UNIVERSITY OF LJUBLJANA | FFA Faculty of Pharmacy

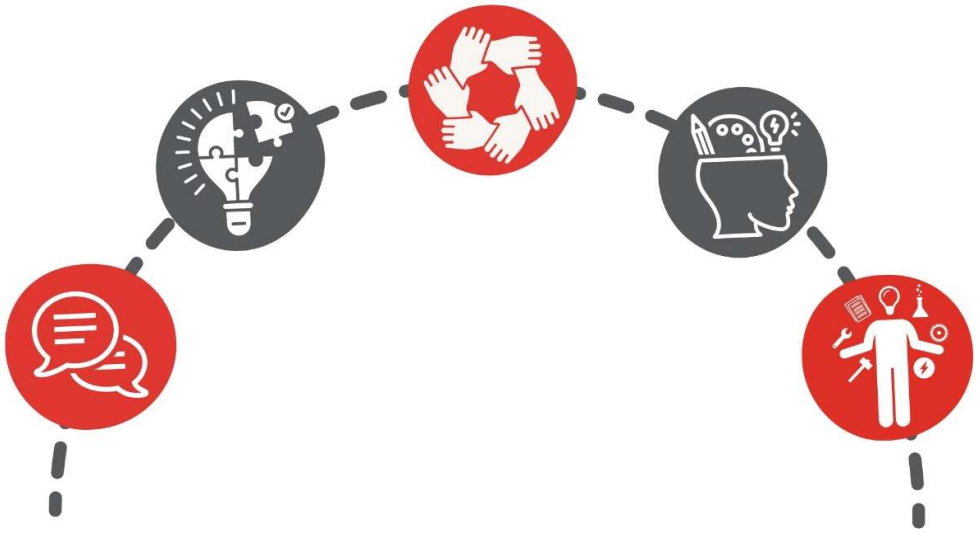
Brunching with Soft- and Research Skills at UL FFA: Unlocking Your Full Potential

1ST UL FFA Graduate Student Research Symposium

BOOK OF ABSTRACTS

University of Ljubljana, Faculty of Pharmacy

April 2024



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FOREWORD

This inspirational event and submitted abstracts highlight the amazing diversity and high quality of research conducted by our doctoral students, with talks covering various clinical and non-clinical topics spanning the fields of pharmacy, chemistry, biology, technology and medicine. The intention of the organising and scientific committee is to foster a dynamic dialogue between doctoral students and academic staff in the exciting field of pharmaceutical sciences, and we sincerely hope that you will enjoy the format of the symposium.

The inaugural version of the CDTC programme and the symposium, where all of you will be able to present your work to colleagues, will expose all participants to new areas of research and will allow for the cross-pollination of ideas. Opportunity to present your results and ideas, ample time for discussion after each of the two guest lectures, opportunities for targeted networking and best flash talk prize ceremony complete this exciting programme.

Best wishes for a fruitful symposium!

dr. Alen Krajnc, MSci Hons

SYMPOSIUM PROGRAMME

12:00 – 12:15	*Opening Address* **Assoc. Prof. dr. Igor Locatelli and dr. Alen Krajnc**
12:15 – 12:40	**Prof. dr. Zdenko Časar** University of Ljubljana, Faculty of Pharmacy and Lek Pharmaceuticals, d.d. *Boron in the Synthesis of Active Pharmaceutical Ingredients*
12:40 – 13:40	**Graduate Student Talks** (numbers 1 – 17) *Coffee Break and Networking*
14:10 – 14:35	**Prof. dr. Lucija Peterlin Mašič** University of Ljubljana, Faculty of Pharmacy *Between Toxicology and Pharmaceutical Chemistry*
14:35 – 15:40	**Graduate Student Talks** (numbers 18 – 38)
15:40 – 16:00	*Awards Ceremony* *Closing Remarks*

ABOUT THE PROJECT

In today's rapidly changing world, the importance of connecting businesses, academia and other stakeholders in the scientific arena is crucial for successful and impactful progress of science. At University of Ljubljana, Faculty of Pharmacy (UL FFA), we recognize the significance of forming strategic partnerships and actively engage in various (inter)national collaborations. We continuously seek new ways of working together with domestic and international partners, and this has resulted in the development of our current innovative career-development training programme (CDTC) titled *"Brunching with Soft and Research Skills at UL FFA: Unlocking Your Full Potential."*

The aim of the CDTC programme is to complement regular doctoral and postdoctoral researchers with additional knowledge and skills that empower individuals to unlock their full potential, positively impact their scientific research excellence, promote peer-to-peer interactions, creativity and innovation, cooperation and facilitate a more successful entry into the job market. The programme includes a combination of technical expertise and soft skills, theoretical knowledge and practical exercises, and merges academic knowledge with entrepreneurship. The project is entirely conducted in English, making it suitable for international students, which allows for a better integration of the international community into the matrixed research environment at our faculty as a whole.

In 2023-24, a total of 6 interactive social skills training sessions were conducted as part of the inaugural version of the CDTC programme, aimed at improving scientific excellence, work efficiency, communication skills, teamwork, whilst concurrently promoting international collaboration and the international reputation of UL FFA. The final milestone of the inaugural event series was the *1st UL FFA Graduate Research Student Symposium*, where every PhD student at UL FFA had a chance to present his/her work in a “Shark-tank” 2 minute pitch style. The carefully selected format enabled that every PhD student got the chance to present their work to the whole cohort of students and academic staff, raising awareness about the depth (and breadth) of research at the faculty and acted as a catalyst for future in-house collaborations.

The project was carried out in collaboration with external partners such as Ljubljana University Incubator, the Knowledge Transfer Office, internationally renowned academics (e.g. NCI/NIH, USA and University of Oxford, UK) and the main industrial partner – Lek Pharmaceuticals d.d. Workshops were designed to integrate technical knowledge with soft skills, theory with practice, science with entrepreneurship, faculty with individual economic partners, contributing to the development of an open innovation space for the advancement of new knowledge, future competencies, and talents. Soft skills training is crucial for the development of research potential and the personal growth of new generations of scientists, as confirmed by the positive feedback from doctoral students who see great advantages in such programmes.

1 Exploring Drug Release Mechanism from HPMCAS Based Amorphous Solid Dispersion Prepared with Hot-Melt Extrusion: and Advanced Microscopy Reverse Engineering Approach

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In the last 20 years, one of the main challenges for the pharmaceutical industry is formulating novel drugs from BCS II/IV classes into dosage forms that enable attaining therapeutic plasma levels for desired period of time. Common approach to overcoming poor solubility/poor permeability drawbacks is preparation of amorphous solid dispersion (ASD)[1]. While ASD approach is well-known and frequently practiced, characterization of formulation bio-performance is not a straight-forward process due to the complex and intertwined processes including drug and polymer dissolution – supersaturation – precipitation interplay, liquid-liquid phase separation (LLPS) which leads to the formation of drug-rich colloidal species and impact on effective permeability[2]. Determining drug release mechanism and establishing biorelevant dissolution method is crucial for the development of safe and effective drug product, compliant with the regulatory requirements. Most widely used technique for ASD preparation is hot-melt extrusion (HME), which consists of (i) melting the drug and polymer carrier (ii) kneading and mixing of the melt and (iii) extrusion, cooling and milling of the extrudate. Even if the influence of specific process parameter on drug dissolution profile is known, building comprehensive scientific knowledge model for establishing meaningful relation between HME critical process parameters, extrudate physico-chemical properties, micro-morphology and dissolution, is lacking due to gaps in understanding complex interaction between the polymer and the drug in ASD system when exposed to biorelevant dissolution media. Thus, elucidation of ASD dissolution behaviour provides the basis for consolidating aggregated data and accelerating development of drug products[3].

The aim of this study is to provide close insight into drug release mechanism from HPMCAS based ASD dispersion prepared with HME in biorelevant dissolution media with the use of advanced Verios-SEM microscopy technique. Dissolution media as well as undissolved extrudate particles were thoroughly studied. Weakly basic BCS II drug was used to prepare ASDs samples via HME with specified drug/polymer ratios using different HPMCAS grades. Samples were evaluated with biorelevant dissolution-permeation methods and Verios-SEM. Relation between HPMCAS grade, extrudate dissolution behaviour, namely size, number, and stability of surface colloidal particles formed, and *in vitro* dissolution studies was made. Furthermore, bioequivalence study was performed and *in vitro* behaviour was further linked with *in vivo* permeation/absorption.

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2 Assessment of Physio-Chemical Characteristics and Appearance of Lyophilizates

Matija Pečnik[a,b], Biljana Janković[a,b], Klemen Kočevar[b], Pegi Ahlin Grabnar[a], Maja Bjelošević Žiberna[a]

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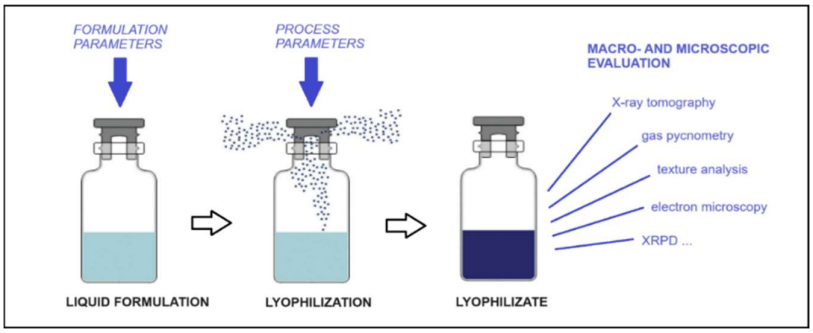
Currently registered parenteral dosage forms exist in solid and liquid state. Due to the instability of active ingredients in aqueous or organic solutions, it is reasonable to prepare a dosage form in a solid state. Freeze-drying or lyophilization is a suitable method to ensure long-term stability of small and biological active ingredients (e.g. peptides, proteins and nucleic acids) by converting liquid formulations into a powder or solid scaffold (cake) by solvent sublimation[1]. Lyophilisation is suitable for thermolabile active ingredients and allows drying without significant degradation. One of the most subjectively assessed characteristics of lyophilised products is the appearance of the cake. Ideally, the lyophilisation product is the same size and shape as the vial-filling solution, with a uniform colour and texture. Defects and changes in the appearance of lyophilisates may lead to increased reconstitution time, poorer physicochemical stability and altogether lower performance of the product. The most common defects in appearance described in the literature are melt back, slanted cakes, puffing, lifted cake, shrinkage, cracking and skin formation[2]. These phenomena affect the inadequate physio-chemical stability of the product and, indirectly, the safety and efficacy of the product.

In our research we will define model parenteral lyophilizate and evaluate impact of formulation and process parameters on cake structure and physio-chemical properties using complementary analytical methods. In-depth study of cake properties at macro- and microscopic level will allow better predictability of the lyophilization process at industrial scale allowing production of homogeneous and intact lyophilized products. In the early phase of our work, we will investigate the possibility of obtaining quantitative data of cake quality by means of micro-tomographic techniques (X-ray μ-CT), electron microscopy (SEM), specific surface area measurements (BET SSA), gas pycnometry and texture analysis[1,3]. We will assess impact of process parameters during lyophilization cycle on mechanical properties of primary packaging by nano- or microindentation techniques. The pre-formulation evaluation of the physio-chemical properties of the cake and the mechanical properties of the primary packaging will be correlated with different process parameters during freezing such as shelf cooling rate, nucleation temperature, annealing, drying etc.



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3 The Journey From Virtual Screening to Potent Antibacterials

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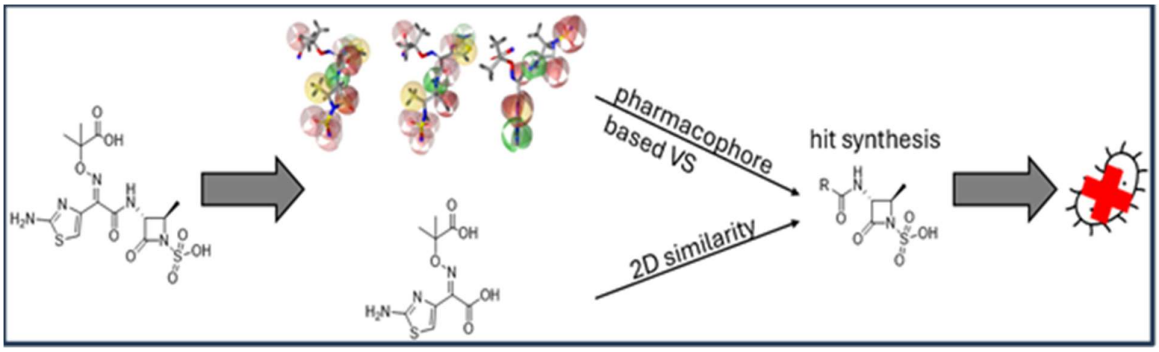
Antimicrobial resistance (AMR) poses a global health crisis due to the uncontrolled use of antibacterial agents. Bacterial infections are increasingly resistant to existing chemotherapies, resulting in at least 1,270,000 deaths annually worldwide. Urgent action is essential to prevent this toll from reaching 10 million deaths per year by 2050[1]. The ESKAPE pathogens, notorious for their resistance, demand effective infection control measures. Additionally, the discovery of novel antibacterials with innovative chemical entities or unique mechanisms of action is critical for combating AMR.

Peptidoglycan (PG) constitutes the primary structural element of bacterial cell walls. It consists of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine units cross-linked by short stem peptides. Penicillin-binding proteins (PBPs) catalyse transpeptidation reactions, forming essential 4→3 cross-links between adjacent pentapeptide chains. Notably, PBPs serve as validated targets for discovering novel antibacterial agents, particularly within the beta-lactam antibiotic class[2]. Crystallographic investigations have illuminated the architecture and mechanisms of PBP transpeptidases. These enzymes typically exhibit a conserved structure within their transpeptidase (or penicillin-binding) domain. The active site crucially involves residues from two subdomains, with a nucleophilic serine residue at its core — such as Ser510 in PBP1b in *E. coli*[1].

Our study aimed to develop novel monobactams that inhibit the model penicillin-binding protein (PBP1b from *S. pneumoniae*) and exhibit potent antibacterial activity. We constructed a database by virtually forming an amide bond between a monobactam scaffold (containing a free amine) and our in-house library of carboxylic acids. Employing three pharmacophore models, we conducted virtual screenings to identify promising hit molecules. As an alternative strategy, we performed a 2D similarity screening, comparing commercially available carboxylic acids against the aztreonam side chain. We synthetised the molecules obtained from both methods and subsequently conducted *in vitro* testing on them. Our efforts successfully yielded PBP inhibitors, some of which also exhibited potent antibacterial effects. Notably, the second approach provided stronger antibacterials, as supported by our results.



