

## Signal enhancement in electronic detection of DNA hybridization

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Electronic detection of the specific recognition between complementary DNA sequences is investigated. DNA probes are immobilized at different lateral positions on a Poly(L-lysine)-coated surface of an integrated silicon transistor array. Hybridization and field effect detection are done with the solid surface immersed in electrolyte solutions. Differential measurements are performed, where DNA hybridization leads to surface potential shifts between the transistors of the array. We experimentally show that these differential signals of hybridization can be enhanced significantly by changing the salt concentration between hybridization and detection.

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DNA microarrays are broadly used for studying gene expression, discovering drugs, and diagnosing disease [1]. The underlying recognition principle, hybridization between complementary strands, is currently detected by fluorescence measurements requiring a labeling step, which is expensive and time consuming. This motivates research on various label-free detection methods [2–4], among which electronic detection with semiconductor field effect structures increasingly appears as a promising future concept [5–10].

Field effect detection in electrolyte/oxide/semiconductor configurations requires a low-salt electrolyte, since the measured quantity, a variation in electrostatic potential induced by molecule binding at the electrolyte/oxide interface, is subject to screening by the mobile ions of the electrolyte solution [5,11]. DNA hybridization on microarrays, however, is typically done at comparatively high salt (50 mM and above) where a better specificity of the base sequence recognition is obtained. For salt concentrations above a few millimolar, the hybridization-induced shift in threshold voltage is only a few millivolts. Experimental drifts often are of comparable amount, making reproducible detection difficult. Most earlier work on field effect detection of hybridization used an *in situ* approach, where the electronic measurement is done during the hybridization [8,12–14]. Two groups showed *in situ* field effect detection of point mutations in oligonucleotide sequences, using low-salt ( $\leq 20$  mM) hybridization. Specificity was achieved by using either positively charged Poly(L-lysine)-coated surfaces [8,15] or special peptide nucleic acid probes [14]. Recently, DNA binders introduced as charged labels between hybridization and detection were used to obtain a signal amplification for transistor-based detection of DNA oligonucleotide single nucleotide polymorphisms [10].

In this paper we consider hybridization detection by field effect transistor arrays, in a configuration where the hybridization and the detection steps are separate in time. This introduces the possibility of performing the hybridization and the field effect measurement in different buffer solutions

and optimizing sensitivity and specificity independently. One potential difficulty of this approach is the fact that a sole buffer change induces an electronic signal and may induce drift. Another is that DNA duplexes formed in a high-salt hybridization buffer could separate during a subsequent measurement at low salt.

We use silicon *p*-channel field effect transistor (FET) arrays, fabricated as described in [16]. Each integrated array is composed of 31 or 96 individual FETs, which are linearly arranged with a period of 20 or 40  $\mu\text{m}$  and exhibit active surfaces of 24, 36, or 100  $\mu\text{m}^2$  covered by a 10-nm-thick

