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Final LDRD Report for the Project Entitled: Biosensors based on the Electrical Impedance of Tethered Lipid Bilayers on Planar Electrodes

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"Biosensors based on the Electrical Impedance of Tethered Lipid Bilayers on Planar Electrodes."

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Abstract

Impedance based, planar chemical microsensors are the easiest sensors to integrate with electronics. The goal of this work is a several order of magnitude increase in the sensitivity of this sensor type. The basic idea is to mimic biological chemical sensors that rely on changes in ion transport across very thin organic membranes (supported Bilayer Membranes: sBLMs) for the sensing. To improve the durability of bilayers we show how they can be supported on planar metal electrodes. The large increase in sensitivity over polyelectrolytes will come from molecular recognition elements like antibodies that bind the analyte molecule. The molecular recognition sites can be tied to the lipid bilayer capacitor membrane and a number of mechanisms can be used to modulate the impedance of the lipid bilayers. These include coupled ion channels, pore modification and double layer capacitance modification by the analyte molecule. The planar geometry of our electrodes allows us to create arrays of sensors on the same chip, which we are calling the "Lipid Chip".

Acknowledgments

This project benefited from the contributions of a number of Sandia Staff and students from UNM. Jeff Brinker worked with us on the sol-gel scaffold concept and his students and postdocs fabricated many materials: Dhaval Doshi worked on the photoresist seals on sol-gels, Darren Dunphy made some of the first sol-gel scaffolds on the lipid chips along with Hongyou Fan. Darryl Sasaki (1141) helped with the lipid vesicle and bilayer formation concepts, Jeb Flemming fabricated lipid chips after Stephen Bello left Sandia, Liz Patrick and Bridgette Ray (UNM students) worked on vesicle formation and lipid chip testing.

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Contents

Acronyms and Abbreviations

AC	alternating current
ASIC	application specific integrated circuits
BLM	black lipid membrane, or bilayer lipid membrane.
CVI	commerially valuable information
DC	Direct current
Hz	Hertz
LDRD	Laboratory-Directed Research and Development Program
LB	lipid bilayer
mV	millivolt
ppm	parts per million
sBLM	supported bilayer membrane
V	Volt

Introduction

Researchers have been trying for years to mimic the exquisite sensitivity and selectivity of biological cell-based chemical sensing. There are a number of reports of single molecule detection in artificial devices that exploit biological ion channels embedded in unsupported lipid bilayers [1,2]. Unsupported means that the very thin (5 nm) bilayers have liquid solutions on both sides and have a seal against ion transport at the edges of a hole in the partition that separates the two aqueous solutions. These systems are often called "black lipid membranes" or "Bilayer Lipid Membranes" (BLMs) [1,2] and their importance as the basic structure of cell walls has been known for 60-70 years. There is a vast literature on experiments done with these BLMs, including the developments in electronics that enables the time resolved recording of the current through single ion channels (often called "patch clamp amplifiers"). Erwin Neher and Bert Sakmann got the Nobel Prize in 1991 for inventing these techniques.

The BLMs require considerable skill to form properly across the hole in the partition and are notoriously fragile; they are basically like soap bubbles. In spite of the interesting data that can be obtained on ion channels when the BLM is intact, they are so unreliable that no one has produced a usable biosensor based on a BLM. Workers for many years have tried many techniques for supporting the lipid bilayer on a solid surface to make a rugged and reliable biosensor. In order to understand the sensor signals and also to characterize coated metal electrodes, a little background in impedance spectroscopy and the definition of the various physical structures that can be represented as simple capacitors and resistors is needed.

Impedance Spectroscopy of Coated Metal Electrodes

Advances in electronics have made the acquisition of frequency dependent electrical impedance much easier and quicker than in the past. With our Solartron (1260/1296) instrument we can obtain spectra over six or seven decades in frequency and some 13 decades in impedance automatically without the tedious corrections that were once needed. In a complicated system like a lipid bilayer supported on a metal electrode, one needs the full spectrum to characterize the system. A good textbook on the details of impedance analysis in electrochemical systems is by Bard and Faulkner [3]. Although there are many possible complexities, the analysis of the supported lipid bilayers can be broken down into a fairly simple lumped circuit which has the elements needed to understand the system. Fig. 1 shows an impedance spectrum of a bare Pt electrode of 500 microns in diameter and the same electrode coated with a lipid bilayer. The fabrication of the electrode array (called the lipid chip) and the formation of the bilayer will be discussed in the experimental section. In its simplest form the impedance spectra consists of a simultaneous measurement of the impedance, Z, in ohms, and the phase in degrees. For the bare electrode at the higher frequencies, $> 10^3$ Hz, the impedance comes

from the resistance of the ions in the aqueous solution, in this case 10 mM Tris buffer. At lower frequencies the ions pile up on the Pt surface giving what is known as the double layer capacitance. The phase in that region shows that it is capacitive (-90 degrees) as opposed to the zero degrees for a resistor. The equivalent circuit for this structure is given in Fig. 2 as a capacitor in series with the resistor from the ionic solution. In this kind of plot (called a Bode plot) a capacitor gives a spectrum which closely follows the formula:
$Z = (2\pi fCs)^{-1}$, where f is the frequency in Hz, and Cs is the double layer capacitance in farads. From the impedance at 10 Hz, we see that Z is $7x10^5$ ohms, and using the formula Cs is $2.3x10^{-8}$ farads. The area of the 500 micron dot is $2x10^{-3}$ cm^2 , so the double layer capacitance is 11 microfarads/cm^2 , which is a typical value from the literature. The upper curve in Fig. 1 shows what happens when a lipid bilayer is spread across the electrode. The bilayer has a well defined capacitance which is about 10 times smaller than a double layer capacitance. This is because the bilayer acts like a dielectric that the ions in solution can not penetrate and its thickness is about 5 nm. Different lipids with longer or shorter tails will have somewhat different capacitances. That capacitance can be extracted from the data between 10^4 and 10^5 Hz in Fig. 1. The equivalent circuit model in Fig. 2 shows that the two capacitors are in series and they are linked by a resistance, Rm, which can be seen in Fig. 1 as being about $5x10^4$ ohms. This is a very important parameter because it tells us how easily ions can leak through the bilayer (or around the edges). For a good sensor the value of Rm should be as high as possible. In order to normalize results that we and others have reported for membrane resistance, we will report the values in terms of the conductance, Gm, per area. In this case, Gm is about 0.01 S/cm^2 . The solid lines in Fig. 1 show mathematical fits to the circuits shown in Fig. 2; although it is fairly easy to estimate the values as we did above from the different regions of the frequency spectrum where one of the four elements dominates the value of Z. The value of the phase in degrees at the particular frequency tells you if Z is mostly from a capacitor (-90) or a resistor (0).

Summary of Prior Work in Supported LBs and Ion Channel Sensors

A recent example by Cornell, et al. [4,5,6] used extremely elegant chemistry of the lipids to form tethers to a gold electrode. They have since formed a company (see http://www.ambri.com/) to exploit their advances and sell biosensors of various kinds. No one outside this Australian group has published a repetition of this work and anecdotal evidence from other workers is that the chemistry is very difficult to perform (to get the lipid tethers). No one has questioned that they have done what they say, but most workers, including our work at Sandia, are trying to find a simpler means to stabilize LBs. They report Gm values of 1 to 10 microS/cm^2 for sBLMs without ion channels. Values of Gm in this range are too high to observe the current fluctuations of single ion channels, so their biosensors are made with multiple ion channels. The impedances in the correct frequency range are used for the sensor measurement (in Fig. 1 it would be about 10^3 Hz).

One of pioneers in BLMs, H. T. Tien, invented a technique for supporting LBs on the end of a silver wire. It has to be freshly cleaved in solution [7] to work and they report low

values of Gm, 0.1 to 1 microS/cm^2 , similar to BLMs. However they report no single ion channel data, and the present authors' are suspicious that their measurement technique is not reading the true Gm. They report no impedance spectroscopy and do not say what frequency they used for their measurement. In fact, since they say they are using a pico ammeter, we suspect that they are in fact measuring the double layer charging current which will give a much larger impedance (in Fig. 1 this would be Z at low frequency). Other workers have tried this technique, but it has not found wide acceptance because of fabrication difficulties.

The Heath group at UCLA [8] used a micromachined pore (50 to 200 micron sizes) and painted a BLM with voltage gated ion channels in it. They discuss the problem of getting high Gm values with supported LBs and propose this method as being more reliable and as a path for making micromachined arrays of ion channel devices. They report Gm values below 0.6 microS/cm^2 , which is good enough to see the currents from single channels. They don't give information about the longevity of their BLMs, but they are probably not better than ones made on perforated partitions [1,2].

A German group from Munster [9] used 9 different methods for forming sBLMs on metal electrodes. They did not use tethering lipids (which are hard to come by), but commercially available self-assembled monolayers (SAMs) and some other surface preparation techniques. They used impedance spectral analysis, but unfortunately their instrument was not capable of measuring high impedances at frequencies below 1 Hz. Therefore they did not get accurate Gm values for their films, but state that Z was probably greater than 10^6 ohms. For their electrode area, this translates to a Gm of less than 8 microS/cm2, which is in the region of other reports for sBLMs. If the Gm was much higher (as in Fig. 1) it would be seen at the higher frequencies they use, so that sets an upper limit on their Gm values. They did not attempt to observe single ion channel behavior, but they did show the activity of multiple gramicidin ion channels.

