Chemoelectromechanical Actuation in Conducting Polymer Hybrid with Bilayer Lipid Membrane

Hao Zhang
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Chemoelectromechanical Actuation in Conducting Polymer Hybrid with Bilayer Lipid Membrane

by

Hao Zhang

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

Doctor of Philosophy

in

Mechanical and Nuclear Engineering

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May 2013
Richmond, Virginia

Keywords: conducting polymer, BLM, ion channel, ion transport

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Chemoelectromechanical Actuation in Conducting Polymer Hybrid with Bilayer Lipid Membrane

Hao Zhang, Ph.D. Candidate
Virginia Commonwealth University, 2013
Advisor: Vishnu Baba Sundaresan

ABSTRACT

Biological and bio-inspired systems using ion transport across a membrane for energy conversion has inspired recent developments in smart materials. The active mechanism in bioderived materials is ion transport across an impermeable membrane that converts electrochemical gradients into electrical and mechanical work. In addition to bioderived materials, ion transport phenomenon in electroactive polymers such as ionomeric and conducting polymers produces electromechanical coupling in these materials. Inspired by the similarity in transduction mechanism, this thesis focuses on integrating the ion transport processes in a bioderived material and a conducting polymer for developing novel actuation systems. The integrated membrane has a bilayer lipid membrane (BLM) formed on a conducting polymer, and the proteins reconstituted in the BLM regulate ion transport into the conducting polymer. The properties of the polymer layer in the integrated device are regulated through a control signal applied to the bioderived layer and hence the hybrid membrane resembles an ionic transistor. Due to the bioderived nature of this device, it is referred to as a bioderived ionic transistor. The research carried out in this thesis will demonstrate the fabrication, characterization and design limitations for fabricating a chemoelectromechanical actuator using the BIT membrane.

The BIT membrane has been fabricated using BLM (DPhPC) reconstituted with protein (alamethicin) to gate Na\(^+\) transport into conducting polymer membrane
(PPy(DBS)). In this membrane, the biodevived layer is fabricated with proteins by vesicle fusion method and conducting polymer is fabricated by electropolymerization. The biodevived layers, the conducting polymer layers and the hybrid membrane are characterized using electrochemical measurements such as cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy.

The fabrication, characterization and design effort presented in this thesis focuses on the integration of ion transport through the biodevived membrane into volumetric expansion and bending actuation. The characterization efforts are supported by empirical and physics-based models to represent the input-output relationship for both PPy(DBS) actuator and biodevived membrane, and design rules for the proposed actuation platforms are specified. The electropolymerized PPy(DBS) actuator is anticipated to be used in a bicameral device with the chambers kept separated by the DPhPC-alamethicin biodevived membrane. The relationship between the gradient potential, ionic current through the gate, ion concentration, ion transport coefficient in the conducting polymer layer, and the induced tip displacement in the polymer has been concluded from experiments and fitted to the actuation system model. This thesis will also address future directions for this research and anticipated applications for this hybrid actuation concept, such as artificial muscle, drug delivery.
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Nomenclature

\[ A \quad \text{Cross-section area of ion channel} \]
\[ A_p \quad \text{Cross-section area of the pore} \]
\[ C \quad \text{Capacitance of the equivalent circuit} \]
\[ D \quad \text{Diffusion coefficient} \]
\[ E \quad \text{Young’s modulus} \]
\[ F \quad \text{External force} \]
\[ I \quad \text{Second moment of area} \]
\[ L \quad \text{Length of the beam} \]
\[ M \quad \text{Bending moment} \]
\[ N_A \quad \text{Avogadro constant} \]
\[ P_o \quad \text{Average fraction of channels that are open (the probability that a given channel will be open)} \]
\[ Q \quad \text{Exchanged Charge through the membrane} \]
\[ \nabla [U] \quad \text{Potential gradient across the membrane} \]
\[ V \quad \text{Applied transmembrane potential} \]
\[ \Delta V \quad \text{Applied voltage through PPy} \]
\[ V_c \quad \text{Nernst equilibrium potential} \]
\[ R \quad \text{Resistance of the equivalent circuit} \]
\[ T \quad \text{Absolute temperature} \]
\[ Z \quad \text{Impedance of the equivalent circuit} \]
\[ \delta_0 \quad \text{Free displacement} \]
\[ \alpha \quad \text{Ion transport coefficient} \]
\[ \sigma \quad \text{Stress induced by exchanged charge} \]
\( \rho \)  
Radius of the beam

\( \theta \)  
Slope of the beam

\( \kappa \)  
Curvature of the beam

\([c]\)  
Ion concentration

\( c(t) \)  
Ion concentration

\( c_o(t) \)  
Ion concentration in outer chamber (outside of the membrane)

\( c_i(t) \)  
Ion concentration in inner chamber (inside of the membrane)

\( c_{o0} \)  
Initial value of ion concentration in outer chamber

\( c_{i0} \)  
Initial value of ion concentration in inner chamber

\( d_p \)  
Length of the pore

\( e \)  
Elementary charge

\( h_1 \)  
Thickness of polycarbonate layer

\( h_2 \)  
Thickness of the beam

\( i_{vg}(t) \)  
Ionic current through the protein transporter via voltage-gated diffusion

\( k \)  
Boltzmann’s constant

\( q_s \)  
Electrical charge of the charged ion species

\( t \)  
Time

\( u_s \)  
Electrical mobility of the charged ion species

\( u(x) \)  
Displacement in 'z' direction

\( u(L) \)  
Free displacement

\( \Delta v \)  
Volume change

\( v_o \)  
Volume of outer chamber electrolyte

\( v_i \)  
Volume of inner chamber electrolyte

\( v_{PPy} \)  
Volume of PPy

\( w \)  
Width of the beam

\( w \)  
Frequency

\( x, y, z \)  
Spatial coordinate

\( z \)  
Valence of the ion
Chapter 1

Introduction and Literature Review

1.1 Conducting Polymers

Conducting polymers are a unique category of polymers that exhibit full range of conductivities from insulator to superconductor depending on their doping state.

Electrical conductivity in polymers was discovered by Walatka et al. in 1973 [219], and superconductivity phenomenon of polysulfur nitride (SN)$_x$ below 0.3K in 1975 [63] led to a wave of interest in the scientific community towards the study of conducting polymers. These investigations resulted in the discovery of changes in electrical conductivity of (SN)$_x$ doped with bromine at room temperature [202]. Subsequently, several new classes of conducting polymers were reported: polyacetylene (CH)$_x$ doped with I$_2$ or AsF$_5$ in 1977 [32], polypyrrole doped with BF$_4$ in 1979 [43], poly(p-phenylene) doped with AsF$_5$ in 1979 [85], poly(phenylene vinylene) doped with AsF$_5$ in 1979 [225], polyphenylene sulfides doped with AsF$_5$ in 1981 [185]. The significance of this discovery and subsequent fundamental understanding was recognized with Nobel Prize in 2000 for Alan MacDiarmid, Hideki Shirakawa and Alan Heeger.

The chemical structures of conducting polymers play an important role in
Figure 1.1: The backbone structure of common conducting polymers.

Figure 1.2: Conductivities of common conducting polymers compared with other classical materials. The red line indicates the conductivity increases with the doping level. The lower value of the line is corresponded to the undoped state, and the upper value is to the doped state.
their electrical properties. The alternating single and double bonds in the backbone of the polymers offer the capability to release or capture electrons and lead to the semiconductor-like response. The single bonds, and one bond of the double bonds are formed as sp² hybridized covalent bonds, called σ bonds, which have low mobility and high stability. The second bond of the double bonds is formed by pₓ orbitals, which are perpendicular to the other three σ bonds around one carbon atom. These two parallel pₓ orbitals, referred to π bonds, have high mobility and low stability.

In the neutral state, conducting polymers have a large energy-gap between their conduction band and valence band (>2eV) and hence behave like an insulator. If a positive potential is applied, electrons egress from the backbone, which are first removed from the pₓ orbital resulting in partial of the orbitals to be emptied. Thus, the electrons become mobile leading to the formation of charge carriers such as solitons, polarons, bipolarons, etc along the chain. The conducting polymer backbone in this state is saturated with charges and become conductive, and this electron-egress process is a oxidation process. When a negative potential is applied, electrons are added to the pₓ orbitals of the backbone. The backbone of conducting polymer accumulates charges until saturated and behaves as a conductor, and this electron-ingress process is called reduction. If the reduction and oxidation (redox) of the conducting polymer is carried out in an electrolyte, the electron egress and ingress drive ion transport between the polymer and the electrolyte to maintain electroneutrality. The ions exchanged by the backbone with the electrolyte serve as dopants and regulate
Conducting polymers are classified into two kinds based on the positive or negative charge on the backbone.

In conducting polymers with positive charge on the backbone, anions (serve as dopants) compensate for the positive charges and lead to a partially oxidized backbone as shown in Figure 1.4(a). This is p-doping by chemical and/or electrochemical processes [121]. When the anion $D^-$ is small and mobile, the anions are incorporated in the polymer during oxidation. During reduction they are dominantly expelled from the polymer, resulting in a contraction at the backbone. The redox process occurring in the polymer backbone is shown in Equation 1.1.

$$P^+ (D^-) + e^- \rightleftharpoons P + D^- \quad (1.1)$$

The volumetric strain generated by the polymer during this contraction depends on the size of the anion and molecular weight of the polymer. In Equation 1.1, $P^+$ represents the positive charged (doped), or oxidized state of the polymer; $D^-$ is
the anion as a dopant to be incorporated in the polymer; \( P \) stands for the undoped, reduced, or neutral state of the polymer.

If a large anion \( D^- \) is incorporated into the polymer during polymerization, it remains trapped in the polymer chain. An electrical field applied to such a polymer leads to cation exchange between the polymer backbone and the electrolyte. A schematic of this mechanism is shown in Figure 1.4(a). The redox reaction as shown in Equation 1.2 due to cation exchange between the anion-doped polymer and the electrolyte results in cyclic volumetric change in the polymer and leads to bulk strain.

\[
P^+(D^-) + C^+ + e^- \rightleftharpoons P(DC)
\]  

Equation 1.2

In Equation 1.2, \( P(DC) \) indicates reduced polymer backbone due to the cation entering the polymer.

In the case of the medium-sized anions the reactions shown in Equation 1.1 and Equation 1.2 occur simultaneously, causing contraction and expansion in the polymer. Since it is highly difficult to regulate ion transport into and out of the polymer, such a selection of monomer and ions is undesirable for engineering applications [192].

In conducting polymers with negative charge on the backbone, cations (serve as dopants) compensate for the negative charges and lead to a partially reduced backbone in Figure 1.4(b). This is n-doping by chemical and/or electrochemical processes [121]. The electrochemomechanical response from cation-doped polymers leads to the exchange of dopant cation (in the case of a small dopant), leads to the exchange of dopant and anions (in the case of medium-size dopant) and anion exchange (in the case of large cation as a dopant) with the electrolyte. This response is similar to anion-doped polymer backbone and couples the electrochemomechanical properties of the polymer.

1.1.1 Synthesis of Conducting Polymers

Conducting polymers can be fabricated by various methods, such as chemical polymerization, electrochemical polymerization, casting polymerization, plasma polymer-
Table 1.1: Synthesis methods of common conducting polymers

<table>
<thead>
<tr>
<th>Synthesis Methods</th>
<th>Conducting Polymers*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical polymerization</td>
<td>PPy</td>
<td>Machida[123], Kudoh[104]</td>
</tr>
<tr>
<td></td>
<td>PANI</td>
<td>Macdiarmid[122], Cao[24],</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sun[204], Jin[92], Alvarez[7]</td>
</tr>
<tr>
<td></td>
<td>PA</td>
<td>Shirakawa[189]</td>
</tr>
<tr>
<td></td>
<td>PTh</td>
<td>Yamamoto[230]</td>
</tr>
<tr>
<td></td>
<td>PPP</td>
<td>Ivory[85]</td>
</tr>
<tr>
<td></td>
<td>PPS</td>
<td>Gagnon[56]</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>Mcdonald[130], Wnek[225]</td>
</tr>
<tr>
<td>Electrochemical Polymerization</td>
<td>PPy</td>
<td>Diaz[42], Penner[160], West[223], Otero[154]</td>
</tr>
<tr>
<td></td>
<td>PANI</td>
<td>Nayak[143], Nedungadi[144], Eftekhari[47], Conroy[36]</td>
</tr>
<tr>
<td></td>
<td>PTh</td>
<td>Lukkari[118]</td>
</tr>
<tr>
<td>Casting polymerization</td>
<td>PPy</td>
<td>Fang[49]</td>
</tr>
<tr>
<td></td>
<td>PANI</td>
<td>Pud[165]</td>
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<td></td>
<td>PPS</td>
<td>Hawkins[73], Mazurek[129]</td>
</tr>
<tr>
<td>Phase polymerization</td>
<td>PPy</td>
<td>Mohammadi[135], Kim[101]</td>
</tr>
<tr>
<td></td>
<td>PANI</td>
<td>Cruz[37]</td>
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<tr>
<td></td>
<td>PTh</td>
<td>Sadhir[177]</td>
</tr>
<tr>
<td>Langmuir-Blodgett technique</td>
<td>PANI</td>
<td>Granholm[62]</td>
</tr>
<tr>
<td>Self-assembly technique</td>
<td>PPy</td>
<td>Onoda[149]</td>
</tr>
</tbody>
</table>

*Abbreviations refer to Figure 1.1

Chemical polymerization is one of most popular methods for the synthesis of conducting polymers. The monomers are oxidized in appropriate solution by these oxidants and are able to drive the chemically active cation to form the polymers. The oxidants of chemical polymerization are relatively strong chemical oxidants like ammonium persulfate ((NH₄)₂S₂O₈) [204], arsenic pentafluoride (AsF₅) [225, 85, 56], iron(III) chloride (FeCl₃) [204], permanganate (MnO₄⁻) [230, 123], dichromate (Cr₂O₇²⁻) [189],...
hydrogen peroxide (H₂O₂) [204]. It can take place as bulk polymerization or surface-localized polymerization [126]. Chemical polymerization carried out in the bulk of the solution typically precipitates the polymer as insoluble solids. If a surface coating of the polymer is preferred, the surface is pretreated with a solution containing either the oxidizing agent or the monomer. Thus, polymerization takes place mainly on the surface rather than in the solution. In some applications, poor adherence of deposited conducting polymer on the surface becomes a major obstacle. In such cases, electrochemical polymerization is preferred.

Polyacetylene (PA) was chemically synthesized in 1967 in the form of a thin film with a high concentration of the Ziegler-natta catalyst, and doping of bromine was carried out in 1976 [189]. Poly(p-phenylene) (PPP) is prepared by oxidative cationic polymerization by using benzene and doped with arsenic pentafluoride (AsF₅) [85]. Poly(p-phenylene vinylene) (PPV) is synthesized via a Wittig reaction [130] and doped with arsenic pentafluoride (AsF₅) [225]. Poly(p-phenylene sulfide) (PPS) is synthesized from the monomer and doped with arsenic pentafluoride (AsF₅), sulfuric acid (H₂SO₄), and sodium naphthalide in tetrahydrofuran (THF) [56]. Polythiophene (PTh) is chemically synthesized by using magnesium in tetrahydrofuran (THF) and nickel(bipyridine) dichloride [230]. Polyaniline (PANI) is synthesized by numerous oxidizing agents like ammonium persulfate ((NH₄)₂S₂O₈), iron(III) chloride (FeCl₃), potassium permanganate (KMnO₄), potassium dichromate (K₂Cr₂O₇), potassium iodate (KIO₃), potassium bromate (KBrO₃), potassium chlorate (KClO₃), hydrogen peroxide (H₂O₂) [122, 24, 204]. Oxidative polymerization is also employed with immobilized horseradish peroxidase (HRP) enzyme to form a water-soluble PANI [92, 7]. Polypyrrole (PPy) is obtained using iron(III) sulfate (Fe₂(SO₄)₃) as an oxidant and an anionic surfactant by chemical synthesis [104].

**Electrochemical Polymerization**

Electrochemical polymerization is preferred as a general method for preparing conducting polymers due to its simplicity and reproducibility. This approach uses oxidation of monomers through oxidative coupling and successive repetitions to form
the polymers. This technique [42] uses a three-electrode configuration as shown in Figure 1.5. A working electrode (WE), a counter electrode (CE), and a reference electrode (RE) are submerged in the electrolytic bath for polymerization [42, 160]. The anodes (i.e., WE) are usually conducting, inert materials such as chromium, gold, nickel, palladium, titanium, platinum, and indium-tin oxide coated glass plates, semiconducting materials such as n-doped silicon, gallium arsenide, cadmium sulphide, and semi-metal graphite. The commonly used cathodes (i.e., CE) are conducting materials similar to the anodes such as gold, platinum, nickel. The RE could be saturated calomel electrode (SCE), Ag/AgCl electrode, and silver wire. The monomer and a supporting electrolyte dissolved in appropriate solvent constitute the electrical bath. The heterocyclic and aromatic monomers that could be polymerized include pyrrole, bipyrrrole, terpyrrole, thiophene, biothiophene, terthiophene, azulene, pyrene, carbozole, fluorene, fluoranthene, aniline. The choice of electrolyte solution depends on solubility, degree of dissociation, and nucleophilicity. Some examples of dopant ions include dodecylbenzene sulfonate (DBS$^-$), tetrafluoroborate (BF$_4^-$), hexafluorophosphate (PF$_6^-$), perchlorate (ClO$_4^-$), chloride (Cl$^-$), bromide (Br$^-$), iodide (I$^-$), arsenic pentafluoride (AsF$_5^-$), hydrogen sulphate (HSO$_4^-$), trifluoromethanesulfonic (CF$_3$SO$_3^-$), tosyl (CH$_3$C$_6$H$_4$SO$_3^-$), sulphate (SO$_4^{2-}$) [118, 223, 47]. Electrochemical polymerization can be operated potentiostatically (i.e., constant potential over time), galvanostatically (i.e., constant current over time), potential scanning/cycling, or potentiodynamic (i.e., time varying potential). Investigations on mechanism of the chemical reaction and kinetics of the growth on a conducting surface have shown that factors, such as composition of the solution, density of the solution, conductivity of electrode material, state of the electrode surface, distances between electrodes, applied voltage, current density, and temperature, are of significance for polymerization.

The electrical hardware required for electrochemical polymerization allows for precise control of applied voltage and currents between the WE and RE. This enables the fabrication of films of desired thickness. Entrapment of desired materials like small molecules, proteins, DNA, etc. in conducting polymer is achievable by electrochemical
method so that it can be applied to a wide range of applications.

Polypyrrole is one of the most popular conducting polymers produced by electrochemical polymerization. Diaz et al. [42] and Penner et al. [160] synthesize PPy by electrochemical polymerization on platinum using tetrafluoroborate (BF$_4^-$) as a dopant. Polypyrrole doped with lithium perchlorate (LiClO$_4$) is electrochemically synthesized and investigated with various parameters such as current density [223], temperature, concentration, etc. [154]. Polyaniline is fabricated by electrochemical polymerization in the liquid-solid interface (one phase comprises a polar solvent with electrolytes and the other consists of a non-polar solvent with monomer) by Nayak and Bhakta [143], Nedungadi [144]. Polyaniline is prepared from aniline in sulfuric acid solution for enzyme-modified coating at an aluminum electrode using electrochemical polymerization [47]. Polyaniline is directly electrochemically deposited at aluminum from a p-Toluensulfonic acid solution [36]. Polythiophene is electrochemically synthesized in solutions of tetrabutylammonium hexafluorophosphate (Bu$_4$NPF$_6$) and 3-methylthiophene in acetonitrile [118].

**Casting Polymerization**

Casting polymerization uses the evaporation of solvent to crosslink monomers into polymers. The solution containing monomers or precursors is spread or spin-coated on the surface of materials such as plastics, glass, metals, as well as micro/nano porous materials. The conducting polymer is formed by evaporating the solvent of the solution. The main difficulty of casting method is that almost conducting polymers

---

**Figure 1.5: Three-electrode configuration of electrochemical polymerization**

[Diagram of a three-electrode configuration with labels: Working Electrode, Counter Electrode, Reference Electrode, and Electrolyte.]
are insoluble, non-melting in most solvents of practical interest. Improvement has been achieved by mixing with other soluble materials, as well as by the chemical modification of parent molecules to get soluble derivatives. Besides the problem of poor solubility, the other difficulty is the uniformity of the coating on the surface, which is of great importance for some situations.

Polyaniline is synthesized mostly by casting polymerization. Monomer is dissolved in solvent such as N-methyl-pyrrolidinone (NMP), and then the solution is spread and evaporated on a solid substrate under an lamp [165]. Polypyrrole film is deposited on silicon substrates by spin coating pyrrole with chemical oxidants FeCl₃ and then irradiated using UV lamps for several minutes at room temperature [49]. The thickness of the films was controllable within the range from hundreds of nanometers to thousands of nanometers, while the surface quality and morphology were modified by change of excitation light wavelength in photolithographic processes. Poly(p-phenylene sulfide) films were spin cast onto interdigitated electrodes patterned on an oxidized silicon substrate and cured to 0.1-0.2 µm thickness[73, 129].

**Vapor/gas-phase Polymerization**

Vapor-phase polymerization makes use of chemical vapor deposition to obtain the coating of conducting polymers on a substrate. It works by exposing the substrates with monomers and oxidants in the vapor-phase. Thin films with good adhesion and conductivity can be achieved, but it needs the pretreatment of the substrates, such as mixing or dipping the substrates in electron acceptors/initiators. Precise control of the pressure and the amount of oxidant is required to achieve the appropriate deposition. Some groups including Mohammadi et al. [135], Machida et al. [123], and Kim et al. [101], demonstrated the vapor-phase polymerization with dry distilled pyrrole using FeCl₃ as a oxidant.

Gas-phase plasma polymerization works by the chemical reaction of various molecules in plasma environments and the deposition of a thin conducting polymer film on a substrate. This plasma assisted deposition process is a solvent-free, room temperature procedure and can be used to deposit a thin film with thickness in the
range from nanometers to microns onto most substrates. It is hard to verify the exact chemical structure presenting in the deposited surface layer due to the various reactive elements in the plasma. Their structures and properties depend on a large number of factors, e.g., the structure of monomer, gas phase composition, flow rate, pressure, the geometry of reaction cells. However, the surfaces of conducting polymer prepared by plasma method are highly cross-linked and higher temperature and chemicals resistance. Polyaniline was synthesized by plasma polymerization and the PANI thin film was adhered to glass and metal surfaces [37]. Sadhir et al. [177] carried out plasma polymerization of polythiophene.

**Langmuir-Blodgett Technique**

Langmuir-Blodgett (LB) films are typically prepared by delivery of monolayers of amphiphilic molecules formed at the air-water interface onto solid substrates. First, the amphiphilic molecules are dissolved in an organic solvent, then the monolayer is formed by compressing and decreasing the area in which the molecules are confined on the water surface. Last, by moving the substrate vertically or horizontally, an ultra-thin film is obtained through transferring onto a solid substrate. The thickness of the thin film is controllable at molecular level. Polyaniline doped by protonic acids such as camphorsulfonic acid (HCSA) or dodecylbenzenesulfonic acid was dissolved in common organic solvents and the PANI film was formed directly by LB technique using this solution [62].

