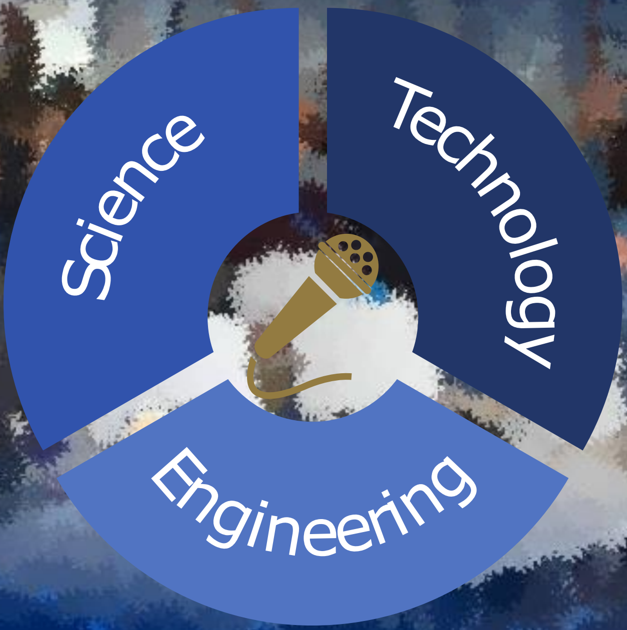
15th May

Institut
"Jožef Stefan"
Ljubljana, Slovenija

## 12th STUDENT CONFERENCE

Jožef Stefan International Postgraduate School
And 14th Young Researchers' Day
(CMBE-Chemistry, Material, Biochemistry, Environmental)
**first Online Conference**

# Book of abstracts



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# 12. ŠTUDENTSKA KONFERENCA MEDNARODNE PODIPLOMSKE ŠOLE JOŽEFA STEFANA IN 14. DAN MLADIH RAZISKOVALCEV (KONFERENCA KMBO)

## Knjiga povzetkov

# *12th JOŽEF STEFAN INTERNATIONAL POSTGRADUATE SCHOOL STUDENTS CONFERENCE AND 14th CMBE DAY*

## *Book of abstracts*

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# First Online IPSSC Conference – May 2020

# or

# *Chronicles of COVID-19*

Dear Participants,

As we can see, times are changing and we have to adapt to them. We are proudly welcoming you to the 1st online conference of International Postgraduate School Jožef Stefan (IPSSC), organized by students for students on the 15th of May, 2020. This conference is merged with the Day of the Young Researchers of Chemistry, Materials, Biochemistry and Environment (CMBE), which has proven to be a good practice. The committee has been selected among members of CMBE for announcing the awards for the best online presentation, the best graphical illustration of presentation and the best abstract.

This brand-new event has gathered students from different disciplines, such as ecotechnologies, nanosciences and nanotechnologies, sensor technologies, communication and computer technologies, medicine, etc. Our conference is a place where you, students, can present your current and future research work. Through networking with your colleagues, researchers, and supervisors it is also a safe environment to discuss breakthrough ideas or just comment on the current research sphere - you never know when someone could challenge you and thus help you connect the missing dots. Moreover, it is also a way for professors of IPS and mentors to see their students present their work and to have a conversation in a friendly and relaxed atmosphere among colleagues of IPS.

In these changing times, we have to be supportive, respectful, tolerant and helpful to each other as colleagues, whatever the gender, research position or age. At the same time, we have to know how to use our knowledge in our devotion to our research vision and project, so that we can make progress in research and society. We have the power of knowledge and it is our duty to use it to its full potential. To support you in achieving one of your goals, we have decided that we will help you by organizing this online conference at which the program of the conference was adapted for the current situation of the coronavirus. The IPS staff have given us support, as organizers, and you, as participants, during the whole preparation and organization of this conference. IPS has also financially supported the conference for which we would like to express our honest gratitude. In the name of all of us, we thank everyone for the generous support and help, with which we would not have been able to conduct a conference at such a level. *Rock on!*

For the end, let us quote our fellow scientist, Marie Curie:

> "I am among those who think that science has great beauty."

Student Council and Organizing Committee of IPSSC

# Beseda predsednika MPŠ: Akad. prof. dr. Vito Turk

Živimo v času in pogojih, ki jih do sedaj še nismo poznali. To ni vojna, pač pa pandemija, ki jo je povzročil novo odkriti agresivni koronavirus (covid-19), ki se je iz kitajskega mesta Wuhan razširil po vseh kontinentih in državah. Tudi ni šel mimo nas. Le hitremu ukrepanju zdravstvenega osebja ter oblastem se moramo zahvaliti, da je covid-19 naši državi le nekoliko prizanesel v primerjavi z nekaterimi drugimi državami. Hitro smo se morali na nov način vsakodnevnega življenja priučiti, kar terja veliko samodiscipline ter odgovornosti na vseh ravneh. Morali smo začasno omejiti stike med sorodniki, prijatelji, študijskimi kolegi in drugimi z namenom, da se čimprej znebimo tega nam nevidnega sovražnika našega zdravja in pričnemo normalno živeti.

Seveda posledice pandemije čuti tudi naša Mednarodna podiplomska šola Jožefa Stefana. Morali smo se hitro prilagoditi novim razmeram, kar je terjalo tako od vodstva šole, profesorjev in mentorjev in naših podiplomcev popolnoma nov pristop k reševanju nastale situacije. Tudi seje študijskih komisij, senata, volitve dekanje in še bi lahko našteval, smo morali izvesti s pomočjo internetnih komunikacij, kar ni šlo brez tudi vsaj manjših občasnih težav. Ravno ob tej priliki pa se je pokazalo, kako je majhnost lahko velika prednost in omogoča veliko fleksibilnost. Prepričan sem, da smo se v tem pogledu najbolje odrezali v Sloveniji! Tudi naši podiplomci s svojim vodstvom pri tem več kot uspešno sodelujejo, za kar zaslužijo izjemno zahvalo.

Dejstvo je, da se je MPŠ že uveljavila v domačem in mednarodnem prostoru. V vsem tem času je več kot uspešno opravljala svoje poslanstvo, kar kažejo njeni dosežki. Objavljeni so bili kazalci, po katerih je naša šola celo prva med vsemi v Sloveniji po kvaliteti in številu objav naših doktorantov. To je rezultat uspešnega sodelovanja podiplomskih študentov in njihovih mentorjev. Potrebno na prvem mestu omeniti Institut Jožef Stefan, ki največ doprinaša k uspešnemu delu te šole z odlično opremo vključno s Centri odličnosti in širokim ter kvalitetnimi mentorji. Vključitev in sodelovanje Nacionalnega inštituta za biologijo – NIB in Inštitut za kovinske materiale in tehnologije - IMT Značilnost te šole je njena interdisciplinarna naravnanost, ki kaže na naše stalno spremljanje razvoja znanosti in inovativnosti v svetu. Pojem "odličnost" se je v svetu uveljavila, še zlasti na področju znanosti. Pri različnih razpisih, še zlasti Horizon 2020, so uspešni le tisti projekti, ki dobijo oceno odlično ali celo izvrstno. Tudi nekaj naših raziskovalcev in profesorjev je med njimi. Rezultati teh osnovnih raziskav pa tudi drugih lahko vodijo s svojimi prelomnimi dosežki do novih inovacij in proizvodov, od katerih je odvisna ekonomska rast in moč naše države. Na tem mestu naj poudarim, da to morajo razumeti tako politiki, ki vodijo državo, in gospodarstveniki. Zato pa je potrebno veliko naporov in finančnih vlaganj vlad, ki razumejo pomen znanosti in raziskav za razvoj celotne družbe. To velja še zlasti v današnjem času, ko se ne bomo mogli izogniti recesiji. Pot iz nje pa nam bo omogočilo znanje! To niso prazne besede, kajti za tem stojijo uspehi mnogih uspešnih držav in njihovih gospodarstev, ki so ob teh prilikah povečevali vlaganja v znanosti in razvoj. Pri nas pa so med prvimi po pravilu na udaru znanosti in raziskave.

Pomembno je enakovredno mednarodno sodelovanje, kar pomeni, da le uspešni sodelujejo z uspešnimi oz. razviti z razvitimi. Če temu ni tako, razviti in bogati izkoriščajo nerazvite in revne s slabo plačano delavno silo. Pred kratkim sta predsednik nemške Max-Planckove zveze institutov dr. Martin Stratmann in predsednik francoskega CNRS dr. Alain Fuchs poudarila pomen odličnih osnovnih raziskav ter inovacij, ki ustvarjajo raziskovalce sposobne razvoja novih idej. To počno razviti, ekonomsko šibkejše države, kamor se uvršča tudi Slovenija, pa bi se morale potruditi , da ustvarijo primerljive pogoje s primernim financiranjem in infrastrukturo. Le tako bodo te države lahko uspešno vzpostavile sodelovanje z industrijo ter razvijalo produktivne partnerske odnose. Za Slovenijo velja, da bi morala njena vlada vlagati vsaj 1% BDP. Prav za prav še več, da bi v doglednem času nadoknadili zamujeno! To je bilo že večkrat obljubljeno, pa nikoli storjeno! Trenutno vlagamo pod 0.5% BDP, kar nas uvršča na dno Evrope. Zmotno je mnenje, da bomo le z sredstvi EU in Horizon 2020 dosegli zaželjene cilje. Glede na navedeno nas ne sme presenečati, da smo priče begu možganov in ne kroženju, kot to želijo nekateri prikazovati. Žal odgovorni vedno znova in znova dokazujejo, da vsega tega ne razumejo, kajti če bi razumeli, bi delali in ukrepali drugače.

Kljub vsemu MPŠ vlaga velike napore za doseganje več kot solidnih, v nekaterih primerih tudi odličnih rezultatov v sicer neprijaznih pogojih na področju raziskovalne in visokošolske dejavnosti. Dejstvo je, da se vpisujejo na področja delovanja naše šole praviloma odlični mladi podiplomci, ki s svojem znanjem, zagnanostjo in dosežki segajo v sam vrh kvalitetnih mladih raziskovalcev.. Seveda teh uspehov ne bi bilo brez odličnih mentorjev in somentorjev, ki so prejeli za svoje delo vrsto domačih ter mednarodnih priznanj. Naj omenim še izjemno vzdušje in kolegialne odnose, ki vladajo med podiplomci in njihovimi mentorji, ter na drugi strani vodstvom MPŠ. Vse to omogoča tudi uspešno vpetost v mednarodne povezave, tako v Evropskem kot tudi v globalnem prostoru. S svojim delovanjem MPŠ prispeva k hitrejšemu prehodu iz vsesplošne krize v družbo znanja. Letošnja predstavitev raziskovalnih dosežkov naših podiplomcev, kljub izrednim razmeram, je ponoven dokaz vaše uspešnosti, ki je posledica trdega dela in talenta. Vse to vam omogoča, ob pomoči mentorjev ter bližnjih sodelavcev, da se boste razvili v kreativne raziskovalce, na katere smo in bomo ponosni. S svojem znanjem boste lahko doprinašali k boljši prihodnosti, kot vam jo ponuja sedanjost. Vso pravico imate, da se uspešno spopadate z izzivi v domačem okolju, ne pa da iščete izpolnitve svojih ambicij in eksistenčnih možnostih z odhodom v tujino.

Ob koncu, bi še enkrat ponovil in kar sem že večkrat izjavil, da je “znanje vrednota, ki omogoča narodu ekonomski razvoj in obstoj. Osnovne raziskave sodijo v ospredje moderne kulture, in nam pomagajo razumeti kdo smo “! Mladi vrhunski raziskovalci so pogoj za uspešen gospodarski in vsesplošen razvoj in so srce družbe znanja. Očitno so potrebne za to spoznanje globoke družbene spremembe, katere pa do sedaj še nismo dočakali. Morda pa bo grozeča gospodarska kriza le odgovorne spametovala. Torej bodimo še naprej optimisti, saj upanje umre zadnje!

## Beseda dekanje MPŠ: Prof. dr. Milena Horvat (slo)

V času pandemije in omejitev socialnih interakcij študentje MPŠ niso obupali nad organizacijo svoje tradicionalne letne konference. Sodobna komunikacijska orodja omogočajo organizacijo virtualne konference in tako učinkovito razširjanje in komuniciranje raziskovalnih rezultatov. Te zahtevne naloge sta se lotila organizacijski odbor in študentski svet. Na to odločitev, zavzetost in odlično organizacijo smo izjemno ponosni. Čestitke vsem – odgovornim za organizacijo in tistim, ki so se prijavili na konferenco!

Skupna organizacija dveh tradicionalnih študentskih konferenc – Študentske konference MPŠ in Dneva mladih raziskovalcev s področja kemije, materialov, biokemije in okolja (KMBO) na Inštitutu »Jožef Stefan« – se je izkazala za zelo učinkovito pri spodbujanju izmenjave znanja in izkušenj med študenti in raziskovalci. Letošnja študentska konferenca zaradi virtualne narave nadaljuje s prakso iz prejšnjega leta, da je treba o znanosti govoriti na način, ki je razumljiv širokemu spektru ljudi.

Izziv ustvarjanja pogojev za MPŠ, kjer študentje lahko izvajajo odlične mednarodne primerljive znanosti, ob tem pa razvijajo svoje ustvarjalne in poslovne veščine, je eden glavnih ciljev MPŠ. V sodelovanju s partnerskimi raziskovalnimi ustanovami in industrijo bomo še naprej podpirali vse dejavnosti, ki ustvarjajo pogoje, ki omogočajo povezovanje programov MPŠ z drugimi slovenskimi in tujimi univerzami, da bi študentom zagotovili najboljše znanje in veščine, ki jih bodo potrebovali v prihodnosti.

Vsem udeležencem študentske konference želim uspešno delo in jih spodbujam, da se učijo iz izkušenj virtualne konference. Združitev tega modela z običajnim pristopom lahko pozitivno vpliva na kakovost in poveča doseg konference z vključitvijo večjega števila študentov in drugih zainteresiranih strani. Trenutno restriktivne pogoje lahko tako spremenimo v izzive in priložnosti za prihodnost!

