# DEPARTMENT OF CHEMICAL & BIOMOLECULAR ENGINEERING

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# 2023 SUMMER RESEARCH REVIEW

## SECOND YEAR TALKS

## JUNE 9, 2023

UNIVERSITY OF DELAWARE

UNIVERSITY OF DELAWARE
**ENGINEERING**
**DEPARTMENT OF CHEMICAL AND BIOMOLECULAR ENGINEERING**

# 2023 SUMMER RESEARCH REVIEW

8:00 AM – 9:00 AM BREAKFAST for Faculty & Presenters ..... 2nd Floor Lobby
8:55 AM Welcome & Opening Remarks ..... 102 Colburn lab

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|---|---|
| 10:40 AM | BREAK |
| **12:40 PM** | **LUNCH** |
| 03:40 PM | REFRESHMENTS |

- **List of Talks in 102 Colburn..... pgs. 03 – 16**

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|---|---|---|---|---|---|
| 9:00 AM | Ross Klauer | 11:00 AM | Caitlin D'Ambrosio | 2:00 PM | Blake Richards |
| 9:25 AM | James Mullin | 11:25 AM | Ming Hun Yen | 2:25 PM | David Le |
| 9:50 AM | Lily Motabar | 11:50 AM | D'Jana Wyllis | 2:50 PM | Anthony Stohr |
| 10:15 AM | Emma Sudduth | 12:15 PM | Avaniek Cabales | 3:15 PM | Jinzhen Hu |

- **List of Talks in 104 Colburn..... pgs. 17 - 29**

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|---|---|---|---|---|---|
| 9:00 AM | Arnav Mittal | 11:00 AM | Dat Huynh | 2:00 PM | Yeonsu Kwak |
| 9:25 AM | Jessie Sun | 11:25 AM | Rucha Railkar | 2:25 PM | Marco Colin Martinez |
| 9:50 AM | Christine Oberhausen | 11:50 AM | Arun Senthil Sundaramoorthy | 2:50 PM | James Buchen |
| 10:15 AM | Alison Shapiro | 12:15 PM | Ching-Mei Wen | | |

- **List of Talks in 109 Colburn..... pgs. 31 – 44**

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|---|---|---|---|---|---|
| 9:00 AM | Matthew Naughton | 11:00 AM | Kiet Pham | 2:00 PM | Tristan Myers |
| 9:25 AM | Ahryeon Lee | 11:25 AM | Thaddeus Egnaczyk | 2:25 PM | Rafael Castro |
| 9:50 AM | William Hartt | 11:50 AM | Matthew Murdock | 2:50 PM | Breanna Huntington |
| 10:15 AM | Sean Farrington | 12:15 PM | Yinkui Yu | 3:15 PM | Stephen Kronenberger |

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- **List of Talks in 102 Colburn**

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# Discovery of polyethylene degrading microbes and enzymes from the gut of the yellow mealworm

Ross Klauer
Advisor: Mark Blenner, Kevin Solomon
Committee Members: LaShanda Korley, Aditya Kunjapur

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Global reliance on recalcitrant plastic materials has led to the production of over 380 million tons of plastic waste annually. Plastic waste handling and recycling economics are unfavorable, leading to environmental issues from the accumulation of over 7 billion tons of plastic waste in the environment since 1950. Biodegradation and bio-upcycling can offer a sustainable and economical plastic waste handling solution by operating near ambient conditions and valorizing waste plastic. Insect larvae have recently been identified as efficient plastic degraders, with insect gut microbiota being a key contributor to polymer deconstruction. Addressing the growing global plastics waste accumulation crisis, we have leveraged the gut microbiome of the yellow mealworm (*Tenebrio molitor*) as a platform for degradation of the most abundant waste plastic, polyethylene (PE). The microbial community in the yellow mealworm gut is expected to be rich in microorganisms and enzymes capable of efficient PE deconstruction due to its rapid PE degradation rate. Although taxa from the mealworm gut have been identified to be upregulated upon the enrichment of PE in the diet, gut microbes responsible for PE degradation remain mostly unidentified. Moreover, enzymes capable of degrading polyethylene at a high rate remain elusive. In this study, PE was fed to mealworms as their primary nutrient source in order to enrich the gut microbiome for PE-degrading organisms. Upon isolation of gut microbiota, growth in a mineral medium with LDPE as the primary carbon source indicated microbial PE assimilation. Potential PE-degrading enzymes from the mealworm gut community and top performing isolates were identified through genomic and proteomic analyses. After heterologous expression and purification of candidate 'PE-ase' enzymes, surface and chemical changes to PE were demonstrated by chemical and physical analyses. The discovery of PE-active enzymes will allow for future work in enzyme optimization and engineering for enhanced PE-degradation. 'PE-ase' enzymes can ultimately be coupled with engineered microbes for the upcycling of PE to value added products.

# Collagen-Mimetic Peptide Based Materials for Tunable Nucleic Acid Delivery and Expression of Therapeutic Growth Factors in 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. Underlying pathologies, such as Diabetes Mellitus or poor circulation, are the primary cause of wound chronicity. Developing effective treatments for chronic wounds is challenging for several reasons, including bacterial infection, prolonged inflammation, increased protease activity, and disrupted cellular signaling. To address these challenges, particularly disrupted cellular signaling, our group has designed extracellular matrix inspired hydrogels which utilize the inflammatory wound environment for controlled release and expression of growth factor encoding plasmid DNA (pDNA). The hydrogels are composed of collagen-mimetic peptide (CMP) decorated polymer-plasmid nanostructures (polyplexes) tethered to a collagen matrix containing fibrin as a secondary component. This formulation enhances the tunability of gene carrier release and expression in the presence of elevated protease activity through CMP-collagen hybridization. We characterized these materials using circular dichroism, dynamic light scattering and gel electrophoresis, determined the retention, release, and expression profiles of the pDNA using confocal microscopy and fluorescence assays *in vitro*, and evaluated wound healing outcomes in diabetic wound models.

The melting temperature of CMP is approximately 45 ºC as determined by circular dichroism, indicating the highest temperature at which the CMP will fold into a triple-helix. Below this temperature, the CMP can hybridize with the native collagen molecules to form a stable tether between the polyplexes and the collagen matrix, enabling tunable control over release and expression through the extent of CMP modification on the polyplexes. Dynamic light scattering results for all polyplex formulations demonstrate a suitable size (<200 nm) and surface charge (~30 mV) for efficient cellular uptake, and complete pDNA condensation is observed through gel electrophoresis. Control of retention, release, and gene expression was confirmed *in vitro*, with more CMP modification resulting in slower release and later peaks in gene expression. In animal models, delayed expression of growth factor pDNA improved wound healing compared to recombinant growth factor delivery and unloaded hydrogels. Taken together, these results show the potential of CMP-based gene delivery from extracellular matrix mimicking hydrogels for treating chronic wounds. Continued work on this project aims to incorporate multiple therapeutic cargo and understand the biological mechanisms of gene carrier release with the goal of optimizing the material for clinical translation.

# Impact of Glycosylation on Monoclonal Antibody Self-Interactions

Lily Motabar
Advisor: Christopher J. Roberts
Committee Members: Abraham M. Lenhoff, Millicent O. Sullivan

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Monoclonal antibodies (MAbs) are a leading class of therapeutic proteins that play a significant role in the growth of the biopharmaceutical market. Due to properties such as high binding affinity and target specificity, MAbs have revolutionized the treatment of chronic diseases including many forms of cancer, diabetes, and autoimmune disorders. Protein self-interactions measured via second osmotic virial coefficients ($B_{22}$) and dynamic light scattering interaction-parameter values ($k_D$) are often used as metrics for assessing the favorability of MAb candidates and formulation conditions during product development. Glycosylation is an important feature of MAbs, playing a critical role in their immunogenicity, FcγR binding kinetics, and overall stability. Model predictions of $B_{22}$ or $k_D$ typically do not account for glycans. Experimentally glycosylation can potentially impact MAb self-interactions, although there is not a systematic assessment of that in the literature. In this work, $B_{22}$ and $k_D$ values of two fully de-glycosylated MAbs and their native (i.e., glycosylated) counterparts were measured by light scattering at a range of pH and ionic strength conditions. Ionic strength was used to modulate the effect of electrostatic contributions. Significant differences between $B_{22}$ and $k_D$ of the native and de-glycosylated forms were observed at a range of solution conditions. Differences were most pronounced at low ionic strength, indicating that electrostatic interactions are a key factor. Although $B_{22}$ and $k_D$ values were statistically equivalent at high ionic strength where electrostatic contributions were fully screened, we observed protein-dependent qualitative differences, which indicate that steric interactions may also play a role in the observed $B_{22}$ and $k_D$ differences. These results demonstrate that protein self-interactions measured via light scattering can be sensitive to changes in glycosylation and thus are critical to assess during molecule development in addition to other biophysical characterization tools such as differential scanning calorimetry and fluorescence spectroscopy. A domain-level coarse-grained molecular model, used in prior work to capture charge distribution differences between MAbs, was also considered to potentially provide additional insight into the impact of electrostatic contributions but was not fully predictive of the behavior across all the solution conditions investigated. This highlights that both the level of modeling and lack of inclusion of glycans limit existing models in making quantitatively accurate predictions of self-interactions.

