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# Indicator free DNA hybridization detection via EIS based on self-assembled gold nanoparticles and bilayer two-dimensional 3-mercaptopropyltrimethoxysilane onto a gold substrate

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**Abstract**

A novel indicator free DNA biosensors fabricated by self-assembling of bilayer two-dimensional 3-mercaptopropyltrimethoxysilane (B2dMPTS), gold nanoparticles and oligonucleotide has been studied on gold substrate. The thiol groups of 3-mercaptopropyltrimethoxysilane (MPTS) serve as binding sites for the covalent attachment of MPTS to gold electrode surface. After hydrolysation and condensation, the polymerized monolayer, one-dimensional network of MPTS (1dMPTS) was combined together into a two-dimensional sol–gel network (2dMPTS). The second silane layer (B2dMPTS) was formed by immersing electrodes back into the MPTS solution overnight, and then the gold nanoparticles were chemisorbed onto the thiol groups of the second silane layer. Finally, the mercapto oligonucleotide was self-assembled onto the surface via the gold nanoparticles. Electrochemical impedance spectroscopy (EIS) was used to characterize the modified process. And we used the impedance spectroscopy as a platform for reagentless DNA sensing assay. The performance and factors influencing the performance of the resulting biosensor were studied in detail. The linear range of the biosensor was from $1.0 \times 10^{-8}$ to $1.0 \times 10^{-6}$ M with a detection limit of $5.0 \times 10^{-9}$ M at $3\sigma$. In addition, the experiment results indicate that oligonucleotide immobilized on this way exhibits a good sensitivity, selectivity, stability and a long-term maintenance of bioactivity.


*Keywords:* Bilayer two-dimensional 3-mercaptopropyltrimethoxysilane; DNA immobilization; Gold nanoparticles; Electrochemical impedance spectroscopy

## 1. Introduction

Due to the major biological role of nucleic acids, reliable analytical techniques are required for their quantitation. Various techniques including radiochemical [1], enzymatic [2], fluorescent [3,4], colorimetric [5], chemiluminescent [6], surface acoustic wave [7], quartz crystal microbalance (QCM) [8,9] and electrochemical methods [10–15] are widely reported. One of the key drawbacks of the DNA biosensor concepts discussed above is the requirement for an indicator to transduce the hybridization event. Increased attention has been given recently to direct label-free electrochemical detection schemes [16–20], in which the hybridization event triggers a change in an electrical signal. Such protocols greatly simplify the sensing protocol and offer an instantaneous detection of the duplex formation. Such direct, in situ detection can be accomplished by monitoring changes in electronic or interfacial properties accompanying the DNA duplex formation event.

Electrochemical techniques are well suited for rapid detection of specific DNA sequences for their high sensitivity, small dimensions, low cost and compatibility with micro-fabrication technology of transducers. To prepare electrochemical DNA biosensors, the immobilization of the DNA probe should be considered. To a large extent the sensitivity, selectivity and reproducibility of a DNA recognition interface will depend on the immobilization of the DNA probe strands. When hybridization occurs the probe and target strands must be free to coil around each other. Immobilization of the probe strand onto a transducer surface will inevitably cause a decrease in configurational freedom.

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Therefore, minimization of the decrease in this freedom is required to achieve efficient hybridization. The present study focuses on the influence of different immobilization techniques for DNA on the surface of solid electrodes. One of the DNA immobilization procedures based on multi-site attachment using simple adsorption methodologies. But neutron reflectivity studies [21] indicated that the ss-DNA was lying flat on the surface of electrode with multiple adsorption points as the DNA bases complexed with the surface. The resultant hybridization efficiency was low, as the probe DNA was too strongly associated with the surface of electrode to hybridize with the target. In order to improve the hybridization efficiency, ss-DNA immobilization on transducer surfaces using single-point attachment is recommended by preventing the nonspecific adsorption of the DNA bases. Self-assembled monolayer (SAMs) is one kind of useful technique, since it can provide simple organizing procedure and regular orientation to immobilize DNA on various metal and oxide surfaces [22]. This technique provides an elegant method to preparation of well-define assemblies on solid surfaces. 3-Mercaptopropyltrimethoxysilane (MPTS) is a bifunctional molecule that contain both thiol and silane functional group, therefore it has been immobilized on glass, glass carbon, Pt, Au and Cu substrates [23–29]. SAMs of thiol moleculars on an electrode surfaces provides a useful means to attach various functional groups, such as nanoparticles. Recently, nanomaterials have been used in bioanalytical chemistry for their unique properties. Colloidal gold is a kind of nanomaterial which possesses tremendous specific surface area and good biocompatibility. And the colloidal gold modified on a solid electrode behaves as an ensemble of closely spaced but isolated microelectrodes. It can strongly adsorb some proteins or DNA without loss of their biological activity [30].

