Surface Charge Modulation and Reduction of Non-Linear Electrolytic Screening in FET-Based Biosensing

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Abstract—We experimentally investigate the influence of non-linear electrolytic screening by the electric double layer (EDL) and its impact on the field-effect transistor (FET) sensitivity to charged (bio)molecules. We use DNA hybridization to a PNA capture probe layer as model system. By co-depositing positively or negatively charged blocker molecules together with the PNA capture probes on the negatively charged SiO₂ surface of the FET, we control the overall surface charge and modulate the strength of local ion concentration within the EDL. We observe a FET signal enhancement of up to 51% when the overall charge including the captured analyte itself swings roughly symmetrically around zero during capture, as confirmed by zeta potential measurements. Surface plasmon resonance measurements rule out a change in captured analyte density as the origin of the enhancement in sensitivity. This confirms that excess screening caused by the large local ion concentration, which increases non-linearly with potential in the EDL, is responsible for a loss in sensitivity in bioFETs with a charged surface. These experimental findings agree with the early theoretical works that find a low surface potential to be desirable for the best bioFET performance.

Index Terms—Biosensing, pH sensing, field-effect transistor, electrolytic screening, surface charge, DNA hybridization, electric double layer.

I. INTRODUCTION

IT IS well established that electrostatic screening of biomolecular charge by electrolyte ions significantly impacts potentiometric biosensing using field-effect transistors (FETs). The screening increases with increase in ionic strength and consequently FET-based sensing is preferentially done in low ionic strength buffers [1]–[3]. However, much less attention has been given to the phenomenon of increased non-linear

electrolytic screening when an electric double layer (EDL) is present at the sensor surface, e.g. in response to surface charges. The local ion concentration increases exponentially with the local potential (Boltzmann distribution), and strongly exceeds the bulk ionic strength as soon as the local potential exceeds a few times $k_B T/q$ ($= 25$ mV). This results in a strong and non-linear increase in electrolytic screening.

Landheer *et al.* [4], [5] identified this phenomenon in a 1-D numerical model for planar devices where the biomolecular layer was modeled as an ion-permeable ‘membrane’ of finite thickness, separated from the surface by a thin Stern layer. Later, Wunderlich *et al.* [6] and Landheer *et al.* [7] presented analytical solutions for the simplified cases where the analytes are positioned directly at the oxide-electrolyte interface, i.e. in a plane with zero thickness or where the biolayer has a finite thickness, respectively. This resulted in a much more accessible analytical treatment, and the simple dependency between biomolecular sensitivity and the pre-existing surface potential for the charge plane [6],

$$\frac{d\Psi_0}{d\sigma_{ana}} \sim \frac{1}{\cosh\left(\frac{zq\Psi_0}{2k_B T}\right)} \quad (1)$$

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with Ψ_0 the potential at the oxide-electrolyte interface with respect to the bulk electrolyte potential, σ_{ana} the analyte charge density (located in the interfacial plane), and z the valency of the bulk electrolyte ($z = 1$ for NaCl). Note that the pre-existing EDL potential can not only result from fixed interface or oxide charges, but also from analyte charges already present on the surface. In other words, the differential sensitivity also tends to go down as more analyte charges are captured. A few other groups also noted this non-linear screening effect [8] and have explored it using numerical analysis [9]. More recently, Shoorideh and Chui [10] modelled the independent co-optimization of the EDL (low potential drop i.e. low integrated charge density) and of the FET (e.g. low channel charge density in the subthreshold regime) using both a front and back gate, and they suggested a practical way to fine-tune the device by engineering the amount of surface charge near the electrolyte/oxide interface. However, there has been very limited follow-up [11] and to the best of our knowledge, no detailed experimental verification of this screening effect has been reported.

In this paper, we provide the experimental demonstration of the non-linear screening phenomenon and we show that an improved FET-based biomolecular sensitivity can be obtained by optimizing the sensor surface charge with charged blocker molecules. For this, we used a peptide nucleic acid (PNA) - deoxyribonucleic acid (DNA) hybridization system as a model in combination with FETs fabricated completely using CMOS technology. In order to modify the surface charge, we co-deposited positively charged ($-\text{NH}_2$ terminated) or negatively charged ($-\text{COOH}$ terminated) blocker molecules together with the PNA capture probes. This was followed by a non-complementary DNA (ncDNA) incubation step (as negative control) and a complementary DNA (cDNA) hybridization step. By varying the surface blocker density for positive and negative polarities we were able to demonstrate the impact of non-linear EDL screening on hybridized cDNA FET signal transduction by controlling the surface charge. Streaming potential measurements were used to determine the zeta potential after various surface modifications. Surface plasmon resonance measurements were used to check for any secondary influence of the blockers on the degree of cDNA hybridization.

