The role of hydrocarbons in cyanobacterial membranes.

Understanding location and function

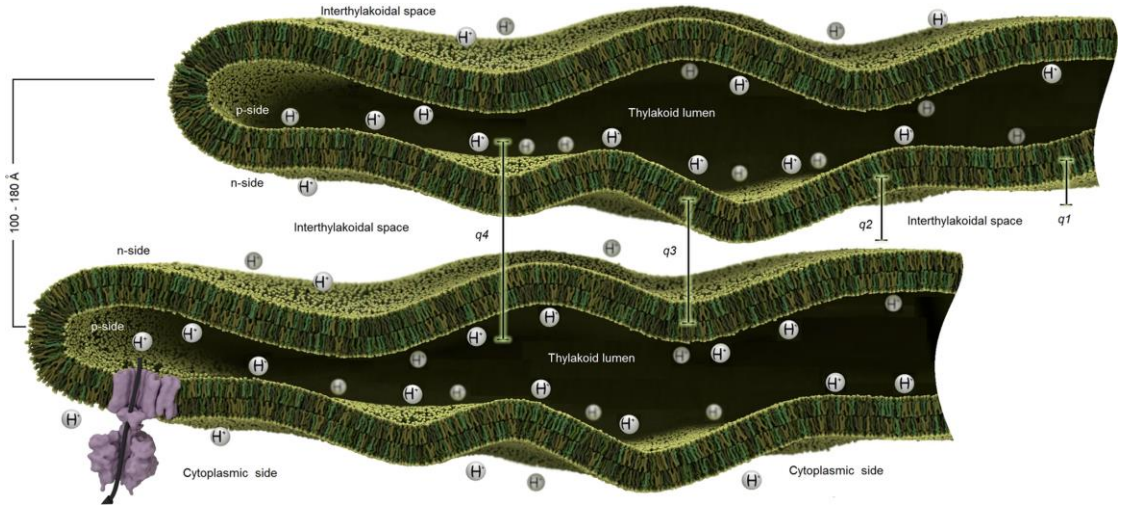
PRESENTED BY DR SOPHIE AYSCOUGH, EUROPEAN SPALLATION SOURCE