**Self-assembly Technique**

Self-assembly technique takes advantage of a strong interaction between the surface of the substrate and a functional group of the adsorbent, e.g., sulphur-gold interaction, to drive self-assembly process. The films are prepared by transferring the aqueous solution containing self-assembling materials to the surface of a solid substrate, and subsequently, a highly organized molecular layer forms spontaneously on the substrate. Various functional groups can be exploited into the monolayer without disturbing the formation of the self-assembly molecules. The aqueous solution usually
contains a positively charged polymer, i.e., polycation, or a negatively charged polymer, i.e., polyanion, by applying appropriate acidic solution. If molecular layer by layer is desired, the substrate is processed by dipping in polycation solution, polyanion solution, successively, or reversely. Eventually, the molecules held by electrostatic attraction of alternately charged polymers are forced to form the bilayer or multiple layers. The growth of polypyrrole on sulfonated low-density polyethylene (SPE) substrate by the self-assembly process was observed by Onoda [149].

Other Nanoscale Techniques

Traditional chemical or electrochemical polymerization methods are modified and used with nanosubstrate formation techniques. Hard-template, e.g., porous membranes, nanofibers, colloidal particles, and soft-template, such as micelles, nano wires, monomer droplets, aligned nanowire arrays, are popular methods for synthesis of conducting polymer nanostructures [229]. Ultrasonic excitation [97], emulsion polymerization [232], vapor deposition [89], electrospinning [140] and co-evaporation electrospinning [221] have also been used as versatile approaches for the preparation of conducting polymer nanoscale structures.

1.1.2 Characterization of Conducting Polymers

Techniques that have been developed to determine the properties and mechanism of conducting polymers are listed in Table 1.2, including electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), chronamperometry (CA), piezoelectric microgravimetry at electrochemical quartz crystal microbalance (EQCM), probe beam deflection (PBD), spectroscopies, microscopies. These intrinsic properties and behaviors of the conducting polymers contain the rate of the charge transfer, the ionic charge transport processes, the chemical reaction mechanism, kinetics and equilibrium of switching, and redox transformation. Despite dedicated efforts by various groups to comprehend the dynamic and static properties of the conducting polymer backbone and dopants, a detailed understanding and a generic model to represent
these system is lacking due to diversity and complexity. The conformation changes induced by dimerization, cross-linking, ion-pair formation, and also polymeric properties derived from backbone chain, segmental motions, morphology, relaxation are not clear [84] owing to the limit of current technique.

**Electrochemical Impedance Spectroscopy**

Electrochemical impedance spectroscopy (EIS) is one of the most effective and reliable methods to extract the electrical equivalents of an electrochemical system. This method is applied by giving a small amplitude of the potential or current such as a single sine wave, and the output is the response of the impedance magnitude and the phase shifts with different frequencies. This technique provides electrical equivalents that represent the double-layer capacitance, solution resistance, diffusion impedance, and the rate of charge transfer. For conducting polymer system, EIS is an effective tool to estimate the resistance and capacitance of each layers or subsystems and provides valuable informations about the ionic and electronic charge transport processes [231, 95, 236]. It was found out that the reduced form of polypyrrole film could be represented by a Randlles-type equivalent circuit [160]. The ion transport in polypyrrole film in solid cells is revealed as a semi-infinite diffusion process [50]. The diffusion coefficient of polypyrrole is estimated by fitting EIS measurement with the admittance model to be $1.4 \times 10^{-12} \text{ m}^2/\text{s}$ [124]. The ionic conductivities of polypyrrole poly(styebesulfonate) (PPy(PSS)) and polypyrrole perchlorate (PPy(ClO$_4$)) films indicates that anion transport is primarily responsible for the ionic conductivity of PPy(ClO$_4$), while cation transport dominates in PPy(PPS), and Na$^+$ in PPy(PSS) is more mobile than ClO$_4^-$ in PPy(ClO$_4$) [169].

**Cyclic Voltammetry**

Cyclic voltammetry (CV) is a useful diagnostic technique for the study of the electrochemical behavior of a system. It works though a potential sweep with recording
<table>
<thead>
<tr>
<th>Characterization Methods</th>
<th>Properties</th>
<th>Conducting Polymers*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIS</td>
<td>Capacitance and resistance (impedance)</td>
<td>PPy</td>
<td>[160],[50],[95],[124]</td>
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<td>CV</td>
<td>Volume change correlated with potential and current</td>
<td>PPy</td>
<td>[160],[169],[154],[188],[237],[64],[194]</td>
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<td>CA</td>
<td>Electrical charges</td>
<td>PPy</td>
<td>[169],[64]</td>
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<td>Spectroscopic method</td>
<td>Visible</td>
<td>Energy band gap</td>
<td>PPy, PTh</td>
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<td>Chemical bond and molecular structure</td>
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<td>[100]</td>
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<td>Quantitative determination</td>
<td>PPy</td>
<td>[31],[92],[100],[113],[80]</td>
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<td>XPS</td>
<td>Atomic composition</td>
<td>PPy</td>
<td>[169]</td>
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<tr>
<td>NMR</td>
<td>Structures, dynamics, reaction state of atoms or molecules</td>
<td>PANI</td>
<td>[134]</td>
</tr>
<tr>
<td>Microscopy</td>
<td>AFM</td>
<td>Surface 3D topography</td>
<td>PPy</td>
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<tr>
<td>SEM</td>
<td>Surface 2D topography and electrical conductivity</td>
<td>PPy</td>
<td>[188]</td>
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<tr>
<td></td>
<td>TEM</td>
<td>Thin specimen 3D image</td>
<td>PANI</td>
</tr>
<tr>
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<td>Mass change</td>
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<tr>
<td></td>
<td>PBD</td>
<td>Ion exchange</td>
<td>PPy</td>
</tr>
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</table>

*Abbreviations refer to Figure 1.1
of the current-voltage curves. The input signal is a cyclic waveform of potential with appropriate linear scan rate ranging from 10 mV/s to 1000 V/s [12]. The scan starts at an initial potential moving in positive or negative direction and reverses at a switching point and returns to the initial potential. The recorded current is a function of potential, and it is useful in obtaining information about complicated reaction systems [175]. Thus, CV is a reliable tool to understand the redox behavior of the conducting polymers [160, 154, 237]. It was proven that the voltammetric peaks in CV measurement for polypyrrole increases with increasing scan rate, and the subsequent calculation indicated that every 4-5 pyrrole units carry +0.2V [169]. Redox peaks for polypyrrole doped with dodecylbenzenesulfonate (PPy(DBS)) films in various pH proved that it is easier to oxidize and reduce the films at higher pH and no ion exchange occurs below pH 3 [188]. CV is used to determine the volume change correlated with potential and current, and 40% of the height change is observed during redox of PPy(DBS) films [194]. Besides, CV is used in electrochemical polymerization of conducting polymers by Zotti et al. [238], Nunziante et al. [146], West et al. [223], and Guadarrama et al. [64].

**Chronoamperometry**

Chronoamperometry (CA) is another technique for monitoring the electronic behavior of a system. It provides the electrochemical information by a potential step with recording of the current-time curves. A series of step potentials is given for a reasonable duration with appropriate time interval. The response curve is required to build the response function and predict the system response in terms of the experimental parameters of time, potential, concentration and transfer coefficients [12]. The electrical charges stored in conducting polymer films can be obtained from CA [154]. In addition to electrochemical characterization, CA is utilized as the potentiostatic way for electrochemical polymerization of conducting polymers by Nunziante et al. [146], Otero et al. [151], and Guadarrama et al. [64].
Spectroscopic Method

Spectroscopic method takes advantage of the relations between radiated energy and corresponded wavelength or frequency, and it is used to study absorption, emission, elastic scattering and reflection, inelastic scattering (Raman and Compton scattering), resonance.

The absorption spectrum of the sample is obtained by visible light offering the energy related information of materials. In 1983, polypyrrole, polythiophene, poly(3-methylthiophene), poly(3,4-dimethylthiophene) was characterized by the visible absorption spectrum showing the maximum sensitivity at 430 nm for polypyrrole, 480 nm for both polythiophene and poly(3-methylthiophene), which corresponded to the energy band gap about 2-2.5 eV [214].

Fourier transmission infrared (FTIR) spectroscopy is used to obtain the infrared spectrum information of absorption, emission, photoconductivity, or Raman scattering in a wide spectral range by Fourier transmission techniques [100]. The comparison of polyaniline and polyaniline/poly(styrene-co-styrene sulfonate) by FTIR spectra demonstrated the successful coating of polyaniline on the surface of poly(styrene-co-styrene sulfonate) [100].

Ultraviolet-visible (UV-Vis) spectroscopy uses the absorption or reflectance of light in the ultraviolet-visible region to analyze the chemicals [31, 92, 100, 113, 80]. Polypyrrole was observed having the absorption bands in the 450-500 nm ranges, which is in accord with dominant $\pi-\pi^*$ transitions [31]. UV-Vis was performed to analyze the synthesis of polyaniline with the immobilized horseradish peroxidase enzyme in different pH solution [92]. The oxidized state and formation of polar on band of polyaniline/poly(styrene-co-styrene sulfonate) nanoparticles were verified by UV-Vis [100]. The pH sensitivity was measured by the UV-Vis spectra with the polyaniline-poly(vinyl chloride) in different buffer solution with pH from 2 to 9 [113']. UV-Vis spectra were obtained to get the information of conjugated structure of polyaniline and its patterns [80].

X-ray photoelectron spectroscopy (XPS) is a chemical analysis technique by
generating a beam of X-ray to irradiate the samples for measuring the kinetic energy and number of electrons escaped under ultra-high vacuum conditions [169, 113]. XPS was performed on polypyrrole to confirm what the ion contents of the composite in equilibrium oxidized states and reduced states [169]. Redox sensitivity of polyaniline-poly(vinyl chloride) was measured by the XPS spectra through monitoring the ionic couples [113].

Nuclear magnetic resonance (NMR) is a phenomenon induced by the magnetic properties of certain atomic nuclei. NMR spectroscopy can obtain the information about structures, dynamics, reaction state of atoms or molecules [96, 134]. Quasi-1D spin diffusion in polyaniline was investigated by NMR spectroscopy, indicating a strong correlation of transport properties and spin dynamics [134]. $^{13}$C solid-state NMR spectroscopy was applied to compare the structures in the different forms (leucoemeraldine base, emeraldine base, emeraldine hydrochloride) of polyaniline [96].

**Microscopy**

Atomic force microscopy (AFM) is a high-resolution microscopy that can image, measure and manipulate samples at the nanoscale. It consists of a cantilever with sharp tip at the end, and the tip is used to scan the specimen surface. When the tip is brought close to the surface, the force between the sample surface and the cantilever tip leads to deflection of the cantilever, and a laser is reflected from the cantilever to detector, thus, the deflection is measurable and translated into the height of the surface. AFM is used for the three-dimensional surface profile without any pretreatment of the conducting polymers with high resolution[194, 101]. AFM was used to monitor the expansion or contraction of polypyrrole doped with dodecylbenzenesulfonate films against different applied voltages. 30% to 40% volume changes were seen between oxidized and reduced states except for the first reduction cycle [194]. The morphologies of PPy films after polymerization was observed by AFM, indicating that polymerization at high temperature (above 80 °C) leads to the more uniform and compact morphologies than that of low temperature (30 °C) [101].

Scanning electron microscopy (SEM) offers an alternative route to scan the
morphology of the surfaces in two-dimension. The high-energy beam of electrons is used to get the response from interaction with sample surfaces. It is operated under the vacuum environment, and the surfaces of most samples are required to be pretreated with gold or platinum coating [238, 146, 188, 25]. Morphology of polyaniline deposited with different strong acid had various forms, e.g. using HClO$_4$ results to a compact structure, while giving H$_2$SO$_4$ leads to an open structure [238]. The morphology and the thickness of polyaniline films were evaluated by SEM, and these films through CV and CA deposition in various solution of aniline, H$_2$SO$_4$, Na$_2$SO$_4$ were used to investigate the factors to effect the thickness of the films including monocular concentration, acid concentration, acid properties, and scan rate [146].

Transmission electron microscopy (TEM) is a specific technique for viewing the ultra thin specimen. A beam of electrons transmitted through the specimen causes interaction, and the interaction is imaged by an expert observation device [100, 25]. Thin layer of polyaniline coated poly(styrene-co-styrene sulfonate) (PS-PSS) was observed by SEM to compare with original PS-PSS latexes to confirm a thin overlayer PANI deposited on the core particles with 30-50 nm diameters [100].

**Electrochemical Quartz Crystal Microbalance (EQCM)**

EQCM measures the mass change associated with the frequency change of a quartz crystal resonator. During electrochemical reaction, it allows the simultaneous measurement of mass change on the electrode and reaction rate under in-situ conditions [83]. The ion movement and the solvent content can be monitored by EQCM during redox process of conducting polymers[150, 141, 11]. The insulator-to-conductor transition was formulated at 0.2V through EQCM measurement during the redox of polyaniline [150]. Naoi et al. [141] found out the mass change of the redox process is small in contrast to the electrochemical deposition, indicating less ions insert or extract from the polymer chain during redox process. Baker et al. [11] demonstrated the mass transport of polypyrrole poly(stybesulfonate) (PPy(PSS)) films increases with addition of cation mass, indicating PPy(PSS) films are cation specific involved. Moreover, the mass change of conducting polymers related to the degree
of doping is also measurable by EQCM. A doping degree of 30% was confirmed by EQCM for dodecylsulfate doped polypyrrole (PPy(DS)) film, and DS\textsuperscript{-} stays immobilized during redox process [162]. Doping degree of polypyrrole doped with dodecylbenzenesulfonate (PPy(DBS)) was reported to be 20% [213]. The formation of columnar structure in both PPy(DS) and PPy(DBS) monitored by EQCM is associated with the abrupt increase in resonance resistance [142].

The early stage of mass change measurement is achieved by in-situ electroravimetry. The variation in weight and volume of the films in relation to charge was estimated by electroravimetry. The weight change of PANI in 1M and 0.2M LiClO\textsubscript{4}/PC non-aqueous electrolytes was 126% and 122%, respectively [147], which is similar to that of polypyrrole [191].

**Probe Beam Deflection (PBD)**

Probe beam deflection, also called optical beam deflection, is a technique that measures deflection or refraction of the probe beam by a simple light source traveling in the refractive index gradient. In principle, the different parts of a beam travel perpendicular to the gradient lines, and traverse by different refractive index causing the deviation. Since the beam from PBD is deflected toward area of higher concentration, ion flow direction is determined in ion transfer process [67, 127]. The cation exchange is verified to be dominant during the redox of PPy(DBS), and the deflection is monitored by PDB with different parameters like refractive index, concentration in various electrolytic solution. The diffusion coefficient was obtained approximately in the range of 2 to 3.6 cm\textsuperscript{2}s\textsuperscript{-1} for both anion and cation [127].

**Other Characterization Techniques**

Radiotracer technique utilizes a radioisotope substance to monitor the movement and the speed of chemical processes for conducting polymers [82, 79]. PANI was investigated by labelled HSO\textsubscript{4}\textsuperscript{-} and SO\textsubscript{4}\textsubscript{2}\textsuperscript{-} to investigate the details of the ion exchange during the redox and overoxidation [79].

Ellipsometry is used to characterize the thickness of a thin film of conducting
polymers by analyzing the change of light polarization. It was shown the thickness of PPy(ClO$_4$) changes by 30% during redox process [33, 68]. Hamnett et al. [68] used ellipsometry to study thickness changes of polypyrrole films in aqueous NaBF$_4$ solutions. The expansion during reduction was initially 12%, and the thickness in the reduced state became smaller than that in the oxidized state with redox cycling.

1.1.3 Mathematical Models of Conducting Polymers

Mathematical models for conducting polymer system are the white-box models and reusable for similar structures. Two configurations have been developed as actuators - one is free-standing linear actuators, the other is multilayer bending actuators. The goal of actuation models is to find a physics-based relation between actuation and electrical properties based on the charge transport, chemical properties related to ion transport, and mechanical properties due to polymer chain movement.

Mathematical modeling of active processes in a conducting polymer structure have been inspired by Berry et al. [18]’s framework on epoxy-based actuator. PPy(TsO)/Au/PE cantilever [156] was recognized as bilayer by ignoring the substrate and electrode (gold). Bending moment required to deform and relax the strip was investigated during reduction. It was proposed and confirmed that cation insertion, salt draining, and phase relaxation contribute to the volume change and control the rate of reduction. Mathematical models of the cation insertion, salt draining, and bending were built and validated with experimental data. However, this model was based on the assumption that strain throughout the PPy thickness is constant over various length scales and bulk Young’s modulus of PPy is applicable at microscale (<10 $\mu$m)[34].

Christophersen et al.[34] investigated the curvature of Au/PPy bilayer film as a function of the thickness of PPy and that of Au. The actuation strain and Young’s modulus of PPy(DBS) determined by the thicknesses was incorporated in the curvature model. It offered reliable Young’s modulus that can be applied to micro-scale actuation. Du et al. [46] produced a multilayer bending model that the strain
<table>
<thead>
<tr>
<th>References</th>
<th>Theory</th>
<th>Classification</th>
<th>Materials*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pei[156]</td>
<td>Bending beam method</td>
<td>Equation of motion</td>
<td>PPy(TsO)/PE</td>
</tr>
<tr>
<td>Otero[152]</td>
<td>Electrochemically stimulated conformational relaxation model (ESCR)</td>
<td>Transducer equation</td>
<td>PPy(ClO_4), PTh(ClO_4), PANI(SO_4)</td>
</tr>
<tr>
<td>Christophersen[34]</td>
<td>Bending beam theory</td>
<td>Constitutive model</td>
<td>PPy(DBS)/Au</td>
</tr>
<tr>
<td>Du[46]</td>
<td>Bending beam method</td>
<td>Constitutive model</td>
<td>PPy(DBS)/Au/kapton</td>
</tr>
<tr>
<td>Rubin[174]</td>
<td>Ionic transfer</td>
<td>Transducer equation</td>
<td>Poly(JUG-co-JUGA)/Au(thin)</td>
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</table>

<table>
<thead>
<tr>
<th>References</th>
<th>Equations</th>
<th>Parameters</th>
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<tr>
<td>Pei[156]</td>
<td>Curvature change against time</td>
<td>Diffusion coefficient of cation insertion and salt draining, linear strain of cation insertion and salt draining</td>
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<td>Bay[15]</td>
<td>Change of strip length under constant external pressure</td>
<td>Ion concentration, osmotic coefficient</td>
</tr>
<tr>
<td>Otero[152]</td>
<td>Conformational relaxation time related to molar enthalpy change</td>
<td>Closing potential, coefficient of cathodic compaction, correlation coefficient</td>
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<td>Alici[5]</td>
<td>Curvature and force output at the tip</td>
<td>Exchanged charge coefficient</td>
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<td>Alici[4]</td>
<td>Thermal strain and mechanical strain related to voltage</td>
<td>Bending angle, bending moment, temperature, coefficient of thermal expansion</td>
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<td>Christophersen[34]</td>
<td>Curvature and strain related to thickness ratio</td>
<td>Bending strain and Young’s modulus</td>
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<tr>
<td>Du[46]</td>
<td>Curvature related to strain</td>
<td>Thickness (ratio) and Young’s modulus (ratio)</td>
</tr>
<tr>
<td>Rubin[174]</td>
<td>Electrochemical impedance, electrogravimetric transfer function</td>
<td>Kinetics constant, electrolyte concentration, film thickness</td>
</tr>
</tbody>
</table>

*Abbreviations refer to Figure 1.1
could be expressed as a function of the curvature without the small angle assumption. Alici et al. [4, 131] proposed a model on a trilayer cantilever bending actuator by the finite element method. The bending angle and the bending moment were in relation to the voltage input in different temperatures. Bay et al. [15] demonstrated the free-standing PPy(DBS) films showing a considerable part of total volume change due to osmotic expansion. Alici et al. [5] developed a nonlinear model for bending actuation of a trilayer actuator. The charge transfer coefficient $\alpha$ was calculated approximately to be $0.1(F/m^2)/(C/m^3)$. Chen et al. [30] developed a model on ionic polymer-metal composite (IPMC), and added a controller for controllable state space modeling to investigate the ionic transfer of conducting polymers. Rubin et al. [174] simulated the ionic transfer through the electroactive film/electrolyte interface in a dynamic response model.

1.1.4 Application of Conducting Polymers

Conducting polymers can produce in-plane strains typically from 2% to 10% with the operating voltage no larger than 2V. Some of them can even reach to over 30% out-plane strains [193]. The Young’s modulus of conducting polymer varies 0.05GPa to 100 GPa, and the tensile strength varies 1 MPa to 1 GPa [193]. Based on the redox mechanism, the bending geometries of a conducting polymer actuator can be either full expansion/contraction, or linearly controlled at a specific value by a precise oxidation/reduction of the backbone. Conducting polymers can be operated in vivo due to their biocompatibility. Conducting polymer may locally deliver electrical stimuli to stimulate specific cell function or trigger cell responses with potential applications in wound healing, or in corporation with neuromuscular junctions to stimulate the muscle activities.

Conducting polymers have novel applications as actuators, sensors, energy harvest system, corrosion protection, electroluminescent and electrochromic devices, and micro-/nano-scale structures. The common conducting polymers, i.e. polypyrrole, polyaniline, polythiophene, poly(p-phenylene), poly(phenylene vinylene), are used
Table 1.4: Applications of conducting polymers

<table>
<thead>
<tr>
<th>Year</th>
<th>References</th>
<th>Conducting Polymers*</th>
<th>Application</th>
</tr>
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<tbody>
<tr>
<td>1980</td>
<td>Chen[29]</td>
<td>PA</td>
<td>Photoelectrochemical photovoltaic solar cells</td>
</tr>
<tr>
<td>1992</td>
<td>Otero[151]</td>
<td>PPy</td>
<td>Artificial muscles</td>
</tr>
<tr>
<td>1993</td>
<td>Smela[196]</td>
<td>PPy</td>
<td>Artificial fingers and corkscrews</td>
</tr>
<tr>
<td>1993</td>
<td>Pei[157]</td>
<td>PPy</td>
<td>Artificial muscles</td>
</tr>
<tr>
<td>1993</td>
<td>Pei[158]</td>
<td>PANI</td>
<td>Artificial muscles</td>
</tr>
<tr>
<td>1994</td>
<td>Hatfield[72]</td>
<td>PPy, PANI, PTh</td>
<td>Electronic nose</td>
</tr>
<tr>
<td>1995</td>
<td>Smela[195]</td>
<td>PPy</td>
<td>Folding of micro-structure</td>
</tr>
<tr>
<td>1995</td>
<td>Gardner[57]</td>
<td>PPy, PANI</td>
<td>Chemical and biosensors, microelectronic devices, bearing materials, and micromechanical actuators</td>
</tr>
<tr>
<td>1995</td>
<td>Kaneto[94]</td>
<td>PANI</td>
<td>Backbone-type and shell-type electrochemical actuator</td>
</tr>
<tr>
<td>1996</td>
<td>De[40]</td>
<td>PPy</td>
<td>Optical pH sensor</td>
</tr>
<tr>
<td>1997</td>
<td>Talaie[208]</td>
<td>PPy, PANI</td>
<td>pH sensor</td>
</tr>
<tr>
<td>1999</td>
<td>Smela[192]</td>
<td>PPy</td>
<td>Patterned microfabrication</td>
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<td>Guadarrama[64]</td>
<td>PPy, PANI, PTh</td>
<td>Volatile compound sensors</td>
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<td>Jager[87]</td>
<td>PPy</td>
<td>Microactuators in cell biology and biomedicine</td>
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<tr>
<td>2000</td>
<td>Jin[91]</td>
<td>PANI</td>
<td>Optical pH sensor</td>
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<td>2000</td>
<td>Stella[201]</td>
<td>PPy, PANI</td>
<td>Electronic nose for characterization of olive oil</td>
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<td>2001</td>
<td>Sotomayor[198]</td>
<td>PANI</td>
<td>Optical pH sensor</td>
</tr>
<tr>
<td>2002</td>
<td>Radhakrishnan[167]</td>
<td>PPy, PANI</td>
<td>Chemical vapor sensors for methanol, ammonia and nitrogen dioxide</td>
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<tr>
<td>2002</td>
<td>Roemer[172]</td>
<td>PPy, PANI</td>
<td>Microactuators</td>
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<td>2003</td>
<td>Shigi[187]</td>
<td>PPy</td>
<td>Imprinted sensors for structural isomer</td>
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<td>2002</td>
<td>Wang[220]</td>
<td>PANI</td>
<td>PANI integrally skinned asymmetric membranes based monolithic actuators</td>
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<td>2004</td>
<td>Hara[71]</td>
<td>PPy</td>
<td>PPy-zigzag metal wire composite film actuator</td>
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<td>2005</td>
<td>Aguilar[2]</td>
<td>PANI</td>
<td>Polymer nanojunction arrays or peptide-modified PANI arrays for detection of heavy metal ions in drinking water</td>
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<td>2005</td>
<td>Ryu[176]</td>
<td>PPy</td>
<td>PPy actuator integrated with a PVDF sensor</td>
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<tr>
<td>2007</td>
<td>Ge[58]</td>
<td>PANI</td>
<td>pH sensor</td>
</tr>
<tr>
<td>2008</td>
<td>Perera[161]</td>
<td>PPy</td>
<td>PPy/PANI based electrolyte/PPy artificial muscles</td>
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<td>2008</td>
<td>Bailey[10]</td>
<td>PPy</td>
<td>Sensors for monitoring the headspace of metabolites produced from superficial wounds and burns</td>
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<td>2009</td>
<td>Ince[81]</td>
<td>PPy, PTh</td>
<td>Chloroform vapor sensor</td>
</tr>
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</table>

*Abbreviations refer to Figure 1.1
as the active components in electrochromic displays, photolithography, rechargeable or light weight batteries, smart windows, light emitting diodes (LEDs), toxic waste cleanup, field effect transistors (FETs), electromagnetic interference (EMI) shielding, electrochemical capacitors [57, 120, 84], corrosion inhibitors, solar energy cells, electroluminescence, microlithography, photoconductors, laser materials, etc[65, 117].

