SWISS PHARMA SCIENCE DAY 2025

SAPhS
Swiss Academy of Pharmaceutical Sciences

Thursday 21 August 2025
Von Roll Campus University of Bern

«Emerging Trends in Pharmaceutical Sciences – Recent Academic Appointments in Switzerland»

Welcome

The SWISS PHARMA SCIENCE DAY (SPhSD) is an annual event of the Swiss Academy of Pharmaceutical Sciences (SAPhS, www.saphw.ch). It offers a unique platform for scientists in the field of pharmaceutical sciences to meet, interact and learn.

The 18th SPhSD is placed under the theme of *«Emerging Trends in Pharmaceutical Sciences – Recent Academic Appointments in Switzerland»*. Seven recently appointed colleagues from the universities of Basel, Bern and Geneva, and ETH Zurich will address pharmaceutical sciences from the perspective of their respective disciplines. As in previous years, MSc and PhD students, and post-doctoral scientists will have the opportunity to present their latest research in a poster session, and three abstracts will be selected for short oral presentations. The scientific part will be followed by the award ceremony and the traditional apéro.

One of the primary goals of the SPhSD is to promote professional and social contacts between students, postdocs and established scientists in academia, industry, hospitals, public health administration, and public pharmacies. For students and young scientists the SPhSD offers a unique platform for learning about opportunities and career paths in various professional fields. Established scientists can meet young scientists who may be recruited for a position in their organisation.

We look forward to seeing you all in Bern!

Organizing Committee:

Prof. Matthias Hamburger, PhD, Prof., SAPhS
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Program

09:00 – 10:00	**Registration, Welcome Coffee**
10:00 – 10:10	**Welcome Address**
	Prof. Dr. Matthias Hamburger, President SAPhS
10:10 – 12:10	**Morning Session**
	Chair: Prof. Dr. Paola Luciani, University of Bern
10:10 – 10:40	Prof. Dr. Carole Bourquin, University of Bern Obesity, Sex, and Cancer Immunotherapy: A Surprising Connection
10:40 – 11:10	Prof. Dr. Amy E. Fraley, ETH Zürich Accessing Therapeutic Natural Products via a Bio-based Approach
11:10 – 11:40	Prof. Dr. Valerie Gabelica, University of Geneva New Mass Spectrometry-based Approaches to Characterize Oligonucleotide Therapeutics
11:40 – 12:10	Prof. Dr. Linda Simmler, University of Basel From Head-twitching to Synaptic Potentiation: Pre-clinical Research on Psilocybin
12:10 – 14:00	**Lunch Break and Poster Session**

Program (cont.)

14:00 – 16:45 **Afternoon Session**

Chair: Prof. Dr. Klaus Eyer, Aarhus University

14:00 – 14:45 **3 Short Oral Presentations of Selected Abstracts (SOPs)**

14:00 – 14:15 Inès Nikolic
University of Geneva:
«Scarring Beyond Healing: Exploring Cyclops Syndrome – A Distinct Form of Knee Arthrofibrosis Following the Anterior Cruciate Ligament Reconstruction»

14:15 – 14:30 Boris Sevarika
University of Basel
«Targeted Mannose-6-phosphate Liposomes for Enhanced Lysosomal Drug Delivery»

14:30 – 14:45 Kaushavi Anil Cholke
University of Bern:
«Deciphering the Targets of Trypanocidal Chalcones in Trypanosoma cruzi»

14:45 – 15:15 Prof. Dr. Samuel Allemann, Dr. Céline Stäuble,
University of Basel
Pharmacogenetics in Pharmaceutical Care: From Translational Research to Real-World Implementation

15:15 – 15:45 Prof. Dr. Nathalie Grob, ETH Zürich
Peptides in Drug Discovery: Challenges, Innovations, and Opportunities

15:45 – 16:15 Prof. Dr. Marie-Paule Schneider Voirol,
University of Geneva
Combining Interprofessional Education and Implementation Science: Keys to Reshaping Pharmacy Practice in Primary Care

Program (cont.)

14:00 – 16:45	**Afternoon Session (cont.)**
16:15 – 16:25	**Break**
16:25 – 16:40	**Award Ceremony**
	SAPhS Fellow 2025
	Poster Prizes
16:40 – 16:45	**Closing Remarks**
16:50	**Farewell Apéro**

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Lectures

L-1

Prof. Dr. Carole Bourquin, University of Bern

«Obesity, Sex, and Cancer Immunotherapy: A Surprising Connection»

Biosketch:

Professor Dr. Dr. Carole Bourquin has been appointed as Full Professor and new Director of the Institute of Pharmacology at the University of Bern since September 2024.
Prof. Bourquin brings extensive experience and a distinguished background to her new role. She studied Medicine in Geneva before earning her PhD in Immunology at the Max-Planck Institute in Munich. She then established her own research group at the Ludwig-Maximilian University of Munich, where she focused on cancer immunotherapy. In 2011, she returned to Switzerland as a Full Professor of Pharmacology at the University of Fribourg. Between 2016 and 2025, she held a professorship at the Institute of Pharmaceutical Sciences (ISPSO) at the University of Geneva while also practicing as a clinical pharmacologist at the University Hospital of Geneva. Prof. Bourquin will combine her academic role as Institute Director with a clinical appointment as Senior Consultant in Clinical Pharmacology at Inselspital, Bern.
Her research bridges fundamental science and clinical applications, with a focus on antitumor immunity and pharmacological strategies to enhance immunotherapy. With her expertise in immunopharmacology and drug development, she has made significant contributions to advancing the field, particularly with the use of nanocarriers for delivering immunoactive drugs -an innovative and rapidly evolving area of research.
As new director of the Institute of Pharmacology, Prof. Bourquin is committed to furthering the institute's mission of excellence in both research and education, continuing to build strong collaborations with academic, clinical, and industrial partners.

Lecture Abstract:

Obesity, Sex, and Cancer Immunotherapy: A Surprising Connection

Obesity is a well-known risk factor for cancer, yet recent findings suggest it may also improve responses to cancer immunotherapy. Understanding the biological mechanisms behind this paradox could improve cancer treatment strategies. One key factor is estrogen, which is found at higher levels in obese patients. Our study suggests that estrogen levels could serve as a predictive marker for response to immunotherapy in male patients. These findings open new avenues for personalized immunotherapy, integrating sex and metabolic factors into treatment decisions.

L-2

Prof. Dr. Amy E. Fraley, ETH Zürich

«Accessing Therapeutic Natural Products via a Bio-based Approach»

Biosketch:

Amy Fraley received her BSc in Chemistry at Millersville University of Pennsylvania in 2014. She then earned her PhD in Medicinal Chemistry in 2019 from the University of Michigan College of Pharmacy working in the laboratories of Prof. David Sherman and Prof. Janet Smith where she studied fungal indole alkaloid biosynthesis and enzymology. She then moved to the ETH Zürich Institute of Microbiology for postdoctoral studies in the laboratory of Prof. Jörn Piel, with a focus on the biosynthesis of anticancer polyketides by marine symbiotic bacteria. In January 2024, she became the Assistant Professor of Medicinal Chemistry in the Institute of Pharmaceutical Sciences and the Department of Chemistry and Applied Biosciences at ETH Zürich. Inspired by the molecular complexity found in nature, her research group is developing and applying new bio-based methods for sustainable chemistry.

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Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, Eidgenössische Technische Hochschule (ETH) Zürich, Vladimir-Prelog-Weg 4,8093 Zürich

Lecture Abstract:

Marine microbial communities are a wellspring of complex biochemical transformations, with discovery fueled by the ever-expanding development of biotechnological methodologies. Meta-omic strategies - from genome-based studies to enzymology and molecular level investigations - have shed light on the involvement of microbial dark matter in the biosynthesis of therapeutic metabolites. Here, we will traverse the marine symbiont world, highlighting our efforts to shed light on new microbial chemistry, and its redesign for new-to-nature chemical diversification. Our focus will be on megasynthases, which act in an assembly line-like fashion to furnish complex anticancer molecules. While the native producers remain uncultivated, we have heterologously expressed components of the pathways and made strides towards the sustainable production of the final bioactive molecules. A key pathway currently under investigation in our lab is that for the anticancer tetrahydroisoquinoline (THIQ)-containing ET-743 which is a marketed drug used to treat soft tissue sarcoma. ET-743 is part of a diverse family of compounds characterized by a bis-THIQ core, however it is the only member to be marketed as an approved drug. Due to low yields from the current production methods, we aim to develop a bio-based production system for ET-743 to increase yields and allow for facile access to therapeutically relevant analogs. In this regard, the biosynthetic development falls into three categories: i.) early-stage modification of precursors, ii.) THIQ core formation, and iii.) late-stage diversification. Across the THIQ family, the early-stage modification of the tyrosine precursor is consistent, and we have validated the enzymatic reactions catalyzing both C- and O-methylation, as well as hydroxylation by a unique class of heme proteins. The formation of the THIQ core depends on a non-ribosomal peptide synthetase that catalyzes two iterative Pictet-Spengler reactions combining the modified tyrosine residues with cysteine and facilitating subsequent late-stage C–S bond formation. This reconfigured THIQ then undergoes a final Pictet-Spengler reaction to incorporate an additional modified tyrosine and build the characteristic tris-THIQ scaffold of ET-743. We envision that these newly characterized enzymes will pave the way toward a biotechnology platform for the sustainable production of the anticancer therapeutic ET-743 and its previously inaccessible analogs.

Keywords: Non-ribosomal peptide synthetase, anticancer, biocatalysis, synthetic biology, tetrahydroisoquinoline

L-3

Prof. Dr. Valerie Gabelica, University of Geneva

«New Mass Spectrometry-based Approaches to Characterize Oligonucleotide Therapeutics»

Biosketch:

Valérie Gabelica obtained her PhD in Chemistry in 2002 at the University of Liège in Belgium. After a postdoc in Frankfurt as Humboldt fellow, she rejoined the Mass Spectrometry Laboratory in Liège where she obtained a permanent position as FNRS research associate in 2005. In 2013, she joined the Institut Européen de Chimie et Biologie (IECB, Bordeaux, France) as research director of the INSERM (French National Institute for Health and Medical Research). She served as the director of the IECB from 2021 to 2023. She obtained an ERC Consolidator grant in 2014 and was awarded several research prizes (French Academy of Sciences in 2018, Liliane Bettencourt Prize for Life Sciences in 2021, Heinrich Emanuel Merck Award for Analytical Sciences as well as INSERM research prize in 2022). In January 2024, she was appointed as Full Professor in Analytical Chemistry at the University of Geneva, in the School of Pharmaceutical Sciences. Her research interests span fundamental aspects of mass spectrometry and its application to characterizing folding and binding interactions in general, and more specifically nucleic acids as targets or drugs.

Lecture Abstract:

Mass spectrometry has become a versatile analysis method for chemistry and biochemistry. But did you know that mass spectrometry is also a powerful method for biophysical characterization? This talk will walk you through the various ways in which advanced mass spectrometry tools can reveal important features of nucleic acid folding, self-assembly, and interactions [1]. Moreover, the advent of oligonucleotide therapeutics, which are highly chemically modified synthetic constructs, pose specific challenges for molecular characterization. We will highlight how novel fragmentation approaches in mass spectrometry can take up the challenge, in the context of oligonucleotide therapeutics process development. The latter part of the work was carried out on a timsOmni™ prototype mass spectrometer, a one-of-its-kind instrument in Switzerland at the moment.

[1] Largy E, Konig A, Ghosh A, Ghosh D, Benabou S, Rosu F, Gabelica V. Mass Spectrometry of Nucleic Acid Noncovalent Complexes. Chem Rev 2022; 122: 7720-7839.

L-4

Prof. Dr. Linda Simmler, University of Basel

**«From Head-twitching to Synaptic Potentiation:
Pre-clinical Research on Psilocybin»**

Biosketch:

Linda Simmler received her degree in Pharmacy from the University of Basel, where she also obtained her PhD in Pharmacology in 2013, investigating amphetamine-type designer drugs in the laboratory of Prof. Matthias Liechti. From 2013 to 2015, Linda Simmler was a post-doctoral fellow at Vanderbilt University in Nashville, USA, in the laboratory of Prof. Randy Blakely and supported by the Swiss National Science Foundation (SNSF). She investigated serotonin-mediated effects of cocaine using a knock-in mouse model with a cocaine-insensitive serotonin-transporter. In 2016, Simmler joined the laboratory of Prof. Christian Lüscher, where she obtained a SNSF Ambizione grant: She continued her research on drugs of abuse, focusing on drug-induced synaptic changes and the potential of psychoactive substances like ketamine for the treatment of psychiatric disorders. In 2022, Linda Simmler obtained a competitive SNSF Starting Grant and since August 2023, she is an Assistant Professor in the Department of Pharmaceutical Sciences at the University of Basel. She directs the Neuropharmacology research group, focusing her research on synaptic plasticity in the context of antidepressant effects of psilocybin and other hallucinogens.

Publist: https://pharma.unibas.ch/de/personen/linda-simmler/publikationen/
Google scholar: https://scholar.google.nl/citations?user=jVAXAM8AAAAJ&hl=en

Lecture Abstract:

Psilocybin is a psychedelic compound which induces hallucination-like acute effects in humans. Psilocybin is converted rapidly into its active metabolite psilocin in the digestion system and blood. Psilocin is a partial agonist at the 5 HT2A receptor and also potently binds the 5 HT2C and 5 HT1A receptors. Recently, psilocybin and related psychedelic compounds have gained much attention since clinical studies found that single doses can induced rapid and sustained antidepressant effects, even in patients with depression resistant to conventional treatment. The long-lasting nature of these clinical effects are understood to be due to neuroplasticity, but on a synaptic level, little is known about psychedelic-induced synaptic plasticity and a causal link to its antidepressant effect has yet to be established. In our research we use pre-clinical mouse models to investigate psilocybin's acute effects, long-term synaptic plasticity and long-lasting effects on a behavioral level. The ultimate goal is to identify the synaptic plasticity and neuronal circuits underlying psilocybin's antidepressant effects, which would pave the road for targeted, mechanism-driven drug development.
In my lecture, I will present our recent findings on acute psilocybin effects in mice and on changes on a synaptic and behavioral level that go beyond acute effects. We observed that head-twitching, a pre-clinical in-vivo proxy for hallucinatory drug effects, is induced by psilocybin in a dose-depended manner. We also measured psilocin levels in the brain. Interestingly, we found a clear mismatch between the head-twitch behavior and psilocin levels, which has high relevance for drug development studies. I will also present our findings on long-lasting forms of synaptic plasticity induced after a single dose of psilocybin, which we assessed using whole-cell patch-clamp recordings of mouse brain slices. We found increased frequency of miniature excitatory postsynaptic currents when recorded from prelimbic cortical cells, indicating a potentiation of synaptic strength. Our ongoing research investigates whether such increased synaptic strength underlies the long-lasting antidepressant effect of psilocybin and related psychedelics.

L-5

Prof. Dr. Samuel Allemann, Dr. Céline Stäuble, University of Basel

«Pharmacogenetics in Pharmaceutical Care: From Translational Research to Real-World Implementation»

Biosketches:

Samuel Allemann:
Samuel Allemann is a clinical pharmacist and assistant professor. Since August 2021, he leads the Pharmaceutical Care Research Group (PCRG) at the Department of Pharmaceutical Sciences, University of Basel. He works with his research group on optimising the use of medicines, with a focus on pharmacogenetics, digital health, and interprofessional collaboration. To develop and evaluate pharmaceutical care services, the group uses a diverse set of quantitative and qualitative health services research and implementation science methodologies. For their research projects, the group routinely collaborates with community pharmacies, other national and international research institutions and companies providing services for pharmacists, e.g. health informatics providers. In addition to research, his team is active in teaching in the Master Pharmacy and provides services to non-university institutions and individuals.
Céline Stäuble:
Céline Stäuble is a clinical pharmacist at the Institute of Hospital Pharmacy at the Stadtspital Zurich and has been a postdoctoral researcher in the Biopharmacy and Pharmaceutical Care research groups at the Department of Pharmaceutical Sciences at the University of Basel since 2023. Her research projects focus on the application of pharmacogenetic data in clinical practice. She investigates how pharmacists can usefully support pharmacogenetic testing as part of an interprofessional service. In particular, she is interested in how a combination of genetic information and drug interaction data can be considered to improve patient-specific therapy optimization.

Lecture Abstract:

Personalized pharmacotherapy requires advances in basic research as well as robust strategies for real-world implementation. Using the case of a woman with dyslipidemia and statin-associated myopathy, we illustrate how pharmacogenetic insights can inform clinical decisions. We review the role of OATP1B1 polymorphisms in drug transport and interactions, present findings from a pharmacovigilance analysis on muscle-related adverse effects and investigations on a biomarker to quantify drug-drug-gene interactions, and eventually discuss their implications for pharmaceutical care. By combining data from completed studies with current research and implementation initiatives, we highlight practical challenges and opportunities for integrating pharmacogenetics into routine clinical practice. This presentation aims to stimulate discussion on the translational and implementation strategies needed to make pharmacogenetics accessible and impactful in pharmaceutical care.

L-6

Prof. Dr. Nathalie Grob, ETH Zürich

«Peptides in Drug Discovery: Challenges, Innovations, and Opportunities»

Biosketch:

Nathalie Grob earned her BSc in Pharmaceutical Sciences and MSc in Pharmacy from the University of Basel in Switzerland. For her MSc thesis, she joined the group of Prof. Ulf Göransson at Uppsala University in Sweden to study structure-activity relationships in complex peptide natural products. She subsequently pursued a PhD at ETH Zürich with Prof. Roger Schibli and Prof. Thomas Mindt to work on the development of radiolabeled peptides for cancer diagnosis and treatment. From 2020–2023, Nathalie Grob was a postdoctoral researcher in the group of Prof. Bradley Pentelute at the Massachusetts Institute of Technology, where she focused on early-stage drug discovery of small molecules and peptides. In April 2024 she returned to the Department of Chemistry and Applied Sciences and the Institute of Pharmaceutical Sciences to launch a new research group for “peptide-based drug discovery” as an Assistant Professor supported by a Starting Grant of the Swiss National Science Foundation. She acts as a board member to the Division of Medicinal Chemistry and Chemical Biology by the Swiss Chemical Society and to the Swiss Academy of Pharmaceutical Sciences.
Nathalie Grob's research aims to develop efficient methods for the discovery of novel drug modalities. The research of her group focuses on the therapeutic modulation of protein–protein interactions by peptide-based modalities. By using combinatorial chemistry and high-resolution mass spectrometry her group follows a multidisciplinary approach to develop robust workflows for hit identification.

