

Electrical characterizations of biomimetic molecular layers on gold and silicon substrates

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Abstract

Electrical impedance technology was used to characterize DNA recognition in a monolayer containing single-stranded DNA probes immobilized on a gold substrate using thiol self-assembly chemistry. Recognition of targeted complementary DNA was principally correlated with an eight-fold increase in the conductance of the monolayer and attributed to electron conduction through double helices formed upon the binding of the DNA targets to the probes. The high recognitive sensitivity was possible without the use of the redox labels or large bias voltages required for recognition using cyclic and Osteryoung square wave voltammetry. The impedance technology also provided atomic resolution of a hybrid bimolecular lipid membrane formed by deposition of a phospholipid:cholesterol monolayer onto a hydrophobic alkyl monolayer covalently attached to a silicon substrate via silicon–carbon bonds. Atomic resolution was achieved through preparation of membranes on surfaces approaching atomic flatness and the performance of impedance measurements over precisely defined areas of the surface in contact with solutions. Principally capacitive properties distinguished between the immobilized (octadecyl) and more fluidic (lipid:cholesterol) leaflets of the hybrid membrane. The lipid:cholesterol leaflets were structurally similar to those leaflets in free-standing bimolecular lipid membranes. The hybrid membrane therefore provides a highly stable and physiologically relevant surface for studying biomolecular interactions with membrane surfaces.

Keywords: electrical impedance spectroscopy, BLM, bilayers, monolayer, DNA, biosensor

Introduction

Electron conduction through DNA has attracted much interest from both physiological and technological points of view. It not only plays a major role as a repair mechanism in DNA damage (Dandliker *et al* 1997) but also has potential applications in the fields of forensics and nano-electronics (Bhalla *et al* 2003). Efforts to probe the mechanisms of DNA conduction (Arkin *et al* 1996, Lewis *et al* 1997) have utilized self-assembly processes for constructing molecular interfaces on solid substrates (Ulman 1991). Similarly self-assembly chemistry is utilized to prepare a variety of other biorecognitive or biomimetic interfaces, such as stabilized lipid membranes.

The substrates provide stability for these fragile interfaces. The substrates also provide the most likely means of monitoring or stimulating biological functions of these interfaces. This is particularly so for substrates comprising silicon and gold as these elements underpin the semiconductor technology that can potentially link biological functionalities of the constructs directly to effective transduction mechanisms and well-established amplification and recording technologies.

Additionally, the interfaces so stabilized may be studied with a wide range of surface-sensitive techniques including x-ray and neutron reflectometry, surface plasmon resonance, ellipsometry, atomic force microscopy and cyclic voltammetry. In combination with techniques commonly applied to free-standing bimolecular lipid membranes (BLMs), such as electrical impedance spectroscopy (EIS), fluorescence microscopy and fluorescence recovery after photobleaching, a detailed understanding of the structural and dynamic properties of the interfaces and incorporated molecules can be obtained.

Electrical impedance spectroscopy (EIS) has proved to be a very effective means of probing the structure of free-standing BLMs (Ashcroft *et al* 1977). For example, a series of EIS studies were able to locate cholesterol (Ashcroft *et al* 1983), oxidized cholesterol (Karolis *et al* 1998), anesthetics (Coster and Laver 1986), growth hormones (Zimmerman *et al* 1977) and immunosuppressant drugs (Karolis *et al* 1999) in these BLMs down to the atomic level. Atomic spatial resolutions were also achieved in an EIS study (Wong *et al* 2007) of a series of alky monolayers on silicon in which thicknesses of the layers were verified using x-ray reflectometry (XRR).

Here we present (1) EIS studies of DNA constructs on gold and (2) biomimetic membranes on silicon. In the first study, the relative sensitivities of EIS, cyclic voltammetry and Osteryoung square wave voltammetry measurements to the formation of helical DNA duplexes are compared. In the second study, the structural and electrical properties of the hybrid membranes are compared with those properties of free-standing BLMs. Finally, the quantity and quality of the information obtained from EIS studies of the biomimetic structures on the different types of substrates are compared and discussed.

Materials and methods

Preparation of DNA-modified Au surface

Details of the preparation of surfaces are described in detail by Chilcott *et al* (2007) and Gooding *et al* (2000). Briefly, cleaned gold electrodes were immersed in 4 μ M thiolated DNA with the sequence 5'-GGGGCAGAGCCTCACAACT-p-(CH₂)₃-SH-3' in an immobilization buffer (1 M KH₂PO₄ (pH 4.5)) for 90 min. The surfaces then were incubated in a 1 mM mercaptohexanol (MCH) solution for 30 min, followed by rinsing with a phosphate buffer. The DNA/MCH-modified electrodes were then immersed in 4 mM complementary target DNA with the following sequence: 5'-AGGTTGTGAGGCTCTGCCCC-3' in the hybridization

