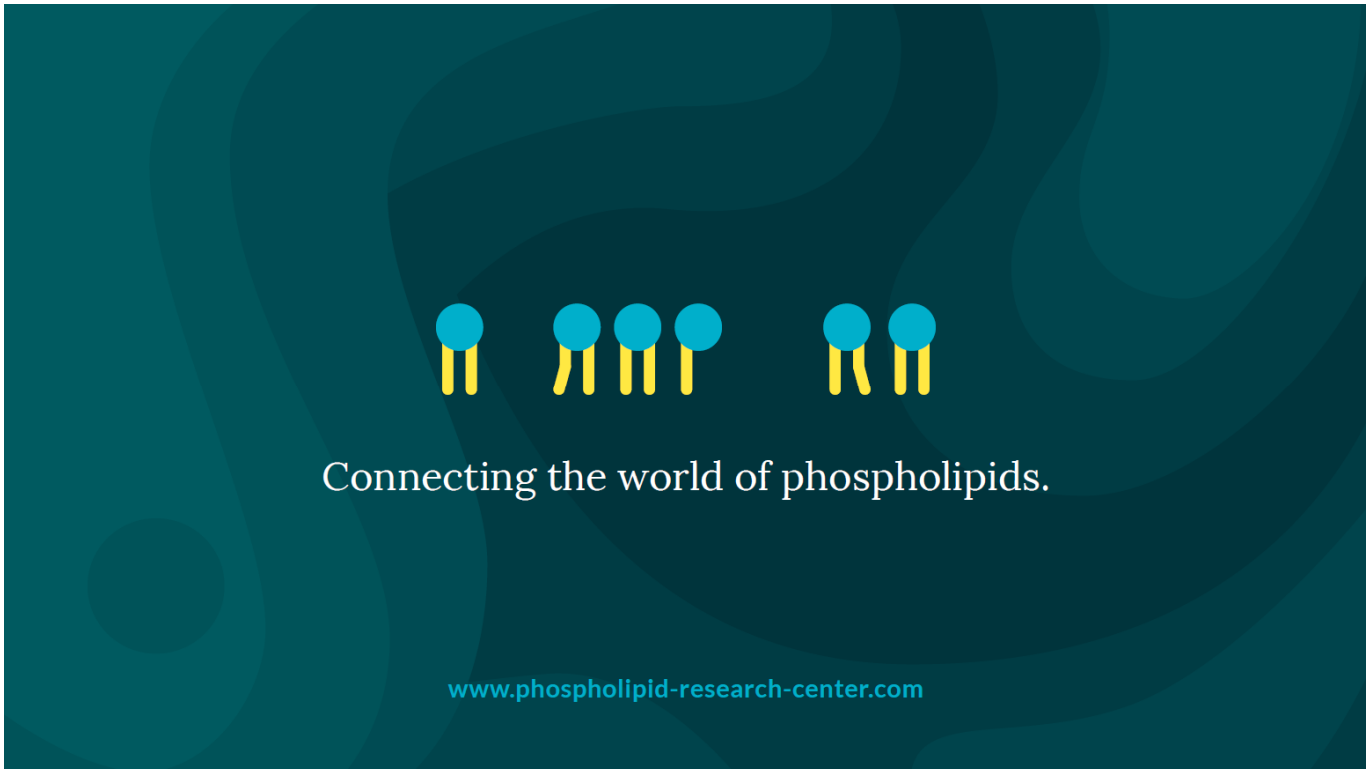
Phospholipid Research Center

International Online Symposium on Phospholipids in Pharmaceutical Research

SEPTEMBER 14, 2021

BOOK OF ABSTRACTS



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Our Plenary Speakers

It is our great pleasure to introduce our keynote speakers. We at the Phospholipid Research Center would like to take this opportunity to thank you for taking the time to attend this event and make a valuable contribution.

Martin Brandl

Professor in Pharmaceutics, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark

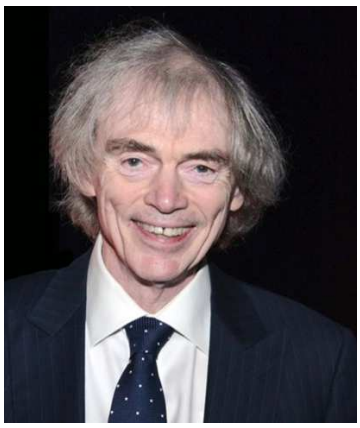
CV

Pharmacist by training

1986 – 1991	PhD student, Albert-Ludwigs-University, Freiburg, Germany
1986 – 1991	Scientific officer, Head of department, GRY Pharma, Kirchzarten (now TEWA), Germany
1991 – 1992	PostDoc, School of Pharmacy, University of London, UK (Prof. Dr. G. Gregoriadis)
1992 – 1998	Lecturer, Albert-Ludwigs-University, Freiburg, Germany
1998 – 2008	Full Professor, Artcic University, Tromsø, Norway
Since 2009	Full Professor, University of Southern Denmark

Research area

- Oral delivery of poorly soluble drugs and mechanistic understanding of candidate-enabling formulations thereof.
- Development of *in vitro* tools for predictive performance ranking of drug formulations through combined dissolution-/ permeation testing.
- Analysis of colloidal and micro-particulate structures in the context of oral drug absorption, spontaneous formation of drug precipitates from supersaturated solutions, human and artificial intestinal fluids, particle separation and characterization by flow field-flow fractionation / multi-angle laser light scattering.



Pieter Cullis

Professor, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada

CV

Physicist by training (University of British Columbia, Vancouver, Canada)

1967 – 1972	PhD student, UBC, Vancouver, Canada
1973 – 1976	PostDoc, University of Oxford, Oxford, UK
1977	PostDoc, University of Utrecht, Utrecht, the Netherlands
1978 – 1985	Assistant and Associate Professor, UBC, Vancouver, Canada
Since 1985	Full Professor, *ibid.*

Research area

Dr. Cullis and colleagues have been responsible the development of nanomedicines employing lipid nanoparticle (LNP) delivery technology leading to five clinically approved drugs for cancer therapies, gene therapies, and vaccines. Two recent examples are Onpattro (the first approved RNAi drug) to treat the hereditary condition transthyretin-induced amyloidosis and BNT162b2, the COVID-19 mRNA vaccine marketed by Pfizer/BioNTech. Dr. Cullis has co-founded ten biotechnology companies that now employ over 300 people, has published over 350 scientific articles and is an inventor on over 60 patents.

Katarina Edwards

Professor, Department of Physics, Department of Chemistry – Ångström Lab, Uppsala University, Uppsala, Sweden

CV

1986	BSc in Chemistry, Uppsala University, Sweden
1991	PhD in Physical Chemistry, *ibid.*
1991 – 1997	Assistant Professor, Physical Chemistry, Uppsala University, Sweden
1992 – 1993	NFR Postdoctoral Fellow, University of Melbourne, Australia
1997 – 2001	Senior Lecturer, Physical Chemistry, Uppsala University, Sweden
Since 2001	Full Professor, *ibid.*

Research area

Edwards' research is focused on fundamental and applied studies of self-assembled lipid systems. The projects are as a rule inspired by issues of biological or medical/pharmaceutical relevance. Past and ongoing activities include projects aiming at the design of novel, improved model membranes/sensor surfaces for biomolecular analysis and drug screening, as well as development of targeting nanocarriers intended for cell-specific drug delivery. Projects belonging the latter category are carried out in close collaboration with national and international partners within the areas of medicine, biology, and pharmacy. Ongoing research with financial support from the Phospholipid Research Center and the Swedish Cancer Society are centered on the development of liposomes and lipodisks for formulation and receptor-targeted delivery of anticancer peptides, chemotherapeutics and radionuclides to tumor cells. Studies of more fundamental character focus on factors affecting the inherent physicochemical properties of lipid bilayers, and on clarification of factors that influence the structure, stability and barrier properties of lipid self-assemblies.

