Gentile group research projects, summer 2024

Background: Malate dehydrogenase (MDH) is an enzyme that catalyzes the reversible conversion of oxaloacetate + NADH to malate and NAD^+. It is an enzyme involved in the citric acid cycle, gluconeogenesis, and the malate aspartate shuttle. We are working towards understanding how MDH binds to other molecules- both small molecules as well as larger proteins. To do this, we propose using tryptophan fluorescence to monitor the conformational change that happens upon binding to MDH. Human mitochondrial MDH (hMDH2) does not have any tryptophan amino acids in its primary amino acid sequence.

1. **Fluorescent hMDH2**: CSBSJU students in my lab have been working to add a tryptophan amino acid to hMDH2 so we can use it to detect and quantitate binding between hMDH2 and other small molecules and proteins. We have created one mutation, H108W (histidine 108 changed to tryptohan) that has good fluorescence and has native-like specific activity. We have used this mutation to detect binding to NADH and have determined its Kd value. We are working to create other tryptophan mutations that will allow us to detect binding to other small molecules that bind to hMDH2.

2. **hMDH2 binding to CS**: Now that we have been able to detect hMDH2 binding to the small molecule NADH, we want to detect and quantitate its binding to citrate synthase (CS), the next enzyme in the citric acid cycle. Detection of hMDH2 and CS protein-protein binding would provide evidence for a metabolon being formed between hMDH2 and CS, which would be an efficient way for a product of one reaction to be handed to the next enzyme in a metabolic pathway to be used as its starting material.

3. **Understanding hMHD2 mutations leading to pediatric epilepsy**: A paper published at the end of 2022 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9676216/pdf/main.pdf) shows mutations in hMDH2 leading to a particular type of pediatric epilepsy. With the work we have done, we aim to ask if these mutations either (i) lead to an enzyme that does not have native-like activity to catalyze its reaction, and/or (ii) lead to an enzyme that cannot bind to citrate synthase (CS), the next enzyme in the citric acid cycle. Either case would cause metabolic problems.

This lab is a protein biochemistry lab. In it you'll learn how to visualize protein structures using PyMOL, mutate plasmids to create specific mutant proteins, over-express and purify proteins, determine specific activity of proteins, measure the fluorescence (and CD) of proteins, and determine binding constants of proteins.

In addition to the opportunity to do summer research, opportunities exist to continue/start research during the academic year (for course credit (Chem-215 and/or Chem-330) or as a volunteer). Students have the option to present their research on campus (CSC Day in April and at the end of summer research), at local meetings (Minnesota Academy of Sciences Winchell Symposium of Undergraduate Research in April), and at regional or national meetings (usually the American Chemical Society Meeting (ACS) or the American Society for Biochemistry and Molecular Biology (ASBMB)) depending on their project progress.