Suspended Polypyrrole Films Supporting Alamethicin
Reconstituted Bilayer Membranes

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Suspended Polypyrrole Films Supporting Alamethicin Reconstituted Bilayer Membranes

by

Robert Northcutt

Thesis submitted to the Faculty of the Virginia Commonwealth University in partial fulfillment of the requirements for the degree of

Masters of Science

in

Mechanical Engineering

Professor Vishnu-Baba Sundaresan, Chair
Professor James T. McLeskey
Professor Gary M. Atkinson

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Keywords: Conducting Polymer, Bilayer Membrane, Bioderived Membrane, Polypyrrole, DPhPC, Alamethicin, Biochemical Sensing

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Suspended Polypyrrole Films Supporting Alamethicin Reconstituted Bilayer Membranes

Robert Northcutt, M.S. Candidate
Virginia Commonwealth University, 2012
Advisor: Vishnu-Baba Sundaresan

ABSTRACT

This thesis presents a novel architecture for a sensing element fabricated from a conducting polymer and a bioderived membrane. The thin film device provides controlled, selective ion transport from a chemical concentration and produces measurable electrical signals, ion storage, and small scale actuation. A chemical gradient applied across a bioderived membrane generates ion flow through protein transporters in the presence of a gating signal. A conducting polymer undergoes ion ingress/egress in the presence of an electrical and chemical potential, which causes a change on the polymers conformal backbone. A ligand (or) voltage gated protein in the bioderived membrane results in ion transport through the bioderived membrane. Integrating the two electroactive materials provides a unique architecture which takes advantage of their similarities in ionic function to produce a device with controlled and selective ion transport. The chemoelectromechanical device is one that couples chemical, electrical, and mechanical potentials through number of ions, dielectric displacement, and strain.

The prototype consists of a stacked thin conducting polymer film and bioderived membrane which form three aqueous chambers of varying ionic concentrations. The top chamber contains an electrolytic solution, and the bottom chamber contains deionized water adjacent to the conducting polymer. The current that passes through a conducting polymer for an applied electrical signal is based on the level of doping/undoping and therefore can be used as a method of sensing protein function in the sensing element. This architecture results in a sensing element applicable in real time chemical sensors, volatile organic compound
detectors, and bioanalytical sensors.

The conducting polymer layer is formed from polypyrrole (PPy) doped with sodium dodecylbenzenesulfonate (NaDBS), and the bilayer lipid membrane is formed from 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) reconstituted with the protein alamethicin. The magnitude of current required to span a 175 µm pore was empirically found to be 326.5 A/cm² and is based on electrode condition, electrode surface area, pyrrole concentration, and electrical potential. A micron-scale pore through a silicon substrate is spanned by a thin PPy(DBS) layer, forming a bridge which supports the bioderived membrane. The bioderived membrane is reconstituted with alamethicin, a voltage-gated protein extracted from *trichoderma viride*.

Ion transport experiments were performed to characterize the PPy(DBS) layer and the bioderived membrane and are represented as electrical equivalents for subsequent analysis. The equivalent impedance of polypyrrole was calculated to be 1.7847 ± 0.1735 Ωcm² and capacitance was calculated to be 1.2673 ± 0.1823 µF/cm². The equivalent impedance of a bioderived membrane was calculated to be 1.654 ± 1.9894 MΩcm², capacitance was calculated to be 1.1221 ± 0.239 µF/cm², and alamethicin resistance was calculated to be 1.025 ± 0.7228 MΩcm². Thus, using impedance measurements in the conducting polymer layer, it is proposed that a scaled up sensing element can be fabricated using the suspended polypyrrole supported bioderived membrane.
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Virginia Commonwealth University
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Chapter 1

Literature Review

1.1 Conducting Polymers

Conducting polymers are organic polymers that exhibit varying magnitudes of electrical conductivity. This phenomena occurs when switching between operating in either a doped state or an undoped state. The ability of conducting polymers to behave similar to metal conductors gives rise to new engineering applications. Material properties for conducting polymers, compared to metals, include light weight, ease of chemical modification, and ease of processing at low temperatures [53].

The first observation of conductivity in a polymer was observed in polysulfur nitride, (SN)$_x$, at a temperature range of 4.2 to 300$^\circ$K [99]. Polysulfur nitride was the first polymeric material to display superconductive behavior, with a transition temperature of (0.26 $\pm$ 0.03)$^\circ$K [33]. The interest in conducting polymers increased with the discovery of highly conductive ($<10^3$ S/cm) organic polymers. Trans-polyacetylene was the first polymer in which the doping process was discovered to influence conductivity [82]. Experiments with thin films of polyacetylene show that the electrical conductivity of conducting polymers increases when doped with halides such as chlorine, bromine, or iodine, and with the arsenic pentafluoride [16]. The polymer polysulfur nitride was also found to have permanently increased electrical conductivity at room temperature by inserting bromide ions into a thin polymer film. Doping polysulfur nitride also caused other morphological changes such as color and mechanical deformation [91]. Therefore, investigations of polyacetylene and polysulfur nitride established the precedence that doping a polymer with an ionic species brought about changes in conductivity and morphology.
The ability of polymers with \( \pi \)-electron conjugated structures to switch between doped and undoped states sparked the creation of several conducting polymers for various device applications. Some examples of widely studied conjugated organic polymers include poly\((p\text{-}\text{phenylene})\), polyaniline, polypyrrole, polythiophene, polyfuran, polycarbazole, polyindole, and polyfluorene. The polymer backbone structure for these common conducting polymers is shown in Figure 1.1.

![Polymer backbone structures](image)

**Figure 1.1:** The polymer backbone structure for common conducting polymers.

The first nonacetylenic hydrocarbon polymer stable in air at room temperature and shown to exhibit electrical conductivity is \text{poly}\((p\text{-}\text{phenylene})\) \cite{41}. \text{Poly}(p\text{-}\text{phenylene}) is typically used as polymer light-emitting diodes due to their conversion efficiency of larger than 1\% photons/charge carrier \cite{8}. \text{Poly}(p\text{-}\text{phenylene sulfide}) can be melt and solution processed without being irreversibly damaged by the doping/undoping process unlike other conducting polymers \cite{81}. \text{Polypyrrole} was the first organic conducting polymer to show high conductivity (between 10 and 100 S/cm) \cite{55}. When electropolymerized, polypyrrole
forms durable films which are strongly adherent to metallic surfaces [23]. The films produced have high environmental stability. The first conducting polymer with a covalently attached functional group for ion conjugation was polythiophene [56]. Polythiophene was demonstrated to be indefinitely stable in air at room temperature, as well as thermally stable under nitrogen up to 400°C due to a highly ordered chemical structure [100]. Polyfuran has received less attention as a conducting polymer due to fabrication difficulties [37]. Polycarbazole is primarily of interest due to optical properties in the visible range for LEDs [58], with interesting photoconductive and electrochromic properties which lead to an application in electroluminescent devices [96]. Polyindole is a unique conducting polymer due to certain sensitivity as a sensor and ability to be a model polymer for biopolymers such as melanine [105]. An example of polyindole’s biosensing capability is the use of the enzyme glucose oxidase in order to determine the presence of glucose using potentiometry, a detection method to immobilize enzymes in the electrodeposited polymer layer [30]. Polyfluorene is typically used for its optical and electrical properties including high photoluminescence, and is the only conducting polymer which can emit light throughout the entire visible spectrum. The photoactive behavior of polyfluorene and power conversion efficiency as high 4.74% makes it ideal for high performance solar cells [39]. Polyaniline was observed to form a stable nitrogen base salt rather than a CH₅⁺ ion common to other conducting polymers.

The phenomena of doping is what separates conducting polymers from all other polymers [17]. Conducting polymers switch between doped and undoped states at oxidation and reduction potentials in the presence of the dopant anion/cation. A conducting polymer in surface contact with an electrolytic solution and varying electrical potential is capable of undergoing oxidation and reduction. At a neutral state (no applied electrical field), conducting polymers behave as insulators.

A conducting polymer can either be doped with a cation or an anion. The case with an anion dopant will be discussed, in which the application of a positive potential causes the removal of electrons, a phenomena known as oxidation. During oxidation, the conducting polymer backbone becomes saturated with electrons and demonstrates increased conductivity. Reduction occurs when a more negative electrical potential is applied, which causes electrons to return to the backbone. When in a reduced state, conducting polymers to behave as insulators due to charge saturation. Strain is induced in the conducting polymer
as the oxidation level is altered and follows an asymptotic response to a step potential. This strain is primarily due to the mass transport of ions causing volumetric expansion or reduction [18].

