BioDrug

CONFERENCE 2023

Recent Advances in Structural Biology and Drug Discovery

September 18-19, 2023

Riga

Latvian Institute of Organic Synthesis

Latvian Institute of Organic Synthesis

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MONDAY, September 18th, 2023

9:00-9:30	Registration
9:30 – 9:45	Welcome from the LIOS Director Osvalds Pugovics
9:45-10:10	**Emilio Parisini** Latvian Institute of Organic Synthesis, Riga, Latvia *"BioDrug: an ERA-chair story"*
10:10-10:40	**Luca Pellegrini** University of Cambridge, Cambridge - United Kingdom *"CryoEM insights into primer synthesis by the human primosome"*
10:40-11:10	**Marco Mazzorana** Diamond Light Source, Oxford - United Kingdom *"Improving reliability, accuracy and turnover of MX data collection campaigns"*
11:10-11:40	*Coffee Break*
11:40-12:10	**Alfonso T. Garcia-Sosa** University of Tartu, Tartu - Estonia *"Beyond protein structure prediction"*
12:10-12:40	**Isabel Uson-Finkenzeller** ICREA at IBMB CSIC, Barcelona - Spain *"Predictions and experiments in structure determination: ARCIMBOLDO and VAIRO"*
12:40-14:15	Lunch break and networking (poster session)
14:15-14:45	**Carlo Matera** University of Milan, Milan – Italy *"Photoswitchable Molecular Tools: Applications to Enzymes, GPCRs and Ion Channels"*

14:45-15:15	**Galyna Maleeva** Institute of Bioengineering of Catalonia IBEC, Barcelona – Spain *"Photopharmacology for the control of the neuronal activity"*
15:15-15:45	**Francesco Calzaferri** Institut des Biomolécules Max Mousseron CNRS, Montpellier - France *"Novel strategies to target epigenetic mechanisms for cancer treatment: from rational compound optimisation to PROTAC development"*
15:45-16:30	**Short talks** (10' each): **Nikhil Agrawal**: "*A coarse-grained molecular dynamics investigation on spontaneous binding of Aβ1–40 fibrils with cholesterol-mixed DPPC bilayers"* (Poster n 1) **Kristīne Krūkle-Bērziņa: "***Cyclodextrin Metal organic frameworks as a drug delivery system for selected pharmaceutical active ingredients"* (Poster n 10) **Pilar M. Luque Navarro:** "Sulphur bioisosterism exerts different antiplasmodial responses" (Poster n 11) **Karlis Pleiko**: "*Targeting triple-negative breast cancer cells with a β1-integrin binding aptamer"* (Poster n 15)
16:30-18:30	Networking & **poster session** & coffee
19:30	**Conference Dinner**

TUESDAY, September 19th, 2023

9:00	Registration
9:30-10:00	**Alfonso Gautieri** Politecnico di Milano, Milan - Italy *"Computer aided redesign of deglycating enzymes for therapeutic and diagnostic applications"*
10:00-10:30	**Rob Meijers** Institute for Protein Innovation, Boston – USA *"Generating synthetic antibodies for the human surfaceome at the Institute for Protein Innovation"*
10:30-11:00	**Piermichele Kobauri** Aqemia, Paris - France *"Rational Design of Light-Controlled Bioactive Compounds for Photopharmacology"*
11:00-11:30	*Coffee Break*
11:30-12:00	**Elena Ishow** University of Nantes, Nantes – France *"Photoactive organic nanoparticles as versatile tools for theranostics"*
12:00-12:30	**Silvia Giordani** Dublin City University, Dublin – Ireland *"Carbon nano-onions for biomedical applications"*
12:30	Poster Prizes – Closing remarks
12:45	**Lunch**
14:00	Visit to LIOS (for registered participants)

INVITED TALKS

CryoEM insights into primer synthesis by the human primosome

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Eukaryotic DNA replication depends on the primosome – a complex of DNA polymerase alpha (Pol α) and primase – to initiate DNA synthesis by polymerisation of an RNA - DNA primer. Primer synthesis requires the tight coordination of primase and polymerase activities. Because of the intrinsic flexibility of the primosome, structural information about the initiation of RNA primer synthesis is still lacking. Here, we capture cryoEM snapshots of the priming reaction to reveal the conformational trajectory of the human primosome that brings the PRIM1 and PRIM2 subunits of primase together, poised for RNA synthesis. Furthermore, we use cryoEM to demonstrate the structural basis for inhibition of Pol α by CD437, a retinoid-like molecule with potent any-tumour activities.

Improving reliability, accuracy and turnover of MX data collection campaigns

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Over the past few decades, Macromolecular Crystallography (MX) has gradually changed from a laborious and time-consuming biophysical method into a rapid and efficient technique used to describe the structure of biological molecules and their interaction with substrates, modulators and inhibitors.

Leading the way in this advancement, synchrotron facilities played a pivotal role in enhancing X-ray sources, sample manipulation, and data analysis capabilities. As a result, they made cutting-edge equipment more accessible, democratizing its usage and significantly boosting the efficiency of both academic and proprietary research endeavours.The growing diversity and increased power of synchrotron beamlines can lead to sample overexposure, causing radiation damage and data deterioration. Strategies like cryogenic temperature data collection[1,2], the use of free radical scavengers, and the adoption of techniques based on exposing fresh sample regions over time or multi-crystal approaches have been adopted to counter this.

Recent integration of automation and sample logistics enables fully unattended data collection. These methods follow standard 'recipes' set by beamline scientists for common scientific cases[3] to which users contribute defining their goals and quality criteria. The result is a beamline-agnostic approach, granting extreme flexibility and versatility and avoiding the most common pitfalls of operator-driven experiments.

They are generally conservative and tend to preserve data crystal quality for a wide variety of samples. However, a better data collection strategy needs to be driven by dose[4] and include the properties of beamline, sample and must consider the final goal of the experiment. I will present here how the integration of live flux calculations, the Raddose3D software[5] and the user requirements can provide valuable data, giving uniformity to experiments conducted at different instruments over time.

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Beyond Protein Structure Prediction

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Recent advancements in scientific methodologies have ushered in an era where the design and assessment of novel compounds as well as novel proteins for addressing neglected diseases and understanding their biological interactions are profoundly transformed. The strategic identification and validation of therapeutic targets are pivotal. It not only infuses innovation into drug design but also potentially mitigates resistance to treatment modalities, as observed in conditions such as leishmaniasis.

Deep learning, a subset of machine learning, is demonstrating its prowess in this domain. It has showcased its potential in generating actionable targets, notably in combating infections caused by the *Plasmodium falciparum* parasite. Such innovations rooted in computational biology and chemistry are rapidly gaining traction, offering significant advantages over traditional approaches.

A testament to the versatility of these methods is their applicability beyond disease treatment. An illustrative example is the utilization of machine learning algorithms to classify compounds based on their affinity to the androgen receptor. Such insights are invaluable in toxicology, paving the way for improved safety profiles and risk assessments of chemical agents, and underscore the transformative role of machine learning in drug design and toxicology, emphasizing its potential in revolutionizing our approach to combating neglected diseases and ensuring safer therapeutic interventions.

