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BOOK OF ABSTRACTS

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2015

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LJUBLJANA, SEPTEMBER 2015

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CUTTING
EDGE 2015
SCIENCE BEHIND THE LIVING
TECHNOLOGIES FOR THE EARTH AND ENVIRONMENT
MATERIALS OF THE FUTURE

An enthusiastic group of young researchers and students at the Faculty of Chemistry and Chemical Technology University of Ljubljana has decided to organize an international scientific conference to tackle the issues regarding last year's changes to the Law on awarding scholarships. The conference is named Cutting Edge 2015 and was held on 22nd of September 2015.

The issue with the changes in the Law on awarding scholarships is that all students who want to get the Zois's scholarship besides good average score of grades now also need to have an additional exceptional achievement. Into this category falls the scientific contribution published in a Book of abstracts which accompanies a scientific conference and is also published in the bibliographic system COBISS. When looking through the events suitable for the students to present their research, we found out, that there is really none events like that. The already very well established conferences all have quite high registration fees which are not suitable for the students because they are often too big of an expense so therefore those conferences are out of reach for them.

This lead the Students' council, Student organization, young researchers and faculty executives to give the students of natural sciences an alternative. We decided to organize a one-day event, international science conference Cutting Edge 2015. Apart from presentations of foreign established experts, our participants will also have the ability to present their research and get it published in this Book of abstracts, which is sufficient for them to be able to apply for the Zois's scholarship.

The conference has been divided into three main sections: *Science behind the living*, *Technologies for the Earth and Environment* and *Materials of the Future*. There were plenary lectures given by renowned experts from foreign countries regarding all three topics.

The conference was attended by more than 100 participants from Slovenia and also a few other countries with researches from different fields. They include environment preservation, pharmacy, construction, materials, as well as medicine and engineering. Most of them showed their work in a poster exhibition, while selected Masters' and Doctoral students got a chance to present their research in the form of oral presentations.

The conference could not have been organized at the level as it was without the help of several students of the Faculty of Chemistry and Chemical Technology. We thank Simona Gričar, Ana Grom, Andrej Hoivik, Tomislav Kostevc, Tina Paljk, Monika Pepelnjak, Špela Peršič, Nina Podjed, Nik Rus and Ciril Štern for their efforts. The credit for excellent graphical design goes to Urška Škrap, a student at the Facutly of architecture. The professional committee members that offered their help, prof. dr. Jurij Svete, prof. dr. Andreja Žgajnar Gotvajn, prof. dr. Marjan Marinšek, doc. dr. Marko Novinec, prof. dr. Marko Anderluh, dr. Gabriela Kalčikova, doc. dr. Anita Jemec, dr. Marjetka Levstek, doc. dr. Irena Kralj Cigić and prof. dr. Boštjan Jančar are also most kindly thanked.

The conference started at 9 AM, with a speech from the Minister of education, science and sport, dr. Maja Makovec Brenčič, the dean of the Faculty of Chemistry and Chemical Technology, prof. dr. Matjaž Krajnc and the president of the organizing committee and president of the Student organization of the faculty, Sara Drvarič-Talian. The opening was followed by plenary lectures and oral presentations. The one day conference ended with a small ceremony, where best poster and best oral presentation awards were given.

	LECTURE HALL 3
7:30-8:00	Registration
8:00-8:30	
8:30-9:00	
9:00-9:30	Opening
9:30-10:00	**Plenary lecture** - Science behind the Living
10:00-10:30	
10:30-11:00	**Oral presentations** - Science behind the Living
11:00-11:30	
11:30-12:00	Coffee break
12:00-12:30	**Plenary lecture** - Technologies for the Earth and Environment
12:30-13:00	
13:00-13:30	**Oral presentations** - Technologies for the Earth and Environment
13:30-14:00	
14:00-14:30	Lunch break
14:30-15:00	
15:00-15:30	
15:30-16:00	
16:00-16:30	**Plenary lecture** - Materials of the Future
16:30-17:00	
17:00-17:30	**Oral presentations** - Materials of the Future
17:30-18:00	
18:00-18:30	Closing ceremony
18:30-19:00	
19:00-19:30	

	chair: Jurij Svete
10:30	**Martin Gazvoda** Transformations between sulfonamides, N-acyl sulfonamides and N-sulfonylformamidines
10:50	**Katarina Šoln** Morphological and biochemical changes of *Raphanus sativus* roots after to exposure aqueous leaf extract of invasive species *Fallopia japonica*
11:10	**Matja Zalar** Structural features of hexanucleotide repeat expansions in *C9ORF72*

	chair: Andreja Žgajnar Gotvajn
13:00	**Vesna Mislej** Characterization of Residues, Resulting from the Thermal Treatment of the Sewage Sludge, with the Purpose of Nutrient Recovery
13:20	**Veno Kononenko** Cytotoxic and genotoxic effects of ZnO nanoparticles, ZnO microparticles and $ZnCl_2$ on MDCK kidney cell line
13:40	**Tea Romih** The use of voltammetry in the analysis of nanoparticle dissolution in various test media

	chair: Marjan Marinšek
17:00	**Matija Gatalo** Stabilization of ordered $PtCu3/C$ electrocatalyst by gold doping
17:20	**Tina Zabukovec Jezovšek** Black titania as alternative catalyst material for fuel cells
17:40	**Tanja Mivšek** Preparation and characterization of methacrylate supports with different porosity

	LECTURE HALL 4
7:30-8:00	Registration
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	Oral presentations - Technologies for the Earth and Environment
11:00-11:30	
11:30-12:00	Coffee break
12:00-12:30	
12:30-13:00	
13:00-13:30	Oral presentations - Materials for the Future
13:30-14:00	
14:00-14:30	Lunch break
14:30-15:00	
15:00-15:30	
15:30-16:00	
16:00-16:30	
16:30-17:00	
17:00-17:30	Oral presentations - Science behind the Living
17:30-18:00	
18:00-18:30	Closing ceremony
18:30-19:00	
19:00-19:30	

chair: Gabriela Kalčikova

10:30
Matej Čehovin
Enhancement of H_2O_2/UV AOP
with Hydrodynamic Cavitation

10:50
Brigita Hočevar
Application of deep eutectic
solvents in biotransformations

11:10
Sabina Bec
Influence of supplements and
pre-treatmentof concentrated
secondary (waste activated) sludge
on the production of biogas

chair: Irena Kralj Cigič

13:00
Gorazd Koderman Podboršek
Fabrication of Flexible
Platinum Thin Films

13:20
Klara Retko
HPC-based photoreduced silver
colloid: a substrate for SERS
study of organic dyestuffs

13:40
Polona Mrak
Mineralized biological matrix
formation – at the meeting point of
developmental biology
and material sciences

chair: Marko Novinec

17:00
Katja Hrovat Arnež
Differential necroptotic activity
of two human MLKL isoforms

17:20
Miha Bahun
The SOS-genes of the opportunistic
oral pathogen *Aggregatibacter*
actinomycetemcomitans

17:40
Živa Marsetič
ComQXPA Quorum Sensing
Systems May Not Be Unique to
Bacillus subtilis: A Census in
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TECHNOLOGIES FOR THE EARTH AND ENVIRONMENT - ORAL PRESENTATIONS

MATERIALS OF THE FUTURE - ORAL PRESENTATIONS

CUTTING EDGE 2015 - POSTERS

PLENARY LECTURE

SCIENCE
BEHIND
THE
LIVING

Applied MCR Chemistry @ Dömling Laboratory

Alexander Dömling[1]

[1] Department of Drug Design, University of Groningen, The Netherlands.

The Dömling laboratory has a focus on MCR chemistry since >20 years, in industry as well as in academia. In this lecture recent developments in applied MCR chemistry will be discussed.[1] 1) Novel small molecular weight scaffolds based on IMCR and MCR for the high throughput synthesis of compound libraries in the frame of the US-based ROADMAP as well as the European Innovative Medicine Initiative – European Lead Factory (IMI-ELF);[2] 2) the creation of a very large billions size virtual library based on MCR scaffolds and the associated free web-based pharmacophore software ANCHOR.QUERY;[3] 3) the application of MCR chemistry to discover novel potent and selective protein-protein interaction antagonists exemplified with p53-mdm2 and the PIF pocket of PDK1;[4] 4) the use of MCR chemistry for the design and synthesis of novel macrocyclic compounds for use in magnetic resonance imaging;[5] 5) the introduction of novel isocyanides which can be cleaved under mild conditions or functionalized to novel heterocyclic compounds; 6) a GOOGLE-MAP based approach to a spacio-temporal survey of the world of MCR.[6]

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3. Koes, D. et al. Enabling large-scale design, synthesis and validation of small molecule protein-protein antagonists *PLoS One* **2012**, *7*, e32839. doi:10.1371/journal.pone.0032839.
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5. PCT/EP2014/002713
6. http://www.drugdesign.nl/

Biography: Alexander Dömling studied Chemistry & Biology at the Technische Universität München and performed his PhD with Ivar Ugi and his postdoc with Barry Sharpless. He is the founder of several biotech companies. He is interested in novel aspects of and the application of MCR chemistry for the discovery of novel drugs in areas of unmet medical needs. He was professor at the University of Pittburgh and since 2011 he is Chair of the Department of Drug Design and professor at the University of Groningen.

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PLENARY LECTURE

TECHNOLOGIES
FOR THE
EARTH
AND
ENVIRONMENT

The Influence of Operating Conditions on Advanced Oxidation Processes Efficiency in Water and Wastewater Treatment

Ewa Maria Siedlecka[1]

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Advanced oxidation processes (AOPs) are technologies based on the intermediacy of hydroxyl and other radicals to oxidize recalcitrant, toxic and non-biodegradable compounds to various by-products and eventually to inert end-products. The environmental applications of AOPs are numerous, including water and wastewater treatment (i.e. removal of organic and inorganic pollutants and pathogens). Generally, AOPs can be divided on electrochemical (under active and non-active electrodes), photochemical (H_2O_2/UV, TiO_2/UV etc.) and chemical (with Fenton reagent) processes. The effectiveness of various AOPs for non-biodegradable pollutants removal from aqueous systems dependence of different key factors such as: pH of solution, wastewater and water composition, heterocatalyst/homocatalyst loading, pollutant concentration, type of irradiation, current efficiency etc. The influence of some selected factors on organic pollutants efficiency removal (sulphonamides, cytostatic drugs, methyl-*t*-butyl ether, ionic liquid) by different AOPs (electrolysis at active and non-active electrodes, photooxidation in presence of TiO_2 and Pt-TiO_2 photocatalyst and reaction with Fenton reagent) will be discussed.

The influence of inorganic ions naturally exist in water on AOPs efficiency was investigated in details, because this fact has been ignored for many years.

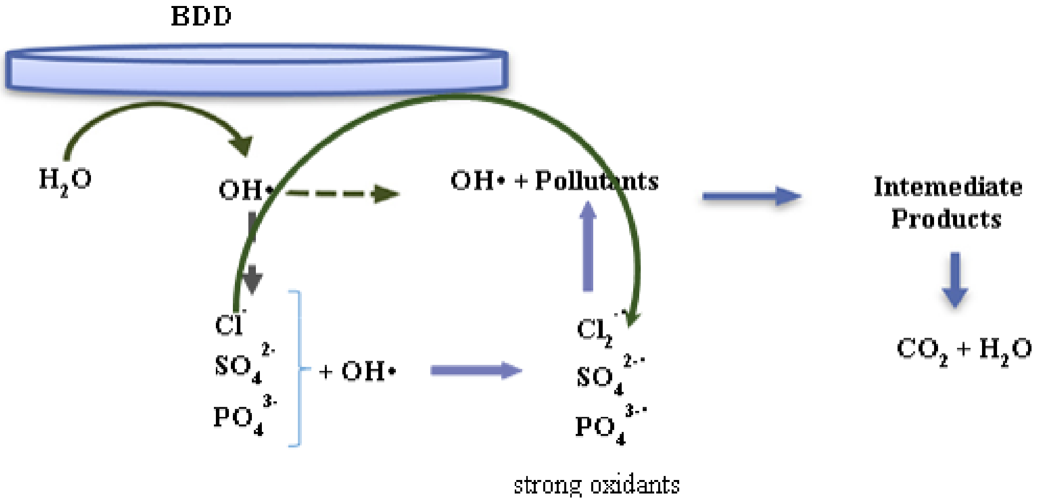


Figure 1: The influence of selected anions on the organic matter oxidation during the electrolysis at boron doped diamond anode.

PLENARY LECTURE

MATERIALS
OF THE
FUTURE

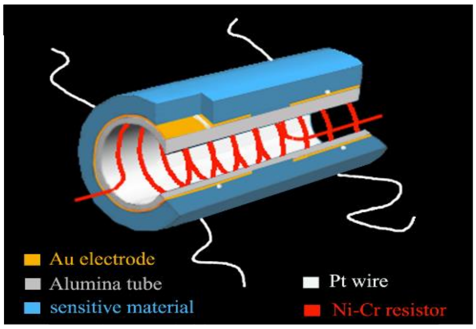
The Application of Metal Oxide Nanoparticles in Gas Sensing Devices and Lithium Ion Batteries

Igor Djerdj[1]

[1] Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia.

Metal oxide nanoparticles constitute an outstanding class of functional materials with potential applications in almost all fields of technology. In occasion of this conference, two important applications of metal oxides nanoparticles will be presented: an active material in gas sensing devices and anode material for lithium ion batteries. Concerning gas sensing research, the focus is on the development of sensing materials with new structures or morphologies to improve sensitivity, selectivity, and stability of sensors, and also on the development of new and better fabrication techniques to ensure reliability, safety, reproducibility, and cost reduction. Typical gas sensor is sketched in Figure 1 with active sensitive material (metal oxide nanoparticles) shown in light blue. Mixed phases SnO_2 nanorods are highly promising for gas sensor applications, as the gas response for isopropanol was significantly enhanced by the presence of orthorhombic phase (S=61.5 to 1000 ppm isopropanol and response time and recovery time of 4 and 10 s).[1] The enhancement in sensitivity is attributed to the presence of small orthorhombic SnO_2 crystals with average radius shorter than the Debye screening length of 7 nm for SnO_2. Pt activated SnO_2 shows the gas response toward 500 ppm of ammonia from 6.48 to 203.44 through the activation by Pt. And the results indicate that the sensor based on Pt activated SnO_2 not only has ultrahigh sensitivity but also possesses good response–recovery properties, linear dependence, repeatability, selectivity and long-term stability, demonstrating the potential to use Pt activated SnO_2 nanoparticle clusters as ammonia gas sensors.[2]

In the second part of this presentation nanoparticulate $CoMn_2O_4$ anode material will be presented. It was found that contrary to the common assumption that nanostructuring of anode material improves the battery performance, $CoMn_2O_4$ with the largest particle size exhibit excellent performance with capacity retention of 104% after 1000 cycles (compared to the 2nd cycle).[3]



Scheme 1: Sketch of the structure of a typical metal oxides nanoparticles gas sensor.

References:

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ORAL PRESENTATIONS

SCIENCE
BEHIND
THE
LIVING

Transformations between Sulfonamides, N-acyl Sulfonamides and N-Sulfonylformamidines

Martin Gazvoda[1], Marijan Kočevar[1], Slovenko Polanc*[,1]

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Sulfonamides **1** are known for their wide range of applications in medicine (Figure 1).[1] *N*-Acylsulfonamides **2** and *N*-sulfonylformamidines **3**, important derivatives of **1**, also have a diverse application in medicine and chemistry.[1]

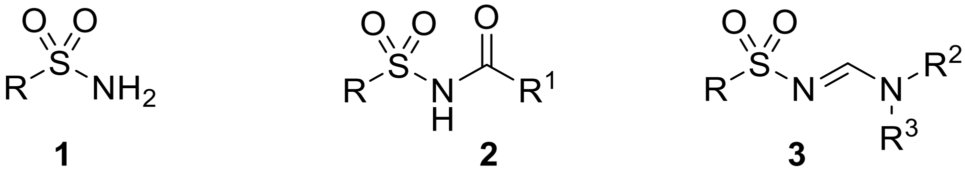

Figure 1: Structures of sulfonamides, *N*-acylated sulfonamides, *N*-sulfonylformamidines

Surprisingly, procedures that enable a selective and high-yielding synthesis of **3** from **1** are scarce, and to the best of our knowledge, there is no report in the literature on the transformation of *N*-acylsulfonamides **2** into *N*-sulfonylformamidines **3**.

We have discovered a gentle, simple, cheap, clean and highly selective procedure for the preparation of *N*-sulfonylformamidines **3** from either sulfonamides **1** or *N*-acylated sulfonamides **2** (as well as sulfonylureas and *N*-sulfonylsemicarbazides).[2] The method is based on clean and quantitative *in situ* formation of the Vilsmeier type of reagent from the appropriate formamide and oxylal chloride. This Vilsmeier reagent in the subsequent step reacts with **1** or **2** to afford **3**. The procedure is transition-metal- and oxidant-free and tolerates sensitive functional groups. Noteworthy, chiral substrates do not undergo racemization during the process. An excess of hydrazine hydrate in ethanol at room temperature enables selective removal of the formamidine functionality from **3**, returning sulfonamides **1** in good to excellent yields.

The described procedure was successfully applied for deacetylation of α-aryl cinnamic acid**,** an inhibitor of the AKR1C enzymes.[3]

References:

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Morphological and Biochemical Changes of Raphanus sativus *Roots after Exposure to Aqueous Leaf Extract of Invasive Species* Fallopia japonica

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Fallopia japonica is very invasive plant species which causes serious problems in Europe and North America. Quick vegetative reproduction and allelopathy are strategies which give *Fallopia* plants advantages against their neighbour plants.[1] In order to study its allelopathic effects, we focused on daily morphological and biochemical changes in roots of radish (*R. sativus*) exposed to aqueous extract of *F. japonica* leaves with concentration 5% (w/v). The control plants were watered with distilled water. Seedlings' roots were sampled one week after the germination. Allelopathic influence of *F. japonica* was shown daily as significantly smaller root length. At the end of the experiment was root growth inhibited up to 25% (Fig. 1) which was correlated with 66% lower mitotic index in roots, exposed to extracts (Fig. 1). Biochemical parameter total antioxidative capacity (TAC) was 3% higher in the exposed roots at the last two days. Also lipid peroxidation slightly increased while the activity of antioxidative enzymes guaiakol peroxidase and catalase showed no significant differences when compared to the control. The correlation between antioxidative acivity and lipid peroxidation is in accordance with previous studies.[2]

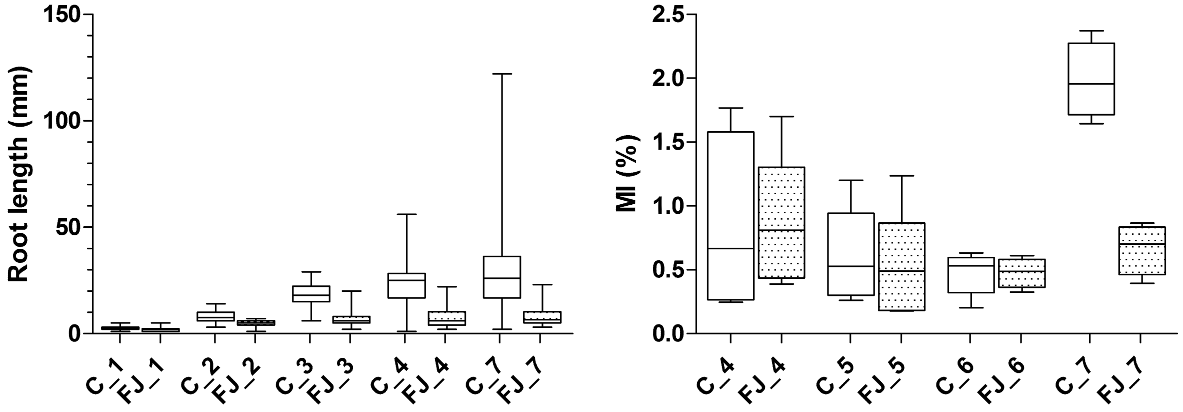

Figure 1: Length (left) and mitotic index (right) of seedlings' roots exposed to 5% extract of *F. japonica* (FJ) and control (C). Symbols on the box plot represent: mean value (—), maximum and minimum value (whiskers: ┴); N = 50.

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Structural Features of Hexanucleotide Repeat Expansions in C9ORF72

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The G_4C_2 hexanucleotide repeat expansion (HRE), located in the *C9ORF72* gene is a common genetic feature of patients suffering from amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD)[1]. Unusually high GC content of both strands in HRE potentially enables the formation of several non B-DNA structures that could affect gene's replication, transcription and translation as well as have impact on ALS and FTLD development[2]. Utilizing NMR and complementary spectroscopic methods, we have shown previously that different types of G-quadruplexes form on G-rich $d(G_4C_2)_n$ sense strand, depending on oligonucleotide's length[3]. On the other hand, it seemed highly improbable that C-rich $d(G_2C_4)_n$ repeats on anti-sense strand remain unstructured. Indeed we show that $d(G_2C_4)_n$ repeats form i-motifs and protonated hairpins which persist even in the presence of complementary strand (Figure 1).

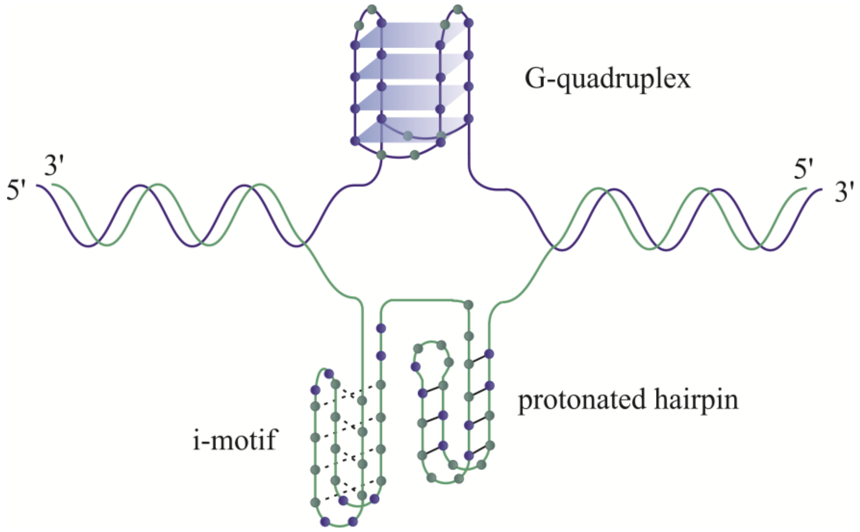

Figure 1: Structural features of hexanucleotide repeat expansion in *C9ORF72* gene

References:

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Differential Necroptotic Activity of two Human MLKL Isoforms

Katja Hrovat Arnež[1,2], Michaela Kindlova[2], Nilesh J. Bokil[2], Matthew J. Sweet[2] and Gregor Gunčar[1]

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Necroptosis is a programmed form of cell death that features morphological hallmarks of necrosis. It occurs under caspase-compromised conditions, when cells are stimulated either by death domain receptor ligands or by Toll-like receptor ligands and has an important role in inflammation and innate immunity. The necrosome complex responsible for necroptosis consists of the kinases RIP1 and RIP3, as well as mixed lineage kinase-like (MLKL). MLKL consists of an N-terminal coiled-coil domain and a C-terminal pseudokinase domain. Recent studies have mapped the death-initiating properties of MLKL to the N-terminal domain. In human, two isoforms of MLKL have been identified in HT-29 cells.[1] The shorter isoform, isoform 2, lacks a part of the C-terminal domain that undergoes RIP3-mediated phosphorylation, a process that enables cell death to be initiated via the N-terminal domain (Figure 1). Here we show that both isoforms are expressed by primary human macrophages. As predicted, isoform 2 was more potent than full length MLKL (isoform 1) at initiating cell death in ectopic expression studies. Current studies are investigating mechanisms of regulated necroptosis and regulated expression of MLKL isoform 2 in primary human macrophages, as well as similarities and differences between human and mouse macrophages in necroptosis pathways.

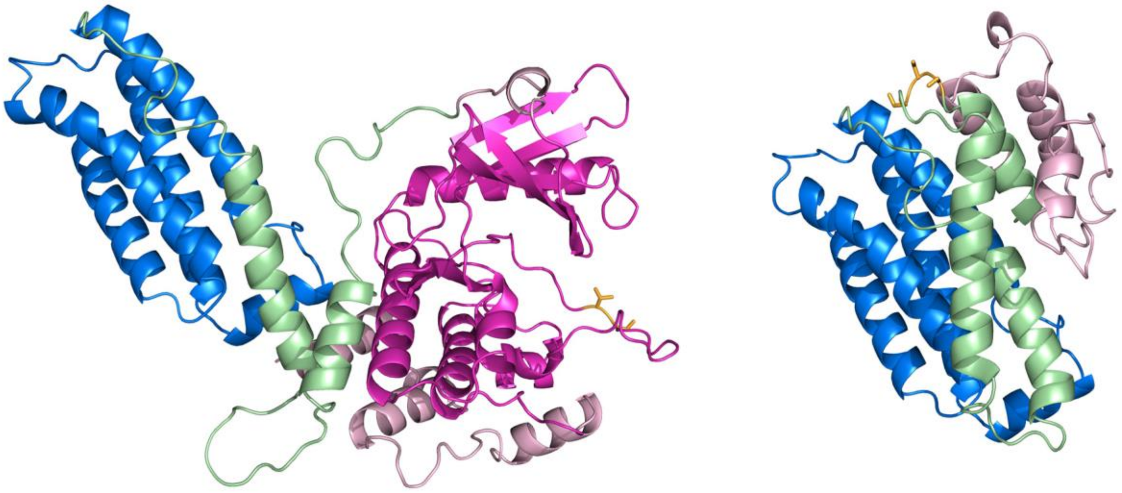

Figure 1: Models of MLKL isoform 1 (left) and MLKL isoform 2 (right). The N-terminal four-helix bundle (4HB) shown in blue, the brace region in green and the C-terminal domain in pink. Activation sites on MLKL1 are presented as orange rods.

