

## **Biophysical studies of KlenTaq DNA Polymerase**

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Abstract:

The ability of DNA polymerases to select correct nucleotide for incorporation is central to fidelity in DNA replication and repair, and thus to genome stability. Crystal structures of DNA polymerase trapped at different stages of the incorporation cycle show a large inwards movement of the nucleotide binding (fingers) subdomain upon binding of correct nucleotide. This movement is thought to be crucial to nucleotide selection, as a complete active site, with steric constraints excluding incorrect base pairs, is only present in the closed form. However the kinetics of this fingers closure have remained obscure to study at the ensemble level – studying the average behaviour of many molecules. The aim of this project was to design a FRET system capable of monitoring movement of the fingers subdomain at single molecule level, and ultimately to investigate its role in nucleotide selection.