Bilayers at the ILL

Wednesday, 11 December 2019 - Friday, 13 December 2019

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Bilayers at the ILL

Book of Abstracts

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Metabolic incorporation of deuterium into nerve myelin

Author: Daniel A. Kirschner[1]

Co-authors: Anne Baumann [2]; Bruno Demé [3]; Andrew R. Denninger [1]; Marek Domin [1]

[1] *Boston College*

[2] *University of Bergen*

[3] *ILL*

Corresponding Author: kirschnd@bc.edu

Myelin sheaths are the differentiated membrane assemblies in the central and peripheral nervous systems (CNS; PNS) that wrap nerve fibers in a jelly-roll-like arrangement. The regular organization along fibers of these electrically-insulating, multilamellar, lipid-rich sheaths is responsible for the rapid conduction of electrical signals from one node of Ranvier to the next node. The disruption of myelin structure is the basis for many demyelinating neuropathies in the nervous system, including multiple sclerosis, Guillain-Barré syndrome and CIDP, and hereditary motor and sensory neuropathies. Understanding what brings about myelin destabilization requires knowledge of its structure, which, because of its paracrystalline order, is an appealing target for diffraction studies [1]. Owing to exceptional improvements in neutron scattering instrumentation for detecting deuterium (2H or D) in myelinated nerves [2,3], we explored the localization of D in myelin after its metabolic-incorporation via drinking water into pregnant dams and their developing embryos. We observed significant differences in the neutron diffraction patterns from the myelinated nerves of H2O- versus D2O-fed dams and their pups. Neutron scattering length density profiles showed a major increase in density in the middle of the myelin membrane bilayer. Mass spectrometry confirmed the presence of D-labeled lipids—phosphatidylserine, sulfatide, phosphatidylinositol, phosphatidylethanolamine, and triacylglycerol—in the nervous tissue from the D2O-fed mice. Whereas the lipids from the D2O-dam were 75-80% deuterated, the lipids from the D2O-pups were ~99% deuterated. Moreover, the deuterated lipids for the pups vs. the dam had different incorporation patterns of the label.

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Session A / **76**

Interactions in the pre-AD mimicking model membranes

Authors: Norbert Kucerka[1]; T. Kondela[2]

Co-authors: T. Murugova [3]; Olexandr Ivankov [4]; E. Ermakova [5]; E. Dushanov [6]; K. Kholmurodov [7]

[1] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, Russia and Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia*

[2] *Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia and Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia*

[3] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, Russia and Moscow Institute of Physics and Technology (State University), Dolgoprudny, Russia*

[4] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research*

[5] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, Russia*

[6] *Laboratory of Radiation Biology, Joint Institute for Nuclear Research in Dubna, Russia and Department of Chemistry, New Technologies and Materials, Dubna State University in Dubna, Russian Federation*

[7] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, Russia and Department of Chemistry, New Technologies and Materials, Dubna State University in Dubna, Russian Federation*

Corresponding Author: kucerka@fpharm.uniba.sk

The Alzheimer's disease (AD) is a devastating neurodegenerative disease caused by the formation of senile plaques, primarily consisting of Amyloid-beta peptides. The crucial role in this process at its pre-clinical stage is likely imparted by peptide-membrane interactions [1], though the further details are yet to be understood. Our recent experimental data for example revealed several intriguing structural properties of biomimetic membranes. First, it is their sensitivity to the charge present in the surrounding environment. The structure of membranes changes with increasing concentration of ions, which appears to be an effect born by peculiar properties of ions and lipid themselves [2]. Interestingly, the differences in lipid interactions with ions have been noted to be underlined by the hydration properties of the ions. The hydration interactions appear to determine also the location of other membrane constituents, such as cholesterol. Moreover, cholesterol increases the order of lipid hydrocarbon chains while increasing the stiffness of membrane, in the contrary to the fluidizing effect of melatonin [3]. Both of the latter effects have been correlated recently with the development of AD. We are particularly interested in investigating the effect of membrane fluidity, that can be controlled by the two additives, on the interactions taking place in such pre-AD mimicking model membranes [4].

This work is being supported by the Russian Science Foundation under grant 19-72-20186.

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Session A / 5

Lipid domain modulation and inhalation anesthesia

Authors: David Worcester[1]; Michael Weinrich[1]

[1] *NIST Center for Neutron Research*

Corresponding Author: worcesterd@missouri.edu

Small membrane lipid domains are important in cell signaling. We investigate modulation of lipid domains by volatile anesthetics. Many volatile compounds produce anesthesia. With no molecular target identified, mechanisms remain puzzling. Large membrane concentrations (~100 mM) are required to produce anesthesia. Hyperbaric pressures (~100 bar) reverse anesthesia.

Using neutron/x-ray scattering, effects of anesthetics on mixing transitions ofternary lipid mixtures exhibiting liquid-liquid phase coexistence were investigated. Multi-layer stacks of DPPC (or brain sphingomyelin)/DOPC/cholesterol (2/2/1) produce two sets of diffraction peaks, giving d-spacings for two lipid phases and their relative amounts which change with temperature and anesthetic concentration. Hydrostatic pressure reverses effects of raising temperature. [1] The contrast variation technique of Pencer gave similar results with small unilamellar vesicles. Clinical concentrations of anesthetics (isoflurane, halothane, xenon, nitrous oxide, chloroform, hexane) shift mixing transitions to lower temperatures. Recently, we demonstrated that hexanol produces similar effects. These neutron/x-ray results for hexanol differ from results with fluorescent dyes that apparently perturb lipid mixing. [2] Volatile anesthetics at clinical concentrations affect lipid mixing, possibly causing changes in membrane lateral pressures or hydrophobic mismatch of membrane-embedded proteins. An elegant example of lipid mixing modulating neural activity is provided by recent work on the potassium channel TREK-1. Hansen and colleagues [3] showed that TREK-1 in planar bilayers is

not modulated by anesthetics, while TREK-1 in cells studied with patch clamp is modulated. Fluorescent labels showed that anesthetics produce migration of phospholipase D from lipid domains to TREK-1 where the phospholipase produces phosphatidic acid, a potent modulator of TREK-1. More examples are anticipated.

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Session A / **29**

Influence of galactoglycerolipids on the chloroplast envelope outer membrane architecture and its adaptation to phosphate deprivation in plants

Author: Stéphanie Bolik[1]

Co-authors: Ignacio Rodriguez Loureiro [2]; Emanuel Schneck [2]; Bruno Demé [3]; Juliette Jouhet [4]

[1] *Laboratoire de Physiologie Cellulaire et Végétale (CEA/CNRS/INRA/UGA)/ILL*

[2] *Max Planck Institute of Colloids and Interfaces*

[3] *ILL*

[4] *Laboratoire de Physiologie Cellulaire et Végétale (CEA/CNRS/INRA/UGA)*

Corresponding Author: stephanie.bolik@cea.fr

Plant cell membranes glycerolipids can be classified in two groups: phospholipids containing phosphate, mainly synthesized in the endoplasmic reticulum (ER), and glycolipids – galactolipids and sulfolipids - without phosphate, synthesized in chloroplasts, and being the major constituents of photosynthetic membranes. When plants are deprived of phosphate, a frequent natural situation that limits plant growth, they adapt to the environment by increasing their phosphate absorption, decreasing their phosphate consumption and mobilizing phosphate cell reserves. Phospholipids contain up to one third of intracellular phosphate [1] and for that reason represent a non-negligible phosphate source. In phosphate deprived plants, phospholipid amount, mainly phosphatidylcholine (PC), decreases and is replaced by digalactosyldiacylglycerol (DGDG) in extraplastidial membrane. Because we demonstrated that PC bilayers do not share the properties of DGDG bilayers [2,3], we are now investigating what the consequences of a PC-DGDG replacement are. During phosphate starvation, to support lipid trafficking, the number of contact sites between chloroplasts and mitochondria is increased by a factor of three and the chloroplast envelope forms extensions called stromules (Stroma filled tubules). We suppose that under phosphate starvation the chloroplast outer membrane is enriched in DGDG and deprived of PC. Our objective is to understand if this change of composition can be partly responsible for the observed change in the chloroplast envelope architecture. To answer these questions, we developed the complete procedure to purify natural plant lipids and we propose to investigate a series of samples as a function of hydration under controlled humidity by neutron diffraction on the D16 instrument at ILL. The measurement of the spacing of the bilayers and the bending rigidity of the different samples indicate that the membranes enriched in DGDG are favoring membrane stacking and are less rigid. These results support the role of DGDG for stromule formation and membrane contact site during phosphate starvation.

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Session B / **3**

Linking membrane structure and dynamics: insights from neutron scattering

Author: Elizabeth Kelley[1]

[1] *National Institute of Standards and Technology*

Corresponding Author: egk@nist.gov

Life places extreme demands on the material properties of the membranes that surrounds cells. These thin bilayers must be both rigid enough to define the cell structure yet flexible enough to undergo dramatic changes in cell shape in processes like endocytosis and cell division. In turn, the properties of the biomembranes are determined by the unique characteristics of the thousands of chemically distinct lipid molecules that make up the membrane. A long-standing challenge in membrane biophysics is to link the complex and highly regulated lipid diversity to the membrane properties and ultimately cell function. This talk will highlight new insights from neutron scattering towards understanding the role of lipid diversity in tuning the properties of model biomembranes. We will demonstrate how subtle changes in lipid composition, such as mixed hydrophobic tail lengths or adding charged headgroups, can have significant and unexpected effects on the membrane dynamic properties. The results reveal the complex and interwoven relationship between lipid membrane composition, structure and dynamics.

Session B / **49**

Curved lipid interfaces studied with GISANS

Authors: Karolina Mothander[1]; Tommy Nylander[1]; Adrian Rennie[2]

[1] *Physical Chemistry, Lund University*

[2] *Uppsala University*

Corresponding Author: karolina.mothander@fkem1.lu.se

Curved cell membranes are important for the function of the cell, both for compartmentalization in organelles within the cell as well as for cellular mitosis. It has also been shown that the curvature of a lipid membrane can affect the concentration of membrane bound proteins. In this project we have used semiconductor nanowires to study the effect of curvature on phospholipid bilayers and membrane proteins. We have previously demonstrated the formation of a supported phospholipid bilayer on nanowires via vesicle fusion, which can be used as a proxy for curved membranes. Based on fluorescence recovery after photobleaching, FRAP, measurements, the phospholipid bilayers were found to follow the contours of the nanowires as continuous and fluid, locally curved bilayers. However, these data do not reveal the coverage, composition and the dimensions of the bilayer. In this project we aim to reveal this structure using grazing incidence small-angle neutron scattering (GISANS).

For this purpose, we have performed GISANS experiments with the KWS-1instrument at FRM II (Germany), with VSANS at NIST (USA) and SANS2D at ISIS (UK) with phospholipid covered nanowires. The nanowires were covered with the bilayer obtained from deposition from a vesicular dispersion of a mixture of 20mol% DOPE in DOPC. In these experiments we have successfully shown the formation of a lipid bilayer and the subsequent binding of membrane protein, streptavadin. These results show that nanowire supported lipid bilayer is an excellent way to study binding of membrane protein to curved lipid interfaces.

Session B / **32**

Partitioning of DNA nanochannel between Lo/Ld phases in a lipid membrane: Relevance of lipid anchors

Authors: Ahmed Sayed[1]; Aleksander Czogalla[2]; Ralf Seidel[3]

[1] *Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany*

[2] *University of Wroclaw, Wroclaw, Poland*

[3] *Peter Debye Institute for Soft Matter Physics, Universität Leipzig, Leipzig, Germany*

Corresponding Author: a.sayed@hzdr.de

DNA origami is emerged as a powerful and versatile method to fabricate highly controllable 3-dimensional structures at the nanometer-scale. One example of those powerful DNA nanostructures is a membrane-spanning DNA channel, which exhibited the ability to mimic the biological channels in transporting of ions and biomolecules across the lipid membrane (Burns et al.2016).
Membrane-spanning DNA channel exists often in a cylinder-like structure of six-helix bundle attached with a lipid anchor in order to lower the energy barrier of DNA insertion into lipid bilayer. At the time of this study, the relevance of the lipid anchor in controlling the preferential interaction of DNA with different lipid compositions has not been studied. *In vivo*, for instance, palmitoylated proteins are enriched in lipid raft; whereas prenylated proteins segregate into non-raft lipids (Melkonian et al. 1999). Here we address a question whether the spatial configuration of the lipid anchor could play a role in targeting the DNA nanochannel to a distinct lipid domain phase.We fabricated DNA nanopores of six DNA double helices arranged in parallel with a 9-15-nm length and decorated with hydrophobic tags derived from fatty acid tail of lipids. These fatty acid chains were chosen based on their potency in partitioning the membrane-bound proteins *in vivo* into lipid rafts (liquid-ordered phase, lo) and non-raft phases (liquid-disordered, ld). The DNA channels with various lipid anchors were incubated with giant unilamellar vesicles (GUVs) with Lo + Ld phase coexistence and the lipid phase preference of DNA channels was visualized under confocal microscope. Moreover, the transport activity of these artificial channels was assessed by a release technique of a fluorescent dye encapsulated into SUV liposomes.

Session B / **70**

The lipid organisation in skin: an integrated approach of lipid model systems and clinical studies

Author: Johanna Aaltje Bouwstra[1]

Co-authors: G.S. Gooris ; E. A. Mojumdar ; D. Groen ; Charlotte Beddoes

[1] *Leiden University*

The skin barrier function is primarily located in the outermost layer of the skin, the stratum corneum (SC) and is comprised of corneocytes (dead cells) and intercellular lipids. The main lipid classes in the SC are ceramides, free fatty acids, and cholesterol. These lipids form two crystalline lamellar phases, with a repeat distance of 13 nm and 6 nm, respectively. The composition and organization of the SC lipid matrix are crucial for a proper skin barrier function. The 13 nm lamellar phase is very characteristic for the SC lipid matrix and is considered to plays a crucial role in the skin barrier function.
For many years our group focusses on the lipid composition and organization in inflammatory skin diseases. These skin diseases are characterized by an impaired skin barrier function. A well-known example is atopic dermatitis (AD). AD is the most prevalent skin disease in children. Nowadays 15-20 % of the children in Western Europe are suffering from this disease and the lipids may be an underlying factor for the mpaired skin barrier. We performed a clinical study aiming to determine the role of SC lipids in the impaired skin barrier function. We observed that changes in the lipid composition and organization correlated with the impaired skin barrier function. Using lipid model systems it appeared that the changes in lipid organization encountered in the clinical studies reduced the lipid barrier. This demonstrates that the changes in lipid properties are an underlying factor of the impaired skin barrier.

As the lipids are important for a proper skin barrier function, more detailed analysis using neutron diffraction focusing on the arrangement and configuration of the lipid subclasses in the unit cell of the 13 nm lamellar phase. By combining D2O/H2O contrast variation together with the use of (partly) deuterated lipids we were able to localize most of the lipid subclasses in the unit cell and revealed the configuration of an abundant ceramide subclass. These studies provided more details on the function of the various lipid classes in understanding the skin barrier function in healthy and diseased skin in more details.