II. MATERIALS AND METHODS

A. Reagents

To prepare 150 mM and 1.5 mM buffers, phosphate buffered saline (PBS) 10X solution (1.5 M) (by InvitrogenTM, purchased from ThermoFisher Scientific, catalog #AM9624) was diluted with ultrapure de-ionized water (DIW). PNA with dibenzocyclooctyne (DBCO) modification purified with high-performance liquid chromatography (HPLC) (DBCO-C6-N term/AAC TCT CCA CTC ACC/C-term), was purchased from Panagene Inc and was suspended in DIW to a concentration of 50 μM , aliquoted and stored at -20°C . Complementary single stranded DNA (5/GGT GAG TGG AGA GTT/3) and non-complementary single stranded DNA (5/AAC ACA CAA CAC ACC/3) purified with HPLC (for hybridization and for negative control experiments respectively) were purchased from Integrated DNA Technologies BVBA (IDT). The

DNA was first dissolved at a concentration of 100 μM in a buffer (pH 8) containing 10 mM tris (product #T1503, Sigma-Aldrich) and 0.1 mM ethylenediaminetetraacetic acid (EDTA) (product #431788, Sigma-Aldrich), aliquoted and then stored at -20°C . Isopropyl alcohol (IPA) was purchased from KMG chemicals (product #135825). The pH adjustments for the buffers were done using 7% HCl solution (product #130471, KMG chemicals) or 2 M NaOH solution (pellets purchased from Supelco, product # 106469). The molecule 11-azidoundecyltrimethoxysilane (N_3 -silane) (95% pure, CAS: 334521-23-2, article no. AB258623) for surface functionalization was purchased from abcr GmbH, Germany. The DBCO modified charged blocker molecules (with polyethylene glycol (PEG) linker), DBCO-PEG4-NH₂ (CAS: 1255942-08-5) and DBCO-PEG4-COOH (CAS: 1537170-85-6) with HPLC purification ($> 95\%$ purity) were purchased from Jena Bioscience GMBH, Germany. These blocker molecules were first dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 mM, aliquoted and then stored at -20°C .

B. Device Fabrication

The FETs in this work were inversion-mode p-channel devices fabricated completely in a CMOS pilot line [12], [13]. They were fabricated on 300 mm silicon-on-insulator (SOI) wafers (supplied by Soitec) with a 145 nm thick buried oxide (BOX) and 88 nm thick silicon device layer (resistivity $\sim 12 \Omega \text{ cm}$). First, the Si device layer thickness was reduced to 26 nm by successive oxidation and wet etching. Fins with widths from 1000 nm to 60 nm and lengths from 3000 nm to 450 nm were defined using an advanced patterning approach based on 193 nm immersion lithography and hard mask shrinking [14], [15]. The channel doping is set at $2 \times 10^{17} \text{ cm}^{-3}$ (phosphorous) and the source/drain regions received high contact implant doping ($> 1 \times 10^{20} \text{ cm}^{-3}$, boron). This was followed by the growth of a 4 nm thick thermal SiO_2 layer as gate oxide. Next, CVD polysilicon was deposited to act as sacrificial layer for final cavity opening for electrolyte access to the Si fin. This was followed by gate patterning and an anneal at 1030°C .

Next, an oxide passivation layer was deposited, followed by opening of the source and drain contact, routing-out the connections towards the bond pads and the final oxide passivation layer deposition. The FET cavity and the bondpads were opened using a combination of dry and wet etch processes to expose the FET gate oxide and the bondpad metal.

C. Surface Chemistry

A self-assembled monolayer (SAM) of N_3 -silane was deposited on the sensor surface using chemical vapor deposition [16]. This was done in a vacuum chamber by vaporizing 120 μl of N_3 -silane at 145°C and at 25 mbar pressure for 2 hours, during which a uniform monolayer of the molecule was deposited on the surface. After the deposition, the dies were first rinsed with acetone and IPA and blow dried with N_2 . The DBCO conjugated PNA with/without blocker molecules were immobilized on the surface by reacting with the azide terminal of the SAM.

