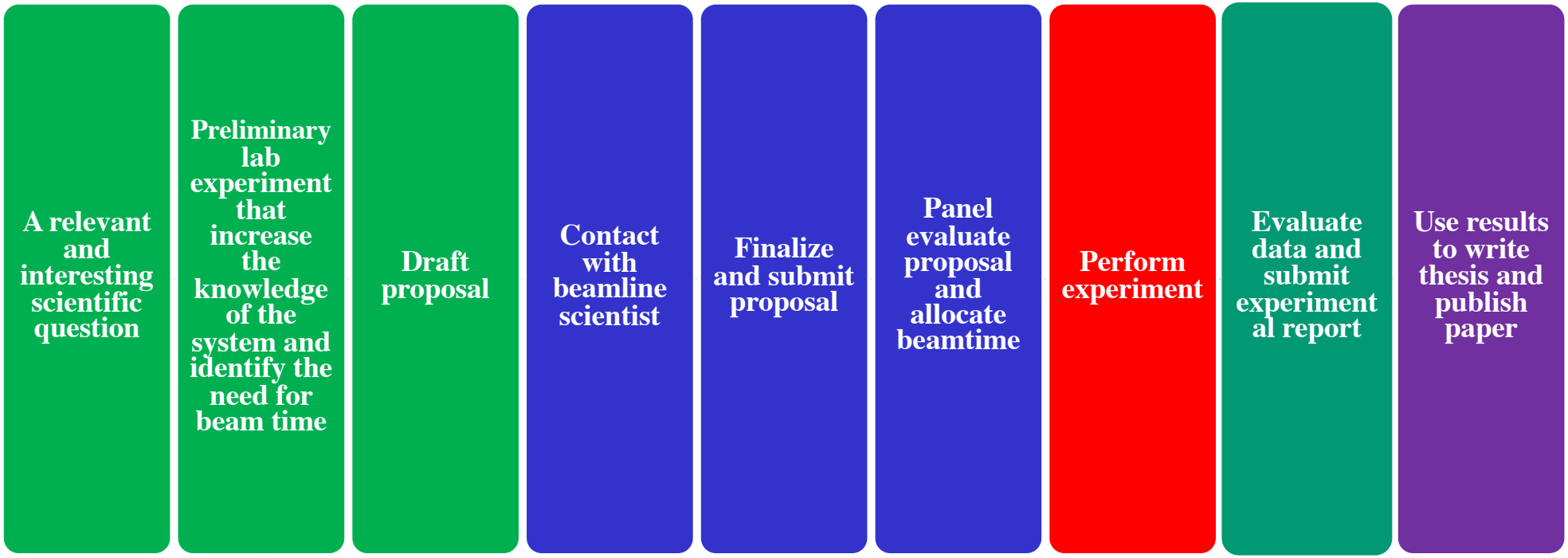
How to write a good beam time proposal!



MANCHESTER 1824
The University of Manchester

Discussion 1

- what are your research interests?

- what lab techniques do you use?

- submission of a beam time proposal before...
  - if so, neutrons or X-rays?
  - if so, reflectivity, bulk, diffraction, spectroscopy etc.?

MANCHESTER 1824
The University of Manchester

Experiment Conception & Planning

- strong scientific case based on extensive literature search
  - neutrons are expensive so research must be novel with a significant advance
- technique and instrument capabilities
  - contact beamline scientist for advice
- test the system with lab techniques first
  - understand the behaviour of the system
  - ensure that measurements are reproducible
  - learn from lab techniques and be able to state what neutrons would add
- test for isotopic effects (added strength)
  - these can be different strengths of interaction or gravity
  - so test deuterated samples in the lab too
- simulate possible data outcomes (added strength)
  - gain confidence that the isotopic contrasts proposed are the best ones
  - explain how the data will be analysed
- write a detailed experimental plan
  - do no inflate the beam time request
- explain the expected outcomes of the experiment and future research direction

Discussion 2

- do you know which facility or instrument to use?

