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# A non-labeled DNA biosensor based on light addressable potentiometric sensor modified with $TiO_2$ thin film*

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**Abstract:** Titanium dioxide ($TiO_2$) thin film was deposited on the surface of the light addressable potentiometric sensor (LAPS) to modify the sensor surface for the non-labeled detection of DNA molecules. To evaluate the effect of ultraviolet (UV) treatment on the silanization level of $TiO_2$ thin film by 3-aminopropyltriethoxysilane (APTS), fluorescein isothiocyanate (FITC) was used to label the amine group on the end of APTS immobilized onto the $TiO_2$ thin film. We found that, with UV irradiation, the silanization level of the irradiated area of the $TiO_2$ film was improved compared with the non-irradiated area under well-controlled conditions. This result indicates that $TiO_2$ can act as a coating material on the biosensor surface to improve the effect and efficiency of the covalent immobilization of biomolecules on the sensor surface. The artificially synthesized probe DNA molecules were covalently linked onto the surface of $TiO_2$ film. The hybridization of probe DNA and target DNA was monitored by the recording of I-V curves that shift along the voltage axis during the process of reaction. A significant LAPS signal can be detected at 10 μmol/L of target DNA sample.

**Key words:** DNA biosensor, Titanium dioxide ($TiO_2$) thin film, Light addressable potentiometric sensor (LAPS), Silanization, Fluorescein label, Gene chip

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## INTRODUCTION

To detect the existence or changes of certain DNA sequences is significant for biological research and medical diagnosis. Field-effect device (FED) sensors for DNA molecule detection have been developed (Souteyrand *et al*., 1997; Ingebrandt *et al*., 2007; Poghossian *et al*., 2007; Shishkanova *et al*., 2007). The detection method of the FED sensor depends on the charge changes before and after the hybridization of DNA strands near the sensor surface. The application of FED sensor for DNA-hybridization detection could omit the need of labels that some conventional DNA detection methods employed (de-los-Santos-Álvarez *et al*., 2004).

Silanization is one of the most frequently employed methods for the surface modification to immobilize the biomolecules to the biosensors, the mechanism of which is considered to be the hydrolysis and crosslink of the silane reagent with hydroxyl groups on the substrate surface (Silberzan *et al*., 1991). Several methods have been studied to introduce the hydroxyl groups or to increase the density of these groups on the substrate surfaces. Wet-chemistry method is frequently employed and reported (Libertino *et al*., 2007; Qin *et al*., 2007), which includes the treatment of the surface with strong acids and other strong oxidants. The effects of different methods have been compared on glasses (Cras *et al*., 1999) and silicons (Han *et al*., 2006). The wet-chemistry method of surface treatment for silanization should be well

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controlled to avoid any damages brought to the surface, especially when the intact surface is important for the readout of the biosensor, such as in the case of field-effect devices (Souteyrand *et al*., 1997).

Titanium dioxide ($TiO_2$) has been found to have the capability to catalyze the oxidization of organic contaminants and exhibit hydrophilicity after ultraviolet (UV) irradiation (Wang *et al*., 1997; Takeuchi *et al*., 2005). The UV-induced hydrophilicity of $TiO_2$ surface was attributed to the attachment of more hydroxyl groups onto the surface (Wang *et al*., 1997; Nakamura *et al*., 2001); however, its mechanism is still under extensive investigations.

Light addressable potentiometric sensor (LAPS) is a type of FED, sharing surface photovoltage property with semiconductor, by which the signal of a very spot on the sensor surface could be read out by focusing a modulated irradiation there. The setup of LAPS is illustrated in Fig.1. After the first report of this technique by Hafeman *et al*.(1988), various applications have also been developed (Mourzina *et al*., 2001; Yoshinobu *et al*., 2005), including Dill *et al*. (1997)'s and Kung *et al*.(1990)'s work to detect DNA. Here we report a novel approach based on the above mentioned techniques and methods to prepare the surface of LAPS for the detection of DNA molecules. $TiO_2$ thin film was deposited on the surface of the LAPS substrate, the artificially synthesized probe DNA molecules were covalently linked onto the surface of silanized $TiO_2$ film.

