Cardiolipin synthesis in bacteria and mitochondria

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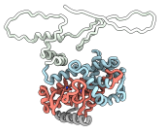
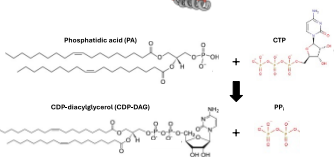
All living organisms depend on the ability to form membrane systems using phospholipids as membrane building blocks. The bacterial plasma membrane and mitochondrial inner membranes contain the unique diphosphatidyl glycerolipid cardiolipin. While the overall process of cardiolipin synthesis is known in terms of substrates, products and enzymes, detailed molecular insights into this pathway are limited, primarily due to the complete lack of structural knowledge of the enzymes. This gap also precludes fundamental understanding of mutations in cardiolipin-forming enzymes that cause severe diseases in affected patients. **The goal of the MIM-Lab is to dissect the molecular nature of bacterial and mitochondrial cardiolipin synthases in order to understand how structural features dictates their functional activities**.

Project 1: Human mitochondrial cardiolipin synthase

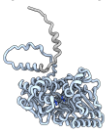
Human mitochondrial cardiolipin synthase CRLS1 belongs to the CDP-alcohol phosphotransferase enzyme family, which utilizes CDP-DAG to transfer phosphatidic acid onto phosphatidylglycerol to form cardiolipin. The whole cardiolipin synthesis process takes place in the mitochondrial inner membrane.

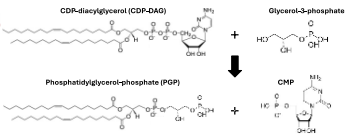
CDP-DAG synthesis by TAMM41



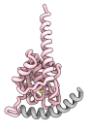


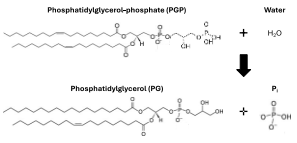
PGP synthesis by PGPS1



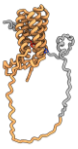


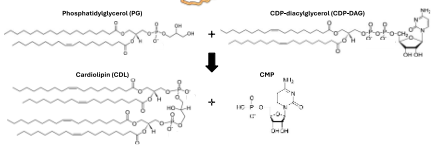
PG synthesis by PTPMT1





CDL synthesis by CRLS1

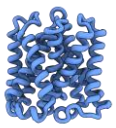


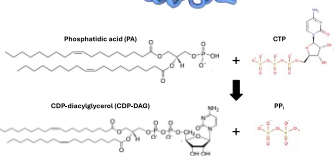


Project 2: Bacterial cardiolipin synthase

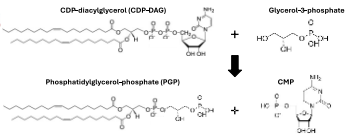
Bacterial cardiolipin synthase ClsA belongs to the phospholipase-D enzyme family, which condenses two molecules phosphatidyl-glycerol to form one cardiolipin. The bacterial cardiolipin synthesis process takes place in the plasma membrane, using a set of enzymes which are very different to the mitochondrial counterparts.

CDP-DAG synthesis by CdsA

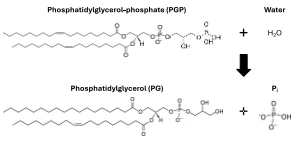




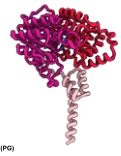
PGP synthesis by PgsA

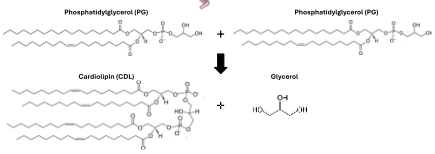


PG synthesis by PgpA



CDL synthesis by ClsA



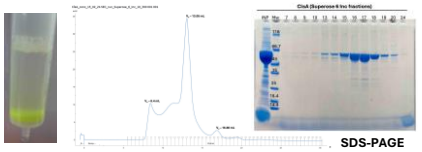


Techniques

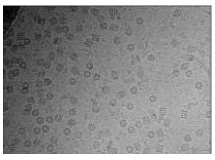
The lab uses standard molecular biology and biochemistry techniques, including but not limited to:

- Design and cloning of recombinant constructs
- Protein expression (bacterial and mammalian cell cultures)
- Protein purification (affinity, size exclusion chromatography, etc.)
- Native protein purification from mitochondria
- SDS and Blue-Native PAGE analysis
- Western blot
- Biophysical characterization (nanoDSF, DLS, mass photometry)
- Structural analysis (negative stain EM, cryo-EM)
- *In silico* structure prediction and design (AlphaFold, RFdiffusion)

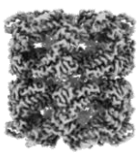
Affinity purification



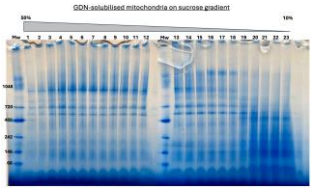
SDS-PAGE



Cryo-EM



Isolated mitochondria



Blue Native-PAGE

3D reconstruction

Further reading on MIM-Lab projects:
https://mbg.au.dk/rasmus-kock-flygaard

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