Reference:

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The SOS-Genes of the Opportunistic Oral Pathogen Aggregatibacter actinomycetemcomitans

Miha Bahun,[1] Vid Skvarča[1], Angelika Vižintin[1], Barbara Umek[1], Majk Banfi[1], Tadej Bosilj[1], Vesna Hodnik[1], Gregor Anderluh[1,2], Rok Gašperšič[3], Matej Butala*[,1]

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Chronic periodontitis is inflammatory disease of the gingiva, periodontal ligament and alveolar bone characterized by progressive tissue destruction and eventual tooth loss which is initiated by dental plaque bacteria.[1] Amongst oral microbiota, opportunistic human pathogen *Aggregatibacter actinomycetemcomitans* (Aa) is strongly implicated in the etiology of aggressive forms of periodontitis with rapidly progressing tissue loss.[2] Here we have used *in silico* and surface plasmon resonance spectrometry analysis to characterize the Aa genes that respond to DNA damage *via* the SOS response. We confirmed dozen DNA targets for LexA repressor, the key regulator of this system. In contrast to the majority of LexA regulons determined in other pathogens,[3] Aa SOS-genes are not involved in virulence. In addition, our *in vitro* results indicate that in Aa inactivation of LexA depends on repressor`s dissociation from the target DNA. Remarkably, among the analyzed Aa serotypes we observed highly variable promoter regions of the error-prone polymerase *polIV*, which implies on diverse gene expression regulation and on the different frequency of mutagenesis among the strains and their ability to adapt to the host.

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ComQXPA Quorum Sensing Systems May Not Be Unique to Bacillus subtilis*: A Census in Prokaryotic Genomes*

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Bacterial quorum sensing (QS) is a cell to cell communication that enables synchronized population behaviors such as swarming, biofilm formation, stress resistance and production of toxins and secondary metabolites.[1] Bacillus subtillis is a member of the family Bacillaceae (some of its members communicate via QS) which is known to perform fundamental roles in soil ecology (cycling of organic matter) and in plant health (suppression of plant pathogens and phosphate solubilization).[2] The *comQXPA* locus of *Bacillus subtilis* encodes a quorum sensing (QS) system typical of Gram positive bacteria. It encodes four proteins ComQ, ComX, ComP and ComA. These are encoded by four adjacent genes all situated on the same chromosome strand. For those genes a comprehensive census of *comQXPA*-like gene arrangements in 2620 complete and 6970 draft prokaryotic genomes has been made. We searched bacterial genomes for candidate loci similar to the *B. subtilis* ComQXPA locus, by combining Hidden Markov Model (HMM) recognizers with filtering criteria based on the structural and organizational properties of ComQXPA QS systems. After manually checking the data for false-positive and false-negative hits, we found 39 novel *com*-like predictions. The census data show that in addition to *B. subtilis* and close relatives, 20 *comQXPA*-like loci are predicted to occur outside the *B. subtilis* clade. Characteristic gene-overlap patterns were observed in *comQXPA* loci, which were different for the *B. subtilis*-like and non-*B. subtilis*-like clades. Pronounced sequence variability associated with the ComX peptide in *B. subtilis* clade is evident also in the non-*B. subtilis* clade suggesting grossly similar evolutionary constraints in the underlying quorum sensing systems.[3]

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ORAL PRESENTATIONS

TECHNOLOGIES
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Enhancement of H_2O_2/UV AOP with Hydrodynamic Cavitation

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H_2O_2/UV Advanced Oxidation Processes, alone or in combination with Hydrodynamic Cavitation (HC, as a promoter of H_2O_2 conversion to radical species and turbulent effects in the fluid[1]), were compared. Methylene-blue dye was used as a model substance due to its controllable reactivity to HO• radicals[2]. Low pressure, monochromatic UV light at 254 nm was used in the experiments with dosages up to 2800 mJ cm^{-2} and H_2O_2 dosages of 5 and 10 mg L^{-1}. Nozzle (n = 1) and orifice plates with n = 4, 8 and 16 holes were used as HC generators, with cavitation number (C_v) ranging between 0.15 and 0.20. Colour of the solution was ranging from 0.358 to 0.375 m^{-1} at the start of experiments. Improvements in colour removal at $\lambda = 610$ nm in the range of 5-20 % were observed (Figure 1) when HC was applied due to improved mass transfer of H_2O_2. UV absorbance at $\lambda = 254$ nm was ranging between 0.144 and 0.192 m^{-1}, with HC performing better the higher the UV absorbance. The results indicate potential improvements of H_2O_2/UV AOPs, necessary for increased removal efficiency of organic matter[3] from water matrices containing scavenging chemical species.

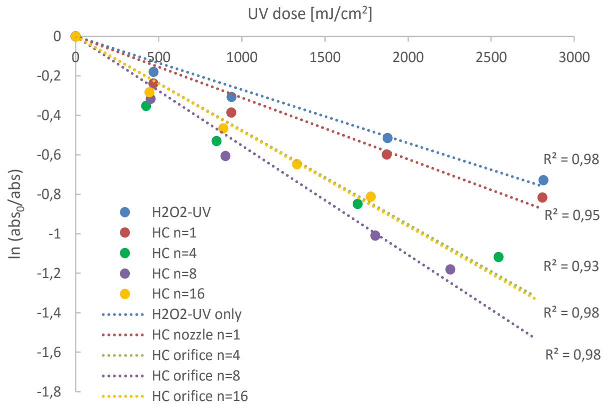

Figure 1: Absorbance reduction at $\lambda = 610$ nm – discolouration with H_2O_2 (5 mg L^{-1})/UV AOP alone and in combination with hydrodynamic cavitation, using different generator geometry

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Application of Deep Eutectic Solvents in Biotransformations

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Deep eutectic solvents (DES) are formed by mixing at least two salts, typically organic salt and hydrogen bond donor such as alcohol, acid or amide, in a proper molar ratio. This new anhydrous solvents offer many new advantages such as biodegradability, thermal stability, low toxicity, low cost and easy synthesis.[1]

In this study, several DES (choline chloride-urea, choline chloride-glycerol, choline chloride-malonic acid) have been prepared. Based on the evaluation of their viscosities, densities and pH of aqueous solutions, choline chloride-urea was chosen as a (co)solvent for biotransformations. First the lipase B catalyzed synthesis of butyl butyrate in DES was performed in a batch reactor and compared with the same reaction performed in organic solvent (*n*-heptane) and ionic liquid ([bmim][BF4]) to confirm the appropriateness of using DES as a solvent (Figure 1a).[2] Furthermore, the influence of selected DES on ω-transaminase- catalyzed synthesis of 4-phenyl-2-butylamine was studied. DES was used as a cosolvent to increase the solubility of the ketone in the aqueous medium, which increased the productivity of the batch process (Figure 1b). The reaction of transamination was also performed in a continuous microreactor system with integrated extraction of the 4-phenyl-2-butylamine.

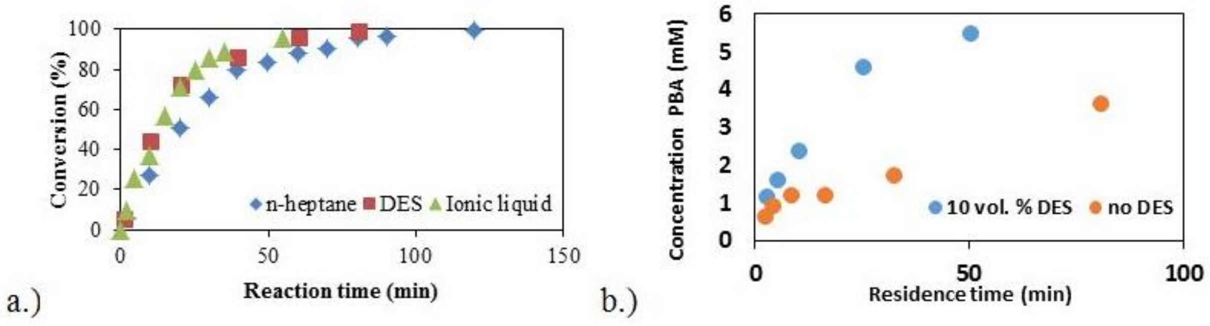

Figure 1: a) Lipase B-catalyzed transesterification in different anhydrous solvents; b) ω-transaminase-catalyzed production of 4-phenyl-2-butylamine (PBA).

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Influence of Supplements and Pre-treatment of Concentrated Secondary (Waste Activated) Sludge on the Production of Biogas

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The disposal of waste sludge is a problem of growing inportance in wastewater treatment plants. It can represent up to 50% of operating costs.[1] Anaerobic digestion plays an important role due to its abilities to transform organic matter into biogas which can be used as a renewable source of energy. Secondary sludge has low degradability and it limits hydrolysis, thus different pre-treatment processes can be used to enhance biogas yield.[2] The aim of the study was to examine whether the addition of supplements or pre-treatment of concentrated secondary (waste activated) sludge could improve the production of biogas from it and also reduce the final content of organic matter in the digestate in Domžale – Kamnik wastewater treatment plant (WWTP). Five experiments were performed in two pilot-scale CSTR bioreactors (one as the experimental and the other as the control reactor) with total volume of 200 L. Digestate from a full-scale anaerobic digester was used as an inoculum. The experiments have been conducted for 30 days; during that 10 L (three times) and 20 L (two times) of concentrated secondary sludge was added as a substrate. At the beginning and at the end the digestate was left to stand still for 5 days. To enhance the biogas production in anaerobic digestion process two different commercial enzymatic/bacterial preparations (MethaPlus L120, Agrovit and BioCat+^{TM}, Citadel) and mechanical desintegration (hydrodynamic cavitation) as a pre-treatment process were applied. During the process pH, alkalinity, Kjeldahl's and ammonium nitrogen, short chain fatty acids, chemical oxygen demand and organic matter were monitored off-line and volume of biogas on-line. The applied mechanical pre-treatment (hydrodynamic cavitation) enhanced the biogas production most successfully, up to 10 %. Analysis of microbial community using PCR-DGGE was performed.

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Characterization of Residues, Resulting from the Thermal Treatment of the Sewage Sludge, with the Purpose of Nutrient Recovery

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Following the thermal treatment of dried sewage sludge, resource recovery of nutrients from the solid residue in the shape of bottom ash or in the case of pyrolysis as a biochar, must be taken into account, continuing their sustainable circulation in nature. Due to the impoverishment of natural sources of phosphorus, it is especially important to find opportunities for recycling it.[1]

In the resulting residues we have determined the content of nutrients (TOC, N, P, K and Mg) and their mineralogical composition by XRD analysis and SEM-EDS technique; the proportion of volatile part of nutrients due to the thermal treatment, the proportion of water-soluble nutrients with the extraction method in accordance with EN 13652 and their leaching behaviour. The water solubility of nutrients in resulting residue is the highest after the thermal treatment of the sewage sludge at 450 ^{o}C. At higher temperatures of the oxidizing thermal treatment, the nutrients get chemically transformed to a shape which is not water-soluble anymore, thereby reducing the possibility of material utilization of residues.

Pyrolysis offers a much greater chance of material utilization of the sewage sludge. A research of residue was performed after thermal treatment of sewage sludge[2] in an inert atmosphere at 450 °C (granules and milled pellets) and at 900 °C (milled pellets). It was discovered that the water solubility of phosphorus in biochar, produced at 450 °C, is higher when compared to the remains from the oxidizing atmosphere; on the contrary, the water solubility of K in Mg is lower.

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Cytotoxic and Genotoxic Effects of ZnO Nanoparticles, ZnO Microparticles and $ZnCl_2$ on MDCK Kidney Cell Line

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ZnO nanoparticles (NPs) are mass-produced NPs with a wide range of applications.[1] Although their toxic effects have been studied intensively,[2] the knowledge about their impact to the excretory system is still limited. In our study, we evaluated cytotoxic and genotoxic potential of ZnO NPs to the Madin-Darby Canine Kidney (MDCK) cells. To assess the importance of particle size and ion release, we treated MDCK cells with ZnO particles of different sizes (ZnO NPs: 30-200 nm; ZnO microparticles (MPs): 100-500 nm) and with a soluble form of zinc ($ZnCl_2$) in the concentration rage of 12.3 μM to 737.2 μM. After 24-hour treatment, we evaluated cytotoxicity applying three different assays: MTT assay, neutral red uptake assay and trypan blue exclusion assay. All zinc compounds induced cytotoxicity at a starting level of 184.3 μM. Furthermore, alkaline comet assay and cytokinesis-block micronucleus (**CBMN**) **assay** were applied for genotoxicity assessment at sub-cytotoxic concentrations. Results showed that all studied zinc compounds induced similar concentration-dependent cytotoxicity, however, only ZnO NPs significantly elevated DNA damage (Figure 1). Our study illustrates that the genotoxicity of ZnO particles depends on particle size and cannot be simply explained just by extracellular ion dissolution.

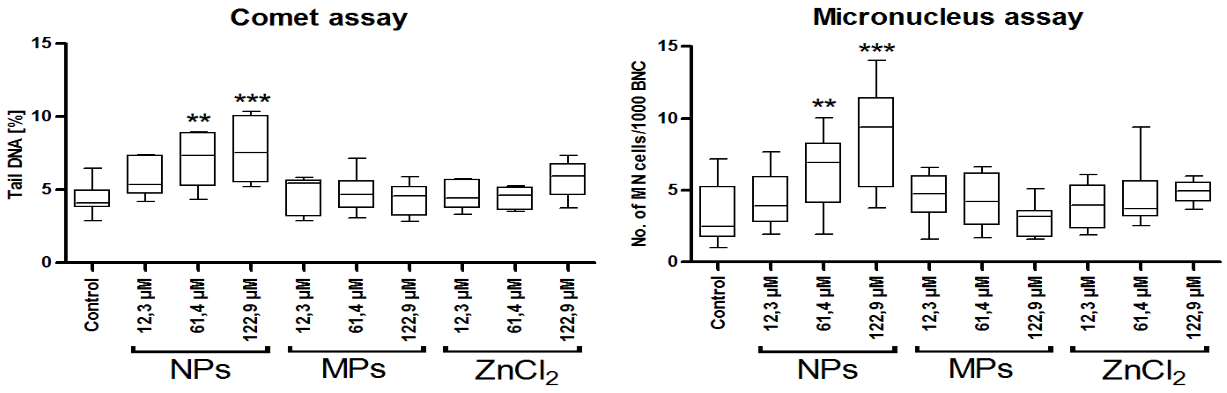

Figure 1: Results of genotoxicity assays indicate higher DNA damaging potential of ZnO NPs compared to that of ZnO MPs and $ZnCl_2$. Results were statistically analysed using ANOVA with Dunnett's multiple comparison post test (****P**<0,01) (***P<0,001).

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The Use of Voltammetry in the Analysis of Nanoparticle Dissolution in Various Test Media

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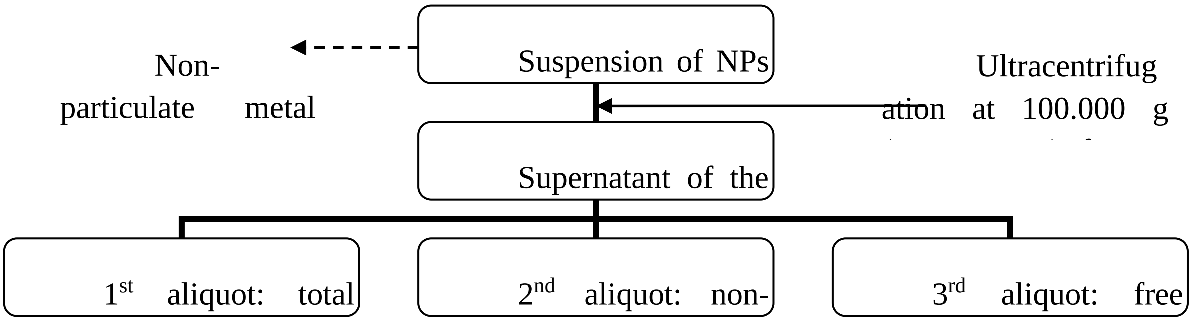
The dissolution of metal-based nanoparticles (NPs) to ions is one of the major ways in which NPs become bioavailable and toxic. Determination of the dissolved metal species in NP suspensions is therefore an important part of NP characterization. However, accurate analysis of NP dissolution is methodologically very difficult, with one of the main obstacles being the efficient separation of NPs from the dissolved metal species. Commonly employed protocols combine a separation step (such as centrifugation, ultrafiltration, dialysis, ion exchange etc.) with a detection step *via* spectroscopic techniques, such as atomic absorption spectroscopy or inductively-coupled plasma mass spectroscopy. Unfortunately, both steps may be a source of measurement errors due to poor NP removal from the solution.

Voltammetry has recently gained interest in the field of nanotoxicology due to inherent insensitivity to NPs. Voltammetry, with its plethora of possible techniques, enables the detection of dissolved metal species in the suspensions of NPs in various test media (such as deionized water and standard artificial fresh water or seawater) as well as in complex mixtures of organic molecules. We will present our latest applications of voltammetry with *in situ* bismuth-film electrode for the detection of dissolved metal species in suspensions of NPs in deionized water and in cell culture media. We have also successfully determined the dissolved metal species in the untreated suspension of PVP-stabilized Ag NPs.



Scheme 1: The sequence of steps in the measurement of nanoparticle dissolution.

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ORAL PRESENTATIONS

MATERIALS
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Fabrication of Flexible Platinum Thin Films

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Electro-catalytically active platinum thin films were deposited on substrates for potential use in flexible dye sensitized solar cells.[1] Due to the low temperature used in preparation process the films were prepared on plastic substrates and their characteristics were determined by measuring electrical resistivity and reflexion and by means of scanning electron microscopy.

First of all, a TiO_2 sol was prepared, which was deposited on the substrate using the dip-coating method. The next step was the deposition of the precursor complex (an ethanol solution of H_2PtCl_6) onto the superhydrophilic TiO_2 layer by the drop coating method and the coating was dried under intense ultraviolet light. The last step was the reduction of the precursor complex at low temperatures by exposing it to vaporized formic acid.[2] The parameters of the deposition method were varied (concentration and volume of the H_2PtCl_6 solution) in order to optimize the prepared platinum films. The resulting layers have good adhesion to the substrate, are electrically conductive and have mirror-like properties.

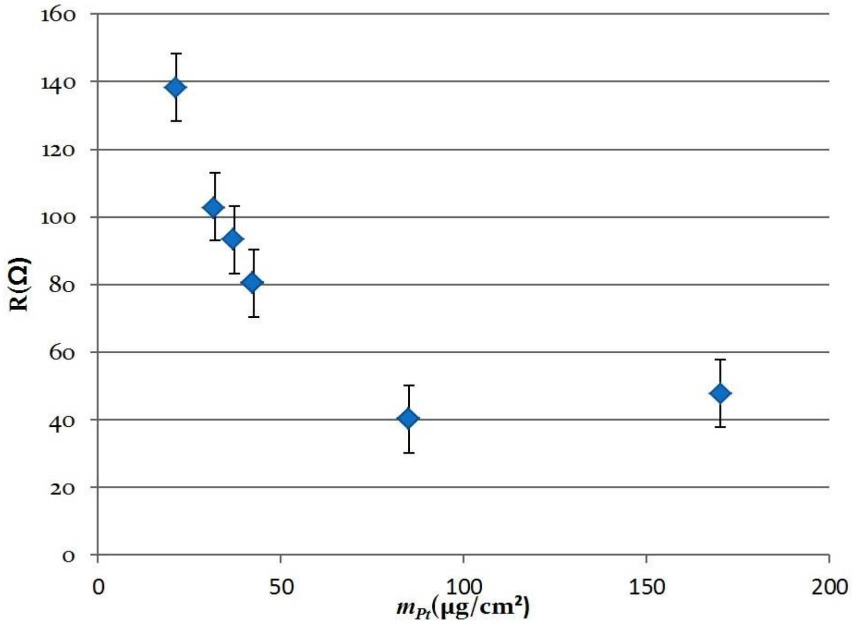

Figure 1: Dependence of resistance of platinum films on plastic substrates on mass of platinum per unit of surface (m_{Pt})

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HPC-based Photoreduced Silver Colloid: a Substrate for SERS Study of Organic Dyestuffs

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Surface-Enhanced Raman Spectroscopy (SERS) has been established in the conservation science as an extremely efficient analytical method for the investigation of organic dyestuffs, present on the samples of cultural heritage importance (*e.g.* fibres, paintings, archaeological objects, polychrome sculptures).[1,2] Weak Raman scattering properties of organic colorants along with fluorescence, which further obscures the Raman signal, limit the use of conventional Raman spectroscopy. Presence of nanoparticles (usually Ag, Au) in SERS substrates amplifies the Raman signal of organic compounds, which are adsorbed or placed in their vicinity. The signal enhancement is, in addition to fluorescence quenching, in general ascribed to electromagnetic enhancement (based on the surface plasmon excitation and the local electric field intensification) with a stronger contribution and the chemical enhancement (involving an interaction between the adsorbed molecule and the surface of the nanoparticle). Regarding sensitivity and selectivity of the technique, SERS allows the detection of organic colorants (present in low concentration, usually combined with organic binders and other compounds) in minute samples with minimal intervention in the cultural heritage objects, thus preserving their integrity. In this work, HPC(hydroxypropyl cellulose)-based photoreduced silver colloid was used as a SERS substrate. Its properties, which were determined by means of different spectroscopic and microscopic techniques, are governed by the initial ratio of silver ions and the polymer, and the incident radiation. The substrate was employed for the characterisation of different colorants, commonly present in the works of art. Examples of vibrational characterisation of several antraquinones (*i.e.* alizarin, purpurin, carminic acid, laccaic acid) and different paint layers are presented. Different excitation wavelengths were employed in order to sufficiently quench fluorescence, to define the optimal parameters for their positive detection and to follow the enhancement of different adsorption forms. Collected spectra will serve as a part of SERS spectral database for the identification of organic colorants on unknown samples from cultural heritage objects.

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Mineralized Biological Matrix Formation – at the Meeting Point of Developmental Biology and Material Sciences

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Mineralized biological matrices are organic – inorganic composites of complex and dynamic structure and composition. Exoskeletal cuticle of crustaceans is a model of mineralized chitin-based matrix and includes different calcium minerals, specifically calcite and amorphous calcium carbonate and calcium phosphate.[1] Formation and biocalcification of chitinous matrices during embryonic development is a poorly understood issue.

In addition to different imaging microscopic techniques, energy-dispersive X-ray spectroscopy (EDS) and Raman spectroscopy were used to reveal ultrastructure, elemental and mineral composition of the cuticle in the embryonic and larval stages of the invertebrate model organism *Porcellio scaber*. Our results show that several epidermal matrices are secreted sequentially during development.[2] The thin early matrices consist of the outermost lamina and loose material, while later in development the matrices with structurally distinctive layers and characteristic helicoidal arrangement of chitin-protein fibrils are formed. The initial accumulation of calcium is evident in the assembling cuticular matrix of the newly hatched larva, in which the elemental and mineral composition considerably differs from that in the advanced larval stages and adults. Changes in matrix structure and composition during development show progressive cuticle formation and calcification and imply establishment of the essential biological functions of the cuticle.

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Stabilization of Ordered $PtCu_3$/C Electrocatalyst by Gold Doping

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While platinum is already the most active metal for oxygen reduction reaction, its alloys in many cases additionally enhance its activity.[1] Although this is a very useful phenomenon, such catalysts mostly lack proper stability. This makes them unviable for an actual application as a cathode catalyst material in a polymer electrolyte membrane fuel cell (PEMFC).

Here we present a possible route toward stabilization of ordered $PtCu_3$/C catalyst by gold doping. Ordered $PtCu_3$/C catalyst is known in literature as a very active material for oxygen reduction reaction[2] and already exhibits better stability than standard commercial Pt/C catalysts. By modifying our catalyst surface with optimal addition of gold, we managed to retain the inherently high oxygen reduction activity, while greatly improving catalyst stability (Figure 1). From TEM imaging we can also observe both platinum and gold surface segregation. This is an important step towards a very stable and active $Pt_{25-x}Au_xCu_{75}$ shell and $Pt_{25}Cu_{75}$ core catalyst with a proper skin type surface and points to the direction of making Pt alloys more stable and therefore viable for real PEMFC application.

