UNIVERSITY OF CALIFORNIA,
IRVINE

Investigation of Nanopores Drop-Casted with Polymers of Intrinsic Microporosity as Ion Selective Systems
And
Curriculum Development for the General Chemistry Laboratory Series

DISSERTATION

Submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY
in Chemistry

by

Taylor Luanna Frey

Dissertation Committee:
Professor Zuzanna Siwy, Chair
Professor Ken Shea
Professor Joe Patterson

2022

DEDICATION

To my parents Joy and Randy Frey
I love you to the moon and back.

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ACKNOWLEDGEMENTS

I would like to begin by thanking my mentor and research advisor, Dr. Zuzanna Siwy, for her support of my research and goals. Without her guidance, this dissertation would not have been possible. Her mentorship and advice when I first joined the lab made it possible to quickly adapt to a new field. Her intelligence and kindness made each meeting a pleasure and a learning experience. She has encouraged my interests both in research and in career development. I have had the opportunity to explore mentorship, teaching opportunities, and technology transfer while working in her lab and I am eternally grateful for her support of these endeavors.

I would also like to thank my committee members, Dr. Ken Shea and Dr. Joe Patterson. Dr. Shea has been my collaborator for the last three years and in every meeting his passion and inquisitive nature have inspired me to become a better scientist. Dr. Patterson has given me advice and encouragement since I was a second year Ph.D. candidate.

I have had the opportunity to work with two fantastic educators, Dr. Kim Edwards and Dr. Renée Link. I became Dr. Edwards' head TA at the start of the pandemic and we, along with Dr. Link and a large instructional team, worked to create an online curriculum to meet the demands of a trying time. Dr. Edwards and Dr. Link have encouraged me throughout my graduate school journey, and I am incredibly thankful for their friendship.

My colleagues both in the Siwy lab and in the department, especially Dan Seith, Dr. Jake Polster, Wilfred Russel, Cody Combs, Dr. Rachel Lucas, Dr. Krista Fruehauf and Dr. Tyler Albin all have been wonderful peers and mentors and without whom I would not be the scientist I am today. Tyler was my mentor for the first two years of my graduate career and his advice and encouragement helped me grow into an independent researcher. When I switched into the Siwy lab, each member welcomed me and helped me integrate into a new setting and Krista gave me ample advice and encouragement while we collaborated on our PIM projects. I am incredibly thankful for their support and friendship.

I am especially grateful for the friendships I have made at UCI. Dr. Will Howitz, Alex Klodt, Dr. Bridgette Kohno, Dan Seith, Natalie Smith, Dr. Leanna Schulte, Collin Hickey, Gretchen Guaglianone, and Sarah Wang all have taken this journey together with me and we have been through thick and thin. We have laughed, cried, and grown together and I look forward to our continued friendship as we enter the next stage in our lives. I am also grateful for my four best friends that I have had since college, Allison Cornelius, Hanna Huss, Mariana Sainz, and Jaelynn Theobalds. These ladies have been my support system for over 7 years, and I am excited to see what our futures hold.

Finally, I want to thank my parents, Joy and Randy Frey, whose love has always been with me regardless time away or distance apart. Words cannot express how much they mean to me or how fantastic they have been as parents.

VITA

EDUCATION

University of California, Irvine
Ph.D., Organic Chemistry
Area of Research (Current) – Organic and Electrical Chemistry – Polymers of intrinsic microporosity casted on silicon wafers with a single drilled nanopore for ion and enantiomer selectivity in aqueous solutions
Area of Research (Previous) – Organic Chemistry – Directed design and analysis of dendronized polypeptides for the use as vaccine adjuvants against *Coxiella Burnetti* pathogen
Current GPA: 3.92

Case Western Reserve University, Cleveland, Ohio
Bachelor of Science, Chemical Engineering
Senior Collaboration (with Procter & Gamble) – Optimization of surfactant testing for use in electrospun fabric quality control
Area of Research – Organic Chemistry – Photoresponsive cobalt and silicon phthalocyanine dyes
Society of Women Engineers (SWE) * American Institute of Engineers
GPA: 3.56

EXPERIENCE

Sep 2017– Present
Graduate Student Researcher • UCI

- Designed ion-selective using thin polymer membranes drop casted onto silicon nitride wafers containing single nanopores. Conducted current-voltage measurements for ion-mobility studies.
    - Organic ion separation system for applications like water purification.
    - Chiral ion separation system for applications in pharmaceutical separations.
- Created single nanopore sensors for cancer biomolecules.
- Previously developed biopolymers as adjuvants for more potent vaccines in collaboration with DTRA.
- Confirmed biopolymer adjuvant activity *in vitro* with human monocytic cell lines and *ex vivo* mouse macrophages.
- Formulated vaccines with biopolymer adjuvants and tested formulations *in vivo* under IACUC and LAOHP regulations.

Jan 2021– Present
Technology Transfer Intern • UCI

- Worked closely with UCI's Research Translational Group to review technologies from principal investigators.
- Created non-confidential disclosure forms, patent landscapes, and potential licensee lists for promising technologies.

Mar 2020– Dec 2021
Head Teaching Assistant • UCI

- Worked closely with the general chemistry laboratory series instructor, led weekly meetings, and managed teams of up to 30 TAs per class, for 1 to 3 classes per academic quarter.
- Developed two new curriculums for the laboratory series to accommodate online learning during quarantine and argument driven inquiry for reopening.

Sep 2020– Mar 2021
Graduate Student Mentor • UCI

- Peer mentor to 3 incoming first year graduate students to help them transition to graduate school.

Oct 2017– Dec 2019
Graduate Student Mentor • UCI

- Taught lab sections for both the general and organic chemistry classes series.
- Guided up to 30 students per lab section in the fundamentals of their chemistry experiments, giving small lectures when necessary.

May 2014– Aug 2017
Summer Intern • Kadant Black Clawson • Mason • Ohio

- Analyzed data taken from inlet and outlet paper fiber streams and the quality of the finished paper product.
- Verified data at the International Paper mill and conducted tests on the mill's underflow solids. Created a model to simulate the mass and energy balance of a full paper recovery system and reviewed the mass and energy transport.
- Created process diagrams for reactors, created bills of materials for assembly guidelines, and reviewed parts list for quality control.

PUBLICATIONS

Electrochemical Probing of Steric, Electrostatic and Hydrophobic Interactions of Large Cations in Polymers of Intrinsic Microporosity • *Journal of The Electrochemical Society* • 2022

Online In No Time: Design And Implementation Of A Remote Learning First Quarter General Chemistry Laboratory And Second Quarter Organic Chemistry Laboratory • *Journal Of Chemical Education* • 2020

Polymer Composites with Photo-Responsive Phthalocyanine for Patterning in Color and Fluorescence • *European Polymer Journal* • 2017

AWARDS AND CERTIFICATES

Management Beyond the Classroom UCI Graduate Division - 2021
DTEI Graduate Fellowship DTEI at UCI – 2021
Extraordinary Graduate Departmental Service UCI Chemistry Department- 2021
Graduate Award for Lab Team Departmental Service UCI Chemistry Department - 2020

ABSTRACT OF THE DISSERTATION

Investigation of Nanopores Drop-Casted with Polymers of Intrinsic Microporosity as Ion Selective Systems
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Taylor Luanna Frey

Doctor of Philosophy in Chemistry

University of California, Irvine, 2022

Professor Zuzanna Siwy, Chair

This dissertation is a compilation of the work done during my graduate career and consists of two sections. The first section, comprised of the first three chapters, will discuss the research I conducted in the Siwy lab on selective polymer and nanopore systems. The second section is comprised of chapters four and five which detail the curriculum I have developed for chemical education in the general chemistry laboratory series.

Chapters 1 and 2 detail the use of polymers of intrinsic microporosity as porous, large-ion selective membranes. Porous membranes have been used for many applications, including separations in biotechnology, the food industry, water purification, and even energy storage devices. The benefit of polymers of intrinsic microporosity (PIMs) is their consistently sized nanopore channels. Inherent functionalities of the PIM structure not only create these channels but are also available for further modifications that can change the interactions of ions and molecules inside of the pore. Chapter 1 describes solid state nanopores on which are drop-casted two different PIMs, functionalized with either a cyano group or a carboxylic acid. Ionic transport through the membranes is investigated based on pore size and charge-charge interactions, as well as steric and hydrophobic interactions. Chapter 2 describes a chiral carboxylic acid PIM drop casted onto a solid

state nanopore and its interactions with chiral small molecules. The ionic transport of the small molecules is investigated based on chiral interactions and hydrogen bonding. Achieving specific ion selectivity with easily processable porous membranes opens new avenues for water purification strategies, energy storage, and pharmaceutical separations.

Chapter 3 describes a single nanopore that can be functionalized with a range of enzymes for biomolecule detection. Biosensors are extremely important in a wide variety of disease diagnostics. However, a separate sensor must be made specifically for the disease model it is detecting. Thus, it is desirable to produce a biosensor that can be adapted for many different diseases with simple modification steps. We report a single 20 nm nanopore functionalized with a biotin linker capable of binding to a range of enzymes that detect cancer molecules.

Chapters 4 and 5 are devoted to curriculum development for an online course in response to the pandemic and for returning to in-person instruction. The instruction of high enrollment general and organic chemistry laboratories at a large public university always have curricular, administrative, and logistical challenges. Chapter 4 discusses how the instructional teams met these challenges in the transition to remote teaching during the COVID-19 pandemic. Chapter 5 discusses how the general chemistry instructional team transitioned to argument-driven inquiry for reopening back to in-person instruction. Both chapters report the reasoning behind the approaches, the utilization of our existing web-based course content, the additions and alterations to our curriculum, replacement of original experimental work with videos or theme-based inquiries, the results of both student and TA surveys, and lessons learned for iterations of these courses in the near future.

Chapter 1: Electrochemical Probing of Steric, Electrostatic and Hydrophobic Interactions of Large Cations in Polymers of Intrinsic Microporosity

1.1 Preface

When I joined the Siwy lab, I took over a collaborative effort between Dr. Shea and Dr. Siwy which investigated the potential of polymer membranes on nanopores as ion selective systems. Dr. Krista Fruehauf in the Shea lab had synthesized two nanoporous polymers with different functionalities within the monomer unit which I later utilized for ion separation with large quaternary ammonium ions. Before I joined the Siwy lab, Dr. Jake Polster and Dr. Rachel Lucas had conducted initial studies on the membrane-nanopore system and had seen some interesting trends with larger cations. I continued the collaboration and expanded the library of ions to include larger inorganic cations and anions, as well as large quaternary ammonium cations that incorporated functionalities designed to interact with the steric, hydrophobic, and charged regions of the polymer. This chapter will outline the interactions between the polymer membrane and the expanded library of cations.

1.2 Introduction

Polymer membranes are applied in a wide variety of industrial processes such as water purifications, energy storage devices and biotechnology.[1–7] The application of polymer membranes in these industries is highly advantageous due to the high processability and low cost of polymer membranes in comparison to traditional methods of separations, particularly as pertains to ion separation and reverse osmosis.[7–12]. The potential for ionic and molecular separations with polymer membranes has led to a desire to better understand the interactions of transported species with the electrochemical properties of the pores.[1,13–15] Thus, polymer membranes are currently being used

in fundamental research to investigate the interactions between ions and molecules with surfaces at nano-confined regions with the aim to ultimately design efficient separation systems.[16–18] There is a growing interest in designing membrane systems that would exhibit excellent ion-ion selectivity, including selectivity between ions of the same charge.[19]

Polymers of intrinsic microporosity (PIMs) are a versatile class of porous organic polymers that show promise for ion-ion specificity.[20–23] Their polymer backbone prevents close packing of polymer chains in the solid state, thereby leading to a uniform pore structure (3 to 4 nm) and high surface area.[24] The effective pore opening of PIMs and surface properties of these pores can be further tuned via chemical modification, cross-linking, and tuning chemistry of PIMs monomers.[24–29] Consequently, PIMs have been employed in a wide variety of areas, including gas separations and storage, catalysis, small molecule capture, chiral separations, and sensors.[21,24,30–32] PIMs are also used in a wide range of electrochemical applications, such as ion selective membranes prepared for ionic diodes and membranes in aqueous organic redox flow batteries.[17,33–35]

Taking advantage of the well-defined diameter of nanopores in PIMs, we used the membranes as a model system to understand how interactions of the transported ions with the pore walls can affect ionic selectivity. We focused on ionic transport under an electric field, rather than pressure difference driven transport typically employed in nanofiltration applications.[36] The electrochemical measurements allowed us to observe current carried by all ions and compare the interactions of various ions with the pore walls. In the experiments, we used two types of PIM polymers, PIM CN containing cyano groups and PIM COOH containing carboxyl groups.

To probe ionic transport through PIM CN and PIM COOH, the membranes were individually drop-casted onto silicon nitride films, each containing a single ~300 nm diameter pore[37,38] The solid-state support was crucial for PIM COOH, which is less mechanically stable

than PIM CN, allowing for both membranes to be examined at the same thickness and under the same conditions. Additionally, the presence of the single SiN pore restricted the measured transport to a small area of the membrane so the voltage applied across the membrane was uniformly focused across the membrane in all measurements. The hybrid PIM/SiN structure became the model system to investigate ion selectivity from both nanoconfinement induced in PIM CN and by charge dependent ionic conductance in carboxylated PIM COOH. We performed electrochemical measurements of ionic conductance of both PIM polymers in salts of varying sizes and chemical characteristics that arise from the different cations and anions. Inorganic salts as well as quaternary ammonium salt derivatives, the cations of which have the same charge but different functional groups, were our primary focus for these studies. The quaternary ammonium salts were chosen because they are easily accessible as chloride salts and their center nitrogens can be functionalized with hydrocarbon groups to produce a wide range of sizes, charge distributions, and hydrophobic effects. These functionalities allow us to easily probe the steric, electrostatic, and hydrophobic interactions of the cations with the membrane's pore walls. Additionally, the largest quaternary ammonium salts contain hydrocarbon chains and benzene rings, which we hypothesized might promote accumulations of the cations in the hydrophobic regions of PIMs, affecting the transport.[39] Finally, the positively charged quaternary ammonium center allowed us to probe specific interactions of these ions with the negatively charged carboxylic acid of PIM COOH.

1.3 Experimental

1.3.1 Reagents

Trizma base (99.9%), ammonium chloride, tetraethyl ammonium chloride, tetrabutylammonium chloride, trimethyl benzyl ammonium chloride, and triethyl

benzylammonium chloride were purchased from Sigma-Aldrich. Potassium chloride, potassium iodide, potassium bromide, sodium chloride, and lithium chloride were purchased from Fisher Scientific.

Ammonium chloride, tetraethyl ammonium chloride (TEACl), tetrabutylammonium chloride (TBACl), trimethyl benzyl ammonium chloride (BzMeACl), and triethyl benzylammonium chloride (BzEtACl) are hereon referred to as the quaternary ammonium salts. Potassium chloride, potassium iodide, potassium bromide, sodium chloride, and lithium chloride are hereon referred to as the inorganic salts. For structures of the quaternary ammonium salts, see Figure 1.1.

3,3,3',3'-Tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol (TTSBI) (Sigma), 2,3,5,6-tetrafluorphthalonitrile (TFPT) (Sigma), potassium carbonate (K_2CO_3) (Sigma), dry dimethyl formamide (DMF), methanol, sulfuric acid, glacial acetic acid, and sodium bicarbonate ($NaHCO_3$) were all used as received.

All reagents were used without further purification.

1.3.2 Preparation of Ionic Solutions

All solutions were prepared in nanopure deionized water (18.2 MΩ·cm, Milli-Q IQ 7000 ultrapure water system). All 1M inorganic stock solutions and 100mM quaternary ammonium salt stock solutions created contained 10mM Tris-buffer and were diluted to create all other solutions. Solutions were buffered to pH 8 with either sodium hydroxide or hydrochloric acid. Each salt solution was prepared for 100mM, 10mM and 1mM concentrations. Inorganic salt solutions were also prepared at 1M.

1.3.3 Synthesis and Characterization of Polymers of Intrinsic Microporosity

1.2.3.1 PIM CN Synthesis

3,3,3',3'-Tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol (TTSBI) (270 mg, 0.871 mmol) was dissolved in dry DMF (5 mL) followed by addition of 2,3,5,6-tetrafluorphthalonitrile (TFPT) (174 mg, 0.872 mmol). Once the mixture was homogenous, excess anhydrous K_2CO_3 (1 g, 7.2 mmol) was added and the heterogenous mixture was heated to 70 °C for 72 h. After about 30 min the reaction mixture turned from brown to bright yellow. Once cooled, the mixture was precipitated into water (50 mL), filtered, rinsed with water, and dried in a vacuum oven o/n at 70 °C. The crude powder was then suspended in methanol (10 mL) and heated to reflux for 3 h. The mixture was filtered hot and rinsed with hot methanol before drying in a vacuum oven o/n at 70 °C. The product was a fine, fluorescent yellow powder (86% yield).[24] (Appendix Figure 1.6)

1.2.3.2 PIM COOH Synthesis

The acid catalyzed hydrolysis experimental methods were the same as used in our previous work.[24] PIM 1 (200 mg) was treated with a solution of H_2SO_4 (3 mL), H_2O (3 mL), and glacial acetic acid (1 mL). The suspension was heated to 110 °C for 48 h. The powder slowly turned from fluorescent yellow into a light brown color. Once cooled, the mixture was diluted into water (50 mL), filtered, rinsed with saturated aqueous $NaHCO_3$, rinsed with copious amounts of water, then dried in a vacuum oven at 70 °C o/n. The product appeared as a light brown powder (86 % yield). FTIR showed full conversion. Incomplete conversion was apparent when temperatures were at 105 °C.[24] (Appendix Figure 1.6)

1.3.4 Fabrication and Characterization of Single Nanopores in Silicon Nitride Chips

In this study, we used single pores prepared in low-stress silicon nitride (SiN_x) films (50μm by 50μm; 30nm thick) purchased from Norcada. Two classes of single pores were fabricated.

Large single pores between 300 and 600 nm in diameter were prepared using Focused Ion Beam (FIB) drilling on a FEI Helios Nanolab 600i FIB/SEM DualBeam System at 30 kV.[37,38] Four independently prepared single pores were used in the project as mechanical supports for the PIMs and were labeled as 9-12. We also used a 5nm in diameter single pore that was drilled by a 200 kV electron beam in the JEOL 2100F TEM.[40] After drilling, the pore was washed in a 1:3 H_2O_2:H_2SO_4 (piranha) solution at 100 °C for 30 minutes and rinsed with nanopure water.

Diameters of all the SiN pores were estimated from the resistance of the pore found by generating current current-voltage (IV) curves in 1M KCl solutions between -1 and 1 V. The pore diameter (D) is estimated using the following equation:

$$D = \frac{G}{2\sigma}\left[1 + \sqrt{\left(1 + \frac{16\sigma L}{\pi G}\right)}\right]$$

where G is the slope (conductance) of the IV curve, σ is the solution conductivity, and L is the pore length.[41] The shape of the pore was assumed to be cylindrical thus all diameters reported are called here effective diameters.

All IV curves in this study were measured with a Keithley 6487 picoammeter/voltage source (Keithley Instruments, Solon, Ohio, USA) and a software program written in MATLAB (MathWorks, Natick, Massachusetts, USA) using a voltage range between -1 and 1 V with 0.1 V voltage steps. Two non-polarizable Ag/AgCl electrodes were used for all current measurements.

1.3.5 Fabrication and Characterization of Single Conically Shaped Nanopores in a Polymer Film

A conically shaped single pore in a polyethylene terephthalate (PET) film was created by the track-etch method previously reported.[42,43] Briefly, the polymer film (3 cm diameter; 12 μm

thick) was exposed to irradiation with a single, energetic, heavy ion that leaves a single latent damage track through the membrane. The irradiation step was performed at the Universal Linear Accelerator (UNILAC) at the GSI Helmholtz Centre for Heavy Ion Research, Darmstadt, Germany. The latent track was subsequently developed into a single nanopore by wet chemical etching. The conical shape of the pore was obtained through asymmetric chemical etching, such that one side of the irradiated film was in contact with 9 M NaOH while the other side was in contact with an acidic medium.[44] The etching process was monitored electrochemically. Once the latent track was completely etched through, a finite current was recorded. The membrane was then washed in nanopure water and tested in 1 M KCl by recording an IV curve. The membrane resistance was then used to estimate the pore opening diameter.

1.3.6 Preparation and Characterization of Drop-Casted PIMs on Solid State Nanopores

Powdered forms of both polymers were used for drop-casting onto the silicon nitride chips with 300 – 600 nm in diameter silicon nitride pores. Thick drop-casts of both polymers were performed by first dissolving 1 mg of polymer into 1 mL of tetrahydrofuran (THF). A 1 mL syringe with a 27-gauge needle was used to drop-cast 1-2 drops of solution onto the silicon nitride samples. Samples 9 and 10 received PIM CN and samples 11 and 12 received the PIM COOH. All samples were allowed to dry overnight and then washed with nanopure deionized water before further experiments. The thickness of these membranes, ~25 μm, was determined by scanning electron microscopy.

After drop-casting, all PIM/SiN samples were tested in each salt by recording current-voltage curves in the voltage range of -1V to +1V with 100 mV steps. Samples 9 and 12 were tested from low to high salt concentrations starting at 1mM of every salt, and samples 10 and 11 were tested from high to low salt concentrations starting from 1M. The order of experiments for

every sample is as follows: KCl, KBr, KI, NaCl, LiCl, NH4Cl, TEACl, TBACl, BzMeACl, and BzEtACl.

1.4 Results and Discussion

1.4.1 Characterization of Ionic Transport in PIMs

PIM CN and PIM COOH were chosen as model systems to probe the steric and electrostatic interactions of transported ions within the membrane's pores (Figure 1.1).[1,45,46] We predicted that because PIM CN lacks charged functional groups, it would highlight any steric selectivity towards a specific ion based solely on the size of PIM's pores. Conversely, PIM COOH would pinpoint any electrostatic effects on ionic transport that result from the negatively charged carboxylic acid groups. We also hypothesized the aromatic and aliphatic functional groups on the polymer backbone present in both polymers could affect ionic transport through hydrophobic interactions. Four different sized quaternary ammonium salt cations were chosen to probe the transport of large ions through the confined pores of PIM CN as well as electrostatic interactions between the quaternary ammonium and the carboxylic acid of PIM COOH. The quaternary ammonium salts act as a good model system to understand ionic transport in nanoconfinement as they can contain cations with a wide variety of different sizes and chemical characteristics. The largest cations we chose, TBA and BzEt, contain hydrophobic parts that we hypothesized might facilitate stacking on the PIMs hydrophobic backbone.[39] As ionic current is known to be sensitive to the interactions of ions with pore walls, the signal of ion current recorded when the membranes are in contact with the salts will indicate to which extent the transport of quaternary ammonium cations is hindered in the porous structure of PIMs.[47–51]

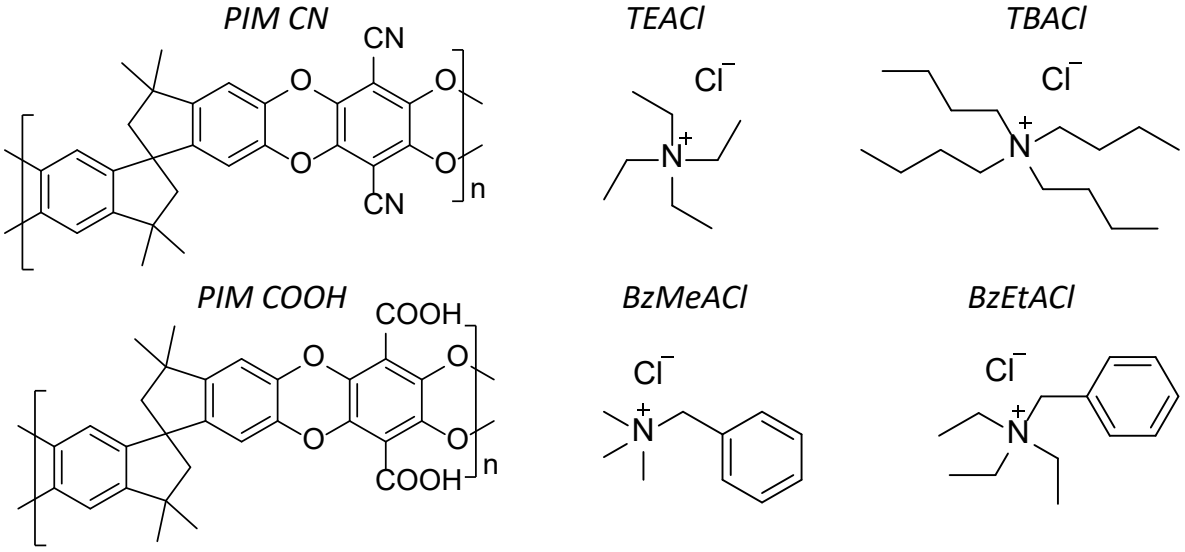

Figure 1.1. a) Structure of PIM CN and PIM COOH. b) Structures of the quaternary ammonium salt derivatives.

To determine the interactions of the membranes with the salts, we first prepared ~100 μm thick drop-castings of both PIM membranes without mechanical support. PIM CN was placed between two chambers of a conductivity cells that exposed ~1 cm^2 of the membrane's surface to the solution. PIM COOH was examined in a PDMS cell, as it was significantly more brittle, where a smaller area of 1 mm^2 was in contact with the solutions. Ionic transport properties of both PIMs were probed with a series of inorganic (KCl, NaCl, LiCl, KBr and KI) and quaternary ammonium salt solutions (Figure 1.1), all buffered to pH 8, to highlight the effects of size, hydrophobicity, and charge on the ionic conductance of the membrane. For each solution and concentration, both chambers of the conductivity cell were filled with a single electrolyte solution and current-voltage (IV) curves were recorded. Measurements for PIM COOH were challenging due to the membrane's mechanical instability as it was especially brittle. Even microcracks in the membrane would significantly increase the conductance, and mask ion transport properties that originate from the nanoporous structure of PIMs. Additionally, both membranes, PIM CN and PIM COOH, are

characterized by low ionic resistance, such that a voltage applied across the system would also drop in the bulk solution, making comparisons between data sets quantitatively difficult. In spite of the difficulties, the results revealed that the transport properties of the PIM COOH were altered when in contact with the 1mM quaternary ammonium salt solutions (Appendix Figure 1.6). Current measurements for each salt were compared with the measurements in KCl, which acts as a reference electrolyte due to the equal mobility of potassium and chloride ions.[52]

1.4.2 PIM Membranes Casted on Silicon Nitride Chips Containing a Few Hundreds nm in Diameter Single Pore

To further explore the effect of the salts on the ionic conductance of the PIMs, the PIMs were drop-casted onto four silicon nitride chips containing ~300 – 600 nm in diameter single pores. Before drop-casting, all as-prepared SiN samples were tested with the inorganic and quaternary ammonium salt solutions to confirm that SiN pores of such size do not exhibit selectivity towards any of the salts (Appendix Figure 1.8). After drop-casting, the chips provided both mechanical support for PIM COOH and an increased resistance that limits the ionic transport of the ions to only a small portion of the membrane. The increased resistance is essential for well-defined electrochemical measurements as the voltage applied drops entirely across the SiN/PIM system, allowing the membrane and pore's conductance to be calculated. Figure 2c shows current-voltage curves that were recorded in 1 M KCl through the silicon nitride nanopore before and after casting the PIM COOH membrane. As expected, the addition of the PIM membrane increases the system's resistance due to the micrometer scale of the PIM thickness (Figure 1.2, Appendix Figure 1.6). The resistance increase, however, was modest, confirming the high porosity and low ionic resistance of the PIMs.

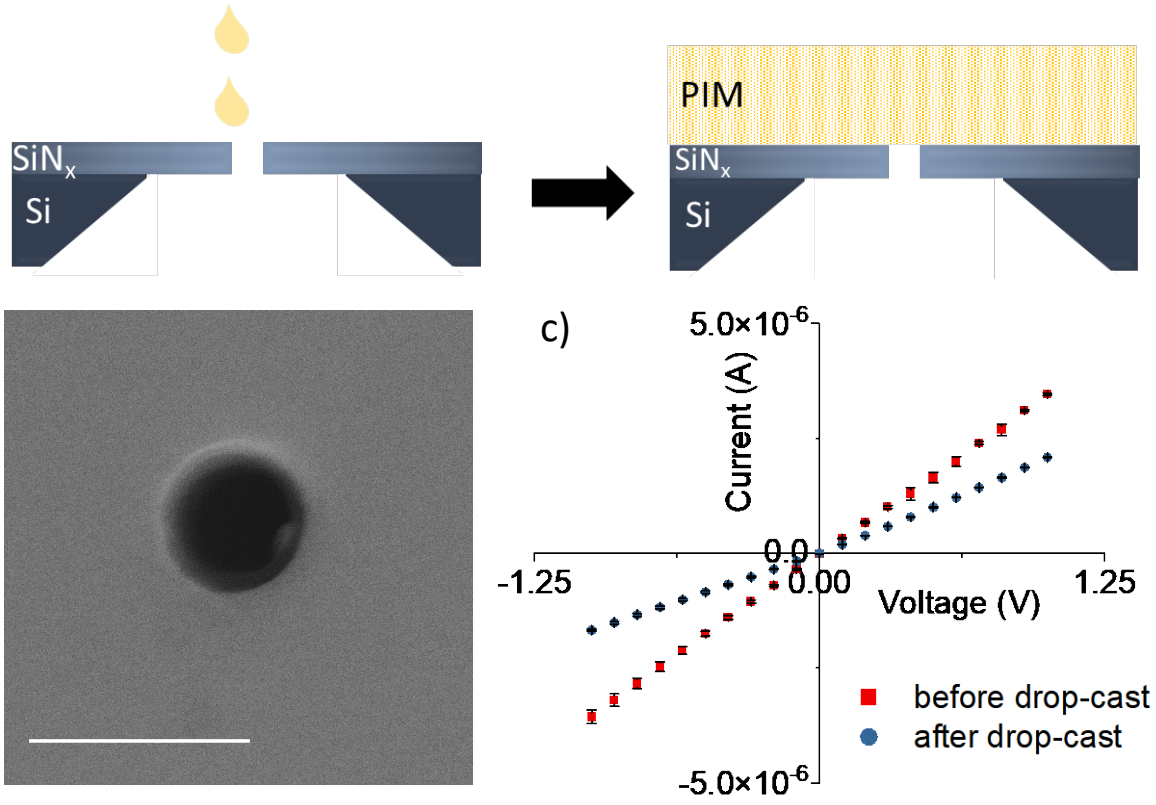

Figure 1.2. a) Scheme for drop-casting PIMs onto a silicon nitride chip with a single pore. b) SEM picture of a ~600 nm SiN pore created by FIB drilling. c) IV curves depicting a decrease in current after PIM COOH is drop-casted onto a single ~600 nm in diameter SiN pore. Both IV curves before and after drop-casting were recorded in 1 M KCl.

1.4.3 Carboxylic Acid Functionality Leads to Hindered Transport of Quaternary Ammonium Derivatives

Both the PIM COOH/SiN and PIM CN/SiN systems were tested with the series of inorganic and quaternary ammonium salt solutions to investigate the PIMs' potential steric and electrostatic effects on ion mobility. Electrochemical characterizations of the SiN/PIMs systems were performed in a PDMS conductivity cell designed and fabricated in-house to accommodate the small size of the silicon nitride chip and facilitate the exchange of a solution between two reservoirs without damaging the chip. Current-voltage curves were recorded for all salts with a

concentration range between 1 mM and 1 M for inorganic salts, and 100mM for the quaternary ammonium salts. We focus here on recordings in 10mM and 100mM.

