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PURIFICATION, CRYSTALLIZATION, AND ANALYSIS OF VARIOUS OMPF MUTANTS AND
THEIR BLOCK COPOLYMER AGGREGATES FOR USE IN THE PRODUCTION OF BIOMIMETIC
MEMBRANES

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ABSTRACT

The use of biomimetic membranes for the treatment and purification of high-salinity water is a novel one that requires a plethora of information on the properties of the membranes being used in the process. For the scope of this project, the membrane proteins of various *E. coli* cells were studied and analyzed so it could be determined which mutated outer membrane protein (in this case OmpF) would be optimal for both polymer conjugation and for eventual water purification. These purifications were performed primarily via ultracentrifugation and fast protein liquid chromatography (FPLC) while critical data on the success of these purifications were provided by various assays including Bradford and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Data on the filtration potential of produced biomimetic membranes was obtained by filtration of various molecular weight dyes. Future work includes performing further purifications and assays on additional OmpF mutants. It is the hope of this project that a sufficient number of OmpF mutants will eventually be analyzed such that biomimetic membranes with the highest possible efficacy can eventually be produced on a large scale.

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Chapter 1

Problem Statement

The purpose of this project was to investigate the purification of several mutants of *E. coli* outer membrane proteins for laboratory-scale use in developing and characterizing protein-polymer conjugates that can be used in the development of biomimetic membranes. Such biomimetic membranes are targeted at efficiently producing fresh water from wastewater and saltwater, which can be used to grow and sustain various forms of life.^{1,2} Due to the rapid growth of both population and industry in the past couple of decades, now more than ever more clean water is needed to sustain prosperous growth, and because of this strong need, water is more and more being pulled from lower-quality water sources.^{3,4} Biomimetic membranes, once produced on a scale large enough to induce economies of scale, would satiate the need for clean water done at a relatively low cost.

As of now this technology does face significant challenges, and thus does not have enough research performed on it, such that it cannot be used as a cost-effective method to produce clean water at a viable level. One of the primary inhibitions to rising to this level of effectiveness is determining which porin would be best at being the biological component in the conjugate used to produce biomimetic membranes. The primary goal of this project then was to provide enough data on purified porins (in this case, *Escherichia Coli*'s Outer Membrane Protein F) to determine which OmpF mutant would react most completely with a polymer to create a conjugated protein-block copolymer (BCP) aggregate with the least amount of unreacted materials, as well as which porin-based conjugate would enable the most effective combination of water purification and energy expenditure.

Chapter 2

Background and Hypothesis

2.1 Preliminary Information and Prior Research

The use of membranes in general to purify water is not a novel idea, as the technology of reverse osmosis has been around for decades.⁵ Reverse osmosis on its own is a very effective way to remove undesirable solutes and contaminants from water and is generally known to the public for its use in desalination plants around the world, particularly in places near the sea with scarce freshwater sources but cheap sources of energy with which to run the (sometimes very energetically demanding) pressure gradient needed in a reverse osmosis membrane system.

A more novel variant on this decades-old idea however is the use of biomimetic membranes made from block copolymers and porins to treat wastewater and saltwater. Until recently, the primary issue with developing these protein-block copolymer (BCP) aggregates was the inability to introduce sufficient levels of the porin into BCPs. A relatively effective method around this was developed by Kumar et al in 2012⁶ which uses membrane dialysis to slowly crystallize the aggregate into a single cohesive unit through the dilution of the concentration of solubilizing detergent used to create the mixture.

Using this dialysis technique with AQP0, a specific kind of water channel similar in general structure to OmpF for the purposes of this project, and a number of BCPs, Kumar et al⁶ determined that some aggregates have unique morphologies dissimilar to the original BCP which can be influenced by the porin inserted into it. Furthermore, some aggregates, upon inspection with electron microscopy, were seen to have two-dimensional crystal lattices which leads to positive results that are twofold: it may be possible to determine the structure of membrane proteins in porin-BCP membranes, and high levels of protein can

in fact be incorporated into BCPs due to the aggregates' planar architecture. OmpF was determined to be one of these materials that forms two-dimensional arrays.