- have you been in touch with a beamline scientist for advice?

- main challenges to write a neutron proposal...
  - making samples?
  - doing characterisation?
  - performing simulations?
  - formulating experimental plan?

MANCHESTER 1824
The University of Manchester

Proposal Example 1

Recent work on anticancer drugs

Drug-lipid interactions determine the passive transport of drugs through phospholipid membranes, which constitute the first barrier on the way to a cell. Deep understanding of the mechanisms responsible for these interactions is important in the view of effective treatment. The Langmuir technique has proved to be very useful in the investigation of the interactions of drugs with simple biomembrane models such as Langmuir monolayers [1]. We have recently employed such an approach to study the interactions of anticancer drugs with models mimicking healthy and cancerous cell membranes [2,3]. Two implementations of neutron reflectometry (NR) – structural and dynamic compositional; latter possible only on FIGARO – combined with Brewster angle microscopy (BAM) allowed us to solve the interaction mechanisms in terms of structure, composition and morphology (fig. 1) [4]. The interactions of doxorubicin (DOx) and idarubicin (IDA) with monolayers of DMPS (as a crude proxy for cancerous cell membranes) are fundamentally different when raised to a physiologically-relevant surface pressure of 30 mN/m. DOx clusters into domains of aggregates and electrostatic interactions diminish as the drug is excluded from the lipid monolayer. We related this surface-induced aggregation to the ability of DOx to self-aggregate mediated by H-bonding [5]. There is no extended layer of IDA yet the drug penetrates the acyl chains region of the monolayer, which we relate to its greater lipophilicity [6], allowing its interactions with rigidly-packed acyl chains. Importantly, the dynamic compositional data revealed loss of lipid from the monolayer upon surface area compression, which could not be inferred from surface pressure data alone. See reports #8-02-797/805.

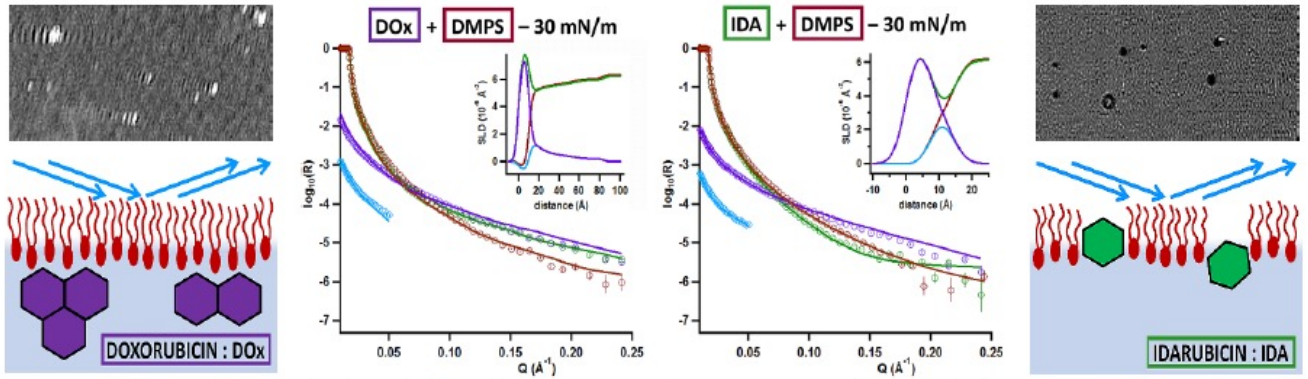


Fig. 1. NR data and optimized model fits for DMPS monolayers at 30 mN/m on subphases containing 10^{-5} mol/L (left) DOx or (right) IDA: (purple) d_{54}-DMPS/ACMW, (blue) DMPS/ACMW, (green) d_{54}-DMPS/D_2O, (red) DMPS/D_2O; insets are scattering length density profiles; corresponding BAM images and schematics.