As the inorganic and quaternary ammonium salts contain ions of different sizes and mobilities, the current recorded is affected by the bulk conductivity of the solutions as well as possible interactions of cations, anions, or both with the porous structure of PIMs. Thus, each solution was first characterized by its bulk conductivity using a conductometer at room temperature and conductivity ratios were then created by dividing the conductivity of a KCl solution of a given concentration and conductivity of another salt solution at the same concentration. Conductivity ratios were found for all salts and concentrations probed. To account for any effects from bulk conductivity on the current measurements, current ratios of each salt were created by dividing the current in a KCl solution by the value of the current in a given salt of the same concentration and at the same voltage.[53–55] If a membrane conducts KCl and another salt to the same extent, the current ratios of these two salts would be equal to their conductivity ratio. Conversely, if the transport of the other salt is either hindered or enhanced due to interactions with the pore's walls, the current ratio will differ from the conductivity ratio. Current ratios higher than conductivity ratios indicate hindered transport whereas current ratios lower than conductivity ratios indicate enhanced transport.

The ion current ratios of KCl and all other salts were analyzed at +0.3V and -0.3V versus the maximum 1 V, because some IV curves of the PIMs in KCl exhibited non-linear behavior at higher voltages due to concentration polarization.[56] Concentration polarization leads to the formation of a zone with depleted ionic concentrations on one membrane surface that limits the recorded current. Thus, to compare conductance of the membranes at different salts, we used the low-voltage part of IV curves. Figure 1.3 a,b shows ratios of ionic currents in KCl and inorganic

salts through PIM CN (Figure 1.3a) and PIM COOH (Figure 1.3b) membranes casted on two separate silicon nitride chips containing single pores. The ratios of currents nearly in all cases were equal to the ratios of bulk conductivities, suggesting the membranes did not exhibit any preference towards any of the ions. We did, however, observe slightly lower conductances of both membranes in KCl compared to KI and KBr. We believe this effect originated from the hydrophobic components of the PIM membranes that could cause an accumulation of large polarizable anions such as Br^- and I^-, shown before for hydrophobic surfaces.[57,58] The only notable discrepancy was observed with the PIM CN membrane in LiCl whose currents were significantly lower than could be predicted based on bulk conductivity. This observation could be caused by a diminished wettability of PIM CN in LiCl, due to the strong hydration shell of both lithium and chloride ions.[59–61] Small ions with a strong hydration shell do not partition easily in a hydrophobic region. PIM COOH is significantly more hydrophilic due to the presence of charged groups, and the ratio of currents in KCl and LiCl was equal to the ratio of bulk conductivities of the two salts in this membrane.

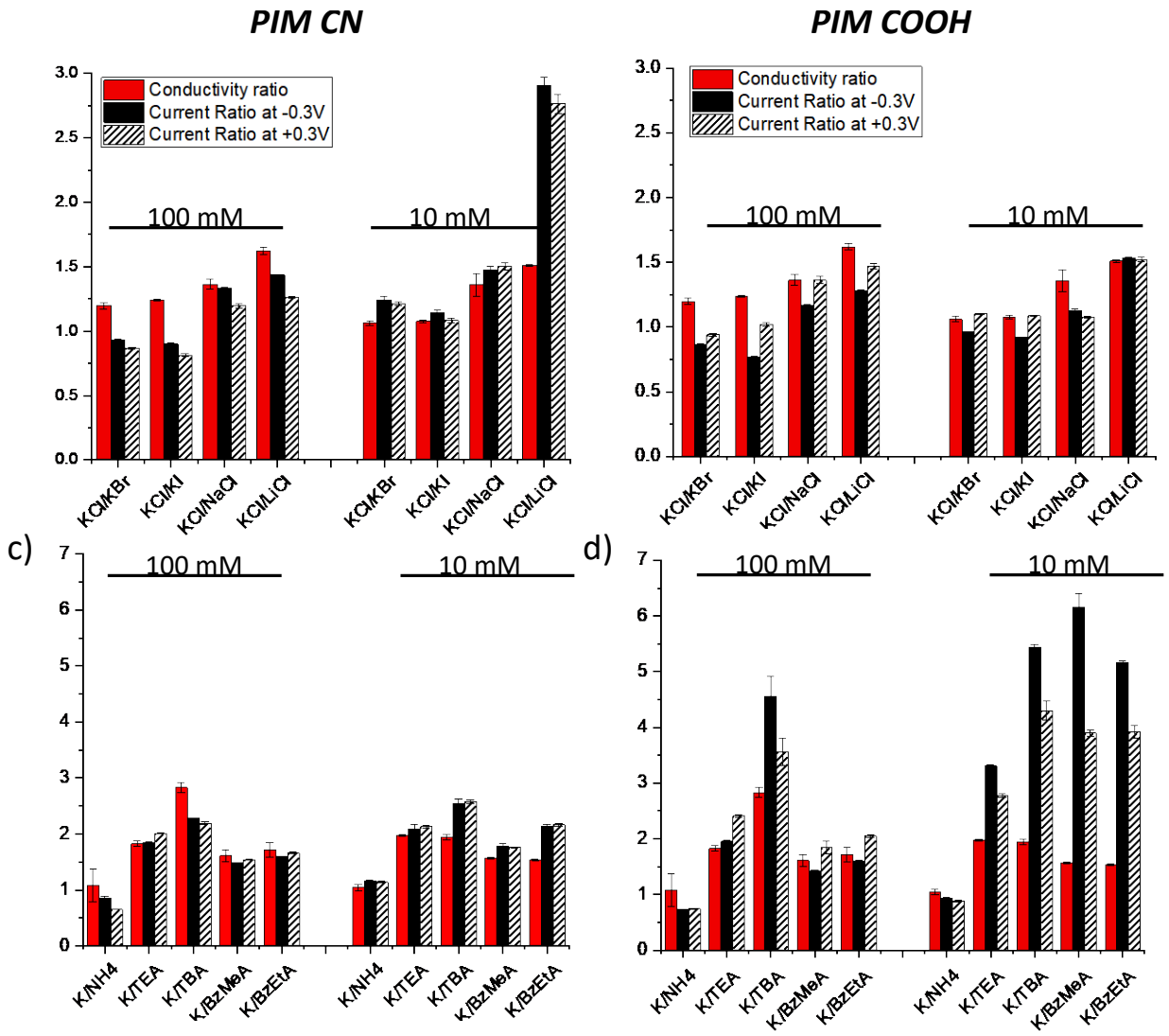

Figure 1.3. a) Ion current ratios of the inorganic salts tested on PIM CN drop casted on a silicon nitride chip with a single pore (sample 10). The current ratios were calculated by dividing current measured in KCl by a current recorded in another salt at the same concentration. b) Current ratios of the inorganic salts tested on sample 12 drop-casted with PIM COOH. c) Current ratios of the quaternary ammonium salts tested on sample 10 drop-casted with PIM CN. Ratios for 10mM TBACl and BzEtACl show an effect of nanoconfinement on their transport within the membrane's pores. d) Current ratios for the quaternary ammonium salts tested on sample 12 drop-casted with PIM COOH. Ratios for all 10 mM quaternary ammonium salts indicate a strong reduction in their transport through the PIM COOH compared to KCl. Red bars in all panels indicate ratios of bulk conductivities of KCl solutions and bulk conductivities of remaining salts at the same concentrations.

The ion-ion selectivity was, however, very evident when the PIMs were probed in quaternary ammonium salts. Figure 1.3 c,d summarizes ionic current ratios of recordings in KCl and in the series of quaternary ammonium ion salts. Both PIMs exhibited diminished transport in these salts, however the effect in PIM COOH was much stronger than the effect in PIM CN. In the case of PIM COOH, ion currents measured in all quaternary ammonium salts at 10 mM were significantly lower than could be predicted based on their bulk conductivities. In 100 mM, the effect of TBACl was especially dominant (Figure 1.3d). Data collected for concentrations of 10 mM and 100 mM of all salts allowed us to probe the effects of the ionic concentrations of the bulk electrolyte on the relative conductances of the membrane. The effects of the salts on ionic transport were stronger in 10 mM versus 100 mM, suggesting that the transport modulations occur due to interaction of cations and not anions within the porous structure of the membrane. In the lower concentration, the negatively charged PIM's pores are predicted to be predominantly filled with cations, which dominate the measured current.[62] Note that the membranes were thoroughly washed with deionized water at least three times when changing a solution to a new salt or concentration. The strong decrease of the current in the quaternary ammonium salts (Figure 1.3d) suggests a possible formation of physical blockages that limit the ionic transport. The current in 10 mM TBACl is ~5 times smaller than in 10 mM KCl, a current decrease which could not be justified by mere cation size. Appendix Figure 1.11 shows current-voltage curves through a PIM COOH membrane in TBACl of different concentrations, and highlights that in the presence of this salt, ionic transport through the membrane can be decreased nearly to zero. We also noticed that after the measurements in TBACl, changing the solution to another salt does not immediately restore the membrane conductance. The membrane's original conductance in KCl was recovered only after repeated rinsing in nanopure water. These observations suggest that the confinement in PIMs

together with carboxyl groups could create conditions that facilitated a strong adsorption of the ammonium salts, formation of ion pairs, and even formation of aggregates or precipitates, reported previously for carboxylated surfaces in contact with ammonium salts.[63–66] Aggregation of quaternary ammonium salts on the surface can be enhanced by their surfactant nature as well as the salt's solubility, hydrophobicity, and the higher local concentration of cations around the negatively charged PIM COOH.[67] The presence of blockages is also suggested by the asymmetry of the positive and negative ion current ratios (Figure 1.3d). The asymmetry would stem from a non-uniform distribution of these blockages along the membrane thickness. The distribution of aggregates/precipitates will be different in different membranes, causing the level of blockage and the asymmetry to vary between membranes.

To test the hypothesis of possible accumulation that could lead to precipitation of the ammonium salts in PIM COOH, we performed X-ray photoelectron spectroscopy (XPS) measurements (Figure 1.4). Four PIM COOH membranes were first exposed to 100mM solutions of one of the four quaternary ammonium salts for an hour, then rinsed well with nanopure deionized water and allowed to dry completely before analysis with XPS (Appendix Figure 1.13). A control membrane that was exposed only to nanopure water was analyzed as well (red line in Figure 1.4 and Appendix Figure 1.13d). In both the control membrane and the TBACl exposed membrane, unhydrolyzed cyano groups are present in the XPS traces at low concentrations (see the red line in Figure 1.4).[68] However, the TBACl exposed membrane also shows an additional peak indicating the presence of a quaternary nitrogen species (blue line in Figure 1.4), confirming the formation of an aggregate or precipitate in the presence of carboxylic acids.[69] This trend is also seen for all other samples exposed to the quaternary ammonium salts to various degrees (Appendix

Figure 1.13). We noticed that the relative intensity of the peaks corresponding to the precipitates correlates well with the extent of ionic current hindrance a given salt induced.

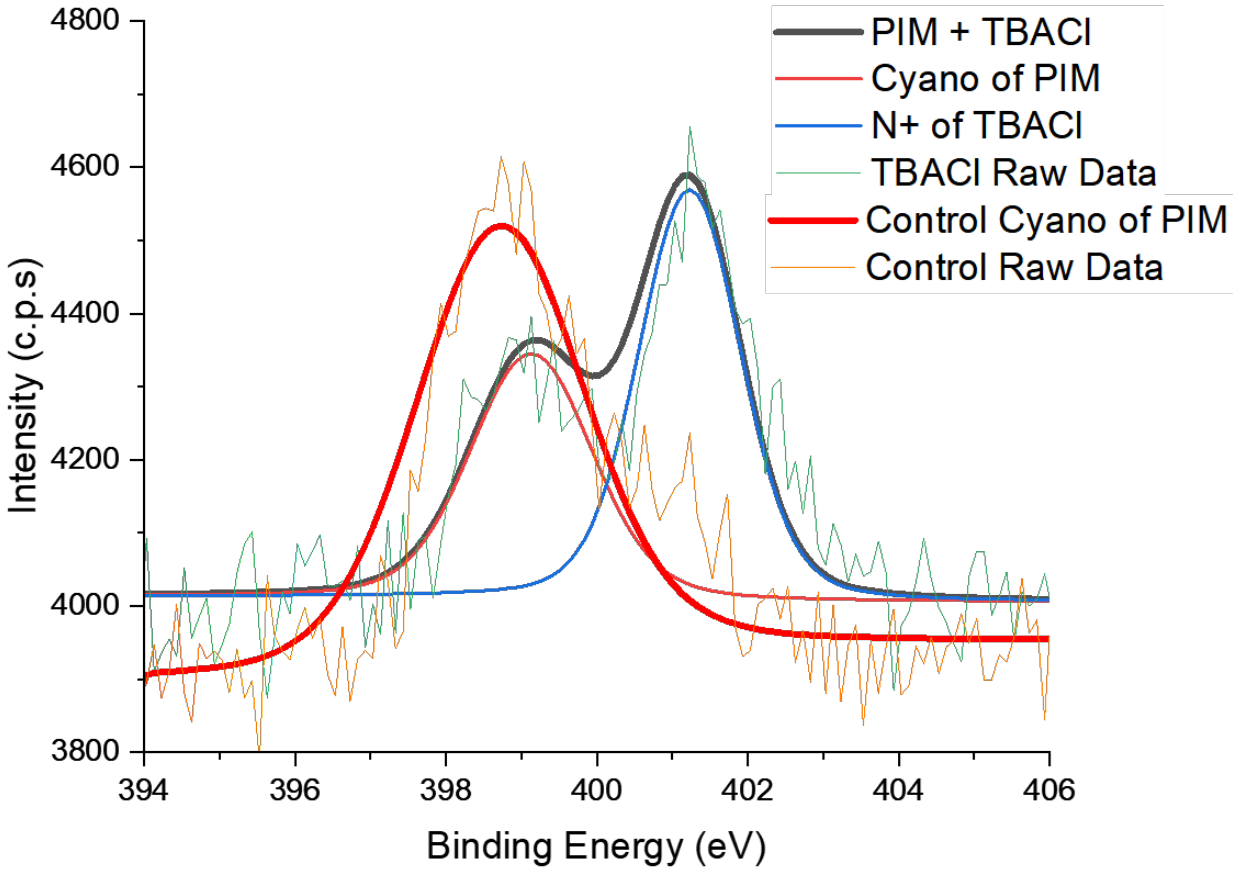

Figure 1.4. XPS measurements of a control PIM COOH membrane exposed only to nanopure water (red line) and of a PIM COOH membrane exposed to a 100mM solution of TBACl (black line). Binding energies of the control membrane only show residual unhydrolyzed cyano groups inherent to the membrane. The binding energies of the second membrane exposed to TBACl shows both the unhydrolyzed cyano groups (red peak) as well as the quaternary nitrogen peak from TBACl aggregates/precipitates around the carboxylic acid groups (blue peak). Both membranes were casted and analyzed without a SiN support.

Note that the effects of quaternary ammonium on PIM COOH are more pronounced when the membrane is supported on the SiN chip versus a free-standing membrane shown in Appendix Figure 1.7. This could be due to tiny microcracks in the free-standing PIM COOH which would dominate the transport and diminish the effects of the nanoporous structure on the measured signal. The results suggest that the application of PIM COOH in separations will require porous supports.

1.4.4 Nanoconfinement from PIM Pores May Contribute to Hindered Transport of Quaternary Ammonium Derivatives

The analysis of ion currents in the PIM CN membrane (Figure 1.3c) suggests that this membrane can also modulate ionic transport of quaternary ammonium salts. Transport of larger cations, such as TBA and BzEtA, in low concentrations seems to be hindered by PIM CN, but to a much smaller degree (~25% in 10 mM TBACl) than seen with PIM COOH (Figure 1.3d). We hypothesized that the conductance decrease in the PIM CN membrane could be caused by the size of the cations, which are significantly larger than potassium ions, as well as by hydrophobic interactions with the pore walls. Lack of carboxyl groups in PIM CN is expected to eliminate blockage of the pores from aggregates as seen with PIM COOH. It is interesting to note that the effects in PIM CN are more pronounced in 10 mM versus 100 mM solutions even though the membrane does not carry any charged groups. We believe the influence of salt concentration on ionic transport in this case stems from the high (~4) dipole moment of the cyano groups which can arrange with the nitrogen atom having a transient negative charge facing the pores. This transient negative charge can render the pores weakly cation selective, so that at lower concentrations, they are mostly filled with cations that undergo steric and weakly electrostatic hindrance.

To separate the steric, hydrophobic, and electrostatic effects that the membrane exerts on cations from the possible aggregation/precipitation that obstructs the pores, we performed

additional experiments with single nanopores in both a polyethylene terephthalate (PET) film and silicon nitride. PET nanopores contain a high density of carboxyl groups on the pore walls whereas SiN nanopores are negatively charged due to the presence of silanol groups.[50,70] Due to lack of hydrocarbon or carboxyl groups in silicon nitride nanopores, this system allows us to understand the role of steric and electrostatic effects only for the cation transport. Comparing the experimental results in PIM CN and the SiN nanopore will allow us to establish which effect dominates the transport.

1.4.5 Single Pore systems show a Hindered Transport of Quaternary Salts

To validate our hypothesis that carboxylic acid functional groups lead to accumulation and possible precipitation of the quaternary ammonium salts, we created a small, 10 nm in diameter, conically shaped, single pore in PET using the track-etching technique.[49,50] The conical geometry, where one opening is on the nanoscale while the other opening is few hundreds of nanometers in diameter, allows us to record measurable currents even though the PET films are 12 μm thick. Etching in sodium hydroxide hydrolyzes the outer layer of esters on PET to carboxylic acids, creating a significantly higher ratio of carboxylic acids per repeat unit than for the PIM COOH.[70] If ionic transport is hindered from the specific interactions of carboxyl groups and the quaternary ammonium cations, recordings for the PET pore were expected to mirror our observations with PIM COOH membranes. Results from the PET pore were then compared with observations in a 5 nm pore prepared in silicon nitride, where carboxyl groups are not present (Figure 1.5).[40,54] The size of the SiN pore was directly comparable to the size of the pores created during drop-casting of the PIMs. Note that the SiN film was only 30 nm thick, which facilitated recording currents through this nanoscopic pore. The silicon nitride pore, after cleaning with acid, also has a negatively charged layer due to the formation of a thin silicon oxide layer with silanol groups. The

quaternary ammonium cations are therefore expected to interact with the pore walls through steric and electrostatic interactions.

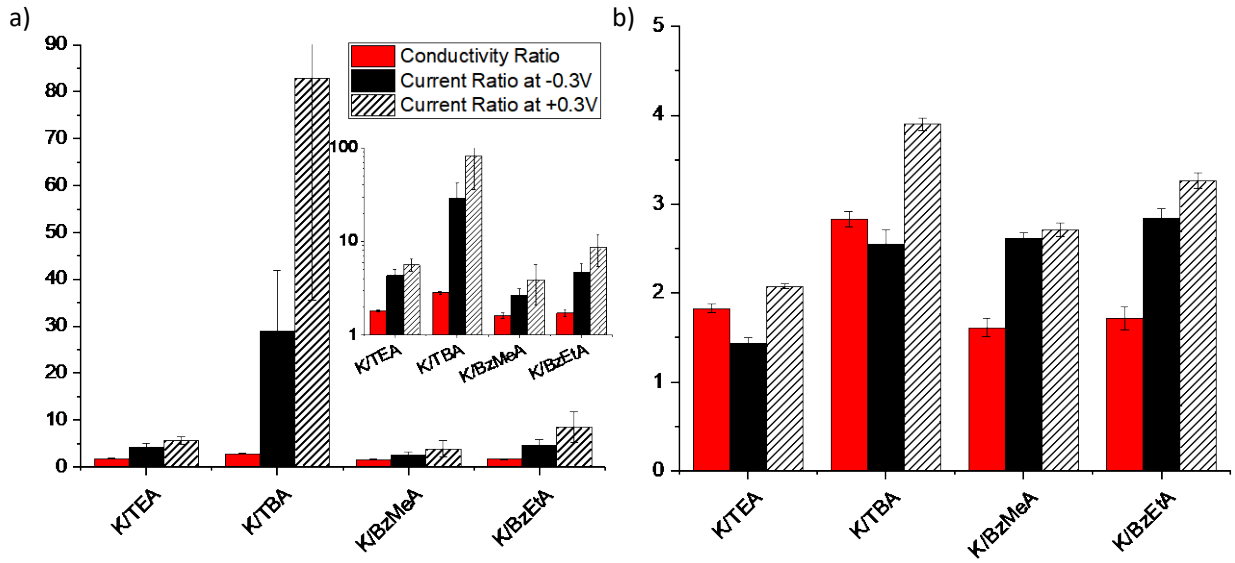

Figure 1.5. a) Ion current ratios for quaternary ammonium salts tested on a 10 nm in diameter conical PET pore. The ratios were calculated by dividing ion current measured in 100 mM KCl by a current recorded in a given salt present in 100 mM. Current ratios in the PET pore confirm the importance of carboxylic acids in formation of ion current blockage, especially for TBACl. The other salts are also transported less efficiently than KCl, but the effect is less pronounced than for TBACl. b) Current ratios for the quaternary ammonium salts tested in a single 5 nm in diameter SiN pore. Ratios for these salts reveal effects of nanoconfinement similar in magnitude to what is seen with PIM CN (Figure 1.3c). Red bars in all panels indicate ratios of bulk conductivity of 100 mM KCl solution and bulk conductivities of remaining salts at the same concentration.

Both pores were tested with 100 mM quaternary ammonium salts and KCl (Figure 1.5). We did not perform experiments in 10 mM solutions, due to small (sub-100 pA) ion current signals that increase the error in the current ratios' calculations. A comparison of the current ratios to the conductivity ratios obtained for the PET and SiN nanopores point to the strong interactions of the carboxylic acid of the PET and the salts. The large discrepancy between the ratios of TBACl and KCl in current and conductivity in the PET pore (Figure 1.5a) indicates that the transport of TBACl is significantly hindered compared to the transport of KCl, similar to what we found with the PIM

COOH membranes (Figure 1.3d). The limited transport of TBACl within the PET pore led to large experimental error due to the exceptionally low current in the system but this further confirms the presence of some physical obstruction (Figure 1.5a). The measurements with the PET nanopore, therefore, supported our hypothesis that the transport of a quaternary ammonium ion is hindered by carboxyl groups that can cause aggregation/precipitation. Note that the transport of the other three ammonium salts was also diminished in the PET pore compared to KCl, but to a lower extent than for TBACl.

Conically shaped PET pores are also known to rectify the current, producing asymmetric IV curves.[49,71,72] The rectification stems from the pore shape as well as presence of negative surface charges. IV curves recorded in both KCl and all quaternary ammonium salts displayed current rectification, even in TBACl (Appendix Figure 1.12). Based on this observation, we concluded that the aggregates/precipitates do not fully cover the pore walls such that zones with exposed, negatively charged carboxyls are still present on the pore walls. We believe the precipitates are mostly forming at the tip region, where due to the confinement, ionic concentrations are the highest, facilitating precipitation.

Figure 1.5b shows recordings for the 5 nm in diameter SiN nanopore. The transport of three ammonium salts with larger cations, TBA, BzMeA, and BzEtA was also lower in this pore compared to predicted trends based on their bulk conductivity. The SiN nanopore is significantly more negatively charged than PIM CN and does not contain any hydrocarbon groups, yet the degree of the transport hindrance is comparable to the recordings in 10 mM salts for PIM CN (Figure 1.3c). These observations led us to conclude that the size of these cations is the main factor responsible for the transport hindrance in both the 5 nm SiN nanopore and PIM CN membrane systems, and the hydrophobic interactions overall played the weakest role.

1.5 Conclusions

We report solid state nanopores drop-casted with polymers of intrinsic microporosity as a model system to probe interactions of passing ions with the nano-confined electrochemical environment. The PIM membranes exhibit a hindered transport of large cations compared to potassium ions at concentrations up to 100mM. We discovered the importance of steric interactions in both uncharged and carboxylated PIMs and that the presence of carboxylic acids in PIMs facilitates the transport of potassium ions while causing aggregation and further hindrance of quaternary ammonium ions. Though the experiments presented here were performed with quaternary ammonium salts, the conclusions on the importance of steric and electrostatic interactions for transport of large ions in nanoconfinement is expected to be applicable to other molecules and even particles.

Our future studies will examine the size and stability of the aggregates we observed here with carboxylated surfaces. Additional experiments with our PIM-nanopore systems will investigate the transport characteristics of chiral membranes and molecules, which may lead to new methods for enantiomeric separation.

1.6 Appendix

1.6.1 Synthesis and Characterization of PIMs

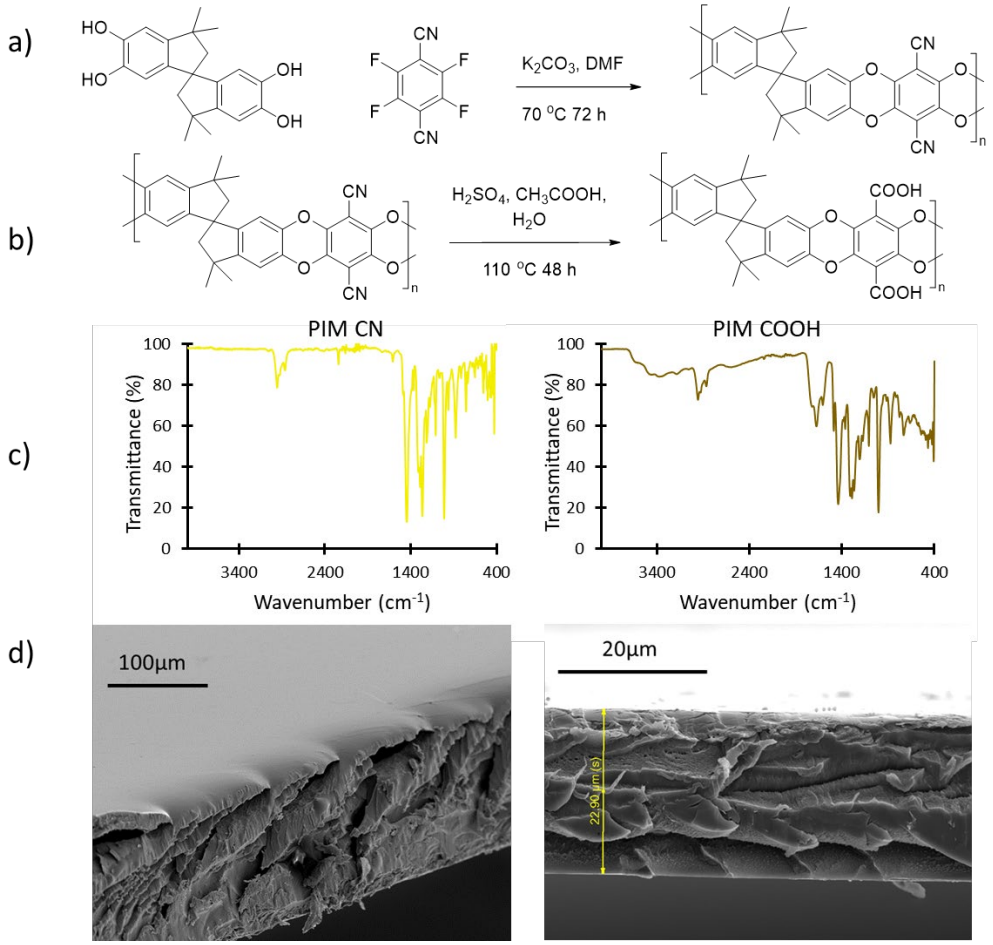

Figure 1.6. a) Synthesis scheme for PIM CN. b) Synthesis scheme for PIM COOH. c) FTIR measurements of PIM CN and PIM COOH membranes. Right: PIM CN. Left: PIM COOH. PIM CN contains the -CN peak around 2200 cm^{-1}, but this disappears after hydrolysis and instead the presence of the carbonyl stretch around 1750 cm^{-1} appears as well as the -OH signal around 3300 cm^{-1}. d) SEM images for thick (left) and thin (right) castings of PIM membranes.

1.6.2 Preparation of unsupported 100 mm thick PIMs

100 µm thick membranes of both PIM CN and PIM COOH were made by dissolving 8 mg of powdered polymer into 1 mL of THF solvent and drop casting the solutions to cover the bottom of a glass petri dish or scintillation vial and allowing the castings to fully dry. The membrane thickness was confirmed by scanning electron microscopy characterization. The castings were then

removed from the glass surface by soaking the membrane with methanol until the membrane could be peeled off the surface. Each PIM membrane was thoroughly washed with nanopure water and allowed to dry before further testing.

A conductivity cell consisting of two PCTFE blocks with fluid reservoirs was used during experiments with PIM CN. The membrane was pressed between the two polymer blocks that exposed ~1 cm^2 of the membrane's surface to the solution. The polymer blocks were then clamped together to prevent any solution leakage. The membrane was first rinsed with nanopure water and then ethanol while in the conductivity cell. Due to the brittleness of the PIM COOH, this membrane was tested in a soft, PDMS-based conductivity cell also used for the PIM/SiN experiments. Both PIMs were exposed to the inorganic and quaternary ammonium salt solutions at concentrations ranging from 1 M to 1 mM. For each solution and concentration, both chambers of the conductivity cell were filled with a single electrolyte solution and current-voltage (IV) curves were recorded. The order of experiments was as follows: KCl, KBr, KI, NaCl, LiCl, NH_4Cl, TEACl, TBACl, BzMeACl, and BzEtACl.

In order to interpret the data, we first measured bulk conductivity of all solutions used in the experiments and calculated the ratios of ionic conductivity of a KCl solution at one concentration and ionic conductivity of a solution of another salt in the same concentration. We also calculated the ratios of ion currents in KCl at a given concentration and all other salts in the same concentration. If a ratio of currents is higher than the ratio of ionic conductivities, a conclusion of hindered transport of the salt versus KCl can be drawn. The same protocol was used to obtain data shown in Figure 3 in the main manuscript. The current ratios for the unsupported PIM CN were equal to the ratio of bulk conductivities. Measurements in PIM COOH, however, indicated a hindered transport for some salts at low concentrations. Figure S2 shows recordings

for unsupported PIM COOH in 1 mM solutions. Ion currents in TBACl and BzEtACl were indeed reduced compared to what could be expected based on bulk conductivities.

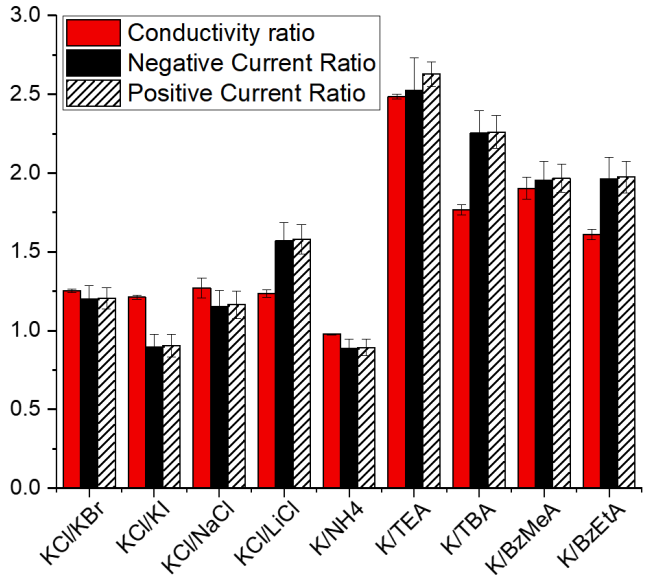

Figure 1.7. Electrochemical characterization of a free-standing ~100 mm in thickness PIM COOH. The bar graph shows ratios of bulk conductivity of KCl and a given salt (red bars) as well as ratios of ion currents in KCl and a given salt at -300 mV (black bars), and +300 mV (dashed bars) at a concentration of 1mM for each solution.

