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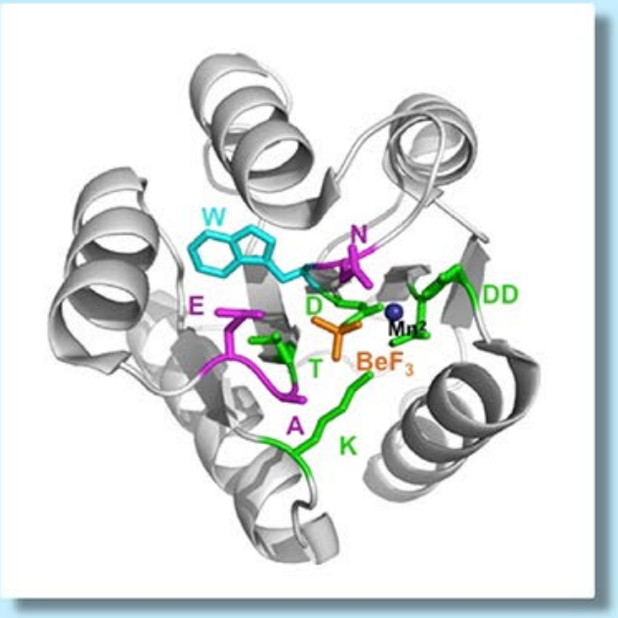

Active Site of CheY
Receiver Domain

Combinations of Variable Active Site Residues Interact to Influence Response Regulator Reaction Kinetics

Signal transduction pathways are responsible for sensing and responding to stimuli. Two-component systems are a common type of signaling pathway found in microorganisms and plants, and are composed of a sensor kinase (SK) and response regulator (RR) protein. Changes in the phosphorylation states of the SK and RR due to environmental stimuli provide a molecular switch to control signal output. RRs possess the catalytic activity for phosphotransfer, but it is not known how closely related RRs self-catalyze phosphorylation and dephosphorylation reactions with rate constants that span up to six orders of magnitude. Variable residues in the RR active site, named according to their position relative to a conserved aspartic acid (D+2) and threonine residue (T+1 and T+2) individually influence autophosphorylation and autodephosphorylation reaction kinetics by one to two orders of magnitude. Our hypothesis is that certain combinations of variable active site residues interact to produce much larger effects on RR reaction kinetics than the sum of their individual effects. To investigate these interactive properties, we examined existing data on the effects of specific D+2 and T+2 combinations, in the presence of the most common T+1 residue (Ala), that resulted in synergistic interactivity. To complete characterization of position T+1, the impact of Gly (the second most abundant residue) on reaction kinetics was tested. Finally, the effects of combinations at all three positions on rate constants were tested. Results show similarity in rate constants between proteins bearing Ala or Gly at T+1, while D+2/T+1/T+2 combination Asn/Thr/Ser had additive properties and Asn/Val/Ser was antagonistic. The T+1 results are consistent with the structural similarity of Ala and Gly, while Asn/Thr/Ser and Asn/Val/Ser combination results suggest an underlying interactivity between these positions. To further elucidate interactive properties, Arg/Gly/His and Arg/Gly/Tyr are currently being investigated.