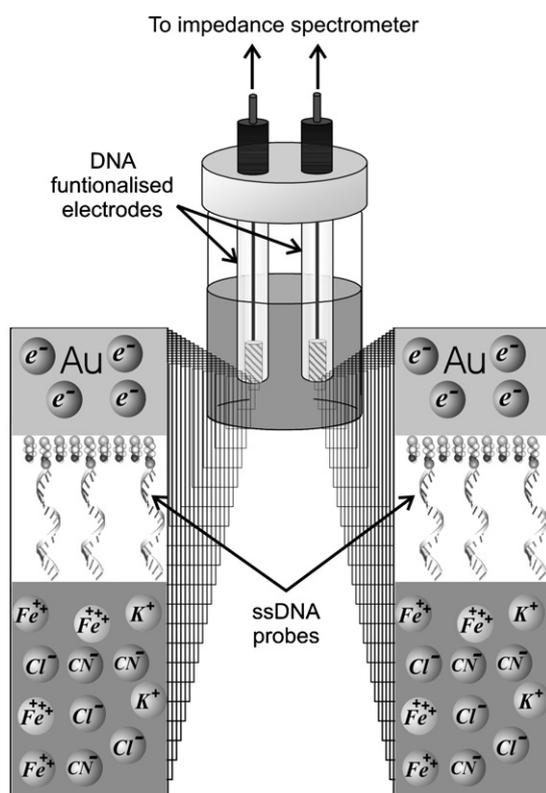


Figure 1. Experimental chamber and two identically prepared electrodes used for DNA hybridization characterizations in a 300 mM KCl solution containing 5 mM ferrous/ferric cyanide. The insets are magnified schematics of the single-stranded DNA probes immobilized in monolayers on the gold surfaces.

buffer (10 mM Tris-HCl, 1 M NaCl (pH 7.0)) for 2.5 h and then rinsed with the phosphate buffer.

Preparation of Si-C linked monolayers

Details of the preparation of these surfaces are described in detail by Böcking (2003). Briefly, Si(1 0 0) wafers (p-type, 0.005 Ω cm) were cleaned using a 'Piranha' solution and rinsed thoroughly with Milli-Q water. 2.5% hydrofluoric acid was used to generate a hydride terminated Si(1 0 0) surface. Hydrosilylation chemistry in deoxygenated octadecene under UV light then linked the octadecane monolayer via Si-C bonds to the surface. The surface was rinsed with hexane, dichloromethane, tetrahydrofuran and ethanol and dried thoroughly under a stream of nitrogen.

Preparation of hybrid bilayer lipid membranes on silicon

Lipid mixtures used were PC⁴:cholesterol (2:1, mol/mol) and POPC⁵:cholesterol (2:1, mol/mol).

⁴ Phosphatidylcholine or lecithin from egg yolk.

⁵ 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine.

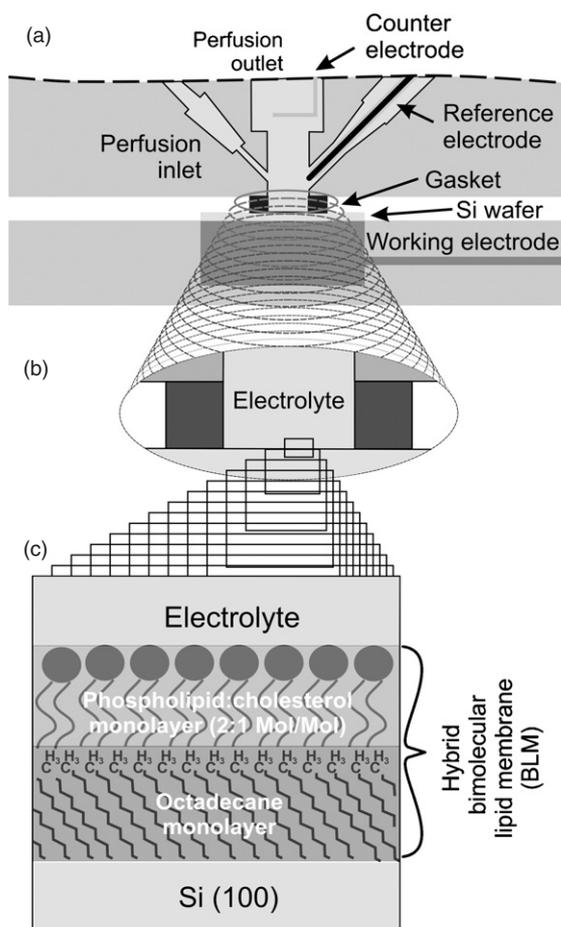


Figure 2. (a) Schematic of the Inphaze chamber used to study hybrid bimolecular lipid membranes (BLMs) on silicon. (b) A gasket of rectilinear cross section that defines the active area of the substrate and volume of electrolyte for impedance measurements (see the appendix and figure A.1). (c) Hybrid BLM.

Solvent-dilution or vesicle-adsorption techniques as described by Böcking (2003) were used to form hybrid bimolecular lipid membranes (figure 2(c)).

Solvent dilution. Phospholipids and cholesterol were dissolved in dichloromethane and mixed in the desired ratios. The solution was evaporated in a gentle stream of argon and dried under vacuum with gentle warming (30 °C) overnight.