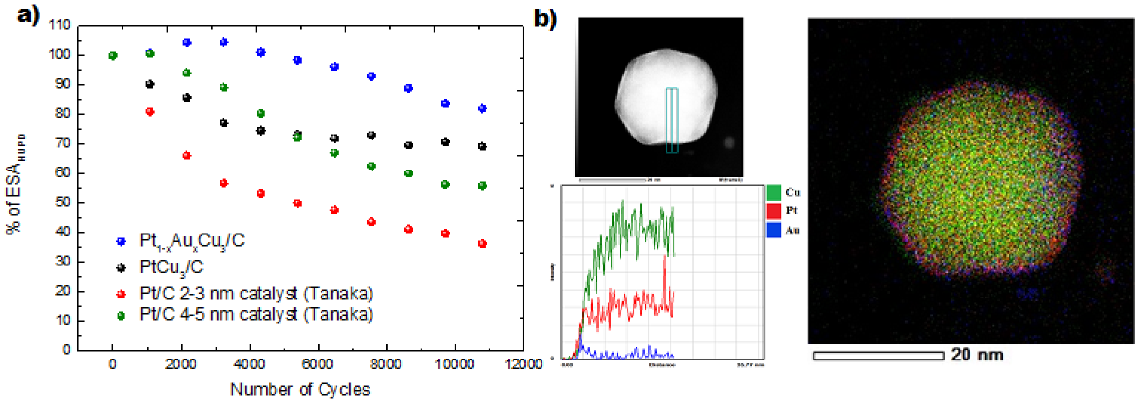

Figure 1: (a) Start-Stop degradation cycling (0.4 – 1.4 V_{RHE}, 1 Vs^{-1}, 10800 cycles) and **(b)** line scan and elemental mapping of a TEM image of gold stabilized $PtCu_3$ nanoparticle

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Black Titania as Alternative Catalyst Material for Fuel Cells

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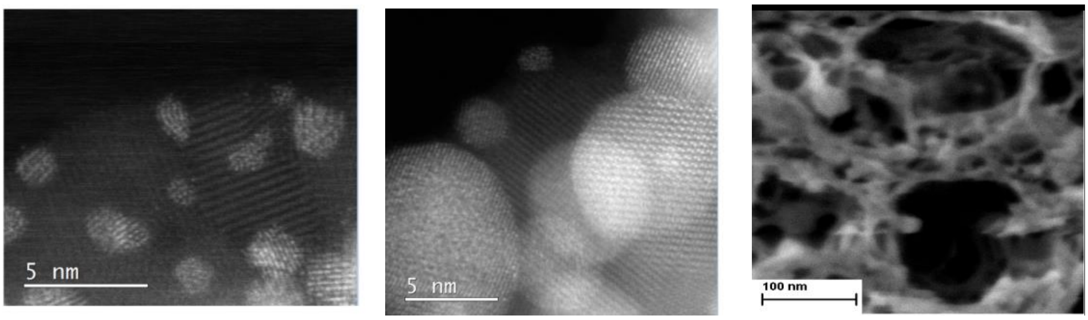
Nowadays, carbon based materials are generally used as a support material in proton exchange membrane (PEM) fuel cells.[1] The main problems for these support materials are primary and secondary degradation.[2] To avoid this degradation we here propose to replace the usual carbon-based support with a titania-based support. The active material of choice was Cu_3Pt particles (Figure 1A). Modified sol-gel procedure is one of the method used to prepare these materials, which allows us to obtain a web-like structure decorated with small Cu_3Pt particles (Figure 1C). A special reducing agent is used to create a conductive material after the sol-gel synthesis. The total percent of metal loading in the final product was 18 wt. %. The final product was lyophilized for 24 h. After filtration, the powder was calcined in furnace at 500 °C in inert atmosphere for one hour, where amorphous material crystallized into anatase. TEM micrographs revealed quite homogeneously distributed Cu_3Pt nanoparticles on reduced titanium dioxide support. The presence of nano-crystals of anatase phase was additionally checked and verified by electron diffraction (Figure 1B).



Figures 1A, 1B, 1C: 1A HAADF-STEM micrograph of titania catalyst material with Cu_3Pt nanoparticles, 1B Selected area electron diffraction pattern of TiO_2 support with labelled 101 anatase planes and 1C SEM micrograph of web-like TiO_2/Cu_3Pt.

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Preparation and Characterization of Methacrylate Supports with Different Porosity

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Large biomolecule drugs made a significant progress towards in recent years, resulting in a need for fast and efficient purification procedures to achieve high purity products. For this purpose liquid chromatography is commonly used. One of the challenges on a preparative chromatographic scale is to enable high resolution high-throughput purification at low to moderate pressure drop[1]. Convection based chromatographic resins seems to represent meaningful alternative to conventional porous matrixes due to their flow-unaffected resolution and dynamic binding capacity. While membranes limited to their inherent thinness are challenged by achieving high resolution separation, monoliths that can be produced in various shape and dimensions seem to be a valuable option. Besides flow unaffected properties, being consequence of convection based transport minimizing any diffusional restrictions, one of their main advantages is also high hydraulic permeability resulting from high porosity. Monolith pores are open and highly interconnected forming a network of channels through which the mobile phase flows. Due to this open architecture even large molecules can access pore surface area utilizing entire monolith available surface resulting in high binding capacity for very large biological molecules. While smaller pore size increases total surface area, it has an opposite effect on monolith permeability, governing pressure drop. It is therefore of utmost importance to be able to precisely characterize monolith structural properties with a non-invasive methods. Monolith porosity can be effected by varying monomer to porogen ratio while pore size distribution is almost independently tailored by temperature of polymerization and porogen composition. By changing a porogen however, surface properties of monolith can be changed too due to possible different orientation of skeleton groups during phase transition resulting in different monolith wettability[2]. To investigate this effect we prepared monoliths with different structure by varying monomer to cross linker ratio, monomers to porogen ratio and nature of the porogen. Porosity was determined by measuring weight difference between dry monolith and same monolith soaked in water, ethanol and cyclohexane.

Figure 1: Dry monoliths

Figure 1: Monoliths soaked in watter

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Development of Synthetic Pathways for 7-Substituted Pyrazolo[1,5-a]pyrimidine-3-carboxamides

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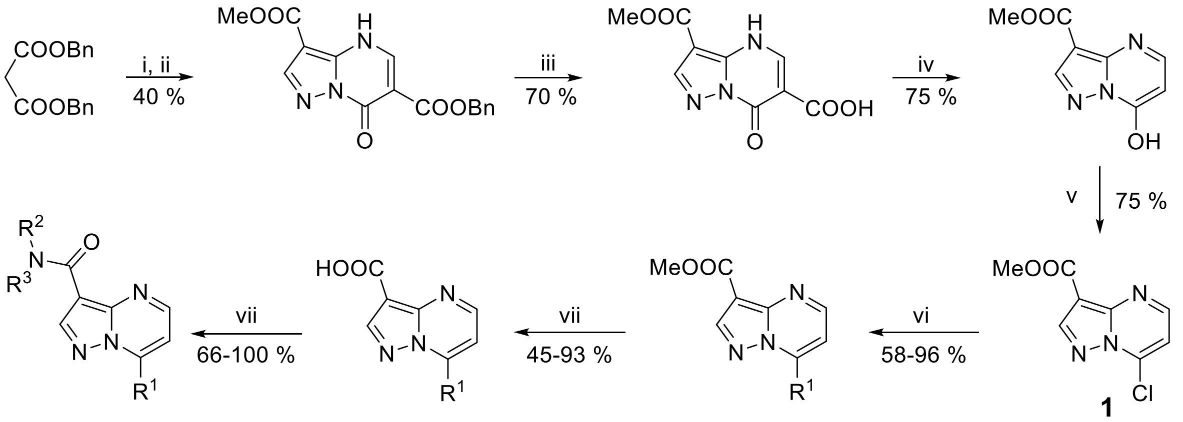
Pyrazolo[1,5-*a*]pyrimidines are very interesting compounds because most of known derivatives exhibit various biological activities. Some derivatives are already been used as active pharmaceutical ingredients. We decided to develop a novel synthesis of methyl 7-chloropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (**1**) as an important intemediate in the synthesis of novel pyrazolo[1,5-*a*]pyrimidines. Since **1** contains two sites of diversity, it is also suitable for preparation of compound libraries. Treatment of dibenzyl malonate with DMFDMA gave dibenzyl 2-[(dimethylamino)methylidene]malonate.

Further cyclization with methyl 3-aminopyrazolo-4(1*H*)-carboxylate in acetic acid gave 6-benzyl 3-methyl 7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3,6-dicarboxylate. Deprotection of the benzyl ester with HBr-AcOH and subsequent decarboxylation with quinoline-Cu gave methyl 7-hydroxypyrazolo[1,5-*a*]pyrimidine-3-carboxylate, which was chlorinated to furnish the highly reactive target molecule **1**. Suzuki-Miyaura reaction with phenylboronic acid and reactions with *N*-benzyl-*N*-methylamine and benzyl alcohol gave the corresponding 7-substituted derivatives. Hydrolysis of the esters followed by amidation gave the corresponding carboxamides. With these transformations some possible derivatization of intermediate **1** were shown.[1]



(i) DFMDMA; (ii) methyl 3-amino-1*H*-pyrazole-4-carboxylate; (iii) HBr-AcOH; (iv) quinoline, Cu; (v) $POCl_3$; (vi) $PhB(OH)_2$, Pd^0 or Bn(Me)NH or BnOH; (vii) NaOH; (viii) BPC, R^2R^3NH.

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Influence of Fiber Composite Reinforcement on Flexural Strength of Timber Beams

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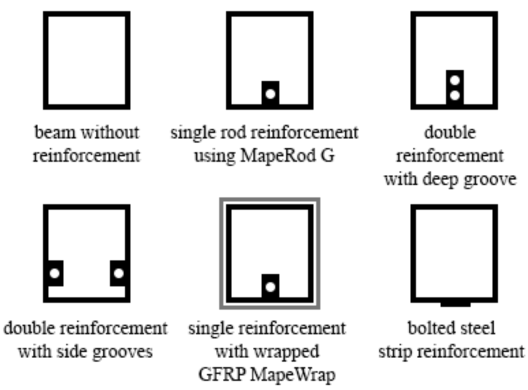
During their lifetime, timber structural elements are exposed to various loads, temperature and moisture cycles and pests. According to Srpčič,[1] exposure combined with wood rheology may often result in large deflections and deformability. Many researches have been made in the recent years to take advantage of highly rigid composites in flexural strengthening.[2] These were already proven to be effective at repairing historic timber roofs and ceilings in old buildings of high importance.[3]

As described by Tajnik et al.[4] fiber-reinforced polymers (FRP) consisting of epoxy or vinylester matrix and glass, carbon or aramid fiber have high mechanical properties (stiffness, compressional strength, tensional strength) and reduce stresses in a cross section. Mechanical behaviour in improved conditions is non-linear and difficult to model. Therefore, an experimental approach has been taken to test several different applications of glass FRP using latest and most promising near-surface mounted (NSM) method described below. Full-scale spruce beams with a length of 4 metres were tested to bending failure using four-point bending apparatus.

The basic idea of the NSM method is to carve rectangular grooves in the tension zone of section stresses. Positive bending moment under distributed load implies tension in bottom part of the cross section and if too high, tension stresses can cause cracking. Different reinforcement configurations which were tested are shown in Scheme 1. The main objectives of the research were to determine the optimal reinforcement configuration and to evaluate at what rate the failure bending moment increases. Different configurations lead to various failure mechanisms, but overall, a big potential of using composite materials in structural engineering was proven.



Scheme 1: Configurations of reinforcement used in an experiment to evaluate the increase of the failure bending moment.

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Synthesis of Pyrazolo[1,2-a]pyrazolecarboxylates

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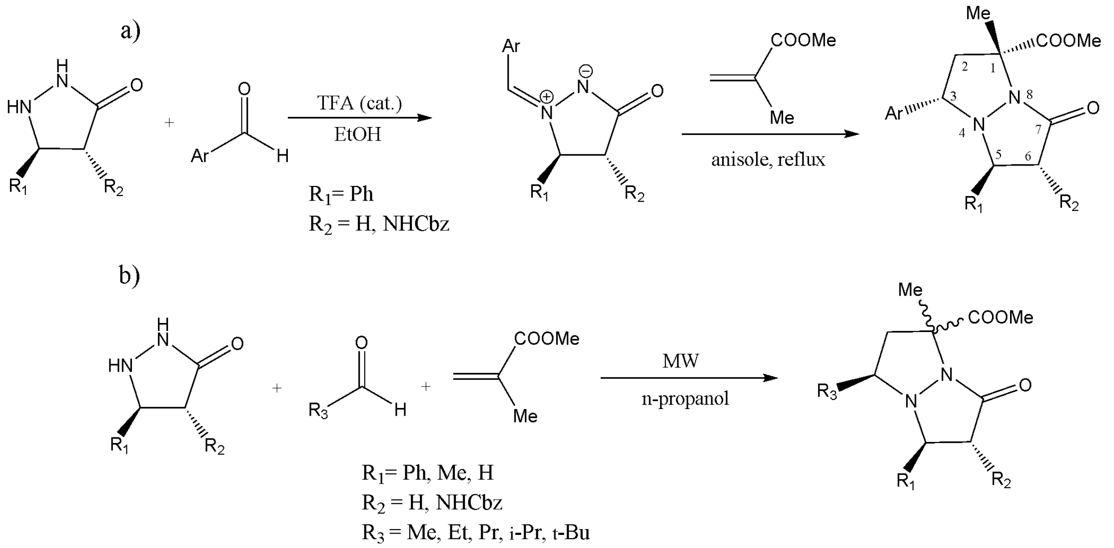
Our research group has been investigating reactions of 3-pyrazolidinone derived azomethine imines with different dipolarophiles leading to pyrazolo[1,2-a]pyrazolecarboxylates. These cycloadducts were prepared by stepwise synthesis from 3-pyrazolidinones, which were first converted with aromatic aldehydes into stable azomethine imines, which were isolated and subsequently reacted with dipolarophiles. In most cases, these cycloadditions were highly regio- and stereoselective.[1] However, azomethine imines derived from aliphatic aldehydes are not so stable and easy to prepare and their reactions have not been studied so intensively. In extension, we decided to develop a simple synthetic method, where 'aliphatic' azomethine imines would be formed *in situ* and reacted with dipolarophiles.



Scheme 1: A stepwise (a) and one-pot synthesis (b) of pyrazolo[1,2-a]pyrazolecarboxylates.

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A Green Synthesis of Possible Multidentate Ligands from Bicyclo[2.2.2]octenes and Hydrazinylpyridazines

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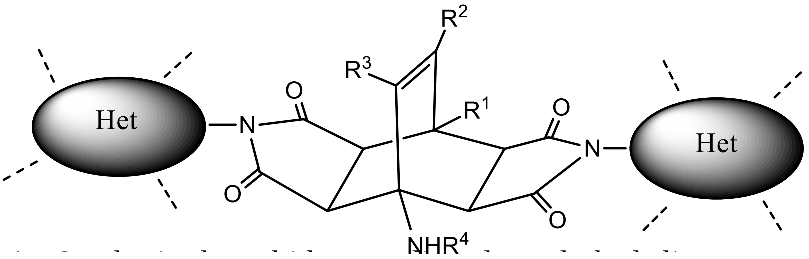
In their structure bicyclo[2.2.2]octenes and their fused derivatives possess various functionalities and thereby might serve as useful building blocks in organic synthesis.[1] Our aim was to prepare fused succinimide derivatives of bicyclo[2.2.2]octenes that could be applied as bi- or multidentate ligands for various transition metal ions, thereby forming various multidimensional coordination assemblies.[2] Our synthesis started with a thermal cycloadition of 2*H*-pryran-2-one derivatives with the maleic anhydride as the dienophile. Such cycloadditions in the majority of cases so far resulted in the formation of exclusively symmetric *exo,exo*-bicyclo[2.2.2]octenes.[3] For the next step we applied the well-known transformation between the anhydride moieties and amines or hydrazines (giving special attention to the application of substituted hydrazinylpyridazines), yielding the corresponding amides or imides. These substitutions were carried out under green reaction conditions,[4] in closed vessels (ACE glass tubes), by using water as the reaction medium and microwaves as the source of heating. This resulted in a simple isolation procedure giving the products in very high yields.[5] Along with the other results, the preliminary tests of the coordination ability of the prepared, presumably multidentate ligands, with various metal cations (Ni^{2+}, Y^{3+} etc.) as a potentially interesting entry into a plethora of different molecular architectures[6] will be presented.



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Sludge and Wastewater Digestion from Graphic Paper Mill with Deinking

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Large amount of sludge generated in recycled fibre paper mill with deinking (170 - 190 kg t^{-1}_{paper}) is the main reason for energy recovery of the sludge as one of the best available techniques for sludge management. To decrease mass ratio of waste activated sludge (WAS) to primary sludge and increase dryness of sludge mixture, anaerobic and aerobic digestion of WAS and mill effluents were investigated. With anaerobic or aerobic WAS digestion total reduction of WAS solids was only 23 - 26%. WAS pre-treatment with alkali hydrolysis and ozone solubilised 5 - 12% of WAS solids and released inhibitory substances for biodegradation and methanogenesis, dehydration properties of solids residue decreased as well. The most efficient process for WAS reduction is anaerobic pre-treatment of deinking plant effluent or mill effluent after primary treatment, which reduces BOD load of aerobic stage for 60 - 78% and biological sludge quantity for 52 - 71% and enables total mill sludge energy recovery and reduction of mill operational cost up to 5 € t^{-1}_{paper}. The effluent emission and biological treatment efficiency stays on the same level with anaerobic pre-treatment as it is only with aerobic stage, volume of aeration tanks can be reduced up to 70%.

Table 1: Digestibility of WAS and mill effluents – test results compared to reference values

	Anaerobic digestion		**Aerobic digestion**
Parameter	**Raw / hydrolysed WAS** **(NaOH, pH 12, 70^{0}C, 4h)**	**Mill effluents**	**Raw / ozone pre-treated WAS (16 mg O_3 g^{-1}VSS)**
WAS TSS reduction (%)	26 / 33	60 - 78	23 - 26 / 20
WAS VSS reduction (%)	31 (21 - 55)[1] / 55 (28 - 62)[3]	/	30 - 31 / 25
COD reduction (%)	/	64,8 - 67,6 (58 - 86)[1]	/
Biomethane potential (m^3 $t^{-1}VSS_{fed}$)	141 (40 - 200)[1] / 120 (11 - 249)[2,3]	386 - 456 (240 - 400)[1] (m^3 $t^{-1}COD_{removed}$)	/

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Ruthenium Quinazoline Complexes

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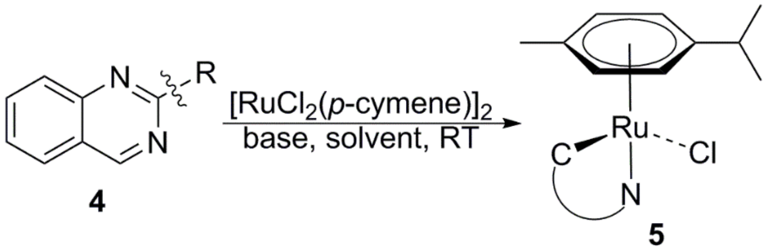
Cyclometallation of ligands by transition metals is a very important reaction since it enables the synthesis of organometallic complexes with a carbon-metal bond. The most commonly used methods for the synthesis of cyclometallated complexes are (a) transmetallation (transfer of ligands from one metal to another) and (b) metallation with selective C−H activation.[1,2] Organometallic reagents that are used in transmetallation reaction are expensive and often commercially unavailable. C−H activation allows the selective and direct functionalization of the non-reactive (hetero)aromatic C−H bonds, which leads to economically and ecologically friendly synthesis.[3]

Our research is based on the synthesis, characterization and evaluation of ruthenium quinazoline coordination compounds. We have successfully synthesized quinazoline derivatives that were further used as ligands for the synthesis of ruthenium complexes 5 (Scheme 1). Complexes wewe fully characterized using X-ray single crystal analysis. A detailed kinetic study of C-H activation reaction was carried out.



Scheme 1: Quinazoline analogues **4** and ruthenium complexes **5**.

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Determination of Fatty Acid Profile of Poppy seed, Walnut and Linseed Oil During UV Ageing

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Different kinds of oils can be distinguished according to their fatty acid (FA) composition. But the content of FA changes with ageing due to several factors. In the presence of triplet oxygen, oil is oxidized via autoxidation. Oil oxidation can also be accelerated by light (photosensitized oxidation) and is influenced by metals, pigments, antioxidants[1]…

FA composition of poppy seed, walnut and linseed oil was determined. After hydrolysis of triglycerides in oils fatty acid methyl esters (FAMEs) were prepared[2] and analysed using GC-FID. FA content of oils influenced by photosensitized oxidation was also examined. Samples of poppy seed, walnut and linseed oil were exposed to UV irradiation for different periods of time. Later on, FA composition of those samples was determined.

Our results show that oxidation caused a decrease in amount of unsaturated fatty acids (C18:2 and C18:3). On the contrary, short-chain fatty acids (C6:0 and C8:0) content increased. Despite the oxidation the mass percentage ratio of saturated fatty acids C16:0 and C18:0 stayed inside the limits characteristic for certain kind of oil.[3] Therefore, that ratio can be used as a criterion to differentiate between these three kinds of oils.

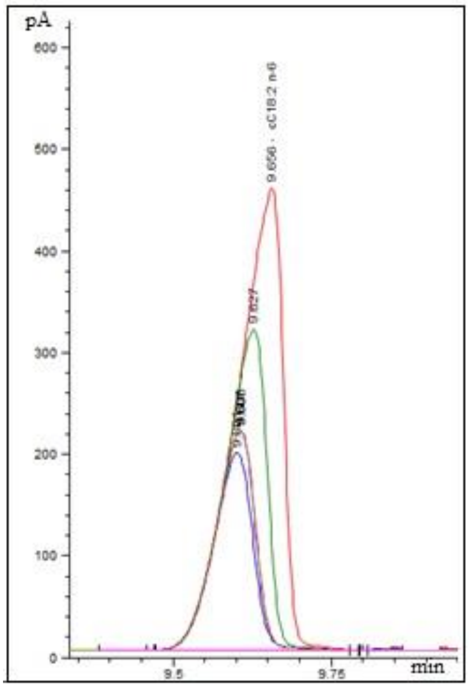

Figure 1: A part of gas chromatograms of the FAMEs that were prepared from samples of linseed oil after being irradiated for different periods of time. Peaks show a decrease in amount of linoleic acid (C18:2).

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Composting of Starch-based Bioplastics

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Composting is Nature's way of recycling, which mimicks what nature does every day and is a result of the action of microorganisms, such as bacteria and fungi.[1] The aim of the work was to check, whether the commercially available organic waste bags made of starch-based bioplastics (KP) can be composted. Their degradation potential was compared to potential of thermoplastic starch prepared in the laboratory (LP). The term TPS (thermoplastic starch) describes an amorphous or semi-crystalline material composted of gelatinized or destructurized starch, containing one or more plasticizers.[2] Plasticized films (with glycerol) show homogeneous surface without pores and cracks compare with unplasticized films. Starch-based bioplastic is brittle without glycerol.[3] Bioplastics were composed at 30 °C. Prior composting experiments the water retention capacity of the compost and the content of carbon in bioplastics were determined. Composting was successful for both bioplastics, because after 15 days for the LP and 68 days for the KP, we did not notice any visible residues of bioplastics in compost. The activity of microorganisms was proved with a scanning electron microscope images (Figure 1).

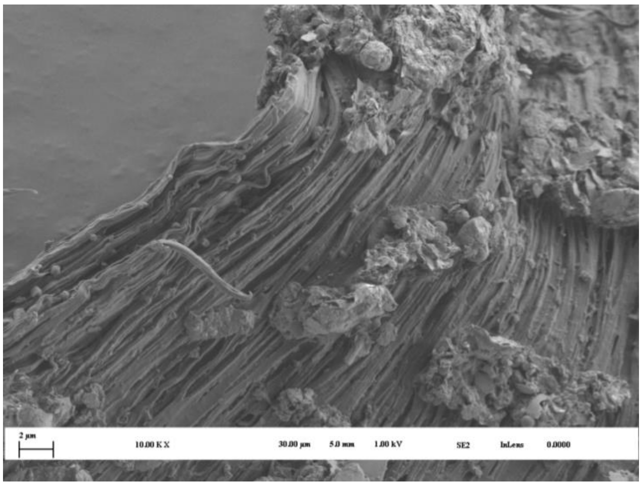

Figure 1: SEM micrographs of surface KP after 30 days of composting test (picture taken by B. Alič, UL FKKT).

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The Effects of Antioxidants on Gene Electrotransfer

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Gene electrotransfer is one of promising non-viral methods for introducing genes into the cell by using high voltage electric pulses. The method enables efficient gene transfer, however high electric pulses can produce free radicals and reactive oxygen species that affect cell survival. The aim of our study was to test the effect of two antioxidants on gene electrotransfer efficiency and cell survival: glutathione and vitamin E. We used CHO cells, cultured in vitro and performed experiments on plated cells and analysed effect of two different antioxidants on cell viability after electroporation. We also used two different pulsing protocols consisiting of longer (ms) and shorter (µs) pulses. Our results indicate that both glutathione and vitamin E didn't have much effect on gene electrotransfer efficiency and cell viability. Further investigations are needed to understand the mechanisms behind our findings.

