Examining Membrane Biochemistry with Neutron Reflectometry

Wednesday, 7 September 2022 - Friday, 9 September 2022

Cosener's House

Programme

Table of contents

Wednesday 07 September 2022

Welcome (09:50-10:00)

- **Presenter: Prof. ECCLESTON, Roger (STFC)**

Meeting Overview (10:00-10:10)

- **Presenter: Dr CLIFTON, Luke (STFC)**

Neutron Reflectivity: Historical perspective and Impact (10:10-10:50)

- **Presenter: Prof. PENFOLD, Jeffery (STFC)**

The development of neutron reflectivity for the study of surfaces and interfaces will be reviewed. The early stages of the development, the associated uncertainties, and the key breakthroughs associated with its application will be charted. Emphasis will be placed upon the importance of the ability to manipulate the refractive index by deuterium labelling, and the impact of this on our understanding of Soft Matter interfaces. Particular emphasis will be placed on the ability to determine detailed structural information and vital compositional data in complex multi-component mixtures. In many ways these developments have all led to the ability to contribute to the study of membrane biochemistry, and could be considered as an ultimate step in the development of the technique.

The Role of Intramembrane Peptide Nanoaggregates in Bacterial Fast-killing (10:50-11:10)

- **Presenter: LIAO, Mingrui (The University of Manchester)**

Recent development of antimicrobial peptides (AMPs) has focused on the rational design of short sequences with less than 20 amino acids due to their relatively low synthesis costs and ease of correlating structure-function relationship. However, there remain gaps in our understanding of how these short cationic AMPs interact with bacterial outer and inner membranes and how these interactions underline their antimicrobial efficacy and dynamic bacterial killing. In this work, we have examined the membrane-lytic actions of three synthetic AMPs including G(IIKK)3I-NH2 (G3), G(IIKK)4I-NH2 (G4) and GLLDLLKLLLKAAG-NH2 (LDKA) and a natural AMP GIGAVLKVLTTGLPALISWIKRKR-NH2 (Melittin). The mechanistic processes of membrane damage and disruption strength of the four AMPs were characterized by both experimental measurements and molecular dynamics simulations. The combined studies revealed that G3 and G4 had higher antimicrobial efficacy and better membrane selectivity than Melittin and LDKA. Their distinctive antimicrobial actions arose from the dual actions of strong binding to outer and inner membranes and formation of intramembrane nanoaggregates. Furthermore, intramembrane binding and aggregation from all AMPs studied intensified as the ratio of peptide to lipid increased, but the most effective membrane insertion of G3 was well in line with its most efficient antimicrobial action.

Examining the interactions of polyphenols with model bacterial and epithelial membranes (11:10-11:30)

- **Presenter: COONES, Ryan (University of Reading)**

This work focuses on understanding interactions of polyphenolic compounds with model membrane systems. We selected a simple bacterial membrane model, a simple epithelial membrane model, and a complex epithelial membrane model (DOPC/DOPG (7:3), DOPC/DOPS (8:2), and DOPC/DOPS/Chol/SM (5:2:2:1) respectively). The interactions of these bilayers with three polyphenols, (-)-EGCg, Tellimagrandin I and Tellimagrandin II, were investigated. Membrane interactions were probed qualitatively using both ATR-FTIR and QCM-D. Neutron reflectometry was used to investigate the interactions that showed promise with the FTIR and QCM-D pre-characterisation by showing where the polyphenols reside in the membrane system post-interaction. These results take steps towards structurally understanding the lipid-polyphenol interactions, with the bacterial membrane model showing agreement with previously published NMR data. The interactions were consistent across membrane models given the polyphenol chemical structure.

Coffee (11:30-11:50)

Mechanism of pore formation by Nep1-like proteins (11:50-12:30)

- **Presenter: Prof. ANDERLUH, Gregor (National Institute of Chemistry, Ljubljana, Slovenia)**

Maintaining the integrity of cellular lipid membranes is critical for proper functioning of cells. Many natural toxins have evolved to damage lipid membranes by forming pores. The mechanisms by which these pores are formed have been intensively studied in recent years because pore-forming proteins have a prominent role in microbial pathogenesis and the immune system. Biophysical and structural biology approaches, and model lipid membrane systems are used to study the structure and function of these intriguing proteins. We will present how we have used neutron reflectometry to study the steps in the pore formation mechanism of necrosis and ethylene-inducing

peptide-1-like proteins (NLPs). NLPs are produced by some of the most pressing phytopathogens and enrolled in diseases of major crops. Their distinctive feature is that they act on lipid membranes containing plant sphingolipids, glycosylinositolphosphorylceramides (GIPCs), one of the major components of the plant plasma membrane. Using neutron reflectometry and other biophysical approaches, we demonstrated that NLPs associate with membranes in a multi step mechanism involving electrostatically driven and plant-specific lipid recognition, shallow membrane binding, followed by protein oligomerization and pore formation. Notably, neutron reflectometry showed that a layer of an NLP protein forms on the surface of the supported lipid membrane.

