UNIVERSITY OF DELAWARE
ENGINEERING

DEPARTMENT OF CHEMICAL & BIOMOLECULAR ENGINEERING

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2020 SUMMER RESEARCH REVIEW

SECOND YEAR TALKS

JUNE 2-4, 2020

2020 SUMMER RESEARCH REVIEW - SECOND YEAR TALKS

TUESDAY, JUNE 2, 2020

TIME	PRESENTER	ADVISOR
9:50 AM	**WELCOME** **Samantha Cassel, Colburn Club President**	
10:00 AM	Jessica Belliveau *"Analyzing the Role of Chinese Hamster Ovary Extracellular Vesicles (CHO-EVs) in Extracellular Communication, Cellular State, and Protein Expression of CHO Cultures"*	E. Terry Papoutsakis
10:20 AM	Erica Green *"Development of a Flexible Mammalian Cell Based Platform Process for Recombinant Adeno-Associated Virus Vector Production"*	Kelvin H. Lee
10:40 AM	Neil Butler *"De Novo Biosynthesis and Incorporation of a Nitro-containing Amino Acid"*	Aditya M. Kunjapur
11:00 AM	**BREAK**	
11:20 AM	Kartik Bomb *"Development of a Mechanically Tunable, Non-degradable Hydrogel Platform to Assess Macrophage Polarization in Idiopathic Pulmonary Fibrosis"*	Catherine A. Fromen April M. Kloxin
11:40 AM	Sabyasachi Sen *"Engineering Targeted Proteolysis in Non-hypoxic Environments for Medical Imaging"*	Aditya M. Kunjapur
12:00 PM	James Forder *"Modeling MAb Interactions and Aggregation Rates with Experimental and Computational Approaches"*	Christopher J. Roberts
LUNCH BREAK		
1:30 PM	Nicholas Oliveira *"Understanding Electric and Non-Electric Field Effects on Electrochemical Double Layer Restructuring for the Model Platinum HOR"*	Yushan Yan Bingjun Xu
1:50 PM	Yung Wei Hsiao *"Design and Characterization of a Microfixed-bed for Reactive Separations of HMF"*	Dionisios G. Vlachos
2:10 PM	Yingjie Chen *"Development of Hybrid Models for Continuous Pharmaceutical Manufacturing Lines under Industry 4.0 Framework"*	Marianthi G. Ierapetritou
2:30 PM	**BREAK**	
2:50 PM	Sophia Kurdziel *"Vibrational Scaling Relationships for Transition States"*	Dionisios G. Vlachos
3:10 PM	Yong Yuan *"Characterization of Ga Speciation in Ga/H-ZSM-5 by In-situ Transmission FTIR Spectroscopy"*	Bingjun Xu
3:30 PM	Montgomery Baker-Fales *"Design and Implementation of a Microwave-Heated Extractive Flow Reactor for Intensified HMF Production"*	Dionisios G. Vlachos

NOTE: To request virtual log-in information, please contact Cinda Younce (cyounce@udel.edu)

2020 SUMMER RESEARCH REVIEW - SECOND YEAR TALKS

WEDNESDAY, JUNE 3, 2020

TIME	PRESENTER	ADVISOR
10:00 AM	Roshaan Surendhran *"Developing a Tandem Strategy for the Direct Conversion of Glucose to Ethylene Glycol"*	Marat Orazov
10:20 AM	Stephanie Matz *"Development of Electrochemically-Driven CO_2 Separator for Transport Hydroxide Exchange Membrane Fuel Cells"*	Yushan Yan
10:40 AM	Xue Zong *"Experimental Data Variability in Heterogeneous Catalytic Reactions"*	Dionisios G. Vlachos
11:00 AM	**BREAK**	
11:20 AM	Lina Lee *"Theoretical Insights Into Heterogeneous Ethylene Hydroformylation on Atomically Dispersed Rh-ReOx/γ-Al_2O_3"*	Dionisios G. Vlachos
11:40 AM	Mingchun Ye *"Oxidative Coupling of Methyl Furoate to Form 5,5'-dicarboxylic-2,2'-bifuran Methyl Esters"*	Raul F. Lobo
12:00 PM	Catherine Weiss *"Determination of Oxygen Transport Resistance Through Limiting Current Analysis in Hydroxide Exchange Membrane Fuel Cells"*	Yushan Yan
LUNCH BREAK		
1:30 PM	Michaela Jones *"Improving Non-Standard Amino Acid Incorporation through Dynamic Codon Reassignment"*	Aditya M. Kunjapur
1:50 PM	Will Thompson *"The Properties and Production of Extracellular Vesicles from Megakaryocytes and Chinese Hamster Ovary Cells"*	E. Terry Papoutsakis
2:10 PM	Ian Woodward *"3D Printed Hierarchical and Anatomical Structures for Approximating the Lung"*	Catherine A. Fromen
2:30 PM	**BREAK**	
2:50 PM	Morgan Sulzbach *"Enzymatic Functionalization of Lignin Depolymerization Products"*	Aditya M. Kunjapur
3:10 PM	Chase Herman *"Modeling Host Cell Protein Retention in Chromatographic Polishing Operations"*	Abraham M. Lenhoff
3:30 PM	Jonathan Otten *"Understanding Electric and Non-Electric Field Effects on Electrochemical Double Layer Restructuring for the Model Platinum HOR"*	E. Terry Papoutsakis

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2020 SUMMER RESEARCH REVIEW - SECOND YEAR TALKS

THURSDAY, JUNE 4, 2020

TIME	PRESENTER	ADVISOR
10:00 AM	Xutao Shi *"Modelling sustained release through non-destructive computed tomography"*	Abraham M. Lenhoff Norman J. Wagner
10:20 AM	Zijie Wu *"Coarse-grained Modeling and Simulation Studies of Macromolecular Materials with Directional Interactions"*	Arthi Jayaraman
10:40 AM	Soham Jariwala *"Developing rheological constitutive models using population balances for thixotropic suspensions"*	Antony N. Beris Norman J. Wagner
11:00 AM	**BREAK**	
11:20 AM	Christian Heil *"Computational Study of Structure and Self-Assembly of Bio-Inspired Colloidal Particles"*	Arthi Jayaraman
11:40 AM	Josh Meisenhelter *"Fragment Assembly of Coiled-coil Peptide Rods"*	Christopher J. Kloxin
12:00 PM	Haeun Shin *"Carbon-Nitrogen Bond Formation on Cu (111) Surface in Electrochemical Carbon Monoxide Reduction"*	Feng Jiao
LUNCH BREAK		
1:30 PM	Haoran Ding *"Expanding on Kolbe Electrolysis for Organic Electrosynthesis"*	Marat Orazov
1:50 PM	Sean Overa *"Two-Step Electrochemical Reduction of CO_2 for the Production of Concentrated Product Streams"*	Feng Jiao
2:10 PM	Jian Pan *"High Temperature Hydrocarbon Upgrading"*	Bingjun Xu
2:30 PM	**BREAK**	
2:50 PM	Michael Abramovitch *"Stakeholder-Driven Multi-Objective Optimization of a Modular Food Waste Valorization Process"*	Marianthi G. Ierapetritou
3:10 PM	Tso-Hsuan Chen *"Computational Insights into the Direct Acylation of 2-Methylfuran with Acetic Acid over Phosphotungstic Acid and H-BEA Zeolite"*	Dionisios G. Vlachos

NOTE: To request virtual log-in information, please contact Cinda Younce (cyounce@udel.edu)

Stakeholder-Driven Multi-Objective Optimization of a Modular Food Waste Valorization Process

Michael Abramovitch

Advisor: Prof. Marianthi Ierapetritou

Committee Members: Prof. Dionisios Vlachos, Prof. Raul Lobo, and Prof. Philip Barnes

Food waste is produced at staggering rates (1.3 billion tons/year), consumes vast amounts of energy (26 exajoules/year), imposes substantial collection and disposal costs, and is a significant source of land, water, and air pollution. One strategy for dealing with food waste is to use it as a feedstock for the production of valuable chemical products as part of the "circular economy". Since food waste is a geographically distributed feedstock, modular processing at the sources (e.g., restaurants, cafeterias, grocery stores, etc.) can minimize overall costs and environmental impact by eliminating the need to transport raw materials to a centralized processing facility. When designing distributed chemical or biological processes for implementation in such public areas, it is critical to work closely with all stakeholders involved; these include business owners and employees, municipal and state policymakers, and members of the general public. Each of these groups has their own set of priorities and constraints which must be met to ensure the process is practical, supported by policy, and safe to implement.

