

XXIII GEM MEETING: Diving into the Secrets of Cellular Membranes

APRIL 1-4 2025

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1-1 Apr 2025

Vesicles and Organelles: The Dynamic Interface of Cellular Compartments

Invited talk: Lipid-lipid interaction in shaping membrane trafficking, cell polarity and signaling in plants

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In eukaryotic cells, intracellular membranes have evolved into specialized compartments that support complex cellular functions, including membrane trafficking, cell polarity, and signaling. Biological membranes exhibit remarkable molecular diversity and complexity, with lipids now recognized as essential regulatory components rather than merely structural elements. Lipids interact synergistically with both proteins and other lipids, playing a crucial role in cellular processes. They not only recruit key protein trafficking machineries to membranes, facilitating the activation of transport and cargo sorting, but also engage lipid-remodeling enzymes. Through these interactions, lipids contribute to maintaining the identity of intracellular compartments or driving their maturation into new functional entities.

The Golgi apparatus serves as a model for studying membrane dynamics. It acts as a central sorting hub that continuously processes a dynamic flow of cargo destined for specific cellular locations while simultaneously evolving to acquire distinct identities. Structurally, the Golgi consists of a stack of cisternae, with the pre-Golgi side receiving incoming cargo and the post-Golgi side facilitating sorting and targeting through the trans-Golgi network (TGN). The TGN plays a dual role as a sorting station and an endosome in plants. Our research has shown that lipid composition at the TGN is critical for sorting proteins like the auxin efflux carrier PIN2. Specifically, sphingolipid biosynthesis, culminating in the glycosylation of ceramides, leads to an enrichment of glycosylated sphingolipids. This enrichment triggers the consumption of phosphatidylinositol-4-phosphate (PI4P), a process that appears to be homeostatically regulated across membrane leaflets.

Beyond the TGN, sphingolipid-dependent mechanisms also operate at the plasma membrane, where they regulate auxin signaling by organizing Rho-GTPase nanodomains. Additionally, we identified at the pre-Golgi interface, a tubulo-vesicular network functioning as an ER-Golgi Intermediate Compartment (ERGIC). This lipid-dependent structure connects the ER to the Golgi and is stabilized by pre-existing Golgi cisternae, enabling its maturation into new Golgi compartments. Ceramides synthesized in the ER are essential for this process.

Overall, these findings highlight how lipid synthesis and homeostasis contribute to the transformation of biological membranes into functional entities. This dynamic process is closely coordinated with protein sorting and trafficking, ensuring efficient cellular organization and signaling.

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Keywords: Membrane Trafficking, Lipid Homeostasis

In hospite response of Photosynthetic Thylakoid Membranes in Symbiodinium to simulated ocean warming using small angle neutron scattering

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Photosynthesis is an important energy input into reef forming corals and this ecological system by virtue of the symbiotic relationship between the single cell dinoflagellates from the genus *Symbiodinium* and the coral polyp. Environmental stress, such as rising seawater temperatures, lead to a phenomena termed "coral bleaching" where the symbiote leaves the polyp terminating the mutually beneficial relationship. Although the ecological implications for the coral reef are as yet not clear, understanding the mechanism of *Symbiodinium* expulsion is important step in developing this knowledge, and if necessary, developing remediation steps. Slavov *et al.*,¹ have proposed, on the basis of electron microscopy and photophysical methods, that a structural rearrangement of the photosynthetic structures, a so-called "super-quenching state", which protects the photosynthetic apparatus from the oxidative stress associated with thermal stress, is responsible the expulsion of *Symbiodinium* from the symbiosis. Previously we have demonstrated the power of the small angle neutron scattering (SANS) technique, where the intensity of scattered neutrons is measured at small angles and compared to intensities calculated from a structural model, in probing the organisation of photosynthetic thylakoid membranes *in hospite*². In the results present here we probe the average 3D organisation of *Symbiodinium* photosynthetic membranes in two symbiotic organisms (staghorn coral and the glass anemone) during thermal stress by the modelling of SANS measurements. We discuss the limitations of the approach of Slavov *et al.*,¹ compared to the SANS approach and the use of SANS measurements in elucidating important structural aspects of photosynthetic machinery during environmental stress.

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Keywords: small angle neutron scattering, thylakoids, coral, symbiodinium

Breast cancer extracellular vesicles : structural profiling and membrane interaction studies using X-ray and neutron scattering techniques

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Extracellular vesicles (EVs) are key vectors in the cell-to-cell communication and play an important role in the metastatic progression of cancer. While the scientific interest for EVs is continuously growing, how EVs interact with and are internalized by the recipient cell remain poorly understood. Recently, the role of the protein corona and the physiological environment of EVs gained in attention but dissecting their contribution remain challenging experimentally. In this framework, we are investigating the structural characteristics and interaction properties of breast cancer EVs obtained from different isolation procedures. Combining scattering of neutrons and x-ray, atomic force microscopy and infrared spectroscopy, we performed an in-depth structural and compositional characterization of EVs. Our results revealed a strong variability in composition of EVs depending on the isolation procedures, mainly related to changes in the soluble protein content.

Then, we investigated EVs' effect on lipid membrane models, analyzing structural changes through scattering and reflectometry of neutrons and x-ray, and impact on lipid dynamic using neutron spin echo and differential scanning calorimetry. While EVs hardly impacted the structure of membrane models, a significant impact on lipid dynamics was evidenced, with an influence of the protein environment.

Overall, our study provides additional facets in the characterization EVs and their effect on lipid membranes, offering a foundation for broader applications in EVs research across different biological contexts.

*Speaker

Keywords: extracellular vesicles, SAXS, neutron spin echo

Fluorescent probes for biomembranes and organelles: from lipids to proteins

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Fluorescent probes are smart chemical tools that enable visualization of biological structures, tracking dynamic processes and detection of (bio)molecules. Lipid membranes are important targets for fluorescent probes because they constitute a natural frontier of the cells in form of plasma membranes and they delimit major intracellular organelles.¹ Previously, we developed a large pallet of plasma membrane probes,¹ but they systematically exploited non-covalent labelling strategy. Therefore, we recently developed lipid-driven covalent labelling of membrane proteins, which enabled permanent labelling of cell surface and long-term multicolor tracking of cells.² On the other hand, in order to achieve super-resolution imaging of plasma membranes, we developed fluorogenic dyes that couple reversible target binding (exchangeable probes) with ON/OFF switching.³⁻⁴ Here, solvatochromic dyes that change their emission color in response to their local environment⁵ enable monitoring lipid organization of biomembranes.⁴ To study lipid organization in membranes of organelles, we developed a series of solvatochromic probes with organelle-targeting ligands that revealed specific signatures of their membrane polarity and their response to oxidative and mechanical stress.⁶ By genetic targeting of a solvatochromic dye to proteins, their nanoscale environment in different organelles was monitored.⁷

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Keywords: fluorescent probes, plasma membranes, lipid organization, organelles, protein, lipid interactions

2-2 Apr 2025

Membrane-Targeting Drugs: Unlocking New Therapeutic Frontiers

Invited Talk: Lipoprotein structure and its role on dysfunction: from atherosclerosis to covid-19

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Fat metabolism involves the production of lipid carrying particles known as lipoproteins. Lipoproteins are nanoemulsion-like particles composed of fats and proteins. There are several types of lipoproteins with distinctive composition not only in terms of the ratio between proteins and fats, but also due to the specific protein type present in them. Two well-known lipoproteins are high density lipoprotein (HDL) and low density lipoproteins (LDL) which are known as the good and bad cholesterol. Misbalance in the net lipoprotein composition (focusing on HDL and LDL) mark an increased risk for developing atherosclerosis as well as several other diseases such as the severity of symptoms in COVID19. Despite an enormous body of work, there remains to be fully understood what in lipoproteins causes dysfunction. We aim to unravel the role of lipoprotein composition on its structure, and how this is related to functionality.

In my group, we use small angle neutron and X-ray scattering to characterise the nanoparticle structure while mapping their function for instance via traditional biochemical and cell biology methods as well as via mapping the lipid exchange between lipoproteins and model membranes using biophysical methods such as ATR-FTIR and neutron reflection. We have done this in the presence and absence of the SARS CoV-2 spike (S) protein.

So far we have updated the structural models for the main lipoproteins LDL (published in 2017) and HDL (unpublished). We have applied these updated models to analyse a range of samples isolated from individuals classified as low vs high risk to develop cardiovascular disease. We have found that, structurally, the greatest differences in lipoprotein fractions across these groups occurs in specific subfractions, suggesting a critical role for these in dysfunction. Functional analysis suggest that lipid cargo is key for the function of lipoproteins.

In summary, by the combination of structural, functional and compositional data we present an approach that should enable discerning key molecular properties differing across cohorts, and in due time could provide means to better classify risk to develop these diseases as well as to drive the development of targeted treatments.

Keywords: lipoproteins, atherosclerosis, covid, 19

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Distribution of two synergistic antimicrobial peptides on the lipid membrane surface and sidechain dynamics: a fluorescence study

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Antimicrobial peptides represent an effective and promising alternative to conventional antibiotics as they attack bacterial cells via different pathways which are less prone for resistance development. A big class of antibiotic peptides are characterized by an in-planar topology of amphipatic helices in their membrane bound form. Aspects of the way of action of this class is best described by the carpet (1) and the SMART model (2). For both models the distribution of peptides on the membrane surface plays an essential role. Here we focus on two members of this family, PGLa and magainin 2, which interact with biological membranes in a synergistic manner

Fluorescence quenching is used to determine the peptides' packing on membrane surfaces. PGLa and Mahainin 2 are labelled at different positions by introducing the artificial amino acid diaminopropionic acid (Dap) with NBD (nitrobenzoxadiazole) attached to the sidechain amide. The labeling scheme allows to refine the supramolecular structures of PGLa on lipid membrane interface previously detected by N-terminal labeled PGLa (3).

Time resolved fluorescence anisotropy measurements allows the characterization of the sidechain dynamics in the ns time range. The orientation of the absorption transition dipole of NBD in combination with the short sidechain of diaminopropionic acid as a linker brings the dynamics into the sensitive range. The side chain dynamics can be an indicator of the local environment. In addition, the dynamics on the ns timescale might be easily correlated with molecular dynamics simulations.

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Keywords: antimicrobial peptide, fluorescence, self, quenching

Membrane partition and structural reorganization induced by antipsychotics with distinct clinical profiles

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Antipsychotics (APs) are used in the treatment of severe mental disorders, such as schizophrenia. Their mechanism of action involves interaction with multiple brain targets, such as the dopamine D2 receptor (D2R). Due to their lipophilic nature, APs also partition and accumulate in lipid membranes, particularly around the D2R and in synaptic vesicles. When intercalated into brain membranes, APs slowly accumulate and act as a reservoir, allowing their rapid release on demand to modulate neurotransmitter signalling. They also modify the physicochemical and mechanical properties of the lipid bilayer, which can subsequently affect the conformational changes of embedded membrane proteins like the D2R. We have investigated the effect of two major APs with different pharmacological and clinical profiles on membranes: chlorpromazine and clozapine, both commonly used in treatment. Surprisingly, although D2R antagonism is usually associated with AP potency, clozapine (with the weakest D2R potency) has repeatedly demonstrated clinical superior efficacy to other APs and is therefore recommended for treatment-resistant schizophrenia. This has been primarily attributed to its binding to other receptors; however, the effect of this AP on membranes has not yet been thoroughly investigated. Therefore, we focused on elucidation of the two APs' effect on membrane remodelling properties, which could impact their distinct profiles, by thoroughly comparing their partitioning and impact on the physicochemical properties of the lipid membrane by using a combination of several biophysical methods and look on their impact on phase transition, thickness, elasticity, phase separation, membrane integrity and charge.

Keywords: Antipsychotics, lipids, membrane partition, membrane remodeling

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Exploring Membrane Interactions: A Comparative Study of Daptomycin and a Novel Cyclic Lipopeptide

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The rise of antimicrobial resistance and the declining efficacy of many antibiotics highlight the urgent need for new antimicrobial agents. Cyclic lipopeptides (CLiPs) are a promising class of antibiotics, produced as secondary metabolites by bacteria such as *Streptomyces*, *Bacillus* and *Pseudomonas*. A clinically relevant example is Daptomycin, currently the only CLiP on the market, which is used as a last-resort antibiotic against gram-positive infections. Here we present Olikomycin A, a novel CLiP from *Streptomyces*, and provide initial insights into the mode of action compared to Daptomycin. We use isothermal titration calorimetry (ITC) to characterize their binding to model membranes, such as liposomes and electroneutral DIBMA-nanodiscs. The membrane binding of both CLiPs is a complex process depending on interactions between the antibiotic, Ca²⁺, phosphatidylglycerol (PG)-containing lipid membranes and various intermediates. Daptomycin’s calcium-dependent changes in stoichiometry are particularly noteworthy. Due to this complex binding behavior, a simple fitting model is insufficient to describe this process. To rationalize and quantify the binding behavior, we are establishing a biophysical model that enables a direct comparison of Daptomycin with the new CLiP.