Experimental Procedures and Results

The Lipid Chip

Our goal in designing a liquid phase biosensor was to create a device that is easy to test and provides a platform that could be used in field applications. A picture of our design is shown in Fig. 3; we called it the "lipid chip". It is a planar array of metal electrodes that could be any metal; we chose Pt and Au because of the vast literature on aqueous phase electrochemical experiments that have been published on thin films of these metals. It has an 8 electrode array with different sized working electrodes, and large counter and reference electrodes. The device is fabricated on Pyrex substrates with 10, 50, 100, 500, and 1000 micron diameter platinum electrodes. The larger working electrodes can be seen in Fig. 3 as round dots and the counters are long strips. The large pads at the two ends are for making electrical contact with the electrodes. The "traces" or leads connecting the pads with the electrodes must be insulated from contact with the ions in the aqueous solution. This was accomplished in the CSRL by patterning a layer of insulating PECVD silicon nitride. An "O" ring seal prevents the liquid samples from reaching the electrode read-out pads, which are contacted with pogo pin spring loaded connectors. Each chip can then be removed for further processing steps on the electrodes

and quickly retested without encapsulation or wire-bonding. The fixture in Fig. 3 shows a flow through cell for quickly exchanging any liquid mixtures contacting the electrodes with a peristaltic pump and valves for switching sample bottles (not shown). The different electrodes can be contacted by plugging into the SMA connectors around the edges of the fixtures. The cables are shielded for low noise in observing high values of impedance.

Our improved approach differs from the electrochemical cells used in Refs [4,5,6,7,9] in its planar structure and rugged wiring scheme. Other workers generally use a planar electrode clamped to the bottom of a cylinder with an "O" ring contacting part of the electrode area. The cylinder (or cell) contains the aqueous solution, the counter electrode and perhaps a reference electrode. Solutions have to be changed by removing and rinsing the cylinder and pouring in a new solution. The size of the "O" rings prevents this kind of apparatus from having electrode areas smaller than about 0.1 cm^2 (the lipid chip has some down to $8x10^{-7}$ cm^2 on the 10 micron diameter electrode), and we also get simultaneous measurement on several different size electrodes without changing solution and from the same preparation of LB films. We had hoped that the smaller areas would give us more perfect LB films with low values of Gm.

A more detailed drawing of the lipid chip fabrication is shown in Fig. 4. The insulating layer did give us some problems with ionic leakage. Fig. 5 shows the impedance spectra of several of the Pt dots on a single lipid chip. On this good chip, the double layer capacitances calculated from the lower frequency data match the areas of the electrodes. The very low frequency roll-off in Z for the smallest electrodes is due to leakage to the pogo pins (10^{10} ohms is very high and would be good enough to observe single channel currents if we ever got Rm to be that high). Chips that had problems with leakage in the insulation of the traces would show roll-off at lower frequencies followed by lower Z with a capacitive phase at still lower frequencies. This is caused by leakage of ions through pin-holes in the insulator and charging of the trace metal; in effect creating a larger area electrode, but very confusing if you are trying to characterize an LB film. We always checked our lipid chips for perfect insulation as shown in Fig. 5 before using them in experiments. We also checked the cleanliness of the Pt on the electrodes. Galvanic cleaning was performed using the SI 1260 and 1287 Solartron Impedance/Gain-phase analyzer (Solartron, Fambrough). A solution of 0.5 M H_2SO_4 was applied to electrochemically clean the electrodes prior to applying the lipid membranes. After cleaning, cyclic voltammetry was performed using the SI 1260 and 1287 Solartron Impedance/Gain-phase analyzer (Solartron, Fambrough). A 5 mM solution of the redox couple $K_3[Fe(CN)_6]/K_4[Fe(CN)_6$ (1:1) was used to verify the cleanliness of the electrodes and to provide a baseline.

Formation of Lipid Bilayer films on Electrodes

Synthetic bilayers were created from vesicles of dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) (Avanti Polar Lipids, Inc., Alabaster), cholesterol (Sigma, St. Louis), dihexadecyldimethylammonium bromide (DHADAB) (Sigma), and gramicidin (Sigma). Vesicles were sized by extrusion through 0.3 mm polycarbonate filters. We usually followed the receipes that can be found on the webpage of Avanti Lipids (http://www.avantilipids.com/) and some receipes found in ref. 9. The LBs formed on the bare metal shown in Fig. 1 had low values of Rm (high values of Gm) and we tried several other structures to try to improve our Gm values.

Lipid Bilayers on Sol-gel Scaffolds

In discussions with Jeff Brinker (1846) , we had the idea that a templated sol-gel scaffold on the electrode might make for a rugged sBLM along with a reservoir of ions under the LB to support membrane-bound proteins (MBPs). Some MBPs are longer than the LB and require room for parts of the protein to extend out from both sides of the LB. A firmly tethered LB on the metal might not incorporate these kinds of MBPs. Fortunately, gramicidin does not require so much space. The templated films were coated over the whole lipid chip and annealed at various temperatures [10]. Fig. 6 shows how the templated film is formed on the lipid chip. It is roughly 500 nm thick and has open pores of 7 nm which are created when the surfactant is removed from the film during annealing (sometimes called "calcining" at 300˚C). The idea is that the LB film will be more robust when it is supported by the silica edges of the pores. The walls of the pores are roughly 2 nm, so care must be taken that the scaffold does not dissolve in water before being sealed with the LB film.

We had some concern that the sol-gel film would completely block the Pt surface and create a capacitor that would prevent us from observing ion channel behavior in an LB film (it would only take about 10 nm of non-porous silica out of the 500 nm film to completely block the Pt surface. Fig. 7 shows that this did not happen. Cyclic voltammograms (C-V) of the Pt surface with redox active ions, in this case ferro/ferricyanide, will show even a small amount of blockage of the Pt sites. A description of C-Vs is given in Ref. 3; the "duck" shapes of the current –voltage curves at different scan rates characterize how clean the Pt is. The sol-gel is apparently not restricting redox ions from the Pt catalytic sites, which is very good. Impedance spectra of the same electrodes show that ions can access almost all the Pt surface without going through any "solid" silica (which would slow them down, producing low frequency signatures in the impedance spectrum).

Fig. 8 shows how vesicle (or liposome) fusion is used to form the LB film on the sol-gel template. The dimensions are not to scale since the sol-gel film is about 500 nm and the LB about 5 nm thick. Also seen in the cartoon are the gramicidin ion channels which are incorporated into the vesicles and thus also in the LB film.

Fig. 9 shows the impedance spectroscopy of the final structure, including the composition of the vesicles used to form the LB film. The lipid mixture ratios were:

DMPC:cholesterol:DHADDAB (45:46:9) mol% and DMPC:cholesterol:DHADDAB: gramicidin (45:46:9:1) mol% for the MBP vesicles.

There are a number of interesting features to this spectrum. First, comparing the Z and the phase about 10^3 Hz shows a capacitance of the LB film about 10x smaller than the double layer capacitance (at 0.4 microF/cm^2 it matches literature values for LB films). We have made other films with much lower capacitance at that frequency which indicates multiple layers of lipids: not good for sensors since ion channels are not functional in multilayers. So this is the first indication that a good LB has formed. Second is the value of Z where the phase tells us that ions are leaking through the LB film and charging the double layer. At very low frequencies like 0.1 Hz, the capacitance (and Z) approaches that of the bare electrode, which is another good check on the structure. Another very important feature in Fig. 9 is the response of the sensor to different ions in solution. Gramicidin ion channels are well known for transporting Cs^+ ions in great preference over larger or doubly charged ions like Ca^{++}. The middle data set shows the drop in Rm for 5 mM Cs^+ ions compared to the background Tris buffer solution. This is very good evidence that the gramicidin channels are functional and spanning the LB properly. We believe that this is the first demonstration of an LB film supported on a mesoporous templated sol-gel scaffold with functional ion channels.

Rough values for the LB film capacitance, the Rm values and the double layer capacitance can be extracted from the spectrum in different frequency regions where each element dominates, as we have shown above. More quantitative values for the lumped circuit elements in an equivalent circuit model can be extracted as shown in Fig. 2 from analytical models.

The lipid chip as a biosensor and the importance of Rm

The value of Rm seen in Fig. 9 is about 10^7 ohms (Gm=50 microS/cm^2) and is good enough to observe functionality of the gramicidin ion channels. In effect this is a biosensor for Cs^+ ions. To create biosensors for other analytes (molecules) there are methods for labeling the gramicidin protein to obtain specific binding of the analyte. These binding schemes result in the blocking of the gramicidin channel when the analyte is present. In this way the impedance at certain frequencies (in Fig. 9 this would be in the 10 to 100 Hz range) can be correlated with the concentration of the analyte. For example we could develop a calibration curve for Cs^+ ion concentration by taking data at different concentrations and plotting it against Z at a fixed frequency. A larger value of Rm would allow us to go to lower Cs^+ ion concentrations and get reliable values. In Fig 9, the Rm is being dominated by non-selective leakage of ions through the LB film through defects or the edges of the film; in this field they refer to this phenomenon as “not having a good seal”. The literature reports cited above often claim values of Gm a factor of 10 lower and the unsupported BLMs more than 100 lower than in Fig. 9. The conductance fluctuations of a single ion channel would be obscured in Fig. 9 by the high value of Gm. We spent a considerable amount of effort in this program seeking methods to get higher values of Rm (lower Gm), in addition to durability of the structures.