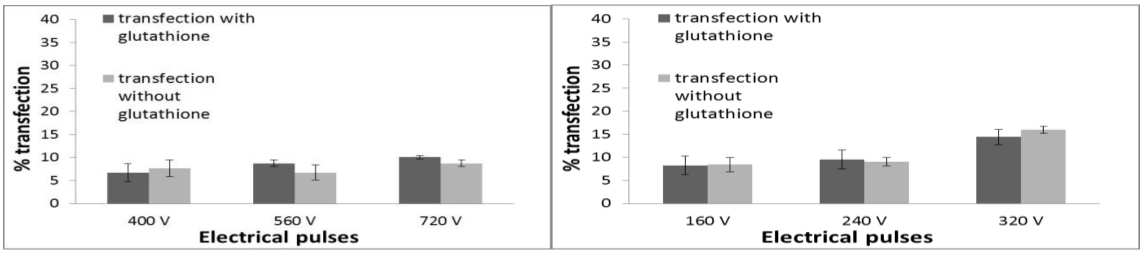

Figure 1: Effect of glutathione on gene electrotransfer efficency. A) with µs pulses of 8 × 200µs, and B) with ms pulses of 8 × 5ms

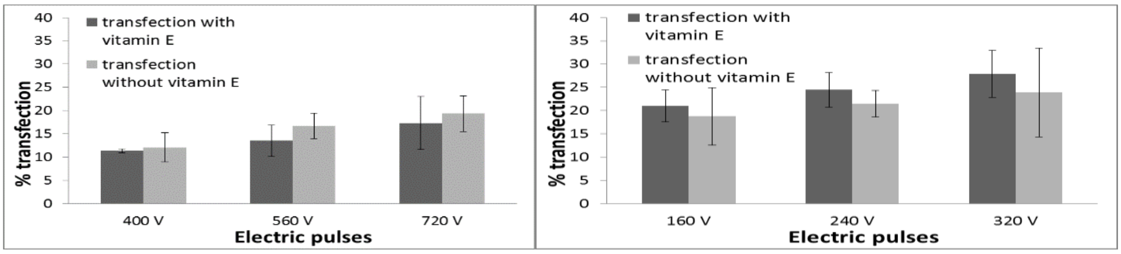

Figure 2: Effect of vitamine E on gene electrotransfer efficency. A) with µs pulses of 8 × 200µs,and B) with ms pulses of 8 × 5ms

Reference:

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Removal of Selected Specific Substances by Ozone Based Processes

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Discharging various micropollutants into aquatic environment in general and in particular substances classified as priority, hazardous and persistent ones generate serious concerns due to their potential adverse effects on human health and the living organisms in the environment[1,2]. Mostly these are synthetic and non-synthetic substances and their effective removal requires non-traditional processes, novel approach and technologies[3]. Ozonation and ozone based processes are considered the prospective ones[4]. The results of examination of the ozonation process, O_3/UV, adsorptive ozonation and combined process of ozone with zero-valent iron nanoparticles (nZVI) and zeolites (ZEO),are presented. Five selected organochlorine pesticides, i.e. hexachlorobutadiene (HCHBD), pentachlorobenzene (PCHB), hexachlorobenzene (HCHB), lindane (LIN) and heptachlor (HCH) in water solution were investigated. Three of these are specified as priority hazardous substances, while four of the pesticides are listed on the Stockholm Convention of Persistent Organic Pollutants[5,6]. Process removal efficiency exceeding 92% was reached for all studied pesticides after 3 min of ozonation; PCHB (50.8% removal efficiency) and LIN (no removal) being the only exceptions. Slightly higher removal rates and efficiencies were measured with O_3/UV process. However, the problem with low removal efficiencies of LIN and HCHBD remains unsolved also for this process. Adsorptive O_3/GAC and O_3/ZEO ozonation processes provided high removal rates and efficiencies for all pollutants, but again, except for LIN. Significant reduction in reaction time resulted from these processes in comparison to O_3 or O_3/UV processes. High removal efficiencies were measured for LIN, PCHB and HCH within about 5 min with nZVI. The highest differences were observed for the combined process GAC/O_3 in comparison with adsorption on GAC.

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Study of Binding Ligand TMPyP4 to Oligonucleotide of Human Telomeric Sequence Tel22 with Spectropolarimetry and Fluorimetry

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The nature of the binding mode and stoichiometry of the TMPyP4 cationic porphyrin to G-quadruplex structures is still very controversial, especially for intramolecular G-quadruplexes from human telomeric sequences.[1] In spite of numerous studies there is no simple general code that would explain the observed binding affinity of directed ligand.

Our work was based on studying the driving forces of binding of cationic ligand TMPyP4 to human telomeric sequence Tel22. Experiments were performed in 100mM K^+ and temperatures 15, 25 and 35°C. Ligand binding was studied using reverse titration method combined with CD-spectropolarimetry and fluorimetry spectral analysis.[2] Our aim was to determine the binding constant and the binding number from thermodynamic analysis of our spectra. Experimental data obtained at different temperatures were successfully described by a global model that takes into account independent binding sites on G-quadruplex.[3] We found that formation of complex is enthalpically and entropically favorable.

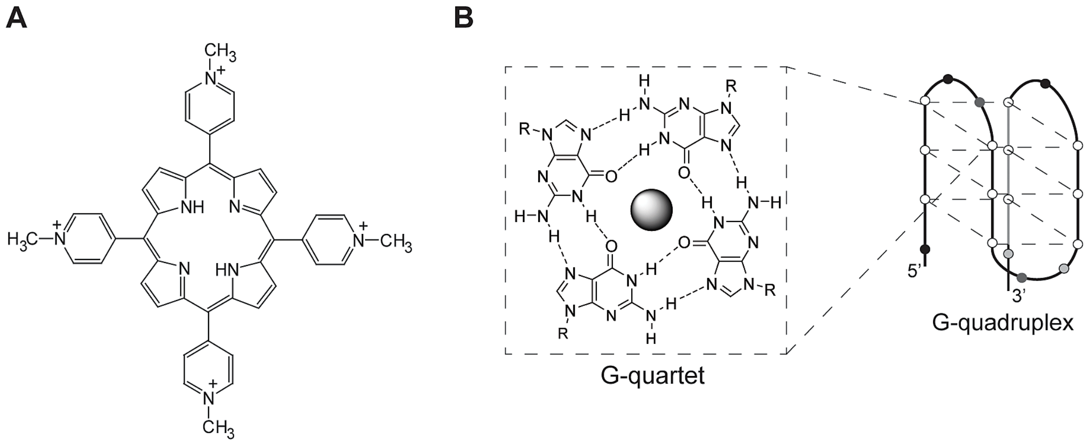

Figure 2: Structure of TMOPyP4 (**A**) and G-quadruplex (**B**)

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Novel (S)-1-(Pyrazolo[1,5-a]pyrimidinyl)ethan-1-amines Synthesis

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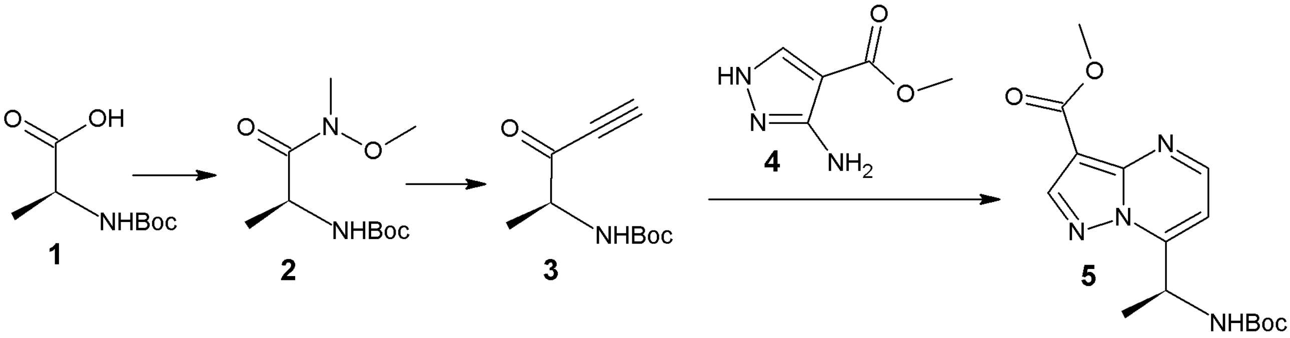
Many derivatives of pyrazolo[1,5-*a*]pyrimidines are biologically active as they are analogues of purine. As enantiomerically pure amines they are useful as ligands, bases and catalysts in asymmetrical synthesis and for separation of racemic mixtures.

Novel (*S*)-1-(pyrazolo[1,5-*a*]pyrimidinyl)ethan-1-amines were synthesized. First 1,3-dielectrophilic ynone **3** was prepared from (*S*)-Boc-alanine (**1**) via Weinreb amide **2**. Ynone **3** reacted with *N*,*N*-1,3-dinucleophillic 3-aminopyrazole **4** to produce target compound **5**. The fluorescent product **5** was separated from the other reaction components by colon chromatography and characterized by NMR.[1] The yield of the main product **5** was at first not satisfactory and was later improved from 46 % to 70 %. According to a known procedure, an extra step of enaminone formation was introduced but was not successful as some impurities were formed. Catalytic hydrogenation of **5** proceeded at the pyrimidine ring to afford two diastereomeric tetrahydropyrazolo[1,5-*a*]pyrimidines, which were separated by colon chromatography and characterized by NMR.[1] Compound **5** and the major diastereomer obtained upon hydrogenation were also deprotected. The ester group of compound **5** has been hydrolysed to form the free acid.



Scheme 2: Synthesis of pyrazolo[1,5-*a*]pyrimidine **5**.

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Consideration of Risks, Connected to Extreme Weather Events, in Spatial Planning of Energy Infrastructure

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The research investigates how to include risks to electric infrastructure due to extreme weather events (EWE) into the spatial planning. The ultimate goal is reduction of societal cost associated to damaged infrastructure, while intermediate goal is proper siting of new infrastructure. The work covers both development and testing of a method for integrating risks and spatial planning. The possibilities of application of the method are wide, ranging from different types of extreme weather events, diverse infrastructure, as well as different geographical scales and regions.

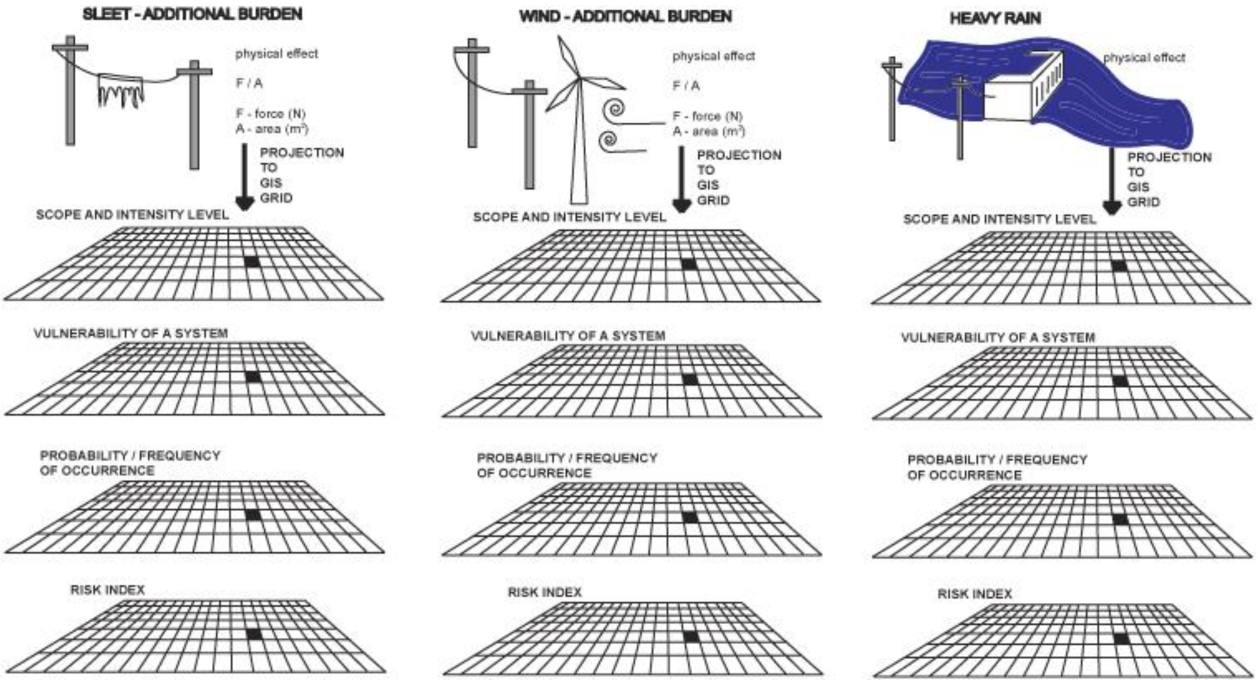

Figure 1: Steps in risk analysis

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Extraction and Characterization of TiO_2 Nanoparticles in Chewing Gums

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Nowadays engineered nanoparticles can be found in agriculture, cosmetics, pharmaceuticals, and also in food. One of the commonly used nanomaterials in food industry is titanium dioxide (TiO2), which is classified as food colorant due to its white color (label E171).[1] Thus, the purpose of this work was to verify if the chewing gums available on the Slovenian market contain nano-sized TiO2 and, further, to characterize the physico-chemical properties of such particles, isolated from the chewing gums.

The sample preparation was accomplished according to modified procedures from the literature[2,3] using only ultra-pure water, ethanol and acetone for extraction and cleaning. The physico-chemical characterization was made in dry (Scanning Electron Microscopy, X-Ray Diffraction and Energy Dispersive X-ray Spectroscopy analysis) and wet (zeta potential analysis and particle size distribution at inherent pH) conditions.

The results confirmed the presence of TiO2 in the tested chewing gums coatings (Figure 1). All samples had at least 5% of particles with dimensions between 130 nm and 170 nm, even though a bigger ratio of those agglomerated when in suspension.

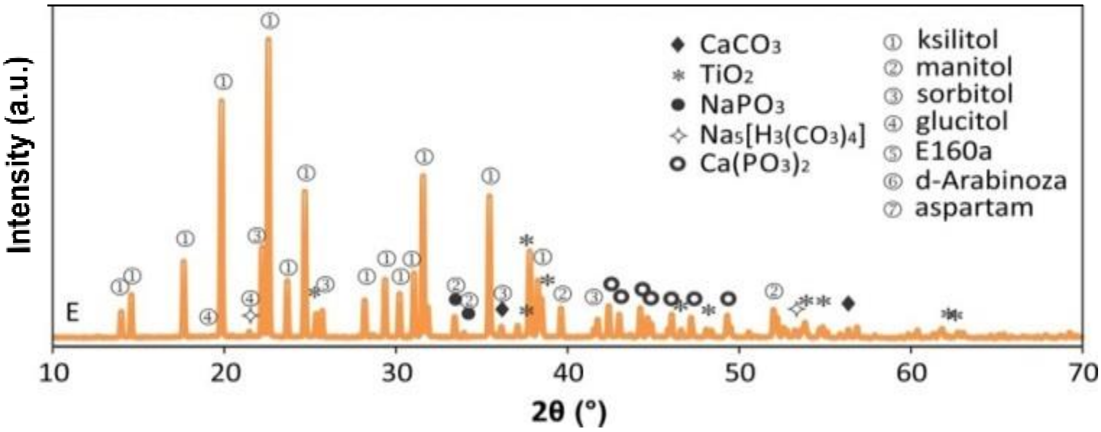

Figure 1: X-Ray Diffraction results for one of samples

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Quantum Mechanical Treatment of the Carcinogenesis of Acrylonitrile and Cyanoetyhlene Oxide

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Acrylonitrile (AN) is a carcinogenic compound. Cyanoethylene oxide (CEO), produced during the metabolism of AN, is a mutagenic and suspectedly carcinogenic compound. Solomon et al.[1] have confirmed the *in vitro* and *in vivo* binding of AN and CEO to nucleic bases as well as isolated the most common products. CEO reacts primarily with dGuo, to a lesser extent with dAdo and to an even lesser extent with dCyd and dThd.

We have performed quantum mechanical calculations of activation barriers for reactions of AN and CEO with nucleobases that produce the most common adducts. The calculations were performed with HF and MP2 *ab initio* methods, with B3LYP, M062X and MPW1K DFT and hybrid methods, as well as with AM1 and PM3 semiempirical methods. Various implicit solvation models were applied. The calculated activation barriers were compared with experimentally obtained reactivities of nucleobases. In the next step we have performed the calculations of activation barriers for reactions of CEO with various natural polyphenolic scavengers, and determined the best potential scavengers. Lastly, we have explored the effect of microwave catalysis on the dGuo alkylation by CEO. We employed the empirical force field and Eyring's transition state theories and compared the calculated thermodynamical parameters for the reaction with and without the influence of microwaves.[2,3]

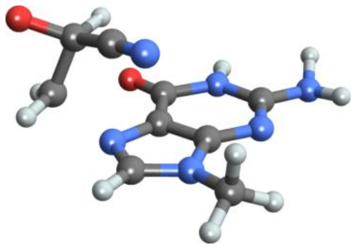

Figure 1: Transition state of CEO-Guo reaction, obtained using the MP2/6-311++G(d,p) level of theory. Ribose is truncated to a methyl moiety.

References:

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Distribution of Oxide Nanoparticles in Stainless Steel

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It is well established that the steel matrix composites commonly have a good combination of hard ceramic reinforcement particles (e.g. TiC, TiB_2, WC and Al_2O_3) and ductile metallic matrix, which make them a promising candidate in wear resistance applications.[1] Generally, there are several methods for fabricating the particulate reinforced steel matrix composites, such as powder metallurgy, conventional melting and casting, reactive sintering and self-propagating high-temperature synthesis.[1] The casting process is more economical than the other available routes for integration of nanoparticles into microstructure of steel. However, it is extremely difficult to obtain uniform dispersion of ceramic nanoparticles in liquid metals due to the poor wettability and the specific gravity difference between the ceramic particles and metal matrix. Furthermore, low concentrations and only few nanometers size limit the possibility of identifying presence, distribution and effect of ceramic nanoparticles in the steel matrix.[2]

The aim of the present work was to identify the distribution of nanoparticles in the steel matrix introduced through conventional melting and casting method, but above all to determine methodology and analyzing techniques suitable for analyzing and identifying nanoparticles incorporated in the steel matrix. In the frame of this work the steels dispersed with Al_2O_3 and TiO_2 nanoparticles were produced by conventional casting method and their microstructure investigated by optical, SEM and TEM techniques. Microstructural analysis shows that distribution of Al_2O_3 and TiO_2 nanoparticles, which show high degree of agglomeration, is very non-uniform. Furthermore, for detailed analysis of nanoparticles specific preparation and characterization by advanced microscopic techniques is required.

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Study of Cellulose and Ethyl Cellulose Dust Explosion Parameters

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Dust explosions are mostly treated as explosions which can occur only in coal mines and grain elevators. Now we know this is not true. It may apply wherever the combustible dusts are stored and the dust explosion conditions are fulfilled (dust explosion pentagram in Figure 1). The necessary conditions are fuel (combustible dust), oxidant (oxygen in air), ignition source (fire/heat), mixing of the fuel and the oxidant and confinement of the resulting dispersion.

Materials used in this study were selected carefully taking into consideration their common use in industry. Cellulose 20 and cellulose 50 were used to study the influence of particle size and specific surface area on dust explosion parameters while ethyl cellulose was chosen to describe the role of functional groups during ignition and progress of dust explosion.

The particle size distribution of powders and BET specific surface was determined by laser diffraction system Microtrac Bluewave and ASAP 2020 Micromeritics, respectively. Maximum pressure (P_{max}) and maximum rate of pressure rise ($(\frac{dP}{dt})_{\max}$) were measured in a 20L explosion sphere (Kühner). Minimal ignition energy (MIE) was measured by standard Hartmann tube.

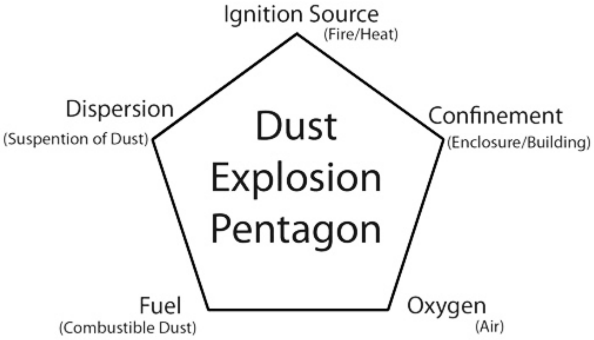

Figure 1: Dust explosion pentagon.

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Potential Biofungicide Effect of Cannabis sativa L. *Extract*

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Cannabis is an annual plant, which belongs to the family *Cannabaceae*. Fiber hemp (*Cannabis sativa L.*) is a sustainable and high yielding industrial crop, which can be grown for fiber and/or seeds in a wide range of geographic zones and climates.[1] The leaves can be used for tea infusions; however their use is not as abundant as in the case of stem and seeds. The phytochemistry of cannabis is very complex. Secondary metabolites present in *C. sativa* are cannabinoids, flavonoids, stilbenoids, terpenoids, lignans and alkaloids. The concentrations of these compounds are usually the highest in the young leaves and racemes, but depend much on tissue type, age, variety, growth conditions (nutrition, humidity and light levels), harvest time and storage conditions.[2]

There have been several studies showing that extracts from *C. sativa* have biopesticide actions.[3] The main purpose of our research was to validate biofungicide properties of extracts from various parts of plants. We tested ethanol extracts of different concentrations (25 g/l, 50 g/l), deriving from leaves and racemes, on parasitic and endophytic fungi. Fungi were inoculated on Petri dishes containing PDA medium and smeared extracts (0,1 ml per Petri dish. After 7 days of incubation the results showed that extracts from *C. sativa* have inhibitory effect on the growth of pathogenic *Epicoccum nigrum*, pathogenic *Botrytis cinerea* I, II, III and opportunistic pathogen *Alternaria alternata*. The highest inhibition rates were measured after treating fungi with 50 g/l racemes extract. However, no growth changes on endophyte *Aureobasidium pullulans* and pathogen *Fusarium oxysporum* were observed. We can conclude that application of C.sativa leaf and racemes extracts to the crop plants may be promising to fight plant fungal disease.

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Bled Lake Water Purification System - Siphon

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Lake Bled is ecologically vulnerable habitat and its self-cleaning ability is due to human activity insufficient. Water drain from the deepest parts of the lake Bled was installed in 1980/81, supported with a flushing water supply from the stream Radovna, should intensify the eutrophic water removal. A siphon rehabilitation was made in 1996, but lake condition is still not improving adequately (algal bloom remains). Total outflow at the end of the siphon is checked at measuring chamber, while actual locations of water intakes can be evaluated only by diving along the siphon, using local sediments as tracers. The research was done in December through diving, monitoring and filming the siphon and both Radovna pipelines and consequently defining the pipelines' location, hydrodynamic conditions at certain intake points and water temperature distribution. Temperature measurements show that autumn regime of the lake has been established, so the siphon impact on eutrophication in lake was weakened, while impact of Radovna water inflow increased. Inventory of all pipes was done and drawn on the lake's map. A webpage with videos and photographs of the research was created to illustrate the siphon's underwater activity. Flow visualization techniques revealed that pipeline A has many well-working inflow points. Inflow into the pipeline B is reduced, as opening is partially covered with a flap, while the flow into the pipeline C was not detectable. Research findings make the proper work of the siphon questionable. On the other hand, explored inlet conditions are significant information, enable a better calculation of the balance of nutrients in the lake.

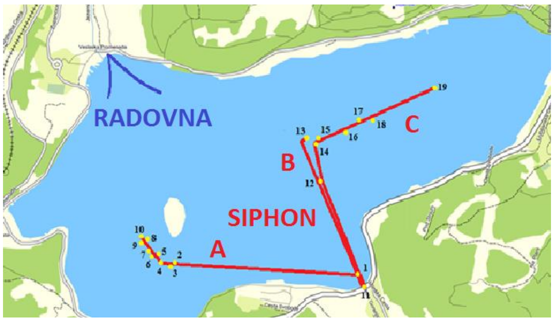

Figure 1: Location of pipelines based on the research

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Can Fungus Botrytis cinerea *Degrade Copper Nanoparticles?*

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Although copper (Cu) is an essential element for all living beings, in higher concentrations it can be one of the most toxic metals.[1] However, some microorganisms synthesize enzymes or produce biogenic oxidants/reductants which result in altered local redox environment and consequently detoxification of toxic compounds.[2,3] The uptake and accumulation of heavy metals and nanoparticles (NPs) by fungal biomass is receiving increasing attention since microbe based technologies may provide an alternative techniques of metal or NPs removal from polluted soils and waste waters or/and in production of different materials.

In our present study we compared two isolates (*Botrytis cinerea* and *Alternaria alternata*) from common buckwheat (*Fagopyrum esculentum*) spermosphere for their tolerance to copper. Their performance in the presence of CuO microparticles (MPs), CuO NPs, Cu NPs and Cu-salt, was tested in liquid media as well as on solid agar. As indicator for lipid peroxidation of membranes, the content of malondialdehid (MDA) in exposed fungal mycelia was determined by TBA spectrophotometric method.

Biomass production showed that *A. alternata* is sensitive and *B. cinerea* is more tolerant to copper treatments. Cu-salt was the most detrimental for fungal growth, followed by Cu NPs, CuO NPs and CuO MPs. In addition, MDA concentrations, analysed to demonstrate the level of stress, were lower in mycelia of *B. cinerea* than in *A. alternata*. The future challenge is the study of changes in Cu oxidation state and its complexation with different ligands.

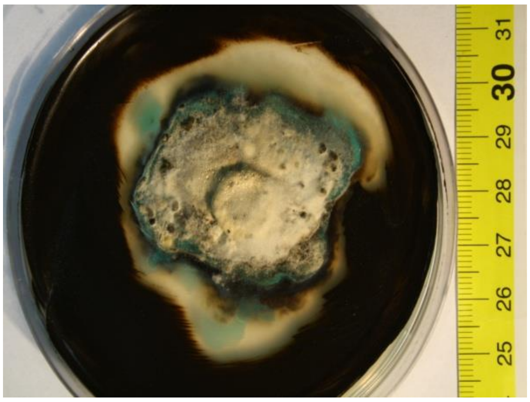

Figure 1: Growth of *B. cinerea* on Cu NPs.

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Humic Acid – an Important Factor in Toxicity of Metals

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Recently, many environmental factors have gained attention to be important parameters in altering metal toxicity. Mostly, pH or water hardness has been assessed as the most important ones. While impact of humic acid – an ubiquitous organic matter in the aquatic environment – has been only rarely investigated[1,2]. Hence the aim of our study was to assess how environmentally relevant concentration of humic acid will affect toxicity of chromium to duckweed *Lemna minor*.

In absence of humic acid, chromium ($0.15\ \mathrm{mg\cdot L^{-1}}$) was presented only as hexavalent chromium (Cr(VI)). It significantly reduced specific growth rate and chlorophyll *a* and *b* content by 18%, 51% and 38%, respectively. High accumulation of chromium has been noticed at the end of the experiment ($359\ \mathrm{mg\ Cr\cdot kg^{-1}}$ of dry weight of plant). In presence of humic acid (TOC = $10\ \mathrm{mg\cdot L^{-1}}$), hexavalent chromium was reduced to trivalent one (Cr(III)). Consequently, no negative effect of Cr on specific growth rate and chlorophyll *a* and *b* content was observed. Humic acid also caused low accumulation of chromium; about 57 % less chromium was accumulated in duckweed. The main reason for differences in chromium accumulation in presence/absence of HA and the observed effect can be explained by the fact, that HA strongly binds chromium, with Cr(III) having higher affinity than Cr(VI)[3]. Therefore the most plausible explanation in our case is, that Cr(III) was complexed by HA and remained unavailable while Cr(VI) was not. Hence, findings of our study suggest that humic substances can be considered as one of the most important environmental factors affecting chromium behavior in nature.

