential. The latter can be calculated from the capacitor model giving $\Delta \psi_s = \sigma/C_0 \exp(-\kappa d)$ for $U_{\mu\mu} = 0$ and $C_0 < C_\infty$. In this case a simple exponential decay of the signal with increasing distance $d$ is obtained. At the same time, the signal is expected to depend linearly on the number of charges given by $\sigma$. In our system the distance $d$ is determined by the length of the complete NTA headgroup including the spacer of 12 carbon atoms plus the polyhistidine tag. Indeed, we find a deviation from the linear behavior as the signal increase for each additional charged residue decreases with increasing number of amino acids. This can be explained by the finite thickness of the charged layer $b$ leading to the expression

$$\Delta \psi_s = \frac{\sigma}{C_D} \exp (-\kappa b) \frac{\exp(-\kappa d) - \exp(-\kappa(d+b))}{\kappa b}.$$

By fitting Eq. (1) to the measured data we find $d = 2.8$ nm and $b = 0.3$ nm per charged amino acid residue for $\kappa^{-1} = 1$ nm$^{-1}$, and $d = 2.3$ nm and $b = 0.1$ nm per charged amino acid residue for $\kappa^{-1} = 0.7$ nm$^{-1}$. At higher salt concentration, the screening of the charges is increased and at the same time the peptides are in a less extended configuration leading to lower values for $d$ and $b$. This demonstrates that using the simple model it is possible to determine quantitatively the charge variations at the sensor surface caused by small molecules.

5 Detection of self-assembled polyelectrolyte multilayers

Next, we consider a complex system consisting of alternately charged polyelectrolyte layers. The build up of polyelectrolyte multilayers (PEMs) consisting of the positively charged poly(allylamine hydrochlo-
ride) (PAH) and the negatively charged poly(sodium 4-styrenesulfonate) (PSS) was observed by the SOI sensor [16] as shown in the inset of Fig. 3. The potential change between adjacent deposition steps $\Delta \psi_s$ was determined from the measured sheet resistance using the calibration data and is plotted against the number of adsorbed monolayers as shown in Fig. 3. The differently charged polyelectrolytes adsorbing to the sensor surface result in defined potential shifts, which decrease with the number of layers deposited.

Applying the capacitor model, the observed decrease can be quantitatively explained by assuming reduced electrostatic screening by mobile charges inside the PEMs compared to the bulk medium outside [16]. We model the polyelectrolyte multilayers sensor system as a series of $N$ alternately positively and negatively charged layers with the volume charge distribution

$$\rho = \pm \frac{\sigma}{d}$$

with a multilayer surface charge $\sigma$ and a constant layer thickness of $d$. Solving the Debye–Hückel equation we obtain the surface potential

$$\psi_s(N) = \frac{1}{C_p} \left( \frac{1}{C_p} \sinh (\kappa N d) + \frac{1}{C_0} \cosh (\kappa N d) \right) + \frac{U_{\text{ion}} - \frac{1}{C_0} (-1)^N \sigma_{\text{eff}}}{(C_p/C_p + C_p/C_0) \sinh (\kappa N d) + (1 + C_p/C_0) \cosh (\kappa N d)}$$

with the effective polyelectrolyte surface charge $\sigma_{\text{eff}} = 1/\kappa d [(1 - \exp (-\kappa d))/(1 + \exp (-\kappa d))] \sigma$ and the Debye capacitance per area $C_p = \epsilon \kappa$ within the polyelectrolyte medium. As we measure the change in sheet resistance between adjacent layers of polyelectrolytes, we calculate $\Delta \psi_s = \psi_s(N-1) - \psi_s(N)$, which can be simplified for $\kappa d \ll 1$ and even number of layers $N$, yielding

$$\Delta \psi_s(N) = \frac{\sigma C_0^{-1}}{(C_p/C_p + C_p/C_0) \sinh (\kappa N d) + (1 + C_p/C_0) \cosh (\kappa N d)}.$$