Lunch (12:30-13:30)

Interactions of antimicrobial peptides with model membranes (13:30-14:10)

- Presenter: Prof. LU, Jian Ren (University of Manchester)

Antimicrobial peptides (AMPs) offer an alternative to currently used antibiotics. Because AMPs kill microbes by physically disrupting their membranes, the deactivation processes are usually faster and less likely to develop resistance. However, there are still many hurdles to be overcome before AMPs could be developed into medicinal uses. Current research challenges lie in understanding how to improve their antimicrobial efficacy and reduce cytotoxicity, thereby enhancing their selectivity. Neutron reflection and SANS in combination with deuterium labelling are important tools to help unravel how designed AMPs interact with membrane models mimicking different pathogenic microbes and host cells. The structural data together with molecular dynamics simulations provide valuable insights in different interfacial processes underlying selectivity and efficacy. Some of our recent studies will be shared during the talk.

Structural kinetics of Hepatitis B virus from small-angle scattering and computational modelling (14:10-14:50)

- Presenter: Dr MAHMOUDI, Najet

The genetic material of viruses is typically protected in an icosahedral capsid, which is primarily assembled from multiple subunits of the same protein in a spontaneous self-assembly process. Similar highly efficient assembly processes are ubiquitous in biological systems, and viral capsids in particular present a unique platform to exploit for therapeutic advances in the targeted cellular delivery of cargo packaged within the capsid. Our research aims to provide a more detailed understanding of how this precise viral capsid protein assembly process occurs from a pool of single building blocks, and specifically how the RNA is incorporated into the capsid. Here, we present results from small-angle neutron scattering (SANS) experiments using contrast variation to reveal the final assembled structural organization of both the protein and nucleic acid components from recombinant full-length Hepatitis B virus (HBV) capsid protein and a synthetically prepared RNA containing the capsid protein binding domain. Time-resolved small-angle x-ray scattering (SAXS) experiments were also used to determine the HBV assembly pathway in the presence and absence of RNA. We employed Bayesian statistics-based computational methods to extract kinetic parameters of assembly and the overall size and shape of the dominant structural intermediates from the SAXS data. The developed framework can be extended to other hierarchical assemblies in biology.

How Do Nonionic Surfactants Interact with Plant Waxy Membrane? (14:50-15:10)

- Presenter: HU, Xuzhi (University of Manchester)

Nonionic surfactants are widely added into commercial pesticide formulation to help enhance pesticide solubilisation, increase droplet coverage on plant surface and transport active ingredients across plant “skin”, the wax film. However, our current knowledge of these interactions at the molecular level still remains very limited. As a result, little is known about how these interactions implicate pesticide solubilisation and efficiency of delivery into plant wax film. We have undertaken extensive investigations to follow a typical agro-spray process where the configurational alterations of surfactant micelles with and without pesticide solubilised upon exposure to plant waxes were examined by small angle neutron scattering (SANS), cryogenic transmission electron microscopy (cryo-TEM) and nuclear magnetic resonance (NMR) whilst the structural changes of the reconstituted wax film before and after exposure to pesticide and surfactant were determined by neutron reflection. Pesticide solubilisation altered micellar micellar length, whilst shrinking and dehydrating their shells, leading to consequent decrease in the cloud points. When waxes were further solubilised into the pesticide-loaded micelles, pesticides were partially released from the micelles, resulting in the adjustment of micellar structures by shortening micellar lengths, whilst expanding and rehydrating their shells. In contrast, the dynamic adsorption of pesticides and surfactants onto model wax films from neutron reflection in combination with deuterium labelling to wax, surfactant and solvent revealed that the hydrophobicity of the substrate influenced wax film morphology and integrity. From stable wax films formed on the hydrophobic substrate, it was feasible to observe how surfactants adsorbed onto and penetrated into the wax film and then altered local wax film structure. These studies altogether have provided a useful rationalisation of the interplay between surfactant structures, pesticide structures and environmental factors that affect pesticide loading and release.

