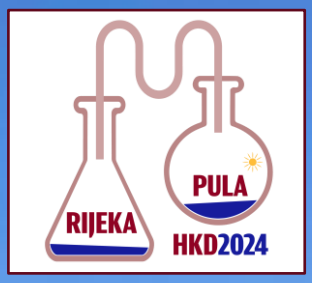

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SUMMER CONFERENCE OF CROATIAN CHEMICAL SOCIETY RIJEKA – PULA 2024

Ljetna konferencija Hrvatskog kemijskog društva Rijeka – Pula 2024

5 – 6 September 2024, Faculty of Biotechnology and Drug Development, University of Rijeka, Rijeka, Croatia

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BOOK OF ABSTRACTS

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On behalf of the Organizing Committee, it is my great joy to present a novel conference which from this year the Croatian Chemical Society and the Faculty of Biotechnology and Drug Development are organizing to gather chemists of different interests once a year, in the summertime, in a beautiful setting of Croatian Littoral and Istria.

After two successful Symposiums, that attracted a great number of scientists, this year we decided to organize a two-day conference in which we have more than two hundred authors from both the academy and industry.

The Summer conference of Croatian Chemical Society Rijeka-Pula 2024 is scheduled for September 5-6, 2024 in Rijeka, Croatia.

We look forward to welcoming you in Rijeka!

Vidorić Nikolina

Asst. Prof. Nikolina Vidović, chair

PROGRAM
THURSDAY

8:30 – 9:00	***Registration***
9:00 – 09:15	Opening addresses
9:15 – 10:00	**Kristina Sepčić** (Biotechnical Faculty, University of Ljubljana) ***Lipid-binding aegerolysin proteins from edible oyster mushrooms as bioinsecticides and membrane lipid markers***
10:00 – 10:30	**Aleksandra Maršavelski** (Faculty of Science, Zagreb) ***Designing Ancestral Enzymes to Understand and Enhance Plastic Degrading Activity***
10:30 – 11:00	**Valentina Bušić** (Faculty of Food Technology Osijek) ***Synthesis of biologically active derivatives of vitamins B6 and B3***
11:00 – 11:15	**Bojan Hamer** (Ruđer Bošković Institute, Center for Marine Research, Rovinj) ***Biomass production and ecosystem services of the mussel species Mytilus galloprovincialis: UPOV Cuvi vs Lim Bay***
11:15 -11:45	***Coffee break***
11:45 – 12:30	**Nađa Došlić** (Ruđer Bošković Institute, Zagreb) ***Strategies for modeling photochemical reactions: achievements, challenges and perspectives***
12:30 – 13:00	**Nikola Bregović** (Faculty of Science, Zagreb) ***Calorimetric characterization of reactions in solid-state - proton transfer, co-crystallization and host-guest complex formation***
13:00 – 13:30	**Petar Kassal** (Faculty of Chemical Engineering and Technology, Zagreb) ***Using Printed Electronics Technologies for Mass Production of Solid-state Ion-selective Electrodes***
13:30 – 13:45	**Nikola Biliškov** (Ruđer Bošković Institute, Zagreb) ***Thermal stability and transport properties of zinc zeolitic imidazolate lattices determined by internal vibrational dynamics***
13:45 – 14:00	**Katarina Lisac** (Ruđer Bošković Institute, Zagreb) ***Catalytic and magnetic study of monometallic and bimetallic MOF-74 materials with terephthalic and isophthalic linkers***
14:00 – 14:10	**Ahmed Dhifaoui** (JEOL EUROPE)
14:10 – 15:15	***Lunch break***
15:15 – 17:00	***Poster session***
17:00 – 18:45	***Trsat Castle guided tour and wine tasting***
19:30 – 22:30	***Dinner***

PROGRAM

FRIDAY

8:30 – 9:00	***Registration***
9:00 – 9:45	**Marin Roje** (Ruđer Bošković Institute, Zagreb) ***Isolation, Synthesis and Structural Characterization of Natural and Synthetic Compounds***
9:45 – 10:15	**Fabio Faraguna** (Faculty of Chemical Engineering and Technology, Zagreb) ***Enhancing the fuel quality with biofuels and additives: summary of the research project findings***
10:15 – 10:30	**Leo Frkanec** (Ruđer Bošković Institute, Zagreb) ***A self-assembled amino acid-based nanofiber hydrogel as a novel hydrolase model***
10:30 – 10:45	**Marko Purić** (Faculty of Science, Zagreb) ***Development of method for the synthesis of chiral multisubstituted cyclopentadienyl ligands***
10:45 – 11:15	***Coffe break***
11:15 – 11:45	**Daniela Kalafatović** (Faculty of Biotechnology and Drug Development, Rijeka) ***Pushing the boundaries of peptide discovery using machine learning-guided generative models***
11:45 – 12:15	**Adrijana Vinter** (Selvita, Zagreb) ***Revolutionizing drug discovery: unleashing the power of AI in Chemistry***
12:15 – 12:30	**Marko Babić** (Faculty of Biotechnology and Drug Development, Rijeka) ***Comparing the effectiveness of Coarse-Grained simulations in peptide self-assembly and aggregation with different structural inputs***
12:30 – 12:45	**Katarina Ćaćić** (Faculty of Science, Zagreb) ***Masked protective group in alkylation of unprotected indoles***
12:45 – 14:00	***Lunch break***
14:00 – 14:30	**Filip Lešić** (JGL d.d., Rijeka) ***Risk assessment and evaluation of potential N-nitrosamine impurities presence in pharmaceutical drug products, starting raw materials and the development of a corresponding mitigation strategy***
14:30 – 14:45	**Marko Gobin** (Ruđer Bošković Institute, Zagreb) ***Customized multisubstituted cyclopentadienes: innovations in design and synthesis***
14:45 – 15:00	**Lovro Vučetić** (Faculty of Science, Zagreb) ***Photoinduced chemo- and regioselective alkylation of phenols***
15:00 – 15:15	**Filip Duplić** (Ruđer Bošković Institute, Zagreb) ***Design and synthesis of chiral amine-based Cp-ligands***
15:15 – 16:45	***Round table*** ***Chemistry in the Age of Artificial Intelligence: Innovations, Collaborations, and Challenges***
16:45 – 16:50	***Closing ceremony***

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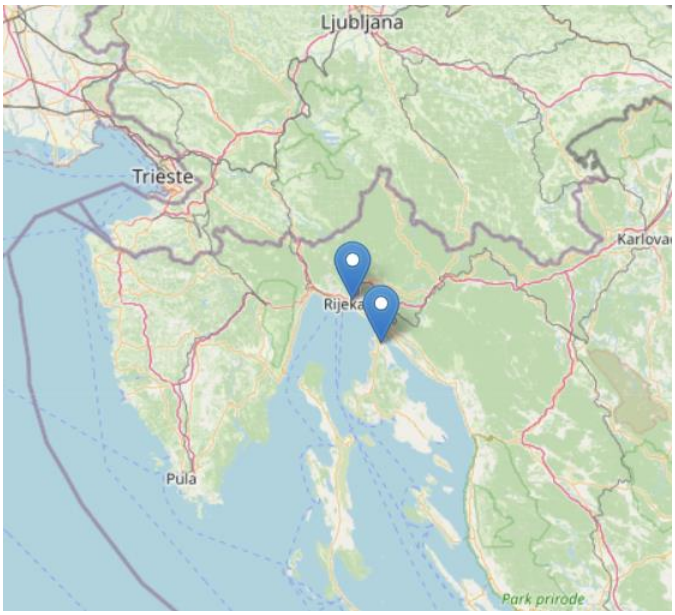

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LECTURES

PREDAVANJA

Lipid-binding aegerolysin proteins from edible oyster mushrooms as bioinsecticides and membrane lipid markers

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Lipid-binding proteins from the aegerolysin family, produced by mushroom genus *Pleurotus* (oyster mushrooms), specifically bind to sphingomyelin/cholesterol complexes in mammalian cell membranes. They also bind with high affinity to ceramide phosphoethanolamine (CPE), which is the major membrane sphingolipid of invertebrates (particularly insects and molluscs). Further, the genomes of *Pleurotus* mushrooms have nucleotide sequences that encode proteins with membrane-attack complex/ perforin (MACPF) domain. In the presence of a protein with a MACPF domain, *Pleurotus* aegerolysins can function as bi-component lytic complexes for target cell membranes.

These lipid-binding specificities propose several uses of aegerolysins or aegerolysin/MACPF lytic complexes. Fluorescent fusion derivatives of *Pleurotus* aegerolysins can be used for tracking raft-like membrane nanodomains composed of sphingomyelin and cholesterol. Moreover, the selectivity of some aegerolysin-based cytolytic complexes for increased membrane sphingomyelin/ cholesterol contents can be exploited for selective killing of urothelial carcinoma cells. Finally, due to their specific interaction with CPE, some cytolytic complexes based on *Pleurotus*-derived aegerolysins could represent a novel promising class of biopesticides for controlling selected economically important coleopteran pests: the Colorado potato beetle and the western corn rootworm. Indeed, transgenic potato plants in which an aegerolysin was targeted to the vacuole or to the cytoplasm, and its MACPF-partnering protein was targeted to the endoplasmic reticulum, showed a pronounced effect on feeding rate and subsequent development of Colorado potato beetle larvae. The best-performing transgenic lines did not differ from the non-transgenic control plants in their growth rate, overall phenotype, and in tuber production. Moreover, gene expression analysis of insect larvae exposed to aegerolysin/MACPF complexes revealed the response indicative of a general stress status of larvae and no evidence of possibility of developing resistance due to the functional inactivation of aegerolysin sphingolipid receptors.

Designing ancestral enzymes to understand and enhance plastic degrading activity

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The growing concern over plastic pollution necessitates innovative approaches for biodegradation [1], particularly for biopolymers such as polylactic acid (PLA) [2]. In this study, we identified a novel enzyme, termed MGY, with PLA-degrading activity from a metagenomic database. Despite demonstrating efficacy in PLA degradation, the MGY enzyme exhibited low solubility and poor yield, posing challenges for structural and functional characterization via crystallization.

To address these limitations, we employed ancestral sequence reconstruction as a strategic design tool. This approach enabled us to infer and produce ancestral variants of the enzyme. The reconstructed ancestral enzymes, representing three distinct evolutionary nodes, exhibited significantly improved solubility and expression yields.

Activity assay (Fig. 1) confirmed that all three ancestral variants retained robust PLA-degrading activity. Moreover, we successfully obtained these enzymes in higher concentrations, facilitating downstream applications and detailed structural studies. These findings underscore the utility of ancestral sequence reconstruction in enhancing enzyme properties and advance our understanding of biocatalysts for sustainable plastic degradation.

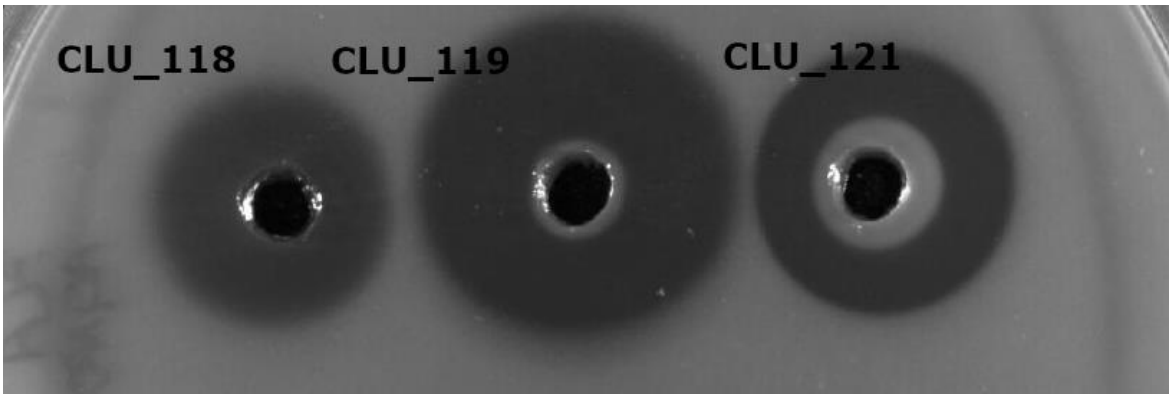

Fig. 1 : All three ancestral variants of MGY enzmye show robust PLA-degrading activity.

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Synthesis of biologically active derivatives of vitamins B6 and B3

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Vitamins B6 and B3 are water-soluble organic compounds that are essential to human life. They are multimodal due to their many and varied physiological functions, metabolism and chemistry. Vitamin B6 is a generic term that refers to pyridoxine, pyridoxal, pyridoxamine and their related phosphorylated forms. Coenzymes such pyridoxal phosphate and pyridoxamine phosphate are catalytically active form of vitamin B6 and acts in more than 140 different enzymatic reactions. They are involved in various biochemical reactions, including the metabolism of amino acids, carbohydrates, fatty acids, lipids, synthesis of nucleic acids, hemoglobin, synthesis of serotonin, neurotransmitters dopamine, noradrenaline and gamma-aminobutyric acid. The vitamin B3 is obtained from the diet in the form of nicotinic acid and nicotinamide which are transformed to nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate. These compounds participate in cellular oxidation – reduction reactions that are critical for energy production. Organic chemists focus their efforts on the development of different structural modifications of vitamin B6 and B3 in order to synthesize new derivatives with different biological activity. Molecules of pyridoxine and nicotinamide provide wide possibilities for chemical modification and enable various synthetic transformations. Therefore, it is not surprising that a large number of pharmacologically significant pyridoxine and nicotinamide derivatives have been synthesized in the past few decades. Numerous research efforts have resulted in the emergence of a number of approved drugs, as well as drug candidates that have reached the stage of clinical trials. Since the pharmaceutical industry is increasingly moving towards green production with the aim of curbing the further increase in costs, new paths in organic syntheses are being explored in accordance with the twelve principles of green chemistry. In this lecture, quaternization reactions on the pyridine nucleus of pyridoxal oxime and nicotinamide, will be presented. The syntheses of vitamin B6 and B3 derivatives have been performed by conventional synthesis and syntheses according to the principles of green chemistry: microwave-, ultrasound-assisted synthesis and mechanosynthesis. The most organic solvents used in conventional synthesis of pyridinium quaternary salts are volatile, hazardous and toxic like benzene, acetonitrile, acetone and DMF. These volatile organic solvents were successfully substituted with a greener variant in our research - non-volatile deep eutectic solvents. Among the numerous biological activities of pyridinium quaternary salts, they stand out as good antibacterial agents. Pyridoxal oxime derivatives are potential antidotes for poisoning organophosphorus compounds, while some nicotinamide derivatives possess antifungal activity.

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Biomass production and ecosystem services of the mussel species *Mytilus galloprovincialis:* UPOV Cuvi vs Lim Bay

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The mussel *Mytilus galloprovincialis* is an important commercial mariculture species and a powerful bioindicator used to monitor the distribution of contaminants in coastal waters. As part of the ERA-NET BlueBio – MuMiFaST project, this study aimed to demonstrate the concept of bioremediation of the local marine environment through ecosystem services provided by mussels near the UPOV Cuvi wastewater outfall and control site in the Lim Bay mariculture area. Mussels not only purify the water column and prevent the spread of contaminants but are also a valuable source of biomass (e.g. mussel meat) and other by-products of the circular economy. From undersized mussels grown from August 2022 to May 2023, we have produced commercial-size (>6 cm) mussels. After harvesting the mussels and determining their vitality and condition index [1], the produced mussel meat was analyzed for its nutrient content (proteins, carbohydrates, lipids) and potential contaminants (heavy metals, PAHs, PCBs) (Fig. 1). The comparison of the results of the nutrient and contaminant analyses leads to the conclusions that the mussel meat of the mussels from Lim Bay has a higher lipid-carbohydrate content (better quality) and that the mussel meat from both sites complies with the EU regulation on permissible contaminant levels in seafood [2].

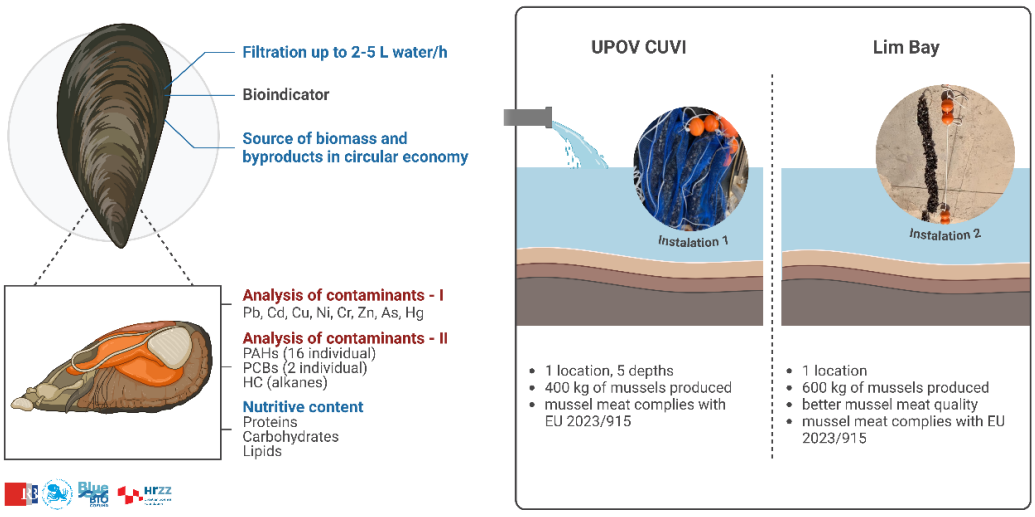

Fig. 1: Shematic diagram of adopted research methodology (created with Biorender.com).

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Strategies for modeling photochemical reactions: achievements, challenges and perspectives

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Photochemical reactions have been the focus of research interest for decades. However, photochemistry has only recently evolved from a relative niche topic to a major research field. From the theoretical side this transition has been driven alike by a plethora new concepts and painstaking advances in photodynamics methods.

Unraveling the mechanism of a photochemical reaction requires monitoring the electronic and structural transformations that the molecular system undergoes after light absorption. Through a series of examples of productive collaborations with experimental partners, we will illustrate how photochemical concepts are manifested in the experiments [1-3] and how the mechanisms of fundamental photochemical reactions, for instance the isomerization of cyclohexadiene, are often more complex than expected [4].

Also, I'll present our efforts to develop computational tools for simulating photoinduced processes and calculating spectroscopic signals, which are more accessible to non-experts. Useful information will be presented that can help interested researchers to perform their simulations and explore photochemical processes at their own pace.

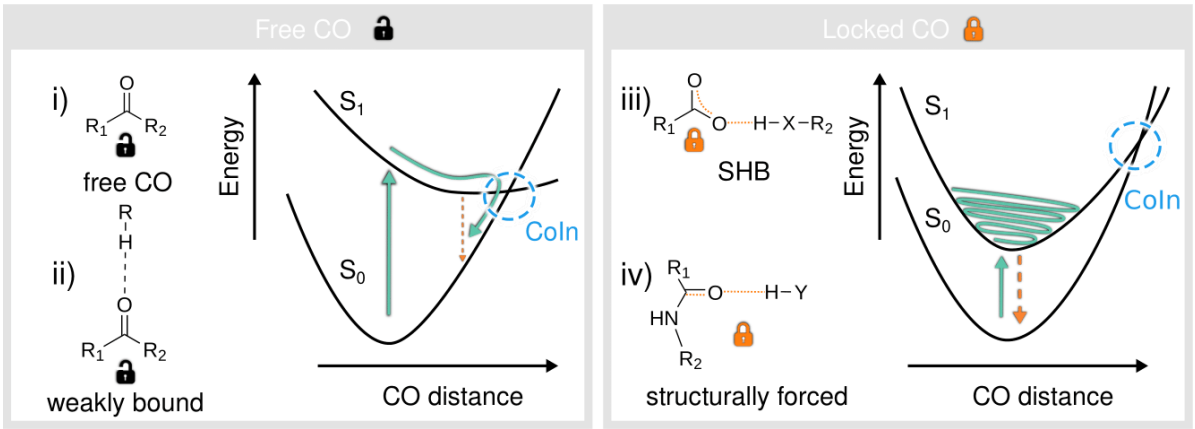

Fig. 1: The CO lock mechanism of non-aromatic fluorescence.

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Calorimetric characterization of reactions in solid-state - Proton transfer, co-crystallization and host-guest complex formation

Nikola Bregović,[1] Marija Cvetnić,[1] Robert Šplajt, Mirta Rubčić, Edi Topić
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During the last decades research of chemical transformations in solid state has received tremendous attention and the related studies provided greener, more efficient synthetic routes to otherwise inaccessible materials [1]. Despite the growing importance of solid-state reactions, their thermodynamic characterization has largely remained unexplored. This is in part due to the lack of well-established methodology for measuring the heat effects related to reactions between solid reactants. Our endeavor to address this research gap will be presented in this lecture, reviewing the achievements made so far, discussing the encountered difficulties and examining the potential further developments [2].

