UNIVERSITY OF LJUBLJANA | FFA Faculty of Pharmacy

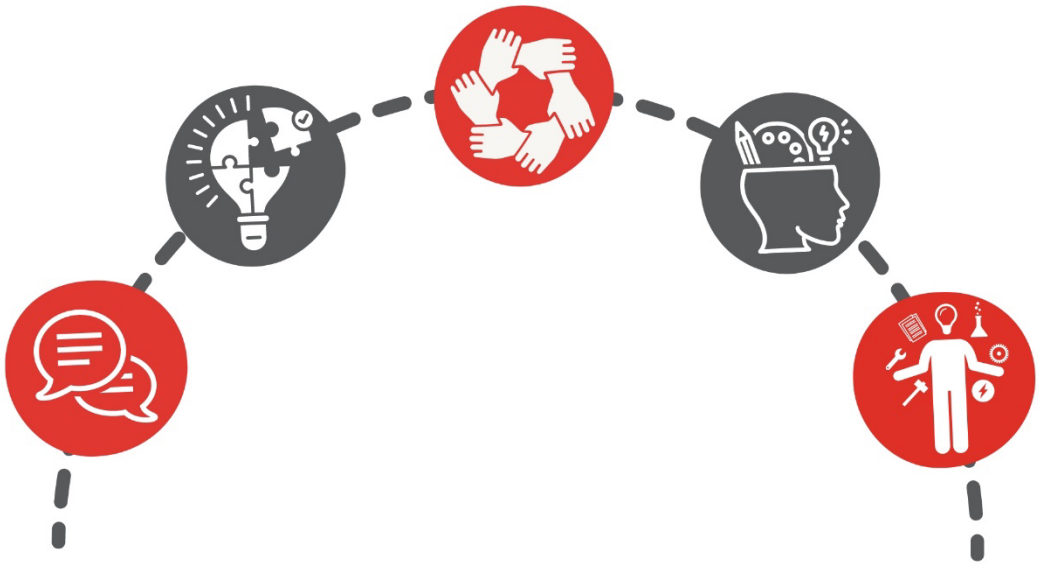
Brunching with Soft- and Research Skills at UL FFA: Unlocking Your Full Potential

2nd UL FFA Graduate Student Research Symposium

BOOK OF ABSTRACTS

University of Ljubljana, Faculty of Pharmacy

31st March 2025



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FOREWORD

This year's symposium once again celebrates the remarkable research achievements of our doctoral students. The submitted abstracts and presentations highlight the exceptional diversity and high calibre of work being carried out across clinical and non-clinical domains, at the interface of pharmacy, chemistry, biology, technology, and medicine.

The organising and scientific committee remain committed to fostering an engaging dialogue between doctoral students and academic staff within the vibrant and evolving field of pharmaceutical sciences. We hope that this year's programme provides an inspiring platform for discussion, collaboration, and the exchange of innovative ideas.

In its second edition, the CDTC programme and symposium once again bring together our research community to share progress, spark new collaborations, and encourage intellectual cross-pollination. Alongside the student presentations, this year's event features an interesting industry guest lecture, targeted networking opportunities, and the best flash talk award, making for an enriching and stimulating experience.

We wish you all an enjoyable and productive symposium!

SYMPOSIUM PROGRAMME

13:00 – 13:05 *Opening Address*
Prof. dr. Lucija Peterlin-Mašič and dr. Alen Krajnc

13:05 – 13:40 **Dr. Rok Sekirnik**
Head of Process Development mRNA/pDNA, Sartorius BIA Separations

Quality by Design in mRNA manufacturing

13:45 – 14:45 **Graduate Student Talks** (numbers 1 – 15)

Coffee Break and Networking

15:00 – 16:00 **Graduate Student Talks** (numbers 16 – 32)

15:40 – 16:00 *Awards Ceremony*
Closing Remarks

1 Validation of a sildenafil physiologically based pharmacokinetic model for prediction of plasma and unbound brain interstitial concentrations

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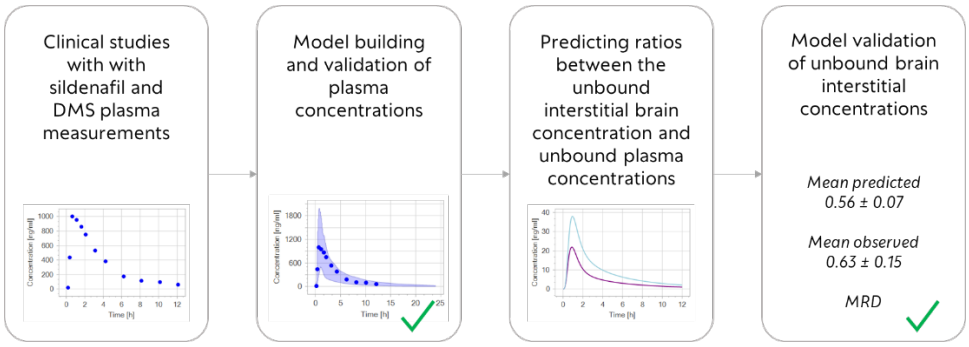
The phosphodiesterase type-5 inhibitor sildenafil is not only effective in the treatment of erectile dysfunction and pulmonary arterial hypertension but may also have neuroprotective and neurorestorative effects.[1] It is a potential drug for central nervous system disorders and a repurposing drug candidate for the treatment of Alzheimer's disease as it reduces tau phosphorylation and has protective effects in iPSC derived neurons of Alzheimer's patients. [1, 2] Sildenafil crosses the blood-brain barrier (BBB) in vivo, making it an interesting drug to predict its concentrations in the central nervous system, which may be responsible for its effects. [3] This abstract aims to describe a physiologically based pharmacokinetic (PBPK) modelling of sildenafil and its main metabolite *N*-desmethyl sildenafil (DMS). Based on a validated PBPK model, it is possible to optimise dosing for the treatment of central nervous system disorders with sildenafil. The PBPK model was developed in PK-Sim software, version 12.0. Clinical studies with sildenafil and DMS plasma measurements were used for model building and validation. Sildenafil is a P glycoprotein (P-gp) substrate and its maximum transport rate was estimated at 114 pmol/min/pmol with Km of 32.2 µM and reference concentration of P-gp of 0.077 µM multiplied by a factor of 3.57 in the intestinal mucosa. The mean relative deviation (MRD) of all predicted plasma concentrations for sildenafil and DMS were 1.89 and 1.99, respectively. Moreover, the geometric mean fold error (GMFE) of all predicted plasma concentration for sildenafil and DMS was 1.22 and 1.35, respectively. The GMFE of all predicted maximum plasma concentrations for sildenafil and DMS was 1.47 and 1.61, respectively. Since the MRD and GMFE values were not higher than 2, a model was assumed to be adequate and was therefore validated. The PBPK model predicted sildenafil crossing BBB, since the predicted ratios between the unbound interstitial brain concentration and unbound plasma concentrations were above 0.1, i.e. above the threshold for drug crossing the BBB. The predicted and observed mean ratio ± standard deviation was 0.56 ± 0.07 and 0.63 ± 0.15, respectively. Furthermore, the MRD value of all predicted ratios was 1.24, thus confirming the predictive performance of the model for unbound interstitial concentrations in the brain.



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2 Short peptides as decoys for autoantibody neutralisation in autoimmune disease

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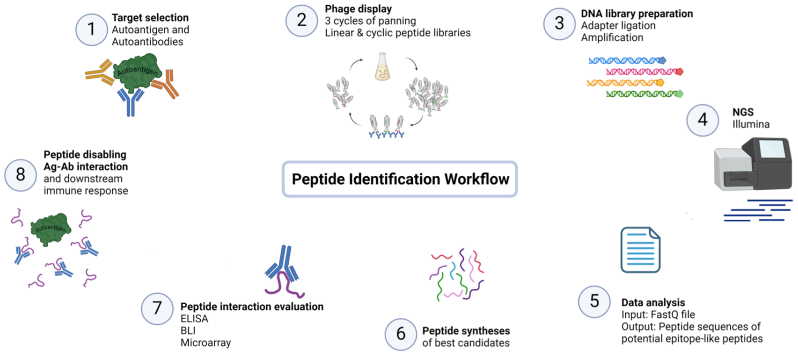
Autoimmune diseases are highly complex; however, a common feature of many of them is the production of so-called autoantibodies, which target molecules naturally present in the body, referred to as autoantigens, leading to an autoimmune response. In such cases, blocking the interaction between autoantibodies and autoantigens could greatly improve the disease and help reduce its symptoms. In our study, we aim to develop short peptides that act as decoys and effectively inhibit the binding of autoantibodies to their autoantigens as a novel therapeutic approach. We are focusing on two autoimmune diseases where autoantibodies directed against known targets are commonly found in patient sera. The first is thrombotic thrombocytopenic purpura (TTP), where the autoantigen is an enzyme involved in blood coagulation process. Autoantibodies inhibit the enzyme function, which leads to dangerous formation of microclots in small blood vessels. The second disease is systemic sclerosis, where the autoantigen we focus on is DNA-topoisomerase I. Binding of pathogenic autoantibodies causes excessive fibroblast activation and leads to excessive collagen production in the skin and organs. Both are life-threatening autoimmune disorders with limited therapeutic options. Screening random phage display peptide libraries in fast and efficient approach that enables us to obtain peptides that bind to a specific target – in our case pathogenic autoantibodies. Mapping the obtained peptides onto the three-dimensional structure of the autoantigen using computational analysis will firstly help us to identify hotspots that are involved in autoimmune response and secondly allow us to design and synthesize peptides that are highly likely to bind to the pathogenic autoantibodies. Different tests using biolayer interferometry, fluorescent enzyme immunoassay and competitive ELISA tests will help us assess the binding efficiency of peptides to patient pathogenic autoantibodies and their ability to act as decoys and efficiently block interaction between autoantibodies and autoantigen. Effective blocking of serum autoantibodies using a combination of multiple peptides would present a novel approach to treating autoimmune diseases that not only blocks the pathogenic antibodies but also prevents further downstream immune response.



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3 *In Vitro* evaluation of nasal spray efficiency with 3D printed nasal cast

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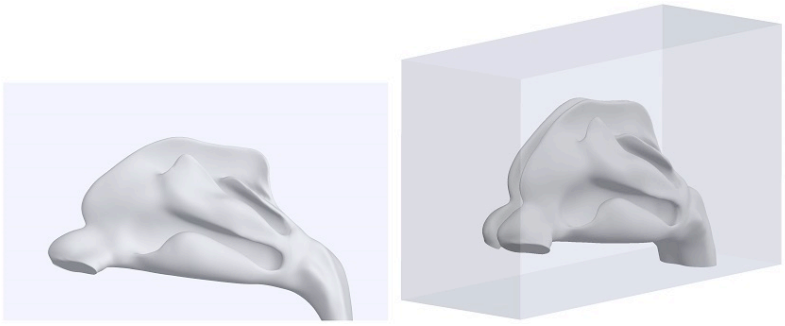
Nasal delivery is an attractive route for both local and systemic drug delivery and has great potential for accessing the brain via the olfactory region, where the blood-brain barrier is absent. Using sprays, aerosolized particles can be directed to the olfactory region, but their specific deposition in this area is complicated by a combination of factors such as formulation properties, delivery device and method of use, and physiological differences between patients.[1] While testing the physical properties of aqueous nasal sprays with various in vitro tests is useful for comparing the efficiency between different sprays, the deposition of nasal spray droplets in the regions of the nasal cavity cannot be easily predicted with such data.[2] Nasal models have advanced into detailed 3D printed replicas that closely mimic real conditions. These can be segmented into regions of interest for deposition quantification, using various techniques for quantification. Nasal models can help us develop patient instructions to ensure deposition at the target site. Additionally, they allow for inexpensive and rapid research on formulations or sprays in real nasal geometry.[3] We are investigating the critical quality parameters of liquid nasal sprays and evaluating their impact on nasal deposition in a realistic in vitro nasal model. The model is divided into five key regions of the nasal cavity, allowing for the determination of optimal conditions for delivering the formulation to the olfactory region, which enables direct delivery of active substances to the central nervous system. We use an idealized 3D model of the nasal cavity based on the average geometry of fifty adult patients. The model is divided into five regions of interest: the vestibular part, the lower and middle turbinates, the upper turbinates, the olfactory region, and the posterior part of the cavity (nasopharynx). With this model, we study the impact of different nasal spray administration methods and spray characteristics on nasal deposition. The model is mounted on a modular stand with an automatic actuator, allowing for the simulation of different head positions and spray administration angles. The model is connected to a vacuum pump to simulate steady or pulsating airflow. So far, we have conducted in vitro tests for various sprays such as particle size distribution, plume geometry, and spray pattern. A hand study was conducted with 8 volunteers to determine the actuation parameters for the automatic actuator for different devices. We also performed preliminary deposition tests at different spray angles. Quantification was performed using liquid chromatography.



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4 Stereodivergent synthesis of fluorinated aminopiperidones as aminoglutarimide mimetics

Boštjan Adamlje[a], Manca Hribar[a], Andrej Emanuel Cotman[a]
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The aminoglutarimide fragment is part of thalidomide, initially developed as an anti-nausea agent but later withdrawn due to teratogenicity. Recently, its derivatives have gained importance as cereblon binders in targeted protein degradation.[1] At our faculty, we study how replacing a carbonyl group with CF_3 affects small-molecule properties and bioactivity by implementing this bioisosteric replacement to relevant bioactive parent molecules. Replacing a single carbonyl group in 2-aminoglutarimide with a trifluoromethyl group leads to 3- or 5-amino-6-trifluoromethyl-2-piperidone, each of which exists in two diastereomeric forms. To synthesize the racemic trans-5-amino derivative, we first reacted a racemic tolylsulfinyl amide with ethyl hemiacetal of trifluoroacetaldehyde. The resulting hemiaminal underwent an aza-Henry reaction with nitromethane, followed by the Michael addition of the β-nitroamine to ethyl acrylate.[2] After cyclization to 5-nitro-2-piperidone and catalytic hydrogenation, we obtained the desired aminopiperidone as a diastereomerically pure product. The cis-5-amino derivative was synthesized via a diastereospecific total reduction of the corresponding nitropyridinol. The mixture of cis- and trans-3-amino-6-trifluoromethyl-2-piperidone, which is configurationally labile, was prepared by hydrogenation of the corresponding nitropyridine under elevated pressure (60 bar) and temperature (60 °C).[3] The obtained stereochemically defined amines were coupled with substituted phthalic anhydrides, yielding thalidomide analogs as pure diastereomers after purification by column chromatography or recrystallization. The relative configuration was determined by NMR spectroscopy using a NOESY experiment. The developed synthetic tools enable the study of the biological activity and physicochemical properties of all eight stereoisomers of aminoglutarimide analogs, where one carbonyl group is replaced with a trifluoromethyl group.