Wine & cheese poster session / 60

Comparing molecular dynamics force fields in bacteria membranem-Models

Author: Antonio Peón[1]

Co-authors: Bábara Claro [1]; Margarida Bastos [1]; Ángel Piñeiro [2]; Rebeca García-Fandiño [3]

[1] *CIQ-UP – Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal.*

[2] *Facultad de Física, University of Santiago de Compostela (USC), Santiago de Compostela, Spain*

[3] *Singular Research Centre in Chemical Biology and Molecular Materials (CIQUS), Organic Chemistry Departament, University of Santiago de Compostela (USC) Santiago de Compostela, Spain*

Corresponding Author: antonio.peon@gmail.com

The cell membrane is a universal component found in both prokaryotic and eukaryotic cells, which is composed mainly of a series of lipid mixtures that act as a physical barrier against various pathogens, in addition to other functions that remain unknown. There is a large difference in the lipid composition of bacterial and mammalians cytoplasmic membranes, a fact that can be used to obtain antimicrobial selectivity. One of the most exciting advances in the scope of Molecular Dynamics (MD) simulations of bacterial membranes in the last years has been the development of molecular-level models that incorporate the heterogeneity of the nonprotein constituents, and now frequently a mixture of phospholipids is used in simulation studies.[1,2] While MD simulations can provide invaluable detailed structural and dynamical information about the studied system, the crucial issue of the reliability of such simulations is the quality of the force field. Whereas much effort has been dedicated to parametrize and optimize the force fields for biomembrane modelling, most of the comparisons have been done for homogeneous bilayers composed of a single phospholipid type, which may not work optimally or even fail when used in description of complex in homogeneous systems.

We will present preliminary results obtained from the comparison of MD simulations of different homogeneous and heterogeneous bacterial membrane models using different force fields (Slipids, CHARMM36, GROMOS 54A7 and Lipid17). Models consisting of five hundred lipids each one was studied, as gram-positive bacteria mimetic model, POPE/POPG (1:3) as gram-negative mimetic model, POPE/POPG (3:1) and POPG/POPC (3:7) as well as homogeneous bilayer models to simulate neutral membrane systems, anionic membrane systems or the outer layer of the membranes will be used respectively POPE, POPG and POPC. For comparation: area per lipid, thickness, number of hydrogen bonds, lateral diffusion, order parameters, lateral density, radial distribution function and number of clusters were used. These results would be useful to understand the behaviour of lipids at atomistic-level at lipid-bilayer/water interfaces and provide a point of reference for making the appropriate decision on the force field in bacterial membrane models MD simulations.

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Wine & cheese poster session / **17**

Understanding the interactions between the intrinsically disordered peptide Histatin 5 and lipid bilayers, the effect of amino acid sequence and electrostatic interactions

Author: Amanda Eriksson Skog[None]

Co-authors: Stéphanie Jephthah [1]; Sandeep Chakane ; Yuri Gerelli ; Marie Skepo

[1] *Lund University*

Corresponding Author: amandaeskog96@gmail.com

Histatin 5 (Hst5) is a histidine-rich, 24 amino acid protein, classified as an intrinsically disordered protein (IDP). It is a cationic salivary protein found to play a crucial role in fungicidal activity, and its activity to inhibit the growth and viability of Candida albicans has been evaluated using a variety of techniques. The underlying mechanism is however not very well known. The aim of this project is to understand the underlying mechanism of the role of the histidines and the interaction with the lipid bilayer system.

The results obtained from neutron reflectometry and QCM-D have shown that the interaction between the peptide and the lipid bilayer is completely governed by electrostatic effects. This was done by changing the charge content in the bilayer and the ionic strength of the buffer. At low ionic strength, the peptide penetrates the bilayer and cumulate close to the solid silica substrate underneath the lipid bilayer. The effect of the number of histidines, and hence the charge of the peptide, have been studied with QCM-D. Results show that the interaction with the bilayer is strongly affected by the number and position of the histidines in the peptide. The peptide adsorption is reduced when the number of histidines is conserved, but the peptide sequence is scrambled, as well as when some selected histidines are replaced by glutamines, which is the pH-insensitive counterpart to histidine. Results on how the different variants of Hst5 adsorb to surfaces and the free energy profiles for the interaction is obtained from coarse-grained Monte Carlo simulations.

UV/Vis-spectrophotometry measurements have shown that the interaction between Hst5 and a lipid bilayer initiates a peroxidation reaction, this is also affected by the number of histidines in the sequence.

Wine & cheese poster session / **69**

Interaction of DDAO surfactant with model membrane - liposome-micelle transition

Author: Jana Gallova[1]

Co-authors: Daniela Uhrikova [2]; Olexandr Ivankov [3]; Katarina Zelinska [4]

[1] *Faculty of Pharmacy, Comenius University in Bratislava*

[2] *Faculty of Pharmacy, Comenius University in Bratislava, Odbojarov 10, 832 32 Bratislava, Slovakia*

[3] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research*

[4] *Institut Laue-Langevin & Department of physical chemistry of drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia*

Corresponding Author: gallova@fpharm.uniba.sk

Surfactant N,N-dimethyl-1-dodecanamine-N-oxide (DDAO) possess a wide range of biological effects (antimicrobial, phytotoxic, antiphotosynthetic, immunomodulatory). Interaction of non-ionic form of DDAO with DOPC and DOPC-CHOL (CHOL:DOPC =0.5:1 mol/mol) model membranes was studied by SANS. Unilamellar liposomes were prepared by extrusion in PBS buffer. Aggregation of

DOPC and DOPC-CHOL unilamellar liposomes at zero and low DDAO concentration was detected. We supposed that it was induced by constituents of PBS buffer. Lamellar paracrystal model was used to analyse the SANS data for samples with DDAO:lipid in the range 0 – 1 mol/mol. The number of bilayers interacting in the aggregate, was found close to 1.4. The intercalation of surfactant molecules into the lipid bilayer caused narrowing of the lipid bilayer. Undistinguished changes in repeat distance after addition of DDAO up to molar ratio DDAO:lipid = 0.5 were followed by an increase at higher DDAO concentration. A transition from bilayer to cylindrical micelles took place around molar ratio DDAO:lipid =1. The SANS data in the range DDAO:lipid = 3 - 5 mol/mol were analysed using a model of rigid cylinder with elliptical cross section. Pore formation in lipid bilayer caused by DDAO was studied by fluorescence probe leakage method. The changes in the size of lipid aggregates upon increasing DDAO concentration were followed turbidimetrically. Structure of DDAO - lipid aggregates, partition coefficient of DDAO between lipid and aqueous phase, as well as effective ratios Re (the amount of DDAO integrated into the bilayer to the amount of lipid at particular DDAO concentration in the sample) are not considerably influenced when one third of DOPC molecules is substituted with CHOL.

This work has been supported by the VEGA grants 1/0916/16 and 1/0228/17, JINR topical themes 04-4-1121-2015/2020 and APVV project 17-0239. This work benefited from the use of the SasView application, originally developed under NSF award DMR-0520547.

Wine & cheese poster session / **53**

Understanding the interfacial structure of pulmonary surfactant films by neutron reflectometry

Authors: José Carlos Castillo Sánchez[None]; Ainhoa Collada Marugán[None]; Antonio Cruz[None]; Jesús Pérez-Gil[None]; Armando Maestro[None]

Corresponding Author: ainhoaco@ucm.es

Pulmonary surfactant consists on an adsorbed phospholipid-based monolayer at the alveolar air-liquid interface connected to a membrane reservoir placed in the aqueous subphase. Its main function is to minimize the surface tension stabilizing the mammalian respiratory surface, enabling breathing dynamics and preventing alveolar collapse during expiration. Pulmonary surfactant is mainly composed of phospholipid, but the hydrophobic surfactant proteins SP-B and SP-C are small proteins that strongly interact with surfactant membranes playing a critical role during the formation and stabilization of surfactant films. SP-B is proposed to be involved in the regulation of surfactant adsorption to the interface forming lipid channels that could allow surfactant phospholipids to adsorb efficiently into the alveolar air-liquid interface. SP-C participates on remodelling of surfactant films along respiratory dynamics inducing curvature and promoting exclusion of unsaturated surfactant lipids when surface pressure increases. To accomplish a quantitative structural characterization of surfactant complexes, neutron reflectometry of adsorbed surfactant films in a dynamic air-liquid interface was performed. Surfactant films composed by model lipids (DPPC/POPC/POPG) with or without SP-B or SP-C were characterized at FIGARO. Experiments were performed using both hydrogenated and deuterated lipids and two contrasts: D2O and Air Contrast Match Water. Neutron reflectometry was recorded at 10mN/m and 35mN/m before and after 5 compression-expansion cycles. Data obtained are compatible with an increase in surface lipid concentration at 35mN/m. Although no significant differences in the reflectivity were detected when using surfactant proteins at their physiological concentrations, normalized 2-D detector scans showed a signal corresponding to a lipid reservoir in bulk as well as an increase in the off-specular signal in the presence of SP-B, meaning that SP- B promotes the formation of a reservoir connected to the material forming the interfacial film. Additionally, the reservoir and the adsorbed material undergo structural alterations as a consequence of compression-expansion cycles.

Wine & cheese poster session / **30**

Quaternized chitosan oriented surfactant self-assembly complexes

Authors: Bin Dai[1]; Leonardo Chiappisi[None]; Marie-Sousai Appavou[2]; Ingo Hoffmann[3]; Michael Gradzielski[1]

[1] *Technische Universität Berlin, Fakultät II, Institut für Chemie, Stranski-Laboratorium für Physikalische und Theoretische Chemie*

[2] *Jülich Centre for Neutron Science JCNS at Heinz Maier-Leibnitz Zentrum (MLZ) Forschungszentrum Jülich GmbH*

[3] *ILL*

Corresponding Author: daibin4@gmail.com

PolyElectrolyte-Surfactant Complexes (PESCs) are interesting colloidal systems characterized by a complex, mesoscopically ordered inter- and intra-particle structure. Depending on their composition such assemblies are often sensitive to environmental stimuli, e.g. T and pH. One building block of such a potentially functional material can be the naturally derived polycation chitosan and the oppositely charged biofriendly surfactant alkylethoxy carboxylate, which were studied in our group previously [1, 2]. Here one has the nm-sized structures formed by the surfactant molecules and the larger sized structures controlled by the longer length scale of the polymer chains. This system has potential for applications in various fields, e.g. cosmetic and pharmaceutical formulations or in detergency, especially as it shows marked pH-dependent behaviour. Previous results showed that the local PESCs structure depends substantially on the surfactants packing parameter and their interactions. By admixing the oppositely charged macromolecule unilamellar vesicles formed by C12E4COO are transformed to multilamellar vesicles (MLVs). However, with normal chitosan the working pH range was limited to below pH 6. By quaternizing the chitosan by methylation with up to 100% permanent charges the working pH range was successfully extended to the full pH range of acidic via neutral to basic. The ionically assembled structures for different types of surfactants and different quaternized chitosan were investigated as a function of the mixing ratio with different methods e.g. turbidity, light scattering, and small-angle neutron scattering (SANS) in order to cover the complete relevant size range of 1-500 nm for a broad range of composition. Different tunable bilayer structures were observed and one has as structural tuning parameters the molecular structure of the surfactant, pH (controlling the charge on the surfactant aggregates) and the mixing ratio of the surfactant and modified chitosan.

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Wine & cheese poster session / **37**

How soap dissolves micelles: solubilisation kinetics of mixtures between anionic surfactants and block copolymer micelles

Author: Synne Synne Myhre[1]

Co-authors: Matthias Amann [1]; Lutz Willner [2]; Kenneth Dahl Knudsen [3]; Reidar Lund

[1] *Chemistry Department University of Oslo*

[2] *Forschungszentrum Jülich GmbH*

[3] *IFE*

Corresponding Author: synne.myhre@kjemi.uio.no

Fundamental understanding of the solubilisation process in self-assembly systems is important for many applications including membrane solubilization and protein extraction. The kinetic processes involved in mixtures of surfactants and block copolymer micelles are not well understood. However, it is commonly known that surfactants exhibit rather fast equilibration kinetics, in the order of micro- to milliseconds, while polymers are much slower, in the order of minutes to months. In this

contribution, we will present a study of the stability and solubilization kinetics of block copolymers micelles upon addition of sodium dodecyl sulphate (SDS) using small angle X-ray scattering (SAXS) and time resolved neutron scattering (TR-SANS). We compare the ability of the surfactant to dissolve and form mixed micelles with three amphiphilic polymers; poly(ethylene propylene)-poly(ethylene oxide) (PEP-PEO) and end-capped PEO (C28-PEO and C21PEO5). The exchange kinetics of C28PEO occurs over 5 seconds and C28PEO occurs on time scales on the order of minutes-hours on ambient temperatures, and that of PEP1-PEO20 is known to be frozen on practical time scales. Addition of SDS to PEP1-PEO20 shows close to no change, even after extended period of time. However, time-resolved SAXS data of addition of SDS to C21PEO5 shows a fast dissolution and formation of mixed micelles over seconds, while under the addition of SDS to C28PEO5 we observe a dissolution over hours. The solubilisation process can be analysed by a combination of two processes. Initially, a fast concentration dependent fragmentation process takes place, before a re-organization step of the polymer chains occurs which rate is dependent on length of the hydrophobic block. The results provide insight into the role of dynamic asymmetry in self-assembly, which is transferable to many multi-component systems.

Wine & cheese poster session / 55

Molecular dynamics simulations with cationic lipids

Authors: Inna Ermilova[1]; Jan Swenson[1]

[1] *Chalmers University of Technology*

Corresponding Author: inna.ermilova@chalmers.se

Cationic lipids are widely used in modern drug delivery systems such as lipid nano-particles (LNPs). These drug carriers are multi-component systems (containing novel cationic lipids, cholesterol, phospholipids, siRNA/mRNA) with strognly pH-dependent structures, which are poorly characterized. Earlier experimental methods could provide the information about optimal sizes of LNPs, but their exact inner structures and mechanisms of actions were not determined.
In this work we use the combination of scattering techniques with multiscale computer simulations in order to refine structures of pharmaceutical LNPs and understand their pharmaco-kinetics. At the first step models for cationic lipids were developed for both neutral and ionized forms using the same methodology as the one which was employed for the derivation of atomistic SLipids force field. Parameters for lipids tails were adopted from the ones which were derived for polyunsaturated phospholipids and head-groups were mainly parametrized. Membranes containing cationic lipids and cholesterol were simulated using classical molecular dynamics (MD) approach employing newly delivered models at the ratio 50:38.5. From the preliminary analysis of MD trajectories it was observed that cholesterol molecules aggregate and water pores were formed in every simulated lipid bilayer. This information will be used for future simulations of more complex structures and the derivation of coarse-grained models.