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Electrocoagulation of Liquid Wastes

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Often in industry hazardous and non-hazardous liquid wastes are produced, that exceed the limit values for discharge into the main sewage system, therefore a proper pretreatment is necessary. The purpose of this thesis was to study the possibility of physico-chemical treatment of liquid wastes to the extent of being suitable for discharge into the main sewage system. Electrocoagulation (EC) has shown to be an appropriate treatment method for some industrial liquid wastes according to the recent studies and literature. There are also a number of manufacturers of wastewater treatment plants that have been successfully including EC in their treatment systems. That is why we tested four different types of liquid wastes with the EC process with iron plates on a laboratory pilot device: liquid wastes from car washes and oil traps, liquid wastes from the metal manufacturing industry, liquid wastes from the industry of paints and varnishes and liquid wastes from the industry of soaps and detergents. The comparison of analysis of samples before and after the EC process demonstrated an effective reduction of organic pollution (COD and BOD_5), removal of suspended solids and reduction of concentrations of mineral oils, heavy metals and surfactants. All of the above mentioned liquid wastes have proven to be appropriate for that kind of treatement before the subsequent treatment on a biological treatment plant.

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Investigation of Imidacloprid Removal from Water Media by Multiwalled Carbon Nanotubes and Magnetite Modified Multiwalled Carbon Nanotubes

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Nanotechnology is a multidisciplinary science that studies the physical, chemical and biological properties of atomic particles and molecules and it is, also, the engineering discipline that investigates the technological processes of design of functional materials, devices and systems at the nanometer scale (1-100 nm).[1] Nowadays different type of nanosized materials find applications in biology, medicine, environmental protection,[2] at the nanometer scale devices, different sensors[3] and many others.

In this study, we examined the removal of a worldwide used neonicotinoid insecticide imidacloprid (IMI) by multiwalled carbon nanotubes (MWCNTs), oxidized MWCNTs and by 20% w/w magnetite modified oxidized MWCNTs in the presence/absence of H_2O_2 from its water suspensions. The investigations were performed by different weight of nanomaterials (10 and 20 mg in 20 mL of water) in the presence/absence of 537.2 µg/mL H_2O_2. Initial concentration of IMI was 2.4 µg/mL either in the case of standard solution or in the case of solution prepared from its commercial formulation. The IMI removal was monitored by a simply HPLC-DAD method from the water phase of the samples which were taken at following time intervals: 0; 1; 2.5; 5; 7.5; 10; 15; 20; 30 and 40 min. It was found that the amount of the used nanomaterials influences significantly the IMI removal and the magnetic behavior of the composite material helped for the removal of adsorbents. The most intensive changes in the IMI concentration were observed in the first 5 min of treatments.

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Changes in ODN Nucleotide Sequence Effect TLR9 Activation

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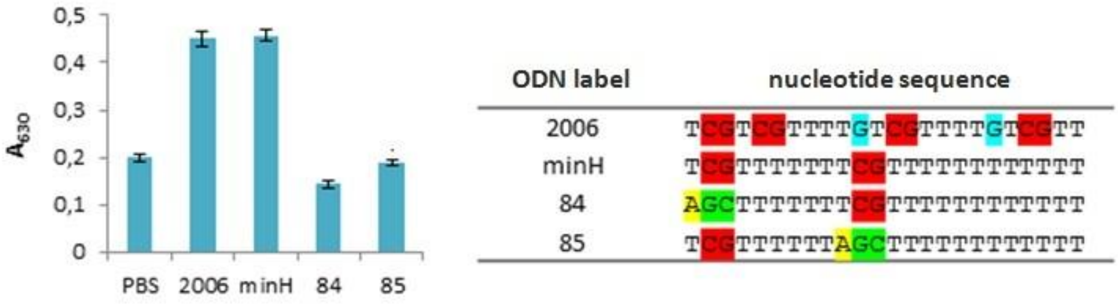
TLR9 receptors play an important role linking innate and adaptive immunity and therefore overall immune response. They are activated by nonmethylated CpG motives in single stranded DNA molecules of microorganisms or oligodeoxynucleotides (ODN).

Previous research on B-cell activation via TLR9 by Pohar J. et al [1] determined an ODN with the minimal sequence requirements to activate human TLR9 (minH). Further work focused on observing how the ability of minH to activate human TLR9 receptor is affected by varying different parameters.

B-cell activation with minH is comparable to that with the standard human TLR9 agonist ODN2006. Collected data show TLR9 receptors are highly specific as slightly altering minH nucleotide sequence results in decreased activation of human TLR9. It was observed CpG motives are essential for successful activation of B-cells as shown in Figure 1. Proper order of motives from 5' end to 3' end and definite lengths of other parts of sequence is also a requirement for successful B-cell activation.



ODN label	nucleotide sequence
2006	TCGTCGTTTTGTCGTTTTGTCGTT
minH	TCGTTTTTTTCGTTTTTTTTTTTT
84	AGCTTTTTTTCGTTTTTTTTTTTT
85	TCGTTTTTTAGCTTTTTTTTTTTT

Figure 2: The effect of replacement of 1st or 2nd motive TCG with a complemetray sequence AGC on B-cell activation. Used sequences are presented in the table on the right accordingly. As negative control an aliquot of PBS was added. Positive controls are ODN 2006 and minH.

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Characterisation of Biochar Produced from Waste Sewage Sludge

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Char is a product of carbonization of organic material in a process of pyrolysis. The term biochar is used for char which is intended for agricultural purposes, mainly for increasing soil fertility. Many studies on biochar have shown that it has many benefits on the environment. It has the impact on pH in soil, water retention capacity and releasing of nutrients. The studies are lately focused on using sewage sludge from wastewater treatment plant as a precursor for biochar production to simultaneously solve a problem of sewage sludge deposition. In presented research dry granulated sewage sludge was characterised and converted to biochar in a small biochar kiln aimed for domestic preparation of biochar. Pyrolysation was accomplished in different conditions and produced biochar was compared. Thermal anylyses of products were also accomplished and they have shown significant difference in behaviour of precursor and biochar (Figure 1). It confirmed that biochar had less moisture and higher C content. Surface of the granulated sewage sludge before and after pyrolysation was examined using confocal microscopy.

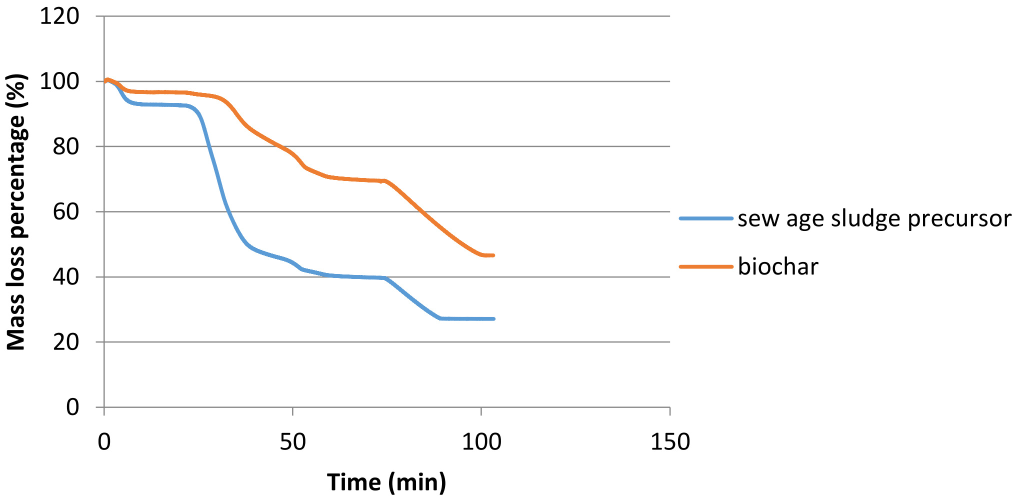

Figure 1: Thermal analysis of dry waste sewage sludge before and after pyrolisation**.**

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Determination of Proteins in Works of Art: Connecting CIM Monolith Chromatography and ELISA

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Analysing the materials present in artworks presents unique challenges, due to their chemical complexity, interactions between the components of the mixture, alteration, exposure to human intervention, etc. Among materials present in art objects, proteins have been widely used, generally in the form of adhesives, coatings, but especially as binding media for colouring materials. The most commonly used were casein, ovalbumin and collagen. An investigation of proteins used for such purpose can be particularly challenging due to their low concentration present in solid matrix, co-presence of different organic substances, pre-existing conservation treatments, etc. Furthermore, propensity to undergo alterations during ageing (fragmentation, alteration of amino acids such as oxidation, deamination and hydrolysis) represents additional difficulty in their specific identification in different works of art. The implementation of immunological analytical techniques, such as ELISA showed the potential to become essential in the determination of proteins in works of art (high sensitivity (in the nanogram range), specificity and micro invasiveness of required sampling).[1] However, despite the advantages of these methods, interference by metal ions present in pigments, lipids and saccharides in other components of paints, grounds and varnishes may cause several problems for incontestable identification of proteins. To overcome the drawbacks of the ELISA technique, the DEAE modified (weak anion exchange) CIM (Convective Interaction Media) monoliths, which are highly cross linked, porous monolithic polymers with a well-defined channel size distribution, were utilized to purify the extracted mixtures to predominantly protein fractions.[2] Utilisation of CIM monoliths greatly enhanced the ELISA detection of proteins in the model paint samples (aged and non-aged), for at least one tenfold dilution of a sample. In several cases the purification enabled detection of the target protein, otherwise not possible with non-purified samples. ELISA procedures were concomitantly co-evaluated by LC/MS-MS to get a better insight on the alterations in the studied protein.

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Influence of Cutting Parameters on Surface Roughness of Steel in Milling

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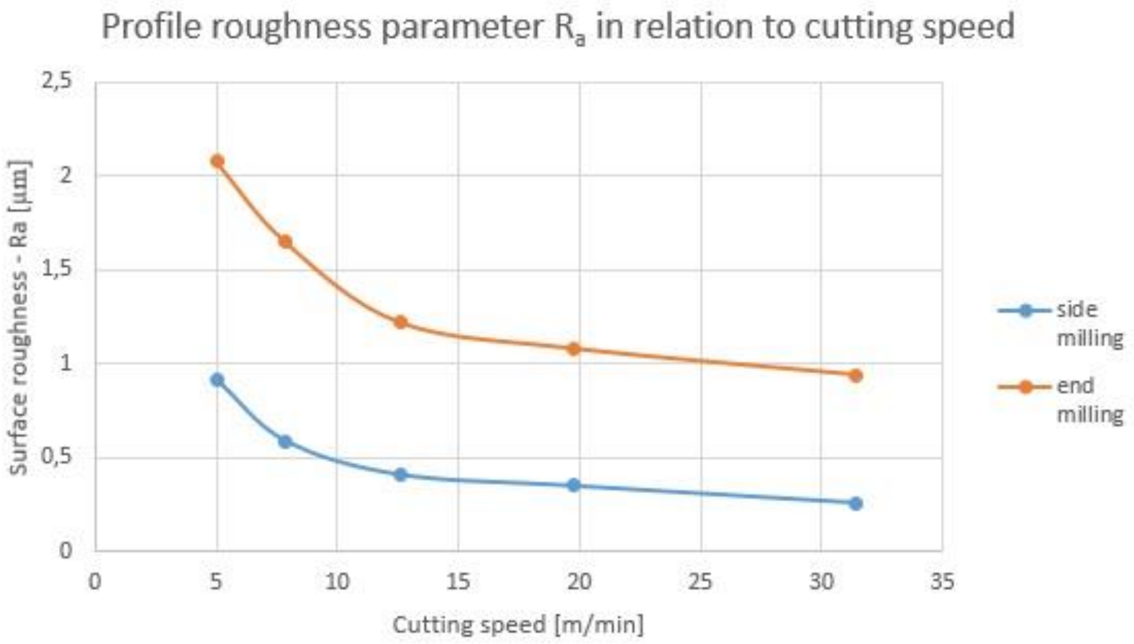
The objective of this work was to analyze the relation between cutting parameters and surface roughness in milling and compare them to theoretical results obtained from already known equations. An TiAlN coated end mill was used in combination with a manual milling machine. Because of backlash of manual milling machines, only up-miling was used, however both side and end milling were tested. Theoretical and practical results showed similar trends, however there were noticeable discrepancies between them, which can be explained by a number of factors influencing cutting efficiency, including machine rigidity and geometric accuracy, absence of cooling and quality of the tool used.



Scheme 1: Profile roughness parameter R_a in relation to cutting speed – experimental results

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Taxonomic Delineation of the Synechocystis Genus (Cyanobacteria) Based on Sequence Analysis of 16 S rRNA and Internal Transcribed Spacer Regions

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Identification and classification of cyanobacteria was traditionally based on morphological features, which often lead to misidentifications and false classification[1]. To overcome variable morphological criteria, DNA sequencing is becoming the most widely applied molecular method in the identification and cataloguing of cyanobacteria, either as the sole method of identification or in combination with morphological, phenotypic, and ecological characterization. Genus *Synechocystis* is especially interesting since no in depth taxonomic classification has been performed up to now, even though *Synechocystis* sp. PCC 6803 is the most popular cyanobacterial model for prokaryotic photosynthesis and metabolic engineering. To clarify the phylogeny of this genus, PCR amplification and sequencing was performed using two regions commonly used in classification: 16S rRNA region and internal transcribed spacer (ITS) located between 16S and 23S. Nine different *Synechocystis* strains were analysed, seven of them being determined to species level. As expected, much greater variability was observed among species in the ITS than 16S rRNA region. Our results clearly suggest that under current classification, *Synechocystis* genus is polyphyletic. Since some strains exhibited higher relatedness to other genera such as *Synechococcus*, *Chamaesiphon*, *Gloeothece* and *Gloeocapsa*, we propose reconsideration of taxonomic classification of the genus *Synechocystis.*

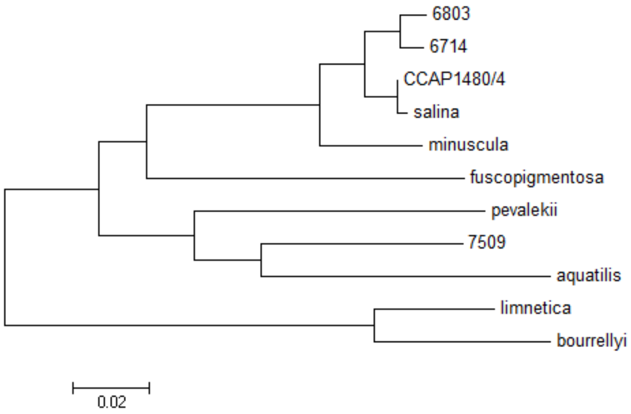

Figure 1: A phylogenetic tree for the analysed members of the *Synechocystis* genus based on sequencing results of the 16S rRNA region and the ITS region combined. Sequence data for PCC 6714 and PCC 7509 are included as deposited in GenBank.

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Extraction of Phenolic Acids from Red Wines Samples, their Simultaneous Identification and

Quantification by GC-MS

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Phenolic acids (PAs) are plant metabolites widely spread throughout the plant kingdom.[1] They have excellent antioxidant activities, higher than those of vitamins C and E, and because of this, PAs can be used to prevent or treat diseases associated with oxidative damage, such as: coronary heart disease, stroke and cancers.[2] High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are the most useful among the chromatographic methods applied for determination of PAs.[3] Aim of this study was to develop a method for the extraction, simultaneous identification and quantification of 5 different PAs (*cis*-caffeic, *trans*-caffeic, vanillic, syringic, trans-p-coumaric) from wine samples using GC-MS. The extraction efficiency of different SPE cartridges was compared. HLB cartridges for water samples have been proven as the most applicable. Stationary phases in such cartridges are made from a specific ratio of two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene. The identification of phenolic acids was carried out using a GC-MS method after derivatization with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). An internal standard (ISTD)-2,5-dichlorobenozic acid was used. The method linearity was tested in the concentration range from 5 to 100 mg L^{-1}. It was confirmed that the method is linear with the correlation coefficients (r^2) between 0.997 and 0.999, for all analyzed PAs. The limit of detection (LOD) and limit of quantification (LOQ) for PAs were determined on the basis of a standard calibration curve. LOD's were between 0.03 and 0.25 mg L^{-1}, and LOQ's between 0.20 and 0.60 mg L^{-1}. The applicability of the developed method was tested on the samples of Slovenian domestic red wines. Identified phenolic acids were present in the concentration range from 2 to 8 mg L^{-1}.

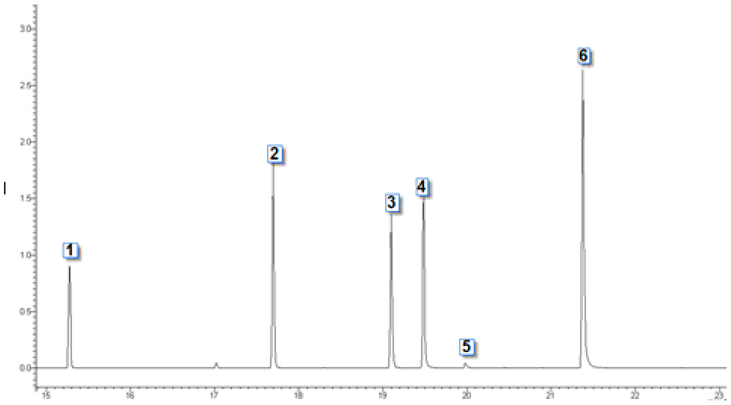

Figure 1: GC-MS chromatogram of PAs from a standard mixture. (1.ISTD; 2. vanillic acid; 3.syringic acid; 4. *trans*-p-coumaric acid; 5. *cis*-caffeic acid; 6. *trans*-caffeic acid)

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Assessesment of Packaging Waste Generation

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In order to develop more efficient waste prevention policies, it is necessary to identify and to assess the driving forces of waste generation. As a solution to the problem of waste prevention measuring, common approach in the literature is to analyse the connections of the generated amounts of waste with the variables describing socio-economic activities[1]. In this work, three methods where used to analyse the household consumption as the cause of packaging waste generation by using the statistical data for the 15 EU member states (2002 – 2012). Panel data analyses (PDA) fixed effects model was used to project the amounts of generated waste. Decoupling factor approach[2] was used to show whether the decoupling between the regression slopes of the observed variables is taking place. Logarithmic Mean Divisia Index (LMDI) analysis[3] was used to analyse whether the changes in the generated amounts are connected with the changes in the intensity of waste generation, with the changes in the overall consumption activity of the member sates or with the changes in the consumptions between different years (structural). The results are shown in the Figure 1.

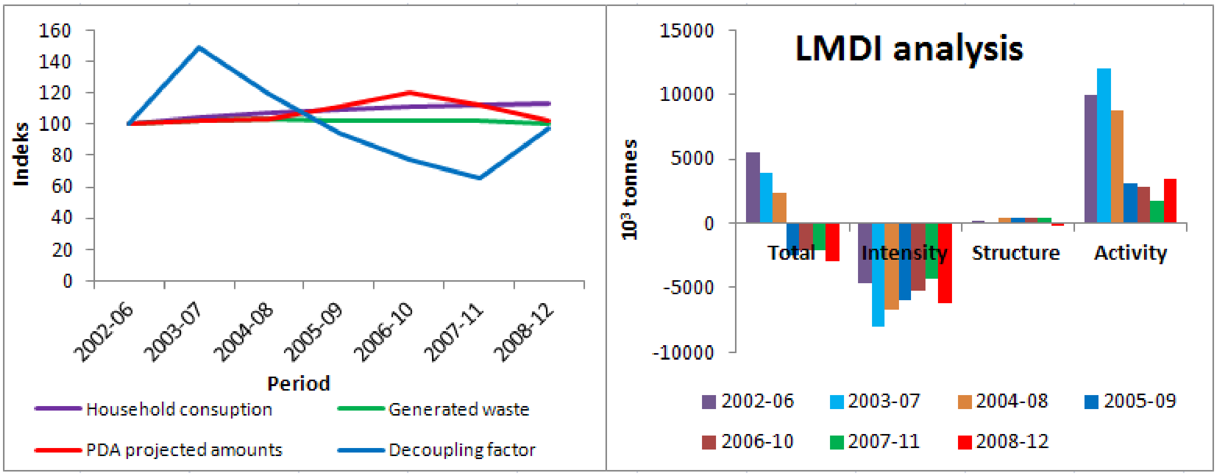

Figure 1: Analyses results of the household consumption as a driver of packaging waste generation

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Synthetic Biology Devices for Improved Biosafety of Genetically Modified Synechocystis sp. *PCC6803*

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Biosafety issues of genetically modified cyanobacteria are widely unexplored, although they can have a fundamental impact on public opinion and acceptance of cyanobacterial biotechnology. Within the EU CyanoFactory project we are developing synthetic biology devices for improved biosafety of *Synechocystis sp*. PCC6803. Cyanobacteria have evolved a network of toxins and antitoxins that play a role in cell survival under stress conditions. We thought that by rewiring at least one of the toxin-antitoxin pairs for conditional expression, it would be possible to achieve cell survival only in the photobioreactor, while the toxin would prevail in the case of accidental release into environment, thus killing the cells. To circumvent the likely event that some of the cells in the population would develop resistance (1) to this suicide switch, we envisaged another kill-switch that would eventually be combined with the first in the same cell. We chose the nonspecific nuclease from *Anabaena sp*. (in combination with its cognate inhibitor) rewired for conditional toxicity. So far, killing has been observed for both switches. Our combination biosafety device is designed to (i) induce controlled killing of recombinant cyanobacteria as well as (ii) degrade bacterial genetic material.

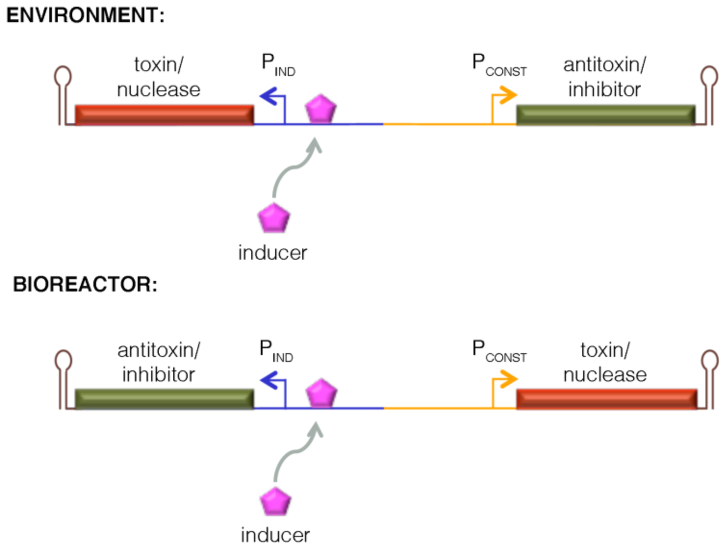

Figure 1: Two possibilities for the combined toxin-nuclease switch. (*environment*): placement of the toxin/nuclease gene under an inducible promoter to achieve cell death upon exposure to environmental inducer, while producing the antitoxin/inhibitor from a low-level constitutive promoter to protect cells from possible leaky toxin/nuclease expression in the bioreactor; (*bioreactor*): expression of the antitoxin/inhibitor from inducible promoter in the bioreactor (the trigger is present at inducing levels only therein), while producing the toxin/nuclease from a constitutive promoter (in the environment, toxin/nuclease expression would take over and cause cell death).

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Characterization of Interactions of Cathepsin K with Physiologic Anions and Polyanions in Acidic Enviroment

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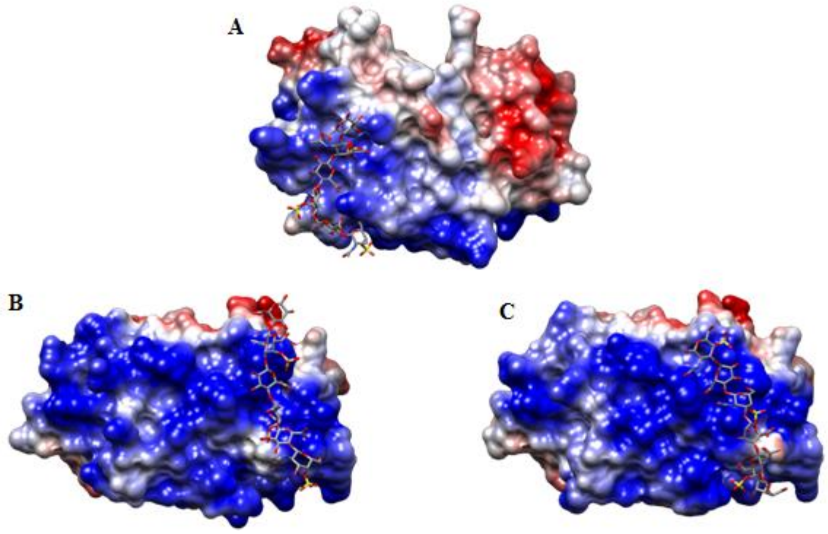
Cathepsin K is an endopeptidase from the papain like cysteine cathepsin family. Its most important physiological function is collagen degradation in bone resorption by osteoclasts[1]. Several glycosaminoglycans, especially chondroitin and dermatan sulfate have been known to regulate of its collagenolytic activity via allosteric mechanisms at neutral pH[2].