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4 Development of Phosphonium-Free Mitochondriotropic Conjugates with Improved Properties for Targeting Kv1.3 Expressing Cancer Cells

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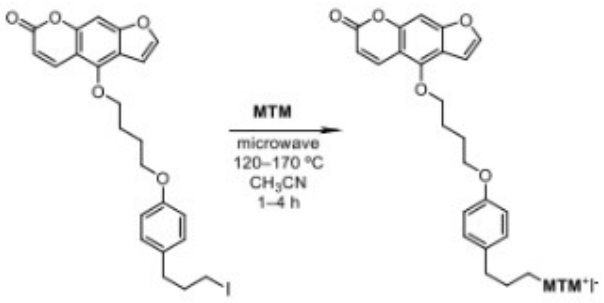
In recent years, the application of mitochondria-targeting technology to anti-cancer drug design has shown to be a promising strategy for the development of future therapeutics. As of 2021, a first-in-human phase I clinical study of a mitochondrial-targeted Hsp90 inhibitor implementing this strategy has been ongoing.[1] The voltage-gated potassium ion channel Kv1.3 is showing potential as a target for cancer therapy. Mitochondrial Kv1.3 is located in the mitochondrial membrane and is involved in the regulation of apoptosis.[2]

Mitochondriotropic Kv1.3-targeting conjugates with *in vivo* anti-cancer activity have been developed, and are based on triphenylphosphonium as a mitochondria-targeting moiety (MTM), which improved their selectivity for cancer cells.[3] One of the highly potent conjugates is PAPTP, a derivative of a high-affinity psoralen-type Kv1.3 inhibitor PAP-1. Firstly, we have developed a phosphonium-free heterocyclic analog of PAPTP with comparable activity, which has shown greater selectivity towards the induction of apoptosis in the cancerous cell line COLO-357, relative to the healthy TERT cell line. To improve physicochemical properties of the otherwise insoluble PAPTP and our new analogue, we have then developed a follow-up series of analogs with broad ranging lipophilicities of substituents, to observe their influence on activity and selectivity, as well as measure their thermodynamic solubilities and distribution coefficients. The series constitutes a platform for effective and reliable manipulation of solubility and distribution parameters, maintaining high potency and selectivity for a subset of the analogues. Based on the obtained findings, the series of analogues has been further expanded in order to additionally exploit the desired properties some of the substituents in the previous series have allowed for.



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5 Elevated ICOS Expression on Peripheral Blood CD4+ T Lymphocytes and its Diagnostic Potential in Sjögren's Disease

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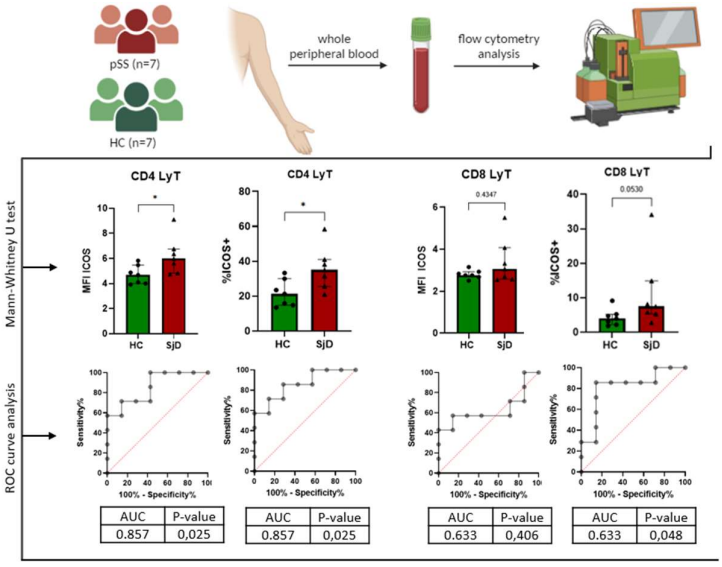
Background and aims: The inducible T cell co-stimulator (ICOS) is a costimulatory receptor expressed on activated CD4+ and CD8+ T lymphocytes. Transcriptomic studies have shown that ICOS is upregulated in the peripheral blood cells of patients with Sjögren's disease (SjD)[1], whereas studies investigating its expression on the surface of T lymphocytes by flow cytometry are scarce. The aim of our study was to investigate the expression of ICOS on the surface of T lymphocytes and to evaluate its diagnostic value in SjD.

Methods: We analyzed surface expression of ICOS on peripheral lymphocytes by flow cytometry in 7 patients with SjD and 7 healthy controls (HCs). Group comparisons were made using the Mann-Whitney test and diagnostic utility was evaluated via ROC curve analysis (Fig. 1).

Results: A trend of increased percentage of ICOS+ cells (%ICOS+) and an increased surface expression of ICOS (MFI) was observed in the lymphocyte populations studied in patients with pSS. The increase was statistically significant in CD4+ T lymphocytes but not in CD8+ T lymphocytes. The ROC curve analysis showed the best discriminatory ability for the %ICOS+ CD4+ T lymphocytes and the level of ICOS expression (MFI) on CD4+ T lymphocytes (AUC 0.857) (Fig. 1).

Conclusion: Our results are consistent with the presumed role of ICOS in the pathogenesis of SjD and suggest ICOS surface expression as a potential biomarker in SjD.



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6 Toolbox for New Approaches Towards Hsp90 Inhibition

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The first N-terminal heat shock protein 90 (Hsp90) inhibitor geldanamycin was discovered in the 1990s. Its anticancer activity prompted the exploration of Hsp90 chaperone family as a target for the treatment of cancer. Potent inhibition of Hsp90 resulted in decreased intracellular levels of several oncogenic Hsp90 client proteins that rely on Hsp90 to obtain their tertiary structure (Her2, CDK4, estrogen receptor α and many others). Despite their potent *in vitro* effect clinical use of the first Hsp90 inhibitors was halted due to heat shock response (HSR) induction that led to ineffectiveness and increased risk for toxicity. Thus, the development of classical N-terminal ATP-competitive Hsp90 inhibitors with equipotent affinity for entire Hsp90 family was proven unsuccessful. Luckily, the research direction has shifted towards C-terminal Hsp90 inhibitors, N-terminal isoform selective inhibitors and protein-protein interaction inhibitors between Hsp90 and its co-chaperons that modulate the chaperone cycle.[1]

The aim of this doctoral thesis was to utilize various design approaches and prepare new Hsp90 inhibitors that belong to all three classes of new modalities. We have successfully prepared N-terminal Hsp90 inhibitors that are selective for the cytoplasmic isoform β over its most similar counterpart Hsp90α. We have designed and prepared compounds meant for PPI inhibition between Hsp90 and Cdc37 – a co-chaperone responsible for delivering protein kinases to Hsp90, thus being relevant to cancer pathophysiology. The most work has been done to prepare new C-terminal Hsp90 inhibitors as we have prepared four libraries of different structural classes that bind the C-terminal domain of the chaperone. Overall, we have shown that all three Hsp90 inhibitor types do not induce HSR while still affecting Hsp90 client protein levels and causing inhibition of cancer growth both *in vitro* and *in vivo*.[2,3]

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7 Evaluation of Medication Reconciliation Service Benefits in Hospitalised Medical Patients

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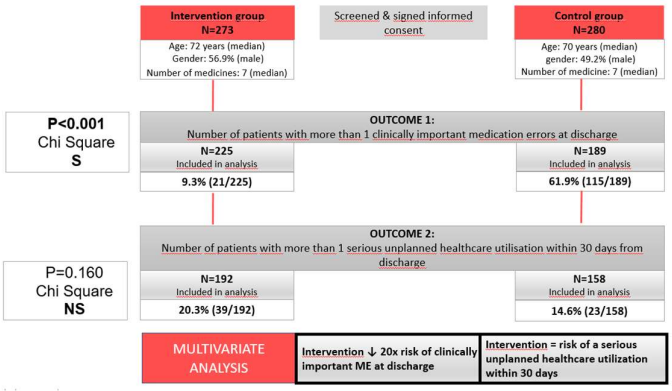
The patient's journey through transitions of care is prone to medication errors that can lead to unnecessary healthcare utilisation. Medication reconciliation is the process of identifying an accurate list of a person's current medicines and comparing it with the current list in use, identifying any discrepancies, and documenting any changes, thereby resulting in a complete list of medicines, accurately communicated.[1] Pharmacist-led medication reconciliation service has been shown to be effective in reducing the risk of medication errors. Clinical studies on the impact on more clinically important outcomes, such as unplanned healthcare utilisation, have produced inconsistent results.[2]

Our aim was to examine the effectiveness of routine pharmacist-led medication reconciliation on healthcare utilisation within 30 days of discharge and on medication errors at discharge and 30 days post-discharge. We conducted a pragmatic, prospective, controlled clinical trial in five general medical wards at the University Clinic Golnik, Slovenia. Patients were assigned to the intervention or control group according to their admission ward, the latter being randomly assigned. The intervention was delivered by clinical pharmacists and included medication reconciliation at admission and discharge, coupled with patient counselling. The study outcomes of clinically important medication errors at hospital discharge and serious unplanned healthcare utilisation within 30 days of hospital discharge were assessed by independent research clinical pharmacists. Overall, a total of 414 patients (53.4% male, median age 71) were included, 225 in the intervention group and 189 in the control group. The intervention significantly reduced, by 20-fold the risk for a patient to have a clinically important errors at discharge (9.3% intervention vs. 61.9% control group: OR 0.050). However, no significant differences were noted in any and serious unplanned healthcare utilisation (20.3 % intervention vs. 14.6% control group) [3]. An analysis of medication discrepancies 30 days after discharge is still pending. Our study is showed an important but not sufficient component of pharmacist-led seamless care programmes aimed at safer transitions of care. Therefore, we expect that the findings from our study will support the further development, implementation and delivery of seamless care at a national level.



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8 Genetic Variants in SERPINC1 and Sensitivity of Activity Assays for Detection of Antithrombin Deficiency

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Antithrombin (AT) deficiency, the most severe form of inherited thrombophilia[1], carries a high risk for venous thromboembolism, therefore, a highly sensitive assay to identify this disorder is essential. Some genetic variants are difficult to detect, and this often depends on the particular AT activity assay.[2,3] We conducted a study, where we aim to compare AT activities measured by different AT activity assays in relation to the genetic background of Slovenian patients with AT deficiency in order to improve recognition of AT deficiency in this population. The study included 220 adult patients, who developed idiopathic venous thromboembolism before the age of 50. 129 patients were consecutive and 91 with previously determined AT activity < 80 %. In all patients, AT activity was measured by five different AT activity assays (Innovance, Stago, Biophen IIa, Biophen Xa, HemosIL). Potential genetic variations in any of the seven exons and flanking intron regions of the AT gene, *SERPINC1*, were examined by Sanger sequencing. In patients in whom genetic variant was not detected, the presence of structural genetic variants was investigated by MLPA.

A genetic variation in *SERPINC1* was found in 81 % of cases with AT activity < 70 %. 13 different point variants (8 missense and 5 nonsense variants) and one whole gene deletion were detected. The most prevalent variant was AT Padua I (c.236G>A, p.Arg79His), which was found in almost half of the patients with AT deficiency. Other variants present in our cohort were AT Basel (c.218C>T, p.Pro73Leu), AT Dublin (c.89T>A, p.Val30Glu), AT Budapest III (c.391C>T, p.Leu131Phe) and AT Denver (c.1277C>T, p.Ser426Leu). In addition, some nonsense and one missense variant causing type I AT deficiency were found in individual families. All assays showed good sensitivity for type I variants, while of the type II variants, only AT Budapest III was detected by all assays. AT Denver (type II variant with reactive site defect (type IIRS)) was detected by all FIIa-based assays (Stago and Biophen IIa) and only by one FXa-based assay (Innovance). On the other hand, the sensitivity of each FXa- and FIIa-based assay was different for different type II variants affecting the heparin-binding site (type IIHBS). Transient variant, AT Dublin, was the most difficult to detect, as all assays measured normal AT activity. Assay with the highest sensitivity was Innovance (95 %), followed by Stago (93 %), Biophen IIa (66 %) Biophen Xa (66 %) and HemosIL (61 %). Since not all variants can be detected by only one activity assay, the introduction of molecular methods in diagnostic algorithm of AT deficiency seems reasonable.

Diagnostic sensitivity of antithrombin activity assays to different types of antithrombin deficiency

AT activity assay / Genetic variant	Innovance (FXa-based)	Biophen Xa (FXa-based)	HemosIL (FXa-based)	Sta-Stachrom (FIIa-based)	Biophen IIa (FXa-based)
Type I	100%	100%	100%	100%	100%
Type IIHBS	100%	52%	4%	96%	48%
Type IIRS	100%	0%	0%	100%	100%
Transient	0%	0%	0%	0%	0%

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9 Human Lymphoblastoid Cell Lines Offer a Good Alternative to Whole Blood for Immunological Testing

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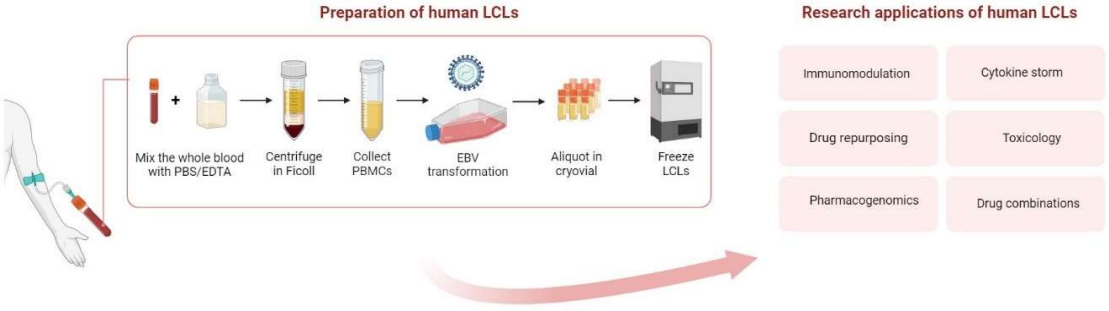
Cytokine storm, a COVID-19 complication, is a life-threatening systemic inflammatory syndrome involving the uncontrolled secretion of cytokines, which in severe cases leads to systemic organ failure and death.[1] Especially in the first year of the COVID-19 pandemic, effective treatments for cytokine storm were not available. With the urgent need for treatment options, drug repurposing came to the forefront as it could cut the normal time from drug discovery to registration by more than two thirds. The first step in drug repurposing is compound screening, for which good *in vitro* cell models are necessary.