Isothermal calorimetry is arguably the method of choice for achieving direct thermodynamic studies of chemical reactions between solid reactants, yet no calorimeter has been specifically configured for such purpose. Therefore, as a first step we explored the potential of using a robust calorimeter with Calvet-type heat detector for the related measurements. Specifically, we used a device equipped with a cell originally designed for studies of dissolution processes demonstrating that it is indeed suitable for direct enthalpic characterization of a variety of mechanochemically relevant processes. This included proton transfer reactions, Baeyer-Villiger oxidation, cationic host-guest complex formation, and molecular co-crystallization showcasing versatility of the devised methodology. Reliability of the method was scrutinized by correlation with the values obtained via solution calorimetry applying the Hess law.

Throughout the lecture the focus will be on practical aspects of the methodology, stressing out implications of the results in the wider context, i.e. understanding reactivity in solid-state, aiming to communicate the importance of thermodynamic data for further methodical development of mechanochemistry.

Fig. 1: Cartoon representation of the calorimetric characterization of reaction between solid reactants.

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Using printed electronics technologies for mass production of solid-state ion-selective electrodes

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Ion-selective electrodes (ISEs) have come a long way from their bulky impractical designs with inner filling solutions: the inner electrolyte has been substituted with a solid contact, which should provide stable ion-to-electron transduction, 2D designs and flexible formats are being implemented to enable new application scenarios, and mass production strategies are being sought out to reduce fabrication costs and facilitate distributed sensing on a large scale [1]. Screen printing of electrodes is the state-of-the-art, but inkjet printing presents a greener and more cost-effective way of fabricating solid-state ISEs. The challenges associated with inkjet printing of electrodes will be highlighted in this talk. Secondly, a flexible inkjet printed ammonium ISE will be presented (Figure 1). The solid contact of the ISE is based on inkjet-printable mechanically exfoliated melamine-intercalated graphene nanosheets [2]. The developed ISE exhibits a linear response over 6 orders of magnitude, an LOD of 0.88 µM and was successfully used for determination of ammonium concentration in landfill leachate water, a very complex sample. However, the conventional ion-selective membrane has unsuitable physicochemical properties and cannot be inkjet printed. In the last part of the presentation, optimization of the ion selective membrane composition and its deposition with an automated fluid dispensing system (spotter) will be presented.

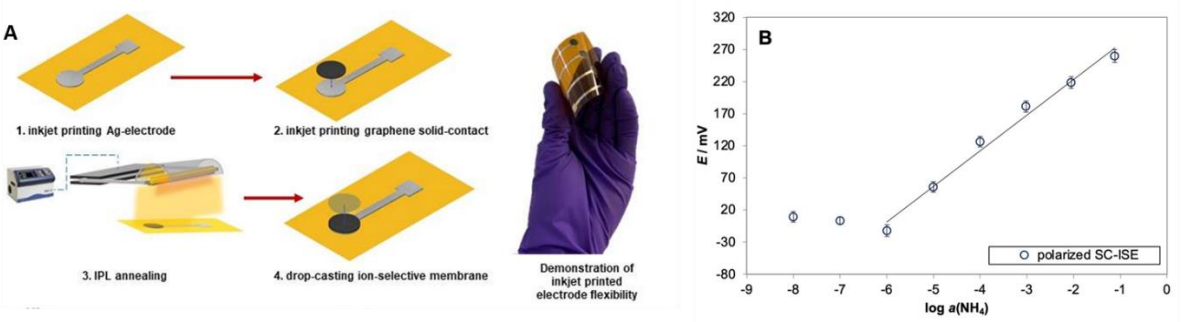

Fig. 1: Ammonium ISE fabrication via inkjet printing (A) and the ISE response (B).

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Thermal stability and transport properties of zinc zeolitic imidazolate lattices determined by internal vibrational dynamics

Nikola Biliškov,[1,2] Yonger Xie,[2] Hatem Titi [2]
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Thermal stability and kinetics of zeolitic imidazolate frameworks (ZIFs) are crucial for their applications as energetic materials. Here, the effect of microscopic vibrational dynamics on the thermal stability of ZIFs is demonstrated by using simple tools. Specifically, we explored the thermal kinetics based on Flynn–Wall–Ozawa and Kissinger's analysis of TGA measurements [1]. The study comprises a combination of structure-related effects such as topology, density, and alkyl substitution with respect to vibrational dynamics in ZIFs. The results exhibit a linear correlation between the vibrational dynamics of the linkers and activation energy, *I.E.* stabilization of ZIFs, in the polymorphic zinc(II) ethylimidazolate ($Zn(EtIm)_2$) series. At the same time, thermal destabilization was observed with the growing alkyl chain and was further probed by IR spectroscopy. Similarly, vibrational dynamics of alkyl functional groups of imidazole moieties plays a decisive role determining transport of bulky molecules encapsulated inside the pores of ZIFs, as illustrated by the example of C_{60}@ZIF-8 [2].

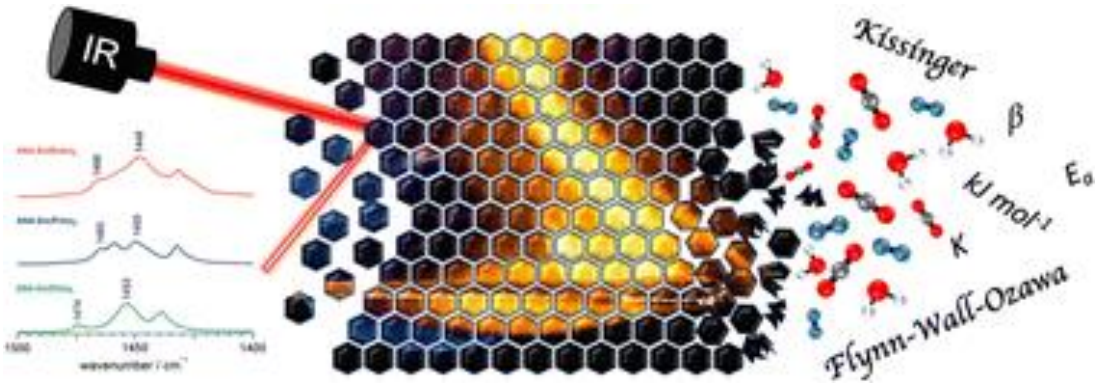

Fig. 1: Properties of ZIF-s as determined by IR spectroscopy and thermal methods.

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Catalytic and magnetic study of monometallic and bimetallic MOF-74 materials with terephthalic and isophthalic linkers

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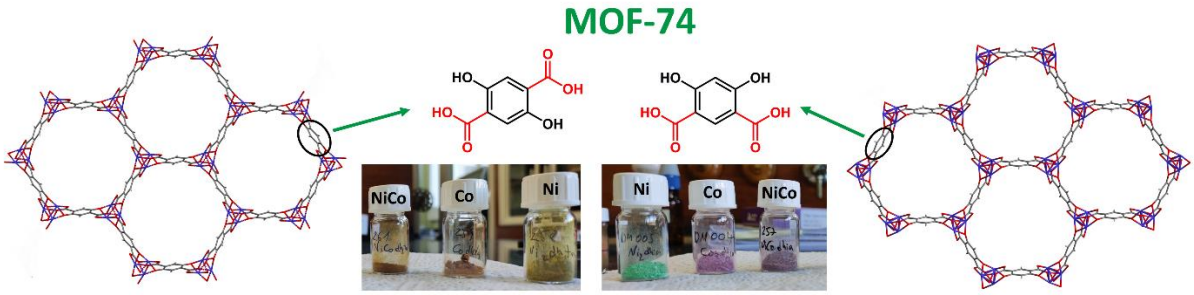
Metal-organic frameworks (MOFs) are a class of porous, crystalline solids constructed from polytopic organic linkers and metal nods. In MOF-74, divalent metal cations are bridged by organic linkers, fully deprotonated 2,5-dihydroxyterephthalic acid, and organized in 1D rod-like oxometallic chains along the crystallographic *c* axis forming a highly porous and stable structure with hexagonal channels in a honeycomb arrangement.[1] These materials are extensively studied because of their outstanding properties and strong application potential (adsorption, gas separation and storage, catalysis, etc.). The mixed-metal MOF-74 materials are particularly interesting due to their improved magnetic and catalytic properties.[2]

Herein, we present a study of monometallic (Co-MOF-74 and Ni-MOF-74) and bimetallic (NiCo-MOF-74) isostructural MOF-74 materials based on 2,5-dihydroxyterephthalic acid (dhta) and its structural isomer 4,6-dihydroxyisophthalic acid (dhia) as organic linkers. We showed that these two groups of isostructural materials have significantly different thermal, magnetic, and catalytic properties. Furthermore, we report the first controllable synthesis of bimetallic NiCo-MOF-74-dhia combining a previously published stepwise method which exploits a nickel-dhia coordination oligomer as an intermediate [3] and thermo-mechanochemical approach. Within this MOF series, bimetallic NiCo-MOF-74-dhia stands out as a superb catalyst for degradation of a lignin model compound compared not only to monometallic MOF-74-dhia and MOF-74-dhta, but also to bimetallic NiCo-MOF-74-dhta.



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Isolation, synthesis and structural characterization of natural and synthetic compounds

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Biologically active compounds isolated from different terrestrial or marine organisms as well as their synthetic analogues, in general represent important structures of biomarkers and potential novel effective therapeutics [1]. With increasing frequency, new drug candidates being introduced into pharmaceutical drug pipelines are chiral. The introduction of molecular chirality along with proper structure determination adds additional bottleneck in drugs development.

The lecture will present the results of research in the field of chemistry of natural products and their synthetic derivatives; (*S*)-lactic acid as a biomarker [2], structures possesing β-lactam or hydantoin ring [3], marine polysaccharides [4], and marinoaziridines A and B from marine bacteria [5]. Since all disclosed molecules are chiral, a special emphasis will be put on methods for their structural characterization and/or enantioseparation as prerequisite for their absolute configuration (AC) determination. Since chirality is closely related to biological activity in living organisms, exact AC determination is of outmost importance in medicinal chemistry. The results of conducted biological tests will be also shared for each of the mentioned structures.

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Enhancing the fuel quality with biofuels and additives: summary of the research project findings

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Focus of the lecture will be on the results of the HRZZ Installation research project Development of functional biofuels and (bio)additives and characterization of blends with mineral fuels (FunBioFA). Results will be presented for each of the five main project objectives: (1) investigation of the influence of the reaction parameters on conversion of transesterification reaction for the synthesis of various alkyl esters; (2) development of the novel purification procedures for biodiesel purification; (3) analysis of the properties of novel biofuels, their blends with conventional fuels and testing compliance with automotive standards; (4) engine performance tests and assessment of the influence of biofuel addition to conventional fuels; and (5) development of new (bio)additives for fuels. Within the project more than 10 different potential biofuels, several bio-additives and more than 10 polymer additives were developed. Further implications of the results for future research and outlooks will be discussed.

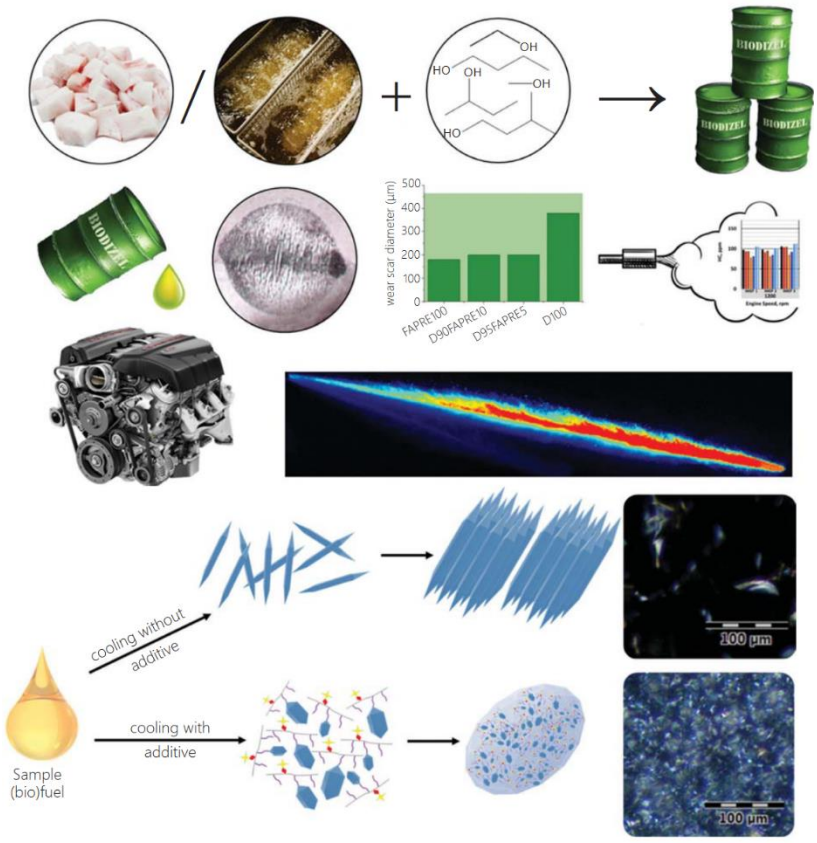

Fig. 1: FunBioFA project objectives: biofuel synthesis (first row), tests for compliance with automotive standards (second and third row); influence of polymer additives on fuel properties (fourth and fifth row).

A Self-Assembled Amino Acid-Based Nanofiber Hydrogel as a Novel Hydrolase Model

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A recent study in the field of supramolecular gels has advanced the development of distinct areas within materials chemistry, particularly focusing on self-organizing soft materials. These materials present significant opportunities for specific applications across various fields, including materials chemistry, pharmaceuticals, the food and cosmetic industries, as well as tissue engineering and regenerative medicine. [1] Natural enzymes are a type of highly efficient biocatalyst. In the past several decades, considerable efforts have been made to employ various strategies to mimic natural enzymes, recently been shown that the matrix of fibrous nanoparticles formed by the self-assembly of peptide-based hydrogels can serve as a highly efficient catalyst for ester hydrolysis. [2-5] A series of lipopeptides (**1** -**4**) was designed to study their organocatalytic properties towards ester hydrolysis and the role of their self-assembled structures in catalysis (Figure 1.).

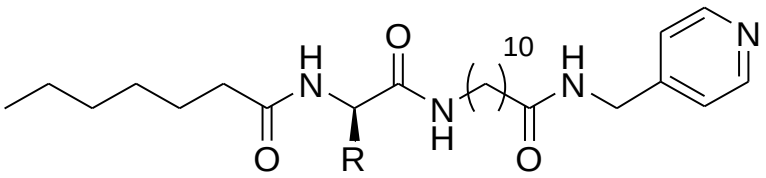

Fig. 1: Structural formulas of low-molecular-weight hydrogelators **1**-**4** (R = benzyl , phenyl, imidazole, indole)

Hydrogelators with catalytic activity were synthesized by incorporating fatty chains on amino acids (L-Phe, L-Phg, L-His, and L-Trp) at the C-terminal position and the N-terminal end, giving them amphiphilic character and self-assembly properties. Histidine and tryptophan were inserted into the compound to enable organocatalytic activity. Self-assembly organization was demonstrated by determining critical aggregation concentrations, followed by characterization of aggregates using spectroscopic and microscopic methods. Variations in the structures (amino acid composition, hydrophobic character) led to the formation of different aggregates, ranging from globular objects to fibers. Derivatives containing histidine showed catalytic activity for the hydrolysis of *p*-nitrophenyl acetate in aqueous solution. The influence of self-organization on catalysis is demonstrated by the different behavior observed between monomers and aggregates.

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Development of method for the synthesis of chiral multisubstituted cyclopentadienyl ligands

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Efficient chiral cyclopentadienyl ligands, whose chirality originates from the 1,2-substitution of a fixed chiral backbone such as mannitol or BINOL, have been known since 2012 and 2013, respectively. Transition metal complexes of these cyclopentadienyl ligands have been successfully used in numerous stereoselective transformations.[1] Recent research suggests that additional substitution of the cyclopentadiene ring positively influences the modulation of the stereoelectronic properties of the catalytic pockets of these complexes.[2] Such a dependence of multisubstitution and the stereoelectronic properties of catalysts indicates the need for parameterization of this relationship, as well as the need for newer and more efficient methods for the synthesis of these cyclopentadienyl ligands. This study approached the development of a methodology with the basic hypothesis that such multisubstituted cyclopentadienyl ligands can be prepared by the reaction of non-terminal diyne with low-valent organozirconium(II) species (Negishi's reagent), generating corresponding zirconabicyclic intermediates.[3] The planned synthetic route begins with the synthesis of the diynes with already decorated sidewalls at the 3,3'-positions, which have been found to be a fundamental prerequisite for the efficiency of chiral cyclopentadienyl ligands. The synthesis concludes with a *one pot* cyclization of the diynes with the Negishi's reagent, followed by cross coupling with an alkyl-diiodide (diiodomethane) catalyzed by copper(I) chloride in the presence of DMPU as shown in Figure 1. The product is a chiral multisubstituted cyclopentadienyl ligand with an extended sidewall and an added frontwall.

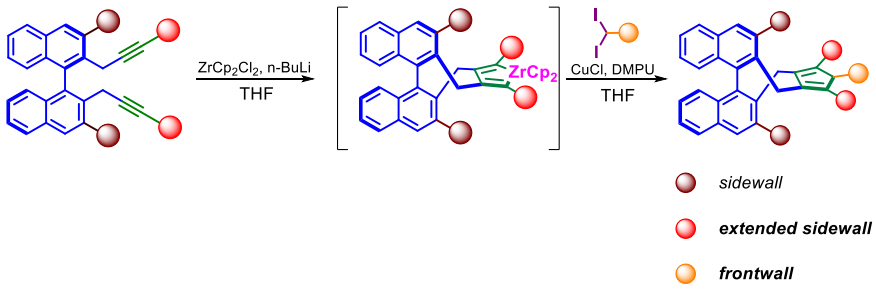

Fig. 1: Planned route for chiral multisubstituted cyclopentadiene ligand synthesis.

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Pushing the boundaries of peptide discovery using machine learning-guided generative models

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The discovery of new active peptides (i.e., antimicrobial, antiviral, catalytic) is challenging, as they are part of a very large search space and the correlation between the peptide sequence and the desired activities and/or functions is not yet fully understood. To avoid expensive and time-consuming guesswork and experimental failure, our strategy is to apply machine learning (ML)-based predictions in combination with genetic algorithm-based optimizations to accelerate peptide discovery. Search-based algorithms allow for a faster exploration of peptide permutation space which grows exponentially with peptide length and whose amount and dimensionality is too overwhelming to rationally comprehend. ML can find patterns or regularities in data, build mathematical models based on the theory of statistics and make up for the lack of knowledge. To date, both strategies have been applied to a variety of chemical problems to maximize the chance of successful and rapid solving of complex issues. One of these complex combinatorial problems is the prediction of peptide self-assembly and the assignment of potential new functions to sequences deriving from unexplored regions of the peptide search space.

One of the challenges in applying ML to peptides is the lack of large, balanced, and well-structured datasets. For this purpose, we manually curated datasets of catalytic [1] and self-assembling peptides and used those of therapeutic ones from the literature. Another challenge is a variety of representation schemes with different levels of information and complexity that can be applied and that yield different prediction results [2]. For this purpose, we developed a new sequential properties representation scheme that combines physicochemical properties with the amino acid order within the sequence [3]. We used both FASTA and SMILES representations and optimized the data preprocessing step by reducing the number of important features necessary for efficient peptide classification based on large and small datasets [4]. Furthermore, we developed a flexible and adaptive model based on MLdriven genetic algorithm that allows for a directed search of the sequence space by promoting catalytic activity [5] or self-assembly propensity [6]. The generated sequences are under investigation using MD simulations and experiments, leading to new knowledge that can be used to amplify the existing datasets and efficiently complements human intuition in the exploration of self-assembling peptides [6]. This research presents an important step in the development of intelligent laboratories for accelerated material discovery.

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Revolutionizing drug discovery: unleashing the power of AI in Chemistry

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Artificial intelligence (AI) is revolutionizing drug discovery, making the process faster, more affordable, and more efficient. This lecture will give an overview of the current market situation and how AI is being used in chemistry to develop new medicines. It will also discuss how companies like Selvita are at the forefront of this transformation.

Traditional drug discovery is often slow, expensive, and prone to failure. AI provides a powerful solution by quickly analyzing large data sets, predicting how molecules will behave, and identifying promising drug candidates with greater accuracy. Case studies will be presented, including also on how AI, although a powerful tool, is not almighty in the drug discovery process.

We will discuss how Selvita applies these AI technologies in drug discovery by optimizing the properties of potential drugs and predicting possible side effects.

This lecture will emphasize the crucial role of collaboration between AI experts and chemists, showing how Selvita's interdisciplinary approach is leading to groundbreaking discoveries. By the end of the session, participants should understand how their expertise, combined with AI, is transforming drug discovery and what the future might hold for this exciting field.

Comparing the effectiveness of Coarse-Grained simulations in peptide self-assembly and aggregation with different structural inputs

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Nowadays, peptide-based materials find applications in nanotechnology, medicine, and industry. Initially, their discovery heavily depended on experimentation and trial and error approach with the aim of understanding sequence and propensity relationships to form supramolecular assemblies, until molecular dynamics (MD) offered a quicker and cheaper opportunity to rationalize their design. Existing coarse-grained (CG) MD methods focus on modeling the aggregation propensity of short peptides, mostly two to six amino acids long. When aggregated, these peptides often form β-sheet-like supramolecular assemblies [1–3]. Consequently, the structural parameter in these simulations is set to an extended β-sheet which might bias results for longer peptide sequences that can exhibit more heterogeneous structural features, leading to inaccurate aggregation propensity (AP) estimations.

