Utilizing Biotin Proximity Labeling to Study Changes in Mitochondrial Dynamics

Mitochondria are responsible for a variety of essential metabolic pathways and perform crucial functions within the cell. Mitochondria are also believed to be involved in the formation and progression of a variety of human diseases. Recent studies have found that more than 1000 proteins are encoded within the mitochondrial genome and are connected in complex and dynamic protein networks. However, the mitochondrial proteins that aid and regulate these key functions within the cell are poorly understood. Characterizing the function and interactions of these proteins within the mitochondrial matrix is essential to understand mitochondrial dysfunctions.

One method commonly used to identify these protein interactions in living cells is BioID. This method uses a biotin ligase fused to your target protein. When expressed in cells, it allows for the covalent tagging of endogenous proteins within a few nanometers of the enzyme. However, current methods of proximity labeling utilizing BioID and BioID2, require an 18–24-hour labeling period. In this study, we are comparatively looking at a mutant of the biotin ligase called miniTurbo. Previous literature states, miniTurbo only requires a ten-minute labeling period with biotin to see an increase in biotinylated proteins. We want to determine if the short labeling period of the miniTurbo can be used to track changes in mitochondrial dynamics.

To integrate the protein environment of the mitochondrial matrix, we were able to make constructs using the mitochondrial matrix targeted sequence (MTS), COX8A. This was generated by using The Gateway recombinational cloning system and the plasmid was then confirmed by Sanger sequencing.