THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

IMPROVING EXTRACTION, PURIFICATION AND CHARACTERIZATION OF MEMBRANE PROTEINS

HYEONJI OH
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Reviewed and approved* by the following:

Manish Kumar
Associate Professor of Chemical Engineering
Thesis Supervisor

Enrique Gomez
Professor of Chemical Engineering
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Membrane proteins (MPs) have gained steady increase in interest due to its critical importance in biomedical research. In current extraction processes, MPs are solubilized and stabilized by specialized non-ionic detergents to stabilize their hydrophobic exteriors. However, free detergent molecules and empty detergent micelles (not containing MPs) are often retained together with the extracted MPs in subsequent ultrafiltration (UF) steps which are required to obtain higher concentration of MPs needed for characterization process and eventual use. This poses a challenge as the retained detergent may destabilize MPs and interfere with their functional/biophysical characterization. To address this challenge, the effect of detergent selection on extraction and ultrafiltration was studied in this work. Also, a flat-bottomed centrifugal filter was designed to concentrate MPs and remove free detergents molecules/empty detergent micelles with higher efficiencies. Two biomedically-relevant MPs, halorhodopsin (pHR) and KR2 were used as models for complex 7 transmembrane helix containing G-coupled Protein Receptors (GPCRs). Common employed MP detergents were used to investigate the influence of detergent selection and to test the performance of this filter over a wide range of salt concentrations. Detergent with longer alkyl chains such as dodecyl maltoside (DDM) which has a 12-carbon alkyl chain showed more efficient extraction than the ones with shorter alkyl chains due to its higher hydrophobicity, larger micelle size, and lower CMC. Detergent passage of flat-bottomed centrifugal filter was significantly higher compared to the commercial centrifugal filters due to its higher concentration polarization (CP).

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ACKNOWLEDGEMENTS

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Chapter 1

Introduction

Membrane proteins refer to the proteins that are part of or interact with the cell membranes. Membrane proteins (MPs) play important roles in biological process as they have several functions such as material transport, enzyme activity, signal transduction, cell-cell recognition, etc. Due to these versatile functions, they proposed for use in many areas including desalination, optogenetics, and physiological/chemical research. Especially, pharmaceutical research is one of the largest areas where MPs are used since more than 60% of current drugs targets MPs.[1] Several examples of membrane proteins are given below :

Water transport membrane proteins - Aquaporins

Aqauaporins (AQPs) are integral membrane proteins that are contained in the cell membrane of various bacteria, fungi, animal and plant cells, facilitating transport of water molecules between cells.[2] In a human body, they regulate and mediate transcellular water transport, influencing urine concentration in the kidney and secretion of saliva from salivary glands, etc[3] (Figure 1). The rate of water transport through AQPs is around a billion to 10 billion water molecules per second and the selectivity is so high that even protons are repelled[2]. Due to these properties, AQPs are studied in water purification studies as well as medical studies.

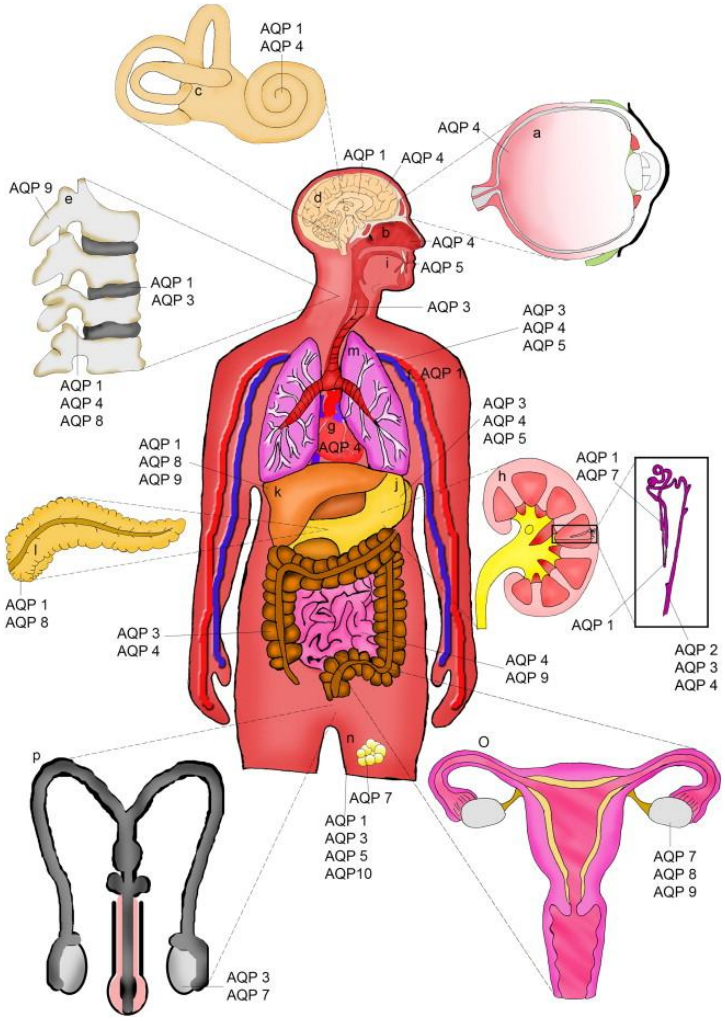

Figure 1. Aquaporins in the human body.