Cyanobacteria

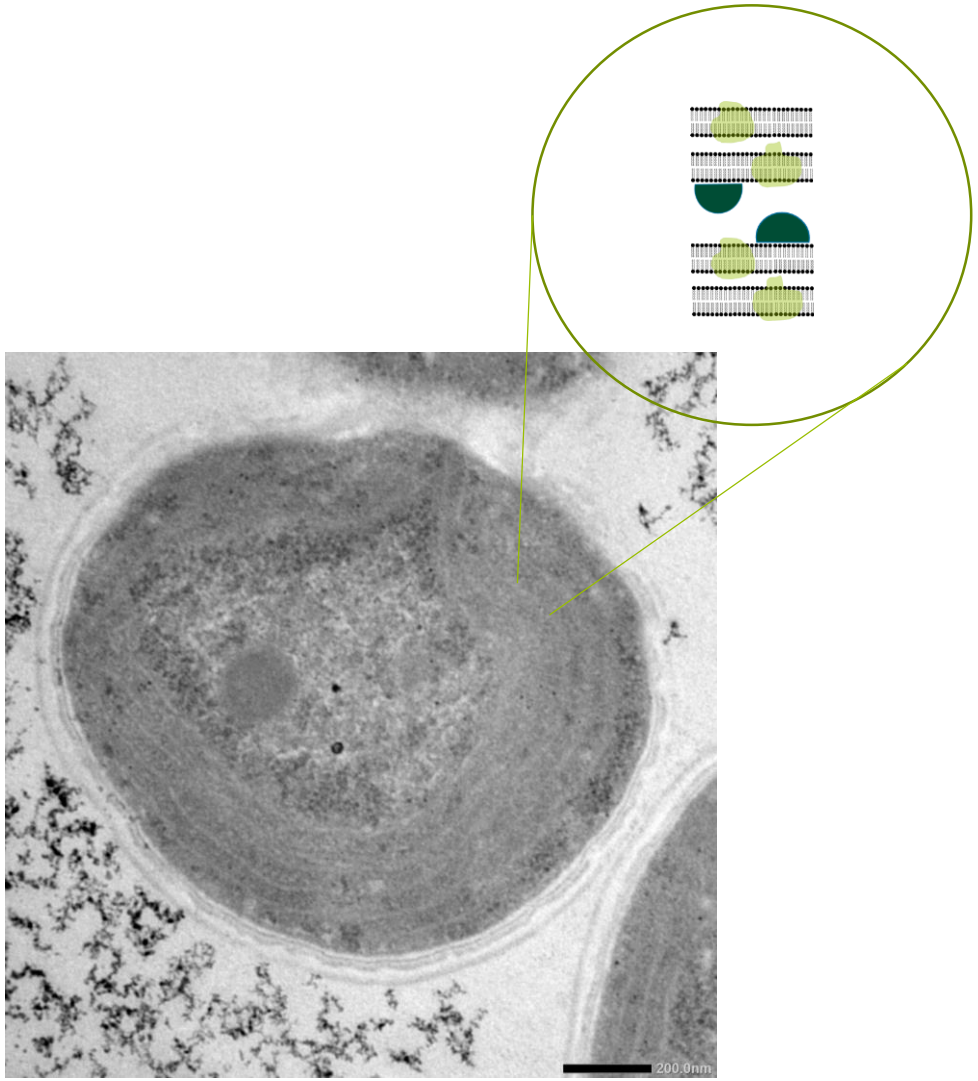
- Bacteria capable of oxygenic photosynthesis
- Highly abundant
- Many pathways/processes are conserved in plants (precursor to chloroplasts)
- Platform for biotechnology applications
    - biofuel production
    - industrial compounds (such as hydrocarbons)
    - food
    - Oil spill clean up

July 11, 2015 (NASA)

Cyanobacteria cell structure: Thylakoid membranes

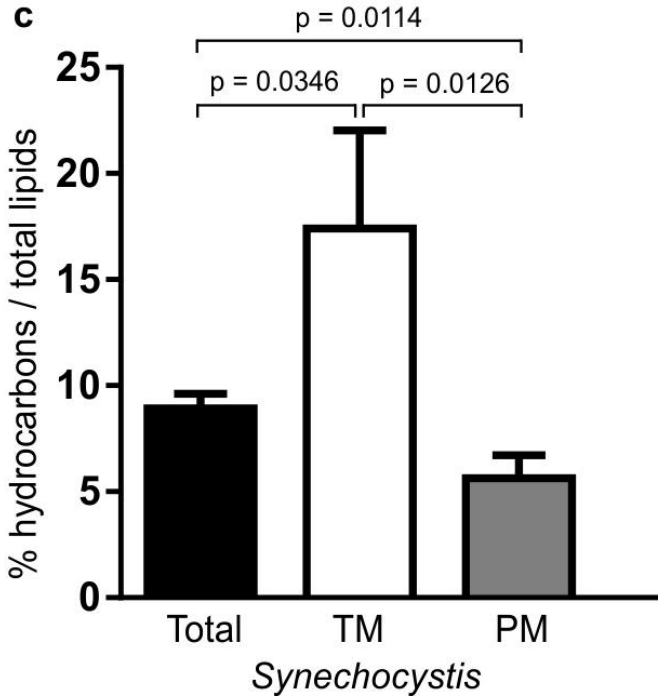


Stingaciu, L, et al., *Scientific reports* 6.1 (2016): 1-6.



