Functional expression of wild type cGMP-dependent protein kinase Iα in *E.coli*

The major serotonin transporter, SERT, modulates the amount of serotonin in the synaptic gap via reuptake into the presynaptic neuron. Serotonin reuptake inhibitors, SSRIs, are the dominant antidepressant prescribed to treat patients with major depressive disorder (MDD). Yet, 30% of those diagnosed have found the current modes of treatment ineffective. SERT is activated by the type Iα guanosine 3',-5' cyclic monophosphate (cGMP) dependent protein kinase (PKG Iα). It is proposed that regulating SERT trafficking by modulating PKG activity in neurons may provide a novel therapeutic target for disorders affected by a deficiency of serotonin in the synapse. Unfortunately, the molecular mechanisms of PKG activation are poorly understood. In order to perform functional analysis of the protein, the full-length wild type PKG must be isolated and characterized in vitro in a soluble state. Historically, PKG produced in bacterial cells is misfolded and insoluble. To increase proper post-translational folding of PKG, Shuffle™ Compentent E.coli Cells were used as they produce recombinant proteins with enhanced disulfide bond formation. To further increase proper folding, the cells were co-transfected with pRARE to mimic mammalian Codon usage. In a addition a detergent (NP40) was used to increase solubility. This will allow the expression of full-length wild type PKG in a soluble form, which can then be used for functional analysis studies and for x-ray crystallography.