DEPARTMENT OF CHEMICAL & BIOMOLECULAR ENGINEERING

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2022 SUMMER RESEARCH REVIEW

SECOND YEAR TALKS

JUNE 2, 2022

UNIVERSITY OF DELAWARE

UNIVERSITY OF DELAWARE
ENGINEERING
DEPARTMENT OF CHEMICAL AND BIOMOLECULAR ENGINEERING

2021 – 2022 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

UNIVERSITY OF DELAWARE
ENGINEERING
DEPARTMENT OF CHEMICAL AND BIOMOLECULAR ENGINEERING

2021 – 2022 SUMMER RESEARCH REVIEW

LOCATION: **102** COLBURN
Listing of Talks and Abstracts

9:25 AM	Grissom, Spencer **"Identification of Heritable Biomarkers that Characterize Resistance to Stress and Improved Productivity in CHO Cell Line Development"** Advisor: Mark Blenner Committee Members: Wilfred Chen & Kelvin Lee
9:50 AM	Gitman, Philip **"Integrating Synthetic Methylotrophy into Y. lipolytica for Carbon Efficient Isoprenol Production"** (Project 1) **& "Elucidating Causes of Cellular Heterogeneity in Engineered Y. lipolytica"** (Project 2) Advisor: Mark Blenner Committee Members: Aditya Kunjapur & Eleftherios Papoutsakis
10:15 AM	Dickey, Roman **"Whole Cell Biocatalysis for the Valorization of PET Deconstruction Products"** Advisor: Aditya Kunjapur Committee Members: Wilfred Chen & Catherine Fromen
10:40 PM	**BREAK**
11:00 AM	Forti, Amanda **"Use of Synthetic Auxotrophy for Control of Growth in Bacterial Co-Cultures"** Advisor: Aditya Kunjapur Committee Members: Mark Blenner and Eleftherios Papoutsakis
11:25 AM	Anderson, Shelby **"Biosynthesis and Incorporation of Photolabile Non-standard Amino Acid Analogs"** Advisor: Aditya Kunjapur Committee Members: April Kloxin & Wilfred Chen

11:50 AM	Gopal, Madan **"Reductive Enzyme Cascades to Valorize the Products of PET Depolymerization"** Advisors: Wilfred Chen & Aditya Kunjapur Committee Members: Mark Blenner, Arthi Jayaraman, & Joshua Michener
12:15 PM	Mayhugh, Christopher **"*Bacillus subtilis* Spore Display of Nitrated Antigens for Immunogenic Modulation"** Advisor: Aditya Kunjapur Committee Members: Catherine Fromen & Kelvin Lee
12:40 PM	**LUNCH for Faculty & Presenters (2nd Floor Lobby)**
2:00 PM	Vaidya, Akash **"*Ex Planta* Production of Barley Stripe Mosaic Virus-Like Particles for Flexible Genetic and Protein Engineering"** Advisor: Kevin Solomon Committee Members: Catherine Fromen & Wilfred Chen
2:25 PM	Leibiger, Thomas **"Development of a Small-scale Transient rAAV Production Bioprocess for Proteomic Analysis of Process-related Impurity Retention and Clearance"** Advisor: Kelvin Lee Committee Members: Abraham Lenhoff & Wilfred Chen
2:50 PM	Hill, John **"Elucidating a Mechanistic Understanding of Interspecies Bacterial Fusion through Transposon Insertion Sequencing and Oligonucleotide Fluorescent Probes"** Advisor: Eleftherios Papoutsakis Committee Members: Aditya Kunjapur & Kevin Solomon
3:15 PM	Raudenbush, Katherine **"Modeling the Effect of Gradients on Cell Culture Performance in Large Scale Bioreactors"** Advisors: Marianthi Ierapetritou & Eleftherios Papoutsakis Committee Members: Christopher Roberts & Christopher Kloxin
3:40 PM	**REFRESHMENTS (2nd Floor Lobby)**

Identification of Heritable Biomarkers that Characterize Resistance to Stress and Improved Productivity in CHO Cell Line Development

Spencer Grissom
Advisor: Dr. Mark Blenner
Committee Members: Dr. Wilfred Chen, Dr. Kelvin Lee

Many therapeutic proteins are produced using the Chinese hamster ovary (CHO) cell line due to their natural genetic plasticity, human-like post translational modifications, and superior production of secreted proteins. This genetic plasticity gives way to heterogenous clones that drive cell line development (CLD) where a monoclonal production cell line is identified based off optimized growth, productivity, and product quality. However, this CLD process represents a time and cost barrier to produce these therapeutics and is biased towards clonal populations that perform well in the scaled-down environment that occurs during screening. It fails to identify optimal clones that perform exceptionally well in a larger production environment and associated stress agents. One approach for improving these CLD limitations involves the narrowing the clonal pool based on biomarkers, which are genetic states that confer a favorable phenotype. This research describes a workflow for the identification of heritable biomarkers that characterize resistance to stress and improved productivity to enhance the clonal pool during CLD.

To identify suitable biomarkers, a population-based RNA sequencing technique, referred to as MemorySeq, was first used to identify gene expression states whose fluctuations continue for several divisions and were distinct from a noise control. These expression states are considered heritable if their variation significantly exceeded the transcriptome-wide variation. This was paired with differential gene expression analysis (DGEA) in the presence of stress agents characteristic of production cycle media. The overlap of heritable expression states from MemorySeq and differentially expressed genes from DGEA with functional analysis may suggest genes that would bias the CLD clonal pool to better performance. The MemorySeq workflow identified nearly 200 heritable expression states and six network communities of co-fluctuating genes, characterized by cellular adhesion, response to chemicals and stimulus, and cell differentiation from GO enrichment analysis. High levels of ammonia, lactate, and osmolality were then introduced in fed-batch format to simulate production cycle media. Day 5 cell samples were used for DGEA and 130 of the heritable genes were differentially expressed in at least one of the stress conditions. Six genes associated with either higher protein secretion, negative regulation of apoptosis, or increased glycosylation were selected from this pool as possible biomarkers for screening. In future work, clones with high expression of one or more of these six genes may be selected and expanded for fed-batch culture to verify the heritability and assess its impact on production performance. If these clones exhibit better performance for extended duration, then this method would significantly reduce the CLD timeline and improve the adaptability of the clones when grown at production-scale.

