1. **Protein engineering of a spectroscopic probe into malate dehydrogenase.** Have you ever wondered how the product of one enzyme catalyzed reaction in a metabolic pathway makes its way to the next enzyme in the pathway to serve as the starting material for the next reaction? A metabolon is a multi-enzyme complex that allows the product of one reaction to be channeled directly to the active site of the next enzyme in the pathway. It has been proposed that malate dehydrogenase (MDH) and citrate synthase (CS) form a metabolon in the citric acid cycle. To be able to detect this, we need to be able to monitor conformational changes in MDH. In proteins, the amino acid tryptophan (trp) is the best spectroscopic handle, allowing for fluorescence visualization. MDH does not have any trp residues. Our goal is to clone MDH mutants that have specific amino acids mutated to trp so that it has native binding activity and can be visualized by fluorescence spectroscopy. Our best effort (work from Sonja Hoversten, Will Yu, and Morgan Fiereck) thus far is the design of a fluorescent mutant from watermelon that is ~25% as active as the wild type enzyme. Using the design principles discovered in that work (distance from the active site, shape/size of the amino acid to be mutated, location in stable secondary structure), our goal is to create a trp mutant of human mitochondrial MDH to be used in metabolomic studies. Students involved in this project will learn how to visualize proteins with PyMOL, design and create protein mutants, over-express and purify proteins, determine protein specific activities, and monitor protein conformational changes via fluorescence spectroscopy.

2. **Determination of the function, and identification of the small molecule inhibitors, of SARS-CoV2 ORF8.** The SARS-CoV2 viral protein encoded from open reading frame 8 (ORF8) is an accessory protein that has been shown to directly interact with major histocompatibility complex-1 (MHC-1) and downregulate their surface expression, thereby weakening immune surveillance. It has also been shown to interact with 47 human proteins. In addition, deletion of the ORF8 gene leads to milder COVID symptoms and better disease outcomes. As such, it is an interesting drug target. Anna Nguyen, a student from my group took a computational approach to defining a pharmacophore for binding to ORF8 and identified 4 lead compounds which could bind to ORF8. One of the repurposed drugs identified, novobiocin, showing a micromolar dissociation constant to ORF8, is particularly promising given its role in disrupting histone-histone association and blocking chromatin formation. In addition, the binding site of novobiocin on ORF8 is shown to include both a critical arginine as well as the histone H3 mimic ARKS (alanine-arginine-lysine-serine) motif. This data suggests that ORF8 has a role as a histone H3 mimic. Our goal is to provide additional evidence for this. Towards that end, Ryan Chiu has made mutants of the arginine in the ARKS motif to explore its role in binding to ORF8. With Ryan graduating, the next student interested in working on this project will over-express and purify these mutant proteins and use fluorescence spectroscopy to determine their binding to ORF8. Results from these studies will likely lead to further computational modeling as well as the design of additional sets of mutants to probe the function/importance of SARS-CoV2-ORF8.

3. **Exploring similarities of enzyme mechanisms for effective use of repurposed drugs.** When SARS-CoV2 became a pandemic in the spring of 2020, it was necessary to test known drugs that had previously been explored/used as therapeutics for other conditions (i.e., repurposed drugs) to see if they would be effective against SARS-CoV2. This led us to ask the question about how close enzyme mechanisms must be to effectively share effector molecules. Jake Minkkinen, Evan Spevacek, Ryley Nelson, and Joe Butterfield have been exploring this question with SARS-CoV2 and HIV-AIDS. The SARS-CoV-2 main protease (Mpro) is a cysteine/histidine protease and the HIV-1 protease is an aspartyl protease. Are there enough similarities between the two that new and repurposed SARS-CoV-2 Mpro inhibitors will also bind to, and inhibit, HIV-1 protease? Jake, Evan, Ryley, and Joe have found many SARS-CoV-2 Mpro inhibitors that computationally show binding
to HIV-1 protease. They have over-expressed and purified the recombinant HIV-1 protease and are exploring binding by fluorescence spectroscopy. Our goal is to continue to explore the question of how close enzyme mechanisms must be to effectively share effector molecules, either with the HIV-AIDS/SARS-CoV2 system or with others of student interest. This is a joint computational/experimental project, which is flexible based on your interests.

4. **Computational exploration of protein-ligand binding/pharmacophore generation/experimental verification of binding.** If you are more advanced in your background and are interested in pursuing an extended research project focused on a particular protein, we can design a project that will allow you to explore things like the structure, conformational changes, ligand binding of wild type vs. mutant proteins. This project could involve computational work with: POCASA (a program that identified potential binding surfaces on proteins), SwissDock (a program that docks particular ligands to a specific protein), LigandScout (a program that helps define pharmacophores for binding to a particular protein binding site), the PDB (protein data base of known protein structures), PyMOL and Chimera (programs to visualize protein structures and intermolecular forces between proteins and bound ligands). Experimental lab work would then be used to verify and further explore computational results.