**Inhalable Particulate Immunotherapy Formulations Using Model PEG-Based Nanoparticles for the Elderly**

Emma R. Sudduth
Advisor: Dr. Cathy A. Fromen
Committee Members: Dr. Millicent Sullivan and Dr. April Kloxin

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The elderly population represents a growing at-risk group for pathogenic infection and chronic immune disorders, who furthermore do not respond well to traditional vaccination. In part, this is due to poor cell-mediated responses that arise via injection-based delivery. The pulmonary route of administration has been shown to activate cellular, humoral, and mucosal immunity, due to the host of specialized immune cells present in this microenvironment. However, design parameters for inhaled nano-immunotherapies to target these cells has yet to be determined. As a model platform, poly(ethylene glycol) diacrylate (PEGDA) nanoparticles (NPs) present highly beneficial characteristics for an inhalable formulation as they offer readily tunable physiochemical properties and have shown non-immunogenic effects 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 towards our overall goal of designing elderly-specific inhalable vaccines.

Both cationic and anionic formulations of PEGDA NPs were fabricated with similar hydrodynamic diameters around ~225 nm and low polydispersity indexes, through the addition of 10 wt% charge-establishing co-monomers. Both formulations demonstrated robust nebulization through a commercial Aeroneb® device via non-significant morphological alterations post-nebulization, ascertained through Cryo-Scanning Electron Microscopy (Cryo-SEM) and ImageJ analysis. To assess phagocytosis capacity of PEGDA NPs using *in vitro* aerosol delivery, a 3D printed adapter was designed that connected the Aeroneb® device to air-liquid interface (ALI) transwell cultures of innate immune cells. As expected, cationic NPs demonstrated significant uptake at all dosing conditions based on electrostatic charges; however, NP uptake overall by these cells was greatly reduced at ALI. To corroborate findings from *in vitro* studies, murine studies of lung distribution following orotracheal delivery of cationic and anionic PEGDA NPs were performed. Cellular uptake in immune cell subpopulations, NP trafficking, and the cellular inflammatory profiles in young (~1 month old) C57BL/6 mice were compared between each of the NP formulations. 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. Future directions look to repeat *in vivo* studies using aged murine models to determine age-dependent NP formulation parameters that could be used to design effective inhalable vaccines for the elderly.

# Controlling chemoenzymatic chemistry for the construction of coiled-coil peptide nanostructures

Caitlin D'Ambrosio
Advisors: April Kloxin, Wilfred Chen, Christopher Kloxin
Committee Members: Millicent Sullivan, Arthi Jayaraman

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The foundation of nature's most impressive materials is protein, a molecule composed of amino acid sequences that can fold and assemble into unique structures. Proteins are untapped natural building blocks that should be explored due to their versatility and exquisite molecular structures. The modularity and precision of these building blocks allows a bottom-up design of complex bioinspired structures. However, limited studies reflect the structure-property or structure-function relationships within proteins due to their chemical heterogeneity and structural complexity. A challenge exists in the design and construction of proteins with specific structures and functionalities through building block-type strategies.

The fusion of chemoenzymatic chemistries and protein engineering afford precise control of site-specific modifications, enabling the production of complex proteins with novel functionalities in a piecewise manner. Sortase ligation, which utilizes the sortase enzyme found in bacteria, is a novel technique that has gained recent attention due to its efficiency and similarity to traditional synthetic click chemistries. Starting with a coiled-coiled peptide motif, or bundlemer, as the molecular building-block, hierarchical structures can be constructed by the incorporation of recognition motifs and ligation sites. These peptides will be recombinantly expressed and designed to contain the bundlemer, a linker, and the reaction handles for sortase ligation (GGG- or -LPETG). This project aims to investigate the impact of linker length and salt concentration on the ligation product and efficiency. By adjusting the salt concentration, the reversibility of the sortase ligation reaction can be controlled. The length of the linker between the bundlemer and the recognition motif will determine the size and dimensionality of the constructed protein. By controlling the size and chemical functionalities of these molecules, precise hierarchical structures can be built from the nano- to macroscale.

**Microbial Foundry for scalable ssDNA production**

Ming Hung Yen
Advisor: Kevin V. Solomon
Committee Members: Catherine A. Fromen, Wilfred Chen, Millicent O. Sullivan, Abraham M. Lenhoff

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Single-stranded DNA (ssDNA) is a versatile genetic material utilized in modern biological research. Applications include aptamers for molecular diagnostics, data storage, and the sequence specificity of ssDNA also guides the formation of larger assemblies (i.e. DNA origami) that can be used for vaccine development and drug delivery. However, scalable synthesis of ssDNA with high sequence fidelity still remains a bottleneck for widespread adoption. To overcome this, I propose retrons as a cost-effective strategy for scalable, *in vivo* production of ssDNA. Retrons are natural gene cassettes that reverse transcribe RNA into ssDNA and have been identified in various strains, including *E. coli*. By using retrons, I hope to leverage the proof-reading capability of living systems to improve sequence fidelity over traditional *in vitro* synthesis while leveraging established bioprocessing principles for truly scalable ssDNA production.

In this talk, I will present strategies to engineer and enhance retron ssDNA production. Retrons consist of a reverse transcriptase and an mRNA with one mRNA region serving as a primer for reverse transcription of the other. ssDNA production yield was increased up to 5-fold by refactoring the retron and increasing expression via promoter and plasmid engineering. To detect ssDNA production, I developed a simple assay using a run-off primer reaction, enabling the determination of product size and sequence fidelity. ssDNA production yield was quantified with an optimized qPCR assay. Additionally, I identified a dispensable retron region that enables production of targeted non-native sequence for scalable ssDNA production. Computational modeling of mRNA secondary structure also highlights additional strategies for optimization of production. These results confirm the ability to detect and examine the ssDNA product from retron and provide deeper understanding of retron ssDNA production mechanisms.

# Bioprospecting L-threonine transaldolases for the synthesis of beta-hydroxylated amino acids

D'Jana Wyllis
Advisor: Dr. Aditya Kunjapur
Committee Members: Dr. Kevin Solomon and Dr. Wilfred Chen

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Beta-hydroxy-amino acids (β-OH-AAs) are an important class of molecules with a vast array of functions from naturally occurring protein building blocks like threonine, to non-standard amino acids like Droxidopa, which is used for the treatment of Parkinson's disease. The synthesis of β-OH-AAs is currently reliant on inefficient, low yield, multi-step chemical synthesis processes that involve harsh chemicals. Since they contain two chiral centers, the result of this synthesis is often a racemic mixture of four diastereomers. Although in some cases this racemic mixture is acceptable, only the L-isomers of non-standard amino acids have been incorporated into proteins. In the case of Droxidopa, stereoselectivity is particularly important as only the L-*threo* diastereomer is biologically active for use as a norepinephrine precursor. This low yield and low stereoselectivity leads to high cost of production and the demand for a more efficient, selective method to synthesize Droxidopa.

A class of enzymes called L-threonine transaldolases (L-TTAs) had been discovered to catalyze the reaction of aldehydes and L-threonine to form β-OH-AAs and acetaldehyde with high diastereomeric excess of the L-*threo* diastereomer. Although published TTAs have been demonstrated to accept benzaldehyde and derivatives with an electron-withdrawing substituent at the ortho-/meta-/para- positions, these enzymes do not show significant activity on the substrates of interest with electron donating groups in those positions. Here, we investigate the renewable, stereoselective capabilities of L-TTAs. We have conducted bioprospecting of putative L-TTAs in the synthesis of Droxidopa. As we explore the substrate specificity of these enzymes, we also seek to synthesize β-OH-AAs from lignin derived compounds as part of a long-term vision of incorporating these non-standard amino acids into proteins for synthetic auxotrophy applications.

# Engineering *Bacillus subtilis* Biocontainment for Control over Persistence and Production

Avaniek Cabales
Advisor: Dr. Aditya Kunjapur
Committee Members: Dr. Mark Blenner and Dr. Wilfred Chen

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The deployment of microorganisms is of interest in many industries for various applications due to the ability of microbes to sense and respond to environments. This can allow them to colonize specific areas and effect their surroundings. Synthetic biology provides the capabilities to engineer these microbes as sensors and treatments. In this work, we will use *Bacillus subtilis,* a spore forming organism which is ubiquitous in the environment and is labelled generally recognized as safe by the FDA. Its safety record and genetic tractability make it an attractive organism to engineer for environmental deployment; however, intrinsic biocontainment is necessary for the safe use of engineered microbes for deployment applications in uncontrolled settings to ensure they do not proliferate beyond their designed purpose. However, the lack of standardization of tools and testing for biocontainment make it difficult to implement. Understanding the persistence of biocontained microbes, or how long they will survive under non-permissive conditions, will allow for better implementation of containment and reduce the risks of using microbes in deployed settings.

