

My research career began with a Post-It. On the note, my freshman advisor had scribbled “FYRE,” a reminder for me to look into the First-Year Research Experience program offered through the honors college at the University of Oklahoma (OU). I was nervous to apply. As I had begun meeting fellow freshmen from other high schools, I realized I had arrived at OU with a disadvantage. A friend from a large school told me her favorite class had been biomedical sciences and I was shocked. I thought to myself, “A biomedical sciences class was offered as a *high school* course?” In contrast, I had come from a low-income rural town with two stoplights, more horses than people, and a high school with a graduating class of 135. Though I was hesitant to apply to FYRE, believing that my background was comparatively too limited, I remembered why I was pursuing a microbiology degree at OU. I had learned of the immortality of cancer cells in my biology class. I was interested in the implications and applications of this cellular endurance, yet unsure of how to channel my fascination—a similar captivation that had led to some of my peers already having done research for years. As a first-generation college student from that small town, I had very few opportunities to develop my curiosity, yet I knew I wanted to study the microscopic world in order to help people. I had left my high school with ambition to become a microbiologist and I thought (naively) that I would be on my way to curing cancer, although I had no real-world experience in the field. Suppressing any doubts, I became determined to take advantage of the research opportunities available at OU and see what I could accomplish. So I applied for and was accepted into the FYRE program.

RELEVANT BACKGROUND AND INTELLECTUAL MERIT

Through FYRE, I worked in the biochemistry lab of Dr. Chaunbin Mao. Although this was not the microbiology lab where I would tackle all of the problems I was hoping to solve, I enjoyed my project creating nanoparticles. I got to see firsthand the gold nanorods I made—albeit misshapen, misaligned, and unable to be used downstream. This was a part of science I grew familiar with: *what went wrong and why?* Each unsuccessful nanoparticle synthesis helped me determine the chemicals in the seeding process that influenced the size and shape of the nanoparticles. As the nanoparticles began to improve, I read about the implications of my research. High-quality nanorods would be slated for “theranostic” purposes—both *therapeutic* and *diagnostic* with regard to cancerous tissue. This is where I began to realize what research really is: small steps toward solving a problem by contributing to the information collective. I found it enthralling that there were countless possible avenues of research, each with its own merit and each available for exploration. When my semester in the Mao lab was over, I knew I had to find a way to keep doing laboratory work and find my own personal research niche.

I began work in the lab of Dr. Elizabeth Karr in August 2014, where I have continued research through to the present. I have worked on several projects with the overall goal of further understanding transcription regulators within microorganisms, including hypervirulent *Clostridium difficile*, a human gastrointestinal pathogen of high medical concern, and methanogenic archaea (methanogens), a comparatively unexplored yet ecologically and economically significant branch on the tree of life.

My first project in the Karr lab was to investigate the importance of cysteine residues in the activity of the *Methanosarcina acetivorans* transcription regulator MsvR, an oxidative stress response protein exclusive to methanogens. Understanding how these anaerobic organ-

isms respond to environmental stress and regulate their metabolism accordingly is important because the methane they produce via methanogenesis accounts for a sizeable portion of atmospheric methane, a potent greenhouse gas contributing to climate change. Moreover, controlled methane production can be advantageous as bioenergy. Thus the manipulation of methanogenesis is critical to develop methods that regulate methane production. This may be accomplished through investigations into the gene regulatory networks that govern methanogenesis in order to determine precise targets for potential modifications. My projects with MsvR were accomplished through site-directed mutagenesis, cloning, recombinant gene overexpression, protein purification, X-ray crystallography, methylotrophic culture maintenance, and *in vitro* functional assays.

As I became more proficient at molecular biology techniques, I was given projects that afforded more independence. My current research for my honors thesis is investigating a two-component regulatory system within *Methanococcus maripaludis*. This organism is a model methanogen with its genetic tractability and relatively fast growth rate. The two-component system I am studying—part of the gene regulatory network of methanogenesis—is hypothesized to regulate the formate dehydrogenase genes in *M. maripaludis*. These genes are required for the use of relatively inert liquid formate rather than combustible hydrogen gas as the electron donor necessary for methanogenesis. Not only may the results of this research influence and improve methods of studying *M. maripaludis*, it will also explore the gene regulatory network that governs methanogenesis. Through this project, I have learned hydrogenotrophic culture maintenance and growth assessments. Once the project is complete, I will have learned *in vivo* cloning, transformation, and RNA preparation and analysis techniques. I presented my preliminary results at the Oklahoma LSAMP (Louis Stokes Alliances for Minority Participation) 22nd Annual Research Symposium and will present at the 2017 annual American Society for Microbiology branch meeting.

