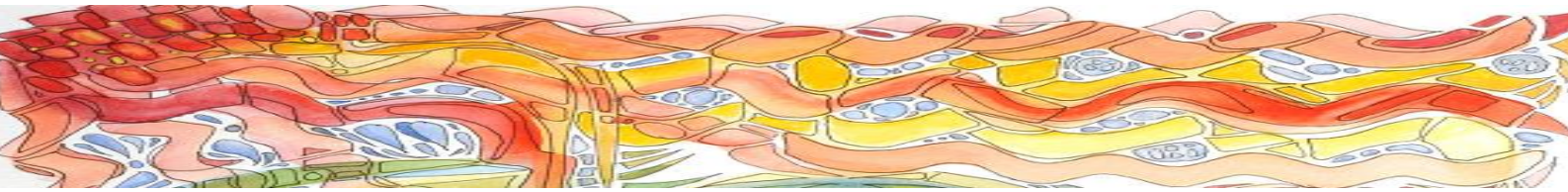

Pre-clinical development of antimicrobial peptides

International Workshop and Symposium 13 May 2022, Strasbourg, France

Programme

12th May 2022 Salle de seminaires (salle verte), Chemistry building, 1 rue Blaise Pascal

16h30 **Gianluigi Veglia**, University of Minnesota, Biochemistry and Biophysics, Minneapolis, USA, guest professor University of Strasbourg,
NMR methods to investigate membrane proteins: applications to the SERCA-Phospholamban membrane complex

19h30 Au Pont Saint Martin, La Petite France **Welcome Dinner** for speakers and guests from far (upon invitation)

13th May 2022, European Doctoral College, 46 Blvd de la Victoire, Strasbourg France

8h00 Registration

Presentations, Chair Bechinger
8h40 Welcome note

8h45 **Philippe Lavalle**, Biomaterials & Bioengineering, U. Strasbourg / INSERM, France
Homopolypeptides as building blocks for supramolecular coatings and hydrogels with antimicrobial properties

9h15 **Marina Rautenbach**, Stellenbosch University, Biochemistry, South Africa
Nano-sculpting with a Trp-rich cyclodecapeptide, tryptocidine C, to create antimicrobial surfaces

9h45 **Maria Hoernke**, Chemistry and Pharmacy, University of Freiburg, Germany
Antimicrobial activity: a network of membrane perturbations

10h15 **Coffee and poster viewing**

Presentations, Chair Heerklotz

10h45 **Sven-Ulrik Gorr,** University of Minnesota, Dental School, Minneapolis, USA
DGL13K- an antibiotic alternative to treat localized drug-resistant bacterial infections and biofilms.

11h15 **Dominique Ferrandon**, IBMC, University of Strasbourg / CNRS, France
Toward a renewed understanding of the functions of peptidic effectors of the innate immune response in the genetic model organism Drosophila melanogaster

Academic Consortium 21

With support from AC21 (www.ac21.corg)

11h45 **Ignacio Casuso**, INSERM, Marseille
Direct molecular-level visualization of the Structure-Activity-Relationship of drugs on biological membrane mimics

12h05 **Fabien Lamret**, Marie Dubus, Halima Kerdjoudj, Marius Colin, Biomatériaux et Inflammation en Site Osseux (BIOS) EA 4691, Université de Reims
Screening of extracted small metabolites from umbilical cord derived-extracellular matrix to Identify Promising Antibacterial Compounds (to be confirmed)

12h25 **lunch break and poster viewing**

Presentations, Chair Gorr

14h00 **Lorenzo Stella**, University Tor Vergata, Chemistry, Rome, Italy,
guest professor of the University of Strasbourg
Quantification of the association of antimicrobial peptides with live bacterial cells: what have we learned?

14h30 **Wilma van Rensburg**, Stellenbosch University, Biochemistry, South Africa
How it's made: self-sterilizing peptide materials

15h00 **Valérie Heitz**, Chemistry, University of Strasbourg / CNRS, France
An antimicrobial peptide connected to a π-extended porphyrin for photoinactivation of bacteria

15h30 **Shuai Shi**, Maria Hoernke, Chemistry and Pharmacy, University of Freiburg, Germany
Leaky membrane fusion: an ambiguous effect induced by antimicrobial polycations

15h50 **Coffee and poster viewing**

Presentations Chair Rautenbach

16h20 **Niels Gaudens**, Organic and Macromolecular Chemistry and NMR Expertise Centre Ghent, Belgium
Structural investigations of antimicrobial lipopeptides from Pseudomonas: challenges and (some) solutions

16h50 **Heiko Heerklotz**, Pharmacy, University of Freiburg, Germany
Parameters controlling the activity and selectivity of cyclic lipopeptides to permeabilize lipid membranes

17h20 **Ahmad Saad**, Chemistry, University of Strasbourg / CNRS, France
Solid-state NMR investigation of the synergistic action of magainin antimicrobial peptides

17h50 – 19h45 **Bretzel/snacks & wine/bee and poster viewing** (20h closure of building)

Academic Consortium 21

With support from AC21 (www.ac21.corg)

POSTERS

1. **RW peptides as potential candidates for antifungal drug development**
   Gamuchirai Mamhende and Marina Rautenbach
   Department of Biochemistry, Stellenbosch University, South Africa

2. **Formulation of natural cyclodecapeptides for surface sterilisation**
   Christopher Borrageiro, Marina Rautenbach

3. **Introducing Metals: Formulations for Directing Peptide Nano-assembly**
   Carmen de Villiers, Marina Rautenbach

4. **Multi-functional activity assay for discovering of antifungal peptides and compounds against planktonic and biofilm forms of *Candida* species**
   Bernice Jenkins Barnard, Wikus Ernst Laubscher, Marina Rautenbach

5. **Making use of intrinsic tryptophan fluorescence for the characterization of membrane-active peptides**
   Iulia Carabadjac, Jessica Steigenberger, Niels Geudens, Yentl Verleysen, José C. Martins, and Heiko Heerklotz

6. **Parameters controlling the activity and selectivity of cyclic lipopeptides to permeabilize lipid membranes (poster accompagnying a talk)**
   Jessica Steigenberger, Niels Geudens, Yentl Verleysen, Vic de Roo, José C. Martins, and Heiko Heerklotz

7. **Vesicle budding as an effect of area asymmetry nduced by lysolipids**
   L. Hua1, M. Kaiser1 and H. Heerklotz

8. **Rapid structural elucidation of cyclic lipopeptides via NMR fingerprint matching**
   Vic De Roo, Yentl Verleysen, Benjámin Kovács, René De Mot, Monica Höfte, Annemieke Madder, José C. Martins, Niels Geudens

9. **Effects of cyclic antimicrobial peptides on various model membranes.**
   Katharina Beck, Janina Nandy, Maria Hoernke

10. ***Drosophila melanogaster CG44404* and *CG45045* encode short peptides, not lncRNAs, that act together against outer membrane vesicles (OMVs) produced by Gram-negative bacteria**
    Chuping Cai, Adrian Acker, Li Zi, Samuel Liegeois, Nicolas Matt, Dominique Ferrandon

11. **A Toll pathway effector protects *Drosophila* specifically from distinct toxins secreted by a fungus or a bacterium**
    Jianqiong Huang, Yanyan Lou, Jiyong Liu , Philippe Bulet, Renjie Jiao, Jules A. Hoffmann, Samuel Liégeois , Zi Li , and Dominique Ferrandon

12. **Insights in the mechanism of Mag2/PGLa synergism by fluorescence techniques**
    Christopher Aisenbrey, Mariana Amaro, Martin Hof and Burkhard Bechinger[1]

13. **Solid-state NMR investigations of the MHC II transmembrane domains: topological equilibria and lipid interactions.**
    Evgeniy Salnikov, Christopher Aisenbrey, Bianca Pokrandt, Britta Brügger, Burkhard Bechinger

14. **Biophysical investigations of antimicrobial peptide mimics for mechanistic studies**
    Kathakali De, Christopher Aisenbrey, Burkhard Bechinger