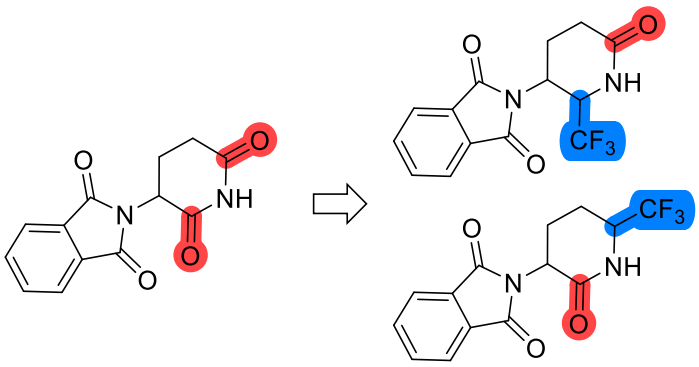

Figure 1: *Fluorinated Thalidomide Analogs*

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5 Exploration of monocyclic β-Lactams as covalent inhibitors of D,D- and L,D-transpeptidases

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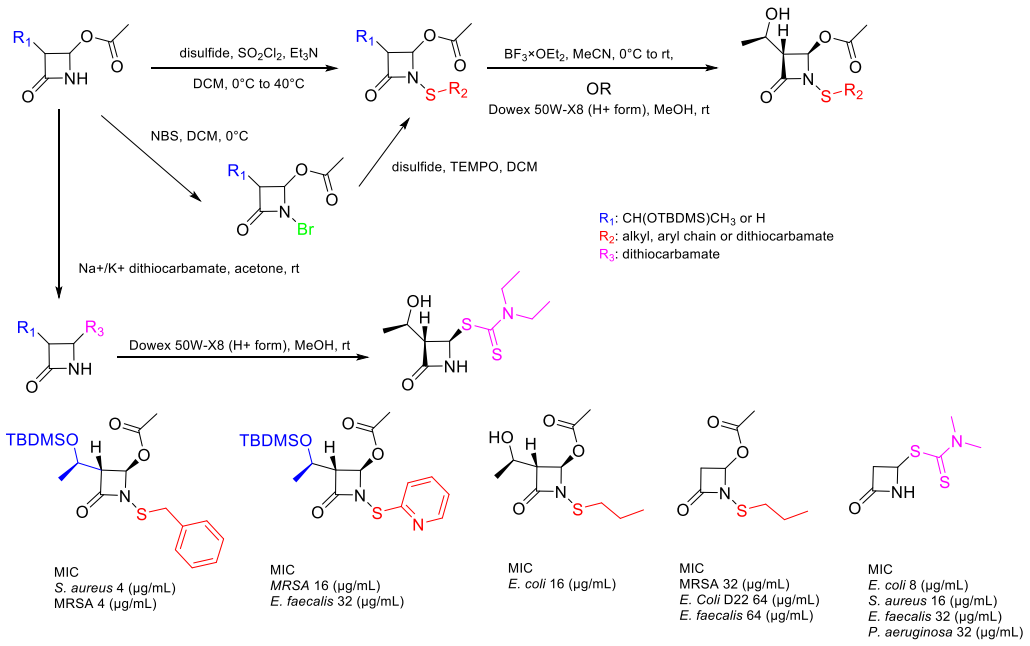
The bacterial cell wall is mainly composed of peptidoglycan (PG), which is crucial for protecting bacteria against osmotic lysis and maintaining cell structure. Penicillin-binding proteins (PBPs) and L,D-transpeptidases (Ldts) are essential enzymes involved in PG cross-linking.[1] Ldts catalyse 3→3 cross-linking between tetrapeptides, contain active-site cysteine and anchor Braun's lipoprotein (Lpp) as the sole covalent link between the outer membrane and PG, thereby maintaining cell envelope integrity. In contrast, PBPs catalyse 4→3 cross-linking between pentapeptides, have active-site serine and are vital for survival for most bacteria. Ldts are upregulated in *E. coli* during β-lactam exposure response, allowing bacteria to circumvent PBP inhibition and survive under stressful conditions.[2] This study focuses on developing inhibitors for *E. coli* LdtB and *S. pneumoniae* PBP1b. A library of monocyclic β-lactams with *N*-thioether and *N*-dithiocarbamate functional groups was synthesized through various reactions, including sulfuryl chloride-mediated *N*-thioether-addition and reactions of *N*-bromo-2-azetidinones, catalysed by TEMPO. We also achieved optimization of TBDMS group deprotection and C3 position dithiocarbamate incorporation. The goal is to enhance the antibacterial efficacy of standard-of-care antibiotics through synergy testing (*checkerboard assay*). We synthesized 51 compounds and among them 30 demonstrated modest antibacterial activity.



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6 AI-Driven drug discovery: a computational pipeline for rational drug design

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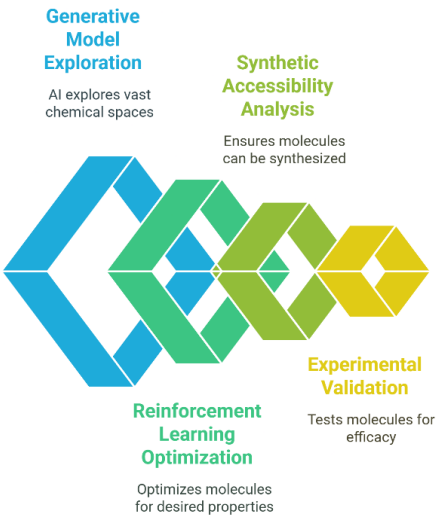
Artificial intelligence (AI) is transforming drug discovery by accelerating the development of new therapeutics and optimizing molecular properties.[1] One promising application is "*de novo*" drug design, where generative models explore vast chemical spaces to propose novel molecules with desired properties. A notable example is AstraZeneca's REINVENT framework, which employs a generative model coupled with reinforcement learning to design new compounds.[2] Reinforcement learning optimizes generated molecules toward objectives such as enhancing binding affinity or improving drug likeness. Another critical aspect of drug discovery is ensuring synthetic accessibility. AstraZeneca's AiZynthFinder addresses this challenge by leveraging machine learning for retrosynthetic analysis, systematically identifying synthetic routes to target molecules.[3] Using a Monte Carlo tree search algorithm guided by a neural network trained on reaction templates, AiZynthFinder decomposes complex molecules into commercially available precursors, enhancing synthetic accessibility and accelerating the transition from virtual hits to tangible compounds. We introduce an integrated computational framework designed to enhance the discovery of novel therapeutics using generative AI models and high-throughput molecular screening. Leveraging the capabilities of REINVENT for molecule generation and AiZynthFinder for assessing synthetic accessibility, our approach ensures that generated compounds are both innovative and synthesizable via a few-step route from commercially available precursors. We plan to validate our pipeline on a few drug targets, emphasizing its broad applicability and potential in streamlining drug development. Our approach aims to demonstrate significant in vitro activity, with validation through comprehensive biochemical and biophysical assays. By integrating advanced computational techniques with experimental validation, this pipeline has the potential to greatly optimize the drug discovery process, enhancing efficiency and reducing resource expenditure.



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7 Transformation and cold-blooded murder: through new cell lines towards a better treatment of chronic lymphocytic leukaemia

Gašper Tomšič[a], Tijana Markovič[a], Maša Kandušer[a], Jernej Repas[a], Irena Mlinarič-Raščan[a]

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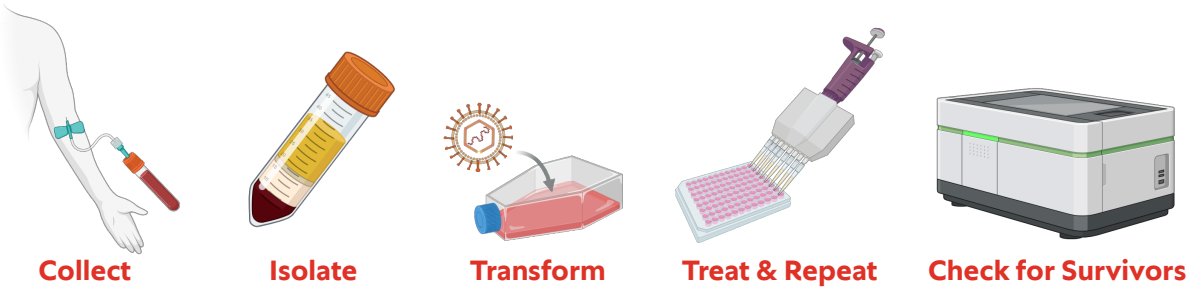
Chronic lymphocytic leukaemia (CLL) is among the most common hematologic malignancies in the Western world. Historically only being treated with IV chemotherapy, advances in medicine and the discovery of the therapeutic potential of Bcl-2 and Bruton tyrosine kinase (BTK) inhibitors have drastically improved patient outcomes and the management of the disease. Regardless, CLL yet remains incurable and while standard of care (SoC) is adequate at first, relapses are common with few treatment options available for CLL with acquired immunities, thus presenting a need for novel therapies.[1] Currently, the standard procedure for anti-CLL drug screening is using primary cells from donor patients for ex vivo experiments. This has inherent drawbacks as CLL cells only replicate in lymphoidal tissue in the body and are not viable for long periods ex vivo. Thus, we have a very limited amount of biomaterial available for testing, effectively limiting major HTS studies, without posing a burden to patients. Additionally, as primary CLL cells do not replicate ex vivo, any drugs targeting the cellular replication process will always show as inefficacious. Lymphoblastoidal cell lines (LCLs) have long been used as cellular models for drug screenings, because of their relatively simple maintenance and relevance as models for cytotoxicity assays and pharmacogenomic analysis.[2] LCL cells can be obtained by infecting B-cells with Epstein-Barr virus (EBV) in vitro and subsequently expanded for several generations. However, CLL cells are resistant to this method of transformation. As new papers continue to be published, emphasizing the role of cytokines and the tumour microenvironment in the prolonged survival and proliferation of CLL cells[3], we are trying to develop a reliable method of CLL-to-LCL transformation by changing the environment in which the cells are infected with EBV. Because we are striving to make our research usable to patients as soon as possible, we've leveraged drug repurposing for the identification of potential novel anti-CLL agents, focusing additionally on the synergistic potential of identified drugs with the current SoC. Preliminary in silico results showed promise with Src family kinase inhibitors, such as saracatinib, as well as NOTCH1 antagonists. In vitro cytotoxicity studies confirmed this theory, with the latter group being especially useful in combination with proteasome inhibitors, which are known novel treatment agents for CLL. Additional research is needed and ongoing, however we are confident, that our research will yield results useful to patients.



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8 Engineered bacteriophage vaccines: a modular platform for precision antigen delivery and immune boosting

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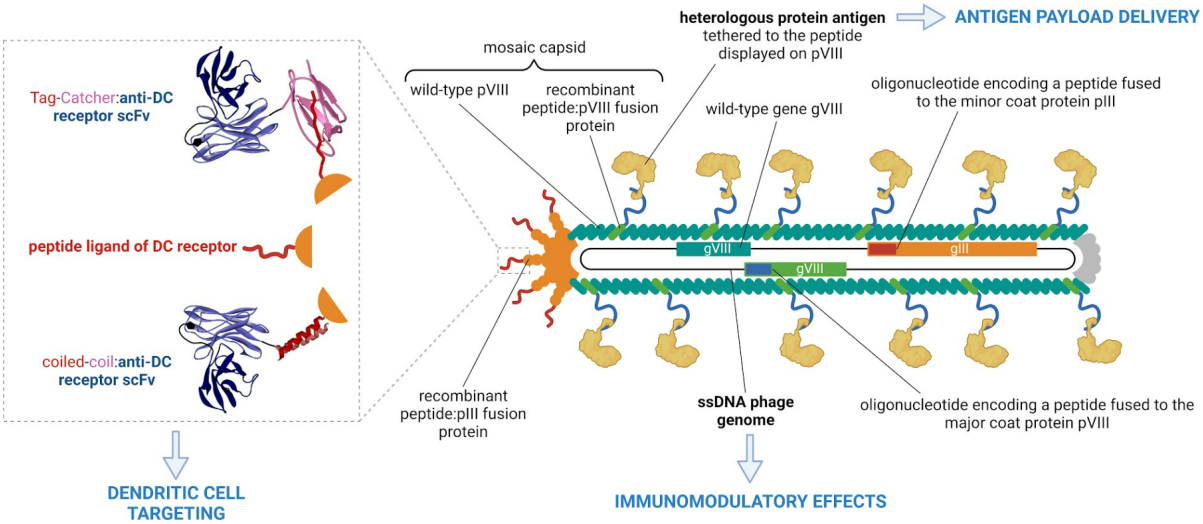
Vaccination remains one of the most cost-effective tools in public health, yet the search for a versatile vaccine platform capable of eliciting broad and robust immune responses continues. Our approach harnesses the unique properties of filamentous bacteriophage – its ability to display heterologous peptides in high valency and its intrinsic immunomodulatory effects – to create a modular vaccine scaffold with built-in adjuvant properties. We have engineered a hybrid bacteriophage vector that simultaneously displays foreign peptides on both the pIII and pVIII capsid proteins. The pVIII-displayed peptides serve as anchors for recombinant subunit protein antigens, while those on pIII are designed to target dendritic cells, amplifying both antibody and cellular immune responses. To demonstrate this concept, we fused the SpyTag peptide to pVIII and optimized its display efficiency, confirming expression levels via ELISA. As a model antigen, sfGFP was expressed in *E. coli* as a SpyCatcher fusion protein. The SpyTag/SpyCatcher system enables spontaneous isopeptide bond formation, allowing precise antigen tethering to the phage capsid.[1] We verified this attachment through immunoblotting and fluorescence intensity measurements, further visualizing decorated phages using total internal reflection fluorescence (TIRF) microscopy. To enhance dendritic cell targeting, we leveraged nanobody technology. Specifically, we displayed nanobodies against Clec9a[2] on pIII using a SnoopTag/SnoopCatcher system,[3] enabling binding to recombinant Clec9a, as confirmed via phage ELISA. This targeted approach holds great potential for fine-tuning immune responses and advancing next-generation vaccine platforms.



