Project Descriptions for RISE 2025

1. **Project:** Catalytic oxidation of 3-hydroxypropionic acid
   Mentor: Seonyeong Kim
   Faculty Member: George Huber
   Location: University of Wisconsin Madison
   Prerequisites: Coursework regarding organic chemistry, catalysis, and reaction kinetics would be helpful.

   Project Description: This project aims to catalytically convert 3-hydroxypropionic acid to malonic acid.

   Skills Overview: The student will gain experience in using batch reactors for catalytic conversion, analytic skills for chemicals, and characterization of metal catalysts.

2. **Project:** Optimization of oleaginous yeast cultivation in lignocellulosic hydrolysate using a scale-down bioreactor
   Mentor: Shufang Huang
   Faculty Member: Brian Pfleger
   Location: University of Wisconsin Madison
   Prerequisites: Basic understanding of fermentation would be helpful.

   Project Description: This project will focus on optimizing oleaginous yeast (*Yarrowia lipolytica*) performance in a scale-down bioreactor. One of CABBI's goals is to transform plant biomass into sustainable energy sources that address societal needs. Achieving this requires scaling up bioprocesses, which involves translating lab-scale research findings into commercial applications. However, strains that demonstrate high performance in a laboratory setting often underperform in industrial environments. Scale-down bioreactors simulate the common stress conditions encountered in industrial processes. Therefore, by optimizing production using a scale-down bioreactor, we can better identify strains that are more resilient and effective at a larger scale.

   Skills Overview: The student will learn to operate various equipment, including bench-top bioreactors, HPLC, and GC-MS. They will develop skills in preparing and designing media, culturing CABBI yeasts at different scales, and optimizing processes (pH, temperature, pO_2) for higher yield and productivity through data analysis from previous experiments.

3. **Project:** Green solvent-assisted one-pot processing of bioenergy crops for production of fermentable sugars
Mentor: Tirath Raj
Faculty Member: Vijay Singh
Location: University of Illinois Urbana-Champaign
Prerequisites: Basic science knowledge and a motivation to learn the basics of chemistry, green chemistry, and biotechnology would be helpful.

    Project Description: Integration of green chemistry and modern biotechnology could help combat current climate issues. Natural deep eutectic solvents are considered "green solvents" and can be derived from natural precursors (e.g., sugars, lactic acid, glycerol, choline chloride) and can be employed for processing of CABBI crops for a safer environment owing to green chemistry principles.

    Skills Overview: The intern will learn to operate laboratory instruments (e.g., HPLC, TGA, UV-Visible, XRD) and software (e.g., Origin, MNova, Excel, Endnote etc.) for data interpretation. Additionally, they will learn project management, technical writing, presentation, and communication skills for academic, professional and scientific career development.

4. **Project:** Investigating genetic variation in Miscanthus for improved photosynthesis
Mentor: Steven Burgess
Faculty Member: Steven Burgess
Location: University of Illinois Urbana-Champaign
Prerequisites: None

    Project Description: The project will focus on using chlorophyll fluorescence imaging to screen Miscanthus genotypes for variation in photosynthesis. This has the potential to identify genomic regions influencing the efficiency of photosynthesis which could inform future efforts to improve biomass production.

    Skills Overview: The student will learn how to design and conduct an independent research project. They will gain experience in taking physiological measurements in a field environment, teamwork, and data analysis using the R programming language.

5. **Project:** Optogenetic control of enzymes in *Issatchenkia orientalis*
Mentor: Jaewan Jang
Faculty Member: José Avalos
Location: Princeton University
Prerequisites: General biology and bioengineering would be helpful.

   Project Description: This project aims to utilize optogenetic tools for metabolic engineering in *Issatchenkia orientalis*. This involves controlling the expression of key enzymes in a metabolic pathway using light.

   Skills Overview: The student will learn basic molecular biology, fermentation, and optogenetic techniques.

6. **Project**: Investigating how mineralogy affects the stability of newly formed mineral-associated organic matter in bioenergy crops (sorghum) grown under different moisture regimes
Mentors: Yuan Liu and Katerina Estera-Molina
Faculty Member: Jennifer Pett-Ridge
Location: Lawrence Livermore National Laboratory
Prerequisites: Courses in general biology or chemistry would be helpful.