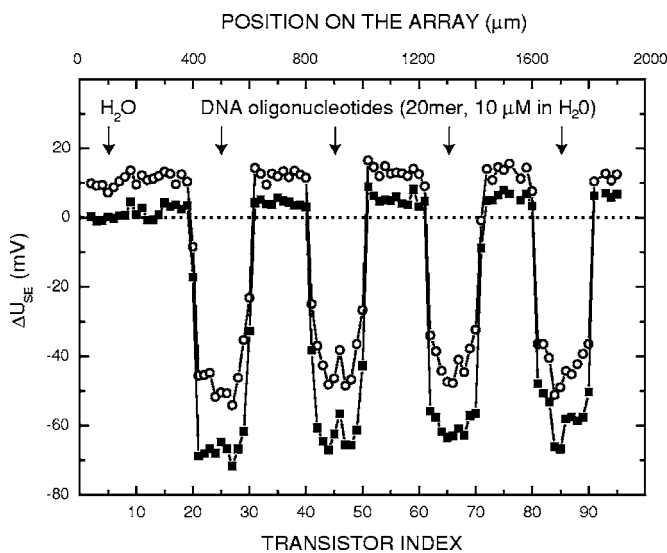


FIG. 1. Transistor-based detection of surface-bound DNA. Four deposits of a DNA oligonucleotide solution (right) and one reference deposit of  $\text{H}_2\text{O}$  (left) are performed across a linear array of 96 FETs integrated with a period of 20  $\mu\text{m}$  in silicon. The data points show, for each transistor of the array (bottom axis) and fixed ( $I_D$ ,  $U_{SD}$ ) working point, the shift  $\Delta U_{SE}$  of the current-voltage characteristics between two measurements performed with a 0.1 mM  $\text{KNO}_3$  electrolyte. The first measurement is done prior to the deposition. The second measurement is done either directly afterwards (full squares) or after an additional rinsing with pure water (open circles). An electrode with ion bridge was used for this measurement.

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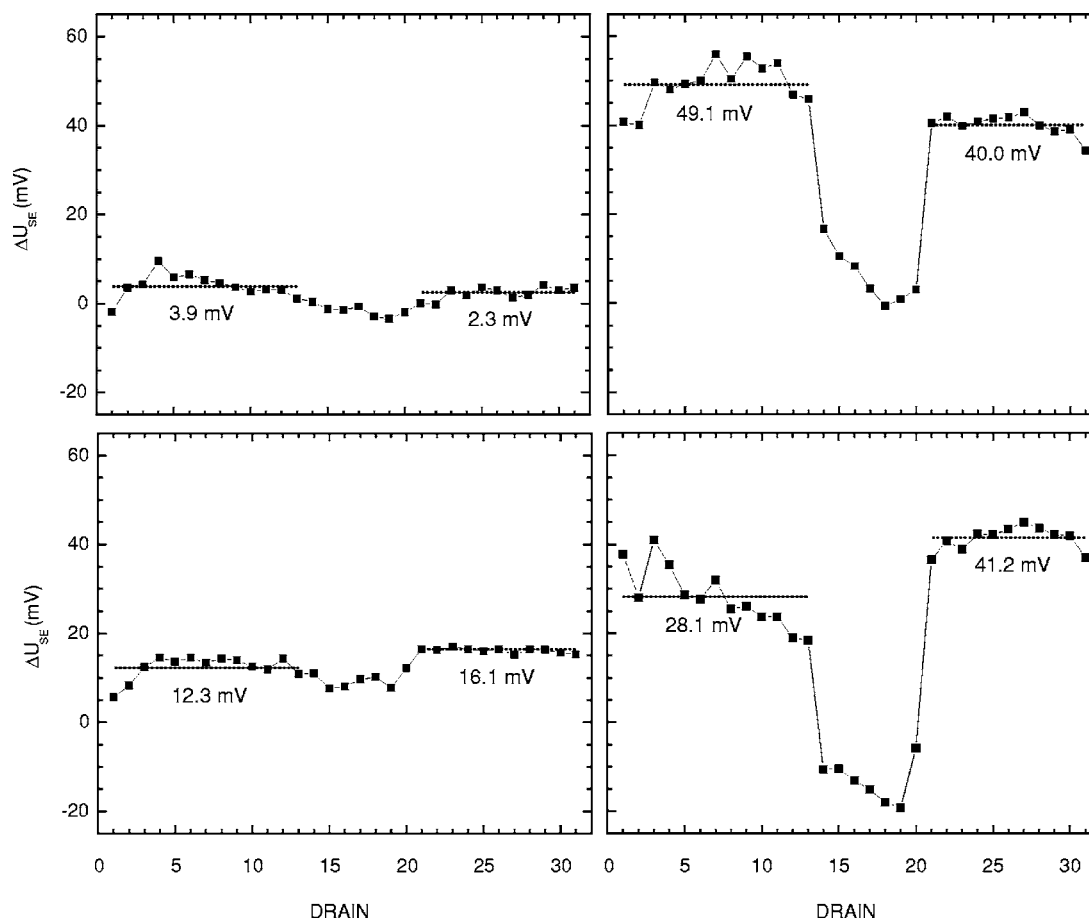


FIG. 2. Electronic detection of specific hybridization between DNA oligonucleotides. Measurements presented in the left (right) column have been performed with a 50 mM (0.01 mM) KCl electrolyte. The upper (lower) graphs show the shifts between a measurement after the first (second) hybridization reaction and a reference measurement done before the hybridizations. Both hybridizations are correctly detected. A smaller average  $\Delta U_{SE}$  is observed where probes and targets are complementary. The reduction in salt leads to an amplified difference signal.

