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CHEMICAL & BIOMOLECULAR ENGINEERING

2024 SUMMER RESEARCH REVIEW

2nd year talks

MAY 29, 2024

UNIVERSITY OF DELAWARE

Chemical & Biomolecular Engineering
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8:00 – 8:50 AM..... Breakfast for **FACULTY** & **PRESENTERS**..... 2ND Floor Lobby
8:55 AM..... Welcome & Opening Remarks.....102 Colburn Lab
9:00 AM..... Talks begin in designated rooms.....CLB 102 / 109 / 104

10:40 AM – 11:00 AM	BREAK
12:30 PM – 2:00 PM	LUNCH for FACULTY & PRESENTERS **only**

102 COLBURN LAB – Synthetic Biology / Drug Delivery / Biomaterials

Moderator: Sofia Alfieri	
9:00 AM	Jodi Graf
9:25 AM	Spencer Wolfe
9:50 AM	Logan Yeager
10:15 AM	Derron Ma

Moderator: Jodi Graf	
11:00 AM	Sofia Alfieri
11:25 AM	Alex Hansen
11:50 AM	Sofia Capece
12:15 PM	Philip O'Dell

Moderator: Logan Yeager	
2:00 PM	Emily Doleh
2:25 PM	Robert Barlow
2:50 PM	Christopher Pirner
3:15 PM	Willliam Rears

109 COLBURN LAB – Catalysis

Moderator: Abigayle Polsky	
9:00 AM	Jacqueline Ngu
9:25 AM	Enerelt Burentugs
9:50 AM	Nicholas Houck
10:15 AM	Rajas Mehendale

Moderator: Jacqueline Ngu	
11:00 AM	Jae Young Kim
11:25 AM	Abigayle Polosky
11:50 AM	Justin Harrington
12:15 PM	Jeffrey Hoffmann

Moderator: Jay Shah	
2:00 PM	Genevieve Yarema
2:25 PM	Pedro Moura
2:50 PM	Izak Minnie
3:15 PM	Sakshi Satyanand

104 COLBURN LAB – Soft Matter / Computation / Synthetic Biology

Moderator: Erik Anderson	
9:00 AM	Jack Rooks
9:25 AM	Venice Magunga
9:50 AM	Jackie Arnold
10:15 AM	Juliana Nam

Moderator: Juliana Nam	
11:00 AM	Hasan Koybasi
11:25 AM	Jay Ashish Shah
11:50 AM	Zhifei Yuliu
12:15 PM	Erik Anderson

Moderator: Anthony Stohr	
2:00 PM	Jessica Rubia Gamba
2:25 PM	Austin Desmarais
2:50 PM	Saloni Gupta
3:15 PM	

Targeting Bottlenecks in rAAV Production: Optimizing Gene Expression for Improved Packaging Efficiency

Sofia Rose Alfieri
Advisor: Dr. Kelvin H. Lee
Committee Members: Dr. Millicent Sullivan and Dr. April Kloxin

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Recombinant adeno-associated virus (rAAV) is a leading viral vector for gene therapy due to its favorable properties such as low immunogenicity, tropism for specific tissues, and long-term episomal expression. However, rAAV production systems produce a large proportion of empty capsids, with typically only 5 to 30 percent of vectors containing the desired therapeutic transgene. This low packaging efficiency results in high manufacturing costs due to extra purification steps needed to remove empty capsids that do not provide therapeutic benefit to patients. Engineering approaches to improve full capsid yield have focused on improving vector genome (VG) titer through plasmid design but neglect the underlying mechanisms affecting complete viral particle assembly. This study investigates critical links between gene expression, VG and capsid titer, and vector design for rAAV systems to better understand the underlying expression patterns that control rAAV genome packaging.

There are three categories of genes required for rAAV production: AAV genes *Rep* and *Cap* that aid in DNA replication and capsid formation respectively, adenovirus (AdV) helper genes *E4ORF6*, *E2A*, and *VA RNA*, and therapeutic transgene flanked by AAV inverted terminal repeats. In this work, different configurations of these genes were assembled and screened transiently in suspension human embryonic kidney (HEK293) cells immortalized in the AdV *E1A/B* gene. Cytotoxicity of key viral genes was identified, prompting the design of polycistronic expression cassettes with either internal ribosome entry sites (IRES) or self-cleaving 2A peptides to control expression levels. These cassettes were shown to significantly impact rAAV titer and packaging. In addition to this, increasing capsid gene expression was shown to increase VG and capsid titer over two-fold. To support this work, a reliable analytical method for packaging efficiency quantification was developed using anion exchange high performance liquid chromatography. Correlations between expression of key viral genes and packaging gained from these studies will provide insight into the mechanisms behind rAAV packaging and will be used to design rAAV production systems with improved packaging, ultimately paving the way for more cost-effective and productive gene therapy strategies.

Modeling and Simulations for Design-Structure-Property Relationships in Soft Materials

Erik J. Anderson
Advisor: Arthi Jayaraman
Committee Members: Dr. Eric Furst, Dr. Abraham Lenhoff, Dr. Victoria Muir, Dr. April Kloxin

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Elastin-like peptides (ELPs) and collagen-like peptides (CLPs) are two classes of thermoresponsive biopolymers of interest to us. ELPs remain soluble in water at temperatures below their inverse transition temperatures T_t and phase separate at temperatures higher than T_t. As compared to ELPs, CLPs are relatively hydrophilic exhibiting a triple-helix structure at temperatures below their melting transition T_m. ELPs can be conjugated to the CLPs to form dual-thermoresponsive amphiphilic macromolecules whose dual phase transition temperatures Tt and Tm can be tuned via design of ELPs and CLPs (e.g., chain length, peptide composition and sequence). Above T_t and below T_m, ELP-CLP conjugates self-assemble in water because of the hydrophobic ELP chains and relatively hydrophilic CLP triple helices. For certain design parameters of the ELP-CLP conjugates these self-assembled structures are vesicles that can encapsulate many small molecule drugs, making them valuable for drug delivery. Recent experiments by Kiick and coworkers show that the size and shape of these vesicles is affected by drug chemistry (hydrophobic vs. hydrophilic) and loading. Furthermore, the changing vesicle morphology affects the drug release profile. As such, the design flexibility of these ELP-CLP vesicles presents a unique opportunity to control the rate of release of encapsulated drugs to improve drug efficacy and minimize off-target effects. For realization of such an opportunity, we aim to develop a fundamental understanding of how the vesicle morphology is affected by the interactions between the ELP-CLP conjugates and the encapsulated drugs. In this work, we perform coarse-grained molecular dynamics simulations to probe drug-induced morphological changes to derive generalized rules for future design of ELP - CLP nanovesicles (ECnV's) for desired drug loading and release. Our work shows that increasing strength of attraction between encapsulated hydrophilic drugs and CLP domains of the ELP-CLP conjugates changes the vesicle morphology. However, similar increases in attraction between encapsulated hydrophobic drugs and ELP domains of ELP-CLP conjugates do not show significant morphological changes to the vesicle. Insights derived from these simulations are valuable to guide the selection of ELP-CLP amino acid sequences to allow controlled rate of release of any chosen drug.

High-performance, lignin-derivable polymers as sustainable alternatives to petroleum products

Jackie Arnold
Advisor: Prof. LaShanda Korley
Committee Members: Prof. Thomas H. Epps, III; Prof. Srikanth Pilla; Prof. Christopher Kloxin

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Polyurethanes (PUs) represent a large sector of functional materials used in everyday life, including coatings, foams, elastomers, and adhesives. A polyurethane is traditionally made up of a polyol and an isocyanate, one of which is typically an aromatic compound. Aromaticity can provide high-performance PUs with tunable properties such as temperature stability, rigidity, and toughness. However, common aromatic monomers such as the polyol, bisphenol-A (BPA), and the isocyanate, methylene diphenyl diisocyanate (MDI), are sourced typically from petroleum feedstocks and pose health risks to humans and ecosystems upon production, use, and disposal. To overcome the toxicity and non-renewable nature of PU precursors, it is necessary to reimagine the polymer backbone chemistry and incorporate sustainable building block replacements. Lignin is an attractive source for biobased bisphenols, termed bisguaiacols, that have similar chemical structures to petroleum-derived bisphenols with the addition of pendent methoxy groups. Moreover, lignin is the largest source of natural aromatics. Additionally, lignin is non-food source competitive and is widely available as a waste material from the paper and pulp industries. Previous work has explored lignin-derivable, non-isocyanate polyurethanes (NIPUs), which have been shown to increase hydrogen bonding and decreased estrogenic activity through the use of lignin-derivable bisguaiacols in place of petroleum-derived bisphenols. NIPUs eliminate the need for isocyanates through the reaction of cyclic carbonates with amines. PUs and NIPUs have structurally similar backbones, though NIPUs display hydroxyl moieties that offer opportunity for additional functionalization and tunable intermolecular interactions.