However, biocontainment strategies often reduce the fitness of the microbe. For single-dose applications, where a microbe is deployed without a survival signal, it will be necessary to improve the productivity of the microbe. As such, we will demonstrate and optimize the ability of a biocontained strain to produce a metabolite of interest. Surfactin is a circular lipopeptide that has been shown to be beneficial in for plants and aid in bioremediation efforts. However, surfactin is non-specific and can exhibit hemolytic properties at higher concentration, and as such, it would be desirable to improve control over its application.

Here, I will discuss our work towards establishing protein degradation tools and surfactin production in *B. subtilis.* We will engineer a kill-switch in *B. subtilis* using degradation of an essential protein for biocontainment. We hypothesize that by tuning the rate of degradation, we can tune how long these microbes will survive. However, many biocontainment strategies result in decreased fitness in deployed settings. As such, we will engineer biocontained surfactin producers in non-permissive conditions for use as a single-dose deployed organism.

# Tunneling a Way Forward: Design of Conditional Proteases to Enable Nanoparticle Penetration in Solid Tumors

Blake Richards
Advisor: Wilfred Chen, Millicent Sullivan
Committee Members: Catherine Fromen, Aditya Kunjapur

Protein nanoparticles have attracted much interest as drug carriers for treating cancer, but their efficacy is significantly limited by the penetration resistance of solid tumors. Due to the overexpression of extracellular matrix components—namely collagen—nanoparticles are unable to penetrate the tumor and instead accumulate at the tumor periphery. Previous research has shown conjugating active collagenases to a nanoparticle's surface can help overcome this barrier through a process akin to invasive tumor cells tunneling out of tumors via focalized proteolysis. Unfortunately, delivering active proteases is acutely toxic and therefore not a viable option. My research focuses on the development of control architectures that inhibit the activity of proteases until they reach the tumor environment. A family of enzymes called matrix metalloproteinases (MMPs) are highly overexpressed in the tumor environment and therefore could be exploited as an activating signal for the inhibited proteases. Upon entering the tumor environment, the proteases on the nanoparticle's surface will become activated by these MMPs allowing for the nanoparticle to penetrate the tumor. Because these MMPs are found throughout the body the control architectures need to differentiate physiological MMP levels and tumor relevant MMP levels, requiring the use of orthogonal control architectures.

Stromelysin-1 was identified as a viable protease due to its activity profile. It was then successfully expressed in E. coli. The protease demonstrated clear activity on the expected substrate. Armed with a soluble and active protease, the next step was to introduce the first control architecture. To control the stromelysin-1's activity, a weak inhibitor was fused to the protease via a linker. Tethered inhibitors have previously shown to significantly increase the inhibitory activity effectively shutting off the protease. This linker contains a cut site that can be cleaved to sever the inhibitor off, this cut site will be specific to a cancer specific MMP. A separate control architecture needs to threshold the activation of the protease to only occur at the elevated MMP concentrations associated with tumors to avoid toxicity concerns. This can be done by conjugating MMP inhibitors to the surface of the nanoparticle to locally inhibit the activating MMPs at low concentrations, but the elevated concentration in tumors overwhelms the inhibitors allowing for the MMPs to cleave the linker and activate the proteases. This concept was demonstrated using MMP-2, which is highly upregulated in the tumor environment. The cleavage of a fluorogenic substrate was suppressed at low MMP-2 concentrations using a soluble inhibitor, the activity was then restored in the presence of elevated MMP-2 concentrations.

These exciting control architectures not only have clear implications with drug delivery but will be translatable to other systems where logic gated control of protease activity is necessary. Exploiting the overexpression of MMPs as a signal to identify the tumor also could be expanded to the many pathologies associated with MMP overexpression such as chronic wounds.

# Investigating Novel Gene Editing Tools for Enhanced Cell Line Development in CHO Cells

David Le
Advisor: Prof. Kelvin Lee
Committee Members: Prof. Wilfred Chen and Prof. Kevin Solomon

Chinese hamster ovary (CHO) cells are the most widely used mammalian cell lines in biotherapeutic production due to their ability to fold complex proteins with human-like post-translational modifications. However, the instability of CHO cell lines poses a significant challenge. The inherently unstable CHO genome is prone to chromosomal rearrangements, gene loss, and gene silencing in the absence of selection pressures. Current cell line development (CLD) strategies employ random integration (RI) of drug transgenes, enabling high volumetric productivities (>10 g L-1) but necessitating rigorous clonal selection and screening to isolate stable and productive clones. In contrast, site-specific integration (SSI) techniques minimize clonal variability by integrating transgenes at specific genomic loci, known as hotspots, which exhibit reproducible gene expression and epigenetic stability. SSI platforms, however, have limited capacity for integrating smaller, yet more stable gene cassettes at fewer integration sites compared to RI-generated hosts.

To overcome these limitations, an ideal SSI CLD method would facilitate simultaneous transgene incorporation at multiple hotspots, simplifying the generation of high transgene copy cell lines and matching the productivity of RI cell lines. In this work, we investigate the potential of a novel non-homology-directed repair CRISPR-Cas9 strategy called prime editing (PE) in CHO cells. PE has demonstrated superior editing efficiency and reduced undesirable editing in various mammalian cell lines, including human embryonic, carcinoma, and murine cells. By employing PE-mediated (PEM)-SSI strategies, the development of high-copy cell line platforms could be accelerated, enhancing gene insertion efficacy while minimizing off-target integration. Notably, the utilization of PE in CHO cell lines has not been previously reported.

We aim to characterize and optimize PE methods to integrate recombination sites at multiple hotspots within CHO. These recombination sites will enable the targeted integration of drug transgene-containing cassettes, thereby generating cell lines with enhanced productivity. Initial investigations focused on the feasibility of employing PE for genome editing in CHO, targeting both small and large sequence editing outcomes. Experimental evaluations utilizing fluorescent protein-based assays and next-generation sequencing confirmed the efficacy of PE methods in manipulating host cell phenotypes at the genotypic level. Ongoing investigations are directed towards the development and optimization of PE techniques for the insertion of large functional gene cassettes encoding proteins such as fluorescent proteins, monoclonal antibodies, and blood clotting factors.

## Metabolite-responsive protein assemblies for dynamic control of microbes

Anthony M. Stohr
Advisors: Wilfred Chen and Mark A. Blenner
Committee Members: Aditya M. Kunjapur and Kevin V. Solomon

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Microbial fermentation is a promising and more sustainable alternative to conventional chemical catalysis and crop-based natural product extraction. Extensive genetic engineering is required to achieve high levels of biochemical production. Engineered microbes often overexpress a host of both native and heterologous enzymes which reroute cellular resources away from growth and towards production of the desired biochemical. This inherent trade-off between growth and production is a fundamental issue in developing robust and economically viable microbial bioproduction platforms. However, dynamic regulation can alleviate this trade-off by cycling between growth and production states, where the expression and activity of product-related enzymes are modulated. Ideally, dynamic regulation would be rapidly responsive to critical changes in the cellular environment, such as the concentration of key metabolites, and have minimal burden on the host.

Here, we present a generalized dynamic regulatory strategy that can enable metabolite-responsive transcriptional activation or post-translational enzyme scaffolding. The metabolite-responsiveness is enabled by the fusion of a CRISPR guide RNA and a dual aptamer. Aptamers are short sequences of nucleic acids that are capable of binding to proteins or small molecules rapidly (on the order of seconds) and with high specificity. Recent work has developed dual aptamers that only are capable of binding to a small virial coat protein (MS2), which can itself be fused to another useful protein, when the metabolite of interest is also bound. We took advantage of this cooperative binding event to demonstrate CRISPR metabolite-based activation (CRISPR-MBA) in bacteria. Using a theophylline-responsive dual aptamer, we achieved 3-fold gene activation upon addition of theophylline. We are currently investigating the optimal spacing between the gRNA and transcription start site to develop the design rules for CRISPR-MBA as well as adapting this regulatory strategy for use in eukaryotic systems. Additionally, we explore the use of these aptamers for the conditional assembly of proteins onto an RNA scaffold. 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.

# Histone-Mimetic PEI Polyplex Based Gene Delivery System for Treatment of Monogenic Lung Disease

Jinzhen Hu
Advisor: Millicent O. Sullivan
Committee Members: Catherine Fromen, April M. Kloxin, Deepthi Alapati (Nemours)

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Neonatal respiratory distress syndrome (RDS) is a severe and often fatal condition among newborns. Monogenic mutations in surfactant production genes have been identified as the causative driver of RDS for at-term infants, as well as severe refractory respiratory failure and partial loss of lung function in diseases with late-onset time. Current treatments for genetic lung disease are non-specific and often result in an undesirable clinical outcome. To address this problem, we propose a novel non-viral gene delivery carrier for targeting neonatal alveolar type 2 (AT2) cells, the surfactant protein-producing cells in the lung.