D. Biacore Setup and Protocol

Surface plasmon resonance (SPR) experiments were performed on a Biacore 3000 system. The substrates were made

Fig. 1. PNA (+ blocker), ncDNA and cDNA immobilization protocol for the reference SPR based Biacore 3000 measurements (on oxide covered Au surface [17]) and for the FET electrical sensing measurements (corresponding to protocol in Table I); the purple layer represents N_3 -silane SAM. (1) $1 \mu\text{M}$ PNA (shown as black single strands) + variable blocker concentration (shown as blue circular dots) in 150 mM PBS for 30 minutes, (3) $1 \mu\text{M}$ ncDNA in 150 mM PBS for 15 minutes as negative control and should not bind to the PNA probe, (5) $1 \mu\text{M}$ cDNA in 150 mM PBS for 15 minutes to obtain (saturated) hybridization with PNA probe. Each incubation step was followed by (2), (4) and (6) thorough rinsing and end-point measurements using 1.5 mM PBS for both FET as well as SPR. This ensured similar incubation as well measurement conditions for FETs and Biacore 3000 reference measurements [13], [17].

from $300 \mu\text{m}$ thick, $1 \times 1 \text{ cm}^2$ borosilicate glass (D 263®T eco Thin Glass, purchased from Schott AG), coated with a stack of 2 nm Ti, 40 nm Au (evaporated), 2 nm Ti and 10 nm SiO_2 (sputtered) [17]. The samples were cleaned with acetone and IPA, followed by a 15 min UV- O_3 treatment ($28 \sim 32 \text{ mW/cm}^2$ at 253.7 nm) prior to N_3 -silane SAM deposition.

The sample injections (PNA/blocker/DNA) were done in 150 mM PBS, while for running buffer (which flows between each successive sample injection) 1.5 mM PBS was used. To quantify the amount of PNA/blocker/DNA immobilized on the surface, end-point measurements were done in 1.5 mM PBS running buffer (before and after each sample injection step). This was done to replicate similar conditions as in the end-point FET electrical measurements [13], where 150 mM PBS was used during PNA/blocker/DNA incubation steps (when no FET electrical measurements were being performed) and 1.5 mM PBS was used during FET electrical measurements (after each PNA/blocker/DNA incubation step) as shown in Figure 1. The flow rate for Biacore 3000 was set at $5 \mu\text{l/min}$. First, $150 \mu\text{l}$ (30 mins) of $1 \mu\text{M}$ PNA along with blocker molecule (concentration varied) in 150 mM PBS buffer was injected into the channel. This was followed by a $75 \mu\text{l}$ (15 min) injection of $1 \mu\text{M}$ non-complementary DNA (ncDNA) and then a $75 \mu\text{l}$ (15 min) injection of $1 \mu\text{M}$ complementary DNA (cDNA), both in 150 mM PBS, which was long enough to obtain a saturated cDNA hybridization signal.

E. Streaming Potential Measurements

Zeta potential (ζ) measurements using the streaming potential technique were performed on a SurPASS™ 3 system (by Anton Paar). We used planar Si samples ($1 \times 2 \text{ cm}^2$)

TABLE I
INCUBATION STEPS AND PROTOCOL FOR FET ELECTRICAL MEASUREMENTS

#	Protocol steps	Incubation time (min)	PBS buffer ionic strength (mM)
1	Capture probe + blocker grafting: $1 \mu\text{M}$ PNA + (variable) blocker ($400 \mu\text{l}$) injection	30	150
2	(Rinsing + I-V measurements) $\times N$	-	1.5
3	Negative control: ($1 \mu\text{M}$ ncDNA ($500 \mu\text{l}$) injection)	15	150
4	(Rinsing + I-V measurements) $\times N$	-	1.5
5	Hybridization: ($1 \mu\text{M}$ cDNA ($500 \mu\text{l}$) injection)	15	150
6	(Rinsing + I-V measurements) $\times N$	-	1.5

with a 100 nm thick thermally grown SiO_2 on top. The zeta potential was measured while varying the pH of the 1 mM KCl electrolyte using HCl and NaOH. To minimize pH drift of the electrolyte, N_2 gas was continuously bubbled through the closed beaker containing the electrolyte. The protocols for surface functionalization and PNA/blocker/DNA immobilization for these samples was same as that used for Biacore (and FET electrical measurements), i.e. N_3 -silane deposition followed by PNA-blocker co-deposition and DNA incubation steps in 150 mM PBS.