To address the sustainability and toxicity challenges of PU materials, we aim to (1) demonstrate the potential for NIPUs as a high-performance material platform, (2) explore the use of lignin-derivable bisguaiacols in traditional PU chemistry, and (3) use bisphenols with varying numbers of pendent methoxy and methyl groups to establish structure-property relationships with PU and NIPU classes. Six thermoplastics, consisting of PUs and NIPUs derived from BPA, bisguaiacol A (BGA) and bisguaiacol F (BGF), were synthesized with comparable molecular weights, dispersities, and film clarity. To realize lignin-derivable PUs, we developed new strategies to ethoxylate bisguaiacols followed by polymerization with hexamethylene diisocyanate (HDI). Comparing these lignin-derivable NIPUs and PUs, the degradation temperatures ($T_{d,5\%}$s) varied between 274 °C and 316 °C as a function of polymer bulk resulting from substituent presence, demonstrating high thermal stability in all cases. The glass transition temperatures (T_gs) of these materials were between 45 °C and 58 °C, showcasing their utility for typical PU applications. Additionally, the molecular structure of BPA-PU, BGA-PU, BGF-PU, BPA-NIPU, BGA-NIPU, and BGF-NIPU provide functional matrices to explore interactions with fillers [*e.g.,* metal-organic frameworks (MOFs)]. We will explore the development and performance of these lignin-derivable NIPU and PU nanocomposites for targeted applications required high chemical resistance, mechanical robustness, and thermal management.

Developing *Clostridium butyricum* Argonaute (CbAgo) for gene editing applications in eukaryotes

Rob Barlow
Advisor: Dr. Kevin Solomon
Committee Members: Dr. Mark Blenner, Dr. Wilfred Chen, Dr. Aditya Kunjapur

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Gene editing has advanced many fields, including research, agriculture, and healthcare/pharmaceuticals, with CRISPR/Cas at the center of this revolution. Despite its ease of use, Cas endonucleases require targets to be next to a protospacer adjacent motif (PAM) site, with an estimated spacing of about 25 nt in *Escherichia coli*. Thus, modifications far from PAM sites pose a significant challenge, limiting the scope of CRISPR/Cas as a research and therapeutic tool. Although "near-PAMless" Cas variants have been engineered, they have limited use due to their reduced efficiency and increased off-target effects, which require mitigation strategies. Argonaute (Ago) proteins are a diverse group of enzymes with nucleic acid-guided endonuclease activities. More importantly, some prokaryotic Agos (pAgos) have been shown to mediate gene editing in *E. coli* with *no* targeting requirements (i.e., PAM sites). pAgo research has primarily focused on *in vitro* and bacterial systems. However, no one has successfully used pAgos in eukaryotes. We lay the foundation for pAgo gene editing in these systems, using the leading mesophilic (~37ºC) pAgo, *Clostridium butyricum* Ago (CbAgo). CbAgo uses short (~20 nt) 5' phosphorylated single-stranded DNA guides to cleave complementary DNA. We demonstrate eukaryotic expression of CbAgo in the model eukaryote, *Saccharomyces cerevisiae*. With the addition of two nuclear localization tags, CbAgo localizes to the nucleus, where it can interact with the host's DNA. These findings show that fusions must be made to the pAgos' N-terminus, as C-terminal fusions likely disrupt proper protein folding and activity. This work developed design rules for pAgo implementation into higher eukaryotes and sets the stage for future experiments to demonstrate CbAgo-mediated gene editing in *S. cerevisiae* that circumvents PAM site requirements.

Development of a Joule-Heated Reactor with Porcupine Coil Heating Element for Endothermic Reactions

Enerelt Burentugs
Advisor: Raul F. Lobo
Committee Members: Raul F. Lobo, Dionisios Vlachos, Dongxia Liu

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Over the past decade, greenhouse gas emissions have reached the highest levels in human history, requiring urgent action. The chemical industry was responsible for around two percent of total CO2 emissions in 2021[1]. As a result, it is important that chemical manufacturing processes are electrified. As compared to conventional thermochemical reactors, Joule-heated reactors (JHR) provide local uniform heating, which maximizes catalyst utilization while limiting unwanted byproducts. Additionally, this reactor configuration allows for a quicker start-up time and can achieve higher temperatures at a higher energy efficiency [2]. While JHR has demonstrated improved reaction yields and efficiencies, many technical aspects remain as challenges, including scalability, temperature control, and mass and heat transfer.

In this study, we develop a JHR that uses a porcupine coil to electrify highly endothermic, high-temperature chemical reactions. The reactor's performance was tested by carrying out a catalytic ethane dehydrogenation reaction (EDH) using highly active Mn-containing ZSM-5 catalyst [3]. With conditions comparable to conventional reactors, the proposed reactor system exhibited excellent catalytic performance at low power inputs. The effects of different operating parameters on the performance EDH reaction are evaluated experimentally and numerically using Computational Fluid Dynamics (CFD) simulation. Preliminary experimental and modeling results indicate that increasing washcoat thickness increases the catalytic rate, while increasing the input power leads to coke formation on the catalyst due to thermal cracking. Moreover, CFD results revealed that porcupine coil geometry produces turbulent-like flow conditions, alleviating mass-transfer limitations. Future work will include quantifying the energy efficiency of the current setup and optimize both the model and the JHR.

References

[1] Priyadarshi R Shukla, Jim Skea, and Raphael Slade. Working Group III Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.
[2] Xinying Li, Ding Yuan, Tian Xie, Quancong Zhang, Wenjun Xu, Ting Fu, Xuyang Chu, Tao Luo, Linjing Wu, and Wei Zhou. UV-Laser ablation enhanced Joule-heating catalyst support for electrified MSR in microreactor. *Chemical Engineering Journal*, 459:141571, March 2023. ISSN 1385-8947. doi: 10.1016/j.cej.2023.141571.
[3] Pan, J.; Lobo, R. F. Ethane Dehydrogenation over Manganese Oxides Supported on ZSM-5 Zeolites. *Catal. Sci. Technol.* **2023**, *13* (9), 2794–2801.doi: 10.1039/D2CY02062A.

Harnessing Syntrophic Microbial Cocultures for Carbon-Neutral Supratheoretical Isopropanol Production

Sofia Capece
Advisor: Dr. Eleftherios Papoutsakis
Committee Members: Dr. Aditya Kunjapur and Dr. Wilfred Chen

The petrochemical industry not only contributes to the shortage of non-renewable fossil resources but negatively impacts the global climate through greenhouse gas effects. To achieve a more sustainable process for chemical production, microbial cell factories can use renewable feedstocks to produce a variety of chemicals and fuels. However, much of microbial fermentation research has been focused on the engineering of singular microorganisms for chemical production, often with processes suffering from low yields, rates, and selectivity of the desired product. To lessen the metabolic burden of chemical production, synthetic microbial cocultures employ a division of labor with specialist microorganisms that each contribute their unique metabolic pathways to a common goal. As proof of concept, we engineered two specialist *Clostridium* species for carbon-neutral isopropanol production. *Clostridium acetobutylicum* has been engineered for acetone production by our group and naturally consumes a variety of sugars, while *Clostridium ljungdahlii* is adept at carbon fixation and reduction of acetone to isopropanol.

In this study, we uncovered the impacts of high-density coculture fermentations and acetone pathway engineering on the promotion of carbon-negative isopropanol production. The engineered coculture achieved a supratheoretical yield of 0.8 Cmol isopropanol/ Cmol glucose, with 7.9 isopropanol/ ethanol molar ratio at 110% carbon recovery. We discovered that metrics such as product selectivity and yield are significantly influenced by species ratio. We also compared genome-wide transcriptomics for both species across different time points in the fermentation and correlated these findings with the coculture's performance in isopropanol selectivity and carbon recovery. This analysis sheds light on the syntrophic interactions and transcriptional regulation that enables the performance of this coculture. Through interspecies interactions and material exchange[1], the coculture achieves supratheoretical yields of isopropanol, substantially outperforming what is possible by one microorganism alone. Alongside the ever-increasing advancements in the genetic engineering of *Clostridium* species and our growing understanding of the dynamic interactions between microorganisms in recent years, engineered microbial cocultures serve as an important step in the decarbonization of the chemical industry.

(1) Charubin, K.; Modla, S.; Caplan, J. L.; Papoutsakis, E. T. Interspecies Microbial Fusion and Large-Scale Exchange of Cytoplasmic Proteins and RNA in a Syntrophic Coculture. *Mbio* **2020**, *11* (5). DOI: ARTN e02030-2010.1128/mBio.02030-20.

Engineering *Bacillus subtilis* to Deliver Nitrated Antigens

Austin Desmarais
Advisor: Aditya Kunjapur
Committee Members: Mark Blenner, Catherine Fromen, Julie Maresca

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Vaccines have constituted a major triumph in global healthcare. However, only a small fraction of all human pathogens can be vaccinated against.[1,2] There are several different vaccine technologies available on the market, from live-attenuated to mRNA vaccines. Yet there is no universal "best choice" platform. Each approach has potential advantages and disadvantages; different methods of administration, shipping and storage requirements, manufacturing considerations, and qualities of the induced immune response may be preferred given contextual factors such as the target pathogen or expected demand timelines. By investigating potential new vaccination strategies, we aim to improve the scope and quality of these essential tools.

One such promising but yet-unrealized platform is the bacterial-vector vaccine, in which bacteria would be engineered to recombinantly express an antigen. *Bacillus subtilis* is a bacterial species with several attractive features for this purpose: it has no known human pathogenicity and is widely considered safe, being naturally found in the human microbiome and already available in commercial probiotics. Additionally, *B. subtilis* can form a spore: a dormant, durable phenotype triggered in response to environmental stress such as nutrient limitations.[4] Others have demonstrated it is possible to anchor exogenous proteins to the surface of these spores. Many examples show that administering recombinant spores orally or nasally to mice elicits a protective immune response toward the displayed protein. However, this often requires repeated dosing,[5] and the method suffers from a lack of effective mucosal adjuvants.[6] We hope to overcome these limitations by further engineering *B. subtilis* to site-specifically incorporate a non-standard amino acid (nsAA) into these displayed proteins. Previous work suggests that incorporating *para*-nitro-L-phenylalanine (pN-Phe) into an otherwise weakly immunogenic protein may stimulate a strong immune response.[7]

During this presentation, I will highlight the steps taken to develop and investigate this technology. I will report data in two distinct areas: the initial efforts to engineer *B. subtilis* to measure the incorporation efficiency of nsAAs within spore-displayed proteins, and progress in determining what effects pN-Phe (and other nsAAs) may have on human tissue through *in vitro* cell culture. This includes results quantifying the potential toxicity of several nsAAs, as well as attempts to assess if pN-Phe may be incorporated into the human proteome by natural protein-synthesis machinery. Future work seeks to optimize pN-Phe incorporation within *B. subtilis* spores and further assess the potential for immunogenic nsAAs to be used in therapeutics.

