Mechanism and sensitivity of the intrinsic charge detection of biomolecular interactions by field effect devices

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For many biotechnological applications a quantitative and label-free detection of biomolecular interactions is becoming of outstanding importance. The here presented analytical description of the direct charge detection by field effect devices introduces a straightforward tool for quantitative analysis. By combined measurements of the *p*H and electrolyte concentration response the absolute amount of adsorbed surface charge can now be determined. © *2007 American Institute of physics*. [DOI: 10.1063/1.2775040]

The label-free detection of biomolecular interactions is a challenging field in biotechnology. Semiconductor based field-effect transistors with a biocompatible surface functionalization (BioFETs) offer a promising approach for the development of new biosensors as they enable the direct electrical detection and real time monitoring of biomolecular interactions.[1–3] Hereby, silicon-on-insulator (SOI) devices are of special interest, because they are based on standard semiconductor technology and thus are open to miniaturization and parallelization for high throughput applications at affordable costs. Different approaches to apply FETs as biosensors have been developed, exploiting the charge sensitivity[1,3–7] as well as the *p*H[8–10] and electrolyte ion concentration sensitivity,[11,12] though it had been suggested that the direct detection of intrinsic charges would not be possible.[13] The BioFET sensitivity depends critically on the *p*H and electrolyte concentration n_0 of the aqueous solution but most importantly also on the gate oxide material properties. These oxide-electrolyte interfaces are generally described by a combination of the Gouy-Chapman theory (optionally extended by a Helmholtz layer) describing the surface potential drop in electrolyte solution and a site-binding model describing the acid-base reactions of (de)protonable sites on the oxide surfaces.[14–17] Until now, most interest was given to the *p*H sensitivity of such devices, and all dependencies are commonly collected in a dimensionless *p*H sensitivity parameter α, describing deviations from the Nernstian response.[17] However, to understand the sensitivity of BioFETs on variations in the electrolyte concentration and on the intrinsic charge of binding biomolecules, the complete set of equations has to be solved analytically as a function of all parameters, which has yet to be done. Only recently, the effect of adsorption of charged biomolecules on BioFET-gates has been studied by simulating the aqueous as well as the semiconductor side of typical sensor devices.[18]

In this letter we derive analytical descriptions for the sensitivity of BioFETs upon variations in the electrolyte concentration and upon binding of analyte charge and show how these are coupled to the *p*H sensitivity parameter α. Thus, by measuring the *p*H and the electrolyte concentration sensitivity of a field effect sensor, the charge sensitivity of the device can be determined without any further knowledge of the surface parameters. As these are not well known and may vary between different devices and even during measurements, we identify a critical prerequisite for an unambiguous interpretation of BioFET signals especially in differential measurement applications: *p*H and electrolyte jump experiments provide an easy method to ensure that the sensitivity of different samples and of differently functionalized structures is equivalent.