1.6.3 Fabrication and Characterization of SiN single pores

All silicon nitride (SiN) chips used in the experiments were 30 nm thick. Single SiN pores numbered as 9-12 and used as mechanical supports for the PIMs were fabricated by FIB drilling and imaged with scanning electron microscopy (SEM). In addition, a single 5 nm in diameter SiN nanopore was drilled and imaged with transmission electron microscopy (TEM). Figure S3 a-c shows images of samples 9-11, and Figure S3 d shows a TEM image of the 5 nm pore.

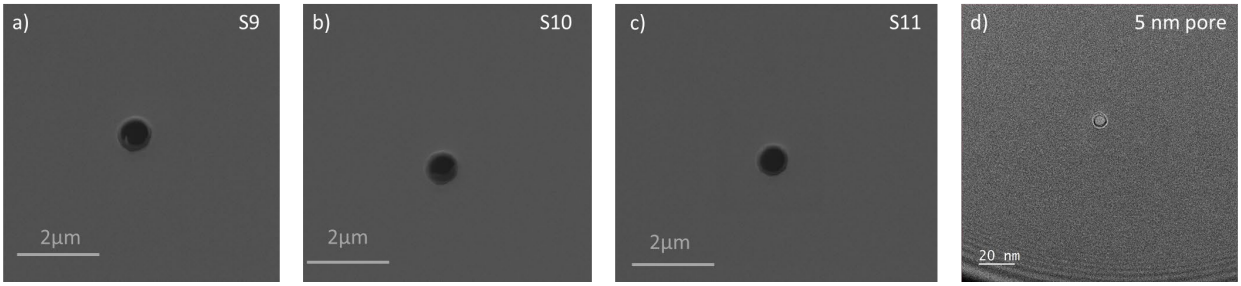

Figure 1.8. SEM and TEM images of single SiN pores. (a-c) Scanning electron micrographs of pores that were used as a support for PIM CN and PIM COOH membranes; these pores were prepared by FIB. (D) Transmission electron microscopy image of a single 5 nm in diameter pore.

1.6.4 Electrochemical properties of as prepared ~300 nm in diameter SiN single pores

SiN pores drilled by FIB were first cleaned with iodine solution to remove the gold coating necessary for the pore fabrication and SEM imaging. The pores were then washed thoroughly with nonpure water and ethanol. The pores were allowed to dry completely before being placed in the PDMS conductivity cell used for the PIM/SiN experiments. The pores were exposed to the inorganic and quaternary ammonium salt solutions at concentrations ranging from 1 M to 1 mM. For each solution and concentration, both chambers of the conductivity cell were filled with a single electrolyte solution and current-voltage (IV) curves were recorded. The resulting current ratios from each salt and concentration were compared to the conductivity ratios, using the same procedure as explained in Section 2 and Figure S2. The order of experiments was as follows: KCl, KBr, KI, NaCl, LiCl, NH4Cl, TEACl, TBACl, BzMeACl, and BzEtACl. Figure S4 shows the results for a representative as-prepared ~300 nm in diameter pore. No interactions were seen between the SiN pore wall and any of the salts tested.

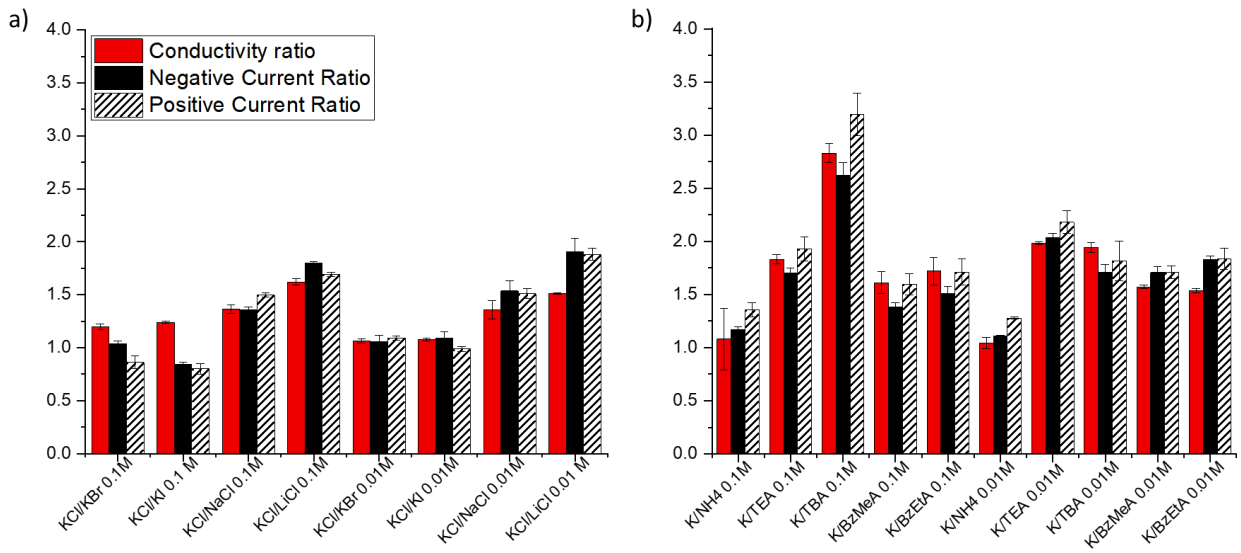

Figure 1.9. Electrochemical characterization of a ~300 nm in diameter SiN pore in (a) inorganic, and (b) ammonium salts. The bar graph shows ratios of bulk conductivities of KCl and a given salt (red bars) as well as ratios of ion currents in KCl and a given salt at -300 mV (black bars), and +300 mV (dashed bars).

1.6.5 Electrochemical characterization of a PIM COOH/SiN sample (sample 11) in 100mM and 10mM salt solutions

Figure 3 in the main manuscript shows properties of a PIM COOH/SiN system, sample 12. A similar set of experiments were performed with another, independently prepared PIM COOH/SiN system, designated as sample 11. Experiments were performed in a PDMS conductivity cell. Sample 11 was exposed to the inorganic and quaternary ammonium salt solutions at concentrations ranging from 1 M to 1 mM. However, unlike sample 12, sample 11 was first exposed to high concentrations of each solution followed by low concentrations. For each solution and concentration, both chambers of the conductivity cell were filled with a single electrolyte solution and current-voltage (IV) curves were recorded. The resulting current ratios from each salt and concentration were compared to the conductivity ratios. The order of experiments is as follows: KCl, KBr, KI, NaCl, LiCl, NH_4Cl, TEACl, TBACl, BzMeACl, and BzEtACl. Figure S5 shows the comparison of current ratios for 100 mM and 10 mM salt solutions.

Similar to the results for Sample 12 (Figure 3), sample 11 highlights the hindered transport of the quaternary ammonium salts through the membrane, especially in the case of TBACl. However, because this sample was first exposed to high concentrations of solutions, the magnitude of the current ratios is smaller than with sample 12, indicating that some residual precipitates may reside from these high concentrations.

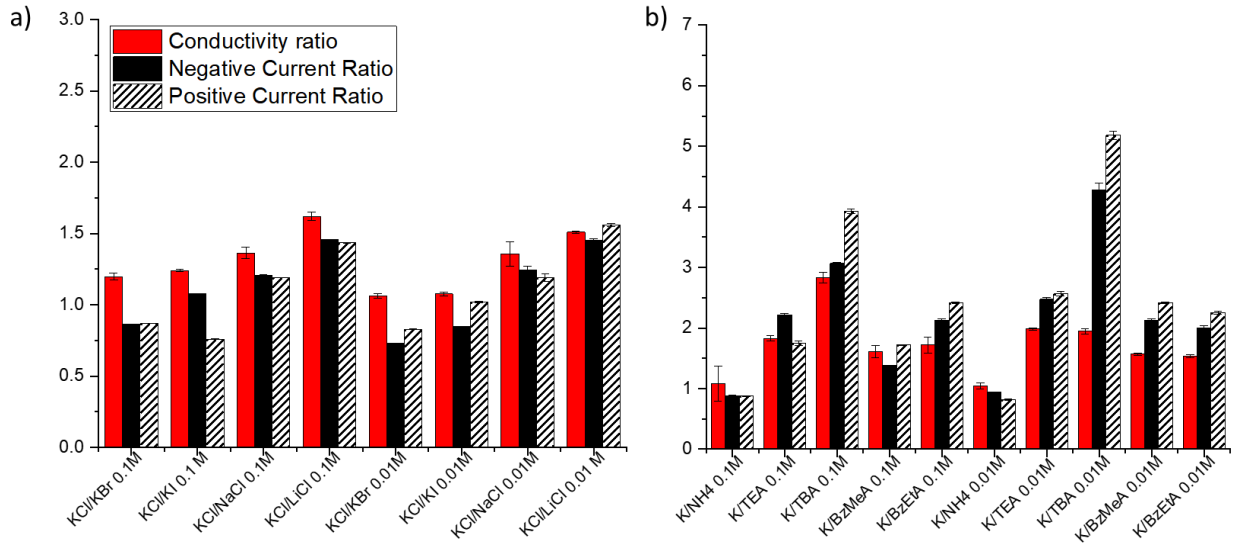

Figure 1.10. Electrochemical characterization of a PIM COOH/SiN system in (a) inorganic, and (b) ammonium salts. This is a different sample than shown in Figure 3 of the main manuscript. Red bars show ratios of bulk conductivities. Ratios of ion currents in -300 mV and +300 mV are shown as black and dashed bars, respectively.

1.6.6 Transport properties of PIM COOH/SiN in TBACl

Following observations of a significant drop of PIM COOH conductance in TBACl, separate experiments were performed with two samples used to collect data shown in Figure 3d and Figure S5. We hypothesized that the current decrease stems from possible accumulation of the salt in the carboxylated membranes. The samples were therefore thoroughly washed with nanopure water beforehand to ensure that residual aggregates from previous experiments would not be present. After a baseline IV measurement in KCl, IV curves were subsequently recorded in TBACl,

followed by recordings in the bulkier quaternary ammonium salts. The samples were intentionally washed with nanopure water only once between changing salt solutions to exchange the salt but preserve any aggregates that could have formed. Figure S6 shows the IV curves of the bulkier quaternary ammonium salts for Samples 11 (shown in Figure S5) and 12 (Figure 3d) after their exposure to TBACl. The IV curves indicate the presence of a blockage possibly due to TBACl aggregates that closed the pores of the membrane and significantly decreased transport, especially for any large, bulky ions. Sample 12 also shows the recovery of the membrane's transport with BzEtACl after thorough washing with nanopure water.

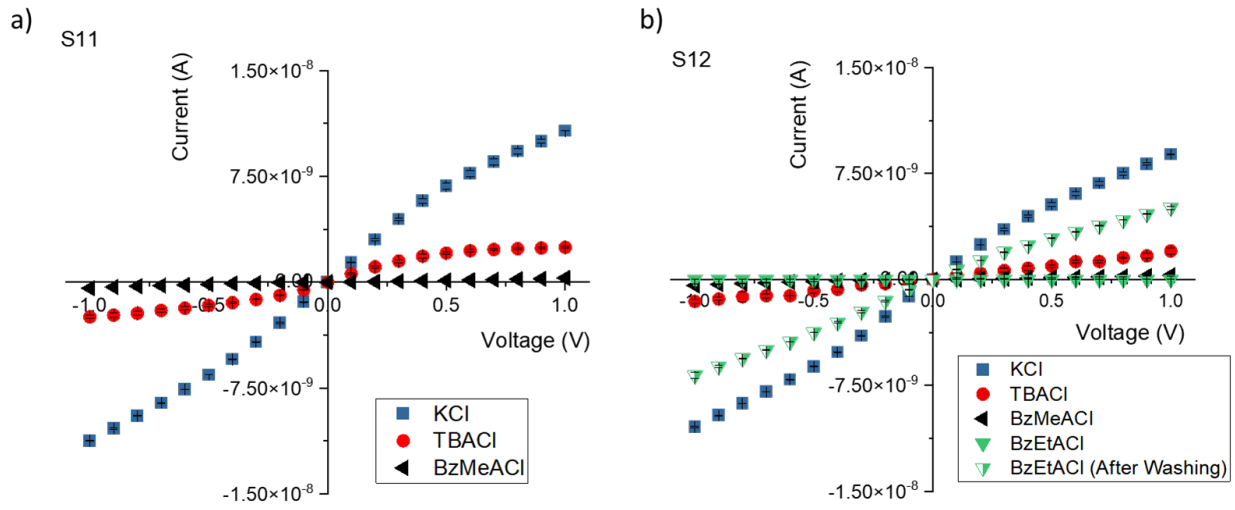

Figure 1.11. (a,b) Current-voltage curves of two PIM COOH/SiN samples exposed to TBACl (red circles) followed by measurements in BZMeACl (black) triangles. The membrane in (b), which was also used to record data in Figure 3, was in addition exposed to BzEtACl, followed by a thorough washing, when a recording in BzEtACl was repeated. The measurements in KCl (blue squares) were recorded first. All salts were present in a concentration of 1 mM.

1.6.7 Electrochemical characterization of a single polyethylene terephthalate (PET) conical pore in quaternary ammonium salts

A single PET nanopore with the tip opening of 10 nm was used in the experiments. The nanopore was prepared by the track-etching technique. IV curves shown in Figure S7 were

collected in KCl and four quaternary ammonium salts. These measurements were used to calculate ion current ratios shown in Figure 5 in the main manuscript. The measurements were done in the following order: KCl, TEACl, TBACl, BzMeACl, and BzMeACl. The pore was thoroughly washed in between each change in solution to remove any aggregates that may have formed in the pore. Note that all IV curves shown in Figure S7 exhibit rectification.

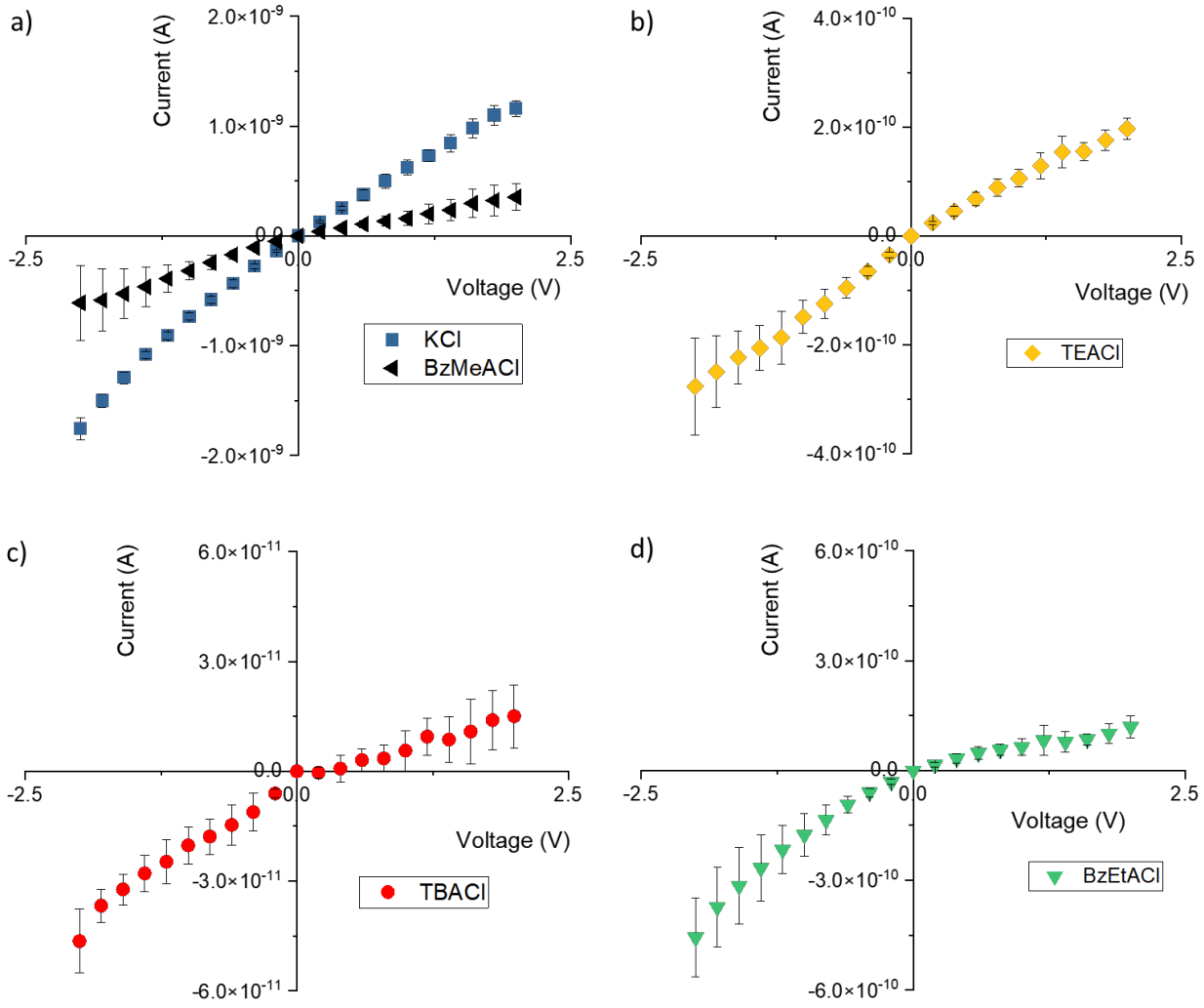

Figure 1.12. Current-voltage curves through a single 10 nm in diameter conical PET pore recorded in KCl and a series of quaternary ammonium salts, as indicated in the legends of panels a-d. All recordings were performed in 100 mM.

1.6.8 XPS studies of PIM COOH exposed to quaternary ammonium salts

All XPS measurements were taken on a Kratos AXIS-Supra under ultra-high vacuum. Figure 8 shows the XPS traces for three quaternary ammonium salts, TEACl, BzMeACl, and BzEtACl. XPS for TBACl is shown in the main manuscript. All salts showed N+ (blue lines) aggregates as well as unhydrolyzed cyano groups from the polymer (marked in red).

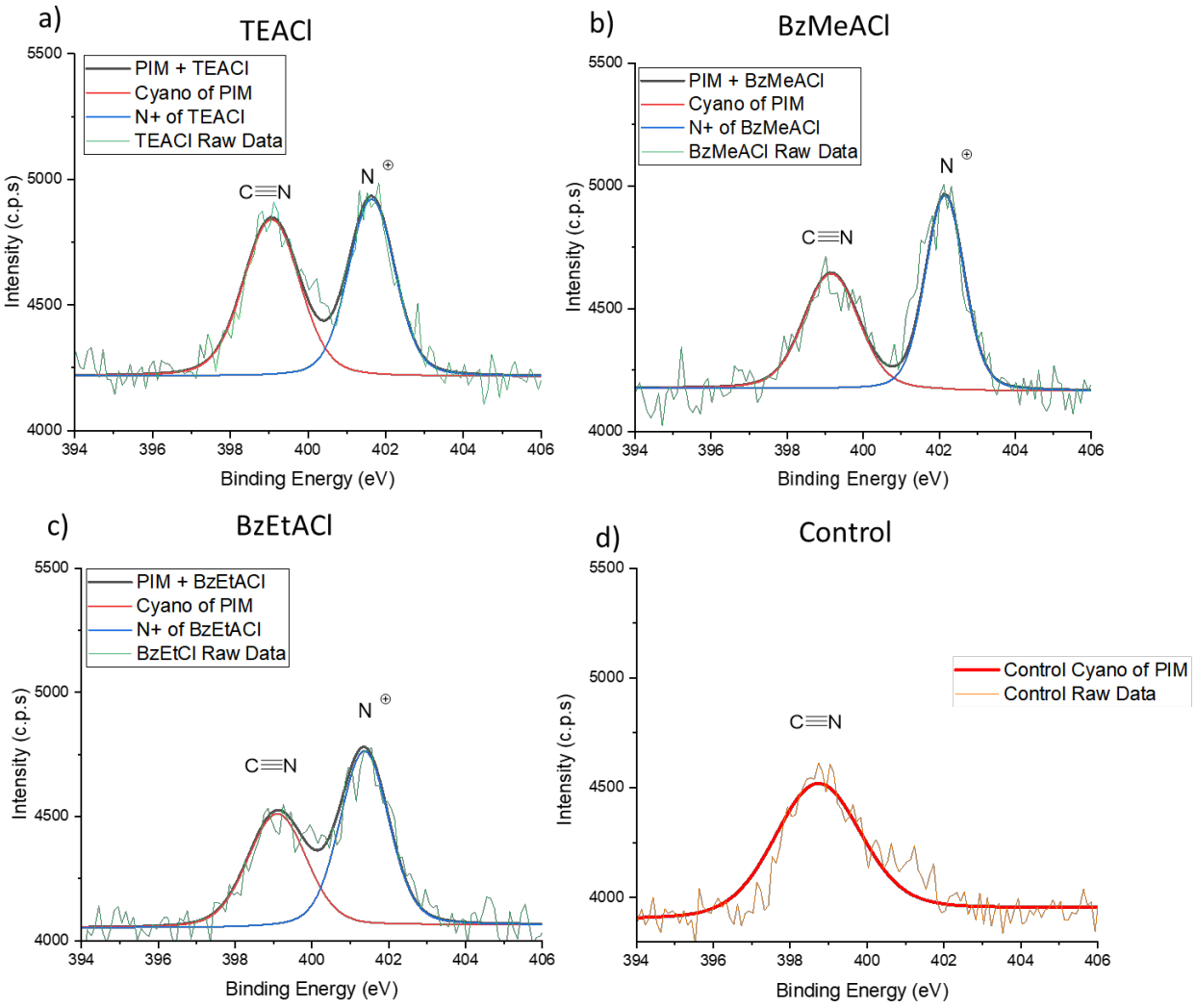

Figure 1.13. XPS spectra of PIM COOH exposed to 100 mM of (a) TEACl, (b) BzMeACl, and (c) BzEtACl. Panel (d) shows a control measurement with a PIM COOH that was not exposed to any of the organic salts.

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Chapter 2: Electrochemical Probing of Chiral Interactions of Large Ions in Polymers of Intrinsic Microporosity

2.1 Preface

After publishing the results of the first study with PIMs, I wanted to investigate the potential for the PIMs to be used as separation membranes for chiral molecules. Dr. Shea and his graduate students had previously published a paper using chiral PIMs to separate chiral molecules using diffusion and a concentration gradient. I became interested in studying the separation of chiral molecules with these PIMs under a voltage driving force. The advantage of using a driving force to perform separation experiments is seen with shorter time scales but also highlights the fundamental interactions as ions move through a membrane. These interactions can be captured via current-voltage curves by observing rectification in the region of voltages applied over the membrane. By exploring the effects of voltage on the chiral membrane-ion interactions, we may be able to utilize chiral membranes to their fullest potential for large scale pharmaceutical separations in the future.

2.2 Introduction

Chiral separations play an extremely important role in the pharmaceutical industry.[1–4] Most medications that are commonly used exist as racemic mixtures before purification.[5–9] While the enantiomers in this mixture are chemically similar, they often have vastly different pharmacological activities and one can be toxic if not removed properly.[1,9–11] However, the separation of the desired drug from its enantiomer can be challenging as the two are nearly identical and common methods of separation, such as extraction or basic liquid chromatography, cannot be used.[4,12,13] Currently, chiral purifications are conducted using either high-performance liquid

chromatography (HPLC) with a chiral stationary phase or simulating moving bed (SMB) chromatography.[14–17] While both methods achieve high enantiomeric ratios, HPLC is expensive on an industrial scale and SMB may not be sufficient for some enantiomers.[1,18] Chiral polymer membranes have emerged as a new method to separate enantiomers.[5,6,19–22] Polymer membranes are advantageous for their high processability, durability, efficiency, and low cost.

Polymers of Intrinsic Microporosity (PIMs) have garnered attraction for membrane separations due to their ability to form small uniform nanopores on the order of 2-4 nm in size.[23–31] The PIM monomer can also be manipulated to have a wide range of charged or hydrophobic groups to enhance separation.[27,32–37] However, PIMs have been relatively unexplored as membranes for chiral separations. The Shea lab has recently published the preparation of chiral PIMs and their ability to perform enantiomeric separations under diffusion and a concentration gradient.[27] Here, we explore the chiral PIMs' enantiomeric separation abilities under a voltage driving force. We have previously reported the ability of the achiral PIMs combined with nanopores to separate complex organic ions under voltage-driven force and now seek to further understand the PIMs' selection capabilities with chiral ions.[31] The advantage to applying voltage over the PIMs during separations is that the experiments are not limited by diffusion and can be done over a significantly shorter time scale.[38,39]

We investigate the mobility of chiral ions through a chiral membrane using a chiral PIM functionalized with carboxylic acid (chiral PIM COOH) drop-casted onto a silicon nitride chip drilled with a 400 nm pore.[40,41] The single SiN pore restricted the measured transport to a small area of the membrane creating a uniform voltage applied across the membrane in all measurements. We performed electrochemical measurements of ionic conductance of chiral PIM COOH in chiral ionic solutions of varying sizes and hydrogen bonding capabilities. Sodium

lactate, mandelic acid, and boc-tyrosine were our primary focus for these studies. These molecules were chosen as they are easily accessible as racemic and chiral forms, are adequate charge carriers, vary in size, and have different hydrogen bonding capabilities. These attributes allow us to easily probe the chiral, steric, and hydrogen-bonding interactions of the chiral ions with the membrane's pore walls.

2.3 Experimental

2.3.1 Reagents

3,3,3',3'-Tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol (TTSBI), potassium carbonate (K_2CO_3), 2,3,5,6-tetrafluorphthalonitrile (TFPT), racemic mandelic acid, (R)-(-)-mandelic acid, (S)-(+)-mandelic acid, racemic sodium lactate, sodium L-lactate, sodium D-lactate, boc-L-tyrosine, and boc-L-tyrosine were purchased from Sigma-Aldrich. Dry dimethyl formamide (DMF), methanol, sulfuric acid, glacial acetic acid, and sodium bicarbonate ($NaHCO_3$) were all used as received. Potassium chloride was purchased from Fisher Scientific. For structures of the racemic molecules, see Figure 1. All reagents were used without further purification.

2.3.2 Preparation of Chiral Ionic Solutions

All solutions were prepared as 1mM stock solutions in nanopure deionized water (18.2 MΩ·cm, Milli-Q IQ 7000 ultrapure water system). Mandelic acid solutions were corrected to pH 3 with hydrochloric acid. All other solutions remained unbuffered. Potassium chloride was prepared for 1M, 100mM, 10mM and 1mM concentrations.

2.3.3 Synthesis and Characterization of Chiral PIM COOH

3,3,3',3'-Tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol (TTSBI) (270 mg, 0.871 mmol) was resolved into its enantiomeric form via formation of a diastereomic complex with (8S,9R)-(-)-N-benzylcinchonidinium chloride and then was dissolved in dry DMF (5 mL) followed by

addition of 2,3,5,6-tetrafluorphthalonitrile (TFPT) (174 mg, 0.872 mmol). Once the mixture was homogenous, excess anhydrous K_2CO_3 (1 g, 7.2 mmol) was added and the heterogenous mixture was heated to 70 °C for 72 h. After about 30 min the reaction mixture turned from brown to bright yellow. Once cooled, the mixture was precipitated into water (50 mL), filtered, rinsed with water, and dried in a vacuum oven o/n at 70 °C. The crude powder was then suspended in methanol (10 mL) and heated to reflux for 3 h. The mixture was filtered hot and rinsed with hot methanol before drying in a vacuum oven o/n at 70 °C. The product was a fine, fluorescent yellow powder (86% yield).[27]

The acid catalyzed hydrolysis experimental methods were the same as used in our previous work.24 Enantiopure PIM CN (200 mg) was treated with a solution of H_2SO_4 (3 mL), H_2O (3 mL), and glacial acetic acid (1 mL). The suspension was heated to 110 °C for 48 h. The powder slowly turned from fluorescent yellow into a light brown color. Once cooled, the mixture was diluted into water (50 mL), filtered, rinsed with saturated aqueous $NaHCO_3$, rinsed with copious amounts of water, then dried in a vacuum oven at 70 °C o/n. The product appeared as a light brown powder (86 % yield). FTIR showed full conversion. Incomplete conversion was apparent when temperatures were at 105 °C.[27]

2.3.4 Fabrication and Characterization of Single Nanopores in Silicon Nitride Chips.

Two large single pores between 300-400nm in diameter were prepared in low-stress silicon nitride (SiN_x) films (50μm by 50μm; 30nm thick) purchased from Norcada. The pores were created using Focused Ion Beam (FIB) drilling on a FEI Helios Nanolab 600i FIB/SEM DualBeam System at 30 kV.[40,41] Both pores were used as mechanical support for PIMs and were labeled as "control" and "chiral".

Diameters of both SiN pores were estimated from the resistance of the pore found by generating current current-voltage (IV) curves in 1M KCl solutions between -1 and 1 V. The pore diameter (D) is estimated using the following equation:

$$D = \frac{G}{2\sigma}\left[1 + \sqrt{\left(1 + \frac{16\sigma L}{\pi G}\right)}\right]$$

where G is the slope (conductance) of the IV curve, σ is the solution conductivity, and L is the pore length.[42] The shape of the pore was assumed to be cylindrical thus all diameters reported are called here effective diameters.

All IV curves in this study were measured with a Keithley 6487 picoammeter/voltage source (Keithley Instruments, Solon, Ohio, USA) and a software program written in MATLAB (MathWorks, Natick, Massachusetts, USA) using a voltage range between -1 and 1 V with 0.1 V voltage steps. Two non-polarizable Ag/AgCl electrodes were used for all current measurements.

2.3.5 Preparation and Characterization of Drop-Casted PIMs on Solid State Nanopores

In this study, two PIMs were used. Both PIMs were functionalized with carboxylic acid and one PIM was synthesized with a chiral monomer. Both polymers were used for drop-casting onto the silicon nitride chips containing pores. Powdered forms of the polymers were first dissolved in tetrahydrofuran (THF) at a concentration of 1 mg per mL. A 1 mL syringe with a 27-gauge needle was used to drop-cast 1-2 drops of solution onto the silicon nitride samples. One SiN pore received PIM COOH (control) and the other received chiral PIM COOH. Both samples were dried overnight and then washed with nanopure deionized water before further experiments. After drop-casting, both PIM/SiN samples were tested in KCl solutions by recording current-voltage curves in the voltage range of -1V to +1V with 100 mV steps.