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9 Optimisation of the baculovirus vector to improve chromatographic purification in the production of biological

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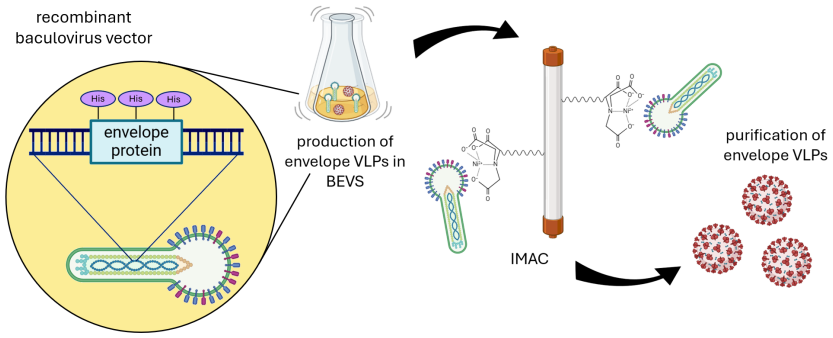
The insect cell-based baculovirus expression vector system (BEVS) is a well-established platform to produce recombinant proteins. There, the recombinant baculovirus transfers heterologous genes to cultured insect cells for large-scale production of biopharmaceutics, vaccines and gene therapy vectors. The baculovirus vector exhibits a high capacity for the insertion of foreign DNA, making this system particularly suitable to produce complex high molecular weight proteins, such as virus-like particles (VLPs). Several BEVS-derived products have been already approved for human use. [2] One of the major drawbacks of BEVS is the efficient removal of baculoviruses, which represent one of the main bioprocess impurities. This is particularly problematic in the production of enveloped VLPs, as their characteristics, including size and surface charge, closely resemble those of baculoviruses. Baculovirus impurities can affect safety, stability and quality of biological nanoparticle-based vaccines and therefore limit their use in biopharmaceutical applications. By integrating protein engineering and viral genome editing, our goal is to develop an optimized baculovirus vector to improve chromatographic purification of biological nanoparticles, in particular enveloped VLPs. Specifically, we will mutate baculovirus envelope glycoproteins by inserting histidine residues into exposed, flexible protein loops. This modification will enable a specific interaction between the engineered baculovirus vector and an immobilized metal affinity chromatography (IMAC) column. One of our primary targets for protein engineering is the baculovirus GP64 fusion protein, a trimeric glycoprotein concentrated at one end of the rod-shaped baculovirus. GP64 is essential for baculovirus attachment to the surface of insect cells and virus entry.[3] However, mutations in GP64 can impair its function, potentially leading to the formation of non-infectious particles or baculoviruses with reduced infectivity. To minimize this issue, we will perform structural analysis of the protein, identifying suitable loops before introducing minor sequence mutations that will preserve protein structure and function while still enabling IMAC-based separation. In addition to GP64, we will also modify other baculovirus envelope proteins that have not been previously mutated and are non-essential for viral replication, therefore minimizing any adverse effect on baculovirus infectivity. We expect the engineered recombinant baculovirus vector to bind to the IMAC column, allowing effective separation from the biological nanoparticles (VLPs) produced in BEVS. Optimized vector would be useful for removing baculovirus impurities during the production of different recombinant protein or protein complexes in BEVS and thus enable greater safety and quality of BEVS-derived vaccines and biotherapeutics.



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10 Evaluation of the prevalence, diagnostic gap, and pharmacotherapy of metabolic dysfunction associated steatotic

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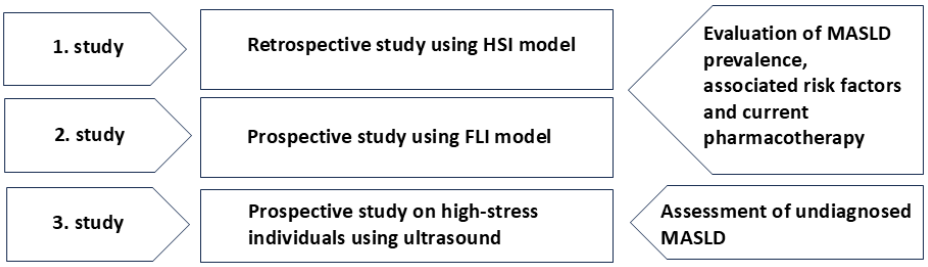
Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), formerly known as Non-Alcoholic Fatty Liver Disease (NAFLD), is defined by liver fat accumulation in the absence of excessive alcohol consumption and in the presence of one or more cardiometabolic risk factors. MASLD is the most common chronic liver disease, affecting over 30% of the global population, with prevalence increasing due to rising metabolic disorders. It is strongly linked to T2D, obesity, and metabolic syndrome, and can progress to steatohepatitis (MASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).[1,2] Despite its serious implications, MASLD often remains undiagnosed due to its asymptomatic nature, with early detection being crucial since lifestyle modifications can reverse the disease in its initial stages.[3] The current European Association for the Study of the Liver (EASL) guidelines recommend weight loss through diet and behavioral therapy as primary treatment. While no pharmacological treatment is widely approved, resmetirom, a thyroid hormone receptor agonist, has recently been introduced. Additionally, some antidiabetic drugs and lipid-lowering agents show potential in improving liver outcomes.[1] Given the increasing burden of MASLD, raising awareness, enhancing early diagnosis, and implementing effective management strategies are essential to reduce disease progression, healthcare costs, and improve patient quality of life. We will conduct three studies in collaboration with ZVD - Zavod za varstvo pri delu, d.o.o. In the first study, retrospective in nature, we will use the Hepatic Steatosis Index (HSI) to predict liver steatosis based on routinely collected data. In the second study, a prospective one, will collect additional MASLD-related parameters beyond routine data, including waist circumference, which is required for prediction of liver steatosis using the Fatty Liver Index (FLI) model. With these two studies we wil evaluate the prevalence of MASLD in the active workforce in Slovenia, assess associated risk factors (e.g., age, lifestyle, comorbidities, and ongoing treatments) and assess the proportion of individuals who are receiving pharmacotherapy in accordance with the current EASL guidelines. The third study, also prospective, will assess the prevalence of undiagnosed MASLD among high-stress working individuals in Slovenia who undergo frequent and detailed preventive health examinations. The doctoral dissertation will comprehensively investigate the epidemiological aspects of MASLD in the working-age population in Slovenia and compare them with global data, providing a deeper understanding of the disease's prevalence and its impact on health and productivity. In addition to assessing the epidemiological burden, it will evaluate current pharmacological treatment practices for MASLD and their alignment with EASL guidelines, which will be crucial for optimizing clinical management and implementing new therapies. A special focus will be placed on identifying undiagnosed MASLD cases among individuals exposed to high work-related stress, who undergo frequent medical check-ups, to enhance screening strategies. The findings of this research will contribute to raising awareness, improving early detection and management of MASLD, and developing a cost-effective strategy for its prevention and treatment in Slovenia.



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11 Statistical models and in-process techniques to target structural properties of lyophilizates

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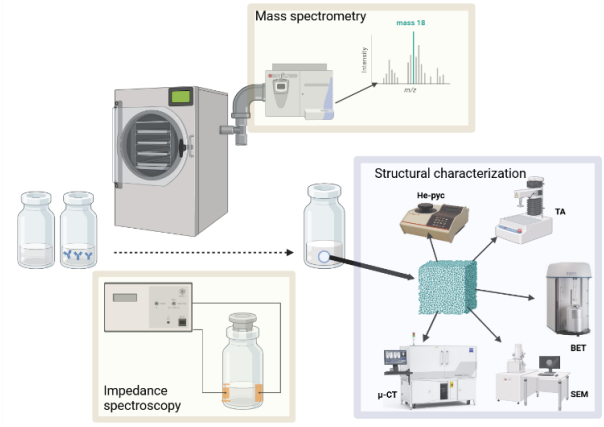
Lyophilization, a drying method conducted at low pressures and temperatures, produces dry material with low water content. This technique is particularly suitable for biological and biosimilar formulations, where compounds are often thermolabile and sensitive to water. Despite being a well-established procedure, lyophilization still faces challenges in process understanding, process control and cost-effectiveness during development and production. To address these challenges our study aims to statistically evaluate the impact of freezing process parameters on the structural properties of freeze-dried cakes, the effect of primary packaging on controlled nucleation effectiveness, and the possibilities for process optimization using novel approaches with mass spectrometry and impedance spectroscopy. During our research, we will develop a statistical model using Design of Experiments (DOE) to assess the effect of freezing process parameters on the structural properties of freeze-dried cakes, that influence critical quality attributes (CQAs) of the product such as reconstitution time, drying efficiency, residual water content, and drug stability during storage. Structural properties of freeze-dried cakes will be determined using complementary approach with microcomputed tomography (μ-CT) and scanning electron microscopy (SEM) in correlation with density (gas pycnometry), specific surface (Brunauer-Emmett-Teller method) and mechanical properties (texture analysis). Our findings will contribute to a better understanding of the freezing step on cake structure, cost-effectiveness, and drying time.[1] Additionally, we will explore the mechanism behind rapid depressurization method (ControLyo®), a widely used method for nucleation temperature control. We aim to determine the effect of fill (headspace) volume, stopper opening diameter and vial size on nucleation efficiency, providing a deeper understanding of the underlying mechanism.[2] In compliance with Quality by Design (QbD) paradigm, we will explore the possibilities of novel process analytical technologies and develop a method for measuring sublimation rates using mass spectrometry. Furthermore, we will evaluate whether the simplified design and additional electrodes improve the homogeneity of the electrical field and therefore enhance accuracy.[3]



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12 Comparison of health outcomes of high-efficacy therapies for the treatment of relapsing-remitting multiple sclerosis based on real-world data

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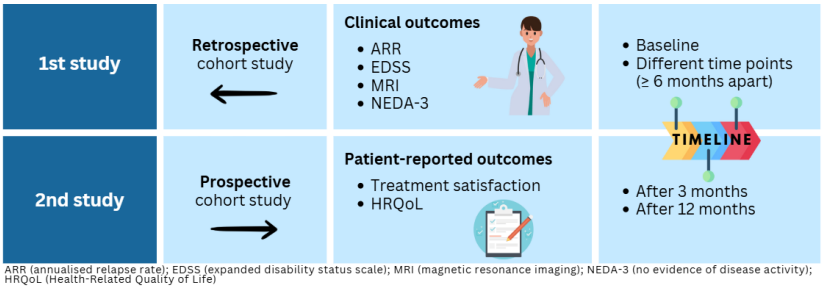
Relapsing-remitting multiple sclerosis (RRMS) is the most prevalent clinical phenotype of multiple sclerosis, accounting for approximately 85 % of cases at diagnosis. It is a chronic inflammatory disease of the central nervous system, characterized by episodes of neurological relapses followed by remission.[1] Although no curative treatment exists, disease-modifying therapies (DMTs), particularly high-efficacy therapies (HETs), have transformed the disease course in recent years. Cladribine, ocrelizumab, and ofatumumab are among the key HETs currently available and are increasingly used to treat highly active RRMS. As the target populations of these three therapies largely overlap, information on their comparability in terms of clinical and patient-reported outcomes (PROs) is needed to support informed treatment decisions.[2,3] However, no randomised controlled trials have directly compared these therapies, and available real-world evidence is limited. Our research aims to evaluate and compare the clinical outcomes and PROs of cladribine, ocrelizumab, and ofatumumab in a real-world setting. For this purpose, we will conduct two observational studies. The first study will be a retrospective cohort study based on clinical data from patients treated at the Multiple Sclerosis Centre at the Division of Neurology, University Medical Centre Ljubljana, who are enrolled in the MSBase registry. Adult RRMS patients who initiated one of the three therapies will be included. Clinical outcomes such as annualised relapse rate (ARR), expanded disability status scale (EDSS) score, MRI lesion activity, and proportion of patients with no evidence of disease activity (NEDA-3) will be assessed at different time points. Propensity score matching will be applied to ensure additional comparability. The second study will be a prospective cohort study focusing on PROs. Eligible adult RRMS patients starting treatment with one of the three therapies will be identified from the MSBase registry. They will be invited to complete validated questionnaires: TSQM (Treatment Satisfaction Questionnaire for Medication) and EQ-5D-5L (a health-related quality of life questionnaire). Data will be collected primarily at 12 months post-treatment initiation, with a subset also at 3 months. Findings from our research will provide real-world evidence on the comparative effectiveness and PROs of cladribine, ocrelizumab, and ofatumumab. The evidence is expected to support a more individualized and patient-centred approach to therapy selection in RRMS.



ARR (annualised relapse rate); EDSS (expanded disability status scale); MRI (magnetic resonance imaging); NEDA-3 (no evidence of disease activity); HRQoL (Health-Related Quality of Life)

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13 New InhA inhibitors

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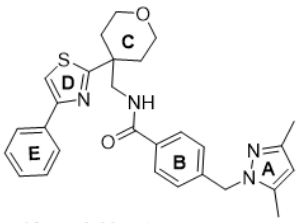
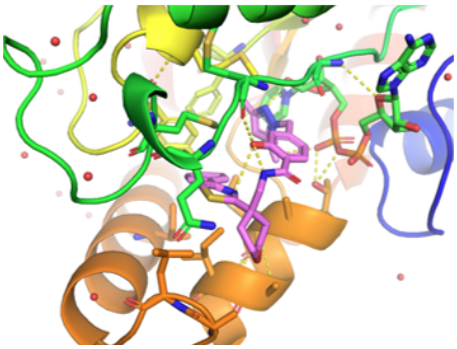
[b]*i3S-Institute for research and Innovation in Health, University of Porto, Porto, Portugal*

Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) remains a significant global health threat.[1] InhA, an enoyl-[acyl-carrier-protein] reductase, is a key enzyme in the fatty acid synthase II (FAS-II) system of *M. tuberculosis*. It is also a target of a frontline drug isoniazid, making it a validated therapeutic target. However, isoniazid as a prodrug requires activation through a series of biochemical transformation, including the KatG enzyme, which is prone to mutations that lead to isoniazid resistance. Directly targeting InhA bypasses this resistance, offering a viable strategy for new antitubercular therapies.[2] Our tetrahydropyran-based inhibitors were identified through high-throughput screening (HTS) in collaboration with GlaxoSmithKline. The binding of our lead compound A (Fig. 1) in the substrate binding site was confirmed with solved crystal structure of the InhA-inhibitor complex. It shows that the lead compound A adopts a U-shaped conformation, engaging hydrophobic interactions in the lipophilic pocket and hydrogen bonds with the enzyme binding site and NADH co-factor.[2] In order to obtain further insight in the chemical space of the binding site we designed a series of inhibitors with a preserved biaryl system (rings E and D) while varying the substituents on rings A and B. While larger ring A substituents reduced activity, adding a hydroxyl group to ring B enhanced physicochemical properties and maintained enzyme inhibition. Replacing the tetrahydropyran (ring C) with other cyclic moieties retained potency, suggesting its primary role lies in maintaining the inhibitor's conformation. In light of that, we replaced it with piperidine to further enhance physicochemical properties. Our inhibitors were tested against various *Mycobacteria* strains (*M. tuberculosis*, *M. avium*, and *M. abscessus*) as well as the isolated enzyme. Additional crystal structures of the InhA in complex with our inhibitors revealed new binding interactions and computational modeling provided new insights that will guide further optimization. Overall, this work lays a strong foundation for developing new antitubercular drugs.