Coffee (15:10-15:30)

Models of Bacterial Membranes: From Simple to Less So (15:30-16:10)

- Presenter: Dr HOLT, Stephen (ANSTO)
Early models of the gram-negative bacterial membrane tended to focus on replicating the negative charge of the system. This approach was rather simplistic and didn't consider either the overall structural or molecular complexity of the membrane. In recent years, while the structural complexity is still challenging, progress has been made at increasing the molecular complexity of model systems. This presentation will take a brief journey from Langmuir monolayers through supported, floating and tethered lipid bilayer systems where journey from simple charged phospholipids such as DPPC to various versions of Lipopolysaccharides (LPS) will be outlined. Finally examples of a tethered bilayer model will be presented, demonstrating the synergistic impact of gold nanoparticles and a 'last resort' antibiotic on LPS/PC mixed model membranes.

Neutron reflection as a tool to address a 'blind spot' in structural biology: The structure of membrane associated protein complexes (16:10-16:50)

- Presenter: Prof. LÖSCHE, Mathias (Carnegie Mellon University)
Surface-sensitive scattering of x-rays or neutrons has long been used to characterize the molecular scale properties of lipid monolayers and bilayers at aqueous surfaces and interfaces,[1,2] but has not been in the standard toolbox for structural biology due to several limitations. Most seriously, the intrinsic resolution of reflectometry in its application to biological membranes is far less than that of more established methods such as x-ray crystallography or NMR spectroscopy [3]. Yet, combining multiple sources of information have leveled this playground, thus unleashing the intrinsic strengths of reflectometry to provide structures of protein complexes on fluid bilayers that closely resemble biomembranes.
We developed a comprehensive infrastructure for biological reflectometry that includes stable sample environments, optimized measurement capabilities and innovative data modeling [4]. Prediction of the information gain expected from measurements at optimized neutron scattering length density (nSLD) contrasts across the samples[5] and the steering of molecular dynamics (MD) simulations using experimental nSLD profiles [6] have augmented the utility of the method further. Because radiation damage is not an issue in neutron reflection even at room temperature (as opposed to X-ray scattering), conformational changes of membrane-associated proteins can be triggered by external cues, and structurally characterized [7]. Using these tools to characterize the membrane-associated structure of the tumor suppressor KRas4B, we observed that disordered segments of the protein let this GTPase function as a membrane-bound conformational switch [8] and allowed us characterize its structures in membrane-bound complexes with downstream binding partners of the KRas signaling cascade.

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Close (16:50-17:00)

Thursday 08 September 2022

Coffee (09:00-09:20)

Studying complex biological systems at the solid liquid interface (09:20-10:00)

- Presenter: Dr KNOWLES, Tim (University of Birmingham)
Our research focuses on developing platforms for studying complex proteinaceous machineries at the solid liquid interface. In this talk I will discuss our advances in the incorporation of active proteinaceous machineries within planar lipid bilayers. How through the use of floating supported bilayers we can tune the distance of these complexes from the surface through solution electrolyte modification, and our recent progress in developing a novel double bilayer mimetic of the gram-negative envelope.

How does oxidative damage change peptide/lipid membrane interactions? (10:00-10:40)

- Presenter: Dr THOMPSON, Katherine (Birkbeck University of London)
The insertion of peptides and proteins into cell membranes is crucial for a huge range of biological functions and has been widely studied. However, real biological membranes are subject to continuous attack by oxidants, changing their chemical composition, structure and biophysical properties. We have used neutron reflection as a tool to study how oxidation changes the structure of both lipid monolayers spread at the air-water interface, and of supported lipid bilayers. We have then investigated how the insertion of different peptides into both model mammalian and model bacterial lipid membranes changes upon partial oxidation of the membrane. The results have implications for peptide/membrane specificity.

Coffee (10:40-11:10)

Lipoproteins and SARS coV2 protein at model membranes: Exchanging fats! (11:10-11:50)

- Presenter: Prof. CÁRDENAS, Marité (Malmö University)
The metabolism of fats including lipids and cholesterol involves the production, in the liver, of lipid carrying particles known as lipoproteins. Lipoproteins are nanoemulsion-like particles composed of fats and proteins (named apolipoproteins). The complexity of lipoproteins is great, with different compositions not only in terms of the amounts of the fat and proteic components, but also on the specific protein type and isoform. Specific apolipoproteins are known to mark an increased risk for developing atherosclerosis where fat accumulation to form plaques occurs at the initial stages of this first class world killer disease.
Fat metabolism is affected upon Covid-19 infection, with consequences in levels of main lipoprotein types, cholesterol and triglyceride. Vaccination with Sars-CoV-2 spike (S) protein producing constructs also induces a change in lipid metabolism.
In this talk, I will present the efforts of my group to explore the role of lipoprotein composition on lipoprotein structure as well as the capacity of lipoproteins to exchange fats. Moreover, we show that the equilibrium between lipids removed and lipids deposited by lipoproteins on model membranes is affected by SARS coV2 spike protein. This works is otherwise impossible to do without contrast matching, biodeuteration and neutron scattering.

