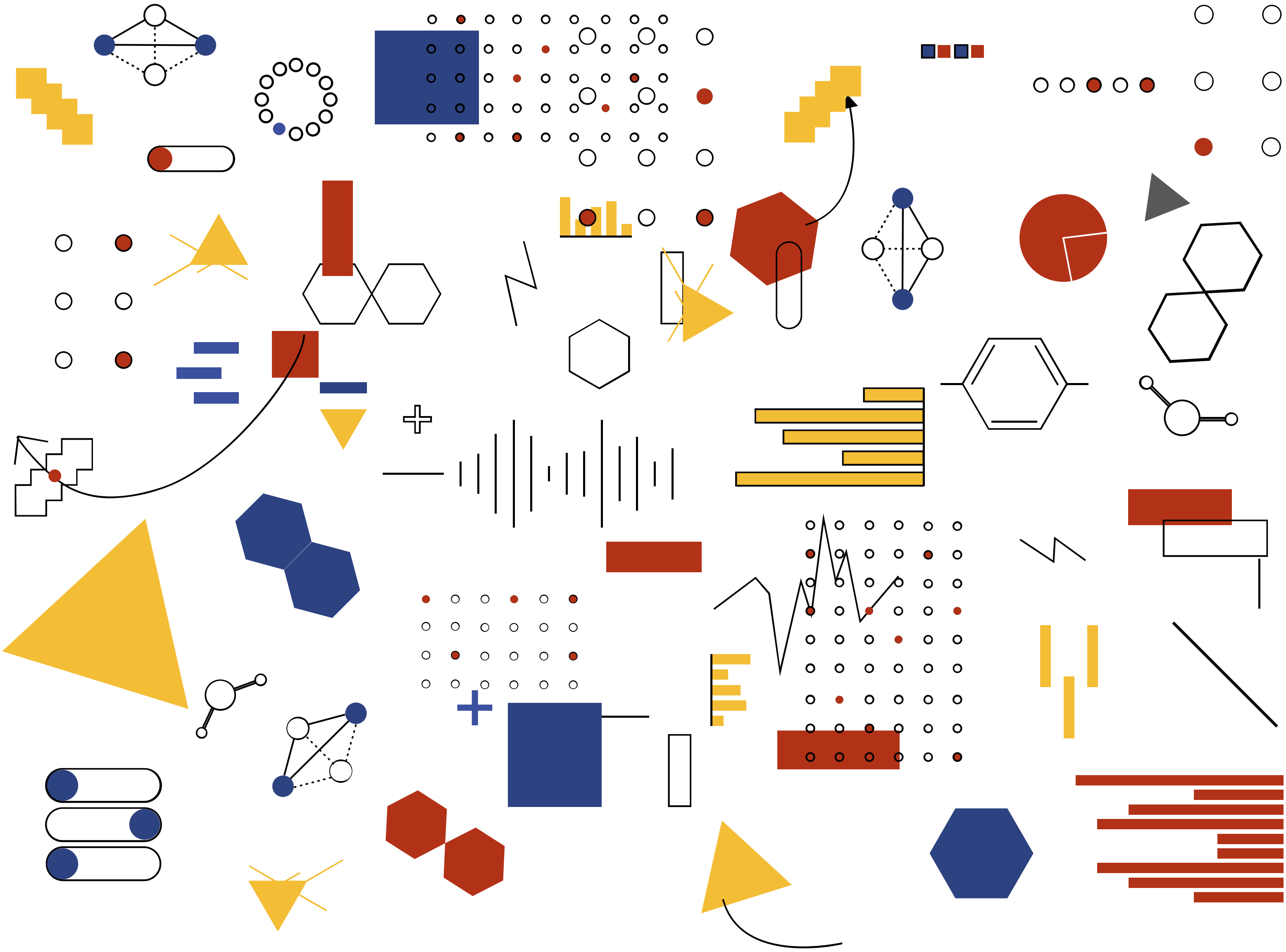

Book of abstracts

CUTTING
EDGE
2021

SCIENTIFIC CONFERENCE
FOR YOUNG RESEARCHERS
Independent in science

Thursday
16/09/21

Virtual
conference

Organizator in založnik zbornika
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani

Kraj prireditve
Spletna konferenca, Fakulteta za kemijo in kemijsko tehnologijo

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Independent, Science Independent

in times when everything depends on your internet connection!

Well, these two years were a bit much, right? Sometimes it feels like the last Cutting Edge conference was only yesterday, while in fact, two years have passed and although one can have an impression everything has changed, we are still here, in fact, we are here to stay, no matter the challenges confronting us!

Lockdown after lockdown has pushed us into the exciting virtual world only to realize a few months later, how important the actual physical presence is. How we miss sitting in the lecture halls chatting with other colleagues that became friends, having fun in the labs, enjoying the coffee breaks with good coffee and actual cookies (not only the one's internet serves you every time you visit a webpage).

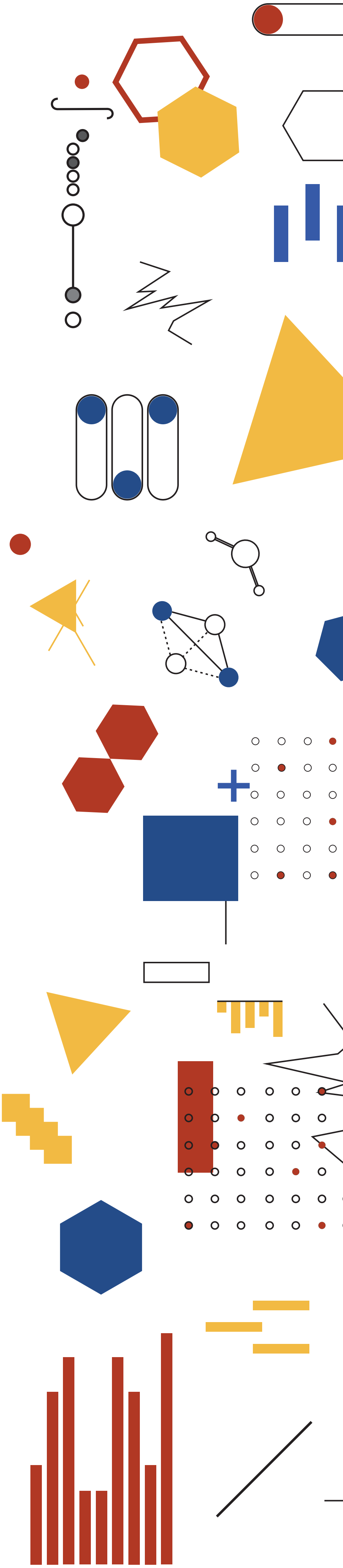
But rather than cooking all that frown, let's turn it upside down and look at the important lessons these extraordinary circumstances have brought forth. We have realized, we can actually make use of this tiny little camera on the laptop, we perfected the chocolate chip cookies recipe and mastered that sourdough yeast. And most importantly we have realized it is absolutely OK to take a break and just be bored and do nothing for a little while.

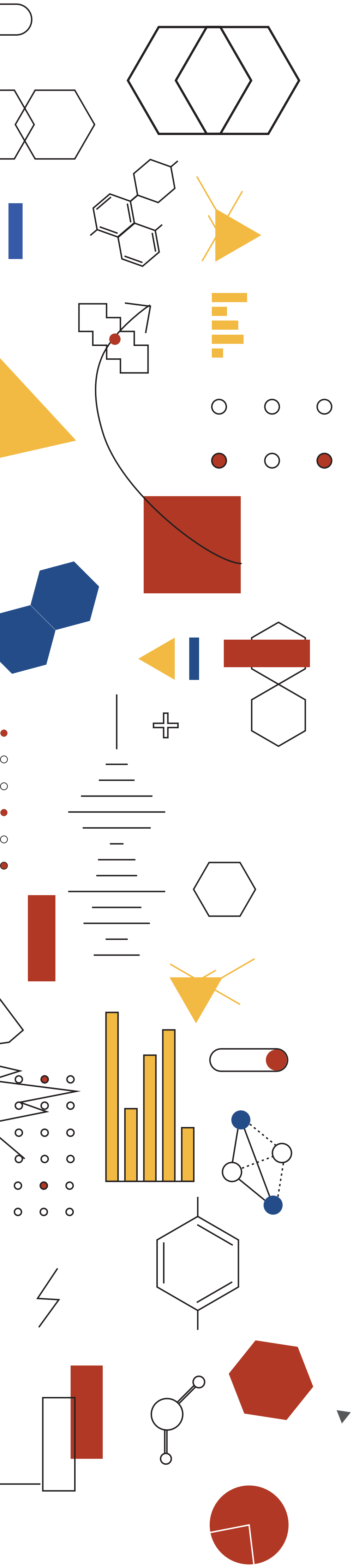
In the end, we are discovering the cutting edge not only in the field of science but also exploring new grounds of online meetings and conferences.

This year's Cutting Edge was organized in collaboration with the Faculty of Chemistry and Chemical Technology, University of Ljubljana, National Institute of Chemistry, National Institute of Biology, and Jožef Stefan Institute.

The unique Cutting Edge 2021 conference took place on Thursday, 16. September 2021 and lasted from 8 am until 5 pm. Due to the constantly changing epidemiological situation, the conference was held online on the world wild web.

This year's topic was Independence in Science. We invited speakers with extensive experience and wonderful advice regarding this topic. Dr Richard Wheeler, European Projects Manager of the European Sustainable Energy and Project advisor at Jožef Stefan Institute, and Tjaša Nabergoj from the Project management office at the University of Ljubljana prepared in-depth talks about different opportunities available to students and young researchers.





Dr Urša Tiringer, an inspiring young scientist that has recently concluded a post-doctorate at Technical University Delft founded from Marie Curie fellowship, Dr Matic Lozinšek, a recent recipient of the ERC Starting Grant project HiPeR-F and a rising star on the horizon of researchers in the field of inorganic chemistry, and Dr Matjaž Humar, who also received the prestigious ERC Starting Grant and recently obtained a Young Investigator grant by The Human Frontiers Science Program, talked about their personal experiences on becoming independent in science.

There were more than 145 participants who joined from their office, desk, couch, bed, favourite cafeteria or in fact any other place you can think of that has an internet connection. Around 117 of them presented their research in three separate sessions – Science behind the living, Materials of the future, and Technologies for the Earth and environments. Even online, we still managed to have chocolate chip cookie coffee breaks! We finished the marvelous day by giving awards for the best posters.

On Wednesday, 15th of September 2021 some of the Cutting Edge 2021 conference participants were able to attend the workshop organized by our friends over at Ljubljana University Incubator. With this workshop we wanted to give our participants the opportunity to learn how to effectively articulate their ideas, which is a valuable lesson for all of us, not only in the field of presenting our research, but also when applying for grants, looking for a job, or just trying to impress someone.

The conference could not have been organized at the level it was without additional help from our collaborators, sponsors, and you, the participant, who is reading this. We cannot find the right humble words to thank you enough, so rather than that, let us finish with a promise:

See you again in 2023!

Join us, we have cookies!

Cutting Edge 2021 Organizing Committee

16/09/21

CUTTING
EDGE
2021

conference schedule

8:00 – 8:30	Opening ceremony
8:30 – 9:30	Tjaša Nabergoj
9:30 – 10:30	**Plenary lecture** Dr. Richard Wheeler
10:30 – 11:00	Coffee break with sponsors Tehnološki park, Ljubljanski univerzitetni inkubator, and Mikro+Polo
11:00 – 11:30	Dr. Matjaž Humar
11:30 – 12:00	Dr. Matic Lozinšek
12:00 – 12:30	Dr. Urša Tiringer
12:30 – 13:30	Lunch break
13:30 – 16:30	**Poster session**
16:30 – 17:00	Closing ceremony and best poster award ceremony

Notes

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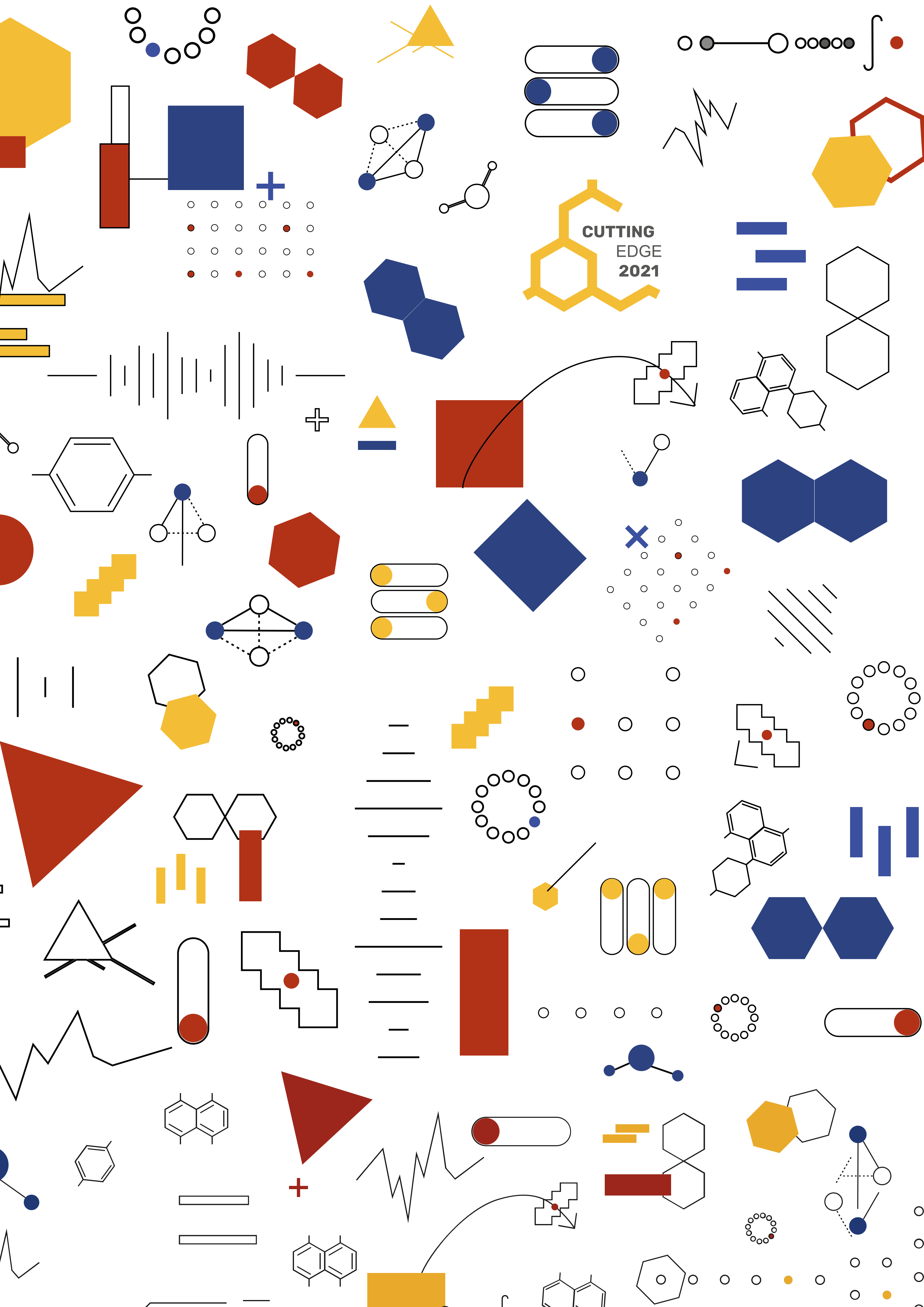
CUTTING
EDGE
2021

Poster presentations

Reliable determination of resistome in sputum samples using targeted HTS

Aleksander Benčič*[1, 2], Nataša Toplak[3], Simon Kopen[3], Alexandra Bogožalec Košir[1], Mojca Milavec[1], Viktorija Tomič[4], Dane Lužnik[4], and Tanja Dreo[1]

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[2]Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia
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[4]University Clinic of Pulmonary and Allergic Diseases Golnik, Laboratory for Respiratory Microbiology, Golnik 36, 4204 Golnik, Slovenia

Slow diagnostic process of antibiotic susceptibility testing is one of the reasons for ineffective treatment of bacterial infections and over prescription of broad spectrum antibiotics in hospital environment. Targeted high-throughput sequencing (HTS) is a promising new method that could help overcome limitations of existing diagnostic methods[1]. To evaluate the reliability of targeted HTS, we determined the resistome in three sputum samples using Ion Torrent sequencing and Ion AmpliSeq™ Pan-Bacterial Research Panel to prepare library. Three different extraction methods were used – CTAB, GXR NA/Arrow and QIAamp DNA mini kit. Extractions were performed on three technical replicates of each sputum sample, and repeated in two consecutive days. Our results show that DNA extraction methods have a significant impact on the diversity of resistome. Results of resistome determination were repeatable for all three extraction methods, however, QIAamp DNA mini kit on average gave the most repeatable results (Fig. 1). Additionally, inter-day variability did not show significant impact on results.

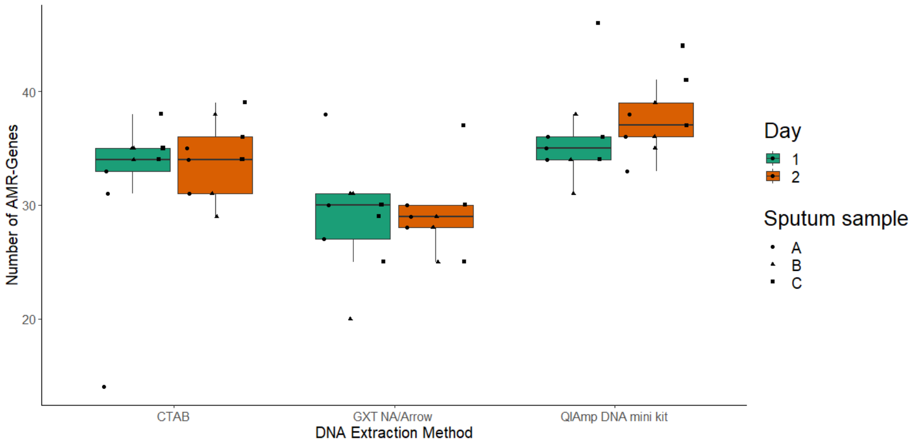

Fig. 1: *Numbers of genes associated with antibiotic resistance (AMR-genes) according to method of DNA extraction and day of extraction in each sample.*

1. Terranova, L.; et al., How to process sputum samples and extract bacterial DNA for microbiota analysis, *Int. J. Mol. Sci.* **2018**, *19*, 3256.

Understanding the role of abiotic factors on naturally occurring transformations and fate of phenolic pollutants in surface waters

Aleksander Kravos[1], Helena Prosen*[,1], and Gabriela Kalčíková[1]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia, helena.prosen@fkkt.uni-lj.si

Gradually, a new vision of twenty-first century as transition era has been arising. There are new strategic plans on the horizon, such as European Green Deal, directed towards redefinition of world's societies. As a result, the role of environmental chemistry is increasing and becoming more complex than ever before; especially when dealing with the introduction of priority pollutants into aquatic ecosystems. Representative family of those are phenolic compounds, *e.g.* phenol and its chlorinated derivates[1]. But despite being regulated and not classified as emerging pollutants, their ubiquitous presence has been shown repeatedly in surface waters (see IP-CHEM database[2]) ranging between 2–2000 ng/L and even reaching an extent of few mg/L[3].

***Fig. 1**: Outline of performed simulations to evaluate the role of certain abiotic and structural factors.*

Environmental chemistry has a duty to understand the fate of pollutants once discharged into environment[1]. Majority of the phenolics are considered to biodegrade slowly. Having additional C–Cl bond(s) makes them even more persistent and toxic, posing a threat to aquatic life. Processes are even more complex, since there are structural and abiotic factors that influence natural degradation which may induce photochemical and physical transformations, as well as intensify or inhibit catalytic oxidations, hydrolysis, reactions on sediments' surface[3] and interactions with external substances[1]. Extensive role of sunlight and air in decomposition of chlorophenols was, for example, demonstrated in seawater without any biological factors present[4]. Consequently, we aimed to provide even more detailed data on chemistry of naturally induced degradation processes of selected phenols, as depicted in Fig. 1. Accordingly, analytical techniques for quantitative (*i.e.* HPLC) and qualitative (*i.e.* UPLC-MS, solid phase microextraction followed by GC-MS) analysis were applied. Moreover, ecotoxicological assessment on *Daphnia magna* organisms allowed us to monitor natural detoxification. Long-term effects and chemical lability in natural waters can be therefore better understood.

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3. Czaplicka, M., Sources and transformations of chlorophenols in the natural environment. *Sci. Total Environ.* **2004**, *322*, 21–39.
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New transition-metal-containing MgFe mixed oxides as highly effective catalysts for the hydrodeoxygenation of benzyl alcohol

Alexandru-Tudor Toderaşc*,[1], Adriana Urdă[1], and Ioan-Cezar Marcu[1]

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Nowadays, hydrodeoxygenation (HDO) is employed for converting oxygenated compounds into hydrocarbons through treatment at moderate temperatures and pressures by means of C-O bond scission[1]. Copper-containing mixed metal oxides seem to be interesting catalysts for this kind of reactions due to their significant hydrogenolysis activity and limited propensity for complete hydrogenation of aromatic substrates[2].

In this work, several transition-metal-containing M-MgFeO mixed oxides, with M = Cu, Ni, Co, Ag or Ce, were prepared, characterized and tested in the HDO reaction of benzyl alcohol. The layered double hydroxides (LDH) precursors, with Mg/Fe = 3 and 10 at. % M or, for M = Cu, from 2.5 to 20 at. %, were prepared through coprecipitation at a constant pH of 10 using Mg, Fe and M nitrates as starting materials. The synthesized LDH were dried and then calcined at 500 ºC for 5 h. The LDH precursors and the mixed oxides were characterized by XRD, N_2 adsorption-desorption, H_2-TPR, CO_2-TPD and SEM-EDX techniques. The obtained mixed oxides were tested in the HDO reaction in an autoclave reactor at 230 ºC, under 5 atm of H_2 for 3 hours, with 1 ml of benzyl alcohol and 50 mg catalyst. The catalytic performance of the mixed oxides strongly depended on both the nature and the content of transition metal. Among them, Cu-MgFeO with 10 at.% Cu gave the best results, with 93,8 % alcohol conversion and 93,8 % selectivity for toluene. Considering the excellent performance of the copper-containing catalyst, the influence of the copper content of the mixed oxides was then studied by preparing a series of Cu_x-MgFeO catalysts, where x = 2,5, 5, 7,5, 15 and 20 at.%. The sample with 10 at.% Cu still showed the best results, and the influence of reaction temperature, reaction time, catalyst weight were studied on this catalyst. Benzaldehyde and benzyl benzoate were considered reaction intermediates, and catalytic tests using them as reactants confirmed this hypothesis. Based on the products observed, a reaction pathway has been proposed.

1. Rizescu, C.; Sun, C.; Popescu, I.; Urdă, A.; Da Costa, P.; Marcu, I-C., Hydrodeoxygenation of benzyl alcohol on transition-metal-containing mixed oxides catalysts derived from layered double hydroxide precursors. *Catal. Today.* **2021**, *366*, 235–244.
2. Deutsch, K.L.; Shanks, B.H., Hydrodeoxygenation of lignin model compounds over a copper chromite catalyst. *Appl. Cat. A Gen.* **2012**, *447–448*, 144–150.

Green ZIF-90 Synthesis

Aljaž Škrjanc[1,2,*], Ciara Byrne[1], and Nataša Zabukovec Logar[1,2]

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Zeolitic imidazolate frameworks (ZIFs) are a subclass of metal-organic frameworks (MOFs) which have shown promising results as functional materials for gas storage[1], separation[2], and catalysis[3] applications. From the wide array of known ZIFs, ZIF-8 and ZIF-90, make up more than half of all articles on ZIFs in the last 10 years. Their potential use in large-scale applications as functional materials also poses the question of atom economy in their synthesis, as well as its environmental impact. However, on the other hand, implementation of greener solvents is also becoming an option in reducing environmental impact of ZIF synthesis. While ZIF-8 has already been synthesised in pure methanol [4], ZIF-90 synthesis still largely relies on *N,N*-dimethylformamide (DMF) as a solvent. The use of green solvents as an alternative to dimethylformamide (DMF) in the synthesis of zeolitic imidazolate framework-90 (ZIF-90) was investigated. Two bio-based aprotic dipolar solvents Cyrene™ and γ-valerolactone (GVL) proved to successfully replace DMF in the synthesis at room temperature with a high product yield. While the Cyrene™ - based product shows reduced porosity after activation, the use of GVL in the synthesis resulted in materials with preserved crystallinity and porosity after activation, without prior solvent exchange and a short treatment at 200 °C. The primary particles of 30 nm to 60 nm in all products further form agglomerates of different size and interparticle mesoporosity, depending of the type and molar ratios of solvents used.

1. Akbari Beni, F.; Niknam Shahrak, M., Alkali metals-promoted capacity of ZIF-8 and ZIF-90 for carbon capturing: a molecular simulation study. *Polyhedron.* **2020**, *178*, 114338.
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3. Xiang, W.; Sun, Z.; Wu, Y.; He, L. N.; Liu, C. jun., Enhanced cycloaddition of CO2 to epichlorohydrin over zeolitic imidazolate frameworks with mixed linkers under solventless and co-catalyst-free condition. *Catal. Today.* **2020**, *339*, 337–343.
4. Hu, L.; Chen, L.; Peng, X.; Zhang, J.; Mo, X.; Liu, Y.; Yan, Z., Bifunctional metal-doped ZIF-8: A highly efficient catalyst for the synthesis of cyclic carbonates from CO2 cycloaddition. *Microporous Mesoporous Mater.* **2020**, *299*, 110123.

Expression of circular RNAs in non-small cell lung cancer: bioinformatic analysis of microarray data

Ana Dolinar*,[1]

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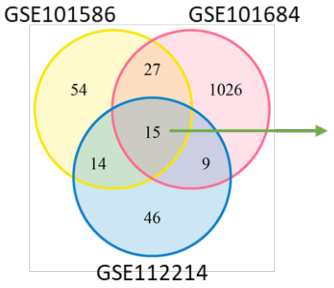
Circular RNAs (circRNAs) are considered a powerful player in many human diseases, including cancer. They are involved in several cellular processes and are becoming a noteworthy type of biomarkers. Lung cancer is responsible for 2.21 million new cases of cancer and 1.8 million deaths in the year 2020[1]. Here, a bioinformatic approach was used to identify differentially expressed circRNAs in tissue samples of non-small cell lung cancer. Three microarray data sets (GSE101586, GSE101684, GSE112214) were obtained from Gene Expression Omnibus Database. Data sets were analyzed using R software and limma package. In total, 15 circRNAs are differentially expressed in all three data sets (Fig. 1). They are encoded in 13 different host genes and some of them were already reported as differentially expressed in lung cancer[2,3]. Host genes are involved in axon guidance, alternative splicing, and dendritic growth. Based on data from circAtlas, half of these circRNAs have putative binding sites for microRNAs which were already implicated in non-small cell lung cancer progression and/or proposed as diagnostic biomarkers. The biological role of differentially expressed circRNAs is not fully understood yet, however, it was shown that circRNA *hsa_circ_0001320* can inhibit cancer cell growth and invasion, and circRNA *hsa_circ_0002346* can inhibit or promote metastasis and promote chemoresistance, depending on the cancer type.



hsa_circ_0000842, hsa_circ_0001072, hsa_circ_0001320, hsa_circ_0002286, hsa_circ_0002346, hsa_circ_0003176, hsa_circ_0004089, hsa_circ_0005013, hsa_circ_0006677, hsa_circ_0007386, hsa_circ_0008234, hsa_circ_0008453, hsa_circ_0009043, hsa_circ_0027033, hsa_circ_0069244

Fig. 1: *Differentially expressed circRNAs in non-small cell lung cancer. Venn diagram shows overlap between three datasets and 15 common circRNAs are listed on the right side.*

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Organocatalyzed synthesis of dihydropyrano[3,2-*b*]pyrroles – a case study of self-disproportionation of enantiomers (SDE)

Ana Gorenc[1], Luka Ciber[1], Jurij Svete[1], Bogdan Štefane[1], Franc Požgan[1], and Uroš Grošelj*,[1]

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Organocatalyzed reaction of *N*-unsubstituted-5-arylidene-Δ^2-pyrrolin-4-ones[1,2] with malononitrile resulted in fused pyranopyrrole derivatives[3] (Fig. 1). The enantiomerically enriched products of this [4+2] annulation were purified with column chromatography. The enantioselectivity of the product in a discrete tube differed from the enantioselectivity of the bulk sample, resulting in unrepeatable results of enantioselectivity. Similarly, sample preparation for HPLC analysis by trituration or by homogeneous dissolution lead to markedly different enantioselectivity results. It appeared that via column chromatography over silica gel or trituration, enantioenrichment has occurred. This phenomenon in also known as *self-disproportionation of enantiomers* (SDE). It can appear via phase transitions solid–liquid, solid–gas, and liquid–gas, achiral chromatography, SEC, and GC. Its occurrence is of great importance and is often overlooked, especially in the field of chromatography, and can lead to errors in reported enantioselectivity results in the literature[4].

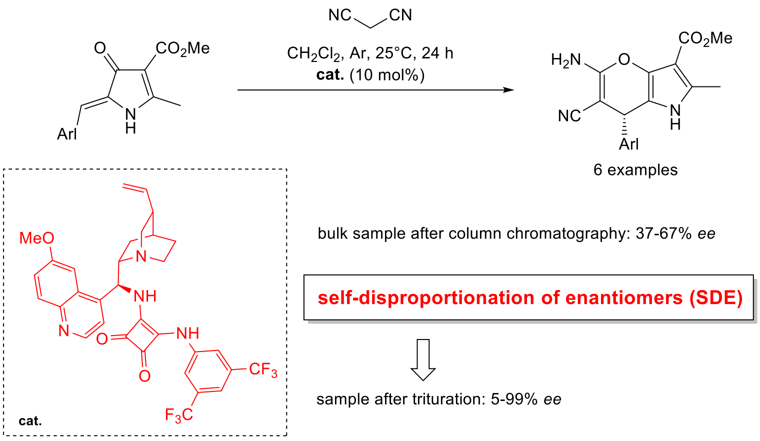

***Fig. 1**: Organocatalyzed annulation of arylidene-Δ^2-pyrrolin-4-ones with malononitrile.*

1. Grošelj, U.; Ciber, L.; Gnidovec, J.; Testen, Ž.; Požgan, F.; Štefane, B.; Tavčar, G.; Svete, J.; Ričko, S., Synthesis of spiro-Δ^2-pyrrolin-4-one pseudo enantiomers via an organocatalyzed sulfa-Michael/aldol domino sequence. *Adv. Synth. Catal.* **2019**, *361*, 5118–5126.
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Competitiveness of *Salmonella enterica* mutants in mixed-species biofilms with *Bacillus subtilis*

Ana Grosek[1], Eva Kovačec[1], Tjaša Danevčič[1], Eli Podnar[1], Andi Erega[1], Sara Goršek[1], Bram Lories[2], Hans Steenackers[2], and Ines Mandić-Mulec[*,1]

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In recent years, biofilms have become an important research subject in the fields of microbiology, medicine and agriculture, since biofilm formation is the most common multicellular behaviour exhibited by bacteria in nature[1]. Influence of social interactions on the spatial distribution of biofilms is crucial to understand the physical stability of biofilms, to help improve cleaning procedures or develop better therapeutics strategies. The main aim of our research was to investigate interspecies social interactions between *Bacillus subtilis* and *Salmonella enterica*, focusing on the role of extracellular matrix components of *S. enterica* in competitiveness and spatial segregation of mixed biofilms containing both species.

To investigate these mechanisms, we used *S. enterica* wild type and mutants with inactivated master regulator of biofilm formation (Δ*csgD*) or production of cellulose (Δ*bcsA*). Biofilms were grown in 96-well microtiter plate for 24 hours at 37 °C. All used strains constitutively produced fluorescent proteins and carried antibiotic marker, which allowed us to monitor their spatial distribution in biofilms. The frequency of strains in multi-species biofilms was determined by colony forming units (CFU) on selective agar. Spatial segregation of cells in biofilms was analysed by confocal laser microscopy. Results show that the strain of *S. enterica* incapable to produce cellulose increase its ability to compete with antagonistic strains such as *B. subtilis*.

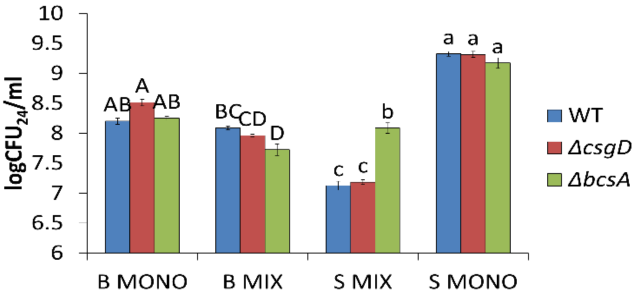

Fig. 1: *Bacterial counts (logCFU$_{24}$/ml) of B. subtilis wild type in mono- (B MONO) and mixed-biofilm (B MIX) and of S. enterica wild type and their mutants in mono- (S MONO) and mixed-biofilm (S MIX). Different upper case letters (A-D) above the columns indicate statistically significant differences (p < 0.05) between B. subtilis, lower case letters (a-d) between S. enterica strains.*

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Formation and characterization of *E.coli* biofilms and its interaction with bacteriophages in a continuous system

Ana Lisac*,[1], Elfi Birsa[1], and Aleš Podgornik[1]

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Bacterial biofilms are a complex heterogeneous and dynamic structures of bacteria.[1] This multilayer bacterial growth is involved in many chronic infections due to their low susceptibility to host immune defenses and antibiotic treatments.[2] Bacteriophages as natural predators of bacteria are gaining attention as means of biofilm control and prevention. Physiological states of bacteria vary in different layers of biofilm, going from the highest metabolic activity in upper layers to reduced activity in deeper layers. Bacteriophage efficacy toward biofilms is influenced by physiological state of their host.[3] To characterize biofilm formation and to study interaction between phages and biofilm, a lytic bacteriophage T4 and bacteria *E.coli* as host were used. In our experiments *E.coli* biofilms were formed under controlled physiological state of bacteria in a continuous system. Phages were introduced to bacteria in different stages of biofilm formation. It was found that at 0.01 m min^{-1} velocity rate through silicone tubular bioreactor biofilm is formed in one hour and phages successfully infect bacteria in biofilm, however the complete eradication of biofilm did not occur.