Maria Fedorova

PhD, group leader, Lipid Metabolism: Analysis and Integration, Center of Membrane Biochemistry and Lipid Research, University Hospital and Faculty of Medicine Carl Gustav Carus of TU Dresden, Germany

CV and Research area

Maria Fedorova studied Biochemistry at Saint-Petersburg State University and obtained her PhD at Faculty of Chemistry and Mineralogy, Leipzig University. Now she is a group leader at the Center of Membrane Biochemistry and Lipid Research, TU Dresden. Her research is focused on implementation of LC-MS methods in discovery lipidomics targeting human lipidome in variety of metabolic disorders.

Jean-Christophe Leroux

Professor, Drug Formulation and Delivery at the Institute of Pharmaceutical Sciences at ETH Zurich, Switzerland

CV and Research area

Dr. Leroux was head of this institute from 2014-16. Over the past 25 years, he has made fundamental and applied contributions to the fields of colloids, biomaterials and drug delivery. He has been involved in the development of polymer therapeutics for Celiac disease, in the evaluation of colloidal lipid-based biodetoxification systems for the treatment of intoxications, and in the design of drug-eluting devices by 3D printing. He has authored more than 250 peer-reviewed articles in chemistry, materials sciences and pharmaceutical technology, and is co-inventor on 22 patents/patent applications.

Dr. Leroux was associate editor of the Journal of Controlled Release from 2012 to 2017, and currently serves in the editorial board of four scientific journals including Biomacromolecules and Molecular Pharmaceutics. He has received several prestigious distinctions such as the Debiopharm Life Sciences Award, the APV Research Award and Grand Prize form the French Academy of Pharmacy for his innovative research in pharmaceutical technology. He is a fellow of the Controlled Released Society, American Association of Pharmaceutical Scientists, European Academy of Sciences, and co-founder of Versantis AG and Inositec AG.

Raymond Schiffelers

Professor of Nanomedicine, University Medical Center Utrecht, the Netherlands

CV and Research area

Raymond Schiffelers studied Bio-Pharmaceutical Sciences at Leiden University (1990-1995). After an industrial traineeship at SmithKline Beecham Pharmaceuticals (UK) he did his PhD in medical microbiology at Erasmus University Rotterdam on liposomal targeting of antimicrobial agents (1996-2001). Subsequently he became post-doc at Utrecht University working on liposomes targeting tumor vasculature. In 2002-2003, at Intradigm Co (USA) he expanded his tumor vasculature-targeting work with polymers for delivery of siRNA. After his return to Utrecht University he became assistant and then associate professor. He received an ERC Consolidator Grant in 2010 to investigate extracellular vesicles as biological drug delivery systems.
After he moved to University Medical Center Utrecht in 2011 he became professor of nanomedicine working on bio-inspired and synthetic drug delivery systems. He coordinates two H2020 projects on this topic, B-SMART and EXPERT, is editor for the International Journal of Pharmaceutics, Journal of Controlled Release and Journal of Extracellular Vesicles, and is founder of EXCYTEX-an extracellular vesicle-based company. Since 2021 he also works part-time for Nanocell Therapeutics as VP Preclinical R&D and is chair of the ETPN.

Carla Schmidt

Jun.-Professor, Interdisciplinary research center HALOmem, Institute of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany

CV

2001 – 2006	Studies of Chemistry, Leipzig University, Germany
2006 – 2010	PhD student, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
2010 – 2011	PostDoc, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
2011 – 2015	Postdoc, University of Oxford, United Kingdom
Since 2016	Young investigator group leader and junior professor, Martin-Luther-University Halle-Wittenberg

Research area

- Protein-lipid-interactions in synaptic vesicles and the neuronal synapse
- Development and application of various mass spectrometric techniques including proteomics, lipidomics, cross-linking and native mass spectrometry

Some Technical Information

ZOOM NETIQUETTE

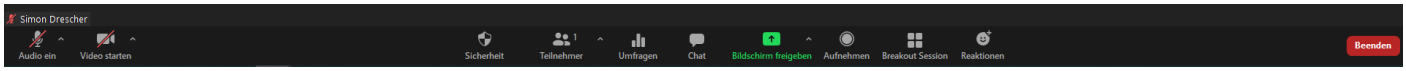
Please adhere to Zoom netiquette so that everyone can enjoy a friendly environment.

- Type your first and last name when joining.
- Be mindful of background noise and distractions around you, as much as possible.
- Adjust your camera and lighting so we can see your face well.
- Raise your hand or type in the Chat during the live discussions if you have a question for the speaker.
- Use Chat to communicate with others.

FOR PRESENTERS AND ATTENDEES

Please use the link sent to you after registration to join our Online Phospholipid Symposium.

If necessary, you would need to install the zoom software on your computer. After the meeting is started, you will see the following screen on the bottom:



Audio and Video are disabled by default. You can start both by clicking the lower left buttons. You may need to check your settings by clicking on the little arrow next to both buttons.

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During breaks, a "break room" will be available for further discussion. To do so, click on the Breakout Session button and select an appropriate room.

FOR PRESENTERS

Please be online in time for your session. After the introduction by the chair, you can share your screen by clicking on Share Screen (Bildschirm teilen). If you have a second screen, it usually makes sense to share this second screen. If not, then you can also share only your presentation.

Please stay in time!

Please stay online at least during the break after your session. There will be a "meet-the-speaker-room" for each presenter to answer any further questions that may arise about your talk. Of course, it is best if you stay online for the entire duration of the Online Phospholipid Symposium and participate in the lively discussions.

FOR CHAIRS

As always, please briefly introduce the presenters and pay attention to keeping within the time limit.

Please also lead the discussion after the talk by presenting the questions raised in the chat or asking the attendees for their question.