Integrating Synthetic Methylotrophy into *Y. lipolytica* for Carbon Efficient Isoprenol Production (Project 1) **&**
Elucidating Causes of Cellular Heterogeneity in Engineered *Y. lipolytica* (Project 2)

Philip Gitman
Advisor: Dr. Mark Blenner
Committee Members: Dr. Aditya Kunjapur & Dr. Terry Papoutsakis

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Project 1 Abstract: Current microbial cell factory strategies for oleochemical production are inefficient due to the release of CO_2, achieving a maximum carbon efficiency of 62%. The focus of this work will be to use synthetic biology and metabolic engineering tools allowing the model oleaginous yeast species, *Yarrowia lipolytica,* to incorporate renewably generated methanol into an oleochemical biosynthesis pathway. We chose *Y. lipolytica* due to its naturally high flux for acetyl-CoA derived compounds, including oleochemicals and isoprenoids. To reach maximum carbon efficiency, we will integrate genes from the synthetic homoserine (HS) pathway into *Y. lipolytica*. This engineered pathway will achieve higher titers, rates, and yields with minimal addition of non-methanol derived carbon source. The HS pathway enables energy and redox efficient ligation of formaldehyde into acetyl-CoA (He et al., 2020; Kim et al., 2021). However, the low activity of key methanol assimilating pathway enzymes in the HS pathway have resulted in insufficient biomass yields on methanol alone in *E. coli* (He et al., 2020). We will leverage the bioinformatics expertise of the Joint Genome Institute for genome and metagenome mining of additional enzyme candidates based on similar homology, genomic context, and phylogenetic similarities with these known enzymes. New candidate enzymes will be selected based on these similarities with their *E. coli* counterparts, synthesized, codon-adjusted and integrated into *Y. lipolytica* to construct the HS pathway *in vivo*. The most active enzymes will then undergo further characterization, evolution, and transcriptomic analysis. Overall, this work will help overcome the kinetic limitations of the HS pathway by selecting for improved enzyme activity and specificity. Constructing the HS pathway *in vivo* will allow us to study the underlying mechanism of improved methanol assimilation efficiency in the engineered *Y. lipolytica* strain.

Project 2 Abstract: The model oleaginous yeast species, *Yarrowia lipolytica,* is a promising biomanufacturing chassis well suited for production of oleochemicals and terpenoids, including biofuels such as limonene, bisabaline and other valuable chemicals of the carotenoid family. Scaleup of these processes, however, has been marred by phenotypic changes, such as loss of titer (or titer instability). This problem is exemplified by an engineered β-carotene producing strain that was developed as a platform to produce β-ionone (Czajka et al., 2018). This engineered *Y. lipolytica* strain was developed by enhancing flux from acetyl-CoA to terpene precursors by overexpressing several upstream mevalonate pathway enzymes (**push**) and by reducing flux (**block**) towards squalene synthesis. Then, *carB* and *carRP* enzymes were overexpressed via genome integration to **pull** flux from GGPP to β-carotene, achieving ~4 g/L using benchtop bioreactors. The β-carotene producing strain was further engineered by overexpression of a novel carotenoid cleavage dioxygenase, resulting in β-ionone fermentation (~1 g/L). However, when moving those engineered strains to larger bioreactors, cell performance significantly dropped. The goal of this work is to investigate the nature of titer instability in this engineered β-carotene producing *Y. lipolytica* strain as a model to understand more broadly the factors that lead to cellular heterogeneity during cell line development and scaleup.

Whole Cell Biocatalysis for the Valorization of PET Deconstruction Products

Roman M. Dickey
Advisor: Aditya Kunjapur
Committee Members: Professors Chen and Fromen

As the majority of high market-share plastics are obtained from nonrenewable and ecologically damaging petroleum/natural gas feedstocks, sustainable and cost-effective strategies for polymer plastic waste recycling need to be developed. Polyethylene terephthalate (PET) is a common consumer plastic that can be deconstruction via chemical or biological to the monomer diacid unit terephthalic acid (TPA). This work seeks to address contemporary bottlenecks in plastic waste recycling by developing valorization strategies to convert TPA into the diamine product, p-xylylenediamine (pXYL), for use in up-cycled materials. The biocatalytic functionalization of TPA aims to increase the economic viability and advance the circularity of plastic's lifecycle through upgrading plastic derived monomers units to form higher value amines that are synthetically challenging to produce. These amines can be utilized to make novel tunable network polymers and resins for 3D-printing applications.

Our strategy focuses on the use of a whole cell two enzyme cascade in *Escherichia coli* using promiscuous carboxylic acid reductases (CARs), for the conversion of acids to aldehydes, and transaminase (TAs), for conversion of aldehydes to amines. Whole cell biocatalysis offers a promising method for large-scale and low-cost production as it contains functional cofactor regeneration systems, eliminates expensive downstream processing required for enzyme purification and separation, and provides increase solvent and substrate tolerance as compared to *in vitro* and cell-free approaches. Upon expression of CARs from *Mycobacterium avium* and *Segniliparus rotundus* on TPA, we observed the rapid over-reduction to the corresponding alcohols due to endogenous activity on the desired aldehyde products. As such, we constructed an enhanced aldehyde accumulating *E. coli* strain, RARE 2.0, with a total of 11 gene deletions of aldehyde reductases and aldo-keto reductases that limits the reduction of our corresponding aldehyde intermediates within our system. Encouragingly, we observed very rapid conversion of the di-aldehyde intermediate to pXYL with the use of a resting whole cell expressing a ω-transaminase (ωTA) from *Chromobacterium violaceum*. A coupled one-pot whole cell CAR and TA reaction with the enzymes modularly expressed in separate cells enabled production of pXYL from TPA and creates the foundation for cost-effective, high concentration and large-scale pXYL production.

Use of Synthetic Auxotrophy for Control of Growth in Bacterial Co-Cultures

Mandy Forti
Advisor: Dr. Aditya M. Kunjapur
Committee Members: Dr. Mark A. Blenner and Dr. Eleftherios T. Papoutsakis

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Genetically engineered microbes can prove beneficial if deployed into the environment for applications such as crop enhancements and bioremediation or into the human body for applications such as tumor fighting agents and vaccines. Safely releasing modified bacteria into either of these spaces requires strategies to prevent uncontrolled spread of the engineered bacteria to avoid unintended consequences. One promising solution for the release of modified bacteria is to design intrinsic biological containment based on synthetic auxotrophy, which means that the microbe is engineered to depend on a synthetic nutrient. However, the use of synthetic auxotrophy creates an additional challenge: How do we allow our designer microbe to survive in target environments that we cannot easily access and do not want to pollute by adding excessive amounts of a synthetic nutrient?

Here, I explore a new concept of having one organism produce a synthetic nutrient that another relies on, which can be referred to as a "synthetic obligate" relationship between the two species. In this model system, the synthetic nutrient is the non-standard amino acid O-methyl-L-tyrosine (OMeTyr). OMeTyr has previously been incorporated within proteins in *Escherichia coli* and *Bacillus subtilis* using specialized machinery. It is also a rare, natural product, reported to be produced by two microbial species, though it has not been the target of engineered biosynthesis to date. In this talk, I will demonstrate that we can engineer *E. coli* to biosynthesize OMeTyr at levels that are, in principle, conducive to incorporation within proteins. Expression of two exogenous enzymes increases *E. coli's* native production of tyrosine, which can then be methylated to produce OMeTyr. In the C321.ΔA strain of *E. coli*, titers of 0.49 mM ± 0.1 mM have been achieved after 24 hours of growth in LB media. As work is continued on this project, we plan to increase the production rate of OMeTyr to achieve titers high enough for incorporation in a shorter period to allow the synthetic auxotroph a chance to thrive during the co-culture. I will also describe our plans to advance towards co-cultures that consist of a sender and receiver, first among microbial species and with an eventual goal of plant-microbe communication.

