7th European Joint

Theoretical/Experimental Meeting on Membranes

(EJTEMM 2021)

BOOK OF ABSTRACTS

April 7 – 9, 2021

Graz, Austria

hosted by

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Natural Sciences

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17:15 - 17:45	*Closing Lecture*
17:45 - 18:00	Famous last words

*celebrating the contributions by Karl Lohner on the occasion of his retirement

Welcome from the Organizers

Dear Attendee,

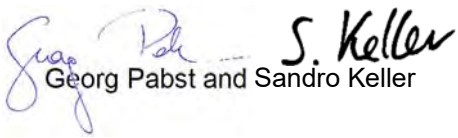
On behalf of the organization committee, we would like to convey our warm and hearty welcome to the 7th European Joint Theoretical/Experimental Meeting on Membranes (EJTEMM 2021). Certainly, we wish to have had the opportunity to welcome you in person in the beautiful city of Graz (Austria). However, we have all been handcuffed for about a year now to remain where we are. Yet, there is no means to constrain our minds and imagination, and as such we invite you to a virtual scientific journey through a meeting focused on membrane biophysics.

Membranes are ubiquitous and diverse on cellular length scales and so is research in this field. In assembling the sessions of the meeting, our idea was to combine a variety of topics, all which in our opinion deserve to be in the spotlight of membrane biophysics research. In particular, we wish to celebrate the contributions of our esteemed colleague and friend Karl Lohner to membrane biophysics and to antimicrobial peptides on the occasion of his retirement. Actually, this was the most important original stimulus for bringing this meeting to Graz, and we are very grateful to Lukas Cwiklik and Hector Martinez-Seara for allowing us to slip this in before their taking terms of organizing EJTEMM in Prague.

This EJTEMM 2021 will differ significantly from all previous EJTEMMs. Although we combined in the spirit of this meeting experimental and theoretical research, online-only means that we will be missing any face-to-face communication, be it after talks, at the posters, over coffee or maybe a glass of beer or wine later in the evening. Yet, meeting the challenge of an online conference also carries many unexpected opportunities. For example, EJTEMM was devised as a European-based meeting, naturally appealing mainly to researchers from Europe and explaining the dominance of invited speakers from this region of the world. EJTEMM 2021 has attendees that most likely would have never found their way to Graz (no matter how beautiful our city is), encompassing 17 (!) time zones. Naturally, this boosts the scientific quality of the meeting, and we were excited to receive so many outstanding abstracts.

Our challenge is to meet all your expectations even if you cannot be here in person. EJTEMM will be hosted by a selection of online tools most of which we have become familiar with during the last year, including WebEx and Slack. This should allow you to communicate across time zones in various ways following some rules that you will find summarized in the guidelines. We intend to provide a platform that brings scientist together to promote research in an open-minded way even if we face barriers in traveling. After all, it will be you making the difference and shaping the character of the meeting. We are looking forward to three exciting days of membrane biophysics and thank you for making this happen.

Sincerely,



Georg Pabst and Sandro Keller

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Acknowledgements/ Sponsors

We thank the advisory board for their support and, in particular, Burkhard Bechinger, Alex Bunker, Lukas Cwiklik, Martin Hof, Alexander Lyubartsev, Hector Martinez-Seara, Norbert Kučerka, Michal Otyepka, Marta Pasenkiewicz-Gierula, Michael Rappolt, and Patrick Trouillas for selecting oral contributions. We have received 126 excellent abstracts giving the selection committee a hard time to narrow down this number to the available slots. We are also indebted to our invited speakers, who all decided to stay with us even in these difficult times. Many thanks to our other members of the organization committee, namely, Sabrina Schröder, Dagmar Zweytick, Nermina Malanovic, and Klaus Groschner for making this meeting possible. Last but not least, we thank Nadine Angerer, Lukas Pein, Birgit Stückler, and Marlies Leopold for their invaluable administrative support.

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General Information

Date & Location

The 7th European Joint Theoretical/Experimental Meeting on Membranes will take place from 7th to 9th April 2021. The University of Graz is going to host the EJTEMM2021 virtually via the platform "Slack".

Of course, this form of conferencing is different and most likely a bit more impersonal when you cannot exchange experiences and information face-to-face, but to create a feeling of "normality", as much interaction as possible is promoted. Therefore, different communication levels are provided, described in detail in the "Guidelines for the virtual EJTEMM 2021".

To be on time for the presentations, keep in mind that **CET** is the corresponding time zone in Graz. The following link tells you what time it is in Graz!

Oral presentations (OP)

Lectures will be given via a provided Cisco WebEx link, which is shared in the Slack Channel "03-live-talks" 10 minutes in advance of the first scheduled presentation every day. All session are colour codes as follows: Complex Biomembrane Mimetics, Membrane Curvature, Non-lamellar Phases, Lipid/Protein Interactions, Lipid Droplets and Monolayers, and Membrane Active Peptides. We have also colour coded our invited talks.

Time slots:

- Invited speakers: **25 minutes**, plus 5 minutes for discussion (30 minutes in total)
- Contributed talks: **10 minutes**, plus 5 minutes for discussion (15 minutes in total).

Poster presentations (PP)

Poster sessions are planned on day 2 and 3 of the meeting. You can find the time schedule below in the section "Poster Presentations – Scheduling" (*page 11*). Each poster abstract contains a number code **PPXX-Y-Z**, where XX just follows the number order and Y (= 1, 2, 3) refers to the allocated session, and Z (= A, B) refers to the time when the poster should be presented live. Code A means first half of the poster session and code B means second half. For example, poster PP07-3-A will be presented live on April 9, 2021 from 11:30 – 12:15.

Posters will be on display during the whole meeting on Slack and open for discussion. Please upload and pin your posters to your session channel on slack and provide a live video chat meeting link using your preferred platform (Zoom, WebEx, BBB, Teams,….) as detailed in a separate email. Of course poster presenters can use the video chats to present their work in form of a short talk (using e.g. Powerpoint). Use threads to discuss posters on Slack, during the off time of the presenting person.

Coffee breaks, lunchtime, dinner break

We will provide break-out sessions on WebEx named after touristic attractions in Graz, where all attendees can meet and chat. Of course, you can also explore Slack and chat privately. One-to-one video calls are also supported on this version of Slack.

Programme – Wednesday, April 7, 2021

13:45 - 14:30	**EJTEMM 2021 Opening** *(Chairs: Sandro Keller & Georg Pabst)*
13:45 - 14:00	Welcome
14:00 - 14:30	**Opening Lecture:** Anthony Watts (University of Oxford, UK) *The importance of water in membrane receptor function*
14:30 - 16:30	**Complex Biomembrane Mimetics** *(Chairs: Marta Pasenkiewicz-Gierula & Norbert Kučerka)*
14:30 -15:00	Rumiana Dimova (Max Planck Institute of Colloids and Interfaces, Germany) *Remodeling of artificial cells: to bud or not to bud*
15:00 - 15:15	Valeria Rondelli (Università degli Studi di Milano, Italy) *Fusion mechanisms of small extracellular vesicles with model plasma membranes*
15:15 - 15:30	Moritz P.K. Frewein (University of Graz, Austria) *Interdigitation-induced interleaflet coupling in asymmetric liposomes*
15:30 - 15:45	Markéta Paloncýová (CATRIN, Czech Republic) *Individual role of artificial lipids in mRNA vaccines*
15:45 - 16:00	Roland Faller (UC Davis, CA) *Controlling lipid printing*
16:00 - 16:30	Siewert Jan Marrink (University of Groningen, The Netherlands) *Modeling complex cell membranes*
16:30 - 17:00	**Coffee Break**
17:00 - 19:00	**Membrane Curvature** *(Chairs: Alex Bunker & Hector Martinez-Seara)*
17:00 - 17:30	Patricia Bassereau (Institut Curie, France) *Membrane curvature influences (or not…) protein distribution and function*
17:30 - 17:45	Anand Srivastava (Indian Institute of Science, India) *Membrane remodeling due to mixture of multiple curvature proteins*
17:45 - 18:00	Danijela Bakarić (Ruđer Bošković Institute, Croatia) *Undulations of phosphatidylcholine lipid bilayers with incorporated palmitic acid are blurred by lateral proton transfer*
18:00 - 18:15	Sebastian Himbert (McMaster University, Canada) *The nanoscopic bending rigidity of red blood cell membranes*
18:15 - 18:30	Luka Mesarec (University of Ljubljana, Slovenia) *Stable discocyte shapes of normal red blood cells explained by membrane's in-plane ordering*
18:30 - 19:00	Primož Ziherl (Józef Stefan Institute, Slovenia) *Vesicle self-replication and aggregation*

Programme – Thursday, April 8, 2021

10:00 - 12:00	**Non-lamellar Phases** *(Chairs: Michael Rappolt & Georg Pabst)*
10:00 - 10:30	John Seddon (Imperial Collage, UK) *Complex non-lamellar inverse phases of lipids*
10:30 - 10:45	Andrea Ridolfi (CSGI, Italy) *Development and structural characterization of inverse bicontinuous cubic phase lipid films*
10:45 - 11:00	Shuo Qian (ORNL, TN) *Water distribution in a fusogenic lipid membrane from grazing-angle neutron diffraction*
11:00 - 11:15	James Jennings (University of Wisconsin-Madison, WI) *Protonation-driven self-assembly of synthetic six-tail lipid A mimics*
11:15 - 11:30	Ondrej Dlouhý (University of Ostrava, Czech Republic) *Lipid polymorphism of subchloroplast membrane particles: Isolated granum and stroma thylakoids*
11:30 - 12:00	Erik Lindahl (Stockholm University, Sweden) *Formation & permeability of the stratum corneum*
12:00 - 14:00	**Lunch Break**
14:00 - 16:00	**Poster Session 1**
16:00 - 18:00	**Lipid/Protein Interactions** *(Chairs: Michal Otyepka & Sandro Keller)*
16:00 - 16:30	Alessandra Luchini (Paul Scherrer Institute, Switzerland) *Investigating membrane proteins with neutron reflectometry*
16:30 - 16:45	Roberto Covino (Frankfurt Institute for Advanced Studies, Germany) *AI-driven computer simulations discover mechanisms of transmembrane assembly*
16:45 - 17:00	Christopher Kelly (Wayne State University, MI) *Nanoscale membrane curvature sorts lipid phases and alters lipid diffusion*
17:00 - 17:15	Hector Martinez-Seara (Czech Academy of Sciences, Czech Repulic) *Improving CHARMM36 simulations by reintroducing the missing electronic polarizability: prosECCo*
17:15 - 17:30	Nina Gubensäk (University of Graz, Austria) *Structural insights into the gram-negative lipid transporter YrbC of hämophilus influenzae*
17:30 - 18:00	Robert Vácha (CEITEC, Czech Republic) *Enhanced Translocation of Amphiphilic Peptides Across Membranes by Transmembrane Proteins*
18:00 - 20:00	**Dinner Break**
20:00 - 22:00	**Poster Session 2**

Programme – Friday, April 9, 2021

10:00 - 11:30	**Lipid Droplets and Monolayers** *(Chairs: Martin Hof & Lukas Cwiklik)*
10:00 - 10:30	Sylvie Roke (École Polytechnique Fédérale de Lausanne, Switzerland) *Molecular level probing and quantification of lipid droplet, liposome and liquid condensate interfaces in aqueous solution, using nonlinear light scattering*
10:30 - 10:45	Fabio Strati (Martin Luther University Halle-Wittenberg, Germany) *Stratum corneum vesicles as promising strategy to treat skin diseases*
10:45 - 11:00	Candelaria Cámara (Universidad Nacional de Córdoba, Argentina) *Dexamethasone and dexamethasone phosphate and their effects on DMPC membrane models*
11:00 - 11:30	Luca Monticelli (CNRS, France) *Molecular simulations of lipids droplets*
11:30 - 13:00	**Poster Session 3**
13:00 - 14:00	**Lunch Break**
14:00 - 15:30	**Membrane Active Peptides I*** *(Chairs: Nermina Malanovic & Burkard Bechinger)*
14:00 - 14:30	Miguel Castanho (Instituto de Medicina Molecular, Portugal) *Diffusion-lysis coupling of antimicrobial peptides in biofilms of S. aureus*
14:30 - 14:45	Adéla Melcrová (University of Groningen, The Netherlands) *An unexpected molecular mechanism unveils the functioning of peptidomimetics as antimicrobials*
14:45 - 15:00	Enrico F. Semeraro (University of Graz) *Partitioning and in-situ kinetics of lactoferricin derived Antimicrobial peptides in Escherichia coli*
15:00 - 16:00	**Coffee Break**
16:00 - 17:15	**Membrane Active Peptides II*** *(Chairs: Dagmar Zweytick & Alexander Lyubartsev)*
16:00 - 16:30	Lorenzo Stella (Università di Roma Tor Vergata, Italy) *A journey in the complex world of host defense peptides: from in silico to cellular studies*
16:30 - 16:45	Mariana Valério (Universidade Nova de Lisboa, Portugal) *Parainfluenza fusion peptide forms oligomeric pore-like structures inside a membrane*
16:45 - 17:00	Bárbara Claro (Universidade do Porto, Portugal) *Self-assembling of antimicrobial cyclic peptides at model membranes: nanotubes or micellar aggregates?*
17:00 - 17:15	Morane Lointier (Université de Strasbourg, France) *Peptide supramolecular assembly as a determinant of biological function*
17:15 - 18:00	**EJTEMM 2021 Closing** *(Chairs: Stefan Knippenberg & Georg Pabst)*
17:15 - 17:45	Closing Lecture: Jesus Perez-Gil (Universidad Complutense, Spain) *Structure-function determinants in pulmonary surfactant protein SP-B, a crucial protein for breathing*
17:45 - 18:00	Famous last words

*celebrating the contributions by Karl Lohner on the occasion of his retirement

Poster Presentations - Scheduling

SESSION 1

Thursday, April 8, 2021 14:00 - 16:00

	Poster Number
"Complex Biomembrane Mimics"	PP01 – PP14
"Membrane Curvature"	PP36 – PP42
"Non-Lamellar Phases"	PP47 – PP50
"Lipid/Protein Interactions"	PP54 – PP62
"Membrane Active Peptides"	PP89

SESSION 2

Thursday,April 8, 2021 20:00 - 22:00

"Complex Biomembrane Mimics"	PP15 – PP31, PP104
"Membrane Curvature"	PP43 – PP44
"Non-Lamellar Phases"	PP51
"Lipid/Protein Interactions"	PP63 – PP76,PP105
"Membrane Active Peptides"	PP90

SESSION 3

Friday, April 8, 2021 11:30 - 13:00

"Complex Biomembrane Mimics"	PP32 – PP35, PP101
"Membrane Curvature"	PP45 – PP46
"Non-Lamellar Phases"	PP52 – PP53
"Lipid/Protein Interactions"	PP77 – PP87, PP102
"Lipid Droplets and Monolayers"	PP88 – PP90
"Membrane Active Peptides"	PP91 – PP100, PP103

Abstracts – Presentations

The order of the abstracts of all presentations follows the conference schedule

Abbreviations:

OP – Oral presentation

PP – Poster presentation

Oral presentations - Overview

OP01, OP02… oral presentation number

"Complex Biomembrane Mimics"	OP01-OP07
"Membrane Curvature"	OP08-OP13
"Non-Lamellar Phases"	OP14-OP19
"Lipid/Protein Interactions"	OP20-OP25
"Lipid Droplets and Monolayers"	OP26-OP29
"Membrane Active Peptides"	OP30-OP38

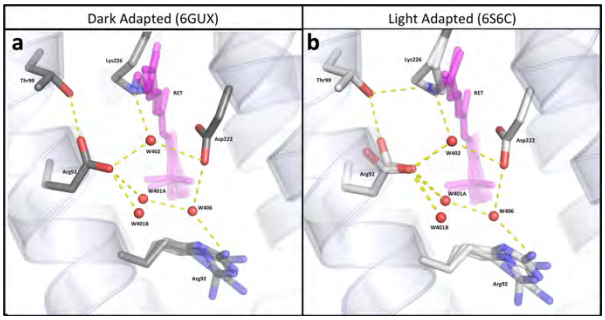
OP01

THE IMPORTANCE OF WATER IN MEMBRANE RECEPTOR FUNCTION

Anthony Watts

Biochemistry Department, University of Oxford, Oxford, OX1 3QU, UK

Resolving conformational changes in membrane receptors in response to a stimulus, and capturing their functionally relevant dynamics, is very challenging. Over the years we have addressed this challenge using a range of spectroscopic approaches[1,2,3] on functionally competent photoreceptors, often in their natural membranes[4] or Lipodisqs™ [5]. More recently, we have complemented this work with very high resolution (1.07Å) crystallography[6] and photo-induced x-ray, free electron laser studies, without the use of detergents and including natural lipids. This high resolution information reveals waters and their importance in both receptor activation-desensitization and QM(SCC-DFTB)/MM MD trajectories give information about the activation process. The system studied is achearhodopsin-3 (AR3), a photoreceptor utilized widely in optogenetics despite the lack of structures until now. We suggest that the different arrangement of internal water networks in AR3 is responsible for the faster photocycle kinetics compared to homologs – AR3 is ~10x more efficient than bacteriorhodopsin at current generation. These insights may well have generic implications for other receptors.



[1] Higman et al., (2011) Angew. Chemie 50(36):8432

[2] Dijkman et al., (2018) Nature Comms. 9:1710

[3] Dijkman et al., (2020) Science Advances, 6:33

[4] Lavington & Watts (2020) Biophys. Rev. 12:1287

[5] Juarez et al., (2019) Chem. Phys. Lipids 221:167

[6] Juarez et al (2021) Nature Comms. 12:629

OP02

REMODELING OF ARTIFICIAL CELLS: TO BUD OR NOT TO BUD

Rumiana Dimova

Max Planck Institute of Colloids and Interfaces, Science park Golm, 14424 Potsdam, Germany

Cellular membranes exhibit a large variation in curvature. It is a common perception that curvature is mainly generated by the activity of specific protein species. However, curvature can be readily generated by various other asymmetries, which plausibly represent a governing factor for defining shapes of membrane organelles and their remodeling. As a workbench for artificial cells, we employ giant vesicles (10-100μm), see "The Giant Vesicle Book" Eds. Dimova&Marques, CRC Press, 2020. Here, we will introduce approaches employing them for the precise quantification of the membrane spontaneous curvature. Several membrane remodelling examples will be considered: asymmetric distribution of ions across the membrane (Nano Lett. 18:7816,2018), insertion/desorption of the ganglioside GM1 (Proc.Natl.Acad.Sci.USA. 115:5756,2018), asymmetric lipid distribution (Sci.Rep. 8:11838,2018) and PEG adsorption and internal droplets (Proc.Natl.Acad.Sci.USA. 108:4731,2011; ACS Nano 10:463,2016). All of these factors can reshape the membrane triggering formation of buds/nanotubes. We will also show how curvature generation by protein adsorption at low surface density is able to modulate membrane morphology and topology to the extent of inducing vesicle fission (Nature Commun, 11:905,2020). The presented examples will demonstrate that even in the absence of proteins and active processes, the membrane is easily remodeled by simple physicochemical factors.

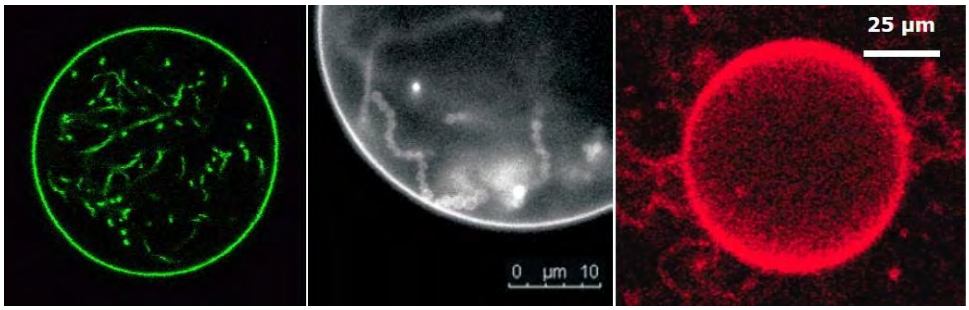


Figure: Membranes can be deformed into tubes (cylindrical, necklace-like, inward and outward, first three images) upon interactions with various molecules and exhibit budding upon wetting by droplets (last snapshot) as evidenced on giant unilamellar vesicles.

OP03

FUSION MECHANISMS OF SMALL EXTRACELLULAR VESICLES WITH MODEL PLASMA MEMBRANES

V. Rondelli[1], P. Brocca[1], F. Perissinotto[2,3], L. Casalis[2], P. Parisse[2,4]

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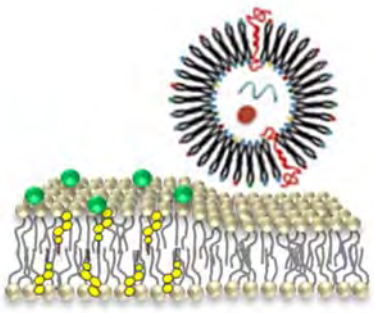
[2] *Elettra Sincrotrone Trieste, Trieste, Italy.*

[3] *Center for Infection and Immunity of Lilli, INSERM U1019, Lille, France.*

[4] *CNR-IOM, Trieste, Italy.*

Extracellular vesicles (EVs) are a potent intercellular communication system. Such small vesicles transport biomolecules between cells and throughout the body, strongly influencing the fate of recipient cells. Due to their specific biological functions they have been proposed as biomarkers for various diseases and as optimal candidates for therapeutic applications. Despite of their extreme biological relevance, their mechanisms of interaction with the membrane of recipient cells are still hotly debated. We performed a multiscale investigation based on AFM, SAXS, SANS and Neutron Reflectometry to reveal structure-function correlations of purified EVs in interaction with model membrane systems of variably complex composition, to spot the role of different membrane phases on the vesicles internalization routes. Our analysis reveals a strong interaction of EVs with the model membranes and preferentially with the borders of protruding phase domains. Moreover, we found that upon vesicle breaking on the model membrane surface, the biomolecules carried by/on EVs diffuse in a way that departs from the expected simple fusion. Our approach has clear implications on the modulation of EVs internalization routes by targeting specific domains at the plasma cell membrane and, as a consequence, on EVs-based therapies.

[F. Perissinotto, V. Rondelli et al., Nanoscale, in press]



OP04

INTERDIGITATION-INDUCED INTERLEAFLET COUPLING IN ASYMMETRIC LIPOSOMES

M.P.K. Frewein[1,2,3,4], F.A. Heberle[5], M. Doktorova[6], Haden L. Scott[5], Enrico F. Semeraro[1,3,4], L. Porcar[2] and G. Pabst[1,3,4]

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[3]BioTechMed Graz, 8010 Graz, Austria
[4]Field of Excellence BioHealth – University of Graz, 8010 Graz, Austria
[5]Department of Chemistry, The University of Tennessee, Knoxville, TN 37996, USA [6]Department of Integrative Biology and Pharmacology, The University ofTexas Health Science Center at Houston, Houston, TX 77030

Cellular envelopes contain a large number of lipid species that are distributed asymmetrically between the two leaflets of the bilayer [1]. One of the enduring questions of plasma membrane architecture and lipid asymmetry concerns the possibility of interleaflet coupling even in the absence of proteins, which may influence a number of physiological processes [2]. Currently conceived lipid-mediated coupling mechanisms consider either intrinsic lipid curvature, headgroup electrostatics, cholesterol flip-flop, dynamic chain interdigitation, or thermal membrane fluctuations [3]. We use asymmetric large unilamellar lipid vesicles (aLUVs), produced via cyclodextrin-mediated lipid exchange [4], to study the effect of interdigitation stress on membrane structure and dynamics, characterized by small-angle neutron and X-ray scattering as well as neutron spin-echo. We present data on vesicle systems containing mixed-chain phosphatidylcholine or sphingomyelin with high chainlength mismatch, and the effect of the packing density in their opposing leaflet, demonstrated by saturated and mono-unsaturated phosphatidylcholine.

[1] J.H. Lorent et al., Nat Chem Biol 16.6, 644 (2020)

[2] K. Simons and D. Toomre, Nat Rev Mol Cell Biol 1, 31 (2000)

[3] J.D. Nickels et al., Chem Phys Lip 192, 87 (2015)

[4] M. Doktorova et al., Nat prot 13.9, 2086 (2018)

OP05

INDIVIDUAL ROLE OF ARTIFICIAL LIPIDS IN MRNA VACCINES

M. Paloncýová, P. Čechová, M. Šrejber and M. Otyepka

Czech Advanced Technology and Research Institute (CATRIN), Palacky University Olomouc, Křížkovského 511/8, 779 00 Olomouc, Czech Republic

Rising interest in liposomal drug delivery in the last years, reached new urgency due to the COVID-19 pandemic. In an unprecedented effort uniting global scientific community, several vaccine mechanisms were proposed, one of them being mRNA vaccines. The immunogenic messenger RNA coding SARS-CoV-2 spike protein is enveloped by a liposome consisting of a mixture of natural and engineered lipids. These liposomes or liposome-derived nanoparticles are tailored to attain desired features of antigen carriers. However, the full nature of the liposome-mRNA interactions is not yet understood - and can become a focus of *in silico* studies for gaining deeper insight.

Both manufacturers of mRNA SARS-CoV-2 vaccines, Pfizer&BioNTech and Moderna use a four lipid mixture. Natural phosphatidylcholine (DSPC) and cholesterol are used together with unique lipid species, such as artificial ionizable cationic lipids and lipids with a polymeric polyethylene glycol (PEG) headgroup modifications. Those have been experimentally shown to influence the liposome size and stability. To evaluate the effect of these clinically designed lipids on the membrane properties, we created *in silico* models of nitrogenated cationic, and PEGylated lipids in form of lipid bilayers, mimicking the presumed vaccine liposome particle composition in all-atom and coarse grained resolution. We focused on the effect of individual lipid species on bilayer stability and overall membrane structural and dynamic parameters. Furthermore, we studied temperature dependence behavior of membrane models as it is one of the key parameters in liposome-based vaccines delivery/storage, and systematically modified membrane composition in order to identify the role of individual lipids.

OP06

CONTROLLING LIPID PRINTING

B. Harris[1], A. Karsai[2], Y. Zheng[2], Y. Huang[2], G.-Y. Liu[2], R. Faller[1]

[1] UC Davis, Department of Chemical Engineering, One Shields Ave, Davis, CA, 95616, USA

[2] UC Davis, Department of Chemistry, One Shields Ave, Davis, CA, 95616, USA

The molecular understanding of 3D printing and controlled assembly of soft materials is hugely important for the future development in many areas from artificial tissue to energy harvesting. Lipid features formed after deposition of ultrasmall aqueous droplets containing lipids depend on the interplay between the lipids, surface, evaporation, and spatial confinement. Here we are presenting an integrated molecular simulation and experimental effort to understand the printing of lipids on different substrates with various hydrophilicities.

We are using the Martini simulation model to study the assembly of lipids on various substrates during the drying process in order to understand the stacking and arrangement as a function of surface chemistry as well as concentration. In parallel experimental efforts, lipids are deposited using microfluidic probes and characterized using AFM to control and reveal lipid assembly, respectively.

We find that very defined multilayer stacks are stable under a variety of conditions. The simulations clearly show that for hydrophobic surfaces the system is less ordered. under spatial confinement and with increasing lipid concentration (i.e., drying) the system becomes more ordered both laterally and along surface normal directions.

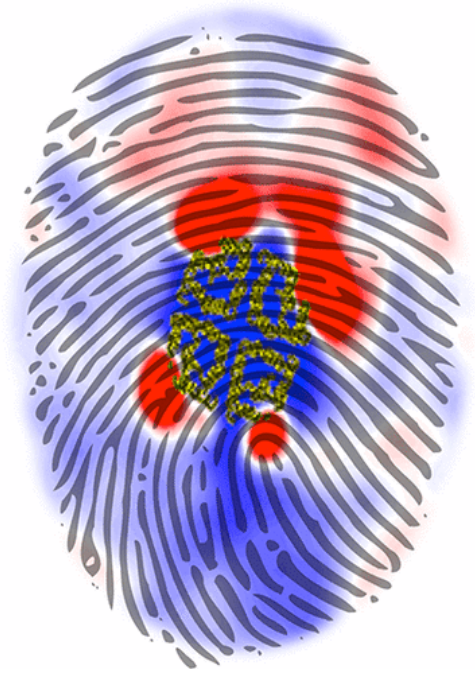
OP07

MODELING COMPLEX CELL MEMBRANES

S.J. Marrink

University of Groningen, The Netherlands

In this lecture I will describe our current efforts to capture the dynamic organization of cell membranes, based on the coarse-grain Martini model developed in our lab [1]. I will illustrate the power of the model by providing a few in-depth examples of large-scale simulations involving membranes with realistic composition, in particular, the lateral organization of lipids and proteins in complex plasma membrane models [2,3].



Proteins embedded in a realistc plasma membrane show distinct protein-lipid interaction patterns. These 'fingerprints' are fundamental to the lateral organizational principles of cell membranes.

[1] S.J. Marrink, D.P. Tieleman. Chem. Soc. Rev. 42, 6801 (2013)
[2] H.I. Ingólfsson et al. JACS 136, 14554 (2014)
[3] V. Corradi et al., ACS Central Sci. 4, 709 (2018)

OP08

MEMBRANE CURVATURE INFLUENCES (OR NOT…) PROTEIN DISTRIBUTION AND FUNCTION

F.C. Tsai[1], A. Damm[1], C. Prévost[1], B. Sorre[1], M. Simunovic[1], A. Mahalka[1], A. Callan-Jones[2] P. Bassereau[1]

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[2] *Laboratoire Matière et Systèmes Complexes, Université de Paris, France*

Cell membranes are highly deformable and have to be strongly curved, for instance during trafficking when small buds form and eventually detach from cell membranes, or during cell migration upon the formation of actin-sustained cellular protrusions (filopodia). These membrane-shaping processes always require the interaction with proteins (for instance BAR domain proteins with an intrinsically-curved shape) and in some cases with the actin cytoskeleton. *In vitro* membrane systems with controlled curvature combined to theoretical models have been instrumental for understanding how proteins and cytoskeleton shape cellular membranes, and conversely how membrane curvature is a cue for the local enrichment of peripheral or integral proteins with non-symmetric shape. Similarly, they help understanding how non-curvature sensing proteins are recruited on curved membranes. In this talk, I will discuss examples of proteins with intrinsically-curved shapes that are enriched in curved membranes due to their coupling to membrane curvature, and also how they assist the recruitment of non-curvature sensing proteins. Finally, I will show that membrane curvature can affect the conformation distribution of trans-membrane proteins and thus their activity.

OP09

MEMBRANE REMODELING DUE TO MIXTURE OF MULTIPLE CURVATURE PROTEINS

G. Kumar and A. Srivastava

Molecular Biophysics Unit, Indian Institute of Science-Bangalore, Bangalore, India

Here, we address membrane remodeling due to a mixture of differently curved proteins using mesoscopic Monte-Carlo simulations where proteins are modeled as nematics adhering to a deformable fluid membrane elastic triangulated sheet. The local orientation of the nematic field is denoted by the unit vector that lies in the local tangent plane of the membrane and is free to rotate in this plane. Protein-membrane interactions are modeled as anisotropic spontaneous curvatures of the membrane and protein-protein interactions are modeled by the splay and bend terms of Frank's free energy for nematic liquid crystals. Our simulation results show different morphologies of deformed vesicles that depend on the curvatures and densities of the participating proteins as well as on the protein-protein and membrane-proteins interactions.

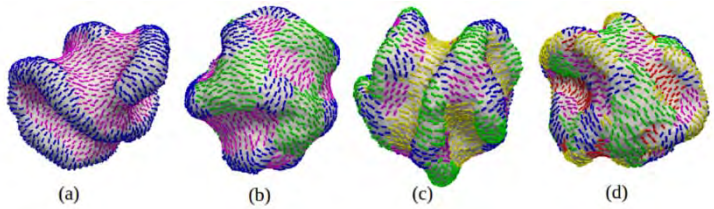

FIG. 1. Deformed vesicle morphologies obtained by Monte Carlo simulation. Panel shows the results for two, three, four and five different curvatures of proteins with curvature values (± 0.5), $(0, \pm 0.5)$, $(\pm 0.5, \pm 0.8)$ and $(0, \pm 0.5, \pm 0.8)$. Different colors

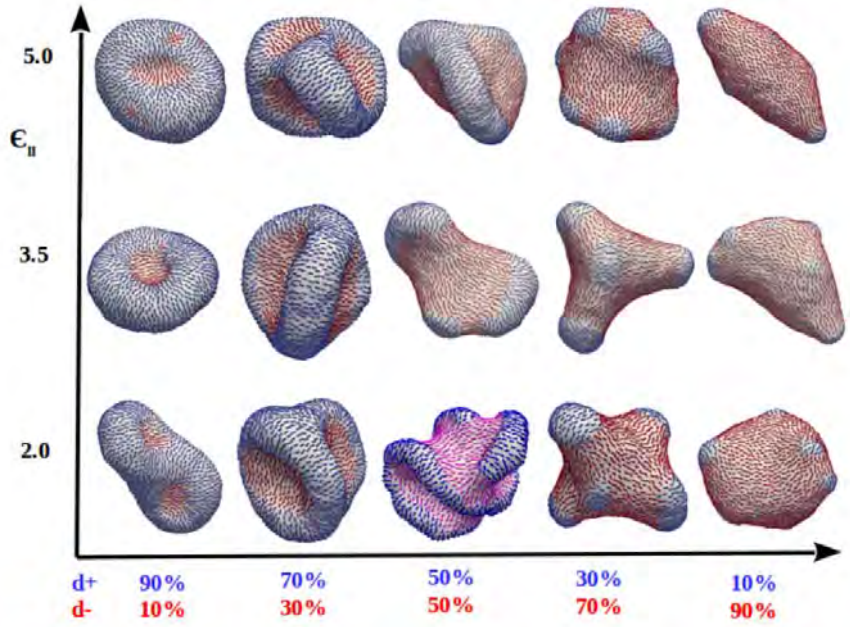

FIG. 2. Panel shows the vesicle deformation due to two different proteins with curvature (± 0.5). Here the different vesicle shapes are obtained with different proteins densities and different protein-protein interactions. On the X axis, density of positively curved protein is decreasing while negatively curved proteins in increasing. On the Y axis, nematic-nematic interaction in increasing. Other parameters are $\kappa = 20$, $\kappa_{\parallel} = 5$ in $K_b T$ unit.

OP10

UNDULATIONS OF PHOSPHATIDYLCHOLINE LIPID BILAYERS WITH INCORPORATED PALMITIC ACID ARE BLURRED BY LATERAL PROTON TRANSFER

Z. Brkljača[1], M. Pišonić, I. Crnolatac[1], M. Vazdar[1,3], D. Bakarić[1]

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[2] *Chemistry Department, Faculty of Science and Mathematics, University of Zagreb, Zagreb, Croatia*
[3] *Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic*

Proton transfer along biological interfaces is exceptionally difficult to unravel from both experimental and theoretical perspective [1]. In addition to rather scarce computational studies, experimental endeavours are mostly focused on the incorporation of membrane-anchored pH-sensitive dyes that detect the arrival of a migrating proton. Evidently, the establishment of a probe-free method would serve as considerable breakthrough in deepening our knowledge on lateral proton transfer. In this regard, we have examined lipid multibilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) in the absence and the presence of small amount of palmitic acid (PA) (2 %) in buffers of pH values 4 ≤ pH ≤ 9 by FT-IR spectroscopy. Depending on the pH value, PA can be protonated (COOH) or deprotonated (COO^-) and, consequently, placed at different depth of a lipid bilayer [3]. The presence of such small amount of PA should not significantly modify characteristic undulations of DPPC multibilayers surface [4], but accompanying proton transfer is expected to leave its signature. The latter are indirectly detected by FT-IR spectroscopy coupled with MCR-ALS with EFA analysis (Fig. 1) that is very sensitive and powerful tool in discerning of coupled events [5]. Experimental observations are corroborated by molecular dynamics (MD) simulations and calorimetric measurements.