**Actuators**

Conducting polymers have been incorporated in actuation systems such as micro-actuator [192], microflaps [87], microscopic valves and pumps [172], artificial muscle [220, 193, 71], vibration suppression[176]. These conducting polymer actuators can be classified into electrode storage actuators and electrolyte storage actuators, or extensional actuators and hydrostatic actuators [21]. In the electrode storage actuators, the ion-intercalated electrodes provides the electrochemical transfer of ions. In the electrolyte storage actuators, the salt stored in electrolyte leads to the charged and discharged state during cycles. Extensional actuators fulfill the linear or biaxial dimension charges of conducting polymers. Hydrostatic actuators accomplish a net volume change of conducting polymers.

Conducting polymer was demonstrated as artificial muscles by Baughman in 1990 [14] and other groups [132, 161]. Bilayer actuator with polypyrrole and a non-conducting and flexible material was synthesized by Otero [151]. Polypyrrole/Au/polyethylene sandwich was formed by Pei at el.[157, 158]. Two types of polyaniline based actuators, i.e., backbone-type(emeraldine salt film/adhesive/emeraldine salt film) and shell-type(cellophane tape wrapped), were fabricated by Kaneto at el.[94]. Smela at el.[196] produced a unimorph micro fingers and corkscrews with polypyrrole/Au.

**Sensors**

Conducting polymers have been widely used as platforms for chemical sensor [20, 168, 88, 9, 106], optical sensors [40, 208, 91, 58, 198]; ion detectors, radiation detectors, biosensors [1, 59, 105, 229, 159, 137], and electronic nose [72, 201, 13]. Take
the biosensors as an example in the sensor application. Conducting polymers can serve as a matrix for enzymes due to their compatibility with lots of molecules in biological media. Moreover, the electrodeposition of conducting polymers can be taken advantage of to control the location, geometry and size of certain enzyme designed for sensors. Immobilization of the enzyme was achievable at the electrode surface and its interaction surface during the direct polymerization of conducting polymer[3, 211].

Other Applications

Conducting polymers have been incorporated in energy harvesting devices such as solar cells [35, 29]. Due to the double-layer capacitance of conducting polymers, it can work as memory backup, light weight batteries, electrochemical displays[197]. It provides the charge-discharge-charge cycle of a battery and the chromatic switching cycle of an electrochemical display. Electroluminescent and electrochromic devices such as light-emitting diodes, electrochromic windows based on conducting polymers have been illustrated by Gurunathan [66].

Corrosion protection is an important project that can be solved by incorporation of conducting polymers. For example, polyaniline, as a metal anti-corrosion additive, was connected with its potential ability to replace other environmentally unfavorable corrosion inhibitors [165, 84, 117]. Drug delivery is a valuable project that can be accomplished by making use of conducting polymers [207, 117]. Biomedical structures have also been developed for surgery. For example, a polypyrrole/Au bilayer film was used as a blood vessel connector. When inserted it curled into a roll, and it expanded and connected the vessels during healing [195, 193, 65].

1.2 Bioderived Active Materials

Bioderived active materials are a category of ionic active materials that are made out of structures and functions of a living organism to serve in a bio-related technology. Bioderived active materials utilize the ion transport properties of cell membranes to couple multiple physical domains and beneficially interact with or effect on any
element across the membranes. Some successful commercialized or well proposed bioderived active materials are involved in implantable heart valves, silkworm silk fibers for sutures[6], chitosan-based polysaccharide for cartilage tissue repair [53], polymeric carrier drug delivery system [107], dental alloys [22], etc.

In early 20th century, the cell membrane was known as a thin "black membrane". Until 1925, Hugo [54] calculated the thickness of the membrane by measuring the capacitance, and Gorter el at. [61] demonstrated that the thickness of the lipid membrane is equal to the height of two lipid molecules. In 1958, lipid bilayer structures of cell membrane were verified by electron microscopy [190]. Cell membranes contain cholesterol and a variety proteins, and some proteins serve as transporters to exchange ions and molecules. These characterizations make cell membranes as selective barriers that keep or prevent specific ions, molecules from the cell cytoplasm.

The current state of the art in bioderived active materials uses the same molecules extracted and purified from the cell membranes of plant and animal cells. These molecules are known as glycerophospholipids. Glycerophospholipids contain a glycerol molecule, two fatty acid chains, a phosphate group, and a simple organic molecule. This composition impart a significant property, i.e., amphiphilicity, and self-assemble into a bilayer. The height of the lipid is a few nanometer, and it can form various structures like micelle, liposome, planar bilayer, bicelle. The bilayer lipid membrane (BLM) serves as the host to various proteins and form the basic structure of bioderived active materials. A variety of proteins can be incorporated in the BLM and serve in their functions such as pumps, gates, switches, catalysts, receptors, reactors, and motors. In the following sections, bioderived active materials are referred to bilayer lipid membrane (BLM) reconstituted with proteins.

Research related to bioderived active materials include the formation of bilayer lipid membrane [55], models for cell membrane [90, 86], molecular mechanisms of cell membrane [109], supported bilayer lipid membranes [199, 27], ion channel reconstitution [128, 203], ion pump [76], etc.
1.2.1 Formation of Bilayer Lipid Membranes and Reconstitution of Proteins

Formation of bilayer lipid membrane utilizes the amphiphilic properties of lipids. (The phosphate group with negative charge considered as polar group is hydrophilic head and attracted to water. The hydrophobic tail consists of long fatty acid chains and are repelled by water. When placed in water, the hydrophobic tails line up against each other with hydrophilic heads of both sides facing the water.) A variety of formation techniques have been proposed or developed during the last fifty years, including falling droplet, lipid folding, Langmuir-Blodgett(LB), vesicle fusion, lipid painting, droplet interface bilayer, microfluidic interface bilayer, liquid-supported lipid bilayer, droplet on hydrogel-supported bilayer (DHB). Gold [8], silicon [38], silicon nitride [203] are most common substrates for anchoring lipid bilayer. Polymers have also been used as direct support of BLM with smooth surface[199], with higher fluidity[226], for biomimetic membrane studies[186], for cell-surface models [210].

Langmuir-Blodgett/Langmuir-Schaefer Technique

Langmuir-Blodgett (LB) method was introduced by Irving Langmuir and Katharine Blodgett in 1930s. A monolayer of amphiphilic molecules is spread on the surface of a water bath. A hydrophilic substrate is vertically lifted from the water bath. Thus, a monolayer is anchored on the substrate with the hydrophobic tail facing out. Then, by dipping the substrate into water bath, a lipid bilayer forms on the previously formed monolayer. Some modifications have been done on Langmuir-Blodgett (LB) technique and become to Langmuir-Schaefer (LS) method. Instead of dipping the substrate vertically, the substrate horizontally contacts with the monolayer on water surface under a constant pressure [209, 200, 115]. LB/LS technique can build up structures layer-by-layer and offer precise control over the thickness. However, the surface pressure that gives best results is required to be established empirically. In addition, a tight control of temperature, the deposition rate, and the type and nature of the solid substrate is required to control thickness and quality of self-assembly.
Figure 1.6: Langmuir-Blodgett/Langmuir-Schaefer technique for formation of BLM. (a) a monolayer on water surface, (b) formation of Langmuir-Blodgett monolayer, (c) formation of Langmuir-Blodgett bilayer, (d) formation of Langmuir-Schaefer bilayer.

Lipid Folding Technique

Lipid folding technique, called Montal-Mueller technique, was developed by Montal and Mueller in 1972 [136]. Two chambers, made with the hydrophobic material and separated by an aperture, are filled with aqueous solution under the aperture level. Then, lipid in an organic solution is dispensed to the surface of the aqueous solution, leading to the formation of a lipid monolayer at the air/water interface. By increasing the level of the aqueous solution in the chambers to the aperture point, two separate lipid monolayers will fold and combine together in the aperture and from the lipid bilayer [218]. Lipid folding technique provides a possibility in formation of asymmetric membranes and allows the simultaneous assembly of lipid and protein
and the probably insertion of proteins into the lipid matrix [136].

Figure 1.7: Lipid folding for formation of BLM. (a) a monolayer on water surfaces of two separate chambers, (b) bilayer is formed after raising the water level above the aperture.

**Vesicle Fusion Technique**

Vesicle fusion technique is the process by which the vesicles spread on the surface and fuse with the surface to form a planar BLM. Phospholipid molecules are dissolved in water or electrolytic solution to form the lipid vesicle followed by sonication, vortexing, and/or extrusion to enhance the uniform-size formation of vesicles [111, 110, 139]. Vesicles are then spread onto the supporting surface so as to incubate for some time to allow the rupture and fusion to form into a lipid bilayer[98, 166, 8]. The drawback of vesicle fusion is that the formation of a stable and complete lipid bilayer takes more than 20-30 minutes [102]. To minimize fusion time and to improve stability of BLM formed from vesicle fusion, ultracentrifugation (at 40,000 rpm for 2 h) [119] and the combination of vesicle fusion and LB technique [199, 226, 93, 184] have been attempted.

**Droplet Interface Bilayer Technique**

Droplet interface bilayer (DIB) method takes advantage of the interface between an aqueous droplet and the lipid organic solution to form a lipid monolayer. As the lipid organic solution serves as bulk phase, two droplets forms two lipid monolayers at
Figure 1.8: Vesicle fusion for formation of BLM. The BLM is obtained by the fusion of vesicles on the hydrophilic surface.

Figure 1.9: Droplet interface bilayer for formation of BLM.

each interface and are brought in contact to form a lipid bilayer[16]. The lipid bilayer formed from DIB can be imaged by microscopes, and complex array of interconnected BLMs is achievable by connecting more than two droplets [45]. However, the stability of BLM formed by DIB requires the precise control on the formation process and the incorporation of electrode for the electrical measurement. To prevent two droplets fusing into a single droplet, two droplets are settled down separately for a while before brought into contact, or confined by tubes. It was demonstrated to form a stable network that lasted for weeks without bilayer rupture and possess the capability to embed proteins [78].

**Droplet on Hydrogel-supported Bilayer Technique**

Similar to DIB method, droplet on hydrogel-supported bilayer (DHB) technique makes use of the two phase interface by applying an aqueous droplet with a hy-
drogel layer to form a bilayer. Hydrogel or agarose substrates are formed and kept in contact with the lipid/oil solution, then a droplet containing water is delivered into the lipid/oil solution. After a stabilization period, the droplet is fused with the hydrated substrates that promotes the self-assembly of lipid bilayers [77]. The hydrogel and the droplet are easy for incorporation of electrodes for measuring the response of BLM, and the interface is more stable for formation of BLM compared to DIB method. However, the dehydration of hydrogel is a problem required to solve by the further modification of the surfaces.

**Falling Droplet Technique**

In falling droplet method, a monolayer formed on water-air interface is fused with a monolayer of lipids on a falling water droplet, and the falling droplet is precisely guided onto the monolayer water-air interface using a thin silver wire. First, a monolayer is formed at the aperture which connects two reservoirs, and the lower reservoir contains water while the upper one is filled with lipid solution (lipid dissolved in n-decane). Then a water droplet is dispensed onto a silver wire, which is pointed to the center of the aperture, and dropped onto the lipid solution covering the aperture. Since the density of water is greater than that of lipid solution, the droplet will deliver the lipid monolayer onto the aperture at the interface to form a lipid bilayer [234]. The silver wire in falling droplet method can serve as an electrode for measurement, and sometimes agarose gel may be applied to the surface of the silver wire to offer
Figure 1.11: Falling droplets method for formation of BLM. (a) a monolayer is formed at the aperture interface, (b) the BLM is obtained by adding a droplet.

Figure 1.12: Lipid painting for formation of BLM. The BLM is formed by the solvent evaporation and brought to contact with water.

good conductivities.

**Lipid Painting Technique**

Lipid painting technique is used for formation of BLM on the hydrophilic surface. Normally the lipid is dissolved in an organic solvent such as n-alkane [69], and the mixture solution is painted and dried on the hydrophilic surface. When the surface is brought to contact with an aqueous medium, self-assembly will occur to form a lipid bilayer. The time of drying the solution on certain substrate area is a key point for good results in lipid painting. For example, Han et al. [69] successfully formed a lipid bilayer by dissolving the lipid in n-decane with a concentration of 10mg/mL, spreading it onto the chip with area of 1, 0.36, 0.0025 cm², and letting it stand for 10 to 30 minutes before adding water.
Figure 1.13: Microfluidic devices technique for formation of BLM. Two microfluidic channels cross each other, the BLM is formed by controlling the pressure on water.

Microfluidic Devices Technique

Techniques using various microfluidic devices to form the BLM are recently developed, involving the application of pressure (air pressure, hydrostatic pressure, or mechanical pressure) to a lipid-monolayer/water/lipid-monolayer sandwich [179, 235]. Let us take a simple example as shown in Figure 1.13, two microfluidic channels are cross each other. The vertical channel is filled with aqueous solution and lipid solution is then injected into the horizontal channel. The lipid bilayer is formed by controlling the water level in the vertical channel to reject the lipid solution through the horizontal channel until a thin bilayer is achieved by appropriate pressure [55]. In this microfluidic device, the assembling and disassembling a lipid bilayer can be controlled by pushing the aqueous phase or the oil phase, thus, ion transport can be analyzed with initial and ending conditions information. The formation of an array of BLM is achievable by fabricating a reliable BLM microfluidic device.

Protein Reconstitution Techniques

Protein reconstitution in BLM can be accomplished simultaneously when forming the BLM by various methods such as vesicle fusion, droplet interface bilayer, lipid painting, etc, or by adding protein, such as alamethicin [224], directly to the formed BLM after the formation of BLM [99]. Proteo-liposome fusion technique, similar
Table 1.5: Reconstitution of protein into lipid bilayer

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Lipid</th>
<th>Protein</th>
<th>Protein to lipid ratio</th>
<th>References</th>
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<tr>
<td>mica</td>
<td>DOPC</td>
<td>gramicidin</td>
<td>1:100 molar ratio</td>
<td>[111]</td>
</tr>
<tr>
<td>ITO treated glass</td>
<td>DOPC, DOPE, DOPS, SYPC, SOPC</td>
<td>bacteriorhodopsin</td>
<td>1:160-1350 molar ratio</td>
<td>[60]</td>
</tr>
<tr>
<td>ITO treated glass</td>
<td>DOPC, DOPE, DOPS, SYPC, SOPC</td>
<td>Ca(^{2+})-ATPase</td>
<td>1:680-5500 molar ratio</td>
<td>[60]</td>
</tr>
<tr>
<td>PMMA, PTFE</td>
<td>POPE, POPG, ergosterol</td>
<td>kscA</td>
<td>1:22000 molar ratio</td>
<td>[233]</td>
</tr>
<tr>
<td>gold</td>
<td>DPhPC</td>
<td>M2</td>
<td>1:500 molar ratio</td>
<td>[217]</td>
</tr>
<tr>
<td>gold</td>
<td>DPhPC, DPhPE</td>
<td>M2(\delta)</td>
<td>1:1000 molar ratio</td>
<td>[98]</td>
</tr>
<tr>
<td>silicon</td>
<td>DOPC</td>
<td>gramicidin</td>
<td>1:100 molar ratio</td>
<td>[133]</td>
</tr>
<tr>
<td>N/A</td>
<td>DPhPC, POPE, POPD</td>
<td>KvAP</td>
<td>2:10 weight ratio</td>
<td>[182]</td>
</tr>
<tr>
<td>PMMA</td>
<td>DPhPC</td>
<td>alamethicin</td>
<td>1:500 weight ratio</td>
<td>[180]</td>
</tr>
</tbody>
</table>
to vesicle fusion technique, is a technique frequently used for protein reconstitution. Proteins, such as M2 [217], gramicidin-A [133], alamethicin [133], KvAP [182] and kscA [233], are mixed with the prepared solution containing lipid vesicles, resulting to the proteo-liposome with a lipid to protein molar ratio range from 100 to 22000 [155, 170, 60]. After the proteo-liposome is spread onto the substrates and the fusion is completed, the protein is reconstituted in BLM. Droplet interface bilayer method are also used for incorporation of proteins, such as α-hemolysin and alamethicin [181], in the droplet, resulting to ion channel formed in the DIB [78].

1.2.2 Characterization Techniques of Bilayer Lipid Membranes and Reconstituted Proteins

Characterization of bilayer lipid membrane is performed to investigate the physical properties of the bilayer lipid membrane reconstituted with proteins. Different physical properties of BLM with or without proteins are obtained by a verity of characterization techniques such as electrical impedance spectroscopy, cyclic voltammetry, chronoamperometry, etc. and are valuable to establish the models and determine the potential applications.

Electrochemical Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) measures the impedance magnitude and phase over a wide frequency range by applying a small vibration of the potential or current. EIS provides a way to probe the charge-transport processes in the bioderived active materials by extracting quantitative information from impedance spectra using fitting methods for the parameters of capacitors and resistors in equivalent circuits. It can be used to investigate the ion permeation through the BLM and the ion transport through BLM reconstituted with proteins and indicate the formation of BLM and/or ion channel[69, 217, 171, 112]. For instance, incorporation of synthetic M2 peptides into tethered BLMs was demonstrated to have the selectivity for small monovalent cations [217]. EIS was applied to model the passive ion transport in tethered BLM
on gold electrode, and the result indicated the mechanism of ion permeation is more similar to intermittent open-pores than diffusion [171]. EIS was used to investigate the electrical properties of lipid bilayer on polymer-cushioned silicon substrate, and the concentration of polymer to lipid ratio of 5.9% was demonstrated to maximize the impedance with a low capacitance [112].

**Cyclic Voltammetry (CV)**

Cyclic voltammetry (CV) provides the current-voltage behaviors by applying a potential sweep to the bioderived active material system. CV provides the information on the cis- or trans- electron transfer and directional ion transport, which are illustrated through the current-voltage response [90, 116, 180], when the cyclic waveform of potential with a proper scan rate is applied to BLM system with or without functional proteins. CV can be used to prove the implant of protein in the BLM and monitor the potential at which lipid bilayers rupture. CV is also useful to investigate the electromechanical responses of proteins and record the potential at which ion channel is activated in the case of voltage-gated protein. For example, alamethicin is a voltage-gated protein that can be incorporated in the BLM to form the ion channel. Depending on which side of BLM alamethicin is added to, polarity of applied voltage, and concentration gradient of ions, the ion channel will choose a direction for ion transport, and CV is the right tool to predict the diode-like behavior of ion channels [218].

**Chronoamperometry (CA)**

Chronoamperometry (CA) offers the current response against time when a step voltage is applied to the bioderived active material system. Typically, the step voltage applied to the BLM system with or without functional proteins is in the range of several hundred of millivolt, and the current recorded in short time duration illustrates the electrochemical response. If the protein has been reconstituted appropriately, the ion channel activities can be observed through current response. In some electrochemical experiments, the transistor function is required to be demonstrate, and CA
offers a good opportunity to monitor the current change associated with proper trigger signal in the form of square-wave current. Let us take the BLM with reconstituted alamethicin as an example. Ion channels formed in BLM are not open simultaneously, in the other word, ion transport across the membrane happens at some protein site for certain duration. CA is useful for recording the ion transport through multiple ion channels [182] and monitoring the gating voltages for voltage-gated ion channel [182].

**Single-Channel Recording (SCR)**

Single-channel recording (SCR), similar to chronoamperometry as the current recording, is used exclusively to observe the protein functions with proper trigger signals by measuring the charge or current of ion through a single protein channel. SCR can examine the gating behavior, the ion selectivity, and the ion sensitivity in the single channel level. Typically, SCR is achieved by patch clamp techniques [178] that place micropipettes onto the surface of the cell membrane and measure the current through the micropipettes. SCR are used to observe single channel activities in hydrated supported lipid bilayer [77], nystatin-ergosterol proteoliposomes [233], falling droplet lipid bilayer [234], painted lipid bilayer on silicon nitride [203], droplet networks [78], tethered bilayer lipid membrane on gold [98].

**Microscopy**

Atomic force microscopy (AFM) is a useful tool for visualization of BLM. Tapping mode is suitable for imaging the BLM without ruining the membrane. Though contacting-mode imaging have the possibility of damaging the soft biological samples by scraping or dragging, the drawback can be overcome by controlling the operative force [139]. AFM in dynamics illustrated the substrate takes only several minutes to be treated by vesicles before rinsing, but the formation of lipid bilayer by fusing and depositing needs several hours [111]. AFM was performed to investigate the surface interaction effect on the formation of lipid bilayer on silicon oxide [102]. AFM tip can be used as an electrode for the electrical measurements, thus, the thickness and the
impedance of the lipid bilayers are recorded simultaneously \[166\]. Cryogenic transmission electron microscopy (Cryo-TEM) was used to visualize the structure of lipid treated with liquid nitrogen cooling \[8\]. High-speed microfluorescence spectroscopy was utilized to capture the entire fast fusion process of lipid bilayer formation by vesicle fusion \[110\]. Ca\(^{2+}\)-ATPase and bacteriorhodopsin were labeled to monitor the protein incorporation and activities by fluorescence spectroscopy\[60\]. Fluorescence microscopy was used to investigate the FomA proteoliposome fusion into planar lipid bilayers, and the lipid to protein ratio of 50 was found to be optimal in the sense of fusion efficiency \[164\]. Surface plasmon resonance spectroscopy (SPR) illustrated the formation of lipid bilayer by the thickness change of surface based on the fact that the stable bilayer supported on the substrate is only several nanometers higher than the substrate baseline \[199, 217\]. Scanning electrochemical microscopy (SECM) was used to map ion channel flux through BLM incorporated with alamethicin in real-time \[224\].