Acknowledgements

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Predictions and experiments in structure determination: ARCIMBOLDO and VAIRO

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Artificial intelligence trained on previously determined structures has yielded protein models approaching experimental accuracy1. While AlphaFold predictions are useful hypotheses about protein structures, experimental information remains essential for creating an accurate model2. Predictions have been incorporated in our structural determination methods3, demand a redefinition of how we integrate prior knowledge in experiment interpretation4 (figure 1a) and open new opportunities to gain a dynamic view by using experimental structures to establish the background of a prediction or impose boundary conditions (figures 1b, 1c).

Figure 1. Our methods: ARCIMBOLDO (a) solves the phase problem systematically verifying the model and VAIRO (b) informs AF predictions with experimental structures, setting boundary conditions to target particular states within a dynamic.

Acknowledgements

We thank Martin Alcorlo and Juan A. Hermoso from IQF-CSIC and Fernando Govantes from UPO for collaborations around interesting cases.

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Photoswitchable Molecular Tools: Applications to Enzymes, GPCRs and Ion Channels

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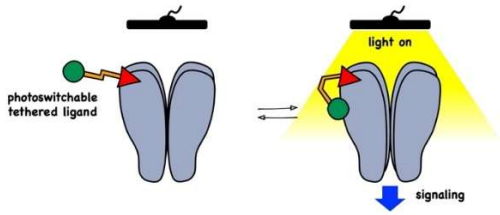
Photocontrolled molecular tools provide powerful means to manipulate and interrogate biological functions with high spatiotemporal precision and low invasiveness. Our research efforts in the field have focused on the design of reversible photoswitchable compounds to photocontrol enzymes, GPCRs, and ion channels. Among others, we have developed phototrexate, the first photoswitchable inhibitor of the human dihydrofolate reductase with demonstrated cytotoxicity in vitro and in zebrafish larvae,[1] and PAI, a light-controlled dualsteric agonist of muscarinic M_2 receptors that enabled photomodulation of cardiac function in tadpoles and brain states in mice.[2] More recently, we have designed a fast photoswitchable tethered ligand of ionotropic glutamate receptors to enable activation of the auditory neurons with light. This compound, named TCP_{fast}, induced photocurrents in untransfected neurons upon covalently tethering to endogenous glutamate receptors and activating them reversibly with blue light. We applied it to the ultrafast synapses of cochlear auditory neurons that encode sound and provide auditory input to the brain. TCP_{fast} functions as a molecular prosthesis that bypasses the neurotransmitter-encoded signal with a photonic signal. Photosensitization of cochlear spiral ganglion neurons (SGNs) by locally administered TCP_{fast} enabled temporally precise light-evoked firing up to a rate of approximately 1 kHz, matching the fastest optogenetic SGN stimulation.[3] Hence, TCP_{fast} shows that photopharmacology might serve as an interesting alternative for the development of optical cochlear implants for hearing restoration. The main results of all these studies will be presented and discussed.



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Photopharmacology for the control of the neuronal activity

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Inhibitory neurotransmission, mediated by γ-aminobutyric acid (GABA) and glycine receptors, is crucial for the control of neuronal activity. Having a goal to develop an efficient photoswitchable modulator of $GABA_ARs$ we have employed several different strategies.

Compounds obtained by azologization of 7-aminonitrazepam with nitrosobenzenesulfonamide and nitrosopyridine (Azo-NZ1 and Glyght respectively) were highly photocontrollable. However, they have demonstrated unexpected activity at GABARs and GlyRs. Using patch-clamp technique, point mutations approach and molecular modelling we were able to demonstrate that Azo-NZ1 is a photo-switchable ion channel blocker of $GABA_{A,C}Rs$ and GlyRs (Maleeva et al., 2019), while Glyght is a photo-switchable negative modulator of GlyRs of various subunit compositions (Gomila et al., 2020). Testing in zebrafish demonstrated high efficiency of Glyght for the photocontrol of their behavior *in vivo*. Thus, we have developed a group of azobenzene/nitrazepam-based compounds that negatively modulate activity of two main inhibitory brain receptors in light-dependent manner. In contrast to nitrazepam, they did not potentiate GABARs currents but induced subunit-specific inhibition of $GABA_ARs$ and GlyRs.

Pursuing the goal to develop a GABARs positive photomodulator, we successfully functionalized the benzodiazepine nitrazepam into a light-controllable molecule via extension by a photochromic fulgimide. The molecule that was obtained, Fulgazepam, is the first photochromic switch-on potentiator of $GABA_ARs$ and its ability to photomodulate neuronal activity and behaviour was successfully demonstrated *in vitro* and *in vivo* in zebrafish (Rustler et al., 2020).

In summary, we have developed a toolbox of photoswitchable modulators of GABARs and GlyRs that can be used for variety of tasks when studying gabaergic and glycinergic neurotransmission and circuits. They were successfully employed for the photocontrol of synaptic transmission and zebrafish behaviour.

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Novel strategies to target epigenetic mechanisms for cancer treatment: from rational compound optimization to PROTAC development

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Epigenetic modifications, like DNA and histone methylation, control gene expression. DNA methyltransferase inhibitors (DNMTi) proved to be effective in anticancer therapy, but they are highly toxic and chemically instable.[2] Histone methyltransferase inhibitors (HMTi) have been less explored so far. For example, the HMTi A-196 induces DNA repair defects in cancer cells but without causing their death, and its mechanism of action remains elusive.[3] Thus, we envisaged novel strategies to target DNMT and HMT for cancer treatment and research.

Our group discovered novel flavanone-based DNMTi[4] and rationally optimized them to increase their potency and chemical stability. We identified a 3-dihalo-2-disubstituted flavanone active in the nanomolar range, with a half-life of more than one week in water, and capable of inducing tumor suppressor gene expression in colon cancer cells.

Moreover, we started synthesizing proteolysis-targeting chimeras (PROTACs)[5] of A-196-targeting HMT. We first functionalized A-196 and coupled it with short alkyl- and long PEG-based linkers, bearing three different E3 ligase ligands to hijack HMT to proteasomal degradation. We assessed compound activity by western blot in prostate cancer cells, identifying potential HMT PROTACs.

Our research provided novel active molecules and chemical tools for epigenetic targeting, leading to new cancer treatment and research opportunities.

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Computer aided redesign of deglycating enzymes for therapeutic and diagnostic applications

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Blood glucose can alter functional proteins through the formation of so-called Advanced Glycation End-products (AGEs). AGEs accumulation is particularly severe in the elderly and in diabetic patients. The glycation process, albeit slow, is cumulative and irreversible and it affects all proteins indiscriminately. It has been related to a long series of adverse clinical outcomes including arterial stiffening, nephropathy, and retinopathy. However, there are currently no effective ways of preventing AGEs formation. A promising strategy to prevent protein glycation involves the use of Amadoriase enzymes, naturally occurring enzymes that are able to cleave glycated amino acids, but are inactive on whole proteins. Starting from the Amadoriase I crystal structure and extensive computational characterization that we have provided[1], I am developing a combined in silico-experimental approach to rationally design protein-deglycating Amadoriase enzymes[2,3]. These engineered enzymes will be able to cleave glycation products on larger molecules (proteins or protein fragments), thus providing a viable therapeutic tool against AGEs-induced tissue stiffening and aging. Additionally, the proposed innovative strategy for enzyme design is expected to boost knowledge-based design of different biological nanomachines for diverse applications, and thus with huge potential impacts in several areas such as health, agriculture, food processing and fuel industry.

