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ABSTRACTS AND SCHEDULE GUIDE

Clayton Hall Conference Center
Newark DE 19716

Wildtype adeno-associated virus productivity and packaging insights towards improving recombinant adeno-associated virus production

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Recombinant adeno-associated virus (rAAV), a viral vector in which the genome of wildtype adeno-associated virus (wtAAV) is engineered to carry a therapeutic gene, is the most commonly used viral vector in clinical applications for gene therapy. wtAAV, a naturally occurring non-pathogenic human virus, is more productive than its recombinant form, reaching over a log-fold higher titer and a content ratio, the proportion of capsids that contain the therapeutic genome, exceeding 95% full capsids. In contrast to wtAAV, rAAV is limited by low titers and a suboptimal content ratio, typically 5-30%, making it difficult to generate the large quantities of vector genomes needed for treatment. The higher productivity of wtAAV is likely due in part to native viral expression feedback loops that optimize capsid assembly and genome packaging but remain poorly understood in the context of rAAV production. Here, a novel wtAAV production platform in suspension human embryonic kidney 293 (HEK293) cells, the dominant production platform for rAAV, is presented for a range of wtAAV to helper adenovirus multiplicities of infection. The impact of viral genome delivery and helper virus interactions on AAV production was characterized by comparing wildtype infection-based approaches to recombinant transfection-based and hybrid infection–transfection systems. The wtAAV and hybrid infection-transfection systems displayed distinct host cell growth patterns and viral gene expression profiles, achieving over an order of magnitude higher titers and 45% greater content ratios than transfection systems.

Nonequilibrium Molecular Dynamics Simulations of Ultrasonic Welding of Thermoplastic Composites

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With the environmental crises posed by both climate change and the accumulating plastic waste, it is critical that we find new methods to create composite materials that we need in a method that is low-energy and amenable to recycling. Thermoplastic composites are a prime candidate for recycling and repair as their matrix can be melted and reformed as needed. However, thermoplastic matrices present challenges with processing due to complex flow behavior and high viscosity, which can make it difficult to fabricate parts with complex geometries. One promising technique to create these parts is ultrasonic welding, whereby complex part geometries can be made by using high frequency sound waves to join the matrices of the two simpler parts, this technique has been proven in applications, but there still remain theoretical gaps in our understanding of this process. It is known that ultrasound impacts the entanglements between polymers in the bulk, and it is, as yet, unclear how the coherent energy provided by this ultrasound affects the formation of the interfacial chain entanglements that ultimately dictate the strength of the weld. Additionally, we cannot directly observe the formation of these entanglements during the welding process with experimental methods. Using a coarse-grained molecular dynamics model, matched to the chain-level mechanical response of polymer chains, the welding process is simulated to derive the chain-level insights to understand the welding process and quantify the interfacial entanglements and chain configurations of the polymers during this process with the goal of optimizing the performance of welded parts. These simulations will provide insights into the role of frequency and amplitude on the formation of interfacial entanglements and ultimate weld strength.

Elucidating the role of pendent functional groups in lignin-derivable polyurethanes

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Non-isocyanate polyurethanes (NIPUs) made with biobased precursors are emerging as potentially safer and more sustainable replacements for traditional isocyanate-based polyurethanes (PUs). However, direct structure–property comparisons are essential to assess their performance characteristics. In this work, four polymers—two PUs and two NIPUs—are prepared from (i) petroleum-derived bisphenol A (BPA) and (ii) lignin-derivable bisguaiacol A (BGA). Characterization of these samples elucidates how methoxy substituents from BGA-based monomers and hydroxyl substituents characteristic of NIPU chemistry govern hydrogen bonding, free-volume effects, and resulting polymer properties. Consistent trends appear across all results: NIPUs with pendent hydroxyl groups exhibit the effects of increased hydrogen bonding between chains, whereas methoxy substitution in BGA-based systems display increased intermolecular interactions as well as molecular weight between entanglements, free volume, and steric bulk. Specifically, attenuated total reflectance–Fourier transform infrared spectroscopy reveals significant differences in hydrogen-bonded –OH/–NH content amongst all samples (BPA-PU: 90%, BGA-PU: 97%, BPA-NIPU: 75%, BGA-NIPU: 89%) and shows that the presence of methoxy groups contributes to the ordering of C=O hydrogen bonding (BPA-NIPU: 3% *vs.* BGA-NIPU: 26%). The hydroxyl-rich NIPUs display glass transition temperatures ~8 °C higher than their PU analogues, consistent with reduced segmental mobility driven by hydrogen bonding. Tensile testing demonstrates a tradeoff between strength and elongation. BPA-PU possesses the highest Young's modulus (2.7 GPa) and ultimate tensile strength (~59 MPa), while BGA-NIPU achieves the largest elongation (~95%) and highest toughness (~7 MJ m^{-3}) in comparison to its BGA-PU and BPA-NIPU analogues. Rheological experiments indicate methoxy and hydroxyl groups slow chain relaxation, although only methoxy groups improved dynamic fragility. Contact-angle measurements indicate that methoxy substitution enables advantages for adhesion to low-surface-energy substrates and improved solvent resistance in lignin-derivable systems, as demonstrated by increases in hydrophobicity and reductions in surface energy (<30 mN m^{-1}) in comparison to petroleum-derived counterparts. Collectively, these findings show that the strategic combination of lignin-derivable monomer design with NIPU chemistry provides a route to sustainable thermoplastics whose thermomechanical, rheological, and interfacial properties can be systematically tuned, highlighting their potential as processable, high-performance materials.[1]

[1] J. R. A. Walker, C. B. Thompson, G. W. Peterson, T. H. Epps, III, and L. T. J. Korley, "Elucidating the role of pendent functional groups in lignin-derivable polyurethanes," 2025. (in revision)

Developing mesophilic prokaryotic Argonautes as a biotechnology tool in eukaryotes for synthetic biology

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Gene editing has revolutionized countless industries, including healthcare, pharmaceuticals, and agriculture, while also serving as an indispensable tool in biotechnology and research. CRISPR/Cas9 is the current gold standard since it is easy to use and highly efficient. However, Cas9 requires a protospacer adjacent motif (PAM) site to identify its targets, limiting edits in regions where PAM sites are sparse (such as in the genome of the malaria-causing parasite, *Plasmodium falciparum*) or poorly positioned relative to the target site. To enable more universal editing, alternative platforms are needed.

Prokaryotic Argonautes (pAgos) are an attractive alternative, as they offer many of the benefits of Cas9 (e.g., easy reprogrammability through short single-stranded DNA guides) without known sequence limitations (i.e., a PAM requirement). To date, pAgos have only been used successfully for gene editing in bacteria, with studies in several eukaryotic systems being inconclusive. Using *Saccharomyces cerevisiae* as a model eukaryote, I evaluate the function of pAgos *in vivo* and develop strategies to enable eukaryotic genome manipulation via pAgos. Native activity of apo-pAgo involves pseudorandom DNA cleavage (“chopping”) of single-stranded or rapidly replicating DNA to generate ssDNA guides. These guides are then loaded to form holo-pAgo, which is then programmed for targeted cleavage of the complementary sequence with high efficiency. Expression of *Clostridium butyricum* pAgo (CbAgo) in *S. cerevisiae* results in chopping and reduced cell growth due to impaired DNA replication, confirming heterologous activity in eukaryotic systems. Currently, for all host species, guides must be produced via chopping or delivered exogenously, resulting in low efficiency, toxicity, and off-target effects. To address this, I am testing T7 RNA polymerases known to generate ssDNA for *in vivo* guide synthesis. To further improve targeting accuracy, I develop pAgos as a base editing platform by fusing an inactivated form of CbAgo to an adenine deaminase. I demonstrate a proof-of-concept pAgo base editor in *Escherichia coli* with preliminary results reaching editing efficiencies of ~6%. This work addresses key challenges in adapting pAgos for eukaryotic systems and positions them as a potential next-generation gene editing technology. pAgos’ unrestricted, precise targeting, coupled with its ease of reprogrammability, may advance biotechnology in areas such as agriculture, disease detection, and therapeutics.