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Interaction between human dihydroorotate dehydrogenase and coenzyme Q10 in model lipid bilayers

Author: Juan Manuel Orozco Rodriguez[1]

Co-authors: Ewa Krupinska [1]; Giovanna Fragneto ; Hanna Wacklin ; Wolfgang Knecht [1]

[1] *Lund University*

Corresponding Author: manuel.orozco@biol.lu.se

In humans, dihydroorotate dehydrogenase (DHODH) is an flavoprotein found in the inner mitochondrial membrane (IMM). DHODH catalyzes the oxidation of dihydroorotic acid with the concomitant

reduction of ubiquinone Q10 (coenzyme Q10), thus acting as a link between the *de novo* pyrimidine biosynthesis and the mitochondrial respiratory chain. DHODH is a well validated target for immunosuppressive and antiproliferative compounds that act as inhibitors of the enzyme[1, 2]. Furthermore, mutations in human DHODH have been identified as the cause of Miller syndrome, a rare autosomal recessive disorder resulting in numerous abnormalities of the head, face and limbs [3]. Despite the abundance of crystal structures for DHODH, the mechanisms by which the enzyme interacts with its lipophilic cosubstrate, ubiquinone Q10, are not well understood. We have investigated the interaction between an N-terminally truncated version of human DHODH (lacking the transmembrane domain) and coenzyme Q10 by means of neutron reflectometry (NR) and Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) under physiologically relevant conditions, using supported lipid bilayers mimicking the composition and structure of the IMM. Our results indicate that DHODH displays higher affinity towards bilayers that incorporate tetraoleoyl cardiolipin (TOCL) compared to bilayers consisting only of phosphatidylcholine (POPC). However, the NR data indicates that the binding between the truncated DHODH and the lipid bilayers studied is weak and reversible, suggesting that the presence of the transmembrane domain might be a prerequisite for stable interaction. We have obtained similar results with bacterial DHODH (originating from *E. coli*), which naturally lacks transmembrane domains.

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How to use lipid models to instruct the design of antimicrobial peptides

Author: Haoning GONG[None]

Co-author: Lu Jian [1]

[1] *Supervisor*

Corresponding Author: j.lu@manchester.ac.uk

Antimicrobial peptides (AMPs) are found in nature to selectively kill microbes by disrupting their cell membranes. Because of the rapid physical action, pathogens are less likely to develop the relative resistance mechanism to the AMPs. Therefore, AMPs are the potential candidates for the multiple-drug resistance (MDR) treatments. We have designed a series of surfactant-like AMPs based on the general formula of G(IIKK)2I-NH2 (denoted as G3). They have strong antimicrobial activity against various strain species including antimicrobial resistant strains, but have relatively mild cytotoxicity to the host mammalian cells.
In this work, we designed a series of de Novo AMPs, and studied their interactions with different lipid models as mimetics of different cell types. Small unilamellar vesicles (SUVs) as composed of specific lipids were used to mimic either the bacterial or the host mammalian cell membranes. The carboxyfluorescein (CF) leakage experiment indicated that AMPs kill bacteria by breaking their cell membranes. Lipid monolayers as constructed on the air/water interface is a simple but efficient model to study the lipid and peptide interactions. Neutron reflection (NR) is a powerful technique to determine the amount and location of the AMPs once bound to the lipid monolayers. The strongly hydrophobic AMP showed potent membrane disrupting activity to either anionic or non-charged SUVs, whilst the weakly hydrophobic AMP disrupted anionic SUVs at higher concentrations and showed litter effect to the electrically neutral SUVs. These results suggest that potent AMPs could be toxic, and weak AMPs have an advantage of benign biocompatibility. All results provide a useful guideline to develop the next generations of AMPs with improved antimicrobial activity but tuned biocompatibility.

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Purification and characterization of perdeuterated lipid mixtures for building biomimetic membranes

Authors: Krishna Batchu[1]; Anne Martel[1]; Yuri Gerelli[1]; Valerie Laux[1]; Juliette Jouhet[2]; Michael Haertlein[1]; Giovanna Fragneto[1]

[1] *Institut laue-langevin*

[2] *CEA*

Corresponding Author: corucci@ill.fr

Deuteration is a useful strategy to modulate the visibility of specific components of the sample using neutron scattering techniques as it offers increased contrast with no significant impact on the physico-chemistry of the membrane. In this context, mass production of biologically relevant deuterated phospholipids of varying head groups and acyl chain compositions is currently the limiting factor in the full exploitation of such experiments. At the Partnership for Soft Condensed Matter (PSCM) in collaboration with ILL's D-lab, we have been investigating methods to biologically produce (*from Pichia pastoris and E.Coli*), further extract and separate the various phospholipid classes thus allowing us to recreate model membranes that could mimic specific cell organelles (*in a healthy or a diseased state*). Thus far, we have had success in being able to separate phospholipid classes that include phosphatidylcholine [dPC], phosphoethanolamine [dPE], phosphatidylserine [dPS], phosphatidylglycerol [dPG] and Cardiolipin [dCL] mixtures. Further, acyl chain compositional determination of such purified fractions was carried out by employing mass-spectrometric analyses. Ultimately, we aim to purify individual molecular species from such complex mixtures by employing reverse-phase chromatographic techniques. All such efforts allow access to well characterized pure deuterated phospholipids for use by the neutron community. Users will then be able to use these lipids to reconstruct model membranes that are more biologically relevant and offer increased contrast.

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Interaction between tethered bilayer membranes and misfolded proteins

Authors: Rima Budvytyte[1]; Vytautas Smirnovas[2]; Evelina Jankaityte [2]; Gintaras Valincius[2]

[1] *Life Sciences Center, University of Vilnius*

[2] *Life Sciences Center, Vilnius University*

Corresponding Author: rima.budvytyte@gmc.vu.lt

A central event in pathogenesis of Alzhaimer's diseases are thought to be intracellular and extracellular accumulation, aggregation and misfolding of low molecular mass peptides such as β-amyloid (Aβ1-42), tau protein (Tau) and s100A9 [1,2, 6]. Small size aggregates-oligomers were found to be extremely neurotoxic in vitro and in vivo with the ability to disrupt the major neuron membranes [3,4] and lead to synaptic dysfunction, mithochondrial dysfunction, neuronal apoptosis and brain damage [5].

In this work different sizes of soluble recombinant s100A9 aggregates were used to investigate their interaction with tethered phospholipid membranes (tBLM). The morphology and size of misfolded protein aggregates were monitored by dynamic light scattering (DLS) and atomic force microscopy (AFM). These protein aggregates exhibited the different membrane damaging properties as probed by the electrochemical impedance spectroscopy (EIS). The function and morphology of s100A9 aggregates were depending on different oligomerisation conditions: temperature and time. The interaction between s100A9 and tBLM was monitored by EIS time series measurements. The observed lag phase of this interaction were significantly decreased at s100A9 aggregates concentration level.

Membrane composition was found to be one of the important factors affecting the interaction of the s100A9 oligomers to phospholipid membranes.

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Location of the general anesthetic n-decane in model membranes

Authors: Pavol Hrubovcak[1]; Bruno Demé[2]; Norbert Kucerka[None]

[1] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna*

[2] *ILL, Grenoble, France*

Corresponding Author: hrubovcak.pavol@gmail.com

The location of n-decane molecules within the model biological membrane consisting of dioleoylphosphocholine (DOPC) bilayers has been investigated via small angle neutron diffraction (SAND) method. Diffraction patterns of the samples containing varied amounts of labeled and unlabeled n-decane have been obtained at various H2O:D2O scattering contrasts and utilized in the reconstruction of their neutron scattering length density (NSLD) profiles. The experimental data analysis based on mutual comparison of contrast varied NSLD profiles revealed unambiguously the location of n-decane molecules within our complex liquid system. They are distributed mostly in the lipid bilayer center while oriented both perpendicular to the bilayer normal, as well as in orientation parallel to the bilayer normal. In this, the mode of n-decane incorporation differs very little for the different concentrations examined (1:1 and 2:1 n-decane:DOPC molar ratios).

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A model of tethered lipid bilayers using anchor-harpoon surfactants on designed electrodes

Author: Ophelie Squillace[None]

Co-author: Guillaume Brotons [1]

[1] *Le Mans Université*

Corresponding Author: squillace.o@gmail.com

Sparsely tethered bilayer membranes (stBLM) provide a particularly advantageous platform to study membrane proteins' functions, ion transport, pores, and therefore to understand fundamental mechanisms, thanks to their stability and amenability of characterization by a wide range of surface-sensitive techniques[1]. As biosensors, they also provide an outreach to diverse biotechnological applications including the development of supported olfactive sensors, of novel antibacterial treatments to reduce drug resistance[2], of organ-on-chips to mimic human in vitro functions[3]. However, maintaining the membrane hydrated, fluidic and close to the substrate without cumbersome chemistry to synthesize and link the "anchor-harpoon"molecules to the substrate[4] can be a challenge. Here, we develop a new experimental approach where a single model phospholipid bilayer is kept fluid and partially tethered to a flat electrode. We proposed an original anchoring surface functionalization that is highly reactive to –OH terminated molecules[5]. In this way we avoid complex organic chemistry and graft commercial Brij non-ionic surfactants chosen for: i) their appropriate hydrophilic chain length that forms an aqueous cushion for the membrane; ii) their hydrophobic alkyl block that anchors the lipid bilayers by insertion in their core. In this way, we keep the membrane fluidity in full immersion and presence of salts. This method appears to be a simple and cheap

way to prepare tethered membranes with tunable anchoring densities on various supporting materials[6]. Electrochemical Impedance Spectroscopy (EIS) has shown that stBLM tethered to Brij58 molecules diluted in PEO9 are well adapted to probe biomimetic or biological membranes under electric fields and the dynamics of surrounding molecules and ions[7]. Using transparent electrodes offers the possibility to simultaneously check the membrane fluidity and lipids dynamics from fluorescence techniques. Functionalized doped silicium enables structural investigations of the stBLM using neutron to shade some light on the role played by the hybrid cushion[8].

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Effect of oxidised lipids on bilayer structure and deposition

Author: Emil Gustafsson[1]

Co-authors: Adrian R. Rennie ; Katherine Thompson ; Maja Hellsing ; Nina-Juliane Steinke ; Tim Bowden

[1] *Uppsala University*

Corresponding Author: emil.gustafsson@physics.uu.se

Lipids are found widely in biological systems because of their unique interfacial properties. They are the primary component of cell membranes, which act as barriers containing the contents of the cells and protecting them from external threats. In fulfilling their function, the lipid membranes are exposed to oxidation processes that change their molecular structure. Such processes occur naturally through the presence of superoxides (O2-) that are released during inflammatory response, or through environmental pollutants. Oxidation can result in the hydrophobic tail region of lipids becoming more hydrophilic. This alters the physical properties of the lipids and the lipid mixture that in turn can affect their biological function. In this study we used a model system to investigate the changes in structure and physical properties that occur when a portion of the lipid in a bilayer is replaced with a lipid containing a damaged tail group, as found following oxidation.

Mixtures of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) that contained a lipid oxidation product, 1-palmitoyl-2-(5'-oxo-valeroyl-sn-glycero-3-phosphocholine (POVPC) have been investigated with a variety of surface and bulk techniques. Neutron reflectivity measured on bilayers revealed an increase in area per headgroup and significantly higher degree of hydration in the tail region when POVPC is present. In contrast, when oxidised lipids with a longer oxidised tail are present, the degree of hydration does not change. Further bilayer characterization with a QCM-D revealed that formation of bilayers containing POVPC is highly sensitive to surface preparation. Surface preparation with basic solutions, which create rough surfaces [1], inhibit vesicle adsorption and prevents bilayers from forming. Light scattering confirms that vesicles used for QCM-D containing POVPC had similar size to those of pure DMPC. Further, the stability of spread monolayers is reduced when POVPC is present.

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Stability of supported lipid bilayers in high adhesive regime

Authors: Pierluigi Bilotto[1]; Maxmilian Lengauer[None]

Co-authors: Jakob Andersson ; Ulrich Ramach ; Laura L.E. Mears ; Markus Valtiner

[1] *Vienna University of Technology*

Corresponding Author: bilotto@iap.tuwien.ac.at

Supported lipid bilayers (SLBs) are often used for investigation in biophysics. They can be a good model system because it is not difficult to compose them at different scales of complexity. In fact, by using mixtures of lipids, or the addition of other proteins, the interactions between those bilayer components can be investigated. Alternatively, polymers can be attached to a defined proportion of the outer layer allowing for a wide range of end functionalities and coverages in order to research particular interactions. The Surface Forces Apparatus (SFA) has been used frequently to understand lipid bilayer interactions primarily under low adhesive loads such as bilayer – bilayer experiments where the stability of the SLB is not in doubt . The aim of this work is to explore how SLBs behave in highly adhesive regimes.
The poster presents how phospholipid bilayers respond in a high adhesive confinement. In particular, we use as a zwitterionic phospholipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and as a positively charged phospholipid 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP). The bilayers are transferred onto the mica substrate by means of a Langmuir Blodgett deposition (LBT) then, to describe the interaction between them and a mica surface; we perform SFA experiments and record topography of the systems utilizing an Atomic Force Microscope (AFM).
Also, we compare thiol based SLBs to the ones discussed previously, focusing on the stability under high adhesion force.
In conclusion, we illustrate the results we obtained allowing us to understand lipid bilayer stability naming the most stable SLB in a highly adhesive regime. Then, using the best model system, we will take a first look at a more complex solid liquid interface.

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Nanomechanical properties of synthetic and natural lipid bilayers through Atomic Force Microscopy (AFM): from Supported Lipid Bilayers (SLBs) to vesicles

Author: Andrea Ridolfi[1]

Co-authors: Marco Brucale [2]; Costanza Montis [1]; Lucrezia Caselli [1]; Lucia Paolini [3]; Paolo Bergese [3]; Debora Berti [1]; Francesco Valle [2]

[1] *Università degli Studi di Firenze*

[2] *Consiglio Nazionale delle Ricerche, Istituto per lo Studio dei Materiali Nanostrutturati (CNR-ISMN), Bologna, Italy*

[3] *Università degli Studi di Brescia*

Corresponding Author: andrea.ridolfi@ismn.cnr.it

Lipid bilayers are self-assembling structures constituting the membranes of every animal cell and many others cell-derived nanoparticles like Extracellular vesicles (EVs). EVs are vesicles with dimensions ranging from 50 to 500nm produced and released by cells to act as cargoes for biological material (proteins, nucleic acids) which play a pivotal role in intercellular communication and in other relevant biological processes. The mechanical properties of EVs regulate fundamental cellular mechanisms like membrane fusion and budding. Their characterization is not trivial, especially due to the many different components that are anchored to or embedded in the lipid matrix, such as proteins, carbohydrates and glycans. Liposomes represent ideal biomembrane models, mimicking their mechanical behavior but featuring a simpler composition. AFM-Force Spectroscopy (AFM-FS)

can be exploited for studying the indentation mechanics of intact individual vesicles and determining their stiffness. Many models in literature relate this parameter to the bending modulus [1,2]; however, none of them seems to work properly for all the phases assumed by lipid bilayers, leaving open the issue. A possible strategy to tackle the problem could be to confine these lipid bilayers on surfaces, realizing SLBs that pattern native lipid membranes in a bidimensional space and still preserve most of their intrinsic mechanical parameters. Moreover, effects like curvature and luminal pressure, which are relevant in vesicles [1], are totally absent in SLBs. In an attempt to rationalize the observed discrepancies, we performed AFM-FS on SLBs obtained from liposomes and EVs and compared these results with the ones on vesicles of similar lipid composition. This parallel approach could provide an experimental strategy for a more robust estimation of the bending modulus of both synthetic and cell-derived lipid vesicles, contributing to address the role of membrane elasticity in biological interactions.