15. **Controlling α-helical peptide fibril/crystal formation and membrane interactions with anions**
    Jian-Qiao Jiang, Burkhard Bechinger

16. **Structural studies of peptide supramolecular self-assemblies used for lentiviral transduction**

    Romuald Manca, Morane Lointier, Jianqiao Jiang, Jésus Raya, Elise Glattard, Burkhard Bechinger

17. **Investigations of the peptide-peptide and peptide-lipid interactions underlying the synergism of antimicrobial peptides in membranes.**

    Adrien Gebus, Elise Glattard, Jésus Raya, Ahmad Saad, Burkhard Bechinger

Presentations by guest professors of the Univeristy of Strasbourg during the week before and after the conference:

Prof. Gianluigi Veglia

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA.

a) NMR methods to investigate membrane proteins: applications to the SERCA-Phospholamban membrane complex

Phospholamban (PLN) is a regulin that controls the cardiac Ca^{2+}-transport response to β-adrenergic stimulation, thus modulating cardiac output during the fight-or-flight response. PLN binds to the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) in the sarcoplasmic reticulum, keeping this enzyme's function within a narrow physiological window. PLN phosphorylation by cAMP-dependent protein kinase A or increase in Ca^{2+} concentration reverses the inhibitory effects through an unknown mechanism. Using solid-state NMR spectroscopy and replica-averaged NMR-restrained structural refinement, we found that phosphorylation of PLN's cytoplasmic domain disrupts the inhibitory interactions at the transmembrane binding interface of the SERCA-PLN complex. These effects are propagated to the enzyme's active site, augmenting Ca^{2+} transport. Our findings unveil a signal transduction mechanism operated by posttranslationally modified bitopic membrane proteins.

b) Solid-state NMR unveils the role of sarcolipin in muscle thermogenesis.
The sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) plays a central role in muscle contractility and nonshivering thermogenesis. SERCA is regulated by sarcolipin (SLN), a single-pass membrane protein belonging to the regulin family. SLN is thought to uncouple Ca^{2+} transport from ATP hydrolysis, promoting futile enzymatic cycles and heat generation. However, the molecular determinants for regulating heat release by the SERCA/SLN complex are unclear. Using thermocalorimetry, chemical cross-linking, and solid-state NMR spectroscopy in oriented phospholipid bicelles, we show that SERCA's functional uncoupling and heat release rate are dictated by specific SERCA/SLN intramembrane interactions, with the carboxyl-terminal residues anchoring SLN to the SR membrane in an inhibitory topology. Systematic deletion of the carboxyl terminus does not prevent the SERCA/SLN complex formation but reduces uncoupling in a graded manner. These studies emphasize the critical role of lipids in defining the active topology of SLN and modulating the heat release rate by the SERCA/SLN complex, with implications in fat metabolism and basal metabolic rate.

c) NMR characterization of functional and dysfunctional allosteric communication within a kinase: implications to disease
cAMP-dependent protein kinase A (PKA) is the archetypical eukaryotic kinase belonging to the AGC-kinase family. The structure of the catalytic subunit (PKA-C) is highly conserved and has represented an ideal model for establishing kinase structure-function-dynamics relationships. PKA-C comprises two lobes: the N-lobe with the ATP binding site and the C-lobe that harbors the substrate-binding groove. A distinct feature of PKA-C is the allosteric binding cooperativity between the nucleotide and substrates. In past decades, several PKA-C mutations have been

linked to the development of adenocarcinomas, leading to Cushing's syndrome and myxomas, rare forms of noncancerous cardiac tumors. We used solution NMR spectroscopy to characterize the structural and dynamic changes of wild-type and mutated PKA-C. For the mutant analyzed, we found that a disruption of the nucleotide-substrate cooperativity correlates with the loss in substrate fidelity and dysregulation of the kinase by the regulatory subunits and the endogenous inhibitor PKI. The loss in allosteric cooperativity may constitute a common trait for both orthosteric or allosteric mutations of PKA-C, leading to disease.

Presentations by Lorenzo Stella during the week after the conference:

From liposomes to cells: filling the gap between biophysical and microbiological studies of the activity and selectivity of host-defense peptides.

Exploring the allosteric mechanism of oncogenic phosphatase SHP2 for the design of peptide inhibitors of its protein-protein interactions.

Speakers Abstracts

NMR methods to investigate membrane proteins: applications to the SERCA-Phospholamban membrane complex

Gianluigi Veglia

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA.

Abstract:

Phospholamban (PLN) is a regulin that controls the cardiac Ca^{2+}-transport response to β-adrenergic stimulation, thus modulating cardiac output during the fight-or-flight response. PLN binds to the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) in the sarcoplasmic reticulum, keeping this enzyme's function within a narrow physiological window. PLN phosphorylation by cAMP-dependent protein kinase A or increase in Ca^{2+} concentration reverses the inhibitory effects through an unknown mechanism. Using solid-state NMR spectroscopy and replica-averaged NMR-restrained structural refinement, we found that phosphorylation of PLN's cytoplasmic domain disrupts the inhibitory interactions at the transmembrane binding interface of the SERCA-PLN complex. These effects are propagated to the enzyme's active site, augmenting Ca^{2+} transport. Our findings unveil a signal transduction mechanism operated by posttranslationally modified bitopic membrane proteins.

Homopolypeptides as building blocks for supramolecular coatings and hydrogels with antimicrobial properties

Philippe Lavalle[1,2], Varvara Gribova[1,2], Lorène Tallet[1,2], Cynthia Calligaro[3], Eloïse Lebaudy[1,2], Florent Barbault[4], Engin Nihal Vrana[2]

[1]Institut National de la Santé et de la Recherche Médicale, Inserm UMR_S 1121 Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg, Strasbourg, France

[2]Université de Strasbourg, Faculté de Chirurgie Dentaire, Strasbourg, France

[3]SPARTHA Medical, Centre de Recherche en Biomédecine de Strasbourg, Strasbourg, France

[4]ITODYS, Université de Paris, CNRS UMR 7086, Paris, France

Abstract:

All implantable biomedical systems face several risks once in contact with the host tissue and the main one is the development of bacterial biofilms. To prevent such infections, a multifunctional surface coating that can address this issue would significantly improve clinical outcomes.

Polyarginine (PAR), polylysine (PLL), or polyornithine (POR) are synthetic highly cationic homopolypeptides that can act as antimicrobial agents due to their positive charges. We developed a family of new supramolecular coatings based on these homopolypeptides assembled with hyaluronic acid (HA). We demonstrate that exclusively coatings constructed with homopolypeptide chains of 30 residues in length (PAR30, PLL30 or POR30) provide a strong antimicrobial activity[1]. These coatings have an inhibitory effect on all pathogenic bacteria associated with infections of medical devices, including antibiotic resistant bacteria. However, PAR30/HA coating appear as the most effective and the most biocompatible coating. No secondary structure of the homopolypeptides is needed to provide the activity and the mechanism is related to a physical damage on the membrane once the homopolypeptide sticks to the bacteria, with no need to interfere with the bacteria metabolism. Moreover, this assembly can also be fabricated in the form of hydrogel[2,3] useful to provide antibacterial properties to porous implants like surgical meshes.

