6th George Olah Conference

XXII Conference of the George Olah Doctoral School

23 September 2024

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Program

EVENT VENUE: BME CH201 ROOM PUNGOR

8^{30} Opening

ORAL PRESENTATIONS

Chairman: Dr. Alfréd Kállay-Menyhárd

8^{35}-9^{05} **Dr. Márton Nagyházi** – Beyond carbenes – versatile use of highly nucleophilic ligands in transition metal complexes – Invited lecturer, George Olah Prize winner in 2023

9^{05}-9^{50} **Prof. Dr. Julius Vancsó** – Where synthetic and natural materials touch: Biointerface chemistry and lessons to be learned for engineering – Invited lecturer from the University of Twente and Sulis Polymers LTD

9^{50}-10^{20} **Prof. Dr. Gábor Laurenczy** – Reflections on hydrogen storage and delivery – Invited lecturer from the Swiss Federal Institute of Technology Lausanne

10^{20}-10^{40} Coffee Break

Chairman: Prof. Dr. László Poppe

10^{40}-11^{10} **Prof. Dr. István Hargittai** – George A. Olah's Magnanimity – Invited lecturer from the Department of Inorganic and Analytical Chemistry

11^{10}-11^{30} **Dr. Hajnalka Pataki** – Heterogenous crystallization in the presence of formulation additives – Invited lecturer from the Department of Organic Chemistry and Technology

11^{30}-11^{50} **Dr. Eszter Makkos** – Modelling surfaces – The role of catalytic support in CO_2 conversion – Invited lecturer from the Department of Inorganic and Analytical Chemistry

11^{50}-12^{10} **Dr. Dénes Berta** – Mechanism and reactivation of Ras GTPases based on virtual reactivity screening – Invited lecturer from the Department of Physical Chemistry and Materials Science

12^{10}-12^{30} **Dr. Dávid Havasi** – From building blocks to virtual chemicals: insights into design and synthesizability – Invited lecturer from the Department of Chemical and Environmental Process Engineering

12^{30}-12^{50} **Dr. Gergely Nándor Nagy** – Structural neurobiology: ligand-receptor and proteoglycan interactions during extracellular signal transduction – Invited lecturer from the Department of Applied Biotechnology and Food Science

12^{50}-14^{20} Break and Poster session – 2nd floor

Jury: Prof. Dr. Julius Vancsó, Dr. Soma Papp, Dr. Evelin Bell, Dr. Márton Nagyházi

P01	Anna Péter-Haraszti	Investigation of relaxations of interacting and non-interacting amorphous solid dispersions with different analytical methods
P02	Dorottya Vaskó	Development of an Inline Monitoring System for Adalimumab Filtration Using Raman and NIR Spectroscopy
P03	Tibor Tamás Novák	Fluorofunctionalizaton of selected functionalized cycloalkene scaffolds through halofluorination/fluoroselenation and aziridination/aziridine opening protocol
P04	Lucy Nyambura Karanja	Preparation, Characterization, and Photocatalytic activity of ZnO Nanorods grown on TiO_2 and ZnO Inverse Opal Structures
P05	Niloofar Bayat	Synthesis and Thermal Analysis of Hexaamminecobalt (III) Dibromide Permanganate
P06	Orsolya Péterfi	Real-time particle size measurement during the pellet layering process using artificial intelligence-aided endoscopic imaging
P07	Sarah Morais Bezerra	Synthesis of SiC nanocrystals for quantum applications
P08	Tibor Viktor Szalai	Experimental validation of water network prediction tools - structure and thermodiynamics
P09	Askar Kholikov	Studying thermostable α-amylase form native B. licheniformis 104.K: Screening, Cloning and Computational design
P10	Kata Buda	Discovering xylanolytic enzyme production of Spencermartinsiella europaea and Sugiyamaella novakii
P11	Emese Sándor	Enantiocomplementary Bioreduction of 1-(Arylsulfanyl)propan-2-ones
P12	Eszter Holub	Changes in Gene Expression and RNA Processing Inducesd by Thymidylate Synthase Inhibitory Drugs
P13	Ghazwan Saleh Ahmed	Enzymatic interesterification of sunflower oil to biodiesel in a solvent-free process

P14	Honvári Máté Gergő	Utilization of Wild Yeasts in the Bioreduction of Butan-2-ones with (Partially) Saturated Heterocyclic Side Chains
P15	Máté Laurinyecz	Effect of Carrier Morphology on Metal Ion Affinity Immobilization—A Case Study With Phenylalanine Ammonia-Lyase
P16	Péter Magyar	Aqueous multicomponent reactions – step-by-step to biocatalysis
P17	Nikolett Emődi	Insights into Zearalenone Degrading Enzymes
P18	Viktória Berta Perey-Simon	Role and effect of uracil metabolism on zebrafish embryonic development
P19	Gabriella Muskovics	Changes of gluten protein composition during sourdough fermentation in rye flour
P20	György Nimród Stoffán	Development of continuous additive-controlled crystallization by DoE-based batch experiments

STUDENTS' ORAL PRESENTATIONS

Section A – CH201

Chairman: Prof. Dr. László Poppe

14^{20}-14^{35} **Orsolya Péterfi** – Artificial neural network-based prediction of in vitro tablet dissolution profile using granulation process parameters and spectroscopic measurements

14^{35}-14^{50} **Anna Bulátkó** – Reduced graphene oxide cryogels and implications for green applications

14^{50}-15^{05} **Gábor Koplányi** – Immobilization of a Potential Therapeutic Enzyme on Magnetic Nanoparticles

15^{05}-15^{20} **Petra Záhonyi** – Investigation of the dehydration of dextrose monohydrate during twin-screw wet granulation and in-line, real-time monitoring of the anhydrous content in granules

15^{20}-15^{35} **Andor Vancza** – Heteroleptic iron(II)-bis-terpyridine complexes: the effect of ligand combinations on the metastable quintet state lifetime

15^{35}-15^{50} **Pradeep Kumar** – Development of edible food packaging using food processing industry side streams

15^{50}-16^{10} Coffee Break

Section B – CH201

Chairman: Dr. Alfréd Kállay-Menyhárd

16^{10}-16^{25} **Barbara Honti** – Explainable deep recurrent neural networks for the batch analysis of a pharmaceutical tableting process in the spirit of Pharma 4.0