SiO<sub>2</sub> layer. To prepare DNA immobilization, we coat the FET arrays with Poly(L-lysine) (PLL), as described elsewhere [11]. A commercial piezo spotter equipped with custom video control is used to locally deposit DNA solutions on the dry PLL-coated surface. Each DNA spot covers a number of neighboring FETs on the array. PLL-mediated immobilization is widely used in the field of DNA microarrays, although the resulting DNA attachment is known to be less stable than the one obtained by covalent immobilization strategies. An advantage is that the electrostatic immobilization of the negatively charged DNA by the positively charged PLL layer allows reusing the FET arrays; we can clean the SiO<sub>2</sub> surfaces after use and repeat the cycles of surface treatment, DNA immobilization, and detection.

In the electronic measurements, the surface of the whole array is covered by an electrolyte solution and an Ag/AgCl electrode is immersed. The drain current  $I_D$  of each FET is measured as a function of a dc voltage  $U_{SD}$  applied between source and drain and a dc voltage  $U_{SE}$  applied between source and the reference electrode [11]. Microscopic fluorescence measurements, performed on dry FET arrays after completion of the electronic detections, use a dual color setup with laser excitation at 532 and 633 nm, submicrome-

ter spatial resolution, and a cooled photon-counting-mode detector (see [11] for details). All manipulations and measurements described in this paper were done at room temperature.

Figure 1 illustrates the electronic detection of adsorbed DNA. Four deposits of an oligonucleotide solution (right) and one deposit of pure water (left) are performed on the dry surface of a PLL-coated array. The 96 individual transistors of this array are arranged along a line of 2 mm in length. The bottom horizontal axis indicates the index of each transistor and the top axis provides the corresponding position along the array. Each deposit exhibits a diameter of about 200  $\mu\text{m}$  and covers about ten transistors. For each transistor and fixed working point ( $I_D, U_{SD}$ ), differences in  $U_{SE}$  between two measurements are shown. The first measurement is done prior to the deposition, while the second measurement is done either directly afterward (full squares) or after an additional rinsing with pure water (open circles). The adsorption of DNA here gives rise to negative shifts  $\Delta U_{SE}$  of about 60 mV, while no significant signal is detected on the transistors subjected to the water deposition.

We observe that, in the case of Fig. 1 the rinsing step leads to a slight reduction of the amplitudes of the DNA

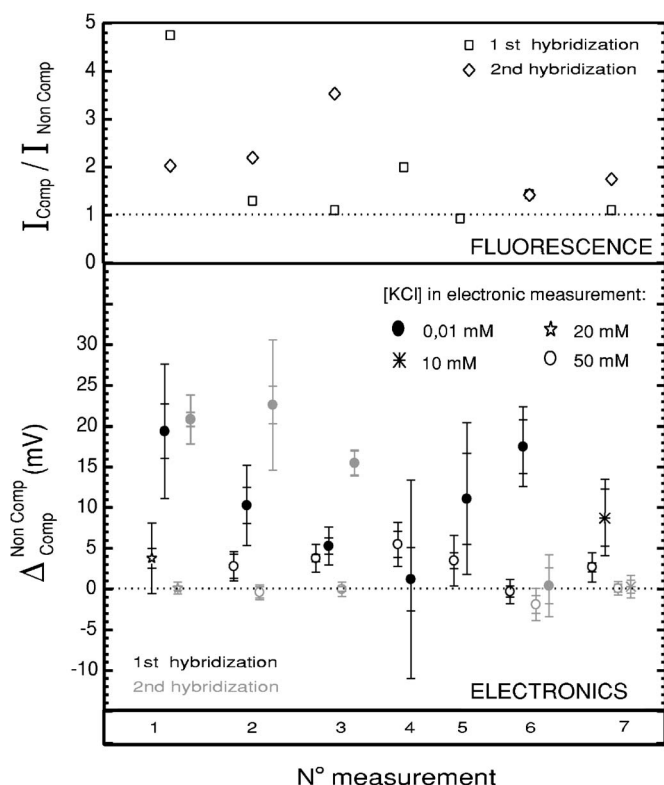


FIG. 3. Overview of results of seven hybridization experiments with electronic and fluorescence detection (see text).

