

## The role of hydrocarbons in cyanobacterial membranes

### Content

Cyanobacteria contain highly-folded photosynthetic membranes called thylakoids, that host the necessary proteins for photosynthesis. It has been shown that these lipid membranes contain up to 17 mol% hydrocarbon in their compositions, which are synthesized by the bacteria.[1] Where the hydrocarbon sits within the lipid leaflets and the effect of hydrocarbon incorporation on thylakoid membrane properties has not previously been investigated. It is postulated that hydrocarbons facilitate curvature of the thylakoid membrane structures.

We have directly observed small angle scattering differences between two live bacterial samples: a wild-type cyanobacterium (WT) and a variant that has been genetically modified to not produce hydrocarbons. The thylakoids form stacked membranes in the cyanobacteria that scatter as Bragg peaks when measured by small angle scattering. Our data analysis suggests that there is a small decrease in thickness of membranes in the hydrocarbon deficient mutant. Furthermore, the hydrocarbon deficient mutant does not undergo the large light to dark membrane restructuring that would be expected, suggesting that the lack of hydrocarbons severely restricted the flexibility of the membrane.

To understand the underlying physics of alkane incorporation on lipid membrane properties we took the approach of investigating lipid bilayer membrane mimics. One of the key questions was to understand where the hydrocarbon sits within the lipid bilayer. We expect that the hydrocarbon will associate with the lipid tails, however, depending on the lipid properties, the alkane could sit parallel or perpendicular to the lipid tails within the bilayer leaflet. SANS and SAXS results show an increase in bilayer thickness of a thylakoid lipid bilayer with increasing hydrocarbon content. By making use of selective deuteration and SANS (using deuterated hydrocarbon and hydrogenated lipid) we were able to observe that at over 10 mol% hydrocarbon content the hydrocarbon separates between the tail layers. A similar experiment was done on planar lipid bilayers with neutron reflectivity which further confirmed the formation of a hydrocarbon layer between the lipid leaflets.

One of the other approaches taken in this project has been determining the changes in dynamic properties of the lipid membranes with increasing hydrocarbon content. This has been investigated using infrared spectroscopy and differential scanning calorimetry of lipid films. DSC results indicated a decrease in the gel to fluid transition of lipids with increasing hydrocarbon content. Infrared analysis of planar lipid bilayers showed a decrease in wavenumber of the CH<sub>2</sub> asymmetric stretching band. Combined these initial results suggest that hydrocarbons increase the fluidity of the lipid bilayer-in line with our conclusions on live bacteria.

### References

- [1] Lea-Smith, D. J. et al. Hydrocarbons are essential for optimal cell size, division, and growth of Cyanobacteria. *Plant Physiol.* 172, 1928–1940 (2016).

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