The simulation setup for testing the impact of input structures on hexa- and longer peptides is based on methods adapted from the literature, that implemented a Martini 2.2p force field, with 120-200 peptides, depending on their length, per 20x20x20 nm box filled with polarizable water. Simulations were performed using four different inputs: two secondary structure DSSP flags: extended β-sheet and unstructured; an input based on cluster analysis; and PepFold3-generated inputs. The cluster analyses found the most dominant structure with most 'near neighbors' of a 10 ns atomistic simulation and used them as the starting structure for CG simulations. The results are analyzed visually and using AP scores based on the change of solvent-accessible surface area (SASA) during simulation and peptide contact maps.

This study showed the impact of using different structure inputs on the aggregation morphology, AP scores, and SASA plateau times. It showed possible advantages of using near-native structures from structure predictions as inputs for CG modeling. This can expand MD applications towards longer peptide sequences and help to extrapolate supramolecular behavior patterns through statistical analysis or machine learning algorithms.

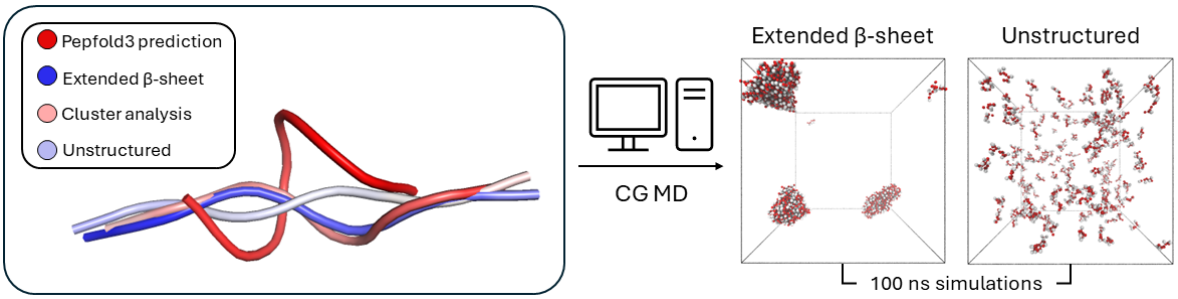

Fig. 1: Different input structures for IMGIIA hexapeptide might result in different aggregation behaviors.

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Masked protective group in alkylation of unprotected indoles

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The C3 position on indole has the highest electron density and is the most nucleophilic site for electrophilic substitution. However, the N-H position can also be functionalized under basic conditions.[1] The competitive C3/N-H regioselectivity is typically resolved by protecting the N-H functionality with standard protective groups, steering functionalization towards the C3 position or cyclopropanation of the double bond depending on their electronic nature. Previous research shows that electron-donating groups at the N-atom increase the nucleophilicity of the C3 atom, resulting in C3 alkylation, while electron-withdrawing groups favor cyclopropanation. This reliable approach requires at least two additional synthetic steps (protection/deprotection), increased material consumption, and lower overall yield. This research aims to circumvent these issues using a masked protective-group approach. ^{1}H and ^{13}C NMR were used to determine the nucleophilicity of the respective indole positions (C2, C3, and N-H). Indole's interaction with certain additives affects the NMR shifts, reflecting the electronic character and nucleophilicity of the atoms of interest. The presence of an additive such as Et_3N results in a downfield shift of the N-H proton and an upfield shift of the C3 signal, indicating increased nucleophilicity at the C3 position. This suggests that an additive could enable regio- and chemoselective alkylation. A reaction between indole and diazoester under blue light irradiation was used to test this methodology. While previous attempts to functionalize unprotected indole under the same conditions were unsuccessful,[2] our results showed C-H and N-H insertion in the reaction between indole and diazoester, and exclusively C3-monosubstituted indole in the presence of an additive (Figure 1).

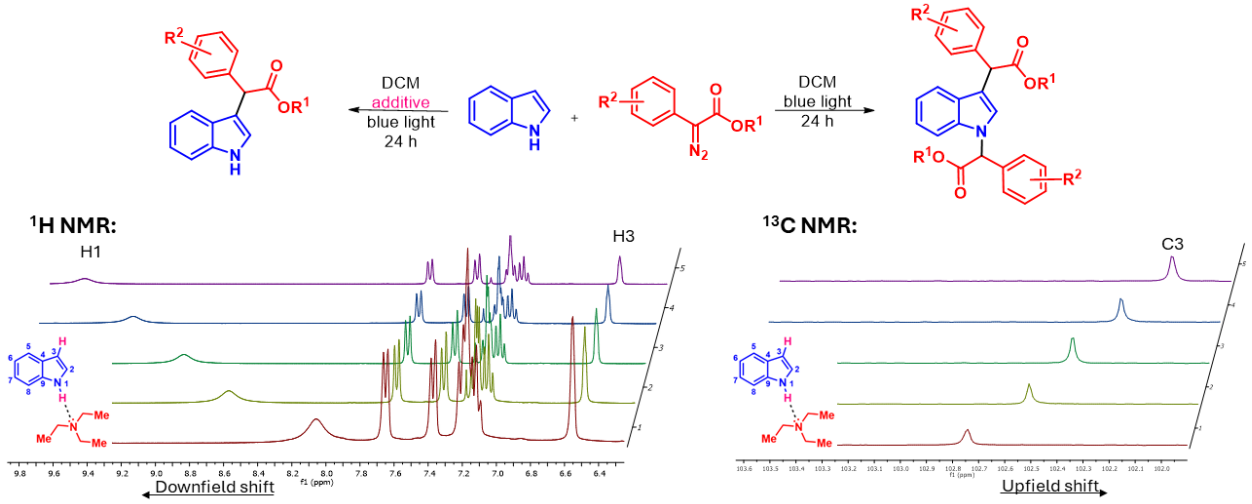

Fig. 1: Additive effect on alkylation of indole.

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Risk assessment and evaluation of potential *N*-nitrosamine impurities presence in pharmaceutical drug products, starting raw materials and the development of a corresponding mitigation strategy

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N-nitrosamines are a class of compounds that are known to be very potent carcinogenic with widespread occurrence throughout the human environment as well as food, water and drugs [1]. They can be formed when an amine and nitrosating agent are combined under favourable conditions although other generation pathways are also possible, such as oxidation and reduction processes from hydrazine-type compounds and *N*-nitro derivatives [2-3]. Recent drug recalls (valsartan and ranitidine) linked to the discovery of nitrosamine impurities have led to increased regulatory scrutiny and vigilance in the pharmaceutical manufacturing of drug products [3]. Hence it is mandatory for every drug product marketing authorisation holder to assess and evaluate the potential risk of *N*-nitrosamine impurities in its final drug products as well as the starting materials that are used in the manufacturing process such as the active pharmaceutical ingredient (API) or inactive pharmaceutical ingredient (IPI), intermediates, reagents and solvents. Besides starting materials and the final drug product, it is of high importance to evaluate and assess the entire manufacturing and packaging process as well, including all of the manufacturing and packaging equipment, cleaning detergents used in equipment cleaning and disinfection and consumables to make sure that there is no possible risk of *N*-nitrosamine presence or contamination. Finally, after all the risk factors are checked and the data is collected risk analysis is either concluded or a mitigation strategy is developed in case a possible *N*-nitrosamine contamination/presence is confirmed.

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Customized multisubstituted cyclopentadienes: innovations in design and synthesis

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The synthesis of certain types of cyclopentadienes requires adjusting the reactivity of specific functional groups, which poses a significant limitation in the selection of available methods. Furthermore, many current methods allow the introduction of only a small number of substituents, while those methods that prepare highly substituted (tetra- or pentasubstituted) derivatives remain a synthetic challenge. Given the diverse applications of cyclopentadienes as substrates in organic synthesis and their importance as ligands in transition metal chemistry, it is crucial to continue developing new methods for their preparation.[1] The chemical reactivity of low-valent organozirconium compounds has been extensively studied in various cyclization reactions of dienes, enynes, and diynes, generating the corresponding cyclic organozirconium precursors that have been used in the synthesis of cyclopentane, cyclopentene, and cyclopentadiene derivatives.[2] Although numerous literature sources describe these reactions, zirconocene-assisted cyclization of dienynes, which would provide a new precursor for further transformations, has not been investigated to date. In this work, a methodology for the preparation of multisubstituted cyclopentadienes through several convergent synthesis steps was developed. This strategy allows selective introduction and size-variation of functionalities on the cyclopentadienyl ring. The synthetic pathway begins with the regioselective bromoallylation of disubstituted alkynes **1**,[3] yielding the corresponding bromodienes **2** with a reactive carbon-halogen bond. This is crucial for performing the Sonogashira reaction with terminal acetylenes to generate non-conjugated dienynes **3**. Cyclization of dienynes with low-valent zirconium compound (Negishi reagent) resulted in cyclopentenes **4** with a pendant *exo*-cyclic double bond. In the final step, acid-catalyzed *exo*-to-*endo* double bond isomerisation afforded cyclopentadienes **5** (Figure 1).

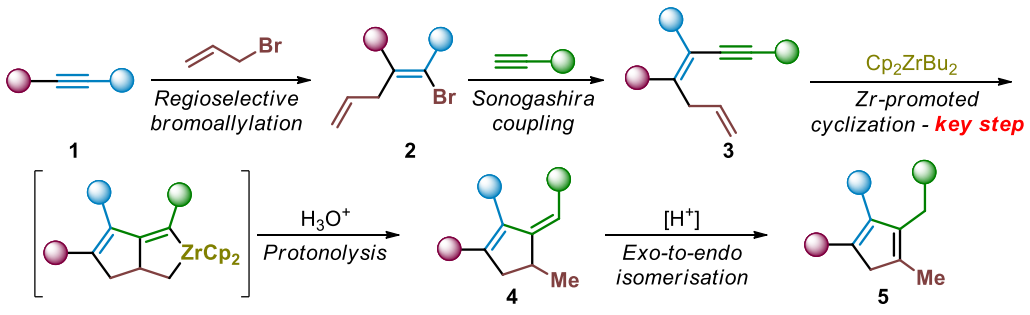

Fig. 1: Proposed synthetic strategy for the preparation of multisubstituted cyclopentadienes

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Photoinduced chemo- and regioselective alkylation of phenols

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Because of importance of phenyl motifs in pharmaceuticals and natural compounds, the transformation of readily available phenols into structurally more complex homologs is of great interest. Despite significant progress in metal carbenoid induced C-H functionalization of arenes, the direct C-H functionalization of free phenols using diazo compounds remains particularly challenging and surprisingly uncommon. This rarity is likely due to the fact that carbenoids often favor competitive O-H bond insertion over C-H functionalization.[1] Reactive diazoester carbenoid intermediates can be generated thermally (impractical due to harsh conditions), using transition metal catalysts, or in recent years photochemically using blue light which allows reactions to occur under mild conditions at room temperature without catalysts, addressing issues with high energy UV-light which compromised chemoselectivity.[2] The aim of this work is to develop the chemo- and regioselective functionalization of phenol and its derivatives by photoinduced C-H alkylation with diazoesters in *para*-position using blue light. This methodology is environmentally friendly and cost-effective. The resulting *para*-C-alkylated phenol can be further transformed into molecules of great interest. One potential synthetic application includes the synthesis of cannabinoid receptor (CB1) antagonists, which have therapeutic effects against drug abuse, addiction and help in regulating body-weight gain. Another potential application is in the synthesis of histone deacetylase inhibitors, which have demonstrated significant potential as a treatment option for various cancerous and non-cancerous diseases.[3,4]

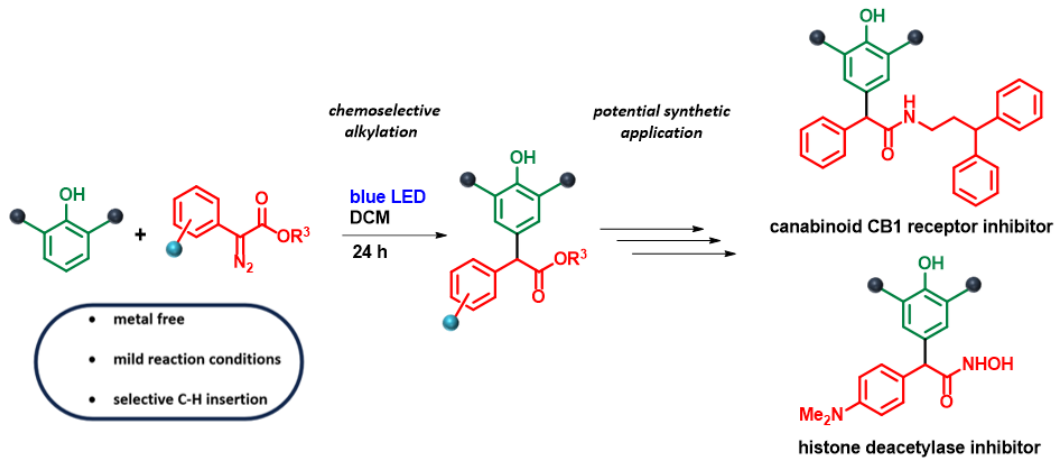

Fig. 1: para-Selective photoinduced alkylation of phenols

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Design and synthesis of chiral amine-based Cp-ligands

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Chiral amines play a pivotal role in catalytic asymmetric synthesis and they are providing chemists with new methods for the synthesis of complex molecules. They are commonly used as chiral ligands and, as such, have shown great promise in catalysing various stereoselective transformations [1]. Transition metals coordinated with cyclopentadienyl (Cp for short) ligands are very important complexes that have played critical roles in catalytic reactions. Cyclopentadiene derivatives can be prepared using Cp_2Zr^{II}- mediated pair-selective reductive coupling of diyne or two alkynes affording zirconacyclopentadiene intermediate. Subsequent intermolecular coupling with CH_2I_2 in the presence of CuCl and DMPU generates the desired cyclopentadiene derivatives.[2] It was shown that substituents on Cp ligands significantly influence the reactivity and catalytic efficiency of these transition metal complexes. Therefore, development of synthetic methods for cyclopentadiene derivatives containing different substituents is in great demand.[3] In this work, a methodology for the preparation of chiral amine-based Cp-ligands was developed. The synthesis begins with bis-propargylation of chiral amines **1**, forming the corresponding terminal acetylenes **2**. This step is followed by Sonogashira coupling reaction which generates diynes **3**. Cyclization of obtained diynes using the Negishi's reagent results in corresponding zirconacyclopentadiene **4**. The final step includes inter/intramolecular coupling with one-carbon unit affording chiral amine-based Cp-ligands **5** (Figure 1).

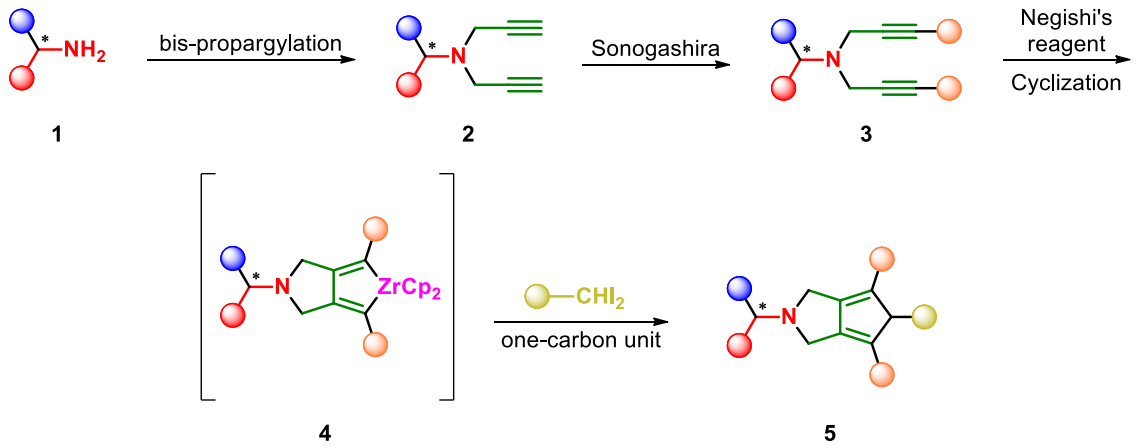

Fig. 1: Proposed synthetic strategy for the preparation of pyrrolidine Cp-ligands.

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ROUND TABLE

OKRUGLI STOL

Round table

Chemistry in the Age of Artificial Intelligence: Innovations, Collaborations, and Challenges

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The round table titled "Chemistry in the Age of Artificial Intelligence: Innovations, Collaborations, and Challenges" will convene experts from various sectors to explore the transformative impact of artificial intelligence (AI) on the field of chemistry. This discussion aims to provide a multidisciplinary overview of the current state, emerging challenges, and future directions for AI applications across industry, education, and research.

Industry experts will delve into how artificial intelligence is revolutionizing manufacturing processes, enabling faster innovation, and improving resource utilization. They will discuss how AI is facilitating the rapid implementation of new technologies, optimizing chemical reactions, and driving the development of novel materials, thus increasing overall efficiency and productivity.

From the perspective of education and pedagogy, professors and educators will examine the significant shifts in curricula resulting from the integration of artificial intelligence into educational processes. The discussion will highlight innovative approaches to teaching chemistry, where AI plays a pivotal role in enhancing students' understanding of complex concepts through personalized learning experiences and advanced simulations.

Researchers will share their insights on utilizing artificial intelligence for sophisticated chemical analyses and predictions, as well as automating routine laboratory tasks. This perspective underscores how AI is accelerating research processes and opening up new avenues for discovering groundbreaking solutions in the field of chemistry.

Lastly, students will provide their viewpoints on how artificial intelligence is influencing their education and shaping their future career opportunities. The conversation will address the challenges they face in acquiring new technological skills and their expectations for the evolving role of AI in the chemical profession.

The round table promises to offer a comprehensive overview of current trends, collaborative efforts, and the challenges associated with integrating artificial intelligence into chemistry. The goal is to inspire further adoption of AI in the field and to contribute to the development of new educational and industrial standards.

POSTERS

POSTERI

Synthesis of a graft copolymer based on cellulose derivatives with vinyl polymers for use as an API dispersant

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Many newly discovered active pharmaceutical ingredients suffer from poor water solubility which greatly diminishes their potential to be used as effective therapies [1,2]. One of the approaches to combat this is developing amorphous solid dispersions (ASDs) which inhibit the crystallization of APIs improving their water solubility [3]. Most common ASD dispersants used are different types of polymers and polysaccharides such as Eudragit (polymethacrylates) and cellulose derivatives such as hydroxypropyl methylcellulose [3,4]. In order to increase the stabilizing properties of the dispersant, grafting of polymers with certain functional groups can be performed. For this purpose, we have synthesized a novel graft copolymer based on a cellulose derivative grafted with vinyl and maleic anhydride (5 mol. %) copolymers with *N*,*N*-dimethylpyridin-4-amine (DMAP) as an acyl transfer reaction catalyst. The reaction was performed in *N*,*N*-Dimethylformamide (DMF) at 50 °C for 4 hours. The synthesized graft copolymers were subsequently characterized using FT-IR, DSC and TGA in order to confirm the grafting of the copolymers onto the cellulose derivative.

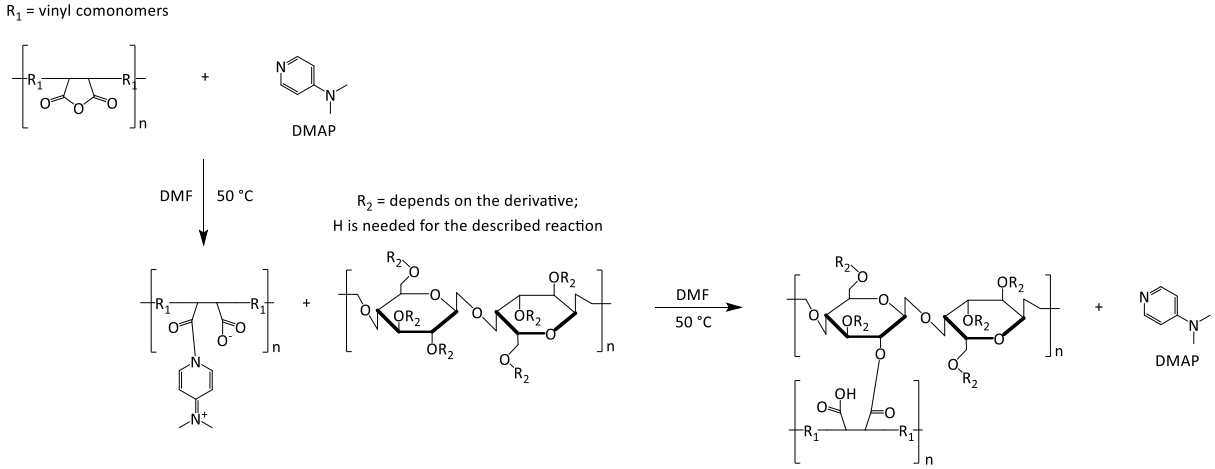

Fig. 1: Cellulose derivative grafting reaction scheme [5].