References:

1. A. Mutschler, L. Tallet, M. Rabinea, C. Dollinger, M.-H. Metz-Boutigue, F. Schneider, B. Senger, N. E. Vrana, P. Schaaf, P. Lavalle; *Chem. Mater.* **2016**, 28, 8700-8709
2. V. Gribova, L. Petit, C. Seguin, S. Fournel, A. Kichler, N.E. Vrana, P. Lavalle ; *Macromol. Biosci.*, **2022**, 22000043
3. V. Gribova, F. Boulmedais, A. Dupret-Bories, C. Calligaro, B. Senger, N. E. Vrana, P. Lavalle, *ACS Appl. Mat. Int.*, **2020**, 12, 19258

Nano-sculpting with a Trp-rich cyclodecapeptide, tryptocidine C, to create antimicrobial surfaces

Marina Rautenbach[1]*, Vikas Kumar[1], Wilma van Rensburg[1], Christopher Borrageiro[1], Carmen de Villiers[1] , Jacky L. Snoep[1,2], Henrich H. Paradies[3]

[1]*Stellenbosch University, Department of Biochemistry, Stellenbosch, South Africa*

[2]*Molecular Cell Biology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

[3] *Jacobs-University, Department of Chemistry and Life Science, Bremen, Germany*

*email: mra@sun.ac.za

Abstract:

Tryptocidine C (TpcC), a Trp-rich cyclodecapeptide is a minor constituent in the antibiotic tyrothricin complex from *Brevibacillus parabrevis*. TpcC possesses a high tendency to oligomerise in aqueous solutions and leads to ion pores in target cells. TpcC tends to stick to various materials forming robust self-sterilising surfaces. Surface-dried TpcC forms distinct self-assembled nanoparticles, that can be manipulated by the solvents system and TpcC concentration. TpcC, dried from a high concentration in 15% ethanol, primarily assemble into small 24.3 nm diameter nanospheres. At 16 □M, a concentration near the CMC and most MICs of TpcC, led to polymorphic architectures such as sheets, small nanospheres and larger nanospheroids, when dried from 20-50% ethanol. These polymorphic surface morphologies correlated with maintenance of fluorescence properties in solution and the surface-derived antibacterial activity against *Staphylococcus aureus* over time. Conversely, there was a significant change in fluorescence over time and loss in activity in the 10% and 75% ethanolic TpcC preparations where 3-D crystals were observed. This indicated that TpcC oligomerisation in solutions with 20-50% ethanol leads to metastable structures with a high propensity for release of antimicrobial moieties such as amphipathic dimers, while those leading to crystallisation limit active moiety release. TpcC nano-assemblies can find application in antimicrobial coatings/materials, surface disinfectants, food packaging and wound healing materials.

Antimicrobial activity: a network of membrane perturbations

Maria Hoernke

Chemistry and Pharmacy, Albert-Ludwigs-Universität Freiburg, Freiburg i.Br. Germany

Abstract:

The interaction of polycations such as antimicrobial peptides or biomimetic polymers with membranes of various lipid compositions can cause a variety of physical-chemical membrane perturbations. These membrane biophysics are therefore widely used for the selection or modification of natural peptides or rational design of biomimetic compounds in the search for alternatives to classical antibiotics. To date, the role of certain types of membrane perturbations in antimicrobial killing is still unclear. My team investigates the principles of activity and selectivity of membrane-active antimicrobial polycations for membranes of different lipid composition (representing different species or microbes). The central perturbation is membrane permeabilization or leakage. However, many observations cannot be explained with leakage alone, even when considering the many different leakage mechanisms. I will show that selectivity for certain lipid compositions depends on properties of the polycation but also on the predisposition of the lipid composition for certain leakage mechanisms. Additionally, membrane fusion and aggregation can both influence membrane leakage or cause unnoticed measurement artefacts. Furthermore, also electrostatic lipid clustering (the local enrichment of negatively charged lipids by positively charged polycations) can modulate both leakage and fusion activity. This type of mechanistic understanding can be used not only in the search and design of antimicrobial compounds, but also for creating drug delivery systems.

DGL13K- an antibiotic alternative to treat localized drug-resistant bacterial infections and biofilms.

Sven-Ulrik Gorr,

University of Minnesota

Abstract:

"Imagine a world in which a paper cut can lead to infection that can't be controlled". Antibiotic resistance is a global problem that can affect anyone in any country. In the United States, more than 2.8 million drug-resistant infections occur each year and more than 35,000 people die as drug-resistant infections threaten advances in surgery, wound healing, cancer treatment, organ transplants and other areas of modern medicine.

Our lab has developed a family of antimicrobial peptides based on the core peptide GL13. The design of this peptide was based on the structure of the salivary protein BPIFA2. Targeted modifications have produced a peptide with broad antibacterial activity. The L-amino acid version of this peptide is active against gram-negative bacteria while the D-amino acid version is also active against gram-positive. The difference appears to be mediated by D-alanylation of the cell wall in gram-positive bacteria. The lead peptide DGL13K is effective against drug-resistant priority pathogens, including *P. aeruginosa, S. aureus. K. pneumonia, E. faecalis and A. baumanii*. The peptide is effective against bacterial biofilms and does not cause de novo resistance in vitro. Unlike traditional antibiotics, GL13K inhibits endotoxin activity and improves survival in a mouse peritoneal sepsis model. DGL13K shows a promising toxicity profile in vitro and in vivo with a therapeutic index (LD50/MIC) of 200 in cell culture experiments. DGL13K is active in saliva, urine and synovial fluid and in vivo experiments have demonstrated efficacy in a mouse skin burn wound infection model as well as an insect infection model. In the latter model, infection and peptide efficacy were monitored in real time in vivo. To improve topical delivery of DGL13K, we have developed peptide hydrogels that retain antibacterial activity. The properties described here make DGL13K an attractive candidate for further preclinical development as a treatment for localized infections.

References:

1. Hirt, H., J.W. Hall, E. Larson, and S.U. Gorr. 2018, PLoS One. 13:e0194900.
2. Gorr, S.U., C.M. Flory, and R.J. Schumacher. 2019, PLoS One. 14:e0216669.
3. Gorr, S.-U., H.V. Brigman, J.C. Anderson, and E.B. Hirsch. 2020. bioRxiv: 2020.2005.2008 085282.

Toward a renewed understanding of the functions of peptidic effectors of the innate immune response in the genetic model organism *Drosophila melanogaster*

Dominique Ferrandon[1-3], Rui Xu[1-3], Jianqiong Huang[1-3], Yanyan Lou[1-3], Chuping Cai[1-3], Li Zi[1], and Samuel Liégeois[1-3]

[1]*Sino-French Hoffmann Institute, Guangzhou Medical University, Guangzhou, China*

[2]*Université de Strasbourg, Strasbourg, France*

[3]*Modèles Insectes de l'Immunité Innée, UPR 9022 du CNRS, Strasbourg, France*

Abstract:

Historically, the first animal antimicrobial peptides (AMPs) have been isolated in insects by Hans Boman in 1980. This ushered a new era of AMP isolation and characterization, in insects as well as in other living organisms. In *D. melanogaster*, the cloning of the corresponding genes led to the study of their promoters and the identification of binding sites for the NF-B transcription factor. Genetic studies performed in Strasbourg established that two distinct NF-β pathways, Immune deficiency (IMD) and Toll, play a critical role in the host defense against Gram-negative bacterial infections (IMD) and Gram-positive as well as fungal infections (Toll). Among hundreds of genes regulated at the transcriptional level are included those belonging to seven families of AMP genes as well as some further putative Toll pathway effectors such as *Bomanins* and *BaramicinA*. The advent of CRISPR-Cas9 has allowed the fine genetic dissection of AMP genes by the laboratory of Bruno Lemaitre thus allowing to partially delineate the exact functions of AMPs in host defense. Unexpectedly, several studies have reported an exquisite specificity of action of some, but not all AMPs, which appear to be required in the host defense only against certain pathogens. Thus, the immune response expresses both "generalist" AMPs, which may synergize in a cocktail effect, and tailored AMPs selected to deal with pathogens that exert a significant evolutionary pressure on the host.

We have ourselves focused on uncharacterized peptidic effectors of these pathways, including Bomanins, BaramicinA-derived peptides, and two other peptides derived from genes thought initially to encode lncRNAs. We have discovered that they selectively play a role in host defense by protecting the host from the action of specific secreted toxins or outer-membrane vesicles (OMVs) and do not directly attack the pathogens. Thus, evolution can select molecules able to neutralize specific virulence factors or their effects. Understanding how these effectors function will represent a novel field of investigations. These findings taken together provide a novel example of a form of immunity that is distinct from resistance and is known as resilience/disease tolerance.