We present here a novel *in vitro* cell model for evaluating the potential of compounds in decreasing the secretion of cytokines: immortalised B-lymphocytes, known as human lymphoblastoid cell lines (LCLs). They are produced by transfecting isolated donor B lymphocytes with Epstein-Barr virus (EBV).[2] For drug screening we selected a set of compounds based on literature search or our previous research. Empirically, we found they secrete various cytokines, and can for that reason be used as an alternative to an established in vitro model of cytokine release, whole blood.[3] We compared human LCL to an established *in vitro* cytokine release model, whole blood.

Whole blood represents phenotypes of different donors, contains different cell types, but it can only be used for one analysis and repeated sampling is needed. While there already are other commercial *in vitro* cell line models for cytokine secretion (e.g. THP-1, Jurkat), they represent only one phenotype. To cover different phenotypes, we have prepared a biobank of LCLs derived from 71 reconvalescent COVID-19 donors with differing severity of disease. Our results on a selected set of compounds indicate that the trend of cytokine secretion suppression is comparable between whole blood and human LCL. This supports the use of human LCLs as a suitable *in vitro* cell model and as a good personalised medicine platform for cytokine-release related compound screening, since our LCL COVID-19 patients derived biobank accounts for interindividual differences.



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10 Unveiling the Interdomain Dynamics of Type II DNA Topoisomerase Through All-Atom Simulations: Implications for Understanding its Catalytic Cycle

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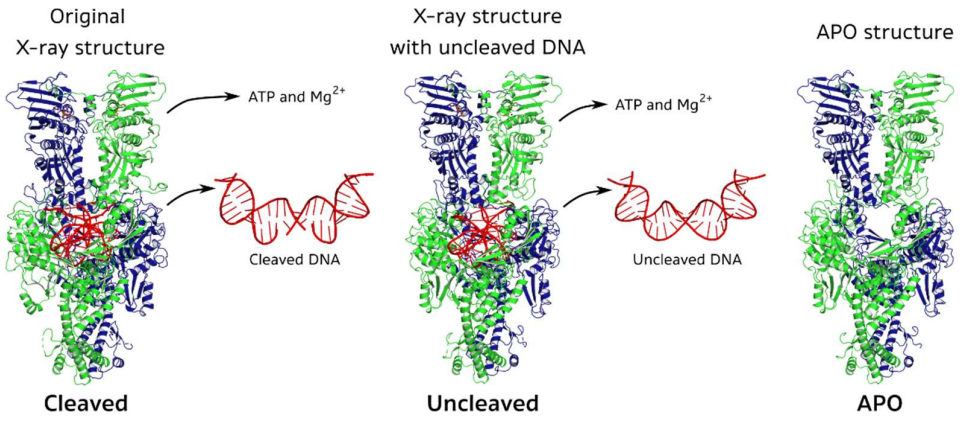
Type II DNA topoisomerases are complex molecular nanomachines that control the topological states of the DNA molecule in the cell and play critical roles in basic cellular processes such as cell division.[1,2] In addition, the human type II topoisomerase α isoform, which is more highly expressed in rapidly proliferating cells, including cancer cells, is considered an established target in cancer chemotherapy.[3]

To better understand the catalytic cycle through which these molecular motors operate, we performed all-atom molecular dynamics simulations. Starting from the available crystal structure of a fully catalytic topoisomerase IIA homodimer from *Saccharomyces cerevisiae*, we constructed three states of this molecular motor primarily changing the configurations of the DNA segment bound in the DNA gate and performed μs-long all-atom molecular simulations. A comprehensive analysis revealed a sliding motion within the DNA gate and a teamwork between the N-gate and DNA gate that may be associated with the necessary molecular events that allow passage of the T-segment of DNA. The observed movement of the ATPase dimer relative to the DNA domain was reflected in different interaction patterns between the K-loops of the transducer domain and the B-A-B form of the bound DNA. Based on the obtained results, we mapped simulated configurations to the structures in the proposed catalytic cycle through which type IIA topoisomerases exert their function and discussed the possible transition events. The results extend our understanding of the mechanism of action of type IIA topoisomerases and provide an atomistic interpretation of some of the observed features of these molecular motors.[4]



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11 Brunner Syndrome Elucidated by Molecular Simulations

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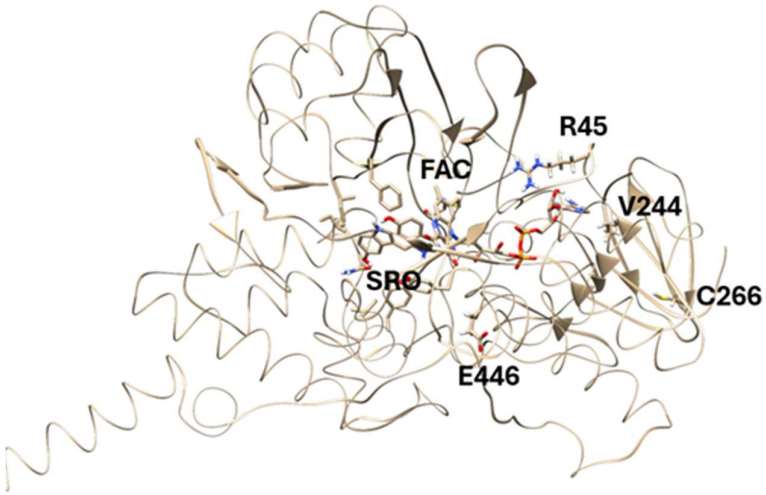
Brunner syndrome is a rare genetic condition known for impulsive aggression and mental disability, associated with genetically driven mutation of the monoamine oxidase A (MAO-A) enzyme. Enzymes are known as biological catalysts and even the slightest change in the amino acid sequence can lead to decreased catalytic performance. For MAO-A the decreased performance results in the slower serotonin metabolism, directly affecting prenatal brain development and leading to neuropsychiatric disorders such as autism, aggression, etc. In line with the hypothesis that catalytic function of enzymes is mainly governed by electrostatic interactions, it can be assumed that the main source of decreased performance of mutants is likely in the changed electrostatic interactions.

In our study, we focus on genetically driven point mutations (E446K, C266F, R45Q, R45W, V244I) of MAO A enzyme, known as Brunner mutants, which have been associated with pathogenic effects, including extreme aggression and intellectual disability. Though comprehensive molecular simulations, including the empirical valence bond approach, we observe a substantial increase in the reaction barriers caused by these mutations, resulting in a significant decrease in serotonin metabolism rates, often equivalent to gene knockout. Moreover, our analysis reveals alterations in electrostatic interactions due to these mutations, with a notable loss of electrostatic stabilization in the transition state. This study provides valuable insights into the molecular mechanisms underlying neuropsychiatric disorders and the employed techniques hold promise for predicting susceptibility to such disorders directly from genomic mutation data. Additionally, the study validates the hypothesis that enzyme catalytic function is rooted in preorganized electrostatics.



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12 Genetic Predictors of Therapeutic and Adverse Side Effects of Rosuvastatin Treatment in Patients After Myocardial Infarction

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Cardiovascular diseases are one of the most common causes of premature death in the developed world. Their occurrence is closely linked to the concentration of LDL cholesterol in the blood, which is primarily reduced by healthy lifestyle habits and statins. Rosuvastatin is considered one of the most effective statins and is commonly used in secondary prevention in individuals with known cardiovascular disease. It successfully lowers the concentration of LDL cholesterol, total cholesterol, and triglycerides, while increasing the concentration of HDL cholesterol. It also has a positive effect on reducing atheroma volume and lowering hs-CRP levels. However, effectiveness varies among individuals. Some of the differences can be attributed to genetic changes, primarily discovered in proteins involved in rosuvastatin transport (SLCO1B1 and ABCG2). The occurrence of side effects during statin treatment poses a significant problem, especially in patients on high doses, so it would be important to discover genetic changes that can lead to higher risk. Many studies have already been conducted in the field of pharmacogenetics of rosuvastatin, and several important polymorphisms that affect intolerance or effectiveness have been discovered. Through next-generation sequencing and microarray genotyping, some new polymorphisms have been identified that could affect statin treatment. However, in none of the studies within our knowledge, have the effects of genetic variations been examined in therapy with exclusively high doses of rosuvastatin in secondary therapy in the Caucasian population.[1,2]

Our research focuses on the field of pharmacogenomics in cardiovascular therapy, particularly in the treatment of post-heart attack patients with rosuvastatin. At the onset, following a heart attack, patients are prescribed rosuvastatin. After 2-month, once the rosuvastatin treatment stabilizes, they are enrolled in rehabilitation, where they undergo regular monitoring for 2 to 3 months. During this period, blood tests are conducted to assess crucial parameters indicative of treatment effectiveness (lipidogram) and markers of intolerance, such as liver and kidney function tests, as well as CK levels. Additionally, plasma concentrations of rosuvastatin are measured, and genetic analysis is performed. Subsequently, a comprehensive statistical analysis will be conducted. By utilizing genetic analyses, we expect to discover genetic changes that may affect concentration of rosuvastatin in plasma, the effectiveness of high-dose rosuvastatin treatment or the occurrence of adverse effects in patients. This will enable more precise identification of patients who may benefit from this medication, as well as those who may experience adverse effects or have an inadequate therapeutic response. Based on the results, we will contribute to the development of more personalized treatment, allowing us to identify patients who may be suitable for other medications (e.g., another statin or biological therapeutics) or dosage adjustments.

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13 Real-Time Insights Into Fluidized Bed Granulation: Utilizing NIR and Raman Spectroscopy for Crystalline Amlodipine Maleate Quantification in Amorphous Solid Dispersion Granulates

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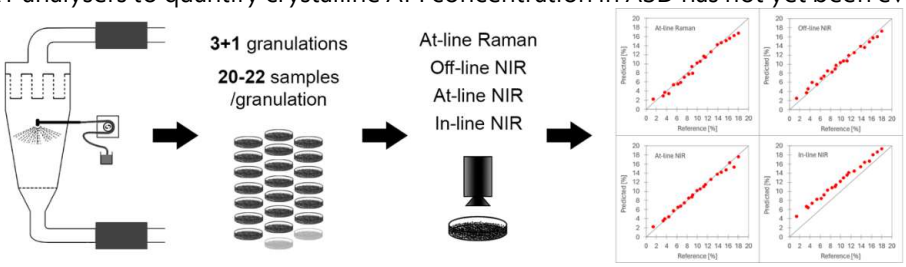
While spray drying and hot melt extrusion dominate discussions regarding the production of amorphous solid dispersions, there is another well-established technology that enables effective industrial-scale production of amorphous solid dispersions (ASDs): fluidized bed granulation (FBG). Active pharmaceutical ingredient (API) and polymer are dissolved together and sprayed onto solid particles, resulting in granules containing ASD. ASDs were developed as a formulation strategy for overcoming API solubility limitations.[1] Any crystalline API residue within the ASDs can have effect on drug bioavailability, making it vital to ensure that formulations contain no crystalline API.[2] Process analytical technology (PAT), introduced by the FDA in 2002, offers a set of tools for real-time process monitoring and quick product analysis inside or at the processing line.[3] NIR and Raman spectroscopy are well-established techniques capable of quantifying crystalline API in ASD in laboratory settings and are also widely used as PAT analysers.[4] However, the ability of NIR and Raman PAT analysers to quantify crystalline API concentration in ASD has not yet been evaluated.



In the current study, four PLS models were developed for quantifying crystalline amlodipine maleate (AM) in ASD granulate produced using FBG. During each of three FBG runs, 20-22 samples were collected. The AM content was determined for all samples using UV-VIS spectrometry. In the next step, a mixture of crystalline AM and polymer in the same ratio as within the granulate was added to the samples to achieve 100% AM content, which equalled 19% of the granulate. Samples spiked with a known concentration of crystalline AM in the granulate (within 0,9% and 18,5%) were analysed using three NIR probes for in-line, at-line, and off-line analysis, in addition to an at-line Raman probe. Using the spectral data, four cross-validated PLS models were developed and validated on an additional granulation. The results revealed that the at-line NIR model performed the best (RMSEP = 0,52 %, RPD = 9,87), followed by the at-line Raman (RMSEP = 0,63%, 8,15), off-line NIR (RMSEP = 0,74%, RPD = 6,93), and in-line NIR (RMSEP = 2,34%, RPD = 2,19) models. According to RPD values, the at-line NIR and Raman models, as well as off-line NIR model, are considered very good, suitable for quantification of crystalline AM in the granulate. These models could be used for quick analysis of final product samples directly at the production site, enabling rapid detection of processing errors (e.g., incomplete dissolution of AM in the granulation liquid). However, the performance of the in-line NIR model is poorer and would require additional experimental work for its improvement.[5]

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14 Deprescribing Proton Pump Inhibitors Among Patients with Lung Cancer

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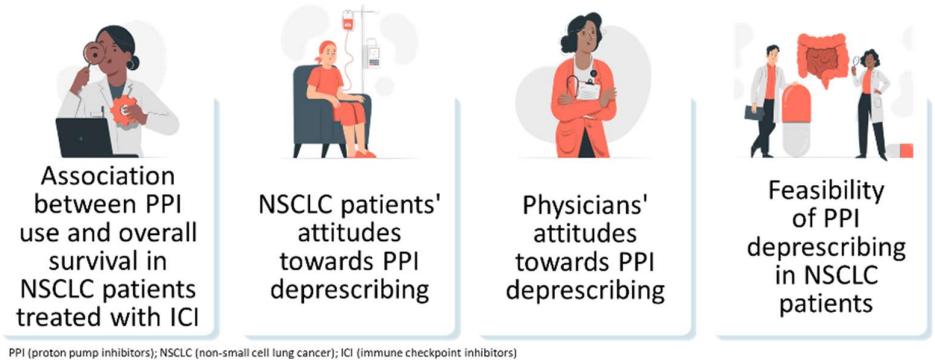
Understanding proton pump inhibitor (PPI) use in patients with lung cancer is critical due to its impact on immune checkpoint inhibitor (ICI) therapy effectiveness, potentially through alterations in gut microbiota.[1,2] Notably, PPI use 30 days before or after ICI initiation has been associated with substantially shorter overall survival in patients with advanced non-small cell lung cancer (NSCLC).[2] The prevalent overuse of PPIs underscores the need for treatment optimization through deprescribing, a planned dose reduction or cessation process of potentially harmful or no longer beneficial medicines.[3] This dissertation aims to enhance understanding of PPI use and its impact on survival outcomes in patients with advanced NSCLC receiving ICI therapy, to investigate the attitudes and perspectives of both patients with lung cancer and physicians towards PPI deprescribing, and develop a tailored PPI deprescribing protocol for this patient cohort for the first time, followed by assessing its feasibility in real-world clinical practice.