## Deans words, Prof. dr. Milena Horvat (eng)

At a time of pandemic and limited social interactions, IPS students did not give up on organizing their traditional annual conference. Modern communication tools allow virtual conferences to take place and enable effective dissemination and communication of research results. This challenging task was undertaken by the Organizing Committee and the Student Council. We are extremely proud of this decision, commitment and excellent organization. Congratulations to all – those in charge of the organization and those who registered for the conference!

Joint organization of two traditional student conferences – the IPS Student Conference and the Day of Young Researchers in Chemistry, Materials, Biochemistry, and Environment (KMBO) at the Jožef Stefan Institute – has proven to be very effective in promoting the exchange of knowledge and experience between students and researchers. Regardless of its virtual nature. This year's student conference continues with the previous year's practice of talking about science in a way that is comprehensible to a wide range of people.

The challenge of creating an IPS environment where students can pursue excellent international comparative sciences while developing their creative and business skills is one of the major goals of IPS. In cooperation with partner research institutions and the industry, we will continue to support all the activities which create the conditions that enable the integration of IPS programs with other Slovenian and foreign universities in order to provide students with the best knowledge and skills they will need in the future.

I wish all the participants of the student conference successful work and encourage them to learn from the virtual conference experience. Combining this model with the conventional approach can have a positive effect on the quality of the conference in the future and help reach more students and other interested parties. Let us learn from the current restrictive conditions and turn them into challenges and opportunities for the future!

## Prof. dr. Gašper Tavčar (CMBE president)

I was given an honour to prepare a few short words for the book of abstracts of 12th Jožef Stefan International Postgraduate School Students' Conference as a member of CMBE. This will be probably one of the most unusual conferences in the whole history of Jožef Stefan International Postgraduate School. Practically the whole world including Slovenia is in a lockdown due to COVID-19 pandemic and all the international conferences I know are being cancelled or postponed during this time. However, the students decided differently and instead of taking the easy way of cancelling the whole event you have decided to proceed with an innovative approach and organized the first IPS online conference. This decision is remarkable and shows your determination to keep the exchange of scientific ideas which is the foundation of the modern science despite the pandemic. Moreover, you have decided to proceed with completely different organizational challenges, that no other IPS students have tried before. There is a chance that you will have plenty of unexpected problems required by a new approach, but you will gain a lot of new knowledge and experience, that you will be later able to implement while you will be developing your career after finishing your study at IPS. Additionally, you will prepare the way for next generations of IPS students that will be able to follow up much more comfortably in this field.

As a member of CMBE I am glad that such generations of students from our field will be coming out in next years and hopefully this momentum will remain with you and lead to new scientific breakthroughs and innovative industrial processes in the future.

At the end I wish you a successful and fruitful conference that you will be able to remember for many years to come.

# Povzetki/Abstracts

# Ekotehnologija (Ecotechnology)

# Aldiminium based poly(hydrogen fluorides) – potential nucleophilic fluorination reagents

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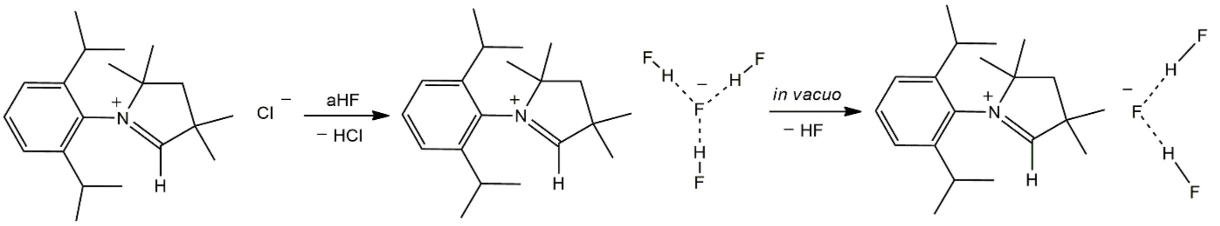
Incorporation of fluorine into organic and organometallic compounds is still considered a challenging task. The use of hydrogen fluoride as a fluorination reagent would be ideal in terms of cost efficiency, but its low boiling point and high corrosivity make it difficult to handle.[1] To overcome this problem, commercially available fluorination reagents, such as pyridinium poly(hydrogen fluoride) and thiethylamine trihydrogen fluoride are employed. Both reagents are liquid and therefore easier to deal with, however they still require careful handling as they fume in air.[1]

During the last decade many safer and more selective fluorination reagents have been proposed. For example, pyrimidone based hydrogen fluoride reagent is considered suitable for reactions in acidic medium,[2] while pyrrolidinium and imidazolium based ionic liquids, are considered safer to handle and have the potential to be used as non-aqueous reaction media.[1]

Imidazolium salts can also be used as precursors for N-heterocyclic carbenes (NHCs). In 2016, three fluorination reagents based on imidazolium fluoride were prepared in our laboratory, specifically an imidazolium fluoride [IPrH][F] and two poly(hydrogen fluorides) [IPrH][$(HF)_nF$] ($n = 1, 2$).[3] They turned out to be versatile and applicable reagents, useful in the fluorination of organic and inorganic compounds. Inspired by this results, we decided to prepare another class of fluorination reagents, based on aldiminium salts. To the best of our knowledge, this has never been done before. Our research focussed on the use of an aldiminium salt, which is a direct precursor of a stable cyclic alkyl amino carbene (CAAC). CAACs also belong to the NHC family, but they express different chemical properties. Nowadays, they are extensively studied as ligands in organometallic chemistry.[4]

During our work, we successfully prepared two aldiminium based poly(hydrogen fluorides), specifically [$^{Me}$CAACH][$(HF)_3F$] and [$^{Me}$CAACH][$(HF)_2F$] by adding anhydrous hydrogen fluoride (aHF) to the aldiminium salt [$^{Me}$CAACH][Cl]. Results of the preliminary studies on aluminium precursors have shown the potential use of these compounds as nucleophilic fluorination reagents.



**Scheme 1**. Synthesis of [$^{Me}$CAACH][$(HF)_3F$] and [$^{Me}$CAACH][$(HF)_2F$].

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# Behaviour of U isotopes in two different karst aquifers, Ljubljanica River and Krka River (Slovenia)

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Uranium (U) isotopic composition has proven to be very valuable to study different chemical and physical processes involved either in estuarine [1], marine [2] or in continental waters [3]. U isotopes can change along the flow path of karstic freshwaters; therefore, can be applied as an important tracer for studying changes in groundwater flow pattern, changes in host rock composition, and variable mixing of waters of different origin (shallow and deeper aquifers). However, to detect minor isotopes and to have high accuracy, this can be achieved only by using advanced analytical tools, such as multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS).

Measurements of U isotope ratios were carried out with Nu plasma II, (Nu instruments Ltd, UK) MC-ICP-MS with the high-efficiency sample introduction system Aridus II™ (Cetac Technologies, NE, USA). For assessing U concentration, a single collector mass spectrometry an Agilent 8800 Triple Quadrupole ICP-MS (ICP-QQQ) (Agilent Technologies, California, USA) was used. Instrument mass bias was corrected with external standard–sample bracketing method. Measured U isotope ratios were calibrated against corresponding U isotope ratios in the certified U standard IRMM-184 that had been measured before and after the applying sample.

U concentrations and its isotope ratios, presented as the $^{234}$U/$^{238}$U activity ratios and $\delta^{238}$U, have been monitored in water samples collected from two Slovenian karstic aquifers, Ljubljanica River and Krka River. The results reveal variations of the U isotope ratios of water samples in the range of 1.10 to 1.79 for the $^{234}$U/$^{238}$U activity ratios and 0.05 to 5.67‰ for $\delta^{238}$U in the Ljubljanica River and in the range of 1.29 to 1.55 for the $^{234}$U/$^{238}$U activity ratios and -0.18 to 1.69‰ for $\delta^{238}$U in the Krka River. The $^{234}$U/$^{238}$U activity ratios show variations for some sampling sites between sampling campaigns inside the area of the same river and between two different rivers. Results of $\delta^{238}$U in two different rivers give us mostly unclear pattern compared to the $^{234}$U/$^{238}$U activity ratios. Minor $\delta^{238}$U fractionations occur, due to weathering and transport of the main river bedrock. Variable results in U isotope ratios between two Slovenian rivers can be interpreted as a different source of lithology that change with the flow path of karstic freshwater. Therefore, U isotopic composition show promise as a tracer of water sources in stream waters at the catchment scale and can be also used as a tracer for seasonal variation along the flow path of karstic water, due to significant difference in U isotopic composition between low-flow and high-flow conditions.

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# Characterisation and attempted geographical differentiation of extra virgin olive oils from Italy and Slovenia using stable isotope ratio analysis

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The traceability of food products and the assurance of their quality are increasingly requested by consumers or required by European laws. Stable and radiogenic isotope ratios have proven successful not only as a means of detecting fraud but also in providing designations of geographical origin for various food products. In this study, bulk $\delta^{13}C$, $\delta^{18}O$ and specific compound $\delta^{13}C$ values were determined in European extra-virgin olive oils using gas chromatography coupled to on-line combustion isotope ratio mass spectrometry (GC-C-IRMS). A varied selection of olive oil samples was crucial to check the robustness of the results: 12 olive oils from 4 locations in Italy at the same altitude but different latitudes were sampled in 2015 and 2019, as well as 24 samples from Eastern Slovenia (taken in 2019). The selected array of samples included mono-cultivar (i.e., Buga, Belica, Leccino, and Maurino) and multi-cultivar olive oils. A fatty acid characterization had previously been carried out with GC-FID (after FAME extraction) in order to verify the quality of the samples and determine which fatty acids were suitable for specific compound analysis. Isotope composition was then used to differentiate samples based on their geographical origin. When the bulk data were combined with fatty acid isotope data, the accuracy of the differentiation improved significantly. The integration of mono-cultivar sample isotope results further increased the resolution. The isotopic fingerprint of a food product remains the only practical way of building a traceability chain and verifying the authenticity of the product.

# Condition of oral microbial communities in individuals from a shared household

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The human oral cavity represents a habitat for many microorganisms forming oral microbiome, which is beneficial for the host. The most numerous group of microorganisms in oral microbiome are bacteria, which form commensal, symbiotic, and pathogenic associations with individuals at different life stages. In contrast to the gut microbiome, the structure of the oral microbiome is regulated on the one hand by the local environmental conditions, which determine niches (viz. niche hypothesis), and on other by the microorganisms that are uptaken through food, water and other sources of microbes (viz. neutral populations). Since human oral ecosystem is open for the immigration of microbes, the core microbiome is relatively small. However, since many microbes can have a similar function, the differences in microbial composition between different oral microbiomes can fulfill functional diversity. Based on these assumptions, the stability of the oral microbiome structure is dependent on the stability of the pool of immigrants. Hence, our hypothesis is that people sharing common households have more similar community in the oral microbiome that those that are part of other households.

The second purpose of this study was to determine the presence of probiotic bacteria in the oral microbiome using aerobic and anaerobic cultivating conditions.

In this study, we isolated DNA from biofilms formed on the teeth, from saliva, and mucosa of individuals shared the same or different households. The diversity of the oral microbiome on the basis of sequences of 16S rRNA gene. The functional diversity was predicted from 16s rRNA sequences as well by sequencing the whole microbiome. In addition, the probiotic strains were also screened for the antimicrobial traits against different oral pathobionts. The most potent bacterial strains were identified on the basis of 16s rRNA genes.

According to our hypothesis, the microbiome taxonomic structure is more similar within the individuals from the same household than from the different. The age and health status should also be taken into consideration for the interpretation of these results. The probiotic strains acting against oral pathobionts are sequences and are forming small populations up to 5% of all isolates.

# Dynamics of the artificially constructed microbial biofilms

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One of the most essential property of all bacterial cells is to attach on various surfaces and form biofilms, which is the surviving strategy coping against the harsh environmental conditions. The most of the research of attached bacterial cells is focused into the understanding of biofilm formation and physiological adaptations of microbial cells. In contrast to this mainstream, based on deductive approaches, our research is focused on the inductive approach where we are engineering the microbial films by combining colloid physics and microbiological analytics to observe microbial responses recursively.

Since colonization of surfaces is dependent on the intrinsic property of the surface of the bacterial cell, we overcome this property by the electrostatic attachment of bacterial cells on surfaces and keeping them alive. This approach enables that the attachment of the cell is not anymore dependent on biological properties but on the (*i*) physicochemical conditions of the surface to which we want to attach cells, such as roughness, hydrophobicity, charge, and (*ii*) properties of the solvent that is used during the attachment, such as ionic strength, pH, temperature. However, the dynamics of the biofilm maturation after the physical attachment of cells are currently not known [1]. Based on our assumption, enabling cells to breach the physical repulsion barrier will keep cells attached, we might force bacteria to form biofilms at any physiological stage and type of the cell.

Here we used bacterial cells that cannot form biofilms *per se.* The efficiency of the initial attachment of cell is following basic physical principles: (*i*) higher ionic strength in the solution leads to a greater force of adhesion of non-covered bacteria to the surface and oppositely for covered ones and (ii) lower temperature as well as treating the inanimate surface with positively charged polyelectrolytes induced higher adsorption. Cells form biofilms only when we attach them. However, the biofilm is weak and in 12 hours is disintegrated. We assume that after the division of cells the surface charge density is lowered due to the increase of the surfaces of the cells resulting in decreased electrostatic forces.

Accordingly, the modification and understanding of these parameters it can result in significant improvement of immobilization of cells and can be exploited as a development of multicellular aggregates with joint metabolic pathways and cell based biosensors employing variety of different cells.