Keywords: Isothermal titration calorimetry (ITC), cyclic lipopeptide (CLiP), liposomes, nanodiscs

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Lipids as Battlegrounds: Breaking the Code of Host-Pathogen Communication

Invited Talk: Cracking Nature’s Recipes to Design Lipid-Targeting Antibiotics

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Antimicrobial resistance is a global health threat, calling for new antibiotics. Good candidates could be peptide-based compounds that target special lipids that only exist in bacterial, but not in human cell membranes. These drugs kill pathogens without detectable resistance, which has generated considerable interest.

Here, using an integrative structural biology approach, we show that drugs that target special lipids in bacterial membranes use sophisticated supramolecular killing mechanisms (1-3). 1. Shukla, R. et al. Teixobactin kills bacteria by a two-pronged attack on the cell envelope. *Nature* 608, 390-396, doi:10.1038/s41586-022-05019-y (2022).

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Keywords: antimicrobial resistance, structural biology, peptides

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Interplay between membrane fluidity and amyloid fibrillation in PSM α 3-induced host membrane disruption, towards *Staphylococcus aureus* virulence

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Phenol soluble modulins α 3 (PSM α 3) are amphipathic peptides that self-assemble into amyloid-like cross- α fibrils and play a key role in *S. aureus* virulence. They notably contribute to host cell lysis *via* membrane disruption, and in a receptor-independent manner. Interestingly, erythrocytes and neutrophils - rich in phosphatidylcholine (PC) and cholesterol - are highly susceptible to PSM α 3-induced lysis, suggesting that membrane fluidity and phase separation influence PSM α 3 activity. However, these contributions remain unclear. Supporting this hypothesis, we previously demonstrated that both electrostatic and hydrophobic interactions drive lipid binding and insertion into membrane fluid phases, concomitant with PSM α 3 interfacial fibrillation *in vitro*.

Here, using infrared spectroscopy on membranes of controlled lipid composition, we show that PSM α 3 induces greater membrane disruption when (i) membrane packing/rigidity is increased, (ii) cholesterol is present or (iii) upon phase separation into microdomains. This highlights the role of hydrophobic core accessibility and membrane rigidity in promoting PSM α 3 deleterious activity. Surprisingly, modifying the peptide N-terminal capping, from a N-formyl-methionine (f-) to N-an acetylated-methionine (ac-), inhibits membrane disruption, except for membranes with high-cholesterol content and/or phase separations. This suggests that, beyond the net charge of PSM α 3 driving its binding to specific lipids, subtle changes in its hydrophobic properties can modulate its ability to permeabilize membranes, with increased hydrophobicity potentially stabilizing membrane defects and reducing depletion. Furthermore, Atomic Force Microscopy reveals fibrillation of both f- and ac-PSM α 3 at membrane interfaces, yet on different timescales depending on the lipid composition, with eventual thinning effects preceding fibril elongation. Despite morphological similarities, fibrils may differ in their secondary structure, particularly when grown at the interface of phase separated membranes. This further emphasizes the role of lipid composition and fluidity in PSM α 3 structuration and fibrillation, and its subsequent impact on cell membranes *via* carpet-like disruption. These *in vitro* mechanisms might underlie the distinct time- and concentration-dependent cytotoxicity observed in HEK cells for f- and ac-PSM α 3.

In conclusion, our approach provides new insights into how membrane properties regulate PSM α 3 fibrillation, structuration, and peptide-lipid interactions. These findings likely contribute to PSM α 3 cytotoxicity and, ultimately, *S. aureus* pathogenesis.

*Speaker

Keywords: amyloid, membrane, host, pathogen interaction, AFM, FTIR

Membrane translocation process of the *Bordetella pertussis* adenylate cyclase CyaA toxin

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The adenylate cyclase CyaA toxin (a 1706-residue RTX protein), is secreted by *Bordetella pertussis*, the causative agent of whooping cough. CyaA plays an essential role in the early stages of colonization of the human respiratory tract. However, the process of cell intoxication by CyaA is still poorly understood. Following secretion by a type I secretion system, CyaA intoxicates human cells by translocating its catalytic domain (ACD) directly across the plasma membrane. Once in the cytosol, ACD binds to calmodulin (CaM) and catalyzes high levels of cAMP, leading to cell death. Using a combination of approaches, we characterize the membrane translocation process of CyaA. Our results illustrate how the structural flexibility of CyaA is crucial for its secretion, folding, translocation across the plasma membrane and intoxication of the target cell. All of these steps involve disorder- to- order conformational transitions that are finely tuned to the environmental conditions to which CyaA is exposed on its journey from the bacterium to the cytoplasm of the eukaryotic cell. These results open up new opportunities for both basic research and biotechnological applications, where recombinant CyaA proteins are used as antigen delivery vehicles and as a potential protective antigen in the next generation of pertussis vaccines.

Keywords: membrane translocation, protein membrane interaction, protein protein interaction, toxin, bacterial toxin, virulence factor

*Speaker

Mode of action of peptides inhibiting lipoprotein modification enzyme Lgt

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Bacterial lipoproteins present in the membrane are crucial to maintain the integrity of the cell envelope and fulfil different functions impacting bacteria physiology and virulence. The first stage of the lipoprotein modification pathway is mediated by the integral membrane enzyme Prolipoprotein Phosphatidylglycerol Diacylglyceryltransferase (Lgt), essential for cell viability and highly conserved across bacterial species. Lgt recognizes a specific sequence within the signal peptide of a pre-prolipoprotein and catalyses the transfer of a diacylglyceryl (DAG) moiety from phosphatidylglycerol to the sulfhydryl group of the invariant cysteine at position +1 of this signal peptide. This study aims to understand and characterize the mechanism of inhibition of Lgt by synthetic peptides and explore Lgt potential as a novel target for antibiotic development. Two types of peptides were evaluated; peptides containing non-natural amino acids (NNAA) 1 and peptides with a modified signal peptide where Cys+1 is substituted by Ser that may act as a putative competitive inhibitor (LppC21S). The properties of the peptides in solution were characterized by dynamic light scattering (DLS) and 1D-NMR. Detergent purified *E. coli* Lgt was used to determine the enzymatic activity through a gel-shift assay, monitoring the DAG modification of synthetic biotinylated peptide substrates. All NNAA containing peptides were shown to inhibit DAG transfer, however the putative competitive inhibitor did not inhibit Lgt activity *in vitro*. Inhibition of *in cellulo* growth of *E. coli* was also observed with the NNAA containing peptides, however, likely following different kinetics. Cell morphology was not affected, and only partial lysis could be detected. Furthermore, overproduction of Lgt did not increase the Minimal Inhibitory Concentration (MIC) of both cyclic and linear peptides, suggesting that the inhibitory effect of peptides might be Lgt independent. Currently, we aim to investigate the mode of action of the inhibiting peptides, by characterizing the interaction with Lgt, and to explore the impact of the peptides on the membrane, using a combination of approaches, including a membrane permeabilization (ANTS:DPX) assay, NMR-based affinity determination

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(B2LiVe), stoichiometry of membrane-bound proteins (dynamic mass photometry) and structural modelling.

Keywords: lipoprotein, Lgt, synthetic peptides, inhibitors

In Silico Frontiers: Exploring the Complexity of Cell Membranes through Simulation and Modeling

Invited Talk: Bacterial membranes - does the molecular complexity hold the key? A computational microbiology perspective

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The outer membrane of Gram-negative bacteria faces the external environment and thus forms the first layer of defence against incoming macromolecules such as antibiotics and therefore plays a key role in antimicrobial resistance. It is composed of lipopolysaccharide (LPS) molecules in the outer leaflet and phospholipids in the inner leaflet. Outer membrane proteins (OMPs) are integral to the outer membrane- these proteins are usually based on a beta barrel architecture. They have a multitude of functions including enzymatic activity, signalling, adhesion and providing active and passive transport pathways for a range of small molecules. Previously unknown details of the molecular-level organisation of OMPs and lipids in the outer membrane of *Escherichia coli* are now coming to light. We use multiscale molecular dynamics (MD) simulations (complemented by a range of experimental methods from our collaborators) to predict the large-scale organization of LPS, phospholipids and OMPs in the outer membrane of *E. coli*. In particular, we focus on the role of LPS in maintaining the organisation of the membrane. The details we uncover are almost impossible to gain from any other (single) experimental method. We also show the action of antimicrobial peptides on the outer membrane and provide a mechanism for the insertion of polymyxins in the outer membrane using the most comprehensive molecular model of that membrane reported to date.

Keywords: Antimicrobial resistance, Molecular dynamics simulations

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Molecular Dynamics Insights into Annexin-Mediated Plasma Membrane Repair

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Cellular membranes are essential for maintaining cell integrity but are constantly subjected to stress and damage, requiring efficient repair mechanisms. Annexins, calcium-dependent membrane-binding proteins, play a central role in plasma membrane repair. Despite their importance, the molecular mechanisms underlying their function remain poorly understood. We present computationally driven investigations using molecular dynamics simulations to explore how annexins interact with lipid membranes, shedding light on the hidden biophysics of membrane repair.

Our research focuses on the role of annexin trimers, such as ANXA4 and ANXA5, in inducing negative membrane curvature, which is a crucial step in plasma membrane resealing. By simulating annexin-membrane interactions at the atomic level, we uncover how cholesterol and anionic lipid headgroups influence annexin-induced curvature, affecting the localization of annexins at membrane rupture sites. Additionally, we engineer ANXA3 to form trimers similar to ANXA4 and ANXA5, offering deeper insights into the structural requirements for effective membrane repair.

Integrating molecular dynamics simulations with experimental validation, our studies provide high-resolution frameworks to understand how curvature-driven protein-lipid interactions regulate membrane healing. We also explore how pharmacological modulation of these interactions can disrupt membrane repair, presenting potential therapeutic strategies, including the inhibition of annexin-mediated membrane repair in diseases such as cancer, where compromising membrane integrity promotes cell death.

Our findings enhance the understanding of the dynamic and intricate processes involved in membrane repair, shedding light on how cells maintain structural integrity under stress. Our studies offer critical insights into the molecular mechanisms of annexin-mediated membrane repair and pave the way for novel therapeutic strategies targeting membrane repair pathways.

Keywords: Annexins, Plasma membrane repair, Molecular dynamics simulations, Membrane curvature, Computational biophysics, Membrane integrity

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Improvements of Passive Permeation Simulations: Accounting for Cell Membrane Complexity

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Passive permeation of xenobiotics, such as drugs, across biological membranes is a fundamental process that influences their disposition within the body. Rationalizing the underlying molecular mechanisms allows to optimize drug design and delivery. Molecular dynamics simulations provide a powerful computational approach to predict permeation coefficients by characterizing the interactions of xenobiotics with the lipid bilayer environment, thereby offering insights into their dynamic behavior.

Our in-house model, MemCross, offers a framework to investigate passive permeation at the atomic resolution. We have been focusing on single-molecule passive permeation using a simple POPC lipid bilayer. To broaden its scope, we aim to extend our model towards new permeation mechanisms that more accurately reflect the complexity of physiological conditions.

Firstly, we explore the interactions between xenobiotics and the surface of transmembrane protein domains. Such interactions could introduce preferential pathways that alter the energy landscape of transmembrane crossing. We also assess the influence of protein sequence on the protein-xenobiotic interactions.

Secondly, we examine the impact of lipid composition on passive permeation. Biological membranes consist of a variety of lipid species, differing across numerous tissues and organs. By simulating xenobiotic diffusion across various lipid mixtures membrane models, we can determine how different lipid environments modulate permeation barriers and drug partitioning.

Lastly, we investigate potential synergistic interactions between xenobiotics that may enhance translocation across the membrane. By analyzing such cooperative effects, we seek to identify conditions that increase the permeation of poorly permeable compounds, potentially informing novel drug delivery strategies.

Through these advancements, we try to provide a more comprehensive understanding of passive permeation mechanisms, to improve correlation with more complex experimental models. These insights would help to guide the rational design of pharmaceuticals with improved membrane permeability, ultimately contributing to more effective therapeutic interventions.

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Keywords: Molecular dynamics, Permeation, Xenobiotics, Lipid composition, Transmembrane peptide

Impact of Cell-Penetrating Peptides on Blood-Brain-Barrier Cellular Membranes: A Coarse-Grained Simulation Study

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The blood-brain barrier (BBB) is a semi-permeable multicellular structure that regulates substance exchange between the bloodstream and the central nervous system (CNS), maintaining homeostasis but restricting the transport of many therapeutic molecules into the brain. Cell-penetrating peptides (CPPs) offer a non-invasive approach to overcoming this challenge, as they can interact with biological membranes without causing significant damage. However, how they interact with the BBB remains underexplored, partly because membrane crossing occurs on timescales beyond the reach of all-atom molecular dynamics (MD) simulations.