To address these challenges, we develop a *stakeholder-driven* multi-objective optimization framework for the design of modular food waste valorization processes. Our novel methodology based on the Fuzzy Analytic Hierarchy Process (FAHP) is the first to both (1) allow public and private-sector stakeholders to propose the metrics to be used as objectives in process optimization and (2) systematically convert qualitative stakeholder priorities into numerical objective weightings. This quantitative approach to "responsible innovation" in chemical process design aims to reduce the non-technological barriers to adoption of the proposed processes and increase the likelihood of beneficial real-world impact. As a proof-of-concept, we apply this method to the optimization of a simplified model for a modular food waste valorization process and demonstrate the effects of different stakeholder priorities on the optimal design.

Design and Implementation of a Microwave-Heated Extractive Flow Reactor for Intensified HMF Production

Montogmery Baker-Fales
Advisor: Dionisios G. Vlachos
Committee Members: Raul Lobo, Abraham Lenhoff, Marat Orazov

5-Hydroxymethylfurfural (HMF) derived from cellulosic sugar is a renewable chemical which provides a platform for production of fuels, monomers, and solvents. Side reactions and multiple – often batch – processes left a need for improvement which has been filled by the use of continuous-flow reactive extractor. With a Lewis/Bronsted acid catalyst system, tandem reactions of glucose to fructose and fructose to HMF can be achieved with favorable yield and selectivity. However, given the economic barriers associated with low-density cellulosic biomass, further improvement and intensification of this process is necessary. Microwaves have strong potential for providing heat both faster and more efficiently than conventional methods. Microwave heating of flow systems has been explored to a modest degree in literature, but design principles have not been adequately developed, thus hindering effective use. In addition to this, the biphasic nature of reactive extractions implies phase-selective temperature gradients – a phenomenon with unexplored applications. In this work, a continuous-flow microreactor is constructed inside a commercial microwave applicator. Design and fabrication of microfluidics were conducted and microwave flow-heating experiments were performed and compared to a computational fluid dynamics model. Good agreement between experiment and model was found for monophasic systems, after which a machine learning approach was used to optimize system geometry for microwave heat absorption. An optimized microreactor was built and demonstrated to heat the process fluid with >90% energy efficiency. A batch-mode sampling reactor was also constructed to probe the effect of microwave heating on HMF distribution in biphasic systems. Finally, initial experiments in biphasic systems will be reported.

Analyzing the Role of Chinese Hamster Ovary Extracellular Vesicles (CHO-EVs) in Extracellular Communication, Cellular State, and Protein Expression of CHO Cultures

Jessica Belliveau
Advisor: Dr. E. Terry Papoutsakis
Committee Members: Dr. Catherine Fromen and Dr. Millicent Sullivan

Chinese hamster ovary (CHO) cells are the primary host cell line for producing protein therapeutics, resulting in the need to better understand and improve CHO cell culture conditions to increase protein production and quality [1]. CHO cells dynamically produce and uptake extracellular vesicles (EVs) in culture to exchange small RNAs, protein material, and small DNA fragments. This exchange of protein and genetic material dynamically regulates the cellular state and protein expression of the target cell. Here, CHO cell derived EVs (CHO-EVs) harvested from various shake flask cultures are characterized in terms of size distribution, morphology, zeta potential, and RNA content. CHO-EVs are separated by differential ultracentrifugation into the heavier EV fraction and in the lighter EV fraction and the size distribution is measured by nanoparticle tracking analysis (NTA). RNA fragment analysis shows CHO-EVs are enriched in small RNAs compared to the parent cells, indicating the CHO-EVs are selectively sorting material into the CHO-EVs to regulate protein expression in target cells. To visualize the dynamic production, protein and genetic material exchange, and uptake of CHO-EVs in shake flask cultures, non-specific protein dyes (carboxyfluorescein succinimidyl ester (CFSE) and CellTracker Deep Red) were used in conjunction with correlative confocal microscopy and scanning electron microscopy (SEM). Correlating confocal images with SEM images showed the localization of exchanged CHO-EVs with the high-resolution imaging of CHO cell morphology. Additionally, flow cytometry and confocal microscopy were used to determine the proportion of CFSE and Deep Red cells in culture and the exchange of fluorescently stained CHO-EVs in culture. The characterization of CHO-EVs produced in shake flask cultures elucidates the native cell communication and material exchange mechanisms via EVs. Future steps in expanding the characterization and application of CHO-EVs include loading specific genetic cargo, such as anti-apoptotic small interfering RNAs (siRNAs), to deliver in CHO cultures to prolong CHO culture health and lifespan.

1.   Fischer, S.; Handrick, R.; Otte, K., The art of CHO cell engineering: A comprehensive retrospect and future perspectives. *Biotechnol Adv* **2015,** *33* (8), 1878-96.

Development of a mechanically tunable, non-degradable hydrogel platform to assess macrophage polarization in idiopathic pulmonary fibrosis.

Kartik Bomb

Advisors: Catherine A. Fromen & April M. Kloxin

Committee Members: LaShanda T. Korley, Millicent O. Sullivan

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrotic disease thought to be initiated by repeated micro-injuries to the alveolar epithelium, resulting in deposition and accumulation of scar tissue and increased tissue stiffness. Currently FDA approved therapeutics for IPF, pirfenidone and nintedanib, only slow the progression of fibrosis and cannot reverse disease pathology. Development of new therapeutics are challenged by poor *in vivo* efficacy despite promising preclinical findings. Consequently, there remains a significant need to better understand the pathobiology of IPF and establish improved model systems for both its study and the development of more effective therapeutics. In particular, insights and approaches are needed for studying the role of macrophages in both initiation and progression, which are interesting therapeutic cellular targets most capable of tissue repair. Upregulation of Th2 cytokines (such as IL-13) is commonly observed in the fibrotic lungs, which is hypothesized to aid in fibrosis progression by activating macrophages (M2 phenotype) that contribute to the pro-fibrotic cascade. While previous studies have shown the role of stiffness and profibrotic microenvironment in activating fibroblasts, little is known about the combined role of extracellular matrix (ECM) stiffness and IL-13 in tuning macrophage response within fibrosis. Understanding how these microenvironment cues direct macrophages phenotype within fibrosis progression would allow for identification of new therapeutic strategies to reverse these effects.

To study macrophage response to microenvironment cues of the lung, we are developing well-defined hydrogel-based synthetic matrices to mimic healthy and fibrotic conditions. Hydrogels with moduli in the range of healthy (Young's modulus (E) ~ 1.5 kPa) to fibrotic lung tissue (E~20 kPa) were prepared using a thiol-ene chemistry by utilizing norbornene functionalized 4-arm PEG and a dithiol functionalized crosslinker. Relevant conditions have been established for independent tuning of matrix mechanical properties, degradability, and biochemical content, as well as promoting consistent alveolar macrophage attachment to these bioinspired substrates. Additionally, assessment of IL-13 stimulation on polarization of alveolar macrophages has been performed in control polystyrene tissue culture plates. We find that IL-13 stimulation of an alveolar macrophage cell line, MH-S cells, leads to a robust M2-like phenotype, with significant increase in CD206 expression and a significant decrease in CD86 expression. These findings suggest the role of IL-13 in upregulating the M2 macrophage phenotype that could aid in fibrosis progression. Future work will compare the degree of M2 polarization of MH-S cells on hydrogel systems mimicking the physiological stiffness of both healthy and fibrotic lung tissue to assess if M2 polarization is enhanced by substrate stiffness in the presence of extracellular IL-13. Further engineering and application of these hydrogel systems will continue to provide exciting opportunities to investigate macrophage response in both the initiation and progression of fibrosis.

De novo biosynthesis and incorporation of a nitro-containing amino acid

Neil Butler

Advisor: Dr. Aditya Kunjapur

Committee Members: Drs. Wilfred Chen and Eleftherios T. Papoutsakis

Live bacterial vaccines in the form of attenuated pathogens or recombinant delivery vehicles are promising technologies for prevention of widespread diseases. However, these in situ antigen-producing platforms are often limited by their inability to elicit long-lasting immune response when attenuated or when provided at low doses required for safety. Coupling these technologies with the biosynthesis of a broad immunostimulant could overcome a major hurdle to vaccine development for several types of pathogens by triggering high, sustained humoral response with low bacterial administration. The molecule para-nitro-L-phenylalanine (pN-Phe) has been demonstrated to act as an immunostimulatory compound when present as a surface residue on multiple proteins, including self-proteins for the purpose of breaking immune self-tolerance. Excitingly, others have shown that pN-Phe incorporation leads to formation of antibodies that predominantly bind to other regions of the protein antigen rather than the pN-Phe containing epitope, thus cross-reacting with wild-type antigen. Although pN-Phe can be incorporated site-specifically within proteins in live cells, there is currently no means to biosynthesize pN-Phe within live cells. Thus, pN-Phe incorporation cannot yet be used to enhance live vaccines.

