



Bilayers at the ILL 2019

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INFORMATION

Workshop Venue: All sessions will take place in ILL4, the principal building of ILL.

Posters (portrait A0) should be on display throughout the duration of the workshop in the ILL4 hall. The main poster session will take place on the Wednesday evening.

Lunches will be available for the participants at the joint ILL/ESRF restaurant. Please make sure to wear your conference badge at the till.

The **conference dinner** will take place at the
Bistrot à l'envers on Thursday evening.
Instruction about how to reach the restaurant from ILL
can be found in the next page.

Alison will be present at the workshop reception desk until the start of the first session and during coffee breaks. Outside these periods, assistance will be available at her office in the **Science Building 3rd floor office 304**. She can also be contacted by phone **0476207635** (if you use an ILL phone you can dial the last 4 digits only).

WiFi Access: You can log on to the EPN VISITORS network

Login: bill2019

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EDUROAM network is also available.

The ILL cannot be held responsible for any loss or damage of personal property or vehicles. On the EPN campus participants MUST respect the campus' safety rules.


Workshop Dinner

Thursday 12 December 2019 at 19h30

Bistrot à l'envers

3 rue d'Alembert 38000 Grenoble

Tel : 04.76.48.62.83

To get to the restaurant you will have to take tramway 
at the Oxford stop
(200m from EPN entrance)
Nearest stop:
Palais de justice

Organisers will provide public transportation tickets for all participants

BILL2019, 11 – 13 December 2019

Institut Max Von Laue – Paul Langevin

GRENOBLE, FRANCE

PROGRAMME

Wednesday 11 December

10:00–12:00 Registration

12:00 **Lunch at the on-site canteen**

take your meal showing the conference badge at the pay desk.

13:30–14:00 Opening address

Session A

14:00–14:40 Keynote Talk: The lipid organisation in skin: an integrated approach of lipid model systems and clinical studies

J.A. Bouwstra, *Leiden University*

14:40–15:10 Invited Talk: Interactions in the pre-AD mimicking model membranes

N. Kučerka, *Frank Lab. of Neutron Physics*

15:10–15:30 Lipid domain modulation and inhalation anesthesia

D. Worcester, *TU Delft*

15:30–15:50 Influence of galactoglycerolipids on the chloroplast envelope outer membrane architecture and its adaptation to phosphate deprivation in plants

S. Bolik, *ILL & UGA*

15:50–16:30 **Coffee Break, ILL4 Hall**

Session B

16:30–17:00 Invited Talk: Linking membrane structure and dynamics: insights from neutron scattering

E. Kelley, *National Institute of Standards and Technology, Gaithersburg*

17:00–17:20 Curved lipid interfaces studied with GISANS

K. Mothander, *Lund University*

17:20–17:40 Partitioning of DNA nanochannel between Lo/Ld phases in a lipid membrane: Relevance of lipid anchors

A. Sayed, *Helmholtz-Zentrum Dresden-Rossendorf (HZDR)*

17:40–18:00 Metabolic incorporation of deuterium into nerve myelin

D.A. Kirschner, *Boston College*

18:00 **Wine & Cheese Poster Session, ILL4 Hall**

Thursday 12 December

Session C

- 09:00–09:30** Invited Talk: Shedding light into membrane receptor functioning and lipid interactions by plasmon waveguide resonance
I. Alves, *CBMN, U. of Bordeaux*
- 09:30–10:00** Invited Talk: Probing the internal structure of nanoparticles for mRNA delivery
A. Dabkwoska, *AstraZeneca*
- 10:00–10:20** DNA-tagged lipid bilayers: novel nano-scaled membrane-mimetic systems
K. Fahmy, *Helmholtz-Zentrum Dresden-Rossendorf*
- 10:20–10:40** A multi-technique approach for the characterization of the self-assembling of cyclic peptides into nanotubes at biological model membranes
B. Claro, *University of Porto*
- 10:40–11:00** **Coffee Break, ILL4 Hall**

