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Biophysical Characterization of the Biofilm Regulating Pseudomonas Aeruginosa Two-component System Bqsrs

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Pseudomonas aeruginosa (Pa) is a Gram-negative bacterium that is a major cause of chronic infections, especially when Pa forms biofilms. These extracellular networks provide a protective quality for Pa by increasing its antibiotic resistance. A two-component signal transduction system, BqsRS, regulates biofilm formation and decay in Pa through the sensing of Fe(II), an important nutrient during the infectious cycle of Pa. Homology suggests that PaBqsS is a transmembrane sensor kinase while PaBqsR is a DNA-binding cytosolic response regulator that alters the gene transcription involved in biofilm formation. These proteins have not been structurally characterized, and the details of their interactions with Fe(II) remain unknown. Using X-ray crystallography, we have solved the structure of the PaBqsR N-terminal phosphorylation domain to 1.3 Å resolution. Using NMR, we solved the PaBqsR DNA-binding domain and found an HXH motif conducive to DNA binding. We found the DNA consensus sequence upstream of the feo operon, the primary Fe(II) transporter in Pa, and EMSAs indicate that PaBqsR is an additional regulator to feo. Different than PaBqsR, we have shown that PaBqsS binds 1 Fe(II) ion per protein dimer, and using site-directed mutagenesis and X-ray absorption spectroscopy, we have characterized the His kinase Fe(II) binding site for the first time.