The major purpose of our work was to characterize the effect of different physiologic anions and polyanions on cathepsin K in acidic environment similar to the osteoclast resportion pit. For this purpose we have expressed recombinant procathepsin K, activated it and measured its kinetic parameters in the presence and absence of effectors. We tested interactions with heparin, chondroitin sulfate, heparan sulfate, dermatan sulfate and hyaluronan as well as fosfate ions. Experiments performed with the synthetic substrate Z-Phe-Arg-AMC have shown that dermatan sulfate and fosfate ions activate cathepsin K, whereas heparin inhibits it. Chondroitin sulfate, heparan sulfate and hyaluronic acid did not show any measurable effect.

Based on known crystal structures of cathepsin K with chondroitin sulfate and dermatan sulfate[3] we constructed mutant cathepsin K variants in which basic residues interacting with glycosaminoglycans were replaced (Arg123Ala, Lys119Ala and Lys119Glu). We then tested the effect of glycosaminoglycans on the mutants with Z-Phe-Arg-AMC and soluble calf skin collagen as the substrates.



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Halosubstituted 8-Hydroxyquinoline Ruthenium Complexes as Potential Anticancer Agents

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In our previous research we have reported the improved synthesis, physico-chemical characterization and biological evaluation of an organoruthenium-clioquinol complex [(η^6-*p*-cymene)Ru^{II}(Cq)Cl] (**1**) which exhibits selective toxicity towards leukaemic cancer cells. In our efforts to 'teach an old dog new tricks', we combined in a single molecule the structural fragment of an established apoptotic agent, clioquinol, with an organoruthenium moiety. The cytotoxicity studies show that the antiproliferative activity of **1** is mediated through caspase activation. Moreover, the cytotoxic activity itself in these leukaemia cell lines is improved in comparison with free ligand CqH, and is copper independent. The mechanism-of-action studies suggest a mechanism of action that is very much different from that of the free 8-hydroxyquinoline ligand.[1] In order to better explore the impact of 5- and 7-substituents and the labile halide ligand we synthesized a series of analogues. The complexes [(η^6-*p*-cymene)Ru^{II}(κ^2-O,N-HyQ)Cl] with $R_5 = R_7 =$ Br, $R_5 = R_7 =$ Cl and $R_5 =$ Cl $R_7 =$ H were already reported by Vidya et al.,[2] while the complex [(η^6-*p*-cymene)Ru^{II}(κ^2-O,N-HyQ)I] $R_5 =$ Br $R_7 =$ H is first presented here.

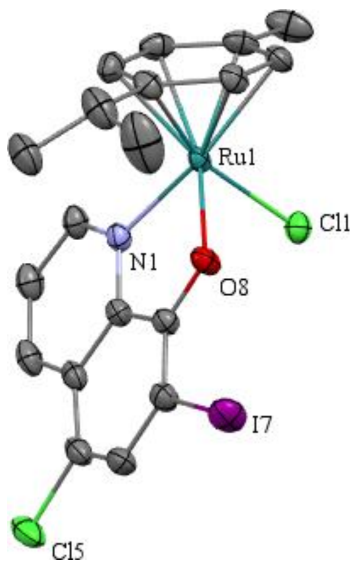

Figure 3: Crystal structure of **1** with labeled heteroatoms. Hydrogen atoms are omitted for clarity. Thermic ellipsoids are shown at 30% probability.

Table 2: IC_{50} for selected cell lines (µM).

Compound\Cell line	MCF-7	PC-3	HOS	Raji	Thp-1	Jurkat
Clioquinol	>50	45±7	28±4	25±5	24±6	22±5
1	>50	42±10	27±2	6±3	6±5	5±2

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The Effect of Magnetic Water Treatment on Calcium Carbonate Precipitation on Heated Surfaces

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Physical water treatment incorporates both conventional and unconventional processes based on a number of different mechanisms. The utilization of latter methods has been increasing steadily for the last few decades and some of them have already received widespread household applications, e.g., magnetic water treatment devices. Yet, we still do not have a full understanding of the mechanisms behind many of the methods and thus predictions in practical problem solving are often difficult due to the lack of a theoretical background.

This project deals with magnetic water treatment (MWT), specifically the calcium carbonate buildup on electric heaters. The buildup is especially problematic in process engineering as it reduces the effectiveness of heat exchangers, cooling towers and other similar equipment by both restricting the liquid flow and reducing the heat flux. MWT is supposed to reduce and possibly prevent $CaCO_3$ buildup although the experts are divided on the matter and the reports of experimental results are often contradictory to one another. MWT allegedly increases the portion of the more favorable precipitate polymorph aragonite[1,2].

Tests were performed on two parallel experimental setups with one setup including a commercially available MWT section "Eko sistemi Mark". In addition to a pump, each setup included two electric heaters of which one operated continuously and the other in alternating on/off mode. Heat exchangers were used in each setup to maintain constant water temperature. Synthetic hard water was used to eliminate the influence of other components and impurities found in tap water[3]. After multiple weeks of continuous operation, scale buildup on the heated surfaces was examined using x-ray diffraction analysis.

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Characterization of Interaction Between The PheThr Dipeptide and Cathepsin B

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Lysosomal cathepsins are synthesized as preproenzymes. The N-terminal proregion assists in the folding of the enzyme and its targeting to the lysosome and acts as an inhibitor to prevent inappropriate proteolytic activity of the zymogen. Propeptides are removed during processing of cysteine cathepsins and have been shown to be potent inhibitors of their cognate enzymes *in vitro*. The synthetic cathepsin B N-terminal propeptide was found to exhibit a limited selectivity of inhibition with K_i = 0,4 nM at pH 6.0, though the inhibition was much weaker at pH 4.0.[1]

The crystal structure of mature recombinant cathepsin B, revealed an unexplained electronic density in the P' sites. Based on computational modeling and experimental analyses by N-terminal sequencing, the electron density was identified as a PheThr dipeptide derived from the propeptide (**Fig. 1**). The conserved cleavage site for cathepsin B during autocatalytic processing is reported to be between Met56 and Phe57.[2] N-terminal sequencing of our recombinant cathepsin B has determined the cleavage site between Thr58 and Glu59, suggesting that the PheThr dipeptide is removed during processing and binds into the primed sites.

Our kinetic analyses with small synthetic and large macromolecular substrates show that the PheThr dipeptide is a competitive inhibitor of cathepsin B with a K_i value of 210 µM at pH 4.5. The collected data point towards PheThr being an intrinsic safety mechanism for the inhibition of cathepsin B peptidyl-dipeptidase activity in the absence of proper substrates in the resting lysosome.

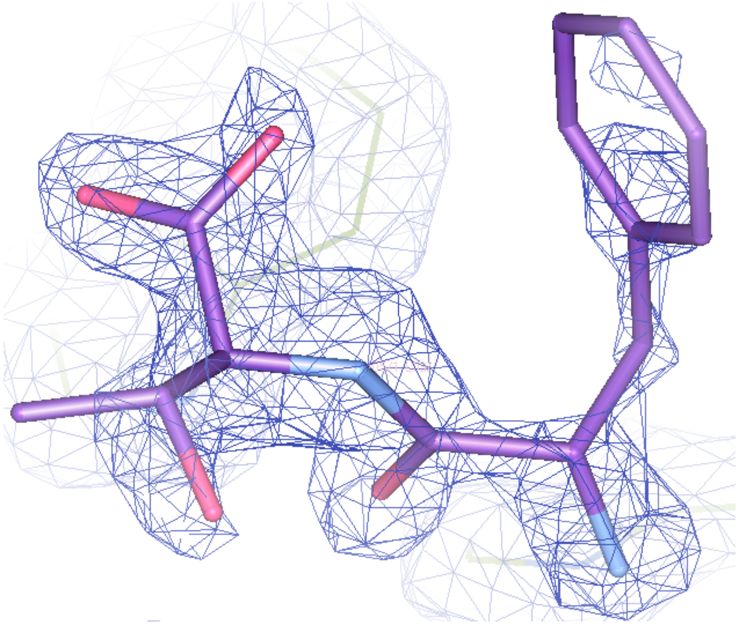

Figure 1: Modeling the dipeptide PheThr into the unexplained electron density at r.m.s.d value 1. The image was prepared using Coot.

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Tuning the Morphology of Poly(HEMA) Prepared via Suspension Polymerization

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The most frequently used form of polymer supports for batch operations are spherical particles prepared by free radical polymerization in a suspension medium.[1] Poly(2-hydroxyethyl methacrylate), poly(HEMA), is a biocompatible polymer, which is commercially used for contact lenses[2], tissue engineering and other biomedical applications.[3] Many studies of preparing porous poly(HEMA) based beads have been reported.[4]

Herein, we report the synthesis of poly(HEMA) beads by inverse suspension polymerization, with N,N'-methylenebisacrylamide (MBAA) as the crosslinker. In order to study the morphology, the amount of crosslinker (25, 10 and 2 w %) and porogens (isopropanol, toluene, THF, n-heptane) have been varied. Polydisperse swellable beads were obtained. With using porogens obtained beads were smaller (Fig.1 left). Measured surface area and pore volume showed a microporous or nonporous structure. Higher surface area was determined only with 25 % MBAA content (Fig.1 right). Open porous nature was not achieved with porogens, so results indicated a closed porosity of poly(HEMA-co-MBAA) beads.

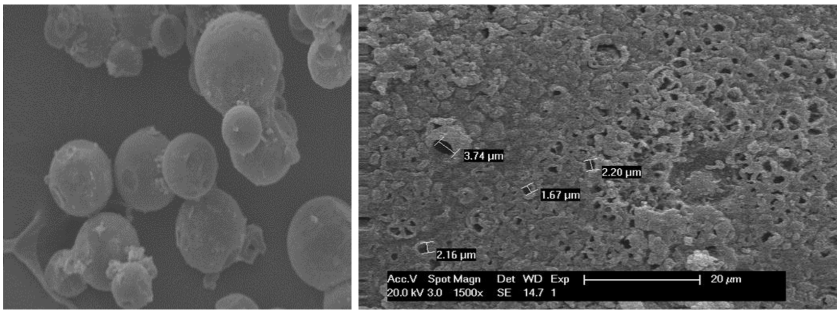

Figure 1: SEM image of poly(HEMA) beads: (left) with isopropanol; (right) bead surface-25 % MBAA

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Free Fatty Acids and Secreted Phospholipase A_2 Can Have Either a Positive or a Negative Effect on Lipid Droplet Formation in Cervical Cancer Cells

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Cells store neutral lipids within lipid droplets (LDs), which act as platforms integrating cell signalling and metabolism with important implications for metabolic diseases and cancer. Secreted phospholipases A_2 (sPLA_2s) are lipolytic enzymes that act in the extracellular environment, hydrolyse cell membrane phospholipids and release a mixture of mono- and polyunsaturated fatty acids (PUFAs). We have shown recently that sPLA_2s induce LD formation in breast cancer cells and prevent their death upon starvation. Here we analysed the effects of sPLA_2, and its individual FA products, in HeLa cervical cancer cells. Surprisingly, we found that the amount of LDs in HeLa cells and the effect of sPLA_2 depends strongly on cell density (Figure 1 left). At lower cell densities, less LDs were present in control cells and sPLA_2 stimulated LD formation, while in very dense, confluent cultures, the enzyme reduced the amount of LDs. A similar differential effect was observed with individual FAs, including omega-3 and omega-6 PUFAs. Importantly, we found that low, physiological concentrations of FAs reduce LD amount, while higher concentrations, typically used in most studies, induce LD formation (Figure 1 right). Therefore, unsaturated FAs, either coming from dietary sources or released from cell membranes by sPLA_2 activity, can have a positive or negative effect on LD formation in cancer cells. Our results suggest the intriguing possibility that LD metabolism is tighly linked to cell growth and proliferation in cancer and possibly other types of cells.

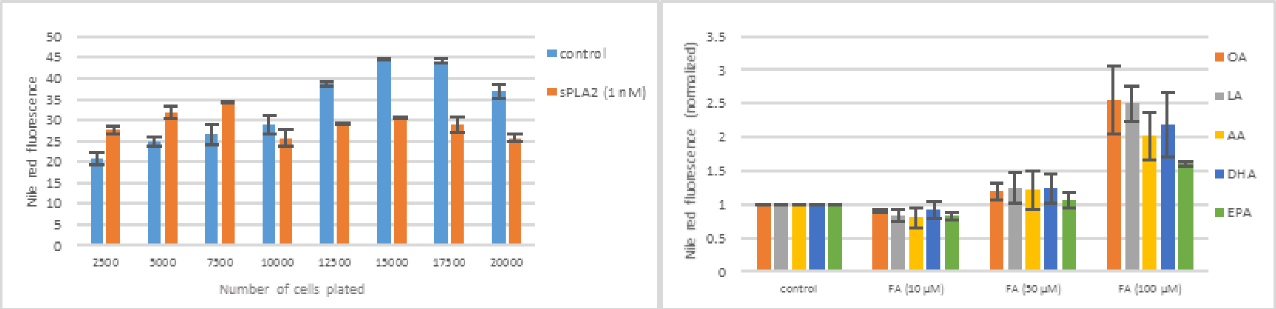

Figure 1. sPLA_2 has a dual effect on LD formation in HeLa cells (left). Oleic (OA), linoleic (LA), arahidonic (AA), docosahexaenoic (DHA) and eicosapentaenoic acids (EPA) reduce LD formation at 10 μM and induce their formation at higher concentrations (right).

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Characterization of Interaction Between Testican-2 and C1q

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Testicans are multidomain proteoglycans assembled of follistatin, thyroglobulin and calcium binding domains.[1] It is proposed that each testican domain with its structural and functional features contributes to a diverse set of different roles and activities. Some of these activities have already been identified in the past, yet without the knowledge of precise structure their significance remains elusive. Besides testican-1 there are two homologs known in humans, testican-2 and testican-3. Most probably they function in extracellular environment where cell-cell and cell-ECM interactions take place. Therefore testicans might be crucial for the adhesion and migration capabilities of cells and may be involved in vast array of physiological and pathological states.[2] The existence of interaction between testican-2 and complement component C1q has already been demonstrated using affinity methods.[3] However, specific features and attributes remain unknown and are under investigation.

For the purpose of studying above mentioned interaction, two different recombinant testican-2 constructs were prepared in Sf9 insect cell line as an N-terminal fusion with modified expression cassette (Figure 1). The first construct $T2^{23\text{-}424}$ represents native form of testican-2 whereas the second construct $T2^{23\text{-}379}$ is truncated form of the first construct in a way that supposedly unstructured negatively charged C-terminal is removed. The two constructs were designed based on an assumption that testican-2 interacts with C1q with its C-terminal domain which was laboriously studied. Our preliminary results suggest aggregation of both testican constructs presumably due to lack of GAG chains in the recombinant proteins. Cross-linking experiment results indicate that interaction between testican-2 and C1q is more prominent for the native construct. Nevertheless, additional experiments are needed to confirm necessity of C-terminal region for testican-2 interaction with C1q.

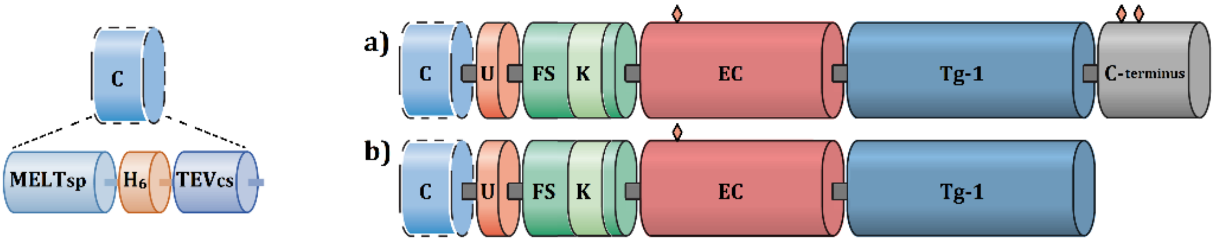

Figure 1: Schematic representation of expression cassette and both testican-2 constructs, a) native and b) truncated. C - expression cassette, MELTsp - honeybee melittin signal peptide, H_6 - histidine tag, TEVcs - TEV protease cleavage site, U - unique, FS - follistatin, K - Kazal, EC - extracellular calcium-binding, Tg1 - thyroglobulin type-1 domain, ♦ - GAG attachment site.

References:

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Use of Bioinformatics in Drug Discovery

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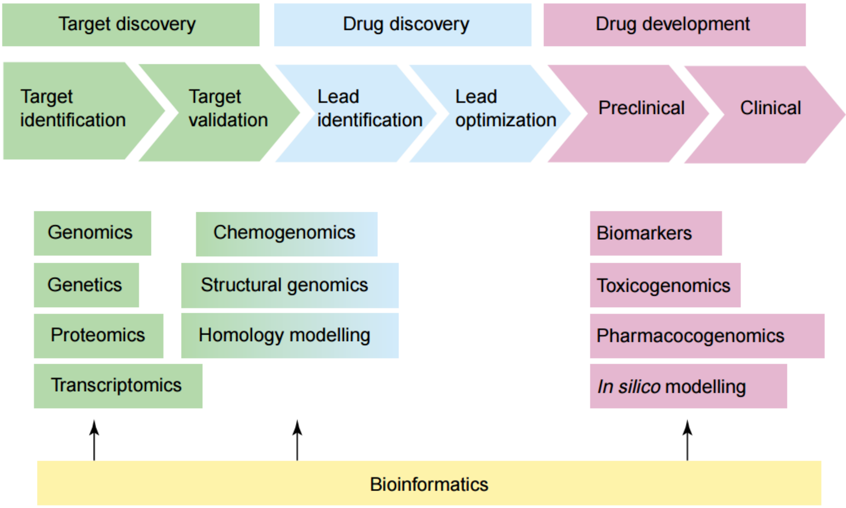
Poster reviews and to some extent describes relevance of bioinformatics in drug discovery. Bioinformatics is an interdisciplinary science, grounded on information technology, programming, mathematics and molecular biology. Certain field of bioinformatics specializes in drug discovery and represents one of the most promising approaches in modern pharmaceutical industry.[1,2,3] Bioinformatic methods can identify and validate new drug targets and new lead compounds, optimize them and discover drugs with multiple targets. The aim is to increase drug safety, reduce the number of animals used in experiments and to shorten the time required for drugs to come on the market. Biomarkers, toxicogenomic tools and farmacogenomic tools can maximize the efficiency of the drug. Several diverse computational programs and methods, either based on ligands or structures, are being developed. *In silico* methods are being used in different stages of drug development and can reduce the cost of development up to 50 %.



TRENDS in Pharmacological Sciences

Figure 3: The current paradigm of drug discovery and development emphasizes the exploitation of genomic and genomic-related knowledge, with bioinformatics playing a key role in 'unlocking' available information.[2]

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Positioning Quality of GPS Sensors in Smartphones

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One of the basic tasks of geodesy is determination of accurate position (coordinates) in terrestrial coordinate systems. Due to a rapid technological and satellite geodesy development, also a common user nowadays is able to find out his position accurately, despite not being familiar with concepts of satellite aided positioning. These concepts are available in respective textbooks,[1] but besides position itself the position quality is of great importance. We may stress that GPS does not provide information regarding quality of acquired position.[2]

Absence of relevant information about positioning quality was the motivation for our testing of few GPS sensors, available in present-day smartphones. Four groups of tests were set up i. e. for testing position quality in static and kinematic mode and in good and difficult conditions for the reception of satellite signal.

Testing of static receiver was performed according to previously determined reference positions and for moving receiver test we developed our own procedure for reference position determination using the tachymeters able to track the moving target which was time-synchronised with GPS time. In both cases the reference positions were determined with cm level position accuracy. Static position quality was estimated according to 70 positions. Moving receiver testing was done by evaluating approx. 600 positions at walking speed.

Table 1: Test results

	Good conditions				**Difficult conditions**			
	Static mode		**Kinematic mode**		**Static mode**		**Kinematic mode**	
	s_{hz} [m]	**s_v** [m]	**s_{hz}** [m]	**s_v** [m]	**s_{hz}** [m]	**s_v** [m]	**s_{hz}** [m]	**s_v** [m]
iPhone 5s	3,59	6,23	1,68	2,79	N/A	N/A	2,15	2,40
LG Optimus G E975	2,83	4,32	0,95	3,30	6,53	7,03	3,74	8,75
Nokia Lumia 620	7,40	4,88	N/A	N/A	8,80	12,70	N/A	N/A

*In Table 1 s_{hz} and s_v represent standard deviations in horizontal and vertical position.

The positional accuracy of GPS sensors proved to be in range of 3-6 meters in good and in range of 6-8 meters in difficult conditions, with maximum deviations up to 5-16 meters (good conditions) and 20-26 meters (difficult conditions). Although some tested GPS sensors were not able to determine position (N/A) in kinematic mode or in difficult conditions, the GPS sensors are of high quality and meet the requirements of common user.

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Synthesis and Coordination Properties of Novel Enaminones

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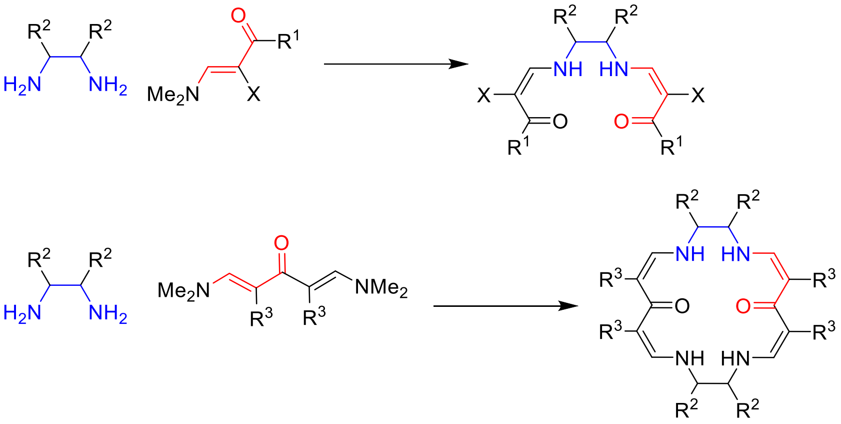
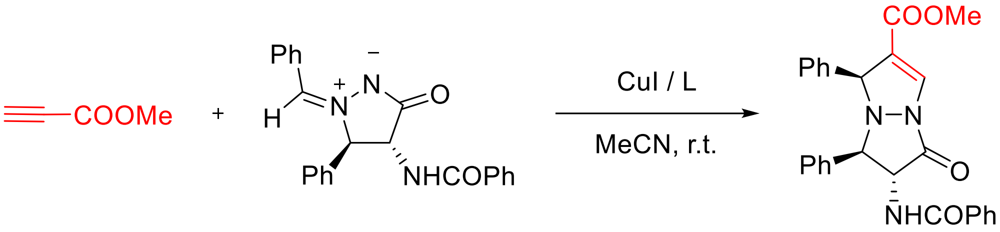
Properties and further transformations of enaminones have been thoroughly studied in the past[1]. However our knowledge of their coordination abilities is limited[2]. By varying the structure of starting ketones and diamines we have created different shapes and sizes of binding space (Scheme 1).



Scheme 3: Synthesis of enaminone ligands

Based on different reaction rates of well studied[3] copper (I) catalyzed [2+3] cycloaddition between methyl propiolate and azomethine imine derivative we were able to investigate enaminones coordination properties (Scheme 2). In all the experiments, the desired complex between copper(I) and enaminone was formed *in-situ*.



Scheme 4: Copper(I) catalyzed [2+3] cycloaddition

References:

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Derivatization of 8-Haloquinolines via Suzuki–Miyaura Cross-Coupling Reaction

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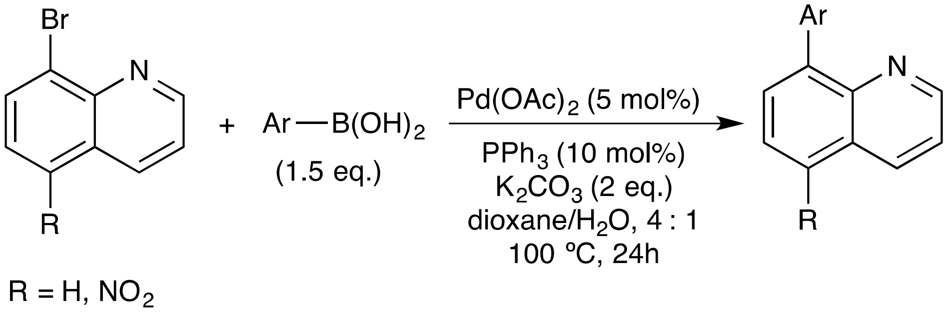
Quinoline and its derivatives present a prominent structural motif found in a wide range of natural products with interesting biological activities.[1] For example, nitroxoline (5-nitro-8-hydroxyquinoline) has proven to be a potent inhibitor for the enzyme cathepsin B.[2] The synthesis of such quinoline derivatives can easily be achieved with the Suzuki–Miyaura cross-coupling reaction.[3] The reaction is carried out between an aryl halide and boronic acid in the presence of a catalytic amount of palladium complex.

Therefore, the Suzuki cross-coupling reaction between the quinoline derivatives (8-bromoquinoline and 8-bromo-5-nitroquinoline) and a number of hetero(aryl)boronic acids gave the desired products in high yields. The reaction was carried out in the presence of a $Pd(OAc)_2$-PPh_3 catalytic system with K_2CO_3 as the chosen base in a mixture of dioxane and water.



Scheme 1: The synthesis of 8-hetero(aryl)quinoline derivatives via Suzuki–Miyaura cross-coupling reaction.

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Preparation of Recombinant N-Terminal Domain of MLKL and AntiMLKL Nanobody and Cloning of their Fusion Protein

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The N-terminal domain of MLKL is responsible for necroptosis, possibly by forming oligomers that can bind to membrane and enable membrane leakage resulting in cell death.[1] The antiMLKL nanobody (M79) is specific for MLKLN-154 and can bind both, its monomeric and dimeric forms,[2] but its precise epitope on MLKL is unknown. The aim of this study is to determine crystal structure of a complex between M79 and MLKLN-154 and explain the complex formation in atomic detail. Therefore, MLKLN-154 and M79 were expressed in a bacterial expression system, isolated with immobilized metal ion affinity chromatography (IMAC) and purified with size-exclusion chromatography. Since the oligomerization mechanism for MLKLN-154 is still unknown, cross-linking under reducing and non-reducing conditions was performed in order to determine, whether oligomerization is caused by disulfide bond formation. Fusion protein was also created by linking M79 and MLKLN-154 with $(GGGGS)_3$ linker and cloned using SLiCE method.[3] Since the structure of M79 nanobody is unknown, a model was prepared, as well as a model of a fusion protein (Figure 4) that is going to be used in crystallization studies.

