



# CHEMICAL AND BIOMOLECULAR ENGINEERING



UNIVERSITY OF DELAWARE  
ENGINEERING

# WINTER RESEARCH REVIEW

4<sup>TH</sup> YEAR TALKS | WED., JAN. 22, 2025

ABSTRACTS AND SCHEDULE GUIDE

CLAYTON HALL CONFERENCE CENTER

# WELCOME TO OUR ANNUAL WINTER RESEARCH REVIEW.

Today's program of research presentations by our fourth-year graduate students provides a wonderful opportunity to learn about the scientific discoveries and training pathways of our senior graduate students and their faculty advisors. Throughout the day, you can also visit research posters presented by our third-year students.

Our graduate program is one of the central foundations of the department's mission towards scholarship and education. We hope that you will enjoy this opportunity to learn more about our department and its activities, as well as to meet the students and faculty. We are pleased that you can join us!



Millicent Sullivan  
Alvin B. and Julie O. Stiles Professor and Department Chair  
Department of Chemical and Biomolecular Engineering



Logan Yeager  
Colburn Club President  
The Graduate Student Organization

Colburn Club is the graduate student organization in the Chemical and Biomolecular Engineering Department, which is comprised of representatives from each year as well as a number of members filling specialized roles. The primary functions of the club are to organize research reviews and social events for the department, in addition to serving as one line of contact between the students and the faculty. We hope you enjoy this event and can join us again in the future.

The Colburn Club  
<https://sites.udel.edu/colburnclub/>

## Hydroxide exchange membrane carbon capture using a nickel hydroxide symmetric battery cell

James Buchen  
Advisor: Yushan Yan

Direct air capture (DAC) has been identified as a key net negative carbon technologies to achieve a net zero future.<sup>1,2</sup> DAC is required to offset continued emissions from dilute CO<sub>2</sub> sources e.g., agriculture and construction.<sup>3</sup> The majority of current DAC technologies at scale (>1 KT·yr<sup>-1</sup>) are sorbent based with significant energy cost.<sup>4</sup> The United States Department of Energy (DOE) has set forth a cost target of 100 \$·tonCO<sub>2</sub><sup>-1</sup> which is not possible to meet with current industrial electricity costs near 70 \$·MWh<sup>-1</sup> and current temperature swing adsorption devices energy costs above 1.5 MWh·tonCO<sub>2</sub><sup>-1</sup>.<sup>5,6</sup> A combination of lower electricity costs and lower DAC energy costs are required to meet the target DAC cost.

Electrochemical carbon capture devices can be a low energy cost solution using renewable electricity. Historically electrochemical carbon-capture has targeted a range of concentrations from atmospheric (400 ppmCO<sub>2</sub>) (DAC),<sup>1</sup> to life-support (5000 ppmCO<sub>2</sub>),<sup>2</sup> to point-source capture (10% CO<sub>2</sub>).<sup>3</sup> The hydroxide exchange membrane nickel hydroxide symmetric battery cell with two identical electrodes has low voltage requirements making it more suitable for DAC than other electrochemical approaches. A 25 cm<sup>2</sup> laboratory cell shows an average energy cost of 1.15 MWh·tonCO<sub>2</sub><sup>-1</sup> and a CO<sub>2</sub> flux of 78 kgCO<sub>2</sub>·m<sup>2</sup>·yr<sup>-1</sup> at 2mA·cm<sup>-2</sup>. The components of this energy cost will be discussed in detail including, pH gradient formation, cyclic state of charge change, cyclic time lag, and nickel hydroxide stability.

We have partnered with an industrial collaborator who has produced a more manufacturable 25 cm<sup>2</sup> cell is durability tested for 5000 hours and achieves an energy of 0.46 MWh·tonCO<sub>2</sub><sup>-1</sup> and a flux of 62 kgCO<sub>2</sub>·m<sup>2</sup>·yr<sup>-1</sup> at the end of the test. A DAC pilot system with a stack of 9 scaled-up 300 cm<sup>2</sup> cells demonstrates an energy of 0.83 MWh·tonCO<sub>2</sub><sup>-1</sup>, a flux of 75 kgCO<sub>2</sub>·m<sup>2</sup>·yr<sup>-1</sup>, and meets the 300 Pa pressure drop required for DAC.<sup>4</sup> Modular design projects improvements in cost from manufacturing and economies of scale, and a pathway to below 100 \$·tonCO<sub>2</sub><sup>-1</sup> from learning rates of past energy technologies.<sup>5</sup>

- 1 UNEP (2017). The Emissions Gap Report 2017. United Nations Environment Programme (UNEP), Nairobi. (2017).
- 2 IEA (2022), Direct Air Capture 2022, IEA, Paris <https://www.iea.org/reports/direct-air-capture-2022>, Licence: CC BY 4.0. (2022).
- 3 Keith, D. W., Holmes, G., St. Angelo, D. & Heidel, K. A Process for Capturing CO<sub>2</sub> from the Atmosphere. *Joule* **2**, 1573-1594 (2018). <https://doi.org/10.1016/j.joule.2018.05.006>
- 4 Fasihi, M., Efimova, O. & Breyer, C. Techno-economic assessment of CO<sub>2</sub> direct air capture plants. *Journal of Cleaner Production* **224**, 957-980 (2019). <https://doi.org/10.1016/j.jclepro.2019.03.086>
- 5 Monthly Energy Review. (U. S. Energy Information Administration, 2024).
- 6 *Carbon Negative Shot*, <<https://www.energy.gov/fecm/carbon-negative-shot>> (2022).
- 7 Shi, L. *et al.* A shorted membrane electrochemical cell powered by hydrogen to remove CO<sub>2</sub> from the air feed of hydroxide exchange membrane fuel cells. *Nature Energy* **7**, 238-247 (2022). <https://doi.org/10.1038/s41560-021-00969-5>
- 8 Bell, W. L. Synthesis and Evaluation of Electroactive CO<sub>2</sub> Carriers. *SAE International* **97**, 544-552 (1988).
- 9 Rheinhardt, J. H., Singh, P., Tarakeshwar, P. & Buttry, D. A. Electrochemical Capture and Release of Carbon Dioxide. *ACS Energy Letters* **2**, 454-461 (2017). <https://doi.org/10.1021/acsenergylett.6b00608>
- 10 Luukkonen, A., Elfving, J. & Inkeri, E. Improving adsorption-based direct air capture performance through operating parameter optimization. *Chemical Engineering Journal* **471** (2023). <https://doi.org/10.1016/j.cej.2023.144525>
- 11 McDonald, A. & Schratzenholzer, L. Learning rates for energy technologies. *Energy Policy* **29**, 255-261 (2001). [https://doi.org/10.1016/s0301-4215\(00\)00122-1](https://doi.org/10.1016/s0301-4215(00)00122-1)

## **Engineering *Bacillus subtilis* Towards Safe and Effective Deployment in the Rhizosphere**

Avaniek Cabales

Advisor: Aditya Kunjapur

Committee Members: Mark Blenner, Wilfred Chen

---

Global food demand is projected to increase; however, modern agriculture relies heavily on fertilizers and pesticides, which negatively impact the sustainability of global food production. Plant growth promoting rhizobacteria provide a more sustainable alternative to chemicals harmful to the environment. Currently, natural isolates can be used to enrich soil, but these strains often have inconsistent field performances. Microbial engineering holds the potential to gain more understanding and control of desired microbial functions. However, little is understood about how engineered functions can affect or be affected by the complex interplay of biotic and abiotic factors in the rhizosphere.

Here, we aim to understand how a model beneficial molecule is affected by culturing conditions. We successfully engineered overproduction of surfactin, a circular lipopeptide with many benefits such as improving colonization and stimulating plant immunity. We observed that our strain can produce surfactin in both rich media and in conditions simulating plant growth environments. We delve into understanding nitrogen assimilation of *B. subtilis* under various conditions in plant hydroponic media. In collaboration with the Bais Lab at the University of Delaware, we examined how wild-type and engineered strains colonize the roots of tomato plants. Given the importance of organic nitrogen sources, we examined the exogenous L-glutamate and surfactin affected colonization. Additionally, we are designing strategies to control survival of engineered *B. subtilis* in soil by introducing dependence on a non-standard amino acid (nsAA). Here, we quantify the abundance of engineered *B. subtilis* in sterile soil and we have successfully engineered nsAA incorporation in these conditions. Overall, we present work moving towards engineering controlled deployment in uncontrolled, complex agricultural environments.

## **Integrating bioactivity and viscoelasticity into covalent hydrogels using multifunctional collagen mimetic peptides**

Rafael Castro

Advisor: Dr. April M. Kloxin

Committee Members: Dr. Arithi Jayaraman, Dr. Millicent O. Sullivan

---

Peptides, functionally encoded by their amino acid sequence, are increasingly being designed within the biomaterials community to create mimics of the extracellular matrix found within human tissues. Collagen-like peptides have been designed to mimic parts of the structure and bioactivity of collagen I, the most prevalent protein in the human body and important in the structure and properties of many tissues. Recently, synthesis methods have been developed for self-assembling multifunctional collagen mimetic peptides (mfCMPs) for the formation of synthetic matrices with robust and tunable properties. There remains a need for self-assembling peptides with tunable properties inspired by native tissues that can be used to create functional biomaterials with tailorable biophysical and biochemical properties and consistency from batch to batch that have the potential to serve as surrogates for harvested collagen I. To address this need, I have designed and synthesized mfCMPs that contain integrin binding motifs within their amino acid sequence that self-assemble into triple helices and fibrils inspired by natural collagen: e.g., GFOGER (available for binding in intact collagen I) and RGD (available for binding in damaged/denatured collagen I). These mfCMPs can be incorporated into polyethylene glycol-based hydrogel matrices to mimic both healthy and diseased tissue by tuning stiffness and number of RGD binding sites. I have verified that these mfCMPs retain their assembly within the hydrogel matrix by imaging them using super-resolution microscopy techniques. I have also shown through stress relaxation experiments that once incorporated, these mfCMPs impart viscoelasticity into otherwise elastic hydrogels. Finally, I have shown that hydrogels containing mfCMPs influence cellular behavior due to their bioactive and viscoelastic properties. When T-47D breast cancer cells are encapsulated in these hydrogels, organoid clusters form when mfCMPs are present and are larger when the mfCMPs contain integrin binding sequences. Overall, mfCMPs are part of a modular system of building blocks that has the potential to be a fully synthetic surrogate for natural collagen I for applications in vitro and in vivo.

## **Carbonate-rejecting ionomer to boost CO<sub>2</sub> removal of a hydroxide exchange membrane carbon capture device**

Marco Colin

Advisor: Yushan Yan

Committee Members: Raul Lobo, Antony Beris

---

The development of negative emission technologies (NETs) is necessary to offset unavoidable CO<sub>2</sub> emissions, but their widespread deployment hinges on advancements in technology cost, energy consumption, and durability to achieve under \$100/ton CO<sub>2</sub> capture and storage. We estimate a material and manufacturing cost of \$23/tonCO<sub>2</sub> for our hydrogen-powered, shorted hydroxide exchange membrane carbon capture (HEMCC) technology owing to its construction of mostly plastic components. Stoichiometrically, maximum electron efficiency (CO<sub>2</sub>/e<sup>-</sup>) is achieved in the HEMCC when only bicarbonate transports carbon through the membrane, while carbonate limits electron efficiency to 50%. Surprisingly, slowing down the ion transport in the membrane accelerates CO<sub>2</sub> capture, leading to savings in hydrogen operating costs. To understand this, we developed a physics-based model and carbon-loss framework, revealing that higher membrane resistance enhances the migration-to-diffusion ratio, improving efficiency. This framework also enabled a breakthrough design featuring a large porous interlayer utilizing a carbonate-rejecting ionomer, advancing toward pure bicarbonate transport. A non-optimized design path is predicted to lower hydrogen operating costs down to \$32/tonCO<sub>2</sub>.