2.2 The Benefits of Using Porin-BCP Aggregates to Purify Water and Biomolecules

It may not initially seem apparent why the development and use of biomimetic membranes is a worthy use of time and resources for the purification of water and biomolecules when a technology such as reverse osmosis already works to a respectable degree with membranes that are developed from just a polymer for water. However, there are a number of benefits that a biomimetic membrane has that typical polymer-based membranes cannot have. The first and foremost of these benefits is the uniform distribution of pore sizes in biomimetic membranes. In polymer-based membranes, the pores vary in size based on the characteristics of the normal curve; when a membrane is said to have a certain size, that size is actually the mean size of all the pores, and the variance of the pores is non-zero, which is not ideal for separations. This is because desired retentate may leak through the larger pores into the permeate and desired permeate may not be able to pass through the pores of the smaller end of the normal curve. For biomimetic membranes however, the polydispersity is very close to zero, so a separation using such a membrane can be performed with a relatively low amount of fouling and a very high amount of product purity.

The reason the variance is close to zero is because the protein mutants being purified are not manmade; they are created by nature and exist in very large, uniform quantities on the cell membranes of *E. coli* cells. Developing membranes as exact as the ones developed in nature is not yet possible using purely manmade techniques, so depending on these proteins generated from living organisms is the only true way to capture this uniformity advantage.⁶

2.3 The Selection and Uses of Outer Membrane Protein F (OmpF) and its Mutants

The two types of porins are currently being studied for their use in protein-BCP conjugates are a series of mutants of aquaporins (AQPs) and a series of mutants of Outer Membrane Protein F (OmpF). The two porins are used for vastly different purposes in the purification of water because of the size of their pores.⁷ The pore size of the aquaporin mutants is roughly 2.8 Å, which is ideal for removing small ions such as salts from water, particularly saltwater—these aggregates are particularly useful in desalinization plants. The protein studied in this project, OmpF, has a larger pore size, on the order of roughly a nanometer. Therefore, wildtype OmpF aggregates are better suited for treating wastewater and saltwater of larger contaminants (greater than 500 Da) but should not be used to remove salts from water, since the salts simply pass through the nanometer-sized hole. However, we aimed to develop various mutants of OmpF have amino acids in the center of their pores which are hydrophobic and reduce the effective pore size via a sort of “clogging” caused by the functional groups. This combination of hydrophobicity, which increases the rate of pure water due to the lack of hydrogen bonding with the pore walls, and reduced effective pore sizes on the order of magnitude of the aquaporins’ makes OmpF mutants superior and thus desirable for use in protein-BCP aggregates.

2.4 Hypothesis

The goal of this experiment was to investigate the purification, aggregation, and characterization of a porin-BCP conjugate using OmpF mutants as the porin of choice. It was hypothesized that membranes developed from this purification and aggregation can be used to remove most if not all impurities in water, and the characterization of these aggregates may give crucial insight into their architecture and the concentration level of protein that can be introduced.

If enough OmpF mutants are purified, aggregated, and characterized via TEM, then the desired balance of mass transfer and purity of water through a porin-BCP aggregate of these mutants can be determined and produced industrially to help purify wastewater and saltwater on a large scale.

Chapter 3

Materials and Methods

Several materials and methods were used to develop the crystallized OmpF-BCP aggregates. These include the growing, inoculating, and harvesting of the *E. coli* strain *BL21(DE3)omp8*⁸, the extraction and purification of the various mutants of OmpF via cell lysis, centrifugation and ultracentrifugation, and fast protein liquid chromatography (FPLC), and the aggregation of the OmpF-BCP subunit via crystallization through dialysis. Transmission electron microscopy was also used to characterize the arrays produced by aggregate crystallization.

3.1 *E. coli* Inoculation, Growth, and Harvesting

E. coli BL21(DE3)omp8 was streaked onto several inoculated plates containing lysogeny broth (LB) and agar. After being grown for at least 18 hours, a few colonies of cells were chosen and added to 30 mL of LB media to grow a starter culture. These cells were inoculated with 50 mg/L ampicillin and grown for at least 24 hours. 5 mL of the starter cultures are then added to 1.5 L LB media cultures and the large mixtures were then again inoculated with 50 mg/L ampicillin and 0.5% glycerol and grown until UV/Vis reads an OD 600nm of 0.5-0.8. IPTG is then introduced at a concentration of 0.4 mM and the cells were then grown at 16 °C to maximize protein expression. Roughly 1 kg of wet cells were finally harvested via centrifugation at 4 °C and 6,000g for 15 minutes. The cells were then frozen at -80 °C until needed for production of OmpF.

3.2 Cell Lysis, Centrifugation and Ultracentrifugation, and FPLC

Frozen cells were thawed and then suspended in 10 mL of 20 mM pH 8.0 Tris buffer and 1 U/ μ L of DNase per 1 g of cells. The cells were then lysed with a sonicator ten times over a period of roughly 30 minutes.