Biosynthesis and incorporation of photolabile non-standard amino acid analogs

Shelby Anderson
Advisor: Aditya Kunjapur
Committee Members: April Kloxin, Wilfred Chen

Biomolecular engineers are increasingly turning to peptides and proteins as part of delivery vehicles for encapsulated cargo. However, a biocompatible stimulus for the release of encapsulated cargo is necessary in the targeted environment. Light is valuable as a chemical-free control mechanism and therefore an attractive stimulus for controlled release. The non-standard amino acid (nsAA) *ortho*-nitro-phenylalanine (oN-Phe) has the property of photo-cleavage of peptide bonds after UV irradiation and can be incorporated within target protein sequences using genetically encoded machinery. Light responsive nsAAs as single-residue mutations offer the added benefit of minimal interference in protein folding and function as compared to other bulky light-responsive domains. However, there are limitations to incorporating nsAAs into proteins due to nsAA commercial availability, chemical synthesis costs, and necessary external provision of the nsAA to the cell. We seek to mitigate these constraints using L-threonine transaldolases (TTAs), which catalyze the conversion of aromatic aldehydes to nsAAs with a hydroxy group at the beta carbon (β-OH nsAAs).

Here, I describe my effort to begin tackling several of these problems through the biosynthesis and eventual incorporation of nitrobenzyl nsAAs with a beta-hydroxy moiety via a characterized and putative TTA. We have demonstrated activity of the TTAs on a range of aromatic aldehydes *in vitro* and observed candidate peaks via HPLC for β-OH nsAA production *in vivo*. As we investigate *in vivo* production, we also seek to understand compound stability in metabolically active cells. Ultimately, we seek to biosynthesize and incorporate the light-responsive nsAA analogs produced by the TTA reaction into proteins. We have screened for aminoacyl-tRNA synthetases capable of incorporating the model photolabile nsAA, oN-Phe, and seek to use this basis to identify variant(s) capable of accepting the beta-hydroxy moiety.

Reductive Enzyme Cascades to Valorize the Products of PET Depolymerization

Madan R. Gopal
Advisor: Dr. Wilfred Chen and Dr. Aditya M. Kunjapur
Committee Members: Dr. Mark Blenner, Dr. Arthi Jayaraman and Dr. Josh Michener

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To incentivize collection of plastic wastes, new chemical transformations must be developed. Polyethylene terephthalate (PET) is a commonly used plastic whose deconstruction through chemical or enzymatic means has received much attention. Here, we explore using terephthalic acid (TPA), a product of PET depolymerization, as a starting material for green synthesis of a value-added diamine, *para*-xylylenediamine (*p*XYL). Green synthesis methods for production of diamines with aromatic moieties remains understudied, and introduction of the aromatic moiety into diamines may prove useful in applications such as formation of novel nonisocyanate polyurethanes and polyamides. In this work, we show the biocatalytic conversion of TPA to its corresponding diamine, *p*XYL, by constructing a 5-enzyme cascade in a cell-free environment.

Using a retrobiosynthetic approach, we show that a promiscuous ω-transaminase from *Chromobacterium violaceum* could efficiently produce *p*XYL from terephthalaldehyde (TPAL). Motivated by this novel result, we hypothesized that we could create TPAL *in situ* from TPA by creating an enzyme cascade initiated by a carboxylic acid reductase (CAR), which belongs to a class of biocatalysts that performs the selective 2-electron reduction of acids to aldehydes. To find a CAR that could reduce TPA to TPAL, we used a bioprospecting approach and generated a protein sequence similarity network. From this network, we screened 17 CAR orthologs to determine their specificity towards carboxylate-containing products of PET deconstruction. We found several CAR orthologs across different domains of life that had the activity we desired on TPA. While our highest performing CAR from *Segniliparus rotundus* was not able to completely convert 10mM of TPA to TPAL at our chosen endpoint of 24-hours, coupling CAR to the ω-transaminase and a biocatalytic NADPH and ATP regeneration cascade drove the conversion of 10mM TPA and resulted in a 70% yield of the target diamine, *p*XYL.

By combining the synergies of PET depolymerization with biocatalytic functionalization, we show, to our knowledge, the first report of enzymatic production of *p*XYL. This work lays the foundation for eventual valorization of waste PET to higher-value materials that can be made from *p*XYL, augmenting the sparse list of closed-loop strategies for diverting PET waste away from environmental accumulation.

Bacillus subtilis Spore Display of Nitrated Antigens for Immunogenic Modulation

Christopher C. Mayhugh
Advisor: Aditya Kunjapur
Committee Members: Drs. Fromen and Lee

In times of nutrient scarcity, the gram-positive bacterium *Bacillus subtilis* undergoes sporulation to form an endospore characterized by metabolic dormancy and a multi-layer proteinaceous spore coat. Over the last two decades, researchers have utilized *B. subtilis* endospores to display proteins of interest for agricultural, therapeutic, and biocatalytic applications. Endospores are highly durable, stable bioparticles that are capable of enduring extreme environmental conditions and enhance the stability of proteins fused to the endospore. Additionally, *Bacillus subtilis* endospores possess natural adjuvant-like properties and exhibit oral bioavailability, making their use advantageous for delivery of immunogenic antigens and therapeutic proteins. However, previous spore-based therapeutics were limited by low efficacy and required doses that were not feasible for commercial use. Through utilizing synthetic biological techniques, we believe it is possible to enhance the efficacy of spore-displayed therapeutics via nitrated nonstandard amino acids (nsAA) incorporation.

We are investigating the development of a novel vaccine modality by exploring the following: (1) whether the nsAA *para*-nitro-L-phenylalanine can increase the immune response generated by immunization with bacterial antigens, and (2) the subsequent display of nitrated bacterial antigen on the surface of *Bacillus subtilis* spores. Nonstandard amino acid incorporation has enabled the engineering of proteins to contain diverse functionalities. Previous research has shown that *para*-nitro-L-phenylalanine can be site-specifically incorporated into self-antigens to increase immunogenicity. We hypothesized that *para*-nitro-L-phenylalanine incorporation could be used to enhance the immunogenicity of foreign bacterial antigens and tested this hypothesis in a mouse model in collaboration with the Fromen Lab. Our preliminary results show saturated, high-titer systemic IgG responses from both nitrated bacterial antigen plus adjuvant and wild-type antigen plus adjuvant groups, and we will conduct additional ELISAs at higher dilution factors to determine the statistical significance of the antibody titer differences between experimental groups. We further hypothesize that *para*-nitro-L-phenylalanine incorporation in *B. subtilis* spore coat-fused bacterial antigens offers a valuable strategy to enhance immunogenicity upon immunization and provide proof-of-concept methodology for designing improved spore-based therapeutics. To verify sporulation and spore display, we have genetically fused fluorescent reporters to *B. subtilis* spore crust proteins, CotY and CotZ, via both rigid and flexible linker sequences, and extracted spore coat proteins for subsequent SDS-PAGE analysis. We will next explore nsAA incorporation in *B. subtilis* spores using spore crust-reporter fusions and amber codon suppression. Long term goals include multi-site *para*-nitro-L-phenylalanine incorporation via synthetase engineering, and subsequent animal studies with increased sample size, different adjuvants, and varied route of immunization.