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Generating synthetic antibodies for the human surfaceome at the Institute for Protein Innovation

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The Institute for Protein Innovation (IPI) is a non-profit organization founded by entrepreneur and Lasker prize winner Tim Springer. Its mission is to advance protein science for the purpose of accelerating research and improving human health. IPI is a hybrid, blending academic exploration with industrial-scale. high throughput capabilities. With technology at IPI's foundation, we have optimized an automated platform—based on synthetic display technologies—and successfully used it to discover and develop antibodies for human cell surface and secreted proteins (a.k.a. as the human surfaceome).

To date, we have produced more than 300 antibodies for over 50 targets and aim to make the majority available to the life science community. In addition, we have created mammalian expression vectors, yeast display libraries and affinity purification raisins, which we also plan to share broadly. By championing such an "open science" approach, IPI strives to enable start-ups and academic laboratories to achieve their scientific objectives and allow them to commercialize their scientific output in a straightforward manner.

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Rational Design of Light-Controlled Bioactive Compounds for Photopharmacology

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Photopharmacology is an attractive approach for achieving targeted drug action with the use of light.[1] In photopharmacology, molecular photoswitches are introduced into the structure of biologically active small molecules to allow for the optical control of their potency. A major challenge for the field is the rational design of photoswitchable drugs that are more active in the light-induced, metastable state, i.e., "*cis*-on" in the case of classical azobenzenes.[2]

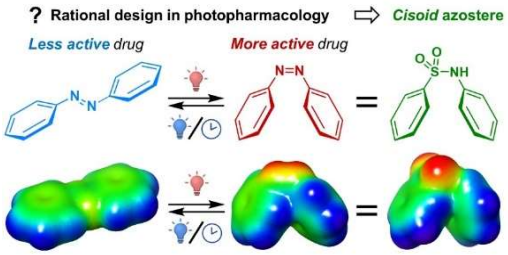

Figure 1. Biaryl sulfonamides as *cisoid* azosteres.

Here we describe the application of computer-aided drug design strategies to develop light-controlled bioactive compounds. In the first study, analysis of structural and electronic similarity between a library of fragments and *cis*-azobenzene led to the identification of biaryl sulfonamide as an excellent *cisoid* azobenzene bioisostere ("azostere", Figure 1).[3] In the second study, we iteratively examined several structure-based hypotheses to guide the design of potent photoswitchable inhibitors of *Escherichia coli* dihydrofolate reductase.[4] Overall, we demonstrated that molecular modeling can provide valuable insights and help photopharmacology to go beyond trial and error.

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Photoactive organic nanoparticles as versatile tools for theranostics

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The strong development of theranostic nanomaterials, advantageously combining therapeutics and diagnostics, has brought to the forefront fluorescent organic nanoparticles (FONs), made out of self-assembled π-conjugated species. There is no area left unexplored by such functional nanomaterials, amenable to multimodal bioimaging, drug delivery, biochemical sensing, or photodynamic therapy to cite only a few. Their "smart" properties actually rely on the high payload of active units (10^4-10^5 molecules per nanoparticle), the tight interactions between the dyes and their ability to self-assemble with hydrophobic drugs, and eventually the large surface-to-volume ratio. The resulting structural confinement leads to highly sensitive response to tiny external change, especially the solvation shell, which can be harnessed for low-concentration analyte detection. After a brief survey of the main classes of emissive nanoparticles and their potential pros and cons, we will show through selected examples how photoactive organic nanoparticles, providing tight matter cohesion and multiple interactions, have been exploited to offer dynamic follow-up of drug release as well as selective and minute-like diagnostics.

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Carbon nano-onions for biomedical applications

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In this presentation, carbon nano-onions (CNOs) will be discussed as a potential vesicle for nanocarrier-type drug delivery systems.[1] CNOs, or multi-layer fullerenes,consist of multiple concentric layers of sp2 hybridized carbon and are emerging as platforms for biomedical applications because of their ability to be internalized by cells and low toxicity.[2] In my research group we have developed methodology for the synthesis of pure, monodispersed CNOs and various chemical functionalization strategies for the introduction of different functionalities (receptor targeting unit and imaging unit) onto the surface of the CNOs. The modified CNOs display high brightness and photostability in aqueous solutions and are selectively taken up by different cancer cell lines without significant cytotoxicity. Supramolecular functionalization with biocompatible polymers is an effective strategy to develop engineered drug carriers for targeted delivery applications. We reported the use of a hyaluronic acid-phospholipid (HA-DMPE) conjugate to target CD44 overexpressing cancer cells, while enhancing solubility of the nanoconstruct. Non-covalently functionalized CNOs with HA-DMPE show excellent in vitro cell viability in human breast carcinoma cells overexpressing CD44 and are uptaken to a greater extent compared to human ovarian carcinoma cells with an undetectable amount of CD44. In addition, they possess high in vivo biocompatibility in zebrafish during the different stages of development suggesting a high degree of biosafety of this class of nanomaterials.[3] Our results encourage further development as targeted diagnostics or therapeutics nanocarriers.

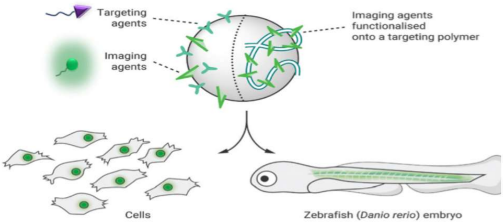

Figure 1. Figure illustrating the carbon nano-onion surface modification with imaging and targeting units and the *in vitro* and *in vivo* biocompatibility studies.

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POSTER SESSION

Poster Presentations

1	Nikhil Agrawal *A coarse-grained molecular dynamics investigation on spontaneous binding of Aβ1–40 fibrils with cholesterol-mixed DPPC bilayers*
2	Vladislavs Baškevičs *Triple Helical Recognition of Cytosine in RNA using Peptide Nucleic Acids: Insights from Molecular Dynamics*
3	Anna Lina Bula *Plant autocatalytic peptide cyclases as tools for customised cyclic peptide production*
4	Anna Lina Bula *Fragment-based drug discovery of SARS-CoV-2 methyltransferase nsp14 inhibitors*
5	Rossella Castagna *Biohybrid electrospun fibers for smart-materials interfaces*
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A coarse-grained molecular dynamics investigation on spontaneous binding of Aβ1–40 fibrils with cholesterol-mixed DPPC bilayers

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Alzheimer's disease is the most common form of dementia. Its aetiology is characterized by the misfolding and aggregation of amyloid-β (Aβ) peptides into β-sheet-rich Aβ oligomers/fibrils. Although multiple experimental studies have suggested that Aβ oligomers/fibrils interact with the cell membranes and perturb their structures and dynamics, the molecular mechanism of this interaction is still not fully understood. In the present work, we have performed a total of 120 μs-long simulations to investigate the interaction between trimeric or hexameric Aβ1–40 fibrils with either a 100% DPPC bilayer, a 70% DPPC-30% cholesterol bilayer or a 50% DPPC-50% cholesterol bilayer. Our simulation data capture the spontaneous binding of the aqueous Aβ1–40 fibrils with the membranes and show that the central hydrophobic amino acid cluster, the lysine residue adjacent to it and the C-terminal hydrophobic residues are all involved in the process. Moreover, our data show that while the Aβ1–40 fibril does not bind to the 100% DPPC bilayer, its binding affinity for the membrane increases with the amount of cholesterol. Overall, our data suggest that two clusters of hydrophobic residues and one lysine help Aβ1–40 fibrils establish stable interactions with a cholesterol-rich DPPC bilayer. These residues are likely to represent potential target regions for the design of inhibitors, thus opening new avenues in structure-based drug design against Aβ oligomer/fibril-membrane interaction[1].