Conductivity in polymers was initially explained using the structure of polyacetylene. The structure of polyacetylene consists of \( \sigma \)-bonded carbons to one hydrogen and two neighboring carbon atoms with a \( sp^2 \) hybridization. The \( \pi \)-electrons delocalize into a conducting band with metallic behavior on the application of small electrical fields (V/m). [16]. Polymers can be either p- and/or n-doped by chemical or electrochemical methods, where p- doping is a partial oxidation of the \( \pi \) backbone and n- doping is partial reduction of the \( \pi \) backbone [49]. They can be doped with either anions or cations in either the oxidized or reduced states. This behavior is characterized by Equation 1.1 for oxidized and equation 1.2 for reduced states.

\[
P^+(A^-) + e^- \rightleftharpoons P + A^- \quad \text{(1.1)}
\]

\[
P^+(A^-) + C^+ + e^- \rightleftharpoons P(AC) \quad \text{(1.2)}
\]

In Equation 1.1, \( P^+ \) represents the positively charged, oxidized state of the polymer, \( e^- \) represents charge transfer, \( P \) stands for the neutral state of the polymer, and \( A^- \) represents the anion, the function \( P^+(A^-) \) indicates the movement of the anion into the polymer backbone. In equation 1.2, \( C^+ \) represents the cation, and the function \( P(AC) \) indicates the movement of a cation inserted during reduction. Ion ingress/egress causes volumetric changes in the polymer, coupling the chemical, electrical, and mechanical domains via ion transport, dielectric displacement, and conformal relaxation. Conductivity of conducting polymers can be varied between the fully doped and fully undoped states based on the level of doping. The equations demonstrate charge neutrality between the conducting polymer and electrolytic solution independent of doping status. The conducting polymer can either oxidize and incorporate anions, reduce and expel anions, or reduce and incorporate cations. Incorporating cations causes the conducting polymer to return to a neutral state. Volumetric expansion of the polymer can occur during either oxidation or reduction and is dominated by the size of the anion [85].

The various conducting polymers operate with different chemical anions and cations as dopants. Early dopants include \( \text{Br}_2 \), \( \text{I}_2 \), and \( \text{ICl} \) to modify the electrical and opti-
cal properties of polyacetylene [91]. Polypyrrole was historically doped with \( \text{BF}_4^+ \) [90], and most common dopants include AsF_5, FeCl_3, ClO_4, LiClO_4, sodium camphorsulfonate (C_10H_15NaO_4S), and sodium dodecylbenzenesulfonate (NaDBS).

1.1.1 Synthesis of Conducting Polymers

Methods for synthesizing conducting polymers include chemical polymerization, casting polymerization, phase polymerization, self-assembly, Langmuir-Blodgett, vapor/gas polymerization, and electrochemical polymerization. **Polypyrrole** can be synthesized using chemical polymerization with a FeCl_3 solution and a methanol solvent [51], electrochemical polymerization [22], casting polymerization [27], and phase polymerization using Fe_3Cl [57]. **Polyaniline** can be synthesized using chemical polymerization by using the oxidative polymerization of aniline in an acidic solution with ammonium peroxydisulfate in hydrochloric acid [50], electrochemical deposition with ferrocene and methyl methacrylate in a benzene or toluene solution [60], casting polymerization [67], phase polymerization using plasma polymerization with RF glow discharges and resistive coupling between stainless-steel electrodes [20], and the Langmuir-Blodgett technique, which involves directly depositing films using acidic subphases of HCl or camphorsulfonic acid [32]. **Polythiophene** can be synthesized using chemical polymerization [100], electrochemical polymerization [48], and phase polymerization [74]. **Polyindole** can be synthesized with electrodeposition by anodic oxidation of indole from tetrabutylammonium perchlorate in dichloromethane [79] and vapor polymerization through chemical vapor deposition methods to create 1D nanostructures [31].

**Poly(p-phenylene)** can only be synthesized using chemical polymerization with the AsF_5 doping of poly(p-phenylene) oligomer crystals [41]. **Polyfuran, polycarbazole**, and **polyfluorene** can only be synthesized with electrochemical polymerization. Polyfuran requires electroreduction of 2,5-dibromofuran in acetonitrile with a Ni(bipy)_3^{2+} catalyst [104]. Carbazole is dissolved in dimethylformamide with sodium hydride and electropolymerized with triethylene glycol mono methyl ether terminated by a p-toluene sulfonate group in an argon atmosphere to form polycarbazole [96]. Polyfluorene can be electropolymerized by oxidative coupling of fluorene units, which can be done on the nano-scale [97].

The simplest method is electrochemical polymerization, or electrodeposition when polymerizing on metals, and can be utilized by most polymerization reactions. Electrodepo-
position uses a three-electrode configuration in which a working electrode, counter electrode, and reference electrode are electrochemically connected through an electrolytic solution. The working electrode and counter electrode are conducting, chemically inert metals, and the reference electrodes are typically Ag/AgCl electrodes or silver wire. Deposition can either be potentiodynamic or potentiostatic and occurs when current passes from the working electrode to the counter electrode. Thickness of the electropolymerized film can be controlled via chemical composition of the electrolytic solution, concentration of the monomer, condition and conductivity of electrodes, distance between electrodes, and magnitude of applied potential. Electrodeposition can be used to create nano-scale polymer films.

The electrodeposition steps of polypyrrole is included in Figure 1.2 as described by Diaz et al. Oxidation of the monomer produces a radical cation with several resonance forms. A stabilization step causes the loss of two protons, which leads to the generation of an aromatic dimer. The dimer then oxidizes to form different resonance forms, which then combine with another monomer form a trimer due to a lower oxidation potential of the dimer compared to monomers. Deprotonation leads to a neutral state. Subsequent oxidation leads to a progressive increase in chain length to create a final polymerized structure for polypyrrole. Doping occurs simultaneously to oxidation during polymerization, in which the oxidation potential of the monomer is lower then the polymer. Formation of long ordered chains or short cross-linked chains occur based on polymerization conditions.
Figure 1.2: The electropolymerization steps for the formation of polypyrrole as explained by Diaz et al.

1.2 Characterization of Conducting Polymers

Conducting polymers are either characterized electrochemically through cyclic voltammetry, electrochemical impedance spectroscopy, or visually through microscopy. Cyclic voltammetry provides useful information such as oxidation and reduction potentials and peak current. Electrochemical impedance spectroscopy yields complex impedances, which can be solved for equivalent electrical impedance values which provides a model of voltage-current relationship. Microscopy gives useful information on surface roughness and morphology of a conducting polymer film, which can verify the structural integrity of devices reliant on polymer placement.

1.2.1 Cyclic Voltammetry of Conducting Polymers

The complete electrochemical behavior of an ionically active system can be obtained by analyzing the current-time curves through a series of steps to different potentials. Cyclic voltammetry refers to an experimental configuration in which the applied electrical poten-
tial is periodic. It can be used to analyze the electrochemical interactions at electrode-electrolytic interface and bulk electrochemical response. A three electrode potentiostat controls the potential between the working and reference electrode. A typical CV begins with zero electrical potential, and applies a linearly ramping electrical potential over time. This increasing potential ends when it reaches a switching potential, in which the ramp potential is inverted until a negative switching point it reached. This inversion can occur multiple times in a single trial.

A representation of a idealized cyclic voltammetry plot for a conducting polymer film is shown in Figure 1.3. An exponential increase in current occurs as the potential is increased from 0V due to oxidation leading to an increase in conductivity. The oxidation processes are nearly complete by 0.4V, where the current falls off due to the concentration of the electrolytic solution being depleted close to the surface. This depletion of the neighboring electrolytic solution reduces the amount of mass transfer and therefore reduces the current. Upon application of a negative potential, an exponential increase in current occurs due to reduction. Since the redox reaction is reversible for conducting polymers, application of a negative electrical potential will cause a reoxidation of the product formed in the first reduction reaction. This reoxidation causes a current of reverse polarity from the forward scan. The processes associated to reduction are complete by -0.4V, with the concentration of the neighboring electrolytic solution being depleted and reducing the amount of mass transfer of ions available. As with oxidation, this causes the current to level off.

Cyclic voltammetry can also be used to find the polymerization potential for a conducting polymer. During electropolymerization, application of an increasing electrical potential will result in an exponential increase in current. The start of this current increase is referred to as the polymerization potential, or the potential in which the polymerization initiates.

The peak current for a reversible reaction can be calculated based on electrode area, diffusion coefficient, and ionic concentration. The governing equation for calculating peak current is shown as equation 1.3.

\[ i_p = (2.69 \times 10^5)v^{1/2}AD^{1/2}C_0 \]  

(1.3)

with \( v \) as the scan rate, \( A \) is the electrode area, \( D \) is the diffusion coefficient of the ion,
1.2.2 Electrochemical Impedance Spectroscopy of Conducting Polymers

The technique where the electrode impedance is measured as a function of frequency ($\omega$) is called electrochemical impedance spectroscopy (EIS). Electrochemical impedance spectroscopy gives a frequency dependent impedance that can be used to calculate analogous electrical equivalents.

The equivalent circuit for a conducting polymer is a resistor and capacitor in parallel, with electrolytic resistance added in series [26]. Using complex impedance analysis, specific
resistance and capacitance values can be fit to an equivalent circuit shown in Figure 1.4.

![Equivalent Circuit](image)

**Figure 1.4: The equivalent circuit for a conducting polymer**

Points from the output bode plot can be input into the complex impedance equation and solved simultaneously for $R$ and $C$ using limits of the impedance. These limits of impedance are: $\omega=0$, $\hat{Z} = R$ and $\omega \to \infty$, $\hat{Z} = 0$. The resistance of the electrolytic solution is of low significance due to being very small and dependent on electrode placement.