Lecture Abstract:

Over the past century, peptides have emerged as promising drug modalities across various therapeutic areas. However, the translation of bioactive peptides from initial hits into successful drug candidates frequently represents a challenge due to their low metabolic stability and the rapid elimination upon administration. To overcome these limitations, medicinal chemists often explore strategic chemical modifications, including the incorporation of non-natural amino acids (nnAAs), during hit-to-lead optimization. The introduction of nnAAs can also be leveraged in the early discovery phase to enable the identification of novel peptides and peptidomimetics with intrinsically improved pharmacodynamic and pharmacokinetic properties by design. Although recent breakthroughs in genetic code expansion have enabled the incorporation of several nnAAs in display technologies, the chemical synthesis of peptides on solid support allows for the facile introduction of a diverse repertoire of nnAAs at defined positions, significantly expanding the structural and functional diversity. The robust synthetic preparation further supports the integration of nnAAs into early drug discovery phases by enabling the generation and screening of large, chemically diverse peptide libraries. Affinity selection-mass spectrometry (AS-MS) has proven particularly effective for the identification of high-affinity peptide binders against proteins of therapeutic interest from synthetic, structure-based and structure-agnostic peptide libraries. This presentation will discuss the application and impact of AS-MS in peptide discovery, highlighting recent innovations and emerging opportunities in novel target spaces and therapeutic strategies.

L-7

Prof. Dr. Marie-Paule Schneider Voirol, University of Geneva

«Combining Interprofessional Education and Implementation Science: Keys to Reshaping Pharmacy Practice in Primary Care»

Biosketch:

Marie P. Schneider, PhD, pharmacist, is associate professor of medication adherence, interprofessionality and health communication at the Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Switzerland. She is also the scientific Director of the living lab, pharma24, an academic community pharmacy located at the exit of the University Hospitals of Geneva. It serves as an academic hub at the interface between the University, and the community & hospital practices. Her research focuses on medication adherence in long-term diseases and on the implementation of interprofessional intervention programmes in outpatient and community care. These programs are built upon interprofessional collaborations between patients, pharmacists, physicians and nurses. In teaching, she represents ISPSO in the Geneva Interprofessional Education Programme, which includes all health students in health of the University of Geneva and Geneva School of Health Sciences. She is a board and honorary member of the International Society for Medication Adherence (ESPACOMP), and a core and executive member of the Swiss Implementation Science Network (IMPACT).

Lecture Abstract:

Healthcare systems must be redesigned to cope with an ageing population, polypharmacy and significant shortages of healthcare professionals. Pharmacy practice has the timely opportunity to transform itself into new roles, enabling it to better meet the evolving needs of patients and society. Achieving real-world impact requires a shared vision among all stakeholders, from undergraduate and postgraduate education to scientific excellence. Changing teaching programs is challenging due to limited curriculum space, financial constraints, and a shortage of teaching expertise. In interprofessional education, another barrier is coordinating schedules across diverse schools and faculties, such as pharmacy, medicine, and nursing. Political vision and careful implementation are essential. The paradigm shift is similar but on a larger scale when integrating new interprofessional activities into community practice. Vision and rigorous research are key drivers of innovation, aiming for measurable improvements in population health outcomes. Implementation science is instrumental in developing scalable, sustainable, and effective healthcare solutions in Switzerland and beyond.

Posters

I. PHARMACEUTICAL BIOLOGY / PHYTOPHARMACOLOGY

P-I-1 **M. Karpouchtsi, UniBS:** Target identification for natural products inhibiting oncogenic PI3K/AKT and MAPK/ERK signaling pathways in malignant melanoma

P-I-2 **T. Balsiger, UniBS:** Drug discovery pipeline unveils ellagic acid as a nanomolar Musashi inhibitor: Latest advances in lactam analogue synthesis

P-I-3 **M. Rakhmanov, UniBS:** More than detoxification: How marine bacteria utilize glutathione to produce sulfur containing antibiotics

P-I-4 **F. Huwyler, UniBE:** Exploring the potential of THC/THCV Cannabis strains in preclinical assays

P-I-5 **D. Markovic, UniBE:** Functional characterization of the *Trypanosoma cruzi* polyamine transporter TcPAT12: A novel drug target for Chagas disease

P-I-6 **K.A. Cholke, UniBE:** Deciphering the targets of trypanocidal chalcones in *Trypanosoma cruzi*

II. PHARMACEUTICAL TECHNOLOGY

P-II-1 **A. Tsalmpouris, UniGE:** Robust determination of mRNA encapsulation in lipid nanoparticles by anion exchange chromatography

P-II-2 **B. Herzog, UniBS:** Monte Carlo simulations of light transport in sunscreen films

P-II-3 **B. Sevarika, UniBS:** Targeted mannose-6-phosphate liposomes for enhanced lysosomal drug delivery

P-II-4 **A. Savoy, FHNW:** Using forward linear scattering for highly material-saving solubility measurements

P-II-5 **O. Majchrzak, UniGE:** Library of lipids in extracellular vesicles

P-II-6 **A. Halmi, UniGE:** Beyond the cold chain: Exploring lyophilization to stabilize mRNA-LNP vaccines

P-II-7 **G. Ulivi, UniGE:** Novel modulable burst-free hydrogel platform technology for drug delivery

P-II-8 **R. Eugster, UniBE:** Thermo-reactive in situ forming liposome depot (TILD): From computational design to *in vivo* efficacy

P-II-9 **N. Salar, UniBE:** Vaginal administration of a dual-acting drug delivery system for endometriosis treatment

P-II-10 **A. Cavegn, UniBS:** Intracellular processing of DNA-lipid nanoparticles: A quantitative assessment by image segmentation

P-II-11 **M. Stierli, UniBS:** Dual targeted lipid nanoparticles for enhanced DNA delivery to breast cancer cells

P-II-12 **E.Ü. Kuzucu, UniBS:** Physicochemical characterization of lipid nanoparticles by microfluidic particle analysis

P-II-13 **C. Zivko, UniBE:** Analyses and correlations between extracellular vesicles and cells across tissues

III. CLINICAL PHARMACY / CLINICAL PHARMACOLOGY

P-III-1 **A.N. Goetschi, University Hospital Bern:** Assessing the risk of falls and fractures in older adults with painful diabetic polyneuropathy initiated on gabapentinoids, SNRIs, or TCAs: An observational study

P-III-2 **A.N. Goetschi, University Hospital Bern:** Trends and outcomes of naloxone use for iatrogenic opioid overdose: A 10-year retrospective case series

P-III-3 **A.N. Goetschi, University Hospital Bern:** Implementing a clinical pharmacy service for older adult inpatients with chronic non-cancer pain: A proof-of-concept study

P-III-4 **G. Bailer, UniBS:** Diabetes in Switzerland: A 20-year comparison of risk factors by sex

P-III-5 **U. Wernli, University Hospital Bern:** Administration of intranasal midazolam for acute anxiety in palliative care (AIM Care Study)

P-III-6 **F. Mulder, UniBE:** Defining an interprofessional co-care service for patients with hypertension: A participatory qualitative study

IV. MOLECULAR PHARMACOLOGY / MOLECULAR MEDICINE

P-IV-1 **M. Bohley Steiger, ETHZ:** Uncovering mucinase inactivation mechanisms to improve oral peptide delivery

P-IV-2 **P. Merkl, ETHZ:** Mucin degrading enzymes – A platform to study their activity

P-IV-3 **S. Liu, ETHZ:** Selective cleavage of mucin-domain glycoproteins by mucinases: An in vitro screening study

P-IV-4 **M. Zhao, ETHZ:** Combining tissue microporation and stretching for enhanced transbuccal peptide absorption

P-IV-5 **S. Pinheiro, UniBS:** Nature-inspired protection: Engineering complement-resistance via surface-tethered M22 peptidomimetics

P-IV-6 **I. Mataradzija, UniBS:** Novel leech-derived dual-inhibitors of the complement and coagulation system

P-IV-7 **F. Meyer, UniBS:** Evaluation of pharmacodynamic properties after Fc-fusion of complement-modulating peptides and proteins

P-IV-8 **S. Vogt, UniBS:** Breaking the boundaries of the clinical C3 inhibitor compstatin: Development of species-tolerant and long-acting analogs

P-IV-9 **J. Felsch, UniBS:** Complement downregulation on complement-activating surfaces by coating with the regulatory peptide 5C6: An elegant combination of metabolic glycoengineering and click-chemistry

P-IV-10 **A.J. Lander, UniBS:** Leech-inspired bivalent peptides for multi-target modulation of host-defense responses

P-IV-11 **A. Blagojevic, UniBS:** Fantastic drugs and where to find them: Investigating and improving drug-like properties of the leech-derived complement inhibitor gigastasin

P-IV-12 **C. Pratesi, UniBS:** mRNA Display screening for the identification of translationally-active analogs of the compstatin class of complement inhibitors with enhanced species specificity profiles

V. PHARMACOLOGY / BIOPHARMACY

P-V-1 **C. Weber, UniBS:** Behavioral and neuronal effects of psilocybin in mice

P-V-2 **L. Potzel, UniBS:** Drug-drug-gene interactions involving OATP1B1 – investigating coproporphyrins as biomarkers for clinical routine

P-V-3 **N. Paloumpis, UniBS:** From potatoes to precision: Developing an analytical method for solanidine-based CYP2D6 biomarkers

P-V-4 **I. Nikolic, UniGE:** Scarring beyond healing: Exploring Cyclops syndrome – a distinct form of knee arthrofibrosis following the anterior cruciate ligament reconstruction

P-V-5 **L. Kempinger, UniBS:** Antidepressant response – a combination of ABCB1 genetics and substrate recognition?

P-V-6 **H. Guo, ETHZ:** (RS)-[^{11}C]HBP2 - A PET radioligand with high specificity, good brain uptake and favorable kinetic properties for imaging monoacylglycerol lipase

P-V-7 **D. Gao, ETHZ:** Localized heat application as a strategy to improve oral peptide bioavailability

P-V-8 **M. Roth, UniBS:** Potential risk of phenoconversion in the Swiss population: A descriptive study using claims data focusing on drugs metabolized by the enzymes CYP3A4/5 and CYP2B6 or transported by OATP1B1 and BCRP

P-V-9 **E. Götzinger, ETHZ:** Characterization of the stability of a GDNF solution for colonic administration in Hirschsprung disease

I. PHARMACEUTICAL BIOLOGY / PHYTOPHARMACOLOGY

P-I-1

Target identification for natural products inhibiting oncogenic PI3K/AKT and MAPK/ERK signaling pathways in malignant melanoma

M. Karpouchtsi[1], L. Dürr[1], M. Dobrzyński[2], S. Radetzki[3], T. Hell[1], R.E. van Diest[1], M. Smieško[1], M. Hamburger[1], J.P. von Kries[3], O. Pertz[2], R. Teufel[1], E. Garo[1]

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Introduction: Malignant melanoma is the deadliest type of skin cancer, characterized by aberrant activation of PI3K/AKT and MAPK/ERK signaling due to high mutation rates within these key oncogenic pathways. While targeted therapies such as vemurafenib (Zelboraf®), a selective BRAFV600E inhibitor, and cobimetinib (Cotellic®), a MEK inhibitor, show initial promise, drug resistance develops quickly, leaving patients with limited options [1]. Novel treatments are therefore urgently needed.
Aim: Target identification for natural products inhibiting oncogenic AKT and ERK signaling and advancement into lead compounds for future drug development.
Method: As part of a broader drug discovery effort, we conducted two screening campaigns: one using our in-house library of 2,576 crude plant extracts, and a second based on large libraries of over 25,000 pure compounds from both natural and synthetic origin. We combined HPLC-based activity profiling with a specifically developed high-content screening (HCS) assay that reports on downstream AKT and ERK activity. This was followed by targeted isolation of the bioactive constituents [2]. Our current efforts focus on identifying the upstream molecular target(s) responsible for this activity. We developed a complementary strategy to explore compound-target interactions that includes computational target prediction, thermostability-based binding assays and selective kinase activity assays.
Results & Conclusion: A total of 42 active compounds were confirmed as downstream inhibitors of AKT, ERK, or both signaling pathways, with IC_{50} values in the low micromolar range. Of these, 23 were natural products that spanned a broad range of structural scaffolds. Most natural product hits modulated the AKT pathway, suggesting that ERK signaling is inherently more robust against perturbations. Challenges encountered in target identification and first insights into potential targets will be presented. Together, our findings highlight natural products as a valuable source of bioactive compounds for melanoma drug discovery.

Keywords: target identification, natural products, PI3K/AKT and MAPK/ERK signaling, protein kinases, high-content screening, melanoma

References:

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P-I-2

Drug discovery pipeline unveils ellagic acid as a nanomolar Musashi inhibitor: Latest advances in lactam analogue synthesis

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Introduction: Forgetting is not a failure of memory as we often claim. It is essential for maintaining physiological brain function. In the context of neurodegenerative diseases such as Alzheimer`s disease, forgetting becomes pathological, affecting cognitive integrity and quality of life. Recently, a protein called Musashi (MSI) has emerged as a crucial player in promoting forgetting [1]. The lack of efficient treatment for age- or disease-related forgetfulness, prompted us to search for new natural product-based MSI inhibitors.
Aim: Discovery of new lead compounds from plant origin inhibiting human MSI.
Methods: Our in-house library of 2,844 plant extracts was screened using a biophysical MSI inhibition assay. HPLC-based activity profiling, which links activity to distinct peaks in an extract, was used to select the most promising hits, such as the MeOH extract of *Freziera candicans* Tul., which was then scaled up for isolation. All isolated compounds were first tested *in vitro*, and one of the most active compounds, ellagic acid (EA), then further subjected to *in vivo* short- and long-term associative memory studies in *Caenorhabditis elegans* (*C. elegans*). Given the metabolic instability of EA, synthetic efforts focused on the preparation of more stable analogs, notably incorporating a lactam in place of the native lactone.
Results & Conclusion: A total of 11 active natural products from *Freziera candicans* Tul. were isolated, inhibiting human MSI in the low micro- to nanomolar range *in vitro*. One of the most active compounds was identified as EA, a polyphenol common in many foods such as nuts and berries. EA significantly improved short- and long-term associative memory in *C. elegans*, which was further confirmed to be mediated through specific MSI inhibition. EA as a promising lead compound is known to have limited bioavailability and is extensively metabolized by the gut microbiota into urolithins. These metabolites were also tested *in vitro* and did not show any MSI inhibition which suggested that the cleavage of the lactone occurring in the first metabolization step is crucial for EA's activity on MSI. Therefore, efforts to synthesize analogues of ellagic acid were initiated. In this work, we present the latest update on the challenges and opportunities in the synthesis of a lactam analogue of EA.

Keywords: Forgetting, natural products, Musashi inhibitors, ellagic acid, *C. elegans*, synthesis

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P-I-3

More than detoxification: How marine bacteria utilize glutathione to produce sulfur-containing antibiotics

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Introduction: Almost all domains of life have developed glutathione (GSH) biochemistry as a major way of dealing with potentially harmful metabolic dead-ends, xenobiotics and oxidative stress. While well studied in eukaryotes, e.g. human drug metabolism, its role in bacteria received far less attention but has at the same time emerged to be more complex than previously assumed [1]. Bioinformatic analyses of bacterial genomes unveiled that GSH-conjugation might play a crucial role in the biosynthesis of secondary metabolites, one example being the structurally unique marine antibiotic tropodithietic acid (TDA), which contains two sulfur atoms that appear to be incorporated via non-canonical ways. With antibiotic resistance globally on the rise, investigating unusual and underexplored antibiotics such as TDA is of imminent importance.
Aim: Deciphering the cryptic sulfur incorporation during TDA biosynthesis with the aim to generate novel antibiotics using the underlying biochemistry.
Method: Enzymes involved in TDA production were chosen based on bioinformatic predictions and knockout studies. These candidate enzymes were heterologously produced, and their biochemical functions assessed using *in vitro* assays as well as biophysical methods. To test biosynthetic hypotheses structural analogues of postulated intermediates were synthesized and used as surrogate substrates for these investigations. Complementary structural studies were conducted using X-ray crystallography to scrutinize the underlying enzymology.
Results: We were able to successfully reconstitute a three-enzyme cascade *in vitro*, leading to the integration of a thiol group into a model substrate. More precisely, it could be shown that a GSH moiety attached to an aromatic backbone *via* a C-S bond is sequentially degraded by a γ-glutamyl-cyclotransferase, dipeptidase and cysteine-S-conjugate-β-lyase. Additionally, the structure of the β-lyase could be elucidated using X-ray crystallography, granting insight into the catalytic mechanism behind the C-S bond cleavage.
Conclusion: These findings corroborate the notion of GSH as the source of sulfur in TDA and pave the way for the biotechnological production of analogous compounds. Interestingly, the degradation of GSH to a reactive thiol has been described in human drug and xenobiotic metabolism before. However, it seems to be a metabolic end-product associated with nephrotoxicity [2]. It appears that bacteria have found a way to leverage this reactivity to expand their repertoire of secondary metabolites.

Keywords: antibiotics, bacterial metabolism, enzymes, glutathione, natural products

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P-I-4

Exploring the potential of THC/THCV Cannabis strains in preclinical assays

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Introduction: Medical cannabis has gained recognition as an effective adjunct therapy in palliative care, particularly for chronic pain management. The development of opioid tolerance has highlighted the need for alternative treatments, making medical cannabis an important option. Tetrahydrocannabinol (THC) is valued for its analgesic properties but is associated with side effects such as cognitive impairment and sedation. Tetrahydrocannabivarin (THCV) may mitigate these side effects by modulating THC's psychoactive impact while preserving its pain-relieving properties.
Aim: This study investigates the potential of a cannabis strain combining THC and THCV to maintain pain relief while minimizing adverse effects.
Methods: *Cannabis sativa* L. strains with elevated THCV levels were screened and cultivated ((AB)-8/5-BetmG - 2022 / 017392). To quantify the activity of CB1 receptors, which are known to mediate THC-induced adverse effects, GRAB_eCB2.0 biosensor was employed [1]. To assess CB2 receptor activity, which has been implicated in the attenuation of inflammatory and neuropathic pain [2], we engineered a CB2-targeted biosensor utilizing a comparable mechanism to the previously described CB1-biosensor. HEK293 cells were transiently transfected with a plasmid encoding the biosensor. Upon subsequent addition of THC and THCV in different ratios, real-time fluorescence measurements were acquired using FLIPR Tetra. Ethanolic extracts were prepared, dried and resuspended in vehicle and tested using a tetrad assay in female C57BL/6 mice. The THC:THCV ratios varied between 1:0.6 and 1:1.9, with a control group receiving artificially added THCV (75 mg/kg). All treatments were administered orally (gavage), and the effects were evaluated two hours after administration.
Results: The cannabinoid composition in flowers from the same plants changed over time, with THCV content increasing more significantly relative to THC and 20 other cannabinoids. In comparison to THC alone, the addition of THCV significantly reduced CB1 activity *in vitro*. Furthermore, THCV decreased catalepsy and enhanced locomotion *in vivo*, without compromising the analgesic effects of THC.
Conclusion: Cannabis strains with balanced THC and THCV levels show promise for effective pain relief with a reduced side effect profile. These results pave the way for an in-human study to validate cannabinoids with high THCV content as alternative analgesics for patients suffering from chronic pain.

