

P024 An investigation into archaeal DNA polymerases and their role in DNA replication using ensemble and single-molecule fluorescence

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The family B DNA polymerases from thermophilic archaea have been extensively studied leading to widespread understanding of their structure and function. However, while crystal structures have been solved for some of the archaeal family B DNA polymerases such as *Thermococcus gorgonarius* polymerase (Tgo Pol) these only give a fixed snapshot of the proteins and their domains when interacting with DNA. Further investigation is required in order to understand the movement of the five domains during DNA replication. After analysis of the amino acid sequence of *Pyrococcus furiosus* polymerase (Pfu Pol) and the crystal structure of the homologous polymerase Tgo Pol it was possible to select specific surface amino acids that can undergo quick change mutagenesis to introduce a unique cysteine residue into each of the five domains of Pfu Pol, creating five separate mutants. These mutants were introduced into a cysteine free variant of the wild type by removing the four naturally occurring cysteines. Surface cysteine residues have been used for conjugation with organic fluorescent dyes. Conjugated proteins are being investigated using rapid mixing FRET analysis and also single molecule multi-parameter fluorescence detection. It is thus proposed to fully characterise the interaction of the polymerase with DNA and to monitor inter-domain motion during catalysis.