Preliminary data from Clinic Golnik reveals that a significant proportion (72.1%; 209/290) of patients with advanced NSCLC treated with ICI from 2015 to 2021 received PPI prescriptions from 30 days before to the completion of ICI therapy. Specifically, 77.0% (161/209) were prescribed PPI within the crucial window of 30 days before or after ICI initiation, and exhibited an average proportion of days covered (PDC) of 70.5%, with over half having access to PPI throughout nearly the entire immunotherapy (55.3% with PDC over 80%). We will further investigate the impact of PPI usage on survival outcomes. In a study involving 120 patients with lung cancer, the majority (89.3% non-PPI users; 100% PPI users) expressed willingness to stop one or more of their medicines if recommended by physician, with 78.6% of PPI users open to stop PPI. Interviews with nine primary care physicians highlighted the need for additional guidance and digital support to facilitate PPI deprescribing, while recognizing the crucial role of clinical pharmacists in this process. This data will guide the creation of a PPI deprescribing protocol for patients with lung cancer. Adopting a comprehensive approach ensures an effective strategy, addressing real-world clinical challenges to enhance patient care, especially for those receiving systemic cancer treatment with ICIs, potentially improving survival outcomes.



PPI (proton pump inhibitors); NSCLC (non-small cell lung cancer); ICI (immune checkpoint inhibitors)

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15 Development and Characterization of Albumin Nanoparticles for Delivery of Drugs with High Affinity for Albumin Binding

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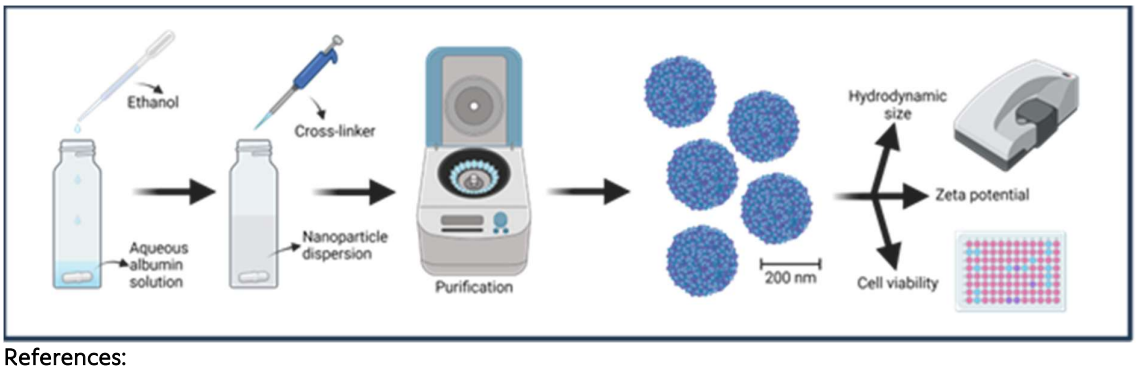
Nanoparticulate drug delivery systems demonstrate enormous potential for efficient drug delivery by active or passive targeting and for achieving prolonged drug circulation times. Amidst rising safety concerns associated with synthetic nanoparticle materials, the exploration of biocompatible materials, such as proteins, has become paramount.[1] In this context, albumin nanoparticles, if prepared from human endogenous proteins, hold many advantages, such as good stability, low toxicity, minimal immunogenicity and potent drug loading capabilities – qualities derived from the natural binding sites within the albumin molecule.[1,2] Such nanoparticles are not only biodegradable and easily purified but also capable of protecting sensitive drug substances due to mild preparation conditions such as coacervation, desolvation, or emulsion techniques.[2]

Our study aimed to refine the coacervation method for preparation albumin nanoparticles for the incorporation of drugs with high affinity for albumin binding and to evaluate their stability and cell compatibility. The nanoparticle preparation was adapted from a previously published procedure by Jithan et al.[3] Our method involved dissolution of bovine serum albumin in purified water, followed by controlled addition of ethanol to precipitate albumin as nanoparticles. Subsequently, the crosslinking phase with glutaraldehyde ensured particle stabilization, while multiple centrifugation cycles allowed the removal of free crosslinking agent and change of dispersion medium. Nanoparticle formation and size were evaluated by dynamic light scattering and their one-month physical stability was determined through zeta potential and size measurements. Thus, we have developed an optimized procedure, which enables the highly reproducible formation of purified albumin nanoparticles with a size of ~220 nm and a polydispersity index < 0.1. The absolute zeta potential values were > 30 mV, indicating good electrostatic stabilization of the prepared formulations. The safety of our nanodelivery system was evaluated *in vitro* utilizing the AlamarBlue cell viability assay on EA.hy926 human endothelial cells. Our preliminary assessments showed no significant cytotoxic effects after short (2 h) and longer (24 h) incubation with produced nanoparticles in concentrations of up to 2 mg/mL. Our findings demonstrate the potential of albumin nanoparticles in drug delivery paradigms. Our future investigations will focus on expanding the applicability of this nanodelivery system for a spectrum of pharmacological compounds with inherent albumin binding affinity, and comprehensive exploration of their efficacy and safety profiles.



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16 Evaluation of the Use of Process Analytical Technology in the Process of Pellet Production Using Extrusion-Spheronization and Film Coating Methods

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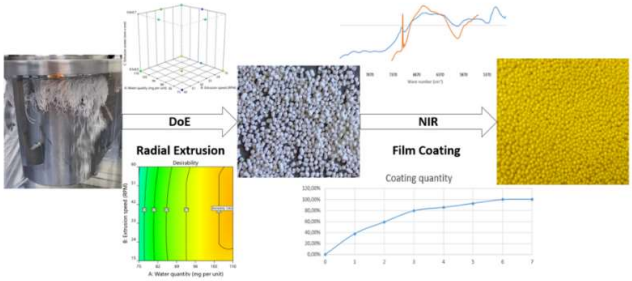
The FDA's initiative Pharmaceutical CGMPs for the 21st century opened the door for the introduction of several risk-based approaches in pharmaceutical industry. One significant advancement is the implementation of process analytical technology (PAT), which has enabled better understanding and control of complex technological processes.[1] Two such processes, radial extrusion and pellet coating, offer a solid foundation for the application of PAT tools due to their numerous critical process parameters.[2]

First part of the concluded study focused on the DoE optimization of the radial extrusion process, aiming to produce pellets with properties desired for further film coating. The desired properties of the pellets, i.e. narrow particle size distribution, high sphericity and high process yield, were successfully achieved. In the second part of the study, pellets were coated in a bottom spray fluid-bed and the coating quantity was predicted in real or near real-time using in-line and at-line NIR probes. The performance of both probes was evaluated. Models for film coating quantity prediction using in-line and at-line NIR probe were successfully calibrated and tested by coating two additional batches. At-line NIR exhibited excellent prediction performance and enabled accurate determination of process end-point. The coating quantity determined by reference method of UV/VIS spectroscopy in both test batches deviated by less than 2.0% from the target value. However, the in-line NIR probe, primarily due to its inferior spectral resolution, displayed a slightly lower quality of the calibrated model and notable coating quantity overprediction for the tested batches.[2] The properties and quality of a product are not solely dependent on its composition, but also on the manufacturing process. Formulations can vary significantly, especially in the initial stages of development, so it cannot be assumed that the same manufacturing process parameters will be suitable for all. Therefore, the future work will be focused on the use of advanced experimental designs for the optimization of radial extrusion-spheronization process. One of more promising approaches is the KCV design, that presents an economical way of optimizing the formulation and process variables in a single study. Due to the complexity of the pellet film coating process, it is advisable to utilize various PAT tools, which enable real-time monitoring of pellet coating and allow for timely and appropriate adjustments in case of deviations. The future work in the field of pellet coating will focus on the simultaneous use of multiple PAT tools such as NIR probe, Raman probe and spatial filtering velocimetry all in combination with multivariate statistical methods to create models for the prediction of coated pellets properties.



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17 Fusidic Acid and Trimethoprim Enhance Cell Death and Cause Cell Cycle Arrest in Folate-Depleted Neuroblastoma Cells

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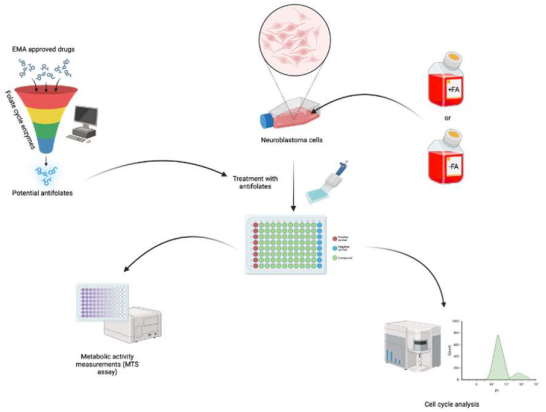
As early neural development is susceptible to changes in folate homeostasis, a reduction in folate levels can lead to neural tube defects.[1] Maintaining adequate folate levels can be affected by numerous factors, including concomitant treatment with drugs that interfere with folate metabolism, known as antifolates.[2] The use of prescribed and over-the-counter medications during pregnancy and childbearing age is increasing.[3] Pharmacotherapy with antifolates may have effects on the folate cycle and may be detrimental to neural development.[1,2] Therefore, we aimed to investigate the antifolate activity of drugs approved by the European Medicines Agency for their effect on the mechanisms of cell proliferation under normal and folate-depleted conditions using *in vitro* cell models for neural development. First, we created a library of all approved drugs (N=2575) and performed a virtual screening for their ability to bind to the folate cycle enzymes of interest, namely DHFR, SLC19A1, MTHFD1, and MTHFR. The »top hits« with the highest binding score in our *in silico* experiment were unanimous among different folate enzymes and comprised: trimethoprim (TMP), dexamethasone, canagliflozin, fusidic acid (FUS), apixaban, vorapaxar, nebivolol, dabigatran, dasabuvir, domperidone, vemurafenib, olmesartan, cefpirome and irinotecan. Next, metabolic activity was measured and cell cycle characteristics of the neuroblastoma cell line SH-SY5Y were analysed after exposure to the identified compounds. Cells were treated under regular and folate-depleted conditions, methotrexate was used as a positive control. We found that FUS and TMP decreased relative metabolic activity, especially in combination with folate-depleted medium (FUS: IC_{50}^{+FA} = 159.8 mM, IC_{50}^{-FA} = 194.8 µM, and TMP: IC_{50}^{+FA} = 18.5 M, IC_{50}^{-FA} = 117.9 µM). This was accompanied by an arrest in the S-phase of the cell cycle in folate-depleted media (p_{FUS} = 0.0001 and p_{TMP} = 0.0008). Regular conditions in the medium showed no significant difference in the cell cycle profile, compared to the control. Our results show the importance of folate supplementation in child-bearing age and during pregnancy, as even supposedly safe drugs can have significantly increased toxicity to neurons in folate-depleted environments.



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18 Prescribing Cascades in the Treatment of Mental *Disorders Across The Lifespan*

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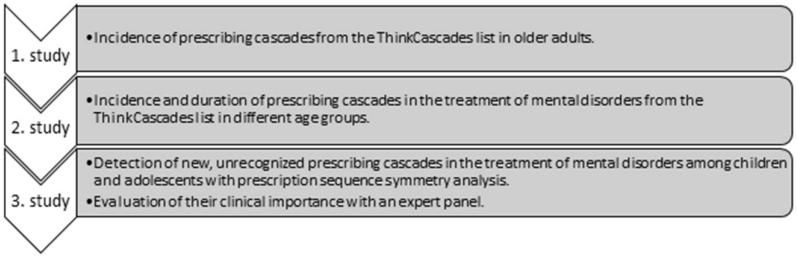
The concept of prescribing cascades, where the adverse effects of medications are misinterpreted as new medical conditions, leading to further unnecessary prescriptions, poses a significant challenge to healthcare systems.[1] This cycle escalates the risk of adverse reactions, drug interactions, and treatment costs. Despite widespread documentation, particularly in cardiovascular and neurological diseases, the occurrence and implications of prescribing cascades in treating mental disorders remain underexplored. Moreover, while there is considerable research focusing on older adults, studies on prescribing cascades among children, adolescents, and even adults are scarce, highlighting a critical gap in our understanding of prescribing cascades in different age groups. [2,3]

We will conduct three national pharmacoepidemiologic studies utilising the Slovenian Outpatient Prescription Medicine Database from the National Institute of Public Health (NIJZ). The first study will examine the incidence of nine clinically important prescribing cascades from the ThinkCascades list among older adults in the year 2017, with a particular focus on assessing whether psychiatric medications contribute to the greatest burden, given that four out of nine cascades are related to treating mental disorders. Our second study will examine the incidence in 2017 and duration of four specific prescribing cascades associated with mental disorders from the ThinkCascades list across different age groups – children, adolescents (0 – 19 years), adults (19 – 64) and older adults (≥ 65 years). In our final study, employing the Prescription Sequence Symmetry Analysis (PSSA) method, one of the most commonly used methods for researching prescribing cascades, we will strive to uncover unrecognised prescribing cascades in treating mental disorders among children and adolescents. Positive signals from the PSSA will undergo further evaluation through a literature review and an expert panel's assessment of their clinical importance. The summary of the research plan is presented in Figure 1.

Our research aims to examine prescribing cascades in the treatment of mental disorders, a significant and increasing burden across all ages. While most published studies target older adults, the impact on children, adolescents, and young adults remains largely unexplored. Our study will be among the first to investigate the incidence and duration of known prescribing cascades across all age groups and identify unrecognised clinically important cascades in children and adolescents. The anticipated findings will enhance the understanding of the impact of prescription cascades across different life stages, which is crucial for improving care for patients with mental disorders.



