Characterization of the outer membrane (the mycomembrane) of Mycobacteria: Biochemical, biophysical and structural studies

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Scientific context:

Tuberculosis, whose etiologic agent is *Mycobacterium tuberculosis*, is responsible for about 1.4 million deaths each year with one person killed every 25 seconds. The emergence of MDR (multidrug resistant) or 'XDR' (extremely drug resistant) strains is partly responsible for the failure of treatment and the resurgence of tuberculosis. The development of new antibiotics against resistant strains is one of the objectives of disease control programs. This requires a good knowledge of the bacillus. The study of the cell envelope of Mycobacteriales is particularly important because of its localization at the interface between pathogens and the host immune system. Many biochemical data are available about the envelope molecular composition. It is a giant tripartite complex constituted of the mycomembrane (MM), arabinogalactan (AG) and peptidoglycan (PG), covered by a capsule. The MM exhibits a conventional 7-8 nm thickness but contains very long-chain, up to 90 carbons, fatty acids (mycolic acids, MAs), covalently linked to AG, which in turn is covalently attached to PG. The inner leaflet of the MM is believed to be formed by a parallel arrangement of MAs. The outer leaflet of the MM is composed of various lipids including trehalose lipids and phospholipids.

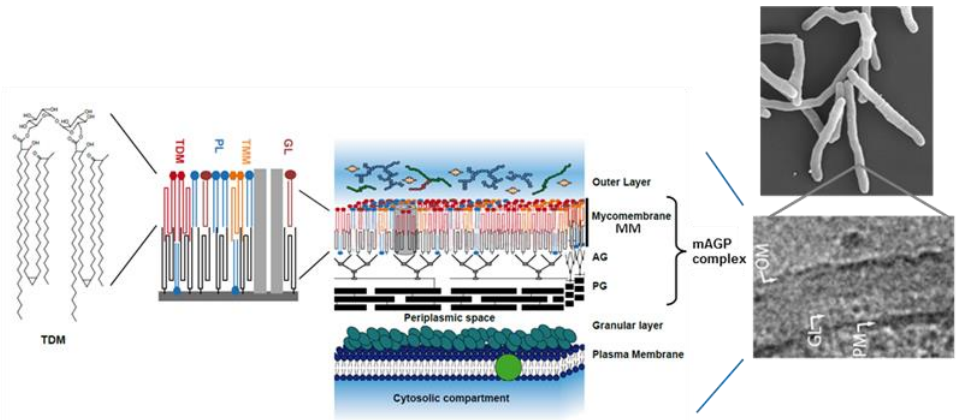


Fig. 1 Mycobacterial envelope (adapted from Chiaradia et al). Scanning electron microscopy (top) and CEMOVIS (right): plasma membrane (PM), granular layer (GL) and outer membrane (OM). Chemical model of the arrangement of cell envelope components: arabinogalactan (AG), peptidoglycan (PG), trehalose dimycolate (TDM in red), trehalose monomycolate (TMM in orange), glycolipids (GL in brown) and polar lipid (PL in blue).

MM rich in MAs appears to contain few porins (compared to *E. coli* for example), an observation that could explain the impermeability of mycobacteria to nutrients and hydrophilic antibiotics (up to 100 to 1000 times less permeable than bacteria to Gram negative *E. coli*). To date, only the MspA protein has been unambiguously identified and characterized as a porin in the MM of *Mycobacterium smegmatis*. However, the identification and characterization of the MM proteins is important for a number of reasons: i) MM proteins are essential to understand the physiology (e.g. nutriment uptake) and pathogenicity (e.g. virulent factors export) of mycobacteria. ii) MM proteins are folded into an atypical lipid membrane and it is interesting to determine their structure and especially to understand their interactions with mycobacterial lipids. iii) Inhibitors of MM proteins necessary for virulence or cell survival do not have to cross the notoriously impermeable MM, a major player in intrinsic drug resistance.

In order to identify the different constituents and characterize their organization in MM, **the "integrative biological NMR" team led by Dr Andrew Atkinson and Dr Olivier Saurel, and "Mycobacterial envelopes: Structure, biosynthesis and function" team led by Dr H. Marrakchi and Dr. M Daffé, from the Institute of**

Pharmacology and Structural Biology of Toulouse, combine their complementary expertise in structural biology and molecular analysis of these envelopes. The specific objectives of this collaboration are:

(i) Isolate and purify MM
(ii) Determine its lipid and protein composition
(iii) Analyze the structure and supramolecular organization of different membrane constituents (protein and / or lipid)

The collected data from these studies will allow the achievement of an important and essential step in the understanding of the cell envelope, notably the mycomembrane, a prerequisite for any future progress in its pharmaceutical targeting in the fight against mycobacterial pathogens as *Mycobacterium tuberculosis*.

Objectives of the research Master internship:

Due to the level 3 biosafety requirements associated with the handling of Mtb strains, studies will be performed on mycomembranes isolated from *Mycobacterium smegmatis* (Msm). This non-pathogenic and fast growing strain is widely used and accepted as a model for the biogenesis and organization of the Mtb cell envelope. This choice will allow the student to participate in all steps of sample preparation.

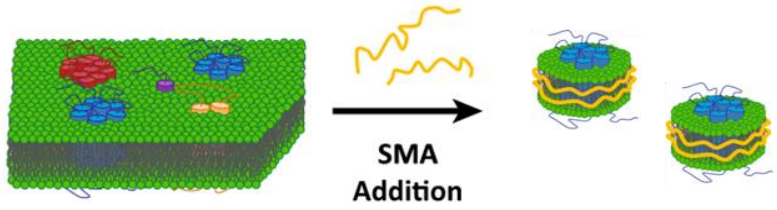
The Master's internship project has two objectives:

- Test the extraction of MM proteins into native-lipid nanodiscs by using co-polymers solubilization
- Study the conformation, spatial organization and dynamics of MM lipids at the molecular level.

Various experimental approaches will be implemented:

A) Bacterial culture, purification of mycobacterial cell envelope, production and purification of several enzymes for cell envelope digestion and MM purification: **all these first steps for the preparation of Msm mycomembrane have already been developed and validated**.

B) Optimization of MM solubilization by co-polymers**, biochemical and biophysical characterizations** of MM co-polymer nanodiscs, **liquid and/or solid state NMR characterization of MM and/or nanodiscs**.



Schematic representation of membrane solubilization in nanodiscs by styrene maleic acid co-polymers: (https://www.biomembranes.nl/membrane-solubilisation-styrene-maleic-acid-copolymers/)

References: [Myco] Daffé (2015) *Tuberculosis*, 95, S155-S158, Chiaradia, L. et al. (2017). Sci Report 7: 12807, Carel *et al* (2017) *PNAS*, 114: 4231-36, **[SMA]** Stroud, Z. et al. (2018) Methods, 147: 106–117, Flegler V. J., *et al* (2020), *PNAS*., 117: 28754, **[ss-NMR]** Kang, X. et al. (2018). *Nature Comm*. 9(1): 2747, Saurel, O. et al (2017) *JACS* 139, 1590.