We acknowledge FETOPEN-801367 evFOUNDRY.

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Understanding properties of bilayers from a study of solid surfactants

Author: Jeremy K Cockcroft[1]

Co-authors: André Shamsabadi [1]; Han Wu [1]; Adrian R Rennie [2]

[1] *University College London*

[2] *Uppsala University*

Corresponding Author: jeremykarl@gmail.com

It has been known for a long time that adsorbed bilayers of *n*-alkyl trimethylammonium bromide surfactants, C_{*n*}TAB, at aqueous solution/hydrophilic solid interfaces are thin, much less than two extended hydrocarbon chains. These materials are bactericidal but the mechanism is not well-understood although differences in behaviour with chain length are established. For these reasons understanding factors that control their properties is important. We have studied the bulk phases of C₁₀TAB to C₁₈TAB systematically over a wide range of temperatures using X-ray diffraction and calorimetry. Small crystals can now be investigated routinely using modern laboratory equipment.[1] Common features in the structures are identified, with packing dominated by the co-ordination of the cationic head groups with bromide ions and interdigitation of the hydrocarbon chains. This arrangement provides an explanation for the thin adsorbed layers observed previously.[2] Molecular volumes and arrangement are comparable with structures of a number of different self-assembled amphiphiles. The alkyl chains are highly mobile and at high temperatures, a plastic phase is found for all materials with a transition enthalpy that is similar to the melting enthalpy of many long alkyl chains. The phase behaviour evidently depends on delicate balances of the various contributions to free energy. It is likely that the assembly and mobility of such surfactants is important in membrane penetration. Similar studies on other materials such as phospholipids are likely to yield further interesting results.
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Interaction of cellulose nanocrystals with lipid bilayers

Author: Yotam Navon[1]

Co-authors: Bruno Jean [2]; Anne Bernheim [3]; Laurent Heux [2]

[1] *Cermav, UGA, BGU*

[2] *Cermav*

[3] *Ben Gurion University of the Negev*

Corresponding Author: yotam.navon@cermav.cnrs.fr

Using elementary building blocks to mimic and reconstruct biological structures is intriguing both from fundamental aspects, providing a simple model to study a complex environment, as well as from the applicative point of view, opening the possibility of utilizing such constructs for the creation of new functional materials. Understanding the key parameters governing the interaction between the building blocks of such systems is highly important.
In the frame work of bio-inspired materials, the plant cell wall provides an interesting platform, since its basic components are abundant, eco-friendly and possess outstanding properties. In plant kingdom, one of the first steps of cell wall constitution is the deposition of cellulose microfibrils on top of the plant plasma membrane.[1] In model systems, cellulose nano crystals (CNCs), prepared by acid hydrolysis of the natural fiber, are an attractive building block since they possess properties similar to the native fiber and have excellent colloidal stability.[2]
In this work we have investigated the interaction between CNCs and lipid membranes using 2D and 3D architectures. Quartz crystal microbalance with dissipation, total internal reflection fluorescence microscope, atomic force microscopy and neutron reflectometry were used for the investigation of the 2D system, in which CNCs were deposited on top of supported lipid membranes (SLBs). The interaction between lipid vesicles and CNCs was studied in suspension using isothermal titration calorimetry, light scattering and transmission electron microscopy.[3] Key parameters governing the interaction were elucidated and the results are discussed in the context of plant cell wall inspired materials.

Acknowledgments: Jean-Luc Putaux, Lilianne Guerente, Franck Dahlem, Giovanna Fragneto, Yuri gerelli

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Interactions of model membranes with surfactants and antimicrobial peptides studied by small-angle neutron diffraction.

Authors: Katarína Želinská[1]; Jana Gallová[2]; Bruno Demé[3]

[1] *ILL, Grenoble, France & Department of physical chemistry of drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia*

[2] *Department of physical chemistry of drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia*

[3] *ILL, Grenoble, France*

Corresponding Author: zelinska@ill.fr

Due to alarming increase in the number of cases of antibiotic-resistant bacterial infections, it is necessary to develop new antibiotics, explore relationships between structure and observed effect and study the mechanism of antimicrobial action. Amphipathic antimicrobial peptides are perspective compounds which display antimicrobial effects. Dermaseptins represent a large group of cationic peptides exhibiting membrane damaging effect. [1] N,N-dimethyl-1-dodecanamine-N-oxide (DDAO)

is an amphiphilic surfactant possessing antimicrobial activity. [2] Moreover, DDAO displays phytotoxic, antiphotosynthetic and immunomodulatory activity and it can play an important role in designing lipid based systems for drug and DNA delivery. Antimicrobial effect is a required attribute of new potential antibiotic drug, but what is even more necessary is its specific selectivity against bacterial membranes, leaving mammalian membranes unaffected. Therefore, both the bacterial membrane mimicking model and mammalian membrane model were studied. Small-angle diffraction experiments were performed at the D16 instrument. Oriented stacks of bilayers with various amount of dermaseptin S1 and DDAO were hydrated from vapour using the dedicated chambers of D16 with humidity controlled at 97% and the data was collected from series of rocking curves (using a rotating sample with 2D detector at fixed position). To vary the scattering contrast between the lipid bilayers and water, measurements were repeated for samples with diverse D2O/H2O mixtures (e.g., D2O volume fractions of 8, 40, 70, 100%). The contrast variation approach allowed us to perform the Fourier reconstruction of the scattering length density profiles.

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This work has been supported by the VEGA grants 1/0916/16 and 1/0228/17, JINR topical themes 04-4-1121-2015/2020 and APVV project 17-0239.

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Aescin-induced conversion of gel-phase lipid membranes into bicelle-like lipid nanoparticles

Author: Ramsia Geisler[1]

Co-authors: Lise Arleth [2]; Martin Cramer Pedersen ; Rajeev Dattani ; Ralf Schweins ; Sylvain Prevost [3]; Thomas Hellweg [4]

[1] *TU Darmstadt*

[2] *Niels Bohr Institute, University of Copenhagen*

[3] *Institut Laue-Langevin*

[4] *Universität Bielefeld, PCIII*

Corresponding Author: ramsia.geisler@gmail.com

Mixtures of the phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and the saponin beta-aescin spontaneously form monodisperse, bilayered discoidal micelles (also known as “bicelles” or “nanodisks”) in aqueous solution. Such bicelles form below the melting temperature of DMPC when the phospholipids are in the rigid state and are precursors of spontaneously formed vesicles. The aescin concentration must be far above the cmc (cmc(aescin) = 0.3-0.4 mM). It was found that the shape and size of the bicelles are tunable by composition. High amounts of aescin decrease the size of the bicelles from diameters of aroun 320 Å at 7 mol% to around 150 Å at 30 mol% beta-aescin. The structures are scrutinized
by complementary small-angle X-ray (SAXS) and neutron (SANS) scattering
experiments. The scattering curves are subsequently analyzed by model-independent
(IFT analysis) and a model based approach where bicelles are described as polydisperse bilayer disks encircled by a beta-aescin rim. Moreover, the monomodal distribution and low polydispersity of the samples were confirmed by photon correlation spectroscopy (PCS). The discoidal structures were visualized by transmission electron microscopy (TEM).

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Metallacarboranes as non-amphiphilic detergents for lipid membrane solubilization

Authors: Damien Rault[1]; Marie-Thérése Paternostre[2]; Franck Artzner[3]; Olivier Diat[4]; Pierre Bauduin[4]

[1] *ICSM - CEA*

[2] *I2BC - CNRS*

[3] *Institute of Physcis - Univesrity of Rennes*

[4] *ICSM CEA*

Corresponding Author: pierre.bauduin@cea.fr

Metallacarboranes are a unique class of inorganic polyhedral clusters containing carbon, boron, hydrogen, and metal atoms. A typical metallacarborane is the nanometric size anion [COSAN]-: a Co3+ metal ion sandwiched between two [C2B9H11]2– (dicarbollide) clusters. In the last ten years we have shown that COSAN and its derivatives have all the properties of classical surfactants (surface activity, foaming, self-assembly in micelles/vesicles and lamellar lyotropic phase formation in water) although COSAN do not have any amphiphilic topology, i.e. a hydrophilic-hydrophobic sequence in their chemical structure. In the present contribution we show that COSAN derivatives are also able to fully solubilize (neutral) model artificial bilayers made of DOPC. SAXS has reavealed that the solubilization of DOPC by COSANs takes place with a continuous evolution in the shape of the DOPC/COSAN aggregates formed. The phase sequence which is obtained follows the evolution of structures with increased curvature from multilayer vesicles, to disc (comparable to bicelles), to elongated rods, to short rods, to core-shell spherical micelles (i.e. DOPC micelles decorated by COSANs) to COSAN micelle containing molecularly dissolved DOPC. In contrast to the classical solubilization process by surfactants (or detergents), which takes place by anchoring of their alkyl chains in the core of bilayers, COSAN derivatives show a different solubilization mecanism first by adsorbing at the bilayer surface and second by strongly disturbing the lipid packing which dramatically increases the curvature. The present results open new opportunities in solubilization and in the general understanding of the biological activity of COSANs and the underlying intermolecular forces involved.

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Nanoscale lipid models to study membrane proteins

Author: Madhumalar Subramanian[None]

Co-authors: Katarina Iric [1]; Karim Fahmy [2]; Thorsten Schmidt [3]; Jana Oertel [2]

[1] *Helmholtz-Zentrum Dresden-Rossendor*

[2] *Helmholtz-Zentrum Dresden-Rossendorf*

[3] *Technical University Dresden*

Corresponding Author: m.subramanian@hzdr.de

Developments in the field of X-ray diffraction, such as XFEL (X-ray Free Electron Laser) has become advantageous in studying Membrane Protein's structure and dynamics. For in-vitro studies, membrane protein needs to be reconstituted in a more native like hydrophobic environment. A novel membrane model system, Nanodisc due to its defined size and low mass-ratio of lipid to protein, proves to be a suitable candidate for XFEL studies. Nanodiscs are nano-sized lipid bilayer patches held intact with the help of a scaffold protein, MSP (Membrane Scaffold Protein). Engineering this protein will help in creating higher order structures, such as disuphide linked oligomers of Nanodiscs in the case of cysteine mutagenesis. This approach of engineering the protein to create well-ordered oligomeric structures will help in avoiding the normal crystallisation process.
Another recent development in the area of membrane model system is the DEBs (DNA Encircled Lipid Bilayer), where the MSP scaffold in Nanodisc is replaced by alkylated DNA, the alkylation provides enough hydrophobicity to accommodate a lipid patch. DNA being a versatile tool for nanotechnological application, DEB system with further development and characterization will help in enhancing the studies in Membrane protein.

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Functionalized membrane domains: an ancestral feature of Archaea

Author: Maxime Tourte[1]

Co-authors: Marta Salvador Castell [1]; Philippe Schaeffer [2]; Vincent Grossi [3]; Philippe Oger [1]

[1] *Microbiology, Adaptation and Pathogeny Lab CNRS UMR5240 Université de Lyon*

[2] *Institut de Chimie de Strasbourg CNRS UMR 7177 Université de Strasbourg, France*

[3] *Laboratoire de Géologie de Lyon, Terre, Planètes, Environnement CNRS UMR 5176 Université de Lyon, France*

Corresponding Author: maxime.tourte@univ-lyon1.fr

The current tree of life is divided into three domains: Bacteria, Eukarya and Archaea. While bacterial and eukaryotic lipids are constituted of sn-3 glycerol on which straight fatty acids are ester-bound, archaeal lipids contain ramified isoprenoid hydrocarbons that are linked through ether bounds on a sn-1 glycerol. As a results, plasma membranes are spatially organized into domains of distinct functions in Bacteria and Eukarya, but membrane functionalization remains a debated question in Archaea. However, the recent reinvestigation of the lipid content of the hyperthermophilic and piezophilic archaeon *Thermococcus barophilus* led to the elaboration of a novel membrane ultrastructure in which monolayered and apolar hydrocarbon-containing bilayered domains may be delineated[1]. To estimate the ubiquity of this novel membrane ultrastructure in and out of the order Thermococcales and all the organizational possibilities it implies, we reassessed the lipid composition of all the Thermococcales type species and all archaea. We show that almost all archaea can synthesize di- and tetraether lipids, which reflect their lifestyle more than their phylogeny and support the universal existence of a functionalized membrane in Archaea. Our findings establish functionalized membrane domains as a universal feature of Archaea, and thus of all living organisms, opening new avenues to understand the membrane physiology and adaptation since the origin of cellular life.

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Wine & cheese poster session / 47

Advances in purification of perdeuterated lipid mixtures for building biomimetic membranes

Authors: Krishna Batchu[1]; Anne Martel[1]; Yuri Gerelli[1]; Valerie Laux[1]; Juliette Jouhet[2]; Michael Haertlein[1]; Giovanna Fragneto[1]

[1] *Institut laue-langevin*

[2] *CEA*

Corresponding Author: fragneto@ill.fr

Deuteration is a useful strategy to modulate the visibility of specific components of the sample using neutron scattering techniques as it offers increased contrast with no significant impact on the physico-chemistry of the membrane. In this context, mass production of biologically relevant deuterated phospholipids of varying head groups and acyl chain compositions is currently the limiting factor in the full exploitation of such experiments. At the Partnership for Soft Condensed Matter (PSCM) in collaboration with ILL's D-lab, we have been investigating methods to biologically produce (*from Pichia pastoris and E.Coli*), further extract and separate the various phospholipid classes thus allowing us to recreate model membranes that could mimic specific cell organelles (*in a healthy or a diseased state*). Thus far, we have had success in being able to separate phospholipid classes that

include phosphatidylcholine [dPC], phosphoethanolamine [dPE], phosphatidylserine [dPS], phosphatidylglycerol [dPG] and Cardiolipin [dCL] mixtures. Further, acyl chain compositional determination of such purified fractions was carried out by employing mass-spectrometric analyses. Ultimately, we aim to purify individual molecular species from such complex mixtures by employing reverse-phase chromatographic techniques. All such efforts allow access to well characterized pure deuterated phospholipids for use by the neutron community. Users will then be able to use these lipids to reconstruct model membranes that are more biologically relevant and offer increased contrast.