Scalable Kolbe Electrolysis of Levulinic Acid via Alternating Polarity on Carbon Electrodes

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The transition away from petroleum-based feedstocks toward renewable alternatives is critical for achieving a sustainable chemical industry. Biomass-derived platform chemicals offer a promising route to produce monomers for next-generation plastics that are both renewable and amenable to chemical recycling. Levulinic acid, readily obtained from lignocellulosic biomass, can be converted to 2,7-octanedione via Kolbe electrolysis. 2,7-octanedione can be hydrogenated to 2,7-octanediol, a secondary diol monomer for polyester synthesis[1,2]. Conventional Kolbe electrolysis requires platinum electrodes and direct current (DC), achieving ~65% yield under optimized conditions[1]. However, platinum's high cost and susceptibility to passivation[3] have contributed to the limited industrial adoption of Kolbe electrolysis despite over a century of study.

Herein, we systematically investigate rapid alternating polarity (rAP) Kolbe electrolysis of levulinic acid using inexpensive reticulated vitreous carbon (RVC) electrodes, examining the effects of alternating frequency, applied voltage, base, and co-solvent addition. Optimization studies reveal that low frequencies (2.5 Hz) and moderate voltages (17 V_{pp}) maximize dione selectivity, achieving 68.5% yield at 98.2% conversion and 69.7% selectivity—surpassing platinum-based benchmarks. Among the bases screened, tetrapropylammonium hydroxide outperforms tetramethylammonium hydroxide and potassium hydroxide. Isopropyl alcohol can be incorporated as a co-solvent without compromising reaction metrics. RVC electrodes can be reused without loss of performance, and ongoing work on semi-batch and flow cell operations aims to facilitate scale-up. These findings demonstrate that rAP electrolysis on carbon electrodes offers a sustainable, cost-effective route to renewable monomers from biomass feedstocks.

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Genome Engineering of *Clostridium acetobutylicum* for Carbon-Neutral Isopropanol Production in a Synthetic Microbial Coculture

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Amidst the ever-increasing global greenhouse gas emissions and the ever-decreasing supply of fossil fuel resources, it is important to develop a carbon-neutral or carbon-negative chemical production platform. *Clostridium acetobutylicum (Cac)* has a long-standing history of biological ABE (Acetone-Butanol-Ethanol) fermentation from renewable sugar substrates, however, a major barrier to its implementation in large-scale fermentations is the loss of carbon sugar from glycolysis. To overcome this barrier, we constructed a mixotrophic coculture of solventogenic *Cac* with acetogen *Clostridium ljungdahlii (Clj)* that uses sugars, as well as CO_2 and H_2 gases released during glycolysis, to synthesize value-added chemicals. Exchange of acetone produced by *Cac* to *Clj* produces isopropanol, a novel product possible only by the coculture system. What remains key in the success of microbial chemical production is how to improve the yield of the desired product over competing by-products.

Genome engineering of *Cac*, including the deletion of competing pathways and enzymes to favor production of acetone, is our strategy. We employ several methods, including screening enzymes for acetone pathway production from multiple-copy plasmids to stable integration of constitutively expressed copies of the acetone operon in the *Cac* genome using CRISPR/Cas9. Through strong expression of key acetone pathway enzymes, particularly CoA transferase, we have seen marked increases in the acetone production in *Cac* monocultures. Our work demonstrates both synthetic biology and metabolic engineering strategies to maximize the reassimilation of CO_2 to favor isopropanol production. While our work on this microbial coculture serves as an example of carbon-neutral production of isopropanol, these same strategies can be more broadly applied to carbon-neutral production of other desirable chemicals.

Development of an inducible stable producer cell line for recombinant adeno-associated virus production

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Recombinant adeno-associated virus (rAAV) is emerging as a popular delivery vehicle for gene therapies. This viral vector is attractive due to its low immunogenicity, wide range of infectivity, and overall safety profile. Traditionally, rAAV is produced through a triple transient transfection of HEK293 cells, allowing for short-term production. Replication and capsid genes, Rep and Cap, respectively, are responsible for the replication and packaging of the payload into a viral capsid. However, problems such as plasmid costs, batch-to-batch variation, and misalignment of gene expression limit production efficiency. Stable integration of the genes required for rAAV production can address some of these issues, but cytotoxicity caused by continuous expression of these viral components has hindered stable cell line development. Ideally, a new inducible plasmid system could address cell line development and misalignment of expression obstacles.

In this work, we developed a proof-of-concept stable inducible producer cell line for rAAV production regulated by a single inducer molecule. We controlled the genes required for rAAV production via a Tet-inducible system. Utilizing site-specific integration, three plasmids were sequentially inserted into HEK293T genomic hotspots. Stable pools were generated, and clones were screened for successful integration and ability to produce virus before proceeding to the next round. A packaging cell line containing all required genes except the payload was created, with viral production within two orders of magnitude of the traditional production method. Similarly, a producer cell line with an integrated GFP payload was created, showing lower production than the packaging cell line but still within two orders of magnitude of the traditional method.

To further increase rAAV production in a stable cell line context, we implemented an oscillating degradation tag to control the toxic expression of Rep. Rep78 and Rep68 are viral proteins necessary for the replication of the payload during production. However, constant expression of these genes results in cell cycle arrest and apoptosis. By including a degron tag fused to the Rep gene, we can control the amount of Rep78 and Rep68 present in the cell and limit the apoptotic effects when they are greatest. This approach allows for rAAV production while reducing cytotoxicity and resuming cell division. We demonstrated the functionality of the oscillating tag with respect to Rep78 and Rep68 expression over time. We also compared rAAV production between cells with a degron-tagged Rep gene and an untagged Rep gene. We have shown there is no negative impact on transient virus production when utilizing the tag. Ongoing work is focused on determining the impacts in a stable expression context. Overall, these studies show the ability to develop inducible stable cell lines for rAAV production with site-specific integration, as well as the ability to control Rep gene expression in a transient manner.

Hybrid bacterial-yeast platform for epitope profiling

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Developing vaccines for antibiotic-resistant pathogens is limited by the low immunogenicity of conserved antigens. The non-standard amino acid (nsAA) *para*-nitro-phenylalanine (pN-Phe) can enhance immunogenicity, though its success depends on incorporation site and genetic background. Here, we present a hybrid platform pairing antigen-displaying bacteria with receptor-displaying yeast (e.g., MHC-II or nanobodies) to profile epitope variants. This approach aims to enable the identification of optimal pN-Phe sites for vaccine design and is extensible to other non-standard amino acids.

Initial constructs using the AIDA-I autotransporter resulted in high self-aggregation under peptide-MHC binding conditions, masking sequence-dependent effects. A systematic evaluation of alternative anchors showed that the intimin anchor provided the highest consistent display and minimized aggregation. Furthermore, while cargo-dependent self-aggregation persisted, we utilized orthogonal fluorescent reporters and microscopy to show that specific cognate antigen-nanobody pairs could be successfully distinguished from non-specific aggregation noise. We also investigated the constraints of nsAA incorporation. Profiling a small library of epitope variants revealed a strong dependence on position and codon context, with earlier positions tolerating pN-Phe better than later ones. Modifications to media composition and vector backbones further optimized these incorporation levels. To validate these findings, we are currently optimizing a workflow to cleave displayed peptides for mass spectrometry analysis. Finally, we addressed platform accuracy by characterizing non-specific bacterial-yeast interactions. We identified the bacterial fimH gene as a primary mediator of background binding to yeast mannose residues. A *fimH* knockout strain reduced non-specific binding to wild-type yeast, though interactions with glycosylated MHC-II remain complex. Overall, this study defines key design rules for using cell-based display to study the immunogenic effects of nsAA incorporation.

Bioinspired Microenvironments for Modulating Innate and Adaptive Immune Cell Responses and the Production of Cell Therapies

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The immune system is responsible for key functions in the human body, from serving as "first-responders" that stimulate downstream cascades for fighting foreign invaders to generating tolerance to non-pathogenic species and promoting tissue regeneration. While two-dimensional (2D) culture on tissue-culture plastic remains standard of practice for studies of immune cell biology, the field is shifting toward compliant 3D culture models. There is a critical need to generate physiologically relevant materials to better understand how the human immune system functions in native microenvironments and inform therapeutic designs. Synthetic bioinspired materials hold potential for generating such systems *in vitro* by stripping the complexity of animal models and providing consistent, cost-effective, and physiologically relevant approaches. In this work, we utilize a well-defined, biocompatible polymer, poly (ethylene glycol) (PEG), to prepare synthetic extracellular matrices with tunable biophysical and biochemical properties for directing immune activation. We specifically look at two essential players–macrophages of the innate immune system and T-cells of the adaptive immune system. Macrophages are key innate immune cells that serve as the first line of defense against foreign pathogens and particulates through phagocytosis and proinflammatory or anti-inflammatory signaling. Macrophages also generate a host of soluble signals that stimulate the adaptive immune system, allowing modulation of T-cells, which hold exceptional promise for cell therapy applications. Biotechnologies that leverage these types of cell-cell interactions in addition to cell-ECM interactions offer great promise for the scalable production of cell therapies.