Ion channel membrane proteins

Ion channels are membrane proteins that allow transport of ions and solute across the cell membrane. They are often referred as the most ancient sensor to the external environment as the aqueous pore on ion channels become accessible to ions after a conformational change responding to chemical or mechanical signals.[4] Individual ion channels are selective; they only

allow certain ions to flow through them. For example, the anion pump halorhodopsin (pHR, Figure 2) transports chloride (Cl^-), bromine (Br^-), iodine (I^-), nitrate ($NO3^-$), and thiocyanate (SCN^-) across the cell membrane responding to light.[5] G protein-gated ion channels affect the flow of potassium (K^+), sodium (Na^+), chloride (Cl^-), and calcium (Ca^+) ion across the cell membrane when they are activated by a family of associated proteins.[6] The ion channels play critical roles in physiological functions such as nerve impulses, muscle contraction, and hormonal secretions[7], therefore used in many research areas such as medical and pharmaceutical researches. Additionally, as the activities of ion channels introduce electronic signals, they are often used in neuroscience research studies.[8]

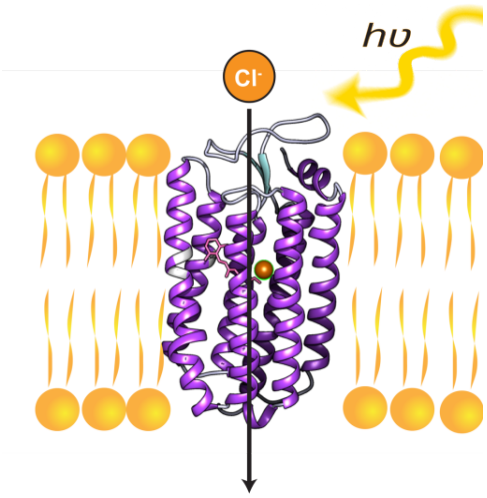

Figure 2. Schematics of halorhodopsin. Halorhodopsin pumps certain anions (Cl^-, Br^-, I^-, NO_3^-, SCN^-) when they are exposed to light.

Characterization of these membrane proteins is essential for understanding their functions and structures. However, only a fraction of membrane proteins is fully characterized; only 0.1% of MPs crystal structure have been determined and less than 2% of the reported crystal structures in the Protein Data Bank (PDB) are made up by MPs.[9] The bottleneck in studying MPs comes from challenges in obtaining high concentration of purified membrane proteins, as these are required for *in vitro* structural and functional characterization.

The current membrane protein purification process can be divided into two parts as shown in Figure 3. First, membrane proteins are extracted from cell membranes. Use of detergents is required here due to amphipathicity of the proteins; membrane proteins have both hydrophilic (both of the end of the protein) and hydrophobic regions (middle exterior of the protein). Once the hydrophobic regions is exposed to hydrophilic solutions, the proteins are prone to denaturtion and loss of functionality. When they are embedded in the lipid bilayer, their hydrophobic cores are protected by hydrophobic tails of lipids while the their hydrophilic heads enable them to be solubilized in aqueous solutions. Detergents can mimic this condition as they share structural similarities with lipid molecules (hydrophilic heads and hydrophobic tails), preventing proteins from hydrophilic environment as shown in Figure 4.

Especially, in purification of integral membrane proteins, which span the entire lipid bilayer detergent, detergent should be used unlike the other classification of MPs such as peripheral membrane proteins. Peripheral membrane proteins are temporarily embedded in the lipid bilayer or integral membrane proteins and they can be dissociated from membranes by using milder techniques, such as use of high salt or high pH solutions.[10]

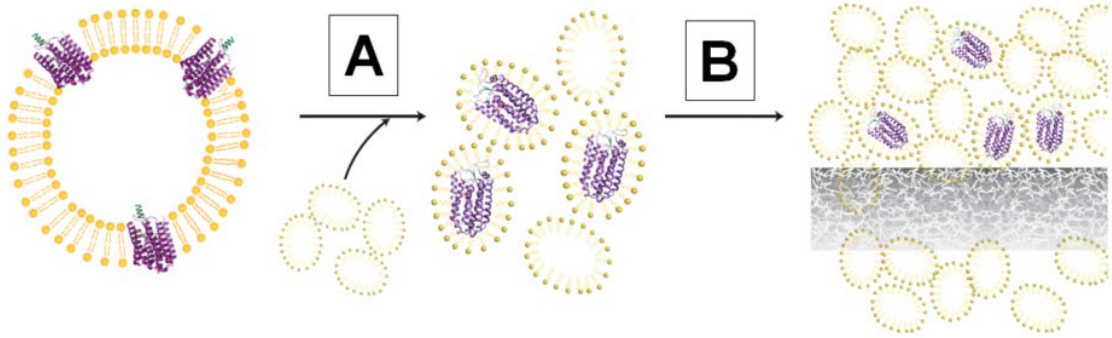

Figure 3. Current process of membrane protein purification. (A) First, membrane proteins are extracted using detergent. (B) After extraction, the membrane proteins-detergent complexes are further concentrated.