Direct molecular-level visualization of the Structure-Activity-Relationship of drugs on biological membrane mimics

Ignacio Casuso

INSERM, Marseille

Label-free visualization of the activity of drugs on membranes at the molecular level has recently become a reality using state-of-the-art high-speed atomic force microscopy (HS-AFM), as shown for Daptomycin [1]. Thanks to the label-free visualization of the activity of drugs, the Structure-Activity-Relationship (SAR) can be assessed in an unprecedented manner: as uniquely the hs-afm assesses, among others, the stoichiometry of the drug-oligomers on the membrane, the energy-landscape of the interaction of the drug-oligomers, the transients at the drug-induced molecular-level structural transitions at the cell membrane during the early, middle, and later stages of the action of the drugs. An additional advantage of our approach is that the event of a permeabilization of the membrane by the drug can be mimicked, as the two sides of the membrane, or only one, may be exposed to drug. At this communication, you will learn that the HS-AFM opens the door to the non-averaged molecular assessment of the SARs of the membrane-interacting drugs and this development presents exceptional opportunities for drug-discovery applications.

References:

1. Zuttion, F., et al., *High-speed atomic force microscopy highlights new molecular mechanism of daptomycin action.* Nature communications, 2020. **11**(1): p. 1-16.

Screening of extracted small metabolites from umbilical cord derived-extracellular matrix to Identify Promising Antibacterial Compounds

Fabien Lamret, Marie Dubus, Halima Kerdjoudj, Marius Colin

Biomatériaux et Inflammation en Site Osseux (BIOS) EA 4691, Université de Reims Champagne Ardenne, 51100 Reims, France.

The field of regenerative medicine has recently seen an emerging trend toward decellularized extracellular matrix (ECM) as a biological scaffold for stem cell-delivery. Human umbilical cord represents a valuable opportunity from both technical and ethical point of view to obtain allogenic ECM and demonstrated a potential application in treatments of wound infections. In a previous study, we demonstrated that a decellularization process, besides having the benefit to prevent rejection from the patient and reduce legislative restrictions, enhanced the antibacterial, antibiofilm and immunomodulatory properties of the Wharton's Jelly derived from umbilical cord. Herein, we sought to identify the post-partum metabolites within the umbilical cord, with the intrinsic antibacterial and antibiofilm properties. After methanol extraction followed by Centrifugal Partition Chromatography (CPC), Nuclear Magnetic Resonance (NMR) and Liquid Chromatography / Mass Spectrometry (LC/MS) techniques revealed the composition of the ten CPC-fractions. The analysis revealed that the main constituent of the umbilical cord derived-ECM might be oligosaccharides, followed by free fatty acid, niacinamide and hypoxanthine. A high diversity of minor sterols, steroids, and prostaglandins were also detected. The antibacterial and antibiofilm effects of the ten fractions are currently under investigation against *Staphylococcus aureus*, first species involved in bone infection as well as in skin and soft tissues infections, to identify the compounds of interest. Preliminary results showed that several fractions displayed antibiofilm properties. To deeply decipher the antibacterial peptides within DC-WJ, fractioning and peptidomic explorations are in progress. Thus, umbilical cord might represent a valuable source of antibacterial and antibiofilm molecules.

Acknowledgments: This work was supported partially by UmbRegen project and 3D4MED Interreg project

Quantification of the association of antimicrobial peptides with live bacterial cells: what have we learned?

M. R. Loffredo[1], C. Troiano[2], S. Bobone[2], B. Casciaro[1], M. L. Mangoni[1], L. Stella[2]

[1]Department of Biochemical Sciences, Sapienza University of Rome, 00185 Rome, Italy;
[2]Department of Chemical Science and Technologies, University of Rome Tor Vergata, 00133 Rome, Italy.
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Abstract:

Antimicrobial peptides (AMPs) are crucial effectors in innate immunity. Since they usually kill bacteria by perturbing their cellular membranes, they are promising molecules to fight drug-resistant microbes. Therapeutic applications and a full understanding of the biological functions of AMPs have been hampered by a chasm between biophysical studies on model bilayers and microbiological experiments. For instance, the affinity of AMPs for their target membranes is an essential determinant of peptide activity and selectivity. This property is well characterized using artificial bilayers but was essentially unexplored for real cells. We developed a spectroscopic assay allowing the quantitative determination of peptide association to live bacterial cells. Characterizing this basic aspect has led to multiple insights into the function of AMPs.[1-7] Our data showed that millions of peptides must bind to each cell to cause its death. This number even exceeds the complete coverage of cell membranes. We observed that peptide affinity for bacteria whose membranes have already been perturbed by sonication is an order of magnitude higher than for live, healthy cells. Therefore, after membrane perturbation, AMPs accumulate inside the cell, binding to intracellular components. This sequestration of peptide molecules by dead cells can protect the remaining bacteria from AMP activity. Based on cell-binding results, we predicted and observed a specific trend for the cell-density dependence of AMP activity. This inoculum effect is significant for cell densities above 5×10^5 cells/mL, while for lower densities the active concentrations are essentially constant, in the micromolar range. As a consequence, AMP activity and selectivity depend on the concentrations of target and host cells. These results question the clinical utility of activity and selectivity determinations performed at fixed, standardized cell densities. Overall, our findings clarified some key aspects of AMP function but also led to several new questions, which will be addressed during the presentation.

References:

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How it's made: self-sterilizing peptide materials

Wilma van Rensburg[1], Marina Rautenbach[1]

[1]Stellenbosch University, Department of Biochemistry, BIOPEP Peptide Group, 7600, Stellenbosch

Abstract:

The propagation of pathogenic microorganisms on various surfaces can be decreased with the use of modified antimicrobial and antifouling material, which in turn, will minimize the occurrence of infections, transfer, and spoilage. There has been a renewed focus on naturally produced antimicrobials as alternative modes of treatment with the increased consumer demand for 'green' solutions. Tyrocidines, cyclodecapeptides naturally produced by a soil bacterium *Brevibacillus parabrevis*, have a broad spectrum of activity against bacteria, filamentous fungi, and yeasts. Continual losses in tyrocidine production highlighted the possible association of peptides to various materials and surfaces. It was observed that tyrocidines readily associates with both synthetic and natural polymeric materials, with a selectivity towards polysaccharide-type materials, such as cellulose. Peptide-treated cellulose was found to remain active after exposure to a broad pH range, high temperature, salt solutions, water washes, and organic solvent washes, with the sterilizing activity partially affected by 1% SDS and 70% acetonitrile. It is hypothesised that the tyrocidines form two distinct types of oligomers which is determined by the environment, one type stabilised by hydrophobic interactions and the other by hydrogen bonds in extended sheets-like structures and small nanoparticles. Both types of oligomers and nanostructures would allow for an electrostatic interaction between the polar groups protruding from the peptide structures and hydroxyl groups of the cellulose-based materials. This first electrostatic interaction is proposed to first form a seeding layer followed by new self-assembly structures on the material's surface. For synthetic polymeric materials such as polypropylene, the initial interaction would depend on hydrophobic interaction for creating a seed layer for self-assembly. However, once associated the hydrophobic force and H-bond stabilised peptide nanostructures do not readily dissociate from the base material. The robust association between the tyrocidines and various materials holds great promise in applications focused on preventing surface contamination and creating self-sterilising materials.

An antimicrobial peptide connected to a π-extended porphyrin for photoinactivation of bacteria

Valérie Heitz

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Abstract:

Antimicrobial photodynamic therapy (a-PDT) is a promising strategy to kill multi-resistant bacteria.[1] The action mode of this localized treatment is based on the generation of reactive oxygen species (ROS) upon light excitation of a photosensitizer that binds to bacteria, leading to their eradication. A major strength of this photodynamic treatment is the absence of development of bacterial resistance and its effectiveness on resistant strains.[2] Nevertheless, the selectivity of a-PDT towards host cells remains an issue as well as the wavelengths of light excitation used to kill cells generally situated outside the optical therapeutic window.[3]
Regarding these issues, our group in collaboration with the group of B. Bechinger have developed a porphyrin-peptide conjugate for a-PDT. It consists of a porphyrin photosensitizer[4] connected to an antimicrobial peptide PGLa[5] designed to target and kill bacteria upon light excitation in the near infrared (Figure 1). The antimicrobial activity of the conjugate on Gram-positive and Gram-negative bacteria will be presented.