Hybrid bilayer films

The method we found that yielded the most reliable high values of Rm and durable structures we call the "hybrid" bilayer. It is not formed by vesicle fusion. We created hybrid bilayers composed of octadecyltrichlorosilane (OTS) (Sigma) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (Sigma). OTS was covalently attached to the substrate and acted as the lower leaf of the bilayer. Gramicidin channels (1 mol%) were incorporated into the lower OTS (99 mol%) leaf prior to applying the upper leaf. The upper leaf was applied using a micelle of POPC (99 mol%) and gramicidin (1 mol%), prepared as a solution of 40 mM solution of 1-O-n-octyl-b-D-glucopyranoside in 10 mM Tris buffer pH 7.4

We were concerned that the gramicidin in the lower OTS leaf would not remain functional because it might be immobilized. Fig. 10 shows the impedance spectra of a hybrid bilayer. The Rm value of the whole structure with gramicidin ion channels is seen in the top curve to be abut $4x10^6$ ohms (Gm= 30 microS/cm^2). The curve showing the response to 1 mM Cs^+ ions indicates that the ion channels are quite functional. A curve showing recovery of the Rm value after Cs^+ ions are removed shows good reversibility of the film. The solid lines through the data points are for fits to the analytical model shown in Fig. 2. The values for the fits are given in Fig. 11 In the hybrid bilayer, the capacitance of the OTS film is similar to the total membrane capacitance of the DMPC, cholesterol, DHDDMAB, gramicidin bilayer. This is attributed to the longer alkane chain of the OTS (C18) as compared to DMPC (C14). The addition of the POPC film on the OTS leaf decreases the capacitance of hybrid bilayer even further.

A further check of the functionality of the gramicidin ion channels is given in Fig. 12 where it is demonstrated that Ca^{++} ions do not go through the channels.

The durability of the hybrid is shown in Fig. 13 where the reversibility of the film was checked for 8 days. Another hybrid film was functional for three weeks. These durability tests are somewhat limited by our patience in tying up the cell for this long a time. We could remove the lipid chip from the cell and preserve it in buffer and check it at some later time, but this has not been done. The bilayer coated electrode must not be allowed to dry out or it will self-destruct.

Discussion and Conclusions

We have demonstrated a biosensor technology that has potential for being deployed in the field. The Lipid Chip is an array of electrodes that can be individually addressed. Its planar geometry in our flow injection cell means that sample volumes as little as 20 microliters can be analyzed by an array of electrodes (which could have different sensors on them). We did not anticipate how hard it would be to find the protocols (recipes) for reliably forming single LBs with low leakage conductance. Our search for better support

structures took longer than expected, so that the work of derivatizing ion channels to make selective sensors for a variety of analytes will have to be done in future programs.

The problem of the ionic sealing of the lipid bilayer

We are encouraged by our demonstration of an LB film supported on a mesoporous templated sol-gel scaffold with functional ion channels. Although our first attempts did not yield low Gm values, there is reason to hope that different processing steps will lower Gm. Dhaval Doshii of the Brinker group(1846) did try an interesting method of sealing the pores at the edges of the electrode. He incorporated photoresist with the sol-gel preforms. He then cured the photoresist everywhere except over the small electrode area. This photoresist was removed to open up the pores, but the pores at the edges would be sealed. We were not able to get lower Gm values on the first try with these lipid chips, but in further work the curing of the photoresist and calcining of the sol-gel should make better blocking of the pores.

We also tried the electrochemical cell method that was used by earlier workers, where the aqueous solution was contained inside the "O" ring and the LB seal occurs where the rubber "O" ring presses onto the chip. We were not able in a few tries to obtain the low Gm values reported by others [4,5,6,7,8] with this cell on either bare Au electrodes or ones with templated sol-gels. We were able to obtain functional LB films as seen from the impedance spectra, but with Gm values no lower than on the lipid chips.

There are a number of methods for improving Gm which should be tried in the future. These include using a "clean" box during cleaning and formation of the vesicles and the vesicle fusion to make sure dust particles are not causing defects in the films; preparing the surfaces to be purposely hydrophilic with surface modification techniques; and varying the lipid compositions with good temperature control during fusion.

Building Biosensors based on Ion Channels

We successfully fabricated sensors shown in Figs. 9, 10 and 12 with gramicidin ion channels. The channels were shown to be functional by introducing Cs^+ ions which preferentially move through the channels. In effect we made a biosensor for the concentration of Cs^+ ions. The structure will act as a biosensor if we can devise means to turn the channel currents on or off in the presence of the analyte of choice. In the time alloted to this study we were able to try one experiment with labeled gramicidin. Dave Wheeler (1746) derivatized the gramicidin protein with biotin. We created an LB hybrid film with these labeled gramicidins and showed that they preferentially conducted Cs^+ ions. Then we tried to interrupt the gramicidin ion channel by introducing a large protein, avidin, that binds strongly to biotin. We tried several concentrations of avidin, but were unsuccessful in interrupting the ion channel. Other workers [4] have reported success in interrupting the gramicidin ion channel with antibodies, so we feel that this should be successful in future work.

The problem of sample collection and sensitivity of single ion channels

There has been considerable discussion of the use of ion channel sensors as single molecule detectors. For sensors with electrical read-out, protein based ion channels have been deployed in artificial cell membranes like lipid bilayers. Optical microsystems use fluorescent tags that indicate occupation of a site by single analyte molecules. However, in order for these detectors to be useful in the real world for detecting very low concentrations of specific analytes, the molecules must find their way to the detector. This is not always easy, because the detector element, like an ion channel, has a receptor for the analyte molecule that is only 10 nm or less in diameter. Since the ion channels must have aqueous, ionic environments on both sides, the analyte molecule's diffusion constant will be less than 10^{-5} cm^2/sec. Experiments with these systems are typically made with high concentrations of analyte, milli to micro molar.[11] To be useful in many applications, it is desired to detect pico or lower molar concentrations in the liquid. Also in many applications, the analyte molecule to be detected is in the air, and a transfer from air to liquid phase is required. Present methods of air sampling for low concentrations of chemicals like explosives and bioagents involve noisy and power hungry collectors with mechanical parts for moving large volumes of air (think DustBuster). In nature the key to detection of extremely low concentrations of molecules in air seems to center around arrays of ion channel detectors that are close enough together, and close to the surface of pores in hair-like sturctures to sample single molecules. A discussion and simulations are given by Futrelle [12] and Berg [13].

This leads us to the conclusion that to make ultra-sensitive detectors we must master the art of fabricating sBLMs with single channels on electrodes and then create closely spaced sensor electrodes for arrays. Each channel will need the electronics for monitoring the opening and closing of the ion channel. We know that this can be done for charge sensitive pre-amps for single x-ray detection on an ASIC (application specific integrated circuit), because we have done it at Sandia for a minuature radiation detector and small spaced duplicates of these circuits can be made on a single Si wafer. The lipid chip is a good start on the array technology since the same photolithographic process we developed can be used to make dozens or 100s of small area electrodes and bring their leads to output bonding pads. The most difficult part will be the formation of the sBLM with an ion channel in it . We envision some form of automated pipetting onto our planar lipid chip array.

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Figure Captions

Fig. 1. Impedence spectra of a synthetic lipid bilayer on a bare Pt electrode that is 500 microns in diameter. Inset shows a sideview of the electrode on the lipid chip with insulating areas defining the metal.

Fig. 2. The equivalent circuit models used to analyze the impedance spectra on the lipid chip.

Fig. 3. The design of the lipid chip biosensor. The upper left has the cell that the chip is mounted in. Electrical contact is made by spring loaded pogo pins to avoid wire bonding. Solutions can be quickly changed over the chip by flow injection. Another version of the cell has an opening in the top over the chip so that solutions can be pipetted in and out.

Fig. 4. A more detailed side view of the lipid chip construction. Several versions were made that had different layers on top of the SiN in attempts to improve lipid sealing.

Fig. 5. A set of impedance spectra from a single lipid chip showing good insulation of the traces and the "O" ring which prevent the solution with ions from reaching any of the "dry" surfaces, including the traces and bonding pads. The lowest Z is for the 1000 micron electrode and in succession to high Z values are the 500, 100, 50 and 10 micron electrodes. The Z values conform to the areas of the electrodes, showing good definition.

Fig. 6. The lipid chip with a mesoporous sol-gel film to serve as a scaffold for the lipid bilayer. The sol-gel film is about 0.5 microns thick with pore dimensions of about 7 nm. Also shown is the flow cell with the lipid chip in place.