Here, I present an integrated heterologous pathway in *Escherichia coli* for the biosynthesis of *para*-nitro-L-phenylalanine (pN-Phe). Expression of four exogenous enzymes, including a previously uncharacterized aryl-amine oxygenase (ObaC), redirects metabolic flux in the shikimate pathway to pN-Phe, achieving titers of 0.75 ± 0.21 mM after 48 h. In parallel, we have screened and identified site-specific incorporation technology that is selective for pN-Phe as compared to nsAA pathway intermediates. As part of our ongoing efforts, I am planning to merge site-specific nsAA incorporation technology with our biosynthetic pathway for the synthesis and testing of immunologically relevant antigens.

Computational Insights into the Direct Acylation of 2-Methylfuran with Acetic Acid over Phosphotungstic Acid and H-BEA Zeolite

Tso-Hsuan Chen
Advisor: Dionisios Vlachos
Committee Members: Raul Lobo, Marat Orazov, Feng Jiao

It has recently been demonstrated that the Friedel-Crafts acylation of furanic compounds is a low-carbon-footprint synthesis of renewable oleo-furan sulfonate (OFS) surfactants;[1] a class of surfactants with enhanced properties.[1] Current industrial acylation processes that use carboxylic chloride or anhydrides as acylating agents have several drawbacks: formation of corrosive side products and significant amounts of carbon waste requiring additional separation/recycling processes. In contrast, carboxylic acid acylating agents only generate water as a by-product and are 100% carbon atom efficient. Thereby, executing the direct acylation with carboxylic acids is of significant interest for developing renewable surfactants and green processes in general.[2]

A major challenge to realizing this in practice is accelerating the acyl formation, which entails dehydration of an acid. In this paper, we perform electronic structure calculations and microkinetically analyze an extensive reaction network to gain insights into the Brønsted acid catalyzed acylation of 2-methylfuran with acetic acid over phosphotungstic acid (HPW), a superacid, and compare with the catalytic activity of H-BEA zeolite. The reaction entails two steps: formation of the acyl group, either as acylium ion or stabilized acyloxy surface species; and formation of the Wheland intermediate by electrophilic aromatic addition (EAS). Among the major predictions from our models are: *(a)* In HPW, the dominant pathway is through the acylium intermediate, which is very unstable and extremely reactive and as result the EAS requires no activation. *(b)* In H-BEA, the preferred pathway is via the stable acyloxy intermediate and the rate-limiting step is the EAS. *(c)* We predict higher turnover frequencies in HPW than in H-BEA even though HPW under low-coverage conditions.

References:
(1)   Park, D. S.; Joseph, K. E.; Koehle, M.; *et al*. *ACS Cent. Sci.* **2016**, *2* (11), 820–824.
(2)   Gumidyala, A.; Wang, B.; Crossley, S. *Science Advances* **2016**, *2* (9), e1601072.

Development of Hybrid Models for Continuous Pharmaceutical Manufacturing Lines under Industry 4.0 Framework

Yingjie Chen

Advisor: Prof. Marianthi Ierapetritou

Committee Members: Prof. R. Bertrum Diemer, Prof. Babatunde Ogunnaike

Continuous pharmaceutical manufacturing (CPM) has been a critical research area for the last decade, aiming to develop more efficient and reliable solid-dosage based manufacturing processes. Current efforts in CPM development have focused on implementing Industry 4.0 technologies to digitalize the manufacturing processes, which leads to an increase in the amount of data available from the entire manufacturing cycle. These data need to be appropriately used to extract process information. Traditionally, CPM processes mainly use white-box (WB) models with limited data for predictive modeling. Although the WB models provide a high level of transparency on addressing the physical significance of the model, the development process is often costly, and the performance largely depends on the mastery of process knowledge. To address these challenges utilizing the availability of data, attempts are currently made to combine data with process knowledge to form hybrid models (HMs). HMs consist of a mix of white-box and black-box (BB) sub-models, where the BB sub-models take in historical data and adopt different machine learning (ML) algorithms to provide input-output relationships. The development of HMs with various structures provides greater flexibility and better predictability in modeling CPM processes.

In this work, a framework from the implementation of Industry 4.0 to HM development in CPM is presented. An integrated data collection and analysis flow is first constructed for a direct compaction (DC) line of CPM using Industry 4.0 technologies to facilitate the centralization of data and the formation of a data bank. Data are compared against predictions from WB models to identify plant-model mismatch in loss-in-weight feeder and blender to provide a direction for HM development. HMs for these two unit operations are then built in serial, parallel, and combined structures. The BB sub-models are trained with the collected data using an artificial neural network (ANN) and support vector regression (SVR). The predictive performances of HMs are compared with the original WB models and the purely data-driven BB models. These cases serve as a proof-in-concept development of HMs, and the potential applications of HMs in system analyses and model maintenance will be discussed, along with the digital twin development in pharmaceutical manufacturing.

Expanding on Kolbe Electrolysis for Organic Electrosynthesis

Haoran Ding
Advisor: Marat Orazov
Committee Members: Bingjun Xu, Feng Jiao, Raul F. Lobo, Dionisios G. Vlachos

C-C coupling reactions are intensively studied by organic chemists for the ability to realize carbon-chain extension, thereby enabling numerous organic synthesis. Many C-C coupling reactions employ organometallic compounds such as Grignard reagents [1] or require noble metal catalysts such as palladium.[2] Thus, these reactions usually involve highly pyrophoric and expensive chemicals and/or elevated temperature, which limits the application to the laboratory synthesis and hinders industrial production. Electrochemical methods for C-C coupling have gained interest as an alternative pathway that avoid these limitations.

Kolbe electrolysis, the first discovered electroorganic reaction, involves the decarboxylative coupling of carboxylic acids at anodes [3] and may serve as a versatile tool in the synthesis of C-C coupling products. The reaction is reported to proceed through a radical coupling pathway.[4] Inclusion of radical acceptors (e.g., C=C double bonds or aromatic rings) expands the scope of this strategy beyond the simple radical self-coupling that yields symmetric products and allows the production of numerous asymmetric C-C coupling products. In this study, we have focused on the radical addition to C=C double bonds of styrene, using alkyl radicals generated electrochemically from carboxylic acids. With this strategy, we are able to produce various aromatic products with an elongated side chain and different functional groups from reagents that are challenging to couple through conventional methods. We have probed this model system through a parametric study, investigating the electrochemical factors involved in radical generation and the reaction-diffusion nature of the coupling reaction. These insights have allowed us to developed reaction protocols that overcome anode deactivation and enhance the selectivity towards desired products.

Reference

[1] Smith, Michael B.; March, Jerry. Advanced Organic Chemistry: Reactions, Mechanisms, and Structure (6th ed.) (2007), New York: Wiley-Interscience.
[2] Heck, R. F. *J. Am. Chem. Soc.* (1969), 91: 6707-6714
[3] Kolbe, H., *Justus Liebigs Ann. Chem.* (1849), 69, 257
[4] Walker, O. J., and J. Weiss. *Transactions of the Faraday Society* (1935) 31: 1011-1017.

Modeling MAb Interactions and Aggregation Rates with Experimental and Computational Approaches

James K. Forder
Advisor: Christopher J. Roberts
Committee Members: Arthi Jayaraman, Eric M. Furst

Formulation development is a key step in the development of monoclonal antibody (MAb) therapies. Formulation conditions set the chemical environment of MAbs (e.g., pH, cosolutes and ionic strength) such that the drug is viable for a multi-year shelf-life. Aggregation is a ubiquitous but not well-understood form of MAb degradation that can lead to detrimental immunogenic response in patients. The lack of a fundamental understanding and accurate predictions of MAb aggregation rates, combined with a large possible formulation space, gives rise to the need for tools that reduce the demand for costly material-intensive experiments when screening potential formulations. Accordingly, methodologies for quantitative prediction of high-concentration MAb protein-protein interactions (PPI) and aggregation rates based on formulation conditions are in development. Prior experimental work has semi-quantitatively connected static light scattering (a measurement of PPI) to aggregation rates for formulations as a function of pH and salt concentration, motivating studies with more breadth that capture a broader range of proteins and have the statistical power to provide quantitative aggregation rate predictions. Additional techniques such as differential scanning calorimetry and dynamic light scattering are used to characterize conformational stability and PPI, providing further insight into the fundamental phenomena influencing aggregation. Moreover, low-concentration static light scattering data are used to parameterize coarse-grained simulations that predict high-concentration static light scattering behavior. This presentation focuses on improved predictions of high-concentration aggregation rates, and low- and high-concentration PPI across a broader range of MAbs and formulation conditions to allow for more efficient and rationally designed formulation development and candidate selection for protein-based therapeutics.

