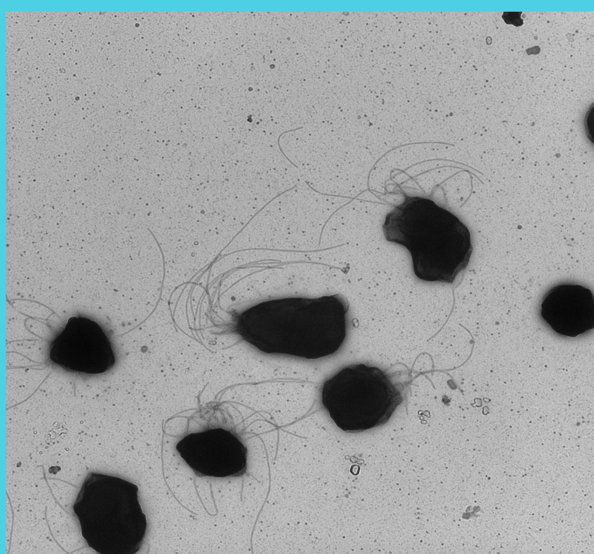
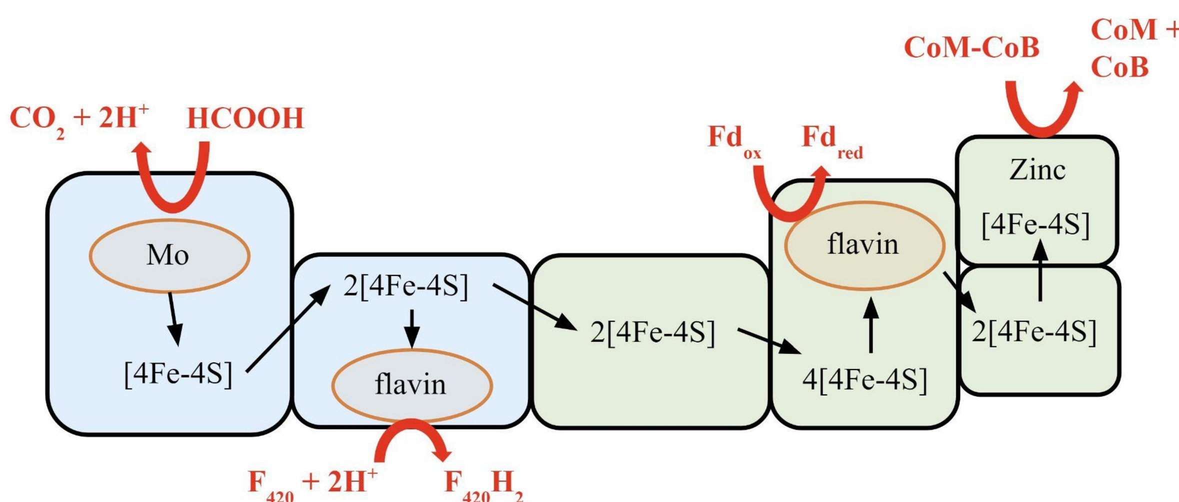


Exploring the Role of Iron-Sulfer Clusters in Electron Bifurcation in Methanogenesis