More specifically, we induce and expand polyclonal regulatory T cells (Tregs), which are of high relevance for promoting tolerance in patients with autoimmune disease or undergoing organ transplantation but remain difficult to obtain due to challenges in biomanufacturing. Here, we present a promising tool for generating a T-cell population with high enrichment of regulatory T-cells using a scalable, bioinspired material functionalized with activating ligands CD3/CD28. Consistent with other reports, we showed that macrophages, specifically immunosuppressive macrophages (treated with IL10/TGFB), can be used to enrich Treg populations; however, activation with bioinspired synthetic matrices outperforms traditional activation platforms. Notably, T-cells activated on functionalized hydrogels have > 9-fold increase in Treg counts and enrich in Treg populations to > 50% compared to traditional activation methods. Our work demonstrates the importance of microenvironment cues in T-cell activation and differentiation and supports the relevance of these engineered biomaterials for production of Treg cell therapies.

Limiting and Controlling Post-Translational Modification of Nitrated Residues in Proteins

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Nitrated residues on proteins are of interest across fundamental and applied science. For example, tyrosine residues are prone to attack by reactive nitrogen intermediates to form *meta*-nitro-L-tyrosine (mN-Tyr), which is associated with inflammatory and autoimmune conditions. In addition, synthetic nitrated amino acids offer utility in biotechnology, and two examples are *para*-nitro-L-phenylalanine (pN-Phe), which can increase the immunogenicity of the protein that it is incorporated within, and *ortho*-nitro-L-phenylalanine (oN-Phe), which is a photo-responsive amino acid that can cleave adjacent polypeptide bonds upon UV irradiation. Each of these nitrated non-standard amino acids (nsAAs) can be supplemented exogenously to bacterial cell cultures and incorporated within proteins at sites of interest using genetic code expansion technology.

One major obstacle when working with nitrated nsAAs is the known reduction of many small molecule nitroarenes when supplemented to bacterial culture media given the existence of enzymes that function as nitroreductases, reducing the nitro group usually fully to an amino group. Relatedly, previous work has noted that certain photo-caged nsAAs incorporated within proteins have been subject to reduction; however, there has been no evidence provided thus far about whether this occurs pre-, co-, or post-translationally.

We recently conducted an extensive characterization of the stability of nitroaromatic compounds in *Escherichia coli* strains that were wild-type or engineered to lack numerous nitroreductases. In this work, we observed that exogenously supplemented nsAAs mN-Tyr, pN-Phe, and oN-Phe are not prone to notable reduction even in wild-type cells. However, we surprisingly observed that supplemented pN-Phe was prone to reduction after ribosomal incorporation within a translated reporter protein. We then showed that the use of *E. coli* hosts lacking key nitroreductases eliminates the reduction of incorporated pN-Phe, presenting the first evidence indicating that bacterial nitroreductases can act on, and may even prefer, nitro groups present on proteins. Through systematic analysis, we characterized the site-dependence of nitro reduction, and identified the specific nitroreductases responsible. We further extended this work to proteins containing *ortho*- and *meta*-nitro phenylalanine, as well as meta-nitro tyrosine, encompassing both photo-responsive nsAA and naturally occurring modifications.

Ensuring homogenous, site-specific incorporation of nitroaromatic amino acids is essential for generating chemically defined nitrated proteins, a requirement for reproducibility and for downstream applications such as vaccine development and photo cleavable protein engineering.

Elucidation of yellow mealworm gut microbiome polyethylene biodegradation pathways

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Only 14% of the 380 million tons of plastics produced annually is recycled, leading to substantial environmental accumulation. Conventional recycling depends on costly sorting, high temperatures, and harsh chemicals, limiting its sustainability. Biological degradation and upcycling offer a promising alternative by converting waste plastics into higher-value products. We investigate the yellow mealworm, which can degrade polyethylene (PE) over days rather than years. Metagenomic and functional evidence shows that PE feeding enriches microbial species encoding DyP peroxidases, an understudied enzyme family capable of initiating PE oxidation, a critical first step in its breakdown. In parallel, the mealworm host alters gut chemistry during PE feeding, enhancing oxidative conditions that facilitate polymer deconstruction. We also demonstrate that yellow mealworm guts can measurably decrease PE molecular weight, consistent with enzymatic oxidation–driven depolymerization. Building on this, we expand the set of candidate oxidative enzymes involved in PE deconstruction, including Baeyer-Villiger monooxygenases (BVMOs), which may participate in downstream oxidation or functionalization steps. Identifying and characterizing these enzymes provides a foundation for reconstructing and engineering a biological PE-degradation pathway outside the mealworm system.

Thermodynamic Limitations of Hydroxide Exchange Membrane Electrochemical Carbon Capture and Removal

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The transition away from a fossil fuel based economy to a circular carbon economy promises to significantly reduce CO_2 emissions, while simultaneously offering the ability to shore up the American manufacturing industry.[1] Circular carbon economies break the linear model of carbon extraction and release, by providing captured or removed CO_2 as a carbon feedstock, instead of fossil fuels. This is then converted into products, and the associated emissions are then further recycled or sequestered.[2] Key to this process is the capturing or removal of CO_2 (from point sources or the atmosphere respectively), without this, the process cannot be fully circular, as such, technologies must be developed that allow for scalable, energy efficient carbon capture and removal.[3]

Electrochemical carbon capture and removal (ECC) is a promising category of technologies that enable highly flexible carbon capture and removal with direct integration into renewable energy sources.[4] Much work has been shown on practical devices, measuring CO_2 flux, energy consumption, and electron efficiency; however, not much work has been done on the thermodynamic limitations of ECC technologies.[4–7] We developed a broad framework to understand the thermodynamic limitations of ECC devices through analysis of the dependence of potential on CO_2 concentrations. We applied this framework to a pH swing hydroxide exchange membrane (HEM) ECC system to determine the maximum electron efficiency and energy efficiency that a HEMECC system can achieve. We then extended this further to how a HEMECC system is limited by the presence of bicarbonate back-diffusion and ohmic overpotentials in a non-ideal case.

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Mediated oxidation in hydroxide exchange membrane water electrolyzers

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

The membrane of a HEMEL bears bound cationic groups, which conduct hydroxide ions from the cathode to the anode. Membrane degradation introduces two HEMEL failure modes: (1) performance loss due to decreased hydroxide conductivity and (2) increased crossover of product gasses between the electrodes – the later of which poses a major safety concern due to the wide flammability range of hydrogen in oxygen. Therefore, membrane durability is among the most important challenges for the viability of HEMELs. We have observed membrane degradation with the greatest severity near the HEMEL anode. This suggests that stress due to the highly oxidative environment at the anode may be a major cause for membrane degradation. Here we demonstrate evidence for mediated oxidation and discuss the possible role of reactive oxygen species in this membrane degradation process.

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Oxidative Dehydrogenation of Ethane using NO/O_2 Mixtures

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Alkane steam cracking is a crucial method of producing ethylene, but it accounts for over 260 MMt of CO_2 annually.[1] Alternatively, ethylene can be produced from the oxidative dehydrogenation of ethane which is less energy intensive and may reduce carbon emissions.

Here, we simulate using nitric oxide (NO) as a radical initiator for the homogeneous gas-phase oxidative dehydrogenation of ethane in a tubular reactor. Simulations are conducted using Cantera[2] and a reaction network, reaction kinetics, and thermodynamic data from an existing propane oxidative dehydrogenation model.[3] From the model, we identify two primary reaction pathways: the reaction is driven by OH radicals formed from H_2O_2 homolysis when NO is < 5% of the feed, and the reaction is driven by OH radicals formed from NO and HO_2 when NO is > 5% of the feed. Additionally, temperature and feed composition are optimized for a maximum ethane conversion of 53% with 90% ethylene selectivity. However, most NO radicals are consumed and form NO_2. Replacing NO in the feed with NO_2 was investigated; NO_2/O_2 mixtures optimized for ethane conversion achieve 38% ethane conversion with 90% selectivity and 5% NO_2 conversion.