Vesicle adsorption methods. A 50 mM solution of lipids in isopropanol (50 μ l) was injected into a vigorously stirred 50 mM KH_2PO_4 buffer (pH 7.4, 1 ml). This vesicle suspension was then diluted 1:10 with the buffer and injected onto the octadecane monolayer. Alternatively, a 15 mM solution of lipids in isopropanol (15 μ l) was deposited onto the octadecane monolayer and an aqueous buffer (50 mM KH_2PO_4 , adjusted to pH 7.4–8) added with a micropipettor.

AC impedance measurements

An Inphaze impedance spectrometer (Inphaze Pty Ltd, Sydney, Australia) performed impedance measurements over a frequency range of 6 mHz to 100 kHz as described in detail by Chilcott *et al* (2007). DNA experiments utilized two identical DNA-modified gold electrodes immersed in 5 mM ferric cyanide and ferrous cyanide in 300 mM KCl (see figure 1). Characterizations of the hybrid BLMs utilized a three-electrode electrochemical chamber containing 100 mM KCl (see figure 2). A detailed description of the cell, the importance of using a gasket of rectilinear cross section as opposed to one of circular cross section and the method for estimating the thicknesses of layers can be found in the appendix.

Results

DNA interface on gold

Measurements of conductance and capacitance as a function of frequency for a gold electrode which was initially bare (■), modified with single-stranded DNA (●) and then double-stranded DNA (◆) are shown in figure 3. The dispersions with frequency are uniquely different from each other except at the extreme frequencies. The convergence of the dispersions at high frequencies can be attributed to the bulk conductance of the KCl/FeCN₂/FeCN₃ electrolyte and that at low frequencies to a gold–electrolyte interface with vastly different conduction properties to the classic ionic double layer. This difference is attributed to the presence of electron donors (i.e. Fe²⁺) and acceptors (i.e. Fe³⁺) in the electrolyte which facilitate electron conduction between the electrolyte and the gold either directly (■) or via the DNA interfaces (● and ◆). Indeed the measurements at 10⁻² Hz, where the conductance dispersions converge, reflect a conductance value for the interface which is some 500 times that reported for a ‘blocking’ ionic double layer (Gaedt *et al* 2002), i.e. one that forms in the absence of redox ionic species.

The variations in the dispersions between these extremes of frequency can be correlated with the immobilization of single-stranded DNA (●) and the subsequent hybridization of the complementary single-stranded DNA (◆). In particular, the decrease in the conductance after immobilization is consistent with previous reports (Steel *et al* 1998) and the explanation that electrons are repelled from the interface by the polyanionic backbone of the immobilized single-stranded DNA. Further, the eight-fold increase in the conductance after hybridization can be attributed to formations of double-stranded DNA which act as molecular wires for electrons (Aich *et al* 1999).

Hybrid bimolecular lipid membranes (BLMs) on silicon

The electrical characterization of the hybrid BLMs was performed using a chamber that facilitated impedance measurements over a precisely defined area of substrate surface and electrolyte solution. Characterizations on a silicon substrate with a native oxide layer in contact with an electrolyte (see the appendix) revealed that the impedance of the silicon–electrolyte interface derived principally from the capacitive properties of its oxide layer and not from the type of double layer that dominates the electrical properties of the gold–electrolyte interface.

Figure 4 shows capacitance and conductance measurements of the octadecane monolayer (○) attached directly to the silicon surface after the oxide layer had been chemically etched away (see inset (c) of figure 4). The Maxwell–Wagner fit to these measurements is also consistent with interfacial properties deriving principally, in this instance, from the capacitive