F. Electrical Measurement Setup

A $60 \mu\text{l}$ silicone flow cell (by Grace Bio-Labs, SKU: 621101-S) was mounted on the sample die and connected to a flow-through true Ag/AgCl electrode (provided by Micro-electrodes Inc. product #16-702), which was used to apply gate-source bias (V_{GS}) through the electrolyte [18]. The semi-automatic measurements were performed using a Keithley 2602 on a nano-probe station (by SUSS Microtech). The measurements were performed in darkness with drain-source bias (V_{DS}) of -50 mV and an integration time of 40 ms . The data reported in this paper were measured on 250 and 1000 nm wide and 1000 and 3000 nm long FETs.

G. Electrical Measurement Protocol

First, a mixture of IPA (75%) and 1.5 mM PBS (25%) was flushed through the flowcell to ensure the proper wetting of the cavities. This was followed by immediate rinsing with 1.5 mM PBS to remove of any leftover IPA in the flowcell. The subsequent steps for PNA + blocker graft and DNA hybridization are listed in Table I.

We used constant current method to quantify the shifts observed after cDNA and ncDNA incubations, where the voltage V_0 was defined as the gate voltage (V_{GS}) for which the drain-source current $I_{\text{DS}} = 1 \text{ nA}$, at $V_{\text{DS}} = -50 \text{ mV}$. The transfer characteristics (I_{DS} vs V_{GS}) of the FETs were measured until I_{DS} crossed 1 nA and the corresponding V_0 value was extracted for each measurement and for each incubation phase. Multiple rinsing (1.5 mM PBS, $500 \mu\text{l}$) and

Fig. 2. PNA-DNA hybridization using Biacore 3000, with different colours indicating the 4 different channels; (0), (2), (4), (6) 1.5 mM PBS was used as running buffer which flows between each successive sample injection, (1) 1 μ M PNA + 1 μ M -NH₂ blocker injection in 150 mM PBS for 30 minutes gave a response of 4607 ± 90 RU (black arrow), (3) 1 μ M ncDNA injection in 150 mM PBS for 15 minutes gave a response of 27 ± 17 RU, (5) 1 μ M cDNA injection in 150 mM PBS for 15 minutes reaches saturation for hybridization (as shown in the inset) and gave a response of 671 ± 13 RU (green arrow). The hybridized cDNA does not de-hybridize when the buffer is returned back from 150 mM PBS to 1.5 mM PBS as shown by a stable response in (6).

end-point electrical measurement (1.5 mM PBS) steps were performed (Steps # 2, 4, and 6 in Table I) after each incubation step, until stable I-V characteristics were obtained. During the PNA/DNA incubation steps (# 1, 3 and 5 in Table I), the FET device was in floating condition.

III. RESULTS AND DISCUSSION

A. Influence of PNA-Blocker Co-Deposition on DNA Hybridization

To understand the impact of non-linear screening on DNA hybridization sensing, we modified the surface potential by co-immobilizing PNA probes with variable concentrations of charged blocker molecules, PEG₄-NH₂ ($pK_{a,NH_2} \cong 9.8$, positively charged at pH 7.2) and PEG₄-COOH ($pK_{a,COOH} \cong 2.4$, negatively charged at pH 7.2) [19]. The dependence of the amount of DNA hybridization on the blocker concentration used during the grafting of the PNA capture probes (with a constant 1 μ M PNA) was measured using SPR (Biacore 3000 system).

Figure 2 shows a typical Biacore 3000 sensogram. The amount of immobilized PNA (+ blocker) or hybridized DNA was quantified by taking end-point measurements in running buffer i.e. the difference of the response units before and after each injection cycle, when the running buffer was flowing.

Multiple PNA-blocker co-depositions were carried out by increasing the bulk blocker concentration, while keeping a constant 1 μ M PNA concentration, followed by ncDNA and cDNA hybridization steps for every PNA-blocker co-deposition. The end-point saturated measurement data for all these experiments is shown in Figure 3.

Fig. 3. (A) Total PNA + blocker signal (where 1 Biacore RU ≈ 1 pg/mm²) as function of the positive (NH₂) and negative (COOH) bulk blocker concentrations with increase in bulk blocker concentration (molecular weight ~ 0.5 kDa) co-deposited with 1 μ M PNA (4.2 kDa). The dash-dot line represents a full PNA layer (deposited without blockers). The error bars represent the standard deviation for PNA (+blocker) signal on 4 different channels. (B) Saturated complementary (cDNA) and non-complementary DNA (ncDNA) hybridization signals. The dashed line represents mean saturated cDNA hybridization of 656 ± 8 RU (equivalent to 8.3×10^4 cDNA/ μ m²) to a full PNA probe layer (without blockers).