Abstracts – Morning Session I – Analytics and more

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Maria Fedorova (Technical University Dresden/Germany)

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Simona Sennato (La Sapienza University of Rome/Italy)

10.00 – 10.35 Mass spectrometry-based identification of protein-associated phospholipids for exploring the importance of protein-lipid interactions
Carla Schmidt (University Halle (Saale)/Germany)

Epilipidome: diversity of lipid modifications in (patho)physiology

Maria Fedorova

Lipid Metabolism: Analysis and Integration, Center of Membrane Biochemistry and Lipid Research, University Hospital and Faculty of Medicine Carl Gustav Carus of TU Dresden, Germany

Abstract

Lipids are characterized by extremely high structural diversity translated to a wide range of physicochemical properties providing different functions. Moreover, lipids can be enzymatically and non-enzymatically modified via introduction of small chemical groups. including oxidation, nitration, sulfation and halogenation, to compose a new level of lipidome complexity (epilipidome) required to regulate complex biological functions. The main challenges in addressing epilipidome diversity are low natural abundances of modified lipids, and the lack of the knowledge on their chemical diversity in biological matrices. Thus, analysis of epilipidome requires simultaneous application of two different analytical workflows – targeted detection to address their low abundance and untargeted/discovery methods to provide high epilipidome coverage. Over last years, we developed several analytical approaches for detection, identification and relative quantification of complex oxidized lipids in biological context specific manner. By combining mass spectrometry analysis and bioinformatics tools specific regulatory signature of modified lipids were identified in the context of human metabolic disorders as well as basic cellular processes such as ferroptotic cell death.

Membrane perturbations induced by biomimetic polymers

S. Shi[1], *A. Stulz*[1], *S. Schmager*[1], *M. Hoernke*[1]

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

Abstract

Membrane-active, biomimetic polymers are designed for diverse therapeutic applications, for example to mimic antimicrobial peptides or to assist in drug delivery or enhance endosomal escape. Similar to their natural prototypes, the polymers are typically short, often amphiphilic and partially charged. The types and properties of the involved lipids often crucially modulate the various membrane perturbations that can be induced. Most typically, biomimetic polymers are designed to induce selective and/or triggered membrane permeabilization. Additionally, biophysical studies of membrane aggregation/adhesion, enhanced fusion, or electrostatic lipid clustering and their interplay are briefly discussed. Each field of application requires selectivity and an appropriate balance of the membrane perturbing effects.

References

[1] Stulz, A.; Vogt, A.; Saar, J. S.; Akil, L.; Lienkamp, K.; Hoernke, M., Quantified Membrane Permeabilization Indicates the Lipid Selectivity of Membrane-Active Antimicrobials. *Langmuir* **2019,** *35* (49), 16366-16376. https://doi.org/10.1021/acs.langmuir.9b01849

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Microfluidic-derived cell-sized vesicles: Implications for development and application

T. Gutsmann[1], *I. Bahrmann*[1], *C. Nehls*[1]

1 – Research Center Borstel Leibniz Lung Center, Division of Biophysics, Borstel, Germany

Abstract

Until a few years ago, all attempts to produce lipid vesicles based on microfluidics failed. A major problem was the high residual oil content in the membrane of the generated water-in-water droplets. In 2016, the Dekker lab introduced the octanol-assisted liposome assembly technique [1], which for the first time enabled the controlled production of nearly solvent-free liposomes within a microfluidic system. We adopted this technique in our lab with the goal of establishing model systems for bacterial membranes and, if possible, envelopes. As antibiotic resistance becomes an increasing problem for the global fight against bacterial infections, we aim to contribute a new tool for compound characterization.

Here we report the optimization of preparation parameters for microfluidic-derived cell-sized vesicles and the extension of microfluidic vesicle generation to bacterial lipid systems, which is a crucial development towards a suitable model system. We present strategies to evolve this system towards a bacterial envelope model and describe options for the use of the vesicles for compound characterization by vesicle trapping [2].

References

[1] Deshpande, S.; Caspi, Y.; Meijering, A. E.; Dekker, C., Octanol-assisted liposome assembly on chip. *Nat. Commun.* **2016,** *7*, 10447. https://doi.org/10.1038/ncomms10447

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A novel approach for determination of entrapment capability of liposomal nanocarriers by Laser Transmission Spectroscopy (LTS) technique

S. Sennato[1], *A.Sarra*[1], *M. Carafa*[2] , *F. Bordi*[1]

1 – CNR-ISC and Physics Dept. La Sapienza University of Rome, Italy
2 – Drug Chemistry and Pharmaceutical Technology Dept. La Sapienza University of Rome, Italy

Abstract

Size and absolute number of particles are of paramount importance for a comprehensive physicochemical characterization of nanosized-carriers and are crucial determinants of their physiological behavior and quality assurance. Unfortunately, existing methods for determining the particle concentration suffer of several limitations since are based on questionable assumptions [1].

We are currently improving the Laser Transmission Spectroscopy (LTS) technique [2] to get an apparatus able to determine the geometrical size distribution (and, in principle, the shape) of the particles in a colloidal suspension in terms of their absolute concentration, with higher sensitivity than other existing techniques [3]. Here we show that LTS can be used as a unique and powerful tool for studying liposomal nanocarriers.

First, we validated LTS by comparison with HPLC data of lipid mass in unilamellar SoyPC:Chol liposomes. Then we investigated novel HSPC-DPPG unilamellar liposomes formulations loading the antitubercular drug isoniazid. For the first time, we used LTS to determine the geometrical size and the particle number concentration of vesicles, thus evaluating the real volumetric drug entrapment capability of the carrier, to be compared with the entrapment efficiency determined by UV spectroscopy. We showed that HSPC-DPPG unilamellar liposomes can load more drug than expected thanks to the presence of drug-lipid interaction, which favors drug accumulation at lipid bilayer [4].

Our LTS-based approach for characterization of liposomes allows the determination of the colloidal properties, i.e. true geometrical size and volume fraction, and the evaluation of the actual drug entrapment capability of the nanocarriers, thus solving an issue left open to date. More, it allows to compare different formulations as if they were composed by the same number of identical vesicles thus unveiling the influence of bilayer properties on drug entrapment.

Notably, the proposed method is conceptually simple and it can be extended to others nanocarrier systems, with different geometry and structure, since it relies on the general Mie scattering theory and hence simply on the a-priori knowledge of the form factor of the nanocarrier.

References

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Mass spectrometry-based identification of protein-associated phospholipids for exploring the importance of protein-lipid interactions

Carla Schmidt

Interdisciplinary research center HALOmem, Institute of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Halle / Saale, Germany

Abstract

Biological membranes separate the aqueous interior of cells and cellular compartments from the mostly aqueous environment. This separation, however, makes the transport of information or material through the membrane necessary. This important task is carried out by the proteins that reside in the membrane or dynamically associate with the lipids. Both, integral and peripheral membrane proteins, therefore, undergo interactions with the surrounding lipids. Consequently, the main purposes of protein-lipid interactions are the stable fixation of the proteins in the membrane as well as attraction of the proteins towards the membrane; however, the functional importance of protein-lipid interactions is gaining attention.

Mass spectrometry is ideally suited to identify proteins and lipids in membrane protein complexes and to unravel their interactions [1]. We, therefore, combine proteomics, lipidomics and structural mass spectrometry and study the importance of lipids on the structure and function of membrane protein complexes. For this, we firstly develop workflows and strategies to identify lipids that associate with proteins and protein complexes in biological membranes. We then often explore the effects of the lipids on the structure of the proteins by native mass spectrometry. Finally, computational modelling provides insights into the structural arrangements of the protein-lipid assemblies.

In addition to analyzing purified protein-lipid complexes, we explore membrane mimetics such as liposomes and nanodiscs to study protein-lipid assemblies in a native-like environment. This includes both the identification of the protein and lipid components of the mimetics as well as their establishment for structural mass spectrometry.

