Experimental report

27/08/2023

Proposal: 9-13-1001 **Council:** 4/2021

Title: Biophysical interaction mechanismsof statins with model lipid membranes: interfacial composition, structure and dynamics

Research area: Soft condensed matter

This proposal is a resubmission of 8-02-939

Main proposer: **Richard CAMPBELL**

Experimental team: Dorota MATYSZEWSKA
Michalina ZABOROWSKA
Glenn COOPE
Richard CAMPBELL

Local contacts: Philipp GUTFREUND
Armando MAESTRO

Samples: DMPC and d54-DMPC Lipids
DMPS and d54-DMPS Lipids
fluvastatin
cerivastatin
pravastatin

Instrument	Requested days	Allocated days	From	To
FIGARO	3	3	17/09/2021	20/09/2021

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.

Experimental Report #9-13-1001

September 2021

Dorota Matyszewska, Michaelina Zaborowska, Glenn Coope & Richard Campbell

This experiment took place on FIGARO in September2021. The scope was to gain insight into the structure and dynamics of interactions involving different statins with model lipid monolayers. The statins were pravastatin (PRA), fluvastatin (FLU) and simvastatin (SIM) and the model lipid monolayers were simply DMPC and DMPS. Measurements were conducted at two starting surface pressures, 10 and 30 mN/m, prior to injection of statin into the subphase. Full-Q_z measurements were recorded in 4 different isotopic contrasts to resolve the interfacial structure while low-Q_z measurements were recorded in 2 contrasts to resolve the dynamic interfacial composition during successive compression/expansion cycles on a Langmuir trough.

We learned about different extents of lipid loss from the interface because of the drug/membrane interaction for the different systems. Key conclusions were: (1) drug is effectively squeezed out by physiological surface pressure for DMPS, (2) less drug is squeezed out for DMPC with minimal squeeze out for DMPC–PRA, (3) interactions of DMPS–SIM are greatest, and (4) drug interaction continues for over an hour for SIM with both lipids whereas the cycles are reproducible for the other drugs.

The experiment was highly successful in the depth of information resolved, but some data remained missing. As a result, a proposal was shortly submitted to ISIS to continue the work on the INTER reflectometer. The proposal that was submitted to INTER is copied on the next two pages as it shows some of the structural and dynamic data recorded on FIGARO in experiment #9-13-1001, provides some data interpretations, and outlines details about the information that was still unknown after the FIGARO experiment. The beam time was awarded on INTER, and the dataset was completed. The data analysis is ongoing.

New insight into drug-lipid interactions

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 with a view to improving effective treatments. The Langmuir technique has proved to be very useful in the investigation of drugs interactions with lipid monolayers [1]. We have recently employed such an approach to gain new insight into the interactions of anticancer drugs (anthracyclines) with lipid monolayers [2,3]. The interactions of doxorubicin and idarubicin with monolayers of negatively charged DMPS were shown to be fundamentally different when raised from 10 mN/m to a physiologically relevant surface pressure of 30 mN/m. Doxorubicin clusters into domains of aggregates while idarubicin penetrates the acyl chains region of the lipids. The drug interactions with DMPS were also quantified to be at least a factor of 5 stronger than with zwitterionic DMPC. This new insight was possible thanks to structural and dynamic implementations of neutron reflectometry (NR), and a comprehensive paper on the structure, composition and morphology of the drug-lipid interactions was published this year in *JCIS* [4].

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 the membrane-associated protein HMG-CoA reductase [5]. These drugs are commonly administered to prevent high risk of cardiovascular diseases despite reports on side effects [6]. Statins comprise a vast group of medicines, both synthetic and natural, which contain the same pharmacophore group of the beta-hydroxy acid chain but differ in their side groups, which determine the lipophilicity and lipid interactions [7]. Incorporation of statins into lipid bilayers has been reported to alter the membrane properties. Our recent Langmuir studies on interactions of statins with lipid monolayers show that pravastatin (PRA), fluvastatin (FLU) and simvastatin (SIM) influence the membranes to different extents (Fig. 1), also modulating the lipid phase as inferred from the compressibility modulus (insets) [8]. Lipids DMPC and DMPS were selected to vary the charge of the headgroups but with the same acyl chains. DMPC provides better fluidity than saturated lipids with longer chains and is available fully deuterated, whilst DMPS has negatively charged headgroups, which strongly influences the statin interactions.