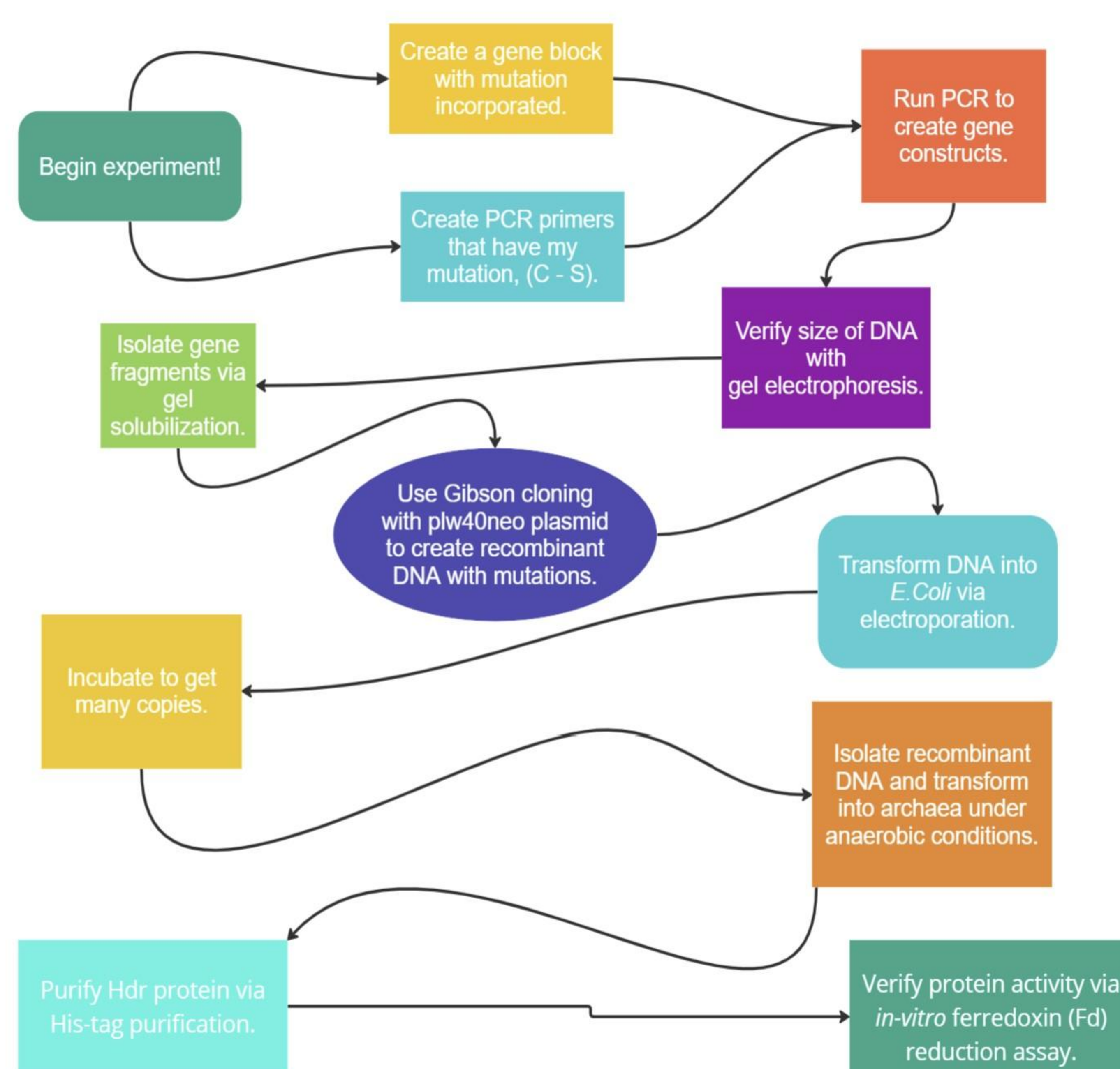
By Theodore Tran, Dr. Kyle Costa, and Dr. Farid Halim

Introduction:

- **Methanogenic archaea (methanogens) are organisms known to reduce carbon dioxide to methane, also known as methanogenesis**. Methanogens produce around seventy percent of the methane on Earth and are crucial in the breakdown of organic materials in environments that lack oxygen.
- Methanogenesis consists of a series of **oxidoreductive** steps. At least one of these reactions depends on the protein complex **Heterodisulfide reductase(Hdr).**
- This protein complex plays a central role in this process by carrying out a process known as a **flavin-based electron bifurcation reaction.**
- How Hdr accomplishes this reaction is unknown but **it contains iron-sulfur clusters, complexes known to be able to catalyze electron transfers to and from Flavin.**
- **To further investigate their role, I constructed** and expressed a **mutant protein complex where certain cysteines are mutated to serine residues,** something **we hypothesize will disrupt iron-sulfur cluster formation.**



Methods:



Figures:

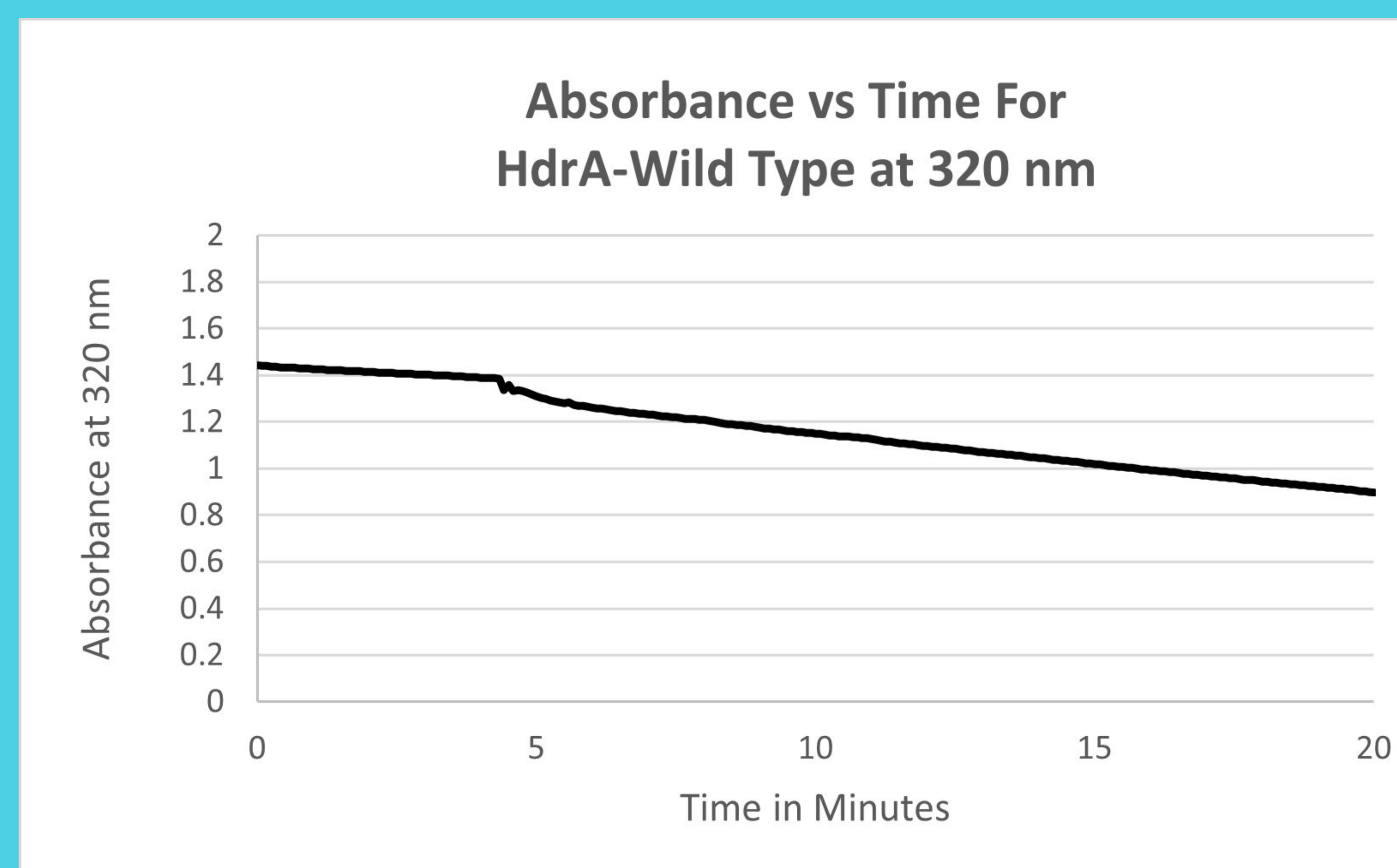


Figure 1: Absorbance graph from results of ferredoxin assay on wild-type Hdr protein. This showcases the general trends of the bifurcation reaction over time facilitated by HdrA wild-type protein. Heterodisulfide was added at 3.33 minutes after data collection began. In this case, loss of absorbance at 320 nm indicates the reaction is proceeding as expected.