A tabletop centrifuge was run for 15 minutes at 4 °C and 4,000g to remove unbroken cells. The membranes were then suspended in 1% (w/v) sodium dodecyl sulfate (SDS) in Tris for 20 minutes at 4 °C and then were spun down with an ultracentrifuge at 4 °C and 200,000g for 1 hour. The pellet of this spin was then resuspended in 0.125% n-Octylpolyoxyethylene (octyl-POE), 20 mM sodium phosphate, pH 7.4 with 5 mL of this buffer being used per 1 g of cells. This suspension was then incubated at 37 °C for 60 minutes. The suspension was again spun down at the same settings as before on the ultracentrifuge, and the pellet was then resuspended once more in 3% octyl-POE, 20 mM sodium phosphate, pH 7.4 with 5 mL of this buffer being used per 1 g of cells. Unsolvated membranes were then spun down with an ultracentrifuge with the same settings but this time for 30 minutes. The supernatant of this spin was collected and moved to undergo two rounds of FPLC.

The supernatant of this spin was loaded onto GE Healthcare Sepharose HiScreen DEAE FF column after the column, which was initially stored in 20% ethanol to water (v/v), was equilibrated with deionized water, then DEAE column buffer 1 (50 mM NaH_2PO_4 , 3 mM NaN_3 , 1% (w/v) octyl-POE, pH 7.6), and then DEAE column buffer 2 (5 mM NaH_2PO_4 , 3 mM NaN_3 , 1% (w/v) octyl-POE, pH 7.6). The suspension was then eluted by DEAE column buffer 3 (5 mM NaH_2PO_4 , 3 mM NaN_3 , 30 mM ethylenediaminetetraacetic acid (EDTA), 100 mM NaCl, 1% (w/v) octyl-POE, pH 8.0) and the eluted peak fractions were concentrated down to 2 mL by using a Millipore protein concentrator and a tabletop centrifuge at 2,000 rpm for 20 minutes at 4 °C. The 2 mL was then run over a GE Healthcare Superose 12 SEC column following a procedure developed by Garavito and Rosenbusch.⁹ Size exclusion fractions were eluted by a gel filtration buffer (10 mM Tris, 100 mM NaCl, 1.2% (w/v) n-Octyl- β -D-glucopyranoside (OG), pH 7.6), collected,

and concentrated. The concentration of the protein in the gel filtration buffer was measured via a Bradford assay, and the purity of the protein was confirmed via SDS-PAGE.

3.3 Crystallization of OmpF-BCP Aggregates

A crystallization buffer of 20 mM 4-(2-Hydroxyethyl)piperazin-1-ylmethanesulphonic acid (HEPES), pH 7.2, 10 mM MgCl₂, 100 mM NaCl, 0.2 mM D,L-dithiothreitol (DTT), and 3 mM NaN₃ was used to incubate 60 μ L dialysis buttons.¹⁰ A series of ratios of OmpF to polymer (for this experiment, polyethylene-*b*-polyethylene oxide (PBPEO)) ranging from 0.1 to 1.0 were crystallized to be analyzed. The crystallization buffer was mixed with octyl-POE to bring the mass percent of octyl-POE to 4% and the overall volume in the buttons to 60 μ L. The concentration of the protein in the buttons was chosen to be 1 mg/mL. The buttons were sealed off with a 12,000-14,000 Da molecular weight cut-off membrane used for dialysis. The system was then sealed with a rubber O-ring to ensure dialysis as the only method of mass transfer.

The dialysis buttons were then placed in 50 mL of 4% by weight octyl-POE in crystallization buffer. To ensure slow crystallization, every 24 hours, crystallization buffer without octyl-POE was placed in the system in such a way that every daily addition halved the concentration of octyl-POE in solution. Upon reaching 0.25% octyl-POE (w/v), 1 L of crystallization buffer without detergent was used as the new buffer to finish the crystallization. This was done three times even over the course of 12 hours for a residence time of 4 hours for each fresh liter of crystallization buffer.