2.4 Results and Discussion

2.4.1 Chiral PIM COOH Casted on a Silicon Nitride Chip Containing a 400 nm in Diameter Pore.

To explore the ion mobility of chiral ions through a chiral membrane, chiral PIM COOH was drop casted onto a SiN chip containing a single nanopore ~400 nm in diameter (Figure 2.1a & b). The as-prepared SiN pore before drop-casting was characterized with a series of KCl solutions ranging in concentration from 1M to 1mM and was subsequently sized in 1M KCl (Figure 2.1c). After drop-casting the chiral PIM COOH onto the pore, the PIM/SiN pore was again characterized with the series of KCl solutions to confirm the presence of the membrane. The presence of the SiN pore provided both mechanical stability as the free-standing membrane was found to be brittle and limited the ionic transport of the chiral molecules to a small portion of the membrane.[27,31] For well-defined electrochemical measurements, the increased resistance provided by the pore is imperative due to the voltage applied decreasing entirely. Figure 2.1c shows the current-voltage (IV) curves before and after drop-casting as recorded in 1M KCl. As expected, the chiral membrane increases the system's resistance, but the increase is small due to the low resistance and high porosity of the membrane itself. Additionally, a control achiral PIM COOH used in the previous study was drop-casted onto another ~400 nm SiN pore and served to distinguish chiral interactions from steric or charge interactions. The SiN/PIM control system was characterized in the same way as the chiral system before and after drop-casting (appendix Figure 2.5).

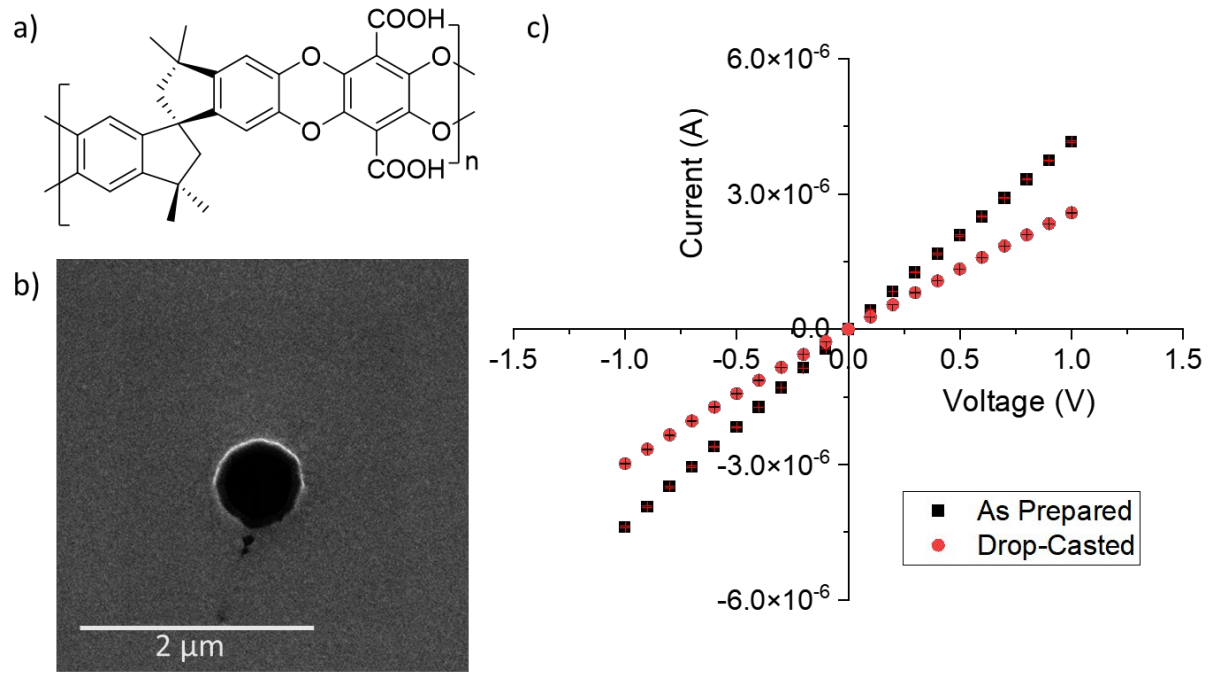

Figure 2.1 a) Structure of chiral PIM COOH. b) SEM picture of a ~400 nm SiN pore created by FIB drilling c) IV curves depicting a decrease in current after chiral PIM COOH is drop-casted onto a single ~400 nm in diameter SiN pore. Both IV curves before and after drop-casting were recorded in 1 M KCl.

2.4.2 Chiral Interactions Between the Ions and the Membrane Rely on Size and Hydrogen Bonding.

Chiral PIM COOH/SiN was exposed to a series of chiral molecules at 1mM concentrations to understand the membrane's potential for chiral interactions. All solutions' conductivities were measured to be between 0.7-0.9 microsiemens per cm. As reported previously, chiral separations are often enhanced through multiple routes of separations such as size, charge, and bonding capabilities. Thus, the three chiral molecules chosen (sodium lactate, mandelic acid, and boc-protected tyrosine) all embody different size and hydrogen bonding characteristics. Electrochemical characterizations for each solution were recorded as current-voltages curves which are used to determine the chiral interactions. Molecules that do not have any interaction with the chiral membrane are expected to generate linear IV curves as the transport from the side

of the pore without membrane to the side of the pore with the membrane remains unchanged without chiral effects. Molecules that are hindered by the membrane are expected to have rectified IV curves and the transport through the membrane will generate smaller current. The positive voltage range characterizes the side of the pore drop-casted with membrane. The ground electrode was placed on the side of the chip drop-casted with membrane thus for positive voltages, the cations were sourced from the membrane side and moved towards the opposite side of the chip.

The chiral PIM COOH/SiN pore was first exposed to sodium D-lactate and sodium L-lactate at 1mM concentration. Sodium lactate was chosen for its chirality, small size, and lack of hydrogen bonding capability. Both solutions were prepared in nanopure water without any adjustments to pH, resulting in a solution pH of 6, at which the molecules cannot hydrogen bond with the PIM's carboxylic acids. We hypothesized that although sodium lactate may have chiral interactions with the membrane, those interactions would be minimized or non-existent under a larger voltage-driven force. Additionally, the small size of sodium lactate allows for minimal interactions with the membrane's pore walls as the molecule can move freely through the pore without steric hinderance. Figure 2.2 b & d shows the current voltage curves of sodium L and sodium D lactate. As predicted, the current-voltage measurements are linear, indicating that neither molecule had significant interactions with the chiral membrane as no rectification at positive voltages was seen. This observation is also confirmed with the control achiral PIM COOH/SiN which also generates linear IV curves in the sodium lactate solutions (appendix Figure 2.6).

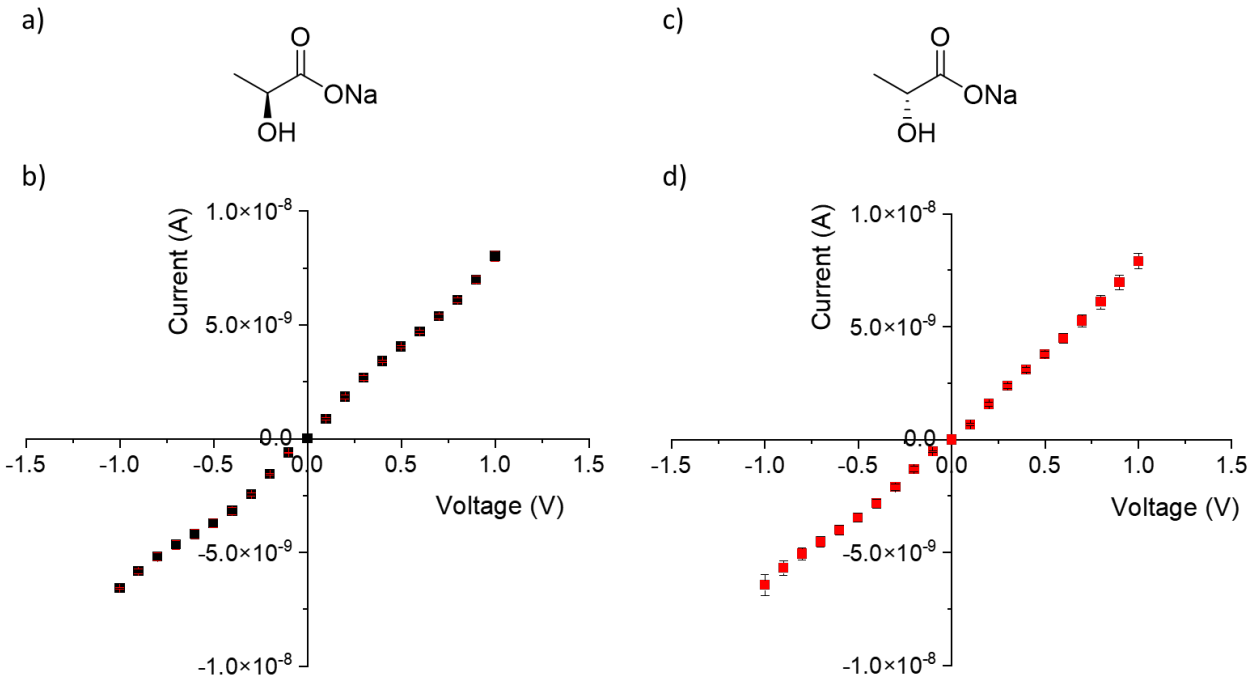

Figure 2.2 a) Structure of sodium L-lactate. b) IV measurements of chiral PIM COOH exposed to 1mM sodium L-lactate. The resulting curve shows a linear trend with little interaction with the chiral membrane. c) Structure of sodium D-lactate. d) IV measurements of chiral PIM COOH exposed to 1mM sodium D-lactate. The resulting curve shows a linear trend with little interaction with the chiral membrane.

The second set of chiral solutions were created with R-(-)-mandelic acid and S-(+)-mandelic acid at a 1mM concentration in nanopure water. Mandelic acid is a larger chiral molecule that, at certain pH values, can undergo hydrogen bonding. The mandelic acid solutions were corrected to pH 3 to protonate a percentage of ions and create a hydrogen bonding pair with the negatively charged carboxylic acid of the membrane. The addition of hydrogen bonding sites along with the larger size was expected to enhance any chiral interactions with the membrane. The larger size allows the molecule to encounter the membrane's pore walls and the hydrogen bonding site can attract the molecule to the membrane. Figure 2.3b & d highlights the rectification of the IV curves in both mandelic acid solutions. The rectification in the IV curves points to potentially hindered and directional ionic transport through the membrane, which is confirmed with the

control experiments on the achiral membrane (appendix Figure 2.7). The achiral membrane, when exposed to the same mandelic acid solutions, does not show any rectification in the positive voltage regions, confirming that the hindered transport seen with the chiral membrane is predominantly due to limited spatial conformations as mandelic acid moves through the membrane. Additionally, mandelic acid solutions were created at pH 2 without pH correction and tested through both the chiral and achiral PIM COOH. Regardless of the chirality of the membrane, the voltage curves with mandelic acid at pH 2 did not rectify as seen at pH 3 (appendix Figure 2.8). This is attributed to mandelic acid being mostly protonated at pH 2 and therefore cannot hydrogen bond with chiral. The pH studies confirm that additional attraction between the molecule and the membrane are needed to enhance chiral interactions, especially under a large driving force.

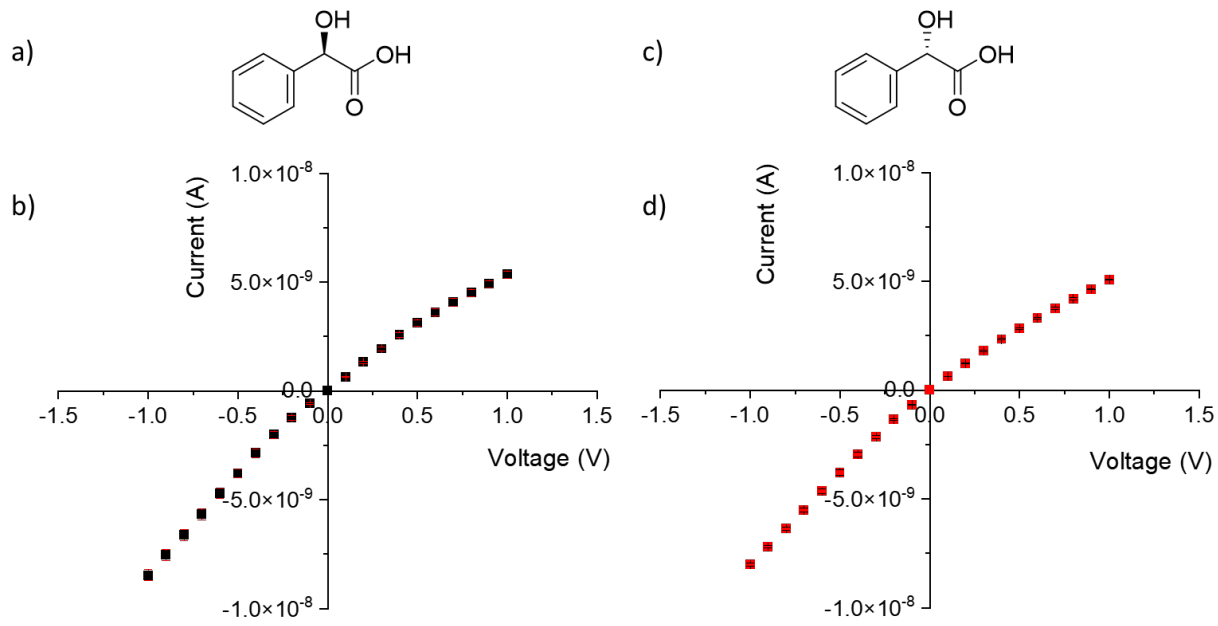

Figure 2.3 a) Structure of (R)-(-)-mandelic acid. b) IV measurements of chiral PIM COOH exposed to 1mM (R)-(-)-mandelic acid. Rectification is seen in the resulting curve indicating directional ionic transport of the chiral ion through the membrane. c) Structure of (S)-(+)-mandelic acid. d) IV measurements of chiral PIM COOH exposed to 1mM (S)-(+)-mandelic acid. Rectification is seen in the resulting curve indicating hindered ionic transport of the chiral ion through the membrane.

The final molecules tested were boc-L-tyrosine and boc-D-tyrosine. The Shea lab previously reported great success with separating fmoc-protected phenylalanine, which we would like to observe under a voltage driving force.[27] However, fmoc-phenylalanine is extremely hydrophobic and cannot be used in aqueous ionic solutions. Instead, boc-tyrosine was selected for the ionic solutions as the chemical structure is similar to phenylalanine, but the additional alcohol group renders the molecule hydrophilic for aqueous solutions. This molecule was also chosen because of its large size, the largest of the three tested, and its inherent positive charge from the protonated amine in the amide group. The solutions were created in nanopure water and were not corrected for pH, resulting in a pH of 4 at which the molecule is neutral but still capable of hydrogen bonding at the N terminus. We predicted that this molecule would have the greatest interaction with the chiral membrane because of its size relative to the membrane's pores and its ability to hydrogen bond. The IV curves shown in Figure 2.4b & d confirm our prediction as the curves display extremely hindered transport of tyrosine through the chiral PIM COOH. The IV curves show not only diminished current values compared to the other two molecules tested but also hysteresis and a sharp transition around voltages close to zero. The low current values characterize the low mobility of tyrosine at high voltages and the hysteresis and sharp transition at 0V potentially indicate the existence of an energy barrier for the tyrosine to enter the membrane's pores. Interestingly, the control achiral PIM COOH, while showing some diminished current, does still show finite current at every voltage and no hysteresis or sharp transitions around 0V. We anticipated some charge-charge interactions with the amide group of the tyrosine and the carboxylic acid of the PIM as demonstrated by our previous findings with quaternary ammonium groups, which accounts for the diminished current in the control membrane.[31] These charge-charge

interactions do not, however, account for the additional characteristics in the chiral membrane's IV curves which must come from the chiral centers in the membrane itself.

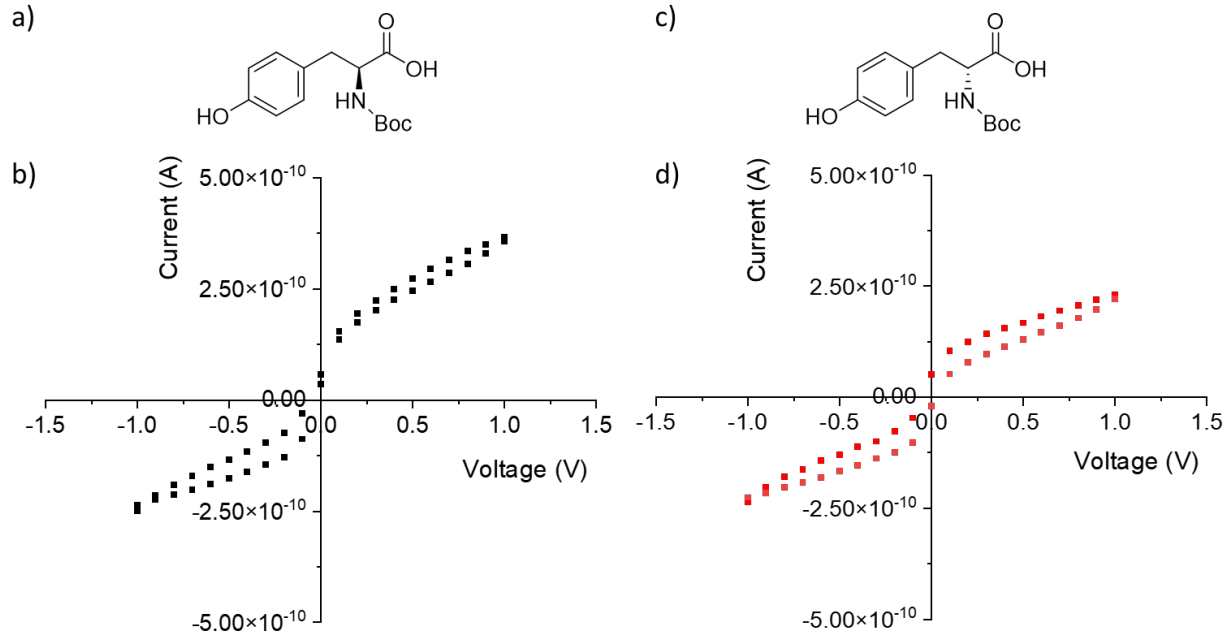

Figure 2.4 a) Structure of boc-L-tyrosine. b) IV measurements of chiral PIM COOH exposed to 1mM boc-L-tyrosine. The resulting curves show hysteresis between scans and a sharp transition around 0V, indicating rejection of tyrosine by the membrane especially at low voltages. c) Structure of boc-D-tyrosine. d) IV measurements of chiral PIM COOH exposed to 1mM boc-D-tyrosine. The resulting curves show hysteresis between scans and a sharp transition around 0V, indicating rejection of tyrosine by the membrane especially at low voltages.

2.4.3 Chiral PIM COOH Favors Specific Spatial Geometries.

While no obvious selectivity for one type of enantiomer over the other is seen in the IV curves for each of the three chiral molecules tested, it is important to note that certain spatial geometries show decreased mobility through the chiral PIM COOH. Specifically, the geometries that correspond to sodium-D-lactate, (S)-(+)-mandelic acid, and boc-D-tyrosine show decreased currents relative to their enantiomer counterparts. The IV curves for Figures 2.2, 2.3, and 2.4 all show slightly diminished current for the molecules listed above compared to their enantiomers, a trend which is not seen in any of the studies conducted on the control membrane. This may indicate

some spatial selectivity towards these geometries from the chiral components of the chiral PIM COOH.

2.5 Conclusions

We report solid state nanopores drop-casted with chiral polymers of intrinsic microporosity as a model system to probe interactions of chiral ions with a spatially confined electrochemical environment. The PIM membranes exhibit a directional transport of mandelic acid and boc-tyrosine compared to sodium lactate at concentrations of 1mM. We discovered the importance of molecular size and hydrogen bonding capabilities on the interactions of chiral separation through a chiral membrane. The presence of hydrogen bonding sites between the chiral molecules and the chiral PIM COOH facilitates the attraction of the ions to the membrane while the size and chiral arrangements cause directional transport of the chiral ions leading to hindered mobility. Though the experiments presented here were performed with a limited set of chiral ions, the conclusions on the importance of chiral interactions for selectivity of large chiral ions is expected to be applicable to other molecules.

Our future studies will examine PIM membrane's capability to select for one enantiomer over another under a voltage driving force. Additional experiments with our PIM-nanopore systems will investigate the transport characteristics of ionic chiral liquids through the chiral membranes, which may lend to further understanding of enantiomeric separations.

2.6 Appendix

2.6.1 Synthesis and Characterization of achiral PIM/SiN Nanopore.

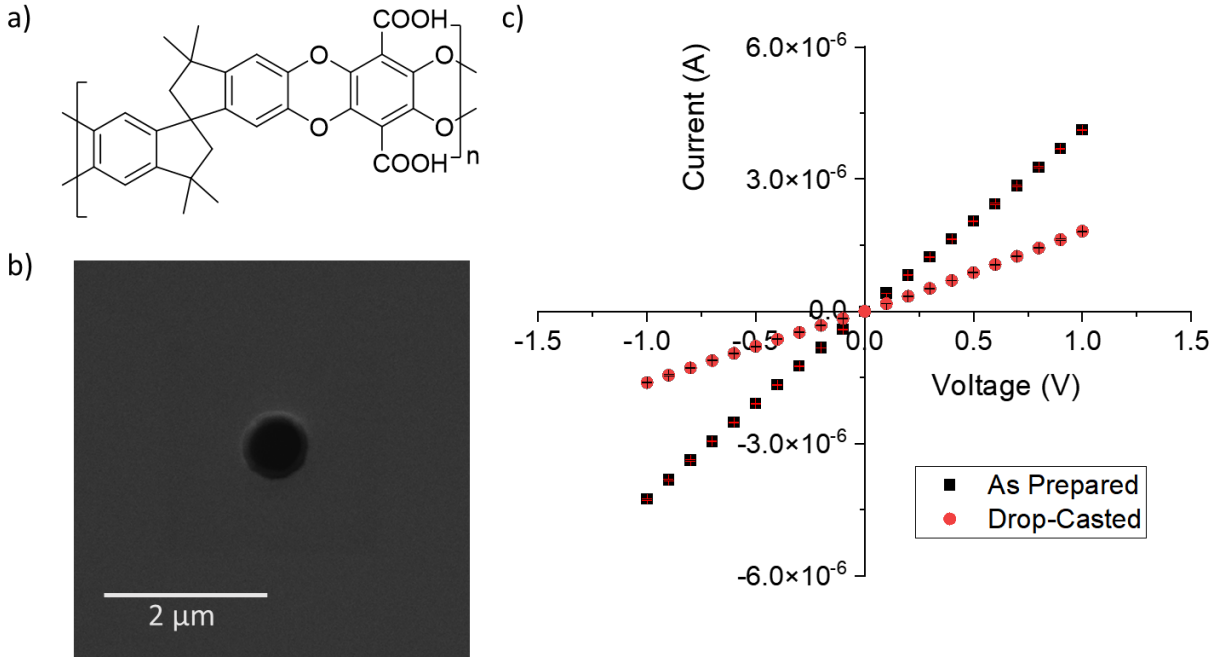

Figure 2.5. a) Structure of achiral PIM COOH used for all control experiments. b) SEM image of a ~400nm pore which was used for all control experiments. c) IV measurements of the SiN pore before and after drop-casting with the achiral PIM COOH. Curves were recorded in 1M KCl.

An achiral PIM COOH used in previous studies (cite paper) was used as a control membrane to investigate the chiral molecules' transport through an achiral membrane. A control pore was fabricated by FIB drilling and imaged with scanning electron microscopy (SEM). The pore was sized as prepared with 1M KCL. The achiral PIM COOH was drop-casted onto the control pore by dissolving 1 mg of powdered polymer into 1mL of THF. The pore was allowed to dry overnight and then tested in 1M KCl to verify the presence of the membrane.

2.6.2 Control Studies of Sodium Lactate through the Achiral Membrane.

The achiral membrane on the SiN pore was exposed to both 1mM sodium L-lactate and 1mM sodium D-lactate. The resulting IV curves show no interaction with the achiral membrane as expected. It is important to note that the shape of the IV curves shown below is not in relation to any interactions with the ions and the membrane. The shape is inherent to the PIM/SiN system and is also present at 1mM KCl.

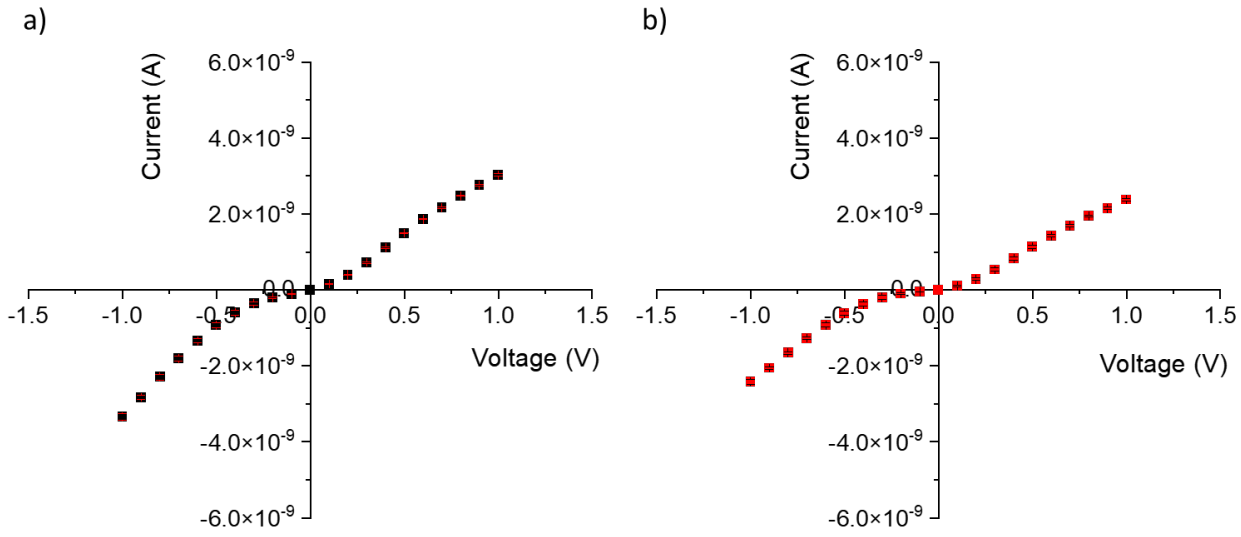

Figure 2.6. a) IV measurements of 1mM sodium L-lactate through the achiral PIM COOH. Sodium L-lactate has no interactions with the achiral membrane. b) IV measurements of 1mM sodium D-lactate through the achiral PIM COOH. Sodium D-lactate has no interactions with the achiral membrane.

2.6.3 Control Studies of Mandelic Acid through the Achiral Membrane

The achiral membrane on the SiN pore was exposed to both 1mM (R)-(-)-mandelic acid and 1mM (S)-(+)-mandelic acid. The resulting IV curves show no interaction with the achiral membrane as expected.

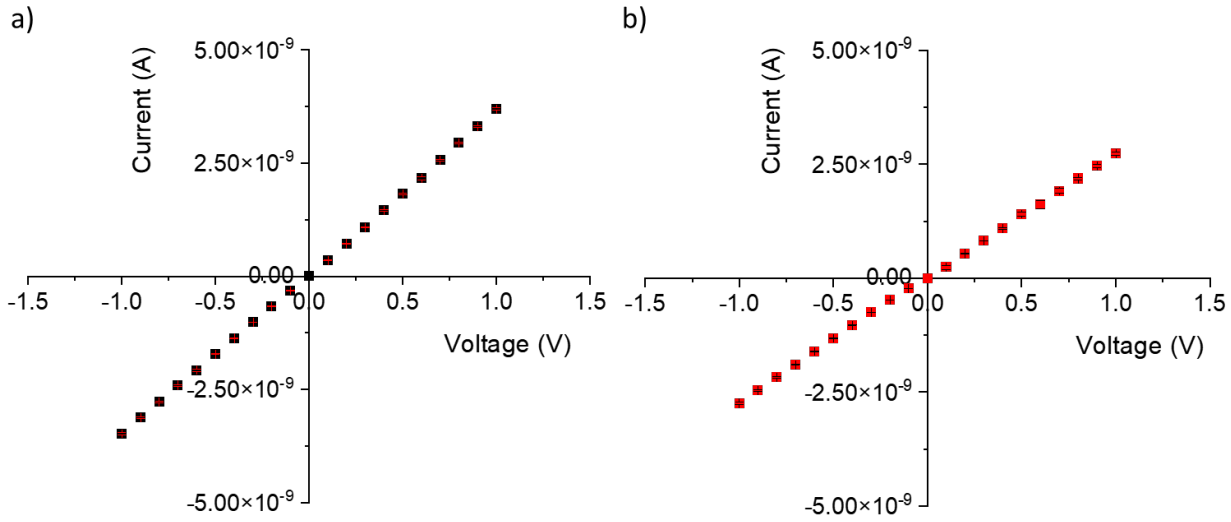

Figure 2.7. a) IV measurements of 1mM (R)-(-)-mandelic acid through the achiral PIM COOH. (R)-(-)-mandelic acid has no interactions with the achiral membrane. b) IV measurements of 1mM (S)-(+)-mandelic acid through the achiral PIM COOH. (S)-(+)-mandelic acid has no interactions with the achiral membrane.

2.6.4 pH Dependent Hydrogen Bonding with Mandelic Acid

Both (R)-(-)-mandelic acid and (S)-(+)-mandelic acid were dissolved in nanopure water to create 1mM solutions. These solutions were left uncorrected at pH 2 to understand the role of hydrogen bonding in the chiral PIM COOH system. Both the chiral and achiral PIM COOH were exposed to the solutions at pH 2. The resulting IV curves show no interaction with either membrane, indicating that hydrogen bonding plays a significant role in the interactions with the chiral membrane.