References:

1. M. E. J. Woolhouse and S. Gowtage-Sequeria, *Emerging Infectious Diseases*. **11**, 12 (2005).
2. U. S. FDA, FDA.gov, "Vaccines Licensed for Use in the United States." Accessed Dec 2023.
3. U. S. CDC, Antibiotic Resistance Threats in the United States. (2019).
4. H. A. Hong, *et al.*, *Research in Microbiology*. **160**, 2 (2009).
5. A. Saggese, *et al.*, *International Journal of Molecular Sciences*. **24**, 13 (2023).
6. E. C. Lavelle and R. W. Ward, *Nature Reviews Immunology*. **22** (2022).
7. J. Grünewald, *et al.*, *Proceedings of the National Academy of Sciences*. **105**, 32 (2008).

Development of a stable inducible producer cell line for recombinant AAV production

Emily Doleh
Advisor: Dr. Mark Blenner
Committee Members: Dr. Kelvin Lee and Dr. Kevin Solomon

Recombinant adeno-associated virus (rAAV) is emerging as a popular delivery vehicle for gene therapies. This viral vector is attractive due to its low immunogenicity, wide range of infectivity, and overall safety profile. Traditionally, rAAV is made through a triple transient transfection of HEK293 cells, allowing for short term production. However, problems such as plasmid costs, batch-to-batch variation, and misalignment of gene expression are limiting production. Stable integration of the genes required for rAAV production can address some of these issues. However, cytotoxicity caused by continuous expression of these viral components has stunted stable cell line developments. Ideally, the development of an inducible system will be able to address cell line development and misalignment of expression obstacles.

Here, we seek to lay the groundwork for the creation of a stable inducible producer cell line for rAAV production. We will control the genes expressed in rAAV production through a redesign of the traditional triple transfection plasmids. Utilizing the Tet system and promoter engineering, we can tune the expression responses while keeping the system under the control of a single inducer molecule. We can achieve differing gene expression levels using the same promoter by adjusting the number of operators in the sequence. The promoters' response times to the inducer molecule can also be influenced by the spacing between operators. We developed several new Tet inducible promoters by combining these two variables and characterized their activity. Currently, we are implementing these promoter variants into our new triple transfection plasmids with the goal of comparing their influence on rAAV production to plasmids containing the industry standard Tet promoter. Future work will consider long term viral component accumulation via degron tag additions. We anticipate this work to directly contribute to the future development of a stable cell line for rAAV production.

Learning immune cell preferences for epitopes containing non-standard amino acids

Jessica Rubira Gamba
Advisor: Dr. Aditya Kunjapur
Committee Members: Dr. Kevin Solomon and Dr. Wilfred Chen

Among the over 1400 known human pathogens, bacterial pathogens are a cause for concern due to a lack of effective vaccines and increasing rates of antibiotic resistance. The challenge in the development of effective vaccines against various classes of bacterial pathogens lies in the fact that many well-conserved antigens across serotypes of a disease are weakly immunogenic. *Para*-nitro-phenylalanine (pN-Phe), a non-standard amino acid, has been shown to function as an immunogenic amino acid and to terminate immune self-tolerance in mouse models when incorporated in autologous proteins. However, the immunogenic effect is dependent on both the site of incorporation and the genetic background of the mouse, specifically its Major Histocompatibility Class II (MHC-II) allele. This study aims to broaden immune recognition toward weakly immunogenic targets by characterizing where pN-Phe should be placed within a protein to enhance immunogenicity for therapeutic design. We plan to accomplish this by using a hybrid microbial-yeast display screening platform to learn the preferences of MHC-II for nitrated epitopes.

For the construction of the screening platform, epitopes with known MHC-II binding affinities were cloned and presented on the surface of E. coli, which were engineered to constitutively express GFP to enable the collection of bound yeast-bacteria through fluorescence-activated cell sorting (FACS). The epitope displays were evaluated using fluorophore-labeled immunostaining and flow cytometry after protein expression. Yeast cell lines displaying the allele HLA-DR401 were generated in collaboration with Prof. Lawrence Stern from the University of South Florida and underwent display verification through the same method. Full display of the epitopes was assessed by Western blot after staining with anti-target antibodies. Further platform characterization will include the quantification of the display expression level in both E. coli and yeast cells. In summary, this work provides insights into the immunogenicity of natural post-translational modifications, and how nsAA incorporation could make target antigens more recognizable by the immune system. Future directions look to optimize the screening conditions after co-incubation of yeast and bacterial cells and identify strong binders after pN-Phe incorporation in the epitopes. Ultimately, we hope the knowledge and tools developed from this project will result in vaccines with increased efficacy, opening the door to protecting against new classes of target antigens.

A high throughput, versatile approach for probing macrophage responses to microenvironment cues in 3D bioprinted synthetic extracellular matrices.

Jodi Graf
Advisor: Catherine Fromen and April Kloxin
Committee Members: Millicent Sullivan, Alexandra Bayles

Macrophages are key innate immune cells that serve as the first line of defense against foreign pathogens and particulates through phagocytosis and proinflammatory, anti-inflammatory, or anti-microbial signaling. While two-dimensional (2D) culture on tissue-culture plastic remains the standard of practice, the field is shifting toward compliant three-dimensional (3D) culture models for testing hypotheses about cell-microenvironment interactions and providing well-defined, tunable, and robust systems that better mimic aspects of the native extracellular matrix (ECM). However, the low throughput of many manually-prepared 3D culture models presents challenges for translation of assays and their broad and accessible use. Bioprinting has the potential to be a consistent and high-throughput method for generating synthetic ECMs in a multi-well plate format for studying cellular interactions and treatment strategies within more physiologically relevant microenvironments. For achieving this, we have established pertinent workflows and demonstrate the relevance of this approach for a model human monocyte cell line (THP-1) that can be differentiated into macrophages, with insights into macrophage responses to different stimuli (e.g., stimulation with biochemical and biophysical cues, pathogens, applied treatments). Our data further support the utility of this innovative methodology and technology for culturing a variety of macrophage types, including primary bone-marrow derived macrophages.

Hydrogels were created with the RASTRUM™ bioprinter, using polyethylene glycol (PEG)- and peptide-based bioinks that react using thiol-maleimide Michael addition chemistry. Inks incorporated integrin-binding peptides RGD, GFOGER, and YIGSR, inspired by the ECM proteins of native human tissues. Human THP-1 macrophages were encapsulated within bioprinted hydrogel-based synthetic ECMs and differentiated into macrophages. Macrophage viability, morphology, phenotype, and inflammatory response to stimuli were assessed within synthetic ECMs with stiffnesses relevant to healthy (storage modulus (G')~0.7 and 1.1 kPa) and fibrotic (G'~3.0 and 4.8 kPa) lung tissues. Hydrogels were shown to support macrophage viability, and flow cytometry and ELISA revealed appropriate macrophage polarization and cytokine secretion in response to microenvironmental stimuli. We then applied our model 3D cultures to study immune response to invasion of a bacterial pathogen implicated in hospital born lung infections and mortality. This work represents a platform for modeling and understanding immune response in physiologically relevant tissue microenvironments, with opportunities for well-defined, yet more complex co-cultures. Future directions aim to further establish healthy and diseased tissue models to understand how immune cells respond to pathogens, particulates, treatments, and other stimuli relevant to chronic lung diseases.

Optimizing the biosynthesis of immunogenic nitrated antigens in *E. coli*

Saloni Gupta
Advisor: Dr. Aditya Kunjapur
Committee Members: Dr. Wilfred Chen, Dr. Mark Blenner

Bacterial vaccines could potentially serve as a solution to the rising antibiotic resistance crisis. Unfortunately, vaccines are only available for 35 of 1400 known pathogenic bacteria. One of the challenges in the development is the poor immunogenicity of some of the conserved bacterial antigens. We envision a platform that could amplify the immunogenicity of these potential candidate antigens to create strong vaccine targets and be manufactured within the patient autonomously by a live bacterial vector. Previous studies have shown that substituting a single amino acid residue with a nitroaromatic non-standard amino acid (nsAA) - *para*-nitro-L-phenylalanine (pNPhe) - in self-proteins leads to the termination of immune tolerance and production of cross-reactive antibodies. To achieve an autonomous synthesis of nitrated proteins, a proof-of-concept metabolic pathway has been established to produce pNPhe and site-specifically incorporate it into model proteins. However, low protein titer and mis-incorporation of amino acids limit the advancement of this platform toward non-model proteins such as antigens.