16^{25}-16^{40} **Apoko Stephen Omondi** – Control over the morphology and catalytic properties of porous multimetallic nanoparticles

16^{40}-16^{55} **Gergely T. Solymosi** – Single Synthetic Ion-Channels as Potentiometric Ion Sensors

16^{55}-17^{10} **Norbert Kovács** – Revealing Molecularly Imprinted Cavities and Pinholes in Electrically Insulating Nanofilms by Gold Electroplating and Conductive Atomic Force Microscopy

17^{10}-17^{25} **Khadijeh Firoozirad** – Achieve advance in crystal nucleation studies through comprehensive experimental and theoretical modeling

17^{35} Closing – CH201

ARTIFICIAL NEURAL NETWORK-BASED PREDICTION OF *IN VITRO* TABLET DISSOLUTION PROFILE USING GRANULATION PROCESS PARAMETERS AND SPECTROSCOPIC MEASUREMENTS

Orsolya Péterfi[1]; Brigitta Nagy[1]; Kovács Béla Kovács[2]; Tibor Casian[3]; Erzsébet Orsolya Tőkés[4] and Zsombor Kristóf Nagy[1]

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In vitro dissolution testing is required during the quality control of solid dosage forms, in order to control product quality, stability, and batch-to-batch consistency. However, traditional analytical quality control methods are time-consuming, labor-intensive, involve the destruction of tablets, and do not allow for 100% inspection of products. [1] The estimation of dissolution profiles can be achieved using artificial neural networks (ANN) based on data obtained from in-line, rapid, and non-destructive analytical sensors. [2,3]

The aim of this study is to develop an artificial intelligence-based method to predict the dissolution profile of tablets prepared with the direct compression of hot-melt granules. The artificial neural network (ANN) models were trained using process parameters registered by the granulation equipment (temperature, air humidity), tableting parameters (lubrication time, compression force), and the near-infrared (NIR) spectra of the tablets. The goodness of the models was characterized by the f_2 similarity factor and the RMSE (Root Mean Square Error) between the measured and estimated dissolutions. Despite the acceptable f_2 values, some of the the ANN models predicted an average dissolution profile. Therefore, the SRD (Sum of Ranking Differences) method was used for model ranking. The model utilizing data from the NIR spectrum proved to be the most suitable for estimating the dissolution profile of the tablets.

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REDUCED GRAPHENE OXIDE CRYOGELS AND IMPLICATIONS FOR GREEN APPLICATIONS

Anna Bulátkó[1*]; Attila Farkas[2]; János Madarász[3]; Miklós Mohai[4]; Lakshmi Shiva Sankar[4]; Zoltán Sebestyén[4] and Krisztina László[1]

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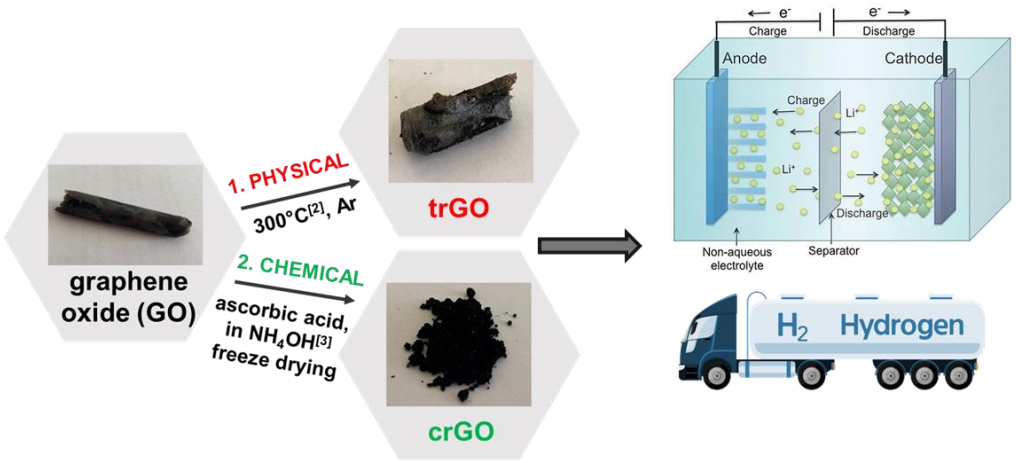

Figure 1. Graphene oxide cryogels and possible green applications

Graphene oxide (GO), formerly considered mainly as an intermediate in wet chemical graphene production, has now become a widely applicable material with expanding possibilities. While traditionally used in a solid, dry form for the production of GO-based electrodes and optoelectronic devices, it is often processed in a suspended state. Despite its beneficial hydrophilic nature, GO tends to lose many of its remarkable graphitic properties. For compensation of that, various reduction methods are available.

Different reduction techniques restore the graphenic properties with different efficiencies. This study investigates the effect of a physical (thermal)[1] and a chemical (ascorbic acid)[2] reduction method on GO. Characterisation using conventional analytical methods highlights the advantages and limitations of each alternative approach. A comprehensive analysis was carried out to reveal the morphology and surface chemistry of the reduced cryogels. Powder X-ray diffraction (XRD) and nitrogen adsorption were used for morphological analysis, while the possible regeneration of the graphenic structure was followed by Raman spectroscopy. The chemical composition was investigated with thermogravimetry/mass spectrometry and photoelectron spectroscopy (XPS). The combined effect of morphology and surface chemistry was investigated in green applications such as energy storage and gas storage capacity.