Keywords: tetrahydrocannabinol, tetrahydrocannabivarin, biosensor, tetrad test, analgesia

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P-I-5

Functional characterization of the *Trypanosoma cruzi* polyamine transporter *Tc*PAT12: A novel drug target for Chagas disease

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Introduction: *Trypanosoma cruzi* (*T. cruzi*), the etiological agent of Chagas disease, follows a digenetic life cycle involving an insect vector (the triatomine bug) and a mammalian host. Currently, only two drugs, benznidazole and nifurtimox, are approved for treatment, but both have limited efficacy and severe side effects. Consequently, there has been an increased interest in targeting the unique metabolic pathways of *T. cruzi* to identify differences from its mammalian host. One critical pathway is the polyamine transport, which is essential for various cellular functions and plays a crucial role in cell growth and development. In *T. cruzi*, putrescine is essential for survival and must be acquired from the host, as the parasite is auxotrophic for this compound. Additionally, the availability of polyamines, and their effects on the parasite, vary across different stages of its life cycle.
Aim: This study investigates the potential of the most abundant polyamine transporter, *Tc*PAT12, as a potential drug target.
Methods: Using CRISPR/Cas9, the polyamine transporter *Tc*PAT12 was knocked out in the *T. cruzi* Y strain. The resulting knockout (KO) parasites were characterized with respect to transport function, virulence, and metabolic alterations. Transport activity was evaluated using a radiolabeled assay with radioactive putrescine as the substrate, while virulence was assessed through fluorescence-activated cell scanning (FACS) to quantify parasite release in parallel with microscopy imaging. Metabolic profiling was performed by LC-MS/MS to quantify amino acids and other metabolites.
Results: Comparing the transport kinetics of wild-type (WT) and KO parasites, we observed a >60% reduction in putrescine uptake and a minor shift in its affinity (WT: V_{max} = 8.50 ± 0.43 pmol/min, K_M = 5.48 ± 0.87 µM; KO: V_{max} = 3.10 ± 0.34 pmol/min, K_M = 12.14 ± 3.31 µM). Infection assays and bright-field microscopy confirmed that the reduced putrescine uptake results in dramatically decreased infection of mammalian cells by the KO parasites. Rescue experiments were performed. Moreover, metabolic profiling revealed that KO parasites had changed levels of most amino acids and several other metabolites compared to WT parasites.
Conclusion: Loss of *Tc*PAT12 disrupts polyamine acquisition and alters central metabolism, ultimately compromising *T. cruzi* infectivity. These findings highlight the transporter's essential role in parasite virulence and support its potential as a drug target for Chagas disease.

Keywords: Chagas disease, *Trypanosoma cruzi*, polyamines, *Tc*PAT12

P-I-6

Deciphering the targets of trypanocidal chalcones in *Trypanosoma cruzi*

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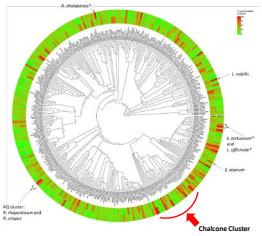
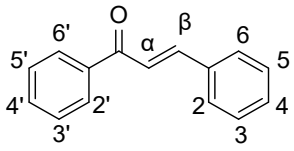
Introduction: The protozoan parasite *Trypanosoma cruzi (T. cruzi)* is the causative agent of Chagas disease (CD) which affects millions of primarily indigenous people in Latin America [1]. Infection with *T. cruzi* is usually life-long, and up to 30% of individuals develop chronic Chagas disease, with symptoms that include cardiomyopathy and/or digestive mega syndromes. Treatment of *T. cruzi* infection with the nitroheterocyclic drugs benznidazole and nifurtimox, introduced in the 1970s, is suboptimal. Both drugs show cross-resistance and can have severe side effects and do not consistently result in sterile cure. This highlights the urgent need for improved drugs. Based on an extensive screening of 775 extracts of botanical drugs used in Bolivia in the context of CD and botanical drugs from unrelated indications from the Mediterranean De Materia Medica (Fig.1A), we investigate the natural product class chalcones (α, β-unsaturated ketones) (Fig. 1B) which are often present in plants together with flavonoids. It was previously shown that chalcones mediate covalent interactions with specific enzymes in *Leishmania* spp. [2]. To identify the molecular mechanism of action of trypanocidal chalcones in *T. cruzi* we employ a chemoproteomics strategy by generating clickable probes. We successfully improved the selective toxicity of chalcones towards *T. cruzi* and established a strategy to identify their targets. The enzymes targeted by chalcones in *T. cruzi* also appear to have a role in benznidazole and nifurtimox resistance. By employing targeted and untargeted LC-MS based metabolomics we have deciphered the role of these enzymes in *T. cruzi* biology.



1A



1B

Figure 1A: Phylobioactive profiling of botanical drugs for their selective trypanocidal effects [3]; 1B: Chalcone chemical structure of basic scaffold

Aims: To identify and characterize the molecular targets of trypanocidal chalcones in *Trypanosoma cruzi* and elucidate their potential role in drug resistance, with the goal of developing improved therapeutic options for Chagas disease.
Methods: A library of 45 chalcones was screened for *Trypanosoma cruzi* infection *in vitro* using fluorescence-activated cell sorting (FACS), and their cytotoxicity was evaluated in RAW 264.7 macrophages. A chalcone-based clickable probe was chemically synthesized and employed in a chemoproteomics workflow to identify molecular targets in *T. cruzi.* CRISPR-Cas9 mediated knockouts of the identified targets were generated to validate their role in parasite biology. To further explore the biological function of these enzymes, LC-MS based metabolomics analyses were conducted. Additionally, cryo-electron microscopy (cryo-EM) was used to resolve the structure of one of the chalcone targets.

Results: The screening successfully evaluated 45 chalcones and assessed their cytotoxicity in RAW 264.7 cells. Chemoproteomics analysis identified molecular targets of chalcones in *T. cruzi*, and metabolomic profiling revealed key roles for these enzymes in parasite biology. Chalcone treatment led to the formation of reactive oxygen species (ROS) in *T. cruzi*. The molecular targets were further validated biochemically and structurally, including structural determination via cryo-EM.
Conclusions: Chalcones interact covalently with the trypanothione-dependent peroxidases via the reactive double bond. Chalcones can increase reactive oxidation species by inhibiting the trypanothione-dependent peroxidases in *T. cruzi.* 2-Cholkene is an activity-based probe which reacts with the active site Cp (Cys 81) of mTXNPx and then binds covalently with the resolving cysteine Cr (Cys 204). Cryo-EM reveals mTXNPx is a pentamer of dimers. Cryo-EM analysis of purified oxidized and reduced mTXNPx is ongoing to confirm the mechanism of action with chalcones. Knocking out cTXNPx affects the growth and infectivity of *T. cruzi*. Trypanothione was significantly upregulated in the cTXNPx KO.

Keywords: *Trypanosoma cruzi*, chalcone, chemoproteomics, metabolomics, Chagas disease, drug resistance

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II. PHARMACEUTICAL TECHNOLOGY

P-II-1

Robust determination of mRNA encapsulation in lipid nanoparticles by anion exchange chromatography

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Introduction: The approval of the first mRNA-lipid nanoparticle (LNP) vaccine a few years ago has signalled the race for novel mRNA-based vaccines and therapeutics. The need for robust analytical strategies to define the critical quality attributes (CQAs) of mRNA has been increasing ever since [1]. Among the CQAs, encapsulation efficiency (EE) is defined as the percentage of total mRNA that is efficiently protected by the LNP from nuclease degradation.
Aims: The EE must be assessed to ensure that sufficient mRNA evades enzymatic degradation and traverses biological barriers to reach the cellular machinery for translation, without triggering unwanted immune responses caused by free mRNA [2]. For this reason, a reliable analytical method must be developed to ensure accurate and repeatable quantification of mRNA in routine analysis of drug products.
Methods: In this study, we developed a strategy based on anion exchange chromatography (AEX) to separate LNPs and free mRNA based on their charge differences. EE was determined by analysing undiluted samples for free mRNA and then measuring total mRNA after LNP disruption using surfactants. During development, we faced mRNA and LNP carryover issues which we mitigated by using a washing step with surfactant, high pH, and high salt concentration.
Results: The method was successfully applied to the analysis of 30 different mRNA-LNP samples to determine EE. The results were compared to those obtained with the RiboGreen assay, a fluorescence-based reference method. Our results revealed significant discrepancies between the two techniques that could be explained by the structural information obtained in AEX conditions. While the RiboGreen assay provides information on the quantification of mRNA accessible to the fluorescent dye, AEX allows relative quantification of mRNA dissociated from LNP, including information on surface-localised and transmembrane mRNA.
Conclusions: Our findings establish AEX as a reliable EE assay, providing information on mRNA distribution within LNPs and advancing the understanding of LNP structure-function relationships. Based on these features, it offers critical guidance for rational design of next-generation mRNA vaccines and therapeutics.

Keywords: encapsulation, mRNA, LNP, anion exchange, liquid chromatography, RiboGreen

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P-II-2

Monte Carlo simulations of light transport in sunscreen films

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Introduction: Sunscreens are used for the protection of human skin against the harmful effects of solar UV radiation. Due to the low thickness of sunscreen films typically applied to the skin, it can be challenging to achieve the strong absorbance needed for good UV-protection, and most efficient sunscreen compositions are desirable. The presence of scattering particles can increase the efficacy of dissolved UV-absorbers in the oil or water phases of the formulation. As many sunscreens contain UV-absorbing particles, it is of interest how much the scattering effect of such materials contribute to the protection of the respective sunscreen.
Aims: The currently available software programs for simulating sunscreen performance are based on a Beer-Lambert law approach for irregular model film profiles, but do not take into account scattering effects of particles. However, Monte Carlo simulations of the UV-light transport through sunscreen films are capable to take scattering from particles into consideration.
Methods: Running the Monte Carlo program, simulated photons are launched towards a sample of a sunscreen film. The photons can be either reflected from the surface, scattered or absorbed in the sample, or transmitted to the skin [1]. Such processes occur with certain probabilities depending on the optical properties of the sunscreen, like the absorption coefficient μ_a and the scattering coefficient μ_s. The fate of a specific photon in the sample is simulated by using random numbers. The absorption and scattering coefficients are calculated form experimental data. Those comprise the complex refractive index of the media and the materials the particles consist of, obtained via ellipsometry, the absorption coefficients of dissolved UV-absorbers measured by UV/vis spectroscopy, and the particle sizes obtained with dynamic light scattering.
Results: Using Monte Carlo simulations, this work shows that the efficacy of absorbance can be increased in the presence of scattering particles. However, this is of limited significance when the particles are UV-absorbers themselves.
Conclusions: In the currently used sunscreen simulating models, the effects from light scattering of particulate UV-filters with respect to amplification of the absorbance or back-scattering of radiation are not taken into account. The results of this study show that this is obviously a reasonable approximation, as such scattering effects seem to be small in comparison to absorption.

Keywords: sunscreens, UV-protection, irregular films, light transport, Monte Carlo simulations

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P-II-3

Targeted mannose-6-phosphate liposomes for enhanced lysosomal drug delivery

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Introduction: Liposomes are a cornerstone of nanomedicine due to their biocompatibility and ability to encapsulate a wide range of therapeutics. However, their effectiveness depends on targeted cellular uptake and precise subcellular delivery. Mannose-6-phosphate (M6P) is a well-established ligand for lysosomal trafficking, as it binds to the cation-independent M6P receptor, facilitating clathrin-mediated internalization and lysosomal delivery. We aimed to exploit this biological pathway to enhance the delivery of therapeutics to lysosomes using M6P-decorated liposomes [1].
Aims: The primary aim of this study was to design, synthesize, and characterize M6P-functionalized liposomes capable of enhancing lysosomal targeting. A secondary objective was to investigate the specificity and internalization pathway of these liposomes *in vitro*.
Methods: M6P ligands were synthesized by covalently attaching M6P to a phospholipid via a polyethylene glycol (PEG) linker. These ligands were incorporated into liposomes using a thin-film hydration method, followed by extrusion to achieve a uniform size of ~100 nm and a surface charge of approximately −40 mV. Cellular uptake was assessed in multiple cell lines using fluorescence microscopy and flow cytometry. Control liposomes containing similar monosaccharides or equally charged ligands were used to evaluate specificity. Co-localization with lysosomes was quantified using confocal microscopy and lysosomal markers.
Results: M6P-functionalized liposomes exhibited a concentration-dependent increase in cellular uptake, achieving up to a 14-fold enhancement compared to non-targeted liposomes. Control liposomes with unrelated sugars or similar charge did not show increased uptake, confirming M6P-specific internalization. Mechanistic inhibition studies revealed that the uptake occurred predominantly via clathrin-mediated endocytosis. Once internalized, approximately 72% of the M6P-liposomes co-localized with lysosomal markers, indicating efficient trafficking to the target organelle.
Conclusions: These results demonstrate the potential of M6P-functionalized liposomes as a modular and highly specific platform for lysosomal drug delivery. The enhanced uptake and lysosomal localization highlight their applicability for enzyme replacement therapies and other treatments targeting lysosomal storage diseases.

Keywords: liposomes, mannose-6-phosphate, lysosomal delivery, targeted nanomedicine, enzyme replacement therapy

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P-II-4

Using forward linear scattering for highly material-saving solubility measurements

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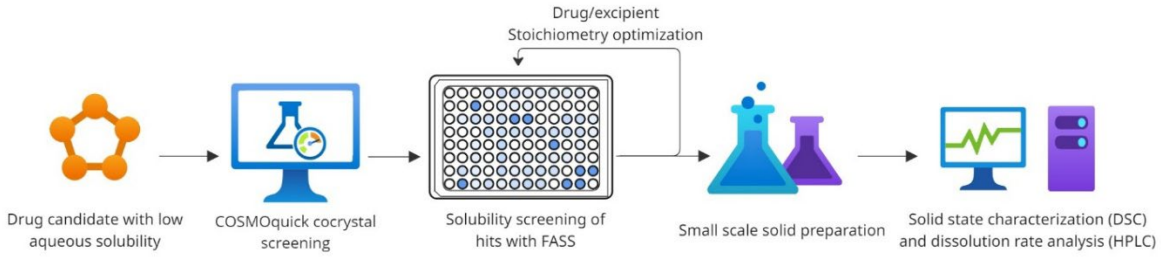
Introduction: A novel pulsed ultrafast laser-based technology has been developed by a innosuisse project (EASY) that will be commercialized by Oryl Photonics. This patented technology combines Second Harmonic Light Scattering (SHS) with Forward Linear Light Scattering (FLS). SHS leverages the solvent redistribution method [1] to provide structural insights into aggregates present in solution. FLS is distinct from traditional linear and dynamic light scattering techniques and detects light in-line with the laser path through a narrow iris. Preliminary results suggest a high sensitivity and further investigations and benchmarks are being conducted within the Fast and Accurate Solubility for Sustainability (FASS) project, a european collaboration funded by a HORIZON grant.
Aim: We aim to find applications within the pharmaceutical and chemical industry for this promising technology, more specifically in pre-formulation, where studies rely on limited quantities of compound. In this specific field, there is a lack of early-screening methods for the selection of suitable excipients in the development of co-crystals or co-amorphous solids [2] with enhanced properties, such as increased dissolution rate, prolonged shelf life or increased solubility in aqueous media. We aim to demonstrate that, by combining computational predictions and efficient screening tools, the pre-formulation of co-crystal and co-amorphous solids will become more sustainable, fast and thorough.
Methods: Combination of computational (COSMOquick) and experimental methods. The novel laser-based instrument is based on forward linear light scattering. The established tools are HPLC and DSC.
Results: We are proposing and studying the relevance and reliability of a new workflow for the screening of excipients for the preparation of co-crystals and co-amorphous solid dispersion. The first step is an optional computational screening. COSMOquick was chosen, as it has been demonstrated to yield accurate prediction for co-crystal screening [3]. The identified promising excipients then undergo further experimental screening. The miniaturized FLS technology would have a high potential as a first experimental screening method for excipient selection and optimization (see Figure below). Preliminary results are promising but it is essential to establish the limitations and restrictions of this novel screening tool. Following steps are the established experimental screening steps, namely DSC analysis and dissolution rate measurement by HPLC from the prepared co-crystal or co-amorphous solid dispersion.



Conclusion: Screening of excipients with FLS requires a minimal amount of drug (as little as 2 µL of a stock solution per replica) with a library of excipients that can be readily available as stock solutions. It would also enable a faster and more thorough screening of excipients. This workflow could, down the line, be automated with technologies that are compatible with the well plate format.

Keywords: workflow, screening, pre-formulation, excipient, solubility, laser-based analytics

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P-II-5

Library of lipids in extracellular vesicles

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Introduction: Extracellular vesicles (EVs) are defined as nano/micro-sized lipid bilayer particles secreted by diverse cell types [1]. EVs are involved in facilitating cell-to-cell crosstalk between donor and recipient cells located either in close vicinity or at considerable distances [2]. Notably, numerous studies have reported that the composition of EV lipid membranes varies significantly depending on the originating cell type [3]. However, the potential for using these lipid profiles as cancer biomarkers remains largely unexplored.
Aims: This study aims to define lipid profiles specific to TNBC-secreted EVs and to highlight potential biomarkers for its detection and further in-depth characterization.
Methods: We studied the lipid composition of EVs secreted by triple-negative breast cancer (MDA-MB-231/453/468) and a non-cancerous breast (MCF10A) cell lines. We performed EV isolation using a double ultracentrifugation. We characterized EVs following MISEV2023 guidelines, meeting the quality control requirements including the quantification of particle number concentration and particle size using NanoTracking Analysis, morphology observation via cryo-Transmission Electron Microscopy, and protein composition assessment via Western Blot. We performed an untargeted lipid profiling using reversed-phase liquid chromatography hyphenated with High Resolution Mass Spectrometry (LC-HRMS). We analysed the data using Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA).
Results: Fundamental characterization defined the EVs' size range between 150 and 300 nm, with a zeta potential of -30 mV at a sample pH of 7.2 and a particle concentration of $5x10^9$ particles/mL consistent across all cell sources from which the EVs were isolated. EVs were positive for CD9, Hsp70, and TSG101. We identified 567 lipid species after quality filtering and curation. PC1 clearly discriminated EVs derived from cancerous versus non-tumorigenic cell lines. PC2 highlighted differences among EVs secreted by TNBC cell lines corresponding to their distinct genetic background. Phosphatidylcholines with alkyl chains 18:0-19:1, 20:1-22:5, 18:1-22:6 consistently emerged as shared lipid species across all TNBC-derived EVs. Furthermore, we defined EV-specific lipid subsets unique to each TNBC cell line.
Conclusions: We identified distinct lipid signatures in EVs associated with TNBC and we suggest candidate lipid-based biomarkers for TNBC detection and characterization. Furthermore, we provided a curated list of biologically relevant lipids that could improve TNBC-targeted drug delivery by expanding the current repertoire of lipids used in lipid-based nanocarriers.