## **Stimuli-responsive assembly from biosynthetic building blocks for the construction of nanomaterials with programmable architectures**

Caitlin D'Ambrosio

Advisor: April Kloxin, Christopher Kloxin, and Wilfred Chen

Committee Members: Millicent Sullivan

---

Nature provides exquisite control of material properties from the bottom up, where the underlying amino acid sequence of proteins give rise to hierarchical nanostructures and related micro- to macro-scale material properties. Examples of this precision range from viral particles to extracellular matrix bottlebrushes. However, the architectural complexity of many hierarchical nanostructures found in nature has made it difficult for scientists to replicate with synthetic building blocks. To address this, we have combined molecular engineering principles with biosynthetic techniques to create coiled-coil peptides as building blocks, known as bundlemers. Bundlemers are computationally designed to possess robust stability and modularity, allowing tailored modifications of residues for hierarchical assembly. Through click and enzymatic chemistry, we can construct these bundlemers into precise protein-like nanostructures with programmable functionalities. By integrating an unnatural amino acid, we used copper-catalyzed azide-alkyne cycloaddition (CuAAC) to attach the pH-responsive peptides responsible for the assembly of rod-like polymer backbone. Further, sortase ligation, which is facilitated by an enzyme and exhibits high reaction efficiency similar to traditional click chemistries, was employed to attach pendant groups, or complementary bundlemer peptides, onto the assembled backbone. We demonstrate approaches of the synthesis and controlled formation of assembled units with programmed structure and responsiveness, providing opportunities for the construction of bottlebrush nanostructures through the manipulation of the grafted peptide sequence and alteration of the reaction conditions. This work establishes design rules and workflows for the creation of hierarchically-structured, proteinaceous nanomaterials. By demonstrating controlled synthesis and structure formation, we lay the foundation for the construction of previously inaccessible protein-based structures relevant for a range of applications, from biolubricants mimicking naturally occurring aggrecan in the human joints, to targeted drug delivery vehicles for dense tissue such as the brain.

## **Designing sustainable geopolymer materials by rheo-kinetic and nanoscale structural evolution measurements of aluminosilicate gels**

Thaddeus M. Egnaczyk

Advisor: Norman J. Wagner

Committee Members: Ryan Murphy, Alexandra Bayles, Raul Lobo

---

The design of cementitious materials for emerging applications like additive manufacturing (AM) requires appropriate rheology for mixing, pumping, extrusion, and deposition with high shape fidelity after extrusion. Reacting materials such as cements and geopolymers have additional kinetic constraints that influence processing parameters and material formulation. Therefore, kinetic structure-property relationships are required to connect an initial material composition to desired final material properties and processing parameters. Alkali-activated aluminosilicate binders (AAB's) are an important class of alternative cement materials (e.g., geopolymers) with the potential to reduce carbon emissions in cement construction by up to 80%. This type of binder can be produced from naturally occurring aluminosilicate clays and waste products including fly ash and slag. Such materials are also candidates for in-situ resource utilization (ISRU) in lunar and Martian construction. To reduce complexity in understanding the proposed AAB polycondensation reaction mechanism, an aluminosilicate gel can be synthesized without the presence of larger scale, heterogeneously composed particles. When sodium is used as the alkali cation, this gel is referred to as the sodium aluminosilicate hydrate (N-A-S-H) gel. The early age properties and gelation kinetics of the model gel system offer insight into the complicated structural evolution in the complete AAB system.

The goals of this work are to (1) connect gelation kinetics measured via rheology to structural evolution via stopped-flow SAXS and (2) understand how relevant compositional changes affect the kinetic evolution of gel structure near the critical gel point. A complete rheological characterization of material property evolution during gelation is captured via small amplitude oscillatory shear (SAOS) rheology paired with optimally windowed-chirp (OWCh) signals to measure time-resolved frequency spectra. Combined stopped-flow SAXS (CHESS) and USAXS (ESRF) enable rapid mixing of gel precursor solutions in the beamline with ~100 ms time resolution, capturing the dynamics of the rapidly evolving gel structure from the ms timescale to hours and length scales from several nm to  $\mu\text{m}$ . Gel structural parameters including primary particle radius of gyration, fractal dimension, and aggregate cluster size are measured during gel formation. A correlation between reaction kinetics and property development is developed through application of the Avrami kinetic model to both the gel volume fraction calculated from the SAXS data and the linear viscoelasticity of the gel. Finally, gel elasticity and structure are connected via the Shih model to define a reaction-limited cluster-cluster aggregation mechanism which transitions from a strong-link regime to a weak-link regime as the gel aggregate volume fraction increases. Furthermore, a lateral study across a range of gel compositions helps to understand the impact of gel composition on the structure and kinetics of material property development, which is necessary for optimized design of processing routes for sustainable geopolymer construction materials.



## **Transient Blood Rheology Induced by Red Blood Cell Aggregation**

Sean Farrington

Advisor: Norman Wagner & Antony Beris

Committee Members: Alexandra Bayles & Abraham Lenhoff

---

Human blood is a colloidal suspension of 7-8  $\mu\text{m}$  deformable biconcave discoids. Rheological characteristics of blood are correlated to cardiovascular disease risk and may be used as an advanced screening diagnostic.<sup>1</sup> One essential rheology experiment is the shear rate step-down, which exhibits a unique transient behavior for blood that has not been fully explained in the literature. A critical feature of blood's microstructure is the formation of red blood cell aggregates at low shear rates, known as rouleaux. Rouleaux formation contributes to transient behaviors of blood rheology such as plasticity, viscoelasticity, and thixotropy.<sup>1</sup> Some animal species, such as sheep, do not form rouleaux, which makes their rheology distinct from species that do. The goal of this work is to determine if the step-down transience is a direct result of rouleaux formation and examine potential mechanisms. To accomplish this aim, blood is separated into rouleaux-forming and non-rouleaux-forming samples by using a polymeric depletant to induce aggregation. Rheology is measured for these samples and shows that a step-down transience at low shear rates occurs as a direct result of rouleaux formation. Sedimentation and radial migration are then examined as potential mechanisms for this transience caused by rouleaux. Static multiple light scattering suggests that sedimentation is unlikely to account for the transience. However, a two-fluid model provides a promising explanation for radial migration, which is validated by microfluidic videos of the blood suspensions. Further exploration of the connection between physiology and rheological behavior may explain why rouleaux structures exist in humans and many other animal species.

<sup>1</sup> Beris, A. N., *Soft Matter* **2021**, 17 (47), 10591-10613.

## Renewable and Recyclable Monomers for Advancing Circularity

Charles Fields

Advisor: Dionisios Vlachos and Raul Lobo

Committee Members: Dongxia Liu, LaShanda Korley, and Alan Allgeier

---

The need to reduce global dependence on petroleum-derived chemical products drives the innovation of renewable alternatives to replace established materials. The plastics industry provides vast opportunities to harness abundantly available biomass to meet the ever-increasing consumer demands while mitigating the environmental footprint associated with the current plastic economy. Herein, we investigate biomass-derived pathways to generate monomers for polyester applications amenable to chemical recycling, promoting a renewable and circular plastics economy.

Recent investigations demonstrated a pathway to prepare 4,4'-dimethylbiphenyl (DMBP), an attractive platform chemical, from readily available biomass precursors. Utilizing this renewable molecule in consumer plastics through oxidation and esterification to dimethylbiphenyl-4,4'-dicarboxylate (BPDC) and subsequent polymerization into high-performance polyesters demonstrates significant advances in sustainable alternatives. The synthesis of DMBP follows a two-step process: (1) 2-methylfuran (MF) oxidative coupling to 5,5'-dimethyl-2,2'-bifuran (DMBF), (2) DMBF tandem Diels-Alder-Dehydration with ethylene to afford the desired DMBP. Earlier research identified conditions achieving 63 % MF conversion and 59 % DMBF yield with a space-time yield of 0.59 mol L<sup>-1</sup>h<sup>-1</sup>, leaving room for improvements in DMBP production. A surface response design identified an initial optimum at 44 °C and a MF/solvent molar ratio of 0.71, improving the space-time yield to 0.75 mol L<sup>-1</sup>h<sup>-1</sup>. Temperature effects at the optimum MF/solvent ratio showed that as the reaction reaches complete conversion, DMBF further reacts to form oligomers. It was found that reducing the time to 1.5 hours at 67 °C led to a MF conversion of 96.2% and a DMBF yield of 77.5 % with a DMBF space-time yield of 1.10 mol L<sup>-1</sup>h<sup>-1</sup>, an 86.4 % increase from the baseline. Scale-up efforts resulted in a 44x increase in DMBF production, bringing the process from milligrams to grams. For the second step, a homogenous Lewis acid catalyst in the Diels-Alder-Dehydration reaction demonstrated a 40x increase in DMBP yield at reduced temperatures compared to the initial protocols. Successful demonstration of the Diels-Alder-Dehydration at a 3 g scale while maintaining selectivity for the DMBP product suggests facile large-scale feasibility. Renewable DMBP was oxidized via the MidCentury process and esterified to BPDC in yields of ~90 % and purity of 97 %, enabling polymerization in copolymers.

Our investigation of renewable monomer pathways is continued through the carbonylation of biomass-derived aryl triflates. Focusing on lignin-derived monophenol substrates, we replace the phenolic group through trifluoromethanesulfonylation using a trifluoromethanesulfonyl pyridinium salt, yielding an aryl triflate attuned for palladium-catalyzed carbonylation. Under this system, trifluoromethanesulfonylation of methyl vanillate occurs within 30 minutes at room temperature, achieving a 72 % isolated yield. Further efforts on the carbonylation of aryl triflates under mild conditions to produce a diester available for application in polyesters will be reported.

## **Advancing sustainable geopolymer construction materials by developing experimental structure-property relationships aided by rheology-informed neural networks**

William H. Hartt V

Advisor: Norman J. Wagner

Committee Members: Alexandra Bayles and Raul Lobo

---

Geopolymer materials are a sustainable alternative to modern cements offering significant reductions in both CO<sub>2</sub> emissions and water usage and are candidates for in-situ resource utilization (ISRU) on Earth, the Moon, and Mars. When properly formulated, these alkali activated materials (AAM) are comparable to or exceed the properties of Ordinary Portland Cement. My research on lunar regolith simulant Black Point 1 (BP-1) supports the formation of geopolymers from ISRU<sup>1</sup>, in line with NASA's drive to have a sustained human presence on the Moon with Project Artemis, requiring the development of novel construction materials for landing pads, habitats, storage facilities, and other structures. In fact, candidate lunar ISRU geopolymers developed in our lab are currently being flow on the MISSE test station (mission MISSE-20) of the International Space Station (ISS) (under support from NASA EPSCoR) to improve their technology readiness level.

My research focuses on the development of molecular structure-property relationships by extending the pseudo-ternary state diagram<sup>2</sup> to additional regoliths, alkali activator compositions, curing protocols including microwave accelerated curing, and regolith physical properties. This includes my current NSF INTERN fellowship at the AFRL to study phosphate based geopolymers within the framework we have developed at UD. Here, I will discuss both the evolution of rheological properties during curing as well as the 7-day compressive strength and how they depend on the particle size distribution for BP-1 lunar regolith stimulant with different regolith particle size distributions. Our hypothesis is, because the dissolution and gel-formation processes during geopolymer synthesis involve surface reactions, property evolution and final materials properties will scale with the Sauter mean diameter ( $d_{3,2}$ ). This is demonstrated for BP-1 geopolymers through rheological and compressive strength measurements on sieved size classes. These experimental results and analyses provide guidance for the development of processing strategies and design of material properties for alkali activated aluminosilicate geopolymers, decreasing a broad material input, the particle size distribution of the aluminosilicate source, into a single parameter.

As geopolymer composition varies with the local source, advanced computational tools are necessary to extend limited experimental results to address the need for ISRU processing strategies. Working with the Jamali group at Northeastern (NSF DMREF Award #2118944), we are developing a rheology-informed neural network (RhINN) to provide optimal composition and processing strategies across a very broad class of source materials. First results for the amount of high-fidelity laboratory data required for predicting rheology on a model, complex rheological sample provides guidance for current efforts to develop a tool to address geopolymer activation and processing strategies<sup>3</sup>.