The saturated cDNA hybridization on a full PNA layer (without blocker molecules) gave a mean response of 656 ± 8 RU, which is approximately equivalent to 8.3×10^4 cDNA/ μ m² (assuming 1 Biacore response unit (RU) = 1 pg/mm² [20], [21]), and is shown as a dashed line in Figure 3 (B). With increase in blocker content, the PNA probe density reduces somewhat (Figure 3 (A)), causing at first an increase in PNA-DNA hybridization (Figure 3 (B)) due to the reduction of steric hindrance [22], [23]. We observed a maximum enhancement of 11 to 16% in DNA hybridization (up to 9.7×10^4 cDNA/ μ m²) at approximately 3 μ M blocker concentration (Figure 3 (B)), compared to DNA hybridization on a pure PNA layer without blockers. With further increase in blocker concentration, the PNA probe density starts to decline significantly, causing a drop in DNA hybridization.

We observe no significant difference in the amount of PNA-DNA hybridization for positive versus negative blocker concentration variation. This indicates that the use of 150 mM ionic strength buffer during the immobilization steps (PNA/blocker/DNA) screens most of the molecular and surface charge, thereby minimizing analyte (DNA) to surface and analyte electrostatic interactions.

Fig. 4. Zeta potential versus pH of the electrolyte (1 mM KCl) for surfaces with various conditions and treatments, with a dotted vertical line at pH 7.2. Curves #4 and #3 show the zeta potentials for bare SiO₂ surface (-90 mV at pH 7.2) and after N₃-silane SAM deposition (-50 mV at pH 7.2) respectively. Curves #1 and #5 show the N₃-silane SAM coated surfaces with saturated positive and negative blocker deposition (without PNA), modulating the zeta potential from $+75$ mV down to -115 mV at pH 7.2 respectively. For the surface saturated only with PNA (without blockers), the zeta potential was very close to zero mV at pH 7.2 (curve #2). Markers: data points, lines: after smoothing using the locally estimated scatterplot smoothing algorithm (LOESS).

B. Impact of Surface Treatments on Surface Potential

The influence of the PNA-blocker co-depositions on the potential distribution close to the surface was evaluated using streaming potential measurements which gives access to the zeta potential (ζ). This is the potential at the slip plane, which we can assume to lie outside the dense functionalization layers, and this gives an indication of the net charge of the surface plus the functionalization layer. The bare SiO₂ surface was measured to be negatively charged with the zeta potential about -90 mV at pH 7.2 (Figure 4, curve #4). After N₃-silane SAM deposition on the SiO₂ samples (and before PNA or blocker grafting), the negative surface charge reduced, and the zeta potential increased to -50 mV (curve #3). By adding a saturated layer of blocker molecules (without PNA), the zeta potential could be tuned to be strongly positive (up to $+75$ mV (curve #1)) using $-NH_2$ terminated blocker or strongly negative (-115 mV (curve #5)) using $-COOH$ terminated blocker, at pH 7.2, as shown in Figure 4. For an N₃-silane SAM coated SiO₂ surface with a saturated density of only PNA molecules (without blockers), the zeta potential was very close to 0 mV (curve #2). These zeta potential measurements demonstrate the strong control of near-surface potentials by the selection of the appropriate type and concentration of charged blocker molecules.

C. Impact of Non-Linear Screening on FET Sensitivity

A typical PNA-DNA hybridization sensing experiment for a 1000 nm long and 1000 nm wide FET with its transfer curves is shown in Figure 5 (A). The gate voltage was swept down from 0 V until the I_{DS} crossed 1 nA (Figure 5 (A)).