3.4 TEM Imaging

For negative staining, crystal suspensions were adsorbed on a glow-discharged carbon-coated electron microscopy grid, negatively stained with 0.75% (w/v) uranyl formate, and imaged with an FEI

Tecnai G2 Spirit BioTwin electron microscope operated at 80 kV and using an FEI Eagle 4K x 4K HS CCD or Gatan Orius 2K x 2K CCD camera. Image analysis of the crystals was performed using the 2dx and ImageJ6 software packages.¹¹

3.5 Biomimetic Membrane Construction

In order to produce mechanically stable biomimetic membranes, Whatman Nuclepore Track-Etched Membrane of 50 nm (serial number 110603) were used as a base for the crystalline protein-BCP aggregates to form their selective membrane on. This was achieved through first using ultraviolet ozonation on the support membrane, and then introducing them and 1 mL of polyethylenimine (PEI) per membrane to an Amicon stirred cell. The solution is allowed to sit for 15 minutes, and then is dumped. A new solution of 20 μ L 2D crystal stock solution (in this case the OmpF Trans mutant's crystals) and 3 mL of HEPES is added over top of the membrane. This was left to sit for 15 minutes. The Amicon stirred cells were filled with HEPES, and then introduced to 4 psi of pressure, forcing the crystals to bind to the support membrane, and forcing the HEPES out of the stirred cell. The above procedure was repeated six times to add six overlapping layers of Trans-BCP membrane alternating with PEI to the support membrane. The final step in the process is to cross-link the crystalline membranes to the support membrane to ensure mechanical stability.

3.6 Biomimetic Membrane Filtration Testing

To test the selectivity of membranes, Millipore Ultrapure water first tested for permeability. The next step was to test the permeability of several dyes of various, increasing molecular weight. Amicon stirred cells were fitted with a magnetized spin bar and loaded with a composite biomimetic membrane and were filled with dye. They were then pressured with 15 psi of pressure until 20 g of dye was pushed out of

the system. Upon 20 g of dye leaving the system, 1 g of permeate and 1 g of retentate were sampled. This was done with two separate membranes (membrane A and membrane B), and then these samples were diluted with ultrapure water and then analyzed with UV/Vis for concentration. A diagram of the system used to obtain these filtration results is shown in Figure 1.

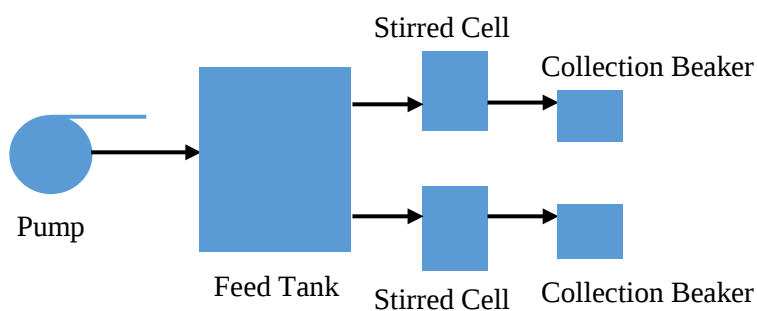


Figure 1. The setup of the filtration unit for biomimetic membrane permeation testing.

Chapter 4

Results and Discussion

4.1 TEM Images of 2D crystals of OmpF-BCP conjugates

Images of crystalline arrays of OmpF-BCP aggregates was the primary goal of the purification steps discussed earlier: having monolayered, 2D arrays ensures that the proteins were purified properly and were dialyzed in such a manner that a lattice was thermodynamically preferred by the molecules. The images below are both pictures of the purified proteins themselves as well as the crystal lattices they form when aggregated with polymer PBPEO. The results of these photos vary based on the mutant that was pictured: for wildtype OmpF, the images are of a successfully crystallized aggregate; for Trans, the images are a nanosheet, a nanostructure that is not quite a 2D crystal because of its lack of crystallinity, but it does have 2D structure to it; for Para, the images are of vesicles, polymer or protein and polymer that has not aggregated properly into two dimensions. Para has not yet been able to be crystallized into even a nanosheet yet, and it will be addressed in future work. Trans has in fact been crystallized (outside these experiments) and the nanopieces may be a result of either flawed dialysis or protein purification. The following images are Tingwei Ren's, and I would like to acknowledge and cite her for sharing her results with me.