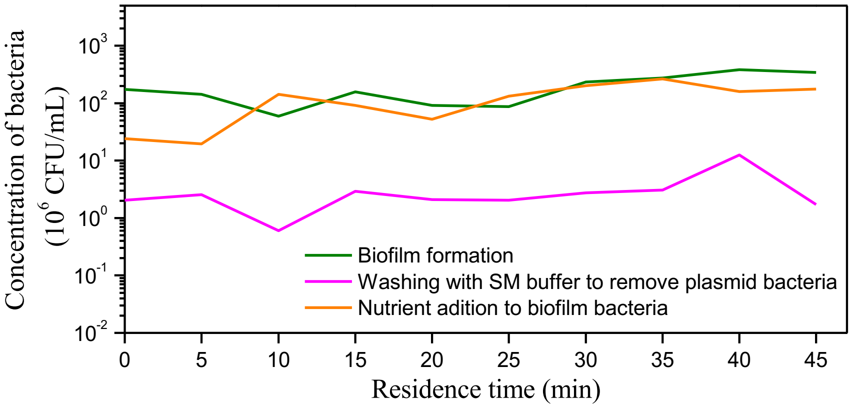

Fig. 1*: Concentration of bacteria in different stages of biofilm formation as a function of residence time in tubular bioreactor.*

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Effects of microplastics from disposable medical masks on terrestrial invertebrates

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The global coronavirus (COVID-19) pandemic crisis has increased the consumption of single-use plastics, such as disposable face masks, which may represent an important source of microplastics in the environment due to improper disposal[1]. We investigated the effects of microplastics on the following terrestrial invertebrates: woodlice *Porcellio scaber*, mealworms *Tenebrio molitor* and enchytraeids *Enchytraeus crypticus.* We obtained microplastics from disposable medical masks by crio-milling and found two types of particles - irregularly shaped fragments and fibers with a mean size of 50 µm – to which we exposed organisms in soil for 3-4 weeks according to established procedures[2]. We observed no changes in survival of tested invertebrates, however we noted some sublethal effects of microplastics: the change of energy allocation in mealworms (Fig. 1), induction of immune response in woodlice and slight effect on the reproduction of enchytraeids.

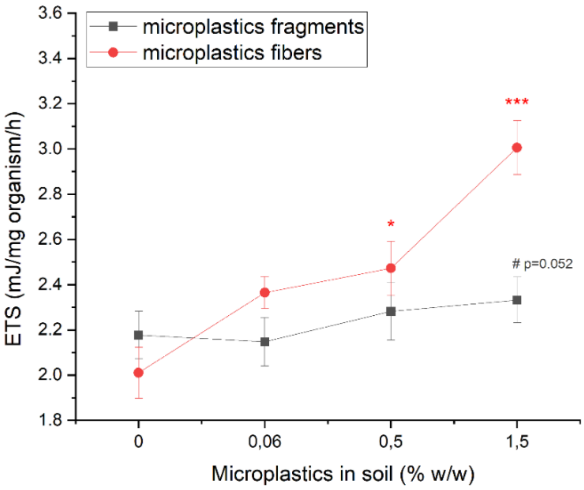

Fig. 1: *Electron transport system (ETS) activity in Tenebrio molitor larvae, exposed to microplastics obtained from disposable medical masks.*

Acknowledgement: We thank Marjan Marinšek for providing data on size and shape analysis of microplastics and Matej Dolenec for access to crio-milling equipment.

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Kinetics of cellulose nanomaterials esterification

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Cellulose, the most abundant polymer in nature, offers a great potential for various applications. With an appropriate treatment, it is possible to extract rod-like nanoparticles, the cellulose nanocrystals (CNCs) or cellulose nanofibrils (CNFs) [1,2]. Cellulose (nano)materials possess a hydrophilic character due to the inter- and intramolecular hydrogen bonding between the surface hydroxyl groups that can limit their application[3].

The aim of this research is to study the kinetics of frequently described esterification reaction with acetic anhydride[4] that reduces the hydrophilicity in cellulose nanomaterials. Firstly, both CNCs and CNFs were evaluated in terms of morphology, crystallinity and surface hydroxyl groups accessibility. The esterification reaction was carried out on both materials at various conditions. The acetyl content was then calculated through the ratio of $I_{C=O}/I_{C-O}$ peaks in ATR-FTIR spectra (Fig. 1). Additionally, crystallinity was followed with XRD.

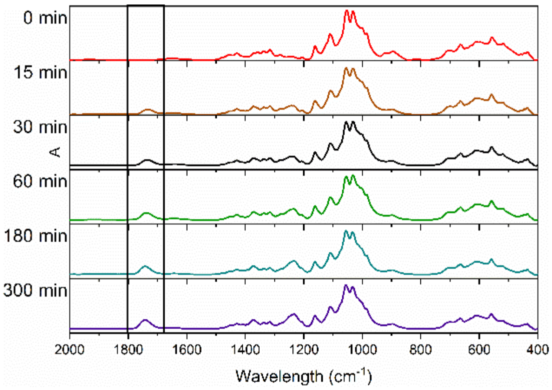

***Fig. 1**: The FTIR spectra of CNFs with increasing acetyl content.*

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CRISPR/dCas9 systems: Prospective RNA-guided genomic tools for transcriptional regulation

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The CRISPR/Cas9 system is a useful tool for genome editing, consisting of single-stranded RNA (sgRNA), complementary to target sequence, and Cas9 endonuclease[1]. Dead Cas9 (dCas9) which lacks endonuclease activity was created by mutating catalytic sites of Cas9 protein. Different transcription factors can be fused with dCas9 to regulate transcription (Fig. 1). Different transcription regulators (*e.g.* KRAB, VP160, VPR) and sgRNA scaffold sequences are available for CRISPR activation (CRIPSRa) and interference (CRISPRi) systems, which vary in gene regulation efficiency[2]. In our experiment, we used dCas9-fused VP160 and VPR activators to induce transcription of the *TLR10* in lung epithelial cells (A549 cell line). After transfection with plasmids, containing different sgRNAs and dCas9 activation fusions, RNA was isolated from cells. Relative transcription of the targeted gene was determined by RT-qPCR. The results show that both fusions were able to induce *TLR10* transcription, however VPR turned out to be a more potent activator than VP160. Level of the activation was highly dependent on the sgRNA sequences used. CRISPR/dCas9 systems are a promising technology for gene regulation with versatile applications in the field of functional genomics.

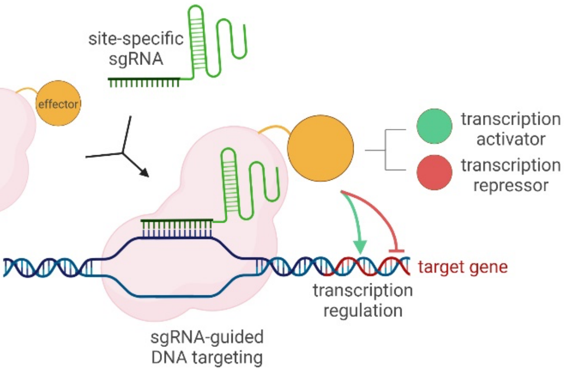

Fig. 2: *CRISPR/dCas9 fused with effectors for targeted regulation of transcription.*

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Organic Bodyguards – sterically shielding N-heterocyclic carbene ligands for monomeric metal hydrides

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N-Heterocyclic carbenes (NHC) have emerged as versatile and widely-employed compounds, most notably used as ligands for metal complexes.[1] The stability of such complexes heavily depends on the electronic and steric properties of the ligand. Sterically shielding NHCs are able to kinetically stabilize highly reactive low-valent transition metal atoms with low coordination number.[1]

Low-nuclearity coinage metal hydrides have been a challenging field of research for many decades.[2] Copper and silver hydrides have the propensity to aggregate and more often than not form polynuclear complexes.[2] The only crystallographically characterized mononuclear coinage metal hydrides are gold complexes. Knowing this, the task of isolating and characterizing copper and silver mononuclear hydride complexes poses a great obstacle.

Herein we present a very sterically hindered *N*-heterocyclic carbene ligand IPr**. The unique combination of steric properties, favorable spectroscopic features, high solubility and tendency towards crystallization make this ligand a great candidate for stabilizing low-coordinate copper and silver hydride complexes. Further work will be aimed at isolating coinage metal hydride complexes with IPr**.

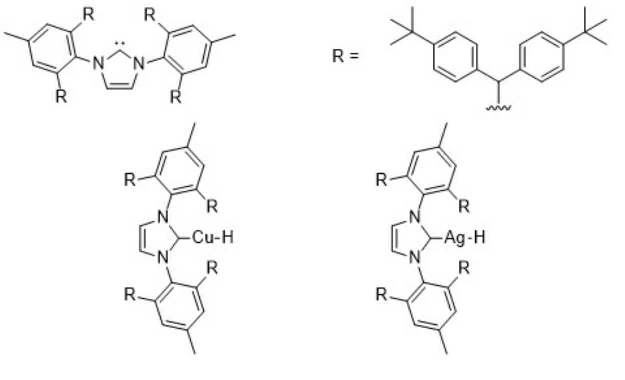

***Fig. 1**: IPr** ligand (above), IPr** copper and silver hydride complexes (below).*

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Computer vision algorithms for electrocatalyst stability assessment

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Proton exchange membrane fuel cells are a promising replacement for internal combustion engines. They convert chemical energy to electrical energy through combining hydrogen and oxygen into water. Structure-activity and structure-stability relationships of electrocatalysts for the hydrogen oxidation reaction and the oxygen reduction reaction are still not fully understood[1], but we can learn more about stability by using identical location transmission electron microscopy (IL-TEM), where catalyst changes can be observed at the atomic scale[2].

I will focus on computer vision algorithms for automating the analysis of atomically-resolved IL-TEM images of catalysts before and after electrochemical treatments[3]. I will present algorithms for determination of atomic column positions and alignment of identical location images to better elucidate the structure-stability relationship of Pt-based catalysts.

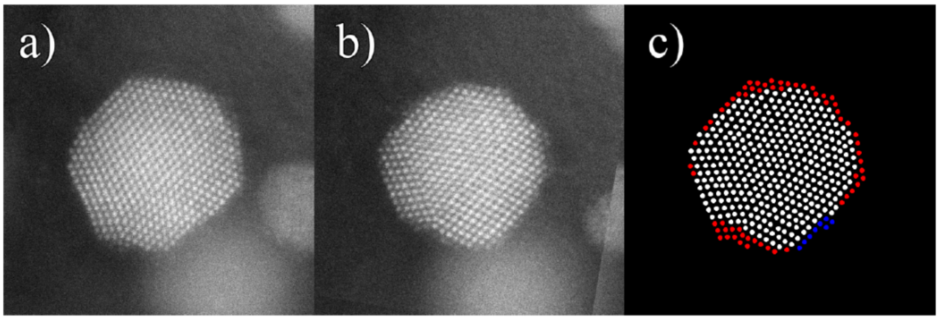

***Fig. 1**: A Pt-Co nanoparticle before (a) and after (b) an electrochemical treatment, and a representation of its evolution (c). White dots represent ever-present atomic columns, red dots represent disappearing ones (dissolution), and blue dots represent appearing columns (redeposition).*

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Characterisation of a three component system for DNA interaction partners identification

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DNA:protein interactions govern many cellular processes, including precise temporal regulation of transcription. Their disruption can lead to diseases[1], therefore the data about DNA:protein interactors can prove very useful in the field of treatment development. While strong interactions are relatively easy to find, weak ones are often overlooked. To overcome this limitation we have recently developed and prepared a three-component system for DNA:protein interaction identification based on proximity labeling of proteins with biotin[2] (Fig. 1), which is suiTab.for the detection of all kinds of interactions. It consists of a nucleotide sequence F-DNA containing a DNA sequence of interest and two chimeric proteins, Tus-TurboID and GAL4(1–147)-sfGFP. The system is designed so that the three components and target interaction partners of the DNA sequence of interest are colocalized. This enables specific biotinylation of all the proteins mentioned since Tus-TurboID is a promiscuous biotin ligase, which biotinylates proximal proteins non-specifically. GAL4(1–147)-sfGFP represents the internal control of the system. Here we present our recent results of electrophoretic mobility shift assays that confirmed the colocalization of the three components. We also determined the approximate ratio of the components for our further experiments which indicate that biotinylation of GAL4(1–147)-sfGFP is specific. Our next step is to optimize experimental conditions to reduce the nonspecific biotinylation.

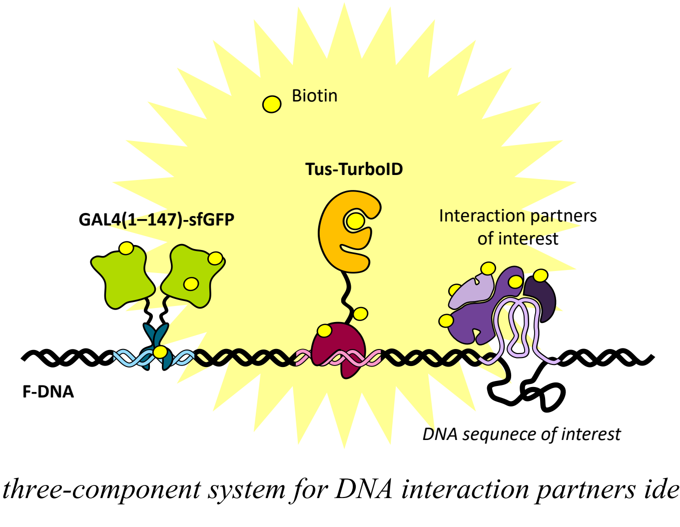

***Fig. 1**: The three-component system for DNA interaction partners identification.*

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On the way to fast »*on-site*« detecion of plant viruses and viroids

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Viruses are very important pests infecting humans, animals and plants, yet one of the most challenging entities to detect due to the lack of conserved genes across taxa. Development of high-throughput sequencing (HTS) technologies has revolutionized detection and discovery of (new) viruses due to their generic sequencing approach[1]. By using HTS we detected different viruses, among others also henbane mosaic virus, which was detected for the first time in Slovenia on a new host – tomato[2]. Currently the most established HTS platforms are very expensive and mostly fit for bigger labs and are not fit for on-site/small labs applications. In this study we compared the most established HTS approach, based on Illumina sequencing, with different nanopore-based sequencing approaches using MinION (Oxford Nanopore Technologies) – small portable device[3] for rapid and possibly on-site HTS applications (Fig. 1). We performed comparison of the methods on a broad range of samples, containing plant viruses (11) and viroids (2) with different genomes' organizations. We detected slight differences in the performance of different tested nanopore sequencing approaches, however, most of the viruses/viroids included in the study were detected with all of the employed nanopore sequencing approaches. The results of this systematic comparison (i) showed that nanopore sequencing is a promising technique for plant virus/viroid detection and (ii) are opening doors for broad and more standardized use of nanopore sequencing for diagnosis of plant virus diseases also in small labs in near future.

***Fig. 1**: Using MinION for plant virus/viroid detection.*

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Preparation and surface functionalization of SiO_2 nanoparticles with proteins

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Silica nanoparticles have great potential for various biochemical and biomedical applications such as biosensors and biomarkers. Not only are they biocompatible and have low toxicity, it is also possible to precisely control their size and other properties. Moreover, we can chemically modify their surface in many different ways to achieve the desired function and prepare them for binding of various biomolecules[1, 2].

We have prepared silica nanoparticles in three different sizes (80, 200 and 500 nm) using the modified Störber-Fink-Bohn method, which is a hydrolysis and condensation of tetraethyl orthosilicate (TEOS) with water or ethanol and ammonia as a catalyst. It allows us to predict the final diameter of the particles by changing the dilution of TEOS in ethanol[3]. First, we modified the hydroxyl groups on the surface to amino groups by grafting the organosilane APTES (3-aminopropyltriethoxysilane)[4]. Then we formed a Schiff base by binding glutaraldehyde to the amino-modified silica nanoparticles[5]. Finally, the desired protein was covalently attached to the modified particles. The particle size was measured on SEM and the presence of different functional groups were confirmed by the IR spectra. In the case of coupling silica spheres with GFP (green fluorescent protein), we also confirmed the binding with confocal fluorescence microscopy.

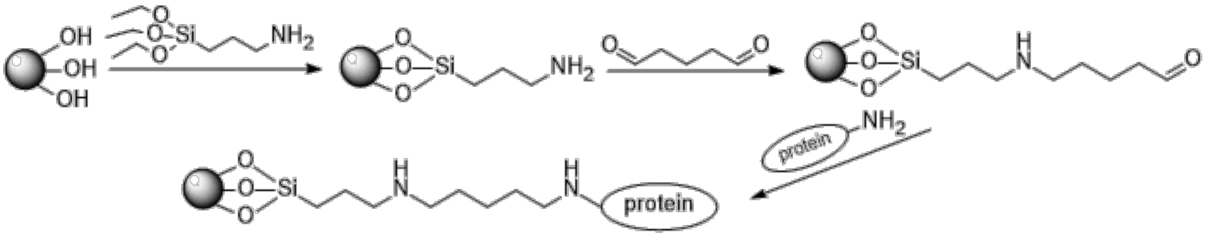

***Fig. 1**: Surface modification of silica nanoparticles for protein binding.*

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Cell cycle dynamics of pre-rRNA nucleolar compartmentalization in embryonic stem cells

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Nucleolus is a hallmark biomolecular condensate, whose formation by liquid–liquid phase separation facilitates the initial steps of ribosome biogenesis and other important functions roles including processing of ribosomal RNA (rRNA). In eukaryotes during open mitosis all condensates disassemble and reassemble as the nucleus itself breaks down and reforms during each cell cycle. Little is known how assembly of biomolecular condensates, including best studied nucleolus, is regulated during cell cycle[1]. The nucleolus is a multilayer condensate composed of the 3 subcompartments: the granular component (GC), the fibrillar centre (FC), the dense fibrillar component (DFC)[2]. Current imaging techniques of RNA molecules are predominantly limited to fixed cells, where the fixation agents can lead to morphological nucleolar changes[3]. Aim of our study is to develop an imaging technique, which will enable us to localize pre-rRNA isoforms during cell cycle in live cells and in parallel apply multiomics approaches. Our research approach is based on application of catalytic inactive CRISPR-Cas13 (dCas13) system coupled with engineered variation of ascorbate peroxidase (APEX2). We have established stable mouse embryonic stem cell lines expressing *dCas13-APEX2* gene. Initial results with transient transfection of gRNAs targeting rRNA precursors enable tracing of precursor rRNA isoforms in live cells and allow application of super-resolution STED fluorescent microscopy. This development of imaging technique opens up the possibility of tracking molecules in live cells leading to insight of dynamics of molecular complexes and present a turning point in understanding of nucleolar organization during cell division.

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Fate of bisphenols during conventional wastewater treatment

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Bisphenols are used in the production of epoxy resins, and polycarbonate[1]. They share a common structure of two hydroxyphenyl functionalities and are known to exhibit endocrine-disrupting effects[1]. They have been detected in surface water, sediment, sewage, sludge, and should be considered contaminants of emerging concern[1]. Our research aim was to understand the fate of bisphenols during conventional wastewater treatment. First, an analytical method for the determination of 16 bisphenols in the activated sludge, based on solid-phase extraction and gas chromatography coupled with mass spectrometry, was developed and validated. Composite samples from different compartments of a conventional wastewater treatment plant were analysed. Concentrations of bisphenols were recalculated into mass flows, and their removal rate and their adsorption to primary and secondary sludge determined (Fig. 1). Their emissions into the environment *via* effluent release or sludge disposal were evaluated. All the bisphenols were removed from the aqueous phase to below the limit of quantification, except BPA (79 ng L^{-1}) and BPS (20 ng L^{-1}). The removal of bisphenols from the aqueous phase ranged from 35% to 94% and adsorption to primary and secondary sludge from 2% to 46%. Calculated daily emissions of bisphenols *via* effluent release and sludge disposal were 0.33-1.48 g d^{-1} (BPA, BPS) and 0.33-4.63 g d^{-1} (BPA, BPS, 24BPF, 44BPF), respectively. Although bisphenols are highly removed during wastewater treatment, their concentrations in sludge should be further examined before the reuse of sludge.

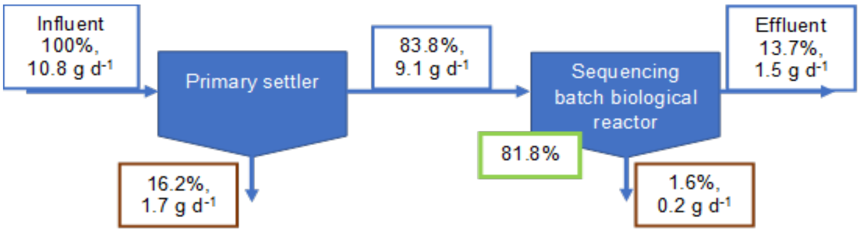

Fig. 1: *Distribution of BPA between aqueous phases (blue rectangles), sludge phases (brown rectangles) and removal of BPA (green rectangle).*

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Japanese knotweed extracts as biochemicals for *in situ* synthesis of ZnO nanoparticles on cotton

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The most common synthesis of zinc oxide nanoparticles (ZnO) for textile applications include classical (and toxic) chemicals[1]. In order to reduce the environmental impact, the "green" syntheses are emerging, with emphasis on the use of waste materials[2]. Here, a completely green method of ZnO *in situ* (direct) synthesis on cotton fabric using plant waste is presented.

The *in situ* synthesis of ZnO was performed by immersing fabric for 1 min in aqueous wood ash extract, 0.1M zinc nitrate hexahydrate and aqueous extract of Japanese knotweed leaves or rhizome, respectively. The samples were dried in a continuous dryer between the immersions and at the end of the synthesis process. The results showed that the extract from the leaves of Japanese knotweed is an excellent reducing agent as it produces numerous ZnO particles on the surface of cotton, which has very good protection against UV radiation UPF = 38.4, while the rhizome extract did not show such excellent results (Fig. 1).

This research presents an example of a green circular economy, where a bio-waste material can be used to produce ZnO directly on cotton at low temperatures and short treatment times without adding classical chemicals and enables production of cellulosic fabrics with excellent UV protection.

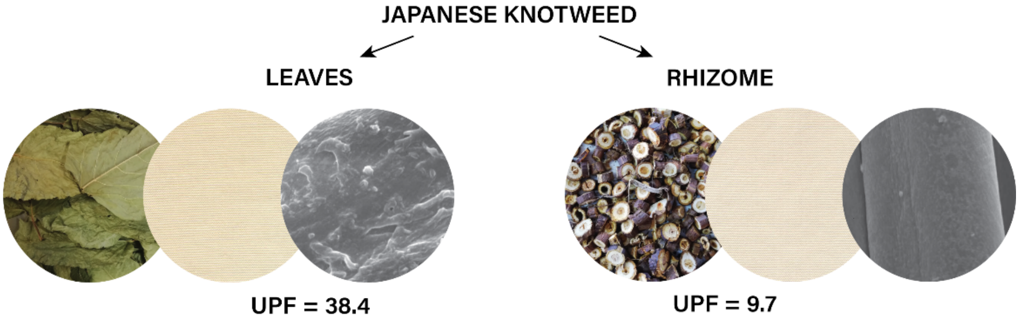

***Fig. 1**: Cotton samples photographed and imaged under scanning microscope with UV protection factor (UPF) shown.*

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Two synthetic paths to triphenylphosphine-based palladium bis(acetylides)

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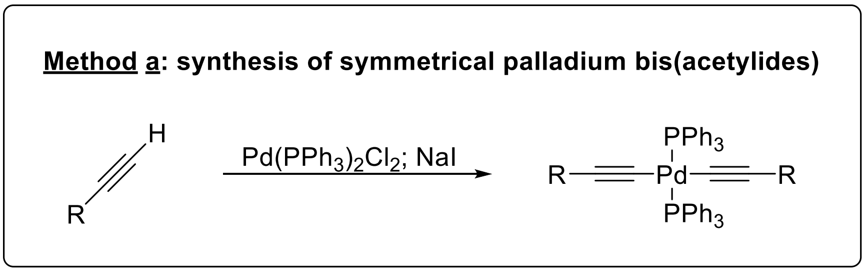
Copper-free Sonogashira reaction is one of the most employed methods for preparation of disubstituted alkynes. Despite playing a vital role in reaction mechanism[1], palladium bis(acetylides) were not systematically investigated yet. Preparation of such complexes usually employs harsh reaction conditions or other transition metals[2]. We developed two different synthetic routes to bis(triphenylphosphine)palladium bis(acetylides) that were prepared using accessible, affordable and non-hazardous reagents.


Method a: synthesis of symmetrical palladium bis(acetylides)

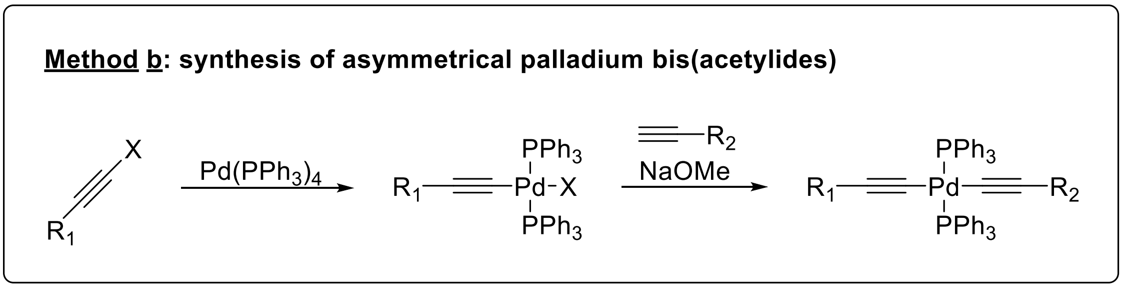

Method b: synthesis of asymmetrical palladium bis(acetylides)

Fig. 1: Two new synthetic pathways to palladium bis(acetylides).

1. (a) Gazvoda, M., Mechanism of copper-free Sonogashira reaction operates through palladium-palladium transmetallation. *Nature Commun.* **2018**, *9*, 4814, (b) Martek, B., Designing homogeneous copper-free Sonogashira reaction through a prism of Pd-Pd transmetalation. *Org. Lett.* **2020**, *22*, 4938–4943.

2. (a) Masai, H., The infrared spectra of the square-planar dialkynyl complexes of nickel(II), palladium(II) and platinum(II). *J. Organometal. Chem.* **1971**, *26*, 271–276, (b) Nelson, J. H., Reactions of palladium(0)-phosphine complexes with terminal α-hyroxyacetylenes. *Inorg. Chem.* **1972**, *13*, 27–33, (c) Sonogashira, K., Novel preparation of σ-alkynyl complexes of transition metals by copper(I) iodide-catalysed dehydrohalogenation. *J. Chem. Soc., Chem. Commun.* **1979**, 291–292.

Diversity of orthobunyaviruses in mosquitos in Slovenia

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Orthobunyaviruses (OBV) belong to one of the four genera in the Peribunyaviridae family and have structurally simple virion, comprising of only four proteins, and tripartite, single-stranded (-) RNA genome. As for the others arboviruses, they are transmitted by blood-feeding arthropod vectors, most commonly mosquitos[1]. Currently known OBV can have devastating effects either by causing disease symptoms in human or by causing abnormalities in livestock and crops[2]. The aim of the study was to test the frequency and diversity of OBV infecting mosquitos, collected on 28 different locations across Slovenia in years 2017- 2019. Out of 1295 tested groups of mosquitos, 153 groups, representing 11,8 %, contained OBV-infected mosquitos. With Sanger sequencing of conserved regions in L segment of OBV genome and further phylogenetic analysis we selected eight closely related sequences from GenBank that were isolated from genera *Orthobunyavirus*, *Phasivirus* and *Goukovirus*. All three genera used to belong to Bunyaviridae family, which was recently divided into separated families in *Bunyavirales* order[3]. Generating phylogenetic trees and networks with obtained nucleotide sequences helped us to confirm the correlation between the diversity of OBV in Slovenia and sampling sites as well as the species of their arthropod host.

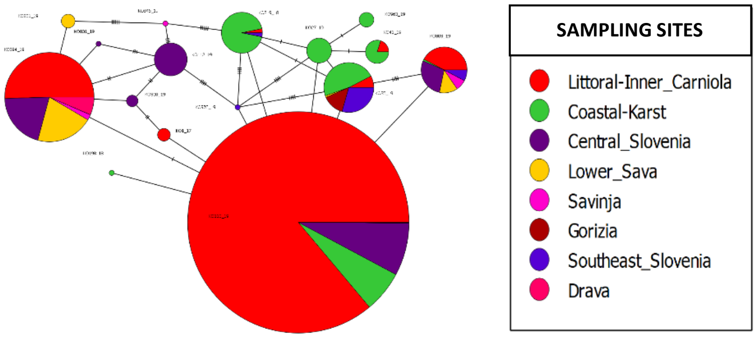

Fig. 1: *Phylogenetic network of 127 nucleotide sequences obtained from different groups of mosquitos collected all across Slovenia.*

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Bakelite: The first plastic material made, but the last investigated in microplastic research

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Bakelite is the first synthetic plastic, a thermosetting phenol formaldehyde resin. Bakelite is quite brittle and can be rapidly degraded into microplastics when entering the environment. Despite the diversity of materials, environmental research on microplastics has focused primarily on thermoplastics (PE, PS) while other polymers (thermosets, elastomers) are severely underrepresented in laboratory studies. Furthermore, most of the studies have been investigating mechanical stress and microplastics ingestion, while additives leaching from microplastics have been greatly overlooked[1]. In our study, ecotoxicity of industrial Bakelite microplastics (MP, 100 mg/L, particle size ~ 8 µm [1]) and its leaching properties were evaluated using four aquatic organisms: crustacean *Daphnia magna*, aquatic plant *Lemna minor*, bacterium *Allivibrio fischeri* and algae *Pseudokirchneriella subcapitata*. Leachates were prepared by dispersing MP into the medium specific for the organism, incubated under the same conditions as ecotoxicity tests with particles were performed and afterwards removed by filtration.

***Tab. 1**: The effects of 100 mg/L Bakelite microplastics/leachates on test organisms.*

Organism	**Criterion**	**Time**	**Inhibition [%]**	
			Microplastics	Leachates
Daphnia magna	immobility	24 h	16 ± 2	100 ± 0
		48 h	37 ± 19	100 ± 0
Lemna minor	root length	168 h	42 ± 7	31 ± 4
Allivibrio ficheri	bioluminiscence	30 min	12 ± 0	29 ± 1
Pseudokirchneriella subcapitata	growth rate	96 h	44 ± 10	11 ± 5

Leachates caused higher inhibition than MP to daphnids and bacterium, while aquatic plant and algae were more affected by MP (Tab.1). It is assumed that the increased inhibition of MP on aquatic plant's root length was due adsorption of particles to the roots, however a much greater effect of MP was observed on algae, probably due to the formation of heteroaggregates between algae and particles.

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Efficiency of advanced oxidation processes for removal of viruses from municipal wastewater

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The presence of viruses in wastewater poses a general health risk due to the infectivity and resistance of viruses to different removal methods. Under certain conditions, viruses can survive without a host cell and transmit infections through aquatic environments[1,2]. One of the reliable and efficient ways of wastewater treatment and removal of viruses are advanced oxidation processes (AOPs), which are based on the formation of a strong oxidant (usually a hydroxyl radical). The latter causes the oxidation of pollutants, leading to a complete deactivation and decomposition[3]. The objective of this research was a literature review of the efficiency of virus removal with a use of different AOPs. A part of results is demonstrated in Tab. 2, which displays investigated viruses, applied AOPs and determined LRVs (logarithm reduction values). By reviewing the available literature and research, we confirmed that all reviewed cases showed sufficient virus decomposition and/or deactivation under various conditions of AOPs. Concluding, AOPs are reliable processes for removal of viruses from municipal wastewater.

***Tab. 2**: Investigated viruses, AOPs and LRVs.*

APPLIED AOP	**DETERMINED LRV [/]**						
VIRUS	NV[1] (Norwalk virus)	PV1[1] (poliovirus 1)	MS2[1,2,3,4,5] (Escherichia virus MS2)	T4[3] (bacteriophage T4)	ΦX174[3]	HAV[5] (hepatitis A virus)	MNV[2] (murine norovirus)
O$_3$	> 3	5	3.9–7	/	/	4.2–6	/
UVA	/	/	0	/	/	/	0
UVB	/	/	4	/	/	/	3
UV	/	/	1.8–3	6	4–6	/	/
H$_2$O$_2$	/	/	0	0	0	0	/
UV/H$_2$O$_2$	/	/	4–6.3	6	6	/	/
O$_3$/H$_2$O$_2$	/	/	3.3–5.5	/	/	3.3–4.8	/
Fenton like process	/	/	3	/	/	/	/
TiO$_2$ photocatalysis	/	/	1-4	/	/	/	4

/…Data not available

0…Virus not detected; negligible LRV

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Effect of microstructure on electrochemical behavior of corn cob derived non-graphitizable carbons

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Sodium ion batteries (NIBs) are considered one of the promising candidates to displace lithium ion batteries (LIBs) in the large scale energy storage application[1]. Unlike in Li-ion batteries, graphite can not form binary compounds with sodium, thus non-graphitizable (NG) carbons are one of the possible host structures. Their advantages are low price, high storage capacity and cycling stability[2]. The choice of the precursor (e.g. sucrose, glucose, cellulose, lignin, etc.) for the NG carbon synthesis play a critical role in the electrochemical behavior, due to different produced microstructure[3]. Additionally, biomass waste can be used as NG carbon precursors. In this study, a two-step carbonization process is employed to enhance the structure ordering and electrochemical performance of corn cob derived NG carbons. We prepared NG carbons at various final temperatures of heat treatment and they were used for the structural and electrochemical characterizations. Electrochemical tests show high reversible capacities of 166.9 mAh g^{-1}, 204.9 mAh g^{-1}, 258.2 mAh g^{-1} and 234.6 mAh g^{-1} after 100 cycles for samples prepared at 900°C, 1200°C, 1400°C and 1600°C, respectively. The best performing material was used for the full cell experiments. We will discuss correlations between physical and electrochemical properties for corn cob derived NG carbons.