Our vector is formulated with histone 3 (H3) tail peptides, which enable improved nuclear unpacking, engaging importin sorting mechanism and target cells with higher caveolin-1 expression by engaging in caveolin-1 endocytic transport. We hypothesize that the H3/PEI polyplex can selectively target the neonatal AT2 cell, where the caveolin-1 expression is high. We plan to optimize the non-viral vector through fine-tuning the H3 and polycation polymer ratio to maximize the transfection efficiency, and employ a polyethylene glycol (PEG) layer for maintaining the integrity of the non-viral vector and assisting transport. Currently, through tuning formulation, we achieved on par transfection efficiency (40-50%) with PEI polyplex counterpart while maintaining lower cytotoxicity for in vitro MLE-12 lung AT2 cells model. To confirm the mechanistic role of caveolin-1 in H3/PEI polyplex uptake, we evaluated the colocalization coefficient between the polyplex and caveolin-1 in MLE-12 cells. To achieve efficient extracellular transport, we will study the transport ability and stability of the PEGylated polyplexes in perfluorocarbon (PFC) solution and patient lung exudate. This study will lay the foundation for developing gene therapies for monogenic lung disease using a H3 incorporated gene carrier that is biocompatible, highly tunable, and efficient for targeting the desired progenitor cells.

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| 2:25 PM | Marco Colin Martinez |
| 2:50 PM | James Buchen |

# Effect of scale-up on mass transfer and flow patterns in liquid-liquid microchannel flows using experiments and computations

Arnav Mittal
Advisor: Prof. Dionisios Vlachos & Prof. Marianthi Ierapetritou
Committee Members: Prof. Raul Lobo & Prof. Yushan Yan

Recent developments in microfluidic technology have enabled process intensification and miniaturization of chemical processes. The small characteristic length scale ($< 1\,mm$) of a microchannel gives large surface-to-volume ratios, resulting in heat and mass transfer orders of magnitude higher than traditional large-scale batch or continuous reactors.[1–3] Combining the short diffusion time with laminar flow allows more precise process windows, residence time, and short reaction times for single and multiphase systems.[2] These traits are manifested in liquid-liquid biphasic microreactors where two immiscible liquids come in contact and generate various flow patterns.[4]

Predicting these flow patterns and mass transfer rates in microchannels as the diameter varies while accounting for solvent effects is important but currently lacking in the literature. We develop random forest and symbolic genetic regression machine learning (ML) models to predict flow patterns and the mass transfer rate, respectively, using our experimental and computational fluid dynamics (CFD) data and literature-mined data, while accounting for solvent properties and channel diameter. To minimize the number of CFD simulations and maximize model accuracy, active learning is used in selecting calculations. The uncertainty of the ML models built on hybrid data is quantified.

References:

1 P. Desir, B. Saha and D. G. Vlachos, *Energy Environ. Sci.*, 2019, **12**, 2463–2475.
2 P. Desir, T.-Y. Chen, M. Bracconi, B. Saha, M. Maestri and D. G. Vlachos, *React. Chem. Eng.*, 2020, **5**, 39–50.
3 T.-Y. Chen, P. Desir, M. Bracconi, B. Saha, M. Maestri and D. G. Vlachos, *Ind. Eng. Chem. Res.*, 2021, **60**, 3723–3735.
4 K. Wang and G. Luo, *Chemical Engineering Science*, 2017, **169**, 18–33.

# Investigation of Ru/C Structure Activity Relationships in Plastic Waste Hydrogenolysis

Jessie Sun
Advisor: Dionisios Vlachos
Committee Members: Raul Lobo, LaShanda Korley

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Plastic waste accumulation is a global environmental issue, posing severe ramifications for the natural world. The current management of plastic waste is unsustainable, resulting in an estimated 79% of all plastic being disposed of in landfills, while only 9% is recycled.[1] Plastic upcycling via hydrogenolysis is an attractive solution to redirect common polyolefin waste from the landfill and into value-added products such as lubricants. Ruthenium (Ru) supported catalysts are highly active for polyolefin hydrogenolysis. However, high catalyst activity and selectivity require high Ru loadings (>4 wt. %) and high catalyst/polymer ratios, increasing overall costs. Thus, this work aims to provide insights that will improve a fundamental understanding of structure-activity relationships governing catalytic behavior, as this is necessary for the development of tunable catalysts and can ultimately be leveraged to enable rational design.

One of the objectives is to correlate particle size, structure, and metal support interactions (MSI) with product selectivity in polypropylene (PP) hydrogenolysis. Previous work in the field has shown that Ru's performance is dependent on particle size with high activity for ultra-small clusters. MSI in reducible supports also play a key role in stabilizing small Ru clusters and creating active $Ru^{\delta+}$ species but details regarding their impact remain unclear. Our initial work focuses on investigating Ru particle size effect on an inert carbon support utilizing various characterization methods including HRTEM, $H_2$-TPR/TPD, XPS, NMR and GPC. Using an activated carbon support and systematic variation of Ru particle size (by changing Ru loadings and different pretreatment protocols), we reveal basic site requirements for PP hydrogenolysis activity, and demonstrate the influence of small Ru nanoclusters on C-C bond scission, polymer tacticity and impact on reaction selectivity.

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# Thermal and Catalytic Pathways in the Deconstruction of Ethylene Vinyl Alcohol Copolymer

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Committee Members: Dr. Raul Lobo, Dr. Dongxia Liu

Multilayer film plastic packaging (MF) is a complex, single-use plastic commonly used in food and medical applications. MFs are comprised of several stacked thin-film polymeric components, such as polyethylene (PE), polypropylene (PP), ethylene vinyl alcohol, (EVOH), polyamides, and polyurethanes. The immiscibility of the various components in the complex structure of MFs makes mechanical recycling infeasible, instead requiring costly and inefficient solvent-assisted processing. Subsequently, 2Mt of post-consumer MF waste is landfilled or incinerated annually. Alternative waste management strategies must be developed.

Chemically deconstructing MFs into valuable chemicals is a promising method to reutilize this carbon resource. Although significant progress has been made for PE and PP deconstruction, EVOH has been largely ignored. Herein, thermal and catalytic hydroconversion processes have been developed for efficient EVOH deconstruction into oxygenated and hydrocarbon products. Thermal deconstruction is facilitated by radical reactions that are initiated by the homolytic fission of C-O bonds to deconstruct EVOH. Thermal deconstruction of EVOH can be achieved at moderate temperatures (200-400 °C) with maximum oil yields of 72%. GC-MS and $^{1}$H NMR of the oil demonstrate the presence of a wide range of products, including alcohol, carbonyl, and aromatic moieties, and gel permeation chromatography (GPC) of the oil shows broad molecular weight distribution. The addition of a hydroconversion catalyst significantly reduces the reaction time and temperature required for EVOH deconstruction. Catalytic hydroconversion refines the width of the molecular weight distribution of the liquid/oil products while shifting the functional group selectivity towards alkanes and ketones. Catalyst and process optimization tunes product selectivity, demonstrating control of both the molecular weight distribution and functionality of products. Reaction products and remaining solids were also characterized to provide insights into structure-property relations. We establish a mechanistic framework for the catalytic hydroconversion of EVOH to explain the formation of liquid products. Moreover, key similarities and differences are identified between the hydroconversion of EVOH and polyolefins. These findings are utilized to design reaction systems for the single-pot conversion of polymer mixtures representative of real multilayer films.

# Leveraging thermogravimetric analysis to screen lignin content for high-throughput lignocellulosic biomass characterization

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Lignocellulosic biomass (LCB) characterization is a critical step in the valorization of LCB as it can inform feedstock selection, guide process decisions, and enable economic/environmental optimization; however, the heterogeneity and compositional variation among LCB feedstocks remains a significant hurdle to accurate biomass compositional measurements, especially with respect to lignin. In this work, we report a high-throughput thermogravimetric analysis (TGA) method to characterize whole lignocellulosic biomass (LCB) accurately and consistently. The current standard approach is a National Renewable Energy Laboratory (NREL) comprehensive laboratory analytical procedure, which has high accuracy/repeatability, but the method's utility as a screening approach is constrained by low throughput and a complex workup procedure. As a higher-throughput alternative, a TGA method was developed to quantify the lignin content using the thermal decomposition profile of the unfractionated woody and herbaceous biomass feedstocks. The method achieved comparable accuracy/repeatability to the NREL procedure for lignin quantification with a significant reduction in the required characterization time, feedstock volume, and sample preprocessing prior to characterization. Additionally, the TGA-derived thermal deconstruction profiles of the biomass were leveraged to evaluate the relationship between the lignin's thermal decomposition behavior and the experimentally measured lignin phenolic yields of native biomass in an intensified reductive catalytic fractionation process. The TGA characterization method developed in this work increases characterization throughput while enabling the measurement of lignin content from whole LCB biomass.

