Probing Biomimetic Molecular Structures on Gold and Silicon(111) with Electrical Impedance Spectroscopy

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Abstract— **Self-assembled monolayers (SAMs) offer an approach for engineering molecular interfaces that mimic biological structures and their processes on solid substrates. Here we demonstrate the ability of electrical impedance spectroscopy to monitor the biological process of DNA recognition in a monolayer comprised of single stranded DNA assembled on a gold substrate and characterize the structure of a hybrid bimolecular lipid membrane (BLM) on an atomically flat silicon surface. These biomimetic examples demonstrate the crucial importance of the impedance phase in being able to distinguish between electrical conductive and capacitive properties of the molecular interfaces.**

Keywords— **Electrical impedance spectroscopy, BLM, bilayers, monolayer, DNA, biosensor**

I. Introduction

Molecular self-assembly is a powerful approach for producing novel biomimetic-architectures on the solid substrates [1]. For example, the gold and sulphur self-assembled monolayers (SAMs) and organosilanes on silica surfaces have been used extensively for the controlled immobilization of biomolecules such as DNA [2]. And lately silicon-carbon linked organic monolayers [3] have become an area of substantial interest because of their potential for bio-fuctionalising semiconductor devices and developing tethered bimolecular lipid membranes (BLM) for studying the modus operandi of pharmaceutical agents and membrane proteins [4].

Early electrical impedance spectroscopy (EIS) studies performed on highly unstable unsupported BLMs resolved the dielectric substructure using four-terminal ultra low frequency EIS with a spatial resolution in the order of Å [5]. Four electrically distinct regions were detected in lecithin BLMs and assigned to the acyl chains, the carbonyl group, the glycerol bridge and the phosphorylcholine headgroup. It was shown that inclusion of cholesterol induced changes in the electrical properties of some of these regions, and the location of the molecule within the bilayer was deduced from these changes [6]. Oxidation of cholesterol in-situ was monitored and was shown to lead to the movement of the oxidised sterol towards the polar headgroup region of the lipids [7]. Changes in the substructure of BLMs have also been correlated with the inclusion of local anaesthetics [8], growth hormones [9] and antibiotics [10].

Here, we demonstrate the use of EIS as a biosensor through the detection of vast increases in conductance when complementary DNA bound to a monolayer of single-stranded DNA assembled on a gold surface. Further, we demonstrate its utilisation for structural studies by monitoring changes in capacitance dispersions with frequency during the self assembly of a lipid molecular leaflet onto an alkyl monolayer attached to an atomically flat silicon (111) surface to form a hybrid BLM.

II. Experimental

A. Preparation of DNA Modified Au Surface

Before the immobilization of ss-DNA, the gold working electrodes were cleaned and prepared as described previously [11]. The cleaned gold electrodes were immersed in 4μM thiolated DNA with the following sequence: 5'-GGGGCAGAGCCTCACAACCT-p-$(CH_2)_3$-SH-3' for 90 min in immobilization buffer (1 M KH_2PO_4 (pH 4.5). Subsequently, the single-stranded DNA modified gold surfaces were incubated in 1 mM mercaptohexanol (MCH) solution for 30 min, followed by rinsing with phosphate buffer. The DNA/MCH modified electrodes were then immersed in 4 mM complementary target DNA with the following sequence: 5'-AGGTTGTGAGGCTCTGCCCC-3' in hybridization buffer (10 mM Tris-HCl, 1 M NaCl (pH 7.0)) for 2.5 hour. The resultant double-stranded DNA modified electrodes were then rinsed with phosphate buffer.

B. Descriptive rules Preparation of Si-C Linked Monolayers

Si(111) wafers (n-type, 0.01-0.1 Ωcm) were cleaned using "Piranha" solution (concentrated H_2SO_4:30% H_2O_2, 3:1, v/v) at 90°C for 30 min and rinsed thoroughly with Milli-Q water. The cleaned wafers were then etched in deoxygenated NH_4F solution (40% v/v) for 20 min to generate a hydride terminated Si(111) surface. The freshly etched Si(111)

surface was then converted to a Si-C linked monolayer through hydrosilylation in deoxygenated alkene (1-decene, 1-dodecene, 1-tetradecene, 1-hexadecene or 1-octadecene) or 1-undecenoic acid solutions by either thermal or photochemical activation under UV light for 2-10 hours (10 hours for 1-decene and 1-dodecene, 3 hours for 1-tetradecene and 2 hours for 1-hexadecene and 1-octadecene). The alkyl functionalized silicon wafer was then rinsed with hexane, dichloromethane, tetrahydrofuran and ethanol prior to drying thoroughly under a stream of nitrogen.