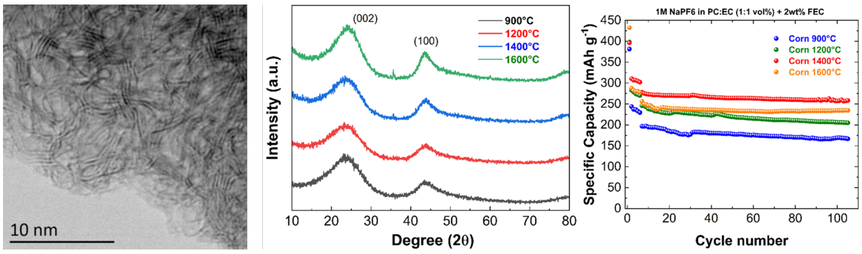

Fig. 1*. Correlation between structure and electrochemical performance.*

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Pd–Pd transmetallation tuned to perfection in Heck alkynylation of aryl bromides

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It was recently postulated[1,2] that the mechanism of copper-free alkynylation operates through a process that is practically identical to the Pd/Cu catalyzed variant, only the role of the copper co-catalyst is taken by yet another palladium complex. Herein we present novel catalytic system for the alkynylation of (hetero)aryl bromides which is based on productive Pd–Pd transmetallation (Fig. 1)[3]. It features simultaneous introduction of two different palladium (pre)catalysts, one tuned to promote oxidative addition to (hetero)aryl bromide and another to activate terminal alkyne substrate. This conceptually novel rational design of copper-free Sonogashira reaction enabled facile identification of the reaction conditions, suitable for the synthesis of alkyl, aryl and heteroaryl substituted alkynes at room temperature with as low as 0.125 mol % total Pd-loading. It was also applicable in the synthesis of the intermediate of active pharmaceutical ingredient (API), and in multi-gram scale synthesis.

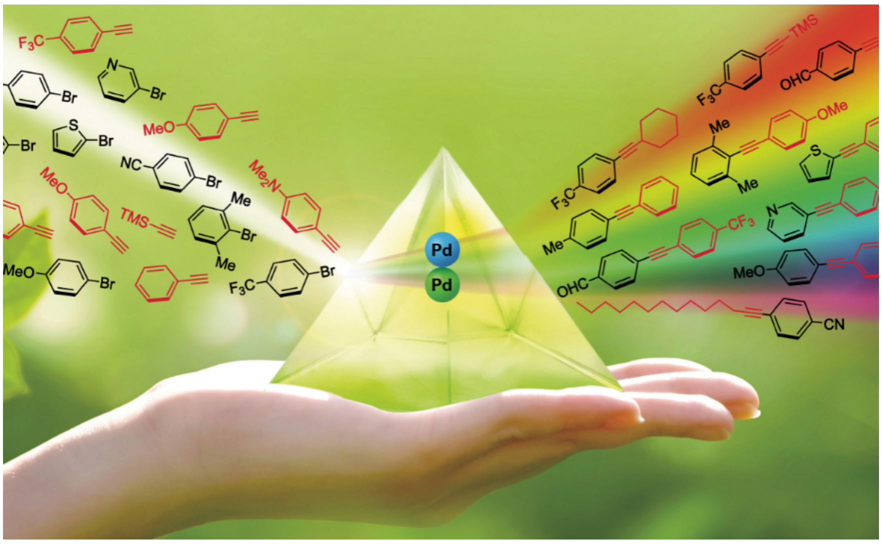

Fig. 1: *Schematic representation of productive Pd–Pd transmetallation.*

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Hybrid sol-gel coating doped with cerium nitrate to enhance the corrosion protection of AA7075-T6

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The aluminium alloy 7075-T6 (AA7075-T6) is widely used in the aerospace industry due to its low weight and its passive behaviour in atmospheric conditions. However, due to the presence of alloying elements such as Zn, Cu, Mg, Si, etc., this alloy is highly sensitive to corrosion in chloride-containing solutions.

The corrosion protection systems for aluminium alloys have mainly been based on chromate conversion treatments, but the use of hexavalent chromium(VI) has been limited in many countries, due to its negative effect on human health and the environment.

Nowadays, several environmentally acceptable surface treatments have been studied and, the sol-gel coating is considered as an alternative. The sol-gel chemistry is based on the hydrolysis and condensation reactions of initial organically modified silica and silane reagents. The active protection of hybrid sol-gel coating can be achieved by the addition of cerium salts, e.g. cerium nitrate [1].

In this study, the hybrid sol-gel coatings without and with cerium nitrate applied on AA7075-T6 were studied. The synthesis was prepared from organic precursors: 3-(methacryloyloxy)propyl trimethoxysilane and methyl methacrylate. The inorganic one was tetraethyl orthosilicate [2]. The characterization was performed by Fourier-transform infrared spectroscopy and scanning electron microscopy. The corrosion properties were evaluated by electrochemical measurements and during exposure in the salt spray chamber according to standard ASTM 117.

The results indicated that synthesized hybrid sol-gel coatings are able to form a continuous few micrometres thick film, necessary to provide excellent barrier and active properties. Thus, coated AA7075-T6 showed better corrosion performance in the studied media.

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Benchmarking machine-learning algorithms for near-infrared spectral classification of polymer types in heritage science

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Diverse types of polymers constitute items of cultural significance. For proper preservation of these items, their chemical composition and polymer type must be determined. When the value and significance of the sample under investigation prohibit the use of destructive techniques, researchers tend to utilise spectroscopic techniques that are fast, reliable and non-destructive[1]. This study represents a procedure to determine the optimal machine-learning (ML) algorithm for the classification of polymers constituting heritage objects based on their near infra-red (NIR) reflectance spectra. k-Nearest neighbours (kNN), support vector machines (SVM), linear discriminant analysis (LDA), naïve Bayes classification (NBC), decision tree (DT) and XGBoosted random forest (XGB) algorithms were evaluated using a spectral database assembled on the basis of 242 samples of six polymer types that are predominantly found in heritage objects: polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), cellulose acetate (CAc) and acrylonitrile butadiene styrene (ABS). The benchmarking procedure was implemented using R and the mlr package[2] with a simultaneous bootstrapping validation method of 500 iterations for all algorithms under investigation. The results are presented in Fig. 1. Overall, the LDA algorithm is the most accurate one with a classification accuracy of 96 %. An investigation into reflectance bands that explain the largest amount of variance was conducted to identify the crucial wavelengths used during ML classification.

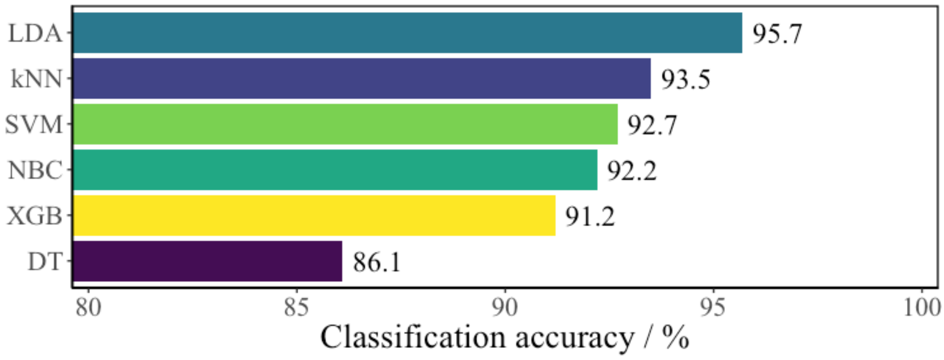

***Fig. 1:** Accuracy the algorithms under investigation.*

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Simple and efficient method for preparation of 4-monosubstituted-1,2,3-triazoles

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1,2,3-Triazoles have found various applications in different fields of science. One of the most noticeable are their biological and medicinal applications. 1,2,3-Triazoles with different substitution patterns were found to have antibacterial, antifungal, antiviral, antiepileptic, anti-inflammatory and antiallergic activities. They are investigated as potential substances for treatment of cancer, tuberculosis, HIV infection, schizophrenia and Alzheimer's disease[1]. Among them, a subclass of 4-monosubstituted-1,2,3-triazoles were found to be inhibitors of human methionine aminopeptidase type 2 (hMetAP2) enzyme and indoleamine 2,3-dioxygenase enzyme, and are therefore investigated for potential cancer treatment[2,3]. This 4-monosubstituted-1,2,3-triazole moiety is also a part of Cefatrazine (BL-S640) a β-lactam cephalosporin antibiotic and azahistidine amino acid (histidine analogue with triazole ring) which can be incorporated into peptides and proteins to modify their properties. Monosubstituted-1,2,3-triazoles are also a great scaffold for further functionalization.

Current synthetic methods for preparation of 4-monosubstituted-1,2,3-triazoles have at least one of the following drawbacks: expensive starting materials and/or catalysts, inert atmosphere, anhydrous reaction conditions, high temperature, long reaction times, limited substrate scope, difficult work-up, multistep procedures. We have developed a new method for preparation of 4-monosubstituted-1,2,3-triazoles. Reactions proceed with terminal acetylene substrates and copper catalysts which are commercially available and not costly. Our method does not require anhydrous reaction conditions or inert atmosphere. Developed one-step method is also atom-economic with easy work-up procedure to afford triazoles in good to excellent yields. By optimizing reaction conditions we are able to enable this reaction with high selectivity in different solvents under mild conditions, showcasing the method on wide substrate scope, including on structurally complex molecules, amino acids and peptides, making it applicable for late-stage modification.

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Preparation of cell models with stable expression of dipeptide repeat proteins (DPR) and evaluation of their role

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by gradual degradation of motor neurons[1]. Most common reason for development of the genetic form of ALS is mutation in gene *C9orf72*. It occurs in the hexanucleotide (GGGGCC) repeats region inside the first intron. Mutation can raise the number of these repeats from about 30 to more than thousand[2]. One of the main hypotheses explaining the pathological effect of extended hexanucleotide repeats is accumulation of toxic proteins with dipeptide repeats (DPR). There are five DPR proteins: poly(GA), poly(GR), poly(PA), poly(PR), poly(GP), which are produced by translation that is independent of the start codon AUG[3].

We aimed to prepare five cell lines that would stably express DPR proteins with 125 repeats. For that purpose, we cloned DNA fragments that code for DPR proteins to expression vector pcDNA5 FRT TO mScarlet-I Myc stop in a way that they would cotranslate with fluorescent marker mScarlet. After that we transfected the Flip-in HEK293T cell line with these constructs. Target DNA integrated in the cell genome via Flp recombinase-mediated DNA recombination at the FRT sites, located on expression vector and cell genome. Successful integration was confirmed with fluorescent microscopy.

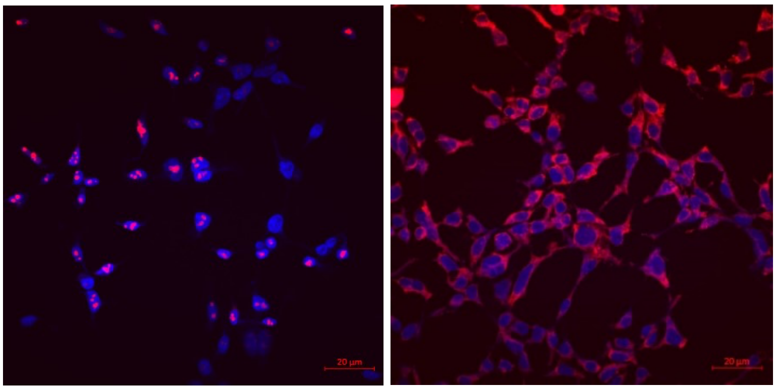

Fig. 1: *Cell line HEK293T transfected with DPR protein PR (left) and GR (right). Nucleus are colored blue (DAPI) and proteins DPR are colored red (mScarlet).*

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Rheology of graphene oxide aqueous suspensions

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In virtue of their unique features and rheological properties displayed in aqueous phases, graphene oxide (GO) suspensions attracted a great deal of attention since they can find several applications in various fields (from industrial to biomedical one)[1]. Due to a relatively large density of oxygen-containing functional groups on GO basal planes and edges it is possible to prepare stable GO suspensions without employing additional surfactants[2]. The effects of GO particle concentration and its properties, such as average particle size, number of GO layers per particle, and amount of oxygen-containing functional groups, on the viscoelastic and flow properties of aqueous GO suspensions are investigated[3]. The experimental data are described using rheological models, such as Cross and generalized Maxwell model, which give parameters useful for the quantitative interpretation and comparison of the experimental results. At higher GO concentrations, which depend on GO particle properties, gel-like properties of suspensions are observed. When interparticle interactions are so effective that they lead to arrested states, the rheological behavior of the suspensions undergo a sharp transition, from quasi-Newtonian to markedly shear thinning behavior with viscoelastic properties.

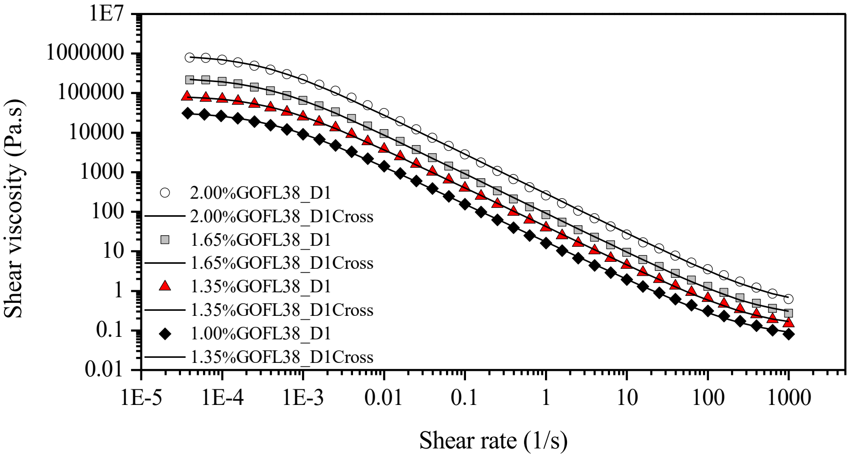

***Fig. 1**: The effect of 2-4 layer graphene oxide concentrations (w/v%) on flow curves of aqueous suspensions. The lines represent data fitting using Cross model.*

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Aggregation studies of annexin A11 and its mutants

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive decline in motor function[1]. The most common genetic causes of familial and sporadic ALS are an intronic hexanucleotide repeat expansion in *C9orf72* and non-synonymous mutations in the *SOD1*, *TARDBP*, and *FUS* genes. Smith and co-workers identified a novel non-synonymous mutation in the *ANXA11* gene and linked it to ALS[2]. The *ANXA11* gene encodes annexin A11 (ANXA11), a calcium-dependent phospholipid-binding protein that belongs to the annexin protein family. Its C-terminus is highly conserved and consists of four annexin repeats, while its N-terminus is long, hydrophobic, intrinsically disordered, and has the ability to bind to several interaction partners, including calcyclin[1, 2]. ALS-linked ANXA11 mutations modify its interactions with calcyclin. This interaction is specifically inhibited by the D40G mutation, which is also the most common ANXA11 mutation in the European population. The altered binding properties lead to accumulation of cytoplasmic ANXA11, promoting its abnormal aggregation[2]. In *E. coli* strain BL21 [DE3] pLysS, we expressed three different forms of the ANXA11 protein (ANXA11^{WT} with complete protein sequence, ANXA11^{D40G} with mutated protein sequence and ANXA11$^{\Delta S119}$ with truncated N-terminal domain at Ser119). We isolated the proteins by nickel affinity and size exclusion chromatography. We then performed fluorescence spectroscopy using thioflavin T as the amyloid dye[3] to understand why the intrinsically disordered N-terminus of ANXA11 tends to aggregate.

Fig. 3: *Graphical representation of annexin A11 with labelled mutants and binding site of calcyclin. ANX – four annexin repeats, Cal – binding site of calcyclin, D40G – mutated protein sequence, ΔS119 – N-terminally truncated at Ser119.*

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Geminal dibromoalkenes: Versatile building blocks for the preparation of terminal acetylenes and bromoacetylenes

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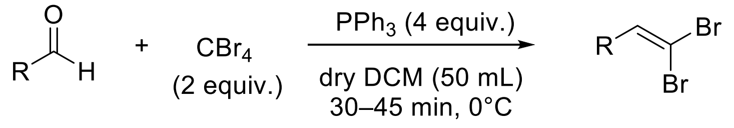
Geminal dibromoalkenes, also known as 1,1-dibromoalkenes, are organic compounds, where two bromine atoms are directly bound to the *sp*^{2} hybridized carbon atom. They serve as versatile building blocks in organic chemistry for variety of cross-coupling reactions and also for the production of terminal acetylenes and bromoacetylenes[1]. Geminal dibromoalkenes can be prepared *via* the first step of the Corey-Fuchs reaction[2], which is also referred to as Ramirez olefination[3].

Herein we describe the synthesis of seven aromatic and heteroaromatic 1,1-dibromoalkenes from the starting, commercially available aldehydes, two of which were monohalogenated benzaldehydes, two were sterically hindered, one contained an electron-accepting and the other electron-donating functional group, and one was a heteroaromatic aldehyde. Ice cold aldehyde solutions in dichloromethane were treated with the ylide, formed *in situ* from tetrabromomethane and triphenylphosphine. Subsequently, the aldehydes were converted into corresponding *gem*-dibromoalkenes (Fig. 1). The products were isolated, purified by column chromatography on SiO_2 and characterized by ^{1}H NMR and IR spectroscopy. The purity of the synthesized products was determined by HPLC chromatography and the melting points of the solid products were also determined. Comparison of the conversions of different substrates revealed that most of the aldehydes were readily converted into the desired products.



1–7 **1a–7a**

Fig. 1: *General representation of the synthesis of geminal dibromoalkenes.*

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Genetic variations in translesion polymerase REV3L are associated with gastric cancer

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Gastric cancer incidence has decreased in the past few decades, however, gastric cancer remains the fourth most common cause of cancer death worldwide. Standard-of-care therapy is based on combination of surgery, systemic chemotherapy and radiotherapy. Commonly accepted chemotherapy regimen is the fluoropyrimidine and platinum combination[1]. DNA damage associated with chemotherapy can generate a replication block, but cells have mechanisms that can bypass such blockage either by error free or error-prone translesion DNA synthesis (TLS) with specialized TLS polymerases. Human *REV3L* gene codes a catalytic subunit of DNA polymerase zeta (Pol ζ), which is one of the known pathways of DNA repair by TLS[2]. Due to the important role of translesion polymerases in interstrand crosslink repair, they might alter the response to chemotherapeutic agents[3]. Our aim was to evaluate the association between genetic variations (SNPs) in translesion polymerase *REV3L* and gastric cancer in Slovene population.

Methods: 175 patients with gastric cancer and 160 healthy controls were genotyped for four SNPs in *REV3L* using competitive allele specific PCR. Kaplan Meier analysis and Cox regression were used to determine the impact of SNPs on survival. The gastric cancer risk and association of SNPs with tumor characteristics were evaluated with logistic regression.

Results: Our results indicated that rs462779, rs455732, rs3204953, and rs465646 were not associated with increased gastric cancer risk, even after the adjustment for gender and age. *REV3L* rs3204953 AA genotype was associated with significantly shorter overall survival (P=0.018, HR=4.19, 95% CI=1.28-13.66). In addition, we observed an association with higher frequency of vascular invasion in patients with rs455732 CT genotype (P=0.003, OR=3.93, 95% CI=1.59-9.74).

Conclusion: Our results showed that genetic variations in *REV3L* were associated with shorter overall survival of patients with gastric cancer (rs3204953) although they did not influence gastric cancer risk. Genetic variations in *REV3L* may also contribute to specific tumor features, indicating that certain genotypes could be associated with worse prognosis.

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Potential use of isolated essential oils as disinfectants

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Essential oils are volatile components of plant species with an intense scent. Essential oils of rosemary, lavender and sweet wormwood from the geographical area of NE Slovenia and essential oil of rosemary from the coastal areas of SW Slovenia were isolated by simple distillation and extraction between the aqueous and organic phases. Synthesized oils were characterized by IR spectrometry. The biological activity of the essential oils was verified by a modified form of the Kirby-Bauer disc diffusion method[1]. We found that in addition to the type of plant, the composition of the essential oil is also influenced by the geographical location where the plant thrives. Biological tests of essential oils have shown that isolated essential oils have an inhibitory effect on the growth of some bacteria – even better compared to five analyzed commercial antiseptics (Fig. 1).

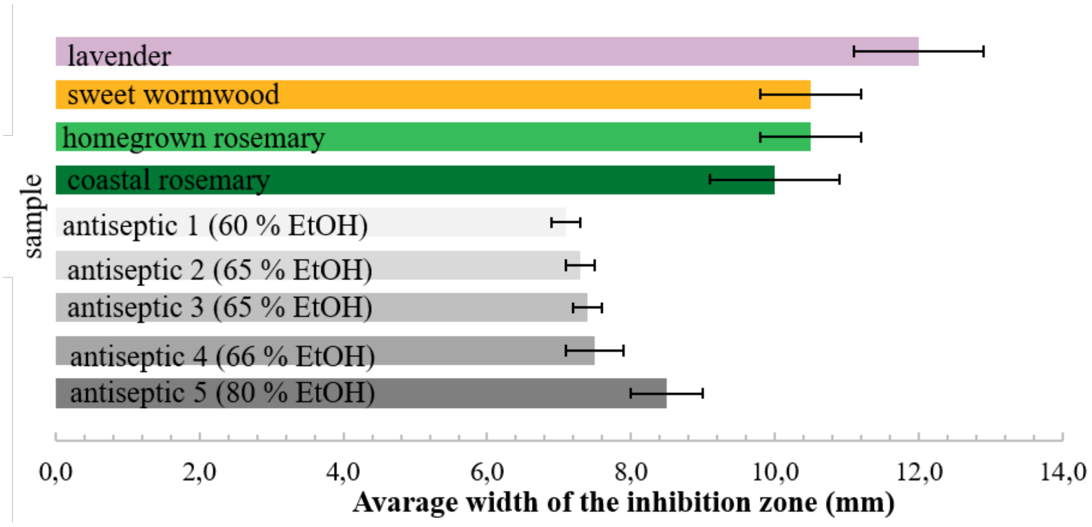

***Fig. 3**: Results of Kirby-Bauer disc diffusion test for five commercial antiseptics and four isolated essential oil from different plants.*

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Crosslinking of cellulose by dicarboxylic acid derivatives

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The development of science and changing lifestyles have led to the replacement of traditional materials with new and modern ones, including hydrogels. These are a three-dimensional network of hydrophilic polymers that can swell in water and absorb a large amount of water while maintaining the structure through chemical or physical cross-linking of the individual polymer chains[1]. By definition, water must make up at least 10% of the total weight or volume for a material to be a hydrogel. Their use is popular in many fields, especially in the pharmaceutical, biomedical and agricultural industries[2]. Due to their characteristic degree of flexibility, hydrogels may be the next generation of materials used for tissue and organ bioengineering. There are a considerable number of different hydrogels known, which differ in their composition, the starting polymer used to form hydrogels, the method of polymerization and other properties[3]. In fact, acrylic-based hydrogels have been in use for a long time. With a strong drive in society to reduce carbon footprint and consequently improve our attitude towards the environment, search for alternative material intensified. As a result, the use of biodegradable materials, including natural forms of hydrogels, has increased. Notable, cellulose-based hydrogels are essential part of this group.

We will present our study on the use of dicarboxylic acid and acid chloride for crosslinking of cellulose as an entry point towards cellulose-based hydrogels. We further studied their properties by IR spectroscopy, thermal analysis, microscopy and determination of degree of crosslinking (Fig. 1).

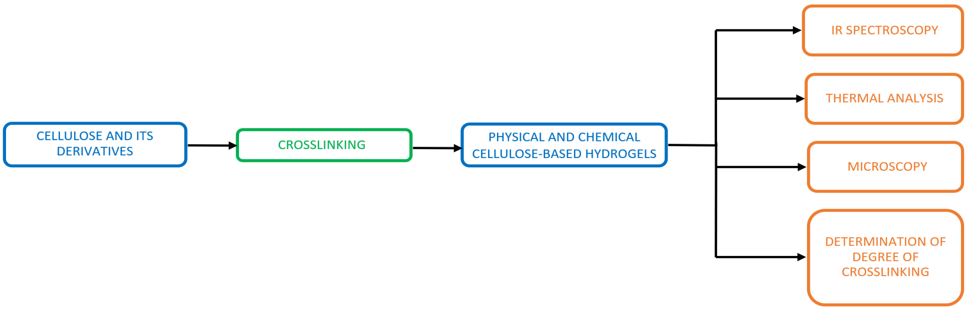

Fig. 2: *Path from cellulose to cellulose-based hydrogels and determination of their properties.*

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A practical approach to novel deoxyfluorinating reagent

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A practical and operationally friendly synthesis in aqueous medium of novel deoxyfluorination reagent based on dihydrogen trifluoride ($H_2F_3^-$) salt was developed. Reagent uses previously reported 1,3-bis(2,6-diisopropylphenyl)-2-chloroimidazolium ($IPrCl^+$) cation known to facilitate deoxyfluorination of phenols[1] and alcohols[2] using a large excess of CsF as fluorine source. With non-inert conditions and cheap reagents, synthesis shown in Fig. 1 provides much more affordable reagent, that already contains fluorine source and is capable of fluorinating phenols bearing electron-withdrawing groups with fair to excellent yields under mild reaction conditions using slight excess of reagent (Fig. 1.). Additionally, it allows late-stage fluorination, giving importance to modern day synthesis of fluorine-containing drugs. Furthermore, side product of fluorination is isolatable and could be regenerated into functional reagent using chlorinating agents providing a more sustainable and economical approach.

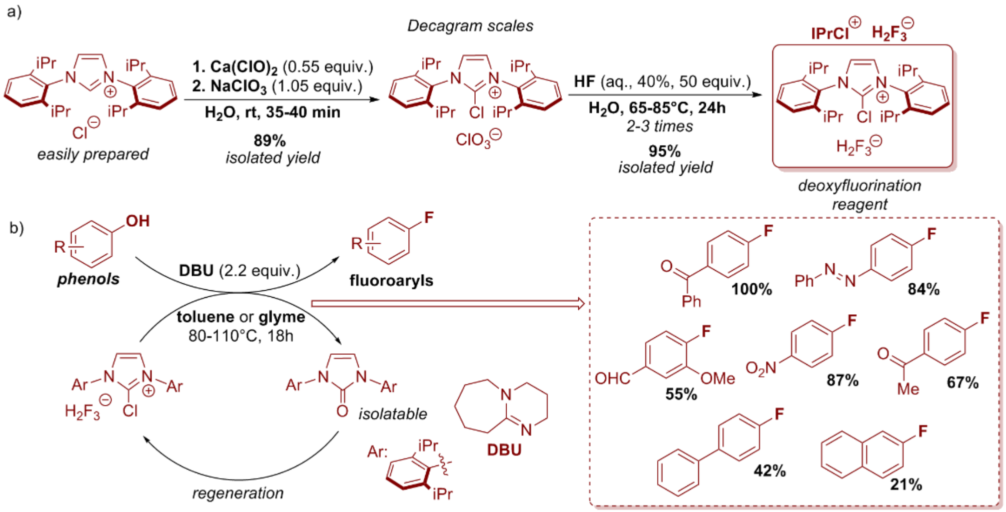

Fig. 1: a) Synthesis of deoxyfluorination reagent IPrCl H_2F_3 applicable on decagram scales using cheap, commercially available reagents and water as a solvent. b) Fig. shows conditions for deoxyfluorination of phenols with regenerable side product (left) and various fluorinated products obtained (right). Yields were determined by ^{19}F NMR spectroscopy using internal standard.

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Establishment of an experimental autofluorescence-based system for tracking the growth of aquatic microorganisms in co-culture

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Co-cultures of algae and cyanobacteria are critical for the study of microbial interactions in the environment and the production of valuable compounds for biotechnological purposes[1]. One of the major challenges in working with co-cultures are the analysis of the system and the determination of the interaction mechanism[2]. In this work, we propose a method to determine the population sizes of cyanobacteria or algae in co-cultures. The method is based on measuring photosynthetic pigment fluorescence and calculating the cell number using a calibration curve. Since different photosynthetic organisms have different pigments[3], the fluorescence of specific pigments could be used to distinguish between species in a mixed culture and to monitor their growth separately.

The autofluorescence spectra of two species of cyanobacteria belonging to the genus *Synechocystis* were measured (Fig. 1). The selected species show sufficiently distinct pigmentation and the proposed method could be applied for tracking their growth in a co-culture.

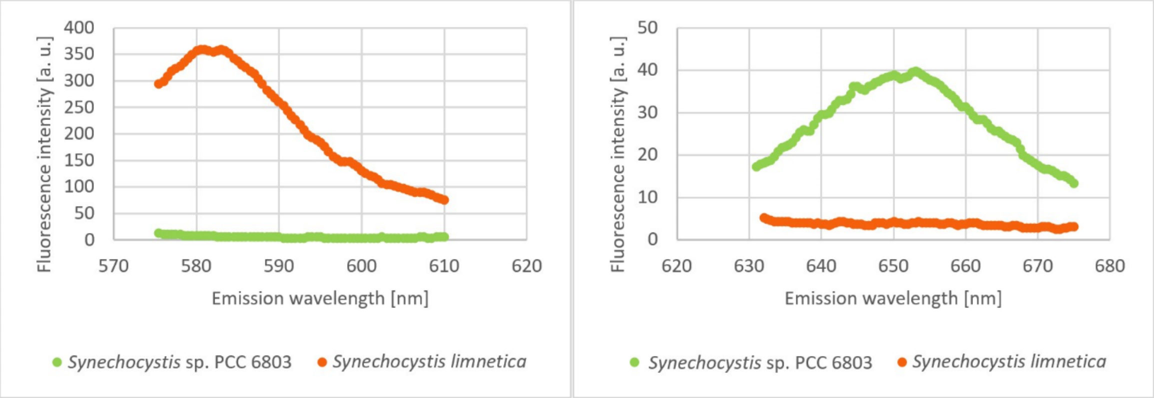

Fig. 1*: In vivo autofluorescence spectra of cyanobacteria Synechocystis sp. PCC 6803 and Synechocystis limnetica monocultures. Left: excitation at 565 nm (corresponding to phycoerythrin). Right: Excitation at 620 nm (corresponding to phycocyanin)*[3].

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Carboxylic acids effectiveness for heavy metal removal from fluvial sediments

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Accumulation of sediments is a problem in river management and therefore justifies its removal from the riverbed. Heavy metal contamination poses a risk when sediments are dredged, disposed of, or reused. One of the solutions for heavy metal removal is sediment washing[1]. Using chemical or biochemical reagents, it is possible to extract heavy metal ions from sediment, the most widely used reagent being EDTA[2].

Our research focused on the comparison of natural and naturally occurring mono- and polycarboxylic acids[2,3] (acetic acid, lactic acid, salicylic acid, succinic acid, malic acid, tartaric acid, and citric acid) as reagents in terms of their ability to remove Pb, Zn and Cd. Sediment washing was performed on two samples – old and newly dredged Drava sediment. The extracted heavy metal concentrations were determined by AAS. Extraction efficiency is given in relation to total concentrations obtained by digestion with nitric(V) acid. Citric and malic acids were found to be most successful at removal of all three metals from river sediments, for example, citric acid was able to remove up to 95 % of Cd (Fig. 1). Future work will focus on optimising the washing procedure (time, concentration, ratio), combining different reagents, and reusing them.

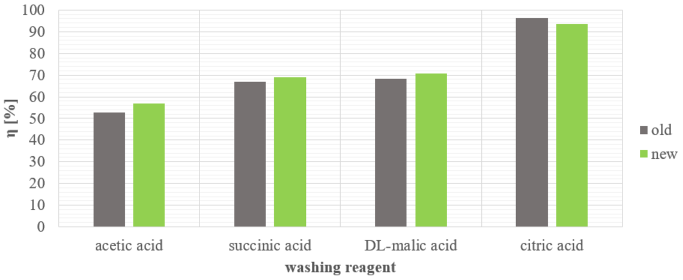

***Fig. 1**: Percentage of removed Cd from sediment samples by sediment washing.*

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Efficient inhibition of cholinesterases, Alzheimer's disease related enzymes, by organoruthenium pyrithione-type chlorido complexes

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Alzheimer's disease (AD) is the most common type of dementia. Apart from certain neuropathologic changes, enzymes such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) play an important role in the development of AD[1]. While the activity of AChE remains or decreases during the progress of AD, the activity of BuChE increases. Therefore, novel drugs with preferably dual inhibition of both enzymes are in high demand[2]. Eight organoruthenium(II) chlorido complexes **1a**–**h** (Fig. 1) with pyrithione **a**, its methyl **b**–**e** and benzo-fused analogues **f**–**h** were prepared and evaluated for their activity towards electric eel acetylcholinesterase (eeAChE) and horse serum butyrylcholinesterase (hsBuChE). Among all tested compounds, complex **1g** with 1-hydroxyquinoline-2-(1*H*)-thione resulted as the best inhibitor of eeAChE as well as of hsBuChE, with low micromolar IC_{50} value on eeAChE ($IC_{50} = 4.9 \pm 0.1$ µM) and submicromolar IC_{50} value on hsBuChE ($IC_{50} = 0.2 \pm 0.05$ µM). Furthermore, docking calculations were performed on *Torpedo californica* AChE, which supported the experimental results with complex **1g** possessing the lowest calculated energy value[3].

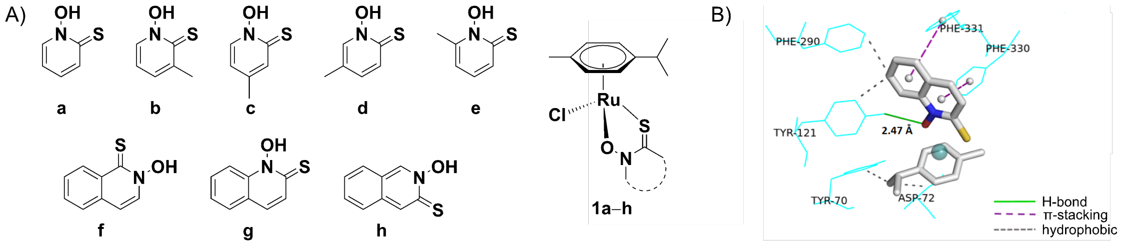

Fig. 1: *A) Pyrithione **a**, its analogues **b–h** and their organoruthenium(II) chlorido complexes **1a–h**. B) Interactions of complex **1g** in the active site of Torpedo californica AChE*[3].