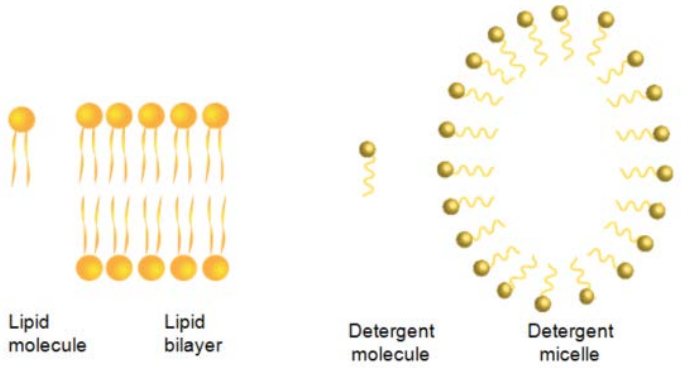

Figure 4. Hydrophobic region of membrane proteins is vulnerable to hydrophilic environment. When proteins are embedded into lipid bilayer or partitioned in detergent micelle, they can be protected and retain their structures and functionalities.

Because the concentration of extracted proteins from current process is orders of magnitude lower than the concentration required for characterization assays, the extracted membrane protein-detergent micelle complex (MPD complex) is further concentrated after extraction. One concern arising while concentrating membrane proteins using retentive membranes is that detergent monomers and empty detergent micelle are also concentrated with the proteins. This can be detrimental to membrane proteins as there is a possibility that the redundant detergent could denature the proteins as well as complicating the subsequent assays.

In the next section, I will talk about our work on improving the extraction and purification of membrane proteins. Especially, (a) to develop more effective extraction procedures, different detergents varying in alkyl chain lengths/head sizes were tried and effect of salt concentration of buffer solutions was studied, (b) to develop the post-purification part (concentrating MPD complex), a new filter was designed with different geometry. In the last chapter, I will talk briefly about the characterizing membrane proteins, specifically halorhodopsin chloride anion transport rate measurement as an example.

Chapter 2

Improvement of membrane proteins extraction

In this project, the goal was to improve the membrane protein extraction via rational detergent selection and optimization of buffer solution composition.

Detergent Selection

Detergents play important roles in membrane protein extraction as mentioned earlier. Once added in, they bind to and solubilize lipid membranes. As more and more detergents are added, they start to lyse membrane and form lipid-protein-detergent complexes. Moreover, the hydrophobic alkyl chains of detergent encapsulate the transmembrane hydrophobic domain of MPs, while the hydrophilic head groups solubilize the micelles in the aqueous environment.

The extraction efficiency of a specific detergent is directly related to the hydrophobicity of its micelle core. In this work, the hydrophobicity of a range of commonly employed non-ionic detergents with different length of alkyl chains and head group size, octyl-β-D glucoside (OG), octyl-β-D maltoside (OM), decyl-β-D maltoside (DM) and dodecyl-β-D maltoside (DDM), were determined experimentally. The structure of these detergents are shown below (Figure 4).

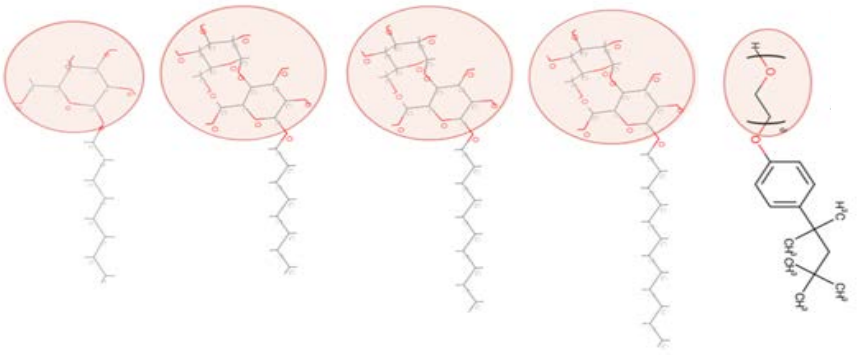

Figure 5. Hydrophobicity of a range of detergent with different length of alkyl chains and head size were measured. octyl-β-D glucoside (OG), octyl-β-D maltoside (OM), decyl-β-D maltoside (DM) and dodecyl-β-D maltoside (DDM), Triton X-100 are shown from left to right.

Relative hydrophobicity of detergent micelles was estimated by observing the emission and absorption maxima of distyryl-stilbene oligoelectrolyte (DSSN+), a small fluorescent molecule. Lippert-Mataga equation (Eq 1) were used to analyze the results.

$$\frac{1}{\lambda_A} - \frac{1}{\lambda_F} = \frac{2}{hc}\left[\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}\right]\frac{[M_E-M_G]^2}{a^3} + \left(\frac{1}{\lambda_{A,S}} + \frac{1}{\lambda_{F,S}}\right) \qquad \textbf{Eq. 1}$$

$$\Delta v = \left(\frac{1}{\lambda_A} - \frac{1}{\lambda_F}\right) - \left(\frac{1}{\lambda_{A,S}} + \frac{1}{\lambda_{F,S}}\right) = \frac{2}{hc}\left[\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}\right]\frac{[M_E-M_G]^2}{a^3} \qquad \textbf{Eq.2}$$

(λ_A is wavelength of absorbance, λ_F is wavelength of emission, $\lambda_{A,s}$ and $\lambda_{F,S}$ are the absorbance and emission wavelengths in absence of solvent, h is Planck's constant, c is the speed of light, a is the radius of cavity where fluorophore resides, ε is dielectric constant of solvent, n is refractive index of solvent, M_E is the fluorophore excited state dipole moment, and $M_G$is the fluorophore ground state dipole moment.)