C. Preparation of hybrid bilayer lipid membranes on silicon

Hybrid bilayer lipid membranes (hBLMs) were formed using vesicle adsorption or solvent-dilution techniques. Phospholipids and cholesterol were dissolved in dichloromethane, mixed in the desired ratios, and the solution was evaporated under a gentle stream of argon. The lipids were then further dried under the vacuum of a rotary vane pump with gentle warming (30°C) over night. The dried lipid mixtures were stored under argon at –20°C . Generally one of the following lipid mixtures was used for bilayer formation: (1) lecithin:cholesterol (2:1, mol/mol), (2) POPC:cholesterol (2:1, mol/mol) or (3) POPC:POPE:cholesterol (1:1:1, mol/mol/mol).

Vesicles were prepared fresh and stored at room temperature for no longer than a few hours. An aqueous 50 mM KH_2PO_4 buffer (pH 7.4, 1 ml) was placed into a 3 ml glass vial and agitated vigorously using a vortex mixer. A 50 mM solution of lipids in isopropanol (50 µl) was injected with a glass microsyringe into the stirred buffer, which yielded a vesicle suspension. The vesicle suspension was diluted 1:10 with buffer and injected into the measurement cell.

Alternatively BLMs were formed by placing a 15 mM solution of lipids in isopropanol (15 µl) onto the derivatised silicon surface followed by addition of an aqueous buffer (50 mM KH_2PO_4, adjusted to pH 7.4 with aqueous KOH or 10 mM TRIS, adjusted to pH 8.0 with HCl) with a micropipettor.

D. AC Impedance Measurements

AC impedance measurements were performed with an Inphaze Impedance Spectrometer (Inphaze Pty Ltd., Sydney, Australia) over a frequency range of 6 mHz to 100 kHz. A 2-electrode electrochemical cell containing 5mM ferric cyanide/ferrous cyanide in 300 nM KCl was used for the DNA experiment using two identical DNA-modified gold electrodes.

Impedance measurements on silicon modified surfaces were performed using the 3-electrode electrochemical cell depicted in Fig. 1 with an Ag|AgCl reference electrode and platinum counter electrode. Gallium-indium eutectic was applied to form a rear ohmic contact between the silicon wafers and the working electrode. The electrolyte was 100mM KCl.

A Maxwell-Wagner model was fitted to the impedance data to obtain the conductance value of the electrolyte between the reference electrode and organic surface as well as the capacitance and conductance values of the leaflets of the bilayers (see insets to Fig.3). Significantly, the capacitance per unit area of a layer, C, is related to the thickness, d, of the layer by;

$$d = \varepsilon_r \varepsilon_o / C \qquad (1)$$

where ε_r is the dielectric constant of the layer, ε_o is the permittivity of free space (=8.85x 10^{-12} Fm^{-1}).

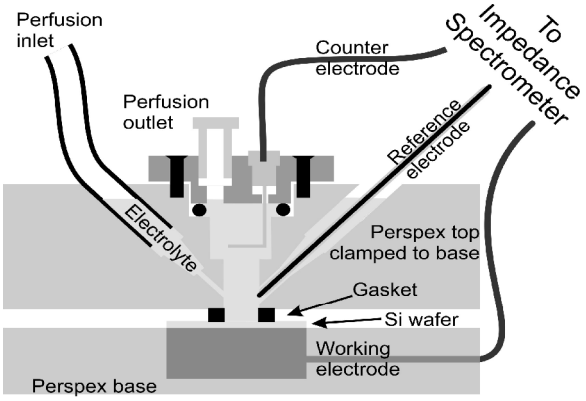

Fig. 1 Electrochemical Cell for hybrid bilayer characterisations

III. Results and Discussion

A. DNA Modified Surface

Whether DNA is able to transport electrons has attracted much interest, particularly as this ability plays a major role as a repair mechanism in DNA damage [12]. Experiments addressing DNA conductivity have involved a large number of DNA strands labeled with intercalated donor and acceptor molecules, and the conductivity has been assessed from electron transfer rates as a function of the distance between the donor and acceptor sites [13, 14].

