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DEPARTMENT OF CHEMICAL ENGINEERING

PHOTOSYSTEM I PROTEIN INCORPORATION IN SELF-ASSEMBLING BLOCK COPOLYMER MEMBRANES

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ABSTRACT

Block copolymer membranes provide high utility in the field of membrane science by being able to provide a biomimetic environment similar to the environment created by lipid membranes. In this project, the goal was to create self-assembling block copolymer membranes incorporated with photosystem I (PSI) protein. This is the first step in a process that could create a device for conversion of solar energy by mounting this protein embedded membrane on a gold electrode, which could generate a photocurrent in the presence of light. Using poly(butadiene)-poly(ethylene oxide) block copolymers and PSI protein purified from cyanobacteria and dialysis, several membrane samples with packed proteins were successfully created. These different crystals utilized different amounts of protein and polymers (protein polymer ratio – PoPR) and were characterized by transmission electron microscopy (TEM). In the end, consistent, stable membrane two-dimensional crystals were unable to be formed. Future work in the field would revolve around finding ideal conditions for crystal formation before continuing on to creating the device for solar energy conversion.

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Chapter 1

Problem Statement

Living cell membranes consist of lipid bilayers with many proteins naturally incorporated to help the cell perform various tasks from transport of large molecules to helping maintain water balance and equilibrium. However, because of their temperature and pH sensitivity, lipid bilayer membranes by themselves cannot be used for technological applications as they become unstable at high temperatures and low pH ranges. Block copolymer membranes, however, provide the potential for many different engineering applications in the field of membrane science due to their increased stability.[1] Therefore, it is potentially useful to incorporate membrane proteins into block copolymer membranes in order to provide more stable and longer-lasting protein incorporated membranes.[2]

The practical applications of biomimetic membranes in the scope of this project focused on eventual use in solar energy conversion. Previous experiments conducted using lipid membranes have shown that the natural transfer of electrons in photosynthetic proteins could be carried out in biomimetic membranes using a gold electrode to drive the electron transfer rather than several proteins in sequence.[2] This could potentially be used to provide a device that could produce hydrogen via photocurrent, where light drives the efficient transfer of electrons through the photosynthetic protein incorporated membrane. If tethered to a hydrogenase enzyme, this transfer of electrons would drive the production of molecular hydrogen all from solar energy.[4] While the application of the project lays the foundation for future work, this project focused on creating the photosystem I (PSI) membranes that will be incorporated into these electrodes.

Block copolymers have applications in the field of biochemical engineering by creating an environment that can mimic the membrane environment created by cells. They have the ability to form bilayer membranes that can readily allow for the incorporation of membrane proteins that occur naturally in lipid membranes. Once PSI has been incorporated into the membrane, it can be mounted onto a gold electrode and photocurrent can be measured. However, before the membrane-mounted electrode can be created, a stable, PSI-incorporated membrane must be created. Therefore, the goal of this project was to find out if it was possible to create sheets or vesicles of block copolymer membrane crystals and then to see if it was possible to get PSI protein to incorporate and remain stable within the membrane.

Chapter 2

Background and Hypothesis

Block copolymers are especially useful in the field of membrane science due to their ability to provide a biomimetic environment by forming bilayer and vesicle structures similar to those formed by lipids in naturally occurring membranes. These biomimetic membranes can then readily incorporate membrane proteins just like natural membranes. In this experiment, Photosystem I protein (PSI) was used. These membranes can then be characterized via transmission electron microscopy (TEM).

2.1 Block copolymers are able to mimic lipids and improve stability of membranes

One of the common features of membranes is their amphiphilic properties. This gives them the ability to form micelles, vesicles, and the bilayer membrane commonly seen in naturally occurring membranes.[5] Experimentation has shown that block copolymers are able to mimic the hydrophobic and hydrophilic properties of lipids and produce these morphological structures that lipids can produce.[5,6] However, the use of block copolymers in biomimetic membranes carry several advantages compared to that of their lipid membrane counterparts. First, engineering the actual block copolymers allows for the control of the structure of the membrane. In this experiment, diblock copolymers were used. The benefit of this is that the blocks can be selected in such a way to achieve two leaflet layers with a hydrophobic core.[5,6,7] The benefit of this structure is that the block copolymers will self assemble into these vesicle or bilayer structures solely due to the nature of hydrophobic-hydrophilic interactions. The basics of the mechanism through which the block copolymer assembles into the bilayer structure, involves

an amphiphilic molecule with a hydrophilic polymer "head" and a hydrophobic "tail" which is a direct parallel to the structure of membrane lipids common in natural membranes.[7] The structure of the membrane and the transmembrane distance, d, can be altered based on choice of the block.[5,7] For the purpose of this experiment, a mixture of poly(butadiene)-12-poly(ethylene oxide)-8 (PB12-PEO8) and a sulfur-functionated PB12-PEO8 block copolymer were used. This choice of the sulfated block copolymer has been shown to give a well-established gold/sulfur chemical interaction, which will lead to immobilization of the membrane on the surface.[8, 9] This property will be useful when applying the PSI-incorporated membrane onto the gold electrode surface. The PB12-PEO8 block copolymer is also useful because it has been proven to form vesicular structures in water, does not exhibit toxicity to living tissue, and is able host membrane proteins.[10,11] These properties made PB12-PEO8 a suitable choice of block copolymer for this project.