Our objective is to leverage the tools of metabolic engineering and synthetic biology to optimize the current system by dividing it into three parts – 1) central carbon metabolism and a heterologous operon to produce the amine precursor, 2) an amine-oxidizing *N*-oxygenase, and 3) an orthogonal amino-acyl synthetase and tRNA pair to incorporate biosynthesized pNPhe site-specifically into proteins. Prioritizing the bottleneck amine-oxidation step, we redesigned the pathway such that the *N*-oxygenase is on a separate expression vector, creating a two-plasmid system, and observed better production of our target pNPhe. We hypothesize that the improved titer is due to a higher gene copy number for the *N*-oxygenase, improving its overall expression. Furthermore, we found that these diiron-containing *N*-oxygenases benefit from overexpression of native *E. coli* flavodoxin reductase (*fpr*), increasing the NADPH pool for diiron core recycling and the pNPhe concentration by 2-fold. We are simultaneously developing a selection platform for the rational engineering of the *N*-oxygenase by coupling the enzyme activity to cell growth to improve the amine-oxidation step in our pathway further. Future work comprises modular optimization by tuning the promoter and ribosome binding site strength and engineering the orthogonal amino-acyl tRNA synthetase to be more specific and selective towards pNPhe, reducing misincorporation. In summary, the current system can benefit from an optimal expression system, efficient *N*-oxygenase, and improved orthogonal amino-acyl tRNA synthetase to boost the production of nitrated antigens.

Elucidation of yellow mealworm gut microbiomes polyethylene biodegradation pathways

Alex Hansen
Advisor: Mark Blenner and Kevin Solomon
Committee Members: Aditya Kunjapur, Eleftherios Papoutsakis

Plastic waste is a significant global crisis, as only 14% of the 380 million tons produced annually is recycled, leading to significant accumulation of plastics in landfills and the environment. Current plastic recycling methods typically require high temperatures, expensive catalysts, or hazardous chemicals, which reduces the economic viability and sustainability of these recycling processes. Biological degradation and upcycling of plastics promise a solution to these problems by enabling a future of turning waste plastics into high-value chemicals. We study the yellow mealworm to discover what allows the yellow mealworm to degrade polyethylene (PE) on a time scale of days, not years, like most biological systems. To discover what pathways, exist in the yellow mealworm for PE biodegradation, we combine a variety of bioinformatics analyses centered around a combination of genomics, transcriptomics, proteomics, lipidomics, and metabolomics. Yellow mealworm guts have shown the capability to decrease PE molecular weight by three orders of magnitude outside of the mealworm system, according to our work. Further, PE diets for yellow mealworms select for microbes that contain a niche promiscuous enzyme family that we have expressed heterologously and shown that at least one member can do first-step chemical changes to PE as proposed in our pathway. Analysis of yellow mealworm gut transcripts has also highlighted potential further steps of the pathway by showing the upregulation of many oxidizing enzymes that are potentially capable of next-step oxidation and potential chain cleavage of PE. This pathway promises PE biodegradation, which enables metabolic engineering and biological upcycling of PE.

Measuring Current Distributions and its Relationship to Efficiency for Hydroxide Exchange Membrane Carbon Capture Devices

Justin Harrington
Advisor: Yushan Yan
Committee Members: Dionisios Vlachos, Raul Lobo, Dongxia Liu, Abraham Lenhoff

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Climate change is one of the most pressing social issues in the modern day. CO_2 emissions are responsible for most of the greenhouse gases which cause temperature rise. The Paris Agreement set a goal of limiting temperature rise to 2.0 °C with a preferred target of 1.5 °C. The implementation of negative emissions technologies will be required to reach these goals[1]. Direct air capture (DAC) is a negative emissions technology that removes CO_2 from ambient air. As such it is a promising tool to negate emissions from hard to decarbonize sectors such as industry, transportation, and agriculture. The Yan group has previously developed a DAC technology that uses hydroxide exchange membrane fuel cell principles to continuously separate CO_2 at ambient temperatures, only requiring hydrogen and air as inlets[2]. The production of current within the cell is used as the driving force for the capture of CO_2.

To save on capital costs, the ionomer membrane was purposefully shorted; however, this removed the ability to control current within the cell using classic electrochemical methods[3]. Instead, hydrogen is fed in limiting flow rates to specify system current. While this allows for the specification of current, carbon capture efficiency decreases for the same device conditions. It is hypothesized that non-uniform current distributions cause this decrease in system efficiency. In a non-uniform system, current is produced in one location that is incapable of capturing CO_2, but if it were produced in a different location, it would be capable of capturing CO_2. The purpose of this talk is to present a unique method for measuring current distributions in a shorted electrochemical cell and investigate the relationship between current distribution uniformity and carbon capture efficiency for a variety of system configurations, namely: kinetic and mass transport control over current distributions.

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The Impact of Progressive Buildup of Trapped Gas Bubbles on the Durability of Oxygen Evolution Reaction Electrodes During Alkaline Water Electrolysis

Jeff Hoffmann
Advisor: Yushan Yan
Committee Members: Dongxia Liu, Raul Lobo, Dion Vlachos, and Norman Wagner

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Hydroxide exchange membrane electrolyzers (HEMELs) stand to be an effective means of achieving low-cost green hydrogen. However, HEMEL durability remains a substantial barrier to realizing this goal. The United States Department of Energy has set a durability target of a voltage increase of 2 μV/hr[1]. We have previously demonstrated constant current degradation rates of 1810 and 560 μV/hr with applied current densities of 500 and 200 mA/cm^2, respectively, thus illustrating the substantial gap between our current achievable HEMEL durability and our target durability[2].

Gaseous products are formed at both electrodes in a HEMEL; by manipulating the wettability of the electrodes, recent studies have shown that the failure of gas bubbles to detach from the electrode surface leads to overpotentials associated with the loss of accessible reaction sites and exacerbated mass transport to those sites that remain accessible[3,4]. While these studies have demonstrated the impact of gas bubbles on acute water electrolyzer performance, we have found little work to investigate the impact of trapped gas bubbles from a durability perspective.

Here we find that electrode wettability can change throughout the lifetime of a device. We investigate the durability of an Fe_xNi_yOOH – nF anode catalyst supported on nickel felt in a three-electrode setup with 1M KOH. We find that at a moderately low current density of 50 mA/cm^2 the performance of this catalyst degrades by tens of mV over several hundred hours and that this performance loss is recoverable with the removal of oxygen bubbles adhered to the anode surface at open circuit. Moreover, when we resume the passage of current, the surface layer of oxygen (and associated performance loss) evolves more rapidly – suggesting that the wettability of the anode has decreased throughout the experiment. This is further supported by contact angle measurements of pre- and post-mortem anodes. Finally, we investigate whether the introduction of hydrophilic ion-conducting polymers can mitigate the emergent hydrophobicity of the anode catalyst layer.

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Oxidative Dehydrogenation of Ethane over Boron MFI Zeolites

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Committee Members:

Ethane steam cracking is the primary method of producing ethylene and is energy-intensive. The oxidative dehydrogenation (ODH) of ethane is a promising alternative to steam cracking. Unlike steam cracking, ODH is exothermic and is not equilibrium limited, meaning that ODH reactors use less energy than steam cracking, and ODH reactions can achieve higher conversions of ethane. ODH produces less coke, leading to less catalyst deactivation. However, high ethane conversion typically comes at the expense of high ethylene selectivity. Recently, Zhou et al[1] showed boron MFI has high selectivity towards propylene for propane ODH with relatively high conversions, but the kinetics of boron catalysis for ODH are not fully understood.

Our goal is to understand the kinetics of ethane ODH over borosilicate zeolites. Understanding the kinetics of the mechanism is key to designing a catalyst that promotes high ethane selectivity and high ethane conversion. We have performed simultaneous ethane and propane ODH, showing that the addition of propane to the feed of ethane ODH increases ethane conversion, thus supporting the idea of a radical intermediate shared by the two reaction mechanisms. Additionally, we show that the boron MFI becomes more active over time similar to the findings in Tian et al[2] due to water formation. Lastly, we discuss the impact of the zeolite framework on ethane ODH.

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Similarity-based machine learning for small datasets; Application in predicting bio-lubricant properties

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Committee Members: Prof. Antony N. Beris and Prof. Marianthi Ierapetritou

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Machine learning (ML) has been successfully applied to learn patterns in experimentally generated chemical data to predict molecular properties. However, experimental measurements can be expensive and, as a result, experimental data for several properties is scarce. Several ML methods face challenges when trained with limited data. Here, we introduce a similarity-based ML methodology to efficiently train ML models on small datasets. We group molecules with similar structures, represented by molecular fingerprints, and use these groups to train separate ML models. We apply the methodology to predict kinematic viscosity of bio-lubricant base oil molecules at 40 °C (KV40). Our method shows noticeable improvement in model performance compared to transfer learning (TL) and standard Random Forest (RF) approach. Our methodology provides a robust framework for scenarios with limited data and can be readily generalized to a diverse range of molecular datasets.

PROPANE DEHYDROGENATION IN AN ELECTRIFIED WASHCOATED REACTOR: COMPUTATIONAL INSIGHTS INTO THE DESIGN AND EFFICIENCY

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Electrified reactors can decarbonize highly endothermic reactions by utilizing renewable electricity. Here we model a propane dehydrogenation reactor with a resistively heated nichrome V wall with an adjacent Pt-Zn/silicalite-1 catalyst layer. The transient pseudo-2D energy and species conservation equations are solved simultaneously using finite differences. Design principles are discussed for the washcoat thickness and reactor inner radius based on pressure drop, mass transport limitations, and washcoat isothermicity. The relative magnitudes of conductive and convective thermal resistances determine the radial temperature uniformity inside the solid layers. The axial temperature varies strongly with the Peclet number. Gas preheating allows a much more uniform axial temperature profile. The achieved temperature uniformity enables a pseudo-homogeneous treatment and a facile way to estimate the steady-state reactor temperature based on applied power and the time required to reach steady-state. The extent of catalyst utilization and energy efficiency correlate with the applied electrical power and inlet gas flow rates. The propane conversion, on the other hand, is maximized at high residence times and high power. The reactor reaches steady-state temperature from ambient conditions in less than 10 min, indicating a fast temporal response. The high surface area to volume ratio due to the small reactor diameter is the main challenge in reaching high energy efficiency. With 15 cm thick thermal insulation, 90% sensible heat recovery, and a maximum catalyst temperature of 700 °C, the reactor can reach 50% energy efficiency.