4.1.1 Images of Wildtype OmpF Crystallized with PBPEO

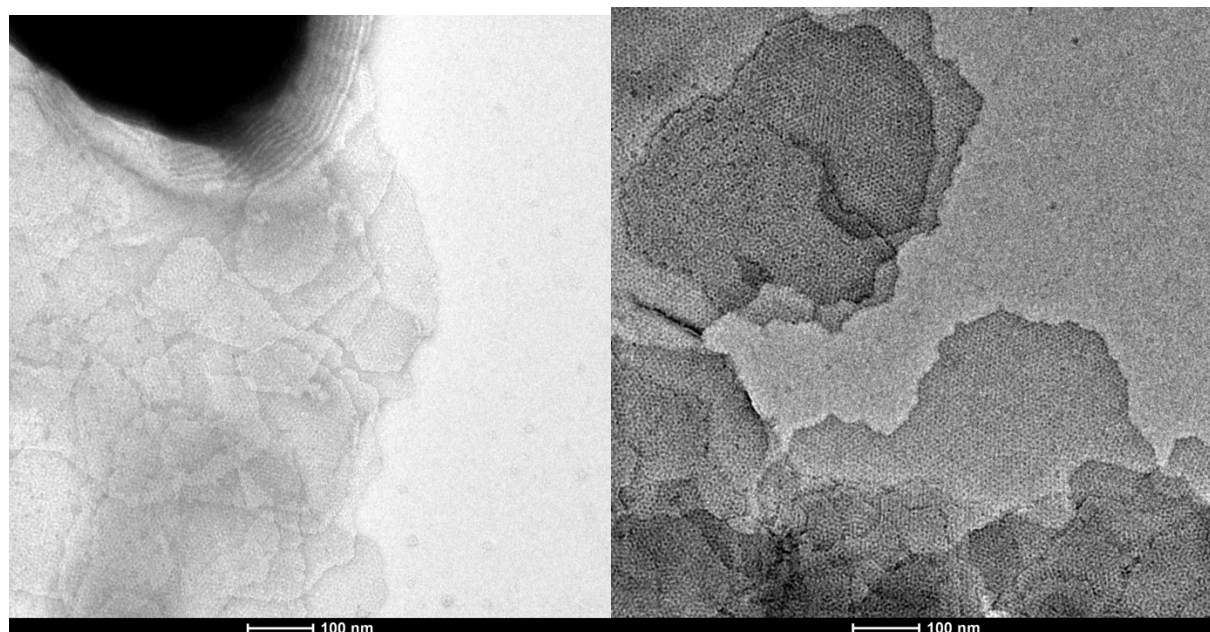


Figure 2. TEM Images of Wildtype OmpF crystallized with PBPEO.

4.1.2 Images of Trans-Mutated OmpF Made into Nanopieces with PBPEO

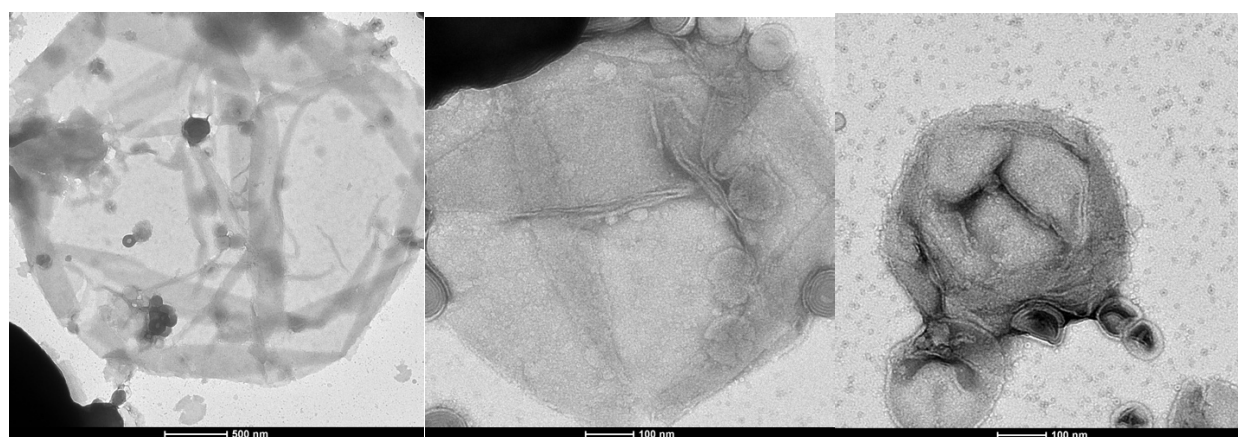


Figure 3. TEM Images of Trans-Mutated OmpF-BCP Nanopieces.