In addition to the ability to select different block copolymers to create different morphological structures, block copolymers also provide for increased stability and shelf life compared to their lipid counterparts. Experimentation at varying block copolymer molecular weights show that the elasticity and transmembrane diameter may be extended to values above those of natural lipid membranes.[12] This allows for enhanced physical stability in the case of block copolymer membranes. The fact that the membranes can be distorted more and still revert back to their original, functioning state means that block copolymers not only can handle more strain during experimentation and characterization than lipids, but also will last longer on the shelf than lipid membranes. **Figure 1** shows the response of block copolymer membrane vesicles to strain and show the slow dynamics with which it is able to revert slowly back to its original state.[12]

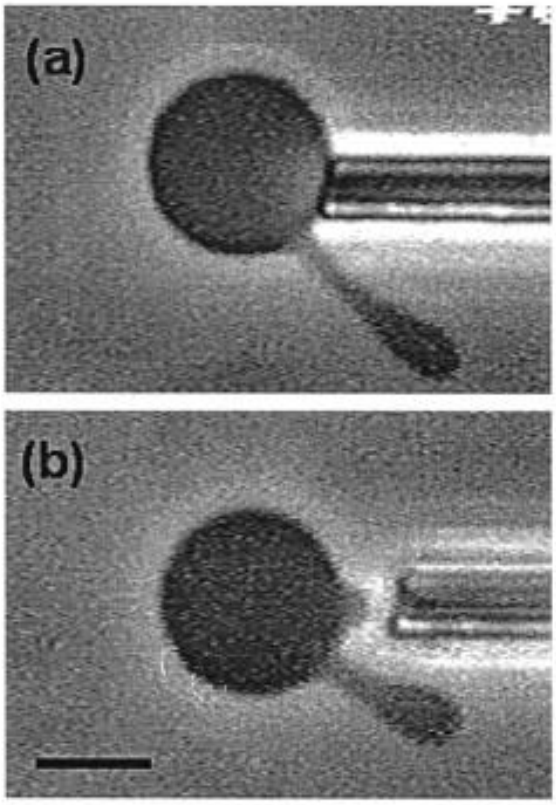

Figure 1 – Response of copolymer vesicle to strain. Reprinted with permission from H. Bermudez, A. K. Brannan, D. A. Hammer, F. S. Bates and D. E. Discher, *Macromolecules*, 2002, 35, 8203–8208. Copyright 2002 American Chemical Society.

Previous work has shown how block copolymer membranes not only provide a stable membrane environment that mimics that provided by biological lipids, but also provide an increased physical stability to strain and torsion, which can help increase the shelf life of these membranes. In order to be useful for this application however, it must be possible to integrate transmembrane proteins into these block copolymer membranes, which has been shown to be successful with the block copolymer PB12-PEO8[10,11].

2.2 Incorporation of photosystem I protein has been done in lipid membranes

The incorporation of PSI protein into lipid membranes has been carried out by Saboe, et. al. (2014) and provides a standard to compare the results for incorporation into block copolymer membranes. The objective of the incorporation is to create a stable 2D crystal, which can be characterized by transmission electron microscopy (TEM). These crystals provide an advantage because it creates an ordered and concentrated set of reaction centers for the transfer of electrons carried out by PSI.[3,13] The crystals produced in the lipids experiment by Saboe, et. al. can help predict what the block copolymer 2D crystals should look like. The importance of these crystals is that they are treated with a conjugated oligoelectrolyte, which increases the conductivity of the crystals when electrons are excited.[13,14] Another important quality of these crystals is the high level of order. These crystals are highly organized and have a size of roughly 2 to 5 μm.[15,16] Using these results from the literature and previous experimentation as a basis, comparisons could be made between the lipid crystals and the polymer crystals due to the similarities previously shown in their structures and functions.

Both past experimentation and literature confirm that PSI can be successfully incorporated into 2D lipid crystals.[3] In addition, literature has shown that lipid behavior can be mimicked with functionalized block copolymers, especially PB12-PEO8.[1] Because of this, it should be possible to incorporated PSI protein into a block copolymer membrane to produce a 2D embedded polymer crystal. Once successfully characterized by TEM, it will be possible to look to incorporate these crystals on gold plates to create photocurrent in a fashion similar to that of Saboe, et. al.[3]

2.3 Photosystem I protein is important due to its role as an electron reaction center

The photosystem I protein (PSI) occurs naturally in the thylakoid membrane of plant cells and play a key role in carrying out the light reactions of photosynthesis. After light strikes photosystem II and the excited electron is carried down the first electron transport chain via cytochrome b_6f, cytochrome c_6 and plastocyanin, the electron is excited again by PSI, which passes the electron to ferredoxin to help reduce $NADP^+$, which aids in the dark reactions of photosynthesis.[17,18] **Figure 2a** shows the native photosynthetic membrane.

In contrast with the natural photosynthetic membrane, a photosynthetic device will be created using the PSI-incorporated 2D block copolymer crystal that will be developed. This process was already done by Saboe, et. al with a 2D lipid crystal.[4,14] PSI serves a strong role in designing this 2D crystal. For photovoltaics, the goal is to provide a mechanism for effective and rapid transfer of electrons from the electrode to the target electron sink. With a large concentration of PSI protein in the 2D crystal, the electron transfer between the electron acceptors and donors is readily facilitated.[4] In contrast, 3D crystals could be used, but in this case, a tethered 2D crystal will suffice.[19] **Figure 2b** shows the idea behind the photosynthetic device that will arise following successful incorporation of PSI into a 2D polymer crystal.

For the application of solar energy conversion mentioned previously, the interest in PSI protein is in its ability to facilitate electron transfer. In natural photosynthesis, electrons are transported from photosystem II to photosystem I via the cytochrome and plastocyanin cofactors in the thylakoid membrane. However, in a biomimetic membrane tethered to an electrode made of a conductive material, such as gold, photosystem I is the only protein that is needed to make an effective electron pathway by utilizing both a current and photons from the surroundings.

Figure 2c shows the comparison between the native electron pathway that utilizes both PSII and PSI and the biomimetic electron pathway that can be used with a PSI-incorporated membrane to create a photosynthetic device.[4] **Figure 2** essentially summarizes the utility of PSI protein and the application of the PSI-incorporated 2D polymer crystal that this thesis research attempted to create.