In this lecture, I will present the available strategies and current developments for identification of lipids in protein assemblies. In addition, I will highlight the application of membrane mimetics for structural mass spectrometry of protein-lipid complexes.

References

[1] Frick, M.; Schmidt, C., Mass spectrometry-A versatile tool for characterising the lipid environment of membrane protein assemblies. *Chem. Phys. Lipids* **2019**, *221*, 145-157. https://doi.org/10.1016/j.chemphyslip.2019.04.001

Abstracts – Morning Session II – Drug Delivery I

11.00 – 11.35 Loading drugs into extracellular vesicles: is it too challenging?
Jean-Christophe Leroux (ETH Zurich/Switzerland)

11.35 – 11.50 Liposomal formulation of resistance breaking vancomycin-derivatives
Philipp Uhl (Heidelberg University Hospital/Germany)

11.50 – 12.05 Light activated liposomes for controlled drug release
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12.05 – 12.20 Spatiotemporal controlled drug release with light to overcome chemotherapy resistance in pancreatic cancer
Tristan Le clainche (University Grenoble-Alpes/France)

12.20 – 12.35 Rifabutin liposomes as a novel approach for the treatment of *Staphylococcus aureus* infections
Magda Ferreira (University of Lisbon/Portugal)

Loading drugs into extracellular vesicles: is it too challenging?

B. F. Hettich, J. J. Bader, J.-C. Leroux

Institute of Pharmaceutical Sciences, ETH, Department of Chemistry and Applied Biosciences, Zürich, Switzerland

Abstract

Owing to their ability to interact and exchange information with cells in a remote fashion, extracellular vesicles (EV) have received in recent years increasing interest in the field of pharmaceutical sciences. While the activity of EV can be manipulated *via* the incorporation of various types of drugs, the loading efficiency of common methods towards hydrophilic low molecular weight compounds, and the influence of these methods on the structural and biological properties of the vesicles have been poorly studied. In this work, several approaches to incorporate hydrophilic non-membrane permeable compounds into stem cell-derived small EV (i.e. saponin, sonication, fusion, freeze-thawing, osmotic shock) have been investigated, and the impact of the loading step on the vesicle characteristics have been analyzed [1]. When comparing the different methods, the loading capacity increased in the following order: saponin ≤ sonication < fusion < freeze-thawing ≤ osmotic shock. It was found that the encapsulation procedure impacted the structural and biological properties of the EV, highlighting the importance of including additional characterization parameters to probe alterations of the biological functionality of EV. Freeze-thawing and the osmotic shock appeared as the most suitable methods for EV loading. They provided the highest entrapment efficiency, while preserving the vesicle structural and biological characteristics. This work was funded by and ETH grant (ETH-10 16-1) and the Phospholipid Research Center (JCL-2018-065/2-1).

References

[1] Hettich, B. F.; Bader, J. J.; Leroux, J. C., Encapsulation of Hydrophilic Compounds in Small Extracellular Vesicles: Loading Capacity and Impact on Vesicle Functions. *Adv. Healthc. Mater.* **2021**, e2100047. https://doi.org/10.1002/adhm.202100047

Liposomal formulation of resistance breaking vancomycin-derivatives

J. Werner[1], *F. Umstätter*[1], *G. Fricker*[2], *W. Mier*[1] *and P. Uhl*[1]

1 – Heidelberg University Hospital, Department of Nuclear Medicine, Heidelberg, Germany
2 – Heidelberg University, Institute of Pharmacy and Molecular Biotechnology, Department of Pharmaceutical Technology, Heidelberg, Germany

Abstract

The emergence of multidrug-resistant bacteria demands innovations in the development of new antibiotics. As an alternative to the generation of completely new substances, novel approaches focus on structural modifications of established antibiotics such as vancomycin to overcome resistance. We developed a highly potent vancomycin-derivative (FU002), able to overcome all common types of vancomycin resistance [1,2].

However, FU002 suffers from low oral bioavailability and poor pharmacokinetics due to rapid hepatobiliary excretion. Novel liposomal formulations of FU002 might enhance both its oral availability and its pharmacokinetics. As our previous findings demonstrated that cell penetrating peptide (CPP)-modification of the surface boosts oral delivery of peptide drugs, the liposomes contain (CPP)-phospholipid conjugates [3]. Moreover, the liposomal formulation contains tetraether lipids that enhance the stability in the gastrointestinal tract. For pharmacokinetics optimization, the formulation shall be further modified by PEGylation strategies.

Prolonged contact between the antibiotic and biofilm-producing bacteria can be achieved by liposomes possessing a surface charge opposite to the negative surface charge of the bacteria. The cell penetrating peptides on the liposomal surface intrinsically lead to a positive charge and might therefore perfectly fulfill these requirements. The project is based on the conscientious preparation of the liposomal formulation and subsequent physicochemical characterization followed by the determination of the antibacterial activity. The enhancement of the pharmacokinetics is monitored by molecular imaging of both the radiolabeled nanocarrier and its cargo.

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Light activated liposomes for controlled drug release

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Abstract

Light-activated drug carriers permit site and time-specific drug delivery to specific targets. The technology enables higher drug compound concentrations at the diseased cells while reducing the off-target exposure, and thus adverse reactions. This is a critical option for highly potent drugs that may have also have serious effects on the healthy tissues, like in the case of cancer. We have developed light-triggered indocyanine green liposomes and combined them with various surface coating materials. These sophisticated nanocarriers have been shown to be biocompatible and very stable in storage conditions. The light triggered release of small drug compounds and macromolecules occur very fast in few seconds. The ICG-liposomes are capable of carrying the cargo into the cells and delivering it upon request to the nucleus. Distribution and triggered contents release from the nanocarrier has been demonstrated *in vivo*. Preliminary *in vivo* anti-tumor treatment studies show effectiveness against PC-3 cancer cell line, although further optimization is required. The ICG-liposomes are a promising option for systemic or ocular treatment enabler for several difficult to treat diseases.

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Spatiotemporal controlled drug release with light to overcome chemotherapy resistance in pancreatic cancer.

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Abstract

Despite its low incidence, pancreatic ductal adenocarcinoma (PDAC) is the fourth-leading cause of cancer-related mortality [1]. Cancer desmoplasia, which forms a protective barrier of stromal cells and extracellular matrix around cancer tissues, is widely known for its implication in the high resistance of PDAC against chemotherapeutic treatments [2,3]. To improve the efficacy and safety of such treatments, we aim to design innovative liposomal vectors to control the release of the drug in a spatiotemporal-controlled manner. In the field of photodynamic therapy, photosensitizers such as benzoporphyrin derivative (BPD) are used to produce reactive oxygen species (ROS) upon light excitation [4]. By including photosensitizers in liposomes composed of oxidation-susceptible unsaturated phospholipids, we provide compelling proof for the spatiotemporal-controlled release of drugs with light (Figure 1) [5,6]. We have systematically optimized the liposome composition and discovered that liposomes surface properties were of major impact on drug release efficiency. In parallel, we are developing new 3D culture models of PDAC on alginate hydrogels that are compatible with state-of-the art, high-content imaging assays [7,8]. These models are used to investigate the uptake and toxicity of the liposomes, and to determine the penetration depth of the released therapeutics. These exciting findings open new possibilities for the controlled release of cancer therapeutics and pave the way to in vivo experiments.

