Experimental report

15/09/2022

Proposal: 8-02-975 **Council:** 4/2021

Title: Using small angle neutron scattering to probe the structural changes induced by hydrocarbons in cyanobacteria

Research area: Biology

This proposal is a new proposal

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Samples: cyanobacteria (Synechocystis sp. PCC 6803) in deuterated media

Instrument	Requested days	Allocated days	From	To
D11	1	1	11/09/2021	12/09/2021
D22	1	0		

Abstract:

Cyanobacteria are known to produce hydrocarbons between 15-22 carbons in length, these hydrocarbons are the ideal length for kerosene and diesel fuel. The role of these hydrocarbons in cyanobacteria is not known. Since the hydrocarbons are located predominantly in the thylakoid membranes of cyanobacteria, it has been suggested that they aid the bacterial membrane in being able to form the complex architectures needed to house the necessary proteins for photosynthesis. Our aim is to directly measure the membrane curvature and thickness of thylakoid membranes in the cyanobacterium Synechocystis sp. PCC 6803 from wild-type and hydrocarbon-deficient mutant cells, under dark and light conditions using small angle neutron scattering.

Experimental report for proposal 8-02-975

Using small angle neutron scattering to probe the structural changes induced by hydrocarbons in cyanobacteria

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Our aim was to understand how hydrocarbons sit within the bacterial membranes and how the presence and absence of hydrocarbons affects the membrane thicknesses and curvature within the cell. Hydrocarbon deficient mutants, i.e. cyanobacteria that have been genetically modified such that they can no longer produce hydrocarbons, have been shown to have significant growth, size and division defects.[1] Small angle neutron scattering has been used to analyse other cyanobacteria and plant thylakoid membranes to discern the repeat distances between the thylakoid membranes. These repeat distances will appear as clear Bragg features in the collected SANS data.[2-5] The neutron scattering measurements were carried out *in-vivo* i.e. on live bacterial cells. The results are part of a HFSP funded, multidisciplinary investigation into the question of whether hydrocarbons induce membrane curvature in cyanobacteria via a novel mechanism previously unobserved in nature. [6]

Cultures of the cyanobacterium *Synechocystis* PCC6803 were measured using small angle neutron scattering on D11 at 2 detector distances (10 m and 1.7 m). Both the wild-type (WT) and hydrocarbon-deficient mutant were investigated with the aim of observing structural differences in the thylakoid arrangement of the bacteria with the absence of hydrocarbons. Cultures were grown to an optical density (OD) of 0.4, pelleted and resuspended in 97% D_2O BG11 media to an OD of 4. Cultures were measured in light and dark conditions, the light condition being 50 µmol photons m^{-2} s^{-1} of warm white light (6000K) provided by LED strips placed in-front of the sample changer and the dark condition being provided by aluminum foil covering the sample position. Cells were measured that had been incubated in the light or dark for at least 6 hours prior to measurement (including 1 hour in-situ on the instrument). Measurements were also made after switching the light environment on/off to observe whether structural rearrangement on the timescale of 3 minutes to 1 hour occurred. Initial fits have been carried out using a Pseudo-Voight peak fitting model in SASview, fitting 5 individual peaks, see the peak models displayed in Figure 1.

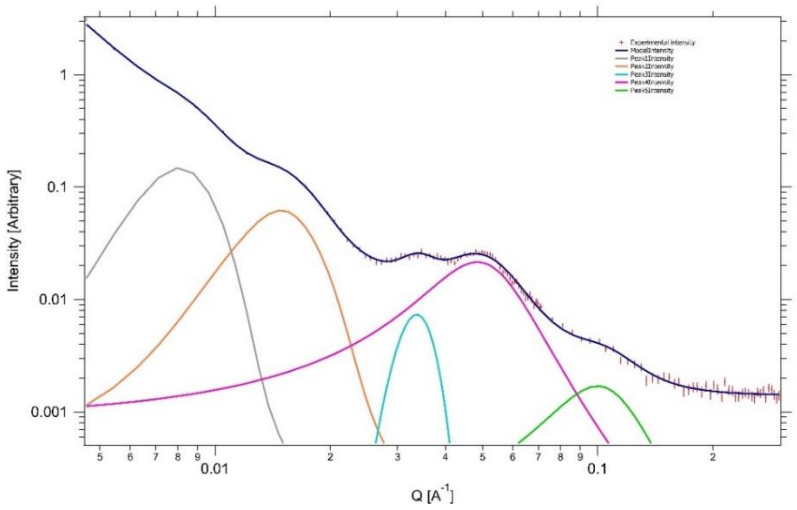

Figure 1: *Pseudo-voight model fitting of small angle neutron scattering of Synechocystis 6803 WT light incubated. The 5 peak models are shown.*