1. T. Egnaczyk, W. Hartt, J. Mills, and N. Wagner; *Adv. Space Res.* **73**, 885 (2024)
2. J. Mills, P. Mondal, and N. Wagner; *Cem. Concr. Res.* **151**, 106618 (2022)
3. M. Saadat, W. Hartt, N. Wagner, and S. Jamali; *J. Rheol.* **68**, 679 (2024)

## **Peptide-DNA Hybrid Lipid Nanoparticles for Targeted Local Lung Delivery in Monogenic Surfactant Lung Disease**

Jinzhen Hu (Hugo)

Advisor: Dr. Millicent Sullivan

Committee Members: Dr. Catherine Fromen, Dr. April Kloxin, and Dr. Deepthi Alapati

---

Monogenic surfactant diseases, resulting from mutations in surfactant genes within alveolar type 2 cells of the pulmonary epithelium, pose a significant clinical challenge with high morbidity and mortality rates. Current treatment options are limited to palliative care or lung transplantation, both of which are associated with poor outcomes. Emerging strategies such as pre-natal gene editing using viral vectors face significant barriers, including stringent equipment requirements and immune responses to viral particles.

To address these limitations, we are developing a novel non-viral delivery system using lipid nanoparticles (LNPs) to transport DNA cargo, which offers several advantages over mRNA-based systems. DNA LNPs provide enhanced cargo stability and prolonged gene expression but lack in therapeutic efficacy. We are optimizing the DNA LNP formulation and design for localized lung delivery, overcoming challenges such as inefficient therapeutic distribution and limited LNP uptake in the absence of serum proteins. By integrating perfluorocarbon-assisted partial liquid ventilation, we aim to improve the distribution of LNPs within the lung. Additionally, pre-identified peptides and serum protein pre-coating strategies have been employed to enhance LNP uptake and targeting specificity.

Our work has yielded several key results: (1) optimized formulations for peptide-inclusive DNA LNPs, (2) significant insights into the role of serum protein coatings in enhancing uptake and gene expression, (3) a 99% transfection rate with a 16-fold increase in model gene expression using optimized peptide-DNA LNPs compares to regular, and (4) demonstrated cell-specific targeting through tailored serum protein coatings, with minimal off-target effects in vitro. We also established a method for isolating coated lipid nanoparticles using functionalized magnetic beads.

Future studies will focus on in vivo testing of serum protein-coated peptide hybrid LNPs for therapeutic efficiency and further proteomic analysis of serum populations interacting with the LNPs. This work represents a promising step toward postnatal gene therapy for surfactant diseases, with potential to improve treatment outcomes significantly.

## **Development of a 3D Bioprinted Skin Microenvironment**

Bree Huntington

Advisors: April M. Kloxin and Eric M. Furst

Committee Members: Catherine A. Fromen and Norman J. Wagner

---

Synthetic, *in-vitro* methods that recapitulate the skin microenvironment for assessing therapeutic approaches for dermal wound healing remain a pressing need in the biomedical field. A unique approach to address this need and associated challenges is to develop a 3D bioprinted hydrogel model system that mimics the human skin microenvironment. Hydrogels have been highly sought after as a 3D cell culture platform, given their biocompatibility, ability to incorporate various bioactive moieties, and tunability of mechanical properties. 3D bioprinting offers many advantages, including ease of use, biomimicry based on material composition, spatiotemporal considerations, and high-throughput sample preparation. In this work, we examined the use of the *Inventia RASTRUM* 3D bioprinter and Normal Human Dermal Fibroblast (NHDF) cells to create a reductionist human dermis microenvironment model system. We used 2 different bioinspired matrix formulations: a more compliant formulation and a stiffer formulation to assess the effects of microenvironmental mechanics on cellular responses. We found that cells exhibit good viability over a 7-day time frame in the selected matrix formulations. Additionally, we observed cellular response to the mechanics of the microenvironment and assessed relevant tissue-specific proteins through various immunostaining assays. To further ramp up the complexity of the model, we have developed an air-liquid interface (ALI) co-culture model system with Normal Human Epidermal Keratinocytes (NHEKs) with NHDF toward recapitulating aspects of both the epidermis and dermis, respectively. Ongoing work aims to further characterize and optimize the co-culture system, introduce immune system components, and introduce triggered degradation of the matrix at the ALI to examine cellular response to injury.

## **Biorefinery superstructure optimization under static and dynamic carbon pricing constraints**

Dat Huynh

Advisor: Prof. Marianthi Ierapetritou and Prof. Dionisios Vlachos

Committee Members: Prof. Raul Lobo

---

With the continued rise in global mean surface temperatures driven by increasing anthropogenic CO<sub>2</sub> emissions, governments are striving to implement policies aimed at mitigating and reducing these emissions. Concurrently, scientists are developing innovative engineering solutions to enhance the efficiency of CO<sub>2</sub>-reduction technologies for existing processes and to advance sequestration technologies that remove CO<sub>2</sub> from the atmosphere. Policymakers play a critical role by enacting regulatory and environmental policies designed to limit emissions. Among these, carbon pricing refers to policy mechanisms that assign a cost to carbon dioxide emissions. This work evaluates three types of carbon pricing policies: carbon tax, cap-and-trade, and carbon cap. Carbon taxes impose a direct cost on CO<sub>2</sub> emissions, calculated as a fixed rate per metric ton of CO<sub>2</sub> emitted. Cap-and-trade systems allocate a set number of emission allowances to CO<sub>2</sub> emitters, which can be traded on the market as needed. The government determines the total number of allowances, setting an overall emissions cap. Market dynamics determine the carbon price based on the trading of these allowances. Carbon cap policies impose strict limits on CO<sub>2</sub> emissions but do not directly involve a carbon price. Emitters are required to stay within specified caps.

In this study, a two-stage stochastic mixed-integer linear programming model is developed to optimize biorefinery process designs under various carbon pricing policies and crediting mechanisms. The model accounts for uncertainties in emissions, feedstock supply, chemical demand, and pricing. Biomass-based feedstocks, which can qualify for carbon credits, demonstrate significant financial savings under carbon tax policies. Sensitivity analysis on varying carbon prices and policies highlights shifts in the Pareto frontier, revealing the impact of policy changes on process design.

The proposed formulation offers a robust decision-making tool for governments implementing environmental policies and businesses adapting to these frameworks. The model is further extended to consider dynamic carbon pricing, reformulating the optimization problem as a capital investment planning problem. To meet the 1.5°C global warming target, carbon pricing policies must become progressively stringent. Under this model, carbon cap policies underscore the need for reducing the costs of carbon capture, utilization, and storage (CCUS) technologies to enable compliance with stricter emission caps over time. Similarly, carbon tax policies result in decreased chemical production due to financial penalties, emphasizing the importance of affordable CCUS technologies for offsetting emissions and achieving net-zero carbon goals.

## **Development of enzyme cascades for polyethylene deconstruction**

Ross Klauer

Advisor: Mark Blenner, Kevin Solomon

Committee Members: Aditya Kunjapur, LaShanda Korley

---

Polyethylene (PE) is the most abundantly produced plastic globally due to favorable material properties such as high ductility, mechanical strength, and UV resistance that make the plastic extremely recalcitrant. This recalcitrance, coupled with low PE production costs from petroleum feedstocks, has led to the environmental accumulation of an estimated 4 million tons of PE annually. Mechanical and chemical PE recycling strategies are hindered by process economics due to high operating conditions and often emit greenhouse gases as side products. Biological PE deconstruction and upcycling offers a sustainable and economically viable alternative by operating near ambient conditions and allowing for product specificity through metabolic engineering, but enzymes for the deconstruction of PE into bioavailable products remain undiscovered. To combat this lack of available enzymes, we mined the guts of Low-Density PE (LDPE) eating mealworms to discover dye decolorizing peroxidases (DyPs) that oxidize LDPE, initiating the biological deconstruction of the polymer. We confirmed the activity of DyPs on PE chains by demonstrating activity on additive-stripped LDPE and the inclusion of cofactor-free control treatments. DyPs with LDPE-oxidase activity are defined by a hydrophobic loop region proximal to the active site of the enzyme that mediates PE binding. LDPE oxidation occurs through solvent exposed aromatic residues near the active site that oxidize PE chains through oxygen radical chemistry, forming ketones on the PE backbone. A secondary PE oxidation to convert ketones to hydrolysable esters is hypothesized to be performed by monooxygenases. We identified putative LDPE-active monooxygenases and confirmed their activity on model ketones as a proxy for oxidized LDPE. DyPs and monooxygenases will be paired with esterases to generate fatty acids for biological upcycling. This work provides robust evidence for an entirely enzymatic PE deconstruction pathway and identifies targets for enzyme engineering and deconstruction products that will allow for further development to realize scalable biological LDPE deconstruction and upcycling.

## Computational Studies of Network Morphologies in Soft Materials

Stephen Kronenberger

Advisor: Arthi Jayaraman

Committee Members: April Kloxin, Alexandra Bayles

---

Soft materials with connected, network morphologies often have enhanced mechanical and transport properties that are desired for different applications. Connected networks of hydrophilic domains in ionomer membranes allow ions to diffuse more quickly than they would without a network of hydrophilic domains. The formation of fibrillar networks of collagen give rise to enhanced mechanical properties that provide structural support to the body's tissues, and fibrillar networks of methylcellulose act as a thickener and stabilizer for food processing applications. My thesis focuses on studying the effect of molecular design on the network structure in a wide range of soft materials. In this talk, I will focus on the thermoreversible self-assembly of methylcellulose (MC) into fibrillar networks. MC is a cellulose derivative with some of the hydroxyl groups along its backbone substituted with methoxy groups. Commercial MC is typically synthesized using techniques that yield a heterogeneous substitution of methoxy groups along the polymer backbone, with a degree of substitution (DS) of around 1.8 methoxy groups per monomer. Commercial MC is soluble at room temperature, and forms a turbid gel at around 50-60°C as individual MC chains aggregate into semi-flexible fibrils. For commercial MC, it has been shown that these fibrils have a consistent diameter of approximately 20nm, independent of concentration and molecular weight. However, through alternative synthesis techniques, one can synthesize MC with a more homogenous substitution pattern or even block copolymers of MC monomers with different substitutions. These changes to the distribution of hydrophobic groups along the MC backbone have been shown to have profound impact on the solubility and gelation properties of MC, but the fibril structural changes that accompany these alternative synthesis methods have not been explored. As synthesis techniques develop further, a strong understanding of the relationship between MC molecular design and solubility and gelation properties would allow for MC synthesis tailored for a given application, but such an understanding has been limited by the large design space of methyl substitution pattern. In this talk, I will share my ongoing work using a combination of data-driven exploration and physics-based coarse-grained molecular dynamics simulations to investigate how the DS and substitution pattern impact the self-assembly of MC chains in aqueous solution.



## **Electrified Biogas Upgrading into Value-Added Chemicals: Challenges and Opportunities**

Yeonsu Kwak

Advisor: Dionisios G. Vlachos

Committee Members: Raul Lobo, Dongxia Liu, Yushan Yan

---

Climate change mitigation demands innovative approaches to valorize methane and CO<sub>2</sub>. Despite their abundance, activating these stable molecules remains challenging. Here, we demonstrate that electrified heating strategies offer unique advantages in overcoming these obstacles. We compared conventional heating (CH), microwave heating (MWs), and continuous/rapid pulse Joule heating (CJH and RPH) across methane conversion processes. While MWs show promise for catalytic reforming, they prove unsuitable for direct methane coupling due to coking and thermal runaway risks. Conversely, RPH emerges superior power efficiency, enhancing C<sub>2</sub> yield and suppressing coke formation in biogas upgrading. Remarkably, RPH facilitates direct biogas conversion to C<sub>2</sub> products with negligible CO formation, achieving efficiency comparable to non-oxidative methane coupling. This contrasts with CH scenarios where direct coupling competes with oxidative reforming when CH<sub>4</sub> is co-fed with CO<sub>2</sub> or H<sub>2</sub>O, yielding substantial CO.

