

# Potentiometric Detection of DNA Molecules Using Field Effect Devices

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## 1. Introduction

Several types of DNA chips and DNA microarrays have been developed and some of them are used in the field of molecular biology (1). Although most of the current DNA chips and DNA microarrays are based on the fluorescent detection method, amperometric detection methods have been developed in combination with redox reagents (2). We investigated another approach to realize an electrochemical detection for DNA chips and the novel concept of a genetic field effect transistor (FET) is proposed in the present study. Detection of DNA molecules using a genetic FET is in principle based on charge density change at the gate insulator. Fundamental characteristics of a genetic FETs are described.

## 2. Concept of genetic field effect transistor

The conceptual structure of a genetic FET is shown in Fig. 1. Oligonucleotide probes are immobilized on the surface of the gate insulator. When complementary DNA molecules are contained in a sample solution, they are hybridized with the oligonucleotide probes at the surface of the gate area. Since DNA molecules are negatively charged in an aqueous solution, it is possible to detect them with field effect devices. In the present study, we make use of ionized characteristic of intercalator in an aqueous solution, while it is used as a fluorescent reagent in the conventional methods. Intercalators are ionized and positively charged as shown in Fig. 2, and introduced into double stranded oligonucleotide probes on the gate surface, which leads to increase in the surface charge density. In this way, the signal of hybridization can be enhanced and detected by the use of field effect devices.

## 3. Experimental

Oligonucleotide probes were immobilized on the  $\text{Si}_3\text{N}_4$  surface using 3-(2-aminoethylamino) propyl trimethoxy silane. After probe deposition, surface groups were blocked with glycine treatment. The detailed procedure for immobilization and base sequences of the oligonucleotide probes used have been reported previously(3). Potentiometric measurements were carried out in a phosphate buffer of pH 6.86 with a

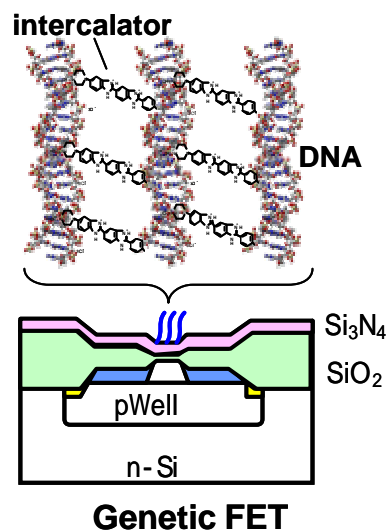


Fig. 1 Concept of genetic FET

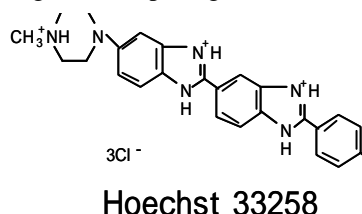


Fig. 2 Chemical structure of typical intercalator

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*Form Approved*  
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1. REPORT DATE <b>00 JUN 2003</b>			2. REPORT TYPE <b>N/A</b>			3. DATES COVERED <b>-</b>		
4. TITLE AND SUBTITLE <b>Potentiometric Detection of DNA Molecules Using Field Effect Devices</b>						5a. CONTRACT NUMBER		
						5b. GRANT NUMBER		
						5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)						5d. PROJECT NUMBER		
						5e. TASK NUMBER		
						5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Biomaterials Research Center, National Institute for Materials Science 1-1, Namiki, Tsukuba 305-0044, Japan; Department of Chemical Materials Science, Japan Advanced Institute of Science and Technology</b>						8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)						10. SPONSOR/MONITOR'S ACRONYM(S)		
						11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release, distribution unlimited</b>								
13. SUPPLEMENTARY NOTES <b>See also ADM001697, ARO-44924.1-EG-CF, International Conference on Intelligent Materials (5th) (Smart Systems &amp; Nanotechnology)., The original document contains color images.</b>								
14. ABSTRACT								
15. SUBJECT TERMS								
16. SECURITY CLASSIFICATION OF:					17. LIMITATION OF ABSTRACT		18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>		b. ABSTRACT <b>unclassified</b>		c. THIS PAGE <b>unclassified</b>	<b>UU</b>		<b>2</b>	

Ag/AgCl reference electrode.