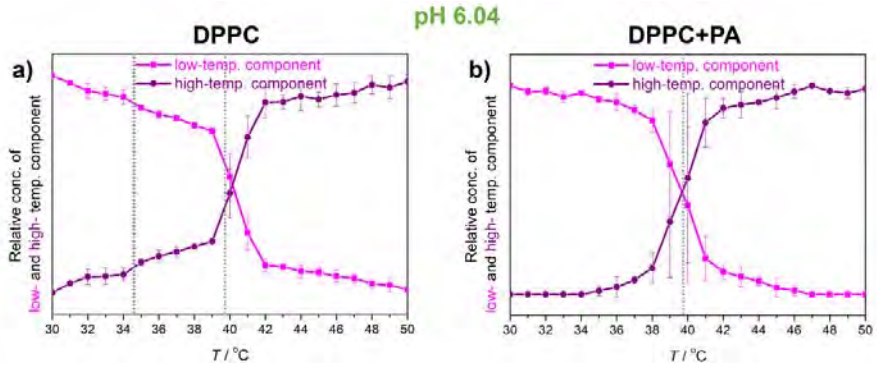


Fig. 1. Determination of T_m and T_p from FT-IR spectra using MCR-ALS with EFA in: a) DPPC and b) DPPC+PA at pH=6.04

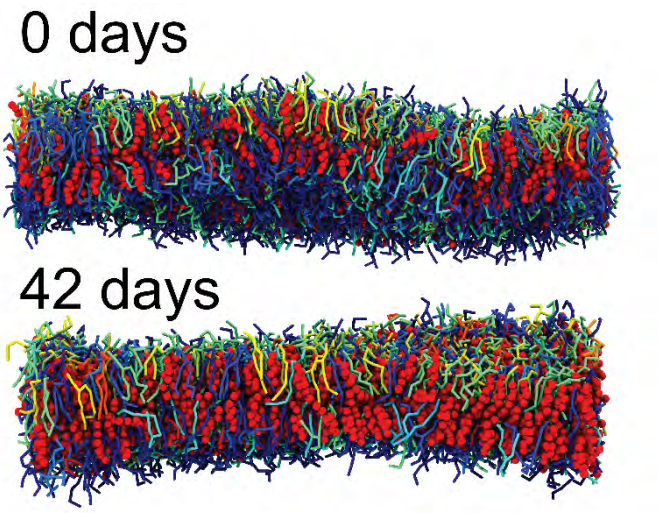
[1] N. Agmon, H. J. Bakker, R. K. Campen, R. H. Henchman,P. Pohl, S. Roke, M. Thämer, A. Hassanali, Chem. Rev. ***116*** *(2016) 7642−7672.*
[2] C. Zhang,1, D. G. Knyazevb, Y. A. Vereshaga, E. Ippoliti, T. H. Nguyen, P. Carlonia, P. Pohl, Proc. Natl. Acad, Sci. ***109*** *(2012) 9744–9749.*
[3] A. A. Pashkovskaya, M. Vazdar, L. Zimmermann, O. Jovanović, P. Pohl, E. E. Pohl, Biophys. J. ***14*** *(2018) 2142–2151.*
[4] K. Akabori, J. F. Nagle,Soft. Matter. ***11*** *(2015) 918–926.*
[5] P. Maleš, Z. Brkljača, I. Crnolatac, D. Bakarić, submitted.

OP11

THE NANOSCOPIC BENDING RIGIDITY OF RED BLOOD CELL MEMBRANES

S. Himbert, M. Rheinstädter

Department of Physics and Astronomy, McMaster University, 1280 Main Street West, Hamilton, Canada.



Blood banks all around the world store blood for several weeks ensuring the availability of blood for transfusion medicine. Although the storage conditions have been optimized for decades it has become evident that red blood cells (RBC) undergo numerous changes when being stored.

We investigated the effect of storage on the nanoscopic bending rigidity of RBC membranes with a combination of Molecular Dynamics simulations, inelastic neutron scattering and diffuse X-ray scattering [1]. Coarse grained (CG) models of RBC membranes were created by matching experimental lipidomic analysis. It was found experimentally that the concentration of fatty acids and cholesterol changes during storage and aged membranes were mimicked by adjusting the lipid composition accordingly.

Solid supported membrane stacks of fresh and stored RBC samples were prepared. X-ray diffraction experiments were then conducted at high relative humidity allowing to reconstruct the membrane surface fluctuations from diffuse scattering signals using a GPU accelerated workstation. These experiments were complemented by Neutron Spin Echo measurements on RBC vesicles which probe the membrane fluctuations directly. Bending moduli of 1.8 k_BT and 15.4 k_BT were measured in excellent agreement with the simulation data.

[1] Himbert et al., "The Nanoscopic Bending Rigidity of Red Blood Cell Membranes", in preparation

OP12

STABLE DISCOCYTE SHAPES OF NORMAL RED BLOOD CELLS EXPLAINED BY MEMBRANE'S IN-PLANE ORDERING

L. Mesarec[1], W. Góźdź[2], A. Iglič[1,3], V. Kralj-Iglič[3,4,5], E. G. Virga[6] and S. Kralj[7,8]

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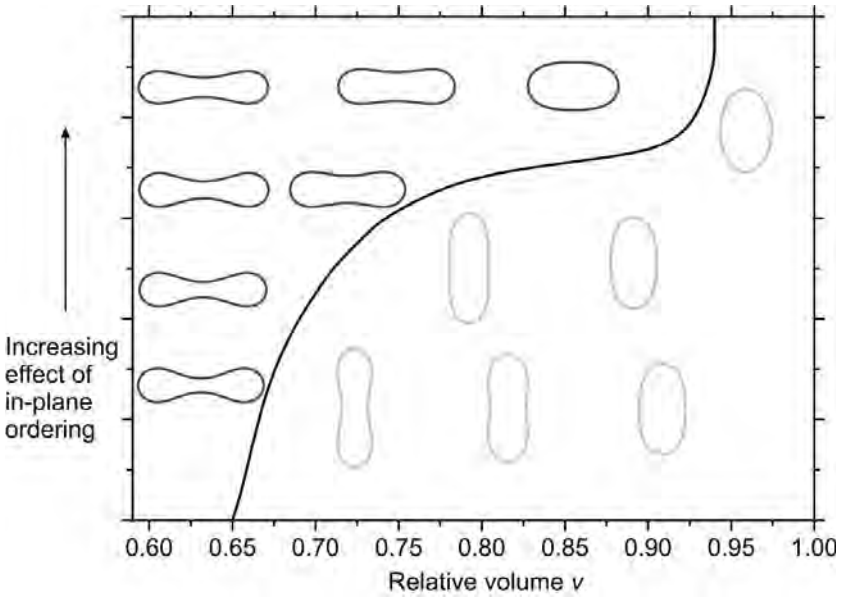
[2]*Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland*
[3]*Laboratory of Mass Spectrometry and Proteomics, Institute of Biosciences and BioResources, National Research Council of Italy, Napoli, Italy*
[4]*Laboratory of Clinical Biophysics, Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia*
[5]*Laboratory of Clinical Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*
[6]*Department of Mathematics, University of Pavia, Pavia, Italy*
[7]*Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia*
[8]*Condensed Matter Physics Department, Jožef Stefan Institute, Ljubljana, Slovenia*

Red blood cells (RBCs) are present in almost all vertebrates and their main function is the transport of oxygen to the body tissues. In almost all mammals in normal conditions, RBCs adopt discocyte (oblate) shape, which optimizes their flow properties in vessels and capillaries. Experimental observations show that stable discocytes range in a relatively broad window of relative volume values between v~0.58 and v~0.8. However, these observations are not supported by existing theoretical models for membrane shapes, which predict stable discocyte RBCs only in a quite narrow interval between v~0.59 and v~0.65. We demonstrate that this interval can be significantly broadened if we take into account membrane's in-plane ordering. In our study, we model RBCs by using a hybrid Helfrich-Landau mesoscopic approach. We show that an extrinsic (deviatoric) curvature free energy term is crucial for explaining experimentally observed stability range of the RBC discocyte shapes.



OP13

VESICLE SELF-REPLICATION AND AGGREGATION

T. Jimbo[1], Y. Sakuma[1], N. Urakami[2], K. Murakami[1], R. Ebihara[1], T. Kono[1], T. Chiba[1], P. Ziherl[3,4], and M. Imai[1]

[1]Department of Physics, Tohoku University, Aoba, Sendai, Japan.
[b]Department of Physics and Information Sciences, Yamaguchi University, Yamaguchi, Japan.
[3]Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia.
[4]Jožef Stefan Institute, Ljubljana, Slovenia.

The understanding of vesicle self-replication and the structure of vesicle aggregates may provide valuable insight into the mechanics of the early stages of embryonic development. We present combined experimental and theoretical studies of two topics in the field. First we discuss self-replicating binary vesicles which, when placed at a temperature-controlled stage, undergo repeated shape deformation to a dumbbell shape and division upon heating and return to a spherical shape upon cooling [1,2]. We use a fast confocal microscope to analyze the morphometry of the dividing vesicles across several generations, showing that the total membrane area is conserved. The deformation pathway is interpreted using the area-difference-elasticity theory, postulating that there exists a tiny asymmetry in the composition of the monolayers. Second, we investigate the shape of vesicle doublets and other aggregates, finding that the contact zones may be either spherical or sigmoidal [3]. We show that the standard model based on the constant-volume and the constant-area constraint is inadequate and that the contact and the non-contact parts of the membranes are generally characterized by dissimilar surface tensions [4].

[1] Y. Sakuma and M. Imai, Model system of self-reproducing vesicles, Phys. Rev. Lett. ***107****, 198101 (2011).*
[2] T. Jimbo, Y. Sakuma, N. Urakami, P. Ziherl, and M. Imai, Temperature-controlled self-reproduction cycle of binary vesicles, Biophys. J. ***110****, 1551 (2016).*
[3] P. Ziherl and S. Svetina, Flat and sigmoidally curved contact zones in vesicle-vesicle adhesion, Proc. Natl. Acad. Sci. USA ***104****, 761 (2007).*
[4] K. Murakami, R. Ebihara, T. Kono, T. Chiba, Y. Sakuma, P. Ziherl, and M. Imai, Morphologies of vesicle doublets: Competition among surface tension, bending elasticity, and adhesion, Biophys. J. ***119****, 1735 (2020).*

OP14

COMPLEX NON-LAMELLAR INVERSE PHASES OF LIPIDS

J. M. Seddon[1], A.I.I. Tyler[2], H.M.G. Barriga[3], A.M. Squires[4], E.S. Parsons[5], S.D. Connell[6], O. Ces[1], R. V. Law[1], N. J. Brooks[1]

[1]*Chemistry Department, MSRH, Imperial College London, London W12 0BZ, UK.*
[2]*School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK.*
[3]*Dept. of Medical Biochemistry and Biophysics, Karolinska Institutet, SE 171 77 Stockholm, Sweden.*
[4]*Department of Chemistry, University of Bath, Bath, UK.*
[5]*London Centre for Nanotechnology, University College London, London, UK.*
[6]*School of Physics and Astronomy, University of Leeds, Leeds, UK.*

Lyotropic liquid crystals of 1-, 2-, or 3-dimensional periodicity spontaneously assemble when lipids are mixed with solvent under various conditions of temperature, pressure and hydration. There are two quite distinct types of inverse cubic phases: *bicontinuous* ones based on underlying periodic minimal surfaces, and *discontinuous* ones based on simple or more complex packings of discreet inverse micelles.

By incorporation of charged phospholipids, we have been able to swell inverse bicontinuous cubic phases to approx. 500 Å, with water channels of approx. 220 Å diameter, potentially expanding the range of usefulness of such phases for applications. We have produced swollen cubosomes and shown incorporation of a large protein (the lectin PHA-L). We have studied the effect of chain branching on the phase behaviour of a series of synthetic β-D-glucosides derived from Guerbet alcohols, whose total hydrocarbon chain length ranged from C_8 to C_{24}. A wide range of liquid-crystalline phases was observed, with the C_{16} Guerbet glucoside (i.e. β-Glc-$C_{10}C_6$) forming an Ia3d bicontinuous cubic phase of space group in excess aqueous solution, which is very unusual – and potentially useful - behaviour. We have been able to produce well-aligned samples of cubic phases on solid substrates for study by Gi-SAXS and AFM (A.I.I. Tyler et al., unpublished data).

We have previously shown that by addition of weakly-polar amphiphiles such as diacylglycerols to phospholipids, we can tune the interfacial curvature to be strongly inverse, leading to the formation of a discontinuous cubic phase of spacegroup Fd3m, with a structure based upon a complex close packing of two types of quasi-spherical inverse micelles. We have investigated the effect of hydrostatic pressure on the structure and stability of this phase, and have discovered a number of novel effects. We have also studied the structure of this phase by contrast variation neutron scattering, and have been able to demonstrate that the more weakly amphiphilic diacylglycerol component is preferentially located in the smaller, more highly curved inverse micelles, and conversely for the phospholipid component (A.I.I. Tyler, unpublished data).

We have dispersed this bulk Fd3m phase into 'micellosomes' by sonication in the presence of the amphiphilic block copolymer F127, and have used x-ray diffraction to compare their structure to that of the bulk Fd3m cubic phase (A.M. Sartor et al., unpublished data).

Some time ago we discovered a lyotropic phase of space group $P6_3/mmc$, whose structure is based upon a 3-D hexagonal packing of quasi-spherical inverse micelles, in a hydrated mixture of dioleoyl phosphatidylcholine, dioleoyl glycerol, and cholesterol. This phase is expected to have a greater chain packing frustration than the Fd3m cubic phase, and it appears that the cholesterol is able to relieve the chain packing frustration within the hydrophobic region of this phase, allowing the $P6_3/mmc$ phase to form.

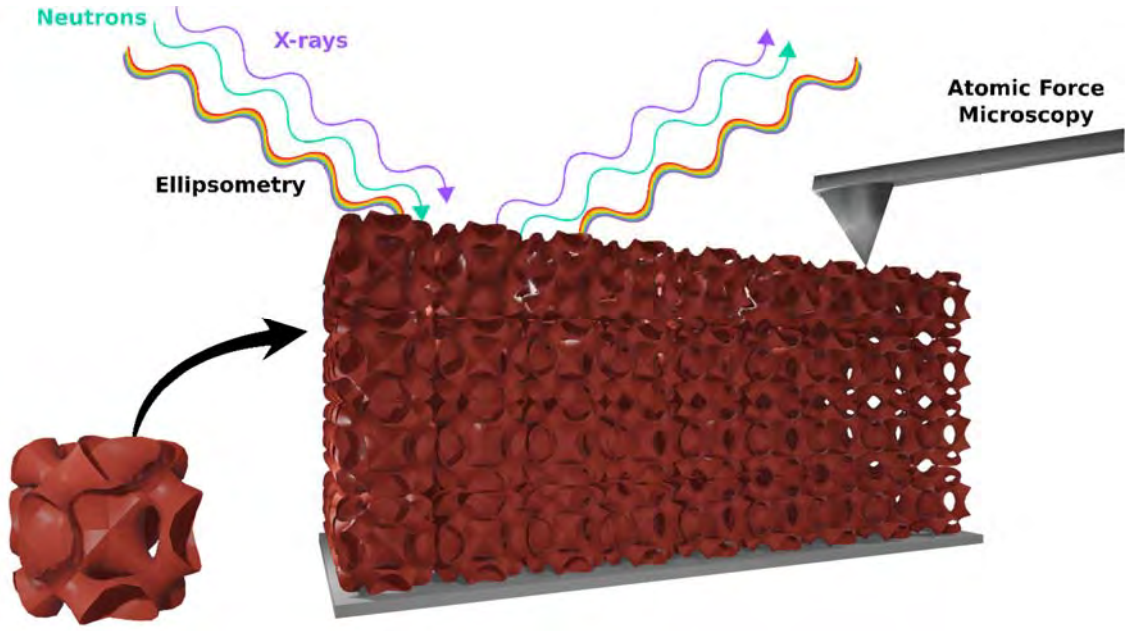
OP15

DEVELOPMENT AND STRUCTURAL CHARACTERIZATION OF INVERSE BICONTINUOUS CUBIC PHASE LIPID FILMS

A. Ridolfi[1,2,3], L. Caselli[1,3], C. Montis[1,3], B. Humphreys[4], G. Mangiapia[5], D. Berti[1,3], T. Nylander[4], M. Brucale[1,2] and F. Valle[1,2].

1) Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase (CSGI), Florence, Italy
2) Consiglio Nazionale delle Ricerche, Istituto per lo Studio dei Materiali Nanostrutturati (CNR-ISMN), Bologna, Italy
3) Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Florence, Italy
4) Department of Physical Chemistry, Lund University, Lund, Sweden
5) GEMS am Heinz Maier-Leibnitz Zentrum (MLZ), Helmholtz-Zentrum Geesthacht GmbH, Garching, Germany.

Non-lamellar lipid membranes are ubiquitous in nature; in particular, inverse bicontinuous cubic phase membranes are thought to be relevant for understanding important biological processes like cell fusion and food digestion (*Nature communications* 6.1,2015:1-8). A lot of studies focused on the development of cubic phase nanoparticles (cubosomes) for drug delivery purposes (*Nanoscale*, 2018,10,3480-3488; *JCIS* 541,2019:329-338); however, few efforts have been made for developing homogeneous supported cubic phase lipid films (*Interface focus* 7.4,2017:20160150). These membrane mimics would allow studying interactions of biomolecules and inorganic/organic nanoparticles (NPs) with these still poorly characterized lipid architectures. In this framework, we herein show the development of supported cubic phase lipid films and the characterization of their properties via multiple techniques. Exploiting different strategies, we functionalize the surfaces with homogeneous lipid films of different thickness and presenting highly oriented cubic architectures. We then characterize these films by employing various techniques, from Neutron and X-ray scattering to a combination of Atomic Force Microscopy and Ellipsometry, which allows evaluating the film structure and stability without relying on large scale facilities. Finally, we present our latest study on the interaction of differently shaped AuNPs with the cubic architecture, providing an example of how these cubic phase lipid films can be employed for studying membrane interaction processes.



OP16

WATER DISTRIBUTION IN A FUSOGENIC LIPID MEMBRANE FROM GRAZING-ANGLE NEUTRON DIFFRACTION

S. Qian

Oak Ridge National Laboratory, Oak Ridge, TN U.S.A.

Grazing-angle neutron diffraction is a valuable technique to study large unit cell structure such as lipid membranes and their morphology under the influence of other molecules. The relatively large scale of the lipidic structure, in orders of several nanometers, necessitates a diffractometer covering small angular range for the application. The film-like sample geometry further requires the neutron beam to be grazing-incident at the sample surface.

With this technique, we obtained the first direct imaging of water distribution between lipid bilayers transiting from a lamellar structure to non-lamellar rhombohedral phase, which depicts the water layer change during the first step of membrane fusion process (*JPCL, 9, 18, 5578*). The D_2O used in hydrating the sample stands out of the structure with much higher neutron scattering length density. The results show the planar water was significantly reduced and was squeezed into pockets around the hemifusion stalk.

The configuration we have implemented expands the application of a regular small-angle neutron scattering instrument, and it can be adopted by other similar instruments. The experiment also demonstrated the recently developed the time-of-flight small-angle neutron beam line is suitable for Grazing-angle neutron diffraction that provides detailed structure of lipid membrane complex.

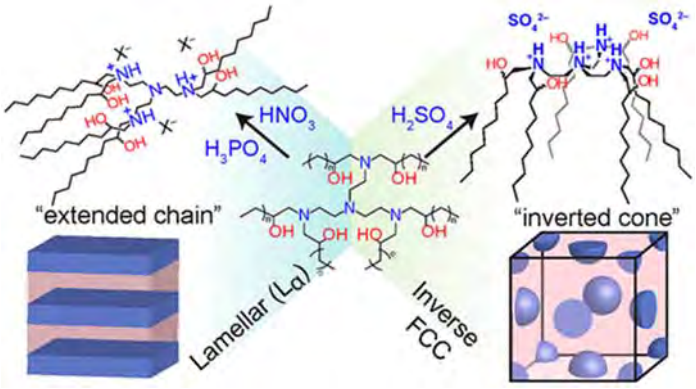
OP17

PROTONATION-DRIVEN SELF-ASSEMBLY OF SYNTHETIC SIX-TAIL LIPID A MIMICS

J. Jennings[1,2], M. C. D. Carter[2], A. L. R. Lopez[2], X. Guo[2], C. Y. Son[2], Q. Cui[2], D. M. Lynn[2], and M. K. Mahanthappa[2]

[1]*Institute of Molecular Biosciences, University of Graz, Humboldtstr. 50/III, 8010 Graz, Austria;* [2]*Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706, United States.*

The self-assembly behaviour of amine-based “lipidoids”, a class of synthetic molecules that mirror essential structural features of the bacterial amphiphile lipid A, is reported. Small-angle X-ray scattering studies demonstrate that protonation of the tetra(amine) headgroups of six-tail lipidoids in aqueous HCl, HNO_3, H_2SO_4, and H_3PO_4 solutions drives their self-assembly into lamellar and inverse micellar lyotropic liquid crystals (LLCs). Like Lipid A, self-assembly is strongly dependant on the aqueous environment, amphiphile tail length, and temperature. Lipidoids protonated in $H_2SO_{4(aq)}$ exhibit inverse body-centered cubic (BCC) and inverse face-centered cubic (FCC) micellar morphologies, the latter of which unexpectedly coexists with zero mean curvature lamellar phases. Atomistic molecular dynamics simulations provide insights into this unusual self-assembly behaviour, demonstrating how lipidoids can adopt both inverse conical and chain-extended molecular conformations depending on the tail length and protonation state. Stable LLC particles formed using lipidoids are a rare synthetic example of isasomes, which are commonly prepared from biological lipids. These synthetic cubosomes and multi-lamellar particles are shown to be responsive to the aqueous environment and can encapsulate and deliver small molecule actives. Further derivatisation of these Lipid A-mimicking molecules imparts antibacterial properties, thought to originate from membrane disrupting pathways.



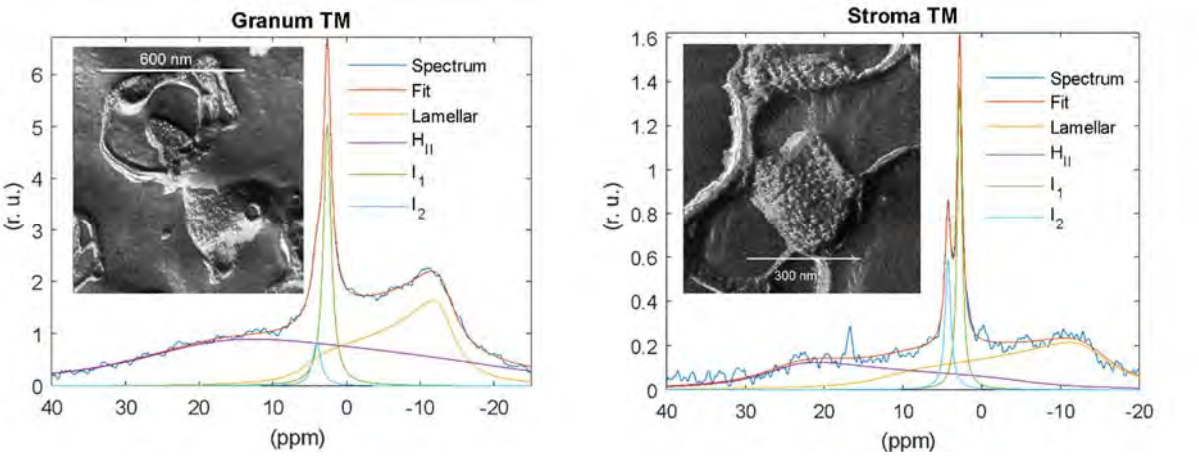
OP18

LIPID POLYMORPHISM OF SUBCHLOROPLAST MEMBRANE PARTICLES: ISOLATED GRANUM AND STROMA THYLAKOIDS

O. Dlouhý[1], U. Javornik[2], O. Zsiros[3], P. Šket[2], A. Bóta[4], I. Domonkos[3], R. Kouřil[5], V. Karlický[1], I. Kurasová[1], J. Plavec[2], V. Špunda[1], G. Garab[1,3]

[1]Faculty of Science, University of Ostrava, Ostrava, Czech Republic; [2]Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia; [3]Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary; [4]Research Centre for Natural Sciences, Budapest, Hungary; [5]Faculty of Science, Palacký University in Olomouc, Czech Republic

Chloroplast thylakoid membranes (TMs) have been shown to contain four well discernible lipid phases: a lamellar, an inverted hexagonal (H_{II}) and two isotropic phases, lending TMs high flexibility (Garab et al. 2017 Scientific Reports). Plant TMs are differentiated into two main substructures. Granum TMs are packed with photosystem-II and light-harvesting proteins and form stacked coin-like structure, whereas the unstacked stroma vesicles, wounding around and interconnecting grana, contain photosystem-I proteins and the ATP synthase. To characterize the polymorphic phase behavior of these particles, we performed ^{31}P-NMR spectroscopy and small-angle X-ray (SAXS) measurements and recorded scanning-, transmission- and freeze-fracture electron microscopy (EM) images. ^{31}P-NMR signatures, the deconvoluted spectra and saturation-transfer experiments revealed very similar polymorphisms as for TMs, albeit with different temperature dependences and higher stability. Presence of H_{II} phase could not be discerned by SAXS or EM, suggesting the absence of long-range order in this phase. EM images showed that the protein-rich membrane patches were interconnected with tubular or narrow membrane networks - probably giving rise to an isotropic phase, which could be selectively destroyed by lipase treatment. These data strongly suggest that non-bilayer lipid phases, via fusing the bilayer membranes, carry the ability of self-assembly of the TM system.



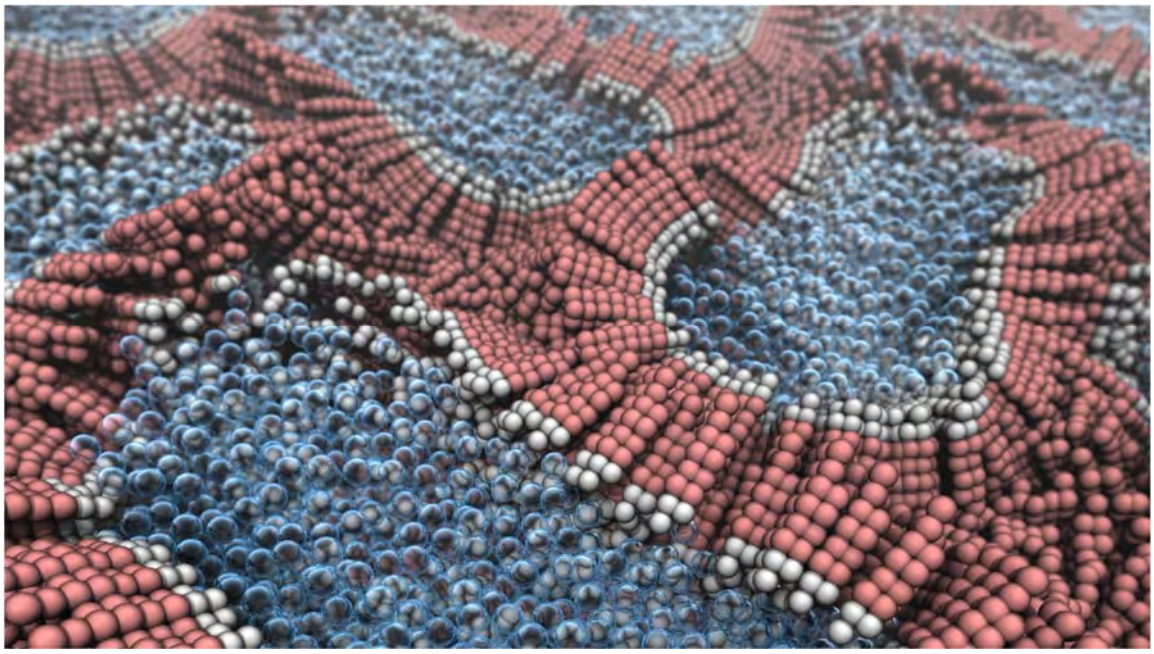
OP19

FORMATION & PERMEABILITY OF THE STRATUM CORNEUM

C. Wennberg[1,2], M. Lundborg[1,2], L. Norlén[3], E. Lindahl[1,4,5]

[1]Science for Life Laboratory, Solna, Sweden, [2]ERCO Pharma AB, Stockholm Sweden, [3]Karolinska Institutet, Stockholm, Sweden, [4]KTH Royal Institute of Technology, Stockholm, Sweden, [5]Stockholm University, Stockholm, Sweden

The stratum corneum is the outermost layer of human skin and our primary barrier towards the environment. Its main component is a large number of stacked layers of saturated long-chain ceramides, free fatty acids and cholesterol, but we do not yet know the molecular structure or formation details. Here, I will present our work on new methods to fit models to low-resolution cryo-EM microscopy vitreous section (CEMOVIS) data, in particular by generating molecular models and using cryo-EM simulation to generate electron diffraction micrographs that can be compared directly to experimental data, and iteratively use these to improve the models. This has enabled us to create a number of alternative models, compare how they fit existing experimental data, and also use coarse-grained simulations to understand the formation process where cubic phases turn into bilayers depending on the lipid composition. These types of models can be highly useful tools for understanding the barrier properties, and we are currently combining it with free energy calculations to explore rapid prediction of permeation properties from CEMOVIS-derived models, which could have important applications in developing new generations of skin-permeating drugs.



OP20

INVESTIGATING MEMBRANE PROTEINS WITH NEUTRON REFLECTOMETRY

A. Luchini [1], F. Grønbæk Tidemand [2], R. Araya-Secchi [2], L. Arleth [2]

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Supported lipid bilayers (SLBs) are commonly used to mimic biological membranes and can be characterized by surface sensitive techniques, such as neutron reflectometry (NR). However, conventional methods for SLB preparation do not enable the incorporation of membrane proteins. Recently, we developed a new protocol which allows membrane proteins with asymmetric structure, i.e. exhibiting a large extramembrane domain, to be reconstituted and oriented in SLBs [1,2]. The method is based on the deposition on a solid support of protein-loaded peptide-discs[3], ca. 10 nm discoidal lipid bilayers stabilized in solution by self-assembled 18A peptide molecules. Subsequently the 18A is removed by rinsing with fresh buffer solution. This method opens new possibilities for the characterization of membrane proteins with surface sensitive techniques. Among all, NR is a powerful technique that provides information on membrane proteins location in SLBa with a few AA resolution. In particular, we discuss the peptide disc mediated formation of a SLB with incorporated tissue factor (TF), the membrane protein responsible for the initiation of the blood coagulation [2]. The analysis of the collected NR data produced for the first time structural information on TF and its complex with the soluble protein FVIIa in a lipid bilayer.

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[3]S. A. Smith, et al., Critical reviews in biochemistry and molecular biology, 50, 4, 2015, 326–336.

OP21

AI-DRIVEN COMPUTER SIMULATIONS DISCOVER MECHANISMS OF TRANSMEMBRANE ASSEMBLY.

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In cellular membranes, lipids and proteins assemble into dynamic complexes that control crucial cellular functions. Despite the biological and biomedical importance, we still lack a mechanistic understanding of how the interplay between proteins and lipids controls the assembly process. We develop a deep reinforcement learning artificial intelligence (AI) that extracts molecular mechanisms from computer simulations. Our algorithm combines transition path sampling (TPS), deep learning, and statistical inference to simulate the dynamics of complex molecular reorganizations while simultaneously learning how to predict their outcome. With this algorithm, we study the spontaneous assembly of a model transmembrane dimer using MARTINI simulations. In less than 20 days and with minimal human intervention, the AI accumulates a total of 5 ms simulation time distributed over 10000 trajectories, collecting approximately 4000 unbiased transition paths. We estimated the dissociation rate as approximately 1/s, making the observation of even a single dissociation event in much longer equilibrium simulations very unlikely. Additionally, interpretable AI methods provide simplified models that reveal distinct assembly mechanisms. In conclusion, we present a computational framework that will facilitate quantitative mechanistic models of complex assembly processes in cellular membranes.

OP22

NANOSCALE MEMBRANE CURVATURE SORTS LIPID PHASES AND ALTERS LIPID DIFFUSION

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Cellular homeostasis requires the precise spatial and temporal control of membrane shape and composition. However, the interplay between membrane curvature and local membrane composition is poorly understood. We employed single-molecule localization microscopy to observe single-lipid diffusion in model bilayers with varying lipid compositions, phase, temperature, and membrane curvature. Engineered, hemispherical membrane buds induced lateral compositional heterogeneity in otherwise homogeneous membranes. Membrane curvature recruited liquid-disordered lipid phases in phase-separated membranes and altered the diffusion of the lipids. The disorder-preferring lipids sorted to the nanoscale curvature at all temperatures, but only when embedded in a membrane capable of sustaining liquid-liquid phase separation at low temperatures. The curvature affected the local membrane composition most strongly when the curvature was locally surrounded by a liquid-ordered phase. The curvature-induced sorting of lipid phases was quantified by the sorting of disorder-preferring fluorescent lipids, single-lipid diffusion measurements, and simulations that couple the lipid phase separation to the membrane shape. Unlike single-component membranes, lipids in phase-separated membranes demonstrated faster diffusion on curved membranes than the surrounding, flat membrane. These results support the hypothesis that the coupling of lipid phases and membrane shape may yield lateral membrane composition heterogeneities with functional consequences.

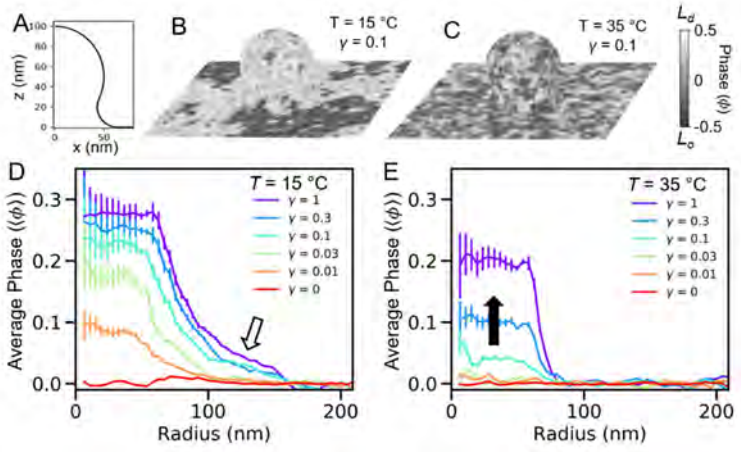

Figure: The experimentally observed phase-curvature coupling was reproduced in computational simulations. A phase-separated membrane was modeled with a fixed membrane geometry (A) at a low (B) or a high (C) temperature and with varying phase-curvature coupling (γ). (B, D) At low temperatures, the curvature-preferring L_d phase spreads to the surrounding, flat membrane (*white arrow*). (C, E) At high temperatures, when no stable phase separation is observed on the flat membrane, the curved membrane may still be more disordered than the average membrane (*black arrow*).

OP23

IMPROVING CHARMM36 SIMULATIONS BY REINTRODUCING THE MISSING ELECTRONIC POLARIZABILITY: PROSECCO

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Molecular dynamics simulations (MD) are a powerful tool in structural biology. They allow studying molecular processes at scales often unreachable by experimental techniques. They, however, suffer because of size and time limitations related to computational power. Still, their main problem is accuracy given by the models they rely on. If a model is faulty, the results are too. MD models have improved significantly, and CHARMM36 is a state of the art force field covering most biomolecules. Unfortunately, classical nonpolarizable MD has limitations on its own. It lacks the electronic polarizability that plays a significant role in the interaction between charged moieties. Membranes, with their lipids and proteins, do not escape from this problem. To improve this deficiency, we have created prosECCo. This lipid, protein, and sugar force field is a derivative of CHARMM36 that includes the missing electronic polarizability in a mean-field way. In practice, we do implement it by simple charge scaling of the atomic partial charges. Therefore, all available MD Softwares can readily use it without modifications. Moreover, it is computationally free. Our new models for lipids show a significantly improved interaction with ions and proteins. The latter also show significant improvement when interacting with other charges. Overall, we show that missing physics in our models can be incorporated for free and that by doing so, we immediately improve the structural behavior.

OP24

STRUCTURAL INSIGHTS INTO THE GRAM-NEGATIVE LIPID TRANSPORTER YrbC OF HÄMOPHILUS INFLUENZAE

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The lipid composition of the outer membrane of Gram-negative bacteria plays a crucial role in pathogenesis and intercellular interactions. Recent studies presented a VacJ/Yrb ABC (ATP binding cassette) transport system which is conserved among Gram-negatives. [1] The periplasmic protein YrbC is essential in this mechanism, since it transports accumulated phospholipids from the outer to the inner membrane thereby maintaining the membrane's barrier function.
By using crystallography and molecular replacement we could solve the structure of YrbC from *Hämophilus influenzae* (2.2Å), including a bound phospholipid. Analysis of the ligand via Nuclear Magnetic Resonance NMR and Mass spectrometry MS showed a clear preference of YrbC for phosphoglycerol over phospahtidylethanolamin. Analysis of lipid ligands from two YrbC orthologues from *Vibrio cholerae* and *Escherichia coli* showed the same outcome.
The structure of YrbC *H. influenzae* reveals new insights into its binding preferences and furthermore the binding cavity and its chemical environment. These findings can be useful to understand how the VacJ/Yrb ABC transport system works, which by itself may have a pathophysical role *in vivo* since proper function of the outer membrane is crucial for bacterial adaption to harsh environments, like colonization of a new host, as well as antibiotic resistance.