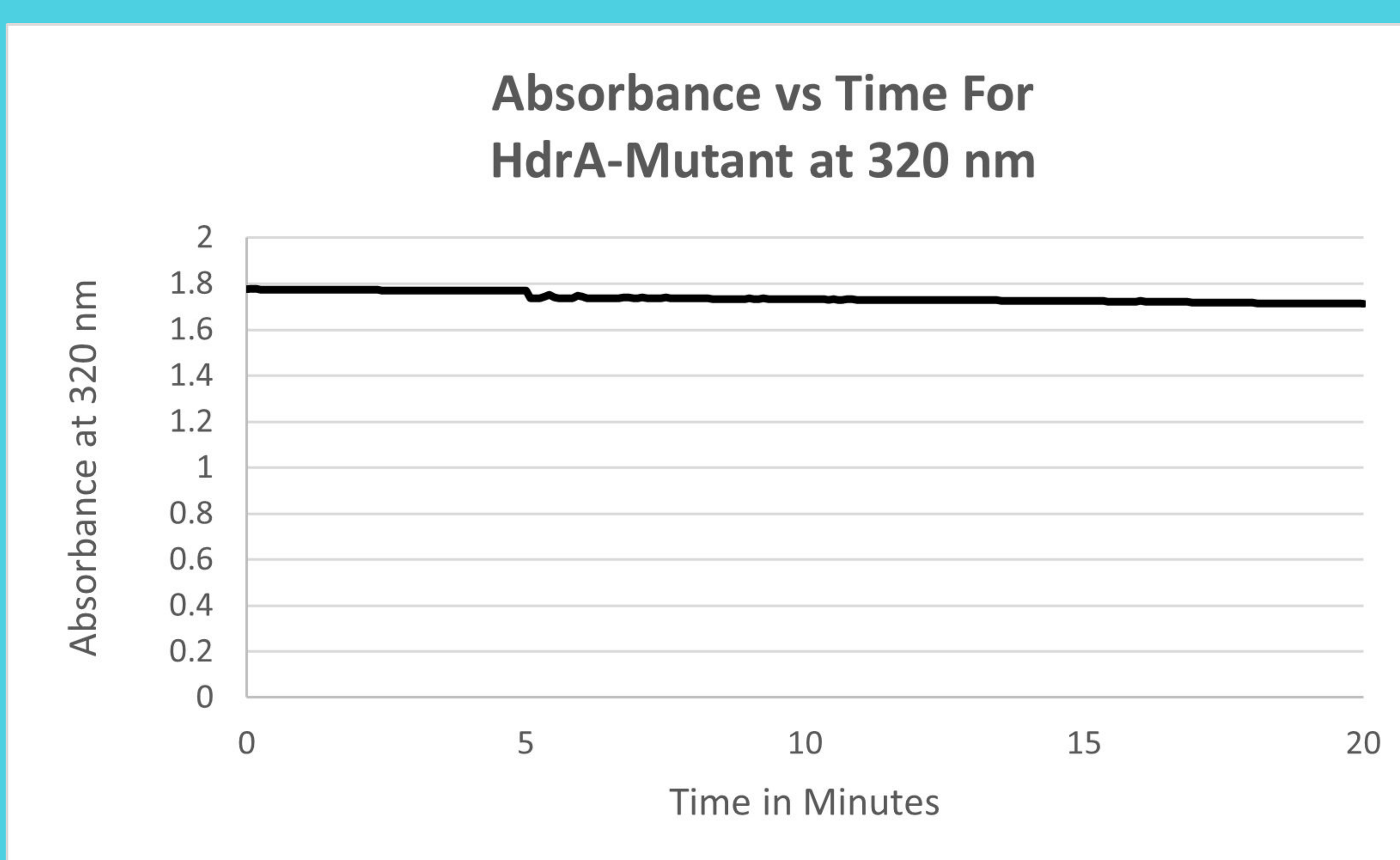


Figure 2:Absorbance graph from results of ferredoxin assay on Mutant (C - S) Hdr protein. This showcases the general trends of the bifurcation reaction over time facilitated by my constructed mutant protein that has a disrupted iron-sulfur cluster. Heterodisulfide was added 4.5 minutes after data collection began. In this case, loss of absorbance at 320 nm indicates the reaction is proceeding as expected.

Results:

- A noticeable decrease in absorbance at 320 nm was observed in the wild-type Hdr-loaded protein sample immediately after the required cofactor was added to the testing medium.
- No noticeable decrease in absorbance at 320 nm was observed in the mutant Hdr-loaded protein sample even after the required cofactor was added to the testing medium.

Conclusions:

- **In Figure 1, the noticeable drop in absorbance coordinated with the approximate time at which the cofactor was added, indicating** that the wild-type Hdr protein **catalyzed the bifurcation reaction.** Therefore, the protein was functional and serves as a baseline for what is expected in a normal flavin-based electron bifurcation reaction.
- **In Figure 2, the constant plateau, even after addition of the cofactor for bifurcation, supports the fact that the mutant HdrA protein is inactive.**
- Loss of activity in the mutant protein could be due to one of several factors:

  1. The mutation resulted in a defective iron sulfur cluster and inhibition of the electron bifurcation reaction. This would suggest that this iron-sulfur cluster is essential for electron bifurcation by Hdr, and for methanogenesis in general.

  2. The mutation destabilized the Hdr protein complex resulting in a lack of activity.

Future Approaches/Avenues:

- Modify each individual cysteine residue into serines and test individual mutation effects on ferredoxin reduction activity.
- Modify distinct pairs of cysteine residues in this iron-sulfur cluster chain and access ferredoxin reduction activity.
- Test under various conditions (variable temperature, growth conditions…etc) and monitor reduction activity.
- Access reduction activity if another distinct iron-sulfur cluster was modified.
- Utilize this same protocol but in another model methanogen.

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