IC_{50} = 0,02 µM
MIC (Mtb) = 11,7 µM
IC_{50} (*M. avium*) = 12,5 µM
IC_{50} (*M. abscessus*) = >50 µM



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14 Discovering butyrylcholinesterase inhibitors among FDA-approved central nervous system drugs

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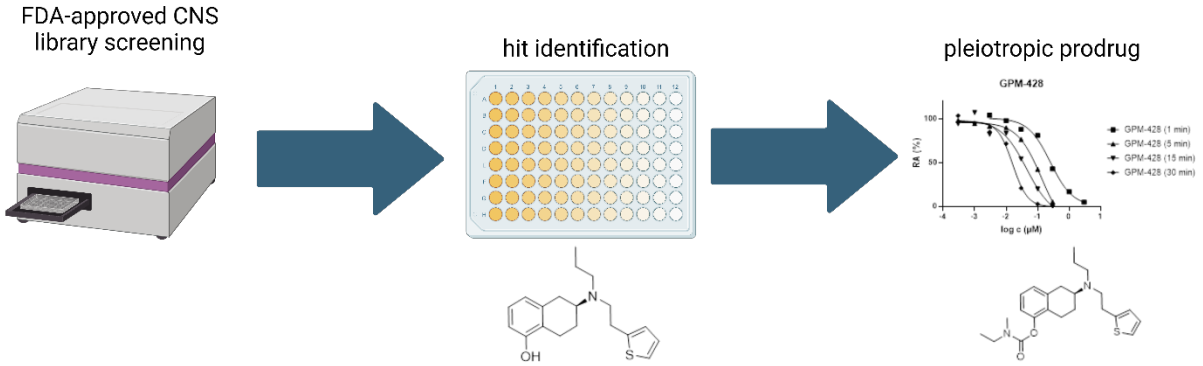
Butyrylcholineesterase (BChE) is an enzyme closely related to the better-known acetylcholinesterase. While its primary substrate is the neurotransmitter acetylcholine (ACh), BChE is also capable of hydrolysing other endogenous and exogenous molecules. BChE is an old and well-established target in the field of Alzheimer's disease research, but recent research shows potential of targeting BChE as a potential treatment for other central nervous system (CNS) disorders as well.
Unpublished results indicate that BChE inhibitors exhibit antidepressant effects in animal models, presumably through the BChE–ghrelin–dopamine axis.[1] Another recent study indicates that the antidepressant duloxetine is a potent BChE inhibitor, raising questions about a potential additional role of this mechanism in mood enhancement, beyond the inhibition of norepinephrine and dopamine reuptake, of course.[2] This findings led us to explore the potential BChE-inhibitory activity of other established CNS drugs. Commercial library of FDA-approved CNS-active drugs was purchased and screened for BChE inhibition *in vitro* using Ellman's assay. Of the 103 compounds in the library, 20 exhibited IC_{50} values below 10 μM. Among those are well established antipsychotic and antidepressant drugs such as clozapine (IC_{50} = 1,284 ± 0,575 μM), aripiprazole (IC_{50} = 1,495 ± 0,561 μM) and amitriptyline (IC_{50} = 4,984 ± 1,85 μM). The most potent hit, the dopaminergic agonist rotigotine (IC_{50} = 0,307 ± 0,108 μM), contains a free hydroxy group, making it an attractive candidate for pleiotropic carbamate prodrug design.[3] A focused library of carbamate analogues was prepared, leading to an order-of-magnitude improvement in potency, as well time-dependent BChE inhibition, suggesting a covalent mode of action. These novel compounds represent interesting lead candidates for treating dementia in Parkinson's disease or general cognitive enhancement, and are powerful pharmacological tools for further research in the field of neurodegeneration.



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15 Role of proteasomes and cathepsins as biomarkers in neurodegenerative diseases

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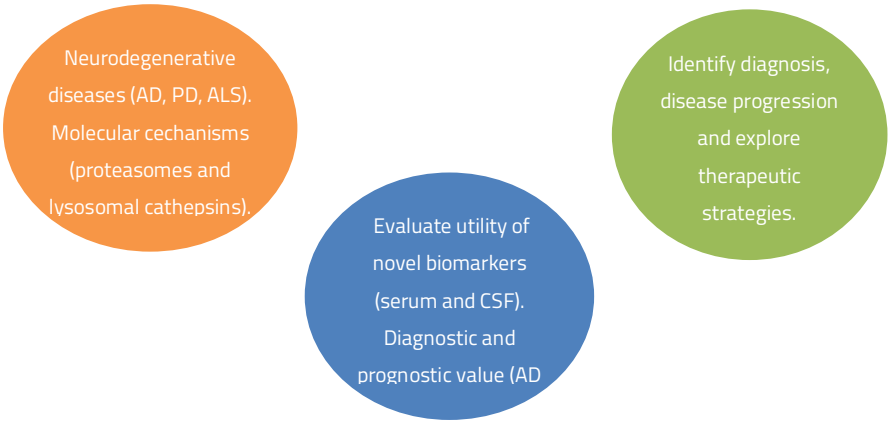
Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and amyotrophic lateral sclerosis (ALS) represent a significant and growing global health burden, with their prevalence projected to more than double by 2050.[1] Despite advances in understanding the molecular mechanisms underlying these disorders, early and accurate diagnosis remains a major challenge, and current therapies offer only limited symptomatic relief.[2] In this study, we investigate the role of two critical proteolytic systems (the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway) in neurodegeneration, with a focus on their potential as sources of novel biomarkers. Specifically, we examine proteasomes and lysosomal cathepsins for their diagnostic and prognostic value in AD and PD. Both proteolytic systems are essential for protein homeostasis and cellular integrity, and their dysregulation is a hallmark of neurodegenerative diseases.[3,4] Cathepsins, key lysosomal peptidases, have been implicated in neuronal damage through their roles in protein degradation, microglial activation, and neuroinflammation.[5] The proteasome, a central component of the UPS, is responsible for the degradation of misfolded and damaged proteins, and its dysfunction is associated with the upregulation of immunoproteasomes, which may exacerbate neuroinflammatory responses.[6,7] In our study, we will assess serum and cerebrospinal fluid (CSF) levels of proteasomes and cathepsins, evaluating both their protein concentrations and enzymatic activities. Samples will be collected at diagnosis, during disease progression, and following therapeutic interventions in AD and PD patients. These biochemical data will be correlated with clinical features and disease severity to evaluate their utility as biomarkers. Ultimately, the goal is to identify reliable, non-invasive biomarkers reflective of underlying pathological processes and to explore therapeutic strategies targeting these proteolytic systems to slow or prevent neurodegeneration.



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16 Investigation of bone-muscle communication through the effect of the muscle secretome on mesenchymal stem cells in osteosarcopenia

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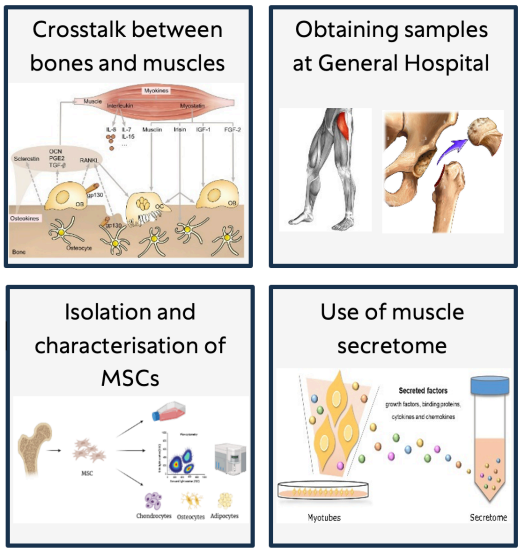
Osteosarcopenia is a syndrome that combines biological and clinical features of osteoporosis and sarcopenia. The prevalence of osteosarcopenia ranges in community-dwelling older adults between 5 and 37 % amongst people of age 65 or more (5–37 % (≥ 65 years)). Osteosarcopenia can be defined as a syndrome whose pathogenesis involves genetic polymorphisms, endocrine alterations, reduction in the mechanical loading or impaired crosstalk between muscles, bones and fat cells. Bone and muscle interact either mechanically or chemically, and bone and muscle homeostasis are closely connected. In particular, bone and muscle crosstalk through the endocrine system secreting myokines such as irisin, IL-6 and myostatin and osteokines such as sclerostin. Our aim is specifically to identify muscle factors affecting osteoporotic bone cells. The samples for our study are obtained at the General Hospital Celje. People who suffer from femur fracture and are thus operated, donate the femoral head and a small piece of muscle. This is where we come into the picture. First, we appropriately process bone and muscle. Then we go to the laboratory, where we isolate and characterise mesenchymal stromal cells. We check adherence to plastic, expression of specific markers by flow cytometry and differentiation to chondrocytes, osteocytes and adipocytes. We also determine the MSC proliferation and will check their transcription. Satellite cells from muscle are isolated in another institution, where a muscle secretome is also prepared. This secretome is intended to be used in assessing MSC proliferation, differentiation and their transcription to see if there are any differences when using secretome. In conclusion, bones and muscles are intricately connected. We plan to find new signalling pathways in bone-muscle crosstalk that may contribute to the future treatment of osteosarcopenia.



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17 Novel compounds targeting mitochondrial Kv1.3 for cancer treatment

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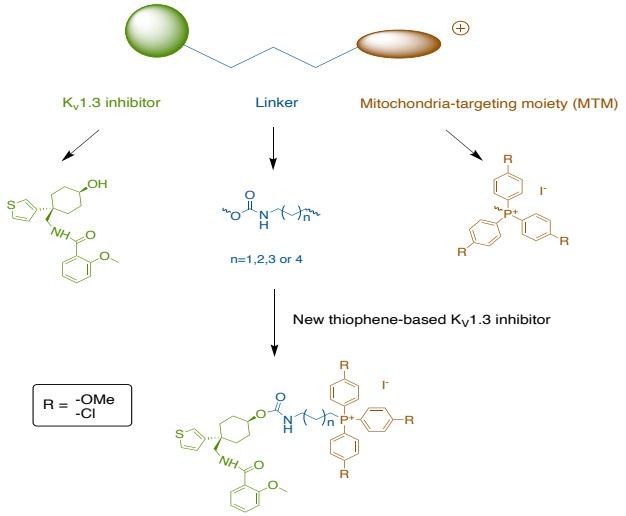
K_V1.3 is a transmembrane protein found in both the cellular and mitochondrial membranes and is part of the voltage-gated potassium channel KV1.x subfamily. It has emerged as promising target for anticancer therapy due to its demonstrated correlation with cancer development. KV1.3 is overexpressed in various tumor types, and its activity plays a key role in regulating cell proliferation and apoptosis. [1,2] In our study, we identified a hit compound, which present a benzamide scaffold[3], a four carbon linker chain and triphenylphosphonium group (TPP^+) as mitochondrial targeting moiety (MTM) with potent cytotoxic activity. We explored structural modifications to enhance its activity. We synthesized and evaluated different analogues with variations in the linker chain length (three to six carbons) and modifications in the mitochondrial targeting moiety (MTM), specifically TPP^+ and its substituted derivatives. The new inhibitors were screened for antiproliferative activity in COLO-357 and SK-N-MC cell lines, demonstrating significant cytotoxicity and cell viability reduction. Among all tested compounds, *cis*-24 emerged as the most promising. Patch-clamp experiments confirmed its potent inhibition on mitoK_V1.3. Moreover, it selectively inhibited tumour cell growth without affecting growth of non-tumour hTERT-RPE1 cells, caused mitochondrial membrane depolarization and induced apoptosis via caspase 3/7 activation in COLO-357. Further investigations revealed that *cis*-24 activates the permeability transition pore (PTP) rather than significantly increasing reactive oxygen species (ROS), distinguishing it from other mitoK_V1.3 inhibitors. To assess the compound's efficacy beyond 2D cultures, *cis*-24 was evaluated in 3D spheroids models of PANC-1 and COLO-357. It induced strong antiproliferative and cytotoxic effects, particularly in COLO-357 spheroids, where significant impact was observed even at low micromolar concentrations. Finally, in an in vivo zebrafish xenograft model of Ewing sarcoma, *cis*-24 significantly inhibited tumour growth a 2 mM, demonstrating promising efficacy in an *in vivo* model.

These findings highlight the potential of *cis*-24 as a novel mitochondrial K_V1.3 inhibitor for cancer therapy, with significant anticancer activity. The observed efficacy in both in vitro and in vivo models suggested that *cis*-24 could serve as valuable lead compound for further development in this field.