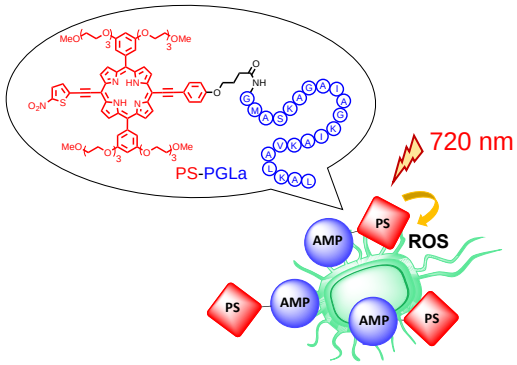

Figure 1. A near infrared photosensitizer connected to an antimicrobial peptide for photoinactivation of bacteria.

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Leaky membrane fusion: an ambiguous effect induced by antimicrobial polycations

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Abstract:

Both, antimicrobial peptides, and their synthetic mimics are potential alternatives to classical antibiotics, as they can induce several membrane perturbations including permeabilization. Especially in model studies, interactions of such polycations with charged vesicles often cause vesicle aggregation and fusion. We show that these side effects cannot be ignored, as they can change the outcome of biophysical studies unnoticed.
We study an antifungal but also antibacterial biomimetic polymer representing the group of highly charged highly selective membrane-active compounds. The interactions with negatively charged PG/PE vesicles were examined and an unexpected leakage mechanism was determined in a combination of methods. By a fluorescence lifetime-based calcein leakage assay, significant leakage was found to occur only above a polymer concentration that neutralizes the lipid charges. At this concentration, vesicle aggregation also evolves into vesicle fusion. Furthermore, membrane fusion and aggregation were prevented by decorating the vesicles with PEG-chains. So that the relation of vesicle aggregation and vesicle fusion with membrane permeabilization was elucidated.
Interestingly, the PEG-chains inhibited not only vesicle aggregation and fusion, but also membrane permeabilization, even though the binding of polymer to vesicles is not affected markedly. Therefore, and with additional experiments, we conclude that leakage is induced by leaky fusion instead of an independent leakage mechanism. This has important implications not only for vesicle aggregation or fusion as unnoticed artefacts in biophysical experiments potentially leading to severe misinterpretations. Regarding biological activity, induced fusion may interfere with intracellular compartments of pathogenic fungi or mammalian cells, but is less relevant to bacteria.

Structural investigations of antimicrobial lipopeptides from *Pseudomonas*: challenges and (some) solutions¶

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Abstract:¶

Pseudomonas are ubiquitous bacteria and outstanding producers of bioactive secondary metabolites in support their eclectic lifestyle (e.g., iron scavenging, swarming motility, biofilm formation, pathogenicity, cooperation or antagonism) [1]. Of these metabolites, cyclic lipopeptides – CLIPs in short – have enjoyed the attention of numerous researchers because of their antimicrobial activity profile and anti-proliferative properties, which holds some potential for biomedical applications [2]. Their biosynthesis through non-ribosomal peptide synthetases creates a large structural diversity, introducing opportunities to learn how Nature uses the same molecular blueprint to generate a swiss-army like diversity of effects.[2]
As always, understanding CLiP structure and linking it to biological activity is considered essential to uncover the molecular mode of action and ultimately design analogues with improved potency while mitigating undesirable properties. Although NMR based approaches to determine the structure and conformation of peptides is well-established and considered rather routine, we found the road to be marred by potholes in the case of CLiPs, requiring non-standard and partly novel approaches. First, the incorporation of non-proteinogenic amino acids with a majority of residues displaying D-configuration through the (currently) unpredictable action of epimerization domains in the non-ribosomal assembly line, magnifies the chemical structure elucidation challenge [3]. This is addressed using an NMR based spectral fingerprinting approach, which can also be used for dereplication approaches [4]. Second, it is generally accepted that CLiPs act through perturbation and/or permeation of the cellular membrane [2]. Thus, the conformation needs to be investigated under membrane mimicking circumstances rather than merely aqueous solution. The determination of the conformation of various CLiPs in such conditions as well as the investigation of location and orientation of the CLiP across the water/lipid interface using NMR and modelling, and insight derived therefrom will be discussed [5]. In particular, the added value generated by the possibility to biosynthetically introduce ^{13}C and ^{15}N isotope labelling using the Pseudomonas own NRPS will be highlighted. Their insertion allows the application of NMR methodologies allowing to extract conformations sensitive scalar couplings while directly identifying and monitor hydrogen bonds under a variety of conditions. Using these, conformational changes upon insertion in DPC or SDS micelles can be investigated. A final challenge, relating to understanding the molecular basis for the generation of well-defined supramolecular structures by certain CLiPs under low polarity solvent conditions may be touched upon, time permitting.

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Parameters controlling the activity and selectivity of cyclic lipopeptides to permeabilize lipid membranes

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Abstract:

We have studied the membrane permeabilizing effect of *Pseudomonas* lipopeptides such as viscosin, pseudodesmin A and tolaasin along with synthetic analogs. The aim of this work was to understand the molecular parameters of peptide and membrane governing activity and selectivity of membrane permeabilization.

First, we checked how a synthetic variation of the acyl chain length of pseudodesmin affects permeabilizing activity. We observed a bell-shaped curve with the maximal activity observed for the native, decyl chain. Shorter chains compromised membrane partitioning whereas longer ones rendered the membrane-bound peptides less effective.

Second, we tested the effect of electrostatic interactions by investigating the neutral pseudodesmin along with its anionic (viscosin) and cationic (viscosin E2K) analogs with respect to their activity against zwitterionic and negatively charged membranes. It turned out that, as expected, the positive charge of the peptide enhanced its partition coefficient, K, into negatively charged membranes but, at the same time, reduced its membrane-permeabilizing effect there substantially.

Third, we investigated how target membrane thickness (as varied in terms of the chain length of symmetric, monounsaturated phosphocholines) affects the activity of tolaasin. Thicker membranes were found more resistant against permeabilization, which can be explained by their increased mechanical stability. This was not considered trivial since thicker membranes should suffer from a stronger mismatch between peptide and lipid chains.

Overall, membrane selectivity seems often governed by a balance of competing effects. What favors membrane partitioning may compromise the activity of the membrane-bound peptide. With lipid concentration increasing beyond 1/K, selectivity switches from the partitioning controlled to the local activity-controlled regime.

Solid-state NMR investigation of the synergistic action of magainin antimicrobial peptides

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Abstract:

Magainin and PGLa are cationic, amphipathic antimicrobial peptides isolated from the skin of *Xenopus Laevis* African frog, known to interfere with the barrier function of the bacterial membrane and cause bacterial killing. They adopt alpha-helical structures in membrane environments and can disrupt the lipid bilayer organization and ordering. When added as a mixture, they show enhanced synergistic activities in both antimicrobial assays as well as biophysical experiments[1,2]. Several models have been proposed to understand the synergistic behavior between PGLa and magainin, however, the mechanism behind this is still elusive. The objective is to elaborate a high-resolution study at molecular level of the peptide-lipid assemblies to decipher the mechanism of synergism between the two peptides.

In the present work, we started a structural analysis of the interaction between magainin 2 and a liposome model that mimics a bacterial membrane composed of POPE:POPG (3/1 molar) lipids using MAS solid-state NMR. We study the secondary structure, insertion, and dynamic of the uniformly ^{13}C-^{15}N labeled peptide on specific positions in the lipid bilayer. Experimental chemical shift analysis of ^{13}C indicates an alpha-helical conformation of magainin 2 in the lipid membrane. While the lack of correlations between peptide and lipid acyl chain signals in HETCOR experiment reveals that the peptide is not inserted in the hydrophobic core of the membrane and relies on the water lipid interface. Furthermore, signal intensities analysis of CP and INEPT experiments show that the peptide undergo isotropic motion in the liquid crystalline phase which is partially removed at the gel phase.