This study investigates the translocation mechanism of three CPPs-R9, (KH), and MPG, a peptide combining a membrane-fusing segment from HIV-1 and a nucleus-targeting sequence from a SV40 virus protein-and how they affect membrane characteristics using coarse-grained MD simulations. These peptides, representing distinct CPP classes, were simulated with a model lipid bilayer mimicking brain microvascular endothelial cell composition. To observe their natural behavior and membranotropic effects, each peptide was simulated for 500 ns. Despite the longer timescales allowed by coarse graining, spontaneous membrane insertion did not occur, but all peptides altered membrane properties, reducing thickness and lateral diffusion.

To further explore peptide-membrane interactions, enhanced sampling simulations were employed to accelerate translocation across the bilayer (akin to the molecular pulling action of an AFM cantilever) and obtain free energy profiles. Multiple simulations captured different translocation stages, providing a detailed view of the process. MPG’s free energy barrier appeared farther from the membrane center than R9 and (KH)9, requiring an initial reorientation before stabilizing in-plane. R9 had the highest free energy and thinned the membrane without structural transitions. (KH)9 required reorientation and induced lipid disorder, especially in SAPI lipids. Radial distribution analysis indicated strong (KH)9-SAPI interactions. These findings emphasize the roles of peptide reorientation and lipid interactions in translocation.

Keywords: Cell Penetrating Peptides, Blood Brain Barrier, Coarse Grained

*Speaker

3-3 Apr 2025

Membrane Mosaics: Deciphering the Language of Protein-Lipid Communication

Invited Talk: Actin dynamics sustains spatial gradients of membrane tension in adherent cells

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Tension propagates in lipid bilayers over hundreds of microns within milliseconds, precluding the formation of tension gradients. Nevertheless, plasma membrane tension gradients have been evidenced in migrating cells and along axons. Here, using a fluorescent membrane tension probe, we show that membrane tension gradients exist in all adherent cells, whether they migrate or not. Non-adhering cells do not display tension gradients. We further show that branched actin increases tension, while membrane-to-cortex attachments facilitate its propagation. Tension is the lowest at the edge of adhesion sites and highest at protrusions, setting the boundaries of the tension gradients. By providing a quantitative and mechanistic basis behind the organization of membrane tension gradients, our work explains how they are actively sustained in adherent cells.

Keywords: membrane tension, cell mechanics, actin dynamics, actomyosin, cytoskeleton, cell migration, biophysics

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Capturing of a new transient conformation of an ABC multidrug efflux pump

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Antibiotic resistance is a major threat in antibacterial therapies and it can be caused by the overexpression of dedicated efflux pumps such as those from the ABC (ATP-Binding Cassette) transporter superfamily. BmrA ("Bacillus multidrug resistance ATP") is an ABC multidrug efflux pump which confers antibiotic resistance in *Bacillus subtilis*. It is also highly homologous to ABCB1, a human transporter involved in MDR phenotype in cancer cells. BmrA constitutes therefore an archetypical transporter particularly useful to understand the mechanism of multidrug ABC transporters. These pumps work according to an alternative access mechanism with an apo state, inward-facing (IF), conformation prone to drug binding and an ATP-bound, outward-facing (OF), conformation that permits drug release. Structural snapshots of these pumps support a 'rigid body' transition from IF to OF states with a stable contact maintained throughout the catalytic cycle between the so-called intracellular coupling domains, ICD1 and ICD2, that protrude from the TransMembrane Domains (TMD), and the Nucleotide-Binding Domains (NBD). We introduced two cysteine residues to form a disulfide bond between ICD1 and a subdomain of the NBD in the apo state (Dalmas et al., JBC, 2005). We subsequently solved by cryo-EM the apo structure of the reduced form of this mutant (~3.2 Å), which is similar to the known structures of related ABC transporters. By contrast, the oxidized form reveals a unique global conformation of the transporter, more compact and dubbed IF*, with a reorientation of the NBD. Biochemical data reveal that this unusual IF* conformation is transient but is stabilized by the formation of the disulfide bond upon displacement of ICD2 from its classical positioning at the NBD/TMD interface. Because this conformation creates a larger cavity at the interface between the two moieties of the transporter, we postulate that this unprecedented conformation might be involved in the binding of large drugs by BmrA.

*Speaker

Keywords: ABC transporter, multidrug transporter, multidrug resistance

Hydrophilic/Lipophilic Balance: A Lighthouse Parameter for Designing Efficient Detergents in Membrane Protein Applications?

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Since the 1970s, the hydrophilic/lipophilic balance (HLB) has been widely considered a key parameter for evaluating the effectiveness of detergents across various fields, including membrane protein applications. Detergents play a crucial role in the characterization of membrane proteins by disrupting the membrane bilayer, creating a stable environment to maintain solubility and native conformation, and facilitating crystallization.¹

Comparing the HLB values of well-known, widely used detergents with those of newly synthesized molecules provides valuable insights into their efficiency in membrane protein studies and drives the design of future detergents.²

Here, we provide an overview of how chemists and biochemists can leverage the HLB parameter to optimize detergent structures. By exploring various detergent series-including hydrogenated and fluorinated, as well as cyclic and linear molecules-we demonstrate that a broad range of HLB values can yield to effective detergents. For instance, solubilization and stabilization properties were fine-tuned by modifying the linker length in fluorinated cyclic maltoside detergents³ or by adjusting fluorination in lactobionamide detergents.⁴

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Keywords: Detergents, hydrophilic/lipophilic balance (HLB), membrane proteins, solubilization, stabilization

Deciphering membrane protein structures through scattering and modeling: insights into TSPO, a neuroimaging key marker

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Membrane proteins play essential roles in cellular function and are prime therapeutic targets, yet their structural characterization remains a major challenge. Despite advances in artificial intelligence, including AlphaFold, accurately predicting their structures is still difficult. These proteins make up 30% of the proteome and 60% of drug targets, yet they are vastly underrepresented in the PDB, with only 3% of resolved structures. This is largely due to the challenge of maintaining their native state in amphiphilic environments, limiting the applicability of classical structural techniques such as X-ray crystallography, NMR, and cryo-EM.

To overcome these limitations, we combine small-angle X-ray and neutron scattering (SAXS/SANS) with *ab initio* modeling, offering a powerful approach to study membrane proteins under near-physiological conditions. We apply this methodology to TSPO (Translocator Protein), a highly conserved transmembrane protein with strong pharmacological relevance, particularly in neuroimaging.

Our study explores the solution structures of mouse TSPO (mTSPO) in various amphiphilic environments to unravel its structure/function relationships: (i) SDS, the detergent used for its solubilization from *E. coli* inclusion bodies, where mTSPO is stable and monodisperse but nonfunctional ; (ii) DPC, the detergent used to determine its NMR structure, where a stabilizing ligand enhances rigidity and induces μ M affinity ; (iii) membrane-mimetic environments (DPC/DMPC bicelles), where optimized refolding restores nM affinity, approaching its native

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functional state.

By deciphering the structural behavior of mTSPO across these conditions, our work provides valuable insights into its function, paving the way for the development of new pharmacological molecules for diagnostics and therapeutics.

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Keywords: small angle scattering, ab initio modeling, transporter, biomimetic systems

Pushing the Limits of Biophysical Analysis: New Tools for Understanding Membrane Function

Invited Talk: Origins and consequences of intrinsic-area asymmetry stress in lipid membranes

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Biological organisms spend considerable energetic and genetic effort to establish, maintain and regulate different types of asymmetry between the inner and outer leaflet of their lipid membranes. Fundamental studies of asymmetric model membranes are needed to understand the multiple functions of this asymmetry, which likely include the regulation of membrane remodeling processes and transmembrane signaling. The asymmetric insertion of amphiphilic membrane-impermeant drugs and biomolecules into the cis leaflet may induce an intrinsic-area mismatch between the leaflets that can initiate vesicle budding and/or transient membrane failure. This is demonstrated for example for lysolipids, antimicrobial cyclic lipopeptides and a saponin. Cholesterol can redistribute between the inner leaflets but this does not inhibit budding, at least as induced by lysolipid insertion. A flip of cholesterol to the trans side may reduce intrinsic-area asymmetry but at the same time, it creates an intrinsic-curvature asymmetry, where both lysolipid-induced positive intrinsic curvature of the outer and cholesterol-induced negative intrinsic curvature of the inner leaflet add up to bend the bilayer. Finally, bilayers with asymmetric distribution of lipid species may expand asymmetrically as temperature or other conditions change. Analogously to a bimetallic couple, this causes reversible membrane bending. Alternatively, the coupling of the leaflet areas can induce a coupling of the transitions between the leaflets. We have been trying to find some orientation and system in these, at first glance potentially confusing, asymmetric expansion effects.

Keywords: membrane asymmetry, lipid bilayer remodeling, membrane curvature

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Invisible nanodiscs: A tool to study structural dynamics of membrane proteins

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Small angle scattering is a low-resolution structural biology technique enabling to follow large conformational rearrangements of macromolecules upon change of their environment. Neutron scattering (SANS), in particular, has the ability to distinguish between different macromolecules according to their scattering length density (SLD), which is tunable by deuteration.

In order to study membrane protein in solution, we propose specifically deuterated nanodiscs which become invisible to neutron scattering in D2O buffer, enabling to visualize the protein of interest in its near-physiological environment. This tool can be used to follow conformational changes of a membrane protein upon addition of a ligand, or change in its physical environment (light, temperature...).

In order to optimize nanodisc size and stability, we produce the partially deuterated version of several belt proteins:

- MSP1D1 (his-tag cleaved) to make 9 nm diameter discs
- csE3 (his-tag cleaved) to make 12 nm diameter discs, stabilized by covalent circularization
- spNW15 (his-tagged) to make 15 nm diameter discs stabilized by covalent circularization
- spNW30 (his-tagged) to make 30 nm diameter discs stabilized by covalent circularization

In term of lipidic environment, one can choose

- d67-DMPC (Avanti polar lipids)
- Full polar lipid mixture of *E. coli*, *B. subtilis* or *P. pastoris*
- Other perdeuterated lipids which can be produced by DEUNet members.

Here, we will present the basis of SANS as well as the sample requirements. As an example, we will show a nanodiscs characterization, and a time resolved study of nanodisc assembly.

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Keywords: Small angle neutron scattering, nanodiscs, membrane protein, protein, lipid interaction

The isomerization of photo-lipids in free-standing membranes forms protein clusters in ordered domains

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Infections with Ab5 toxins producing bacteria are responsible of hundreds of thousands of deaths each year as they are responsible of various diseases (1) for which no treatment is available although vaccines are under development (2). Therefore, investigating the interactions between glycolipids receptors, bacterial toxins and membranes is of great interest to better comprehend the early processes leading to toxins internalization to help solve this issue. We recently reported the synthesis of light-sensitive globotriaosylceramide glycolipids (photo-Gb3), which constitute natural receptors for bacterial Shiga Toxins (STxB) (3). *In vitro* studies performed with model membranes have shown that photo-Gb3 can greatly alter domain organization.

Here, we have investigated the influence of the azobenzene group of photo-Gb3 on lipid and lipid-protein interactions. By means of surface tension, we evidenced that the speed of isomerization of photo-Gb3s and the miscibility for lo phase lipids increase when the azobenzene is deeply embedded in the membrane, suggesting a preferential partitioning of photo-Gb3 in lo domains. To negate the impact of the substrate on light-induced domain rearrangement, we studied photo-Gb3 isomerization using pore-spanning membranes, which possess a supported part (s-PSMs) and a free-standing part (f-PSMs). Interestingly, fluorescence microscopy evidenced the size and dynamics of domains was greatly reduced on f-PSMs. Upon light-irradiation, we observed a modification of domain morphology and the formation of permanent protein clusters on the surface of the membrane, which were suggested to be a pre-requisite for the internalization of bacterial toxins into cells (4). This system may help to further understand domain dynamics as it allows a non-invasive and reversible control of membrane lateral organization.