   Project Description: Mineral-associated organic matter (MAOM) formation is fundamental to soil carbon (C) stabilization, and the stability of MAOM depends on the type of minerals that the carbon is associated with. This project aims to evaluate the biological stability of MAOM associated with four types of minerals that were harvested from a ^{13}C-labeling greenhouse experiment with oil-enhanced sorghum under two moisture treatments (drought vs. non-drought). We will conduct a laboratory incubation experiment to investigate the stability of newly ^{13}C-labeled MAOM on mineral surfaces and evaluate how the oil-enhanced sorghum and moisture regimes affect MAOM stability.

   Skills Overview: The student will gain experience conducting jar incubation experiments, processing isotopically labeled (^{13}C) mineral and gas samples, conducting statistical analyses, and analyzing data from jar incubation experiments and a ^{13}C greenhouse labeling experiment.

7. **Project:** Quantifying variance in water fluxes among bioenergy crops

Mentor: Michael Benson
Faculty Member: Carl Bernacchi
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: This project aims to understand how the biology of different crops affects spatial variability of sensible and latent heat fluxes. To accomplish this, we will install a series of Li-710 evapotranspiration sensors in miscanthus, sorghum, switchgrass, and maize fields at the University of Illinois Energy Farm.

Skills Overview: The student will learn and gain experience designing experiments and setting up research infrastructure, as well as in processing, analyzing, and interpreting data.

8. **Project:** Antisolvent crystallization to recover high purity bio-organic acids from real fermentation broth
Mentor: Somesh Mishra
Faculty Member: Vijay Singh
Location: University of Illinois Urbana-Champaign
Prerequisites: Basic mass and energy balances, chemical calculations, exposure to lab, and introductory computing skills would be helpful.

Project Description: The project aims to identify and optimize key process parameters to recover organic acid in high purity from real fermentation broth in batch reactors using antisolvent crystallization.

Skills Overview: The student will learn process design principles and apply them to the recovery of organic acids. They will also learn to select and apply appropriate analytical methods for the purification and quantification of organic acids. They will gain experience making relevant calculations and in using applications of R, Minitab, SuperPro designer, and potentially other programs.

9. **Project:** Lignin removal from biomass utilizing produced water- a byproduct of oil and gas production
Mentor: Somesh Mishra
Faculty Member: Vijay Singh
Location: University of Illinois Urbana-Champaign

Prerequisites: Basic mass and energy balances, chemical calculations, exposure to lab, and introductory computing skills would be helpful.

Project Description: Modern sustainability R&D policy promotes green extraction techniques. In this context, lignin removal from lignocellulosic biomasses utilizing produced water is the right call. Produced water is a by-product of oil and gas production units and is 10 to 15% saltier than seawater. Apart from that, the primary anions present in produced water are chloride (Cl^-), sulfate (SO_4^{2-}), and carbonate (CO_3^{2-}), while the primary cations are Na^+, K^+, Ca^{2+}, Mg^{2+}, and Ba^{2+}. Other constituents include soluble and non-soluble oil/organics; trace amounts of precious metals, particularly platinum group metals like platinum, palladium, and rhodium; suspended and dissolved solids; and other chemicals applied during production. Due to this, it is toxic, raising environmental concerns, and thus, it is a point of concern for states like Illinois, Texas, California, Pennsylvania, Colorado, and Oklahoma. Here, we will explore the use of produced water to removing intact lignin from lignocellulosic biomass as a practical application of this industrial biproduct.

Skills Overview: The student will learn about lignocellulosic biomass composition and valorization and water quality testing methods. They will gain hands-on experience with biomass pretreatment and learn about optimization techniques and their application in the context of process development. They will gain experience with R, Minitab, SuperPro designer, and other relevant software.

10. **Project:** Evaluation of nitrogen use efficiency (NUE) with engineered sorghum genes under nitrogen deficiency
Mentor: Lalit Dev Tiwari
Faculty Member: Li-Qing Chen
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: This project will focus on the analysis of sorghum nitrogen metabolism-associated or transport genes and on understanding how root and shoot communicate about nutrient availability at the whole-plant level and adjust nutrient acquisition to plant growth requirements and their influence on overall growth.

Skills Overview: The student will learn some molecular and phenotyping skills, such as polymerase chain reaction (PCR), gel electrophoresis, DNA isolation, stress treatment, and cloning (if required).

11. **Project:** Screening a CRISPRi library of *Issatchenkia orientalis* under various stress conditions to identify relevant genes
Mentor: Thasneem Banu Frousnoon
Faculty Member: Yasuo Yoshikuni
Location: Lawrence Berkeley National Laboratory
Prerequisites: Molecular biology would be helpful.