MANCHESTER 1824
The University of Manchester

Proposal Example 1

New project on statins

Statins are well-known therapeutic agents for cardiovascular diseases and lipid disorders. They lower the level of LDL cholesterol by inhibiting a membrane-associated protein HMG-CoA reductase [7]. Incorporation of statins into lipid bilayers has been reported to alter the bilayer properties and this effect is of remarkable importance since changes in lipid bilayer conformation can alter the function of membrane receptors and proteins. Our recent Langmuir studies have shown that the statins pravastatin (PRA), fluvastatin (FLU) and cerivastatin (CER) influence the properties of lipid monolayers at the air-water interface (fig. 2) [8]. Lipids DMPC and DMPS were again chosen to vary the charge of the polar headgroups whilst conserving the same acyl chains; DMPC was selected as it provides better fluidity than saturated lipids with longer chains and is available in a fully deuterated form, whilst DMPS is has negatively charged polar headgroups of relatively low hydration, which strongly influences the interactions with statins.

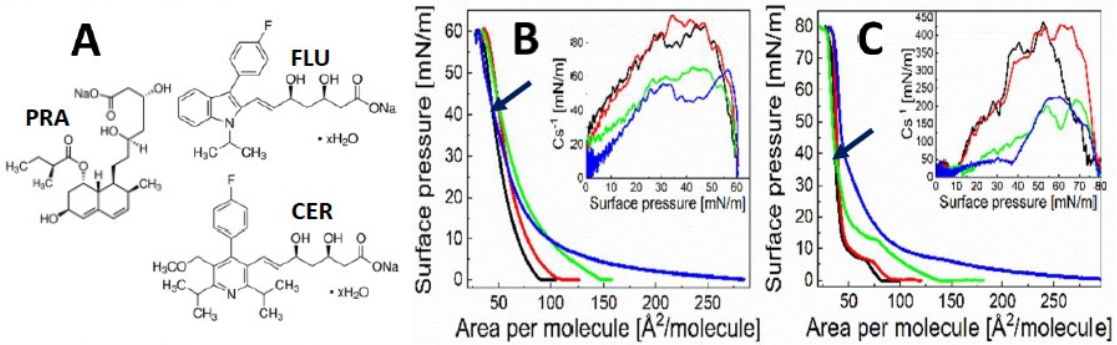


Fig. 2. (A) Statin structures; surface pressure isotherms of (B) DMPC and (C) DMPS monolayers formed on pure water (black) and water containing 10^{-5} M pravastatin (red), fluvastatin (green) and cerivastatin (blue).

It has been previously indicated that the location and extent of interactions of statins in lipid biomembranes depend both on the drug hydrophobicity and the degrees of charge and hydration of the lipid polar headgroups [9,10]. The most hydrophilic statin, pravastatin, was inferred to interact only with the headgroups of the monolayer and to affect its organization by increasing the headgroup hydration. The contribution of electrostatic interactions between the negatively charged headgroups of DMPS and the drug was observed, and a strong dehydration effect of cerivastatin was inferred from drug incorporation into the acyl chains region of the monolayer. Nevertheless, inferences made to date have been indirect, and the fact that the surface pressure data in the presence of the statins cross those of the pure lipids (see arrows) suggests lipid loss from the interface, which would render invalid the area per lipid molecule abscissa (calculated on the common assumption of lipid insolubility). This is a matter that we believe merits further investigation – and attention to the biophysics community – as the composition, structure and dynamics of statins in lipid monolayers at the air-water interface are resolved directly for the first time.

MANCHESTER 1824
The University of Manchester

Proposal Example 1

Current objectives

We plan to acquire direct quantitative information about the composition and structure of the interactions at the air-water interface of the three statins (pravastatin, fluvastatin and cerivastatin) with the two different lipids (DMPC and DMPS). The choice of drugs is motivated by their different hydrophobicity. The choice of lipids is motivated by the fact that PC and PS are the main types of lipids present in the intestinal cell membranes. The focus is to understand better specific drug-lipid interactions through varying the charge and hydration of the polar headgroup, which strongly affects resulting drug interactions.