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Keywords: membrane, photo, lipids, domains, glycolipids, Gb3 receptor, Shiga Toxin

Discovering the Membrane Core: New Frontiers in NMR of membranes

Invited Talk: Structures & Mechanisms of Virus Ion Channels from Solid-State NMR

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Many viruses encode ion channels that cause pathogenicity to cells and that are required for the virus lifecycle. One of the essential proteins of coronaviruses is the highly conserved envelope (E) protein, whose ion channel activity is a virulence factor and a target of antiviral drugs. The small size and high hydrophobicity of E proteins have made them resistant to structure determination by X-ray crystallography and electron microscopy. In comparison, solid-state NMR spectroscopy is well suited for investigating the structure, dynamics and drug binding of these E proteins in phospholipid bilayers. We have determined the atomic-resolution structures of the transmembrane domain of the E proteins of both SARS and MERS coronaviruses using solid-state NMR. Combining ¹³C, ¹⁵N labeled proteins with sparsely fluorinated proteins, we measured conformation-dependent chemical shifts, inter-atomic distances, helix orientations and oligomeric states of these proteins. These high resolution structures provide detailed insights into how the SARS E channel is activated, how protons and Ca ²⁺ are recruited by a polar network, how small molecules bind SARS E to inhibit its channel activity, and how the MERS E channel structure and ion conduction mechanism differ from SARS E.

Keywords: Solid, state NMR, viruses, structural biology

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Membrane Interaction of small molecule peptidomimetics of antimicrobial peptide: does the size matter?

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Antimicrobial resistance has become a growing global health concern. In response, there has been an increased emphasis on developing antimicrobial peptides (AMPs) and their various mimics. Understanding their mechanisms of action against pathogens is crucial for the ongoing development of new active agents. In this discussion, we focus on a specific category of AMP mimics: cationic amphipathic small molecules. We investigate the biophysical behaviors exhibited by these mimics on membranes using biophysical tools. To tackle the challenge of understanding how these membrane-active compounds function, the small molecule mimics were introduced to liposomes that mimic bacterial membranes. Using deuterium and phosphorous solid-state nuclear magnetic resonance spectroscopy, the effect of the compounds on the lipid membrane were evaluated. Furthermore, several fluorescence studies were conducted using both bacterial and mammalian model membranes. These studies included dye-release assays, which provided insights into the pore-forming abilities of membrane-active compounds. Interestingly, bacterial and mammalian not only differ in their surface charge, but also in their intrinsic curvature. Here the size of molecules becomes important. Further, quenching studies were done to determine the localization of these agents within the lipid bilayer and binding studies to assess the compounds' affinity for bacterial model membranes.

Keywords: AMP, Peptidomimetics, Solid state NMR, Fluorescence, Lipid membrane, Mechanism of action

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Solid-state NMR structural investigations of a tandem dimer peptide apolipoprotein A1 mimic

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High density lipoproteins (HDL) are complex nanostructures that play important roles in cell function, lipid metabolism, and the progression of various diseases. The main components of lipoproteins are phospholipids and apolipoproteins, particularly apolipoprotein A1 (apoA-I). HDL has a key role in the transport of fats within the human body. Over time, numerous apo-AI mimetic compounds have been presented. Among these, the most extensive group of peptide mimetics comprises sequences that are 18 amino acids in length. These sequences differ in the number of phenylalanine residues, ranging from two (2F) to seven (7F) on the non-polar face of amphipathic helices, thus exhibiting varying degrees of hydrophobicity. Among these, 4F (DWLKAFYDKVAEKLKEAF) has garnered the most attention, likely due to its good solubility in water. Moreover, a dimer composed of 4F connected by a proline linker (4F-proline-4F), resulting in a 37-mer sequence, is even more biologically effective than the monomeric peptide¹. In this study, we investigate the 4F-P-4F topology using oriented solid-state NMR spectroscopy within two distinct environments: (1) in the presence of lipid bilayers, and (2) in the presence of disc-like bicelles. The 4F-P-4F peptide was chemically synthesized by solid-phase peptide synthesis using the Fmoc strategy, purified by HPLC, and characterized by mass spectrometry. Aligned membrane samples made from DMPC lipids and 4F-P-4F peptide were investigated at different peptide:lipid ratios, where the peptide:lipid ratio (1:250, mol:mol) corresponds to amphipathic peptides reconstituted into oriented lipid bilayers², whereas in higher P/L ratio (1:40, mol:mol) mixtures of phosphatidylcholines with derived apolipoprotein A-1 forms disk-like structures^{3,4}. The quality of oriented membrane was controlled by the ³¹P experiments. When 4F-P-4F was labeled (¹⁵N-A11), at low molar concentration, the value obtained is consistent with helix alignments parallel to the membrane surface. The ²H solid-state NMR spectrum of Ala-30 in the presence of oriented lipid bilayers exhibits two different quadrupolar splittings from the peptide. Further investigations are still ongoing at low and high molar ratio, and will provide information to determine the topology and structural features of 4F-P-4F for both supramolecular environments.

Keywords: High density lipoproteins, peptide, lipid interaction, solid, state NMR

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Asymmetric Membranes Suitable for Solid-State NMR Reveal Pronounced Changes in Membrane Elastic Properties and Acyl Chain Order

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Most of our biophysical membrane models ignore the most characteristic aspect of biological membranes: their asymmetry. The impact of this energetically very costly asymmetry is so far not fully clear. The production of asymmetric vesicles can be achieved through methyl- β -cyclodextrin-mediated exchange of outer leaflet lipids between acceptor and donor vesicle populations. However, studying lipid dynamics by solid-state NMR spectroscopy has been limited due to the incompatible small size of asymmetric large unilamellar vesicles. To address this problem, we developed a protocol to produce multilamellar vesicles (MLV), consisting of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (POPG) in 9:1 molar ratio, and exchanged only the outermost leaflet to either 1-palmitoyl-*d*31-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC-*d*31) or 1-palmitoyl-*d*31-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS-*d*31).

Analyzing the 2H NMR spectra of the outermost leaflet, we found that the acyl chain order parameter (S) profiles in the asymmetric membranes were significantly different from those measured in symmetrical controls of pure POPC-*d*31, POPS-*d*31, or symmetrical POPC-*d*31/POPE/POPG or POPS-*d*31/POPE/POPG membranes. Further on, the elastic properties of membranes have previously been characterized by plotting the 2H NMR Zeeman order relaxation rates ($R1Z$) to the square of the order parameter (S^2) in so-called square-law plots. From such studies, we found that the outer leaflet of the asymmetric membranes is more rigid than the symmetrical controls even if the same lipid composition was compared, displaying a more linear (rather than convex) relationship between $R1Z$ and S^2 . This membrane rigidification is likely independent of interdigitation of the acyl chains.

We speculate that the differences in lipid packing densities between the inner and outer leaflets lead to stiffer membranes through interleaflet coupling, which may be relevant for membrane protein function, fusion, lateral domain formation, and other membrane-related phenomena. By producing asymmetric MLVs, we have enabled further studies of biologically relevant asymmetric model membranes using solid-state NMR, potentially including investigations of membrane proteins.

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Keywords: asymmetric membranes, NMR, fluidity

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Lipids in Motion: Understanding the Regulation and Function of Cellular Lipid Transport

Invited Talk: Studying lipid transport pathways in the budding yeast model

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A hallmark of eukaryotic cells is their highly diversified membrane system, crucial for organizing cellular functions. Membrane diversity arises from differences in bulk lipid composition, driven by localized lipid synthesis and cytosolic lipid transfer proteins (LTPs) that selectively transport lipids between organelles. For instance, phosphatidylserine (PS) is enriched at the plasma membrane (PM) by specific LTPs, in budding yeast Osh6 and Osh7. Lipid diversity also occurs within the same membrane, either as lateral inhomogeneity or trans-bilayer asymmetry. The PM exhibits a well-studied asymmetry, with the cytosolic leaflet enriched in negatively charged phospholipids like PS. By contrast, the endoplasmic reticulum (ER) cytosolic leaflet is less charged, but its precise lipid organization remains poorly understood. The yeast protein Ist2, which localizes to the ER-PM contact sites, represents a striking example of a protein that may integrate different lipid transport activities. In collaboration with the group of Guillaume Drin, we have demonstrated that Osh6/7 interact with a short segment of the cytosolic tail of Ist2, and this interaction is required for efficient PS transport to the PM. Using a wide array of Ist2 mutants, we ask how the rest of the Ist2 tail, which is very long and disordered, influences Osh6 localization and function, and, more generally, why membrane tethering is important for lipid transfer between cellular organelles. Ist2 also contains an ER-embedded transmembrane domain with similarity to the TMEM16 protein family, whose members function as phospholipid scramblases, decreasing trans-bilayer membrane asymmetry. In collaboration with the groups of Guillaume Lenoir and Luca Monticelli, we show that Ist2 itself is a phospholipid scramblase in the ER, and that its scramblase activity affects vesicular transport, lipid droplet biogenesis and lipid transport from the ER. Together, our work provides a mechanistic description of a protein machinery that can coordinate lipid transport within and between membranes, enabling precise control of membrane function.

Keywords: lipid transport, membrane asymmetry

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Translocation of cell-penetrating peptides involving calcium-dependent interactions between anionic glycosaminoglycans and the PC bilayer

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Cell-penetrating peptides can internalize ubiquitously in many, if not all, cell types. To explore the specific targeting issue of cell-penetrating peptides (CPPs), we studied GAG-binding peptides previously identified in Otx and En2 homeoproteins (HPs), alone or extended with the penetratin-like third helix (H3) of En2. HPPs are indeed known to internalize in specific cells, thanks to their GAG-targeting sequence. We quantified the capacity of these peptides to internalize into various cell lines known to express different levels and types of heparan sulfates (HS) and chondroitin sulfates (CS) glycosaminoglycans. We also analyzed by calorimetry (DSC, ITC) and fluorescence spectroscopy, the bipartite and tripartite interactions between heparin (HI), (4S, 6S)CS (CS-E), zwitterionic phosphocholine (PC) model membranes and those peptides. Altogether, our results demonstrate the existence of Ca²⁺-dependent interactions between CS-E or HI and PC lipid bilayers, the major phospholipid found in animal cell plasma membrane. Importantly, we show that CS-E can act as a Ca²⁺-dependent bridge with PC membranes that can be exploited by a chimeric CS-E-recognition motif-H3 peptide to bind and cross the membrane lipid bilayer and get access directly to the cytosol of cells. Altogether, this study brings further information uncovering the molecular mechanism of the translocation process of CPPs that implies specific GAGs at the cell-surface. It also covers the homeoprotein field by shedding light on the role of GAGs in the paracrine activity and cell specificity of HPs.

Keywords: Cell, penetrating peptides, glycosaminoglycans, Calorimetry

*Speaker

The yeast lipid flippase Drs2/Cdc50 transports multiple negatively charged phospholipids besides phosphatidylserine

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In eukaryotic membranes of the late secretory/endocytic pathway, anionic phospholipids like phosphatidylserine (PS) or phosphatidylinositol (PI) are confined to the cytosolic leaflet, which is essential for e.g. vesicular trafficking or signalling. P4-ATPases (flippases) maintain this asymmetry by transporting lipids from the exoplasmic to the cytosolic leaflet at the expense of ATP hydrolysis. Dysfunction of P4-ATPases may cause severe diseases, highlighting the importance of transbilayer lipid asymmetry in normal cell function. For example, mutations in the human P4-ATPase ATP8B1 are directly linked to intrahepatic cholestasis, a rare inherited disorder. Despite recent advances in structural characterization of P4-ATPases, it remains unclear how P4-ATPase dysfunction leads to the observed pathological conditions, primarily due to the difficulties in accurately assessing their substrate specificity and selectivity.

We study the yeast flippase Drs2, a homolog of human ATP8B1, as a model system. Drs2 tightly associates with another transmembrane protein, Cdc50, and is autoinhibited by its long, partially unstructured N- and C-terminal extensions. *In vitro*, Drs2 autoinhibition release and activation requires truncating its N- and C-terminus and phosphatidylinositol-4-phosphate (PI4P). The principal substrate of Drs2 is PS. However, no yeast flippase has been identified for other anionic lipids, and PS is not strictly required for Drs2 function, raising the question of whether Drs2 can translocate other phospholipids – a possibility not yet thoroughly explored.

To address this question, we purified the Drs2/Cdc50 complex and measured ATPase activity of N- and C-terminally truncated Drs2 (NC Drs2) in presence of various phospholipids. We next successfully reconstituted NC Drs2 into proteoliposomes containing PI4P and fluorescently labelled phospholipids, and employed a fluorescence-based transport assay to directly monitor lipid translocation across proteoliposome membranes. In addition, we obtained three-dimensional structures of Drs2/Cdc50, revealing that PS, PG and PI can be occluded in the substrate binding pocket. Collectively, we have strong evidence that Drs2/Cdc50-mediated lipid transport is not limited to PS. Our data show that Drs2 exhibits a much broader substrate specificity, especially for anionic phospholipids such as PI. These results suggest a previously unrecognized and critical role of P4-ATPases in the turnover of phosphoinositides - lipids playing a key role in intracellular signalling and membrane dynamics.