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19 New Series of Benzamide-Based Mitochondrial Kv1.3 Inhibitors with Anticancer Activity

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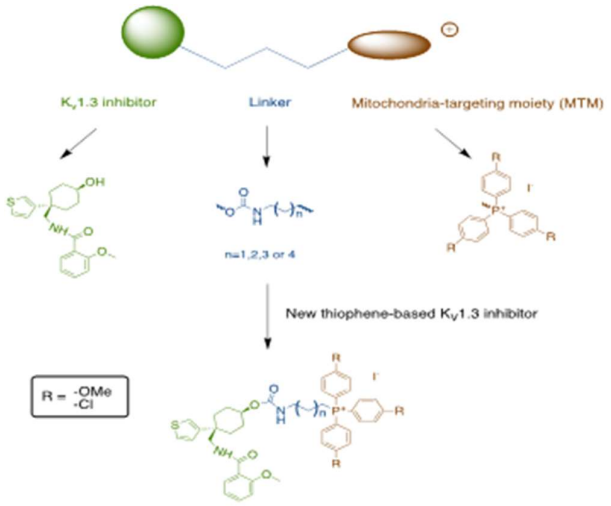
K_V1.3 is a transmembrane protein, expressed in cellular and in mitochondrial membrane, belonging to voltage-gated potassium channel K_V1.x subfamily. K_V1.3 has become an interesting target for anticancer therapy because a correlation between its expression and the development of cancer was demonstrated. It is overexpressed in different types of tumors and its activity is involved in cell proliferation and in the process of apoptosis.[1,2]

The aim of our work is to develop new inhibitors of the mitochondrial K_V1.3 (mitoK_V1.3) channel that would induce the apoptosis of cancer cells. We recently designed, synthetized, and evaluated a new series of benzamide-based mitochondrial K_V1.3 inhibitors[3] composed of the thiophene-based K_V1.3 inhibitor, a lipophilic alkyl linker and different mitochondria-targeting moieties such as cationic triphenylphosphonium group (TPP^+), substituted triphenylphosphonium group or different pyridinium groups.

The anticancer activity of new compounds was evaluated and compared in different cancer cell models in which a significant toxicity and induction of apoptosis was observed. Moreover, the channel inhibition was investigated by patch-clamp electrophysiology and safety in non-cancer cells. Further biological evaluation is currently in progress.



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21 Oxidative Stress-Related Biomarkers as a Potential Tool for Monitoring Inflammatory Bowel Disease Activity: A Systematic Review and Meta-Analysis

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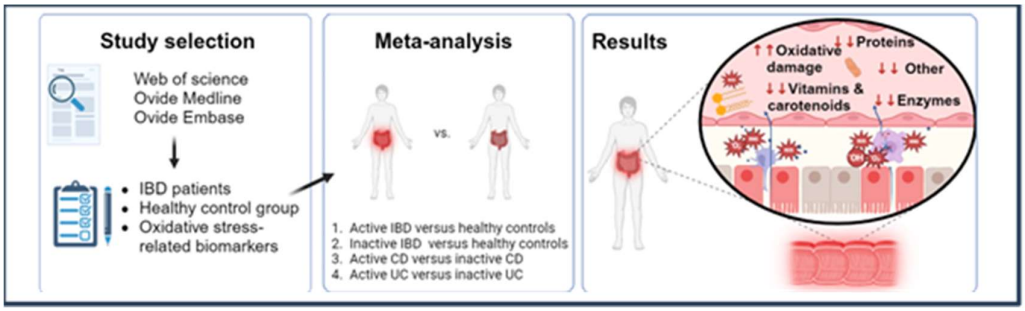
Emerging evidence suggests the involvement of oxidative stress in the pathogenesis of inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC).[1,2] Oxidative stress-related biomarkers offer a novel and minimally invasive approach for detecting underlying inflammation processes, sparing patients from unpleasant endoscopic procedures.[3] This meta-analysis aimed to identify and quantify the IBD-related redox imbalances and their associations with disease activity. Bibliographic databases, including Ovid Medline, Ovid Embase, and Web of Science, were systematically searched for relevant studies assessing blood levels of oxidative stress-related biomarkers in both individuals with IBD and healthy controls. Standardized mean differences (SMDs) with 95% confidence intervals were calculated for each biomarker in the following comparisons: (I) active IBD versus healthy controls; (II) inactive IBD versus healthy controls; (III) active CD versus inactive CD; and (IV) active UC versus inactive UC.

A total of 52 studies were included in the meta-analysis, revealing the accumulation of oxidative damage to biomacromolecules and reductions in various antioxidants among both active and inactive IBD patients compared to healthy controls. Additionally, our study identified biomarkers differentiating between active and inactive CD, including malondialdehyde (SMD: 0.85), paraoxonase-1 (SMD: -1.20), catalase (SMD: -0.50), albumin (SMD: -1.00), transferrin (SMD: -0.58), and total antioxidant capacity (SMD: -0.55). Similarly, levels of paraoxonase-1 (SMD: -0.96), erythrocyte glutathione peroxidase (SMD: -0.78), catalase (SMD: -0.65), albumin (SMD: -0.74), transferrin (SMD: -0.72), and free thiols (SMD: -0.76) differed significantly between active and inactive UC patients. Vitamins and carotenoids also emerged as potential activity biomarkers for both CD and UC, however, their intake should be closely monitored to obtain meaningful results.

Our findings highlight the potential of oxidative stress-related biomarkers as an additive tool for monitoring IBD activity, offering valuable insights into disease management and improving patient care.



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22 Unveiling Platelet Power: Exploring Proteasome Subunits for Insights into Platelet Function

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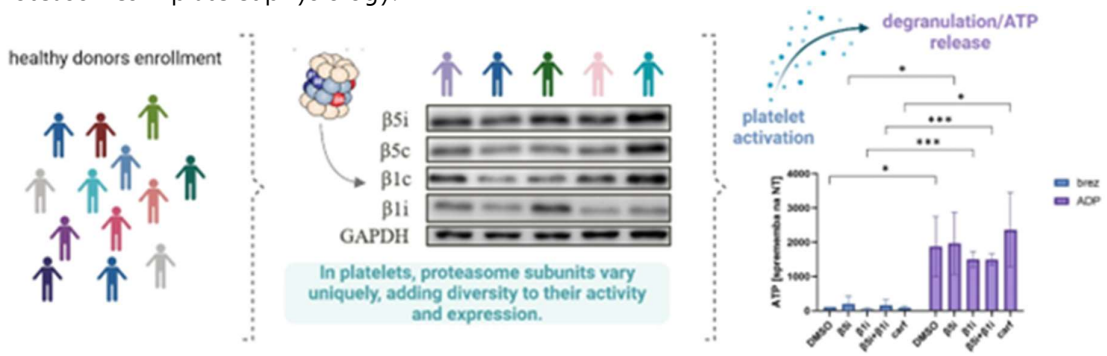
Platelets, which are essential for hemostasis, are also involved in various processes such as inflammation, metastasis, and tumor proliferation, which is promoted by numerous immune-related molecules within them.[1] Recent research indicates the presence of active proteasome and immunoproteasome subunits in platelets. Proteasomes are enzyme complexes responsible for intracellular proteolysis, that includes catalytic subunits (β1, β2, β5).[2] Immunoproteasomes, a specialized form, replace these with cytokine-inducible homologs (β1i, β2i, and β5i) [2,3]. Proteasomes play a central role in the processing of MHC class I antigens and contribute significantly to the shaping of both innate and adaptive immune responses by regulating cytokine production, differentiation, survival, and proliferation of immune cells.[3] However, the exact involvement of (immuno)proteasomes in platelet function and their potential influence on the microenvironment remain unclear.

In our study, differences in the expression of the catalytically active proteasome and immunoproteasome subunits β1, β2, and β5, as well as variations in their activity, were observed in platelets from healthy donors. Moreover, preliminary results suggest that specific proteasome inhibitors may influence signaling pathways within platelets and regulate the release of chemokines after degranulation induced by specific platelet aggregation activators such as ADP and TRAP-6. Following the degranulation process, differences in RANTES chemokine release were detected after inhibition of the β1i and β5i subunits, while secretion levels of IL-8, MIG, MCP-1, and IP-10 were unaffected.

The expression and activity of catalytically active proteasome and immunoproteasome subunits in platelets show interindividual differences between donors. Modulation of these subunits by selective inhibitors leads to altered *in vitro* platelet function, which affects aggregation and chemokine release. Nevertheless, further studies are needed to elucidate the indispensable role of proteasomes in platelet physiology.



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23 Gold Nanoparticles in Biomedicine: Synthesis Strategies for the Shape, Size, and Surface Modification of Nanoparticles

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In recent years, there has been significant advancement in the synthesis of metal nanoparticles (NP), expanding their potential applications across diverse fields such as healthcare research, microelectronics, and food packaging. Among the various nanotechnology approaches in medicine, gold nanomaterials stand out as particularly promising due to their unique optical and physical properties, including surface plasmon resonance. Gold NPs can be functionalized and conjugated with a wide range of molecules, including polymers, surfactants, ligands, dendrimers, drugs, DNA, RNA, proteins, peptides, and oligonucleotides.[1] In medical research, gold nanoparticles offer distinctive features that can be used in various areas, including electroporation, drug delivery, hyperthermia and more.

To explore these potential applications, we synthesized gold NP of varying sizes and shapes. Initially, we adapted the citrate reduction method described by Turkevich in 1951 to produce spherical gold NP.[2] By reducing chloroauric acid with sodium citrate, we successfully synthesized NP with an average diameter from 10 to 130 nm. These NPs were further functionalized with thiol-terminated polyethylene glycol. Additionally, we synthesized gold nanorods with different aspect ratios using the seed-mediated method, which was first described by Nikoobakht and El-Sayed in 2003.[3] This synthesis involved the initial generation of gold nano-seeds, followed by their introduction into a growth solution containing a gold precursor, a surfactant, and a mild reducing agent. In our experiments, we used hexadecyltrimethylammonium bromide (CTAB) as both a surfactant and a stabilizing agent. However, due to its cytotoxicity, CTAB must be replaced with a more biocompatible stabilizer for biomedical applications. Therefore, we conducted additional syntheses to produce biocompatible nanorods by substituting CTAB with thiol-terminated polyethylene glycol as an alternative stabilizer after the initial synthesis. Dynamic light scattering and transmission electron microscopy (TEM) were used to characterize colloidal stability and morphological aspect of AuNPs.

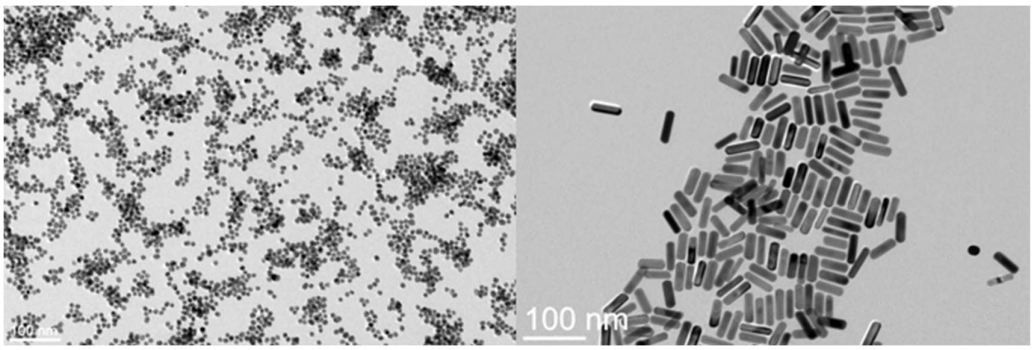

Figure 1: TEM image of spherical gold nanoparticles (left) and gold nanorods (right).

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24 Introducing PROTAKs: First-in-Class TAK1 PROTACs for Targeted Cancer Therapy

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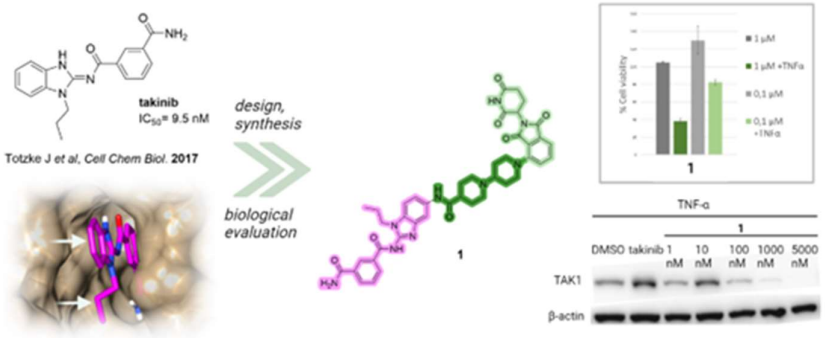
TAK1 kinase (transforming growth factor-β-activated kinase 1) is a member of the protein kinase kinase kinase (MAP3K) family.[1] It is involved in intricate signaling networks, including the tumor necrosis factor-alpha (TNF-α) signaling cascade, the interleukin-1 (IL-1) pathway, the toll-like receptor (TLR) pathway and the transforming growth factor-β (TGF-β) pathway, which makes TAK1 an important regulator of cell proliferation, cell differentiation, apoptosis and immune response. Dysregulation of TAK1 is associated with the onset and progression of various cancers and autoimmune diseases. In 2017, the first selective and potent inhibitor takinib was reported by Totzke et al.[2]

Over the last ten years, there has been remarkable progress in the field of small molecules as modulators of pharmacologically important targets. Such example are PROTAC molecules (proteolysis targeting chimeras) that utilize the ubiquitin-proteasome system to degrade the target instead of merely inhibiting it. These heterobifunctional compounds consist of a target protein binder, a compatible linker and a ligand for the E3 ligase.[3]

We report the design, synthesis and biological evaluation of nineteen PROTACs using takinib as a ligand for TAK1 together with different linkers and ligands for the E3 ligases VHL, CRBN and IAP. The first series of PROTACs had long, flexible linkers connected to takinib at two different anchoring points. All synthesized PROTACs retained high inhibition of TAK1, with IC_{50} values ranging from 3 nM to 1 μM. In the first series of compounds, the IAP-hijacking PROTACs induced TAK1 degradation at a concentration of 1 μM in MDA-MB-231 cells. In the second series of compounds, we introduced rigid linkers, leading to the development of compound **1**, a CRBN-hijacking PROTAC. Remarkably, **1** showed effective depletion of TAK1 at 1 μM in both MDA-MB-231 and THP-1 cells, as well as a significant effect on the viability of TNF-α stimulated THP-1 cells. TNF-α-dependent degradation of TAK1 and apoptosis represent a promising approach for the treatment of various inflammation-related cancers.