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# Elemental bioimaging by laser ablation inductively coupled plasma mass spectrometry

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Bioimaging is a term for acquiring, processing and visualizing data (information) of a biological sample or a living object and forming it into an image. With the rapid development of relevant analytical instruments and methods, new possibilities for bioimaging have been developed. Among them, laser ablation connected to inductively coupled plasma mass spectrometry (LA-ICP-MS) is frequently used. It is a sensitive microanalytical elemental imaging technique applied to determine the quantitative distribution of elements with high lateral resolution (up to 1µm) and low limit of detection. The main advantage of LA-ICP-MS compared to other mass spectrometry methods is that it offers solid sample elemental mapping with very little interferences compared to conventional ICP-MS analysis, thus being able to measure concentrations down to femtograms per pixel. This method was originally created for analysis of geological samples, but due to its capabilities, it is being developed for other fields as well. As a new method for determining elemental distribution in cells and tissues, it has a long way to go for development of standards and reference material in order to provide precise quantification. Due to these limitations, this method is not a commercial technique. Besides geological and biological samples, LA-ICP-MS (triple quadrupole) can measure almost every element in any solid sample. Currently, we are developing several analytical methods for biological samples (tissues, tumorous spheroids [1] and cells [2]) that are comprised of quantification by matrix matched standards and isotope dilution technique. So far, the most precise quantification method is isotope dilution, as it also serves as an internal standard for instrumental drift correction.

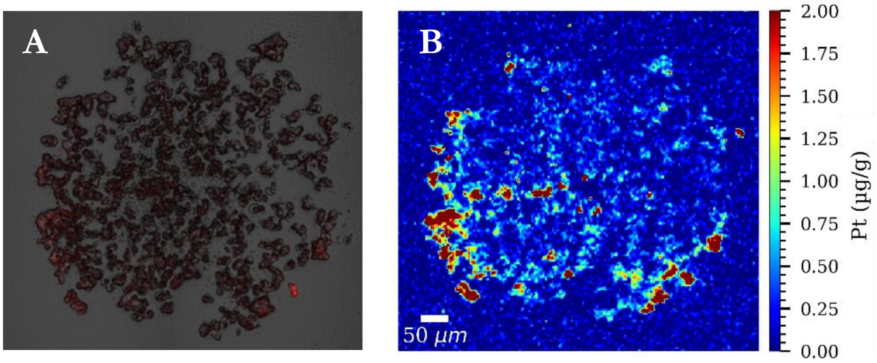

**Figure 1**. A) Platinum distribution via confocal microscopy of a tumorous spheroid treated with Texas-Red cisplatin. B) Platinum quantification via isotope dilution LA-ICP-MS of a tumorous spheroid treated with Texas-Red cisplatin.

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# First Slovenian study about detecting potentially toxigenic cyanobacteria in freshwater bodies by quantitative PCR

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Cyanobacterial blooms and a subsequent release of cyanotoxins into the environment pose a threat to all ecosystem services as well as human and animal health. Moreover, they cause significant economic damage due to their negative impact on touristic, recreational and industrial activities, agriculture and drinking water supply. Increased frequency of cyanobacterial blooms caused by rising temperatures and eutrophication is creating a growing need for reliable methods of early cyanotoxin threat detection in water bodies. Currently, risk assessment is based on traditional methods of identification and quantification of cyanobacterial cells and their toxins, such as morpho-taxonomic analysis and various analytical methods (e.g. HPLC, LC-MS, ELISA or PP1A). These analytical methods can be expensive and technically demanding, while microscopic analysis has a low sensitivity and is often unreliable due to taxonomic inconsistencies. In addition, all of these methods are very time-consuming. Thus, our aim is to develop a fast, cost-effective and sensitive tool for early detection of potentially toxic cyanobacteria.

By using environmental DNA (eDNA) extracted from water bodies and a qPCR method, we are targeting genes involved in crucial parts of cyanotoxin synthesis. Detection and quantification of these genes, if incorporated in regular monitoring, might serve as an early warning in case of an increased potential for cyanotoxin production. Toxins and genes of interest in this study are microcystins (*mcyE*), saxitoxins (*sxtA*) and cylindrospermopsins (*cyrJ*). The analyses will be carried out on 28 phytoplankton and 23 phytobenthos samples from 15 different freshwater bodies in Slovenia. Results from qPCR will serve for estimating the number of potentially toxin-producing cells. This will be compared with microscopically-determined cell number of known toxin-producing species, chlorophyll *a* content and cyanotoxin concentration measured by LC-MS/MS. By exploring correlations between these parameters, we aim to evaluate the potential of the qPCR method for implementation in biomonitoring programs.

Preliminary results show that the qPCR assays are highly specific and efficient in monocultures of cyanobacterial strains, whereas for complex environmental samples a cautious interpretation of results will be crucial. The expected research outcome is optimized protocol for early cyanotoxin threat detection based on eDNA and qPCR along with recommendations for implementation of these tools in water monitoring programs. Highly sensitive molecular methods can provide more in-depth information about the dynamics of toxic cyanobacterial populations in water bodies and thus help with adopting appropriate mitigation strategies. In the future, this approach could be upgraded with next-generation sequencing methods, functional genomics and metatranscriptomics in order to expand our understanding of cyanotoxin production even further.

# GC-MS analysis of contaminants of emerging concern: are their silylated derivatives stable enough?

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Structural elucidation of small molecules such as contaminants of emerging concern (CECs) and their transformation products is the major challenge in non-targeted mass spectrometry-based eco-exposomics. Chromatographic separation techniques (gas chromatography (GC) or liquid chromatography (LC)) coupled to high resolution accurate mass - tandem mass spectrometry (MS) are the ultimate analytical platforms used. Their robustness and reproducibility, complemented with the power of the cutting-edge computational methods, offer satisfying confidence during compound identification. In the context of GC-MS analysis, we often employ silylation in order to decrease polarity, improve thermal stability and chromatographic peak shape, thus making the resulting CEC derivatives more amenable for this type of analysis. Yet, the degradation of the silylated derivatives during their storage and analysis can seriously impair compound identification accuracy and confidence, supporting false negative results. Despite its importance, this issue has not been addressed to date.

This study examines the stability of trimethylsilyl (TMS) derivatives of 71 CECs in ethyl acetate, under the relevant conditions of their storage and analysis. In this respect, we performed three separate experiments, including short-term stability at the room temperature and in the fridge, long-term stability in the fridge and in the freezer, and freeze-thaw stability (Figure 1).

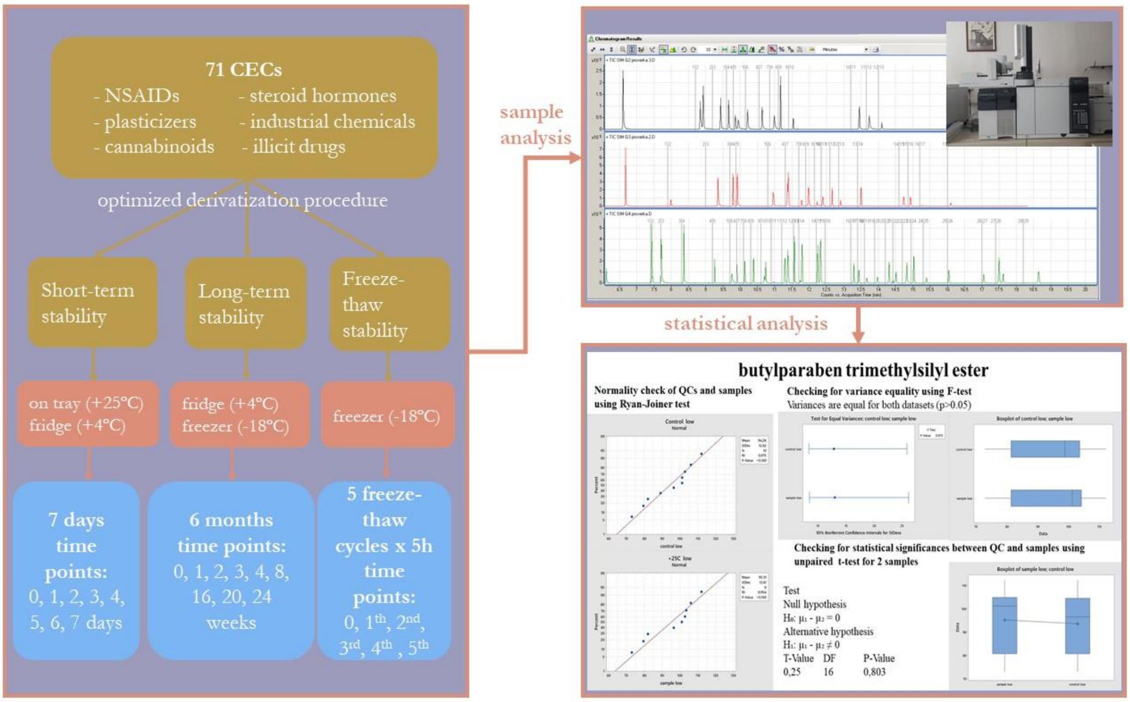

**Figure 1. Workflow of stability studies of TMS derivatives of CECs**

We divided the 71 CECs into three groups, according to the optimized derivatization conditions. Their stability was examined at the two concentration levels: 0.1 µg/mL and 1 µg/mL. Aliquots of mixed solutions in amber glass vials were derivatized using N, O-bistrifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and stored under above-described conditions. Samples were prepared in triplicates (n=3). At each sampling point, quality control (QC) samples in triplicates (n=3) at the two concentration levels were freshly prepared and used to monitor the method performance. Samples were analysed using an Agilent 7890B/5977A series GC-MSD. Method performance was evaluated by determination of linearity, accuracy, limits of detection and quantification, sensitivity, precision and measurement uncertainty. Multi-level experimental design and conventional statistical analysis were employed to assess the effect of temperature, storage time and concentration, and to establish their correlation.

Our results show that the stability of the TMS derivatives examined is within method's measurement uncertainty under all experimental set-ups. Overall, this study proves that identity confirmation of CECs and their transformation products using reference standards and GC-MS is possible without problems associated with degradation of TMS derivatives.

# Hourly sampling of tap water at Jožef Stefan Institute with mixed groundwater resources origin

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Water circulates in nature differently from urban environments, and supplying high-quality drinking water from various water resources of different quality parameters is a significant challenge. The knowledge about urban water circulation is, therefore, essential. Investigations of tap water have shown that isotopic composition also includes information on the geographical origin of water and some hydrological processes in the human-managed hydrological systems. In this context, environmental isotopes have become a well-established tool as they can propagate the isotopic "signatures" of water sources from natural or artificial mixing of different waters from various origins.

The first 24 hours experiment on tap water was performed in April 2019 with an emphasis on the hourly variability in the water sample. Samples were collected in the main building at Jožef Stefan Institute, where water from two different wellfields and aquifers (i.e. from Kleče at Ljubljansko polje and Brest from Ljubljansko barje) is mixed. In-situ measurements of temperature, electrical conductivity, and pH were performed every hour and in between every hour. Moreover, 25 water samples were collected hourly for determination of the isotopic composition of oxygen ($\delta^{18}O$), hydrogen ($\delta^{2}H$) and carbon in the dissolved inorganic carbon ($\delta^{13}C_{DIC}$), major (Ca, K, Mg, and Na) and trace elements (Ag, Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, U, V, and Zn). $^{87}Sr/^{86}Sr$ isotope ratio was determined only in 5 samples.

The diurnal variations of parameters determined in tap water were not very large, however some specific characteristics can be observed. Based on the observed temporal differences during the experiment we could identify three different patterns: i) higher values in the beginning and at the end and lower in between ($\delta^{18}O$, $\delta^{13}C_{DIC}$, Ca, Na, B, Ba, Cr, Li, Sr); ii) lower values in the beginning and at the end and higher in between (K, Mg, As, Mn, V); and iii.) higher values at the beginning of the experiment (Cd, Co, Fe, Mo, Ni, Pb, Sb, Zn). The first and the second pattern (i., ii) indicate that during the experiment proportion of water from two different sources (Kleče and Brest) changed and that in the beginning and at the end of the experiment higher share of drinking water was supplied from Kleče and in between from Brest. Besides, by applying simple linear mixing model element concentration (i.e., Mg) by considering data from wells sampled in 2018, we estimate that the proportion of water from Kleče changed from 60 to 100 % during the 24 hours experiment at selected sampling location. The third pattern (iii), however, indicates the influence of the leaching of specific elements (e.g., Fe, Ni, Pb, Zn), probably due to corrosion of the pipeline of the water supply system, at the beginning of the experiment, when drinking water starts flowing.

Founding: ARRS (Slovenian Young Researcher Program, Program P1-0143), and IAEA CRP 33024.

# Identification of volatile organic compounds in dry-cured ham

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The aroma is an important quality parameter of dry-cured ham and results from the biochemical and enzymatic processes that occur during different stages of ripening namely, as proteolysis, lipolysis, oxidation reaction, Maillard reaction and Strecker degradation, which contribute to flavor development. This study aims to develop a method that can distinguish the aroma profiles of four dry-cured hams with Protected Geographical Indication (PGI), i.e., where at least one of the stages of processing, production or preparation occurs in a specific geographical area, while the raw materials can come from somewhere else. The samples of dry-cured ham, analyzed in this study include two from Slovenia (Kraški pršut and a dry-cured ham prepared from the Krškopoljski pig, the only endemic pig breed in Slovenia), an Italian dry-cured ham (Prosciutto di Parma) and a Spanish dry-cured ham (Jamón Ibérico), which dominate the European market. The goal is to develop a gas chromatographic mass-spectrometric method (GC-MS) for the detection of volatile aroma compounds in dry-cured hams. Aromatic compounds will be isolated using solid-phase microextraction (SPME), which adds value to the analysis of aroma quality by detecting the most common volatile compounds belonging to various chemical families: aldehydes, phenols, aromatic hydrocarbons, aliphatic hydrocarbons, alcohols, ketones, esters, and terpenes [1,2].