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Investigating Biophysical Interactions in Pollen-Induced Thunderstorm Asthma using Neutron Reflectometry (11:50-12:10)

- Presenter: Mr SIDDIQUE, Arslan (University of New South Wales Australia)
Epidemic thunderstorm asthma triggered by the unique combination of grass pollens and thunderstorm activity affects a large population within a local vicinity.^{1} The inhalation of a high load of respirable aeroallergen proteins released under the influence of a storm has been associated with asthma exacerbation.^{2} Particularly, the

biophysical interactions of such allergens with the lung surfactant monolayer and alveolar epithelium can elicit serious downstream immune and inflammatory reactions^3,^{4} leading to the occurrence of an asthma attack.^{5} However, the precise causative mechanism of this phenomenon is under-reported. We propose that thunderstormborne aeroallergens fetch reactive oxygen nitrogen species (RONS) from the atmospheric plasma activated water (PAW), which can potentially enhance their allergenicity.

In this talk, we elucidate the effect of PAW-treatment on the allergens and its subsequent biophysical impact using a biomimetic model system, and employing a non-invasive technique, neutron reflectometry (NR). We used the dipalmiptoylphosphatidylcholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3 phosphocholine (POPC) to mimic the lung surfactant monolayer and alveolar epithelial bilayer, respectively. The biointerfacial interactions were examined using the model allergen, *Wisteria floribunda* lectin (WFL) and a rye grass (*Lolium perenne*) pollen allergen (Lol p 1) in the presence and absence of PAW. We observed that the PAW-treated allergens undergo conformational alterations and exert stronger non-specific interactions on the DPPC monolayer and POPC bilayer at both room (25 $^\circ$C) and physiological temperature (37 $^\circ$C), which implies the characteristic role of RONS in potentially escalated allergenicity. Furthermore, we examined that PAW-treatment results in the partial insertion of allergen into the monolayer and upper leaflet of the bilayer in contrast to the untreated allergen. Such a detailed mechanistic insight into this phenomenon coupled with the ongoing clinical studies will enable effective diagnostic and therapeutic strategies for the treatment of thunderstorm asthma.

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Combining Neutron Reflectometry with Molecular Dynamics Simulations to Investigate a Versatile Model Membrane System (12:10-12:30)

- Presenter: JOHN, Laura (University of Oxford)

Resolving structural and functional aspects of interactions between proteins and membranes requires experiments on membranes which mimic cellular characteristics as closely as possible. In this respect, previous methods which tethered or bound the membrane to a subjacent substrate showed significant deficiency. However, the so-called free-floating bilayer (FFB) model membrane system promises an important step forward. In this FFB system the membrane is surrounded on both sides by water and no longer in contact with the subjacent substrate. This results in better agreement with native membrane dynamics and fluidity. Nevertheless, the acting forces within this system are not fully understood. Ca2+ ions, essential for the self-assembling of the system, seem to play a crucial role. Also, the tightly bound water layers on top of a carboxylate terminated oligo (ethylene glycol) alkanthiol self-assembled monolayer (OEG-SAM), which covers here the substrate surface, are suggested to be a key element for generating FFBs. In this study, molecular dynamics simulations alongside with neutron reflectivity measurements were used to systematically investigate FFB systems consisting of differently charged phospholipid bilayers in presence of Ca2+ and Na+ ions, respectively. The results provided interesting insights in cation- and water-interactions with OEG-SAMs and phospholipid bilayers and enabled to understand the processes within the FFB system. The predicted model suggests that cations bind preferably to the OEG-SAM, hereby attracting the FFB. Nevertheless, structured water layers on top of the OEG-SAM maintain a water layer in between OEG-SAM and FFB via repulsive hydration forces. This knowledge is crucial to use the FFB system for the structural and functional analyses of the interactions between membrane proteins and lipid bilayers.

