

Glu445Ala in DesD Crystallization and ATP/AMP binding with Kinetics

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Iron is vital for living organism since it plays a role to allow protein's biological activity in metabolic processes such as aerobic and anaerobic ATP biosynthesis. One of the many organisms that requires iron to keep themselves alive are bacteria specifically bacteria that cause diseases also known as pathogenic bacteria. The way pathogenic bacteria acquire iron is by a family of protein called NIS synthetases, the specific protein in the family being look at currently is DesD. The mechanism used by Pathogenic bacteria to acquire Iron and transport it back to the microorganism are call siderophores. NIS synthetases is plays a key role because one of its members of the family is present in many of the pathogenic bacteria so one model can be use for all. Current Pathogenic bacteria has become very resistance to antibiotics. This is concerning since eventually antibiotics will become useless leaving individuals vulnerable to common pathogenic bacteria. We predict that ATP binding association is diminished and will see that in the thermodynamics assay. As well it is predicted that this will have an impact on the kinetic rate, but mostly due to an increased KM (dissociation constant) when ATP is limiting while also finding new crystal structure substracts binded to Glu445. It is expect significantly diminished kinetics and binding thermodynamics due to an increase in dissociation constants (KD or KM). Also testing the role of Glu445 in specifically coordinating ATP by comparing our ATP binding curves (both the wild type and the Glu445Ala) to AMP or adenine as well. Since the structure indicates contacts to the ribose ring and alpha phosphate, which AMP has but adenine does not, we expect these tests to bind poorly and well, respectively, compared to the same thermodynamics in the wild-type protein.