### 1.2.3 Microscopy of Conducting Polymers

Scanning electron microscopy gives high resolution two dimensional images due to scanning a sample with an electron beam in a raster scan. A thin film specimen is placed in a vacuum under an electron gun and the high energy beam of electrons interacts with the sample surface. Signals produced by a scanning electron microscope include secondary electrons, back-scattered electrons, and transmitted electrons. Backscattered electrons are typically used in organic materials or biological systems due to the intensity required to produce secondary electrons. Conducting polymers are first coated with a very thin (nanoscale) layer of either gold or platinum to increase conductivity. In order to reducing damaging a conducting polymer sample, the electron beam is run at low accelerating voltages (0.3-4kV).

### 1.3 Conducting Polymers as Sensors

Chemical sensors convert concentrations of analytes to measurable signals, such as current. A physical alteration of the sensing material is quantified via instrumentation [3]. Conducting polymers have been used as optical sensors, chemical sensors [4, 40], pH sensors [87], electronic noses, and biosensors [1, 66, 59].
1.3.1 Conducting Polymer Chemical Sensors

Chemical sensors rely on having a measurable signal being output from a molecular binding event. Conducting polymers specifically take binding events and produce electrical signals. Different analytes can also be detected using conducting polymers by chemically modifying their functional groups for specific analyte affinity \[2\]. Common analytes include carbohydrates, proteins, amino acids, and metabolites in blood, while more recent work studies include neurotransmitter molecules \[103\].

Real-time detection of Cu$^{2+}$ and Ni$^{2+}$ ions was accomplished using a nanoscale conducting polymer junction array of polycarbazole and polyaniline \[45\]. The peptide-functionalized array was synthesized by Aguilar et al. using polyaniline with a $<60$nm spacing between nanoelectrodes. As a gas or vapor sensor, conducting polymers undergo redox reactions in the presence of certain gases which introduces variations in the doping level for gases such as NH$_3$, NO$_2$, and H$_2$S \[7\]. High sensitivity nitrogen dioxide vapor sensors were fabricated using polyaniline, polypyrrole, and polythiophene functionalized with copper phthalocyanine \[68\]. Overoxidized PPy films can be used as to detect target molecules, such as endocrine disrupters, based on shape-complementary cavities.
1.3.2 Conducting Polymer pH Sensors

Conducting polymers that possess acidic or basic groups can be protonated or deprotonated and thus used as pH sensors. Examples of conducting polymers used as pH sensors include polyaniline, polypyrrole, polyindole, polycarbazole, and polyazines [45]. Protonation or deprotonation of a conducting polymer leads to changes in the electrical and optical properties, which then lead to a change in the oxidation/reduction properties. A direct relationship therefore exists between absorption and conductivity in conducting polymers used as pH sensors. Protonation enhances conductivity where deprotonation leads to lower conductivity [65]. As an example, polypyrrole chains were subjected to a pH shift from 6pH to 12pH, causing deprotonation and therefore increasing the absorbance between 600nm and 900nm while shifting the minimum from 600nm to 500nm [45].

Ultrathin polyaniline films deposited on indium-tin oxide were characterized as pH sensors over a 3-9 pH range [29]. The films were described as suitable substrates for supporting phospholipid bilayers due to their hydrophilic nature. Polyaniline porous vycor glass nanocomposites were used as a sensing phase of an optical fiber pH sensor [87].

The parameter of interest in creating a conducting polymer pH sensor is the acid dissociation constant $pK_a$. Using a plot of the absorption spectra of a conducting polymer, the $pK_a$ can be determined using equation 1.4 as given by Demarcos et al. [21].

$$pK_a = pH + \log \left( \frac{A_x - A_b}{A_a - A_x} \right)$$

(1.4)

where $A_x$ is the absorbance based on a defined pH near the $pK_a$, $A_b$ is the absorbance of the base form, and $A_a$ is the absorbance of the acid form.

1.3.3 Conducting Polymer Electronic Noses

Arrays of conducting polymers can provide an increased level of selectivity for both gaseous and liquid (artificial nose and tongue, respectively) analytes. A conducting polymer array been assembled by Hatfield et al. that act as an artificial nose. This nose behavior is based on heterocyclic molecules in conducting polymers which display reversible conductivity in the presence of polar volatile chemicals [36].

Conducting polymer noses have been fabricated to detect benzene, toluene, ethyl benzene, xylene compounds using polypyrrole [6]. Various types of olive oil were detected
using polyaniline and polypyrrole based artificial noses [89]. Polyaniline was used to characterize wine [34]. Polypyrrole and polythiophene based conducting polymer noses can also be used to detect fires based on chemical markers in smoke [80], aromatic hydrocarbons [6], certain types of bacteria and fungi [11, 12, 14], and water pollutants [10].

1.3.4 Conducting Polymer Biosensors

Conducting polymers can have a sensitivity to organic molecules based on an affinity to the polymer backbone, side groups, or immobilized receptors. Biosensors convert biological processes into a measurable signals.

The most extensively used conducting polymer for bioanalytical sensors is polypyrrole. Polypyrrole is able to be used as an immobilization matrix for immunosensors and DNA sensors [7]. The ability to undergo redox reactions, form nanowires, perform ion exchange and ion discrimination, display strong absorption properties for gases, proteins, and DNA, and electrochromatic processes makes polypyrrole suitable for a large number of bioanalytical sensing capacities [69].

Conducting polymers can also be used for the immobilization of biologically active molecules during electrochemical deposition and allow for electron transfer from redox enzymes [42]. Some examples of this include using polypyrrole to detect glucose, D-alanine, atrazine, cholesterol, choline, glutamate, gructose, and urea through chronoamperometry or using polyaniline to detect glucose, hemoglobin, L-lactate, lipids, uric acid, and triglycerides [30]. Conventional methods for immobilization procedures are physical adsorption, entrapment, cross-linking, and covalent bonding [30].

1.3.5 Conducting Polymer Sensors Review

There are several advantages to using conducting polymers as sensing materials. Conducting polymer sensors have improved characteristics when compared to commonly available sensors such as high sensitivities and short response time, ease of fabrication, and good mechanical properties [3]. Conducting polymers can be synthesized to nanoscale sized devices, allowing for investigation of nanoscale level molecular binding. Disadvantages of using conducting polymers as sensors include a lack of specificity, requirement of an eternal energy source, and complicated post processing in the case of biosensors. This thesis presents a conducting polymer chemical sensor for sodium ions in salt solutions. The presence of
sodium cations in polypyrroles conformal backbone changes the conductivity which can be directly measured.

1.4 Bioderived Active Materials

Bioderived active materials are a category of ionic active materials which are fabricated from biological macromolecules. They utilize ion transport properties of cell membranes to couple chemical, electrical, mechanical, and optical domains. Phospholipid molecules can be extracted from nature and reconstituted into a synthetic cell membrane. A synthetic cell membrane provides selective transport for ions from one aqueous solution to another.

Cell membranes are biological membranes which separate the interior of a cell from the outside environment. They are comprised of phospholipid molecules and are selectively permeable to ions and organic molecules which protect the cell from undesirable molecules. Cell membranes host a variety of proteins that provide structural rigidity as well as controlled transport through the membrane. Proteins serve as ion transporters through a bilayer and are highly specific in their ion transport function. They selectively transport ions for an applied stimulus.

Phospholipid molecules have a hydrophilic head group and a hydrophobic tail group. The hydrophilic head group tends towards the outer section due to a negatively charged phosphate group, and the hydrophobic tail group tends towards the inner section due to fatty acid hydrocarbon chains. This amphipathic structure is ideal for forming micelles, vesicles, and bilayer membranes. Phospholipids can be extracted from nature and reconstituted in a lab into a bilayer lipid membrane. A bilayer lipid membrane is an ideal structure for embedding proteins, which serve as functional units for both cell structure and metabolic processes. The bilayer is a thin, polar membrane consisting of two layers of lipid molecules. They are particularly impermeable to ions as well as most hydrophilic molecules.

Protein can also be extracted from nature and reconstituted into a bilayer lipid membrane. The combination of a bilayer lipid membrane with embedded protein is referred to as a bioderived membrane. A bioderived membrane has useful engineering applications due to controlled, selective ion transport. Applications include sensors, actuators, and energy conversion membrane systems. An example of a bioderived membrane as an actuator is generating strain due to moving fluid through a membrane into an enclosed cavity [92].
Protein in a bioderived membrane can be used to generate power by using the hydrolysis of adenosine triphosphate (ATP) from an (ATP)ase enzyme [93]. Pore forming proteins can be engineered to give control for membrane transport properties, which allows for sensing of analytes at single molecule level [15].

The first instance of a bilayer lipid membrane was reported in 1961 at the Symposium on the Plasma Membrane. The symposium introduced a black lipid membrane, described as a 60-90Å thick construct similar to a cell membrane by Mueller and Rudin, which separates two aqueous solutions [63]. An early description of the bilayer membrane with embedded protein was given by Singer. Singer presented a fluid-mosaic model comprising of a lipid matrix which allows for globular transmembrane protein being randomly distributed throughout the structure [83]. A similar representation is shown below and visualized as protein molecules floating in a sea of lipids as shown in Figure 1.6.