Hydrocarbons accumulate in membranes

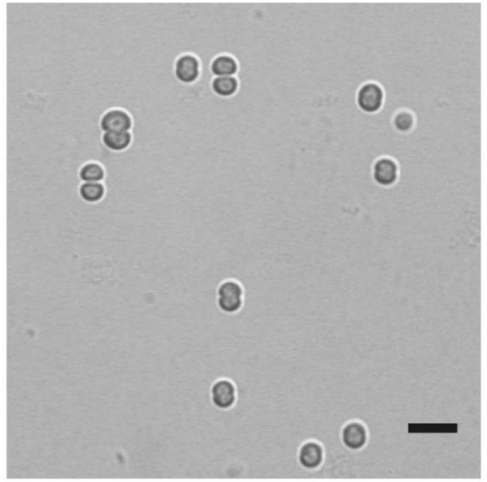
- Purified total membranes (TM) from *Synechocystis*
- Purified thylakoid (TM) and plasma membranes (PM) from *Synechocystis*
- % hydrocarbons as total lipids by GC-MS



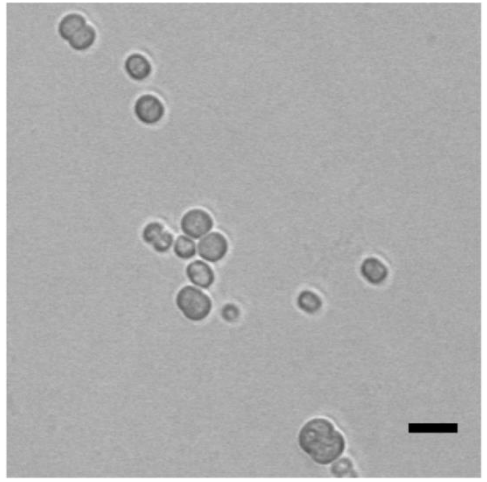
- 5 mol% heptadecane in the plasma membrane.
- 17 ml% heptadecane in the thylakoid membrane.

Lea-Smith, David J., et al. , *Plant Physiology* 172.3 (2016): 1928-1940.

Hydrocarbon deficient mutants developed that have increased cell size and division defects