Session D

- 11:00–11:30** Invited Talk: The rich phenomenology of glycolipids and membrane-anchored polysaccharides - Insights from scattering techniques and complementary computer simulations
E. Schneck, *TU-Darmstadt*
- 11:30–11:50** Using suspended bilayers as a novel bacterial membrane mimetic for investigating mechanosensitive ion channels with neutron reflectivity
S. Titmuss, *University of Edinburgh*
- 11:50–12:10** Mucin thin layers on top of model membranes as a model environment for mucosal delivery
V. Rondelli, *University of Milan*
- 12:10–12:30** Oxidation of saturated and unsaturated bilayers by reactive oxygen species
M. King, *Royal Holloway University of London*
- 12:30** **Lunch at the on-site canteen**
take your meal showing the conference badge at the pay desk.

Session E

- 14:00–14:30** Invited Talk: What do we really measure when we track the diffusive dynamics in biomembranes?
I. Vattulainen, *University of Helsinki*
- 14:30–15:00** Invited Talk: Investigation of membrane and proteins short wavelength collective dynamics from MD simulations. Connection to scattering techniques
M. Tarek, *CNRS-Université de Lorraine*
- 15:00–15:20** Coarse-grained molecular dynamics simulations of antimicrobial peptides against membrane models
G. Tolufashe, *University of Porto*
- 15:20–15:40** Coupling of leaflet structure in asymmetric lipid vesicles
M.P.K. Frewein, *ILL & University of Graz*
- 15:40–16:00** Lipid sponge-phase nanoparticles as enzyme carriers - structure and intermolecular interaction controlling the enzyme encapsulation
T. Nylander, *Lund University*

16:00–16:30 Coffee Break, ILL4 Hall

Session F

16:30–17:00 Invited Talk: Drug delivery of antimicrobial peptides to lipid bilayers

K. Browning, *University of Copenhagen*

17:00–17:20 Elucidating the mode of action of antimicrobial peptides using small-angle X-ray and neutron scattering techniques: the lipids point of view

R. Lund, *Oslo University*

17:20–17:40 Lipoproteins at model cell membranes, and the transport of fats

M. Cardenas, *Malmö University*

17:40–18:00 Structural changes of pulmonary surfactant induced by bacterial lipopolysaccharide and by Polymyxin B

D. Uhríkova, *Comenius University in Bratislava*

18:00–18:30 Round Table

19:30 Workshop dinner

Friday 13 December

Session G

09:00–09:30 Invited Talk: Contribution of galactoglycerolipids to the three-dimensional architecture of thylakoids

J. Jouhet, *CEA-Grenoble*

09:30–10:00 Invited Talk: Lateral organization in bacterial cells and model membranes.

J. Nickels, *University of Cincinnati*

10:00–10:20 Apolar lipids, the membrane adaptation toolbox of extremophiles

M. Salvador Castell, *INSA Lyon*

10:20–10:40 Effects of alcohol addition on a fatty acid membrane

L. Misuraca, *ILL*

10:40–11:00 Coffee Break, ILL4 Hall

11:00–11:20 pH-induced rearrangements in lipid bilayer causing in drug release from pH-sensitive liposomes

O. Zaborova, *Moscow State University*

11:20–11:40 Role of membrane sphingolipids in the interaction with amyloid beta-peptide

R. Carrota, *National Research Council of Italy*

11:40–12:00 Adsorption and interactions of polymer stabilised lipid nanodiscs with a bilayer at the solid-liquid interfaces

T. Arnold, *European Spallation Source*

12:00–12:30 Invited Talk: Dynamic polymer-based nanodiscs for membrane biophysics

S. Keller, *TU-Kaiserslautern*

12:30–12:40 Closing Address

12:40 Lunch at the on-site canteen

take your meal showing the conference badge at the pay desk.