**Other Techniques**

Woodbury and Miller \[227\] proposed a method that can estimate the number of functionally reconstituted ion channels by observing the different conductance levels. Surface forces apparatus was used to provide the force-distance relationship between two polymer-cushioned lipid bilayers, which indicated the pressure and energy for vesicle fusion\[226\]. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to measure the liposome size after sonication \[228\]. A software tool using saw-tooth voltage stimulation was developed to analyze the resistance and capacitance of the bilayer lipid membrane \[145\]. Molecular dynamics (MD) was used to simulate the lipid bilayer with/without proteins in atomic details \[212, 163, 128\]. Nuclear magnetic resonance (NMR) and molecular dynamics (MD) simulations were combined to study the chemical composition and configuration of alamethicin in DMPC/DHPC bilayers\[44\]. Quartz crystal microbalance with dissipation (QCM-D) monitoring, combined with atomic force microscopy (AFM), was used to monitor the fusion process and determine the adsorption and rupture of lipid
vesicle [8]. Electrophysiology measurements were utilized to determine the electroporation threshold voltage [8], that of pure DPhPC has a value of 80mV, indicating the electrical stability of the DPhPC bilayer remains good until 80mV. Fluorescence recovery after photobleaching (FRAP) was used to investigate the mobility of the lipid [112].

1.2.3 Modeling of Ion Transport

The active response of bioderived active materials is realized from controlled ion transport through the bioderived membrane. The mathematical models for ion transport attempt to develop a physics-based input-output relation for ion transport. The mechanism of ion transport across protein reconstituted BLM is investigated and brought into models so that ion transport can be controlled by proper input.

Ion transport in BLM is achieved by the incorporation of proteins as ion pumps, ion channels, or ion exchangers. Ion pumps act in the way of active transport against the gradient of selective ions and are motivated by the hydrolysis of ATP. Ion channels behave by means of passive transport that allow selective ions go through the membrane along the gradient direction. Ion exchangers are combination of ion pumps and ion channels that moves one kind of ions against its gradient powered by energy, and subsequently results in another type of ion to be transported along the gradient.

Ion transport can be classified into four categories: simple diffusion (passive transport), facilitated diffusion (passive transport), primary active transport, second active transport [173]. Simple diffusion needs neither ion carrier nor energy to allow small ions passing through the membrane along their electrochemical gradient. Facilitated diffusion requires carriers or receptors to transport certain ions along the gradient. Primary active transport utilizes the carriers as well as metabolic energy to transport selective ions against their electrochemical gradient. Second active transport makes use of one type of carriers to allow certain ion species flowing through the membrane against the gradient, and takes advantage of the concentration change due to the previous flowing to fulfill another ion species transporting along its gradient by a different type of carriers.
Simple diffusion can be modeled by Fick’s diffusion laws. Facilitated transport employs a chemical or an electrical stimulus to realize the selective ion transport, and the theory for its modeling includes the simple carrier mechanism and two-site carriers mechanism [26], passive permeation described by the integrated Nernst-Planck equation [171].

Active transport requires energy to against ion concentration gradient, and one of the simplest descriptions was illustrated by Hansen in the enzyme reaction scheme that assumes the number of fuel molecules is linear with the number of transported ions[70]. In Lauger’s model [108], a ion route image is illustrated as a ATP utilizing ion pump, and this ion pump with two different conformational states has a ion-binding site for the unidirectional ion transport. This energization-relaxation channel model has been employed into multiple ion-binding sites [138]. The non-equilibrium energy process in active transports is demonstrated by Hamiltonian function that evaluates the effective potential and the energy required to transport one particle [51]. The ion exchangers behaved in the manner of secondary active transport are modeled with reaction rates in the energy balance [205, 206].

1.2.4 Application of Bioderived Active Materials

Bioderived active materials as a functional component in the application can be utilized to generate a sensitive input or output. This input or output could be the trigger signals for stimulating proteins or the changed ion concentration induced by ion transport. The examples of sensing application include voltage sensors [183], glucose sensors [28]. Recently, the artificial cilia sensors [114] inspired by the stimuli-responsive behavior of nature hair cells use strain gauges or force-sensitive resistors to generate an electrical response to sense touch or flow.

Ion transport occurred in bioderived active materials is typically associated with conformational change in proteins. The mechanical motion due to conformational change of proteins is the linear or rotational displacement of certain subunits of few angstroms and difficult to be coupled with the external systems. By incorporating the electroactive polymers that generate volumetric strain resulting form
ion transport through the bioderived membrane, a membrane-based micro hydraulic actuation concept was developed [205].

Other applications of bioderived active materials include electrochemical transistors [17], devices of solar energy conversion by water photolysis [39], electrical devices such as a current limiter, a half-wave rectifier and a full-wave rectifier [125].

1.3 Motivation for Hybrid Bioderived Ionic Systems

Conducting polymers are a unique category of polymers that contain mobile ions in the polymer matrix and respond to an applied electric field. The transport of ions through the polymer matrix under the influence of the applied field results in force generation and deformation of the soft polymer backbone and is applicable to an actuator. In addition, the transport of ions due to an external stimulus (mechanical shear, chemical analyte or light) results in charge separation in the polymer and is utilized for its application as a chemical sensor. Biological processes in living organisms use ion transport to maintain life functions. Bioderived active materials utilize this property in the design of actuators, sensors and energy harvesting devices. In these materials, the bilayer lipid membrane (BLM) with proteins is supported on a synthetic substrate to provide electrolytic access, structural support and shape. Various examples of device concepts using a lipid membrane with proteins include the chemomechanical actuator, droplet interface bilayer devices, and various sensor designs. The actuation and sensing functions in conducting polymers and bioderived active materials are driven by the same fundamental process, ion transport, and hence present an opportunity to integrate these two materials into a hybrid bioderived ionic system. This hybrid system may potentially provide a path to overcome the individual limitations of electroactive polymers and bioderived active materials, and benefit from the unique application-specific advantages of the individual materials.
Chapter 2

Hybrid Bioderived Membrane

2.1 Objective

Inspired by the similarity in ionic function between a conducting polymer membrane and cell membrane, this work presents a thin-film laminated membrane in which protein-reconstituted lipid bilayer membrane is supported on a conducting polymer membrane. The objective of this work is to demonstrate the conducting polymer membrane can be incorporated with protein-reconstituted lipid bilayer membrane to realize the ionic function so that this hybrid bioderived membrane can be used in the application such as actuators, sensors, or energy harvesting devices. This dissertation focuses on a conducting polymer based actuator that can be controlled by ion transport through the protein in the lipid bilayer membrane. The energy required for actuation is still provided by the gradient applied across the conducting polymer membrane and the architecture developed in this thesis provides a direct means to regulate actuation in the conduction polymer.

2.2 Physics of Operation

The hybrid bioderived membrane is fabricated by direct assembly of BLM reconstituted with proteins on the conducting polymer. The BLM reconstituted with proteins is formed on the conducting polymer and thin film of aqueous medium separates the
two layers. The proteins reconstituted in BLM serve as the gateway for ion exchange at the conducting polymer-BLM-electrolyte interface and are either voltage-gated or ligand-gated. The ligand-gated proteins conduct ions into the conducting polymer in the presence of a chemical messenger and the voltage-gated proteins conduct ions into the polymer for an applied bias-potential. Once the proteins are triggered with appropriate signals, the regulation of the chemical potentials across the bilayer lipid membrane results in active control of strain in the conducting polymer. In the presence of cations (or) anions required for redox reactions in the conducting polymer, a cyclic field applied to the conducting polymer produces cyclic strain in the conducting polymer. Thus, in the presence of an appropriate gating signal, the hybrid actuator generates a response in the conducting polymer. Due to the nature of the interaction between the chemical, electrical, and mechanical domains, the hybrid membrane resembles a chemo-electro-mechanical transistor that can be turned ON/OFF using a chemical signal or transmembrane potential applied to the BLM. The hybrid membrane is referred to as a bioderived ionic transistor (BIT) due to the mechanism of operation with a gate, source, and drain ports. Each BIT device resembles an electronic transistor and is designed to have two inputs to regulate the output from the device. The gate input is the electrical or chemical signal to the protein in the BLM, the other input is the ionic gradient across the hybrid membrane, and the output is the mechanical response of the conducting polymers. The conducting polymer-BLM-electrolyte interface and an equivalent circuit representation of the active components are shown in Figure 2.1. The electrode inserted in the electrolyte is recognized as gate, the electrode on the other side of bioderived membrane are considered as drain, and the electrode connected with the other side of conducting polymer serves as source. Two requirements are needed for operation of hybrid bioderived membrane: the gate input is applied between gate and drain, and the ionic gradient exists between electrolyte and the conducting polymer. The hybrid bioderived membrane in operation will generate a mechanical response of the conducting polymer, which is induced by the egress and ingress of ion. These ion movement may be observed through current change between source and drain. In the presence of an appropriate gating signal and
Figure 2.1: A hybrid bioderived membrane formed from protein reconstituted bilayer lipid membrane and a conducting polymer. (a) Schematic of the hybrid bioderived membrane in which ion transport into the polymer is gated by proteins, (b) equivalent circuit representation as a bioderived ionic transistor, (c) operation features of the hybrid bioderived membrane.

The choice of conducting polymer, the output from the hybrid bioderived membrane could be tailored to present itself as change in volume, color, conductivity, capacitance, etc. This feature of the hybrid bioderived membrane presents a multitude of options for versatile sensors, actuators and energy storage applications.

2.3 Modeling Methods

Based on the physics of operation described in the previous section, this chapter will present a first order model for actuation using the hybrid bioderived membrane. In the presence of an appropriate gating signal, the charge transport through ion channels in BLM is related to the ion concentration gradients, which is illustrated in the electrodiffusion model. In the conducting polymer actuator, the relationship between exchanged charge and the tip displacement is demonstrated through bending actuation model.

1. Bioderived Membrane Layer Model

In voltage-gated ion channels, ion transport is regulated by the presence of ions, concentration gradient and the applied trans-membrane potential. The
equilibrium potential of a particular ion due to different concentrations using
the Nernst equation is given by
\[ V_c = \frac{kT}{ze} \ln \frac{c_o(t)}{c_i(t)} \] (2.1)
where \( k \) is the Boltzmann’s constant \((1.381 \times 10^{-23} \text{ J} \cdot \text{K}^{-1})\), \( T \) is the absolute temperature, \( z \) is the valence of the ion, \( e \) is the elementary charge \((1.602 \times 10^{-19} \text{ C})\), \( c_o(t) \) and \( c_i(t) \) is concentration of ion on either sides of the membrane.

By Fick’s law, the flux of ions due to diffusion is
\[ \vec{\phi} = -D \nabla [c] = -\frac{u_s kT}{q_s} \nabla [c] \] (2.2)
where \( D \) is the diffusion coefficient, \( q_s \) is the electrical charge of the charged ion species, \( u_s \) is the electrical mobility of the charged ion species, \([c]\) is the ion concentration.

By Ohm’s law, the flux of ions due to electric forces is
\[ \vec{\phi} = -\frac{u_s z e}{q_s} \nabla [U] \] (2.3)
where \( \nabla [U] \) is the potential gradient across the membrane.

The Nernst-Planck equation for the total flux of ions due to diffusion and electric forces is
\[ \vec{\phi} = -\frac{u_s kT}{q_s} \exp \left( -\frac{zeU}{kT} \right) \nabla \left( [c] \exp \left( \frac{zeU}{kT} \right) \right) \] (2.4)

The flux is related to the ionic current by the relation
\[ \vec{\phi} = \frac{i_{vg}}{N_A ze} \] (2.5)
where \( N_A \) is Avogadro constant, and \( A \) is the cross-section area of ion channel.

Simplifying Equation 2.4 and Equation 2.5 described by Endresen et al. [48], the ionic current through the protein transporter via voltage-gated diffusion is given by
\[ i_{vg} = \frac{2N_A ze u_s kT}{q_s} \sqrt{c_o c_i} \frac{A_p}{d_p} P_o \sinh \frac{ze(V - V_c)}{2kT} \] (2.6)
where $V$ is the applied transmembrane potential, $P_o$ is the average fraction of channels that are open (the probability that a given channel will be open), $A_p$ is the cross-section area of the pore, $d_p$ is the length of the pore (assuming an ion channel having a constant area except for a short and narrow constriction or pore in its middle [48]).

2. Conducting Polymer Layer Model

The PPy-based actuator is considered to have small tip displacements and hence assumed to behave like an Euler-Bermouli beam. According to the schematic in Figure 2.2, the length of beam is $L$, the width of beam is $w$, and the thickness of PPy layer and substrate layer is $h_2 - h_1$ and $h_1$, respectively.

$$M = EI \frac{d^2u}{dx^2}$$

(2.7)

where $u(x)$ is the displacement in ‘z’ direction, $E$ is the Young’s modulus, $I$ is the second moment of area. The relationship of dimensional parameters is expressed as

$$\frac{1}{\rho} = \frac{d\theta}{dx} = \frac{d^2u}{dx^2} = \kappa$$

(2.8)

where $\rho$ is the radius of the beam, $\theta$ is the slope of the beam, $\kappa$ is the curvature of the beam.

The area moments of inertia with respect to the geometric dimensions for PPy layer and substrate layer, respectively, are expressed as

$$I_{PPy} = \frac{w(h_2^3 - h_1^3)}{3}$$

(2.9)

$$I_{pc} = \frac{wh_1^3}{3}$$

(2.10)

By substituting we have

$$EI = E_{PPy}I_{PPy} + E_{pc}I_{pc} = \frac{w}{3} \left[ E_{PPy} (h_2^3 - h_1^3) + E_{pc}h_1^3 \right]$$

(2.11)

The strain in conducting polymers is linearly proportional to the exchanged charge density. Thus, the stress induced by exchanged charge can be shown
Figure 2.2: Bending actuation model for PPy based actuator following Alici et al. [5] as

\[ \sigma = \alpha \frac{\Delta Q}{v_{PPy}} \]  

(2.12)

where \( \Delta Q \) is the exchanged charge, \( v_{PPy} \) is the volume of PPy layer, \( \alpha \) is the ion transport coefficient that can be calculated through experimental data.

In this actuation system, an applied voltage to the conducting polymer layer is the source for actuation. Thus, an expression that relates this applied voltage to the exchanged charge of the PPy layer must be established. Here, we utilize the classical relation for a steady-state response between voltage and charge in the conducting polymer and we relate this to strain developed in the conducting polymer. The charge exchanged with the electrolyte is

\[ \Delta Q = \Delta VC \]  

(2.13)

where \( \Delta V \) is the applied voltage, and \( C \) is the capacitance of PPy.

The bending moment due to this stress can be derived as

\[ M_1 = \int_{h_2}^{h_1} \sigma zdA = \sigma w \int_{h_2}^{h_1} zdz = \frac{1}{2} \sigma w (h_2^2 - h_1^2) \]  

(2.14)

By substituting Equation 2.12 and 2.13 into Equation 2.14 we obtain

\[ M_1 = \frac{\Delta VC}{2v_{PPy}} \alpha w (h_2^2 - h_1^2) = \frac{\Delta VC}{2L} \alpha (h_2 + h_1) \]  

(2.15)
The bending moment due to the external force can be expressed as

$$ M_2 = F (L - x) \quad (2.16) $$

Thus, the total bending moment due to the exchanged charge and external force is

$$ M = M_1 + M_2 = \frac{\Delta VC}{2L} \alpha (h_2 + h_1) + F (L - x) \quad (2.17) $$

By substituting Equation 2.17 and 2.11 into Equation 2.7 we obtain

$$ \frac{d^2 u}{dx^2} = \frac{3\Delta VC \alpha (h_2 + h_1) + 6FL(L - x)}{2Lw [E_{ppy} (h_2^3 - h_1^3) + E_{pc}h_1^3]} \quad (2.18) $$

Integrating the above expression over $dx$, the displacement in 'z' direction at length 'x' from the root of the cantilever is given by

$$ u(x) = \frac{[3\Delta VC \alpha (h_2 + h_1) + 6FL^2]x^2 - 2FLx^3}{4Lw [E_{ppy} (h_2^3 - h_1^3) + E_{pc}h_1^3]} \quad (2.19) $$

The tip displacement ($x = L$) is

$$ u(L) = \frac{3\Delta VC \alpha (h_2 + h_1) L + 4FL^3}{4w [E_{ppy} (h_2^3 - h_1^3) + E_{pc}h_1^3]} \quad (2.20) $$

The free displacement ($F = 0$) at the tip is

$$ \delta_0 = \frac{3\Delta VC \alpha (h_2 + h_1) L}{4w [E_{ppy} (h_2^3 - h_1^3) + E_{pc}h_1^3]} = \frac{3\Delta Q \alpha (h_2 + h_1) L}{4w [E_{ppy} (h_2^3 - h_1^3) + E_{pc}h_1^3]} \quad (2.21) $$

In the PPy based actuator, the capacitance of PPy is measured by EIS. The tip displacement $\delta_0$ corresponding to the step voltage can be mounted by video under the microscope. The dimensional information of the actuator is gathered by SEM or AFM. By substituting the values into Equation 2.21, the ion transport coefficient $\alpha$ is obtained.

### 2.4 Constructional Features

Constructional features of the hybrid actuator illustrated in Figure 2.3 are based on a framework that uses the conducting polymer as a supporting substrate for the
self-assembled bilayer lipid membrane with reconstituted proteins. This framework requires the conducting polymer to have a smooth surface with features that are conducive to support the BLM with proteins. In addition, the interfacial region between the conducting polymer and the bilayer lipid membrane should maintain a concentration gradient for ion transport through the proteins. Since moist or aqueous environments is required for the formation of the bioderived membrane, the membrane needs a confined layer or a porous gel layer to restrict the area for formation. The electrolyte in our original design is in the form of aqueous solution and is either restricted in the confined layer or contained in the porous gel layer. The hybrid actuator in Figure 2.3(a) will be developed into a micro cantilever, and the hybrid actuator in Figure 2.3(b) can be developed into a extensional actuator.

![Figure 2.3: Constructional features of the hybrid bioderived ionic actuator. (a) Fixed-ending actuator, (b) free-standing actuator.](image)

### 2.5 Selections of Materials

**Conducting Polymer layer:** Polypyrrole (PPy) as a conducting polymer is well-understood and has excellent mechanical properties such as low Young’s modulus, wear resistant and is chemically inert. It undergoes millions of cycles of redox reactions and does not show any significant drop in its performance. When DBS\(^-\) is used as a dopant in PPy, the backbone PPy(DBS) demonstrates the ability to exchange cations (specifically sodium ions) with the surrounding electrolyte. In general, PPy(DBS) film is near black due to having an absorption band in visible-near infrared region. The conductivity of PPy(DBS) film depends on the thickness, the conduc-
tivity is $10^{-1}$ to $10^0$ S/cm for 20 -100 nm, nearly linear increasing between 200 and 500 nm, staying 600 S/cm for 600 -1000 nm [101]. PPy(DBS) is so sensitive to the anions, applied voltages, and concentrations.

**Bioderived Membrane Layer:** DPhPC [215], which is short form of 1,2-di-(3,7,11,15-tetramethylhexadecanoyl)-sn-glycero-3-phosphocholine, has often been used to form bilayer lipid membrane reconstituted with ion channels. The chemical structure is shown in Figure 2.4. It has choline incorporated as a polar head group, a phosphate group connected to glycerol with two saturated fatty acids. These two16-C fatty acids with no double bond are the non-polar tails. Artificial membranes can be formed by the self-assembly of DPhPC bilayers due to its amphiphilic molecular structure.

![Figure 2.4: The chemical structure of DPhPC](image)

Alamethicin, isolated from trichoderma viride, is identified as linear peptide antibiotic with the sequences of:

$$\text{Ac} - \text{Aib} - \text{Pro} - \text{Aib} - \text{Ala} - \text{Aib} - \text{Ala(Aib)} - \text{Gln} - \text{Aib} - \text{Val} - \text{Aib} - \text{Gly}$$

$$-\text{Leu} - \text{Aib} - \text{Pro} - \text{Val} - \text{Aib} - \text{Aib} - \text{Glu} - \text{Gln} - \text{Phl}$$

The N-terminal residue is acetylated (Ac$^-$) and the C-terminal residue is L-phenylalaninol(Phl) [218]. Alamethicin is a 20-residue peptide, which belongs to the family of peptaibols. It contains a high proportion of the helix-inducing aminoisobutyric acid (Aib) residue not belonging to the 20 standard amino acids and ends in an amino alcohol as the C-terminus. Since the discovery of the ion conducting properties of alamethicin in the early 1970s and the X-ray structure of alamethicin by Fox and Richards in the early 1980s [52], alamethicin has been one of the most frequently studied voltage-gated ion channels[23, 163]. Alamethicin has been demonstrated to form an ion-conducting pore by placing the $\alpha$-helical peptides perpendicular to the
lipid bilayer. The hydrophobic ends of multiple peptides connect with the hydrophobic tails of the lipid bilayer, while the hydrophilic faces align to form the channel. Barrel-stave model is popular to represent the alamethicin channel structure [128]. A key feature of this structure model is that the $\alpha$ helices are paralleled to one another. By applying the electrostatic field across the bilayer their helix dipole repulsions would be overcome to allow the pore forming. Given this effect, once alamethicin is added to one side of a lipid bilayer, only cis-positive voltage will allow the ion channel formation, which results in the asymmetry current behavior by voltage. Earlier work on alamethicin was tested within the lipid bilayer formed by lipid folding showing the asymmetry in current-voltage curve.

![Chemical structure of alamethicin]

**Figure 2.5: The chemical structure of alamethicin**

**Interfacial and Supporting Layers:** To polymerize the PPy, a flat gold layer is required for chemoelectrical deposition. The choice for this gold layer is either the thin gold foil, or the polycarbonate filter paper sputtered with gold. The gold foil provides an excellent platform for investigating the ion exchange properties of the conducting polymer and the bilayer lipid membrane, but is comparatively rigid. The polycarbonate filter paper sputtered with gold is flexible but less conductive. The formation of bioderived membrane is localized to a region for targeted measurement of ion transport properties on the conducting polymer. A fixture as shown in Figure 2.3(a) is fabricated from PMMA (or) Teflon, and the porous substrate for interfacing the conducting polymer with bilayer lipid membrane is formed from PMMA and PVDF as shown in Figure 2.3(b). The bilayer lipid membrane is supported on the gel layer with electrodes microfabricated in place. This architecture will be used to
characterize the ion exchange capabilities of the hybrid membrane in this thesis and scaled up to build an actuator.