Following the same approach as our recent publication on anticancer drugs [4], we plan to use the FIGARO reflectometer in two modes to resolve structure and composition. Equilibrium structural measurements will be made at a surface pressure of 30 mN/m in 4 contrasts (d-lipid/drug and h-lipid/drug each in ACMW and D_2O) over the full Q-range. These data will allow us to resolve the location of the drug in the model biomembrane with varying drug hydrophobicity and lipid headgroup charge. Dynamic compositional measurements during compression/expansion cycles will then be made in the 2 ACMW contrasts only at low Q [11]. These data will allow us to quantify the lipid loss with changing area per lipid molecules, re-scale the area per lipid molecule abscissa in the surface pressure isotherms, and provide novel quantitative information about the relative extents of interactions of the 3 drugs with the 2 lipids.

Experimental plan

First, we will measure DMPC and DMPS monolayers on subphases of 10^{-5} M pravastatin, cerivastatin and fluvastatin at a surface pressure of 30 mN/m in 4 isotopic contrasts. From experience, the 24 measurements will take 2 h each allowing for sample changes, surface pressure changes, system equilibration and data acquisition = **48 h**. We have reference measurements on DMPC and DMPS already [4,12], the prior investment of which will improve the efficiency of this experiment. Next, we will measure the same 6 systems over a compression/expansion cycle using the low-Q approach in 2 contrasts. From experience, the 12 measurements will take around 1.5 h each including sample changes etc. = **18 h**. Set up, direct beams and calibrations = **6 h**. As such, 3 days of beam time on FIGARO are required to complete this experiment.

MANCHESTER 1824
The University of Manchester

Proposal Example 1

Expected outcomes

The proposed new compositional, structural and dynamic data on drug-lipid interactions will provide a complete dataset for publication to follow up our recent FIGARO publication on anticancer drugs [4]. It is planned that the data will also motivate a follow-up neutron experiment on the interactions of statins with mixed cholesterol-containing phospholipid monolayers, which are important given the cholesterol-inhibiting function of the drugs [7], and for which lab data acquisition is almost complete. Please note that a previous draft of this proposal was submitted to college 8 in September 2020, and whilst no specific concerns were raised by the Reviewers (remark A1), the Chair recommended re-submission to college 9.

References

[1] L. Clifton et al., *Adv. Colloid Interf. Sci.* 277 (2020) 112118; [2] D. Matyszewska, *BBA Biomembranes* 1862 (2020) 183104; [3] D. Matyszewska et al., *Electrochim. Acta* 280 (2018) 229; [4] D. Matyszewska et al. *J. Colloid Interf. Sci.* 581 (2021) 403-416; [5] E. Tasca et al. *Chem. - A Eur. J.* 24 (2018) 8195; [6] L. M. Hollingshead et al. *Drugs* 42 (1991) 690; [7] E. S. Istvan et al. *Science* 292 (2001) 1160; [8] M. Zaborowska et at., *J. Molec. Liquids* 313 (2020) 113570; [9] G. Larocque et al., *Eur. Biophys. J.* 39 (2010) 1637; [10] L. F. Galiullina et al, *BBA Biomembranes* 1861 (2019) 584; [11] L. Braun et al. *Adv. Colloid Interf. Sci.* 247 (2020) 130; [12] R. A. Campbell et al., *J. Colloid Interf. Sci.* 531 (2018) 98.