Another project I am assisting with in Dr. Karr's lab is the structural and functional investigation of transcription regulators within hypervirulent *Clostridium difficile*—proteins that are predicted to regulate genes that allow *C. difficile* to thrive in the human gut. Three-dimensional protein structures of two transcription regulators I worked with were obtained using X-ray crystallography. **This work will result in a publication.** I presented this research at two symposia hosted at the University of Oklahoma: the 4th Annual Symposium on Structural Biology and the 1st Annual Undergraduate Research Summer Symposium. This project was part of a collaboration between the University of Oklahoma and the Albert Einstein College of Medicine (AECOM) funded by the Price Family Foundation. Through this collaboration, **I was a summer research intern at AECOM**, learning high-throughput sample processing and anaerobic X-ray crystallography.

BROADER IMPACTS AND FUTURE GOALS

Methanogens have closely held my research interest. I came across my specific research goals while doing a growth curve for *M. maripaludis*. I had been in the lab for 19 hours, waiting for my cultures to reach stationary growth phase, when I thought, “I wish these grew faster!” I plan to optimize methanogens for research and industry by researching varied genetic and environmental manipulations (such as identifying ways in which to increase growth rate). **My current end goal is to be a professor at a research university**, where I would be in charge of a molecular biology lab course to teach students problem-solving and

laboratory skills and prepare them to be self-sufficient scientists. In my own research lab, I will be able to mentor students and promote outreach for science and research. Based on my current efforts and future goals, I believe that effective outreach should encompass initiation, advancement, and broadening of scientific interest. **As a female scientist from a low-income high school and of Indian heritage, I am also committed to extending that outreach to students from underrepresented groups.**

The Karr lab encourages outreach to high schools and undergraduates, which includes high school class visits to the lab and mentoring students from high school, FYRE, and NSF Research Experiences for Undergraduates (REU). In my 2.5 years in the lab, **I have helped advise and train these students**, and plan to continue this legacy throughout my graduate and postdoctoral career and into my future independent lab. Because I am passionate about offering research opportunities to those who otherwise may not be able to have the experience, I will host NSF-REU and undergraduate students and connect with local high schools to initiate class visits, shadowing opportunities, and internships.

In order to encourage students interested in sciences to pursue research experiences, I am also involved in the newly established Scientific Research Discovery and Discussion group at OU, where students are exposed to research options and can ask questions about undergraduate research from upperclassmen like myself. Because of my positive experience in FYRE, I help in outreach efforts to get more students interested in the program. I also participate in outreach for the OU branch of LSAMP, an organization that promotes STEM involvement for minority students. Moreover, I intend to be an LSAMP mentor in my future lab.

Research dissemination is important in promoting sharing of techniques, recipes, methodologies, and research impacts. This sharing of research sparks curiosity and aids in the advancement of research. One way in which I help share scientific knowledge developed in the Karr lab is by assisting in **updating the Karr Lab website with information about our research and methodologies** in anaerobic microbiology and molecular biology. I plan to develop this mode of outreach to include instructional videos, discussion forums, and interactive lessons. Broadening and advancing interest in research is also why I attend the Norman Public Library science café, an organization where people of all backgrounds participate in discussions about various topics in science.

I will speak about my research with methanogens to the biology classes at the high school from which I graduated. The exposure to research, especially performed by an alumna at the university level, would be beneficial to those who may not have known of research opportunities otherwise. I hope that these students will see higher education as an option and become interested in its pursuit. I also plan to continue visiting these classes each year throughout my graduate career.

I am confident that the skills I have attained and continue to develop—both inside and outside the lab—will help me succeed in a research career. Receiving the NSF-GRFP will greatly facilitate this process by allowing me to spend more time focused exclusively on my research rather than splitting time between research and serving as a teaching assistant. I am excited about the possibility of utilizing the Graduate Research Opportunities Worldwide (GROW) program to collaborate with international labs studying methanogens. I also look forward to the professional development opportunities available through the Graduate Research Internship Program (GRIP) which will broaden my research experiences.

INTRODUCTION

Biogenic methane (biomethane) accounts for approximately three-quarters of atmospheric methane, a potent greenhouse gas that is increasing in parts per million annually¹. Production of biomethane (methanogenesis) is due primarily to methanogenic archaea². While biomethane is a contributor to climate change, and its reduction in the atmosphere could prove beneficial, it can also be utilized as a biofuel³. The manipulation of methanogenesis is thus an important tool for developing methods to control biomethane production.

A crucial first step is elucidating the gene regulatory network (GRN) that governs methanogenesis. Prior studies have proposed a GRN within *Methanococcus maripaludis*⁴. The focus of my proposed research is on the regulation of formate dehydrogenase genes (*fdh*) in *M. maripaludis* (MMP), whose genome contains two sets of functional *fdh*^{5,6}. These genes are required for the use of formate rather than hydrogen gas (H₂) as the electron donor in reducing carbon dioxide (CO₂) to biomethane⁶ (Fig. 1). Shortly upstream of one set of *fdh* is a two-component regulatory system⁵. Two-component systems, which sense and transmit environmental signals, are comprised of a sensory transduction histidine kinase and a response regulator receiver protein, corresponding to MMP1303 and MMP1304, respectively⁷. This system is not involved in chemotaxis⁵; thus **the biological role of the MMP1303-1304 two-component system is unclear** and is the primary focus of this research plan.