# Optimal Biomass Conversion Technology Investments Considering Uncertainty and Environmental Policy

Dat Huynh
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Committee Members: Prof. Feng Jiao, Prof. Raul Lobo, Prof. Marat Orazov

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The replacement of petrochemical feedstocks with biobased chemicals is a significant area of research aimed at reducing greenhouse gas emissions and creating a sustainable economy[1]. Despite extensive research on converting biomass into drop-in or alternative chemicals, such as bioethanol or polyethylene furanoate (PEF), commercialization of biomass-based processes often fails[2-4]. This partly due to the risk associated with unproven technologies and unknown market conditions for these feedstock streams and product demands[4]. Policymakers can play a role in facilitating this transition toward a green economy by establishing carbon emissions policies. Certain countries have begun implementing these policies to reduce emissions and incentivize greener processes[5].

This work developed a framework to assist decision-makers in assessing a wide range of technologies for making investments while considering policy and uncertainty. The framework analyzed the changes in technological investment decisions made across various carbon policies such as carbon tax, cap and trade, cap, and offset[6]. For each scenario, the framework generated a Pareto-optimal curve that considered economic and environmental metrics, including profit and Global Warming Potential, while also considering risk metrics to understand the trade-offs between risk, profit, and emissions. To mitigate uncertainty, the superstructure consisting of biomass transformation pathways obtained from literature was utilized in a two-stage mixed integer linear stochastic programming problem. The two-stage formulation captured the first stage "here-and-now" decisions and second stage "wait and see" decisions, operating and production levels under different realizations of uncertainty. Risk metrics, such as conditional value at risk and downside risk were considered to manage uncertainty[7].

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**Towards understanding the effects of dynamic electrification**

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The production of high-volume chemicals, such as hydrogen, ammonia, methanol, and small olefins, needs significant energy, traditionally met through the burning of natural gas, which yields considerable greenhouse gas emissions. In response, electrification methods such as Joule heating are emerging as an alternative, providing a means for greener, rapid heating and greater control over kinetics. Several efforts have experimentally demonstrated the impact of dynamic electrification.[1,2] However the development of a comprehensive modeling framework capable of accommodating rapid Joule heating with nonlinear temperature control functions, for reactors employing detailed microkinetic models, remains outstanding. This study seeks to assess the impact of a dynamic operation via rapid pulse heating on the performance of gas phase systems, avoiding any nonlinearities due to catalyst coverages.

The study demonstrates that dynamic electrification of even simplistic gas phase reactions can substantially improve conversion and selectivity along with energy efficiency compared to isothermal operation by maintaining the system away from a steady state. We also employ a data-driven approach to formulate experimental recommendations for rapid pulse heating. This study thus provides a framework for applying pulse heating to other important catalytic reactions. In summary, we highlight the potential of electrification in chemical manufacturing and the importance of dynamic operation to achieve enhanced performance.

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# Modelling for Optimal Operation of Modular Integrated Methane Dehydroaromatization Process

Arun Senthil Sundaramoorthy

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Committee Members: Dr. Antony N. Beris, Dr. Marianthi Ierapetritou, Dr. Marat Orazov, Dr. Feng Jiao

Natural gas is a viable alternative to crude oil feedstocks to meet energy and chemical manufacturing demands. Many of these deposits are in remote locations, motivating the development of on-site manufacturing approaches, known as modular technologies, for effective and economical production of more valuable products [1]. Herein, a novel modular methane dehydroaromatization process, integrating dehydroaromatization (DHA), chemical looping (CL), and temperature swing adsorption (TSA), proposed by [2] is considered for selective conversion of natural gas into aromatics by overcoming thermodynamic equilibrium. Experiments have proven the integrated process to be a promising technology in terms of aromatics yield and energy consumption compared to the conventional process technologies [3]. However, the dynamics of the modular process operated in semi-batch units, involving interactions due to material re-circulation, transport parameters, and non-linear rate kinetics, are yet to be understood from an operations perspective. Understanding the process operation is challenging because process variables are not easily measured, limiting the amount of process data needed to verify the process of interest.

This work aims to develop a dynamic model for the modular integrated dehydroaromatization process to describe and verify the safe and optimal operating regime by elucidating the process dynamics. In this presentation, we first discuss the development of a reference model and its verification using experimental data. Then, we describe challenges with the simulation of the integration of this model [4]. Finally, I describe our short-term research goals and the future work of this research.

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# Techno-economic Analysis and Life Cycle Assessment of Isopropanol-Ethanol Fermentation via Novel Co-Culture Systems

Ching-Mei Wen
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Isopropanol (IPA) plays a vital role as a disinfectant and antiseptic in the medical field, emphasizing its significance in public health and safety. Biosynthesis of IPA offers a sustainable and potentially more efficient alternative to traditional energy-intensive and environmentally unfriendly chemical synthesis methods, enabling the use of renewable resources and reducing dependency on fossil fuels, possibly lowering production costs. Conventional IPA production relies on propylene as feedstock, while innovative Isopropanol-Ethanol (IE) fermentation strains can utilize alternative carbon sources (typically sugars), such as syngas and $CO_2$ directly, without the need for feedstock pretreatment. In this study, co-culture microbial communities producing IE from sugar and $CO_2$ are investigated at an industrial scale of 18,000 metric tons of sugar per year to evaluate potential economic viability and environmental impacts. While previous techno-economic studies have concentrated on the commercial feasibility of biochemical IE production, this study presents comprehensive comparisons of technology purification designs and environmental life cycle performance. Techno-economic analysis (TEA) is conducted for two scenarios of the fermentation process combined with a pressure swing distillation (PSD) and extractive distillation (ED) purification systems. Life cycle assessment (LCA) evaluates the global warming potential (GWP) in terms of $CO_2$-equivalent emissions using simulated input inventories and a dynamic multispecies metabolic modeling framework (DMMM). The DMMM combines genome-scale dynamic flux balance analysis (dFBA) and an ordinary differential equation-based growth model to assess dynamic changes, predict product titers, and identify viable CO2 cross-feeding ranges in co-culture systems. The estimated minimum selling price of IE using extractive separation process is more favorable than those using the pressure swing designs ($929.8 and $774.8 per metric ton, for the PSD and ED scenarios, respectively). Raw material costs and byproduct prices are identified as critical parameters for biorefinery improvement. The co-culture systems exhibit negative carbon emissions based on the predicted maximum theoretical $CO_2$ uptake assumptions. Overall, the results indicate that the novel co-culture microbial system using ED purification is the most promising scenario for achieving the future sustainable bulk chemical goal.

## Microwave-assisted, performance-advantaged electrification of propane dehydrogenation

Yeonsu Kwak
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Committee Members: Prof. Raul Lobo, Prof. Yushan Yan, Prof. Marat Orazov

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Non-oxidative propane dehydrogenation (PDH) is an essential process for producing propylene, a fundamental component of value-added chemicals. Despite its commercial viability, PDH faces challenges in selectivity and significant catalyst deactivation, limiting process efficiency and requiring lower operating temperatures. Furthermore, the high temperatures and energy fluxes involved make PDH a highly environmentally detrimental chemical process. In this study, we introduce a microwave (MW)-assisted PDH approach using $PtSn/SiO_2$ catalyst pellets loaded in a silicon carbide (SiC) monolith, which serves as both a MW susceptor and heat spreader. We revisit the importance of accurate bulk temperature measurement under MWs through direct fiber optics and computational investigations. Maintaining conditions comparable to conventional reactors, our time-on-stream experiments reveal active and stable MW reactor operation at 500 °C without necessitating hydrogen addition. Upon increasing temperature or feed partial pressure at high space velocity, MW-heated catalysts demonstrate exceptional coke resistance, improved activity, and superior selectivity, in stark contrast to conventionally heated reactors that experience significant catalyst deactivation. We attribute this to nanoscale temperature gradients between active sites and support, which help elucidate the reduced coke formation and delayed active site agglomeration under MW conditions. Our results highlight the immense potential of electrifying highly endothermic catalytic reactions to promote a more sustainable future.

# Investigating the effect of membrane properties on the performance of the hydrogen-powered electrochemically driven $CO_2$ separator (EDCS)

Marco Colin Martinez
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Committee Members: Raul Lobo, Marat Orazov, Dionisios Vlachos, Antony Beris

The broadening gap between emissions and capture of carbon dioxide symbols the decreasing likelihood of achieving the Paris Climate Agreement. Accordingly, there is a growing need for new carbon capture avenues such as Direct air capture (DAC). Although traditional carbon capture technology has relied on adsorbent/absorption technology, they pose prohibitively large energy and capital costs for dilute $CO_2$ feeds. Recently, an alternative $CO_2$ capture approach has been demonstrated by exploiting the alkaline environment generated by anion exchange membrane (AEM) hydrogen fuel cells. Here, hydroxides are electrochemically generated at the cathode which chemically scrub $CO_2$ out of the air, producing (bi)-carbonates that transport across the AEM and accumulate at the anode. There, the local pH environment lowers until $CO_2$ generation is thermodynamically favorable resulting in a concentrated $CO_2$ stream. From the stoichiometry of the proposed acid-base mechanism, the system has a theoretical upper limit of 1 $CO_2$ per e- (mol captured/mol consumed), typified as 100% electron efficiency. This metric can be used to indicate the energy demands for $CO_2$ capture in the EDCS. Previous work on the electrochemically-driven $CO_2$ separator for DAC applications has encountered a soft limit of roughly 40% electron efficiency. For widespread commercialization of carbon capture technology, reducing energy costs is essential.