Figure 1.6: A three-dimensional representation of a fluid mosaic model for a bilayer membrane with transmembrane protein.

1.4.1 Formation of Bilayer Lipid Membranes and Bioderived Membranes

The planar bilayer membrane that serves as a host for protein measures approximately 6-10nm in thickness and can be self-assembled by various methods due to the amphipathic nature of phospholipids. Methods for producing black lipid membranes include lipid painting [61, 62], and lipid folding [72] and involve forming a membrane over a small aperture (1mm), resulting in a bilayer separating two aqueous chambers [13]. This configuration
is called a suspended bilayer membrane, which has an inherent difficulty in transporting a suspended bilayer onto/into robust material platforms. The lifetime of a suspended bilayer is also decreased due to poor stability and fully mobile transmembrane proteins [13].

Supporting a phospholipid bilayer with a solid substrate increases stability. Substrate selection for bioderived membranes is limited due to selective requirements for bilayer membrane formation. Examples of good substrates include borosilicate [52], borosilicate [19], mica [102, 25], and oxidized silicon [94]. Examples of various self-assembly techniques on solid substrates include Langmuir-Blodgett [101], [78], and Langmuir-Schaeffer [94], both of which involve forming monolayers and combining them into bilayers through the use of hydrophilic substrates [88]. The disadvantage of these techniques is that it is difficult to reconstitute protein into the bilayer membrane [13].

Vesicle fusion is one of the easiest means of forming either a suspended or solid supported phospholipid bilayer. Vesicles are hollow-spherical structures comprised of a lipid bilayer forming a continuous outer shell. Vesicles can be created by extruding multilaminar vesicles through polycarbonate membranes under pressure in order to form small unilaminar vesicles [54]. Another method to form vesicles is through sonication, vortex mixing, and ultracentrifugation of aqueous lipid suspensions [5]. The vesicles are deposited in an aqueous solution on top of a solid support and allowed to rupture and refuse into a bilayer membrane [47], [46], [44]. A vesicle fusion based bilayer membrane allows for easy transmembrane protein insertion due to accessibility of both sides of the membrane.

A major shortcoming of self-assembled bilayer membranes is fragility and limited lifespan. One solution to this problem is the use of the droplet interface bilayer method (DIB), which reconstitutes phospholipids into a bilayer membrane inside of a solid structure to add support. This technique was first introduced and described by Funakoshi, et al. [28]. Major advantages of the droplet interface bilayer method are simplicity, stability, and ability to support transmembrane proteins. The disadvantage is in the difficulty of manipulating individual droplets on small scale apertures.

This difficulty can be overcome through utilization of the regulated attachment method developed by Sarles and Leo [76, 77]. A flexible substrate is used to support phospholipid coated droplets in adjacent compartment which are brought together through an applied force to the container. The result of the regulated attachment method was a stable, highly electrically resistive membrane necessary for measuring single and multiple
channel ion currents through protein transporters.

1.4.2 Formation and Function of Ion Transporting Protein Channels

Transporter proteins are biological macromolecules consisting of polypeptides folded into a globular or fibrous form. Polypeptides are formed from single, linear amino acid chains bonded together by peptide bonds between the carboxyl and amino groups. There are roughly twenty standard amino acids which compose the majority of protein transporters. The combined effect of the side chains throughout the protein determine the three dimensional structure and chemical reactivity. The arrangement of amino acid side chains in the two-dimensional and three-dimensional structure causes hydrogen bonding, van der Waals and steric interactions in the presence of an external stimuli.

Protein, when embedded in a bilayer membrane, converts electrical, mechanical, or chemical stimuli into ion transport by opening conductive pathways. Formation of a conductive pathway allows a specific ion to travel across a membrane. This ionic transport causes an ionic current through both the protein transporter and host cell membrane.

Ion transport through a bioderived membrane can be classified as simple diffusion, facilitated diffusion, primary active transport, and secondary active transport. For simple diffusion, the protein transporter resembles open channels and allows ion transport along a concentration gradient without expending energy. In facilitated diffusion, a gating signal to the protein causes the opening of ion transporting channels. This gating signal can be electrical, mechanical, chemical, or optical. For primary active transport, the protein transporter breaks down molecules such as adenosine triphosphate (ATP) to produce the required energy for transporting an ion across the membrane against the chemical gradient. Secondary active transport can occur in instances when active transporters provides the necessary environment for facilitated diffusion to occur simultaneously in a neighboring area.

1.5 Characterization of Bioderived Membranes

Bioderived Membranes are characterized electrochemically through cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy. All electrochemical measurements apply a very small (mV scale) electrical potential across the bioderived mem-
brane and measure current or complex impedance. It is important in all cases to establish a baseline measurement of ion transport through the BLM and charge separation across the membrane without the protein transporter.

1.5.1 Cyclic Voltammetry of Bioderived Membranes

The first characterization step is to confirm the formation of a bioderived membrane with cyclic voltammetry. A typical cyclic voltammetry plot of a bilayer membrane represents ohmic behavior with a high resistance (gigaohms). The addition of active protein results in an asymmetrical current-voltage curve [98].

1.5.2 Chronoamperometry of Bioderived Membranes

Chronoamperometry (CA) is used to demonstrate protein function. A bilayer membrane will quickly reach a steady state response to an applied electrical potential, with minor perturbations due to electrostriction causing ionic noise. Alamethicin function is demonstrated by multiple conductance states. Simultaneous calcium flux imaging and single channel recording of alamethicin demonstrate multiple conductance states attributed to a single protein channel diffusing in the bilayer [35]. Determining the occurrence of a single channel pore is based on the pattern of conductance levels. A stationary fluctuation state after reaching steady state implies a single channel measurement [9].

1.5.3 Electrochemical Impedance Spectroscopy of Bioderived Membranes

The equivalent circuit for a bioderived membrane is two resistors in parallel with a capacitor, in which the bilayer membrane contributes a resistor \( R_b \) and capacitor \( C_b \) and the embedded protein contributes a resistor \( R_a \). In the case of a solid supported bioderived membrane, a thin layer of deionized water is predicted to exist between the membrane and substrate and is modeled by the inclusion of a resistor \( R_{DI} \) in series. Using the complex impedance analysis, specific resistance and capacitance values can be fit to an equivalent circuit in the same manner as conducting polymers. The complex impedance for a bilayer membrane formed from DPhPC was found have a capacitance of \( 0.65 \pm 0.2 \mu F/cm^2 \) and a resistance of at least \( 1.6 \times 10^8 \Omega cm^2 \) [70].
1.6 Bioderived Membranes as Sensors

The bioderived membrane can be used as a sensing element by converting the response to an applied chemical, electrical, or mechanical stimulus to a measurable electrical signal. A bioderived membrane is fabricated such that electrodes are accessible on either side of the membrane on a synthetic substrate. Electrode placement relative to the bioderived membrane allows for monitoring electrical current, transmembrane voltage, or complex electrical impedance. Protein can be selected based on appropriate stimuli and transporter ability which allows the overall device to respond to chemical analytes, bioelectrical sensors, mechanical strain, light, and temperature. An example of this includes drug screening applications, which require investigating the function of membrane proteins. Membrane proteins are currently >50% of drug targets, which shows the importance of understanding transmembrane protein function.

1.7 Hybrid Active Material Systems

The common function between conducting polymers and a BLM with embedded protein is ion transport in response to an external stimulus. Sackmann originally proposed the idea of supporting a bioderived membrane with solid materials such as polymer cushions or ultrathin water layers. Sackmann reported identical structural and dynamic properties of suspended bilayers compared to free bilayers and presented applications to surface-sensitive techniques as well as biosensors. The most commonly found transporter proteins in biological membranes transport sodium, potassium, or calcium ions. Polypyrrole is able to exchange sodium ions with an electrolyte due to an applied electric field, whereas a bioderived membrane formed from DPhPC and reconstituted with the voltage gated alamethicin protein is also able to transport sodium ions in response to an electrical stimulus. The result of ion flux into or out of a conducting polymer is a change in electrical resistance as well as a deformation of the polymer backbone inducing strain. Advantages of using conducting polymers as sensors compared to metals and other conventional materials include being lightweight, easy to manufacture, inexpensive, and disposable, while disadvantages include lacking specificity and require complicated post-processing for chemical and biological sensing applications. Ion transport through protein has inspired the development of sensing and actuation concepts due to their highly specific function.
and selectivity [73, 95], despite being too fragile when supported on solid substrates or suspended in pores. This common functionality is enhanced by the ability to combine conducting polymers and bioderived membranes in a thin film arrangement which augments their advantages while mitigating their limitations. A conducting polymer film combined with a bioderived membrane spanning a pore is a unique configuration which allows for a number of sensing applications due to having electrical access along the polymer film. The framework allows for real time chemical sensing, volatile organic compound detection, small scale actuation, and a hybrid computing element.