Co-feeding CO<sub>2</sub> or H<sub>2</sub>O in RPH systems enhances the C<sub>2</sub>/(C<sub>3</sub>+C<sub>4</sub>) ratio by inhibiting secondary reactions. Importantly, fluctuations in CO<sub>2</sub>/CH<sub>4</sub> feed ratios minimally impact C<sub>2</sub> yield, highlighting RPH's robustness. Our findings provide insights into electrified catalysis methods for biogas valorization, offering a framework for developing energy-efficient, selective processes for methane and CO<sub>2</sub> conversion. This work paves the way for more sustainable chemical industries, potentially revolutionizing shale and biogas utilization strategies.

## **Improving Bispecific Antibody Titer and Quality in Single CHO Cell Hosts**

David Le

Advisor: Prof. Kelvin Lee

Committee Members: Prof. Wilfred Chen and Prof. Kevin Solomon

---

Bispecific antibody (bsAb) drugs have significant clinical and commercial importance due to their therapeutic advantages over traditional monoclonal antibodies (mAbs), such as dual-antigen targeting, improved specificity, and cytotoxic effector cell recruitment. These core characteristics of bsAbs enhance treatment efficacy, which in turn reduces toxicity and cost by minimizing dosing regimens. Robust, high-titer production platforms are needed to meet the growing clinical demand for bsAbs and other multispecific antibody formats.

Current cell line development (CLD) strategies in CHO cells can result in high volumetric productivities (>10 g/L) but are not fully transferable to bsAb production. mAb expression involves the transcription of two genes, whereas bsAb expression requires the transcription of three or more genes derived from two distinct antibodies. As a result, bsAb production in single host cell formats faces challenges related to product heterogeneity and lower yields, primarily due to antibody chain mismatches that lead to the generation of non-functional or monospecific molecules. Although significant efforts are focused on addressing this issue at the protein design and purification stages, much of the knowledge applied to producing bsAbs at the CLD stage still relies heavily on applied principles from traditional mAb production.

To better understand bsAb CLD bottlenecks, we used transposase-mediated integration techniques to develop high-productivity (high mg/L to g/L) reference cell pools. Two FDA-approved bsAbs, emicizumab (Hemlibra) and faricimab (Vabysmo), representing two different IgG-like formats, were used as model molecules to evaluate titer and product quality. In-gel digestion and peptide mapping were used to validate proper bsAb assembly and expression. Using an established site-specific integration (SSI) method and landing pad cell line, we generated preliminary cell pools to assess production limitations when using SSI. Establishing cell line development workflows for these model molecules provide insights for rational designs of transgene cassettes to improve production, including cassettes for multi-landing pad systems.

## **Dynamic Operation of Carbon Monoxide Electrolysis**

Ahryeon Lee

Advisor: Prof. Feng Jiao, Prof. Yushan Yan

Committee Members: Prof. Dongxia Liu, Prof. Raul F. Lobo

---

Electrochemical carbon monoxide (CO) reduction is a promising strategy for converting CO<sub>2</sub> into value-added products through tandem CO<sub>2</sub> reduction. While CO<sub>2</sub>/CO electrolysis has traditionally operated in a steady state, continuous operation presents significant challenges due to the mismatch between renewable electricity availability/pricing and constant power demands. Dynamic operation has emerged as a potential solution, but achieving stable performance under intermittent conditions – particularly during startup and shutdown cycles based on renewable energy availability – remains challenging due to catalyst degradation.

Here, we present a strategy that maintains copper catalysts within a protective voltage window during shutdown periods. By preserving copper catalysts under slightly reducing conditions with less than 1% of full operational power, catalyst oxidation can be prevented. This approach enables stable CO electrolysis under dynamic conditions for over 300 hours. Our techno-economic analysis shows that this operational strategy can lower acetic acid production costs by utilizing low-cost electricity periods compared to continuous operation.

## **Investigating CHO Cell Metabolism in Perfusion Culture Through Experimental and Modeling Techniques to Advance Continuous Biomanufacturing**

Nikola Malinov

Advisors: Marianthi G. Ierapetritou, Eleftherios T. Papoutsakis

Committee Members: Kelvin Lee, Mark Blenner

---

Recombinant monoclonal antibodies (mAbs) have transformed medicine since the 1980's providing life-saving therapeutics for a broad range of diseases. Chinese hamster ovary (CHO) cells are the preferred mammalian cell platform for mAb production given their robust growth in defined media, efficient transfectability, and innate capacity to induce complex post-translational modifications such as N-linked glycosylation. Increasing global product demand, coupled with the emergence of an ever-expanding biosimilars market, have prompted the industry to explore continuous manufacturing; a trend consistently witnessed across numerous sectors of chemical engineering. Perfusion platforms facilitate continuous upstream cell culture and process intensification by enabling high viable cell densities (VCDs), increased productivity, smaller equipment sizes, and improved product quality profiles. However, the interdependency between the large number of critical process parameters (CPPs), coupled with their complex effects on production hosts, challenge continuous bioprocess development. This work seeks to address these challenges through a combined experimental and computational approach to investigate CHO cell metabolism in continuous culture and provide insights towards process advancement.

A pseudo-perfusion platform was designed for the CHO-K1 VRC01 cell line to assess metabolic demands and mAb production across a range of industrially relevant conditions. Pseudo-perfusion cultures reduce the number of large-scale experiments required to characterize an appropriate design space by approximating continuous operational dynamics in a semi-continuous non-instrumented setting. Subsequent perfusion bioreactor validation experiments confirmed the pseudo-perfusion platform as a reliable tool to both enable metabolic steady state and emulate metabolic phenotypes observed under continuous operation. Comparative analysis of growth, nutrient uptake, and metabolite production rates between pseudo-perfusion and bioreactor cultures, and across culture conditions, demonstrated significant agreement.

Mechanistic models are a valuable tool for the bioprocess industry as they systematically link CPPs with the key process outputs, reducing process development times by informing subsequent experiments and design decisions. With respect to continuous mAb production, modeling cell metabolism is imperative to assess nutrient utilization and physiological objectives under varying operating setpoints to propose improved medium formulations. A comprehensive Dynamic Metabolic Flux Analysis (DMFA) model was developed for the CHO-K1 VRC01 cell line from prior fed-batch applications. The framework was trained on the pseudo-perfusion dataset to describe the effect of limiting nutrients, identify key ammonium sinks, and make successful predictions of continuous perfusion performance. Future work aims to apply the DMFA model towards *in silico* medium optimization studies, followed by validation experiments.

## Computational Design Insights into Joule Heated Reactor

Arnav Mittal

Advisor: Prof. Dion Vlachos and Prof. Marianthi Ierapetritou

Committee Members: Prof. Raul Lobo and Prof. Antony Beris

---

Manufacturing is the world's largest energy consumer and a major greenhouse gas emitter, with process heating accounting for over 36% of its energy use. Therefore, there's a demand for highly efficient reactors powered by renewable energy to aid decarbonization efforts. Joule-heated reactors, which use electrification to generate heat, offer promise. However, it is crucial to understand their dynamics and optimize designs complicated by varied heating element materials. Our study employs 3D Computational Fluid Dynamics (CFD) to analyze Joule-heated reactors' power distribution, temperature profiles, and flow patterns. We conduct a parametric analysis to optimize heating rates and responsiveness by varying heating element materials and properties. Our findings corroborate previous experimental results, emphasizing the importance of material selection for achieving rapid and uniform heating while avoiding unwanted chemical reactions. We provide insights into system design, advocating high voltage and elements with high electrical conductivity and low heat capacity for faster heating. The study also introduces analytical formulae to guide experimental reactor design. We further analyze gas-phase propane pyrolysis and provide key insights to design Joule heated reactors for catalytic propane dehydrogenation.

**Keywords:** Electrification, Joule heating, flow patterns, heat transfer, dynamics, timescales, material selection

## **Impact of Electrostatic Interactions on the Aggregation, Capsid-Capsid Interactions, and Capsid-Genome Integrity of Adeno-Associated Virus Vectors**

Lily Motabar

Advisor: Christopher J. Roberts & Susana C.M. Teixeira

Committee Members: Abraham M. Lenhoff & Millicent O. Sullivan

---

While adeno-associated viruses (AAVs) have seen great commercial success as delivery vectors for gene therapy in recent years, their rapid growth within the pharmaceutical industry has outpaced the understanding of product formulation stability and degradation. Electrostatic interactions, modulated by solution conditions including pH and ionic strength, are known to play a role in the stability of AAV formulations. These interactions impact both intra-capsid self-assembly forces and capsid-capsid interactions, suggesting a significant influence on aggregation propensity and genome release. In this work, we explore the impact of electrostatic interactions on the aggregation behavior and capsid-genome integrity of AAV9 capsids carrying an enhanced green fluorescent protein (EGFP)-expressing transgene by modulating solution pH and ionic strength and employing several biophysical characterization techniques. Thermal stress studies revealed pH-dependent differences in genome release and extent of aggregation. Dynamic light scattering and transmission electron microscopy measurements demonstrated that a decrease in the ionic strength to 5 mM led to the formation of reversible aggregates, and the amount of salt needed to reverse aggregates to monomeric capsids varied significantly with pH. Furthermore, static light scattering measurements demonstrated that capsid-capsid self-interactions are driven by strong electrostatic attractions at 150 mM, with stronger attractions at lower solution pH. Overall, our data supports the significant role of electrostatic interactions in driving AAV9 capsid stability, particularly in relation to aggregation, genome release, and self-interactions.

## **Collagen Mimetic Peptide-Based Materials for Therapeutic Nucleic Acid Delivery to Chronic Wounds**

James Mullin

Advisors: Dr. Millicent Sullivan and Dr. Kristi Kiick

Committee Members: Dr. Catherine Fromen and Dr. April Kloxin

---

Chronic wounds affect millions of individuals each year, with poor patient outcomes and high treatment costs placing a major burden on the global healthcare system. Infection, prolonged inflammation, and impaired cell signaling disrupt the highly coordinated healing process and are major obstacles in creating effective treatments. To restore the normal healing cascade, our group has designed biopolymer hydrogels which rely on the inflammatory wound environment to deliver therapeutic plasmid DNA. The hydrogels are composed of extracellular matrix proteins (primarily collagen) and loaded with collagen-mimetic peptide (CMP) decorated polymer-plasmid nanostructures. CMPs are thermoresponsive, with monomers folding into stable triple helices below their transition temperature. CMPs also form triple helices with collagen and gelatin molecules, enabling them to serve as physical crosslinks between polyplexes and the hydrogel. Our strategy relies on interactions between the hydrogel, wound milieu, and target cells (fibroblasts) for degradation of these crosslinks, cargo release, and gene expression. Current work is focused on understanding how wound conditions and hydrogel composition influence cell behavior and gene delivery.

Cell behavior changes significantly in the inflammatory wound environment and understanding these changes is critical in designing effective therapeutics. We cultured human dermal fibroblasts in conditioned media from macrophages to simulate the wound and study how it affects cell behavior and gene delivery. Compared to normal conditions, inflammatory conditions caused decreases in cell viability and gene transfection. Hydrogel composition also heavily influences cell behavior. Specifically, we observed that human dermal fibroblasts transfect poorly on collagen substrates, but adding gelatin to the hydrogel improves gene transfection. Understanding the complex interplay between the hydrogel, cells, and wound environment will enable us to design more effective biomaterials for treating chronic wounds. Future work includes testing multi-gene delivery strategies and demonstrating therapeutic efficacy in animal models to determine which formulations may be suitable for clinical translation.