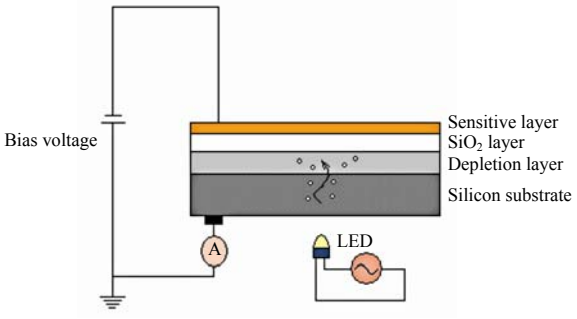


**Fig.1 Illustration of the setup of LAPS**
LED: light-emitting diode

## MATERIALS AND METHODS

### Materials

Si for the LAPS preparation was *n*-type, with a specific resistance of 3~8 Ω·cm and a thickness of 0.5 mm. Tetrabutyl orthotitanate (TBOT) and dimethyl sulfoxide (DMSO) were from Beijing Chemicals Co. Ltd., China. The artificially synthesized probe DNA (CCAAGAGTTGCAGTTCCTGA, 5′-end $C_6$-$NH_2$ modified), complementary target DNA (TCAGGAACTGCAACTCTTGG), and randomly sequenced DNA (GTGATCGAGTAGGTGA GCTA) were purchased from Takara Biotechnology Co. Ltd., Dalian, China. They were all in phosphate-buffered saline (PBS) with desired concentration when samples were made. Bovine serum albumin (BSA) was provided by Sino-American Biotechnology Co. Ltd., China. 3-Aminopropyltriethoxysilane (APTS), fluorescein isothiocyanate (FITC), and glutaraldehyde were purchased from Sigma-Aldrich, USA. All reagents were of analytical degree and were used without further purification. PBS (0.01 mol/L, pH 7.4) and carbonate (0.1 mol/L, pH 9.0) buffers were homemade. High purity water (Millipore, 18 MΩ·cm) was used to prepare the solutions.

### Sample preparation

(1) Preparation of LAPS

LAPS was prepared by extensively cleaning the Si substrate to remove possible contaminants, followed by thermal oxidization to grow a 50-nm thick layer of $SiO_2$ under 1180 °C for 40 min. After the deposition of $TiO_2$ film on the surface, the backside of the Si was treated with HF to remove the $SiO_2$ layer and deposited with a layer of aluminium by evaporation. Openings of 2 mm in diameter on a mask were for ohmic contact and back illumination during measurement.

(2) Preparation of $TiO_2$ sol-gel and its deposition onto the LAPS surface

$TiO_2$ film was deposited onto the LAPS surface by spin-coating of $TiO_2$ sol-gel. 4 ml TBOT was dissolved in 16 ml ethanol as solution A; 4 ml ethanol, 4 ml water, and 2 ml concentrated nitric acid were mixed together as solution B. Solution B was water-bathed at 70 °C while solution A was added dropwise with extensive stirring. The solutions were stirred for 3 h after the mixing, and kept sealed under room temperature for 24 h to form $TiO_2$ sol-gel.

The LAPS substrate was spun in the speed of 3000 r/min for 10 s, with 200 μl of $TiO_2$ sol-gel on the surface for the spin-coating. After spin-coating, the substrates were dried in a furnace at 120 °C for 20 min

and sintered at 500 °C for 60 min in air with the temperature raised at 10 °C/min.

**Characterization of $TiO_2$ thin film and measurement of fluorescence**

(1) Wettability property of the $TiO_2$ surface under UV treatment

The wettability of the $TiO_2$ surface under UV treatment was observed using a commercial mercury-arc lamp, with filter glass to eliminate the most visible and infrared irradiation. The substrate surface was in the dimension of 18 mm×18 mm, covered by a mask with square openings of 5 mm×5 mm, and intervals of 5 mm between the openings as illustrated in Fig.2. The $TiO_2$-deposited surface was irradiated with UV for 2 h in the ambient condition. The measurement of the contact angles of the seeding layer was conducted before and after the UV treatment (DSA 10-MK2, Kruss, Germany).

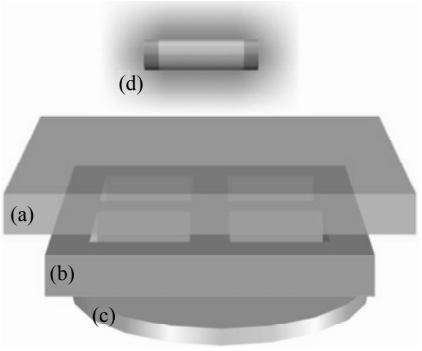


**Fig.2 Diagram of the UV treatment apparatus to the $TiO_2$ thin film-deposited substrate**
(a) UV-filter glass; (b) Opaque mask; (c) Si substrate; (d) UV light source

(2) Silanization and fluorescein labeling of $TiO_2$ surface under UV treatment

The influence of the UV treatment of $TiO_2$ surface on the silanization effect of the film was investigated by fluorescein labeling and then measuring the intensity of the emitted fluorescence. The $TiO_2$-deposited surface was irradiated with UV for 2 h as described for wettability test introduced above. The substrates were kept under ambient environment after UV treatment briefly before silanization. Silanization of the surface was carried out by dipping the substrates into the toluene solutions with APTS concentration of 0.1% (v/v) for 1 h and sealed under room temperature. After being rinsed completely with toluene and ethanol, the substrates were cured at 120 °C for 1 h.