The differences of Stoked shifts in the presence of detergent micelles and respective aqueous solvent were calculated (Eq 2). Next, the shift for the difference in size of detergent micelles was corrected to evaluate $-a^3\Delta v$, the relative stoke shift, which provides a measure of the relative polarity of the micellar environment; Larger stokes shift indicates higher hydrophobicity/apolarity of the detergent micelle. These experimental hydrophobicity values were compared to the theoretical HLB (hydrophilic-lipophilic balance) values which were calculated using the Griffin and Davies method[11,12]; Higher HLB value indicates higher hydrophobicity. As shown in Figure 4, our experimental hydrophobicity values were well aligned with theoretically calculated HLB values (R^2 > 95%), establishing the credibility of the experimental measurements.

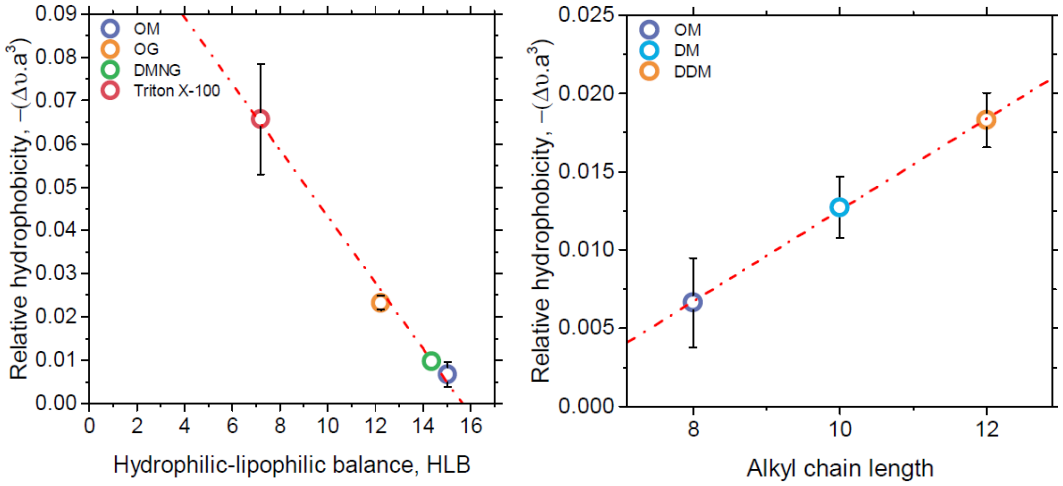

Figure 6. (a) Relative hydrophobicity of detergent micelles of different detergents were measured experimentally and compared to theoretical HLB values. (b) It was determined that detergents with longer alkyl chain are more hydrophobic.

From Figure 6a, it was observed that OG is more hydrophobic than OM indicating the detergent with smaller head group is more hydrophobic when they have the same length of alkyl chains. This could be explained by noting the larger packing parameter of OG and its lower steric repulsion, allowing tighter packing and better exclusion of water in the hydrophobic core.

Detergents with longer alkyl chain are more hydrophobic (Figure 6b), as the relative hydrophobicity of DDM, which has alkyl chain composed of 12 carbon atoms was the highest followed by DM and OM, whose alkyl chain is composed of 10 and 8 carbons respectively. This is possibly due to the changes in micellar morphology, for example, micelle of DDM would have thicker hydrophobic regions compared to the other ones.

To prove the principle of detergent hydrophobicity having an effect on protein extraction, KR2, a MP of the rhodopsin family was expressed in *E.coli* and solubilized using OM, DM, and DDM with the same concentration of 1.2%. The result showed that DDM, the detergent with longest alkyl chain, was the most efficient at extracting KR2 followed by DM and OM as expected (up to 5 fold improvement) for the reason mentioned earlier.

Additionally, dynamic light scattering (DLS) size measurement showed that the detergents with longer alkyl chain form larger micelles, therefore offering greater accessible volume for MPs.

Even though Triton X-100 and OG showed high hydrophobicity values previously, they were not used here; we did not use Triton X-100 due to impurities such as polyethylene glycol and degradation products such as peroxides which can affect the final MP yield.[13] OG was not used since it was reported that rhodopsins such as pHR was degraded in presence of it.[14]

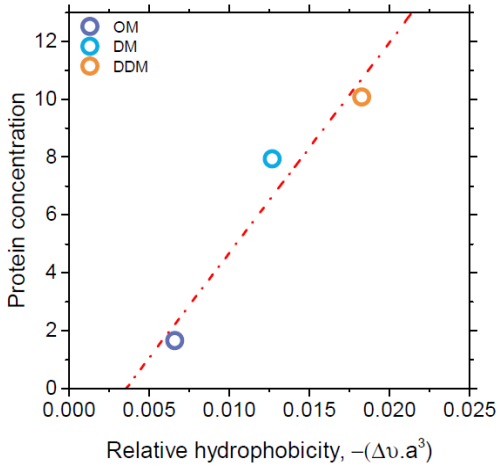

Figure 7. KR2, a sodium pump, was extracted using different detergents including OM, DM, DDM. It was determined that greater extraction efficiency in order of DDM > DM > OM, proving longer alkyl chain helps efficient extraction of proteins.