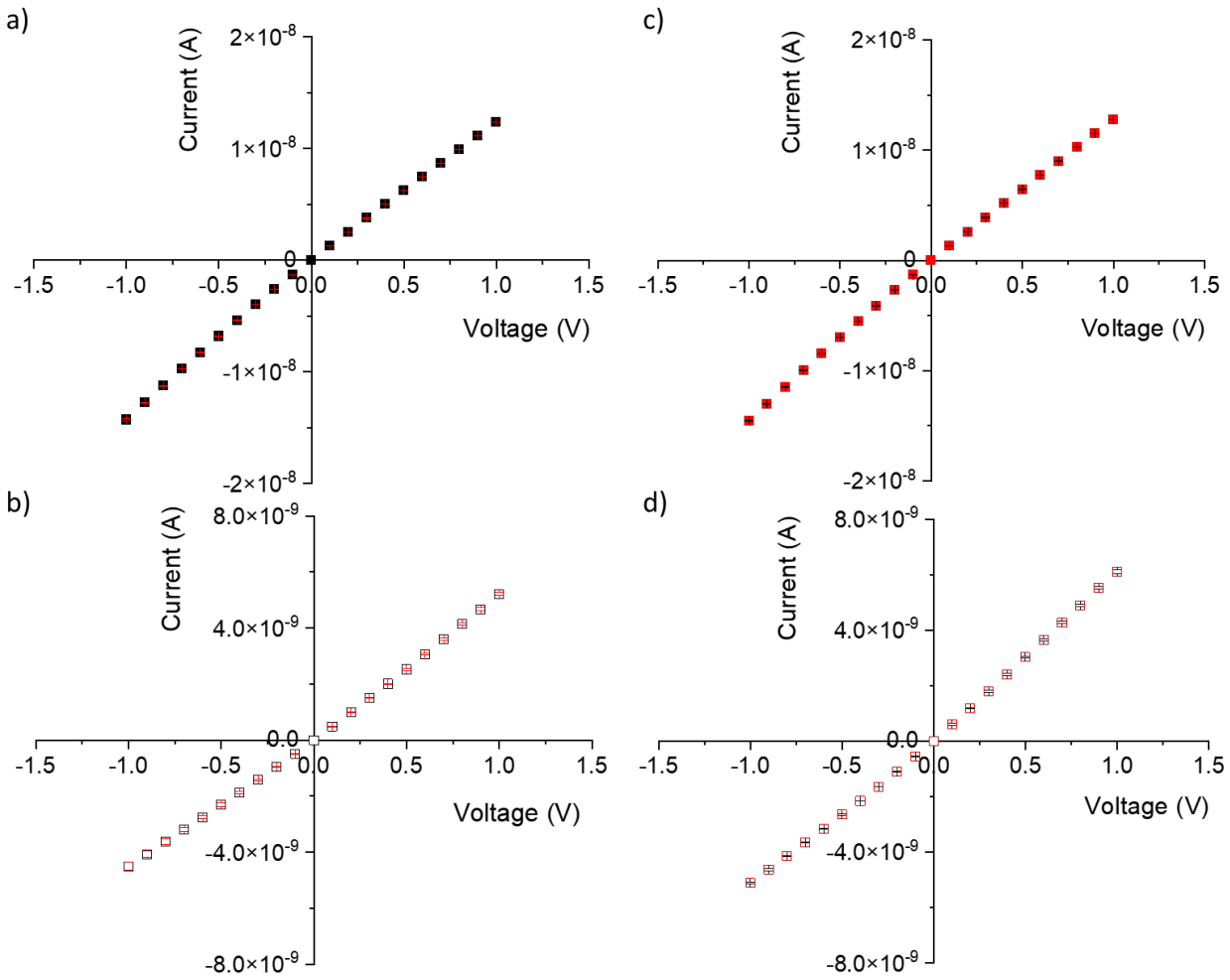

Figure 2.8. a) IV measurements of 1mM (R)-(-)-mandelic acid through the chiral PIM COOH at pH 2. b) IV measurements of 1mM (R)-(-)-mandelic acid through the achiral PIM COOH at pH 2. c) IV measurements of 1mM (S)-(+)-mandelic acid through the chiral PIM COOH. d) IV measurements of 1mM (S)-(+)-mandelic acid through the achiral PIM COOH. Both (R)-(-)-mandelic acid and (S)-(+)-mandelic acid at pH 2 have little interaction with the chiral membrane, unlike at pH 3, indicating that hydrogen bonding plays a role in the mobility of the chiral ions through the membrane. Neither have interactions with the achiral membrane as expected.

2.6.5 Control Studies of Boc-Tyrosine through the Achiral Membrane

The achiral membrane on the SiN pore was exposed to both 1mM boc-L-tyrosine and 1mM boc-D-tyrosine. The resulting IV curves show some interaction with the achiral membrane from the quaternary ammonium coordination with the PIM COOH as expected from, previous studies. However, unlike the chiral PIM, the achiral PIM IV curves show discrete current

measurements at every voltage and no hysteresis. This reconfirms that hydrogen bonding capabilities are important for separation but also that the addition of chiral interactions ultimately causes the rejection of tyrosine from the membrane. It is important to note that the shape of the IV curves shown below is not in relation to any interactions with the ions and the membrane. The shape is inherent to the PIM/SiN system and is also present at 1mM KCl.

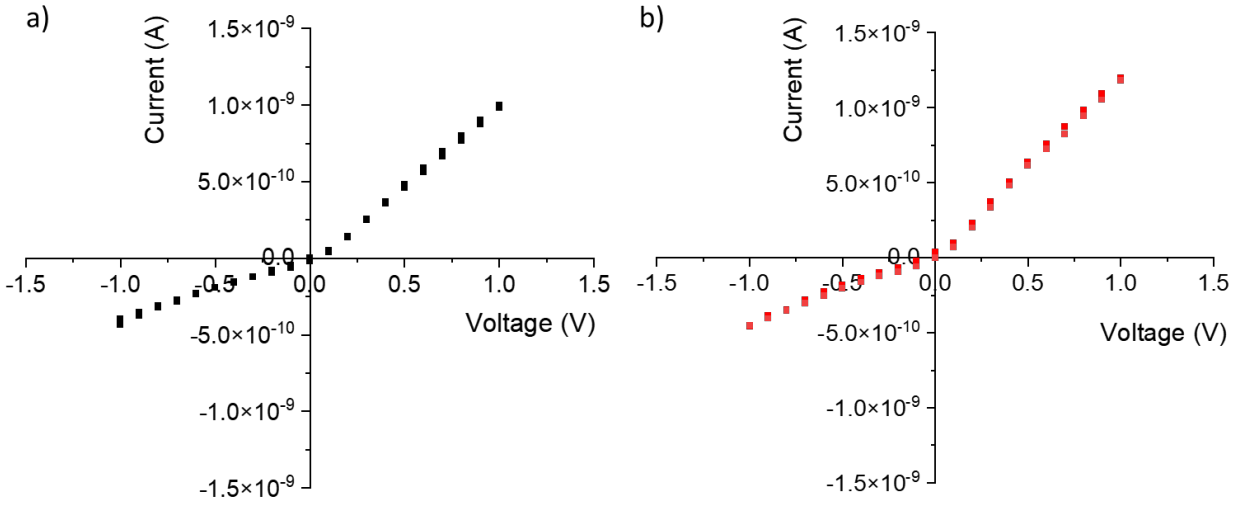

Figure 2.9. a) IV measurements of 1mM boc-L-tyrosine through the achiral PIM COOH. Boc-L-tyrosine has small interactions with the achiral membrane. b) IV measurements of 1mM boc-D-tyrosine through the achiral PIM COOH. Boc-D-tyrosine has small interactions with the achiral membrane.

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Chapter 3: Design and Electrochemical Characterization of a Nanopore Sensor for Cancer Biomolecules

3.1 Preface

While working in the Siwy lab, I also had the opportunity to collaborate with the Dr. Spitale and his graduate student. Dr. Spitale was interested in creating a multifunctional biosensing device, the idea for which he posed to our lab as we have extensive knowledge with all things nanopore-related. I was interested in taking on this project as my background is in biochemistry, and I was excited to design a nanodevice that could be used as a diagnostic tool for cancer molecule detection. Dr. Spitale's graduate student, Abigail Vandewalle, and I collaborated to create a small silicon nitride pore that was functionalized first with a layer of free amines and then with a PEG-linked biotin. The initial idea was to use biotin as a standard linker that could bind to any avidin-enzyme combination and detect a range of cancer biomolecules with the second enzyme. This later will adapt to more complex linkers that can bind niche enzymes specific to certain cancer types and the molecules that mark the onset of these diseases. The studies presented here detail the surface chemistry needed to create a bio-sensing nanodevice and the electrochemical characterizations that confirm the presence of each new functionalization. The electrochemical characterizations can subsequently be adapted for any linker that binds any enzyme.

3.2 Introduction

Recently, the precise and specific detection of biomolecules for genetic diseases and cancer have garnered great interest in the field of clinical diagnostics.[1,2] The advantage to diagnostic methods that are specific to a certain disease is that the likelihood of a false-positive or false-negative result for these diseases is miniscule.[1–3] However, most reported methods for specific

disease detection can require complex platforms, rigorous procedures, expensive materials, and may not be sensitive to low concentrations of biomolecules.[2] Nanodevices have emerged as a new technology for diagnostic purposes as their small size (1-100 nm) render them highly sensitive to single molecules and even atoms and thus low biomolecule concentration in bulk samples is not an issue.[2,4–7] Here, we present the initial studies used to create a nanodevice capable of detecting both small molecule functionalizations and large enzymatic bindings. The nanodevice was created from a ~20 nm single pore fabricated in a silicon nitride chip and modifications with a biotin linker and avidin-HRP within the pore. Current-voltage measurements taken at each modification step are capable of detecting nanometer size decreases due to surface modifications and the 1 to 1 binding of a biotin linker and Avidin-HRP enzyme complex. The work presented here is intended to be initial concept studies for the later development of a nanodevice capable of detecting pancreatic cancer biomolecules.[8]

3.3 Experimental

3.3.1 Reagents.

(3-aminopropyl)trimethoxysilane (APTMS) and Trizma base (99.9%) were purchased from Sigma-Aldrich. NHS-PEG$_4$-Biotin, Avidin-Horseradish Peroxidase, and potassium chloride were purchased from Fisher Scientific. All reagents were used without further purification.

3.3.2 Preparation of Potassium Chloride Solutions.

KCl electrolyte solutions were prepared as 1M stock solutions in nanopure deionized water (18.2 MΩ·cm, Milli-Q IQ 7000 ultrapure water system). 1M KCl contained Tris-buffer at 10mM and was buffered to pH 8 with hydrochloric acid.

3.3.3 Fabrication and Characterization of Single Nanopores in Silicon Nitride Chips.

A ~20 nm single pore was used in the project as the solid construct of the nanodevice. The single pore was drilled by a 200 kV electron beam in the JEOL 2100F TEM in low-stress silicon nitride (SiN_x) films (50μm by 50μm; 30nm thick) purchased from Norcada.[9] After drilling, the pore was washed in a 1:3 H_2O_2:H_2SO_4 (piranha) solution at 100 °C for 30 minutes and rinsed with nanopure water. The diameter the single pores was estimated from the resistance of the pore found by generating current current-voltage (IV) curves in 1M KCl solutions between -1 and 1 V. The pore diameter (D) is estimated using the following equation:

$$D = \frac{G}{2\sigma}\left[1 + \sqrt{\left(1 + \frac{16\sigma L}{\pi G}\right)}\right]$$

where G is the slope (conductance) of the IV curve, σ is the solution conductivity, and L is the pore length.[10] The shape of the pore was assumed to be cylindrical thus all diameters reported are referred as effective diameters.

All IV curves in this study were measured with a Keithley 6487 picoammeter/voltage source (Keithley Instruments, Solon, Ohio, USA) and a software program written in MATLAB (MathWorks, Natick, Massachusetts, USA) using a voltage range between -1 and 1 V with 0.1 V voltage steps. Two non-polarizable Ag/AgCl electrodes were used for all current measurements.

3.3.4 Modification of ~20 nm Single Pore with APTMS.

APTMS was prepared as a 1% silane solution in ethanol in a 10 mL beaker. The single nanopore was the placed into the solution and was allowed to react with the silanes for 30 minutes. The pore was removed from the solution and washed with copious amounts of ethanol. The pore was then placed in a clean 10 mL beaker and heated to 70 ºC for 60 minutes. The pore was cooled

slowly while remaining on the hot plate after the heat was turned off.[11,12] The pore was then removed and placed into a conductivity cell for characterization with KCl.

3.3.5 Addition of NHS-PEG$_4$-Biotin to the Aminated ~20 nm Single Pore.

NHS-PEG$_4$-Biotin was dissolved in nanopure water at a concentration of 60mM in a 10mL beaker. The pore was placed into the solution and was allowed to react with the biotin overnight at room temperature.[13] The following day, the pore was removed from the solution and washed with copious amounts of nanopure water. The pore was placed into a conductivity cell for characterization with KCl.

3.3.6 Binding of Avidin-HRP to the Biotinylated ~20 nm Single Pore.

5 mg of lyophilized Avidin-HRP was dissolved in 3mL of nanopure water in a 10mL beaker. The pore was placed into the solution and was allowed to react with the avidin-HRP for three hours at room temperature.[13] The pore was removed from the solution and washed with copious amounts of nanopure water. The pore was placed into a conductivity cell for characterization with KCl. See Figure 3.1 for the full modification scheme of avidin-HRP to the pore wall.

3.4 Results and Discussion

3.4.1 Characterization of an As-Prepared ~20 nm SiN Pore and Modification Scheme of a Nanoscale Biosensor.

The nanodevice was created by drilling a ~20 nm single pore into a silicon nitride chip using TEM. We elected to create a small nanopore for the device as small size changes due to modifications of the pore wall are easily detected on this scale rather than in a large nanopore. Before any surface chemistry was done to the silicon nitride, the pore was cleaned in piranha acid to hydrolyze the pore walls to a layer silicon oxide and create free alcohols for easy modification. The pore was

sized in 1M KCl to determine its electrochemical characteristics as-prepared. Figure 3.1 shows the TEM image of the drilled nanopore and the resulting IV curve from recordings in 1M KCl. As seen in the IV curve in Figure 3.1b, the pore displays a linear trend indicating a symmetrical, cylindrical geometry before modification.

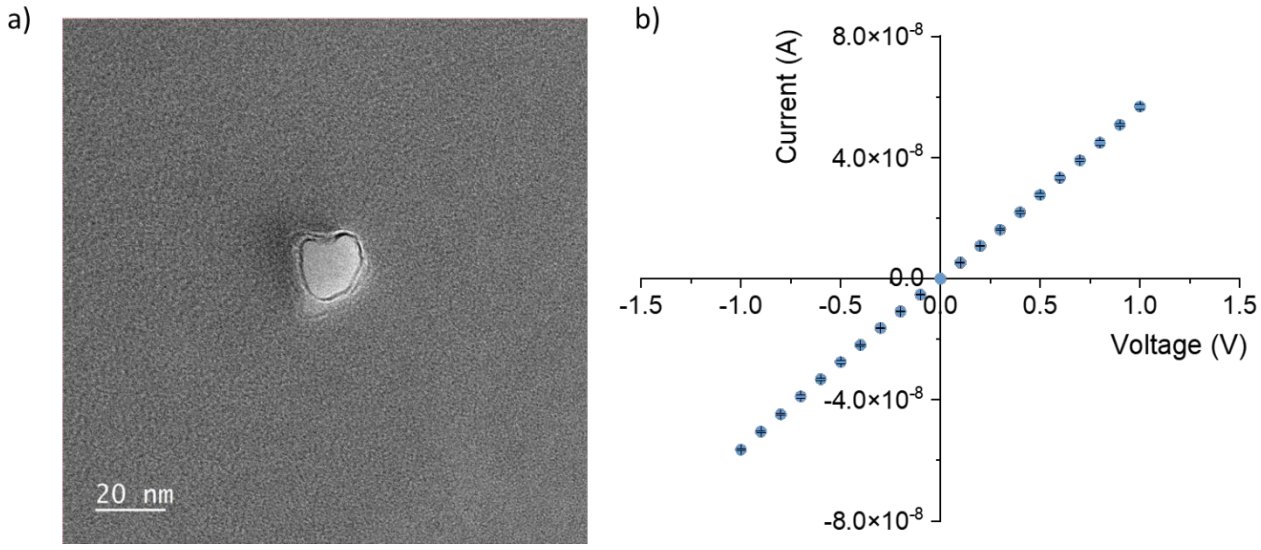

Figure 3.1 a) TEM image of ~20 nm pore drilled in silicon nitride. b) IV measurements of ~20 nm pore in 1M KCl.

The initial concept for the biosensor was to create a biotin linker inside of the pore that could bind to an avidin-enzyme complex. We chose biotin-avidin binding to complete the sensor as the complexation is well documented, can be done in an aqueous solution, and is nearly irreversible once finished ensuring that the complete sensor remains intact after exposure to electrolyte solutions and voltages.[14–16] The challenge was then to functionalize these to the silicon nitride pore. As stated, the pore was cleaned in acid to generate free alcohols at the surface. This enabled us to functionalize the free alcohols with an amino silane, APTMS, to generate free amines.[11] The free amines allowed for NHS ester chemistry to attach the biotin. The avidin-enzyme complex was added as the final step to complete the biosensor. Avidin-HRP was chosen for this

step as the complex was commercially available. Figure 3.2 shows the complete modification scheme for creating a nanoscale biosensing device. All modifications are shown relative to the pore wall on one side. In practice, the modifications would happen on every surface of a cylindrical pore.

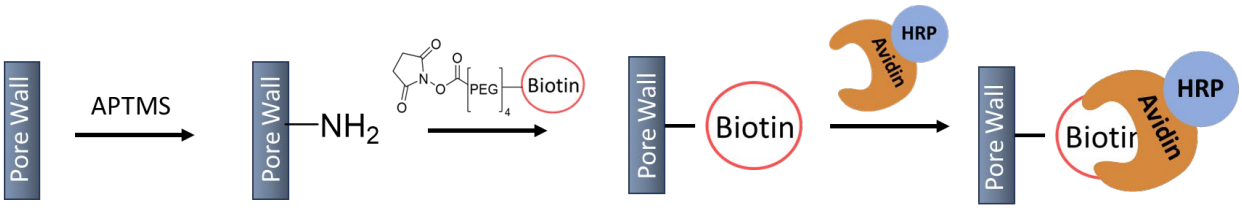

Figure 3.2 Modification scheme of a SiN nanopore wall with avidin-HRP. The pore wall is first modified with ATPMS to generate free amines on the pore surface. The free amines are then functionalized with biotin NHS ester with a PEG spacer. The final modification is the binding of avidin-HRP to the biotin.

3.4.2 Confirmation of Modifications to the Single Pore through Current Decrease in IV Curves.

The surface chemistry with ATPMS was previously reported by our lab on silicon nitride pores and the procedure was adapted for the nanodevice.[11] ATPMS is advantageous over other amino silanes as the reaction happens on a short time scale and the molecule is large enough to detect with current-voltage (IV) measurements. After modification, IV curves were recorded in 1M KCl to confirm the presence of the amino silane addition. Though not intentional, the amino silane asymmetrically functionalized the nanopore on the chip's side facing the beaker in solution. The side facing the beaker received one layer of the amino silane as seen with the size decrease in the positive voltages of about 2 nm, whereas the side exposed only to solution was functionalized with multiple layers as the size decrease by 11 nm (Figure 3.3a).[17] The asymmetric functionalization later became beneficial to definitively confirm the presence of the enzyme in the

final functionalization step. Future iterations of this functionalization will not need asymmetric layers.

The next functionalization was the biotin addition. NHS-PEG$_4$-Biotin was chosen for this step specifically because the PEG linger provided additional size to the molecule for better detection with IV curves and separated the biotin for the pore wall that may contain unfunctionalized free amines. Figure 3.3b shows the IV measurements conducted in 1M KCl after the biotin addition. Because of the uneven distribution of charge and hydrophobic regions inside the pore after biotin was added, hysteresis occurred within the pore over multiple measurements so the size decrease from biotin alone could not be calculated.

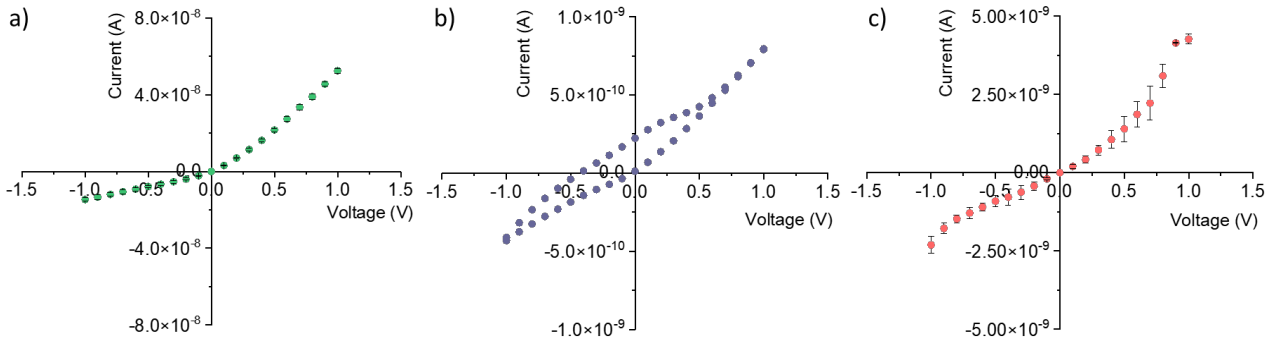

Figure 3.3 a) Modification of ~ 20 nm pore with ATPMS to functionalize pore surface with free amines. IV measurements were conducted in 1M KCl. The resulting size decrease on the top side of the pore is ~ 2 nm and the bottom side is ~11 nm, indicating an asymmetric modification of multiple functionalizations of ATPMS to the pore. b) Modification of ~ 20 nm pore with biotin to the free amines. Due to the nature of biotin, a defined current was not able to be measured as it rendered the pore hydrophobic in 1M KCl. c) Modification of ~ 20 nm pore with avidin-HRP to the biotin. IV measurements were conducted in 1M KCl. The resulting size decrease on the top side of the pore is ~ 12 nm and the bottom side is ~9 nm, indicating an asymmetric modification the enzyme to the top of the pore only.

The final functionalization was the complexation of the biotin and avidin-HRP. The avidin-HRP complex is approximately 9 nm, and the biotin-PEG is approximately 3 nm. In Figure 3.3c, the size decrease in the positive voltage range is roughly 12 nm which is the size of biotin bound

with avidin-HRP. The size decrease in the negative voltage region is roughly 3 nm, indicating that only biotin is attached. This is expected due to the multiple additions of the amino silane in the first modification.

The results of these experiments verify the surface chemistry needed to create a biosensor with an enzyme complex inside of a small nanopore. Further experimentation with the biosensor would expose the nanodevice to a solution of cancer biomarkers and track the binding of the enzyme to the biomolecule. The sensor again would show a current decrease in the IV curves recorded in 1M KCl and therefore an additional size decrease.

3.5 Conclusions

We have demonstrated the modification steps necessary to create a biosensor on a nanoscale. The presence of a biotin linker inside of a small nanopore facilitates the binding of an avidin-HRP enzymatic complex which can be used as a model system for future iterations of the nanodevice. Our future studies will examine a new linker using the same NHS ester chemistry that can is functionalized with a caged molecule that is cleaved when a SNAP enzyme binds to the linker. We intend to use this new sensor to detect pancreatic cancer biomarkers.

3.6 Appendix

3.6.1 Layered IV Curves Displaying the Current and Size Decrease After Each Component's Addition.

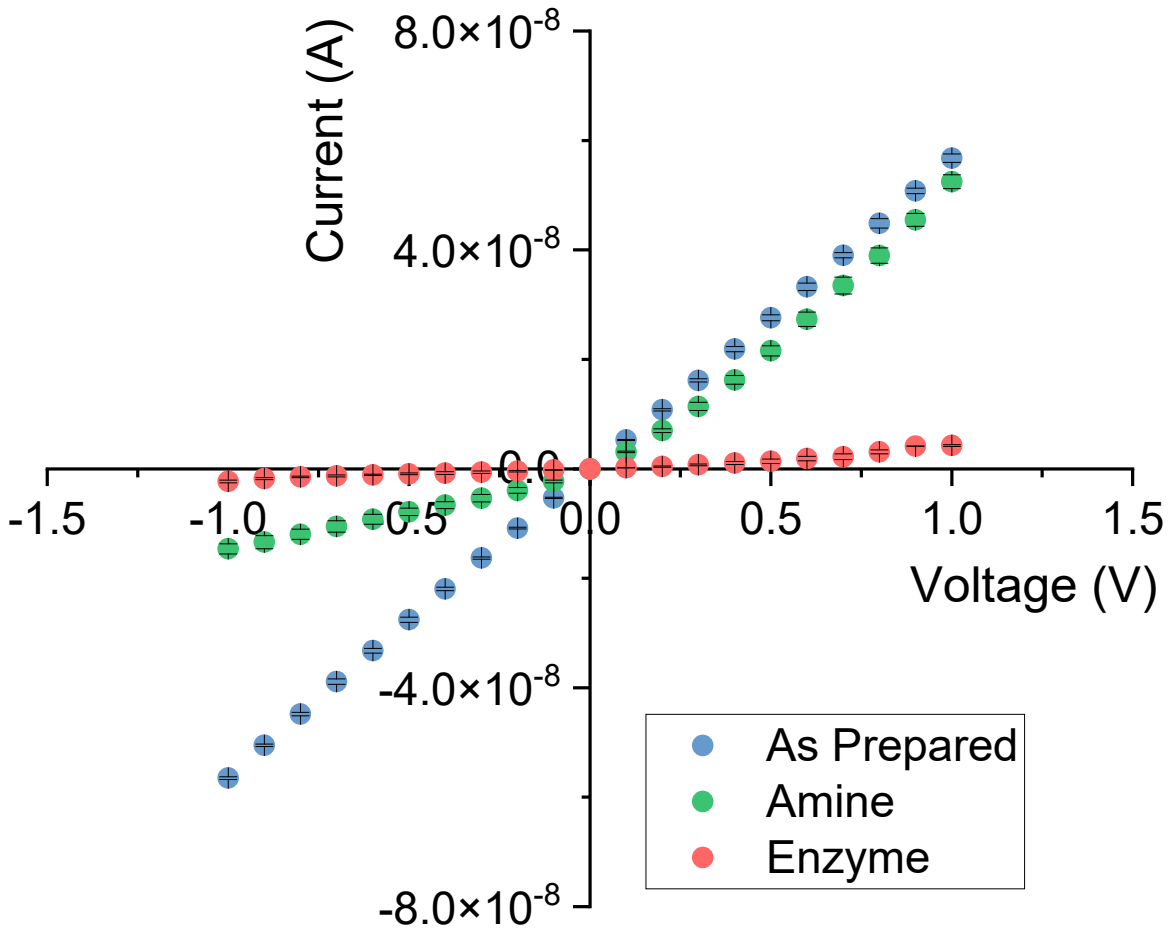

Figure 3.4 Layered IV curves from Figure 3.3 to show the decrease in current and size of pore relative to the pore as-prepared.

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Chapter 4: Online in No Time: Design and Implementation of a Remote Learning First Quarter General Chemistry Laboratory and Second Quarter Organic Chemistry Laboratory

4.1 Preface

During my time in graduate school, I had to opportunity to become the head teaching assistant for the general chemistry laboratory series. I was first mentored by my friend and colleague, Dr. Will Howitz, who had acted as the head teaching assistant before me. When my head TAship started, we had just entered a global pandemic and had 3 weeks until the next quarter to develop an online laboratory class. Will and I worked tirelessly with our fearless leader and the general chemistry instructor, Dr. Kim Edwards, to meet this challenge. We also had the opportunity to work with Dr. Renee Link and the instructional team for the organic laboratory series. Over three weeks, we filmed 8 experimental videos, built entire online structure in Canvas, created comprehensive quizzes to engage student learning, and redesigned the final exam. This chapter will describe the combined efforts from both the general and organic chemistry instructional teams to convert what is traditionally not recommended to be an online experience, into an online course.

4.2 Introduction

The onset of the global COVID-19 pandemic forced chemistry laboratory courses to rapidly shift from hands-on, experiential learning courses to remotely delivered courses.[1] For the lower division laboratory courses at the University of California, Irvine (UCI), this emergency pivot to remote instruction occurred at the end of our winter term, requiring us to create a full quarter of chemistry laboratory courses for more than one thousand students in two weeks. Unlike schools on semester terms where instructors and students already had an established relationship,

the students in our largest laboratory course (general chemistry) began their laboratory experience in this new remote format. We leveraged our existing course infrastructures, including extensive online tools, to create remote learning experiences as similar to our hands-on courses as possible. Both courses took very similar approaches, deviating only where needed to account for differing student needs.

4.2.1 In-Person Course Structure

UCI is a quarter-system school with three, 10-week terms per academic year. Chemistry laboratory courses for non-chemistry majors at UCI are offered in quarters offset from lecture courses (Table 4.1).[2] The total approximate enrollment for these laboratory courses is 1,400 students for General Chemistry Lab I (GCL-I) and 1,000 students for Organic Chemistry Laboratory II (OCL-II). These students are spread across laboratory sections consisting of 20–24 students supervised by graduate student teaching assistants (TAs). Students attend their assigned laboratory section for a single 3-hour 50 minute session each week in the first eight weeks of the term and laboratory practical exams are given in the last two weeks of the term. The general chemistry laboratory courses contain weekly instructor lecture videos but no in-person lecture component. In contrast, the organic chemistry laboratory courses include both prelaboratory lecture videos and a 50-minute weekly interactive laboratory lecture taught by the instructor and offered in multiple sections of approximately 200-400 students.

Table 4.1: Structure of General and Organic Chemistry Courses

Year	Fall Quarter	Winter Quarter	Spring Quarter
First Year	General Chemistry Lecture I	General Chemistry Lecture II	General Chemistry Lecture III
	No laboratory course	No laboratory course	General Chemistry Laboratory I (GCL-I)
Second Year	Organic Chemistry Lecture I	Organic Chemistry Lecture II	Organic Chemistry Lecture III
	General Chemistry Laboratory II	Organic Chemistry Laboratory I	Organic Chemistry Laboratory II (OCL-II)

GCL-I is the first college laboratory course taken by undergraduate science majors, primarily from biological sciences, public health, pharmaceutical sciences, and engineering. For undergraduate students, the first laboratory course can be a difficult transition as this may be their first experience with four-hour laboratory sections, electronic laboratory notebooks (ELNs), new laboratory techniques, and weekly reports. The large enrollment of these inexperienced students is challenging under normal circumstances. In a remote environment where instructional content was developed and implemented right before use, a large instructional team of seven development TAs and three learning assistants (LAs) was needed in addition to the 28 section TAs that would normally be assigned to the course. The development TAs supported the instructor by developing course material, while the LAs provided additional support for students through message boards and office hours.

OCL-II is the last chemistry laboratory course many students complete, typically at the end of their second year. They have already completed three chemistry laboratory courses and are familiar with the rigor, course policies, and technology requirements. Students enrolled in this laboratory course are also familiar with the instructor from a previous laboratory course experience. While the enrollment for this course typically approaches 1,000 students, a smaller offering with only 104 students was required in this scenario because the instructor (RDL) was also supporting colleagues who were converting organic chemistry lecture courses into an emergency remote format. A smaller instructional team of five development TAs and four instructional TAs was needed for this course.

4.2.2 Laboratory Course Objectives and Existing Online Infrastructure

In designing our emergency remote delivery course structures, we focused on maintaining as many of our existing course objectives as possible (Table 4.2). The objective of students performing techniques with chemicals, glassware, equipment, and instrumentation could not be achieved.[3,4] Therefore, we focused on other objectives typically assessed throughout a laboratory course by laboratory reports and during laboratory final exams: data interpretation and calculation, theory behind experiments, conceptual understanding of techniques/procedures, and laboratory safety.[5]

Table 4.2. Course Learning Objectives for General and Organic Chemistry Laboratory Courses

General Chemistry Laboratory	Organic Chemistry Laboratory
1. Prepare solutions using volumetric glassware and calculate solution concentration. Use burette to perform titrations. Demonstrate understanding of the procedures and calculations associated with these techniques.	1. Perform fundamental organic chemistry techniques in the context of laboratory experiments.
2. Operate temperature, conductivity and voltage probes, a simple visible spectrometer, and digital balance to acquire data.	*2. Demonstrate understanding of concepts underlying fundamental techniques by proposing solutions to actual or potential problems encountered during an experiment.
*3. Proficiently use an electronic laboratory notebook to record qualitative observations in detail and quantitative data with the correct number of significant figures.	*3. Accurately draw reaction mechanisms for reactions conducted in laboratory sessions.
*4. Interpret experimental data and calculate results to develop scientifically sound conclusions.	*4. Use spectroscopy data to determine structures of unknown molecules.
*5. Employ basic computational chemistry to explain resonance, acid strength, and reaction coordinate diagrams.	*5. Use data collected from an experiment to make claims supported by evidence.
*6. Demonstrate understanding of basic safety symbols, safety data sheets, corrosives, handling of chemical waste, fire safety, and chemical spill response.	*6. Identify safe and unsafe practices related to techniques used in laboratory sessions.

*Course learning objectives prioritized in designing the remote delivery format.