Acknowledgements

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Triple Helical Recognition of Cytosine in RNA using Peptide Nucleic Acids: Insights from Molecular Dynamics

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Peptide nucleic acids (PNA) are potentially powerful tools in research and diagnostics and are being actively developed as therapeutic agents.[1] However, only the purine nucleobases can be recognized on a double-stranded RNA (dsRNA) Hoogsteen surface by canonical bases. In addition, pyrimidine heterocycles extend further out in the major groove making the Hoogsteen surface irregular and creating steric hindrance for triplex forming PNAs.

To accelerate development of PNA for recognition of any dsRNA sequence we constructed a triplex model (Fig. 1A). Several known and new nucleobases as well as linkers connecting nucleobases to the PNA backbone were examined in our model.

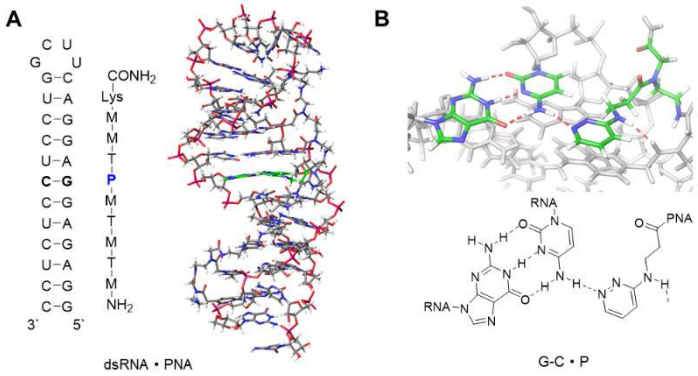

Figure 1. PNA-dsRNA triple helical model (A). G-C•pyridazine triplex (B).

During the course of this study, a new perspective pyridazine nucleobase with an aminoethyl linker for recognition of cytosine (Fig. 1B) was identified, incorporated in PNA, and the binding affinity was determined.

Acknowledgements
This work was supported by internal student grants of Latvian Institute of Organic Synthesis IG-2023-08 (to V.B).

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Plant autocatalytic peptide cyclases as tools for customised cyclic peptide production

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BURP domains are a class of plant proteins that catalyse the cyclisation of small peptidic motifs called core sequences[1,2]. These recently described cyclases have the potential to be used as biosynthetic catalysts for the preparation of cyclic peptide therapeutics, antibacterials and insecticides. Our goal is to produce functional recombinant BURP domain proteins from various plant species in order to perform cyclisation of various peptide substrates, both encoded as core peptides N-terminal of the BURP domain (*cis* cyclisation) and linear peptides added separately to the BURP domain protein (*trans* cyclisation). This could become a useful platform to produce cyclic peptides for various application purposes.

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Fragment-based drug discovery of SARS-CoV-2 methyltransferase nsp14 inhibitors

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The Covid-19 pandemic, caused by the highly infectious SARS-CoV-2, has highlighted the urgent need to create effective antiviral therapies. The nonstructural protein 14 (nsp14) is a key component of the viral replication machinery - an S-adenosyl-L-methionine-dependent guanine-N7 methyltransferase necessary for the capping of viral mRNA, which ensures its stability and translation[1]. It is therefore an appealing target for drug development.

Using the DSI-poised library, we performed a ligand-observed NMR fragment screening of nsp14, specifically exploring the SAM binding pocket. The known SAM analog, sinefungin, was used in the competitive binding experiments. We identified 47 compounds that showed binding to nsp14, two of which were competitive binders[2]. These fragments represent promising starting points for hit-to-lead optimization towards the development of potent and selective nsp14 inhibitors.

Acknowledgements

This work was done as part of the Covid19-NMR consortium and was financially supported by the Latvian Council of Science, grant numbers: VPP-COVID-2020/1-0014 and VPP-EM-BIOMEDICĪNA-2022/1-0001.

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Biohybrid electrospun fibers for smart-materials interfaces

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Biohybrid materials are formed by the combination of biogenic and non-biogenic components. Indeed, biomolecules, cells or tissues can be combined with synthetic polymers or inorganic elements to generate active biohybrid materials, provided that the bio component retains its biological function during material processing and fabrication.

The electrospinning technique has proven very effective for incorporating delicate biomolecules into fibrous nanostructures for different applications. For instance, biohybrid nanofibrous membranes can serve as innovative solutions for sequestering drugs and their metabolites from wastewater. We electrospun poly(vinyl alcohol) (PVA)/bovine serum albumin (BSA) blended nanofibers starting from a bi-component feed solution. By demonstrating that our mats can remove ketoprofen from water, we showed that the combination of a BSA-induced biofunctionality with a nanostructured fibrous material allows for the development of an efficient biohybrid filtering device for the large and widely used family of nonsteroidal anti-inflammatory drugs (NSAIDs), which are often found in wastewaters as contaminants. Finally, we provided the crystal structure of the complex between BSA and ketoprofen, confirming the interaction between the two species[1].

The Biotechnology group at LIOS has acquired a state of the art equipment to produce particles and fibers by means of the technique of electrospray and electrospinning. The currently available setup allows producing single and multicomponent materials with a random or aligned fiber disposition.

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Aggregation Condition–Structure Relationship of Mouse Prion Protein Fibrils

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A peculiar aspect of amyloid formation is the ability of one type of protein/peptide to form multiple, structurally distinct fibrils[1]. Lack of insight into this, seemingly generic, property of protein fibrillization may be one of the reasons why there is still no complete understanding of amyloid aggregation and, in turn, very few effective treatments for their respective diseases. In this work, we investigate mouse prion protein folding domain (MoPrP(89-230))[2]. We prepare fibrils in three different denaturing conditions and study them using FITR, AFM and MAS NMR. In all the samples, rigid core is formed by residues 165-223. The differences for residues preceding His177 and succeeding Tyr218 are negligible, indicating that the structure of N- and C-terminal regions of the fibrillar core is not affected by the aggregation condition. Both samples produced with GdnHCl as sole denaturant are more similar to each other and hence show small chemical shift deviations, except for the residues between Lys194 and Glu196. In contrast, in comparisons with the chemical shifts of the sample with urea, fibrils show larger differences, particularly in the regions Asp178–Asn181 and Lys204–Met213. Analysis of methyl group region of the NMR C(HH)C spectra propose that the denaturant ionic strength plays a major role in determining the structure of fibrils obtained in a particular condition by stabilizing fibril core interior-facing glutamic acid residues (Figure 1).