FITC was dissolved into DMSO (1 mg/ml), and then diluted with carbonate buffer into 0.1 mg/ml before labeling. Silanized substrates were placed into the fluorescein solution under room temperature in dark for 10 h, and then rinsed completely to remove any unattached reactants.

Measurements of fluorescence were carried out by the Maestro In-Vivo Imaging System (CRI, USA), with the excitation wavelength at 488 nm and the photographed chip samples in the dimension of 18 mm×18 mm.

**Preparation of DNA sensor based on $TiO_2$-LAPS**

DNA probes were immobilized on LAPS by a covalent crosslink method (Fig.3). The deposited $TiO_2$ thin film was silanized by the method as described above, without the usage of the mask. The introduced amine residues reacted with glutaraldehyde overnight under room temperature. After rinsing with PBS thoroughly, probe DNA solution was added and the amine residues on the end of the probe DNA molecules reacted with the aldehyde residues on the $TiO_2$ film under room temperature for 12 h. After the immobilization reaction, the LAPS chip was rinsed again and 1% (v/v) BSA in PBS was added to block any unreacted aldehyde residues and other non-specific binding sites on the $TiO_2$ film. The sensor was rinsed with PBS and kept under 4 °C before measurement.

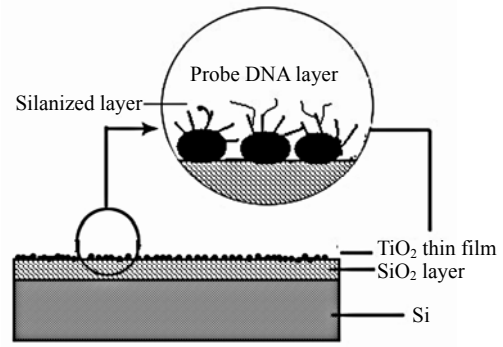


**Fig.3 Diagram of the structure of the DNA sensor based on $TiO_2$-LAPS**

**Measurement setup of DNA sensor based on $TiO_2$-LAPS**

The measurement setup is illustrated in Fig.4 and introduced elsewhere (Xu *et al*., 2005), with a

potentiostat (EG & G M273A Princeton Applied Research, USA) to supply the bias voltage between the LAPS substrate and Ag/AgCl reference electrode, and with a thin Pt wire as counter electrode. The photocurrent was amplified before entering the lock-in amplifier (Stanford Research System, SR830, USA) for alternating current (AC) signal extraction. The photocurrent was generated by the separation of carriers in the space charge region (SCR) of LAPS when illuminated from backside by light-emitting diode (LED). A wavelength of 830 nm, a power of 70 mW, and an intensity of 10 kHz were used by the oscillator output of the lock-in amplifier. The recorded data were plotted with photocurrent vs. bias voltages, and referred to as I-V curve.

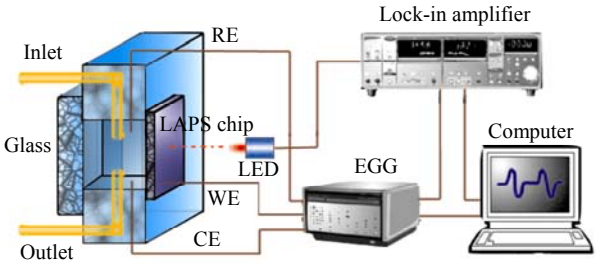


**Fig.4 Diagram of the measurement setup of the DNA sensor based on $TiO_2$-LAPS**
RE: reference electrode; WE: working electrode; CE: counter electrode; LED: light-emitting diode; EGG: potentiostat EG & G M273A

The DNA sensor based on $TiO_2$-LAPS was sealed as an outer part onto a test chamber, with channels to introduce and expel test samples. The test chamber was put into a faraday cage to avoid any electromagnetic interference. All measurements were conducted in dark and ambient temperature. The control software was homemade using LabView (National Instrument Co. Ltd., Austin, TX, USA).