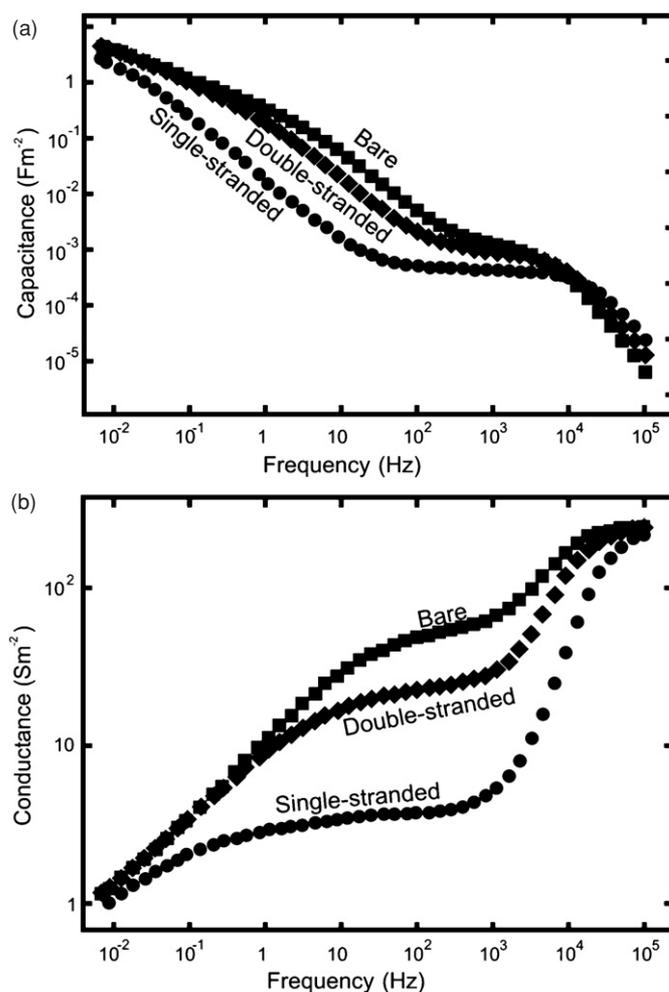


Figure 3. Capacitance (a) and conductance (b) data obtained from the impedance measurements of bare (■), single-stranded DNA (●) and double-stranded DNA-modified (◆) gold electrodes (adapted from Chilcott *et al* (2007)).

properties of the monolayer. The capacitance values for this interfacial layer were $18 \pm 2 \text{ mF m}^{-2}$, in agreement with the values of 16.8 mF m^{-2} reported by Yu *et al* (2000) and $15 \pm 1 \text{ mF m}^{-2}$ reported by Wong *et al* (2007).

Addition of a lipid:cholesterol vesicle suspensions to the hydrophobic Si-C linked monolayer led to a substantial decrease in the capacitance and conductance of the system. This decrease, which was attributed to the formation of the hybrid BLM (hBLMs) by the spreading of vesicles on the hydrophobic alkyl monolayer, occurred within approximately 15–20 min after injection of the vesicle suspensions. Similar kinetics have been observed for the preparation of hBLMs on alkanethiol monolayers on gold (Silin *et al* 2002).

After the initial fast decrease, the vesicle suspension was removed and exchanged with a fresh buffer. The capacitance and conductance of the system continued to decrease at a slow rate for more than 1 h, which might reflect small defects in the lipid monolayer ‘healing’ slowly.

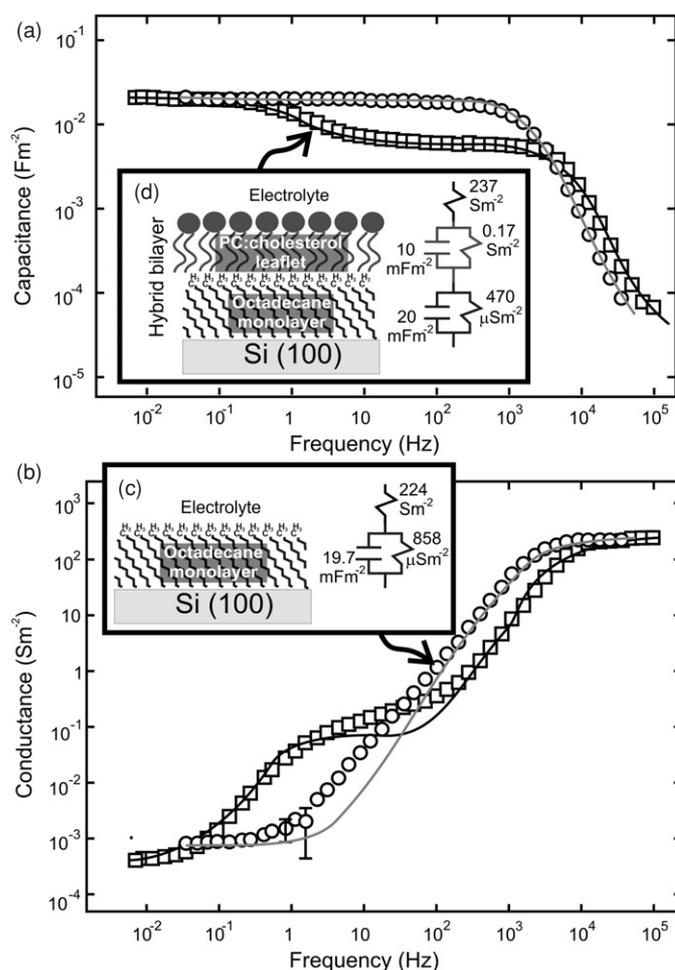


Figure 4. Capacitance (a) and conductance (b) dispersions with frequency obtained from the impedance measurements of the octadecane monolayer (○) and partially formed hybrid BLM (□) on a Si(100) surface (adapted from Chilcott *et al* (2007)). Insets (c) and (d) depict the stages of formation of the hybrid and the Maxwell–Wagner circuit elements for the electrolyte, monolayer and PC:cholesterol leaflet that provided theoretical fits to the data (see the appendix and footnote 6).

Figure 4 shows the conductance and capacitance measurements of a hybrid BLM (□) comprising PC:cholesterol and octadecane leaflets. The theoretical fit to these data yielded three Maxwell–Wagner elements. The conductance and capacitance values of two of the elements are of similar magnitude to values obtained for the electrolyte and octadecane leaflet prior to the addition of PC:cholesterol (e.g. see inset (d) of figure 4). The third element therefore was justifiably attributed to the upper PC:cholesterol leaflet⁶.

