Centre for BioSystems Science and Engineering

THESIS COLLOQUIUM

at 04:30 PM on May 15, 2017
MRDG Seminar Hall

Molecular determinants of self-assembly for the pore forming toxin Cytolysin A

Mr. Pradeep Sathyanarayana

Advisers: Prof. Ganapathy Ayappa(ChE) and Prof. Sandhya Visweswariah (MRDG)

The membrane is a complex two dimensional fluid matrix comprising of lipids and proteins. It serves as the primary barrier to insults from the external environment. Many microorganisms have evolved mechanisms to disrupt this barrier by formation of nanopores on membrane surface. This aids in establishing infection and result in diseases such as Pneumonia, Cholera and Anthrax to name a few. This class of proteins, called pore forming toxins (PFTs), spontaneously convert from soluble to membrane bound forms upon membrane exposure without the aid any extraneous processes.

This, being an intrinsic property of the protein, raises questions regarding the underlying design principles conferred by sequence and structure. We explore these questions in an a-PFT, Cytolysin A from E. coli, which assembles as a dodecamer upon exposure to the plasma membrane. ClyA pore has a significant portion (>80 % residues) that is solvent exposed, and the membrane inserted segment is composed of 12 transmembrane helices. Here, we set out to understand how the solvent exposed and transmembrane domains impinge on spontaneous membrane insertion of ClyA.

In the first part of the work, we explore how the solvent exposed C-terminal loop of 11 amino acids, devoid of any inter/intra-protein contacts, is indispensable for activity. We examine the different steps of pore formation using biochemical experiments and molecular dynamics simulations to understand the underlying molecular mechanism. We show that the C-terminal loop is an important stabilization factor for the helical bundle in ClyA and also governs fidelity of conformational transition upon membrane exposure. This further results in aberrant oligomerization leading to formation of dysfunctional pores. Therefore, regions of PFTS, not directly involved in the pore structure, assist in transitioning through intermediary steps of assembly, leading to successful pore formation in a membrane environment.

In the second part of the work, we examine how membrane-protein interactions plays a role in stabilization of the membrane inserted structure. We use single molecule tracking (SMT) and spectroscopy to understand the dynamics of ClyA on artificial lipid membranes. Diffusional analysis of particle trajectories revealed the existence of two discrete mobility states exhibiting 'fast' and 'slow' dynamics. We prove that this heterogeneity of motion is due to the conformational transition between a peripherally associated protein conformation and a membrane inserted conformation respectively. We show, for the first time, the existence of a cholesterol binding motif in the TM helix and validate by molecular dynamics, mutational studies and SMT. We propose a molecular mechanism for selective pore formation in eukaryotic membranes driven by conformational selectivity in the presence of cholesterol.

In summary, this study has provided molecular insights into hitherto unknown regulatory mechanisms of ClyA pore formation. Albeit seemingly disparate, the solvent exposed and transmembrane domains are intricately coupled in ClyA, and both are indispensable for generation of a successful membrane-inserted structure. These results can be extrapolated to design novel 'anti-toxin' therapies to be used in case of pathogenesis. Furthermore, structural analysis presented here can be used for the rationale design of nanopore based biosensors towards specific molecules, as well as improve fidelity of existing ones.

Poster presentation depicting short snippets from this work will start from 3:30 PM

For more information and artwork depicting the research (http://www.be.iisc.ernet.in/seminars.html)