We were fortunate that our courses were well positioned for the remote environment because we had already built the necessary internet-accessible framework of curriculum and instructional tools.[6] Manuals, technique videos, readings, and instructor videos were already embedded in the ELN, LabArchives, and/or the learning management system, Canvas.[7–22]

Prelaboratory work consisted of online homework and completion of select portions of the ELN.[10,13,23,24] During the laboratory session, students also utilized the ELN to enter procedures, observations, and data. Rubrics for grading on Canvas were already built, and Gradescope, an assignment submission and grading platform, had been used for laboratory practical exam grading for two years.[25] Additionally, we had an existing means of communication with students through the message board, Piazza.[13,26–29] Finally, we recognized we could compile authentic experimental data from the student ELNs of previous iterations of the courses.

4.2.3 Determining Our Emergency Pivot Approach

When converting our courses to a remote delivery format, both instructional teams were guided by principles grounded in the existing chemistry and STEM education literature. Courses were designed in a highly structured format to provide students with accountability for asynchronous coursework and regular formative assessments.[30–37] A combination of asynchronous work and synchronous meetings were included to provide students with a connection to the instructor and TAs while also accommodating their rapidly changing schedules.[38–41] We aimed to keep the course workload similar to the previous course format (or lighter if possible) for students and TAs.

In considering the best approach to transition our courses into an online format, we evaluated known replacements for experimental work. While simulations exist for general chemistry laboratory courses, we determined that we could not develop a rich online framework around such simulations comparable to the existing curriculum of GCL-I. Furthermore, we could not find simulations to cover half of the topics within GCL-I (Table 4.3). Vendor-supplied kits for home experiments were not considered because of their cost and the lead time required to customize kits.[42] Far fewer resources exist for virtual organic chemistry laboratories. The resources that do exist focus mainly on introducing laboratory techniques typically covered in a first-term course[43]

or incorporate verification experiments at odds with our standard curriculum (Table 4.3).[44] For both courses, we felt that the instructional tools present in our current electronic course content (i.e., lecture videos, online homework, computational studies) were essential for student understanding of the content of whatever modality we chose to replace in-lab experimentation.[6] We also felt that developing supporting curriculum and summative assessments for new content would add significant effort to an already challenging quarter. We concluded the more expedient and pedagogically appropriate choice was to film experiments and use previously obtained data for both laboratory courses.[18–20] Access to a public Google Drive folder containing our instructional materials and experiment videos is available in the Supporting Information.

Table 4.3 Descriptive Summaries of GCL-I & OCL-II

GCL-I Experiments	OCL-II Experiments
1. Enthalpy of Formation: Coffee cup calorimetry and Hess' Law used to find the enthalpy of reaction.	1. Clove Oil Steam Distillation: Eugenol is distilled from cloves. Purity is assessed by TLC and ^{1}H NMR.
2. Equilibrium and Visible Spectroscopy: The iron thiocyanate equilibrium constant is found using visible spectroscopy and LeChatelier's principle.	2. Electrophilic Aromatic Substitution: Relative reactivities determined by bromination of aromatic rings bearing various substituents.
3. Computational Study of the Thiocyanate Ion: Spartan is used to investigate the actual structure of thiocyanate by looking at bond lengths and orbitals. Diatomic molecular orbitals are determined in the process	3. Wittig: Each lab section selects a variable to explore (Wittig salt, aldehyde, or base) from a list of available chemicals. The focus is on how the selected variable might affect the E/Z selectivity of the products. Students run proposed reactions during the second week of the experiment. ^{1}H NMR analysis of all products within a lab section are shared to all students in that section. Students identify any trend present and address how this trend corresponds with their initial hypothesis.
4. Dissolution Thermodynamics: The enthalpy and entropy change for the dissolution of borax is determined by acid-base titration of borate ion samples taken at different temperatures.	4. Oxidation and Reduction: Oxidation of 4-t-butylcyclohexanol to 4-t-butylcyclohexanone. Reductions of 4-t-butylcyclohexanone to 4-t-butylcyclohexanol using sodium borohydride and Meerwein-Pondorff-Verley conditions. ^{1}H NMR spectroscopy of resulting product mixtures. Spectra for product mixture resulting from reduction using L-selectride provided. Analysis requires explaining the differences in product mixtures under three different sets of reduction conditions. Help with analysis is provided in video and in person during lab lecture.

5. Electrical Conduction of Solutions:
The conduction of various electrolytes is measured as a function of increasing atomic mass, acid strength, and increasing concentration. The equivalence point of a double displacement reaction is determined by conductometric titration.

6. Acid-Base Buffers:
Preparation and the investigation of the effects of acid or base addition and buffer dilution on pH. Spartan investigation of dissociation as a function of acid strength.

7. Electrochemical Cells:
Measurement of cell potentials, creation of reduction potential table, and investigation of the effect of concentration on cell potential.

8. Rate Law Determination and Visible Spectroscopy:
Visible spectroscopy is used to measure the disappearance of crystal violet as a result of hydroxylation. The rate law, rate constant, and half-life are determined. Spartan investigation of a reaction coordinate diagram.

5. Determining Absolute Configuration Using CEC Method:
Experiment developed from work in Rychnovsky lab. Students qualitatively and quantitatively determine which reaction proceeds faster in a matched and mismatched case of acetylation of an alcohol with a chiral catalyst and determine absolute configuration of unknown chiral alcohol. Review of assigning R/S and optical rotation included.

6. Aldol Condensation:
Double aldol condensation with unknown aldehyde and ketone. Differentiate aldehyde and ketone by IR spectroscopy. Determine structures of unknowns by first determining structure of product by ^{1}H and ^{13}C NMR.

4.2.4 Creating Video Versions of Our Existing Experiments

Both the GCL-I and OCL-II course teams filmed video content during spring break and the beginning of the spring quarter while following all public health guidelines. The GCL-I videos were filmed and edited by the development TAs, whereas the OCL-II videos were filmed and

edited by the university media team. TAs wrote the scripts and served as actors in the videos. During the editing process, videos were segmented into approximately 15 minute portions to maintain student attention and increase comprehension.[45] Automatically graded Canvas video quizzes promoted student accountability and engagement.[46,47] TAs in the general chemistry videos narrated their actions in detail to guide less experienced students through the basic techniques and data collection performed. In contrast, the more advanced students in OCL-II were already familiar with fundamental laboratory techniques, so video narration required less detail.

4.3 Structuring the Remote Versions of the First-Term General Chemistry and Second-Term Organic Laboratory

4.3.1 Scheduling

To achieve a high structure format for the remote versions of both GCL-I and OCL-II, modifications from the in-person versions of the courses were required. Many of the structural similarities between the two courses allowed for equivalent alterations to scheduling and ELN use. The typical experiment schedule for both courses was delayed by one week to expand the time available for curriculum development and provide students with a structured introduction to the online laboratory format. In the GCL-I course, the first week of the quarter introduced students to the online tools required for the course (i.e. Zoom video conferencing tool, Piazza message boards, ELN, Canvas, Spartan computational software, and Sapling Learning online homework) through webinars.[21,22,26,48–50] Because most students in the OCL-II course were already familiar with the online tools, the first week was devoted to a writing workshop in which students critiqued and revised one of their laboratory reports from a previous course. Delaying experimental work also allowed us to support the technological needs of students and TAs. Laptops with cameras were

loaned to students from our teaching laboratory stockroom. Writing tablets, webcams, and smartphone holders were distributed to TAs to enable remote teaching.

For the in-person version of both courses, assignment due dates were scheduled to correlate to the day and time of a student's laboratory section. To provide a clearer course structure for students enrolled in the remote courses, the availability of weekly content and assignment due dates were made the same for all students, regardless of the day and time of their scheduled laboratory section (Figure 4.1).[47,51] To provide additional clarity, both courses utilized the announcement function of Canvas on a weekly basis to connect due dates to assignment expectations.[29,52]

Week	Monday	Tuesday	Wednesday	Thursday	Friday
3	GCL-I SAPLING DUE 11:59 PM	GCL-I EXP 2 VIDEO AND CANVAS QUIZ OPEN 12 PM		GCL-I EXP 1 POST LAB DUE 11:59 PM	GCL-I EXP 2 PRE/IN-LAB AND CANVAS QUIZ DUE 11:59 PM
	OCL-II EXP 2 VIDEO OPEN 10 PM	OCL-II PRE-LAB VIDEO QUIZZES, SAPLING, AND EXP 2 PRE-LAB DUE 1 PM		OCL-II EXP 1 POST LAB DUE 11:59 PM	OCL-II EXP 2 IN-LAB AND CANVAS QUIZ DUE 11:59 PM
4	GCL-I SAPLING DUE 11:59 PM	GCL-I EXP 3 VIDEO AND CANVAS QUIZ OPEN 12 PM		GCL-I EXP 2 POST LAB DUE 11:59 PM	GCL-I EXP 3 PRE/IN-LAB AND CANVAS QUIZ DUE 11:59 PM
	OCL-II EXP 3 VIDEO OPEN 10 PM	OCL-II PRE-LAB VIDEO QUIZZES, SAPLING, AND EXP 3 PRE-LAB DUE 1 PM		OCL-II EXP 2 POST LAB DUE 11:59 PM	OCL-II EXP 2 IN-LAB AND CANVAS QUIZ DUE 11:59 PM

Figure 4.1. Representative two-week schedule for GCL-I (green) and OCL-II (blue).

4.3.2 Electronic Laboratory Notebook and Data Sets

In previous quarters, students completed all sections of a blank ELN page weekly. To account for the lack of in-person communication this quarter, scaffolding was added to the ELN page and gradually reduced as the course progressed. In initial experiments, prefilled sections were added as examples for students to reference in later weeks when the scaffold was removed. This modification was included in both courses to ease the ELN learning curve for the new laboratory students in GCL-I and provide added direction in OCL-II.

Student ELN entries from previous iterations of the course were also leveraged to provide unique data sets to minimize academic dishonesty. These data sets were distributed to each section at the end of the Canvas video quizzes (see Appendix for data delivery instructions). The two instructional teams had different goals when selecting data. The general chemistry team provided "good" data that approximated ideal results to ease new students into the teaching laboratory course environment. The organic chemistry team, however, provided their more experienced students with imperfect data to provide opportunities for rich discussion around limitations of experiments and their outcomes.

4.3.3 Staff Meetings, Office Hours, and Class Meetings

Although both courses retained similar course structures, differences in enrollment and student demographics required course-specific approaches to laboratory lectures, teaching staff meetings, office hours, and online homework. Some synchronous class meetings were held for each course, although the approach to these meetings differed. During weeks when challenging concepts were introduced in GCL-I (e.g. graphing, calculations with significant figures, etc.), multiple live webinars were held to supplement instructor lecture videos. Students in OCL-II attended one 50-minute weekly, interactive laboratory lecture on Zoom. Students engaged with

material using PollEverywhere to earn participation credit and communicated using Zoom's chat feature.[53] The laboratory lecture was recorded and provided along with a make-up assignment on Canvas for students who could not attend. A similar online lecture format has been offered in previous years. This experience enabled us to easily shift to a fully online laboratory lecture and expand existing instructional techniques in OCL-II.

Management of the TAs in both courses was handled using a weekly one-hour staff meeting on Zoom. During previous in-person meetings, a group of 3–4 TAs who performed the current week's experiment beforehand would present various procedural tips and tricks which they believed would help fellow TAs in the laboratory. For GCL-I this quarter, these TAs could not perform the experiments, so presentations focused on contextualizing the laboratory material and explaining the theory underlying the laboratory techniques and instrumentation. Because OCL-II had fewer TAs due to lower enrollment, staff meetings required less structure.

The GCL-I TAs held one, two-hour office hour weekly over Zoom in pairs. Each pair's office hours were scheduled on the same day and time as their assigned laboratory section to ensure students could meet with their designated TA. Student attendance was encouraged, but not required. More experienced TAs were strategically paired with less experienced TAs. Within the pair, one TA responded to questions by speaking while the other TA responded to questions by typing into the chat window.

The OCL-II TAs met with students online during the first half of their regularly scheduled laboratory section and held two, one-hour office hours each week. Like GCL-I, student attendance at these scheduled meetings was encouraged, but not required. TAs began synchronous class meetings by giving a short summary of the experiment. Then TAs played the in-laboratory videos using screen share and stopped the video at strategic points to engage students in a discussion of

key steps or concepts. Class meetings ended with a question and answer session. In addition to the class meeting, OCL-II TAs held two unstructured office hour meetings. Pairing TAs was unnecessary for office hours as there were fewer attendees. Office hours for the organic chemistry laboratory course were also held over Zoom using the Canvas integration.[54]

4.3.4 Monitoring Online Homework

Although both courses use online pre-laboratory homework, the GCL-I team developed a computer script, titled BigBrother, to streamline TA responsibilities. In a typical academic term, TAs would log into Sapling and manually check for incomplete assignments. In GCL-I, a course with 28 TAs for 56 lab sections, BigBrother identified students with incomplete online homework and sent a student list by section directly to the appropriate TA's account in a messaging platform (Slack).[55] The annotated code is provided in the Supporting Information.

4.4 Replacing the In-Person Laboratory Practical Exams

Both the GCL-I and OCL-II courses typically conclude with a practical exam.[56–59] Different approaches were taken by each course to replace these exams because GCL-I uses a traditional points-based grading system, whereas OCL-II uses a specifications grading system.[60,61] However, the widespread social uprising that occurred in late May and early June of 2020 in response to the deaths of Ahmaud Arbery, Breonna Taylor, George Floyd, and others necessitated alterations to our plans. Many of our students were directly impacted by the widespread protests and media reporting. We include both the intended exam replacement plans and our emergency adjustments here for clarity and discussion.

The traditional, in-person format of the GCL-I practical exam consisted of students performing two short wet-laboratory exercises taken directly from experiments conducted during the quarter, analyzing data collected from a computational study, and answering multiple-choice questions

pertaining to safe laboratory practices. In the remote version of the course, the new exam consisted of two parts: a Canvas quiz, requiring the Respondus LockDown browser and Monitor AI, and two "take-home" essays submitted to Gradescope.[62] The Canvas quiz assessed the understanding of chemical theory and data analysis. The goal of the essays was to encourage students to demonstrate conceptual understanding of two general chemistry laboratory techniques.[63–65] Students selected and responded to two of six possible essay prompts. They then researched and described the procedures of their two chosen laboratory techniques in detail. A table of essay prompts, response rates, and averages can be found in the

4.4.1 Supporting Information.

GCL-I final exams began Monday, June 1, 2020. However, campus guidance for changes to final examinations was announced two days later as administrators attempted to respond to the evolving social uprising and its impacts on our students. Because the exams had already started for about half of the 1,403 students, the alteration of exam content or conditions could be perceived as unfair by those who had already taken the exam. However, students needing accommodations throughout the week were allowed to take the online exam or turn in the essays at later dates.

The in-person final exam structure for OCL-II consisted of three required assignments for all students to earn passing grades and additional assessments to achieve an A or B letter grade. The initial plan for final assessments in this remote format retained all of the in-person components with two adjustments. Two of the mandatory assessments, a safety exam and an exam covering concepts and data analysis, would be administered as automatically graded Canvas quizzes. These quizzes were intended to evaluate understanding of laboratory safety and overall understanding of course content. A third mandatory assessment on thin layer chromatography would be converted

from a hands-on activity to an online quiz using both Canvas and Chemix, a chemistry diagramming software.[66]

To earn more than a passing grade in the course, students would have completed additional technique assessments using Canvas and Chemix on liquid-liquid extraction and recrystallization (Figure 2). Students would also have completed a mastery project where they develop a hypothesis and analyze experimental data related to a previously studied reaction. The project, designed to replace open-ended questions on typical practical exams, could either be presented as a lab report for a B grade or as a journal-style article or research poster for an A grade.[67,68]

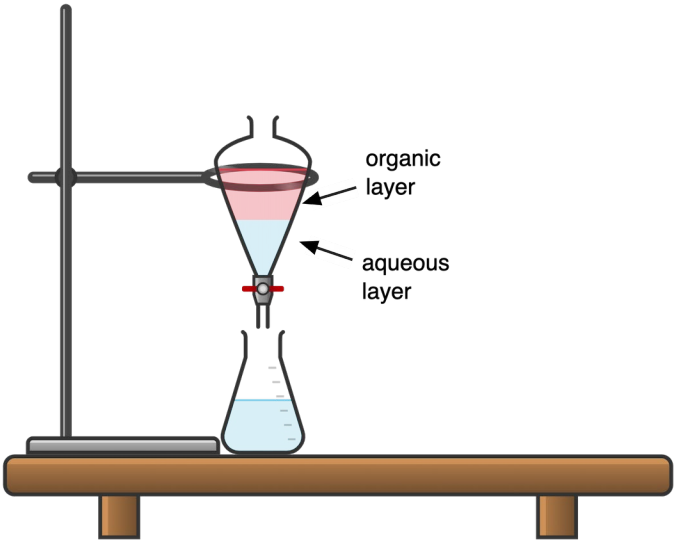

Figure 4.2. Chemix drawing of liquid-liquid extraction.

Although we created a comprehensive set of exam replacements, the social upheaval that impacted our students necessitated a rapid change in plans. The following adjustments were made to the final examination for the organic chemistry laboratory: technique tests required for A and B grades were already completed, but the remainder of the planned assessments were cancelled. A set of alternative assignments were introduced. All students chose from one of the three following options: 1) complete the mastery project they had already started, 2) write advice to students

attempting to study during times of great trauma, or 3) create a multimedia presentation of their choice connecting chemistry to something they were experiencing. All three assessment options were graded on a complete/incomplete basis with credit awarded for any good faith effort. Students appreciated the accommodations, and several welcomed them as a safe space to express their struggles in dealing with traumatic experiences.

4.5 Student and TA Feedback

Surveys were administered to determine how students and TAs perceived the remote course structure. The GCL-I team administered two surveys: a mid-quarter and a post-quarter. Of the 1,403 students and 28 TAs in the GCL-I course, 79% of students and 64% of TAs responded to the mid-quarter survey, respectively (Table 4). A total of 67% of students and 82% of TAs completed the post-quarter survey. The OCL-II team administered one student survey late in the quarter. Of the 104 students in the OCL-II course, 84% of students responded. Because this survey was completed later in the quarter than the corresponding survey in the GCL-I course, a final student survey was not conducted. Informal feedback was collected from TAs weekly, and a TA survey was conducted at the conclusion of the term. Survey questions are included in the Supporting Information.

Table 4.4 Feedback collection methods and response rates for remote delivery GCL-I and OCL-II.

Feedback Collection	GCL-I Response Rate	OCL-II Response Rate
Student mid-quarter	1,101 (79%)	87 (84%)
TA mid-quarter*	18 (64%)	4 (100%)
Student post-quarter	943 (67%)	NA
TA post-quarter	23 (82%)	4 (100%)

*Feedback from OCL-II TAs was solicited through conversations in weekly staff meetings.
Students: N = 1,403 for GCL-1, N = 104 for OCL-II, TAs: N = 28 for GCL-I, N = 4 for OCL-II.

4.5.1 GLC-I Student Feedback

Student responses to the mid-quarter and post-quarter surveys were mostly positive. Students valued Canvas, citing its modular set-up and summary of assignment due dates. They also appreciated the video demonstrations of the experiments and taking associated quizzes. Perceptions of the ELN and Piazza in the mid-quarter survey were mixed. Actions were taken to address these concerns and post-quarter survey responses indicated the changes made were well received.

While students liked the scaffolding of the ELN, they wanted more direction for its use. We subsequently recorded an instructional video describing the use of the ELN functionalities, especially how to properly download and submit the page for grading to aid students navigating the ELN for the first time.[69,70] Students also expressed frustration and anxiety about the time consuming nature of filling out the ELN, a sentiment which is not unique to this remote course. Students are often surprised by the workload in their first laboratory course. The instructor and two development TAs filmed short videos addressing these and other student concerns from the

mid-quarter survey which was intended to enhance student perceptions of instructor presence in the course.[18,40]

Students also identified the Piazza message board as a source of anxiety. The number of message board posts was ten times higher than the previous year (Table 4.5). Many students felt they needed to read all responses to ensure understanding of assignment expectations. Conversely, the redundant questions indicated many other students were not reviewing answered posts before making their own. This behavior was partially encouraged by a faster average response time compared to the prior year.

Table 4.5. GCL-I Piazza Statistics for 2019 & 2020

Comparison of In-Person & Remote Instruction	Spring 2019	Spring 2020
Questions asked	903	7,131
Posts, responses, edits, follow ups, comments	2,615	29,806
Average response time	33 minutes	6 minutes
Percentage of students with at least one contribution	32%	62%

To reduce the number of posts and student anxiety, many question-by-question responses were curtailed. The most commonly asked questions each week were compiled and answered in a single announcement. This reduced the overall number of posts and provided the TAs with a set of talking points to address during office hours. Immediately following the first announcement, the number of posts was almost cut in half, but the number of users (viewers) remained very high (Figure 4.3).[71,72] In the final course survey, students indicated the changes to Piazza reduced anxiety by making answers easy to find.

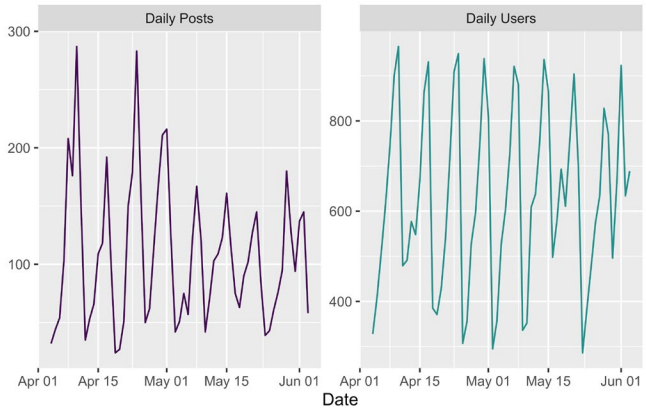

Figure 4.3. GCL-I Remote Instruction Piazza Message Board Users and Posts

4.5.2 GCL-I TA Feedback

GCL-I TAs overwhelmingly agreed that the most positive moments they experienced with students were running office hours through Zoom. TAs noted having a partner to split work between vocal and written (chat) response was an optimal arrangement. However, TAs did indicate occasional difficulty fielding a large volume of student questions through the chat function. TAs appreciated the ability to screen share to guide students through online tools and subject matter questions. They also noted the regular attendance of a sizable number of motivated students, in contrast to much lower attendance of typically 5-10 students for in-person office hours in previous quarters. Weekly attendance at each office hour started at over 100 students on average and then dropped to about 25 students toward the end of the quarter (Figure 4.4). While TAs commented positively about the use of Zoom, they also voiced concerns about the lack of connection to their students because of the absence of face-to-face contact.

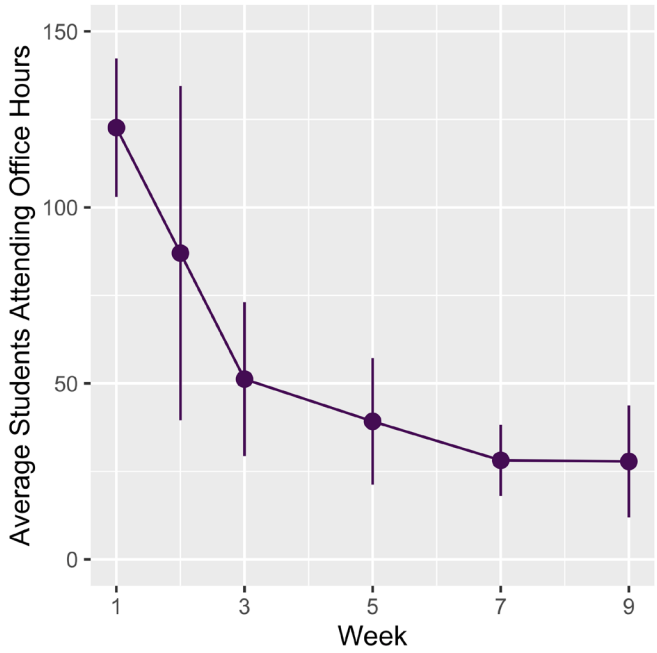

Figure 4.4. Average GCL-I office hour attendance by week. Error bars are ± standard deviation.

The changes to in-person staff meetings that were adopted for the remote setting were described by TAs as insufficient preparation for teaching during office hours. Most of the student questions pertained to assignment rubrics and grading rather than the theory underlying the laboratory techniques and instrumentation. TAs expressed that going over the rubrics during the staff meeting would be better preparation for their office hours. This change was made following the mid-quarter survey and was received positively based on TA responses to the post-quarter survey. TAs voiced that the change lessened the time spent on grading overall so it was easier to meet the weekly grading deadlines set by the instructor. TAs also unanimously praised the integration of the BigBrother code with Sapling and Slack, commenting that the code lightened their workload because it simplified checking the Sapling pre-laboratory requirements, which was done manually in previous quarters.

4.5.3 OCL-II Student Feedback

Students in OCL-II were surveyed once in the latter part of the term before final exams. No immediate course changes were made because the survey was administered after the final experiment week concluded. We planned a post-course survey to gather student feedback on the exam components, but this survey was abandoned when exams were cancelled. When asked what they liked best about the online lab sections, many students remarked they could more easily ask questions in this format. Students attributed this difference to greater ease of getting the TA's attention and a lack of time pressure to complete laboratory work. Many students valued watching the videos together with their TA and classmates. However, they suggested that the TAs should have more structure in guiding the class discussion around what was happening in the experiment videos. Students appreciated the overall structure and organization of the course, especially the consistent weekly deadlines. Most comments on improvement for the course organization addressed issues of Canvas structure that cannot be altered. Students felt that the lab lecture component of the course was helpful, and those who had experienced the in-person laboratory lecture in previous courses thought the online version was similar. Based on student comments, we succeeded in establishing a sense of connection between the students and the instructors in this new course format, but many students felt disconnected from their classmates in the online environment.

4.5.4 OCL-II TA Feedback

At the conclusion of the course, the OCL-II TAs completed a survey comparing the remote teaching experience to their prior in person experience. TAs felt that the remote lab required a smaller time commitment due to the lack of in-person, four-hour lab periods. An average of 13 students attended weekly "in-lab" meetings where TAs led viewing and discussion of the video

with students, and office hours attendance was less than for in-person courses they had taught previously. Typically, students needed more guidance when interpreting data and performing error analysis as compared to TAs' previous experiences.

OCL-II TAs also commented on some of the benefits and challenges associated with remote learning. TAs cited an increased focus during the remote "in-lab" meetings on theory and concepts associated with the experiment in comparison with prior in-person teaching experiences. This change may have resulted from a decreased cognitive load required to watch experiment videos instead carrying out experimental procedures.[73,74] The increased flexibility of the online format also allowed students to contact their TAs more easily compared to attending in-person office hours. In contrast, TAs felt the biggest challenges, aside from lack of hands-on experience, were associated with TA-student interactions. Although some students were more engaged, this was not true for all students. TAs perceived an overall decrease in student participation and struggled to assess the gaps in students' knowledge.

4.6 Lessons Learned and Planned Changes for Future Iterations

Despite the limited time frame to enact our emergency pivot to a new remote delivery environment for the GCL-I and OCL-II courses, the students and instructional teams for both courses felt the endeavor was a success. The positive student response to our emergency remote laboratory courses will inform the creation of additional laboratory courses while the global pandemic necessitates continued remote learning. Future courses, currently in development, will retain the same overall structure, consistent due dates, and synchronous class meetings with asynchronous options for students experiencing scheduling challenges.

The instructional teams of the general and organic chemistry laboratories historically have worked together, adopting many of the same web-based tools that served us well during this

pandemic. Our similar approaches allow us to address the challenges we encountered during the first quarter of remote instruction in ways that will improve future iterations of both laboratory series. Based on the GCL-I team's experience, staff meetings will be restructured to help TAs focus on student needs in the remote delivery of the course. Because the experimental videos are now complete, TAs will be required to watch the experiment video and fill out an electronic survey before each meeting. This survey will have two goals: (1) to actively engage the TAs in video experiments and, (2) to generate talking points for office hours with students. A group of TAs will also be assigned to lead a discussion of the survey responses and rubrics for the experiment running that week. All large-scale courses will manage message boards with daily instructor posts. Because course content has now been prepared, we plan to open modules earlier in each term to allow students greater flexibility in managing their weekly workload. Finally, we endeavor to create more connections between students during Zoom lab sessions by strategically employing tools such as polling and emoticon use to encourage full participation, using the new Live Chat function in Piazza to structure discussion, and instituting group work where applicable in break out rooms.[75–78]

Regardless of the successes we have had in creating online laboratory courses, we still strongly assert that this emergency replacement does not meet the primary objective of any laboratory course — performing fundamental laboratory techniques. To enhance all aspects of learning chemistry, hands-on interaction with chemicals and laboratory instruments are essential.[3] While we were able to challenge our students with assignments that required conceptual understanding and critical analysis, we could not assess their ability to manipulate laboratory glassware or use laboratory instrumentation.[5] We look forward to the return of in-person laboratory courses.

4.7 Appendix

4.7.1 IRB Statement

This study was approved by the University of California, Irvine, Institutional Review Board as exempt (IRB 2018-4661) including FERPA compliance.

