

Detection of DNA Hybridization Using a Field-Effect Device

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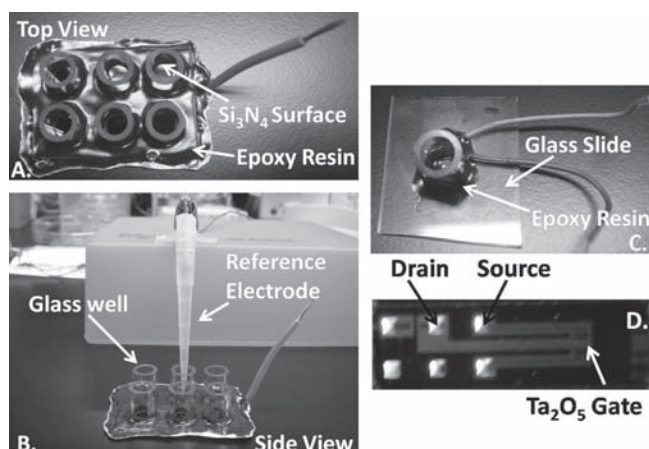


Figure 1: (A) Top view and (B) Side view of capacitive device. (C) Top view of FET. (D) Transistor used.

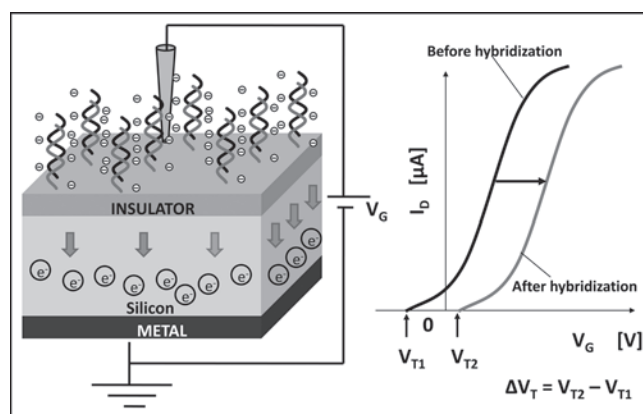


Figure 2: Concept of field-effect devices.

Abstract and Introduction:

In this study, two field-effect devices, a capacitive device with a silicon nitride (Si_3N_4) surface (Figures 1A, 1B) and a field-effect transistor (FET) with a tantalum oxide (Ta_2O_5) gate (Figures 1C, 1D) were fabricated to detect deoxyribonucleic acid (DNA) hybridization by taking potentiometric measurements using the intrinsic charge in DNA. DNA hybridization biosensors, which utilize bio-recognition between two single-stranded complementary DNA, can be used for genetic testing, clinical diagnosis, and forensic science.

The surface of each device was treated with poly(2-methacryloyloxyethylphosphorylcholine-co-3-methacryloxypropyltrimethoxysilane) (PMSi) to increase the charge density at the surface of the gate, 3-aminotriethoxysilane (APTES) to introduce a reactive amino group, and glutaraldehyde to act as a bifunctional cross-linking agent [1]. Next, DNA was immobilized at the surface, and treated with glycine to block any free aldehyde groups. Contact angles were taken after every modification to ensure that the surface had been uniformly coated.

A reference electrode was used to control and fix the potential of a measurement solution and gate voltage [1]. When DNA molecules adsorb onto the surface of an insulator, electrons in the silicon substrate electrostatically interact with the immobilized DNA, affecting the electrical characteristics

of the field-effect device [2]. Detection of hybridization is indicated as a shift in the flat-band voltage (V_{FB}) for the capacitive device, and a shift in interface potential (V_T) for the FET due to the increased charge density (Figure 2). Results show that the capacitive device was not sensitive enough to detect hybridization; however, the FET showed a significant shift in interface potential, successfully detecting DNA hybridization.

Experimental Procedure:

Device Fabrication. The Si_3N_4 surface of the capacitive device was washed in acetone, ethanol and water, and dried with nitrogen (N_2) gas. Six glass wells were attached to the surface using epoxy resin and baked at 120°C for 30 minutes. A wire was attached to the bottom of the device using silver paste and covered in aluminum foil. The remaining Si_3N_4 surface was covered with epoxy resin and baked for 2 hours.