To optimize performance, a thorough understanding of ion transport in the EDCS is vital. The Nernst-Plank equation is a widely used tool to describe ion transport, revealing that diffusion and migration are the two primary modes of transport. Hydroxides and (bi)-carbonates are driven by migration toward the anode where carbon dioxide is released. However, the accumulation of carbonates near the anode results in a (bi)-carbonate back-diffusion which negatively impacts performance. Moreover, the Nernst-Plank equation suggests that membrane resistance can be utilized as a control handle to enhance performance. This is because ion migration remains roughly constant with membrane resistance, whereas diffusion exhibits an inverse relationship. Consequently, back-diffusion can be decreased by increasing membrane resistance through lower membrane conductivity or thicker membranes. However, the latter would in higher capital costs, and thus the lower membrane conductivity route is preferred. To do this, the ion exchange capacity of the membrane can be reduced. Previous experiments have shown that exposing PAP-TP-85 to high temperatures in its carbonate form can result in cation site deactivation, whereas the halide form of the membrane exhibits greater stability. This difference in ion stability can be leveraged as a method for selectively degrading ion exchange sites by subjecting PAP-TP-85 to various carbonate-to-halide ratios at elevated temperatures. After degradation, the membranes are characterized by employing potentiometric titration to determine IEC and electrochemical impedance spectroscopy to measure membrane resistance. The impact of membrane resistance on electron efficiency is examined in the EDCS on a range of IECs that have. Preliminary experimental and modeling results indicate that membranes with lower conductivities can achieve electron efficiencies over 50%.

**Direct air capture of carbon dioxide using nickel hydroxide electrodes**

James Buchen
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Committee Members: Prof. Raul Lobo, Prof. Feng Jiao, Prof. Dion Vlachos, Prof. Marat Orazov

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Direct air capture (DAC) is a growing field responding to the need to remove carbon dioxide from the atmosphere. Several technologies are being developed today attempting to meet this need. The solutions furthest in development are temperature swing adsorption technologies have moved out of the pilot plant scale with an energy cost of 1.8 $MWh \cdot ton^{-1}$ $CO_2$.[1] This research looks to present an electrochemical device as alternative to these technologies. The biggest hurdle for this electrochemical approach is increasing the flux of $CO_2$ to allow for more compact, lower capital cost, devices. Strategies for managing the transient charge and discharge behavior of the battery system will be discussed. Focus will be on improving flux to reduce the overall cost of the device.

The electrochemical device proposed is a pair of nickel hydroxide ($Ni(OH)_2$) battery electrodes which produce hydroxide ($OH^-$). The hydroxide reacts with carbon dioxide ($CO_2$) to create carbonate ($CO_3^{2-}$) and bicarbonate ($HCO_3^-$). These carbonates can be transported from the cathode across an anion exchange membrane to the anode where they are evolved back into $CO_2$ due to a pH reduction at the anode. This builds on work in the Yushan Yan group that optimized a Hydroxide Exchange Membrane Fuel Cell (HEMFC) for $CO_2$ capture, showing the efficacy of an electrochemically mediated pH swing carbon capture device.[2,3] The benefit of the nickel hydroxide battery approach is the low energy cost associated with separation. Because a pair of nickel hydroxide batteries undergo the same electrochemical reaction, low potentials, and thus low energy cost is required. Most of the energy required by the device produces the pH gradient used to capture and release $CO_2$. Experiments have shown device level energy requirements of less than 1 $MWh \cdot ton^{-1}$ $CO_2$.

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# 2023 SUMMER RESEARCH REVIEW

- **List of Talks in 109 Colburn**

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| 9:00 AM | Matthew Naughton |
| 9:25 AM | Ahryeon Lee |
| 9:50 AM | William Hartt |
| 10:15 AM | Sean Farrington |

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| 11:00 AM | Kiet Pham |
| 11:25 AM | Thaddeus Egnaczyk |
| 11:50 AM | Matthew Murdock |
| 12:15 PM | Yinkui Yu |

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|---|---|
| 2:00 PM | Tristan Myers |
| 2:25 PM | Rafael Castro |
| 2:50 PM | Breanna Huntington |
| 3:15 PM | Stephen Kronenberger |

# Reproducible Synthesis of Proton-Conducting Solid Oxide Electrochemical Cells Through Low-Cost Dip-Coating Method

Matthew Naughton
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Committee Members: Dr. Raul Lobo & Dr. Dionisios Vlachos

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High temperature solid oxide electrochemical cells utilize earth-abundant materials to achieve unrivalled performance thanks to inherent kinetic and thermodynamic advantages over low temperature systems. However, the >800°C operating temperature required to achieve sufficient oxide ion conductivity induces high balance of stack costs which limits practical scalability. Proton-conducting solid oxide cells avoid these high balance of stack costs with intermediate temperature (350-600°C) operation enabled by high proton conductivities. Thus, proton-conducting solid oxide cells are attractive devices for fuels, chemicals, and electricity production as they directly address the critical challenges facing low and high temperature electrochemical systems. State of the art materials used in proton-conducting solid oxide cells, yttrium and ytterbium doped barium-cerate-zirconate perovskites, are notoriously difficult to sinter. This makes the production of thin (<20 micron) and dense electrolyte layers, a requisite for electrochemical operation, a multifront challenge. Here, a low-cost and reproducible dip-coating method for producing lab-scale proton-conducting solid oxide cells based on a $BaCe_{0.7}Zr_{0.1}Y_{0.1}Yb_{0.1}O_{3-\delta}$ electrolyte is presented. Scanning electron microscopy (SEM) and experimental testing are used to demonstrate the effectiveness of the proton-conducting solid oxide cells produced with this method.

# Electrochemical $N_2O$ Reduction on Transition Metal Oxides Catalysts

Ahryeon Lee
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Committee Members: Dr. Raul Lobo and Dr. Yushan Yan

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The electrochemical reduction of $NO_x$ ($NO_xR$) is a promising method for producing value-added or benign products (e.g., ammonia, hydroxylamine, and dinitrogen) operating at near-ambient conditions coupled with renewable electricity. While considerable effort has been devoted to developing selective transition metal catalysts in $N_2OR$, their practical applications have been restricted by the high overpotential (> 1.77 V) required. Thus, it is crucial to understand why transition metal catalysts inherently have high overpotential to produce $N_2$ from $N_2O$ and discover potential effective mechanisms with catalysts that can enable this.

In this work, Density Functional Theory (DFT) suggested that the proposed $N_2O$ dissociation pathway can reduce the overpotential compared to the continuous protonation of the $N_2O$ pathway, which has been a widely accepted mechanism for transition metal catalysts. Furthermore, the theory suggested that transition metal catalysts with sub-oxide layers can enable to follow the $N_2O$ dissociation mechanism. The theory was then proved by experiments, where electrochemical $N_2O$ reduction to $N_2$ on oxide-derived cobalt catalysts showed 50 mV lower overpotential than cobalt catalysts with over 87% $N_2$ selectivity at 400 mA $cm^{-2}$. This work provides alternative pathways for $N_2O$ reduction to $N_2$ with lower overpotential and design principles of optimal catalysts.

# Composition-property relationships of BP-1 lunar regolith simulant binders for in-situ resource utilization as planetopolymers

William H. Hartt V
Advisor: Norman J. Wagner
Committee Members: Alexandra V. Bayles & Raul F. Lobo

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In situ resource utilization (ISRU) is required for long-term human habitation on the Moon and Mars, with the need to construct environmental protection and critical infrastructure foremost. Analogous to terrestrial construction materials formed from aluminosilicates, i.e., geopolymers, binders formed from lunar aluminosilicate regolith (planetopolymers) are necessary to create construction materials for lunar landing pads and habitats. A previous lateral study performed by Mills et al. investigated multiple lunar and Martian regolith simulants for ISRU with the analysis centred on the ability of the various simulants to form a solid binder at different curing conditions [1]. For end-use applications it is vital to go further to understand the relationships between the binder's material properties and their composition.

In this work we perform an in-depth study on Black Point-1 (BP-1) lunar regolith simulant geopolymers exploring the connection between the material properties and composition [2]. The material properties investigated include the 7-day compressive strength, strain to failure, and secant modulus with initial compositions varying the water content, activating solution sodium and silica content, and solids weight percent. The water loss in the system and the chemical composition of the materials elucidated through NMR is also utilized to connect observed trends. The ability to predict material properties with respect to the initial composition would allow for the intelligent design of geopolymer systems with desired material properties, which will be of critical importance for use in extra-terrestrial construction in support of human exploration of our solar system.