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IMMOBILIZATION OF A POTENTIAL THERAPEUTIC ENZYME ON MAGNETIC NANOPARTICLES

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Tyrosine hydroxylase (hTH) plays a crucial role in the synthesis of catecholamine neurotransmitters in human body. The structure, function and regulation of hTH has lately been extensively researched area and the possibility of enzyme replacement therapy (ERT) involving hTH and nanocarriers has also been raised. This research focuses on investigating the immobilization of a recombinant hTH on magnetic nanoparticles (MNPs) functionalized with various surface linkers, from both quantitative and mechanistic perspectives. Our findings demonstrates that the activity of hTH retains after the immobilization procedure in case of secondary and covalent interactions, as well. As a result, a homogenous enzyme layer could be achieved, that is demonstrated by Raman mapping. Furthermore, the analysis of physico-chemical properties of the different surface linkers of MNPs provides additional information about the possible enzyme-carrier interactions. The covalent attachment of hTH on MNPs via aldehyde or epoxy linkers provides irreversible immobilization with recovery of the native enzyme activity. Operational stability of hTH attached to MNPs in simulated nasal electrolyte solution (SNES) is also investigated with promising retaining enzyme activity. This outcome highlights the relevance of immobilization and applying MNPs as potential formulation tool of sensitive therapeutic enzymes, thereby presenting new possibilities for ERT in the treatment of neurodegenerative disorders.[1]

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INVESTIGATION OF THE DEHYDRATION OF DEXTROSE MONOHYDRATE DURING TWIN-SCREW WET GRANULATION AND IN-LINE, REAL-TIME MONITORING OF THE ANHYDROUS CONTENT IN GRANULES"

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As the solid-state form of a crystalline material is a key quality attribute influencing the physical and physicochemical properties of the substance, the investigation of its various crystalline transformations is crucial in the pharmaceutical field, where most organic compounds can exist as different polymorphs or solvatomorphs[1,2]. While the examination of the active pharmaceutical ingredient (API) is regularly conducted during the development phase, it is equally important to investigate excipients, which often constitute the majority of the final drug product, especially in formulations with modern, potent APIs. These excipients largely determine the properties of the final product. Numerous studies have shown that the solid-state form of the excipients can influence bulk and tap densities, flow properties, moisture absorption, lubrication, caking, compactibility, and tablet characteristics (including tensile strength, disintegration, and dissolution) etc.[3,4]

The solid-state behaviour of D-glucose (dextrose) — a widely used agent in both the food and pharmaceutical industries — is also a relevant topic that needs further exploration because of its increasing application. Dextrose can exist in various crystal forms, most commonly as anhydrous α and β-D-glucose or α-D-glucose monohydrate[5], and the solid-state form can significantly impact physical properties such as flowability, tabletability, and disintegration. [6] The transformation between the anhydrous and hydrated α forms (both generally stable at room temperature) is particularly important, as it can easily occur during various formulation process steps, potentially affecting the further processing and the quality of the final product.

The goal of this research was to investigate the dehydration of dextrose and explore the relationship between process parameters and the resulting solid-state form. Additionally, we aimed to develop a method for monitoring the crystal form of dextrose in line and in real time to ensure product quality. A continuous granulation process — consisting of twin-screw wet granulation, continuous drying, and milling — was examined using various analytical methods to determine the anhydrous content of granules and its link to drying parameters. Both off-line and in-line methods were compared, with in-line Raman spectroscopy enabling real-time monitoring. Given the importance of the crystal form of dextrose, this technology proves highly valuable for ensuring adequate product quality.

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HETEROLEPTIC IRON(II)-BIS-TERPYRIDINE COMPLEXES: THE EFFECT OF LIGAND COMBINATIONS ON THE METASTABLE QUINTET STATE LIFETIME

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Transition-metal complexes have been the topic of research in the past decade to lay the foundation of their application as molecular devices. Up until now our group has primarily studied the homoleptic $[Fe(terpy)_2]^{2+}$ system as a potential spin-state switch and light-sensitive compound, as well as the effect of electron donating (ED) and withdrawing (EW) substituents in various positions[1-5] on its ultrafast photorelaxation dynamics. As the next step, we systematically explore the effect of substituents in heteroleptic complexes, and potentially provide a combined description for both homo- and heteroleptic complexes with 4'Y and 5,5"diX substituents.

This presentation will show results of transient absorption measurements carried out at Wigner RCP and at the ELI-Beamlines facility. I shall present the quintet-state lifetime of complexes with different ED/EW character and compare them with the Swain-Lupton substituent parameters. Additionally I propose a semi-quantitative method to describe the correlation of such parameters with the lifetime of the metastable quintet state for both substituent positions discussed.

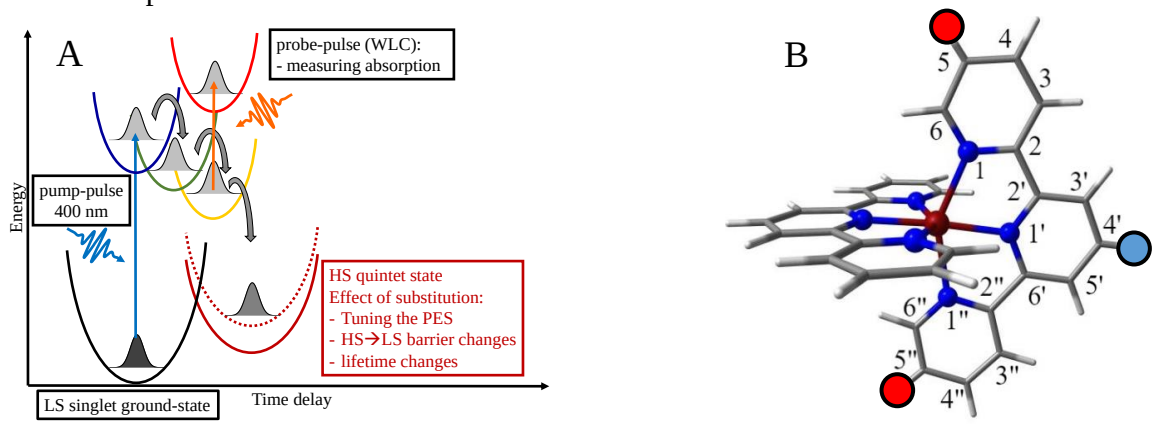

Figure 1. A) Measurements scheme of the pump-probe method, B) Schematic representation of the studied gorup of molecules

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DEVELOPMENT OF EDIBLE PACKAGING VALORIZING FOOD PROCESSING INDUSTRY SIDE STREAMS

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The increasing environmental concerns regarding plastic waste have driven research towards sustainable packaging solutions, including edible packaging materials derived from food processing industry side streams. My presentation aims to explore innovative methods and technologies to develop edible packaging by valorizing side streams, such as deoiled walnut pomace, gelatin, pectin, and other bio-based materials. The focus is on utilizing these underexploited resources to create sustainable packaging alternatives that reduce waste, lower environmental impact, and contribute to a circular economy.

