# Direct DNA detection using ion-sensitive field effect transistors (IS-FETs) based on peptide nucleic acid

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#### Introduction

Molecular recognition **DNA** based on hybridization an important reaction is biotechnology. Bio-devices that are discriminate between double-stranded nucleic acids and single stranded nucleic acids with high efficiency and specificity are useful tools in this post-genome sequence era. In this study, we focused on a direct detection system for DNA hybridization that combines device function with efficient molecular recognition of heterogeneous nucleic acid hybridization.

Direct DNA detection has been demonstrated using ion-sensitive field effect transistors (ISFETs) based on peptide nucleic acids (PNAs). PNA is a structural **DNA** analogue with neutral a N-(2-aminoethyl)-glycine-based psedopeptide backbone replacing the negatively charged phosphate backbone of DNA<sup>1-3</sup>. When compared with the DNA duplex, PNA strongly discriminates mismatched DNA and has a strong binding affinity for complementary DNA due to the lack of repulsion between PNA and DNA. Moreover PNAs resistant to enzymatic degradation and binds independently of salt concentration. Therefore PNA is a highly practical molecule in the biotechnology field. ISFETs have been proven to be sensors that are very sensitive to any kind of electrical interaction at or nearby the gate insulator / electrolyte interface<sup>4-6</sup>. Therefore, well-constructed ISFETs are widely used as pH sensors and are commercially distributed at low-cost. In this study, the gate substrate was constructed from Si/SiO<sub>2</sub>/Si<sub>3</sub>N<sub>4</sub>/Ta<sub>2</sub>O<sub>5</sub> and the ionsensitive thin film was Ta<sub>2</sub>O<sub>5</sub>, which has a high dielectric constant. We modified the surface of Ta<sub>2</sub>O<sub>5</sub> for PNA immobilization and we demonstrated that PNA modified Ta<sub>2</sub>O<sub>5</sub> plays a role in the specific molecular recognition of complementary DNA present in solution. Accordingly, PNA-immobilized ISFETs are expected to undergo

hybridization and enable direct detection of DNA without any modification, such as fluorescence probe molecule. (Figure 1)

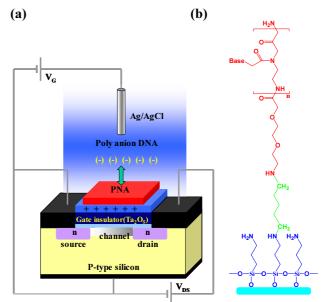


Figure 1 (a) Schematic representation of an ISFET sensor device whose gate electrode is modified with PNA. (b) Chemical structure of a PNA molecule on  $Ta_2O_5$ .

### Results and discussion

Table 1 shows the comparison of the  $T_m$  of PNAs/DNAs with that of DNAs/DNAs and their dependence on the salt concentration. The  $T_m$  value of the probe PNAs and the complementary DNA is 72.4°C in 2×SSC (300mM sodium chloride-30mM sodium citrate) containing 1mM EDTA and the  $T_m$  value of the probe DNA and the complementary DNA is 68.2°C in 2×SSC-1mM EDTA. The  $T_m$  of PNAs/DNAs is higher than that of DNAs/DNAs. And the PNAs/DNAs is binding independence on the salt concentration. These results suggest that the probe PNAs have the advantage of the strong

binding affinity compared with probe DNA under any condition.

Changes in the drain current during DNA hybridization were monitored in real time and we demonstrated an I-V measurement method for direct detection of DNA. Figure 2 shows representative *in situ* DNA hybridization result of I-V measurements. We performed continuous monitoring of the drain current at a 5 V drain source bias and a 4 V gate bias during exposure with complementary target DNA solution and pure buffer solution as a control. DNA hybridization induces an exponential decrease in the drain current for up to 1.5 h. In contrast, the control experiment showed no changes in the drain current. We were thus able to demonstrate a real-time decrease in the drain current during DNA hybridization.

Table 1 Comparison of the  $T_m$  value of the PNAs/DNAs with that of DNAs/DNAs and their dependence on the salt concentration.

	probe PNA		probe DNA	
	<sup>a</sup> 300mM	b30mM	300mM	30mM
target DNA	72.4°C	72.1°C	68.2°C	52.7°C

 $^a300 mM: 2\times SSC-1 mM EDTA$   $^b30 mM: 0.2\times SSC-0.1 mM EDTA$ 

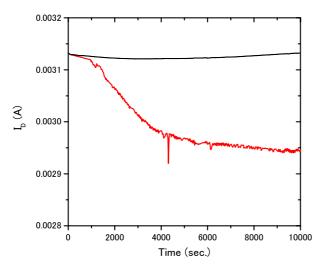


Figure 2 *In situ* hybridization results of I-V measurement. Continuous monitoring of drain current at a 5 V drain source bias and a 4 V gate bias during exposure to complementary target DNA solution (line) and pure buffer solution as a control (broken line).

## **Conclusions**

Using an ISFET based on PNA, we observed through the I-V characteristics that the hybridization of surface-immobilized PNA with complementary DNA induces a decrease in the saturation current and a positive shift in the threshold voltage. These variations correspond to changes in the surface potential at or near the gate insulator / electrolyte interface induced by complementary recognition. These results demonstrate possibility of using an ISFET based on PNA as a DNA sensor and we expect potential applications in medical diagnostics and molecular biology. In subsequent studies, we will design and fabricate the ISFET micro-array and control the amount of gate surface-immobilized PNA in order to ensure reproducibility.

## Reference

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