Synthetic acetyl-CoA-responsive biological toolbox for dynamic control of metabolism

Derron Ma
Advisor: Wilfred Chen and Mark A. Blenner
Committee Members: Aditya M. Kunjapur and Kevin V. Solomon

Sustainable manufacturing through metabolic engineering enabled the conversion of low-value feedstocks into value-added chemicals by highly accurate biological processes. Optimizing cellular resources for microbial cell growth and product synthesis remains a major challenge. Dynamic regulation offers a promising method to fine-tune metabolic flux between cell growth and product synthesis. Rapid and reversible metabolic state sensing and enzyme activity modulation can provide autonomous regulation in a heterogeneous fermentation microenvironment. However, regulating desired metabolic pathways with precision remains challenging due to the long developmental time for metabolite-specific biological parts catered to definitive pathways.

Here, we present a one-for-all strategy by developing a synthetic biological toolbox revolving around the key central metabolite Acetyl-CoA, a precursor to diverse, high-commodity chemicals such as fatty acids and terpenoids. We have successfully developed Acetyl-CoA Transient Sensor (ACTS) by repurposing the native PanD-PanZ protein pair with the splitFAST fluorescence reporter to generate real-time fluorescence responses based on acetyl-CoA changes. We achieved a second-scale 12-fold fluorescence increase at saturated Acetyl-CoA concentration from in vitro assays. Perturbating *E. coli* native metabolism shows at least a 2-fold fluorescence increase on a minute scale. In current works, we are investigating the reversible nature of ACTS, particularly the OFF rate of our system. Furthermore, we are expanding the functionality of PanD-PanZ into Acetyl-CoA Transcriptional Regulators (ACTR) by coupling with CRISPR activation/interference systems. We envision that our work will result in dynamic regulation via a universal node applicable to multiple organisms and a wide range of metabolic pathways.

Influence of Pendant-Group Chemistry on the Lithium-ion Mobility of Poly(oligo-oxyethylene methyl ether methacrylate)/Poly(ethylene oxide) Polymer Blend Electrolytes

Venice Magunga
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Committee Members: Prof. LaShanda T. Korley and Prof. Norman J. Wagner

Lithium-ion batteries (LIBs) have been widely used as energy storage systems due to their high energy and power densities, long cycle life, and low self-discharge properties; however, use of LIBs has resulted in catastrophic accidents such as fires and explosions because of the incorporation of highly flammable liquid electrolytes.[1] Solid polymer electrolytes (SPEs), which employ a polymer that can efficiently conduct lithium ions without the need for organic solvent,[1] present a less flammable alternative to liquid electrolytes. Polyethylene oxide (PEO)-based SPEs have been heavily studied due to their effective solvation of lithium ions (Li^+). Unfortunately, PEO-based SPEs suffer from low room temperature ionic conductivity, because of the crystalline nature of PEO, and exhibit low Li^+ mobility [i.e., lithium transference number (t_{Li+})~0.2], thereby limiting their practical applications. A low t_{Li+}, which indicates that the majority of the total ionic conductivity is not associated with the transfer of Li^+ but rather its counter anion,[2] leads to ion concentration polarization within the battery cell. This concentration gradient leads to high internal resistances that ultimately limit charge density, charge and discharge capacity, and cell lifetime.[3] Poly (*oligo*-oxyethylene methyl ether methacrylate) (POEM) exhibits similar lithium solvation in comparison to PEO with reduced crystallinity, resulting in higher room temperature ionic conductivity; yet, it still possesses poor Li^+ conductivity. Moon Park and colleagues demonstrated that incorporating diol functional end groups on PEO (doped with lithium bis(trifluoromethanesulfonyl)imide (LiTFSI) salt) resulted in a two-fold increase in t_{Li+}.[4] This enhancement was attributed to hydrogen bonding interactions between the hydroxyl groups of the diols and $TFSI^-$ resulting in slow diffusion of the anions, thereby increasing t_{Li+}.[4]

The aim of this work is to investigate the impact of chemical composition and blending ratio of pendant-functionalized PEO on the ionic conductivity and t_{Li+} of a LiTFSI-doped PEO/POEM blend polymer electrolyte with the ultimate goal to improve t_{Li+} while maintaining high solid-state ionic conductivity. Ionic conductivity and t_{Li+} will be explored using a combination of direct current polarization test and AC impedance spectroscopy. Furthermore, pulse-field gradient nuclear magnetic resonance spectroscopy will be leveraged to give insights into the relative diffusivity of Li^+ and $TFSI^-$. The understanding gained from this work will be beneficial in the pursuit of high-performance LIBs with solid polymers as electrolytes.

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Multiscale Modeling Studies of the Effects of Catalyst Surface Morphology on Polymer Adsorption for Polyethylene Hydrogenolysis

Rajas Milind Mehendale
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Committee Members: Prof. Arthi Jayaraman, Prof. Raul F. Lobo

As the global production of plastics skyrockets, it is critical to mitigate the generated plastic waste for a sustainable and circular economy. Heterogeneous catalytic reactions like hydrogenolysis are a powerful approach in the chemical upcycling of plastic waste to useful products like fuels and lubricants.

The interactions of the polymer (plastic) melt with the catalyst surface significantly influence the product distributions. It is difficult to experimentally study polymer adsorption on catalytic surfaces at reaction conditions, especially since catalysts surfaces are not smooth. Since the rate of the hydrogenolysis of linear alkanes is surface morphology dependent, this lack of understanding of the adsorption behavior limits our ability to optimize the yields of the desired products.

Here, we present a multiscale modeling approach combining Density-Functional Theory (DFT) calculations and Replica Exchange Molecular Dynamics (REMD) simulations to study the effect of catalyst surface morphology on the adsorption of polyethylene melts at platinum surfaces of varying termination.

We developed an atomistic forcefield to describe platinum–polymer interactions for different platinum terminations. The forcefield was trained on DFT data and deployed for the simulation of surrogate chains of polymer melts over platinum. We show that the conformations of the polyethylene chains on the surface are determined by surface morphology, and that they adsorb in shorter segments and at longer intervals on high-index surfaces than on flat surfaces. The polymer chains do not display any preference for absorption of terminal or medial segments. However, statistical analysis shows that the adsorbed terminal segments of the polymer are shorter than the medial ones.

This surface dependent distribution of polymer conformations has implications for the hydrogenolysis of polymers on metal surfaces and thereby for the product distribution; the shorter the adsorbed segments the lighter the gaseous products. The change in surface conformations on the more active high-index surfaces suggests that the adsorption behavior will dictate the size of the hydrogenolysis products.

We thus argue that catalyst surface morphology is a macroscopic parameter that could be leveraged to tune the product distribution. The presented methodology can be applied to other polymer-catalyst systems to develop a better understanding of the conformations of polymers on catalyst surfaces and thus product distributions.

Keywords: Plastic waste upcycling, catalyst morphology, Replica-Exchange Molecular Dynamics, Density Functional Theory.

Modification of GDE composition and assembly for stable CO_2 electrolysis

Izak Minnie
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Committee Members: Prof. Raul Lobo, Prof Yushan Yan

The continued emission and build-up of CO_2 in the atmosphere has led to significant global warming effects. This has prompted a global effort towards carbon capture, utilization, and storage (CCUS) that comprises many different types of technologies. One of these technologies is CO_2 electroreduction (CO2R), where CO_2 is electrochemically transformed into useful products such as CO, formic acid, and ethylene. The realization of this technology has the potential to enable a circular CO_2 economy.

In order to become a commercially viable process, CO2R must reach industrially relevant levels in four key parameters: 1) Current density (production rate), 2) Overpotential (energy efficiency), 3) Faradaic efficiency (selectivity), and 4) Durability. The first three of these metrics have mostly been achieved via zero-gap membrane electrode assembly (MEA) cells, gas diffusion electrodes (GDEs), and catalyst design. The field is now turning its focus towards the final parameter: durability.

CO2R systems are subject to a range of physicochemical mechanisms[1] that cause their performance to degrade over time. Most commonly, electrode flooding or loss of active catalyst area decreases the amount of suitable triple phase boundary locations where the CO_2 reactions take place. Mitigation of these degradation mechanisms requires optimization of the interplay between all the different cell components including the cell hardware, GDEs, catalyst, catalyst support, binding ionomers, and membranes. In this study we use a systems level approach to investigate the use of 1) porous 3D-ordered carbon as a catalyst support 2) crosslinked membrane-ionomer interfaces and 3) Cu_xPd catalysts to extend system durability in a zero-gap MEA cell.