Lunch (12:30-13:30)

The structure of membrane-bound KRas from neutron reflectometry and molecular simulation. (13:30-14:10)

- Presenter: Dr HEINRICH, Frank (Carnegie Mellon University and NIST Center for Neutron Research)

KRas4B is a membrane-anchored signaling protein and a primary target in cancer research. Predictions from molecular dynamics simulations have shaped our mechanistic understanding of KRas signaling but disagree with recent experimental results from neutron reflectometry [1]. Therefore, we implemented restrained and bias-free molecular simulations for a quantitative comparison with NR and complementary nuclear magnetic resonance and thermodynamic binding data. Our results show that KRas4B approximates an entropic ensemble of configurations at model membranes, which is not significantly affected by interactions between the globular G-domain of KRas4B and the lipid membrane [2]. These findings promote a model of KRas, in which the G-domain explores the entire accessible conformational space while being available to bind to effector proteins. Our results deemphasize a mechanism in which KRas4B purposefully assumes discrete configurations at the membrane that modulate

signaling activity.

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Structural determination of the mechanism of action of synthetic antimicrobial polymers using model interfacial membranes (14:10-14:30)

- Presenter: Dr HALL, Stephen (STFC)

Rising antimicrobial resistance represents an ongoing global challenge. Antimicrobial peptides have shown promise in overcoming some of the limitations of traditional small molecule antimicrobials, where resistance can be typically easily acquired, yet can still be targeted by secreted proteases, typically display high cytotoxicity against mammalian cells and are synthetically expensive to produce. We have developed synthetic polyacrylamide-based polymers with chemical functionality mimicking that commonly found amongst antimicrobial peptides. These polymers have been shown to be active against clinically relevant Gram-negative bacteria, and simultaneously non-toxic towards mammalian cells. With such a wealth of synthetic possibilities offered by modern controlled radial polymerisation techniques such as reversible addition-fragmentation chain transfer (RAFT) polymerisation, there emerges a requirement to understand the structure-function relationship of synthetic antimicrobial polymers in order to design the most optimal polymeric architectures for the clinical setting.
In this talk, we present our recent work to link the molecular properties of synthetic cationic polymers to their ability to disrupt bacterial membranes. We have synthesised a small library of cationic antimicrobial polymers differing in block segmentation, molecular weight and cationic moieties. Using a variety of model membranes at the solid-liquid and air-liquid interfaces, we have used neutron reflection to gain a detailed molecular understanding of the interaction of these polymers with mimics of bacterial membranes. These results, corroborated by in vivo assays and a range of complementary biophysical techniques, will enable the optimal design of the next generation of synthetic antimicrobial polymers.

Phase-separation in Gram-negative bacterial outer membranes (14:30-15:10)

- Presenter: Prof. HOOGENBOOM, Bart (University College London)

Gram-negative bacteria are surrounded by a protective outer membrane with phospholipids in its inner leaflet and lipopolysaccharides (LPS) in its outer leaflet. The outer membrane is also populated with many β-barrel outer-membrane proteins (OMPs), some of which have been shown to cluster into supramolecular assemblies. Using atomic force microscopy on living bacteria, we have shown that the outer membrane of *E. coli* is phase-separated into LPS-enriched and OMP-enriched domains, and that additional phases appear when the LPS-phospholipid asymmetry of the outer membrane is perturbed. I will discuss how we have discovered and characterised these phases, and conclude with an outlook how studies of nanoscale in-plane membrane heterogeneity may be complemented by neutron reflectometry for studying out-of-plane order in the membrane.

Coffee (15:10-15:30)

Investigating model influence on the analytical resolution of neutron reflectometry (15:30-15:50)

- Presenter: SHIAELIS, Nicolas (University of Oxford)

[1]Investigating model influence on the analytical resolution of neutron reflectometry

Neutron reflectometry is an invaluable tool for the study of biological samples due to its sensitivity to atomic nuclei and their penetrative capabilities [2] [3] [4]. The resolution of the technique as de-fined by the diffraction limit is at about 10 Å. However, evidence from literature would suggest that this is not the case, as articles on this technique routinely describe resolving parameters which are smaller than this [5]..

Neutron reflectometry data is rarely analysed without any prior knowledge of the system i.e. what layers are present in the sample. Therefore, any analysis should always be viewed in the context of the model used. In this work, we investigate the relationship between the resolution and the choice of different models used, each with different numbers of parameters based on reasonable and justi-fied assumptions, to describe the system under consideration. Bayesian model selection [6] [1] is used to quantify the relative quality of the models relative to each other for varied number of con-trasts and the direct effect our ignorance or knowledge of the system has on the resolution.