Wild-type *Synechocystis*



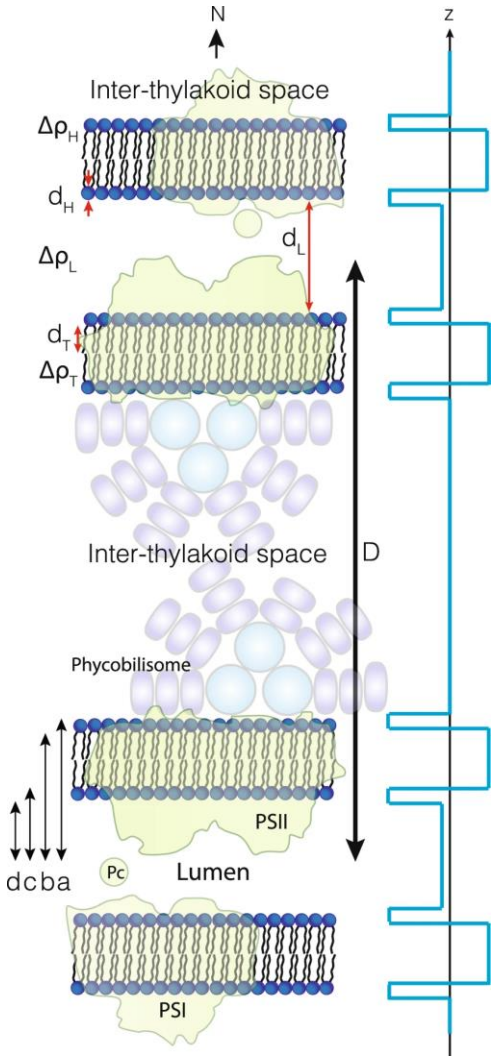
Hydrocarbon deficient mutant

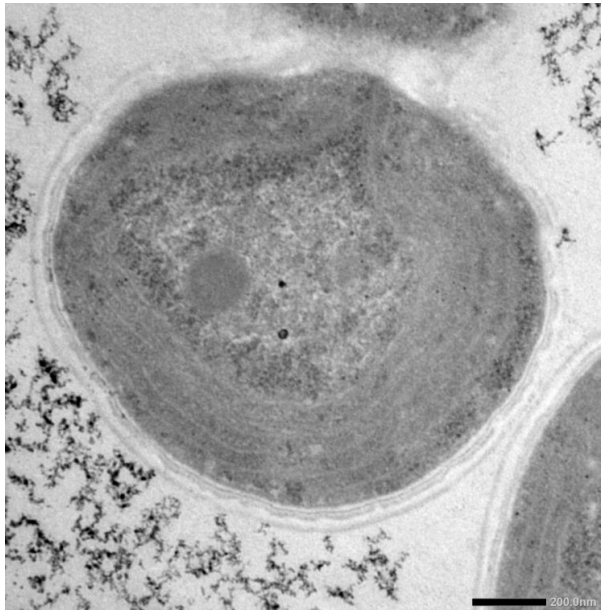
- WT Synechocystis synthesizes heptadecane in its cells.
- A genetically modified strain (a mutant) was developed that cannot produce heptadecane.
- The mutant cells have increased cell size and division defects.

SOPHIE AYSCOUGH, ESS, SOPHIE.AYSCOUGH@ESS.EU

Lea-Smith, David J., et al. , *Plant Physiology* 172.3 (2016): 1928-1940.

SANS of live cyanobacteria





Small angle neutron experiment at ILL on D11

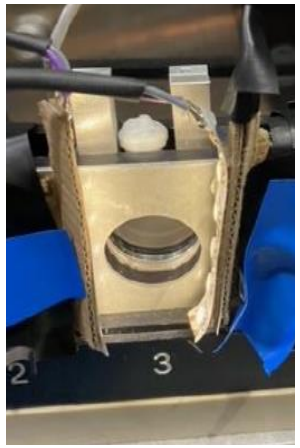
Measured two species:

- Wild-type Synechocystis
- Mutant of Synechocystis -modified to be hydrocarbon deficient.

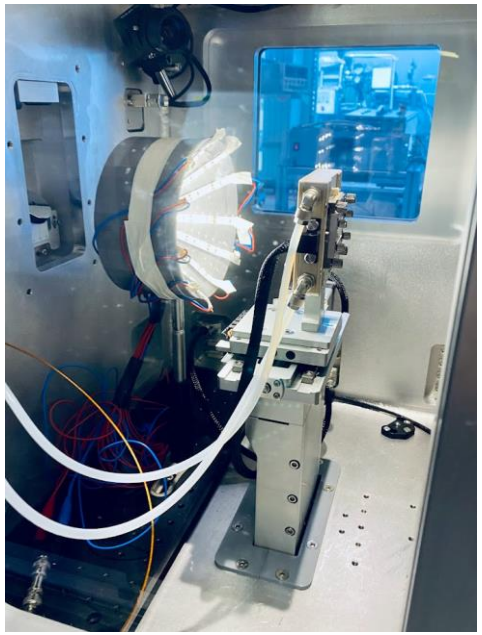
- The repeat spacing between thylakoid membranes results in Bragg peaks in the SANS curves.
- Thylakoid spacings adapt to light conditions.

Image taken from: Jakubauskas, Dainius, et al. "Ultrastructural modeling of small angle scattering from photosynthetic membranes." *Scientific reports* 9.1 (2019)

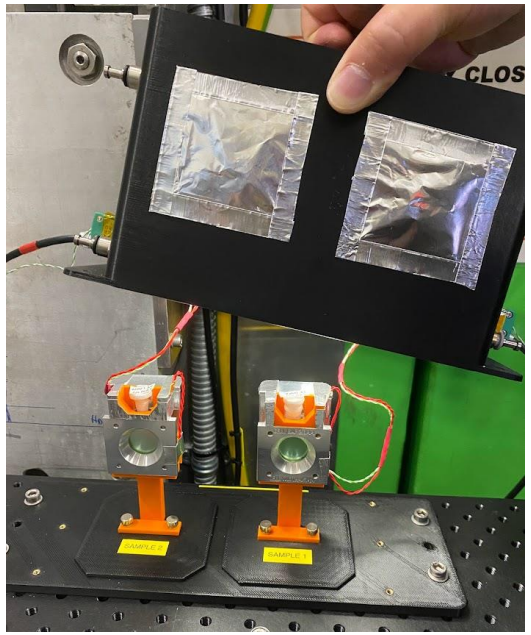
Small angle scattering light set-ups



D11, ILL
SANS
Olga Matsarskaia (ILL)
Harald Schneider (ESS)
Katrin Michel (ESS)