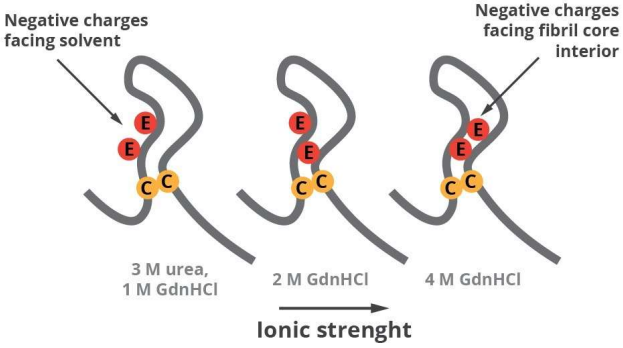

Figure 1. Dependence of glutamic acid location in the prion protein fibrils on the ionic strength of denaturation agent.

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Effects of different medicinal substances on the secretion of cytokines in HepG2 cell fatty liver model *in vitro*

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Non-alcoholic fatty liver disease (NAFLD) is a growing burden on the global health system and is estimated to affect approximately 25% of the population. NAFLD has been recognized as a manifestation of the metabolic syndrome in the liver with a strong association with obesity, insulin resistance, increased systemic inflammation and progressive atherosclerosis. However, the pathogenesis of NAFLD has not been fully elucidated. The mechanism is usually explained by the classical theory that lipid accumulation causes hepatic steatosis, leading to inflammation, lipotoxicity, and dysregulation of glucose and lipid metabolism. Previous studies have reported several inflammatory mediators involved in the development and progression of NAFLD, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and others. However, clinical trials have found an inconsistent relationship between inflammatory cytokines and NAFLD. Moreover, the effects of drugs on lipid accumulation and inflammatory cytokine production in fatty liver cells have been less studied.

The goal of the study was to study the effect of selected drugs on the production of several cytokines in the NAFLD model of HepG2 cells, using *in vitro* assays of cell viability, lipid accumulation, and TNF-α, IL-6, IL-8 and CRP secretion. HepG2 cells have a low metabolic capacity, so we assumed that the obtained results refer to the studied substances and not to their metabolites. Liver steatosis model was obtained by adding mix of the oleic and palmitic acids to the cell medium for the 24-48 h. All tested compounds at concentrations not influencing the cell viability were added simultaneously with lipids. In our study, HepG2 cells under the influence of fatty acids produced more cytokines than control cells. Fatty acids caused a 3-4-fold increase in IL-8 production and an 8-9-fold increase in IL-6 secretion. The results of the study showed that silymarin, metformin, simvastatin, diclofenac, and celecoxib reduced lipid accumulation in the cells and reduced the elevated by fatty acids production of TNF-α, IL-6 and Il-8. Silymarin is well known as a liver protector. However, IL-6 secretion was most actively inhibited by celecoxib whereas IL-8 secretion by atorvastatin and celecoxib.

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Effect of calcium-cadmium substitution on E- cadherin dimerization

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Cadherins belong to a large superfamily of calcium-dependent transmembrane proteins that are known to mediate cell−cell adhesion. All classical cadherins share a high degree of sequence and structural similarity. They comprise an extracellular region, which features five tandemly-arranged immunoglobulin-like extracellular domains (EC1-5), and a cytoplasmic tail, which connects dynamically with the actin cytoskeleton via the interaction with α-catenin, β-catenin and p120. Three calcium ions are bound at each inter-domain junction to help stabilize the extracellular portion and maintain protein functionality (1). Cadherin dimerization follows a dynamic trajectory whereby the protein reaches a so-called strand swap dimer conformation going through a conformational intermediate called X-dimer.

In this study, we aim to assess the effect of calcium substitution with other divalent cations on E-cadherin dimerization. Cadmium, which is known for its toxicity in tissues, seems to be a potential candidate for binding site competition assays, as it can easily interact with cadherin molecules in the epithelium, the first line of defense in several organs. Apart from altering the activity of many enzymes and inducing oxidatitive stress in cells, cadmium has been shown to antagonize the activity of other metal ions, including calcium (2). In light of the well-established correlation between cadherin expression levels and cancer development, it is interesting to study the effect of the exchange of calcium with other metal ions on the cadherin dimerization mechanism.

So far, our findings confirm previous studies that suggested that cadmium is able to compete with calcium to occupy the binding sites at the EC1-EC2 junction, resulting in a change in the dimerization constant (Kd) of E-cadherin (3). We now plan to prove this change by determining the crystal structure of E-cadherin in complex with cadmium.

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Development of irreversible covalent inhibitors for plasmodium serine protease SUB1

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Malaria is a life-threatening disease caused by the *Plasmodium* parasite which is responsible for over 600 000 deaths annually.[1] Due to a widespread resistance of plasmodium strains to available chemotherapeutics new anti-malarials with novel modes of action are urgently needed. The *Plasmodium* parasite is a single-celled organism which infects and replicates in the human red blood cells. Escape from the red cell *via* process called egress is triggered by an essential subtilisin-like serine protease SUB1 which renders this enzyme as a perspective anti-malarial drug target.

Boronic acid **1** is known to have an inhibitory effect of SUB1 by forming covalent reversible bond with catalytic site serine of the enzyme.[2] In our follow up studies, the boronic acid moiety in peptidomimetic inhibitors has been replaced by other warheads able to form covalent irreversible bonds[3] with the serine residue of SUB1. Here we present the synthesis of a small library of structures which have the potential to be effective SUB1 inhibitors (**Figure 1**).

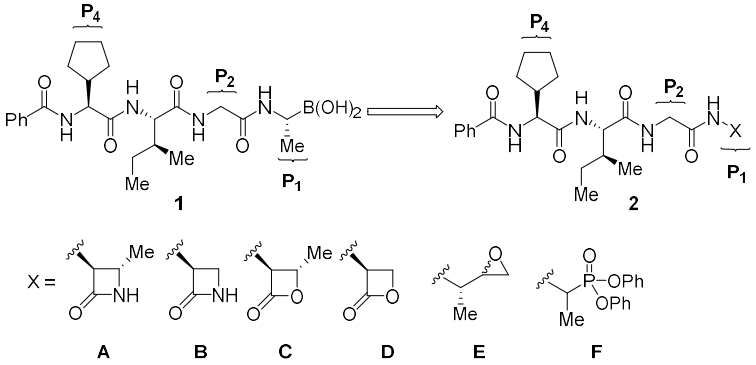

Figure 1. Potential peptidiomimetic SUB1 inhibitors comprising covalent warheads

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Cyclodextrin Metal organic frameworks as a drug delivery system for selected pharmaceutical active ingredients

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Metal–organic frameworks (MOFs) have been known for decades, and they continuously have gained increased interest because of their application potential in various fields - pharmacy, medicine, technology etc.[1] Porous architecture and adjustable properties of cyclodextrin (CD) MOFs allow them to be considered as promising drug carriers. Modification of the properties of an existing active pharmaceutical ingredient (API) without changing its biological role can be much faster and more effective.[2] Several papers have already reported successful encapsulation of APIs in CD MOFs by adjusting the bioavailability properties of the drug.[3]

In this study we explored encapsulation process of APIs in several newly obtained MOFs with structures and properties not yet reported in the literature. For the encapsulation process we selected model drugs - carmofur (HCFU), 5-fluorouracil (5-FU) and salicylic acid (HBA). The efficiency of encapsulation was monitored using different crystallization protocols. To evaluate the API encapsulation process in the CD MOFs different methods were applied - differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) and solid-state NMR spectroscopy (ssNMR). We also evaluated the toxicity and biocompatibility of the API:CD-MOF carriers using *in vitro* and *in vivo* methods.