1.8 Suspended Polypyrrole Films Supporting Bioderived Membranes as Sensing Devices

1.8.1 Architecture of the Integrated Element

The integrated ionic device consists of a suspended polypyrrole film supporting a bioderived membrane and suspended from by two gold pads. The advantage of this architecture is the ability to electrically access the component layers in the thin-film integrated device. The layers are electronically coupled and have source, gate, and drain similar to an electronic transistor. An electrical field is applied across the conducting polymer which causes ion ingress/egress and forms the source and drain ports. The chemoelectrical gradient applied across the bioderived membrane regulates ion transport through the protein and hence forms the gate and drain ports. A schematic for the integrated ionic device is shown in Figure 1.7.

1.8.2 Chemical Sensing

A schematic of a chemical sensing unit fabricated from suspended conducting polymer films supporting bioderived membranes is shown in Figure 1.8. As a chemical sensor, a solution carrying an analyte of unknown concentration is brought into contact with the bioderived membrane. A gating electrical signal (from the source to the gate) causes protein transporters to open channels for ion transport through the bioderived membrane and into a suspended conducting polymer film. Ions are therefore transported from the solution and into the conducting polymer film. A concurrent electrical potential from the source to
the sink would supply a current through the conducting polymer film. This electrical signal would give information about the concentration of ions in the solution. By electrochemically characterizing a suspended conducting polymer film, the electrical activity in response to an applied electrical signal is known for various levels of ionic concentration through peak current calculations.

1.8.3 Volatile Organic Compound Detection

As a volatile organic compound detection device, a ligand-gated protein (or) system of proteins with affinity to a volatile organic compounds is used in the bioderived membrane. A conceptual schematic is shown in Figure 1.9. The protein selection is dependent on the
volatile organic compound (VOC) that needs to be detected by the sensing element. In the presence of the VOC, ion transporting protein channels transport ions through the bioderived membrane and into the suspended polypyrrole film. The polypyrrole film will then become doped, causing a measurable change in the conductivity. This change would be monitored through the use of an electrical potential applied across the polypyrrole film.

![Chemoelectrical Sensor/VOC Detection](image)

**Figure 1.9: A conceptual schematic of a VOC detection unit.**

In order to fabricate and test these sensing elements, the following research goals were designed for this work.

### 1.9 Research Goals

The overall goals of this project and how they will contribute to the scientific and engineering community are as follows.

- **Fabricate a suspended conducting polymer film**
  
  A micron scale pore will be fabricated through a silicon substrate to create a platform for a suspended conducting polymer film. Gold pads on either side of the pore will allow for the electrodeposition of polypyrrole across the span, creating a polypyrrole bridge.

- **Demonstrate a supported bioderived membrane**
  
  A bioderived membrane, created with DPhPC vesicles reconstituted with alamethicin, will self assemble on top of the suspended polymer film via vesicle fusion. This bioderived membrane will be verified through electrochemical impedance spectroscopy.
Ion transport through alamethicin channels will be verified through chronoamperometry and cyclic voltammetry.

- *Electrochemically characterize integrated components*

An equivalent circuit based on electrochemical theory will be produced as a model for the integrated ionically active device. Through complex impedance based mathematical methods based on information from electrochemical impedance spectroscopy, equivalent impedance values for each component of the equivalent circuit will be calculated.
Chapter 2

Microfabrication of Silicon Substrates and Suspended Polypyrrole Films

In the previous chapter, we discussed the individual layers of an integrated, ionically active device. We introduced the motivation for integrating the electroactive layers into a thin film membrane and provided the physics of operation for novel sensing applications. In this chapter, the procedure developed through this research to fabricate the device is presented.

2.1 Microfabrication of Silicon Substrates

In order to form an insulated platform for electrochemical substrates, silicon wafers were processed including etched through pores to contain thin film layers. Microfabrication of silicon substrates was chosen in order to produce high resolution pores on the micron scale in a highly repeatable manner. Micron scale pores are required for electropolymerization of a conducting polymer film across the pore as well as the self-assembly of a bioderived membrane. A conceptual schematic detailing the fabrication steps is shown as Figure 2.1.
Figure 2.1: A six step conceptual schematic for the fabrication of silicon substrates to be used with suspended polypyrrole films and bioderived membranes.

(1) Four inch, P-type silicon wafers with $<100>$ orientation, and $525 \pm 25 \mu m$ thickness were purchased from the Virginia Wright Microelectronics Center. The architecture requires a through pore and thus requires dual-sided processing during etching. To achieve higher tolerances during etching wafers with smoothed-finish on both sides are preferred. The $<100>$ orientation was chosen such that KOH etching would form trapezoidal wells with no depth limit more common with $<111>$ oriented wafers. (2) To pattern the etch on the silicon wafers using KOH, a SiO$_2$ mask was created through oxidation. Due to poor selectivity between Si and SiO$_2$ at relatively low temperatures, a loss of 2$\mu m$ of SiO$_2$ was expected in order to etch 525 $\mu m$ of Si. As a result, silicon wafers were baked for a 21 hour period at a temperature of 1000$^\circ$ C until 2.4 $\mu m$ thick sacrificial SiO$_2$ layer was formed.

3' To remove a selected area of the SiO$_2$ mask, a buffered oxide etch using a chemically inert photoresist mask provides a patterning method. Five inch sodalime lithography masks were patterned with a Heidelberg $\mu pg$ 101 laser mask writer. SPR 3012 photoresist was spun
onto the backside (defined as the side where the etch would begin) of a wafer at 4000rpm for 30 seconds to ensure a uniform and smooth 1 $\mu$m thick film. The wafer was then prebaked at 90$^\circ$ C to adhere the mask to the wafer. Optical lithography was then performed on the photomask such that an array of 700 $\mu$m squares were formed. Photoresist was then applied to the front and sides of the wafer and hard baked in an oven for 10 minutes at 120$^\circ$ C. The oven was chosen instead of a hot plate for the hard bake in order to ensure the photoresist was not scratched in the processing at the cost of increased bake times. A buffered etch was performed at room temperature, with an expected etch rate of roughly 600 Å/min. Etching for 45 minutes, the SiO$_2$ layer was completely removed to form trapezoidal divots. A (2 $\mu$m expansion of the 700$\mu$m squares occurred due to the etch but was considered negligible. The photoresist was then thoroughly removed using acetone and isoproponal in order to not contaminate the KOH solution.

(4) The etch rate of $<25$ $\mu$m/min was expected with a 20% KOH solution at 60$^\circ$ C. The 60$^\circ$ C was required at the cost of processing time in order to reduce undercut, which was significant at temperatures of 70$^\circ$ or higher, and improve the $<100>, <111>$ selectivity. In order to form through pores, the etch was performed for 20 hours to ensure removal of the front side SiO$_2$ layer over the finished pores. The wafer was flipped 180$^\circ$ halfway through the etch to improve uniformity. Roughly 44$^\circ$ walls were etched with little to no undercut to form a trapezoidal-well shaped pore with a roughly 175 $\mu$m cross section. The wafers were then reoxidized for a 2 hour period at a temperature of 1000$^\circ$ to form an insulating SiO$_2$ layer along the pore walls roughly 3,000 Å thick.

(5) To synthesize a thin gold film to be used for electropolymerization, an adhering chromium layer was chosen to bind the gold to the silicon surface. Due to the difficulties in removing gold via etching, a lift-off technique was selected to pattern the gold film. The limiting condition of photoresist for lift-off is the thickness of the resist must be $<4x$ thicker than the deposited metal. SPR3012 photoresist was selected due to ease of use, as the deposited metal layers were of nanoscale thickness.

Photoresist was spun at 4000rpm for 30 seconds to ensure a uniformly thick 1 $\mu$m layer and prebaked at a temperature of 90$^\circ$ C. A photomask was formed on the front side of the wafer via optical lithography using a second sodalime lithography mask such that resist was removed only where the metal deposition was desired. The silicon wafer was then postbaked at a temperature of 120$^\circ$ to ensure adherance and to ensure the photoresist
walls formed from photo development were as straight as possible to increase lift-off success. A 15 nm thick chromium layer was deposited underneath of a 65 nm thick gold layer in a single process by electron beam evaporation at 1E-6 Torr. The lift-off procedure required submerging the wafer in an acetone bath and sonication for roughly 1 hour in order to remove all photoresist and leave only the patterned metal films.

The overall configuration of the front face consisted of two 3000 µm diameter pads with a 500 µm wide gold arm spanning between them and a 10 µm gap in the center of the arms disrupting the span, with a single 175 µm pore is wedged between the two pads in the 10 µm gap. An image of the fabricated wafer is shown in Figure 2.2

![Figure 2.2: A picture of a processed silicon wafer as a platform for an integrated electroactive device.](image)

2.2 Fabrication of a Suspended Polypyrrole Film

Among conducting polymers, polypyrrole (PPy) has an affinity with Na\(^+\) doping and therefore shares ionic function with the selected bioderived membrane. It has been demonstrated that PPy doped with sodium dodecylbenzenesulfonate (NaDBS) can be electropolymerized on thin, evaporated gold films [84]. Due to the growth pattern of PPy(DBS), this work shows that a PPy(DBS) bridge can be formed across a micron scale pore with gold film on either side of the span.
2.2.1 Microfabrication of Polypyrrole

Polypyrrole is electropolymerized using a three electrode configuration as described by Smela et al.\cite{84}. The two gold pads on the chip were used as working electrodes, a 5μm thick gold film (99.99% pure from Sigma-Aldrich) was used as a counter electrode, and a 0.1mm thick Ag/AgCl (Sigma-Aldrich) wire was used as a reference electrode. The counter and reference electrode were both placed 5 mm above the gold pads and spaced 1 cm apart, equidistant from the working electrodes to aid uniform growth. The counter electrode was cleaned using isopropanol (Sigma-Aldrich), and both counter and reference electrodes were soaked in bleach to purify their surfaces. The electrolyte consisted of solution with 0.3M NaDBS and 0.1M pyrrole in deionized (DI) water (with resistivity 18.2MΩcm filtered by Thermos Scientific). Cyclic voltammetry, performed with EcoChemie AUTOLAB 128N, gave a potentiodynamic response of the system which provided details of the polymer growth region, which was then used to determine the potentiostatic voltage required for polymer growth. The CV scan ranged from -0.8V to +0.8V with a step potential of 0.00106V and scan rate of 0.009 V/s.