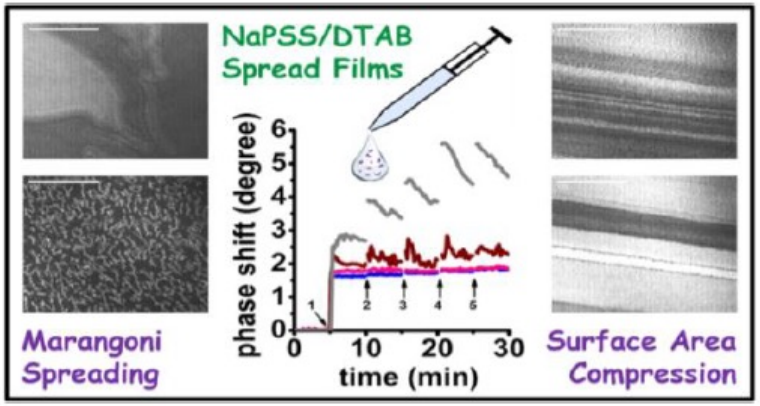
MANCHESTER 1824
The University of Manchester

Proposal Example 2

Scientific Background

Oppositely charged polyelectrolyte/surfactant (P/S) mixtures have been studied extensively because of their common use in everyday life products,[1] as well as their applications in materials science varying from pharmaceutics[2] to energy harvesting.[3] In recent years, considerable efforts have been invested into understanding the relation between the interfacial properties of P/S mixtures and their bulk phase behaviour.[4–6] We started a few years ago to exploit non-equilibrium effects in preparing spread films of poly(sodium styrene sulfonate) (NaPSS) and dodecyltrimethylammonium bromide (DTAB) at the air/water interface.[7] Films are formed when a small aliquot of liquid crystalline aggregates, formed in the bulk of a mixture, is dropped onto the surface of pure water. Key mechanisms in film formation are Marangoni spreading from the dissociation of the aggregates as they contact the water and film trapping associated with the entropy of counterion release. The spread films have a much higher surface excess than layers formed from the adsorption of bulk complexes.

Work on NaPSS/DTAB Spread Films

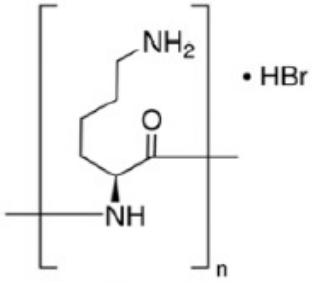


We published a paper recently on the dynamic properties of spread NaPSS/DTAB films with respect to the charge of aggregates used in their preparation.[8] Neutron reflectometry (NR), ellipsometry and Brewster angle microscopy (BAM) were the key techniques used. We showed that reservoirs of extended structures can be formed at the air/water interface upon spreading or surface area compression (see ellipsometry data & BAM images in figure, above), but only when nucleated by embedded aggregates of positive charge. While observations about the presence of monolayer versus multilayer structures have been made for P/S mixtures previously,[9] this was the first time a key parameter to control and tune their formation had been identified; see report #9-12-461 and ref. 8. In spite of the progress described above, important questions about the nature of the extended structures (e.g. loops, attached vesicles, multilayers…) and the optimal physicochemical factors of the aggregates that nucleate their formation (e.g. charge, size, hydration…) remain.

MANCHESTER 1824
The University of Manchester

Proposal Example 2

Extension to PLL/SDS Spread Films



An ILL PhD student aims to resolve the nature of tuneable extended P/S structures at the air/water interface by extending the spread films methodology to systems of potential use in pharmaceutical applications. Mixtures of poly-*L*-lysine (PLL; right) and sodium dodecyl sulfate (SDS) were chosen, which are versatile as in future work we plan to tune the P/S interaction strength with the solution pH,[10] and are biocompatible.[11] In proposal #9-12-614, zeta potential measurements had been used to characterise the bulk SDS concentration with 100 ppm PLL to produce undercharged (0.50 mM; less SDS than PLL), neutral (0.63 mM; stoichiometric PLL and SDS) and overcharged (0.80 mM; more SDS than PLL) aggregates. The aggregates were used to form spread films, which were examined during compression/expansion cycles on a Langmuir trough. Strong hysteresis, consistent with the formation of extended structures during surface area compression, was exhibited for films formed from undercharged *and* overcharged PLL/SDS aggregates, even though extended structures are produced only by overcharged NaPSS/DTAB aggregates. Ellipsometry data were also revealed film collapse, yet the structure and composition of the films remained unknown.