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**Physiology-Based Parameterization of Human Blood Shear Rheology via Machine Learning**

Sean Farrington
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Committee Members: Dr. Abraham Lenhoff and Dr. Alexandra Bayles

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Hemorheology is the study of blood flow and the mechanical stresses and kinematics involved.[1] In this research we connect hemorheology models to blood's components using machine learning. The Casson constitutive equation is a popular and simple model used to describe the steady shear rheology of blood, with only two parameters that specify an infinite shear viscosity and a yield stress that depend on blood physiology. To extend toward transient blood rheology a structure parameter thixotropic constitutive model is chosen, which reduces to the Casson behavior at steady shear.[2] The hemorheology constitutive models can be parameterized with blood components to make the connection of physiology to blood flow. Previous literature identified red blood cell volume fraction (hematocrit) and a plasma protein (fibrinogen) as the two most important physiological factors that affect blood flow. However, previous parameterizations of the Casson model may not be reliable due to the use of non-standardized data sets. This study uses machine learning and the largest standardized dataset[3] to improve the parameterization of shear rheology with respect to hematocrit and fibrinogen concentration for healthy individuals. The study also employs machine learning to identify an additional factor, mean corpuscular hemoglobin (MCH), that affects blood rheology. The proposed parameterization could be used to predict blood rheology parameter ranges for healthy individuals useful for cardiovascular diagnostics.

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## Relationship between interfacial properties and long-term stability of therapeutic proteins

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Monoclonal antibodies (mAbs) play an indispensable role in various disease treatments, including asthma, autoimmune diseases, and cancer. The success of mAbs stems from their high specificity, potency, and ability to be engineered for various targets. However, instabilities during manufacturing and in formulation and delivery limit their use. In particular, the aggregation propensity of mAb is a longstanding issue for biopharmaceutical development. Indeed, the presence of aggregates in the final product could reduce therapeutic efficacy, trigger unwanted immune responses, and even cause life-threatening complications. Therefore, the ability to predict the aggregation propensity of these protein-based therapeutics during the early stage of formulation development is crucial and currently attracts much research from both industry and academia.

MAbs are surface-active species that can adsorb and form viscoelastic gel-like layers at the air-water interface. The formation of the gel-like interface is relevant to the exposure of buried hydrophobic moieties through some degrees of conformational change. On the other hand, the aggregation mechanism of therapeutic proteins also involves the exposure of hydrophobic moieties when proteins undergo partial unfolding. We hypothesize that the interfacial properties of mAb interfacial layers are correlated to mAb aggregation propensity. To test this hypothesis, the interfacial properties of four different mAbs with different bulk stability according to visible particle counts from a 3-year stability study are measured. MAb interfacial films are characterized by dynamic surface tension, interfacial shear rheology as well as X-ray reflectivity. The measured interfacial properties are compared to the bulk stability measurements to identify correlations between the interfacial properties and the aggregation propensity of the therapeutic formulation. We find that the interfacial elastic modulus measured at 8 hours of adsorption correlates with the long-time stability within a particular class of mAb.

# Connecting the rheology and kinetics of sustainable geopolymer cements from metakaolin for additive manufacturing applications

Thaddeus Egnaczyk
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In cementitious systems, well established connections between cement chemistry, reaction kinetics, and material properties enable material design for targeted applications including the additive manufacturing of large structures.[1] However, cement production is one of the leading contributors to global $CO_2$ emissions and industrial water consumption.[2] Geopolymers are ceramic-like binders which provide a sustainable alternative to cement with the potential to reduce up to 80% of $CO_2$ emissions and enable in situ resource utilization (ISRU) for both terrestrial and lunar applications. While cement binders form from a calcium-silicate system, geopolymer binders form from an alumino-silicate system, resulting in unique rheological and kinetic curing profiles. The geopolymer formation process includes several characteristic steps including the dissolution of precursor species and the formation of a heterogenous gel via polycondensation reaction. A quantitative connection between geopolymer binder chemistry, reaction kinetics, and dynamics of structure formation via rheology does not currently exist for geopolymer binders, yet is essential to enable material design for applications like additive manufacturing.

This work aims to quantitatively connect geopolymer material property development via bulk rheology to reaction kinetics via isothermal calorimetry, and chemical composition via nuclear magnetic resonance spectroscopy (NMR). The Avrami kinetic equation is used to model kinetic and rheological extent of reaction.[3] Geopolymer binders are synthesized via the alkaline activation of metakaolin using a sodium silicate activating solution. A model binder system with nominal elemental ratio of 2/1/1/7 ($Si/Al/Na/H_2O$) is chosen for optimal mechanical properties after cure and for comparison with relevant literature. Small amplitude oscillatory shear (SAOS) experiments characterize the microstructure development of the reacting system. Isothermal calorimetry data provides a time-dependent extent of reaction for a given composition. NMR spectroscopy reveals the aluminosilicate binder composition at a given reaction extent. Successful modelling of geopolymer property development will allow for design of binders with optimized rheological properties, like time-dependent yield stress, for future construction-scale additive manufacturing in terrestrial and lunar applications.

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[2] J.L. Provis, *Cement and Concrete Research.* **114**, 40 (2018).
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# Probing Operation Limits of Advective Assembly in Additive Manufacturing using Digital Twins

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Multi-material additive manufacturing (MMAM) is a 3D printing technique that produces structured composites by arranging chemically distinct inks within a single part. Customizable control over the internal long-range order is useful in a vast range of applications, including programming shape transformations in soft actuators,[1] mimicking hierarchical tissues in bioprinting,[2] and even realizing texture in sustainable food alternatives.[3] Traditionally, MMAM composites are constructed by layer-by-layer direct ink writing where nozzles or streams are swapped each time a new constituent is introduced. This sequential method affords precision, but suffers from low volumetric throughputs, long build times, and poor interfacial adhesion. These limitations constrain the inks that can be used and subsequently the composites that can be realized by layer-by-layer construction. In contrast, the promising new technique of advective assembly (AA) has the potential to expand the material design space and production capacity beyond layer-by-layer MMAM. Resembling static mixers, advective assembly nozzles force co-flowing inks through a series of addition, rotation, and splitting flow elements. The elements sculpt laminar streamlines, multiplying the flowing pattern and shrinking its characteristic dimension. The AA nozzles efficiently structure multi-material filaments prior to their extrusion onto the print bed. The modular combination of the flow elements allows operators to create voxelated, designer architectures provided that the flow remains stable and predictable.

Here, we use computational fluid dynamics to systematically investigate how ink rheology affects flow stability. Polymeric 3D printing inks are often both shear thinning and viscoelastic. As a result, when the inks are forced through successive multiplicative elements, complex stress gradients arise and compromise fidelity of the extruded architecture.[4] Alternatively, recent AA particle image velocimetry experiments[1] have shown that viscoplastic, shear thinning microgel inks assemble with high fidelity. We hypothesize that the reliable assembly is due to the yield stress of the granular ink which limits deformation of the architecture to regions near the wall. To systematically probe this hypothesis, we build digital twins of advective assembly nozzles in ANSYS Fluent 2023 R1. We investigate operating regimes for different Newtonian and Non-Newtonian fluids over a range of inlet velocities, yield stresses, power law indices, and viscosities. The effect of these parameters on the outlet structure is reported via a computational framework which compares the simulated output to a Boolean-inspired first-order prediction. Distortions are measured through matrix comparison metrics such as the Jaccard index, matrix norm, and a modified Frobenius norm. Pressure drop calculations in high-throughput operating regimes illustrate the potential for AA to increase production capacity of MMAM while achieving small characteristic dimensions. Future work will include further investigation into global solution convergence to evaluate the impact of wall shear and slip. Overarchingly, quantitative identification of stability windows is critical for the operation and optimization of advective assemblers for MMAM.

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# Fundamental investigation of aerosol deposition in additive manufactured lattice structure

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Aerosol filtration is a crucial process for various applications such as air purification, medical equipment, and industrial processes. The pressure drop during a filtration process is a critical metric in determining the performance of filters, as higher pressure drops during filtration indicate higher operational cost. Improvements in filtration efficiency for traditional fabric filters also come at the cost of higher pressure drop. Additive manufacturing (AM) and parametric design may afford unique opportunities to overcome this tradeoff. AM can allow for the production of highly controllable and customizable designs that can be tailored to specific applications, enabling fast prototyping and both time and cost savings. The lattice, a hierarchical structure porous metamaterial enabled by AM, has shown high potential in fluid applications due to its highly ordered and scalable structures. Despite the potential of using AM lattices as aerosol filters, there is a lack of fundamental research in this field needed to optimize lattice designs for given applications. Owing to significant differences in length scales when compared to conventional filters, current filtration theory has yet to be validated on these lattice materials. The main challenges addressing aerosol performance in these materials include the difficulty in quantifying particle deposition in a 3D geometry and the multitude of design parameters to investigate, including lattice geometry, lattice surface area, aerosol flow rate, and aerosol particle size. This study aims to identify key design parameters affecting deposition performance and to formulate engineering correlations that can give good predictions on filtration performance across a wide range of design and operating parameters of lattice filters. We hypothesize the controllable pore size and high surface area of AM lattice designs can be exploited in aerosol filtration processes to offer improved deposition performance while maintaining lower pressure drops as compared to traditional fabric filters. Our results to date have shown that lattice designs can be tuned to function through an impaction-dominating mechanism that can provide high deposition efficiency (>95%) with high throughput while maintaining a low pressure drop. Using a custom apparatus in the Fromen lab, AM lattices were fabricated using the Carbon M1 printer, and bulk deposition efficiency was evaluated for monodisperse 1 μm polystyrene aerosol particles using an Optical Particle Sizer (OPS). We find that overall deposition efficiency increases with increasing flow velocity, but peak aerosol collection varies with the unit cell geometry; under the same inlet flow velocity of 10 m/s, the Weaire-Phelan unit cell geometry has a maximum deposition efficiency of 95% and significantly outperforms the Kelvin unit cell geometry (max deposition efficiency of 80%) and Cubic unit cell geometry (max deposition efficiency of 40%). These differences in deposition efficiency are somewhat unexpected, as the prior 3 geometries had consistent cell length size and porosity, and thus the same number of unit cells and the same amount of void volume within the filter. Continued work in this area will provide a comprehensive understanding of aerosol deposition and pressure drop in lattice structures.

