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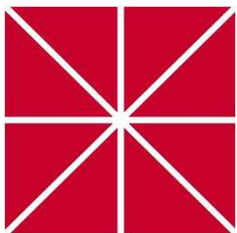
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Protein Protection: Characterizing how CowN Protects Nitrogenase

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Introduction

Nitrogen Fixation:

Nitrogen fixation is the process that converts atmospheric dinitrogen into ammonia. This occurs via a biological catalyst called Nitrogenase, which reduces nitrogen using energy in the form of ATP (Equation 1). Nitrogenase is a multi-subunit protein consisting of iron protein (FeP) and Molybdenum iron protein (MoFeP) (Figure 1) (Katz et al. 2016).

Inhibition:

Carbon monoxide (CO) is a known non-competitive inhibitor of nitrogenase. In the presence of CO, CO will displace a sulfur located at the FeMoco active site of nitrogenase making it unable to reduce substrate. The mechanism in which CO inhibition occurs is currently unknown (Spatzal et al. 2014).

Recently, a small protein called CowN was characterized to protect Nitrogenase from CO (Figure 2). This prediction was first reported in *R. capsulatus*. Our group then determined that CowN lowers the binding affinity of CO to nitrogenase, thus enabling nitrogenase to keep reducing substrate

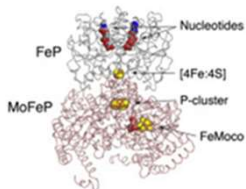
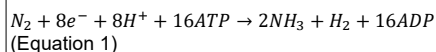


Figure 1: Structure of Nitrogenase

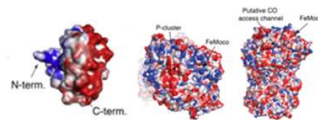


Figure 2: Charge Structure of CowN (left) and Femoco site (middle and right)

Evidence of Nitrogenase and CowN Interactions

Crosslinking with Diazirine showed that under conditions containing light, a band was found at 70 kDa, which shows a possible crosslink between MoFeP of Nitrogenase and CowN. Mass spectroscopy of the 70 kDa band confirmed characteristic bands of a CowN at 1307.69 m/z and of a beta chain at 1293.67 m/z (Figure 3).

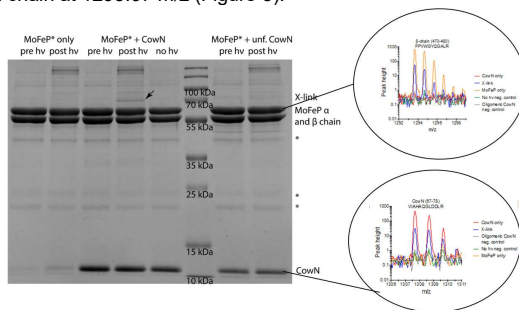


Figure 3: SDS Page gel of crosslinking and Mass Spectroscopy data of the 70 kDa band

C90S Mutant Affects Activity

We don't know where CowN binds to MoFeP. We hypothesize that a cysteine in the 90 position on CowN may play a role in binding as it is conserved (Figure 4). We analyzed the protective activity of a C90S mutant CowN and compared it to wild type CowN. C90S protected less suggesting the C90 residue may be important in CowN function (Figure 5).

Figure 4: Sequence alignment for CowN

```
sp|D5V218|COML_ARCNC  -----NSHFETVNIYESYFHNIDCFEHAQVVDMLNVLNENHWNIYVNIKIVPH  51
sp|Q2RN15|COML_RHORT  -----NTIDGPAHPRRYVYFQGVNVEGLSQLIARLFLHYADPAISMAFNEHFCAK  52
sp|A711E0|COML_XAHIP2  MDQPAAGFPALHLELPDRYLYTFQGDIFEGMRRVLAHLRYVIDDPAHGAHFDFICAR  60
                                                                    * : * : * : * : * : * :
                                                                    * : * : * : * : * : * :

sp|D5V218|COML_ARCNC  IPKAYYDRPHSDTKEELLYVCSNFSYLDLFEKAEADQNALSKCEGEC  104
sp|Q2RN15|COML_RHORT  LAD-----ADKTLARTADSLCLLGGATGYIDELFEDINDDEEGLTLRLRLEDELC  101
sp|A711E0|COML_XAHIP2  LQA-----AEGAATICDKLLLLHSHYYVVDLFEQDEDELADLKLTEECFC  109
                                                                    * : * : * : * : * : * :
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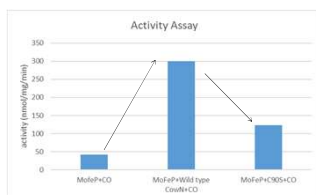


Figure 5: Activity assay for nitrogenase showing nitrogenase activity being affected by CowN

Weak Diffraction Found with Crystallography

To determine the structure of the CowN-MoFeP complex, we ran an X-ray diffraction. Crystal diffraction was found and the unit cell was determined, however diffraction was not good enough to determine the protein complex (Figure 6).

Unit cell parameters:

$a, b, c = 97.66, 188.73, 216.05$ Angstrom
 $\alpha, \beta, \gamma = 65.38, 78.72, 66.74$ degrees

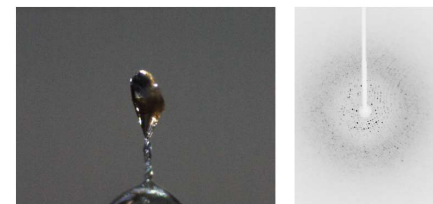


Figure 6: Crystal in loop (left) and Crystal diffraction (right)

Future Directions

- Run Cross-linking on C90S CowN with MofeP
- Conduct crystallography experiments with other possible conditions to crystalize MofeP and CowN

References

1. Cedric P. Owens, Faith E. H. Katz, Cole H. Carter, Victoria F. Oswald, and F. Akif Tezcan. *Journal of the American Chemical Society* 2016 138 (32), 10124-10127. DOI: 10.1021/jacs.6b06783
2. Hoffmann M-C, Pfander Y, Fehringer M, Narberhaus F, Masepohl B. 2014. NifA- and CooA-Coordinated cowN Expression Sustains Nitrogen Fixation by *Rhodobacter capsulatus* in the Presence of Carbon Monoxide. *J Bacteriol.* 196(19):3494-3502. doi:10.1128/JB.01754-14.
3. Howard JB, Davis R, Moldenhauer B, Cash VL, Dean D. 1989. Fe-S Cluster Ligands Are the Only Cysteines Required for Nitrogenase Fe-Protein Activities. *Journal of Biological Chemistry.* 264(19):11270-11274. doi:10.1016/S0021-9258(18)60459-4.
4. Katz FEH, Owens CP, Tezcan FA. 2016. Electron Transfer Reactions in Biological Nitrogen Fixation. *Israel Journal of Chemistry.* 56(9/10):682-692. doi:10.1002/ijch.201600020.
5. Medina MS, Bretzing KO, Aviles RA, Chong K, Espinoza A, Garcia CNG, Katz BB, Kharwa RN, Hernandez A, Lee JL, et al. 2021 Mar. CowN sustains nitrogenase turnover in the presence of the inhibitor carbon monoxide. *Journal of Biological Chemistry.* 100501. doi:10.1016/j.jbc.2021.100501.
6. Spatzal T, Perez KA, Einsle O, Howard JB, Rees DC. 2014. Ligand binding to the FeMo-cofactor: Structures of CO-bound and reactivated nitrogenase. *Science.* 345(6204):1620-1623.

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