Investigation of DFG Motif Activation of Aurora A Kinase
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Abstract
Aurora A kinase (AurA) belongs to the serine/threonine protein kinase family and found in all eukaryotic organisms. To succeed at the job of activating G2 phase of the cell cycle for cell growth and preparing for mitosis, AurA is activated by autophosphorylation and TP22 binding. TP22 and ADP change the conformation to the kinase’s active state. In particular, the DFG motif is of interest for research. The DFG motif is a catalytic triad that consists of aspartate-phenylalanine-glycine residues. The regulation of activation for AurA is based on interchanging conformations of DFG-in and DFG-out. When AurA is considered in DFG-in state, the aspartate residue faces in toward the active site which is favored when ADP binds. This allows for the interaction of the aspartate residue and magnesium ions associated with ATP. In the inactive state or DFG-out conformation, the aspartate rotates outward away from the magnesium ions. This study is focused on this major activation motif and how it functions when a residue in the activation loop is mutated to a different residue. In this case, the glycine residue was mutated to an alanine residue to investigate whether the DFG-motif will still work and how it functions when a residue in the activation loop is mutated.

Background
Aurora A is a serine/threonine protein kinase that regulates cell cycle progression through the control of chromosome segregation during mitosis. It belongs to the serine/threonine protein kinase family and is found in all eukaryotic organisms. To succeed at the job of activating G2 phase of the cell cycle for cell growth and preparing for mitosis, AurA is activated by autophosphorylation and TP22 binding. TP22 and ADP change the conformation to the kinase’s active state. In particular, the DFG motif is of interest for research. The DFG motif is a catalytic triad that consists of aspartate-phenylalanine-glycine residues. The regulation of activation for AurA is based on interchanging conformations of DFG-in and DFG-out. When AurA is considered in DFG-in state, the aspartate residue faces in toward the active site which is favored when ADP binds. This allows for the interaction of the aspartate residue and magnesium ions associated with ATP. In the inactive state or DFG-out conformation, the aspartate rotates outward away from the magnesium ions. This study is focused on this major activation motif and how it functions when a residue in the activation loop is mutated to a different residue. In this case, the glycine residue was mutated to an alanine residue to investigate whether the DFG-motif will still work and how it functions when a residue in the activation loop is mutated.

Methods
We have successfully mutated a AurA CL (C290A) G276A plasmid by Quikchange Lightning Mutagenesis kit using primers that encoded the G276A mutation. Also we have utilized a Quikchange Lightning Mutagenesis kit using primers that encoded the G276A mutation. The Wild-Type AurA plasmid was a hexahistidine-tagged AurA. A Quikchange Lightning Mutagenesis kit using primers that encoded the G276A mutation was used to create the mutant G276A AurA plasmid.

Results
The circular dichrosmolar ellipticity content (Figure 3) showed that both AurA CL2 S284C (WT) and AurA CL2 (C290A) G276A mutant has similar helical content. The thermal melt (Figure 4) of both AurA WT and G276A mutation resulted in about a 10 degree shift between the two. AurA CL2 (C290A) G276A experienced the higher melting point of the two. The two proteins can be said to similarly folded, but the G276A mutant is more thermally stable.

References
Quikchange Lightning Site-Directed Mutagenesis Kit: Data Sheet. Agilent Technologies.