Experimental report

31/10/2023

Proposal: 8-02-962 **Council:** 4/2021

Title: New information about the structure and dynamic composition of lipid reservoirs mediated by lung surfactant protein B at low surface tension

Research area: Biology

This proposal is a new proposal

Main proposer: Richard CAMPBELL

Experimental team: Javier CARRASCOSA TEJEDOR
Yixuan YAN
Richard CAMPBELL
Glenn COOPE
Margaret Jayne LAWRENCE

Local contacts: Thomas SAERBECK
Philipp GUTFREUND

Samples: Lipids: DPPC and d62-DPPC
Lipids: POPC and d31-POPC
Lipids: POPG and d31-POPG
Recombinant peptide: Mini-B (FPIPLPYCWLCRALIKRIQAMIPKG)

Instrument	Requested days	Allocated days	From	To
FIGARO	3	3	23/09/2021	26/09/2021

Abstract:

Neonatal respiratory distress syndrome is an acute condition suffered by premature babies who have suppressed amounts of lung surfactant protein SP-B. The protein is understood to function by nucleating lipid reservoirs at the air/water interface as the surface of the lungs is compressed during expiration, allowing surplus lipid confined close to the surface monolayer to spread rapidly and maintain very low surface tension during inhalation. Only intuitive physical pictures of the reservoirs have been proposed to date due to the extreme experimental challenge of resolving structures at fluid interfaces under physical conditions that mimic respiration. This proposal builds on lab data where we have done exactly that as phosphatidylglycerol lipids appear to modulate the squeeze out of unsaturated lipids from the surface monolayer by an SP-B analogue called Mini-B at low surface tension during multiple cycles of the surface area. Structural and compositional neutron reflectivity data are proposed to resolve unique information under dynamic cycles of the surface area at low surface tension for the first time. The work will lead to future studies on more advanced lung surfactant models.

FINAL EXPERIMENTAL REPORT: #8-02-962

New information about the structure and dynamic composition of lipid reservoirs mediated by lung surfactant protein B at low surface tension

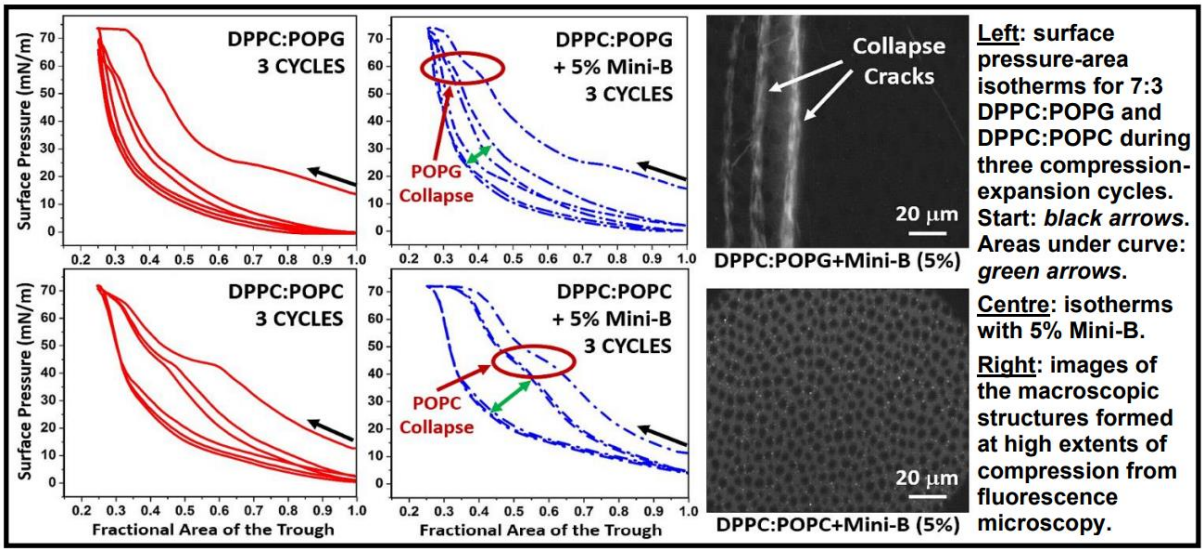
Glenn Coope, Yixuan Yan, Javier Carrascosa, Jayne Lawrence & Richard Campbell

FIGARO, 26–29 September 2021

Abstract

Neonatal respiratory distress syndrome is an acute condition suffered by premature babies who have suppressed amounts of lung surfactant protein SP-B. The protein is understood to function by nucleating lipid reservoirs at the air/water interface as the surface of the lungs is compressed during expiration, allowing surplus lipid confined close to the surface monolayer to spread rapidly and maintain very low surface tension during inhalation. Only intuitive physical pictures of the reservoirs have been proposed to date due to the extreme experimental challenge of resolving structures at fluid interfaces under physical conditions that mimic respiration. This proposal builds on lab data where we have done exactly that as phosphatidylglycerol lipids appear to modulate the squeeze out of unsaturated lipids from the surface monolayer by an SP-B analogue called Mini-B at low surface tension during multiple cycles of the surface area. Structural and compositional neutron reflectivity data are proposed to resolve unique information under dynamic cycles of the surface area at low surface tension for the first time. The work will lead to future studies on more advanced lung surfactant models.