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Keywords: P4, ATPase, reconstitution, lipid transport

Lunatin-1 peptide-functionalized alumina nanoparticles: structural characterization and membrane interaction studies

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This study encompasses the characterization, antimicrobial activity assay and interaction analysis of Lunatin-1 (Lun-1) peptide-alumina nanostructure (NP-Lun-EC and NP-Lun-EN) with POPC:POPG (3:1, mol:mol) LUVs. Addressing the pressing global health threat posed by multidrug-resistant bacteria (1), this innovative biomaterial integrates the biocompatibility of alumina nanoparticles (2) with the antimicrobial efficacy of Lun-1 (3), offering a promising system for advanced applications. The study focuses on how the functionalization position (C-terminal vs. N-terminal) influences the peptide's interaction with membrane surfaces and its antibacterial efficacy. Characterization using TEM, FTIR, and ¹³C ssNMR confirmed the successful synthesis of Lun-1-Al₂O₃ nanofilaments. Antibacterial assay against *S. aureus* demonstrated that functionalization at the C-terminal position did not significantly alter the peptide's efficacy, whereas N-terminal functionalization markedly reduced its antibacterial activity. CD analysis revealed that Lun-1 has 70% α -helical content in the presence of LUVs. While a decrease in helical content was observed for the peptide-nanoparticle structures, the NP-Lun-EC conjugate exhibited a higher helical content (55%) compared to NP-Lun-EN (40%). Interaction studies employing CD, ITC and ²H ssNMR further substantiated the main role of functionalization position. The negative Gibbs free energy values confirmed that Lun-1, NP-Lun-EC and NP-Lun-EN, interaction with LUVs occurs spontaneously, predominantly driven by entropy. The lower entropy observed for NP-Lun-EN suggests a higher proportion of free peptides in solution, indicative of reduced membrane binding affinity. Conversely, NP-Lun-EC, characterized by an unbound N-terminal region, exhibited higher values for the binding constant, Gibbs free energy, and entropy. ²H ssNMR experiments revealed that Lun-1 functionalization reduced order parameters of methylene groups of lipids. Comparisons of ²H quadrupolar couplings showed that NP-Lun-EC induced higher structural perturbations as evidenced by the lower relative order parameters for NP-Lun-EN.

Keywords: Lunatin, 1 (Lun, 1) peptide, alumina nanostructure, antimicrobial activity

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BBA PhD Prize

Analysis of the Factors Regulating the Activity of the PLA1-1 isoform : A Neutron Reflectivity and Mass-Spectrometric study

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Glycero-phospholipids are amphipathic molecules constituted by a polar head and hydrophobic chains. These molecules are involved in several pathways (such as signal transduction) and are also fundamental in the structural containment of cells and their organelles. In fact, lipid membranes are mainly made up of phospholipids and contain other critical elements such as triglycerides, sterols, proteins, etc. For the membrane to be structurally and chemically functional, its fluidity must be retained when changes occur in its surroundings. Therefore, several remodelling and degradation pathways for the phospholipid are constantly active. Within those pathways, phospholipases (PLs) play a key role; whether they are activated or expressed in response to stimuli from the external environment or directly from the lipid membrane (such as fluidity or elasticity).

Phospholipase group A (PLAs) is taken into consideration and analysed in its complex kinetics and interaction with model lipid membranes. PLAs are lipolytic enzymes that hydrolyse phospholipid substrates at specific ester bonds. They are widespread in nature and play very diverse roles, from signal transduction and lipid mediator production to membrane phospholipid homeostasis. Phospholipases are very diverse in their structure, function, regulation and mode of action. Therefore, a deeper understanding of their dynamics and kinetics is crucial. The present study involves employing neutron reflectivity, mass spectrometry and other physico-chemical techniques as to better understand the principles underlying the phospholipases substrate specificity.

PLA reactions occur in multiple steps, some involving the specificity of the PLA under examination. More specifically, efflux propensity and active site accommodation are the two restricting reaction steps and within these two steps the preferred substrate is selected to be cleaved. The efflux propensity is the ability of a phospholipid molecule to move out of its membrane. This property is related directly to the physical properties of the phospholipid molecule and the membrane, such as hydrophobic interactions, which play a key role in this process. The second crucial step is the active site accommodation: describing how well a phospholipid molecule adapts in

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the PLA active pocket site. In this work, PLA1-1, sourced from *Aspergillus oryzae*, was used as model enzyme to address these main questions.

Keywords: Phospholipases, Phospholipids, Natural lipids, Neutron scattering, Mass spectrometry, Kinetics

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Amyloid-Cationic Peptides for Antimicrobial Applications: relation to their membranotropic activities

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Antimicrobial resistance has become a significant challenge, prompting the need for novel strategies in drug discovery. Here, we present a new class of antimicrobial peptides (AMPs) based on chimeric designs that combine amyloidogenic and cationic sequences. Among the wide variety of AMPs, some naturally contain amyloidogenic sequences and are capable of self-assembling into amyloid-like fibrils (1). Interestingly, amyloid-beta ($A\beta$) peptides, typically associated with Alzheimer’s disease, have been shown to exhibit antimicrobial activity comparable to the well-known human AMP LL-37 (2). Building on the interplay of AMPs and amyloid proteins as membrane-active peptides, we designed new chimeric peptides, named Amy-Cat-Peps, by combining a highly amyloidogenic sequence from $A\beta$ with a short cationic sequence (3). The cationic sequence of the first generation of Amy-Cat-Peps was chosen for its cell-penetrating properties (4). These peptides displayed significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, with minimal hemolytic effects at antimicrobial concentrations. The secondary structures of the peptides were studied in solution and in interaction with bacterial and mammalian membrane models using circular dichroism (CD) and FTIR-ATR spectroscopy. The FTIR-ATR setup developed at CPCV allowed us to monitor the peptide binding and conformational changes at the buffer/membrane interface at bulk micromolar concentrations (5). Membrane-disruptive activity was further investigated using calcein leakage assays and conductance measurements on lipid-only membranes. These studies helped clarify the mode of action of the Amy-Cat-Peps, differentiating between mechanisms on prokaryotic and mammalian models. These findings provide new insights into the design of AMPs with potential low cytotoxicity.

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Keywords: antimicrobial peptides, lipid partitioning, conformation, pore, formation

Osh6 Mediates Lipid Transport in a Membrane Composition-Dependent Manner

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Non-vesicular lipid transport is essential for maintaining cellular lipid homeostasis and the distinct identities of individual membrane organelles. Oxysterol-binding protein-related proteins (ORPs) is a large family of proteins that primarily function in this process. These proteins typically act at membrane contact sites, shuttling lipids from donor to acceptor membranes through the cytosol via their lipid-binding hydrophobic pockets. In our study, we focus on a member of this family, Osh6, which is involved in yeast in the transport of phosphatidylserine (PS) from its site of synthesis in the endoplasmic reticulum (ER) to the plasma membrane against its concentration gradient. The key feature of the transport mechanism is the exchange between two cargo molecules: PS and phosphatidylinositol-4-phosphate (PI4P), with the latter being shuttled back from the plasma membrane to the ER. However, the exact transport mechanism remains poorly understood.

To investigate this process, we developed an in vitro fluorescence cross-correlation spectroscopy (FCCS) assay using large unilamellar vesicles (LUVs) to monitor lipid transfer. Our study demonstrates that cargo lipids and membrane properties, such as charge and fluidity, significantly influence Osh6-mediated transport. Additionally, we show that the binding of a specific cargo lipid to Osh6 affects its recognition of a particular target membrane, thereby determining the directionality of transport. These findings provide insights into the mechanisms and regulation of lipid transport processes in cells.

Keywords: Nonvesicular lipid transport, Osh6, FCCS

*Speaker

Strong membrane permeabilization activity can reduce selectivity of cyclic antimicrobial peptides

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Selectivity is a key requirement for membrane-active antimicrobials to be viable in therapeutic contexts. Therefore, the rational design or suitable selection of new compounds requires adequate mechanistic understanding of peptide selectivity. In this study, we compare two similar cyclic peptides that differ only in the arrangement of their three hydrophobic tryptophan (W) and three positively charged arginine (R) residues, yet exhibit different selectivities. This family of peptides has previously been shown to target the cytoplasmic membrane of bacteria, but not to act directly by membrane permeabilization. We have systematically studied and compared the interactions of the two peptides with zwitterionic phosphatidylcholine (PC) and negatively charged phosphatidylglycerol/phosphatidylethanolamine (PG/PE) model membranes using various biophysical methods to elucidate the mechanism of the selectivity. Like many antimicrobial peptides, the cyclic, cationic hexapeptides investigated here bind more efficiently to negatively charged membranes than to zwitterionic ones. Consequently, the two peptides induce vesicle leakage, changes in lipid packing, vesicle aggregation, and vesicle fusion predominantly in binary, negatively charged PG/PE membranes. The peptide with the larger hydrophobic molecular surface (three adjacent W residues) causes all these investigated effects more efficiently. In particular, it induces leakage by asymmetry stress and/or leaky fusion in zwitterionic and charged membranes, which may contribute to high activity but reduces selectivity. The unselective type of leakage appears to be driven by the more pronounced insertion into the lipid layer, facilitated by the larger hydrophobic surface of the peptide. Therefore, avoiding local accumulation of hydrophobic residues might improve the selectivity of future membrane-active compounds.

Keywords: permeabilization, leakage, antimicrobial activity, selectivity

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Interactions of endosomal model membranes and biomimetic, pH-sensitive polymers in the context of drug delivery

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The interaction of biomimetic, pH-sensitive polymers with membranes of various lipid compositions can cause a variety of physical-chemical membrane perturbations. These can be exploited to damage or overcome membranes in the context of drug delivery, e.g. assisting endosomal escape. Systematic and mechanistic understanding of the underlying membrane biophysics is crucial for the rational design of biomimetic compounds. The lowering of the endosomal pH, and the occurrence of the negatively charged lipid BMP during endolysosomal development, can be used to selectively permeabilize endosomal membranes.

To reveal the membrane perturbations, the polymer pKa, the pH, and lipid composition are systematically varied, representing cytoplasmic or early/late endosomal membranes. Using various methods such as fluorescence spectroscopy, monolayer methods, as well as microcalorimetry, we characterized membrane perturbations caused by the pH-sensitive polymers. Membrane leakage, electrostatic lipid clustering (the local enrichment of negatively charged lipids by positively charged polycations), and membrane fusion can play important roles for membrane permeabilization and thus for the endosomal escape. We find that the pH-sensitive polymers do not insert into lipid membranes, but rather induce local heterogeneities, as well as limited membrane permeabilization. Our data indicates that the polymers interact electrostatically especially with negatively charged, i.e. endosomal-like, model membranes.

This dominance of polymer and membrane charge emphasizes the role of the negatively charged endosomal lipid BMP, while the pH change contributes indirectly to endosomal escape by increasing the polymer charge.

Keywords: endosomal escape, membrane permeabilization, pH sensitive polymer, interactions

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Mechanism of perception of cyclic lipopeptides from soil bacilli by the monocotyledon plasma membrane in relation with their plant defense eliciting activity

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Elicitors, defined as compounds capable of stimulating the natural defenses of plants, are a promising alternative to conventional chemical pesticides to limit the invasion of pathogens in a context of sustainable agriculture.

Cyclic lipopeptides (CLPs) secreted by soil beneficial bacilli, have been shown to be promising candidates for stimulating natural plant defenses. Due to their amphiphilic nature and unlike most elicitors which are perceived via high-affinity membrane protein receptors, CLPs are likely to interact directly with the lipids of the plasma membrane (PM) of plant cells, resulting in dynamic changes in the lipid organization. This may explain why a particular CLP does not have the same impact on different plants, as the composition of the plant PM lipids varies between species.

While new insights into the molecular basis of plant defense-eliciting mechanisms by surfactin (Srf), one bacilli CLP, on *Arabidopsis thaliana* (a dicot model), was recently obtained, there is a gap of knowledge on the eliciting activity of Srf and other bacilli CLPs on monocots, as well as on the molecular mechanism of CLP perception by monocot cells.

The general goal of this project is to investigate, at a molecular level, the capacity of CLPs from *Bacillus* to be perceived by the PM of monocotyledon in relation with their plant defense eliciting activity. By using a rational combination of plant biological assays with appropriate biophysical analyses on monocotyledon PM, three specific objectives are targeted:

(a) Identify the activity of each CLP family (Srf, fengycin, iturin) and their synergies on monocotyledon defense stimulation.

(b) Study the impact of CLPs on monocotyledon PM mechanics and organization.

*Speaker

(c) Determine the significance of specific monocotyledon PM components/organization in CLP-PM interaction and defense elicitation.