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25 Micro-Managing Osteoporosis: Unraveling Molecular Secrets for Unbreakable Bones

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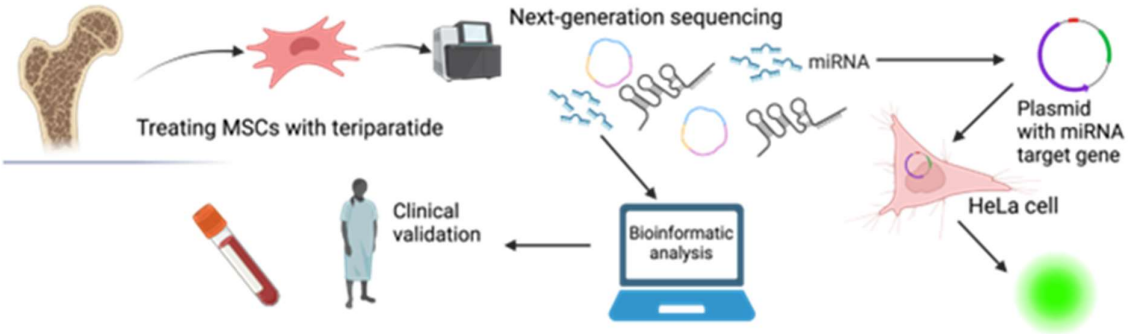
Osteoporosis, the most common metabolic bone disease, is characterized by low bone mass and weakened bone microarchitecture, increasing fracture risk. It arises from an imbalance between bone resorption by osteoclasts and formation by osteoblasts. The condition poses a significant health burden, leading to reduced quality of life, elevated mortality rates, and substantial treatment costs. Available treatments include antiresorptives like bisphosphonates and denosumab, which can inhibit bone resorption but may have adverse effects. Osteoanabolic agents like teriparatide offer promising alternatives, but their efficacy varies among individuals. Despite effective antiresorptive treatments, efforts to find anabolic therapies continue.[1,2]

Epigenetic factors, including non-coding RNAs (ncRNAs) like micro RNAs (miRNA), are increasingly recognised to contribute significantly to osteoporosis and may affect treatment response. Understanding signaling pathways they regulate during osteoblastogenesis is crucial for improved diagnostics and novel anabolic treatment approaches. Limited studies have investigated miRNAs in patients, particularly in response to teriparatide treatment. *In vitro* studies on teriparatide have shown promising results, but comprehensive analyses of ncRNAs in treated cells are lacking.[1,3]

Our study aims to fill this gap by using whole-genome sequencing and bioinformatic analysis to identify RNA transcript differences between teriparatide-treated and untreated mesenchymal stem cells (MSC). This will uncover new RNA molecules associated with teriparatide's action and bone remodeling. Our objectives include discovering ncRNAs linked to teriparatide's effects on MSC cell differentiation *in vitro* and identifying key miRNAs associated with its osteoanabolic effects. Through experimental validation of miRNA-mRNA interactions using luciferase reporter assays, we aim to elucidate the molecular mechanisms underlying teriparatide's effects. Ultimately, our research aims to provide novel insights into the molecular pathways involved in teriparatide's action, potentially leading to improved osteoporosis treatments and possible markers of treatment efficacy.



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26 Optimization of NOD2 Agonistic Activity Through Acylation of Desmuramylpeptides

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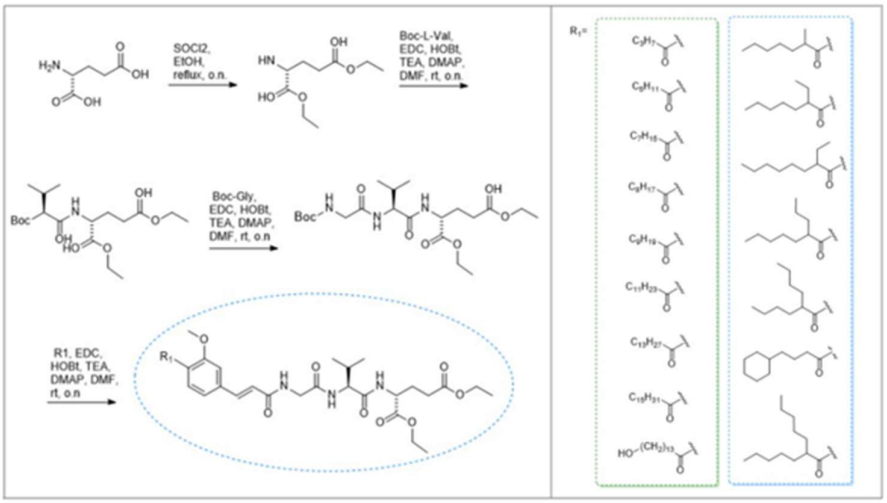
The innate immune receptor nucleotide-binding oligomerization domain-containing protein 2 (NOD2) represents a promising target for modulating immune responses. Its ligands have potential applications as vaccine adjuvants or immunostimulatory agents. Muramyl dipeptide (MDP), a fragment of bacterial peptidoglycan, activates NOD2, promoting both innate and adaptive immunity. However, the clinical use of MDP is hindered by its strong pyrogenicity, rapid clearance, and metabolic instability. In previous studies, the MDP analogue SG8, which substitutes the N-acetylmuramic acid moiety with a trans-feruloyl-glycine moiety, showed potent NOD2 stimulatory activity in the mid nanomolar range. However, insufficient membrane penetration and poor liposomal encapsulation limited its in vivo efficacy. To address these issues, we introduced lipophilic acyl groups to the trans-feruloyl portion of SG8 to enhance membrane permeability and possibly liposomal encapsulation as well as fine-tune the NOD2 agonistic activity.

The synthesized desmuramylpeptides were assessed for their NOD2 activity using the HEK-blue NOD2 reporter cell line in a dose-dependent manner. The butyryl-tail analog emerged as the most potent agonist, with an EC50 of 4 nM, exhibiting a more than 20-fold improvement over SG8. Interestingly, NOD2 activity dropped with longer aliphatic chain lengths and increased chain branching. Additionally, introducing a hydroxyl group or a double bond into the alkyl side chain enhanced NOD2 agonistic activity compared to unsubstituted derivatives. These findings contribute to our understanding of the structure-activity relationship of desmuramylpeptide NOD2 agonists.



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27 Tailoring Polymeric Nanofiber Formulations with Live Therapeutic Bacteria to Improve Bacterial Preservation and Modify Release

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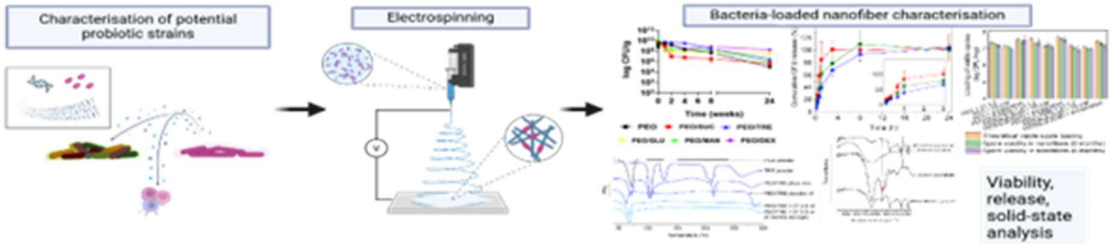
Live bacteria are widely used in biomedical applications, including as therapeutic entities that can be utilized under the scope of either probiotic products or their more strictly regulated counterpart – live biotherapeutic products.[1] While electrospun nanofibers represent a promising approach for immobilization of live bacteria due to the possibility for simultaneous drying and preparation of a bacterial delivery system, electrospinning is often associated with significant reductions of bacterial viability.[2,3] We developed multiple hydrophilic nanofiber formulations incorporating various potentially probiotic species to address two different key aspects of probiotic delivery system design: improved preservation and controlled release of bacteria.

Vegetative bacteria belonging to various genera were incorporated into poly(ethylene oxide) (PEO)-based nanofibers with different excipients and the stabilizing effects of the latter on bacterial viability during processing and storage were evaluated. We showed that addition of saccharides improved preservation of vegetative bacteria in nanofibers during electrospinning and/or storage in a species-dependent manner. Crystallization of semi-crystalline excipients during storage was associated with a trend of accelerated bacterial death, demonstrating that crystallization-prone excipients should be considered critically. Conversely, preservation of bacteria with sporulating ability is less challenging which we showed in a separate study, wherein spores of two *Bacillus* strains were incorporated into PEO-based nanofibers with different proportions of alginate. Bacterial viability was completely preserved during 6 months of storage. Bacterial release from nanofiber mats was evaluated and PEO/alginate mats underwent swelling, thus prolonging spore release with alginate acting as a possible release-modifying excipient as a trend of slower release kinetics was observed at higher alginate proportions. Such swellable delivery systems can be employed to prevent dose dumping and immediate probiotic washout. Moreover, to present a comprehensive work-flow, the studied *Bacillus* strains were genotyped and their probiotic potential for use in periodontitis treatment evaluated in *in vitro* studies of antibacterial and immunomodulatory activity.

Our studies show both the potential of nanofibers as a local delivery system for probiotics as well as the need for optimization and tailoring of formulations for specific strains or a desired effect (i.e., controlled release).



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28 Subunit Antigen-Decorated Filamentous Bacteriophage as a Modular Vaccine Platform

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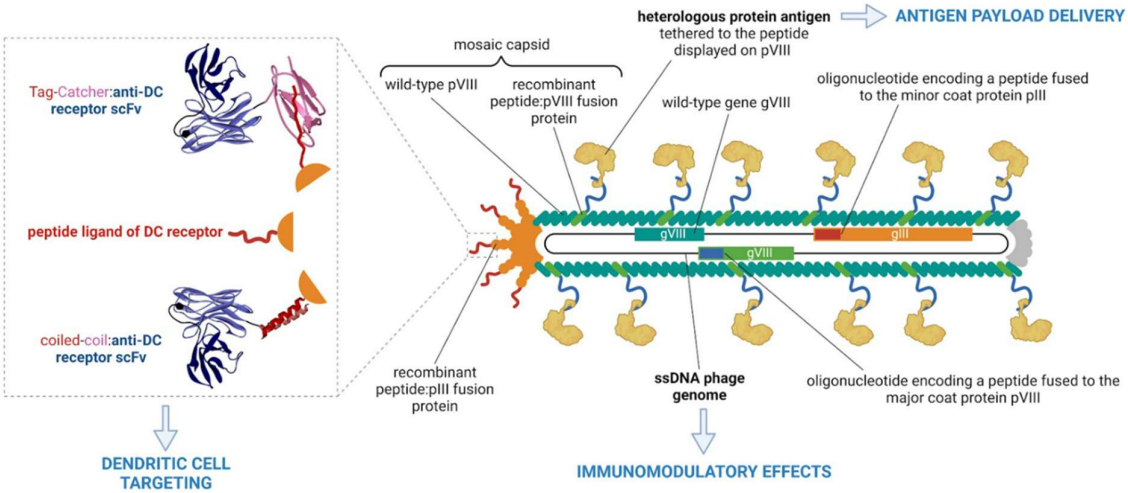
Vaccination is one of the essential and most cost-effective public health interventions. A general robust vaccine platform capable of triggering a broad range of immune responses is considered the holy grail of vaccinology. We propose to exploit the high valency heterologous peptide display capacity and unique immunomodulatory properties of filamentous bacteriophage to design a modular vaccine scaffold with adjuvant properties (Fig. 1). We designed a hybrid phage vector capable of simultaneously displaying foreign peptides on pIII and pVIII capsid proteins. Those on pVIII are exploited to tether recombinant subunit protein antigens, while peptides fused to pIII are used to target dendritic cells with the goal of further potentiating antibody and cellular immune responses.

Specifically, we have displayed the SpyTag peptide fused to the pVIII and monitored display levels with ELISA. Different signal peptides for periplasmic transport and SpyTag variants were combined to optimize display valency. Display was further improved by gentle induction of the recombinant SpyTag-pVIII fusion gene. Finally, a model antigen (GFP) was expressed in *E. coli* fused to the SpyCatcher protein. The SpyTag/SpyCatcher split protein spontaneously assembles and forms an isopeptide bond.[1] We have verified that GFP is tethered to the phage capsid by immunoblotting. Dendritic cells can be targeted via a number of endocytotic receptors.[2] As proof of concept, we have shown by phage ELISA that a nanobody against Clec9a, expressed as a fusion with SnoopCatcher and conjugated to bacteriophage surface through pIII protein displaying SnoopTag [3], enables phage binding to recombinant Clec9a.



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29 First-In-Class Nanomolar Inhibitors of Galectin-8 N-Terminal Domain

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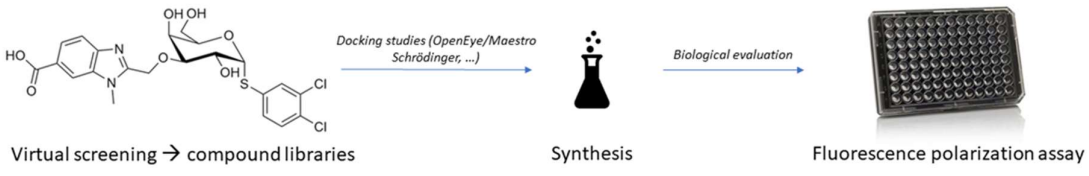
Galectins are carbohydrate-binding lectins with a high affinity for β-galactosides.[1] They are involved in many cell processes, such as cell adhesion, migration, proliferation and differentiation and are involved in a wide range of diseases, such as fibrosis, cancer and heart disease.[1,2] The galectin family has 16 members, which can be classified into three groups: a) prototype galectins (galectin-1, -2, -5, -7, -10, -11, -13, -14, -15, -16) associated as homodimers with a single CRD; b) chimera-type galectins (galectin-3) with only one CRD, which can form oligomers upon binding to bivalent/multivalent glycoconjugates; and c) tandem-repeat galectins (galectin-4, -6, -8, -9, -12) with two homologous, but non-identical CRDs linked by a peptide linker (the length of 5-50 amino acids).[3] Galectin-8, a member of tandem-repeat group of galectins, is present in both primary and secondary lymphoid organs. It plays an important role in both innate and adaptive immune response. The most interesting role of galectin-8 is the regulation of cancer growth and metastasis. Since high affinity selective ligands are known only for galectin-3, we decided to design and synthesize selective ligands that bind to the N-terminal domain of galectin-8. *N*-methylbenzimidazole-galactoside ligand[2] (further referred to as ligand **1**), synthesized by Hassan M. *et al.*, is the most potent selective galectin-8N ligand up to date with a K_d of 1,8 μM. In our quest for selective and potent galectin-8 ligands, we prepared different compound libraries using a free and open-source KNIME synthetic protocol Workflow starting from the ligand **1** as a core structure. Chemical compound libraries were prepared using aliphatic halogenides, azides and consequently triazoles as moieties that were virtually attached to the solvent-exposed position 2 in the galactopyranoside structure to improve the K_d values and reach selectivity over galectin-3. Since X-ray crystal structures of galectin-8N with the co-crystalized ligands are known, all the compounds from chemical libraries were docked to the chosen galectin-8N crystal structure (PDB ID: 7AEN) to explore the possibility of substituents to make favourable contact with protein surface and help select candidate for synthesis and biochemical evaluation. From each chemical compound library, 40 top ranked/scored compounds (according to the FRED Chemgauss4 score values) were chosen and analysed via TPSA and logP calculations. Thresholds for compounds that are suitable for synthesis were set to TPSA < 200 Å^2 and logP < 5 (6). Compounds were tested for their binding affinities against galectin-1, -3, -8N, and –8C. The binding properties of our most potent galectin-8N inhibitors were determined with ITC to obtain their thermodynamic fingerprint. Importantly, we obtained a crystal structure of selected galectin-8 inhibitor in complex with the protein, which helped us to decipher the interaction nature of substituents at position 2.