Project Description: *Issatchenkia orientalis* is a non-model yeast valuable for producing high-value products such as organic acids due to its tolerance to environmental stressors such as low pH. Besides low pH, *I. orientalis* can also thrive under other stress conditions, making it beneficial for industrial applications. However, the mechanisms these yeasts use to overcome stress conditions are not well understood. This study aims to subject a CRISPRi library-transformed SD108 to multiple stress conditions, assess the strain's fitness, sequence samples at various time points, and analyze the sequencing data to uncover the pathways that enable *I. orientalis* to tolerate various stressors.

Skills Overview: The student will learn to prepare different stress conditions such as osmotic stress and the presence of toxic compounds (HMF, furfural), transform CRISPRi library into *I. orientalis*, expose the subsequent culture to various stress conditions, measure yeast growth, extract DNA from yeast, run gels, amplify guide RNA regions, and map guides to target genes to identify candidates for various stress tolerances.

12. Project: Analysis of expression of NAD+ related genes in tandem with metabolic changes in *R. toruloides*
Mentor: Alexandria Murphy
Faculty Member: Melanie R. McReynolds
Location: Penn State University
Prerequisites: Biochemistry and basic microbiology would be helpful.

Project Description: This project aims to understand how expression of NAD-dependent genes changes in yeast over time. As these cells age, their bioproduct output does not remain consistent and this project will give insight into why.

Skills Overview: The student will learn basic microbiology and molecular biology techniques like qRT-PCR and RNA extraction. They will also gain experience with biochemistry assays such as metabolite extraction.

13. **Project:** Screening autonomously replicating sequences (ARS) for episomal plasmid development in non-model yeasts
Mentor: Hao Xu
Faculty Member: Huimin Zhao
Location: University of Illinois Urbana-Champaign
Prerequisites: Molecular and cellular biology and biochemistry is required.

Project Description: This project aims to screen for genomic autonomously replicating sequences (ARS) for the development of episomal plasmids, an invaluable genetic tool, in non-model yeasts.

Skills Overview: The student will gain experience in basic molecular cloning and yeast genetic manipulation.

14. **Project:** Analysis of expression of flowering time genes in *Miscanthus sacchariflorus*
Mentor: Dessiree Patricia Zerpa Catanho
Faculty Member: Erik Sacks
Location: University of Illinois Urbana Champaign
Prerequisites: Any lab class in which the student has been taught to use micropipettes or do PCR would be helpful.

Project Description: The project aims to validate genes that regulate flowering time in *Miscanthus sacchariflorus* in response to photoperiod changes. Candidate genes previously obtained by transcriptome analysis will be validated by qPCR.

Skills Overview: The student can expect to gain experience with total RNA extraction, cDNA retrotranscription, and qPCR. The student will learn to analyze the results and interpret them.

15. **Project:** Virus infection method to regenerate engineered tobacco to increase lipid
Mentor: Jia Dong
Faculty Member: Matthew Hudson
Location: University of Illinois Urbana-Champaign

Prerequisites: Experience with plant transformation and regeneration, gene cloning, and mass spectrometry would be helpful.

Project Description: This project focuses on regeneration of plants using a viral infection method to increase lipid production.

Skills Overview: The student will learn transformation, regeneration, gene cloning, and agrobacterium infection.

16. **Project:** Stomatal function on stem and pod of soybean
    Mentor: Shellie Wall
    Faculty Member: Tracy Lawson
    Location: University of Illinois Urbana-Champaign
    Prerequisites: None

    Project Description: This project will focus on determining the number and function of stomata on non-foliar (non-leaf) tissue in soybean. Stomata are the small pores on the leaf that adjust aperture to allow CO_2 uptake for photosynthesis, as well as water loss for evaporative cooling and the movement of nutrients up from the roots. However, the role of stomata on tissues other than leaves is unknown. The student will use thermal imaging and infrared gas exchange analyses to determine the role of stomata in the stems and pods of the soybean plant.

    Skills Overview: Students will gain experience using microscopy, thermal imaging, chlorophyll fluorescence imaging, and infrared gas exchange analyses, as well as data analysis and scientific presentation skills.

17. **Project:** Building genetic designs to improve crop water use efficiency
    Mentor: Hyun Jin Jung
    Faculty Member: Andrew Leakey
    Location: University of Illinois Urbana-Champaign
    Prerequisites: Basic knowledge of genetics and molecular biology would be helpful.