⁶ Maxwell–Wagner models utilizing an additional element yielded better fits to the conductance measurements in figure 4(b) but did not improve substantially the exceptionally good fits to the capacitance data. Further, the additional elements did not alter substantially the capacitance and conductance values deduced for the monolayers or the conductance values for the electrolytes used to generate the theoretical curves in figure 4. The origin of the additional elements is unknown but could be attributed to the manifestation of interfacial polarization layers in the electrolyte arising from the dependences of the Born energies for ions and electrons on the dielectric properties of Si.

The conductance of the upper leaflet was several orders of magnitude higher than that of the lower leaflet, which indicates that the phospholipid and cholesterol molecules were more loosely packed than the immobilized octadecane molecules; the lower packing density correlating with an increased ionic permeability and hence conductance. The differences in packing densities also indicate that there was little interdigitation of the hydrocarbon (acyl chain) regimes of the PC leaflet and octadecane monolayer as occurs between the hydrocarbon regimes of the two leaflets in free-standing BLMs (Silin *et al* 2002). On this basis, a thicker PC leaflet would be expected to form on the densely packed monolayer anchored to the silicon than leaflets of free-standing BLMs. Indeed the capacitance value of 10 mF m^{-2} , obtained from the data shown in figure 4, is less than the values of $13.2 \pm 0.4 \text{ mF m}^{-2}$ for PC:cholesterol leaflets in free-standing BLMs (Karolis *et al* 1998) (see equation (A.4)).

Analyses of data from POPC:cholesterol leaflets on octadecane monolayers yielded similar trends. Further, hybrid BLMs with similar properties to those formed by vesicle adsorption were also prepared by the solvent-dilution technique. Hybrid bilayers formed spontaneously after an aqueous buffer was added to the chamber. The impedance spectra of hybrid BLMs prepared on the same substrate by both techniques were usually indistinguishable.

Discussion

Self-assembled monolayers (SAMs) of alkylthiols on gold or of alkylsilanes on silicon dioxide surfaces have been utilized in many applications ranging from the controlled immobilization of biomolecules such as DNA (Levicky *et al* 1998) to the development of biomimetic membranes (Plant *et al* 1994). Although the gold and silicon substrates fulfill an important structural role in stabilizing the otherwise fragile biomimetic interfaces, they do not necessarily link directly to the functionality of the interfaces. For example, the interface that gold forms with aqueous environments alone, called the ionic double layer, represents a substantial electrical barrier to any electronic transduction of a biological signal as does the oxide layer that forms on silicon.

DNA interface on gold

A typical strategy to reduce the barrier of the ionic double layer in DNA-on-gold applications, utilizing cyclic and Osteryoung square wave voltammetry as a means of characterizing electron conduction, has been to employ redox labels, e.g. 2,6-disulfonic acid anthraquinone (AQDS), to facilitate the detection of electron conduction through the DNA into the gold substrate (Wong and Gooding 2003). An alternative strategy has been to use ferric cyanide as an electrocatalyst (Kelley *et al* 1999) in the aqueous environment where the targeted complementary DNA is located. A variation in the latter strategy is described herein, resulting in an almost three orders of magnitude decrease in the double layer barrier and an eight-fold increase in the conductance of the interface upon hybridization of the DNA (see figure 3(b)).

The eight-fold increase in the conductance after hybridization is consistent with reported two-fold increases in the peak-to-peak current of cyclic voltammograms obtained from similar interfaces after hybridization (Wong and Gooding 2003). The magnitude of the increase represents a significant improvement in recognitive sensitivity which was achieved without complications involving the use of redox labels and bias voltages of large magnitude ($\sim -400 \text{ mV}$) required by cyclic and Osteryoung square wave voltammetry.

Hybrid bimolecular lipid membranes (BLMs) on silicon

The characterization of the electrical properties of alkyl monolayers formed by silane chemistry on oxidized silicon substrates is complicated by the contributions of the silicon dioxide layer to the capacitance measured in the low frequency regime. This complication can be overcome by utilizing silicon–carbon linked monolayers without the intervening oxide layer (Linford and Chisley 1993, Böcking *et al* 2004). The capacitance measurements for the octadecane (figure 4(a), ○) monolayer directly attached to the silicon surface atoms reveal an interfacial capacitance that was independent of frequency over some four decades of frequency and which was fully consistent with theoretical expectations for an octadecane layer with homogeneous dielectric properties. This consistency justified the use of the theory to provide an unambiguous and precise estimate of the thickness of such a layer in good agreement with published values using electrical impedance spectroscopy (EIS) (Wong *et al* 2006) and other technologies (Yu *et al* 2000) including x-ray reflectometry (Wong *et al* 2007). The EIS characterization was successfully extended to characterization of an additional lipid layer (figure 4, □), the dielectric and geometrical properties of which were very similar to that of lipid leaflets in physiologically relevant free-standing bimolecular lipid membranes (Karolis *et al* 1998).

Limitations in resolution of characterizations of molecular constructs on a gold substrate

EIS studies of the DNA interface on gold yielded substantial capacitive changes correlated with hybridization (see figure 3(a)) that are indicative of structural and/or dielectric changes expected during the formation of the double helices. However, a specific characterization of such structural and dielectric changes is complicated by the persistence of a modified ionic double layer in the vicinity of the DNA interface as well as the three-dimensional geometries of the electrochemical apparatus (figure 1) and rough surfaces of the gold substrate used in these experiments.