Development of a Flexible Mammalian Cell Based Platform Process for Recombinant Adeno-Associated Virus Vector Production

Erica A. Green
Advisor: Kelvin H. Lee
Committee Members: April M. Kloxin and Millicent O. Sullivan

Gene therapy (GT) is an emerging therapeutic approach that introduces, removes, or changes genetic material in a patient's cells with the potential to cure ailments ranging from rare genetic diseases to cancer. Recombinant adeno-associated virus (rAAV) is the vector of choice for therapies in the pipeline because wild type AAV does not cause any known disease in humans, and rAAV vectors can be engineered to target specific cell types or organs *in vivo*. While there are rAAV GTs that have been brought to the commercial market, there remain many unsolved issues surrounding the development of scalable processes, particularly with respect to achieving high titers and accurately assessing rAAV vector quality.

To address these problems, we are working to develop and optimize a more robust production platform for the manufacturing of rAAV therapies and complementary analytical methods to inform process development. Preliminary work focuses on the development of a high throughput transient system that can be used to screen for optimal host cell configurations and culture conditions. In parallel, analytical methods will be developed to more accurately assess vector quality and product titer, and will be used to inform process development decisions. Identification of the optimal production conditions in a transient configuration will inform the design of a stable rAAV platform cell line that can be customized to develop product-specific cell lines or used to screen host cell configurations to improve rAAV titer.

Computational Study of Structure and Self-Assembly of Bio-Inspired Colloidal Particles

Christian Heil

Advisor: Arthi Jayaraman

Committee Members: Dr. Catherine Fromen & Dr. Eric Furst

Directed and self-assembly of colloidal particle mixtures is a proven route to precisely engineer organic, inorganic, or hybrid materials with controlled optical properties. During the design of such optically active materials, an important step is the structural characterization of the assembled particle mixture. This thesis aims to develop computational approaches to describe the self-assembly and structure of assembled bio-inspired materials containing melanin for optical nanomaterials applications. By leveraging low-cost, high-throughput modeling and simulations, we sought to rapidly identify the key parameters in controlling structural characteristics of these self-assembled supraparticles with potential applications in areas ranging from paint to camouflage. Our results have demonstrated how particle chemistry, particle size, particle mixture composition, assembly timescale, and spherical confinement (i.e., supraparticle) cooperate or compete to control the spatial distribution of particles on the surface and within the supraparticle. We have also developed genetic algorithm (GA) based approaches to analyze the output from small angle scattering based structural characterization of assembled supraparticles, without depending on off-the-shelf analytical scattering models. Future work in this area will focus on 1) expanding the above approaches to non-spherical particles to understand the impact of particle shapes such as rods on self-assembled supraparticle structure, 2) extending computational methods to analyze assembled supraparticles to more complex particle types and shapes, and 3) adapting simulation approaches that mimic particle self-assembly with explicit consideration of hydrodynamic forces.

Modeling Host Cell Protein Retention in Chromatographic Polishing Operations

Chase Herman
Advisor: Abraham Lenhoff
Committee Members: Eric Furst and Kelvin Lee

Contemporary bioprocess design is largely empirical, despite numerous incentives to adopt model-based approaches. Mechanistic chromatography models could improve the design and control of preparative separations, but their utility is often limited by insufficient adsorption equilibrium data. This study seeks to develop approximate models to estimate dilute-solution adsorption equilibria of proteins on ion-exchange resins. Such models would be applicable to designing flow-through polishing operations, where a variety of host cell protein (HCP) impurities must be retained. The relatively weak adsorption of some HCPs can lead to inadequate clearance, and this may be amplified by intrinsic differences among resins. Estimating the effects of solution conditions and resin differences on HCP retention would be useful. This is attempted for model proteins on the basis of continuum electrostatics, using structural information and idealized representations of both the protein and resin. Predictions from molecular-scale calculations are compared to experimental retention in column chromatography. Experimental data are separately modeled at the column scale to extract transport and isotherm information from elution profiles. Future efforts will be directed toward the improvement of HCP retention predictions, with the goal of obtaining sufficiently accurate sensitivity estimates for integration into column-scale models.

DESIGN AND CHARACTERIZATION OF A MICROFIXED-BED FOR REACTIVE SEPARATIONS OF HMF

Yung Wei (Jessie) Hsiao
Advisor: Dionisios G. Vlachos
Committee Members: Raul Lobo, Abraham Lenhoff, Feng Jiao

5-Hydroxymethyl-furfural (HMF) synthesized from biomass derived sugar molecules is an important platform molecule for the production of renewable fuels and chemicals. Acid-catalysed fructose dehydration into HMF has been extensively studied, but the overall HMF yield is limited by the subsequent degradation into side products such as levulinic and formic acids, and further hampered by slow, batch processing. Reactive adsorption of HMF in a single reactor shows potential in reducing the number of subsequent downstream separation steps without compromising the purity of valuable product. Selective HMF adsorption onto porous activated carbon materials has been previously demonstrated in batch systems, but few studies have established their use in a continuous fashion, and even less have combined catalysis with adsorption. In this work, we present a two-stage, continuous flow micro-fixed bed reactive adsorber using polymer derived spherical activated carbon (PBSAC) as the adsorbent for HMF. The adsorption performance of the column is first characterized using the major components of the fructose dehydration reaction as a function of temperature, and the adsorption kinetics is derived using adsorption models. Fructose dehydration reaction is then performed using homogeneous HCl catalyst (pH = 0.7) between 130 – 200 ºC, where the breakthrough curves of the as-produced HMF product are used for optimizing the fixed-bed geometry and operating conditions. The recovery of HMF through desorption at high temperatures is demonstrated, and the reusability of the carbon adsorbent is discussed. Finally, conventional and microwave-based heating are compared. This design highlights the potential of modular design for future biorefineries.

Developing rheological constitutive models using population balances for thixotropic suspensions

Soham Jariwala
Advisors: Antony N. Beris, Norman J. Wager
Committee Members: Eric M. Furst, R. Bertrum Diemer

Aggregating colloidal suspensions exhibit rheology with distinctive features such as thixotropy, viscoelasticity, and yield stress, much of which can be linked to the complex interactions and kinetics of their mesoscale structures. Population balances offer a more structurally informed particle-based approach to model these rheological features using physically meaningful and independently measurable parameters, such as aggregate volume fraction and fractal dimension. The rheological predictions from this framework are comparable to the structure kinetics approach; however, the model takes a viewpoint of particle/aggregate size as opposed to that of bonds. Going forward, it is of interest to establish a correspondence between this particle level information and the model predictions of structure and rheological properties.

As a first step in establishing this connection, the population balance-based constitutive model first proposed by Mwasame *et al.* [AIChE Journal, 63, 517-531 (2017)] is applied to thixo-elasto-visco-plastic (TEVP) systems of human blood and carbon black suspensions that form intricate hierarchical structures that involve multiple length scales. We demonstrate that well-validated colloidal physics theories of aggregation and breakage at the particle cluster level can be used to build *ab initio* models for rheology to predict the fractal dimension as well as the evolution of particle cluster size. This work is an attempt towards a general approach to describe macroscopic complex fluid flows utilizing the coarse-grained details of the mesoscale structures.

Improving Non-Standard Amino Acid Incorporation through Dynamic Codon Reassignment

Michaela Jones

Advisor: Dr. Aditya Kunjapur

Committee Members: Dr. Wilfred Chen, Dr. Millicent Sullivan

Non-standard amino acid (nsAA) incorporation into proteins expands the chemical repertoire available to biology, expanding the potential functions and applications of proteins and peptides. One such example is the incorporation of catechol groups into proteins of interest. Catechol containing amino acids naturally exist in mussel peptides that provide for under water adhesion but there are further unnatural applications like bioconjugation and biorthogonal labeling, that make catechols useful for protein functionalization. The current state of nsAA technology, especially for nsAAs that diverge in structure and chemical properties from sAAs, is limited by low incorporation efficiencies which result in low protein yield, a significant impediment to industrial production. Current research to improve incorporation efficiency is not scalable for incorporating multiple nsAAs and not translatable to other organisms beyond *Escherichia coli*. To address the limitations of current nsAA incorporation systems, we propose dynamic codon reassignment (DCR). DCR will be developed by placing all components surrounding nsAA incorporation under artificial, inducible control. Thus far in the development of DCR, we have successfully demonstrated the complementation of endogenous translation machinery with a degradable, inducible counterpart. Additionally, we have begun probing the complexities of intracellular protein degradation through experimental and computational approaches. Finally, we are beginning to expand the screening of synthetases for incorporation of unique catechol containing nsAAs and others.