[1] Lea-Smith et al, *Plant Physiology* 2016 172(3)1928; [2] Liberton et al, *J. Biol.Chem.* 2013 288(5) 3632; [3] Stingaciu et al, *Nature Scientific Reports* 2016 6:19627; [4] Ünnep et al, *Open Biol.* 2020 10200144 [5] Jakubauskas et al. *Scientific reports* 9.1 2019 1-13. [6] HFSP grant RGP0031/2019

Results and Analysis

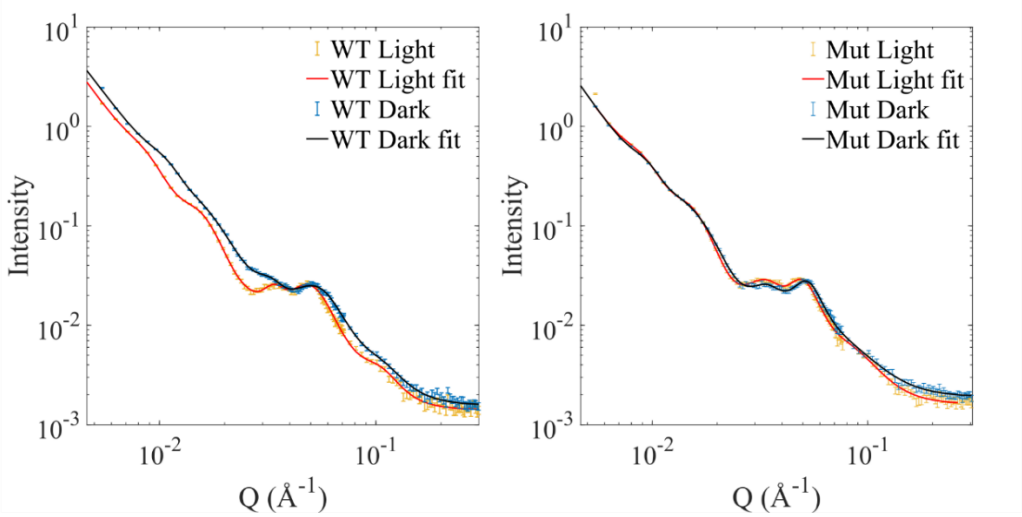

Figure 2: *Difference in thylakoid spacing after light and dark incubation for WT Synechocystis (Left) and the hydrocarbon deficient mutant (right), all scattering curves are fit to a 5 peak pseudo-voight model.*

The scattering observed from wild type *Synechocystis* 6803 shifts depending on whether the bacterial culture has been incubated in the light or the dark, see the left panel of Figure 2, which is consistent with observations from other researchers. The shift in peak position is equivalent to a 10 Å decrease in distance between the thylakoid lumens after dark incubation, this is more than observed in previous work possibly due to our use of warm rather than cool white light. [3] Other changes to the scattering curve are apparent other than a simple shift in peak positions, the low-Q peaks after dark incubations are broader indicative of a broader polydispersity of distances between the thylakoids. The peak shape is also dependent on the membrane rigidity, i.e. flat thylakoid sheets would result in more clearly defined peaks and undulating thylakoids would result in peak blurring. For the hydrocarbon deficient mutant, only a minimal shift in peak position in the SANS scattering is observed between light and dark incubation suggesting that the hydrocarbons are necessary for rearrangement of the thylakoids in response to light.

As well as comparing light and dark incubation, we investigated exposing dark incubated samples to light to determine whether thylakoid rearrangement could be observed within a few minutes, similar to that observed by Ünnep et al. for monstera leaves.[4] WT *Synechocystis* undergoes a transition from when exposed to light within 3 minutes with some shifts still occurring after 48 minutes, see Figure 3. We did not observe the scattering returning to that of the light incubated culture, although the peak positions are similar. This is likely due to the phycobilisomes (Large, ~1 MDa light antennae between the thylakoid membranes) being down regulated during dark incubation. The hydrocarbon-deficient mutant *Synechocystis* did not undergo any thylakoid rearrangement on exposure to light, as no shift was observed in the peak positions, see Figure 4.