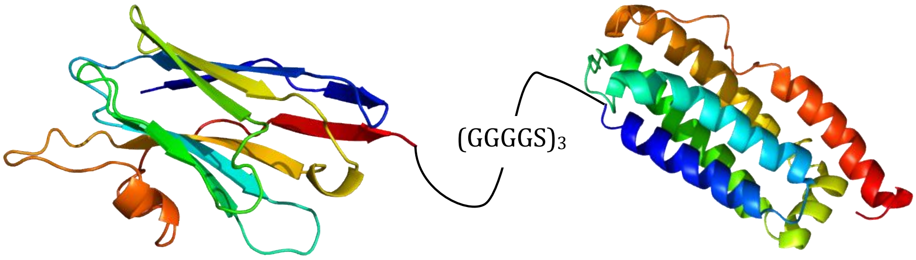

Figure 4: Model of fusion protein created by linking nanobody (PDB: 1MEL) and MLKLN-154 (PDB: 2MSV) with the flexible linker $(GGGGS)_3$.

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Cytotoxic vs. Cytostatic Properties of Organoruthenium Chlorido- and Pta-Complexes with β-Diketonates

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Current statistics indicate that one in every three people will develop some kind of cancer during their life time.[1] Platinum-based drugs are so far the most used chemotherapeutic agents and are used in nearly 50% of all cancer treatments.[2] Unfortunately, both cancerous and non-cancerous cells are being killed this way. With the use of ruthenium based complexes more selective and more efficient drugs can be developed.[3]

It is known that even very small changes in the chemical structure can have a major effect on their biological activity. We decided to use of the diketonate scaffold, which offers various possibilities for structural modifications in order to obtain the desired physicochemical properties. A series of organoruthenium(II) chlorido complexes with fluorinated O,O-ligands $[(\eta^6\text{-}p\text{-cymene})\mathrm{Ru}(\mathrm{F_3C\text{-}acac\text{-}Ar})\mathrm{Cl}]$ and their respective 1,3,5-triaza-7-phosphaadamantane (pta) derivatives $[(\eta^6\text{-}p\text{-cymene})\mathrm{Ru}(\mathrm{F_3C\text{-}acac\text{-}Ar})(\mathrm{pta})]\mathrm{PF_6}$ were synthesized and fully characterized in both solution and solid state.[4]

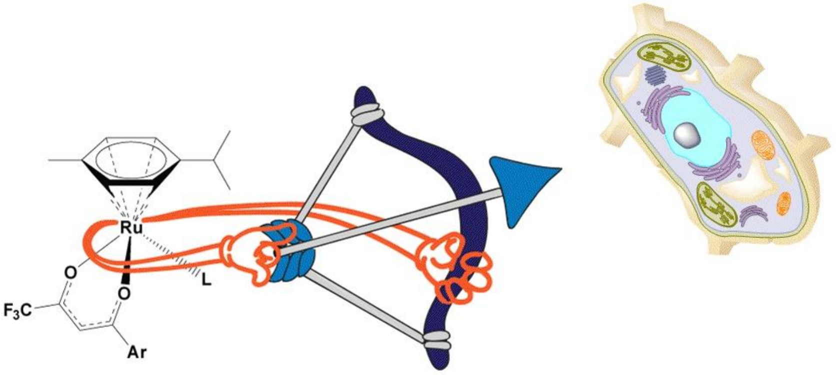

Figure 1: Newly synthesized organoruthenium(II) complexes exhibit potent antiproliferative activity toward a range of cancer cells.

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The Role of Nutritional Science in Cardiovascular Disease Prevention

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Nutrigenomics is the science that explores how nutrients and bioactive food components affect gene expression whereas nutrigenetics tries to identify the role of genetic variability on dietary response. Nutrients can alter gene expression directly as transcription factors or indirectly by activating different hormones, for example insulin, glucagon, glucocorticoids, and thyroid hormones. By altering gene expression nutrients can change an individual's phenotype. In contrast, SNPs (single nucleotid polymorphisms) can influence metabolic response to our diet. Nutritional science, including nutrigenomics and nutrigenetics, has led to the introduction of personalized nutrition aimed at each individual and specific dietary needs based on our genetic differences. The field of nutritional science could be applied to cardiovascular diseases. Dietary interventions on a population level have largely been unsuccessful, the same dietary intervention has caused several inter-individual differences on the organism. For example, in some individuals supplementing fish oil (more than 2 g per day) which is rich in omega – 3 fatty acids has caused an unexpected increase in the level of LDL cholesterol for 5 to 10%. This is typical for ApoE4 carriers that represent 25% of the population. Another example of gene-nutrient interaction that might have an impact on cardiovascular disease prevention is TCF7L2 gene and the Mediterranean diet. Consistent consumption of the Mediterranean diet has neutralized the effect of TT genotype which has an increased risk of cardiovascular disease. Moreover, folic acid supplementation may also reduce the risk of CVD in patients with C677T genotype which carries an increased risk of cardiovascular disease. Practical application of this knowledge has not been possible yet while there are some restrictions to this approach. For instance, dietary supplementation of a specific nutrient may have a protective effect on the development of one disease and a harmful effect on another. Therefore, more studies are necessary to assess future possibilities in the setting of personalized nutrition and its use in clinical practice.

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Structural Insight into the Z-disc Protein Interactions

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Although all types of muscle cells use actin and myosin for contraction, only in skeletal and cardiac muscle these proteins are organized into sarcomeric units. These units are delimited by Z-discs, which are intricate webs of various proteins including α-actinin 2, F-actin and myotilin. At the focus of our research is protein myotilin, which consists of two immunoglobulin-like domains flanked by a unique serine-rich N-terminus and a short C-terminal tail[1]. In order to gain insight into its remarkable interaction capabilities we investigated its actin-binding properties from the structural point of view.

First, we determined binding affinities for interaction of different myotilin constructs with G-actin using microscale thermophoresis (MST) measurements, all showing relatively weak binding in the micromolar range. We additionally confirmed the binding of myotilin to G-actin using chemical cross-linking coupled with mass-spectrometry (XL-MS) which enabled us to identify amino acid residues likely involved in interaction. Furthermore, ^{15}N-HSQC titration NMR experiments were performed for the Ig1 domain. Based on the gathered experimental data we propose a model in which single Ig-domain of myotilin, specifically with the β strands B, D and E, binds to one subunit on actin filament. Consequently, full-length myotilin, anchored on an actin filament with C-terminal Ig-like domains, is able to connect two adjacent α-actinins in the sarcomere with its N-terminus (Figure 1).

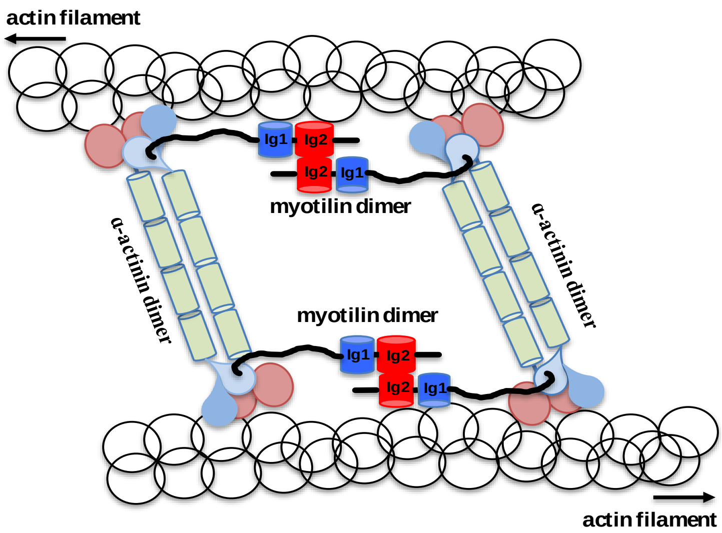

Figure 1: The model of sarcomeric Z-disc structure including myotilin, α-actinin and actin filament.

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Production of HRP-Conjugated anti-His scFv for Easier Immunodetection of C-Terminal His-Tagged Proteins

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The ever-growing demand for molecules with antibody-like properties has driven scientists to develop novel ways to produce small functional antigen-binding fragments in large quantities. One way to do this is to generate single-chain fragment variable (scFv), which consists of variable regions of heavy (V_H) and light chains (V_L) joined together by a flexible peptide linker.[1] ScFv antibodies can be expressed in easily accessible bacterial cultures.

In order to achieve high level expression of mutant form of the scFv[2] against C-terminal hexahistidine tags (His_6-tag) we optimized the key production parameters. The process of expression and purification is both faster and cheaper than the original one[2] in a way that it uses a more commonly available strain of *E. coli* BL21[DE3] and omits the affinity chromatography step. Binding to recombinant His_6-tagged proteins was verified by size-exclusion chromatography using recombinant testican-2 as a target protein. Additionally, the interaction was confirmed by surface plasmon resonance (SPR).

Moreover, we generated horseradish peroxidase-conjugated (HRP) anti-His tag scFv antibodies, which were then directly used to detect small amounts of His_6-tagged proteins by Western blot analysis. Our results demonstrate that low-cost production of anti-His scFv offers very specific and sensitive detection of C-terminal His_6-tagged proteins in immunoassays, which can be achieved with one-step probe incubation.

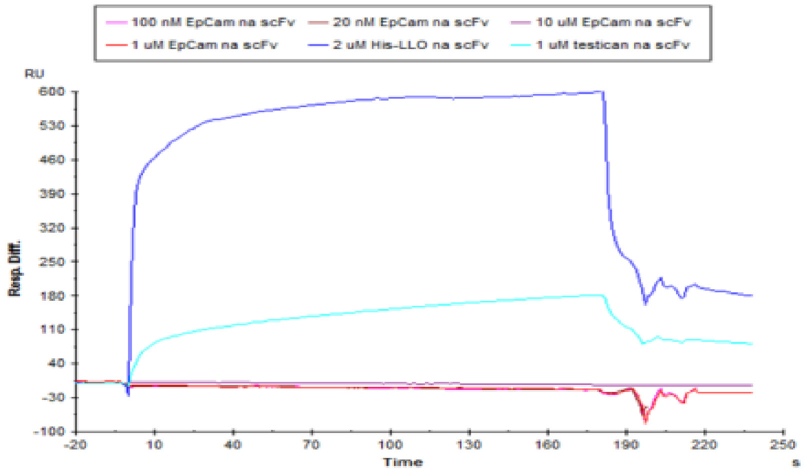

Figure 1: SPR interaction analysis between anti-His scFv and testican-2.

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The Structure and Calcium Regulation of Human α-Actinin 1

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α-Actinin 1 is one of the calcium sensitive non-muscle cytoskeletal isoforms of the α-actinin, a member of the spectrin superfamily of actin binding proteins. All isoforms of α-actinin are functional as antiparallel dimers (Figure 1a) and share a common basic structure with N-terminal actin-binding domain (ABD), connected via a flexible neck region to a rod domain composed of four spectrin-like repeats (SR) in metazoans, followed by a C-terminal calmodulin-like domain (CAMD). CAMD is composed of four EF-hand motifs organized in two side-by-side pairs (EF1-2 and EF3-4).[1] In non-muscle cells, calcium binding to EF hands triggers a conformational change within the CAMD of α-actinin 1 (Figure 1b), resulting in reduced actin bundling capacity of α-actinin dimer[2]. We have determined a high-resolution NMR structure of CAMD in both apo and holo form (i.e., without and with bound Ca^{2+} ions). Using SAXS we provided complementary information on the molecular envelope in solution and dynamics of the molecule. Furthermore, by using ITC and MS, we showed that CAMD has only one calcium binding site, located in EF1 hand with K_d of approx. 100 μM. Moreover, we succeeded to crystallize half dimer of α-actinin 1 comprised of all domains. Structure determination is still in progress and will give us insight into the most important intramolecular interactions.

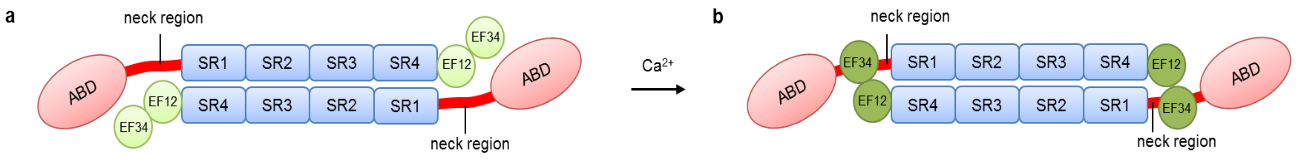

Figure 5: Domain structure of α-actinin 1 antiparallel dimer (**a**) and proposed changes accompanying calcium binding to EF hands of CAM domain (**b**).

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The Effect of Acute Ibogaine Treatment on the Survival of Dopaminergic Neurons in Organotypic Nigrostriatal Co-Cultures

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Parkinson disease (PD) is characterized by selective loss of dopaminergic neurons in substantia nigra compacta. Glial cell line-derived neutrophic factor (GDNF) represents a potent therapeutic agent for the treatment of PD due to its ability of protecting dopaminergic neurons and restoring motor functions in animal models of PD. There is some evidence in the literature that we can induce GDNF expression in dopaminergic neurons by ibogaine, a psychoactive indole alkaloid. The effects of ibogaine have so far been studied mainly for the purpose of alcohol, nicotine and opiates addiction treatment. Recent studies have shown that a single application of ibogaine results in long-lasting reduction of drug craving and alcohol intake and increased GDNF expression in the midbrain. Thus, single dose of ibogaine (10μM) induces a persistent increase in GDNF expression in dopamine-like cell line SHSY5Y suggesting that increased GDNF expression mediates the long lasting actions of ibogaine that reduces drug and alcohol self-administration in rodents. Aim of this study was to investigate if a single dose of ibogaine contributes to dopaminergic neurons survival *in vitro*. We utilized organotypic nigrostiatal co-cultures from 3 weeks old rats. Dopaminergic degeneration was induced by the simple cutting of dopaminergic fibers. Nigrostriatal co-cultures were exposed to a single dose of ibogaine (10μM) and cultivated for 2 weeks. Brain slices were immunolabeled with tyrosine hydroxylase (TH), which represents a good marker for dopamine neurons. Our study demonstrated that a single application of ibogaine does not improve survival of TH-positive neurons. In our further experiments we will treat the nigrostriatal cultures chronically with ibogaine and compare the results of dopaminergic neurons survival to nigrostriatal cultures treated with recombinant GDNF.

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α-Aminoalkyl Ynones in 1,3-Dipolar Cycloaddition Reactions

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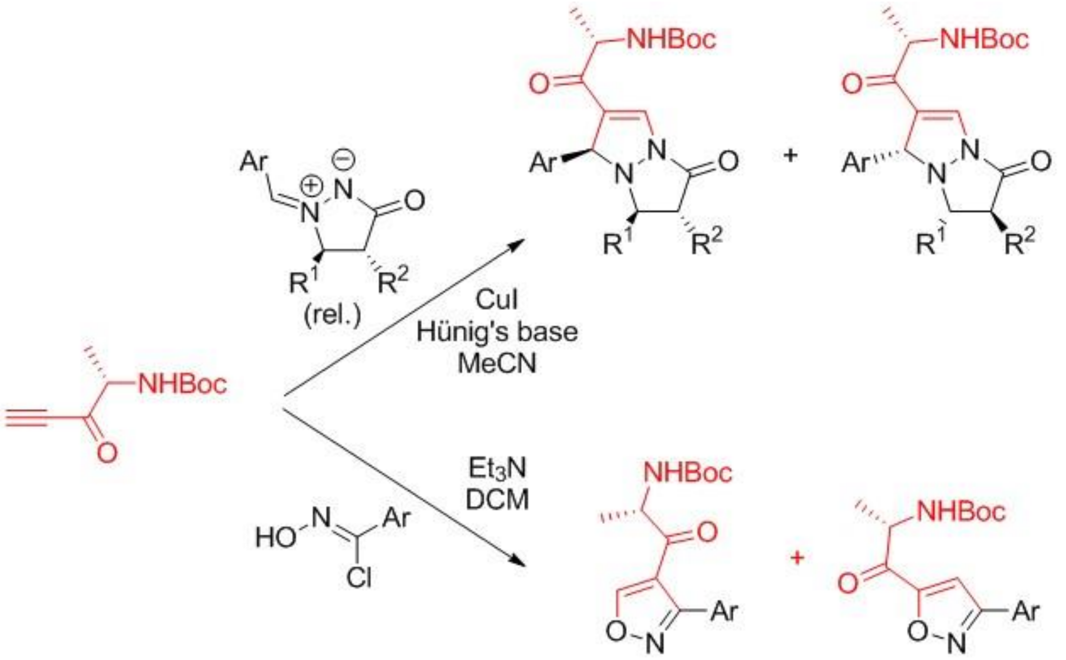
α-Aminoalkyl ynones, synthesized in very good yields from *N*-protected amino acids, are versatile reagents and have already been used in cyclocondensation reactions[1] and to prepare new vinylogous peptides.[2] Now they are being used as dipolarophiles in reactions with different dipoles, such as azomethine imines,[3] nitrile oxides, azides and others.



Scheme 1: 1,3-Dipolar cycloaddition of *tert*-butyl (*S*)-(3-oxopent-4-yn-2-yl)carbamate with racemic azomethine imines and nitrile oxides, generated *in situ*.

Because *tert*-butyl (*S*)-(3-oxopent-4-yn-2-yl)carbamate is enantiomerically pure, reactions were carried out under mild conditions, so no racemization took place and products were enantiomerically pure as well. In this way new heterocyclic derivatives of *L*-alanine have been prepared.

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Spectroscopic Investigation of Environmental Influences and Surface Pre-treatment of Exterior Cultural Heritage Objects at Dornava Manor

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Objects of cultural heritage, which are inappropriately preserved, are often subjected to uncontrolled decaying. Environmental influences (*e.g.* humidity, temperature, air pollution, etc.) affect not only chemical composition, but can also interfere with physical properties and in some cases offer suitable conditions for biological overgrowth (*e.g.* algae, lichens, cyanobacteria). In order to plan safe conservation and restoration processes, the relation between environmental effects and current state of the historical site needs to be established. In this study, an important Slovenian historical site, a baroque manor Dornava, showing a high level of decaying was investigated within HEROMAT European project. Five major types of lichen, as well as their metabolic products were discovered in the investigated specimens by optical and Raman microscopy[1]. Before the application of newly developed HEROMAT materials (protective coatings and consolidants), surface pretreatment was necessary. After the mechanical cleaning mineral composition of render, concrete and stone, as well as the remains of biodeterioration processes was determined by transmission, reflection infrared (FTIR) and Raman spectroscopy. Spectral features ascribable to chitin[2] (main component of cell walls in plants, bacteria, lichens, etc.), chlorophyll, carotenoids, scytonemin[3] confirmed the presence of biological overgrowth.

Acknowledgements: The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 282992; project acronym: HEROMAT; project title: PROTECTION OF CULTURAL HERITAGE OBJECTS WITH MULTIFUNCTIONAL ADVANCED MATERIALS; coordinator: Faculty of Technology, University of Novi Sad, Novi Sad, Serbia; research area: ENV-NMP.2011.3.2.1-1 Development of advanced compatible materials and techniques and their application for the protection, conservation and restoration of cultural heritage assets; type of funding scheme: SME targeted Collaborative Project; web address: http://www.heromat.com/

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Bifunctional Thiourea Camphor-Derived Organocatalysts

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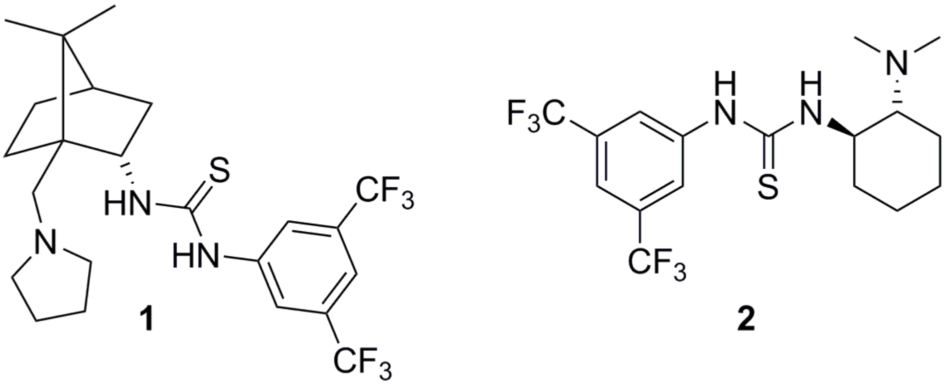
Organocatalysts are green and environmentally friendly alternative as opposed to the traditionally used transition metal-based catalysts, which are usually rather toxic and sensitive to air and moisture. An efficient way to access new organocatalysts is to prepare them from easily attainable and affordable chiral pool molecules, such as amino acids, terpenes and sugars. Camphor, as a member of terpenoids, is an interesting starting material for the synthesis of new potential organocatalysts because of its unique chemical reactivity and commercial availability of both enantiomeric forms.

The majority of non-covalent organostalaysts are represented by thiourea organocatalysts. By interaction with the substrate through hydrogen bonds they promote and stereochemicaly control known organic transformations.

We have not found any compounds in the literature where camphor was used as the only chiral scaffold in combination with a tertiary amino group and 1-(3,5-bis(trifluoromethyl)phenyl)thiourea group. We decided to synthesize aforementioned systems and test their catalytic activity in a model *Michael* addition of nucleophiles to *trans*-β-nitrostyrene.[1,2,3,4]



1

2

Figure 1: Bifunctional camphor derived organocatalyst **1** and commercially avaliable catalysts **2**.[4]

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Influence of Redox Mediators and Co-Cultures on Decolourisation of Acid oorange 7 in a Microbial Fuel Cell

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Azo dye-containing industrial wastewaters need to be properly treated due to toxicity, of their reaction products and the dyes themselves. It has been suggested that an anaerobic-aerobic two stage treatment process should be used for their complete mineralization. Treatment of azo dyes in microbial fuel cells (MFC) could represent the first stage in the treatment process, with an additional benefit of green electricity production. The decolourisation of an azo dye Acid orange 7 (AO7) in the anode of a MFC was studied. The effect of addition of two redox mediators, anthraquinone-2,6-disulfonic acid (AQDS) and anthraquinone-2-sulfonic acid (AQS), and two cultures, *Pseudomonas trivialis* P513/19t and *Pseudomonas putida* 8251, to the basic anode culture *Shewanella oneidensis* MR-1 was explored. AQDS exhibited enhanced AO7 decolourisation (100% within 18.25 hours excluding lag phase), similarly as the co-culture of *S. oneidensis* and *P. trivialis* (100% within 37.5 hours excluding lag phase). This work highlights the possibility of using microbial fuel cells to achieve enhanced AO7 decolourisation with the aid of redox mediators and some defined co-cultures.

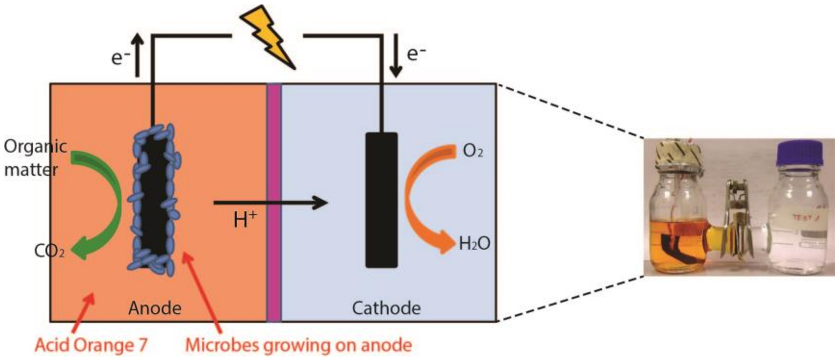

Figure 1: Schematic presentation and photography of a microbial fuel cell for simultaneous Acid Orange 7 decolorisation and electricity generation.

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The Water-Gas Shift Reaction Over CuZnGaO$_x$ Catalyst

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The water-gas shift reaction is an important industrial reaction used for H_2 enrichment and/or CO reduction. During the reaction, CO and H_2O are converted to CO_2 and H_2.

$$CO + H_2O \leftrightarrow CO_2 + H_2 \ \Delta H^{\circ}_{298\,K} = -41{,}1\ kJ/mol$$

The reaction is slightly exothermic and equilibrium-limited, favoring the production of H_2 at lower temperatures. Operation at lower temperatures requires a highly active catalyst. The aim of the work was to synthesize the catalyst, which would provide high conversion rates of CO at low temperatures (180 – 250 °C). For this, a CuZnGaO$_x$ catalyst was synthetized by a coprecipitation method. The prepared catalyst was analyzed by SEM, EDS, BET and XRD analytic methods. The catalyst activity was tested with experiments in a packed bed reactor, with varying CO and H_2O steam feed concentrations, temperatures and flow rates.

A mathematical model was developed to describe the process, which includes the convectional and diffusional mass transfer, longitudinal diffusion and dispersion, mass transfer through the catalyst layer, reaction on the catalyst active sites, pressure drop, etc.[1-4] The properties of the gas were calculated according to its constitution at each segment of the packed bed reactor. The reaction kinetics were determined by nonlinear regression analysis. Matlab was used for the calculations.

The developed model can be used for process optimization, for the prediction of gas constitution for various process parameters and for providing a deeper understanding of the underlying phenomena of the water-gas shift reaction inside a packed bed reactor.

