Research statement

Before considering the potential of life on other planets, it is crucial to understand the foundation of life on Earth. This entails investigating the evolution of microbial metabolisms and, consequently, of nutrient cycling on Earth. Information describing the conditions of life on the early Earth is found in two main datasets: the rock record (e.g., fossils, isotope fractionations, etc.) and extant biological molecular diversity (e.g., genomes, protein sequences, etc.). Both sources of data come with shortcomings, making it imperative in the field of astrobiology to integrate them to develop an understanding of the evolution of metabolisms on Earth. Proteins studies integrate these two datasets: enzymes catalyze reactions that produce biosignatures identifiable in the rock record, while enzyme protein sequences are preserved in modern-day microbial genomes. While studies have been able to utilize protein molecular sequences to estimate the timing and evolution of different metabolic pathways, these have relied on data collected in the present-day. Little work has been done to investigate the function of ancestral enzymes in relationship to the changing environment of the early Earth. More research is thus needed to determine if life has been using the same set of metabolic strategies throughout history, or if these mechanisms varied in the past.

Dr. Betül Kaçar's lab, based at the University of Wisconsin-Madison and part of the NASA Center for Early Life & Evolution, is spearheading the research investigating this question using methods of protein reconstruction and resurrection. In this approach, molecular models of protein evolution are applied to phylogenetic gene trees to infer ancient protein sequences (a method termed "reconstruction"). Once the sequences are determined, these ancestral proteins sequences can be synthesized in the lab and inserted into the genomes of a bacterial model using CRISPR (a method termed "resurrection"). The phenotypes of these ancestral proteins can then be studied *in vivo* under a variety of conditions mimicking those of the early Earth.

At Dr. Kaçar's lab, I will be joining a new project involving close collaboration with a group of PhD students and postdoctoral researchers in the lab. Specifically, my work will involve synthesizing a variety of ancestral enzymes involved in nitrogen cycling and reengineering these enzymes into the genome of the bacterial model *Azotobacter*. The phenotypes expressed by ancestral enzymes will be investigated by growing microbes in a variety of cultures that reflect chemical and thermal changes in the Earth's early environment. These chemical changes include altering iron, zinc, molybdenum, and copper abundances, all metals incorporated by important proteins in nitrogen metabolism whose abundances changed with the oxygenation of the early atmosphere. This work expands on my integrative senior exercise (where I investigated the influence of early changes in metal abundance on the timing of metalloprotein evolution) by testing *in vivo* the effect of altered metal abundances on ancestral proteins. Through this project, I will gain experience using wet lab techniques, which will supplement my computational background in research on the early Earth.