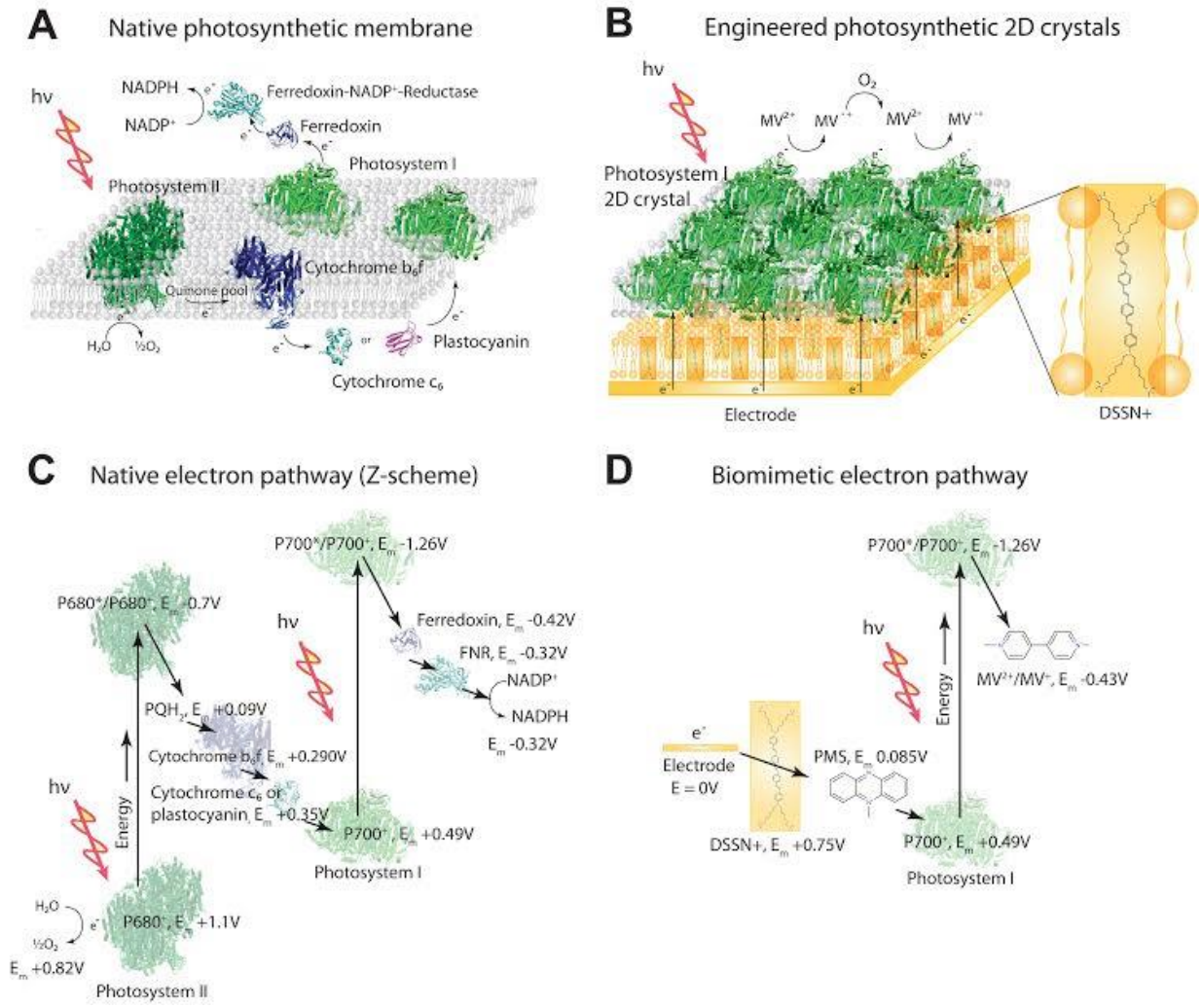

Figure 2 - PSI's role in nature and photosynthetic devices. Raw image reprinted with permission from Patrick Saboe, Saboe, P.; et. al. *Advanced Materials*, 2014.

The goal of this research is to create a self-assembling block copolymer crystal that has PSI protein incorporated into it and to characterize it using transmission electron microscopy. PSI is useful because it can act as a highly efficient electron pump and can be incorporated into biological membranes. This crystal can be tethered to an electrode to facilitate rapid electron transfer from a current to an electron acceptor to create a photovoltaic cell.

2.4 PSI incorporation prior to tethering provides more flexibility

Once the PSI-incorporated polymer crystal is created, the next step is to tether it to the gold electrode to create the photosynthetic device. The tethering is done using a special functionalized block copolymer with a sulfur group at the end. A common functional group for achieving the desired gold/sulfur chemistry is lipoic acid.[20] There are two methods used to create a tethered, solid-supported bilayer membrane (TSSBM). The first method was done by Zhang, et. al. (year) used gold/sulfur chemistry via lipoic acid functionated block copolymers to tether the copolymer membrane to the gold surface prior to protein incorporation. The second method was done by Saboe, et. al. in their lipid experiment where the 2D crystal was created. In order to understand why the second method is the one that will be utilized for the copolymer photosynthetic device, a comparison must be done between the two methods.