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

Johanna Aaltje Bouwstra, G.S. Gooris, E. A. Mojumdar, D. Groen, C. M. Beddoes
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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 play 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 impaired 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.

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Interactions in the pre-AD mimicking model membranes

T. Murugova, O. Ivankov, E. Ermakova, E. Dushanov,

K. Kholmurodov, T. Kondela, **N. Kučerka**

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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.

REFERENCES:

- [1] A. Martel, et al. JACS 139, 137-148 (2017).
- [2] N. Kučerka, et al. Langmuir 33 (2017) 3134-3141.
- [3] E. Drolle, et al. Biochim. Biophys. Acta 1828, 2247-2254 (2013).
- [4] T. Murugova, et al. Gen. Physiol. Biophys.39/2 (2020).

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Lipid Domain Modulation and Inhalation Anesthesia

David Worcester, Michael Weinrich

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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 of ternary 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.

1. M Weinrich & DL Worcester, *Acta Cryst D* 74 (2018) 1169-1177.
2. CE Cornel et al., *Biophys J.* 113 (2017) 1200-1211.
3. MA Pavel et al., *BioRxiv* (2018) 31973.

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

Stéphanie Bolik, Ignacio Rodriguez Loureiro, Emanuel Schneck,
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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.

Références.

- [1] Poirier Y, Thoma S, Somerville C, Schiefelbein J. 1991. Plant Physiol 97, 1087–93.
- [2] Demé B, Cataye C, Block MA, Maréchal E, Jouhet J. 2014. FASEB J. 28:3373-83.
- [3] Kanduc M, Schlaich A, de Vries AH, Jouhet J, Maréchal E, Demé B, Netz RR, Schneck E. 2017. Nature Com. 8:14899

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Linking membrane structure and dynamics: insights from neutron scattering

Elizabeth Kelley

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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.

[illegible]

Curved lipid interfaces studied with GISANS

Karolina Mothander, Tommy Nylander, Adrian Rennie

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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, streptavidin. These results show that nanowire supported lipid bilayer is an excellent way to study binding of membrane protein to curved lipid interfaces.

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

Ahmed Sayed, Aleksander Czogalla, Ralf Seidel

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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.

Notes

Metabolic Incorporation of Deuterium into Nerve Myelin

Daniel A. Kirschner, Anne Baumann, Bruno Demé,

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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 H_2O - versus D_2O -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 D_2O -fed mice. Whereas the lipids from the D_2O -dam were 75-80% deuterated, the lipids from the D_2O -pups were ~99% deuterated. Moreover, the deuterated lipids for the pups vs. the dam had different incorporation patterns of the label.

1. H Inouye, DA Kirschner. In: *Molecules: Aggregation, Nucleation, Crystallization – Beyond Medical and Other Implications* (Eds. J Sedzik, P Riccio), World Scientific Publishing (Singapore), 2009, pp. 75-94.
2. AR Denninger, B Demé, V Cristiglio, G LeDuc, et al., *Acta Cryst D70* (2014):3198-3211
3. AR Denninger, A Breglio, KJ Maheras, G LeDuc, et al., *Biophys J.* 109 (2015):1387-1397.

Notes

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

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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.

[1] E. Harte, N. Maalouli, A. Shalabney, E. Texier, K. Berthelot, S. Lecomte, I. Alves, Chemical Communications 2014, 50, 4168-4171.

[2] P. Calmet, M. De Maria, E. Harte, D. Lamb, M. Serrano-Vega, A. Jazayeri, N. Tschammer, I. D. Alves, Sci Rep 2016, 6, 36181.