Keywords: lipidomics, extracellular vesicles, biomarkers, lipid formulations

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P-II-6

Beyond the cold chain: Exploring lyophilization to stabilize mRNA-LNP vaccines

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Introduction: In this study, we investigated the freeze-drying of blank lipid nanoparticles (LNPs) as a strategy to reduce reliance on ultracold storage, supporting the development of more accessible mRNA vaccines [1].
Aims: This research focuses on investigating freeze-drying as a strategy to stabilize two mRNA lipid nanoparticles (mRNA-LNPs), offering a potential alternative to costly ultracold storage.
Methods: Two LNP formulations were investigated: one containing a cationic ionizable lipid (cil1a) and one containing a cationic lipid (cl2). The LNP formulations were manufactured using ethanol injection method (EIM), with lipids dissolved in ethanol and injected into 50 mM citrate buffer (pH 4.5) at a 3:1 organic-to-aqueous phase ratio under stirring. The ethanol and citrate buffer were exchanged to DPBS 1x. Particle size (Z-average diameter), polydispersity index (PDI) of cil1a and cl2 were analyzed by DLS using a Malvern Instruments Zetasizer Nanoseries. This study evaluates the effect of six cryoprotectants - sucrose, trehalose, mannose, glucose, mannitol, and sorbitol - at three different concentrations (5%(m/v), 10%(m/v), and 20%(m/v)) on the stability of both LNP formulations post freeze-drying.
Results: The aim was to obtain a particle size under 200 nm, a PDI below 0.3, a pH between 5 and 8, an osmolarity between 300 mOsm/kg and 600 mOsm/kg and a good quality cake. Among the tested conditions, sucrose seems to be a good cryoprotectant for both formulations, but at different concentrations: 5%(m/v) for the cl2-LNP formulation and 20% (m/v) for the cil1a-LNP formulation.
Conclusion: Sucrose emerged as an effective cryoprotectant for both LNP formulations, although the optimal concentration is different: 5% (m/v) for the cl2-LNP formulation and 20% (m/v) for the cil1a-LNP formulation. These findings provide a valuable basis for further freeze-drying and formulation optimization.

Keywords: nanoparticles, freeze-drying, ultracold storage, mRNA vaccines

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P-II-7

Novel modulable burst-free hydrogel platform technology for drug delivery

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Introduction: A primary obstacle in hydrogel's drug delivery is the initial "burst release", where a portion of the incapsulated Active Pharmaceutical Ingredient (API) is rapidly released upon administration. This phenomenon leads to significant drawbacks such as drug overdosage, reduced therapeutic efficacy and shorter treatment duration. While current strategies to mitigate this effect exist, they often introduce process complexity, reduce drug loading capacity, and require additional synthetic steps which increase costs and narrow the range of applicability, often implying a tailored optimization for a specific application. Our work introduces a novel, simple and elegant platform technology that directly solves this long-standing challenge while opening endless new possibilities for the use of hydrogels.
Aims: The primary goal was to develop and validate a tunable one-pot synthetic route for functionalized hydrogels and demonstrating the lack of burst release *in vitro*. We aimed to demonstrate tunable rheological properties and achieve predictable, controllable release profiles of a hydrophilic anti-inflammatory drug, i.e. diclofenac sodium salt.
Methods: Hydrogels were prepared from functionalized hyaluronic acid (HA), a crosslinker, and a library of aromatic compounds in a one-pot, solvent-free process, with diclofenac sodium salt incorporated as the model drug after the hydrogel synthesis. Formulations were characterized structurally by NMR and FTIR. Rheological properties were analyzed to assess viscosity and cohesivity. Antioxidant capacity was measured using the CUPRAC assay. Drug release profiles against control formulations were monitored via UV-Vis spectroscopy. To confirm in vitro biocompatibility was assessed on the L929 fibroblast cell lines degrading gel samples over 72 h. A WST-1 assay was used to assess the sample's toxicity.
Results: The platform successfully produced a range of HA-hydrogels with distinct and tunable rheological profiles. All formulations demonstrated significant antioxidant activity as measured by the CUPRAC assay. Critically, the release studies of diclofenac sodium salt showed highly controlled, near zero-order kinetics, eliminating the initial burst effect that was characteristic of the control formulations. Furthermore, the WST-1 assay performed on the degraded gel products confirmed excellent cell viability (>95%), proving the biocompatibility of the system.
Conclusions: We have successfully achieved a scalable platform that overcomes the critical challenge of burst release in drug delivery. Our one-pot approach provides a superior, cost-effective method for producing hydrogels with tunable rheology and zero-order release kinetics. We are actively seeking collaborations to apply this platform to new therapeutic challenges and to advance our formulations toward *in vivo* evaluation.

Keywords: hydrogels, hyaluronic acid, burst release, controlled drug delivery, tunable rheology

P-II-8

Thermo-reactive in situ forming liposome depot (TILD): From computational design to *in vivo* efficacy

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Introduction: Frequent drug administration burdens patients and caregivers, leading to poor adherence and suboptimal outcomes, particularly in geriatric, incapacitated, and veterinary populations [1,2]. While long-acting injectable systems could alleviate this issue, many existing formulations face limitations in scalability, injectability, and achieving both a rapid onset and sustained effect [2].
Aims: This study aimed to develop and validate a thermoresponsive, *in situ* forming liposomal depot (TILD) that enables biphasic subcutaneous (s.c.) drug release, combining computational design with experimental and *in vivo* testing across species to address limitations of current prolonged-acting delivery systems.
Methods: TILD was developed on the basis of computational atomistic and coarse-grained molecular dynamics simulations, identifying key interactions between a model drug (buprenorphine) and charged lipid components, predicting surface association, membrane embedding, cation-triggered release, and depot formation. Comprehensive *in vitro* characterizations - including SAXS, NMR, DSC, DLS, cryo-TEM, and HPLC - were performed to confirm the simulations and assess membrane structure, drug encapsulation, stability, depot formation, and controlled release [2]. Pharmacokinetics and biodistribution studies in rats assessed depot localization and drug release kinetics, while pharmacokinetics and safety were evaluated in beagle dogs. A pharmacodynamic incision pain model was used, where rats underwent surgery followed by drug administration, and pain relief was measured using von Frey filaments to assess the pain threshold.
Results: Simulations of vesicles and membrane (Fig. 1a-b) and experimental analyses confirmed drug localization within and on the liposomal membrane, further revealing structural transitions upon exposure to physiological conditions. These changes drive depot formation and enable a favorable release profile - an initial physiological ion-triggered onset followed by body temperature-induced sustained release (Fig. 1c-d). *In vivo* studies demonstrated rapid onset, prolonged drug availability (Fig 1 e-f) and effective analgesia over several days in rats (Fig. 1g), with large-animal pharmacokinetic data supporting translational relevance (Fig 1h).
Conclusions: TILD leverages a rational computer-guided liposome design to implement physiological triggers, overcoming key challenges in sustained drug delivery. The system enables scalable, dual-phase release with translational relevance, offering a practical alternative to existing depots. These findings highlight TILD as a potential modular platform suitable for further development in both veterinary and human medicine.

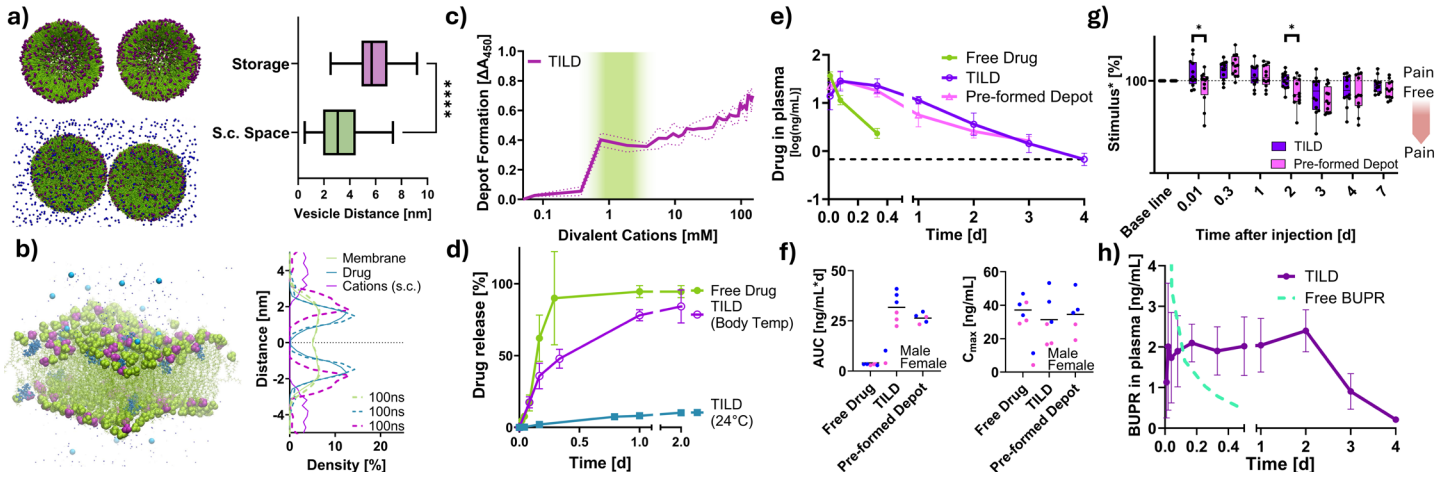

Fig. 1 a) Coarse-grained simulation of liposomes demonstrating s.c. depot formation as indicated by vesicle proximity. b) Atomistic simulation of a drug-loaded vesicle bilayer under s.c. conditions revealed drug localization within the bilayer and a fraction associated with charged headgroups; Ca^{2+} ions competed for these sites, displacing surface-bound drug and initiating a burst release as shown by density plot. c) Aggregation studies demonstrate vesicle formation at physiological cation concentrations within s.c. space (green). d) In vitro release studies confirmed temperature-triggered release at body temperature. e-f) Pharmacokinetic analysis showed sustained release over 3–4 days, exceeding free drug performance and matching pre-formed depots. g) Mechanical pain threshold after incision: in situ-formed depots reduced pain for 2–3 days, surpassing pre-formed depots and aligning with clinical reassessment at day 4. h) Sustained release over 3-4 in beagle dogs, compensating for 12 consecutive injections of the free drug.

Keywords: liposome, *in situ* forming depot, thermoresponsive, computational formulation design

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P-II-9

Vaginal administration of a dual-acting drug delivery system for endometriosis treatment

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Introduction: Endometriosis, a chronic condition affecting 10% of women worldwide, is characterized by the presence of endometrial tissue outside of the uterine cavity on pelvic organs such as the fallopian tubes, ovaries, bowel or bladder [1]. A common feature of endometriosis is fibrosis [2], an abnormal wound healing process which is characterized by accumulation of the extracellular matrix proteins [3]. Pirfenidone (PFD) can be applied as a repurposed drug against endometrial fibrosis by inhibiting TGFβ [4]. Moreover, cannabidiol (CBD), incorporated into liposomes, can be applied for pain management [5] in endometriosis, providing higher bioavailability.
Aim: Aim of this work was to prepare a composite drug delivery system (DDS), comprising mucopenetrating CBD liposomes incorporated in a mucoadhesive, thermosensitive gel in which PFD was solubilized. Due to the first uterine pass effect [4], we expect that the vaginal administration of the gel leads to elevated concentrations inside and surrounding the uterus. This would allow PFD to exert its antifibrotic action on endometriotic lesions and CBD to provide a local pain relief, bypassing the first-pass metabolism.
Methods: We established an *in vitro* mucin-binding assay via dynamic light scattering (DLS) and we identified the best liposomal formulation for mucopenetration, CBD encapsulation and CBD release. The mucoadhesivity of the formulation was investigated by mixing liposomes decorated with polyethylene glycol (PEG) lipids having different acyl chain lengths with porcine mucin, evaluating also the effect of PEG concentration at the liposomal surface. The release profiles of CBD and PFD within the stand-alone and combined DDS were tested in simulated vaginal fluids [4]. Further, the gel was characterized in detail with regard to its rheological properties.

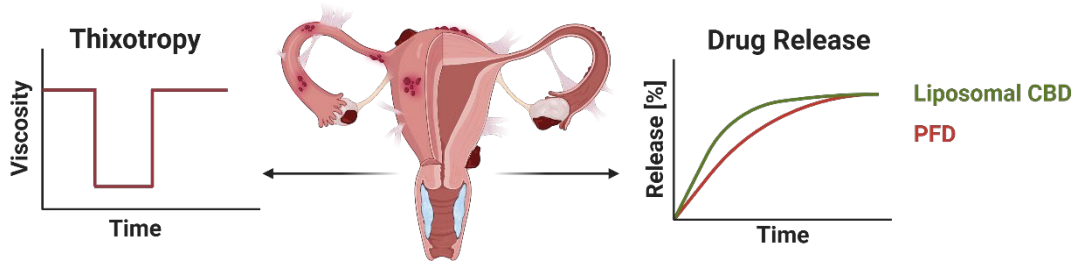

Figure 1: Schematic illustration of the applied gel with drug release and thixotropy profile

Results: Liposomes containing DMPE-PEG_{2000} (1,2-dimyristoyl-*sn*-glycero-3-phosphoethanol-amine-N-[methoxy(polyethylene glycol)-2000) showed lower mucoadhesion than the ones formulated with DSPE-PEG_{2000} (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000), independent of the PEG concentration. The composite system, containing CBD liposomes (DMPE-PEG_{2000}:DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine)) and PFD, showed a 96% and 93% release after 8 h, respectively. The rheological properties of the gel remain nearly unaffected, by the different liposomal formulations with the exception of thixotropic recovery. The gel containing the DMPE-PEG_{2000}:DOPC liposomal formulation showed a recovery of 84%, while when formulating liposomes with DSPE-PEG_{2000}:DOPC, only a recovery of 73% could be obtained.

Conclusion: We developed a patient-centric, dual-acting drug delivery system with easy applicability and a suitable release profile. Our non-hormonal composite system has the potential to become a therapeutic option for pain relief and for addressing fibrosis in endometriosis. Our findings provide a solid foundation for the *in vivo* studies to analyze the pharmacokinetics of the first uterine pass effect, but also the pharmacodynamic behavior of both drugs.

Keywords: endometriosis, CBD liposomes, PFD, thermosensitive gel, pain, fibrosis management

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P-II-10

Intracellular processing of DNA-lipid nanoparticles: A quantitative assessment by image segmentation

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Introduction: Carriers such as lipid nanoparticles (LNPs) have emerged as promising tools for the efficient transport of nucleic acids in gene therapy. However, achieving effective intracellular delivery remains a significant challenge, largely due to the complexity of intracellular transport pathways. Upon cellular uptake, LNPs are internalized into endosomes, from which they must escape to avoid degradation. Current LNP formulations exhibit low endosomal escape rates, and existing methods to quantify endocytic events, including escape, are often complex and inconsistent [1].
Aims: This study aimed to establish an automated, high-throughput imaging assay to quantify endosomal escape and recycling of Cy5-labeled DNA-LNPs in live cells. Using engineered HuH7 cell lines overexpressing EGFP-tagged Gal9 or Rab4a, we compared trafficking behavior of DNA-LNPs formulated with either SM-102 or ALC-0315 ionizable lipids.
Methods: Stable EGFP-HuH7 cell lines expressing Gal9 (escape marker) or Rab4a (recycling marker) were generated and characterized. DNA was encapsulated into LNPs by microfluidics formulated with the two indicated ionizable lipids. Cells were treated with Cy5-labeled DNA-LNPs (0.5 µg/mL), and intracellular trafficking was monitored by confocal live-cell imaging. A custom Python script integrated with Ilastik-based segmentation enabled automated quantification of >150 cells per time point. Lysosomal co-staining was applied to assess additional trafficking events including degradation.
Results: Gal9- and Rab4a-overexpressing HuH7 cells were generated and showed distinct fluorescence patterns, reflecting endosomal escape and recycling upon activation. Automated image analysis revealed differences in intracellular trafficking between SM-102 and ALC-0315 formulations, with quantification of Cy5-DNA signal overlap with EGFP puncta over time. Moreover, upon treatment, only subpopulations of the HuH7 target cells could be activated with respect to escape or recycling. Co-localization with lysosomes captured additional insights into cargo fate, covering approximately 92% of detectable intracellular events.
Conclusions: The developed imaging and automated analysis platform enabled quantitative assessment of intracellular trafficking; revealing significant differences in the endosomal escape rates of two DNA-LNP formulations. This evaluation provided valuable insights into the endocytic dynamics of DNA-LNPs, underscoring the critical role of formulation optimization in improving intracellular delivery of nucleic acid carriers. The assay may be extended to other nucleic acids such as mRNA or even viral vectors, offering broad applicability for gene delivery research.

Keywords: gene delivery, lipid nanoparticles, intracellular trafficking, endosomal escape, endosomal recycling

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P-II-11

Dual targeted lipid nanoparticles for enhanced DNA delivery to breast cancer cells

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Introduction: Since the success of COVID-19 mRNA vaccines, lipid nanoparticles (LNPs) have emerged as a promising non-viral gene therapy platform due to their adaptability, low immunogenicity, safety, large payload capacity, and cost-effectiveness. LNPs can be engineered to target specific tissues by adjusting their properties (e.g., pH, charge, vascular structure), improving efficiency, circulation, and targeting. While LNPs passively target solid tumors, their effectiveness is limited in early metastases, which lack certain vascular features. Active targeting, through antibodies or receptor ligands on the LNP surface, can enhance delivery to metastatic cancer cells, reducing off-target effects. For example, trastuzumab (Herceptin), a monoclonal antibody targeting the Her2 receptor, improves survival in Her2-positive breast cancer by causing cell arrest during the G1 phase. However, resistance to trastuzumab, due to factors like Her2 downregulation, can limit its efficacy. Combining trastuzumab with other targeting strategies, such as folate receptor targeting, could help overcome this resistance. Folate receptors are overexpressed in many cancers, including breast, ovarian, and lung, and can be targeted with folate-conjugated therapies that are internalized into cancer cells, enabling more precise drug delivery.
Aims: This study aimed to design dual-targeting LNPs encapsulating a DNA expression plasmid and evaluate their effectiveness compared to single-targeted LNPs in different breast cancer cell lines: MCF7 (low Her2 expressing), MDA-mb453 (moderate Her2 expressing), and SKBR3 (strongly expressing Her2). The LNPs were synthesized using a microfluidic mixing method, followed by ligand attachment through Michael addition. Characterization was performed using dynamic light scattering (DLS), while performance was evaluated via fluorescence-activated cell sorting (FACS) analysis and confocal microscopy.
Methods: LNPs were synthesized using the microfluidic mixing method, followed by ligand attachment to their surface through Michael addition. The particles were characterized using DLS and their performance was evaluated via FACS analysis and confocal microscopy. To validate the *in vitro* findings, a zebrafish xenograft model was employed.
Results: *In vitro* tests showed that Dual-LNPs demonstrated significantly faster and more sustained internalization across all cell lines compared to single-targeted LNPs, indicated by increased mean fluorescence intensity (MFI) over time. *In vivo* xenograft experiments using a zebrafish model demonstrated the targeting capacity of LNPs under physiological conditions. Dual-LNPs showed greater cellular interactions in MDA-mb453 and SKBR3 cells compared to single-targeted formulations. After 48 h, Dual-LNPs achieved higher transgene expression even in MCF7 cells.
Conclusion: It is demonstrated that Dual-LNPs, modified with both Her and FA targeting moieties, outperformed single-targeted LNPs in targeting and transfecting breast cancer cells both *in vitro* and *in vivo*. Notably, they were effective not only in Her2-overexpressing SKBR3 cells but also in Her2-negative MCF7 cells, showcasing their versatility.