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Protein interactions of matrin 3

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Matrin 3 (MATR3) is a poorly understood DNA/RNA-binding nuclear matrix protein[1]. The protein consists largely of intrinsically disordered regions, with the exception of two tandem RNA recognition motifs and two zinc finger domains[2]. This essential nuclear matrix protein has important functions in RNA metabolism, maintenance of mRNA stability, transcription, alternative splicing and mRNA transport[2,3]. MATR3 has been implicated in several pathogenesis processes. About a dozen missense mutations in MATR3 have been associated with amyotrophic lateral sclerosis (ALS)[3]. Among them, S85C is the most frequent[2]. Recent studies have shown that MATR3 interacts with SFPQ, NONO, TDP-43 and FUS (Fig. 1)[1-3]. However, protein interactions with MATR3 and its mutants are poorly characterized. Our aim was to compare the interactomes of wild-type MATR3 and its mutant form ALS-MATR3 (S85C). To this end, we produced mammalian cell lines expressing recombinant fusion matrin 3 variants with the biotin ligase BioID2. To identify interaction partners of MATR3 and ALS-MATR3, we performed pull-down assays. With our results, we aim to contribute to a better understanding of the role of MATR3 in ALS.

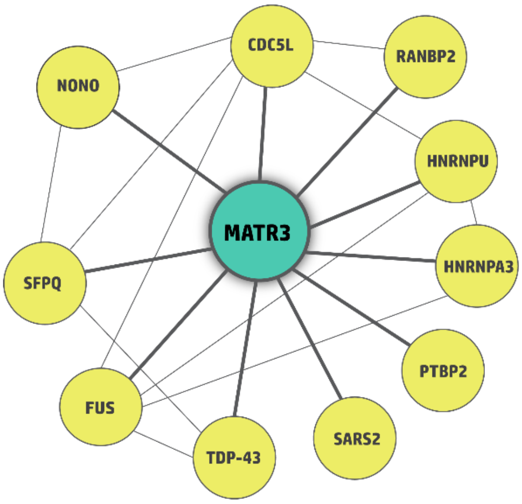

***Fig. 1**: Simplified graphic presentation of the protein-protein interactions for MATR3.*

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Simultaneous removal of arsenic and toxic metals from contaminated soil: A pilot-scale demonstration of novel technology

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Contaminated soils are recognized as one of the most pressing global environmental problems. As is one of the most hazardous elements: chronic exposure to arsenic has devastating effects on health, cardiovascular diseases, cancer and eventually death. Pb, Zn and Cd are very highly toxic metals which affect almost every organ in the body. With this in mind, new technologies for soil remediation processes are urgently needed.

Calcareous artificially contaminated soil containing 231 mg kg-1 As and historically contaminated with Pb, Zn and Cd was washed with a 1:1.5 solid-liquid ratio of 90 mM EDTA, 100 mM oxalic acid and 50 mM sodium dithionite to remove 59, 75, 29 and 53% of As, Pb, Zn and Cd, respectively. To reduce emissions of residual EDTA and chelated metals from the remediated soil, zero valent iron (ZVI) was added (1% w/w) to the slurry of the washed soil immediately prior to rinsing. Experimental controls were conducted without the addition of ZVI after remediation. The use of ZVI, reduced metal leachability and minimized toxic emissions 21 days after remediation. After this time, NH4NO3 extraction was performed to determine the mobility of toxic elements in soil. In addition, Unified Human Bio-accessibility Method (UBM) was performed to quantify the bio-accessibility levels of metals in stimulated human gastric and gastrointestinal phases.

Fig. 1: *Graphical abstract of the process.*

Direct trifluoromethylthiolation of aromatic amino acid derivatives and peptide analogues

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The incorporation of fluorine-containing moieties into organic frameworks has been increasingly recognised as highly promising for the discovery of novel biologically active compounds[1]. Due to the inherent high lipophilicity parameters and the strong electron-withdrawing effect of the trifluoromethylsulfanyl group (SCF_3)[2], the development of direct C–H bond trifluoromethylthiolation methods and late-stage functionalization strategies has been an active research area in recent years[3].

Although there are several reports on preparation of SCF_3-containing amino acids and peptides, the synthesis of trifluoromethylthiolated aromatic amino acid systems has not been reported up to date. Our group recently reported a *p*-chloro analogue of the trifluoromethanesulfenamide reagent ($PhNHSCF_3$) as a more stable source of the electrophilic SCF_3 group[4]. The reagent is also more efficient and enables trifluoromethylthiolation of diverse (hetero)arenes in combination with an activator (strong Lewis or Brønsted acid).

We will present our research on the synthesis of trifluoromethylthiolated amino acids as building blocks in peptide chemistry and potential use of the studied methodology for late-stage functionalizations of peptides.

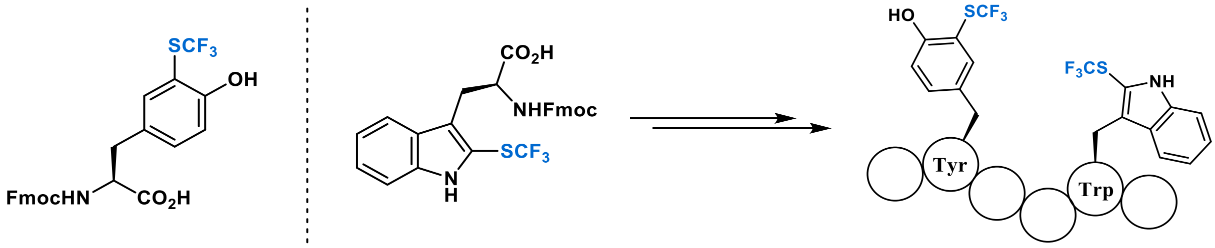

***Fig. 1**: Fmoc-protected trifluoromethylthiolated tyrosine and tryptophan products as potential building blocks in peptide chemistry.*

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Expression and isolation of TACE inhibitory peptide, designed on the basis of the propeptide sequence

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TNFα Converting Enzyme (TACE) or ADAM17 is a sheddase, correlated with inflammation and cancer progression. TACE is generated as a zymogen with an auto-inhibitory propeptide that inhibits TACE's sheddase activity[1]. It has been demonstrated that a double mutant of the prodomain (TPD-DM) can be expressed, isolated and used as an *in vivo* inhibitor of TACE[1]. Our goal was to utilize the TPD-DM as a ligand for the purification of the recombinant TACE ectodomain (TACE-EX) from insect cells. We conducted two different purification processes in various experimental conditions that resulted in the TPD-DM with an intact or cleaved off HIS-tag (Fig. 1). Our goal was to achieve the highest efficiency in the purification process, that would in turn enable testing of different immobilization methods of TPD-DM to the matrix. We found out that the TPD-DM product that has its HIS-tag region intact is more soluble than its counterpart without the HIS-tag. We also successfully immobilized the TPD-DM to a matrix and used it for TACE-EX purification. Our experimental results provide an important improvement in the purification process of TACE.

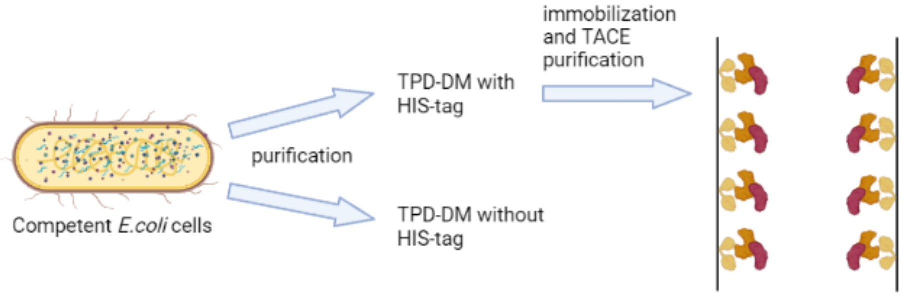

***Fig. 1:** Our main experimental steps (Created with BioRender.com).*

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Characterisation of cannabinoid receptors RNA expression on breast cancer cell lines

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Breast cancer (BC) is the most common form of malignancy in women, with 2.3 million women with positive diagnoses and 685 thousand deaths worldwide in 2020[1]. Based on the expression of the hormone receptors (HR) and human epidermal growth factor receptor 2 (HER2), three types of BC are distinguished: HR-positive, HER2-positive, and triple-negative BC[1,2]. Cannabinoid receptor type 1 (CB1R, gene *CNR1*) and type 2 (CB2R, gene *CNR2*) are G-protein coupled receptors that bind cannabinoids in neural and immune cells, respectively. Their expression has been confirmed also in BC cells and several studies have shown the antitumor effect of cannabinoids[3].

Our study aimed to characterize the RNA expression of cannabinoid receptors on breast cancer cell lines. We selected six breast cancer cell lines that differ in expressing HER2 and HR. First, we overviewed existing transcripts of the genes *CNR1* and *CNR2* (Fig. 1) using the protein and nucleotide banks. Three isoforms of CB1R (CB1, CB1a, and CB1b) and two isoforms of CB2R (CB2A and CB2B) are known[3]. We then reviewed existing TaqMan assays for the genes of interest and the reference genes and selected assays covering most of the known individual gene transcripts. Further we isolated RNA from breast cancer cell lines and reverse transcribed it into cDNA. We determined the expression of gene *CNR1* level with quantitative PCR and used the relative method for quantification. Contrary to our expectations, we were unable to detect *CNR1* gene expression in any analysed breast cancer cell line. The research will be extended to characterization on protein level to fully confirm the absence of *CNR1*/*CNR2* expression.

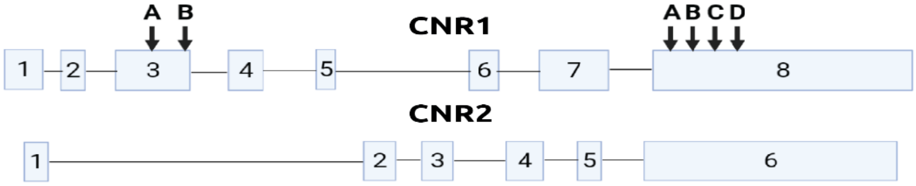

***Fig. 1**: Structure of genes CNR1 and CNR2. Legend: 1-8: exons; A-D: splicing sites.*

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Modification of electronic properties of mesoporous carbon materials using oxygen plasma treatment

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Increasing energy demand, increasing carbon dioxide pollution together with the limited resources of fossil fuels lead to growing interest in the development of research into the use of alternative energy sources. Hydrogen is being considered as one of the energy carriers that will play a key role in future mobile energy conversion devices. It can be produced using renewable energy sources such as sun, wind, hydropower. Hydrogen can also be obtained renewably by electrochemical splitting of water[1].

This work is part of the research, which aims to develop the composite material for the aforementioned process. The composites will be based on the mesoporous carbon materials which will stabilize transition metal compounds. The main role of carbon is to provide enhanced surface area for active centres exposition and high electrical conductivity[2].

Mesoporous carbon materials are widely used, among others in adsorption or catalysis. This is because of their unique textural properties. The properties of mesoporous carbon materials can be further adopted to the selected application by modifying e.g. electron properties. It is possible, among others by an oxygen plasma modification.

Plasma, popularly known as the fourth state of matter, is an ionized electrically charged gas that contains a high concentration of electrons, ions, radicals, photons. Plasma enables the functionalization of a material by introducing polar functional groups onto its surfaces. This leads to a change in the material's work function (F), which is a parameter depending on its electronic structure. Hence, the degree of functionalization or amorphization of the surface of the carbon material can be tracked by measuring the work function changes (DF)[3].

In this study, the influence of the oxygen plasma modification on the electronic properties of three mesoporous carbon materials with different nitrogen content (0,13%, 5,14%, 7,78%) was investigated. The performed measurements confirmed the strong influence of the plasma action on the value of the work function of the tested materials (work function increases upon plasma treatment). Moreover, it has been observed that the electronic properties of studied carbon materials strongly depends on the nitrogen content.

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Emodin derivatives as photoredox catalysts

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Naturally occurring quinones are important pigments, while quinone ring has essential role in natural redox processes[1]. Anthraquinone (Aq) and derivates are colored, their anion radicals possess moderate reduction potentials in ground state, excited states of radical anions of Aq derivates are strong reductants[2,3].

Natural anthraquinone emodin is colored compound with wide biological activity. Emodin core is interesting starting point for further modification through introduction of different functional groups. Variety of electrophiles (halogenation, nitration, sulfonation) were introduced to emodin ring in one-step synthesis under simple conditions and good yields. Structural and photophysical properties (absorption maximum, molar extinction coefficient, fluorescence emission, fluorescence quantum yield, fluorescence lifetime, radiative and nonradiative rate constants, cyclic voltammetry) of these derivates were examined. Understanding the correlation between structural modifications and photophysical properties are necessary for design of potential photocatalysts. We will present study of photochemical properties of a library of emodins and their use as photocatalysts for reductive activation of aryl halides.

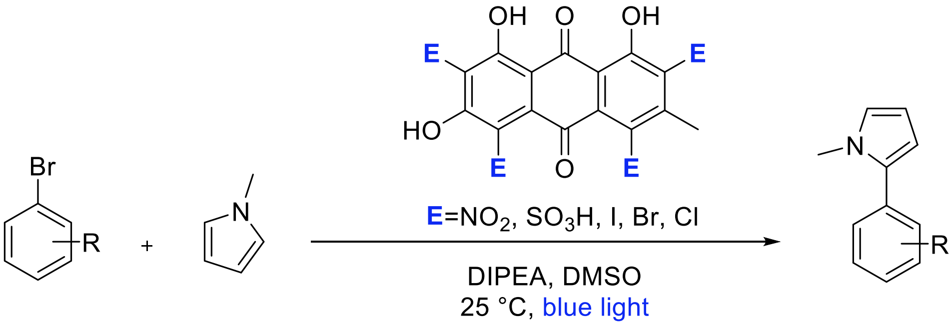

Fig. 1: *Model reaction for emodin derivates as photocatalysts.*

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Quantification of biofilm on microplastics

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Plastic has become an indispensable material in our lives. Consequently, enormous amounts of plastics end up in our environment every day where they break down into smaller particles called microplastics. By entering the environment microplastics encounter microorganisms, which form a biofilm on the particle surface[1].

As a part of our study, we developed biofilm on microplastics under laboratory conditions. Polyethylene particles extracted from cosmetic products were used as a sample of microplastics. The microorganisms present in the surface water sample began to form and evolve a biofilm on particles over time (Fig. 1). After 12 weeks, microplastics with biofilm development were used for analyzes to determine the amount of biomass on particles, photosynthetic pigment chlorophyll, extracellular polymeric substances (EPS), and urease activity. The amount of biomass was determined by digestion with Fenton's reagent and represented one-third of the mass of the total sample.

The specific concentration of chlorophyll *a* was 0,660 ± 0,050 mg/g and of chlorophyll *b* 0,241 ± 0,026 mg/g. That indicated the presence of algae, which are autotrophs, organisms that are able to produce food on their own. Moreover, in the process of photosynthesis, oxygen is formed, which is also a source of life for other microorganisms that build a biofilm. Extraction of EPS was achieved physically by raising the temperature. The concentration of EPS was 0,156 ± 0,025 mg/g. EPS are responsible for protection against hazards in the environment and also serve as a supply of nutrients and energy. Biofilm viability was demonstrated by the activity of urease enzymes, which catalyze the hydrolysis of urea. Urease activity was determined indirectly through spectrophotometric analysis of the formed ammonia. The concentration of hydrolyzed nitrogen was 1,904 ± 0,191 mg/g.

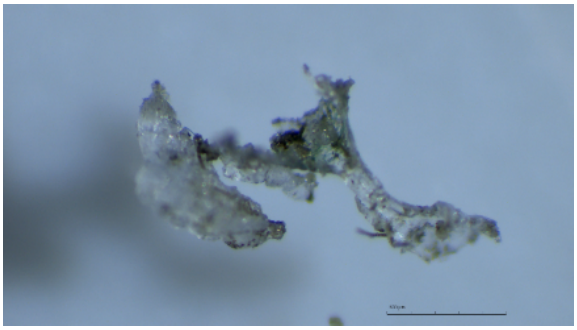

***Fig. 1**: Biofilm formation on the surface of microplastics.*

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Molecular cloning of antitoxins MazE and YefM from the bloom-forming cyanobacterium *Microcystis aeruginosa*

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Toxin-antitoxin (TA) modules are genetic elements that encode a relatively stable protein toxin that inhibits cell growth and an unstable antitoxin that inhibits a deleterious effect of the cognate toxin. TA finder - a web-based tool to identify type II TA loci, predicted as many as 121 TAs the in genome of the cyanobacterium *Microcystis aeruginosa* PCC 8706SL. Despite their abundance in this genome, only a few have been experimentally characterized so far.

We selected two PCC 7806SL TA pairs for detailed analysis, a MazEF-type and one pair of YefM/YoeB-type. In *E. coli*, the antitoxin MazE binds to the endoribonuclease MazF to form an inactive heterohexamer. On the other hand, YefM binds to the ribonuclease toxin YoeB in a 2:1 ratio. However, almost nothing is known about this type of TAs in cyanobacteria.

After completing the initial cloning steps we are attempting to express the recombinant cyanobacterial proteins MazE and YefM in *E. coli*. These would allow further research to gain insight into protein function, structure and regulation of TA gene expression.

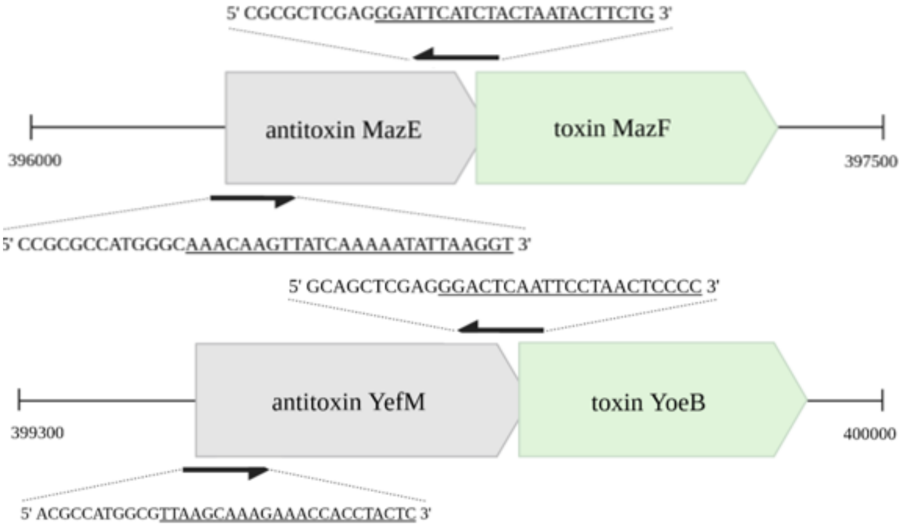

Fig. 4*: Schematic genomic arrangement of TA loci with marked primers used for PCR.*

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A comparison of a computational and experimental investigation of a microwave-assisted Diels–Alder reaction

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The Diels–Alder reaction is one of the most important and widely used transformations in synthetic organic chemistry. During our research, we investigated a microwave-assisted Diels–Alder reaction using 3,5,6-trisubstituted 2*H*-pyran-2-one derivatives as the diene and maleic anhydride as the dienophile[1] (Fig. 1). Besides the expected bicyclo[2.2.2]octene adduct, an unexpected side product was detected. Under similar conditions (conventional heating) it was shown that an aromatic byproduct can be formed[1–3]. We therefore theorized that this might be the side product in this case as well. A DFT geometry optimization was completed using the B3LYP functional with a 6-31G* basis set, while the vibrational analysis was achieved using a 3-21G* basis set at the Hartree–Fock level of theory. The computational study was implemented[4] to try to explain the formation of aromatic compounds during the microwave-assisted cycloaddition. The initial theoretical investigation did not provide conclusive results, but those that we obtained, suggest that the aromatic compound could be thermodynamically more stable and therefore the favoured product. Consequently, the main bicyclic product could be the kinetic product.

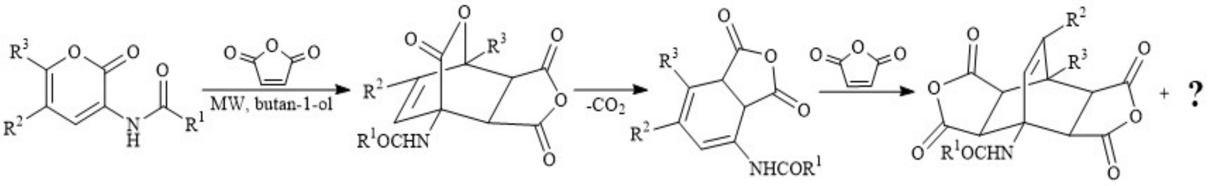

***Fig. 1**: Diels–Alder reaction under investigation.*

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Phylogenetic analysis of the FET protein family in mammals and conservation analysis of the EWS QGSY domain in vertebrates

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The FET protein family consists of three DNA- and RNA-binding proteins named FUS, EWS, and TAF15[1]. Mutations in these proteins have been associated with several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD)[2]. Bioinformatic analysis revealed that all FET proteins contain prion-like domains, one of which spans the QGSY-rich domain. These domains could be associated with cytoplasmic aggregation and pathology[3]. In this work, we analysed the evolution of FET proteins in mammals and found that the phylogenetic relationships of each FUS, EWS, and TAF15 are largely consistent with the evolution of mammalian species. Through multiple sequence alignment, we identified positions within sequences that could be the cause of unexpected results. We also discovered that genes encoding FET proteins were duplicated prior to mammalian species diversification. In addition, we analysed the conservation of the EWS QGSY domain in vertebrates with multiple sequence alignment. We identified tyrosine as the most conserved amino acid compared to other amino acids with similar frequency of occurrence.

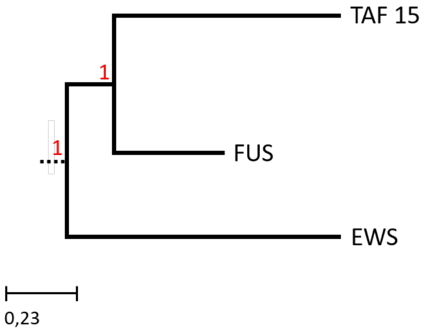

***Fig. 1**: Phylogenetic relationships of FET proteins in mammals. The phylogenetic tree was originally constructed from 33 mRNA coding sequences. Red numbers on branch points are bootstrap values. Scale bar represents 0,23 nucleotide substitutions per site.*

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In vitro biotinylation of AviTagged cathepsin S using expressed recombinant BirA protein

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Robust protein immobilization is an important step when performing selections of native or engineered proteins using methods such as ribosome display or phage display. Due to a very strong binding of biotin with streptavidin, immobilization using this interaction is widely used[1,2]. By using enzymatic biotinylation as opposed to chemical biotinylation, the reaction occurs at a specific lysine amino acid residue within a 15 amino acid long sequence, called AviTag. This allows directional, efficient and stable immobilization of AviTagged substrates on streptavidin-coated surfaces while preserving their native conformation[1,3]. Here we describe the preparation of recombinant BirA enzyme, which enables us to attach a single molecule of biotin by selectively targeting the AviTag attached to the target protein. Using molecular cloning, we prepared a His_6-MBP-3C-BirA containing vector and induced expression of the recombinant protein in *E. coli* BL21[DE3] cells. After expression, we purified the recombinant BirA protein using Ni-affinity and size-exclusion chromatography. Finally, we demonstrated that the purified recombinant protein is active and catalyzes the biotinylation of AviTag fused substrates and can therefore be used for specific biotinylation.

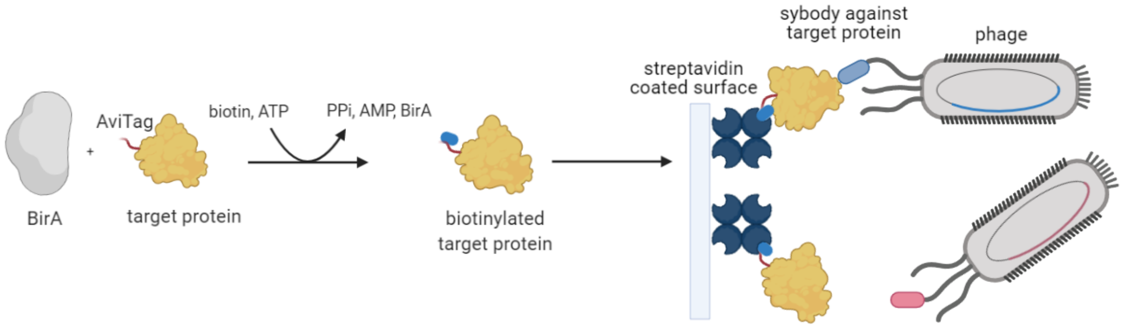

***Fig. 5:** Enzymatic biotinylation of AviTagged target protein for further immobilization using streptavidin-coated surfaces for phage display selection of sybodies against target protein.*

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Long-term exposure of duckweed to microplastics

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Plastic particles smaller than 1 mm are known as microplastics[1]. The majority of research about the effects of microplastics on the environment is focused on animals. Therefore, less is known about the interactions of microplastics with aquatic plants, especially in long-term exposure[2].

In this context, the aim of our study was to investigate the long-term effects of microplastics on duckweed (*Lemna minor*), which is a small floating plant with simple morphology and is known to quickly respond to various environmental conditions[3]. Duckweed was exposed to microplastics (100 mg/L), extracted from a cosmetic product, for 12 weeks. The specific growth rate and root length were determined every week, while the impact on chlorophyll *a* and *b* content and wet to dry mass ratio were determined every two weeks. Microplastics did not have a significant impact on any of the parameters within 12 weeks, however, we observed microplastics adsorption on duckweeds roots (Fig. 1). Thus, duckweed has potential for phytoremediation of microplastics, however, more detailed studies need to be conducted for determining the binding mechanism between microplastics and plants surface.

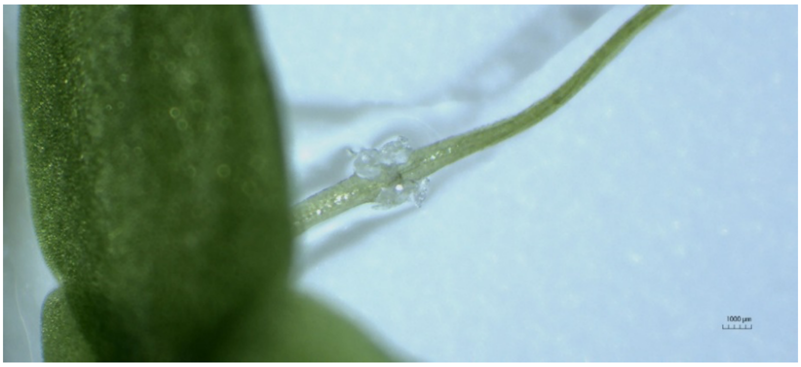

***Fig. 6**: Microplastic adsorbed on a root of common duckweed taken by Motic Stereo zoom microscope SMZ-171-TLED.*

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Impact of thermal post-treatment on the mechanical properties of 3D-printed cellular materials with triply periodic minimal surface

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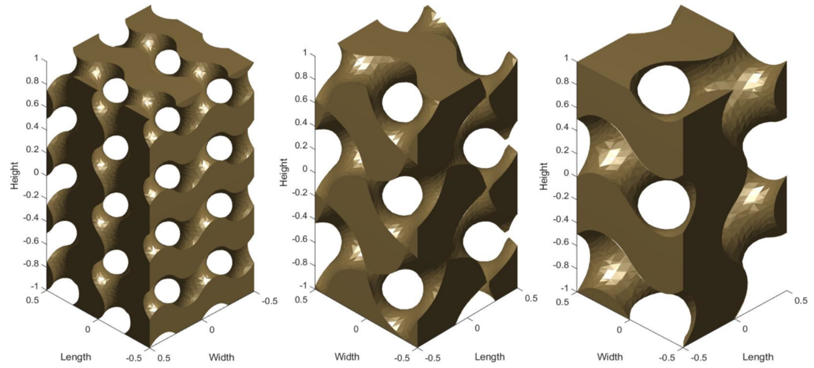
Cellular materials with tailored porosity combine high strength with a relatively low mass[1]. Tailoring can be made by the use of triply periodic minimal surface (TPMS) lattices, using Gyroid, Primitive, Diamond, etc. unit cells[2]. The aim of this research was to observe the impact of temperature on volume changes and mechanical properties of the Gyroid unit cell structure with different cell sizes. For this purpose, CAD models were prepared and manufactured by binder jet 3D printing (BJ3DP). The mechanical properties of the cellular TPMS were evaluated by mechanical flexural and compression test after temperature post-treatment (up to 500 °C). Different analytical techniques (SEM and microCT) were used for microstructural characterization of the cellular materials. The results of our research have shown changes in the microstructural and mechanical properties of the designed Gyroid unit cell structures caused by the increased temperature. Post-treatment has demonstrated the usable temperature range of such materials designed for several applications in civil engineering.



a) b) c)

***Fig. 1**: CAD models of a) 0.5, b) 0.8 and c) 1.0 Gyroid cell size.*

Acknowledgement: This research work was performed under the Slovenian Research Agency (Programme No. Z2-1861).

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Characterization of biochar from lignocellulosic biomass

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Biochar is carbon-rich, porous material produced by thermochemical conversion of biomass in oxygen-limited or anoxic conditions. The most widespread technology for such production is pyrolysis, as it is relatively simple, efficient, safe and environmentally friendly process. Biochar has received increasing attention due to its unique properties (high carbon content, large specific suface area and cation exchange capacity – CEC) which are affected by several technological parameters, mainly pyrolysis temperature, and type of biomass. Biochar's porous structure and large specific surface area allow for greater soil airiness, softness and ability to retain water and nutrients in the soil. Biochar improves soil CEC by retaining cations in a form accessible to plants. Water, nutrients and minerals are also retained in the micropores of biochar where beneficial microorganisms find ideal conditions for growth and reproduction. All of this contribute to higher soil quality and fertility, resulting in larger and healthier crops. In addition, biochar can also reduce greenhouse gas emissions from agricultural sources.

This research focuses on determining the characteristic parameters of biochar used as a soil amendment in agriculture. Three main methods were established to characterize the following biochar parameters: specific surface area, CEC and polycyclic aromatic hydrocarbons (PAHs) content (Tab.1). The first method allowed us to determine the specific surface area based on the Brunauer-Emmett-Teller (BET) theory[1]. For CEC determination was modified the existing ammonium acetate (NH_4OAc) method, in which NH_4^+ displaces base cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) initially adsorbed on active sites on the biochar surface[2]. CEC was additionally determined by a consequent exchange of adsorbed NH_4^+ with KCl solution. The third method was accomplished to determine PAHs in biochar using Soxhlet extraction[3]. Samples were extracted with toluene and PAHs were analyzed by GC-MS.

Tab. 4: *Methods used for the biochar characterization.*

Method	***BET analysis***	***NH_4OAc/KCl method***	***Soxhlet extraction***
Determined characteristic parameter	Specific surface area	CEC: $Na^+, K^+, Mg^{2+}, Ca^{2+} / NH_4^+$	PAHs

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Investigation of asymmetric organocatalyzed synthesis of dihydropyrano[3,2-*b*]pyrroles

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Asymmetric synthesis of chiral molecules attracts considerable attention in organic synthesis[1]. In this work, arylidene pyrrolin-4-ones were employed in the synthesis of new chiral dihydropyrano[3,2-*b*]pyrroles. Pyrrolone (Δ^2-pyrrolin-4-one) is a core motif found in potentially bioactive small molecules[2]. Pyrrolone derivatives also represents an alternative building block to well-established pyrazolone derivatives in asymmetric synthesis. We already showed that arylidene pyrrolin-4-ones give good results in asymmetric sulfa-Michael/aldol reactions[3].Asymmetric tandem Michael addition/cyclization reaction of *N*-methyl substituted arylidene pyrrolin-4-ones with malononitrile gave dihydropyrano[3,2-*b*]pyrroles with moderate enantioselectivity (12 examples, *ee* up to 77%). Reaction enantioselectivity is solvent and substrate (*E*/*Z* configuration) dependant (Fig. 1), therefore enantioselective formation of the desired dihydropyrano[3,2-*b*]pyrrole could be achieved. We also investigated mechanistic aspect of this reaction with control experiments and DFT calculations.

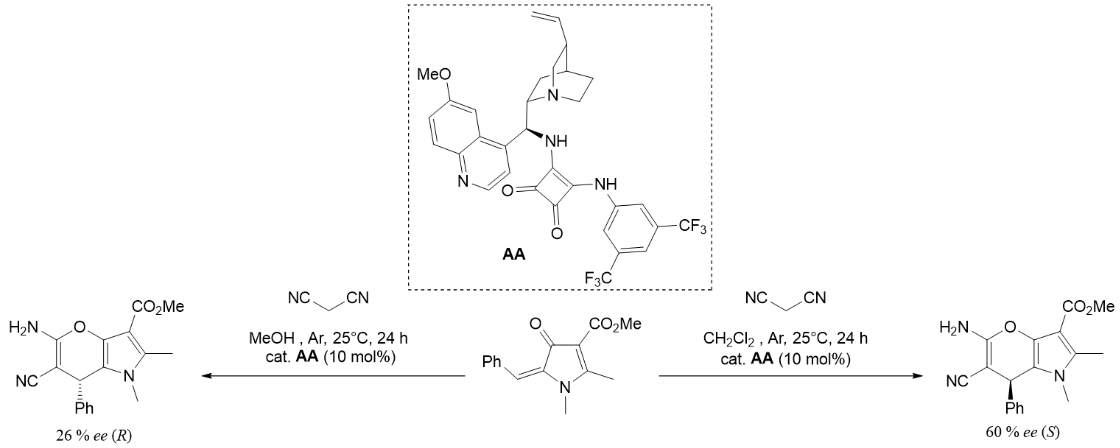

***Fig. 1**: Solvent dependant, organocatalyzed formation of dihydropyrano[3,2-b]pyrroles.*

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Size- and time-dependent nanoparticle concentration measurements in Trojane tunnel

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The aim of this work was to determine the concentration of nanoparticles in one of the busiest sections of the highway in Slovenia. The measurement was performed on two consecutive working days in the Trojane tunnel from 12:00 on the current day to 12:00 on the last day. The size- and time-dependent nanoparticle concentration was measured with a Scanning Mobility Particle Sizer (SMPS, TSI)[1]. At the same time, we also collected samples with the Dekati® low-pressure cascade impactor (DLPI; Dekati Ltd.)[1]. The results provided us with the mass concentration in each size fraction (stage). Stages 1 (30 nm), stage 2 (270 nm) and stage 8 (1 mm) were also analysed by a scanning electron microscopy and Energy-dispersive X-ray spectroscopy, which gave us data on the morphology and elemental composition of the samples.