Deoiled walnut pomace, a by-product from walnut oil extraction, offers significant potential as a raw material for edible packaging due to its high fiber content, antioxidants, and bioactive compounds. The utilization of walnut pomace not only adds value to this agro-industrial side stream but also enhances the nutritional properties of the packaging. Research indicates that walnut pomace can be effectively integrated into packaging films, providing both functional properties, such as mechanical strength and barrier functionality, and active properties like antioxidant activity.

Gelatin and pectin are natural biopolymers widely used in the development of edible films and coatings due to their excellent film-forming capabilities, biodegradability, and compatibility with other materials. Gelatin, a protein derived from animal collagen, offers flexibility, strength, and transparency in packaging films. Its application in combination with walnut pomace can produce robust edible packaging materials with enhanced physical properties and bioactivity. Pectin, a polysaccharide obtained from fruit peels and other plant sources, is recognized for its gelling properties, which are valuable for forming stable and flexible films. Combining pectin with deoiled walnut pomace could improve film integrity, provide additional nutritional benefits, and create a synergistic effect that enhances the overall performance of edible packaging materials.

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EXPLAINABLE DEEP RECURRENT NEURAL NETWORKS FOR THE BATCH ANALYSIS OF A PHARMACEUTICAL TABLETING PROCESS IN THE SPIRIT OF PHARMA 4.0

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The pharmaceutical industry has seen a dramatic transformation as a result of Industry 4.0. Due to the continuously increasing Cost of Goods Sold, the pharmaceutical industry has faced several challenges, and the Right First-Time principle with data-driven decision-making has become more pressing to sustain competitiveness. Thus, in this work, three different types of artificial neural network (ANN) models were developed, compared, and interpreted by analyzing an open-access dataset from a real pharmaceutical tableting production process. First, the multilayer perceptron (MLP) model was used to describe the total waste based on 20 raw material properties and 25 statistical descriptors of the time series data collected throughout the tableting (e.g., tableting speed and compression force). Then using 10 process time series data in addition to the raw material properties, the cumulative waste, during manufacturing was also predicted by long short-term memory (LSTM) and bidirectional LSTM (biLSTM) recurrent neural networks (RNN). The LSTM network was used to forecast the waste production profile to allow preventive actions. The results showed that RNNs were able to predict the waste trajectory, the best model resulting in 1096 and 2174 tablets training and testing root mean squared errors, respectively. For a better understanding of the process, and the models and to help the decision-support systems and control strategies, interpretation methods were implemented for all ANNs, which increased the process understanding by identifying the most influential material attributes and process parameters. The presented methodology is applicable to various critical quality attributes in several fields of pharmaceutics and therefore is a useful tool for realizing the Pharma 4.0 concept.

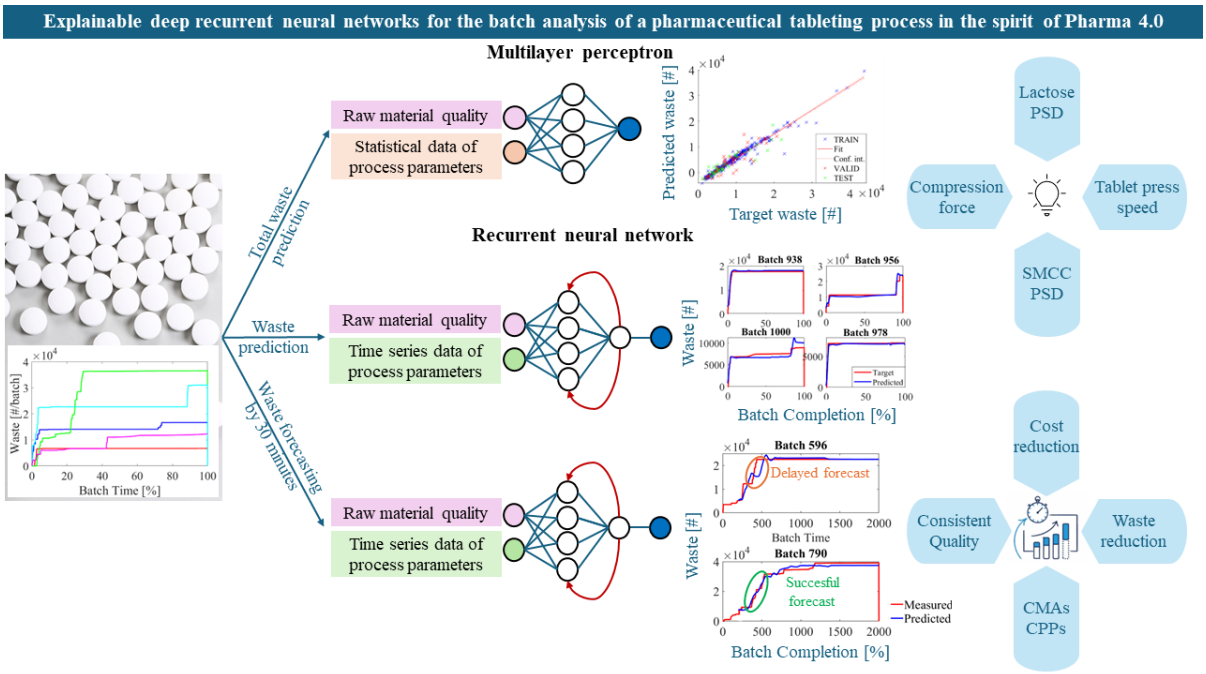

Figure 1. Graphical abstract.