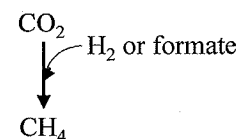


Figure 1: A simplified pathway for methanogenesis.

CH₄ = biomethane.

PRELIMINARY DATA AND HYPOTHESIS

My preliminary data have shown that part of the MMP1303-1304 system is vital for the growth of *M. maripaludis* on formate. A strain with the MMP1304 gene deleted (Δ MMP1304) showed a marked inability to grow on formate in comparison to wild-type (WT), yet this impairment is not demonstrated when grown on H₂ (Fig. 2).

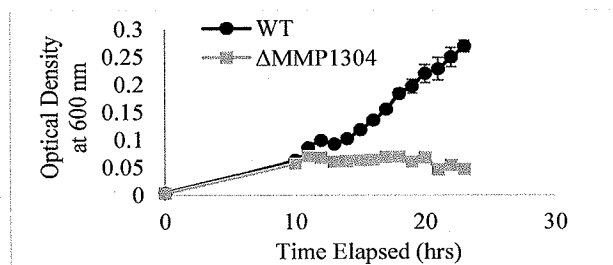


Figure 2: A growth curve of WT and Δ MMP1304 grown on formate.

Previous studies have shown that *fdh* genes are repressed by both the presence of H₂ and the transcription regulator MMP1100^{4,6}. **I hypothesize that MMP1303-1304 represent the initial step in the GRN by controlling expression of *fdh*.** My proposed mechanism of regulation is shown in Fig. 3. MMP1303 would sense H₂ concentration and phosphorylate MMP1304 accordingly. As MMP1304 does not have a DNA-binding domain necessary for direct interaction with DNA, it is possible that phosphorylated MMP1304 induces a conformational change in or directly interacts with MMP1100, which results in the derepression of *fdh*.

RESEARCH PLAN

Aim I: Determine the relationship between MMP1304 and MMP1100.

As shown in Fig. 3, *I hypothesize that MMP1304 directly interacts with MMP1100 to modulate transcription regulation.* To determine this potential interaction, affinity purification using tagged MMP1304 and subsequent mass spectrometry will reveal if MMP1100 is

in complex with MMP1304 during growth on formate.

Aim II: Explore the role of MMP1304 in the *M. maripaludis* gene regulatory network.

RNA-seq will be used to show levels of gene expression for each *M. maripaludis* strain. Comparisons between the expression patterns of WT with those of Δ MMP1304 will reveal the genes that are differentially expressed due to lack of regulation by MMP1304. The differential expression of these genes will be corroborated by qPCR. *I predict that these genes include fdh.*

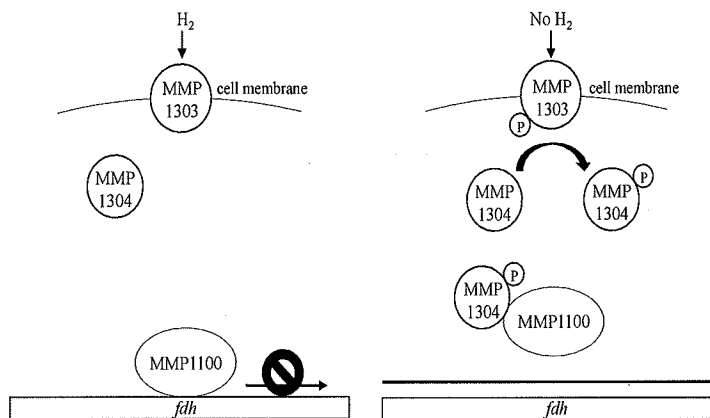


Figure 3: The proposed mechanism of *fdh* regulation during growth on H₂ (left) and on formate (right). P = phosphate; arrow along *fdh* = transcription.

BROADER IMPACTS AND INTELLECTUAL MERIT

M. maripaludis is a model organism for investigations into methanogenesis due to its relatively quick growth rate, fully sequenced genome, and genetic tractability⁸. It also possesses industrial applications in the metabolic production of other valuable compounds (e.g. geraniol, corrinoids, vitamins, amino acids^{8,9}). My research may contribute to understanding and unraveling the GRN of methanogenesis. It may also impact use of *M. maripaludis* in research and industry, as liquid, inert formate is often the growth substrate of preference over gaseous, combustible H₂.

I have successfully completed gene recombination and affinity purification, so I will be able to use these skills during the proposed research. The University of Oklahoma has facilities to perform mass spectrometry. I will prepare RNA for RNA-seq and learn how to examine the results under the guidance of a postdoctoral researcher in the lab.

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