\section{Conclusion}

A hybrid bioderived ionic device proposed here combines the bioderived membrane and the conducting polymer and utilizes the similarity of their ionic function to convert energy. This device works as a transistor that once a gating signal is applied, the chemical energy transfers into mechanical energy. This device will be eventually developed into a actuator. This actuator could be either fixed-ending or free-standing. The preliminary selections of the materials and design requirements of the component layer are illustrated. Following these requirements, the fabrication of this hybrid bioderived ionic actuator is described in the next chapter.
Chapter 3

Fabrication of Hybrid Bioderived Membrane

3.1 Introduction

The concept for a hybrid bioderived ionic actuator that consists of the conducting polymer and the bioderived active materials has been provided in chapter 2. In this chapter, the proposed concept is translated into a membrane, and a layer-by-layer method developed to fabricate the membrane is presented. The layer-by-layer fabrication method includes the synthesis of conducting polymers layer, the formation of bioderived membrane layer, and the assembly of the layers. The synthesis of conducting polymer layer is conducted by electrochemical polymerization of PPy(DBS) on the substrate of gold foil or gold sputtered polycarbonate filter paper. The formation of bioderived membrane layer is accomplished by vesicle fusion of DPhPC and reconstitution of alamethicin. The assembly of the layers are associated with the layer-by-layer configuration that once the separate layers are formed in sequence, the assembly is simultaneously achieved in the layer-by-layer configuration. In such a way, a hybrid bioderived membrane is fabricated and ready for characterization.
Figure 3.1: Assembly structures of alamethicin reconstituted BLM supported on PPy(DBS) film. (a) Schematic of layer-by-layer configuration. 1,2,3 are electrical terminals for electrical measurement. (b) Equivalent circuit representation of bioderived ionic transistor. (c) Various real structures, from left to right: PPy(DBS) film attached to PMMA, teflon, epoxy confined by silicon o-ring.

### 3.2 Prototype Design

The concept of bioderived ionic transistor (BIT) has been illustrated in chapter 2, and the prototype of the hybrid device is designed to be able to achieve the similar function as BIT. A multi-layer device as shown in figure 3.1(a) is designed as a prototype. In this layer-by-layer configuration, PPy(DBS) due to its hydrophilicity is used for direct supporting BLM reconstituted with proteins. Three electrical terminals are incorporated for demonstrating the transistor function: terminal 1 serves as gate, terminal 2 as drain, and terminal 3 as source. In such a structure, once a trigger signal is applied between gate and drain, the ion channel formed across the BLM will conduct ion flowing between the PPy(DBS) and the electrolyte due to different ion concentrations. Subsequently, the ion ingress or egress of PPy(DBS) resulting from the ion transport leads to a current increase or decrease between source and drain. This layer-to-layer configuration is used as a prototype for fabrication.
3.3 Conducting Polymer Layer

Polymerization of PPy(DBS) on Gold Foil

Figure 3.2: Three-electrode deposition method for PPy(DBS). (a) Electrical terminals are connected to Autolab 128N. These terminals include a working electrode (WE) holding the gold substrate that PPy(DBS) is deposited on, a counter electrode (CE) close (10 mm distance) to WE, and a reference electrode (RE) relatively far away from WE. The electrolyte contains 0.1 M Pyrrole and 0.1 M NaDBS in deionized water. (b) Schematic of the three-electrode deposition setup.

3.3.1 Materials

Deionized water (resistance = 18.2 MΩ-cm) is obtained by running tap water into a Barnstead deionizer system (Thermos Scientific) and used for preparation of solution. 0.1 M sodium dodecylbenzenesulfonate (NaDBS, technical grade, Sigma-Aldrich) was dissolved in deionized water and stirred sufficiently. Then, 0.1 M monomer pyrrole (reagent grade, 98%, Sigma-Aldrich) was added in the NaDBS solution and shaken well to accelerate the dissolution. Since the pyrrole solution was stored at 2C to 8C, it is allowed to stand on the counter for 1 hour to reach room temperature. Due to the amphiphilic characteristic of DBS\(^-\) containing solution, foam forms during mixing and vanishes eventually. The mixture of aqueous solutions of pyrrole and NaDBS is used as the electrolyte for electrodeposition. The cut stripes of gold foil (20mm long, 5mm wide, 10\(\mu\)m thick, 99.99%, Sigma-Aldrich) are prepared by cleaning with isopropanol, deionized water and drying under nitrogen in sequence. The polycarbonate track etch (PCTE) membrane (18 \(\mu\)m thick, 10 \(\mu\)m pore diameter) is prepared by sputtering
gold for 40 s (ca. 40 nm), cleaning with isopropanol, deionized water and drying under nitrogen in sequence. A silver-silver chloride (Ag/AgCl) half-cell (2 mm diameter, 4 mm long, World Precision Instruments Inc.) is rinsed in deionized water, dried with wipers (Kimberly-Clark), and stored in dark place after use.

### 3.3.2 Experimental Setup

A three electrode polymerization cell was used for the deposition of PPy onto gold foil in aqueous solutions [192] as the schematic shown in Figure 3.2(b). The polymerization cell was rapid prototyped and holds three electrodes that are connected with EcoChemie Autolab 128N. The electrodes arranged in the cell as shown in Figure 3.2(a) include one strip of gold foil serving as a working electrode (WE) and another strip of gold foil as a counter electrode (CE). The two electrodes are placed parallel and separated by 10 mm to ensure a uniform electric field. All electrical measurements were recorded with respect to a Ag/AgCl half-cell served as a reference electrode (RE) that provides an equilibrium reaction. The equilibrium potentials versus the reference in electrochemical cell can be easily interpreted. The submerged area of the gold foil served as the working electrode in the electrolyte is required to be controlled with extra precaution so that the conducting polymer deposits on the constant-limited surface that is used to support BLM and proteins by the layer-by-layer approach.

### 3.3.3 Methods

Cyclic voltammetry (CV), is performed to understand the current-voltage response of the electrochemical three-electrode cell. The potential required for electrochemical polymerization is obtained from the CV measurement as shown in Figure 3.3(a). The CV scans run from $-0.8 \text{ V}$ to $+0.8 \text{ V}$ between the working electrode (gold foil) and the counter electrode (gold foil). The step potential is 0.00106 V and the scan rate is 0.009 V/s. The applied voltage starts at 0 V, and the current remains flat until the voltage increases near to 0.5 V. The sudden raising current implies the start of polymerization, and the deposition rate exponentially increases with the applied
voltage after this point. When the potential returns below 0.5 V and reaches until near to $-0.6$ V, a small current peak is observed as a sign of reduction of PPy. Na$^+$ cations enter the polymer to compensate backbones to the neutral state. When the potential reaches to the lower limit and raises back near to $-0.35$ V, another current peak is observed, indicating the polymer is oxidized by forcing the Na$^+$ cations exit the polymer. The reduction-oxidation (redox) reactions are related with charge transport and volume changes.

The electrodeposition of PPy(DBS) is achieved potentiostatically - chronoamperometry (CA). The higher applied voltage can be used for quick deposition, and the lower applied voltage provides a uniform surface of polymer. The cell is operated at 0.6 V for 600 s and current is recorded in Figure 3.3(b). As the cell is turned on, it is observed that nucleation sites begin to appear and rapidly cover the surface of the working electrode (gold foil) with a black PPy(DBS) layer. Normally, within 600 s of CA deposition, a complete coverage of PPy(DBS) on the surface of the gold electrode is observed. The current-time response indicates when a certain voltage higher than the critical point of polymerization was applied, a spike of current due to the double-layer capacitive changing would appear. The current would rise slowly after the minimum was reached until to a steady state value. Slow increase of current presents the period of nucleation, and when the current reaches to a constant value, the growth of the polymer should be linear with time.

The gold foil with PPy(DBS) deposited on is stored in a vial filled with the 0.1 M NaDBS electrolyte. The gold foil is retained with the polymer to provide structural support and to serve as an electrode for the subsequent characterization. As shown in Figure 3.1(c) of two structures from the left, the PPy(DBS) polymer is attached to the bottom of a custom designed and rapid prototyped fixture (PMMA or teflon) which provides electrical access to the polymer and the gold electrode on the bottom. The first structure from the right in Figure 3.1(c) is fabricated by bringing a silicon o-ring into contact with gold foil by epoxy and leaving a 2 mm diameter circular area for deposition of PPy(DBS). These fixtures also provide electrolytic access above the
polymer for electrochemical characterization of the polymer and subsequent BLM formation.

To investigate the deposition rate and the polymer growth rate during electrodeposition of PPy(DBS), samples of gold foil in the same condition are used as the substrates, and the deposition is performed under 0.7 V for 600 s. The gold foils are weighted before and after polymerization to calculate the weight of deposited PPy(DBS). The thickness of the samples are measured before and after deposition as well, and the differences are the thickness of deposited PPy(DBS). The deposition area, cumulative charge, and duration of electrodeposition are used to calculate the parameters including deposition rate and growth rate. More than 25 samples have been fabricated, and the representative date from three candidate samples are listed in Table 3.1. The thickness of PPy(DBS) could be controlled by knowing the exact growth rate.

Figure 3.3: Typical measurements of CV and CA for electrodeposition of PPy(DBS) on gold foil. (a) CV and there are redox regime and polymer growth regime due to different applied voltages. (b) CA and the applied voltage is 0.6 V.
Table 3.1: Electrodeposition parameters of PPy

<table>
<thead>
<tr>
<th>Sample</th>
<th>Au thickness (mm)</th>
<th>PPy thickness (mm)</th>
<th>PPy average area (mm×mm)</th>
<th>PPy weight (g)</th>
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<tr>
<td>1</td>
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<td>0.05</td>
<td>2*5.5</td>
<td>0.0008</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>0.05</td>
<td>2*4</td>
<td>0.0004</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>0.05</td>
<td>2*4.25</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cumulative charge (C)</th>
<th>Deposition rate (C/cm²)</th>
<th>PPy growth rate (nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5819</td>
<td>5.290</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>0.3769</td>
<td>4.710</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>0.4087</td>
<td>4.810</td>
<td>80</td>
</tr>
</tbody>
</table>

3.4 Bioderived Membrane Layer

3.4.1 Vesicle Preparation for Vesicle Fusion

In the next step, the BLM is formed on the polymer membrane using the vesicle fusion method. Vesicle fusion method provides a simple preparation procedure and is suit for self-assembly of the membranes in our fabricated structures. 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids) is purchased in powder form (molecular formula C₄₈H₉₆NO₈P, molecular weight 846.252) and prepared in a concentration of 5.0mg/mL in deionized water. The solution is then sonicated (B1500A-DTH, VMR) for periods of 10 min to get a small size of vesicle in solution, and vortexed (digital vortex mixer 14005-824, VWR) under 1500 rpm for periods of 5 min to get a uniform distribution of vesicle in solution. A 15 min interval as the rest time is applied between each sonication-vortexing period to cool the solution to the room temperature. The solution is repeated three times with the sonication-vortexing-rest cycles and seen less cloudy after each cycle. (To obtain a homogeneous size of vesicles, syringe filter (Puradisc 4, 0.2 µm, nylon, Whatman) could be used to extrude the solution.) The final solution was scattered onto the surface of PPy(DBS)-coated gold film. The vesicles prepared using sonication-vortexing-rest method are shown in the optical micrograph in Figure 3.4. Dynamic light scattering (Zetasizer Nano-zs90, CLSE, VCU) is used to determine the size distribution of lipid vesicles.
upon repeating numbers of sonication-vortexing-rest cycle. The size distribution by intensity are shown in Figure 3.5 and 3.6, both of which are measured with 1 mg/mL DPhPC concentration. The vesicles after three cycles have the most 73.9% population around 600 nm diameter and a minority of 26.1% around 100 nm diameter. The diameters from ten cycles are seen to be a wide distribution between 40 nm and 1400 nm, which is not desired for the uniform requirement of vesicles.

Figure 3.4: Microscopy of DPhPC vesicles after 18 min sonication and 5 min vortex

Figure 3.5: DPhPC vesicles after 3 cycles of sonication-vortex-rest treatment
Figure 3.6: DPhPC vesicles after 10 cycles of sonication-vortex-rest treatment

3.4.2 Protein Reconstitution

Alamethicin (from trichoderma viride 98%, HPLC, Sigma-Aldrich) is purchased as a lyophilized powder (molecular weight 2kDa) and stored in ethanol at 0.5% (w/v) at 2 °C to 8 °C. There are two ways to reconstitute the protein into the lipid membrane. In the first method, vesicles containing only DPhPC are prepared using the sonication-vortexing-rest procedure described earlier, added to the PPy(DBS) surface, and equilibrated for a while to form a planar BLM. Then, 1µL of the alamethicin stock solution (alamethicin dissolved in ethanol) is diluted further by mixing it with 1 mL of 100 mM NaCl solution. 1–2µL of the mixture is added directly to the BLM supported on PPy(DBS) surface. In the second method, the proteins are first reconstituted into the vesicles using sonication-vortexing-rest method by one additional cycle. The vesicles containing the BLM and the alamethicin are added to the PPy(DBS) membrane and allowed to fuse onto the surface. In both methods, membranes are allowed to stand in air for 15-30 min and rehydrated with 500µL of 100 mM NaCl for electrochemical characterization. Both the methods yield successful reconstitutions of protein in the BLM and will be demonstrated in the next chapter.
3.5 Conclusion

This chapter translates a novel concept into an electroactive ionic device by integrating a conducting polymer with a protein-reconstituted bilayer lipid membrane. The experimental work demonstrates the fabrication of this device for further investigation on its functionality as an ionic transistor. The conducting polymer layer is a polypyrrole membrane doped with dodecylbenzenesulfonate (PPy(DBS)) that is easy fabricated by electrodeposition method and can be controllably doped/undoped in the presence of sodium ions. The thickness of PPy(DBS) is controllable by knowing the growth rate that can be calculated based on the different applied voltage. The BLM is formed from DPhPC lipids on to the surface of PPy(DBS) by vesicle fusion method. The voltage-gated protein alamethicin that transports sodium ions beyond an operating voltage is reconstituted into the BLM. The fabricated device will be characterized in next chapter to prove it provides a novel mechanism to regulate the concentration of the mobile cation in the vicinity of the polymer and hence reversibly control the doping/undoping process in the polymer.
Chapter 4

Characterization of Hybrid Bioderived Membrane

4.1 Introduction

The hybrid bioderived membrane has been fabricated using a layer-by-layer assembly method. Gold foil or gold sputtered polycarbonate track etch (PCTE) membrane has been used as the substrate for adding the component layers to build the membrane. In this chapter, the experimental methods to characterize the component layers and the integrated membrane is presented in detail. Ion transport properties of the conducting polymer are investigated under different ion concentrations using electrochemical measurements such as EIS, CV, and CA, and the surface morphology are investigated through AFM and SEM.

4.2 Equivalent Circuits of Hybrid Bioderived Membrane

The prototype of hybrid bioderived membrane is illustrated in Figure 4.1(a). Gold foil measuring 10µm in thickness serves as the support for deposition of PPy(DBS) and works as an electrode for characterization. On top of the gold, a thin layer
of PPy(DBS) of thickness 50µm is formed and supports the DPhPC lipid bilayer reconstituted with alamethicin. The electrolyte in the top chamber has an Ag/AgCl electrode that serves a port to access the BLM. Once a voltage appropriate to activate the protein to form a ion channel is applied between the Ag/AgCl and gold electrode, alamethicin monomers in the BLM aggregate to form a pore and conduct ions down an applied gradient. The ion ingress or egress in the PPy(DBS) layer is regulated by the ion transport across the BLM, leading to a mechanical response of the PPy(DBS) membrane.

To illustrate the mechanism of component layers and the function of the hybrid bioderived membrane, two equivalent circuit representing the hybrid bioderived membrane are presented in Figure 4.1(b) and (c). If the PPy(DBS) is in oxidized state when the voltage \( V \) is applied, and the polarity of the applied voltage is in the same direction as along the ion concentration gradient, i.e., \( c_2 < c_1 \), the Na\(^+\) ion will flow from the electrolyte to the vicinity of PPy(DBS) across the BLM, as well as the current. With ions entering the PPy(DBS), the volume of PPy(DBS) increases. If the PPy(DBS) is in reduced state when the applied voltage \( V \) is given, and the polarity of applied voltage is in the same direction as along the ion concentration gradient, i.e., \( c_2 > c_1 \), the Na\(^+\) ion will flow from the vicinity of PPy(DBS) to the electrolyte across the BLM, and so does the current. With ions exiting the PPy(DBS), the

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**Figure 4.1:** Hybrid bioderived membrane for characterization. (a) Schematic of hybrid bioderived membrane. (b) Equivalent circuit of hybrid bioderived membrane in the oxidized state of PPy(DBS). (c) Equivalent circuit of hybrid bioderived membrane in the reduced state of PPy(DBS).
volume of PPy(DBS) decreases. In both cases, the exchanged charge $\Delta Q$ between the PPy(DBS) layer and the electrolyte is regulated by ion transport through the protein-reconstituted BLM.

As proved by preliminary experiments, the impedance of BLM is much higher than that of other components in the hybrid bioderived membrane and slightly lower than that of whole hybrid membrane. When a voltage $V$ is applied to the whole hybrid membrane between the Ag/AgCl and gold electrode, the voltage $V_b$ across the BLM could be considered same as the voltage $V$. In the other word, the voltage $V$ applied between the Ag/AgCl and gold electrode is taken as a gate signal across the BLM and used to trigger the alamethicin to form the ion channel in the characterization experiments.

Most physical systems have the electrical impedances as functions of frequency, indicating capacitors and conductors contribute to the circuit model. And these physical systems usually consist of several subsystems, for which the circuit model is not known or complex. EIS provides a method to measure the total complex impedance of such systems varies with frequency. Thus, the equivalent circuits of the hybrid bioderived membrane and its components are required to investigate the electrical properties through the electrical characterization methods such as EIS.

An equivalent circuit for analysis of RC networks for gold-supported PPy(DBS) is shown in Figure 4.2. $R_e$ is resistance of electrolyte solution, $R_P$ and $C_P$ account for the polymer resistance and capacitance. Equation 4.1 represents the total impedance of the PPy(DBS)-coated gold film.

\[
R_{\text{electrolyte}} \quad R_{\text{ppy}} \quad C_{\text{ppy}}
\]

Figure 4.2: Equivalent circuit of PPy(DBS) measurement
\[ Z = R_e + \frac{R_P}{\omega^2 C_P^2 R_P^2 + 1} - j \frac{\omega C_P R_P^2}{\omega^2 C_P^2 R_P^2 + 1} \] (4.1)

An equivalent circuit presenting the RC networks for gold-supported PPy(DBS) with BLM is shown in Figure 4.3. \( R_B \) and \( C_B \) account for the BLM resistance and capacitance. Equation 4.2 represents the total impedance of the BLM supported by PPy(DBS)-coated gold film.

\[ Z = R_e + \frac{R_P}{\omega^2 C_P^2 R_P^2 + 1} + \frac{R_B}{\omega^2 C_B^2 R_B^2 + 1} - j \left( \frac{\omega C_P R_P^2}{\omega^2 C_P^2 R_P^2 + 1} + \frac{\omega C_B R_B^2}{\omega^2 C_B^2 R_B^2 + 1} \right) \] (4.2)

Alamethicin is modeled as a pure resistor \( R_A \), and is included in the equivalent circuit as shown in figure 4.4. And the total impedance for the alamethicin reconstituted BLM supporting by PPy(DBS)-coated gold film is shown in Equation 4.3.

Figure 4.3: Equivalent circuit of measurement on BLM supported by PPy(DBS) film

Figure 4.4: Equivalent circuit of measurement on alamethicin reconstituted BLM supported by PPy(DBS) film
\[ Z = R_e + \frac{R_p}{\omega^2 C_p^2 R_p^2 + 1} + \frac{R_B R_A (R_B + R_A)}{\omega^2 C_B^2 R_B^2 R_A^2 + 1} - j \left( \frac{\omega C_p R_p^2}{\omega^2 C_p^2 R_p^2 + 1} + \frac{\omega C_B R_B^2 R_A^2}{\omega^2 C_B^2 R_B^2 R_A^2 + (R_B + R_A)^2} \right) \]

In the above equations, the total impedances for the hybrid bioderived membrane and its components are functions of frequency \( \omega \), which is associated with the measurement from EIS.

### 4.3 Actuation in Conducting Polymer Layer

#### 4.3.1 Electrochemical Measurements of the Gold-supported PPy(DBS) Layer

Figure 4.5: Experimental setup for CV measurement of PPy(DBS) film. Electrical terminals include the PPy(DBS)-coated gold foil that is connected to Autolab 128N through working electrode and source, and Ag/AgCl half cell through counter electrode and reference electrode.

All the electrochemical measurements are carried out using Autolab/PGSTAT128N electrochemical analyzer system (ECO Chemie BV) as shown in Figure 4.5. This system can provide a variety of electrochemical measurements including electrochemical impedance spectroscopy(EIS), cyclic voltammetry(CV), chronoamperometry(CA), etc. The experimental setup is placed on a vibration isolation table to
Figure 4.6: CV of PPy(DBS) film in different electrolytes

isolate the setup from structural disturbances.

Cyclic Voltammetry (CV) Measurement

A baseline CV test is performed on PPy(DBS) film to demonstrate the current-voltage relationship in varying concentration (0 mM, 10 mM, 100 mM, 1 M) of Na$^+$ ions in the electrolyte. Voltage is varied between 0.3 V and −1 V up to 50 times or until the response is stabilized for the variations in applied voltage. The CV data of the stabilized cycle from representative samples are shown in Figure 4.6. The CV data obtained from the polymer resemble the behavior shown by other groups [192] (at 1 M) that use it as an actuator and exhibit the regimes expected from a (DBS)$^-$-doped PPy. It is observed that in the absence of mobile ions in the electrolyte (in the case of deionized water), the impedance of the conducting polymer is fairly high and the currents measured are negligible (∼pA). With more Na$^+$ ions in the electrolyte, the impedance of conducting polymer decreases and the currents increase, indicating the egress and ingress of mobile Na$^+$ ion contribute to the redox of conducting polymer and mechanical response of conducting polymer likely occurs in high concentration of Na$^+$ ion.
Electrochemical Impedance Spectroscopy (EIS) Measurement

For EIS characterization, the PPy(DBS) membrane is prepared by attaching gold-supported PPy(DBS) to the rapid prototyped fixture described in chapter 2 and rinsed in deionized water. As an example shown in Figure 4.7, the gold-supported PPy(DBS) attached to epoxy confined by silicon o-ring has a chamber above the polymer filled with 100 mM NaCl solution. The working electrode and source of Autolab 128N are connected to a silver wire (cleaned with ethanol and water, and immersed in common household bleach for 15-30 min) contacting with the 100 mM NaCl solution, while the reference and counter electrodes are connected to the gold film. EIS is conducted at the beginning point of 100 KHz and ending point of 0.01 Hz, and has 20 points measured in approximately 9 min. An alternating signal of sinusoid with amplitude 10 mV is applied between the two electrical terminals and the frequency-dependent impedance response of the polymer is measured. As a representative, measurements from six data sets are shown in Figure 4.8. This EIS measurement serves as the baseline for the formation of BLM above PPy(DBS) membrane.

Using the expression of total impedance in Equation 4.1 and experiment data from Figure 4.8, the general values based on the representative samples are calculated. The resistance of PPy(DBS) in 2mm diameter circle area is $2.9(\pm 1.0)$ MΩ and the
capacitance of that is 0.067 (± 0.022) µF. The specific resistance of PPy(DBS) is 91 (± 32) kΩ·cm² and the specific capacitance of PPy(DBS) is 2.1 (± 0.7) µF/cm².