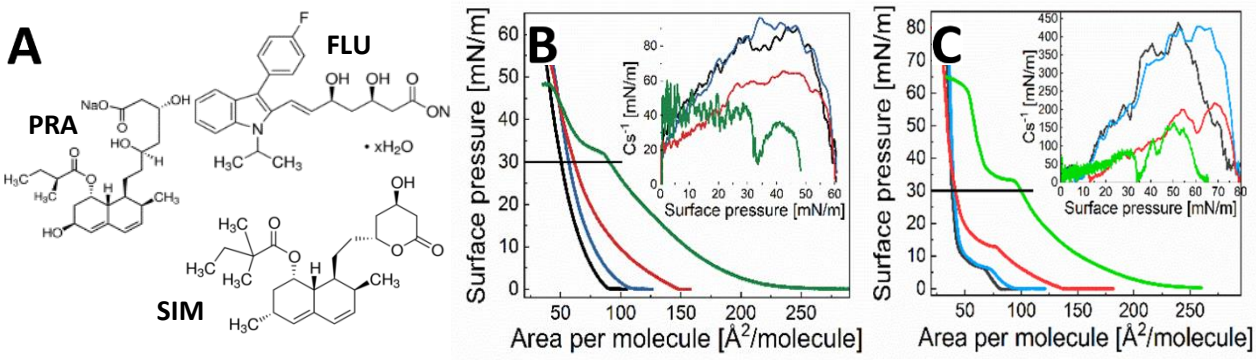

Fig. 1. (A) Statin structures; surface pressure isotherms of (B) DMPC and (C) DMPS monolayers formed on pure water (black) and water containing 10^{-5} M PRA (blue), 10^{-5} M FLU (red) and 10^{-6} M SIM (green).

It has been indicated that the location and extent of statin interactions with lipids depend both on the drug hydrophobicity and the degrees of charge and hydration of the lipid headgroups [9,10]. The most hydrophilic statin PRA was inferred to interact only with the lipid headgroups, modulate its organization by increasing the headgroup hydration, and interact more strongly with negatively charged headgroups. FLU was found to interact with the lipids headgroups, while aromatic moieties partitioned into the nonpolar part of the bilayer. SIM, due it its greater hydrophobicity, was suggested to penetrate the layers in the most effective way and occupy a position between the hydrocarbon chains influencing the fluidity of the lipid bilayers and affecting their phase behavior [11]. Nevertheless, such indications were indirect. We believe this topic merits further investigation and attention raised in the biophysics/biomedical communities, so we have started to directly resolve the structure and dynamics of statins with lipid monolayers for the first time.

We were supported to conduct a 3-day experiment, #9-13-1001, on the FIGARO neutron reflectometer at the ILL during 17th–20th September 2021 to study the dynamic and structural interactions of PRA, FLU and SIM with DMPS and DMPC monolayers with respect to the surface pressure. The choice of the 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 where there is uptake of the drugs.

Fig. 2A presents our recent dynamic analysis for lipid spread on drug solutions where after an equilibration period (dashed line), two compression-expansion cycles (0–40 mN/m) were conducted (max. compressions = down arrows + max. expansions = up arrows); data were analysed with the low-Q method [12]. Only the drug surface excesses are shown due to space constraints, but we also learned different extents of lipid loss upon increasing surface pressure (i.e. 19% for DMPC–SIM and DMPS–FLU versus just 4% for DMPC–PRA). Note that it was possible to perform 5 repeats, showing that uncertainties in quantifying the drug surface excesses are < 0.05 μmol/m^2, hence the larger differences observed are statistically significant. We learned from these data that: (1) drug is effectively squeezed out by physiological surface pressure for DMPS, (2) less drug is squeezed out for DMPC with minimal squeeze out for DMPC–PRA, (3) interactions of DMPS–SIM are greatest, and (4) drug interaction continues for over an hour for SIM with both lipids whereas the cycles are reproducible for the other drugs. *What a great depth of new information!* We had time also to record structural data on DMPC–FLU (Fig. 2B), DMPS–PRA (not shown due to space constraints) and DMPS–SIM (Fig. 2C) at 10 and 30 mN/m. Interestingly, at 10 mN/m, the drug is associated with the head groups in the first two cases and forms an extended layer in the latter case (purple arrow), indicating additional information about fundamentally different lipid interactions of these statins.