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Active fluctuations in model membranes

Author: Tetiana Mukhina[1]

[1] *ILL*

Corresponding Author: mukhinat@ill.fr

Membranes exhibit thermal fluctuations, but transmembrane protein activity breaks the fluctuation-dissipation theorem leading to out-of-equilibrium fluctuations. Active fluctuations have been widely described theoretically [1], but to a lesser extent experimentally.
We will present our recent results on the investigation of out-of-equilibrium fluctuations of phospholipid membranes induced by transmembrane protein activity. Transmembrane protein Bacteriorhodopsin (BR) was used as a light-driven proton pump, which activity was triggered by visible light to induce active membrane fluctuations. In this context, model systems such as solid-supported single and floating phospholipid bilayers were used for the protein reconstitution studies and to investigate phospholipid membranes and their interactions.
A detergent-mediated incorporation method [2] was adapted to perform the BR reconstitution into the phospholipid bilayer at the interfaces.
The combination of neutron reflectometry, QCM-D, fluorescence microscopy and AFM allow us to develop the robust protocol of BR reconstitution and to demonstrate that it is possible to insert BR in model bilayer systems without losing their structural integrity. An activity of the incorporated proteins through its effect on the structure and on the fluctuations of a double bilayer system and the reversible effect of light illumination on BR activity was demonstrated by specular and off-specular synchrotron radiation reflectometry experiments.
The preliminary analysis of the synchrotron measurements is consistent with the magnification of membrane shape fluctuations induced by BR activity. The obtained results open the way to investigate, for the first time, the fluctuation spectrum of a planar membrane-protein system at the nanoscale and to access the physical properties of the system such as bending modulus, surface tension and interaction potential between adjacent membranes.

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Wine & cheese poster session / **58**

Transmigration of non-ionic synthetic polymers through lipid membranes

Authors: Ekaterina Kostyurina[None]; Jürgen Allgaier[1]; Ralf Biehl[None]; Margarita Kruteva[None]; Stephan Förster[2]

[1] *JCNS*

[2] *Forschungszentrum Jülich*

Corresponding Author: e.kostyurina@fz-juelich.de

Polymers with balanced hydrophilicity can translocate through biological membranes without doing damage. In the case of synthetic polymers there are only few reports of translocation using charged polymers [1-2]. For non-charged polymers translocation phenomena were predicted theoretically [3] but not verified experimentally.
We have synthesized such balanced, alternating hydrophobic/hydrophilic polymers and studied their translocation properties as well as the interactions with lipid membranes using Pulsed Field Gradient (PFG) NMR, QCM-D and scattering techniques.
The investigated polymers contain polyethylene glycol (PEG) as a hydrophilic part and dicarboxylic acids as the hydrophobic one. As a model cell membrane we used unilamellar liposomes of different lipids (DOPC, POPC, DMPC).
Translocation properties were measured by PFG NMR and analyzed by a two-phase diffusion model [5]. Two dynamical phases of the polymers were observed in their mixture with liposomes (Fig. 1). One phase is attributed to the free polymers and the other one characterizes their interaction with liposomes. The time dependence of the ratio between these two phases indicates the translocation process. The exchange process was observed in the time range from 50 to 900 ms.
Using the two-phase diffusion model, the dependence of translocation time on the polymer molecular weight and liposome composition was studied. The calculations show a slight increase of the exchange time with increasing of polymer molecular weight and strong dependence of the exchange time on thickness of lipid bilayer.
Currently neutron scattering experiments are in progress to understand the mechanism of polymer translocation through the membrane. First SANS results under different contrast conditions indicate distinct interactions between the polymers and the lipid membrane.
Because of the non-ionic nature of the polymers only small interactions with biological compounds are expected and the materials might be interesting candidates for drug delivery and other applications.

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Structural and functional characterization of a complex involved in lipid transport to mitochondria during plant adaptation to phosphate starvation

Author: Sébastien Leterme[1]

Co-authors: Catherine Albrieux [1]; Juliette Jouhet [2]; Morgane Michaud [1]

[1] *Laboratoire Physiologie Cellulaire & Végétale*

[2] *Laboratoire de Physiologie Cellulaire et Végétale*

Corresponding Author: letermesebastien@gmail.com

Phosphate starvation is a frequent nutrient stress encountered by plants to which they adapt by exerting different mechanisms. The partial degradation of phospholipids, a common constituent of cellular membranes, is a widespread response observed in plants to increase the intracellular phosphate availability. To maintain membrane integrity, the degraded phospholipids have to be replaced by a non-phosphorous plastid-synthetized lipid, the galactoglycerolipid digalactosyldiacylglycerol (DGDG). This replacement implies a lipid transport by an unknown mechanism from the plastid to other organelles. A large set of studies has revealed that lipid transport between intracellular membranes can effectively take place at membrane contact sites (MCS) where it is enabled by specific proteins. Recently, the mitochondrial transmembrane lipoprotein (MTL) complex, a huge complex enriched in lipids, has been identified and one of its components, AtMic60, was shown to play a role

in DGDG trafficking to mitochondria. We hypothesize that this complex plays a major role in lipid transport between the chloroplast and the mitochondria at specific MCSs in response to phosphate starvation. However, the transport mechanism used by the MTL complex as well as its structure and its functions remain unknown. To dissect the role of the MTL complex in lipid transport and plant adaptation to phosphate starvation, its composition and structure will be characterized by a combination of innovative multi-disciplinary approaches including co-immunoprecipitations, proximity dependent biotin identification and single particle cryo-electron microscopy. Further investigation by reverse genetics and lipidomic analyses in phosphate deplete and replete conditions will enable to dissect the cellular function of the MTL complex and its role in plant adaptation to phosphate starvation. The results obtained will not only shed light on a huge gap of fundamental knowledge in the field of membrane biogenesis and lipid transport between organelles but will also pave the way for agricultural improvement in plant phosphate usage.

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NhaA protein incorporation in tethered lipid bilayer membranes studied by neutron reflectometry and impedance spectroscopy

Author: Sebastian Koehler[1]

Co-authors: Marco Maccarini [2]; Giovanna Fragneto [1]; Donald Martin [2]

[1] *ILL*

[2] *Université Grenoble Alpes*

Corresponding Author: koehler@ill.fr

The study of active membrane proteins requires an environment which is as close as possible to their natural environment to retain protein function, while at the same time keeping the system as simple as possible to allow for an experimental characterization and to be able to identify factors which influence the system. Tethered lipid bilayers (tBLMs) represent an experimentally accessible and stable model for biological membranes that offers a high level of control over the structure and can form a more natural environment for membrane protein incorporation than the widely used solid supported bilayers [1]. We report the use of a tBLM system to investigate how the structural factors of the surrounding membrane influence the incorporation and subsequently the activity of the NhaA protein, which is the main sodium proton antiporter of Escherichia coli. NhaA serves as the means for E. coli to maintain sodium homeostasis and for pH control [2]. Here we present a combined neutron reflectometry (NR) and electrochemical impedance spectroscopy (EIS) study on the incorporation of NhaA into PEG-tBLMs on gold surfaces.
We describe how lipid composition and incorporation methods affect the resulting tBLM-protein system. NR allowed us to determine the nanostructure of the membrane-protein system to monitor bilayer dimensions, completeness and to precisely determine the amount of incorporated NhaA protein. EIS provided functional characteristics like electrophysiological properties related mainly to ion permeability and indicated defects induced by protein incorporation.
The combination of these two methods enables us to correlate structural and functional information of the NhaA-membrane system in order to understand the mechanisms behind these dependencies.
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Wine & cheese poster session / **63**

Calixarene ”flowers” blossoming captured with cryoTEM imaging

Author: Nadia Lengweiler (Opara)[1]

Co-authors: Mina Moradi [2]; Patrick Shahgaldian [2]; Henning Stahlberg [3]

[1] *C-CINA, Biozentrum, University of Basel*

[2] *School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland*

[3] *C-CINA, Biozentrum, University of Basel*

Corresponding Author: nadia.opara@gmail.com

We are presenting transmission electron microscopy (TEM) imaging as an advanced tool for characterization freestanding crystalline metalorganic networks (MONs) [1]. As an example of creation of ultrathin material, the two-dimensional (2D) layer of the monomolecular thickness amphiphilic derivatives of calix[4]arenes are selected [1, 2]. The methyl-carboxy-functionalized calix[4]arenes can undergo mono to bilayer transition from nucleated and gradually grown second layer islands. The TEM imaging performed in the low dose mode and at cryo-conditions allows for obtaining micrographs capturing observation of this process on freestanding membranes suspended over lacey-carbon-covered copper grids.

The financial support of the Swiss Nanoscience Institute through the ORACLE grant P1308 and project P1305 is gratefully acknowledged.

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Interdomain flexibility within NADPH oxidase revealed by SANS using LMNG stealth-carrier

Authors: Christine Ebel[1]; Annelise Vermot[1]; Isabelle Petit-Härtlein[1]; Cécile Breyton[1]; Aline Le Roy[1]; Michel Thépaut[1]; Corinne Vives[1]; Martine Moulin[2]; Michaël Härtlein[2]; Serguei Grudinin[3]; Susan M. E. Smith[4]; Anne Martel[2]; Franck Fieschi[1]

[1] *IBS*

[2] *ILL*

[3] *INRIA*

[4] *Kennesaw State University*

Corresponding Author: christine.ebel@ibs.fr

Membrane protein solubilization implies the use of amphipathic detergents that form protective belts around hydrophobic patches to maintain the protein’s structural integrity outside of the lipid environment. Consequently, the resulting SANS signal includes both protein and detergent belt signatures leading to a global outer shell larger than the envelope expected for the protein alone; masking the detergent signal represents an important issue. Moreover, specific detergent characteristics can disrupt the proper folding of the membrane protein, greatly impacting the structural parameters of the SANS envelope and thus necessitating significant effort in the preparation of an optimized sample for SANS. A strategy to overcome these specific issues was tested using the LMNG detergent, recently characterized as a strongly stabilizing detergent. We studied the membrane protein SpNox, a prokaryotic homolog of NADPH oxidase originally identified in phagocytic cells where it produces Reactive Oxygen Species. Detergent screening confirmed the improved thermostability of SpNox and limited aggregation when solubilized in LMNG. Through contrast variation experiments, we were able cancel the LMNG contribution to the SANS signal, and we determined the percentage of D20 corresponding to the buffer matching point. Then, to improve protein contrast, we produced deuterated SpNOX and subsequently solubilized and purified it in LMNG. Finally, since SANS studies are very sensitive to the presence of aggregates or oligomers in the sample, the development of a

new in-situ size exclusion chromatography (SEC) system on a SANS instrument enabled data collection on line from an aggregation-free homogeneous sample. This strategy led to the determination of a low-resolution envelope of SpNox confirming the monomeric state of the protein in solution. Parallel to the structural study, a homology model of SpNox was developed; the model was successfully docked in the SANS envelope. The general strategy may be applied to many other membrane proteins in the future.

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AMP monomers or aggregates - Which is more effective on antimicrobial: an insight from coarse-grained simulations and experiments

Authors: Mingrui Liao[None]; Haoning GONG[None]

Corresponding Author: mingrui.liao@postgrad.manchester.ac.uk

Over the past few decades, extensive studies have been devoted to the use of peptides as antimicrobials, with a focus on rationally designed short peptides composed of a sequence shorter than 20 amino acids, due to their relatively low synthesis costs and ease of manipulation1,2. A more complete understanding of how these short cationic antimicrobial peptides brings about bacterial cell death is needed to further their optimization and development for practical applications.
Several synthetic antimicrobial peptides (AMPs), including G(IIKK)3I (G3), G(IIKK)4I (G4), GLLDLLKLLLKAAG□LDKA□and naturally existing Melittin were studied using both molecular dynamics simulations in MARTINI coarse-grained models and experimental approaches. Among them, G3, G4 were shown to have better membrane selectivity than Melittin and LDKA. Meanwhile, their aggregate behaviours in the bulk solution also effect their interaction modes with lipid bilayers in some degree.
Among them, melittin shows a “detergent-like” action to disrupt DPPG membrane, while LDKA self-assembles to form long fibres which become inserted into membranes by means of a non-pore carpet mechanism. Simulations revealed that G3 molecules are widely distributed on the DPPG bilayer in single or oligomer states, divide the whole outer membrane into small parts, but do not penetrate through the bilayer in the timescale of modelling. On the other hand, G4 molecules interact with the DPPG bilayer in a manner similar to melittin, with G4 nano-assemblies disrupting the lipid membrane in a “detergent-like” mode. LDKA are more hydrophobic with less net positive charges than G3 and G4. As a result, they have more preference to self-assemble on the DPPG bilayer but they interact with the membrane differently: LDKA’s fibre aggregation can even roll up lipid micelle from DPPG bilayer under a high peptide concentration. These observations provide a rich information base for devising systematic studies in future work.

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Wine & cheese poster session / **75**

A neutron reflectometry approach to investigate factors regulating the substrate specificity of the mitochondrial phospholipase - iPLA2-gamma

Author: Giacomo Corucci[1]

Co-authors: Krishna Chaithanya Batchu [1]; Robert Jacobs [2]; Giovanna Fragneto [1]

[1] *ILL*

[2] *Oxford University*

Corresponding Author: corucci@ill.fr

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Session C / **1**

Shedding light into membrane receptor functioning and lipid interactions by plasmon waveguide resonance

Author: Isabel Alves[None]

Corresponding Author: i.alves@cbmn.u-bordeaux.fr

G-protein coupled receptors (GPCRs) are a large receptor protein family that sense molecules outside the cell and activate intracellular signal transduction pathways and modulate cellular responses. Since they are activated by extracellular stimuli of varied size and nature such as light, odors, hormones, and neurotransmitters, these receptors are extremely important therapeutic targets. Over the last decades, the comprehension of their activation mechanism provided by the growing number of high resolution structures has allowed significant advances. However, key aspects in the functioning of these proteins remain obscure, especially with respect to the role of the membrane lipid environment in the activation and signaling events. This can be in part explained by the lack of approaches that allow to follow such processes in a very sensitive and direct manner. We have developed an innovative technique named plasmon waveguide resonance (PWR) that is ideal to study thin anisotropic films such as proteolipid membranes[1].
PWR can be applied both to the study of GPCRs in their native cell membrane (whose membrane lipid composition can be altered) and in reconstituted model lipid systems of controlled lipid composition. Recent studies on the chemokine receptors CCR5 and CXCR3 will be presented concerning: 1) direct monitoring of CCR5 reconstitution in lipid model membranes and the role of cholesterol in receptor/ligand interaction[2]; 2) the complexity of CXCR3 pharmacology in the context of cancer development[3].
Additionally the impact of polyunsaturated fatty acids in Dopamine D2 receptor activation, in the context of lipid dysfunctions observed in psychiatric disorders will be discussed.

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Session C / **77**

Probing the internal structure of nanoparticles for mRNA delivery

Author: Aleksandra Dabkowska[1]

Co-authors: Marianna Yanez Arteta [1]; Lennart Lindfors [1]

[1] *Advanced Drug Delivery, Pharmaceutical Sciences, R&D, AstraZeneca, Gothenburg, Sweden*

Corresponding Author: aleksandra.dabkowska@astrazeneca.com

New therapeutic modalities, such as RNA-based drugs, have shown promising results in treating diseases that are currently difficult to tackle with standard small molecule drugs. One type of RNA therapeutic, mRNA, is especially promising due to its ability to induce protein production in target cells, where it can replace damaged or missing proteins. However, clinical progress is often limited by the mRNA molecule's innate properties: a large size, hundreds of negative charges and a propensity for rapid degradation in serum. Hence, successful application usually requires an advanced delivery system. Lipid nanoparticles (LNPs) are among the most advanced delivery vehicles for mRNA. Physico-chemical characterization of mRNA-containing LNPs reveal a structured core enveloped by a defined outer shell. By varying LNP size and surface composition we demonstrated that both size and structure have significant influence on intracellular protein production. To design better LNPs for improved therapies, we seek to understand how specific components affect the structure of these nanoparticles as well as their function.