Buffer solution conditions - salt concentration

Aside from detergent selection, the effect of buffer solution condition was studied to optimize the membrane protein extraction.

Fluorescence assays (using DSSN+) and DLS size measurement showed that the size and relative hydrophobicity increase as the salt concentration increases, possibly resulting in the salting out effect (Figure 8). This salting out effect also causes a decrease in CMC and earlier onset of micelle formation, which would be useful for MP extraction at low detergent concentrations.

To prove this principle, extraction of a light-driven chloride ion pump, halorhodopsin was tried at different salt concentrations (Figure 9). It was demonstrated that protein extraction is more effective at higher salt concentrations. Also, in agreement with the previous discussion, detergents with longer alkyl chain were more effective at all different salt concentrations.

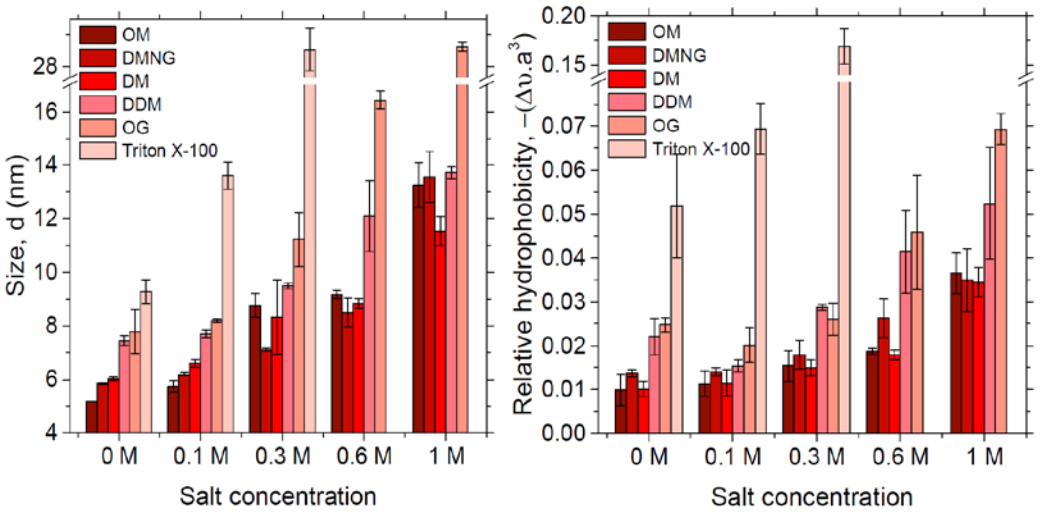

Figure 8. Through DLS and fluorescence assay, it was determined that the size and relative hydrophobicity of detergent micelles increase as salt concentration of buffer solution increases. This helps MP extraction because it protects MP more effectively from hydrophilic environment and offer larger volume that MP can access.

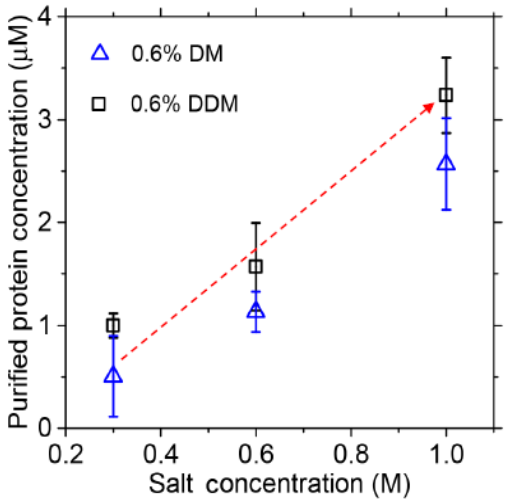

Figure 9. Both of detergents were more effective at protein extraction at higher salt concentration. Also, in consonance with the previous discussion, the detergent with longer alkyl chain (DDM) was yielded higher protein concentration.

Chapter 3

Improvement of membrane protein post-purification techniques

As mentioned before, the concentration of membrane proteins, which was extracted using detergents, is frequently lower than what is required for the biophysical characterization. This requires the next process, concentration of the membrane protein-detergent micelle complex (MPD) solutions to be implemented.

Ultrafiltration is commonly employed as the protein concentration technique among several different methods such as lyophilization, precipitation, dialysis against polymer solutions, and ion-exchange chromatography as they may lead to drying/chemical/thermal-induced degradation of proteins as well as contamination with excipients/polymers/salts.[15,16]

However, the concomitant increase in the concentration of free detergent monomers and empty micelles is another concern with ultrafiltration, as they may complicate the aforementioned assays and denature MPs. From previous work of the Kumar group, it was determined that increasing concentration polarization (CP) would be beneficial to concentrate MPs without retention of detergent.[17] Following the study, our group tried to design a new filter with different geometry promoting CP and minimizing the concentration of the empty detergent micelles and detergent monomers.

CP refers to the accumulation of retained solutes on the membrane surface, which can lead to greater protein and detergent passage, membrane fouling, and a reduction in the driving force for filtration. The commercial centrifugal filters have inclined vertical surfaces which generates natural convection induced recirculation which minimize CP and hence improves rejection of proteins and filtrate flux as shown in Figure 8.