4.7.2 GCL-I Mid-Quarter Survey Questions for Students

1. What times zone are you in? *short answer*

2. Who is your TA? *drop down*

3. Are lab reports returned in a timely manner and graded fairly? Do they contain useful feedback? Provide specific examples. *paragraph*

4. How are office hours? What have TAs done well during this time? What could be done better? Please share any suggestions you have. *paragraph*

5. Does your TA respond in a timely manner to email? Is the response helpful? *paragraph*

6. My TA's expectations are clear and the enforcement of those expectations is consistent. *5-point Likert scale: strongly agree to strongly disagree*

7. My TA is well organized and provides clear explanations. *5-point Likert scale: strongly agree to strongly disagree*

8. Please use this space to provide any other comments about your TA that you would like to include. *paragraph*

9. What has been the most positive moment for you in this course so far and why? *Paragraph*

10. What has been the most challenging moment for you in this course so far and why? *paragraph*

11. What is your primary source of information in this course? How can it be improved? *paragraph*

12. What technology challenges have you had? If there is something you think we could do to help, please describe your issue. *paragraph*

13. What do you like about the LabArchives ELN? What could be better? Is the prompting on the Pre/In lab pages helpful? *paragraph*

14. What do you like about the course content in Canvas? What could be better?

15. Please use this space to provide any other comments about the course that you would like to include. *paragraph*

4.7.3 GCL-I Mid-Quarter Survey Questions for TAs

1. What time zone are you in? *short answer*

2. What year of your program are you in? *short answer*

3. Have you taught a general chemistry lab before? *multiple-choice: yes or no*

4. Select the classes you have taught before. *multiple answer*

5. Describe what worked well on Zoom. *paragraph*

6. What has been the most positive moment for you in this course thus far? *paragraph*

7. What has been the most challenging moment for you in this course thus far and why? *paragraph*

8. What would you change about this course and why? *paragraph*

9. How would you change this course? *paragraph*

10. Please use this space to provide any other comments about the course that you would like to include. *paragraph*

4.7.4 OCL-II Mid-Quarter Survey Questions for Students

1. Are you attending the online equivalent of lab section time with your TA on Zoom? *Likert: never- always*

2. What is helpful about attending the online equivalent of lab time? *paragraph*

3. What suggestions do you have for improving the online equivalent of lab time? *paragraph*

4. Are lab reports returned in a timely manner? *Likert: never-always*

5. Do graded lab reports contain useful feedback? *Likert: never-always*

6. Please provide specific examples about timeliness of graded work and/or feedback on graded work. *paragraph*

7. Do you attend your TA's office hours? *Likert: never-always*

8. Describe something that is working well in your TA's online office hours. *paragraph*

9. Describe any suggestions you have to improve office hours. *paragraph*

10. Does your TA respond to email in a timely manner? *Likert: never-always*

11. Are email responses from your TA helpful? *Likert: never-always*

12. Please use this space to provide any other comments about how your TA is supporting your learning. Remember to be specific and professional with your feedback. *paragraph*

13. How do you normally attend lab lecture? Choose the option you use most often. *Multiple choice*

14. What aspects of lab lecture do you feel have worked well for you? *paragraph*

15. What aspects of lab lecture would you change? How would you change them? Please be specific. *paragraph*

16. What has been the most positive moment for you in this course so far and why? *paragraph*

17. What has been the most challenging moment for you in this course so far and why? *paragraph*

18. What is your primary source of information in the course? How can it be improved? *paragraph*

19. What technology challenges have you had? If there is something you think we could do to help, please describe your issue. (Note: Results from this survey are anonymized. If you need help with a specific issue, please reach out to Dr. Link so we can help you find a solution!) *paragraph*

20. What do you like about the LabArchives ELN? *paragraph*

21. What about the ELN could be better? *paragraph*

22. Was the template for the Pre/in lab pages in the early experiments useful? *Likert: not at all useful - extremely useful*

23. What is helpful about the class organization in Canvas? *paragraph*

24. What aspects of the class organization on Canvas could be improved? Please provide specific suggestions. *paragraph*

25. How often do you use the captions provided with the in-lab videos? *Likert: never-always*

26. Choose the most recent Chem 51L class you took before this one. *Multiple choice*

27. If you have taken a previous Chem 51L class at UCI, how does the workload in the remote learning format compare to your previous class experience? *Likert: much lower - much higher*

28. Please tell us about any new challenges/responsibilities you have taken on during the pandemic (if you are comfortable sharing). This might include changes to job situation, new or changed responsibilities in caring for children or other family members, food or housing insecurity, or any other major change that impacts your ability to complete your class work. (Reminder: These survey responses are NOT connected with your name. We are using this question to get a sense of what challenges our students are dealing with.) *paragraph*

29. Please use this space to provide any additional feedback. *paragraph*

4.7.5 GCL-I Post-Quarter Survey Questions for Students

1. Who is your TA? *drop down*

2. What, if anything, did your TA do differently in the way they conducted their office hour after the mid-quarter survey? *paragraph*

3. If your TA changed what they were doing, did you like the change? Why or why not? *paragraph*

4. Describe TA-led activities that worked well during office hours. *paragraph*

5. Did grading change after the mid-quarter survey? If so, how? *paragraph*

6. What could be done differently to make grading expectations clearer? *paragraph*

7. Please use this space to provide any other comments about TA grading or office hours that you would like to include. *paragraph*

8. What aspects/topics/techniques of general chemistry laboratory do you feel the MOST confident about after taking this course? *paragraph*

9. What aspects/topics/techniques of general chemistry laboratory do you feel the LEAST confident about after taking this course? *paragraph*

10. What have you found beneficial about taking this lab remotely? *paragraph*

11. What has been challenging about taking this lab remotely? Propose ways you think might help change the experience for future students. *paragraph*

12. How did the changes made to Piazza after the mid-quarter survey affect your use of the message board? *paragraph*

13. What was the easiest electronic tool to use (Canvas, ELN, Spartan, Sapling, Zoom, or Piazza)? *paragraph*

14. What was the hardest electronic tool to use (Canvas, ELN, Spartan, Sapling, Zoom, or Piazza)? *paragraph*

15. How many times this quarter have you encountered technological problems which adversely affected your work in the course? *multiple choice: 0 - 10*

16. Did you use the captioning of the experiment videos? If so, why?

17. Please use this space to provide any other comments about the course that you would like to include. *Paragraph*

4.7.6 GCL-I Post-Quarter Survey Questions for TAs

1. What year are you in the Ph.D. program? *drop down*

2. Which general chemistry laboratory course(s) have you taught previously? Please select all that apply. *multiple answer*

3. Please estimate the average number of students that would come to your in-person office hours when you taught a general chemistry laboratory class previously. *drop down*

4. Indicate the extent to which you agree with the following. *5-point Likert scale: strongly agree to strongly disagree*

a. I feel that the structure of the experiment videos with associated video quizzes supported student learning of the laboratory content.

b. I feel that the due dates set for student assignments were appropriate.

c. I feel that the due dates by which assignments had to be graded helped me stay up to date with grading.

d. I feel that the due dates set for when assignment grading had to be completed were fair.

e. I feel that the instructor digest posts on Piazza were helpful.

f. I feel that the instructor digest posts on Piazza were an improvement over the way Piazza posts were previously answered.

g. I feel that the structural changes made to the TA meeting since mid-quarter were beneficial.

h. I feel that checking Sapling pre-lab completion was easier using BigBrother integrated with Slack than it would have been without it.

5. Did you like the changes made to the TA meeting format in the second half of the quarter? Why or why not? *paragraph*

6. What would you change about the TA meeting format in the future, if anything, and why? *paragraph*

7. How did you feel about BigBrother? What would you like to see changed about how sapling pre-lab completion is assessed in the future and why? *paragraph*

8. What is better about teaching remotely compared to your previous in-person experience and why? *paragraph*

9. What is more challenging about teaching remotely compared to your previous in-person experience and why? *paragraph*

10. What recommendations do you have for improving the future? *Paragraph*

4.7.7 OCl-II Post-Quarter Survey Questions for TAs

1. Compared to previous quarters, was this class more/less work than holding this TA position in person? *paragraph*

2. On average, how many students show up to your "in lab – video watch session" each week? *paragraph*

3. On average, how many students show up to your office hours each week? *paragraph*

4. Did your students need more/less guidance in interpreting data and understanding techniques? *paragraph*

5. What do you feel are the benefits of a remote lab experience? *paragraph*

6. What do you feel are the downfalls of a remote lab experience? *paragraph*

7. Did you borrow technology from the department? If so, what did you need? *paragraph*

8. In your opinion, what was the biggest challenge teaching remotely? *paragraph*

9. Use this space for anything else you want to include. *Paragraph*

4.7.8 GCl-I Guide to Filming

4.7.8.1 Equipment:

The general chemistry labs used simple consumer-grade video cameras to record videos. Videos were recorded and acted out by TAs assigned to the course. The combined cost of the equipment was <$1,000.

-Sony alpha 6100 mirrorless camera

-16-50 mm lens included with camera

-Several 64gb SanDisk Extreme memory cards

-Manfrotto tiltable tripod

The camera used in our experiments supports 4k video recording, however recording at such high definition has the downside of consuming more storage space and being difficult to edit on most computers. As most students do not have 4k displays and most educators do not have the computer processing power to edit 4k, footage was shot on 1080p in a properly angled shot. The dedicated zoom feature was useful when getting shots where the tripod could not get close enough. Note that the digital zoom used on phone cameras, action cameras, etc. is not a substitute for a dedicated zoom lens. When choosing a videography equipment, it was important that the video cameras accept memory cards as opposed to cell phones/tablets that do not have the internal storage to film a complete experiment.

4.7.8.2 Production:

As the videos were a large part of the student's education, it was important to tell a complete story that brings together the most important aspects of the experiment. Without a well thought out plan, the videos may seem disjointed, incomplete, or completely unusable requiring a re-shoot. Before shooting experiments, a director's script was written which ensured that the story

was well thought out and there were no missing segments. The director's script included all necessary reagents, correct experimental values, and side notes for when to introduce new chemicals/equipment.

In most experiments, the TA was facing the camera and the equipment was in front of the TA. If

a fume hood was required, the video was recorded at an angle so the viewer could clearly observe the regent handling. The TA was asked to use the furthest arm from the camera to perform the bulk of the work so that they are not blocking the shot. The TA stood in an "open" position where the front of their body faced the camera as much as possible. This gave a warmer feeling to the video as opposed to a TA that had their back to the camera.

When filming, the camera needed to be close enough to see the details, ensuring that everything

was in the shot with no unused space. If there was unused space, the camera was brought closer and the equipment rearranged to make it fit within the shot. Once the equipment was set up, strips of tape were placed around the workspace so that the TA knew the edges of the shot. The videos were almost exclusively shot using a tripod. It was beneficial to have a tripod tall enough to rest on the ground and film into the fume hood. We encourage using a tripod as it is easily reproducible, will reduce shakiness, and will make postproduction a cleaner process. The automatic video settings were used on the camera and resulted in consistent video quality. Consistency in both style and quality were important for maintaining the student's expectations.

4.7.8.3 Post-Production:

We used Apple iMovie® to edit the videos due to its ease of use and native integration in Apple

laptops. Videos were broken down into the following sections: Title, Introduction, Safety, Chemicals & Equipment, Part A, B, C, etc, Waste and Equipment Cleanup, and Final Notes for a report. While editing, title slides were used to clearly separate the important sections of a video. Subtitles were added at the bottom of the screen where applicable.

The first video in the series began by panning around the laboratory to introduce the students to

the university's chemistry labs. After showing the whole lab, the safety features were pointed out to familiarize the students with the locations of the fire extinguishers, eye wash stations, and safety showers. Each experimental video started by going over safety and ended by showing the TAs cleaning glassware and returning it to storage. Along with the main videos, a supplemental 'Safety Moment' video was recorded. Less than two minutes long, the safety moment detailed small aspects of chemical safety and hygiene that the students would normally pickup from attending the lab in person such as the contents of a spill kit, how to dispose of gloves, solvent safety, etc. A photograph of a white board detailing the main concepts, formulas, and equations was added at the beginning of each video along with a voice-over narration for the experiment's introduction. Then, highlighted arrows were added post-production to emphasize talking points.

The voice-over narration followed a pre-written script that highlighted the important aspects of

the video. As this was the first general chemistry laboratory for some students, the narration was key in describing the techniques used for the experiments. When repeating similar processes such as weighing samples, pipetting liquids, or titrating solutions a fast forward effect was used to speed up the shot and reduce overall video time. Each video was targeted to be less than 20 minutes to maintain student attention. Videos longer than 30 minutes were broken into multiple shorter

videos. *Crossblur* transitions were used in between different conceptual shots. If there wasn't a conceptual change, no transition was used. The completed videos were sent to the head TAs and instructor for final edits and then exported in 1080p high quality compression.

4.7.9 OCl-II Guide to Filming

4.7.9.1 Equipment:

If possible, employ a two-camera system for filming, one on a stable wide shot, and one that is your mobile close-up camera. This can be achieved with only one camera operator, as the wide only needs to have the record button pressed at the beginning of filming. Having two cameras, one recording the whole experiment and one recording a close angle ensures that no part of the process will be missed. Using two cameras also meant we never had to pause the experiment to adjust the camera zoom or angle/ the experiment could be carried out in real time as it would in the classroom. Our main camera was a cinema-grade camera with xlr inputs so that audio could be run through it/monitored through headphones, and our close-up was a dslr for portability.

-Canon C200 (used for wide) w/24mm lens

-Nikon D500 (used for close-up) w/24-70mm lens

-Lectrosonic wireless Lavalier (with XLR inputs run through C200)

-2 Manfrotto tripods

-2 sd cards (each 128g due to the file size of 4k footage)

Our videos were filmed in 4k. While this does require a large amount of storage space, having the ability to punch into certain aspects of the experiment (such as TLC plates drying) without breaching the fume hood was important. Using 4k footage downscaled to 1080p meant that we could zoom in much more than our standard zoom lens allowed and gave the greatest overall picture quality.

4.7.9.2 Production:

We found it beneficial to film each video with two TAs, one to perform the experiment, and one to narrate what was happening. A script was written prior to filming, and the TA reading from the script would be positioned at a distance where they could still see the experiment, but far enough away to eliminate as much noise from the fume hood as possible. If the fume hood proved to be too noisy at any point, portions of the script would be read after the experiment and synced up in post-production to the point in the video where the original lines were read. Having someone read the script allowed the person performing the experiment to concentrate on the experiment to execute it properly.

The wide and close up camera would start recording at the same time, and the camera operator

would operate the close up camera, adjusting for angles, making sure to capture labels and markings on beakers and vials, etc. We used a higher angle for the wide shot, and a low angle for the close up camera, as it needed to be able to film underneath the fume hood.

Cameras were cut during processes that would take long amounts of time that weren't necessary to be shown (ex., a solution stirring for 15 min+, condensation processes, etc.) This saved us storage space on the SD cards and helped eliminate portions we would have had to cut down in post-production. The TAs would keep the camera operator well-informed about stages of the process, key images to be captured, etc. Insert shots would sometimes be filmed during down time to later be added to the final video.

4.7.9.3 Post-Production:

All videos were edited in Premiere Pro. We would cut between the wide shot and the close-up so students could routinely see the entire experiment and extremely detailed shots of steps being

carried out- much clearer than they would with the naked eye if they were observing in the classroom. Video would be color corrected, audio would be mastered to eliminate as much background noise as possible, and a light, instrumental music track was placed on the video to keep some audio running during otherwise silent parts of the experiment. We chose meditative music to be calming and non-disruptive, while keeping the video from becoming boring if there were long stretches without narration.

The most common editing techniques we employed, though, were to either fast-forward during certain processes or cross-dissolve between two shots. For example, we would fast-forward when TLC plates were in solution, so students would see the plate absorb the solution in a matter of seconds. If a process would take a lot of time, such as stirring a solution or waiting for a solution to boil, we would film short segments every 10-15 minutes, and dissolve between them so that students could see the change in the experiment but not take up large amounts of time. This means an experiment that would take up to 4 hours in the lab could be fully seen and demonstrated in a 20-minute video. We would overlay text on the video when time would pass to let the students be aware of the time taken in the experiment.

Depending on the length of the experiment, we also chose to chunk up the full video into several

parts (each about 10 minutes each). If the experiment had two main components, we would create a video of each component to make viewing easier for the students. The end of the experiment would include a sign off from the TA and a reminder to clean up workspaces, so students would know they were at the last video in the experiment.

4.7.10 GCl-I and OCL-II Guide to Post-Lab Data Distribution

1. Create a practice quiz that does not count towards the students' grades in Canvas.

2. Upload PDFs of the requisite data to the Files section of Canvas. Once uploaded, change the access settings so that only students *with the link* are able to access the file.

3. Create a question in the practice quiz asking students to choose their lab section.

a. GCL-I: The files were named by experiment title and course ID number. Ex. Electrochemical Cells 40202.

b. OCL-II: The files were named by course ID number, scheduled time, and TA name. Ex. 40700 Tuesday 8AM, Jane Smith.

4. Enter answer choices.

5. Click the three horizontal dots below an answer choice. The alt-text is: Click to enter comments for the student if they choose this answer. This opens a rich text editor.

6. Click into the rich text editor and then scroll up until you see the Links toolbar on the right side of the page. Click to the Files tab and find the data PDFs you previously uploaded. Click on the relevant PDF you wish to attach.

7. Your comment should now contain a blue hyperlink to download the relevant PDF.

8. Update the question.

9. Save the quiz.

10. Preview the quiz.

4.7.11 GCl-I Essay Response Statistics

Essay Prompts	**Number of Students**	**Average** *out of 50 pts* **(±Std Dev)**
Group I		
Acid Dilution: Describe the procedural steps for dilution of 3.20 M HCl(aq) to make 100 mL of 0.800 M HCl(aq).	1152	36.36 (±5.11)
Filtration: Describe the procedural steps for collecting solid $PbSO_4$ precipitate from a liquid mixture contained in an Erlenmeyer flask and how an accurate mass of the precipitate is obtained afterward.	151	36.33 (±5.39)
Visible Spectroscopy: Describe the procedural steps for obtaining an absorbance spectrum of a crystal violet solution.	71	40.49 (±6.14)
Group II		
Electrochemistry: Describe the procedural steps for setting up a copper / zinc electrochemical cell based on the standard reduction potentials.	537	43.62 (±4.71)
Solution Formation: Describe the procedural steps for the formation of 10 mL of 0.200 M KSCN(aq) solution from solid KSCN.	448	38.83 (±5.39)
Titration: Describe the procedural steps for the titration of a 5 mL borax solution, sampled at 55°C, with a 0.52 M HCl solution. (Pictures of initial and final volumes in burette given.)	384	36.89 (±6.89)

4.7.12 Link to Public Google Folder

The following link will take you to a public Google folder containing the following: https://bit.ly/2YOnw8J

1. A readme directory file.

2. Full class schedules for GCL-I and OCL-II.

3. Copies of all experiment handouts for GCL-I and OCL-II.

4. Links to all experiment videos from GCL-1 and OCL-II.

5. Sample video quizzes used in GCL-I and OCL-II.

6. Sample lab final Canvas quiz from GCL-I

7. Sample “take-home” essay prompt from GCL-I.

8. Sample concept and data analysis exam from OCL-II.

9. Sample safety exam from OCL-II.

10. Sample technique exam from OCL-II.

11. Mastery project instructions from OCL-II.

12. Sample poster and journal article mastery projects from OCL-II.

13. Code for the BigBrother Python script as a .py file. (The most recent version can be located in the script author’s GitHub Repository.

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Chapter 5: A Specifications-Graded, Sports Drink-Themed General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach

5.1 Preface

After UCI's transition to remote instruction, Dr. Kim Edwards, Dr. Will Howitz, and I began developing a new general chemistry laboratory curriculum to help students reengage in education once laboratories transitioned back to in-person. The new curriculum adopted the structure of argument-driven inquiry (ADI) first popularized by Joi Walker Phelps in 2011. ADI differs from traditional laboratory experiences as it seeks active student engagement through scientific argumentation with peers through a mock poster session. Additionally, we added a theme-based component to the course that used Gatorade for every experiment. The decision to use Gatorade added a layer of fun for the students as they were working with a "chemical" that they encounter daily. This chapter will describe the four new experiments developed for the first quarter of the general chemistry laboratory series using the principles of ADI and their implementation during the 2021-2022 academic year.

5.2 Introduction

A traditional approach to instructing general chemistry laboratory courses requires students to verify known scientific principles through experiments that provide a diverse, but often disconnected, review of lecture topics. While this approach exposes students to laboratory techniques and hones their data analysis skills, it is ineffective at developing science process skills or improving student attitudes toward the subject matter.[1,2] A guided inquiry approach to laboratory instruction is an improvement over a traditional one because students have the opportunity to investigate a problem by developing and testing out their own solutions.[3] Adding

argumentation — a chance for students to share and constructively critique claims, evidence, and justifications with each other — improves the guided inquiry model with sense-making. Sense-making is the process by which people use communication and contextualization to rationalize their world. Laboratory experiments incorporating argumentation allow students to develop sense-making by proposing and defending scientific ideas with peers.[4] This approach, called argument-driven inquiry (ADI), has seven steps:[5]

1. Teams of students are introduced to a task and given a research question.
2. Each team designs a procedure to address the research question and collects data following that procedure.
3. The team comes up with a claim, evidence, and justification to answer the research question based on the collected data.
4. The team then presents their findings to other teams in an argumentation session.
5. Individually, students write reports based on the findings.
6. Each student participates in an anonymous peer review of other students' reports.
7. The student then revises their report to reflect the comments of their peers and submits it to the instructor for final evaluation.

Steps two, three, and four differ substantially from traditional settings as they require the students to apply higher order cognitive skills such as designing a procedure, analyzing and evaluating results, and proposing an answer to a research question. Students who took a laboratory course taught using ADI show a more positive attitude toward science and demonstrate increased conceptual understanding, critical thinking, and scientific processing

skills than those who took a more traditional expository course.[2,6] Furthemore, because students work in teams and share resources, they build important interpersonal skills needed to constructively engage in discussions of scientific issues with the general public.[7] A growing number of laboratory courses using the ADI approach have been developed including high school chemistry, general chemistry, organic chemistry, and physical chemistry.[2,5,6,8-12]

Herein, we describe the adaptation of a thematically connected, ADI approach to laboratory instruction for a large-scale general chemistry laboratory course at the University of California at Irvine (UCI). UCI operates on the quarter system with three, 10-week terms. Non-chemistry majors (predominantly biological sciences, public health, pharmaceutical sciences, and engineering majors) take the chemistry laboratory courses in quarters offset from the lecture courses (Table 5.1). Laboratory sections meet weekly for 3 hours and 50 minutes and each section serves 24 students supervised by one graduate student teaching assistant (GTA). During high demand quarters, more than 50 laboratory sections serving 1300+ students are held each week.

Table 5.1: Structure of On-Sequence General Chemistry Courses

Year	Fall Quarter	Winter Quarter	Spring Quarter
First Year	General Chemistry Lecture I	General Chemistry Lecture II	General Chemistry Lecture III
	No laboratory course	No laboratory course	**General Chemistry Laboratory I (GCL-I)**
Second Year	Organic Chemistry Lecture I		
	General Chemistry Laboratory II (GCL-II)		

Before the adoption of the new course content, the General Chemistry Laboratory I (GCL-I) course contained eight traditional expository-type experiments which addressed a diverse list of topics derived from the corequisite lecture course (e.g., equilibrium, computational chemistry, thermodynamics, buffers, kinetics, and electrochemistry). During the laboratory, students worked in pairs to complete the procedures outlined in the laboratory manual. After completing experimentation, each student worked independently to answer a series of post-laboratory questions requiring students to perform calculations with their collected data.

Instead of the traditional, broad expository coverage of topics, the new course is structured around four Gatorade-themed projects: determining the concentration of sugar in Gatorade, using visible spectroscopy to determine the concentration of dye(s) in Gatorade, measuring the buffer capacity of Gatorade with titration, and determining the kinetics of the degradation of dyes commonly found in Gatorade.[13-17] We chose theme-based instruction because it provides a conceptual framework that increases student perception of their own understanding and their actual assessed comprehensive understanding.[18-22] In addition, it incorporates connections between experiments to make the content more relevant to the students, increasing their engagement and motivation.[19,20,23] Multiple examples of general chemistry laboratory courses with themes can be found in the literature.[24-29]

In conjunction with theme-based instruction, we converted GCL-I's four projects into ADI experiments in the hope of improving students' conceptual understanding of course content and ability to use evidence to justify conclusions.[6] Similar to the processes reported by other ADI practitioners, each of our projects takes place over two laboratory sessions (two weeks). During the first session, a team of three to four students is given a foundational activity requiring the students to practice new laboratory methods or techniques. Before the end of this first session, the

team uses knowledge obtained during the foundational activity to plan an experiment aimed at answering a provided research question. During the second session, the team collects and analyzes data to find evidence which answers the research question. At the end of this session, each team presents their claim (answer to the research question), evidence, and justification in an academic poster session, referred to as an argumentation session. In this student-led argumentation session, members from different teams discuss the validity of evidence and the accuracy of claims with each other. Students complete a laboratory report based on their findings before the start of the next project.

While the approach of Walker, et al, incorporates a peer review process for the laboratory reports, we did not include this process in our course because we adopted a specifications grading system.[5] Specifications grading is an alternative grading system, first popularized by Linda Nilson in her 2014 book, "Specifications Grading: Restoring Rigor, Motivating Students, and Saving Faculty Time".[30] Under this grading system, students earn their letter grade by completing instructor-specified bundles of assignments. These assignments are commonly assessed as satisfactory or unsatisfactory using rubrics in which students must meet set criteria which define a passing threshold. Consequently, there is no partial credit. However, students are eligible to revise and resubmit assignments assessed as unsatisfactory, often in exchange for tokens. Because the number of tokens available to students is limited, this limits the number of assignments students may revise and resubmit. We believe our feedback process provided by GTAs is an appropriate alternative to the peer feedback students use in Walker et al's course to revise their reports.

5.3 ADI Implementation and Laboratory Course Objectives

Our primary motivation for redeveloping the curriculum for the general chemistry laboratory series was to increase student engagement and interpersonal interactions, especially in

the wake of the COVID-19 pandemic. The ADI approach was especially appealing because it actively promotes student communication and collaboration as well as peer-to-peer learning from student-led argumentation. We also felt the ADI format aligned well with the course learning outcomes (Table 5.2).

Table 5.2: GCL-I Learning Outcomes

GCL-1 Learning Outcomes (LO)
LO1: Engage in experimental design, scientific argumentation, scientific writing, and revision.
LO2: Interpret experimental data and calculated results to develop scientifically sound conclusions.
LO3: Proficiently use an electronic lab notebook to record qualitative observations in detail and quantitative data with the correct significant figures.
LO4: Utilize a variety of laboratory glassware (beakers, flasks, pipets, and burets). Correctly use a digital balance to mass samples.
LO5: Operate a simple visible spectrometer to acquire absorbance measurements.
LO6: Demonstrate basic understanding of safety symbols, safety data sheets, corrosives, handling chemical waste, fire and chemical spill response.

During the redeveloped GCL-I course, a team of three to four students work together on four ADI projects. Each project spans two laboratory sessions, which are referred to as the fundamental skills session and the original investigation session (LO1). Both laboratory sessions are initiated with separate guiding questions (Table 5.3). Answering the guiding question of the fundamental skills session is necessary to engage in the original investigation session. Not only does the fundamental skills session serve as training experience, but it also provides foundational knowledge, as well as data and results for the original investigation session the next week. The understanding and evidence compiled during the two sessions are combined to form the team's poster and each individual's summative laboratory report for each project. As the students proceed through the quarter, each project increases in complexity, and it is expected that students are

applying concepts and skills learned earlier in the quarter to later projects. For example, the solution preparation skills and visible spectroscopy concepts learned during the first two projects are expected knowledge for the last two projects.

Table 5.3. Guiding Questions for GCL-I

Project	Fundamental Skills	Original Investigation
1	What glassware provides the most precise data for the calibration curve? Students create aqueous sucrose solutions of known concentration to create a density calibration curve with four different pieces of glassware.	Is the mass of sugar in Gatorade comparable to what is listed on the nutrition label provided by the manufacturer? In other words, which glassware gave the most accurate result (the smallest percent error)? Students use their calibration curve to answer the guiding question.
2	What is your dilution plan for creating standard dye solutions and how are these standard solutions used to create a Beer's Law Plot? Students dilute aqueous dye solutions to known concentrations to create a Beer's Law plot.	What dye or combination of dyes is present in your Gatorade sample and what is/are the concentration(s)? Students use their Beer's Law plot to answer the guiding question.
3	How well does the Henderson - Hasselbach equation predict the pH of acetic acid / acetate and ammonia / ammonium buffer solutions? What is the buffer capacity of these solutions? Students create and titrate buffer solutions.	Which titrant and what concentration is appropriate for a sufficient data set? What is the buffer capacity and HA/A^- or B^-/HB ratio of your Gatorade sample? Students use concepts and techniques to determine the buffering capacity of Gatorade.
4	What are the optimal conditions to observe the kinetics of the crystal violet hydroxylation? What is the reaction order of crystal violet? Students use absorbance spectroscopy to determine optimal concentrations and reaction order.	What are the optimal conditions to observe the kinetics of the bleaching of your chosen dye? What is the reaction order of that dye? Students use concepts and techniques to determine the reaction order of a dye in Gatorade.

Before the fundamental skills session each student individually completes a pre-laboratory quiz through the course learning management system (LMS) which includes safety, conceptual, procedural, and calculation questions based on short instructor videos, expository laboratory manual instructions, and information about laboratory techniques which are hyperlinked into the laboratory manual. The student is then required to provide an objective,

chemical and safety tables, and a draft of expected procedures in their electronic laboratory notebook (ELN) before entering the laboratory (LO3).

At the beginning of the fundamental skills session, the GTA leads the students in their laboratory section in an interactive safety moment activity connected to the pre-laboratory safety reading and quiz (LO6).[31] The GTA then provides a brief demonstration of instrumentation or glassware set up which is central to the session's procedures. Students work with their team during this session to learn techniques, concepts, and calculations that will help them with their original investigation (LO4, LO5). Though working in a team, each student has a set task to accomplish which answers one part of the guiding question and can use the laboratory manual, their peers, and the GTA for additional assistance if necessary. At the end of the fundamental skills session, students share their individual findings with their teammates and, collectively, the team then analyzes the data and answers the fundamental skills guiding question (LO2). Students enter or attach their work to a scaffolded ELN page. The team then reviews the original investigation guiding question and creates an outline of an experimental procedure for the next laboratory session, the original investigation session.

Before the original investigation session, each student individually completes a second pre-laboratory quiz which includes questions related to the assigned safety reading and requires them to summarize information from the fundamental skills session, evaluate sample claims, evidence, and justification, and use a computational chemistry program to better understand chemical behavior. Students are expected to provide an objective and a draft of expected procedures (which was outlined by the team at the end of the last laboratory session) in their ELN before entering the laboratory. During the laboratory session, the team revises their procedure as they perform experimentation, make observations, perform calculations and/or

create relevant graphs, and analyze data to propose an answer to the original investigation guiding question. All work is recorded in the ELN.

The original investigation session finishes with a student-led argumentation session. The expectation for the argumentation session is that each team of students will create a poster with three main components: claim, evidence, and justification (Figure 1). The claim is typically the team's answer to the guiding questions for the original investigation session. For example, if the original investigation guiding question is to determine the identity and concentration of the dye in Gatorade, then the team would make a claim stating "The dye in the orange Gatorade is Yellow-6. The concentration of the dye is 79.7 μM." Next, the team would provide any evidence that supports their claim, which could include calibration curves, spectra, calculations, error analysis, etc. Lastly, the team would craft a justification section, linking the evidence to their claim. This section allows the students to explain how their evidence supports their claim by using scientific concepts and theory (LO1, LO2). While "correct" answers (or claims) to the original investigation guiding question exist, the team or student does not need to arrive at one of these answers. Furthermore, a variety of procedural approaches and potential answers to an original investigation guiding question encourages more discussion during the argumentation session. As the quarter proceeds, the complexity of each project increases, leading to even more varied approaches during the original investigation session.

