Expression and Purification of Recombinant Hemoglobin HbS/Providence

Christopher Anjorin[3], Jayashree Soman[1], George Phillip[1], John S. Olson[1].

[1] Department of BioSciences, Rice University, Houston, USA.
[2] Department of STEM, Houston Community College, Houston, USA.
[3] Department of Bio-Medical Science, Texas A&M University,College Station,USA.

Sickle Cell disease is due to a mutation in the hemoglobin protein from glutamate to valine at the 6th position of the β-subunit; this mutation results in many alterations in the biochemical properties and structural properties of the protein leading to a damaged vascular system in Sickle Cell patients. I am working with a specific providence mutation of HbS, β{K82D} which has proven to partially obscure the severity in SCD phenotype caused by β{E6V} by forming resistance to oxidative degradation based on recent research.

I did not obtain Sickle hemoglobin cells from SCD patients; Thus, my lab project was to express and purify recombinant HbS Providence using E.Coli bacteria cells with the goal of growing protein crystals for X-Ray diffraction. In order to perform this project, additional mutation of recombinant Hb genes was done in pHE2 vector using a PCR kit. Then we performed transformation and expression of the HbS providence protein in E.Coli bacteria cells with appropriate antibiotic and heme. After harvesting the cells, cells were thawed, lysed, and further purified using several preparative chromatography methods to initiate maximum purity. Batch Method Crystallization was then used to grow crystals due to past successes with native Hemoglobin. The idea of having crystals of this specific protein at homogeneity is to use the concept of X-Ray Crystallography to better characterize the structure of the mutated protein, and understand its exact function in order to create better clinical therapies for Sickle Cell Disease patients.