Microscopies of the Gold-supported PPy(DBS) Layer and PPy(DBS) Deposited on Gold Sputtered Polycarbonate Filter Paper

The gold-supported PPy(DBS) membrane is imaged under an AFM (Asylum Research, Dr Yadavalli, VCU) and an SEM (Hitachi SU-70 FE-SEM, Nanoscale Core Characterization Lab, VCU). The sample shown in Figure 4.9 is scanned using a PicoPlus Molecular Imaging controller at the room temperature in appropriate aqueous environments. The image illustrated in Figure 4.10 is observed under 7kV. The image of the PPy(DBS) delaminated from the gold sputtered polycarbonate filter paper is also observed by SEM as shown in Figure 4.11.
4.3.2 Coupling Coefficient of PPY(DBS) Deposited on Gold Sputtered Polycarbonate Filter Paper

Coupling coefficient that relates the electrical properties with the mechanical properties for conducting polymers is an important parameter that can illustrate the sensitivity of the transformation between the physical domains and be used in modeling the hybrid bioderived membrane. The gold sputtered polycarbonate filter paper (resistance is ca. 4.5 Ω·cm) deposited with PPY(DBS) is used to demonstrate the coupling by measuring the field-dependent bending deformation in the actuator. The movements of PPY(DBS) deposited on gold sputtered polycarbonate filter paper in 1M NaCl recorded by video is driven by the applied voltages in a step function between 0.5V and −1.5V with a frequency of 50 mHz. The step input lasting 60 seconds can be used to demonstrate if the response reaches steady state, and the cumulated charge regarding to the ion transport in 60 seconds can be obtained from the current response. According to the previous description in chapter 2, the Equation 2.18 is used to calculate the coupling coefficient $\alpha$ by substituting the values of material pa-
Figure 4.10: SEM of PPy(DBS) deposited on gold foil

Table 4.1: Parameters used for calculation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
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<td>Thickness of polycarbonate filter paper</td>
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<td>Thickness of the whole film</td>
<td>$h_2$</td>
<td>40 µm</td>
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<tr>
<td>Width of the whole film</td>
<td>$w$</td>
<td>3 mm</td>
</tr>
<tr>
<td>Length of the whole film</td>
<td>$L$</td>
<td>17 mm</td>
</tr>
<tr>
<td>Elastic modulus of PPy(DBS)</td>
<td>$E_{PPy}$</td>
<td>80 MPa</td>
</tr>
<tr>
<td>Elastic modulus of polycarbonate filter paper</td>
<td>$E_{pc}$</td>
<td>2 GPa</td>
</tr>
<tr>
<td>Exchanged charge through PPy(DBS)</td>
<td>$\Delta Q$</td>
<td>0.1083 C</td>
</tr>
</tbody>
</table>

Parameters listed in Table 4.1 and estimating the geometry from bending strip shown in Figure 4.12(b). The coupling coefficient $\alpha$ of the PPy(DBS) deposited on gold sputtered polycarbonate filter paper is 0.0897 (F/m²)/(C/m³), which is quite similar to Alici’s result [5].
4.4 Ion Transport in Bioderived Membrane Layer

Electrical Impedance Spectroscopy (EIS) Measurement

The gold-supported PPy(DBS) membrane attached to the rapid prototyped fixture described earlier is washed with deionized water and dried under nitrogen, and vesicles prepared following the procedure in Section 3.4 are added on top of the PPy(DBS) membrane and allowed to fuse with the surface. If lipid vesicles are prepared without any protein, the formation and presence of a BLM on PPy(DBS) can be validated by EIS. The experimental setup is the same as the one for EIS characterization of the gold supported PPy(DBS), and same condition is applied using EIS with the frequency-dependent impedance measured. The measured impedance response from PPy(DBS) supporting DPhPC BLM is shown in Figure 4.13. It is observed that the impedance in the presence of the BLM increases by $10^3$ to $10^4$ orders of magnitude by comparing the plots in Figure 4.8 and 4.13. This increase is consistent and remains stable for at least 24 h.
Figure 4.12: Actuation of PPy(DBS) on gold sputtered polycarbonate filter paper. (a) Schematic of actuation model, (b) bending actuation.

Cyclic Voltammetry (CV) and Chronoamperometry (CA) Measurements

In the next step, alamethicin is reconstituted in the BLM following the procedure described in chapter 2. Upon reconstitution of the alamethicin in the BLM, no significant difference in the impedance of alamethicin reconstituted BLM is observed by EIS. The amplitude of the alternating signal in EIS characterization (10 mV) is insufficient to form pore forming channels from alamethicin in DPhPC BLM. Hence, the similarity in impedance response is repeated between the DPhPC BLM with and without alamethicin. Due to the voltage-gated nature of alamethicin reconstituted in the BLM, the peptide units assemble into a pore forming channel and allow the transport of sodium ions occur over a range of voltages along the applied concentration gradient. The electrical activity of alamethicin in BLM is observable by CV and CA using appropriate voltages. The DPhPC to alamethicin ratio is 2.36:1 in Figure 4.14(b), (c), (d). The other Figures ??, 4.16, 4.17 is in the condition of 9.44:1 lipid to protein ratio.

The CA characterization on alamethicin activity is performed by applying two different voltages 50 mV and 100 mV in both situations, BLM with and without alamethicin, as shown in Figures 4.14. It is observed that the currents of the BLM with alamethicin (in Figure 4.14(c)) increase compared to that of without alamethicin (in Figure 4.14(a)) when 100mV is same applied. The varying current behavior is seen from BLM with alamethicin, while the smooth current response is from BLM
without alamethicin. This is a clear evidence for the formation of the pore forming channels. It is also noticed that the currents of BLM with alamethicin under 100 mV (in Figure 4.14(c)) increase and vary in a wider range compared to that of 50 mV (in Figure 4.14(b)). This indicates the formation of pore forming channel occurs more often under 100 mV than that of 50 mV. The zoom in part shown in Figure 4.14(d) has been processed by 4th order butterworth lowpass filter with 0.2 cutoff frequency. The varying magnitude of ionic current could be considered as multiple states of the activated ion channels and non-activated state. It is obvious that the ion channels are not constantly activated and the number of activated ion channel is varied with time. Ionic currents in discrete states correspond to different population of the activated ion channels. A histogram of ionic current is derived from the CA characterization under 100mV as shown in Figure ???. This plot represents the accumulative effect for multiple ion channels. The most population of activated ion channels occurs in the current range from 180 pA to 230 pA.

The current-voltage response of DPhPC with alamethicin is analyzed by CV
Figure 4.14: CA of DPhPC BLM supported on PPy(DBS) film with and without alamethicin under different voltages. (a) BLM without alamethicin under 100 mV, (b) BLM with alamethicin under 50 mV, (c) BLM with alamethicin under 100 mV, (d) zoom in 5s from (c) with lowpass filter.

in the voltage range from 80 mV to −80 mV and represents the behavior of the alamethicin-reconstituted BLM along the applied gradient for different applied transmembrane potentials. The membranes tested as shown in Figures 4.16 are reconstituted with 1mg/mL of protein and allowed to sit for 24 h before rupturing. The plots in Figure 4.16 (a), (b), (c) are measured in time sequence, and it is observed that the larger ionic current through the membrane are measured in the voltage ranges of −80mV to −50mV. Despite the noise measured in these plots, the activity of the protein and voltage-dependent conductance change are clearly observed.

To demonstrate the voltage-dependent conductance change, the CA characterization performed in 1 h after reconstitution of alamethicin in DPhPC BLM is analyzed as shown in Figure 4.17. The increased conduction beyond an electrical gradient of −50 mV applied across the BLM with alamethicin supported on PPy(DBS) is due to the presence of alamethicin in the BLM. This observation resembles the
behavior of alamethicin reported by various groups in DPhPC BLM [218, 90, 180]. The conductance of the BLM reconstituted with alamethicin increases by 3 to 4 times above the operating voltage.

As described earlier, the oxidized or reduced state of PPy(DBS) determines the direction of ion transport when a trigger signal is applied to the alamethicin reconstituted BLM along the ion concentration gradient. This is the reason of some electrochemical measurements with positive current and other negative, thus, the controlling of PPy(DBS) film in appropriated state is required for the repeatability of the characterization. Another obstacle attributes to the variations in the BLM formed on top of a rough (relative to the thickness of the BLM) PPy(DBS) surface.
Figure 4.17: Voltage-dependent conductance change of alamethicin reconstituted DPhPC BLM supported on PPy(DBS) film. The operating voltage refers to the voltage above which an increased conductance is observed.

4.5 Conclusion

It has been proved that the architecture of supporting a BLM with proteins on a conducting polymer provides a novel mechanism to regulate the concentration of mobile cations available in the vicinity of the polymer. The redox state of the conducting polymer can be regulated by a mechanism besides electrical field that is external to the conducting polymer. The regulation of concentration in the interfacial region produced by the protein can have the influence of concentration on the electrical properties of the conducting polymer through redox processes. The current experimental arrangement of our device does not allow for accessing the interfacial region between the conducting polymer and the BLM and hence experimental data are not available to demonstrate the function of the hybrid bioderived membrane as a transistor. Nonetheless, a ionic actuator developed from the hybrid bioderived membrane will be proposed in the following chapter.
Chapter 5

PPy(DBS) Actuator

5.1 Introduction

The concept of hybrid bioderived ionic actuator developed from hybrid bioderived membrane has been described in Chapter 2. PPy(DBS) actuator is fabricated and characterized to enable the design of the bioderived hybrid ionic actuator. PPy(DBS) actuator is demonstrated as a actuation element that converts chemo-electrical energy into mechanical energy in the presence of appropriate sodium ions and an applied electrical field. A volumetric actuation element and micro-cantilever actuation element are fabricated and coupling coefficients are obtained from characterization experiments. The characterization coefficients are used to identify the design constraints for the hybrid bioderived ionic actuator.

5.2 Fabrication of PPy(DBS) Cantilever Actuator

The polycarbonate track etch (PCTE) membrane (18 \( \mu \text{m} \) thick, 10 \( \mu \text{m} \) diameter pore), or called polycarbonate filter paper, is used as the substrate for PPy(DBS) actuator due to its high compliance. A 50nm thin platinum layer is sputtered on the polycarbonate membrane for the electrodeposition of PPy(DBS). A three electrode polymerization cell similar to the one described in chapter 3 and redesigned as shown
Figure 5.1: Electrodeposition of PPy(DBS) on gold/platinum-sputtered polycarbonate filter paper. (a) Experimental setup for electrodeposition of PPy(DBS) on gold/platinum-sputtered polycarbonate filter paper, (b) three electrode polymerization cell, (c) cyclic voltammetry indicating the voltage range for electrodeposition, (d) chronoamperometry is conducted under 0.65V for polymerization.

in Figure 5.1(b) is used for electropolymerization. In this cell, the platinum-sputtered PCTE is used as the working electrode (WE). Gold foil is used as the counter electrode (CE) and Ag/AgCl half-cell is used as the reference electrode (RE). The electrolyte used for electropolymerization is 0.3 M pyrrole (reagent grade, 98%, Sigma-Aldrich) mixed with 0.1M NaDBS solution. The increase in concentration of pyrrole from 0.1M to 0.3M enhances the deposition of PPy(DBS) on less conductive sputtered-platinum layer (in comparison with the gold-sputtered/gold-film used in experiments described in Chapter 3). All the electrical terminals are connected to Autolab 128N as shown in Figure 5.1(a) for electrodeposition and characterization, including electrochemical impedance spectroscopy, cyclic voltammetry and chronoamperometry as shown in Figure 5.1(c)(d). Electropolymerization of PPy(DBS) on the platinum-sputtered PCTE filter paper is achieved by potentiostatic deposition at 0.65 V for 1200s. The
currents measured during deposition is shown in Figure 5.1(d) and used to calculate the amount of PPy(DBS) deposited on PCTE filter paper.

Figure 5.2: PPy cantilever actuator. (a) A dark layer of PPy(DBS) deposited on the gold/platinum sputtered polycarbonate filter paper, (b) PPy(DBS) cantilever actuator with 1mm by 5mm effective PPy(DBS) area, (c) schematic of PPy(DBS) cantilever actuator as a sandwich assembly of gold foil, gold leaf, and PPy(DBS) deposited on the gold/platinum sputtered polycarbonate filter paper.

The samples are cut into an appropriate size and assembled into a sandwich to form the cantilever actuation element. This sandwich, as shown in Figure 5.2(c) contains two outer gold foil layers for electrical connection, an inner layer of PPy(DBS) on gold/platinum-sputtered polycarbonate filter paper, and two intermediate gold leaf layers for conduction between gold foil and PPy(DBS) film. The actuation element shown in Figure 5.2(b) measures 1mm by 5mm and is ready for characterization experiments.

5.3 Characterization of PPy(DBS) Cantilever Actuator

PPy(DBS) cantilever actuator undergoes volumetric expansion for an applied electric field in the presence of sodium ions in the electrolyte. The mechanical response resulting from the applied electrical field is quantified by electrochemical measurements and optical microscopy. The electrochemical measurements include electrochemical impedance spectroscopy and chronoamperometry. The deformation of the actuation
element is monitored under an optical microscope and the actuation data is correlated with the applied electrical stimulus to characterize chemomechanical coupling. Sample micrographs of the actuator observed under 4X magnification in MeijiTechno ML2700 microscope is shown in Figure 5.3 and the setup for characterization is shown in Figure 5.4 and Figure 5.6.

![Figure 5.3: Microscope image of PPy(DBS) on platinum sputtered polycarbonate filter paper](image)

5.3.1 Electrochemical Impedance Spectroscopy (EIS) Measurement

![Figure 5.4: Experimental setup for EIS measurement. PPy(DBS) cantilever actuator is held as a cantilever in the teflon chamber containing the electrolyte, and is connected to working electrode and source. The Ag/AgCl half cell is connected to counter electrode and reference electrode. EIS measurement is collected by Autolab 128N between electrical terminals.](image)

In the Chapter 4, EIS has been measured on PPy(DBS) supported on gold
foil. Here, PPy(DBS) on platinum-sputtered polycarbonate filter paper, as the main part of the PPy(DBS) cantilever actuator, is measured to characterize the electrical equivalents of the PPy(DBS) cantilever actuator. The experimental setup is shown in Figure 5.4. A teflon rectangular chamber with a glass slide attached as the bottom is used for stabilized holding the PPy(DBS) cantilever actuator and containing the electrolyte. PPy(DBS) cantilever actuator serves as the working electrode and source, and Ag/AgCl half cell is connected to the counter electrode and reference electrode. The equivalent impedance of the PPy(DBS) cantilever actuator in different electrolytic solutions is characterized from electrical impedance spectroscopy. Sample results from these measurements is shown in Figure 5.5. Different sample with different dimensions are also investigated and shown within the similar results (see appendix A.1 for more information).

![Graph showing impedance and phase against different frequencies.](image)

Figure 5.5: EIS of PPy(DBS) cantilever actuator showing impedance and phase against different frequencies.
Table 5.1: Resistance and capacitance of PPy(DBS) cantilever actuator in the electrolyte with different ion concentration

<table>
<thead>
<tr>
<th>c_{NaCl} (mM)</th>
<th>R_{PPy} (kΩ)</th>
<th>ρ_{PPy} (kΩ·cm²)</th>
<th>C_{PPy} (µF)</th>
<th>ε_{PPy} (µF/cm²)</th>
<th>R_{electrolyte} (kΩ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92.0</td>
<td>2.9</td>
<td>1.7</td>
<td>53.8</td>
<td>5.1</td>
</tr>
<tr>
<td>10</td>
<td>45.5</td>
<td>1.4</td>
<td>1.9</td>
<td>58.9</td>
<td>2.5</td>
</tr>
<tr>
<td>100</td>
<td>19.5</td>
<td>0.6</td>
<td>7.4</td>
<td>236.6</td>
<td>0.5</td>
</tr>
<tr>
<td>1000</td>
<td>3.5</td>
<td>0.1</td>
<td>15.5</td>
<td>493.6</td>
<td>0.07</td>
</tr>
</tbody>
</table>

c_{NaCl} - Concentration of NaCl electrolyte  
R_{PPy} - Resistance of PPy(DBS)  
ρ_{PPy} - Specific Resistance of PPy(DBS)  
C_{PPy} - Capacitance of PPy(DBS)  
ε_{PPy} - Specific Capacitance of PPy(DBS)  
R_{electrolyte} - Resistance of Electrolyte

Resistance and capacitance of PPy(DBS) cantilever actuator and the corresponding resistance of electrolyte are calculated using the expression of total impedance in Equation 4.1. The values are obtained using Autolab software (fit and simulation). The PPy(DBS) cantilever actuator has a effective PPy(DBS) dimensions in 1mm width, 5mm length, and 0.013mm thickness. Thus, the specific resistance and specific capacitance in area are calculated as listed in Table 5.1. Generally, the higher the sodium ion concentration in the electrolyte, the smaller the specific resistance of PPy(DBS) cantilever actuator and the larger the specific capacitance of PPy(DBS) cantilever actuator.

5.3.2 Ion Transport Coefficient from Chronoamperometry (CA) Measurement with Tip Displacement Recording

The ion transport during the redox of PPy(DBS) cantilever actuator has been demonstrated by CV measurement. The quantity of ion or charge involved in the redox process is a further step for the extension of characterization. A good way to quantify the charge is to monitor the current by applying step voltages, i.e., CA measurement. The current is more likely to reach or near to a steady-state value when a constant step voltage is applied, thus, the tip displacement staying longer in a static state is
Figure 5.6: Experimental setup for electrochemical and mechanical characterization of PPy(DBS) cantilever actuator.

easily captured by the camera through microscope. The experimental setup of CA measurement is the same as shown in Figure 5.6. PARSTAT 4000 (Princeton Applied Research, Ametek) and ML2700 microscope (Meiji Techno) are used to perform CA measurement and record the tip displacement, respectively. In Figure 5.7, 1M NaCl solution is used as electrolyte. 0.5V and -0.1V are applied as the step voltages with duration 15s, repeating for three times, and the current shows spikes due to the capacitative discharge and die out to reach a steady state value, as shown in Figure 5.7(a). The corresponding charge is plotted in Figure 5.7(b), and the tip displacement is recorded through camera. The tip displacement in Figure 5.7(c) corresponds to largest positive charge in the end of last 0.5V step period, and the tip displacement in Figure 5.7(d) accords to the largest negative charge in the end of last -0.1V step period.

CA measurements for PPy(DBS) cantilever actuator in 1M NaCl are repeated five time, and the average values are calculated. The largest positive charge is 0.0041 C with the tip displacement 410.6 µm, while the largest negative charge is 0.0030 with the tip displacement 428.9 µm. Different ion concentration (150mM, 200mM, 250mM, 500mM, 1M) (see appendix A.3 for details) are analyzed using the same procedure as that of 1M NaCl to have a more general view on the exchanged charge, as well as the tip displacement, along with the ion concentration of the electrolyte. Lower ion concentration (1mM, 10mM, 50mM, 100mM) (see A.3 for details) mainly
Figure 5.7: CA for PPy(DBS) cantilever actuator showing the current-time response. (a) The red line represents the applied voltage, and the blue line is the current-time response, (b) the corresponding charge results from the current-time response, (c) tip displacement image is recorded simultaneously with the point of largest positive charge, (d) tip displacement image is recorded simultaneously with the point of largest negative charge.

Models for PPy(DBS) cantilever have been developed in Chapter 2. Through the characterization, specific resistance and specific capacitance are obtained by electrochemical impedance spectroscopy, ion transport phenomena is demonstrated by cyclic voltammetry, exchanged charge is calculated by the chronoamperometry, tip displacement is recorded by microscope, and dimensional information is achieved by microscope. All the parameters required in the model are available from the characterization experiments. From the experimental characterization discussed in this chapter, ion transport coefficient and its relationship to ion concentration will be developed.
Table 5.2: PPy(DBS) cantilever actuator parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of polycarbonate filter paper</td>
<td>$h_1$</td>
<td>18 µm</td>
</tr>
<tr>
<td>Thickness of the whole film</td>
<td>$h_2$</td>
<td>31 µm</td>
</tr>
<tr>
<td>Width of the whole film</td>
<td>$w$</td>
<td>1 mm</td>
</tr>
<tr>
<td>Length of the whole film</td>
<td>$L$</td>
<td>5 mm</td>
</tr>
<tr>
<td>Elastic modulus of PPy(DBS)</td>
<td>$E_{PPy}$</td>
<td>80 MPa</td>
</tr>
<tr>
<td>Elastic modulus of polycarbonate filter paper</td>
<td>$E_{pc}$</td>
<td>2 GPa</td>
</tr>
</tbody>
</table>

Table 5.3: Ion transport coefficient for PPy(DBS) cantilever actuator in 1M NaCl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium ion concentration</td>
<td>$c$</td>
<td>1M</td>
</tr>
<tr>
<td>Exchanged charge</td>
<td>$\Delta Q$</td>
<td>0.0041C (Ion ingress)</td>
</tr>
<tr>
<td>Tip displacement</td>
<td>$\delta_0$</td>
<td>410.6µm</td>
</tr>
<tr>
<td>Ion transport coefficient</td>
<td>$\alpha$</td>
<td>0.0011(F/m$^2$)/(C/m$^3$)</td>
</tr>
</tbody>
</table>

PPy(DBS) cantilever actuator, as a main functional part in this project, has been modeled as a unimorph cantilever that involves exchanged charge based on applied voltage, tip displacement related to ion transport coefficient. According to the equation 2.21, the ion transport coefficient $\alpha$ can be obtained by knowing the exchanged charge $\Delta Q$ and tip displacement $\delta_0$. The data from CA measurements are used here. The NaCl electrolyte with different ion concentration (1mM, 10mM,

Table 5.4: Ion transport coefficient with ion concentrations from 150mM to 1M

<table>
<thead>
<tr>
<th>Notation</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$</td>
<td>mM</td>
<td>150 200 250 500 1000</td>
</tr>
<tr>
<td>$\Delta Q$ (Ion ingress)</td>
<td>C</td>
<td>0.0057 0.0066 0.0073 0.0094 0.0116</td>
</tr>
<tr>
<td>$\delta_0$</td>
<td>µm</td>
<td>808.4 812.5 804.0 739.5 684.0</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>(F/m$^2$)/(C/m$^3$)</td>
<td>0.0027 0.0023 0.0021 0.0015 0.0011</td>
</tr>
<tr>
<td>$c$</td>
<td>mM</td>
<td>150 200 250 500 1000</td>
</tr>
<tr>
<td>$\Delta Q$ (Ion egress)</td>
<td>C</td>
<td>-0.0065 -0.0076 -0.0081 -0.0105 -0.0124</td>
</tr>
<tr>
<td>$\delta_0$</td>
<td>µm</td>
<td>805.3 812.5 813.8 749.7 687.7</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>(F/m$^2$)/(C/m$^3$)</td>
<td>0.0023 0.0020 0.0019 0.0013 0.0010</td>
</tr>
</tbody>
</table>
Table 5.5: Ion transport coefficient with ion concentrations from 1mM to 100mM

<table>
<thead>
<tr>
<th>Notation (Unit)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>c (mM)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>(Ion ingress) α ((F/m²)/(C/m³))</td>
<td>100</td>
</tr>
<tr>
<td>mean</td>
<td>0.0054</td>
</tr>
<tr>
<td>standard deviation</td>
<td>0.00021</td>
</tr>
<tr>
<td></td>
<td>0.0055</td>
</tr>
<tr>
<td></td>
<td>0.00021</td>
</tr>
<tr>
<td></td>
<td>0.0039</td>
</tr>
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<td></td>
<td>0.00004</td>
</tr>
<tr>
<td></td>
<td>0.0031</td>
</tr>
<tr>
<td></td>
<td>0.00007</td>
</tr>
<tr>
<td>(Ion egress) α ((F/m²)/(C/m³))</td>
<td>mean</td>
</tr>
<tr>
<td></td>
<td>0.0045</td>
</tr>
<tr>
<td>standard deviation</td>
<td>0.00007</td>
</tr>
<tr>
<td></td>
<td>0.0046</td>
</tr>
<tr>
<td></td>
<td>0.00019</td>
</tr>
<tr>
<td></td>
<td>0.0034</td>
</tr>
<tr>
<td></td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>0.00003</td>
</tr>
</tbody>
</table>

50mM, 100mM, 150mM, 200mM, 250mM, 500mM, 1M) are investigated by measuring CA along with tip displacement recording. Both of these data are used for evaluation of the ion transport coefficient, and both ion ingress and ion egress cases are taken into consideration. The values of PPy(DBS) cantilever actuator parameters are listed in Table 5.2. According to the measurements shown in Figure 5.7 in 1M NaCl (measurements are repeated for five time), the average value of concentration-dependent ion transport coefficient are listed in Table 5.3. The results in the electrolyte with different sodium ion concentration from 150mM to 1M are included in Table 5.4, while the statistical value for three time repeating with different sodium ion concentration from 1mM to 100M are complied in Table 5.5.