Session C / **24**

DNA-tagged lipid bilayers: novel nano-scaled membrane-mimetic systems

Authors: Madhumalar Subramanian[1]; Jana Oertel[2]; Karim Fahmy[2]

[1] *Helmholtz-Zentrum Dresden Rossendorf*

[2] *Helmholtz-Zentrum Dresden-Rossendorf*

Corresponding Author: k.fahmy@hzdr.de

Lipid bilayers and lipid-associated proteins play a crucial role in biology. Since studies and manipulation in vivo are inherently challenging, several in vitro membrane-mimetic systems have been developed to enable the study of lipidic phases, lipid-protein interactions and membrane protein function. Controlling the size and shape or introducing functional elements in a programmable way is, however, difficult to achieve with common systems based on polymers, peptides or membrane scaffolding proteins. We have combined DNA-nanotechnology with lipid bilayer self-assembly to create DNA-encircled bilayers (DEBs) as a novel nano-scaled membrane-mimetic. For this, alkylated oligonucleotides were hybridized to a single-stranded minicircle (ssMC) to provide an inner hydrophic surface for lipid attachment. DEBs open new routes to membrane biophysical studies, enabling improved size control, stability and programmability[1]. Here, we present further developments of DNA-associated lipid bilayers for the reconstitution of membrane proteins and for the generation of self-assembled higher order structures. The latter may be ultimately used for the structural biology of membrane proteins in Cryo-EM or in diffraction experiments using advanced X-ray sources, such as synchrotrons or XFELs.

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Session C / **19**

A multi-technique approach for the characterization of the self-assembling of cyclic peptides into nanotubes at biological model membranes

Author: Barbara Claro[1]

Co-authors: Eva Freire [2]; Juan Granja [2]; Erik Goormaghtigh [3]; Tiago Ferreira [4]; Rebeca Garcia-Fandiño [2]; Margarida Bastos [1]

[1] *CIQ-UP Dep. Chemistry & Biochemistry, FCUP, Porto, Portugal*

[2] *CIQUS Singular Research Centre in Chemical Biology and Molecular Materials, USC, Santiago de Compostela, Spain*

[3] *3 Université Libre de Bruxelles, Structure and Function of Biological Membranes, Center for Structural Biology and Bioinformatics, Bruxelles, Belgium*

[4] *NMR – Institute for Physics, Martin-Luther University Halle-Wittenberg, Germany*

Corresponding Author: barbaramariaclaro@gmail.com

Bacterial resistance is presently a major public health concern, due to excessive and misuse of antibiotics. This has stressed the research on new antibiotics with new mechanisms of action [1]. Antimicrobial peptides are part of our innate immune system and represent a new antibiotic paradigm, as they aim the bacterial membrane, have been studied in the past decade [2]. Within this research effort, a new class of potential antimicrobial peptides has emerged [3] - Cyclic Peptides (CP) with an even number of alternating D and L-α-amino acids that assume a planar conformation and form the active species, Self-assembling Cyclic Peptide Nanotubes (SCPNs), when in contact with bacterial membranes. We used different biophysical experimental techniques (DSC, Fluorescence, solid state NMR, ATR-FTIR) together with an in-silico approach to characterize the interaction of these antimicrobial SCPNs with different model membranes, aiming ultimately at unveiling their possible mechanism of action.
Results from these techniques will be shown and compared, allowing us to characterize the formation and orientation of SCPNs at different membranes, and to discriminate the most important factors ruling these peptides/membrane interactions.

Acknowledgement: FCT, Portugal UID/QUI/0081/2013, IF/01133/2015, POCI-01-0145-FEDER-030579, PD/BD/135095/2017, and FEDER (COMPETE 2020) for Norte-01-0145-FEDER-000028
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Session D / **33**

The rich phenomenology of glycolipids and membrane-anchored polysaccharides - Insights from scattering techniques and complementary computer simulations

Author: Emanuel Schneck[1]

[1] *TU Darmstadt*

Corresponding Author: schneck@fkp.tu-darmstadt.de

Biological membranes often contain considerable amounts of glycolipids or membrane-anchored polysaccharides. Both can strongly influence the membrane characteristics in terms of their interactions with ions [1] and molecular components of the aqueous medium [2], their interactions with adjacent membranes [2, 3], and their in-plane organization [1], among others. We use various scattering techniques with x-rays and neutrons and complementary computer simulations to elucidate these phenomena on the molecular level.

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Session D / **8**

Using suspended bilayers as a novel bacterial membrane mimetic for investigating mechanosensitive ion channels with neutron reflectivity

Author: Simon Titmuss[1]

Co-authors: Sophie Ayscough [1]; Maximilian Skoda [2]

[1] *University of Edinburgh*

[2] *STFC*

Corresponding Author: simon.titmuss@ed.ac.uk

In Gram negative bacteria, the inner membrane is suspended from a thin peptidoglycan layer and contains embedded membrane proteins. This composite layer controls the shape of the cell and the flux of materials into and out of the cell. Mechanosensitive ion channels act as safety valves, protecting bacteria from osmotic shock, by opening when the membrane is subject to a stress. In addition to osmotic shock, this stress can be triggered by insertion of amphipathic molecules. We are investigating whether these alternative triggers could make mechanosensitive ion channels an Achilles heel to bacteria. Specifically we are using neutron reflectivity and small angle scattering to investigate whether the insertion of the antimicrobial molecules lyso-PC and pexiganan (an amphipathic antimicrobial peptide) into POPC/POPG bilayers triggers prolonged opening of mechanosensitive ion channels of large conductance (MscL). Such channel opening could explain the antimicrobial behaviour of these molecules, without the requirement for them to induce pore formation in the membrane. In addition to using solid-supported, tethered POPC/POPG bilayers, we have developed a novel mimetic in which the POPC/POPG bilayer is suspended beneath a DODAB monolayer at the air/water interface. In this approach the bilayer is formed by vesicle rupture of either liposomes or proteoliposomes. In the latter case, the ion channels are incorporated into the proteoliposomes by cell free protein expression. In addition to mimicking the manner in which the inner cell membrane is suspended from a peptidoglycan layer in a bacterial cell, the approach offers some advantages over solid-supported mimetics such as floating bilayers. The implementation of this approach, the characterization of the resulting lipid-only and protein-containing lipid bilayers using neutron reflectivity, as well as what has been learned about the way these layers repsond to challenge by lyso-PC and pexiganan, will be described.

Session D / **20**

Mucin thin layers on top of model membranes as a model environment for mucosal delivery

Author: Valeria Rondelli[1]

Co-authors: Emanuela di Cola [1]; Alexandros Koutsioubas [2]; Paola Brocca [1]

[1] *Università degli Studi di Milano*

[2] *Forschungszentrum Juelich GmbH*

Corresponding Author: valeria.rondelli@unimi.it

Mucus is a highly viscoelastic secretion, covering the epithelia surfaces of the gastrointestinal, pulmonary, oral, nasal and genital tracts. Its function and composition differs at different locations of our body, but the general task of mucus is to protect mucosal tissues from dehydration, mechanical stress, and to act as barrier against microorganisms and toxic substances. Mucus is mainly composed of water (90%), lipids, small proteins and nucleic acids, but its mechanical and viscoelastic properties are due to the presence of high molecular weight glycoproteins, identified as mucin. Mucin can establish adhesive interactions with particulates via electrostatic interactions, van der Waals forces, hydrophobic forces, hydrogen bonding, or chain entanglement. Therefore, the development of mucosal drug delivery vehicles is a great challenge because little is still known about the interactions between mucin and other macromolecules: they can either penetrate rapidly or establish prolonged

contact with mucus, depending on their specific formulation. We worked [1] on the development of model mucus environments to deepen the understanding of mucin interactions with polymers used in pharmaceutical formulations by applying complementary techniques. Beside SAXS and SANS characterization in the bulk, we carried on investigations also on thin mucin layers depositions, by applying QCM-D and neutron reflectivity. Further, we developed a bio-inspired complex model system consisting in a mucin layer deposited on top of a single supported model membrane, structurally investigated by neutron reflection. Since complexation between mucins and biomacromolecules takes place close to the cell membrane surface, the present model is potentially predictive of the fate of nanodrugs intended to cross mucus and enter epithelial cells.

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Session D / **10**

Oxidation of saturated and unsaturated bilayers by reactive oxygen species

Author: Martin King[1]

Co-authors: Katherine Thompson ; Toby Robson [2]; Rebecca Welbourn [3]; Adrian R. Rennie ; Philipp Gutfreund ; Thomas Arnold [4]

[1] *Royal Holloway University of London, UK*

[2] *Royal Holloway University of London*

[3] *ISIS*

[4] *ESS*

Corresponding Author: m.king@rhul.ac.uk

Oxidation of membrane lipids in biology is a very important field because it may impact ageing, cell apoptosis and cancer[1]. It is unclear what the chemical identity of the oxidant is and there is plenty of discussion in the literature. Consequently, the term ROS (reactive oxygen species) is invoked and that may include the oxidants OH, $O_2(^1\Delta_g)$, HO_2, O_2^- etc. Studies of the oxidation of lipid bilayers (as a proxy for biological membranes), generate mixtures and unknown amounts of ROS and describe typically what happens to either the morphology of the bilayer/lipid (e.g [2]) or report the resultant products (e.g [3]). We will report on the chemical mechanism, kinetics and morphology gained from the oxidation of DPPC and POPC lipid bilayers with OH radicals and aqueous ozone. We generate known amounts of OH radicals or aqueous ozone by photolysis and with deuterium labelling we have (a) highlighted the location on the lipid molecule of initial attack (head, tail or both?), (b) determined the site-specific rate constants of the bilayer attack and (c) in real time recorded the change in bilayer morphology (film thickness) by neutron reflection.

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Session E / **73**

Investigation of membrane and proteins short wavelength collective dynamics from MD simulations. Connection to scattering techniques

Session E / 25

Coarse-grained molecular dynamics simulations of antimicrobial peptides against membrane models

Author: Gideon Tolufashe[1]

Co-authors: Martin Calvelo [2]; Alicia Muñoz [3]; Ángel Piñeiro [4]; Margarida Bastos [1]; Rebeca Garcia-Fandino [5]

[1] *Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, R. Campo alegre, 687, P4169-007 Porto, Portugal*

[2] *Department of Organic Chemistry, Center for Research in Biological Chemistry and Molecular Materials, Santiago de Compostela University, CIQUS, Spain*

[3] *Soft Matter and Molecular Biophysics Group, Departamento de Física Aplicada, Facultade de Física, Universidade de Santiago de Compostela, Campus Vida s/n, E-15782 Santiago de Compostela, Spain*

[4] *3Soft Matter and Molecular Biophysics Group, Departamento de Física Aplicada, Facultade de Física, Universidade de Santiago de Compostela, Campus Vida s/n, E-15782 Santiago de Compostela, Spain*

[5] *Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, R. Campo alegre, 687, P4169-007 Porto, Portugal and Department of Organic Chemistry, Center for Research in Biological Chemistry and Molecular Materials, Santiago de Compostela University, CIQUS, Spain*

Corresponding Author: gideon.tolufashe@fc.up.pt

The increasing emergence of resistant bacteria is a great concern in terms of public health as available conventional antibiotics drugs are not able to kill them. One strategy proposed is the use of bacterial membranes as a therapeutic target so that their basic properties are perturbed, altering the membrane potential and inhibiting the control functions on the signalling, communication or production bioenergy processes. In this sense, antimicrobial peptides (AMPs) exhibit unique properties, which include broad-spectrum activity, rapid action and difficult development of resistance. They are part of the innate immune system in a large number of species, where they form the first line of defence against pathogenic invasion, still maintaining its effectiveness after being present in nature for thousands of years. Despite these advantages, AMPs have, in general, a small therapeutic window, and can hardly be used systemically because of their high toxicity. A detailed understanding of the molecular details of the membrane permeabilization process would allow the rational design of new molecules with the same mechanism of action, but with improved activity, selectivity, and bioavailability.

Computational studies play an increasingly important role in understanding the structure and dynamics of biomolecular systems. For example, Molecular Dynamics simulations using coarse-grained (CG-MD) resolution is able to systematically explore events that take place into ranges where direct comparison and experimental testing are starting to be feasible. In this study, we performed CG-MD simulations (5 μs) of series of AMPs in the presence of different membrane models containing different mixtures of1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1-Palmitoyl-2oleoyl-sn-glycero-3-phosphoethanolamine (POPE), imitating bacterial, and 1-palmitoyl-2-oleoyl-snglycero-3-phosphocholine (POPC) representing mammalian membranes. The outcome of this study should provide the basis to design better AMPs that will disrupt the bacterial membrane and ultimately cause the death of their cells.

Keywords: Antimicrobial peptides (AMPs), membranes, molecular dynamics, antimicrobials, coarse grained.

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Session E / **54**

Coupling of leaflet structure in asymmetric lipid vesicles

Authors: Moritz Paul Karl Frewein[1]; Haden L. Scott[2]; Milka Doktorova[3]; Frederick A. Heberle[2]; Yuri Gerelli[None]; Lionel Porcar[None]; Georg Pabst[4]

[1] *Institut Laue - Langevin and University of Graz, Graz, Austria*

[2] *Univ Tennessee, Knoxville, TN, USA*

[3] *University of Texas Health Science Cente, Houston, TX, USA*

[4] *University of Graz, Graz, Austria*

Corresponding Author: frewein@ill.fr

Lipid asymmetry is a hallmark of biological membranes [1]. In particular, prototypical mammalian plasma membranes are known to be composed of an outer leaflet enriched in cholinephospholipids, while the majority of the aminophospholipids are confined to the inner leaflet [2]. Asymmetric large unilamellar lipid vesicles (aLUVs), produced via cyclodextrin-mediated lipid exchange [3], are a new platform for more realistic mimics of biological membranes . These systems were shown to be stable over several days [4] and have already been investigated by elastic scattering techniques (small-angle neutron and X-ray scattering; SANS/SAXS), providing insight into structural properties of the individual leaflets [5]. One of the enduring questions concerning plasma membrane architecture and lipid asymmetry is the possibility of bilayer leaflets being coupled to each other, which may influence a number of physiological processes that require communication between interior and exterior of the cell [6]. However, in the physiologically relevant fluid phase no evidence of structural coupling has yet been reported from scattering studies. In this work, we explore the role of hydrocarbon chain interdigitation as a potential trigger for transleaflet coupling.
We use combinations of dipalmitoylphosphatidylcholine (DPPC) in the inner leaflet and mixed lipids with varying chain length mismatch in the outer leaflet, in particular C16:0/C18:1 PC (POPC), C18:0/C18:1 PC (SOPC), C18:0/C14:0 (SMPC), C14:0/C18:0 (MSPC) and C16:0/C14:0PC (PMPC). This entails different interdigitation states of the mixed-chain lipids into the inner leaflet. We present consequences on transbilayer coupling as observed from leaflet specific structural data and thermotropic behavior of these systems.