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# Isolation and characterization of lignin degrading bacteria from soil and combining them in aggregates

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Lignin is a complex organic compound composed of different aromatic molecules and it is found in plants and algae. In plants lignin is forming the lignocellulose composite and its function is to give mechanical support as well as to prevent degradation of cell wall by microbes [1]. Since lignin is not easy to disintegrate it has been long considered as a waste product in papermaking, biofuel and other industries. Due to aromatic monomers forming lignin, it has been recently shown that many valuable aromatic products can be obtained. One of the option is to use microbial cells that degrade lignin polymer and release monomers. Oxidative enzymes, ligninases, excreted by microbes are involved in lignin decomposition and modification. The enzymes are capable of oxidising phenolic aromatic substrates that are often assisted with $H_2O_2$, which is acting as an oxidative agent and is produced by accessory enzymes [2]. Although fungi are predominant lignin degraders, their enzymes are less stable and extremely complex, which does not allow to produce them in high quantities in heterologous bacterial expression system. Although bacterial degradation shows great potential, valuable lignin-derived degradation products at industrial scale are still obtained by classical chemical methods [3]. According to the complex composition of lignin it is necessary to combine two or more different enzymes produced by several isolates to improve degradation and release lignin monomers. Bacterial enzymes chop lignin polymer at different points so by combining different bacterial cells together to form an aggregate we could attain faster lignin degradation and produce wider variety of aromatic compounds. To optimize enzymatic oxidation of lignin and achieve higher efficiency we propose a method that apply use of bacterial aggregates.

Therefore, in this study we sampled soil and we used ABTS screening test to get bacterial isolates producing laccase-like activity. The lignin degrading capacity of isolates was confirmed using $FeCl_3$ and $K_3[Fe(CN)_6]$ test. Strain growth optimization was carried out measuring growth and enzyme activity using ABTS assay. The phylogenetic placement of isolates was determined according to the 16S rRNA genes sequence. Whole genome was sequenced using MinION sequencing flow cell and annotated using PROKKA Genome Annotation. To determine the strain capacity to degrade lignin, after bacterial digestion its amount was measured using acetyl bromide method.

We found five strains isolated from soil showed lignin degrading capacity and carried genes for three lignin degrading enzymes: multicopper oxidase, polyphenol oxidase and blue copper oxidase. The optimal growth conditions for enzyme production is growth in minimal media LBM supplemented with copper and manganese ions at 30 °C at pH 5. 16S rRNA analysis showed all strains are phylogenetically very close relatives of *Burkholderia jirisanensis.* Further studies are going to determine strain capacity of lignin degradation and how combining different bacterial cells in aggregates can bring out different aromatic compounds.

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# Uncertainty associated with assessing personal exposure to particulate matter with high temporal resolution using low-cost portable sensors

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## Summary

Combining particulate matter (PM) concentration and heart rata (HR) data from low-cost sensors can provide personal exposure information with high temporal resolution. Uncertainty associated with low-cost sensors was determined by collocating them with reference instruments, and using this data to model personal exposure to PM. Four models were used with different levels of complexity (in reference to the variables implemented in the model). The results showed that the more complex models, using HR, sex, ethnicity and other variables as proxies to determine minute ventilation, responded better to changes in activity and provided data with less uncertainty. Low-cost sensors could be used for modelling personal exposure to particulate matter.

## Introduction

Exposure to PM has been linked to several health issues and a key step in understanding, and subsequently reducing its impact on health, is to accurately measure exposure on a personal level. Low-cost sensors facilitate extensive studies which can provide data with high-temporal and spatial resolution, but come with certain shortcomings stemming from simplified sensing technologies. Personal exposure calculations from this data have uncertainties which differ according to the type of model used. Several models were used to calculate personal exposure from heart rate and PM concentration data. Uncertainties associated with these models were compared to determine which variables have the highest impact on the uncertainty in each approach.

## Methodology and Results

A portable low-cost PM sensor was used to measure concentrations with high temporal resolution (1 min) for one week in the spring of 2019. Prior to using this sensor in field conditions, it was validated and the results showed that the $PM_1$ data had relatively low uncertainty. HR was used as a proxy for calculating minute ventilation, and was measured by a low-cost activity tracker.

Four models were used to determine personal exposure to PM (from most to least complex): 1. using HR, sex, ethnicity and age (Greenwald et al., 2019); 2. using HR and sex (Zuurbier et al., 2009); 3. using sex, age, body weight, microenvironment characteristics (Madureira et al., 2018); 4. using age and sex, and determining minute ventilation by using average values for specific age groups. Two models did not use HR as input data. The results showed that models 1 and 2 had similar patterns and were mostly in agreement, except at elevated concentration levels. Model 3 mostly followed the pattern from models 1 and 2, but also had some high deviations, which were even more evident at higher concentrations of PM. Model 4 did provide some correction to the raw PM data, but proved to be relatively unresponsive to changes in activity, compared to other models.

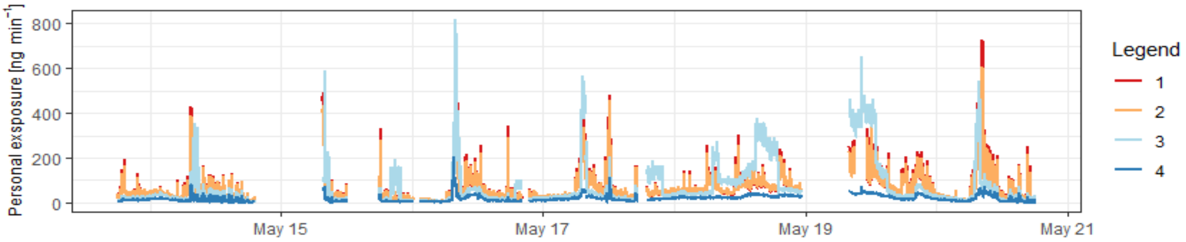

*Figure 1: Time series for all models, numbered from 1 to 4*

## Conclusions

The models used for calculating personal exposure proved to differ, mostly based on the number of considered variables. Models 1 and 2, which used HR as a proxy for minute ventilation, provided data that mostly corresponded with changes in activity and PM concentrations, and the models that did not use HR, showed less response to changes. This research showed that models which use data for multiple variables, which can also be obtained from non-intrusive low-cost sensors, have less uncertainty. Further research is also needed to validate modelled exposure with directly measured personal exposure.

## Acknowledgement

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# Use of Zirconium Isotopes for Strontium Isotopic Ratio Correction in Multi-Collector Mass Spectrometer

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Strontium isotope ratios, especially $^{87}$Sr/$^{86}$Sr give some key data to many studies from different scientific fields e.g. geology, biology. It is important to know these ratios as accurately as possible, to make the most informed conclusions for the relevant studies. One way of measuring the ratios of Sr isotopes is by Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS). MS devices exhibit internal isotope fractionation (IIF) sometimes referred to as mass bias. These are corrected using the correction procedures e.g. [1]. Corrections using standard sample bracketing (SSB) method and correction using the $^{88}$Sr are the most common. However, they might still be improved with by spiking the samples with an internal standard, e.g. Zr. Some authors have reported great improvements of the instrument's precision [2], while some found the opposite to be true [3]. It seems that only $^{91}$Zr/$^{90}$Zr and $^{92}$Zr/$^{90}$Zr have been used in the experimental studies in the literature, but not $^{92}$Zr/$^{91}$Zr, which are the closest according to the fractionation law. The corrections are carried out by linear, power or Russell's law which are all designed for mass dependant fractionation. It is very likely that the IIF in MC-ICP-MS is not just mass dependant but also mass independent [1].

In our experiment, we used the Nu plasma II (Nu instruments Ltd, UK) MC-ICP-MS. The measurements were performed on the biogenic carbonate and volcanic silicate. We used NIST 987 [4] and Zr standard material by Sigma-Aldrich (Buchs, Switzerland) as reference materials. For correction of $^{87}$Sr/$^{86}$Sr, we used $^{88}$Sr/$^{86}$Sr, $^{91}$Zr/$^{90}$Zr, $^{92}$Zr/$^{90}$Zr & $^{92}$Zr/$^{91}$Zr. The signal intensities were equalised between the $^{87}$Sr, $^{86}$Sr, $^{92}$Zr & $^{91}$Zr with appropriate amount of added spike. We used linear mass independent and Russell's mass dependent law for the corrections.

As per our results, we do not recommend any such studies without SSB or any internal corrections as they were completely erroneous. When it comes to the uncertainties of the measurements, use of $^{92}$Zr/$^{91}$Zr & $^{91}$Zr/$^{90}$Zr corrections had the lowest uncertainties amongst Zr pairs. Overall Sr pairs gave better results but this could be changed by additional removal of the interferences from the Zr standard. There was no significant difference between mass dependent and independent corrections. We conclude that signal intensity equalization is a more important factor than the equation used for IIF correction. With additional fine-tuning, this method of correction might be used for more precise Sr isotope determination with MC-ICP-MS.

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# Informacijske in komunikacijske tehnologije (Information and Communication Technologies)

# Analysis of Data on the Presence of Mould in the Celje Ceiling Paintings

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This work presents the analysis of data obtained from samples of damaged paintings on the Celje Ceiling in the Celje Regional Museum. Because of the old age of the paintings, and due to some micro-climate conditions, the paintings have started to deteriorate. The goal of the analysis is to build predictive models for the damage of the paintings, built from the existing data on those paintings, and score different factors for affecting the deterioration (moulding). The data on the paintings' samples was provided by the Institute for the Protection of Cultural heritage of Slovenia. It was pre-processed using some Python scripts and the data mining tasks classification and feature ranking were solved in Weka. Analysis was performed on all available data collectively and for each painting separately. Nine different classification methods were applied to each data set. Those are: decision trees, rules, bagging (with decision trees and rules), random forest, naïve Bayes, logistic regression, support vector machines and k-nearest neighbours. Two feature ranking approaches were tried: InfoGain and Relief. All models were built on the entire data set with 10-fold cross validation. The models were evaluated on their accuracy, per class precision and recall values, and their performance was estimated using area under ROC scores.

Due to the imbalanced class distribution, models built on individual painting data sets perform badly. Their precision and recall values extremely favour one class over the other (mouldy vs not mouldy samples) and their AUC values are very close to or even less than 0.5. Best results are observed when looking at the collective data set. All methods outperform the default classifier by at least 10% and output interpretable models that can be communicated with a domain expert.

Initial results show that the location of the sample has great effect on the mould i.e. that northern samples are very likely to have mould. Moreover, there are some attributes that often appear in trees and rules, and rank high in feature rankings, so they can be deemed important for the presence of mould, such as: goethite, dolomite, the coating of the paintings etc.

In preliminary consultations with experts in the domain we have concluded that these results are consistent with their knowledge. In the future, more data will be available on more historic paintings across Slovenia. Consequently, further analysis will be conducted.

# Finding an Optimal Landing Site for a Lunar Lander

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One of the main tasks in planning lunar lander missions is finding an optimal landing site where (1) the lander needs to target areas of special scientific interest, (2) the site has continuous Earth visibility to enhance the communication with ground stations, (3) the site is well illuminated to provide a good visibility of the lunar terrain, and (4) potentially hazardous terrains are avoided not to damage sensitive equipment. While (1) is normally decided before the launch, the objectives (2–4) are not fully computable in advance and can be analyzed just before the landing. This demands a high degree of autonomy and immediate response in case of critical situations [1, 2].

Finding an optimal lunar landing site with respect to objectives (2–4) can be mathematically formulated as an optimization problem and solving it requires to compare various sites of interest. The quality assessment (evaluation) of landing sites is highly computationally expensive. For this reason, it is required that a lander is capable of finding good sites while performing as few evaluations as possible [1, 2].

The optimization problem studied in our work was presented in the competition held at the Evolutionary Computation Symposium (ECS 2018) and provided by the Japan Aerospace Exploration Agency [3]. The problem originated from selecting a landing site in the south pole area of the Moon from the data recorded by the Kaguya lunar orbiter. The task was to find the latitude and the longitude of landing sites that represent good trade-offs between reliable communication with Earth, continuous periods of illumination, and small landing site inclination that reduces risk for the lander.

We solved the given problem using optimization methods based on evolutionary computation. First, we used visualization techniques to derive problem characteristics. Then, by matching these characteristics with the optimization method properties we were able to select the best performing evolutionary optimization method for our problem. Finally, since evolutionary methods involve various parameters controlling their operation, a sequential optimization approach was used to select appropriate parameter values. This way we were able to reproduce the best results obtained at the competition at a lower number of landing site evaluations. We see the results as beneficial for planning lunar lander missions in the future.

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# Personalized Eating Detection Using a Smartwatch

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Understanding the dietary habits of people plays a crucial role in interventions promoting healthy lifestyle. Obesity, which is a consequence of bad nutritional habits and increased energy intake, can be a major cause of cardiovascular diseases, diabetes or hypertension. Monitoring eating habits of overweight people is an important step towards improving nutritional habits and weight management. Another group of people that require monitoring of their eating behaviour are people with mild cognitive impairment and dementia. They often forget whether they have already eaten and, as a result, eat lunch or dinner multiple times a day or not at all, which might cause additional health problems. Proper treatment of these issues requires an objective measurement of the time at which the meal takes place, the duration of the meal and what the individual eats.

This work addresses a part of the technical challenges for a practical and reliable automated food intake monitoring system, specifically we focus on detecting eating activities. The main goal of our study is to develop a system that uses unobtrusive sensors that can be easily used during daily life activities. For this study we recorded data from 9 subjects in real-life scenarios, using a commercially available smartwatch containing 3-axis accelerometer and gyroscope. Each of the subjects had to record their activities including meals for at least two days, wearing the smartwatch on their dominant hand. Overall, the dataset contains 67 meals. There were no limitations about the type of activities that the subjects can perform during the day, nor the type of meals they can have.

The developed method relies on machine learning, following the established activity-recognition paradigm. It consists of three stages. The first two aim at training an eating detection models on an appropriate amount of representative eating and non-eating data. The first stage builds eating-detection models using all instances of eating and a subset of non-eating data (because there is much more non-eating data). The second stage adds some non-eating training data that is difficult to classify with the first-stage models. The third step takes into account the temporal information between predictions and smooths them using Hidden Markov Model. For this study, along the time-domain and frequency domain features, we developed some eating-specific features based on auto-correlation, which significantly improved the accuracy.

The experiments we performed showed that eating styles vary from person to person to a large degree. So, we decided to investigate the effect of personalized models. We evaluated the personalized models using leave-one-day-out cross-validation technique. In other words, in the training dataset for each subject we included data from all other subjects, and all days that the subject recorded except one, on which we later tested the performance of the trained model. The same procedure was repeated for each subject's day. For this study, we used the Random Forrest classifier, because it has been proven to be effective in the field of activity recognition. We analyzed the following evaluation metrics: recall, precision and F1 score. We achieved the precision of 0.7, recall of 0.85 and F1 score of 0.77. These results are calculated as a mean value from all subjects. The achieved precision and recall are encouraging for further work on this problem if we have in mind that the presented results are obtained on real-life recordings without any limitations on the subjects' daily activities.