### Measurement procedure of DNA sensor based on $TiO_2$-LAPS

PBS (0.01 mol/L, pH 7.4) was first introduced into the test chamber and I-V curves were recorded as blank control. To evaluate the specificity of this DNA detection method, 10 μmol/L randomly sequenced DNA sample solution in PBS was added to the test chamber with DNA sensor based on $TiO_2$-LAPS and underwent the reaction for 12 h under the room temperature. I-V curves were recorded. After the process was finished, the test chamber was rinsed again with PBS and I-V curves were recorded under this condition.

Then 10 μmol/L target DNA sample solution was added to the test chamber to substitute the PBS solution and the hybridization of DNA was monitored continuously by recording the I-V curves during the reaction process. After the reaction was finished, the test chamber was rinsed completely by PBS to make sure no reactants left and I-V curves were recorded again under this solution environment.

## RESULTS AND DISCUSSION

### Wettability characteristics of $TiO_2$ thin film

Fig.5 shows the photographs of the contact angle measurement of the $TiO_2$ thin film coated on the LAPS substrate surface before and after the UV treatment. The contact angle changed from around 65° to less than 10°, indicating the attainment of the hydrophilicity of the $TiO_2$ thin film after the UV irradiation.

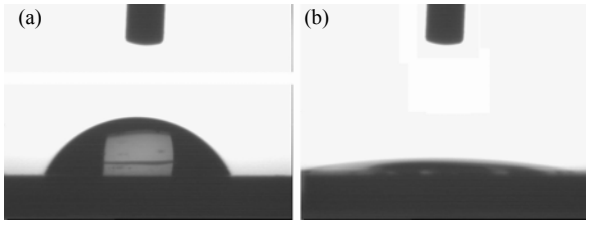


**Fig.5 Contact angle measurement of the $TiO_2$ thin film (a) before and (b) after the UV treatment**

### Silanization level of $TiO_2$ thin film characterized by fluorescence measurement

The UV treatment effect on the silanization of $TiO_2$ thin film was illustrated in Fig.6. The uniformity of the surface could not be guaranteed very definitely due to the preparation of $TiO_2$ thin film, so it is more explicit to exam the distribution of the fluorescence intensity with relation to the UV treatment process. The difference of the fluorescence intensity of the individual UV-treated area was compared with that of the non-UV-treated area adjacent to it. The maintaining period after UV treatment before silanization process did not affect the appearance of the fluorescence emission of the substrate. Fig.6a indicates the fluorescence distribution on the substrate maintained for 20 min in the ambient condition after UV treatment

and before silanization. It was clear that, under this situation, the fluorescence intensity was stronger in the UV-treated areas of the $TiO_2$ thin film, compared with that in the adjacent areas without any UV treatment. However, with the substrate to which the silanization was carried out right after the UV treatment, the pattern of the fluorescence emission on the substrate was quite different, with the intensity much lower in the UV-treated area than in the non-UV-treated area (Fig.6b). The possible explanation may rely on the ability of $TiO_2$ thin film to produce free radicals after UV irradiation, a mechanism of photocatalysis to decompose organic materials (Fujishima *et al*., 2000). After UV irradiation ceased, some of the produced free radicals may still be active to react with the silanization solution composed of organic components, so the rate of silanization process would be slowed down on the UV-treated areas. After the free radicals lost their activities, no decomposing reaction will happen in the silanization solution. So the UV-induced hydroxyl groups on the surface of $TiO_2$ film will exhibit their function, and the extent of silanization increases, which could be presented with the increased intensity of fluorescence on the UV-treated areas, compared with that of the adjacent non-UV-treated areas.

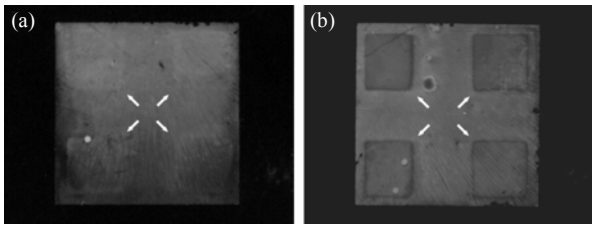


**Fig.6 Photographs of fluorescence emission of fluorescein-labeled silanized $TiO_2$ thin films deposited on Si substrate. The substrates were maintained under ambient condition for different time after UV treatment and before silanization. The white arrows point to the UV-treated areas. The photographed area is 18 mm×18 mm for both samples. (a) 20 min; (b) 0 min**

## Measurement results of DNA hybridization by DNA sensor based on $TiO_2$-LAPS

Fig.7 shows the time evolution of the I-V curves during the hybridization process. I-V curves move towards more negative voltage as hybridization is in process, because of the negatively charged characteristics of DNA molecules and the *n* type feature of the LAPS substrate.