Ex Planta Production of Barley Stripe Mosaic Virus-Like Particles for Flexible Genetic and Protein Engineering

Akash J. Vaidya
Advisor: Prof. Kevin Solomon
Committee Members: Prof. Catherine Fromen, Prof. Wilfred Chen

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Virus-like particles (VLPs) are non-infectious protein nanoparticles that lack viral genomes and can be engineered for diverse applications including electronics, sensing, vaccination, and drug delivery. Rod-shaped plant viruses such as Barley Stripe Mosaic Virus (BSMV) represent a particularly attractive nanomaterial platform due to their precise hierarchical self-assembly, tunable size and aspect ratio, single-stranded messenger RNA genomes, and high-density surface sites for functionalization. VLP analogs can be constructed by substituting native RNA content with non-genomic RNA templates containing a short origin-of-assembly (OAS) sequence. However, traditional *in planta* preparation methods restrict protein engineering flexibility since they require retention of host infectivity. We overcame this constraint by preparing BSMV VLPs in bacteria for the first time. The recombinantly expressed viral coat proteins spontaneously self-assembled into disk-shaped multimers *in vivo*. Upon transcription of OAS-containing RNA templates, the disk intermediates further combined to form the expected nanorod structures. Capitalizing on the flexibility of our new production platform, we generated mutant VLPs with single-residue substitutions to stabilize protein-protein interactions. The mutant proteins self-assembled into full-length VLPs even without the presence of OAS-containing RNA templates. We further modified the surface of these mutants to modulate particle physicochemical properties and functionality. Despite the demonstrated flexibility of bacterial VLP production, some limitations remain. Firstly, *in vivo* preparation limits control over protein:RNA stoichiometry and assembly kinetics, which may influence assembly completion and particle dispersity. Furthermore, bacterial transcription of RNA templates does not support important features such as protective polyadenylation and modified base substitutions to modulate RNA stability, immunogenicity, and translation efficiency. To address these limitations, we extended our BSMV VLP production pipeline to *in vitro* particle assembly with enhanced control over composition and kinetics. These advanced, *ex planta* VLP preparation methods will enable flexible VLP engineering at the RNA and protein levels.

Development of a small-scale transient rAAV production bioprocess for proteomic analysis of process-related impurity retention and clearance

Thomas Leibiger
Advisor: Dr. Kelvin H. Lee
Committee Members: Dr. Abraham Lenhoff and Dr. Wilfred Chen

Gene therapy is a class of medicine that aims to cure disease through introduction or modification of genetic material within a patient's cells. Recombinant adeno-associated virus (rAAV) is the most common vector in gene therapy clinical development pipelines and is preferred over other viral vectors due to its ability to confer long-lasting transgene expression in specifically targeted tissue types with low risk of adverse events. However, significant challenges persist in the development of scalable and well-characterized rAAV biomanufacturing platforms. To meet clinical and commercial demand, upstream processes have begun incorporating scalable suspension culture systems with increased virus titers, requiring accompanying changes to downstream purification strategies. A significant challenge in bioprocess purification is the removal of process-related impurities including host cell proteins (HCPs), which can have immunogenic effects in patients and impact product stability.

To inform development of future platform rAAV biomanufacturing processes, we are establishing optimal conditions for a small-scale transient rAAV production bioprocess followed by downstream purification with affinity chromatography and density gradient separation and developing analytical tools for measurement of viral genome titer and vector packaging efficiency. Downstream unit operations will then be evaluated for their ability to remove product-associated and co-purifying HCPs across different rAAV serotypes using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Leveraging this proteomic approach will contribute to more streamlined process development, resulting in the manufacture of safer, more potent rAAV gene therapies.

Elucidating a Mechanistic Understanding of Interspecies Bacterial Fusion through Transposon Insertion Sequencing and Oligonucleotide Fluorescent Probes

John Hill
Advisor: Dr. Eleftherios Papoutsakis
Committee Members: Dr. Kunjapur and Dr. Solomon

Atmospheric levels of CO_2, a green-house gas, have risen steeply over the last century as a by-product of the world's increased demand for energy and commodities. Chemical engineering practices, in addressing these growing demands, must now consider their environmental impact more seriously and develop renewable carbon sources aiming to achieve a cyclical economy. Acetogens are autotrophic organisms using the primordial Wood-Ljungdahl Pathway (WLP; responsible for fixing ~20% of earth's CO_2) to fix CO_2 in the presence of a suitable electron donor such as H_2. Acetogen-based biotechnologies have grown in importance over the last few years, but their slow growth and inability to consume common biomass sugars or produce high titers of metabolites longer than 2-C limits their industrial impact. To overcome these difficulties, researchers have explored synthetic, syntrophic co-cultures of the fast growing *C. acetobutylicum* (*Cac*, which uses all biomass sugars) with the acetogen *Clostridium ljungdahlii* (*Clj*). *Clj* uses no glucose. In glucose fermentations, *Clj* depends on the CO_2 and H_2 produced by *Cac* during glucose utilization, thus creating a stable syntrophy. *Cac* and *Clj* form bacterial fusions when co-cultured under laboratory conditions. Direct cytoplasmic coupling circumvents traditional limitations to metabolite mass transfer resulting in more efficient substrate utilization and decreased CO_2 emissions. This behavior has never been observed before in Gram-positive bacteria and provides a promising new platform for the production of solvents from biomass feedstocks.

A mechanistic understanding will be needed to leverage this interaction industrially. During my talk, I intend to cover my progress on two simultaneously ongoing approaches: Transposon Insertion Sequencing (*Tn-Seq*) and oligonucleotide fluorescent probes. *Tn-seq* is a forward genetic approach. I will describe the theory of *Tn-Seq* and report the creation a novel transposon insertion mutant library in *Clj*. Transposon insertion libraries are rare in acetogens, and mine is the first to be constructed in *Clj*. This library will be used to uncover the genetic bases of fusion. I will also describe the application of oligonucleotide fluorescent probes to clostridial research. Ultimately, libraries of nucleic acid probes will be used to track the exchange of chromosomal DNA in *Clj*-*Cac* fusion. Furthermore, the evolution of the fusion cell's genomic makeup will be tracked generationally. I will report the design of species specific rRNA-FISH probes in characterizing co-culture and fusion dynamics.