To derive analytical expressions for the BioFET sensitivity we discuss the following model for the functionalized sensor surface: The biomolecules to be detected bind with high affinities to specific receptors on the surface and thus irreversible binding of the analyte charges can be assumed. These receptors are assumed to be located in the same plane as the acid-base charge σ_{prot} originating from the (de)protonation of titratable surface groups, e.g., amphoteric OH groups on SiO_2 gate BioFETs (see Fig. 2 in the supplement[19]). Thus, the total charge σ_s in the surface plane is made up of the amount of bound analyte charge σ_{ana} and the acid-base charge: $\sigma_s=\sigma_{\mathrm{ana}}+\sigma_{\mathrm{prot}}$. The acid-base charge $\sigma_{\mathrm{prot}}(p\mathrm{H}_s)$ is a function of the local surface $p\mathrm{H}_s$ and the gate material properties such as the surface site density and the equilibrium constants of the acid-base reactions. All these important material parameters are collected in the buffer capacity $-e_0\beta_{\mathrm{prot}}=\partial\sigma_{\mathrm{prot}}/\partial p\mathrm{H}_s$ of the surface. The surface $p\mathrm{H}_s$ is determined by the bulk *p*H and the surface potential $p\mathrm{H}_s=p\mathrm{H}+(e_0\Psi_s/\ln 10k_BT)$, which turns $\sigma_s(p\mathrm{H},\Psi_s,\sigma_{\mathrm{ana}})$ into a function of the *p*H, the surface potential, and the amount of bound analyte charge. Neglecting charges inside the semiconductor, the double layer potential $\Psi_s(\sigma_s,n_0)$ is a function of the total surface charge σ_s and the ionic strength n_0 of the electrolyte so that $\Psi_s(p\mathrm{H},n_0,\sigma_{\mathrm{ana}})$ is determined as an implicit function of the *p*H, the electrolyte concentration, and the adsorbed analyte charge. Forming the total differential $d\sigma_s=d\sigma_{\mathrm{ana}}+d\sigma_{\mathrm{prot}}$, reordering and inserting $(\partial\sigma_s/\partial n_0)_{\Psi_s}=-(\partial\Psi_s/\partial n_0)_{\sigma_s}(\partial\sigma_s/\partial\Psi_s)_{n_0}$, β_{prot} and the differential capacitance of the electric double layer $C_{dl}=(\partial\sigma_s/\partial\Psi_s)_{n_0}$ gives the *p*H, electrolyte concentration, and analyte charge sensitivity of the sensor (see supplement[19]),

$$\frac{d\Psi_s}{dp\mathrm{H}}=-\frac{\ln 10k_BT}{e_0}\alpha=\left(\frac{\partial\Psi_s}{\partial p\mathrm{H}}\right)_{p\mathrm{H}_s}\alpha, \tag{1}$$

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$$\frac{d\Psi_s}{dn_0} = \left(\frac{\partial \Psi_s}{\partial n_0}\right)_{\sigma_s} (1-\alpha), \tag{2}$$

$$\frac{d\Psi_s}{d\sigma_{\text{ana}}} = \frac{1}{C_{dl}}(1-\alpha) = \left(\frac{\partial \Psi_s}{\partial \sigma_s}\right)_{n_0} \left(\frac{\partial \sigma_s}{\partial \sigma_{\text{ana}}}\right)_{\sigma_{\text{prot}}} (1-\alpha), \tag{3}$$

with the dimensionless sensitivity parameter $0<\alpha<1$ introduced in Ref. 17,

$$\alpha = \frac{1}{1+(\ln 10 k_B T C_{dl}/e_0^2 \beta_{\text{prot}})}. \tag{4}$$

Apparently, the electrolyte concentration and the charge sensitivity are connected to the *p*H-sensitivity via the sensitivity parameter α, a high *p*H sensitivity reduces both the other sensitivities. This is because the acid-base reactions on the surface, which give rise to the *p*H sensitivity, partly compensate signals generated under constant bulk *p*H, e.g., a positive signal caused by the adsorption of positive charge induces a rise in the surface potential. This rise in the surface potential is accompanied by a decrease in the surface $p\text{H}_s$, which causes more surface groups to dissociate—the surface becomes more negative and the surface potential is consequently decreased, so that the original charge adsorption signal is reduced.

As $p\text{H}_s = p\text{H} + (e_0\Psi_s/\ln 10 k_B T)$, any signal $\Delta\Psi_s$ detected by the sensor under constant bulk *p*H involves a change in the surface $p\text{H}_s$ of $\Delta p\text{H}_s = e_0 \Delta\Psi_s/\ln 10 k_B T$. The corresponding change in the surface charge is $\Delta\sigma_s = -e_0\beta_{\text{prot}}\Delta p\text{H}_s = -e_0^2\beta_{\text{prot}}\Delta\Psi_s/\ln 10 k_B T$, so that the induced "acid-base" reaction signal" $\Delta\Psi_s^{re}$ is in the opposite direction to $\Delta\Psi_s$: $\Delta\Psi_s^{re} = \Delta\sigma_{\text{prot}} C_{dl} = -e_0^2\beta_{\text{prot}}\Delta\Psi_s/\ln 10 k_B T C_{dl}$. As the combined signals are detected by the sensor, the acid-base reaction signal causes the charge and electrolyte concentration signals to be smaller than they would have been with the charge from the titratable groups σ_{prot} kept constant,