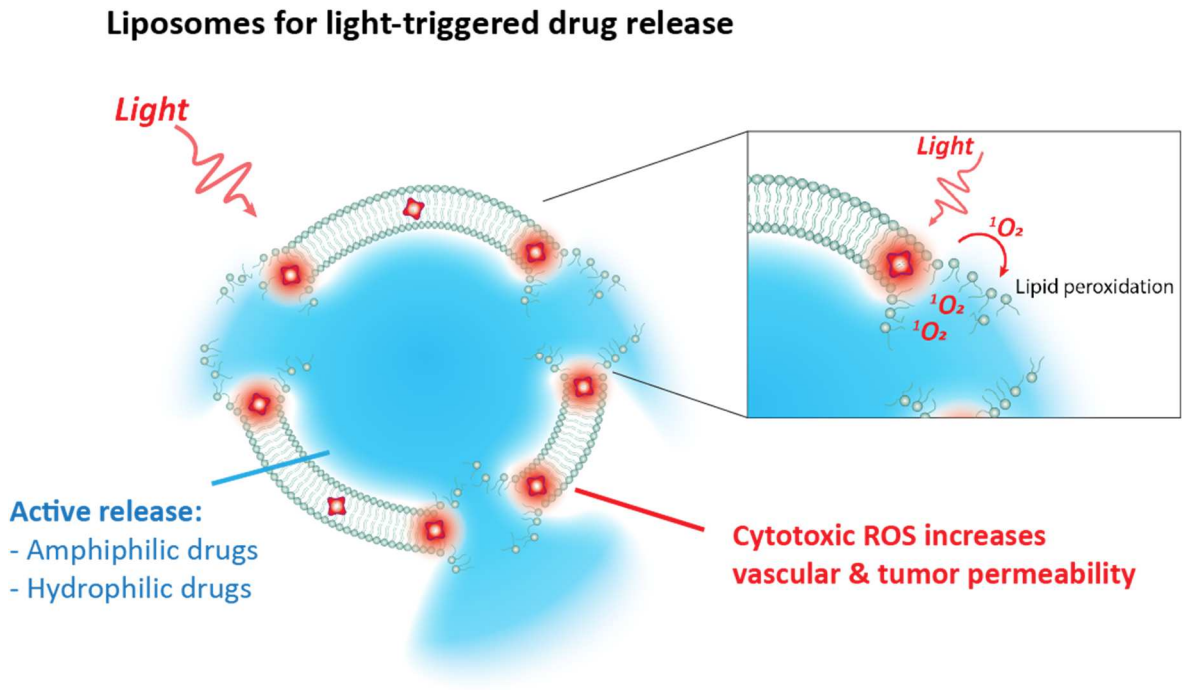

Figure 1. Light-sensitive liposomes for spatiotemporal-controlled drug release.

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Rifabutin liposomes as a novel approach for the treatment of *Staphylococcus aureus* infections

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Abstract

Staphylococcus aureus is one of the most infectious bacterial pathogens worldwide being particularly associated to hospital-acquired infections. Conventional treatment has been hampered by the emergence of multi-drug resistant strains and the innate ability of *S. aureus* to form biofilms and evade the immune system [1]. A potential therapeutic alternative is the repurposing of antibiotics in combination with nanotechnological platforms [2]. Among them, liposomes, are one of the most appealing approaches, due to their ability to specifically target infected areas and interact with biofilms, releasing the incorporated antibiotic at therapeutic levels within the infection site. In the present work, the potential therapeutic benefit of rifabutin (RFB) was assessed in both free and liposomal forms.

RFB was efficiently encapsulated in liposomes with different lipid compositions, by dehydration-rehydration method, and the obtained loading values ranged from 24-57 µg/µmol of lipid, with a mean size of 100 nm. Susceptibility assays to free and liposomal RFB were performed for *S. aureus* reference strain (ATCC®25923™) in planktonic and biofilm forms. Free RFB displayed a high antibacterial potential with minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration (MBIC50) below 0.006 µg/mL. RFB incorporated in liposomes preserved its antibacterial activity against both planktonic and biofilm forms of the reference strain. In a biofilm transwell model the positively charged RFB liposomes demonstrated the highest interaction with *S. aureus* biofilms. Nevertheless, RFB incorporated in negatively charged liposomes displayed lower MBIC50 [2]. These results were confirmed by confocal scanning laser microscopy analysis. Preliminary *in vivo* studies were also performed, aiming to establish a murine infection model, using Balb/c mice infected with MSSA strain, demonstrating an inoculum dose-depend behaviour. Overall, negatively charged RFB liposomes are a promising approach against *S. aureus* infections and further *in vivo* studies should be performed to validate our proposal.

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Abstracts – Afternoon Session I – Drug Delivery II

13.30 – 14.05 Do Phospholipids Boost or Attenuate Oral Drug Absorption? *In Vitro* and *In Vivo* Studies on Mono- and Diacyl Phospholipid-Based Solid Dispersions of Celecoxib

Martin Brandl (University of Southern Denmark, Odense/Denmark)

14.05 – 14.20 Development of effective ligands for liposomal targeted drug delivery in rhabdomyosarcoma

Dzhangar Dzhumashev (University of Bern/Switzerland)

14.20 – 14.35 Controlled diffusion of corticosteroid within an artificial skin membrane through phospholipid-based multilamellar liposomes

Antoine Bernasqué (University Bordeaux/France)

14.35 – 14.50 Protective role of sphingomyelin in eye lens membrane against oxidative stress during aging

Mehdi Ravandeh (University Greifswald/Germany)

14.50 – 15.25 PEG-stabilized lipodisks – from discovery to targeted drug delivery

Katarina Edwards (Uppsala University/Sweden)

Do Phospholipids Boost or Attenuate Oral Drug Absorption? In Vitro- and In Vivo- Studies on Mono- and Diacyl Phospholipid-Based Solid Dispersions of Celecoxib

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Abstract

Despite the fact, that phospholipids are generally recognized as promising excipients for oral drug delivery, systematic studies on oral absorption are limited. The model drug Celecoxib (CXB) is a BCS class II drug bioavailability is limited by poor aqueous solubility. Amorphous solid formulations aiming to increase the solubility of CXB have been studied. Based on an in vitro dissolution-/permeation method a systematic comparison of phospholipid-based solid dispersions was established. By formulating CXB solid phospholipid (PL) dispersions with various PL-to-drug ratios using freeze drying, it was illustrated that the enhancement of CXB solubility does not proportionally translate into enhanced permeability; both parameters are highly dependent on the PL-to-drug ratios as well as the dispersion media (i.e., the presence of 3-mM sodium taurocholate). The in vitro screening revealed: 1) none of the formulations with high phospholipid content increased permeation, 2) phospholipid content was negatively correlated with permeation, and 3) mono and diacyl-phosphatidylcholine formulations performed equally. The in-vivo study revealed, that at low phospholipid content, absorption was enhanced, phospholipid content was negatively correlated with absorption, and monoacyl and diacyl phosphatidylcholine formulations performed equally. This highlights the importance of evaluating both, solubility and permeability, and the use of biorelevant media for testing the candidate-enabling performance of such formulations. Molecular mechanisms that may explain the effect of PL formulations on the permeability of CXB are discussed.