Acknowledgements

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Sulphur bioisosterism exerts different antiplasmodial responses

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Plasmodium falciparum kinases have been widely studied due to their potential as an alternative to Artemisinin-combined therapies. Their role in the parasite blood-stage replication and their homology with human kinases has led to the use of already tested antitumoral kinase inhibitors as antiplasmodial drugs [1]. Vial et al. first pointed out the *Pf.* Choline kinase (CK), a cytosolic enzyme involved in phospholipids synthesis, as a promising target for parasite resistance strains [2]. *Pf.* CK uses the host choline and catalyzes its transformation in phosphocholine, a key step for the formation of new lipidic membranes that cover the new parasite progeny inside the erythrocyte. In this work, two families of bioisosteric *Pf.* CK inhibitors based on sulphur replacement have been synthesized, based on *disulphide* and *dithioethane* linkers assembled with two pyridine, thienopyrimidine and quinoline -substituted cationic heads [3].

Interestingly, while the *disulphide* (FP) library shows *Pf.* CK inhibition in the nM range independently of the cationic head, these compounds did not show any effect over the infected erythrocytes. On the other hand, the *dithioethane* (PL) library showed the opposite trend with in vitro activity, but not over the isolated enzyme. These results could be due to the *disulphide* degradation in-vitro, and on the other hand, because of an off-target effect in the *dithioethane* library.

Figure 1. New libraries of sulphur-containing *Pf.* CK bioisosteres.

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Targeting trans-dimerisation of type II classical cadherin

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Cadherins are calcium-dependent adhesion molecules that mediate contacts between cells at adherens junctions. Different tissues express different cadherins subtypes, ensuring adhesion specificity. Classical cadherins contain an elongated extracellular portion consisting of five immunoglobulin-like extracellular (EC) domains, of which the outermost N-terminal domains EC1 and EC2 mediate the protein-protein interaction (PPI) between cadherin protomers on opposing cells, called trans-dimerisation. A central feature of this interaction is the strand-swapping of one or two tryptophan residues between the EC1 subunits, in type I and type II cadherins, respectively (**Figure 1**). Type II cadherins, such as cadherin-11 and VE-cadherin, are potential therapeutic targets in a range of conditions, including metastatic cancer, arthritis and fibrosis.

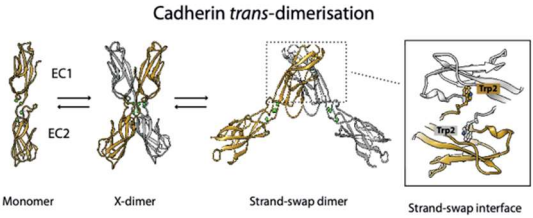

Figure 1. Cadherin trans-dimerisation

In this work we aim to pharmacologically modulate trans-dimerisation of classical type II cadherins. For this we have engineered constructs of the EC1-EC2 domains that form predominantly the X-dimer and present a more accessible Trp pocket for detecting weak interactions. We have established a crystallographic and biophysical screening platform to identify small molecule fragments that bind the EC1-EC2 domains. We identify a fragment that interacts at the X-dimer interface, at a novel binding pocket formed by the two protomers. The resulting fragments can serve as starting points for lead-like compound design.

Acknowledgements

The work is funded by the ERDF grant Nr. 1.1.1.5/21/A/002 “STARCAT: Selective targeting of Cadherin trans-dimerisation”. We acknowledge the Diamond Light Source synchrotron for providing access to the XChem screening facility (proposal LB33144).

Rational Design of Fructosyl Peptide Oxidase enzyme

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Fructosyl Peptide Oxidases (FPOX) are deglycation enzymes used as key enzymatic components in diabetes monitoring devices. At present, FPOX used in enzymatic assays is not able to directly detect whole glycated proteins. This requires to perform a preliminary proteolytic treatment of the target protein to generate small glycated peptides that can act as viable substrates for the enzyme. This is an expensive and time-consuming step. In this work, we applied a Rational Design approach to an already modified FPOX, X02C from Estiri et al. [1], to further widen the access tunnel leading to the active site of the enzyme, thus improving its catalytic activity towards large substrates. We identified a potentially expendable loop (Fig.1) that limits the access to the active site. Using Rosetta Remodel we shortened the loop (from 9 amino acids to 2-5 amino acids) generating a library of 2064 enzyme variants. We then performed molecular dynamics simulations at 300K, 350K and 400K to assess their stability. Our approach allowed the selection of 5 mutants, which will be studied *in vitro* to assess their stability and their activity towards large ligands and possibly whole proteins. This may represent a significant step towards the development of *in vitro* HbA1c diagnostic devices based on a direct enzymatic oxidation system.

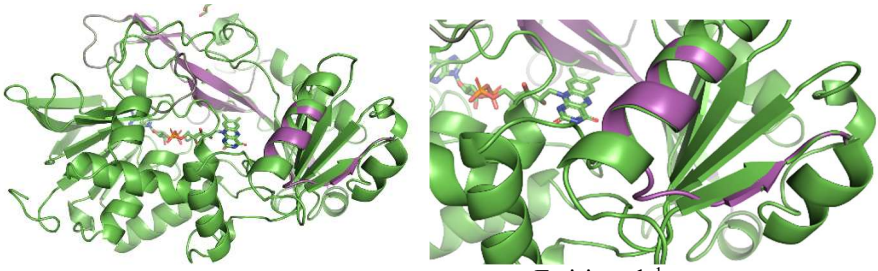

Figure 1. Comparison of protein structures of X02C Estiri et al. [1] in green and one of its mutants with reconstructed loop of two residues in violet.

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Synthetic Application of Monofluorocyclopropylsulfinate

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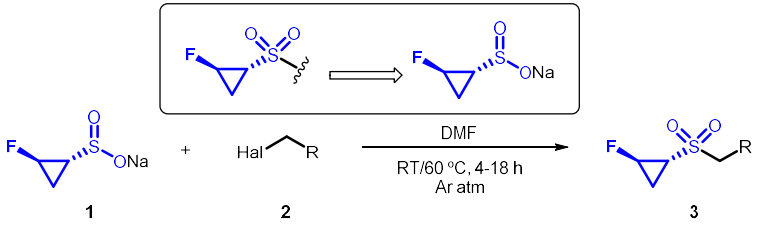
Fluoroalkyl containing compounds (e.g. $–CF_3$, $–CF_2H$, $–CFH_2$) are of high significance in research of pharmaceuticals[1], agrochemicals[2] and advanced materials[3] as fluoroalkyl groups can alter physiochemical properties of a molecule, for example, metabolic stability and bioavailability.[4] Specifically, *Langlois* reagent ($NaSO_2CF_3$) has been used in a variety of trifluoromethylation reactions[5] as a $–CF_3$ source. Monofluorocyclopropyl group is an intriguing moiety with potential application in medicinal chemistry, therefore, monofluorocyclopropylsulfinate **1**, being similar to *Langlois* reagent, could be an attractive, yet little explored, source of this moiety in fluorine chemistry.