Vibrational Scaling Relationships for Transition States

Sophia Kurdziel
Advisor: Prof. Dionisios G. Vlachos
Committee Members: Prof. Raul F. Lobo & Prof. Marat Orazov

Semiempirical energy relations provide means of estimating thermodynamic properties and simplifying underlying theoretical trends among classes of reactions. Specifically, Brønsted-Evans-Polanyi (BEP) and transition state scaling (TSS) relationships correlate reaction energies with activation barriers and electronic energies of local minima with transition states, respectively. Scaling relations are not limited to electronic energies; recently, vibrational frequencies of adsorbed surface species were shown to scale across transition metal surfaces.[1] However, scaling between vibrational modes of adsorbed local minima and transition states, which influence temperature corrections to free energies and reaction rate constants, is lacking. We present density functional theory calculations for AH_x (A = C, N, O) diffusions and dehydrogenations on transition metal surfaces, where normal modes are classified in relation to adsorbate-surface interactions. Vibrational scaling relationships (VSRs) correlating such normal modes between local minima and transition states are developed, and we fundamentally derive the slopes of the VSRs from corresponding BEP/TSS scaling. We extend BEP/TSS relationships across different surface sites to VSRs. Furthermore, we demonstrate how vibrational thermodynamic quantities scale across surfaces and resulting effects on thermodynamic/kinetic model projections.

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Theoretical insights into heterogeneous ethylene hydroformylation on atomically dispersed Rh-ReOx/γ-Al₂O₃

Seungyeon (Lina) Lee
Advisor: Dr. Dion Vlachos
Committee Members: Dr. Raul Lobo, Dr. Marat Orazov

Hydroformylation is an important industrial process for synthesis of aldehydes from olefins and synthesis gas.[1] It is performed homogeneously over Rh or Co complexes, a process that is cost-intensive due to recycling and loss of catalyst through leaching.[1] An approach to mitigate these drawbacks and maximize metal efficiency is to utilize atomically dispersed Rh atoms on supported oxides.[2] Recent work has demonstrated that singly dispersed Rh atoms supported on ReOx-modified γ-Al₂O₃ can enhance selectivity for ethylene hydroformylation to propanal, whereas Rh atoms on pristine γ-Al₂O₃ selectively catalyze hydrogenation to ethane.[3] The enhanced selectivity and activity of the ReOx-modified γ-Al₂O₃ surface has been attributed to weaker binding of CO to the Rh atoms, manifesting itself in blue-shifted CO stretching vibrations relative to those of Rh-CO complexes on unmodified γ-Al₂O₃. It has been hypothesized that Rh atoms vicinal to ReOx particles are more cationic.

Here we first characterize the electronic properties of Rh atoms on γ- Al₂O₃ and ReOx-modified γ-Al₂O₃ surfaces. We explore numerous binding sites, compute IR spectra and compare to the experimentally observed blue-shift in the CO spectra. By analyzing the electron density and the density of states, we conclude that there is indeed less Rh to CO π-back-donation in the presence of ReOx. Furthermore, we perform mechanistic studies and microkinetic analysis of an extensive reaction network to gain insights into the enhanced selectivity of Rh atoms on ReOx-modified γ-Al₂O₃ surfaces for ethylene hydroformylation. We reproduce experimental selectivity trends and kinetic parameters and reveal that selectivity to hydroformylation is due to an interplay between coordination geometry and electronic structure.

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Development of Electrochemically-Driven CO_2 Separator for Transport Hydroxide Exchange Membrane Fuel Cells

Stephanie Matz
Advisor: Yushan Yan
Committee Members: Bingjun Xu, Raul Lobo

Hydrogen fuel cell vehicles are a promising, emerging alternative to the internal combustion engine but the commercialized proton exchange membrane fuel cells (PEMFCs) are comparatively cost prohibitive. Hydroxide exchange membrane fuel cells (HEMFCs) are a potentially lower cost hydrogen fuel cell technology under development; however, ambient levels of CO_2 in air are detrimental to HEMFCs resulting in lower performance and impeding HEMFCs from becoming competitive with the incumbent fuel cell technology. The hydroxide produced during the electrochemical reaction in a HEMFC reacts readily with CO_2 in air due to its acid-base equilibrium, essentially scrubbing it from the air. By optimizing a HEMFC for CO_2 capture, rather than power production, an electrochemically-driven CO_2 separator (EDCS) was developed. The EDCS has the potential to enable HEMFCs to operate efficiently with ambient air.

This presentation will demonstrate the ability of the EDCS to effectively and continuously remove CO_2 from air at ambient levels using minimal hydrogen flow to power the separation. This work will explore the effect of operating conditions such as anode flow, cathode flow, and current density on CO_2 separation from ambient air. Additionally, the impact of design parameters such as flow fields, gas diffusion layers, catalyst layer, and the addition of a carbon-ionomer interlayer on CO_2 separation performance will be discussed.

Fragment Assembly of Coiled-coil Peptide Rods

Joshua Meisenhelter
Advisor: Christopher Kloxin
Committee Members: April Kloxin, LaShanda Korley, Darrin Pochan

The coiled-coil motif, commonly found in proteins, is a self-assembling structure that has been used to create hierarchical materials with unique functions. It has been used to drive the self-assembly of different structures, including hydrogels, nano-cage assemblies, and self-assembling nano-tubes. In previous work, peptides were computationally designed to form tetrameric coiled-coils and chemically crosslinked to form rigid-rod structures. The 29 amino acid peptides were modified at the N-termini with a click functional group, which enabled selective covalent linking between assembled coiled-coil units, or bundlemers. Here we have explored the effect of peptide length on bundlemer assembly using circular dichroism, finding that peptides having fewer than 15 amino acids were an insufficient length for coiled-coil assemblies. It was hypothesized that by conjugating the 15 amino acid sequences at the N-termini a stable bundlemer would form. The N-termini was functionalized with a thiol or maleimide functional group to rapidly form dimer conjugates via the thiol-Michael click reaction. Bundlemer rod formation was observed, presumably due to overhanging residues, referred to as "sticky" ends, that stabilize the assembly. The covalently linked bundlemers were observed to grow into rod-like bundlemer-polymers that were microns in length, as characterized through electron microscopy, dynamic light scattering, and infrared spectrometry. Through this work we demonstrate a new method of forming peptide-based rigid-rods through peptide self-assembly rather than previously reported chemical reactions. Additionally, we improved the scalability and ease of synthesis as demonstrated by improvement of yields from previously reported values of ~15% to ~45%.

Understanding Electric and Non-Electric Field Effects on Electrochemical Double Layer Restructuring for the Model Platinum HOR

Nicholas Oliveira
Advisor: Prof. Bingjun Xu, Prof. Yushan Yan
Committee Members: Prof. Marat Orazov, Prof. Feng Jiao

Fuel cell electrochemical devices have the potential to significantly impact emissions in the transportation sector by providing a means to move vehicles with hydrogen and oxygen, while producing water as the only waste product. Hydroxide exchange membrane fuel cells (HEMFCs) have shown recent experimental promise over their acidic proton exchange membrane (PEM) counterpart, but a decrease in the rate of the anodic hydrogen oxidation reaction (HOR) by two orders of magnitude from acidic to basic conditions on platinum has severely limited their implementation. Various theories have been proposed to explain this, ranging from adsorbed cation effects to changes in the electric field strength. One leading argument is the transition in chemical environment contributes to strong changes in electric field effects, which serve as the culprit to the decreased activity. In this work, we challenge this notion using a combination of traditional electrochemical and spectroscopic techniques to highlight the importance of considering both electric field and non-electric field effects on reaction rates. Further, we use this distinction to understand how each factor contributes to water structure re-arrangement in the double layer, which we hypothesize to be the pivotal factor in the aforementioned activity decrease from acid to base.

The electrochemical double layer is traditionally defined by specifically adsorbed species (inner helmholtz plane, IHP), the next layer of hydrated ions (outer Helmholtz plane, OHP) and further layers (diffuse region) protruding and mixing into the bulk electrolyte. It has been shown that the stark tuning rate, which is a function of electric field strength, is dictated by hydrated cation size, and provides a metric with which to measure the thickness of the electrochemical double layer. By altering cation size through chelating ethers, the stark tuning rate, and thus the electric field, can be affected up to a 0.5:1 ratio of crown ether to cation. However, changes in HOR activity are observed beyond this threshold, providing strong evidence for different electric and non-electric field effects. Linear ethers of similar molecular weight are also employed to demonstrate different stark tuning effects but similar activity changes beyond the 0.5:1 ratio, helping to further decouple the electric and non-electric effects contributing to the activity.