Figure 3 shows the results of Zhang, et. al.'s experiments with PB-PEO block copolymers tethered to a gold surface followed by incorporation of α-Haemolysin protein.[20]

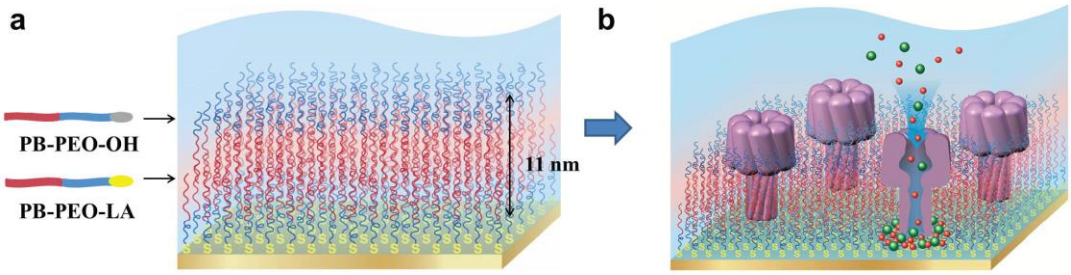

Figure 3 - Creating TSSBMs by tethering the copolymer first. Reprinted with permission from Zhang, X., et. al. *Scientific Reports*. 2013, 3, **2196 under a Creative Commons license (http://creativecommons.org/licenses/by-nc-nd/3.0/). Copyright 2013 Macmillan Publishers Limited.**

The major advantage of this process is that no detergent is needed. Detergents are small, organic molecules that help solubilize membranes and stabilize membrane proteins extracted from biological membranes. However, since these block copolymers have one end tethered to a solid surface, it reduces the need for detergents. Despite this advantage, there are a few disadvantages for this method. First of all, because the polymers are already tethered prior to protein incorporation, as the distance from the plate decreases, the flexibility also decreases. With only a flexible top layer, it could become difficult to incorporate larger, bulkier proteins. However, for the α-Haemolysin protein, conductance measurements as seen in **Figure 4** before and after insertion of the protein into the membrane shows the integrity of the membrane was preserved during protein incorporation and a functional, protein-incorporated TSSBM was created.[20]

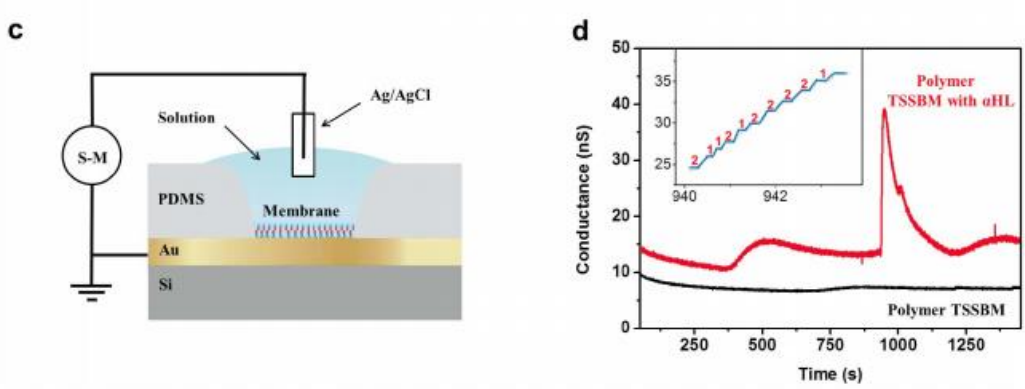

Figure 4 - Zhang measured of conductance using the setup in C revealed that after insertion of aHL protein at 1000s, the TSSBM was still functional (D). Reprinted with permission from Zhang, X., et. al. *Scientific Reports*. 2013, 3, **2196 under a Creative Commons license (http://creativecommons.org/licenses/by-nc-nd/3.0/). Copyright 2013 Macmillan Publishers Limited.**

The second method, as utilized by Saboe, et. al., is the method that was chosen for this experiment. By incorporating the protein into the 2D polymer crystal prior to tethering to the gold surface, a higher protein density is achievable due to the flexibility of both the upper and lower parts of the bilayer.[4] Another key advantage is the ability to incorporate other elements into the bilayer instead of just protein. In this case, a conjugated oligoelectrolyte (COE) can be used to make the entire membrane functional and able to transport electrons rather than just restricting electron transport to PSI. COEs also are useful in this experiment because PSI needs a highly conductive environment to facilitate its electron transporting function. The need to incorporate these COEs to provide this environment for PSI makes this method of incorporation prior to tethering highly favorable.[4]

The major disadvantage to this method is that once the crystal is created, it must be tethered in bulk to the electrode. With individual block copolymers tethering provides more flexibility and room for error to correct.[20] However, with a large crystal, there is not much flexibility in tethering the crystal to the electrode. Despite that disadvantage, because PSI needs

an electron conducting environment, the ability to incorporate COEs into a membrane prior to tethering makes this method highly favorable.

2.5 Hypothesis

The goal of this experiment is to create a PSI-incorporated, self-assembling 2D polymer crystal that can be characterized by transmission electron microscopy. This crystal has a tremendous application in the field of photovoltaics by being able to be tethered to a gold electrode to create a TSSBM integrated with COEs to create a highly conductive membrane that can facilitate the rapid transfer of electrons produced from a current. As the electrons travel through the device, an enzyme, such as hydrogenase, could use them to produce molecular hydrogen.

Because experimentation by Saboe, et. al., has shown that PSI can be readily incorporated into lipid membranes, the goal is to see if PSI can also be incorporated into block copolymer membranes. Because block copolymers can be specially designed to have specific functional groups, they possess a high versatility. In addition, copolymers have been shown to have a longer shelf life, and show resistance to torsion and stress. Finally, a specific block copolymer, PB12-PEO8 has been shown to readily accept proteins into their bilayer membrane, creating a biomimetic environment for the protein. Because of this, PSI should be able to be incorporated into a block copolymer membrane. This membrane should then be able to be crystalized via dialysis and analyzed using transmission electron microscopy.

Chapter 3

Materials and Methods

The objective of this thesis research was to produce a 2D, PSI-incorporated block copolymer membrane crystal that could be characterized by transmission electron microscopy. The overall procedure for creating the crystal is a three-part process that includes the production of the polymer film, isolation of the PSI protein and creation of the PSI-incorporated polymer crystal. After the crystal is created, it must be prepped and stained for characterization with transmission electron microscopy.

