Experimental report

31/10/2023

Proposal: **EASY-1082**

Council: 10/2022

Title: First structural resolution of model lung surfactant layers near zerosurface tension

Research area: Biology

This proposal is a new proposal

Main proposer: **Richard CAMPBELL**

Experimental team: Imre VARGA
Richard CAMPBELL
Glenn COOPE
Pinchu XAVIER

Local contacts: Philipp GUTFREUND

Samples: DPPC, POPC and POPG lipids

Instrument	Requested days	Allocated days	From	To
FIGARO	48	48	30/06/2023	02/07/2023

Abstract:

An intuitive physical picture of the mechanism of lung surfactant has been built up over the last two decades: lipid is expelled into reservoirs upon compression of the surface area, and surfactant protein B facilities its re-spreading to the surface monolayer during expansion when we breathe, preventing lung collapse. Nevertheless, there has not been direct resolution of the surface structures created in these systems at anything like a realistic surface tension, which is close to zero. We have demonstrated that we are now in a position to prepare and hold model systems at a surface tension close to zero for long enough to measure with neutrons, but a recent experiment on INTER at ISIS was beset with sample trough stability problems. A PhD student whose project was delayed significantly due to restrictions from the covid-19 pandemic is now on the verge of making this breakthrough, but we are missing key data, as a manuscript is drafted but misses data recorded under optimal conditions. The FIGARO instrument responsible (Dr. Gutfreund) is supportive of this application, which will allow us to support the student to complete their thesis and submit a rapid high impact publication.

EXPERIMENTAL REPORT: EASY-1082

First structural resolution of model lung surfactant layers near zero surface tension

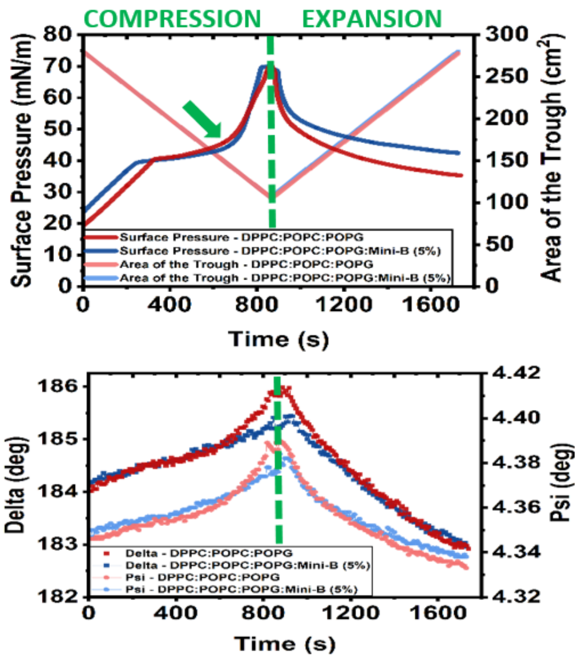
Glenn Coope, Javier Carrascosa, Pinchu Xavier, Imre Varga & Richard Campbell

FIGARO, 30 June–2 July 2023

Abstract

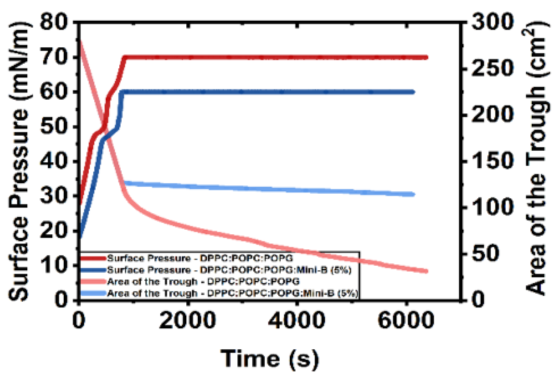
An intuitive physical picture of the mechanism of lung surfactant has been built up over the last two decades: lipid is expelled into reservoirs upon compression of the surface area, and surfactant protein B facilities its re-spreading to the surface monolayer during expansion when we breathe, preventing lung collapse. Nevertheless, there has not been direct resolution of the surface structures created in these systems at anything like a realistic surface tension, which is close to zero. We have demonstrated that we are now in a position to prepare and hold model systems at a surface tension close to zero for long enough to measure with neutrons, but a recent experiment on INTER at ISIS was beset with sample trough stability problems. A PhD student whose project was delayed significantly due to restrictions from the covid-19 pandemic is now on the verge of making this breakthrough, but we are missing key data, as a manuscript is drafted but misses data recorded under optimal conditions. The FIGARO instrument responsible (Dr. Gutfreund) is supportive of this application, which will allow us to support the student to complete their thesis and submit a rapid high impact publication.