# Robotic Camera Motion Optimization for Minimal Image Quality Degradation

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In the factories of the future, it is expected that the operations related to quality inspection will be done using the robots that will rely on visual feedback. However, setting up the visual feedback can be a tedious and demanding task for the robot programmer. In this paper, we present how robotic learning can be applied for learning a velocity profile of robotic motion used in quality inspection. Velocity optimization (that is usually done only at the start of the production) is expected to increase productivity by shortening the cycle times. Path used for the quality inspection of an object can be easily extracted from the CAD model of an object, but the velocity usually needs to be carefully hand-tuned by the robot operator. In order to make this hand-tuning process automated, we used two learning algorithms: Iterative Learning Control (ILC) and Policy Learning by Weighting Exploration with the Returns (PoWER). In our approach, trajectory obtained from the CAD model was encoded in parametric representation of Cartesian Dynamic Movement Primitives (CDMPs) with separated weights that represent the velocity profile to achieve a reduction of the number of parameters that need to be learned. To evaluate the sharpness of the image, we used squared gradient focus measure. Our approach was tested on UR10 robot and an object with curved surface. The initial velocity profile of the trajectory obtained from the CAD model was set to be minimum-jerk. The trajectory was first executed slow (in 60s) to obtain the reference sharpness. Same trajectory, executed in 10s, was then used as the initial trajectory of ILC algorithm. By following the changes in sharpness and changing the velocity weights, ILC algorithm reached 90% of the reference sharpness. PoWER algorithm used the results from ILC for further improvements. As the results in Figure 1 show, self-adaptation algorithms can be used to save both time and money by optimizing the production processes.

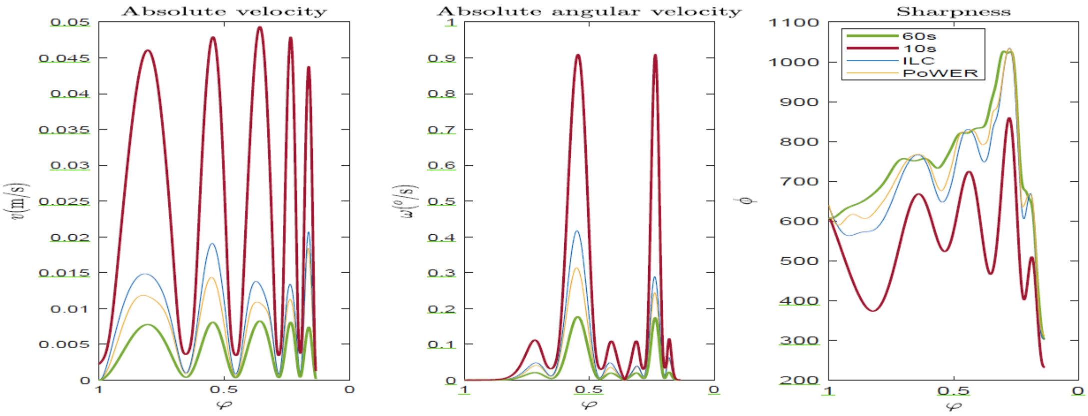

Figure 1: Figure 1: Absolute velocities, angular velocities and their corresponding sharpness in the phase domain of CDMPs. Green line represents quasi-static reference (minimum-jerk trajectory executed in 60s). Red line represents the initial trajectory used for learning (minimum-jerk trajectory executed in 10s). Blue line corresponds to the solution found by using ILC (execution time was 27s) and yellow line corresponds to the solution found by PoWER algorithm that was using ILC solution as the initial state for learning (execution time was 33s)

# Semantic analysis of lyrics for genre labeling and improving music recommendation

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Music as a cross-cultural phenomenon has been an integral part of human history. On the long run, average music listening in the USA is increasing, even though it fell from 32.1 hours per week in 2017 to 26.9 in 2019 Nielsen Music's Music 360 report, which is still an impressive number[1] [2]. Digitalization and the rise of streaming platforms have undoubtedly been key for this trend, as it is now easier than ever to access and discover music.

In principle, a large segment of musical work is instrumental. However, lyrics dominate in popular music and it is obvious that there they are organized in repetitive structures. The analysis of lyrics would lead to discovering features that would aid in tasks such as thematic analysis, genre classification, automatic tagging, sentiment analysis, music recommendation, etc. [3]. The combination of knowledge derived from the textual aspect of the musical piece with digital signal features could even further improve performances in the aforementioned tasks [4] [2]. Features can be engineered with the use of the Stanford CoreNLP library and can follow several dimensions of the lyrics, such as vocabulary, style, semantics, orientation, song structure and sentiment [1] [3]. Another useful resource for acquiring music tags is the last.fm service[4]. The music tags can add another layer of understanding how people interpret the musical piece. This opens the path for experimenting with multi-target classification models in the field of genre recognition, as music is fluid and can rarely be described with only one genre.

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# Nanoznanosti in nanotehnologije (Nanosciences and Nanotechnologies)

# A DFT study of co-adsorption between azole-type corrosion inhibitor molecules and corrosive relevant species on copper surfaces

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Azoles are five-membered cyclic aromatic compounds containing one nitrogen and at least one other heteroatom (N, O or S). They are known for their ability to decelerate corrosion processes on copper surfaces, i.e., they often act as corrosion inhibitors. The formation of a strong inhibitor−surface bond is believed to be an essential step in achieving inhibition of corrosion. However, as important as it might be, other factors, such as lateral cohesive intermolecular forces or effects of inhibitor adsorption on surface electronic properties (e.g. work-function, band-gap), should be considered as well.

In this study we utilized DFT calculations to investigate how co-adsorption of corrosion relevant species, such as $O_{(ads)}$, $H_{(ads)}$, $OH_{(ads)}$, and $Cl_{(ads)}$, affect intermolecular interactions and the strength of the inhibitor−surface bond. The first three species are typically involved in cathodic corrosion reactions, either oxygen reduction or hydrogen evolution, while $Cl_{(ads)}$ is a well-known corrosion activator. Our calculations show that co-adsorption of an overlayer of $O_{(ads)}$ can substantially increase the adsorption bonding of azole molecules on Cu(111) by up to 1 eV. According to our analysis, two factors contribute to this stabilization. The first is the formation of a H-bond between $O_{(ads)}$ and the NH fragment of the azole molecule. The second is the $O_{(ads)}$ induced enhancement of a direct covalent-like bond between the N-atom of the chemisorbed molecule and the surface Cu-atom. The latter enhancement is related to adsorption induced increase of the work-function of copper, that is, the larger is the work-function the stronger is the molecule−surface bond. Chemisorbed $Cl_{(ads)}$ also increases the work-function and enhances the inhibitor−surface bonding, but to a significantly lesser extent. In contrast, $OH_{(ads)}$ reduces the work-function and consequently weakens the adsorption bonding of inhibitors, whereas the presence of $H_{(ads)}$ does not significantly affect neither the work-function nor the molecular adsorption bonding.

Calculations further reveal, that in some cases co-adsorbed $O_{(ads)}$ is able to promote inhibitor's N−H bond cleavage, resulting in an even more stable and strongly adsorbed inhibitor molecule. These findings raise intriguing questions regarding the nature and extent of the effect that some corrosion relevant species have on the adsorption of inhibitor molecules.

# Crosstalk Between Autophagy and Lipid Droplets During Severe and Mild Starvation in Cancer Cells

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Organisms use lipids to build cell membranes, synthesize hormones and produce energy when other nutrient supplies are limited. In most cells, lipids are stored in the form of triacylglycerols (TAGs) and sterol esters (SEs) in lipid droplets (LDs) [1]. For a long time considered only inert fat reservoirs, these newly recognized organelles are now emerging as major regulators of lipid metabolism, but they have also been implicated in the protection of cells and tissues against stress. In cancer cells, LD biogenesis is induced in response to various kinds of stress, including nutrient deprivation or excess. Autophagy, a major cellular recycling and stress response mechanisms, is activated under similar conditions and emerging findings suggest multiple ways of crosstalk between LDs and autophagy [2–4]. The main goal of this study is to uncover how autophagy and LDs cooperate in driving the resistance of cancer cells to nutrient stress. We found here that LDs are dynamically formed and broken down in cancer cells depending on the severity and length of nutrient stress. Intriguingly, LD biogenesis is upregulated within hours of short-term, severe starvation, whereas a milder nutrient deficiency stimulates LD breakdown. Using inhibitors of TAG synthesis and LD formation, we show that the observed increased levels of LDs are a consequence of activation of LD biogenesis. Furthermore, we found that autophagic flux is significantly elevated in acutely starved cancer cells and that blocking autophagy leads to inhibition of LD biogenesis. Importantly, both LD formation and autophagy were essential for cancer cell survival during acute starvation. Surprisingly, we found that increased LD accumulation occurs also after a prolonged (5-day) serum deprivation. However, blocking autophagy led to a further increase in LD content, indicating that it participates in the breakdown of LDs (via "lipophagy") under these conditions. In summary, our results suggest that, depending on the specific nutrient stress conditions, autophagy drives LD biogenesis and/or LD breakdown and that this relationship is essential for the protection of cancer cells against metabolic stress.

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# Deep Cryogenic Treatment as the Next Processing Step in Steel Industry

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Steel industry has been important from the early years to nowadays for production of tools for every day's life. Throughout the years the chemical composition, purpose and shape have been changing, but certain procedures are insufficient or costly to provide the necessary steels needed in today's industry. In recent years, cryogenic treatment has been conservatively applied to improve properties of the steels. A common type of cryogenics is deep cryogenic treatment (DCT), where steel is subjected to temperatures below -160 °C. DCT is a technique used to transform austenite in daughter phases (mainly martensite) and consequently to change properties of the steel. DCT has been reported to change properties of steels such as corrosion and wear resistance, hardness, toughness, better machinability etc. The benefits are not only in changing the properties, but it also allows replacement of 2-3 cycles of tempering, which reduces productions costs[1]–[3]. This study had taken under consideration a group of high-speed steels, tool steels and stainless steels, which are commonly used in various industries. Selected groups of steel were systematically investigated for the influence of DCT on their microstructural, mechanical, and tribological properties. The study provides systematic data of DCT influence on the properties of selected steels showing that DCT is evidently an effective method for applications used in industry. However, its effect differs depending on the steel composition.

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# Design and modeling of arbitrary-shape permanent magnets and the stray-field calculation

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Additive manufacturing is a recently developed technique, which makes us possible to produce specially designed, single-unit permanent magnets. Its potential is to create magnets on the basis of certain surrounding stray fields, which can be determined by means of the magnetization modeling distribution. The main challenge for a producer of magnets, as the key parts of the conversion interfaces between electrical and mechanical energy, is to maintain the efficiency and performance of the various environmental condition and the presence of external magnetic fields, which might occur in electric motor or electric generator. The design of the magnet and its properties are modeled within the frame of the magneto-static problem by using the Free-Fem++ software.

# Determination of resistome using next-generation sequencing

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Antimicrobials are among the most important drugs. They made previously deadly infections curable and helped save millions of lives all across the world. However, ever since the introduction of antimicrobials there has been a growing concern of antimicrobial resistance (AMR). Many antimicrobials are now becoming ineffective against resistant bacterial strains. This results in longer hospitalizations and higher mortality which is an ever-larger burden for the health system and society as a whole [1].

The key step in the fight against AMR is antimicrobial susceptibility testing (AST). Knowing to which antimicrobials bacteria are resistant allows us to choose the correct antibiotic treatment and helps us slow down the spread of resistant strains and genes. In the clinical environment AST is mostly done by traditional microbiological methods of phenotypic determination such as disc diffusion and broth dilution after the isolation of pathogen bacteria. However, these methods require pure culture of bacteria and are thus time-consuming. The development of high-throughput DNA sequencing opened new possibilities in determining the resistome of bacteria. Resistome is the collection of all the antibiotic resistance genes and their precursors in an environment. We can determine resistome of either isolated bacterial cultures or in complex samples. There are two main approaches to how using next-generation sequencing (NGS) for determining resistome. The first is whole-genome sequencing (WGS) and the other is targeted sequencing. A few different platforms can be used for high-throughput sequencing one of which is also ion semiconductor sequencing [2, 3].

The aim of our study is to assess target sequencing approach in complex samples, namely of clinical sputum samples from University Clinic Golnik. We wish to determine resistome of bacteria in the sample through the detection of antibiotic resistance genes and support the analysis with metagenomic characterisation of microbial population. Whole DNA from the sputum samples was extracted using Hain GXT NA Extraction Kit and targeted regions were sequenced Ion Torrent next-generation sequencer Ion S5™ System. The obtained results will be compared to the results obtained by conventional methods of growing bacteria in the presence of an antibiotic. The method for detection of resistome developed as part of this research can later be applied to both clinical and environmental samples.

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# Liquid Crystal Elastomers as Soft Elastocaloric Materials

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Caloric effects are manifested in the heating or cooling a caloric material due to the application or removal, respectively, of the external field under nearly adiabatic conditions. Materials with large caloric effects, such as the elastocaloric (eC) effect, have the promise of realizing new solid-state refrigeration techniques [1]. Soft materials, called liquid crystal elastomers (LCEs), are good candidates exhibiting a large elastocaloric effect with potentially better elastocaloric responsivity than shape memory alloy wires, in which the eC temperature change of 40 K was observed at 0.8 GPa stress field [2], [3]. In this contribution a review of recent direct measurements of the eC effect in LCEs will be given. The eC temperature change of about 1 K was observed in MCLCEs at relatively small stress field of 0.6 MPa [4]. Soft materials can play a significant role as active cooling elements and parts of thermal diodes or regeneration material in the development of new cooling devices.

