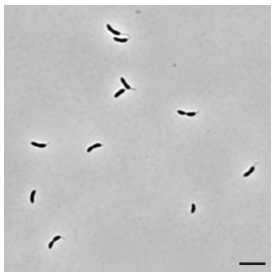
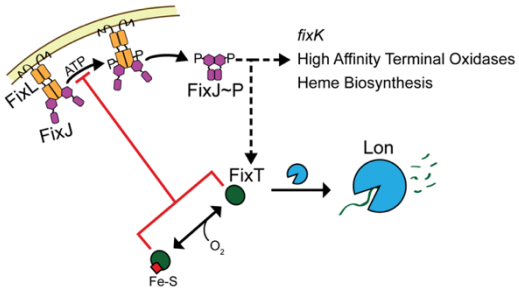
Towards a detailed understanding of sensory systems in *Caulobacter crescentus*

Dr. Benjamin Stein, URP 2022



Bacteria are microscopic organisms that inhabit almost every environment around us, including our own bodies. All bacteria must sense and respond to changes in their environment to survive. To achieve these responses, bacteria employ protein modules called two-component signaling systems. These systems sense a signal(s) through a histidine kinase, which autophosphorylates and passes its phosphoryl group to a response regulator. The response regulator then affects cell physiology, usually via changes in gene expression. My laboratory uses the model bacterium *Caulobacter crescentus* to study the regulation of two-component systems. As these systems are conserved in other bacteria, including pathogens and symbionts, our results will provide broad insight into how bacteria can adapt and thrive in their habitats.

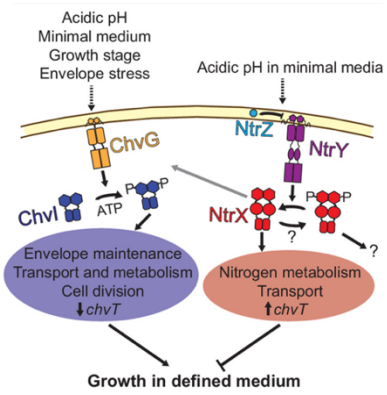
Project 1: Regulation of the FixL kinase by the FixT inhibitor



The FixL kinase is an oxygen sensor that allows certain bacteria, including *C. crescentus,* to respond to low oxygen levels. I previously characterized FixT as an inhibitor of FixL autophospohorylation activity. How, though, does FixT inhibit the FixL kinase? The research student will streamline a binding assay to identify residues important for the interaction between these proteins. Through this project, the student will gain experience in a variety of fundamental techniques critical in both academic and industrial biochemistry research (e.g., PCR/molecular cloning, protein purification, SDS-PAGE, and gel filtration chromatography).

Background reading: Stein, B. J.; Fiebig, A.; Crosson, S., Feedback Control of a Two-Component Signaling System by an Fe-S-Binding Receiver Domain. *mBio* **2020,** *11* (2).

Project 2: Regulation of the NtrY phosphatase by the uncharacterized protein NtrZ



NtrXY is a two-component system employed by many bacteria to regulate properties of their cell envelope, motility, and virulence. I have identified an uncharacterized protein, NtrZ, that appears to inhibit NtrY phosphatase activity, allowing phosphorylation of NtrX. NtrZ has no known domains and has never been studied. This project will investigate the mechanism by which NtrZ regulates NtrY activity. The research student will gain critical experience in techniques commonly used in academic and industrial biochemistry research (e.g., PCR/molecular cloning, pull-down assays, SDS-PAGE, and western blotting).

Background reading: Stein, B. J.; Fiebig, A.; Crosson, S., The ChvG-ChvI and NtrY-NtrX Two-Component Systems Coordinately Regulate Growth of Caulobacter crescentus. *J Bacteriol* **2021,** *203* (17), e0019921.