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Optimization of Rutin Hydrolysis in Subcritical Water Using Response Surface Methodology

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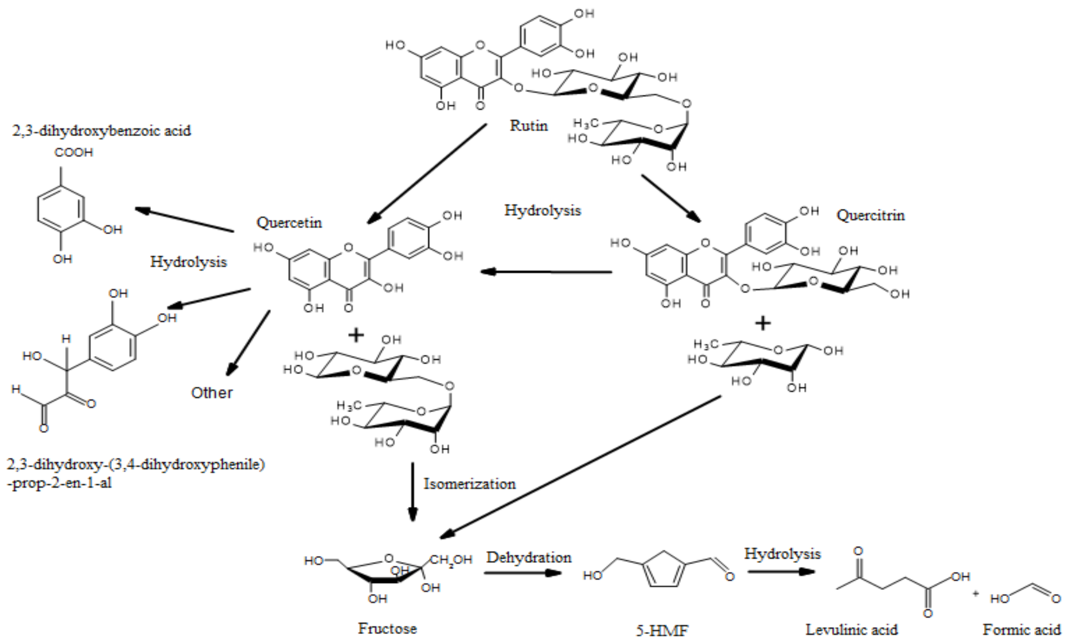
Recent studies suggest that subcritical water (SubCW) could play an important role in chemical processes of the future. Besides its attractive properties as a green industrial solvent, the medium is also interesting in fields of biomass conversion and fuel production, due to its strong hydrolytic nature. [1] Furthermore, SubCW can also find applications in the food industry, e.g. as an extraction solvent or even reaction medium for hydrolyzing food stuffs.

In this study, SubCW was used as a medium for hydrolyzing the glycoside bonded antioxidant quercetin-3-O-rutinoside - rutin into its free aglycone form. Effect of reaction parameters, such as temperature, time and concentration of rutin, were studied and the best possible combination of reaction parameters was established using response surface methodology in a three-variable, three level central-composite design. Optimal reaction conditions for maximal quercetin yield were determined. Additionally, products of hydrothermal degradation that can form from the quercetin or the sugars (**Scheme 1**) were observed and attempted to be minimized, since their formation could lower the biological activity of the hydrolyzed product. Quantification of quercetin and the degradation products was determined using high performance liquid chromatography. [2]



Scheme 1: Possible hydrothermal degradation products of rutin in subcritical water.

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Escherichia coli *Isolates from Incoming Water, Water of the Middle Pool and Outcoming Water of a Wastewater Treatment Plant*

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The aim of the study was to evaluate how many *Escherichia coli* (*E. coli*) strains possessing either type 1 fimbriae and/or curli fimbriae are present in different water samples of a wastewater plant at several time points. Water from three different pools of the wastewater plant in Brežice, Slovenia, was sampled: the water in the incoming pool, the water in the middle pool and water in the outcoming pool. The samples were taken at four different time points, in March, April, May and June 2015. Samples were first incubated on MacConkey plates, then up to 150 red colonies were transferred to Uriselect plates. Lysogeny broth (LB) plates were used to further cultivate the bacterial isolates and to determine the colony size and the ability to grow as single colonies. All plates were incubated over night at 37 °C. From colonies on LB plates, bacterial lysates were made and further investigated with PCR for the presence of *fimH* gene (type 1 fimbriae gene) and *crl* gene (curli fimbriae) with primers specific for these two genes. Finally, ERIC-PCR method was used to determine which isolates belong to the same *E. coli* strain[1]. Our results showed that the most *E. coli* isolates were isolated from the incoming water sample, to a lesser extent from the water of the middle pool and only a few isolates were isolated from the outcoming water.

Table 1: Number of *E. coli* colonies from incoming water, water of the middle pool and outcoming water sample transferred from MacConkey plate to Uriselect and then to LB plates at four different time points.

	Samples taken in							
	March		April		May		June	
	Number of colonies		Number of colonies		Number of colonies		Number of colonies	
Water sample	Uriselect	LB	Uriselect	LB	Uriselect	LB	Uriselect	LB
Incoming pool	100	22	150	51	100	36	100	35
Middle pool	100	18	150	42	100	22	100	38
Outcoming pool	21	14	6	2	100	36	48	14

Acknowledgement: This project was supported by the Slovene Human Resources Development and Scholarship Fund, Republic of Slovenia, Ministry of Education, Science and Sport, and European Social Fund – call "Po kreativni poti do praktičnega znanja".

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Dopaminergic Modulation of Synaptotagmin IV and Substance P Expression and Characterization of Striatal Alterations During Culture of Organotypic Whole Brain Slices from Young Rats

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Parkinson's disease (PA) is neurodegenerative disease which results primarily from the death of dopaminergic (DA) neurons in the substantia nigra pars compacta. The loss of nigrostriatal DA neurons produces a marked deficit of dopamine content in the striatum. Dopamine depletion is followed by hypersensitivity of dopamine receptors to DA agonists that induce expression of several immediate early genes such as synaptotagmin IV (SytIV) and genes encoding neuropeptide transmitters (substance P). In this study we have examined if substance P and Syt IV protein expression can be dopaminergically modulated in the organotypic striatal brain slice cultures (OSBC) from young rats (30 days old). Dopamine depletion in the striatum is inherent to this model due to cutting of DA fibers during preparation of OSBC. In first part of the study we assessed the viability of OSBC. We have performed immunohistochemical staining of neurons, astrocytes, microglial cells, axons and dendrites to characterize the striatum in OSBC cultivated for 0 – 7 days *in vitro* (DIV). In the second part of the study, we have treated control and 2 DIV OBSC with apomorphine (ApoM), a non-selective dopamine agonist that acts on the D1 and D2 dopamine receptors. We expected that stimulation of the dopamine receptors with ApoM increases the level of substance P and Syt IV in 2 DIV cultures compared to control slices due to developed hypersensitivity of dopamine receptors. We will discuss the utility of OSBC from adult rats as the *ex vivo* model of DA hypersensitivity.

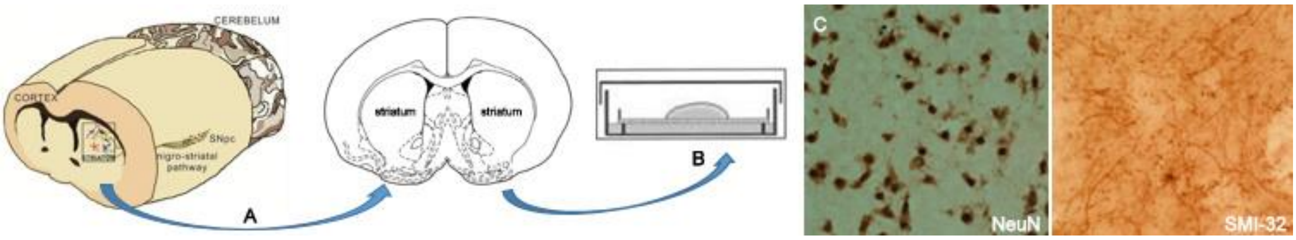

Figure 1. (**A**) Coronal 300 μm striatal brain slices were placed on (**B**) a porous membrane at the interface between the culture medium and the atmosphere. (**C**) Characterization of OBSC utilizing neuronal marker (NeuN) and neurofilament marker (SMI-32) immunohistochemistry.

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Identification of Functional Polymorphisms within Ultraconserved Regions (UCRs) and their Association with Diseases

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Ultraconserved regions (UCRs) have been defined as genomic segments which are absolutely conserved (100% identity with no insertions or deletions) between orthologous regions of the human, rat, and mouse genomes and longer than 200 base pairs. A large fraction of genomic ultraconserved regions encode a particular set of ncRNAs whose expression is altered in human cancers. Genome-wide profiling revealed that UCRs have distinct signatures in human leukemias and carcinomas. UCRs are frequently located at fragile sites and genomic regions involved in cancers. Although these sequences have been defined as identical in the reference human, mouse, and rat genomes, numerous polymorphisms within these regions in the human population have been identified. There was a need to newly define these regions due to several novel genome assembly releases. We checked Pubmed databases to find polymorphisms within these newly identified UCRs and reviewed the literature to analyze if these polymorphisms have previously been associated with diseases or phenotypic traits. Out of 45829 single nucleotide polymorphisms (SNPs) located within 13736 UCRs, 19 SNPs were previously described in 47 scientific papers. Out of 19 SNPs, four had only negative associations and 15 SNPs had been associated with 33 phenotypic traits and diseases, including different types of cancer, Marfan syndrome, responses to various therapeutics, schizophrenia, coronary artery disease and Alzheimer's disease. Polymorphism rs605059 is located within the *HSD17B1* gene and has been associated with the highest number of diseases/phenotypic traits. It has been described in 14 scientific articles and is associated with 12 diseases/phenotypic traits which include: tumorigenesis of uterine leiomyoma, sperm concentration and sperm motility, with increased risk of colorectal cancer in women and others. UCRs are currently defined as genomic segments which are absolutely conserved (100% identity with no insertions or deletions) between orthologous regions of the human, rat, and mouse genomes. However, our study revealed a high number of SNPs within 13736 UCRs longer than 100 bp. Further functional studies are needed in order to obtain a better understanding of UCRs.

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How can Drones Help us Save the Environment?

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Unmanned aerial systems (UAS) or so-called drones are becoming increasingly present in our everyday life. In comparison with satellites drones are cheaper, operative in almost any weather and can provide us with a good look at a field almost plant-by-plant – that is why they have taken over agriculture in the past few years.

Drones can be equipped with many different sensors, such as visible light, infrared or thermal. These help us assess the crop regarding disease pressures, potential damage, plant stress, nitrogen requirements, weed types and even yield potential. Orthophoto and digital surface model, made from visible light sensors, provide farmers with a better view of their property. Thermal and infrared images enable us to determine the moisture content. Combining all of the above enables farmers to improve the precision of irrigation and pesticide spraying, thus conserving water and decreasing the amount of pesticides polluting the environment.

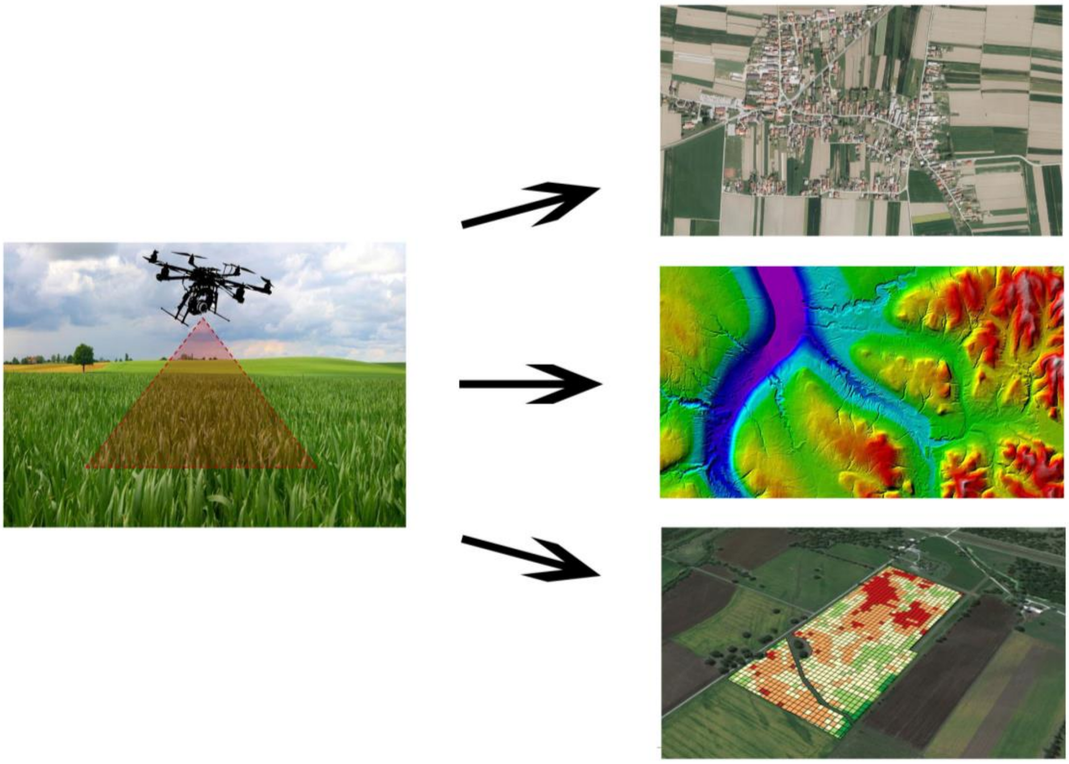

Figure 1: Possible drone use outputs – orthophoto, digital surface model, crop classification

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Identification and Characterization of Allosteric Effectors of Dipeptidyl-peptidase I

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Dipeptidyl-peptidase I (DPPI) is a ubiquitously expressed tetrameric lysosomal enzyme with important roles in the immune system. Recent studies have shown that excessive or insufficient activity of DPPI is connected with various inflammatory and autoimmune diseases, identifying DPPI as an important target in drug development.[1] The aim of our work is to identify effectors that will regulate the enzyme through allosteric mechanisms and will enable us to fine-tune its activity. At the same time, this approach will leave more room to improve other important drug characteristics such as metabolic stability and target selectivity, which are major setbacks of active site-directed DPPI inhibitors.[2] By now, we have successfully isolated and activated recombinant DPPI. We have tested a number of potential allosteric effectors that were predicted by computational methods. Using activity measurements we have identified different mechanisms of inhibition. Apart from several effectors that showed competitive inhibition typical of binding into the active site, we have observed hyperbolic inhibition consistent with allosteric mechanisms with compounds bamnidazole and his analogue ronidazole. *In silico* analyses indicated that these compounds inhibit the enzyme by binding at the interface between two subunits. In preliminary analyses we have also observed negative and positive cooperativity with two other compounds from our in-house compound library. These results are a good basis in our efforts to prove that DPPI is an allosteric enzyme.

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Synthesis of Pure Hydrogen Trioxide (HOOOH) and its Application

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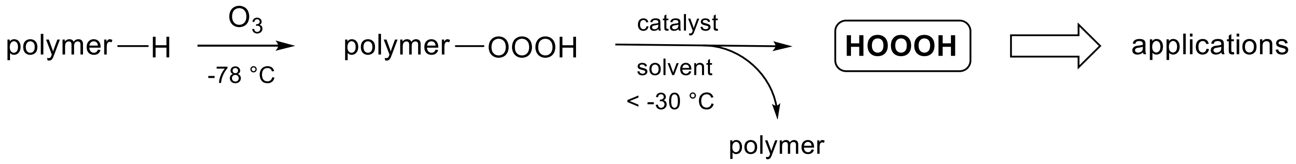
Hydrogen trioxide (HOOOH)[1,2] solutions in diethyl ether, free of other oxidative contaminants and reaction byproducts can be obtained via a simple and efficient methyltrioxorhenium(VII) (MTO)-catalyzed transformation of ozonized resin-bound dimethylphenylsilane.[3,4] In addition, HOOOH can be concentrated and isolated in a highly pure form by removing the solvent *in vacuo* at low temperatures or re-dissolved in other solvents for other purposes. This novel synthetic methodology, which greatly enlarges the research possibilities for this compound in different areas of chemistry,[5] together with some of the possible applications, will be presented.



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Influence of Mathematical Model Describing Pressure Drop on Estimation of Adsorbed Layer Thickness

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Downstream processes represent main part of manufacturing costs in production of biological products, reaching up to 80%. One of the most commonly used separation techniques is liquid chromatography, therefore especially for biological macromolecules, structure of a stationary phases, is of the utmost importance. Chromatographic stationary phases can have different microstructures, which are described with different mathematical models predicting their hydrodynamic properties.

Chromatography principle is based on adsorption of molecules to the stationary phase. Adsorption causes decrease of stationary phase pores and porosity, therefore resulting in a pressure drop increase. On the other hand, this data can provide information about adsorption layer thickness. This is important, since adsorbed layer thickness can give us an insight into how biological molecules behave, when they are adsorbed onto pore surface. To estimate adsorbed layer thickness from a pressure drop data, novel mathematical model based on pressure drop mathematical model has to be derived. From existing literature data there is no information how selection of mathematical model for pressure drop affects estimated adsorbed layer thickness.

In this work we implemented four frequently applied mathematical models describing pressure drop for calculation of adsorbed layer thickness. Firstly, from measured pressure drop data, estimation of pore size using selected mathematical model for pressure drop prediction was calculated. In parallel, equation for estimation of adsorbed layer thickness for each mathematical model was derived. Finally, assuming certain increase of pressure drop during adsorption, thickness was estimated from derived mathematical models.

Results show that pore diameters calculated from different models, interestingly, are the same at porosity around 0,6. However, calculated layer thicknesses based on derived mathematical models differ significantly, depending on porosity and pore size of the bed. Because of that, one has firstly to select the model best describing porous bed and afterwards the adsorbed layer thickness from this model can be calculated.

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Biomonitoring of Water Lice Asellus aquaticus *from Cave and Surface Freshwater Environments*

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The waterlouse, Asellus aquaticus (Crustacea: Isopoda: Asellidae) is a ubiquitous freshwater invertebrate, included in biological processes in the benthic community1. The goal of this research is to investigate selected biochemical parameters (biomarkers) of water lice from cave and surface environments. The animals were collected from 3 locations arranged along the sinking river Pivka: a surface section at the entrance of Postojnska jama Cave, its underground section in Planinska jama Cave and a surface section called the Unica on Planinsko Polje. An additional sample was taken from a swampy forest in Ljubljana. Physical and chemical parameters of water (temperature and pH) at the site of sampling were measured. We aim to establish the differences between different populations in connection to their environment and season of collection. The studied biomarkers are energy reserves (lipids and carbohydrates, and enzyme activities of acetylcholinesterase (AChE) (involved in numerous neuronal and non-neuronal functions) and glutathione S-transferase (GST) (xenobiotic biotransformation enzymes). We will only present the spring 2015 sampling results. They show significant differences between the biomarkers in isopods sampled at different locations, except in the case of carbohydrates. Particularly interesting are the differences in enzyme activities. The population from the Planinska jama has a significantly lower acetylcholinesterase activity than the others. Together with the population from the Unica they also exhibit significantly lower glutathione S-transferase activity than the ones from Ljubljana and Postojna, indicating their less intensive metabolism. We discuss the observed differences in biochemical parameters with respect to the differences in the habitat, cave versus surface.

Figure 6: The waterlouse Asellus aquaticus. On the left, a male from the underground population and on the right, a male from a surface population. (Foto: Žiga Fišer)

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The Potential Impact of Microplastic on the Freshwater Test Organisms

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Microplastics (MPs); plastic fragments smaller than 5 mm; are considered an emerging global issue by various experts and international institutions.[1,2] The main concern is that these smaller particles may be ingested throughout the food web more readily than larger particles. Studies in this field focused in particular on marine environment, but recent papers already report the presence of MPs in freshwater ecosystems.[3] Despite this there is a significant lack of data regarding the potential effect of MPs on freshwater biota.

The aim of our study was to investigate the effect of MPs extracted from a personal care product (crème peeling) on two freshwater test systems: crustacean water flea *Daphnia magna* and floating plant duckweed *Lemna minor*. The particles are made of polyethylene. The size of the particles is in a wide range up to 1000 μm, the majority of particles being in range from 30-100 μm with mean size 71.3 μm (according to number distribution). Both test organisms were exposed to 100 mg/L of MPs according to the existing standards. Afterwards the immobility of daphnids and growth of plants (chlorophyll, number of leaves and root length) were evaluated. Furthermore, the organisms were inspected under the light microscope to evaluate the ingestion/adsorption of MPs onto the organisms. The preliminary results show that the tested MPs have no effect on the daphnids, while some adverse effects on the growth of duckweed roots were found. Further experiments with environmental factors affecting the bioavailability of MPs, such as natural organic matter, are underway.

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The Impact of Copper Nanoparticles on the Composting Process

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An increasing use of products containing nanoparticles (NPs) leads to higher concentrations of NPs in the environment. Most of recent studies focus on evaluating of the effects of NPs on aquatic organisms, whereas a lot of NPs are also introduced into the soil. They get there either for remediation purposes or with mineral/ organic fertilizers and as such have potential impact on composting organisms and other animals. Besides, this soil can be used as a natural fertilizer in the fields, which means that there are more NPs introduced into the natural environment and consequently into crops.[1,2]

The aim of our study is to assess the impact of Cu-salt ($Cu(NO_3)_2 \cdot 3H_2O$) and CuO NPs decomposition processes in the compost. Furthermore we would like to examine if Cu-salt and CuO NPs can be leached out from compost during rainfall. Cu salt and CuO NPs were applied on lettuce leaves that were allowed to degrade in compost for 14 days. Our preliminary results showed that composting process is not affected by addition of Cu salt and CuO NPs. Namely, the degradation of lettuce leaves was the same as in control. Similarly, we have not observed impact on soil microorganisms by measurement of enzyme activities of dehydrogenases and ureases. The possible leaching of tested Cu^{2+}, and CuNPs from the compost by simulating rainfall is currently under investigation. Our future work will be focused on the effect and accumulation of Cu^{2+} in common woodlouse (*Porcellio scaber*) fed by the compost.

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The Influence of Phosphorus on Anatase to Rutile Phase Transformation

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The purification of water and gaseous pollutants by semiconductor catalysis is rapidly growing area. Titanium dioxide is the catalyst with a broad range of use because of its low cost, non-toxicity and stability. The most catalytically active form of TiO_2 is anatase, but it is thermally unstable. The aim of this study was the preparation of anatase particles which would transform to rutile at temperatures above 800 °C.

Anatase was synthesized from commercially accessible metatitanic acid. To the primary suspension different quantities of phosphate ions (in range between 0 to 25 mol%) were added. All prepared samples were characterized by thermogravimetry and dynamic scanning calorimetry. Evolution of the morphology and the size of the particles with increasing temperature were determined by scanning electron microscopy and x-ray diffraction. Distribution and quantity of the dopant, incorporated into in the samples were analyzed by energy dispersive spectroscopy. The results showed that the optimal concentration of dopant is between 10 in 15 mol% with regard to TiO_2.

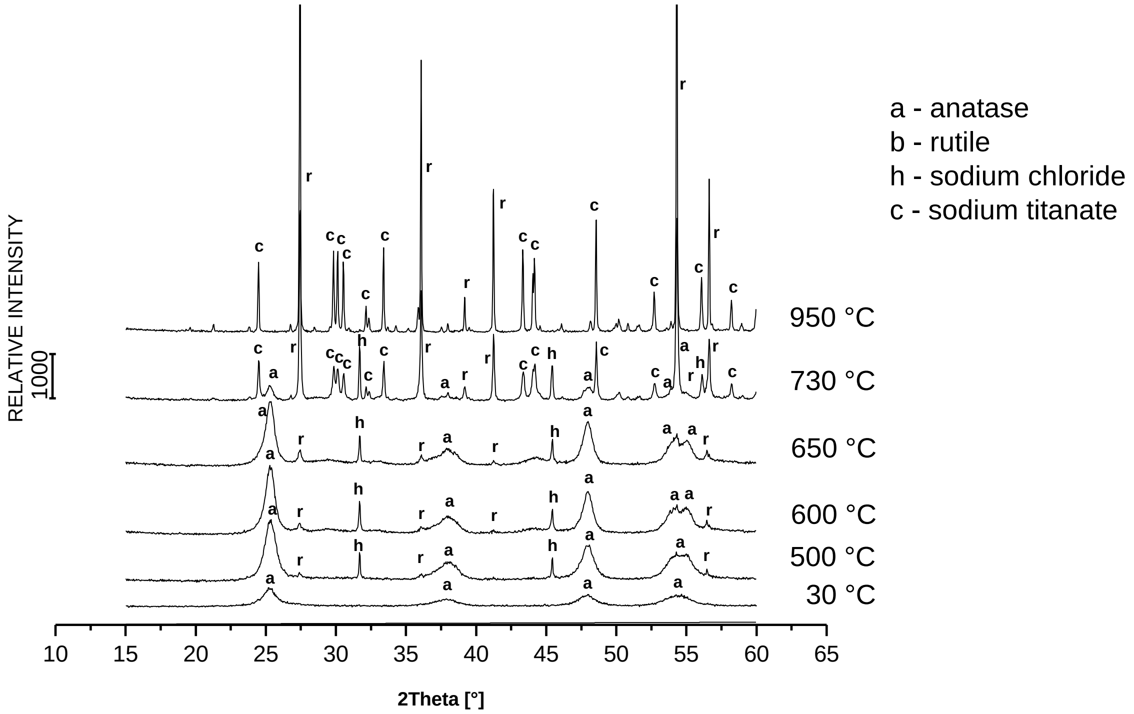

Figure 1: Evolution of diffractograms with an increasing annealing temperature.

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