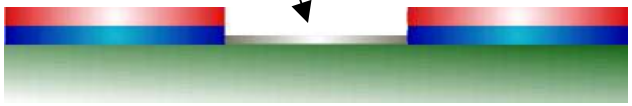
Fig. 7. Cyclic voltammograms of a lipid chip electrode with and without the mesoporous sol-gel film. The current profiles tell us that the Pt surface has not been poisoned or coated by solid silica. This means that a good ionic reservoir can be formed under a lipid bilayer.

Fig. 8. Cartoons showing the steps in using liposomes to form lipid bilayers on a mesoporous (templated) sol-gel film. The gramicidin ion channel proteins are also shown. The sizes are not to scale, since the LB is only about 4-5 nm thick compared to the sol-gel film which is 500 nm, and the diameter of the electrode, which is 500 microns.

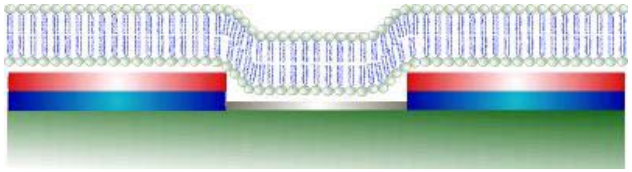
Fig. 9. The impedance spectra of a LB film supported on a mesoporous, templated sol-gel film. The labeled curves show the sol-gel film on the metal as the lowest in Z, the higher Z values with the LB film blocking ions, and the intermediate Z values due to excess conductance of Cs^+ ions. This shows that the ion channels are functional. The solid lines show a fit to the analytical equivalent circuit model.

Impedance Spectroscopy of Bilayer on Bare Substrate

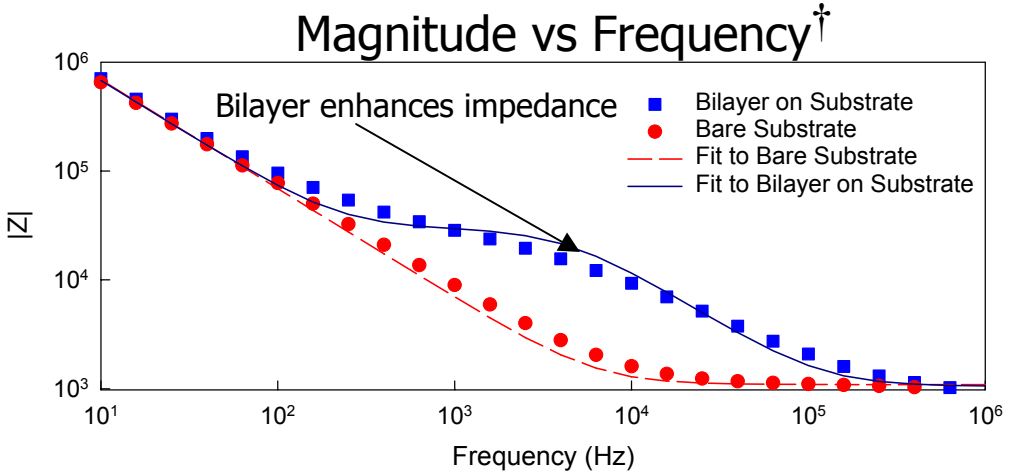
500 μm Electrode Diameter



Apply Lipid Bilayer



†Impedance measurements done in 0.1M KCL



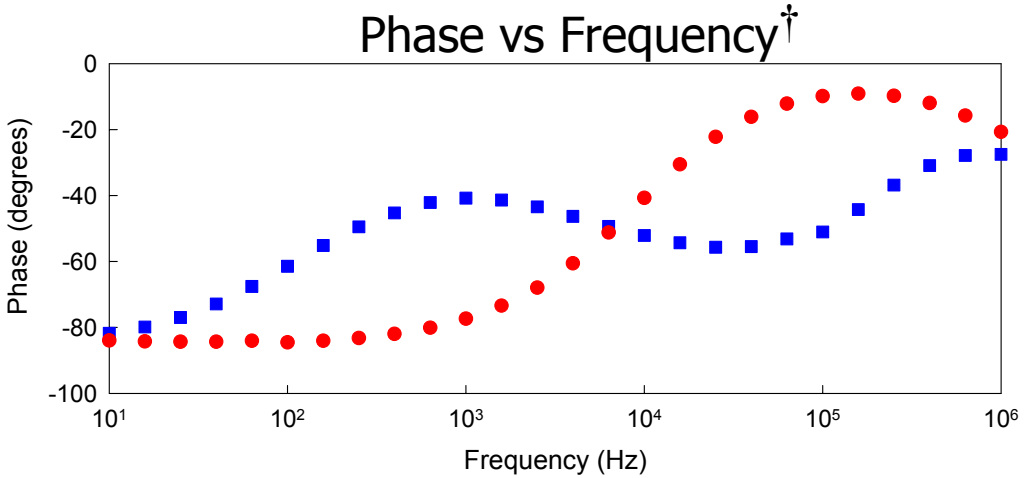
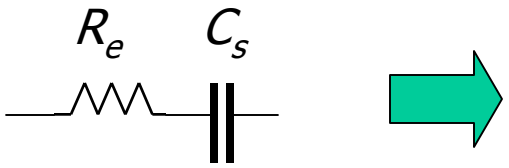


Fig. 1

Equivalent Circuit Models

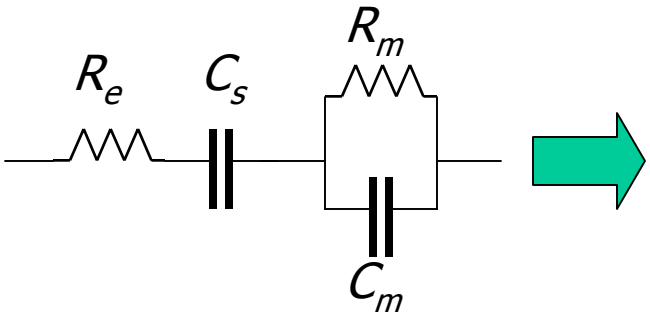
Lipid Chip with Permeable Film



$$Z_{es}(\omega) = R_e + \frac{1}{j\omega C_s}$$

C_s: interface capacitance
R_e: electrolyte resistance

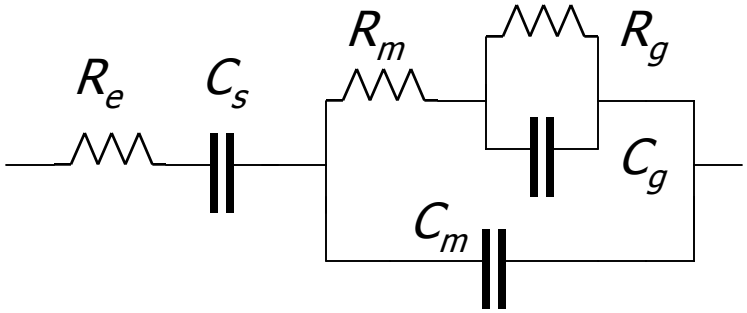
Bilayer on Permeable Film



$$Z_{esm}(\omega) = Z'_{es}(\omega) + \frac{R_m}{1 + j\omega C_m R_m}$$

R_m: membrane resistance
C_m: membrane capacitance

Bilayer + Gramicidin on Permeable Film



$$Z_{gesm}(\omega) = Z_{es}(\omega) + \left[\frac{\frac{R_g}{1 + j\omega R_g C_g} + R_m}{1 + j\omega C_m \left[\frac{R_g}{1 + j\omega R_g C_g} + R_m \right]} \right]$$

R_g: gramicidin resistance

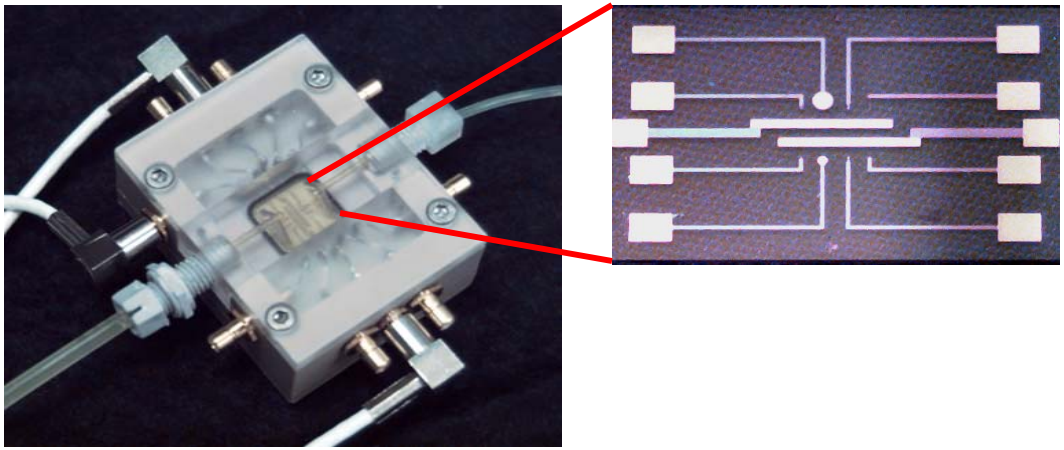
C_g: gramicidin capacitance

Fig. 2

Impedance Based Biosensor

Approach

Lipid Chip



- A modified channel in a lipid membrane will allow transport of ions across the membrane to the electrode surface upon analyte binding.
- Solutions and analytes can be delivered to surface of the device while impedance or current is simultaneously monitored.