Keywords: breast cancer, DNA gene delivery, folate, herceptin, lipid nanoparticles, targeting, trastuzumab

P-II-12

Physicochemical characterization of lipid nanoparticles by microfluidic particle analysis

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Introduction: Lipid nanoparticles (LNPs) are widely used to deliver nucleic acids, especially in gene therapy and mRNA vaccines [1, 2]. To improve their design and use, it is important to understand their physical and chemical properties. However, many current methods cannot fully describe the complexity of LNPs.
Aims: The goal of this study is to present a new method for analyzing DNA-loaded LNPs. We combined two techniques, capillary zone electrophoresis (CZE) and Taylor dispersion (TD), to better understand important features of LNPs. This combination is called electrophoretic Taylor dispersion (eTD).
Methods: We used standard capillary electrophoresis (CE) equipment to study LNP formulations. TD gave information about particle size and how nucleic acids are spread inside the particles. CZE helped us measure the ζ-potential and find out where the DNA is in the particles. We also tested free DNA and RNA, and their mixtures with LNPs, under the same conditions.
Results: TD showed the size of the LNPs and how the nucleic acids were distributed. CZE gave information about the charge of the particles and the location of the DNA. The new eTD method showed clear differences between different LNP formulations and helped detect unencapsulated (free) DNA. We also saw different signals for single-stranded mRNA and double-stranded DNA, showing that the method can separate these molecules.
Conclusions: Combining CZE and TD is a useful way to study LNPs loaded with nucleic acids. This method can give more detailed information about particle size, charge, and how nucleic acids are encapsulated. It can help improve the design and quality control of LNP-based drug delivery systems.

Keywords: **l**ipid nanoparticles, capillary electrophoresis, Taylor dispersion, nucleic acids, ζ-potential, gene therapy, DNA

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P-II-13

Analyses and correlations between extracellular vesicles and cells across tissues

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Introduction: Extracellular Vesicles (EVs) are released by cells across all biological domains, playing crucial roles in many intercellular interactions under physiological and pathological conditions [1]. They are thus increasingly studied for diagnostic and therapeutic purposes [2].
Aims: The new Center for EV Research (EVR) in Bern [3] aims to standardize and provide a full-service platform for processing and analysis of any EV.
Methods: EVs were isolated from 6 distinct human or bovine model tissues (hepatic, ureteral, intervertebral disc, adipose mesenchymal, umbilical cord mesenchymal, and induced pluripotent stem cells). Isolation was performed by ultracentrifugation followed by size exclusion chromatography. The resulting EVs were characterized in terms of yield, size distribution, and surface charge. Membrane blotting of the purified EVs was performed to recover proteins for untargeted shotgun proteomics. Proteins were also isolated from the donor cells, to evaluate selective molecular enrichment within and across the 6 model tissues. Large and small RNA from the same cells and EVs were also selectively isolated and sequenced.
Results: EV isolation yielded 10^8-10^{11} particles per sample, indicating successful recovery but also differences across tissues. Nanoparticle tracking analysis confirmed heterogeneous but consistent vesicles' size distribution profiles (180±100 nm) and zeta potential values (-29±11 mV). Proteomics and RNA analyses revealed highly distinctive yet reproducible signatures for each tissue-derived EV population. It was powerful enough to also discriminate between different treatment conditions within the tissue of interest. Comparative analysis between EVs and their donor cells uncovered selective molecular enrichment patterns, supporting the hypothesis that EVs actively package specific biomolecules rather than merely reflect cellular content.
Conclusions: We provide reproducible evidence of EVs' specialized functionalities with potential biological significance, consistent across 6 different tissues of origin. Analysis of correlations between cells and EVs in terms of proteins and RNAs shows diverse selective enrichment profiles. We lay a thoroughly standardized foundation for future clinical and biotechnological applications, emphasizing tissue-specific integration into therapeutic strategies. Translational correlations between proteomic and transcriptomic analyses are currently underway.

Keywords: extracellular vesicles, proteomics, RNA, liver, urethra, intervertebral disc, adipose mesenchymal stem cells, umbilical cord mesenchymal stem cells, induced pluripotent stem cells, human, bovine

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III. CLINICAL PHARMACY / CLINICAL PHARMACOLOGY

P-III-1

Assessing the risk of falls and fractures in older adults with painful diabetic polyneuropathy initiated on gabapentinoids, SNRIs, or TCAs: An observational study

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Introduction: Painful diabetic polyneuropathy (PDPN) affects up to a quarter of patients with diabetes and has serious implications on their quality of life. First-line treatments for pain management include gabapentinoids, serotonin-noradrenaline reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs). All three groups of drugs carry important risks, especially in older adults. A major concern is their potential to increase the risk of falls and fractures – major causes of morbidity and mortality in older adults. There is currently a lack of comparative safety data for these first-line drugs.
Aim: To compare and assess the risk of falls and fractures in older patients with PDPN initiating gabapentinoids versus SNRIs and TCAs. A secondary objective is to describe whether age, sex and frailty modulate these risks.
Methods: We emulated a new user, active comparator, randomised clinical trial using the Danish nationwide healthcare and administrative registries from 2005-2024. We included patients with diabetes, aged ≥ 65 years, who were initiating either pregabalin, gabapentin, duloxetine, venlafaxine, amitriptyline or nortriptyline. Patients with contra-indications or alternative indications for these drugs were excluded. Patients were followed up for 6 months. We emulated randomisation using a list of pre-specified covariates to calculate propensity score weights and estimated the average treatment effect among the treated patients (gabapentinoid initiators). These weights were used to estimate weighted cumulative incidence rates. Cox regression models were used to estimate hazard ratios, and the robust sandwich variance estimator was used for confidence intervals. We used a set of negative control outcomes to assess the plausibility of the results.
Results: Our cohort consisted of 22,716 patients, with a median follow-up time of 180 days. Weighted incidence rates for falls and fractures were 94 for gabapentinoids per 1,000 person years (95% CI, 87–101) *vs* 101 for TCAs (95% CI, 85–118) and 92 for gabapentinoids (95% CI, 85–99) *vs* 115 for SNRIs (95% CI, 80–150). Gabapentinoid initiators had lower hazards than SNRI initiators (hazard ratio 0.80, 95% CI 0.58-1.09) and TCA initiators (hazard ratio 0.92, 95% CI 0.77-1.11). However, the confidence intervals for both comparisons included 1. Analyses of control outcomes were not significant, but confidence intervals were wide and the point estimates imprecise. Exploratory analyses indicated a higher risk for frail, older and female patients in the comparison gabapentinoids *vs* SNRIs. Sensitivity analyses showed similar results.
Conclusion: In contrast to earlier studies that lacked active comparators, and consistent with more recent, methodologically robust observational research, this study found no increased risk of falls and fractures associated with gabapentinoids compared to SNRIs and TCAs. These findings contribute to the ongoing discussion about the safety profile of gabapentinoids in PDPN and other

neuropathic pain conditions and may support clinicians in making informed, shared decisions with their patients.

Keywords: older adults, medication safety, chronic pain, gabapentinoids, antidepressants, falls, fractures

P-III-2

Trends and outcomes of naloxone use for iatrogenic opioid overdose: A 10-year retrospective case series

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Introduction: Opioids are important therapy options in the multimodal approach to pain management. However, they carry an inherent high risk for adverse drug events (ADEs). These ADEs are generally predictable and avoidable through proper management and monitoring. The most severe ADEs associated with opioids include respiratory depression, loss of consciousness, stupor, and hypothermia. Naloxone, an opioid antagonist, is used to counteract severe opioid overdoses.
Aim: The aim of this study was to assess the frequency of naloxone use at the Bern University Hospital, and to describe the patient population requiring it, the circumstances leading to opioid overdoses, and the outcomes of its application.
Methods: We conducted a ten-year single-centre, retrospective case series of naloxone application in in-patients of the Bern University Hospital from January 2014 to December 2023. Patients were included if they were 18 years or older and if they received naloxone after opioid administration. We excluded patients receiving naloxone during surgery. We conducted an exploratory and descriptive analysis. To calculate the incidence rate of naloxone use, we used the number of patients treated with opioids per year. The study was approved by the Ethics Committee of the Canton of Bern (2024-01506).
Results: Of the 671 naloxone applications, 121 (18.0%) met the inclusion criteria. The incidence rate of naloxone use was 11.0 applications per 10,000 in-patients treated with opioids and showed a slight increase from 2014 to 2023. The median daily opioid dose was 73.6 morphine milligram equivalents (MME). The most frequently involved opioids were morphine, oxycodone and fentanyl. According to the Schumock & Thornton criteria, we considered 82 (67.8%) of the 121 opioid overdoses to be potentially preventable. Reasons for preventability included drug interactions with opioids, unsuitable doses in relation to patient characteristics, and previous adverse reactions to opioids. Patients received higher opioid doses before hospital admission (30.0 MME) than at discharge (15.0 MME). Nevertheless, 71 of the 104 discharged patients (68.3%) still received at least one opioid.
Conclusion: This study found that iatrogenic opioid overdoses are relatively uncommon and occur at rates similar to those reported in other countries. However, the considerable number of preventable opioid overdoses indicates that improvements are advisable. Potential measures include implementing opioid stewardship programs that use trigger tools to identify inappropriate drug combinations or dosing errors.

Keywords: opioids, naloxone, opioid overdose, medication safety

P-III-3

Implementing a clinical pharmacy service for older adult inpatients with chronic non-cancer pain: A proof-of-concept study

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Introduction: Chronic non-cancer pain (CNCP) affects 28-88% of older adults and is associated with a decreased quality of life. Effective management of CNCP requires a multimodal approach. Pharmacological therapies are generally considered a second-line treatment for CNCP. However, older adults frequently receive them. As these drugs carry a high risk of medication-related problems, older adults with CNCP are at increased risk of experiencing medication-related harm.
Aim: This proof-of-concept study aimed to implement a multimodal clinical pharmacy service for older adult inpatients on a geriatric ward, in order to assess the feasibility of including pharmacists more closely in specialised care.
Methods: From January to May 2025, we conducted a single-arm feasibility study. Patients aged 65 years and over who were hospitalised on a geriatric ward at a tertiary care hospital in Switzerland and suffering from CNCP were included in the study. The intervention consisted of a semi-structured interview about the patients' pain history, as well as patient-reported outcome measures (PROMs) and therapy goals. Pharmacists then conducted a medication review using a trigger tool that had been previously developed and validated. The findings were discussed during an interprofessional ward round. The final decision was made jointly with the patients. One month after hospital discharge, we followed up with patients by telephone. This study was exempt from full ethics approval by the Ethics Committee of the Canton of Bern (Req-2024-01252).
Results: We included 48 patients in the study: 28 (58%) were interviewed and 18 (38%) underwent telephone follow-up. Pharmacists suggested 56 therapy changes, of which 29 were identified using the trigger tool and 27 during the regular medication review. The acceptance rates were 78% and 41%, respectively. Pain frequency, highest pain and lowest pain in the last seven days decreased after hospital discharge. The emotional impact of the patients' CNCP was also lower. However, other pain-related PROMs, such as basic and instrumental activities of daily living and quality of sleep, showed no change. Patients reported that their mobility had slightly worsened after hospital discharge.
Conclusion: This proof-of-concept study demonstrated the feasibility of a multimodal pharmacist service for older adult inpatients with CNCP. Using a standardised approach with a trigger tool showed promising results for the efficient detection of medication-related problems. These results suggest that involving pharmacists more closely in standard care could be beneficial.

Keywords: older adults, medication safety, chronic pain, clinical pharmacy

P-III-4

Diabetes in Switzerland: A 20-year comparison of risk factors by sex

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Introduction: Although there are multiple known partially modifiable risk factors for diabetes - such as obesity, smoking, low education, nutrition, sedentary behavior and age - diabetes prevalence is increasing in both men and women globally, with higher prevalences observed in men compared to women. In absolute numbers, however, more women are affected by diabetes.
Aims: To assess gender-stratified changes in diabetes prevalence and associated risk factors in the Swiss population between 2002 and 2022.
Methods: We included all individuals >15 years that specified their diabetes status (Yes/No) in the Swiss Health Surveys (SGB) 2002 (n=18'654) and 2022 (n= 21'906). We assessed diabetes prevalence in 2002 and 2022, stratified by age and sex. Body mass index (BMI) distribution among non-diabetic and diabetic men and women were visualized using violin plots and we used logistic regression to quantify the association between diabetes and age, BMI, smoking status, alcohol consumption, education, and physical activity in men and women, separately. The odds ratios (OR) were calculated with a 95% confidence interval. Using a non-linear Oaxaca-Blinder decomposition we quantified the contribution of changes in population characteristics versus changes in risk factor effects on the diabetes prevalence shift.
Results: Diabetes prevalence increased from 4.78% to 7.38% in men and 4.11% to 5.52% in women between 2002 and 2022. Increasing age was associated with an increasing risk of diabetes in men and women in 2002 (>75 years *vs.* 35-44 years, OR men: 5.15 [4.10-6.49], OR women: 2.67 [2.18-3.29]), but in 2022 this trend was mainly observed in men (OR men: 6.33 [5.01-8.00], OR women: 0.91 [0.77-1.07]). Trends for the other assessed factors were similar in both years and in men and women, except for tertiary education being more protective and smoking carrying higher risk for men in 2022. Comparison of the two survey populations in 2002 and 2022 revealed a shift toward older age (mean 60.3 years in 2002 *vs.* 62.1 years in 2022) and higher BMI (mean 26.7 kg/m^2 in 2002 *vs.* 27.7 kg/m^2 in 2022) among individuals with diabetes, accompanied by an increase in tertiary education in the whole population, especially in women (2002: 9.1% *vs.* 2022: 35.5%), and a rise in physical activity across all groups. The Oaxaca-Blinder decomposition attributed 75.4% of the increase in prevalence to a change in population characteristics (such as higher age and higher BMI) and 24.6% to the impact of these risk factors on the outcome.
Conclusions: Diabetes prevalence increased in both men and women, but BMI distribution and the impact of risk factors varied by sex. Women appeared less affected by age-related risks but exhibited greater susceptibility towards obesity and low education. These findings facilitate the identification of population sub-groups that may benefit the most from targeted prevention interventions.

Keywords: diabetes, gender, sex, cross-sectional, Swiss Health Survey, Oaxaca-Blinder, obesity

P-III-5

Administration of intranasal midazolam for acute anxiety in palliative care (AIM Care Study)

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Introduction: Intranasal midazolam (MDZ) is used off-label to manage anxiety, a common and highly distressing symptom in palliative care (PC). The intranasal route is minimally invasive, with a fast onset and acceptable tolerability. Unit-dose nasal sprays (UDNS) facilitate administration and avoid nasopharyngeal drainage. Evidence on anxiolytic effects, safety, pharmacokinetics (PK), and pharmacodynamics (PD) of low-dose intranasal MDZ in PC remains limited, hence, its clinical use is primarily based on experience.
Aims: This study evaluates the effects and safety of low-dose intranasal MDZ for the treatment of acute anxiety in PC. We hypothesize a significant treatment effect with unequal mean anxiety scores across the three treatment groups (placebo: 0 mg; MDZ_{total}: 0.9 mg or 1.8 mg).
Methods: AIM Care is a double-blind, randomized, placebo-controlled multicenter pilot study with a nested PK analysis (patients with available venous access only). The study design follows a pragmatic approach to ensure feasibility in clinical practice. Using UDNS, 36 PC patients receive a single dose administered as one spray per nostril (placebo: 0 mg, $MDZ_{single\ dose}$: 0.45 or 0.9 mg). Since nasal irritation has been reported with MDZ, all formulations have similar pH ranges to safeguard blinding. A one-way ANOVA will be performed, followed by post-hoc tests if significant. Serum MDZ concentration will be analyzed using LC-MS/MS and further data is collected in *REDCap* and analyzed using *R* and *Monolix*®.
Results: Outcomes are assessed at baseline ($t_{0\ min}$) and 30 min post-intervention ($t_{30\ min}$), with certain secondary outcomes assessed up to 24 h post-intervention. Primary outcome is the change in patient-reported anxiety levels on a visual analogue scale (VAS) from baseline, measured in mm (0-100 mm). Secondary outcomes include oxygen saturation, sedation (Richmond Agitation Sedation Scale Palliative Version), salivary cortisol, and among others, PK parameters (t_{max}, C_{max}, $t_{1/2}$, AUC_{0-T}, $AUC_{0-\infty}$) in a subgroup. Recruitment started in December 2024 and was initially planned for 6 months. Due to unforeseeable challenges, an additional study site was opened and recruitment extended until September 2025. Currently, 9 patients (n = 4 female, n = 5 male, mean age 65 ± 8.2 years) have been enrolled. Preliminary data reveal mean anxiety levels at $t_{0\ min}$ (48.6 ±19.1 [20-75] mm) and $t_{30\ min}$ (24.8 ±19.4 [0-60] mm), resulting in a mean change on the VAS of -23.8 ± 25.6 [-70-0] mm. Sedation levels and SaO_2 indicate no safety concerns, which is supported by the absence of severe adverse event occurrence and presentation of only mild adverse drug reactions such as nasal irritation, fatigue, and rhinorrhea, as expected.
Conclusions: Despite the need for evidence to support decision-making, various challenges severely limit the conduct of trials in PC. This study will provide preliminary PK and PD data to guide the use of intranasal MDZ in PC. The findings will help estimate effect size and variability, informing the design of future studies and supporting evidence-based use of MDZ for anxiety management in this setting.

