Dependence of field-effect biosensor sensitivity on photo-induced processes in Si and its conductivity type

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Abstract. The effect of photoelectron processes in n-Si and p-Si on the glucose sensitivity of a capacitive field-effect biosensor based on electrolyte/oxide/semiconductor structure was investigated. We obtained that illumination of n-Si/SiO₂/PEI structure during the GOx adsorption increases the glucose sensitivity by three times compare to GOx adsorption in the dark. In contrast, p-Si illumination during the GOx adsorption led to a decrease in sensor sensitivity from 2.9 mV/mM to 2.2 mV/mM. The result is explained by a change in the density of immobilized GOx molecules due to a change in the electrostatic force of attraction under illumination and stabilization of the photo-generated charge on the surface electronic states of the Si/SiO₂ and SiO₂/PEI interfaces after illumination.

1. Introduction
Electrolyte/oxide/semiconductor (EOS) structures are widely used to detect nano- and bio-objects ionized in solution. In addition, EOS-structures are used to recognize enzymatic reactions [1], viruses [2], RNA/DNA sequencing. The parameters of enzyme biosensor based on EOS-structures can be improved by increasing the concentration of enzyme molecules adsorbed onto a semiconductor transducer surface. Ionic strength or pH of solution are commonly used to modulate the electrostatic interactions between enzyme molecules and semiconductor surface. However, variation of pH or ionic strength leads to a nonmonotonic and unpredictable change in the surface density of enzyme molecules.

Previously [3], we report on the influence of the white-light illumination of Si substrate during glucose oxidase (GOx) adsorption on the surface concentration of immobilized GOx molecules. This method is called photo-stimulated adsorption (PSA). The PSA efficiency of charged nano- and bio-objects significantly depends both on a Si conductivity type and on a buffer poly(ethylene-imine) (PEI) layer on Si surface [4]. However, studies on the effect of PSA of enzyme molecules onto a semiconductor transducer surface on the sensitivity of a capacitive field-effect biosensor have not existed before. In this work, we studied the effect of photo-stimulated layer-by-layer adsorption of GOx on the glucose sensitivity of field-effect-based capacitive EOS-sensors.

2. Experimental Section
2.1. Materials and Methods
The capacitive field-effect EOS structures were fabricated using single-crystal (100) Si wafers (400±10 μm thickness) of n-type (ρ = 8-10 Ω cm) and p-type (ρ = 9-15 Ω cm). Initially, the wafers
were boiled in a peroxide–ammonia solution and rinsed in deionized water. This treatment leads to “reconstruction” of a native oxide layer to \( \text{SiO}_2 \) (~2 nm thickness). It can be noted that isoelectric point (IEP) of the \( \text{SiO}_2 \) surface is ca. pH=2-3 [5] and above this pH value the surface is negatively charged. Afterwards, wafers were cut into substrates of \( 10 \times 10 \text{ mm}^2 \).

The enzyme glucose oxidase (GOx) from \textit{Aspergillus niger} were used as enzyme molecules. In a wide pH range of the solution, the GOx molecule have an effective negative charge. The size of the GOx molecule is \( 6.0 \times 5.2 \times 7.7 \text{ nm}^3 \) [6]. Branched poly(ethylene-imine) (PEI) with a molecular weight of 25 kDa was used as cationic polyelectrolyte to increase the adsorption of negatively charged GOx onto the silicon substrates. The PEI molecules were adsorbed onto the silicon substrates from the 1 mg/ml aqueous solution during 10 min followed by rinsing in deionized water (\( \rho \approx 18.2 \text{ M}\Omega \text{ cm} \)) during 10 min and drying in nitrogen flow. The glucose solutions were prepared by dissolving D-glucose in the working buffer. As working buffer, a 0.2 mM potassium phosphate buffer solution (pH = 7.3) containing 150 mM NaCl as an ionic strength adjuster was used. The photo-stimulated layer-by-layer adsorption technique suggested in [7] was used to adsorb GOx from the 0.5 mg/ml aqueous solution onto silicon substrates covered with PEI. A halogen lamp (Philips 13186 EPX/EPV) was used to activate photoelectric processes in a silicon wafer during adsorption of polyelectrolyte molecules. The Si substrate was either in the dark or under illumination during the GOx adsorption, other things being equal. Thus, the Si/\text{SiO}_2/PEI/GOx biosensor structures were fabricated.