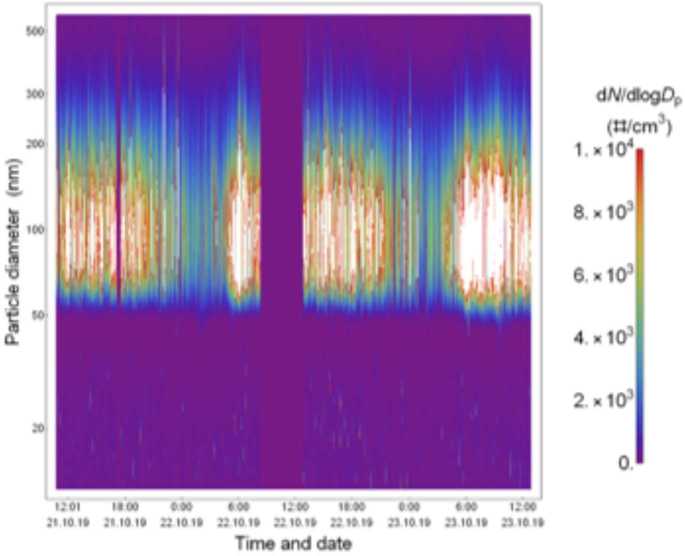

***Fig. 1**: Normalized concentration of nanoparticles given as diameter in log scale vs time.*

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Alkali-aggregate reactions and aging of concrete constructions

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It is known that concrete is in aging process liable to different chemical and physical changes, which can lead to the degradation of material. In the process of our research, we collected concrete samples from Vallo Alpino and from Rupnik line which were systems of fortifications built after World war I from Italians and Yugoslavs. Among chemical reactions I would like to expose so called alkali-aggregate reactions (AAR) especially alkali-silicate reaction (ASR) and alkali-carbonate reaction (ACR)[1]. ASR describes reaction between alkalis and reactive SiO_2 in concrete aggregate which consequently leads to emergence of expansion gel. ASR can in time lead to complete physical concrete degradation because it forms cracks in concrete – making it structurally unstable. ACR on the other hand includes dedolomitization – breakdown of dolomite aggregate in concrete (Fig. 1) and leads to formation of so called secondary $CaCO_3$ and some other gel Mg-Al-Si phases. Although we do not have an exact answer what kind of danger ACR possesses to concrete degradation we know that if dolomite aggregate contains reactive SiO_2 then ACR can also lead to structural failure of concrete construction. However, if SiO_2 is not present ACR can still cause changes in concrete. Newly formed gel phases can form cracks in concrete due to expansion or as it was recently proven secondary $CaCO_3$ can precipitate in formed cracks and can self-heal cracks in concrete[2].

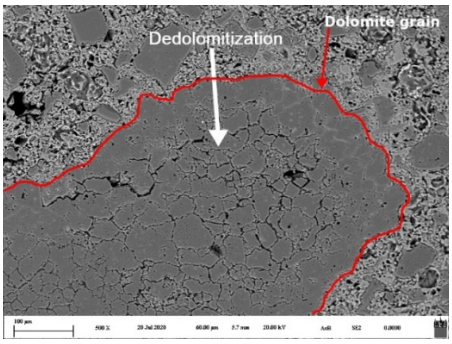

Fig. 1: *Dolomite grain and aggregate breakdown of it because of dedolomitization.*

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Behaviour of microplastics in a laboratory constructed wetland

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In recent decades, small plastic particles called microplastics have received considerable attention, due to their possible impact on the environment and human health. Microplastics can be found virtually everywhere, they are in atmosphere, soil, freshwater, and oceans and come from a variety of sources (fisheries, textiles, personal care products, agriculture, industry, waste, litter…)[1]. Many microplastics are found in wastewaters in form of small plastic fragments or fibers and wastewater treatment plants (WWTP) are the only barriers before microplastics enter the environment. WWTP can retain up to 99% of microplastics, but due to the high volume of treated wastewater the overall load of microplastics into the environment is very high[2]. In less densely populated areas small wastewater treatment plants are often used to treat domestic wastewater. Constructed wetlands (CW) are an economically and energy efficient type of small WWTP and the use of constructed wetlands in Slovenia is growing year by year.

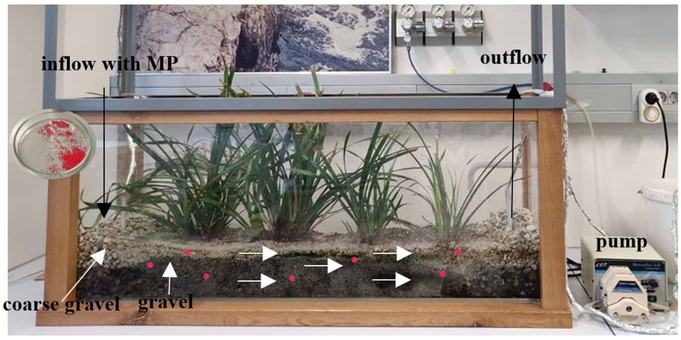

***Fig. 1**: Laboratory constructed wetland*

Mechanisms of the removal of microplastics by CW and the impact of various parameters are still poorly understood. In our research we are aiming to provide more information about the fate of microplastic in CWs by using highly engineered laboratory CW (as seen in Fig. 1). In the first step we will adapt plants to model municipal wastewater. Then we will contaminate the wastewater by various microplastics, follow the capacity of CW to remove microplastics from wastewater and monitor the overall treatment efficiency. Consequently, we will evaluate the fate of microplastic in the CW.

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Mechanical and water-related properties of new sustainable polysaccharide based bioplastics

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Sustainability is a concept worthy of attention that should be seen as a destination and not a journey[1]. Industry of processed hides and skins, known as leather, generates solid, liquid, and gaseous wastes which are prone of leaving negative social, environmental, and economic consequences[1]. While artificial leather is being promoted as more sustainable and eco-friendly solution, materials used for it are made of plastics and still harm the environment to disturbing extent[2]. Aiming to find better ecofriendly materials, testing of the mechanical and water-related properties of novel polysaccharides-based bioplastics material, which is both bio-based and biodegradable, were analyzed. Mechanical properties were evaluated on the bioplastic material after exposed to different temperatures for 2h. Additional test were performed to determine water-related properties of the material coated with different waxes, which can significantly affect the moisture content, swelling degree and total soluble mater presence in the polysaccharide-based bioplastics material (Fig. 1).

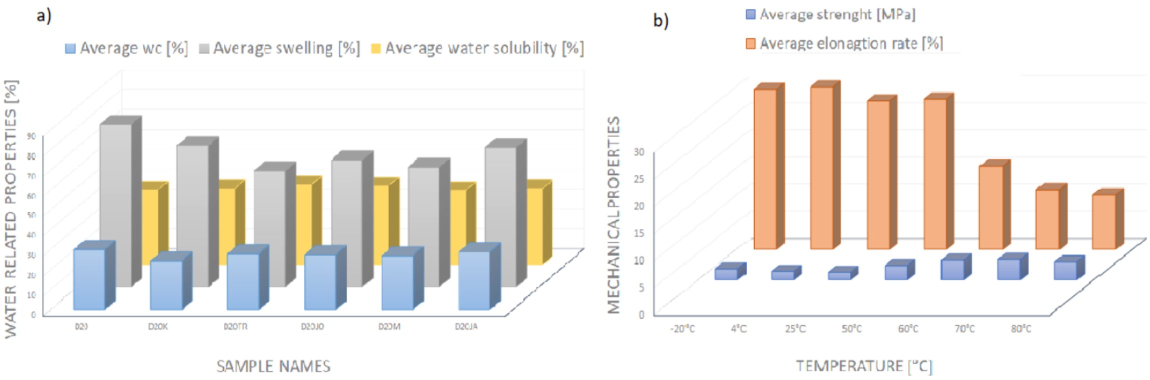

Fig. 7: *Average values of the measured a) water-related properties and b) mechanical properties.*

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Biotic aging changes microplastics properties

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Plastics are often found in the environment in the form of small pieces called microplastics (MPs). The properties of MPs in the environment alter with time, mainly due to the presence of microorganisms that bind to the surface and create a biofilm. This process is often called biotic aging of microplastics. The formation of biofilm causes changes in physical properties, as well as affects physical, biological, and chemical interactions of microplastics with the surrounding environment, including changes of microplastics adsorption properties[1]. Since microplastics often occur in wastewaters, they can coexist with various pollutants that can adsorb on their surface[2]. This is especially important in connection with heavy metals, as they have a high ecotoxicological potential in the environment[3].

The purpose of this research was to determine changes in MPs properties that are initiated by biotic aging. MPs were aged in stream water under laboratory conditions for 12 weeks. Due to biofilm formation, the mass of MPs significantly increased, consequently, the density of particles increased as well and the number of particles per mass of MPs decreased (Tab.1) probably due to the aggregation of aged particles. Adsorption properties of pristine and aged MPs were also compared by testing Pb(II) adsorption on respective particles. Aged MPs adsorbed approximately 10 % more Pb(II) than pristine ones (Tab.1). Our results suggest that biotic aging significantly changes MPs properties and consequently changes MPs fate in the environment.

***Tab.** 5: Properties of pristine and aged microplastics (MPs).*

	Pristine MPs	**Aged MPs**
Density (g/cm3)	0,88	0,95
Number of particles per mg of MPs (particles/mg)	68	50
Adsorbed Pb(II) (%)	82,7	91,3

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3D Printed molecular models – redesigned tool for chemistry education

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Molecular models are indisputably the tool that chemistry lecturers turn to when they need to represent chemical structures in three-dimensional representation[1]. Traditional ball and stick models represent the majority of available molecular sets; balls represent atoms, and sticks represent bonds between them. The Chemistry classroom is the first place students learn about molecules, atoms, and chemical bonds. For their future education in science, it is important to visualize these concepts appropriately. Due to its affordability and commercial availability, 3D printing has become the best tool for creating educational gadgets. It found its use in integrating nanoscience concepts into the classroom, improving intuitive understanding and introducing students to experimental methods for determining 3D atomic structures[2,3].

We will present a 3D-printed molecular set of atoms and chemical bonds with unique magnetic bonding for easier construction of molecules and increased durability of atom-bond connections (Fig. 1).

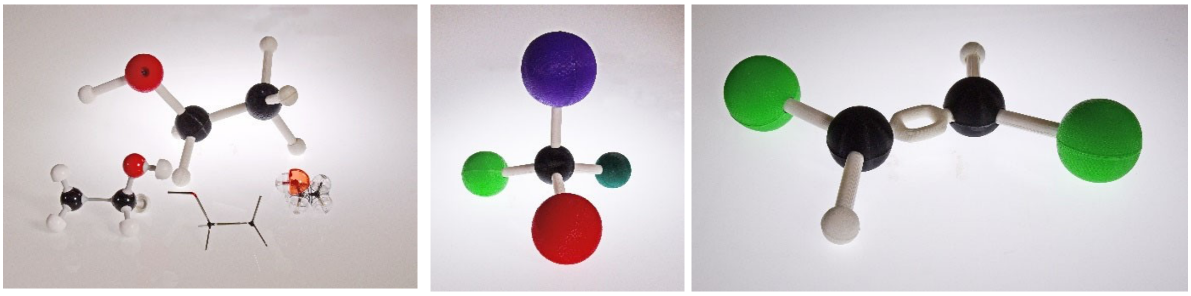

***Fig. 1**: Comparison of 3D-printed models with commercial ones (left), real-size ratios of atoms (middle) and restriction of rotation of double bonds (right).*

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Aerobic iodination of arenes catalyzed by $NaNO_2$

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Iodinated aromatic compounds are invaluable in organic synthesis as intermediates for carbon-carbon and carbon-heteroatom bond formation, specifically in transition metal-catalyzed cross-coupling reactions and in preparation of organometallic reagents. The preferred synthetic approach for these compounds is oxidative iodination, which is cheaper, safer, and more environmentally friendly compared to electrophilic iodination, as it enables 100 % iodine atom economy[1]. The use of molecular I_2 in iodination reactions proves to be difficult and requires activation by an oxidant or an acid. Two environmentally acceptable oxidants are especially compelling for oxidative iodination, namely H_2O_2 and O_2. Additional activation by generating a hypervalent iodination reagent is difficult with these two oxidants. A synergy was observed when systems $I_2/H_2O_2/HCl$[2] and $I_2/O_2/HCl/NaNO_2$[3] were combined with template catalysis by fluorinated alcohols, creating two methods for oxidative iodination of arenes.

In our work, we present a method for aerobic oxidative iodination of arenes using I_2 as iodinating agent, oxygen as external oxidant, and nitrogen oxides as catalysts. By replacing HCl with sulfuric acid, the reaction can also proceed in classical polar solvents, thus eliminating the need for fluorinated solvents.

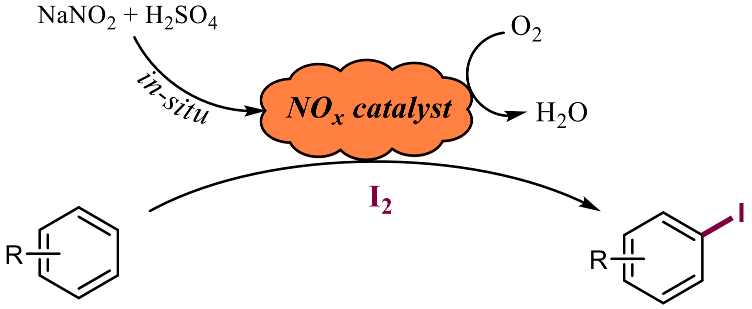

***Fig. 1**: NO_x catalysed oxidative iodination of arenes using elemental iodine and oxygen.*

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Hif3a genetic variability in the Fat and Lean selection mouse lines

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In obesity, expanding visceral adipose tissue is not well vascularized, leading to hypoxia and inflammation. A direct role of hypoxia in adipose tissue dysfunction (ATD) has been demonstrated; however, the molecular events triggering ATD by hypoxia are poorly characterized[1]. Changes in gene expression mediated by hypoxia-inducible transcription factors alpha (HIFA) are critical for adaptive response to hypoxia. There are three inducible isoforms: HIF1a, HIF2a, and a less studied HIF3a that is suggested to have distinct biological roles in adaptation and response to hypoxia[2]. Therefore, the aim of our study was to analyze genetic variants within the *Hif3a* gene of two unique polygenic mouse models of obesity (fat line, F) and leanness (lean line, L)[3]. The analysis showed that F and L have a distinct SNPs profile in the *Hif3a* gene (F, n = 39 SNPs; L, n = 50 SNPs). In F line, SNPs were located mainly in the introns, whereas in L line, they were predominantly in the 3' UTR region of transcript variant ENSMUST00000108492 (Fig. 1). The F had one synonymous and L one missense variant. SNPs in F line overlap with regulatory elements such as open chromatin and gene enhancer. Meanwhile, L line contains SNPs at the predicted miRNA target sites in the 3' UTR region. The present study suggests that *Hif3a* genetic variability could be related to obesity; however, additional bioinformatics and functional analysis are needed to decipher the mechanism by which *Hif3a* affects body fat content.

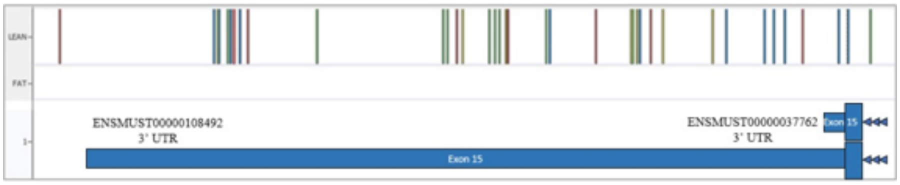

Fig. 1*: Two Hif3a transcripts and genetic variants within the Lean and the Fat line.*

Acknowledgments: The authors acknowledge the study was financially supported by the Slovenian Research Agency under the postgraduate research program Young researchers, J4-2548 research project and P4-0220 research program.

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Codon usage optimization based on translation velocity

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Translation and protein folding are coupled processes that can regulate each other by influencing the velocity of a ribosome[1]. In particular, codon usage has a major impact on translation velocity with access to synonymous codons associated with different average translation times specific to each organism[2]. It has been shown that modifying the codon sequence with only two synonymous substitutions leads to higher rates of misfolding, while faster translation not is always preferable[3]. Therefore, codon usage optimization is an important process in recombinant gene expression as it affects translation time and protein folding[2]. Our research focuses on codon usage optimization by introducing translation velocity as a key factor in the algorithm. Using machine learning and translation simulation approaches, we go beyond traditional optimization techniques and focus on specific properties of individual proteins.

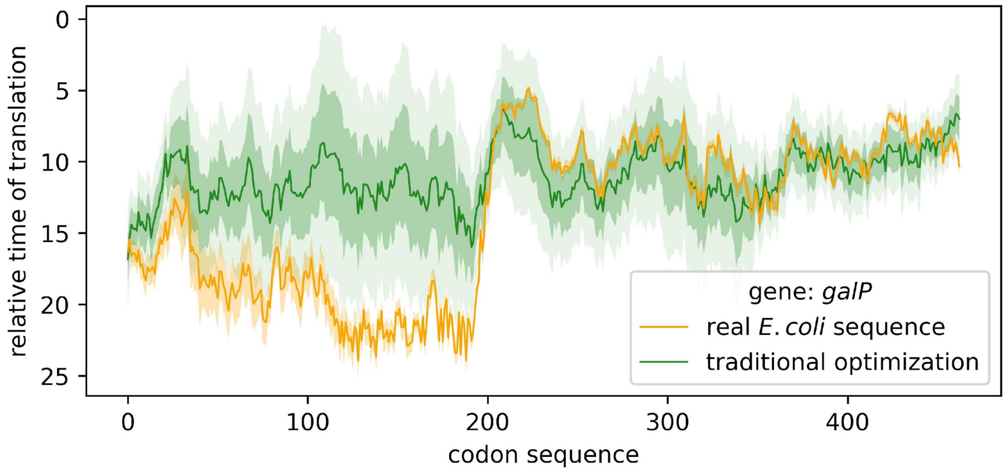

***Fig. 1**: Relative translation time for the real (orange) and traditionally optimized (green) E. coli gene galP. Lighter shades represent standard deviation (1, 2) over multiple iterations.*

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Inhibitory potential of *B. subtilis* isolates from tartar buckwheat on the growth of *S.* Typhimurium

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Salmonella enterica serovar Typhimurium is a foodborne pathogen affecting the gastrointestinal tract in humans. Biofilm production is an important virulence factor in *Salmonella*, however antibiotic treatments often fail to eradicate them successfully, resulting in spread of antibiotic resistance. A promising new alternative to traditional antibiotic treatments is the use of probiotics[1]. *Bacillus subtilis* is a ubiquitous bacteria, most commonly isolated from soil, where it can come into contact with different pathogens. Lately, its use as a probiotic is expanding rapidly due to many advantageous properties including the production of numerous antimicrobial compounds. These have also been proven to have an antagonistic effect against *Salmonella*[2].

The purpose of this study was to investigate the inhibitory potential *B. subtilis* isolates from tartar buckwheat on *S.* Typhimurium growth. To study interactions, *B. subtilis* strains (TA7-1 and TA19-1) were grown with and *S.* Typhimurium SL1344 in 1:1 ratio in liquid TSB medium. After 24 hours of incubation, interactions were quantitatively evaluated by determining CFU on agar plates and determining the thickness of *Salmonella* biofilm using confocal laser scanning microscopy.

The results indicate that selected strains inhibit *S.* Typhimurium growth and reduce its biofilm thickness.

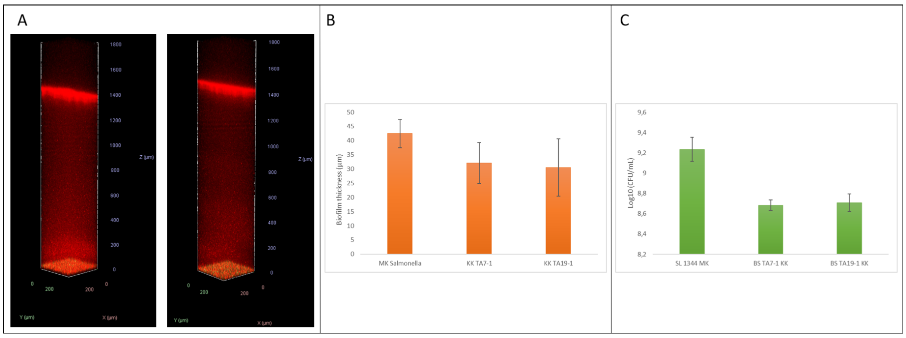

Fig. 8: *Cell distribution in cocultures (A), Salmonella biofilm thickness in monoculture and cocultures (B), Salmonella growth in monoculture and cocultures (C).*

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The use of tridentate Schiff bases for the synthesis of Ni (II) polynuclear coordination clusters

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Schiff bases are one of the largest groups of ligands used in coordination chemistry, with general formula $R^1R^2C{=}NR^3$. Due to their almost unlimited structural and electronic possibilities they can stabilize complexes with almost all transition metals. They are important in the field of polynuclear coordination clusters as they can simultaneously bridge and chelate metal ion centres[1]. In this work two new Ni^{II}/Na^{I} (**2**) (Fig. 1a) and Ni^{II} (**3**) (Fig. 1b) polynuclear coordination clusters with novel coordination modes of the ligands were synthesized and characterized[2]. The Schiff base *N*-salicylidene-4-methyl-*o*-aminophenol was used as chelating-bridging ligand. Each oxygen atom in the deprotonated ligand is capable of bridging two to three Ni^{II} ions, while the position of the nitrogen atom favours the formation of two chelating rings per ligand. The decanuclear cluster **2** was the first structurally characterized Ni^{II}/Na^{I}/CO_3^{2-} coordination cluster, displaying an extremely rare 10.334 coordination mode (Harris notation) of CO_3^{2-}, connecting all metal centres. Metal centres in the hexanuclear cluster **3** are linked by the $H_2C(O)_2^{2-}$ ligand (formed *in situ*), uncommon in 3d-metal chemistry, displaying a novel 6.33 coordination mode.

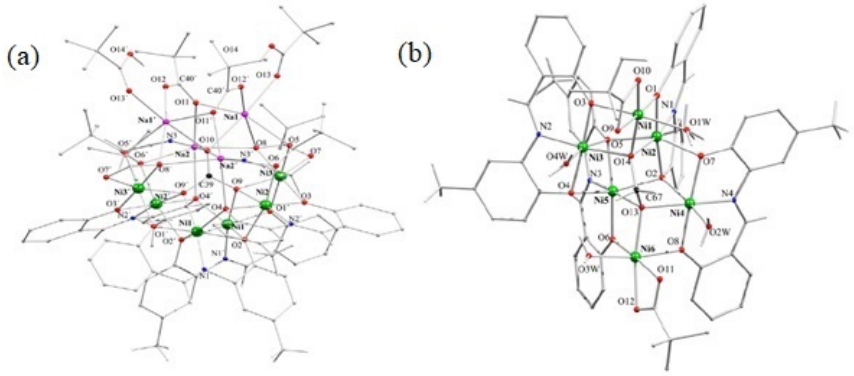

Fig. 9: *Partially labelled structures of (a) cluster* ***2*** *and (b) cluster* ***3****.*

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Intermolecular hydroamination of alkynes catalyzed by palladium complex $[Pd(Py\text{-}tzNHC)_2]^{2+}$

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One of the most important and atom-economic approaches to produce imine present hydroamination of acetylene[1]. Different metals were used for numerous catalysts, which promote the intermolecular catalytic hydroamination of terminal acetylene. Relatively harsh reaction condition such as elevated temperature and addition of additives, are still required in most reported systems[2].

Herein we present an effective method for palladium catalyzed intermolecular hydroamination of terminal acetylene to afford imines. Described method takes place at room temperature and is demonstrated on a broad range of substrates (Fig. 1a). In addition to the developed method, we demonstrated the unique dual role of palladium catalyst $[Pd(Py\text{-}\,tzNHC)_2]^{2+}\,2(BF_4^-)$ during the reaction trajectory (Fig. 1b)[3].

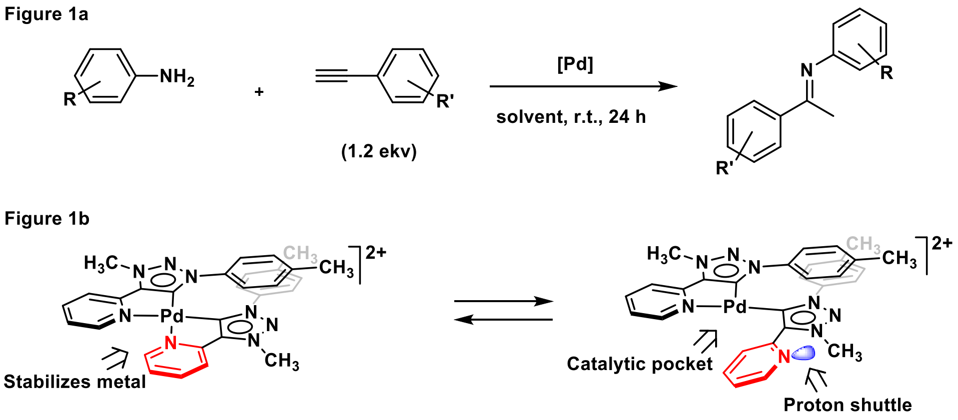

Fig. 1: a) Hydroamination reaction. b) Dual role of $[Pd(Py\text{-}\,tzNHC)_2]^{2+}\,2(BF_4^-)$.

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Biomass pyrolysis – a simple way for climate change mitigation

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Climate change is mainly due to the increasing concentration of greenhouse gases (GHGs) in the atmosphere, especially CO_2. In 2019, we added nearly 9 Gt of carbon (in the form of CO_2) to the atmosphere by burning fossil fuels[1]. Reducing GHG emissions by more than 90% is no longer sufficient to limit the rise in global temperature below 2 °C, considering pre-industrial levels (Paris Agreement, 2016). Therefore, more than 200 Gt of carbon must be removed from the atmosphere globally by the end of the century and safely stored in the long term through the creation of massive carbon sinks[2].

In December 2019, the European Commission announced the "European Green Deal". It addresses the issue of climate change and environmental degradation. The primary goal is to become the world's first carbon-neutral continent by 2050[1]. As a result, European Climate Law is being thoroughly revised. In July 2021, the climate package called "Fit for 55" will be published, mandating a reduction of greenhouse gas emissions to at least 55% below 1990 levels by 2030[3].

Slow pyrolysis is a thermo-chemical conversion of biomass at temperatures above 300 °C, low heating rates and in the absence of oxygen. Unlike combustion, pyrolysis is a carbonization process in which only portions of the biomass are devolatilized, leaving much of the carbon in the form of char. In addition to a solid product, gases and liquids are also produced that can be burned to produce energy. Since (bio) char is not produced to be burned for energy, but is used in such a way that the carbon it contains is retained. Therefore, pyrolysis is considered a carbon-negative technology and a viable solution to contribute, at least in part, to the removal of carbon (sequestration) from the atmosphere to mitigate climate change. Applying pyrolysis instead of combustion reduces CO_2 emissions by almost half due to biochar production. Energy output is reduced by half, meaning that twice the amount of biomass must be pyrolyzed to achieve the same energy output as combustion. Due to the simultaneous carbon sequestration, energy generation and production of useful solids, pyrolysis should receive more attention than combustion in the future[1].

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Discrimination between owlet moth caterpillars based on DNA barcode

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The family of owlet moths (*Noctuidae*) consists of numerous species, many of which are classified as pests[1]. To prevent as much damage as possible, it is crucial to distinguish them while they are still at the larval stage; however, many larvae of different owlet moth species look very similar, making it difficult to discern them morphologically[2]. DNA barcoding is a molecular tool that can be used to circumvent this problem. It is based on the idea that every organism contains a short universal segment in its DNA that can be used to distinguish it down to the species level. The method consists of: (1) DNA isolation from the collected organism, (2) barcode amplification using PCR, (3) barcode sequencing, and (4) comparing the barcode sequence to a database (Fig. 1)[3].

In this work, we used DNA barcoding to distinguish between different species of noctuid larvae. As the barcode of choice, we used the "Folmer" region of the mitochondrial gene *COI*, which is the most common barcode used in animals[3]. We also performed a bioinformatic analysis, in which we compared barcode sequences from four noctuid species and one from the family Lycaenidae. Using this approach we could identify the pestiferous *Chrysodeixis chalcites* (tomato looper) caterpillar several weeks before it hatches into the adult moth.

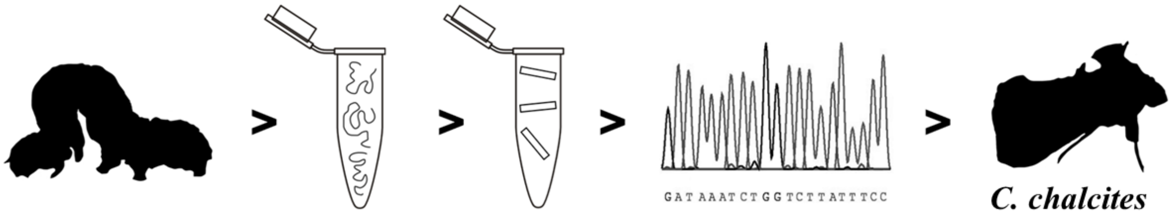

***Fig. 1**: Determining the species of a larva using DNA barcoding.*

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Development of analytical method for determination of phytoestrogens in beer and hops

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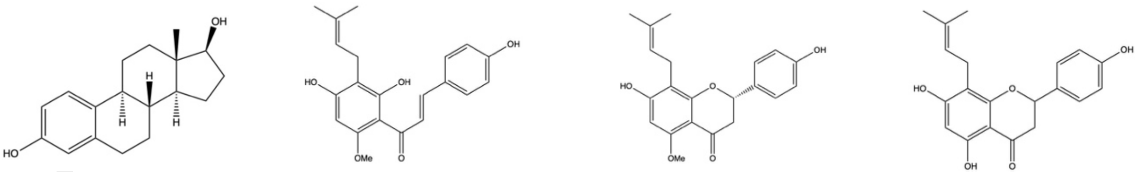
Phytoestrogens are compounds that mimic the effect of estrogen in human body. They are naturally present in various plants such as hops, soya beans, etc. As their structure is similar to 17β-estradiol (Fig. 1), they can bind to human estrogen receptors (ERα and ERβ). They have very different modes of action in our body, some act like hormone agonists, others like antagonists. They have many applications, for example in hormone replacement therapies as they are supposed to reduce cholesterol level, maintain healthy cardiovascular system, and lower the possibility of developing breast cancer in women and prostate cancer in men[1].

Xanthohumol (XH) is the most prevalent phytoestrogen in hops, during boiling of the wort it isomerizes to isoxanthohumol (IX). 8-Prenylnaringenine (8-PN) is a product of microbiological transformation of IX and is also formed in human digestive system by microflora. 8-PN is also the most potent phytoestrogen known[1,2].

I developed an analytical method for qualitative and quantitative determination of three different phytoestrogens that occur in hops and later in beer: XH, IX, and 8-PN. HPLC with C8 column was used for separation and DAD for detection. 8-PN was present below the limit of detection. Using UHPLC-MS/MS system with ESI(-) ionization*,* presence of 8-PN was confirmed in all beer and hop samples.



Estrogen XH IX 8-PN

Fig. 1*: Structural similarity of estrogen, XH, IX and 8-PN.*

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Generation of adenoviruses expressing SARS-CoV-2 spike variants for further use in enhancing vaccine efficacy and thermostability

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The emergence of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in Wuhan at the end of 2019 and the subsequent outbreak of the COVID-19 pandemic left the world seeking for an effective countermeasure. Extensive research of the new coronavirus and high financial support have prompted fast development of various vaccination platforms resulting in accumulating number of approved vaccines in the clinic. Yet their efficacy can be further improved, especially in the terms of effective protection against various viral strains, vaccine reactogenicity, storage and distribution of the vaccine and a thorough protection of diverse groups of population[1]. The aim of our research was to develop a quick and effective method for generating adenoviral vectors encoding full-length spike glycoprotein (S) variants (Fig. 1). We show that the desired genetic modification of the Ad5ΔE1 vector can be done using Gibson Assembly method, avoiding the time consuming and labor-intensive homologous recombination generally used[2]. Furthermore, after transfection of HEK293 and 3T3 cells S is expressed and translocated to the cell membrane. This gives us the opportunity to continue our work coating the isolated adenoviruses with MHC-I SARS-CoV-2 epitopes and engulfing them in nanocellulose matrix, by which we could tackle vaccine reactogenicity, efficacy, and cold-chain demand problems.

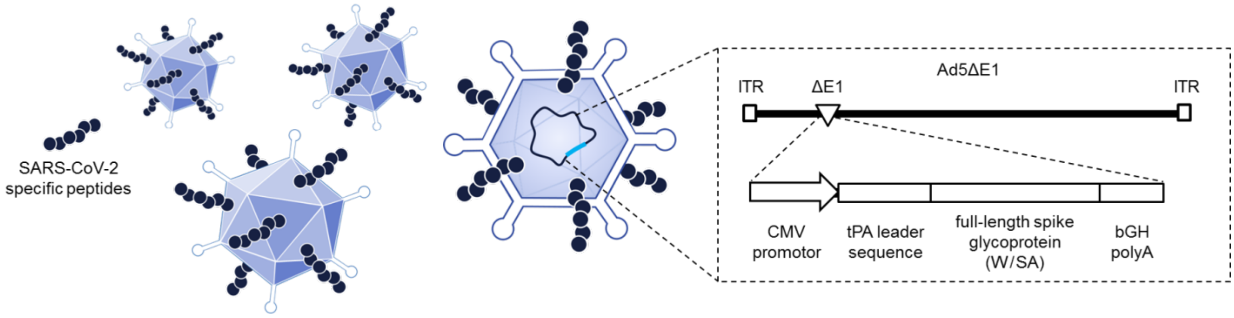

***Fig. 1**: Construction of the recombinant Ad5ΔE1 vector encoding one of the spike glycoprotein variants (W – Wuhan, SA – South African).*

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Sustainable renewal of artistic glass production

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Sustainability of the glass sector relies on various, yet very interlinked factors: industry, environmental aspects, marketing, tourism, chemistry of the glass production and forgery. New environmental directives that target the reduction of emission of hazardous substances have directed the glass industry all over the world toward a more sustainable and environmentally friendly production. There is a positive trend in the individuals' awareness of negative environmental impacts of glass production, especially among young glassmakers who strive for a more sustainable and resilient renewal of the artistic glass sector[1].