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Session E / **40**

Asymmetric membranes and the study of lipid movement across single lipid bilayers

Authors: Ursula Perez-Salas[1]; Lionel Porcar[None]

[1] *University of Illinois in Chigaco*

Corresponding Author: ula@anl.gov

Over the past few years, our lab has put forth an effort to measure the rate and energetics of the diffusion of cholesterol and lipids. Using small angle neutron scattering (SANS), we found that the diffusion rate of cholesterol was much slower (hundreds of minutes) rather than the accepted value of under a second. Our group's work showed that the discrepancy was likely due to artifacts produced by differences between cholesterol and the analogues used by other experiments or by the use of extraneous compounds such as cyclodextrin, which our group argued, disrupts the membrane itself. However, the dispute has remained unresolved since our group's measurement of cholesterol's diffusion within membranes was occurring while cholesterol was also diffusion between different membranes (a process we called exchange). So, our team has been taking steps to eliminate exchange from the measurement and therefore measure the intra-membrane diffusion rate directly. We are using a new non-disruptive method to craft an asymmetric distribution of cholesterol and lipids in the lipid bilayers of unilamellar vesicles to then measure the rate at which they diffuses through the bilayer. To track the diffusion rates we are using nuclear magnetic resonance spectroscopy (NMR), and small angle neutron scattering (SANS).

The great variety of lipid molecules in the cell membrane suggests their complex and unique role in cell function. The cell has further established unique lipid composition indifferent membranes within the cell for directed functionality. In addition, in membranes like the plasma membrane(PM), there is an asymmetric distribution of lipids between the outer or exoplasmic and the inner or cytoplasmic leaflets and the physiological fate of cells depends on the strict maintenance of this asymmetry. However, the
exact mechanisms and energetic toll by which these lipids arrive and particularly remain at their locations, such as in the PM, are not fully
understood. Reliable values of passive lipid translocation rates are a necessary starting point for a detailed mechanistic understanding of
the lipid distribution landscape in cellular membranes. However,
obtaining these values has been hampered by artifacts emerging from different
methodologies. As a result, the reported rates for similar
lipids can vary by several orders of magnitude, from less than a second to
hours. Work in the last few years by several groups including ours, have shown that only those techniques free of artifacts can free of artifacts can potentially be used in the study of the transport of lipids across membranes.
Here we present our recent results on the production of asymmetric membranes
without the requirement of molecules such as cyclodextrin to study the
intra-membrane flip-flop of lipids and cholesterol across a single lipid bilayer using Small Angle Neutron Scattering, NMR, and fluorescence techniques.

Session E / 43

Lipid sponge-phase nanoparticles as enzyme carriers - structure and intermolecular interaction controlling the enzyme encapsulation

Authors: Maria Valldeperas[1]; Najet Mahmoudi[2]; Susana C.M. Teixeira[3]; Martynas Talaikis[4]; Ieva Matulaitienė[5]; Gediminas Niaura[6]; Justas Barauskas[7]; Tommy Nylander[1]

[1] *Physical Chemistry, Department Chemistry, Lund University, Lund, Sweden*

[2] *ISIS, STFC, Rutherford Appleton Laboratory, Didcot, UK*

[3] *Dep. of Chemical & Biomolecular Eng., Colburn Lab., University of Delaware 150 Academy Street, Newark, DE 19716, USA; NIST Center for Neutron Research, 100 Bureau Drive, Gaithersburg MD 20899 – 6102, USA*

[4] *6Department of Bioelectrochemistry and Biospectroscopy, Institute of Biochemistry, Life Sciences Center, Vilnius University, Sauletekio av. 7, LT-10257, Vilnius, Lithuania*

[5] *Department of Organic Chemistry, Center for Physical Sciences and Technology, Sauletekio av. 3, LT-10257, Vilnius, Lithuania*

[6] *Department of Bioelectrochemistry and Biospectroscopy, Institute of Biochemistry, Life Sciences Center, Vilnius University, Sauletekio av. 7, LT-10257, Vilnius, Lithuania; 7Department of Organic Chemistry, Center for Physical Sciences and Technology, Sauletekio av. 3, LT-10257, Vilnius, Lithuania*

[7] *Camurus AB, Ideon Science Park, Lund, Sweden*

Corresponding Author: tommy.nylander@fkem1.lu.se

Non-lamellar lipid aqueous phases, such as reverse cubic or hexagonal phases, can be used to entrap smaller biomolecules. The curvature of the lipid aqueous interfaces in these phases and hence the size of the aqueous cavities depends on the composition, water content and temperature. Normally the size of the cavities is similar or smaller than biomacromolecules, such as large enzymes. This poses a challenge when lipid phases as matrices for enzymes and other functional biomacromolecules. Here, we will present a lipid system, based on mixtures of acylglycerides and acyldiglycerides, which are able to form highly swollen sponge phases (L3), with aqueous pores up to 13 nm of diameter. The structure and composition of the particles were revealed by combining small angle neutron scattering (SANS), light scattering, cryo-TEM, size exclusion chromatography and Raman spectroscopy. The Raman spectroscopy results for the sponge phases show large similarities in lipid chain confirmation and head group interactions as in the lamellar and reverse bicontinuous cubic phase in the same lipid system [3]. This might be expected as all three structures are formed by lipid bilayers, albeit of different curvature. The L3 phase was found to be easy to disperse into sponge-like nanoparticles (L3 NPs) in excess aqueous solution by simply shaking [2]. We investigate encapsulation of two key types of enzymes of different sizes, used in food processing, namely Aspartic protease (34 KDa) and Beta-galactosidase (460 KDa). They are today delivered into the process as solutions with a considerable amount of preservatives and still with limited shelf-life and limited control of the enzyme activity. The SANS results reveal differences in the L3 NPs with and without enzyme that can be interpreted as inclusion of the protein in the liquid crystalline phase. We also performed neutron reflectometry, which verified the enzyme lipid interaction and enzyme penetration in the bilayer. These findings are verified by size exclusion chromatography, Raman spectroscopy and the enzymatic activity of the encapsulated enzyme, which surpasses the storage stability of pure enzymes in solution.

Session F / 44

Drug delivery of antimicrobial peptides to lipid bilayers

Author: Kathryn Browning[1]

Co-authors: Randi Nordström [2]; Lukas Boge [3]; Elisa Parra-Ortiz [1]; Liv Elinor Sofia Damgaard [1]; Richard Campbell [4]; Armando Maestro ; Mario Campana [5]; Joshaniel Cooper [6]; Martin Andersson [7]; Martin Malmsten [1]

[1] *University of Copenhagen*

[2] *Uppsala University*

[3] *RISE research institutes of Sweden*

[4] *University of Manchester*

[5] *ISIS*

[6] *ISIS neutron and muon source*

[7] *Chalmers University*

Corresponding Author: kathryn.browning@sund.ku.dk

The rise of antimicrobial resistance is a major challenge for future healthcare needs. To date antibiotic resistant bacterial strains have been reported in every country with prevalence growing annually. One promising line of treatment is antimicrobial peptides (AMPs), small cationic peptides, similar to the innate antimicrobial peptide defense in humans. It has been shown that AMPs can be highly selective and potent towards bacterial membranes through the disruption of the lipid envelope. However, AMPs are particularly susceptible to proteolytic degradation and decreased bioavailability, thereby challenging their widespread therapeutic use. It is important to protect AMPs from

proteolytic degradation whilst maintaining a potent release profile.
In this project, we have focused on two possible drug delivery vehicles, lipid cubosomes and microgels. Both vehicles protect the AMPs from degradation whilst maintaining the desired bacteria killing effects. We have used a common model AMP, LL-37, and studied its interaction, as a function of concentration, with model lipid bacterial membranes composed of dimyristoylglycerophosphocholine (DMPC) and dimyristoylglycerophosphoglycerol (DMPG). At low concentrations LL-37 inserted into the tail region of the bilayer, predominately in the outer leaflet. At therapeutically relevant concentrations, LL-37 was found to span the lipid bilayer, removing lipids and causing pore formation. We compared this effect to that of the AMP loaded into either glycerol monoleate cubosomes or poly(ethyl acrylate-co-methacrylic acid) (MAA) microgels. Both drug delivery vehicles have shown promising results in bacteria killing assays, however, the effect of the vehicle on the AMP mode of action was not previously known. In neutron reflection experiments AMP-loaded cubosomes were found to bind directly to the bilayer, inserting both cubosome material and AMPs to the lipid bilayer.[1] MAA microgels acted as passive protective containers, lowering the free LL-37 concentration but not interacting directly with the bilayer. [2]

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Session F / 38

Elucidating the mode of action of antimicrobial peptides using small-angle X-ray and neutron scattering techniques: the lipids point of view

Authors: Josefine Eilsø Nielsen[1]; Håvard Jenssen[2]; Reidar Lund[3]

[1] *Department of Chemistry, University of Oslo*

[2] *Roskilde University*

[3] *Department of Chemistry, Unversity of Oslo*

Corresponding Author: reidar.lund@kjemi.uio.no

It is generally believed that antimicrobial peptides, AMPs, are able to evade much of the bacterial resistance because they disturb the fundamental integrity of the entire cell by interfering with the life-defining cell membrane. However, there is no clear general consensus for the molecular basis by which AMPs act, although various structural modifications such as membrane deformation or pore formation have been suggested. [1,2] However, other factors may contribute such as changes in the lipid dynamics, changes in the lateral and transversal lipid composition and enhanced proton/ion transfer.
In order to fully understand the mechanism, we embarked on a study to investigate both the structural and dynamic effects on model membranes. To this end we employed state-of-the-art Small-angle X-ray and neutron scattering (SAXS/SANS) methods which are capable to probe the structure of both lipids and peptide on nanometer length scales[3]. In addition, by using H/D contrast variation scheme we could determine the lipid dynamics extracting both the transversal flip flop motion as well as lipid exchange.. The results further show that indolicidin inserts on the interface between lipid tail/head on the outer leaflet perturbing the lipid packing causing an acceleration in the dynamics. A similar acceleration is found for other AMPs although the structure differs. We speculate that the change in dynamics may cause effects that are detrimental to the bacterial cell.

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Session F / 39

Lipoproteins at model cell membranes, and the transport of fats

Author: Marité Cárdenas[1]

[1] *Malmö University*

Corresponding Author: marite.cardenas@mau.se

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 terrible disease. In this talk, I will present the efforts of my group to explore the role of lipid dynamics in their transport throughout the body by lipoproteins. Contrast matching, biodeuteration and neutron scattering are key to highlight the characterise not only the lipoprotein structure but also their ability to remove and deposit fats as a function of the membrane composition: saturated, unsaturated fats and cholesterol. In this way, we provide unprecedented information on the effect that cholesterol have on fat uptake by lipoproteins.

Session F / 41

Structural changes of pulmonary surfactant induced by bacterial lipopolysaccharide and by Polymyxin B

Authors: Daniela Uhrikova[1]; Nina Kanjaková[2]; Lukáš Hubčík[2]; Andrea Čalkovská[3]; Sophie Combet[4]; José Teixeira[5]

[1] *Faculty of Pharmacy, Comenius University in Bratislava, Odbojarov 10, 832 32 Bratislava, Slovakia*

[2] *Faculty of Pharmacy, Comenius University in Bratislava*

[3] *Department of Physiology, Jessenius Faculty of Medicine in Martin*

[4] *Laboratoire Leon Brillouin*

[5] *Laboratoire Leon Brillouin*

Corresponding Author: uhrikova@fpharm.uniba.sk

Pulmonary surfactant (PS) is a mixture of lipids (~90 %) and 8-10 % specific surfactant associated proteins. PS lines the interior of the lung alveoli and acts to lower interfacial tension. The absence of PS due to prematurity, or its damage, is treated by exogeneous PS in neonatal medicine. Curosurf (Cur) is one such clinically used replacement surfactants. It is an extract of porcine lung tissue consisting of at least 50 different phospholipids and contains a small amount of the essential protein SP-B (~2 wt%). Structurally, Cur is a mixture of uni- , oligo- and multilamellar vesicles. After inhalation, bacterial endotoxin, lipopolysaccharide (LPS) interferes with PS. We evaluated functional and structural changes of Cur in the presence of LPS using pulsating bubble surfactometer, optical microscopy, small angle neutron (SANS) and X-ray scattering (SAXS/WAXS). LPS bound to the lipid bilayer of Cur and disturbed its lamellar structure by swelling. The structural changes were attributed to the surface charge unbalance of the lipid bilayers due to LPS insertion. Polymyxin B (PxB) is an antimicrobial peptide primarily used in clinical practice to treat infections by resistant Gram-negative bacteria. In addition, PxB improves the surface properties of exogenous pulmonary surfactant [1]. Our SAXS experiments revealed that PxB acts as an inhibitor of structural disarrangement induced by LPS and restores original lamellar packing [2]. The lipid bilayer thickness was determined from SANS curves using the model of vesicles.

Acknowledgement. SAXS experiments were performed at BL11-NCD-SWEET beamline at Alba Synchrotron with the collaboration of Alba staff. Experiments were supported by projects APVV-17-0250, JINR 04-4-1121-2015/2020 and VEGA 1/096/16.

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Session G / **18**

Contribution of galactoglycerolipids to the three-dimensional architecture of thylakoids

Author: Juliette Jouhet[1]

[1] *Laboratoire de Physiologie Cellulaire et Végétale*

Corresponding Author: juliette.jouhet@cea.fr

Photosynthetic membranes, also called thylakoids, have a unique and unusual lipid composition. They contain an extremely high amount of unique classes of glycolipids, constituted of galactolipids, i.e. mono- and digalactosyldiacylglycerol (MGDG and DGDG) and of a sulfolipid, i.e. sulfoquinovosediacylglycerol (SQDG). A remarkable feature of the evolution from cyanobacteria to higher plants is the conservation of MGDG, DGDG, SQDG and phosphatidylglycerol (PG), the only phospholipid present in thylakoids. Using neutron diffraction, on reconstituted thylakoid lipid extracts, we observed that the thylakoid lipid mixture self-organizes as a regular stack of bilayers. The natural mixture of thylakoid lipids was shown to switch from hexagonal II toward lamellar phase upon hydration. This transition and the observed phase coexistence are modulated by the fine-tuning of the lipid profile, in particular the MGDG/DGDG ratio, and by the hydration. Our analysis followed by Molecular Dynamics simulation highlights the critical role of DGDG as a contributing component to the membrane stacking via hydrogen bonds between galactose polar heads of adjacent bilayers. DGDG cohesive interactions balance the repulsive electrostatic contribution of charged lipids like PG and SQDG and allow the persistence of regularly stacked membranes at high hydration. The membrane binding of MGD1, the committing enzyme of galactolipid biosynthesis in Arabidopsis, is also dependent of the membrane lipid composition and sensitive to the presence of DGDG. Altogether, these results show that galactolipids are determinant factors for the nonvesicular/nonlamellar biogenesis and for the three-dimensional architecture of nascent thylakoids. A model of biogenesis of photosynthetic membrane is proposed.
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Session G / **68**

Lateral organization in bacterial cells and model membranes.

