

Department of Chemistry

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Over-expression and Characterization of
Guanosine 3',5'-bis(diphosphate) Synthetase

Guanosine 3',5'-bis(diphosphate) (ppGpp) has been found to be a regulatory molecule affecting the stringent response and programmed cell death in bacteria. Under starvation conditions bacterial cells experience a shortage of amino acids; as a result the cells are unable to charge tRNAs. When an uncharged tRNA is loaded into the A site of a ribosome the ribosome stalls and is unable to elongate the protein being synthesized. ppGpp Synthetase (RelA) binds to the stalled ribosome and begins to synthesize ppGpp. ppGpp is released and binds to RNA polymerase changing the expression profile and inducing the stringent response. Recombinant RelA was over-expressed in *E. coli* and purified via His-tag affinity chromatography. The physical and kinetic properties of RelA were determined.