Keywords: anxiety, intranasal midazolam, off-label use, palliative care, pharmacokinetics

P-III-6

Defining an interprofessional co-care service for patients with hypertension: A participatory qualitative study

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Introduction: In Switzerland around 20% of people are either diagnosed with hypertension or receiving hypertension treatment. Of those receiving treatment, about 60% have uncontrolled blood pressure. Other countries have national interprofessional services that involve various healthcare professionals (HCPs), such as pharmacists, general practitioners (GPs), and other medical specialists, that support patients to manage their hypertension. These collaborative approaches have positive effects on patient health outcomes. However, Switzerland does not have a dedicated national interprofessional co-care service for patients with hypertension. Co-care is an approach that highlights the complementary role of healthcare HCPs in supporting individuals' self-management and using tools to create, share, and apply knowledge among all actors involved.
Aims: This study aims to define the structure of an interprofessional co-care service between Swiss pharmacists and GPs to improve patient management of chronic hypertension.
Method: Semi-structured interviews were conducted with 6 pharmacists and 6 GPs as well as a focus group with 6 patients, that self-reported a hypertension diagnosis. The HCPs were recruited by email and patients through various sources using a convenient and purposeful sampling method. Participants were asked about current care practices, their vision for a new co-care service, and their views on communication between HCPs and patients. Transcribed audio-recordings were coded with MAXQDA® software using a step-by-step thematic analysis.
Results: The interviews with HCPs and the focus group with patients gave insights into the key components, challenges, and opportunities to outline a useful co-care service. A clear structure was described as essential to implement a realistic co-care service in Switzerland. Communication was seen as a key aspect by both HCPs and patients, with the need for the implementation of an electronic patients' records allowing effective data sharing. All participants expressed interest in enhancing the competencies of pharmacists within this co-care service, but additional training for pharmacists was seen as crucial by HCPs to prepare them for this responsibility. In addition, trust emerged as a key theme in both the interviews and the focus group discussions. HCPs emphasised the importance of being able to define the collaboration locally (i.e., among HCPs working in close proximity), often facilitated by already existing relationships between pharmacists and GPs. Patients also expressed the need for a trusted point of contact within the pharmacies. To clarify role differentiation, participating HCPs saw the development of clear referral recommendation guidelines as necessary that would also be defined locally. Participants perceived the implementation of a co-care service, supported by effective collaboration between HCPs in Swiss pharmacies and GP practices, as offering potential benefits for patient care.
Conclusion: The structure of this co-care service will depend on the local way of communication and collaboration among HCPs, enabling the development of trust, combined with a holistic patient-centred approach, leading to improved patient care in chronic hypertension.

Keywords: co-care service, hypertension management, interprofessionality, participatory research, development of service, Swiss pharmacies, Swiss GPs

IV. MOLECULAR PHARMACOLOGY / MOLECULAR MEDICINE

P-IV-1

Uncovering mucinase inactivation mechanisms to improve oral peptide delivery

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Introduction: The intestinal mucus barrier, primarily composed of mucins, heavily O-glycosylated glycoproteins, presents a significant challenge to the oral delivery of macromolecular therapeutics [1]. Bacterial mucin-specific proteases (mucinases), including the secreted protease of C1 esterase inhibitor (StcE) [2], represent a promising strategy to transiently degrade this barrier, reduce mucus viscosity and improve drug penetration [3]. However, our recent studies using porcine gastrointestinal mucus revealed considerable variability in StcE's mucolytic activity across individual samples, which may compromise therapeutic efficacy and limit clinical translation.
Aims: This study aimed to elucidate the molecular mechanisms underlying the variability in StcE mucolytic activity observed across different porcine gastrointestinal mucus samples.
Methods: Porcine small intestinal mucus samples were collected and incubated with StcE. Mucolytic activity was evaluated by rheological measurements of the elastic (G′) and viscous (G″) moduli. To investigate potential inactivation mechanisms, StcE was recovered after mucus incubation, analyzed by SDS-PAGE and mass spectrometry, and its proteolytic activity assessed using recombinant human C1 inhibitor (C1 INH) as a model substrate. Additional experiments with increased StcE concentrations and zinc supplementation were conducted to assess the influence of cofactor availability on enzymatic activity.
Results: StcE exhibited substantial variability in mucolytic activity, achieving significant mucus fluidization in only 7 out of 20 samples. Mass spectrometry confirmed that StcE remained structurally intact following mucus exposure, with no evidence of proteolytic degradation or stable inhibitory binding, relative to the untreated control. *In vitro* activity assays demonstrated that StcE retained its enzymatic activity after incubation with mucus, suggesting that inactivation within mucus was linked to local environmental factors rather than intrinsic instability. Increasing StcE concentration did not enhance mucolysis, whereas zinc supplementation significantly restored mucolytic activity in previously unresponsive samples, indicating that local zinc availability was a critical limiting factor.
Conclusion: The study identifies zinc ion availability as a critical factor influencing StcE-mediated mucus degradation, suggesting that local cofactor depletion rather than enzymatic degradation accounts for variability. These findings underscore the potential of cofactor supplementation and mucinase combinations to overcome GI mucus barriers and enhance oral delivery of macromolecules.

Keywords: oral delivery, absorption promotor, gastrointestinal barriers, mucus, mucinases

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P-IV-2

Mucin degrading enzymes – A platform to study their activity

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Introduction: Mucus and surface bound mucins are one of the first physical barriers to systemic delivery for oral and pulmonary therapeutics [1]. The high clearance rate of mucus means that trapped species are quickly excreted emphasising the need for fast transport through this barrier. The significance of this for new modalities such as peptides, oligonucleotide and protein therapeutics is increasingly being recognised. A specific class of enzyme, mucinases, can degrade this mucin backbone to further break the long mucin filaments down into small fragments. We have recently shown the promise of these mucinases, for the first time, in oral drug delivery [2]. However, to better target the degradation of mucins to achieve therapeutic delivery, without broad non-specific degradation of other mucins, new tools to study and engineer these mucinases are needed.
Aims: To establish a validated assay for evaluating mucinases activity and to perform directed evolution to engineer mucinases with desired activity profiles.
Methods: Mucins with a fluorescent tag were transfected into mammalian cell culture lines of HeLa and HEK293 enabling the display on the cell surface of model mucin substrates representing different subtypes of mucins found at epithelial barriers. Mucinases were incubated in a 96 well-plate with these cells, and specific proteolytic activity against the mucin subtypes was measured by release of the fluorescent tag. Site-directed mutagenesis of five active site residues of one mucinases (HC4) was performed by PCR to investigate the role of selected active site residues of the mucinases, using the developed fluorescent cell-display assay.
Results: The assay was established with cells displaying mucins MUC1, MUC5AC and MUC4 and validated by incubation with known mucinases with defined cleavage patterns. This identified sequence and glycosylation dependent cleavage of the mucins in agreement with literature. The glycosylation of displayed mucins was evaluated by mass-spectrometry confirming intact core 3 sialylated glycans. The importance of 5 active site residues of the mucinase HC4 on cleavage was evaluated and allowed identification of key residues for enzyme binding and permissive mutations.
Conclusion: This study established a cell display based assay for the evaluation of mucin subtype and glycosylation dependent cleavage of mucinases. This was further applied for the evaluation of engineered mucinases in a 96 well-plate format.

Keywords: Mucins, epithelial barriers, mucus, mucinases, enzymes

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P-IV-3

Selective cleavage of mucin-domain glycoproteins by mucinases: An *in vitro* screening study

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Introduction: Mucus forms a protective barrier on epithelial surfaces, limiting the penetration of macromolecules and nanoparticle-based therapeutics. This barrier is primarily structured by mucins – large, heavily O-glycosylated proteins that exist in both secreted and membrane-tethered forms [1]. Through extensive glycosylation and cross-linking, mucins form dense, adhesive meshworks that impede drug diffusion. Conventional mucolytics show limited ability to disrupt these networks, whereas mucinases – enzymes that cleave the protein backbone of mucins – may offer a more selective and mechanistically targeted strategy to modulate mucin-rich environments [2].
Aims: This exploratory study aims to identify candidate mucinases for targeted mucolysis by evaluating the mucin-degrading activity and substrate specificity of selected enzymes.
Methods: Selected mucinases (BT4244, OgpA, StcE, AM0627, AM0908, SmE, and HC4) [2,3] were recombinantly expressed in *E. coli* and purified by affinity chromatography. Mucolytic activity was evaluated using C1 esterase inhibitor (C1-INH), a mucin-domain containing glycoprotein, at a 1:50 enzyme-to-substrate molar ratio. Reactions were performed at 37 °C, with or without prior removal of terminal sialic acids by *Vibrio cholerae* sialidase (VC Sia) to assess sialylation-dependent cleavage efficiency. Substrate cleavage was analyzed by SDS-PAGE. To evaluate substrate specificity, mucinase activity was further tested against the non-mucin glycoproteins fetuin (O-glycosylated) and transferrin (N-glycosylated) at a 1:25 molar ratio.
Results: OgpA, AM0627, StcE, and SmE efficiently cleaved C1-INH under the tested conditions, while BT4244, AM0908, and HC4 showed no detectable activity. Co-incubation with VC Sia had no effect on cleavage patterns. OgpA and AM0627 also degraded fetuin, while none of the enzymes cleaved transferrin. StcE and SmE emerged as the most promising candidates, displaying selective activity against a mucin-like glycoprotein without affecting non-mucin substrates. Cleavage efficiency was preserved in the presence of sialic acids, indicating their ability to process mucins bearing complex O-glycans.
Conclusion: Our findings support further investigation of mucinases as targeted mucolytic agents for modulating mucin barriers, such as those encountered in the pulmonary context.

Keywords: Substrate specificity, mucolysis, mucus, mucinase, therapeutic enzymes

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P-IV-4

Combining tissue microporation and stretching for enhanced transbuccal peptide absorption

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Introduction: The buccal mucosa is a promising, non-invasive route for systemic peptide delivery, offering accessibility, high vascularization and permeability. However, achieving clinically relevant bioavailability (BA) remains difficult due to the epithelial barrier and limited residence times [1,2]. Our group previously developed a suction-based transbuccal delivery device enabling double-digit BA *in vivo* for selected peptides when co-formulated with chemical permeation enhancers [3,4]. While effective, this approach is limited for very large molecules, underscoring the need to reach more effectively the subepithelial layer.
Aims: Building on the original suction-based patch design, we integrate solid stainless steel microneedles (MN) to combine tissue stretching with microporation, aiming to improve mucosal permeability. We hypothesize an additive – ideally synergistic – effect on peptide access to the bloodstream. This approach aims to expand the applicability of this platform to broader spectrum of macromolecular drugs.
Methods: Mechanical changes resulting from MN integration into the suction patch were assessed via compression, adhesion and negative pressure tests on silicone patches of varying Shore A (ShA) hardness. Compression to 50 N and adhesion to a ShA0 silicone surface – mimicking buccal tissue – were measured using a Texture Analyzer. Separately, negative pressure was recorded for 1 min. To evaluate the impact on permeability, freshly excised porcine buccal tissue served as a physiologically relevant model. Patches were loaded with sulfonated cyanine (Cy5), a fluorescent, poorly permeable macromolecular surrogate [3]. Following the patch application, the tissues were cryosectioned, fixed, stained (phalloidin and Hoechst), and imaged via fluorescent microscopy to assess Cy5 diffusion in the tissue.
Results: ShA50 silicone was chosen to conduct further *ex vivo* experiments due to its balanced mechanical performance after MN integration. This also ensured consistency with prior data in this proof-of-concept phase [2]. Preliminary *ex vivo* data obtained with porcine buccal mucosa, showed that using either MN or ShA50 device alone resulted in over 50% of the permeated drug surrogate, being concentrated in the superficial layer of the mucosa. In contrast, combining MN with the cup led to broader distribution of the probe, with over 50% detected beyond 500 µm.
Conclusions: Integrating MN into the suction-based device enhanced surrogate diffusion to subepithelial layers. As the epithelium, averaging 200 µm in porcine buccal mucosa, constitutes the primary permeation barrier, these results indicate that this barrier can be at least partially overcome [5]. This observation lays the foundation for future *in vivo* studies to validate the potential for enhanced BA.

Keywords: non-invasive peptide delivery, buccal delivery, microneedles

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P-IV-5

Nature-inspired protection: Engineering complement-resistance via surface-tethered M22 peptidomimetics

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Introduction: The complement system plays a crucial role in immune defense, contributing to both innate and adaptive immunity. However, because complement can also harm healthy human cells, its activity is tightly regulated by various membrane-bound and soluble factors. Complement activation can also trigger unintended attacks on non-self surfaces, such as transplanted organs. Preventing complement-mediated injury to cells - for instance, during transplant rejection - is a pressing clinical challenge, yet current therapeutic options have shown limited success.
Aims: This work aims to develop novel strategies for protecting biosurfaces from complement-mediated cell damage, taking inspiration from natural mechanisms used by pathogens. Specifically, it seeks to design artificial surface coatings that recruit complement inhibitors, thereby preventing immune attack, such as in the context of transplant rejection.
Methods: This study utilizes a synthetic homodimeric peptide (M22-N) derived from the M22 protein, a virulence factor of *Streptococcus pyogenes*, which is known to bind C4b Binding Protein (C4BP), a natural inhibitor of the complement system [1,2]. The peptide was synthesized via Solid Phase Peptide Synthesis (SPPS), site-specifically labelled and immobilized onto artificial surfaces to mimic bacterial presentation. Flow cytometry was employed to assess C4BP recruitment from human serum and evaluate complement inhibition. Additionally, interaction kinetics, using Surface Plasmon Resonance (SPR), between immobilized M22-N and soluble C4BP were characterized.
Results: M22-N-decorated surfaces effectively recruited C4BP from normal human serum and successfully inhibited complement-mediated attack on model biosurfaces. Flow cytometry confirmed functional recruitment, while kinetic analysis revealed that the interaction between immobilized M22-N and soluble C4BP has a nanomolar affinity, indicating a strong and specific binding interaction. These results validate the ability of the synthetic peptide to mimic bacterial complement evasion mechanisms.
Conclusion: This study demonstrates that artificial surfaces functionalized with a synthetic M22-derived peptide can effectively recruit C4BP and, hence, inhibit complement activation. By mimicking a natural immune evasion strategy, this approach offers a basis for developing protective coatings. Future efforts will focus on structure guided design of shorter peptides, optimization of its stability and binding properties to ultimately enhance the potential for biomedical surface protection.

Keywords: complement system, C4b-binding protein (C4BP), surface protective coating

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P-IV-6

Novel leech-derived dual-inhibitors of the complement and coagulation system

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Introduction: Ill-controlled activation of the complement and coagulation systems drives disorders such as ischemia reperfusion injury or microangiopathies. Simultaneously targeting the lectin (LP) [1] and intrinsic pathway (IP) [2] harbors therapeutic potential, especially considering the intricate crosstalk between complement and coagulation, including the kallikrein-kinin system [3]. As part of a phylogenetic analysis of antistasin-like protein homologs, our group recently discovered and named a previously undescribed protein in freshwater leeches: helostasin. Preliminary data showed selective and potent inhibition of the LP, suggesting potential mitigation of thromboinflammatory disorders while preserving classical pathway complement activation.
Aims: We aim to develop a small leech-derived inhibitory protein that selectively targets key proteases in both the LP and IP. By engineering helostasin for dual specificity, and characterizing the structural basis for protease binding, our goal is to lay the foundation for a novel class of multi-target serine protease inhibitors with translational potential.
Methods: Following recombinant expression in *E. coli*, helostasin's activity was assessed empirically in complement activation assays with human blood serum, competitive inhibition assays, and direct binding experiments. To support experimental data, *in silico* immunogenicity predictions, molecular dynamics (MD) simulations, and in-depth analysis of intermolecular interactions were conducted to gain structural insights into binding mechanisms with target proteases.
Results: Successful expression and purification yielded pure, functional protein. Helostasin binds both plasma kallikrein and coagulation factor XIa with nanomolar affinity, over 100-fold stronger when compared to other proteases in the coagulation system. Complement activation assays confirmed LP inhibition, with IC_{50} values in the nanomolar range. Analysis of MD simulations identified residues essential for binding to active sites via the antistasin-like domain, and revealed binding to an exosite via the flexible C-terminal region of helostasin.
Conclusions: Helostasin demonstrates potent inhibition in both the LP and IP. Predictions of MHC II binding regions suggest low immunogenicity, and selectivity screens show no inhibition of thrombin, supporting its therapeutic potential for thromboinflammatory disorders by reducing the risk of bleeding events. Taken together, helostasin provides a structural framework for "multi-targeting" of serine proteases in the complement and coagulation system.

Keywords: serine proteases, enzyme inhibition, anti-inflammatory, anticoagulant, structural biology

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P-IV-7

Evaluation of pharmacodynamic properties after Fc-fusion of complement-modulating peptides and proteins

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Introduction: Despite an impressive increase of complement-targeted therapeutics in the clinic, medical needs remain and dosing regimens of drugs and candidates are not ideal. Latter is particularly true for peptides and small proteins, which feature high potency and selectivity but face challenges regarding pharmacokinetic (PK) properties. Among PK-improving strategies, fusion of proteins/peptides to the crystallizable fragment (Fc) of IgG4 immunoglobulins provide an elegant option to enhance plasma half-life and potentially modulate target binding without activating complement.
Aims: The aim was to generate Fc-fusion constructs with a peptide of the compstatin family and the leech-derived protein gigastasin, and evaluate the impact on complement inhibition.
Methods: Genes encoding compstatin analogue Cp01-V3I or gigastasin were cloned into the pFUSE-hIgG4-Fc2 vector and transfected into HEK293F cells for expression. The fusion proteins were purified using Protein-A affinity chromatography. Complement inhibition was measured with an in-house ELISA and/or chromogenic substrate assays. For the compstatin peptibodies, C3b binding was analyzed using surface plasmon resonance (SPR).
Results: In the case of gigastasin, Fc-fusion enabled mammalian expression that could hitherto not be achieved for the non-tagged protein. Fc-Gigastasin potently inhibited complement initiation pathways albeit with reduced activity; interestingly, the impact was more pronounced for the lectin than the classical pathway. Similarly, the inhibitory potency of compstatin peptibodies *in vitro* was reduced when compared to the free peptides, yet SPR indicated an enhanced target residence on surface-bound C3b.
Conclusion: This proof-of-concept study demonstrate that Fc-fusion of complement-inhibiting proteins/peptides provides a suitable strategy to tune therapeutic properties. Although the initial fusion constructs showed somewhat lower activities, they remained potent inhibitors and activity may be regained by optimizing fusion formats. Importantly, our findings indicate that Fc-fusion may facilitate the production of therapeutics and impact target and/or location selectivity. Finally, it is expected that PK properties of Fc-fusions are improved; their increased molecular size reduces renal clearance, while FcRn-mediated recycling may further extend half-life. In subsequent stages, it will be important to explore the *in vivo* profiles of fusion constructs to assess their impact on both pharmacodynamic and -kinetic properties, optimize formats accordingly, and compare them to other PK-enhancing strategies such as lipidation.