$$\Delta\Psi_s = \Delta\Psi_s|_{\sigma_{\text{prot}}} + \Delta\Psi_s^{\text{re}} = \Delta\Psi_s|_{\sigma_{\text{prot}}} - \frac{e_0^2\beta_{\text{prot}}\Delta\Psi_s}{\ln 10 k_B T C_{dl}}. \tag{5}$$

Solving for $\Delta\Psi_s$ yields in

$$\Delta\Psi_s = \Delta\Psi_s|_{\sigma_{\text{prot}}}(1-\alpha). \tag{6}$$

Thus, both the sensitivity toward changes in the electrolyte concentration [Eq. (2)] and the sensitivity toward binding analyte charge [Eq. (3)] are reduced by a factor of $(1-\alpha)$ compared to the signals with the acid-base charge σ_{prot} kept constant. Effectively the surface groups are buffering the surface $p\text{H}_s$. For $p\text{H}_s = \text{const} = p\text{H} + (e_0\Psi_s/\ln 10 k_B T)$ the sensor shows the Nernstian response to variations in *p*H and α equals 1: $(\partial\Psi_s/\partial p\text{H})_{p\text{H}_s} = -\ln 10 k_B T/e_0$. With $p\text{H}_s = \text{const}$ the surface potential is fixed for measurements under constant bulk *p*H, so that a sensor with $\alpha = 1$ is indifferent to changes in n_0 or binding analyte charge. Hence, a sub-Nernstian *p*H response is a prerequisite for a BioFET to detect proteins or other biomolecules by their intrinsic charge.

The obtained relations can be used for quantitative analysis of BioFET signals, for this purpose a combination with a concrete model is necessary. Using a Gouy-Chapman model for the potential drop in the diffuse layer, $\sigma_s(\Psi_s, n_0)$ is determined by the Grahame equation for $z{:}z$ electrolytes (the description for a Gouy-Chapman-Stern model is producing equivalent results and is added in the supplement[19]),

$$\sigma_s = \sqrt{8\varepsilon\varepsilon_0 n_0 k_B T}\sinh\left[\frac{z e_0 \Psi_s}{2 k_B T}\right]. \tag{7}$$

Now an explicit expression for the electrolyte concentration sensitivity can be given,

$$\frac{d\Psi_s}{dn_0} = -\frac{\sigma_{\text{prot}} + \sigma_{\text{ana}}}{2 n_0 C_{dl}}(1-\alpha) = -\frac{k_B T}{z e_0 n_0}\tanh\left[\frac{z e_0 \Psi_s}{2 k_B T}\right](1-\alpha). \tag{8}$$

As $d\Psi_s/dn_0$ depends on the sign of Ψ_s, the direction of a BioFETs response to a concentration jump reveals the sign of Ψ_s and $\sigma_s = \sigma_{\text{prot}} + \sigma_{\text{ana}}$; a surface with $\sigma_s = 0$ will be indifferent to n_0. Thus a series of concentration steps at different *p*H values can be used to determine the isoelectric point of the surface.[20] With replacing α from Eq. (1) the surface potential can be determined by measuring the *p*H and the electrolyte concentration sensitivity,

$$\Psi_s = \frac{2 k_B T}{z e_0}\operatorname{arctanh}\left[\frac{(d\Psi_s/dn_0)_{\text{norm}}}{(d\Psi_s/dp\text{H})_{\text{norm}} - 1}\right], \tag{9}$$