Syntrophic Co-Cultures in *Clostridium* Species Improve the Production of 4-8 Carbon Acids and Alcohols

Jonathan K. Otten
Advisor: Eleftherios T. Papoutsakis
Committee Members: Wilfred Chen, Cathy Fromen, Aditya Kunjapur

Clostridium species, especially *C. acetobutylicum*, have long been used in solvent production. However, they have largely been replaced by cheap petrochemical means of solvent production, albeit at an environmental cost. Co-cultures of *Clostridium* species can produce both economic and environmental benefits through combining the best enzymatic features of all the organisms, allowing solvent production to efficiently resume through fermentation of biomass feedstocks. This project involves the co-culture of *C. acetobutylicum* (*Cac*), long used to produce acetone, butanol, and ethanol, with *C. ljungdahlii* (*Clj*) and *C. kluyveri* (*Ckl*). *Clj* contains the Wood-Ljungdahl pathway, which fixes CO_2 using H_2 as an electron donor. This allows the co-culture to reach carbon recovery at the theoretical limit. *Ckl* is capable of chain elongation, which converts the products of *Clj* and *Cac* (acetate and ethanol) to the higher-value compounds butyrate, hexanoate, and octanoate. These fatty acids can then be converted by *Cac* into their respective alcohols, which are then condensed from the bioreactor. Two- and three-member syntrophic co-cultures (*Cac/Ckl* and *Cac*/*Clj*/*Ckl*) have been analyzed in both serum bottles and laboratory bioreactors. The system is capable of 6-8C solvent production from a glucose medium, and the carbon-capture benefits of *Clj* are readily apparent.

While the syntrophic co-culture is capable of 6-8C solvent production without genetic modification, tracking metabolite concentration reveals the potential of genetic engineering to improve product yields. A genetically modified strain of *Cac* that produces 3 times more ethanol than the wild-type shows a corresponding increase in the chain-elongation performance of *Ckl*. This project is the first to demonstrate short-timescale 6-8C alcohol production from a *Clostridium* triple co-culture. Areas of further research include population dynamic analysis, ^{13}C metabolite tracking, advances in product separation and purification, and further genetic refinements. This research also revealed novel extracellular vesicles (EVs) produced by all three co-culture species, which were found to contain active enzymes. Future work will use these EVs for biocatalysis and provide a framework for *Clostridium* species in co-culture as a modular and viable bioproduction platform.

Two-Step Electrochemical Reduction of CO_2 for the Production of Concentrated Product Streams

Sean Overa

Advisor: Feng Jiao

Committee Members: Raul Lobo, Dionisios Vlachos

The electrochemical conversion of CO_2 to value added products is a promising approach for the mitigation of rapidly rising CO_2 levels. Electrochemical CO_2 conversion has been shown capable of producing both single carbon (C_1) and multi-carbon (C_{2+}) products. Of these, C_{2+} products are the more interesting, as potential products such as ethylene and acetic acid have significant market potential. Electrochemical production of the C_1 products, formic acid and carbon monoxide, has already been achieved at high selectivity and efficiency, whereas C_{2+} products directly from CO_2 are still far from being commercially viable. These products require a highly alkaline pH environment, typically greater than 1M KOH, to ensure good selectivity over the competing hydrogen evolution reaction. However, this basic electrolyte will spontaneously react with the fed CO_2, leading to consumption of fed CO_2 and KOH, reducing the sustainability of the process. Direct CO reduction, however, has been demonstrated to have both high selectivity towards C_{2+} products as well as significantly improved stability. This is due to the inertness of CO in the presence of highly alkaline environments. Therefore, a two-step system where CO_2 is first converted to CO in neutral conditions and then a subsequent reactor to convert CO to C_{2+} products in alkaline conditions is a highly promising alternative to direct CO_2 reduction. Here we present our recent work into developing a selective two-step electrochemical system for the production of concentrated C_{2+} product streams, specifically acetate and ethylene, from a direct CO_2 feed.

We first demonstrate a CO_2 electrolyzer for the production of CO. This electrolyzer was designed to operate as an anion exchange membrane (AEM) membrane electrode assembly (MEA). By utilizing the AEM MEA, >90% conversion of potentially reduced CO_2 was achieved, while maintaining >80% Faradaic efficiency towards CO. Next, we demonstrate a CO electrolyzer also configured as an AEM MEA. This electrolyzer was capable of maintaining >70% Faradaic efficiency towards C_{2+} products at industry relevant current densities and cell voltages. By utilizing the AEM in place of a conventional cation exchange membrane or three compartment flow-cell, the target product of acetate is shuttled through the membrane and collected in the anode compartment. This design allows for the production of a >0.7:1 ratios of acetate to electrolyte, facilitating significantly easier downstream separation or utilization. We then demonstrated the feasibility of the system by operating the two electrolyzers in series. This system achieved the highest to date conversion of fed CO_2 to C_{2+} products, as well as the highest acetate production rate with a direct CO_2 feed.

High Temperature Hydrocarbon Upgrading

Jian Pan
Advisor: Bingjun Xu
Committee Members: Dr. Abraham M. Lenhoff,
Dr. Feng Jiao, Dr. Marat Orazov, Dr. Aditya M. Kunjapur

The source of ethane is very abundant in natural gas which can be used to produce a lot of useful products in our life, for example ethylene. For now, most of ethane is upgraded into ethylene through steam cracking. Dehydrogenation is also applicable, especially the oxidative dehydrogenation method. However, these approaches are energy intensive and have thermodynamic limitations. Therefore, electrochemical approach is proposed to realize the upgrading of ethane, which is likely to obtain a high faradaic efficiency, improve the energy efficiency, and break the thermodynamic limitations.

To realize electrochemical upgrading of ethane, a proton conducting solid oxide fuel cell has been fabricated and realized ~86% of the highest performance reported. However, C_2H_4 yield of C_2H_6 dehydrogenation at 600 ^oC is very low without clear change under current. Coke formation is high which could lead to pellet structure damage. To minimize the catalytic coking from Ni, the pellet is flipped to avoid Ni as the anode, which leads to higher C_2H_4 yield. At the same time, no big leaking happens, and effects of current are apparent. However, the yield does not show clear increase as expected under current. Therefore, more characterizations of anode after test and modifications of the pellet to increase both C_2H_4 yield and C_2H_6 conversion under current are needed in the future.

Engineering targeted proteolysis in non-hypoxic environments for medical imaging

Sabyasachi Sen
Advisor: Dr. Aditya Kunjapur
Committee Members: Dr. Wilfred Chen, Dr. Kelvin Lee, and Dr. Sharon Rozovsky

The development of diagnostic tools to profile the heterogenous tumor microenvironment is essential for the development of precise and personalized cancer treatment plans. Of particular interest is profiling tumor oxygenation, as highly hypoxic tumors resist standard treatment practices, and ultimately influence prognosis negatively if not accounted for during treatment. The usage of facultative anaerobic bacteria, natural colonizers of poorly oxygenated tumors, for imaging purposes is reliant on tight genetic control of protein generation (on-switches) and protein depletion (off-switches). While oxygen-responsive transcriptional control of synthetic constructs provides a valuable on-switch for expression, there is no similarly responsive posttranslational control mechanism to deplete these proteins in model bacterium such as *E. coli*.

In this talk I will discuss ongoing work to expand the *E. coli* N-degron pathway to include the destabilization of N-terminal cysteine residues in the presence of oxygen. We hypothesize that the import of two proteins associated with eukaryotic post-translational oxygen response, a cysteine dioxygenase and an arginyltransferase, will introduce a set of posttranslational modifications into *E. coli* that would convert a protein carrying N-terminal cysteine into a substrate for ClpAPS-mediated proteolysis in normoxic conditions. To begin to validate this hypothesis, we have developed high-throughput degradation assays and subsequently tested the compatibility between various N-terminal motifs and endogenous proteolytic machinery. Ongoing efforts have focused on characterizing the aforementioned heterologous proteins and optimizing their performance within the proposed proteolytic cascade. Proposed future work will integrate our technology within bacteria engineered for tumor imaging.