Fig. 5. (A) Transfer characteristics of a 1000 nm long and 1000 nm wide FET (at $V_{DS} = -50$ mV) coated with N₃-silane SAM, measured in 1.5 mM PBS buffer (pH 7.2) using a flow-through Ag/AgCl as gate electrode. The plot also shows transfer characteristics after (1 μ M PNA + 3 μ M positive blocker) grafting, ncDNA and cDNA incubation phases (also measured in 1.5 PBS buffer). The transfer curve shifts were quantified using constant current method, where a voltage V_0 (dotted vertical line) was defined as the gate voltage V_{GS} for which the drain-source current $I_{DS} = 1$ nA (dotted horizontal line). The FET gate voltage was swept down from 0 V until I_{DS} exceeded 1 nA (e.g. $V_{GS} = -0.5$ V in this case) and the corresponding V_0 value (i.e. V_{GS} at $I_{DS} = 1$ nA) was extracted for each measurement and for each incubation phase. (B) Post PNA/DNA incubation steps (in 150 mM PBS) and a thorough rinse (in 1.5 mM PBS), multiple rinsing steps followed by I-V measurements were done (both in 1.5 mM PBS) until stable transfer curves were obtained. The plot shows the extracted ($V_0 - V_{ref}$) from each I-V measurement after PNA – blocker co-deposition and ncDNA and cDNA incubation steps, where $V_{ref} = V_0$ for measurement #0.

V_0 (V_{GS} for $I_{DS} = 1$ nA) values were extracted for each transfer curve and for each incubation step. These are shown in Figure 5 (B). Since the PNA and DNA incubations were done in 150 mM PBS buffer and the electrical measurement in 1.5 mM PBS (Table I), we made sure that complete buffer exchange took place by performing multiple rinsing steps followed by an I-V measurement (numbered successively in Figure 5 (B)) until stable transfer curves were obtained. The transfer curve after ncDNA incubation in Figure 5 (A) (for which the corresponding V_0 is shown as measurement #12 in Figure 5 (B)) almost overlaps with the transfer curve after

Fig. 6. FET signal (V_0 shifts) for cDNA hybridization and for ncDNA as negative control, versus bulk blocker concentration (co-deposited with $1\mu\text{M}$ PNA) for both positive (NH_2) and negative (COOH) blockers. The error bars are the standard deviations over 4 device measurements.

PNA-blocker co-deposition (measurement #5, Figure 5 (B)), while after cDNA hybridization we observe a rigid shift in the transfer curve as shown in Figure 5 (A) (corresponding to measurement #20, Figure 5 (B)). For the particular device shown in Figure 5, with $3\mu\text{M}$ positive ($-\text{NH}_2$) blocker co-deposited with $1\mu\text{M}$ PNA, we observed a negligible shift in V_0 of $\sim 3.5\text{ mV}$ for ncDNA incubation and about 29 mV rigid V_0 shift after cDNA hybridization.

The influence of changes in surface charge on the FET signal is shown in Figure 6. With increase in positive blocker concentration, we observed an increase in cDNA signal peaking at about $10\mu\text{M}$, and then a decrease in signal for higher blocker concentrations (Figure 6). The cDNA signal (V_0 shift) at the peak is about 51% enhanced ($\sim 44\text{ mV}$) compared to the cDNA signal without blocker molecules ($\sim 29\text{ mV}$). When a negative blocker concentration was used, the V_0 shift was not only less compared to the V_0 shift for each corresponding positive blocker concentration but the V_0 shift also continuously decreased with the increase in negative blocker concentration.

The peak observed in the FET signal (51%) versus positive blocker concentration is much larger than the maximum increase in the bound DNA as measured by the Biacore (11% for positive blocker and 16% for the negative blocker) and occurs at a different blocker concentration ($10\mu\text{M}$ vs. $3\mu\text{M}$). Moreover, the FET signal shows a peak only for positive surface modification and decreases monotonously for negative surface modification. By contrast, the binding measurements using Biacore show a nearly identical trend for both positive and negative blockers (Figure 3 (B)). This confirms that the dominant effect causing the peak in the FET signal is related to the charge of the blockers and the effect of screening (on FET signal transduction), and not a simple modulation of the amount of bound DNA.

With increase in positive blocker density on an initially negatively charged surface, the absolute potential drop over

the EDL starts to decrease. This causes a decrease in overall ion concentration in the EDL until it becomes comparable to the bulk ion concentration. At that point the excess screening, caused by the strong, non-linear build-up of local ion concentration, vanishes, and the FET signal transduction is maximized. When the positive blocker concentration increases further, an oppositely charged EDL forms, and the screening increases again. Together with the gradual decrease in the amount of hybridized DNA, this results in a strong decrease in signal beyond the peak. For negative blockers, the absolute potential drop over the EDL keeps increasing, causing an increase in the total ion concentration in the EDL and a continuous increase in electrolytic screening of the hybridized cDNA, resulting in a continuous drop in FET signal transduction.