We demonstrate that using monofluorocyclopropylsufinate **1** significantly expands the potential application of our group's developed approach to monofluorocyclopropylsulfones **3** using the Johnson-Corey-Chaykovsky reaction[6], since now not only aromatic, but also aliphatic monofluorocyclopropylsulfones **3** can be obtained.

Herein, we report a simple one step synthesis of monofluorocyclopropylsulfones **3** by reacting monofluorocyclopropylsulfinates **1** with primary or secondary alkyl halides **2**.



Scheme 1. Synthetic application of monofluorocyclopropylsulfinate **1**

Acknowledgements
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Targeting triple-negative breast cancer cells with a β1-integrin binding aptamer

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Targeted therapies have increased the treatment options for triple-negative breast cancer patients. However, the paucity of targetable biomarkers and tumor heterogeneity have limited the ability of precision-guided interventions to live up to their full potential. As affinity-targeting ligands, aptamers show high selectivity toward target molecules. Compared with antibodies, aptamers have lower molecular weight, increased stability during transportation, reduced immunogenicity, and increased tissue uptake. Recently, we reported discovery of the GreenB1 aptamer, which is internalized in cultured triple-negative MDA-MB-231 human breast cancer cells. We show that the GreenB1 aptamer specifically targets β1-integrin, a protein linked previously to breast cancer cell invasiveness and migration. Aptamer binds to β1-integrin with low nanomolar affinity. Our findings suggest potential applications for GreenB1-guided precision agents for diagnosis and therapy of cancers overexpressing β1-integrin.

Styrylpyridinium derivatives for fluorescent cell imaging

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Styrylpyridinium (SP) salts are used as fluorescent probes for biochemical, biophysical and molecular biology studies.[1,2] The development of a new class of dyes with desirable photophysical properties is a challenge for researchers working in this field.

The aim of the study was the synthesis and evaluation of physicochemical, self-assembling and biological properties of a set of styrylpyridinium derivatives as prospective cell imaging agents.

Set of original styrylpyridinium dyes with variation of aldehyde and N-alkyl moieties was obtained (Fig. 1A).

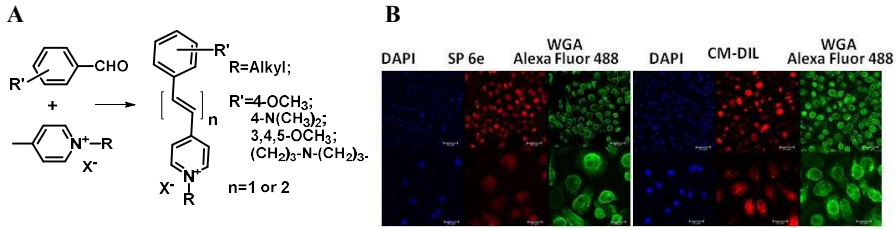

Figure 1. **A**: Synthetic scheme for preparation styrylpyridinium dyes; **B**: Confocal microscopy images of bone marrow-derived macrophages pre-stained with SP **6e** (5 μM), DAPI, (blue) CM-Dil and WGA-Alexa-Fluor 488 (green) dyes.

Compounds have rather high cytotoxicity to cancerous cell lines HT-1080 and MH-22A; at the same time, basal cytotoxicity to NIH3T3 fibroblast cell line is in range from toxic to harmful. Compounds showed large Stokes' shifts from 195 nm to 352 nm. Cell staining with SP **6e** revealed the strong fluorescent signal localised in the cell cytoplasm, whereas the cell nuclei were not stained (Fig. 1B). SP **6e** possesses self-assembling properties and forms liposomes with an average diameter of 118 nm. Obtained novel data on near-infrared fluorescent probes could be useful for the development of biocompatible dyes for biomedical applications.

Acknowledgements: Funded by the Latvian Council of Science project *lzp-2021/1-0283*

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An agrocin 84 toxic moiety (TM84) analogue is a malarial threonyl tRNA synthetase inhibitor

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More than half of the world's population lives in malaria endemic areas, rendering this disease a global health problem and highlighting the urgent need for new drugs to prevent and cure malaria.[1] Aminoacyl t-RNA synthetases have received considerable attention as possible targets for the development of anti-infective agents,[2,3] including antimalarial drugs.[4,5] However, *Plasmodium falciparum* ThrRS (PfThrRS) inhibitors are still limited to borrelidin and its analogues.

Hence, to expand the range of putative malarial ThrRS inhibitors, we designed a threonine-containing analogue (**4**) using a toxic moiety of agrocin 84 (TM84, **3a**), which is a known *E. coli* LeuRS inhibitor, as a scaffold. Analogue **4** showed submicromolar inhibitory potency against PfThrRS (IC50 = 440 nM), which shows its potential as malarial PfThrRS inhibitor. Comparison of the obtained crystal structure of the *E. Coli* ThrRS (EcThrRS) in complex with inhibitor **4** and EcLeuRS in complex with compound **3a** revealed several structural differences between the binding of the two compounds to their host proteins. These findings provide important structural information that can be used for the future design of novel ThrRS inhibitors.

Acknowledgements

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Tailoring FPOX Enzymes for Enhanced Stability and Expanded Substrate Recognition

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Enzyme engineering is a tailoring process that allows the modification of naturally-occurring enzymes to provide them with improved catalytic efficiency, stability or specificity. By introducing partial modifications to their sequence and to their structural features, enzyme engineering can transform natural enzymes into more efficient, specific, resistant biocatalysts and render them suitable for industrial processes.In our lab, we focus on a class of enzymes called Fructosyl Amino Acid Oxidases (FAOX), which are flavoproteins that catalyze the oxidation of fructosyl amino acids to form glucosone, amino acid and hydrogen peroxide which has major role in the management of diabetes, and specifically in the detection of glycated hemoglobin (HbA1c).However, naturally occurring FPOX are not able to detect HbA1c directly because these enzymes show no significant activity on intact proteins due to the buried active site and to the narrow tunnel that provides access to their catalytic pocket, depicted by the crystal structures of FAOX and FPOX enzymes. Hence, the need to expand their substrate range by enzyme engineering. We applied a rational design approach to engineer a novel enzyme with a wider access tunnel to the catalytic site, using a combination of Rosetta design and molecular dynamics simulations.

We have been successful in designing several mutants shows a significantly wider and shorter access tunnel, relative to the wild-type (WT) enzyme. Upon experimental testing, engineered enzyme shows good structural stability and maintains significant activity relative to the WT. Also, the thermal stability of WT enzyme has been improved with the variants that have increased salt bridges, improved RMSF, improved native contacts, and disulphide bonds. We are determined the structures by X-Ray Crystallography of the engineered enzyme, studied its biophysical properties and determined its activity We are currently working to test the enzymes on glycated proteins and to produce nanofibers embedding them in a biomaterial for diagnostic application.