Modelling sustained release through non-destructive computed tomography
Xutao Shi
Advisor: Abraham Lenhoff, Norman Wagner
Committee Members: Christopher Roberts, Antony Beris, Aditya Kunjapur, Arthi Jayaraman

Sustained-release systems improve the bioavailability of biopharmaceutical products and eliminate the necessity for a frequent administration schedule of the drug. A better understanding of such delivery systems, and therefore a more efficient system design, can be achieved by using mathematical models capable of predicting the drug release profiles *a priori*. However, these models are often formulated phenomenologically, and morphological details such as the early-stage pore formation of the sustained-release system are often neglected or assumed without proper experimental characterization. In this work, we utilized non-destructive X-ray computed tomography (CT) to quantitatively characterize the internal structure of a PLGA-based sustained release system. We modified an existing mechanistic sustained-release model and incorporated the structural characteristics obtained from segmentation of the three-dimensional CT reconstructions. The model predictions were compared to the release profiles obtained from experimental studies. The results demonstrate the importance of structural porosity in sustained release and the potential of a CT-aided mechanistic model to predict the drug release profiles of a PLGA-based sustained-release system.

Carbon-Nitrogen Bond Formation on Cu (111) Surface in Electrochemical Carbon Monoxide Reduction

Haeun Shin
Advisor: Feng Jiao
Committee Members: Bingjun Xu, Dionisios Vlachos

Electrochemical carbon monoxide reduction (COR) to multi-carbon products is one of the promising methods in converting carbon dioxide (CO_2) into valuable chemicals using renewable energy sources. However, most of products from conventional electrochemical systems are restricted to carbon-carbon bonded chemicals limiting the range of product diversities in CO_2 utilization. Here, this study examines the formation of Di-/acetylethylenediamine which contains carbon-nitrogen (C-N) bonds by introducing ethylenediamine in electrolyte which can extend the possible acylation reactions in COR. In the mechanistic view, Di-/acetylethylenediamine can be formed by nucleophilic addition of ethylenediamine to ketene-like intermediate on the catalyst surface. It is recently reported that ketene-like intermediate is favored on Cu (111) surface more than other intermediates leading to ethylene and alcohol production due to its low catalytic activity to those intermediates while the ketene-like intermediate is less dependent on the Cu surfaces[1]. Herein, this study will also compare the selectivity toward Di-/acetylethylenediamine on copper nanosheet where the mainly exposed surface of the catalyst is Cu (111) and on polycrystalline copper to propose the efficient electrocatalyst for desired products.

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Enzymatic Functionalization of Lignin Depolymerization Products

Morgan Sulzbach
Advisor: Dr. Aditya M. Kunjapur
Committee Members: Dr. Thomas H. Epps, III, and Dr. E. Terry Papoutsakis

Lignin is the second most abundant source of biomass and the largest renewable source of aromatics, yet only an estimated 2% is utilized for specialty products where the rest is burned as waste. Conversion of waste lignin to higher value chemicals by catalytic or microbial means will increase carbon neutrality and decrease reliance on petroleum. While polymers are attractive research targets for this reason, lignin-derived monomers will need a broader repertoire of functional groups to advance towards the range of properties that petroleum-derived polymers contain. Covalently cross-linked networks, which are useful in thermosets or separations, often rely on a primary amine for polymerization; however, large scale production of amines lacks selectivity and suffers from high energy and safety demands. To help make these monomers, biocatalysis can offer a more cost-effective production method with non-toxic, ambient condition processing and high selectivity.

This project explores the application of carboxylic acid reductases (CARs) and transaminases (TAs) in converting the carboxylic acid products from lignin depolymerization through an aldehyde intermediate to primary amines for use in cross-linking polymer networks. CARs have a broad diversity in substrate specificity and activity, so the first aim of the project was to refine a previously developed, genetically encoded aldehyde biosensor to screen for the CAR with the highest activity on the lignin depolymerization products. The second aim is engineering the TA for activity on the bulkier substrates associated with lignin depolymerization. Protein homology modeling and molecular docking studies were used to hypothesize target residues for protein engineering in later experimental testing with a previously developed screening method. Future directions of the project include assembling the pathway in *Escherichia coli* for lab scale production of aromatic amines.

Developing a tandem strategy for the direct conversion of glucose to ethylene glycol

Roshaan Surendhran
Advisor: Dr. Marat Orazov
Committee Members: Dr. Raul Lobo, Dr. Bingjun Xu, Dr. Feng Jiao, Dr. Antony Beris

With a global production capacity of 34.8 million tons in 2016 and a global market value of 30.4 billion USD in 2018[1], monoethylene glycol (MEG) is a high volume chemical used primarily as a reagent in the synthesis of polymers, as a solvent, and as a heat-transfer fluid. The most prevalent process for the production of MEG currently involves epoxidation of ethylene, followed by the hydration of the ethylene oxide intermediate, which requires multiple reactors and separation units[2]. Over 95% of the global MEG supply is produced through this method utilizing ethylene sourced, predominantly, from non-renewable (natural gas, naphtha, and coal), and, more recently, renewable (biomass) feedstocks[1]. The overall financial and environmental cost for sourcing the ethylene and subsequently converting it to MEG incentivizes the development of a technology that can directly convert the feedstock to MEG. To this end, the direct conversion of cellulose or glucose to MEG has been the subject of research in the recent years, with a sequential retro-aldol–hydrogenation strategy being particularly appealing.

The retro-aldol reaction required for this strategy is equilibrium limited and favors the hexoses (glucose) over the smaller fragments (glycolaldehyde and erythrose). To overcome this limitation, we propose a tandem strategy that irreversibly transforms the glycolaldehyde to MEG via hydrogenation. Such a strategy requires the simultaneous use of two catalysts, one for the retro-aldol reaction, and one for hydrogenation. Molybdenum-based catalysts (e.g., MoO_3) are known to epimerize sugars via a 1,2-carbon shift mechanism and have been shown to catalyze retro-aldol reactions of aldohexoses[3] and ketohexoses[4]. In contrast, Lewis acidic zeotypes that catalyze 1,2-hydride shift reactions of sugars enable transfer hydrogenation of retro-aldol fragments. Furthermore, the inherent microporous nature of zeotypes enables us to develop size-selective transfer hydrogenation catalysts that limit side reactions of larger species. In this work, we have explored the use of such catalysts individually for the retro-aldol and transfer hydrogenation reactions, and have begun to investigate their pairing for the tandem, one-pot process.

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The Properties and Production of Extracellular Vesicles from Megakaryocytes and Chinese Hamster Ovary Cells

Will Thompson
Advisor: Eleftherios T. Papoutsakis
Committee Members: Catherine Fromen, April Kloxin

Extracellular vesicles—lipid bilayer-bound particles of less than one micron in diameter—are produced by every cell type and increasingly thought to be key to intercellular communication. Subcategorized as "exosomes" (derived from late endosomes) and "microparticles" (derived from the plasma membrane), these vesicles transport proteins, lipids, and nucleic acids between cells. Small RNA cargo—thought to be most responsible for inducing phenotypic change in target cells—is of particular note.

Chinese hamster ovary (CHO) cells are the workhorses of the biopharmaceutical industry, responsible for the production of numerous protein therapeutics. However, the properties of extracellular vesicles derived from CHO cells are poorly understood. Preliminary work must investigate the effects of these vesicles on CHO cells during co-culture. Initial experiments suggest a significant role for "healthy" extracellular vesicles in "rescuing" CHO cells from apoptotic stress. Future studies will determine the extent of the vesicles' protective properties, examining how vesicle efficacy varies with production methods and co-culture conditions. The small RNA cargo informing vesicle function must also be elucidated.

The properties of extracellular vesicles derived from megakaryocytes are well-defined. These vesicles promote the proliferation and megakaryopoiesis of hematopoietic stem and progenitor cells, even in the absence of thrombopoietin. Further, this function is primarily due to the action of two small RNAs: hsa-miR-486-5p and hsa-miR-22-3p. The challenge in this case involves developing proof-of-concept for large-scale vesicle production. Such a challenge requires the discovery of structure/function relationships that can inform quality control assays. To this end, correlations have been identified between parent cell agitation and vesicle number, size, uptake (by target cells), and megakaryopoietic capability. In order to quantify the levels of key miRNA cargo, RT-qPCR assays have also been established. These assays suggest that vesicles derived from microparticle-driven megakaryopoiesis are more effective in promoting subsequent megakaryopoiesis than those derived from cultures containing thrombopoietin. Further, preliminary work on the mid-culture "recycling" of undifferentiated HSPCs suggests a promising method for improving vesicle yield. Future work must identify additional structure/function relationships—especially those implicating miRNA content—and further develop strategies for the large-scale production of vesicles from megakaryocytes. In this endeavor, cytokine and vesicle concentration, perfusion, culture agitation, vesicle harvest time, and vesicle isolation method are all variables of interest.