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Development of effective ligands for liposomal targeted drug delivery in rhabdomyosarcoma

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Abstract

Rhabdomyosarcoma (RMS) is the most frequent pediatric soft tissue sarcoma. Surgery and conventional multimodal therapy are not efficient for patients with recurrence or metastases. Encapsulation of therapeutic agents into actively targeted nanoparticles can increase delivery to tumors and decreased side effects. Here, we aim to evaluate peptides as targeting ligands for nanoparticles.

Experiments were performed on RMS and control cells, human primary myoblasts and embryonal lung fibroblasts MRC-5. We used streptavidin-labeled quantum dots (QD) conjugated with biotinylated peptides. We quantified the binding by flow cytometry and verified internalization by microscopy. Protein expression of the targets in RMS cell lines was analyzed by semi-quantitative FACS with validated antibodies to the target receptor.

Results revealed a remarkable binding and internalization of two peptides: F3 [1] and NTP [2]. The target of F3 peptide, Nucleolin, was detected in RMS cell lines, but not in MRC-5 and human myoblasts. Furthermore, the specificity of F3 was confirmed with two alternative synthetic ligands (aptamer AS14114 [3], pseudopeptide N6L [4]). The number of surface molecules of NCAM-1, the target of NTP peptide, was evaluated by semi-quantitative FACS, and was found to be very high.

We formulated PEGylated fluorescent liposomes using microfluidic technique and coated the surface with F3 or NTP peptides. Their stability was verified, and the binding and internalization to RMS cell lines was evaluated by FACS and microscopy. Both ligands were able to increase binding of fluorescent liposomes by at least 30-fold and to drive internalization.

In conclusion, F3- or NTP-mediated nanotargeting is a promising approach for targeted drug delivery to RMS.

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Controlled diffusion of corticosteroid within an artificial skin membrane through phospholipid-based multilamellar liposomes

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Abstract

Corticosteroids are anti-inflammatory molecules largely used for topical treatment of skin diseases such as atopic dermatitis or psoriasis. However, current formulations do not prevent blood stream transfer which is responsible for nocuous side effects such as hormonal or growth disruption. [1]

The structural succession of lipidic bilayers characteristic of multi-lamellar liposomes (MLLs) makes them efficient tools for hydrophobic molecules encapsulation. Moreover, the control of their physico-chemical properties allows to predict their penetration depth into skin. [2] Hence, corticosteroids encapsulation within MLLs appears as a solution for drug efficiency improvement, blood transfer diminution and therefore side effects risk decrease in skin diseases treatment.

Phospholipid based MLLs are obtained by a shearing method allowing a better homogeneity of liposomes in terms of size and structures (Fig.1). MLLs composition dictates their size, charge, and elasticity. The influence of those three parameters on skin permeation has been demonstrated previously.[2] This presentation will focus on artificial skin penetration of encapsulated hydrocortisone. Firstly, influence of the corticosteroid's encapsulation on MLLs properties will be investigated. Then, the fate of the encapsulated drug within an artificial skin model, Strat-M®, will be analyzed owing to an imaging technic: Raman spectroscopy (Fig. 2). Finally, UPLC will be used to quantify and compare free and encapsulated corticosteroid permeation through the membrane.

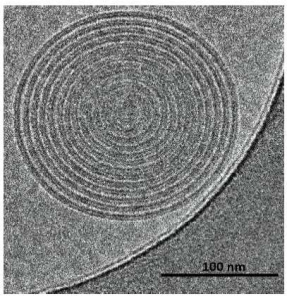

Fig. 1: CryoTEM image of corticoid containing MLLs

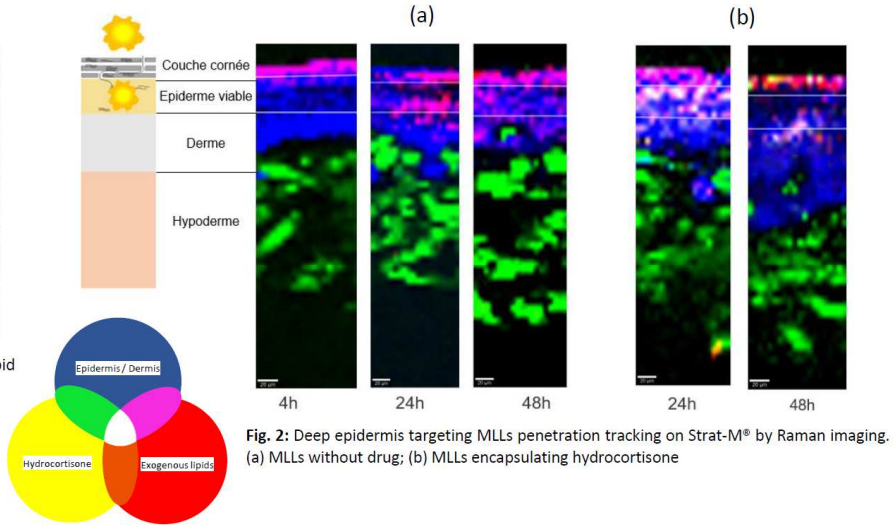

Fig. 2: Deep epidermis targeting MLLs penetration tracking on Strat-M® by Raman imaging. (a) MLLs without drug; (b) MLLs encapsulating hydrocortisone

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Protective role of sphingomyelin in eye lens membrane against oxidative stress during aging

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Abstract

Cataracts, as an age-related disease, can result from oxidative damage in the eye lens. In the eye lens cell membrane, the lipid composition changes during the aging process: the proportion of sphingomyelins (SM) increases while that of phosphatidylcholines decreases [1]. In this study, the protective role of the different ratio of SM in the eye lens membrane against oxidative damage was investigated using state-of-the-art analytical techniques such as electrochemistry, high-resolution mass spectrometry (HR-MS) and atomic force microscopy (AFM). Supported lipid bilayers (SLB) were prepared to mimic the lens cell membrane with different fraction of PLPC/SM (PLPC: 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine). Cold physical plasma was used to generate reactive oxygen species (ROS) including the hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($^{\bullet}O^{2-}$), and H_2O_2 [2], since these ROS reportedly contribute to cataract formation [3]. After plasma treatment of SLB, a protective effect of 30% and 44% in the presence of 25% and 75% SM in the bilayer was observed, respectively. PLPC and SM oxidation products were determined via HR-MS for SLBs after plasma treatment. The yield of fragments gradually decreased as the SM ratio increased. Topographic images obtained by AFM of PLPC-bilayers showed SLB degradation and pore formation after plasma treatment, no degradation was observed in PLPC/SM bilayers [4]. The results of all techniques confirm the protective role of SM in the membrane against oxidative damage and support the idea that the SM content in lens cell membrane is increased during aging to protect the eye from oxidative damage in the absence of effective antioxidant systems and to prolong lens transparency.