Cross-Section of Lipid Chip

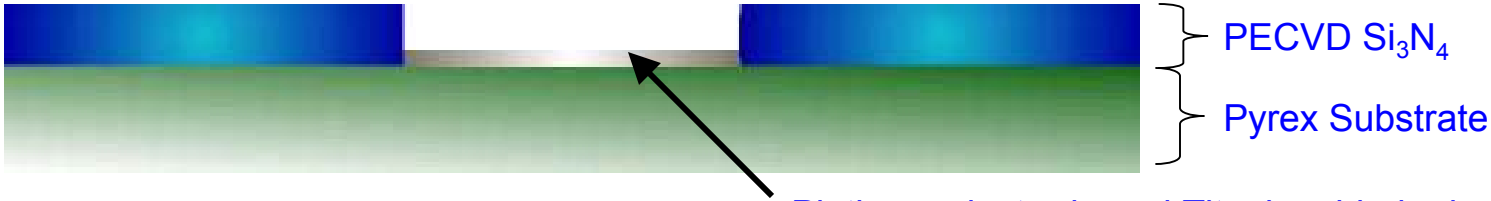


Fig. 3

Lipid Chip Cross Section

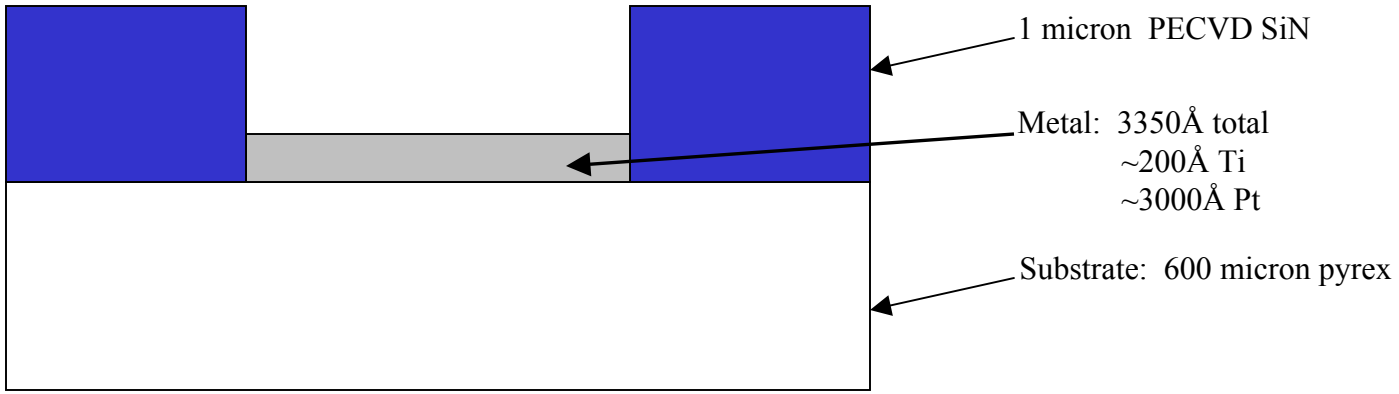


Fig. 4

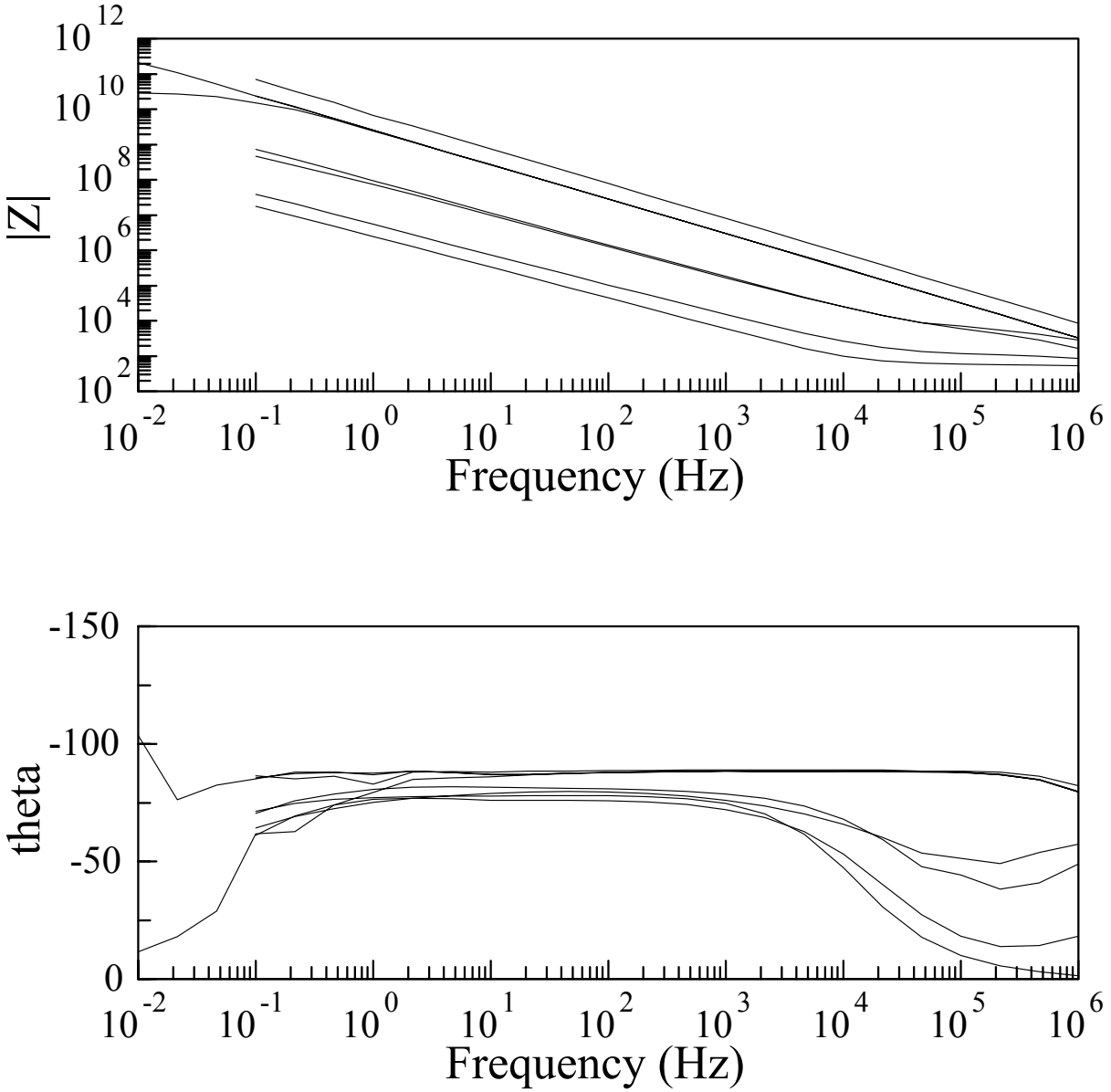
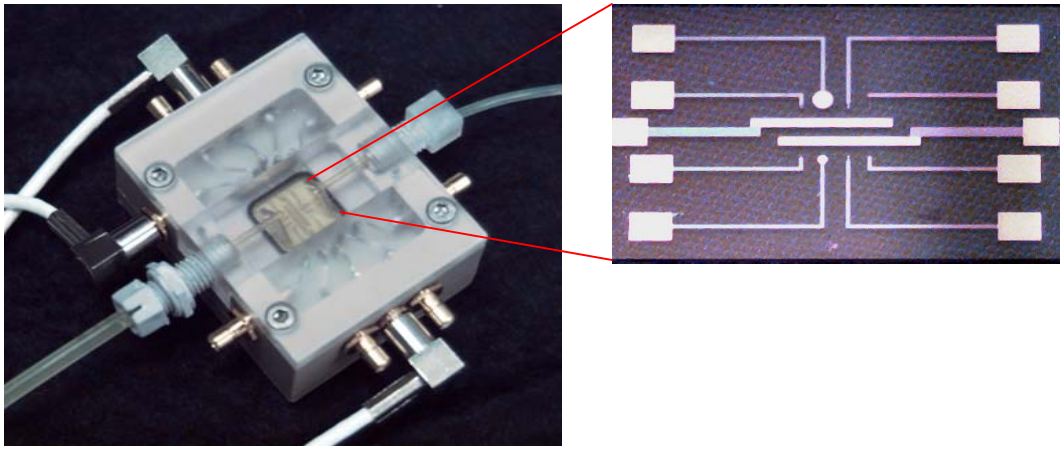

|Z|
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6}
Frequency (Hz)
theta
-150
-100
-50
0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6}
Frequency (Hz)

Fig. 5

Impedance Based Biosensor

for BW agent and toxin detection



Approach

- A synthetic channel in a lipid membrane allows transport of ions across the membrane to the electrode surface upon analyte binding.
- The membrane is deposited on top of a self-assembling sol gel matrix which serves as an ionic reservoir.

