Research experience
Shuvankar Patra

BSc Research experience:

During my B.Sc program, I had the privilege of participating in a summer internship program at the University of Kalyani, West Bengal, under the supervision of Dr. Arunima Biswas. The primary focus of my summer internship revolved around unravelling the intricate roles played by cAMP, a pivotal secondary messenger, in breast cancer stem cells. We directed our research toward two distinct cell lines: MCF-7 (an estrogen receptor/progesterone receptor-positive cell line) and MDA-MB-231 (a triple-negative cell line). Our initial observations revealed notable disparities in the levels of phosphodiesterase, an enzyme responsible for the degradation of cAMP, between cancer cell lines and normal cells. The increase in the down regulator of cAMP is proportional to reducing levels in cAMP. We employed a pharmacological approach targeting the phosphodiesterase enzyme to explore the therapeutic potential. Subsequently, we conducted comprehensive cAMP quantitative assays and cell cytotoxicity assessments, explicitly performing the MTT assay.

The results of our experiments indicated the successful inhibition of phosphodiesterase activity by the administered drugs. This inhibition, in turn, resulted in a substantial increase in cAMP levels, ultimately leading to the selective eradication of cancer cells. Throughout my two-month summer training, I acquired valuable expertise in essential laboratory techniques, including proficient cell culture methodologies, precise western blot analysis, and the meticulous execution of in-vitro assays, notably the MTT and cAMP quantitative assays.

Expertise in Techniques: Western Blotting, Cell culture techniques, Cell cytotoxicity assays (MTT assay), cAMP quantitative assay.

MSc Research Experience:

My master's program involved conducting my dissertation research under Prof. Deepak T. Nair's expert guidance within the Genomic Integrity and Evolution laboratory at the Regional Centre of Biotechnology, Faridabad. The primary objective of my dissertation was to clone three genes, namely tau, delta, and delta prime, which encode the clamp loader complex in Mycobacterium sp. Subsequently, we aimed to purify these proteins to facilitate the reconstitution of the complex.

Initially, our strategy involved cloning the three genes into the pDJN1 expression vector. The Tau protein exhibited robust expression levels within the pDJN1 expression vector. However, the expression of the other two proteins, Delta and Delta prime, proved elusive. To overcome this challenge, we employed an alternative expression vector, the pGEX-6P-1 vector. With this vector, we achieved successful expression of each protein. Nonetheless, delta prime was predominantly found within the inclusion bodies, indicating issues with its solubility.

To address the solubility problem associated with delta prime, we mix the lysate of both proteins (Delta and Delta prime) and then purify them. The idea was that the soluble Delta protein would pull the insoluble Delta prime in the soluble fraction. This experimental tactic yielded fruitful results, as we were able to successfully purify all three proteins and subsequently reconstitute the clamp

loader complex. We performed mass spectrometry to confirm the identity of our complex.

To visually confirm the formation of the reconstituted complex, we employed Negative Stain Electron Microscopy. This technique allowed us to visualise and analyse the structural characteristics of the complex. Notably, we identified distinct ring and horse-shoe-shaped particles in the electron micrographs, which we considered indicative of our putative clamp-loader complex. This finding provided valuable insights into the structural attributes of the complex and underscored the success of our reconstitution effort.

Expertise in Techniques: Molecular cloning, Protein expression check, Mass spectroscopy (Triple-TOF), Protein purification using various liquid chromatography techniques (AKTA-PURE), Negative stain Electron Microscopy (TEM grid preparation and visualisation).

Present research:

Currently, we are trying to elucidate the high-resolution structure of the clamp loader complex of *Mycobacterium tuberculosis* with the help of Cryo-EM. We are using Cryo SPARC to refine the resultant data.