In order to survive, cells must be able to recognize and respond to rapid changes in their environments. These molecular recognition events occur when membrane-spanning receptors physically interact with a stimulus on the outside of the cell and initiate the activation of downstream effectors. In order to uncover how information about the extracellular milieu is converted into a cellular response, we are performing x-ray crystallographic analysis of several model systems to determine the atomic details of protein interactions important for molecular recognition.

![Image of a scientific diagram](image1.png)

**Figure 3:** Evenmominycin and Orf36 enzyme
A. Structure of evenmominycin.
B. Structure of Orf36, a protein catalyzing the formation of the unusual nitrososugar on evenmominycin.

**Antibiotic Development**

The emergence of bacterial antibiotic resistance requires the investigation of novel antibiotic scaffolds. The natural product evenmominycin has potent antimicrobial activity, but this promising scaffold is chemically challenging to synthesize. The enzyme Orf36 catalyzes a key step in the biosynthesis of evenmominycin.

**The Role of Surface Receptors in Pathogen Invasion**

Pathogenic bacteria commonly recognize cell surface carbohydrates to bind specific tissue as the first step in infection. Various pathogens use serine-rich repeat surface receptors to bind plasmin and soligary proteins. We study the molecular details of how these receptors bind.

![Image of a scientific diagram](image2.png)

**Figure 4:** Serine-Rich Repeat Receptor, GspB
A. Crystals of a subdomain of the receptor GspB (GspBα) 
B. A sample X-ray diffraction image of a GspBα crystal. Diffraction was observed to 1.4Å resolution.
C. Structure of surface glycoprotein GspBα.

**Cellular Bioenergetics and Respiration**

Respiration provides cells with usable energy in the form of ATP. We focus on the complex II superfamily and use the E. coli complex II enzyme quinol: fumarate reductase as a model system. We are studying the catalytic mechanisms, inhibition and dysfunction, and novel interactions with flagellar motor proteins.

![Image of a scientific diagram](image3.png)

**Figure 2:** Substrate binding in a complex II superfamily
A. A combination of x-ray crystallography and biochemical analysis suggests that interdomain movement accompanies catalysis in Complex II. Visible absorption spectra from the FAD cofactor when different substrates are bound are shown in the background.
B. The substrate fumarate is shown with [Fe]-[Fe] electron density.

**Protein Engineering**

In collaboration with the Bachmann Group, we are characterizing the structures of potentially tunable enzymes. One example is phosphopentomutase, which normally acts on ribose phosphates but can be tuned to act on dideoxy substrates. This change in chemistry could reduce the cost of manufacturing of the reverse transcriptase inhibitor didanosine, a dideoxynucleotide substrate used to treat HIV.

![Image of a scientific diagram](image4.png)

**Figure 5:** Phosphopentomutase
A. Structure of Bacillus cereus phosphopentomutase.
B. The ribose-5-phosphate binding site.
C. Binding of the unnatural substrate didanosine.
Rotation project 1 (available rotations 2-4): Antibiotic Development. Antibiotic resistance in pathogenic bacteria has quickly emerged as a serious health threat. As the incidence of resistant bacteria increases, the development of new antibiotics to combat infections continues to be of major importance. Most of our antibiotics are derived from natural products, and understanding their biosynthetic pathways will help us fully develop molecules as effective therapeutics.

The natural product antibiotics evenomycin, rubradirin, kijanimicin, daunorubicin, and doxorubicin all contain an unusual nitrosugar moiety. We are interested in the enzymes responsible for biosynthesis of these nitrosugars, and in particular, the nitrososynthases that oxidize the aminosugar to the final nitrosugars. We have begun structural studies of the nitrososynthases in order to understand their mechanism and determinants of substrate selectivity.

We have recently determined the structure of ORF36 from the evenomycin biosynthetic pathway (see Figure3B on the back). Now we would like to characterize DnmZ, which is predicted to be a nitrososynthase in daunorubicin biosynthesis. This project will involve crystallization of protein provided by our collaborators in Brian Bachman’s lab. You will set up crystallization experiments using our Mosquito robot and optimize any resulting crystals. If you grow diffracting crystals, you will collect diffraction data at the synchrotron. Crystallization of DnmZ with cofactors and substrates will also be an important aim in these studies.

Rotation Project 2 (available rotations 3-4): Directed evolution of enzymes suitable for use in the synthesis of the HIV drug didanosine. In collaboration with Brian Bachman’s laboratory, we are developing enzymes that can be used as biocatalysts for the synthesis of didanosine, a reverse transcriptase inhibitor commonly part of the HIV drug cocktail. The three enzymes - ribokinase, phosphopentomutase (PPM; see Figure 5A on back) and purine nucleoside phosphorylase - work in sequence to convert 2,3-dideoxyribose into the final product. However, each enzyme has evolved to act on substrates that contain the 2 and 3 hydroxyls and are only marginally active with the dideoxysubstrates. Our goal is to understand the parts of the enzyme that confer substrate specificity and to suggest mutations that will improve activity with the unnatural substrates.

Sub-project 1: Optimization of crystals of a PPM from Francisella tularensis. In an effort to further characterize variations in domain angles, this project will focus on optimization of crystallization conditions of *F. tularensis* PPM by using diffraction based feedback to find conditions which allow us to collect a high resolution dataset. Once conditions are identified, the student will then focus on refining the model of *F. tularensis* PPM and will compare it to the previously determined structure of *B. cereus* PPM.

Sub-project 2: Co-crystallization of B. cereus PPM with substrate analogs of ribose-5-phosphate. To further characterize substrate binding, analogs of ribose-5-phosphate have been synthesized and the goal of this project will be to characterize their binding. This project will focus on using high throughput crystallization techniques to identify conditions for co-crystal structures of PPM with the substrate analogs.

Rotation Project 3 (available all rotations): G protein signaling. We are working to understand mechanisms of G protein activation and are using several complementary techniques to understand the structural constraints of the GPCR-G protein complex.

Sub-project 1: Design, create, and crystallize mutants of the Gα subunit that mimic the activated state. This project will use site-directed mutagenesis of the Gα subunit of G proteins and will make mutants that have enhanced nucleotide exchange in the absence of receptor. Mutants with interesting biochemical properties will be subjected to crystallization and diffraction data will be collected at the synchrotron.

Sub-project 2: Identification of conditions to stabilize and crystallize the rhodopsin-G complex. This project will focus on using bicelles as membrane-mimics to stabilize the rhodopsin-G complex. All conditions that enhance the half-life to 1 week or better will be subjected to crystallization trials and diffraction data will be collected at the synchrotron.