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OP25

ENHANCED TRANSLOCATION OF AMPHIPHILIC PEPTIDES ACROSS MEMBRANES BY TRANSMEMBRANE PROTEINS

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Cell membranes are phospholipid bilayers with a large amount of embedded transmembrane proteins. Some of these proteins, such as scramblases, have properties that facilitate lipid flip-flop from one membrane leaflet to another. Scramblases and similar transmembrane proteins could also affect the translocation of other amphiphilic molecules, including cell-penetrating or antimicrobial peptides. We studied the effect of transmembrane proteins on the translocation of amphiphilic peptides through the membrane. Using two very different models, we consistently demonstrate that transmembrane proteins with a hydrophilic patch enhance the translocation of amphiphilic peptides by stabilizing the peptide in the membrane. Moreover, there is an optimum amphiphilicity because the peptide could become ‘overstabilized’ in the transmembrane state, in which the peptide-protein dissociation is hampered, limiting the peptide translocation. The presence of scramblases and other proteins with similar properties could be exploited for more efficient transport into cells. The described principles could also be utilized in the design of a drug delivery system by adding a translocation-enhancing peptide that would integrate into the membrane.

OP26

MOLECULAR LEVEL PROBING AND QUANTIFICATION OF LIPID DROPLET, LIPOSOME AND LIQUID CONDENSATE INTERFACES IN AQUEOUS SOLUTION, USING NONLINEAR LIGHT SCATTERING.

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Biomolecular condensates and liquid-liquid phase separated systems are very important players in a plethora of cellular processes. Together with compartmentalized membrane bound systems, such as liposomes and lipid droplets they participate in a wide variety of biological processes. In order to understand the role of these organelles in biology it is necessary to understand the physical and interfacial chemistry of these structures as well as their dynamical interactions. This requires understanding the role of water and the molecular interfacial chemistry and biophysics of these (sub)micron-sized objects. This requires probes of molecular (interfacial) structure that can be applied in-situ.

Vibrational sum frequency scattering and angle resolved second harmonic scattering are two unique nonlinear light scattering techniques that can quantify interfacial structure on the molecular level (see [1] for a perspective). Properties such as the molecular conformation, orientational distribution, relevant chemical interactions and the electrostatic surface potential can be determined in-situ. In this presentation I will introduce these methods and show applications to understand and control the properties of model lipid droplets as well as unilammelar lipid vesicles in aqueous solution.

[1] H. I. Okur, O. B. Tarun, S. Roke, J. Am. Chem. Soc (2019), 141, 31, 12168-12181

OP27

STRATUM CORNEUM VESICLES AS PROMISING STRATEGY TO TREAT SKIN DISEASES

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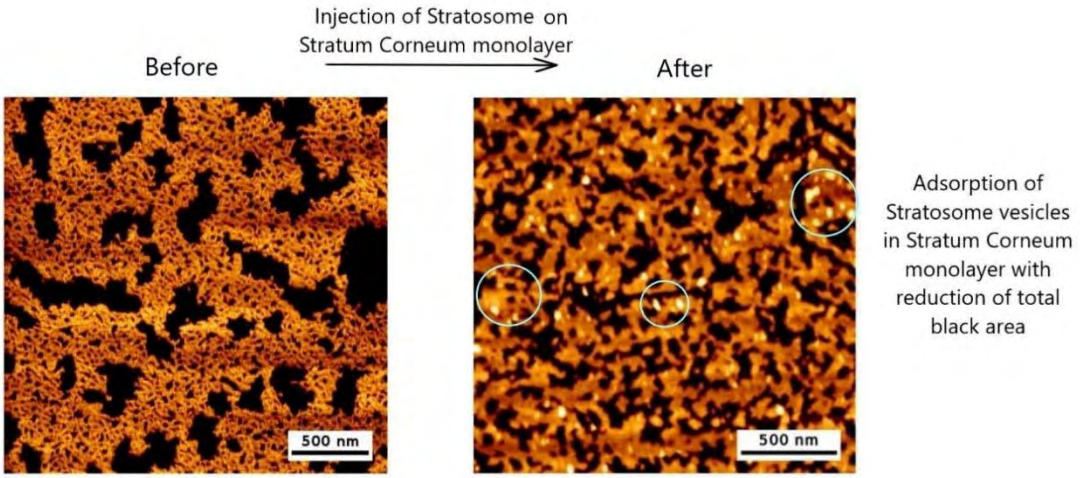

The Stratum Corneum (SC) is the outermost layer of the skin with the mission of physical and chemical protection of dermis and epidermis. It is constituted of a brick and mortar system where the bricks are the corneocytes while the mortar is a dense lipid matrix, composed of a mixture of ceramides, cholesterol and free fatty acids, which is responsible for the properties of the SC. In our project, we devised and characterized a SC lipid matrix model system, composed of a 1:0.7:1 molar mixture of Ceramides:Cholesterol:Free fatty acid, with the objective to have on hand a simple and reliable model to study *in-vitro* the effect of different chemicals relevant for pharmaceutical formulations on the SC. Differentsurface sensitive techniques based on Langmuir monolayers have been applied. Furthermore, a SC liposomal formulation (Stratosomes), designed to treat skin lipid deficiencies, was used to investigate its potential incorporation into the SC model system. The Stratosomes have been injected underneath the SC monolayer. Atomic Force Microscopy and Grazing Incidence X-ray Diffraction experiments prove the potential of the vesicles to interact with and get incorporated into the SC monolayer to change the lipid arrangement and to reduce the areas area of defects.

OP28

DEXAMETHASONE AND DEXAMETHASONE PHOSPHATE AND THEIR EFFECTS ON DMPC MEMBRANE MODELS

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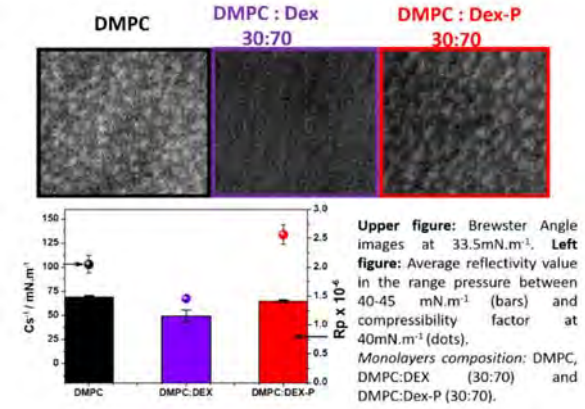
Dexamethasone (Dex) and Dexamethasone phosphate (Dex-P) are synthetics glucocorticoids with a high anti-inflammatory and immunosuppressor action [1]. Both drugs are used in a wide range of diseases from tumor to asthma attack [2–4]. Recently, Dexamethasone has become more renowned because it reduces the mortality in critical patients with SARs-COVID-19 connected to assisted breathing [5,6].

We studied the variation of the properties of DMPC monolayers as Dex and Dex-P inserted into the film in different molar proportions. With this aim, we used complementary techniques such as compression isotherms, Brewster angle microscopy (BAM), and insertion experiments. Vesicle shape fluctuations were also analyzed.



Upper figure: Brewster Angle images at 33.5mN.m^{-1}. **Left figure:** Average reflectivity value in the range pressure between 40-45 mN.m^{-1} (bars) and compressibility factor at 40mN.m^{-1} (dots). *Monolayers composition:* DMPC, DMPC:DEX (30:70) and DMPC:Dex-P (30:70).

Results: The hybrid films DMPC: Dex had lower compressibility modulus in the liquid-condensed (LC) phase, as well as higher reflectivity than pure DMPC monolayers. When Dex molar fraction was higher than 0.5, the liquid-expanded (LE)/LC phase transition blurred, and above 0.8, BAM images showed the presence of aggregates. In contrast, the presence of Dex-P in DMPC monolayer increased the compressibility modulus of the LC phase without affecting its reflectivity, after 0.7 of Dex-P some aggregates were also found. Insertion experiments indicates that Dex induced higher surface pressure changes, with a higher exclusion pressures than Dex-P.

Conclusion: Both drugs penetrate DMPC monolayers up to high surface pressures. Dex, due to its hydrophobic character, disrupts the lipid surface organization of DMPC, altering the nucleation process and making the monolayer more compressible. This effect was opposite to that observed with Dex-P.

[1] *P. Rohdewald, H. Mollmann, J. Baktii, J. Reiider, I. Derendorf, Pharmacokinetics of dexamethasone and its phosphate., BIOPHARMACEUTICS & DRUG DISPOSITION. 8 (1987) 205–212. https://doi.org/https://doi.org/10.1002/bdd.2510080302.*

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OP29

MOLECULAR SIMULATIONS OF LIPIDS DROPLETS

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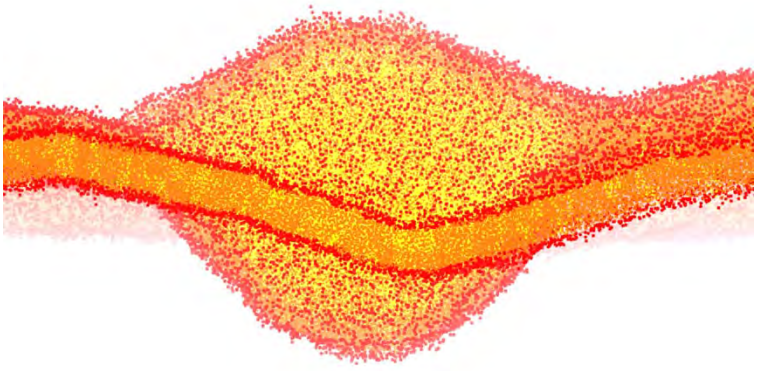
Lipid droplets (LDs) are key cellular organelles regulating energy metabolism. LD biogenesis occurs in the ER membrane, and starts with the phase separation of of synthesized neutral lipids (substantially hydrophobic) from phospholipids (amphipathic, enriched in the ER bilayer), leading to the formation of droplets, which then bud off from the ER. The fate and biological function of LDs are largely determined during their formation, but the driving forces and mechanism of LD formation remain elusive. Here, we use molecular dynamics simulations at the coarse-grained level to gain insight into the driving forces for LD budding, and particularly the relationship between the shape of nascent LDs (that is a proxy for the tendency to bud off), its size, and its chemical composition. We also show that budding directionality can be determined simply by an asymmetry in bilayer surface coverage, with no requirement for specific membrane-bending proteins or lipids favoring positive curvature.



OP30

DIFFUSION-LYSIS COUPLING OF ANTIMICROBIAL PEPTIDES IN BIOFILMS OF *S. AUREUS*

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Antimicrobial peptides (AMP) have been intensively studied in artificial lipid vesicles. The fundamentals of peptide-lipid bilayer intermodulation have been extensively unravelled in detail using a plethora of biophysical techniques. Recently, several attempts to translate these findings into biological samples of bacteria have been published. The role of membrane saturation, peptide clustering, and peptide conformational dynamics, for instance, have been addressed. In addition to effects associated to membrane targeting, intracellular effects have been characterized, such as macromolecular condensation.

A step forward in the cellular biophysics of AMP is the study of peptide diffusion is biofilms, which consist in bacterial colonies living in a gel-like environment formed by an extracellular matrix of polysaccharides, nucleic acids, and proteins formed by the bacteria themselves. This extracellular matrix protects physically and chemically the bacterial colony from deleterious agents, including antibiotics. Because biofilms account for more than 80% of all hospital-related infections in humans, it is urgent to address biofilm penetration in modern antibiotic development strategies. Peptides are relatively big compared to most conventional antibiotics but they are also flexible, which makes diffusion in gels plausible for this class of molecules.

The biofilm matrix diffusion-membrane lysis coupling has been characterized by us using biophysical techniques capable of reporting peptide diffusion through a S. aureus biofilm, killing bacteria along the way.

OP31

AN UNEXPECTED MOLECULAR MECHANISM UNVEILS THE FUNCTIONING OF PEPTIDOMIMETICS AS ANTIMICROBIALS

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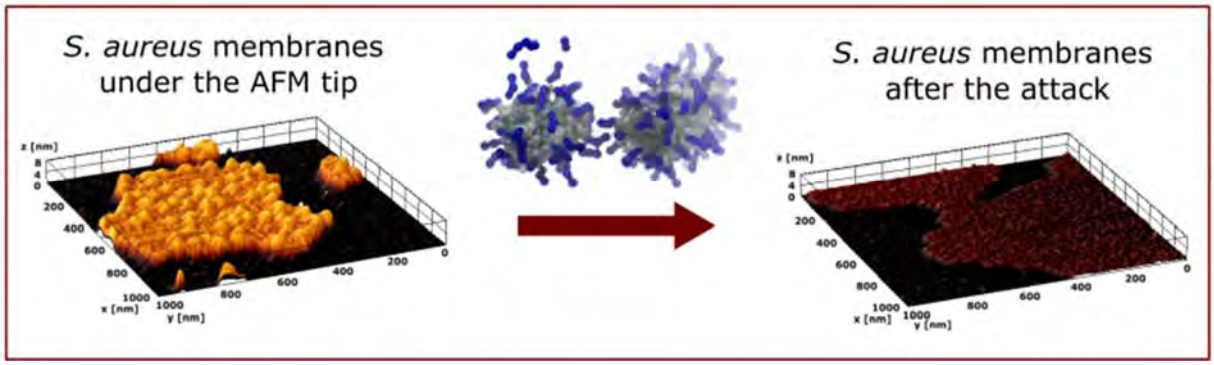

The increasing ability of bacteria to develop resistance against existing antibiotics is a growing concern in medical care. Antimicrobial peptides and their mimics—peptidomimetics—are promising scaffolds for the development of new antibiotics. Here we scrutinize the functional mechanism of the antimicrobial action of a tetrapeptide based peptidomimetic with a potential in clinical use by combining Atomic Force Microscopy (AFM), and Molecular Dynamic (MD) simulations. In particular we study its activity on the anionic plasma membrane of *Staphylococcus aureus*. Our AFM experiments reveal the high impact of the compound on membranes extracted from *S. aureus*. Fast rupture of large unilamellar vesicles is followed by lateral growth of supported membranes without poration activity. Instead of poration, the compound largely influences lateral organization and dynamics of the membrane as revealed by High Speed AFM. MD simulations show aggregation of the compound into stable micelles, which selectively attack the anionic, e. g. bacterial, membranes. The micelles dissolve into the membrane changing its dynamical and mechanical properties. The lack of pores makes the action of this peptidomimetic largely different from natural antimicrobial peptides. Its mechanism rather resembles the activity of small disinfectants, which have been in use for many years without generating bacterial resistance.

OP32

PARTITIONING AND IN-SITU KINETICS OF LACTOFERRICIN DERIVED ANTIMICROBIAL PEPTIDES IN *ESCHERICHIA COLI*

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Membrane active antimicrobial peptides (AMPs) are promising candidates for defending the spread of diseases caused by multi-resistant pathogenic bacteria. Over the years, significant insight on the molecular mode of AMPs activity has been derived from lipid-only mimics of bacterial membranes. However, achieving equally detailed understanding in live cells remains challenging, due to the broad range of time and length scales which need to be bridged for a much more complex system.

In order to overcome this barrier, we combined quantitative studies of AMP partitioning using bio-screening and zeta-potential measurements with time-resolved (ultra) small-angle X-ray scattering (USAXS/SAXS). This allowed us to interrogate the activity of human lactoferricin derivatives in *E. coli* on the micro- to nanometer length scales with a time resolution of few milliseconds. Specifically, we used a multiscale scattering model to retrieve bacterial structural parameters by jointly analyzing differently contrasted (very) small-angle neutron scattering (VSANS/SANS), USAXS/SAXS and transmission electron microscopy data. This enabled us to constrain the analysis of the time-resolved USAXS/SAXS data using a statistical optimization algorithm.

Results revealed a weak cytoplasmic and periplasmic leakage starting after about 3 seconds, alongside affecting the packing of the lipopolysaccharide leaflet. In the subsequent 2-10 minutes, the peptide-induced damage evolved continuously at all length scales, including shrinking of the cell body, loss of positional correlations between inner and outer membranes, phase-separation of the nucleoid region, and outer membrane vesiculation or tubulation. In the final state, bacteria show impaired cell membranes and, strikingly, peptides are accumulated in the cytosol up to concentrations several orders of magnitude higher than in the medium, suggesting intracellular targeting as a key for bactericidal activity.

OP34

A JOURNEY IN THE COMPLEX WORLD OF HOST DEFENSE PEPTIDES: FROM IN SILICO TO CELLULAR STUDIES

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Host-defense peptides (HDPs) kill bacteria mainly by perturbing their cell membranes, with low toxicity towards host cells, and are promising compounds to fight drug-resistant microbes. However, rational design of new molecules with the same activity of HDPs, but improved pharmacological properties, has proven difficult. This is due to the fact that multiple phenomena modulate their properties, such as conformational transitions, aggregation processes, water-membrane partition equilibria and association to different cell components. By considering these aspects, and by combining molecular dynamics simulations, spectroscopic experiments on liposomes and live cells, we showed that peptide conformation and aggregation propensity can be exploited to optimize HDP selectivity. Quantitative peptide/cell association studies allowed the determination of the minimum number of peptide molecules that must bind to a single bacterium to kill it, the cell-density dependence of HDP activity and selectivity and the differential peptide affinity for various cell components. Overall, these studies question some common beliefs in the field of HDPs, and provide useful indications for the rational design of new antibiotic molecules.

BBA 2020,862:1832912019; "Antimicrobial Peptides: Basics for Clinical Application" Springer 2019; Chem Comm 2018,54:4943; Pept Sci 2018, 10:e24041; ACS Chem Biol 2017,12:52; BBA 2015,1848:581; ACS Chem Biol 2014,9:2003; J.Pept.Sci 2013,19:758; BBA 2013,1828: 1013.

OP35

PARAINFLUENZA FUSION PEPTIDE FORMS OLIGOMERIC PORE-LIKE STRUCTURES INSIDE A MEMBRANE

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The parainfluenza virus (PIV) belongs to the large family of paramyxoviruses. Annually, these viruses contribute significantly to the global burden of disease in humans by infecting millions of individuals worldwide and leading to many deaths in areas with poor health care resources. During the infection process the virus must enter the host's cells by fusing its membrane with the host membrane. In the case of the parainfluenza virus, the cell entry process starts with the identification and attachment to target receptors, followed by proteolytic cleavage of the fusion glycoprotein (F) protein, exposing the fusion peptide (FP) region. The FP is responsible for binding and disturbing the target membrane. It is believed to play a crucial role in the fusion process, however, the mechanism by which the parainfluenza FP peptide promotes membrane fusion is still unclear. To elucidate this matter, we performed coarse grain (CG) and atomistic molecular dynamics (MD) simulations, together with spectroscopic experiments of the parainfluenza FP in membranes. The combination of both these approaches led to the pinpointing of the most important residues for the membrane fusion and the novel finding that this peptide, at high concentrations, induces formation of a pore-like structure. Our findings are a step further in the understanding of the membrane fusion process induced by the parainfluenza FP.

OP36

SELF-ASSEMBLING OF ANTIMICROBIAL CYCLIC PEPTIDES AT MODEL MEMBRANES: NANOTUBES OR MICELLAR AGGREGATES?

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An urgent search for antibiotics, particularly with new mechanisms of action, is presently mandatory, due to the worldwide increase of resistant pathogens [1].
Antimicrobial peptides (AMPs) are small peptides essential for the innate immune response of a wide range of organism, presenting activity against a wide range of pathogens, like bacteria, fungi, and viruses. They represent a new antibiotic paradigm, as they specifically target the bacterial membrane with lower toxicity to normal tissues [2]. An alternative to the natural AMPS involves the use of cyclic peptides (CPs) made of sequences of alternating *D,L-α*-amino acids, which are expected to have a higher resistance to protease degradation due to the presence of *D*-amino acids. These CPs would form the active species, ideally a self-assembled cyclic peptide nanotubes (SCPNs), only under some specific conditions, i.e., in the presence of membranes. [3, 4]
We will present results obtained with two biophysical experimental techniques (DSC, ATR-FTIR) together with an *in-silico* approach (CG-MD), to characterize the interaction of three new *D*,*L*-α-CPs, with known antimicrobial activity, with different bacterial model membranes, in an attempt to understand their possible mechanism of action. These studies allowed us to characterize the formation nanotubes/aggregates of these CPs at different membranes and to discriminate the most important factors ruling these peptides/membrane interactions. Although the results suggest the formation of SCPNs with some of the systems, the presence of an alkyl chain connected to the peptide ring induce the formation of micellar aggregates.

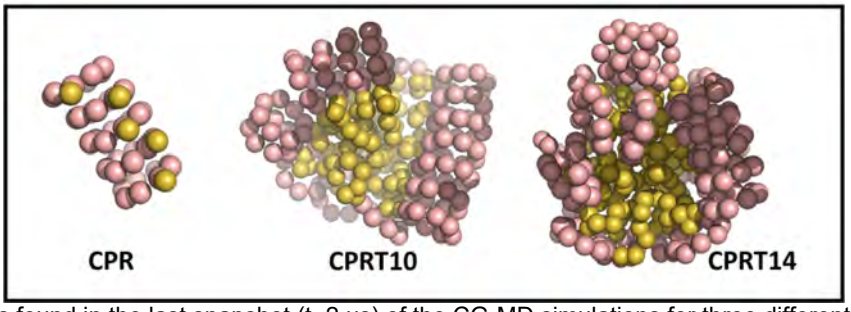

Fig .1 - Structures found in the last snapshot (t=2 μs) of the CG-MD simulations for three different CPs in study

Acknowledgement: Funded by national funds through FCT, Portugal (Fundação para a Ciência e Tecnologia) and european funds through FEDER/Compete2020, under the project with reference PTDC/BIA-FS/30579/2017, POCI-01-0145-FEDER-030579 and PD/BD/135095/2017.

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OP37

PEPTIDE SUPRAMOLECULAR ASSEMBLY AS A DETERMINANT OF BIOLOGICAL FUNCTION

M. Lointier[1], E. Glattard[1], J. Raya[1], A. Marquette[1], P. Bertani[1], A. Kichler[2], A. Galy[3], B. Bechinger[1].

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The LAH4 model peptide was designed as antimicrobial peptide (AMP)[1] composed of 26 residues using 4 different amino acids: two lysines at each extremity ensure good solubility in the aqueous environment, the hydrophobic core consist of alanines and leucines, interrupted by four histidines. Therefore, these histidine-rich peptides exhibit a pH-dependent amphipathic character. In solution, peptides of the LAH4 family adopt an α-helical structure at neutral pH and a random conformation at acidic pH. In membrane environments, the LAH4 α-helix adopts an in-planar orientation along the membrane surface at acidic conditions whereas at neutral pH the peptide is transmembrane.
Besides these structural properties, LAH4 peptides were shown to not exhibit powerful antimicrobial but also gene and siRNA transfection activities as well as to help the cell penetration of proteins, peptides, nanodots, adeno associated and lentiviruses[2]. To enhance this latter transduction activity, derivates of LAH4 were designed and analysed. Two members of the peptides of this family LAH4-L1 and LAH4-A4 enable transfection[2] [3] and enhance transduction[4] [5], respectively. To better understand the mechanism and differences, these peptides of the LAH4 family have been studied and compared. Furthermore, the fiber formation of some peptides has been shown important for lentiviral transduction [5]. The fiber formation, stability and activity are investigated. Furthermore, the fibers are studied by solid state NMR spectroscopy to determine the structure.

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OP38

STRUCTURE-FUNCTION DETERMINANTS IN PULMONARY SURFACTANT PROTEIN SP-B, A CRUCIAL PROTEIN FOR BREATHING

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The delicate structure of mammalian alveoli can only support breathing in the presence of pulmonary surfactant, a lipid-protein complex in charge of forming a multi-layered lipid-based film at the respiratory surface. This film reduces surface tension of the thin water layer coating alveoli, but also provides mechanical stability along breathing dynamics. On top of that, pulmonary surfactant integrates additional elements establishing an innate barrier against the numerous pathogens entering the lung within the thousands litters of air required to provide the oxygen required for body functions.

SP-B is the most important protein in surfactant. Its absence is lethal due to impossibility to maintain the lungs open. SP-B is extremely hydrophobic and is permanently associated with surfactant complexes. From there, SP-B promotes a rapid and efficient transfer of surface-active phospholipid species to the air-liquid interface, and through the establishment of protein-protein contacts, it is the basis of a highly cohesive membrane multi-layered structure providing maximal mechanical estability. It has been revealed that SP-B-promoted membrane-membrane contacts are also highly dynamic, facilitating rapid intermembrane movement of hydrophobic (lipids, drugs) and polar (ions, small peptides) molecules.

This talk will summarize current models derived from experimental and simulation work, on the structure of supramolecular organizations of SP-B and how they could simultaneously sustain formation of multi-layered surfactant films and a proper dynamics at the alveolar spaces.

Poster Presentations – Overview

PP01,02…: poster number

***1,2,3**: Poster Session*

***A, B**: Time Slot for Live Presention*

(A…first half, B…second half of poster session)

"Complex Biomembrane Mimics"	PP01-PP35, PP101
"Membrane Curvature"	PP36-PP46
"Non-Lamellar Phases"	PP47-PP53
"Lipid/Protein Interactions"	PP54-PP87, PP102
"Lipid Droplets and Monolayers"	PP88-PP90
"Membrane Active Peptides"	PP91-PP100

PP01-1-A

DETERGENT-FREE MEMBRANE PROTEIN RECONSTITUTION INTO HYBRID POLYMER-LIPID VESICLES

R. Catania[1,4], J. Machin[1,4], R. Seneviratne[2,4], M. Rappolt[3], S. Muench[1,4], P.A. Beales[2,4], L.J.C. Jeuken[1,4]

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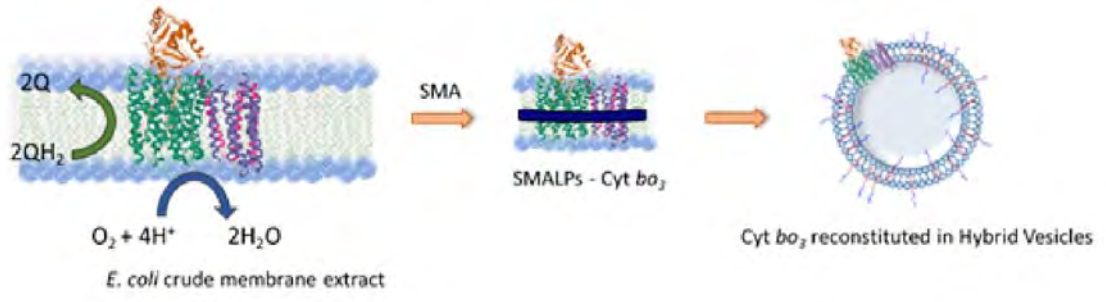
Amphiphilic polymers have shown promise in resolving stability limitations encountered when studying membrane proteins in detergents or liposomes. First, amphiphilic polymers like styrene-maleic acid copolymer (SMA) are able to extract or solubilise membrane proteins without the need of detergents.[1,2] Second, hybrid vesicles (HVs) that consist of mixtures of copolymers and lipids have shown to be robust biomimetics of liposomes, extending the lifetime of reconstituted membrane proteins.[3] Previously, we reconstituted the membrane protein, cytochrome *bo*$_3$ from *Escherichia coli*, in HVs after purification in detergent.[3] Here, we show for the first time that it is possible to functionally reconstitute cytochrome *bo*$_3$ directly from SMA lipid particles (SMALPs) into HVs without the use of any detergent. Interestingly, reconstitution directly into liposomes from SMALPs was not possible for this protein under the same experimental conditions. This suggests that the copolymer-lipid mixtures are more amenable to membrane protein incorporation than liposomes. Finally, we show that this transfer method is not limited to cytochrome *bo*$_3$ and can be performed with complex membrane protein mixtures.



[1] Dorr et al Eur. Biophys. J. (2016) ***45****, 3-21*

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PP02-1-B

ACYL-CHAIN SATURATION REGULATES THE ORDER OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE NANODOMAINS

L. Borges-Araújo [1,4], M. M. Domingues [3], A. Fedorov [1], N. C. Santos [3], M. N. Melo[4], F. Fernandes [1,2]

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PI(4,5)P_2 plays a critical role in the regulation of a plethora of plasma membrane processes and signalling pathways in eukaryotes. A significant amount of cellular resources is spent on maintaining the dominant *1*-stearoyl-*2*-arachidonyl PI(4,5)P_2 acyl-chain composition, while the less abundant and more saturated species have been shown to become more prevalent in response to certain stimuli, stress or aging. Through a combination of fluorescence spectroscopy, atomic force microscopy, and coarse grained molecular dynamics simulations, we report the impact of acyl-chain structure on the biophysical properties of cation-induced PI(4,5)P_2 nanodomains. We observe that in the presence of calcium, PI(4,5)P_2 species with increasing levels of acyl-chain saturation cluster in progressively more ordered nanodomains, which culminate in the formation of gel-like nanodomains for fully saturated species. This is to our knowledge the first report of the impact of PI(4,5)P_2 acyl-chain composition on cation-dependent nanodomain ordering. Our work provides important biophysical clues to the motives for the conserved pattern of enrichment of PI(4,5)P_2 with polyunsaturated acyl-chains as the presence of highly ordered PI(4,5)P_2 domains could lead to dysregulation and disease. Additionally, we also show how calcium induced PI(4,5)P_2 nanodomains are able to generate local negative curvature. a phenomenon that is likely to play a role in membrane remodelling events directly regulated by the presence of PI(4,5)P_2.

PP03-1-A

DMPC-CnOH SYSTEM: THERMODYNAMIC AND STRUCTURAL BEHAVIOURS

M. Klacsová[1], A. Bóta[2], P. Westh[3], S.S. Funari[4], D. Uhríková[1], P. Balgavý[1]

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The thermodynamic and structural behaviours of lamellar dimyristoylphosphatidylcholine-alkanol (DMPC-CnOH, n = 8 – 18) systems were studied by using DSC and SAXD/WAXD methods at a 0 – 0.8 CnOH:DMPC mol/mol range. Up to n ≤ 10 a significant biphasic effect in the dependence of main transition temperature t_m on CnOH concentration was observed. Two breakpoints were revealed: turning point (TP), corresponding to the minimum, and threshold concentration (c_T), corresponding to the end of the biphasic tendency. These breakpoints were observed also in the alkanol concentration dependent change in enthalpy of the main transition ΔHm. In case of CnOHs with n > 10 we propose a marked shift of TP and c_T to very low concentrations, consequently only increase of t_m is observed. A partial phase diagram was constructed for a pseudo-binary DMPC-C12OH system. We suggest a fluid-fluid immiscibility of the DMPC-C12OH system above c_T with a consequent formation of domains with different C12OH content. At a constant CnOH concentration, effects of CnOHs on ΔHm and bilayer repeat distance were found to depend predominantly on the mismatch between CnOH and lipid chain lengths. Observed effects are suggested to be underlined by a counterbalancing effect of interchain van der Waals interactions and headgroup repulsion.

Acknowledgement: This project was supported by APVV-17-0250 and VEGA 1/0223/20 grants and JINR project 04-4-1142-2021/2025.

PP04-1-B

BINDING KINETICS OF HUMAN NOROVIRUSES TO HISTO-BLOOD GROUP ANTIGENS PREDICT SUSCEPTIBILITY OF HUMAN INTESTINAL ENTEROIDS TO INFECTION

K. Thorsteinsson[1,2], I. Rimkute[3,4], M. Henricsson[5], V. R. Tenge[6], X. Yu[6], S. C. Lin[6], K. Haga[6], R. L. Atmar[6,7], N. Lycke[4], J. Nilsson[3,8], M. K. Estes[6,7], G. Larsson[3,8] and M. Bally[1,2]

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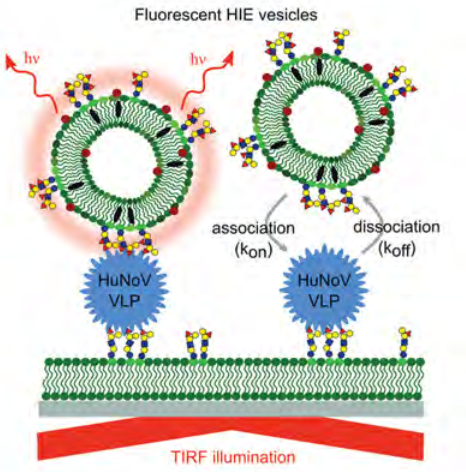

Human norovirus (hNoV) infections are a leading cause of gastroenteritis worldwide. However, the study of hNoVs has long been hampered in lack of adequate in-vitro culture systems, and most knowledge limited to binding studies revealing that histo-blood group-antigens (HBGAs)-carrying glycosphingolipids on intestinal cells, are involved in the infectious cycle. Recently, the development of a culture system based on human intestinal enteroids (HIEs) has facilitated the study of norovirus infections ex-vivo. This culture system allows us to test how HBGA expression correlates to infectious behavior. We characterized lipid extracts from seven HIE cultures with varying HBGA expression. We then used these extracts to study the binding kinetics of norovirus virus-like particles to membranes made from these lipids using total internal reflection fluorescence microscopy. This allows us to study the dynamics of virus-membrane interactions to gain knowledge on the attachment and detachment behavior of the virus(1). We see that the virus has higher affinity for membranes of susceptible cells, but even membranes from non-susceptible cells show intermediate binding. We also observe that disassociation correlates better with susceptibility to infection than association(2). This project demonstrates the importance of host-membrane interactions in norovirus infections and the potential of HIEs in studying hNoVs.

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PP05-1-A

HOW AND HOW MUCH HAS IT FUSED? DETECTING EFFICIENCY, INTERMEDIATES, KINETICS AND COMPOSITIONAL PHASE IN MEMBRANE FUSION

R. B. Lira[1,2], R. R. M. Cavalcanti[2,3], K. A. Riske[3], R. Dimova[2] and W. H. Roos[1]

1 Moleculaire biofysica, Zernike Instituut, Rijksuniversiteit Groningen, The Netherlands
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3 Universidade Federal de São Paulo, Biophysics, São Paulo, Brazil

Membrane fusion is a fast and highly dynamical cellular process that also qualifies as an efficient biotechnological strategy for intracellular delivery. Using advanced imaging and manipulation techniques along with charged-based membrane fusion between LUVs and GUVs, we (i) detect fusion intermediates, (ii) quantify fusion efficiency and (iii) reveal real-time kinetics at the level of single vesicles. Single LUV fusion is extremely fast, it delivers membrane and aqueous contents to acceptor membranes and thousands of LUVs fuse to a single GUV within seconds. When several LUVs fuse, fusion is leakage-free and the GUV area increases, a property which can be measured using electric fields. When many LUVs fuse, their area leaflet asymmetry is incorporated into the GUVs leads to budding as a result of increased spontaneous curvature, ultimately resulting in GUV membrane permeabilization. In order to determine the most efficient LUV composition for fusion, we use FLIM-FRET to construct a phase diagram of fusion efficiency based on LUV composition. The most efficient composition involves a combination of intermediate to high fractions of cationic and non-bilayer forming lipids. Fast and efficient fusion overcomes many limitations of drug-delivery systems and are key to successful, wide-spread application of lipid nanoparticles in medicine.

PP06-1-B

EXPERT SYSTEM TOWARDS *IN SILICO* ASSESSMENT OF DRUG-MEMBRANE CROSSING

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Most xenobiotics (drugs and nutrients) are capable of crossing several biological membranes to reach their active sites. Membrane crossing can occur either through membrane proteins (mainly ABC proteins or solute carriers) or by passive permeation. Therefore, understanding the ability of xenobiotics to permeate spontaneously through lipid bilayer membranes is of utmost importance as this process is key to decipher their pharmacokinetics and pharmacodynamics. We aim at building a robust and efficient method based on MD simulations to predict xenobiotic permeation coefficients in a mammal-prototypical POPC bilayer. These coefficients can be predicted by computing both free energy and diffusivity profiles through the membrane, along the normal to its surface. Two different approaches were used to simulate the free energy profiles, namely alchemical free energy and Accelerated Weight Histogram (AWH) methods. Fractional diffusivity profiles[1] were evaluated from the AWH trajectories. The predicted permeation coefficients for a series of xenobiotics can be compared to experimental results, with the further goal to screen large datasets in a reasonable time.