Cross-Section of Lipid Chip

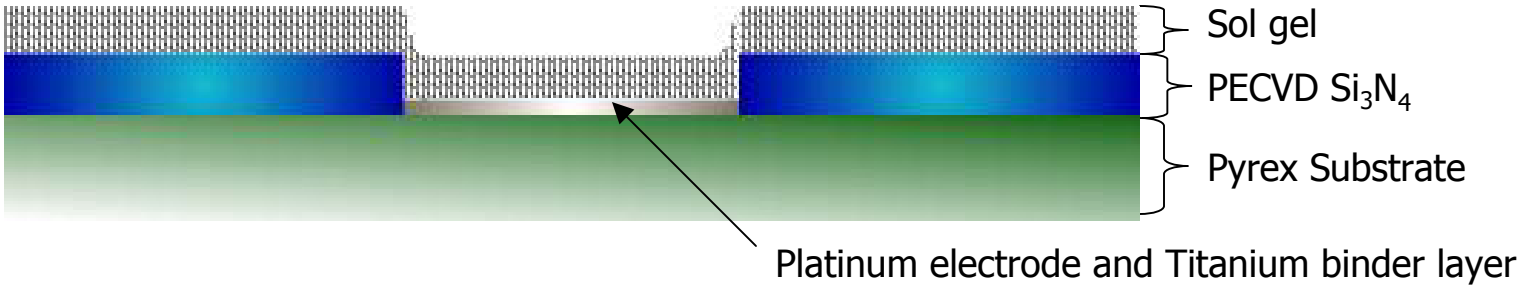
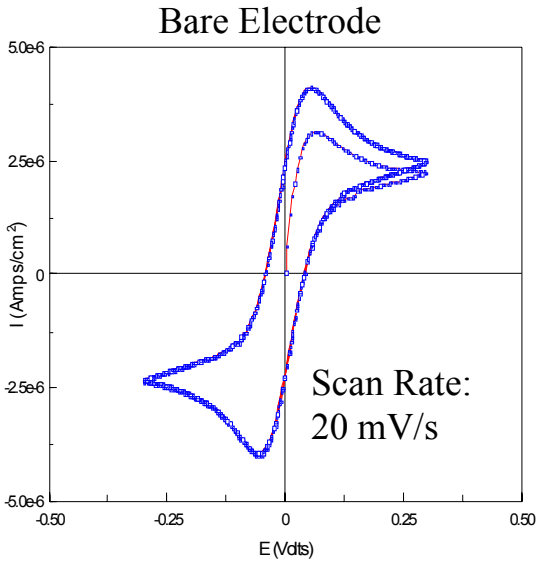
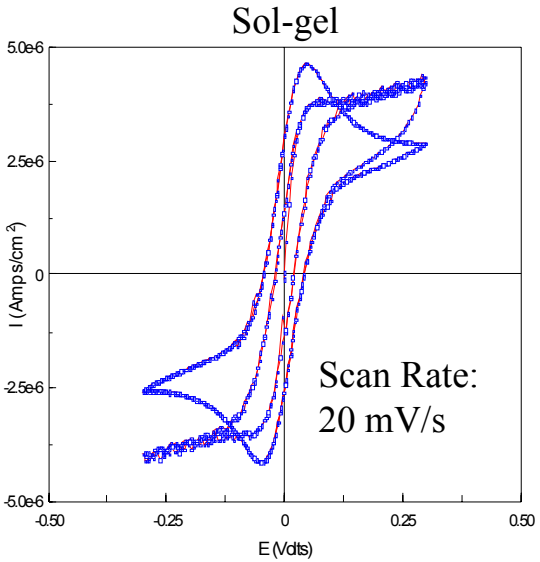
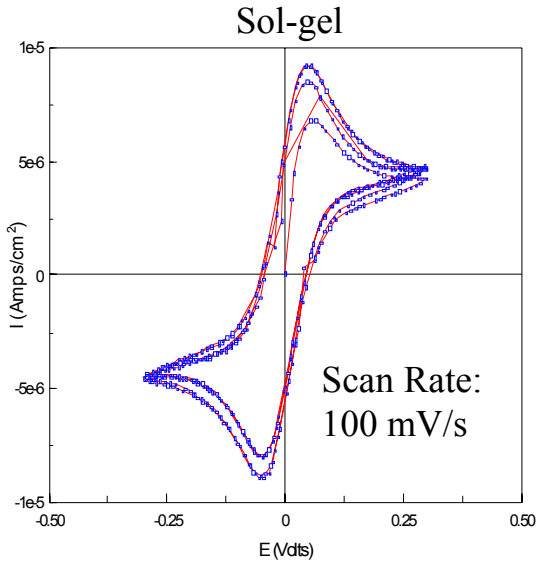


Fig. 6

Cyclic voltammograms of ferro/ferricyanide through sol gel matrix







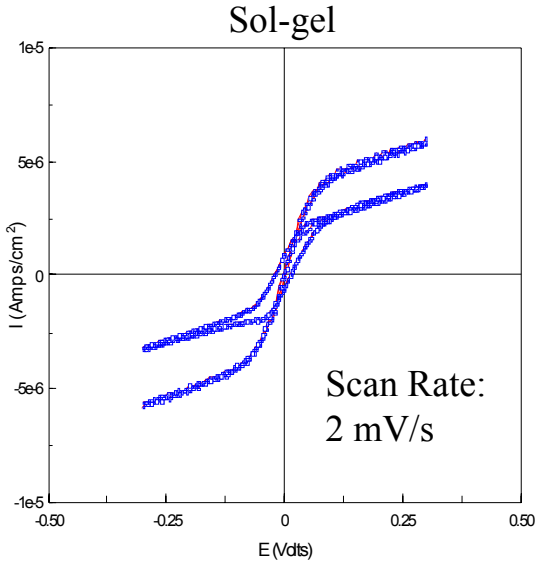
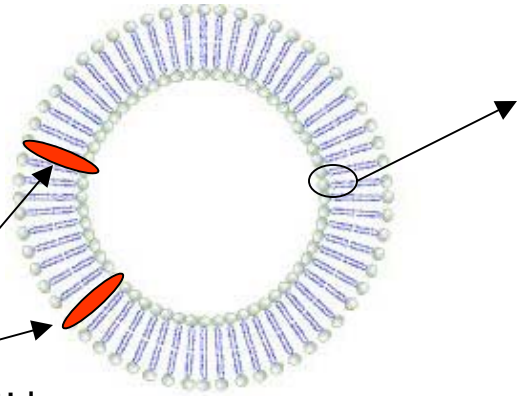
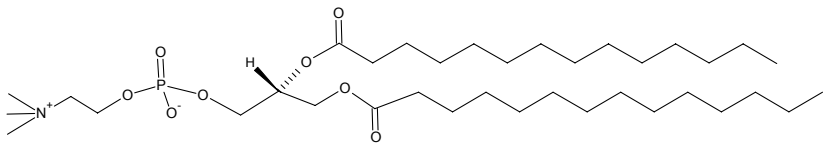


Fig. 7

Preparation of Lipid Bilayers using Liposomes



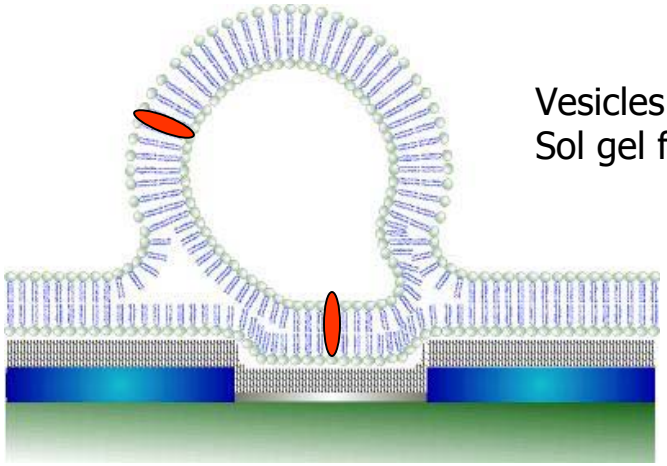
Channel polypeptides



1,2 Dimyristoyl-*sn*-glycero-3-phospocholine (DMPC)