For example, a comparison of capacitance data for the bare silicon–electrolyte (figure A.1, ■) and gold–electrolyte interfaces (figure 3(a), ■) reveals differences in the extent to which the capacitance disperses with frequency at low frequencies. The capacitance data for the former are essentially constant in frequency which yields a capacitance value for the silicon dioxide layer and an estimate of its thickness based on its known homogeneous electrical properties (see the appendix). In stark contrast, the dispersion of the gold–electrolyte interface is very pronounced and cannot be characterized using a Maxwell–Wagner model in which the individual circuit elements describe homogeneous electrical properties of layers. This type of dispersion is commonly observed in inert metal–electrolyte systems (Coster and Chilcott 1999) and attributed to the non-transference of charge at the interface and the topography of the surface (Wang and Bates 1986). Figure 3(a) (■) shows that the dispersion persists even in the presence of an electrocatalyst ($\text{FeCN}_2/\text{FeCN}_2$) which promotes transference of charge (electrons) and increases the conductance by almost three orders of magnitude. In this instance, the persistence of the dispersion can therefore be principally attributed to a surface topography for the gold that was rougher than that for silicon. Indeed, the absence of the dispersion for capacitance data for octadecane and hybrid BLM constructs on silicon (figure 4(a)) is consistent with an atomically flat topography which, in conjunction with the Inphase electrochemical chamber (figure 2) and spectrometer, provided dimensions of the hybrid BLMs down to the atomic level.

Conclusions

The DNA hybridization in a monolayer on gold was correlated with an eight-fold increase in the conductive properties of the monolayer.

Dielectric and structural characterizations of hybrid BLMs on silicon were consistent with other types of characterizations and estimates from the literature.

Electrical impedance spectroscopy measurements over precisely defined areas of biomimetic layers on atomically flat surfaces in contact with electrolyte solutions provided structural resolution of the layers down to the atomic level.

Acknowledgments

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Appendix. Achieving atomic resolutions of molecular layers on Si surfaces

A schematic of the experimental chamber for electrically characterizing molecular layers adsorbed to or self-assembled on flat surfaces is depicted in figure A.1 with the insets featuring two different types of gaskets used to define the active surface areas of the substrates. Conventional chambers employ Nitrile gaskets of circular cross section ('O-ring') whereas the 'Inphaze' chamber employs a gasket of rectilinear cross section comprising a 1 mm thick slice of flexible polymer Tygon of internal diameter 4.8 mm. Impedance measurements of the interface that silicon forms with an electrolyte solution were performed using the rectilinear gasket and a Nitrile O-ring of internal diameter 4.8 mm and cross-sectional diameter 0.8 mm.

The silicon surface readily oxidizes when exposed to air to form a native silicon dioxide (SiO₂). The bulk electrical properties of the SiO₂ layer (and any electrolyte–SiO₂ interfacial layer) are commonly modeled as a single capacitance component C and those properties of the electrolyte as a single resistance component of conductance G . The total impedance of the series combination is

$$Z(\omega) = \frac{1}{G} + \frac{1}{j\omega C} \quad \text{with} \quad j \equiv \sqrt{-1} = e^{j\pi/2}, \quad (\text{A.1})$$

where ω is the angular frequency of the alternating current used for the impedance measurements.

It can be readily shown that the total capacitance per unit area of the measured impedance is given by

$$c(\omega) \equiv \left(\frac{G^2 C}{G^2 + C^2 \omega^2} \right) \frac{1}{A}, \quad (\text{A.2})$$

where A is the known sealed area of the working electrode. At sufficiently low frequencies equation (A.2) approaches a constant value of

$$c(\omega \rightarrow 0) \approx \frac{C}{A}, \quad (\text{A.3})$$

which is the capacitance per area of the interface. And if the interfacial capacitance derives exclusively from the electrically homogeneous oxide layer of known dielectric constant $\epsilon_r =$