D. Zeta Potential Measurements for Confirmation of Non-Linear Screening

To further confirm the origin of the FET signal enhancement, we measured the zeta potentials before and after cDNA hybridization for a surface with a full layer of only PNA molecules (no blockers) and for a surface with $10\mu\text{M}$ positive blocker + $1\mu\text{M}$ PNA concentration (i.e. at the peak of the FET signal) (Figure 7 (A)). At the peak FET signal, the zeta potential swings almost symmetrically around zero, with a total swing of 105 mV . The initial surface charge is opposite to the analyte (DNA) charge but smaller, such that the net charge seen by the FET varies roughly between minus and plus half of the analyte charge. By contrast, for the PNA-only capture surface, the initial zeta potential is close to zero, such that the net charge seen by the FET varies between roughly zero and full analyte charge. Although there is a significant difference between the initial zeta potentials, the final zeta potentials seem to saturate, and the swing is reduced to 60 mV .

This can be understood qualitatively by considering the simplified case of a biomolecular layer with zero thickness (i.e. all the charges sit on the surface, and the slip plane coincides with the surface) and negligible charge inside the semiconductor (i.e. the EDL charge is exactly opposite to the surface charge). In this situation, the surface potential (which now coincides with the zeta potential) as a function of the surface charge is given by the Grahame equation [24] as shown in Figure 7 (B). We see that the maximum rate of change in zeta (or surface) potential occurs within a limited window around zero. When the zeta potential exceeds a few times $k_B T/q = 25\text{ mV}$, the sensitivity of the zeta potential to further changes in the charge decreases (the Debye-Hückel model based on a linearized Poisson-Boltzmann theory breaks down), giving rise to the excess non-linear screening. For a biomolecular layer of finite thickness, the analysis is more complex [4], [5] but the trend for the excess non-linear screening remains the same.

E. Discussion and Outlook

Here we experimentally investigated the impact of surface charge on the sensitivity of FETs to a large biomolecular density. In such a case, the analyte charge itself contributes to the

Fig. 7. (A) Zeta potential versus pH of the electrolyte (with dotted vertical line at pH 7.2), before and after cDNA hybridization for two different surface conditions: PNA only (curves #2 and #4) and $1 \mu\text{M}$ PNA + $10 \mu\text{M}$ positive blocker (curves #1 and #3). The black solid (#2) and dashed curve (#4) show surface after saturated PNA-only immobilization and cDNA hybridization respectively, giving a shift of about 60 mV (black arrow with triangle head). Similarly, the green solid (#1) and dashed curve (#3) show surface after a mixed deposition of $1 \mu\text{M}$ PNA + $10 \mu\text{M}$ positive blocker and cDNA hybridization respectively, giving a larger shift of about 105 mV (green arrow with rhombus head). (B) Plot for zeta potential (ζ) versus calculated surface (and biomolecular) charge density (σ) using the Grahame equation (inset), where σ is the calculated areal charge density at oxide-electrolyte interface, c_0 is the bulk ionic strength, ϵ and ϵ_0 are absolute and relative permittivity constants, and z is the valency of the bulk electrolyte ($z = 1$ for NaCl).

creation of a strong EDL, which gives rise to the excess non-linear screening. Therefore, maximum sensitivity is obtained when the initial sensor surface is charged approximately half and opposite in polarity, of the expected biomolecular charge, such that the EDL potential swings roughly symmetrically around zero. In case of a small biomolecular density or even single molecule sensing however, the analyte itself does not significantly influences the EDL, and the sensor surface potential should be close to zero mV (i.e. an uncharged surface), allowing the ion concentration in the EDL to be the same as the bulk ionic strength.

One last point merits further discussion. The change in the zeta potential in response to the DNA hybridization is significantly larger than the signal picked up by the FET, both at the peak (105 mV vs 44 mV) and on the pure PNA capture probes without additional surface charge control (60 mV vs 29 mV). As the FET measures the surface potential Ψ_0 , this points towards an additional negative feedback (or screening) mechanism that suppresses the change in surface potential compared to the changes at the slip plane deeper inside the liquid. This mechanism has also been identified in some early works [4]–[7], but has been largely ignored since then. It is likely related to screening by the *pH-sensitive surface states*.