Modeling the effect of gradients on cell culture performance in large scale bioreactors

Katherine Raudenbush
Advisor: Professor Marianthi Ierapetritou and Professor Terry Papoutsakis
Committee Members: Professor Christopher Roberts and Professor Christopher Kloxin

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Scale-up of bioreactors is necessary for industrialization of monoclonal antibody (mAb) production but can lead to the formation of spatial gradients in important culture parameters, such as dissolved gases, metabolites, and pH. Different concentrations or values of these culture parameters can have adverse effects on cell culture dynamics, resulting in lower cell density and adverse effects on productivity and product quality (N-linked glycosylation) [1,2]. Cells experience fluctuations of environmental conditions as they move throughout large scale bioreactors, exposing them to not only transient suboptimal conditions, but also oscillating conditions over time, which has shown negative effects of its own [3-6]. Modeling the interplay between fluid dynamics and bio-phase kinetics in large scale mixing tanks can provide insights into expected effects of the oscillating and suboptimal conditions observed in large scale bioreactors, minimizing expensive scale-up experiments [7]. This objective is achieved through 1) small scale experiments of Chinese hamster ovary (CHO) cell growth and mAb production under constant and oscillating conditions, 2) kinetic modeling of CHO metabolism and mAb glycosylation under these conditions based on experimental data, and 3) computational fluid dynamics (CFD) integrating hydrodynamics, multiphase mixing, mass transport and the developed kinetic reactions to predict mAb production and quality under heterogeneous conditions in large scale bioreactors. The work described in this talk sets the foundation for the combined CFD-kinetic model.

References

1. Brunner, M., et al., Investigation of the interactions of critical scale-up parameters (pH, pO2 and pCO2) on CHO batch performance and critical quality attributes. Bioprocess and Biosystems Engineering, 2017. 40(2): p. 251-263.
2. Ivarsson, M., et al., Evaluating the impact of cell culture process parameters on monoclonal antibody N-glycosylation. Journal of Biotechnology, 2014. 188: p. 88-96.
3. Anane, E., et al., Scale-down cultivation in mammalian cell bioreactors—The effect of bioreactor mixing time on the response of CHO cells to dissolved oxygen gradients. Biochemical Engineering Journal, 2021. 166.
4. Brunner, M., et al., The impact of pH inhomogeneities on CHO cell physiology and fed-batch process performance - two-compartment scale-down modelling and intracellular pH excursion. Biotechnology Journal, 2017. 12(7): p. 1600633.
5. Gao, Y., et al., Combined metabolomics and proteomics reveals hypoxia as a cause of lower productivity on scale-up to a 5000-liter CHO bioprocess. Biotechnology Journal, 2016. 11(9): p. 1190-1200.
6. Serrato, J.A., et al., Heterogeneous conditions in dissolved oxygen affect N-glycosylation but not productivity of a monoclonal antibody in hybridoma cultures. Biotechnology and Bioengineering, 2004. 88(2): p. 176-188.
7. Haringa, C. An analysis of organism lifelines in an industrial bioreactor using Lattice-Boltzmann CFD. Engineering in Life Sciences, 2022. p.1-16.

LOCATION: **104** COLBURN
Listing of Talks and Abstracts

9:00 AM	Andini, Erha **"Production of Neo Acids from Biomass Waste"** Advisor: Dionisios Vlachos Committee Members: Raul Lobo & Marat Orazov
9:25 AM	Kararinopoulou, Nefeli **"Plasma-Assisted Nitrogen Fixation – Nitric Acid Production"** Advisor: Dionisios Vlachos Committee Members: Raul Lobo & Feng Jiao
9:50 AM	Chen, Eric **"Data-Driven Investigation of Density Functional Theory-Calculated Adsorption Energies"** Advisor: Dionisios Vlachos Committee Members: Raul Lobo & Antony Beris
10:15 AM	Worrad, Alfred **"Investigating the Structure of Molybdenum Oxide on Alumina via Computational Raman Spectroscopy"** Advisor: Dionisios Vlachos Committee Members: Antony Beris & Raul Lobo
10:40 AM	**BREAK**
11:00 AM	Goculdas, Tejas **"Cross-Ketonization of Biomass-derived Furans and Fatty Acids for Renewable Surfactants"** Advisor: Dionisios Vlachos Committee Members: Raul Lobo & Marat Orazov
11:25 AM	Crane-Moscowitz, Kenneth **"Solution Behavior and Self-Assembly of Cylindrical Coiled-Coil Domains into Discrete Nanoparticles, Soluble Aggregates, and Rigid Rods"** Advisors: Eric Furst, Darrin Pochan, & Christopher Kloxin Committee Members: Arthi Jayaraman & Millicent Sullivan

11:50 AM	Conradt, Jason **"Dissipative Self-assembly of Paramagnetic Colloidal Suspensions in Microgravity"** Advisor: Eric Furst Committee Members: LaShanda Korley, Abraham Lenhoff, Christopher Roberts, & Norman Wagner
12:15 PM	Posey, Tessa **"Coiled-Coil Peptides as Molecular Building Blocks"** Advisor: Christopher Kloxin Committee Members: Millicent Sullivan, April Kloxin, & Darrin Pochan
12:40 PM	**LUNCH for Faculty & Presenters (2nd floor Lobby)**
1:30 PM	Gee, Michelle **"Modeling and Analysis of the Intrinsic Cardiac Nervous System in Closed-Loop Cardiovascular Control"** Advisors: Abraham Lenhoff, Babatunde Ogunnaike, & Rajanikanth Vadigepalli Committee Member: Aditya Kunjapur
1:55 PM	Cherniack, Luke **"Reactor Design of a CO2 Electrolyzer for Pure Formic Acid Production"** Advisor: Feng Jiao Committee Members: Dionisios Vlachos & Yushan Yan
2:20 PM	Crandall, Bradie **"Techno-economics and Scale-up of Electrochemical CO2 Reduction to Carboxylic Acids"** Advisor: Feng Jiao Committee Advisors: Yushan Yan & Dionisios Vlachos
3:40 PM	**REFRESHMENTS (2nd Floor Lobby)**

Production of Neo Acids from Biomass Waste

Erha Andini
Advisor: Dionisios Vlachos
Committee Members: Raul Lobo and Marat Orazov

The global rapid population growth has increased the demand for food and other essential resources, which has resulted in intensified agriculture and industrial manufacturing activities.[1] The large quantity of waste generated has become a growing issue, especially agricultural waste. Most of the waste is burnt or left to decompose in the field due to inefficient utilization and management practices, resulting in adverse environmental impacts. One possible way to increase the value of biomass waste is through the catalytic production of valuable commodity chemicals from biomass waste-derived platform molecules, such as neo acids. Neo acids are commercially available and have diverse industrial applications due to their excellent properties. However, neo acids are currently synthesized from fossil fuels, and the process involves harsh reaction conditions, including high temperatures and pressures, corrosive catalysts, and complicated product purification.[2,3]

A sustainable alternative is producing neo acids from abundant renewable resources such as biomass waste. In this work, biomass waste is utilized to synthesize bio-based neo acids. The proposed synthesis pathway consists of C-C coupling of furans with bio-oil derivatives followed by hydrodeoxygenation. In the first step, we show successful C-C coupling over Brønsted acidic catalysts. Catalyst screening and multi-parameter optimization using machine learning were implemented to obtain the optimum yield and elucidate the correlation between variables and outcomes. In the second step, we employ a Pd/C and metal triflate catalyst. This work provides an alternative to neo acid synthesis from biomass waste and enables multiple opportunities for producing renewable commodity chemicals.