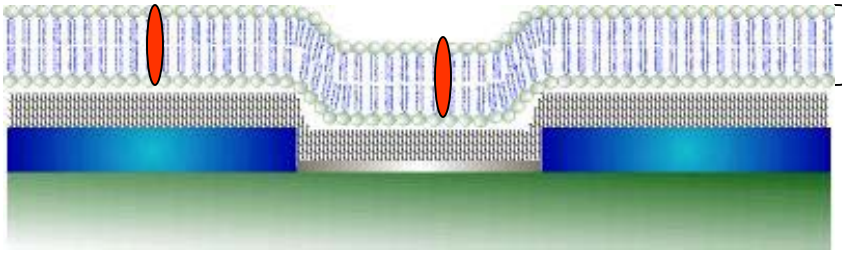
Vesicle Recipe

-DMPC
-cholesterol
-charged lipids
-channel polypeptides



Vesicles fuse to Sol gel film

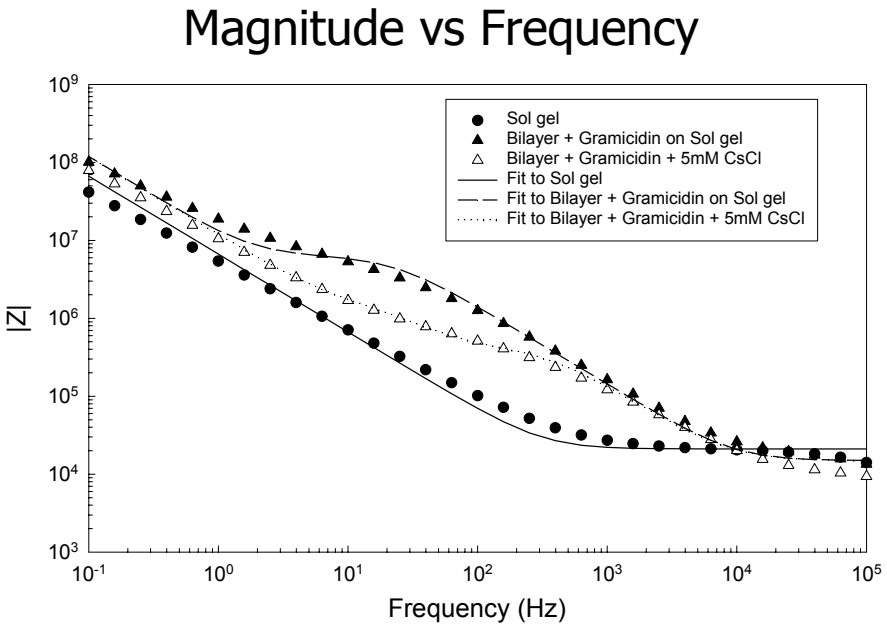
Lipid Bilayer on Sol gel

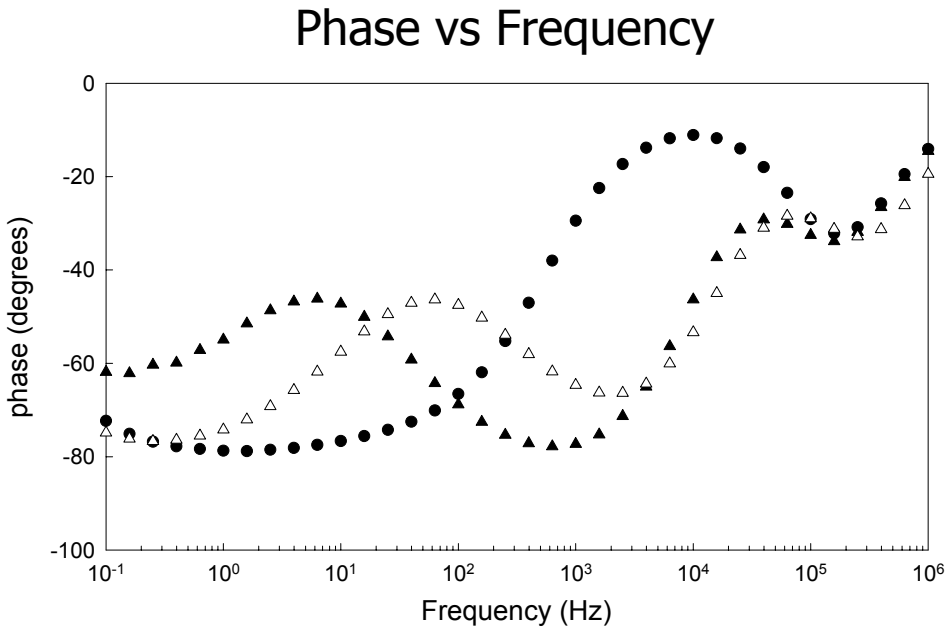


4nm

Fig. 8

Impedance Spectroscopy of DMPC, Cholesterol, and DHDDMAB Bilayer on Sol gel





Electrode Diameter: 500 μm
Buffer:10 mM Tris Buffer pH 7.4

DMPC: dimyristoyl-L-α-phosphatidylcholine
DHDDMAB: dihexadecyldimethylammonium bromide, $^{+}1$ charge

Fig. 9

Impedance Spectroscopy of Hybrid Lipid Bilayers

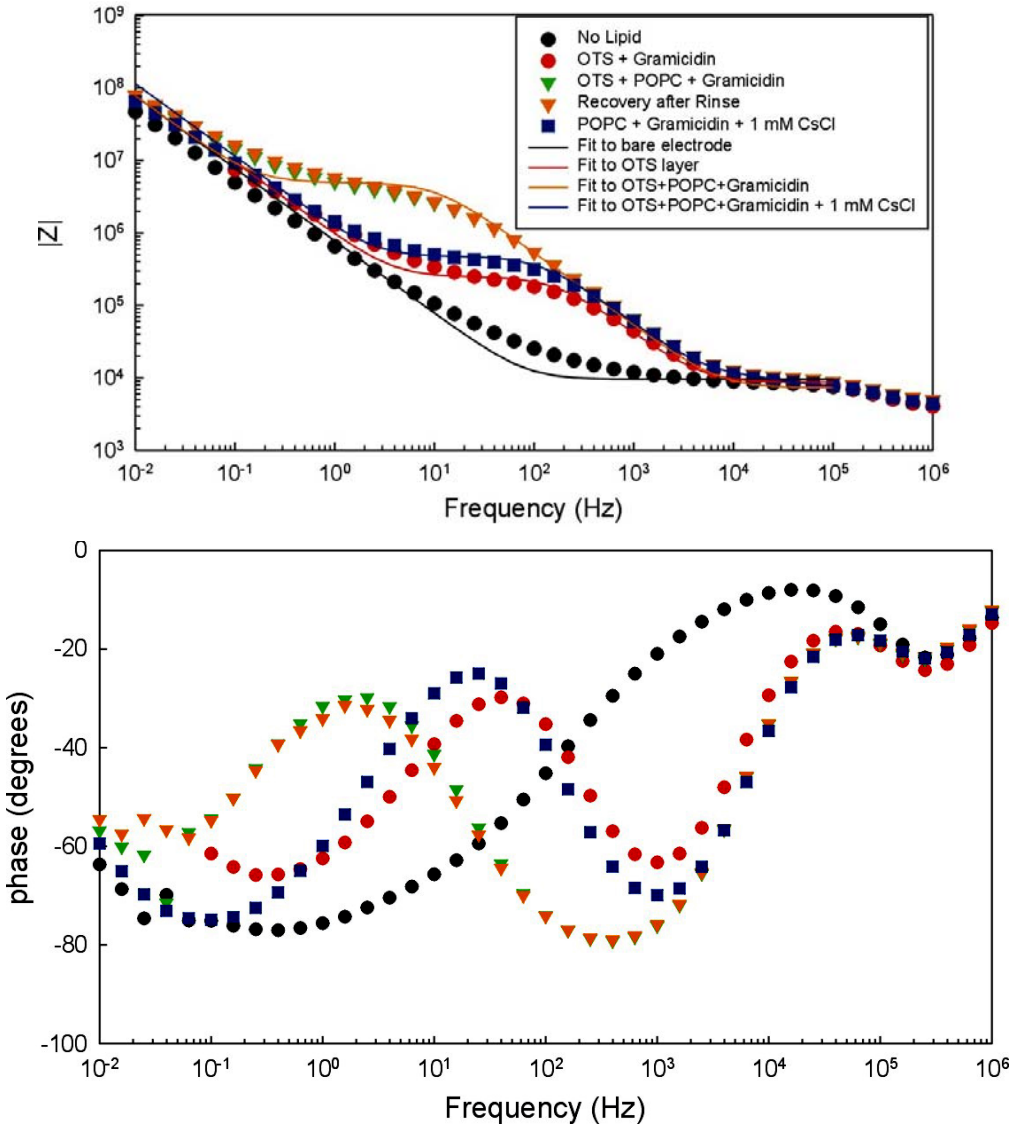


Fig. 10

Results of Fitting Procedures using the Equivalent Circuit Models

DMPC, Cholesterol, DHDDMAB, and Gramicidin Bilayer

	R_e Ω cm2	C_s μF cm^{-2}	R_m kΩ cm^2	C_m μF cm^{-2}	R_g Ω cm^2	C_g μF cm^{-2}
No bilayer	41.3	12.1	-	-	-	-
Bilayer	29.3	6.8	11.2	0.62	-	-
Bilayer + 5mM CsCl	29.3	6.8	0.82	0.66	1240.7	6.5
Bilayer + 10mM CsCl	29.3	6.8	0.55	1.03	397.9	22.4

OTS, POPC, and Gramicidin Hybrid Bilayer

	R_e Ω cm2	C_s μF cm^{-2}	R_o kΩ cm^2	C_o μF cm^{-2}	R_m kΩ cm^2	C_m μF cm^{-2}	R_g Ω cm^2	C_g μF cm^{-2}
No bilayer	75.2	25.8	-	-	-	-	-	-
OTS (lower leaf)	57.6	17.6	1.86	0.52	-	-	-	-
OTS + POPC	64.2	17.6	-	-	33.9	0.37	-	-
OTS + POPC + 1 mM CsCl	64.2	17.6	-	-	1.49	0.48	221.4	0

In the hybrid bilayer, the capacitance of the OTS film is similar to the total membrane capacitance of the DMPC, cholesterol, DHDDMAB, gramicidin bilayer. This is attributed to the longer alkane chain of the OTS (C18) as compared to DMPC (C14). The addition of the POPC film on the OTS leaf decreases the capacitance of hybrid bilayer even further.