## Computational Design of Block Copolymers with High Thermal Conductivity

Tristan Myers

Advisor: Prof. Arthi Jayaraman

Committee Members: Profs. Alexandra Bayles, Antony Beris, Christopher Kloxin, Ulf Schiller

---

Polymeric materials with high thermal conductivity ( $k$ ) may offer advantages over traditional high- $k$  materials (e.g. metals and crystalline solids) in terms of tunability, anisotropy, and reprocessability. Block copolymers (BCPs) are polymers composed of blocks of one of two monomers (A and B), and they may microphase-separate into periodic structures, most commonly lamellae, double gyroid, and regular arrangements of cylinders and spheres. BCPs are a promising candidate for  $k$  enhancement and control because their material properties are influenced by this domain-level as well as chain-level ordering, which in turn are controlled by several molecular design parameters, including the BCP monomer composition and chemistries and the connectivity and relative size of blocks. This large design space makes BCPs an attractive candidate for thermal property tuning but also makes naïve experimental search unfeasible, and therefore there is a need for *in silico* prediction of multiscale ordering in BCPs to select promising design parameters for synthesis and characterization.

In this talk, I will detail my work using molecular dynamics (MD) simulation to study the chain- and domain-level ordering of BCPs of varying chain design and monomer characteristics. I use the novel computational method RAPSIDY (Rapid Analysis of Polymer Structure and Inverse Design strategY) with a coarse-grained BCP model to efficiently evaluate the stability of several microphase-separated morphologies for various BCP designs for comparison with self-consistent field theory (SCFT). Specifically, I investigate melts of pentablock copolymers of varying fraction of monomer A and relative size of the middle block as well as thermodynamic incompatibility (segregation strength) and relative Kuhn length of the monomers. I also relate these design parameters to the conformations of individual BCP chains, which are nontrivial to determine with SCFT but readily available in MD. This work serves as a template for efficiently optimizing multiscale ordering across large material design spaces with the goal of controlling macroscopic material properties.



## Engineering Proton-Conducting Solid Oxide Electrolytes Towards Efficient Green Hydrogen Production

Matthew Naughton

Advisor: Prof. Yushan Yan

Committee Members: Prof. Raul Lobo, Prof. Dionisios Vlachos, Prof. Feng Jiao

---

Proton-conducting solid oxide electrolysis cells (p-SOECs) leverage their low activation energy for proton conduction to achieve high water electrolysis performance in the intermediate temperature regime (400-600°C). Within this sought after temperature zone critical challenges that face high-temperature systems (>700°C) including balance-of-plant costs, degradation, and oxidation may be alleviated while still retaining favorable reaction kinetics with earth-abundant materials. The electrolytes in p-SOECs play a vital role in full cell performance and efficiency. These electrolytes must exhibit high ionic conductivity, low electronic conductivity, and exceptional durability under high steam atmospheres. 20% yttrium-doped barium zirconate (BZY20) and yttrium/ytterbium co-doped barium cerate zirconate (BZCYYb) are the most widely used p-SOEC electrolyte materials to date. However, the practicality of these materials is in question due to the low protonic conductivity of BZY20 and poor durability of BZCYYb in high steam environments. These drawbacks necessitate innovative solutions to produce next-generation p-SOEC electrolytes. In the first part of this study, we investigate the feasibility of  $\text{BaZr}_{0.6}\text{Sc}_{0.4}\text{O}_{3-\delta}$  (BZSc40) as a p-SOEC electrolyte material. We demonstrate that BZSc40 exhibits elevated NiO diffusion during high-temperature sintering, compared to other p-SOEC electrolyte materials. This increased NiO diffusion in BZSc40 leads to decreased cell performance, thus demonstrating the importance of suppressing excessive NiO diffusion during sintering of p-SOEC half cells. We find BZSc40 reaches a faradaic efficiency of 76% at 0.2 A/cm<sup>2</sup> and 600°C compared to 54% for BZY20, in addition to achieving more favorable current-voltage characteristics.

In the second part of this work, we consider the inherent inefficiencies experienced by barium zirconate systems and pivot to investigate the use of a gadolinium-doped ceria (GDC) interlayer to block electron hole leakage in p-SOECs. p-SOECs employing this GDC interlayer experience a reduction in total cell resistance from 0.74 to 0.55 Ω\*cm<sup>2</sup> and a 20% boost in faradaic efficiency at 0.8 A/cm<sup>2</sup> and 600°C. This work concludes with a discussion of future research directions for p-SOEC interlayer design.

## Hydroconversion of EVOH-Containing Multilayer Plastic Films Over Heterogeneous Catalysts

Christine Oberhausen

Advisor: Prof. Dionisios Vlachos

Committee Members: Prof. Raul Lobo, Prof. LaShanda Korley

---

The search for a solution to the growing accumulation of plastic waste remains a pressing challenge for society, in part due to the heterogeneous nature of the plastic waste stream. The diverse mixture of various polymers and multilayer materials introduces specific challenges to the development of strategies for waste management. In particular, multilayer films (MF) contribute to this problem. MFs are complex, single-use plastics commonly used in food and medical applications, often comprised of several stacked thin-film polymeric components, such as polyethylene (PE), polypropylene (PP), and ethylene vinyl alcohol (EVOH). The immiscibility of the various components in the complex structure of MFs makes mechanical recycling infeasible. Subsequently, 2Mt of post-consumer MF waste are landfilled or incinerated annually. Alternative waste management strategies must be developed.

Catalytically deconstructing MFs into valuable chemicals is a promising method to reutilize this carbon resource due to the high polyolefin content of MFs. For example, in hydroconversion, polyolefins can be converted to lubricant and fuel range hydrocarbons over mono- or bi-functional catalysts at relatively mild reaction conditions. However, EVOH thermally degrades into water and polyaromatic species under hydroconversion conditions, which complicates the application of these processes to MFs. Therefore, in this work, new mechanistic frameworks are uncovered for the catalytic hydroconversion of EVOH and LDPE/EVOH mixtures used as surrogates for MFs. First, ruthenium on metal oxide (Ru/MO<sub>x</sub>) catalysts are utilized for EVOH and LDPE/EVOH hydrogenolysis. Catalyst design and reaction conditions are varied to tune the product selectivity, demonstrating control over product functionality. Extensive characterization of the catalyst, products, and remaining solid is completed to explain reaction and deactivation pathways. The impact of EVOH thermal degradation products on catalyst activity is also probed. Second, hydrocracking of LDPE/EVOH is investigated over platinum deposited on BEA zeolite (Pt/BEA). The reaction pathway of EVOH over a bifunctional metal/acid catalyst to form water and hydrocarbons is elucidated. In this way, the critical role of Brønsted and Lewis acid sites in the dehydration of EVOH is unveiled, as well as the role of water as a co-catalyst in LDPE hydrocracking. High yields (58%) of isomerized C5-C12 hydrocarbons are achieved from EVOH/LDPE mixtures in short reaction times (2 h) and mild conditions (250 °C), highlighting the promise of hydrocracking catalysts as a solution for MF waste valorization.

## Competitive adsorption of monoclonal antibodies and non-ionic surfactants at hydrophobic air-liquid interface: Order of addition matters

Kiet G. Pham

Advisor: Norman J. Wagner

Committee Members: Eric M. Furst, Christopher J. Roberts

---

Monoclonal antibodies (mAb) are the main component for cancer therapy and immune disorder treatments. In mAb formulations, the non-ionic surfactant Poloxamer 188 (P188), an FDA approved excipient, has been considered as an alternative for polysorbate surfactants PS20/80 to protect antibodies from the negative impacts of interfaces that are present in drug manufacture, shipping, long-term storage, and administration.<sup>1-3</sup> Although the usage of non-ionic surfactants is widely accepted for stability enhancement, the mode of action for each type of surfactant is not fully understood which still requires case-by-case investigations for different protein therapeutics in formulation development. In this work, a systematic study was conducted to understand the capability of P188 to protect the antibody from the interfacial stresses at the air-liquid interface. Particularly, interfacial properties of the formulations with and without surfactant were characterized through interfacial tension as well as shear and dilatational rheology. Furthermore, the in-plane and out-of-plane surface structure of various formulations were probed by Brewster angle microscopy (BAM), and neutron reflectometry (NR). We found out that the pre-mixed mAb-P188 formulations show no sign of the presence of mAb at the air-water interface while the sequential formulation (mAb was allowed to adsorb first, then P188 was introduced into the subphase) shows the presence of mAb at the interface, implying that the order of addition of components of therapeutic formulations must be considered. Moreover, by using NR, the structure and composition of the mAb-P188 layer at the air-water interface was resolved on a molecular level. Our results show the partial removal of mAb from an air-water interface after the addition of P188 into the subphase below the pre-formed mAb monolayer. Furthermore, the mAb films show a significant loss of mechanical strength, which can provide insight on the interactions between these two molecules at the hydrophobic air-water interface. This same method could possibly be extended to other types of non-ionic surfactants to eventually understand the stabilization mechanism of these excipients.

### Reference

- (1) Lin, G. L.; Pathak, J. A.; Kim, D. H.; Carlson, M.; Riguero, V.; Kim, Y. J.; Buff, J. S.; Fuller, G. G. Interfacial dilatational deformation accelerates particle formation in monoclonal antibody solutions. *Soft Matter* **2016**, *12* (14), 3293-3302, 10.1039/C5SM02830B. DOI: 10.1039/C5SM02830B.
- (2) Bollenbach, L.; Buske, J.; Mader, K.; Garidel, P. Poloxamer 188 as surfactant in biological formulations - An alternative for polysorbate 20/80? *Int J Pharm* **2022**, *620*, 121706. DOI: 10.1016/j.ijpharm.2022.121706.
- (3) Bee, J. S.; Schwartz, D. K.; Trabelsi, S.; Freund, E.; Stevenson, J. L.; Carpenter, J. F.; Randolph, T. W. Production of particles of therapeutic proteins at the air-water interface during compression/dilation cycles. *Soft Matter* **2012**, *8* (40), 10329-10335, 10.1039/C2SM26184G. DOI: 10.1039/C2SM26184G.

## Intensifying hydrogen production through dynamic electrification

Rucha Railkar

Advisor: Dionisios Vlachos

Committee Members: Raul Lobo, Marianthi Ierapetritou

---

Dynamic electrification, particularly employing high-frequency temperature pulses through joule heating<sup>1-3</sup>, can significantly improve performance and decarbonize chemical manufacturing processes. We model these systems to understand how rapid pulsing temperature influences reaction kinetics. We start with comprehensive prototypical models<sup>4</sup> and move up to full microkinetic models, toward applications like ammonia decomposition and steam methane reforming<sup>5</sup>. We also validate the models experimentally using a millisecond pulse reactor, allowing for a rigorous examination of the dynamic pulse heating's efficacy in surpassing conventional steady-state and thermodynamic limits.

We elucidate enhancement in decomposition, reforming rates, and H<sub>2</sub> selectivity in competing reactions. Pulse heating influences endothermic and exothermic reactions, leveraging their interplay to boost H<sub>2</sub> productivity. Detailed mechanistic insights explain the factors driving this enhanced performance. Comparative metrics demonstrate the advantages of pulsed heating over conventional methods in energy efficiency and performance. This work underscores the technical intricacies of dynamic electrification and its transformative role in revolutionizing catalytic processes for a decarbonized chemical industry.

### References

- (1) Yu, K.; Wang, C.; Zheng, W.; Vlachos, D. G. Dynamic Electrification of Dry Reforming of Methane with In Situ Catalyst Regeneration. *ACS Energy Letters* **2023**, *8* (2), 1050-1057. DOI: 10.1021/acseenergylett.2c02666.
- (2) Yu, K.; Sourav, S.; Zheng, W.; Vlachos, D. G. Dynamic electrification steers the selectivity of CO<sub>2</sub> hydrogenation. *Chemical Engineering Journal* **2024**, *481*, 148528. DOI: <https://doi.org/10.1016/j.cej.2024.148528>.
- (3) Dong, Q.; Yao, Y.; Cheng, S.; Alexopoulos, K.; Gao, J.; Srinivas, S.; Wang, Y.; Pei, Y.; Zheng, C.; Brozena, A. H.; et al. Programmable heating and quenching for efficient thermochemical synthesis. *Nature* **2022**, *605* (7910), 470-476. DOI: 10.1038/s41586-022-04568-6.
- (4) Railkar, R.; Vlachos, D. Enhanced Performance of Dynamic Temperature Pulsing: Modeling Insights from Simple Reaction Networks. *Industrial & Engineering Chemistry Research* **2024**, *63* (1), 130-138. DOI: 10.1021/acs.iecr.3c03198.
- (5) Wittreich, G. R.; Liu, S.; Dauenhauer, P. J.; Vlachos, D. G. Catalytic resonance of ammonia synthesis by simulated dynamic ruthenium crystal strain. *Science Advances* **2022**, *8* (4), eabl6576. DOI: 10.1126/sciadv.abl6576.