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30 Transcriptomics Reveals Dysregulated Pathways in Severe Preeclampsia

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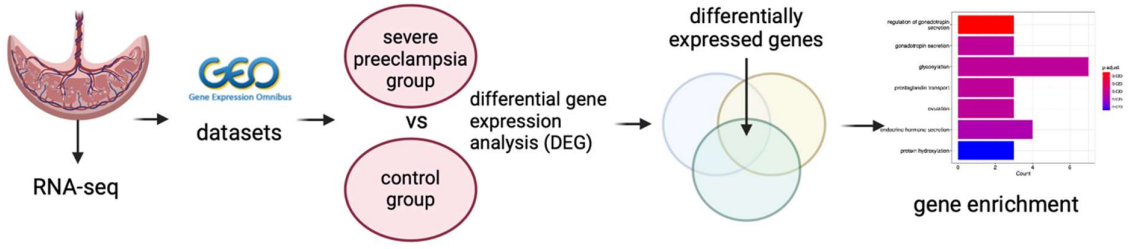
Preeclampsia, among the most severe complications during pregnancy, affects 2 - 8 % of all pregnant women worldwide. It is characterized by the sudden onset of hypertension after 20 weeks of gestation along with other complications, for instance, proteinuria, maternal organs, or uteroplacental dysfunction. The effect of this complex disease can be seen in reduced life expectancy with increased risk of stroke, cardiovascular disease, and diabetes in women who survived preeclampsia. It also has an effect on newborns leading to preterm births, neurodevelopmental delay, cardiovascular and metabolic diseases later in life and even perinatal death.[1] Extensive research is underway to understand the pathophysiology of the disease, yet a definitive explanation remains uncertain.[2] Therefore, effective pharmacological interventions continue to be deficient in preventing and reducing severity of disease. To address this issue, employing more sophisticated and advanced approaches, particularly those involving omic technologies is crucial. The number of transcriptomic studies in this field is constantly increasing; however, studies on severe preeclampsia are down-numbered, and usually done in smaller sample sizes.

For this study we searched and selected placental RNA sequencing datasets relevant to severe preeclampsia and matching controls from the NCBI GEO database.[3] Differential gene expression analysis was conducted using the DESeq2 package and Venn Diagram in RStudio. The results revealed differentially expressed genes specific to severe preeclampsia compared to placental samples from healthy pregnant women. Additionally, gene enrichment analysis was performed using KEGG, GO and REACTOME databases. Upregulation of genes was observed in AMPK signaling, glucagon signaling, gonadotropin secretion and extracellular matrix organization. While downregulation was noted in metabolic pathways and efferocytosis. These findings not only enhance our understanding of the pathogenesis of this complex disease but also offer provide potential targets for more effective diagnostic and therapeutic ultimately improving outcomes for both mothers and their babies.



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31 Investigating Proline Analogues as Viscosity-Reducing Excipients in Monoclonal Antibody Formulations for Subcutaneous Administration

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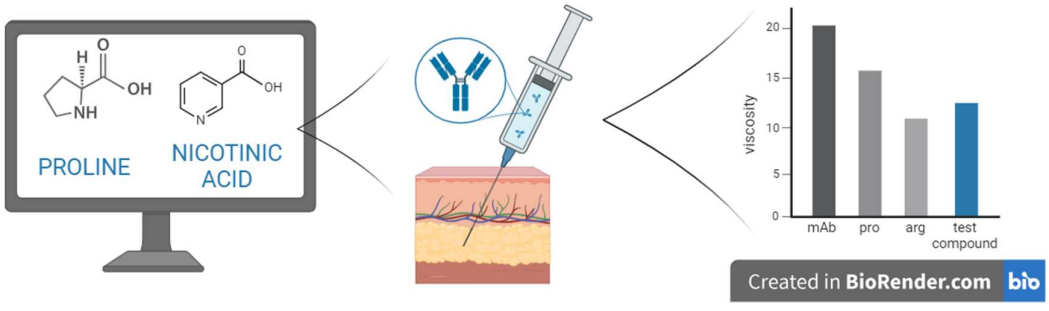
Monoclonal antibodies (mAbs) are the leading class of biopharmaceuticals due to their high specificity. Many mAbs are being developed for subcutaneous (SC) administration, which is less painful, faster, and more cost-effective than intravenous administration. However, SC administration is limited by the small injection volume (≤2 mL) and therefore high concentrations (>100 mg/ml) of mAbs are required. Protein-protein interactions forming in such formulations can increase the viscosity of the solution, hindering the manufacturing and injection. Commonly, viscosity-reducing excipients such as proline and arginine are added to inhibit these interactions, but they are not necessarily generally applicable.[1]

Investigation of novel proline and nicotinic acid analogues as potential viscosity-reducing excipients was based on viscosity measurement and physical stability analysis. Initially, chemical space of proline was explored and test compounds were selected. Then, mAb solutions containing the test compounds (25 mM) were prepared and their viscosity was measured with a viscometer-rheometer on a chip (m-VROC, RheoSense, USA) at 25 °C. In addition, size exclusion chromatography (SEC) was used to assess mAb physical stability. Next, commonly used excipients (sucrose, histidine, polysorbate 80) were added to mAb solutions and the effect of model viscosity-reducing excipients proline and arginine at 25 and 200 mM concentration was evaluated.

Several test compounds effectively reduced the viscosity of the model mAb aqueous solution by more than 20 %, without promoting mAb aggregation. Besides electrostatic interactions, hydrophobic and aromatic interactions probably contributed to the viscosity-reducing effect of these compounds. The mAb formulation containing histidine buffer, sucrose, and polysorbate 80 exhibited higher viscosity compared to an aqueous solution, likely due to increased solute concentration.[2] The addition of proline and arginine reduced formulation viscosity. Specifically, the viscosity-reducing effect of arginine was concentration-dependent, while with proline the reduction of viscosity was less efficient and not concentration-dependent, which is possibly the result of different interactions that each of the amino acids forms with the mAb. In conclusion, we discovered several new viscosity-reducing excipients and lay foundation for further investigation, including study of viscosity-reducing effect of the compounds in combination with other excipients.



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32 Development Of Chimeric Innate Immune Agonists As Vaccine Adjuvants

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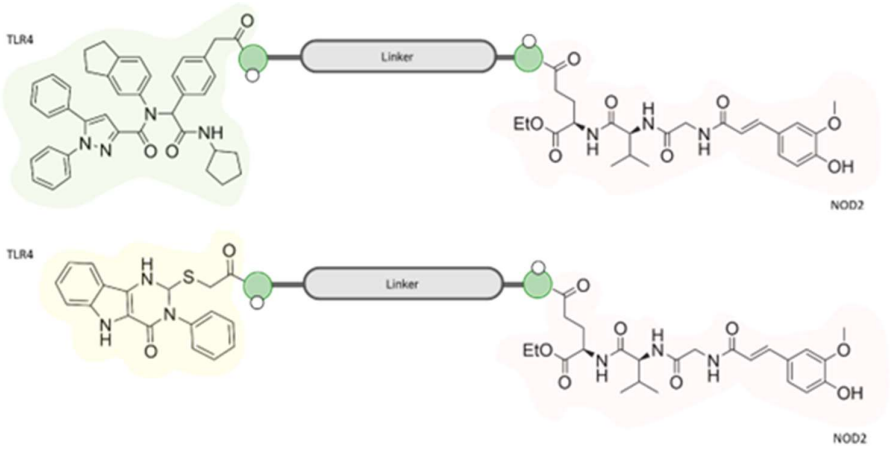
Vaccines have revolutionized public health by substantially diminishing morbidity and eradicating once-prevalent diseases. While recombinant antigen-based vaccines offer enhanced safety profiles over live attenuated vaccines, their weaker immunogenicity necessitates the incorporation of adjuvants to bolster immune responses[1]. This presentation will explore the pivotal role of pattern recognition receptors (PRRs) in shaping both innate and adaptive immunity, highlighting their significance in vaccine adjuvant development. Synthetic PRR agonists, mimicking pathogen-associated molecular patterns (PAMPs), have emerged as promising adjuvants due to their ability to orchestrate robust and tailored immune responses. Co-administration of multiple PRR agonists demonstrates synergistic signal amplification, akin to the immunogenicity of live attenuated vaccines[2]. Rational targeting of specific PRR combinations enables fine-tuning of immune responses, optimizing protection against diverse pathogens. Furthermore, covalent conjugation of multiple PRR agonists enhances immunomodulatory activity, ensuring precise delivery and potentiation within the same cell. This strategy not only improves immune responses but also enables dose-sparing, enhancing adjuvant safety profiles[3]. Thus, the design of dual agonists holds immense potential in augmenting vaccine efficacy and advancing global health initiatives.



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33 Intoxication/Prescribing Ratio and Trend Correlation: Quantitative Comparison of the Risk For Intoxication with Psychotropic Prescription Drugs

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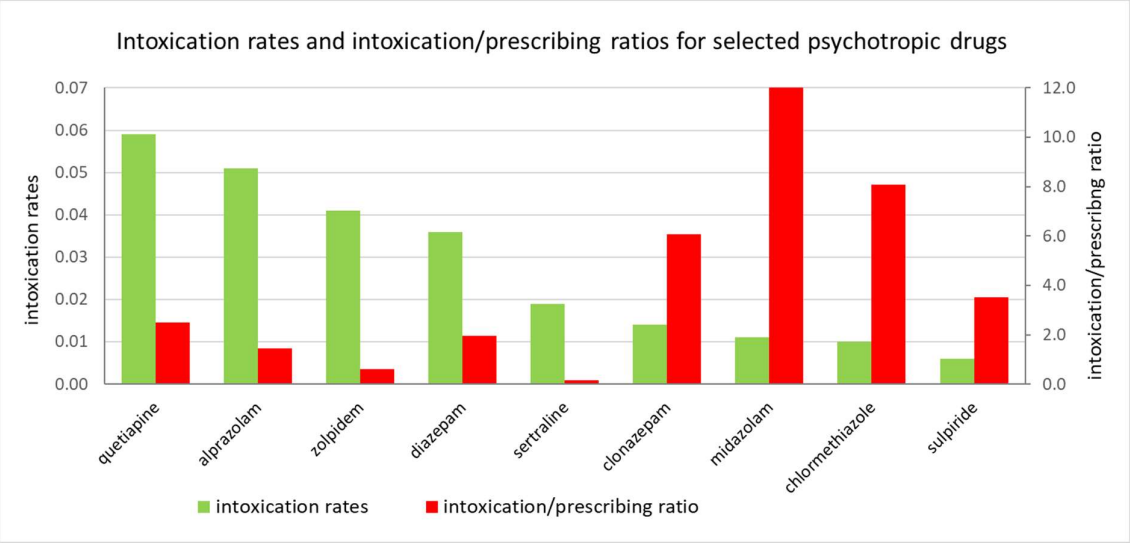
Prescription psychotropic drugs represent 70-80% of medication-related intoxication cases and an increasing cause of hospitalization in this context.[1,2] We conducted a 7-year nationwide observational study in Slovenian adults to quantitatively determine the association and trend correlation between the prescribing and intoxication rates for individual prescription psychotropic drugs. Anticonvulsive, antipsychotic, antidepressant, anxiolytic, and hypnotic/sedative drug groups were included. Intoxication/prescribing ratio values were calculated, and time correlation between trends in prescribing and intoxication rates was assessed using Pearson correlation coefficient.

Anxiolytics and antipsychotics presented the majority of the intoxication cases. As individual drugs, midazolam, chlormethiazole, clonazepam, sulpiride and quetiapine demonstrated the highest intoxication/prescribing ratio values, presenting the highest risk for intoxication. The best correlation was found for the prescribing period of two years before the intoxication events, suggesting that stockpiling unused psychotropic drugs may be a relevant factor leading to intoxications. Intoxication/prescribing ratio provided a useful and comprehensive tool for a quantitative comparison of the risk for intoxication in relation to the prescribing rates for prescription psychotropic drugs.



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34 Exploring Signal Transduction Through the NKG2A/CD94 Receptor with Molecular Dynamics Simulations

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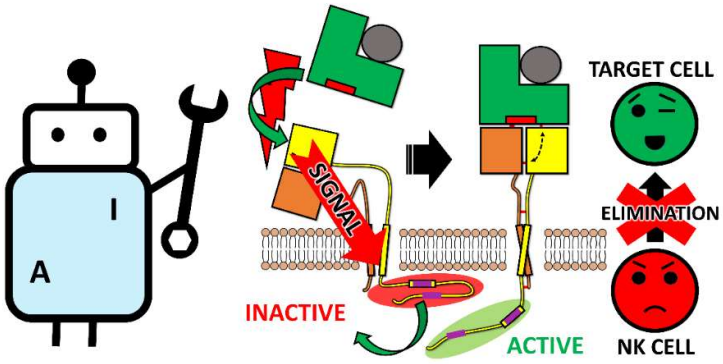
Natural killer cells (NK cells) play an important role in the innate immune response against tumours and various pathogens such as viruses and bacteria. Their function is controlled by a wide range of activating and inhibitory receptors expressed on their cell surface. The dimeric NKG2A/CD94 inhibitory transmembrane receptor binds specifically to the non-classical MHC I molecule HLA-E, which is frequently overexpressed on the surface of senescent and tumour cells. Inhibition of cytotoxic activity by NKG2A occurs via a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM), which can be phosphorylated upon binding to HLA-E. The modulation of this interaction represents a promising therapeutic strategy to combat age-related diseases and cancer.