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Influence of sodium hyaluronate on the physical stability of hyalurosomes

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Hyalurosomes are nanoscale vesicles, similar to liposomes, composed of hyaluronate molecules and phospholipids [1]. These vesicles incorporate sodium hyaluronate (NaHA) within the phospholipid bilayer, forming an inter-lamellar hybrid structure. The initial phase of hyalurosomes consist of liposomes, lipid-soluble vesicles, with an aqueous core surrounded by one or more circular phospholipid bilayers [1]. NaHA in hyalurosomes is utilized for its strong intermolecular interactions and structured network, making it an effective drug carrier with biocompatibility, biodegradability, bioadhesive properties, non-toxicity, low immunogenicity, and viscoelasticity [1,2]. Therefore, it is hypothesized that hyalurosomes can enhance drug delivery compared to liposomes.

There are several ways to produce hyalurosomes and liposomes. The most common methods are: a thin-film hydration method that uses an organic solvent to dissolve lipids, a hydration method using a sonication bath, and a hydration method using ultrasonication. In this work we tested these three methods and found that a simple, eco-friendly method, free from any organic solvent employing ultrasonication produced the most uniform vesicles in terms of size, polydispersity index (PDI) and zeta potential. This method was used to prepare liposomes and hyalurosomes. To obtain hyalurosomes, phospholipids were hydrated with an aqueous dispersion of NaHA at room temperature (20-28°C), while liposomes were formed by directly hydrating phospholipids with purified water [1].

To compare the stability of these vesicles their average size, PDI, and zeta potential were monitored at 5°C and 25°C over a period of 30 days. The obtained results provide new insights important for understanding the use of hyalurosomes for drug delivery.

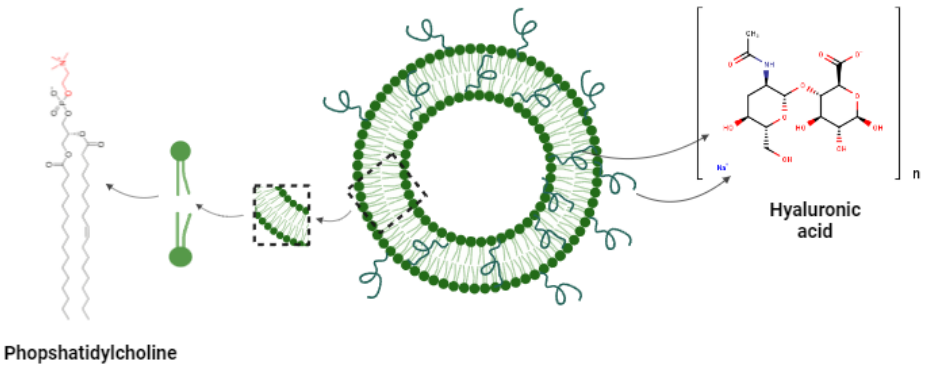

Figure 1. Schematic representation of hyalurosomes.

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The Physiological Effects of DPP3 Overexpression and Silencing in Hela Cells

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Dipeptidyl peptidase 3 (DPP3) is a zinc-dependent aminopeptidase that is widely present in various species and tissues [1]. It has been implicated in several physiological processes, including protein turnover, oxidative stress, pain, and inflammation [1]. DPP3 is highly expressed in cardiovascular organs such as the heart and blood vessels, and it has been associated with cardiovascular diseases like hypertension and heart failure. Additionally, DPP3 has been identified as a potential therapeutic target for cardiovascular diseases. It also regulates blood pressure by interacting with the renin-angiotensin system and plays a role in pain signalling [2]. Furthermore, DPP3 expression has been shown to promote cell proliferation, migration, and survival in vitro, as well as tumor growth and invasion in vivo, particularly in cancers such as esophageal carcinoma and colorectal cancer [3].

We examined the effect of DPP3 overexpression and silencing on the migration of HeLa cells using transwell and wound healing migration assays. In the transwell assay, cells were suspended in serum-free medium and placed in the upper wells of the transwell chamber, while the lower chamber was filled with medium containing FBS and EGF. Cell migration was evaluated by counting the number of cells that migrated through the membrane. The wound healing migration assay involved creating a linear wound in a cell monolayer, exposing the cells to specific conditions, and observing cell migration across the wound area. These assays provided insights into how DPP3 influences cell migration and proliferation in HeLa cells. Overexpression of DPP3 increased cell migration, indicating that DPP3 enhances cell motility. Conversely, when DPP3 was silenced, HeLa cell migration was reduced, suggesting that DPP3 plays a role in facilitating cell migration.

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Characterization of microorganisms adapted to petroleum hydrocarbon contamination

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Population growth and the intensive development of industrial activities have led to an increase in energy demand. Oil remains one of the most important sources of energy, and it is predicted that the daily demand for crude oil will reach 106 million barrels worldwide by 2030 [1]. Compared to traditional and expensive remediation methods, bioremediation technologies, which utilize the enzymatic capabilities of microorganisms to biodegrade contaminants in soil, have proven to be effective, economically viable and safe [2]. Petroleum hydrocarbons not only make the soil toxic and an unsuitable habitat, they also change the properties of the soil and most microorganisms cannot survive under such stressful conditions. However, some bacterial strains have developed adaptive mechanisms to thrive in unfavourable conditions, allowing their community to grow and multiply effectively [3]. To further optimize the bioremediation process, it is essential to characterize the microbial community capable of tolerating oil contamination and possessing the metabolic capabilities for hydrocarbon biodegradation.

In this study, bacterial strains were isolated from soils contaminated with petroleum hydrocarbons. In addition to morphological analyses, a series of biochemical tests were performed to verify the presence of certain enzymes in the isolated microorganisms. After 28 days of testing, the total number of viable microorganisms increased by 24%, indicating that the characterized bacterial strains can grow effectively in the petroleum hydrocarbon-contaminated substrate and are suitable for soil bioremediation.

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Removal of sulfamethoxazole using TiO_2-based molecularly imprinted polymers: characterization, adsorption, and photocatalysis

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Pharmaceuticals, especially antibiotics such as sulfamethoxazole, have become a common problem in the environment and have raised major concerns of environmental and health impacts. Sulfamethoxazole is a widely prescribed antibiotic which was found to be resistant to the conventional treatment methods for wastewater and thus tends to remain in the aquatic systems. This means that the presence of such antibiotics in water bodies leads to the emergence of antibiotic-resistant bacteria, which are dangerous to the health of both ecosystems and human beings. Traditional water treatment methods often fail to completely remove these contaminants, which creates the need to design new and advanced water treatment technologies. In order to overcome this problem, molecularly imprinted polymers (MIPs) have been investigated for their ability to selectively remove sulfamethoxazole from contaminated water sources. These recognition sites are created by polymerizing functional monomers in the presence of the target molecule, which acts as a template. After the polymerization process, the template molecule is removed, leaving behind holes that match the size, shape, and functional groups of the target pharmaceutical. In this research, TiO_2-MIPs were obtained via microwave-assisted synthesis using sulfamethoxazole as the template molecule on titanium dioxide (TiO_2) as the photocatalytic material. Inorganic-organic composite (TiO_2-MIP) has photocatalytic properties, allowing the degradation of adsorbed sulfamethoxazole under light irradiation. The combined action of adsorption with photocatalytic degradation was investigated in the dark and under UV-A light. Detailed characterization of the synthesized TiO_2-MIPs was done using various analyses to confirm the structure and activity of the material.

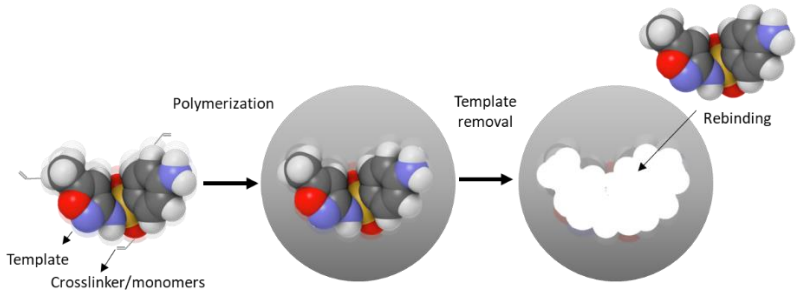

Fig. 1: Schematic representation of the imprinting process of sulfamethoxazole.

Acknowledgments: This work was funded by the Croatian Science Foundation under the project [HRZZ IP-2022-10-4400]: Development of molecularly imprinted polymers for use in the analysis of pharmaceuticals and during advanced water treatment processes (MIPdePharma).

Inclusion complexes of cinnarizine with β-cyclodextrin and its derivatives in solution: ITC study

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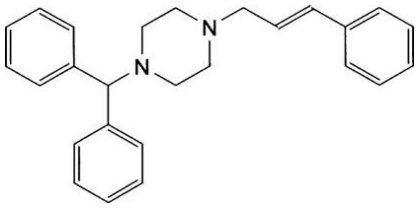
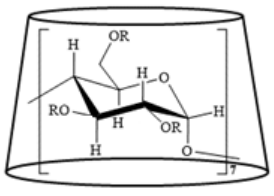
Cinnarizine (CIN) is a piperazine derivative with antihistaminic, antiserotonergic, antidopaminergic, and calcium channel blocking activity. The Biopharmaceutical Classification System (BCS) classifies it as a BCS class II drug, meaning it dissolves poorly in water and has high permeability [1]. These two properties of drugs for oral use are crucial for their bioavailability. Due to its low solubility, stability, and poor bioavailability, CIN is not an effective drug molecule from a physicochemical point of view. The low aqueous solubility of CIN can be improved by using biocompatible and biodegradable materials such as cyclodextrins (CDs) and their derivatives. β-CDs bind lipophilic drug moieties into their central cavity, forming inclusion complexes and thus improving the water solubility and bioavailability of drugs [2]. However, before the new formulations can be implemented, a comprehensive and detailed investigation of the system is required. As part of our ongoing research on supramolecular drug complexes, the complexation of CIN by β-cyclodextrin (β-CD) and its derivatives (hydroxypropyl-β-CD (HP-β-CD), randomly methylated β-CD (RM-β-CD) and sulfobutylether β-CD sodium salt (SBE-β-CD)) in aqueous medium at a pH of 4.5 (simulated duodenal medium) using isothermal titration calorimetry. The formation of 1:1 (CIN:CD) inclusion complexes was observed for all derivatives, and the corresponding reactions were thermodynamically characterized by determining and discussing the stability constants.





β-CD: R = H_{21}

HP-β-CD: R = H_{21-n} or $(–CH_2CH(OH)CH_3)_n$, n = 2–8; DS = 4.5

RM-β-CD: R = H_{21-n} or $(–CH_3)_n$, n = 9–15; DS = 12

SBE-β-CD: R = H_{21-n} or $(–(CH_2)_4SO_3Na)_n$, n = 3–7; DS = 6.5

Fig. 1. Structures of cinnarizine and β-cyclodextrins.

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Anion-binding competition between *p-tert*-butylcalix[4]arene derivatives: urea versus thiourea

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The molecular recognition of anions is a branch of supramolecular chemistry that is still blooming due to its high application potential.[1] Incorporation of functionalities providing hydrogen bonds (such as amine, amide, or urea) for binding anions is one of the key strategies for the preparation of efficient anion receptors. On the other hand, the calixarene backbone is an exceptional scaffold for building receptors for a variety of species due to ease of its functionalization.[2] Therefore, (thio)urea-derivatives of calixarenes have been designed as anion receptors, and they are still used in investigations oriented towards many biochemically interesting (an)ions.[3,4]

In the scope of this work, *p-tert*-butylcalix[4]arene bearing thiourea moieties was prepared (Figure 1), and its pK_a values were determined in acetonitrile. Complexation of several anions (Figure 1) was thermodynamically investigated by ^{1}H-NMR, UV/Vis, and ITC titrations, and the results were compared with anion-binding results for similar calix[4]arene bearing urea moieties (which we recently used for building pH-switchable heterodimers based on the urea-carboxylate interaction).[5] Thiourea won the competition for binding some anions, while for the others binding affinities of urea and thiourea were almost equal. Complexes of several different stoichiometries (receptor: anion = 1:1, 1:2, 1:3, 2:1) pointed out the importance of using different methods in anion-binding research. The possibility of proton transfer from the receptor to the anion was also accounted for in this investigation.

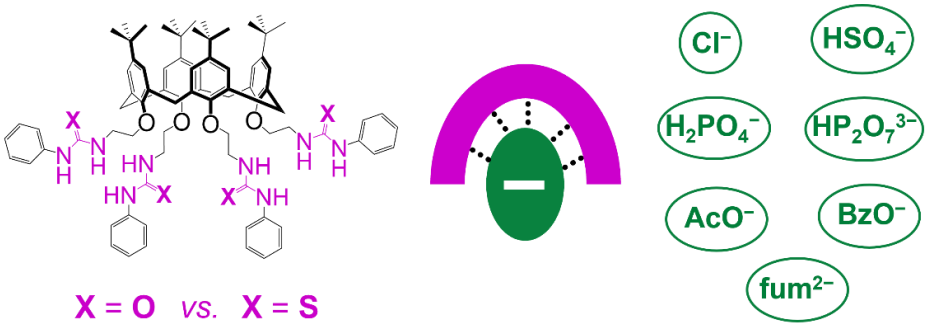

Fig. 1: (Thio)urea-derivatives of calix[4]arene in the role of anion receptors.

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Valorization of biowaste as a potential resource for biological recycling

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Waste is an unavoidable by-product of the modern economy. Organic waste is a resource that must be utilized. Dealing with organic waste is a major challenge when it comes to creating added value. When thrown away, it is a source of pollution, a threat to public health and the environment and a loss of value. Global consumption and demand for raw materials is increasing. The increase in human population has led to an enormous production of biowaste. A new look at known substrates can give new meaning to biowaste. Due to its high amount of organic and biodegradable components that can be recycled, biowaste is not only a major source of contamination, but also a huge source of useful materials [1,2].
In this work, the characterization of real biowaste samples was carried out to determine the potential for valorization. The COD and BOD_5 values were up to 25.6 g/L and 19.9 g/L, indicating a high content of organic matter. The average biodegradability index was 1.3±0.07, indicating that the biowaste can be biologically recycled. Biodegradable substrates contribute to the circular economy and sustainable practices by reducing the amount of waste, minimizing pollution and reducing the need for non-renewable resources.

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Spectrophotometric determination of pK_a values of new resveratrol analogs

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In our previous research [1], certain thiophene analogs of *trans*-resveratrol, known biologically active molecules, showed pronounced inhibition of the cholinesterase enzyme and antioxidant activity. In addition to the proven positive effects, further analysis of their properties is needed in order to investigate their functionality in more detail. Knowledge of the ionization of new drugs is extremely important because of the prediction of their behavior in physiological conditions when the ionization state strongly affects the solubility of drug at the site of application and its ability to diffuse through biological membranes [2]. The ionization constant is an important parameter in drug design and pharmacology as it affects four important aspects of drug development: molecular interactions, drug design factors, biopharmaceutics, and formulation [2]. pK_a values and related properties (log$D$ and solubility) has a profound impact on the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile of drug and are of great importance for the design of excipients and drug carriers [2, 3]. In this work, the ionization constants and corresponding pK_a values of two resveratrol analogs were determined in buffer solutions using the UV-VIS spectrophotometric method. Among the various methods for pK_a determination, UV–VIS spectrophotometry is undoubtedly the method of choice in terms of accuracy and reliability of results.

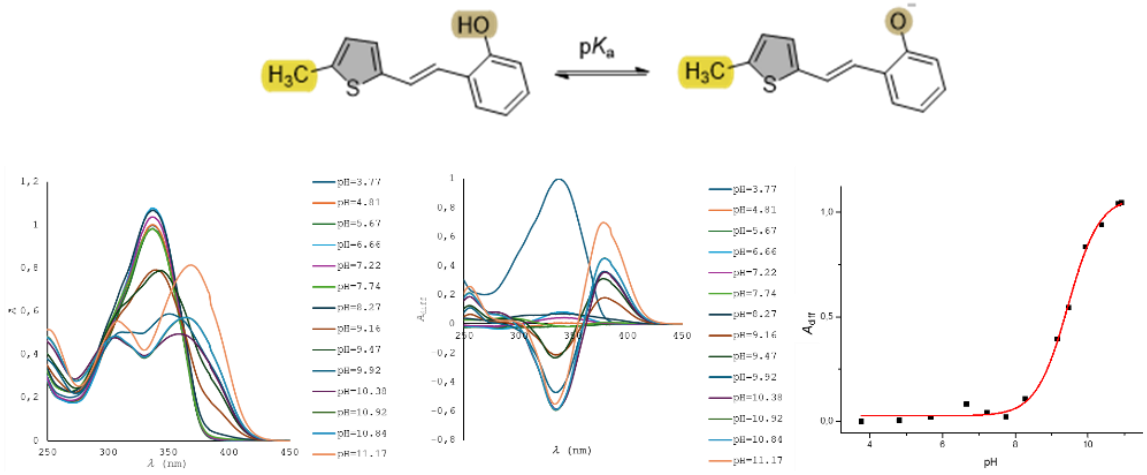

Fig. 1: Spectrophotometric determination of new resveratrol analogs.

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Structural characterization and ADME predictions of novel copper(II) complexes

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Knowledge of the structural characteristics of newly synthesized compounds is the basis for determining their physicochemical properties, such as the lipophilicity of the compound. Lipophilicity is a physicochemical property that affects the compound's solubility, absorption from the digestive tract, passage across the cell membrane, transport and passage across the blood-brain barrier, entry into the target organ and metabolization/excretion of the compound molecule (absorption, distribution, metabolism and excretion - ADME). Knowledge of the lipophilicity of new molecules, especially those exhibiting biological activity, is of great importance as it allows the control and adaptation of the synthetic pathway to develop the potential of the compound towards orally applicable drugs [1]. In this work, the ADME properties of three copper(II) complexes with chromone-2-carboxylic acid (K_1 – K_3) and one with maltol (K_5) were investigated (Fig.1). The log P values determined for the compounds (K_1, K_2 and K_3) were 1.35, 1.98 and 2.33 while the log P value for K_5 was 1.62. In principle, molecules with a log P value between 1.35 and 1.8 are candidates for oral administration with good absorption in the digestive system, while compounds with a log P value around 2 pass the blood-brain barrier well.

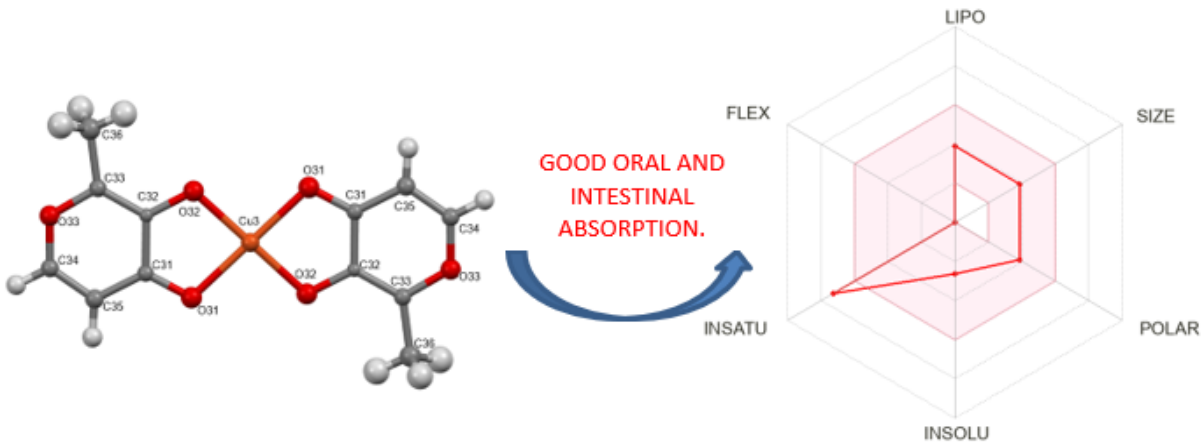

Fig. 1 : Bioavailability radar for the K_5.

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Covalent organic polymers with azo, azoxy and azodioxy linkages for CO_2 adsorption: a computational study

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Industrial development has led to an increase in carbon dioxide levels in nature, which has a negative environmental impact. The design of new materials for CO_2 adsorption is one of the forefronts in new functional systems research. Covalent organic frameworks and covalent organic polymers have shown wide applicability for selective CO_2 adsorption. A part of this research was done computationally, where six new compounds were built from two building blocks (triphenyl substituted amine and pyridine) connected with three nitrogen-nitrogen linkages (azo, azoxy and azodioxy) into 2D frameworks [1,2]. The stability of the crystal structures at different neighboring stacking configurations was examined computationally using periodic DFT methods in the CRYSTAL17 program. Optimized structures were subjected to grand-canonical Monte Carlo (GCMC) simulations in the RASPA program at temperatures of 298 and 273 K, and adsorption isotherms were compared. Using the VTK program, the distribution of CO_2 and N_2 molecules inside examined structures was visualized and the given data was compared with the results of the electrostatic potential analysis. While the analysis of electrostatic potentials can provide an insight into local binding sites, GCMC methods can give a lot of information about the adsorption properties of porous material. With the given results, azodioxy linkage appears to be a promising candidate in the synthesis of covalent organic polymers with enhanced CO_2 adsorption properties [3].

