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Poster Presentation P32

THE CONFORMATIONAL CHANGES IN FNR IN RESPONSE TO ANAEROBIC CONDITIONS

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The goal of our research is to understand the conformational changes that take place as FNR goes from its inactive to active form. FNR is a protein found in facultatively anaerobic bacteria, which forms an active dimer when oxygen is not present. This dimerization occurs after a [4Fe-4S] cluster is assembled. The dimerized FNR binds to the DNA and activates the transcription of genes necessary for anaerobic metabolic pathways. Since traditional methods of determining protein structure, such as x-ray crystallography or NMR spectroscopy, have not been successful with FNR, we will be using fluorescence to interpret its structure. Fluorescence wavelength varies upon exposure to solvent and will be indicative of the location of the fluorescing amino acid (tryptophan) in the FNR protein.

Since FNR contains no tryptophan and has only minimal fluorescence, due to its tyrosine, we replaced one codon in the fnr gene with the codon for tryptophan, the most fluorescent amino acid. All our work was done in plasmids, which allowed us to look at the proteins in vivo and induce overexpression. Our work was done primarily in the dimerization helix, which included residues from 140-160. Of the many mutants made, we selected Q141W and D154W to look at the differences in their fluorescence. Q141W and D154W were both induced with IPTG, to produce FNR protein. The cells were then anaerobically collected, sonicated, ultracentrifuged and run on a Biorex-70 cation exchange column in order to isolate the FNR. Gels show that this process was successful and fluorescence data shows that D154W has a fluorescence emission maximum at 341nm while Q141W emits at 349nm. Unfortunately, UV-vis data suggest no iron sulfur cluster presence in the majority of purified protein in both cases.

Future work involves doing concentration studies to better understand the changes in fluorescence during the dimerization process and looking at the fluorescence of more mutations.