CONTROL OVER THE MORPHOLOGY AND CATALYTIC PROPERTIES OF POROUS MULTIMETALLIC NANOPARTICLES

Apoko S. Omondi[1,2]; Dávid Kovács[1,2]; András Deák[1] and Dániel Zámbó[1*]

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The effectiveness of noble metal nanocatalysts can be improved by the integration of multiple types of metals into a properly designed core-shell nanoparticle (NP). The available active sites necessary for effective (electro)catalysis can be ensured by depositing a multimetallic shell possessing micro- and mesoporosity on a well-designed core NP to exploit the synergy in the multi-elemental system.[1] Here, we present the robust synthesis of tetrametallic nanoparticles consisting of a gold core and a shell of platinum, palladium and iridium. To reveal the impact of the morphology of the cores on the overall activity of the nanocatalysts, five different core morphologies (spherical, rod, prism, octahedral and bipyramidal) were carefully synthesized to ensure an identical volume and surface chemistry for all core NPs preceding the deposition of the trimetallic shell metals. The strict control over the synthesis parameters ensures equivalence in atomic compositions to leave the core morphology as the only variant to investigate its effect on the catalytic properties. The detailed structural characterization (SEM, TEM-EDS, XRD) of the Au@PdPtIr nanoparticles revealed that the well-defined Au cores are surrounded by a porous trimetallic shell with permeable pore system. To set an identical catalyst loading in the investigated reactions, ICP-OES was used. The impact of symmetry breaking on the effectiveness of these nanoparticles was tested through heterogeneous catalytic degradation of p-nitrophenol to p-aminophenol. Moreover, their electrocatalytic activity was assessed by the electrooxidation reactions of methanol, ethanol and formic acid in a three-electrode electrochemical setup. The results depict superiority in activity of the symmetry-broken nanoparticles as opposed to their spherical counterparts that have similar core volumes, elemental concentrations and particle loading in every test study conducted.[2]

Acknowledgement: We acknowledge the support of Pro Progressio and József Varga Foundation. The work was financially supported by the NRDI fund of Hungary under the grant of FK 142148. Project no. TKP2021-NKTA-05 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund (NRDI), financed under the TKP2021 funding scheme.

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SINGLE SYNTHETIC ION-CHANNELS AS POTENTIOMETRIC ION SENSORS

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Functionalized nanoporous membranes, containing large numbers of synthetic ion-channels, had been shown to offer a highly selective and robust alternative to conventional, polymer-based potentiometric membranes.[1,2] Here, we show that a single synthetic ion-channel can also serve as a potentiometric ion sensor, emerging as the smallest ionophore-based ion-selective electrode ever reported. Synthetic single ion-channels were made by the chemical modification of ca. 10-nm-radius single nanopores fabricated by focused ion beam milling in submicron-thick SiN_x/Au membranes. We could produce both "ideally non-selective" and highly Ag^+-selective cation-exchanger single ion-channels by adjusting the chemical modification of the nanopores, demonstrating the versatility of the proposed synthetic approach. The Ag^+-selective ion-channels showed selectivities exceeding 6 orders of magnitude for most tested ions, including strongly lipophilic organic ions. The immobilization of all active components and the high ionic conductance made the ion-channels remarkably resistant to organic solvents and current-induced polarization, thus overcoming most critical disadvantages of conventional micropipet-type ion-selective microelectrodes.

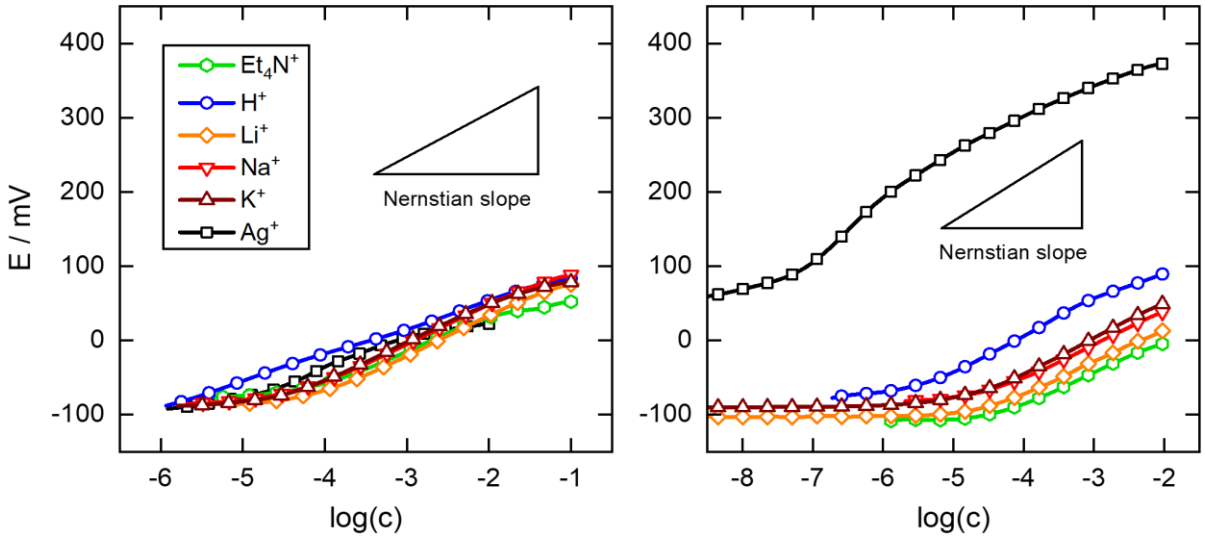

Figure 1. Potentiometric calibration curves of "ideally non-selective" (left) and highly Ag^+-selective (right) cation-exchanger single ion-channels.

The results presented here are under preparation for submission to Nature Nanotechnology.

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REVEALING MOLECULARLY IMPRINTED CAVITIES AND PINHOLES IN ELECTRICALLY INSULATING NANOFILMS BY GOLD ELECTROPLATING AND CONDUCTIVE ATOMIC FORCE MICROSCOPY

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Molecularly imprinted polymers (MIPs) are one of the most promising fully synthetic class of affinity ligands for selective molecular recognition: The generic concept of imprinting enabled the development of MIPs for small ions and molecules, macromolecules as well as synthetic and biological origin nano- and microparticles (e.g. viruses, cells). They have a wide range of applications including separation, drug delivery, affinity assays and chemical sensing.[1] In addition to their robustness and thermal stability, the preparation of MIPs benefits from highly sophisticated methods to control the selectivity, accessability of the imprinted binding sites as well as to generate high-throughput microarrays [2].

However, essential fundamental assumptions regarding the generation of imprints by molecular templates lacks direct experimental evidence, such as the confirmation of their physical presence and distribution. Mainly the functionality of the MIPs in terms of template binding is used to support indirectly the formation of molecular imprints. Indeed, imaging of molecular size imprints/cavities in a polymer nanofilm with a marked surface roughness is hardly possible even by the highest resolution imaging techniques. This is particularly true for small molecule templates, such as peptide imprinted polymers where only a characteristic short peptide of the parent protein is used as a template instead of the whole protein.