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# Local piezoelectric and magnetic properties of $(Bi_{0.88}Gd_{0.12})FeO_3$ ceramics

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For more than two decades, bismuth ferrite (BFO) has been a central material for fundamental multiferroics research, primarily because its intrinsic room-temperature multiferroicity, characterized by a spontaneous ferroelectric polarization along the $[111]_{pc}$ with a Curie temperature of 825 °C and G-type antiferromagnetic order with a Néel temperature of 370 °C [1, 2]. While the ferroelectric polarization of bismuth ferrite is promising the antiferromagnetic response is problematic due to its effectively zero remanent magnetization and low magnetic susceptibility. According to the literature it has been shown that breaking this antiferromagnetic ordering has been achieved by substitution of the A-site of perovskite lattice with a rare earth element – gadolinium [3].

In this work local piezoelectric and magnetic properties of gadolinium modified bismuth ferrite $Bi_{0.88}Gd_{0.12}FeO_3$ ceramics were investigated. Local measurements were performed using atomic force microscope (AFM) equipped with a piezoresponse force microscopy (PFM) and magnetic force microscopy (MFM) modes. PFM and MFM images show that the matrix possesses uncorrelated ferroelectric/ferroelastic and ferromagnetic domain structures, which indicates the multiferroic nature of $Bi_{0.88}Gd_{0.12}FeO_3$. Furthermore, Fe-rich secondary phases are not piezoelectric, but are ferromagnetic, as indicated by their weak magnetic domain structure (Figure 1). To conclude, in this contribution the local piezoelectric and magnetic properties in correlation with microscopic properties of $Bi_{0.88}Gd_{0.12}FeO_3$ ceramics will be discussed.

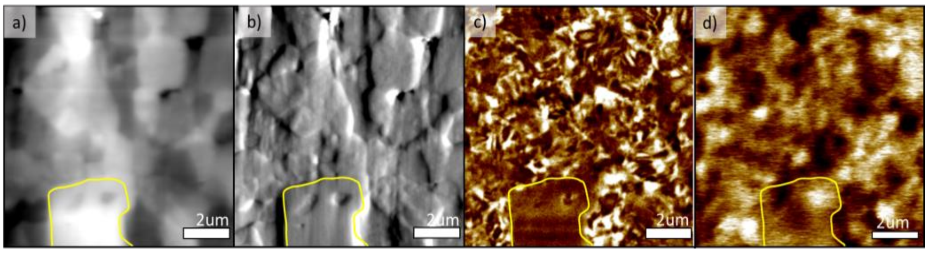

**Figure 1**. AFM topography a) height, b) deflection, c) PFM amplitude and d) MFM frequency images of a region with an inclusion of Fe-rich secondary phase. The yellow line traces the perimeter of the Fe-rich phase as a guide to the eye.

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# Metal pre-treatment by Zr and Ti conversion coatings as an eco-friendly alternative

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The return of a metallurgically processed metal to its initial state of ore, better known as corrosion, led to the establishment of a broad range of corrosion protection methods, with conversion coatings (CC) standing out as the most feasible ones. The reason for that is their ease of formation, as CCs are made by a chemical reaction between the metal and a conversion bath, resulting in a more corrosion-resistant surface [1].

The number of studies on CCs has increased in the last decades, owing to the environmental and economic issues regarding common chromate and phosphate conversion coatings. To date, CCs based on Zr/Ti salts have shown to be the most promising replacement, even maturing to several commercial applications on industrially important metals, namely aluminium, steel and galvanized steel.

Unfortunately, the observed performance of Zr/Ti coatings seems to be highly dependent on the substrate, reaching the poorest values on steel. To develop a multi-metal coating, a comprehensive optimization of numerous parameters (such as bath composition, pH, immersion time, temperature, mixing, etc.) for various metal substrates as well as on each system individually is essential. Besides, using various additives in the conversion bath is desired. Reportedly, Mo, Mn or Zn can enhance coating homogeneity, whereas Ce can upgrade the coating with a self-healing property.

A full insight into the protection mechanism, chemical composition and coating morphology requires a whole set of modern instrumental methods to be correlated, ranging from electrochemical tests (Open Circuit Potential measurements, Electrochemical Impedance Spectroscopy, Linear and Potentiodynamic Polarization, Salt Spray Tests), spectroscopy methods (X-ray Photoelectron Spectroscopy, Secondary Ion Mass Spectroscopy, and the like) to different microscopy techniques.

It is noteworthy that CCs present a pre-treatment layer in a multi-coating system (Figure 1). So, even before providing corrosion protection, a CC should promote adhesion of the subsequent layers. Thus, the evaluation of adhesion between the conversion and upper organic coating should serve as the ultimate indication of the overall corrosion performance [2].

After all of the above, the hitherto investigations on CCs with emphasis on improvements crucial for real applications on steel substrates will be covered in this work.

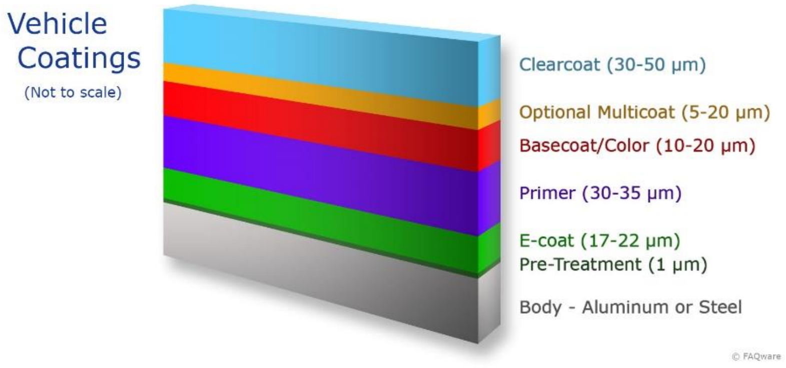

**Figure 1**. Vertical stack of a multi-coating system [3].

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# Phase behaviour of the suspensions of magnetic nanoplatelets

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Magnetic nanoplatelets dispersed in isotropic solvents form very interesting kinds of ferrofluids. For example, by increasing the concentration of nanoplatelets the paramagnetic ferrofluid transforms into a ferromagnetic nematic phase [1], making a unique example of a dipolar fluid – a liquid magnet. The interactions between the nanoplatelets, such as long-range dipolar magnetic and screened anisotropic electrostatic interactions, lead to the formation of the ferromagnetic nematic phase (Fig. 1). The magnetic nanoplateletes are ferrimagnetic Sc-substituted barium hexaferrite (BHF) of which surface is modified by dodecylbenzenesulfonic acid that forms an electrical double layer and provides for their colloidal stability in alcohol. The parameters of the electrostatic interactions between the BHF nanoplatelets are controlled with the fraction of the dodecylbenzenesulfonic acid [2].

The phase diagram was constructed as a function of concentrations of the dodecylbenzenesulfonic acid and size distribution of the platelets. These parameters affect magnetic and electrostatic interactions between the nanoplatelets and, thus, determine the phase behaviour of the suspensions and the formation of the liquid magnet. To distinguish between the birefringent ferromagnetic nematic phase and optically isotropic paramagnetic phase, we characterized suspensions with polarized optical microscopy. From constructed phase diagram of the suspensions, we determined the concentration at which the ferromagnetic nematic phase appears.

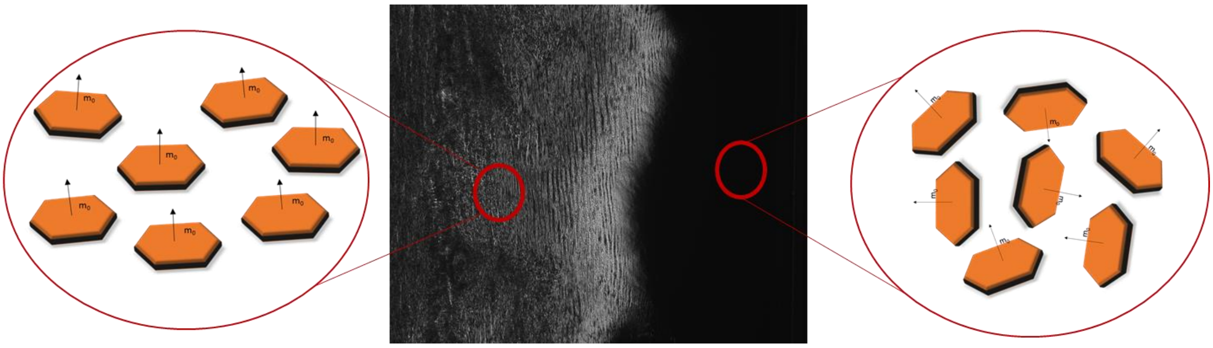

**Figure 1.** Optical microscopy image of the phase boundary between the nematic (left) and isotropic (right) phase in the concentrated suspension of the BHF nanoplatelets in 1-butanol.

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# Pore evolution in water-containing glass

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Cellular materials, natural or artificially created, are used on daily basis. Their usage ranges from simple- to high-tech-applications as they can be found almost anywhere from a disposable coffee cup to a crash-padding of an aircraft cockpit [1]. The process through which a cellular material is obtained is of upmost importance for its cellular structure, which inevitably affects its final properties and possible applications.

Formation of the cellular structure of foamed glass via sintering was investigated. Focus of interest was the initial stage of pore formation (i.e. nucleation). Foamed glass was prepared by heat-treating waste cathode ray tube (CRT) panel glass which was hydrated beforehand. Sintering temperature, expansion, relative density, pore size and type of porosity were followed by means of heating stage microscope, Archimedes density measurements, SEM and thermo-gravimetric measurements coupled with mass spectroscopy. Pore evolution during heat-treatment was investigated in a low-temperature range, where pore nucleation is normally dominant over other processes such as growth or coalescence (< 700 °C). Afterwards, a combination of low- and high-temperature experiments was set. Effect of initial pore evolution on final foam structure was evaluated from the comparison between the pore structure of foams prepared in initial and latter experiments.

CRT glass was hydrated so that it contained ~ 10 wt.% of $H_2O$. Direct foaming of such hydrated glass at 820 °C resulted in expansion, achieving porosity of 83 vol.%. During low-temperature stage of the heat-treatment (650 °C), porosity of the foam increases with time (up to ~ 75 vol.%), while the pore size remains relatively small (< 50 μm). Density decrease and pore growth become more pronounced with increasing temperature. Expansion gas in the case of hydrated CRT is evolving at wide temperature range and is overlapping with sintering temperature. Extended low-temperature step thus postpones the closing of the pores resulting in more expansion gas escaping into the atmosphere. Extending the time of low-temperature step before the high-temperature foaming resulted in denser foams with smaller and more homogeneously distributed pores. Such structure has a positive effect on the properties of foamed glass and is desired, while increased density is not. Therefore, the feasibility of a foaming process with extended low-temperature step should be further evaluated based on foams' thermal and mechanical properties.

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# Room temperature fabrication of strontium titanate ceramics

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Room temperature fabrication (RTF) of bulk ceramic upside-down composites relies on incorporation of high loading of filler material with required functional properties, and corresponding binder partly in aqueous solution. During the pressing and drying of the ceramic body, the binder crystallizes on the surface of the filler particles and thus physically binds them together. In this way lower porosity and good packing density can be achieved, resulting in increased functional properties. The method itself is based on soft solution processing, as a combination of mixing, moulding with steel dyes, pressing and drying. A novel and innovative technique of ceramics densification at room temperature is beneficial since as-prepared ceramics exhibit well-developed electrical characteristics without the need of their sintering at high temperatures, thus considerably reducing ceramics fabrication costs.

Our research encompasses synthesis of the upside-down composites from strontium titanate (STO) and lithium molybdate for their use in various electronic applications. In the scope of this investigation, our aim is to investigate and determine the effect of various synthesis parameters, such as starting materials combinations on ceramics microstructural and functional properties. We determined that by proper particle size distribution and higher packing density mechanical stability and dielectric behaviour can be improved. Although it is clear that the dielectric permittivity is lower than measured for pure STO due to the presence of the binder, the achieve properties are feasible for several applications. Thus, our results demonstrate that the RTF of the upside-down ceramics paves the way to a novel processing of electroceramics without substantially compromising their functionality and enables further integration possibilities in various electronic devices [1,2].

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# Senzorske tehnologije (Sensor Technologies)

# In Altruism, Size Doesn't Matter: The Heroic Case for *Kosakonia* vs *Erwinia* in Papaya

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Bacterial crown rot (BCR) is a disease of papaya (*Carica papaya*) in Southeast Asia caused by a bacterium known as *Erwinia*. Observations using electron microscrope revealed colonization of an unknown bacterial community common in plants tolerant to crown rot, before its infected tissues regrow. This led to the hypothesis that these papaya-associated bacteria might play an altruistic or symbiotic role in fighting against *Erwinia* in exchange for nutrients and its dwelling inside the papaya tissues. Isolation, characterization and identification using 16S rRNA sequence of these bacteria resulted to the discovery of two endophytic bacteria, *Kosakonia* and *Sphingomonas,* and three root-associated *Bacillus* species. Separate inhibition assays indicated that papaya latex and culture metabolites from these bacteria, most notably from *Kosakonia* (isolate EBW), promptly (within 18-30h) and efficiently (60-65%) inhibited *Erwinia* proliferation. Moreover, when metabolites from EBW were incorporated in papaya seeds, it variably retarded seed germination (20-60%), but significantly stimulated plant growth and biomass accumulation, at around two-fold increase. Interestingly, EBW metabolites significantly reduced crown rot disease incidence and severity in susceptible genotype at around two-fold as well. Overall, these results indicated that papaya's fight against *Erwinia* infection is not only influenced by host innate characterstics such as tissue regrowth and pathogen inhibition by plant latex, but also with external help from *Kosakonia* for antibacterial activity, growth promotion and defense biopriming using its beneficial metabolites. Finally, these results also suggest possible utility of culture metabolites from altruistic bacteria in plants as potent and cheaper substitute for chemical pesticides that impose harmful risks to human and animal health and are known to contaminate the enviroment.

# Colors and colored overlays in dyslexia treatment

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Colors are an important factor for the emotional and psychological states of the body, they are impacting the body state also with colors of the learning environment. There is a lot of discussions in previous literature about the color influence on the reading, especially on children with and without dyslexia. In some papers, we can find that the use of colors can enhance the reading of children with dyslexia.