## Mechanistic Modeling of Depth Filtration in Biopharmaceutical Production

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Depth filtration (DF) is a solid/liquid separations process that provides primary clearance of suspended solids; in biopharmaceutical production these are predominantly culture cells. Process performance, including temporal profiles of impurity clearance and trans-filter pressure, is typically determined on a solely empirical basis. We seek to develop mechanistic models of depth filtration that, in contrast, make predictions on the basis of the underlying process mechanisms and interactions relevant to the filtration process. This modeling methodology has been demonstrated for a variety of DF operations but not for biopharmaceutical applications, so the mechanisms presently recognized in the literature are likely to require significant adaptation to describe biological feed streams. For example, physical entrapment or "sieving" is typically the dominant mechanism of particle retention, but our initial analysis of yeast cell filtration indicates that adsorption can contribute significantly.

The approach to develop DF models for cell removal will include actual DF measurements as well as structural characterization of the filters and the process streams (feed and filtrate). Filtration experiments performed with various commercial depth filters and representative cell culture fluid (CCF) provide measures of filtration performance, including trans-filter pressure and filtrate turbidity; flow imaging and dynamic light scattering also provide information on particle size distributions. X-ray computed tomography is employed to analyze both clean and fouled samples of filter material to characterize the morphology of the intra-filter pore space and the resulting distribution of retained solids.

# Design and synthesis of multifunctional collagen mimetic peptides

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Peptides, functionally encoded by their amino acid sequence, are increasingly being designed within the biomaterials community for a range of applications, from fundamental biological studies to therapeutics, with significant interest in creating mimics of the extracellular matrix found within human tissues. In particular, 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 a key protein in the structure and properties of many tissues. Recently, synthesis methods have been developed for self-assembling multifunctional collagen mimetic peptides (mfCMP) for the formation of synthetic matrices with robust and tunable properties. These mfCMP sequences integrate 1) hydrogen bonding with the most common repeat unit of human collagen I (proline-hydroxyproline-glycine)$_n$ [(POG)$_n$], where increasing n increases the stability of the triple helices formed; 2) 'sticky ends' with amino acid substitutions (K and D for O and P) within repeats on the ends of the sequences for forming long fibrils and networks; and 3) non-natural reactive handles for integration of these assembled structures within synthetic polymer networks to better mimic the mechanical and biochemical properties of collagen-rich ECMs.

Building from these advances, there remains a need for self-assembling peptides with well-defined, tailorable properties and increased complexity to better mimic and potentially serve as a replacement for harvested collagen I biomaterials, where desired functionalities include triggered matrix stiffening (e.g., modulus), tunable stability (e.g., melting temperature), and designed bioactivity (e.g., receptor binding). These properties can be modulated to mimic different types of networks and tissues in the human body for biomedical applications of interest, including the study and promotion of wound healing. For example, increased matrix stiffness and tunability in melting temperature can be achieved through an increase in crosslink density in peptide networks or within helices and fibrils, respectively, to study scarring after injury and initiation and progression of diseases. I will address these needs through the design and synthesis of multifunctional collagen mimetic materials in three main Aims. The first aim is to design mfCMP sequences with reactive handles for triggering intra-fibrillar crosslinking and inducing matrix stiffening with light, building from an established polymer-peptide materials system. The second aim is to design mfCMP sequences to assemble, stabilize, and stiffen purely peptide-based materials using light-activated inter- and intra-fibrillar crosslinking. The third aim is to design mfCMPs coassembled with integrin binding sequences and with tunable melting temperatures for controlling material structure and biochemical content, creating a modular system of building blocks that has the potential to be a fully synthetic surrogate for harvested collagen I biomaterials. Overall, this work will result in responsive and tunable mfCMPs and networks for healing applications.

# Assessing Cellular Responses in Viscoelastic Supramolecular Hydrogels for Bioinspired Three-Dimensional Cell Cultures

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The demand for more complex, well-defined pre-clinical *in-vitro* cell culture and delivery platforms continues to grow. Hydrogels are advantageous for cell culture and delivery, owing in part to their ability to incorporate biophysical and biochemical cues akin to the native target cellular microenvironments. Given the soft, viscoelastic, dynamic, and 3D nature of hydrogels, cellular assays and mechanical characterization analyses become increasingly difficult. Thus, a unique approach to assess the dynamic reciprocity between matrix-cell and cell-matrix interactions is needed. My dissertation aims to meet this need by integrating an innovative cellular assaying toolbox, including techniques such as reporter cells, immunostaining, and flow cytometry, and a unique materials characterization approach, including bulk rheometry, atomic force microscopy (AFM), and an array of microrheological techniques.

In this subproject, specifically, we use physically assembled, supramolecular hydrogels, which are formed by weak bonds, such as hydrogen bonding or van der Waals interactions, and typically have low moduli in the range of soft tissues, like the brain, or harvested extracellular matrices, like collagen I or basement membrane extract hydrogels. Synthetic supramolecular hydrogels also can possess unique viscoelastic properties, such as self-healing and shear-thinning, making them reminiscent of more complex soft tissues within the human body and interesting for a variety of biomedical applications. Collectively, these properties make these materials a unique and relevant option for cell culture and delivery. In this work, we collaboratively established an approach for utilizing supramolecularly assembling hydrogels, developed within the Besenius group[1], as a 3D cell culture platform. Here, we developed strategies for the encapsulation and culture of human pulmonary fibroblasts and well-defined extracellular matrix proteins within these physically assembled hydrogels. A process for degradation of the hydrogel was developed through exploitation of the thermoresponsive and chemical properties of the gel such that cellular viability could be assessed using flow cytometry. Furthermore, fibroblast activation was monitored using a dynamic alpha smooth muscle actin ($\alpha$SMA) lentiviral reporter. The unique viscoelastic properties of these gels, such as thermoresponsiveness and shear-thinning, were assessed using rheometry. Altogether, this work is fundamental toward establishing these physically assembling hydrogels as 3D cell culture systems, with future work planned to exploit the viscoelastic nature of these materials toward injectable hydrogels for delivery of cells for biomedical applications.

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# Computational Studies of Network and Gel Structures in Macromolecular Soft Materials

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Network morphologies can be incorporated into soft materials as one method of tuning the physical properties of the material. In particular, the structure of network morphologies is known to affect the mechanical and transport properties of such materials. For example, the structure of networks of hydrophilic domains in ionomer membranes affects the transport of ions through the membrane, and the topology and connectivity of networks of synthetic peptides impact the mechanical strength when such materials are used as "tissue" mimics.

In this talk I will focus on a specific class of soft materials called collagen-like peptides (CLPs), which are synthetic biopolymers that mimic natural collagen, the protein that adds mechanical strength to the extracellular matrix. CLPs are an active research topic for their potential applications in drug delivery and tissue engineering due to their ability to self-assemble into triple helices which further assemble into fibrils and network morphologies via covalent or noncovalent stabilizing mechanisms. In this work [1], we study the self-assembly of CLP triple helices made of CLP strands of different lengths (i.e., heterotrimer), resulting in "sticky ends" with exposed hydrogen bonding groups that drive the triple helices to physically associate and assemble into fibrils and networks. Specifically of interest is how the interplay of CLP design (number of sticky ends) and solvent quality affect the resulting assembled network morphology.

We use a computational approach involving coarse-grained (CG) molecular dynamics (MD) simulations to investigate the self-assembly of collagen-like peptide (CLP) triple helices into fibrillar structures and percolated networks as a function of solvent quality. We use a validated CG model [2,3] for CLP in implicit solvent and capture varying solvent quality through changing strength of attraction between CG beads representing the amino acids in the CLP strands. I developed computational methods to quantify various relevant features of network structures that can be used for additional calculations to predict properties (e.g. strand length and diameter, fractal dimension, and lacunarity). Our CG MD simulations show that, at higher CLP heterotrimer concentrations, decreasing solvent quality causes i) the formation of heterogeneous network structures (increasing lacunarity) with a lower degree of branching at network junctions (decreasing fractal dimension) and ii) increases in the diameter of network strands and pore sizes.

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