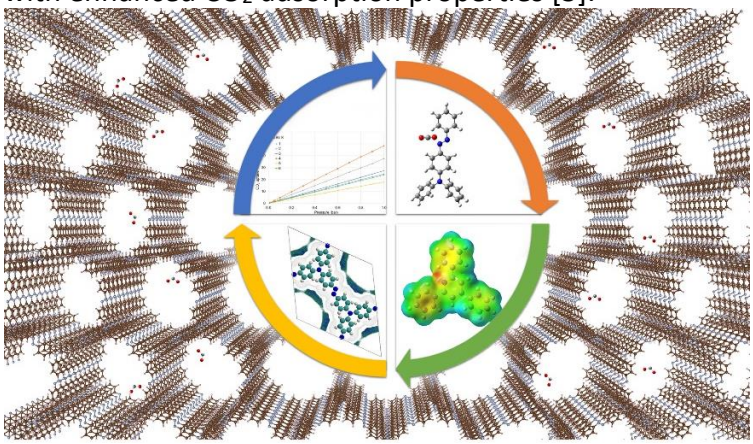

Fig. 1: Suggested computational strategy of porous organic material for selective CO_2 adsorption.

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The Influence of the Reaction Parameters on the Synthesis of Fatty Acid Fusel Esters from Waste Cooking Oil

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Environmental concerns have led scientists to explore renewable alternatives to widely used fossil fuels, such as biodiesel. Also known as fatty acid alkyl esters, biodiesel is synthesized via transesterification reaction from a feedstock (usually vegetable oil or animal fat), and an alcohol (most commonly, methanol or ethanol), in the presence of a catalyst (e.g. NaOH or KOH). [1] To further contribute to the sustainability of the process, it is welcomed to use waste materials as reactants in the reaction, therefore reducing the produced waste and converting it to high-added value chemicals. [2] In this study, waste cooking oil reacted with waste alcohol mixture from fermentation processes in alcohol distilleries (fusel oil) [3], in the presence of potassium hydroxide, as a catalyst, to yield fatty acid fusel esters (biodiesel). The influence of temperature (40-80 °C), time (30-90 min), molar ratio of the reactants (4:1-12:1), mass fraction of KOH (1-3 %), and mixing speed (250-500 rpm) on the reaction conversion was studied, using Response Surface Methodology. Initial results showed that the most significant reaction parameters for this system were temperature, molar ratio of the reactants, mass fraction of the catalyst, and mixing speed. The increase in the molar ratio of the reactants, mass fraction of the catalyst, and mixing speed generally led to the increase in the reaction conversion.

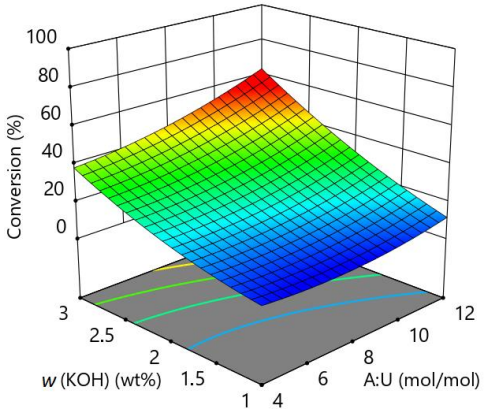

Fig. 1: The influence of the molar ratio of the reactants and the mass fraction of KOH on the synthesis of fatty acid fusel esters at 60 °C, 60 min, and 500 rpm (obtained using Response Surface Methodology)

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Physico-chemical assessment of the quality of marine sediments near the municipal outfall UPOV Cuvi: Coastal area of Rovinj, NE Adriatic Sea, Croatia

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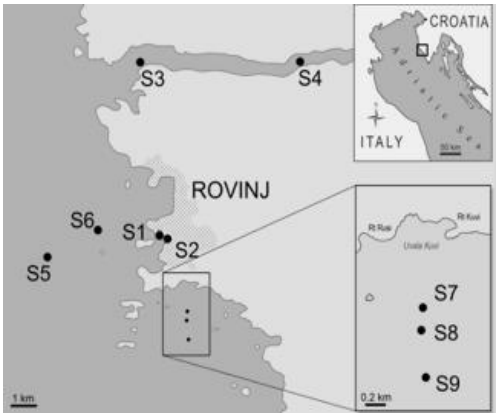
Prior to the final commissioning of the municipal wastewater treatment plant (UPOV Cuvi, Rovinj), marine sediments were sampled in the immediate vicinity of the underwater outlet to assess their quality. The surface sediments (<3 cm) were sampled in May 2022 at the control site (S6) near Rovinj and at stations at 50 m (S7), 200 m (S8), and 1000 m (S9) from the municipal outfall of UPOV Cuvi. The sampled sediments were analyzed for water content, total organic carbon (TOC), 8 heavy metals, 16 polycyclic aromatic hydrocarbons (PAHs), and total polychlorinated biphenyls (PCBs) (Fig. 1). The probability of a toxic effect on marine biota was assessed by calculating P_{avg} and P_{max} values [1], the cumulative risk quotient (ΣQ_{N1}) and the average risk quotient (Q_{PECm}) [1]. The measured content of heavy metals, individual and total PAHs and total PCBs was within the natural values for all samples. All samples analyzed belong to the first category of marine sediments [1,2]. The ecological risk and toxicity assessments show that the analyzed sediments are in a good ecological status and pose no ecological risk to the environment and marine organisms.



Measured parameters	RV001 (S6)	UPOV Cuvi 1 (S7)	UPOV Cuvi 2 (S8)	UPOV Cuvi 3 (S9)
Water content (%)	28.99	35.87	39.86	39.31
Total organic carbon (%)	0.96	1.79	1.94	1.59
As (mg/kg DW)	4.1	7.1	6.5	5
Cd (mg/kg DW)	0.05	0.12	0.09	0.07
Cu (mg/kg DW)	6.5	15	13	10
Ni (mg/kg DW)	8.4	20	17	17
Pb (mg/kg DW)	9	21	13	13
Zn (mg/kg DW)	34	66	49	45
Hg (mg/kg DW)	0.06	0.2	0.1	0.1
Cr (mg/kg DW)	19	30	29	28
∑PAHs (mg/kg DW)	0.07	0.07	0.04	0.04
∑ PCBs (mg/kg DW)	0.1	0.1	0.1	0.1
Σ_{QN1}	1.4	2.78	2.19	2.02
Q_{PECm}	0.14	0.28	22	0.2
P_{avg}	0.18	0.22	0.19	0.19
P_{max}	0.15	0.23	0.21	0.21

Fig. 1: Map of the investigated area (left) and results of sediment chemical analyses (right) conducted within the ERA-NET BlueBio MuMiFaST project (HRZZ).

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Problems of microplastic analysis by Raman microscopy in seawater during sea blooms

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Microplastics (MPs), defined as plastic particles ranging from 1 µm to 5 mm in size, are now ubiquitously present in aquatic and terrestrial environments. Raman microspectroscopy, a powerful technique for chemical identification, characterization and enumeration of small particles provides all the prerequisites for quick detection, delivering size-correlated compound distribution and morphological characterization at the single particle level [1]. However, the complex matrixes and sample composition can make analysis challenging, underscoring the importance of sample preparation. Focusing on sample collection and isolation, aquatic samples are known to be the easiest to handle, requiring no additional manipulation of the sample [2]. The aim of this study was to investigate effect of algal blooming on MPs analysis in seawater. Also, the efficacy of filtering seawater through different pore size filters for microplastic removal and decreasing the number of particles was tested. Two sets of seawater samples were analysed; the first from the time of the sea algal blooming in July, and second gathered in September, when algal blooming was not observed. The results of the study highlight the complex interplay between microplastics and beta carotene under algal blooming conditions in microplastic analysis of the seawater. The results show that the first sample set contained a significant amount of beta carotene which obscured the detection of microplastic particles. It can also be concluded that filtering seawater through several filters is an effective way of purifying seawater to achieve a satisfying consistent quality for use in products for human usage. Overall, having in mind natural source of sea water and its variability- process steps design and in process controls till Finished Product- are essential to assure and maintain pharmaceutical quality of produced products.

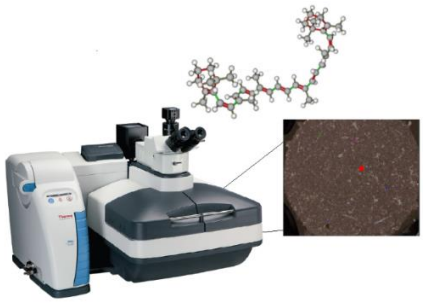

Figure 1. Raman micro spectroscopy – illustration of apparatus and results of microplastic analysis.

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Synthesis, characterization and *in vitro* study of novel asymmetric *meso*-(*N*,*N*-diethylaminophenyl)porphyrins for use in photodynamic therapy

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Photodynamic therapy (PDT) is a clinically approved, minimally invasive, selective treatment that targets malignant cells and shows minimal cytotoxic effects on healthy tissue [1,2]. A photosensitizer (PS), light of a specific wavelength, and molecular oxygen are used in PDT to generate reactive oxygen species (ROS), which causes oxidative stress and, consequently, tumor destruction. The key characteristics of an ideal PS that scientists are trying to achieve include compound purity, cost-effectiveness, strong absorption in the red light spectrum, (photo)stability, minimal toxicity without irradiation and efficient production of singlet oxygen [1,3].

Porphyrins have proven to be an excellent group of PSs, and their properties have been investigated on different types [4]. However, *meso*-(*N*,*N*-diethylaminophenyl)porphyrins as PSs in PDT research have not yet been investigated.

We present here a mixed Adler-Longo synthesis of novel asymmetric *meso*-(*N,N*-diethylaminophenyl)porphyrins and their potential for use as PSs for PDT. All new structures and their purity were confirmed by ^{1}H and ^{13}C NMR spectroscopy. The UV-Vis and fluorescence properties of the new compounds were recorded and compared with commercially available 5,10,15,20-tetra(*N,N*-diethyl-4-aminophenyl)porphyrin. Also, (photo)stability of the PSs, and their singlet oxygen production were quantified by measuring the photodegradation of 1,3-diphenylisobenzofuran (DPBF). Phototoxicity of the PSs was investigated using an MTT assay with red light (λ = 643 nm, 2 mW/cm^2) on two tumor cell lines MDA-MB 231 and HeLa and HFF as a normal cell line.

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Determination of phenolic acids in *Urtica dioica* extracts obtained by deep eutectic solvents

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Urtica dioica L., commonly known as stinging nettle, is a wild herbaceous perennial plant rich in fiber, minerals, vitamins, and antioxidant compounds such as polyphenols and carotenoids. It exhibits antiproliferative, anti-inflammatory, analgesic, anti-infectious, hypotensive, and antiulcer properties [1]. The leaves of *Urtica dioica* contain phenolic acids, flavonoids, and anthocyanins [2].

This study aimed to identify the optimal choline chloride-based deep eutectic solvent for extracting phenolic acids from stinging nettle and to optimize the extraction conditions using magnetic stirring and bead mill homogenization. Screening revealed choline chloride:lactic acid (1:2) as the most effective solvent among 17 tested. Subsequently, the effects of extraction temperature, time, water content and beads speed on phenolic acid extraction using this solvent were investigated.

Comparing the two extraction techniques yielded similar results, favoring mechanoextraction due to its shorter time and lower temperature. The statistically significant influence of the tested extraction parameters varied depending on the component, highlighting the method's selectivity. The optimal conditions for achieving the highest amount of phenolic acids through mixing and heating on a magnetic stirrer were 26.21% water, 30 minutes, and 69.99 °C, whereas for extraction using a bead mill homogenizer they were 44.99 % water, 3.88 minutes, and 5 m/s.

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Control of chitosan-carboxymethyl cellulose polyelectrolyte multilayer properties through pH variation

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Polyelectrolyte multilayers (PEMs) are thin films obtained through the self-assembly of oppositely charged polyelectrolytes (PE) using a layer-by-layer (LbL) method [1]. The properties of such structures can be finely tuned for diverse applications by controlling the adsorption process through parameters such as pH, ionic strength, and concentration [2]. In our study, we explore the properties of a multilayer film composed of chitosan (CS) and carboxymethyl cellulose (CMC) on silica and apple surfaces under different pH conditions. Chitosan, a natural and biocompatible weak polycation, contains amino groups that can help prevent bacteria and mold growth [3]. Surface roughness of multilayer films has proven to be a crucial parameter in preventing bacterial growth and can be controlled by adjusting solution parameters. The morphology of the multilayers, prepared under varying pH conditions of the polyelectrolyte solutions, has been characterized using Atomic Force Microscopy (AFM), revealing a clear dependence of their surface properties on pH. Both CS and CMC are weak polyelectrolytes whose charge depends on the degree of ionization, an important factor in multilayer buildup [4]. The influence of pH on PE's electrophoretic mobility has been demonstrated through microelectrophoresis experiments. PE charge impacts the adsorption process and final properties of the multilayer film, including water contact angles measured on both apple and silica substrates.

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Environmentally sustainable high-speed analysis of basic amino acids in dietary

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Conventional analytical strategies for determining amino acids are high-priced and often demand modification of their polarity or adding aromatic moiety to provide their detectability. The present study aims to develop a new perspective and fast microfluidics method for analyzing L-arginine, L-ornithine, and L-lysine in dietary supplements in an environmentally sustainable manner without the use of hazardous chemicals and the need for chemical modification of amino acids. Microchip electrophoresis conjugated with capacitively coupled contactless conductivity detection (MCE-C^4D) was used. A solution consisting of 0.3 mol dm^{-3} acetic acid and 1×10^{-5} mol dm^{-3} iminodiacetic acid has been identified as the optimal background electrolyte. The established run time of 120 s was long enough for the analysis of all real samples without the influence of the EOF signal. The minimized sample preparation process and short migration times resulted in high-speed analysis. Thus, the method's simplicity resulted in high analysis throughput. The method's analytical performance revealed high precision and linearity ($R^2 \geqslant 0.9971$). To our knowledge, this is the first study that successfully utilizes MCE-C^4D instrumentation to analyze L-arginine, L-ornithine, and L-lysine in real samples. The result of the analysis showed insignificant disagreement with declared values which ranged from 2.55 to 7.52 %. The analytical features of the developed method were compared to the CE-UV-VIS and HPLC-DAD methods.

New sensing material for potentiometric surfactant sensor fabrication

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Surfactants, or surface active agents are widely used for cleaning, washing, and disinfection. Usually, they consist of a hydrophobic tail and a hydrophilic head. The type of the sensor depends on the head charge; cationic surfactants, anionic surfactants, amphiphilic and nonionic surfactants (with no charge). The global demand for surfactants is in constant growth caused by high industrialization and population growth, with an expected CAGR of 4.9 % from 2022 to 2028 [1]. Except the positive sides, they could have some negative effect on human health and environment. Classical methods for surfactant analysis are expensive, time-consuming, use toxic organic solvents and require experienced personnel. For this reasons there is a need to develop fast and low-cost analytical tools. Here we presented a new sensing material based on quaternary ammonium imidazolium tetraphenylborate ion-pair for fabrication of low-cost potentiometric sensors. The surfactant sensor was characterized through potentiometric response, interference study and potentiometric titrations on model samples and showed a near-Nernstian response in a broad range of concentrations. Measurements on technical grade surfactants showed good recoveries in the range from 98.2 to 99.8 %. Measurements of real samples showed good agreement with the referent method. The developed surfactant sensor represents an interesting and fast alternative to existing methodologies for surfactant sensor quantification in different liquid and dry samples.

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Inclusion complexes of cinnarizine with β-cyclodextrin and its derivatives in solution: phase-solubility study

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Cinnarizine (CIN) is a piperazine derivative with antihistaminic and calcium channel blocking activity which is widely used for the treatment of vertigo and prevention of motion sickness. Due to its extremely poor aqueous solubility which is also pH-dependent, CIN is classified as a Class 2 drug substance according to The Biopharmaceutical Classification System [1]. The low aqueous solubility of CIN can be improved by complexation with cyclodextrins (CDs) and their derivatives [2]. CDs can encapsulate lipophilic drug moieties into their central cavity, form inclusion complexes, and thus improve the water solubility and bioavailability of drugs [3]. This study aimed to evaluate the influence of β-CD and its hydroxypropyl (HP-β-CD), sulfobutylether sodium salt (SBE-β-CD) and randomly methylated (RM-β-CD) derivatives on intrinsic solubility of CIN in water and in buffered aqueous solution (simulated duodenal medium, pH 4.5) using phase-solubility analysis according to the existing Higuchi-Connors method [4]. To evaluate total drug solubility changes in the presence of increasing CD concentration phase-solubility diagrams were constructed. Stability constant and complexation efficiency values were calculated as well. Spectrophotometric and spectrofluorimetric methods were developed for the quantitative determination of CIN in the presence of the mentioned cyclodextrins. The methods were validated according to the guidelines prescribed by the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceutical Products for Human Use. Concentration of CIN was determined by HPLC method. Based on the results of phase-solubility analysis it was concluded that CIN forms complexes with all studied β-CDs in 1:1 stoichiometric ratio resulting in significant solubility enhancement of CIN both in water and simulated duodenal medium.

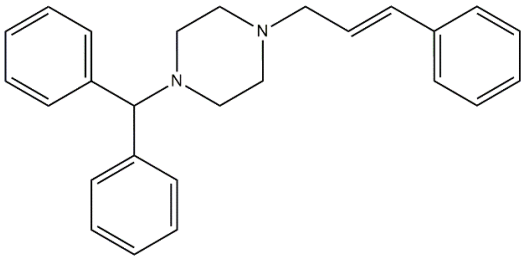

Fig. 1. Structure of cinnarizine

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Synthesis of cyclic organic carbonates *via* carboxylative C-C cross coupling reactions of propargyl alcohols and CO_2

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Capture of carbon dioxide (CO_2) and transformation to highly valued organic compounds are particularly challenging due to its increased kinetic and thermodynamic inertness. However, the direct use of CO_2 as a C1 synthon represents an attractive approach in modern organic synthesis for the valorization of this atmospheric gas responsible for the greenhouse effect [1]. The catalytic capture of CO_2 by propargylic substrates -alcohols or amines- affords α-alkylidene cyclic carbonates or carbamates, respectively, which are structural motifs often found in pharmaceuticals, polymers, chiral auxiliaries, etc [2]. The combination of CO_2 capture with C-C cross-coupling reactions can provide direct access to complex products that otherwise require multi-step syntheses [3].

We present three-component carboxylative C-C cross-coupling reactions with various propargylic alcohols catalyzed by palladium or copper salts. Our efforts are focused on allylation reactions using allyl halides, propargylic alcohols, and CO_2 under atmospheric pressure (Fig. 1). The optimization of the reaction conditions is currently underway, as well as the elucidation of the reaction mechanism by combining experimental and computational methods, i.e. control experiments, *in situ* NMR monitoring and density functional theory (DFT) calculations.

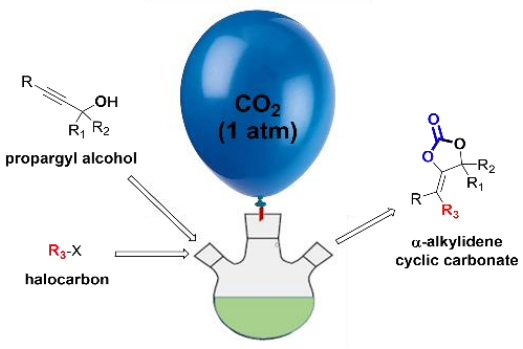

Fig. 1: Illustration of carboxylative C-C coupling reactions for the synthesis of cyclic organic carbonates from CO_2 at atmospheric pressure.

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Upgrade the first-generation biorefinery for the possibility of production of second-generation biofuels and advanced fuels

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The need to use biofuels stems from European legislation and social pressure to preserve the environment. The largest contributors to CO_2 emissions are the energy sector, agriculture, transport, and industry. Biofuels are an alternative to fossil fuels and have less impact on the increase of CO_2 content in the atmosphere because they are produced from biomass that consumes CO_2 during its life cycle.
First-generation biofuels (FAME) are obtained by processing from biomass such as sugars, edible oils, corn, and wheat. The mentioned raw materials are also a source of food, therefore the amount of use of first-generation biofuels is limited by European regulation. Biofuels obtained by processing used cooking oil (UCOME) belong to the second generation of biofuels. UCOME provides the highest greenhouse gas (GHG) savings. Controlled production plants across the EU produce used cooking oil (UCO)-based FAME with more than 90 % greenhouse gas savings compared to fossil fuels, and the energy of biofuels produced from raw materials such as UCO is counted twice the value of the actual energy consumed. The main problem in using UCO as a source of biodiesel are its free fatty acid and water content. These impurities have a very adverse effect on the transesterification process.
Challenges need to be overcome in the production process to transform the first-generation processing plant into a plant capable of processing second-generation biofuels will be presented. In addition, other upgrade options will be investigated to reduce waste production and create the possibility of producing advanced biofuels.

