Expressing βF99H & βF99H/βS188A Variants of Nitrogenase in *Azotobacter vinelandii*

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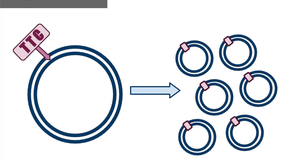
Introduction & Abstract

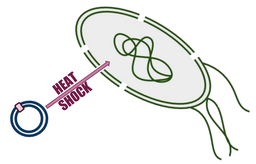
Nitrogen is essential for the biosynthesis of nucleic acids and proteins. Although N_2 is the most abundant gas in Earth's atmosphere, organisms are unable to access this Nitrogen until it is fixed into ammonia (NH_3) or other reduced forms. There is only one enzyme known to catalyze the N_2 fixation process: Nitrogenase, a metalloenzyme produced by diazotrophs. In order for Nitrogenase's active site, FeMo-cofactor (FeMoCo), to be activated, a series of electron transfers must relay electrons to FeMoCo, which is located in MoFe protein (MoFeP). Surprisingly, evidence suggests that the 8Fe-7S P-cluster, also found in MoFeP, relays electrons to FeMoCo prior to being reduced by the 4Fe-4S cluster in Fe protein (FeP). However, despite this, the P-cluster cannot perform this "deficit spending" of electrons in the absence of FeP, suggesting that conformational gating may play a role in Nitrogenase's electron transport chain. O-based amino acid residues that can ligate the P-cluster upon its oxidation have been suggested to play a role in this possible conformational gating mechanism, so, in order to help elucidate the significance of these residues in Nitrogenase, we induced point mutations to express βF99H and βF99H/□S188A in *Azotobacter vinelandii*. Ultimately, we plan to harvest and purify these variants of Mo Nitrogenase in order to observe the effects that changes in the β99 and β188 positions have on Nitrogenase's structure and function.

Method for Making Mutants

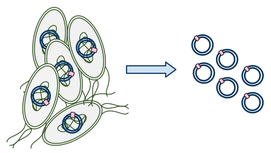
1.) PCR



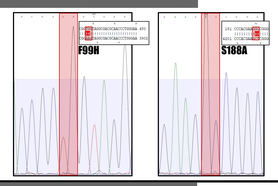
2.) Transformation into *E. coli*



3.) Miniprep

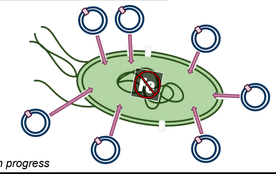


4.) Sequencing



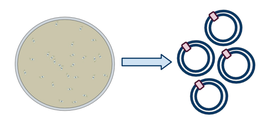
5.) Fe starve *A. v.*

6.) Transformation into *A. v. cells*



In progress

7.) Colony PCR



Soon-to-be completed

8.) Sequencing

Soon-to-be completed

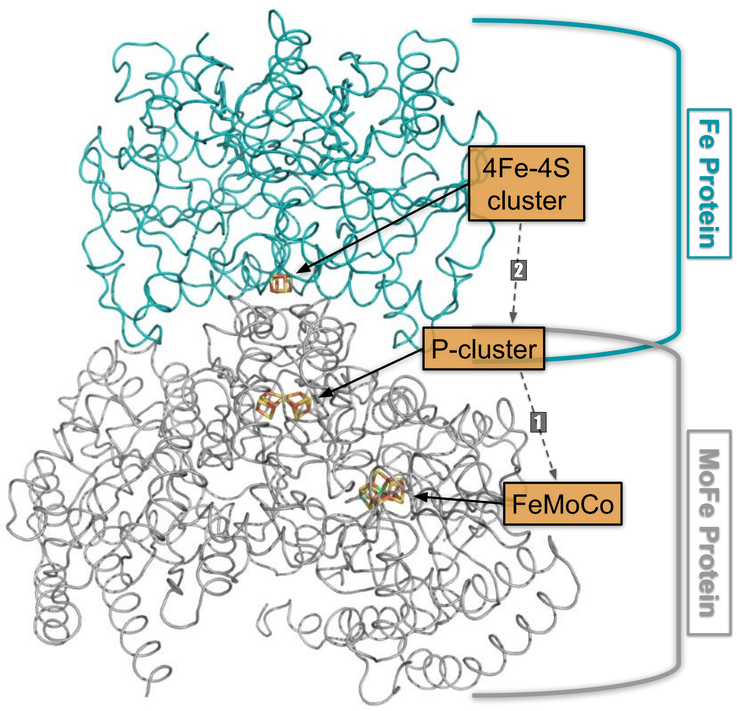
9.) Expression

Soon-to-be completed

Future Directions & Experiments

- Purification of βF99H and βF99H/βS188A
- Crystal structures of βF99H and βF99H/βS188A
- Assays on βF99H and βF99H/βS188A
  - ATP hydrolysis assay
  - H_2 production assay
  - NH_3 production assay
- Once the ATP, H_2, and NH_3 assays have been completed, we will have the data necessary to determine the relationship between ATP hydrolysis and N_2 fixation in the βF99H & βF99H/βS188A variants. This will help elucidate the role of O-based amino acids that ligate the P-cluster as a part of Nitrogenase's unconventional electron transport pathway.

Nitrogenase Structure & Mechanism

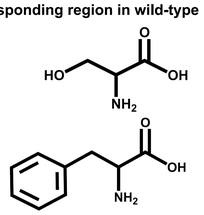


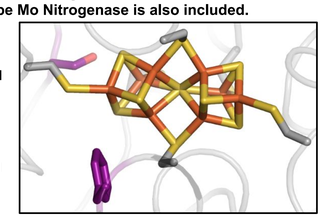
Results & Predicted Structure of the Variants

We successfully induced mutations that code for F99H and F99H/S188A variants of Mo Nitrogenase in the nifK gene. We are currently in the process of transforming these mutations into *Azotobacter vinelandii*, which will express the two variant enzymes. Pictured below are the predicted structures of the P-cluster & surrounding amino acid residues in F99H & F99H/S188A. For reference, the structure of the corresponding region in wild-type Mo Nitrogenase is also included.

Wild-Type Structure

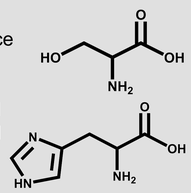
- Serine acts as a hard, O-based ligand that is hypothesized to reduce the P-cluster's reduction potential after it is oxidized. This would make the "deficit spending" electron transport pathway more favorable.
- Across many WT versions of Nitrogenase, when Serine is present in the β188 position, Phenylalanine is present in the 99 position.

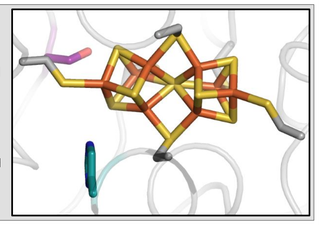




βF99H

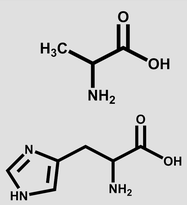
- Has previously been determined to reduce substrates using reducing agents other than FeP.
- This may be due to the fact that two hard ligands are present, decreasing the need for FeP to reduce the P-cluster.

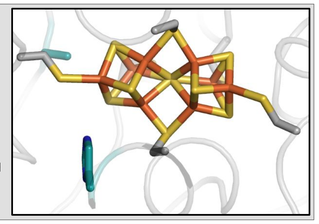




βF99H/βS188A

- Removal of Serine, a hard oxygen ligand, is important in order to observe how the βF99H variation alone affects Nitrogenase's function.
- Will FeP be necessary for catalysis?





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