Compared to other sectors, the artistic glass industry is much more sensitive to environmental, socio-economic and health related issues, since it requires a variety of environmental and purchased inputs, along with a good and stable finances (Fig. 1). For small glass companies and artists who work on their own the struggle for financial survival is even tougher. Therefore, taking into account emergy is of great importance. Emergy is a scientific measure representing all of the directly or indirectly used energy to make a product or service in units of one type of energy. It includes direct and indirect renewable or non-renewable inputs, labour, purchased resources and services, which altogether form the total emergy yield (Y). Other indicators which are also used when evaluating the performance of the systems are emergy yield ratio (EYR), transformity (Tr), environmental loading ratio (ELR) and environmental sustainability index (ESI) [2].

The aim of the research is to point out the benefits of the environmentally-friendly renewal of the artistic glass sector. Glass industry/companies, but also individual glass artists should be aware of the positive effects that environmentally-oriented glass production has on the overall used emergy in the working processes and on the sustainability of the sector in general.

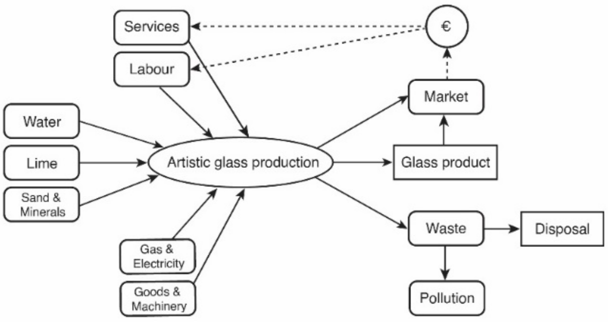

***Fig. 1:** The environmental and purchased inputs required for the operation of a artistic glass furnace[1].*

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Expression and purification of mutated versions of antitoxin 1067 from cyanobacteria *Microcystis aeruginosa* PCC 7806

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Microcystis aeruginosa is cyanobacterium known also for the production of toxic compounds. Toxin-antitoxin systems are small genetic elements present in most bacteria. Functions of toxin-antitoxin systems are inhibition of cell growth, plasmid maintenance, programmed cell death and persistence. Presence of antitoxins neutralizes toxin activity. Toxin, when not in complex with antitoxin, results in growth arrest, possibly even cell death. Toxin-antitoxin represent potential target for antimicrobial drugs. We focused on antitoxins 1067 in cyanobacterium *Microcystis aeruginosa* PCC 7806. It was discovered that in the genome of *M. aeruginosa* PCC 7806 gene coding for MaOC1, a caspase homologue protease, is followed by a toxin-antitoxin module[1]. The orthocaspase was able to cleave the antitoxin from toxin-antitoxin complex. Target of the MaOC1 was most likely C-terminus of the antitoxin with two sections of basic amino acid residues and our suspicion was 3 residues of arginine in α3 helix or 2 residues in the α4 helix[2]. We therefore investigated all 3 possibilities of mutations (Fig.1). After we received constructed plasmids, containing antitoxin with mutations, we analyzed cleavage patterns levels of the four antitoxin variants with the orthocaspase MaOC1.

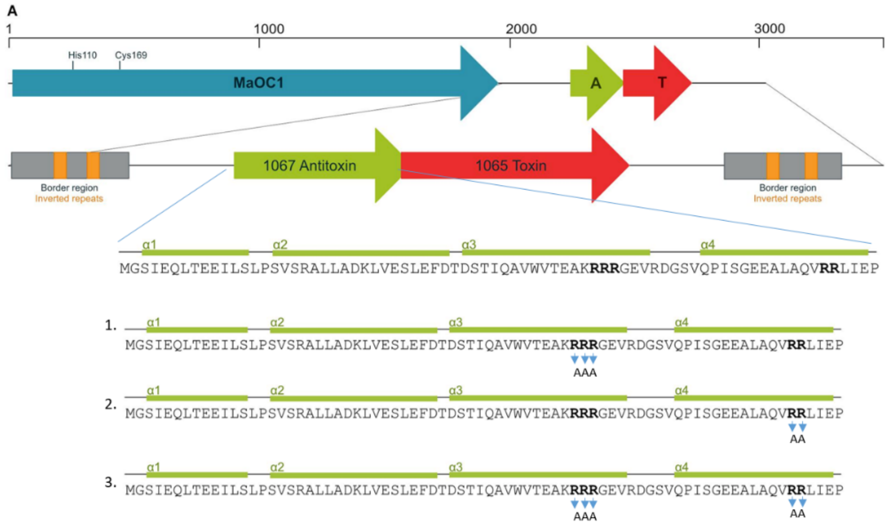

***Fig. 1**: Genome sequence of antitoxin 1067 and 3 mutated forms.*

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Oxygen reduction reaction activity comparison between quasi-1D and 2D N-doped heat-treated graphene oxide

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The comparison study is focused on aspect ratio, N-doping and metal impurities influence on ORR electroactivity between quasi-1D graphene oxide nanoribbons and 2D graphene oxides in acidic and alkaline electrolytes.

In acidic electrolytes, we have shown, that a higher aspect ratio and N-doping play an important role in improving ORR activity. The present study also revealed that inherent metallic impurities have a significant influence on the ORR activity. Furthermore, when comparing N-doped derivatives with non-doped derivatives the ORR activity in alkaline, the N-doping effect is overshadowed by inherent transition metal impurities[1].

The present study will aid in the critical assessment and development of non-metallic catalysts for electrochemical energy conversion devices.

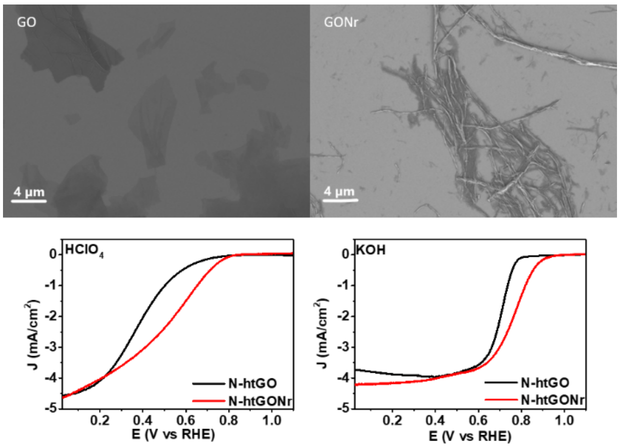

Fig. 1: *SEM images of GO and GONr morphology and ORR polarization curves in alkaline and acidic electrolytes.*

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Conversion of hydrated titanium gel to white TiO_2 pigment

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The aim of this research is to describe the process in a rotary furnace during the calcination of industrial samples of hydrated titanium dioxide gel. The work was focused on the first two processes of titanium (IV) dioxide calcination: drying and desulfurization (Fig.1)[1]. During drying the water is eliminated from the samples in two intervals: initially, surface water and then in the second interval pore water evaporates from the samples. The second process: desulfurization also occurs in two intervals. The first one involves the evaporation of freely bound sulfuric acid where the last remaining water is eliminated and at the same time, due to high temperature, sulphur trioxide is converted to sulphur dioxide. In the second interval, the sulphate bridges disintegrate in the dried sample, causing a rutilization process[2].

The non-isothermal/isoconversion method was performed for both processes[3]. This method resulted in calculated activation energies using several different heating rates representing a reasonably consistent starting point for the mathematical model description of the hydrated titanium dioxide calcination process.

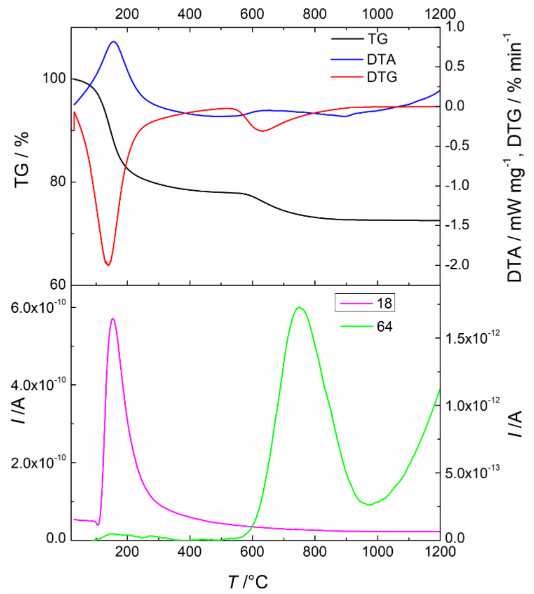

Fig. 1: *Results of thermal analysis (above) and QMS measurements (below) using ß = 10K min^{-}.*[1]

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Green synthesis of zinc oxide nanoparticles using a Japanese knotweed extract

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In recent years, photocatalytic degradation of organic pollutants in water has become a popular topic of research[1]. Titanium dioxide still remains the most popular of these photocatalysts, however more and more research is being done with zinc oxide due to its comparable electronic, physical and chemical characteristics[2]. In the process of the photocatalytic reaction, the photocatalyst absorbs photons that have higher energy than its band gap[3]. This process creates pairs of electrons and holes, which can then migrate to the surface of the catalyst and transfer to adsorbed water and oxygen molecules forming reactive species such as hydroxyl radicals and superoxide anion radicals. These reactive species can then degrade most organic pollutants and in best cases even mineralize them completely. This makes them a popular tool for wastewater treatment and other cleaning applications.

Herein, we present a green synthesis of zinc oxide, using an extract from an invasive plant species *Fallopia Japonica* or Japanese Knotweed. The extract was used to aid the reduction of Zn atoms during the synthesis and to add additional functional groups on the surface of the nanoparticles. The synthesis procedure was optimized and ZnO was then tested as a photocatalyst in photocatalytic degradation of antibiotics as a model for contaminants of emerging concern.

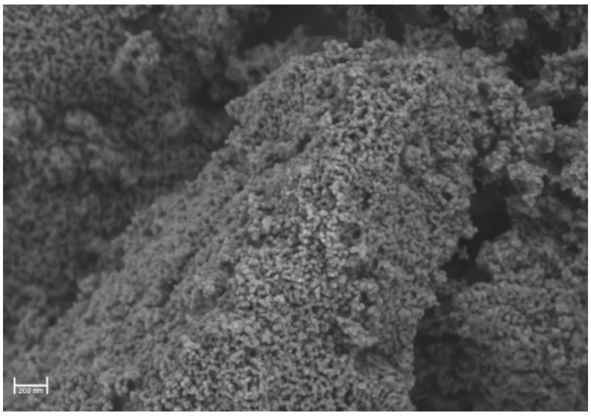

***Fig. 1**: SEM image of the prepared zinc oxide particles.*

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A computational study of hydroamination of alkynes catalysed by Pd-NHC complex

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Understanding of reaction mechanisms is a fundamental part in the rational design of reaction conditions and development of catalytic systems. It allows for better yields, expansion of substrate scope, minimisation of starting material costs and reduction of problematic wastes. Efficient mechanistic studies are performed via an interplay of experimental and theoretical studies that offer information about the relations between intermediates, substrates and products. Experimental findings can be supported well by implementation of computational techniques, such as DFT simulations. These help to identify the elusive transition states that often cannot be characterized by other means.

The reaction between alkynes and amines, also termed hydroamination of alkynes, allows for the formation of C–N bond and results in imines as reaction products. In our recent endeavour in the field, employing palladium complex with pyridyl-mesoionic carbene ligands offered some interesting mechanistic insights. Employing the *in silico* approach to evaluate the structures and properties of transition states revealed a key intrinsic feature of the pyridine sidearm. In addition to the general stabilization role at the transition metal centre, pyridine dent also actively participates in the key reaction stages as a proton shuttle, which mimics the function of enzyme-like architectures by assisting entropically favoured proton transfers[1].

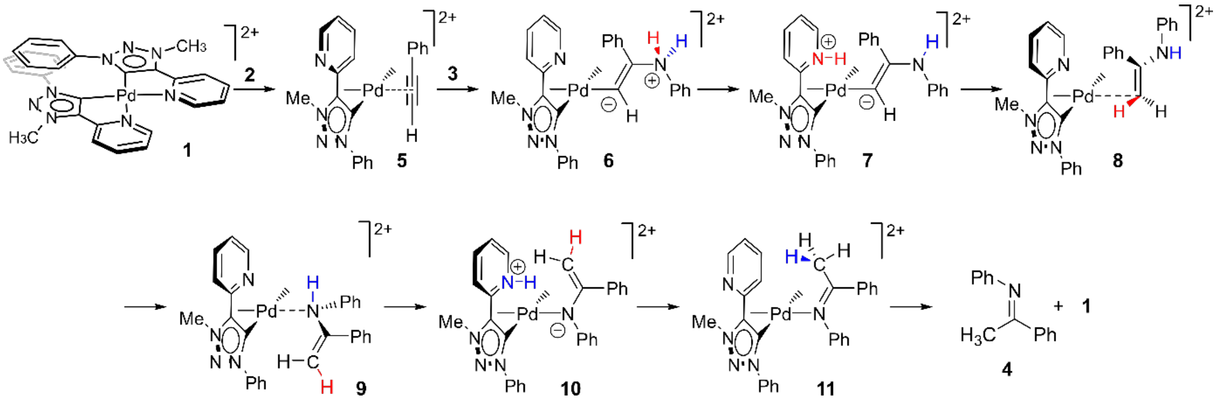

***Fig. 1**: The mechanism of Pd-NHC catalysed hydroamination reaction[1].*

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Tetramic acids as substrates in organocatalyzed transformations

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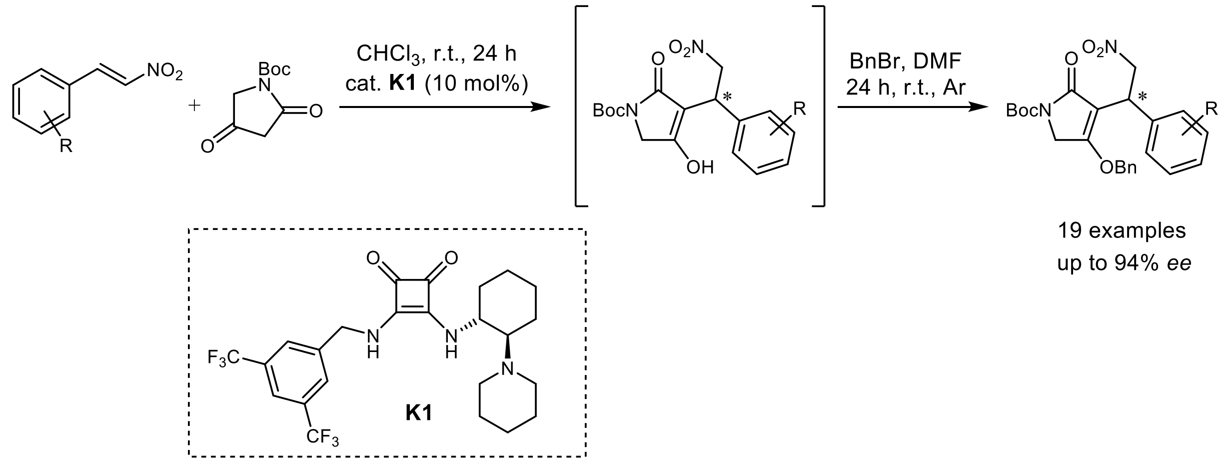
Asymmetric synthesis is as important as it has ever been in synthetic chemistry. To perform enantioselective reactions, chemists usually turn to catalysts. Chiral bifunctional organocatalysts are an example of organic catalysts. They work by bonding to nucleophiles and electrophiles through hydrogen bonds and simultaneously activate them[1]. In this work, squaramide based organocatalysts were used to stereoselectively perform Michael additions using *N*-Boc protected tetramic acid and different *trans*-β-nitrostyrene substrates (*Fig. 1*). Tetramic acids were prepared according to literature[2]. The initial products were alkylated with benzyl bromide and subsequently enantiomeric excess was determined. All reactions were performed using an achiral and chiral catalyst, the latter was chosen based on previous results[3].



19 examples
up to 94% *ee*

***Fig. 1**: Organocatalyzed functionalization of N-Boc-tetramic acid.*

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Analysis of *JAK2* variants associated with erythrocytosis

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Erythrocytosis is a condition with an increased mass of erythrocytes. Congenital erythrocytosis has a diverse genetic background, however, the role of *JAK2* gene in its development is still poorly understood[1]. Several new *JAK2* variants with unknown pathogenicity were discovered in a preliminary NGS analysis[2]. The aim of the present study was to overview and identify regions of *JAK2* gene potentially associated with erythrocytosis and to analyse their genetic variability in selected cohort of patients.

We reviewed potentially causal *JAK2* variants for erythrocytosis development in PubMed database. The data from Ensembl browser was used to allocate those variants on specific gene regions (Fig. 1). Sanger sequencing analysis of selected regions (exons 12, 13, 14, 19 and 24) was established for the subsequent clinical research. We aspire to find additional new variants and confirm the previously described variants on selected cohort of patients.

There still are variants of the *JAK2* gene, which show considerable potential for further functional analysis based on their location in previously reported regulatory regions. With the ongoing research we strive to validate some new crucial ones involved in eryhtrocytosis development.

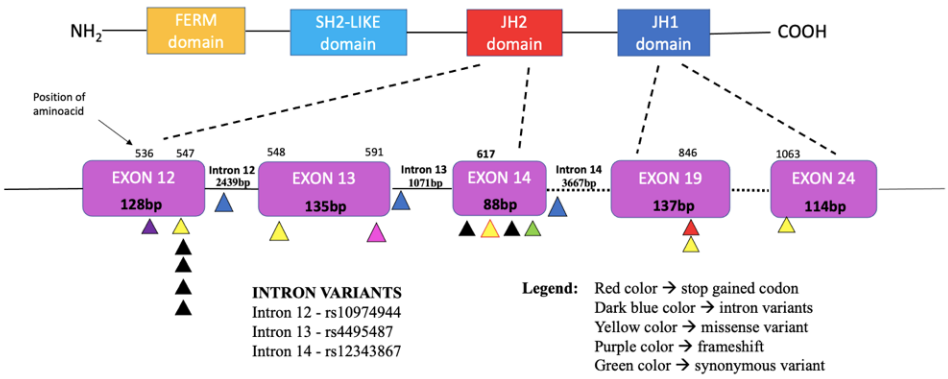

Fig. 1: *JAK2 variants reported to be involved in erythrocytosis development.*

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Characterization of bacteriophage production in continuous stirred tank system

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Bacteriophages are viruses that infect bacteria. Phages use bacterial metabolism for production of new progeny viruses for further infection[1]. Production rate of bacteriophages is phage fitness (λ) and incorporates three growth parameters burst size, latent period and adsorption rate[2]. Phage development depends on metabolic activity of their host, therefore efficacy of phages used for bacterial infection treatment differs between physiological states of bacteria[3]. In our experiments we applied selective pressure on phages with dilution rate changes in a continuous system bioreactor to produce bacteriophage subpopulations with higher fitness parameters. For this study a model bacteriophage T4 and bacteria *E.coli* were used. Growth parameters of individual subpopulation of phages were determined in one step growth experiments using bacteria in controlled physiological states. Our results indicated that burst size and latent period parameters differed between phage subpopulations produced at various dilution rates.

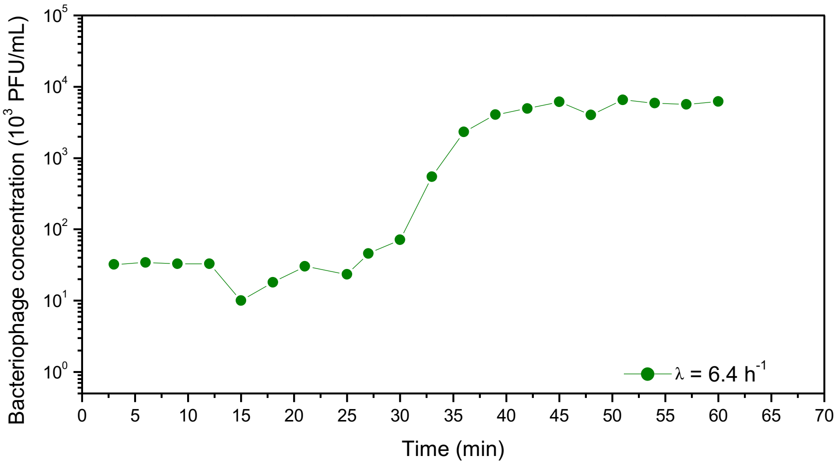

Fig. 1: *Bacteriophage concentration as a function of time after infection of bacteria with phage subpopulation produced at dilution rate of 6,4 h^{-1} in one step growth experiment.*

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Analysis of oligomeric state and enzymatic activity of human ISOC2

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Enzymes from the isochorismatase-like (IHL) protein superfamily mostly catalyse the breaking of C–N or C–O bonds but recognise diverse substrates. They are homo-oligomeric proteins with different numbers of subunits, depending on their identity[1]. Isochorismatase domain-containing protein 2 (ISOC2) is an IHL superfamily protein found in vertebrates including humans. The function of the protein has not been elucidated, but it is known to interact with the tumour suppressor p16[2]. Our goal was to determine the oligomeric state of human ISOC2 and uncover its putative enzymatic activity. Since the structure of ISOC2 is unknown, we first generated homology models of tetrameric and dimeric ISOC2 structures. Next, we docked the human metabolome library to the model structures and discovered that nucleotides fit well into the presumed active site of the protein (Fig. 1). We performed a cross-linking experiment with recombinant ISOC2 and showed that the protein is mainly a dimer, but a small amount of tetrameric ISOC2 was also observed. We also performed basic enzymatic characterization of the protein. Our experiments showed that the protein has no esterase or nuclease activity. In addition, an electrophoretic mobility shift assay showed that the protein does not interact with deoxyribonucleic acids.

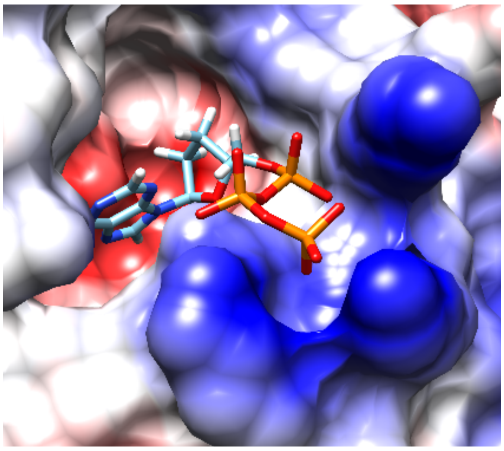

Fig. 10: *The structure of human ISOC2 with docked 2',3'-dideoxyadenosine 5'-triphosphate.*

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Reactions of pyrazolo[1,2-*a*]pyrazolones under visible light

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Pyrazolo[1,2-*a*]pyrazolones are heterocyclic compounds, that exhibit strong fluorescence. Upon irradiation, they reach an excited state that is sufficiently long lived for a photoinduced electron transfer to occur[1]. We were able to exploit this property to achieve multiple selective oxidation reactions involving pyrazolo[1,2-*a*]pyrazolones by diligent optimization of reaction conditions. In the presence of an electron acceptor, such as diethyl bromomalonate, oxidation of substrates to pyrazoles takes place, which is accompanied by ring opening. In the presence of base or nucleophilic reagents, products with varied functional groups can be synthesized, including carboxylic acids, amides, esters and N1-acryloyl-substituted pyrazoles (Fig. 1)[1]. Morever, in the absence of an electron acceptor, aldehydes were formed upon irradiation. Meticulous research into reaction mechanism revealed, that homolytic C–N bond cleavage took place, followed by intramolecular 1,5 hydrogen atom transfer. Our methods provide a sufficient and simple route for the synthesis of richly substituted pyrazoles. The photochemical reactions take place at room temperature without an external photocatalyst such as iridium(III) and ruthenium(II) complexes[2]. Such methods are more cost-effective and environmentally friendly in comparison to the reactions that require the presence of a catalyst[2].

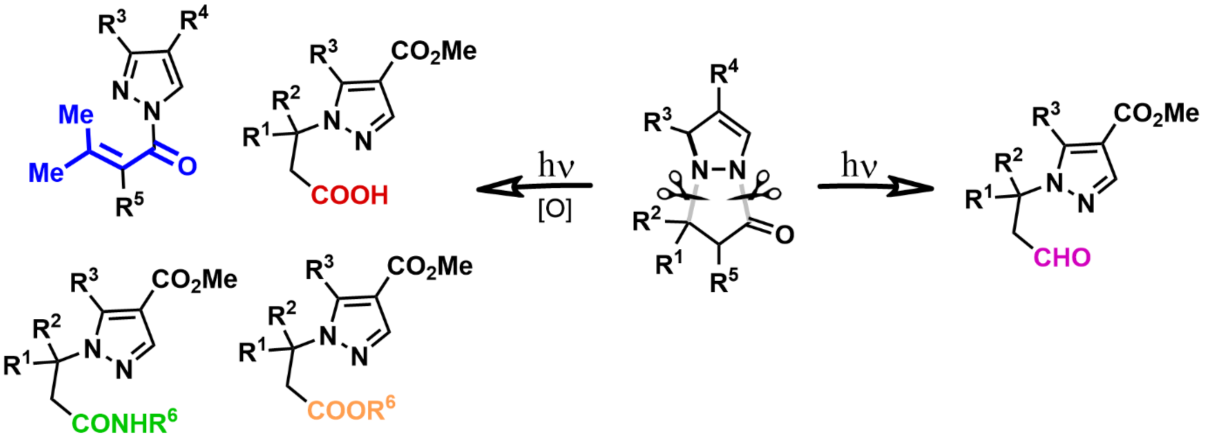

Fig. 1: *Pyrazoles, synthesized by irradiation of pyrazolo[1,2-a]pyrazolones in the presence of suitable reagents[1].*

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Degradation of different types of plastic with extremophilic fungi isolated from household appliances

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Biodegradation is a promising solution to reduce plastic waste with minimal negative environmental impacts. It involves the use of microorganisms to use plastic as their carbon source[1]. Fungi, for example are known as such natural decomposers. The degradation of natural and synthetic polymers by fungi happens due to physical pressure exerted from the hyphae and enzymatic activity of oxidoreductases and hydrolases[2]. We used fifty-nine strains of fungi isolated from plastic parts of washing machines and dishwashers. Exposure to several extreme conditions in household appliances leads to the polyextremotolerant fungi development[3]. We screened the selected fungi for their ability to use five selected plastics as a sole carbon source; low density polyethylene, polyamide, polyethylene terephthalate, polypropylene and polyurethane. We identified 43 new species that grew on at least one type of plastic and had not previously been shown to degrade plastic. According to previous studies on fungal degradation of plastic, only a few strains are able to degrade polyamide and polypropylene. We observed 44 strains colonizing polyamide and 43 strains colonizing polypropylene.

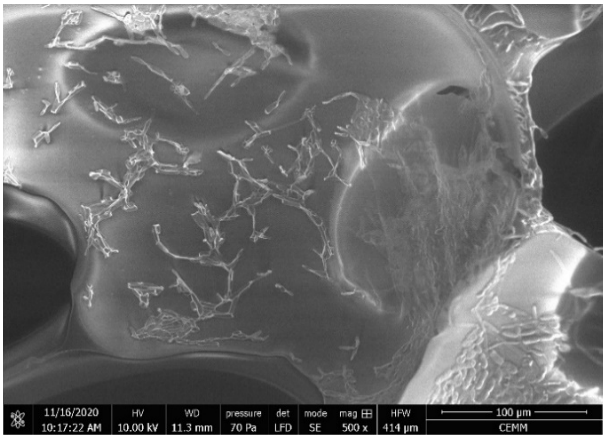

***Fig. 1**: SEM micrograph of fungi Trichosporon ovoides colonizing low density polyethylene.*

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Ozonation of model wastewater containing antibiotic Vancomycin

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Vancomycin (Fig.1) is a bactericidal antibiotic, used for treatment of serious staphylococcal infections, when penicillins cannot be used. It can also be used for other gam-positive and gram-negative cocci bacterias. Vancomycin is relatively nontoxic with predominant toxic neurotoxicity[1].

Ozone is used as oxidant in wastewater treatment. Two paths of its action are known: direct molecular ozone reactions and indirect pathway leading to ozone decomposition and the generation of hydroxyl radicals ($OH^{\bullet}$). Reactions of ozone with organic matter usually led to the formation of aldehydes and carboxylic acids – none of them reacts with ozone, therefore this is the limitation, and the total mineralization of organic pollutant cannot be achived[2].

Oxidative reactions with ozone are slow and selective method. Ozone decomposition in water is strongly pH dependent and with increasing of pH, it's faster. Reaction of formed OH• radicals with organic and inorganic molecules are fast and non-selective. Catalytic ozonation allows for the effective formation of hydroxyl radicals also at a low pH[2].

The aim of my work was to perform ozonations of model wastewater containing Vancomycin at ambient temperature. Different ozonations were accomplished (nonmodified pH, higher pH (9.5), addition of Fe^{2+} ions, UV light, addition of H_2O_2). Two different analytical methods were applied to follow the oxidation processes – TOC (Total Organic Carbon) and COD (Chemical Oxygen Demand) values. Obtained results depend upon the method used.

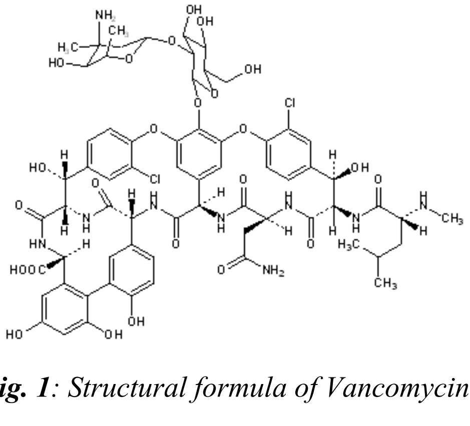

***Fig. 1**: Structural formula of Vancomycin[3].*

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Design of novel constructs for expression of recombinant EGFR ectodomain in mammalian cell lines

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The epidermal growth factor receptor (EGFR) is a tyrosine kinase that is involved in the proliferation of cells as well as in cancer development. EGFR belongs to the ErbB family, it has an extracellular domain, a hydrophobic transmembrane domain and a cytoplasmic tyrosine kinase domain. Signalization is typical for these transmembrane protein. EGFR initiates signaling cascade through ligand binding and undergoes a transition from inactive monomer to an active homodimer[1].

Because the extracellular domain of EGFR is heavily glycosylated (Fig. 1) and has a lot of other post-translational modifications that directly modulate the signaling and trafficking of the EGFR, we wanted to express it in mammalian cell line CHO and HEK with flip-in system. It was found that signal peptides and WPRE exceptionally increase the yield of recombinant protein. We prepared vector pcDNA/FRT/TO with signal peptide albumin and element WPRE in 3' UTR region. These constructs were used generated stable cell lines and determine the recombinant protein expression level by western blot[2]. Our results provide a starting point for large scale expression of EGFR in mammalian cells, which will help to clarify the impact of posttranslational modifications on EGFR's function.

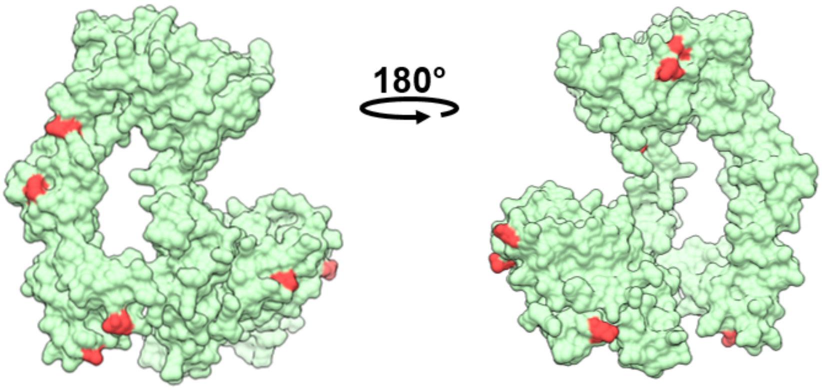

Fig. 1: *Structure of EGFR extracellular part. N-linked glycosylation sites are shown with red.*

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Pizza-helix protein cage: synthetic self-assembly protein

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Designer proteins are novel or modified existing proteins that can have new structures, functions, properties, and purposes. By creating such proteins, we can better understand existing proteins and how to build new proteins with desired properties in the future. In this study, we designed a novel self-assembly protein. In a monomer, we combined one-third of the synthetic beta-propeller pizza protein (Pizza2) and a pair of synthetic α-helix bundles, which dimerizes by cobalt coordination[1,2]. In the hexamer form, designed protein will most likely form a protein cage in the shape of a triangular prism, with the pizza protein as the base (Fig. 1).

The synthetic gene of Pizza2 and the helices were cloned into the vector pET-22b CPD (MATRIX toxin cysteine protease domain) from *Vibrio cholerae* and His6 tag, using a seamless cloning technique called IVA (*In Vivo* Assembly) cloning[3]. Upon addition of $InsP_6$ reagent, CPD becomes a self-cleaving protease[4]. We isolated our synthetic protein via CPD and His-Trap HP purification columns.

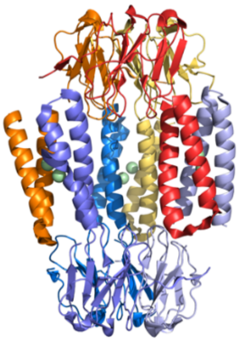

Fig. 1: *Model of Pizza2 and the helices in hexamer assembly forming the shape of a triangular prism.*

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Modelling of pilot plant recarbonization fluidized bed reactor

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Drinking water quality has direct importance for human health. Indicators of drinking water quality also include calcium and magnesium. If the level of Ca^{2+} and Mg^{2+} in drinking water is low, their content has to be increased technologically.

The paper presents some results achieved by the participation of the researchers from the Institute of Chemical and Environmental Engineering in the solution of the LIFE[1] project. The aim of the work was to examine the feasibility of a recarbonization process in a continues-flow fluidized-bed reactor. The improvement of the quality of drinking water was performed by half-calcined dolomite (Magno Dol) and CO_2. The influence of treated water and CO_2 velocity flows on calcium and magnesium concentration, their sum and molar ratio was study. A very good agreement between the experimental and calculated values of dependent variables was achieved with a multiple quadratic regression model with interrelationships between the independent variables. Based on a determination coefficient values it can be concluded that experimental dependent variable values were by 87 - 99% influenced by the CO_2 and treated water velocity flows. From optimization of regression models within boundaries of experimental data the maximum values of the dependent variables correspond CO_2 flow rate 0.4 $l \cdot min^{-1}$ and treated water rate 118 $l \cdot h^{-1}$. Optimal maximum values of dependent variables summarized in Tab.1. Another aim of the work was to compare performance of recarbonization fluidized bed reactor with different half-calcined dolomites, i.e., Magno Dol and Semidol with similar chemical and physical properties. The criterion for comparison was the increasing Mg^{2+} by 10 $mg \cdot l^{-1}$ in an annual treated water flow of 11 000 m^3. The annual consumption of Magno Dol was lower by 5% but the annual consumption of CO_2 was higher by 27% compared to the use of Semidol. At the same time, a higher amount of Ca^{2+} by 38% will be released from Magno Dol.