The electrodeposition of a suspended polypyrrole structure is characterized using potentiodynamic characterization. As the electrical potential is increased from 0V to 0.8V, a sharp increase in current occurs around 0.6V. This current increase defines the polymer growth region as reported by Diaz et al.\cite{23}. In the polymer growth region, a current of significant magnitude for the electropolymerization of the polymer exists. The polymer reduces as the voltage is decreased past -0.6V as shown by an increase in current. As the applied voltage is brought back to 0V, the polymer reoxidizes at roughly -0.5V. A plot of a typical CV for the characterization of the deposition for a suspended polypyrrole film is displayed in Figure 2.3

2.2.2 Electropolymerization of a Suspended Polypyrrole Film

Polymerization of PPy was performed potentiostatically at 0.63V over a period of 10 minutes. A plot of a typical CA for the electrodeposition of a suspended polypyrrole film is displayed in Figure 2.4

Polypyrrole growth during polymerization follows a nucleation pattern in which nod-
Figure 2.3: A typical cyclic voltammetry plot characterizing the electrodeposition of PPy(DBS).

Nodules start forming. Clustered growth expands horizontally until the nodules collide, causing them to grow laterally and form a bridge spanning the pore. The polymer layer spanning the Au-arms extending from the pads and covering the pore was formed by CA over 10 minutes at 0.63V. The PPy(DBS) surfaces were then washed using deionized water to ensure a clean surface. A conceptual graphic displaying the suspended polypyrrole film layer is shown in Figure 2.5.
Figure 3 (a) A typical cyclic voltammetry plot characterizing the electrodeposition of PPy(DBS): (b) A typical chronoamperometry plot characterizing the electrodeposition of PPy(DBS).

The potentiostatic current during electrodeposition of PPy(DBS) shows a steady state current being reached, where the magnitude of the current was required to be higher than 326.5 A/cm$^2$ in order to have PPy(DBS) form across the pore. SEM imaging shows that PPy(DBS) has fully spanned the pore, with some PPy(DBS) growth along the side walls. A closer image shows the clustered growth of PPy(DBS) formed across the pore and between gold arms extending from the pads.

Figure 2.4: A typical chronoamperometry plot characterizing the electrodeposition of PPy(DBS).

Figure 2.5: A conceptual schematic for the placement of a suspended polypyrrole film on a silicon substrate.

A Valco CP-DSM2 pump was used with a 50 µL/sec flow rate to produce droplets that cycled over the PPy(DBS) surface for a 30 minute period. A 18.2 MΩ-cm rated deionized water feed with a wastewater outlet ensured ionically only ionically clean water washed the device. The PPy(DBS) surface was tested using cyclic voltammetry to show an ionically clean surface, which is represented by an ohmic response in the presence of deionized water as seen in Figure 2.7. An image of the experimental configuration for
washing the silicon chip with a PPy(DBS) film is shown as Figure 2.6.

Figure 2.6: The experimental configuration for water droplet cleaning a silicon chip with a suspended PPy(DBS) film.

2.3 Characterization of Polypyrrole Films

The magnitude of current required to span a 175 µm pore was empirically found to be 326.5 A/cm². SEM imaging shows that PPy(DBS) has fully spanned the pore, with a small amount of creep along the side walls. Figure 2.8 shows the silicon-silicon dioxide chip with a SEM close up of the microfabricated well in addition to a SEM of the PPy(DBS) nodular growth at the bottom of the pore. An image of the front face and back face of the PPy(DBS) film is shown in Figures 2.9 and 2.10, respectively. The PPy(DBS) film grew along the side walls of the pore during electrodeposition as shown in Figure 2.11.
The potentiostatic current during electrodeposition of PPy(DBS) shows a steady state current being reached, where the magnitude of the current was required to be higher than 326.5 A/cm$^2$ in order to have PPy(DBS) form across the pore. SEM imaging shows that PPy(DBS) has fully spanned the pore, with some PPy(DBS) growth along the side walls. A closer image shows the clustered growth of PPy(DBS) formed across the pore and between gold arms extending from the pads. Figure 2.8: SEM characterization of PPy(DBS) deposited across a pore. (a): silicon-silicon dioxide chip with Au-pads on the reverse. (b): Close up of the microfabricated well. (c): PPy(DBS) formed at the bottom of the pore shown with clustered PPy(DBS) growth. Figure 2.9: A SEM image of the Au pads with a suspended PPy(DBS) film spanning across a pore. Figure 2.10: A SEM image of the pore side of a suspended PPy(DBS) film.
Electrochemical measurements of a PPy(DBS) film were performed using the experimental configuration shown in Figure 2.13.

The PPy(DBS) film was impregnated in solutions of varying NaCl concentrations and characterized through CV. The CV ranged from -1.0V to 0.3V with an 0.5mm thick Ag/AgCl wire reference electrode. This characterization shows oxidation/reduction potentials after the DBS molecules are removed from the PPy backbone. An increase of current was observed as the ionic concentration of the electrolytic solution increased, demonstrating an increased amount of Na\(^{+}\) doping and undoping as shown in Figure 2.14.

The PPy(DBS) film was washed under a water drip before EIS characterization over several trials. EIS was performed from 100KHz to 10 mHz with 100mM electrolytic solution and a Ag/AgCl wire reference electrode. PPy(DBS) acts as a resistor at high frequencies.
and tends toward capacitor behavior at low frequencies (0.01Hz). The resistor in parallel with the capacitor for the PPy(DBS) equivalent circuit is found to be significantly small.

2.3.1 Interpretation of Experimental Data for Suspended Polypyrrole

A suspended PPy(DBS) film was fabricated using a 0.63V voltage and 0.3M pyrrole and 0.1M NaDBS electrolytic solution. DBS is a large anion and trapped in the polymer backbone, therefore, sodium cations will ingress/egress the polymer backbone. The switching of oxidation/reduction states for a suspended PPy(DBS) film was demonstrated by applying an appropriate voltage. As the polymer reached an oxidation potential, the conductivity of the polypyrrole increased and is considered doped. As the polymer is reduced from an oxidized state, the conductivity of the polypyrrole decreased and is considered undoped. A PPy(DBS) film exposed to an electrolytic solution will have a direct relation between ionic concentration and peak current in the presence of an electric potential. PPy(DBS) was demonstrated to behave similar to a resistor and capacitor in parallel, shifting from resistive to capacitive behavior as the applied frequency is decreased.
2.3.2 Conclusion

In this chapter, a procedure to microfabricate a silicon wafer as a suitable platform for the electrochemical deposition of PPy(DBS) is demonstrated. A method of electropolymerizing PPy(DBS) on evaporated Au pads was introduced. The ability of a PPy(DBS) film to span a pore is shown through SEM imaging. A relationship between conductivity of polypyrrole and levels of doping was demonstrated.
Chapter 3

Bioderived Membranes and Characterization

The previous chapter demonstrated the fabrication technique used to create a suspended polypyrrole film across a silicon substrate. Polypyrrole was shown to respond electrochemically based on ionic concentration of the mobile cation. In this chapter, we demonstrate the fabrication process for reconstituting a bilayer lipid membrane from DPhPC lipids and voltage gated proteins to form the integrated membrane. The bioderived membrane will be investigated for protein function, and the entire integrated ionic device will be characterized via electrically equivalent values.

3.1 Fabrication of a Bioderived Membrane

The bioderived membrane is self-assembled from 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) lipids. A DPhPC bilayer membrane is able to support the reconstitution of the transmembrane protein alamethicin, that is permeable to Na\(^+\) ions. Alamethicin is an antibiotic peptide produced by the fungus *Trichoderma viride* and follows the barrel-stave mode of action. A number of individual hydrophobic peptide molecules, or staves, are arranged in a barrel structure. The hydrophobic surface of the barrel interacts with the hydrophobic fatty acid chains of the bilayer lipid membrane. A single peptide unit will not enter a membrane bound state due to energy requirements; therefore, peptides congregate at the surface of the bilayer lipid membrane until a required concentration is achieved. At transmembrane potentials of 60-80mV or greater, four to six units of the peptide assemble...
into pore-forming channels [24]. The channel is formed by the peptide molecules undergoing a conformal phase transition which force the phospholipid head groups aside. Peptide groups collect at the thinned membrane section and stabilize/increase the size of the pore [64]. These pore-forming channels are believed to be comprised of α-helical peptides which form perpendicular to the bilayer membrane.