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Poster Abstracts

RW peptides as potential candidates for antifungal drug development

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Abstract:

In recent years, fungal infections have become a severe global healthcare challenge, causing an estimated 1.6 million mortalities every year[1]. This is attributable to an unprecedented rise in the population of immunocompromised individuals who are highly susceptible to fungal infections, as well as the emergence of antimicrobial resistance towards existing antifungal drugs. The dire situation is exacerbated by a drug arsenal constrained with respect to the mechanisms of action[2]. Thus, there is a great need for the development of effective, yet safe antifungal agents with novel mechanisms of action. To this end, antimicrobial peptides (AMPs) are attractive candidates for antifungal drug development due to their multiple modes of action which limit the development of antimicrobial resistance. Accordingly, this motivates the characterisation of the antifungal activity of a library of Arg and Trp rich (RW) hexapeptides with known antibacterial activity[3]. Most of the RW peptides in the library exhibited potent activity against the two notorious human pathogens – *C. albicans* and *A. fumigatus*, albeit with different activity profiles between the two organisms. More importantly, the peptides induce little to no haemolysis, highlighting their suitability for development as systemic drugs. While the elucidation of their mechanism of action is still ongoing, we showed that the RW peptides are non-lytic towards their targets and ROS may be involved in their killing mechanism[4]. Additionally, more potent antifungal activity was observed for cyclic analogues in comparison to the linear analogues, while the distribution of the Arg and Trp in the sequence also influenced the activity. Therefore, analysis of structure activity relationships is crucial to understanding peptide activity and in turn antifungal peptide structural optimisation.

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Formulation of natural cyclodecapeptides for surface sterilisation

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Abstract:

Microbial surface colonization and adhesion in both the medical and industrial sectors are becoming a greater threat. Surface contamination can be prevented by treatment of surfaces by antimicrobial formulations or creating materials incorporating antimicrobial peptides (AMPs) as the active ingredients. The tyrocidines (Trcs), a cyclodecapeptide produced by the soil bacterium *Brevibacillus parabrevis*, have broad-spectrum activity against various bacteria, filamentous fungi, and yeast. However, the Trcs readily form higher order oligomers and aggregates resulting in a decrease in selectivity and activity. The influence of six different solvents and additivities on the Trcs aggregation and activity was assessed to determine the most optimal formulation conditions. Findings showed that formulations containing 1% glycerol (Glr) with $CaCl_2$ had improved or maintained activity against *L. monocytogenes* and *S. aureus* in the presence of propylene glycol (PG) or tertiary butyl alcohol (TBA). However, Ca^{2+} formulations only enhanced activity against *L. monocytogenes*, indicating that Ca^{2+} has a selective influenced mode of action, but may act as a stabiliser for the peptide against *S. aureus.* The 1% Glr + $CaCl_2$ TBA formulation of Trc mix had the highest activity against both targets compared to other formulations which may be related to the chaotropic ability of the solvents and additives. The improvement may be related to disrupting large oligomers, but not the amphipathic dimers required for activity. This study highlighted that solvents and additives must be optimised when depositing antimicrobial peptide such as the tyrocidines on surfaces to ensure optimal activity.

Introducing Metals: Formulations for Directing Peptide Nano-assembly

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Abstract:

The propensity of tyrocidines to adhere to a variety of surfaces makes these naturally produced antimicrobial peptides promising agents for the fabrication of robust antimicrobial nanomaterials. Previous studies indicate that the structured self-assembly of the tyrocidines into dimers, tetramers, and other higher oligomers in aqueous solutions assists their association and surface adhesion[1]. It is hypothesised that the smaller oligomers, particularly dimers, serve as both the building blocks for peptide self-assembly and membrane-active moieties. Excessive oligomerisation and unwanted aggregation could lead to the trapping of these active moieties and diminished activity of the otherwise potent tyrocidines. The arrangement and/or rearrangement of formulations to favour amphipathic dimers and small oligomers in solution, or release of such moieties from surfaces, could therefore greatly enhance the biological activity whilst maintaining the robustness of the "sticky" tyrocidines. Pilot studies successfully fabricated metal nanoparticles using tyrocidines as a reducing agent. This work suggested the presence of peptide-metal interactions capable of dictating tyrocidine oligomerisation. Travelling wave ion mobility mass spectrometry studies revealed that the formulation of tyrocidines with metals alters the peptides' self-assembling behaviour to favour different oligomers in the presence of different metals. Furthermore, antimicrobial assays confirmed positive shifts in bioactivity against both Gram-negative and Gram-positive bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, respectively. The capability to direct peptide self-assembly and dictate nanostructure formation via the formulation of tyrocidines with selected metals holds the potential for the development of novel antimicrobial nanodrugs against stubborn pathogens and biofilms.

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Multi-functional activity assay for discovering of antifungal peptides and compounds against planktonic and biofilm forms of *Candida* species

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Abstract:

Candida albicans (*C. albicans*) is an opportunistic, ubiquitous fungus, of which some strains have the ability to form biofilms on various surfaces of the medical, industrial and agricultural sectors. In this poster, we describe a high-throughput assay format that can be used to study various fungal life-cycle stages simultaneously. This assay is an inexpensive and reproducible 96-well pin-lid assay[1], using metabolic activity to indicate *C. albicans* for planktonic cell susceptibility, biofilm prevention and biofilm eradication as well as biofilms dispersed planktonic cell susceptibility [2].

The assay is used to confirm the activity of known anti-*Candida* compounds such as gramicidin S, caspofungin, amphotercin B and the tyrocidine mix on the various *C. albicans* forms[3,4]. Furthermore, it was optimized by testing the anti-*Candida* activity of antimicrobial compounds extracted from known producer organisms, such as *Brevibacillus parabrevis*, *Bacillus subtilis* and *Aneurinibacillus migulanus* among others[5]. Results demonstrated activity against *C. albicans* planktonic and biofilm forms, ranging from very active to non-active compounds, all which were extracted from the known producer organisms using an organic solvent process. Lastly, the multi-functional assay is used to characterise extracts of unknown compounds from soil samples and to test for activity against planktonic and biofilm forms of *C. albicans*, to identify new antimicrobial compounds to treat *C. albicans* biofilm infections. Advantages of this assay includes ease of adaptability to various laboratories, test compounds and test various *Candida* strains in the discovery of anti-*Candida* compounds.

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Making use of intrinsic tryptophan fluorescence for the characterization of membrane-active peptides

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Abstract:

Cyclic lipopeptides (CLiP) interact with cell membranes of various organisms and may display plant beneficial and antimicrobial properties. However, their mode of action is still poorly understood. Improving our biophysical understanding of how CLiP induce membrane permeabilization could aid a rational development of new and selective antimicrobials and pesticides.

This work aims to explore the membrane partitioning behavior and the molecular vicinity of membrane-active CLiP in the lipid bilayer by using the intrinsic fluorescence properties of the amino acid tryptophan.

In more detail, we investigate four CLiP-analogs with different membrane activities, in which one specific hydrophobic amino acid is exchanged for tryptophan, which fluorescence properties are highly dependent on the details of its local surrounding.

In order to exploit this special ability of tryptophan, we measure time-resolved emission spectra (TRES) to monitor continuous solvent relaxation. Moreover, we measure time-resolved anisotropy to gain information about speed and constraints of rotation of the tryptophan molecule itself. Additionally, we measure steady state fluorescence of tryptophan to analyze the Trp-CLiPs membrane partitioning behavior.

The information of these three independent experiments together allow us to draw conclusions about a Trp-CLiP's immediate environment in the bilayer and the integrity of the lipid bilayer. This will be of great help in order to explain a CLiP's mode of action responsible for its membrane activity.