## **Fluorescent Proteolytic Activity Probe with Logic Gating Capabilities**

Blake Richards

Advisor: Wilfred Chen, Millicent Sullivan

Committee Members: Catherine Fromen, Aditya Kunjapur, Emily Day

---

Proteases play a vital role in homeostasis. They are responsible for maintaining and restructuring tissue, processing and relaying cellular information, and regulating the activity of other proteases, among other roles. Accordingly, the dysregulation of protease activity is implicated in a vast and growing number of diseased states, from chronic wounds to cancer progression. Probing protease activity is highly desirable for applications such as screening therapeutic inhibitors, detecting viral infection, and more recently, probes of protease activity have garnered interest as prognostic tools for cancers and chronic wounds. One of the most widely used methods is Förster resonance energy transfer (FRET), while these probes are useful, they have several inherent limitations. Of particular concern, limitations in quenching efficiency result in substantial background fluorescence which reduces the dynamic range, additionally, the stringent spacing requirements between the two moieties disallow modifications for logic gating.

Herein, we present a recombinant protease detection platform with lower background, an improved dynamic range and demonstrated logic gating capabilities. The probe uses a split fluorescent protein called fluorescence and absorbance shifting tag (FAST). Unlike other split fluorescent proteins, splitFAST's fragment complementation is entirely reversible with rapid kinetics. The design reconstitutes these low affinity fragments and upon proteolytic cleavage they dissociate abolishing fluorescence. Each variant achieves an >20-fold reduction in fluorescence compared to commercial FRET peptides which generally respond with a 4-8-fold change in fluorescence. This 'turn-off' design relies on splitFASTs unique reversibility; because it does not use the FRET phenomena it doesn't have the same limitations and enables building off the design to incorporate logic-gates so that it can detect multiple inputs (proteases) and transduce them into a single output (fluorescence).

The first logic developed was a NOR-gate, where we demonstrated similar fold changes in fluorescence if any one of multiple proteases were incubated with the probe. Next, we built an additional architecture on both termini of the protein to construct a NAND-gate that can detect simultaneous protease exposure achieving an >25-fold change in fluorescence only if both proteases were present and a <2-fold change in the presence of one or the other. We next combined the above logic types to construct a mixed logic system capable of OR-gating certain proteases and simultaneously requiring the presence of an orthogonal protease to satisfy the NAND-gate portion of the system. These logic-gates have direct applications in current drug screening processes, as well as prognostic and diagnostic applications. This probe's reduced background, improved dynamic range, and modular logic capabilities makes it a competitive addition to the protease characterization toolbox that expands our ability to probe proteolytic networks.

## **Leveraging feedstock selection and characterization in lignocellulosic biorefineries for the production of sustainable polymers**

Alison J. Shapiro

Advisor: Prof. Thomas H. Epps, III

Committee Members: Profs. LaShanda T.J. Korley, Dionisios G. Vlachos, and Delphis F. Levia

---

To mitigate the environmental concerns associated with fossil fuel-based products, lignocellulosic biomass (LCB) has emerged as a promising alternative. Underutilized LCB residues (*e.g.*, forestry and agricultural byproducts) are optimal biorefinery feedstocks because these residues are available in large quantities at low costs with minimal environmental impact. Yet, compositional variability among feedstocks and heterogeneity remain major hurdles for LCB valorization (especially for LCB residues), necessitating reliable and rapid LCB characterization methods. In this work, higher-throughput advanced LCB characterization approaches have been developed, and harvest optimization for informed feedstock selection has been evaluated to maximize the environmental and economic viability of LCB biorefineries to produce sustainable polymers. Two LCB screening strategies were established to quantify LCB compositional information and predict LCB deconstruction outputs at higher throughputs than traditional LCB characterization methods. One strategy employs a thermogravimetric analysis approach and the other harnesses the optical properties of dissolved organic matter in stemflow; both approaches enable proactive process optimization and biorefinery inventory/output management. To assess harvest optimization of underutilized LCB residues, forestry residues (specifically bark, twigs, and foliage of multiple seasons across phenophases) were characterized to determine structural carbohydrate (cellulose, hemicellulose) content. A conceptual framework was then developed for sugar cycling using patterns identified in structural carbohydrate content and neutral sugar analyses from stemflow samples to understand and potentially optimize forestry residue harvesting. In addition to the characterization of carbohydrates and neutral sugars, lignin content and phenolic deconstruction monomeric yields and distributions from reductive catalytic fractionation (*i.e.*, a popular lignin-first deconstruction approach) were also quantified for the same set of forestry residues to enable informed decisions about harvest optimization for complete biomass valorization. Finally, to highlight the importance of biorefinery feedstock selection, residues with significantly different compositions were evaluated in a biorefinery model to assess the impact on sustainability and cost. The strategies herein inform LCB feedstock selection, harvest, characterization, and valorization, together enabling the optimization of lignocellulosic biorefinery performance.

## **Engineering the Secretion and Surface Display of Heterologous Proteins in *Yarrowia lipolytica***

Rachel Silvestri

Advisor: Dr. Mark Blenner

Committee Members: Dr. Cathy Fromen, Dr. Aditya Kunjapur

---

Bio-production can be broken down into two phases- upstream and downstream. The upstream phase includes cell-line development, fermentation, and filtering, and the downstream phase includes separating, purifying, and finishing the final product. Downstream bioprocessing is much more expensive; for biopharmaceuticals, downstream processing can take up over 80% of production costs. Upstream engineering of a yeast cell line to increase its secretion capabilities can decrease the need for extensive downstream purification, thus, decreasing overall cost of manufacturing biopharmaceuticals.

Hydrophobins are small proteins natively produced by filamentous fungi that self-assemble at hydrophobic/hydrophilic interfaces. These proteins can be used to make stable emulsions, coatings, and biocatalysts. There are two classes of hydrophobins: Class 1 form stable, amyloid-like membranes only soluble in TFA and formic acid, and Class 2 do not form the amyloid-like membrane and are soluble in ethanol and SDS. The protein of interest for this work is the Class 1 hydrophobin, RodA, natively found in *Aspergillus fumigatus*, a known pathogen. Here we report successful production and purification of secreted RodA from the GRAS yeast *Yarrowia lipolytica*. Protein bands of the expected size were detected by SDS Page (Silver Stain), and the identity of the protein was confirmed by mass spectrometry. The functionality of the recombinant hydrophobins was confirmed by showing the ability to flocculate plastic, a trait of the hydrophobins natively produced by *A. fumigatus*. To increase the titers of hydrophobins secreted by *Y. lipolytica*, seven heterologous secretion signals were tested as well as a solubility tag.

## **Metabolite-responsive scaffold RNAs for dynamic CRISPR transcriptional regulation**

Anthony M. Stohr

Advisors: Wilfred Chen and Mark Blenner

Committee Members: Kevin Solomon, Aditya Kunjapur, and Jeffrey Mugridge

---

Engineered microbial cell factories have the potential to be a more sustainable alternative to conventional chemical synthesis or crop-based natural product extraction. However, there is an inherent trade-off between growth and production and striking an appropriate balance between the two requires proper allocation of cellular resources. Dynamic regulation can alleviate this trade-off by cycling between growth and production states by modulating the expression of production enzymes when the cell has sufficient resources.

Existing dynamic regulation approaches are highly context dependent and require extensive reengineering to regulate the production of new biochemicals or function in new hosts. Conversely, CRISPR activation (CRISPRa) is programmable to act on a variety of gene targets and has been demonstrated to work in bacterial, yeast, and mammalian systems. In bacteria, gene-specific recruitment of the transcriptional activator, SoxS, was enabled by translational fusion to a small viral coat protein (MCP) which binds to the 3' end of a CRISPRa scaffold RNA (scRNA). We further modify the 3' end of the scRNA with a structure-switching aptamer that conditionally bind to MCP only when the metabolite of interest is also bound. We took advantage of this cooperative binding event to demonstrate metabolite-responsive CRISPR activation (MR-CRISPRa) in bacteria. We first screened the best performing structure-switching aptamers for activity in *Escherichia coli*. Using a theophylline-responsive structure-switching aptamer, we achieved >10-fold gene activation of a fluorescent reporter upon addition of theophylline. We also demonstrated the selectivity of MR-CRISPRa for the target metabolite. MR-CRISPRa offers similar levels of gene activation with "PAM-less" dCas9 variants, such as dSpRY, which greatly expands the number of activatable genes. By employing a tryptophan-responsive structure-switching aptamer, we observed tryptophan-regulated CRISPRa with our system indicating that MR-CRISPRa could be used to regulate genes in response to physiologically relevant inputs. We used our metabolite-responsive scRNAs for dynamic regulation of a five gene biosynthesis pathway. We are currently investigating the design rules for MR-CRISPRa as well as adapting this regulatory strategy for use in yeast. We envision that this work will lay the foundation for generalizable metabolite-responsive dynamic control that can be used in multiple organisms and for a variety of metabolic pathways.



## **Designing Formulations for Inhalable Particulate Immunotherapies via PEG-Based Nanoparticles**

Emma R. Sudduth

Advisor: Dr. Catherine A. Fromen

Committee Members: Dr. April Kloxin, Dr. Millicent Sullivan, and Dr. Catherine Grimes

---

Natural aging leads to higher susceptibility to severe respiratory infection due to altered pulmonary immune cell functions that is currently not well treated with traditional therapeutic and prophylactic vaccine approaches. Pulmonary delivery offers an alternative delivery method to directly target local immune cells in the lung and overcome the challenges of needle-based routes of administration. To this point, pulmonary antigen-presenting cells (APCs) are abundant in the lung tissue and are ripe targets for nanoparticle (NP)-based designs given their basic functionality to engulf local inhaled debris and activate global, mucosal immunity. However, design parameters to target these cells across various ages utilizing inhaled delivery mechanisms have yet to be established. Thus, there is clear motivation to study inhaled NP drug delivery platforms within various aged models to promote effective, long-term systemic immunity via precise targeting of pulmonary APCs. As a model platform, poly(ethylene glycol) diacrylate (PEGDA) hydrogel NPs present highly beneficial characteristics for an inhalable formulation as they offer readily tunable physiochemical properties, high biocompatibility, and relative nonimmunogenic responses in the lung. Herein, we characterize the aerosol and lung trafficking properties of model PEGDA NPs for inhalable immunotherapeutic applications through the modification of surface charge and formulation.

Both cationic and anionic formulations of PEGDA NPs were fabricated with similar hydrodynamic diameters of roughly 250 nm and high monodispersity, through the addition of 10 wt% charge-establishing co-monomers. Thus far, both formulations demonstrate robust aerosolization through a commercial Aeroneb<sup>®</sup> device with aerodynamic size (MMAD = ~5  $\mu\text{m}$ ) capable of reaching airway deposition and non-significant morphological alterations post-nebulization. Murine studies of lung immune cell distribution following orotracheal delivery of cationic and anionic PEGDA NPs have been performed using two different age groups signifying young and aged adults. Four distinct populations of APCs were isolated from whole-lung tissue utilizing multicolor flow cytometry analysis and were found to demonstrate unique phenotypical expression across ages. Cellular uptake across the APC subpopulations, NP trafficking, and the cellular inflammatory profile were compared between the two groups for each of the particle formulations. Of note, aged mice demonstrated nearly 3-fold decrease in quantity of NP uptake by key lung debris-clearing APCs known as alveolar macrophages, but a nearly 10-fold increase by a less well-studied class of cells known as interstitial macrophages. In comparison, conventional dendritic cells, APCs mainly responsible for activating T cells, showed minimal significant change in uptake of NPs with some even demonstrating increased NP engulfment. Overall, this work revealed specific age-dependent differences in pulmonary APCs that suggest the need for distinct nanoparticle design rules for the different age groups. Current work utilizes this knowledge to create NP vaccines that employ ligand conjugation at differing surface densities to control immune activation. Thus, this work outlines an overall approach to formulate particulate-based inhalable therapeutics that can target specific immune cells in the pulmonary microenvironment for a broad range of inhaled immune engineering applications and the rising need to tune formulations to specific age-based immune population differences.

