User experience: Using national Cryo EM centers towards studying lipid transport across the bacterial cell envelope

Gira Bhabha, Damian Ekiert, Nicolas Coudray, Georgia Isom, Mark MacRae and Rachel Redler

New York University School of Medicine, United States

Cryo EM is one of the main tools that our lab uses to study mechanisms of lipid transport across the cell envelope in bacteria. Transport of lipids across the periplasmic space between the inner and outer membranes is important for building and maintaining the outer membrane in double-membraned bacteria, which serves as a barrier to protect the bacteria from its environment. Consequently, it can also serve as a barrier for antibiotics to enter the cell, leading to antibiotic resistance. Our work focuses on understanding the structures and function of multi-protein complexes that facilitate the movement of lipids across the cell envelope. In particular, we study the MCE (Mammalian Cell Entry) family of proteins, which has been implicated in phospholipid transport, and in virulence of pathogenic bacteria such as Mycobacterium tuberculosis. We aim to study the role of this protein family in the maintenance of the cell envelope, and in pathogenicity. In order to study this protein family, it is necessary to solve structures of multiple proteins and complexes in many different conditions, and at reasonably high resolutions. Having access to adequate and affordable microscope time is key for enabling our lab to study the multiple components of protein families important for this process. Use of national Cryo EM centers made it feasible for us to begin our Cryo EM work prior to the establishment of a Cryo EM facility at our institution, and currently we use both NCCAT and PNCC together with our institutional cryo EM facility to maximize the throughput of our work. The efficient, high-throughput pipelines that we have experienced as users have been instrumental in our research progress. Currently, national Cryo EM facilities provide much-needed training and microscope availability towards engaging new users and democratizing the use of Cryo EM. I will discuss our experience as users of PNCC and NCCAT, highlighting some of the data that we have collected at these national centers.

References