Keywords: fusion-proteins, antibodies, complement therapeutics, protein expression

P-IV-8

Breaking the boundaries of the clinical C3 inhibitor compstatin: Development of species-tolerant and long-acting analogs

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Introduction: The compstatin family of complement C3 inhibitors has been continuously optimized, finding broad applications in biomedical research and as therapeutics. Pegcetacoplan (Empaveli/Syfovre), a PEGylated compstatin derivative, has been approved and non-PEGylated candidates are in clinical development. However, compstatin's narrow species specificity for human/primate C3 prevents broader evaluation in preclinical disease models, potentially limiting indication expansion. Moreover, there remains room for improvement regarding the pharmacokinetic properties of this class [1,2].
Aim: The aim of this study is to develop new analogs of the clinically approved cyclic peptide, compstatin, that are active in rodent models and possess improved pharmacokinetic properties, thereby overcoming the limitations of species specificity and enhancing their potential for translational research and therapeutic applications.
Methods: Structural insights from experimental and homology models of rodent C3b was used to identify molecular determinants of compstatin's species specificity and guide *in silico* redesign of the peptide to enhance drug-target interactions for human and rat C3. The insight also enabled strategies to improve compstatin's pharmacokinetic profile, either by increasing target residence or via lipidation and fusion proteins. Compstatin analogs with improved properties were tested for inhibitory activity and target affinity.
Results: Structure-guided prediction and experimental validation of target-binding kinetics and complement inhibition profiles led to the discovery of a lead peptide featuring both affinity and inhibitory activity for rodent C3. Compstatin modifications that previously led to improved affinity for human C3 are currently being investigated for their impact on the rodent-active lead. As compstatin's PK properties are largely driven by tight binding to the highly-abundant plasma protein C3, the observation that addition of a single methyl group at position 4 resulted in a 30-fold increase in target residence may enable long-acting derivatives. Along the same path, we could show that lipidation of the peptide to exploit plasma protein binding for half-life extension can be achieved without jeopardizing compstatin's activity.
Conclusion: The development of species-tolerant and long-acting analogs of compstatin has opened the door to fine-coarse SAR studies, crystal structures, and *in vivo* models. This insight will guide further pharmacodynamic and -kinetic optimization of the class and may extend research, therapeutic, and diagnostic applications.

Keywords: complement system, peptides, compstatin, pharmacokinetic

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P-IV-9

Complement downregulation on complement-activating surfaces by coating with the regulatory peptide 5C6: An elegant combination of metabolic glycoengineering and click-chemistry

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Introduction: Upon contact of biomedical surfaces with the human immune system complement activation can cause inflammation and loss of function, severely impacting patient's health. So far, downregulation of the complement activation upon contact is restricted to systemic treatment due to the lack of site-specific inhibitors or modulators. However, Factor H (FH), the soluble natural regulator of complement amplification, can be recruited onto such vulnerable surfaces by coating with FH-binding peptides like the cyclic peptide 5C6 [1]. However, until now evaluation of such peptides on living surfaces was restricted by the lack of biocompatible strategies.
Aims: Herein, we described the modification of porcine endothelial cells (PIEC) with 5C6 using a biocompatible modification strategy by combining metabolic glycoengineering and click-chemistry.
Methods: 5C6-Coating was accomplished by incorporating azide-groups into the glycocalyx upon supplementing the cell culture medium with azide-sugars. A clickable 5C6 derivative was prepared and clicked to the azide-modified cells. FH-Recruitment from human serum and complement downregulation on coated cells was analyzed with fluorescence microscopy and flow cytometry.
Results: Upon exposure of 5C6-coated PIEC to serum, FH was selectively recruited onto the cells, which was verified by flow cytometry and fluorescence microscopy. Moreover, 5C6-coating of PIEC was able to prevent complement activation, which was confirmed by a decrease of opsonization as well as the downstream complement effectors to control levels.
Conclusions: Our newly established model validates using 5C6-coating on surfaces to recruit complement-regulating molecules, thereby impairing immune-mediated complications. Due to the simple decoration with strained alkynes, different peptides can be tested in the established assay system. This can support the evaluation of protective peptide coatings and promote the therapeutic outcome of allo- and xenotransplantations.

Keywords: complement system, factor H, metabolic engineering, peptides

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P-IV-10

Leech-inspired bivalent peptides for multi-target modulation of host-defense responses

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Introduction: The complement and coagulation systems are blood-based proteolytic cascades that are essential for innate immunity and maintaining hemostasis. Though tightly controlled by the action of various serine proteases, dysregulation of both systems can occur simultaneously and lead to thromboinflammation - creating a need for new therapeutic strategies [1]. Leeches have evolved to secrete proteins that inhibit complement and/or coagulation through simultaneous blockade of multiple proteases but tuning the selectivity profile of such proteins is restricted. Inspired by nature, we have utilized structural insights of gigastasin, a protein from the giant Amazon leech, in complex with a protease target as a template to design bivalent peptides capable of mimicking its mechanism of action [2].
Aims: This work entails the design and characterization of novel bivalent peptides for precision, multi-target modulation of disease-relevant blood proteases.
Methods: A design-make-test (DMT) system was implemented for the design macrocyclic peptides (SCIPs) capable of blocking the protease active site in a substrate-like binding mode. A known substrate served as a template for probing protease subsite specificity, while structural insights from gigastasin inspired focused synthetic peptide libraries and macrocycle design. The macrocyclic peptides are first characterized for enzyme selectivity using competitive substrate assays, along with target interaction analysis and functional bioassays (complement ELISA/coagulation assay). Precision tuning of selectivity profiles towards disease-relevant proteases is then enabled by further *in silico*-guided design and the use of non-proteinogenic amino acids. Bivalent constructs were also designed (gigalirudins, Gil) by fusing candidate macrocycles with a peptide derived from the sulfotyrosine-containing tail of gigastasin, which was previously shown to block a functional anion-binding exosite (ABE) on multiple blood proteases [2].
Results: First focusing on the complement protease target C1s, we developed numerous macrocycles (SCIPs) displaying nanomolar inhibitory potency. One peptide, SCIP-34, showed functional and selective inhibition of the classical complement pathway in human serum. While highly selective for C1s (K_i = 200 nM), some inhibition was observed against coagulation FXIa (K_i = 2 µM). Fusing SCIP-34 into the bivalent peptide scaffold (Gil-2) simultaneously boosted potency against both the complement and coagulation proteases (15-42 fold) and enabled efficient modulation of both host defense systems *in vitro*.
Conclusions: Inspired by gigastasin, we have designed a family of both macrocyclic and bivalent peptides capable of mimicking its mechanism of action. Precision control enabled by synthetic peptide chemistry enables fine tuning of the macrocycle selectivity to address one or two protease targets while retaining nanomolar potency. Meanwhile, fusing peptides into the bivalent gigalirudin scaffold served as a versatile affinity enhancement strategy that can enable potent modulation of a broader target scope. Collectively, our leech-inspired peptide platform can enable “tailor-made” inhibitors for use as tools or therapeutic options in complex thrombo-inflammatory indications.

Keywords: peptide design, macrocycles, screening, protease inhibitors, complement, coagulation.

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P-IV-11

Fantastic drugs and where to find them: Investigating and improving drug-like properties of the leech-derived complement inhibitor gigastasin

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Introduction: Despite the rapid expansion of complement-targeted therapeutics, medical needs remain for the treatment of complex thromboinflammatory conditions such as COVID-19 or stroke. Given that parasites share the need to control several host defense pathways, their evasion proteins may inspire the development of therapeutic multi-pathway inhibitors. Gigastasin, a leech-derived protein, was previously described as serine protease inhibitor with activity for C1s, MASP, and FXIIa but it remained unclear whether gigastasin or derivatives thereof may be suitable as therapeutics [1].
Aim: Explore gigastasin's target selectivity and uncover structure–activity relationships to design improved derivatives with enhanced biochemical and -physical properties.
Methods: Here, we employed structural models, *in silico* and experimental methods to assess and extend gigastasin's properties. Several expression systems were evaluated for the production of gigastasin and proteins were tested for purity, stability, activity, and selectivity. Structure-guided, site-directed mutagenesis enabled the tailored optimization of gigastasin.
Results: While mammalian expression was unsuccessful, recombinant gigastasin featuring 10 disulfide-bridges could be produced in optimized bacterial systems. The thermal stability of the secreted protein enabled HPLC processing to enhance purity and remove endotoxins. *In vitro* evaluation revealed potent inhibitory activity for the classical and lectin pathways in serum of different species (e.g., human, mouse, rat). Chromogenic substrate and surface binding plasmon resonance (SPR) assays extended selectivity profiling to include (*e.g.,* C1r, FXIa) or exclude (*e.g.,* C2, FXa) relevant proteases; whereas *in silico* simulations corroborated selectivity for active over zymogen forms, protein engineering revealed unexpected activity determinants for distinct pathway inhibition. Computational methods predicted a low overall immunogenicity profile and identified few risk-residues. Combined insight from structural and experimental studies allowed for the generation of gigastasin derivatives with improved expression yields, stability, activity and target-binding properties.
Conclusions: Our studies suggest that gigastasin itself may be suitable for preclinical development but that, by employing structure-guided mutagenesis, it may provide an intriguing template to tailor its properties for specific applications. The insight gained may also improve our understanding of parasite evasion, target selectivity, and complement activation mechanisms.

Keywords: complement inhibitor, gigastasin, serin proteases, leech protein, complement system

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P-V-12

mRNA Display screening for the identification of translationally-active analogs of the compstatin class of complement inhibitors with enhanced species specificity profiles

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Introduction: Compstatin is a cyclic tridecapeptide that acts as protein-protein interaction inhibitor of complement component C3. Earlier optimization efforts improved its activity and led to an approved compstatin-derived drug (pegcetacoplan) and a clinical candidate (AMY-101) for complement-mediated diseases. Despite their clinical success, the narrow species specificity of these peptides for human and non-human primate C3 limits the possibility to perform preclinical studies in rodent models. The development of compstatin analogs with broader species tolerance is therefore considered crucial for translational research and indication expansion.
Aims: This study aims to identify novel compstatin analogs with high affinity and improved inhibitory potential against both human and rat C3 using mRNA display technology.
Methods: Two mRNA display libraries were designed with randomized amino acids at selected positions. One library also featured a three-amino acid N-terminal extension to explore potential gains in binding affinity. Six consecutive selection rounds were performed, alternating between rat and human C3 as target proteins. Candidate sequences retrieved from next-generation sequencing will be synthesized via solid-phase peptide synthesis and assessed by SPR and ELISA for binding affinity and inhibitory potency.
Results: mRNA display selections using N-terminally extended libraries yielded peptides with IC_{50} values ranging from 20-200 µM against rat C3 and 3-40 µM against human C3, as determined by ELISA. These results were comparable to the parent compstatin analog (IC50 = 20 µM for rat; 3.6 µM for human), supporting the feasibility of using mRNA display to identify functional cross-species inhibitors.
Conclusions: This study demonstrates that mRNA display can be an effective platform for the identification of novel compstatin analogs with improved target affinity and species tolerance, as supported by the proof-of-concept screening against rat and human C3 with the six-amino acid extension library. Further library refinement, including targeted randomization and incorporation of short N-terminal extensions, will facilitate the identification of sequence motifs associated with dual-species binding. These optimized peptides may support the development of rodent-compatible compstatin analogs to enabling preclinical studies and expand therapeutic applications of complement inhibitors in the future.

Keywords: complement system, compstatin, peptide inhibitors, mRNA display

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V. PHARMACOLOGY / BIOPHARMACY

P-V-1

Behavioral and neuronal effects of psilocybin in mice

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Introduction: Psilocybin, a classic psychedelic, has demonstrated the potential to act clinically as an antidepressant. Psilocybin is rapidly metabolized into its active metabolite psilocin, which acts as a partial 5-HT_{2A} receptor agonist. Psilocybin-induced rapid antidepressant effects can last for weeks after a single dose, distinguishing it from conventional antidepressants that require chronic administration. Evidence suggests that these effects involve changes in neuronal activity and increased structural plasticity in the frontal cortex. However, psilocybin is also known to induce hallucinations, and the precise neurobiological mechanisms underlying both the therapeutic and hallucinogenic effects remain unclear.
Aims: This study aims to investigate the behavioral and neuronal effects of psilocybin in mice.
Methods: To assess hallucinogenic-like behavior, we recorded mice for 90 min in an open field following intraperitoneal injections of saline, 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, or 2 mg/kg psilocybin. Locomotion and head-twitching response in the open field was analyzed using EthoVision XT. We evaluated brain areas where psilocybin induced acute neuronal effects by immunostaining brain slices from mice sacrificed 90 min after a single injection of 1 mg/kg psilocybin or saline for the immediate early genes c-Fos, NPAS4 and EGR1. Synaptic plasticity was assessed 24 h after a single injection via whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) in layer 5 pyramidal cells of fresh brain slices, using artificial cerebrospinal fluid with picrotoxin and tetrodotoxin.
Results: We found that psilocybin elicited a dose-dependent increase in characteristic head twitching. High-dose psilocybin (2 mg/kg) induced hypolocomotion in the open field, while lower doses did not alter locomotion. Psilocybin increased the number of c-Fos-, NPAS4- and EGR1-positive cells in cortical regions and the amygdala, suggesting enhanced neuronal activity and activation of plasticity-related pathways. Patch-clamp data revealed increased mEPSC frequency in the prelimbic cortex and increased amplitudes in the infralimbic cortex, indicating synaptic potentiation. A reduced decay time of mEPSCs in the prelimbic cortex was observed, suggesting altered AMPA receptor composition (possibly increased GluA1/GluA2 ratio), consistent with synaptic remodeling.
Conclusions: Psilocybin induces behavioral, molecular, and synaptic changes consistent with neuronal plasticity. Ongoing work aims to link these mechanisms with long-lasting antidepressant and anxiolytic behavioral effects. This research may guide the development of novel psychiatric treatments.

Keywords: psilocybin, pharmacology, antidepressant, neuronal plasticity, immediate-early genes, animal studies

P-V-2

Drug-drug-gene interactions involving OATP1B1 – investigating coproporphyrins as biomarkers for clinical routine

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Introduction: Personalizing drug therapy remains a major challenge, as various factors can contribute to the interindividual variability in drug response. Statins, widely used to lower cholesterol and to prevent atherosclerotic cardiovascular disease (ASCVD), illustrate this issue. Over 20% of patients experience statin-associated musculoskeletal symptoms (SAMS), leading to poor adherence and therefore increased cardiovascular risk. One factor assumed to influence statin tolerability is the hepatic uptake transporter OATP1B1, encoded by the *SLCO1B1* gene. Reduced-function variants of *SLCO1B1* are linked to increased systemic statin levels and consequently a higher risk of SAMS. Beyond genetics, non-genetic factors such as co-medications also affect statin pharmacokinetics, contributing to complex drug-drug-gene interactions (DDGIs). Despite growing recognition of these factors, their integration into clinical practice remains limited. Since OATP1B1 transports not only exogenous compounds such as statins, but also endogenous molecules like coproporphyrins (CPs), measuring CP levels as a biomarker of OATP1B1 activity has emerged as a promising approach to assess transporter function *in vivo*.
Aims: This study investigates the potential of endogenously formed coproporphyrins (CPI and CPIII) as biomarkers for assessing OATP1B1 transporter function and identifying DDGIs in clinical settings. Additionally, it investigates the *in vitro* interaction between OATP1B1 and the most commonly used drugs within the study cohort.
Methods: Archived whole blood and serum samples from a case series study, involving patients with adverse drug reactions or therapy failure and suspected genetic associations, were further analyzed. Genotyping for *SLCO1B1* was conducted using real-time PCR and CP levels were quantified using solid-phase extraction together with UPLC-MS/MS. To assess the effects of metformin, atorvastatin, and semaglutide on OATP1B1-mediated uptake of CPs, an *in vitro* transport study was conducted using MDCKII cells expressing OATP1B1.
Results: A correlation was observed between genotype-predicted OATP1B1 phenotypes and CP levels, with higher CP concentrations corresponding to reduced transporter activity, consistent with previous findings. Importantly, among patients with the same genotype-predicted OATP1B1 phenotype, those taking OATP1B1-inhibiting drugs (e.g., atorvastatin) had higher CP levels, suggesting that co-medications can further reduce transporter activity through DDGIs. In addition, interaction between metformin, atorvastatin, semaglutide and OATP1B1 was verified *in vitro*.
Conclusion: Despite the use of serum samples drawn in clinical routine settings, CPI levels reflected OATP1B1 function, supporting their potential as biomarkers to determine transporter activity and phenoconversion in clinical practice.

Keywords: coproporphyrins, biomarker, OATP1B1, personalized drug therapy, drug-drug-gene interactions, clinical routine, pharmacogenetics, phenoconversion

P-V-3

From potatoes to precision: Developing an analytical method for solanidine-based CYP2D6 biomarkers

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Introduction: Cytochrome P450 2D6 (CYP2D6) is a key drug-metabolizing enzyme that plays a critical role in the biotransformation of a broad range of commonly prescribed medications. At the same time CYP2D6 is also one of the most polymorphic CYPs contributing substantially to variable drug disposition [1]. This variability has a profound effect on the efficacy and safety of numerous clinically used drugs that are metabolized by CYP2D6. Recent findings indicate that solanidine, a dietary steroidal alkaloid found in potatoes, and its CYP2D6-dependent metabolites 3,4-seco-solanidine-3,4-dioic acid (SSDA) and 4-OH-solanidine hold potential as biomarkers for CYP2D6 phenotyping [2,3]. However, accurate quantification is hindered by the lack of authentic reference standards.
Aim: This study aims to develop a simple and cost-effective method to isolate pure SSDA and 4-OH-solanidine from human urine. These metabolites will serve further as standards for the establishment and validation of a bioanalytical method involving LC-MS/MS quantification, enabling accurate measurement in serum, plasma and urine samples in order to advance phenotyping of CYP2D6.
Methods: Pooled human urine samples were centrifuged, and the supernatants were subjected to column purification using Diaion® HP-20 resin. Solid-phase extraction with a cation-exchange sorbent was subsequently employed to recover solanidine metabolites. The eluate was further purified using semi-preparative HPLC to isolate the target fractions.
Results: The downstream process successfully enabled the extraction and isolation of the solanidine metabolites SSDA and 4-OH-solanidine from pooled human urine samples. Extracted ion chromatograms (EICs) and mass spectrum data confirmed the presence and identity of the target metabolites in the purified fractions.
Conclusions: This study established a workflow using Diaion® HP-20, solid-phase extraction, and semi-preparative HPLC to isolate SSDA and 4-OH-solanidine from human urine. The purified fractions are suitable for structural analysis and will serve as reference standards for LC-MS/MS method development and validation.