3.1.1 Self-Assembly of Bioderived Membranes

Bilayer membranes with and without reconstituted protein were individually self-assembled over several trials. Between trials, the PPy(DBS) film was washed using the deionized water drip setup described in Chapter 2. An aqueous solution was formed with a concentration of 5 mg/ml DPhPC in deionized water. The solution was sonicated and vortex mixed for 3 cycles of 10 and 5 minutes, respectively, to prepare vesicles. Subsequently, the vesicle solution can be used to form a bilayer membrane. A conceptual graphic displaying the placement of a bilayer membrane on a polypyrrole bridge is displayed in Figure 3.1.

![Figure 3.1: A conceptual schematic for the placement of a bilayer membrane supported by a suspended polypyrrole film.](image)

Alamethicin of concentration 1 mg/ml in ethanol can also be added to the vesicle solution in order to create DPhPC vesicles reconstituted with protein. The solution is then vortex mixed for 5 minutes and sonicated for 10 minutes. The molarities of DPhPC and alamethicin was chosen to achieve a molar ratio of 1000:1.

A 0.25 µl droplet of the lipid vesicle reconstituted with protein solution was added into the pore and allowed to settle for 30 minutes. In this time, the vesicles would rupture and fuse together with surrounding vesicles to self-assemble into a bilayer membrane. A 0.45 µl droplet of 100mM NaCl was added to the pore and given 5 minutes to settle.
3.2 Electrochemical Characterization of Bioderived Membranes

A bioderived membrane is characterized using EIS, CA, and CV. The experimental configuration for the electrochemical characterization of a bioderived membrane is shown in Figure 3.2.

Figure 3.2: The experimental configuration for the electrochemical characterization of a bioderived membrane.

3.2.1 Cyclic Voltammetry and Electrochemical Impedance Spectroscopy of a Bioderived Membrane

A CV of a BLM without protein demonstrates ohmic behavior with significantly higher resistance than that of a PPy(DBS) film. This demonstrates the bilayer lipid membranes selective permeability by undergoing little to no ion transfer. It is observed from the CV that a BLM reconstituted with alamethicin shows an increase in current compared to a BLM without alamethicin and displayed in Figure 3.3. As the electric potential is increased during the CV to the transmembrane potential of roughly 50mV, the conductance increases by a magnitude of 3. This increase in conductance is due to the hydrophobic peptide molecules reaching the required amount of energy to form ion transporting channels following the barrel-stave mode of action.
EIS was performed from 100KHz to 10mHz with 100mM NaCl solution and an Ag/AgCl wire reference electrode on a bilayer membrane as well as a bioderived membrane. A compilation of EIS measurements for several devices is compared to a PPy(DBS) film in Figure 3.4.

The impedance of a BLM without alamethicin is one order of magnitude higher than that of a BLM reconstituted with alamethicin. The impedance of a PPy(DBS) film is 3-4 orders of magnitude lower than a BLM without alamethicin. A BLM with and without alamethicin demonstrates RC behavior at high frequencies and resistive behavior at low frequencies (10Hz). The crossover frequency for the BLM reconstituted with alamethicin shifts to the right approximately by 1 KHz in comparison with a plain BLM supported on PPy(DBS).

3.2.2 Chronoamperometry of a Bioderived Membrane

Chronoamperometry was performed across the BLM with and without alamethicin to demonstrate protein activity. A step function was input from an Ag/AgCl electrode in the electrolytic solution across the BLM. Protein activity is demonstrated by an increase in fluctuation of electrical signals, and the magnitude of current for the same applied electrical potential is roughly 4 magnitudes higher for a BLM reconstituted with alamethicin than a BLM without reconstituted alamethicin, as shown in Figure 3.5.
Multiple conductance states for the bioderived membrane are observed due to the formation of ion-transporting channels as peptide molecules interact with the bilayer lipid membrane. These conductance states can be observed via CA with the results displayed in Figure 3.6(a). Due to the size of the pore spanned by the bioderived membrane, multiple protein channels exist concurrently. Figure 3.6(b) shows the aggregate conductance states (due to multiple channels) for a 1000:1 molar ratio of BLM:alamethicin.

Figure 3.4: (a): A compilation of PPy(DBS) and BLM EIS data over several trials showing trends. (b): A compilation of PPy(DBS) and BLM+ALM EIS data over several trials showing trends.
Chronoamperometry was performed across the BLM with and without alamethicin to demonstrate protein activity (Figure 7). A step function was input from an Ag/AgCl electrode in the electrolytic solution to the Au pads. From the current vs. time plot in Figure 8, we observe different conductance state for the BLM with alamethicin due to the formation of pore-forming channels. The histogram in Figure 8(b) shows the aggregate conductance states (due to multiple channels) for a 1000:1 molar ratio of BLM:alamethicin.

An equivalent circuit was created to explain and characterize the behavior of the ionic device. The model circuit for a BLM with an embedded protein is two resistors and a capacitor in parallel. A parallel capacitor and resistor were added in series to account for the PPy(DBS). The lumped resistance of the pore and electrolyte are taken into account by additional resistors in series. A resistor was added between the PPy(DBS) and BLM with embedded alamethicin components to represent the thin layer of DI water with low Na+ concentration between the layers. The equivalent circuit diagram of the integrated ionic device with its component layers is shown in Figure 9.

Figure 3.5: A comparison between a bilayer membrane (a) and a bilayer membrane with embedded protein (b) via chronoamperometry analysis

3.2.3 Interpretation of Experimental Data for Bioderived Membranes

A bioderived membrane formed with DPhPC and reconstituted with alamethicin can be self-assembled on a suspended PPy(DBS) film. The bilayer membrane provides selective ion transport as demonstrated by the increased ionic resistance. Active transport of ions through the bioderived membrane occurs as a result of protein activity in the presence of an electrical potential. Multiple conductance states are present in a bilayer membrane reconstituted with alamethicin, with a positive electrical bias. A BLM behaves similar to a resistor and capacitor in parallel, and a BLM with reconstituted alamethicin behaves similar to a resistor, resistor, and capacitor in parallel.

3.3 Characterization of Hybrid Active Material Systems

3.3.1 Equivalent Electrical Analysis of a Hybrid Active Material System with Polypyrrole and Bioderived Membranes

Characterization through EIS provides a direct method of analyzing an electrochemical system by providing information on complex impedances. These impedance values can be
Chronoamperometry:
• Steady State behavior
• Multiple protein channels opening and closing
• Multiple conductance states

Figure 3.6: (a) A chronoamperometry analysis of a BLM layer reconstituted with alamethicin: (b) A histogram showing the various stages of alamethicin gates

found by creating an equivalent circuit and fitting values to this circuit. The electrically equivalent circuit for a conducting polymer is a capacitor denoted $C_p$ in series with a resistor denoted $R_p$. The lumped resistance of the pore and the electrolyte, denoted $R_l$ is accounted for by adding a resistor in series to the polymer circuit. A schematic of the equivalent circuit is shown in Figure 3.7

![Schematic of the equivalent circuit](image)

Figure 3.7: An equivalent circuit for the integrated ionic device.

Equation 3.1 shows the total impedance for a suspended PPy(DBS) film

$$Z = R_l + \frac{R_p}{\omega^2 C_p^2 R_p^2 + 1} - j \frac{\omega C_p R_p^2}{\omega^2 C_p^2 R_p^2 + 1} \quad (3.1)$$

By taking various values for $\omega$, Equation 3.1 can be solved for $R_l$, $R_p$, and $C_p$ over a range of frequencies. The calculated value for $\bar{R}_p$ is $1.7847 \pm 0.1735 \Omega \text{cm}^2$, and the calculated
value for $\bar{C}_p$ is $1.2673 \pm 0.1823 \mu F/cm^2$.

A similar process is used to calculate the equivalent circuit values for a BLM. The model circuit for a BLM is a resistor $R_b$ and a capacitor $C_b$ in parallel. A thin layer of deionized water is expected to exist between the PPy(DBS) film and the BLM and is modeled as a resistor $R_{DI}$ in series. The total impedance for a BLM supported by a suspended PPy(DBS) film is shown as Equation 3.2.

$$Z = R_l + R_{DI} + \frac{R_p}{\omega^2 C_p^2 R_p^2 + 1} + \frac{R_B}{\omega^2 C_B^2 R_B^2 + 1} - j \left( \frac{\omega C_p R_p^2}{\omega^2 C_p^2 R_p^2 + 1} + \frac{\omega C_B R_B^2}{\omega^2 C_B^2 R_B^2 + 1} \right)$$ \hspace{1cm} (3.2)

Equation 3.2 was solved simultaneously for various values of $\omega$ for $R_b$, $C_b$, and $R_{DI}$. The calculated value for $\bar{R}_b$ is $1.654 \pm 1.9894 M\Omega \text{cm}^2$, for $\bar{C}_b$ is $1.1221 \pm 0.239 \mu F/cm^2$, and the value for $\bar{R}_{DI}$ is $1.7398 \pm 0.6407 \Omega \text{cm}^2$. The capacitance value for a BLM in literature are reported in the range of $0.3 - 1.5 \mu F/cm^2$, with a resistance of $0.4 M\Omega \text{ cm}^2$ for a BLM and a BLM reconstituted with alamethicin.