3.1 Creation of the polymer film

20 mg of poly(butadiene)-12-poly(ethylene oxide)-8 (PB12-PEO8) and 20 mg of a thiolated PB12-PEO8 copolymer synthesized by Dr. Ian Sines in the Kumar lab, were dissolved in 1 mL of chloroform in their own respective flasks. The flasks were each placed in a Buchi B-491 Water Bath at 42^{o}C using a Buchi R-124 Rotovapor and decompressed with a Buchi V-800 vacuum controller to around 50 millibars. Flasks were covered with foil and left in a Labconco Centrivap Console vacuum chamber overnight. 1 mL of OTG detergent, 1 mL of distilled water, and 4 μL of sodium azide (NaN_3) were added to both flasks and 2 μL of the reducing agent DTT was added to the thiol PB12-PEO8 to prevent self-binding between the sulfur groups. Stir bars were placed in each flask and stored at 4^{o}C while stirring overnight. Polymers were filtered over a 0.2 micron membrane and flushed with water before being mixed together to form a mix of the non-sulfur and sulfur PB12-PEO8 block copolymer.

3.2 PSI protein isolation from cyanobacteria

Figure 5 summarizes the procedure of isolating PSI protein from the cyanobacteria used in this experiment. Undergraduate student Stanley Chan developed this image for the Saboe, et. al. lipids experiment.

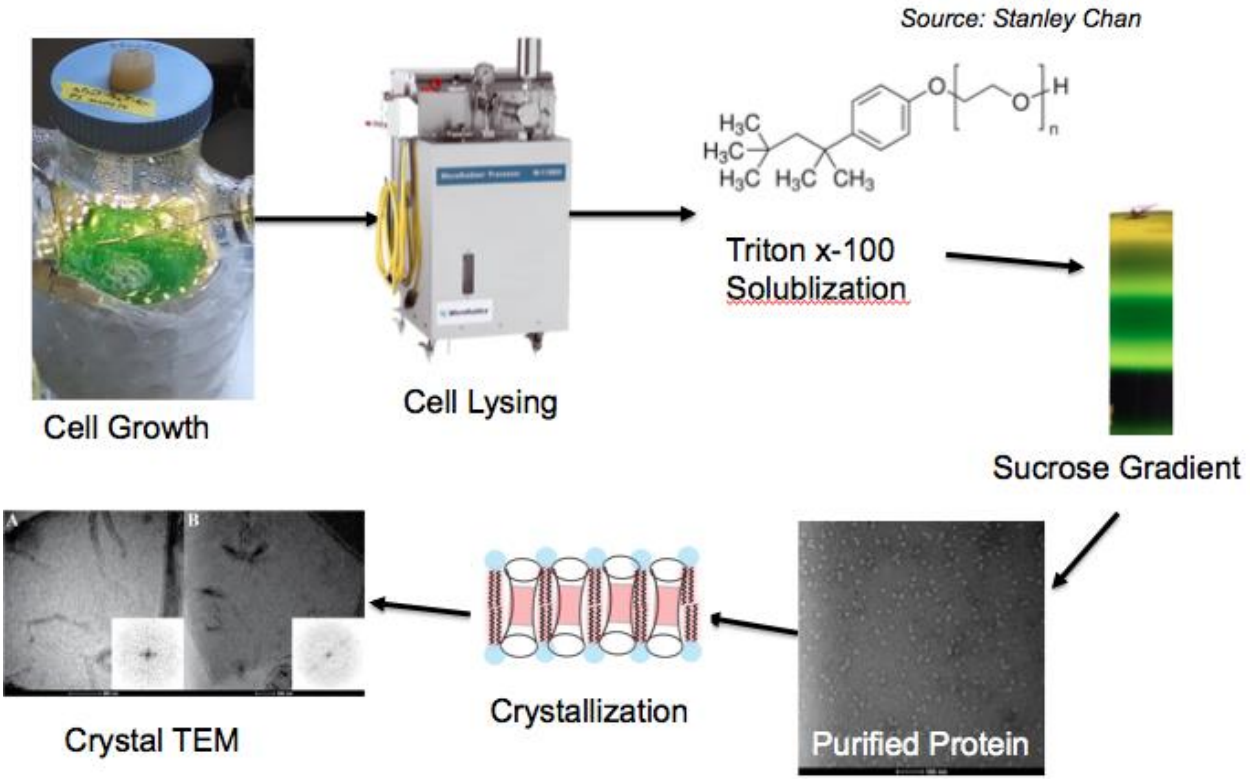

Figure 5 - Overview of PSI isolation procedure. Reprinted with permission from Stanley Chan.

100 mL of cyanobacteria cells were broken using the microfluidizer and then filtered using a 50 mM Tris buffer. Broken cells were centrifuged with the Thermo Scientific Sorvall ST-16R table top centrifuge at 4000 g for 15 minutes. The thylakoid membranes were then spun down using the Thermo Scientific Sorvall WX Ultra Series Ultracentrifuge (45k rpm, 1 hr, 4^{o}C). The resulting supernatant was removed and the pellet was suspended in 50 mM Tris pH 8.3 buffer. Concentration of cell cultures was then measured using UV-Vis spectroscopy. After

solubilizing overnight, the thylakoids were centrifuged again (12k rpm, 30 min). During the centrifugation, 5%-20% sucrose gradients were prepared in 50 mM Tris pH 8.3 buffer with 0.05% Triton X-100. The thylakoids were loaded dropwise on top of the prepared sucrose gradients and centrifuged for 17 hours at 24000 rpm.