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Small-angle scattering elucidates the incorporation of Outer membrane protein F into lipid nanodiscs

(15:50-16:10)

- **Presenter: Dr SOLOVYOVA, Alexandra (University of Newcastle)**

One of the most abundant proteins in gram-negative bacterial membrane is outer membrane protein F (OmpF). In recent years the mechanisms of OmpF interaction with antimicrobial agents and other membrane components were actively investigated [1-4]. This research demonstrated that OmpF has a substantial potential in the number of biotechnology applications such as vaccines and biosensor development [5,6]. Another important implication of these studies on OmpF organization inside the outer bacterial membrane could be a development of drug delivery system. In present work we explored a possibility to incorporate OmpF into lipid nanodiscs and characterised these particles by small angle scattering methods (SAXS and SANS) amongst other complementary techniques such as electron-microscopy and analytical ultracentrifugation. The results clearly demonstrated significant enlargement of lipid nanodiscs in response to OmpF incorporation.

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What do Biology and Medicine need from NR? (16:10-17:00)

- **Presenter: Prof. LAKEY, Jeremy (Newcastle University)**

When I belatedly started using neutrons in my career my research interests included the Gram negative outer membrane (OM) and surface bound proteins. Both leant well to NR research and since then I have been helped by supportive colleagues to answer some fundamental questions about my research areas. These include the structure of the OM, its dynamics and interactions with proteins and antibiotics. For biosensors we showed how antibodies interact with immobilised antigens in a commercial application and how proteins can be stabilised within self-assembling monolayers. I will discuss these and, more generally, the areas where biology and medicine still lack critical information which I think neutrons could supply. I have always been optimistic about NR's sensitivity, speed and information content so my ideas for the future may be overly ambitious but we ought to try.

Break (17:00-19:30)

Conference Dinner (19:30-21:30)

Friday 09 September 2022

Coffee (09:00-09:20)

Reflecting on Langmuir films. Bio-membrane research with neutrons, one layer at a time (09:20-10:00)

- Presenter: Dr PARACINI, Nico (Malmo University)

2022 marks the 90th anniversary of the Nobel prize awarded to Irwin Langmuir for his seminal work on surface chemistry, which remains to this day a central pillar of interface science. An example of his lasting legacy are the insoluble monolayers that bear his name and their many applications in biological membrane research. Langmuir monolayers represent versatile building blocks to assemble complex biological membranes using methods such as Langmuir-Blodgett (LB) and Langmuir-Schaefer (LS) depositions that transfer insoluble lipid monolayers from the air-water interface onto solid substrates. Examples shown here draw from research on asymmetric membranes and nanoparticle-supported lipid bilayers which constitute primary examples of the unique possibilities offered by the combination of grazing incidence neutron scattering techniques and LB/LS assembly methods. If a certain degree of lipid asymmetry is a feature of all biological membranes, in the outer membrane of Gram-negative bacteria the asymmetric distribution of lipids between the inner phospholipid and outer lipopolysaccharide bilayer leaflets is the defining characteristic, crucial for its function. LB and LS methods provide an ideal approach for reproducing this highly asymmetric bilayer in vitro and thanks to selective deuteration of the individual leaflets, specular neutron reflectometry has revealed important insights on the structure of the Gram-negative cell envelope and its interaction with antimicrobials discussed here. In addition to lipids, LB and LS methods offer the possibility of assembling highly ordered monolayers of nanoparticles (NP) which can be used as substrates for the formation of curved NP-supported lipid bilayers. Using a modified LS deposition and a combination of specular and off-specular neutron and X-ray scattering methods we characterised the in plane and out of plane structure of NP monolayers as well as the curved lipid bilayer formed around the NP, which provided insights on the effects of curvature on lipid packing. Finally, some of the limitations of LB and LS methods are outlined together with ongoing improvements in sample environment that could help move forward the applications of these versatile methods.