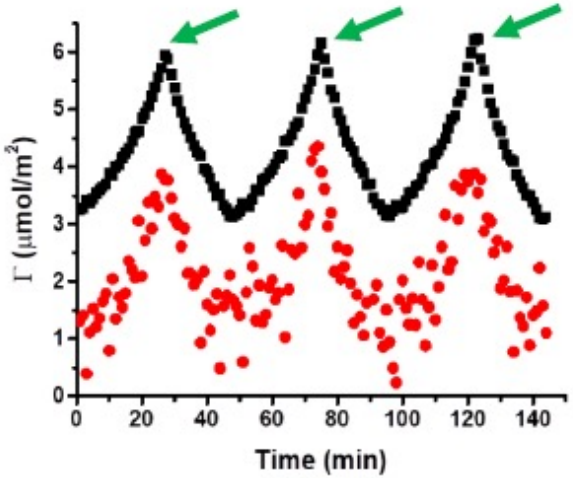
Experiment #9-12-614 on PLL/SDS Spread Films (17–20 August 2020)

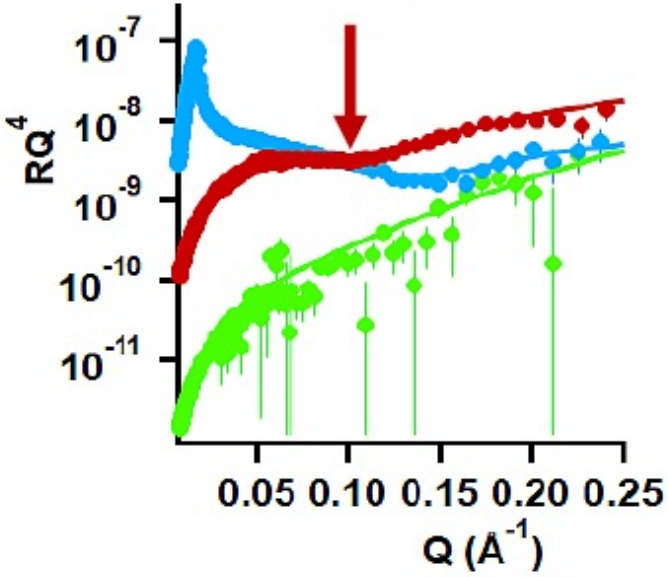
The scope was to perform a dynamic compositional analysis of PLL/SDS films formed from undercharged, neutral and overcharged aggregates during 3 compression/ expansion cycles (1 system; 3 compositions; 2 contrasts of d- and h-surf in ACMW; low Q^7) as well as an equilibrium structural analysis of PLL/SDS and NaPSS/DTAB films at maximum compression (2 systems; 3 compositions; 4 isotopic contrasts of d- and h-surf in ACMW and D_2O; full Q). Report #9-12-614 fully details the context and results. In short, the data acquired were highly insightful and motivate the acquisition of some more samples, yet the full experimental plan was not completed due to difficulties encountered, and it was not possible to record the planned data on neutral aggregates and one measurement on overcharged aggregates.

MANCHESTER 1824
The University of Manchester

Proposal Example 2

The dynamic compositional analysis (figure right for overcharged aggregates) hinted that extended structures form for films prepared with both undercharged and overcharged aggregates. Here the SDS surface excess (black squares) reaches 6.0 μmol/m^2 at maximum surface area compression (green arrows), which exceeds full monolayer coverage of 4.2 μmol/m^2.[12] The interfacial stoichiometry of SDS to PLL (red circles) was also higher for the films prepared from overcharged than undercharged aggregates. The structural analysis (figure left again for overcharged aggregates) confirmed this inference as the optimum layer structure involves an SDS monolayer, a PLL layer bound to the headgroups, and low coverage of a surfactant bilayer (or hemimicelles). Interestingly, this interfacial structure is similar to one reported for an adsorbed P/S film earlier this year.[13] Unfortunately, we did not observe the extended structures in NaPSS/DTAB films prepared with overcharged aggregates as expected, which may be due to their transient nature. Even so, we have made clear steps in the process to gain a systematic understanding of the nature and underlying reasons for the formation of extended structures in P/S films.