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Photoactive beads for scalable wastewater treatment

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Bacteria constantly threaten human health, and research into new antibacterial materials is key towards providing pure drinking water for future generations. Photoactive materials provide an environmentally-friendly method for destroying bacteria through light-mediated reactive oxygen generation. Because reactive oxygen species (e.g. singlet oxygen) are capable of disrupting cell membranes indiscriminately, using them as heterogeneous antibacterial materials is a promising approach towards water purification. However, new scalable design strategies are required to efficiently incorporate photocatalysts onto solid supports. In attempt to meet this need, our group has synthesized antibacterial photoactive materials based on the organic photocatalyst tetrakis(4-carboxyphenyl)porphyrin by crosslinking it into particle-tethered polymer brush coatings. Successful coating on SiO_2 surfaces was confirmed via tensiometry, ellipsometry, and X-ray photoelectron spectroscopy. Singlet oxygen generation of the resulting photocatalytic materials was measured by the oxidation of dimethyl sulfoxide under blue light irradiation. Bactericidal activity against *E. coli* was verified through live/dead assays and cell viability tests.

Current efforts are focused on scaling these photoactive materials and efficiently leveraging the entire visible spectrum in wastewater remediation. Photoactive bead synthesis has been scaled up over twenty-fold since the material was first reported while simultaneously improving process safety and speed. A prototype packed bed photoreactor was constructed to show the photoactive beads can catalyze light-mediated pseudo-click chemistry in flow. Photocatalytic bead sizes were varied and mixed to study the effects of packing on reagent conversion. The second iteration of the packed bed reactor featuring metered flow control, broad chemical resistance, and modular process design will lead studies into packing, reaction concentration, and recycle optimization towards high overall conversion. In addition, new photoreactor designs are under development in collaboration with mechanical and biomedical engineers working on their respective capstone projects. Finally, new green light absorbing photoactive beads were developed based on perylene diimide photocatalysts. These green light-active beads were used successfully to degrade tetracycline hydrochloride, a drug linked to reproductive harm and long-term aquatic toxicity.

Distance-Aware Molecular Property Prediction in Nonlinear Structure−Property Space

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Molecular property prediction with limited data in novel chemical domains remains challenging. We introduce an approach based on the hypothesis that prediction difficulty increases systematically with distance from well-characterized regions in an appropriately defined structure−property space. Our framework combines nonlinear structure−property space embedding with distance-aware domain classification and un- certainty quantification. We create a structure−property embed- ding connecting molecular similarity with prediction difficulty, implement distance-aware classification balancing precision and true positive rate, and provide distance-based uncertainty estimates scaled by molecular similarity. Across four ecotoxicity data sets, our local models reduced root mean squared error by 28−48% for truly in-domain molecules compared to global models, with strong correlations (r = 0.40−0.62) between distance and prediction error. In a biolubricant base oil property application, our approach reduced prediction error by 29% compared to a global model and outperformed transfer learning and standard machine learning approaches. This framework's focus on relevant domains and distance-calibrated uncertainty estimates for limited, heterogeneous chemical data makes it broadly applicable across applications, such as toxicity prediction, drug discovery, and materials design.

Techno-economic assessment of propane dehydrogenation powered by solar heat

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Committee Members: Raul Lobo, Antony Beris

The energy-intensive chemical sector can benefit from diversified and localized energy resources amid rising demand. Concentrating solar power with thermal energy storage (CSP-TES) is a promising option that has been demonstrated at pilot scale for various endothermic processes, including steam-methane reforming[1] and ethylbenzene dehydrogenation[2]. In this modeling study, we examine the economics of a hybrid propane dehydrogenation plant that alternates between CSP-TES and natural gas combustion based on solar availability. We evaluate how plant location and CSP-TES capacity affect process-heat costs, the curtailment of solar energy (wasted solar input due to insufficient thermal storage), and the resulting contribution of natural gas. We assess whether the energy savings enabled by membrane reactors justify the higher capital cost and identify the minimum membrane performance (H_2 permeance) required to match the profitability of the non-membrane (fixed-bed-only) case. Finally, we compare heat-only use of CSP-TES with electricity cogeneration to assess whether onsite power generation improves profitability, which also lowers reliance on grid electricity. Our work highlights the key technical and economic limitations of solar-powered chemical processes and outlines future directions to enable their widespread adoption.

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A Real-Time Fluorescent Sensor for Visualizing Intracellular Acetyl-CoA Level Dynamics

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Committee Members: Aditya M. Kunjapur and Kevin V. Solomon

Sustainable manufacturing through metabolic engineering enabled the conversion of low-value feedstocks into value-added chemicals by highly accurate biological processes. Optimizing cellular resources for microbial cell growth and product synthesis remains a major challenge. Rapid and reversible metabolite sensing can reveal dynamic metabolic states with critical information that is key to optimizing pathway fluxes. While analytical methods can accurately quantify metabolite levels in cells, they exhibit slow turnaround time and lack feedback control modulations.

Here, we present a one-for-all strategy to develop a real-time fluorescent sensor for the key central metabolite acetyl-CoA, a precursor to diverse commodity chemicals such as fatty acids and terpenoids. We have successfully developed an acetyl-CoA Transient Sensor (ACTS) by engineering acetyl-CoA-responsive protein-protein interaction to generate real-time fluorescence responses based on acetyl-CoA changes. We achieved a 12-fold fluorescence increase at saturating acetyl-CoA concentrations in *in vitro* assays. Expression of ACTS in *E. coli* enabled real-time detection of acetyl-CoA dynamics in live cells. Calibration of ACTS signals with analytical measurements facilitates high-throughput real-time quantitative analysis of acetyl-CoA in cells. Real-time measurements allow investigation of acetyl-CoA dynamics under different metabolic states and troubleshooting of biosynthesis pathways during fermentation.

Understanding polymer-catalyst interactions for modeling plastics deconstruction

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

About 400 million tons of polymers are produced annually worldwide of which 30% contain polyethylene (PE) [1,2]. Plastics are often incinerated or discarded after use, with ~ 76% ending up in landfills or the oceans [1]. Mechanical recycling is a low-energy technology but degrades the polymer's quality and value. Catalytic conversion provides a path for tackling the global problem of plastics waste, allowing us to produce high-value products like gasoline, lubricants and waxes. Hydrogenolysis over metal sites and hydrocracking over metal and acid sites can convert PE waste by reacting the polymer melt with hydrogen over a catalyst surface. The product distributions depend on the location of C-C scission on a catalyst and, thus, on the conformation of the polymer on the catalyst surface. However, the effects of catalyst surface morphology on polymer melt adsorption are poorly understood.

Here, we study Pt surfaces of different terminations and polymers of varying chain sizes to probe the impact of terrace width on polyethylene (PE) conformations on the catalyst surface. We parametrize an atomistic force field using Density Functional Theory (DFT) calculations and perform Replica-Exchange Molecular Dynamics (RE-MD) simulations on the PE-catalyst systems. We find that the surface morphology alters the conformations of PE. Stepped surfaces favor shorter adsorbed segments but more such segments adsorb per chain. They also induce more ordering in the adsorbed segments than the flat surface, which has implications for the use of mean-field models for polymer adsorption on surfaces and their subsequent reaction. Even small length alkanes show a distribution in conformations on these stepped surfaces, unlike their behavior on flat surfaces. We discuss the ramifications of these findings for modeling polymer deconstruction processes on heterogeneous catalysts.

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

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Microenvironment Management of Cathode Catalyst Layer to Improve Electrochemical CO_2 Reduction

Izak Minnie
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Committee Members: Raul Lobo, Yushan Yan

The electrochemical reduction of CO_2 has the potential to address the growing issue of greenhouse gas emissions by converting the unwanted CO_2 into more useful products such as CO, formic acid, ethylene and acetate while utilizing the renewable energy sources such as wind and solar that are becoming cheaper and more widely available than ever before. While great strides have been made to get the current density (productivity), potential (energy efficiency) and faradaic efficiency (selectivity) to commercially viable levels, system stability and durability are still lacking. Much of the system instability is the result of poor water/gas management in the catalyst layer of the cathode gas diffusion electrode (GDE), where flooding and salt precipitation disrupt the triple phase boundary resulting in loss of system performance.