[3] aK. Boye, C. Billottet, N. Pujol, I. D. Alves, A. Bikfalvi, Sci Rep 2017, 7, 10703; bY. P. Chen, H. L. Wu, K. Boye, C. Y. Pan, Y. C. Chen, N. Pujol, C. W. Lin, L. Y. Chiu, C. Billottet, I. D. Alves, A. Bikfalvi, S. C. Sue, ACS Chem Biol 2017, 12, 2767-2778; cK. Boye, N. Pujol, D. A. I, Y. P. Chen, T. Daubon, Y. Z. Lee, S. Dedieu, M. Constantin, L. Bello, M. Rossi, R. Bjerkvig, S. C. Sue, A. Bikfalvi, C. Billottet, Nat Commun 2017, 8, 1571.

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Probing the internal structure of nanoparticles for mRNA delivery

Aleksandra P. Dabkowska, Marianna Yanez Arteta, and Lennart Lindfors

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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.

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

Madhumalar Subramanian, Jana Oertel, **Karim Fahmy**

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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 hydrophobic 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.

1. K. Iric, M. Subramanian, et al. Nanoscale 10 (2018) 18463-18467

[illegible]

A multi-technique approach for the characterization of the Self-Assembling of Cyclic Peptides into Nanotubes at Biological Model Membranes

Barbara Claro, Eva Freire, Juan Granja, Erik Goormaghtigh, Tiago Ferreira, Rebeca Garcia-Fandiño, Margarida Bastos
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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

References

- 1 L. Fernández, R. E. W. Hancock, Clin. Microbiol. Rev. 25 (2012) 661-681
2. T. Silva, B. Claro et al Langmuir 34 (2018) 2158-2170
- 3 S. Fernandez-Lopez et al Nature 412 (2001) 452–455

[illegible]

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

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

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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 respond to challenge by lyso-PC and pexiganan, will be described.

[illegible]

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

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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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Oxidation of saturated and unsaturated bilayers by reactive oxygen species

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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, O₂(¹Δ_g), HO₂, O₂⁻ 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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Notes

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

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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 in 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.

Notes

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

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Function of membranes and membrane proteins depend on their dynamics and relaxation behaviour. These may be in principle probed using neutron and x-ray- scattering techniques, yet, inelastic data on membranes and their associated proteins are very scarce, and still difficult to interpret. In this presentation I focus on the short-wavelength collective dynamics of these systems and show how they might be linked to functional modes of membrane proteins and to macroscopic elastic properties of membranes.

Notes

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

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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 μ s) of series of AMPs in the presence of different membrane models containing different mixtures of 1-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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Coupling of leaflet structure in asymmetric lipid vesicles

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

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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 conformation 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.

Notes

Drug delivery of antimicrobial peptides to lipid bilayers

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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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Elucidating the Mode of Action of Antimicrobial Peptides using Small-angle X-ray and Neutron Scattering Techniques: the lipid's point of view

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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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Lipoproteins at model cell membranes, and the transport of fats

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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.

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

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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 exogenous 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.

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Notes

Contribution of galactoglycerolipids to the three-dimensional architecture of thylakoids

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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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Lateral organization in bacterial cells and model membranes

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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.

[illegible]

Apolar lipids, the membrane adaptation toolbox of extremophiles

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Most of Earth's biotopes are held 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°C, 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.

[illegible]

Effects of alcohol addition on a fatty acid membrane

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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.

References

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

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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 $q=1.15 \text{ nm}^{-1}$. 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.

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

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The early impairments appearing in Alzheimer's disease are related to neuronal membrane damage. Both, aberrant A β 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 A β 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 A β , 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 A β -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 A β -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 A β -peptide. However, the co-presence of sphingomyelin, probably due to the higher rigidity of the matrix, has an inhibiting effect on GM1- A β interaction, increasing the concentration of GM1 needed to appreciate a perturbation on the bilayer, which propagates down to the interior of the membrane.

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

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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 bilayers. 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 adsorption for polymer stabilized nanodiscs and has important implications for future applications that would use SMALP technology to deliver membrane proteins to interfaces.

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Dynamic Polymer-Based Nanodiscs for Membrane Biophysics

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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 membrane 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.

Notes