## Development of Hydroconversion Catalysts for Plastic Waste Deconstruction

Jessie A. Sun

Advisor: Dionisios G. Vlachos

Committee Members: Raul F. Lobo, LaShanda T.J. Korley

---

Plastic materials have become an irreplaceable commodity of the 21st century; however, their widespread use has led to the unsustainable management of post-consumer plastic waste. Consequently, chemical recycling *via* hydroconversion (hydrogenolysis and hydrocracking) has become an appealing strategy to upcycle plastic waste into lower molecular weight hydrocarbons. Hydrogenolysis of plastic waste using Ru-based catalysts has shown promising results for deconstructing polyolefins (PO) into waxes and lubricant-range products. Yet, the influence of catalyst atomic structure and size on activity and product selectivity is poorly understood. Herein, we explore the effect of metal particle size and atomic structure on isotactic polypropylene (*i*-PP) hydrogenolysis over Ru supported on carbon. We reveal that *i*-PP hydrogenolysis involves an interplay of C-C bond scission and stereoisomerization. Small, disordered nanoclusters are effective in C-C bond scission, whereas larger metal nanoparticles promote stereoisomerization. We demonstrate that a heterogeneous distribution of metal active sites is essential for both deconstruction and product (lubricant base oil) quality control. The significant structure-property insights demonstrated here expose exciting opportunities for tuning hydrogenolysis catalysts to generate valuable hydrocarbon products for applications in plastic waste upcycling.

Alternatively, PO hydrocracking over bifunctional metal and Brønsted acid catalysts is another potential strategy for deconstructing plastic waste into high-value petrochemical feedstock, such as naphtha (C<sub>5</sub>-C<sub>12</sub>) and jet fuel (C<sub>8</sub>-C<sub>16</sub>). Current hydrocracking strategies predominantly utilize precious metal-based catalysts, as earth abundant metals (EAM) are limited by higher activation energy requirements and stability. Herein, we improve upon the stability of nickel supported on Beta zeolite (Ni/BEA) with the incorporation of a ceria promoter for the deconstruction of low-density polyethylene (LDPE). The Ce-promoted Ni/BEA catalyst achieved over 80% selectivity toward naphtha products with maximum naphtha productivity, outperforming previously reported noble-metal (Pt/Ru) and EAM (Ni/Co) catalysts alike. The promotional effect of ceria directly influences overall catalyst morphology, reducibility, stability, and hydrogen storage ability. The advancements in this work highlight exciting progress towards the optimization and application of cheaper, EAM hydrocracking catalysts for PO upcycling.

## Modelling and Optimization of Integrated Methane Dehydroaromatization Process

Arun Senthil Sundaramoorthy

Advisor: Dionisios G. Vlachos and Raul F. Lobo

Committee Members: Antony N. Beris and Marianthi Ierapetritou

---

The efficient design and operation of energy systems are pivotal in the reduction of global CO<sub>2</sub> emissions and in driving green, and sustainable manufacturing technologies<sup>1</sup>. Methane, a low carbon footprint fuel, can be processed through steam/dry reforming, partial oxidation, nonoxidative coupling, and oxidative coupling. The high temperature, pressure, energy requirements, and capital costs of syngas motivate the exploration of direct processes<sup>2</sup>. Moreover, over 30% of natural gas wells are placed in remote areas, making transportation to centralized locations expensive<sup>3</sup>. In response, on-site modular dehydroaromatization (DHA) has been proposed as a potential alternative<sup>4</sup>.

This work models an intensified dehydroaromatization process to increase product yield and methane conversion by coupling the reactor with a chemical looping unit that effectively separates hydrogen through a redox cycle and a temperature swing adsorption process to remove the aromatics and water and recycle the unconverted methane<sup>5</sup>. We postulate dynamic models and machine learned surrogate models to analyze and optimize the production of the aromatic products in terms of operation and economics. The optimum methane conversion of 48% and the aromatic yield of 42% occurs at a recycle ratio of 0.47 and a reactor temperature of 725°C.

### References

- (1) Kittner, N.; Lill, F.; Kammen, D. M. Energy storage deployment and innovation for the clean energy transition. *Nature Energy* **2017**, 2 (9), 1-6.
- (2) Tso, W. W.; Demirhan, C. D.; Floudas, C. A.; Pistikopoulos, E. N. Multi-scale energy systems engineering for optimal natural gas utilization. *Catalysis Today* **2020**, 356, 18-26.
- (3) Zichittella, G.; Pérez-Ramírez, J. Status and prospects of the decentralised valorisation of natural gas into energy and energy carriers. *Chemical Society Reviews* **2021**, 50 (5), 2984-3012.
- (4) Brady, C.; Debruyne, Q.; Majumder, A.; Goodfellow, B.; Lobo, R.; Calverley, T.; Xu, B. An integrated methane dehydroaromatization and chemical looping process. *Chemical Engineering Journal* **2021**, 406, 127168.
- (5) Sundaramoorthy, A. S.; Kim, S.; Ogunnaike, B. A.; Lobo, R. F.; Vlachos, D. G. Modeling and Optimization of Methane Dehydroaromatization Coupled with Chemical Looping and Temperature Swing Adsorption. *Industrial & Engineering Chemistry Research* **2024**.

## **Nanoparticle Internalization Induces Release of Macrophage Extracellular Vesicle**

Michael Trautmann-Rodriguez

Advisor: Dr. Catherine A. Fromen

Committee Members: Dr. April M. Kloxin, Dr. Brian Kwee, Dr. Millicent O. Sullivan

---

Macrophages, the immune system front-line antigen presenting cells, secrete a whole host of specialized biologics in response to external stimuli. Of these secreted biologics, extracellular vesicles (EVs) have shown potential for immunomodulation, drug delivery vehicles, and diagnostic markers. However, the biogenesis mechanism for these EVs remains elusive and there are limited strategies for inducing release of EVs. In this work, we showed a link between nanoparticle (NP) internalization and EV secretion in primary and immortalized macrophages. Using a poly(ethyl glycol) diacrylate (PEGDA) based hydrogel NP, we showed up to a 14 fold increase in isolated macrophage EVs following NP phagocytosis. We then used transmission electron microscopy to show the formation of multi-vesicle bodies, a crucial step to the release of small EVs, in bone marrow derived macrophages (BMMs) with internalized particles. We proceeded to explore functionality of these NP-driven macrophage EVs, demonstrating their ability to increase BMM survival and metabolic activity. Additionally, we used confocal microscopy to show transfer of NP material through the NP-driven macrophage EVs, highlighting the potential for a novel method for loading therapeutics into EVs. Our studies expand the understanding of EV biogenesis and the mechanism underpinning particle internalization by macrophages. Furthermore, this PEGDA NP platform shows promise as a method for controlled release of macrophage EVs for *in situ* immunomodulation and biomanufacturing.

## Engineering Barley-Stripe Mosaic Virus-Like Particles as Modular Nanovaccines

Akash Vaidya

Advisor: Prof. Kevin Solomon

Committee Members: Catherine Fromen, Wilfred Chen

---

Nanoparticle therapies can reprogram tumor-associated macrophages from cancer-supportive to cancer-suppressive (M1) phenotypes. Therapeutic outcomes depend on nanoparticle size, shape, cargo, and surface structure, but these parameters cannot be precisely tuned in most particle platforms. The special class of rod-shaped plant viruses (RSPVs) create a unique opportunity to control all these properties.<sup>1</sup> RSPVs such as tobacco mosaic virus (TMV) are already under investigation for vaccine applications, but their efficacy is limited by the presence of anti-TMV antibodies in human serum. We show that a related RSPV species, barley-stripe mosaic virus (BSMV), does not suffer from preexisting immunity and thus has immense potential for vaccine development. However, traditional manufacturing in plants is highly limited by the need to maintain host infectivity. We leverage a bacterial platform to produce BSMV VLPs, non-infectious analogs in which the viral genome is replaced by a user-defined RNA template, to enable flexible particle engineering. We also present methods for cell-free assembly of bacterially derived BSMV coat protein onto *in-vitro* transcribed RNA templates. By decoupling VLP production from native host infectivity, we enable high-density surface functionalization with diverse ligands via direct fusion and post-assembly conjugation methods.<sup>2</sup> Through the introduction of specific residues at the surface-exposed C-terminus, we achieve control over surface charge and other physicochemical properties. By inserting cysteine, specifically, which is not present in native BSMV, we enable robust functionalization with fluorescent dyes and validate VLP uptake by murine macrophages. We also decorate the VLPs with peptidic ligands including toll-like receptor agonists, cell-penetrating peptides, and model antigens. Furthermore, we demonstrate that BSMV VLP size and aspect ratio can be tuned by varying the length of the RNA template.<sup>3</sup> The finely tuned, functional VLPs are highly immunogenic and lead to robust M1 activation of murine macrophages. We investigate the mechanism of BSMV VLP immunogenicity, which is partly mediated by toll-like receptors. These scientific and technological advances set the stage for further BSMV VLP development as a nanoparticle platform for cancer immunotherapy and broader vaccine applications.

- (1) Vaidya, A. J.; Solomon, K. V. Surface Functionalization of Rod-Shaped Viral Particles for Biomedical Applications. *ACS Appl. Bio Mater.* **2022**. <https://doi.org/10.1021/acsabm.1c01204>.
- (2) Vaidya, A. J.; Rammohan, M.; Lee, Y.-H.; Lee, K. Z.; Chou, C.; Hartley, Z.; Scott, C. A.; Susler, R. G.; Wang, L.; Loesch-Fries, L. S.; Harris, M. T.; Solomon, K. V. Engineering Alkaline-Stable Barley Stripe Mosaic Virus-like Particles for Efficient Surface Modification. *Biochem. Eng. J.* **2023**, *199*, 109062. <https://doi.org/10.1016/j.bej.2023.109062>.
- (3) Solomon, K.; Lee, K. Z.; Vaidya, A.; Rammohan, M. Nanoparticles and Biotemplates with Tunable Length and Methods of Manufacturing the Same. US20240209382A1, June 27, **2024**. <https://patents.google.com/patent/US20240209382A1/en>.

## **Optimization of Bio-Based Isopropanol Production through Techno-Economic and Life Cycle Assessment**

Ching-Mei Wen

Advisor: Marianthi Ierapetritou and Eleftherios Papoutsakis

Committee Members: Dionisios Vlachos and Raul Lobo

---

The shift towards sustainable chemical production requires comprehensive frameworks to evaluate, optimize, and guide the development of renewable technologies. While advancements in using biomass (e.g., first-generation biomass) as feedstocks show promise, many technologies face scalability challenges, emphasizing the need for early-stage, system-level cost and environmental impact analyses. This work integrates techno-economic analysis (TEA) and life cycle assessment (LCA) to evaluate the feasibility and environmental impacts of bio-based isopropanol (IPA) production. By identifying cost and emission bottlenecks, these analyses inform the development of sustainable production systems and improve the utilization of renewable feedstocks.