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18 Mitochondriotropic conjugates with improved properties for targeting cancer cells, part 2: dielectric boogaloo

Ivan Džajić[a], Natalija Trunkelj[a], Jernej Repas[a], Lovro Žiberna[a], Nika Dremelj[a], Tihomir Tomašič[a], Lucija Peterlin Mašič[a], Andrej Emanuel Cotman[a]

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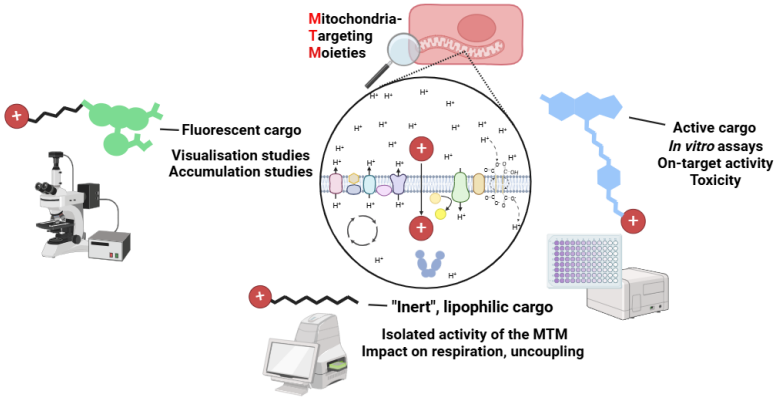
The targeting of cancer cell metabolism has been shown to be one of the most important strategies for drug development in the current era of precision oncology.[1] Mitochondria-targeting moieties (MTM) in the form of delocalized lipophilic cations harness mitochondrial charge separation as a source of free energy to reach the mitochondrial matrix and are currently among the leading technologies for delivering small molecules to their mitochondrial targets.[2] At the previous year's GSR Symposium, we presented our novel additions to the toolbox of MTM. The series was used primarily to target the mitochondrial ion channel mtKv1.3, an anti-cancer target that is being actively explored in recent years.[3] However, none of the compounds in the series successfully maintained all three critical parameters: *in vitro* activity, thermodynamic solubility (TS, experimentally determined), and selectivity (the ratio of healthy and cancer cell cytotoxicity). We have also introduced a newly produced series of MTM designed to bridge the gap between seemingly distant structural types while maintaining all three parameters. Three of the most promising compounds from our series have advanced into *in vivo* studies, and two patent applications are pending. Before proceeding with more labor- and cost-intensive studies and in order to validate all newly synthesized MTM with a rational drug design approach, we have devised a platform that consists of the following three equally important steps. Accumulation in mitochondria is measured as the colocalization of a fluorescent dye-MTM conjugate coincubated with one of the commercially available mitochondria-labeling dyes. Biological activity of inhibitor-MTM conjugates is determined by screening cytotoxicity, apoptosis, and on-target activity in both normal and cancer cells. Intrinsic MTM activity is evaluated by synthesizing a hydrocarbon chain-MTM conjugate as a control, with any detected biological activity attributed to the MTM, as a function of oxygen consumption rate (OCR) using the Seahorse XF assay to assess mitochondrial respiration disruption and uncoupling effects. Using insights from the previous compound series, we are also constructing a QSAR lipophilicity ($logD_{7.4}$) and TS prediction model based on experimental measurements. A new generation of compounds has been synthesized, and its validation using our newly established platform will follow in the subsequent months.



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19 Biophysical approaches in the optimization of novel inhibitors of human and bacterial type II topoisomerases

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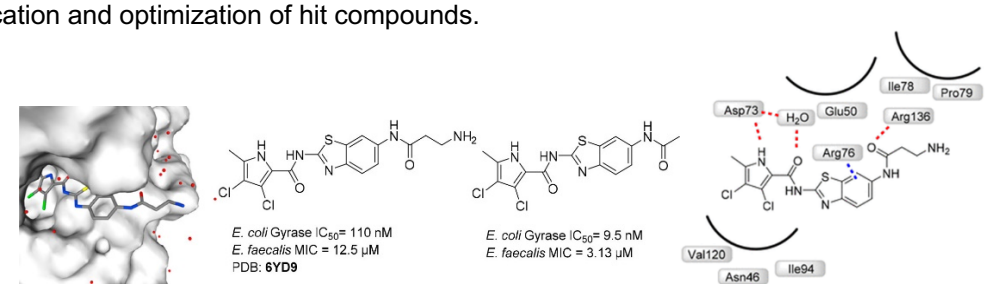
Topoisomerases are essential nuclear enzymes involved in resolving topological issues arising during transcription and replication because of the need for double-stranded DNA chains to unwind. As one part of the molecule is unwinding, a part of DNA molecule elsewhere must overwind to compensate. To eliminate overwinding DNA topoisomerases introduce transient double stranded breaks into DNA chains. Due to their crucial role in DNA replication and cell survival, topoisomerase inhibitors represent a significant class of antibacterial and anticancer drugs. [1] We are focused on synthesis of bacterial gyrase and human topoisomerase IIα inhibitors. In order to enhance target activity, increase selectivity, and optimize physicochemical properties of our topoisomerase inhibitors, we are using biophysical methods such as biolayer interferometry (BLI) and isothermal titration calorimetry (ITC). Simultaneous investigation of structurally similar compounds targeting highly related molecular sites in different organisms (bacteria and mammalian cells) provide deeper insights into fundamental processes and key obstacles in target binding and cellular activity. With BLI we are optimizing kinetic parameters (K_{on}, K_{off}) to improve binding affinity (K_d) and extend the lifetime of the complex between the designed inhibitor and its biological target. A long complex lifetime is a strong indicator of sustained pharmacological response, allowing for less frequent dosing, lower doses, and reduced adverse side effects. [2] To improve potency and physicochemical properties of designed compounds, polar groups are added to solvent-exposed parts of inhibitors to increase their solubility. Partial desolvation of polar groups upon protein binding is inevitable and results in energy loss. It has been shown that this can negatively affect binding enthalpy and reduce ligand binding affinity if the protein's binding site does not facilitate sufficiently strong interactions to compensate for desolvation losses.[3] With ITC we are analysing of thermodynamic parameters such as binding entropy and enthalpy, which provide a deeper understanding of the binding mechanism of designed inhibitors to the selected target. Data obtained from BLI (intrinsic binding mechanism), complemented with ITC data (intrinsic binding mechanism and solvent interactions) offer a comprehensive view of the overall binding mechanism and allow identification and optimization of hit compounds.



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20 Anisotropic magnetic nanoparticles: a new strategy against resistant biofilms

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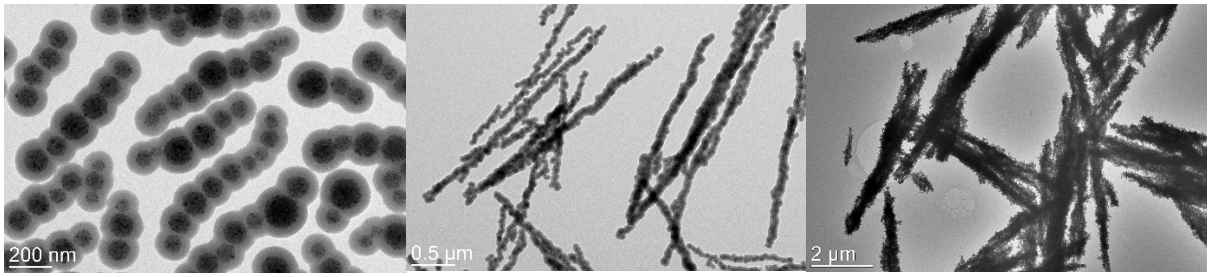
Biofilms exhibit strong resistance to chemical treatments due to their extracellular polymeric substances (EPS) matrix, which blocks antibiotics and disinfectants from reaching embedded microorganisms. This resilience makes biofilms a major challenge, particularly in medicine, where they frequently form on medical devices like contact lenses, heart valves, pacemakers, dental implants, and catheters. Treating biofilm-associated infections, such as periodontitis, osteomyelitis, endocarditis, chronic wounds, and cystic fibrosis-related lung infections, often requires high doses of antibiotics, surgical removal, or localized antimicrobial treatments.[1] To overcome these challenges, iron oxide nanoparticles (IONPs) have gained increasing attention for their antimicrobial and antibiofilm properties. When conjugated with therapeutic agents, they offer a promising strategy for biofilm disruption. Their magnetic properties allow them to be precisely guided using external magnetic fields, enhancing their penetration into biofilms. This targeted approach not only disrupts biofilm structure but also improves the delivery and effectiveness of antimicrobial agents, leading to more efficient bacterial eradication.[2] To address the challenge of biofilm removal, we developed an innovative approach using various anisotropic magnetic nanoparticles (AMPs). When exposed to a rotating magnetic field, these nanoparticles rotate, mechanically disrupting and loosening the biofilm. This process partially decomposes the biofilm by creating channels within its structure, improving the penetration and effectiveness of antibiotics and disinfectants. To achieve this, we synthesized elongated anisotropic magnetic nanostructures in three different length ranges: < 2 μm (nanochains), between 2–5 μm (nanorods), and > 5 μm (microrods). The synthesis is based on the self-assembly of several small superparamagnetic iron oxide nanoparticles (~10 nm) as building blocks.[3] To improve mechanical stability of nanostructures, we coated the formed nanostructures with a silica shell, which is suitable for its further functionalization. The synthesized nanoparticles were characterized using transmission electron microscopy (TEM) and zeta potential measurements. Additionally, their colloidal stability in different cell media was evaluated using dynamic light scattering.



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21 Fight against bacteria: development of novel bacterial topoisomerase inhibitors as new antibacterials

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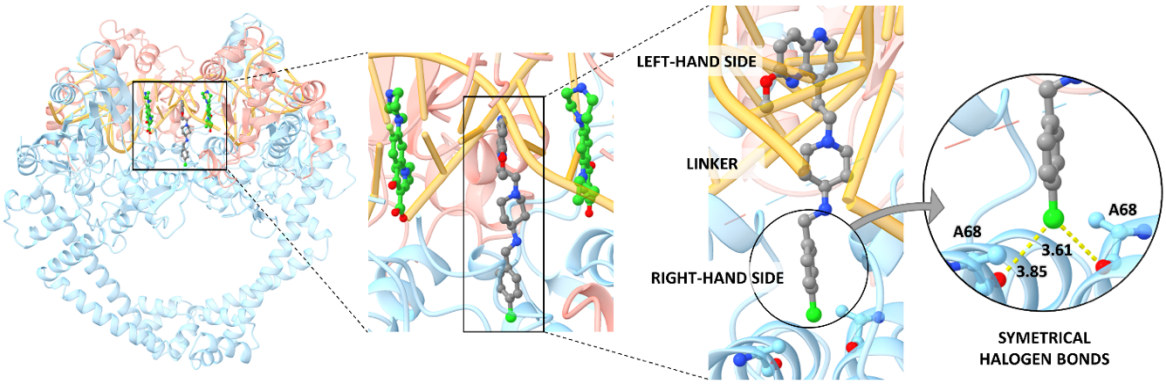
The constant mutation and spread of multidrug-resistant bacteria present a significant threat to human welfare. Bacterial type II topoisomerases, such as DNA gyrase and topoisomerase IV (topoIV), are well well-established and validated targets in the treatment of bacterial infections. Fluoroquinolones are one of the most commonly used antimicrobial agents targeting these enzymes. Unfortunately, their extensive, and often inappropriate, use has led to the wide spread of bacterial resistance. Nearly two decades ago, a new class of antibacterial agents, novel bacterial topoisomerase inhibitors (NBTIs), was introduced. Like fluoroquinolones, they inhibit the same biological targets in bacteria, but avoid cross-resistance to fluoroquinolones by binding to an alternative, but not overlapping binding site on DNA gyrase/topoIV.[1] They are composed of three main parts: a DNA-intercalating left-hand-side (LHS) moiety, an enzyme-binding right-hand-side (RHS) moiety, and an interconnecting linker. We recently solved and published a crystal structure of *S. aureus* DNA gyrase in complex with DNA and an NBTI compound (AMK-12) (Figure 1), which revealed that the compound, which contained a chlorine atom at the para position of the phenyl RHS, forms strong symmetric bifurcated halogen bonds in the binding side. We believe this interaction is key for establishing strong on-target activity.[2] Encouraged by this information, we prepared several libraries of NBTIs with p-halogenated fragments as the RHS moieties that showed significantly enhanced on-target activities, which resulted in balanced dual-targeting inhibitors of DNA gyrase and topoisomerase IV. The good dual on-target inhibition translated into improved antibacterial activity with MIC values as low as 0.004 µg/mL in *S. aureus* and MRSA strains. Besides Gram-positive antibacterial activity, our compounds showed good antibacterial activity against a broad spectrum of bacteria, including Gram-negative ESKAPE pathogens. Furthermore, we showed that the compounds have the ability to inhibit formation of biofilms and cause the disintegration of mature biofilms.[3] Finally, we also assessed the safety and efficacy of some of our most promising compounds in zebrafish embryos and compared them to gepotidacin, the only NBTI that passed phase III clinical trials.



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22 Optimizing subcutaneous mab formulations: novel viscosity-reducing excipients and their stabilizing effects

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The subcutaneous administration of therapeutic monoclonal antibodies (mAbs) offers significant advantages over intravenous administration, including faster administration, the possibility of self-administration, and reduced injection pain. These benefits contribute to decreased healthcare costs and time burdens, making treatment more convenient for patients.[1] However, achieving the necessary therapeutic effect often requires high concentrations of mAbs, leading to high viscosity of the formulation and low protein stability. High viscosity, namely above 20 mPas, can hinder manufacturing and injection processes, while instability can result in protein aggregation, reducing efficacy and potentially increasing immunogenicity.[1,2] To address these challenges, our study aimed to develop an optimal formulation for a model mAb using newly discovered viscosity-reducing agents, which are analogues of proline.[3] We investigated these test compounds individually at concentrations up to 200 mM and in combinations to assess their viscosity-reducing effects. Our results demonstrated that all individual test compounds reduced the viscosity of the mAb formulation by more than 30%, with six test compounds outperforming proline. When aromatic test compounds or those which can form aromatic interactions were added at higher concentrations (up to 200 mM), a concentration-dependent viscosity-reducing effect at sufficiently high mAb concentrations was observed. Furthermore, the viscosity of the formulation was reduced below the 20 mPas threshold using either a combination of two test compounds or a single compound at a concentration above 25 mM. An accelerated stability study indicated stabilization effects of single compounds and their combinations, with aggregate percentages remaining below 5% in most formulations, as observed by size-exclusion chromatography. These viscosity-reducing and stabilization effects were also supported by dynamic light scattering results, which showed reduced attractive forces between the mAb molecules in formulations with test compounds. Our findings suggest that the addition of viscosity-reducing excipients is a useful approach in the development of highly concentrated mAb formulations. Further research of such excipients and their underlying mechanisms could facilitate the development of mAb formulations for subcutaneous administration, ultimately improving patient therapy and outcomes.