[1] Lea-Smith et al, *Plant Physiology* 2016 172(3)1928; [2] Liberton et al, *J. Biol.Chem.* 2013 288(5) 3632; [3] Stingaciu et al, *Nature Scientific Reports* 2016 6:19627; [4] Ünnep et al, *Open Biol.* 2020 10200144 [5] Jakubauskas et al. *Scientific reports* 9.1 2019 1-13. [6] HFSP grant RGP0031/2019

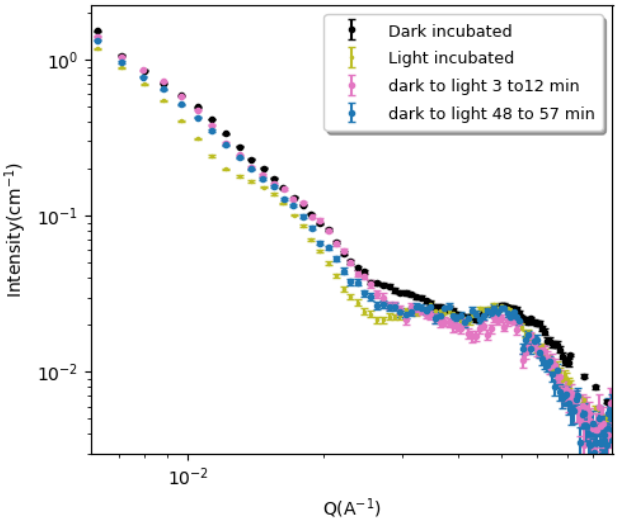

***Figure 3**: SANS curves of light and dark incubated WT Synechocystis 6803 and dark incubated Synechocystis 3 minutes and 48 minutes after being exposed to light.*

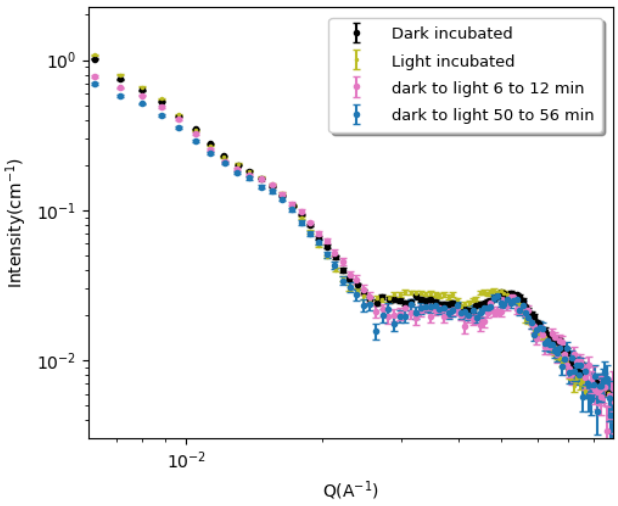

***Figure 4**: SANS curves of light and dark incubated Mutant Synechocystis 6803 and dark incubated Synechocystis 6 minutes and 50 minutes after being exposed to light.*

Conclusions

In the WT cyanobacterium we calculated a 10 Å decrease in spacing between the thylakoid lumens of cultures that had been dark rather than light incubated, calculated from the peak at 0.05 Å^{-1}. The change in thylakoid spacing was not observed in the hydrocarbon deficient mutant which we expect is due to the decreased flexibility of the membranes preventing rearrangement. Initial shown fits have fitted each of the peaks individually however recent publications that fits cyanobacterial SANS data to simulated SANS curves from 3D models of the *Synechocystis* thylakoid suggest that the peaks are resultant of a repeating thylakoid structure, i.e. are a result of 4 repeat thylakoid structures. [5] Our next step is to apply complex thylakoid model fits to the data set but also to separately constrain the peak fitting such that repeat laminar spacings are constrained, the second option reduces any overfitting of the data.

[1] Lea-Smith et al, *Plant Physiology* 2016 172(3)1928; [2] Liberton et al, *J. Biol.Chem.* 2013 288(5) 3632; [3] Stingaciu et al, *Nature Scientific Reports* 2016 6:19627; [4] Ünnep et al, *Open Biol.* 2020 10200144 [5] Jakubauskas et al. *Scientific reports* 9.1 2019 1-13. [6] HFSP grant RGP0031/2019