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Catalytic Deconstruction of Ethylene Vinyl Acetate Copolymer and Polyethylene Mixtures via Hydroconversion

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We explore hydrogenolysis over ruthenium supported on zirconia (Ru/ZrO_2) and hydrocracking over platinum (Pt) supported on zeolites as an effective end-of-life strategy for ethylene vinyl acetate (EVA)—a widely used performance heat-sealant in hard-to-recycle multilayer packaging. For Ru/ZrO_2 hydrogenolysis, EVA reacts slower than low-density polyethylene (LDPE), and the catalyst deactivates due to carbonaceous deposits originating from polyenes generated *in-situ* during EVA thermal degradation. High H_2 pressures and temperatures can overcome catalyst deactivation; however, CH_4 yields are excessive due to cascade hydrogenolysis stemming from strong C=C/metal interactions. Polyene hydrogenation allows chains anchored by C=C to desorb from Ru, shifting product selectivity from CH_4 to higher-value liquids. Hydrogenolysis of mixed EVA and linear low-density polyethylene (LLDPE), mirroring typical frozen food packaging formulations, results in comparable catalyst activity and CH_4 yield as the pure EVA resin. For Pt/zeolite hydrocracking, pure EVA and EVA:LLDPE mixtures are deconstructed to propane or light naphtha with minimal CH_4 production. Among catalysts tested, Pt/HY gives the highest liquid productivity ($g_{C5+\ products}/g_{cat}\cdot hr$). These findings showcase the recalcitrant nature of EVA and its associated mixtures for Ru/ZrO_2 hydrogenolysis, highlighting that hydrocracking catalysts may be superior for complex packaging waste.

Increasing the Throughput of Bioprinting Using Machine Learning Optimized Advective Assembly

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Committee Members: Arthi Jayaraman, Millicent Sullivan

Replicating natural tissue hierarchy is becoming increasingly important in many fields, especially in medicine, biotechnology, and synthetic meat production. One emerging technology for assembling three-dimensional, cell-laden constructs is extrusion-based multi-material additive manufacturing (MMAM), which incorporates multiple species within a single 3D printed object. Typically, 3D-printed "tissues" are constructed in a layer-by-layer manner, where fine nozzles are swapped each time a new bioink is introduced. Although this approach has been successfully used to fabricate emblematic assemblies (e.g., vasculatures, muscle fibers), individual nozzles must have rather large diameters (>100 μm) to prevent high shear stresses from killing cells prior to deposition, which subsequently constrains the size of biomimetic features. In the Bayles Laboratory, we are using principles of advective assembly (AA) to engineer the next generation of high-resolution 3D printing nozzles. Advective assemblers contain sequences of fluidic junctions that align and multiply material by splitting, rotating, and adding laminar flows. The sequences contour co-flowing materials along streamlines and quickly extrude composite filaments with fine, flow-templated internal architectures. To date, advective assembly sequences have been engineered to assemble simple structures, such as stacks of layers and grids of fibers, as well as designer asymmetric structures, such as interdigitated layers and voxelated patterns. In principle, architectures with complex, biomimetic hierarchy can be achieved by combining splitting, rotating, and adding junctions in unlimited unique sequences. Efficient inverse design tools will help 3D printing users tap into this expansive design space. In this work, we use machine learning (ML), specifically convolutional neural networks (CNNs), to design the entire advective assembly process required for a user-specified structure. Defining the process requires users to specify the junction sequence, the ink type fed into each inlet, and the feed flow rates that tune feature size. To generate a data set that spans the design space, we used an efficient forward prediction tool based on Boolean logic. The formalism casts junctions as AND, OR, and NOT logic gates, connects them in circuits, and computes a 2D truth table, which reflects the resultant architecture. The Boolean-logic tool eliminates the need to perform expensive computational fluid dynamics simulations to generate training data. We calculated an initial training set of tens of thousands of structures from practically realizable processes of four combinations of junctions, four inlets, and modulated flow rates. With this dataset, we were able to train and test a CNN model that classifies structures and processing conditions with >80% accuracy. Notably, this classification specifies not only the combination of junctions but also the feeds require to tune the distribution of features at a resolution of 4 μm. Future work will involve expanding the training set to include more junction sequences. The final anticipated inverse design tool will be of substantial practical utility to MMAM, and, in the scope of this project, will allow users to achieve replication of (m)any complex biomimetic structures. This project benefits from the support of the NRT: Computing and Data Science Training for Materials Innovation, Discovery, and AnalyticS (NRT-MIDAS) program at UD.

Light Olefin Production via Catalytic, Melt, Electrified Pyrolysis of Polyethylene

Jacqueline Ngu
Advisor: Dionisios Vlachos
Committee Members: Prof. Raul Lobo and Prof. Dongxia Liu

Plastic waste has become a huge burden on our environment, with less than 10% being recycled and the remaining incinerated or thrown into landfills(1). Most recycled plastic is mechanically recycled. This process cannot handle mixed plastics. Catalytic pyrolysis is an appealing method for plastic deconstruction as it can be feedstock agnostic. However, converting plastic into light olefins (C_2-C_4) is challenging, sometimes requiring a two-step process(2).

Here, we introduce an electrified pyrolysis slurry reactor operate at low temperatures to produce a narrow distribution of light olefins at high yield. Increasing the operating temperature increases the selectivity of light olefins (C_2-C_4). We demonstrate an optimum flow rate to tune product distributions. We also explore reactor optimization via process intensification to further control light olefin yields. Increasing the temperature increases selectivity toward light olefins. At 370 °C and 100 mL/min of N_2, the LDPE conversion was 58% with 22% and ~100% selectivity to C_2-C_4 and C_2-C_{12} olefins, respectively. At 400 °C, the overall yield to olefins of $\leq C_{12}$ is a remarkable $\leq$ 90+%. This slate of products is very valuable because light olefins are used for various products like lubricant base oils, cosmetics, etc. The effect of N_2 flowrate on light olefin selectivity at 400 °C at low conversion is shown in Figure 1b. Decreasing the flowrate increases the residence times of the heavier products in the reaction zone, leading to more cracking and a higher selectivity toward lighter products. At low flowrates, conversions are low due to ineffective mixing, causing inefficient heat and mass transfer. Hence, an optimal flowrate exists for light olefin production and high conversion. To tune the product distribution, we introduce an intensified hybrid setup to recirculate heavy products and further improve the yield to light olefins. To tune the product distribution, we introduce an intensified hybrid setup to recirculate heavy products and further. A condenser enables heavier products to condense back into the reaction zone to react further. We have found that once the products are light enough, such that they cannot be further condensed, they exit the reaction zone. We will also present results on the effect of acid catalyst on light olefins production and the effects of various plastic additives on selectivities for different solid acid catalysts.

Toward a whole pathway integration tool in *Yarrowia lipolytica*

Phil O'Dell
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Committee Members: Dr. Kevin Solomon, Dr. Aditya Kunjapur, Dr. Wilfred Chen

Plant natural products (PNPs) represent a large and vital class of medicinal compounds regularly used to treat a variety of ailments. PNPs can be extremely complex molecules, with great regio- and stereo-selectivity required for their synthesis, prohibiting conventional chemical synthesis from meeting the demand. Additionally, the plants that natively produce these compounds generally grow relatively slowly and produce a very small amount of the product of interest, making extraction from plant matter very expensive and inefficient. Heterologous biosynthesis of PNPs is the most promising avenue for a reliable and cost-effective method of production. However, the metabolic pathways that facilitate production of PNPs are generally as long as the products are complex. One class of medicinally relevant PNPs, monoterpene indole alkaloids (MIAs), includes the anti-malarial quinine, the anti-addiction ibogaine and the anti-cancer vincristine and vinblastine as well as many others. All MIAs are derived from a single compound, strictosidine, itself fairly complex. The heterologous biosynthesis of strictosidine requires the stable overexpression of 20 genes, with more genes necessary for production of any product downstream of strictosidine. The rapid generation of strain libraries capable of producing MIAs requires the integration of these pathways in as few integration events as possible. Serine integrases have been used to integrate extremely large DNA fragments (>50 kb) in mammalian cells and have demonstrated activity in a variety of other organisms such as *S. cerevisiae* and *E. coli*, but their use has not been demonstrated in *Y. lipolytica*. The serine integrase under investigation here (ΦC31), recognizes several different attachment sites, allowing for the design of a marker recycling system that is not predicated on other enzymes or plasmid curing, further enhancing the throughput. Additionally, a three fluorescent protein system for identification and quantification of recombination outcome has been developed and is being optimized.

Dynamic Tryptophan Production in CHO Cells

Topher Pirner
Advisor: Mark Blenner
Committee Members: Kelvin Lee and Kevin Solomon

Chinese hamster ovary (CHO) cells are used for producing numerous biotherapeutics because of their ability to assemble and fold complex proteins with human-like post-translational modifications. Another aspect of CHO cells that makes them desirable is the ability to grow them in suspension cultures in chemically defined (CD), serum-free media. CHO cells cannot produce nine of the 20 standard amino acids, making amino acid supplementation necessary in CD media. Of these amino acids, tryptophan (Trp) is of particular interest because it is often prone to oxidation in media. Upon oxidation of Trp, the oxidized products have been shown to significantly reduce CHO cell growth, titers, and product quality. To address the issue, previous researchers have tried to limit the amount of Trp added to media, resulting in reduced cell growth and productivity. To address the double-edged sword nature of Trp in CD media, we plan to engineer CHO cells to produce their own Trp to eliminate the need for it in media.

Previous studies have suggested that Trp metabolism in CHO cells often results in reduced performance from forming unwanted catabolic byproducts. Therefore, Trp production in CHO cells must be tightly regulated to prevent a reduction in cell growth and titer. In this talk, I will discuss a rational approach to engineering the CHO cell to produce its own Trp. First, we elucidated the impact of excess Trp in the NIST CHO cell line to gain insight into how to regulate Trp production in CHO cells rationally. Preliminary studies indicate that excess Trp reduces monoclonal antibody titers and decreases cell growth four-fold. Then, the Trp and its associated metabolic and oxidative products from the cell pellet and media were analyzed on a LC-MS which informed us why excess Trp can negatively impact CHO cell performance. Additionally, I will be discussing how we plan to engineer the CHO cell for stable, dynamic production of Trp by integrating the Shikimate pathway from *Corynebacterium glutamicum*.