The role of histidines in antimicrobial peptides (10:00-10:20)

- Presenter: ERIKSSON SKOG, Amanda (Lund University)

Antimicrobial peptides (AMPs) are characterized as small, cationic peptides which, as the name suggests, show antimicrobial properties; something that has received considerable attention in recent years. These peptides can, according to structure, be classified into four different groups: extended AMPs, □-hairpin or loops, □-sheet, and amphipathic □-helical [1-2]. In this work the focus is directed at the extended AMPs with sequences rich in glycine, arginine or histidines, with little or no secondary structure [1]. As a model peptide we are working with the histidine rich, intrinsically disordered peptide Histatin 5 (Hst 5), a small saliva peptide of 24 amino acids with a net positive charge. The sequence consists of seven histidines and is known to have antimicrobial properties and it acts as the first defense against oral candidiasis caused by Candida albicans [3]. Hst 5 has been studied by our group over the past ten years and is well characterized in bulk conditions. We now wish to move further and characterize the peptide in vicinity of a solid surface as well as lipid bilayers to understand the structure-function relationship. In a previous study [4] we have shown that Hst 5 can traverse the solid supported bilayer at low ionic strength without disrupting the internal structure of the bilayer. However, at physiological ionic strength, the peptide penetrates and resides both within the bilayer, as well as below it. Our hypothesis is that the number of histidines in the sequence is important for the mechanism of interaction, due to its possibility to charge titrate and charge regulate [5]. To investigate this, a number of variants have been designed, and the interaction with a negatively charged phospholipid bilayer is investigated using surface active techniques such as neutron reflectivity and QCM-D. This will be complemented with atomistic and coarse grained molecular simulations.

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Biophysical investigations of the mechanism of action and lipid-mediated synergistic interactions of antimicrobial peptides within membranes (10:20-10:40)

- Presenter: Prof. BECHINGER, Burkhard (University of Strasbourg, Chemistry, France)

Biophysical and structural studies of peptide-lipid interactions, peptide topology and dynamics have changed our view how antimicrobial peptides insert and interact with membranes. Clearly, both the peptides and the lipids are highly dynamic, change and mutually adapt their conformation, membrane penetration and detailed morphology on a local and a global level. As a consequence, the peptides and lipids can form a wide variety of supramolecular assemblies in which the more hydrophobic sequences preferentially, but not exclusively, adopt transmembrane alignments and have the potential to form oligomeric structures similar to those suggested by the transmembrane helical bundle model. In contrast, charged amphipathic sequences tend to stay intercalated at the membrane interface, where they have been found to adopt mesophase structures in a lipid dependent manner. Although the membranes are soft and can adapt, at increasing peptide density they cause pronounced disruptions of the phospholipid fatty acyl packing. At increasing local or global concentrations the peptides result in transient membrane openings, rupture and ultimately lysis.
Interestingly mixtures of peptides such as magainin 2 and PGLa which are stored and secreted naturally as a cocktail exhibit considerably enhanced antimicrobial activities when investigated together in antimicrobial essays but also in pore forming experiments applied to biophysical model systems. Our investigations including solid-state NMR, fluorescence spectroscopy and neutron reflectvity experiments reveal that these peptides do not form stable complexes but act by specific lipid-mediated interactions and through the nanoscale properties of phospholipid bilayers. Notably, a quantitative idea about the strength of the lipid packing interactions can be obtained when comparing the peptide topologies in DMPC and POPC bilayers.

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Can NR help us observe the MscL ion channel in action? (10:40-11:00)

- Presenter: Dr SKODA, Maximilian (STFC)
Neutron reflectometry (NR) is an ideal tool for studying biological membrane models, and many different models, mimicking bacterial and mammalian cells have been developed in recent years. Often, the focus of such NR studies is on the interaction of various molecules, e.g. proteins, polymers, peptides etc with the membrane model. Another interesting use of such models is the characterisation of membrane proteins under physiological conditions. This is in contrast to other techniques, such as NMR or cryo-EM, which often do not allow observation under physiological conditions.
Here, we present a body of work dedicated to the characterisation of the pore-forming membrane protein MscL (Mechanosensitive Ion Channel of Large Conductance). Our interest in this protein is the fact that MscL is responsible for translating physical forces applied to cell membranes into electrophysiological activities. MscL has a relatively large conductance, 3 nS, making it permeable to ions, water, and small proteins when opened. MscL acts as stretch-activated osmotic release valve in response to osmotic shock.
We discuss the benefits and limitation of two different membrane models, including a novel "suspended" one, with aim of not only determining the protein's location within the membrane, but also attempting to observe the actual opening (or gating) of MscL triggered by exposure of the membrane to the antimicrobial peptide (AMP) pexiganan (PXG). Our hypothesis is that MscL could serve as an Achilles heel that increases antimicrobial activity of AMPs.