where $(d\Psi_s/dp\text{H})_{\text{norm}} = (d\Psi_s/dp\text{H}) e_0/(\ln 10 k_B T)$ and $(d\Psi_s/dn_0)_{\text{norm}} = (d\Psi_s/dn_0) z e_0 n_0/(k_B T)$ are the *p*H and concentration sensitivities normalized to the Nernstian responses. In the same manner the surface charge can be experimentally acquired,

$$\sigma_s = \frac{-\sqrt{8\varepsilon\varepsilon_0 n_0 k_B T}(d\Psi_s/dn_0)_{\text{norm}}}{\sqrt{(1-(d\Psi_s/dp\text{H})_{\text{norm}})^2 - (d\Psi_s/dn_0)_{\text{norm}}^2}}. \tag{10}$$

Furthermore, the sensitivity toward binding analyte charge can be represented by

$$\frac{d\Psi_s}{d\sigma_{\text{ana}}} = \frac{1}{\sqrt{2\varepsilon\varepsilon_0 z^2 e_0^2 n_0/k_B T}\cosh\left[\frac{z e_0 \Psi_s}{2 k_B T}\right]}(1-\alpha) = \frac{\sqrt{(1-(d\Psi_s/dp\text{H})_{\text{norm}})^2 - (d\Psi_s/dn_0)_{\text{norm}}^2}}{\sqrt{2\varepsilon\varepsilon_0 z^2 e_0^2 n_0/k_B T}}. \tag{11}$$

Thus with Eqs. (9)–(11) surface potential, surface charge, and charge sensitivity of the BioFET sensor surface can be determined experimentally by simply performing small steps in *p*H and n_0 to acquire $d\Psi_s/dp\text{H}$ and $d\Psi_s/dn_0$. Notably, as β_{prot} has been eliminated from Eqs. (9)–(11) by inserting the experimentally measured responses, this is possible without further specification of the model for the acid-base reactions on the surface (e.g., it does not matter if there is only one species of titratable groups ore several). Therefore the results for the determination of Ψ_s, σ_s, and $\mathrm{d}\Psi_s/\mathrm{d}\sigma_{\text{ana}}$ are free of any assumptions on the material parameters contained in β_{prot} such as the site density of the surface groups and the local equilibrium constants of the (de)protonation reactions, which strongly influence the BioFET sensitivity (see the comparison of the *p*H, concentration, and charge sensitivity for different gate oxides in Fig. 3 in the supplement[19]). It is obvious from Eq. (11) that the best charge sensitivity is achieved for $\alpha \to 0$ and $\Psi_s \to 0$, where