The paper presents a user study with 50 children whose readability was measured via a sensor hub in order to detect the effect of background and overlay text colors on reading possibilities.

Comparing to the previous studies with children with dyslexia there is a correlation between background colors such as Peach, Orange, or Yellow and benefits for readability. We have found the same correlation in children without dyslexia in the same color range.

Also, comparing the results in children with dyslexia and their readability in cool background colors, in particular Blue Grey, Blue, and Green, we found a correlation with decreased text readability. The results show that using certain background and overlay colors have a significant impact on children with and without dyslexia.

# Wastewater viromics and infectivity tests shed light on the infective plant viruses released in the environment

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In the recent decade, viral metagenomics studies revealed high diversity of viruses in environmental water samples. Water can be an important source of human, animal and plant infections, caused by pathogenic viruses. Wastewater may act as a transmission route for some stable viruses, possibly enabling the spread of the disease to distant environments. Transmission of plant pathogenic viruses is especially relevant in the case of reclaimed wastewater usage for irrigation purposes, which is a common practice in many arid regions worldwide. Our aim was to assess the wastewater virome with special focus on plant viruses, the relevance of such viruses in terms of infectivity and the ability of traditional wastewater treatment for their inactivation. For this purpose, we analysed samples of the influent and effluent of a wastewater treatment plant, representing a possible source for the release of pathogenic plant viruses into the environment. First, we concentrated viruses using Convective Interaction Media monolithic chromatography, a method that can efficiently concentrate viruses from high-volume water samples. Then, we used shotgun high-throughput sequencing to detect the presence of a wide array of viruses from different taxonomic groups. We detected the most common plant viruses by quantitative PCR and confirmed their integrity using transmission electron microscopy. Following, we focused on assaying the infectivity of plant viruses from the genus *Tobamovirus* (family *Virgaviridae*) that are highly stable and were abundant in investigated wastewater samples according to high-throughput sequencing analysis. Using test plants mechanically inoculated with concentrated samples of influents and effluents of the wastewater treatment plant, we were able to confirm the infectivity of some tobamoviruses before and after wastewater treatment.

The findings shed light on the poorly explored diversity of plant viruses in wastewater viromes and their potential impact. Plant viruses remain infective even after conventional wastewater treatment, which is rising concerns on the uncontrolled use of reclaimed water for irrigation purposes.

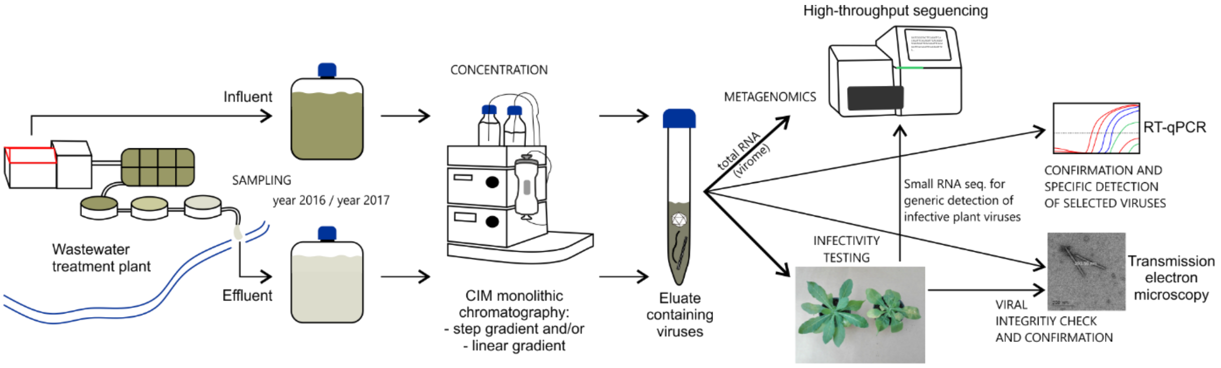

**Figure 1**. A scheme of the main steps of the study including wastewater sampling, viral concentration, metagenomic analysis and infectivity testing.

# Water Virome, why do we care?

**Olivera Maksimović Carvalho Ferreira[1, 2], Katarina Bačnik[1, 2], Denis Kutnjak[1], Maja Ravnikar[1, 3], Ion Gutierrez-Aguirre[1]**

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The global water shortage is looming on the horizon, which is causing a growing interest in the field of water reuse for industrial and cropland applications. However, commercial sewage wastewater treatment systems, do not accommodate for sufficiently efficient virus removal, especially with high influent loads. This means that human, animal and plant pathogens can be recirculated in the area of effluent discharge or reuse [1]. Plant viruses have a big influence on environment, agriculture and economy. Estimations speculate that up to 40% of crop losses, especially in developing countries is consequence of diseases and pests [2]. Furthermore, there is sufficient evidence in support of the concern that water can be a transmission route for infections of viral origin [3].

Water represents a challenging matrix for testing. Depending on its origin, it may contain multiple contaminants that can influence the downstream analysis. In regards to viruses, they are generally present in low amounts and multiple steps of concentration have to be employed order to detect them. A recently developed workflow that allows deep and efficient research into water virome comprises concentration by chromatographic monolith columns and pre-amplification of available genomic material prior to shot-gun high-throughput sequencing (HTS) and inoculation of test plants [4].

One of the doctoral projects that will run in the frame of the recently awarded MSCA-ITN INEXTVIR action coordinated by the National Institute of Biology (Fig.1), includes a study of irrigation waters used in European countries. The study aims to give us an answer on how does the virome change depending on the source of water, what population of plant (and other) viruses are present in the water and what is the probability of transmission of viruses directly by irrigation or indirectly by viruses entering broader environment. The study will also ensure that all steps involved in the analysis of water are optimized (Fig. 2), including viral concentration, HTS platforms and bioinformatics data analysis.

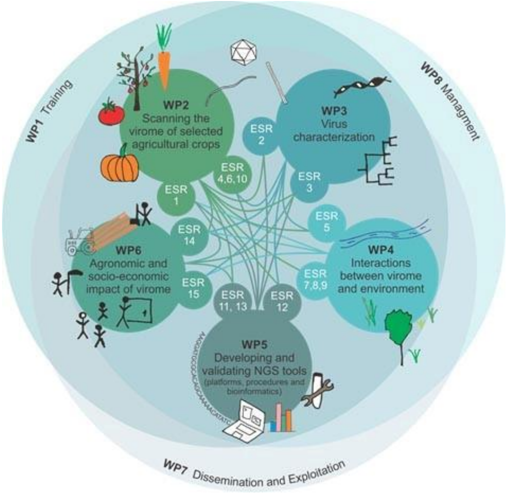

**Figure 1**. Overview of INEXTVIR structure.

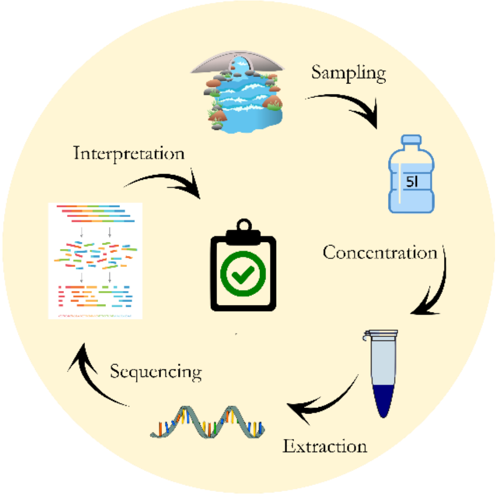

**Figure 2**. Overview of water analysis structure.

References:

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# Drugi programi (Other programs)

# A tunable synthetic procedure for mesoporous silica shells on magnetic core nanoparticles

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Core-shell nanoparticles consisting of a magnetic core and highly porous shell are interesting materials due to the combined abilities of remote magnetic manipulation and transport of various molecular cargos in significant amounts. Such type of nanomaterials found use in biomedical fields as drug delivery vehicles and also as supports for catalysis.

Iron oxides are a well-known material for producing biocompatible and chemically stable magnetic nanoparticles. Additional functionalities can be achieved by applying suitable coatings on their surfaces. A popular example are mesoporous silica shells. Silica exhibits high chemical stability, biocompatibility, and enhanced colloidal stability. The large density of surface hydroxyl groups can be used for various chemical modifications. Mesoporous silica is considered as suitable and universal drug carrying material as well as catalyst support. Its mesoporous structure, shell thickness and pore size play a significant role to reach desired properties that are needed for specific applications. These parameters can be adjusted by modifying the reaction conditions in the soft-templating method which is the most common method for mesoporous silica preparation. The soft-templating method consists of a mixture of a surfactant, catalyst, organic solvent as pore expander, silica precursor and iron oxide nanoparticles as core material.

The main goal is to develop a precisely tunable procedure for mesoporous silica shell synthesis with diverse morphological properties. Additionally, the process should allow complete avoidance of the formation of homogeneously deposited silica. Therefore, we designed a series of experiments to study the effects of several parameters that affect the shell properties including i) surfactant concentration, ii) catalysts, iii) organic solvents, iv) silica precursor and v) reaction temperature. We optimized the synthesis to construct the mesoporous silica shell on different types of magnetic core nanoparticles successfully (Figure 1). The shell was highly porous with radially aligned pores of sizes up to 40 nm and coating thickness of up to 90 nm. Such nanostructures show great potential in drug delivery and catalysis.

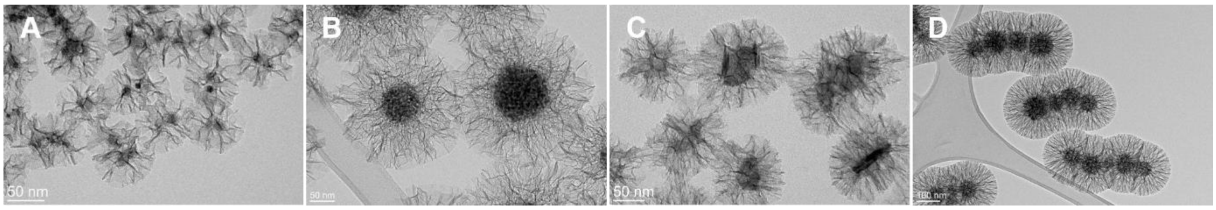

**Figure 1**. Transmission microscope micrographs of mesoporous silica coatings on (A) superparamagnetic iron oxide nanoparticles, (B) superparamagnetic iron oxide nanoclusters and (C) ferromagnetic barium hexaferrite nanoplatelets and (C) superparamagnetic iron oxide nanochains.

# Changes in cystatin F N-glycosylation impact natural killer cell cytotoxicity

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**Background:** Cystatin F is an endogenous inhibitor of cysteine peptidases called cathepsins, expressed in immune cells. It can be secreted but is only active intracellularly in monomeric form. N-glycosylation of cystatin F is important for its internalization into cells and trafficking to endosomes/lysosomes. Monomeric N-terminally truncated cystatin F is a potent inhibitor of cathepsin C which is an important regulator of the cytotoxicity of natural killer cells (NK). Cathepsin C activates granzyme B from the precursor form. Supercharged NKs are ex vivo expanded primary NKs with increased cytotoxic ability and secretion of IFNγ.

**Objectives:** We aim to analyze N-glycosylation of cystatin F in primary and supercharged NKs and determine their differential effect on the function of NK cells.

**Methods:** Primary NKs will be isolated from healthy donors PBMCs. Supercharged NK cells will be prepared by culturing primary NKs with allogeneic osteoclasts and sonicated probiotic bacteria sAJ2. Cystatin F and granzyme B expression will be determined by proteomics and SDS-PAGE and western blot. Glycosylation will be analyzed using deglycosylation enzymes PNGF and ENDO H. Granzyme B activity in lysates will be analyzed by measuring fluorescent degradation products after substrate cleavage. NK cytotoxicity will be measured using chromium-51 release assay.

**Results:** Recombinant wild type cystatin F can be internalized into primary NKs and causes decreased cytotoxicity. The glycosylation profile of endogenous monomeric cystatin F is different in primary NKs versus supercharged NKs. Supercharged NKs express increased protein levels of granzyme B with increased activity compared to primary NKs. This is reflected by increased cytotoxicity of supercharged NKs compared to primary NKs.

**Conclusion**: Noted differences in the N-glycosylation of cystatin F in supercharged NKs are likely to impact their functional activity. We speculate that the differences in N-glycosylation of cystatin F will render it dysfunctional, resulting in an increase in NK cell effector molecule granzyme B, and increased NK cell cytotoxicity.

# Effects of α7-nicotinic acetylcholine receptor antagonists on cancer cells overexpressing these receptors

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Lung cancer still represents a major problem in healthcare and is statistically the second most common cancer in both men and women, whereas cigarette smoking is the leading risk factor of lung cancer. Although nicotine is not a carcinogen itself, tobacco contains thousands of chemicals and several compounds have been recognised as human carcinogens. Furthermore, it has been demonstrated that continuation of smoking during therapy of tobacco-related cancers is associated with lower response rates to chemotherapy and/or radiotherapy. In some cancer types, including lung cancer, elevated expression levels of nicotinic acetylcholine receptors (nAChRs) have been reported. These receptors can be stimulated by locally synthesized or circulating ACh (forming an autocrine cholinergic loop), as well as by nicotine and its derivatives - nitrosamines. Binding of agonists to specific subtypes of nAChR (mainly α7 and α9) was shown to increase intracellular $Ca^{2+}$ levels. Moreover, it leads to activation of signalling pathways that trigger cell division, prevent apoptosis and drive angiogenesis, thereby supporting tumour growth and metastasis. On the contrary, antagonists of these receptors have demonstrated opposite effects, suggesting their potential in cancer therapy [1]. To explore this possibility, the main goal of our study is to analyse signalling pathways of several naturally occurring α7-nAChR antagonists, such as snake venom secreted phospholipases $A_2$ ($sPLA_2s$), α-conotoxins and 3-alkylpyridinium polymers (poly-APS) on selected cancer cell lines. Currently we are in the preliminary phase of our study, preparing the molecular tools – we expressed and isolated recombinant human group V and X $sPLA_2s$. Moreover, since it has been suggested that $sPLA_2s$ act as allosteric ligands of nAChRs and are able to suppress nAChR-signalling independently of their enzymatic activity [2], we have also prepared their enzymatically inactive mutants, harbouring a H48Q point mutation in the active site. These proteins were fully characterized in terms of the molecular mass, N-terminal amino acid sequence and enzymatic activity.