DLSAXS, Diamond
SAXS
Sam Burholt (Diamond)
Harald Schneider (ESS)

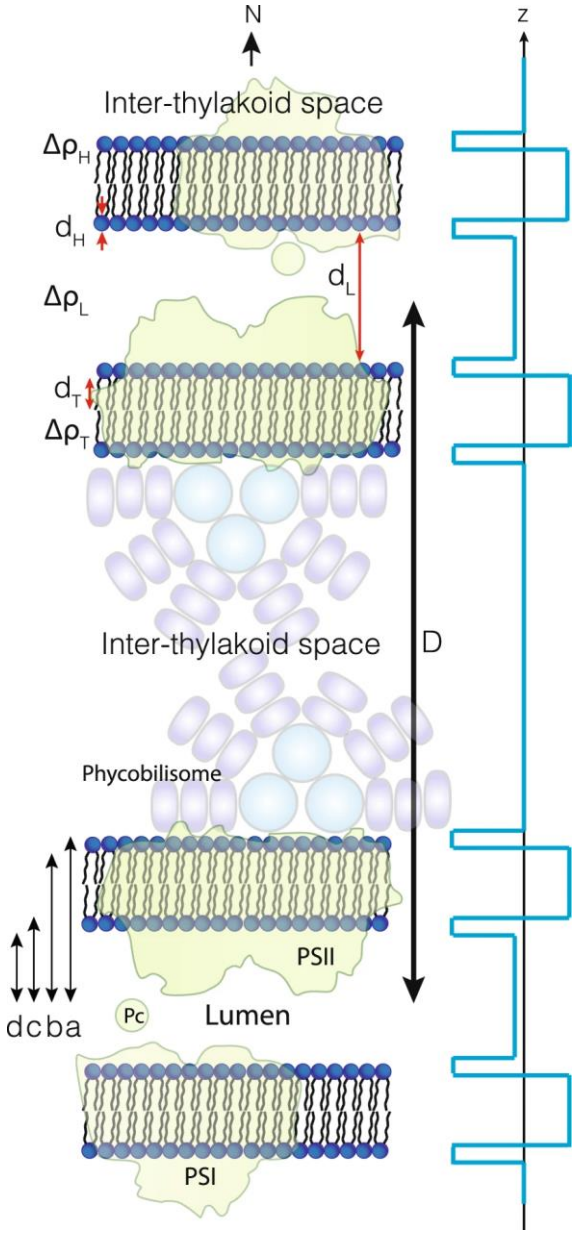


SANS2D
SANS
Najet Mahmoudi (ISIS)
Maksim Schastny (ISIS)

Cyanobacteria measured in light and dark conditions as membrane spacings of WT are known to be light responsive.

Light:
- 50 µmol photons m^{-2} s^{-1}
- Warm white LED light
- Grown in light over 2 days.
- Incubated on SANS/SAXS instrument for 1 hr before measurement.

Dark:
- Kept in dark for 6 hours before measurement.
- Covered in aluminium foil on beamline.



What affects ‘peak appearance’ in Small Angle Scattering

Peak position in Q dependent on a repeat distance in a sample.

Peak shape is dependent on the polydispersity of that repeat distance.