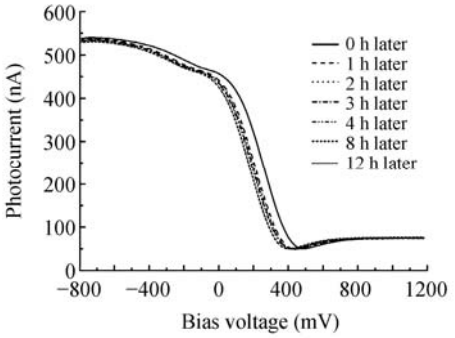


**Fig.7 Measurement characteristics of the DNA sensor based on $TiO_2$-LAPS. 10 μmol/L of target DNA sample was introduced into the test chamber containing DNA sensor based on $TiO_2$-LAPS and I-V curves were recorded during the reaction of hybridization**

In our experimental condition, the back of the LAPS, which is the opposite of the gate in the sense of an FED, is considered as the positive site, through which the work electrode is connected. When the voltage between the back and the reference electrode is positive and increased, the gate is negatively biased if considering the back as 0 potential. Thus, the depletion layer is enhanced, and the impedance of the substrate is increased. This way the photocurrent will decrease as the voltage over the substrate increased in the positive direction along the axis. The Debye length is around 1 nm under the condition of 0.01 mol/L PBS (Russel *et al*., 1989). Some of the surface charge change can be detected by the LAPS that works following the mechanism of FEDs (Poghossian *et al*., 2005).

The shifts of the I-V curves along the voltage axis finally reached a saturation point after 8 h of reaction, indicating the completion of the hybridization. The voltage difference measured from the beginning to the end of the reaction was around 100 mV, consistent with others (Souteyrand *et al*., 1997). This value of voltage shift can be attributed to the positive effect of the $TiO_2$ modification on the surface of LAPS, which will increase the density of the surface hydroxyl groups after UV treatment for increased immobilization of the probe DNA. However, it should be noted that, with the deposition of $TiO_2$ on the surface of LAPS, the detection sensitivity would be decreased due to the increase of the thickness of the insulator layer. This negative effect must be considered and a better detection can be expected.

Fig.8 illustrates the result of the specificity test of this measurement method. Comparing the position of the I-V curves along the voltage axis obtained under the same solution environment and measurement condition, it is nearly the same before and after the randomly sequenced DNA sample was added to the test chamber, revealing a good specificity of this measurement system to the target DNA.

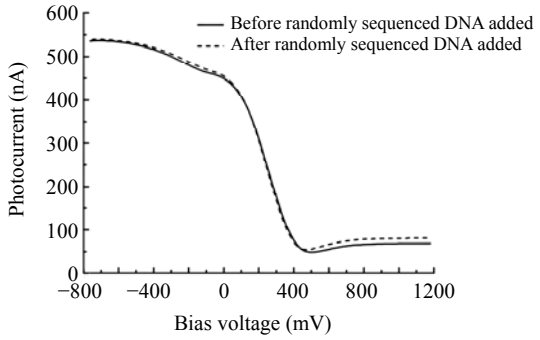


**Fig.8 Comparison of I-V curves recorded before and after the randomly sequenced DNA sample was added to the test chamber with LAPS immobilized with probe DNA on the surface**

## CONCLUSION

We have demonstrated that, with the $TiO_2$ thin film deposition on the surface of substrates for biosensor preparation under well-controlled conditions, the silanization level increased after UV treatment of the $TiO_2$ thin film. This increase may be contributed to the UV-induced hydrophilicity of $TiO_2$ thin film, which has been proposed to be the increase of hydroxyl group intensity on the surface. When coated with the $TiO_2$ thin film, the surface of $SiO_2$ does not need to be treated with strong acids or oxidants to destroy the organic contaminants and to increase the density of hydroxyl groups on the surface for the silanization as one way to increase the attachment of probe biomolecules for biosensor preparation. This simple process will cause little damage to the sensor surface, especially when the field-effect method is applied. LAPS has the ability to detect the hybridization of DNA molecules and the light-addressable characteristics in a time-saving and economic manner, and may become a potential candidate for a new type of non-labeled gene chip.

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