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PEG-stabilized Lipodisks – from Discovery to Targeted Drug Delivery

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Abstract

Lipid-based nanostructures, and in particular liposomes, have due to their non-toxic and bio-similar properties found frequent use in various biotechnical and biomedical applications. Although liposomes over the last few decades certainly have proven their worth as convenient model membranes and effective vehicles for drug delivery, there are some limitations to their use. Importantly, liposomes are hollow, closed structures and the fact that the inner membrane surface is shielded from direct contact with the surrounding media creates potential problems in partition and binding studies. To complicate matters further, most liposome preparations contain an unknown fraction of bi- and multilamellar structures. Moreover, although liposomes constitute excellent transporters for many conventional water-soluble drugs, their use as stable and well-defined carriers for lipophilic drugs, membrane-interacting peptides and genetic material is restricted. Further, accumulating evidence suggest that the use of liposomes, and other nanocarriers with spherical shape, might not be ideal from the perspective of biodistribution, immunological response and tumour accumulation.

During our investigations of different factors affecting liposome structure and transformations we have discovered an alternative type of lipid nanoparticles with promising characteristics. More specifically, we have found that nano-sized membrane disks can be produced from lipid mixtures containing well-balanced amounts of polyethylene glycol (PEG)-lipids [1].The disks have a planar and circular shape, and the PEG-lipids, which favour the rim of the disks, offer steric protection against fusion and self-closure. Several studies show that the disks are remarkably robust and function very well as biomimetic membranes in drug partition studies. Membrane proteins can be reconstituted in the disks, and, since the disks can be stably attached to chromatographic materials, as well as to various sensor surfaces, the proteodisks may be utilized in analyses based on HPLC, SPR and QCM techniques [2,3]. Previous investigations and ongoing studies suggest furthermore that the disks have characteristics that make them highly interesting for formulation and targeted delivery of several important classes of anticancer agents, such as conventional chemotherapeutics, anticancer peptides, genetic material, and therapeutic radionuclides [4-6]. Ongoing projects in our lab include studies focused on the use of lipodisks as a versatile platform for the co-delivery of chemotherapeutic drugs and membranolytic anticancer peptides.

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Abstracts – Afternoon Session II – LNPs and more

16.00 – 16.35 Lipid nanoparticles are enabling gene therapies

Pieter Cullis (University of British Columbia, Vancouver/Canada)

16.35 – 16.50 The role of helper lipids in the design of lipid nanoparticle technology for nucleic acid delivery

Dominik Witzigmann (NanoVation Therapeutics and NanoMedicines Innovation Network, Vancouver, British Columbia/Canada)

16.50 – 17.05 Lyso-phosphatidylcholine as an Interfacial Stabilizer in Parenteral Protein Formulations

Eleni Papadopoulos (University Munich/Germany)

17.05 – 17.40 Natural *vs* synthetic lipid nanoparticles for the delivery of RNA

Raymond Schiffelers (UMC Utrecht/The Netherlands)

Lipid Nanoparticles are Enabling Gene Therapies

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Abstract

Gene therapies employing genetic drugs such as small interfering RNA (siRNA) for gene silencing and mRNA for gene expression have the potential to cure most diseases. However, sophisticated delivery systems are required to enable clinical use of nucleic acid polymers as they are readily broken down in biological fluids, do not accumulate at sites of disease and cannot penetrate target cells even if they arrive at target tissues. Lipid nanoparticle (LNP) technology is increasingly enabling the clinical potential of genetic drugs by packaging the nucleic acid polymer in well-defined nanoparticles that protect the nucleic acid payload in vivo and facilitate intracellular delivery following uptake into target cells by endocytosis. This approach has received clinical validation with the approval of Onpattro by the FDA in 2018. Onpattro consists of an LNP containing siRNA to silence transthyretin in hepatocytes, thereby arresting and reversing the disease transthyretin induced amyloidosis (hATTR), a disease that was previously untreatable and was fatal within five years of diagnosis. In this talk I will describe the design features that were followed to develop Onpattro and how related technology is being employed to construct mRNA-based drugs that are enabling gene therapies generally. A notable example is the development of the Pfizer/BioNTech mRNA vaccine, which is playing a leading role in alleviating the Covid-19 pandemic.

The role of helper lipids in the design of lipid nanoparticle technology for nucleic acid delivery

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Abstract

Lipid nanoparticle (LNP) systems are currently one of the most sophisticated non-viral nucleic acid delivery technologies enabling gene therapies. Decades of designing LNP systems has culminated in the approval of Onpattro in 2018 [1], the first-ever siRNA therapeutic for treating a devastating genetic disorder, and most recently in LNP-mRNA COVID-19 vaccines [2]. LNP-RNA systems are poised to have a revolutionary impact and will increasingly become integrated in mainstream medicine. Approved LNP-RNA systems consist of four lipid components (ionizable cationic lipid, DSPC, cholesterol, and PEG-lipid). The ionizable cationic lipid has been optimized for RNA encapsulation and intracellular delivery, and the PEG-lipids have been engineered to regulate LNP size and transfection potency. The roles of the other the helper (phospho)lipids remain less clear. We have investigated the impact of helper (phospho)lipids in modulating LNP stability, nucleic acid entrapment, uptake rate, and gene delivery potency. The presence of internalized helper lipid is vital to the stable encapsulation of RNA in the LNP and thus to LNP-RNA function [3]. Replacing DSPC with different helper lipids impacts cellular tropism suggesting a potential role of helper lipids in modifying the affinity to distinct target receptors [4]. Improving our fundamental understanding of LNP-RNA systems will be crucial for designing next-generation gene therapies and to extend nucleic acid delivery to extrahepatic tissues [5].

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Lyso-phosphatidylcholine as an Interfacial Stabilizer in Parenteral Protein Formulations

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Abstract

Therapeutic proteins have become one of the fastest growing fields of pharmaceutical research and production. Formulated as aqueous solutions for parenteral use, these proteins experience physical and chemical instability. Typically, surfactants, such as polysorbate (PS) 20 and 80, and poloxamer 188 (PX188), are added for protection against interfacial stress. However, in recent years, it has been shown that the hydrolysis and oxidation of polysorbates in parenteral formulations can lead to adverse effects, including anaphylaxis and other immunogenic responses, a loss of protein protection, and even fatty acid particle formation upon storage [1]. It is therefore vital to find a suitable alternative, such as lyso-phosphatidyl-choline (LPC).

Literature shows that lysolecithins disrupt erythrocyte membranes already at low µM concentrations [2]; therefore, we tested the hemolytic activity of LPC. In our study, various concentrations of surfactant were incubated for 1 h at 37°C in a solution of 2 % whole blood diluted in plasma. Compared to previously described tests with isolated erythrocytes diluted in buffer, the hemolytic activity is several orders of magnitude lower in biologically relevant plasma. With decreasing plasma concentration, the HC5 decreased from 3.2 to 0.005 mg/ml.

Shaking studies with a monoclonal antibody (mAb) indicated the same protective effect against interface-induced particle formation as PS80. Moreover, the concentrations at which LPC is stabilizing are 3 orders of magnitude below the HC5 within 95 % plasma.

Dilatational rheometry with an oscillating drop system indicated the formation of a viscoelastic LPC surface film at concentrations above the CMC. In co-adsorption studies, higher LPC concentrations better kept the mAb off the interface. At concentrations close to CMC, the interfacial tension drops substantially, and the viscoelasticity increases at high frequencies, implying an interaction between the surfactant film and mAb. Similar results can be seen for both PS80 and PX188, although the effects of co-adsorption are less significant.