IV - intravenous, mAbs - monoclonal antibodies, SC - subcutaneous

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23 Proteome analysis of minor salivary glands identifies patient subgroups with distinct clinical phenotypes in Sjögren's syndrome

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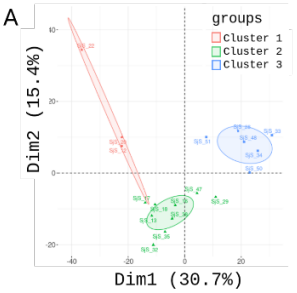
Sjögren's disease (SjD) is a systemic autoimmune disorder characterized by lymphocyte infiltration of the salivary and lacrimal glands, leading to oral and ocular dryness (*sicca* symptoms). SjD is highly heterogeneous, with some patients developing systemic complications affecting multiple organs and an increased risk of B-cell lymphoma. Additionally, patients vary in presence of autoantibodies (anti-Ro, anti-La, rheumatoid factor), cryoglobulins, and hypergammaglobulinemia. Differences in lymphocyte infiltration and abnormal structures in the salivary glands, such as germinal centres, further contribute to disease variability.[1] Identifying subgroups of patients with similar clinical and pathobiological features could help us identify biomarkers for disease outcome, improve our understanding of disease mechanisms, and guide the development of targeted therapies. Despite this potential, there is limited information on clustering SjD patients based on tissue characteristics, such as those in the salivary glands. In this explorative study, we aimed to investigate the proteome of minor salivary glands (MSG) of SjD patients and identify potential subgroups of patients with SjD. Flash frozen MSG from 18 patients with SS, fulfilling the 2016 ACR/EULAR classification criteria[2] were analysed. Clinical data was collected. Proteome analysis was performed by tandem mass spectrometry. Proteins were identified and quantified using the Spectronaut software. Principal component analysis (PCA) was performed based on log2-transformed Spectronaut intensities (SI). Interferon (IFN) signatures were calculated according to Gottenberg et al.[3] Differences in clinical characteristics between clusters were analysed using the Mann-Whitney U test. Proteome analysis detected 8192 proteins in MSG tissues. PCA identified three separate clusters of patients with SjD (Figure 1 A). Patients from cluster 1 (n=3) had significantly higher lymphocyte infiltration measured by focus scores, higher number of germinal centres (GC), significantly higher IFN signatures and lower unstimulated salivary flow (USF) compared to patients from cluster 2 (n=9) and cluster 3 (n=6). In addition, cluster 1 had a higher percentage of patients testing positive in salivary gland ultrasound (SGUS), rheumatoid factor (RF) and cryoglobulines (Figure 1B). In conclusion, SjD patients in our cohort clustered in 3 subgroups based on MSG proteomes, with the first subgroup showing the most prominent glandular damage, and the highest IFN scores.

A



B

	C1 (n=3)	C2 (n=9)	C3 (n=6)	C1 vs C2 p - value	C1 vs C3 p - value
Focus score (median (IQR))	3.3 (9.8)	1.5 (3.0)	1.2 (0.5)	0.0318	0.0238
Number of GC (median (IQR))	4.5 (3.0)	0.5 (2.0)	1.0 (1.3)	0.0444	0.0714
USF (mL/min) (median (IQR))	0.0 (0.0)	0.2 (0.3)	0.1 (0.3)	0.0727	0.1667
IFN signature (Geometric mean of SI) (median (IQR))	295.0 (176.6)	182.4 (19.5)	178.9 (30.6)	0.0091	0.0238
SGUS n positive (% positive)	3 (100)	5 (56)	3 (50)	/	/
Anti-Ro n positive (% positive)	2 (67)	7 (78)	4 (67)	/	/
Anti-La n positive (% positive)	1 (33)	3 (33)	3 (50)	/	/
RF n positive (% positive)	3 (100)	2 (22)	2 (33)	/	/
Cryoglobulins n positive (% positive)	2 (67)	1 (14)	0 (0)	/	/

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24 Understanding severe preeclampsia through omics approach

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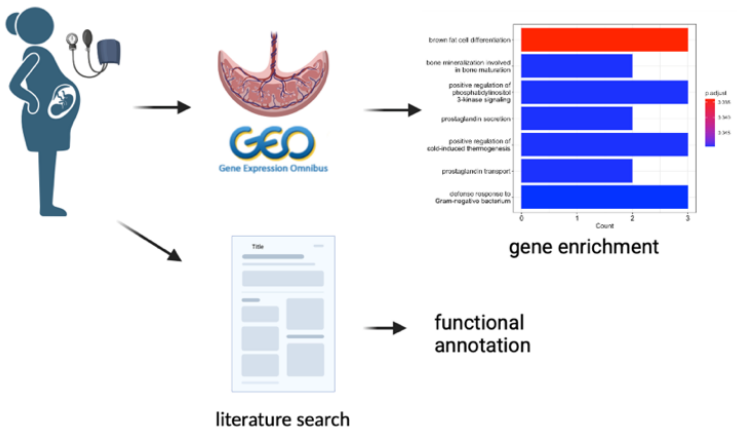
Preeclampsia is one of the most severe pregnancy complications, affecting 2-8 % of pregnant women worldwide. It is characterized by sudden onset of hypertension after 20 weeks of gestation, often accompanied by complications such as proteinuria, maternal organ dysfunction, or uteroplacental impairment. This complex disorder has long-term consequences, including reduced life expectancy and an elevated risk of stroke, cardiovascular disease and diabetes.[1] Any woman diagnosed with preeclampsia has the potential to develop severe symptoms, regardless of the disease's initial presentation. Severe preeclampsia is defined by a blood pressure of ≥160/110 mmHg measured at least four hours apart after 20 weeks of gestation. Additionally, it may be accompanied by complications such as thrombocytopenia, elevated liver enzymes, or acute liver impairment. Women with severe preeclampsia often experience intractable headaches, persistent visual disturbances, severe right upper quadrant pain and pulmonary edema.[2] Despite advancements in the understanding of severe preeclampsia, a significant knowledge gap persists. For this study we conducted differential gene expression using multiple combined publicly available placental RNA sequencing datasets relevant to severe preeclampsia. The results revealed several upregulated pathways, including brown fat differentiation, prostaglandin secretion, inhibition of nitric oxide production, adipogenesis, interleukin-4 and interleukin-13 signalling. On the other hand, the most significant downregulated pathways are mineralocorticoid and glucocorticoid biosynthesis, metabolism of steroid hormones, cell-cell junctions, cell junction organization, cell-cell communication and alpha-defensins. A literature review of studies investigating other omics technologies, including genomics, proteomics and metabolomics was performed. The key findings were analysed and functionally annotated. Several pathways identified in the analysis exhibited overlap with transcriptomic findings, highlighting their potential significance in the underlying biological mechanism. These findings contribute to a more comprehensive understanding of the pathogenesis of this complex disease while also identifying potential targets for more effective and therapeutic strategies.



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25 Ticagrelor is associated with increased rosuvastatin blood concentrations

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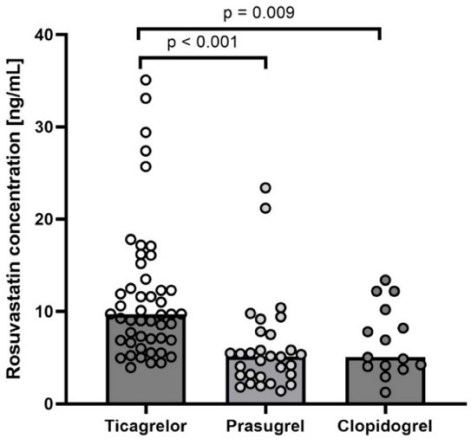
For patients recovering from myocardial infarction (MI), secondary prevention strategies are critical to reduce future cardiovascular risks. These typically include high-intensity statin therapy (e.g., rosuvastatin 20–40 mg daily) to aggressively lower cholesterol and P2Y12 receptor antagonists (e.g., ticagrelor, prasugrel, or clopidogrel) to prevent platelet aggregation.[1] Unlike other statins, rosuvastatin undergoes minimal hepatic metabolism. Its distribution and clearance are mediated by transport proteins such as organic anion-transporting polypeptides (OATP1B1, OATP1B3, OATP2B1) and the efflux transporter ABCG2 (BCRP). Genetic polymorphisms in these transporters—notably *ABCG2* rs2231142—significantly modulate its pharmacokinetics, contributing to variability in drug exposure across populations.[2] Multiple reports have documented severe adverse effects following the coadministration of ticagrelor and rosuvastatin, indicating a potential drug-drug interaction.[3] This study investigates whether ticagrelor alters rosuvastatin plasma concentrations compared to prasugrel/clopidogrel in post-MI patients on high-dose rosuvastatin, aiming to optimize safety and efficacy. A prospective observational study was conducted on 93 post-myocardial infarction (MI) patients receiving oral rosuvastatin 40 mg/day alongside an antiplatelet agent (ticagrelor, prasugrel, or clopidogrel). Blood samples were analysed for standard biochemical markers, and rosuvastatin concentrations were measured. Rosuvastatin concentrations grouped by P2Y12 antagonists were analysed and multivariate linear regression was performed to determine whether ticagrelor independently impacts rosuvastatin concentrations. Patients receiving ticagrelor exhibited rosuvastatin plasma concentrations approximately twice as high (9.7 ng/mL) as those on prasugrel (5.1 ng/mL) or clopidogrel (5.0 ng/mL), suggesting a clinically significant drug-drug interaction. This association persisted after adjusting for age, sex, renal function, and liver function ($P < 0.05$). The interaction is likely mediated by ticagrelor's inhibition of the ABCG2 transporter, which facilitates rosuvastatin efflux. A static drug interaction model predicted a 2.1-fold increase in rosuvastatin exposure due to ABCG2 inhibition[2], consistent with our findings. Potential interactions between ticagrelor and rosuvastatin should be considered in the clinical practice, as both medications are frequently prescribed together as part of guideline-directed therapy in patients after MI.



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26 Expanding monobactam activity: structural and functional insights into PBP and biofilm inhibition

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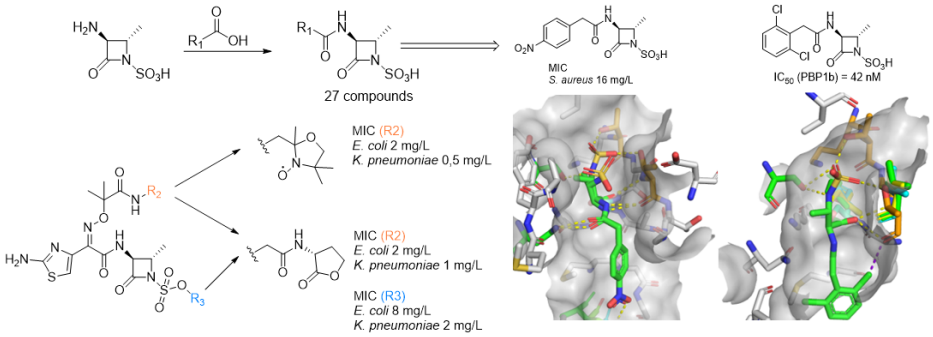
Antimicrobial resistance is a critical global health challenge, with drug-resistant bacteria responsible for 4.7 million deaths annually. β-Lactams remain the most widely used antibacterials, but resistance arises from PBP mutations and β-lactamase activity. Monobactams, a subclass with a monocyclic core, resist hydrolysis by metallo-β-lactamases (MBLs), with aztreonam being the only approved drug in this class.[1] Targeting biofilms is a promising strategy to combat resistance, as they contribute to infection persistence and antibiotic tolerance. These structured bacterial communities, encased in a protective extracellular polymeric substance (EPS) matrix, enhance survival against antimicrobial agents.[2] Our goal was to develop potent inhibitors of PBP1b from *Streptococcus pneumoniae*, which served as a model PBP, by synthesizing a focused library of monobactams with diverse R1 side chains. Structural analysis of obtained co-crystal structures revealed that additional hydrogen and halogen bonding with a key threonine residue could enhance enzyme inhibition. Despite achieving nanomolar PBP inhibition, these compounds did not exhibit strong antibacterial activity. To improve efficacy, we synthesized two series of aztreonam chimeras. The first featured nitroxide conjugates, mimicking nitric oxide's biofilm-dispersing effects, while the second included acyl homoserine lactone (AHL) and acyl homocysteine lactone conjugates, designed to inhibit quorum sensing. Conjugates were synthesized using diverse linkers, forming either amide bonds (R2, nitroxides) or both amide bonds and sulfonic acid esters (R3, AHLs). As shown, some compounds exhibited activity against Gram-positive bacteria) an uncommon property for monobactams (while others demonstrated MIC values comparable to aztreonam against ESKAPE pathogens. Additionally, they inhibited biofilm formation in clinical isolates of *Escherichia coli, Pseudomonas aeruginosa* PAO1, and *Acinetobacter baumannii,* highlighting their dual mode of action.