MANCHESTER 1824
The University of Manchester

Proposal Example 2

Continuation Experiment Scope

Whilst we have characterized the films prepared with charged aggregates, we propose to acquire the missing data on films formed from neutral aggregates. This information is necessary to decouple effects of aggregate charge and structure on the film structures formed. In addition, the Kiessig fringe in the d-surf/ACMW data (red arrow above left) is key in identifying if extended structures are present or not. We propose to acquire a small amount of additional dynamic data at high Q (2 min scans are sufficient) in this contrast only to: (1) resolve finally the nature of the extended structures for NaPSS/DTAB films prepared with overcharged as opposed to undercharged aggregates, and (2) understand the nature of the strong surface pressure hysteresis in the PLL/SDS films during compression versus expansion (i.e. the barriers to monolayer expulsion of surfactant during expansion versus resupply during expansion).

Experimental Plan

Dynamic compositional analysis of PLL/SDS films from neutral aggregates in 2 contrasts = **9 h**. Equilibrium structural analysis of PLL/SDS and NaPSS/DTAB films from neutral aggregates in 4 contrasts + 1 missing measurement of PLL/SDS from overcharged aggregates = **15 h** (inc. sample changes/equilibration). Dynamic structural analysis at high Q in 1 contrast (d-surf/ACMW) for PLL/SDS and NaPSS/DTAB films from undercharged and overcharged aggregates = **18 h**. Setup, direct beams and calibrations = **6 h**. In total, we request 2 days of beam time on FIGARO to complete the initial experiment and perform a small number of additional measurements that will strongly optimise the scientific insight gained into film formation.

MANCHESTER 1824
The University of Manchester

Proposal Example 2

Impact & Context

The discovery of tuneable reservoir formation in films spread from P/S aggregates was an exciting result. The completion and short extension of the experiment on the comparison of PLL/SDS and NaPSS/DTAB films, parts of which only FIGARO can deliver, will result in a publication in a high impact factor journal, and pave the way for the first global description of controlled formation of extended P/S films of broad interest to materials science. Please note that this work forms the ILL PhD project of Javier Carrascosa and represents one of the last opportunities for beam time on FIGARO before a long shutdown.

References

[1] Guzmán, E. ... *Adv. Colloid Interf. Sci.* **2016**, *233*, 38; [2] Barreiro-Iglesias, R. ... *J. Controlled Release* **2003**, *93*, 319; [3] Sweet, M. L. ... *Appl. Surf. Sci.* **2014**, *289*, 150; [4] Campbell, R. A. ... *J. Phys. Chem. Letters* **2010**, *1*, 3021; [5] Campbell, R. A. ... *Langmuir* **2014**, *30*, 8664; [6] Varga, I. ... *Langmuir* **2017**, *33*, 5915; [7] Campbell, R. A. ... *Soft Matter* **2016**, *12*, 5304; [8] Tummino, A. ... *Langmuir* **2019**, *269*, 43; [9] Li, P. ... *Adv. Colloid Interf. Sci.* **2016**, *233*, 38; [10] Choi, J-H. ... *J. Phys. Chem. B* **2015**, *119*, 105544; [11] Lin, P. Y. ... *J. Controlled Release* **2017**, *259*, 168; [12] Campbell, R. A. ... *J. Colloid Interface Sci.* **2018**, *531*, 98; [13] Uhlig, M. ... *Chem. Commun.* **2020**, *56*, 952.

MANCHESTER 1824
The University of Manchester

Discussion 3

- what did you learn from reviewing these proposals?
- how to present different sections?
- how to make visually accessible?
- how to format to optimise space?

MANCHESTER 1824
The University of Manchester

Abstracts

Need to describe all aspects of the research concisely!