Determination of Oxygen Transport Resistance through Limiting Current Analysis in Hydroxide Exchange Membrane Fuel Cells

Catherine Weiss
Advisor: Yushan Yan
Committee Members: Marat Orazov and Bingjun Xu

Development of Hydroxide Exchange Membrane Fuel Cells (HEMFC) is motivated by the promise of significantly reduced overall module costs compared to Proton Exchange Membrane Fuel Cells (PEMFC). By transitioning from a low-pH to a high-pH environment, less expensive materials become stable. Specifically, platinum-group metal loading can be greatly reduced or potentially eliminated from the catalyst formulations, and the bipolar plates can be manufactured more cost effectively. However, HEMFCs are currently in the early stages of development, and improvements in performance and durability are needed.

A key challenge in the design of PEM and HEM fuel cells is maximizing O_2 transport from the inlet air stream to the triple phase boundary in the cathode catalyst layer. O_2 transport resistance causes voltage losses at high current densities, limiting maximum power density. Limiting current analysis has been used extensively to experimentally determine O_2 transport resistance in PEMFCs, and we demonstrate that this technique is valid for HEMFCs and produces similar results. Our analysis quantifies the individual O_2 transport resistance contributions from several factors: molecular diffusion through the gas diffusion layer (GDL), Knudsen diffusion through the microporous layer (MPL) and catalyst layer, and diffusion through ionomer in the catalyst layer. Importantly, PEMFC and HEMFC have a different water balance. In PEMFC, an MPL is added to relieve the cathode GDL of flooding but also contributes to O_2 transport losses. Conversely, in HEMFCs, the cathode dries-out and the anode floods; therefore, the MPL is an unnecessary component on the cathode GDL. We report that the elimination of the MPL significantly decreases the O_2 transport resistance and improves the performance under air, especially at high current density.

3D Printed Hierarchical and Anatomical Structures for Approximating the Lung

Ian Woodward
Advisor: Catherine Fromen
Committee Members: April Kloxin, Abraham Lenhoff, Jason Gleghorn

The lung is one of a few organs able to exchange material between the body and the environment, making it an interesting site for both local and systemic drug delivery. However, for over 60 years, inhalable therapeutics have been developed using devices that do not replicate airway morphology or transient breathing behavior, and the complexity of the airways gives rise to a number of the leading causes of death. Recent developments in medical technology and 3D printing now enable investigators to use non-invasive techniques to recreate patient-specific airways for computational studies and *in vitro* testing. Despite these advances, current 3D printed lung replicas are rigid and typically suffer from limitations in imaging or printing resolution, truncating printed lung geometry to less than 10% of the total airway volume. To bridge the gap between legacy aerosol testing platforms and the next stage of lung models, we have developed an approach to approximating the full lung space that combines advances in medical technology with unique additive manufacturing capabilities.

In this talk, I describe our approach to creating a dynamic lung replica from patient-specific CT scans and 3D printed triply periodic structures. Current progress includes lobe-level control of 3D printed parts to generate physiologically relevant flow rates; a generative design strategy for creating and 3D printing structures to mimic lung dimensions; empirical pressure gradient investigation; and differential particle collection between representative lattice geometries. Future work focusing on continued characterization of these 3D printed architectures and aerosol tracking within the lung replica will build towards a next-generation platform for engineering aerosols and personalized inhalable therapeutics.

Coarse-grained modeling and simulation studies of macromolecular materials with

directional interactions

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Directional molecular interactions like hydrogen bonding and π-π stacking play an important role in driving structure and thermodynamics in synthetic and bio-derived macromolecules (e.g. polymers, polysaccharides, peptides, etc.). Molecular simulation studies of macromolecules with dominant directional interactions require models that can simultaneously capture local, small scale directionality exhibited by hydrogen bonding and π-π stacking interactions as well as large length scale and long time scale structural arrangements that are a signature of macromolecules. In my thesis, I have been developing such coarse-grained (CG) models to enable simulations that capture structures driven by hydrogen bonding interactions exhibited by cellulose chains and cellulose based nanoparticles. First, we developed a CG model that reproduced experimentally-observed features of cellulose structure ranging from single-chain geometry to an assembled state of multiple chains with the correct hydrogen bonding pattern, interchain distance and orientational order. Next, we applied the CG model to study aggregation behavior of chemically modified cellulose chains (e.g., methylcellulose), using "silenced" hydrogen bonding interactions to mimic substituted hydrogen bonding sites. We are currently extending the coarse-graining methodology to simulate polymer nanocomposites with cellulose nanocrystals (CNC) as fillers. In this talk I will present some of the results from the above studies.

Oxidative Coupling of Methyl Furoate to form 5,5'-dicarboxylic-2,2'-bifuran Methyl Esters

Mingchun Ye

Advisor: Raul Lobo

Committee Members: Bingjun Xu, Dionisios Vlachos and Marat Orazov

To mitigate the environmental impact of plastic waste and reduce our dependence on fossil fuels, renewable alternatives with desirable combinations of physical and chemical properties must be found. Recent investigations have shown that bifuran-based polyesters could afford such materials: poly (ethylene bifuranoate), for example, has shown attractive UV blocking and gas barrier properties. To scale up the synthesis of bifuran polymers, alternatives for the selective C-C homocoupling of methyl furanoate via the Heck reaction need to be found. In this work we will investigate and optimize the oxidative coupling of methyl furoate using homogeneous Pd(II) catalysts. We found that the reaction of methyl furoate, palladium acetate and sodium pivalate as catalyst and additives, in dimethylacetamide (DMAc) solvent under mild conditions (60 to 80 $^{\circ}$C) generates acceptable yields (~10% in 2 hours) of the product. The reaction kinetics were investigated, and a possible mechanism and rate determine step (RDS) are proposed. We found that the amount of catalyst, the concentration of reactant, and reaction temperature have significant impact on the reaction rate and yield, while oxygen pressure shows no influence, which states that the Reductive elimination is not the RDS. Concentration of sodium pivalate and temperature have an influence on catalyst stability. The transmetalation of two Pd-activated furoates is suggested as the most likely RDS. Ligands with suitable size and nucleophilicity can be chosen to further increases the reaction rate and catalyst stability. Further studies on solvent-less reaction conditions also showed promising results.

Characterization of Ga Speciation in Ga/H-ZSM-5 by In-situ Transmission FTIR Spectroscopy

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Ga supported on H-ZSM-5 (Ga/H-ZSM-5) has long been recognized as a promising catalyst for nonoxidative dehydrogenation of alkanes, and propane in particular. However, Ga speciation under reaction conditions in this catalyst remains controversial. In this work, in situ transmission Fourier Transform infrared (FTIR) spectroscopy is employed to systematically investigate Ga speciation in Ga/H-ZSM-5 with three Si/Al ratios (15, 28 and 39) and a wide range of Ga/Al ratios (0-1.7). Quantitative FTIR results with pyridine reveal that one Ga atom roughly replaces one Brønsted acid site (BAS) at Ga/BAS ratio up to 0.7, however, only up to ~80% of the BAS on the H-ZSM-5 can be exchanged even with excess amounts of Ga. At a low Si/Al ratio, the intensity of GaH_x bands on the reduced Ga/H-ZSM-5 at 550 °C increases almost linearly at low Ga loadings (Ga/Al < 0.13), then levels off up to a Ga/Al ratio of 0.7, and falls gradually at higher Ga loadings. In contrast, no detectable GaH_x bands is observed on Ga/H-ZSM-5 with a high Si/Al ratio, with Ga/Al ratios up to 1.7. The dependence of GaH_x bands on both the Si/Al and the Ga/Al ratios shows that Ga speciation varies with BAS density in the zeolite. We hypothesize that paired BAS sites are preferentially exchanged with Ga^+, leading to the formation of Ga^+-H^+ pairs, while the exchange of isolated BAS form isolated Ga^+ species. Using water as a probe molecule, we show that isolated Ga^+ and Ga^+-H^+ pair sites have distinct properties, i.e., the former can be easily oxidized by water to form GaOOH species, while the latter is inactive under the same conditions. Results in this work provide direct experimental evidence for the existence of two types of Ga species on reduced Ga/H-ZSM-5, highlighting the possibility that they have different catalytic activities in alkane dehydrogenation reactions.

Experimental data variability in heterogeneous catalytic reactions
Xue Zong
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Published experimental data have long served as a valuable resource for assessing catalytic performance, facilitating catalyst discovery and understanding complicated reaction mechanisms. However, the reported literature data are heterogeneous in nature and often exhibit large differences for identical systems. This fact complicates meaningful comparisons across sources and hinders the utilization of prior knowledge. In order to alleviate this problem, it is important to identify the causes and interpret the discrepancies. In this work, we develop a creative methodology to do this. Specifically, a descriptor-based microkinetic model is established that provides the possibility to evaluate scenarios at a dramatically reduced computational cost compared to first-principles calculations. We demonstrate this approach for the complete methane oxidation as a case study. Our methodology provides a novel approach to assess and analyze prior published data.