Preliminary Results



LEFT. *Surface pressure (top) and ellipsometric changes in psi and delta (bottom) for the ternary lipid mixture in the absence (red) and presence (blue) of Mini-B.*

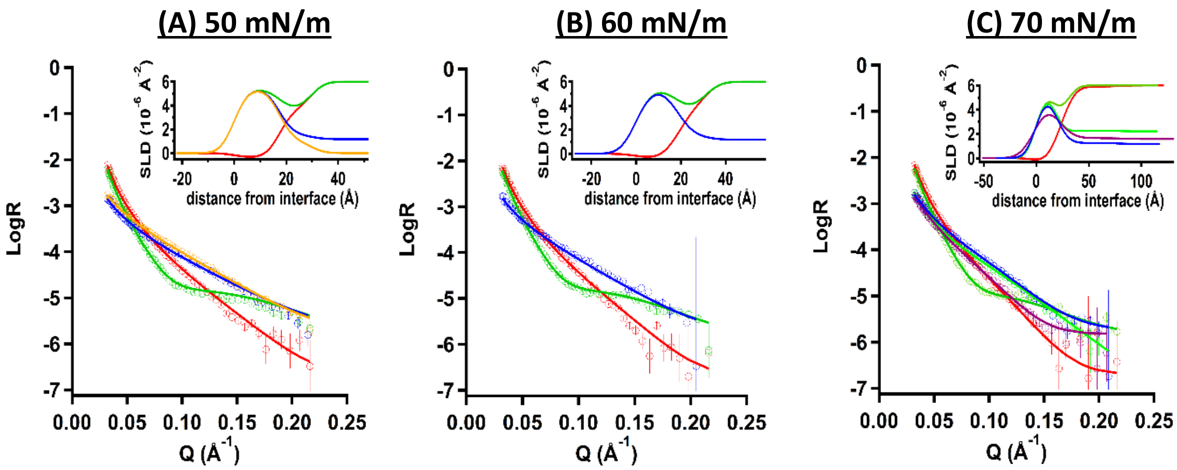
BELOW. *Surface pressure while holding ternary lipid mixtures in the absence (red) and presence (blue) of Mini-B at a fixed high surface pressure.*



This experiment followed on from #8-02-962 (see final report), in which we performed a structural resolution of binary DPPC:POPC and DPPC:POPG (7:3 by mole) systems at a surface pressure of 50 mN/m, and an unprecedented dynamic mid-Q_z resolution of the latter up to 70 mN/m. Here we focussed on structural resolution of a more advanced model, the ternary DPPC:POPC:POPG (5:3:2 by mole) system, at surface pressures of 50, 60 and 70 mN/m. Preliminary data above evidenced that we could achieve stable films for an extended period. It was an extreme challenge, however, to prepare stable films in some isotopic contrasts. Even so, the experiment succeeded in meeting its objectives.

Experimental Results

Neutron reflectivity measurements were performed to reveal the molecular structure of the ternary lipid system at a range of surface pressures: 50, 60 and 70 mN/m. The target surface pressure was held constant over an extended period of time to allow neutron data acquisition. Data were recorded in multiple isotopic contrasts by performing the same experiment with subphases of air-contrast matched water (ACMW; 8.1% D_2O in H_2O), D_2O and – unusually – other proportions of D_2O mixed with H_2O, where the lipids were either all hydrogenous or all deuterated. While h-lipid/H_2O and d-lipid/D_2O were stable enough to reach a target surface pressure of 70 mN/m, we experienced regular premature collapse of monolayers for d-lipid/ACMW. Nevertheless, this contrast is important as a Kiessig fringe is key to resolve the presence of extended interfacial structures. Therefore, we recorded data in d-lipid/25% D_2O, 31% D_2O and 40% D_2O as a compromise. Initially, we believed that the instability may be related to the relative densities of the lipid and subphase. However, the isotopic contrast that was the next least stable was h-lipid/D_2O. This led us to conclude that the situation was more complex than we had thought initially, and that the instability may be related to isotope-specific effects related to unfavourable interactions between hydrogenous and deuterated species.



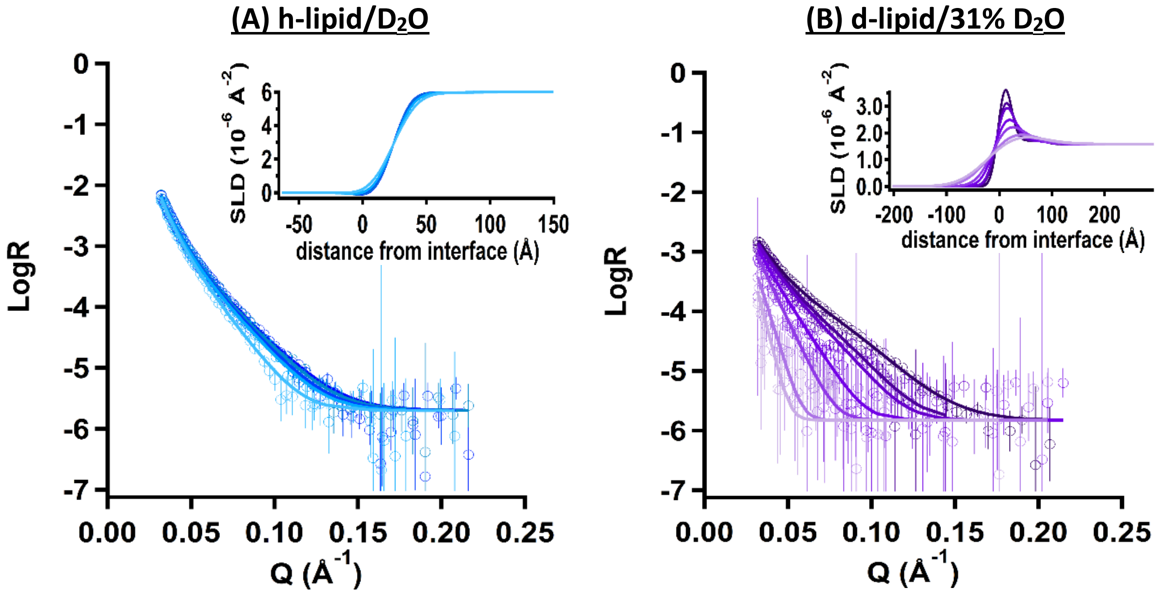
Neutron reflectivity profiles for DPPC:POPC:POPG at surface pressures of (A) 50 mN/m, (B) 60 mN/m and (C) 70 mN/m, with h/D_2O (red); d/D_2O (dark green); d/40% D_2O (light green); d/31% D_2O (purple); d/25% D_2O (blue). SLD profiles are presented as insets for 2 layers at 50 and 60 mN/m and 3 layers at 70 mN/m.