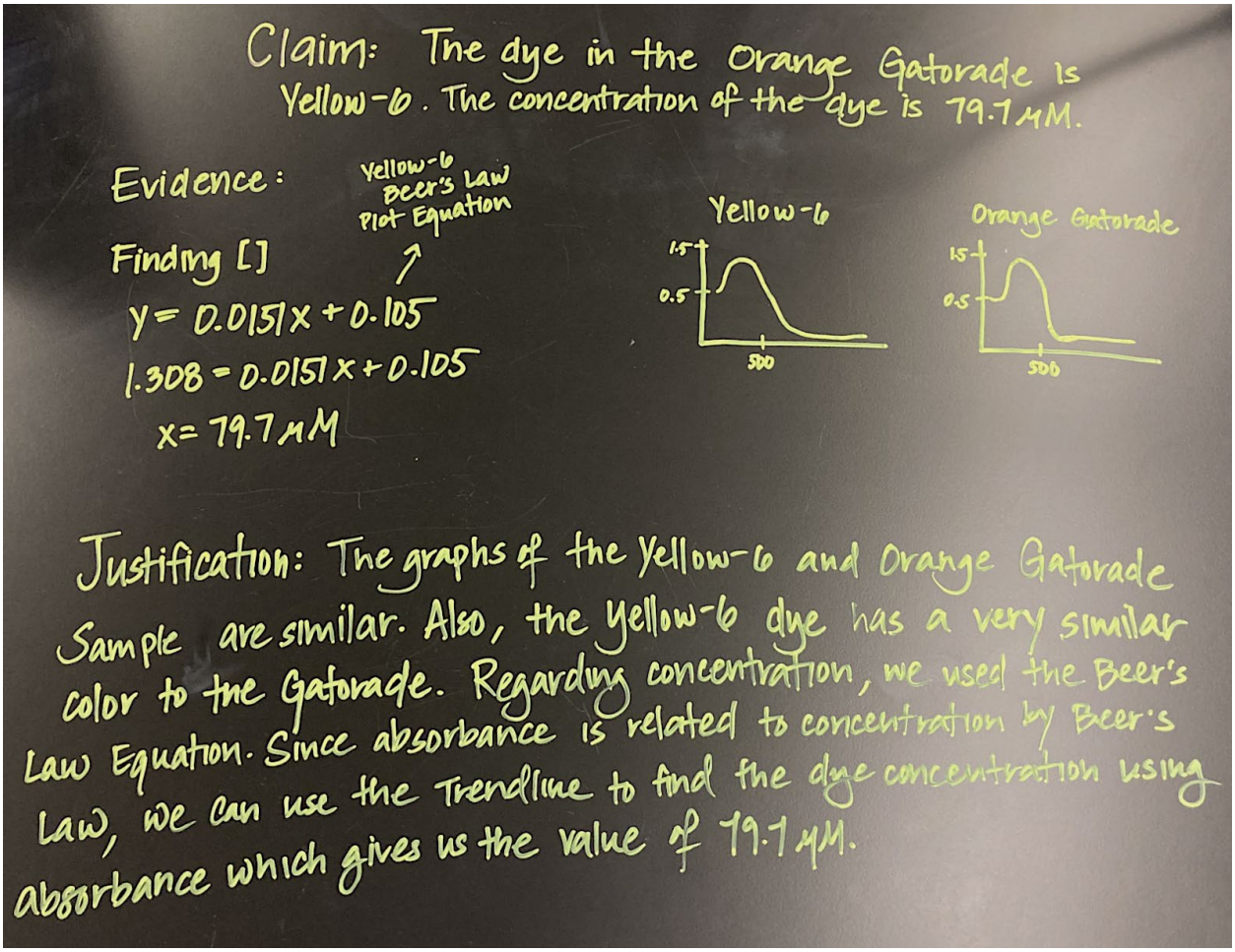

Figure 5.1 Benchtop Poster from Project 2. Students create their poster on the laboratory benchtop with chalkboard markers and then present their poster which engages the students in scientific argumentation.

At the beginning of the argumentation session, the team splits up. One student, the team leader, stays next to the poster to answer questions from other teams. The remaining students, the travelers, visit other teams' posters as a group and ask questions. Students are encouraged to ask their own questions to each team, but if conversation is lacking, a set of questions is available to stimulate discussion. After this session, the team reconvenes to critique their own poster in light of any new understandings discovered from the posters of, and discussions with, the other teams. The team can then determine if a new claim is justified or, if time permitting, more data needs to be collected.

After the entire project is completed, students individually write a formal laboratory report describing their conceptual, experimental, and analytical understanding of the original investigation process (which may include pertinent fundamental skills information) and the content of their team's poster (their claim, evidence, and justification). Students who change their claim after the argumentation session are encouraged to include further justification and an error analysis if warranted.

5.4 Final Exam

A final exam is given during the last full week of instruction (either ninth or tenth week of the quarter). The two-hour exam covers safety, technique, and argumentation topics. The safety portion consists of selected questions from the pre-laboratory quizzes and is delivered through the course LMS with a lockdown browser. This portion of the exam is given during the first 30 minutes of the practical session. Students spend the remaining exam time working on a packet containing two technique questions and two argumentation questions. All four of the ADI projects are represented between the two question types. Students must answer one technique question and one argumentation question. The ability to choose which question to answer is intentionally introduced to reduce student anxiety.[32] Technique questions require students to assemble a set of glassware or equipment and collect and analyze a small set of data to display their technique knowledge. Argumentation questions provide a set of data which must be analyzed to identify evidence that will be used to create and justify a claim. An assessment of student performance on argumentation is ongoing and will be evaluated in subsequent laboratory courses.

5.5 The Role of Instructional Staff in the Laboratory

Each laboratory section is run by one GTA and at least one undergraduate learning assistant (ULA). Most GTAs are first year graduate students who have received a week-long general GTA training before their first quarter of graduate school. Former GCL-I students who did well in the course are invited to apply to be ULAs in subsequent quarters. Once selected by the instructor, incoming ULAs take a learning assistance certification course. ULAs are primarily used to support the GTAs, but do not grade any student work.

Because few, if any, of our GTAs and ULAs have prior teaching experience in laboratory courses using an ADI approach, we developed a three-hour ADI-specific training session that they take at the start of the quarter to prepare them for their roles in GCL-I. This training starts with a brief lecture introducing the basic ideas of ADI instruction. Then sample data from one of the course's projects is provided for teams of GTAs and ULAs to analyze from which they develop a poster displaying their claim, evidence, and justification. The GTAs and ULAs subsequently engage in an argumentation session as previously described so they know what to expect during the original investigation sessions.

The role of the GTA and the ULA differs in each laboratory session. During the fundamental skills session, GTAs and ULAs demonstrate techniques and answer most student questions directly. The only prohibition given to them is that they cannot answer the fundamental skills guiding question for the students. During the original investigation session, they are instructed to act as facilitators. They ask leading and redirecting questions prompting peer-to-peer interaction. They are also instructed not to interfere unless directly addressed. We intentionally trained our GTAs and ULAs in this fashion as the intention of ADI is for students to develop their own process, ask their peers questions, and engage in lively conversations about

the experiments. Thus, GTAs and ULAs take a backseat role while most of the argumentation is student-led.

5.6 Role of Specifications Grading

Students must pass a set number of assignments in each category to earn a specific grade (Figure 5.2). All assignments in GCL-I were evaluated using the specifications grading system. These assignments included laboratory notebook assignments associated with the fundamental skills and original investigation sessions, and laboratory reports for each project. Each assignment was assessed as satisfactory or unsatisfactory depending on the number of rubric criteria a student met relative to the instructor-defined passing threshold. Most of the course rubrics have between 10 and 15 criteria. The passing threshold for the laboratory notebook assignments is set near 80% of the rubric items, as recommended by Nilson.[30] The laboratory reports were assessed as satisfactory with either a high-passing or low-passing threshold, specified by 80% or 60% of the rubric items respectively. We implemented this high-pass and low-pass system in order to differentiate students' grades. Students under this system must pass the majority of assignments to earn grades above a C. Rubrics are designed so that assignments of the same type often use very similar or identical language and rubric criteria (such as those for observations, data analysis and justification). Each assignment rubric and associated passing threshold are posted on the LMS for students to view. Letter grade requirements are also posted in the syllabus on the course LMS (Figure 5.2).

A	B
• **Fundamental Skills:** pass 4/4 • **Original Proposals:** pass 4/4 • **Lab Reports:** high pass 3/4 *or* high pass 2/4 & low pass 2/4 • **Final Exam** (Safety Knowledge, Technique and Argumentation): pass 3/3	• **Fundamental Skills:** pass 3/4 • **Original Proposals:** pass 3/4 • **Lab Reports:** high pass 2/4 & low pass 1/4 *or* high pass 1/4 & low pass 3/4 • **Final Exam** (Safety Knowledge, Technique and Argumentation): pass 2/3
C	**D**
• **Fundamental Skills:** pass 3/4 • **Original Proposals:** pass 2/4 • **Lab Reports:** high pass 1/4 & low pass 2/4 *or* & low pass 4/4 • **Final Exam** (Safety Knowledge, Technique and Argumentation): pass 2/3	• **Fundamental Skills:** pass 2/4 • **Original Proposals:** pass 2/4 • **Lab Reports:** high pass 1/4 & low pass 1/4 *or* & low pass 3/4 • **Final Exam** (Safety Knowledge, Technique and Argumentation): pass 1/3
Plus (+) Meet all the criteria for a letter grade and • **Pre-lab Quiz** total ≥ 80%	Minus (-) Meet all the criteria for a letter grade and • **Pre-lab Quiz** total < 65%

Exception: Earning an A+ requires a 4/4 pass (and high pass) in all categories and a Pre-lab Quiz total ≥ 95%.

Figure 5.2 Breakdown of Assignments at a Passing Threshold for Each Grade

Students have the ability to earn up to six tokens throughout the quarter. One token can be used to revise and resubmit an assignment that was assessed as unsatisfactory or three tokens can be used to attend a makeup laboratory section in the case of an unexcused absence. The first three tokens are earned by completing introductory course assignments. These include quizzes assessing foundational chemistry knowledge, specifications grading and ADI information, and academic integrity and laboratory safety understanding. Additional tokens are earned for completing surveys for education studies and mid-quarter GTA evaluations.

Peer cooperation is essential to the ADI process, therefore, competitive grading processes may work against full student participation. Specifications grading emphasizes attaining competency in specific areas, encouraging students to work collaboratively together and learn

from each other. The students' ability to use tokens for review and resubmission is a substitute for the ADI peer review step. Students are able to leverage token use for the revision of up to half of the assignments in the course with the benefit of TA grading and feedback. Because of the short time scale of the quarter system in conjunction with insufficient LMS peer grading tools for a large enrollment multi-section laboratory course, peer review was not possible for GCL-1.

5.7 Laboratory Course Assessment Survey

A modified version of the **Laboratory Course Assessment Survey** (LCAS) was given to GCL-I students near the end of the quarter while students were engaged in the fourth and final project.[33] LCAS is a 17-item survey designed to measure the effectiveness of course-based undergraduate research experiences (CUREs). It contains three sections by assessing student perception of collaboration with peers, generation of new knowledge, and work revision and repetition. We chose to use the LCAS tool because, in addition to the above-mentioned activities, it also measures student perception of the course activities central to the ADI process: student engagement in scientific practices (experimental design, data collection and analysis and engaging in argumentation). Our modification was the use of a 5-point scale (see Table 4).

In the fall quarter of 2021, 99 students participated in this study. In the spring quarter of 2022, 1225 students participated in this study. More than half of the students in the course are biological sciences majors (54%), followed by undeclared / unaffiliated students (12%), engineering (12%) and pharmaceutical sciences majors (9%). Available GCL-I student demographic breakdown is 32% first generation college students (campus average 46%), 30% low-income students (campus average 33%) and 7% international students (campus average 14%) (Table 5.4).

Table 5.4. GCL-I Student Demographics

Quarter	Fall 2021	Spring 2022	Campus Average
Enrollment	99	1225	N/A
Number of G-TAs	3	28	N/A
Biological Sciences Majors	16%	54%	
Undeclared/Unaffiliated Students	26%	12%	
Engineering Majors	13%	12%	
Pharmaceutical Sciences Majors	3%	9%	
First Generation College Students	32%	32%	46%
Low-income Students	39%	30%	33%
International Students	10%	7%	14%

The averages and standard deviations for each LCAS prompt are provided in Table 5. For the large enrollment spring quarter course, there are a few notable results. The team structure of the ADI approach resulted in more than 75% of the respondents choosing that every survey item happened weekly in the collaboration section (C1-C6). For two of the discovery and relevance statements approximately 80% of respondents somewhat agree or strongly agree that they are expected to formulate a hypothesis & develop new data-based arguments during the course (D3 & D4). This result is indicative that the students connected with the central aspects of the ADI process in the GCL-I curriculum: answering of the guiding question (i.e., the creation of a hypothesis) and the justification of how the data collected is evidence for their claim (i.e, the hypothesis). The result from the discovery and relevance section which the students disagreed with more than any

other is the statement: “In this course, I was expected to generate novel results that are unknown to the instructor that could be of interest to the broader scientific community or others outside of class” (D1). This result is reasonable for the first college chemistry course taken by nonmajors and is most likely connected to the thematic nature of the course. The students are familiar with the overarching theme of the course (the chemistry of Gatorade) and, therefore, would not be as likely to perceive the science as novel. A clear indication of the team-based inquiry nature of the GCL-I course is evident in the response to the third statement in the iteration section. The majority of students (86%) either somewhat agreed or strongly agreed with the statement that they were expected to “share and compare data with other students” (I3). The statements in the iteration section which students disagreed with the most are that they were expected to “revise/repeat work to fix errors” and “change the methods of investigation” (I1,I2). These results are most likely due to the more expository structure of the fundamental skills sessions and the limited knowledge that first quarter general chemistry laboratory students have of laboratory methods. Hopefully, in future laboratory courses students will gain more skills and confidence leading to revision and a deeper understanding of fundamental laboratory techniques that will lead to changing methods.

Table 5. Modified LCAS Results for GCL-I

Quarter (Enrollment)	F21 (99)		S22 (1224)	
	Avg	*SD*	Avg	*SD*
Collaboration	22.8	*0.6*	22.1	*0.6*
C1. Discuss elements of my investigation with classmates and instructors	3.9	*0.5*	3.8	*0.8*
C2. Reflect on what I was learning	3.8	*0.7*	3.6	*0.8*
C3. Contribute my ideas and suggestions during class discussions	3.9	*0.5*	3.6	*0.7*
C4. Help other students collect or analyze data	3.9	*0.5*	3.7	*0.7*

C5. Provide constructive criticism and challenge each other's interpretations	3.7	*0.6*	3.7	*0.6*
C6. Share the problems and seek input on how to address them	3.8	*0.5*	3.7	*0.7*
Discovery / Relevance	19.7	*1.0*	18.4	*0.9*
D1. Generate novel results that could be of interest the community	3.2	*1.2*	3.0	*1.2*
D2. Conduct an investigation to find something previously unknown	3.9	*1.1*	3.6	*1.1*
D3. Formulate my own research question or hypothesis to guide an investigation	4.1	*0.9*	4.0	*1.0*
D4. Develop new arguments based on data	4.4	*0.8*	4.1	*1.0*
D5. Explain how my work has resulted in new scientific knowledge	4.2	*1.0*	3.8	*0.9*
Iteration	24.0	*1.1*	20.9	*1.0*
I1. Revise and repeat work to account for errors or fix problems	3.9	*1.1*	3.2	*1.3*
I2. Change methods of investigation if it was not unfolding as predicted	3.7	*1.2*	3.1	*0.9*
I3. Share and compare data with other students	4.5	*0.9*	4.0	*1.0*
I4. Collect and analyze additional data to address new questions	3.9	*1.2*	4.1	*1.0*
I5. Revise and repeat analyses based on feedback	4.0	*1.2*	4.1	*1.0*
I6. Revise drafts of papers or presentations based on feedback	4.0	*1.2*	3.5	*1.0*

Collaboration was measured on a four point scale: weekly (4), monthly (3), 1 or 2 times (2) and never (1). Discovery / Relevance and Iteration were measured on a five point scale: (5) strongly agree, (4) somewhat agree, (3) neither, (2) somewhat disagree and (1) strongly disagree.

The more than ten-fold enrollment increase going from fall to spring resulted in the lowering of the summed averages for all three LCAS sections. The values for the collaboration & discovery sections both moved slightly lower with the most significant deficit occurring with the statement, "In this course I was expected to explain how work has resulted in new scientific knowledge (D5). Students disagreed with more of the statements in the Iteration section during the large enrollment spring GCL-I course than the smaller enrollment fall course. The only statement to

receive comparable agreement levels was the expectation to "share and compare data with other students." Besides the significant difference in enrollment, the notable differences between the two courses are:

- the lower percentage of first generation students in the the spring (32%) versus fall (52%)
- the higher percentage of biology majors in the the spring (54%) versus fall (16%)
- the lower percentage of unaffiliated / undeclared majors in the spring (12%) versus the fall (26%)
- the larger number of GTAs in the spring (28) versus the fall (3)

The potential roles played by student demographics and GTA numbers (and training) warrant further investigation.

5.8 Lessons Learned and Future Implementation

The ADI-driven GCL-I theme-based format incorporates a structured review of fundamental concepts with a hands-on application of new concepts. The thematic connection offers a conceptual framework connecting projects and increasing the relevance of the content covered. Furthermore, the iterative application of methods and skills from previous projects gives student teams increasing responsibility and freedom to collaboratively develop experimental design skills.

Specifications grading supports ADI because it is not a competitive grading system. This fosters collaboration within and between teams. Assignment revision and focus on specific repeated important rubric criteria encourages students to take an iterative approach to course material. It should also be noted, that while specifications grading has grown in popularity, especially in STEM over the past 5-6 years, most of the published examples to date have been in

lecture courses. In fact, the only published example of a laboratory-only course served as the inspiration for the design of the system used in this course.[34]

LCAS results suggest that GCL-I may provide similar benefits of CURE for an introductory chemistry laboratory course (self-efficacy in lab skills, enhanced identification as a scientist, and higher passing rates in STEM for women & URM). CURE similarities to our course: engagement in scientific inquiry (utilizing scientific practices, engaging with the collaborative and iterative nature of research, and making novel discoveries with broader relevance.

Despite a challenging return to in-person instruction after a pandemic-induced yearlong remote setting, both the students and instructional team felt the endeavor was a success. The positive student response to our redeveloped ADI laboratory experience will help to inform the creation and implementation of additional general chemistry laboratory courses, specifically the implementation in the second quarter of the general chemistry laboratory series. Future courses, currently in the early stages of implementation and development, will retain the same overall structure, scaffolded approach, and active engagement.

5.9 Appendix

5.9.1 IRB Statement

This study was approved by the University of California, Irvine, Institutional Review Board as exempt (IRB 2018-4661) including FERPA compliance.

5.9.2 Project Manual Links

1. Project #1: Does the Quantity of Sugar in Gatorade Match the Nutrition Label?
2. Project #2: What dyes are in Gatorade? What are their concentrations?
3. Project #3: What is the Buffer Capacity of Gatorade?
4. Project #4: What are the Kinetics of the Dyes in Gatorade?

5.9.3 Survey Questions

The end of quarter student survey was used verbatim from:

Corwin, L.A.; Runyon, C.; Robinson, A.; Dolan, E. The Laboratory Course Assessment Survey: A Tool to Measure Three Dimensions of Research-Course Design. *CBE-Life Sci. Educ.* **2015**, (14), 1-11.

1. Collaboration. In this course, I was encouraged to… *Weekly, Monthly, 1 or 2 times, Never*
    1. Discuss elements of my investigation with classmates and instructors.
    2. Reflect on what I was learning.
    3. Contribute my ideas and suggestions during class discussions.
    4. Help other students collect or analyze data.
    5. Provide constructive criticism to classmates and challenge each other's interpretations.

6. Share the problems I encountered during my investigation and seek input on how to address them.

2. Discovery/Relevance. In this course, I was expected to… *Strongly Disagree, Somewhat disagree, Neither agree nor disagree, Somewhat agree, Strongly agree*
    1. Generate novel results that are unknown to the instructor and the could be of interest to the broader scientific communityor others outside of class
    2. Conduct an investigation to find something previously unknown to myself, other students, and the instructor.
    3. Formulate my own research question or hypothesis to guide an investigation.
    4. Develop new arguments based on data.
    5. Explain how my work has resulted in new scientific knowledge.
3. Iteration. In this course, I had time to… *Strongly Disagree, Somewhat disagree, Neither agree nor disagree, Somewhat agree, Strongly agree*
    1. Revise and repeat work to account for errors or fix problems.
    2. Change the methods of investigation if it was not unfolding as predicted.
    3. Share and compare data with other students.
    4. Collect and analyze additional data to address new question or further test hypotheses that arose during the investigation.
    5. Revise and repeat analyses based on feedback.
    6. Revise drafts of papers or presentations about my investigation based on feedback.

The question below was added at the end of the above survey and was excerpted from:

Melvin, J. Personality Type as an Indicator of Learning Style *University of Rochester, Center for Excellence in Teaching and Learning.* **2013**.

4. In general, I *Strongly Disagree, Disagree, Neither agree nor disagree, Agree, Strongly agree*

a. Like getting my energy from active involvement in events

b. Often understand a problem better when I can talk out loud and what other have to say

c. Take time to reflect so that I have a clear idea of what I'll be doing when I decide to act

d. Often prefer doing things alone or with one or two people I feel comfortable with

5.9.4 Sample Poster

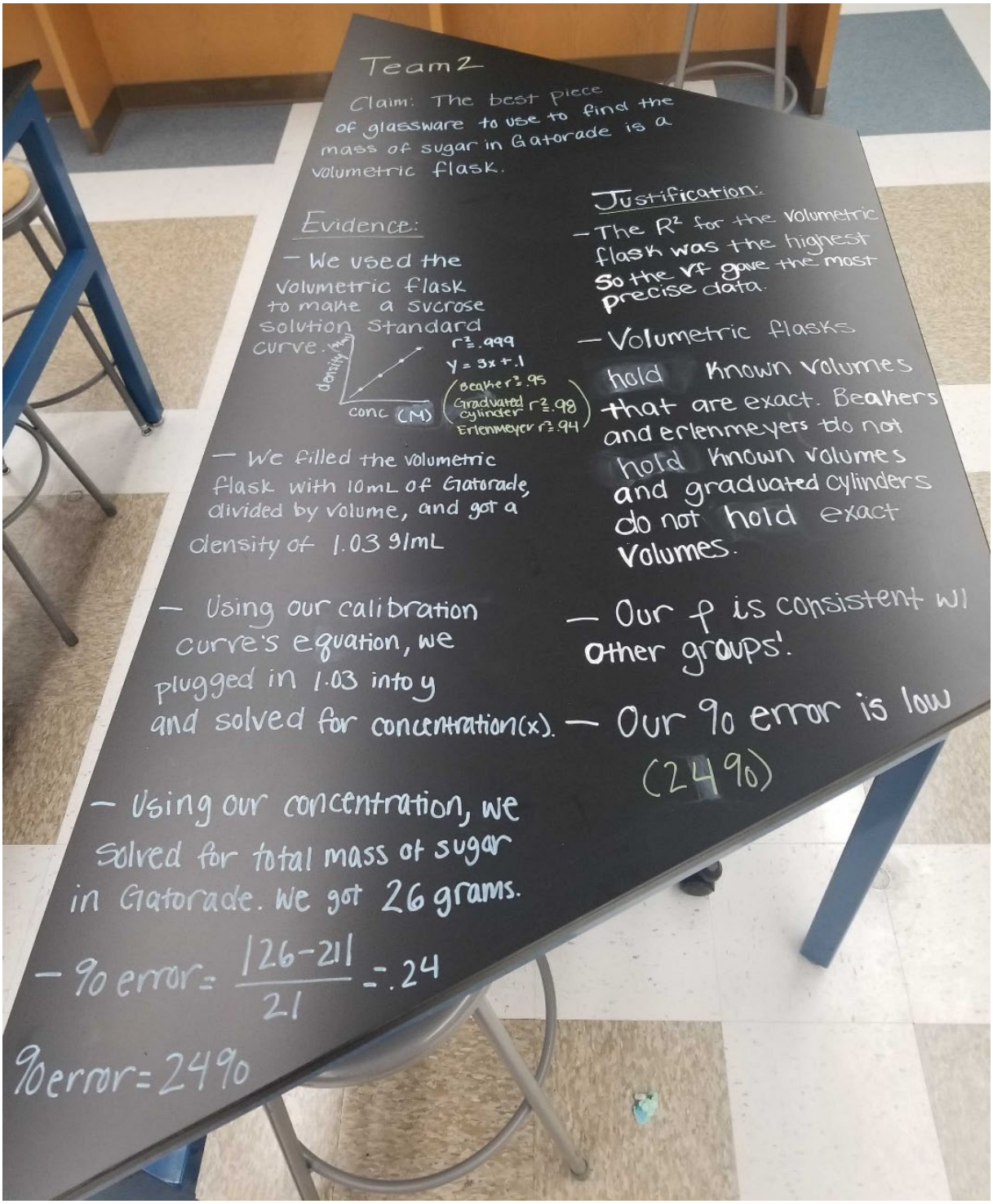

Figure 5.3 Example of "correct" poster for project 1.

5.9.4 Role of Gradescope

Students submit laboratory notebook assignments associated with the fundamental skills sessions and the original investigation sessions and laboratory reports for each project to Gradescope which is integrated with the course LMS. This is so a consistent team of the same 3-4 GTAs (which includes their laboratory section GTA) grade and leave feedback on rubric items the students do not pass. We have found that team grading and the ability to monitor rubric item statistics help ensure grading consistency between TAs in our high enrollment multi-section courses. This grading approach has also reduced each TA's grading time by approximately a third.

5.9.5 LCAS Results

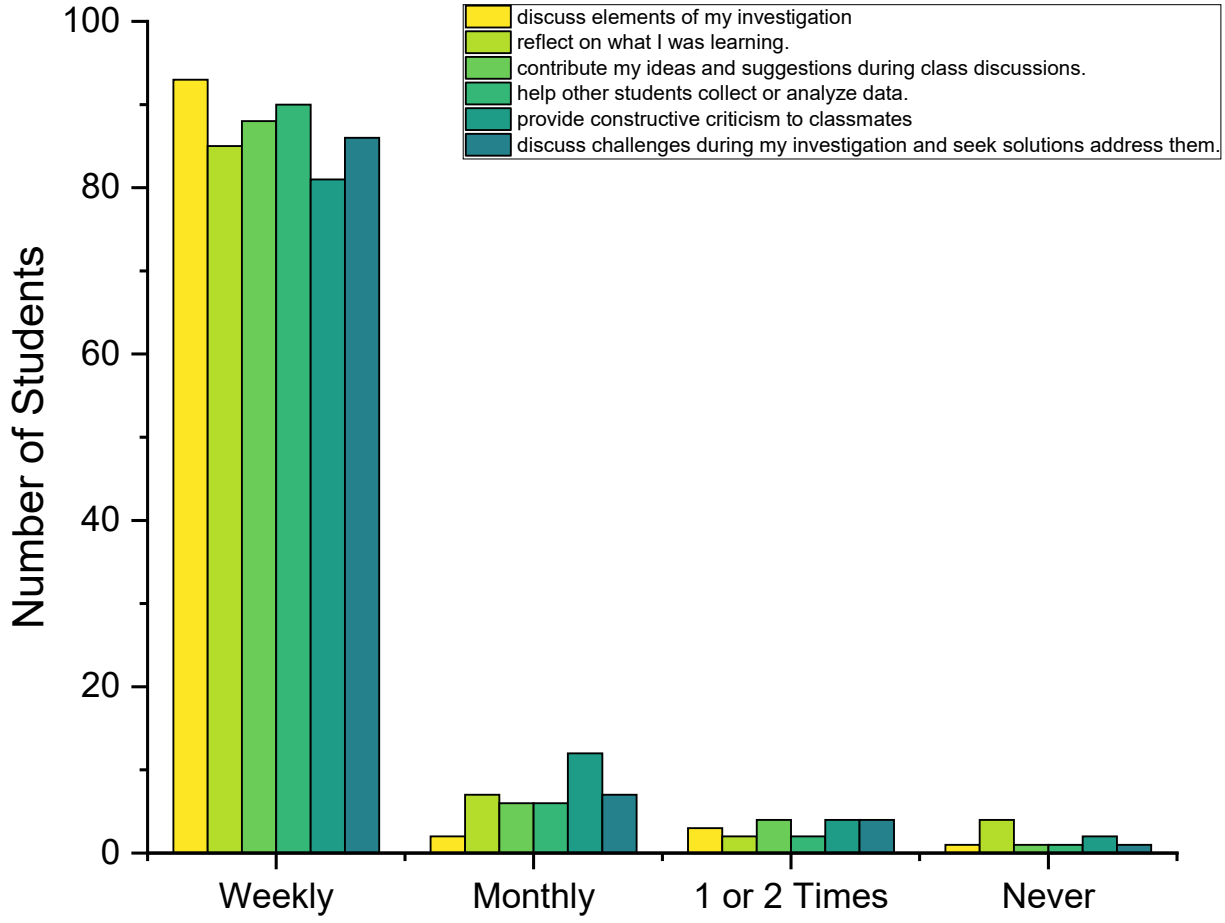

Figure 5.4 Student Attitudes towards Collaborating with Peers

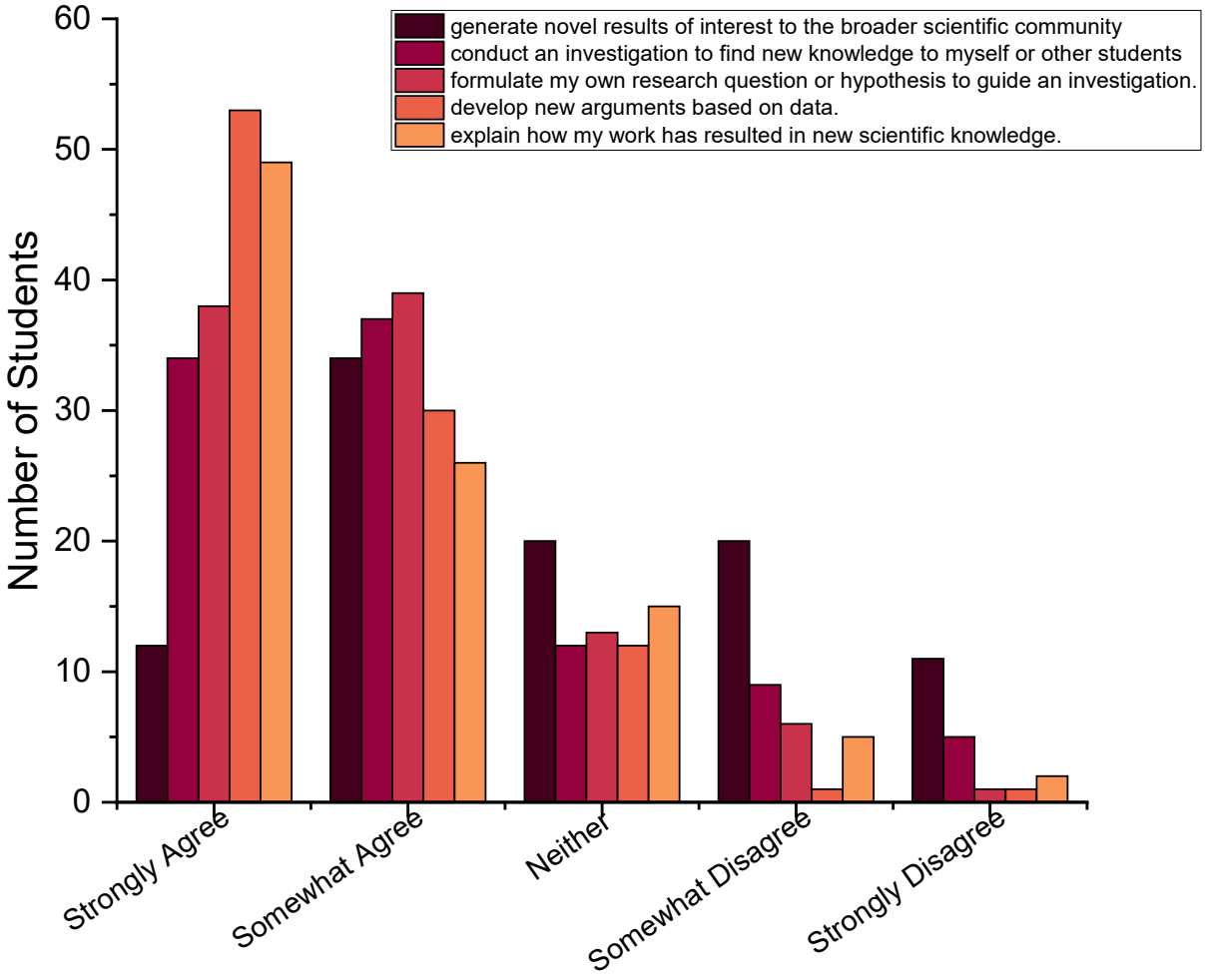

Figure 5.5 Student Attitudes towards Generation of New Knowledge

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