***Tab. 6**: Maximum values of parameters calculated by the regression model.*

$c\ (Ca^{2+}+Mg^{2+})$ $[mmol \cdot l^{-1}]$	$c_M\ (Mg^{2+})$ $[mg \cdot l^{-1}]$	$c_M\ (Ca^{2+})$ $[mg \cdot l^{-1}]$	$c\ (Ca^{2+})\ /c\ (Mg^{2+})$
7.6	104.5	141.5	1.1

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Wastewater treatment from the pulp and paper industry by adsorption

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Wastewater from the pulp and paper industry is one of the most heavily polluted wastewater[1]. Contamination of water with dyes causes not only their significant coloration, but also inhibitory effects on activated sludge microorganisms and the aquatic environment. It is not only a threat to the environment, but can cause chronic diseases, insomnia, cancer, diarrhea, nausea, vomiting, chronic asthma, headaches and many more[2].

Two model solutions of contained dyes (Direct Blue 290, Direct Yellow 132) and two samples of the real wastewater (WP, WB) were studied[3]. The WP wastewater was sampled at the outlet of the paper machine and the WB wastewater was sampled at the inflow into the biological stage of the industrial wastewater (WW) treatment plant. The adsorption process was performed by using Mistron®75-6 KA adsorbent to reduce of dye concentration from WW samples. The results of the work show high adsorption efficiency for the model dye, while for real wastewater there was observed lower efficiency of color elimination. The lower efficiencies for real wastewater samples may be due to the fact that these samples also contain cellulose residues, resins. The best fit of experimental data was achieved by the Freundlich isotherm according to the correlation coefficient values. The values of the adsorption isotherms parameter were determined by nonlinear regression analysis[4].

The work was also focused on the study on immediate biodegradability of studied dyes. Solution of Direct Blue 290 showed readily biodegradability after the adsorption process, while the solution of Direct Yellow 132 has an inhibitory effect on the respirometric rate of microorganisms. Adsorption reduced the concentration of inhibitory substances of the WP sample and eliminated the inhibition effect on the respirometric rate of microorganisms. The sample of WB showed an inhibitory effect on the respirometric rate of microorganisms at higher concentrations.

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Does PES and PET microplastics affect biogas production of anaerobic sludge?

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Microplastics (MP) is an insoluble, solid particle, found everywhere in the environment[1]. One of the important sources of MPs are also Wastewater Treatment Plants (WWTPs), where after treating, more than 90% of MP could be retained in the sludge[2]. Waste sludge can be due to its properties and the amount of useful substances used for several purposes, also for production of biogas. Anaerobic digestion of the excess sludge is one of the most common processes of its utilization and helps to reduce its volume and operating costs of WWTP[3].

To evaluate the impact of MPs on the anaerobic process, the production of biogas of anaerobic sludge with different added concentrations and types of MP was monitored. The inhibition of anaerobic microorganisms was determined by modified standard procedure (SIST EN USO 11734:1999), OxiTop® method, where biogas production was measured in terms of increased pressure (hPa). As shown in Fig. 1, presence of PET decreased biogas production at all investigated concentrations from 0.005 g L^{-1} to 2.38 g L^{-1}, while PES promoted CH_4 production at concentrations from 0.005-0.5 g L^{-1}, while at higher concentrations, 1-2.38 g L^{-1}, inhibition of CH_4 yield was shown.

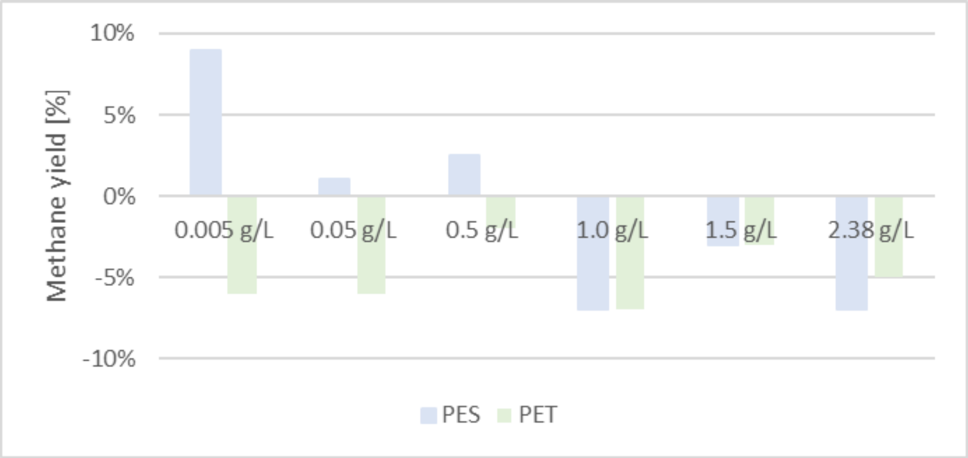

Fig. 1: *CH_4 yield at investigated concentrations of PET and PES.*

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Ceramic upside-down composites for energy-storage applications

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In attempt to meet the needs and expectations of global society and environmental standards, novel technologies for production of dielectric ceramics with improved functional properties have drawn interest in recent years. Room-temperature fabrication (RTF) is a promising alternative to time- and energy-consuming high-temperature sintering of electroceramics[1,2]. The process is based on the incorporation of high loading of functional ceramic filler and a small amount of binder. A part of the binder is admixed to the ceramic particles and another part is added as a saturated aqueous solution, which crystallizes during pressing and drying. In our study, bulk ceramic upside-down lithium molybdate-strontium titanate (LMO-ST) composites were fabricated at room temperature[3]. As the LMO binder is deposited on the surface of ST filler particles, the action of densification takes place. By virtue of that, a sufficient binding with 76 to 84% relative density was achieved. Furthermore, RTF has been investigated under different processing aspects. Its optimization resulted in improved functional properties including relative permittivity in the range of 65-78 and dielectric loss tangent values from 0.002-0.05 in the low frequency measurement range (1 MHz). RTF entails almost infinite possibilities of combining different materials and designing new composites with wide applicative value in the electronics industry.

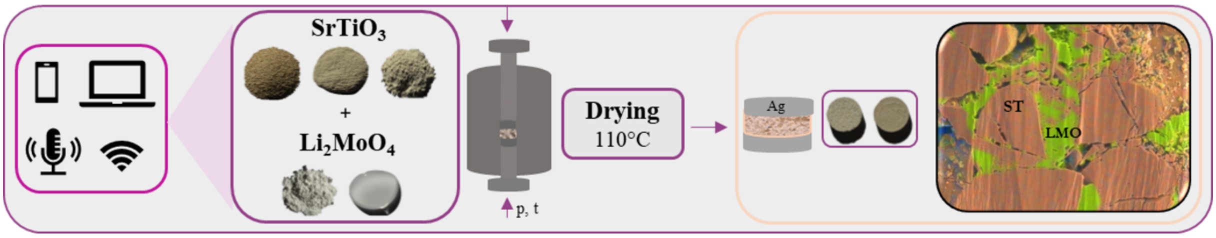

Fig. 11: *The processing scheme of upside-down LMO-ST composites.*

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Zinc(II) coordination compounds with the *in situ* formed amidines

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The importance of amidines in the synthesis of heterocyclic compounds[1] and our aim to explore the structural diversity of zinc(II) coordination compounds were the main motives for studying zinc(II) chemistry with quinaldinate and secondary cyclic amines. Quinaldinate (abbreviated as quin$^-$) is the anion of quinoline-2-carboxylic acid and it was used because it preferentially binds in a bidentate chelating manner. A control over the reaction outcome can be thereby imposed. However, our previous study has shown that reactions with selected secondary cyclic amines (piperidine and pyrrolidine) in acetonitrile led to different products: zinc(II) quinaldinate complexes with amines, amidines and also $[Zn(quin)_3]^-$ compounds with protonated amines or amidines as countercations[2]. In continuation of this study, propionitrile and benzonitrile were used instead of acetonitrile to investigate whether the same types of products formed with different nitriles. Using pyrrolidine and benzonitrile, not only the coordinated amidine species, $[Zn(quin)_2(pyrrolidinobenzoamidine)]$, and the compound with protonated amidine, pyrrolidinobenzoamidineH$[Zn(quin)_3]$, but also a cocrystal containing both were obtained. The ORTEP drawing of pyrrolidinobenzoamidineH$[Zn(quin)_3]\cdot[Zn(quin)_2(pyrrolidinobenzoamidine)]$ is shown in Fig. 1.

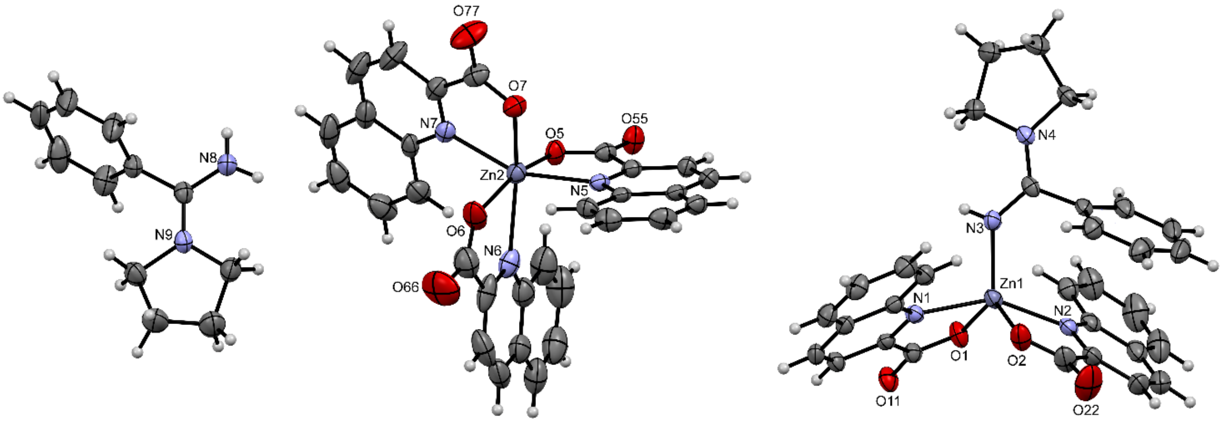

Fig. 1: *ORTEP drawing of pyrrolidinobenzoamidineH*$[Zn(quin)_3]\cdot[Zn(quin)_2(pyrrolidinobenzoamidine)]$*. Displacement ellipsoids are drawn at the 50% probability level. Hydrogen atoms are shown as spheres of arbitrary radii.*

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Evolutionary analysis of dipeptidyl peptidase I

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Human dipeptidyl peptidase I (DPPI), also known as cathepsin C, belongs to the family of papain-like cysteine proteases. Its characteristic features are the exclusion domain, which enables its exopeptidase activity as well as tetramerization[1], and its dependence on chloride ions for enzymatic activity. The evolution of DPPI most likely began with the addition of the exclusion domain to form a monomeric exopeptidase. A functional monomeric form reminiscent of this putative ancestor was described in *Plasmodium*[2]. The aim of this work was to investigate the evolution of DPPI with special focus on structural features that enabled its oligomerization. By examining subunit interfaces, we first determined amino acid residues that form hydrogen bonds between subunits. We then analysed 41 sequences of DPPI and its homologs. The amino acid residues that bind the chloride ion are highly conserved in all species, leading us to conclude that the binding of the chloride ion is an evolutionarily conserved feature of DPPI. After constructing the phylogenetic tree, building model structures of DPPI of organisms at key nodes in the tree, and analysing the hydrogen bonding between subunits, we concluded that oligomerization most likely evolved with the emergence of vertebrates.

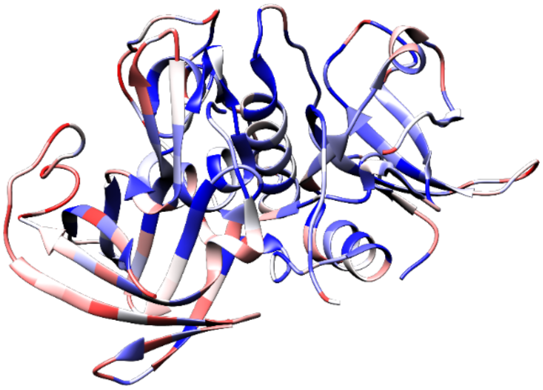

Fig. 2: *Structure of DPPI monomer colored by the conservation of amino acid residues across all analyzed sequences: blue-most, red-least (PDB access code:1K3B).*

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Assessment of lithium-ion batteries fire safety

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A lithium-ion battery is a type of rechargeable battery commonly used as a power source in portable electronics and electric vehicles[1]. Fire safety analysis of lithium-ion batteries is justified by accidents of electrical vehicles in recent years. The continuation of this work provides an overview of safety analysis and simulations performed to illustrate failure mechanisms, highlighting the hazards of ignition, and associated accidental events. For this purpose, we designed and manufactured a puncture testing device for lithium-ion batteries, which identified the vulnerability of batteries to mechanical abuse and a high level of fire risk on 25 commercial lithium-ion batteries. With that in mind we conclude that lithium-ion batteries pose a unique ignition source which can lead to complex fire scenarios in unprecedented manner. In terms of market trends, safety is still seen as one of the main constraints to find acceptable trade-offs in the overall efficiency and cost of lithium-ion batteries in the future[2].

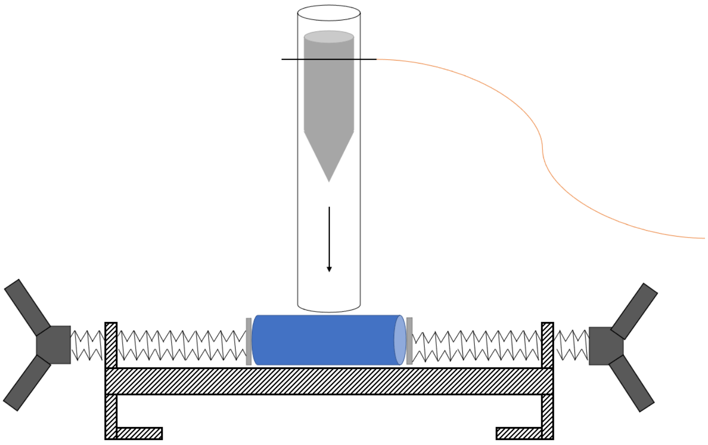

Fig. 12. *The puncture test experiment Fig.[2].*

Tab. 1: *Results of the experiment.*

Number of tested batteries	Fire/explosion	No fire/explosion
25	15	10

1. Scrosati, B., History of lithium batteries. *J. Solid State Electrochem.* **2011**, *15*, 1623–1630.
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An automated high-throughput screening platform to optimize gene expression in β-carotene biosynthesis

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A central challenge in the field of metabolic engineering is the efficient identification of a metabolic pathway genotype that maximizes biosynthesis of valuable molecules. Since the traditional way to tackle this problem by sequential optimization turns out to be time-consuming and expensive[1], our group developed an automated high-throughput screening platform to optimize gene expression in a biosynthetic pathway based on a combinatorial construction of plasmids. We focused on a gene regulation in the biosynthesis of β-carotene, which is used industrially in the manufacture of pharmaceuticals, nutraceuticals, animal feed additives, functional cosmetics, and as colorant in foods[2,3]. Here we present the workflow used in our automated platform and the results of β-carotene production in the acquired *Escherichia coli* strains based on a combinatorial plasmid library (Fig. 1).

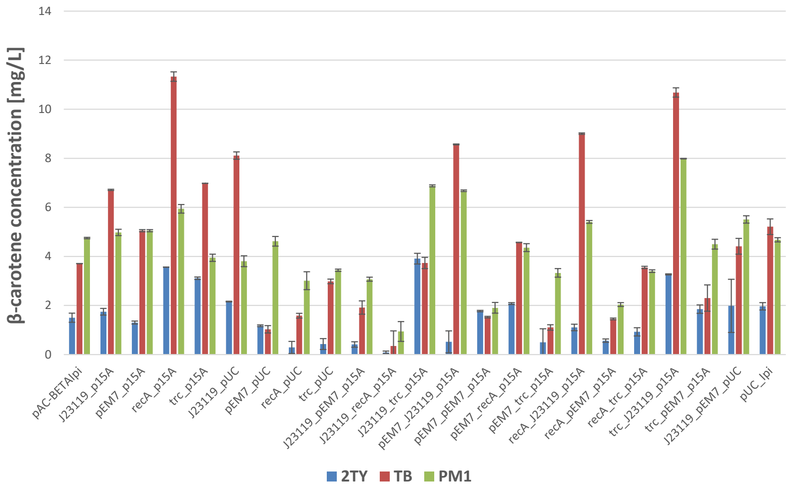

***Fig. 1**: **β-carotene yields in different E. coli strains.** The Fig. shows the graph of β-carotene content in the acquired E. coli strains in three different growth medium after 42 hours of cultivation.*

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Introduction to the sustainable bioplastic materials

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Sustainability is known as "the development that meets the needs of the present without compromising the ability of future generations to meet their own needs" according to the Word Commission[1]. With the fashion industry being the second largest polluter of the environment, radical changes need to be done in its environmental, ethical and social aspect in order to leave our planet with same for our descendants. Synthetic fibers are most often seen as most harmful due to their use of non-renewable oil source and their insufficient biodegradable properties. Commonly used natural materials are also harmful due to a large amount of water and chemicals used in order to grow the plant for the fiber. Environmental and health issues can be also a consequence of fabric pigments and post-treatments[2]. Based on these known facts new bioplastic materials for clothes that could potentially replace harmful animal and faux leather were produced and tested. Different polysaccharides (corn starch, tapioca starch, agar) were used in order to find the best composition to be further used as a clothing material (Fig. 1). Additional functionalities were added as natural additives to the bioplastic to improve its mechanical as well as water repellent properties. A very important feature of these new bioplastic materials is their biodegradability, which is shown for 3 selected bioplastic compositions in Fig. 2.

***Fig. 1**: Visual presentation of bioplastic material samples.*

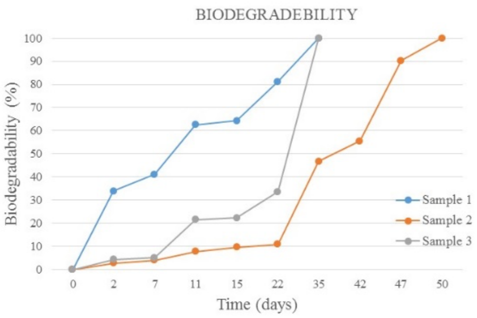

***Fig. 2**: Biodegradability selected formulations of bioplastic materials.*

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Effects of nitrogen and oxygen cold plasma pretreatment on germination of buckwheat seeds

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With further development of technologies in agriculture, a novel, cold plasma technology is slowly being introduced in the field. Cold plasma technology could serve as a way for decontamination of foods and seed material. On the other hand, this novel technology has a potential in improving plant germination, growth, development and even crop yield [1–3].

We investigated the effects of cold plasma treatment of common buckwheat seeds (*Fagopyrum esculentum*) on germination rate and germination speed. Seeds were pretreated with oxygen (O_2) and nitrogen (N_2) cold plasma at power of 75 W for 5 and 10 s. The results indicate, that oxygen cold plasma treatments cause lower mean germination rate of seeds. Cold plasma treatment of buckwheat seeds also affects the speed of germination, causing the seeds to germinate slower when comparing to untreated seeds.

Tab.*7: Germination parameters of untreated and plasma treated buckwheat seeds (G – germination rate (%), MR – mean germination rate, MT – mean germination time, T_{50} - the time to reach 50% germination). Values are the means ± SEM of three replications. Different letters represent statistically significant differences ($p < 0{,}05$) between the control group and all cold plasma treatments of buckwheat seeds.*

	G (%)	MR (day $^{-1}$)	MT (day)	T_{50} (day)
Untreated	68,67 ± 5,74 a	0,357 ± 0,02 a	2,82 ± 0,15 c	2,20 ± 0,05 b
Oxygen, 5 s	67,28 ± 4,15 a	0,252 ± 0,02 bc	4,08 ± 0,53 a	2,70 ± 0,40 a
Oxygen, 10 s	61,35 ± 6,44 a	0,238 ± 0,03 c	4,31 ± 0,49 a	2,74 ± 0,20 a
Nitrogen, 5 s	70,55 ± 5,02 ab	0,308 ± 0,03 b	3,31 ± 0,32 bc	2,47 ± 0,19 ab
Nitrogen, 10 s	70,68 ± 6,10 ab	0,289 ± 0,03 bc	3,58 ± 0,40 ab	2,54 ± 0,17 ab

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Effect of zinc salts and pyrithione on cathepsin L activity

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We are living in a time of one of the biggest epidemics of this century and the whole world is working to discover potential cures for COVID-19. So far, quite a few studies have mentioned the involvement of cathepsins in SARS-CoV-2 production. One of these enzymes is cathepsin L, a member of C1 family of cysteine peptidases, which plays an important role in normal cellular protein degradation and turnover, antigen processing and apoptosis[1]. Metal complex zinc pyrithione (Fig. 1b) has been long known for its antimicrobial properties and it is a common component in commercially available anti-dandruff shampoos. Many researchers have also reported its antitumor and antidiabetic activity[2]. However, little is known about its effect on cathepsin L or any other enzyme from the cathepsin family. On the other hand, zinc ions are known to inhibit cathepsin B, which is also cysteine protease[3].

Therefore, we decided to examine the effect of zinc salts along with pyrithione (Fig. 1a) on cathepsin L activity. First we checked the stability of tested compounds in reaction buffer by UV-Vis spectroscopy and then preformed the enzymatic assays by luminescence spectroscopy using fluorogenic substrate. From obtained data we determined EC_{50} values for each component. Used zinc salts are inhibiting cathepsin L in the micromolar range and the addition of pyrithione augments the inhibitory effect. We believe that such knowledge would be useful in designing experiments and would speed up the search for potential inhibitors of cathepsin L.

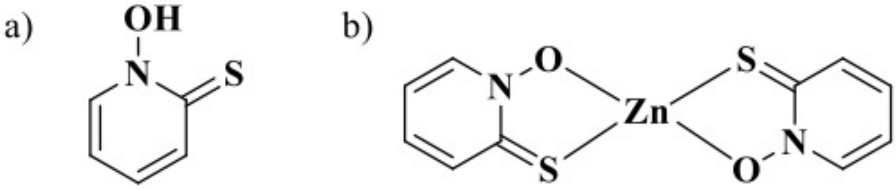

***Fig. 1**: Chemical structure of a) pyrithione and b) its zinc complex.*

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Genome editing of *Brassica* protoplasts using CRISPR/Cas9

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Genome editing technologies have evolved into powerful genetic tools for accelerating plant breeding. CRISPR-Cas is the preferred genome editing tool because it is both versatile and easy to design, requiring only the exchange of sgRNA to achieve a new target specificity[1].

In this study, protoplast transformations were performed on different *Brassica* species *(Brassica napus* L., *Brassica rapa* L., *Brasica oleracea* L. var. *capitata*, *Brasica oleracea* L. var. *botrytis*) by introducing CRISPR/Cas9 ribonucleoprotein complexes (RNPs) or plasmids into protoplasts with PEG, targeting two endogenous genes *FRI* and *PDS*. The FRIGIDA gene (*FRI*) is the vernalization determinant gene associated with the temperature flowering response and has been previously targeted in *Brassica* protoplasts[2]. Phytoene desaturase gene (*PDS)* is the most commonly used model gene, as mutations in *PDS* gene lead to an albino phenotype in plants[3]. There are many reports on PEG-mediated protoplast transformation of DNA, but there are only few reports on PEG-mediated protoplast transformation with RNPs[2, 4]. PEG -mediated transformation was performed in 96-well plates, where we tested the efficacy of different plasmids and RNP complexes. The results showed differences between transformation of protoplasts with plasmids or ribonucleoprotein complexes and there were also differences between transformation rates of *Brassica* species.

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Experimental extraction of 1*H*-benzotriazole from soil using hot water

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Benzotriazoles are heterocycles widely used in households and various industries, mainly as UV stabilizers and corrosion inhibitors. Since 2008 they are classified as emerging organic pollutants due to their toxicity to plants and aquatic organisms, endocrine disrupting properties, potential carcinogenicity and, for certain compounds, their bioaccumulation properties. Due to growing anthropogenic activities and global pollution, an interest in more environmentally friendly analytical methods operating on a smaller scale or with non-toxic solvents has emerged[1, 2, 3].

In my work, I focused mainly on the extraction of 1*H*-benzotriazole from soil, using hot water as the greenest solvent there is. Due to a lack of research using mentioned method on this analyte, I conducted experiments with the aim of optimizing the extraction parameters. After the experiment, additional cleaning with solid phase extraction was used (shown in **Fig. 1**). For analyte quantification, HPLC/DAD method, which was optimized beforehand, was used.

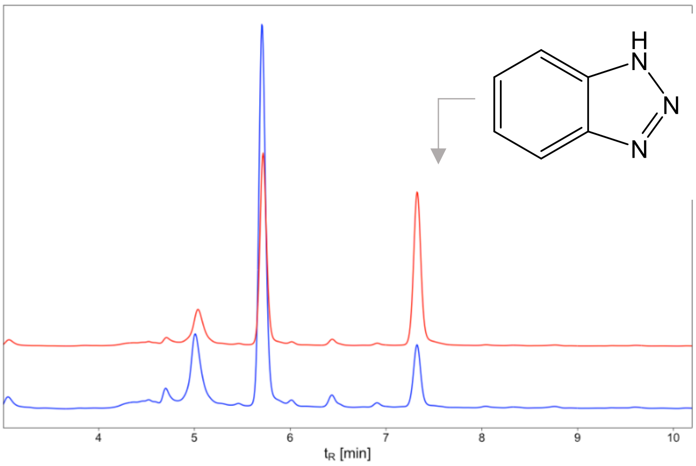

***Fig. 1**: Chromatogram of extract after hot water extraction (blue) and after an additional solid phase extraction (red) with structure of 1H-benzotriazole (t_R= 7.2 min).*

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TiO_2 and Ru/TiO_2 clusters as an attractive photocatalysts for N_2 fixation: a theoretical study

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Light-driven nitrogen fixation as a promising alternative to the conventional Haber-Bosch process makes it possible to carry out the nitrogen reduction reaction (NRR) under mild conditions. Titanium dioxide (TiO_2) exhibits alluring properties for photocatalytic N_2 fixation, especially when its structure is altered by doping[1]. Density functional theory calculations, being the predominant way of modelling at the atomistic level to study the structures, electronic and optical properties of materials, were used to study the clusters and their activity for nitrogen adsorption and the mechanism of NRR[2,3].

Density functional theory (DFT) calculations were conducted to obtain the electronic and optical properties of different TiO_2 clusters, $(TiO_2)_n$, n=1-12. We investigated all stable clusters of a particular size and identified the most stable one. To improve its catalytic properties and to fixate the nitrogen molecule more effectively, a ruthenium (Ru) atom can be loaded on the TiO_2 clusters (Ru/TiO_2). The corresponding changes in properties and adsorption modes of nitrogen and hydrogen were calculated. Moreover, ab initio molecular dynamic (AIMD) simulations were performed to probe the stability of TiO_2 clusters. The calculations were carried out using the GPAW software with the PAW approach[4] in the plane wave regime. While a GGA functional (PBE) sufficed to obtain the catalyst structure and adsorptions, a hybrid functional was required for a more accurate determination of the electronic properties[3].

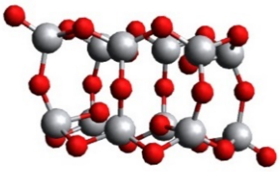

***Fig. 1**: Optimized structure of $(TiO_2)_{12}$ cluster.*

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Synthesis of a library of 1,2,4,5-tetraoxanes for their potential herbicidal activity

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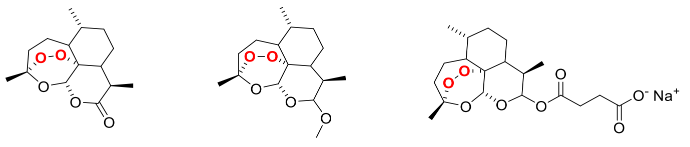
One of the strategies for development of new herbicides is using isolated natural compounds as a model for discovering biologically active compounds[1]. Studies show that artemisinin (compound isolated from the plant *Artemisia annua L.)* and its derivatives inhibit plants' growth (Fig. 1)[2]. The potency depends on the number of peroxide bonds in the structure[3]. Stemming from the peroxide bond being the culprit for the phytotoxicity of artemisinin, 1,2,4,5-tetraoxanes (TO) are potential herbicidal candidates. 1,2,4,5-tetraoxanes' structure has a cyclohexane ring containing two peroxide bonds (Fig 2). In addition to their antimalarial activity, the main field for TO research, new studies emerge that raise the possibility of utilizing 1,2,4,5-tetraoxanes as a new class of herbicides.[3] Functionalization of the side rings enables bonding of compounds with different mechanism for unwanted plants' growth inhibition.

We are going to present a study on the synthesis of a library of 1,2,4,5-tetraoxanes by direct synthesis from carbonyl compounds and hydrogen peroxide and modification of their side chains with the aim to further investigate their potential herbicidal activity.



Artemisinin (ART)   Artemeter   Artesunat

Fig. 1*: Molecular structure of artemisinin and its derivatives.*

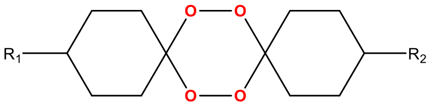


Fig. 2*: Basic structure of 1,2,4,5-tetraoxanes.*

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The impact of ozone dose on degradation of antibiotic amoxicillin

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In recent years antibiotics are considered to be a significant group of pharmaceuticals which cause a serious hazard to the environment and human health[1]. Conventional wastewater treatment plants are unable to remove majority of antibiotics from wastewater. Because of their hydrophobic and lipophilic nature, they are recalcitrant to degradation and cannot be completely removed from the environment. Consequently, they contribute to their presence in drinking water and water bodies[2]. A promising way for this degradation is the application of advanced oxidation processes such as UV radiation, ozonation, H_2O_2, Fenton reactions and various combinations of them[3].

The aim of this study was to determine the optimal ozone dose for the degradation of amoxicillin. The initial concentrations of amoxicillin were 100, 200, 400 and 800 mg L^{-1}. Experiments were performed in a 300 mL semi-batch reactor for 2 hours at room temperature. The nominal inflow of ozone was 4.2 g h^{-1} and 4.5 g h^{-1}. All experiments were repeated three times. The removal efficiency was determined by total organic carbon (TOC) and chemical oxygen demand (COD) measurements. Results show that most efficient COD removal was achieved at lower concentrations of amoxicillin with lower ozone inflow, whereas by TOC the difference in removal efficiency is negligible (Tab. 1, Tab. 2). We can conclude that further research is needed to determine the optimum operating conditions.

***Tab. 1:** COD removal efficiency [%] for amoxicillin.*

Ozone inflow	100 mg L^{-1}	200 mg L^{-1}	400 mg L^{-1}	800 mg L^{-1}
4.2 g h^{-1}	62.13	53.18	51.89	48.07
4.5 g h^{-1}	52.96	50.38	48.80	43.82

***Tab. 2:** TOC removal efficiency [%] for amoxicillin.*

Ozone inflow	100 mg L^{-1}	200 mg L^{-1}	400 mg L^{-1}	800 mg L^{-1}
4.2 g h^{-1}	26.26	24.77	24.09	21.15
4.5 g h^{-1}	24.05	21.55	22.39	20.30

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Applications of *gem*-dibromoalkenes

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Recently, we have been concerned with the synthesis of aliphatic *gem*-dibromoalkenes. These compounds were prepared from commercially available aliphatic aldehydes as starting materials by the first step of Ramirez-Corey-Fuchs reaction[1,2]. Thus, the combination of tetrabromomethane with triphenylphosphine in dichloromethane gave the corresponding ylide, which then reacted with aldehydes into the desired products, as shown in Fig. 1.

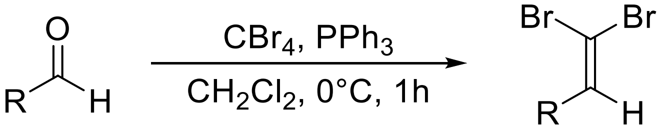

***Fig. 3**: The Ramirez-Corey-Fuchs synthesis of gem-dibromoalkenes.*

Being interested in the applicability of these products, we conducted an extensive literature search and found that they play an important role as precursors for biologically active molecules (e.g., Sanshool derivatives, a class of polyunsaturated fatty acid amides[3]), drugs (e.g., antibiotics α- and β-mycin[4]), reagents in cross-coupling reactions[5] (e.g., Sonogashira reaction), and precursors for thioamides[6], which can be used in biochemical studies, to name a few. Herein, we will also more thoroughly present synthetic paths of thioamides and Sanshool derivatives, and cross-coupling reactions resulting in various 1,3-diynes.

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Optimization of quaternization of cellulose nanofibers reaction

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In recent years, numerous modifications of cationic biopolymers have been developed[1]. The same applies for the cationic functionalization of cellulose, where the modification with epichlorohydrin-ammonia reaction and grafting with quaternary ammonium salts (quaternization of cellulose shown in Fig. 1A) predominate[2], whereby significantly higher reaction efficiencies were achieved by the quaternization of cellulose (epichlorohydrin may enhance the surface area, porosity and hydrophobic character of the hydrogel). Furthermore, the control of the concentration of reactants during this reaction allows the design of the product with desired properties. NaOH catalyzes the conversion of alcohol groups of cellulose into more reactive alcoholate groups[2]. Obviously, an excessive concentration of NaOH inhibits the modification of reactive hydroxyl groups (Fig. 1B). Therefore, the optimal NaOH concentration in the quaternization of cellulose nanofibers was determined at this point, where the OH groups are in the most reactive form. Next, the increased addition of GTMAC to the reaction mixture increases the trimethylammonium chloride content (TMAC) on the surface of cellulose nanofibers (Fig. 1C). The high reaction efficiency compared to other studies can be attributed to the optimized concentration of the NaOH catalyst in the reaction mixture. Additionally, the TMAC concentration significantly affects the interactions between the polymer chains as well as the water molecules during swelling in the aqueous QCNF dispersion, which consequently has an impact on the rheological properties of the product[2]. To conclude, the equation shown in Fig. 1C can be used to easily control the properties of the final product (during synthesis) depending on the desired application.