Using Alphafold, an artificial intelligence program, we constructed the missing segments of the NKG2A/CD94 receptor and generated its complete 3D structure. This served as a starting point for the multi-microsecond all-atom molecular dynamics simulations of the receptor with and without the bound HLA-E ligand and its nonameric peptide. The simulated models showed that a complicated interplay of events takes place between the extracellular and transmembrane regions. These include changes in the network of hydrogen bonds and the positioning of the extracellular region of the NKG2A/CD94 receptor, which lead to a reorganisation of the linker and a repositioning of the transmembrane helix. Ultimately, this affects the intracellular ITIM regions, which harbour the point at which the signal is transmitted further in the inhibitory signalling cascade after binding of HLA-E, by changing their positioning and increasing their exposure to the solvent.

Understanding the atomistic details of the cells' protective mechanism against NK cells and expanding our knowledge of transmembrane signalling of ITIM-bearing receptors may lead to subsequent structural and biochemical experiments leading to new therapeutic approaches for the treatment of various pathologies such as viral infections, cancer and senescent cells.



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35 Dual Inhibitors Targeting BChe And P38α MAPK: A Novel Strategy for Alzheimer's Disease Therapy

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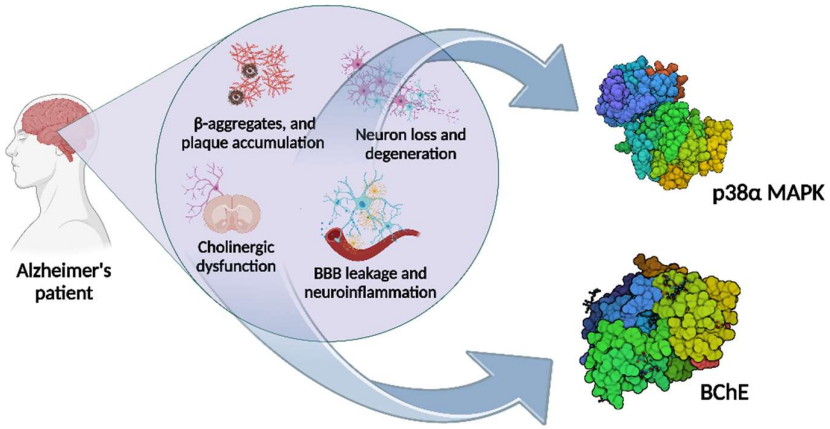
Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia. Current therapeutic options for AD are limited, and restricted to mild-to-moderate dementia, with adverse effects hindering the progress of novel biological drugs in clinical trials. Developing innovative small-molecule drugs targeting key proteins involved in the early pathophysiology of AD therefore presents a daunting task for medicinal chemists.[1] The neuroinflammation hypothesis is extensively studied in AD pathophysiology, suggesting that amyloid beta plaques alongside phosphorylated tau proteins lead to hyperactivated microglia, triggering the expression of various enzymes that exacerbate the imbalance between anti- and proinflammatory cytokines. Among these enzymes, p38α MAP kinase (p38α MAPK) has gained attention for its role in enhancing proinflammatory cytokine production, facilitating Aβ accumulation, and catalysing the hyperphosphorylation of neurotoxic tau proteins.[2]

Our aim is to develop a dual inhibitor targeting both the neuroinflammatory pathway and the traditional cholinergic hypothesis, which states that forebrain cholinergic neuron loss characterizes AD.[3] This multifaceted approach seeks to address the complex interplay of neuroinflammation and cholinergic dysfunction, offering a promising strategy for AD intervention. A library of small molecules with confirmed activity against p38α MAPK was generated and docked into the BChE active site gorge. ARRY-371797, a p38α MAPK inhibitor from Pfizer Inc., showed promising activity against BChE and was further optimized leading to two different series of compounds. All synthesized compounds were tested for in vitro activity against p38α MAPK and BChE using ADP-Glo kinase assay and Ellman's method, respectively. The two best ligands were further analysed for kinome-wide selectivity and cytotoxicity, and tested in vivo on scopolamine and LPS mouse models.



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36 Biophysical Approaches In The Optimization Of Novel Inhibitors Of Human And Bacterial Type II Topoisomerases

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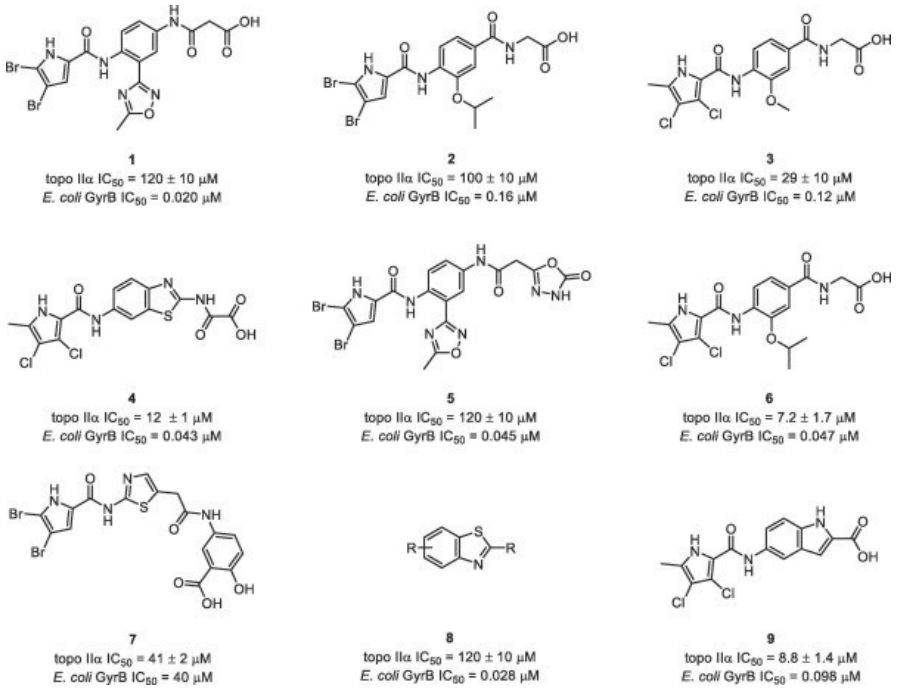
DNA topoisomerases are nuclear enzymes present in both eukaryotic and prokaryotic cells and they are crucial for resolving topological problems that arise during DNA replication and transcription. In order for a DNA molecule to duplicate, a double helix must first unwind which causes tension in remaining parts of the molecule. Topoisomerases create transient breaks in the DNA chain, allowing the molecule to be untangled or unwound. Due to their essential role in cell replication, they are important targets for antibacterial and anticancer drugs. Our research is directed towards bacterial DNA-gyrase and human DNA-topoisomerase IIα.[1]

Bacterial DNA-gyrase and human DNA-topoisomerase IIα both belong to GHKL ATPase superfamily so they share a very high 3D structural similarity. Through screening of an in-house library of ATP-competitive inhibitors of bacterial DNA-gyrase B, new human DNA-topoisomerase IIα were discovered.[2] Their selectivity, potency, physico-chemical, pharmacodynamic and pharmacokinetic properties still need to be improved which will be done with biophysical approaches such as isothermal titration calorimetry and biolayer interferometry.



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37 Development of co-Processed Mesoporous Silica for the Production of Amorphous Solid Dispersions and Orodispersible Tablets

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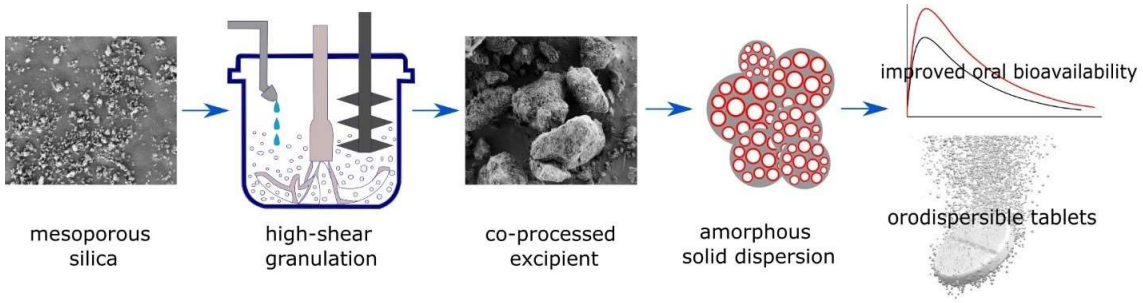
Poor water solubility of active pharmaceutical ingredients (APIs) represents a great challenge in pharmaceutical industry, which is why it is crucial to develop approaches to improve API solubility. One of the approaches to improve dissolution properties is formulation of solid dispersions (SDs) with mesoporous silica as a carrier, onto which the API is adsorbed in an amorphous state. However, an important drawback of these formulations are poor compression and flow properties, which makes it challenging to formulate a final dosage form.[1,2] The aim of our study was to develop a free flowing and compressible co-processed mesoporous silica, and furthermore, to use this material to formulate amorphous SDs with improved dissolution properties of a model API (fenofibrate) in comparison to its dissolution from physical mixtures with the same composition. In the next step, these SDs were formulated into orodispersible tablets (ODTs), which are considered as one of the most patient-friendly final dosage forms.

A co-processed excipient consisting of mesoporous silica and isomalt as a binder was successfully prepared by high-shear granulation using water as a granulation liquid. A Design of Experiment approach determined optimal process parameters, yielding a material with high specific surface area to allow for subsequent drug loading, and suitable particle size to ensure good tabletting properties. In the next step, fenofibrate was loaded onto this material by rotary evaporation method, using acetone, isopropanol or ethyl acetate as a solvent, at two different temperatures of evaporation. Differential scanning calorimetry showed that completely amorphous SDs can be prepared at 30% drug loading, while higher drug ratios resulted in a partially crystalline drug, leading to poor physical stability. Additionally, it was observed that the temperature at which the solvent evaporates exerts a greater influence on the physical state of fenofibrate compared to its solubility in a specific solvent. Dissolution experiments showed significant improvements of dissolution rate from amorphous SDs compared to the physical mixtures and crystalline API. Finally, a 30% SD was used to formulate an ODT with croscarmellose sodium as a superdissintegrant, mannitol as a wetting agent and magnesium stearate as a glidant. The dissolution rate of fenofibrate from ODTs was somewhat lower than from pure SD, indicating that further studies are needed to optimise the tablet formulation.



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38 Role of γ-Enolase in the Development and Degeneration of the Central Nervous System Cells

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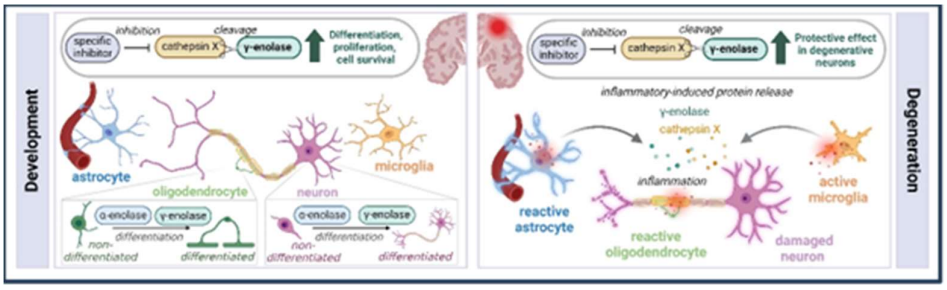
Neuronal development and differentiation rely on the significant expression of neurotrophic factors. Neurotrophic factor-like activity has also been demonstrated for a glycolytic enzyme γ-enolase.[1,2] Its neurotrophic activity is regulated by the lysosomal peptidase cathepsin X, which cleaves the C-terminal end of γ-enolase.[3] So far, γ-enolase has been recognised as a well-known neuronal marker, with a less-known role in neuronal differentiation and the maturation of other central nervous system cells. These cells are crucial for maintaining normal brain function, but they have not been fully studied and are believed to contribute to neurodegeneration. Our research aims to investigate the neurotrophic and neuroprotective roles of γ-enolase within the cells of the central nervous system, focusing on its regulation by cathepsin X.

Using an *in vitro* model of neuronal differentiation, we observed significant expression of γ-enolase within different neuron-specific subtypes and phenotypes upon differentiation. The co-localization of γ-enolase with cathepsin X was observed, suggesting a role for cathepsin X in regulating the neurotrophic activity of γ-enolase in differentiated cells. Furthermore, our research established an oligodendrocyte differentiation model, which demonstrated a significant shift in expression from ubiquitously expressed α-enolase to neuron-specific γ-enolase. Additionally, specific inhibition of cathepsin X led to upregulated levels of the active form of γ-enolase compared to its total form in differentiated oligodendrocytes. Moreover, the inhibition of cathepsin X showed a protective effect in the *in vitro* inflammatory model of differentiating oligodendrocytes. Specifically, cytokine stimulation downregulated the expression level of γ-enolase, suggesting a possible release of the enzyme in the extracellular space. We also examined the role of γ-enolase in an *in vivo* inflammatory animal model of experimental autoimmune encephalomyelitis, observing a significant upregulation of cathepsin X during the disease peak. Similarly, the expression level of γ-enolase altered at the peak of the disease and diminished at the end of the disease. Additionally, we set up an indirect co-culture model to examine the effects of activated microglia on γ-enolase expression in differentiated neuronal cells. We observed changes in expression of γ-enolase in response to pro-inflammatory stimuli, with a dose-dependent effect. Our findings underscore the significant role of γ-enolase in neuronal cells and supporting neuroglia cells during development and degeneration, with cathepsin X as an important regulator and a contributor to inflammation-induced neurodegenerative processes. Therefore, our insights enhance our understanding of central nervous system cell development mechanisms and offer novel strategies to slow the progression of neurodegenerative diseases through the modulation of γ-enolase activity.



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