Keywords: CYP2D6, solanidine, SSDA, 4-OH-solanidine, biomarkers

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P-V-4

Scarring beyond healing: Exploring Cyclops syndrome – a distinct form of knee arthrofibrosis following the anterior cruciate ligament reconstruction

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Introduction: Cyclops syndrome is a form of localized knee arthrofibrosis (excessive scar tissue formation) following the anterior cruciate ligament (ACL) reconstruction. It is typically recognized by a loss of terminal knee extension, discomfort or pain, and a distinctive “clunk” sound at full extension. This complication affects approximately 10% of patients undergoing ACL reconstruction. The standard treatment involves arthroscopic resection of the fibrous nodule (usually within 12 months after the initial ACL reconstruction) followed by structured physical therapy [1]. Given the high incidence of ACL tears, especially in young and active individuals, Cyclops syndrome remains a significant clinical concern.
Aims: The aim of this research is to investigate the histological composition of the Cyclops lesions, and identify key molecular biomarkers involved in their development, supporting the design of targeted treatment strategies capable of preventing or reversing excessive fibrotic tissue formation, without interfering with the ligamentization process.
Methods: Cyclops lesions and synovial fluid were collected during arthroscopic surgery. For histological analysis, Cyclops biopsies were treated and stained (Hematoxylin and Eosin, Masson's Trichrome, Sirius Red), to evaluate tissue architecture. The remaining biopsy material was used for cell isolation (enzymatic digestion with collagenase). Cell populations were characterized using flow cytometry (FACS) to identify the types of cells present within the lesion. A subset of isolated cells was cultured in vitro and used to assess the effect of lysyl oxidase (LOX) inhibition using β-aminopropionitrile (BAPN) on cell viability (WST-1 assay), in order to explore potential antifibrotic treatment strategies. Synovial fluid samples were analyzed to assess the concentration of proinflammatory cytokines (TNF-α and IL-1β) using enzyme-linked immunosorbent assay (ELISA).
Results: Cyclops lesions were characterized by abundant collagen deposition, with disorganized collagen fibers observed in the central region and more organized structures at the periphery. Fibroblasts were present throughout the tissue, along with some immune cells and visible blood vessels. FACS confirmed the presence of $CD45^+$ immune cells, predominantly $CD11b^+$ (myeloid cells), along with a smaller population of $CD3^+$ (lymphocytes). Fibroblasts represented the dominant cell population and were characterized by the expression of fibroblast activation protein, indicating an activated phenotype [2]. ELISA analysis of synovial fluid revealed elevated concentrations of IL-1β and TNF-α, supporting the inflammatory nature of the condition. Measured values in Cyclops samples ranged from 48.81 ± 0.2 pg/mL to 93.56 ± 4.06 pg/mL, while literature reports IL-1β levels in healthy individuals to be approximately 10 pg/mL [3]. Isolated cells were exposed to BAPN at concentrations ranging from 12.5 mM to 0.025 mM. No cytotoxic effects were observed at any time point (6 h, 24 h, 72 h, and 7 days), suggesting its safety for further investigation.
Conclusions: This study is the first to reveal the detailed histological architecture and cellular composition of Cyclops lesions. Our findings suggest that Cyclops syndrome originates from a persistent inflammatory response, sustaining fibroblast activation, leading to abnormal ECM deposition. To explore potential preventive/therapeutic strategies, future research should focus on the encapsulation of BPAN and evaluation of its ability to reduce ECM deposition in *ex vivo* tissue biopsies or in advanced 3D models. Specific ILs may serve as prognostic biomarkers for identifying patients at risk of developing Cyclops syndrome.

Keywords: cyclops Syndrome, knee arthrofibrosis, inflammation, fibroblasts, lysyl-oxidase

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P-V-5

Antidepressant response – a combination of ABCB1 genetics and substrate recognition?

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Introduction: Antidepressant treatment response varies significantly between individuals. One mechanism proposed to contribute to this interindividual variability is the efflux transporter P-glycoprotein (P-gp). P-gp limits brain exposure of its substrates and is encoded by the *ABCB1* gene. Genetic polymorphisms in *ABCB1* are assumed to impact the transporter's functionality and might therefore affect the clinical efficacy of substrate drugs. In line with this, clinical guidelines of the Swiss Society of Anxiety Disorders and Depression recommend genotyping of the *ABCB1* variants rs2032583 and rs2235015 in patients exhibiting drug resistance. It is advised to avoid certain antidepressants in carriers of the reference variants.
Aims: We aim to challenge this recommendation to genotype ABCB1 in antidepressant-resistant patients by re-evaluating the role of P-gp in antidepressant efficacy.
Methods: For this, we start with the characterization of commonly applied antidepressants for their interaction with human P-gp using the SB MDR1/P-gp PREDEASY™ ATPase Kit. Based on our *in vitro* observations we will classify the tested molecules as substrates, inhibitors or non-substrates. Our results will be compared with currently available literature data gathered through a systematic review of the Certara® DIDB database. Subsequently, we aim to determine whether the adapted P-gp substrate classification of antidepressants, changes the predictive value of the *ABCB1*-genotyping in patients with major depression. For this ad-hoc analysis, we will apply data of a prospective clinical study in which patients, genotyped for *ABCB1,* were assessed for treatment efficacy. Within the study, antidepressant response was systematically monitored weekly after treatment initiation applying the Hamilton Depression Rating Scale (HAMD).
Results: The *in vitro* classification of antidepressants according to their interaction with P-gp will be presented and compared with literature-based data. Based on this updated classification, exploratory analyses will be conducted to examine potential associations between ABCB1 genotypes and antidepressant treatment outcomes.
Conclusions: The findings may contribute to a better understanding of the clinical relevance of P-gp-mediated transport and ABCB1 genetic variability in antidepressant response. This could inform future recommendations regarding the utility of pharmacogenetic testing in treatment-resistant depression.

Keywords: P-glycoprotein, ABCB1, antidepressants, pharmacogenetics, treatment resistance, ATPase assay, major depressive disorder

P-V-6

(*RS*)-[^{11}C]HBP2 - A PET radioligand with high specificity, good brain uptake and favorable kinetic properties for imaging monoacylglycerol lipase

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Introduction: Pharmacological inhibition of monoacylglycerol lipase (MAGL) has emerged as a promising therapeutic strategy for various diseases such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury [1]. The development of effective MAGL-targeting PET tracers is essential for advancing the understanding of disease mechanisms and supporting the development of MAGL inhibitors. In collaboration with Hoffmann La-Roche, we identified (*SR*)-HBP2 and (*RS*)-HBP2 from a series of 183 novel bicyclopiperazine-based MAGL inhibitors as promising candidates for PET tracer development based on their half-maximal inhibitory concentration values and multiparameter optimization scores [2].
Aims: To develop ^{11}C-labeled PET tracers for MAGL imaging with high specificity, good brain uptake and favorable kinetic properties.
Methods: (*SR*)-HBP2 and (*RS*)-HBP2 were synthesized via a multi-step chemical pathway. Chiral separation was achieved using supercritical fluid chromatography. Radiolabeling was performed via *O*-alkylation of a phenol precursor with [^{11}C]CH_3I. *In vitro* autoradiography was conducted on brain sections from Wistar rats, MAGL knockout mice, and corresponding wild-type controls. Dynamic PET imaging studies were carried out in anesthetized mice, with blocking experiments using the irreversible MAGL inhibitor PF-06795071 to assess tracer specificity. Radiometabolite analysis was performed on mouse brain and plasma samples 20 min post-injection.
Results: Both (*SR*)-[^{11}C]HBP2 and (*RS*)-[^{11}C]HBP2 were successfully synthesized and radiolabeled. *In vitro* autoradiography revealed a heterogeneous distribution pattern in rodent brain slices, consistent with known MAGL expression. The radioactivity was significantly reduced in rat brain slices under blocking conditions and in slices from MAGL knockout mice, confirming the specificity and selectivity *in vitro*. PET imaging showed that both tracers exhibited high brain uptake and specific binding, as evidenced by substantial signal reduction following administration of MAGL inhibitor PF-06795071. Notably, (*RS*)-[^{11}C]HBP2 exhibited faster washout kinetics than its (*SR*)-enantiomer. Radiometabolite analysis indicated that over 99% of the brain radioactivity was attributed to the intact compounds for both tracers.
Conclusions: Both (*SR*)-[^{11}C]HBP2 and (*RS*)-[^{11}C]HBP2 are promising PET tracers with high selectivity and specificity for imaging MAGL. Of the two, (*RS*)-[^{11}C]HBP2 shows superior kinetic properties, making it better suited for *in vivo* visualization of MAGL and for supporting MAGL-targeted drug development.

Keywords: monoacylglycerol lipase, carbon-11, PET imaging, kinetic properties

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P-V-7

Localized heat application as a strategy to improve oral peptide bioavailability

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Introduction: Macromolecular therapeutics, including peptide and protein drugs, are now employed in the treatment of a wide array of diseases due to their potent therapeutic effects. However, their clinical administration is largely confined to injections, as oral delivery is severely hampered by gastrointestinal barriers such as enzymatic degradation, the mucus layer, and the epithelial lining [1]. While chemical permeation enhancers (PEs) are used to overcome some of these hurdles, their efficacy is relatively limited. We propose the application of heat as an alternative novel approach to improve the oral delivery of macromolecular therapeutics. Previously, we demonstrated the efficient reduction of epithelial integrity and permeation of macromolecules under heat application.
Aims: This work will elucidate the permeation enhancement mechanism of heat application and investigate synergistic effects with commercial permeation enhancers.
Methods: Heat application at 39 °C and 42 °C was applied to Caco-2 monolayers to investigate its effects on the intestinal barrier. Tight junction protein expression and localization were analyzed via western blotting, immunohistochemistry, and gene expression. Cytotoxicity studies assessed the impact of different conditions on barrier modulation. To explore potential synergies, heat was combined with commercial permeation enhancers and evaluated using transepithelial electrical resistance (TEER) and permeability assays with Cy5-labeled poly(ethylene glycols) (1, 5, and 20 kDa).
Results: The observed increase in intestinal permeability was attributed to stress-induced delocalization of tight junction proteins, rather than irreversible cytotoxic damage. TEER recovery following both short- and long-duration heat exposure confirmed barrier reversibility and supported the minimal cytotoxicity observed. Combining heat with commercial permeation enhancers resulted in an additive effect, further increasing both TEER reduction and macromolecule permeability when both are applied simultaneously.
Conclusion: We found that modest heat application of 42 °C for 2 h could significantly reduce the intercellular barrier properties of the epithelium in a reversible fashion and increase the macromolecular permeation. We thereby propose heat as a novel physical mode to improve oral peptide absorption in the gastrointestinal tract by itself and in combination with existing PEs.

Keywords: oral delivery, physical mode, permeation enhancer, peptide delivery

Reference:
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P-V-8

Potential risk of phenoconversion in the Swiss population: A descriptive study using claims data focusing on drugs metabolized by the enzymes CYP3A4/5 and CYP2B6 or transported by OATP1B1 and BCRP

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°These authors contributed equally to this work

Introduction: Drug-drug-gene interactions can affect drug responses and increase the risk of adverse effects or treatment failure. Despite their emerging relevance in clinical research, drug-drug-gene interactions remain understudied and are often ignored in clinical practice. Our objective was to assess the risk of phenoconversion by identifying potential drug-drug-gene interactions involving the transporters OATP1B1 and BCRP and the enzymes CYP2B6 and CYP3A4/5 in the Swiss population.
Aims: Our objective was to assess the risk of phenoconversion by identifying potential drug-drug-gene interactions involving the transporters OATP1B1 and BCRP and the enzymes CYP2B6 and CYP3A4/5 in the Swiss population.
Methods: Using claims data from the Helsana basic health insurance, we identified all persons of all ages with at least one drug claim between 2017 and 2021 and with Helsana basic health insurance coverage for at least one full year. For the 5-year analysis, only persons with insurance for the entire five-year period were included. Within this study population, we assessed and ranked the frequency of potential drug-drug-gene interactions of a pharmacogenetic substrate and an inhibitor/inducer of OATP1B1, BCRP, CYP2B6, and CYP3A4/5. Potential drug-drug-gene interactions were defined as the co-occurrence of a pharmacogenetic substrate and an inhibitor/inducer within 30- or 5-days windows.
Results: During the entire five-year period, 18'523 (2.1%) and 12'645 (1.4%) individuals were exposed to potential drug-drug-gene interactions using the 30-day and 5-day windows, respectively. Potential drug-drug-gene interactions most frequently involved CYP3A4/5 (81.0% and 85.3%), followed by CYP2B6 (10.9% and 8.7%) and OATP1B1 (8.7% and 13.3%). The top 3 drug classes involved were nervous system drugs (75.1%), cardiovascular drugs (10.6%), and dermatologicals (4.0%). Quetiapine ranked first in the number of participations, with quetiapine – metamizole being the predominant drug pair every year.
Conclusions: In Switzerland, 2 out of 100 persons taking drugs metabolized or transported by OATP1B1, BCRP, CYP2B6, and CYP3A4/5 are at risk of a potential phenoconversion, highlighting the importance of considering genetic and clinical data to reduce the risk of potential adverse drug reactions.

Keywords: pharmacogenetics, drug-drug-gene interactions, DDGI, Switzerland, claims data, Helsana Group

P-V-9

Characterization of the stability of a GDNF solution for colonic administration in Hirschsprung disease

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Introduction: Hirschsprung disease is a rare intestinal disorder characterized by the absence of enteric neural ganglia, which can cause neonatal bowel perforation, bowel obstruction, constipation, and enterocolitis, potentially leading to the death of the patient [1,2]. Up to now, the "pull-through surgery", a surgical procedure where the entirely aganglionic part of the bowel is removed, is the only treatment [3,4]. Recently, it was shown that colonic administration of the glia cell-derived neurotrophic factor (GDNF) can significantly prolong survival, induce intestinal neurogenesis, and improve the colon structure and motility in different mouse models [1].
Aim: The aim of this study is to evaluate methods for assessing the stability of GDNF to support subsequent formulation development for colonic administration in neonates with Hirschsprung disease.
Methods: Different concentrations of glycosylated human GDNF (34 kDa) were stored over 12 weeks at room temperature and at 4°C. Analytical methods to assess stability after defined timepoint were established. Aggregation was assessed by size exclusion chromatography and transmission electron microscopy, bioactivity was tested with a CHO-RET-luc cell assay, folding and secondary structure was monitored by circular dichroism spectroscopy and concentration was determined by bicinchoninic acid assay. The bioactivity of GDNF after incubation at body temperature with artificial colonic fluid was also evaluated.
Results: Stability testing revealed that GDNF exhibited a tendency to aggregate after 12 weeks, independent of storage temperature. Aggregation did not affect bioactivity, and the protein remained fully active under both storage conditions after 12 weeks. A slight decrease in protein concentration was observed after 6 weeks, likely due to adsorption, as no precipitation was detected. Circular dichroism spectroscopy analysis indicated that the α-helical secondary structure remained intact over the storage time. Compatibility testing under physiological conditions revealed that bioactivity was retained under simulated colonic conditions.
Conclusion: These findings demonstrate that the protein retains its structural integrity and bioactivity despite aggregation, indicating good stability under storage and physiological conditions. This stability profile supports its suitability for further formulation development in applications such as Hirschsprung's disease.

Keywords: GDNF, neurotrophic factors, protein stability, Hirschsprung

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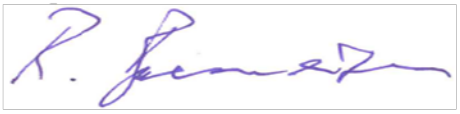

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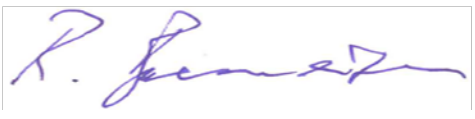

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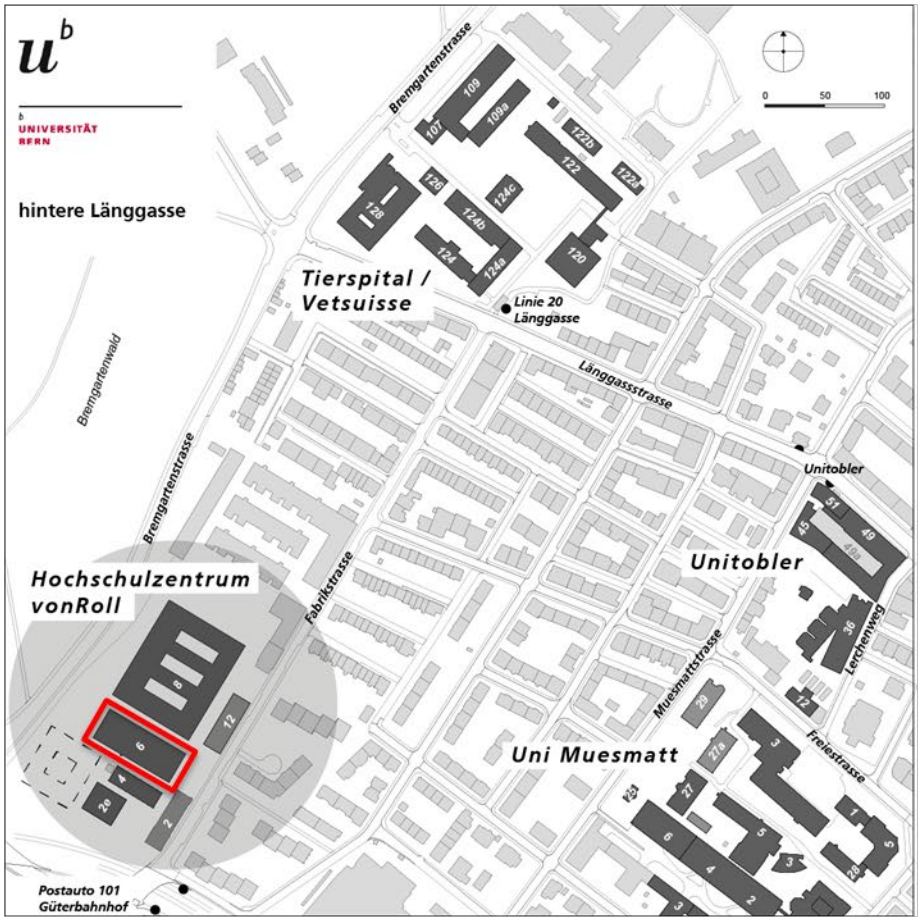

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