1. *Tse, C. H., Comer, J., Sang Chu, S. K., Wang, Y. & Chipot, C. Affordable Membrane Permeability Calculations: Permeation of Short-Chain Alcohols through Pure-Lipid Bilayers and a Mammalian Cell Membrane. J. Chem. Theory Comput.* ***15****, 2913–2924 (2019).*

PP07-1-A

COMPUTATIONAL MODELING REVEALS THE INFLUENCE OF TAILS LENGTHS AND LIPID BILAYER MEMBRANE PHASES ON THE ISOMERIZATION OF EMBEDDED PROBES

M. Paloncýová[1,2], S. Osella[3], G. Aniander[2], E. Larsson[2], M. Sahi[2], J. Piguet[4], J. Widengren[4], S. Knippenberg[1,2,5]*

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[2] *Department of Theoretical Chemistry and Biology, KTH Royal Institute of Technology*
[3] *Biological Systems Simulation Lab, Centre of New Technologies, University of Warsaw, Poland*
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Since fluorescing probes in lipid bilayers tend to partition in different phases, the consequent changes in their orientation and location are both theoretically and experimentally of interest. First, we focused on a DOPC (Liquid disordered phase, Ld) membrane phase and performed molecular dynamics (MD) simulations of the indocarbocyanine DiD probes by varying the length of the attached alkyl tails and also the length of the cyanine backbone [1]. The photoisomerization scheme of DiD was investigated. Starting from the trans conformation, the photoisomerization path to the cis conformation and its dependence on the acyl tail lengths of the probe were evaluated [2].
For the diphenylhexatriene (DPH) probe, the influence of different membrane environments is investigated. The transition dipole moments and one-photon absorption spectra obtained in a 2:1 mixture of sphingomyelin (SM) and Cholesterol (Chol) in the liquid ordered (Lo) phase differ largely from the ones calculated in DOPC (Ld) and DPPC (solid gel phase, So). The molecular conformation of DPH in SM/Chol is found to differ from the other environments [3]. A stringent comparison of the fluorescence anisotropy decay and the fluorescence lifetime confirm the use of DPH to gain information upon the surrounding lipids and lipid phases. DPH might thus open the possibility to detect and analyze different biological environments based on its absorption and emission properties.

*[1] M. Paloncýová, G. Aniander, E. Larsson, S. Knippenberg, Spectrochim. Acta A **2020**, 224, 117329.*

*[2] M. Paloncýová, J. Tornmalm, S. Sen, J. Piguet, J. Widengren, and S. Knippenberg, J. Phys. Chem. C **2020**, 124, 5829.*

*[3] S. Osella, M. Paloncýová, M. Sahi, S. Knippenberg, Molecules **2020**, 25, 4264.*

PP08-1-B

PRODUCTION OF HIERARCHICAL ARTIFICIAL CELL MIMICS USING MICROFLUIDICS

N. Yandrapalli[1] and T. Robinson[1]

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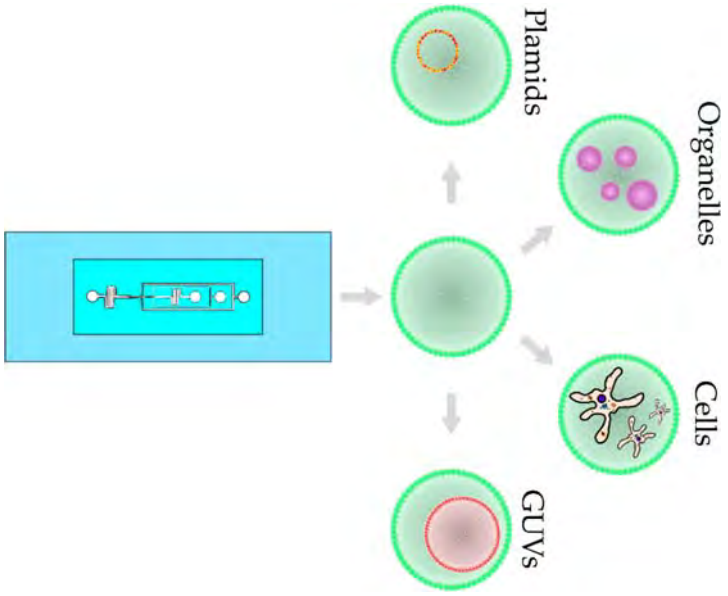
The bottom-up construction of biomimetic artificial cells is key to unravelling the evolutionary significance of various structural and functional aspects of cells. The assembly of giant unilamellar vesicles (GUVs) from individual lipid molecules to cellular mimics is possible through microfluidics technology, but current methods require additional non-biomimetic chemical additives. Unlike electroformation and the inverted emulsion phase transfer method, microfluidics can be used to build GUVs with high uniformity of size and content – allowing for the encapsulation of proteins, enzymes, plasmids, coacervates, cascade reactions and even smaller liposomes. Here, we present a microfluidic platform that can produce complex configurations of artificial cells from GUVs incorporating a diverse range of bio-macromolecules in a high-throughput manner. All without the need for surfactants or additives. Furthermore, PDMS-based microfluidics for the one-step production of GUVs-inside-GUVs structures will be demonstrated as a proof-of-concept for complex compartmentalized systems mimicking eukaryotic cells.



PP09-1-A

COMPUTATIONAL SCREENING OF CANDIDATE MOLECULES FOR LIPOSOME FORMULATION

M. Balouch[1,3], M. Šrejber[2,3], M. Šoltys[1], P. Janská[1], F. Štěpánek[1,*], K. Berka[3,*]

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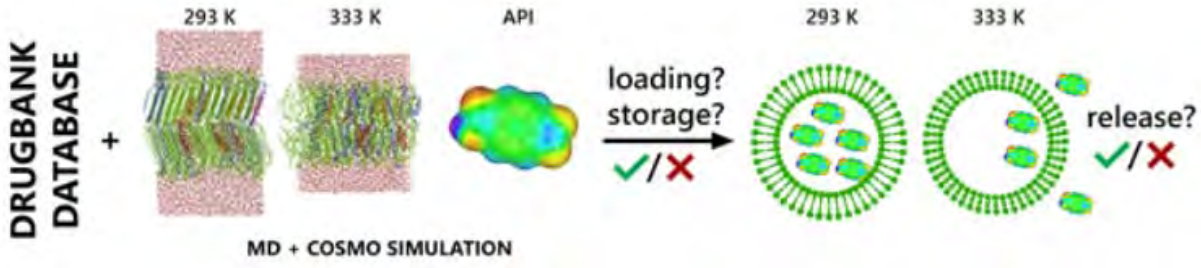
Liposomal formulations are famous as a carrier for Covid-19 mRNA vaccine but that is not their only purpose. Liposomes consists of lipid bilayer surrounding the aqueous cavity that can carry water soluble cargo. Liposomes reduce the systemic toxicity of highly potent Active Pharmaceutical Ingredients (APIs), prolong the systemic circulation of API or prevent API from the body environment. However, not all APIs are suitable for encapsulation in liposomes. One of the main issues with API/liposome compatibility is the permeation of the API through lipid bilayer of liposome. On the other hand, too low permeability affects the preparation procedure of encapsulated molecule. The most reliable way to test API/liposome compatibility so far has been excessive experimental study and therefore, computational model capable of predicting API behaviour in liposome formulation is needed.
In this work, a new *in silico* approach is introduced. Based on COSMOperm approach, the molecular dynamics simulation of chosen membrane at various tepmeratures was performed. Then, σ-profile representing the charge and electron density of candidate molecules from DrugBank database was calculated. COSMOperm calculation of chemical potential of all candidate molecules was performed and partitioning and permeation rate was calculated. Based on the experimental validation on fluorescent dyes, the most potent cantidates were identified and one molecule was tested in anti-microbial assay.



PP10-1-B

EFFECT OF GLYCANS ON LIPID MEMBRANE AT THE NANOSCALE

P. Winkler[1], B. Gumí-Audenis[2,3], F. Campelo[1], M. F. García-Parajo[1,4], M. I. Giannotti[2,3,5]

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Membranes have unique physical properties allowing cells to rapidly change shape, squeeze, stretch, pinch off smaller units, fuse. The lipid bilayer, whose building blocks are held together by weak interactions, is in part responsible for the physical and dynamic properties of the membrane, and lateral compartmentalization at multiple spatiotemporal scales regulates key functions. Due to membrane complexity, mimetics like supported lipid bilayers (SLB) are convenient to identify individual contributions, while tuning the environment can simulate different scenarios. Seeking to understand the role of membrane environs on the membrane properties, we have first shown that SLBs may represent an intermediate scenario between a free membrane (blebs) and a cytoskeleton-supported membrane.[1] The extracellular glycocalyx has recently emerged as an important player in modulating membrane organization. We investigated the influence of hyaluronic acid (HA) on the nanoscale organization of SLBs. We evaluated the effect on the morphology and mechanical properties at the nanoscale using atomic force microscopy and spectroscopy, and photonic nanoantenna arrays combined with fluorescence correlation spectroscopy was used to assess dynamics. Overall, we found that HA has a profound effect on the dynamics, nanoscale organization and mechanical properties of lipid bilayers enriched in sphingolipids and/or cholesterol, such as those present in living cells.[2]

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2. *Winkler et al. J. Phys. Chem. Lett. 12, 1175, 2021.*

PP11-1-A

GM1 LEAFLET ASYMMETRY STABILIZES MEMBRANE PORES

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For effective cell transfection and electrochemotherapy treatments, the cell plasma membrane needs to be transiently porated by electric fields and reseal afterwards. Model membranes are used to understand electroporation phenomena, but their pore lifetimes are much shorter than they are in cells. Cell membranes are highly asymmetric. Here, we investigated the influence of membrane asymmetry on electroporation of synthetic model membranes. We employed giant unilamellar vesicles (Dimova Ann.Rev.Biophys.48:93,2019) and introduced membrane asymmetry by GM1 leaflet distribution (Dasgupta et al.,Proc.Natl.Acad.Sci.USA.115:5756,2018). GM1 is a ganglioside which exhibits strong asymmetry in its distribution across neuronal plasma membranes. In asymmetric GM1-doped vesicles, we identified series of membrane remodeling events which were distinctly different from poration of symmetric membranes. After electroporation, asymmetric vesicles displayed a high density of nanotubes which resulted in slowing down of pore resealing and leading to lower values of the effective edge tension. Simple energy balance considerations allowed us to establish the stability conditions for the membrane pores. Membrane nanotubes were found to obstruct pore closing. Furthermore, we observed the formation of a large number of membrane defects on the vesicles after pore closure and subsequent increased membrane permeability to small molecules suggesting the generation and stabilization of pores with suboptical resolution.

PP12-1-B

MULTISCALE MODELING OF LIPID BILAYER ADSORPTION ON TITANIA NANOSURFACES

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TiO_2-based nanomaterials are ubiquitous in important technological applications, yet their toxicological properties are uncertain[1,2]. Modeling interactions of nanomaterials and biomolecules, such as membrane phospholipids, may help predicting nanotoxicity pathways. However, there is no practical way to model such interactions for sufficiently large nanoparticles with a representative fraction of the cell membrane if one uses an atomistic model. Thus, a more efficient and reliable model is required[3]. In this work, we develop a coarse-grained model of TiO_2-lipid systems using structural data from atomistic molecular dynamics simulations with the inverse Monte Carlo method[4] implemented in the MagiC software package[5]. Molecular dynamics simulations are carried out using the GROMACS 2019 software package[6].

The atomistic simulations have revealed that DMPC and POPE phospholipids can adsorb on different anatase and rutile surfaces through the polar headgroups. Several different binding modes with varying adsorption strength have been identified. The resulting atomistic trajectories are mapped to the coarse-grained coordinates. During the coarse-grained bead mapping, the phospholipid molecules are reduced from ~100 atoms to ~10 coarse-grained sites, and the two surface layers of the TiO_2 slabs are reduced to the beads containing 5-7 atoms. Water molecules and the bulk TiO_2 are accounted for implicitly in the TiO_2-lipid effective potentials. Preliminary data show that the structural features of the TiO_2-lipid interface are well-reproduced in our coarse-grained model. However, the diffusion in the system is ~20 times faster due to the absence of water molecules.

This work has been supported by the EU Horizon 2020 research project SmartNanoTox (No 686098) and the Swedish Research Council. We acknowledge the Swedish National Infrastructure for Computing (SNIC) for granting access to high-performance computing facilities.

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PP13-1-A

THE TWO FACES OF LIQUID ORDERED PHASE

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The well-known phase diagram of some phospholipid bilayers with cholesterol includes three phases: gel, liquid disordered (L_d) and liquid ordered (L_o). The L_o phase, which serves as a model for ordered domains in biomembranes, only appears at the inclusion of sufficient amount of cholesterol and is generally related to the vanishing of the first order L_d-gel transition.
Previously, some experiments reported changes in membrane properties in the L_o region of the phase diagram as a function of temperature for binary mixtures. Certain studies suggested that this observation is related to structural changes that could not be fully observed at the time.
Now, using all-atom MD simulations, we can observe and characterize a continuous transition with temperature for both binary (DPPC\CHOL) and ternary (DPPC\DOPC\CHOL) mixtures within the L_o region. Specifically, for the ternary mixture, this transition seems to be only expressed by changes in the properties of DPPC lipids that reside in hexagonally packed regions of DPPC chains and correspond the melting of said regions. Furthermore, we provide relevant experimental support of such structural transition in membranes with corresponding compositions using DSC and fluorescence spectrometry.
The results of this study may be relevant for the understanding of the structure of L_o domains in L_o\L_d coexistence and of the hypothesized rafts in biomembranes.

PP14-1-B

UNVEILING LATERAL ORGANIZATION AND TRANSVERSAL ASYMMETRY IN PLANT MEMBRANES

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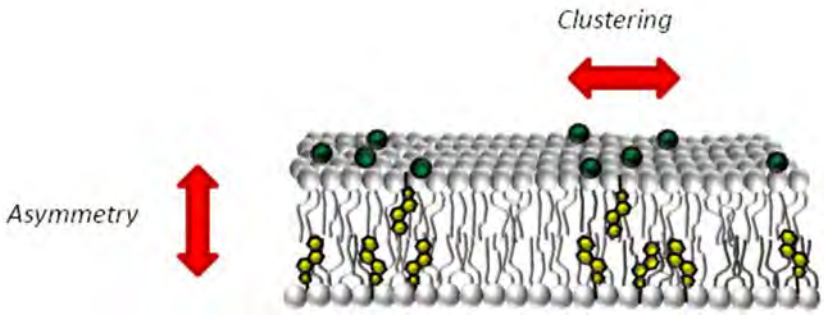
The different properties of plasma membrane depend on the presence, local structure and relative distribution assumed by the thousands of components it is made of. As for animal cells, plant membranes have been demonstrated to be organized in subdomains with different persistence lengths and times. In plant cells, sitosterol has been demonstrated to confer to phospholipid membranes a more ordered structure, similarly to the well characterized cholesterol in animal membranes. Among lipids, glycosphingolipids are claimed to form rafts where they tightly pack with sterols, which strongly interact with saturated phospholipids and sphingomyelin. Glucosylceramides are sphingolipids involved in plant signalling and are essential for viability of cells and whole plant. We investigated the glucosylceramide-sitosterol structural coupling within PLPC membranes by Langmuir films, in silico simulations (M. Deleu et al., Coll. Surf B, 2019) and neutron reflectometry, unveiling that a strong direct interaction between the two molecules exists and governs their lateral and transversal distribution within membrane leaflets. The understanding of the driving forces governing specific molecules direct or indirect clustering and segregation in subdomains, such as glucosylceramide and sitosterol, beside impacting on the local mechanical membrane properties, is of interest since specific molecules pairing could reflect in other molecules partitioning within the membrane.



PP15-**2-A**

MEMBRANE STABILITY AFTER ELECTROPORATION: INFLUENCE OF ANIONIC LIPIDS

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Membrane stability is of vital importance to cells. When subjected to strong stimuli, such as electric pulses, pores open in the membrane. Usually, pores reseal and membrane integrity is restored, as observed in neutral PC (phosphatidylcholine) giant unilamellar vesicles (GUVs). However, GUVs containing charged lipids may display a different response to strong DC electric pulses: Some micron-sized pores open indefinitely leading to vesicle burst while GUVs that apparently restore their integrity after macropore closure can exhibit a long-lasting high permeability revealing the persistence of sub-microscopic pores minutes after the end of the pulse. Here, the stability of GUVs composed of PC and increasing fractions of other physiologically relevant anionic lipids, such as cardiolipin (CL), phosphatidylinositol (PI) and phosphatidylinositol 4,5-bisphosphate (PIP2), is investigated with phase contrast optical microscopy. First, the occurrence frequency of disturbing events is quantified in a large population of GUVs. Then, the dynamics of vesicle contrast loss due to long-lasting permeability is assessed on individual GUVs. Finally, the pore edge tension, which reflects the energy penalty per unit length to arrange lipids in the pore rims, is measured from the dynamics of macropore closure. Overall, membranes containing higher fractions of charged lipids are more unstable and prone to disturbances, as a result of a reduced pore edge tension. More specifically, the pore edge tension decreased significantly for membranes containing 50% of anionic lipid.

PP16-**2-B**

INTERACTIONS OF TOPICAL DRUGS WITH TEAR FILM LIPID LAYER: A BIOPHYSICAL VIEW

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The Tear Film Lipid Layer (TFLL) is an oily phase stabilizing the aqueous tear film covering the cornea. TFLL decreases the tear film surface tension and improves its viscoelastic properties. Destabilization and rupture of the tear film occur in dry eye disease and are closely related to alternations of TFLL. Topical eye drops containing oil-in-water emulsions are used to supplement lipids and, hence, stabilize the tear film. We study biophysical aspects of topical drug components' interactions present in oil-in-water emulsions with TFLL. Our main goal is to understand the macroscopically observed eye drops–tear film interactions, and rationalize these observations at the molecular level. We use a multi-scale approach combining experiments on meibomian lipid extracts from humans, experiments using synthetic lipid films, and molecular dynamics simulations. We show that specific interactions of the studied drugs with TFLL enhance tear film stability and elasticity. These interactions can also be modulated by varying the material packing at the tear–air interface.

PP17-2-A

MODEL STUDY OF INTERACTIONS IN THE LIPID-ION SYSTEM

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The membrane structure changes with increasing ion concentration, which is a peculiar property of the ions and lipids themselves. Here, we modelled the interactions of the divalent cations Ca^{2+} and Mg^{2+} with the zwitterionic lipid bilayer systems, such as a fully saturated dipalmitoylphosphatidylcholine (DPPC) and a di-monounsaturated dioleoylphosphatidylcholine (DOPC) membranes. The effect on the bilayer structural properties confirms the direct interactions in all cases studied. We have observed a small difference when studying DPPC bilayers in the gel and fluid phases, with somewhat larger effects in the latter case. The hydration proved to be a factor in the case of DOPC bilayers, with the larger effects in the case of less hydrated systems. Our interaction model proposed according to the MD results, corroborated also by the neutron scattering experimental results, suggests an existence of the limiting area of about 65 $Å^2$. When the initial lipid area is larger than that, the ions have some fluidizing effect. On the other hand, a strong condensation occurs in the systems with the larger area per lipid.

This work is being supported by the Russian Science Foundation under grant No. 19-72-20186.

PP18-2-B

HOW DID FIRST LIFE EMERGE ON TERRESTRIAL PLANETS?

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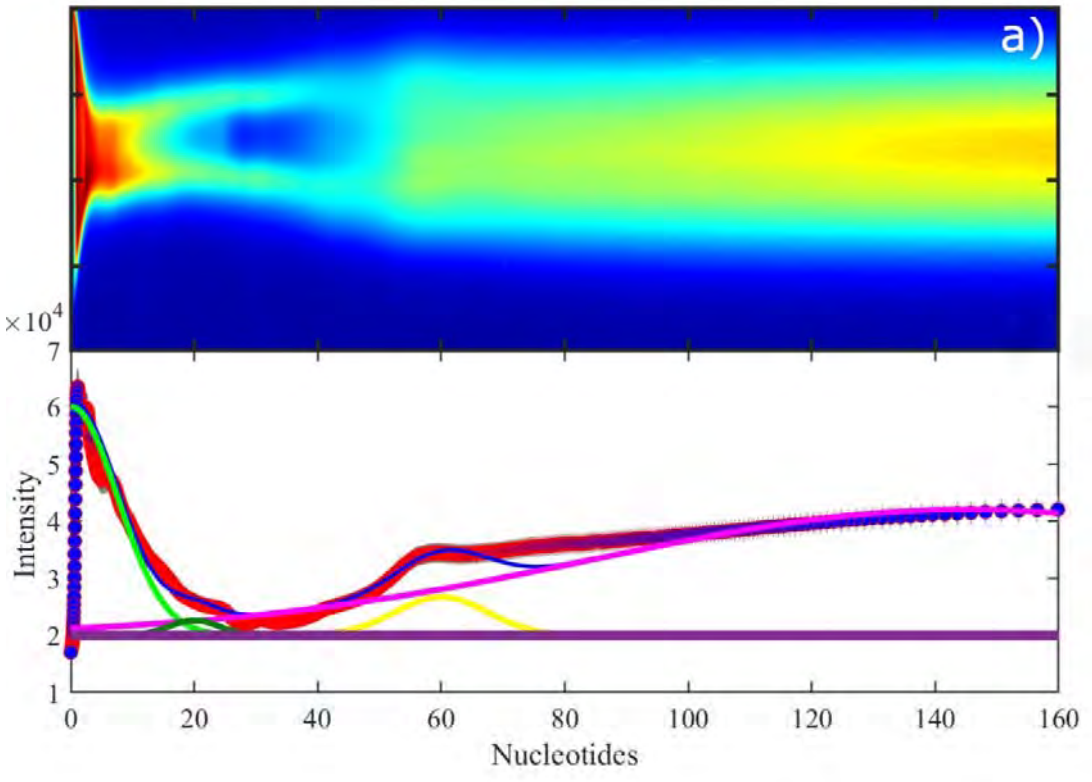
How did the first genetic code and the first forms of cellular appear in the early life, about 3.5 billion years ago, of terrestrial and Earth-like exoplanets? This question has become especially timely with the discovery of an ever-increasing number of rocky exoplanets where liquid water is present.

We investigated how specific conditions on terrestrial planets, such as water, temperatures, radiation, atmospheres, and the presence of certain minerals and organic molecules, can potentially drive polymerization of RNA-like polymers. Experiments were conducted using the Planet Simulator, a custom-built simulation chamber.

Our current results show that the formation of the first genetic assemblies would have occurred in shallow wells that undergo hot-cold and wet-dry cycles. Nucleic acids may have evolved in contact with salt, such as ammonium chloride, and in particular phospholipids and simple membranes, for the formation of protocells when the concentration of those elements would have been optimal. We found that long RNA polymers form spontaneously in the presence of membranes in these warm little ponds and that these polymers are spontaneously incorporated into liposomes. We observe RNA-chains of hundreds of nucleotides whose length depends on the exact temperature and humidity cycles due to daily and seasonal change.



PP19-**2-A**

IMPEDIMETRIC MEMBRANE-BASED BIOSENSOR: A MACHINE LEARNING APPROACH

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This study explores the potential of using Machine Learning (ML) to improve the accuracy and specificity of biosensors based on Red Blood Cell (RBC) biomembranes. Previous studies have shown the potential of using RBC biomembranes for detection of pathogen contamination in liquid samples using Electrochemical Impedance Spectroscopy (EIS) of biomembranes, which allows the detection of a variety of processes in biomembranes linked to active contamination, such as chemical binding of proteins, nucleic acids, cells, antibodies, and antigens among others.
For industrial applications speed, ease of use, and cost remain challenging. ML has the potential to improve the sensitivity and specificity of the EIS method due to the increased ability to filter out the noise and consequently detect minor changes of structure. Additionally, the processing on the server allows the continuous improvement and growth of the range of applications without the need to modify the electronics.
Preliminary simulation results show that non-linear change in the structure of the membrane due to contamination processes provides sufficient data for training ML models, which allow fitting complex alternative circuits, characteristic to different types of biomembranes damage. Further fitting of experimental data is expected to define the range of business applications of ML in RBC biomembranes sensors.

PP20-**2-B**

THE RICH PHENOMENOLOGY OF PHOSPHOLIPID MEMBRANES CONTAINING GLYCOLIPIDS: INSIGHTS FROM SCATTERING TECHNIQUES AND SIMULATIONS

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

PP21-2-A

LIPID MEMBRANE PHASE TRANSITIONS INVOLVE STRUCTURAL REDISTRIBUTION OF INTERFACIAL WATER

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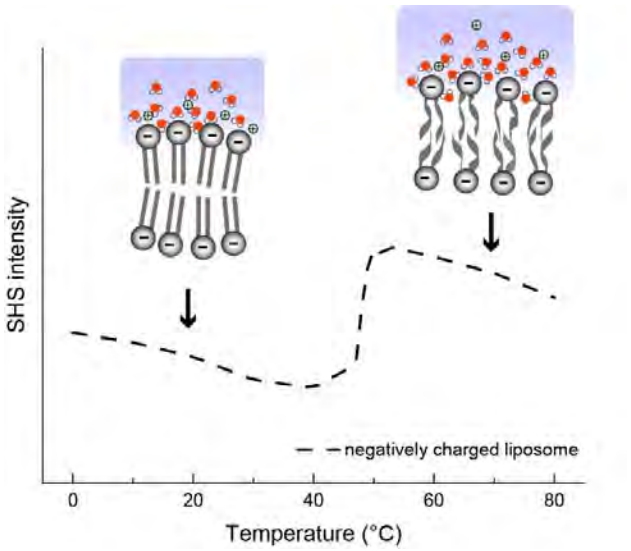
Morphological transitions of lipid membranes are thought to depend primarily on the chain length diversity of its components. However, the role of different lipid head groups, their counter ions and hydrating water molecules in the close proximity of the membrane on the gel-to-liquid phase transition of lipid is mostly unknown. We employed second harmonic (SH) scattering measurements to probe asymmetric distribution of water molecules between the lipid leaflets. Measurements on the same acyl chain length and single lipid component liposomes consisting of 1,2-dimyristoyl-*sn*-glycero-3-phosphate (sodium salt) (DMPA), 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (sodium salt) (DMPS), 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DMPG) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) showed a significant lipid hydration alteration as a function of temperature. More specifically, we observed a > 20% increase in the second harmonic intensity at the phase transition temperature for liposomes made of charged lipids. Zwitterionic DMPC lipids on the other hand, display only smaller (~ 8%) changes. This data implied that an increasing number of water molecules anisotropically oriented towards surface normal above the phase transition and the charge state of the lipids play key role on the gel-to-liquid phase transition. Our data demonstrate that lipid phase transitions not only lead to structural changes in the bilayer itself but also in the adjacent aqueous phase.



PP22-2-B

HeLa MODEL LIPID MEMBRANE USED AS EVIDENCE OF LIPOSOME FUSION

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We engineered a lipid membrane model that mimics the HeLa plasmatic cell membrane. We characterized the model using atomic force microscopy (AFM) and fluorescence. HeLa liposomes, constituted by 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) and cholesterol (CHOL) (0.29:0.31:0.06:0.34, mol/mol/mol/mol) were deposited onto mica surface to form supported lipid bilayers (SLBs). The addition of liposomes of POPC, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and CHOL (0.65:0.15:0.20, mol/mol/mol) or POPC:DOTAP (0.80:0.20, mol/mol) on the HeLa SLBs, as observed by means of Force Spectroscopy, suggests that fusion takes place, with the lipid components of the liposomes becoming inserted into the resulting planar bilayer. To ascertain the fusion mechanism against liposomes mimicking the HeLa lipid membrane, Förster resonance energy transfer was used. Moreover, the characteristics of the fused systems were characterized by measuring fluorescence anisotropy of 1,6-diphenylhexatriene and 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene, which allows the obtention of the bilayers microviscosity. All results suggest that there is an electrostatic interaction between DOTAP borne by the liposomes and the POPS of the HeLa model, and that presence of CHOL in both liposomes enhances the fusion process.

PP23-2-A

ERYTHRO-VLPS: EMBEDDING SARS-COV-2 SPIKE PROTEINS IN RED BLOOD CELL BASED PROTEOLIPOSOMES LEADS TO PRONOUNCED ANTIBODY RESPONSE IN MOUSE MODELS

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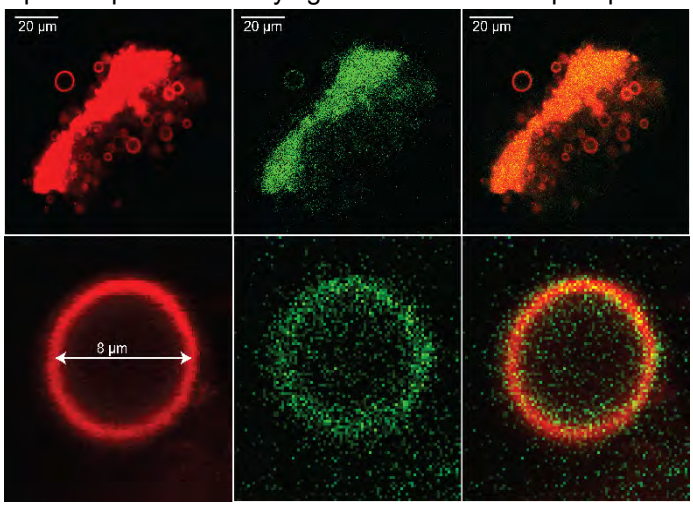
Novel therapeutic strategies are urgently needed to control the SARS-CoV-2 pandemic. Here, I present the fabrication and characterization of Erythro-VLPs: Erythrocyte-Based Virus Like Particles, i.e., red blood cell (RBC) based proteoliposomes carrying the SARS-CoV-2 spike protein.



Erythrocytes can present antigens to the immune system when senescent cells are being phagocytized in the spleen. This capacity together with their high biocompatibility make RBCs effective vehicles for the presentation of viral immunopathogens, such as the SARS-CoV-2 S-protein, to the immune system. Epi-fluorescent and confocal microscopy, dynamic light scattering (DLS), and Molecular Dynamics (MD) simulations were used to characterize the liposomes and the insertion of the S-proteins. The Erythro-VLPs exhibit dose-dependent binding to ACE-2 (angiotensin converting enzyme 2) in biolayer interferometry assays.

We present experimental evidence of a pronounced immunological response in mice after 14 days, and the production of antibodies was confirmed in ELISA. These antibodies were found to be specific for the S-protein RBD sub-domain. This immunological response was observed in the absence of any adjuvant which is usually required for protein-based vaccines.

[1] Himbert et al., "Erythro-VLPs: Embedding SARS-CoV-2 spike proteins in red blood cell based proteoliposomes leads to pronounced antibody response in mouse models", submitted.

PP24-2-B

REVISITING THE SINGER NICOLSON MODEL OF LIPID MEMBRANE: HOW DOES THE 2D LIQUID REALLY LOOK LIKE.

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The Singer Nicolsen model describes the lipid membrane as a two dimensional liquid. Liquids are on the one hand a very familiar concept which gives us the illusion to well understand the system, on the other hand liquids are inherent difficult to describe theoretically. In literature few attempts were made to describe the lipid bilayers with the concept of the theory of liquids e.g. mean field theory[1-4]. This includes the elucidation of the effect of different lipids on the interaction of membrane compounds[2,3]. Those approaches didn't get much attention probably due to their high theoretical manner and few direct experimental verifications.
We will present methods and result for the experimental investigation the effect of lipids on the distribution of peptides on the membrane surface. Those results give evidences that this lipid controlled distribution is key in the activity of the antibiotic peptides PGLa and magainin 2[5] of the African clawed frog Xenopus laevis. Ultimately the lipids play a more "active" role in the activity of antibiotic peptides. We try to make to make the link between those experimental findings and the theoretical descriptions of the membrane as a real 2D liquid.

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PP25-2-A

OPTIMIZATION OF DIHEDRAL TERMS IN THE SLIPIDS FORCE FIELD

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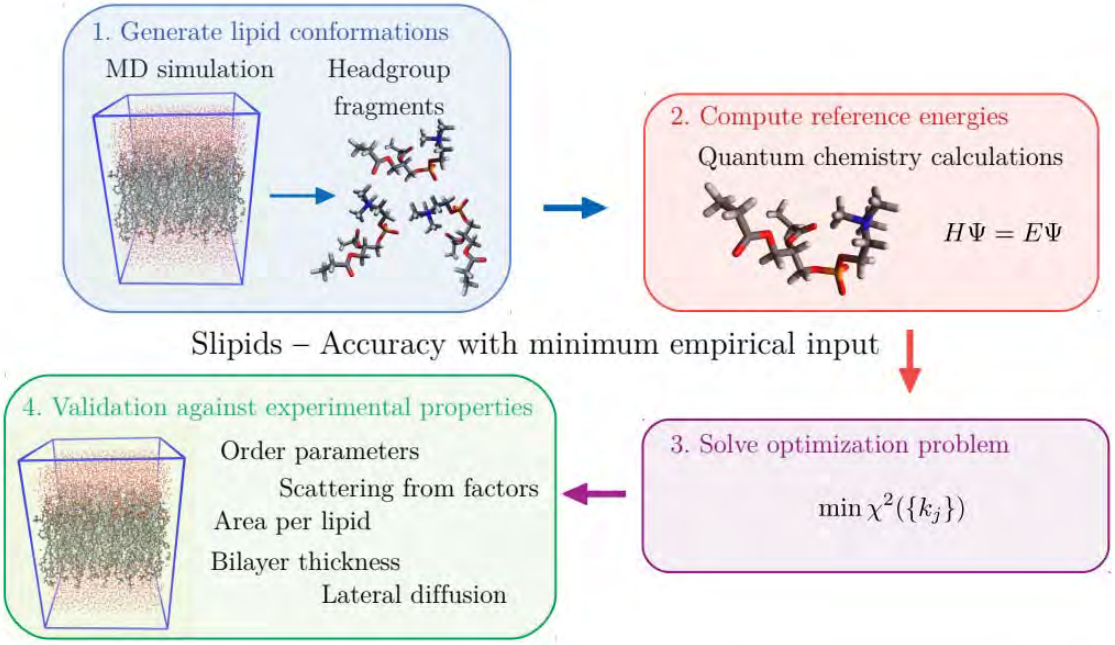
Slipids molecular mechanics force field for simulations of lipid bilayers developed by Jämbeck and Lyubartsev [J. Phys. Chem. B, 2012, 116, 3164 - 3179; J. Chem. Theory and Comput., 2012, 8 2938 - 2948] have been shown to reproduce several experimental properties. These include area per lipid, scattering form factors, order parameters of C-H vectors in lipid tails and lateral diffusion coefficients. However, it has also been shown that order parameters of C-H vectors in the headgroups of phosphatidylcholine lipids, in particular those in the glycerol moiety, are in poor agreement with results from NMR spectroscopy [J. Phys. Chem. B, 2015, 119, 15075-15088]. In this work the Slipids force field has therefore been reparametrized in order to improve the agreement with order parameters from NMR. The new force field was obtained by fitting dihedral parameters against quantum chemical energies for a set of lipid headgroup conformations extracted from an MD trajectory. The new parameter set was validated for three different one component lipid bilayers and two bilayers with different cholesterol content. Good agreement with several experimental properties is obtained and the new force field gives significantly better agreement with NMR order parameters for C-H vectors in the glycerol moiety.



PP26-**2-B**

TETHERED LIPID BILAYERS USING ANCHOR-HARPOON SURFACTANTS.

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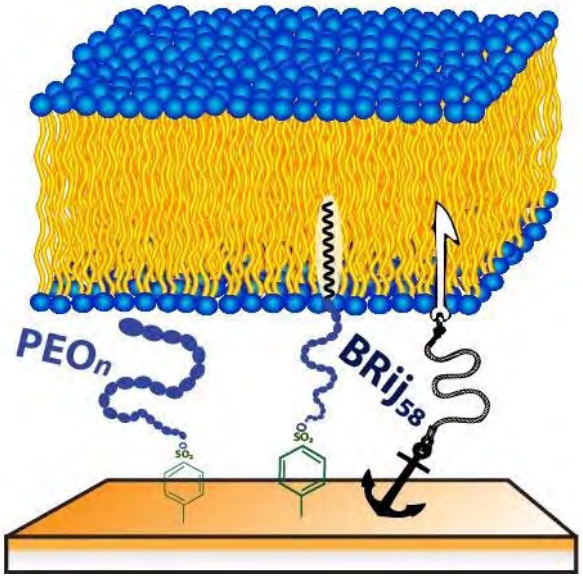
Sparsely tethered bilayer membranes (stBLM) provide a particularly advantageous platform to study membrane proteins' functions, ion transport, pores, and therefore to understand fundamental mechanisms, thanks to their stability and the possible use of a wide range of surface-sensitive techniques (Electrochemical Impedance Spectroscopy, Neutron & X-ray Reflectivity, Fluorescence Recovery After Photo-Bleaching…). Maintaining the membrane hydrated, fluid and close to the substrate without using cumbersome chemistry to synthesize "anchor-harpoon" molecules can be a challenge. Here, we developed a new experimental approach where a single model phospholipid bilayer is kept fluid and partially tethered to a flat surface from an original anchoring functionalization that is highly reactive to –OH terminated molecules. In this way we avoid complex organic chemistry and graft commercial Brij or CiEj (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. Thus, 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 tuneable anchoring densities on various materials.