peaks. This illustrates a noticeable feature of the electronic measurement of DNA hybridization: desorption of surface-bound DNA leads to signal. This contrasts with the standard microarray configuration, where desorption of the unlabeled probe molecules is not detected by the fluorescence technique, but nevertheless can induce erroneous results. The figure also illustrates that rinsing and buffer replacement can easily induce shifts of a few millivolts. Our array-based approach helps in this respect; it is observed that the spatially differential signals (difference signals between transistors of the same array) are less affected by this difficulty than the signals of the individual transistors.

In Fig. 2 we present electronic detection of DNA hybridization with a PLL-coated array of 31 FETs. Four oligonucleotide solutions are used: two different probe sequences  $A$  and  $B$  and the corresponding complementary targets  $A^\dagger$  and  $B^\dagger$  [17]. Target  $A^\dagger$  is dye labeled with Cy5, target  $B^\dagger$  with Cy3. Probes  $A$  are spotted on transistors 21–31, probes  $B$  on transistors 1–13 (0.2  $\mu$ l spots of 1  $\mu$ M solutions in 50 mM KCl). Incubation after spotting is 15 min, followed by  $H_2O$  rinsing and drying.

Subsequently, two different hybridization reactions were done on this sample, the first with target  $A^\dagger$  and the second with target  $B^\dagger$ . Both reactions were allowed for 5 min with 100 nM targets in a 1 ml volume of 50 mM KCl. Electronic measurements were conducted before, between and after the hybridizations, with 50 mM and 0.01 mM KCl electrolyte buffers. After completion of the cycles of hybridizations and electronic measurements the sample was scanned for fluorescence with 532 nm and 633 nm excitation.

In the measurements at 50 mM KCl (left column of Fig. 2), hybridization 1 leads to a slightly lower average value in  $\Delta U_{SE}$  on the right (2.3 compared to 3.9 mV), while for hybridization 2 a lower value is observed on the left (12.3 compared to 16.1 mV). At 0.01 mM KCl (right column) these differences are amplified significantly, their magnitudes increase from 1.6 to 9.1 mV for hybridization 1 and from 3.8 to 13.1 mV for hybridization 2. The signs of the shifts are the same for 0.01 and 50 mM and are consistent with specific hybridization in both directions, namely, hybridization 1 (target  $A^\dagger$ ) preferentially occurs on FETs 21–31 (probes  $A$ ) and hybridization 2 (target  $B^\dagger$ ) on FETs 1–13. The signs and high-salt amplitudes are also consistent with the  $\sim 3$  mV shifts observed for oligonucleotide hybridization at 23 mM monovalent salt on PLL-coated capacitive field effect devices [8].

The electronic hybridization detection is confirmed by the fluorescence results. Scanning the active regions of FETs 1–13, an average count of 1700 Hz is observed for Cy3 (excitation wavelength of 532 nm) and 1450 Hz for Cy5. On FETs 21–31, we find 760 Hz for Cy3 and 1920 Hz for Cy5. The transistor to transistor variations observed in the electronic and the fluorescence techniques are similar.

Our experimental protocol contains no blocking step to neutralize the positive charges of the PLL molecules. Therefore nonspecific interactions are expected on FETs 14–20 and, to a smaller extent, also in the probe regions. The more negative  $\Delta U_{SE}$  observed on the central part of the graphs are thus attributed to nonspecific adsorption of DNA targets by the PLL layer. This signal is, however, not directly comparable to the signals on the probe regions, since the molecular coatings are different.

The fluorescence results show that nonspecific adsorption occurs also in the probe regions. Cross hybridization is negligible in the present case, since sequences  $A$  and  $B$  are strongly different. We can therefore interpret the total fluorescence intensity of 1920 measured for hybridization 1 as the sum of a specific part of 470 and a nonspecific part of 1450. For hybridization 2, we find a specific contribution of 940, to be compared to a nonspecific one of 760. The amounts of nonspecifically adsorbed and specifically hybridized target molecules are thus of the same order of magnitude.

