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Using Fluorescence to Monitor Structural Changes in the Dimerization Helix of the Transcription Factor FNR

Kate McCulloch Illinois Wesleyan University

Laura Moore, Faculty Advisor Illinois Wesleyan University

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USING FLUORESCENCE TO MONITOR STRUCTURAL CHANGES IN THE DIMERIZATION HELIX OF THE TRANSCRIPTION FACTOR FNR

Kate McCulloch and Laura Moore* Chemistry Department, Illinois Wesleyan University

Fumarate nitrate reductase (FNR) is an oxygen-sensing transcription factor in the facultative anaerobic bacteria E. coli. Although FNR is a widely-studied protein, little is known about its structure. FNR is a sequence homologue to catabolite receptor protein (CRP) in E. coli, and its structure is likely similar to CRP. When oxygen is absent, FNR is in an active dimeric form and contains a [4Fe-4S] cluster. Upon exposure to oxygen, the [4Fe-4S] cluster degrades to a [2Fe-2S] cluster and the protein subunits dissociate to two monomers. The structural changes that occur between these two forms are not well characterized. In this study, we use fluorescence spectroscopy to better understand the conformational changes between the active and inactive forms of FNR. Tryptophan, an amino acid with intrinsic fluorescent properties, has been used to replace some amino acid residues within the proposed dimerization helix of FNR. Changes in fluorescence emission wavelength indicate changes in the environment around the tryptophan, and are used to determine relative positions of amino acid residues within the dimerization helix. From preliminary data, the mutant FNR protein with tryptophan at position G149 does not have any significant change in emission wavelength upon exposure to oxygen, suggesting the environment around this residue does not change. However, the emission wavelength of the mutant protein at position M143 differs significantly between the anaerobic and aerobic forms. These data suggest that position 143 becomes exposed to solvent in the monomeric form and is isolated from solvent in the active dimeric form of the protein.