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DESIGNING FNR MUTANT PROTEINS TO PROBE CONFORMATIONAL CHANGES

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The goal of our research is to understand the conformational changes that take place as a transcriptional factor protein (FNR) goes from its inactive to active form. FNR is a protein found in facultatively anaerobic bacteria. It becomes active and dimerizes 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 the anaerobic 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 tryptophan fluorescence to better define the conformational changes. The wavelength of tryptophan fluorescence emission varies upon exposure to solvent and can be used to infer the change of position of an amino acid between aerobic and anaerobic states. As a first step, tryptophan residues were inserted at different amino acid locations along the dimerization helix and near the N-terminus. The FNR mutants R48W, L34W, R140W, Q141W, Q142W, M143W, M144W, S148W, G149W, K152W, G153W and G156W were created by site-directed mutagenesis, in the pET11a plasmid. Of these mutations, Q141W, M143W, M144W, G149W and E150W retained activity similar to the wild type protein. Wild type activity, for our purposes, is defined as at least 50% the activity of the unmutated protein. The retention of wild type activity is crucial to our study because only functional proteins, which are able to dimerize properly, will give insight regarding the actual conformational changes and structure of FNR. In an additional experiment, L146W, M147W and M157W were cloned into the pACYC184 plasmid in order to investigate dimerization of the mutant at FNR concentrations similar to the concentrations likely to be used for in-vitro experiments. Of the three, L146W and M157W retained wild type activity. Currently, we are attempting to purify the mutant proteins using column chromatography.