Therefore, we were interested to develop an imaging technique able to reveal the binding cavities and their surface density in MIP nanofilms.[3] We approached this aim by combining gold electroplating and conductive atomic force microscopy (c-AFM) imaging. Gold electrodeposition forms gold nanowires in the imprinted cavities revealing their presence, while c-AFM confirm that they are in contact with the substrate electrode. As such we can discriminate these from contingent non-specific gold depositions onto the polymer surface. For validation, we created artificial cavities using nanoindentation in an insulating polyscopoletin polymer nanofilm. We determined the optimal gold deposition parameters and also the cavity size-dependency of gold growth. Moreover we noticed that this gold electroplating method is suitable for the detection of pinholes in electrically insulating nanofilms.

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Achieve advance in crystal nucleation studies through comprehensive experimental and theoretical modeling

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Crystal nucleation is crucial in various fields of science and technology, ranging from materials synthesis to pharmaceutical production. Our research aims to determine the nucleation rates from parallel induction time measurement experiments followed by low-cost external bulk video imaging. Stirred conditions are applied, making our results industry-relevant where the crystallizers are also mixed. The L-Glutamic Acid-water system (L-GA/water) is a surrogate. The study is conducted through a series of parallel, small-scale stirred experiments that explore the effects of supersaturation, temperature, and polymer additives on nucleation rates. The methodology involves careful data collection through controlled experimental setup and analysis of induction time distributions that the stochastic nature of nucleation explains the induction time distribution. The discussion section interprets the findings within the context of the research question, highlighting the implications of varying temperature and supersaturation levels as two experimental parameters and polymers on crystal nucleation rates. The specific parameters of supersaturation and temperature dependencies are in the range of those reported in the literature, which validated the developed rapid nucleation rate measurement platform. Stepping outside of these established measurements, analyzing the influence of polymers with high potential in controlling particle size, shape, and polymorphism was also touched. As an unexpected result, the nucleation rates appeared to depend not only on the chemical structure and amount of the polymer but also on the polymer solution preparation method. The remarkable influence of polymers on nucleation rates was shown, and the solubility-altering effect of the polymers alone cannot explain this [1,2].

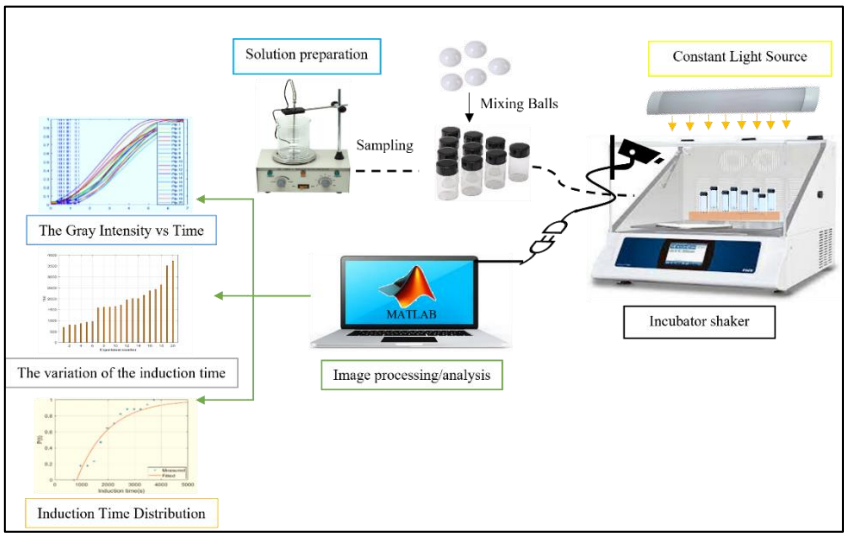

Figure 1. Short description.

References

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INVESTIGATION OF RELAXATIONS OF INTERACTING AND NON-INTERACTING AMORPHOUS SOLID DISPERSIONS WITH DIFFERENT ANALYTICAL METHODS

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To improve the dissolution of active pharmaceutical ingredients (APIs) with poor solubility, developing amorphous solid dispersions (ASDs) can be a promising solution. In the case of preparations containing such systems, it is important to verify the stability, for which it is essential to examine the material properties that affect stability. The presented research was concerned with the comparison of ‘interacting’ and ‘non-interacting’ amorphous solid dispersions (ASDs) based on the thermal analysis of their relaxation transitions. When choosing the tested APIs, the presence or absence of the H-donor group was the fundamental consideration. The two model systems were made using APIs naproxen (NAP) with higher and spironolactone (SPIR) with lower interacting potential combined with polyvinylpyrrolidone vinyl acetate (PVPVA64) copolymer. In the case of the NAP system, the glass transition temperature (T_g) and the relaxations below the T_g (sub-Tg transitions) determined by MDSC and TSDC measurements suggested that this is an 'interacting' system concerning the H-bonding. Besides, the role of the naphthalene ring of the NAP in the sub-T_g relaxation was confirmed by the temperature-dependent Raman spectroscopy. On the other hand, The SPIR system was confirmed to be ‘non-interacting’ by all three analytical methods. This study emphasizes that the simultaneous application of different thermoanalytical and spectroscopic methods aids in understanding the molecular mechanisms behind relaxations in ASDs. This approach supports the intentional design of stable amorphous solid dispersions already at the beginning of the research and development phase.

DEVELOPMENT OF AN INLINE MONITORING SYSTEM FOR ADALIMUMAB FILTRATION USING RAMAN AND NIR SPECTROSCOPY

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Real-time monitoring was performed during the final stage of monoclonal antibody purification, specifically during the continuous diafiltration process, using inline Raman and Near-Infrared (NIR) spectroscopy. The development and implementation of spectroscopy-based models enabled rapid feedback on buffer concentrations, providing precise, real-time control over the purification process. This approach is essential for maintaining tight process control and ensuring high-quality assurance standards. The study investigated three distinct formulation buffer systems. The first system utilized mannitol as the primary component, the second combined histidine and sucrose, and the third involved hydroxypropyl-β-cyclodextrin (HPβCD). Each buffer system plays a crucial role in the final drug formulation.