4.1.3 Images of PBPEO Vesicles from Para-Mutated OmpF-BCP Imaging

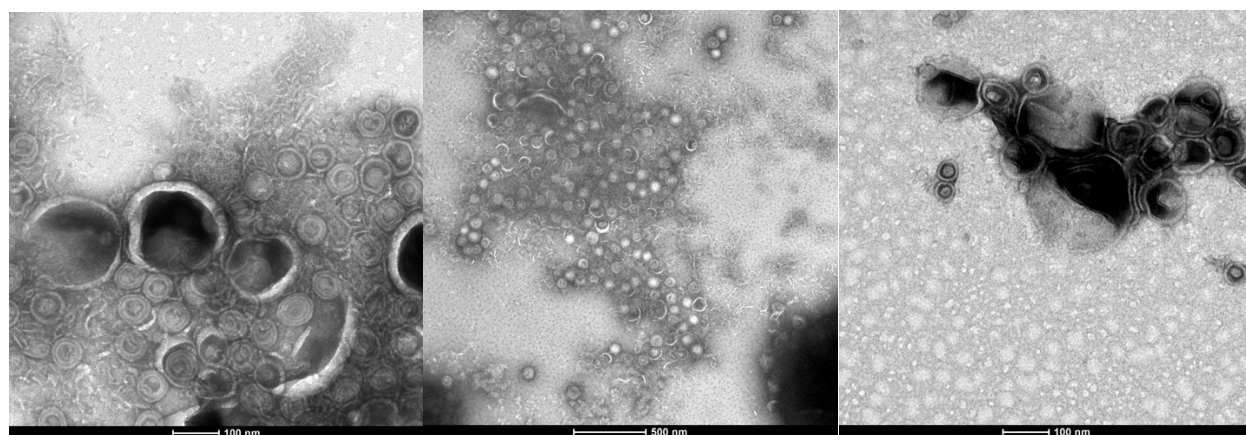


Figure 4. TEM Images of Para-Mutated OmpF with PBPEO vesicles that may contain some Para-OmpF.

4.2 Filtration Data and Results and Discussion (Trans)

The following data is Tingwei Ren's and I would like to acknowledge and cite her for providing it to me. The results of dye permeation of Trans-mutated biomimetic membranes for two separate membranes (membranes A and B) follows. The six dyes used were Rose Bengal (RB) with a molecular weight of roughly 972 Da, Acid Fuchsin (AF) with a molecular weight of roughly 539 Da, Methyl Orange (MO) with a molecular weight of roughly 304 Da, Methyl Blue (MB) with a molecular weight of roughly 754 Da, Crystal Violet (CV) with a molecular weight of roughly 373 Da, and Chrysoidine G (CG) with a molecular weight of roughly 212 Da. These dyes were chosen because their molecular weights are fairly far apart and thus important to study for a comprehensive analysis of membrane permeation.

Table 1. Flux Data for Membrane A for RB, AF, and MO.

Sample	Mass (g)	Liter	m ²	Minute	Hour	PSI	bar	LMH/bar	Ave LMH/bar
Pure Water								37.41	37.41
RB(971.68Da)	9.70	0.00970	0.000410	21	0.350	15.30	1.055	64.08	63.07
	9.80	0.00980	0.000410	20	0.333	15.30	1.055	67.98	
	10.30	0.01030	0.000410	25	0.417	15.30	1.055	57.15	
AF((539.04Da)	10.15	0.01015	0.000410	24	0.400	15.10	1.041	59.45	58.05
	11.67	0.01167	0.000410	28	0.467	15.15	1.045	58.39	
	9.68	0.00968	0.000410	24	0.400	15.20	1.048	56.32	

MO(304.02Da)	10.69	0.01069	0.000410	26	0.433	14.95	1.031	58.37	59.47
	10.60	0.01060	0.000410	25	0.417	15.00	1.034	60.00	
	10.25	0.01025	0.000410	24	0.400	15.10	1.041	60.03	

The most interesting piece of information that can be gleaned from this data is the general flux of water across the membrane. As one of the primary objectives of biomimetic membranes is to purify water, the rate of mass transfer of water across one such membrane is an important piece of data to consider. As far as adjusted, normalized (in LMH/bar) permeation values go, this value of 37.4 L/m²/hr/bar for water is relatively high.¹² For membrane B, this number is even higher, as can be seen in Table 2 below, going as high as almost 48 LMH/bar. Both these sets of data quantify the ability of these biomimetic membranes to quickly allow water through their pores.

Table 2. Flux Data for Membrane B for MB, CV, and CG.

