INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title:
Structural and Functional Studies of a Phospholipid Transporter Involved in the Maintenance of the Outer Membrane Asymmetry in Gram-negative Bacteria

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SHORT ABSTRACT

Gram-negative bacteria are more resilient than Gram-positive bacteria due to the presence of an outer membrane (OM). Unlike the inner membrane (IM), the OM is asymmetric in nature because of the presence of lipopolysaccharide (LPS) and phospholipid (PL) in the outer and inner leaflets, respectively. This asymmetric organization shields the bacteria from antibiotics, toxins, etc. However, the PLs have the tendency to flip back and accumulate in the OM, leading to the formation of patches and disruption of this barrier function. In order to restore the OM permeability, bacteria utilize the highly conserved inter-membrane ATP-binding cassette (ABC) transporter system, viz. maintenance of lipid asymmetry (Mla). The Mla system consists of three sub-complexes- OM-associated MlaA-Osmoporin F/C complex, periplasmic MlaC and IM-associated MlaFEDB complex. The components of the Mla system are involved in the movement of PLs between the membranes, thereby maintaining OM asymmetry. Owing to this, the system has been suggested to be an excellent drug target although in-depth studies highlighting the mechanism of action are still lacking. Thus, in this study, all the Mla proteins from *Escherichia coli* were computationally and structurally characterized. Computational studies of the Mla components reveal their unique features which are not observable in typical ABC transporters. These include the identification of conserved motifs, distinct evolutionary relationships, interaction profiles and interfacial residues. Structure elucidation of MlaC and MlaD (*Ec*MlaC and *Ec*MlaD) through X-ray crystallography reveals that both these proteins do not possess the conserved architecture of N-terminal and C-terminal domains present in substrate-binding proteins (SBPs). Instead, *Ec*MlaC comprises two different domains that are arranged in a discontinuous fashion. On the other hand, *Ec*MlaD forms a homo-hexameric ring with a central hydrophobic channel which is continuous but has varying dimensions. Owing to these structural peculiarities, these two proteins have been classified as non-canonical SBPs. Extensive structural analyses of these proteins have led to the proposition of two novel mechanisms of ligand binding that have not been reported in case of any SBP to date. Furthermore, through this study, global and local motions in *Ec*MlaC have been identified that are critical for ligand binding. Altogether, the findings provide significant mechanistic insights into the functioning of the Mla system in *E. coli* and other Gram-negative bacteria.