- System context and interest.
- Previous knowledge or measurements.
- What is missing.
- Aim of neutron experiment.
- Beam time request.
- What will be measured.
- What insight will be provided.
- Implications.

→ all in about 7 or 8 sentences!

MANCHESTER 1824
The University of Manchester

Abstracts

Abstract

Statins are commonly used therapeutic agents for cardiovascular diseases and lipid disorders that lower the LDL cholesterol level. Our recent Langmuir studies have shown that pravastatin, fluvastatin and cerivastatin influence the properties of lipid monolayers at the air-water interface and the observed fluidizing effects are related to the interaction in the headgroup region. However, direct information about the composition, structure and dynamics of statins in lipid monolayers at the air-water interface is still missing. Therefore, we propose 3 days of beam time on FIGARO to acquire quantitative information on the three statins with DMPC and DMPS monolayers representing key lipid characteristics of intestinal cell membranes. Structural measurements will allow us to resolve the changing location of the drug in the model membrane with varying drug hydrophobicity and lipid headgroup charge. Dynamic compositional measurements will provide novel quantitative information about the relative extents of such interactions and lipid loss from the interface. These results can significantly enhance our understanding of the molecular interactions important in the view of future drug design.

Abstract

Our recent discovery of how to control the formation of extended structures in spread polyelectrolyte/surfactant (P/S) films at the air/water interface marked an exciting advance. It was achieved by tuning the charge of aggregates used in film preparation, which nucleate reservoirs of material upon surface area compression. Even so, the nature of the extended structures (loops, attached vesicles, multilayers) and the aggregate properties required for their nucleation (charge, hydration, size) remain unresolved. We performed a FIGARO experiment in August 2020 on the poly-l-lysine/sodium dodecyl sulfate system. Interestingly, reservoirs were shown to be nucleated by aggregates of different charge, with patches of surfactant bilayer or hemimicelles adhered to the primary P/S structure. Additional time of 2 days is required to record missing data as well as complete a short extension to provide optimal insight into the nature and underlying reasons for extended structure formation. This work will result in a high quality standalone publication and represents a key step towards finally resolving why extended structures form in certain P/S mixtures of broad interest to materials science.

MANCHESTER 1824
The University of Manchester

Discussion 4

- what did you learn from reading these abstracts?
- how to survey the whole proposal in just a few sentences?
- how not to miss key aspects of the research?
- hot to make every sentence count?

MANCHESTER 1824
The University of Manchester

Review Panel Evaluation Reports

- The aims and likely outcome are insufficiently detailed.
- Better characterisation of sample necessary.
- The method and analysis of the proposed experiment need to be clearer.
- The results of the proposed experiment will not further improve the understanding of the subject.
- Details on proposed experimental programme should be given explicitly.
- The proposal is imprecise or ambiguous about the samples to be measured.
- The scientific case did not justify an allocation of beam time.
- Already attempted / Done by others.
- A better estimate of the likely contribution to the field of study is needed.
- The proposal must take more account of existing work in the field.
- The proposal must be related to the earlier results and new information clarified.
- The case for the use of neutrons was not well stated.
- Other techniques are more appropriate and/or should be attempted first.
- Waiting for report on previous experiments.

MANCHESTER 1824
The University of Manchester

Good luck with your proposals ☺

- strong scientific case based on extensive literature search
  - neutrons are expensive so research must be novel & make a significant advance
- technique and instrument capabilities
  - contact beamline scientist for advice
- test the standard system with lab techniques first
  - understand the behaviour of the system
  - ensure that measurements are reproducible
  - learn from lab techniques and state what information neutrons would add
- test for isotopic effects (added strength)
  - these can be different strengths of interaction or gravity
  - so test deuterated samples in the lab too
- simulate possible data outcomes (added strength)
  - gain confidence that the isotopic contrasts proposed are the best ones
  - explain how the data will be analysed
- write a detailed experimental plan
  - do no inflate the beam time request
- explain the expected outcomes of the experiment and future research direction