

P001 PcrA helicase ATPase mechanism
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Helicases catalyse the unwinding of double stranded DNA or RNA for a variety of functions through various mechanisms. Helicases are classified into six Superfamilies (SF) through conserved sequences. The ATPase mechanism of the highly characterised SF1 bacterial helicase PcrA has been investigated. The Helicase is a monomeric enzyme with a role in plasmid replication. This is one of the most characterised helicases and it is known to translocate ssDNA and move with discrete steps of one base per ATP. A variety of biophysical techniques have been applied to determine the ATPase mechanism and the individual rate constants. This includes utilising fluorescent analogues of ATP, 2'(3')-mantATP/ADP (2'(3')-O-N-methylantraniloyl ATP/ADP), to monitor fluorescence intensity in rapid-reaction kinetic experiments, allowing the initial binding and release kinetics to be explored. These analogues are further used for analysis of the hydrolysis step using quenched-flow measurements. Additionally, the fluorescent phosphate binding protein (MDCC-PBP) independently measures the phosphate release step.