2.2. Measurement set-up

Figure 1b schematically shows the experimental set-up, which has been utilised for the characterisation of the EOS sensors with the adsorbed PEI and GOx layers. For the measurements, the EOS sensor was mounted into an electrochemical cell, sealed by an O-ring and contacted on its front side by the electrolyte and Ag/AgCl reference electrode, and on its rear side by a gold-plated pin. The measurements have been performed at room temperature.

![Figure 1. (a) - Schematic energy band diagram of the Si/SiO_2/electrolyte contact before (left) and after (right) equilibrium: \( D_{\text{red}} \) and \( D_{\text{ox}} \) - density distribution of states of the oxidation-reduction system in the electrolyte, \( \lambda \) - energy of reorganization, \( \Phi, \chi \) and IE are the work function, electron affinity, and ionization energy, respectively, of the semiconductor (index S) and electrolyte (index E); (b) - Schematic of a capacitive EOS-sensor.](image-url)

EOS glucose biosensors have been characterised in glucose solutions with different content of D-glucose from 1 mM to 10 mM by means of Capacitance-Voltage (C-V) method using a semiconductor device analyzer (Agilent B1500A). The C–V measurements have been performed at a frequency of 1 kHz. Before the adsorption of PEI and GOx, the pH-sensitive behaviour of the as-prepared Si/SiO_2 structure has been investigated in buffer solutions of pH 3.8–7.5 by C–V method. For operation, a DC voltage is applied via the Ag/AgCl reference electrode and a small AC voltage (20 mV) is applied to the system in order to measure the capacitance of the sensor. For the measuring procedure, about 0.5
mL of the working buffer or particular glucose solution was applied to the EOS gate surface, and C-V curve has been read out for after 10 min. After each measurement, the EOS gate region was rinsed with buffer solution.

3. Results and Discussion

3.1. Effects of pH on EOS sensor
As the working principle of the enzyme-modified EOS sensor is based on the detection of pH changes near the Si/SiO₂ surface induced by the enzymatic reaction, the pH sensitivity of the SiO₂ layer has been proven before GOx immobilization. Figure 2 depicts a typical C-V curves (a) and calibration curve (b) of EOS sensor recorded in 0.1 M sodium-acetate (pH 3.8-4.5) and 0.1 M potassium phosphate (5.9-7.5) buffer solution. The EOS sensor shows a nearly linear pH response between pH 3.8 and pH 7.5 and an average sensitivity of about 42-43 mV/pH at 1 kHz. It didn't depend on the silicon conductivity type.

![Figure 2](image)

**Figure 2.** (a) - C-V curves of EOS sensor before immobilization of PEI and GOx measured in 0.1 M sodium-acetate (pH 3.8-4.5) and 0.1 M potassium phosphate (5.9-7.5) buffer solutions; (b) - the calibration curve derived from the values of the flat band potential shifts.

This change occurs because with an increase in pH of the raster on the semiconductor surface, a negative charge increases. These leads to electrons repelled from the depletion region of Si, which in turn reduces the width of space charge region (SCR) in p-Si and increases it for n-Si. As a result, a total capacity either increases (p-Si) or decreases (n-Si) [8].

