IITB-Monash Research Academy

Indian Institute of Technology Bombay | MONASH University

An Indian-Australian research partnership

Project Title:	**Role of bacterial sugar-based lipids in mediating membrane-induced cell death.**	
Project Number	**IMURA0785**	
Monash Main Supervisor (Name, Email Id, Phone)	Professor Mibel Aguilar, mibel.aguilar@monash.edu	*Full name, Email*
Monash Co-supervisor(s) (Name, Email Id, Phone)		
Monash Head of Dept/Centre (Name,Email)	Prof. Roger Daly, roger.Daly@monash.edu	*Full name, email*
Monash Department:	Department of Biochemistry & Molecular Biology	
Monash ADRT (Name,Email)		*Full name, email*
IITB Main Supervisor (Name, Email Id, Phone)	Prof. Shobhna Kapoor, shobhnakapoor@chem.iitb.ac.in,	*Full name, Email*
IITB Co-supervisor(s) (Name, Email Id, Phone)	NA	*Full name, Email*
IITB Head of Dept (Name, Email, Phone)	Prof. K.P. Kaliappan, kpk@chem.iitb.ac.in,	*Full name, email*
IITB Department:	Department of Chemistry	

Research Clusters:

Highlight which of the Academy's CLUSTERS this project will address?
*(Please nominate JUST **one.** For more information, see www.iitbmonash.org)*

1	*Material Science/Engineering (including Nano, Metallurgy)*
2	*Energy, Green Chem, Chemistry, Catalysis, Reaction Eng*
3	*Math, CFD, Modelling, Manufacturing*
4	*CSE, IT, Optimisation, Data, Sensors, Systems, Signal Processing, Control*
5	*Earth Sciences and Civil Engineering (Geo, Water, Climate)*
6	*Bio, Stem Cells, Bio Chem, Pharma, Food*
7	*Semi-Conductors, Optics, Photonics, Networks, Telecomm, Power Eng*
8	*HSS, Design, Management*

Research Themes:

Highlight which of the Academy's Theme(s) this project will address?
(Feel free to nominate more than one. For more information, see www.iitbmonash.org)

1	*Advanced computational engineering, simulation and manufacture*
2	*Infrastructure Engineering*
3	*Clean Energy*
4	*Water*
5	Nanotechnology
6	*Biotechnology and Stem Cell Research*
7	*Humanities and social sciences*
8	Design

The research problem

The complex and unique cell wall of Mycobacteria species-*causative agent of Tuberculosis*-contains the free lipid sulfolipid, SL-1 and phthiocerol dimycocerosates, PDIM in their outer membrane, which are crucial for virulence but their function are not very well understood. The hydrophobic nature of the free extractable lipids in the outer bacterial membrane allows them to insert into the host lipid membrane, however how such lipids present in the envelope insert into the plasma membrane remains unclear. Membrane insertion of these lipids is expected to disturb the lipid composition of host membranes thereby altering their physical properties and hence modulating its cellular functions. Interestingly, we have recently made a novel finding that purified SL-1 displays anti-proliferative activity on eukaryotic mammalian host cells and induces cell death and identifies a yet-unknown function of SL-1 in the infection process. Furthermore, recent reports shed light on the role of mitochondrial reactive oxygen species during the host-pathogen interactions, and raise the question of direct involvement of mitochondrial membrane in mediating the effect of SL-1 in causing cell death. Unravelling the cross talk of these virulent lipids with host cell membranes would undoubtedly improve our understanding of the mechanism of action of SL-1 and related lipids in the virulence mechanism of the pathogen for therapeutic targeting.

Project aims

This project aims to gain molecular level insights into the mode of action of SL-1 and related lipids from the bacterial outer membrane with the host membrane. We will investigate the association between insertion of such lipids into the plasma membrane (PM) of live cells and changes in the PM organization and function in real time using live-cell fluorescence ratiometric imaging and fluorescence spectroscopy. Using specific cell membrane probes we will report on the dynamic changes in the host PM organization induced by lipid insertion. We will explore the effect of this lipid on the mitochondrial membrane organization and function using cell biology and molecular biology tools to elucidate the role of mitochondria in mediating these effects. To obtain mechanistic details on the mechanism of insertion of SL-1 into host cell membrane, we would perform dual polarization interferometry and surface plasmon resonance experiments on solid supported membranes derived from PM of host cells and compositionally simpler lipid model systems with varying charge, headgroup and chain lengths. The membrane localization of Laurdan in live host cells and model systems would allow us to map the distribution of membrane order and hydration on the bilayer surface during the interaction with Sl-1 to develop a model for host cell membrane insertion and offer significant insights into the modulation of ensuing membrane associated cellular functions. Finally, using proteomics approaches (both cytosolic and membrane-bound) we would like to uncover host cell proteins involved in this cross talk for discovering novel targets for therapeutic intervention.

Expected outcomes

Highlight the expected outcomes of the project

1. Understand the effect of bacterial outer membrane free lipids SL-1 and others on the lateral organization and/or disruption of PM and mitochondrial membrane.

2. Characterize the downstream cellular changes induced by the modulation of host cell membrane properties, specifically the induction of apoptosis and/or necrosis and underlying mitochondrial mechanisms using functional cellular assays.

3. Biophysically characterize bilayer order, hydration and organization using fluorescence microscopy, spectroscopy and dual polarization interferometry in live host cell and model membrane systems mimicking PM and mitochondrial lipids.

4. Identify host cell proteins mediating the plasma membrane action of SL-1 as potential therapeutic targets using chemical proteomics.

5. Develop proteome wide map to elucidate interconnected cellular signalling pathways modulated

during the host cell–SL-1 lipid cross talk.

How will the project address the Goals of the above Themes?

The detailed study of the link between the cell wall lipid SL-1 and host cell death pathway would provide important insights into the molecular mechanism of host cell manipulation by the pathogen. An improved understanding of the host membrane modulation by virulent lipid-based effector molecules from Mycobacterium species using quantitative measures and proteomic-based approaches will facilitate discovery of novel host-directed therapeutic targets for tackling tuberculosis. In addition, the project will lay down the foundation for answering pertinent questions related to modulation of specific and selective eukaryotic membrane-associated signalling events in diseased states for identifying therapeutic points of interventions.

Capabilities and Degrees Required

Masters in Biology, Biophysics, Chemistry (Physical or Organic) and/or Biochemistry. Basic knowledge of Mass spectroscopy (MALTI-TOF, LCMS, ESI-MS), and hands-on-experience in microscopy. Having experience in handling mammalian culture and basic cell biology/biochemistry tools is preferred. Proficient in written and spoken English.

Potential Collaborators

NA

Select up to **(4)** keywords from the Academy's approved keyword list **(available at http://www.iitbmonash.org/becoming-a-research-supervisor/)** relating to this project to make it easier for the students to apply.

BioScience, Biochemistry.