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27 Heterogeneous activity of proteasome subunits in human platelets and the regulatory role of β1i in signaling and granule

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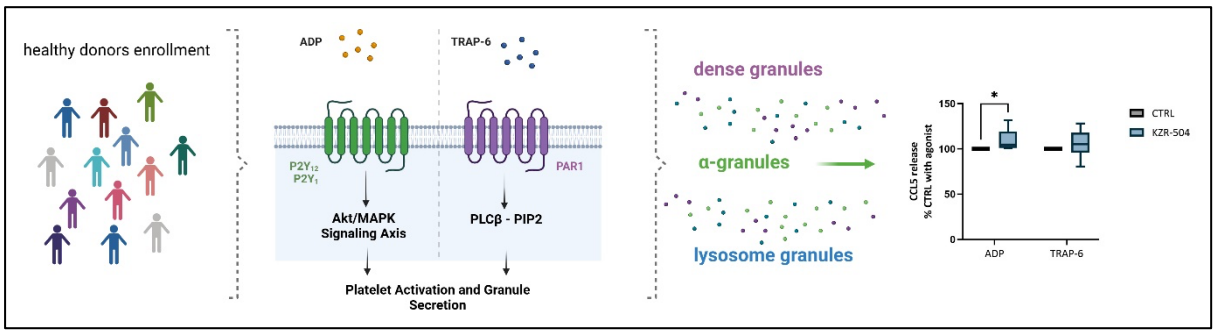
Platelets are essential for hemostasis, but they also contribute to various immune-related processes, including inflammation, cancer metastasis, and tissue repair.[1] Recent findings indicate that proteasomes, enzyme complexes responsible for intracellular proteolysis, are present and active in platelets. These complexes include the constitutive proteasome and its immune-inducible counterpart, the immunoproteasome, which replaces the standard catalytic subunits β1, β2, and β5 with β1i, β2i, and β5i, respectively. [2,3] While proteasomes are well-characterized in immune cells and cancer, their role in platelet biology remains poorly understood. Given that proteasomes influence immune responses by regulating cytokine production, cell survival, and differentiation[3], their presence in platelets suggests a potential role in platelet-mediated immune functions. The expression, activity, and functional significance of proteasome catalytic subunits in human platelets were investigated in our study. Analysis of platelet samples from 31 healthy donors revealed significant interindividual variability in the expression and activity of constitutive and immunoproteasome subunits, with predominant expression of β5i, β5c, β1i, and β1c. Notably, kinetic analysis of platelet lysates showed no detectable catalytic activity of the β2i subunit, suggesting its absence or inactivity in platelets. Using selective proteasome inhibitors, we demonstrated that inhibition of β1i significantly alters platelet signalling pathways, particularly by reducing p38 MAPK and NF-κB phosphorylation. Furthermore, β1i inhibition enhanced α-granule release, notably increasing CCL5 secretion upon ADP activation, suggesting a role in platelet activation and immune modulation. These findings highlight the heterogeneous activity of proteasome subunits in platelets and underscore the potential impact of proteasome inhibitors on platelet function. Nevertheless, further studies are needed to elucidate the indispensable role of proteasomes in platelet physiology.



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28 The effect of membrane composition on the signaling of the NKG2A/CD94/HLA-E immune receptor

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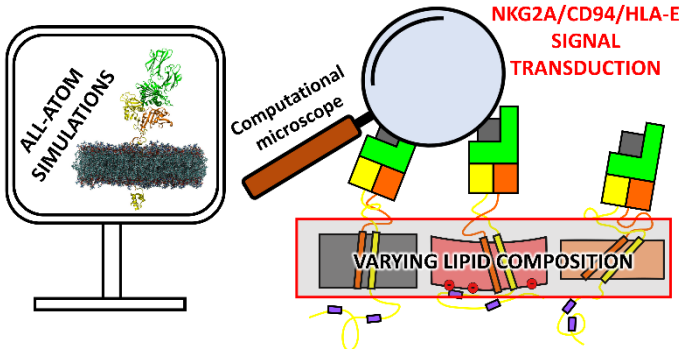
Understanding how membrane composition influences the dynamics and function of transmembrane proteins is crucial for uncovering cellular signaling pathways and developing new strategies to regulate signaling processes. Despite its significance, the comprehensive study of lipid contributions to the conformation and functionality of large protein systems is still in its early stages. In this study, we employed all-atom molecular dynamics simulations to explore how different membrane compositions impact the conformational dynamics of the NKG2A/CD94/HLA-E immune receptor complex.[1] This receptor complex plays a vital role as a negative regulator of natural killer cell cytotoxicity, transmitting inhibitory signals through the ITIM regions in the intracellular domain of NKG2A upon ligand binding.[2] It serves as an ideal model for investigating how membrane properties influence receptor-mediated signaling. Our findings reveal distinct variations in the behavior of the immune receptor complex across five representative membrane compositions: POPC, POPA, DPPC, DLPC, and a mixed POPC/cholesterol system. These differences are particularly evident in the intracellular domain, affecting mobility, tyrosine exposure, and interdomain communication. For instance, the POPA membrane, with its high negative surface charge density, increased lipid interactions, leading to a significant reduction in the exposure of NKG2A ITIM regions to water molecules, which could hinder signal transduction. In contrast, the DPPC membrane, characterized by its high transition temperature and gel-like properties, induced curvature effects that altered the exposure of one ITIM region. The reduced thickness of the DLPC membrane caused a pronounced tilt in the transmembrane domain, which disrupted the hydrogen bonding network in the extracellular domain and modified the linker protrusion angle. Beyond these localized effects, broader shifts in receptor dynamics were observed. For example, POPA was associated with lower overall flexibility, while correlation analyses indicated that domain communication was disrupted in several models. These findings highlight the critical role of membrane composition in shaping transmembrane protein behavior. By demonstrating how lipid environments influence receptor function, our study provides a foundation for exploring lipid-based approaches to modulate signaling.



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29 Targeting human H_V1 proton channels: discovery of a new series of inhibitors with promising anticancer activity

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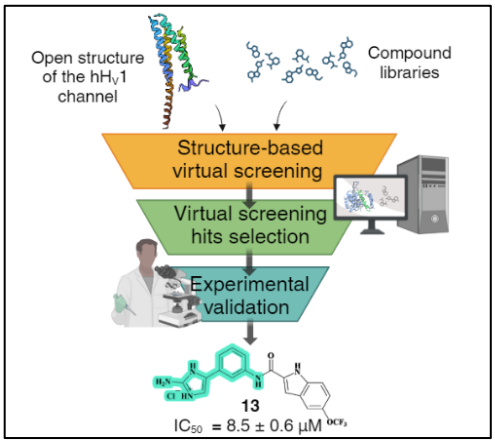
Voltage-gated proton channels (H_V1) are proton-selective voltage-dependent channels that have been found in various mammalian cells as well as in cancer cells. They play an important role in many signalling pathways by regulating the intracellular pH and preventing intracellular acidification.[1] In physiological conditions, at the resting membrane potential, the channels are closed; however, when various pathological conditions occur, these channels can open even at the resting membrane potential. In this acidic microenvironment, tumour cells can adapt extremely well, while immune cells functions are impaired. The aim of our work is to discover and evaluate new H_V1 inhibitors. At present, there are no selective inhibitors specific for H_V1 proton channels. An open structure of the human H_V1 channel was used to perform virtual screening (VS) of an in-house library of compounds and selected known H_V1 inhibitors. Compounds were docked to the binding site of guanidine derivatives, such as 2-guanidinobenzimidazole (2GBI), on the voltage-sensing domain.[2] Virtual screening results were evaluated, and a series of most promising hits were selected to be tested by patch-clamp electrophysiology on H_V1 channels. With an IC_{50} value of 8.5 μM, compound 13 exhibited a significant block of the proton current. Six additional compounds were found to block channel activity by more than 50% at 50 μM concentration when twenty-three analogues of compound 13 were biologically evaluated. These results allowed us to study the structure-activity relationship and to discover a new series of molecules with a 5-phenyl-2-aminoimidazole core as a new structural class of inhibitors of human H_V1 channels. Moreover, the antiproliferative activity of the seven hits was investigated by testing the compounds in two tumour cell lines, MDA-MB-231 and THP-1, in which H_V1 channels are highly expressed. Most of the molecules were found to be potent growth inhibitors, with compound 13 showing the lowest IC_{50} values (MDA-MB-231 IC_{50} = 9.0 ± 1.0 μM, THP-1 IC_{50} = 8.1 ± 4.3 μM).[3] By bringing together the knowledge and the results from ligand- and structure-based drug design, biophysical and pharmacological characterization, and medicinal chemistry methods, we have obtained promising hits that can be used for further hit-to-lead optimization and serve as chemical tools to better understand the role of H_V1 in tumours and other relevant diseases.



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30 Proton pump inhibitor exposure and overall survival in metastatic non-small cell lung cancer treated with immune checkpoint

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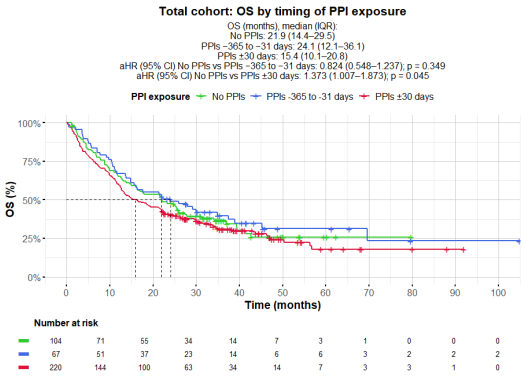
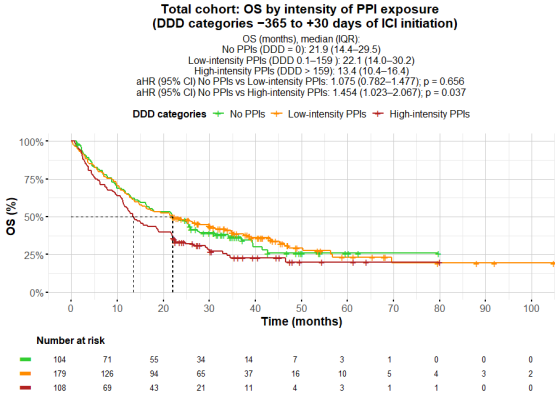
Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of metastatic non-small cell lung cancer (mNSCLC), yet nearly two-thirds of patients still fail to respond. Emerging evidence suggests that proton pump inhibitors (PPI) exposure may negatively impact ICI response by altering the gut microbiota, leading to shorter overall survival (OS) in mNSCLC.[1,2] While most studies focus on PPI exposure within 30 days before or after (±30 days) ICI initiation, the impact of prolonged and more intense PPI exposure remains unclear. Therefore, our study aimed to evaluate the association between the timing and intensity of PPI exposure, including exposure up to one year prior to ICI initiation, and OS in mNSCLC patients in a real-world setting. A retrospective cohort study was conducted at Clinic Golnik (2015–2022), including 391 mNSCLC patients treated with ICIs, who were stratified by timing of PPI exposure: (1) No PPIs (N = 104), (2) PPIs within ±30 days of ICI initiation (N = 220), and (3) PPIs exclusively in the pre-treatment period (−365 to −31 days before ICI initiation; N = 67). Group 2 could include earlier PPI use, while group 3 had no PPIs during the ±30-day window. Second, patients were stratified by intensity of PPI exposure (-365 to +30 days of ICI initiation), measured in defined daily doses (DDD): (1) No PPI exposure (DDD = 0; N = 104), (2) Low-intensity exposure (DDD 0.1–159; N = 179), and (3) High-intensity exposure (DDD > 159; N = 108). Median OS (mOS) was estimated using Kaplan–Meier, and Cox regression assessed associations with PPI exposure, adjusting for prognostic factors (sex, age, body mass index, performance status, histology, brain or liver metastases, PD-L1 expression, therapy line, addition of chemotherapy). Patients with PPIs within ±30 days of ICI initiation had significantly shorter mOS (15.4 months) compared to those with no PPIs (21.9 months; aHR: 1.37; CI: 1.01–1.87; p 0.045), while pre-treatment exposure had no significant effect. High-intensity exposure was also associated with shorter mOS (13.4 months) compared to no exposure (21.9 months; aHR: 1.45; CI: 1.02–2.07; p 0.037), while low-intensity exposure had no significant effect. Both timing and intensity of PPI exposure were associated with poorer outcomes, with the ±30-day window emerging as a key target for deprescribing interventions in oncology care. We are now developing a feasibility study to implement PPI deprescribing 30 days before ICI initiation in patients undergoing lung cancer diagnostics. If feasible, this could inform future prospective studies evaluating whether deprescribing PPIs in this critical window could enable this breakthrough therapy to extend patient survival to its fullest potential.





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31 γ-Enolase in oligodendrocyte differentiation: Implications for demyelinating disorders

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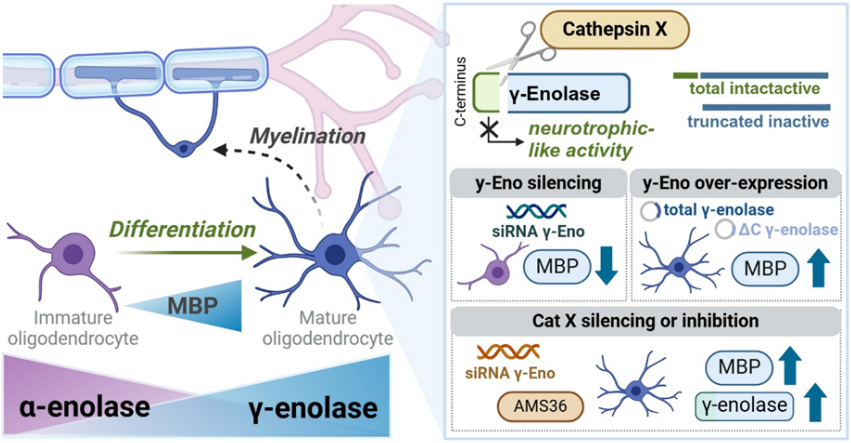
Oligodendrocytes are specialized glial cells in the central nervous system responsible for producing myelin to accelerate signal transmission and for providing trophic support to neurons. Oligodendrocyte differentiation is a process crucial for proper myelination, and its impairment is a hallmark of demyelinating neurodegenerative disorders such as multiple sclerosis.[1] γ-Enolase, a glycolytic enzyme recognized for its neurotrophic and neuritogenic properties, may be involved in oligodendrocyte differentiation, thereby promoting effective myelination.[2] While its neurotrophic properties and regulation by the lysosomal cysteine peptidase cathepsin X are well-documented in neurons, its role in oligodendrocyte differentiation remains poorly understood. By employing a differentiation protocol for the human oligodendroglial cell line, we demonstrated that as oligodendrocytes progressed toward a mature, myelinating phenotype, there was a significant increase in γ-enolase expression, correlating with the upregulation of myelin basic protein (MBP), a marker of mature oligodendrocytes. Interestingly, during differentiation, the expression of α-enolase isoform decreased while γ-enolase expression increased, eventually becoming the dominant isoform. Notably, overexpression of γ-enolase significantly enhanced oligodendrocyte differentiation, as evidenced by elevated MBP levels and morphological changes characteristic of mature oligodendrocytes, including extensive branching and myelin-like membrane formation. Conversely, silencing of γ-enolase expression through small interfering RNA significantly impaired differentiation, reducing MBP expression, decreasing process outgrowth, and leading to a less complex cellular morphology, thus underscoring the trophic role of γ-enolase in oligodendrocyte development. Importantly, cathepsin X acted as a regulator of γ-enolase activity as its inhibition or silencing elevated active γ-enolase levels, promoting oligodendrocyte differentiation and enhancing its neurotrophic effects.[3] Our findings highlight the importance of γ-enolase in supporting the differentiation of oligodendrocytes and in providing trophic support, a process that is regulated by cathepsin X. Therefore, exploring the trophic role of γ-enolase could provide therapeutic strategies for enhancing myelin repair and protecting neurons in inflammation-associated neurodegenerative diseases.