PP27-**2-A**

FUSION OF CATIONIC LIPOSOMES WITH PHASE-SEPARATED CHARGED VESICLES: A COMBINED CALORIMETRIC AND CONFOCAL MICROSCOPY STUDY

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[3]*Groningen University, Groningen, Netherlands*

Cell membranes are believed to be heterogeneous and to exhibit lipid domains (or rafts), which govern cellular functions and processes, including membrane fusion. We investigated membrane fusion of homogeneous cationic liposomes with phase separated negatively charged giant vesicles, in which the charges are either in the gel or in the fluid phase. The stability and shape of domains were observed using confocal and epifluorescence microscopy at room temperature and the membrane phase transition was assessed with differential scanning calorimetry (DSC). Confocal microscopy showed that cationic liposomes mainly docked onto phase-separated giant vesicles exhibiting charged-gel and neutral-fluid domains, but were able to efficiently fuse with vesicles exhibiting neutral-gel and charged-fluid domains. High concentration of fusogenic liposomes led to the dissolution of the domains and suppression of demixing in both cases. The DSC studies confirmed that after the addition of fusogenic liposomes to both systems, the phase transition was shifted to lower temperatures. A completely fluid phase was reached at around 25 °C, in agreement with the microscopy results. These findings have the potential to unravel an important role in the regulation of the interactions between cells and liposomes that are used in drug delivery systems.

This work was supported by FAPESP (2017/093675)

PP28-**2-B**

CREATING ASYMMETRIC VESICLES USING LIPID-COATED SILICA NANOPARTICLES.

Y. Liu[1], K.C. Batchu[2], L. Porcar[2], U. Perez-Salas[1]

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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. Here we present our recent results on the production of asymmetric membranes using lipid coated silica nanoparticles to study the intra-membrane flip-flop of lipids and cholesterol across a single lipid bilayer using Small Angle Neutron Scattering and NMR.

PP29-2-A

CATION–ZWITTERIONIC LIPID INTERACTIONS ARE GOVERNED BY THE LATERAL AREA PER LIPID

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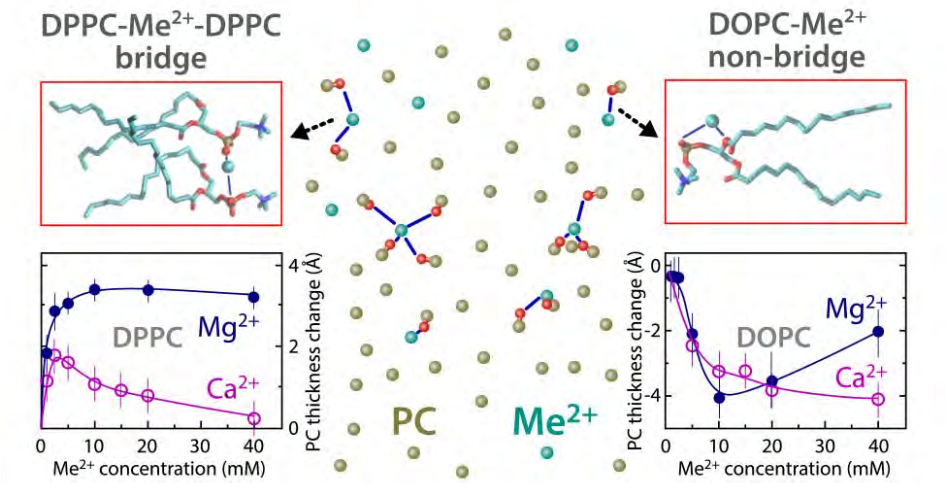
Interactions of the divalent cations Ca^{2+} and Mg^{2+} with the zwitterionic lipid bilayers prepared of DPPC or DOPC proved to influence the bilayer thickness and lateral area. The extent of structural changes suggests various mechanisms of the cation−lipid interactions. There was a qualitative difference between the results of the fully hydrated DOPC bilayers and the others examined. Our observations prompted us to suggest an interaction model that is plausibly governed by the lateral area of lipid. Namely, when the interlipid distance is small enough to allow for the multiple lipid−ion interactions, the lipid−ion−lipid bridges are formed. The bridges impose strong attractions that increase the order of lipid hydrocarbon chains, resulting in the bilayer thickening. In the other case, when the interlipid distance extends beyond a limiting length corresponding to the area per lipid of ~65 $Å^2$, ions continue to interact with the lipids by forming the separate ion−lipid pairs. Since these interactions affect the lipid membrane structure in the lateral direction, they may prove to play their role in other mechanisms lying within the complex membrane systems.

Acknowledgement: NK, EE, KTK, SK were supported by the RSF grant 19-72-20186, DU acknowledges grants from VEGA 1/0223/20 and APVV-17-0250.



PP30-**2-B**

THE EFFECT OF DIVALENT IONS ON THE BILAYER STRUCTURE OF DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLES

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The biologically relevant divalent metal cations such as Ca^{2+} and Mg^{2+} impose consimilar changes in the structural parameters of lipid bilayers in the DMPC unilamellar vesicles at a concentration range 0–30 mM. The bilayer structural parameters (thickness and area per lipid) obtained at different concentrations of cations in the gel and fluid phases of the lipid systems by means of small-angle neutron scattering change in the cases of both Ca^{2+} and Mg^{2+} ions. At the concentrations of 0–1 mM, the lipid bilayers demonstrate clearly a drastic increasing in the bilayer thickness. In the concentration range of 1–30 mM, a weak tendency to a thickness decrease of ~ 1 Å is observed. In the case of Co^{2+} ions, on the contrary, all changes are extremely weak. A model of electrostatic interactions for these systems that assumes the formation of ion bridges between lipid headgroups may be adopted to our systems when considering the typical average distances between DMPC lipids, adsorbed ions, and the Debye screening length. The developed model is of interest to the future studies of bilayer interactions with various peptides and proteins embedded.

Acknowledgement: the authors were supported by the RSF grant 19-72-20186

PP31-2-A

SOLUBILIZATION OF BIOMIMETIC MEMBRANES BY DETERGENTS WITH DIFFERENT PHYSICAL-CHEMICAL CHARACTERISTICS

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Detergents are used to solubilize biomembranes and extract their components. Previously, our group has shown that, in giant unilamellar vesicles (GUVs) of ternary biomimetic compositions, the detergent Triton X-100 promotes Lo/Ld phase separation followed by solubilization of the Ld phase only, while Lo phase remains unimpaired. The present work extends the study to detergents with different physical-chemical characteristics: Triton X-165, Dodecyl Maltoside (DDM), C10E5, Octyl Glucopyranoside (OG), Tween 20, CTAB, SDS and Chaps. The membrane compositions studied were Ld phase (pure POPC), Lo phase (SM:cholesterol 7:3), and the ternary mixture POPC:SM:cholesterol 2:1:2. The solubilization profile of each detergent was followed by turbidity measurements on large unilamellar vesicles (LUVs). Then, optical microscopy was used to observe GUVs in the presence of the detergents. Except for CTAB and SDS, all detergents could completely solubilize vesicles in the Ld phase. On the other hand, Lo vesicles were completely or partially insoluble to all detergents. Two different interactions with Ld phase were determined: increase in GUV surface area and turbidity before solubilization (C10E5, TX165, Chaps, Tween 20 and OG) and vesicle rupturing/bursting (DDM, CTAB and SDS). The results are discussed in terms of the flip-flop rate and characteristics of the polar head of detergents.

PP32-3-A

BACTERIAL MIMETIC SYSTEMS FOR STUDYING BACTERIAL INACTIVATION AND INFECTION

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Gram-negative bacteria are equipped with a cell wall composed of a complex matrix of lipids, proteins and glycans, which forms a rigid protective layer against the environment. One major component of the outer membrane is the glycolipid lipopolysaccharide (LPS). The unique properties of the large and amphiphilic LPS molecules have a major impact on Gram-negative outer membrane characteristics and understanding their role is important for various aspects such as electric-field based inactivation of bacteria, exploration of new antibiotics or bacteriophage treatments. Due to the high complexity of the Gram-negative outer membrane, there is a great demand for reduced and well-defined models. Giant unilamellar vesicles (GUVs) are synthetic membrane systems that allow the investigation of membrane properties, while controlling parameters such as membrane composition, surrounding media and temperature. In contrast to eukaryotic membranes, models of the Gram-negative outer membrane are scarce. In this project, we aim at generating an *in vitro* GUV model of the highly asymmetric Gram-negative bacterial outer membrane. We use an adapted method based on inverted emulsions to integrate LPS in the GUV outer leaflet and present a detailed analysis of the resulting LPS-GUVs.

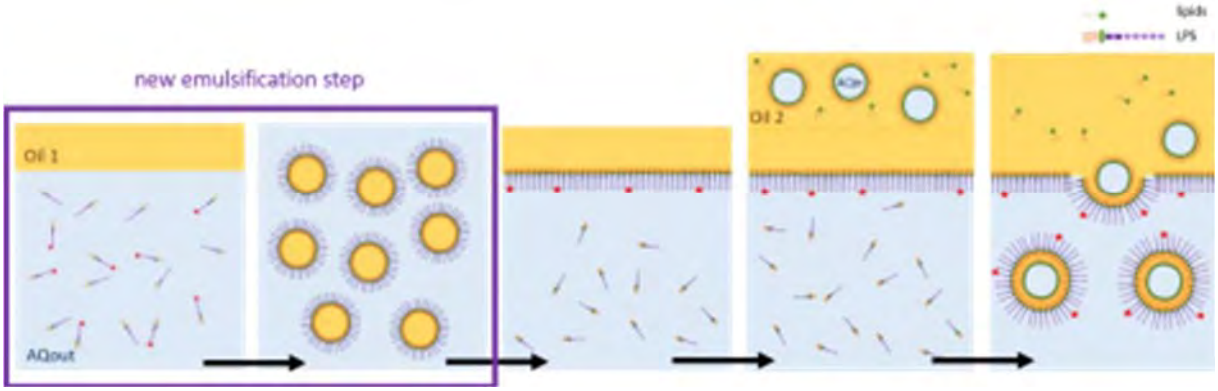

Figure 1: Schematic illustration of the protocol for LPS-GUV preparation employing an inverted emulsion technique.

PP33-3-B

INCREASE IN MEMBRANE TENSION AND LIPID DIFFUSION COEFFICIENT OF ADHERING LIPOSOMES VIA ELECTROSTATIC INTERACTIONS AND OSMOTIC DEFLATION

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Membrane adhesion is a common phenomenon in cells and known to be related to a variety of biological events such as migration, morphogenesis, and differentiation. For the understanding of the physicochemical features of membrane adhesion, liposome–liposome and liposome–substrate adhesion have been studied. Although the membrane adhesion has been shown to increase membrane tension and hinder lipid diffusion, the correlation between these changes and the extent of membrane adhesion has not been quantified. Here, we analyzed the dependence of membrane tension and lipid diffusion on the extent of membrane adhesion, i.e., the area fraction of the adherent region. For this purpose, we developed a concise method to obtain adhered liposomes through simple electrostatic interactions between the membranes followed by osmotic deflation. Using these adhered liposomes, we found that the membrane tension of the adhered liposomes slightly increases with an increase in the area fraction of the adherent region. Furthermore, the diffusion coefficient of lipids is larger for the adhered liposomes than isolated liposomes, which is consistent with the theoretical prediction. The analysis might provide a basis for the understanding of the relationship between cell adhesion and bio-membrane physicochemical properties such as membrane tension and molecular diffusion [1].

*[1] A. Oda, C. Watanabe, N. Aoki, M. Yanagisawa, **Soft Matter**, 2020,**16**, 4549-4554*

PP34-3-A

THE INFLUENCE OF LIPID MEMBRANES ON FLUORESCENT PROBES' OPTICAL PROPERTIES

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Organic fluorophores embedded in lipid bilayers can nowadays be described by a multiscale computational approach. We developed over the years a methodology to characterize probes in view of their different positions, orientations, conformational versatilities, and (non) linear optical as well as fluorescence properties when they are embedded in model membranes. Combining different length and time scales, a full description of the probe led to novel insights into the effect of the environments. In this talk, computations on Laurdan, indocarbocyanine and azobenzene derived probes are presented, sketching how a multiscale approach based on extended molecular dynamics and hybrid quantum mechanics-molecular mechanics frameworks can predict probes' optical properties like two-photon absorption, second harmonic generation, and fluorescence lifetime and time resolved fluorescence anisotropy. We show not only how computer simulations can explain particular confocal experiments by analysing the localization and orientation of probes in different membrane phases, but also how computation can uncover novel functionalities of well-used probes, which might change the accepted view upon them. We conclude that multiscale modelling can assess a priori novel probes' optical properties and guide the analysis and interpretation of experimental data. The properties can be used to gain information on the phase and condition of the surrounding biological environment.

PP35-3-B

RAFT LIKE DOMAINS IN PHOSPHOLIPID-DECANOL MODEL MEMBRANES

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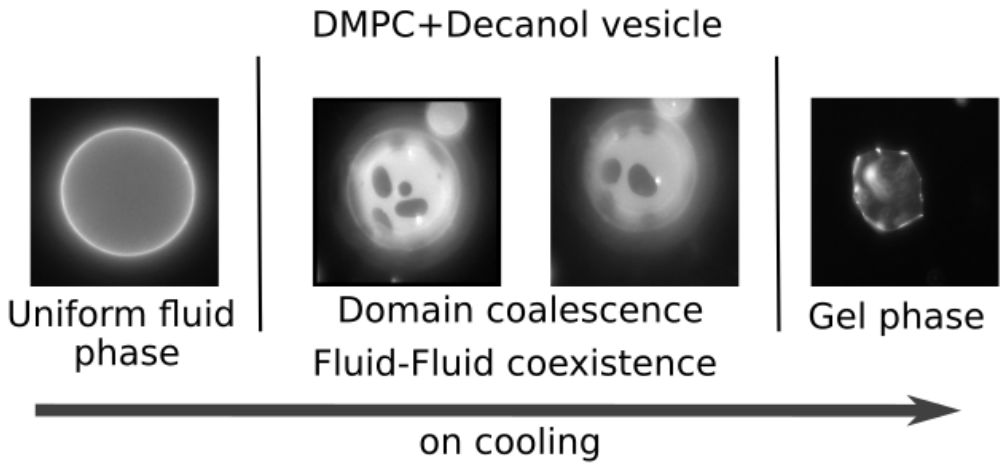
We have studied the effect of 1-decanol on the phase behavior of dimyristoylphosphatidylcholine (DMPC) membranes using a variety of experimental techniques. 1-decanol is found to induce fluid-fluid coexistence in DMPC membranes over a temperature range just above the chain melting transition temperature. The decanol-rich phase is found to have a higher bilayer thickness and a lower diffusion coefficient compared to the decanol-poor phase. On cooling, a long-wavelength ripple phase is observed, which transforms into a gel phase at lower temperatures. This phase behavior is reversible on heating. Although earlier experimental studies and computer simulations of phospholipid membranes have shown an enhancement in lipid chain ordering in the presence of long-chain alcohols, resulting in an increase in the bilayer thickness, fluid-fluid coexistence in these systems has not been hitherto reported. The system presented here can serve as a simple model to understand physical mechanisms that can drive fluid-fluid coexistence in membranes, which is believed to be important in the functioning of cell membranes.



PP36-1-A

DETERMINANTS OF MEMBRANE DOMAIN SIZE: LINE TENSION VERSUS MONOLAYER CURVATURE

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Thermally-induced membrane shape changes may result in phase separation since local curvature and local composition are coupled[1]. Such coupling could explain raft formation in symmetric bilayers: As the curvature of the first leaflet is counteracted by the opposing leaflet's equally large disposition to bend the bilayer in the other direction, small domains in the order of 10nm could be stabilized[2]. Here we exploit photo-switchable lipids capable of rather large spontaneous monolayer curvature changes to test the hypothesis. We used domain tracing and confocal fluorescence microscopy to observe domain size distribution and domain fusion kinetics in free-standing planar lipid bilayers.[3] In contrast to the theory, we did not observe the predicted curvature-driven dispersion of micrometer-sized domains. Instead, we found the line tension between liquid-ordered domains (LODs) and liquid disordered domains (LDDs) to govern domain size. Theoretical calculations show a local minimum of elastic energy at a distance between domain boundaries of ~ 8 nm. At closer quarters, both neighboring domains affect the intervening lipids. Depending on the conformational state of the photolipid, the elastic energy reaches its peak at a distance of ~4-5nm, which LODs must overcome to merge. Akin to the photolipids in our model experiments, line active substances may regulate LOD's size in biological membranes by kinetically hampering small domains' coalescence.

1 *Seifert, Phys.Rev.Lett.* ***70****, 1335 (1993).*

2 *Meinhardt, et al, PNAS* ***110****, 4476 (2013).*

3 *Saitov et al. Phys.Rev.Lett.* ***124****, 108102 (2020).*

PP37-1-B

DYNAMICS OF SPONTANEOUS WRAPPING OF MICROPARTICLES BY FLOPPY LIPID MEMBRANES

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In vivo, proteins are most probably the main actors of membrane deformation. The adsorption of inert particles could enable reproduction of the essential physics of membrane deformation by bound proteins.
In a recent study we have observed experimentally how micron sized particles adhere and are subsequently enveloped by lipid membranes (Spanke et al., Phys. Rev. Lett. 125, 198102). The lipid membranes are characterized by a bending rigidity κ_b and a membrane tension σ, which is near zero in our experiments. The polystyrene microparticles we use experience depletion interactions introducing an adhesion energy. Both the adhesion energy and particle size can be varied continuously in our system without changing the underlying composition.
At high adhesion strengths, the particles are enveloped spontaneously. We measure the velocity of this spontaneous wrapping process as well as the forces experienced by the particle using an optical trap. These results offer insight into the energy landscape of the wrapping process as well as the contact line dynamics.

Funding Information:

Grant number 172824 of the Swiss National Science Foundation

PP38-1-A

SMALL-ANGLE SCATTERING AND DENSITOMETRY APPROACH FOR RIPPLE PHASE INVESTIGATION

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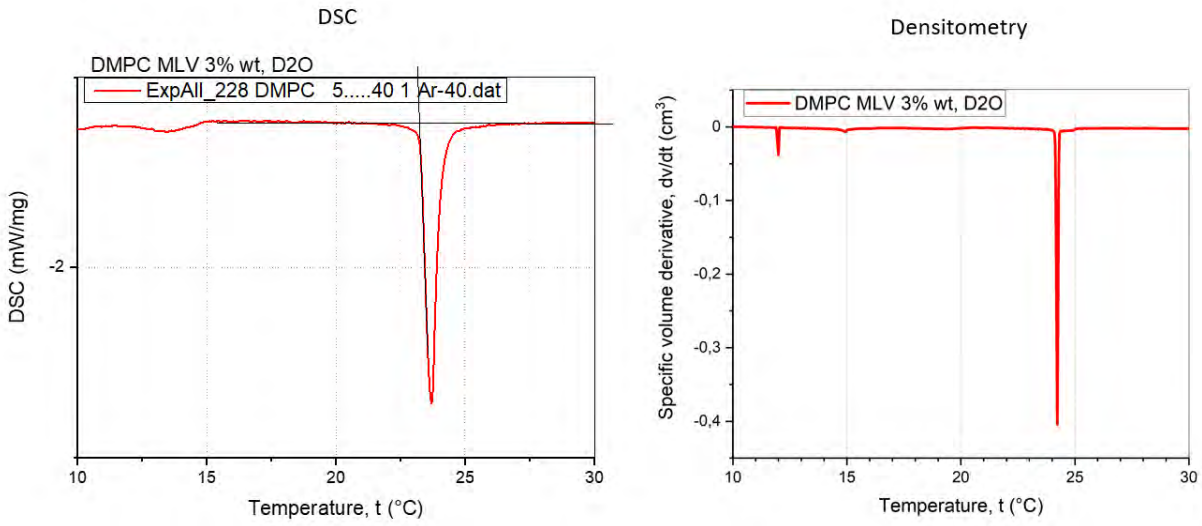
Besides the well studied ordered gel phase Lβ' and disordered liquid phase Lα in a wide range of lipids, mainly phospholipids, the so-called ripple Pβ' phase is observed. In this state, the surface of the lipid bilayer, in contrast to Lβ' or Lα states, is wavy. Pβ' phase might be of great interest: for example, the presence of membranotropic agents, including peptides, affects the local curvature of the membrane, and the curvature of the membrane, in turn, can affect the incorporation of proteins. There are at least two models of Pβ' phase. Earlier data show that the lipid hydrocarbon chains in Pβ' phase are highly ordered. In this case, the density of the lipid bilayer during the Lβ'-Pβ' transition should not undergo significant changes. The second model implies that the ripple phase is a disordered intermediate state of the membrane. In that case the bilayer density is expected to change.

Based on the studies of DMPC vesicles aqueous dispersions by densitometry, differential scanning calorimetry and small-angle scattering methods, an assumption about the possible predominant conformation of the hydrocarbon chains in the ripple phase is made.

Acknowledgement: This work has been supported by the Russian Science Foundation under grant 19-72-20186.



PP39-1-B

MONTE-CARLO STUDIES OF DETERGENT AND LIPOSOME INTERACTIONS

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It is known that giant unilamellar vesicles undergo dynamic morphological changes when exposed to a detergent. The solubilization process may take multiple pathways. In this work we identify lipid vesicle shape dynamics prior to the solubilization of DOPC giant unilamellar vesicles with Triton X-100 detergent. The violent lipid vesicle dynamics was observed with laser confocal scanning microscopy and was qualitatively explained via a numerical simulation. A 3D Monte-Carlo scheme was constructed that emulated the non-equilibrium conditions at the beginning stages of solubilization, accounting for a gradual addition of Triton X-100 detergent molecules into the lipid bilayers. We suggest that the main driving factor for morphology change in lipid vesicles is the associative tendency of the Triton X-100 molecules, which induces spontaneous curvature of the detergent inclusions, an intrinsic consequence of their molecular shape. The majority of the observed lipid vesicle shapes in the experiments were found to correspond very well to the numerically calculated shapes in the phase space of possible solutions. The results give an insight into the early stages of lipid vesicle solubilization by amphiphilic molecules that is nonequilibrium in nature and very difficult to study.

PP40-1-A

INTRINSIC LIPID CURVATURE FROM BAYESIAN DATA ANALYSIS OF INVERTED HEXAGONAL PHASES

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The intrinsic lipid curvature C_0 is given by the inverse bending radius of unstressed lipid monolayers and is an important parameter for the stored elastic stress energy of lipid bilayers. It thus plays a pivotal role in many membrane properties, including lipid/protein interactions or overall membrane curvature to name but a few. We extended a recently reported model-based approach to determine C_0 via a full q-range small-angle X-ray scattering data analysis of inverted hexagonal structures [1]. In particular we studied a series of phosphatidylcholines and sphingolipids, including ceramide as a function of hydrocarbon chain length and composition. The C_0 values of the investigated lipids were derived by determining their effects on the inverted hexagonal template structure of dioleoyl phosphatidylethanolamines. Based on the overall shape of the participating lipids a nonlinear addition of the partial curvatures was considered.
We discuss challenges and constraints of our approach and present current results.

[1] M. Frewein, M. Rumetshofer, and G. Pabst. J Appl. Cryst 52; 403 – 414 (2019).

PP41-1-B

LABEL-FREE AND CHARGE-SENSITIVE SECOND-HARMONIC IMAGING OF GIANT VESICLE HYDRATION

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A biological membrane forms a dynamic and complex barrier between compartments of the living cell and its environment. However, its in vivo studies are difficult because it consists of a high variety of lipids and proteins and is continuously reorganized by the cell. Giant unilamellar vesicles (GUVs) are a powerful model system of the cell membrane due to their comparable size and membrane curvature. The majority of studies carried out on GUVs utilize fluorescence microscopy in combination with fluorescent markers. However, these methods of membrane imaging typically neglect molecular level details. As a consequence, there is virtually no knowledge on the role of membrane hydration, even though it is clear that without water lipid bilayer membranes cannot exist. A recent improvement in imaging throughput has resulted in the construction of a second harmonic imaging device that can non-resonantly and dynamically image interfacial water molecules [1,2]. This microscope was subsequently used to image the hydration of macroscopic free-floating membranes in aqueous solutions [3]. Here, we envision to extend our approach to SH image the interfacial hydration of GUVs. By varying the ionic strength of the adjacent solutions and lipid composition of the vesicles, we show that the non-resonant SH response of water molecules aligned by charge−dipole interactions with charged lipids can also be used as a label-free probe of membrane structure of GUVs.

*[1] Carlos Macias-Romero et al., Optics express, **2014**, 22 (25), 31102–31112.*

*[2] Carlos Macias-Romero et al., Science, **2017**, 357 (6353), 784–88.*

*[3] Orly B. Tarun et al., PNAS, **2018**, 115 (16), 4081–4086.*

PP42-1-A

EFFECT OF LIPOPOLYSACCHARIDE AND POLYMYXIN B ON MEMBRANE FLUIDITY OF PULMONARY SURFACTANT MODEL SYSTEMS

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Pulmonary surfactant is a mixture of lipids and proteins produced by type II alveolar cells. It forms a thin film on the surface of alveoli and reduces surface tension at air/liquid interface in the alveoli. This reduces work of breathing and prevents the alveolar collapse at expiration. Bacterial lipopolysaccharide (LPS) can inhibit the function of pulmonary surfactant and causes severe respiratory diseases. Antimicrobial peptide PolymyxinB (PxB) has shown the ability to interact with both LPS and pulmonary surfactant and prevent its inactivation by LPS.We studied the effect of LPS with and without PxB on the membrane fluidity of lipid model systems of pulmonary surfactant prepared from dipalmitoylphosphatidylcholine (DPPC), and as a mixture of DPPC and unsaturated and anionic phospholipids or fatty acids. The changes in the membrane fluidity were monitored through changes in fluorescence anisotropy using diphenylhexatriene.
LPS increases the fluidity in membranes prepared from lipid mixtures, while we detected an opposite effect in the pure DPPC. The addition of PxB resulted in partial suppression of the LPS effect in all studied model systems.

Acknowledgement. This work was supported by grants VEGA 1/0223/20, APVV 17-0250 and JINR project 04-4-1142-2021/2025

PP43-2-A

BIOMEMBRANE SHAPE REMODELLING BY COOPERATIVE ACTION OF PROTEINS

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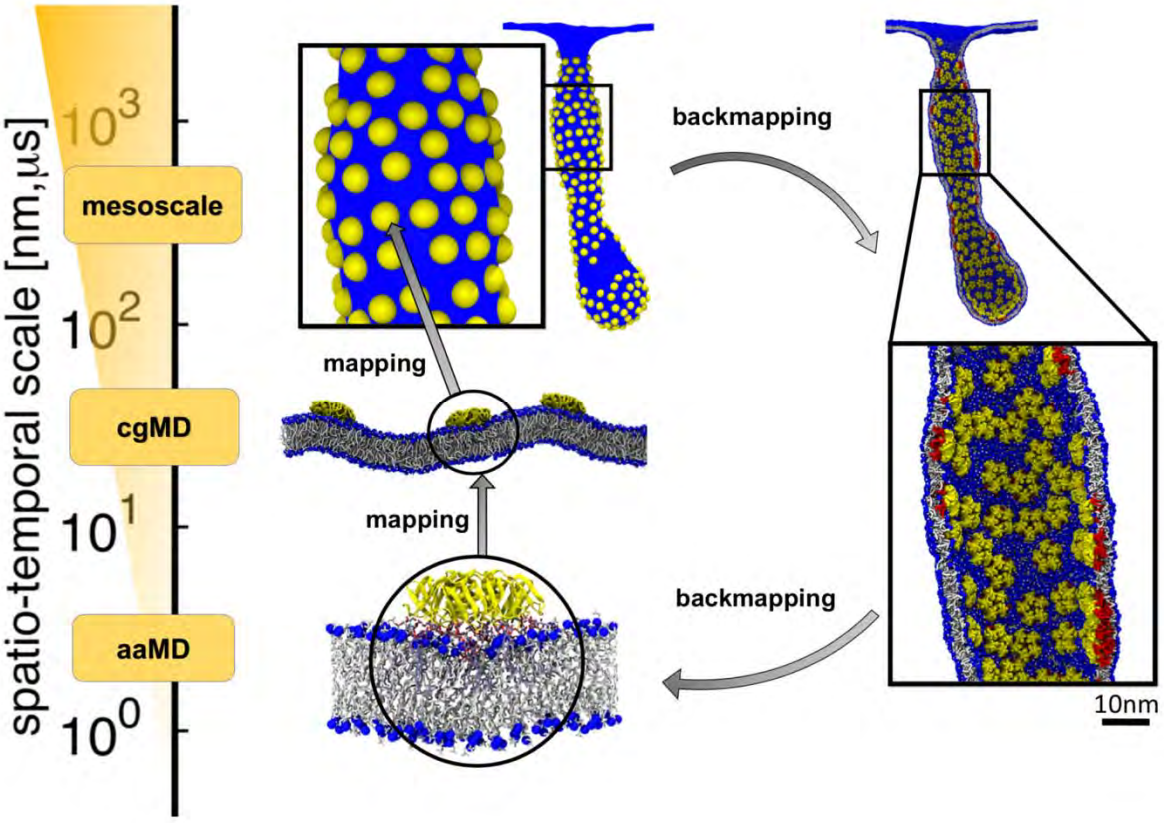
Biological membranes are important functional elements of the cell architecture, actively participating in many cellular processes that are essential for cell survival. A central feature of eukaryotic membranes is their flexible shape that undergoes constant transformations at a length scale much greater than their thickness. Uncovering the mechanisms that underlie the shape remodeling of these macromolecular structures is essential for understanding their biological functions and the rational design of drug delivery vehicles that must navigate the cell membrane to deliver their therapeutic payload. The cooperative action of membrane proteins is one of the significant drivers of membrane remodeling. Nevertheless, it remained unclear how exactly proteins play roles in achieving or stabilization the characteristic shape of cellular membranes. I will present our recent advances in exploring the coupling between membrane shape and lateral protein organizations using multiscale simulations.



PP44-2-B

REGULATION OF LIPID SIGNALING EVENTS BY MEMBRANE SHAPE

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Changes in membrane physical or chemical properties play an active role in signal transduction. Contrary to other membrane physical properties (*e.g.*, intrinsic curvature, lipid domains, fluidity), only recently membrane shape has emerged as actively modulating signal transduction. We show how perturbations in membrane shape can trigger specific lipid signalling responses. Diacylglycerol kinases (DGK) catalyze the phosphorylation of diacylglycerols (DAG) to produce phosphatidic acids (PA). Some of the 10 mammalian DGK isoforms are specific to certain DAG molecular species, suggesting that different isoforms might regulate the levels of different molecular species of DAG/PA and, therefore, different signaling pathways. Since membrane binding is a requirement for DGK catalytic turnover, it is hypothesized that DGK are regulated by the properties of the membrane they bind to. To address this, a systematic investigation of the regulaton of the substrate acyl chain specificity of two DGK isoforms by membrane properties was conducted. By using model membranes with variable physical and chemical properties it is shown that the substrate acyl chain specificity of these DGK isoforms depends on both the enzyme structure and the shape of the membrane they bind to. It is proposed that a hierarchic coupling of membrane physical (shape) and chemical (lipid molecular species) properties synergistically regulate membrane signaling events.

PP45-3-A

ESTABLISHMENT OF A MORPHOLOGICAL ATLAS OF THE *CAENORHABDITIS ELEGANS* EMBRYO USING DEEP-LEARNING-BASED 4D SEGMENTATION

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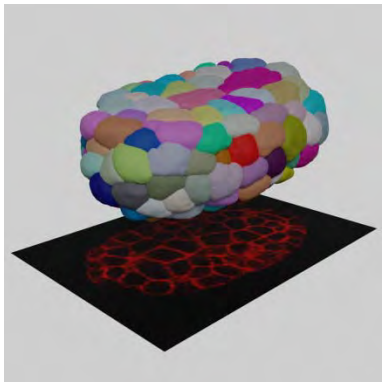
The invariant development and transparent body of the nematode *Caenorhabditis elegans* enables complete delineation of cell lineages throughout development. Despite extensive studies of cell division, cell migration and cell fate differentiation, cell morphology during development has not yet been systematically characterized in any metazoan, including *C. elegans*. This knowledge gap substantially hampers many studies in both developmental and cell biology. Here we report an automatic pipeline, CShaper, which combines automated segmentation of fluorescently labeled membranes with automated cell lineage tracing. We apply this pipeline to quantify morphological parameters of densely packed cells in 17 developing *C. elegans* embryos. Consequently, we generate a time-lapse 3D atlas of cell morphology for the *C. elegans* embryo from the 4- to 350-cell stages, including cell shape, volume, surface area, migration, nucleus position and cell-cell contact with resolved cell identities. We anticipate that CShaper and the morphological atlas will stimulate and enhance further studies in the fields of developmental biology, cell biology and biomechanics (Cao†, Guan†, Ho†, et al. *Nat. Commun.*, 2020, 10.1038/s41467-020-19863-x).



PP46-3-B

COMPUTABLE EARLY *C. ELEGANS* EMBRYO WITH A DATA-DRIVEN PHASE FIELD MODEL

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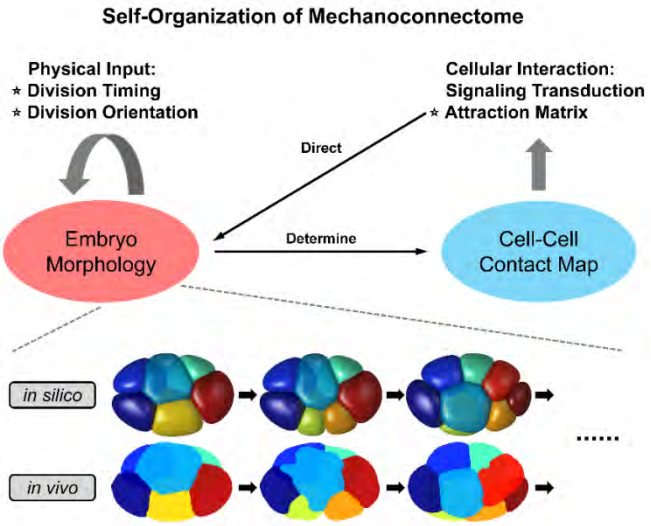
Morphogenesis is a precise and robust dynamic process during metazoan embryogenesis, consisting of both cell proliferation and cell migration. Despite the fact that much is known about specific regulations at the molecular level, how cell proliferation and migration together drive the morphogenesis at the cellular and organismic levels is not well understood. Here, using *Caenorhabditis elegans* as the model animal, we present a data-driven phase field model to compute the early embryonic morphogenesis within a confined eggshell. By using three-dimensional time-lapse cellular morphological information generated by imaging experiments to set the model parameters, we can not only reproduce the precise evolution of cell location, cell shape and cell-cell contact relationship *in vivo*, but also reveal the critical roles of cell division and cell-cell attraction in governing the early development of *C. elegans* embryo. In brief, we provide a generic approach to compute the embryonic morphogenesis and decipher the underlying mechanisms (Kuang†, Guan†, et al. *bioRxiv*, 2020, 10.1101/2020.12.13.422560).



PP47-1-A

EVALUATION OF MECHANISM OF PHASE TRANSITION OF PH-SENSITIVE LIPOSOMES UPON PH CHANGE THROUGH MOLECULAR DYNAMICS SIMULATION.

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Liposome based drug delivery systems composed of DOPE stabilized with cholesteryl hemisuccinate (CHMS) have been proposed as a drug delivery mechanism with pH triggered release as the anionic form (CHSa) is protonated (CHS) in reduced pH; PEGylation is known to decrease this pH sensitivity. In this study, we set out to use molecular dynamics (MD) simulation with a model with all atom resolution to provide insight into phase transition of liposomes upon pH change and also answering this question that why incorporation of polyethyleneglycol (PEG) into DOPE-CHMS liposomes reduces their pH sensitivity; we also address two additional questions: 1) how CHSa stabilizes DOPE bilayers into a lamellar conformation at physiological pH of 7.4 and 2) how the change from CHSa to CHS in acidic pH triggers the destabilization of DOPE bilayers. We found that A) CHSa stabilizes the DOPE lipid membrane through increasing the hydrophilicity of the bilayer surface, B) when CHSa changes to CHS through pH reduction, DOPE bilayers are destabilized due to 1- reduction in bilayer hydrophilicity and 2- reduction in the area per lipid and C) PEG stabilizes DOPE bilayers into the lamellar phase, thus reducing the pH-senstivity of the liposomes through increasing the area per lipid through penetration into the bilayer.