Comparing the results of this project with recent findings on *A. thaliana* will provide valuable insights into the varying effectiveness of CLPs in triggering defense mechanisms in dicots and monocots, facilitating their biotechnological application. In addition, this project will strengthen the basic knowledge on the structural organization of monocotyledon PM, barely described so far

Keywords: Monocotyledon, lipopeptides

Study of Polystyrene Presence in Membranes Using Neutron Diffraction and Reflectometry

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Microplastics are ubiquitous in the environment, polluting ecosystems from ocean surfaces to extreme depths, such as the Mariana Trench. Their interaction with biological membranes raises concerns about their effects on membrane physicochemical properties. Our previous research demonstrated modifications in these properties in the presence of microplastics. In this study, we use a styrene oligomer as a model microplastic to further investigate its incorporation and structural impact on model membranes. Neutron diffraction and reflectometry provide nanometric resolution to characterize the induced changes. Our results reveal significant alterations in membrane organization, suggesting interaction mechanisms that could influence their functional properties.

Keywords: Microplastics, Neutrons, Membrane

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Impact of hydrophilic H-4 amino acid substitution in Piscidin-4 on peptide-membrane interaction: Insights from solid-state NMR, calcein release assay, and tryptophan fluorescence spectroscopy

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Antibiotic-resistant bacterial infections pose a critical global health challenge, severely limiting treatment options and placing a substantial burden on healthcare systems. This growing crisis highlights the urgent need for novel antimicrobial therapeutics that combine high efficacy, low toxicity, and minimal propensity for resistance development. In this context, antimicrobial peptides (AMPs) have garnered significant interest as potential alternatives to conventional antibiotics due to their broad-spectrum activity and unique mechanisms of action.

Our research focuses on Piscidin-4s (FFRHIKSFWKGAIAIFRGARQG-NH₂), a potent AMP. Considering that the His-4 residue is localized at the membrane-aqueous interface, we hypothesized that substituting this hydrophilic residue with a hydrophobic phenylalanine (His-4 → Phe-4) would modulate its interaction with lipid membranes, potentially altering both its structural dynamics and antimicrobial efficacy. To investigate these effects, we employed oriented solid-state NMR (ssNMR), calcein release assay, and tryptophan fluorescence spectroscopy in a phosphatidylcholine:phosphatidylglycerol (POPC:POPG) (3:1, mol:mol) membrane-mimetic environment.

Our findings indicate that the positive charge of histidine may initially facilitate peptide-membrane interactions by enhancing electrostatic attraction. Notably, ²H ssNMR quadrupolar splitting data revealed greater lipid chain disorder induced by the native peptide under acidic conditions (36.4 kHz for His-4 vs. 23.4 kHz for Phe-4), suggesting stronger membrane perturbation. However, the apparent dissociation constant derived from tryptophan fluorescence assays indicated a weaker interaction under the same conditions (228 μM for His-4 vs. 108 μM for Phe-4), suggesting that despite the increased lipid disorder, the native peptide exhibits lower overall membrane affinity at acidic pH.

*Speaker

Conversely, the (F4)ecPis-4s analog (His-4 $\rightarrow$ Phe-4) exhibited a weaker apparent dissociation constant, particularly at neutral pH (140 μ M for His-4 vs. 90 μ M for Phe-4 at pH 7.5). This trend aligns with calcein release assays, which demonstrated a modest yet significant increase in membrane disruption for the more hydrophobic analog, particularly at higher peptide-to-lipid molar ratios.

Taken together, these results provide critical insights into the structure-activity relationship of piscidin-derived AMPs, underscoring the role of residue-specific modifications in tuning membrane interactions and antimicrobial potency. This knowledge may contribute to the rational design of next-generation peptide-based therapeutics aimed at combating antimicrobial resistance with enhanced efficacy and selectivity.

Keywords: Piscidin, antimicrobial peptide, peptide membrane interactions, solid state NMR, tryptophan fluorescence, calcein release.

Quantitative lipid analysis of brain, myelin-enriched and corpus callosum fractions in mice, by multinuclear NMR and mass spectrometry

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Myelin is a key component of the central nervous system. It is a lipid and protein lamellar membrane structure that tightly envelops the axon in a concentric fashion (cochlear structure). Present in the central and peripheral nervous systems of vertebrates, it gives its name to white matter due to its high concentration (80%) of phospholipids, glycosphingolipids and cholesterol. It plays a key role in signal transmission and neuron survival in the central nervous system, and its loss or lack of integrity can lead to a number of neurodegenerative diseases in particular Multiple Sclerosis. Although myelin preserves several aspects of biological lipid bilayers, its peculiar membrane organization is still not fully understood. To decipher compositional and dynamics properties of myelin, we proposed a combined NMR and mass spectrometry approach to quantitatively investigate lipid composition. We compared various samples ranging from mice whole brain, corpus callosum (the largest commissural white matter bundle in the brain,) and extracted myelin-enriched fraction to study their lipid composition. By comparing control samples, specimens extracted from animals in pathological conditions and in vitro reconstituted lipid membranes, we provided a detailed description of lipid dynamics. * ANR ULTIMO ANR-22-CE18-0041

Keywords: myelin, lipid extraction, brain, NMR, Mass Spectrometry, membrane reconstitution

*Speaker

Analytical Ultracentrifugation as a Tool for Exploring COSAN Assemblies

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COSAN anion ((Co(C₂B₉H₁₁)₂)⁻) is composed of two bulky dicarbollide semicages, each of them bearing two negative charges that "sandwich" a metal cation Co³⁺. COSAN derivatives are considered for applications in various fields, for example, in the medicine field, as potent inhibitors toward HIV protease, or as a source of boron for boron neutron capture therapy treatment of cancer tumors. COSAN derivatives spontaneously cross biological membranes to accumulate in living cells, halting their proliferation and growth. COSAN is a "nanoion", with one charge delocalized over the nanometric hydrophobic structure (11 Å long and 6 Å large). The low charge density (≈ 2.5 charge/nm) confers to COSAN the possibility to bind with an unexpectedly strong affinity to hydrophilic neutral interfaces, a water-mediated effect we called the superchaotropic effect, and to apolar surfaces (hydrophobic interactions). These and possibility to make intermolecular di-H bonds (B-H...H-C), and electrostatic interactions, provide multiple ways for intermolecular interactions. COSAN have most surfactant properties in water: it is surface active, forms vesicles and micellar-like aggregates at moderate concentration. COSAN specifically binds to the hydrophobic cavity of BSA at low stoichiometry, and binds non-specifically with BSA surface at high stoichiometry. COSAN interacts with Octyl Glucoside (OG) monomer. Above OG micellar concentration, COSAN at low concentration adsorbs on the micelle surface; at higher concentration COSAN disrupts OG micelles. COSAN is able to solubilize membrane protein from E. Coli cells (unpublished). We aim to describe the interactions of COSAN with proteins (Fakhouri et al, under revision, EBJO). We will present our first results with Analytical Ultracentrifugation to characterize the interactions with COSAN and the soluble Myoglobin, and the detergent-solubilized membrane protein FhuA. We will discuss them in complementarity to SAXS and DLS –for Myoglobin- and biochemical analysis –for FhuA.

Keywords: COSAN, nanoion, micelles

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Asymmetry Stress in Model Membranes: A Driver of Biophysical and Dynamic Changes

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Asymmetry stress in model membranes originates from an intrinsic area discrepancy between the inner and outer leaflets. This stress is not merely a structural irregularity but rather a crucial catalyst of membrane remodeling processes and may contribute to the antimicrobial activity of cyclic lipopeptides (CLPs). In biological systems, asymmetry stress plays a fundamental role in processes such as vesicle formation and signal transduction. Here, we present two opportunities to induce asymmetry stress and to investigate its role in membrane biophysics and dynamics. The first approach involves the insertion of CLPs (e.g. surfactin) into the outer leaflet of large unilamellar vesicles (LUVs) while minimizing the translocation of CLPs to the inner leaflet. This creates a controlled area imbalance between both leaflets. The resulting overpopulation of the outer leaflet leads to a quantifiable difference in intrinsic area of each leaflet, which enables the analysis of membrane stress responses.

The second approach focuses on synthesizing asymmetric LUVs (aLUVs), where the outer leaflet contains an additional lipid component compared to the inner leaflet. This lipid remains in the gel phase during aLUV production. Upon heating, it undergoes a phase transition to the fluid phase, resulting in an increased area per lipid.

In both approaches, asymmetry stress leads to the formation of smaller daughter vesicles due to membrane fission driven by stress gradients. Differently sized vesicles are separated and analyzed using Asymmetric Flow Field-Flow Fractionation (AF4). This allows the precise characterization of vesicle populations and the biophysical effects of induced asymmetry stress.

Keywords: Asymmetry stress, cyclic lipopeptides, asymmetric LUVs, Asymmetric Flow Field, Flow

^{*}Speaker

Sfh3 is a Sec14-homolog protein with a PI/PI4P transfer function controlled by sterol

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Sfh3 is a yeast protein of the Sec14 superfamily, able to bind phosphatidylinositol (PI) and sterol in a mutually exclusive manner. Sfh3 is recruited at the lipid droplet (LD)/vacuole contact site by LDO45 (Lipid Droplet Organization) and seems involved in regulating the utilization of energy stored in LDs by the yeast. To date, how Sfh3 translates its ability to bind PI and sterol into a cellular function remains elusive. To gain insights into this, we analyzed in real-time by fluorescence how it captures and transfers lipid ligands in controlled in vitro systems using artificial membranes and recombinant proteins. We found that Sfh3 does not have the features of an efficient sterol/PI exchanger but can transfer PI between membranes to promote PI4P synthesis. We also found that, unexpectedly, it can transfer PI4P between membranes. We next found that ergosterol and lanosterol levels regulate the ability of Sfh3 to transfer PI and PI4P. We conclude that Sfh3 distinguishes itself from the well-known PC/PI exchanger Sec14 as it has the unique capacity to regulate PI4P signaling processes in a sterol-dependent manner. The biochemical characterization of several Sfh3 mutants associated with particular phenotypes in yeast supports this idea. Notably, our data suggests that Sfh3 might provide yeast with resistance against azole treatment via PI4P-dependent signaling processes. Collectively, our data indicate that Sfh3 ensures functional links between sterol metabolism and PI4P signaling by unanticipated lipid transfer modalities.

Keywords: Sfh3, Sec14, lipid transfer protein, sterol, phosphatidylinositol, PI4P, membrane contact sites

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Mechanobiological characterization at different scales of biological samples using AFM

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In recent years, there has been a significant rise in the study of cellular and extracellular matrix (ECM) mechanics, driven by the recognition of its crucial role in physiological and pathological processes. Atomic force microscopy (AFM) has emerged as a powerful technique for investigating the mechanical properties of living cells, membranes and ECM, offering measurements of stiffness, elasticity, adhesion, and viscoelasticity. However, AFM’s effectiveness in biological research is limited by its reliance on skilled users and its low throughput. To overcome these challenges, researchers have been working on enhancing AFM’s throughput to improve efficiency and enable the acquisition of larger data sets.

Since several years, we have developed an automated system based on object detection that incorporates algorithms to ensure the quality of the acquired data and includes an open-source software package for measuring elastic and viscoelastic properties from AFM data. To demonstrate the viability of our developed automated AFM system, we conducted tests on several samples commonly studied using AFM, each at a different size scale.

Among several applications, we conducted experiments on artificial nanovesicles (NVs) with three different lipid mixtures and three different extrusion sizes. The development of automated data acquisition and data analysis protocols, pave the way for establishing a methodology that can be applied to cell-derived extracellular vesicles under both pathological and non-pathological conditions.

By integrating a tracking algorithm, we monitored the mechanical properties of migrating NIH3T3 fibroblasts on glass over time. We observed that the front-to-back E ratio and the leading edge/following edge E ratio of migrating NIH3T3 cells are weakly correlated with the migration speed but not with the migration angle or changes in the migration angle of the cell. Suggesting that even when cells are moving in a particular direction, they are constantly probing alternative paths.

Furthermore, we adapted the system to measure the mechanical fingerprint of rat bladder tissue revealing the three distinct mechanical regions and obtaining values consistent with previous studies.

This work contributes to the advancement of biophysical research by introducing a novel automated AFM system capable of characterizing the mechanical properties of diverse biological

*Speaker

samples at several scales.

Keywords: nanovesicles, young modulus, automation, AFM

Membrane Interactions of QBP1 Derivatives: Toward Dual Anti-Amyloid and Antibacterial Peptides

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Neurodegenerative diseases affect millions worldwide and are the second leading cause of death after cardiovascular diseases. Amyloid-related disorders, such as Huntington’s and Parkinson’s, are characterized by the aggregation of misfolded proteins into oligomers and fibrils, which may contribute to neuronal death and neurodegeneration. Interestingly, misfolded amyloid proteins share structural similarities with antimicrobial peptides: they are poorly structured in solution but adopt well-defined conformations when interacting with biological membranes. This resemblance suggests a previously unrecognized antimicrobial role for amyloid proteins (1), supporting the hypothesis of an infectious origin of neurodegeneration.