Combined these initial findings show the stabilizing potential of LPC at concentrations far below the parenterally safe limit. Further studies will include chemical stability testing of LPC in mAb solutions, as well as interfacial rheology in silicon oil. This opens up an entirely new field of research for lecithins and possibly provides an alternative to the currently used controversial surfactants in protein formulation.

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Natural vs synthetic lipid nanoparticles for the delivery of RNA

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Abstract

Synthetic lipid nanoparticles composed of ionizable lipids, helper lipids and stabilizing lipids have firmly established themselves as frontrunners for the delivery of nucleic acid therapeutics. Onpattro, Comirnaty, and Spikevax are examples of marketed products based on this technology. Interestingly, in our body, natural lipid nanoparticles (known as extracellular vesicles) can be found filled with nucleic acids. This begs the question whether these extracellular vesicles are able to functionally deliver their nucleic acid cargo and if so, whether they are any good at it.

We have developed a system for the highly sensitive read-out of nucleic acid delivery based on sgRNA and CRISPR-Cas9 machinery [1]. When we use this system for a head to head comparison of synthetic lipid nanoparticles and extracellular vesicles, the vesicles appear much more efficient in delivering sgRNA, up to 1000-fold. Important caveat, the extracellular vesicles contain approximately a million-fold less sgRNA than their synthetic counterparts [2].

As a result, synthetic lipid nanoparticles remain the method of choice for delivery of nucleic acid therapeutics. Yet, our research demonstrates that the study of the uptake, internalization and cytoplasmic release of extracellular vesicles offers opportunities to further enhance lipid nanoparticle delivery efficiency.

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Upcoming Events

October 11–13, 2021

Lipids 2021
PRC Workshop

Moscow & Online

The **Lipids 2021** conference will take place as a hybrid event in Moscow, October 11-13, 2021 at the Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry. Conference languages are Russian and English. Visual materials for oral and poster presentations are to be made in English.

As part of this conference, the Phospholipid Research Center will organize a half-day **workshop** entitled **"Progress in Pharmaceutical R&D on Phospholipids"** on October 12, 2021. This workshop will be held as online event.

So far, we have commitments from the following reputed scientists giving a seminar at this event:
Prof. Dr. Chezy Barenholz (Hebrew University of Jerusalem, Israel)
Prof. Dr. Gerald Brezesinski (Institute of Applied Dermatopharmacy, Halle, Germany)
Prof. Dr. Jörg Huwyler (University of Basel, Switzerland)
Prof. Dr. Judith Kuntsche (University of Southern Denmark, Odense, Denmark)
Prof. Dr. Avi Schroeder (Technion – Israel Institute of Technology, Haifa, Israel)
PD Dr. Peter van Hoogevest (PRC Heidelberg, Germany)

Further information on this event will follow soon – so please check out our webpage https://www.phospholipid-research-center.com/ for more information or follow us on LinkedIn @phospholipid-research-center-heidelberg or on Twitter @PRC_Heidelberg to stay in the loop.

Funding of Phospholipid-Related Projects

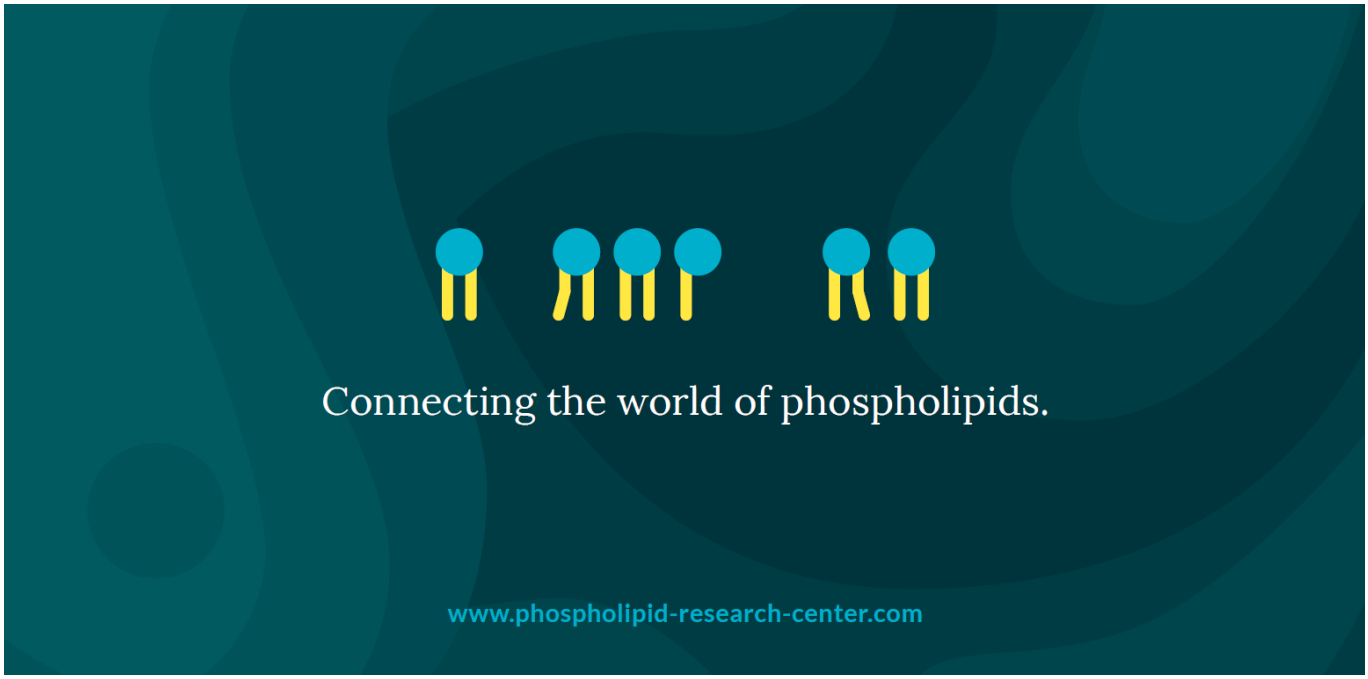
Since 2006, the Phospholipid Research Center has been funding research into phospholipid excipients for pharmaceutical use. The aim is to expand the knowledge of the pharmaceutical and technical application of phospholipid excipients, their ability to improve, for example, the bioavailability and tolerability of drugs in oral, topical, pulmonary, and parenteral dosage forms, and their use as active ingredients.

Individual researchers and research groups from all around the world are encouraged to submit a research proposal covering one or more of the research areas mentioned above to apply for funding of research for non-commercial purposes. Especially PhD and Postdoc projects at academic institutes are in focus.

For more information, please visit:
https://www.phospholipid-research-center.com/funding/research-proposal/

If you want to see what exciting projects the Phospholipid Research Center has already funded, look at the Funded Projects section of our webpage:
https://www.phospholipid-research-center.com/funding/funded-projects/

Do you have a topic you would like to report on and discuss with other researchers? Do you have ideas for an event, such as a workshop? Then you are cordially invited to submit an event proposal.
https://www.phospholipid-research-center.com/funding/event-proposal/



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