Sample	Mass (g)	Liter	m²	Minute	Hour	PSI	bar	LMH/bar	Ave LMH/bar
Pure Water	1.69	0.00169	0.000410	5	0.083	15.00	1.034	47.83	47.99
	12.93	0.01293	0.000410	38	0.633	15.00	1.034	48.15	
MB(753.84Da)	12.40	0.0124	0.000410	22	0.367	14.80	1.020	80.83	77.09
	8.91	0.00891	0.000410	17	0.283	14.65	1.010	75.93	
	10.80	0.0108	0.000410	21	0.350	14.65	1.010	74.51	
CV(372.55Da)	9.99	0.00999	0.000410	26	0.425	14.65	1.010	56.76	52.73
	14.37	0.01437	0.000410	40	0.667	14.60	1.007	52.23	
	6.61	0.00661	0.000410	19	0.323	14.70	1.014	49.20	
CG(212.26Da)	10.63	0.01063	0.000410	26	0.433	14.55	1.003	59.64	67.47
	8.10	0.0081	0.000410	18	0.292	14.70	1.014	66.83	

The more interesting and primary data of this experiment comes in Tables 3 and 4, which have the rejection of each of the dyes for each membrane. This data is so important because it is with it that it can be determined whether these biomimetic membranes are actually going to be effective at letting pure water through but keeping molecules of substantial molecular weight (biomolecules, dyes, salts) in the retentate.

Table 3. Rejection Data for Membrane A for RB, AF, and MO.

Dye		Dilution Factor	Conc (g/L)	Conc (g/L)	Conc (g/L)	Conc (g/L)	Rejection
RB(971.68Da)	Feed	10	0.599	0.597	0.587	5.943	
	Permeate	10	0.105	0.103	0.099	1.023	0.828
AF(539.04Da)	Feed	10	0.359	0.369	0.357	3.617	
	Permeate	10	0.129	0.131	0.129	1.297	0.641

MO(304.02Da)	Feed	10	0.153	0.155	0.155	1.543	
	Permeate	10	0.098	0.1	0.106	1.013	0.343

Table 4. Rejection Data for Membrane B for MB, CV, and CG.

Dye		Dilution Factor	Conc (g/L)	Conc (g/L)	Conc (g/L)	Conc (g/L)	Rejection
MB(753.84Da)	Feed	10	0.599	0.597	0.587	5.94	
	Permeate	10	0.105	0.103	0.099	1.02	0.83
CV(372.55Da)	Feed	10	0.475	0.474	0.47	4.73	
	Permeate	10	0.274	0.268	0.262	2.68	0.43
CG(212.26Da)	Feed	5	0.05	0.047	0.051	0.25	
	Permeate	5	0.043	0.044	0.045	0.22	0.11

The rejection potentials for each dye were relatively expected based on their molecular weights: as the molecular weight of the dye went up, its rejection from the membrane also went up. This is to be expected from any membrane and proves that the multi-layered biomimetic ones do in fact do a substantial job of blocking large molecules from entering the permeate. For the purposes of this experiment, nothing can be concluded about the salt blocking potential of these membranes, as most salts have a molecular weight of less than 100 Da, but these membranes do effectively block all molecules above the order of 300 Da, meaning that blocking biomolecules would be a fairly effective use for these membranes, as most biomolecules' weights do go over that threshold. Figure 5 below shows the comparison between rejection and the molecular weight of the dye in the feed solution.