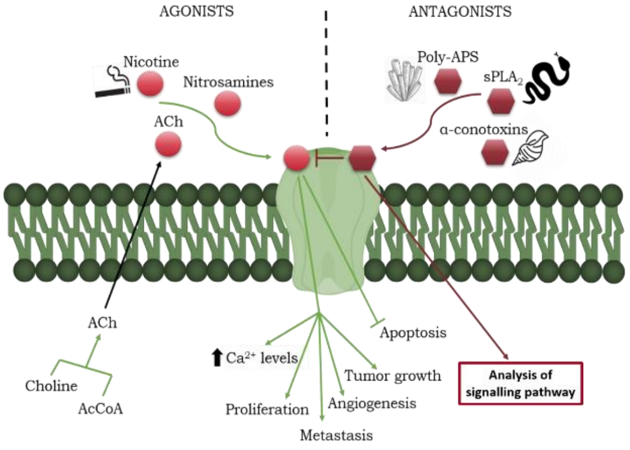

**Figure 1**. Agonists and antagonists of nAChRs and their effects

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# Engineered *Lactococcus lactis* for tumor cell lines targeting

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Lactic acid bacterium (LAB) *Lactococcus lactis* is considered an attractive host for recombinant protein expression and a promising vector for *in vivo* delivery of bioactive proteins by secretion or surface display. Display of recombinant proteins on the surface of LAB has already been exploited in therapy to prepare mucosal vaccines, to display binding molecules directed against pro-inflammatory cytokines in inflammatory bowel disease and to deliver antioxidant molecules in prevention of colorectal cancer. By displaying proteins targeting tumor antigens on the bacterial surface, directed binding of *L. lactis* to cancer cells could be achieved, essentially enabling targeted treatment with fewer side effects.

In the present study, we focused on the development of a system for targeted binding of *L. lactis* to colorectal tumor cell lines. We applied proteins with affinity for three tumor antigens, which are typically overexpressed in tumor cells of colorectal cancer, EpCAM, Her2 and Gb3. Genetic constructs for surface display included genes for affitin with affinity towards EpCAM, for affibody with affinity towards Her2 and for B subunit of Shiga toxin with affinity towards Gb3. Besides specific binding to colorectal cancer cell lines by using targeting proteins, we aimed at concomitant imaging of bound bacteria. We therefore simultaneously expressed infrared fluorescent protein (IRFP) in bacterial cytoplasm, by using plasmid for double protein expression. Surface display of FLAG-labelled targeting proteins was confirmed by flow cytometry, while expression of IRFP fluorescent protein was determined by measuring fluorescence. Furthermore, we confirmed, with flow cytometry, binding of soluble tumor antigens, EpCAM and Her2, to bacteria displaying their respective targeting proteins. *L. lactis* displaying targeting proteins and IRFP were able to selectively recognize selected colorectal human tumor cell lines, indicating their promising targeting ability.

# Interaction between group IIA secreted phospholipases $A_2$ and mitochondria

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Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are among the leading causes of mortality and morbidity in the developed countries with their prevalence continuing to rise as the population ages. Currently, they are incurable and the only available treatment is symptomatic. Although diverse in their pathophysiology - some causing memory and cognitive impairments and others affecting a person's ability to move, speak and breathe - they are all characterized by neurodegeneration, the progressive loss of structure and function of neurons. Despite many hypotheses, their exact etiologies and molecular mechanisms remain unclear. One of the events leading to neuronal degeneration is damage to mitochondria, caused by, among others, elevated activity of the secreted phospholipase $A_2$ group IIA (GIIA), an enzyme that hydrolyses glycerophospholipids to *sn-2* lysophospholipids and fatty acids. While the exact molecular mechanisms of action of GIIA in these diseases are far from being unravelled, the damage it inflicts on neuronal mitochondria is characteristic and very similar to that induced by structurally homologous β-neurotoxins (β-ntxs) that can be found in snake venoms. Therefore, a description of the mode by which β-ntxs encounter and affect neuronal mitochondria on the molecular level would be expected to suggest the role of endogenous GIIA in mentioned pathological conditions. Using ammodytoxin (Atx), a β-ntx from the venom of the nose-horned viper (*Vipera ammodytes ammodytes*), we detected a high affinity $sPLA_2$ membrane receptor in neuronal mitochondria and identified it as the subunit II of cytochrome C oxidase (CCOX-II), an essential constituent of the respiratory chain complex [1]. We have shown that Atx inhibited the enzymatic activity of CCOX in both isolated mitochondria and on rat brain tissue sections. Interestingly, the observed inhibitory effect of Atx was not dependent on its phospholipase activity, as also the enzymatically inactive mutant of Atx was able to elicit it. Using heterologous competition assay, we demonstrated that the mammalian GIIA binds to CCOX-II with a 100-fold lower affinity than Atx. Homologous competition assay using $^{125}$I-GIIA, however, revealed that the endogenous $sPLA_2$ primarily targeted another binding protein in mitochondria with an apparently smaller molecular mass than CCOX-II. Taken together, our results suggest the explanation of the mechanism by which β-ntxs hinder the production of ATP in the poisoned nerve ending and open an important direction of study to advance the understanding of the involvement of the mammalian GIIA in mitochondrial function and dysfunction.

Reference:

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# LC-MS/MS analysis of bacterial lignin degradation products

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Lignin is a complex and heterogenous aromatic biopolymer that can be found in plant cell walls as one of the components of lignocellulose. Large quantities of lignin are daily generated as a by-product in paper and biofuel industries. To date, it is still treated as waste and is mostly burned as a low-quality fuel, even though it could potentially be a great source for producing value-added chemicals and fuels as it is an abundant source of renewable aromatic carbon.

As a result of the energy crisis, the interest in lignin and its valorization has grown considerably in recent years, mainly focusing on more environmentally friendly lignin degradation by microorganisms. The majority of research has so far been concentrating on fungal lignin degradation. However, fungi unfortunately seem to be unsuitable for large-scale application because of the complexity of their enzymes, hence challenging heterologous expression in bacterial strains that are easy to cultivate and produce biomass. Ligninolytic enzymes are very rare in bacteria, but those that possess this physiological trait present new better option than using fungi to valorize lignin.

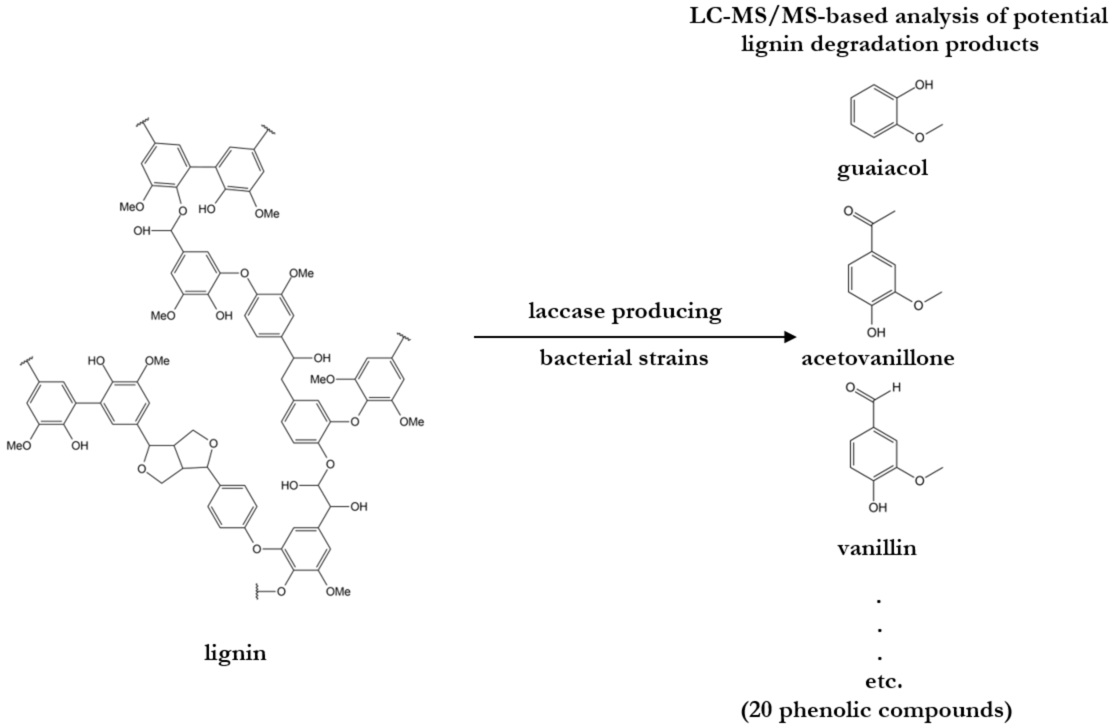

**Figure 1**. Potential lignin degradation products

Our research focuses on bacterial lignin degradation, with the objective to study the ligninolytic activity of 30 laccase-producing bacterial strains, laccase being an important lignin-modifying enzyme. Based on the existing literature we defined 20 phenolic compounds, which could potentially appear as products of bacterial lignin degradation. These compounds (Figure 1) were then included in our analytical method for targeted screening of lignin degradation products. The analytical method is based on determination by reversed-phase liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS). For the chromatographic separation we used the Shimadzu Nexera X2 ultra-high performance LC system with the 2,1 x 100mm (1,7µm) HSS T3 Acquity (Waters Corp.) analytical column. The detection was performed in the multiple reaction monitoring mode on the Sciex Qtrap 4500 MS in the negative electrospray ionization mode. Prior to the LC-MS/MS analysis we purified the samples by solid-phase extraction using the wide-polarity range Oasis HLB sorbent (60 mg, 96-well plates, Waters Corp.). In the next step, the analytical method is going to be tested on real samples, containing lignin treated with either of the laccase-producing bacterial strains, in order to screen for the strains with the ligninolytic activity.

Acknowledgements: The authors acknowledge the financial support from the Slovenian Research Agency (research core funding No. P1-0143) European Urban Initiative Actions founded project "Applause" (Grant agreement UIA02-228) and European Commission project Greener (Grant no. 826312).

# Protein binders of tumor-associated antigens and proinflammatory cytokines simultaneously displayed on *Lactococcus lactis* for targeted therapy of colorectal cancer

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Proinflammatory cytokines interleukin (IL)-6, tumor necrosis factor (TNF)-alpha, and IL-8 are involved in the promotion and progression of colorectal cancer (CRC). The safe probiotic lactic acid bacteria engineered to target CRC and remove cytokines from its environment represent a possible therapeutic approach in inflammation-associated CRC.

We constructed six bi-functional *Lactococcus lactis* strains, each one of them simultaneously displays two small protein binders on their surface: a ligand for tumor-associated antigen (HER2-binding affibody or EpCam-binding affitin) and a ligand for proinflammatory cytokine (IL-6-binding affibody, TNF-α-binding affibody or IL-8-binding evasin). Probiotics exert intrinsic beneficial effects in CRC prevention including induction of cancer cell apoptosis, neutralization of reactive oxygen species and microbiota regulation. Cytokine-binding proteins displayed on *L. lactis* surface are aimed at improving these beneficial effects through anti-inflammatory activity. The proteins directed towards tumor-associated antigens serve for homing of bacteria to the CRC, thus enhancing their natural tendency to accumulate in the tumor. Simultaneous surface display of two proteins was achieved by cloning genes into a lactococcal plasmid for dual protein expression, which was constructed in our previous study by doubling the nisin promoter. The protein binders were fused with secretion signal and AcmA anchor thus enabling the attachment of protein binders onto the surface. The expression of HER2- and EpCam-binding ligands was demonstrated by Western blot or flow cytometry. To determine if the cytokine-binding ligands on the bacteria surface are functional we tested their capacity to bind cytokines from spiked solution using ELISA. Engineered bacteria sequestered from 80% to 100% of the corresponding cytokine compared to control. The most efficient strain was *L. lactis*-displaying IL-6-binding affibody. The ability of engineered bacteria to remove cytokines secreted by colorectal cells is currently being tested on Caco-2 and HT29 cell lines primed with IL-1-beta, lipopolysaccharide, and TNF-alpha.

# Proteins binding to sense and antisense transcript from *C9orf72* gene mutation

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Mutation in *C9orf72* gene is the most common genetic cause of two neurodegenerative diseases - amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Both ALS and FTD are incurable and progressive diseases. ALS is the disease of motor neurons, while in FTD neurons in frontal and temporal cortex are most affected. Even though, these diseases present differently in regard to patients` symptoms, on genetic and neuropathological spectrum they are very close. The mutation in *C9orf72* gene causes up to 40% of hereditary ALS cases and 25% of hereditary FTD cases, it occurs in non-coding region of the gene and is presented as increased number of polymorphic hexanucleotide repeats of GGGGCC. There are up to 23 repeats in healthy individuals, while patients have several hundred or several thousand repeats.

There are three pathological mechanisms proposed for the *C9orf72* mutation. First, reduction in expression of the gene can lead to haploinsufficiency of C9orf72 protein. Second, hexanucleotide repeats can be transcribed to RNA, which is proposed to be toxic for the cell on the account of sequestering RNA binding proteins important for normal cell functioning. Third, RNA transcribed from repeats can be transcribed into proteins with dipeptide repeats (DPRs), which are also to be toxic for the cell.

Our aim is to investigate impact of sense and antisense RNA transcripts from the mutation on the development and progression of ALS and FTD. The extended hexanucleotide repeats of GGGGCC are transcribed into RNA in the sense ($G_4C_2$) and antisense ($C_4G_2$) form, both of which form mostly nuclear RNA foci in spinal cord and brain neurons of C9 ALS/FTD patients. These RNA foci are proposed to sequester RNA binding proteins and, therefore, interfere with their function. In our research we have identified several proteins binding to sense and antisense transcripts, which are involved in various cell processes. We will present latest findings on the impact these interactions could have on cell function, and, therefore, on disease development and progression.

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