Here, thiolated single-stranded DNA was immobilized onto gold electrodes utilizing gold-sulphur self assembly chemistry. Two-terminal AC impedance measurements of the single-stranded DNA modified gold surface were performed prior to and after exposure to complementary DNA.

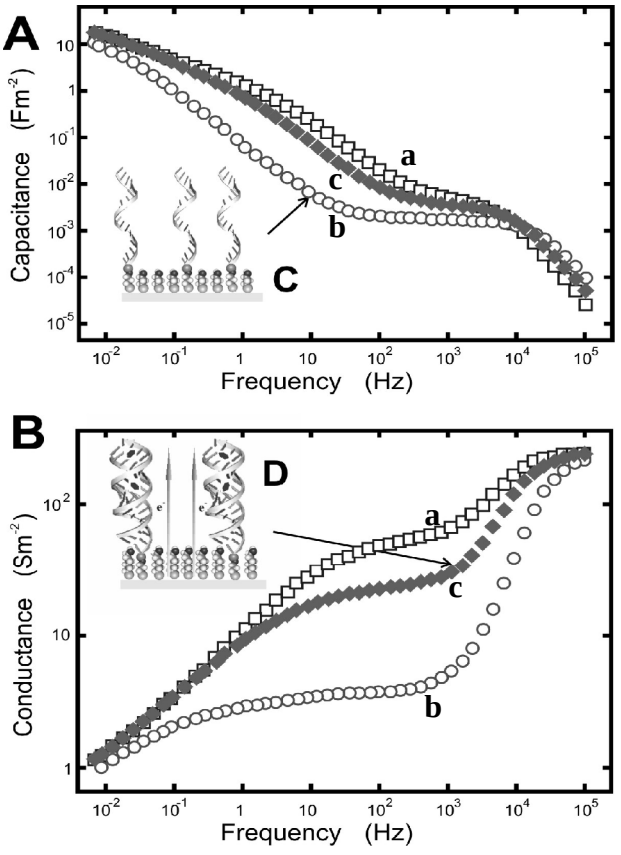

Fig. 2 Capacitance (A) and conductance (B) plot obtained from the impedance measurements performed at (a) bare (squares), (b) single-stranded DNA (circles) and (c) double-stranded DNA modified (diamonds) gold electrodes.

The resultant conductance plots obtained at the bare and DNA modified gold electrodes are shown in Fig. 2. A decrease in conductance was observed after single-stranded DNA was immobilized onto the gold surfaces, which is consistent with the previous reports [15] where electrons, which were present in the measuring solution as consequence of the presence of both ferrous (electron donor) and ferric (electron acceptor) cyanide salts, were repelled away by the polyanionic backbone of the immobilized single-stranded DNA. However, an increase in the conductance was observed after exposure to complementary target single stranded-DNA (Fig. 2). This increase was due to the formation of double-stranded DNA which can act as molecular wire and thus allowed the access of electrons in the solution back to the electrode surface.

B. Hybrid bilayers on silicon

The fitting of the Maxwell-Wagner model to impedance spectra of biomimetic structures on silicon (e.g. Fig. 3) revealed that the capacitance of alkyl (octadecane) monolayers varied between 16 mF m^{-2} to 20 mF m^{-2} which was considerably higher than the expected value of 11.5 mF m^{-2} for these layers calculated using Eqn (1) and a dielectric constant of ε_r=2.2 for the alkyl region. However, Yu *et al.* [16] have determined the capacitances and thicknesses of Si-C alkyl monolayers and obtained a dielectric constant of ε_r=3.3±0.6 for these layers. This value yields an expected value of 16.8 mFm^{-2} for the capacitance in better agreement with the experimentally determined values.