Vesicule Budding as an effect of area asymmetry induced by lysolipids

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Abstract:

The lipid membrane undergoes continuous dynamic changes in a multitude of cellular processes. Exo- and Endo-vesiculation, asexual reproduction or the forming of organelles require a change in local curvature. Although various protein-centered mechanisms have been identified to induce these changes, the mechanical properties of the lipid bilayer must be considered to play a role in these remodeling processes. The overpopulation of one leaflet leads to area asymmetry of the inner and outer leaflet, creating spontaneous curvature [1]. Lysolipids dissolved or dispersed in the outer medium insert into the accessible membrane leaflet and expand its area but show only verry slow translocation in the trans leaflet. This resulting curvature stress is partially relaxed by vesicle budding even in the absence of proteins and contributes to the multiple biological functions of lysolipids and other membrane-impermeant amphiphiles.

Using Asymmetric Flow Field Flow Fractionation (AF4), the extent of vesicles budded from liposomes can be quantified and assessed. Budding can proceed at sufficient lysolipid concentration until the mother vesicle has become a sphere. In addition, further area can bud off as the mother vesicle is compressed by water outflux, but this limited by the internal osmotic pressure created this way.

Our studies provide a detailed picture of the lipid-related phenomena and parameters governing membrane remodeling.

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Rapid structural elucidation of cyclic lipopeptides via NMR fingerprint matching

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Abstract:

Cyclic lipopeptides (CLiPs) are secondary metabolites that are produced and secreted by a range of bacterial genera including *Pseudomonas* and *Bacillus.* They are composed of an oligopeptide, cyclized through a lactone (depsi) bond, and capped at the N-terminus by a fatty acid moiety. Well over 100 CLiPs originating from *Pseudomonas* spp. have been described at varying levels of structural and biological activity details and their numbers keep rising. Structural variations are very diverse, including total amino acid sequence length, size of the macrocycle, amino acid identity and stereochemistry (e.g. D- vs. L-amino acids). In general, CLiPs are involved in several secondary functions and have been reported to display a range of antagonistic properties. Recently, the antimicrobial activities of *Pseudomonas* CLiPs were thoroughly reviewed. [1]

CLiPs and their producing bacteria are ubiquitous in Nature and reports detailing the discovery of novel or already characterized CLiPs from new sources appear regularly in literature. However, the lack of characterisation detail threatens to cause considerable confusion. Using NMR fingerprint matching, we have introduced a rapid and facile way of characterizing existing CLiPs coming from novel bacterial sources. [2] Using this approach, the identity of CLiPs can be established by simple comparison of their NMR spectral fingerprint recorded under standardized conditions. Future integration of the spectral fingerprints into a publically accessible database should allow structure elucidation to be readily achieved by the wider scientific community involved in CLiP research.

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Effects of cyclic antimicrobial peptides on various model membranes.

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Abstract:

Constantly growing antibiotic resistance underlines the importance of alternatives to classical antibiotics. For that reason, we examine trivalent cyclic hexapeptides with antimicrobial activity. In particular, cRRRWWW has previously been found to interact with lipid membranes in multiple ways [Finger2020]. To analyse the mechanism of action, we investigate the effects of the peptide on binary model membranes containing various anionic and zwitterionic phospholipids. We use the self-quenching dye calcein to monitor the vesicle membrane leakage over a wide concentration range and long incubation times.

The lipid composition of the model membrane determines both, the binding of the peptide and the induced vesicle leakage. In monolayer adsorption measurements and isothermal titration calorimetry (ITC) we found binding selectivity for negatively charged membranes over zwitterionic membranes and simultaneous differences in membrane leakage. This is in agreement with the observed selectivity for bacteria over mammalian cells.

Furthermore, we examine various binary mixtures containing negatively charged phosphatidylglycerol (PG) and zwitterionic phosphatidylethanolamine (PE) or phosphatidylcholine (PC) lipids by differential scanning calorimetry (DSC) in order to reveal the thermotropic behaviour, such as lipid chain melting. We use this as an indicator for the influence of peptides on the lateral lipid arrangement, that is, for instance, to detect electrostatic lipid clustering. We find that the changes in thermotropic behaviour induced by the antimicrobial peptide have varying impact on membrane leakage.

In conclusion, the lipid composition is decisive for the membrane behaviour in response to peptide binding.

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Drosophila melanogaster CG44404 and *CG45045* encode short peptides, not lncRNAs, that act together against outer membrane vesicles (OMVs) produced by Gram-negative bacteria

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Abstract:

It has recently become apparent that some genes have been mis-annotated as long noncoding RNAs (lncRNAs) and actually encode short functional peptides. Here we demonstrate that *CG44404* (*CR44404*) and *CG45045* (*CR45045*) encode highly similar short secreted peptides. Both genes were highly induced in the fat body (a functional equivalent of the mammalian liver and adipose tissue) upon Gram-negative bacteria septic injury and dependent on the *immune deficiency* pathway. The double *CG44404-CG45045* knock-out mutants, but not the single mutants, were susceptible to *Pseudomonas aeruginosa (PAO1)* septic injury, suggesting that both peptides play redundant role(s) against *PAO1*. Our data do not support a role for these two peptides in canonical immune responses against Gram-negative bacteria (AMPs production, melanization, phagocytosis and ROS reaction) and indeed the two peptides do not attack the bacteria directly. Instead, both peptides function against the outer membrane vesicles (OMVs) produced by Gram-negative bacteria (*PAO1* and *Serratia marcescens*). OMVs contain virulence factors such as a major secreted protease. Our data suggest that the role of these two peptides in host defense is to detoxify secreted virulence factors, most likely proteases, whether carried by OMVs or not.

A Toll pathway effector protects *Drosophila* specifically from distinct toxins secreted by a fungus or a bacterium

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Abstract:

The *Drosophila* systemic immune response against many Gram-positive bacteria and fungi is mediated by the Toll pathway. How Toll-regulated effectors actually fulfill this role remains poorly understood as the known antimicrobial peptide (AMP) genes it controls are essentially active only against filamentous fungi and not against Gram-positive bacteria or yeasts. *BaramicinA* gene expression is transcriptionally regulated by the Toll pathway. *BaraA* encodes a polyprotein precursor that releases processed proteins into the hemolymph upon immune challenge. Here, we demonstrate that *BaraA* is required specifically in the host defense against *Enterococcus faecalis* and against the entomopathogenic fungus *Metarhizium robertsii*. It does so by protecting the fly from the action of distinct toxins secreted by Gram-positive and fungal pathogens but not by directly attacking them. Thus, in complement to the current paradigm, innate immunity can cope with toxins, effectively, through the secretion of peptides that are not AMPs, independently of xenobiotics detoxification pathways.

Insights in the mechanism of Mag2/PGLa synergism by fluorescence techniques

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Abstract:

Mag2 and PGLa are antibiotic peptides discovered in the skin of the African clawed frog (Xenopus laevis). The mechanism of the synergism of the two peptides is still under discussion[1,2]. We present the results of fluorescence experiments, which give new insights into the interaction of both peptides. The labeling schemes, prerequisites of fluorescence experiments are presented. Whereas self-quenching experiments investigate the packing of the peptide on the membrane surface at high peptide to lipid ratio[3], fluorescence cross correlation experiments detects direct interactions at low peptide to lipid ratio where statistic contacts become scarce.

Instead of competing for surface space the peptides adsorb into the membrane in a cooperative manner and the complex of the two peptides remains stable upon high dilution on the membrane surface[4].

The experiments suggest a mutual shift of the equilibrium towards the membrane bound form of the two peptides. Hence the cooperatively has a strong thermodynamic component.

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Solid‑state NMR investigations of the MHC II transmembrane domains: topological equilibria and lipid interactions.

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Abstract:

The major histocompatibility complex class II (MHC II) membrane proteins are key players in the adaptive immune response. The protein assembles from the DQ alpha-1 and DQ beta-1 subunits where the transmembrane domains of these type I membrane proteins have been shown to be involved in homo- and heterodimer formation. Furthermore, the DQ alpha 1 chain carries a sequence motif that has been first identified in the context of p24, a protein involved in the formation of COPI vesicles of the intracellular transport machinery, to specifically interact with sphingomyelin-C18 (SM-C18). Here, we investigated the structure, membrane interactions and dynamics of the DQA1 and DQB1 transmembrane helical domains by ^{15}N solid-state NMR spectroscopies. The ^{15}N resonances are indicative of a helical tilt angle of the membrane anchor sequence around 20°. Two populations can be distinguished by their differential dynamics probably corresponding the mono- and homodimer (Figure 1).