Our experiments and the discussion so far have focused on the role of the *electrolyte* screening i.e. the accumulation and depletion of mobile electrolyte ions when the arrival of an analyte charge (tries to) modify the local potential. This accumulation/depletion forms a negative feedback loop that opposes (or suppresses) the change in potential. We have studied the non-linear increase in electrolyte screening when a large potential drop is present in the EDL in response to a large density of surface charge (and/or functional layer and analyte charges). Up till now we have implicitly assumed that these charges are fixed, i.e. that they don't change in response to the change in local potential.

However, this is not true for the silanol (Si-OH) groups on the SiO_2 surface. Their protonation (or charge state) is in equilibrium with the surface pH, and the surface pH varies with the surface potential Ψ_0 through the Boltzmann relation, $a_{H_s^+} = a_{H_b^+} \exp(-q\Psi_0/k_B T)$, with $a_{H_s^+}$ and $a_{H_b^+}$ the surface and bulk proton activities [25], [26]. Similar to the mobile electrolyte ions, when the arrival of an analyte charge (tries to) modify the surface potential, the charge in the surface states will change, forming a negative feedback loop that opposes (or suppresses) the change in potential. This results in screening of the analyte charge by the *pH-sensitive surface states*. The resulting tendency to pin the surface potential by the pH-sensitive surface groups is clearly visible in e.g. Fig. 3 of Landheer *et al.* [4], where the potential profiles without and with the analyte charge diverge from the bulk solution towards the outer edge of the analyte layer (approximate position of the slip plane where our zeta potentials are measured), and converge again towards the surface (where our FET signal is obtained) when pH sensitive surface states are present. This pinning effect is strongest for surfaces with the highest pH sensitivity, i.e. surfaces where the surface potential is tied the strongest to the pH of the bulk solution [4]–[7]. Hence, as already concluded by Wunderlich *et al.* [6] “a quantitative detection of charged molecules by FETs is best achieved on surfaces with both low pH sensitivity and low surface potential”. The negative correlation between pH sensitivity and bio-sensitivity has recently been observed experimentally [27].

Hence, in future work, it will be important to consider both, the sensor surface potential (or charge) and the pH sensitivity at the same time, such that the optimization for high FET sensitivity is implemented not only by reducing the absolute surface charge but also by minimizing pH sensitivity, by either selecting appropriate surfaces with low intrinsic pH sensitivity or by using dedicated surface chemistries to

passivate or compensate the effect of the surface (silanol) groups.

IV. CONCLUSION

We experimentally investigated the impact of excess non-linear electrolytic screening on the sensitivity of bioFETs, when the local potential within the EDL exceeds a few times $k_B T/q = 25$ mV, and the linear Debye-Hückel screening model is no longer accurate. We used variable concentrations of either positively or negatively charged blocker molecules, co-deposited with the PNA capture probes, to modulate the overall surface charge of SiO₂-based FET and demonstrated an improvement in FET sensitivity to hybridized cDNA when the net charge is minimized.

Reference measurements using surface plasmon resonance showed only minor changes in the amount of cDNA hybridization (11 to 16%), related to small changes in steric effect and capture probe density, and identical for positive and negative surface modifications. The electrical FET measurements gave a clear difference in response between positive and negative surface modifications. Positive blockers, with their opposite charge to SiO₂ surface and DNA analyte (both negative), resulted in an increase of up to 51% in the FET signal, followed by a decrease when the positive blocker concentration was further increased. Negative surface modification on the other hand resulted in a monotonic decrease in FET sensitivity. Zeta potential measurements confirm that the peak in the FET sensitivity is obtained when the overall charge (or potential) in the EDL is close to zero. For small analyte charge, e.g. in single molecule measurements, the maximum signal would be obtained when the net charge in the double layer is zero. This is in agreement with earlier theoretical predictions [4]–[6] and can be understood as excess or non-linear electrolyte screening when the linear Debye-Hückel model breaks down, i.e. when the potential in the double layer exceeds a few times $k_B T/q = 25$ mV. In such a situation, electrolyte screening is no longer related to the bulk ionic strength only, but the local ionic concentrations in the double layer need to be taken into account as well.

We further found that the bioFET signal (the change in surface potential) is smaller than the change in zeta potential, which may be a signature of additional screening by the pH-sensitive surface states that was predicted in the same theoretical studies and observed experimentally by Kumar *et al.* [27]. Surface treatments to maximize the bioFET response should aim at minimizing both the double layer potential and the pH sensitivity of the sensor.

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