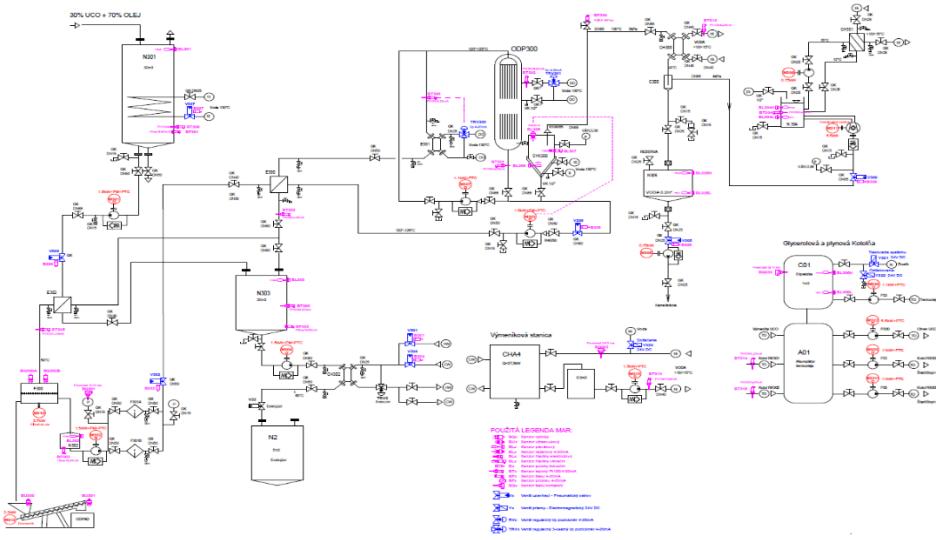

Fig. 1: Technological scheme of UCO preparation for production.

Anonymous testing of samples for the presence of drugs or psychotropic substances

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According to the estimates of the European Monitoring Center for Drugs and Drug Addiction (EMCDDA), citizens of the European Union spend more than 24 billion euros on illegal drugs every year. Drug abuse has a negative impact not only on the individual who consumes drugs (impairment of mental and physical health) but also on the whole society. Since 2017, the City of Zagreb has implemented a program of anonymous testing of samples for the presence of drugs or psychotropic substances and Andrija Stampar Teaching Institute of Public Health (Institute) is collecting and analyzing unknown samples. After sample preparation, analyses are performed on a Liquid chromatography/Direct sample analysis Time of Flight mass spectrometer (LC/DSA-TOF) or/and on a coupled system gas chromatography-mass spectrometry (GC-MS), where the unknown substances are identified by comparison with the NIST, Wiley and TOX databases. After the samples are submitted to the Institute, the results of testing are displayed anonymously on the website of the Institute. About half of the analyzed samples contain some of the illegal substances. So far, THC, cocaine, amphetamine, MDMA, diazepam, caffeine and other illegal substances have been detected. In 2023 THC, cocaine, and amphetamine were detected in the largest number, 15, 18, and 12%, respectively. The results for 2023 are following testing results during the period 2017-2023 with THC, cocaine, and amphetamine as the most detected substances in percentages of 16, 10, and 9 %, respectively. Cocaine use is increasing rapidly, amphetamine a little and THC use is decreasing.

Acknowledgment: This work was carried out within the project "Food Safety and Quality Center" (KK.01.1.1.02.0004). The project is co-financed by the European Union from the European Regional Development Fund.

Molecular recognition of tRNA^{Ile} by the *Priestia megaterium* isoleucyl-tRNA synthetase type 2

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Aminoacyl-tRNA synthetases (aaRS) are essential enzymes that play a key role in protein synthesis by covalently linking cognate amino acids and tRNAs. Aminoacylation proceeds in two steps; an amino acid is activated by ATP to form aminoacyl-adenylate (aa-AMP) followed by the transfer of aminoacyl moiety to the tRNA. The fidelity of protein biosynthesis relies on the high specificity of aa-tRNA synthesis. Isoleucyl-tRNA synthetase (IleRS) is an enzyme that charges tRNA^{Ile} with isoleucine. In bacteria, two types of IleRS have been found, IleRS1 and IleRS2. We have recently solved the first structure of IleRS2 with a fully resolved C-terminal domain [1]. The structure revealed that in IleRS2, like in IleRS1 [2], the C-terminal domain comprises three subdomains (SD1-3). The SD1 and SD2 subdomains in both IleRS types are structurally homologous, except for an SD2-insertion, which is present only in IleRS2. The SD3 subdomain adopts different folds, questioning whether in IleRS2 it recognizes tRNA anticodon as in IleRS1. Here we present a structural model of IleRS2 bound to tRNA^{Ile} at 5.5 Å resolution and supporting kinetic analysis. The model unveils that the strictly conserved Arg964 from SD3 establishes an interaction with the tRNA^{Ile} anticodon base G34. The mutation of this residue completely inactivated IleRS2 aminoacylation while the activation step was not compromised. This demonstrates that the SD3 subdomains, despite different folds, are used in both IleRS types to recognize the first anticodon base. Mutation of residues Lys862 and Phe865 from the IleRS2-specific SD2-insertion resulted in at least a 10-fold increase in K_M for tRNA, confirming the interaction with the D-loop of the tRNA observed in the structural model. Comparison with *S. aureus* IleRS1:tRNA complex [2] shows the two IleRS types significantly differ in the recognition of the D-arm of tRNA^{Ile}. These findings may have some interesting implications in IleRS evolution.

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Integrated millisystem for continuous synthesis and separation of resveratrol analogues: a step towards sustainable production

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In recent years, resveratrol and its analogues have been shown to have numerous bioactivities in the prevention and treatment of diseases. Therefore, the production of larger quantities is gaining more and more interest over time, so that the importance of chemical synthesis is consequently increasing [1]. One way to synthesise these stilbene-like compounds is usually via the Wittig reaction, which can be carried out in a two-phase system as phase transfer catalysis (PTC). This approach avoids the use of aggressive and hazardous bases [2]. In addition, hazardous solvents normally used for these reactions, such as dichloromethane (DCM), can be replaced by natural deep eutectic solvents (NADESs). Further improvements in terms of sustainability and productivity of the process can be achieved by using flow chemistry, as mass transfer is more efficient in continuous multiphase reactions than in batch reactions [1,2].
In this research, a continuous PTC-Wittig reaction was carried out in a millireactor with a serially connected continuous product separation using the Zaiput membrane separator. Integrated production processes were developed in DCM and a suitable hydrophobic NADES as reaction solvent. To maximise conversion, changes in temperature, residence time, tube diameter and pressure were tested. The results obtained in both solvents were compared in terms of productivity and sustainability of the process. After selecting the best reaction conditions, the product-rich organic phase was separated in a Zaiput separator with a suitable membrane.

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Photocatalytic properties of the spinels Co_2CrO_4, $CoCr_2O_4$ and $CoCrFeO_4$ prepared by molecular precursor route

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The properties of mixed metal oxides, an important class of modern materials, can be strongly influenced by crystallinity, particle size, phases present and morphology. These can be partially adjusted by changing and modifying the synthesis methods. Heterometallic oxalate complexes are used as single-source precursors that provide simplified synthetic routes for the formation of mixed metal oxide materials by thermal decomposition in one step. The advantage of a solid phase transition is the retention of the elemental composition defined by the molecular precursor with only a loss of volatile decomposition products, allowing excellent stoichiometric control of the intermetallic ratio in the oxide products [1]. Metal-organic complexes do not always have the appropriate stoichiometry to be used as molecular precursors for the preparation of the desired single-phase oxide materials by thermal decomposition in a single step. Therefore, an oxide with two or more metals could be prepared by combining two or more suitable precursors in the proper ratio before thermal treatment [2].

Based on the appropriate metal ratio, the spinel oxide Co_2CrO_4 was prepared by thermal treatment of the heterometallic complex in one step. In addition, the spinels $CoCr_2O_4$ and $CoCrFeO_4$ were produced by a modified single-source molecular precursor route by thermal processing of mixtures of known and structurally characterized complexes. We have focused on the structural, optical and photocatalytic properties of the crystalline products prepared in this way, successfully bypassing prolonged heating at high temperatures and repeated grinding (Fig. 1).

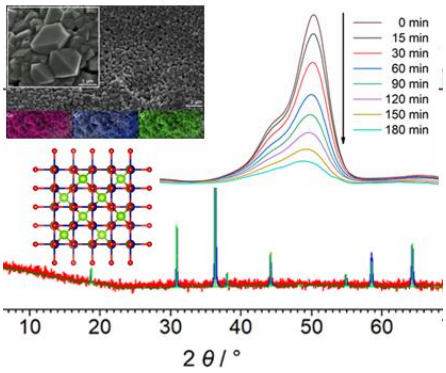

Fig. 1: Characterization of spinel oxides prepared by molecular precursor route.

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Near infrared spectroscopy coupled with artificial neural network modelling for analysis of *Spirulina plantains* aqueous extracts

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Spirulina platensis, a blue-green microalga, is renowned for its rich nutrient profile and bioactive compounds, which offer various health benefits. According to the literature, key bioactive compounds found in Spirulina platensis include phycocyanin, polysaccharides, gamma-linolenic acid, proteins and peptides, vitamins and minerals, phenolic acids, and tocopherols and carotenoids [1,2]. Therefore, an efficient analytical method is required for the rapid and reproducible analysis of *Spirulina platensis* extracts. In this study, ultrasound-assisted aqueous extraction of bioactive molecules from Spirulina platensis was performed across 30 independent experiments. Near-Infrared (NIR) spectroscopy was utilized for the quantification of total protein concentration, phycocyanin concentration, and total polyphenol concentration in the *Spirulina platensis* aqueous extracts. NIR spectroscopy is recognized as a powerful analytical technique due to its capacity to provide rapid, non-destructive, and precise measurements of various components [3]. In this work, NIR spectra of the *Spirulina platensis* extracts were obtained in the wavelength range of 904-1699 nm. The raw NIR spectra were subjected to Principal Component Analysis (PCA) and Artificial Neural Network (ANN) modeling. Results indicated that ANN models developed based on the NIR spectra accurately described the total protein concentration, phycocyanin concentration, and total polyphenol concentration with high precision ($R^2 > 0.75$). Overall, the findings demonstrate significant potential for using portable NIR spectroscopy in the analysis of blue-green microalga extracts.

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Anion Binding Affinity of Penta- and Hexaleucine Cyclopeptides

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During the past few decades there has been a growing interest in research of cyclopeptides as anion receptors. These compounds, besides their metabolic stability and bioavailability, exhibit enhanced binding affinity towards substrates compared to their more flexible linear analogs[1,2] which make them perfect candidates for antibiotics and membrane transport carriers. In this work the stability constants as well as reaction enthalpies and entropies of anion complexation by cyclohexaleucine in acetonitrile and methanol, and cyclopentaleucine in methanol (Figure 1) were determined by means of ^{1}H NMR spectroscopy and microcalorimetry. By using these data and the results of our previous research[3], we compared the effect of the cyclopeptide ring size on its affinity towards various monovalent anions (Cl^-, Br^-, I^-, HSO_4^-, $H_2PO_4^-$, NO_3^-, SCN^-). The enthalpic and entropic contributions to the Gibbs energy of complexation reactions were discussed as well. We also performed MD simulations of the free receptors and their complexes to get an insight into the structural characteristics of these species.

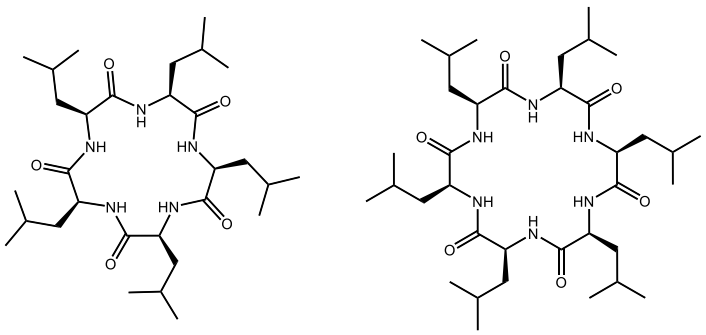

Fig. 1: Structures of cyclopentaleucine and cyclohexaleucine.

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Coffee ring effect on sodium chloride crystallization

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The coffee ring effect, a phenomenon observed when a liquid containing particles evaporates and leaves a ring-like deposit at the perimeter, has garnered significant interest for its implications in material science and fluid dynamics. This effect is particularly relevant in the study of crystallization processes where the distribution and morphology of the residue can provide insights into the dynamics of particle aggregation and sedimentation under evaporative conditions. Understanding this process is crucial for applications ranging from the formulation of paints and inks to the development of diagnostic assays and the manufacturing of advanced materials as well in development of drug.

Recent experiments with sodium chloride have displayed considerable variability in results, necessitating measures to minimize this unreliability. To improve reproducibility, a controlled humidity chamber was designed to ensure stable relative humidity throughout the experiment and meticulous cleaning of the glassware was conducted to reduce impurity presence. These controls can aid in optimizing conditions to achieve consistent patterns (Figure 1) that are reflective of intrinsic interactions rather than environmental variations. Through rigorous control and methodology optimization, these experiments aim to establish more predictable and reproducible conditions for studying the coffee ring effect in sodium chloride crystallization, shedding light on the complex dynamics of crystal formation and deposition under varying ambient conditions.

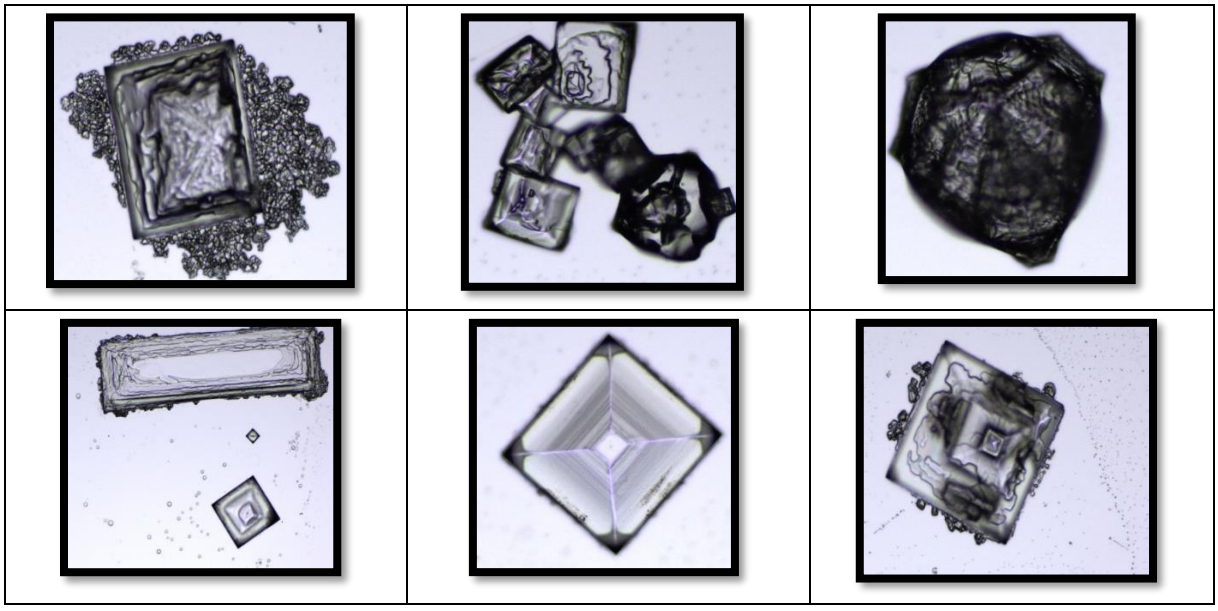

Fig 1. Microscopic Insights into NaCl Crystal Formation from Microdroplets.

Studying the aggregation of protoporphyrin IX and mesoporphyrin IX and their peptide conjugates by molecular dynamics and UV/Vis spectroscopy

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Porphyrins exhibit therapeutic effects such as antimicrobial activity and are widely utilized as photosensitizers in photodynamic therapy due to their unique chemical properties [1-2]. However, their potential for treating microbial infections is limited by their aggregation tendency and inability to cross cell-membrane barriers. To address these limitations, conjugation to peptides able to cross cell barriers, known as cell-penetrating peptides (CPPs), is a viable option that can enhance porphyrins' cell delivery and antimicrobial efficacy [2]. Here, we investigated the aggregation behavior of two known antimicrobial porphyrins (protoporphyrin IX – PPIX and mesoporphyrin IX – MPIX) and their peptide conjugates using a combination of all-atom molecular dynamics simulations and UV-Vis spectroscopy. Simulations were conducted at different concentrations by altering the simulation box size and the number of molecules. Two box sizes were used: a 216 nm^3 cube containing 10 molecules of the respective porphyrin to elucidate aggregation mechanisms, and a 1728 nm^3 cube with 40 molecules to analyze larger aggregation morphologies. The solvent was modeled using the simple point-charge water model, with physiological Na^+ and Cl^- concentrations at 310.15 K. Automated topology builder was used to generate the topology files and custom GROMOS 54A7 force field was employed. UV-Vis spectroscopy was performed by analyzing serial dilutions of porphyrins in environments of different polarity, based on the DMSO and water ratio. Simulations showed concentration-dependent aggregation for both MPIX and PPIX, with PPIX showing a greater propensity to aggregate. Dimerization was identified as the preferential and preceding step to all other oligomerizations in the aggregation process. In the smaller PPIX system, 50 ns of aggregation resulted in the formation of a single aggregate composed of dimers with their polar carboxyl groups oriented to face the solvent, creating a hydrophobic core. The larger system exhibited local aggregation, favoring the formation of dispersed dimers. UV-Vis spectroscopy indicated varying critical aggregation concentrations in different solvents and revealed how porphyrin spectral properties change due to the environment. After quantifying porphyrin aggregation and nucleation using dynamic distance measurements, we plan to expand our work to peptide-porphyrin conjugates to explore their potential pharmacokinetic properties.

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Design and the analysis of ligands binding to dopamine D3 and D4 receptors based on a common pharmacophore

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Dopaminergic receptors (DRs) are a class of GPCR receptors, divided into D1-like subfamily, comprised of DRD1 and DRD5; and D2-like subfamily, comprised of DRD2, DRD3 and DRD4. D2-like subfamily of receptors couples to G_i/G_0 protein, thus deactivating adenylyl cyclase and decreasing the production of cellular cAMP. The aim of this research is to present the rationale of the design and synthesis of different ligands binding to DRD3 and DRD4, as well as their analysis. All ligands were based on the structure of a common pharmacophore, consisting of 4 linked sections, namely: a lipophilic scaffold, a linker, a basic function and an aromatic terminal (Figure 1A). Ligands' binding and functional profiles to their respective receptors were assessed.

We successfully managed to synthesise DRD4 and DRD3 selective antagonists, employing different approaches of organic synthesis. Based on this, the following results were obtained:

1. DRD4: the quinolinone nucleus of **1** can be replaced by an N-indole or an N-tetrahydroquinoline moiety without the loss of DRD4 selectivity. The most DRD4 selective compounds were the ones with *p*-substituted aromatic terminals. The *p*-substituted ligands **13**, **16** and **19** behaved as antagonists toward G_i protein activation and the β-arrestin recruitment. The 2-pyridyl derivative **24** had an interesting, biased profile, being a partial agonist toward DRD4 G_i-protein activation, but an antagonist toward *β*-arrestin recruitment.
2. DRD3: the ligands characterized by an unsubstituted butyl chain between the pharmacophores (ligands **29**, **32**, **35**) exhibited the highest DRD3 affinity. **29** had a DRD3-preferential profile, while **32** and **35** behaved as potent DRD2 antagonists, 5-HT_{1A}R and D4R agonists, as well as potent DRD3 partial agonists. They also behaved as low potency 5-HT_{2C}R partial agonists and 5-HT_{2A}R antagonists. This multitarget profile is often exhibited by drugs used in schizophrenia treatment.

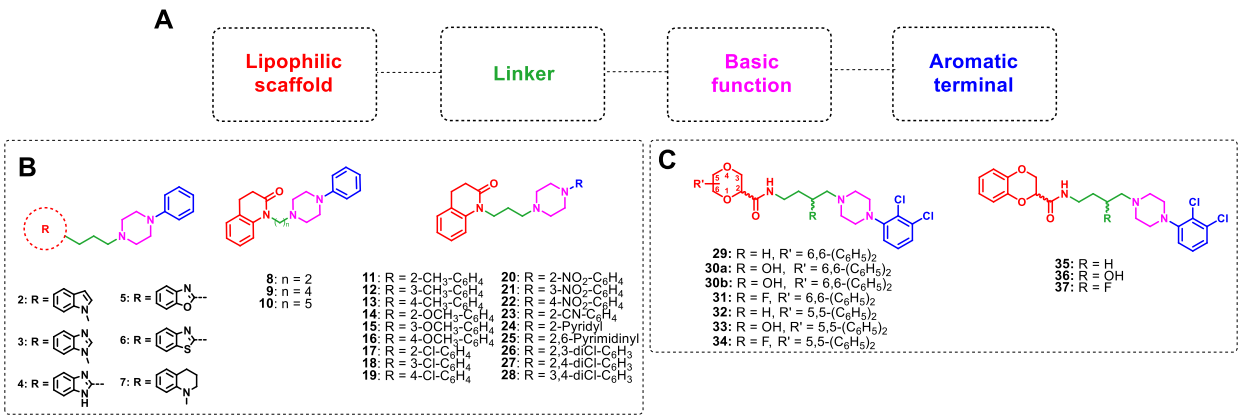

Fig. 1: A) General pharmacophore model shared by: B) DRD4 ligands **2**-**28**, C) selective or multitarget DRD3 ligands **29**-**37**.