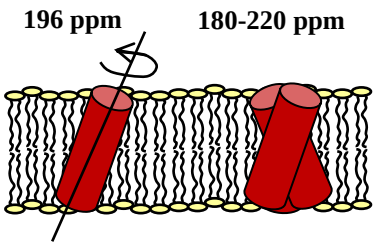

Figure 1. Schematic representation of an equilibrium between a fast-moving monomer and a less dynamic dimer of DQB1 is shown although the exact size of the complexes remains unknown from the experiments presented in this paper. The corresponding ^{15}N chemical shifts are indicated on top of the membrane.

Furthermore, the DQ alpha-1 and DQ beta-1 transmembrane helical domains were reconstituted into POPC or POPC/SM-C18 lipid bilayers where the fatty acyl chain of either the phosphatidylcholine or of the sphingolipid have been deuterated. Interestingly in the presence of both sphingolipid and polypeptide a strong decrease in the innermost membrane order of the POPC palmitoyl chain is observed, suggesting that the ensemble of transmembrane polypeptide and sphingolipid exerts positive curvature strain. In contrast, for the first time the polypeptide interactions were monitored by deuteration of the stearoyl chain of SM-C18.

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Biophysical investigations of Antimicrobial peptide mimics for mechanistic studies

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Abstract:

With a relentless rise in the growth of antimicrobial resistance, more and more groups are now focusing on Antimicrobial peptides (AMP) and the development of AMP mimicking agents to combat this nefarious issue. However, to this date, very few reports come up with adequate reasoning behind the driving force of the membrane perturbing properties. Here, we shall be discussing the two branches of AMP mimics, a. Peptoids [1] and b. Small molecule [2]. Further, we explore the mode of action behind the antimicrobial properties displayed by the library of peptoids and small molecule mimics.

So, to address this big challenge of understanding how the membrane active compound behaves, each of the compounds were introduced to the liposomes, mimicking the bacterial model membranes, and their interactions was recorded using solid state nuclear magnetic resonance spectroscopy (ssNMR)[3]. We shall show that each of the mimics interact with the liposome as the global shape of the vesicles gets deformed. Also we observed a definite interaction with the fatty acyl chain as changes in order parameters were observed with respect to that of pure lipids.

To develop a thorough understanding of the pore forming abilities of these compounds, a fluorescent dye release studies[3] was executed using the bacterial model membrane. The calcein dye release test revealed that the small molecule mimics undergo cooperative effect. For the peptoids investigated, the extent of release could be correlated with their antimicrobial activity.

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Controlling α-helical peptide fibril/crystal formation and membrane interactions with anions

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Abstract:

LAH4 is a family of synthetic peptides known for their versatility: with minor changes in the peptide sequence, they can achieve antimicrobial, DNA/mRNA transfection, and lentiviral transduction enhancement among other biological activities.(Lointier et al., 2020) As a representative of this family, vectofusin (VF), a viral entry enhancer, was found to form fibrils at neutral pH despite its α-helical structure.(Vermeer et al., 2017) In the quest of understanding such unusual fibril formation, we discovered the indispensable role phosphate plays in the process: VF only forms aggregations when a critical phosphate concentration (~10 mM) is reached. While in parallel experiments, VF remains soluble in the presence of 0.1 M Cl^-. With such knowledge in mind, we first identified the amino acid residue interacting with phosphate thus allowing fibril formation; and in a further step, controlled the peptide aggregation into fibrils/sheets/crystals through manipulating anion-peptide interactions. The applications of such interaction aren't limited to the understanding and control of peptide nano assemblies. As an example, in a fluorescent dye release experiment, PGLa-Mag2a 1:1 mixture was found to be more potent for disrupting PE/PG vesicle membrane in the presence of certain anions.

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Structural studies of peptide supramolecular self-assemblies used for lentiviral transduction

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Abstract:

Gene transfer using lentiviral vectors has therapeutic applications spanning from monogenic and infectious diseases to cancer. Such gene therapy can be improved by enhancing transduction levels of target cells or reducing the amount of lentivirus for greater safety and reduced costs. Vectofusin-1, a cationic amphipathic peptide with a viral transduction enhancing capacity, strongly promotes the entry of several retroviral pseudo types into target cells when added to the culture medium. Vectofusin-1 rapidly forms spherical complexes that further assemble into annular and extended nanofibrils in culture medium. These associate with viral particles allowing them to be easily pelleted for optimal virus-cell interaction. These fibrils have been shown to have a unique coiled-coil α-helical structure whereas most other viral transduction enhancers form β- amyloid fibrils. The coiled-coil fibril formation is reversible which bears considerable advantages in handling the peptide for example in scalable gene therapy protocols. Since fibrils made from helical polypeptides are rather rare and requires much effort to produce, our aim is to decipher the intermolecular interactions that stabilize the fibers using a variety of techniques including solid-state NMR spectroscopy.

In the present work, we show how changing some parameters like pH and the solvent used for solubilization in the fibrillation protocol of Vectofusin-1 allows us to obtain different supra molecular structures, modifying the environment in which the peptides are assembled have a direct effect on the shape taken by those supramolecular assemblies.
We also conducted a structural analysis of the secondary structure of fibrils formed by Vectofusin-1 using MAS solid-state NMR. Experimental chemical shift analysis of ^{13}C indicates an alpha-helical conformation of Vectofusin-1 fibrils.

The data obtained from selectively labelled Vectofusin-1 peptides prepared by solid-phase peptide synthesis have not revealed the interaction sites between helices. We plan to scan the full peptide including the histidine and lysine residues that are expensive to label by the chemical approach. Therefore, bacterial overexpression allowing to obtain uniformly labelled ^{13}C ^{15}N Vectofusin-1 has been established with good yields. Ultra-fast MAS solid state NMR using a 0.7mm probe will allow us to make proton detected 3D experiments with high resolution, enabling us to decipher the 3D structure of the peptide's supramolecular assemblies.

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Investigations of the peptide-peptide and peptide-lipid interactions underlying the synergism of antimicrobial peptides in membranes.

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Abstract:

The increasing resistant of more and more pathogenic microorganisms to conventional antibiotics requires new measures to be taken [1]. Our biophysical investigations aim to discover new concepts how multiresistant bacteria could be eliminated. Two antimicrobial peptides discovered in the skin of the African clawed frog, PGLa and magainin 2, interact with lipid bilayers [2-5] where these peptides show remarkable synergistic antimicrobial activities [6] which is optimal at a 1:1 molar ratio [4]. From oriented ^{15}N solid-state NMR investigations the topology in lipid bilayers of these peptides alone and in combination has been studied [6]. Other of our investigations have shown that these peptides form supramolecular complexes along the microbial membrane, enhancing their pore-forming capacity. [7]. In a next step, we plan to analyze more precisely how PGLa and magainin 2 interact with each other within a membrane system, and how the lipid bilayer can be impacted by them.

We firstly produced $^{13}C/^{15}N$ labelled peptides with excellent yield (between 7 and 18 mg of peptide per liter of culture). These peptides will be reconstituted into POPE/POPG (ratio 3:1) lipid bilayers, a close mimetic of bacterial membranes, and analyzed by MAS solid-state NMR spectroscopy. Such experiments are capable to reveal at an atomic level secondary structures, intra- and intermolecular distances and thus the peptide-peptide and peptide-lipid interactions driving synergism [7]. Moreover, to deepen the dynamics of these interactions, we modeled these molecules *in silico* and calculate their trajectory by molecular dynamics. It appears that in some cases, a supramolecular complex can be form, and a processivity of action can be identified. The combination of NMR data with molecular dynamics simulations promises insights into the key interactions at high resolution.

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