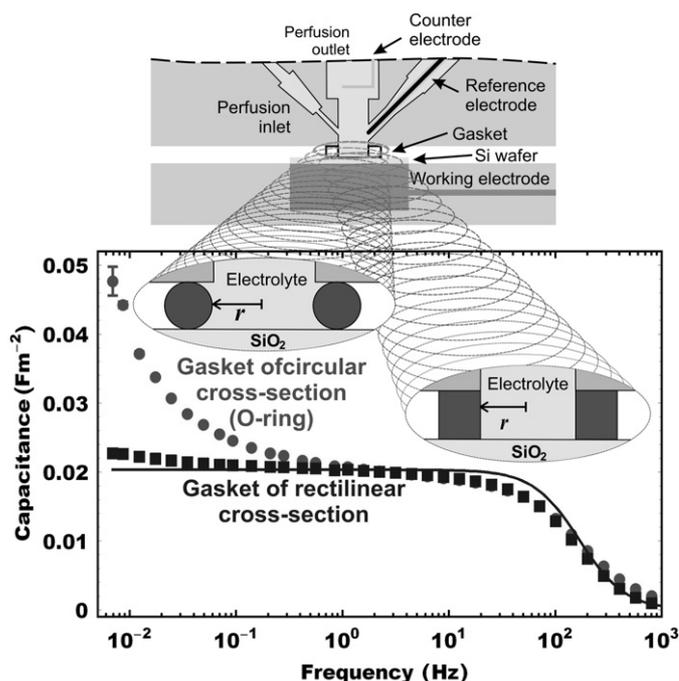


Figure A.1. Schematic of the Inphase chamber used to study the silicon–electrolyte interface and capacitance measurements of a SiO_2 layer made using a gasket of rectilinear (■) or circular (●) cross section. The curve is the dispersion expected for a SiO_2 layer of capacitance 0.02 F m^{-2} (yielding a thickness of 1.9 nm) attached to a Si(100) surface in contact with a 3 mM KCl electrolyte of conductance per area of 21.3 S m^{-2} .

4.41 (*CRC Handbook*), then the capacitance per unit area at low frequencies will yield the thickness t of the layer according to

$$t = \frac{\varepsilon_r \varepsilon_0}{C/A}, \quad (\text{A.4})$$

where ε_0 is the dielectric permittivity of free space ($= 8.85 \times 10^{-12} \text{ F m}^{-1}$).

It can also be shown that the total conductance per unit area of the measured impedance is given by

$$g(\omega) \equiv \left(\frac{GC^2}{G^2 + C^2\omega^2} \right) \frac{1}{A},$$

which, at sufficiently high frequencies, will approach

$$g(\omega \rightarrow \infty) \approx \frac{G}{A}, \quad (\text{A.5})$$

which is the conductance per area of the electrolyte. Note that the total capacitance (equation (A.2)) approaches

$$c(\omega \rightarrow \infty) \rightarrow \left(\frac{G^2}{C\omega^2} \right) \frac{1}{A} \approx 0. \quad (\text{A.6})$$

Figure A.1 shows that capacitance measurements using the rectilinear gasket (■) are generally consistent with theoretical expectations described by equations (A.3) and (A.6) and

represented by the curve in figure A.1 using a value of 21.3 S m^{-2} for the conductance per unit area of the electrolyte (i.e. G/A) and a value of 0.02 F m^{-2} for the capacitance per unit area of the SiO_2 layer (i.e. C/A). Importantly, the measured capacitance at low frequencies is substantially independent of frequency consistent with the known homogeneity of SiO_2 layers. Further, equation (A.4) yields a thickness of 1.9 nm for the layer which is of similar order of magnitude expected for native SiO_2 layers.

Whilst capacitance measurements of the same surface using the ‘O-ring’ (●) are also consistent with theoretical expectations at frequencies above 1 Hz, as described by equation (A.6), they diverge exponentially with decreasing frequency from the expected constant value at low frequencies given by equation (A.3). This divergence could be attributed to the impedance network formed at the contact between the O-ring and the surface (a thin film of solution with a high impedance is formed between the O-ring and the surface).

These results show that experimental chambers that employ gaskets of rectilinear cross section to isolate and seal the active surface areas of a flat surface are better suited to characterizing the electrical and geometrical properties of other molecular layers attached to these surfaces.

The principles outlined above for the insulating silicon dioxide layer can be extended to the characterization of a sandwich of electrically homogeneous layers with distinctively different conductive and capacitive properties. In this instance, the total impedance of the system is given by the Maxwell–Wagner model, which for a planar system is given by

$$Z(\omega) = \frac{1}{G} + \sum_{i=1}^N \frac{1}{G_i + j\omega C_i}, \quad (\text{A.7})$$

where G_i and C_i are the conductance and capacitance, respectively, of the i th layer in the sandwich. Note that equation (A.7) simplifies to equation (A.1) for $N = 1$, $G_1 = 0$ and $C_1 = C$. Inphaze software was used to fit the Maxwell–Wagner model to the impedance data. The capacitances of the layers and the known dielectric properties of the layers were then used to estimate layer thicknesses via equation (A.4).