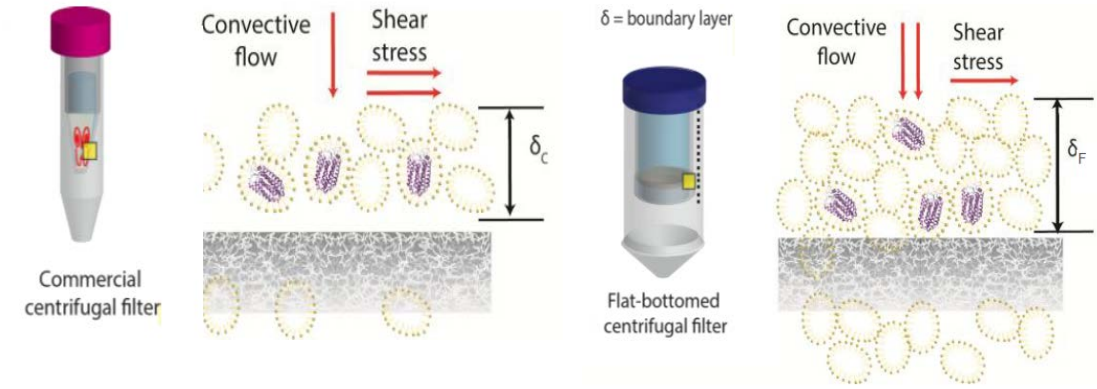

Figure 10. a) Schematics of commercial centrifugal filter with inclined vertical surfaces, generating backflow sending the empty detergent micelles and detergent monomers back to the body solution. (b) Schematics of newly designed flat-bottomed centrifugal filter. Due to the flat bottom, CP was increased leading more effective removal of empty detergent micelles. The boundary layer thickness, δ, of commercial centrifugal filter is lower than the one of flat-bottomed centrifugal filter indicating higher CP of flat-bottomed centrifugal filter.

A new filter was designed with a flat bottom as shown in Figure 8b. Lack of vortices led to greater CP and provided effective passage of empty detergent micelles and detergent unimers to pass through the membrane filter.

To prove this experimentally, different detergents with different alkyl chain length and head size, OM, DM, DDM, and OG were used. (Figure 9)

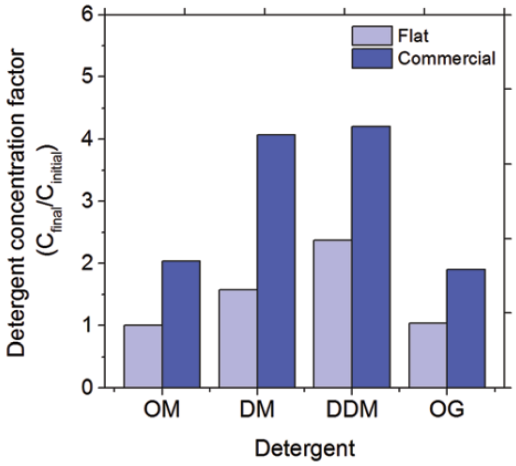

Figure 11. UF experiments on four detergents with varying length of alkyl chains and head groups were tried with both commercial and flat-bottomed filters.

For all four detergents, detergent concentration factors (C_{final} / C_{intial}) were higher when commercial centrifugal filter was used indicating natural convection-induced secondary vortices led to higher shear rates and lower CP, thus minimizing passage of empty detergent micelles across the membrane.[20] It was also shown that the detergent transmission decreases as the chain length increases possibly due to the larger micelle sizes as mentioned in previous chapter. The transmission of OG and OM was determined to be similar despite OM detergent micelle size being larger than OG. This is because the greater steric repulsion of OM causes to form spherical micelles with lower packing parameter while OG forms cylindrical micelles.

It was also determined that the transmission of detergent micelle can be affected by the salt concentration. We conducted experiments with 0.27% OG at different salt concentrations. At low salt concentrations, 0 and 0.1M, the sieving coefficient (S_o) was 1 for both the commercial and flat-bottomed filter, meaning all the detergent passed through the membrane. This was as expected because 0.27% is lower than the CMC of OG (0.7%) where it does not form micelles but stays as unimeric detergent molecules and the molecular weight of OG unimer (292.4 Da) is much below the molecular weight cut-off of the ultrafiltration cut-off (30 kDa). However, at higher salt concentrations of 1M signification OG retention in both commercial and flat-bottomed centrifugal filter was observed. This can be explained by invoking the salting out effect, which reduces the CMC of detergent.

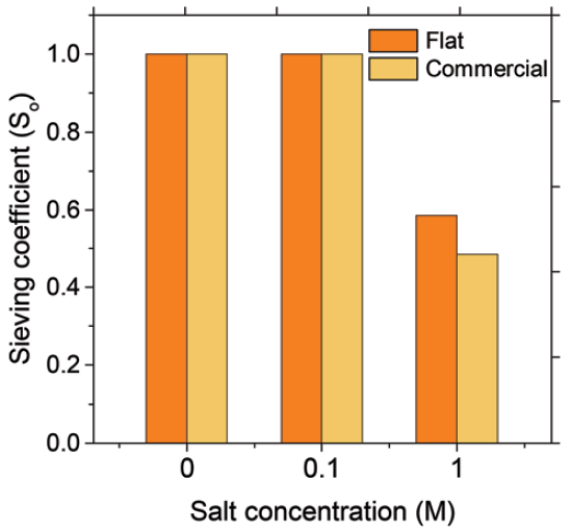

Figure 12. UF experiments were conducted with OG at different salt concentrations. Since the concentration of OG (0.27%) was below its CMC, OG did not form micelles, resulting in sieving coefficients of 1 at low salt concentrations. At high salt concentrations (1M), significant amount of detergent was retained due to salting out effect, lowering the CMC.