Coffee (11:00-11:20)

Unravelling Bcl-2 proteins´ functioning at mitochondrial membrane level (11:20-12:00)

- Presenter: Prof. GRÖBNER, Gerhard (Umeå University)
Programmed cell death (apoptosis) is essential for human life. In its intrinsic apoptotic pathway opposing members of the B-cell lymphoma 2 (Bcl-2) protein family control the permeability of the mitochondrial outer membrane (MOM) and any release of apoptotic factors. Any imbalance can cause disorders including cancer, where often upregulation of cell protecting (anti-apoptotic) Bcl-2 members such as the Bcl-2 membrane protein itself plays a notorious role by blocking membrane perforating apoptotic proteins such as Bax which normally will cause cell death. Here, we apply neutron reflectometry (NR) on supported lipid bilayers which mimic MOM environment and solid state/liquid state NMR spectroscopy to unravel the molecular basis driving opposing proteins to interact with each other at the MOM; a mechanism which is not really understood yet due to lack of high-resolution structural insight. Based on our central hypothesis that Bcl-2 drives its cell-protecting function at a membrane-embedded location as revealed by NR (1), we focus i) to determine the structure of human Bcl-2 protein in its membrane setting by combining solution and solid-state NMR; ii) use NR to study the kinetics and lipid/protein pore assemblied upon binding of Bax to mitochondrial membranes and its membrane destroying activities there; and iii) unravel the nature of direct

interaction between Bcl-2 and Bax to neutralize each other. Knowledge generated here, will be indispensable in understanding the regulative function of the Bcl-2 family at mitochondrial membranes.

Molecular Simulation of Model Biological Membranes (12:00-12:20)

- Presenter: Prof. WINN, Martyn (STFC)

Classical molecular dynamics (MD) provides atomic level detail on the structure and dynamics of biological molecules, as well as allowing *in silico* experiments on the system of interest. In particular, MD has been applied to biological membranes where historically there has been a lack of high resolution experimental probes. There are now increasing efforts to combine the level of detail available from MD with reflectivity scattering curves from real samples. In this talk, I will cover our work to develop a computational pipeline at ISIS that allows the inclusion of MD trajectory data into the reflectivity analysis provided by Rascal/RAT. An innovative part of this pipeline is feedback from the fit to experimental data back to the molecular modelling via applied restraints. The pipeline will be made available through an iDAaaS environment.

This work is part of a wider effort in Scientific Computing to develop molecular simulation methods, to elucidate biological structures, and to support the large facilities at Harwell. I will also summarise some of this other work, in order to provide some of the wider context.

Fitting Reflectivity Data Using Molecular Dynamics Simulations (12:20-12:40)

- Presenter: Dr HUGHES, Arwel (STFC)

Reflectivity data is traditionally analysed using simple, layered approximations of the interface. Whilst these give a guide to the structure, fine details of the interface are often unresolved. Over the past 20 years, there has been a move to using more sophisticated models, using information from atomistic simulations to construct more refined descriptions of the interface. We have recently shown that excellent agreement between theory and experiment can be achieved by incorporating MD simulations directly into the model construction. Here we review those developments, and discuss how advancements in computing power and simulation sophistication mean that these methods are increasingly feasible approaches for data analysis over the timescale of a typical facility experiment, which from proposal to publication can span many months. In many cases, atomistic modelling could be considered as an analysis method for many experiments, but the barrier for users is often access. We discuss efforts at ISIS and SCD to facilitate these analysis approaches for the user community more widely.

Cryo Electron Microscopy of Small Membrane Proteins (12:40-13:00)

- Presenter: Dr QUIGLEY, Andrew (Diamond Light Source)

The resolution revolution in cryo electron microscopy, led by the introduction of direct electron detectors, has let to unprecedent gains in structural biology. However, despite advances in data collection and image processing, single particle cryoEM of small proteins, in particular small membrane proteins remains a challenge.

The Membrane Protein Laboratory at Diamond Light Source is funded by Wellcome to assist the user community in the study of membrane proteins. Working closely with beamlines and the electron bio-imaging centre at Diamond, we optimise sample preparation for experiments (from cloning/expression through to experimental set-up), assist in data collection and data processing.

We have been working closely with eBIC to develop optimised workflows for the study of small membrane proteins (<100 kDa). This includes the development of high-throughput assays and screening to assess protein quality before vitrification, and optimised methods for data processing. Our aim is that these optimised parameters will feed into the eBIC pipeline to assist users to the facility. We demonstrate the benefit of these pipelines with data from two proteins which have been studies in house in the MPL.

Lunch (13:00-14:00)