References

- Aich P, Labiuk S L, Tari L W, Delbaere L J T, Roesler W J, Falk K J, Steer R P and Lee J S 1999 M-DNA: a complex between divalent metal ions and DNA which behaves as a molecular wire *J. Mol. Biol.* **294** 477–85
- Arkin M R, Stemp E D A, Holmlin R E, Baron J K, Hormann A, Olson E J C and Barbara P F 1996 Rates of DNA-mediated electron transfer between metallointercalators *Science* **273** 475–80
- Ashcroft R G, Coster H G L and Smith J R 1977 The molecular organisation of bimolecular lipid membranes. The effect of benzyl alcohol on the structure *Biochim. Biophys. Acta.* **469** 13–22
- Ashcroft R G, Coster H G L, Laver D R and Smith J R 1983 The effects of cholesterol inclusion on the molecular organisation of bimolecular lipid membrane *Biochim. Biophys. Acta.* **730** 231–8
- Bhalla V, Bajpai R P and Bharadwaj L M 2003 DNA electronics *EMBO Rep.* **4**, 5 442–5
- Böcking T 2003 Organic and biofunctional layers on silicon *PhD Thesis* University of New South Wales, Australia
- Böcking T, James M, Coster H G L, Barrow K D and Chilcott T C 2004 Structural characterization of organic multilayers on silicon (1 1 1) formed by immobilization of molecular films on functionalized Si-C linked monolayers *Langmuir* **20** (21) 9227–35
- Chilcott T C, Wong E L S, Böcking T and Coster H G L 2007 Probing biomimetic molecular structures on gold and silicon(1 1 1) with electrical impedance spectroscopy *IFMBE Proc. ICEBI 2007 (Graz, Austria)* ed H Scharfetter and R Merva vol 17 pp 308–11
- Coster H G L and Chilcott T C 1999 The characterisation of membranes and membrane surfaces using impedance spectroscopy *Surface Chemistry and Electrochemistry of Membranes* ed T S Sørensen (New York: Dekker) pp 749–92
- Coster H G L and Laver D R 1986 The effect of benzyl alcohol and cholesterol on the acyl chain order and alkane solubility of bimolecular phosphatidylcholine membranes *Biochim. Biophys. Acta.* **861** 406–12

- Lide D R (Editor-in-Chief) 2005 *CRC Handbook of Chemistry and Physics* (Boca Raton, FL: CRC Press)
- Dandliker P J, Holmlin R E and Barton J K 1997 Oxidative thymine dimer repair in the DNA helix *Science* **257** 1465–8
- Gaedt L, Chilcott T C, Chan M, Nantawisarakul T, Fane A G and Coster H G L 2002 Electrical impedance spectroscopy characterisation of conducting membranes, II Experimental *J. Membr. Sci.* **195** 169–80
- Gooding J J, Erokhin P and Hibbert D B 2000 Parameters important in tuning the response of monolayer enzyme electrodes fabricated using self-assembled monolayers of alkanethiols *Biosens. Bioelectron.* **15** 229–39
- Karolis C, Coster H G L and Chilcott T C 1999 Effects of cyclosporin-A on the molecular organisation of lecithin bilayers by electrical impedance spectroscopy *J. Med. Biol. Eng. Comput.* **37/2** 136–7
- Karolis C, Coster H G L, Chilcott T C and Barrow K H 1998 Differential effects of cholesterol and oxidised cholesterol in egg lecithin bilayers *Biochim. Biophys. Acta.* **1368** 247–25
- Kelley S O, Boon E M, Barton J K, Jackson N M and Hill M G 1999 *Nucleic Acids Res.* **27** 4830–7
- Levicky R, Herne T M, Tarlov M J and Satija S K 1998 Using self-assembly to control the structure of DNA monolayers on gold: a neutron reflectivity study *J. Am. Chem. Soc.* **120** 9787–92
- Lewis F D, Wu T F, Zhang Y F, Letsinger R L, Greenfield S R and Wasielewski M R 1997 Distance-dependent electron transfer in DNA hairpins *Science* **277** 673–6
- Linford M R and Chidsey C E D 1993 Alkyl monolayers covalently bonded to silicon surfaces *J. Am. Chem. Soc.* **115** 12631–2
- Plant A L, Gueguetchkeri M and Yap W 1994 Supported phospholipid/alkanethiol biomimetic membranes: insulating properties *Biophys. J.* **67** 1126–33
- Silin V I, Wieder H, Woodward J T, Valincius G, Offenhausser A and Plant L 2002 The role of surface free energy on the formation of hybrid bilayer membranes *J. Am. Chem. Soc.* **124** 14676–83
- Steel A B, Herne T M and Tarlov M J 1998 Electrochemical quantitation of DNA immobilized on gold *Anal. Chem.* **70** 4670–7
- Ulman A 1991 *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self-Assembly* (New York: Academic)
- Wang J C and Bates J B 1986 Impedance of metal-solid electrolyte interfaces *Solid State Ion.* **18–9** 224–8
- Wong E L S and Gooding J J 2003 Electronic detection of target nucleic acids by a 2,6-disulfonic acid anthraquinone intercalator *Anal. Chem.* **75** 3845–52
- Wong E L S, Chilcott T C, James M and Coster H G L 2006 Electrical impedance spectroscopy characterizations of alkyl-functionalized silicon (1 1 1) *Biophys. Rev. Lett.* **1, 3** 301–8
- Wong E L S, James M, Chilcott T C and Coster H G L 2007 Characterisation of alkyl-functionalised Si(1 1 1) using reflectometry and AC impedance spectroscopy *Surf. Sci.* **601** 5740–3
- Yu H-Z, Morin S, Wayner D D M, Allongue P and de Villeneuve C H 2000 Molecularly tunable ‘organic capacitors’ at silicon/aqueous electrolyte interfaces *J. Phys. Chem. B* **104** 11157–61
- Zimmerman U, Ashcroft R G, Coster H G L and Smith J R 1977 The molecular organisation on bimolecular membranes. The effect of KCl on the location of indoleacetic acid in the membrane *Biochim. Biophys. Acta.* **469** 23–32