A value for the screening length $\kappa^{-1}$ inside the PEMs can be obtained from the measured potential change $\Delta \psi_s(N)$ taking the thickness $d$ of the polyelectrolyte layers into account, which was determined independently by ellipsometry [16]. As can be seen in Fig. 3, the measured $\Delta \psi_s$ can be fitted for $\kappa d$ by Eq. (2), yielding $\kappa^{-1} = 6.5 \pm 1.0$ nm for deposition from 500 mM bulk solution. A similar fit results in $\kappa^{-1} = 6.3 \pm 1.0$ nm for the build up of the PEMs at 50 mM bulk solution. Equation (2) also provides an estimate for the surface charge density $\sigma$ of the adsorbed polyelectrolyte layers, assuming that $C_p$ is much smaller than $C_0$. This leads to $\sigma = 0.020$ C/m$^2$ for the adsorption from 50 mM and $\sigma = 0.022$ C/m$^2$ from 500 mM bulk solution. We demonstrate that the capacitor model allows to determine the screening length $\kappa^{-1}$ inside the PEMs. The screening and thus the concentration of mobile ions in the PEMs was found to be considerably reduced compared to the values of the corresponding bulk solutions. These results are in agreement with the fact that the counterion concentration inside the PEMs was found to be below the detection limit [17].
6 Detection of bovine serum albumin

In a next step the adsorption of molecules with a more complicated charge distribution is studied. The protein bovine serum albumin (BSA) was adsorbed to the blank silicon oxide sensor surface in various concentrations ranging from 0.01 mg/ml to 10 mg/ml. We find an increase of the surface potential with increasing protein concentration as shown in Fig. 4, with a maximum value of $7.2 \pm 1.6 \text{ mV}$. However, from the negative charge of the protein (approximately 15 $e^-$ per molecule at neutral pH) we would expect a decrease of the surface potential. The adsorption of a protein layer with a dielectric constant lower than the bulk electrolyte solution to a negatively charged surface should lead to a decrease in the surface potential according to the capacitor model. Therefore, the observed increase cannot be attributed to a dielectric effect. A possible explanation is that the increase of the surface potential can be ascribed to a dipole moment of the adsorbed layer as a consequence of an oriented protein adsorption. Using the capacitor model we can estimate whether this hypothesis is reasonable. The crystal structure of the human homologue, human serum albumin (HSA), is known, which shares 90% sequence homology with BSA. Using the “Web Server to Calculate Dipole Moments of Proteins” (Clifford Felder and Joel Sussman, Dept. of Structural Biology Weizmann Institute, 761000 Rehovot, Israel) HSA (Protein Data Bank ID 1e7i [18]) we find a total of 97 negatively charged and 82 positively charged residues. When we assume a maximum surface density for BSA of 60 nm$^2$ per molecule [19], the model allows us to calculate the potential change evoked by the oriented adsorption of the protein. A separation of only 20 negatively charged residues and 5 positively charged residues at a distance of 2.1 nm, positive residues pointing towards the negative silicon oxide (Fig. 4 inset), can result in an increase of the surface potential of approximately 7 mV. This is in good agreement with the value obtained from the measurements. It corresponds to a dipole moment of approximately 1400 Debye, which is in the same order of magnitude as the value given by the Web Server (1233 Debye) with respect to the center of mass of the protein. So the increase of the surface potential caused by the adsorption of a protein that is negatively charged in total can be explained within the model by the directed adsorption of a dipolar molecule.

7 Conclusion

The recently introduced field effect device based on SOI technology is well suited for the quantitative determination of charge distributions at complex interfaces. We make use of a capacitor model that takes into account electrostatic screening of charges by mobile ions in the electrolyte solution. The presented theoretical description allows the analysis of various functionalizations of the sensor device. We are able to detect single charge variations of peptides in electrolyte solution and to show the distance dependence of the sensor signal. Polyelectrolyte multilayers were used as a model system to study the electrostatic properties of complex interfaces. The adsorption of BSA to the sensor surface was detected at various concentrations. The sensor signal can be explained in terms of an oriented adsorption of a dipolar protein. The presented SOI resistors based on standard semiconductor technology represent a sensitive tool.
for quantitative biosensing applications and offer the possibility of the label-free detection of small molecules.

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