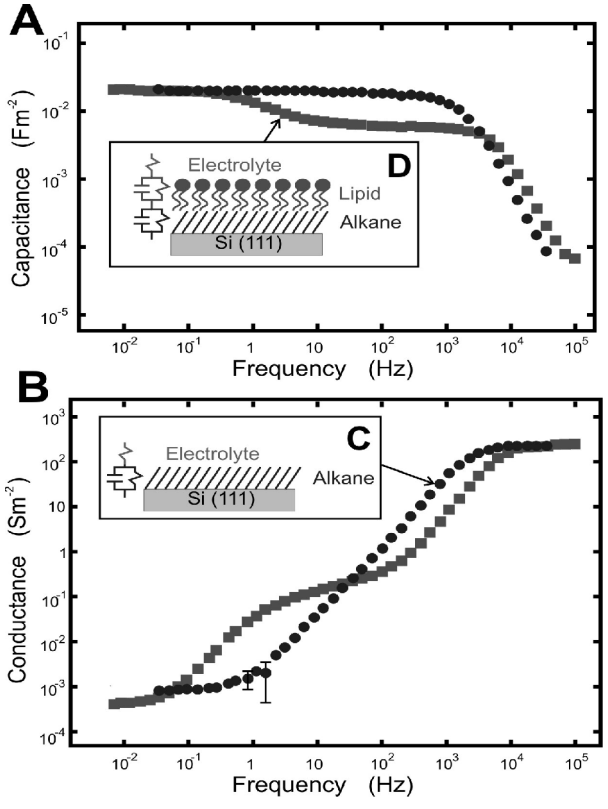

Fig. 3 Capacitance (A) and conductance (B) dispersions with frequency obtained from the impedance measurements of an alkyl (C18) monolayer (circles) on Si(111) surface (inset C) and a hybrid BLM (inset D) comprised of the alkyl monolayer (lower leaflet) and a POPC lipid monolayer (upper leaflet).

Addition of a lecithin:cholesterol vesicle suspension to the hydrophobic Si-C linked monolayer (see inset D in Fig. 3) led to a substantial decrease in the capacitance and conductance of the system in the frequency range 10 to 10^4 Hz. This decrease, which was attributed to the formation of the

hybrid BLM (hBLM) by the spreading of vesicles on the hydrophobic alkyl monolayer, occurred within approximately 15 - 20 min after injection of the vesicle suspension. Similar kinetics have been observed for the preparation of hBLMs on alkanethiol monolayers on gold [17].

The conductance of the top leaflet deposited onto the monolayer was several orders of magnitude higher than that of the lower leaflet, which indicated that the phospholipid/cholesterol monolayer was poorly packed and therefore leaky. The differences between these two types of layers may be related to the more rigid structure of the underlying monolayer [17].

The capacitance of the hBLM (C_{hBLM}) with insulating phospholipid/cholesterol leaflet was 8.2±1.9 mF m^{-2} similar to that of a free standing lecithin BLM [7]. The capacitance of the "upper leaflet" was 14.7 mFm^{-2} smaller than the value of ~ 21 mF m^{-2} obtained for hBLMs formed on gold [16].

Additionally, the capacitance of a POPC monolayer (assuming d_{hc} = 13.5Å [18] and a dielectric constant of ε_{hc} = 2.1) was 13.8 mF m^{-2} only slightly lower than the capacitance determined for the PC/cholesterol monolayer in the hBLM system on silicon ($C_{upperleaflet}$ = 14.7 mFm^{-2}).

The impedance spectra of hBLMs prepared by the solvent-dilution technique on the same substrate were usually indistinguishable from those formed by vesicle adsorption.

III. Conclusions

The recognitive properties of the DNA monolayer on gold were shown to be dependent on principally the conductive properties of the monolayer. In contrast the structural characterisations of the hybrid bimolecular lipid membranes (BLMs) were dependent principally on the capacitive properties of these BLMs. The characterisations of these biomimetic structures illustrate the importance of electrical impedance measurements being able to distinguish between the conductive and capacitive components of impedance, which demands an accurate measurement of the impedance phase. Such is a feature of the Inphaze impedance spectrometer used in these studies.

Acknowledgment

The Australian Research Council funded this research (DP0452447).

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