The electronic measurements do not give the nonspecific contribution, since the baseline shifts induced by the changes in ionic strength exhibit too much variation. The reliable information is the difference between the two probe regions, but not the absolute position on the vertical  $\Delta U_{SE}$  axis.

Regarding the reproducibility of the presented electronic hybridization detection, we performed a number of measurements employing different FET arrays and salt conditions. Results of seven cycles with buffer changes between the hybridization and detection steps are compiled in Fig. 3. The quantity  $\Delta I_{Comp}^{NonComp}$  presented in the lower part corresponds to the difference between the average shift in  $\Delta U_{SE}$  on the region where probes and targets are not complementary and the region where they are complementary. A positive  $\Delta I_{Comp}^{NonComp}$  means correct prediction of specific hybridization. For instance for the 0.01 mM measurement of hybridization 1 of Fig. 2, we have  $\Delta I_{Comp}^{NonComp} = 9.1$  mV. For each manipula-

tion, the first dark point corresponds to the  $\Delta_{Comp}^{NonComp}$  value obtained at high salt and the second dark point to the value at low salt. Results of the second hybridization are represented in gray (here second means second in time, the first hybridization can be with either target  $A^\dagger$  or target  $B^\dagger$  to avoid bias). The hybridization buffer is always identical to the buffer used in the high-salt electronic measurement, its salt concentration is given by the symbol type. In the upper part of the figure the ratios  $I_{Comp}/I_{NonComp}$  of the average fluorescence intensities are shown for each hybridization.

At  $[KCl]=50$  mM, the  $\Delta_{Comp}^{NonComp}$  of the first hybridization reaction are all positive and of the order of a few millivolts, while the  $\Delta_{Comp}^{NonComp}$  of the second reaction are less conclusive at high salt, close to zero or even slightly negative. The low-salt results are more convincing, all  $\Delta_{Comp}^{NonComp}$  values are in the positive range between a few and 20 mV. The second hybridizations of experiments 6 and 7 however gave close to zero values, even at low salt.

Our hybridization measurements used deposited DNA probe surface densities of  $10^4$  to  $10^5$  molecules per  $\mu\text{m}^2$ . We evaluated the ratio of hybridized targets to deposited probes by fluorescence and found that a hybridization-induced shift of 10 mV at 0.01 mM salt corresponds to 100–1000 surface bound targets per  $\mu\text{m}^2$  [18]. For comparison, reverse transcription allows one to label target molecules with about one fluorophore per 20 bases and the reported limit of fluorescence detection is about 1 fluorophore/ $\mu\text{m}^2$  [19]. In terms of surface density, we thus estimate that our label-free technique is presently 2 to 3 orders of magnitude less sensitive

than the fluorescence technique. In terms of the total quantity of hybridized DNA needed for electronic detection with a FET of  $40 \mu\text{m}^2$  active surface, we estimate  $(100-1000) \times 20 \times 40 \approx 10^5-10^6 \approx 0.1-1 \times 10^{-18}$  mol of DNA bases.

In conclusion, we have studied DNA hybridization detection by Poly(L-lysine)-coated silicon transistor arrays, in a configuration where the specific recognition and the electronic detection are separated in time. Exploiting the spatial information of the arrays by a differential measurement, it is possible to use different salt concentrations for recognition and detection in order to improve the specificity of the hybridization (requiring high salt) and the sensitivity of the field effect detection (requiring low salt to reduce screening by mobile ions). The presented experimental configuration is quite robust with respect to buffer changes: specific hybridization is detected even after repeated cycling between low and high salt. While DNA immobilization by the cationic PLL polymer layer allows reusing the array several times, residual nonspecific adsorption of the target DNA still is an important open issue.

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- [17] Base sequences are CAg gCg gCA ggg CTg ACg TT for oligo A, and CCg CgA ACT gAC TCT CCg CC for oligo B. Oligo  $A^\dagger$  ( $B^\dagger$ ) is complementary to oligo A (B) and is Cy5 (Cy3) labeled at the 5' end.
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