The FET was fabricated using a transistor with a Ta_2O_5 gate adhered to a glass slide. Two wires were connected to the source and drain of the transistor using silver paste. A glass well was attached over the gate of the transistor. The rest of the transistor was covered with epoxy resin and baked for two hours at 120°C .

Immobilization and Hybridization of DNA. The chambers were immersed in 0.02 wt% of PMSi in ethanol for 30 minutes, dried in an ethanol atmosphere for 24 hours, and incubated at 120°C in a vacuum for one hour. Next, they were filled with 2 v/v% APTES in ethanol for 24 hours and incubated for another hour.

The chambers were then filled with 25 wt% glutaraldehyde containing 0.01 g/mL sodium cyanoborohydride (NaBH_3CN) for three hours. Overnight, 100 μM probe DNA in TE buffer, containing 0.01 g/mL NaBH_3CN , was incubated in each chamber at 50°C. Finally, chambers were incubated at 50°C for one hour with 1M glycine in TE buffer.

The probe was then hybridized with 100 μM of target DNA in 4 \times SSC, 0.1% SDS buffer and incubated at room temperature for one hour.

Measurements. Contact angles were taken after each surface modification using the Kyowa Dropmaster and FAMAS software. To observe the changes in electrical characteristics, the reference electrode was submerged in the chamber solution (pH 6.86 standard).

For the capacitive device, an Agilent Precision Impedance Analyzer was used to take capacitance-voltage measurements from -5 to 3 volts. The V_{FB} was interpolated from the data at a capacitance ratio of 0.57. This predetermined value is dependent on the thickness of the insulator, carrier density, and dielectric constant of the semiconductor [3].

For the FET, a lab-developed transistor measurement system was used to measure V_{T} . The source-drain current was kept constant. Measurements were taken before and after hybridization, and ΔV was determined for both devices.

A. Surface Contact Angles:	
Surface treatment	Contact Angle
Si_3N_4	11.6°
PMSi	41.1°
APTES	57.1°

B. ΔV_{FB} for Capacitive Device:			
Trial	V_{FB} Before Hybridization (mV)	V_{FB} After Hybridization (mV)	ΔV_{FB} (mV)
Control	-668.92	-663.69	5.23
Trial 1	-608.75	-612.4	-3.65
Trial 2	-589.51	-592.98	-3.47

Table 1: (A) Contact angles after surface modification. Values are an average of six individual measurements. (B) ΔV_{FB} is the difference in V_{FB} before and after DNA hybridization.

Results and Conclusions:

Results from measurements are tabulated in Table 1. The changes in contact angle confirm that the surface had been modified. (Table 1A)

The results for the capacitive device were inconclusive because of the low, negative shift of ΔV_{FB} . ΔV_{FB} should be in the positive direction (Table 1B). This could be due to experimental error, or because the DNA had not immobilized on the surface. However, there was a significant negative

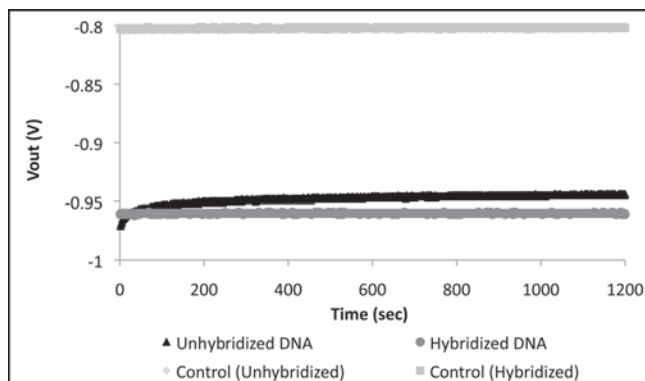


Figure 3: Time course of interface potential before and after DNA hybridization.

shift of -15 mV in V_{T} for the FET, while the control remained constant at -0.802 mV (Figure 3). This significant negative shift confirmed the hybridization of DNA due to the increased negative charge at the gate's surface.

Future Work:

Ta_2O_5 surfaces are rougher than Si_3N_4 surfaces and could have contributed to the unsuccessful immobilization of DNA on the silicon nitride surface. Future work could involve taking measurements regarding the surface roughness and its effects, as well as the reproducibility of the results taken from the FET device.

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