The Impact of Relative Humidity on Electrode Ionomer Deposition for Hydroxide Exchange Membrane Electrolyzers

Abigayle Polsky
Advisor: Yushan Yan
Committee Members: Antony Beris, Dongxia Liu, Raul Lobo, Dionisios Vlachos

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Hydrogen is a multifaceted tool that is prospected to be a key alternative energy source in the fight against climate change as it is energy dense, can be used directly as a fuel source in hydrogen fuel cells, and its storage & energy capacity are temperature independent. Only about 5% of H_2 is made by green hydrogen, or electrolysis, which produces up to 10x less $CO_{2,eq}$ than its counterpart, grey hydrogen[1]. The Hydroxide Exchange Membrane Electrolyzer (HEMEL) is a contemporary green hydrogen device that uses a highly efficient solid electrolyte and is compatible with abundant metals, such as nickel, to be utilized for catalysis. HEMELs function with a coupled set of reactions—the hydrogen evolution reaction (HER) and oxygen evolution reaction (OER)—that produce hydrogen and oxygen gas through electrolysis (electrochemical water separation). At the cathode, H_2O splits at Pt active sites to form H_2 and OH^-. The H_2 bubbles out of the system and is collected. The OH^- is shuttled across an anion-selective membrane and meets FeNiOOH-F active sites on the anode side to satisfy the full-cell reaction.

Industrial-scale manufacturing is the ultimate goal of HEMELs. Since the technology is maturing, production research must be closely studied to prepare for the transition from lab-scale research to large-scale processing. This study will focus on the ionomer deposition of the anode and how the relative humidity (RH) of that environment impacts HEMEL performance. The catalyst-covered anode is dip-coated in an ionomer solution and let to dry at room temperature and varying RHs, between 9% and 92%. Nickel felt and nickel foam anode porous transport layers are tested as well as three ionomer types. Through full-cell polarization curves, electrochemical impedance spectroscopy, and scanning electron microscopy, RH is found to be a substantial factor in HEMEL performance. Up to a 30% increase in HEMEL performance could be attributed to lowering the RH of the environment that the anode is prepared in. Through analysis, it is suggested that the higher RH environments do not allow the ionomer to fully dry and form a strong, interlocked resin film. Thus, when the ionomer comes into contact with water flowing through the HEMEL, it swells substantially and obstructs the substrate's pores. In turn, this hinders gas, water, and hydroxide transport—leading to high mass transport overpotentials.

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Coiled-Coil Peptide-Polymer Star Conjugates with Cleavable and Clickable Arms

Will Rears
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Committee Members: Dr. Arthi Jayaraman and Dr. Alexandra Bayles

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Coiled-coil peptides are an attractive class of molecular building blocks for applications such as drug delivery and nanomaterial design due to their chemical tunability and well-defined sequence-structure relationship. Previously, our group demonstrated the ability to graft synthetic polymers from computationally designed coiled-coil peptides, "bundlemers," creating bundlemer-polymer star conjugates; however, the chemical complexity of these conjugates hindered complete conjugate characterization. Furthermore, the original synthetic design only allowed for one type of polymer to be grafted from the bundlemer, limiting the tunability and diversity of conjugates. I seek to address these limitations by incorporating a selectively cleavable linker between the peptide and polymer to deconvolute polymer characterization; and designing a small-molecule, clickable polymerization initiator to enable conjugates with two different polymer species. Solid phase peptide synthesis was used to create the initiator-containing peptides by employing orthogonal chemistry and on-resin modifications. Amino-alcohol linkers were incorporated between the peptide backbone and polymerization initiator sites to enable oxidative cleavage post-polymerization. A maleimide-functionalized small-molecule initiator was synthesized to attach a second initiation site to the peptide *via* the furan-maleimide Diels-Alder click reaction. Photoinduced atom transfer radical polymerization (ATRP) was used to graft polymers from the initiator-functionalized peptides. The linker was completely cleaved after 5 minutes upon addition of sodium periodate, allowing for fast and facile cleavage of the polymer from the peptide. This approach allows us to completely characterize the polymer independent of the peptide and enables synthesis of more complex bundlemer-polymer conjugates.

Slowed segmental dynamics around nanoparticles in attractive polymer nanocomposites

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Polymer nanocomposites (PNC) enhance the mechanical, optical, and electrical properties over either pure component, but the mechanism for this enhancement is not well understood. Despite this lacking mechanistic understanding of PNCs, they see use in applications such as reinforcement of rubber in automobile tires. Mechanical reinforcement in well-dispersed PNCs is thought to originate from a slowing of dynamics resulting from the interactions between the polymer and nanoparticle, which is amplified by the high surface area of nanoscale objects. The dynamics of interest in PNCs cover many orders of magnitude in length and time scales; from the shortest to longest of these is the polymer segmental dynamics, polymer whole chain sub-diffusive dynamics, polymer diffusion, and nanoparticle diffusion [1, 2].

In our present work we investigate the polymer segmental dynamics with Quasi-Elastic Neutron Scattering (QENS) at the angstroms and tens of picoseconds length and time scales in terms of the Rouse model. Previous work has found that that the average Rouse dynamics slow with nanoparticle addition and that there is an interfacial layer with a gradient in glass transition temperature around the nanoparticles [1, 3]. Through a careful analysis of the QENS data, we are able to i) separate the measured dynamics into a bulk and interfacial component, ii) confirm both can be described as Rouse-like, iii) quantify the slowing of the dynamics in terms of the Rouse rate to be of about one order of magnitude, and iv) estimate the extent of the interphase region to be ≈ 1 nm. We also investigated the effect of nanoparticle shape, allowing us to selectively vary nanoparticle surface area to polymer volume ratio, on the scaling on Rouse dynamics. Successfully linking the scaling of the polymer dynamics, including the Rouse dynamics, with the macroscale mechanical properties will improve the design of PNCs.

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On the mechanisms of ethane dehydrogenation on isolated Fe/SiO_2 catalyst

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Sustainability imperatives have spurred the development of atomically dispersed metal atom catalysts on a variety of supports. In addition to high atom utilization, these catalysts have demonstrated remarkable activity and selectivity for a variety of reactions of economic importance. One such pivotal reaction is the dehydrogenation of ethane to ethylene, which has garnered renewed interest owing to the shale gas revolution. The commercial chromium- and platinum-based catalysts raise concerns over toxicity and cost, underscoring the need for research into employing earth-abundant metal catalysts. Transition metals grafted on amorphous SiO_2 supports, such as Fe/am-SiO_2, emerge as promising candidates for their activity and selectivity for non-oxidative ethane dehydrogenation. The nature of the Fe/am-SiO_2 active site and the C-H activation mechanism are debated due to the heterogeneity in the structure and composition of the amorphous support. Understanding these materials at the atomic level is key to designing novel catalysts tailored for C-H activation chemistry.

In this work, we employ electronic structure calculations to elucidate the nature of the active site and identify dominant reaction pathways for ethane dehydrogenation on Fe/am-SiO_2. We consider atomically dispersed Fe centers in different oxidation states (2+ and 3+) and coordination environments consisting of a combination of silanol (SiOH) and silanolate (SiO^-) ligands. We report that high-spin (quintet) Fe d^6 centers paired with basic silanolate ligands activate the C-H bond heterolytically and that the ensuing β-hydride elimination from the metal-alkyl intermediate by the Fe d^6 center requires spin-crossing into the triplet spin state. We further settle an outstanding question by providing evidence that σ-metathesis, namely ligand exchange between ethane and the metal-hydride (product of the β-H elimination), is not a viable catalytic pathway as it is energetically demanding. On Fe(+3), we propose a novel redox mechanism for ethane dehydrogenation, in which the Fe d^5 active site reduces to d^6 by accepting β-electrons from the silanolate ligands, leaving the α-electrons delocalized over the oxygens of the silanolate ligands. Upon heterolytic cleavage of the first C-H bond, Fe reoxidizes to d^5 by donating the electrons back to the oxygens of the silanolates and quenching their spins as a result. This reoxidation then sets stage for the β-hydride elimination over the low spin state (quartet) of d^5 during the second C-H activation. We further show that our proposed redox mechanism is energetically competitive with the heterolytic C-H activation mechanism previously identified for other transition metals.