Chapter 4

Characterization of membrane proteins

After being isolated from the cell membrane and concentrated, MPs are generally characterized using several characterization methods such as vesicle-based reconstitution, structure determination via MS, NMR or X-ray crystallography.[18,19,20,21] For example, ion transport rate of light driven chloride pump, halorhodopsins from *Natromonas pharaonis* (NpHR) was recently measured by Kumar research group.[22]

The current conflicting data on ion transport rates of HR came from its slow transport rate compared to well-studied light-gated channels. The range of estimated chloride transport rates is very wide, from less than 1 ion/protein/s[23,24] to as high as 1245 ions/protein/s[25]. This discrepancy arises from (a) semiquantitive or indirect measurements, (b) challenges in estimating the directionality of proteins in *in vitro* systems, (c) differences in illumination conditions.

Kumar group tried a direct and quantitative measurement of *Np*HR by inserting them with an N-terminal Myc-tag in *Xenopus laevis* oocytes directionally, enabling *in vivo* ion transport rate measurement. The current induced in *Np*HR-expressing oocytes was determined using two electrode voltage clamp (TEVC) on oocytes during illumination by a 589 nm laser (Figure 13a) and the protein expressed per oocytes was measured by quantifying the genetically fused Myc-tag using Western blot assays (Figure 13b). In Western blot assays, a calibration curve of a range of known concentrations of denatured *Np*HR-antibody complex was used to determine the concentration of *Np*HR per oocyte. The curve was obtained from a calibration curve of the band intensity from a Western blot analysis on independently purified Myc-tagged *Np*HR proteins. Only concentrations for which the calibration curve was linear were used

to obtain the concentration of *Np*HR expressed in oocytes since the band intensity was observed to reach saturation at the higher concentrations of purified *Np*HR.

By normalizing the current at zero potential from voltage clamp experiment and corresponding *Np*HR from western blot assays, the average chloride transport rate of *Np*HR per protein was determined to be 219 (± 98) Cl^-/protein/s at a photon flux of 630 photons/protein/s or 0.35 (± 0.16) Cl^-/photon (Figure 13) [22].

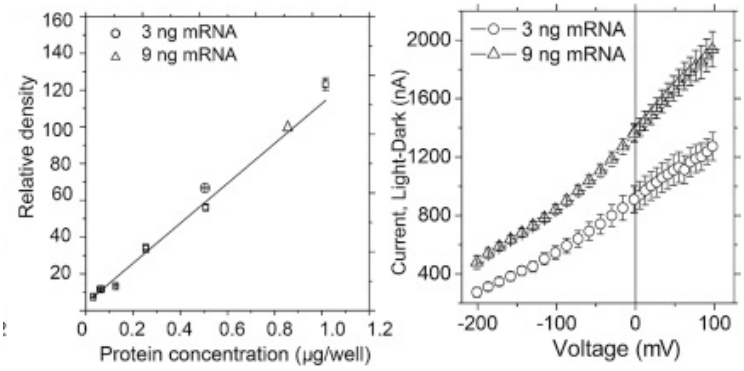

Figure 13. (a) The determination of Myc-tagged *Np*HR expression in oocyte membranes using Western blot analysis is shown.. Two different cRNA concentrations were used per batch of oocytes to induce different levels of *Np*HR expression. Each cRNA concentration was treated as a replicate. Two different batches of oocytes were thus tested, resulting in four replicates. (b) The ion transport in oocytes was quantified using two electrode voltage clamp (TEVC) experiment.

Chapter 5

Conclusions

In this thesis, a process for membrane protein extraction, post-purification, and characterization was discussed. This work was important to unlock the power of membrane proteins, which could be exploited in many areas such as desalination, optogenetics, and pharmaceutical research. First, the detergent with longer alkyl chain and smaller head group size was more effective at protein extraction compared to other detergents due to its higher hydrophobicity, larger accessible free volume, and larger packing parameter. In addition to that, we found that higher salt concentration of buffer solution helps protein extraction via salting out effect. Second, following to the previous work by Kumar group, flat-bottomed centrifugal filter was designed and used to demonstrate its capability in terms of removing empty detergent micelles and concentrating membrane protein-micelle (MPD) complex. Lastly, I talked about ion transport rate measurement of halorhodopsin expressed in oocytes as an example of characterization of membrane proteins.