References

1. Tripathi, N., Hills, C. D., Singh, R. S. & Atkinson, C. J. Biomass waste utilisation in low-carbon products: harnessing a major potential resource. *npj Clim. Atmos. Sci.* **2**, (2019).
2. Mueller, W. & Hartmann, M. Production of Tertiary Carboxylic Acids. U.S. Patent 5,342,979, Aug 30, 1994.
3. Fefer, M. Neo acids: Synthetic highly branched organic acids. *J. Am. Oil Chem. Soc.* **55**, 3–6 (1978).

Plasma-Assisted Nitrogen Fixation – Nitric Acid Production

Nefeli Kamarinopoulou
Advisor: Dionisios G. Vlachos
Committee Members: Raul Lobo and Feng Jiao

Nitric acid, primarily used in the form of NH_4NO_3, is an invaluable compound for agricultural activity. Increasing fertilizer demand in developing regions along with the considerable carbon footprint associated with the H-B process, highlight the need for a greener, more flexible HNO_3 production process. Cold atmospheric plasma NOx fixation from nitrogen and air feedstocks has recently emerged as a potential candidate. A few operating parameters, such as applied voltage and plasma treatment time, and reactor configurations have been studied with regards to their effectiveness on HNO_3 production. However, a systematic study of geometric, electrical and process parameters has not been conducted and the correlation between these parameters and specific chemical pathways is yet to be elucidated. A suitable comparison metric for plasma NOx fixation has not been established, convoluting the comparison of reported processes and process optimization. In this work, we propose the following two metrics of performance evaluation: average nitric acid production rate (umol/min) and energy efficiency, E (mol NO_3^-/MJ). The effect of plasma treatment time, applied voltage, reactor diameter, air gap and water height on NO_3^- production rate and energy efficiency are investigated. It is found that increasing the plasma treatment time or reactor diameter has a positive effect on both E and average NO_3^- production rate. Variation of the applied voltage or the air gap does not seem to impact energy efficiency but positively affects the rate, while increasing the water height decreases both. Determining the effect of the remaining operating parameters, such as gas composition, gas and liquid flow rate, gas and liquid temperature and pressure, is essential in understanding plasma assisted NOx fixation and will be the subject of our future work.

Data-Driven Investigation of Density Functional Theory-Calculated Adsorption Energies

Eric Chen
Advisor: Dr. Dionisios Vlachos
Committee Members: Dr. Raul Lobo and Dr. Antony Beris

Over 90% of chemicals are produced using heterogeneous catalysis[1]. Such chemical reactions take place on the surface of the catalyst, so probing and optimizing the reaction conditions to improve the reaction throughput is crucial, which can be achieved using Density-Functional Theory (DFT). However, DFT calculations exhibit systematic error that can propagate significantly throughout a microkinetic model. In this work, we propose a data-driven model to map lower-levels of exchange-correlation functional (xc) theory to higher-level xc to predict error in adsorption energy (E_{ads}) and determine the most important descriptors to incorporate into a model predicting E_{ads} error relative to experiment.

We begin with mapping chemisorption binding energies between the Local Density Approximation (LDA) xc[2] and the Perdew-Burke-Ernzerhof (PBE) xc[3]. By exploring a chemical space of small hydrocarbons and oxygenates, we built a dataset of 98 adsorption systems consisting of unique adsorbate-site combinations, we build a feature set consisting of geometric and electronic descriptors from the LDA calculations. These descriptors are then used to train a model that predicts the difference in adsorption energy between the LDA and PBE xc (ΔE_{ads}). Through the XGBoost algorithm[4], information gain is calculated for each descriptor to determine their importance in predicting ΔE_{ads}. This model uses five descriptors, the most importance being gas-phase adsorbate valency, gas-phase adsorbate molecular orbital energies, and active-site charge change, to predict ΔE_{ads} with a validation mean absolute error of 0.09 eV.

Sources:

(1) Rothenberg, G. *Catalysis: Concepts and Green Applications*; WILEY-VCH: Weinheim, Bergstr, 2008.
(2) Perdew, J. P.; Wang, Y. Accurate and Simple Analytic Representation of the Electron-Gas Correlation Energy. *Phys. Rev. B* **1992**, *45* (23), 13244–13249. https://doi.org/10.1103/PhysRevB.45.13244.
(3) Perdew, J. P.; Burke, K.; Ernzerhof, M. Generalized Gradient Approximation Made Simple. *Phys. Rev. Lett.* **1996**, *77* (18), 3865–3868. https://doi.org/10.1103/PhysRevLett.77.3865.
(4) Chen, T.; Guestrin, C. XGBoost: A Scalable Tree Boosting System. **2016**. https://doi.org/10.1145/2939672.2939785.

Investigating the Structure of Molybdenum Oxide on Alumina via Computational Raman Spectroscopy

Alfred Worrad
Advisor: Dionisios G. Vlachos
Committee Members: Antony N. Beris, Raul F. Lobo

Transition metal oxides are a key class of heterogeneous catalysts that have numerous applications in industrial processes due to their ability to functionally modify material surfaces and provide excellent catalytic performance. These oxide overlayers are often quite dissimilar to the supporting bulk structures and play an important role in acid-base, selective- and total-oxidation processes. The ability to quantify key structural parameters of these overlayers allows for expert knowledge-based optimization of active sites. An often-utilized set of tools for understanding the nature of these sites is vibrational spectroscopy, however, the heuristic approach typically taken for peak assignments can give rise to ambiguities for complex materials.

In our work, we present a framework for generating computational Raman spectra via static density functional theory (DFT) and *ab initio* molecular dynamics (AIMD) to investigate molybdenum oxide supported on γ-alumina. To quantify the degree of oligomerization, static DFT calculations are combined with a non-negative linear least-squares approach to fit individual component synthetic spectra to experimental spectra where the Lorentzian broadening is a fitted parameter determined by minimizing the residual error. We further utilize AIMD spectra computed from Fourier transformed polarizability autocorrelation functions to explain how frequency shifts present in experimental spectra correspond to the system's dynamics under varying degrees of surface hydroxylation.