Understanding morphology of polysulfamide and mixture at various isomer ratios: Insights from atomistic informed coarse grained molecular dynamics simulation

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Polysulfamides are a new class of polymers that have the potential to serve as sustainable alternatives for commodity plastic like polyurea due to similar chemical structures differing only in carbonyl groups (polyurea) and sulfamide groups (polysulfamide). Michaudel and coworkers have synthesized oligomers of polysulfamides that exhibit high thermal stability, adjustable glass transition temperatures with changing polymer backbone structure, and are degradable in green conditions.[1, 2] Due to these desirable properties, there is significant motivation to create design-structure-property relationships. To achieve design-structure relationships, Jayaraman and coworkers have used phenomenological coarse-grained (CG) models for polysulfamide and molecular dynamics (MD) simulations to link polysulfamide chain design to various features of the assembled structure.[3] From CG MD simulation, they calculate the positional and orientational order within the assembled chains as a function of the polysuflamide chain design as a proxy to the crystallinity from experimental characterization.[3] They also calculate strength of hydrogen bonding (H-Bonding) that is needed for chains to assemble. Currently we are working to extend this past phenomenological CG model of polysulfamides to be atomistically informed. We use atomistic MD simulations of polysulfamide chain to quantify relative spatial arrangement of hydrogen donor and acceptor species within sulfamide group and distributions of bonded angle and dihedral potentials between various groups of atoms; this information is then programmed into the 'newer' polysulfamide CG model. Then, using the same simulation protocol we compare the output of polysulfamide assembly MD simulations for the 'older' CG model of Wu et al. and atomistically informed 'newer' CG models.[3] We observe that while the interaction strength of hydrogen-bonding that is needed for chains to assemble is higher for the 'newer' model than the 'older' model, overall trends in the global structural arrangements with varying design is qualitatively the same between the two models. However, there is different arrangements of the H-Bonding at the local level to form the assembled structure.

Using these CG models, we are now simulating blends of polysulfamides with components differing in placement of the sulfamide group along the backbone, backbone chemistry adjacent to the sulfamide group, and chain lengths. Through systematic variation in the design parameters of the component polysulfamides and observing the effect of those parameters on blend morphology (mixing and demixing, orientational order and positional order) we are establishing design rules for controlling semi-crystallinity within polysulfamide materials.

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Macrophage Internalization of PEGDA NPs Drive Secretion of EVs and Promote APC and T cell Survival

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Nanoparticles (NPs) offer a highly tunable platform that can have their formulations adjusted to change the charge, shape, size, and material to fit various applications. Through these modifications, NPs can be designed to target specific cell populations, such as macrophages. Macrophages are innate immune cells that internalize, or phagocytose, foreign pathogens and debris to then recruit or activate other immune cells. Researchers have shown that NP material can drive changes in macrophage behavior by activating the cell type. While the state of macrophage activation is inconsistent between NP material, recent work in the Fromen Lab has shown that NP phagocytosis promotes cell survival independently of NP material. Through poly(ethylene glycol) diacrylate (PEGDA) NPs, we showed *ex vivo* macrophage lifespan in the absence of cellular activation.

In this work, we show that the phagocytosis of PEGDA NPs leads to an increase in small extracellular vesicle (EV) secretion in a dose-dependent manner. Small EVs are cell-derived NPs that are critical for intercellular communication. We demonstrate that these EVs promote survival in BMMs with no cellular activation. Moreover, these EVs are shown to influence other cell types by promoting the survival of bone marrow-derived dendritic cells and CD3+ splenocytes. Additionally, we demonstrate that these EV secretion phenomena are consistent across immortalized macrophage cell lines. Similar to EVs derived from BMMS, these EVs promote BMM survival with minimal cellular activation. Furthermore, CD63+ EVs originating from PEGDA dosed BMMs are shown to contain PEGDA NP fragments. These EVs are shown to increase MHCII expression in BMMS. These findings reveal a new response to particle internalization and point to the potential engineering for this EV secretion response as a method for *in situ* EV mediated transfer of NP therapeutics.

Joule Heatable Molten Salt Membrane for Ammonia Synthesis Membrane Reactor

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Ammonia is one of the most widely produced chemicals in the chemical industry, used to manufacture fertilizers and many other chemicals. It is also a promising energy vector for hydrogen transportation and storage[1]. However, the Haber-Bosch process, which is the traditional method of producing ammonia, is one of the most energy-expensive chemical processes and is responsible for nearly 1% of global CO_2 emissions annually[2]. This is because the reaction is exothermic and reversible, so the process involves a reactor that operates at high pressure and temperature with a low conversion rate, and a cryogenic separation is needed to remove ammonia from the unreacted hydrogen and nitrogen[3]. Using a catalytic membrane reactor to integrate the reaction and separation steps into a single unit would remove the ammonia as it is made, increasing the equilibrium conversion and eliminating the need for energy-expensive separations. Immobilized molten salt membranes, such as $LiNO_3$, operate at elevated temperatures and can separate ammonia from hydrogen and nitrogen with high selectivity even at low ammonia concentrations[4], making them promising for use in ammonia synthesis membrane reactors.

Joule heating, also known as resistive or ohmic heating, produces heat from the resistance of applying an electric current to a conductive material. In addition to reducing CO_2 emissions by using renewable electricity to produce heat instead of burning a fuel, joule heating the membrane could also increase the ammonia permeability of the membrane. Joule heating, unlike traditional furnace heating, only provides local heat instead of uniformly heating the entire reactor, creating a sharper temperature profile that provides a stronger driving force for the absorption and desorption transport mechanism of ammonia through the membrane. This project investigates how temperature and ammonia concentration affects the separation performance of the membrane. The membrane performances under furnace heating and joule heating are also compared with the goal of determining the optimal conditions for ammonia separation.

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Designing Conditionally Disassembled Protein Nanoparticles for Directed Cancer Therapy

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Cancer continues to be the second leading cause of death in the United States year after year. One of the most common treatment methods for cancer, chemotherapy, yields largely negative side effects as a result of a lack of specificity, with significant adverse impacts on both healthy and cancer cells. Protein therapeutics offer a promising potential solution given their high specificity of function; however, membrane impermeability and rapid degradation limits the activity of therapeutics by decreasing the amount that reaches the site of activity. Nanoparticles can robustly protect delicate protein therapeutics by preventing rapid clearance and proteolytic degradation, but often release their protein cargoes in a nonspecific fashion. This lowers the therapeutic yield, and in the case of pro-apoptotic therapies, increases non-specific cell death. Currently, nanocarriers typically only use one input to offer site-selectivity; however, we hypothesize that utilizing protein nanoparticles embedded with multi-layered protein logic can increase the selectivity of oncolytic therapeutics given tumor microenvironment cues.

Encapsulin is a class of protein nanoparticles which can be loaded with non-native protein cargoes, and a variant was previously created with the ability to disassemble in response to slightly acidic conditions (pH = 6.0). This variant offers a promising release mechanism; however, with preliminary results I have confirmed that this pH responsive variant is unstable even at a neutral pH. This instability may result in premature disassembly in acidic domains of the tumor stroma, necessitating increased stability of this variant and introducing a secondary selectivity measure. Thus far, I have successfully expressed and purified 3 encapsulin variants including the pH responsive encapsulin, all with a C-terminal SpyTag (ST), demonstrating 100% reactivity with a SpyCatcher (SC) protein fusion without affecting self-assembly. In addition, intact nanoparticles can be purified utilizing the lower critical solution temperature of elastin like polypeptide (ELP) covalently conjugated to less than 10% of the monomers, despite the non-covalent nature of encapsulin self-assembly. This permits pH responsive encapsulin to interface with receptor binding moieties through ST/SC conjugation to the 220 unreacted sites, offering a secondary selectivity measure: receptor mediated endocytosis. I have also loaded over 100 molecules of green fluorescent protein (GFP) into the largest encapsulin and pH responsive encapsulin, which would deliver more cargo per nanoparticle than previously studied protein based platforms while also demonstrating input responsive release. Overall, for the first time, I have combined 3 drug delivery functionalities into one nanoparticle: loading, disassembly, and selective surface chemistry. This new material offers the potential to improve the selective delivery of protein therapeutics using multiple inputs.

Constrained black-box optimization for stochastic simulations with polynomial-chaos-based stochastic Kriging

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Surrogate modeling techniques have gained popularity in different engineering fields when an accurate closed-form formulation is not available or when the computational cost for the problem of interest is prohibitively high. Among various surrogate models and optimization algorithms, the Kriging-based model with the Bayesian optimization framework has become increasingly common. This approach assumes the behavior of a computational model to be a realization of a Gaussian random process and uses some infill criterion to propose new sample points while maintaining the balance between exploration and exploitation.

The Kriging-based models consist of two parts: the mean (also known as the trend) and the variance of the Gaussian process. Based on the choice of the trend, Kriging models can be divided into three categories: simple Kriging, ordinary Kriging, and universal Kriging. Simple Kriging assumes a known constant value for the trend, ordinary Kriging assumes an unknown constant value, and universal Kriging uses some deterministic model for the trend function to capture the global behavior. Polynomial chaos expansion has been as the trend of a universal Kriging, and its performance was found to be at least as good as ordinary Kriging for a deterministic simulator. Stochastic Kriging was proposed to extend the deterministic case to stochastic simulations by explicitly accounting for the uncertainty inherent to the simulator. Recently, polynomial-chaos-expansion-based stochastic Kriging was proposed, and a superior performance compared to the ordinary stochastic Kriging was reported. Despite these promising findings, the use of polynomial-chaos-expansion-based stochastic Kriging in Bayesian optimization remains unexplored. This work proposes a tailored framework that capitalizes on the strength of this novel surrogate model in constrained black-box optimization for stochastic simulations.

In this work, we use the polynomial-chaos-based stochastic Kriging as the surrogate model given its advantages in accommodating the intrinsic uncertainty of the simulation and its capability to capture both the global and local behavior of the simulator. A tailored framework that combines the Bayesian optimization with polynomial-chaos-based stochastic Kriging is applied to search for the near-optimal solution. Different infill criteria are applied for adaptive sampling, and the computational performances are compared with the case in which the ordinary stochastic Kriging is used as the surrogate model. Different benchmark problems are used to evaluate the ability of the proposed method to find a feasible solution, locate the global optimum, and make accurate predictions around the optimum. A case study in the chemical recycling of plastic waste is included to illustrate the application of the proposed method in real-world chemical engineering problems.

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