The QBP1 peptide (polyQ binding peptide 1) has shown promise in inhibiting amyloid aggregation across multiple amyloidogenic proteins, including α -synuclein, TDP-43, polyQs, and Sup35NM-implicated in Parkinson’s disease, amyotrophic lateral sclerosis, Huntington’s disease, and prion diseases, respectively. Recently, we identified a moderate antimicrobial activity of QBP1 through sequence-property alignment using the ADAPTABLE webserver (2), which we later confirmed by antimicrobial activity assays.

In this study, we enhanced QBP1’s antimicrobial properties by arginine end-labeling (3), a modification that also improves blood-brain barrier permeability (4). Furthermore, we used molecular dynamics simulations and solid-state NMR to investigate the mechanism of action of these derivatives. Given their activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, we focused on the interaction of two QBP1 derivatives-QBP1 mutR (8R-QBP1) and RWR-QBP1-RWR-with bacterial membrane mimics. Their modes of action were thoroughly analyzed to optimize their therapeutic potential for both bacterial infections and neurodegenerative diseases.

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Keywords: antimicrobial peptide, neurodegenerative diseases, protein amyloidogenesis, NMR, MD

Influence of Cardiolipin on E.coli-Mimicking Model Membranes in the Absence and in the Presence of the Intramembrane Protease GlpG

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Recent studies have shown that the lipid environment of membrane proteins plays an important role in the regulation of important biochemical processes such as signaling or channel activity. The rhomboid protease GlpG, an integral membrane enzyme found in *Escherichia coli*, cleaves single helical transmembrane substrates. While the lipid environment is known to affect GlpG activity and GlpG thins POPE/POPG membranes, the role of cardiolipin (CL) in this process has not been investigated. Cardiolipin could likely influence membrane properties because it has two phosphates as a headgroup and four acyl chains, introducing a strong negative charge and negative intrinsic curvature. CL is also known to affect membrane protein function in both *E. coli* and mitochondrial membranes. In this study, we examine how CL influences the structure and function of POPE/POPG model membranes and whether it affects GlpG-catalyzed proteolysis of the transmembrane substrate LacYTM2. Using ²H solid-state NMR, we measured membrane thickness and found that CL leads to a small decrease in membrane thickness. Furthermore, the presence of CL significantly increases GlpG proteolysis kinetics, suggesting that this lipid plays a role in regulating the protease. These findings help to better understand membrane-protein interaction and highlight that specific lipid species influence enzyme function. In ongoing experiments, we investigate CL dynamics and how it affects GlpG function.

Keywords: GlpG, Cardiolipin, Membrane, protein interaction, Solid state NMR, Proteolysis kinetics

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Electrochemical and Morphological Insights into Colistin-Induced Reorganization of Tethered Bilayer Lipid Membranes

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A greater challenge looms large on the horizon—the antibiotic resistance crisis. If left unaddressed, antibiotic resistance is projected to cause up to 10 million deaths annually by 2050(1). With the discovery of new antibiotics stagnating, the need for alternative antimicrobial strategies is urgent. One promising avenue involves antimicrobial peptides (AMPs), which predominantly relies on bacterial membranes targeting and disruption via few different mechanisms of action yet still maintaining an immunomodulatory effects(2). Colistin, a cationic AMP, is one of the last-resort treatments for multidrug-resistant Gram-negative infections. However, the rising incidence of colistin-resistant bacteria necessitates a deeper understanding of the mechanisms underlying resistance and strategies to overcome it(3). To address this, we employ tethered bilayer lipid membranes (tBLMs) as model systems that can be compositionally modified to mimic either bacterial or mammalian membranes. This, in turn, provides a less complex yet versatile platform for study compared to cellular membranes, while still preserving the robustness of the lipid bilayer and the functionality of the submembrane compartment(4). These biomimetic platforms allow us to investigate colistin's effects using atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS)(5). Our findings reveal that colistin induces a decrease in bacterial membrane mimicking tBLMs conductivity and exhibits different modes of phase shift depending on the bilayer composition. In contrast, its effects on eukaryotic-like bilayers differ markedly, indicating a selective mechanism of action. AFM analysis further demonstrates the appearance of additional layers upon colistin interaction with bacterial membrane mimicking tBLMs, suggesting structural remodeling of the membrane. These insights enhance our understanding of colistin's biophysical effects and may contribute to the both development of novel antimicrobial peptides and improvement of already existing ones.

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Keywords: tBLM, colistin, antimicrobial peptides, atomic force microscopy, electrochemical impedance spectroscopy

Unexpected asymmetric distribution of cholesterol and phospholipids in equilibrium model membranes

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Lipid compositional asymmetry across the leaflets of the plasma membrane is a ubiquitous feature in eukaryotic cells. How this asymmetry is maintained is thought to be primarily controlled by active transport of lipids between leaflets. This strategy is facilitated by the fact that long tail phospholipids and sphingolipids diffuse through the lipid bilayer slowly – taking many hours or days. However, a lipid like cholesterol – which is the most abundant lipid in the plasma membrane of animal cells – has been harder to pin-point in terms of its favored side. In this presentation we show that when a saturated lipid is added to a mix of the unsaturated lipid palmitoyl-oleoylphosphatidylcholine (POPC) and cholesterol, both cholesterol and the long tail phospholipids organize asymmetrically across the membrane’s leaflets naturally. In these extruded unilamellar vesicles both cholesterol and the saturated lipids – dipalmitoylphosphatidylcholine (DPPC) and sphingomyelin (SM) – segregated to the inner leaflet while POPC preferentially localizes in the outer leaflet. This asymmetric arrangement generated a slight phospholipid number imbalance favoring the outer leaflet and thus opposite to where cholesterol and the saturated lipids preferentially partitioned. These results were obtained using Magic Angle Spinning (MAS) NMR in combination with Small Angle Neutron Scattering (SANS) using isotope labeling to differentiate lipid species. We suggest that sidedness in membranes can be driven by thermodynamic processes and could diminish the need of constant active transport across leaflets to achieve compositional asymmetry. These results will trigger a revision of the existing concepts of the origin of plasma membrane asymmetry as well as in bioengineering applications.

Keywords: membrane asymmetry, NMR, SANS, cholesterol

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Structural and Dynamic Insights into the Loss of Activity of Tolaasin I, an antimicrobial cyclic lipopeptide.

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Cyclic lipodepsipeptides (CLiPs), secondary metabolites produced by non-ribosomal peptide synthetases (NRPSs), are found predominantly in *Pseudomonas*, *Bacillus*, and *Streptomyces* species. These compounds exhibit diverse biological functions, such as promoting bacterial motility and demonstrating significant antibacterial and antifungal activities(1). Due to these characteristics, CLiPs are being actively explored for two key applications: combating multi-drug resistant pathogens in clinical settings and serving as biocontrol and biostimulant agents in agriculture. Among these, tolaasin from *Pseudomonas tolaasii*, an 18 amino acid CLiP featuring a 5 amino acid macrocycle at its C-terminus, is known for causing brown blotch disease in mushrooms and for its inhibitory effects against fungi and Gram-positive bacteria(2). Notably, tolaasin’s antagonistic properties can be neutralized by a cohabiting bacterium which causes enzymatic hydrolysis of the depsi (ester) bond closing the macrocycle(3). Given the significance for biological activity, we aim to investigate how the structure, conformational dynamics and membrane interactions of native and hydrolyzed tolaasin is affected by this change, using NMR spectroscopy in SDS micelles. By growing *Pseudomonas tolaasii* on minimal medium, we produce ¹³C- and ¹⁵N-labeled tolaasin, enabling multidimensional NMR analysis. The isotopically enriched hydrolyzed form was obtained through controlled alkaline hydrolysis. Following complete resonance assignment of both forms, we recorded 2D HNHA experiments to access backbone conformation dependant ³JHNHa scalar couplings, while long-range 2D HNCOC experiments allowed to assess long-lived hydrogen bonds through ³hJNC’ scalar couplings. This revealed significant differences in the hydrogen bond networks between native and hydrolyzed tolaasin. Additionally, ¹⁵N relaxation experiments provided insights into peptide backbone dynamics, with order parameters (S²) elucidating molecular motions on the ns-ps timescale, while relaxation dispersion experiments confirmed the existence of μ sec dynamics. These findings allowed us to identify residues with distinct rigidity and flexibility profiles in both forms, guiding the 3D structure determination of hydrolyzed tolaasin using nOe data. The structure reveals that opening of the macrocycle through ester bond hydrolysis affects the native structure well beyond the macrocycle, introducing additional dynamics in the exocyclic residues.

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Keywords: NMR, Cyclic Lipopeptides, Membrane interactions, Antimicrobial peptides, Biocontrol agents

Exploring the interactions of elastin peptides with model membranes by numerical simulations

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Elastin is an essential extracellular matrix protein found in vertebrates. It is present in the elastic fibers that provide elasticity and resilience to many tissues (1). Over time, elastin accumulates damage, which can lead to loss of function but also promote pathological conditions (2). Peptides derived from elastin degradation are hydrophobic. Among elastin-derived peptides (EDPs), those with the XGXXPG or XGXPXGXXG motif are biologically active, as they can interact with dedicated receptors on the cell surface (1). This is also the case for the C-terminal part of the molecule (domain 36). By their very nature, it is possible that EDPs can interact directly with cell membranes.

To test this possibility, numerical simulations with 3 replicas were carried out. Analyses were carried out on the structure of the simulated peptides (secondary structures) during the trajectory, as well as on the percentage of time spent at the membrane, to determine if and how they interact with it. The most promising peptides tested were synthesized, and this work is currently completed by an experimental biophysical study.

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Keywords: elastin, elastin peptides, membrane, numerical simulations, circular dichroism

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Integrating the Role of Membranes in Lipid Signaling in Plants

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Lipid signaling is a crucial process in plant cells, regulating various physiological responses to environmental stimuli, development, and stress. Membranes, both at the plasma membrane and within intracellular organelles, serve as key platforms for lipid-mediated signaling. Lipids such as phosphoinositides, sphingolipids, and fatty acids participate in diverse signaling pathways that influence cell communication, growth, and adaptation to both abiotic and biotic stresses. In *Arabidopsis thaliana*, I have shown that phosphatidic acid (PA) is produced in response to cold stress and peptides like flagellin, forming an integral part of the signaling cascade and triggering relevant plant cell responses. Additionally, I have demonstrated that salicylic acid, a plant hormone central to defense mechanisms against pathogens, induces the production of phosphatidylinositol-4,5-bisphosphate (PIP₂), which may act as a cofactor for the activation of specific phospholipases D (PLDs).

While my research has primarily focused on biochemical and reverse genetics approaches to explore lipid signaling, it has not yet fully considered the membrane aspect of these processes. In this study, I will present examples showing how signaling lipids also play critical roles in membrane trafficking and serve as structural components of membranes, thus altering our understanding of lipid signaling. This will be exemplified through studies of mutants of phosphatidylinositol-4-kinases (PI4K). Moreover, while the plasma membrane is the primary site for lipid-protein and protein-protein interactions involved in lipid signaling, questions remain about the role of dynamic membrane properties, including microdomains, in plant signal transduction. Could molecular dynamics help provide deeper insights into this aspect?

Understanding the intricate interplay between lipid signaling and membrane dynamics is crucial for advancing strategies to improve crop resilience, optimize growth, and manage plant responses to environmental challenges. Ultimately, elucidating the role of membranes in lipid signaling represents an exciting frontier for plant biology research.

Keywords: lipid signaling, membrane trafficking, membrane dynamics

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How does the saturation of lipids influence the alignment of membrane peptides?