In this work we investigate how the use of porous additives in the cathode catalyst layer can lead to improved performance and delayed system failure. We consider two types of systems: a series of carbon additives ranging from non-porous Vulcan Carbon to highly porous lab synthesized three-dimensionally ordered (3DOm) carbon applied to a silver based CO_2 to CO electrolyzer. For the second system we consider zeolites, a well studied and tunable class of materials, as additives to the GDE. By changing the type, size and silica to alumina ratio of zeolites we can tune their porosity and hydrophobicity thus modifying the gas/liquid balance and catalyst microenvironment in the GDE. We find that zeolite addition can stabilize the system and result in lowering the competing hydrogen evolution reaction (HER), but that there are some tradeoffs in CO_2 product faradaic efficiency.

Inverse Design of Bioprinting Nozzles by Machine Learning

Juliana E. Nam
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Replicating natural tissue hierarchy is becoming increasingly important in many fields, especially in medicine, biotechnology, and synthetic meat production. One emerging technology for assembling three-dimensional, cell-laden constructs is extrusion-based multimaterial additive manufacturing, which incorporates multiple species within a single 3D printed object. Typically, 3D-printed "tissues" are constructed in a layer-by-layer manner, where fine nozzles are swapped each time a new bioink is introduced. Although this approach has been successfully used to fabricate emblematic assemblies (e.g., vasculatures, muscle fibers), individual nozzles must have rather large diameters or be operated at slow flow rates to prevent high shear stresses from killing cells prior to deposition, thereby constraining the size of biomimetic features. In the Bayles Laboratory, we are using principles of advective assembly (AA) to engineer the next generation of multimaterial 3D-printing nozzles. Advective assemblers contain sequences of fluidic junctions that align and multiply material by splitting, rotating, and adding laminar flows. The sequences contour co-flowing materials along streamlines and quickly extrude composite filaments with fine, flow-templated internal architectures. To date, advective assembly sequences have been engineered to assemble simple structures, such as stacks of layers and grids of fibers, as well as designer asymmetric structures, such as interdigitated layers and voxelated patterns. In this work, we demonstrate how architectures with complex, biomimetic hierarchy can be achieved by combining modular splitting, rotating, and adding junctions in novel sequences. To tap into the vast design space, we use convolutional neural networks (CNNs) to design the AA sequence required for a user-specified structure. Training datasets are generated using a rapid forward prediction methodology based on Boolean logic. The formalism casts junctions as AND, OR, and NOT logic gates, connects them in circuits, and computes a 2D truth table that maps the extruded architecture, eliminating the need for computationally expensive fluid dynamic simulations. The Boolean formalism reveals that over $5 \cdot 10^5$ unique architectures can be constructed from an AA nozzle composed of combinations of four\ junctions and inlets, each fed with matched viscoplastic inks at coarsely modulated flow rates. We trained and tested a CNN that specifies the combination of junctions and feeds to tune the feature distribution at a resolution of 4 μm with >80% accuracy. The application of this platform is illustrated for rapid printing of biomimetic constructs. Emblematic demonstrations include fabricating skin rete ridges from gelatin methacryloyl microgels laden with fibroblasts and macrophages and producing microbial mat mimetic cultures from viscoplastic poly(acrylic acid) suspensions inoculated with *Chlorobaculum tepidum*. It is envisioned that the modular AA printing platform will be a valuable addition to the bioprinting toolbox, unlocking new opportunities in engineered living systems.

Enhancing Ethylene and Propylene Production from Polyethylene Deconstruction via Rapid Pulse Joule Heating

Jacqueline Ngu
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Committee Members: Raul Lobo and Dongxia Liu

The growing volume of plastics waste, compounded with a low recycling rate, has led to an alarming amount of plastics ending up in landfills or being incinerated. While pyrolysis offers a route for plastic waste deconstruction, its product distribution is often broad and poorly controlled due to unselective radical chemistry at high temperatures. We recently demonstrated that rapid pulse Joule-heated catalytic cracking over HZSM-5, combined with small fractions of steam, can achieve high selectivity (>80 %) toward C_2-C_4 olefins, while significantly reducing coking compared to continuous Joule heating. Here, we investigate how acid catalyst properties, such as silica/alumina ratio, zeolite topology, and catalyst porosity, influence light olefin selectivity during polyethylene deconstruction via rapid pulse Joule heating. We demonstrate that silica-to-alumina ratios of ~30 yield high light olefin selectivity, and small-pore zeolites favor light olefins at the expense of increased coke formation. To mitigate coking, we synthesize HZSM-5 nanosheets and hierarchical zeolites (MFI, FAU, and CHA). These catalysts achieve an ethylene selectivity of approximately 35 %, a twofold increase over prior catalytic pyrolysis. Additionally, co-feeding steam and incorporating hierarchical porosity reduce coke formation and enhance catalyst stability

Developing the Non-Conventional Oleaginous Yeast *Yarrowia lipolytica* as a Monoterpene Indole Alkaloid Production Platform

Philip O'Dell
Advisor: Dr. Mark Blenner
Committee Members: Dr. Wilfred Chen, Dr. Aditya Kunjapur

Pediatric cancer medications, such as vincristine and vinblastine, are chronically in shortage[i] despite being listed on the WHO's List of Essential Medicines for Children[ii]. These compounds, among many other medically relevant chemicals, are part of a class of plant-natural-products called monoterpene indole alkaloids (MIAs). Current MIA production methods rely on precarious supply chains and yield expensive products. This not only leads to shortages of cancer medicines but also restricts researchers' ability to discover and characterize new compounds that may be medically useful for myriad ailments. Current engineering efforts to stabilize the supply of these compounds involve porting the metabolic pathways from the native plants into yeast cell factories[iii,iv,v] with most of the efforts being focused on the model yeast *Saccharomyces cerevisiae* because of its genetic tractability. However, the oleaginous yeast *Yarrowia lipolytica* is more naturally advantaged to produce these compounds, because of its high flux of precursor molecule acetyl coA, robust NADPH recycling system, and efficient cytochrome P450 enzyme expression ability [vi]. Therefore, developing *Y. lipolytica* as an MIA production platform may enable high-level production. This work seeks to engineer a strain of *Y. lipolytica* to biosynthesize strictosidine, the precursor to all MIA compounds, using an iterative random integration approach of the strictosidine pathway that leverages the native non-homologous end-joining (NHEJ) ability of the yeast. We seek not only to biosynthesize strictosidine but also halogenated derivatives to enable the production of halogenated MIAs, as halogen atoms are commonly used in drug development to improve the qualities of drug candidates [vi]. We will accomplish this by leveraging the dual input biosynthetic pathway of strictosidine that utilizes tryptophan. Tryptophan halogenases and promiscuous decarboxylases will be used in concert with the strictosidine biosynthetic pathway to generate halogenated strictosidine. This work advances the development of *Yarrowia lipolytica* as an MIA production platform, pushing toward a stable supply chain for critical medicines.

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Interfacial rheology and structure of a monoclonal antibody at air-water interface

Kiet Pham
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The structure and properties of the protein-enriched layers is of interest in many fields of science and technology, such as foamability and coalescence in food and emulsion science as well as stability in biopharmaceutical formulations. Due to the amphiphilic nature, protein molecules, specifically, monoclonal antibodies (mAb) adsorb to the hydrophobic air-liquid interfaces and often form viscoelastic films. As shown in previous studies, the interface induces the structural modification – such as unfolding, multilayer formation which generally cause destabilization and undesired aggregation in the formulation. Although the amount of adsorbed protein is relatively small compared to the total amount protein in the bulk solution, interfacial stresses coming from agitations or flows shed aggregates into the solution which could further serve to nucleate visible particles.

