AUSTRALIAN SOCIETY FOR BIOPHYSICS

Abstract: 100O

Effect of membrane composition on lipid packing and solute permeability

Lauren A. Lowe,[ab] Rifah Riyadh[ab] and Anna Wang[ab]

[a] *School of Chemistry, UNSW Sydney, NSW 2052, Australia*
[b] *Australian Centre for Astrobiology, UNSW Sydney, NSW 2052, Australia*

This project is part of efforts to build a propagating minimal synthetic cell. A core feature of this synthetic cell is having the enzymes required to grow the membrane (Figure 1). A vital part of this is ensuring that an external feedstock of nutrients is available to ensure that a high yield of phospholipid synthesis and subsequent membrane growth can occur.[1] The growing membrane could have a range of different lipid compositions depending on gene expression levels, but it is not yet understood how this range of lipid compositions could impact membrane permeability. Improving the permeability of membranes of mixed lipid composition to the nutrients required for phospholipid synthesis, some of which are largely impermeable, will help ensure that membrane growth and subsequent division can successfully occur.

Electrical impendence spectroscopy and a shrink-swell assay were used to monitor lipid packing and the permeability of bilayers composed of a mixture of lipids, some of which are important intermediates in phospholipid synthesis. We found that vesicles composed of a blend of POPC and POPG were permeable to sugars such as glycerol and glucose but impermeable to larger species such as sucrose. Blended POPC and POPG bilayers were also permeable to glycine, the simplest amino acid, but impermeable to slightly more complex amino acids such as lysine. We also found that these membranes were largely impermeable to a range of other solutes including AMP, ATP and NaCl, likely owing to their charged nature or size. Cataloguing the permeability of blended membranes to solutes such as these helps us to tune gene expression levels to improve bilayer permeability to nutrients that are vital to the function of the synthetic cell. This will help us work towards the tantalising goal in membrane biophysics of building a propagating synthetic cell that can grow and divide using the simplest components possible.

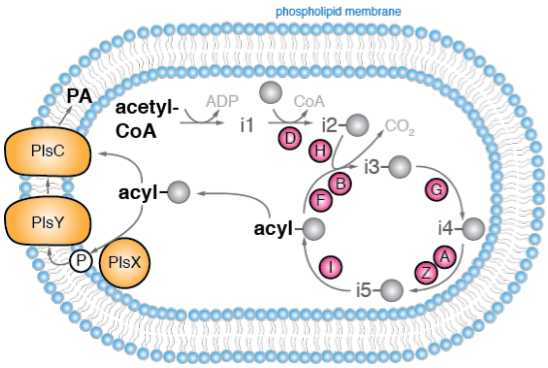

Figure 1. Schematic showing the initial phospholipid synthesis pathway. Enzymes are shown in orange (Pls) and pink (Fab). Acyl-carrier protein is shown in gray. Intermediates are labeled i#. Courtesy Kuruma, Rogers, Wang, HFSP grant.

(1) Eto, S.; Matsumura, R.; Fujimi, M.; Shimane, Y.; Berhanu, S.; Kasama, T.; Kuruma, Y. Reconstruction of Phospholipid Synthesis by Combing in Vitro Fatty Acid Synthesis and Cell-Free Gene Expression. bioRxiv 2021.08.03.454925