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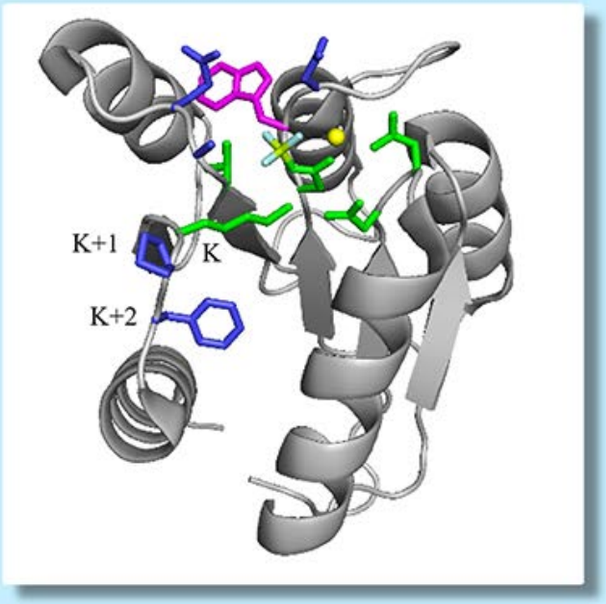

Structure of *E. coli* CheY with variable residues in color

Examining the Influence of Positions K+1 and K+2 on Response Regulator CheY Phosphorylation and Dephosphorylation Reactions

In microorganisms, two-component systems (TCSs) serve as control mechanisms that translate extracellular stimuli into intracellular responses such as movement and gene transcription. TCSs employ a sensor kinase that supplies a phosphoryl group to a response regulator, leading to downstream activity. Because the response times of TCSs span one million-fold from seconds to weeks, it is believed that the rate constants governing phosphotransfer vary across a similar range. However, since tertiary structure varies only slightly among response regulators, these differences are unlikely to explain the rate constant range. Rather, variable site residues adjacent to the active site are most likely responsible for regulating reaction kinetics. Currently identified primary sequence variations only account for 0.01% of the million-fold difference, indicating that additional undiscovered influencing factors exist. Primary sequence covariation showed that residues adjacent to the active site lysine termed K+1 and K+2 (PF in wild-type) strongly covary, suggesting functional importance. Therefore, phosphorylation and dephosphorylation rate constants were measured *in vitro* for mutants in the model response regulator CheY from *Escherichia coli*. The AF, AA, PL, and LL mutants had autophosphorylation rate constants similar to that of wild-type, whereas the constants for the PA and PG mutants were 12 and 7-fold faster, respectively. The LL mutant had a dephosphorylation rate constant 4-fold larger than that of wild-type, whereas the other mutants were similar to wild-type. The mechanisms(s) by which K+1/K+2 residues influence phosphoryl group reaction kinetics are currently unknown. Continuing to examine how the K+1/K+2 residues affect response regulator kinetics will improve understanding of overall TCS rate activity.