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32 Halogenated phenolic ingredients of household and personal care products modulate thyroid receptor signaling

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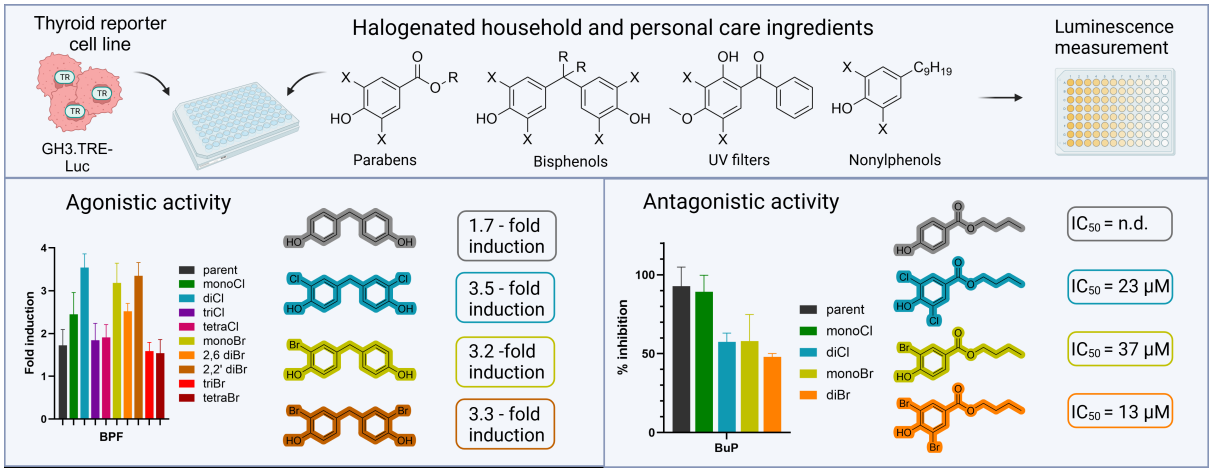
Endocrine disruptors affect thyroid hormone signalling, potentially leading to serious health consequences. Among such compounds, halogenated derivatives of household product and personal care product ingredients are particularly interesting due to their structural similarity to thyroid hormones. In this study, we evaluated a comprehensive library of 125 halogenated transformation products of parabens, bisphenols, UV filters, and nonylphenols for their ability to modulate the thyroid receptor using a luciferase reporter assay in GH3.TRE-Luc cells. Both agonistic and antagonistic activity was assessed at multiple concentrations. Among the tested compounds, halogenated derivatives of bisphenol F exhibited the most pronounced agonistic effects on the thyroid receptor (TR), with ClBPF and BrBPF inducing more than a threefold increase in activity compared to controls. Conversely, di-halogenated parabens, particularly dibrominated derivatives, displayed strong antagonistic effects on the thyroid receptor, with Br_2PeP and Br_2BuP reaching IC50 values of 9.5 µM and 13 µM, respectively. It is important to emphasize that halogenated ingredients of household and personal care products may disrupt thyroid function through additional mechanisms beyond direct thyroid receptor modulation. Given the widespread human exposure to these compounds and their transformation products in various environmental and consumer contexts, the observed TR-modulating effects highlight potential health risks associated with disruptions in thyroid hormone signalling. Our study on halogenated endocrine disruptors provides valuable insights into structure-activity relationships of halogenated HPCP ingredients on TR, serving as a foundation for future toxicological assessments of these and structurally related halogenated endocrine-disrupting chemicals.



Razvoj mehkih veščin na UL FFA kot temelj znanstvene odličnosti: pomemben prispevek k oblikovanju kompetenc prihodnosti

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V znanstvenih in gospodarskih okoljih 21. stoletja se soočamo z izzivi, ki zahtevajo več kot le tehnično usposobljenost. Digitalizacija, globalizacija, interdisciplinarnost in etična vprašanja sodobne znanosti ustvarjajo potrebo po strokovnjakih, ki niso vešči le v metodologijah svojega področja, temveč tudi v umetnosti sodelovanja, komunikaciji, inoviranju in vodenju. S tem postajajo mehke veščine (*angl.* soft skills) bistvena dimenzija znanstvene odličnosti. Predstavljajo sposobnosti, ki omogočajo uspešno integracijo znanja v širše družbene, gospodarske in kulturne procese. Raziskovalcem pomenijo most med idejo in njenim vplivom, med znanostjo in prakso, med individualnim prispevkom in kolektivnim napredkom. Na Fakulteti za farmacijo Univerze v Ljubljani (UL FFA) to prepoznavamo kot strateško usmeritev razvoja prihodnjih generacij raziskovalcev in pedagogov. Z inovativnim projektom ***Brunching with Soft and Research Skills at UL FFA: Unlocking Your Full Potential*** smo zato namensko ustvarili prostor, v katerem prepletamo znanstveno usposobljenost, osebnostno rast in družbeno odgovornost. Projekt smo zasnovali kot platformo za sistematično krepitev mehkih kompetenc doktorandov in podoktorandov, s ciljem razvijati raziskovalce in pedagoge prihodnosti, ki znajo, razumejo in dobro vodijo.

1. **Celostni pristop k razvoju kompetenc.** Sodobno izobraževanje mora preseči tradicionalno razumevanje znanja kot kopičenja informacij. Ključna naloga univerz je razvijati *celostne kompetence*, ki združujejo znanstveno odličnost z osebnostno rastjo. Izobraževanja, usmerjena v mehke veščine, omogočajo študentom, da pridobljeno znanje prevedejo v prakso, gradijo sposobnost refleksije in razvijajo prožnost v nepredvidljivih razmerah. Tak pristop spodbuja *aktivno učenje* in *odgovornost posameznika* za svoj razvoj. Udeleženci postajajo soustvarjalci znanja, ne le njegovi prejemniki. Razvijajo se v posameznike, ki razumejo, da odličnost ne pomeni le znanstvenih rezultatov, temveč tudi način, kako sodelujejo, vodijo, komunicirajo in prispevajo k skupnosti.

2. **Komunikacija kot temelj sodelovanja in vpliva.** Komunikacija je srčika vsakega raziskovalnega procesa. Sposobnost jasnega, spoštljivega in učinkovitega sporočanja je enako pomembna kot vsebina, ki jo prenašamo. Usposabljanja s področja komunikacijskih veščin doktorandom omogočajo, da razvijejo *miselno strukturo, argumentacijsko moč in empatično poslušanje*. Učinkovita komunikacija gradi zaupanje, spodbuja interdisciplinarnost in povečuje družbeni vpliv znanosti. V času dezinformacij in hitrega kroženja podatkov je sposobnost jasne, preverljive in etične komunikacije ena najdragocenejših kompetenc mladih.

3. **Znanstveno pisanje kot izraz zrelosti in natančnosti.** Znanstveno pisanje ni zgolj tehnična spretnost, temveč odraz *intelektualne discipline in sposobnosti sintetiziranja kompleksnih informacij.* Dobre pisne spretnosti so ključne za objavljanje, diseminacijo znanstvenih rezultatov in sodelovanje v mednarodnih raziskovalnih mrežah. Takšna usposabljanja doktorande opremijo z znanjem o strukturiranju člankov, etiki objavljanja, recenzentskem procesu in znanstvenem jeziku. Poudarek ni le na pisanju, temveč tudi na *kritičnem branju* in ocenjevanju drugih del, kar spodbuja razvoj raziskovalne integritete. Krepitev kompetenc znanstvenega pisanja zato neposredno prispeva k prepoznavnosti in verodostojnosti institucije ter k dvigu kakovosti raziskovalne produkcije.

4. **Kreativnost, inovativnost in interdisciplinarno razmišljanje.** V raziskovanju ni napredka brez radovednosti in ustvarjalnosti. Usposabljanja, ki spodbujajo kreativno mišljenje, pomagajo raziskovalcem preseči rutinske miselne vzorce in razviti *strategije inovativnega reševanja problemov*. Z uporabo pristopov, kot so *design thinking*, vizualno razmišljanje in problemsko učenje, se udeleženci učijo, kako strukturirano obravnavati nejasne izzive, razvijati alternativne hipoteze in oblikovati inovativne raziskovalne pristope. Takšne metode krepijo *odpornost na neuspeh*, kar je ključno v raziskovalnem procesu, kjer je poskus in napaka sestavni del napredka. Kreativnost je tako razumljena kot kompetenca, ki povezuje racionalnost in domišljijo, analizo in intuicijo.

5. **Umetna inteligenca in digitalna pismenost.** Med sodobnimi izzivi, ki jih naslavljajo tovrstna izobraževanja, izstopa tudi *umetna inteligenca* (UI). Njena uporaba v znanosti odpira izjemne priložnosti, hkrati pa postavlja pomembna vprašanja o etiki, transparentnosti in odgovornosti raziskovalcev. Delavnice, posvečene razumevanju in uporabi umetne inteligence, udeležence uvajajo v temeljne koncepte *digitalne pismenosti, podatkovne etike in odgovorne rabe tehnologije*. Poudarjajo, da UI ni nadomestek za človeško presojo, temveč orodje, ki jo lahko okrepi, če jo spremlja ustrezno razumevanje in kritično mišljenje. Takšna izobraževanja krepijo zmožnost posameznikov za delo v digitalnih okoljih, razumevanje algoritmov in njihova omejitev ter razvoj reflektirane drže do tehnologij, ki vse bolj sooblikujejo znanstveno prakso. Vlaganje v digitalno kompetenco pomeni vlaganje v prihodnost, v znanstvenike, ki bodo znali združevati *tehnološko moč in humanistično odgovornost.*

6. **Sodelovanje in vodenje v interdisciplinarnih skupinah.** V središču sodobne znanosti je *timsko delo*. Raziskovalni uspeh je vse redkeje rezultat individualnega napora in vse pogosteje dosežek skupin, ki presegajo disciplinarne, geografske in kulturne meje. Usposabljanja s področja sodelovanja in vodenja razvijajo sposobnost prepoznavanja različnih vlog v skupini, gradnje zaupanja in upravljanja konfliktov. Udeleženci se učijo, kako ustvarjati okolje, ki spodbuja odprtost, spoštovanje in delitev odgovornosti. Takšna znanja so temelj trajnostne znanstvene kulture; kulture, v kateri uspeh ni rezultat tekmovanja, temveč *sinergije in skupne vizije*. V raziskovalnih timih se tako krepi organizacijska odpornost, inovativnost in zavest o pomenu skupnega dobrega.

7. **Podjetniški miselni okvir in prenos znanja v prakso.** Znanost svojo družbeno vrednost uresniči, ko njeni rezultati najdejo pot v prakso. Usposabljanja, ki razvijajo *podjetniško razmišljanje*, raziskovalcem omogočajo, da razumejo procese komercializacije znanja, vrednotenje intelektualne lastnine in načine povezovanja z industrijskimi partnerji. V središču ni profit, temveč sposobnost *prepoznati potencial lastnih idej* in jih uresničiti v obliki rešitev, ki prispevajo k javnemu dobremu. Takšna usposabljanja krepijo ustvarjalnost, proaktivnost in strateško mišljenje ter spodbujajo prehod od raziskovalnih rezultatov k družbenemu učinku. Na UL FFA s tem gradimo kulturo sodelovanja med akademskim in gospodarskim sektorjem, ki temelji na odgovornem inoviranju in odprtem prenosu znanja.

8. **Javno nastopanje in znanstvena prezentacija.** Posameznik mora biti tudi *ambasador znanosti*, sposoben predstaviti svoje delo razumljivo, zanimivo in prepričljivo. Usposabljanja na področju javnega nastopanja krepijo samozavest in sposobnost strukturiranega predstavljanja idej. Poudarjajo pomen pripovedovanja (angl. *scientific storytelling*), uporabe vizualnih pripomočkov in avtentičnega stika z občinstvom. Sposobnost javnega nastopa ne prispeva le k osebni prepoznavnosti raziskovalca, temveč tudi k zaupanju javnosti v znanost. Znanstvena prezentacija je tako orodje znanstvene odličnosti kot tudi instrument odgovorne komunikacije (sredstvo, s katerim znanost stopa v dialog z družbo).

Prispevek UL FFA: znanje, odgovornost in sodelovanje. S projektom *Brunching with Soft and Research Skills at UL FFA: Unlocking Your Full Potential* na fakulteti utrjujemo svojo vlogo vodilnega akademskega okolja, kjer razumemo, da je prihodnost znanosti v povezovanju disciplin, generacij in vrednot. Prispevek projekta je zato trojen:

- *akademski prispevek* (izboljšanje kakovosti doktorskega izobraževanja z integracijo mehkih in raziskovalnih kompetenc);
- *inovacijski prispevek* (razvoj odprtega okolja, kjer se znanost in gospodarstvo dopolnjujeta, izmenjujeta ideje in ustvarjata nove priložnosti);
- *družbeni prispevek* (vzgoja raziskovalcev, ki razumejo svojo vlogo v širši skupnosti, kot nosilci znanja, odgovornosti in sodelovanja).

Z vlaganjem v razvoj mehkih in digitalnih veščin na UL FFA ustvarjamo pogoje za ekonomijo znanja in sodelovanja, temelječo na dialogu, kreativnosti, znanstveni odličnosti in etiki. Mehke veščine, digitalna pismenost in razumevanje UI postajajo ključni elementi znanstvene usposobljenosti prihodnosti. Z izvajanjem tovrstnih podpornih izobraževalnih programov na fakulteti dokazujemo, da znanstvena odličnost ni le rezultat znanja, temveč tudi odnosa, sodelovanja in poguma razmišljati drugače. Gre za vlaganje v ljudi, v raziskovalce in pedagoge, ki znajo povezati razum in empatijo, tehnologijo in odgovornost, znanost in družbo. Takšne kompetence niso le orodja uspeha, temveč temelj zaupanja v znanost kot gonilo napredka in sooblikovanja naše skupne prihodnosti.