Dark green (PSI trimer layer) was pumped out and collected for dialysis. Trimers were placed in a dialysis membrane and left to sit for 4 hours in 50 mM Tris pH 8.3 buffer, replacing the buffer solution after 2 hours. 5%-20% sucrose gradients in 50 mM Tris and no Triton X-100 were prepared. The dialyzed solution was loaded dropwise onto the new gradients and centrifuged for 17 hours at 24000 rpm. The resulting supernatant was removed and the pellet was suspended in 2 mL 10 mM Hepes (0.5% OTG). The resulting solution was made into 6 dilutions of protein in 0.5% OTB, Hepes buffer (two of 5x, 10x, and 20x dilutions of 5 μL protein). The 6 tubes and 2 standards were incubated at 37^{o}C in water for 30 minutes then ran through the nanotube BCA program to determine the protein concentration.

3.3 Button dialysis to form PSI-incorporated membranes

Using an Excel spreadsheet, 60 μL samples were made at varying polymer/protein ratios (PoPR) using **Equation 1**.

$$PoPR(wt) = PoPR(mol)\frac{MW(polymer)}{MW(protein)} \quad (1)$$

Samples were placed in the incubator at 26^{o}C overnight. Buttons were labeled and placed in DI water before being sonicated with the Symphony VWR sonicator for 5 minutes. Samples with 2% OTG detergent were added to the buttons and left to incubate in the Echotherm Chilling

Incubator in 50 mL of dialysis buffer at 26^{o}C for three days. Buffer was diluted to 100 mL after one day, 200 mL after the second, and 1 L after the third day and ran using the following program shown in **Table 1**.

Table 1 Temperature program for button dialysis

Button Dialysis Incubator Program		
Time (hr)	**Temp (^{o}C)**	**Ramp**
24	26	99.9
12	26-37	0.9
24	37	99.9
12	37-26	0.9
24	26	99.9

After the dialysis incubation program was ran, the button samples were removed using a pipette and diluted in nanotubes to at most 150 µL of dialysis buffer and stored at 4^{o}C before being characterized with TEM.

3.4 Transmission electron microscope grid preparation

A large cylindrical container was filled to the brim with distilled water. The surface of the water was wiped using a kimwipe taking care not to disturb the surface of the water after cleaning it. 2 drops of amyl acetate were quickly dropped into the center of the water surface. TEM grids were loaded face down onto the amyl acetate film in a 10x10 pattern. A slice of paper was placed over the grids and the film was slowly ripped away from around the paper using tweezers. The grid paper was slowly lifted and placed into a petri dish.

The petri dish was transported to the carbon coating facility in the Millennium Sciences Complex. The grids were carbon coated using an Electron Microscopy Sciences Carbon Coater with an evaporation time of 500 ms with one pulse. The grids were then stored for future use.

3.5 Uranyl Fluoride (UF) stain prep and staining procedure

6 mL of distilled water was placed in a small beaker on a hot plate. The beaker was wrapped in foil and left to boil. Meanwhile, 37.5 mg UF (radioactive) was weighed and added to another beaker wrapped in foil. Boiling water was added to the dry UF followed by 5 μL of 5M NaOH. The resulting solution was then transferred into a Falcon tube using a filtered syringe to remove clumped particles.

Grids for sample screened were obtained and subject to glow discharge with a PELCO easiGlow machine for two rounds on a parafilm covered slide. After discharging the grids, the polymer crystal sample was placed on the grid for 45 seconds and then the disk was dried using filter paper. Then, the grid was washed with 3 drops of water, drying the disk with filter paper after each washing. Finally, the grid was washed twice with a drop of prepared UF stain drying after each drop. For the second drop, the grid was placed upside-down for 15 seconds before drying the grid. The stained grid was then placed in the TEM box for screening. **Figure 6** shows the UF stain as well as the FEI Tecnai BioTwin Spirit transmission electron microscope that was used during the course of this research.