Fig. 11

Selectivity of a Hybrid Bilayer for Cs^+ over Ca^{2+} Divalent Cations using Gramicidin Channels

Permeability of gramicidin to monovalent cations is in the order: $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$
In this study, gramicidin channels would not let $CaCl_2$ pass through. This illustrates the channel specificity for monovalent cations.

Electrode Diameter: 1000 um
Buffer:10 mM Tris Buffer pH 7.4

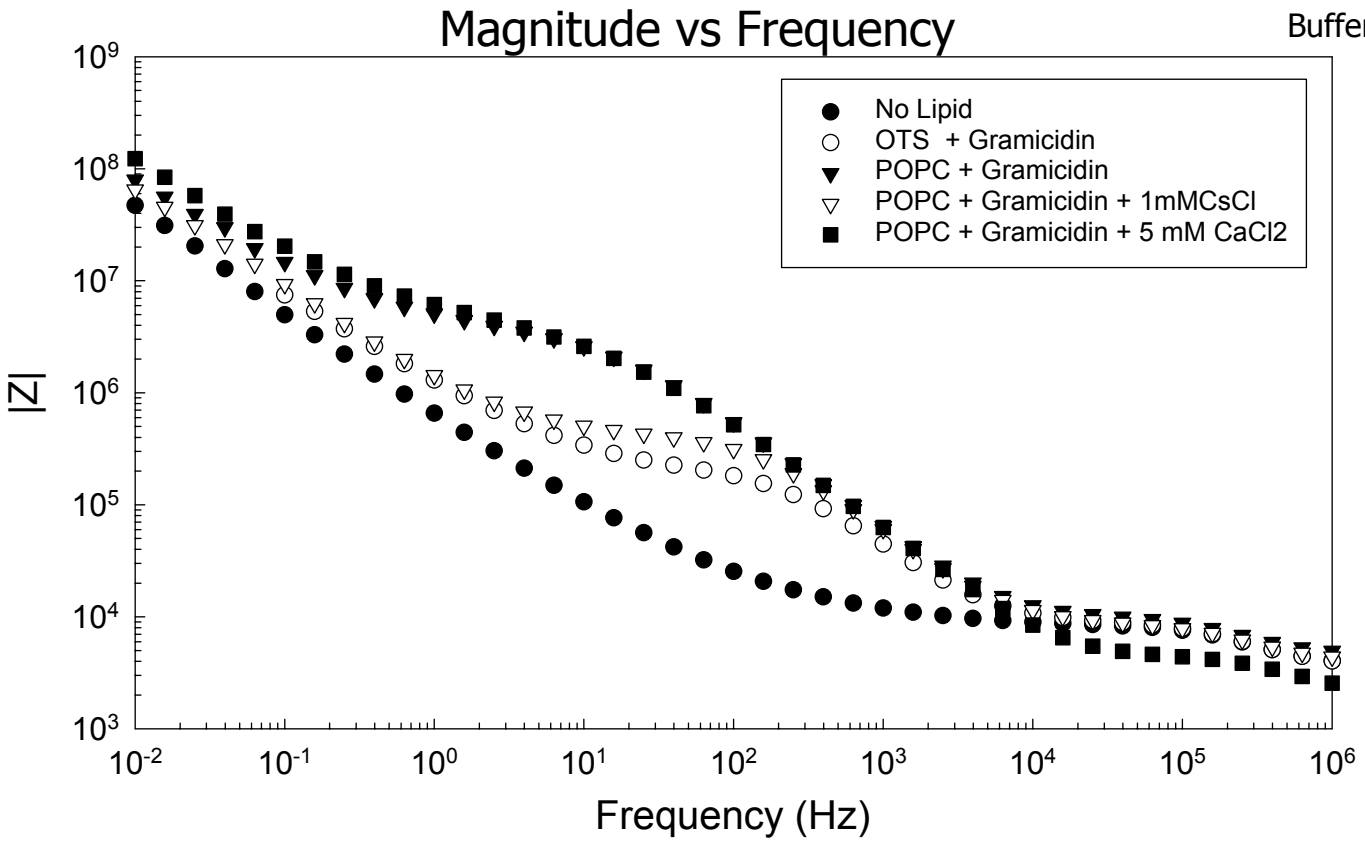


Fig. 12

Hybrid Bilayers Maintain Function for over a Week

Electrode Diameter: 1000 μm
Buffer:10 mM Tris Buffer pH 7.4

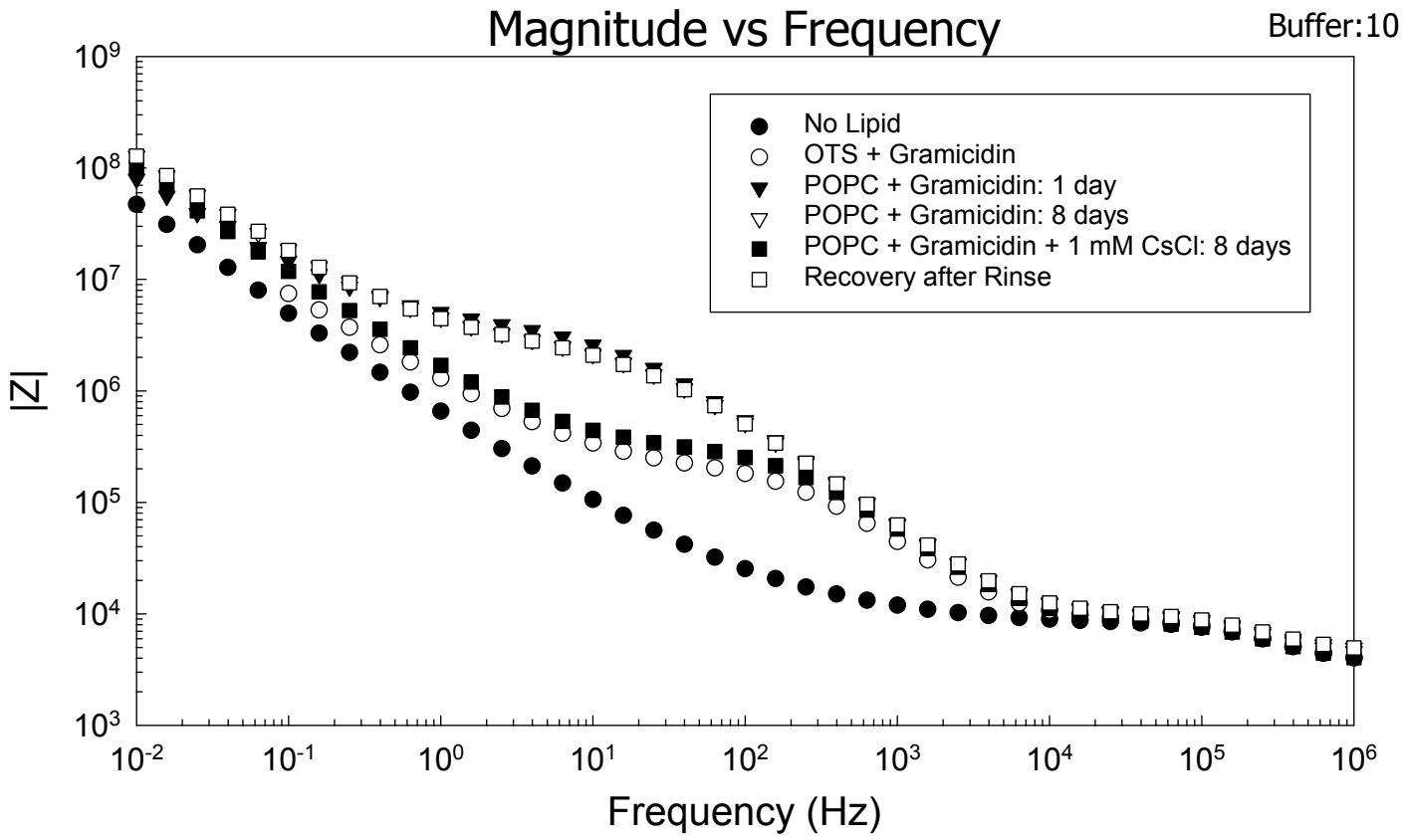


Fig. 13

Distribution:

10	MS 1425	R. C. Hughes, 1744
1	MS 9018	Central Technical Files, 8945-1
2	MS 0899	Technical Library, 9616
1	MS 0612	Review & Approval Desk, 9612
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