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Oxygen Sensing in the FNR Transcription Factor: Creation and Characterization of Variants with Aerobically Stable [4FE-4S] Clusters

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Poster Presentation P7

OXYGEN SENSING IN THE FNR TRANSCRIPTION FACTOR: CREATION AND CHARACTERIZATION OF VARIANTS WITH AEROBICALLY STABLE [4FE-4S] CLUSTERS

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Activity of the FNR protein, a transcription factor in the facultative anaerobe Escherichia coli, is contingent upon the integrity of a [4Fe-4S] cluster incorporated by the protein's effector domain. The cluster is ligated by cysteine residues at positions 20, 23, 29, and 122. In wild-type FNR, this [4Fe-4S] cluster degrades upon exposure to molecular oxygen, and the resulting conformational changes cause the homodimer to separate into monomers, deactivating the protein. A previous study has identified an FNR variant, Leu28His, which has an oxygen-stable [4Fe-4S] cluster. This mutant protein retains activity under aerobic conditions. Using beta-galactosidase assays to quantify in vivo FNR activity, our lab has explored the oxygen sensitivity of several other FNR variants with amino acid substitutions adjacent to ligating cysteines. While many of these proteins exhibit oxygen sensitivity similar to that of wild-type FNR, the mutants Leu28Lys, Leu28Asn, Leu28Gln, Leu28Ser, and Leu28Tyr all retain some activity under aerobic conditions. Western blot analysis suggests that this increase in activity as compared to wild-type is not due to an increase in protein concentration. These mutant proteins have been isolated and characterized by absorption spectroscopy. The spectra indicate that the [4Fe-4S] clusters of these FNR variants do exhibit some degree of oxygen stability. As only substitutions of amino acids with amine, alcohol, or amide side chains have so far resulted in aerobically active FNR, oxygen stability of the [Fe-4S] cluster may result from donation of a hydrogen bond at position 28.