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Understanding the mechanism of action (MOA) of antimicrobial peptide (AMP) offers ways to design alternatives to classical antibiotics.(1) Two well-known AMPs are magainin and PGLa were discovered in African clawed frog skin. Their enhanced bioactivity when combined is referred to as synergism, which is most effective at a 1:1 molar ratio.(2) Several studies on the membrane topology of PGLa have shown that its orientation depends on the saturation level of phospholipids.(2,3) Saturated lipids pushes the equilibrium towards a transmembrane orientation whereas unsaturated lipids stabilize an in-planar orientation of PGLa and the model peptide LAH4.(2,3)

The perturbation capability of the peptides on the membrane can be characterized by the order parameter (4) which is determined for each carbon atom along the deuterated aryl chain. Generally, a high order parameter corresponds to a well-ordered structure, while a low value indicates reduced order. The membrane models used in this study were composed of both fully saturated and lipids with one saturated and one unsaturated chain (e.g., POPE, POPG, DMPE, and DMPG), however exclusively measuring the saturated acyl chain(s) by 2H solid-state NMR. In-planar peptides show a more pronounced effect on the order parameter than transmembrane peptides.(3)

For non-saturated lipids the energetic penalty from chain dis-ordering for hosting in-planar peptides is probably lower than for saturated lipids. In order to test this hypothesis, we need access to the order parameter also of the non-saturated chain. This can be achieved by measuring time averaged ¹³C-¹H dipolar coupling using the natural abundance of ¹³C. The R-type Proton-Detected Local Field (R-PDLF) pulse sequence⁵ enables atomistic resolution in the isotropic chemical shift dimension allowing to assign the dipolar coupling in the second dimension.(2,3)

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Keywords: Antimicrobial Peptide, NMR, Order parameter

Cathelicidin-BF: A Potent Antimicrobial Peptide to Combat Multidrug-Resistant Bacteria

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Cathelicidin-BF (CatBF), an antimicrobial peptide (AMP) derived from *Bungarus fasciatus* and related to LL-37, exhibits a remarkable spectrum of antimicrobial, antiviral, antifungal, and anticancer activities. Unlike many AMPs, it possesses a favorable pharmacokinetic profile, remaining stable in serum for at least one hour and effectively treating bacterial infections in mice. To assess its potential against drug-resistant hospital-acquired infections, we tested its efficacy against 81 clinically relevant multidrug-resistant bacterial isolates. Notably, CatBF demonstrated minimum inhibitory concentrations (MICs) as low as 0.5 μ M against carbapenem-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*.

Given its broad activity, independent of resistance mechanisms or bacterial classification, we explored its mechanism of action at the molecular level. Through a combination of NMR spectroscopy, paramagnetic probes, and molecular dynamics (MD) simulations, we examined its structural properties, depth of insertion, and orientation in various membrane models, including micelles, bicelles, oriented bilayers, and vesicles. Our results indicate that CatBF’s potent antimicrobial effect is driven by its strong cationic charge, which facilitates membrane neutralization at low peptide-to-lipid ratios and preferentially interacts with charged phospholipids. At higher concentrations, its orientation shifts, leading to membrane deformation and the formation of transient pores, a process that likely contributes to bacterial cell death.

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Keywords: antimicrobial peptides, membrane pore formation

Antimicrobial peptide mechanism of action studied by in-cell solid-state NMR

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Staphylococcus aureus is a Gram-positive pathogenic bacterium that is resistant to a wide range of antibiotics. DMS-DA6-NH₂ (DA6) is a novel antimicrobial peptide (AMP) that has high efficacy on various bacterial strains (1). *In vivo* 2H solid-state Nuclear Magnetic Resonance (NMR) is used to study AMP mode of action that disrupt bacterial membranes (2-3). We studied the bacterial membrane lipid profile (head groups and fatty acids) and then optimised the bacterial culture conditions with deuterated palmitic acid (PA-d₃₁), in order to label the lipids. The *in vivo* 2H solid-state NMR spectrum is then characterised by a central peak surrounded by rotational bands on either side, whose spectral moment M₂ can be measured and is related to membrane rigidity (2). It is then observed that the membrane rigidity decreases progressively when DA6 concentration is increased. These results were compared with those of AMPs whose mode of action are already known (3). It is deduced that AMP DA6 has a pore effect on the membrane of *S. aureus*.

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Keywords: Bacteria, Peptides, solid, state NMR, fatty acids, Membranes

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Analysis of the Factors Regulating the Activity of the PLA1-1 isoform : A Neutron Reflectivity and Mass-Spectrometric study

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Glycero-phospholipids are amphipathic molecules constituted by a polar head and hydrophobic chains. These molecules are involved in several pathways (such as signal transduction) and are also fundamental in the structural containment of cells and their organelles. In fact, lipid membranes are mainly made up of phospholipids and contain other critical elements such as triglycerides, sterols, proteins, etc. For the membrane to be structurally and chemically functional, its fluidity must be retained when changes occur in its surroundings. Therefore, several remodelling and degradation pathways for the phospholipid are constantly active. Within those pathways, phospholipases (PLs) play a key role; whether they are activated or expressed in response to stimuli from the external environment or directly from the lipid membrane (such as fluidity or elasticity).

Phospholipase group A (PLAs) is taken into consideration and analysed in its complex kinetics and interaction with model lipid membranes. PLAs are lipolytic enzymes that hydrolyse phospholipid substrates at specific ester bonds. They are widespread in nature and play very diverse roles, from signal transduction and lipid mediator production to membrane phospholipid homeostasis. Phospholipases are very diverse in their structure, function, regulation and mode of action. Therefore, a deeper understanding of their dynamics and kinetics is crucial. The present study involves employing neutron reflectivity, mass spectrometry and other physico-chemical techniques as to better understand the principles underlying the phospholipases substrate specificity.

PLA reactions occur in multiple steps, some involving the specificity of the PLA under examination. More specifically, efflux propensity and active site accommodation are the two restricting reaction steps and within these two steps the preferred substrate is selected to be cleaved. The efflux propensity is the ability of a phospholipid molecule to move out of its membrane. This property is related directly to the physical properties of the phospholipid molecule and the membrane, such as hydrophobic interactions, which play a key role in this process. The second crucial step is the active site accommodation: describing how well a phospholipid molecule adapts in

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the PLA active pocket site. In this work, PLA1-1, sourced from *Aspergillus oryzae*, was used as model enzyme to address these main questions.

Keywords: Phospholipases, Phospholipids, Natural lipids, Neutron scattering, Mass spectrometry, Kinetics

Membrane Interaction of small molecule peptidomimetics of antimicrobial peptide: does the size matter?

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Antimicrobial resistance has become a growing global health concern. In response, there has been an increased emphasis on developing antimicrobial peptides (AMPs) and their various mimics. Understanding their mechanisms of action against pathogens is crucial for the ongoing development of new active agents. In this discussion, we focus on a specific category of AMP mimics: cationic amphipathic small molecules. We investigate the biophysical behaviors exhibited by these mimics on membranes using biophysical tools. To tackle the challenge of understanding how these membrane-active compounds function, the small molecule mimics were introduced to liposomes that mimic bacterial membranes. Using deuterium and phosphorous solid-state nuclear magnetic resonance spectroscopy, the effect of the compounds on the lipid membrane were evaluated. Furthermore, several fluorescence studies were conducted using both bacterial and mammalian model membranes. These studies included dye-release assays, which provided insights into the pore-forming abilities of membrane-active compounds. Interestingly, bacterial and mammalian not only differ in their surface charge, but also in their intrinsic curvature. Here the size of molecules becomes important. Further, quenching studies were done to determine the localization of these agents within the lipid bilayer and binding studies to assess the compounds' affinity for bacterial model membranes.

Keywords: AMP, Peptidomimetics, Solid state NMR, Fluorescence, Lipid membrane, Mechanism of action

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Solid-state NMR structural investigations of a tandem dimer peptide apolipoprotein A1 mimic

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High density lipoproteins (HDL) are complex nanostructures that play important roles in cell function, lipid metabolism, and the progression of various diseases. The main components of lipoproteins are phospholipids and apolipoproteins, particularly apolipoprotein A1 (apoA-I). HDL has a key role in the transport of fats within the human body. Over time, numerous apo-AI mimetic compounds have been presented. Among these, the most extensive group of peptide mimetics comprises sequences that are 18 amino acids in length. These sequences differ in the number of phenylalanine residues, ranging from two (2F) to seven (7F) on the non-polar face of amphipathic helices, thus exhibiting varying degrees of hydrophobicity. Among these, 4F (DWLKAFYDKVAEKLKEAF) has garnered the most attention, likely due to its good solubility in water. Moreover, a dimer composed of 4F connected by a proline linker (4F-proline-4F), resulting in a 37-mer sequence, is even more biologically effective than the monomeric peptide¹. In this study, we investigate the 4F-P-4F topology using oriented solid-state NMR spectroscopy within two distinct environments: (1) in the presence of lipid bilayers, and (2) in the presence of disc-like bicelles. The 4F-P-4F peptide was chemically synthesized by solid-phase peptide synthesis using the Fmoc strategy, purified by HPLC, and characterized by mass spectrometry. Aligned membrane samples made from DMPC lipids and 4F-P-4F peptide were investigated at different peptide:lipid ratios, where the peptide:lipid ratio (1:250, mol:mol) corresponds to amphipathic peptides reconstituted into oriented lipid bilayers², whereas in higher P/L ratio (1:40, mol:mol) mixtures of phosphatidylcholines with derived apolipoprotein A-1 forms disk-like structures^{3,4}. The quality of oriented membrane was controlled by the ³¹P experiments. When 4F-P-4F was labeled (¹⁵N-A11), at low molar concentration, the value obtained is consistent with helix alignments parallel to the membrane surface. The ²H solid-state NMR spectrum of Ala-30 in the presence of oriented lipid bilayers exhibits two different quadrupolar splittings from the peptide. Further investigations are still ongoing at low and high molar ratio, and will provide information to determine the topology and structural features of 4F-P-4F for both supramolecular environments.

Keywords: High density lipoproteins, peptide, lipid interaction, solid, state NMR

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Exploring Membrane Interactions: A Comparative Study of Daptomycin and a Novel Cyclic Lipopeptide

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The rise of antimicrobial resistance and the declining efficacy of many antibiotics highlight the urgent need for new antimicrobial agents. Cyclic lipopeptides (CLiPs) are a promising class of antibiotics, produced as secondary metabolites by bacteria such as *Streptomyces*, *Bacillus* and *Pseudomonas*. A clinically relevant example is Daptomycin, currently the only CLiP on the market, which is used as a last-resort antibiotic against gram-positive infections. Here we present Olikomycin A, a novel CLiP from *Streptomyces*, and provide initial insights into the mode of action compared to Daptomycin. We use isothermal titration calorimetry (ITC) to characterize their binding to model membranes, such as liposomes and electroneutral DIBMA-nanodiscs. The membrane binding of both CLiPs is a complex process depending on interactions between the antibiotic, Ca²⁺, phosphatidylglycerol (PG)-containing lipid membranes and various intermediates. Daptomycin's calcium-dependent changes in stoichiometry are particularly noteworthy. Due to this complex binding behavior, a simple fitting model is insufficient to describe this process. To rationalize and quantify the binding behavior, we are establishing a biophysical model that enables a direct comparison of Daptomycin with the new CLiP.

Keywords: Isothermal titration calorimetry (ITC), cyclic lipopeptide (CLiP), liposomes, nanodiscs

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Sponsor presentations

Sponsor presentation - Eurofins Cerep capabilities overview

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Keywords: Eurofins

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Sponsor presentation - A New AFM Toolkit for Nanoscale Investigations of the Role of Cellular Membranes in Biology and Pathology

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Cellular membranes are fundamental to life, governing essential biological functions and playing key roles in various pathological processes across bacterial, plant, and animal systems. Atomic Force Microscopy (AFM) provides an unmatched capability to visualize membrane structures and their associated molecular dynamics at nanometer resolution under near-physiological conditions.

Our newly developed high-speed AFM scanner technology enables real-time investigation of membrane mechanics, structural reorganization, and dynamic interactions with unprecedented temporal and spatial resolution. Its advanced capabilities and compact design allow seamless integration with commercially available light microscopy techniques, bridging the gap between AFM and high-resolution optical imaging.

We will showcase how these innovations facilitate the study of a wide range of membrane-related biological processes, from the molecular architecture of bacterial and plant membranes to the dynamic remodeling of mammalian cell membranes and tissues. High-speed AFM enables the nanoscale resolution of individual biomolecules, such as DNA structures with specific termini, providing insights into molecular binding mechanisms. Furthermore, we will present studies on membrane-associated cytoskeletal reorganization in living cells and automated topographical mapping of cell cultures across entire microscope stages.

Beyond fundamental biological insights, we will discuss applications in medical research, including drug-membrane interactions and dissolution studies of pharmaceutical compounds. Additionally, we highlight the full suite of BioAFM modes and accessories for studying the nanomechanical properties of membranes, cells, and tissues, offering direct correlation of multiparametric AFM data with super-resolution (STED) microscopy.

By advancing our ability to probe the mechanics, structures, and dynamics of cellular membranes at the nanoscale, this new AFM toolkit opens pathways to deeper understanding in both fundamental biology and disease research.

Keywords: Atomic Force Microscopy, High, speed AFM, cellular membranes

*Speaker

Sponsor presentation - Mass Photometry for Membrane Protein Characterisation

Frédérique Galetto ^{*† 1}

¹ Refeyn – sponsor – France

Mass Photometry is a powerful technic which measures mass with light of single bio molecules, without labels nor coating. You can quickly study agregation , oligomerisation, purity of your sample is native state in solution.

Keywords: Refeyn

^{*}Speaker

[†]Corresponding author:

Sponsor presentation - Smart Tools for Studying Membrane Transport

Artem Kondratskyi ^{*† 1}

¹ Nanion – Germany

Keywords: Nanion

*Speaker

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