Cross-Ketonization of Biomass-derived Furans and Fatty Acids for Renewable Surfactants

Tejas Goculdas
Advisor: Dionisios Vlachos
Committee Members: Raul Lobo, Marat Orazov

The demand for linear alkyl benzene (LAB) class of anionic surfactants is at $8 billion and is projected to rise 4.5% annually. This poses a significant environmental threat as industrial methods for producing LABs utilize non-renewable petroleum based feedstocks and homogeneous acid catalysts. Therefore, it is desirable to develop a sustainable method for producing LABs. Recent work has demonstrated performance-based biomass-derived oleo-furan sulfonate (OFS) surfactants. The C-C coupling reaction to form 2-alkylofuran is considered the bottleneck in this synthesis. In this context, our group developed a new synthesis of 2-alkylofurans via cross-ketonization of 2-furoic acid and lauric acid using earth-abundant, commercially-available iron oxides catalysts. The cross-ketonization strategy offers ease of separation and recyclability of solid catalysts and produces only water and carbon dioxide as byproducts. However, the iron oxide catalyzed ketonization reaction resulted in modest product yield (~41%) due to the decarboxylation of the 2-furoic acid. There is also limited understanding of the ideal catalyst properties since basic and mixed metal oxides have not been studied for this chemistry.

In this work, we investigate the activity of various alkaline earth metals and mixed metal oxides, e.g., MgO, CaO, BaO, etc. as potential catalysts for cross-ketonization. We show that MgO is a highly active catalyst, minimizes the decarboxylation side reaction and achieves ~90% yield to the desired product. Catalyst deactivation was observed but regeneration over multiple runs was possible. We studied the role of molecule structure on the cross-ketonization reaction and the role of complex formation over alkaline-earth metal oxides. Mechanistic insights will also be discussed. This research shows demonstrates the effectiveness of heterogeneous catalysts for cross ketonization toward the development of renewable surfactants.

Solution Behavior and Self-Assembly of Cylindrical Coiled-Coil Domains into Discrete Nanoparticles, Soluble Aggregates, and Rigid Rods

Kenneth Crane-Moscowitz
Advisor: Eric M. Furst, Darrin Pochan, Christopher J. Kloxin
Committee Members: Arthi Jayaraman and Millicent O. Sullivan

Many archetypal colloidal particle systems, like polystyrene and silica, exhibit excellent stability in aqueous suspensions; however, these systems are limited by their smallest achievable size, lack of homogeneity of functionalized domains, isotropic form, and size polydispersity. An alternative to inorganic and polymer-based colloidal particles is synthetic computationally designed peptide sequences which self-assemble in aqueous solutions into 4 nm long, 2 nm diameter cylindrical coiled-coil domains. These peptide bundles, or "bundlemers", can be functionalized to undergo end-to-end colloidal polymerization, creating micron-length 1-dimensional rigid rod structures. This breadth of achievable length-scales, from a few nanometers to a few micrometers, is afforded to the bundlemer particles based on their propensity to self-assemble, anisotropic shape, and spatial control over the location of reactive functional groups. The sequence specificity of peptides also enables the *a priori* knowledge of the exact location and number of chemically reactive moieties on each bundlemer, as opposed to the sparse or patchy distribution of functional groups on traditional nanoparticles.

Peptide nanoparticles may be an attractive alternative to traditional nanoparticles, due to their small size, anisotropic structure, and exact spatial and numeric control of functionalized residues. However, little is known about their solution stability as proteins are known to aggregate or disperse depending on their formulation conditions. Additionally, colloidal polymerization of the bundlemers into rigid rods remains unclear. TEM micrographs have demonstrated the presence of rigid rod structures after 24 hours of reaction between complementary functionalized bundlemers, yet little data confirms the ensemble behavior, evolution of length-scales, and polydispersity of these rods in solution. In the present work, the self-assembly behavior of a previously synthesized peptide bundle nanoparticle, BNDL+4, is examined in solutions at pH 2, 6, and 10 aqueous suspensions via dynamic light scattering (DLS). A reaction, using the Thiol-Michael addition, of two variants of BNDL+4 is carried out to form rigid rods. This reaction was monitored using DLS for 24 hours to observe the changes in particle size distribution. The non-reacting peptide bundles form into two distinct size populations with hydrodynamic radii of 1.7 nm and the other at 60 nm. The populations are taken to be indicative of the individual coiled-coil peptide bundles and collections of bundles forming soluble aggregates. The solution pH also dictates which of the two populations will become the dominant species as the sample ages. The distribution between three observed populations of functionalized particle sizes at 1.5 nm, 100 nm , and 4 microns does not change after three hours of elapsed reaction time. The investigation probed the solution stability of such peptide-based bundles and provided initial estimates of the solution behavior of functionalized bundlemers transitioning into rigid rods. The results will be crucial to future experiments examining the potential of these bundlemers and bundlemer rods to be utilized instead of traditional nanoparticles and in the construction of 2D and 3D microscopic structures.

Dissipative self-assembly of paramagnetic colloidal suspensions in microgravity

Jason Conradt
Advisor: Eric M. Furst
Committee Members: Norman Wagner and Arthi Jayaraman

Colloids of paramagnetic spheres exhibit phase separation in the presence of sufficiently powerful magnetic fields. By toggling the field, suspensions can be directed toward self-assembling into highly anisotropic, dynamic phases. On Earth, experimental investigations of magnetically driven self-assembly are affected by catastrophic sedimentation. Experiments performed on the International Space Station provided insight into the kinetic evolution and terminal behavior of self-assembling paramagnetic colloids in microgravity.

Suspensions of spherical particles (0.26, 0.48, and 1.02 μm diameters) were subjected to toggled magnetic fields with amplitudes ranging from 627 A/m to 2276 A/m over a frequency range of 0.25 Hz to 20 Hz and a duty ratio range of 0.10 to 0.50 in a microgravity environment. At long experiment times (>50 minutes) five unique microstructure types were identified: unstructured, columns I, columns II, anisotropic, and sheet-like. Especially dynamic, active behavior was observed for aggregates in the sheet regime. Aggregates in these fields engage in motion, splitting, merging, ejection, and coalescence, even as times exceed four hours. Additionally, we find that the transmitted light intensity, a reciprocal measure of the extent of aggregation, exhibits a power-law dependence on time during the initial coalescence. The microstructures formed in these experiments appear to be unique to the dissipative self-assembly of magnetic colloids in toggled fields.

Coiled-Coil Peptides as Molecular Building Blocks

Tessa Posey
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Committee Members: Millicent Sullivan, April Kloxin, Darrin Pochan

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The coiled-coil peptide motif unit can act as a molecular building block, or bundlemer, to build higher ordered structures. Its inherent stability and capacity for selective modification make it an excellent tool for a variety of applications. One interesting potential application is its functionality as a complex polyelectrolyte. By attaching a negatively charged molecule, such as a polymer or nucleic acid, to the exterior of the bundlemer, it can be given a controlled charge distribution and charge density. Additionally, a positively charged molecule could be attached to a separate bundlemer unit and multiple bundlemers with varied exterior charge states can be covalently linked at their N-termini, making a complex structure with similar behavior to a bottlebrush polymer. This project aims to investigate the packing density of a charged side chain achievable on a bundlemer unit and explore the complex structures that can be synthesized by linking bundlemers with varied side chains and charge states.