The quasi-2D mAb film encounters two types of interfacial deformation known as shear and dilatation. The viscoelastic response of the film to these deformations is relevant to the protein-protein interaction which is correlated to aggregation propensity of this type of protein. An important advance in interfacial rheology is the development of the Quadrotrough developed by Tein et al., an improved version of which, known as RheoSurfR is utilized in this study. The unique advantage of this instrument is the ability to measure linear rheological response of mAb film in both shear and dilatation modes *independently* in one sample environment, at multiple compressed states. We find out that the mAb is primarily elastic (solid-like) and the 2-dimensional Poisson ratio ν_{2D} decrease 0.9 to 0.4 upon compression indicating the low-shear resistance of the film. In complementary to rheological characterization, X-ray reflectivity (XRR) and Brewster Angle Microscopy (BAM) are used to study the out-of-plane and in-plane structure of the film, respectively. The BAM images confirms the homogenuity of film upon compression while the XRR data shows the extension and densification of the interfacial layer, along with significant desorption to the bulk solution of mAb driven by compression. These results provides insights into mAb behaviors at the air-water interface - commonly observed from the headspace or bubbles in vials, intravenous (IV) bags and syringes, which could possibly related to surface-mediated aggregation of this protein family.

Development and Engineering of the JumpIN Transposase for Improving Antibody Titers in Chinese Hamster Ovary Cells

Christopher Pirner
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Committee Members: Kelvin Lee and Kevin Solomon

Biologics, such as monoclonal antibodies, are novel drugs that can treat a variety of diseases and disorders. Chinese hamster ovary (CHO) cells are the preferred biological chassis for expressing these biologics because of their ability to perform human-like post-translational modifications, produce antibodies at g/L titers, and scale across different culture conditions. There are three common methods for integrating DNA into CHO cells: random integration (RI), site-specific integration (SSI), and transposase-mediated integration (TMI). RI can be used to make high-productivity CHO cell lines, but the resulting cell lines are often heterogeneous and unstable. SSI can be used to make stable cell lines, but they are often less productive than cells generated from RI. TMI offers a balance of high-copy, semi-targeted integration, enabling the generation of stable, high-producing CHO cell lines. However, the development of commercially viable, hyperactive transposases is often resource and time intensive.

We started with a novel, weak transposase from the brown plant hopper that we found to be active in CHO. To improve the transposition efficiency and gene expression, nuclear localization (NLS) tags were added to the N and/or C terminus of the transposase. We found that using both N- and C-terminal NLS tags improved the transposition efficiency threefold and increased gene expression 10-fold. Then, the mass ratio of transposase to donor plasmid was varied to optimize transfection conditions. To benchmark therapeutic production using JumpIN-mediated integration, a payload that expresses cNISTmAB was integrated using JumpIN and RI. JumpIN-mediated integration was found to improve antibody titers six-fold and specific productivity two-fold compared to RI. To further improve cNISTmAb expression, a small library of plasmids was constructed where the relative position of the selection marker was varied. Placing the selection marker between the light and heavy chain cassettes was found to increase the copy number and specific productivity two-fold.

In nature, transposases are often found in a structure known as a transposon, where the protein is flanked by its DNA recognition sites. If a transposon harbors an active transposase, the transposon integrates itself into the cell, whereas transposons with inactive transposases do not. We are looking to leverage this transposon structure to screen a large library of transposases that allow for a high-throughput screen of hyperactive JumpIN mutants. This work provides a framework for various optimization strategies that can be used to increase antibody titers using TMI, along with the groundwork to develop a high-throughput method to efficiently screen many transposase mutants.

A simple, low-cost technique to tailor structural and transport properties of ion-conducting polymer in anion exchange membrane water electrolyzers

Abigayle Polsky-Dowell
Advisor: Yushan Yan
Committee Members: Profs. Dongxia Liu, and Dion Vlachos

Hydroxide exchange membrane electrolyzers (HEMELs) enable hydrogen production using low-cost, earth-abundant materials. A central component of these devices is the ion-conducting polymer (ICP), which plays distinct roles depending on where it is incorporated within the device: at the electrodes, it functions primarily as a catalyst binder that protects fragile particles from mechanical degradation, while at the membrane it acts as the sole medium for hydroxide conduction and water transport. Relying on a single polymer to fulfill both functions is challenging, as effective catalyst adhesion requires a tightly interconnected polymer network, whereas efficient ion and water transport benefits from more open pores. This work provides a simple and low-cost processing strategy that tailors ICP networks for both needs. By using relative humidity (RH)-stabilized saturated salt solutions during polymer processing, we can modulate the amount of atmospheric water incorporated into the ICP, yielding polymer systems with unique electrochemical and physical traits, including ion conductivity, water diffusivity, and mechanical strength.

Bundle Up and Build: Coiled Coil Peptide-Polymer Building Blocks for Hierarchal Assembly

William Rears
Advisor: Dr. Christopher Kloxin
Committee Members: Dr. Alexandra Bayles and Dr. Arthi Jayaraman

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Bundlemers are a class of computationally designed coiled coil peptides that function as tunable molecular “Legos” due to their well-defined cylindrical geometries, interaction patterning, and charge states in aqueous solution. Although the innate charge of bundlemers provides a strong assembly driving force through ionic interactions, selective chemical modification of specific residues enables access to broader interaction landscapes. Grafting polymers from these modified sites further expands this tunability by allowing control over both new and existing molecular interactions through the choice of polymer identity, size, and grafting location. Using orthogonal and click-chemistry strategies, we synthesized an antiparallel, homotetrameric, positively charged bundlemer decorated with atom transfer radical polymerization (ATRP) initiators (one per peptide, four per bundlemer). Sodium pyruvate–mediated aqueous photoinitiated ATRP (SP-PhotoATRP) was then used to grow water-soluble polymers from the bundlemer core, yielding well-defined homopolymer-templated bundlemer stars. Polymerization control and kinetics were quantified by ^{1}H NMR spectroscopy and native aqueous size-exclusion chromatography with UV detection and multi-angle light scattering (SEC-UV-MALS), producing conjugates ranging from 20–300 kDa with low dispersity (<1.3). Absolute molecular weights were determined using a peptide–polymer compositional analysis protocol (ASTRA 8), allowing the peptide and polymer contributions to be decoupled.

After establishing the homopolymer-templated system, we targeted a more complex two-polymer-per-peptide “miktoarm” architecture. The bundlemer core was functionalized with disulfide-protected cysteine residues at the N-termini (top and bottom faces) and ATRP initiators at the same sites used in the homopolymer system (side faces). Nonresponsive 2-hydroxyethyl acrylate (HEA) was polymerized from the side positions and the resulting active chain ends were quenched. The protected cysteines were then deprotected, and maleimide-functionalized ATRP initiators were attached to the N-termini via thiol–maleimide click chemistry. Thermoresponsive N-isopropylacrylamide (NIPAM) was polymerized from these positions, generating an “ABA”-templated conjugate, where A is NIPAM and B is the bundlemer bearing HEA arms. The purified conjugate exhibited a thermoreversible lower critical solution temperature (LCST) of 34 °C as measured by variable-temperature UV-Vis spectroscopy and dynamic light scattering. Cast-film, negative-stain TEM revealed lamellar structures with ~6 nm spacing when cast below the LCST (25 °C) and micelles when cast above it (37 °C). Future work will explore the phase space associated with different polymer combinations and miktoarm bundlemer architectures.

Hydrolysis of Polyamide 6 to ε-Caprolactam over Titanium Dioxide

Pedro Moura
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Committee Members: Dr. Raul Lobo, Dr. LaShanda T. J. Korley, Dr. Dongxia Liu, Dr. Scott Wasserman

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Polyamides (PAs) are an important component of discarded textiles and food packaging. Chemical recycling can recover PA monomers, enabling repolymerization to produce virgin-grade PA. However, contemporary PA chemical recycling methods employ homogeneous catalysts that are hard to separate. We report anatase TiO2 as a catalyst for PA6 hydrolysis at 270 °C for 0.5 h, achieving a maximum ε-caprolactam (CL) yield of 81% (limited by thermodynamic equilibrium). The CL yield decreases upon catalyst reuse, due to loss of catalyst surface area induced by significant changes in catalyst crystallinity and texture. Pretreating the catalyst hydrothermally stabilizes it against morphological changes, yielding repeatable CL yields. Overall, this study discloses a heterogeneous catalyst capable of producing repeatable equilibrium CL yields via PA6 hydrolysis under industrially relevant reaction temperatures and times (<3 h, 250-330 °C).