Distinctive chromic properties of supramolecular inter-ionic charge-transfer complexes between pyridinium oximes and ferrocyanide

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Supramolecular materials are continuously attracting considerable attention because of the modularity of their structural components, inherent reversibility, and overall versatility. The utility of supramolecular metalo-organic electron donor/acceptor materials stems from the noncovalent interactions from which they assemble, endowing them with dynamic properties that can be tailored and controlled. The incorporation of charge-transfer interactions between an electron-donor and an electron-acceptor brings a multitude of advantages on account of their stimuli-responsive optical (thermochromism, hydrochromism and piezochromism) and intramolecular redox (charge separation via electron transfer) properties through the adjustment of the HOMO-donor and LUMO-acceptor orbitals. Such metal-organic frameworks with united physical and chemical properties of different organic/inorganic components are used for many applications, including catalysis, magnetism, sensing, and gas storage. Focused on electron- and charge-transfer processes, pyridinium-4-oxime cations are recognized as potent electron acceptors for the formation of supramolecular charge-transfer complexes with the ferrocyanide anion as electron-donating anion.

Here, we present the comparative solid-state study of the intricate relationship between structural features of selected mono- and bis-pyridinium oximes and the chromic and redox properties of their supramolecular ferocyanide complexes. The XRD structural analysis and various spectroscopic techniques employed in our research provided the insight into observed reversible hydrochromic, vapochromic and thermochromic properties of isolated complexes. In the case of *N*-benzylpyridinium-4-oxime (BPA4) complex with ferrocyanide, the chromic properties are result of distinct interplay between existing charge-transfer and dehydration-/pressure- induced electron-transfer, which led to the formation of small quantities of BPA4 radical and ferricyanide species within a material (Fig 1).

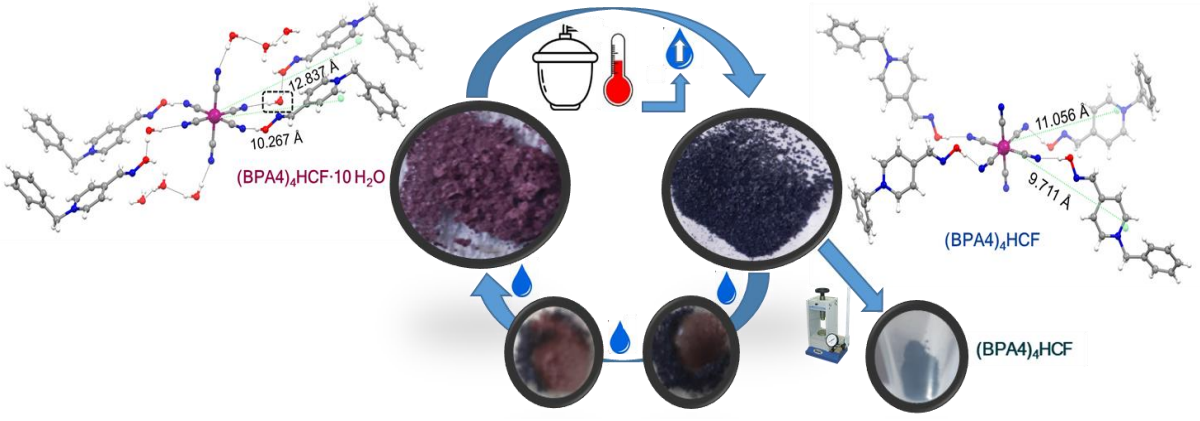

Fig. 1: Reversible chromism of supramolecular complex between *N*-benzylethylpyridinium-4-oxime and ferrocyanide.

Synergy in Science: Collaborative Phytochemical Studies in Croatia

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Phytochemical research in Croatia is somewhat limited, yet there is fruitful collaboration within the small plant science community. To further enhance this collaboration, we have signed a cooperation agreement among three currently active research and installation research projects funded by the Croatian Science Foundation: GinkoBiFlav (UIP-2019-04-1018) [1], NATURALLY (IP-2020-02-6899) [2], and TEMPHYS (IP-2020-02-7585) [3]. All these projects are based on phytochemistry and aim to address several important research questions related to contemporary challenges and the potential use of plant resources to improve the quality of everyday life. Here, we will present the results of these projects through answers to some of the questions we have explored: What is the role of dimeric flavonoids - biflavonoids in plants? Could invasive species offer valuable phytochemicals for application in the pharmaceutical industry? Do climate changes affect plant phytochemical composition, altering their nutritional value and biological effects on mammalians' health? We will also present the joint activities that have been achieved and planned. We believe collaboration and networking synergise resource utilisation and project success, making this a potential future scientific communication and cooperation model in Croatia and beyond.

Fig. 1: Visual Representation of project collaboration.

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Ex situ bioremediation with indigenous microbes from food waste stream

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Bioremediation is a technology that utilizes microbial capabilities to remove contaminants from the environment, recognized today as a green and sustainable approach to addressing ecosystem contamination. Biostimulation is a bioremediation technique that involves altering the biological environment by modifying its physicochemical properties, thereby increasing the activity of indigenous microbes. Widespread in nature, microbes can use a variety of substrates as carbon sources, enabling them to inhabit diverse and unusual environments where they can interact with various pollutants. Their ability to thrive in specific conditions further enhances their efficiency. Additionally, regarding their degradation potential microbes are favored primarily for their rapid growth and ease of manipulation, improving their effectiveness as bioremediation agents [1].
The aim of the presented study was to evaluate the capability of indigenous microbes from a food waste stream to remove organic matter from highly loaded wastewater through the application of biostimulation. The wastewater with a high organic load was obtained from a real sample of the organic fraction of municipal solid waste. The experiment was conducted in a batch reactor under submerged conditions at 160 rpm and a temperature of 25 °C. During the experiment, the concentration of organic matter and biomass, changes in pH values, and the concentration of dissolved oxygen were determined. Biomass process development was monitored by microscopic analysis. The maximum biostimulation efficiency achieved was 87.3 % on the 8th day of the experiment.

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Diamondoid ammonium salt inclusion complexes with cyclodextrins

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Diamondoid ammonium salts (DAS) are guest molecules capable of forming ultra-stable complexes with cucurbit[*n*]uril hosts in aqueous environment, with binding constants achieving up to 10^{15} mol^{-1} dm^3 [1]. Intrigued by these properties, we next turned our attention to binding between DAS and another class of hydrophobic cavity hosts – cyclodextrins (CDs) [2]. Here we present the synthesis, characterization and binding capabilities of various DAS regioisomers and evaluate the influence of different nitrogen-based functional groups on CD complex strengths. Stability constants were determined by ITC and ^{1}H NMR titrations in aqueous solutions and diffusion NMR ^{1}H DOSY method was applied to obtain the corresponding diffusion coefficients. Structural features of the complexes and acting key interactions were elucidated by combining ^{1}H-^{1}H NOESY NMR experiments and computational analysis. The obtained results therefore provided a deeper insight into both the thermodynamics of hydrophobically driven complexation as well as into the structural characteristics of the formed supramolecules.

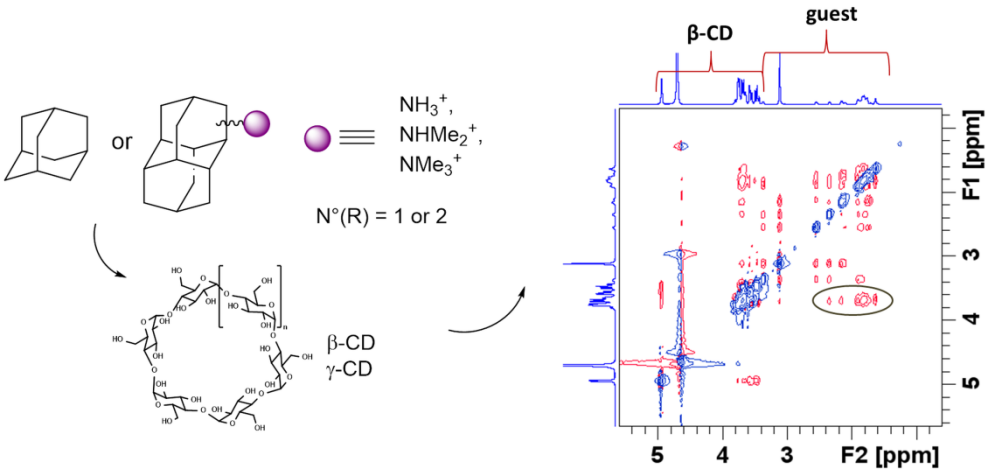

Fig. 1: DAS and CDs (left) and ^{1}H-^{1}H NOESY NMR spectrum of the β-CD complex with *N*,*N*,*N*-trimethyldiamantane-3-aminium iodide (right).

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Collagen extraction from jellyfish: method optimization

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Jellyfish are a novel source for collagen extraction, and it is derived from multiple *Medusozoa* species, including *Rhizostoma pulmo*. Their collagen is evolutionary older and simpler than mammalian collagens making them compatible with human biology. Its specific response to macrophages - a lower M1 macrophage response and a higher M2 macrophage response - triggers regeneration in tissue, making jellyfish collagen a candidate biomaterial for tissue engineering. To compare the collagen composition, multiple sequence alignment was performed between different types of human collagen and between different species using SALIGN. Sequence alignment of human fibrillar collagens showed similarities in characteristic glycine and proline regions, while amino acid analysis showed several differences in composition between different collagen sources.

The extraction of collagen from jellyfish consists of three main steps: sample preparation, extraction, and recovery, and can last up to 7 days. This lengthy procedure can lead to collagen degradation and a decrease in final yield. Current methods consist of tissue cutting and chemical pretreatment during sample preparation, acid and enzymatic extraction, and dialysis [1]. The goal of this research was to see how changes in the extraction steps affect collagen yield, purity, and the methods duration. For this purpose, samples from *Rhizostoma pulmo* were first lyophilized, followed by acid extraction with 0,5 M acetic acid, salting out with NaCl, and purification with C18 cartridges. The obtained collagen powder was analyzed using MALDI to confirm the successful extraction of collagen. In addition, FTIR and SDS-PAGE were performed to further confirm the presence of collagen. The collagen yields that were obtained are similar to those recorded in previous studies [2]. Based on these results, it can be concluded that the use of C18 cartridges can be an alternative for dialysis and could shorten the extraction process.

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Studies towards the synthesis of the antimicrobial natural product enceleamycin A using a formal [3+2] cycloaddition reaction

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The development of antimicrobial resistance has been highlighted as one of the greatest threats to human health in the 21st century by the world health organisation [1]. To combat the development of antimicrobial resistance we are interested in the development of new classes of antimicrobial compounds. Enceleamycin A is a furo-naphtoquinone isolated from a culture of *Amycolatopsis sp.* MCC0218 in 2022 along with two related compounds, enceleamycins B and C [2]. The enceleamycins displayed promising antibacterial activity towards gram-positive bacteria including four MRSA strains with enceleamycin A being the most active (MIC for *S. Aureus* of 2 μg/mL). The enceleamycins also showed *in vitro* anticancer activity by inhibition of AKT2 [3]. Enceleamycin A posesses a novel oxeto-furo-furo-naphtoquinone pentacyclic ring system and contains five adjecent stereocenters, two of which are quaternary, as well as an unprecedented oxetane acetal. Taking into account these structural features enceleamycin A poses a considerable synthetic challenge, motivating us to start a campaign towards its total synthesis with an approach that would enable the synthesis of analogs for structure-activity relationship studies. To this end, several approaches based on the disconnection of the central furan ring using formal [3+2] cycloadditions between the naphtoquinone fragment and an appropriately substituted furan have been explored.

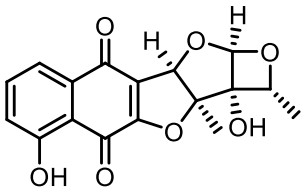

Fig. 1: Structure of enceleamycin A.

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Integrating molecular dynamics and DFT to study polymer-drug interactions

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This study presents a comprehensive computational investigation of interactions between 19 pharmaceutical molecules and 21 different polymer monomer units using molecular dynamics (MD) and density functional theory (DFT). The goal of this research is to narrow the chemical space and selectively identify suitable monomer subunits for the design of functional polymers.

Systems were prepared with an in-house script utilizing ACPYPE and PACKMOL, where each pharmaceutical molecule was surrounded by 30-50 monomer subunits in a 4 x 4 x 4 nm box. These systems were relaxed and simulated in explicit water solvent over 30 nanoseconds with constant temperature and pressure using the GROMACS software. The main conformations of the polymers and pharmaceuticals extracted from these simulations were further analyzed with DFT via Gaussian 16, employing the B3LYP functional and 6-311++G(d,p) basis set. DFT calculations provided insights into the electronic properties of the formed complexes and their intermolecular interactions. Additionally, we employed an MMPBSA approach using the gmx_MMPBSA package for GROMACS to gain further understanding of the changes in interaction energies during the simulation.

Our approach demonstrates the power of computational methods in high-throughput screening, significantly accelerating the identification of potential candidates for experimental validation. This methodology is a valuable tool for the design and optimization of functional polymers, showing promise for advancing material development in pharmaceuticals and other fields.

Acknowledgement

This study was supported by the Croatian Science Foundation under the project number HRZZ-IP-2022-10-4400 entitled *Development of molecularly imprinted polymers for use in analysis of pharmaceuticals and during advanced water treatment processes* (*MIPdePharma*)

Synthesis of new indene derivatives and evaluation of their antimicrobial activities

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Due to their medical, antimicrobial and industrial applications, heterocyclic compounds have taken a significant place in the field of synthetic organic chemistry [1]. The ability to modify the heterocyclic core enables the synthesis of a wide range of derivatives with different functional groups, thereby adjusting chemical properties and biological activity. Also, heterocyclic compounds have shown selective action on target bacterial cells, making them promising candidates in the fight against bacterial infections, especially those caused by antibiotic resistance. Indene derivatives are one of common structural units in marketed drugs, and in synthetic chemistry as targets in the drug discovery process, with its numerous biological activities [2]. The substituted aromatic and aliphatic ring system in the framework of indene and its analogues provides an extensive evaluation of diverse substituent patterns and functionalities. In this study the preparation and characterization of several heterocyclic and non-heterocyclic indene derivatives will be presented, with evaluation of its antimicrobial activities. The synthesized compounds were screened for *in vitro* antimicrobial activity against microbial species *Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*, respectively. Most of the compounds showed good antimicrobial activity against *B. subtilis, C. albicans* and *A. niger*, while all derivatives were inactive for *P. aeruginosa*.

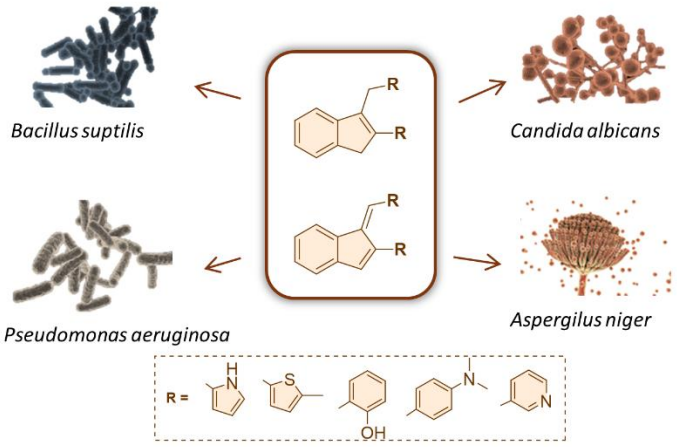

Fig. 1: Structures of synthesized indene derivatives

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Fluorescence in microplates: indirect attenuation of the secondary inner filter effect by enhanced primary inner filter effect

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In our recent publication [1], we presented the added absorber method (AddAbs) for correcting the Inner Filter Effect (IFE) in microplates. By adding an equal amount of a strongly absorbing chromophore to each sample, the method increases IFE-induced fluorescence quenching, effectively mitigating the primary Inner Filter Effect (pIFE) in strongly absorbing samples. In this follow-up study, we show that the proportional increase in pIFE achieved by the addition of a chromophore can also correct the nonlinear fluorescence response caused by the strong secondary Inner Filter Effect (sIFE). The strong and uniform pIFE prevents the excitation light from penetrating deep into the samples, so that most fluorescence events occur in a thin layer near the surface of the liquid sample. Despite significant variations in optical density at the emission wavelength, the fluorescence response remains linear due to the short optical paths travelled by the emitted light (Fig. 1). Testing the method for fluorescein using different concentrations of potassium dichromate as an added absorber resulted in a very linear response even under extreme sIFE conditions ($R^2 > 0.99$; A_{em} up to 79.08, normalized to the path length l = 1 cm). The presented approach is simple, as no additional absorbance measurements or mathematical procedures are required to obtain IFE-corrected results. The method was developed specifically for microplate readers but could also be promising for front-face fluorescence measurements in conventional fluorometers.

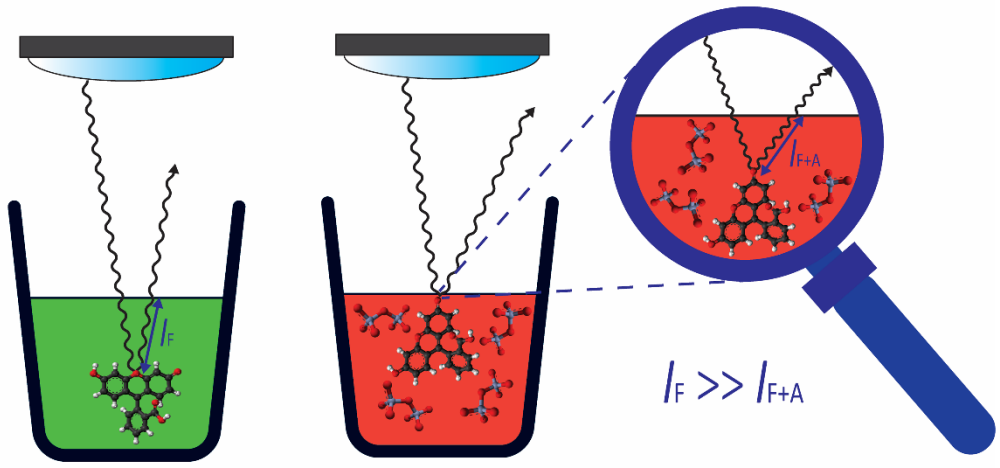

Fig. 1: Schematic representation of the presented IFE correction in microplate wells: In the pure fluorophore solution (left well), longer effective optical paths (l_F) lead to a significant sIFE. In contrast, the added absorber increases the optical density (right well), reducing the effective optical path length (l_{F+A}) for both excitation and emission, improving the linearity of the fluorescence-concentration response.

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Validation of chromatography method after pharmaceuticals extraction from sediment

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The pharmaceutical industry plays a key role in the development and production of medicines, the release of which into the environment through wastewater represents a significant environmental problem today. A large part of pharmaceutical wastewater originates from manufacturing processes, although the industry also discharges untreated wastewater into sewers from other areas. Most drugs are resistant to removal processes in wastewater treatment plants due to their low biodegradability and high solubility in water, which allows them to pollute the aquatic environment and accumulate these substances in sediment. Considering the negative impact of pharmaceutical residual compounds on aquatic ecosystems, as well as the pollution of natural sources of drinking water by release from sediment, it is necessary to research and apply advanced analytical technologies for the determination of pharmaceuticals in order to effectively remove possible toxic substances before release into the environment [1, 2].

The main goal of the work is the development of a specific chromatographic method that enables easy detection and quantification of certain pharmaceuticals in sediment for the purpose of monitoring them. In this work, the method for HPLC-DAD analysis was validated and optimization of extraction by shaking was carried out for certain pharmaceuticals of different structural groups. When optimizing the conditions, the following optimal parameters were determined: sample mass, solvent volume, temperature and duration of shaking extraction. The best solvent used during the extraction turned out to be a mixture of methanol and ethyl acetate in a ratio of 1:1 (5 mL).

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Acknowledgments

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Targeting neurotoxic sites of ammodytoxin A: Binding affinity of sense and antisense peptides

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Snakebites are a significant public health problem in many tropical and subtropical regions, causing high morbidity and mortality. Conventional snake antivenoms face numerous problems, including allergenicity, high production costs and logistical difficulties, highlighting the urgent need for new therapeutic approaches. This study investigates the potential of oligopeptides as therapeutic inhibitors targeting the neurotoxic sites of ammodytoxin A (AtxA, PDB code 3G8G) from *Vipera ammodytes*. We selected two sense oligopeptides representing critical neurotoxic regions of AtxA as targets for inhibition by complementary antisense peptides. Using a heuristic antisense peptide design (HAPD) based on molecular recognition theory, we modelled two antisense oligopeptides as complementary counterparts for each sense oligopeptide. The HAPD algorithm significantly improves the efficiency of peptide library screening by reducing the solution space by a factor of 7×10^5 compared to the random peptide library approach [1]. The modelled sense and antisense peptides were commercially synthesized, and their binding affinities were determined by spectrofluorometric titrations (K_D > 1 µM for all sense-antisense peptide pairs). Confirmation of the binding of the sense-antisense peptide pairs led to further investigation of their potential binding to the native target protein by global docking simulations using the PepATTRACT web server (Fig. 1) [2]. The results emphasize the applicability of molecular recognition theory in the development of antisense peptides. Further studies are needed to investigate the therapeutic efficacy and broader applications of the peptides obtained by the HAPD algorithm.