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Engineered thermostable PETase enzymes for plastic degradation

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The fast and uncontrolled accumulation of plastic waste in the environment has long begun to impact on the natural ecosystems and to pose an existential threat to all forms of life on our planet. Based on the evidence that, owing to the massive usage of plastic products worldwide and to their persistence in the environment, this rapid buildup will likely continue to escalate, advanced technical solutions to the plastic waste management problem are in urgent demand. In this respect, enzymatic degradation of polymeric materials holds great promises as new and more efficient enzymes are constantly being developed. We aimed at applying a computer-aided enzyme engineering approach to improve the efficiency of enzymatic PET degradation. Based on available crystal structures, we carried out molecular dynamics simulations to identify flexible regions of the enzyme and use this information to define enzyme variants with improved thermal stability, which we will test and validate in our laboratory.

We described an engineered Leaf-branch Compost Cutinase (LCC) that features enhanced PETase activity and thermal stability relative to the current gold standard (ICCG). Our LCC mutant shows a Tm > 98 °C and measurable activity beyond 6 days.

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Physical-chemical characterisation of chitosan-coated liposomes as putative delivery systems

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Various drawbacks of drugs, such as rapid clearance, suboptimal biodistribution, low intracellular absorption and toxicity which may limit their therapeutic efficacy, can be diminished by loading the drug into delivery systems (i.e. liposomes).[1] Decoration of liposomes with chitosan is one of the approaches to enhance of their physical-chemical and biochemical properties.[2] The coating mechanism of liposomes with chitosan is mainly based on electrostatic forces.[3] Our previous works demonstrated that polyfunctional pyridinium derivatives on the 1.4-dihydropyridine (1.4-DHP) scaffold formed liposomes and efficiently acted as gene delivery agents.[4] The influence of lipid head-groups, linker structure, and remotion of cationic moieties on transfection activity was also studied.[5]

Liposomes were formed by 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,4-DHP amphiphile composition with various lipids ratios (19:1; 10:1; 2:1). Chitosan derivatives for coating of nanoparticles were synthesised using various surface modifications, such as formation of carboxymethyl[6] or *N,N,N*-trimethylated chitosans[7]. The obtained chitosan derivatives were used to coat liposomes formed by DOPE and 1,4-DHP. Liposomes with various lipids ratios and chitosan-lipid weight ratios (1% - 50%) were studied with DLS and ITC methods. The influence on the stability and size distribution of formed nanoaggregates was evaluated. Thus, chitosan can be adsorbed on liposomes as much as 1:5 lipid:chitosan mass ratio according to ITC; the increase of the ratio of chitosan to total lipids (1:1 w/w) led to the rise of the particle average size comparing to the uncoated ones; stability studies were also carried out.

Acknowledgements
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Bridging the N-terminal and middle domains in FliG of the flagellar rotor

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Flagella are complex organelles that propel bacteria through the environment and contribute to virulence. Flagella consist of multiple ring structures that have self-assembly properties. The rotor traverses the cell membrane, and contains MS-ring built of FliF and C-ring, built of FliG, FliN and FliM. The FliG protien is central in the assembly of the rotor and subsequently the entire flagellum, torque transmission from stator to rotor and linking the C-ring to MS-ring. With no high resolution C-ring structure available the conformation of FliG in assembled state unclear due to variations in available crystallographic data. Molecular dynamics (MD) in this study is used to explore the conformation and dynamics of FliG in different environments. Our data shows that in the linker between the FliG N-terminal and middle domain likely adopts an extended helical conformation *in vivo* unlike what the contracted conformation observed in some of the known previously known crystal structures. We use our results to perform integrative modelling to create full-lenght model of FliG that is compatible with cryo-electron tomography (cryo-ET) and electron microscopy (EM) densities of the C-ring. Collectively, our study contributes to a better mechanistic understanding of the flagellar rotor assembly and its function.

Exploring the Molecular Recognition of Heart-Type Fatty Acid Binding Protein: Thermodynamics and Binding Specificity

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Heart-type fatty acid binding protein (FABP3) is a vital cytosolic lipid transport protein predominantly found in cardiomyocytes. FABP3 as an intracellular lipid chaperone also regulates the density of lipids in the tissues and affects metabolic pathways.

Recent studies propose that FABP3 is potent to bind both fatty acids (FA) and long-chain acylcarnitines (LCACs), thus reducing the toxicity of LCAC *in vitro*. In this work, we focused on the characterization of binding mechanism of LCAC and corresponding long-chain FAs (LCFAs) by nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC). We revealed that monounsaturated LCACs cause ligand-induced dimerization of FABP3. As well, significant deviations in heat capacity changes, ΔC_p, for LCFAs and LCACs were discovered. Thus, combination of ITC and NMR data revealed two distinct binding mechanisms for LCFAs and LCACs interaction with FABP3. Nevertheless, the binding affinities of the unsaturated LCACs were by one order of magnitude higher than those of the corresponding LCFAs (K_D of 2.2 and 0.2 μM, respectively), indicating that FABP3 has a significant preference toward binding of LCFAs and can easily replace LCACs, as was shown by our competitive binding studies.

Acknowledgements
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Photocrosslinked azo nanoparticles as potential photoacoustic imaging markers

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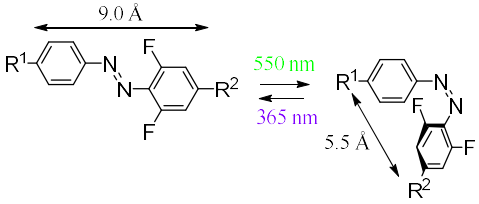
Photoacoustic imaging (PA) is an emerging and non-invasive technique that exploits the known "photoacoustic effect", based on the generation of acoustic waves after light excitation of chromophores. In order to dynamically follow up biologically events, high concentration of absorbing molecules is required.[2]

To overcome this limit, organic nanoparticles, comprising a high payload of dyes, have appeared as a promising alternative to molecular dyes in solution[3], but the corresponding signals can become very difficult to distinguish from the biological background. Thus, we focused our interest on azo photochromic compounds characterized by high photostability, significant geometry and vibrational motions in the excited state, to allow an enhanced PA imaging contrast.



Scheme 1. Volume changes in the photoisomerization process.

Azo NPs implied crosslinked architectures, to avoid disassembling of the nanoparticles, issued from dye self-assembling in water. Moreover, several crosslinkers, namely dithiol and dithiane compounds[4,5], have been synthesized to modulate the mechanical properties of the final nanostructures, and the photoacoustic signal thereof. We tested the photocrosslinking ability of the linkers with the azo chromophore in the solid-state, observing the formation of a polymer using dithiane ligands, while no reaction did occur for dithiol compounds.

Photochromism of azo dyes has been performed with dyes in solution or processed as nanoparticles. As a prospect, combined photochromic and photoacoustic investigations need to be performed to assess the impact of each photoreaction on the generation of acoustic waves.

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