The research aimed to monitor the concentrations of these key components using inline spectroscopy throughout the purification process. Moreover, to develop a robust process analytical technology (PAT) tailored for the filtration stage of protein purification. By employing multivariate data analysis techniques, calibration models were successfully constructed to track the concentrations of critical components in real-time.

It was demonstrated that Raman and NIR spectroscopy could effectively quantify the concentrations of the three different buffer systems. The integration of inline monitoring technology for all three buffer systems proved to be highly efficient and automated, significantly enhancing the robustness and efficiency of the overall purification process.

FLUOROFUNCTIONALIZATON OF SELECTED FUNCTIONALIZED CYCLOALKENE SCAFFOLDS THROUGH HALOFLUORINATION/FLUOROSELENATION AND AZIRIDINATION/AZIRIDINE OPENING PROTOCOL

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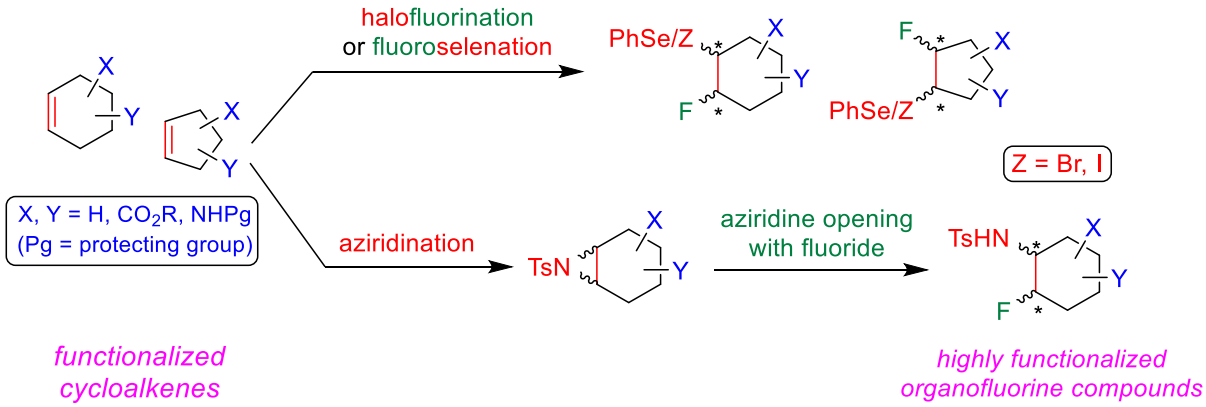
Due to their special properties that are beneficial in pharmaceutical chemistry and drug design, organofluorine compounds have had high importance in organic synthesis in the past years. Many synthetic routes and developing methods have been discovered as they have high interest in versatile fluorinated molecules.[1-3]

Fluorofunctionalization of functionalized cycloalkenes (such as cyclic beta-amino acid derivatives) is also a well-investigated field in synthetic chemistry owing to their biological effects. [4] Our main goal was to attempt various protocols to incorporate fluorine into functionalized cycloalkene scaffolds (such as lactams, esters, amino esters) with the generation of novel chiral centers. This work details reactions of halofluorination and fluoroselantion reactions on some functionalized cycloalkenes.[5] Aziridinations and aziridine opening protocol with fluorine of cycloalkanes have also been investigated.[6]



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PREPARATION, CHARACTERIZATION, AND PHOTOCATALYTIC ACTIVITY OF ZNO NANORODS GROWN ON TIO_2 AND ZNO INVERSE OPAL STRUCTURES

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Rapid industrial and urban development has led to the discharge of chemical pollutants, such as synthetic dyes, into water bodies, posing environmental and health risks. This study investigates the preparation, characterization, and photocatalytic (PC) activity of ZnO and TiO_2 inverse opals (IOs) and ZnO nanorods (NRs) grown on these IOs. Polystyrene nanospheres served as sacrificial templates to form opal structures, followed by thermal atomic layer deposition (T-ALD) of ZnO and TiO_2. Subsequent annealing at 500°C removed the templates, allowing hydrothermal growth of ZnO NRs on ZnO and TiO_2 IOs and a silicon substrate. Characterization techniques included TG-DTA, SEM, EDX, XRD, Raman spectroscopy, UV-Visible spectroscopy, and photoluminescence. Photocatalytic activity was assessed by degrading methylene blue under UV and visible light.

Thermal analysis confirmed complete removal of the PS template at 500°C. SEM revealed well-ordered opal structures and interconnected spherical shells of TiO_2 and ZnO post-template removal. ZnO NRs grew uniformly on ZnO IO substrates, while growth was less dense on TiO_2 IO and silicon. EDS analysis indicated complete removal of PS and elemental differences across samples. XRD and Raman confirmed the crystallinity and phase purity of ZnO and TiO_2. UV-Visible spectroscopy showed absorption peak shifts due to ZnO NRs interaction with IO substrates, while photoluminescence provided insights into recombination processes.

ZnO IO showed higher photocatalytic activity than TiO_2 IO due to better electron-hole pair generation and mobility. ZnO NRs on ZnO IO (ZnO NRs@ZnO IO) had the highest degradation rate (K_app = 0.0044 $min^{-}1$ under UV light), due to synergistic effects providing high surface area, charge transport, and light management. ZnO NRs on TiO_2 IO (ZnO NRs@TiO2 IO) showed a higher rate (K_app = 0.0036 $min^{-}1$) than TiO_2 IO alone (K_app = 0.0026 $min^{-}1$). The PC degradation followed pseudo-first-order kinetics, with higher rates under UV light. These results suggest ZnO-based photocatalysts, particularly ZnO NRs@ZnO IO, are promising for environmental remediation applications.