To accomplish this goal, interior covalent linkages within the bundlemer have been explored to further stabilize its structure and increase its modification limits. The thiol-Micheal click chemistry reaction has been used to connect interior positions within the bundlemer and has been confirmed using liquid chromatography-mass spectrometry. The impact these linkages have on bundlemer stability has been observed using circular dichroism. Additionally, a negatively charged peptide sequence has been attached to the exterior of the bundlemer at multiple locations and the bundlemer's ability to remain in the coiled-coil structure with this negative side chain has been evaluated using circular dichroism. Future work will look into the modification limits of the bundlemer and explore the variation of molecules that can be attached, as well as the structures that can be created by linking multiple bundlemers with varied side chains together.

Modeling and Analysis of the Intrinsic Cardiac Nervous System in Closed-Loop Cardiovascular Control

Michelle Gee
Advisors: Dr. Abraham M. Lenhoff, Dr. Babatunde A. Ogunnaike, and Dr. Rajanikanth Vadigepalli
Committee Member: Dr. Aditya Kunjapur

The baroreceptor reflex is a multi-input, multi-output physiological control system of high clinical importance that regulates short-term blood pressure by modulating parasympathetic and sympathetic nerve activity between the brainstem and the heart. The opposing effects of parasympathetic and sympathetic nerve activity work in conjunction to maintain cardiovascular homeostasis, with imbalances in activity associated with cardiovascular disease. Recently, attention has focused on the contributions of the "the little brain of the heart", the intrinsic cardiac nervous system (ICN), to local control of nervous regulation of the heart and its role in balancing parasympathetic and sympathetic nerve activity. However, knowledge of how the ICN network structure contributes to integrative control of the heart is insufficient for modulating beat-to-beat cardiac control. We have developed a quantitative, closed-loop computational model of the baroreceptor reflex by incorporating a high-fidelity representation of the ICN to evaluate the impact of altered ICN network structures on overall cardiovascular control. We formulated multiple alternative ICN network options based on the anatomical, molecular, and physiological evidence. The computational model consists of (1) a system of ordinary differential equations to represent blood flow in the cardiovascular system, and (2) transfer function representations of sensory neurons, brain stem neuronal groups, and ICN neuronal groups, connected in a closed-loop control circuit. We use this model to investigate, via simulation, the role of the ICN in overall cardiovascular control in response to mean arterial pressure and lung tidal volume perturbations, integrating local cardiac sensory information and cardiovascular efficiency. Our results show that the local circuit neurons that integrate sensory information into the ICN strengthen the response of ICN neuron activity, especially at low blood pressures. In addition, we found that alternative ICN model structures that integrate local sensory information are more efficient at pumping blood. Taken together, these results suggest that integration of local cardiac sensory signals in the ICN amplifies the brainstem's response to perturbations and may contribute to increased cardiac efficiency.

Reactor Design of a CO_2 Electrolyzer for Pure Formic Acid Production

Luke Cherniack
Advisor: Feng Jiao
Committee Members: Dionisios Vlachos, Yushan Yan

Rapidly increasing global temperatures require an immediate strategy to reduce greenhouse gas emissions. CO_2 electrolysis has proven to be a commercially viable technology in converting harmful waste CO_2 emissions into useful fuels and chemicals. A convenient product synthesized from CO_2 electrolysis is formic acid. Formic acid is a promising candidate as a liquid hydrogen carrier through its high volumetric capacity, ease of storage, and low toxicity. Thus, producing formic acid would allow CO_2 electrolyzers to close the carbon loop for a green hydrogen economy. The current state-of-the-art CO_2 electrolyzer that produces formic acid utilized a unique porous solid electrolyte middle interlayer to remove the need of downstream separations. However, this electrolyzer has only been tested at the lab scale (5 cm^2) with insufficient selectivity (<90% Faradaic efficiency). The porous solid electrolyte is a key component in the electrolyzer; however, it introduces a large pressure drop and is very inconsistence to load. Therefore, scaling the porous solid electrolyte in this electrolyzer remains a major challenge. In this presentation, efforts in improving the performance of this unique reactor design are explored in order to create a commercially competitive reactor. Potential ways to improve the selectivity of formic acid are investigated. Also, alternative interlay designs and challenges to creating a conductive interlayer are discussed.

Techno-economics and Scale-up of Electrochemical CO_2 Reduction to Carboxylic Acids

Bradie S. Crandall
Advisor: Prof. Feng Jiao
Committee Members: Prof. Yushan Yan and Prof. Dionisios Vlachos

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The cost of renewable electricity has declined rapidly in recent years; solar photovoltaics and onshore wind are now the cheapest means to produce electricity in the United States. Carboxylic acid production (formic acid and acetic acid) via CO_2 electrolysis allows cheap green electrons to be leveraged to help decarbonize society. Formic acid, the simplest of the CO_2 derived carboxylic acids, can be used as a hydrogen carrier to reduce the costs associated with the storage and transmission of green hydrogen. As both a chemical feedstock and an energy carrier, the affordable delivery of green hydrogen is critical for multi-sector decarbonization. The storage and transmission costs associated with the delivery of gaseous hydrogen currently pose significant barriers to economic feasibility. These limitations may be overcome with the aid of one-way liquid green hydrogen carriers, such as ammonia and methanol, which have been frequently studied, as well as formic acid, which has received less attention. A detailed supply chain analysis across a range of scales was performed to elucidate which liquid green hydrogen carrier is best. Green formic acid, produced via the electrochemical CO_2 reduction reaction, was the cheapest hydrogen carrier (16.3 USD/kg H_2) to meet the typical demand for hydrogen fuel (1,000 kg H_2/day). This hydrogen carrier comparison provides timely insight to guide future decisions related to hydrogen distribution that will be made by researchers, policymakers, and investors alike. Another carboxylic acid that can be produced via CO_2 electrolysis is acetic acid, one of the highest volume chemicals produced in the United States. Tandem electrolysis, where CO_2 is first converted to CO then CO is converted to the final product, offers a promising strategy to electrochemically produce multi-carbons like acetate with improved performance relative to a one step process. However, the downstream costs associated with this process for sustainable acetic acid production are prohibitively expensive due to high energy usage. A cost-effective strategy to produce concentrated acetate (>7.5 M) at high purity (>99%), protonate the acetate to acetic acid, and recover the electrolyte is offered to pave the way for commercialization at scale by significantly reducing downstream energy usage. Techno-economic assessment of carboxylic acid production via CO_2 electrolysis provides vital insight into accelerating the commercialization of electrochemical CO_2 utilization to help affordably decarbonize society at the rate needed to avert imminent climate catastrophe.

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