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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 predictions 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

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP$$ (Equation 1)

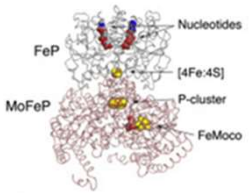

Figure 1: Structure of Nitrogenase

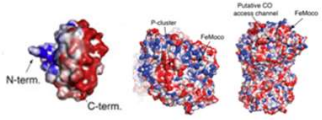

Figure 2: Charge Structure of CowN (left) and Femoco site (middle and right)

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).

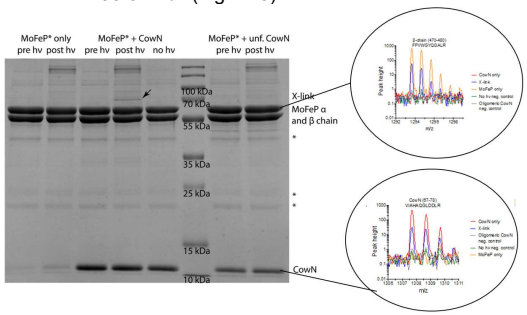

Figure 3: SDS Page gel of crosslinking and Mass Spectroscopy data of the 70 kda band

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

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sp|D5V2I8|COWN_ARCNC    ---------MSNFEIRVNESYESFKNIDCFENACVVIDNMLRVLENPKNMNIYKKKIVPM  51
sp|Q2RNI5|COWN_RHORT    ---------MTMDGPAHMPRRYVTFQGVNVEGLSQQLIARILFHVADPAKSNAFKEHFKAK  52
sp|A7IIE0|COWN_XANP2    MDQPAAGFPALHFELEQPDRYLTFQGIDFEGNMRRVLAHLYRYIDDPAMSNAFKDRFKAR  60
                                . .  * :*.::.    :: .:  : :* : * :*.::

sp|D5V2I8|COWN_ARCNC    IPKAYYDRDPKSDTKEELLYLVCSNSFYLDELFEKAEDEQAINALSKCEQECC 104
sp|Q2RNI5|COWN_RHORT    LAD-----ADKTLARTADSLCLLCGAIGYIDELFEDNDDEEGLTILRRLEDELC 101
sp|A7IIE0|COWN_XANP2    LQA-----AEAGAATICDKLLLLHSHVYYMVDLFEEQEDEIALADLKTLEEECF 109
                        :       :  * *: .   *: :***. :** .:  *   *:
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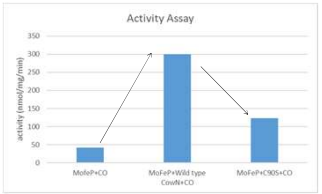

Figure 5: Activity assay for nitrogenase showing nitrogenase activity being affected by CowN

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
α, β, γ = 65.38, 78.72, 66.74 degrees

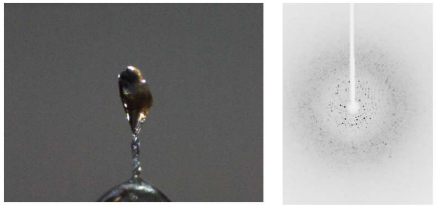

Figure 6: Crystal in loop (left) and Crystal diffraction (right)

Future Directions

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

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