3.2. Effects of photoelectron processes in Si on the sensitivity of EOS biosensor
The glucose sensitivity of EOS biosensors was studied by means of C–V measurements. Figure 3 shows exemplarily a typical set of C–V curves of the EOS biosensor, where GOx deposited in the dark (Figure 3a) and under illumination of n-Si during GOx adsorption (Figure 3b) measured in work buffer and in D-glucose solutions of different concentrations from 1 to 10 mM. The C–V curves exhibit a high-frequency behaviour with typical accumulation, depletion and inversion region. With increasing glucose concentration in the solution, the C–V curves are shifted along the voltage axis in the direction to more negative voltage values. This is due to an increase in the H⁺ concentration on the EOS sensor surface after the enzymatic reaction.

It was found that for EOS sensor, where the GOx molecules were deposited in the dark, the sensitivity to glucose is 1.90 and 2.87 mV/mM for n-Si and p-Si, respectively. In the case of PSA - 5.8 and 2.23 mV/mM for n-Si and p-Si, respectively. These data are in good agreement with the previously obtained results on PSA and are explained using the model of recharging of surface.
electron states under illumination of Si and adsorption of charged molecules from a solution, which is partially presented in [3, 4]. The change in the sensitivity of EOS sensor in the case of photo-stimulation of the Si/SiO\(_2\) structure during GOx adsorption occurs due to a change in the density of the adsorbed enzyme.

Figure 3. Typical C–V curves of the EOS sensors based on \(n\)-Si measured in potassium phosphate buffer and in glucose solutions of different concentrations from 1 to 10 mM; (a) – GOx adsorbed in the dark, (b) – GOx adsorbed under illumination. Inset: calibration curves.

The influence of illumination on the GOx adsorption can be explained as follows. Under equilibrium conditions (i.e., in the absence of illumination and external electric fields), the charge of the space-charge region (SCR) is equal in magnitude and opposite in sign to the charge of surface electronic states (SES). The sign of SES charge usually corresponds to that of main carriers in semiconductor. The negative sign of SES in \(n\)-Si leads to electrons depleted region in the near-surface layer and the corresponding upward bending of the energy bands. The PEI deposition leads to a decrease in band bending. The total charge of SES, traps in SiO\(_2\), and PEI in structures based on \(n\)-Si will be close to zero. When Si/SiO\(_2\)/PEI structure is immersed into an aqueous solution of anionic PE (e.g., GOx), the negatively charged molecules are adsorbed and held on the PEI surface mostly due to electrostatic interactions. The illumination of Si/SiO\(_2\)/PEI structures leads to rectification of their energy bands due to the generation of electron–hole pairs and their separation in the SCR field. As a result, the charge of SES at the Si/SiO\(_2\) interface decreases. In \(n\)-Si/SiO\(_2\)/PEI structure illumination, the SCR field facilitates the drift of holes toward the \(n\)-Si/SiO\(_2\) interface, which leads to an increase in the effective positive charge in this structure at the PEI/GOx interface and results in increasing amount of adsorbed GOx molecules as compared to that adsorbed in the dark. In [3], a similar model for the PSA of GOx molecules onto \(n\)-Si/SiO\(_2\)/PEI structure was presented and the experimental results showed a significant increase in adsorbed GOx molecule number on \(n\)-Si substrate compared to GOx deposition in the dark. In the case of \(p\)-Si/SiO\(_2\)/PEI structure illumination, the drift of electrons toward the \(p\)-Si/SiO\(_2\) interface due to SCR field leads to a decrease in the effective positive charge at the PEI/GOx interface and, as a result, the adsorbed GOx molecule number is decreases compared to adsorption in the dark. The change is not so significant for \(p\)-Si, since the positive charge of PEI does not change under illumination.

4. Conclusion
Thus, an increase in the sensitivity to glucose of an enzymatic biosensor based on n-Si fabricated with PSA of enzyme molecules onto a semiconductor substrate has been experimentally and theoretically proved. This sensitivity exceeds the sensitivity of biosensors both without PSA and based on p-Si,
regardless of the fabrication conditions. Our research is useful in fabrication of capacitive field-effect biosensor since the growth of enzyme molecules density on the surface of the semiconductor transducer increases both sensitivity and selectivity of the sensor.

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