PP48-1-B

POLYMORPHIC LIPID PHASE BEHAVIOR OF CHLOROPLAST THYLAKOID MEMBRANES. DEM, THE DYNAMIC EXCHANGE MODEL

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Energization of thylakoid membranes (TMs) and the synthesis of ATP require a bilayer organization of their lipids. Nevertheless, their most abundant lipid species is the non-bilayer lipid MGDG. In vitro, MGDG, which assumes inverted hexagonal (H_{II}) phase, could be forced, by light-harvesting proteins, to form lamellae (Simidjiev et al. 2000 PNAS). Lipids with non-bilayer propensity were proposed to regulate the protein-to-lipid ratio of membranes, via segregation from and re-entering the membrane (Garab et al. 2000 TIPS). This, the dynamic exchange model (DEM) suggested the co-existence of bilayer and non-bilayer lipid phases, which, on intact, functional membranes, was first shown by Krumova et al. (2008, BBA) using mainly ^{31}P-NMR spectroscopy. Recent experiments revealed two isotropic phases and an H_{II} phase, beside the bilayer (Figure 1a). DEM of TMs contains fusion channels and water-soluble, lipid-binding lipocalins, such as the violaxanthin de-epoxidase (VDE), and membrane-bound segregated lipids (Figure 1b) (Garab et al. 2017, Scientific Reports). Lipid phases of TMs have been shown to undergo different, largely reversible reorganizations, affecting the macroorganization of proteins (Kotakis et al. 2018 Photosynthetica), fine-tuning the permeability of membranes (Ughy et al. 2019 Physiologia Plantarum) and regulating the photoprotective activity of VDE (Dlouhy et al. 2020 Scientific Reports).

a ***b***

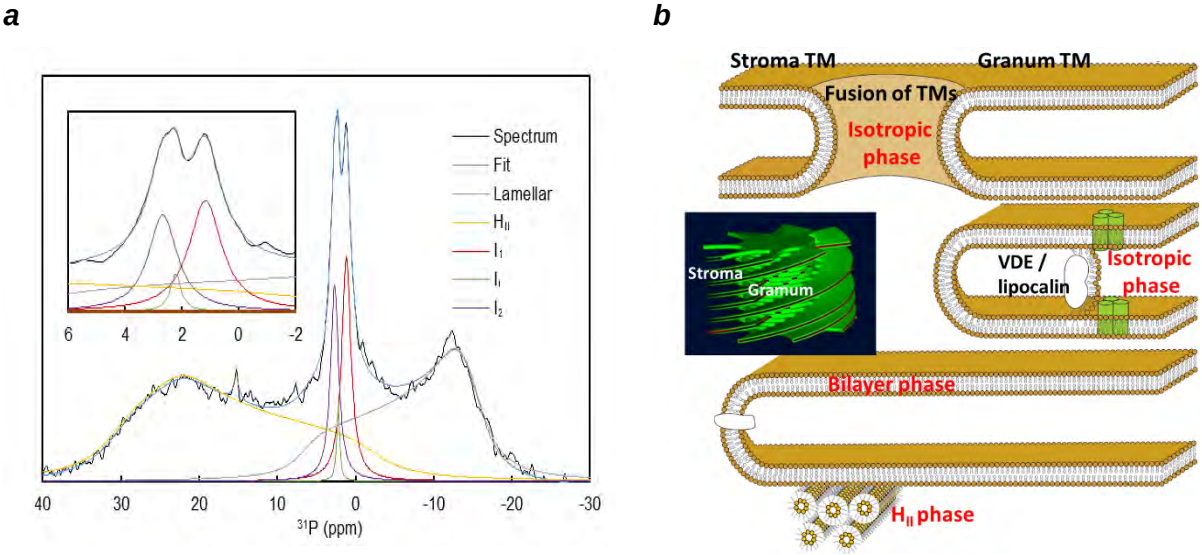

Figure 1a&b

PP49-1-A

Relation of Plant Thylakoid Lipid Phases to Membrane Permeability and their Accessibility to Lipases

B. Ughy[1,2], V. Karlický[2,3], O. Dlouhý[2], U. Javornik[4], Z. Kmecová Materová[2], O. Zsiros[1], P. Šket[4,5], J. Plavec[4,5,6], V. Špunda [2,3], G. Garab[1,2]

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While the functional state of the thylakoid membranes is the bilayer, the major lipids in these membranes are non-bilayer lipids. We have shown by using ^{31}P-NMR, that, in addition to the lamellar phase, three non-bilayer lipid phases are present in freshly isolated, fully functional thylakoid membranes (Garab et al. 2017 Scientific Reports). All the four lipid phases appear to exhibit remarkable structural flexibility, manifested in significant variations in ^{31}P-NMR signatures during storage at 5 °C and/or changing the physico-chemical environment of the membranes. We observed substantial gradual enhancement of the isotropic phases and diminishment of the bilayer lipid phase in time. These changes compared well with the gradually increasing membrane permeability (Ughy et al. 2019 Physiologia Plantarum). No specific changes could be discerned in the ^{31}P-NMR spectra during proteinase treatments, suggesting that the observed changes in the lipid phases in time do not closely related to proteinase activity. In contrast, lipase treatments caused diminishments of the lamellar and inverted hexagonal phases, indicating that different resonances exhibited by the thylakoid membranes originate from different structural entities. Our observations suggest that non-bilayer lipids and non-lamellar lipid phases play significant roles in the structural dynamics and functional plasticity of thylakoid membranes.

PP50-1-B

NONLAMELLAR MEMBRANES IN PLASTIDS UNDER NATURAL CONDITIONS

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Plastids are semi-autonomous organelles of cyanobacterial origin, which contain special lipids such as MGDG, DGDG and SQDG. Out of these lipids, MGDG has a conical shape and is potentially involved in the formation of nonlamellar phases or curvatures. Plastids often accommodate so-called tubular complexes in which, instead of lamellar membranes, branched tubules are joined together. We have shown that such, highly regular cubic phase membrane structures termed prolamellar bodies are widely distributed in light-deprived photosynthetic tissues of various angiosperm plants already under natural light conditions. In addition, we demonstrated that nonlamellar membranes may also occur in chloroplasts as vesicle clusters. Similarly, such membranes were observed in the plastids of several tissues involved in secretory processes (e.g. production of essential oils, secondary metabolites of lipophilic nature or made from isoprenoid building blocks). However, literature data did not clearly show or demonstrate similarities and differences between these structures. In this presentation ultrastructural comparison of such structures will be presented along with a systematic comparison of the structure of the prolamellar bodies of etioplasts and the tubular complexes in the leucoplasts of the neck cells of the peltate glandular hairs of rosemary (*Rosmarinus officinalis*).

PP51-2-A

RELEVANCE OF CUBIC PHASES IN DNA DELIVERY

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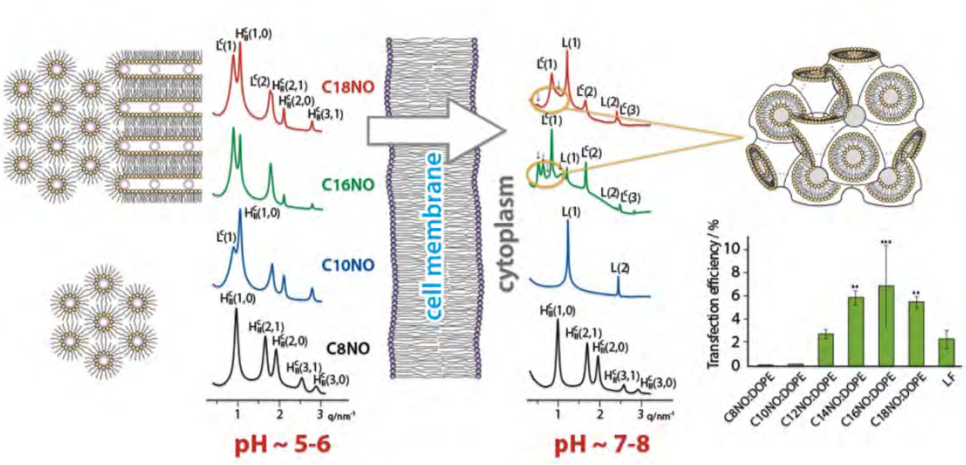
pH sensitive liposomes composed of homologues of series of *N,N*-dimethylalkane-1-amine *N*-oxides (C*n*NO, *n* = 8-18, where *n* is the number of carbon atoms in the alkyl substituent) and neutral phospholipid dioleoylphosphatidylethanolamine (DOPE) were tested for their *in vitro* transfection activity. Transfection efficiency of C*n*NO/DOPE for plasmid DNA into U2OS cells follows quasi-parabolic dependence on C*n*NO's alkyl substituent length with maximum at *n* = 16. C*n*NO/DOPE (*n* = 12-18) are more efficient in DNA delivery than commercially available Lipofectamine 2000. Several techniques (SAXS/WAXS, UV-VIS, zeta potential measurements, confocal microscopy) were applied to characterize the system. A small angle X-ray scattering (SAXS) shows large structural diversity depending on the complex's composition and pH. Transfection efficiencies mediated by two structures, either a condensed lamellar (L_α^c) or epitaxially connected L_α^c and condensed inverted hexagonal (H_{II}^c) phase (L_α^c&H_{II}^c) were found very similar. The change of pH from acidic to neutral induces phase transition L_α^c&H_{II}^c Q_{II}+L_α, with cubic phase Q_{II} of *Pn3m* space group. Q_{II} detected in the most efficient lipoplexes of composition C*n*NO/DOPE (*n*=16 and 18) facilitates DNA release and promotes its internalization in the cell [Liskayová et al., *Langmuir* 35 (2019) 13382-13395].

Projects APVV-17-0250, JINR 04-4-1142-2021/2025, VEGA 1/0223/20 are acknowledged.

Lipoplexes DNA – C*n*NO/DOPE



PP52-3-A

MODULATION OF NON-BILAYER LIPID PHASES AND THE STRUCTURE AND FUNCTIONS OF THYLAKOID MEMBRANES: EFFECTS ON THE WATER-SOLUBLE ENZYME VIOLAXANTHIN DE-EPOXIDASE

O. Dlouhý[1], I. Kurasová[1,2], V. Karlický[1,2], U. Javornik[3], P. Šket[3,4], N. Z. P etrova[5], S. B. Krumova[5], J. Plavec[3,4,6], B. Ughy[1,7], V. Špunda[1,2], G. Garab[1,7]

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The role of non-bilayer lipids and non-lamellar lipid phases in biological membranes is an enigmatic problem of membrane biology. Non-bilayer lipids are present in large amounts in all membranes; in energy-converting membranes they constitute about half of their total lipid content – yet their functional state is a bilayer. *In vitro* experiments revealed that the functioning of the water-soluble violaxanthin de-epoxidase (VDE) enzyme of plant thylakoids requires the presence of a non-bilayer lipid phase (Latowski et al. 2004, Biochemistry). ^{31}P-NMR spectroscopy has provided evidence on lipid polymorphism in functional thylakoid membranes (Garab et al. 2017, Scientific Reports). In this work, we reveal reversible pH- and temperature-dependent changes of the lipid-phase behaviour, particularly the flexibility of isotropic non-lamellar phases, of isolated spinach thylakoids (Dlouhý et al. 2020 Scientific Reports). These reorganizations are accompanied by changes in the permeability and thermodynamic parameters of the membranes and appear to control the activity of VDE and the photoprotective mechanism of non-photochemical quenching of chlorophyll-a fluorescence. The data demonstrate, for the first time in native membranes, the modulation of the activity of a water-soluble enzyme by a non-bilayer lipid phase.

PP53-3-B

ROLE OF NON-BILAYER STRUCTURES IN MITOCHONDRIAL MEMBRANES

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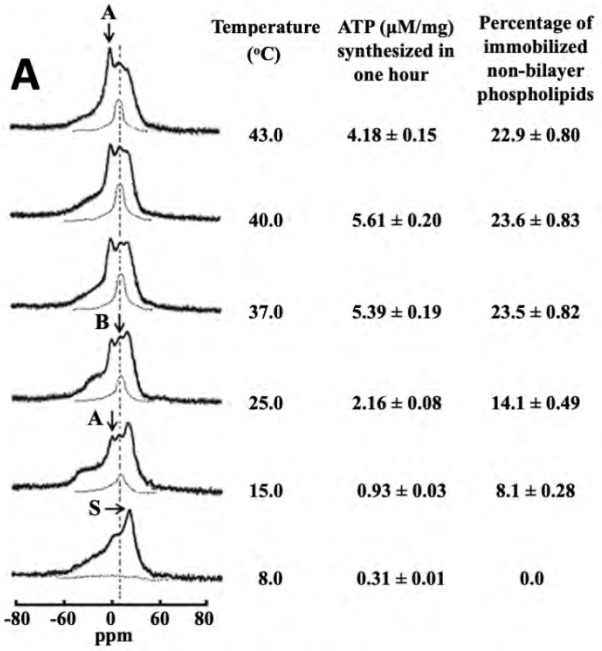
STEM Program, Science Department, Chaoyang KaiWen Academy, Beijing, China and [2]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

Non-bilayer packed phospholipids were observed for the first time in the inner mitochondrial membranes (IMM) four decades ago (Cullis et al. 1980, BBA). Recently, two types of non-bilayer structures made of phospholipids with high isotropic mobility (Fig. 1A signal A) and immobilized phospholipids with non-bilayer packing (Fig. 1A signal B) were identified by ^{31}P-NMR in intact mitochondria with high ATP synthase activity (Gasanov et al. 2015 PlosOne). Increase in temperature, decrease in pH and treatment with basic cardiotoxins promotes the growth in population of immobilized non-bilayer phospholipids and ATP synthase activity (Gasanov et al. 2018 BBA). It has been suggested that H^+ and cardiotoxins interact electrostatically with cardiolipin (CL) in the IMM to dehydrate the polar head of CL and increase its reverse wedge molecular shape to facilitate transition from bilayer to non-bilayer packing of CL. This leads to formation of compartments with an increased surface curvature in the apex of cristae which helps to segregate ATP synthase dimers and entrap higher concentration of protons inside the compartment (Fig. 1B). This promotes higher flow of protons through the F_o channel of ATP synthase to elevate the rate of ATP production.



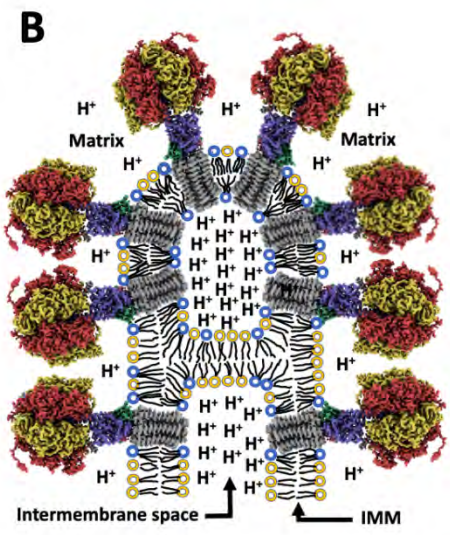


Figure 1. Increase in immolized non-bilayer phospholipids promotes ATP production (A) in compartments in the apex of cristae which segregate ATP synthase dimers and entrape higher concentration of protons (B). ATP synthase structure is modified from Spikes et al. 2020 Proc Natl Acad Sci USA.

PP54-1-A

ANISOTROPIC DIFFUSION IN SINGLE PARTICLE EXPERIMENTS FROM MOLECULAR SIMULATIONS

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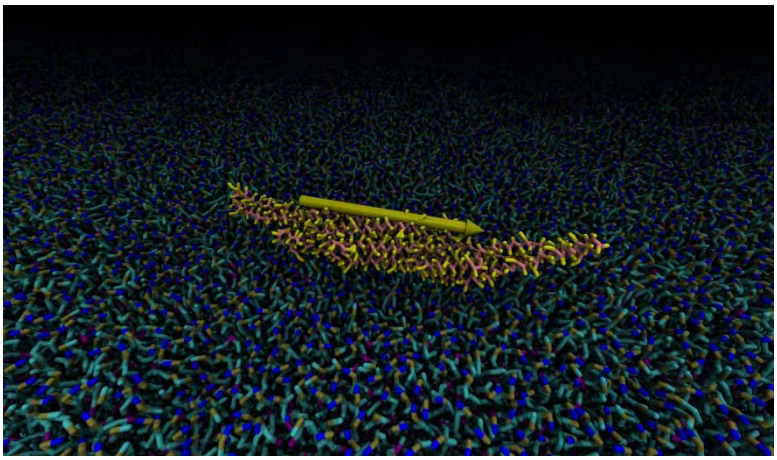

Single particle tracking experiments of lipids and membrane proteins provide information about the properties of biomembranes. Careful analysis of SPT trajectories reveals deviations from ideal Brownian behavior. This includes confinement effects and anomalous diffusion, which are manifestations of the nanoscale structure of the underlying membrane. With the increase in spatiotemporal resolution of experimental methods, anisotropic diffusion might soon provide an additional proxy to the structure and topology of biomembranes. Unfortunately, the theoretical framework for detecting and interpreting anisotropy effects is far from complete. Here, we provide a computational method to evaluate the degree of anisotropy directly from molecular dynamics simulations and indicate connections to SPT experiments . In order to probe the effects of anisotropic diffusion on the motion of the particle, we performed coarse-grained molecular dynamics simulations of FBAR, B_1AR-monomer and B_1AR-dimer in flat and curved bilayers. In agreement with the theoretical basis, our results indicate that anisotropy can persist up to the characteristic time of diffusion, after which isotropic diffusion is observed. The underlying topology of the membrane can couple with the geometry of the particle and extend the spatiotemporal domain over which this type of motion can be observed.

PP55-1-B

PREPARATION OF DIPALMITOYLPHOSPHATIDYLCHOLINE BASED RED FLUORESCENT VESICLES

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Biocompatible nanoparticles with tuneable fluorescence - due to their great potential in biology, medicine and sensor development - are widely studied nowadays. One of the most investigated systems is the red-emitting bioconjugate of Bovine Serum Albumin (BSA) with gold. The red emission BSA - gold bioconjugates is related to the conformational changes of BSA. In our previous study [1] we showed that the BSA structure is not reversible after a neutral - alkali - neutral pH cycle and this behaviour is more pronounced in the presence of the gold salt ($HAuCl_4$). Our results showed that the red fluorescence of the bioconjugate under alkali conditions (it is required for the appearance of red fluorescence) are not affected by the readjustment of pH to neutral. This observation is a big step in the direction of biocompatible fluorescent bioconjugates, however further enhancement of biocompatibility and fluorescent efficiency is a well-established task. Our goal was to produce BSA-$HAuCl_4$ bioconjugates bonded to unilamellar vesicles (DPPC). Calcium ions were used to assure the BSA binding on DPPC. We have executed a wide range structural studies extending from the atomic scale up to several hundreds of nm (ATR-infrared, fluorescence spectroscopy, small angle X-ray and neutron scattering).

[1] Fehér et al. Journal of Molecular Liquids, Volume 309, 1 July 2020, 113065, https://doi.org/10.1016/j.molliq.2020.113065

PP56-1-A

PROTEIN GUIDED VESICLE-FORMATION BETWEEN ARTIFICIAL LIPIDS AND GHOST-DERIVED PROTEINS

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During the nanoerythrosome-preparation, proteins are released from the precursor human red blood cell derived ghosts. These proteins are in solubilized form in the aqueous medium and hardly contain lipids as it was witnessed by infrared spectroscopy (Deák, Bóta et al. 2020. Mat.Sci. & Eng. C.). The protein identification, using LC-MS(MS) showed that the most frequent typical proteins were the same in the precursor ghost, in the aqueous medium and in nanoerythrosomes, formed after the sonication treatment. However, the conformation pattern of the proteins was different in the three matrices, as revealed by infrared and CD spectroscopies. The globular conformation was typical for the solubilized proteins, but it was altered in the cases of membrane-like ghosts and nanoerythrosomes. After adding small amount of an adequate lipid, found to be DPPC, a loose association of proteins was observed. The further addition of guest lipids resulted in the appearance of a random 2D-network. In presence of the increased ratio of the lipids, unilamellar vesicles with protein scaffolding (revealed by small-angle X-ray (SAXS) measurements and freeze-fracture combined transmission-electron microscopy (FF-TEM)) were formed, underlying the importance and possibilities of the ghost proteins in the manipulations of nanoerythrosomes.

PP57-1-B

THE MEMBRANE-ACTIVE AND CALMODULIN-BINDING PROPERTIES OF THE CyaA TOXIN TRANSLOCATION DOMAIN ARE CRITICAL FOR CELL INVASION

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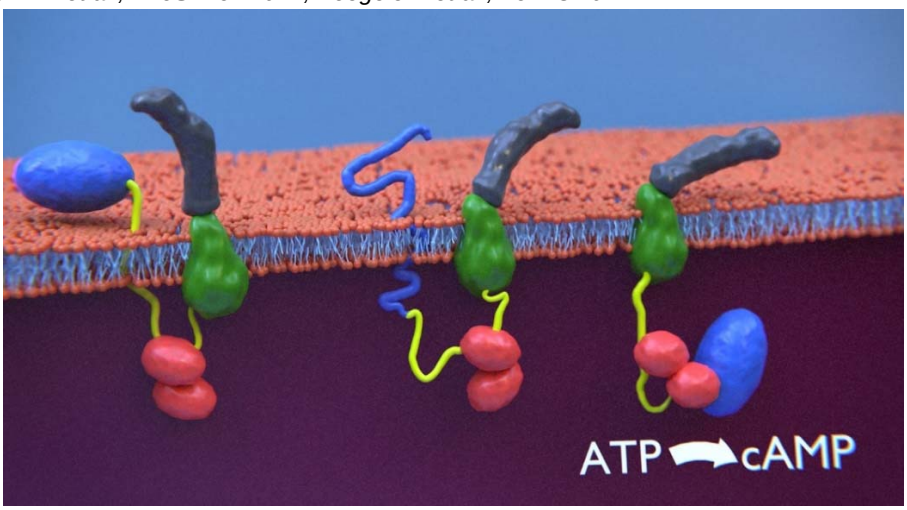
The molecular mechanisms and forces involved in the translocation of bacterial toxins into host cells are still a matter of intense research. *Bordetella pertussis*, the causative agent of whooping cough, produces an adenylate cyclase (CyaA) toxin that plays an essential role in the early stages of respiratory tract colonization. CyaA displays a unique intoxication pathway of human cells via a direct translocation of its catalytic domain (AC) across the plasma membrane. Once in the cytosol, AC impairs the physiology of immune cells, leading to cell death. We show that the P454 peptide (CyaA residues 454–484) is able to translocate across membranes and to interact with calmodulin. The key residues involved in membrane-active and calmodulin binding properties have been identified. Mutational analysis demonstrates that these residues play a crucial role in CyaA translocation into target cells. We proposed that after CyaA binding to target cells, the P454 segment destabilizes the plasma membrane, translocates across the lipid bilayer and binds calmodulin. Trapping of CyaA by the CaM:P454 interaction in the cytosol may assist the entry of AC by converting the stochastic motion of the polypeptide chain through the membrane into an efficient vectorial chain translocation into host cells.

The abstract is based on the following articles: Voegele et al., Advanced Science, in press; O'Brien DP et al., FASEB J. 2019; O'Brien DP et al., PLoS Biol. 2017; Voegele A et al., Toxins 2017.



The membrane-active segment (yellow) of the CyaA toxin translocates across the plasma membrane and binds calmodulin (red), which assists the entry and refolding of the catalytic domain (blue) into host cells while the hydrophobic and acylation domains (green) interact with the membrane and the C-terminal Repeat-in-Toxin domain (grey) remains in the extra-cellular milieu. The cAMP production ultimately leads to cell death.

PP58-1-A

THE STIM1 SOARα2 DOMAIN CONTROLS MULTIPLE STEPS IN THE CRAC CHANNEL ACTIVATION CASCADE

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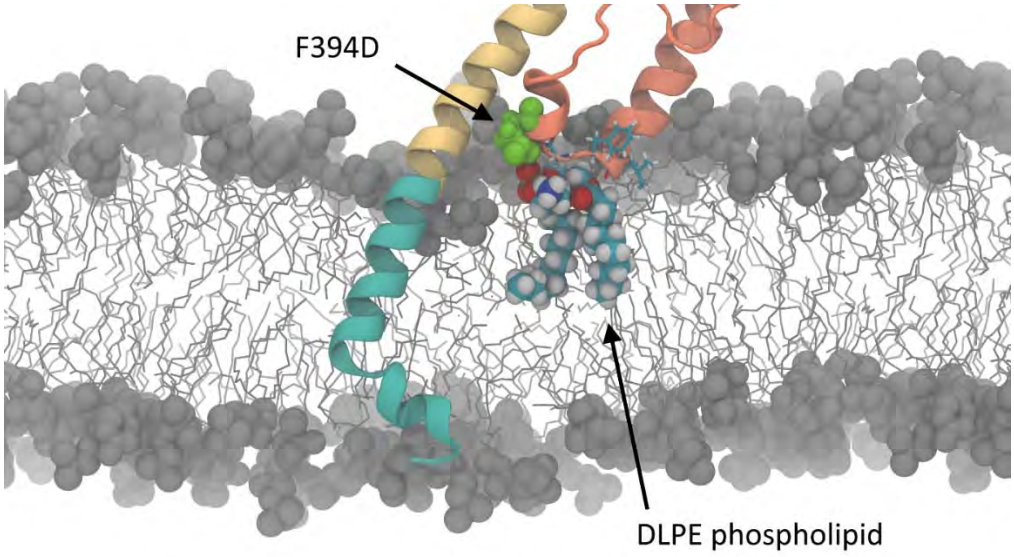

The universal calcium release-activated calcium (CRAC) channel is composed of STIM1, a Ca^{2+} sensor in the endoplasmic reticulum (ER), and Orai1, the Ca^{2+} ion channel in the plasma membrane. Ca^{2+} store depletion triggers a conformational transition within the STIM1 protein, leading to STIM1 homomerization as well as coupling to and gating of Orai1. When activated, the STIM1 C-terminus switches from a folded to an extended conformation, thereby exposing the so-called CAD/SOAR region for coupling to Orai1.

In this study, we discovered the multiple roles of a small alpha helical segment (STIM1 α2) within CAD/SOAR. It contributes to the stabilization of the tight resting state of STIM1 and controls the transition of STIM1 C-terminus to the active conformation, STIM1 homomerization as well as STIM1-Orai1 coupling.

Using molecular dynamics simulations, we found that a loss-of-function mutation F394D in the α2 region impairs STIM activation due to electrostatic interactions between F394D and positively charged head groups of ER membrane phospholipids. Experimentally, we observed that homomerization was impeded only in STIM1 F394D fragments that were directly attached to the ER membrane, whereas in soluble cytosolic STIM1 F394D fragments it remained unaffected. Taken together, our findings provide evidence that STIM1 activity is fine-tuned by interactions between CAD/SOAR and the ER membrane.

PP59-1-B

A NEUTRON REFLECTOMETRY STUDY OF THE INTERACTION OF GLYCEROPHOSPHOLIPIDS AND THE PHOSPHOLIPASE PLA1-1 ISOFORM OF ASPEGILLUS ORIZAE

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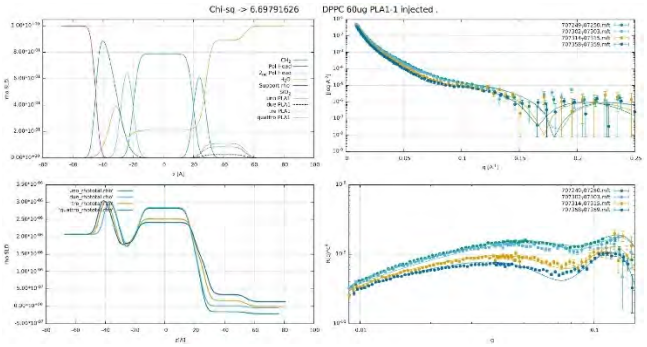
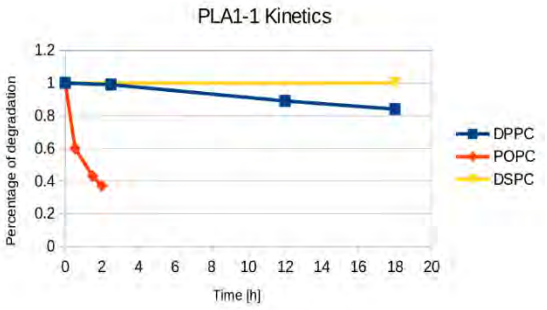
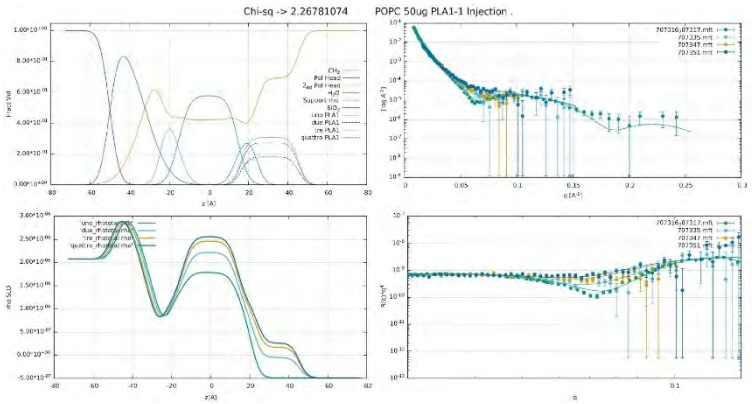
Phospholipases (PLAs) are lipolytic enzymes that hydrolyze phospholipid substrates at specific ester bonds. They are widespread in nature and play very diverse roles right from signal transduction and lipid mediator production to membrane phospholipid homeostasis. Phospholipases vary considerably in their structure, function, regulation, and mode of action therefore a deeper understanding of their dynamics and kinetics can be very crucial. The present study encompasses employing neutron reflectivity including other physical/chemical techniques to better understand the principles underlying the substrate specificity of phospholipases. We here, studied in detail the effect of the acyl chain length and unsaturation of phospholipids on their hydrolysis by type A1-PLA (sourced from Aspergillus oryzae), that was expressed in E.Coli and purified in its pure form thus allowing us to understand the key factors that regulate its activity. Some neutron reflectivity results:







PP60-1-A

LABEL-FREE PROTEIN AND LIPID QUANTIFICATION OF EXTRACELLULAR VESICLES BY INFRARED SPECTROSCOPY

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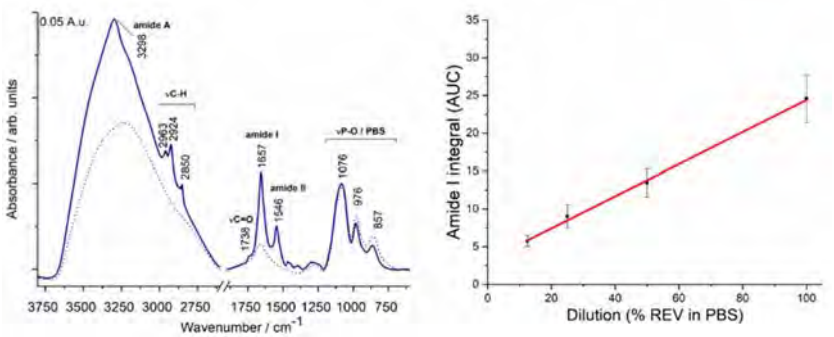
Extracellular vesicles (EVs), emitted by cells spontaneously, play a significant role in intercellular communication. Infrared (IR) spectroscopy, completed with standardized measurement conditions and data processing procedures, was recently introduced to characterize EVs. Since IR spectroscopy provides information about proteins and lipids and/or other EV components simultaneously, a single assay quantification protocol for both proteins and lipids (phospholipids) might be feasible.

The large sized EVs (microvesicles) formed ex vivo in erythrocyte concentrates were examined by IR spectroscopy. The integrated area of the amide I band proved to be proportional to the protein quantity in the EV samples (up to 1 mg/ml), regardless of its secondary structure.



Our results based on a calibration with bovine serum albumin was further affirmed also by multivariate modelling on raw spectra using Partial Least Squares regression. Lipids are essential molecular components of EVs, but at the moment only limited knowledge about their quantification is available. To extend the possibilities of IR spectroscopy, an effort has been made to elaborate an adequate lipid calibration, by using reference vesicles of bovine serum albumin and synthetic lipids.

Acknowledgement:

This study was funded by the National Research, Development and Innovation Office, Hungary under grants NVKP_16-1-2016-0007, K131594 (J.M.) and K131657 (A.B). ZV is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

PP61-1-B

LIPID SPECIFICITY OF VIRAL PROTEINS

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Viral fusion proteins drive fusion of viral and host cell membranes in a series of complex structural transition events. Although the structure of several fusion proteins have been solved, the characterization of viral protein-membrane interactions at atomistic resolution is still missing. Membrane interactions of fusion proteins are conserved and occur via fusion peptides (FPs) in class I and fusion loops (FLs) in class II/III proteins. Previously, we had characterized the glycerophospholipid binding in class II fusion protein glycoprotein C (gC) of Rift Valley fever virus (RVFV) [2] and the studies revealed specific binding pockets for PC lipids. Now we aim to understand if specific lipid binding site also exists in class I and III viral proteins and any preference for lipid type. Molecular dynamics (MD) simulations is an excellent technique to understand how proteins associates with lipid membrane at atomistic resolution and here we make use of MD simulations to gain structural insights into lipid contact sites and membrane insertion of FP / FL residues.

[1] M. Vallbracht et al., J Virol 92: e01203-17, (2018)

[2] P. Guardado-Calvo et al., Science 358, 663-667, (2017)

PP62-1-A

THE INTERACTION OF THE LACTOBACILLI SURFACE LAYER PROTEINS WITH THE LIPOTHEICHOIC ACIDS FROM THE CELL WALL

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Surface layers (S-layers) are 2D crystalline lattices of proteins which cover the whole surface of many archaeal and bacterial cells. Since these proteins are in close contact with their environment they fulfil many vital tasks like bacterial adherence to other cells, protection against life-threatening conditions, maintenance of the cell shape and auto-coaggregation. These S-layer proteins are attached to the cell wall by interaction with lipoteichoic acids (LTA). The domain involved in this interaction, the LTA-binding domain, is found in a variety of bacterial cell surface proteins.
Our goal is to structurally characterize the LTA-biding domain and its interaction with the LTA. The soluble fragments of Lactobacili S-layer protein, containing the LTA-binding domain, were purified and subjected to crystallization. The obtained crystal structures show a unique domain with phosphate molecules bound in the putative binding regions. Isothermal titration calorimetry and thermoflour with LTA and synthetized fragments as well as mutagenesis experiments were performed to characterize the binding. Via Nuclear Magnetic Resonance titration experiments residues affected upon binding of LTA-fragments could be determined, thereby locating the interaction area on the LTA-binding domain.

Acknowledgments: This work has been supported by the Austrian Science Fund (FWF, Project P29432).

PP63-2-A

INSIGHT INTO MECHANISM OF PROTON TRANSLOCATION ASSISTED BY MEMBRANE PROTEINS

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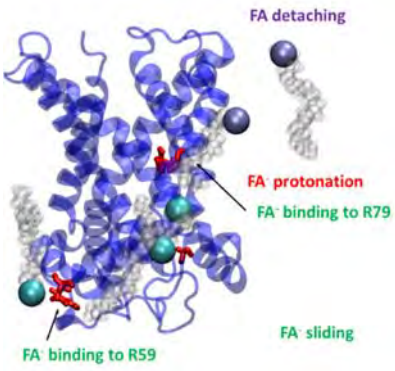
Molecular dynamics simulations are a powerful tool in investigation of complex biological systems, such as DNA, proteins and model biological membranes. In this study we focus on the latter subject, particularly on the phenomenon of membrane permeation. In this respect, while the neutral species can readily permeate across the cell bilayer, the unmediated transport of charged species is usually slow and does not occur on biologically relevant timescales. [1] The transport of such species is thus generally occurring via various supporting mechanisms which commonly involve membrane proteins, used to arbitrate and assist their passage through lipid bilayers. [2]



One of the most prominent membrane proteins located in the inner mitochondrial membrane is adenine nucleotide transporter 1 (ANT1). While its main function is transport of ADP and ATP nucleotides, ANT1 is also found to catalyze translocation of other charged species through inner mitochondrial membranes in the ATP independent manner. [3] In this work, we use classical MD simulations to obtain a detailed insight into the translocation of protons assisted by fatty acids across model bilayers with embedded ANT1 protein. In particular, we use both unbiased MD simulations and “state-of-the-art umbrella sampling along the pathway” technique to investigate this phenomenon.