The PPy(DBS) cantilever actuation model has been evaluated in above section by associating the applied voltage ΔV and ion transport coefficient α to the tip displacement δ₀ of PPy(DBS) actuator. Then, the relation between ion transport coefficient α and ion concentration c is another important link between PPy(DBS) cantilever actuator and the bioderived membrane. The experimental data is plotted in Figure 5.8. The plotted c vs. α curve can be used to demonstrating the actuation of the PPy(DBS) cantilever actuator in the assembled BLM ion channel system. We will focus our experiments on hybrid bioderived ionic actuator in the range of 1mM to 100mM NaCl solution, because larger ion transport coefficient are observed in this range. In the range of 100mM to 1M NaCl, higher ion concentration correspond to
lower ion transport coefficient, which means less efficiency of energy transformation when using higher ion concentration (>100mM) for actuation of PPy(DBS) cantilever actuator. Thus, average value and standard variation of ion transport coefficient are analyzed in the range of 1mM to 100mM NaCl solution.

Figure 5.8: Relation between ion transport coefficient and ion concentration in electrolyte. (a) from 1mM to 100mM with error bar, (b) from 100mM to 1M.

5.4 PPy(DBS) Microsphere Actuator

Figure 5.9: Fabrication of PPy(DBS) microsphere actuator. (a) Experimental setup, (b) microsphere before PPy(DBS) deposition, (c) microsphere after PPy(DBS) deposition as a actuator.

Conformal relaxation of the PPy(DBS) actuator results from an applied elec-
trical field in the presence of appropriate ions in the solution. In the hybrid bioderived ionic actuator, ion transport across the bioderived membrane for an applied signal provides the ions and the electrical field applied to the PPy(DBS) actuation element couples the chemoelectrical gradient with mechanical deformation. To build such an actuator in micro-scale is the next step for the integration with bioderived materials. A microsphere form of PPy(DBS)-based actuator has been developed here due to the difficulty in fabricating a micro-scale PPy(DBS) cantilever. The tip of a thin silver wire (100µm diameter) is shown in flame to form into a ball-shaped end. This ball end serves as a substrate for deposition of PPy(DBS), as shown in Figure 5.9(b). Silver wire electrodes are chlorided in undiluted sodium hypochlorite solution for 1 hour until they turn dark gray in color and then rinsed with water.

The fixture used for electrodeposition is prototyped by curing a thin layer of PDMS with punched holes shown in Figure 5.9(a). Sylgard 184 silicone encapsulant kit clear (Dow Corning) is mixed (10:1 for two part) and poured into a flat bottom cup. PDMS is degased in vacuum oven at -28 inHg for 30 mins. The pressure in the chamber is cycled between negative and atmosphere until the air bubbles are completely popped. The vacuum oven is opened and resealed, and PDMS is heated at 120 °C for 20 minutes. After the PDMS is cured, two overlapping holes are punched out and three small slits are made on the fixture. The fixture is attached on a glass slide to create small chambers for filling with the electrolyte. The electrodes and the wires are inserted into the slits and connected to chemoelectrical analyzer as counter electrode, reference electrode, and working electrode, respectively.

The deposition process is recorded under microscope showing by applying 0.475 V for 10 minutes a dark layer of PPy(DBS) wrapped the silver ball, as shown in Figure 5.9(c). The diameter of the PPy(DBS) microsphere is estimated to be 0.5mm.

In the next step, a simple demonstration on the actuation of PPy(DBS) microsphere actuator is required. The microsphere is monitored under microscope when chemoelectrical measurements are performed. As shown in Figure 5.10, the images are recorded during chronoamperometry conducted by applying -1V and 0.01V each
Figure 5.10: CA for PPy(DBS) microsphere actuator showing the current-time response. The red line represents the applied voltage, and the blue line is the current-time response, the back line corresponds to charge. Images of volume change are recorded simultaneously with the point of largest negative and positive charge. (a) Under reflection mode, (b) under transmission mode.

with 5 second duration. The PPy(DBS) microsphere is seen obviously shrink and swell along with the alternatively applied voltage. The exchanged charge is ca. 0.0003 C, and the radius change is around 5% (assuming volumetric expansion state results from ion transport) (i.e., 0.0125% volume change) for both ion ingress and ion egress cases, as shown in Figure 5.11.
Figure 5.11: The volume change of PPy microsphere under microscope. (a) Shrink under reflection mode, (b) swell under reflection mode, (c) volume change under reflection mode, (d) shrink under transmission mode, (e) swell under transmission mode, (f) volume change under transmission mode

5.5 Conclusion

PPy(DBS) actuation element, as a main part of hybrid bioderived ionic actuator, has been fabricated by electrodeposition of PPy(DBS) on gold/platinum-sputtered polycarbonate filter paper. EIS and CA measurements illustrate the redox of PPy(DBS), and the exchanged charge of PPy(DBS) in the redox process are associated with the tip displacement recorded by a camera attached to the microscope. Subsequently obtained ion transport coefficient relates the chemoelectrical domain to the mechanical domain. Moreover, the ion transport coefficient are investigated under different sodium ion concentration electrolytes, which give a empirical model for relating ion transport coefficient to ion concentration in electrolyte for the future use in hybrid bioderived ionic actuator. A PPy(DBS) microsphere actuator is also illustrated to show the PPy(DBS) actuator in micro-scale. In the following chapter, the ion transport through the bioderived membrane will be discussed and design rules for the hybrid bioderived ionic actuator will be developed using the knowledge developed
and discussed in this chapter.
Chapter 6

Hybrid Bioderived Ionic Actuator

6.1 Objectives

The hybrid bioderived membrane - alamethicin reconstituted BLM supported on PPy(DBS) has been fabricated and characterized in previous chapters. This chapter focuses on developing the design rules for actuator controlled by ion transport through the bioderived membrane. The (micro-)actuator is made of either PPy(DBS) deposited on gold or platinum sputtered polycarbonate track etch (PCTE) membrane or PPy(DBS) deposited on a ball-ended silver wire. The ion channel embedded in bioderived membrane is a pathway for ions traveling from one reservoir to another by the chemical gradient. The subsequent change of ion concentration in the reservoir affects the conversion between the chemoelectrical energy and the mechanical energy. The technical objectives for this chapter is to use the hybrid bioderived membrane as a framework to design a PPy(DBS) actuator in a BLM ion channel system. PPy(DBS) actuator has been evaluated as a functional device and will serve as the main part of the hybrid bioderived ionic actuator. The other important part is the BLM ion channel system that can control the ion transport through the protein reconstituted lipid bilayer and subsequently affect the ion concentration in the vicinity of PPy(DBS) actuator, which will have the influence on the ability of transformation from chemoelectrical energy to mechanical energy. In one word, the BLM ion channel system controls the gating signal for the actuation of PPy(DBS) actuator.
6.2 Architectures

A schematic of the configuration for the hybrid bioderived ionic actuator shown in Figure 6.1 and Figure 6.4 is discussed in this section. Two configurations are discussed and design calculations for the two approaches are developed. In the first approach, the PPy(DBS) cantilever actuator is assumed to function in one of the chambers of a two-chambered fixture in which ion transport between chambers is regulated by the bioderived membrane. In the second approach, the PPy(DBS) microsphere actuator is used in a droplet interface bilayer (DIB) and the concentration of ions in the actuator side is regulated by the bioderived membrane.

1. PPy(DBS) cantilever actuator in cup/chamber BLM ion channel system

![Figure 6.1: Prototype design of PPy(DBS) cantilever actuator in cup/chamber BLM ion channel system. (a) Schematic, (b) cup/chamber fixture consisting of a chamber holder with glass window and a cup with incorporated aperture, (c) microscopy image of the aperture with the diameter 150 µm.](image)

PPy(DBS) cantilever actuator fabricated by the polymerization of PPy(DBS) depositing on the gold/platinum-sputtered polycarbonate filter paper same as described in chapter 3 is assumed to be incorporated in the chamber as a cantilever. The polycarbonate track etch (PCTE) membrane (18 µm thick, 10 µm diameter pore) is suggested as a substrate and a thin gold/platinum layer approximately 50nm thick is sputtered on the polycarbonate membrane. For the
BLM ion channel system, a bilayer formation kit purchased from the Warner Instrument is assumed to be used for the formation of BLM ion channel and the assembly of the hybrid bioderived ionic actuator as shown in Figure 6.1(b). The chamber is made from black Delrin and the cup used here is made from translucent either polystyrene or polysulfone. A glass window is for viewing and the aperture is imaged under microscope in Figure 6.1(c) showing the diameter is 150 µm. The painting method is proposed here for the formation of BLM ion channel. The aperture on the cup wall is pre-coated with lipid with protein solution before assembling the cup/chamber fixture. Then, the cup is mounted in the chamber holder and electrolyte with different concentration is filled into chamber and cup. After the formation of BLM ion channel, the PPy(DBS) cantilever actuator is planned to be inserted in the chamber solution for the actuation demonstration. The dimensions of PPy(DBS) cantilever actuator incorporated in the BLM ion channel system are suggested around 1mm by 5mm. The Ag/AgCl half cells are incorporated to conduct the chemoelectrical measurements. Different ion concentration of electrolyte are applied between the chamber and the cup, which is consistent in each case of formation. Once the BLM ion channel is activated (open), the sodium ion moves from the cup to the chamber. With increased ion concentration in chamber, the PPy based actuator in chamber exhibits stronger capability to transform the chemoelectrical energy to the mechanical energy, which is presented as the tip displacement.

Experimental Verification for Cup/chamber Architecture

Prior to the formation of ion channel embedded in BLM, the cup/chamber fixture must be cleaned. The cleaning procedure is following the wash protocol developed by Warner Instruments. Three squirt bottles are labeled and filled with following solutions: 40-50 mM trisodium phosphate (Na₃PO₄, TSP, Sigma-Aldrich), 0.1% (by volume) hydrochloric acid (HCl, Sigma-Aldrich), deionized water (18.2 MΩ, Milli-Q direct water purification system). The cup is taken out of the chamber holder and cleaned with TSP, DI water, HCl, DI water,
sequently. The inside of the cup is cleaned by squirting the solution hard so that no bubble is attached to the inside wall. The aperture is cleaned by filing the cup with the solution, sealing the top of the cup with your figure, applying pressure to squirt a stream of fluid out of the hole, and wiping the outside of the aperture with a solution soaked cotton swab. The chamber is washed in the same way. The cup/chamber fixture is dried with Kimwipes or an air jet and stored dry.

The formation of lipid bilayer is described as follows. DPhPC (Avanti Polar Lipids) is purchased as 10mg in one division bottle. Lipid solution comprises of DPhPC at 40 mg/mL in hexadecane for best performance (10 mg DPhPC is dissolved in 0.25 mL hexadecane). The aperture is first pre-coated with lipids before assembling the cup/chamber fixture. This is achieved by adding the lipid solution directly to the hole. The technique is using capillary tube for 0.5 or 1 mL lipid solution and touching the solution to the hole from the outside of the cup. The advantages of this technique are that it is contamination-free, straightforward, and the covered area can be visually confirmed as roughly 1mm area around the hole. Once the hole has been coated with lipid solution, the cup is mounted in the chamber holder and a set-screw is used to securely hold the cup in place. 1mM NaCl solution is filled in the chamber while 1M NaCl solution is added in the cup. The lipid bilayer membrane should have been formed in the 150 µm aperture.

The ion channel is formed spontaneously with the formation of lipid bilayer. This is achieved by pre-mixing the lipid solution and protein solution prior to applying the solution to the aperture. Alamethicin is purchased in 5mg per bottle from Sigma-Aldrich. DPhPC at 40mg/mL in hexadecane while alamethicin at 1mg/mL in ethanol are used as the stock solution. The pre-mixing is obtained by adding 1 volume portion of protein solution to 5 volume portion of lipid solution. Thus, the weight ratio of lipid to protein is 200:1 and the molar ratio is 535:1 (Molecular weight of DPhPC and alamethicin are 737.039 g/mol
and 1964.31 g/mol, respectively). Then, 0.5 µL pre-mixed solution is applied to the aperture as the same way as described in the procedure of formation of BLM.

Following the formation BLM ion channel system, the chemoelectrical analysis is conducted by using the electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and chronoamperometry (CA).

Figure 6.2: EIS measurement using PARSTAT 4000 for BLM system and BLM ion channel system. (a) Experimental setup, (b) EIS measurement.

Electrochemical impedance spectroscopy (EIS) measurement has been proven as a good tool for demonstration of BLM formation in previous characterization. The experimental setup of EIS measurement for BLM system and BLM ion channel system is illustrated in Figure 6.2(a). Bilayer formation kit is made
the connection with PARSTAT 4000, placed on the vibration isolation stage, and covered with secondary faraday cage and prime faraday cage. The data is collected from computer via PARSTAT 4000. Working electrode and source are connected with Ag/AgCl half cell placed in the cup, while counter electrode and reference electrode are connected with Ag/AgCl half cell placed in the chamber. 1mM and 1M NaCl electrolyte are used in chamber and cup, respectively. The measured data are plotted in Figure 6.2(b).

Figure 6.3: Ionic current measurement using dSPACE and BC-535 for BLM system and BLM ion channel system. (a) Experimental setup, (b) Ionic current.

Ionic current through ion channel has been demonstrated as a important parameter in the modeling of the entire system. It can be effectively measured by
cyclic voltammetry (CV) as proven in the previous characterization. The experimental setup of CV measurements for BLM ion channel system is illustrated in Figure 6.3(a). Bilayer formation kit is made the connection with BC-535 bilayer clamp amplifier via headstage, placed on the vibration isolation stage, and covered with secondary faraday cage and prime faraday cage. The data is collected from computer via dSPACE data acquisition board that is connected to BC-535. Input of headstage is connected with Ag/AgCl half cell placed in the cup, while ground of headstage is connected with Ag/AgCl half cell placed in the chamber. 1mM and 1M NaCl electrolyte are used in chamber and cup, respectively. The ionic current is plotted in Figure 6.3(b).

2. PPy(DBS) microsphere actuator in droplet interface bilayer ion channel system

![Figure 6.4: Prototype design of PPy(DBS) microsphere actuator in droplet interface bilayer ion channel system. (a) Schematic, (b) experimental structure of PPy(DBS) microsphere actuator, (c) experimental structure of droplet interface bilayer.](image)

PPy(DBS) microsphere actuator is suggested to be fabricated by the polymerization of PPy(DBS) depositing on ball-ended silver wire (100 µm diameter) as shown in Figure 6.4(c). The diameter of PPy(DBS) microsphere is designed
around 0.5 mm. DIB method is proposed here to form BLM and ion channel. DIB is formed in the lipid-out method or in the lipid-in method. The lipid-out is by dissolving the lipids in the oil phase, and the lipid-in is by incorporating the lipid vesicles in the aqueous droplets. The lipid-out DIB and lipid-in DIB are both suggested to be formed using procedures same to Sarles et al [180]. For oil phase, DPhPC in lyophilized powder form (Avanti Polar Lipids, Inc.) are dissolved in hexadecane with a concentration of 10mg/mL. For aqueous droplets, one contains 10mM MOPS, 10mM NaCl, and pH7 buffer solution, and the other contains 10mM MOPS, 1mM NaCl, and pH7 buffer solution. The droplets are ready for use until DPhPC and alamethicin are incorporated in both droplets. Silver-silver chloride (Ag/AgCl) electrodes are obtained from 100 µm thick silver wires by chloriding for 30-60 minutes in household bleach until they turn dark gray in color and then rinsing with deionized water. A single DIB is created in the solid substrate made with Sylgard 184 PDMS. Two connected ball-shaped wells are formed in the PDMS molding as shown in Figure 6.4(b). The lipid solution is added completely filling the wells. And then, the droplet with low ion concentration is added to the well that contains the PPy(DBS) microsphere actuator, and droplet with high concentration is added to the other. The DIB ion channel is assumed to be formed after droplets are in contact and equilibrated for a while. PPy(DBS) microsphere should be inserted in the chamber prior to the formation of DIB ion channel system.

6.3 Mathematical Models

The PPy(DBS) actuator in BLM ion channel system can be modeled by assembling all the subsystems as shown in Figure 6.6. In the BLM ion channel system, modeling work can be found including passive permeation model [171], and ionic current model by Poisson equation and Nernst-Planck equation[38]. In this proposed architecture, the inputs of the ion channel
system are an applied voltage $V$ that triggers the protein, and ion concentration $c_o(t)$. The magnitude of charge transport $Q$ can be related to ionic current $i_{eg}(t)$ and will be used to compute the ion concentration $c_o(t)$ in the solution near the PPy(DBS) actuator. The coupling coefficient of electrical properties to mechanical properties has been identified in chapter 4 as the ion transport coefficient $\alpha$, which will be used to develop the transducer equations related to different ion concentration $c_o(t)$.

For PPy(DBS) actuator, electrochemically stimulated conformational relaxation (ESCR) model [153, 222], models based on cation ingress/egress and salt drain-
ing [156] can be used. In this architecture, the PPy(DBS) actuator will be modeled as a unity with multiple inputs including the applied voltage $\Delta V$ through the PPy(DBS) and the ion transport coefficient $\alpha$ related to the ion concentration, and single output of tip displacement $\delta_0$ or volume expansion $\Delta v$. Following the Equation 2.21 developed in Chapter 2, we could easily relate the tip displacement $\delta_0$, similar as volume expansion $\Delta v$, to the ion transport coefficient $\alpha$. Subsequently, the ion transport coefficient $\alpha$ obtained in different ion concentration $c(t)$ could be modeled empirically. Once enough time is given for activation of the ion channels, the sodium ion concentration in the outer chamber reaches the desired value, the PPy(DBS) actuator under appropriate voltage will generate the tip displacement or volume expansion.

The purpose of following section is to develop equations for ion transport through the BLM using electro-diffusion model.

1. **Time dependent ion concentration for a single ion channel**

The total sodium ion in two chambers is constant, we have

$$c_o(t)v_o + c_i(t)v_i = c_o0v_o + c_i0v_i$$  \hspace{1cm} (6.1)

where $c_o(t)$ is sodium ion concentration in the outer chamber, $c_i(t)$ is sodium ion concentration in the inner chamber, $c_o0$ and $c_i0$ are the initial value. $v_o$ and $v_i$ are the electrolyte volume of outer and inner chamber. Thus,

$$c_i(t) = c_i0 + c_o0\frac{v_o}{v_i} - c_o(t)\frac{v_o}{v_i}$$  \hspace{1cm} (6.2)

The exchanged charge is related to the change of sodium ion as

$$Q = (c_o(t) - c_o0)v_ozeN_A$$  \hspace{1cm} (6.3)

The current derived from the exchanged charge is

$$i = \frac{dQ}{dt} = \frac{dc_o(t)}{dt}v_ozeN_A$$  \hspace{1cm} (6.4)

The ionic current through the protein transporter via voltage-gated diffusion $i_{vg}$ is given by Equation 2.6, and the Nernst equilibrium potential (total flux is zero) $V_c$ is given by Equation 2.1.
The current from Equation 6.4 is equal to that of Equation 2.6. It leads to the relationship between the time $t$ and ion concentration $c_o(t)$ as

$$
t = \int \frac{v_o z e N_A}{i_{eg}} dc_o
$$

(6.5)

Equation 6.5 is suitable for a single ion channel presented in a BLM between two chambers. Some constant parameters are listed in Table 6.1.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Value</th>
<th>Unit</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>1.38065812 × 10$^{-23}$ J·K$^{-1}$</td>
<td>Boltzmann's constant</td>
<td></td>
</tr>
<tr>
<td>$T$</td>
<td>310.15 K</td>
<td>Absolute temperature</td>
<td></td>
</tr>
<tr>
<td>$z$</td>
<td>1</td>
<td>Valence of sodium ion</td>
<td></td>
</tr>
<tr>
<td>$e$</td>
<td>1.60217733 × 10$^{-19}$ C</td>
<td>Elementary charge</td>
<td></td>
</tr>
<tr>
<td>$V$</td>
<td>0.1 V</td>
<td>Applied transmembrane potential</td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>6.022 × 10$^{23}$ mol$^{-1}$</td>
<td>Avogadro constant</td>
<td></td>
</tr>
<tr>
<td>$u_s$</td>
<td>5 × 10$^{-8}$ ([103]) m$^2$·V$^{-1}$·s$^{-1}$</td>
<td>Electrical mobility of sodium ion</td>
<td></td>
</tr>
<tr>
<td>$q_s$</td>
<td>1.60217733 × 10$^{-19}$ C</td>
<td>Electrical charge of sodium ion</td>
<td></td>
</tr>
<tr>
<td>$A_p$</td>
<td>9π</td>
<td>Å$^2$</td>
<td>Cross-section area of the pore</td>
</tr>
<tr>
<td>$d_p$</td>
<td>5</td>
<td>Å</td>
<td>Length of the pore</td>
</tr>
</tbody>
</table>

The controllable parameters include the volume of chambers, the initial ion concentration, and the opening possibility for a single ion channel, as listed in Table 6.2. When a single ion channel is triggered by the applied voltage, the ion concentration in the outer chamber starts to increase from 1mM to 100mM.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Value</th>
<th>Unit</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_i$</td>
<td>10, 50, 100 µL</td>
<td>Volume of inner chamber electrolyte</td>
<td></td>
</tr>
<tr>
<td>$v_o$</td>
<td>10, 50, 100 µL</td>
<td>Volume of outer chamber electrolyte</td>
<td></td>
</tr>
<tr>
<td>$c_{i0}$</td>
<td>200, 500, 1000 mM</td>
<td>Initial ion concentration in inner chamber</td>
<td></td>
</tr>
<tr>
<td>$c_{o0}$</td>
<td>1 mM</td>
<td>Initial ion concentration in outer chamber</td>
<td></td>
</tr>
<tr>
<td>$P_o$</td>
<td>10%, 50%, 95%</td>
<td>Average fraction of channels that are open</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.7: Ion concentration in the outer chamber against time required for ion transport through a single sodium ion channel, by varying (a) volume of electrolyte $v_o$ (or $v_i$). (b) initial ion concentration in inner chamber $c_{i0}$, (c) opening possibility $P_o$. 