π (mN/m)	Backing roughness	1-thick	1-SLD	1-solv	2-thick	2-SLD	2-solv	3-thick	3-SLD	3-solv	Linked roughness	χ^2
50	4.3	17.5	-0.35; 5.36	0	12	1.88	43	-	-	-	Yes	4.74
60	5.7	18.5	-0.35; 5.36	0	12	1.88	40	-	-	-	Yes	2.71
70	9.3; 7.9; 7.6; 11.7; 8.3	19.0	-0.35; 5.36	0	10	1.88	26	50.6	0.26; 6.21	98.5	No	1.84

Parameters for neutron reflectivity plots; thickness/rough units as Å; SLD units as 10^{-6} Å^{-2}.

The structural models at 50, 60 mN/m involved a monolayer with two layers: chains as layer 1 and solvated headgroups as layer 2. At 70 mN/m, the model exhibited an improved χ^2 value by 40% with the presence of a third layer of lipid bound to the surface monolayer. As the surface pressure increases, the interfacial roughness also increases. For the data at 70 mN/m, a requirement in the model was to fit individual contrasts with their roughness values not linked. This may be related to isotope-specific effects or small errors in the calibration of the surface pressure, which above 70 mN/m results in significant differences in the surface tension, which affects the capillary wave amplitude.

Incremental measurements to reach zero surface tension were performed in two isotopic contrasts. As the optimum fit at 70 mN/m required a third layer related to extended structure formation, a third layer was also included in this analysis. The data were modelled with the layer 3 thickness and volume fraction parameters fixed to minimise the number of free fitting parameters, yet the interlayer roughness was left as a free fitting parameter, as above. Satisfactory fits are produced in both isotopic contrasts. Increasing roughness with increasing incremental pressure to near-zero surface tension that allows an optimum fit, which is consistent with the inverse square root dependence of the capillary wave amplitude with the surface tension. No evidence from the detector images was gained of macroscopic buckling as was observed for the DPPC:POPG (7:3 by mole) system; see report #8-02-962.



Neutron reflectivity profiles for 5:3:2 DPPC:POPC:POPG at or above 70 mN/m surface pressure in 0.5 mN/m incremental increases. (A) h-lipid/D_2O from dark blue to light blue (70.2, 70.7, 71.2, 71.7, 72.2 mN/m); (B) d-lipid/31% D_2O from dark purple to light purple (69.8, 70.3, 70.8, 71.3, 71.8, 72.3, 72.8 mN/m).

Outlook

These data are currently being written up as a chapter of PhD student Glenn Coope's thesis and a manuscript for publication. This includes ellipsometry data where there is an overturn in the psi and delta parameters, symptomatic of extended structure formation or film buckling, only above 69 mN/m. A key challenge is to reconcile the different processes occurring at different length scales including increasing capillary wave roughness versus extended structure self-assembly versus macroscopic film buckling. The data analysis and writing are being actively developed at the moment.

Looking forward, EPSRC funding in the UK has been secured for a two-year Postdoctoral Research Assistant, Dr Pinchu Xavier, to work on a project to use hard and soft nanoparticles of different size and charge as crude models of lung surfactant protein SP-B to see if we can tune the nucleation of extended structures. This work is being carried out in conjunction with a more advanced lung surfactant model in the form of a quaternary lipid mixture including cholesterol. A beam time application was submitted to FIGARO in September 2023 proposing to resolve the structure formed from the interactions involving different hard nanoparticles. These data will lay the foundation for the final phase of the project involving peptide-grafted soft nanogels. For this work, scheduled for the Spring/summer of 2024 before the grant finishes, we will use a thermostated chamber at 37 degrees, further enhancing the technical novelty of the work in addition to the very low surface tensions obtained using an underfilled Kibron G2 Langmuir trough with Delrin barriers.