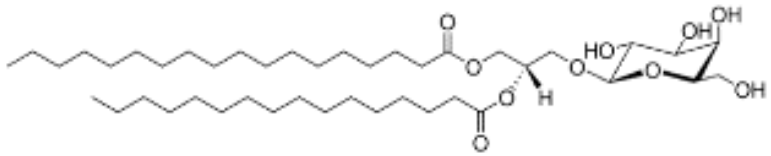
AND

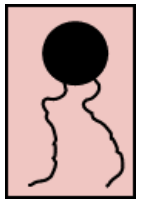
Is dependent on the membrane flatness/disorder.

(difficult to completely differentiate the two even with complex 3D modelling)

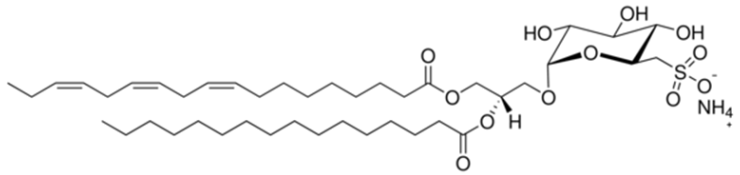
Thylakoid Lipids

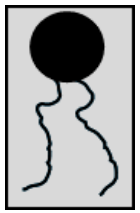
MGDG



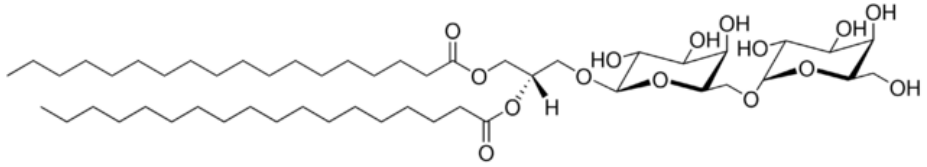


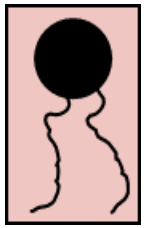
SQDG



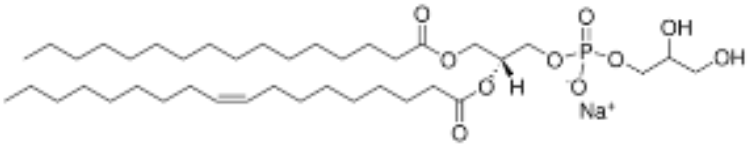


DGDG

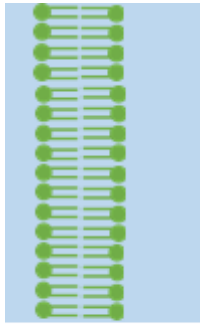


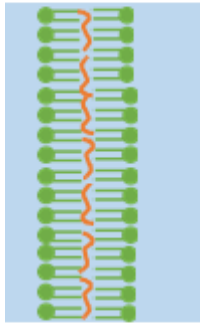


POPG

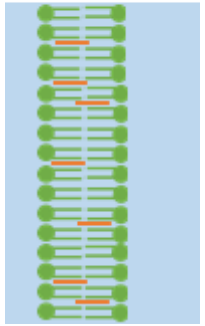


SANS locating the hydrocarbon in liposomes





Hydrocarbon between the lipid leaflets.



Hydrocarbon parallel with the lipid tails.

Unpublished results and conclusions removed.

Please contact me at sophie.ayscough@ess.eu with any questions or for discussion on this research.

Joint project: Understanding the role of hydrocarbons in cyanobacteria.

THE UNIVERSITY OF AUCKLAND
Te Whare Wānanga o Tāmaki Makaurau
NEW ZEALAND

Shamim Shadfar Jane Allison

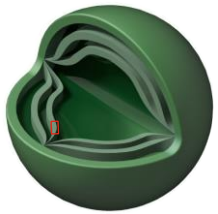
Membrane modelling

In vivo and in vitro scattering measurements

Melissa Sharp Me

EO4

HFSP HUMAN FRONTIER SCIENCE PROGRAM



Imperial College London

Oscar Ces Chi L. Chan

UEA University of East Anglia

David Lea-Smith Andrew Curson

Physical analysis of Membrane extracts and lipids.

Analysis of cyanobacterial and algal mutants