First, a flowsheet model was developed based on the experimental results to provide preliminary findings for TEA and LCA. To improve the reliability of LCA, metabolic network modeling, specifically Flux Balance Analysis (FBA), plays a crucial role in systems biology by providing insights into cellular behavior. While FBA is the primary tool for predicting flux distributions, it often faces challenges in capturing variations under different conditions. Therefore, selecting an appropriate objective function is essential for accurately representing performance. To address the challenge, a Topology-Informed Objective Find (TIObjFind) framework that imposes both Metabolic Pathway Analysis (MPA) and FBA is introduced. TIObjFind identifies Coefficients of Importance (CoIs), which quantify the contribution of each reaction to the objective function and align the optimization results with experimental flux data. By analyzing CoIs, this framework enhances the interpretability of complex metabolic networks and reveals adaptive cellular responses. TIObjFind achieves sum of squared errors (SSE) between 0.32 and 0.09 for key metabolic titers (e.g., isopropanol, butanol, ethanol), demonstrating good alignment with experimental data. Additionally, the framework aids in estimating LCA performance by integrating gas consumption metrics from metabolic modeling, enhancing the understanding of environmental impacts.

Second, the TEA and LCA results support the development of a three-tiered supply chain optimization model for bio-IPA production, integrating environmental and economic goals (e.g., production cost and the carbon dioxide intensity). A sugar beet-to-bio-IPA supply chain is designed for Minnesota, considering geographical information (e.g., transportation, site locations, resource availability, etc.). The results indicate that optimizing production scale (55,800 metric tons/year) and logistics configuration achieves a 70% cost reduction compared to the market price and reduces transportation emissions by 30% relative to fossil-based IPA. Additionally, the potential profits from bio-based IPA are estimated to be nearly double the market price of its primary raw material, sugar, demonstrating the economic feasibility of converting the first-generation biomass for sustainable IPA production.

## **Discovery of an L-threonine transaldolase for enhanced affinity for L-threonine**

D'Jana R. Wyllis

Advisor: Aditya M. Kunjapur

Committee Members: Wilfred Chen, Kevin V. Solomon

---

Beta-hydroxy non-standard amino acids ( $\beta$ -OH nsAAs) are a class of molecules with a broad range of applications such as precursors for  $\beta$ -lactone antibiotics, building blocks for antimicrobial peptides and use as small molecule therapeutics. However, their sustainable production is often hindered by conventional chemical synthesis strategies involving harsh chemicals, multi-step processes and racemic products. Enzymatic approaches, like the use of the enzyme class L-threonine transaldolases (TTAs), offer a more efficient alternative for the synthesis of these compounds with broad substrate scope, high stereoselectivity and reduced environmental impact. This class of enzymes has demonstrated the thermodynamically favorable conversion of aldehydes and L-threonine to produce  $\beta$ -OH nsAAs and acetaldehyde. A key hindrance to their application in live cells is their low L-threonine affinity, requiring the supplementation of excess L-threonine. Therefore, the theoretical advantage of the thermodynamic favorability of the TTA-catalyzed reaction relative to other reactions cannot be realized until there is no longer a need to supply large excesses of L-threonine. This requires identification or engineering of a TTA with sub-millimolar affinity for L-threonine.

This work focuses on engineering an L-threonine transaldolase with enhanced affinity for L-threonine. Our approach combines bioprospecting for promising TTA candidates, with sequence and structural analyses and targeted loop engineering to improve substrate affinity. Through this, we have yielded sub-millimolar TTA L-threonine  $K_m$  values, improving enzyme performance and reducing the need to supplement excess L-threonine to live cultures. By overcoming the limitations of low L-threonine affinity in TTAs,  $\beta$ -OH nsAAs can be synthesized more efficiently, paving the way for broader applications in cell-free and cellular biocatalytic contexts.

## Microbial Foundry for Scalable ssDNA Production

Ming Hung Yen

Advisor: Kevin Solomon

Committee Members: Wilfred Chen, Catherine Fromen, Millicent Sullivan, Abraham Lenhoff

---

Single-stranded DNA (ssDNA) production is critical for modern biological applications including molecular diagnostics, data, storage, and genome editing. Despite the increasing demand, large-scale ssDNA synthesis continues to face significant challenges. Traditional column-based phosphoramidite chemistry offers rapid production of pure short oligo (<150nts) but struggles with the low sustainability. Stepwise base addition drastically reduces the fidelity and yields of long sequences and produces more than 4000 liters of hazardous reagents and solvents per kg of product. Therefore, we seek *in vivo* biosynthesis platforms to leverage intracellular proofreading systems and achieve extended ssDNA production with high fidelity. *In vivo* biosynthesis can also help realize scalable production in an environmentally friendly manner with established bioprocessing technologies. In this work, we explore two common *in vivo* methods for ssDNA production. In the first method, retrons synthesize multicopy ssDNA/RNA hybrids. The RNA template *msd* sequence forms complex secondary structure with a fused *msr* sequence, initiating reverse transcription by an encoded reverse transcriptase (RT) to generate ssDNA. We increased the yield five-fold through plasmid and promoter engineering and identified a dispensable region of *msd* that can be swapped for a desired target ssDNA sequence without reducing ssDNA synthesis yield. However, non-native sequences can form secondary structures that sterically hinder enzyme binding and inhibit ssDNA expression. To enhance sequence design flexibility, we also investigate plasmid-encoded rolling circle replication (RCR). The RCR initiator and terminator (RCORI) sequences flank the target sequence in the plasmid to be produced, which can produce kilobase-length circular ssDNA via an encoded replicase at high fidelity with minimum backbone residue. This work elucidates the design rules for producing non-native sequences from these two established *in vivo* platforms and paves the way for the development of scalable *in vivo* ssDNA production.



|                             |  |
|-----------------------------|--|
| <b>Sofia Alfieri</b>        | “Targeting Bottlenecks in Recombinant Adeno-associated Virus Production: Optimizing Gene Expression for Improved Content Ratio”<br><i>Advisor: Kelvin Lee</i>                              |
| <b>Jackie Arnold</b>        | “Non-Isocyanate Polyurethanes from Lignin-Derivable Monomers: A Safer and More Sustainable Alternative for High-Performance Applications”<br><i>Advisor: LaShanda Korley</i>               |
| <b>Rob Barlow</b>           | “Developing <i>Clostridium Butyricum</i> Argonaute (CbAgo) for Gene Editing Applications in Eukaryotes”<br><i>Advisor: Kevin Solomon</i>   |
| <b>Sofia Capece</b>         | “CRISPR/Cas9-Based Acetone Pathway Integration in <i>Clostridium acetobutylicum</i> for Isopropanol Production in a Synthetic Coculture System”<br><i>Advisor: Eleftherios Papoutsakis</i> |
| <b>Emily Doleh</b>          | “Development of a Stable Inducible Producer Cell Line for Recombinant AAV Production”<br><i>Advisor: Mark Blenner</i>  |
| <b>Jessica Rubira Gamba</b> | “MHC-II Epitope Profiling Using a Bacteria-yeast Screening Platform”<br><i>Advisor: Aditya Kunjapur</i>  |
| <b>Jodi Graf</b>            | “Modulating Macrophage Response in Well-defined Microenvironments”<br><i>Advisors: April Kloxin and Catherine Fromen</i>   |
| <b>Saloni Gupta</b>         | “Optimizing the Biosynthesis of Immunogenic Nitrated Proteins in <i>E. coli</i> ”<br><i>Advisor: Aditya Kunjapur</i>   |
| <b>Alex Hansen</b>          | “Elucidation of Yellow Mealworm Gut Microbiome Polyethylene Degradation Pathways”<br><i>Advisors: Kevin Solomon and Mark Blenner</i>   |
| <b>Justin Harrington</b>    | “Technoeconomic-Analysis of a HEMCC Plant”<br><i>Advisor: Yushan Yan</i>   |
| <b>Nicholas Houck</b>       | “Oxidative Dehydrogenation of Ethane via NO”<br><i>Advisor: Raul Lobo</i>  |
| <b>Jae Young Kim</b>        | “Similarity-based Machine Learning for Small Datasets: Predicting Bio-lubricant Base Oil Viscosities”<br><i>Advisor: Dionisios Vlachos</i>   |
| <b>Hasan Koybasi</b>        | “Process Intensification of Propane Dehydrogenation (PDH) Through Joule Heating and Membrane Separation”<br><i>Advisors: Dionisios Vlachos and Dongxia Liu</i>                             |
| <b>Derron Ma</b>            | “ACTS: A Real-Time Fluorescent Sensor for Visualizing Dynamic Intracellular Acetyl-CoA Levels”<br><i>Advisors: Wilfred Chen and Mark Blenner</i>   |

|                           |   |
|---------------------------|---|
| <b>Venice Magunga</b>     | “Effect of Pendent-Group Chemistry on Ion Transport Properties of Polymer Blend Electrolytes”<br><i>Advisor: Thomas Epps</i>  |
| <b>Rajas Mehendale</b>    | “Effect of Metal Catalyst Facets on Polyethylene Adsorption”<br><i>Advisor: Dionisios Vlachos</i>   |
| <b>Izak Minnie</b>        | “Investigation into Periodic Variations in CO <sub>2</sub> Electrolyzer Stability at the hand of Different Carbon Supports ”<br><i>Advisor: Dongxia Liu</i>                     |
| <b>Pedro Moura</b>        | “Heterogeneous Hydrolysis of Polyamide 6 to Monomer over Metal Oxide Catalysts”<br><i>Advisor: Dionisios Vlachos</i>  |
| <b>Juliana Nam</b>        | “Designing Modular Additive Manufacturing Coextrusion Nozzles Using Machine Learning”<br><i>Advisor: Alexandra Bayles</i>   |
| <b>Jacqueline Ngu</b>     | “Upcycling of Additive-Containing Waste”<br><i>Advisor: Dionisios Vlachos</i>   |
| <b>Philip O’Dell</b>      | “Developing <i>Y. Lipolytica</i> as an MIA Biosynthesis Platform”<br><i>Advisor: Mark Blenner</i>   |
| <b>Christopher Pirner</b> | “Rational Engineering Strategies for Optimizing Bispecific Antibody Production”<br><i>Advisor: Mark Blenner</i>   |
| <b>Abby Polsky</b>        | “The Impact of Relative Humidity on Electrode Ionomer Deposition for Hydroxide Exchange Membrane Electrolyzers”<br><i>Advisor: Yushan Yan</i>                                   |
| <b>Will Rears</b>         | “Bundlemers as Tunable Cores for Miktoarm Peptide-Polymer Star Conjugates via Click Chemistry and ATRP”<br><i>Advisor: Christopher Kloxin</i>                                   |
| <b>Jack Rooks</b>         | “Modelling Polymer Segmental Dynamics in Nanocomposites from Quasi-elastic Neutron Scattering”<br><i>Advisors: Norman Wagner and Antonio Faraone</i>                            |
| <b>Sakshi Satyanand</b>   | “Mechanism of CO Induced Restructuring on Sub-nm Supported Transition Metal Clusters”<br><i>Advisor: Dionisios Vlachos</i>  |
| <b>Jay Shah</b>           | “Understanding the Self-assembly of Polysulfamides from Molecular Dynamics Simulations Using an Atomistically-informed Coarse-grained Model”<br><i>Advisor: Arthi Jayaraman</i> |

**Eric Slaughter**

“Harnessing Tangential Flow Filtration and Hydrogels to Enhance Transduction and Select Distinct Populations for CAR T Manufacturing”  
*Advisors: April Kloxin and Catherine Fromen*

**Genevieve Yarema**

“Ammonia Permeable Immobilized Molten Salt Membrane Reactor for Ammonia Production”  
*Advisor: Dongxia Liu*

**Logan Yeager**

“Developing Methods for Tuning a pH Responsive Protein Nanoparticle”  
*Advisors: Millicent Sullivan and Wilfred Chen*

**Zhifei Yuliu**

“Design of Plastic Waste Chemical Recycling Process Considering Uncertainty”  
*Advisor: Marianthi Ierapetritou*



UNIVERSITY OF DELAWARE  
**ENGINEERING**

---

# DEPARTMENT OF CHEMICAL AND BIOMOLECULAR ENGINEERING

WINTER RESEARCH REVIEW | 4TH YEAR TALKS  
ABSTRACTS & SCHEDULES GUIDE  
JANUARY 22, 2025