$c_{i0} = 200\text{mM}; c_{o0} = 1\text{mM}; P_o = 10\%$

$v_i = v_o = 10\mu\text{L}; c_{i0} = 1\text{mM}; P_o = 10\%$

$v_i = v_o = 10\mu\text{L}; c_{i0} = 200\text{mM}; c_{o0} = 1\text{mM}$
The time required for this ion transport process is monitored by varying the changeable parameters as shown in Figure 6.7. For simplicity and practicality, the electrolyte volume in outer chamber \( v_o \) is the same as that of inner chamber \( v_i \).

2. Time dependent ion concentration for multiple ion channels

The number of alamethicin monomers comprising one ion channel is 6 to 11. The cross-section area of an adsorbed protein monomer is \( \Gamma = 7w \) \([74]\), where \( w \) is the cross-section area of a lipid molecule, for DPhPC \( w=80\text{Å}^2 \) \([75]\). Thus, \( \Gamma = 560\text{Å}^2 \).

![Figure 6.8: Cross section of one barrel-stave channel. (a) 6 alamethicin monomers comprising one ion channel, (b) 11 alamethicin monomers comprising one ion channel](image)

Assuming 6 protein monomers each with a cross section of 560Å² comprise one barrel-stave channel, the number of lipid monomers each with a cross section of 80Å² that surround one channel is

\[
m = \frac{\pi \left( \sqrt{\frac{560\text{Å}^2}{\pi}} + \sqrt{\frac{80\text{Å}^2}{\pi}} \right)}{2 \sqrt{\frac{80\text{Å}^2}{\pi}}} \times 6 \times 2 \approx 69
\] (6.6)

Thus, 6 protein monomers and 69 lipid monomers are required to comprise an ion channel. The total area of lipids and proteins comprising one ion channel is

\[
A_t = \pi \left( \sqrt{\frac{560\text{Å}^2}{\pi}} + \sqrt{\frac{560\text{Å}^2}{\pi}} + \sqrt{\frac{80\text{Å}^2}{\pi}} \right)^2 \approx 6390\text{Å}^2
\] (6.7)
If the molar ratio of DPhPC to alamethicin is 535:1 and one ion channel is formed, 6 protein monomers are used, and $6 \times 535 = 3210 (>69)$ lipid molecules are available. Considering all the spare lipid monomers are surrounding the outside of 69 lipid monomers, the area of these spare lipid monomers is $(6 \times 535 - 69) \times 80 \text{Å}^2 = 251280 \text{Å}^2$. So the total cross section of lipids and proteins including channel area for one ion channel is $6390 \text{Å}^2 + 251280 \text{Å}^2 = 25.1280 \times 10^{-4} \text{µm}^2$.

If we have a pore with 150 µm diameter, and the radius ratio for the formation of BLM is 80%, the number of ion channels will be

$$p = \frac{\pi (\frac{150}{2} \times 80\%)^2}{25.1280 \times 10^{-4}} \approx 4389232 \quad (6.8)$$

![Graph](image)

$v_i = v_o = 10 \µL; \ c_{i0} = 1 \text{M}; \ c_{o0} = 1 \text{mM}; \ P_o = 95\%$

Figure 6.9: Ion concentration in the outer chamber against time required for ion transport through multiple sodium ion channels. Minimum and maximum refer to 2384400 and 4389232 ion channels formed in a 150 µm pore, respectively.

6 protein monomers comprising one channel could be considered as maximum case for the number of formed ion channel. For the minimum case, 11 protein monomers comprising one channel is analyzed as same. $m \approx 126, A_t \approx 13597 \text{Å}^2$, the area of these spare lipid monomers is 460720Å² and the total cross section of lipids and proteins including channel area for one ion channel is $47.4317 \times 10^{-4} \text{µm}^2$, 107
\( p \approx 2384400 \). The number of ion channels formed in a 150 \( \mu m \) pore is between 2384400 and 4389232.

The relationship between the time \( t \) and ion concentration \( c_o(t) \) for multiple ion channels formed in a 150 \( \mu m \) pore is

\[
t = \int \frac{v_0 z e N_A}{i_{eg} P} dc_o
\]

which is plotted in Figure 6.9. The time for ion transport to cause the ion concentration raising from 1 mM to 100 mM is realistic.

The expected tip displacement of PPy(DBS) cantilever actuator is plotted against time as shown in Figure 6.10.

6.4 Design of Hybrid Bioderived Ionic Actuator

The consistency of ion transport through BLM ion channel and PPy(DBS) actuator is required in quantity analysis. The ion concentration required for the appropriate actuation of PPy(DBS) actuator is limited by the ion transport through BLM ion channel. The time allowing the ion transport through BLM ion channel is related to the current and chamber volumes. The chamber volume should be in accord with the
number of single ion channel to achieve the decent quantity of ion transport. Multiple ion channels is better used for large quantity of ion transport. Based on the previous calculation, the chamber volume would be traded off the surface area for formation of ion channels.

1. Design of PPy(DBS) cantilever actuator incorporated in the cup/chamber BLM ion channel system

Figure 6.11: PPy(DBS) cantilever actuator in BLM ion channel array system. (a) Front view, (b) top view, (c) the spherical cup wall has multiple apertures, and each is 200 \( \mu \text{m} \) diameter and 50 \( \mu \text{m} \) apart, (d) side view with PPy(DBS) cantilever actuator and electrodes.

Here, we design an array of BLM ion channel to bring large ion transport and to be fitted to the PPy(DBS) cantilever actuator in millimeter dimension due to the difficulty of manually making a PPy(DBS) cantilever actuator fitted in a micro-scale chamber. It consists of a spherical cup and a rectangular chamber. An array of apertures are on the wall of spherical cup and each aperture is
used for formation of one single BLM ion channel. The spherical cup has a 5mm radius and is cut by a plane that is 2.5mm distant to sphere center. The rectangular chamber has 13mm by 13mm by 10mm dimensions. PPy(DBS) cantilever actuator is inserted in the cup, while a Ag/AgCl half cell is paralleled to PPy(DBS) cantilever actuator. The other Ag/AgCl half cell is immersed in the chamber. The volume of spherical cup is $v_{\text{cup}} = \frac{4}{3}\pi r^3 = 0.4418 \text{ mL}$, and the surface area of spherical cup is $s_{\text{cup}} = \frac{12}{4}\pi r^2 = 255.25 \text{ mm}^2$. Each aperture covers 0.04 mm$^2$ area, thus, the number of possible ion channel is 6381000, if 1000 ion channels are formed in each aperture. The volume of rectangular chamber excluded the cup is $v_{\text{ch}} = 13 \text{ mm} \times 13 \text{ mm} \times 10 \text{ mm} - 0.4418 \text{ mL} = 1.2482 \text{ mL}$. According to the previous modeling calculation, we have $v_o = 0.4418 \text{ mL}, v_i = 1.2482 \text{ mL}$. It is more reasonable to active the ion transport through these ion channels leading to the actuation of PPy(DBS) cantilever actuator in millimeter scale. If the initial electrolyte concentration in the chamber and the cup is 1M and 1mM, respectively. The opening possibility of ion channel is around 95%. The tip displacement of PPy(DBS) cantilever actuator is expected to be near 800 $\mu\text{m}$ in 7 minutes along with the ion concentration in the cup reaching 10 mM.

2. Design of PPy(DBS) microsphere actuator incorporated in droplet interface bilayer ion channel system

Another option for achieving the actuation of PPy(DBS) actuator is to utilize DIB to minimize the volume of electrolyte so that the number of ions required to be transported through the ion channel are limited and executable in reasonable time. PPy(DBS) microsphere actuator is possibly fitted in the micro-scaled droplets.

6.5 Conclusion

In this chapter, architectures developed from the concept of a PPy(DBS) actuator controlled by ion transport through the synthetic membrane have been proposed. It
provides the necessary procedure for fabricating the hybrid bioderived ionic actuator. The design rules and transducer equations based on these architectures have been build upon available models for ion transport through a PPy(DBS) actuator in a BLM ion channel system and could be applied for using this bioderived ionic actuator in various applications.
Chapter 7

Summary

This thesis has developed the concept of a hybrid bioderived membrane and its applicability for designing a hybrid bioderived ionic actuator. The experimental work presented in this thesis demonstrates the procedure to fabricate a hybrid bioderived membrane and characterization of the component layers for designing the hybrid bioderived ionic actuator. The achievements presented in this thesis furthers our understanding of PPy(DBS) actuator and its application in bioderived environment. The contributions and result are addressed below and the future directions as well as anticipated applications are prospected.

7.1 Summary of Contributions

1. Hybrid bioderived membrane fabricated by integrating a conducting polymer with a protein reconstituted bilayer lipid membrane has been proposed. To the best of our knowledge, this is the first report of an alamethicin-reconstituted BLM supported on PPy(DBS) membrane that uses a common mobile cation between the two electroactive ionic materials.

2. A layer-by-layer assembly technique to fabricate the hybrid bioderived membrane have been developed.

3. Experimental methods to characterize the hybrid bioderived membrane has
been developed.

4. PPy(DBS) actuator has been fabricated and its ion transport (coupling) properties have been characterized.

5. Design equations and prototypal architectures for the hybrid bioderived ionic actuator has been developed.

### 7.2 Significant Results

The main concept expanded in this work focuses on hybrid bioderived membrane and its derivative design of hybrid bioderived ionic actuator.

1. Hybrid bioderived membrane

   Electrodeposition of PPy(DBS) on gold have been investigated. The electrodeposition rate and the PPy(DBS) growth rate are quantified. The redox process monitored in different concentration of electrolyte demonstrate the ability of cation transport in and out of PPy(DBS). The reduction peak and oxidation peak are observed. The energy transformation from the chemoelectrical domain to mechanical domain are quantified by coupling coefficient.

   In the next step, assembly of PPy(DBS) film and alamethicin reconstituted BLM was achieved by vesicle fusion. The diameter of vesicles after different cycles of sonication-vortex-rest have been compared. The specific resistance and capacitance of PPy(DBS) film are obtained according to the equivalent circuits. The magnitude of impedance is seen to increase by the order of $10^3$ to $10^4$ after formation of BLM. The BLM in the system behaves as a capacitor and resistor in both low frequency and high frequency, and in the middle range of frequency, it behaves more like a capacitor. The conductance of the alamethicin reconstituted DPhPC BLM increases by 3 to 4 times above the operating voltage.

2. Hybrid bioderived ionic actuator

   PPy(DBS) cantilever actuator is fabricated as a gold/PPy(DBS)/gold sandwich.
The specific resistance of PPy cantilever actuator decreases and the specific capacitance increase with the raise of ion concentration in the electrolyte. The voltage-current response of PPy(DBS) cantilever actuator in the electrolyte of different ion concentration demonstrates the ion transport capability, and the obvious redox is related to the applied voltage and the scan rate. The conformational change is monitored along with applied voltage, current, and exchanged charge. The ability of PPy(DBS) acuator to convert the chemoelectrical energy to mechanical energy is quantified by the ion transport coupling coefficient.

The statistical analysis shows the higher ion concentration in the electrolyte, the lower the value of ion transport coefficient. It is also noticed in the same electrolyte the larger scale of PPy(DBS) film, the higher coupling coefficient. PPy(DBS) microsphere actuator is fabricated as a micro-actuator, and its volumetric actuation is demonstrated by the radius change along with the different exchanged charges.

The design equations for hybrid bioderived ionic actuator are developed based on the electro-diffusion model and ionic current model. Design rules for prototypal architectures incorporating either PPy(DBS) cantilever actuator or PPy(DBS) microsphere actuator are proposed.

### 7.3 Future Directions

The accomplishments from this work can inspire the further suggestions for continuing this work.

1. Fabrication of the hybrid bioderived ionic actuator and demonstration of actuation
   
   We have developed the design equations and prototypal architectures for the hybrid bioderived ionic actuator. The fabrication of this actuator is essentially the next step that could be achieved by MEMS/NEMS fabrication techniques. The demonstration will address on the formation of stable lipid bilayers, the
appropriate ionic current through ion channels, and the efficiency of PPy(DBS) actuator in coupling the different domains. These demonstrations ultimately will prove the actuation of PPy(DBS) actuator could be controlled by the ion transport through biological ion channels.

2. Development of the distributed actuator network

Our research has investigated the capability of chemo-electro-mechanical actuation of the hybrid bioderived ionic device. By varying the controllable parameters each hybrid bioderived ionic actuator could respond distributably, such as generate different amplitudes of tip displacement. Distributed actuator networks can be deployed to collaboratively sense and affect the environment.

3. Implementation of different forms of gating for controlled actuation

The similarity between conducting polymer and bioderived membrane is the ion transport. Our current work has focused on the voltage-gated proteins where the applied voltage is a stimulus. Ligand, as well as light, could serve as a trigger for triggering the ion transport. The physics of operation behind these hybrid devices using ligand-gated protein or light-gated protein are similar except for the different types of stimulus.

4. Development of application specific actuation platforms

The chemoelectromechanical actuation in the hybrid device could be used in other applications such as energy harvesting, sensing and drug delivery.
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Appendix A

Supplement of Electrochemical Measurements of PPy(DBS) Cantilever Actuator

PPy(DBS) cantilever actuator has been illustrated in chapter 6. Electrical properties, as well as the quantity of ion transport, is one of significant factors if PPy(DBS) cantilever actuator is intend to be used into any applications. Some supplementally electrochemical measurements of PPy(DBS) cantilever actuator are provided under various parameters that may affect the ion transport or electrical properties of PPy(DBS) cantilever actuator.

A.1 Electrochemical Impedance Spectroscopy (EIS)

In chapter 6, all the results are based on the PPy(DBS) cantilever actuator in size of 1mm by 5mm from same stripe of PPy(DBS) deposited on platinum sputtered polycarbonate filter paper (sample 1). However, PPy(DBS) cantilever actuator exhibits a high chance working in the micro scale. Thus, another size of PPy(DBS) cantilever actuator in 0.5mm by 2mm and another stripe of PPy(DBS) deposited on platinum sputtered polycarbonate filter paper (sample 2) are used to compare the
Figure A.1: Sample 1 and sample 2 of PPy(DBS) deposited on platinum sputtered polycarbonate filter paper are fabricated in the same manner. Two different sizes, 1mm by 5mm, 0.5mm by 2mm, are cut from sample 1 and sample 2 and made into PPy cantilever actuators.

EIS measurements.

EIS of PPy(DBS) cantilever actuator in NaCl electrolyte with different concentration 1mM, 10mM, 100mM, 1M are obtained with sample 1 in 1mm by 5mm, sample 1 in 0.5mm by 2mm, sample 2 in 1mm by 5mm, and sample 2 in 0.5mm by 2mm, shown in Figure A.2. Higher concentration of sodium ion in the electrolyte, lower impedance of PPy cantilever actuator.

A.2 Cyclic Voltammetry (CV)

The capability of ion egress and ion ingress during the redox of PPy(DBS) cantilever actuator is investigated in different scan rates and electrolyte with different ion concentrations.

PPy(DBS) cantilever actuator are always activated through several redox cycles that are provided by CV repeating cycle. 10 cycle of redox for PPy(DBS) cantilever actuator in electrolyte with different ion concentrations are shown in Figure A.3.

CV measurement has been used to illustrate the voltage-current response of PPy(DBS) on gold foil in Chapter 4, which demonstrated the ion transport during the reduction-oxidation of PPy(DBS) in a proper voltage range. Here, CV is not only utilized on PPy(DBS) on gold/platinum sputtered polycarbonate filter paper to investigate the ion transport through voltage-current response, but also the tip displacement accompany with CV measurement is recorded to show the conversion
Figure A.2: EIS of PPy(DBS) cantilever actuator in NaCl electrolyte with different concentrations (1mM, 10mM, 100mM, 1M). (a) Sample 1 in 1mm by 5mm, (b) sample 1 in 0.5mm by 2mm, (c) sample 2 in 1mm by 5mm, (d) sample 2 in 0.5mm by 2mm.

from the chemoelectrical domain to mechanical domain. PARSTAT 4000 (Princeton Applied Research, Ametek) is used as a potentiostat/galvanostat/EIS analyzer to perform the CV measurement. ML2700 microscope (Meiji Techno) is used to record the tip displacement during the multiple cycles of CV measurement. The experimental setup for CV measurement as well as tip displacement recording is shown in Figure 5.6. PPy(DBS)-based actuator is assembled in a teflon-wall and glass-bottom chamber and placed onto the stage of ML2700 microscope. CV measurements are collected by PARSTAT 4000 between electrical terminals. The glass slide is placed on the microscope stage for real-time recording of the tip displacement of PPy(DBS)-
based actuator during the electrochemical measurements. The electrical connection to PARSTAT 4000 is made by connecting PPy(DBS)-based actuator to working electrode and source, and connecting the Ag/AgCl half cells to counter electrode and reference electrode. The teflon chamber contain electrolytes used here are 1mM, 10mM, 50mM, 100mM, 150mM, 200mM, 250mM, 500mM, 1M NaCl solution. The sample of PPy(DBS) cantilever actuator is cycled between -1 V and 0.5 V for 100 cycles of CV, allowing ion egress and ingress in the PPy(DBS) backbone. The last cycle of CV from each electrolyte is plotted in Figure A.4. Not only electrolyte concentrations but also scan rates may affect the redox of PPy(DBS) cantilever actuator. With the increase of sodium ion concentration the slope (conductance) raises and the area under current-voltage curve increases, indicating more ions entering and existing the PPy(DBS). In Figure A.4(a) an obvious reduction peak around -0.65V and a clear oxidation peak around -0.05V are observed in the current-voltage response with 150 mM NaCl solution. However, there are no evident redox peaks in that of 1M NaCl solution due to a large number of ions movement limited by the time. The scan rate is 0.1 V/s in Figure A.4(a) and different scan rates (0.01V/s, 0.005V/s) are also investigated as shown in Figure A.4(b)(c), but obvious redox peaks are absent in lower scan rate.

A.3 Chronoamperometry (CA)

PPy(DBS) cantilever actuator activated by step voltages through CA are monitored by microscope for the tip displacements. CA measurements are conducted by applying 0.5V and -1V each for 60s. The applied voltage and current, as well as exchanged charge, with different ion concentrations in 150mM, 200mM, 250mM, 500mM, 1M are shown in Figure A.5, while that of 1mM, 10mM, 50mM, 100mM are shown in Figure A.6.
Figure A.3: CV (10 cycles) of PPy(DBS) cantilever actuator in NaCl electrolyte with different concentrations (a) 1mM, (b) 10mM, (c) 100mM, (d) 1M.

Figure A.4: CA (one cycle) between -0.1V and 0.5V for PPy(DBS) cantilever actuator in NaCl electrolyte with different concentrations (1mM, 10mM, 50mM, 100mM, 150mM, 200mM, 250mM, 500mM, 1M) and different scan rates (a) 0.1V/s, (b) 0.01V/s, (c) 0.005V/s.
Figure A.5: CA (with exchanged charge) of PPy(DBS) cantilever actuator in NaCl electrolyte with different concentrations (a) 150mM, (b) 200mM, (c) 250mM, (d) 500mM, (e) 1M.

Figure A.6: CA (with exchanged charge) of PPy(DBS) cantilever actuator in NaCl electrolyte with different concentrations (a) 1mM, (b) 10mM, (c) 100mM, (d) 1M.
Appendix B

Painting Method and Falling Droplet Method

To demonstrate the fabrication and characterization of bilayer lipid membrane as a good candidate of the functional hybrid structure, some preliminary work has been paid attention to the different methods and materials to figure out the better, simpler, and stabler procedure to fulfill the proposed objective.

B.1 Formation of a Bilayer Lipid Membrane and Protein Reconstitution

The structures were machined with the PMMA or teflon, and a hole of 0.1 inch diameter was in the center of bottom. Then a silicon nitride chip with a square pore was attached to the structures as in figure B.1.

In this section, 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS) and 1-palmitoyl-2-oleoyl-phosphoethanolamine (POPE) were used as self-assembly lipids. POPS has a polar head group of serine, and the non-polar tail consists of a saturated fatty acid chain and a unsaturated fatty acid chain with one double bond. Same tail components are POPE except for ethanolamine as a head group. A combination of POPS/POPE at weight ratio of 3:1 was obtained by adding 30 mg POPS and 10 mg POPE in 1 mL
of n-decane. Alamethicin was stored in ethanol at 0.1%(w/v), then the stock solution was diluted with 100 mM NaCl to a concentration of 1µg/mL alamethicin. In this subsection, all the electrolyte used was 10mM KCl.

Painting method carried in this fixture as figure B.1 has been taken out in the followed procedure. First, the well was filled with electrolyte, since the pore on the silicon nitride chip was so small that the aqueous solution wouldn’t leak through it. Then the fixture was inverted to add 1 µL of lipid organic solution at the back of the silicon nitride chip covering the square pore area. The aqueous solution would been hold even if it was upside down because of the surface tension. We waited 2-3 min for the formation of monolayer of lipid. If alamethicin was intended to be incorporated, the protein solution should been added immediately. Then, the fixture was brought back to upside up and placed in the petri dish containing the electrolyte, and after 3-5 min, the top chamber was filled with electrolyte gently.

Falling droplet method shown in figure B.1 has also been attempted as follows. Silver wire was cleaned by ethanol, deionized water, and dried with nitrogen. This was followed by the coating with 1% (w/v) agarose gel. Then the well was filled with 1µL lipid n-decane solution, and silver wire was placed vertically above the conical cavity of the chip. 1µL 10mM KCl droplet was added to the silver wire leading to the droplet falling exactly on the conical cavity and fully covering it. After 2-3 minutes, protein can be reconstituted by adding 1µL alamethicin solution to the silver wire.
as well. Given equilibrium time 3-5 minutes, the fixture was placed in the petri dish containing the electrolyte, and the top chamber was filled up gently with silver wire as one electrode.

Figure B.3: Falling method for the formation of lipid bilayer on the silicon nitride chip
B.2 EIS Measurements of a Bilayer Lipid Membrane with/without Reconstituted Protein

The measurement for verifying the formation of lipid bilayer is carried out by EIS, the silicon nitride chip was measured as a baseline after adding the lipid. The evidence of formation could be obtained by comparison the impedance value of the EIS measurements. Normally, the magnitude will increase $10^4$ to $10^6$ orders if the BLM is formed. The EIS on the silicon nitride chip and BLM formed by above two methods are shown in figure B.4. On the other hand, EIS after adding the proteins was also monitored by EIS. Since not only the amount of protein reconstituted is much less than the lipid itself, but the single ion channel was reported having a small current flow, it turns out the impedance after protein embedded have no significant change compared to the pure BLM. This is supported by the EIS plot of BLM reconstituted with alamethicin in figure B.5, which could be brought to compare with the BLM in figure B.4.

![EIS plot of BLM reconstituted with alamethicin](image)

Figure B.4: EIS of the formation of lipid bilayer on the silicon nitride chip (the silicon nitride chip as baseline)
Figure B.5: EIS of alamethicin reconstituted lipid bilayer on the silicon nitride chip

B.3 Interpretation of Experimental Data

Two different formation techniques for artificial lipid bilayer have been investigated in this section. Both of them have been demonstrated to be reliable with this structure. Through the observation of EIS before and after adding lipid, the BLM can be verified to form. Unfortunately, there is no much difference in the EIS measurement have been observed before and after adding the proteins. A probable reason could be the single channel of alamethicin is supposed to allow pA to flow in or out, thus, the magnitude change of the impedance should not be obvious through the measurement EIS after reconstitution of alamethicin. Other methods, like CV, CA, SCR, need to be operated to verify the ion channel formation.