In this article, we use bilayer two-dimensional network of MPTS (B2dMPTS) and gold nanoparticles to self-assemble 5′-thoil-capped ssDNA for fabricating a DNA biosensor. In comparison to cysteamine, the B2dMPTS increases the surface area of the electrode, enrichs the immobilization and reduces the nonspecific adsorption of ssDNA, enhances the sensitivity of the modified electrode. Such protocol for DNA immobilization offers several major advantages such as simple, providing improved mechanical properties, single-point attachment onto the miniaturized surface. We also exploit the impedance spectroscopy as a platform for reagentless DNA sensing assay. The indicator free detection avenue greatly simplifies the sensing protocol and offers an innovatory and high-speed detection of hybridization reaction.

## 2. Experimental

### 2.1. Reagents and apparatus

The oligonucleotides used in this study was purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China). Oligonucleotide probe with a mercaptohexyl group at its 5′-phosphate end, abbreviated HS-ssDNA, is the sequence as follows: 21-mer 5′-HS-$(CH_2)_6$-ACT GCT AGA GAT TTT CCA CAT-3′; the complementary target oligonucleotide have the sequences: 5′-ATG TGG AAA ATC TCT AGC AGT-3′; three-mismatch containing oligonucleotide sequences: 5′-ATG AGG AAA ACC TCT AGG AGT-3′; and the non-complementary control is the same sequence as the 21-mer HS-ssDNA only without the HS-attachment at the 5′ end. 3-Mercaptopropyltrimethoxysilane and cysteamine were purchased from Sigma (St. Louis, MO, USA) and used as received. Tris(2,2′-bipyridyl)cobalt(III) perchlorate ($Co(bpy)_3^{3+}$) was prepared according to a published procedure [31]. $AuCl_3HCl{\cdot}4H_2O$, $Na_3$ citrate, $K_3Fe(CN)_6$ and other chemicals were all of analytical reagent grade. The following buffers were used, 0.1 M PBS: 0.1 M KCl + 0.1 M phosphate buffer (pH 7.0); 0.3 M PBS: 0.3 M KCl + 0.1 M phosphate buffer (pH 7.0); 0.75 M PBS: 0.75 M KCl + 0.1 M phosphate buffer (pH 7.0); the pH (pH 5.0–8.5) was adjusted with additional $Na_2HPO_4$ solution. Water used was twice distilled and sterilized. The 16-nm Au colloid was prepared according to the literature [32].

Electrochemical impedance spectroscopy (EIS) measurements were performed with a Model IM6e (ZAHNER Elektrick Co., Germany). Cyclic voltammetry (CV) were performed with a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Co., China). The Electrochemical cell consisted of a three-electrode system where bare or modified gold electrodes were used as a working electrode, platinum wire as an auxiliary electrode and a saturated calomel as a reference electrode (SCE). All measurements were carried out at a temperature of 25 °C. All solutions were deaerated with nitrogen and kept under a nitrogen atmosphere throughout the entire procedure. The size of gold nanoparticles was estimated from transmission electron microscopy (TEM) (H600, Hitachi Instrument Co., Japan).

### 2.2. Preparation of working electrodes

The steps of preparing the modified electrode are shown schematically in Scheme 1. The gold electrode was polished to a mirror-like surface with 3.0, 0.3-μm alumina slurry on microcloth pads, and sonicated in ethanol and water. The freshly polished electrodes were pretreated before each experiment by cleaning with piranha solution (7:3 mixture of concentrated sulfuric acid and 30% hydrogen peroxide), according to the literature [33] to self-assemble bilayer two-dimensional network of MPTS. After electrochemical cleaning by several potential cycling between −0.3 and 1.5 V versus SCE in 0.1 M $H_2SO_4$ (the real area, 0.126 $cm^2$, of the gold electrode was calculated by the integration of the cathodic peak in 0.1 M $H_2SO_4$ [34,35]), the gold electrode was immersed in a 40 mM solution of MPTS in methanol for 3 h, so to produce a self-assembled monolayer (1dMPTS). After thorough rinsing, the silane units were interconnected into a two-dimensional network by dipping into aqueous 0.01 M NaOH for 2 h. A second silane layer (B2dMPTS)