**The Expression, Purification, and Crystallization of Myoglobin.**

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As we have begun several weeks of laboratory work in Dr. Olsen's lab, we have been spending our time expressing, purifying, and crystalizing the H64F/V68F myoglobin protein. We began growing the recombinant protein in *E. Coli* in attempts to manifest large amounts of bacteria, thus generating more protein prior to inducing it with Terrific Broth and adding an antibiotic. After harvesting and spinning down the collected cells (which became dark red in pellet color), we began the process of purification to remove unwanted substances such as the cell wall and *E. Coli* proteins to ensure our protein was in its most pristine state before imaging.

We performed an Ammonium Sulfate cut three times, and used a dialysis buffer to resuspend the final red pellet. After concentrating the collected protein we ran it on 2 columns, the DEAE column followed by the CM 52 column, enabling the protein to stick to the top. After performing a gradient wash, equilibrating a pH 6 and pH 9.5 buffer onto the second column and collecting the remaining protein, we used the batch diffusion method to crystallize our final protein. After allowing the Mb to grow for several days we added the precipitant mixture of Ammonium Sulfate and Tris and. The crystals were then mounted for imaging and a beautiful diffraction pattern was captured.

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