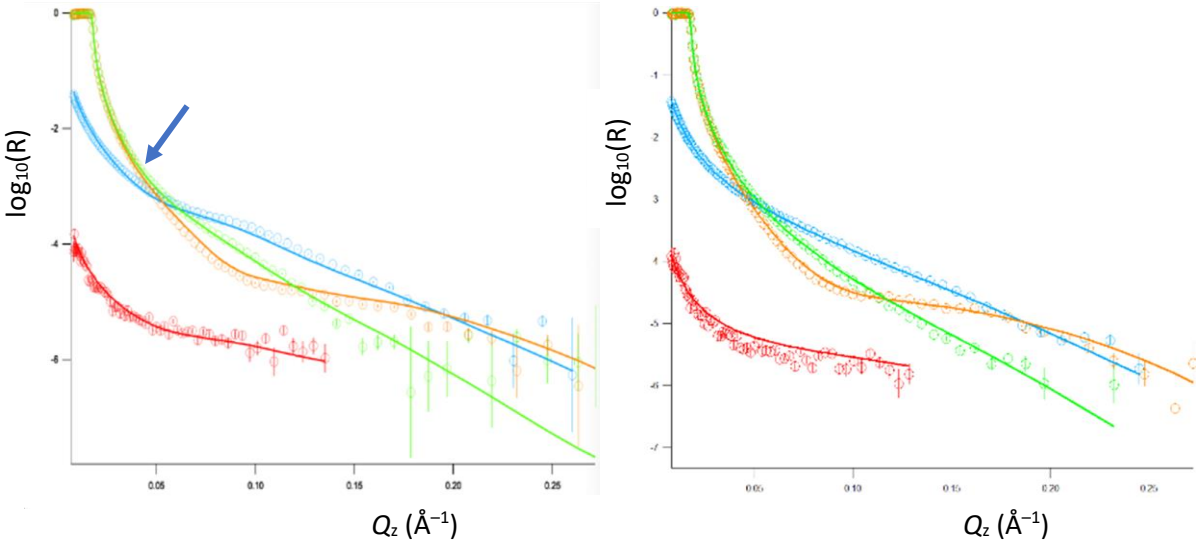
Preliminary Results



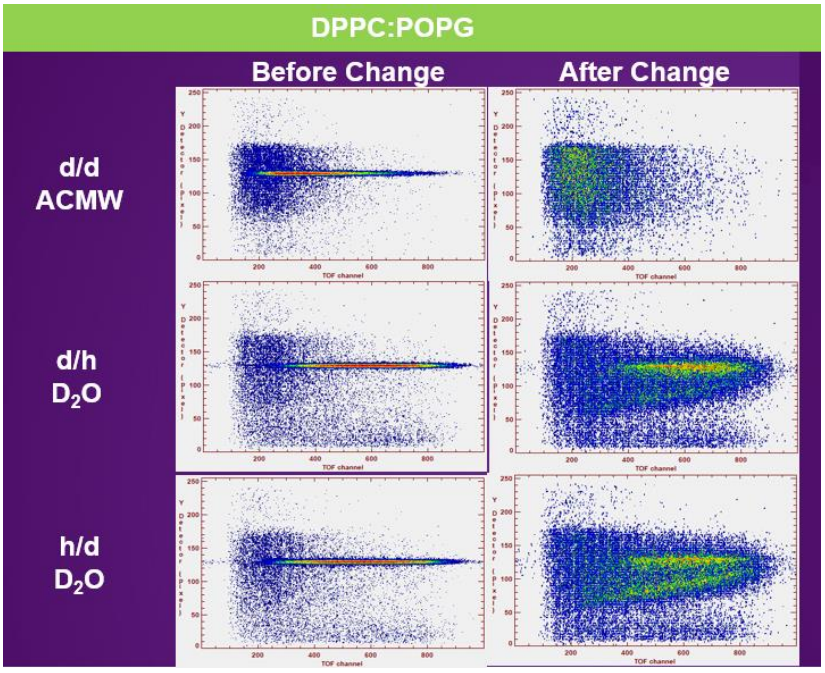
The key results that motivated this neutron reflectivity experiment were on the DPPC:POPC and DPPC:POPG (7:3 by mole) systems. We had recorded surface pressure–area isotherms as well as ellipsometry and Brewster angle microscopy data in our lab prior to the beam time application. Kinks in the surface pressure–area isotherms motivated a plan to resolve the interfacial structure for both systems at a surface pressure of 50 mN/m. Furthermore, thanks to use of our Kibron G2 trough, underfilled and with Delrin barriers, which we installed on the instrument, we did unprecedented mid-Q_z dynamic measurements up to 70 mN/min one system, as described below.