Author: Jonathan Nickels[1]

[1] *University of Cincinnati*

Corresponding Author: jonathan.nickels@uc.edu

The existence and role of lateral lipid organization in biological membranes has been studied and contested for more than 30 years. Lateral lipid domains, or rafts, are hypothesized as scalable compartments within biological membranes, providing appropriate physical environments to their resident membrane proteins. This implies that lateral lipid organization is associated with a range of biological functions, such as protein co-localization, membrane trafficking, and cell signaling; to name just a few. A 'classic' model of lipid rafts as sterol and sphingomyelin rich regions has emerged as a result of a 'mammalian' centric focus. However, lipid rafts also appear to be key features of microbial cell membranes, with recent results illustrating a functional connection between raft disruption and antibiotic resistance. Moreover, we have recently suggested that lipid rafts may also act to buffer membrane physical properties from changes in temperature and environmental perturbations – such as amphiphilic solvents.

Today, we will consider lateral lipid organization, primarily in bacterial cell membranes and bacterial cell membrane mimics. Though there are numerous approaches which are useful to investigate lateral organization, neutrons provide unique information about the structure and dynamics of biological systems on relevant time and length scales. Beyond this, neutrons possess a powerful sensitivity to the isotopes of hydrogen, enabling biodeuteration strategies to resolve lipid rafts in complex experimental systems. There has been significant progress in recent years observing lipid organization in these systems, with neutron scattering featuring as a particularly powerful approach for the characterization of biomolecules in model systems and in vivo. I will present the results of these direct observations of cell membrane transverse and lateral structure in the Bacillus subtilis cell membrane, and update these result with ongoing studies of the biological impacts of such deuteration strategies. Other studies utilizing membrane mimics and other neutron based techniques have isolated the structure and dynamics of lipid domains in greater detail. These results inform the physical mechanisms of domain formation and demonstrate the proposed buffering effect of bilayer physical properties, suggesting a physically based function for these biological structures.

Session G / **31**

Apolar lipids, the membrane adaptation toolbox of extremophiles

Authors: Marta Salvador Castell[1]; Maksym Golub[2]; Nelli Erwin[None]; Bruno Demé[None]; Nicholas Brooks[None]; Roland Winter[None]; Judith Peters[None]; Philippe Oger[None]

[1] *INSA Lyon*

[2] *Tartu Ülikool, 50090 Tartu, Estonia*

Corresponding Author: marta.salvador-castell@insa-lyon.fr

Most of Earth's biotopes are hold under extreme environmental conditions, namely distant from the optimal life conditions of humans. Nevertheless, a large biological diversity of organisms inhabit such environments, i.e. extremophiles. For instance, many living organisms reside at hydrothermal sources of deep oceans: temperatures above 100℃, high concentrations of reduced metals, absence of oxygen and high hydrostatic pressures, without an understanding of the molecular mechanisms enabling them to sustain such extreme conditions. The cell membrane is particularly sensitive to external conditions, but at the same time, it must maintain specific physical properties, such as fluidity and permeability, to preserve cell's integrity and functionality. This work seeks to understand how the lipid bilayer can remain functional at high temperatures and high pressures and thus, allows life under extreme conditions.

The results presented here determine novel membrane components, apolar lipids from the polyisoprenoid's family, that play the role of membrane regulators and confer stability to the lipid bilayer, along with dynamism and heterogeneity, essential properties for an optimal functional membrane. By neutron diffraction, we demonstrated that apolar lipids are placed in the midplane of the lipid bilayer, even at high temperatures and high hydrostatic pressures. Moreover, they establish membrane

lateral heterogeneity by inducing lipid phase separation. Furthermore, SAXS results demonstrated that polyisoprenoids adjust membrane curvature under extreme conditions, enabling essential cell functions that require high curved membrane domains, such as fusion and fission. Moreover, because their specific placement, apolar lipids modify lipid bilayer permeability and reduces proton membrane permeability at high hydrostatic pressures, as demonstrated by fluorescent approaches. All the results demonstrate experimentally a new cell membrane architecture of extremophiles in which the presence and the quantity of polyisoprenoids play a key role and constitute a new adaptation pathway to extreme conditions applicable to life origin.

Session G / **50**

Effects of alcohol addition on a fatty acid membrane

Authors: Loreto Misuraca[1]; Bruno Demé[None]; Judith Peters[None]; Philippe Oger[None]

[1] *ILL*

Corresponding Author: misuraca@ill.fr

Given the probable extremely contrasted environmental conditions at the origins of life (high temperature, pressure and pH), the origin and nature of the first cell membranes is still an open question. Due to complex organic carbon limitations, the first membranes were most likely composed of simpler, single chain fatty acids [1], which raises questions as how they could withstand the very variable and extreme surrounding environment [2].
Our current project considers two possible architectures for protocell membranes: a) a bilayer made of decanoic “capric” acid; b) a mixture of capric acid with a fatty alcohol of equal chain length, decanol.
Several complementary techniques have been employed to characterize these model single-chain amphiphiles vesicles. Among them, Static / Dynamic Light Scattering allowed to observe vesicle appearance, characteristics and time stability. Differential Scanning Calorimetry was employed to detect the membrane phase transitions and stability with temperature. Solid State NMR spectroscopy allowed assessing the bilayer rigidity for both models at different temperatures. Small Angle Neutron Scattering allowed to quantify the vesicle amount, size, lamellarity and membrane thickness.
The results allow defining the substantial role of the fatty alcohol presence in modifying the membrane characteristics and behavior at both ambient and high temperatures; they will serve as a basis to study the combined high temperature – high hydrostatic pressure effects, as these are the mandatory physical parameters to test the validity of the protomembrane model architectures.
The latest results, obtained with the above-mentioned techniques, will be presented.

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Session H / **12**

pH-induced rearrangements in lipid bilayer causing in drug release from pH-sensitive liposomes

Author: Olga Zaborova[1]

[1] *Lomonosov Moscow State University*

Corresponding Author: olya_z_88@mail.ru

The development of stimuli-sensitive, particularly pH-sensitive, liposomal nanocontainers for targeted drug delivery is of great value nowadays. The pH-sensitivity of liposomes can be achieved by

embedding the pH-switcher into the lipid bilayer, thus the decrease of the pH value would result in release of the entrapped compound.
In this study we examined the pH-dependent kinetics of changes in liposomal membrane containing two types of pH-switchers by SAXS technique combined with the stopped-flow apparatus.
The first type of pH-switcher was the lipid-like compound based on morpholinocyclohexanol. With the acidification of the media the pH-sensitive lipid undergoes the conformational change that results in thinning of the lipid bilayer (creating "defects") and thus increases the permeability of liposomal membrane. Kinetic release experiments showed that the entrapped compound began to release from the pH-triggerable liposomes just after decreasing the pH value of the media. The notable changes in the SAXS curves appeared at 11 seconds after the pH change from 7.0 to 4.0 and resulted in formation of a peak at q=1.15 nm-1. Over time the peak became more pronounced, what could be attributed to the formation of ordered structure. Only correlation peak was changing with time implying that the vesicular structure of the liposomes was not disrupted in agreement with previous DLS and cryo-TEM results.
The second type of pH-switcher was represented by the derivative of cholesteric acid. The protonation leads to the rotation of the molecule in the lipid bilayer. The initial state of liposomes with switcher of the second type was the same as for the first ones. But the changes in liposomal structure in the second case became to be evident from the 2 second after pH change from 7.0 to 4.0.

The reported study was funded by the Russian Science Foundation (project 18-73-00076)

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Role of membrane sphingolipids in the interaction with amyloid beta-peptide

Author: Rita Carrotta[1]

Co-authors: Fabio Librizzi [1]; Maria Rosalia Mangione [1]; Silvia Vilasi [1]; Claudia Cossu [1]; Oscar Moran [1]

[1] *CNR, Istituto di Biofisica*

Corresponding Author: rita.carrotta@cnr.it

The early impairments appearing in Alzheimer's disease are related to neuronal membrane damage. Both,aberrant Aβ species and specific membrane components play a role in promoting aggregation, deposition and signal dysfunction. Ganglioside GM1, present with cholesterol and sphingomyelin in lipid rafts, seems to be able to initiate Aβ aggregation on membrane [1]. In general, sphingolipids have a crucial role both in the physico-chemical properties of the membrane matrix and in the signaling paths. Based on our previous studies highlighting the fundamental role of GM1 embedded in large unilamellar vesicles (LUV) in the interaction with Aβ, the dependence of this interaction on GM1 fraction was investigated by SAXS at 20°C [2,3]. LUV containing different amount of ganglioside in the presence of sphingomyelin were extruded and their structure as a function of the matrix composition, per se and with the addition of the Aβ-peptide, was monitored using SAXS. The analysis of the SAXS spectra by a multi-gaussian model evidences structural differences in the bilayer electron density among the liposomes of different composition. Results show the expected asymmetry due both to the natural membrane curvature and to the different matrices. While the presence of sphingomyelin as well as ceramide, without GM1, does not prompt significant interaction of the bilayers with the Aβ-peptide, results confirm the fundamental role of the ganglioside for such interaction. Moreover, the analysis highlights the concentration-dependent effect of GM1 in the interaction with the Aβ-peptide. However, the co-presence of sphingomyelin, probably due to the higher rigidity of the matrix, has an inhibiting effect on GM1- Aβ interaction, increasing the concentration of GM1 needed to appreciate a perturbation on the bilayer, which propagates down to the interior of the membrane.

Session H / 21

Adsorption and interactions of polymer stabilised lipid nanodiscs with a bilayer at the solid-liquid interfaces

Author: Thomas Arnold[1]

Co-authors: Stephen Hall [2]; Luke Clifton [3]; Karen Edler [4]; Tim Dafforn [2]

[1] *ESS*

[2] *University of Birmingham*

[3] *STFC*

[4] *University of Bath*

Corresponding Author: tom.arnold@esss.se

Styrene-maleic acid lipid particles (SMALPs) are self-assembled discoidal structures composed of a polymer belt and a segment of lipid bilayer, which are capable of encapsulating membrane proteins directly from the cell membrane. In recent years a number of different nanodisc forming polymers with varying properties have been developed and characterised. For example, Styrene-maleic imide lipid particles (SMILPs) are stable over a different pH range but are still able to solubilize membrane proteins.
Here we will present recent results from a detailed investigation into the interaction of SMALP and SMILP nanodiscs with phospholipid bilayers at the solid-liquid interface. Using Neutron Reflectometry and ATR-FTIR we have examined the kinetics of lipid exchange between nanodiscs and biliayers. While lipid exchange is seen in each case, the kinetics and extent to which this occurs are considerably different for each polymer. Further, under certain conditions and highly dependent on the polymer, it is possible to adsorb discs at the solid-liquid interface. This is the first evidence of such ad-sorption for polymer stabilized nanodiscs and has important implications for future applications that would use SMALP technology to deliver membrane proteins to interfaces.

Session H / **67**

Dynamic polymer-based nanodiscs for membrane biophysics

Authors: Sandro Keller[1]; Rodrigo Cuevas Arenas[2]; Bartholomäus Danielczak[2]; Anne Grethen[2]; Carolyn Vargas[2]; Anne Martel[3]; Lionel Porcar[None]; Cécile Breyton[4]; Christine Ebel[4]; Annette Meister[5]

[1] *Molecular Biophysics, Technische Universität Kaiserslautern (TUK)*

[2] *Molecular Biophysics, TUK*

[3] *Institut Laue-langevin*

[4] *IBS*

[5] *ZIK HALOmem, Martin Luther University Halle–Wittenberg*

Corresponding Author: sandro.keller@biologie.uni-kl.de

Amphiphilic copolymers enable a fundamentally new approach for investigating membrane proteins, as they obviate the use of conventional detergents. These polymers extract proteins and surrounding lipids directly from cellular membranes to form nanosized discs, where the polymer wraps around a lipid-bilayer patch. Such nanodiscs are amenable to a broad range of methods requiring nanosized particles, which sets them apart from traditional bilayer systems such as vesicles. In this talk, I will focus on styrene/maleic acid (SMA) and diisobutylene/maleic acid (DIBMA) co-polymers as well as derivatives thereof that display improved properties over existing polymers.

Particular attention will be paid to the dynamic nature of polymer-encapsulated nanodiscs, which exchange their lipid contents rapidly through collisional transfer. This was shown by both temperature-dependent stopped-flow small-angle neutron scattering (SANS) experiments at ILL and concentration-dependent stopped-flow Förster resonance energy transfer (FRET) assays (Cuevas Arenas et al. *Sci. Rep.* **2017**, *7*, 45875; Grethen et al. *J. Membr. Biol.* **2018**, *251*, 443; Danielczak et al. *Eur. Polym. J.* **2018**, *10*, 206). Moreover, we used differential scanning calorimetry (DSC), Raman scattering, and

time-resolved fluorescence spectroscopy to demonstrate that DIBMA does not disturb the order, dynamics, and hydration of the solubilised mem-brane fragments (Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919; Grethen et al. *Sci. Rep.* **2017**, *7*, 11517; Oluwole et al. *Langmuir* **2017**, *33*, 14378). Finally, new, modified polymers offer additional advantages in that they carry no charge and have been optimised for membrane-protein extraction from popular expression hosts.

Session E / **4**

What do we really measure when we track the diffusive dynamics in biomembranes?

Author: Ilpo Vattulainen[1]

[1] *Department Physics, University of Helsinki*

Corresponding Author: ilpo.vattulainen@helsinki.fi

There are numerous techniques able to gauge diffusion in biomembranes. For instance, quasi-elastic neutron scattering measures diffusion in a non-perturbative manner over nanosecond time scales, yet sampling is space is here done over large distances. Meanwhile, single-particle tracking allows one to track the dynamics of individual molecules in almost nanometer resolution, but these measurements are based on the use of markers that may interfere with the system under examination, either very little or unexpectedly much. Here we discuss recent nanoscale computer simulation studies that were designed to explore the diffusion mechanisms of lipids and membrane proteins, and the effects of streptavidin-functionalized Au nanoparticles on the lateral diffusion of lipids in biomembranes. The results show that lipids diffuse in a concerted fashion as clusters of lipids whose motion is highly correlated, and membrane proteins move as dynamical complexes with tens of lipids dynamically bound to the protein. Meanwhile, lipids linked to a streptavidin-nanoparticle complex also move in a concerted manner but as a complex with the linker protein and numerous non-labeled unlabeled lipids, and it turns out that this can slow down the motion of the probe by about almost an order of magnitude. Altogether, the results highlight the view that prior to using any technique and/or probe, it makes sense to understand the physical basis of the diffusion process that one aims to measure. Otherwise, interpretation of experimental data can be a surprisingly difficult task.