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ACADEMIC VITA

Hyeonji Oh
614hyeonji@gmail.com

EDUCATION

Bachelor of Science **June 2014 – Present**

Department of Chemical Engineering, The Pennsylvania State University, University Park, PA
Schreyer Honors College, The Pennsylvania State University, University Park, PA
Expected graduation: December 2018

RESEARCH EXPERIENCE

Research Assistant - Dr. Manish Kumar's Biomimetic membrane Lab **May 2017 – Present**
Department of Chemical Engineering, The Pennsylvania State University, University Park, PA

- ***Fabrication of thin-film composite membranes***
  Fabricated thin-film composite membranes via interfacial polymerization and solvent-resistant support membranes via wet film casting / phase inversion

- ***Development of Detergent Scale for Enhanced Membrane Protein (MP) Purification***
  Generated scale of size and relative hydrophobicity of commonly used surfactants for protein extraction and purification using fluorescence assay

- ***Vesicle formation***
  Fabricated lipid/block copolymer vesicles incorporating the light driven protein, halorhodopsin (pHR), to measure ion transport rate using light-sensitive dye (pegylated MQAE) and port-a-patch setup

Research Assistant - Dr. Ralph Colby's Rheology Lab **December 2015 – April 2017**
Department of Material Science and Engineering, The Pennsylvania State University, University Park, PA

- ***Rheological study of cellulose and chitosan solutions***
  Studied rheological behavior of cellulose and chitosan in ionic liquids with addition of water using Discovery Hybrid Rheometer 3

PUBLICATIONS

1. Feroz, H., Kwon, H., Peng, J., **Oh, H.**, Ferlez, B., Baker, C. S., ... & Kumar, M. (2018). Improving extraction and post-purification concentration of membrane proteins, *Analyst*, 143(6), 1378-1386.
2. Song, W., Tu, Y., **Oh, H.**, Samineni, L., Kumar, M. (2018). Hierarchical optimization for high performance biomimetic membranes, *Langmuir* – submitted (10/31/2018)

3. Feroz, H., Ferlez, B., Ren, T., Baker, C.S., Brezovec, J., **Oh, H.,** ... & Kumar, M. (2018). Liposome-based measurement of light-driven chloride transport kinetics of halorhodopsin (co-author) – in preparation
4. **Oh, H.** (2019). Improving extraction, purification and evaluation of light-driven membrane proteins – writing (Honors thesis)

PROFESSIONAL EXPERIENCE

Manufacturing Technology Co-op **January 2018 – June 2018**
Bristol-Myers Squibb, Devens, MA

- Capability Analysis on Nova Bioprofile FLEX
- Lab-scale experiments to support upstream aspect of manufacturing process
- Historical Data Analysis using advanced statistical methods
- Lean Six Sigma Yellow Belt Certified

ADDITIONAL WORK EXPERIENCE

Proctor **August 2016 – May 2018**
Department of Chemistry, The Pennsylvania State University, University Park, PA
Department of Mathematics, The Pennsylvania State University, University Park, PA

Grader **August 2016 – present**
Department of Mathematics, The Pennsylvania State University, University Park, PA
Department of Chemical Engineering, The Pennsylvania State University, University Park, PA

Teaching and Administrative Assistant **November 2012 – May 2014, Summer 2015, 2016**
Miracle Language Institute - Korean English School, Daegu, Republic of Korea

- Lectured for students with different level of English proficiency, held office hours, graded homeworks and exams
- Managed financial operations and coordinated event schedules

PRESENTATIONS

- **Oh Hyeonji**, Enhancement of membrane proteins extraction and post-purification, AIChE Undergraduate Poster Competition , Pittsburgh, PA, October 2018
- **Oh Hyeonji**, Enhancement of membrane proteins extraction and post-purification, Future leaders in Chemical engineering symposium, North Carolina State University, October 2018
- **Oh Hyeonji**, Is Nova capable of measuring ammonium in media?, Bristol-Myers Squibb Co-Op poster session, Devens, MA, June 2018
- **Oh Hyeonji**, Kwon Hyeyoung, Detergent Selection Criteria for Enhanced Membrane Protein (MP) Purification, Summer Research Symposium, The Pennsylvania State University, August 2017
- Koscelansky Connor, **Oh Hyeonji**, James Robinson, Julie Vitola, Characterization of biodiesel fuel and the effects of anti-chelating additive in low temperature conditions, Chemistry Laboratories

Poster Symposium, The Pennsylvania State University, May 2017

- **Oh Hyeonji**, Solutions of ionic liquids and chitosan, Undergraduate Research Symposium, The Pennsylvania State University, April 2017

SKILLS

Technical Skills:

Bradford Protein Assay, Dynamic Light Scattering (DLS), Spectrophotometry, Fluorescence Assay, UV Spectroscopy, Gas Chromatography-Mass Spectroscopy (GC-MS), Nucelar magnetic resonance Spectroscopy (NMR), Infrared Spectroscopy (IR), Differential Scanning Calorimetry (DSC), Fluorescence spectroscopy, Laser photolysis, Luminescence spectroscopy, TFC (film casting) & solvent resistent membrane fabrication, Vesicle formation, BioProfile FLEX, Cedex Bio HT, Discovery Hybrid Rheometer 3

Software Skills:

Wolfram Mathematica, MATLAB, CAD SolidWorks, CAD SketchUp, Adobe Photoshop, Minitab, JMP, Discoverant

AWARDS

Fall 2018

- Larry Duda Research Award (LDUDA)
- Future Leaders in Chemical Engineering Award (A National Award Symposium for Undergraduates)

Summer 2017

- Chemical Engineering Research Experience for Undergraduate Students (REU) Biofellowship

Spring 2017

- College of Engineering Research Initiative (CERI) scholarship
- Special International Grant-in-Aid (SIGIA)

Fall 2016 - Spring 2018

- Leighton and Lorene Riess Scholarship in Chemical Engineering (RIESL)