Experimental Results

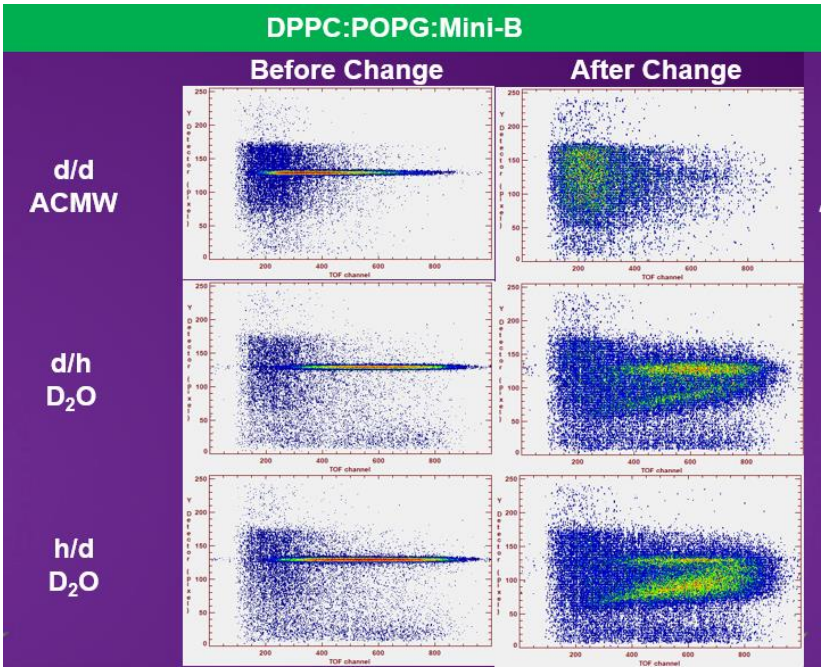
First, we resolved the interfacial structure of the DPPC:POPC (left) and DPPC:POPG (right) systems. The Kiessig fringe (blue arrow) only in data on the DPPC:POPC system is consistent with an interfacial model with an extended structure of lipid in a reservoir bound to the surface monolayer. The absence of the fringe for the DPPC:POPG system was consistent with a surface monolayer only. This result can be linked to the kink in the surface pressure–area isotherm on page 1 of this report.



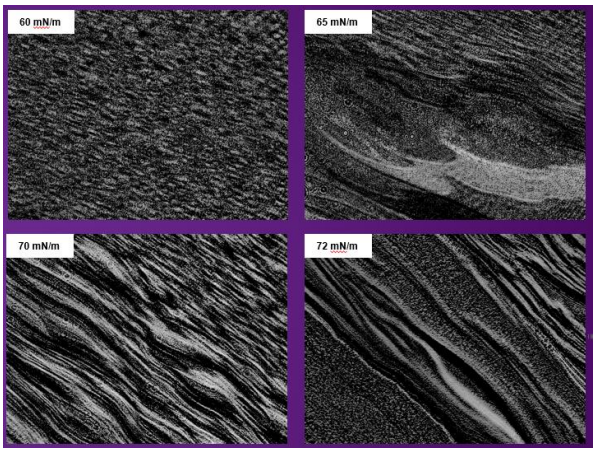
We were unable to perform measurements on the DPPC:POPC system right up to 70 mN/m because the monolayer collapsed prematurely and was not stable enough for data acquisition. However, were able to do so on the DPPC:POPG system in an unprecedented experiment for neutron reflectometry. The dynamic scans were performed in the mid-Q_z range (theta = 2 degrees) during compression. In this case, there was a marked change in the film properties in the range 63–68mN/m according to the isotopic contrast, where d/d = dDPPC/dPOPG, d/h = dDPPC/hPOPG and h/d = dDPPC/dPOPG. A pronounced Yoneda peak appeared at the expense of the specular peak, even though the surface pressure continued to rise up to 70 mN/m. The effect was attributed to a macroscopic buckling of the film, which, notwithstanding that the mixture is a very crude model of lung surfactant, is an observation we feel is interesting to probe further in experiments on more advanced model systems.



We went on to perform these measurements also with 5%wt Mini-B, which is a 25 amino acid sequence that mimics the SP-B protein that is known to facilitate the lipid reservoir dynamics during respiration. The off-specular scattering looks more pronounced in the d/h and h/d measurements, which hints that segregation of saturated versus unsaturated lipid may play a role in the macroscopic changes observed. We are working with the Instrument Responsible on the analysis of these data.



These data prompted us to record Brewster angle microscopy images of the films in our lab following the FIGARO experiment, where pronounced macroscopic collapse cracks were observed in real space.



Outlook

These data are currently being written up as a chapter of PhD student Glenn Coope's thesis and a manuscript for publication. Although the significance of collapse cracks for the DPPC:POPG system is unclear, measurements on more advanced model systems are needed to disentangle the interplay between self-assembled reservoir structures on the molecular level and macroscopic collapse cracks, both of which can be significant physiologically. The project has progressed with an additional neutron beam time experiment to resolve the properties of the DPPC:POPC:POPG (5:3:2 by mole) system. Also, we are working to build a thermostated chamber so that future measurements can be conducted at 37 degrees, further enhancing the technical novelty of the work in addition to the very low surface tensions obtained using a conventional yet underfilled Langmuir trough with Delrin barriers.