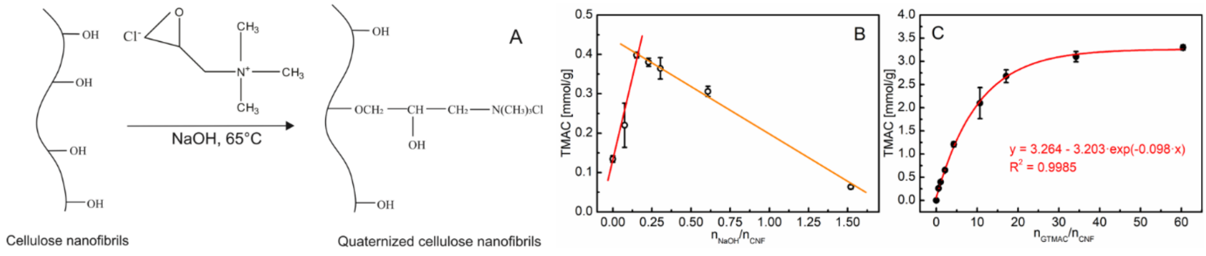

Fig. 1: *Quaternization of cellulose nanofibrils reaction using GTMAC in addition of NaOH catalyst.*

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Design of novel constructs for expression of recombinant EpCAM exodomain in mammalian cell lines

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EpCAM (Epithelial Cell Adhesion Molecule) is a transmembrane glycoprotein recognized as a tumor antigen. It plays a key role in cell proliferation, differentiation and is involved in many signaling pathways. RIP (regulated intramembrane proteolysis) causes degradation of EpCAM (Fig. 1). Initial cleavage results in the soluble extracellular domain (EpEX) which is a ligand for EGFR (Epidermal Growth Factor Receptor)[1]. Previous attempt to characterize the EpEX-EGFR complex was unsuccessful. We hypothesize that post-translational modifications have a crucial role in the interaction between EpEX and EGFR, so we designed 4 different constructs for the expression of EpEX in mammalian cell lines (CHO and HEK), because the glycosylation and other post-translational modifications in insects can differ from the one in mammals. In two of them, we integrated albumin signal peptide instead of native one to improve secretion efficiency[2]. Two constructs include WPRE (Woodchuck hepatitis virus Post-transcriptional Regulatory Element) which enhances transgene export of mRNA from the nucleus to cytoplasm[3]. Our work provides the basis for the expression of larger amounts of EpEX in mammalian cell lines. This will aid to elucidate the effect of post-translational modifications on EpEX-EGFR interaction.

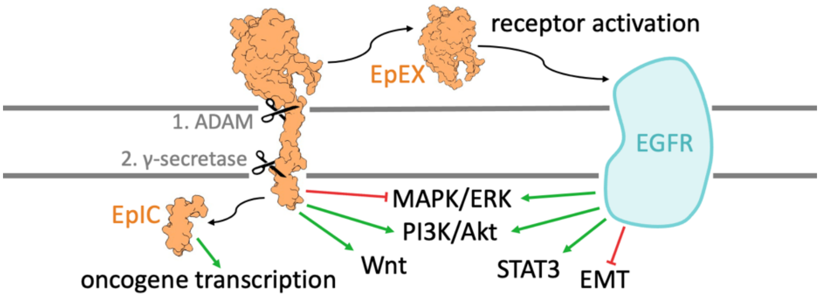

***Fig. 1**: RIP and activation of EGFR. EMT – epithelial-to-mesenchymal transition.*

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Approaches for analysis of non-coding regulatory regions of erythropoietic genes

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Familial erythrocytosis is a rare inherited disease characterized by elevated red blood cell count[1]. Despite sequential analysis of gene coding regions, 70% of patients remain undetermined. In a recent study variants in the *VHL* cryptic exon were detected in seven families with erythrocytosis[2]. Based on these data, we hypothesized that several additional non-coding regions of erythrocytosis-causing genes must be involved in the disease development.

Within our study, we used different databases (NCBI, Ensembl, Encode, UCSC) and tools (MethPrimer, dbSNV search, Human Splicing Finder, and IGV) to analyze the noncoding region of 11 genes associated with erythrocytosis. We reviewed the theoretical background, determined the locations of the noncoding regions and located CpG islands in the promoter and the first intron. The variants identified within the NGS analysis of erythrocytosis patients[1], were systematically reviewed and compared with the results obtained during our study. The set of variants that matched the NGS data was further analyzed using several tools to determine the frequency of the variants in the population and among patients, the coincidence of the location with the location of the cryptic exon and pathogenicity predictions by Combine Annotation Dependent Depletion (CADD) score (Tab.1). As no variant met the pathogenic criteria, we could not confirm any non-coding pathogenic variant in the selected group of patients. The future analysis will be extended to 3'UTR regions.

Tab. 8: *Number of variants identified in genes VHL, SH2B3, and BPGM. The CADD score was determined for each variant; pathogenicity is indicated when the score > 20.*

	VHL	***SH2B3***	***BPGM***
Number of variants	5	1	1
Max CADD	<6	<1	<1

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Suzuki–Miyaura cross-coupling of halotriazoles

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1,2,3-Triazoles are an important class of heterocyclic compounds and have found numerous applications in organic synthesis, transition metal catalysis, medicine, material sciences and supramolecular chemistry[1]. New synthetic pathways towards substituted triazoles are therefore being developed. Recently 4- and 5-halo-1,2,3-triazoles became more synthetically accessible[2]. Functionalization of these substrates could be achieved via cross-coupling reactions.

The Suzuki–Miyaura reaction between aryl halides and boronic acids has proven to be one of the most commonly used palladium-catalyzed cross-coupling reactions. This is due to a wide commercial availability of organoboranes and their stability, tolerance to different functional groups, mild reaction conditions and simple separation of inorganic boron from the reaction mixture[3]. So far most of the reported procedures for coupling halotriazoles and boronic acids have narrow substrate scopes, moderate yields and use relatively high palladium catalyst loadings.[2] Despite these drawbacks Suzuki–Miyaura reaction could become a convenient approach for the preparation of substituted triazoles.

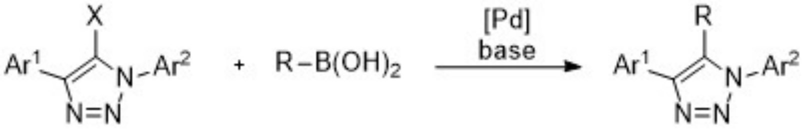

***Fig. 1**: Palladium-catalyzed Suzuki–Miyaura reaction of halotriazole and boronic acid.*

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Synthesis of (+)-isocampholenic acid derived flavors and fragrances

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There is sustained interest with the fragrance industry in developing new fragrance substances in order to enable the creation of new perfume oils. Such substances are used either to replace costly natural materials that have limited supply and high cost or to provide new fragrances of perfumed types, which have not been available. Compounds with a woody odor are indispensable components in perfumery (fragrance) and as food additives (flavor). One particularly valuable class of these woody fragrance substances are compounds with a sandalwood odor. Some synthesized derivatives of campholenic aldehyde such as esters and alcohols with a lengthened side chain have organoleptic properties that could make them useful in fragrance and flavor compositions, especially fragrances of the woody, sandalwood, fruity and floral types[1-3]. On the other side, (+)-isocampholenic acid derived flavors and fragrance have not been researched so fare. Results in the synthesis of (+)-isocampholenic acid derivatives with potential organoleptic properties will be presented.

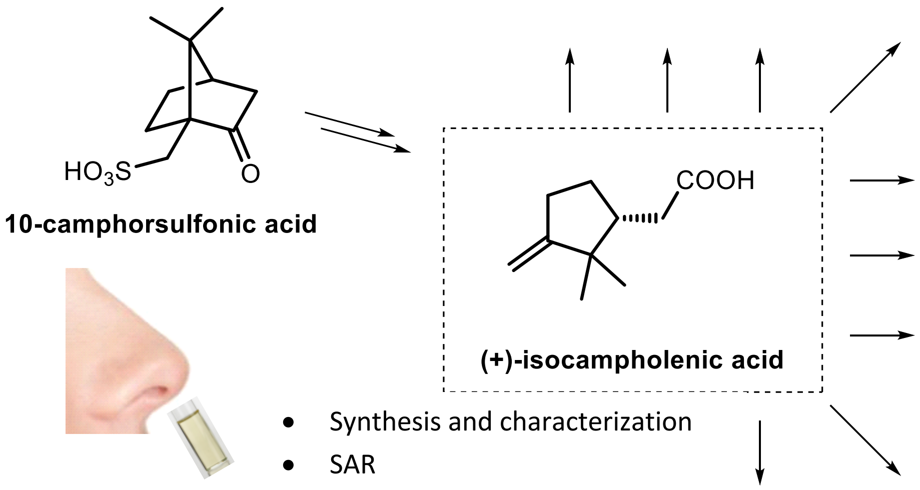

***Fig. 1**: Synthesis of (+)-isocampholenic acid derived flavors and fragrances.*

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Influence of synthesis approach on electrochemical performance of alkoxyaluminate-based electrolytes in Mg rechargeable batteries

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Mg batteries are a promising energy storage system because of the physicochemical merits of Mg metal as an anode material. However, the lack of suitable Mg electrolytes impedes the development of Mg batteries, which is why the development of Mg electrolytes remains an open challenge. One of the state-of-the-art electrolytes are Mg alkoxyaluminates, on which different synthesis approaches have been reported over the last few years[1-3]. Reported electrochemical performances vary greatly; however, direct comparison is not reliable, since the presence of impurities in salts was not characterized, and not all prepared electrolytes were tested under the same conditions. Therefore, $Mg[Al(hfip)_4]_2$ salt has been synthesized using different approaches and electrolytes' performance was tested systematically, under the comparable (abovementioned) conditions. Influence of selection of synthesis approach was studied with NMR, IR and XRD, in terms of detailed product composition analysis, including identifying possible side products and/or impurities. In addition, a new synthesis approach of $Mg[Al(hfip)_4]_2$ is introduced. Optimized electrolyte shows improved electrochemical performance in terms of Coulombic efficiency and overpotentials, confirming the importance of the selection of a synthesis path that is time-efficient and leads to a highly pure product.

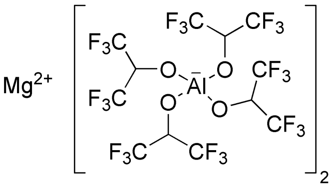

Fig. 1: Schematic of an $Mg[Al(hfip)_4]_2$ salt.

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Towards the development of a damage function for poly(vinyl chloride)

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Poly(vinyl chloride) (PVC) is a polymeric material widely used in everyday and specialty objects, as well as a material for modern and contemporary art and design objects[1]. PVC is prone to degradation by elimination of HCl, resulting in the formation of polyene sequences. PVC objects represent a significant part of heritage collections. Research in the field of PVC degradation in heritage collections was initiated upon observation of rapid progression of degradation, evidenced as discolouration (as a consequence of the formation of polyene sequences), surface leaching and cracking[2]. We are interested in studying the parameters that have the greatest influence on the degradation of PVC objects indoors[3]. For this purpose, 20 PVC samples were subjected to accelerated degradation at elevated temperatures (50 °C and 70 °C) and at two humidity levels (30% and 80% relative humidity). The identity and content of plasticizers, antioxidants and heat stabilizers in the samples were also analysed to determine their influence on degradation. Degradation was modelled using Multiple Linear Regression (MLR; Eq. 1). The rate of yellowing ($k = \frac{\Delta b*}{t}$) is presented as a function of temperature (T [K]), relative humidity (RH [%]) and plasticizer content (w_{plast} [%]). This can be used to prioritise the parameters with the greatest influence on degradation and thus help in establishing conservation guidelines for museums. Preliminary results show that temperature has the greatest influence on the rate of degradation, while relative humidity and plasticizer identity and content have a lesser influence.

$$lnk = b_3 \frac{1}{T} + b_2\, RH + b_1\, w_{plast} + b_0 \qquad (1)$$

Eq. 1: *Multiple linear regression equation, where the rate of degradation (lnk) is predicted from three variables: temperature (T), relative humidity (RH), and plasticizer content (w_{plast}); b_3, b_2, b_1, and b_0 are the modelled regression coefficients.*

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Expression and purification of recombinant human proteins FHL2 and Lef-1

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Epithelial cell adhesion molecule (EpCAM) is a transmembrane protein, which is often overexpressed on the surface of various carcinoma cells, where it is involved in cell proliferation and cancer progression[1]. One of the mechanisms how EpCAM promotes tumor growth is regulated intramembrane proteolysis, which causes the release of EpCAM intracellular domain (EpIC) in the cytosol, where it forms a complex with proteins FHL2 and β-catenin. After its nuclear translocation, the complex binds to Lef-1, which activates the transcription of several oncogenes (Fig. 1)[2]. In order to better understand the mechanism behind the described signaling pathway and to clarify the role of individual components of the complex, the structural characterization of complex is crucial. The first step in solving the structure is expression and purification of all four individual proteins. While purified β-catenin and a GFP fusion of EpIC had already been obtained, the quest of expressing and purifying FHL2 and Lef-1 remained. Expression of FHL2 in bacteria is especially problematic, probably due to partially unstructured nature of protein and high content of zinc finger motifs. Here we present the results of expression of FHL2 and Lef-1 in *Escherichia coli* and various chromatographic techniques, which were used in purification of both proteins.

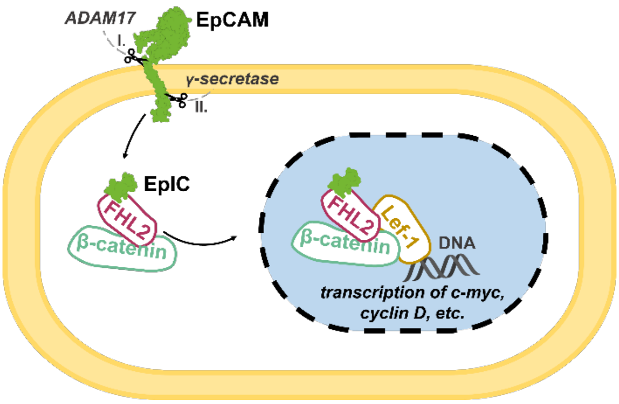

Fig. 1: *Regulated intramembrane proteolysis of EpCAM and activation of downstream signaling pathway. The crucial step in the signaling activation is the formation of complex of EpIC, FHL2, β-catenin and Lef-1, which induces transcription of several oncogenes.*

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The role of NONO and PCBP2 in the expression of selected circRNAs

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Circular RNAs (circRNAs) are an abundant group of RNA, which can function as oncogenes or tumor suppressors and are often dysregulated in cancer, also in hepatocellular carcinoma (HCC). Studies showed that different circRNAs have different mechanisms of action, they can act as microRNA sponges or interact with various proteins[1]. CircRNAs are synthesized by a non-canonical process called back-splicing, which requires canonical spliceosomal machinery. The process is regulated by both cis-acting and trans-acting factors such as RNA-binding proteins (RBPs) (Fig. 1)[2]. The aim of this study was to examine the effect of two RBPs, NONO (Non-POU Domain Containing Octamer Binding) and PCBP2 (Poly(RC) Binding Protein 2), on the expression of circRNA in HCC. Based on the preliminary bioinformatics analysis and determination of binding sites of these proteins, we identified circRNAs, the expression of which could be modulated by NONO and PCBP2. Next, we performed *in vitro* analysis in the human hepatocarcinoma cell line Huh-7. We overexpressed both splicing regulators, measured the expression of selected circRNAs by RT-qPCR and confirmed that RBPs can alter the expression of selected circRNA.

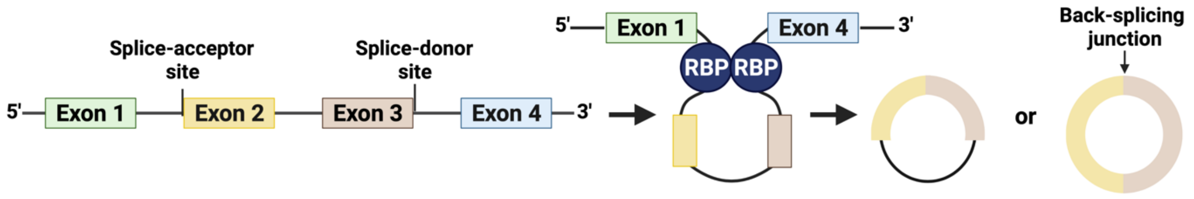

Fig. 1*: RBP driven circRNA biogenesis.*

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Corrosion protection of cast aluminium alloy AlSi7Mg0.3 using zirconium conversion treatment and hybrid sol-gel coating

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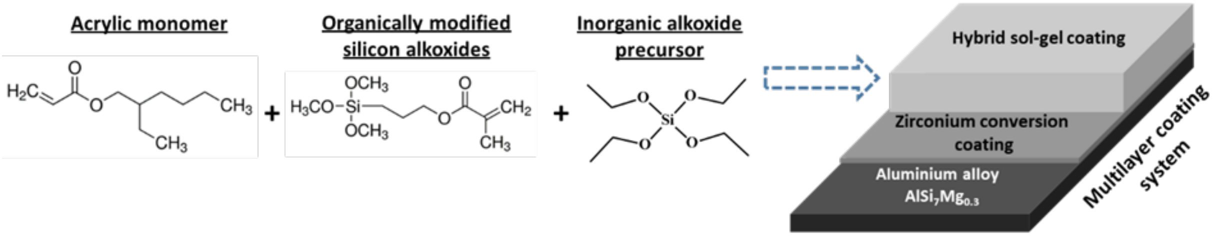
Aluminium alloy AlSi$_7$Mg$_{0.3}$ is an important lightweight metal in the transportation industry. The use of this alloy is limited due to low corrosion resistance due to alloying elements. Therefore, there is a need to develop environmentally acceptable corrosion protection.

Effective corrosion protection can be achieved with chromate conversion coatings, but such surface treatment contains hexavalent chromium, which has been limited due to its harmful effect on humans and the environment. As an alternative, zirconium conversion coatings (ZrCC) as a pretreatment for aluminium surface[1] and sol-gel coatings[2] and their multilayer coating system are presenting novel corrosion coating system (see Fig.).

In this study, the corrosion performance of zirconium conversion coating (ZrCC), hybrid sol-gel coating and their multilayer system were studied. The ZrCC was formed in three steps (degreasing, desmutting and passivation) using commercial reagents distributed by SurTec. The synthesis of hybrid sol-gel coating included organic precursors: 2-ethylhexyl acrylate and 3-(trimethoxysilyl)propyl methacrylate and an inorganic compound: tetraethyl orthosilicate. The synthesis of sol-gel was evaluated at various stages using *real-time* infrared spectroscopy. The corrosion characterisation was performed in 0.1 M NaCl using electrochemical impedance spectroscopy and salt spray test according to the ASTM B117 standard.

The results indicate that ZrCC and hybrid sol-gel coating form a continuous, smooth multilayer system, several micrometres thick, that provide long-term corrosion resistance.



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Effective way of obtaining triacylglycerol molecules from engineered yeast cells

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The growing demand for biofuels, oil-based food supplements and pharmaceuticals is focused on finding sustainable solutions for their production. Microbial production of fatty acids-derived molecules from nonedible biomass feedstocks is a promising alternative to production of such substances from animal fats and plant oils. To realise this quest, metabolic engineering strategies have been implied to non-oleaginous and oleaginous microorganisms, including yeasts. When engineered properly – with mutations in specific genes, even a non-oleaginous yeast *Saccharomyces cerevisiae* can accumulate high amount of fatty acids, stored predominantly as triacylglycerol (TAG)[1,2].

In yeast cells, TAG molecules are surrounded by a monolayer of phospholipids, which form lipid droplets[3]. Our aim is to develop efficient and economically feasible procedures for extraction of TAG from yeast cells with an ultimate goal to obtain large amounts of microbial-produced fatty acids, and with this diversify production of oils.

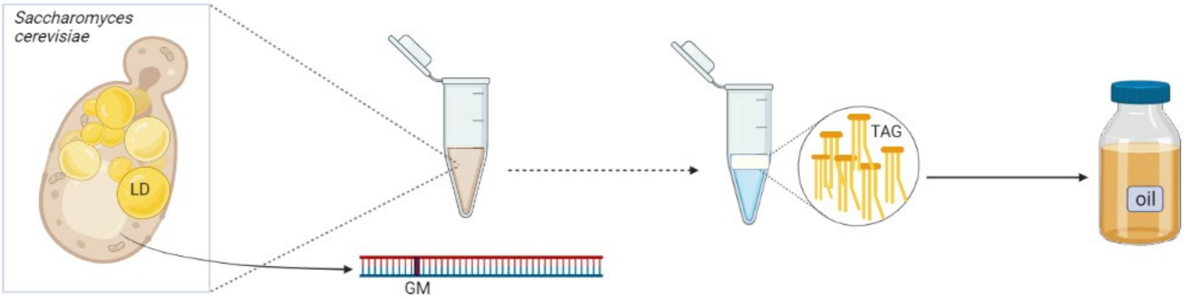

Fig. 1*: The hypothetical Fig. of obtaining TAG from yeast cells. GM, gene modification; LD, lipid droplets; TAG, triacylglycerol. Created with BioRender.*

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Study of phenytoin and oxytetracycline adsorption on ZnO nanoparticles

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Pharmaceuticals are undesirable in nature because of their effect on biological systems[1]. Classical wastewater treatment plants degrade their concentration inadequately[2]. A good alternative is photocatalysis-based degradation using semiconductors. Since photocatalysis is preceded by adsorption, a thorough study of adsorption is crucial for a its better understanding. Herein, we synthesized ZnO nanoparticles via a solvothermal method. Characterizations showed that the ZnO nanoparticles are 10 nm in size, while in suspension they form porous 500 nm aggregates with a positively charged surface in the pH range 6-9 (Fig. 1a). Computational electron surface potential simulations were performed to gain better insight into the polarity of the model pharmaceuticals oxytetracycline (OTC) and phenytoin (PT) (Fig. 1b). Fig. 1c shows the kinetics of adsorption of OTC and illustrates that adsorption on the outer surface of the agglomerates is a very fast process, in contrast to diffusion and adsorption into the pores, which are much slower. Since PT has a large non-polar fraction, it is adsorbed only on the surface of the agglomerates and thus shows only the fast kinetic profile, while OTC is a highly charged molecule that allows stronger interactions and diffusion into the pores. Therefore, it is a better adsorbent with slower kinetics.

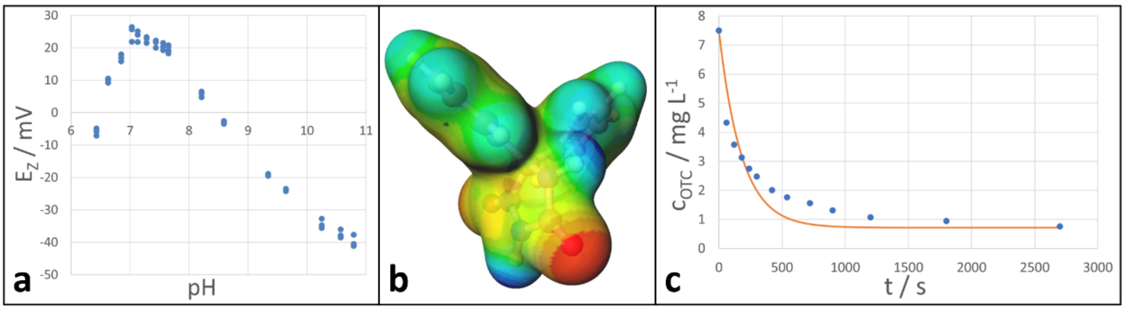

***Fig. 13**: a) Zeta potential measurement b) Phenytoin electron surface potential c) Kinetics of oxytetracycline adsorption with a proposed kinetic model.*

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Corrosion protection of AA2024-T3 with nanostructured oxide film using atomic layer deposition (ALD)

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Aluminium alloy (AA) 2024-T3 is commonly used in the aerospace industry due to its light weight and high mechanical strength. The disadvantage of this alloy is high susceptibility to corrosion in chloride-containing solutions due to alloying elements.

In the past, chromium(VI) conversion coatings have been used to enhance corrosion protection. These compounds have been strictly regulated in various countries due to their harmful effects on humans and the environment. One of the alternatives is atomic layer deposition (ALD), which allows a formation of a nanometers thin oxide film on the surface[1].

In this study, a 100 nm aluminium oxide (Al_2O_3) film was deposited with ALD on AA2024-T3 at different temperatures (120 °C, 160°C and 200 °C)[2]. The films were evaluated using a field emission scanning electron microscope coupled to an energy dispersive spectrometer to determine surface topography, coating thickness and composition. The corrosion characterization was performed in 0.1 M NaCl using electrochemical impedance spectra (EIS) and salt spray test according to the ASTM B117-07A standard.

Results confirm that the ALD films deposited at higher temperatures (above 160 °C) present durable barrier corrosion protection of the AA2024-T3, Fig. 1.

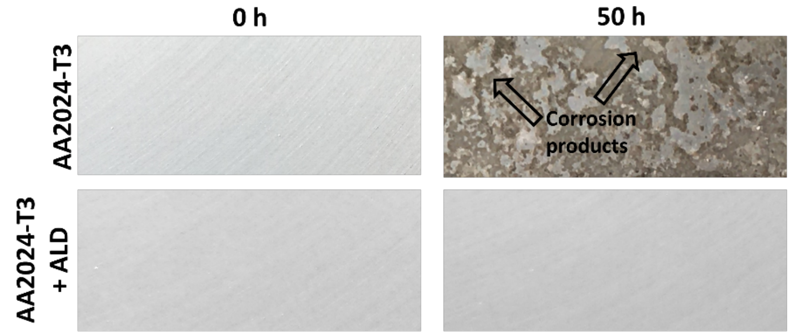

Fig. 1: *The surface appearance of ground AA2024-T3 without (top row) and with ALD coating (bottom row) after testing in a salt spray chamber.*

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Furfural conversion over MoO_2 and MoO_3 surfaces from first-principles

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For the purpose of dehydrogenation, molybdenum oxide based catalysts have already been frequently used[1], hence MoO_2 and MoO_3 were picked as studied catalysts. Dehydrogenation of different compounds such as methane[2] and propane[3] have already been conducted. Subsequently we decided to investigate dehydrogenation of furfural, which has in recent years sparked interest in converting it to higher value chemicals, due to it being a green and renewable product of sugar dehydrogenation.

We utilized *in-silico* studies (DFT) to gain insights into the reaction mechanism. The adsorption energies of furfural and successive intermediates were evaluated on MoO_2 (001), (111) and MoO_3 (101), (111) surfaces. These surfaces were chosen according to Wulff construction which provides us with thermodynamically most stable shape of crystal. Additionally potential energy surface (PES) was constructed to study the reaction mechanism and identify possible bottlenecks. Theoretical calculations were done in Quantum-Espresso[4] package, with projector augmented wave method (PAW), using Perdew-Burke-Ernzerhof (PBE) functional. Grimme-D3 correction to van der Waals forces was incorporated, as to properly describe adsorption.

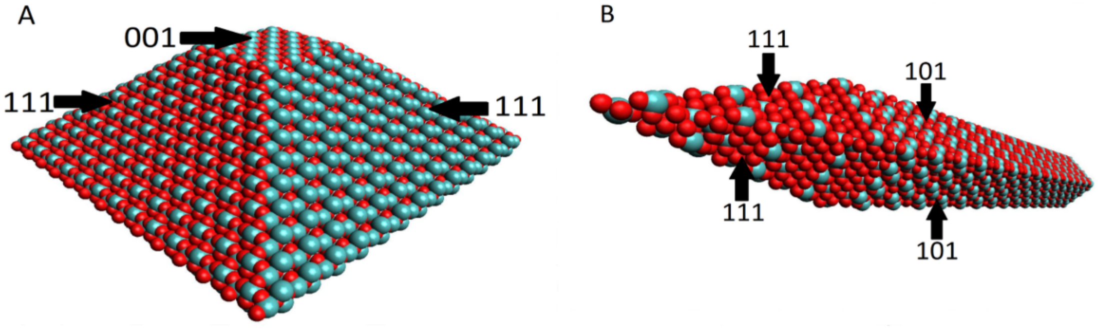

***Fig. 1**: Wulff construction of A) MoO_2 and B) MoO_3.*

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Structural characterization of $LaTaTiO_6$-$La_4Ti_9O_{24}$ based solid solution by X-ray powder diffraction

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Various lanthanum titanates are known for their pronounced dielectric properties, which are of interest to the electronics industry, especially for wireless communication applications. In the search for new ceramics with improved microwave properties, ternary systems with the addition of various third oxides have been studied. The structural characterization of the $LaTaTiO_6$-$La_4Ti_9O_{24}$ based solid solution is a part of investigation of the La_2O_3-Ta_2O_5-TiO_2 ternary system.

By the conventional solid-state reaction technique using La_2O_3, Ta_2O_5 and TiO_2 we prepared four solid solutions $La_{1+3x}Ta_{1-x}Ti_{1+8x}O_{6+18x}$ for x=0.015, 0.030, 0.05 and 0.065 (S1, S2, S3 and S4, respectively). Powder XRD data were collected on Panalytical X'Pert PRO MPD diffractometer using Cu$K\alpha_1$ and structurally characterized by Rietveld refinement. Solid solutions S1, S2, S3 and S4 are isostructural with the monoclinic modification of $LaTaTiO_6$, and crystallize in *C*2/*c* space group[1].

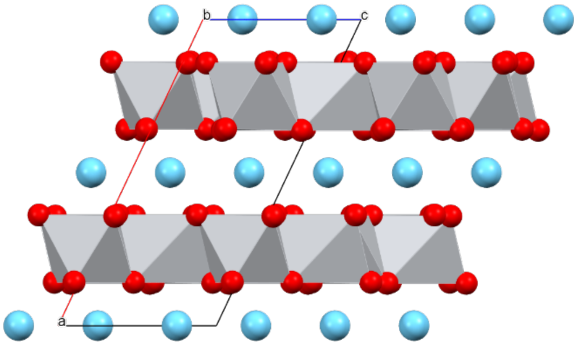

Fig. 14: *Representation of structure of S4 with formula* $La_{1.195}\ Ta_{0.935}Ti_{1.52}O_{7.17}$ *(view along b axis).*

The structure consists of layers, formed by distorted $(Ta/TiO_6)^{3-}$ sharing edges octahedra. Ta^{5+} and Ti^{4+} are randomly distributed on the same crystallographic site with an occupancy of 50% each. In-between octahedra layers there are La^{3+} ions. In the solid solutions the Ta/Ti site remains to be fully occupied with occupancy of Ti^{4+} increasing and Ta^{5+} decreasing with the increase of x. The La sites and O sites in the solid solutions are not fully occupied. Their occupancies and the volume of the unit cell of the solid solutions decrease with the increase of Ti^{4+} content.

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Notes

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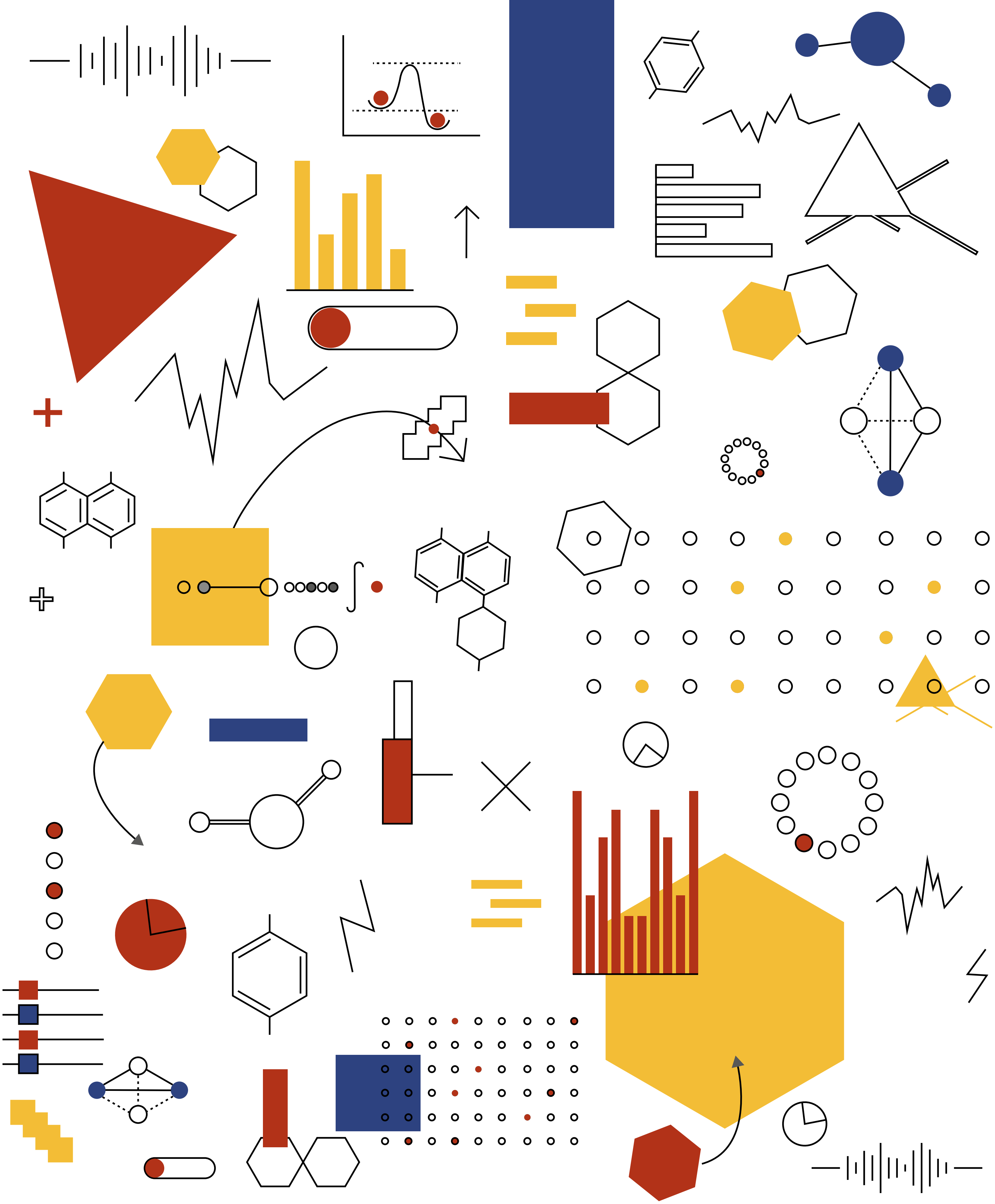

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