Connecting Polymer Segmental Dynamics in Nanocomposites to Rheology with Dynamic Neutron Scattering

Jack Rooks
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Committee Members: Alexandra Bayles, Eric Furst

Polymer Nanocomposites (PNC) can enhance the mechanical properties over the host polymer, partially due to the effect of the polymer-nanoparticle interaction being magnified by the large surface area to volume ratio of nanoscale objects. Greater understanding of their microstructure and dynamics is needed to effectively design PNC with improved characteristics. A variety of polymer and nanoparticle dynamics are present in a PNC, and using a model system of well-dispersed elongated silica nanoparticles in strongly adsorbed poly(ethylene oxide) we explore the short time polymer dynamics as described by the Rouse model. The elongated nature of the nanoparticles is used to investigate the shape effects of nanoparticles with comparison to literature data for other silica particle systems. Quasi-elastic neutron scattering is used to obtain information about the polymer at the interface between the nanoparticles and bulk polymer. Interfacial polymer Rouse dynamics and the extent of the interface is obtained from two possible modifications of the Rouse model for the interface. The first of these suppresses longer range Rouse modes to account for additional topological constraint imposed by adsorption to the nanoparticle. The second of these modifications treats the interface as experiencing the same type of Rouse dynamics as the bulk, but on a longer timescale, indicated a higher relaxation time due to experiencing higher local friction. These results are connected to rheology to explain the large effect of a small amount of nanoparticles commonly observed in PNC.

Atomistic insights into the stability and reactivity of single atom catalysts

Sakshi Satyanand
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Committee Members: Prof. Antony N. Beris, Prof. Raul F. Lobo

Sustainability imperatives have spurred the development of isolated metal catalysts for a variety of reactions of economic importance. Transition metals (TM) deposited on thermally stable SiO_2 and γ-Al_2O_3 supports emerge as promising candidates for their activity, selectivity and efficient atom utilization. In this work, we employ electronic structure calculations via Density Functional Theory (DFT) to investigate the fundamental driving forces of reactivity and stability of three representative model systems: Fe@am-SiO_2, Rh/γ-Al_2O_3 and Pt/γ-Al_2O_3. The broad industrial relevance of these catalysts underscores the need for a molecular level understanding of their reaction energetics and morphologies–Fe@am-SiO_2[1] for alkane dehydrogenation to value-added alkenes, and Rh/γ-Al_2O_3[2] and Pt/γ-Al_2O_3[3] for their central roles in reacting with CO and NO in automotive fume exhausts.

For Fe@am-SiO_2, we consider the impact of Fe centers in oxidation states 2+ and 3+, and coordination environments consisting of silanol (SiOH) and silanolate (SiO^-) ligands on C-H activation. We report heterolytic C-H bond activation to be the dominant reaction pathway regardless of the metal's oxidation state, and that the rate determining step involves spin-crossing from the quintet into the triplet spin state. We further establish that σ-metathesis, namely ligand exchange between ethane and the metal-hydride, is not a viable catalytic route as it is energetically demanding. We also propose that synthesizing Fe3+ sites provides an energetically competitive route for C-H activation.

In supported TM/γ-Al_2O_3, we investigate the thermodynamics of support restructuring under varying coverages of carbon monoxide (CO), which is often used as a probe for catalyst surface characterization. We use DFT calculations to study the underlying mechanism of CO induced structural transformations of single atom and sub-nm clusters of Rh_n and Pt_n (n=1,4), supported on dry and hydroxylated γ-Al_2O_3. We show that the differences in the electronic interactions between the supported metal species and CO drive agglomeration or dispersion depending on the metal. CO forms a super-monolayer coverage on the TM cluster, and the resulting dipole repulsion leads to nanoparticle reconstruction. We further show that surface hydroxyls are not mere spectators but participate in the reaction with CO, which eventually influences the morphology of nanoparticles after carbonylation.

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Structural comparison of polysulfamide and polyurea melts: assessing similarities and differences using coarse-grained simulation

Jay Shah
Advisor: Arthi Jayaraman
Committee Members: Alexandra Bayles and April Kloxin

Semi-crystalline polymers contain both crystalline (rigid) and amorphous (flexible) domains, giving rise to unique thermal and mechanical properties. Commodity plastics such as polyurea exhibit this semi-crystalline morphology; however, they often pose challenges for easy degradation, highlighting the need for new, more sustainable alternatives. One such alternative for polyurea is polysulfamide. Polysulfamides represent a new class of polymers in which the carbonyl group (–CO–) in polyurea is replaced with a sulfonyl group (–SO –), producing a backbone that is structurally analogous to polyurea but chemically distinct. Owing to chemical similarity and the presence of hydrogen-bonding groups in both polymers, researchers are exploring polysulfamides as sustainable alternatives to polyurea. Our collaborators, Michaudel and coworkers at Texas A&M University have synthesized polysulfamides and demonstrated their high thermal stability, tunable glass transition temperatures, and environmentally friendly degradability. [*ACS Polymers Au* 2023, 3, 3, 259–266] To complement their experiments with computational work, a former member of my research lab developed a phenomenological coarse-grained (CG) model with implicit solvent to simulate polysulfamides and understand how hydrogen bonding between sulfamides drives chain assembly into structures with varying crystallinity depending on the choice of backbone segments. [*Macromolecules* 2023, 56, 13, 5033–5049] In my thesis work, I have extended this computational work towards improving the polysulfamide model from being phenomenological to being more realistic by mapping to atomistic details. Using such atomistically informed CG models, I have conducted simulations of polysulfamides in solutions [RSC Applied Polymers 2025, 3, 453–468] and melts [manuscript in preparation], and compared the structure and viscoelastic properties of polysulfamides with those of analogous polyurea systems. In this talk, I will present this molecular-level framework for understanding the similarities and differences between polysulfamide and polyurea melts, and how it provides insights essential for the design of polysulfamide-based materials with tunable structural and viscoelastic properties.

Harnessing Tangential Flow Filtration and Hydrogels to enhance Transduction and Select Distinct Populations for CAR T Manufacturing

Eric Slaughter
Advisors: April Kloxin and Catherine Fromen
Committee Members: Millicent Sullivan and Abraham Lenhoff

Autologous chimeric antigen receptor (CAR) T-cell therapies have become an important tool for the treatment of hematological cancers. Despite this, CAR T has faced limitations in usage clinically, owing to challenges in their manufacturing process resulting in high costs and variable product quality. Current manufacturing results in inefficient and costly use of viral vectors, with minimal control over viral integrations per cell—causing heterogeneous CAR expression and variable therapeutic outcomes. Moreover, existing methods lack precise control of the CD4/CD8 T-cell ratio, a critical determinant of overall treatment efficacy. In this work, we use tangential flow filtration (TFF) with a ligand functionalized hydrogel coated membrane (HCM) to evaluate the impacts of cell density and flow patterns for enhancing and optimizing transduction and selection.

To demonstrate the value of our platform for lentiviral transduction, we transduced Jurkats with a model lentivirus. Modulation of cell density enhanced transduction: 5x and 1.5x in the device in comparison to static and spinfection, respectively, at low multiplicities of infection. Furthermore, TFF transduction reduced the mean fluorescence intensity of transduced cells, indicating that the TFF enhances transduction while minimizing vector integrations per cell. Additionally, the scalability of this platform was examined, demonstrating the ability to decouple operating parameters for optimal transduction.

To evaluate selection with our platform, we tested enriching CD8 T-cells from a heterogeneously mixed population of primary human pan T-cells and transducing the selected population *in-situ* with CAR lentivirus. In the selected population CD8+ T-cells were enriched ~20-30% and the %CAR+ cells were enriched ~2x. Killing efficacy of these cells were then assessed and compared to static and non-enriched controls.

In summary, the use of TFF/HCMs enables more efficient use of lentiviral vectors and better control of cell therapy CD4/CD8 ratios, offering the potential to lower costs, increase accessibility, and increase efficacy of the therapy.