*[1] S. Škulj, M. Vazdar, Phys. Chem. Chem. Phys., **2019**, 21, 10052-10060.*
*[2] N. P. Bethel, M. Grabe, Proc. Natl. Acad. Sci. U. S. A., **2016**, 113, 14049-14054.*
*[3] Y. Wang, E. Tajkhorshid, Proc. Natl. Acad. Sci. U. S. A., **2008**, 105, 9598-9603.*

PP64-2-B

DYNAMICS OF RESPIRATORY COMPLEX I AND QUINONE

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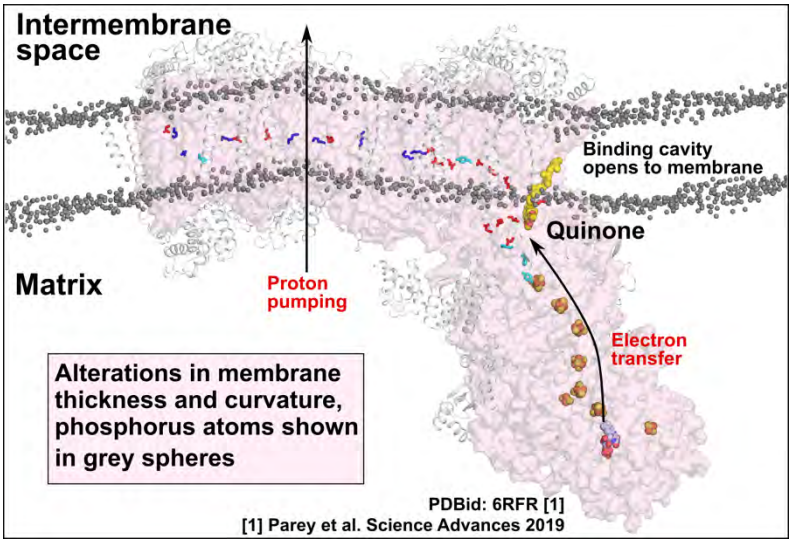
Respiratory complex I has crucial role in the generation of proton electrochemical gradient, which is utilised by respiratory complex V to generate ATP, the energy currency of cells. Mutations and dysfunction of complex I are associated with several neurodegenerative diseases, such as Alzheimer's disease.



Complex I has two domains, one resides in the cytosol or the mitochondrial matrix and the other one in the bacterial or inner mitochondrial membrane (see Figure). The fun
ction of complex I consists of two tightly coupled reactions: electron transfer and proton pumping. Despite extensive structural and biochemical data, the coupling between these two reactions remains unknown.

Complex I binds a hydrophobic molecule, substrate quinone, whose binding cavity is located at the interface of the two domains and which opens to the membrane phase. The dynamics of quinone between the membrane milieu and the protein cavity is likely influenced by protein-lipid interactions. The lipid membrane attains a unique architecture near the cavity opening in which lipid tails are highly tilted and membrane thickness is partly perturbed, caused by the short horizontal helices of protein subunits [1]. We studied these interactions by molecular simulations and hypothesize that lipid-protein interface is crucial for the function of complex I.

[1] Parey et al. Science Advances 2019 (DOI: 10.1126/sciadv.aax9484)

PP65-2-A

HIV-1 P6: A SELECTIVE LIPID-INTERACTING PROTEIN

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The human immunodeficiency viruses (HIV-1 and -2), over time, cause acquired immunodeficiency syndrome, in which progressive failure of the immune system allows opportunistic infections and cancers to thrive. Despite huge efforts have been devoted on the study of these viruses, some mechanisms of its action are still not clearly understood.
The Gag polyprotein Pr55, composed by a matrix, capsid, nucleocapsid and the small protein p6, is necessary for the formation of virus-like particles in HIV-1[1,2]. Even though p6 consists of only 52 amino acids, it regulates the detachment of budding virions from the cell surface by the action of its two distinct late assembly domains [3]. Although free p6 can be found in progeny virions, it is hardly detectable in infected cells. p6 possess a mostly random structure in aqueous solution but its known functions are suggested to occur under hydrophobic conditions near the cytoplasmic membrane, where the protein exhibits a structured state [4]. The environment surrounding the protein is therefore crucial for its conformation and behaviour. Interestingly, we observed, with QCM-D and Neutron Reflectometry, a specific interaction of the protein with the lipid membrane, modulated by the composition of the lipid layers. The study of the interaction of p6 with the different elements of a biomimetic membrane can give insight on the mechanisms of p6 incorporation into cellular membranes and thus of virus spreading.

[1] W.I. Sundquist et al., Cold Spring Harb. Perspect. Med. (2012).
[2] E.O. Freed, Nat. Rev. Microbiol. (2015).
[3] B. Müller et al., J. Virol. (2002).
[4] S.M.Ø. Solbak et al., Biochim. Biophys. Acta - Biomembr. (2013).

PP66-2-B

SPONTANEOUS LIPID BINDING TO THE NICOTINIC ACETYLCHOLINE RECEPTOR IN A NATIVE MEMBRANE

L. Sharp, G. Brannigan

The nicotinic acetylcholine receptor and other pentameric ligand-gated ion channels are native to neuronal membranes with an unusual lipid composition. While it is well-established that these receptors can be significantly modulated by lipids, the underlying mechanisms have been primarily studied in model membranes with only a few lipid species. Here we use coarse-grained molecular dynamics simulation to probe specific binding of lipids in a complex quasi-neuronal membrane. We ran a total of 50-microseconds of simulations of a single nAChR in a membrane composed of 36-species of lipids. Cholesterol selects for concave intersubunit sites and PUFAs select for convex M4 sites, while monounsaturated and saturated lipids are unenriched in the nAChR boundary. Characterizing binding to specific sites, we present a novel approach for calculating a ``density-threshold affinity'' from continuous density distributions. We found affinities for M4 weakens with chain rigidity, suggesting flexible chains may help relax packing defects caused by the conical protein shape. For any site, PE headgroups have the strongest affinity of all phospholipid headgroups, but anionic lipids still yield moderately high affinities for the M4 sites as expected. We observe cooperative effects between anionic headgroups and saturated chains at the M4 site in the inner leaflet. We also analyze affinities for individual anionic headgroups.

PP67-2-A

THE INTERACTION OF HEAT SHOCK PROTEINS WITH LIPID MEMBRANES: A NOVEL DIAGNOSTIC TARGET

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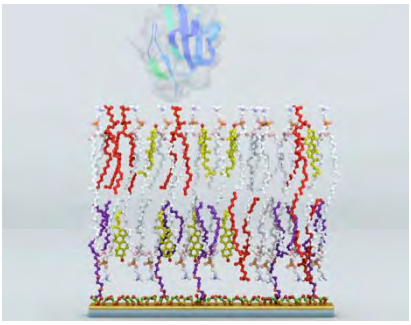
Many members of the underlying heat shock proteins (HSP) are expressed at low levels under physiological conditions and act as chaperones, while others emerge only during stressful conditions to assure cell protection by reassembling protein homeostasis and interfering with apoptotic pathways [1]. As an intracellular polypeptide HSP70 and HSP90 can be exposed on the plasma membrane and/or released into the circulation. However, the role of HSP70 and HSP90 in various nondisease and disease conditions remains unknown.

Our experiments were designed and performed with heat shock proteins (HSP90 and HSP70) that play key role in the cell membrane damage mechanisms identified in Acute Pancreatitis (AP) and cancer and etc., cases [2], which later can be used as biomarkers for AP and cancer detections.

We have developed tethered bilayer lipid membranes (tBLMs) as a long-term stable and versatile experimental model for protein reconstitution and for lipid-protein interaction studies [3]. In this work, different isoforms of HSP's were used to investigate their interaction with tBLM. These HSP's proteins exhibited the membrane damaging properties as probed by the electrochemical impedance spectroscopy (EIS). Membrane composition was found to be one of the important factors affecting the interaction of the HSP proteins to phospholipid membranes.



1pav. Representative image of tethered lipid bilayer membranes (tBLM) interaction with HSP70.

1. Arya R. et.al., J Biosci. 2007;32:595–610. 2. Wei-Chih Liao et.al. *Pancreas*, 2009;38(4):422-426.

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PP68-2-B

SPATIOTEMPORAL IMAGING OF WATER IN OPERATING VOLTAGE-GATED ION CHANNELS REVEALS THE SLOW MOTION OF INTERFACIAL IONS

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Ion channels are responsible for numerous physiological functions ranging from transport to chemical and electrical signaling. Although static ion channel structure has been studied following a structural biology approach, spatiotemporal investigation of the dynamic molecular mechanisms of operational ion channels has not been achieved experimentally. In particular, the role of water remains elusive. Here, we perform label-free spatiotemporal second harmonic (SH) imaging and capacitance measurements of operational voltage-gated alamethicin ion channels in freestanding lipid membranes surrounded by aqueous solution on either side. We observe changes in SH intensity upon channel activation that is traced back to changes in the orientational distribution of water molecules that reorient along the field lines of transported ions. Of the transported ions, a fraction of 10^{-4} arrives at the hydrated membrane interface, leading to interfacial electrostatic changes on the time scale of a second. The time scale of these interfacial changes is influenced by the density of ion channels and is subject to a crowding mechanism. Ion transport along cell membranes is often associated with the propagation of electrical signals in neurons. As our study shows that this process is taking place over seconds, a more complex mechanism is likely responsible for the propagation of neuronal electrical signals than just the millisecond movement of ions.

PP69-**2-A**

THE MOLECULAR RECOGNITION OF PHOSPHATIDIC ACID BY AN AMPHIPATHIC HELIX IN OPI1

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A key decision in cellular physiology is the decision between membrane biogenesis and lipid storage. The regulatory pathways underlying these cellular processes must be carefully coordinated and tightly controlled. The lipid metabolite phosphatidic acid (PA) represents the branch point between glycerophospholipid production for cell proliferation and triacylglycerol synthesis for fat storage (1). The underlying mechanisms how the cell manages to orchestrate these two pathways remain yet elusive.
Using the soluble membrane sensor and transcription repressor Opi1 from the baker´s yeast *Saccharomyces cerevisiae*, we here illustrate that this selective membrane recognition goes beyond sensing total PA content and that Opi1 additionally probes bulk ER properties such as membrane curvature and desaturation degree. We show that the existence of an amphipathic helix (AH) in the PA binding site of Opi1 enables the acyl chain sensing of the hydrophobic core of the bilayer after initial electrostatic membrane recruitment (2). Directed tuning of the AH by introducing single point mutations enables modulations in membrane binding of Opi1 *in vivo in vitro* and *in silico*. We here provide evidence that this AH feature allows Opi1 to sense bulk physical membrane properties and to subsequently regulate the transcriptional program of glycerophospholipid biosynthesis.

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2. Giménez-Andrés, M., Čopič, A., and Antonny, B. (2018) The Many Faces of Amphipathic Helices. Biomolecules. ***8****, 45*

PP70-2-B

NOVEL DRUG DELIVERY SYSTEM FOR ANTIBIOTIC THERAPY USING MODIFIED ERYTHROCYTE LIPOSOMES

H. Krivic, R. Sun, S. Himbert, & M. Rheinstadter.

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As a result of the growing world-wide antibiotic resistance crisis, many currently existing antibiotics have been shown to be ineffective due to bacteria developing resistive mechanisms. There are a limited variety of potent antibiotics that are successful at suppressing microbial growth, such as polymyxin B, however are deemed as a last resort due to being highly toxic to healthy cells. Previous literature has focused on the development of an effective drug delivery system that can inhibit bacterial growth while minimizing negative side effects. In particular, nanoparticles have been of interest as they can be conjugated to a drug of interest, allowing for effective drug transport to the target. Despite their potential, an antibiotic delivery system has yet to be established, presumably due to the nanoparticles lacking specificity. Here, we present a novel antimicrobial drug delivery method that uses modified red blood cells that are encapsulated around polymyxin B. The nanoparticles are made specific to E.coli through the addition of corresponding antibodies on the exterior. We investigate whether this drug delivery system is effective at inhibiting E.coli growth while minimizing the negative side effects seen with conventional polymyxin B treatment. This RBC based platform is potentially advantageous to nanoparticle based approaches because of their biocompatibility and bioavailability, resulting in a longer retention time in the human body.

PP71-2-A

ATOMIC FORCE MICROSCOPY STUDY OF THE INTERACTION OF S100A9 PROTEIN WITH LIPID MEMBRANES

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Amyloid plaque formation and neuroinflammatory processes are the main features of Alzheimer's disease pathology. S100A9 is pro-inflammatory, calcium-binding protein, belonging to the S100 family [1]. S100A9 protein levels are increased in many inflammatory disorders, including Alzheimer's disease [2]. In Alzheimer's disease, S100A9 serves as a junction between amyloid and neuroinflammatory cascades [3]. Due to its amyloidogenicity, together with β-amyloid S100A9 forms neurotoxic amyloid plaques [3], which results in neuronal death and memory impairment. However, the exact mechanism of the interaction of S100A9 with lipid membranes is still unknown.
The aim of this work is to investigate the mechanisms of S100A9 protein aggregation and interaction with lipid bilayer. We used the solid supported membrane and unilamellar vesicles models to mimic the fundamental chemical and physical properties of a cell membrane. To visualize the protein-membrane interaction and to determine the nanomechanical properties of lipid bilayer, we used atomic force microscopy (AFM). We demonstrate that protein S100A9 induce local thinning of the membrane composed of brain total lipid extract (TLE) (Fig. 1).

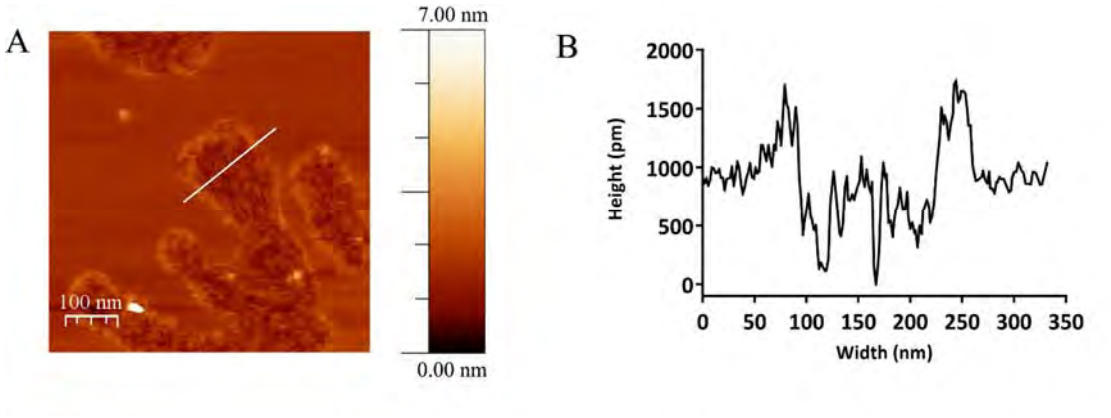

Figure 1. AFM observation of S100A9 disruption on solid supported membrane. (A) AFM topography image showing the local thinning of a TLE membrane. (B) Height profile along the line in the AFM image.

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PP72-2-B

DOES MECONIUM AFFECT THE STRUCTURE OF PULMONARY SURFACTANT?

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Pulmonary surfactant is a mixture of lipids (~90wt%) and specific proteins decreasing surface tension at air-liquid interface in alveoli. Meconium, the first stool of newborn contains substances capable to inactivate pulmonary surfactant.

Two systems were compared: porcine pulmonary surfactant (PS) used in therapy (Curosurf®) composed of phospholipids (~98wt %) with a small portion of specific surfactant proteins and a model system (MS) prepared from phospholipids DPPC/POPC/PLPC/POPG.

Small angle X-Ray scattering on PS at 40°C shows a lamellar phase Lα with the repeat distance d=9.37±0.48nm and within experimental uncertainty does not change up to 10wt% of meconium. Lamellar structure swells at high contamination by meconium (50wt%). For MS, d=9.96±0.25nm, and decreases with increasing content of meconium. The lipid bilayer thickness dL=3.70±0.03nm and 3.62±0.03nm for PS and MS, respectively, were determined from SANS data. In both systems, the lipid bilayer thickness increases in the range 0.06-0.08nm when the lipid bilayer is contaminated by meconium (up to 50wt%).

To conclude, our experiments show that high content of meconium induces structural changes in the lamellar packing and affects the lipid bilayer thickness. The effect is stronger in model system (MS) composed of phospholipids only, in comparison to natural PS containing proteins.

Acknowledgement: Small angle X-Ray scattering (SAXS) experiments were performed at BL11-NCD beamline at Alba Synchrotron with the collaboration of Alba staff. Small angle neutron scattering (SANS) at PACE spectrometer, Laboratoire Leon Brillouin (CEA-CNRS), CEA Saclay, France Experiments were supported by projects APVV-17-0250, VEGA 1/0223/20, JINR project 04-4-1142-2021/2025 and FaFUK/8/2019. Meconium collected from term-born infants and lyophilized was provided by Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava.

PP73-2-A

ASSAYING THE ACTIVITY OF OMPLA IN SYMMETRIC AND ASYMMETRIC LIPID MEMBRANES

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The outer membrane phospholipase A (OmpLA) is an integral membrane enzyme located in the outer membrane of Gram-negative bacteria, which catalyzes the hydrolysis of phospholipids. We reconstituted OmpLA in symmetric liposomes composed of palmitoyl oleoyl phosphatidylcholine (POPC) and in asymmetric large unilamellar vesicles (aLUVs), which we achieved by exchanging the outer leaflet by phosphatidylethanolamine (POPE) using methyl-β-cyclodextrin. First, we focused on the effect of lipid asymmetry on protein activity using a well-established colorimetric assay. Results indicated a significantly reduced activity of OmpLA in aLUVs. However, the assay may lead to increased lipid flip/flop affecting this observation. We therefore developed a second assay based on high performance thin layer chromatography (HPTLC) sensitive to unsaturated hydrocarbons. We report first insights into the differential activity of the protein in symmetric and asymmetric membranes using this assay, as well as signatures of OmpLA-mediated lipid hydrolysis as observed by small-angle X-ray scattering.

PP74-2-B

ENDOCYTOSIS ACROSS SCALES: FROM MOLECULAR STRUCTURES TO A FUNCTIONAL PROCESS

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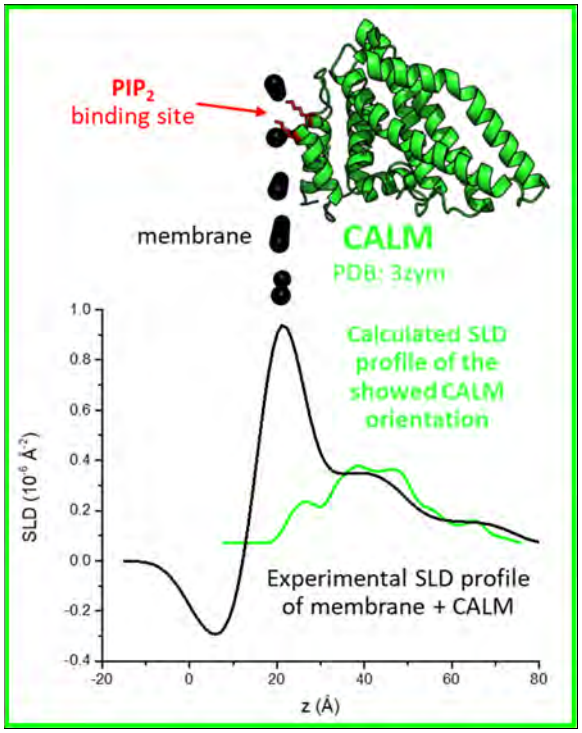
Clathrin-mediated endocytosis (CME) is the main mechanism by which eukaryotic cells internalize and recycle most membrane proteins. Mutations affecting endocytosis have been directly linked to cancer as well as to Alzheimer[1] and Stiff-man[2] diseases. The CME is driven by different Adaptor and Modulator Proteins, that solely interact with the inner leaflet of the cell membrane. By exploiting techniques such as ellipsometry, pressure–area isotherms and Neutron Reflectometry (NR), the aim of this work has been to investigate the binding and resultant structures formed by the adaptor protein CALM and by the modulator FCHo2 on association with lipid monolayers enriched in either phosphatidyl-inositol-4,5-diphosphate (PIP_2), or 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS). In particular, Neutron Reflectometry allowed us to determine the position and orientation of both CALM and truncated versions of FCHo2 with respect to the membrane. The resultant position of the CALM atomic structure[3] on the membrane made biological sense. Regarding FCHo2, a 4-layer-model, which fit well to the NR data, allowed the general orientation of the protein as well as the relative positions of the protein's individual domains to be determined.



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PP75-2-A

BEHAVIOR OF LIPID TAILS OF PALMITOYLATED TRANSMEMBRANE PEPTIDES IN PHOSPHOLIPID MEMBRANES: A MOLECULAR DYNAMICS STUDY

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Palmitoylation is one of the post-translational lipid modifications of proteins in which the cysteine's -SH group was esterified. Protein palmitoylation was proven as essential for many aspects of the cell organization, such as the trafficking of proteins from the intracellular organelles toward the cell membrane and the localization into it.
Phosphoprotein associated with glycosphingolipid micro-domains (PAG) is a transmembrane protein for which palmitoylation was demonstrated to be crucial for its accumulation into the ordered regions of the cell membrane.
What is not well explored is how palmitoyl lipid tails behave in the cell membrane and whether or not these tails are responsible for the stability of the protein within the bilayers. Furthermore, the palmitoylated protein's propensity in different membrane phases, named liquid-ordered (Lo) and liquid disordered (Ld), hasn't been fully explored yet.
In this work, we employ atomistic molecular dynamics simulation to assess the behavior of the palmitoylated PAG transmembrane domain into several model membranes mimicking Lo and Ld phases and Lo/Ld interface. We focus on molecular-level aspects of palmitoyl accumulation at the water-membrane interface and in the well-hydrated headgroup region.

PP76-2-B

OPTICAL LIPID CLAMP ANALYSIS OF TRPC3 ACTIVATION/DEACTIVATION KINETICS SUGGESTS ASYMMETRY OF LIPID-GATING

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Coordination of membrane lipids within channel complexes maintain their structural integrity and cellular regulation. Transient receptor potential canonical (TRPCs) constitute a family of tetrameric cation channels, which are gated by diacylglycerols (DAGs). Recent single-particle cryo-EM studies identified two lipid interaction sites, termed L1 and L2, which can accommodate not only DAGs, but also sterols or PIP_2. To explore the role of L1 and L2 in TRPC3 homotetramers, we combined electrophysiology with molecular dynamics (MD) simulations as well as structure-guided mutagenesis. The activation/deactivation kinetics of TRPC3 was analyzed by Optical Lipid Clamp to gain mechanistic insight into DAG-TRPC3 interactions. MD simulations indicated a strong propensity of DAG (1-stearoyl-2-arachidonoyl-sn-glycerol) to accumulate within the L2 domain. A mutation in the L2 region (G652A) significantly altered activation kinetics in response to the first OptoDArG photoisomerization. This initial (1^{st}) activation kinetics was sigmoidal and best fitted by power exponential functions with a τ of about 500 ms and N(power)>4 in wild type, while τ was <100 ms and N=2-3 in the G652A mutant (L2). In contrast, several mutations in L1 retained wild-type-like activation/deactivation kinetics. TRPC3 activation during consecutive (2^{nd}) photocycling, displayed power exponential kinetics with N=2. Our data suggest the existence of an initial priming process by DAG interaction with L2 that is promoted in G652A. Changes in opening kinetics of TRPC3 indicate two independent DAG-mediated conformational transitions, suggesting a potential asymmetry of the gating structures.

PP77-3-A

ADDITION OF TRANSPORTER DATA TO MOLMEDB

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Biological membranes act as barriers or reservoirs for many compounds within a human body. They play an important role in the pharmacokinetics of drugs and other molecular species. In the past we have collected interactions of small molecules with artificial and biological membranes in MolMeDB (http://molmedb.upol.cz) [1], where we collected data about interactions such as partitioning, penetration, and free energy profiles of drug crossing the membrane. In the case of biological membranes, these passive interactions do not provide a complete picture of membrane transport. Cells use myriads of transporters driving active transport of necessary compounds into and out of the cell. To complement passive interactions data stored in MolMeDB, we have employed a previously generated KNIME workflow [2,3] to gather data about ligand bioactivities on transporters from five publicly available data sources. Currently we have over 14,000 and 449,000 compounds with data on active or passive transport, respectively, with almost 7,000 compounds with overlap.
Here we report the first results from comparison between individual groups of compounds facilitated by MolMeDB.

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PP78-3-B

LIPOSOMES AS CARRIERS OF MEMBRANE-ASSOCIATED PROTEINS AND PEPTIDES FOR MASS SPECTROMETRY ANALYSIS

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Liposomes are phospholipid bilayer vesicles which resemble cellular organelles and membranes. Due to their variability in size, composition and amphiphilic character they are promising mimetics of natural membranes. However, due to their heterogeneity and ability to form large clusters in the gas-phase, liposomes were not employed for mass spectrometric analysis to-date.
While the analysis of integral membrane proteins by mass spectrometry is established, our aim was to utilize liposomes as carriers of peripheral membrane proteins and peptides for mass spectrometric analysis. For this, we first analysed “empty” liposomes varying in composition and concentration and found that liposome membranes dissociate in the gas-phase of a mass spectrometer. We then employed peptides and proteins that associate with liposome membranes of defined composition and show that, upon dissociation of the liposome membrane, the peptides and proteins are released in the gas-phase of the mass spectrometer allowing their structural analysis. Following this approach, we were able to study peptide/protein-lipid interactions as well as peptide/protein oligomerisation in the presence of a phospholipid bilayer. We verified our results by independent approaches including liposome flotation assays, chemical cross-linking and film balance measurements employing phospholipid monolayers. The complete workflow of liposome analysis is shown in the figure below.

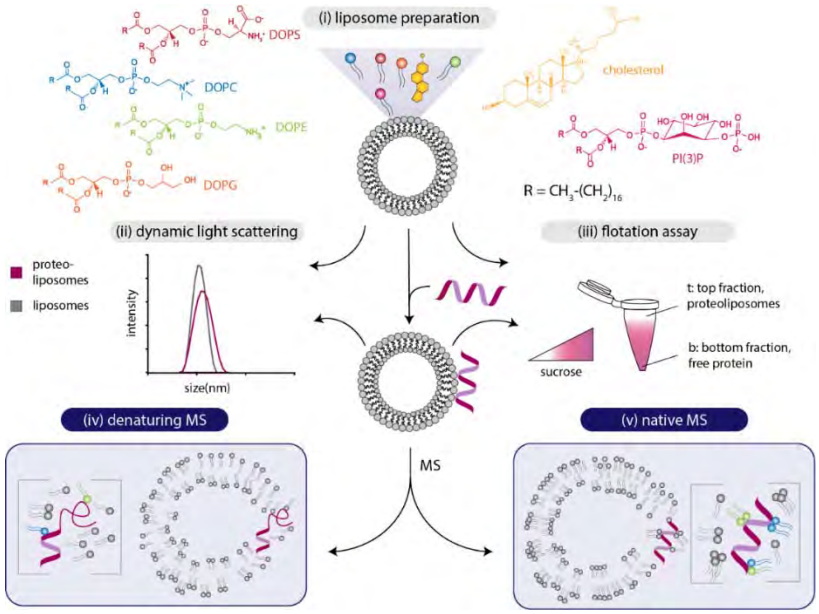

Figure: Workflow of liposome preparation (i) and analysis (ii-v). (i) Liposomes composed of various lipids are prepared as described. Lipid structures are highlighted. **(ii)** Liposomes or proteoliposomes are analysed by dynamic light scattering (DLS) determining the mean size distribution of the vesicles. **(iii)** Liposomes and proteoliposomes are separated from free protein employing a sucrose density flotation assay. **(iv** and **v)** Liposomes are analysed by MS under denaturing (iv) or under native (v) gas-phase conditions.

PP79-3-A

RELATING OLIGOMERIZATION NUMBERS OF PROTEINS TO FORMATION OF MEMBRANE PORES

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Oligomerization of membrane proteins into multicomponent units is often critical for their function (or dysfunction). To give an example for all, apoptosis, also known as programmed cell death, is induced at the molecular level by a change in the aggregation behavior of proteins, subsequent formation of protein complexes with a broad distribution of oligomerization numbers, and finally opening of functional pores in the mitochondrial membrane. Despite this knowledge, it is unclear which of these complexes are functional, i.e. capable of opening membrane pores, and which of them are dysfunctional.
In this contribution, we present a statistical single molecule and single vesicle assay determining the brightness of individually diffusing in-membrane oligomers and correlating their oligomerization state with membrane pore formation. We demonstrate the applicability of the method by investigating membrane translocation of Fibroblast Growth Factor 2 (FGF2). Based on mainly biochemical assays, it has been suggested that FGF2 oligomerizes into permeable pores at cellular plasma membranes from which it is released into the extracellular space. The new approach reveals FGF2 oligomers with about 7 to 8 subunits to represent the functional entities of productive membrane insertion. Moreover, by monitoring the oligomeric state of FGF2 on the same lipid vesicle over time, we detected sudden increases in the FGF2 oligomeric state accompanied by increased membrane permeability. In this way, in-membrane oligomerization of FGF2 was linked directly to the formation of membrane pores in one experiment under physiological conditions. Our findings demonstrate that quantifying oligomeric states alone does not allow for a deep understanding of the structure−function relationship of membrane-inserted oligomers.

PP80-3-B

REGULATION OF ACTIN DYNAMICS BY PHOSPHOINOSITIDES

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The actin cytoskeleton powers membrane deformation during many cellular processes, such as migration, morphogenesis, and endocytosis. Phosphoinositides, especially phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2], regulate the activities of many actin-binding proteins (ABPs), including profilin, cofilin, Dia2, N-WASP, ezrin, and moesin; however, the underlying molecular mechanisms have remained elusive. Here, we applied a combination of biochemical assays, and photobleaching/activation approaches to uncover the molecular principles by which ABPs interact with phosphoinositide-containing membranes. We show that, despite using different lipid-binding domains, these proteins associate with membranes through similar multivalent electrostatic interactions, without specific binding pockets or penetration into the lipid bilayer. Our experiments reveal that these proteins display enormous differences in the dynamics of membrane interactions and in the ranges of phosphoinositide densities that they sense. Profilin and cofilin display transient, low-affinity interactions with phosphoinositide-containing membranes, whereas F-actin assembly factors Dia2 and N-WASP reside on phosphoinositide-containing membranes for longer periods to perform their functions. Ezrin and moesin, which link the actin cytoskeleton to the plasma membrane, bind membranes with high affinity and slow dissociation dynamics. Unlike profilin, cofilin, Dia2, and N-WASP, they do not require high 'stimulus-responsive' phosphoinositide density for membrane binding. Together, these findings demonstrate that membrane-interaction mechanisms of ABPs evolved to precisely fulfil their specific functions in cytoskeletal dynamics.

PP81-3-A

A CRITICAL ROLE FOR CHOLESTEROL IN PI(4,5)P_2-DEPENDENT UNCONVENTIONAL SECRETION OF FIBROBLAST GROWTH FACTOR 2

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Fibroblast Growth Factor 2 (FGF2) is a cellular survival factor involved in tumor-induced angiogenesis. It is one of the most prominent examples for extracellular proteins that lack signal peptides and are secreted by ER/Golgi-independent secretory pathways, processes collectively termed unconventional protein secretion (UPS). Biochemical reconstitution experiments and imaging in living cells have shown that FGF2 is secreted by direct translocation across the plasma membrane (Type I UPS). This process is initiated by PI(4,5)P_2-dependent FGF2 recruitment at the inner plasma membrane leaflet. This in turn results in the formation of membrane-spanning FGF2 oligomers within toroidal membrane pores. FGF2 translocation into the extracellular space is completed by cell surface heparan sulfate prorteoglycans that disassemble FGF2 oligomers at the outer leaflet, a process that makes FGF2 available in the extracellular space for both autocrine and paracrine signaling. Here, using both biochemical reconstitution experiments and live cell imaging, we demonstrate that PI(4,5)P_2-dependent FGF2 recruitment at the inner plasma membrane leaflet is positively modulated by cholesterol in both . Based on extensive molecular dynamics simulations and free energy calculations, we propose cholesterol to increase the negative charge density of the membrane surface and to induce clustering of PI(4,5)P_2 molecules stabilizing FGF2 binding through increased avidity. Our findings have general implications for phosphoinositide-dependent protein targeting membranes and explain the highly selective targeting of FGF2 towards the plasma membrane.

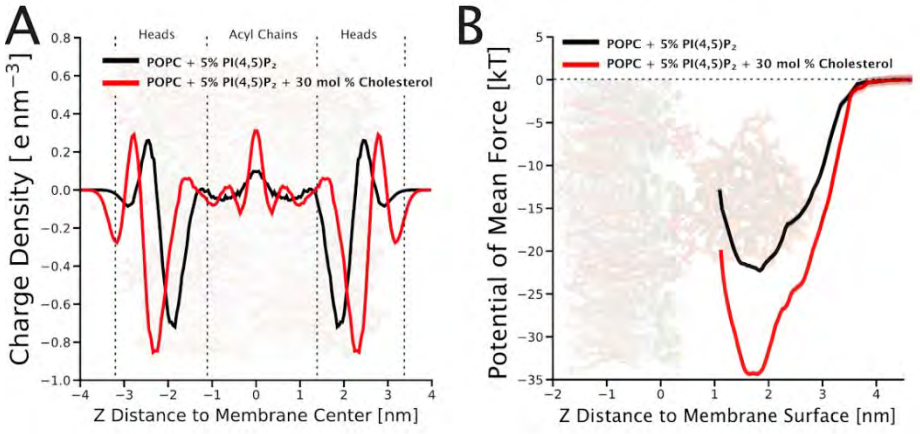

Figure 1. Panel A shows the charge density profiles along the normal of the bilayers averaged over the last 700ns of the pure bilayer simulations (no FGF2) without (black curve) and with 30 mol % of cholesterol (red). Panel B shows the potential of mean force along with the z component of the distance between the center of masses of the protein and the P atoms in the closest leaflet for membranes without (black curve) and with 30 mol % of cholesterol (red).

PP82-3-B

SYNERGETIC APPROACH OF RIGOROUS SIMULATION AND EXPERIMENT HELPS AVOID PITFALLS PREVIOUSLY OVERLOOKED IN PKC C1 DOMAIN TARGETING

S. Lautala[1], R. Provenzani[2], I. Tarvainen[3], Artturi Koivuniemi[1], W. Kulig[4], V. Talman[3], T. Róg[4], R. Tuominen[3], J. Yli-Kauhaluoma[2] and A. Bunker[1]

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Mild activation of the enzyme protein kinase C (PKC) is of potential therapeutic value [1, 2]. The desired activation occurs when PKC C1 domain interacts with an intracellular membrane that incorporates the lipid second messenger diacylglycerol (DAG). Designing these therapeutics is however not without pitfalls, largely due to the complex binding environment of the lipid-water interface, as we have discovered previously [3]. We identified that the reason for failure of pyrimidine analogs designed to improve on the previous isophthalate derivatives were exactly the challenges caused by the lipid-water interface binding environment [3,4,5]. We observed that the propensity of harmful internal hydrogen bonding in the pyrimidine analogs is enhanced by the lipid environment, which then both obstructs binding and leads to unfavorable reorientation, and therefor prevents the correct accessing of the PKC C1 domain [3].
Interestingly, this crucial harmful interaction was only picked up by the more rigorous molecular dynamics simulations (MD), as structure-based docking predicted both isophthalate and pyrimidines both to bind well to the C1 domain [4,5]. Our investigation therefor highlighted the need for *in silico* simulations in the relevant environment already in the design phase. We have now by implementing lipid environment simulations in tandem with *in vitro* assays discovered a new promising generation of compounds, which readily demonstrate the desired behavior of possible therapeutics.

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PP83-3-A

THE EFFECT OF PHARMACEUTICAL COMPOUNDS ON LIPOSOMAL SYSTEMS

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Liposomes represent a simplified model of a cell membrane, herewith facilitating the understanding of fundamental biological processes, such as the influence of pharmaceutical compounds. Thus, three 2-amino-1,3,4-thiadiazole analogues (TS, TSF and TB)(Fig. 1), with promising anticancer and neuroprotective, anti-oxidative, antifungal and antibacterial activities are promising candidates for the drug industry [1].

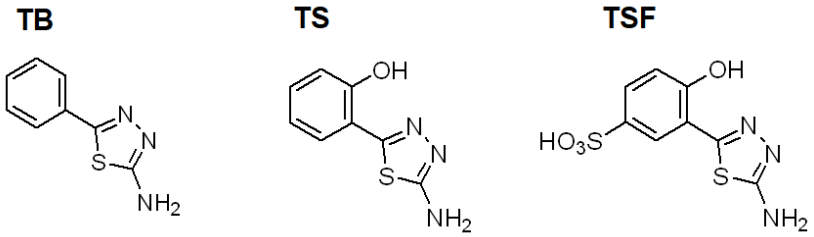

Fig. 1. Chemical structure of: benzoic thiadiazol (TB), salicylic thiadiazol (TS) and sulfosalicylic thiadiazol (TSF).

In this work, the effect of 1,3,4-thiadiazole analogues on lipid membranes has been studied in the model liposomal system prepared from DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine). The effect of these compounds on the phase pre-transition and main-transition temperature of DPPC has been studied at the surface of a gold sensor chip using two label-free methods: surface plasmon resonance (SPR) and electrochemical impedance spectroscopy (EIS). Similar light adsorbing tendencies were observed using SPR and classical spectroscopic methods (UV-vis absorption, fluorescence), whereas SPR could be easily correlated with EIS, which highlighted the transport phenomena at the liposomal membrane in the presence of the pharmaceutical compounds.

