Protein kinase G (PKG) is a cyclic 3', 5'-guanosine monophosphate (cGMP)-dependent enzyme that becomes activated by the binding of two cGMP molecules. Upon activation, PKG has the ability to effect downstream signaling events by subsequently phosphorylating serine/threonine residues on other proteins. The function of PKG is to maintain blood vessel tone, therefore making this kinase an attractive drug target in treating vascular disorders. Although extensive biochemical studies have significantly added to understanding the structure/function relationship of PKG, the molecular mechanism of activation is still largely unknown. Current purification strategies expose PKG to high levels of cAMP and cGMP, thereby making it difficult to obtain and evaluate the apoenzyme. In order to delineate the mechanism of activation, purification methods need to be improved. We propose that by adding a hexahistidine tag to the N-terminus of the protein, expressing the protein in mammalian cells, and by purifying with nickel-affinity chromatography, we will be able to purify PKG without cyclic nucleotide already bound to the regulatory domain. These improvements will allow us to strategically mutate key residues in the cyclic nucleotide binding sites, and obtain K_a and K_D values for the mutant kinase. These values will enable use to assess the individual contributions of each binding site to PKG activation, and ultimately will allow us to develop a comprehensive molecular mechanism of PKG activation.