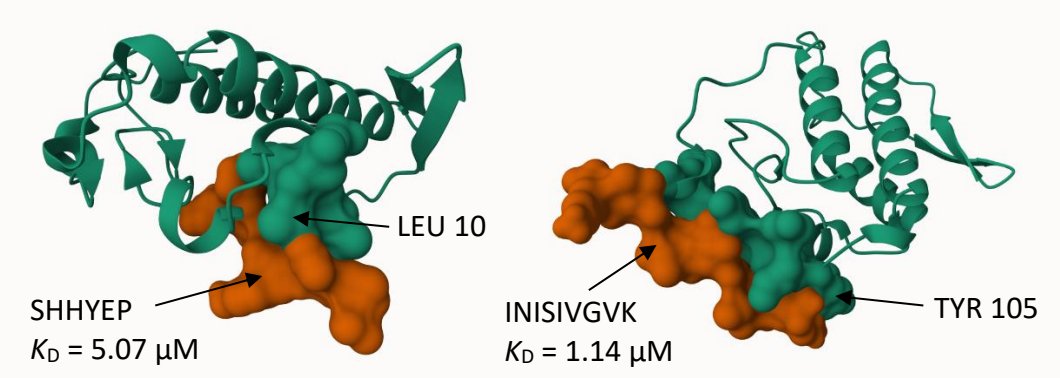

Fig. 1: Global docking simulations of AtxA (PDB code 3G8G) with modelled antisense peptides obtained using the PepATTRACT web server. [2] The values of the dissociation constants K_D were determined by spectrofluorometric titrations of sense-antisense peptide pairs: GMMILG- SHHYEP (left) and YIYRNYPDF-INISIVGVK (right).

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Synthesis of polymer additives for diesel fuel based on methacrylate monomers using FAME biodiesel as a solvent

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Diesel fuel obtains its satisfactory performance properties primarily through the addition of additives. The additives are usually based on polymers such as ethylene vinyl acetate, methacrylate-based polymers, alpha-olefin copolymers, etc. [1,2]. Biodiesel (usually FAME biodiesel) is blended with diesel fuel in a volume fraction of up to 7 % in order to meet environmental standards during use [3]. Most of the difficulties in using diesel fuel occur in the winter period, because the paraffin contained in diesel fuel crystallizes at lower temperatures and it would be difficult to use diesel fuel during this time without the addition of additives. In our research, we have developed new polymer additives to improve the low-temperature properties of diesel fuel based on methacrylate monomers. We used FAME biodiesel as a solvent, which was not removed after the reaction but remained an integral part of our additive. The synthesized additives were added to diesel fuel and showed an improvement in the low-temperature properties of diesel fuel by increasing the pour point by more than 20 °C without negatively affecting other application properties such as density or viscosity.

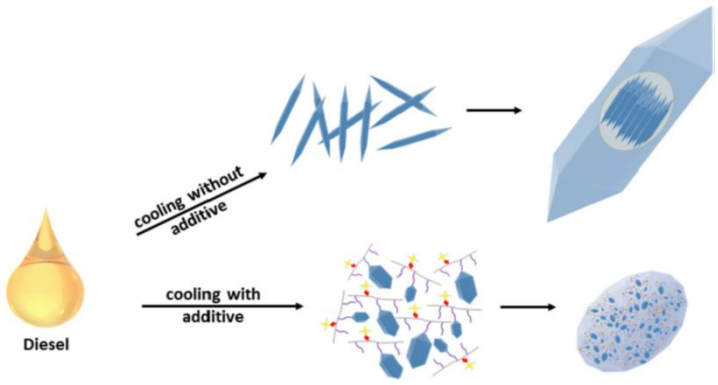

Fig. 1: Proposed mechanism of additive influence on crystallization of paraffinic waxes in diesel.

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Application of Cu nanoparticles in antimicrobial coatings

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Copper is one of the most widely recognized antimicrobial metal linked to multiple antimicrobial mechanisms such as: plasma membrane permeabilization, membrane lipid peroxidation, alteration of proteins, inhibition of protein assembly and activity, or denaturation of nucleic acids [1-3]. Moreover, copper in a form of nanomaterials shows grater antimicrobial activity so is an emerging class of nano-antimicrobials providing complimentary effects and characteristics, as compared to widely used silver or zinc oxide nanoparticles. Metal nanoparticle-loaded yarns can be used in a number of biomedical and textile applications such as medical devices, burn or wound dressings, healthcare materials (including disposables), personal care products, veterinary, military and bio-defense items, protective suits or clothing. This work therefore presents application of Cu nanoparticles in functionalization of the surface of medical material by deep coating method. Electrospun biocompatible yarns were modified by using sol-gel procedure during which the Cu nanoparticles were incorporated within the coating. The materials were characterized before and after the modification by FTIR, UV-VIS, SEM and DLS methods. The results proved efficient formation of a novel antimicrobial coating homogenously distributed on the surface of the yarns. Additionally, antimicrobial tests of Cu nanoparticles proved the antimicrobial property of Cu nanoparticles in coating, enabling future application of novel medical materials.

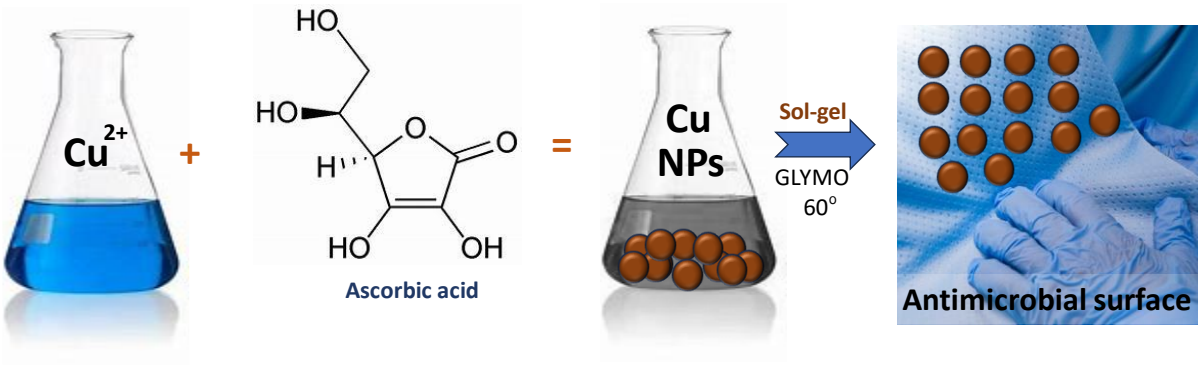

Fig. 1: Cu nanoparticles obtained in reaction with ascorbic acid and applied on medical materials by sol-gel procedure.

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Electrochemical synthesis of 2-imino-1,3-oxazolidines and cyclic carbamates fused onto azithromycin

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Heterocyclic compounds like oxazolidines and thiazolidines are interesting molecular moieties due to their appearance in a number of molecules endowed with various biological activities. It was previously shown that macrolide derivatives having 2-substituted-1,3-oxazolidine and 1,3-thiazolidine moieties fused to the desosamine sugar or azalide macrocyclic ring show potent anti-inflammatory properties [1,2]. We were interested in designing and developing novel, economical, fast, and environmentally friendly electrochemical synthesis of 2-substituted-1,3-oxazolidine fused to macrolide scaffold as an alternative to commonly used classical chemical methods. Macrolides are considered a challenging target for electrochemistry due to the presence of tertiary amine that is easily oxidized and usually aren't regarded as potential substrates for electrochemical transformations. This work will show transformations that occur at lower potential than oxidation of these tertiary amines and how electrochemistry can be a useful method in synthesis of complex molecules derived from natural products. Thiourea and carbamate intermediates were prepared in one step from demethylated azithromycin derivatives. Various electrochemical reaction conditions were probed, and the most promising conditions were further optimized to obtain desired 2-substituted-1,3-oxazolidine fused derivatives in good yields. We will be trying out same/similar conditions on small molecules. Further efforts will be made to develop one pot procedure for these cyclizations to increase overall yields.

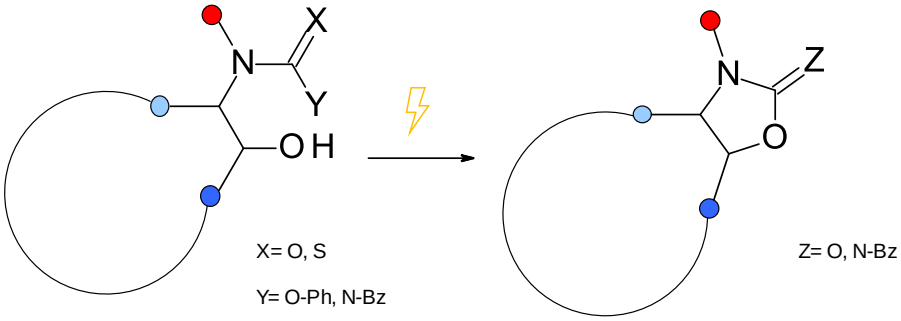

Fig. 1: General scheme of cyclization.

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Identification and quantification of biflavones in ginkgo leaf extracts prepared by convenient and assisted extraction methods

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Ginkgo (*Ginkgo biloba* L.) is a well-known medicinal plant. With 110 identified flavonoid structures, ginkgo leaves contain biflavonoids that show promise as antimicrobial, antiviral, anticancer, and neuroprotective agents [1]. We developed an HPLC-DAD method for the simultaneous identification and quantification of five major biflavonoids: amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin in ginkgo extracts [2]. We compared the effects of various extraction methods — convenient solvent-based (70 % ethanol) and enzyme-assisted, ultrasound-assisted, mechanical-assisted, and chemically assisted — on the biflavonoid content. All extracts contained the five biflavonoids, with sciadopitysin being the most abundant in all analyzed extracts. Except for the chemically assisted extraction, similar biflavonoid contents were achieved after 45 minutes of extraction. Notably, enzymatic and mechanical-assisted extractions yielded significantly higher biflavonoid content within only 5 minutes, indicating these methods could shorten extraction times and improve the efficiency of target compound retrieval [3].

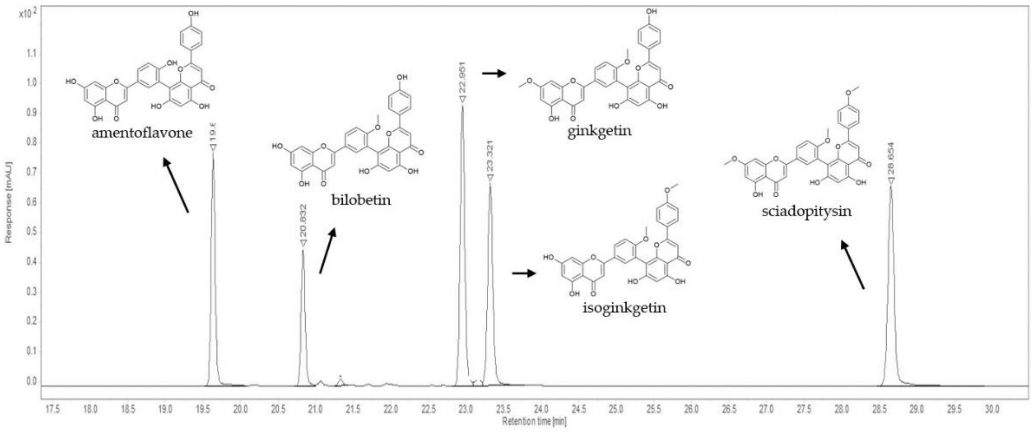

Fig. 1: Representative chromatogram of five biflavones recorded at 330 nm [2].

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A trade-off between ferulic, sinapic and *L*-ascorbic acids in broccoli microgreens grown under temperature stress

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Climate change leads to shifts in temperature patterns, prompting plants to adjust their chemical composition for survival. In our study, we examined how low (LT) and high (HT) growing temperatures affect the concentration of free and conjugated forms of major phenolic acids and vitamin C (*L*-ascorbic acid) in broccoli microgreens (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.). Extracts were prepared in 70 % ethanol. Free forms were analyzed in original extracts, while conjugated forms were analyzed in the extracts hydrolyzed using 1.2 M hydrochloric acid at 80 °C. Using LC-MS/MS and statistical methods, we recorded that hydroxycinnamic acids ferulic and sinapic reacted oppositely to HT and LT. Ferulic acid, free and conjugated, was susceptible to both; HT significantly increased it, while LT decreased it. HT also showed a significantly stronger effect than LT, which altogether might indicate an important role of ferulic acid in the adjustment of broccoli to elevated environmental temperatures. HT significantly reduced the concentrations of both free and conjugated sinapic acid. On the other hand, under LT conditions, free sinapic acid was not affected significantly, but its conjugated form/s was/were increased. This suggests an inverse relation between ferulic and sinapic acid biosynthesis in broccoli microgreens under temperature stress. We assume that ferulic acid has a significant role in the protection of broccoli microgreens from HT, while sinapic acid serves for protection against LT. Depending on the type of temperature stress, broccoli microgreens will direct the biosynthetic pathway either toward ferulic or sinapic acid. This result also suggests that ferulic and sinapic acid might help to mitigate the effect of temperature stress in broccoli microgreens, if exogenously applied. Both HT and LT significantly changed the free and conjugated *L*-ascorbic acid concentrations in the broccoli microgreens. HT significantly increased the concentration of free-form *L*-ascorbic acid, and LT decreased. On the contrary, conjugated *L*-ascorbic acid was decreased in the HT group and increased in the LT group. This suggests a trade-off scheme among the free and conjugated form/s of *L*-ascorbic acid and their involvement in the acclimation of broccoli microgreens to HT/LT.

Use of carbon dioxide for the synthesis of cyclic carbamates

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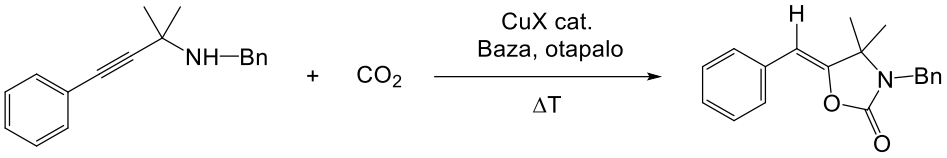
Carbon dioxide (CO_2) is an inert and non-toxic gas. The main source of CO_2 emissions into the atmosphere comes from the burning of fossil fuels used in industry, energy generation and transport sectors. Its excessive emission has serious negative effects on the environment, manifested in ecological disruption, global warming and climate change. Current research is focused on the development of new catalytic reactions and processes that enable the conversion of CO_2 into products and fuels with added value [1]. As part of the above, over the past decade, there has been a significant increase in interest in the use of CO_2 as a renewable C1-source of carbon for the purpose of obtaining carbonates and carbamates, the importance of which is additionally emphasized by the possibility of their multiple uses [1].
For example, oxazolidinones are cyclic carbamates that have significant applications in the pharmaceutical industry and are especially important as structural motifs of antibiotics [2]. These compounds can be successfully synthesized in catalyzed reactions of CO_2 and propagyl amines. With the aim of improving these CO_2-fixation processes, a wide range of organometallic catalysts based on noble metals, including Ru, Pd, Au and Ag, have been developed [3]. Our research aims at the synthesis of oxazolidinone **2** from *N*-benzyl-2-methyl-1-phenylbut-3-yn-2-amine **(1)** in one step, without the use of expensive catalysts (Fig. 1). To optimize the reaction conditions, various cheap copper salts were used as catalysts, and the influence of different bases, solvents and temperatures on the reaction yield was also investigated.



1 **2**

Fig. 1: General reaction for the synthesis of oxazolidinones from CO_2.

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Upotreba velikog jezičnog modela u rješavanju stehiometrijskih zadataka: Usporedba s tradicionalnim pristupom rješavanu

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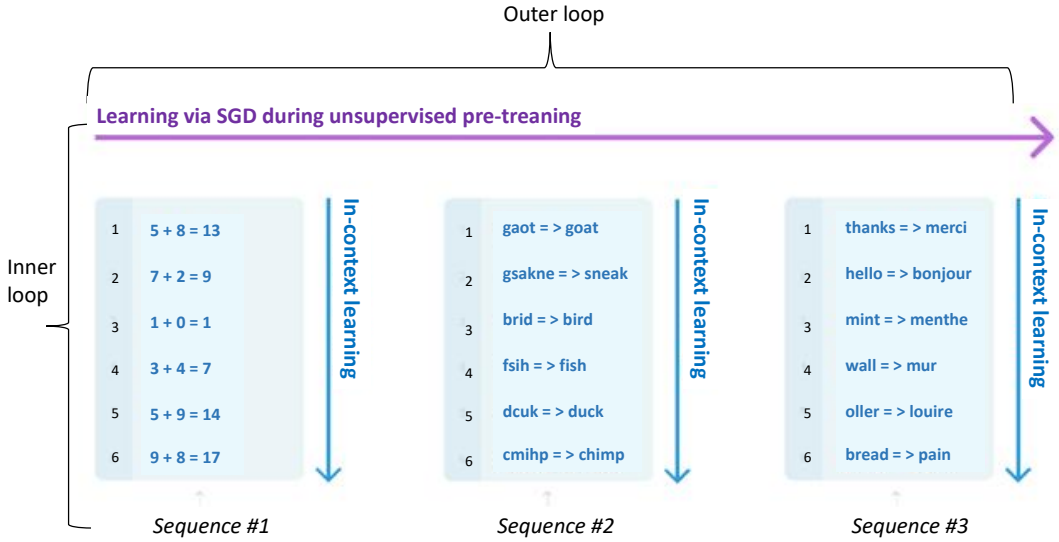
U ovom radu prikazane su mogućnosti koje nudi veliki jezični model, konkretno GPT-4o, u rješavanju stehiometrijskih zadataka u usporedbi s tradicionalnim pristupom. Model GPT-4, razvijen od strane OpenAI, koristi napredne algoritme za obradu prirodnog jezika kako bi razumio i generirao ljudski jezik, omogućujući primjenu u različitim znanstvenim i edukacijskim domenama [1].

Za potrebe ovog istraživanja korištena je zbirka zadataka "Stehiometrija" autora M. Sikirice [2], pri čemu su zadaci birani prema tipu i vrsti. Svaki zadatak rješavan je na dva načina: tradicionalno, od strane autora, i uz pomoć GPT-4 modela. Cilj je bio analizirati točnost, brzinu i učinkovitost rješavanja zadataka te usporediti rezultate između dva pristupa. Naš rad obuhvaća detaljan pregled načina na koji veliki jezični model obrađuje zadatke, uključujući postupak razumijevanja problema, formulaciju odgovora te identifikaciju potencijalnih prednosti i ograničenja u odnosu na tradicionalno rješavanje. Posebna pažnja posvećena je metodologiji odabira zadataka i evaluaciji rezultata.

Rezultati istraživanja pokazat će u kojoj mjeri veliki jezični modeli mogu biti korisni alati u edukaciji i znanstvenom radu, pružajući uvid u potencijalne smjernice za daljnji razvoj i primjenu ovih tehnologija. Kroz usporedbu uspješnosti rješavanja zadataka, naš rad doprinosi boljem razumijevanju mogućnosti i izazova integracije umjetne inteligencije u obrazovne procese



Slika 1. Primjer učenja koji je temelj GPT modela (preuzeto iz [1])

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Sorption of sulfamethoxazole onto molecularly imprinted polymer – kinetic study

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Progress in science and industry is increasingly raising the level of humanity's awareness to preserve natural resources that are contaminated with once invisible, but hazardous substances. Pharmaceuticals, which are among the emerging pollutants, and whose negative effects on biota have already been proven, continue to pose a threat to the both ecosystem and human health through drinking water contamination, antibiotic resistance and the problem of bioaccumulation. Considering that around 3000 pharmaceutically active substances have been declared in the EU, it is not surprising that many of them enter the environment in unchanged form and as metabolites. One of the many pharmaceuticals detected in the water is sulfamethoxazole – an antibiotic commonly prescribed to treat various bacterial infections. In this research, a newly synthesized material – a polymer imprinted with a sulfamethoxazole molecule was tested as a sorbent to improve its detection in complex environmental matrices. To better understand the binding of sulfamethoxazole to molecularly imprinted polymer, the sorption kinetics were described in detail using various kinetics models such as the pseudo-first-order model, the pseudo-second-order model, and the intraparticle diffusion model. Based on the data obtained, the effect of contact time was also described by performing the experiment at different time intervals over 24 hours between the tested molecule and the molecularly imprinted polymer. In order to obtain more realistic data for MIP efficiency in the case of sulfamethoxazole removal of, the kinetic study was also performed in parallel for non-molecularly imprinted polymer. As expected, both materials showed similar kinetic sorption behavior, although the selectivity of the imprinted material was better.

Acknowledgement
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