Immobilized Molten Salt Membrane for Ammonia Synthesis Membrane Reactor

Genevieve Yarema
Advisor: Dongxia Liu
Committee Members: Raul Lobo, Yushan Yan

Ammonia is one of the most widely produced chemicals in the chemical industry, used to manufacture fertilizers and many other chemicals. It is also a promising energy vector for hydrogen transportation and storage. However, the Haber-Bosch process, which is the traditional method of producing ammonia, is very energy-expensive. Although the reaction is thermodynamically favorable at high pressures and low temperatures, the process operates at high temperatures in order to be kinetically favorable. This results in a low single-pass conversion rate, so high pressures are required to shift equilibrium towards the product side and to separate the ammonia product by condensation. These harsh operating conditions require costly infrastructure and energy input. Membrane reactors, which integrate chemical reaction and selective separation in a single unit, offer a promising approach to overcome these limitations, by selectively removing the product to drive the reaction forward and permit product separation at lower pressures. Immobilized molten salt membranes operate at elevated temperatures and can separate ammonia from hydrogen and nitrogen with high selectivity even at low ammonia concentrations, making them promising for use in ammonia synthesis membrane reactors. This project investigates how ammonia concentration and temperature affect the separation performance of the immobilized molten salt membrane under different heating formats, with the goal of determining the optimal conditions for ammonia separation, and how the membrane affects the overall reactor efficiency.

Engineering a protease responsive protein nanoparticle for increased drug delivery specificity

Logan Yeager
Advisor: Wilfred Chen and Millicent Sullivan
Committee Members: April M. Kloxin and Catherine A. Fromen

Achieving cell specific cytosolic delivery of protein therapeutics would greatly expand druggable targets in cancer treatment. However, short serum half-life and membrane impermeability limit effective delivery. Protein nanoparticles help overcome these barriers by encapsulating the therapeutic of interest while also providing a modular material with tunable surface chemistry and stimuli responsiveness to enhance cell specific uptake. Encapsulin is a novel class of protein nanoparticles with high potential in protein drug delivery because the expression of the protein cargo and structural unit can be individually tuned by leveraging a non-covalent targeting peptide (TP)-based loading process. This TP motif has since been repurposed to load heterologous cargo including therapeutic proteins. In addition to its internal loading capability, the external surface of many variants of encapsulin has been decorated using C-terminal fusions of either whole proteins or bioconjugation handles for further decoration. These decoration techniques have enabled display of cell recognition motifs and enzymatic cascades, enhanced by the multivalency of the nanostructure.

With this work, we seek to expand the toolbox of encapsulin functionality by introducing protease-responsiveness to induce selective disassembly in response to the tumor microenvironment. Tumor relevant proteases such as matrix metalloproteases (MMPs) can introduce site specific cleavage of nanomaterials through incorporating one of the native peptide substrates of MMPs within the structural subunits. To enable larger cargos to be encapsulated, we selected one of the largest and most well characterized encapsulin variants to date as a starting point, which self assembles into a 42nm nanoparticle composed of 240 monomers. We then screened three possible locations in the monomer for cut site incorporation, assessing how this impacted the monomer's solubility during protein expression. To confirm assembly and surface modification capabilities the expressed variants were decorated with elastin like polypeptide (ELP) at a 10% density and purified through inverse transition cycling (ITC) purification. Based on gel densitometry, assembled capsids were recovered in the final purification product indicated by the presence of only unconjugated monomers and ELP conjugated monomers. This assembly was confirmed through negative stain transmission electron microscopy (TEM). We then assessed the proteolytic cleavage efficiency and how they were impacted by the location of the cut site within the monomer, identity of the protease, and sequences directly adjacent to the cut site to explore how we could modulate and potentially titrate this proteolytic susceptibility. Interestingly, upon cleavage of one of the cut sites, we observed that the nanoparticle remained intact, creating two new modifiable termini per monomer. Although unintended, we plan to harness this behavior to increase the overall valency of the nanoparticle for various applications including enzyme clustering and cell uptake epitope patterning to increase specificity of delivery. We also anticipate proteolytic susceptibility of encapsulin, in combination with other engineering approaches being actively explored in the lab, can be leveraged to induce controlled disassembly to facilitate highly specific cytosolic delivery of therapeutic proteins.

Resilient Supply Chain Design for Mixed Plastic Waste Management – an Adjustable Robust Optimization Approach

Zhifei Yuliu
Advisor: Marianthi Ierapetritou
Committee Members: Dionisios Vlachos, Athony Beris

Systematic approaches to designing plastic waste management networks are imperative to achieving sustainable treatment of post-consumer plastics and mitigating their environmental impacts. Prior studies have proposed optimization-based supply chain models for plastic waste for individual polymers and mixed plastic streams[1, 2]. However, most of these formulations treat system parameters as deterministic and do not explicitly account for disruptions, such as temporary facility shutdowns, interruptions in transportation and processing capacity.

In this work, we formulate the design of a regional mixed-plastic waste management network as an adjustable robust optimization problem[3] that explicitly models disruption-driven uncertainty. The resulting tri-level mixed-integer linear program follows a two-stage structure: "here-and-now" design decisions select the locations, capacities, and technologies of facilities at the first level before disruptions occur, while the "wait-and-see" recourse decisions optimize material flows at the third level in response to worst-case disruption scenarios, which is identified in the second level. Due to its tri-level nature, this kind of problem cannot be solved from off-the-shelf commercial solvers. We solve this problem using the column-and-constraint generation algorithm[4] and extend it to a bi-objective framework that captures trade-offs between economic and environmental performance. The methodology is demonstrated on a case study for the Greater Philadelphia region, including Philadelphia and neighboring counties in Pennsylvania, New Jersey, and Delaware. The network encompasses collection at municipal solid waste transfer stations, county-level sorting and preprocessing hubs, and end-of-life options—landfilling, incineration, mechanical recycling, and chemical recycling—for major plastic types. Disruption scenarios are modeled as county-level node failures, in which all facilities located within an affected county are forced to shut down.

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Shivam Barodiya	"From Complexity to Clarity: Simplifying CHO Metabolic Models with Model Based Design of Experiments" **Advisors: Marianthi Ierapetritou and Eleftherios Papoutsakis**
Maxwell Bobbin	"Microwave Heating Characteristics of Reduced Metal Oxides" **Advisor: Dionisios Vlachos**
Ming-Chen Chien	"Behavior of Heteroaggregates in Antibody Products" **Advisors: Abraham Lenhoff and Kelvin Lee**
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Reynold Gao	"Synthesis and Evaluation of Novel Gallium Hydrides for Near-Ambient Hydrogen Storage" **Advisor: Raul Lobo**
Raine Hagerty	"Developing Platforms for Characterization and High Expression of Cellulolytic Anaerobic Gut Fungal Proteins" **Advisor: Kevin Solomon**
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Pragati Muthukumar	"Platforms for Logic-Gated and Programmable Targeted Protein Degradation (TPD) Circuits in Mammalian Cells**"** **Advisor: Wilfred Chen**
Hong Nguyen	"The Impact of Extracellular Vesicles (EVs) on Cell Culture Performance: An Overlooked Component in Conditioned Media" **Advisors: Kelvin Lee and Eleftherios Papoutsakis**
Jacob Otolski	**"**Growth Factor Tethering with Collagen Mimetic Peptides for Chronic Wound Healing" **Advisor: Millicent Sullivan**
Hayeon Park	"Metabolite-responsive Protein Condensate for Dynamic Control of Metabolic Pathway" **Advisors: Wilfred Chen and Kevin Solomon**
Matthew Pitell	**"**Measuring Rough Particle Contacts with Optical Tweezers" **Advisor: Eric Furst**
Sriram Tendulkar	"Advancing Nanoparticle Surface Engineering for Enhanced Delivery to Triple-Negative Breast Cancer" **Advisors: Emily Day and April Kloxin**
Daniel Ude	"Developing Real-Time N-Glycosylation Control for mAbs Produced in CHO Cells" **Advisor: Kelvin Lee**
James Van Antwerp	"Engineering a CHO Cell Line Optimized for Non-Standard Amino Acid Incorperation" **Advisors: Aditya Kunjapur and Kelvin Lee**

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Jimmy Vu	**"**Developing and Modeling Recombinant Adeno-Associated Virus Production in Suspension HEK293 Stable Producer Cells" **Advisor: Kelvin Lee**
Alexander Wang	"Utilizing Bioprinted Synthetic Extracellular Matrices to Model Breast Cancer Dormancy and Probe Microenvironmental and Cellular Signaling Mechanisms" **Advisor: April Kloxin**
Jianbo Yang	"A Comparative Study on Polystyrene Decomposition via Radical Initiators in Batch and Semi-batch Reactors" **Advisor: Raul Lobo**
Samiha Zaman	**"**Developing Biosensing Platforms for Rapid Detection of Foodborne Pathogens" **Advisor: Kevin Solomon**
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