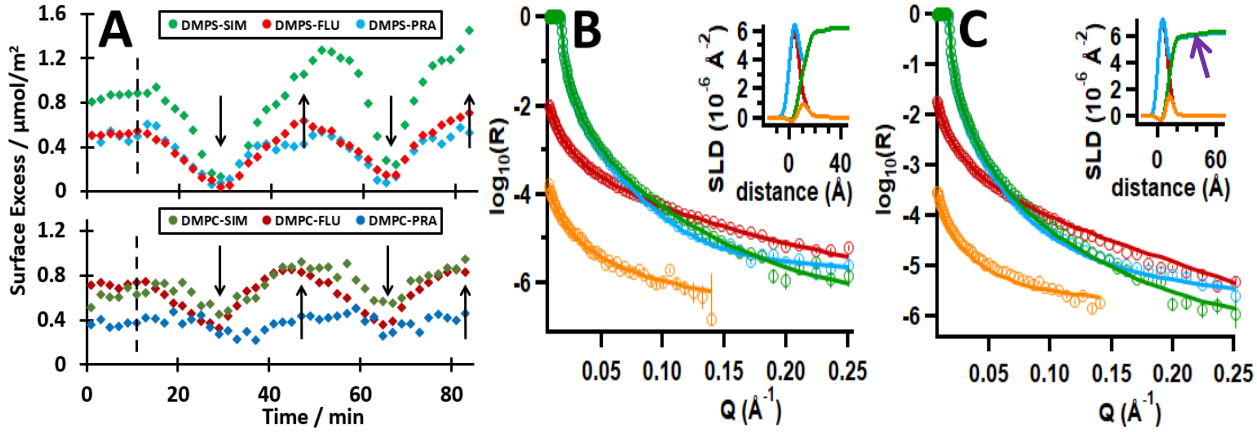

Fig. 2. (A) NR dynamics of drug surface excesses during two compression-expansion cycles (see text), and NR structures at 10 mN/m of data from (B) DMPC–FLU and (C) DMPS-SIM systems in 4 isotopic contrasts.

Current objectives & experimental plan

We need to complete our structural dataset on understanding these statin–lipid interactions through varying the charge and hydration of the headgroup and the hydrophobicity of the drug, both of which strongly affect the interactions. Still, we do not know answers to important questions with systems that exhibit extremes in lipid loss with changing surface pressure: does PRA rearrange structurally to avoid squeeze out at physiological surface pressure with DMPC, and is there an extended structure also for DMPC–SIM? The proposed new structural data on drug-lipid interactions will complete a comprehensive dataset for publication. Given the cholesterol-lowering mechanism of statins, it is planned that this work will motivate research into mixed lipid monolayers involving cholesterol in the PhD project of Michalina Zaborowska.

Without this information, our new research contribution will be incomplete.

The missing structural data needed are DMPC–PRA and DMPC–FLU at 10 mN/m and 30 mN/m, and DMPS–FLU only at 10 mN/m. Also, while we have 3 lipid references to date [4], we are missing DMPC at 30 mN/m, and this information is important to allow us to define changes in thickness and hydration of the headgroup layer resulting from the drug interaction. Data for the 6 systems will again be recorded in 4 isotopic contrasts involving h- and d-lipid in null reflecting water and D_2O. Each of the 24 measurements will take between 1 h 30 min and 1 h 50 min – *including sample changes* – and depending on the contrast, i.e. 40 h in total. Allowance of 8 h for careful trough setup/calibration, as well as transmissions and solvent references, means that we can complete this study with an allocation of 2 days of beam time on INTER.

References

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