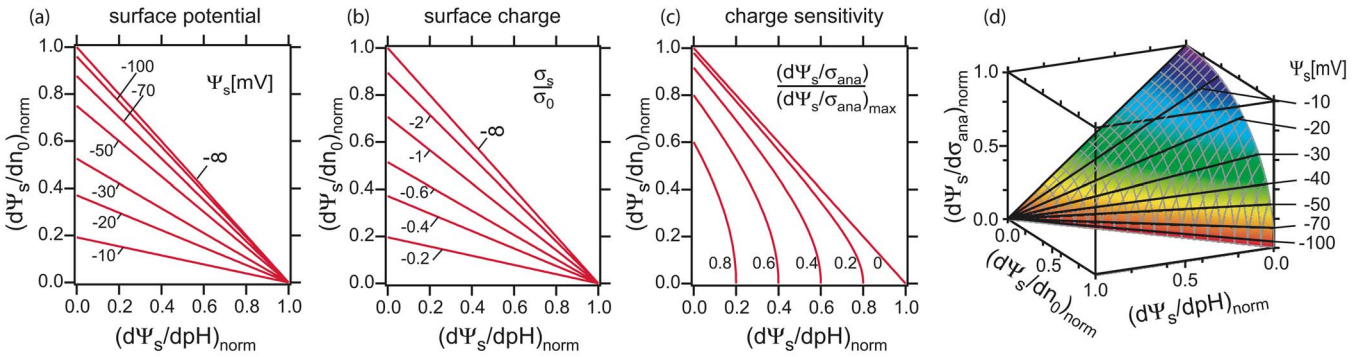

FIG. 1. (Color online) Combined measurements of the pH and the electrolyte concentration sensitivity $d\Psi_s/dp\mathrm{H}$ and $d\Psi_s/dn_0$ allow for the determination of (a) the surface potential Ψ_s, (b) the total surface charge σ_s, (c) the sensitivity toward binding analyte charge $d\Psi_s/d\sigma_{\mathrm{ana}}$ and (d) the interdependency of all sensitivities. $d\Psi_s/dp\mathrm{H}$ and $d\Psi_s/dn_0$ are normalized to the Nernstian responses, the surface charge is normalized to $\sigma_0=\sqrt{8\varepsilon\varepsilon_0 n_0 k_B T}$, and the charge sensitivity is normalized to the maximum sensitivity $(d\Psi_s/d\sigma_{\mathrm{ana}})_{\max}=1/\sqrt{2\varepsilon\varepsilon_0 z^2 e_0^2 n_0/k_B T}$. For surfaces with $\Psi_s,\sigma_s>0$ the electrolyte concentration response is negative, and graphs (a)–(c) are mirrored at the pH-sensitivity axis. For illustration the surface equipotential lines Ψ_s=const are drawn in (d).

$\cosh[e_0\Psi_s/2k_BT]\to 1$. $\Psi_s=0$ corresponds to an uncharged surface (implying $p\mathrm{H}=p\mathrm{H}_{\mathrm{iep}}$ at the isoelectric point) with a vanishing electrolyte concentration sensitivity, so that a sensor with both a low pH and a low concentration sensitivity is most favorable for the detection of binding analyte charge. Figure 1(a)–1(c) shows the contour lines for surface potential, surface charge, and charge sensitivity as a function of the pH and electrolyte concentration sensitivity. Figure 1(d) shows a threedimensional graph of the relation between charge, pH, and electrolyte sensitivity. Note that in practice for charge detection applications it will be difficult to bind σ_{ana} directly into the surface plane, so that in most cases there will be additional screening effects between σ_{ana} and the surface due to the mobile electrolyte ions.[4] Hence, Eq. (11) can be regarded as an upper limit for the charge sensitivity. Due to the inevitable uncertainties in measuring $(d\Psi_s/dp\mathrm{H})$ and $(d\Psi_s/dn_0)$ the application of Eqs. (9)–(11) is practically limited to a potential range $|\Psi_s|\lesssim 50$ mV, where $|\tanh[ze\Psi_s/(2k_BT)]|<1$, which limits the applicability to a pH range not too far away from the isoelectric point. This is also illustrated in Fig. 1(a) by the small separations of contour lines for $|\Psi_s|\gtrsim 50$ mV. Furthermore, the specific binding of counterions, which has not been considered here, will modulate the results,[11,21] but qualitatively the interdependence of the sensitivities should be conserved. However, for a sensor with a reasonably high charge sensitivity $|\Psi_s|\lesssim 50$ mV should be fulfilled and surfaces revealing the same responses to variations in pH and electrolyte concentration can be considered to be equally charge sensitive. This is of particular importance for differential measurement configurations, where signals on differently functionalized surfaces are directly compared.[2,3] In such cases, knowledge of the charge sensitivity on both surfaces is a prerequisite for an unambiguous interpretation.

In conclusion we have shown that a quantitative detection of charged molecules by FETs is best achieved on surfaces with both low pH sensitivity and low surface potential, which corresponds to a low pH and a low electrolyte concentration response. Therefore, combined measurements are mandatory for an unambiguous quantitative interpretation of BioFET charge detection signals.

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