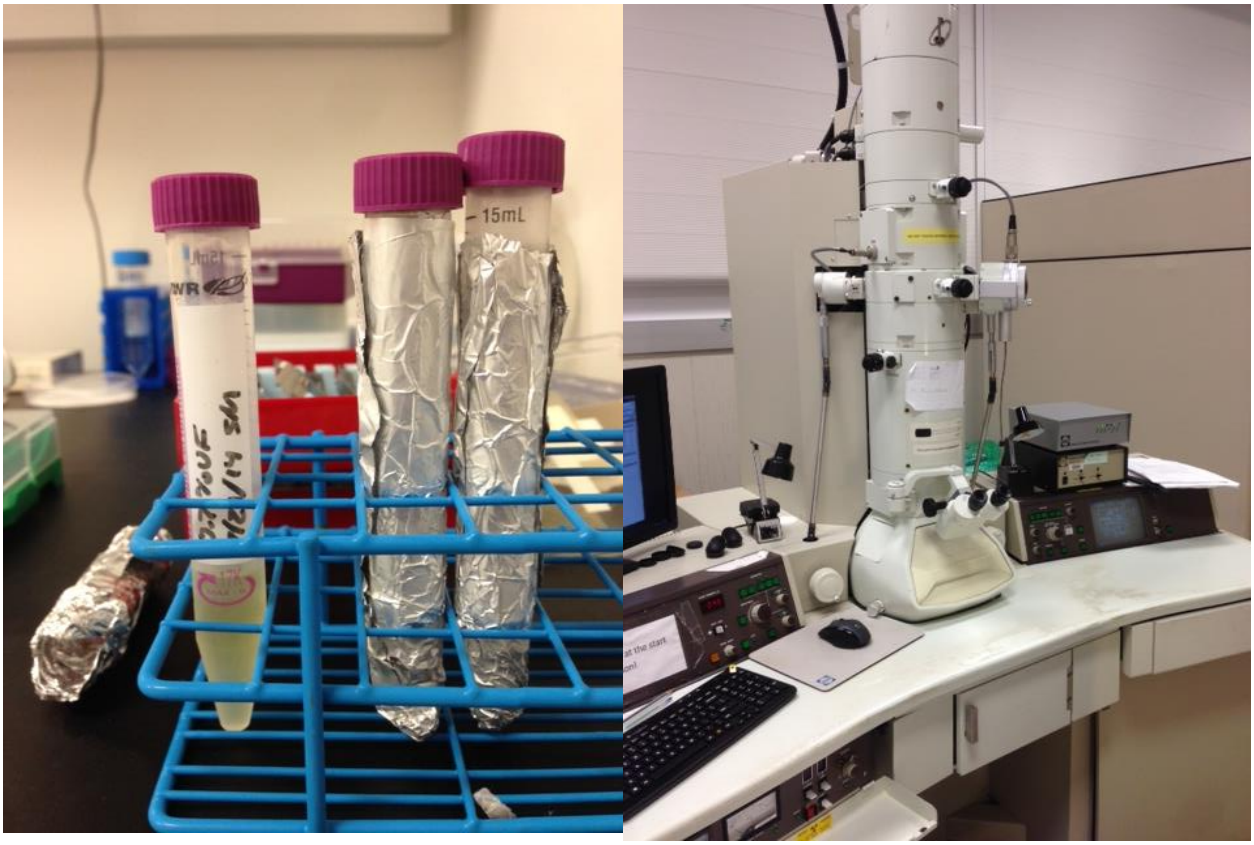

Figure 6 - UF stain (left) FEI Tecnai BioTwin Spirit TEM (right)

Chapter 4

Results and Discussion

The objective of this research was to consistently create a 2D PSI-incorporated block copolymer membrane. While consistent results were not achieved during the research period, there were a few instances where polymer films were successfully created. However, sometimes PSI was not successfully incorporated into the film, or the film was damaged or ripped from either poor handling of the TEM grid or from damage from the high energy electron beam used in TEM imaging.

Figure 7 shows a polymer film with little PSI-incorporation. With no contrasting spots throughout the sheet, there is little evidence that there is high-density PSI incorporation occurring.

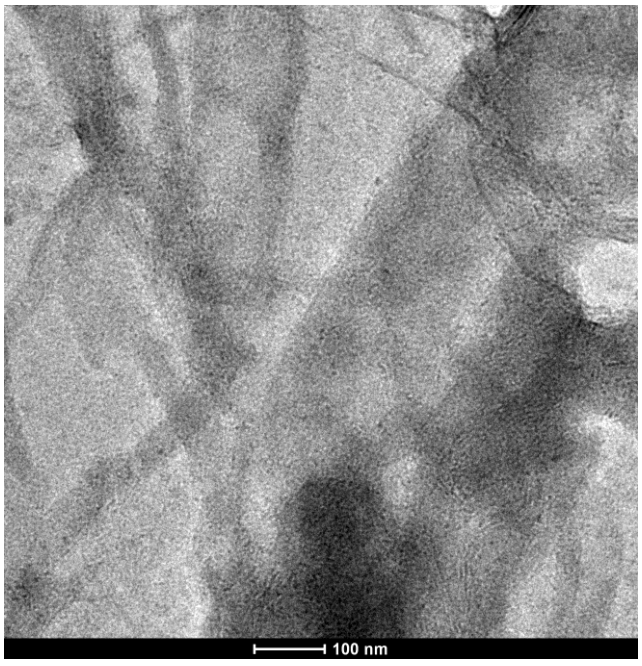

Figure 7 - Polymer film showing little if any PSI incorporation

The above image shows a film that is approximately 1 by 3 microns, which is on the same scale as the 2 by 5 micron films seen in the lipids experiment, which shows that the production of polymer films was just as successful as the formation of lipid films done prior.[4] However, at the polymer protein ratio (PoPR) of 0.60 that was used in this imaging, there was no contrast that could indicate PSI was present in this film.

Another phenomenon commonly witnessed in TEM imaging of these crystals was the formation of circular vesicle-like structures. Because block copolymers can behave like lipids and be amphiphilic, it is possible to form circular vesicles as opposed to bilayer membranes.[5] **Figure 8** shows the aggregation of different vesicle-like structures that was seen with a PoPR of 0.55.

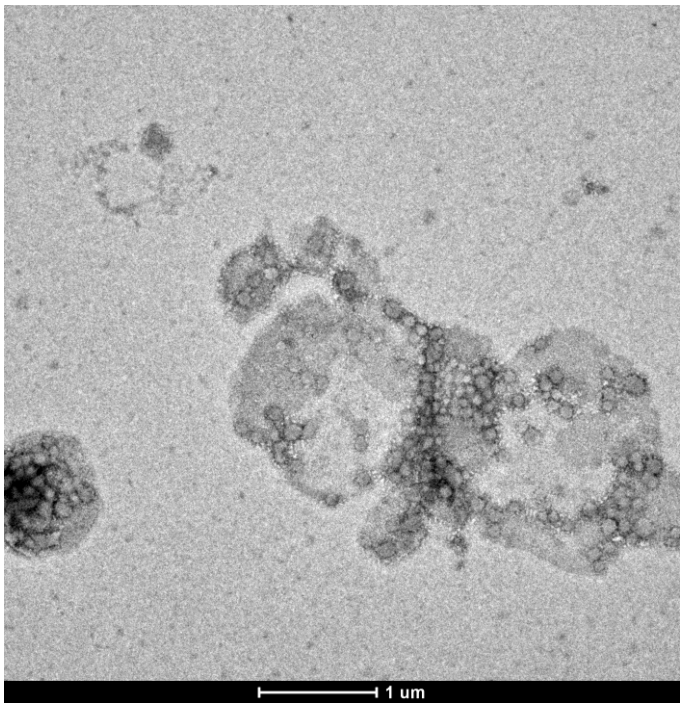

Figure 8 - Vesicle structures organized around a thin polymer film

These vesicles are about 50-100 nm in thickness, which presents a problem. Because PSI has a size on the order of 10 by 10 nm, it would be difficult to achieve high-density incorporation

when the vesicles themselves are only on the order of 50-100 nm.[21] At the PoPR of 0.55 and BCA protein concentration of 2.5 mg/mL, it looks like PSI was not successfully incorporated into polymer vesicles. At a BCA concentration of 2.5 mg/mL and a PoPR of 0.60, a polymer sheet was created, but PSI was still not successfully incorporated.