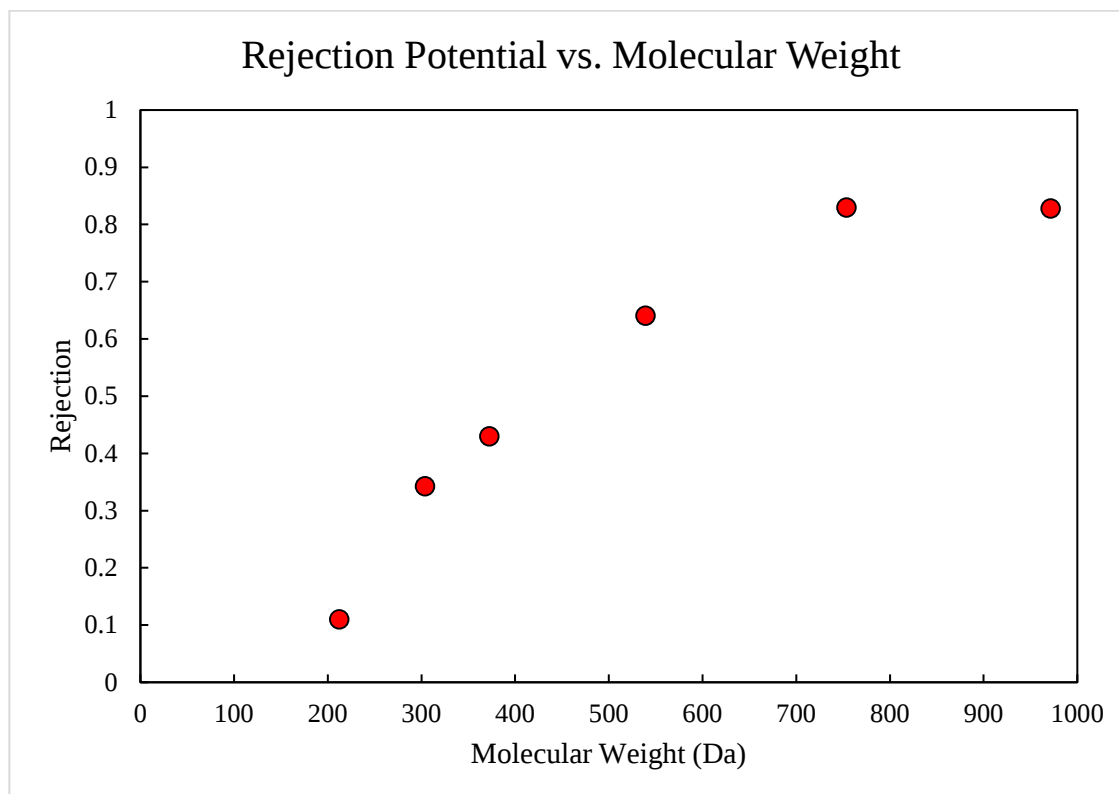


Figure 5. Rejection Potential of Dyes versus Molecular Weight for Membranes A and B.

Figure 5 shows a logarithmic-trending curve for the relationship between molecular weight and rejection. This is to be expected because lower molecular weight molecules will have a very easy time moving through the membrane as their weights get lower and lower because there will be few or no pores that can stop their mass transfer. At higher molecular weights, we can see that there will always be a few pores that allow the molecules within a substantial range through the membrane, and there is some degree of molecular weight independence with regard to rejection (eventually, at extreme molecular weights, this rejection would hit 1, but this is outside the scope of this experiment). Tables 3 and 4, along with Figure 5, show that these biomimetic membranes basically function as normal membranes, albeit with a much higher permeate flux for pure water, one of the primary goals of this project.

Chapter 5

Future Work and Conclusion

The objective of this project was twofold: to determine if various OmpF mutants could be crystallized into 2D arrays, and to test the filtration and permeation abilities of the biomimetic membranes of those OmpF-BCP aggregates that could be crystallized. The results given show that parts of this project were a success and parts need further work to determine possibility. It is certainly possible to crystallize and run filtration data on wildtype OmpF, but its molecular structure lends itself to being permeable for large molecules. On the other hand, it is known that if Para and to some extent TRP and Trans were to be crystallized their water permeability and large molecule rejection would be good, but in this paper, we only see a Trans nanosheet, and Para vesicles, while TRP has not yet even been tested for crystallization.

Future work would definitely include working on crystallizing Para, which preliminary analysis shows is the most promising OmpF mutant and running filtration experiments on it with dyes to see how well it blocks larger and smaller dyes from crossing into the permeate. TRP also shows great promise, and working to purify and crystallize it would be a good step after the work on Para has been completed. One final goal for the future work on this project would be to perform dye-filtration tests on each of the mutants several times to check and confirm the efficacy of the mutants and be able to definitively say which biomimetic membrane is desirable for whatever molecule needs to be removed from water.

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Thesis: PURIFICATION, CRYSTALLIZATION, AND ANALYSIS OF VARIOUS OMPF MUTANTS AND THEIR BLOCK COPOLYMER AGGREGATES FOR USE IN THE PRODUCTION OF BIOMIMETIC MEMBRANES

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Work Experience

May 2015 – May 2018

Undergraduate Researcher

Worked on various undergraduate projects for both Dr. Andrew Zydney and Dr. Manish Kumar, including the research performed for a thesis for the Schreyer Honors College on OmpF purification for use in biomimetic membranes.

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Manish Kumar, Associate Professor of Chemical Engineering;

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