The threshold voltage ( $V_t$ ) of the genetic FET was measured with a semiconductor parameter analyzer (Agilent) and  $V_t$  shifts were evaluated at each step of surface treatment and reaction with a target DNA and an intercalator.

#### 4. Results and discussion

Typical  $V_g$ - $I_d$  characteristic of the FET is shown in Fig. 3. The FETs used were n-channel and depletion mode devices. Fig. 4 shows the  $V_g$ - $I_d$  characteristics with small scales, measured after each step of treatment. The  $V_g$ - $I_d$  characteristic No. 1 is the same as that shown in Fig. 1. When the surface of the gate was treated with aminosilane and modified with oligonucleotide probes, the  $V_t$  was shifted in the positive direction (No.2). This indicates that the negative charges of the oligonucleotide probes were induced at the surface of the gate insulator of the FET. Then, the target DNA molecules were annealed and hybridized at the genetic FETs. A small  $V_t$  shift in the positive direction was observed as shown in Fig. 4(No. 3). This also indicates the increase of the negative charge as a result of hybridization at the gate surface.

When an intercalator such as Hoechst 33258 was introduced to the gate surface, the  $V_t$  was shifted in the negative direction, which indicated the existence of the positive charges at the gate surface. The larger shift of the  $V_t$  was obtained by using the intercalator as compared with hybridization only.

#### 5. Conclusions

We developed a new method for detecting DNA molecules using FETs and intercalators. Since most of intercalators have charges, the signal based on the hybridization can be enhanced by introducing intercalator into the double stranded DNA. Preliminary experiments showed that the signal based on hybridization could be enhanced with the use of intercalator and potentiometric detection of DNA molecules has been successfully demonstrated.

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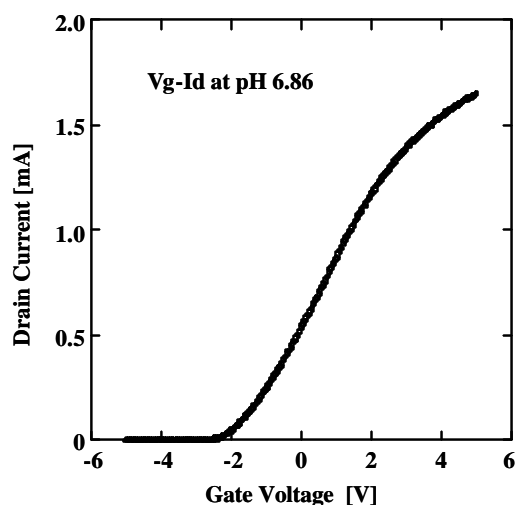


Fig. 3 Typical  $V_g$ - $I_d$  characteristic of the genetic FET

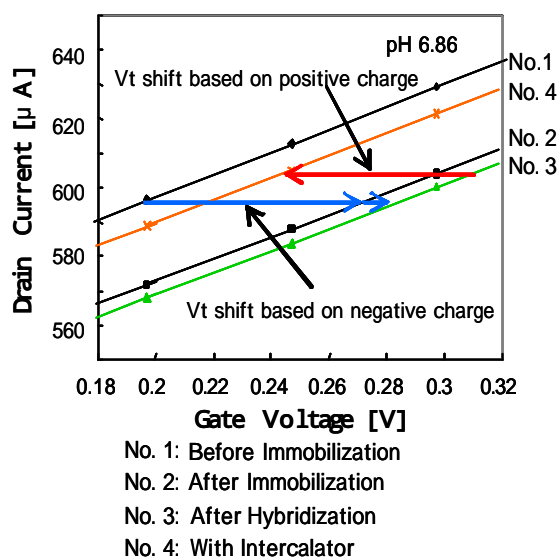


Fig. 4 Threshold voltage shifts of the FET at each step of treatment