By increasing the PoPR to 0.80 with the same BCA concentration, a polymer film that had some contrasting spots that could potentially indicate the presence of PSI was created. **Figure 9** shows the polymer film created with a PoPR of 0.80 and BCA concentration of 2.5 mg/mL.

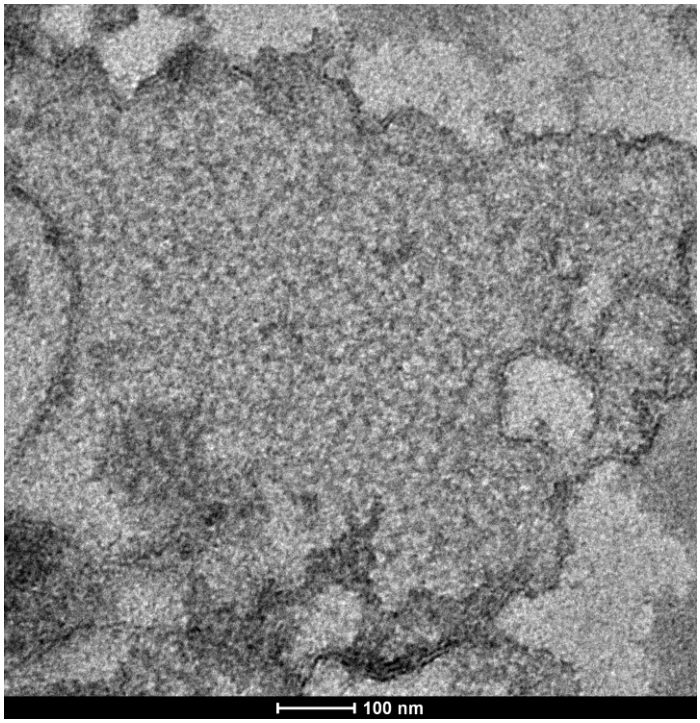

Figure 9 - Contrasting spots in the film might indicate the presence of PSI

While there are lighter spots within the polymer film seen in **Figure 9**, there is one major issue that was also seen with many other films: the films are often ripped or contain a lot of dark spaces that may be outside contamination or other films. While the presence of the lighter spots indicates PSI may be successfully incorporated, further repetition was not done to prove that PSI

could be successfully incorporated to block copolymer membranes on a regular basis prior to the end of the research window.

There were a lot of procedural mistakes that could have been made to cause poor images to result. First of all, poor handling of the TEM imaging grids could have led to ripped film. Because the TEM grids are incredibly fragile, poor handling resulting in even the slightest bending or torsion of the grid would result in some of the image grids to be torn, thus reducing the quality of the image. Another issue was in the staining. If too much UF stain was used, the TEM would show large sections of stain that could be mistaken for films. In addition, excess stain could distort the images of polymer films and create large dark streaks across the field of view, thus making those images worthless.

The objective of this experiment was to create 2D PSI-incorporated block copolymer membrane crystals that could be characterized with TEM. While some images showed the formation of a polymer film or vesicles with no PSI incorporation, a PoPR of 0.80 and a BCA protein concentration of 2.5 mg/mL showed a promising image where a polymer film had lighter contrasting spots located in the film, which are characteristic of embedded proteins. However, this experimentation could not be repeated in the research window. Therefore, further experimentation would be needed to confirm that PSI could successfully be incorporated into a block copolymer membrane.

Chapter 5

Future Work and Conclusion

The objective of this research was to create a 2D PSI-incorporated block copolymer membrane crystal. TEM characterization of the samples created during the research period could not confirm that PSI could be incorporated consistently into polymer membranes. However, the appearance of a polymer film with size on the same order of magnitude as with lipid crystal films confirm that block copolymers do behave in a similar manner as biological lipids.[4] In addition, the formation of vesicles with polymers also showed that block copolymers can form vesicles in a manner similar to lipids. However, the fact that only a few images showed the successful incorporation of PSI into block copolymer membranes means that improvements need to be made in experimentation.

Future work in incorporating PSI into block copolymer membranes could involve dilutions of the protein solution in order to decrease the overall dirtiness of the image samples, which could corrupt the quality of the image. In addition, changing storage temperatures or the incubation program could also benefit in promoting incorporation of protein. Once PSI can be incorporated into PB12-PEO8 block copolymer membranes consistently, different copolymers could be used to find one that provides maximum stability and utility. With a polymer selected, the next steps in the project would be to work on mounting the PSI-incorporated polymer crystal on to an electrode to measure photocurrent in the same ways as was done in the lipids experiment.[4] Once the crystal is successfully mounted to an electrode and the transferring of electrons and current are observed and measured, working with hydrogenase proteins or other

enzymes could be used to help generate hydrogen energy from this process and to design a complete photovoltaic device.

While the experimentation could not be completed in the research period, some images did confirm that block copolymers do behave in a manner similar to lipids forming both polymer bilayer membranes and vesicles. Images with PSI-incorporated crystals showed that the incorporated of transmembrane proteins into block copolymer membranes is possible, but further experimentation and imaging is needed to confirm that the process can be repeated on a consistent basis.

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