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Acknowledgments: *This work was supported by a grant of the Romanian Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-1285, within PNCDI III and the structural funds project PRO-DD (POS-CCE, O.2.2.1., ID 123, SMIS 2637, No 11/2009) for providing some of the infrastructure used in this work.*

PP84-3-B

UNREVEALING THE INTERPLAY BETWEEN CA2+, CALMODULIN AND A MODEL LIPID MEMBRANES IN EARLY CALCIUM SIGNALING EVENTS

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Calmodulin is an ubiquitous calcium-dependent protein that has been found to bind and regulate many others signaling proteins. Calmodulin is highly negatively charged and can bind up to four calcium ions using its EF-loops. This loading occurs during signalling events where calcium concentration increases, and it triggers extensive conformational changes. Another effect of the calcium loading in calmodulin is the charge shielding that can readily affect its interaction with other proteins and the cellular membranes. The latter is the focus of this work. In resting conditions no binding of Calmodulin to the membrane is expected. Still, it is tenable to hypothesize that to interact with signaling membrane-proteins a calcium spike can help the interaction between calmodulin and the membrane. In order to shed light on how calcium can trigger this interaction, we coupled fluorescent measurement and extensive classical molecular dynamic simulations. Furthermore, to understand the role of different lipid components we investigate different membrane composition. The results of experiments and MD simulations together seem to converge on the identification of the main actors in this cross-talk modulating the calmodulin interaction with the membrane. Interestingly, we found a preferential propensity of calmodulin for POPE over POPC. Cholesterol content up to 20% doesn't seem to play an important role on the binding propensity of calmodulin. Finally, POPS effect is currently controversial.Our study shed light on a different aspect of calmodulin function not previously addressed, stressing even further its chameleonic nature.

PP85-3-A

ELECTROCHEMICAL STUDY OF THE INTERACTION OF S100A9 PROTEIN WITH TETHERED BILAYER LIPID MEMBRANES

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S100A9 protein belongs to the S100 family of proteins and is important factor in the regulation of most cellular processes and immune response [1]. It is associated with the development of cancer cells and neurodegeneration. S100A9 protein has amyloid-like properties and is found in extracellular senile plaques together with beta-amyloid in Alzheimer's brain. S100A9 is involved in aggregates formation and inflammatory processes associated with this disease [2].
In this work the interaction between S100A9 and membrane was studied and tethered bilayer lipid membranes (tBLM) [3] were used as simplified membrane model for these studies. The aim of this work was to form tBLM and to optimize their electrical properties in order to use them in the study of the interaction of S100A9 protein with phospholipid bilayer and its mechanism of action. By using electrochemical impedance spectroscopy and dynamic light scattering methods, we demonstrate that smaller S100A9 oligomers are more toxic to the membrane compared to larger aggregates.

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PP86-3-B

INSIGHTS OF ORGANIC ANION TRANSPORTER 1 PROVIDED VIA MOLECULAR MODELING

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Xenobiotic crossing through membranes is key in pharmacology since it affects both pharmacodynamics (PD) and pharmacokinetics (PK). This is even more important regarding the recent attention paid to local PK and bioavailability (i.e., drug concentration close to its target) in the field of pharmacology. Membrane transporters play a central role in membrane crossing events. Despite the importance of transporters in clinical pharmacology and recent advances about local PK/PD relationship, the role of membrane transporters at the cellular scale on *e.g.*, drug-drug interactions or pharmacogenomics (PGx) still remains unclear. This is particularly true for transporter located in liver and kidneys since they are strongly involved in drug metabolism and elimination.

The present study focuses on the Organic Anion Transporter 1 (SLC22A6/OAT1) belonging to the Solute Carrier (SLC) and is expected to adopt the Major Facilitator Superfamiliy (MFS) fold. OAT1 is involved in the intracellular uptake of a broad range of endogenous substrates and xenobiotics (e.g., antiviral drugs) in kidney proximal tubular cells. However, there exist

no resolved structure by means of X-ray crystallography nor Cryo-EM techniques. In the present project, we propose a molecular dynamics (MD)-refined protein threading model of OAT1 in which lipid-protein interactions are investigated by embedding our model in different lipid bilayer models made of PC, PE lipids and cholesterol.

Relying on recent findings about MFS as well as experimental and clinical observations about OAT1, insights about OAT1 structure as well as thorough investigations on protein lipid, protein-substrate interactions are proposed by means of ms-scaled MD simulations. Furthermore, mapping of relevant single-nucleotide polymorphism (SNP) variants and *in vitro* experimental mutants provide hints of OAT1 subdomain functions.

PP87-3-A

ON THE INTERPLAY BETWEEN COMPLEX LIPID BILAYER MEMBRANE AND MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 (MRP1) BY MEANS OF MOLECULAR DYNAMICS

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Membrane crossing events by xenobiotics are key processes in pharmacology, especially in pharmacokinetics (i.e., Absorbtion, Distribution, Metabolism and Elimination – ADME). Particular attention should be paid to membrane transporters located in liver and kidneys since these organs are involved in 95% of drug elimination. In the present project, the focus is on multidrug-resistance associated proteins (ABCC/MRPs) which have been pointed out as ”emerging clinical importance” by the International Transporter Consortium (ITC). Owing to the absence of resolved human MRP structures, bovine MRP1 (*b*MRP1) is used as a prototype for ABCC family.

µs-Scale molecular dynamics (MD) simulations were performed on inward facing (IF) *b*MRP1 conformers in presence or absence of ATP and/or substrate as well as on ATP-bound outward facing (OF) conformation; all being embedded in different lipid bilayer models made of POPC, POPE and/or cholesterol. Internal structural variabilities of *b*MRP1 domains exhibits strong impact upon ATP and/or substrate binding. For instance, MD simulations reveals the *in situ* spontaneous closing of IF conformations as suggested for nucleotide binding domain (NBD) degenerated ABC transporters. Structural parameters reveal relatively weak dependence on lipid bilayer membrane composition. However, lipid distribution analysis exhibits hot-spot for cholesterol-binding to MRP1.

Keywords: ABCC, MRP1, molecular dynamics, lipid-protein interactions

PP88-3-B

PURIFICATION AND BIOPHYSICAL CHARACTERIZATION OF THE LIPID-DROPLET ASSOCIATED G0/G1 SWITCH PROTEIN (G0S2)

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Most organisms store excess energy in form of triacylglycerols (TGs) in lipid droplets (LDs). These LDs have a unique ultrastructure consisting of a core of neutral lipids that is surrounded by a phospholipid monolayer. Numerous different proteins are associated to the LDs. These energy stores can be mobilized in a process termed intracellular lipolysis, which generates glycerol and fatty acids. Enzymatic mobilization of TGs occurs via consecutive actions of three lipases, namely adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoacylglycerol lipase (MGL). The activity of ATGL is regulated by direct interaction with the LD-proteins, especially the co-activator CGI-58 and the inhibitory protein G0/G1 switch protein 2 (G0S2). Determining the structures of proteins involved in lipolysis is of great interest to understand their mechanism of action and interactions.
Our studies focus on G0S2 using an integrated structural biology approach. Due to highly hydrophobic patches G0S2 tends to aggregate in aqueous buffer. We embed G0S2 in circular nanodiscs and DPC micelles, which serve as a LD-mimicking system. We outline the protocol for purification and first steps towards biophysical characterization using small angle X-ray scattering (SAXS), circular dichroism spectroscopy (CD-spectroscopy), and dynamic light scattering (DLS).

PP89-**3-A**

SPECTROSCOPIC INVESTIGATION INTO THE MICELLAR SYSTEMS MODIFIED WITH USE OF THE SELECTED 1,3,4 - THIADIAZOLE DERIVATIVES

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1,3,4-thiadiazole derivatives are class of compounds, which demonstrate a broad array of biological activities, including antitumor, anti-neurodegenerative, antifungal or neuroprotective [1]. Also, these compounds are known to exhibit an extraordinary spectral features such as the ESIPT-induced dual fluorescence [2-5] or environment (solvent) polarizability-induced keto-enol tautomerism[2]. Numerous 1,3,4-thiadiazole derivatives are characteristic of crystals polymorphism and solvatomorphism [6]. Moreover, due to their relatively high affinity to *d*-block transitition metal ions 1,3,4-thiadiazoles may act as ligands in the synthesis of highly biologically-active metal-based agents [7].

Although the biological activities of various 1,3,4-thiadiazole analogues are well-documented, their spectroscopic and biophysical properties often require a more detailed examination. Therefore in this work a series of UV-Vis, fluorescence, FTIR, and Raman spectroscopic together with DSC studies were performed in order to investigate a model liposomal systems incorporating 1,3,4-thiadiazole additives. The fluorescence spectroscopic measurements performed revealed an interesting changes in the emission spectra associated most likely with the ESIPT processes. The results obtained suggest that incorporation of the biologically active 1,3,4-thiadiazole derivatives into the liposomal systems may find a potential practical application in development of novel therapeutic agents and may prove beneficial in terms of the research on the new drug delivery systems.

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PP90-3-B

STUDY OF CALMODULIN INTERACTION WITH MONOLAYERS.

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Calmodulin (CaM) is a messenger protein that is part of the calcium signal transduction pathway, where it plays an important role regulating many physiological processes. It is known that some of its targets are located close to the membrane, and therefore it is important for CaM to be able to approach it. However, the underlaying mechanism for such process is still unknown and far from trivial.
In this work, we use monolayers with several compositions as proxies to study CaM-membrane interaction. For this, we monitor the changes on the surface pressure after the addition of CaM or calcium to monolayers containing mixtures of POPC and POPS with different ratios. This method in principle has a high sensitivity, and the advantage of being label-free. In order to interpret the data, we used Molecular dynamics simulations that provide structural insights on the interaction.
First results show that the interaction of CaM with monolayers highly depends on the packing of the membrane. In monolayers with low surface pressure (~10 mN/m), CaM shows a strong surfactant behavior. It approaches the surface and increases the pressure independently of the lipid composition or the presence of calcium. This behavior is found both in experiments and simulations. With packed membranes (~30 mN/m) the results are harder to interpret. Experiments at shuch pressure show reproducibility issues and non-trivial features. At this packing level, the surface pressure in experiments either drops after the addition of CaM or remains unaltered, indicating a complex mechanism. When it drops, the possible explanations implie the removal of lipids from the interfase, or the formation of nanostructures in the surface. When no changes are observed, it is not clear if it means that there is no interaction, or simply a lack of sensitivity. This is in agreement with the results obtained from molecular dynamics simulations, where the interaction of CaM with the membrane does not alter the surface pressure. This hints to the possibility that the measure of surface pressure of monolayers could not be the apppropriated technique for the study of intermolecular interactions on high packing systems.

PP91-**1-A**

EXTENSIVE INFORMATION ABOUT MEMBRANE PERMEABILIZATION FROM ONE EXPERIMENT

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Membrane permeabilization is often characterized by vesicle leakage experiments. However, it is difficult to compare models to microbes or to draw conclusions about the mechanism.
We show how an adjusted experiment and data analysis can yield more extensive information about the membrane permeabilization behavior from a single leakage experiment.
I. The efficiency of individual membrane leakage events can be quantified using a self-quenching fluorescent dye. Our model then distinguishes weak from efficient types of leakage events.
II. Long leakage kinetics can indicate membrane permeabilization mechanisms: pores that reform in equilibrium or only occur once upon binding of the membrane-active compound.
We analysed leakage by various antimicrobial molecules and revealed that one molecule can cause different types of leakage events over time or concentration range. For example, strong but rare leakage events (such as cracking-in or leaky vesicle fusion) are followed by weaker defects that continue to form in equilibrium.
The classification and quantitative description of leakage behaviour and understanding of leakage mechanisms aids the design and improvement of membrane-active compounds, for example for antimicrobial treatment or in drug delivery.

PP92-**2-B**

THE EFFECT OF CHARGED LIPIDS ON AN AMYLOID-BETA PEPTIDE IN A MODEL PHOSPHOLIPID MEMBRANE: SANS AND MD DATA

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Amyloid-beta (Aβ) is a peptide whose physiological role is not fully understood. Usually, after performing its functions, the peptide is utilized. However, in some cases, the molecules of this peptide begin to bind to each other, forming toxic complexes called fibrils. The accumulation of Aβ peptide in brain cells provokes the development of Alzheimer's disease. The peptide-membrane and peptide-lipid interactions are believed to be critical in this process.
We investigated a model membrane consisting of mixture DMPC with anionic molecules of DMPS (up to 3% molar concentration) and incorporated Aβ (25-35) peptide at several temperature values. Samples of lipid systems as unilamellar vesicles were studied by the SANS. The main parameters were determined, such as the bilayer thickness etc.
There was no significant change in bilayer thickness with the addition of charged lipids. However, during experiments with temperature scan, changes in the shape of particles in the samples were found. The incorporation of Aβ peptide in the DMPC bilayer at temperatures below the phase transition temperature leads to the formation of bicelles. The addition of charged lipid even at low concentrations (from 0.5% mol) inhibits the formation of bicelles.
Molecular dynamics simulation and experimental data are in good agreement.

This work has been carried out under support of the Russian Science Foundation grant 19-72-20186

PP93-**3-A**

INTERACTION OF TWO ANTITUMOR PEPTIDES WITH MEMBRANE LIPIDS – INFLUENCE OF PHOSPHATIDYLSERINE AND CHOLESTEROL ON SPECIFICITY FOR MELANOMA CELLS

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R-DIM-P-LF11-322 and DIM-LF11-318, derived from the cationic human host defense peptide lactoferricin show antitumor activity against human melanoma. While R-DIM-P-LF11-322 interacts specifically with cancer cells, the non-specific DIM-LF11-318 exhibits activity against non-neoplastic cells as well.
Recently we have shown that cancer cells expose the negatively charged lipid phosphatidylserine (PS) in the outer leaflet of the plasma membrane, while non-cancer cells expose only zwitterionic or neutral lipids, such as phosphatidylcholine (PC) or cholesterol. Calorimetric and zeta potential studies with R-DIM-P-LF11-322 and cancer-mimetic liposomes composed of PS, PC and cholesterol indicate that the cancer-specific peptide interacts specifically with PS. Cholesterol, however, reduces the effectiveness of the peptide. The non-specific DIM-LF11-318 interacts with PC and PS. Cholesterol does not affect its interaction. PS depletion of cancer cells by the enzyme PS-decarboxylase confirmed the dependency of R-DIM-P-LF11-322 on the presence of exposed PS. Furthermore, cholesterol depleted melanoma plasma membranes showed an increased sensitivity to R-DIM-P-LF11-322, whereas the activity of DIM-LF11-318 was unaffected.
In conclusion, the specific interaction of R-DIM-P-LF11-322 with PS and sensitivity to cholesterol seem to modulate its specificity for cancer membranes (1).

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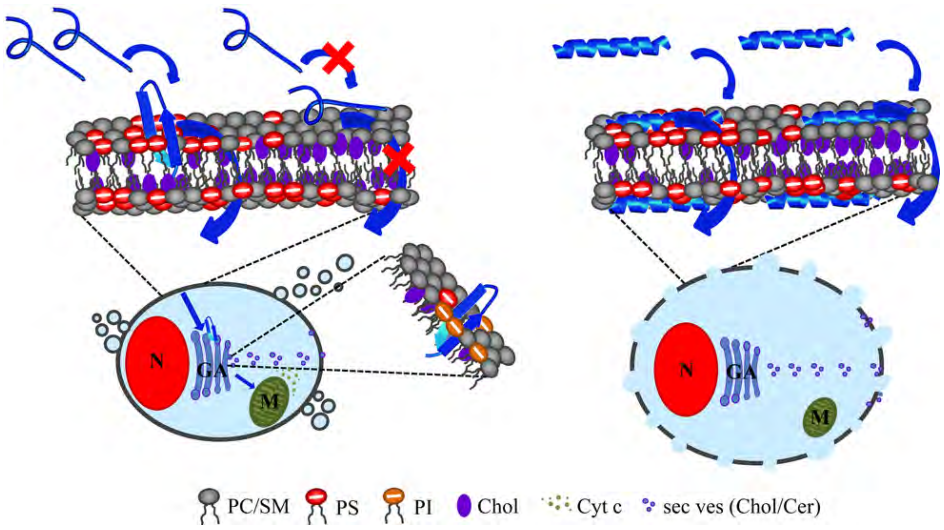

PP94-**3-B**

LIPID CLUSTERING INDUCED BY ANTIMICROBIAL PEPTIDES IN MIXED PE/PG: THE EFFECT OF PEG-LIPIDS

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Constantly increasing antibiotic resistance increases the importance of alternatives to classical antibiotics. Therefore, we examine two different trivalent cyclic hexapeptides with varying sequences and antimicrobial activity which interact with lipid membranes in multiple ways. To analyse the mechanism of action, we investigate their effect on model membranes composed of phosphatidylethanolamine (PE) lipids and anionic phosphatidylglycerol (PG) lipids. Previously, the peptides were shown to induce electrostatic lipid clustering which correlated with their antimicrobial activity [1]. Here, we show the effect of PEG-lipids on electrostatic lipid clustering.

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PP95-**3-A**

FREE ENERGY SIMULATIONS OF PORE FORMATION

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Lipid membranes define biological cells by establishing a semi-permeable barrier. Pore formation plays a role in processes such as membrane fusion and fission, the killing of bacterial cells with antimicrobial peptides, and others. Experiments have provided first hints on metastable pores almost 40 years ago. Although pores are heavily studied with a variety of methods, the free energy landscape of the initial stages of the pore formation is still not fully understood. We use molecular dynamics simulations to study the mechanisms and energetics of pore formation. We overcome the challenge of exploring the free energy landscape using umbrella sampling along a recently developed reaction coordinate [1], as previously applied to tension-free pure-lipid membranes [2]. Here, we here study the effects (i) of electric fields on the free energies of pore formation, as applied during electroporation to allow cellular uptake of drugs or genes, and (ii) of the common small antifungal drug itraconazole[3]. Due to itraconazole low solubility in water there are several liposome-based formulations but the release mechanisms remain unclear. The potentials of mean force (PMFs) show that electric fields greatly stabilize open pores and lower the barrier for pore formation. Interestingly, whereas itraconazole has only a small effect on the structure of planar, intact membranes, it strongly stabilized open pores[3]. In near future, we will use these simulation to study pore formation by membrane-active peptides.

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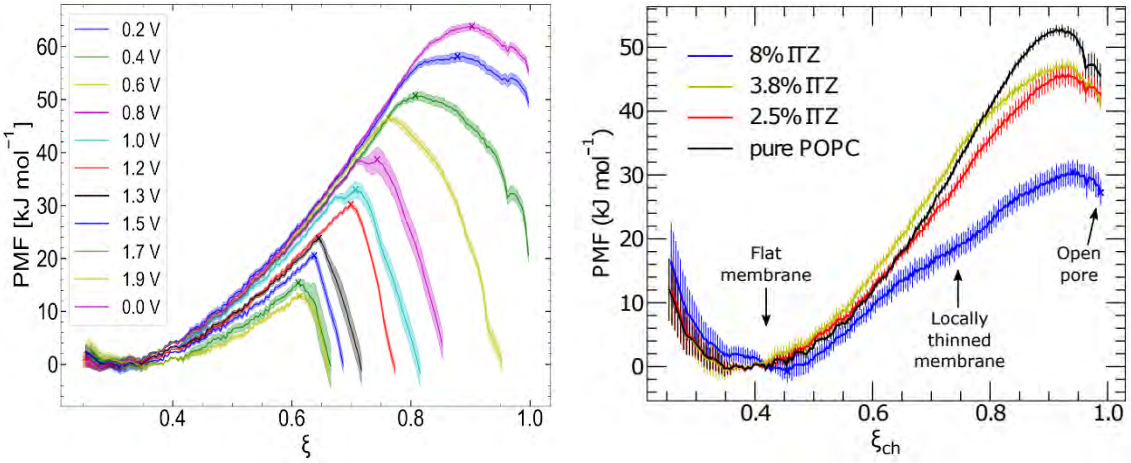

PP96-**3-B**

MEMBRANE PERMEABILIZATION BY ANTIMICROBIAL POLYCATIONS: CONTRIBUTIONS OF VESICLE FUSION AND AGGREGATION

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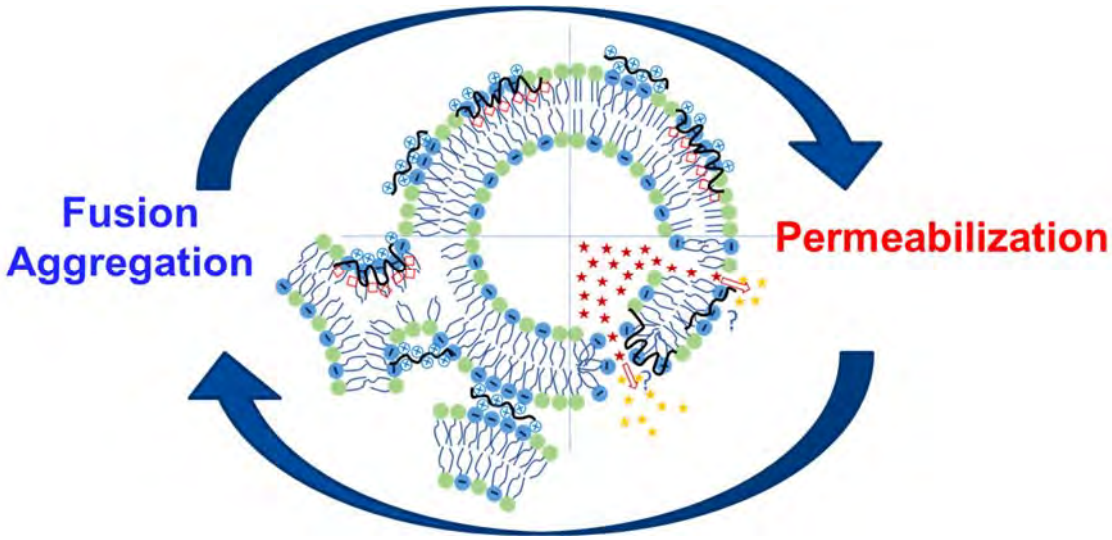
Synthetic polycations, such as nylon-3 copolymers, are regarded to be good functional mimics of antimicrobial peptides acting mostly by permeabilizing bacterial membranes. Understanding more complex membrane behavior behind permeabilization, e.g. vesicle aggregation and fusion, has major implications for the better interpretation of different mechanisms of actions.
The interactions of two types of nylon-3 copolymers containing different cationic/hydrophobic subunits with zwitterionic or negatively charged vesicles were evaluated. Cryo-electron microscopy visually shows the complex membrane behaviors (i.e. permeabilization, aggregation, and fusion of vesicles) after adding both copolymers. Fluorescence lifetime-based calcein leakage assay was used to characterize the various types of membrane permeabilization. Even more, the fusion process of vesicles was detected by lipid mixing assay (NBD-Rhodamine resonance energy transfer assay) and content mixing assay (ANTS-DPX Assay).
Interestingly, the relationship between membrane permeabilization and aggregation or fusion differs for the two nylon-3 copolymers. Both vesicle aggregation and fusion occur often when polycations such as AMPs and their synthetic mimics interact with charged vesicles.



PP97-**3-A**

CANDIDALYSINS ARE A NEW FAMILY OF CYTOLYTIC FUNGAL TOXINS

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Candidalysin is the first peptide toxin identified in the opportunistic fungal pathogen *Candida albicans.* Candidalysin causes both mucosal and systemic fungal infections and activates host defence mechanisms. However, *Candida* infections are also caused by non-*C. albicans* species. Here, we identify orthologues of *C. albicans* candidalysin in *C. dubliniensis* and *C. tropicalis* by comparative genomics, and characterise their cytotoxic activity using biological and biophysical approaches. Comparative functional analysis demonstrates that each candidalysin is highly alpha-helical, causes membrane rupture and calcium influx, and activates epithelial damage, intracellular signaling pathways and cytokine secretion. Importantly, the *C. dubliniensis* and *C. tropicalis* candidalysins appear to be more potent than *C. albicans* candidalysin. This is likely due to more efficient binding of candidalysin orthologues to lipid bilayers membrane which stimulates a more robust host epithelial response. Paradoxically, *C. dubliniensis* and *C. tropicalis* fungi cause less damage to epithelial cells when compared to *C. albicans*. This study identifies the first family of peptide cytolysins in human pathogenic fungi with differences in potency and mechanism of action.

PP98-**3-B**

PARTITIONING KINETICS AND STRUCTURAL CHANGES INDUCED BY TWO LACTOFERRICIN DERIVED PEPTIDES IN LIPID MEMBRANE MIMICS OF VARYING COMPLEXITY

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We used a common thermodynamic framework for antimicrobial peptide partitioning to compare the activity of two well-studied lactoferricin derivatives, LF11-215 and LF11-324, in different lipid-only vesicular mimics of the cytoplasmic membrane of Gram-negative bacteria composed palmityol-oleoyl-phosphatidylethanolamine (POPE), palmitoyl-oleoyl-phosphatidylglycerol (POPG), and tetra-oleoyl-cardiolipid (TOCL) mixtures using tryptophan (Trp) fluorescence and determined their membrane activity using a dye leakage assay and small-angle X-ray scattering (SAXS). Time-resolved Trp fluorescence experiments revealed different partitioning kinetics in mimics containing PE opposed to pure PG vesicles. Generally, partitioning of either peptide was shown to be the highest in CL-systems. Membrane permeability investigations showed peptide-induced, lipid/peptide concentration-dependent dye leakage in all studied membrane mimics coinciding with the formation of large aggregates especially for membranes including PE. SAXS experiments suggested that the aggregated structures contained collapsed multibilayers with sandwiched peptides in the interstitial space between membranes. PG-mimics exhibited only slight structural changes and required the highest peptide concentration to induce vesicle leakage. In leakage experiments of PE-containing systems, we additionally observed an effective shielding of the fluorescent dyes from leakage even at highest peptide concentrations, suggesting the occurrence of vesicle fusion mediated by the intrinsic curvatures of PE and CL. Our results thus show that stored elastic stress makes membranes more vulnerable to peptide activity.

PP99-**3-A**

STRUCTURAL CHANGES IN LIPID MEMBRANE CAUSED BY THE INCORPORATION OF Aß PEPTIDE

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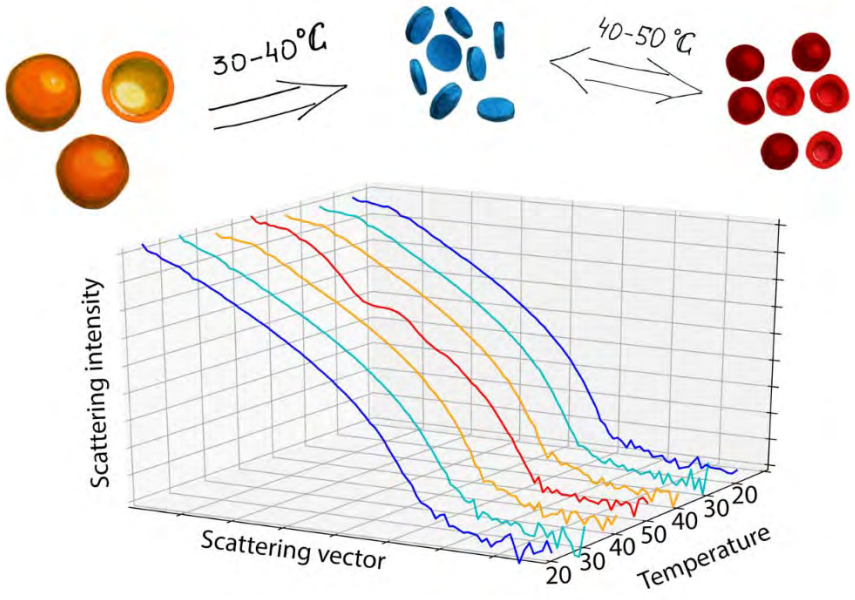
The self-association of amyloid beta-peptides (Aβ) is considered to be one of the initial steps of Alzheimer's disease. Therein, the question about the influence of temperature as well as surrounding environment on the self-aggregation process is still open. We have examined the structural characteristics of the non-charged model lipid membranes with incorporated Aβ25-35 peptide by means of small angle neutron scattering. The number of structural parameters such as radius and thickness of the unilamellar vesicles (ULVs) were obtained as a function of temperature. The small concentration of Aβ was chosen there to neglect the influence of peptide self-aggregation processes. Intriguingly, we have observed unambiguously the changes in membrane structural properties driven by the incorporation of Aβ25-35 to the system. During the temperature changes, the system of ULVs experienced changes from large ULVs to the bicelles-like objects and small ULVs. The membrane shape changes were also accompanied by the dramatical changes in the membrane thickness.

Acknowledgement: This work has been supported by the Russian Science Foundation under grant 19-72-20186.



PP100-**3-B**

SAXS STUDY OF LIPIDATED PEPTIDE PACAP-DHA SUPRAMOLECULAR ASSEMBLIES

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The neuroprotective potential, immunomodulatory effects, and impact of omega-3 polyunsaturated fatty acids (O3-PUFA) in Alzheimer's and Parkinson's diseases have been well recognized. However, the structural organizations of supramolecular assemblies involving O3-PUFA have been scarcely studied. Here we investigated a synthetic construct of the pituitary adenylate cyclase-activating polypeptide (PACAP38) coupled to a docosahexaenoic acid (DHA: an O3-PUFA) in order to create liquid crystalline assemblies from neuroprotective compounds. The hormone PACAP38 is a ligand of the class B PAC1 G-protein-coupled receptor (GPCR), whereas DHA is a lipid trophic factor. The lipidated peptide PACAP-DHA is co-assembled into hierarchical nanostructures elaborated from hybrid vesicle-micelle reservoirs as well into PEGylated cubosomes composed of multiple neuroprotective building blocks. The resulting nanostructures are determined by small-angle X-ray scattering (SAXS) and cryogenic transmission electron microscopy (cryo-TEM). Multicompartment topologies are obtained in a two-fold approach: (i) intriguing compartmentalized vesicles, which embed pep-lipid micelles, and (ii) multidomain pep-lipid cubosomes. The organizational complexity of the scaffolds involving the lipidated high-molecular weight peptide hormone is beyond the one that has been reached with small lipid-like peptide surfactants.

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PP101-3-A

PARTITION COEFFICIENT OF ANTIVIROTICS BETWEEN PHOSPHOLIPID BILAYER AND AQUEOUS PHASE UV-VIS STUDY

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Liposomes are widely used as drug delivery systems, which involve hydrophilic and hydrophobic regions. Distribution of the drugs between the two phases is controlled by the partition coefficient (P), which is the ratio of concentrations of a drug in a mixture of two immiscible solvents at equilibrium. One of the solvents is usually water, while the second is a hydrophobic liquid, such as 1-octanol, according to European Pharmacopoeia standard. However, for a targeted drug delivery, partitioning of the drug between water and lipid bilayer is more relevant. Unilamellar liposomes, prepared from phosphatidylcholines (DOPC, DMPC), were used to determine the partition coefficient of two antivirotics (zanamivir, oseltamivir) between the liquid-crystalline lipid and the aqueous phase (150 mM of NaCl solution, 25°C) using UV-VIS spectroscopy. A set of samples was prepared at different concentrations of lipid, keeping constant concentration of the drug. The spectra were analysed applying the Lambert-Beer's law adapted for a two-phase system. The contribution of the scattered light on the liposomes to the drug's spectra was eliminated numerically using a scattering function. The partition coefficient's value (P) and the molar attenuation coefficient (ε) of the antivirotics in the lipid phase were obtained from the analysis.

Acknowledgements: This work was supported by grants VEGA 1/0223/20; APVV-17-0239; PP-COVID-20-0010 and JINR 04-4-1142-2021/2025.

PP102-3-B

DETERMINATION OF THE ACTUAL SIZE OF PROTEINS IN THE LIVE CELL PLASMA MEMBRANE

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It is well established that lipids and proteins are not just independent components of the plasma membrane of eukaryotic cells but that their arrangement, dynamics and function are interdependent. Besides specific lipid-protein interaction, transmembrane proteins are thought to bind a shell of annular lipids, which are more or less tightly associated with the proteins. Furthermore, highly ordered nanoscopic membrane domains have been proposed to act to compartmentalize proteins and their interactions, but have thus far not been directly observed.

Here, we use a combination of single molecule tracking and protein micropatterning to examine these interactions directly in the plasma membrane of living cells. In our experimental approach, different proteins of interest are immobilized within defined patterns in the plasma membrane, where they act as steric obstacles to the diffusion of lipid tracers and thus locally decrease their mobility. In the presence of lipid-protein interactions of any type, lipid mobility will be even further decreased within protein patterns. We used different types of proteins for patterning – single- and multi-spanning transmembrane proteins, a multi-subunit protein and a GPI-anchored protein. For all proteins we found that lipid tracer diffusion within protein patterns was slowed down only by steric hindrance since the size of the protein "sensed" by the diffusing lipid corresponded well with the size estimated from the protein crystal structure. These findings indicate that none of the examined proteins influence their membrane environment beyond their physical size, neither via tightly associated annular lipids nor via more ordered membrane domains.

PP103-**3-A**

A MICROFLUIDIC PLATFORM FOR THE STANDARDISATION OF GIANT VESICLE EXPERIMENTS AIMED AT THE CHARACTERISATION OF MEMBRANE ACTIVE PEPTIDES

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Membrane Active Peptides (MAPs) constitute a promising class of antimicrobial agents that can target microbial membranes. Studying MAPs' modes of action is critical for developing novel MAPs however has not been a modest task. Hence, there is need for platforms that can evaluate MAPs in a systematic and standardised manner. Here, we report a bespoke multilayer microfluidic platform to quantify the membranolytic efficacy and characterise MAPs. The platform is a biomimetic vesicle-based screening assay, which integrates an element for high throughput generation of Giant Unilamellar Vesicles (GUVs) in physiologically relevant buffers on demand. Thousands of GUVs are individually immobilized downstream in hydrodynamic traps connected to separate perfusion inlets that facilitate total fluid exchange of the vesicle surrounding and controlled continuous peptide administration. Membranolytic efficacy is expressed as a function of the time needed for encapsulated dye to leak out of single GUVs as a result of membrane permeabilization or lysis. The platform has been used to study a number of native and *de novo* synthesized peptides at different concentrations in parallel. The generated results can provide an insight on the capability of the lab-on-a-chip system to differentiate membranolytic modes of action in correlation to the behaviour of the vesicle population.

PP104-2-A

INVESTIGATING THE COMPETITIVE EFFECTS OF CHOLESTEROL AND MELATONIN IN MODEL LIPID MEMBRANES

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Cholesterol and melatonin incorporate into a lipid bilayer at different positions. It has been proposed that melatonin resides in a head-group region of bilayer and thus makes it more fluid. On the other hand, cholesterol should incorporate parallel into hydrocarbon chain region with its hydrophilic head close to the lipid`s head-group and making the membrane stiffer and thicker, because of increasing the order of hydrocarbon chains. These two additives then obviously impact the membrane in opposing directions [Drolle et al. (2013), BBA 1828:2247–2254]. It is however not clear how the membrane should behave when the two are added simultaneously.

We expand the previous works by studying the concurrent effect of cholesterol and melatonin on the structure and dynamics of DOPC and DPPC membranes by SAND, SANS and molecular dynamics calculations. In a general conclusion, we rule out a direct interaction between cholesterol and melatonin when added to the DOPC bilayers, as their mutual effects are clearly additive. In the case od DPPC bilayers, melatonin modulates the influence of cholesterol marginally.

Acknowledgement: This work has been supported by the Russian Science Foundation under grant 19-72-20186.

PP105-2-B

ACTIVATION OF GPROTEIN COUPLED RECEPTORS IS THERMODYNAMICALLY LINKED TO LIPID SOLVATION

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Preferential lipid solvation of the G-protein coupled A_{2A} adenosine receptor ($A_{2A}R$) is evaluated from 35 sec of all-atom molecular dynamics simulation. A coarse-grained transition matrix algorithm is developed to overcome slow equilibration of the first solvation shell, obtaining estimates of the free energy of solvation by different lipids for the receptor in different activation states. Results indicate preference for solvation by unsaturated chains, which favors the active receptor. A model for lipid-dependent GPCR activity is proposed in which the chemical potential of lipids in the bulk membrane modulates receptor activity. The entropies associated with moving saturated and unsaturated lipids from bulk to $A_{2A}R$'s first solvation shell are evaluated. Overall, the acyl chains are more disordered (i.e., obtain a favorable entropic contribution) when partitioning to the receptor surface, and this effect is augmented for the saturated chains which are relatively more ordered in bulk.