The equivalent circuit can also be modified to include alamethicin reconstituted into the BLM. Alamethicin is represented as a resistor in parallel with the BLM circuit and denoted as $R_a$. The total impedance of the integrated ionic device is shown in Equation 3.3

$$Z = R_l + R_{DI} + \frac{R_p}{\omega^2 C_p^2 R_p^2 + 1} + \frac{R_B R_A (R_B + R_A)}{\omega^2 C_B^2 R_B^2 R_A^2 + 1} - j \left( \frac{\omega C_p R_p^2}{\omega^2 C_p^2 R_p^2 + 1} + \frac{\omega C_B R_B^2 R_A^2}{\omega^2 C_B^2 R_B^2 R_A^2 + (R_B + R_A)^2} \right)$$ \hspace{1cm} (3.3)

Equation 3.3 was solved simultaneously for various values of $\omega$ for $R_a$. The calculated value for $R_a$ is $1.025 \pm 0.7228 M\Omega \text{cm}^2$. A table of all equivalent impedance values is compiled in Table 3.1. These values were obtained using Nova Software from EcoChemie.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Equivalent Circuit Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypyrrole(DBS)</td>
<td>$R_p$</td>
<td>$1.7847 \pm 0.1735 \Omega \text{cm}^2$</td>
</tr>
<tr>
<td></td>
<td>$C_p$</td>
<td>$1.2673 \pm 0.1823 \mu F/cm^2$</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>$R_{DI}$</td>
<td>$1.7398 \pm 0.6407 \Omega \text{cm}^2$</td>
</tr>
<tr>
<td>DPhPC Bilayer Membrane</td>
<td>$R_b$</td>
<td>$1.654 \pm 1.9894 M\Omega \text{cm}^2$</td>
</tr>
<tr>
<td></td>
<td>$C_b$</td>
<td>$1.1221 \pm 0.239 \mu F/cm^2$</td>
</tr>
<tr>
<td>Alamethicin</td>
<td>$R_a$</td>
<td>$1.025 \pm 0.7228 M\Omega \text{cm}^2$</td>
</tr>
</tbody>
</table>
3.4 Results and Discussion

A DPhPC bilayer membrane with reconstituted alamethicin has been supported by a suspended polypyrrole film and electrochemically characterized. In the absence of a voltage gated protein, it is observed that the bilayer membrane has a higher electrochemical impedance. A bilayer membrane reconstituted with alamethicin provides a method of controlling ion transport across a membrane through an applied electrical potential. The entire integrated ionic device has been represented with an equivalent circuit and electrically characterized through equivalent impedance values.
Chapter 4

Conclusions

This thesis has demonstrated a fabrication of a suspended conducting polymer film supporting a bioderived membrane, and the electrochemical characterization of the integrated components. The completion of the primary research goals furthers the scientific understanding of conducting polymers and bioderived membranes. The research goals are reiterated below and expanded upon as a result of this work.

4.1 Summary of Accomplishments and Contributions

• Fabricate a suspended conducting polymer film

The fabrication steps for a silicon substrate as a platform for a suspended conducting polymer film was demonstrated in Chapter 2. A micron-scale pore was etched through a silicon chip and processed to form an electrochemically insulating surface with evaporated gold arms designed for a suspended polypyrrole film. The suspended polypyrrole bridge was electrodeposited between the gold arms, across the span and created two distinctly separate aqueous chambers. The architecture of the device was verified via optical microscopy, and morphology of the suspended polymer was analyzed via scanning electron microscopy. The polypyrrole films were characterized via cyclic voltammetry and electrochemical impedance spectroscopy in order to determine oxidation and reduction potentials, and demonstrate the relationship between ionic concentration and electrochemical response. Further characterization included electrochemical impedance spectroscopy to determine equivalent resistance and capacitance.
• *Demonstrate a supported bioderived membrane*

In chapter 3, bilayer membranes were self-assembled and analyzed for their ability to provide selective ion transport. A bilayer membrane consisting of DPhPC lipid molecules, was self-assembled on top of the suspended polypyrrole bridge via vesicle fusion. The bilayer membrane formation was verified through electrochemical impedance spectroscopy and cyclic voltammetry and demonstrated to have an increased complex resistance several orders of magnitude higher than a polypyrrole film. Successful membrane formation demonstrates the validity of suspended polypyrrole films as platforms for an integrated ionic device based on surface smoothness of the polymer film, hydrophilic interactions between the bilayer membrane and polymer layers, and surface interactions between the constituent layers and the silicon substrate. A bilayer membrane was then reconstituted with alamethicin and reformed on top of the conducting polymer film via vesicle fusion. The bioderived membrane was verified with electrochemical impedance spectroscopy and cyclic voltammetry as well as analyzed for ion transport through protein channels via chronoamperometry and cyclic voltammetry. A comparative analysis between a bioderived membrane and a bilayer membrane demonstrated a lower effective impedance, a positive electrical bias, and an increased current for an applied voltage across the membrane. Multiple conductance states in the bioderived membrane were shown through chronoamperometry.

• *Electrical equivalents for component layers*

An equivalent circuit based on electrochemical theory was produced and presented as a model for the integrated device in Chapter 3. The original equivalent circuit models for a conducting polymer and bioderived membrane were combined in series with a pore, electrolyte, and deionized water layer resistance in order to more accurately model the device architecture. Electrochemical impedance spectroscopy over several trials provided data to solve for equivalent resistance and capacitance values for each constituent layer using complex impedance theory. The calculated equivalent values were comparable to those in current literature for similar materials.

In addition to achieving the primary goals of fabricating and characterizing each layer of an integrated ionic device, this thesis presented a novel architecture for an electrochemical sensor. The function of each layer in the presence of an electrolytic solution with Na⁺ ions
and applied electrical potential was confirmed. We showed an excellent agreement between the experimental values and previous studies on the chemoelectroactive response for each layer. A critical result was presented in Chapter 2 in which a suspended polypyrrole film showed a direct relationship between ionic concentration and maximum peak during cyclic voltammetry, demonstrating sensing capabilities. Chapter 3 provided a result validation by demonstrating the ability of a bioderived membrane to provide selective transport when being supported by a conducting polymer film.

4.2 Recommendation for Continuation and Further Improvement of this Work

This work shows a significant accomplishment and can be expanded upon to further progress the scientific field. Research suggestions are listed below as methods for continuing this work.

- **Perform validation experiments to show concurrent ionic activity between the polypyrrole film and bioderived membrane**

  A direct next step from this work is the experimental validation of transistor function. Concurrent measurement of ion transport through a bioderived membrane and causing doping/undoping reactions in conducting polymers allows for highly sensitive electrochemical and bioanalytic sensing applications. This series of experiments would also validate the concept of an ionic computing element, which could be expanded to the creation and integration of other ionic computing elements.

- **Expand the electrochemical model**

  The equivalent circuit characterization for the integrated ionic device is sufficient to understand the input-output relationships between applied electrical potential and induced currents but does not cover the biophysics of membrane transport, transistor function, or behavior based on thermodynamic principles. A model based on membrane biophysics would give a clear understanding of how ion transport through alamethicin transporters functions as a result of intermolecular forces acting on the molecules involved in transmembrane protein transport. This model would further
validate the existence of single or multiple alamethicin channels in the integrated device as well as provide an accurate estimate of the amount of electrochemical activity across the membrane based of the magnitude of electrical potential and ionic concentration required to form ion-transporting channels. The molecular visualization program VMD with the molecular dynamics simulator NAMD provides a platform to create and test the model.

Further modification of the equivalent circuit for the integrated ionic device through the addition of a transistor element would allow for an increased understanding of ionic transistors. It would provide a method of validating the current equivalent circuit by analyzing the overall transistor function of the device and comparing it to the equivalent complex impedances of the individual layers. This understanding would allow for the prediction of hybrid computing element behavior in integrated hybrid circuits.

- **Investigate the miniaturization of device fabrication**

Producing a nanoscale device with similar constitutive layers would validate bioanalytical applications as well as *insitu* chemical detection applications. It is well established that conducting polymers can be synthesized as small as 1D nanowires, and NEMS fabrication methods allow for nanoscale pores etched into silicon substrates. The fabrication of this device would allow for novel sensing applications such as in situ measurement for drug delivery systems and monitoring sodium concentrations with a noninvasive sensing element. Appropriate scaling studies would predict the behavior of the sensing element based on operative surface area.

- **Investigate the behavior of various types of protein and polymer combinations**

There is shared ionic activity in a number of proteins and conducting polymers. As shown in Chapter 1, reconstituting a ligand-gated protein into a bilayer membrane allows for the creation of various sensing elements based on the physics of operation for conducting polymers and bioderived membranes. Integrated devices using conducting polymers and appropriately functionalized protein would a method to detect volatile organic compounds. The investigation of a multiple combinations of proteins and conducting polymers with common physics of operation would create a wide-scale set of real time sensing capabilities.
Bibliography