    Project Description: This project focuses on generating deletion and complementation lines for key stomatal and water use efficiency genes in tobacco.

Skills Overview: Students will gain hands-on experience in plant tissue sampling, DNA/RNA extraction, and various PCR techniques. The project will also involve CRISPR-Cas9 gene editing for precise genetic modifications and tobacco agroinfiltration methods to study gene function and expression. Students will acquire a comprehensive understanding of molecular tools and techniques used in plant genetic engineering.

18. **Project:** Metabolic engineering of *Rhodotorula toruloides* for triacetic acid lactone production
    Mentor: Longyuan Shi
    Faculty Member: Huimin Zhao
    Location: University of Illinois Urbana-Champaign
    Prerequisites: None

    Project Description: This project aims to enhance the production of triacetic acid lactone (a CABBI target chemical) in the oil-producing yeast *Rhodotorula toruloides* through genetic modification and fermentation optimization.

    Skills Overview: The student will gain hands-on experience in genetic engineering, optimization of experimental conditions, and interpretation of results.

19. **Project:** Detecting Miscanthus phenology and yield dynamics using satellite time series
    Mentor: Shah-Al Emrah
    Faculty Member: Emily Heaton
    Location: University of Illinois Urbana-Champaign
    Prerequisites: Beginner-level experience in remote sensing or GIS and programming would be helpful. The most important things are a willingness to learn and an interest in remote sensing, bioenergy, and sustainability.

    Project Description: This project aims to evaluate the phenology and growth cycle of Miscanthus by analyzing time series data from the PlanetScope satellite. It will characterize spatial and temporal trends across different experimental treatment levels and examine the relationship between phenological stages and variations in treatments. Additionally, the project will investigate how changes in spectral signatures correlate with yield, providing insights for optimizing sustainable bioenergy production.

Skills Overview: The student will gain experience analyzing remote sensing data, specifically by working with PlanetScope satellite imagery to process and interpret time-series data. They will develop skills analyzing vegetation indices. Additionally, the student will learn how to identify crop phenological stages and explore the relationship between spectral signatures, experimental treatments, and crop yield.

20. **Project:** Metabolic engineering and scale-up fermentation of *Issatchenkia orientalis*
Mentor: Daniel Tiem Bun
Faculty Member: Zengyi Shao
Location: Iowa State University
Prerequisites: Molecular biology would be helpful.

Project Description: This project will focus on engineering the yeast *Issatchenkia orientalis* to better produce a valuable compound more sustainably from sugar and then performing larger scale fermentation to assess scale-up potential and problems to be addressed.

Skills Overview: The student will learn techniques to culture and engineer a yeast strain to produce a specific compound, how to quantify the compound, and how to operate a bioreactor.

21. **Project:** Extracellular lipid production by oleaginous yeasts: a sustainable approach for industrial biorefineries
Mentor: Sujit Jagtap
Faculty Member: Chris Rao
Location: University of Illinois Urbana-Champaign
Prerequisites: Basic biology is required.

Project Description: This project aims to screen and identify oleaginous yeasts capable of producing extracellular lipids, specifically polyol esters of fatty acids (PEFAs), which are used as biofuel precursors and biosurfactants. Oleaginous yeasts usually store lipids intracellularly as triglycerides, necessitating energy-intensive extraction processes. Extracellular lipid secretion, on the other hand, provides a more sustainable alternative by simplifying downstream processing, lowering energy demands, and improving scalability. The study will investigate the structures of secreted lipids, confirm a proposed biosynthetic pathway for extracellular lipid formation, and engineer *Rhodotorula* yeasts to increase extracellular lipid production. These findings will emphasize the ability of renewable substrates to drive scalable and sustainable lipid production for industrial applications.

Skills Overview: The student will gain experience in microbiology, biotechnology, and synthetic biology, with an emphasis on molecular biology and metabolic engineering. They will learn important laboratory techniques such as electrophoresis, qPCR, DNA assembly methods like Golden Gate and Gibson assembly, genome editing with CRISPR-Cas9, and transcriptomics and metabolomics analysis. This will provide hands-on experience with advanced instruments such as HPLC, GC-MS, LC-MS, and high-throughput microbioreactors. Students will work on metabolic engineering projects that involve modifying bacterial or yeast genomes to improve biofuel and bioproduct production efficiency. They will also improve their data analysis skills by interpreting complex datasets to identify patterns in gene expression, metabolic pathways, and substrate utilization.