Synthesis and Thermal Analysis of Hexaamminecobalt (III) Dibromide Permanganate

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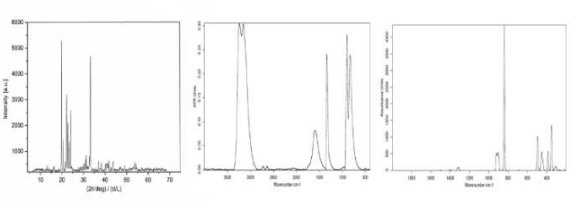
This study focuses on the synthesis, thermal analysis, and characterization of in-air decomposition products of $[Co(NH_3)_6]Br_2MnO_4$, as the thermal decomposition of transition-metal complexes is among the simplest and least expensive techniques for preparing nanosized transition-metal oxides. The cobalt complex was initially prepared from following starting materials, $[Co(NH3)_6](MnO_4)_3$ and $[Co(NH_3)_6]Br_3$ with the yield of 84.26% then characterized by spectroscopic techniques, including Infrared spectroscopy (IR), Powder X-ray diffraction (PXRD), Single crystal X-ray diffraction (SC-XRD) and Raman[1]. The thermal behavior of the complex was investigated using thermogravimetric analysis (TGA) followed by mass spectroscopy and differential scanning calorimetry (DSC). The thermal analysis revealed that the complex has undergone a multi-step decomposition process upon heating. The compound's low decomposition point and the possibility of redox reactions between ammonia ligands and permanganate ions made it a suitable candidate for investigating its solid-phase decomposition products [2]. To determine the nature of decomposition intermediates, the compound was studied with stepwise isothermal decomposition in the solid state. The final product at 500 °C was Co-Mn oxide spinel compound, which was analyzed by some techniques including XRD and IR spectroscopy. The results provide valuable insights into the thermal stability of $[Co(NH_3)_6]Br_2MnO_4$ and its subsequent decomposition products for understanding its potential future applications in various fields, such as catalysis for environmental remediations [3].



Fire1: From left to right: XRD, IR and Raman Spectra

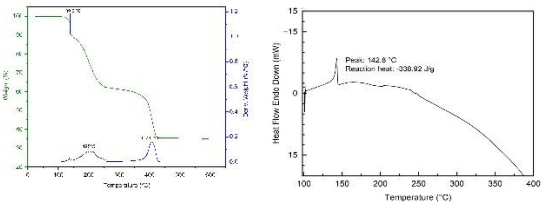


Figure3: From left to right: DTG and DSC analysis

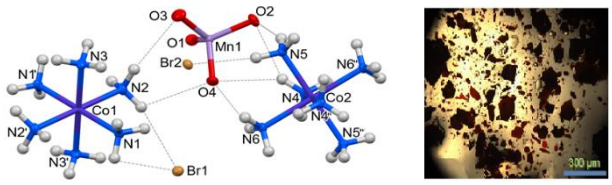


Figure3: From left to right: Structure and Microscopic Picture in air

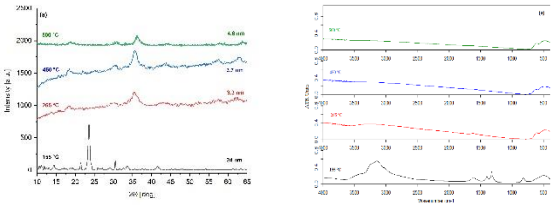


Figure4: XRD and IR spectra of decomposition products

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REAL-TIME PARTICLE SIZE MEASUREMENT DURING THE PELLET LAYERING PROCESS USING ARTIFICIAL INTELLIGENCE-AIDED ENDOSCOPIC IMAGING

Orsolya Péterfi*; Kincső Demeter[1]; Nikolett Kállai-Szabó[2]; Ádám-Tibor Barna[2]; István Antal[2]; Zsombor Kristóf Nagy[1] and Dorián László Galata[1]

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In this study, an artificial intelligence-based machine vision system was developed for in-line particle size analysis during the pellet layering process. In-line process monitoring using Process Analytical Technology (PAT) tools provides real-time information about pellet size and layer uniformity, enabling timely intervention in the case of out-of-specification products. The direct imaging system, consisting of a rigid endoscope, a light source, and a high-speed camera, allowed for the real-time monitoring of the particle size and morphology. A convolutional neural network-based instance segmentation algorithm was employed to detect the pellets and determine their particle size. After training the model, the performance of the developed system was assessed by analysing the particle size distribution of various spherical starter pellets within the 250–850 μm size range. The endoscopic system was tested in-line at a larger scale during the drug layering of inert pellet cores. The developed imaging system is promising; in-line measurements showed good correlation with the off-line reference methods.

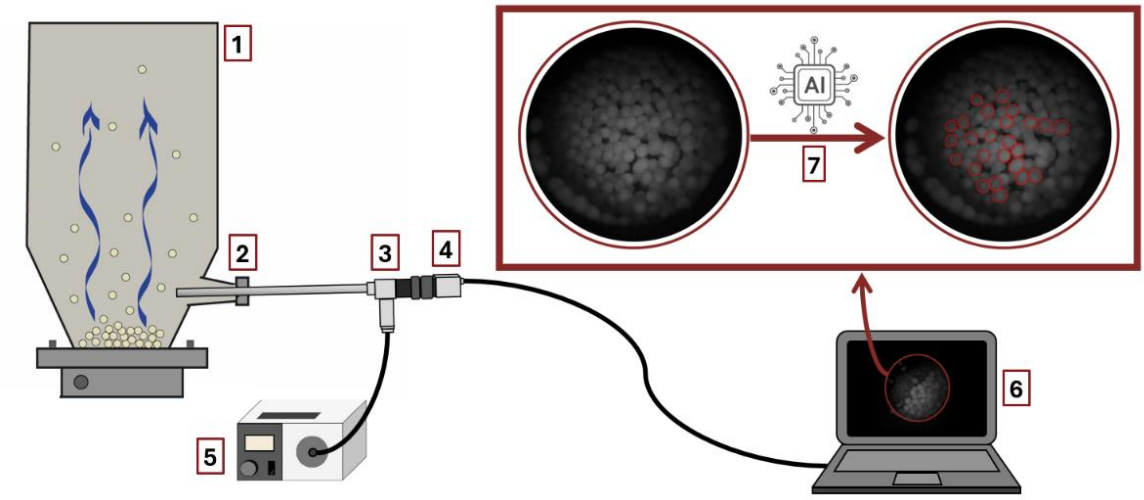

Figure 1. Experimental setup: (1) fluid-bed apparatus, (2) sampling port, (3) endoscope, (4) camera, (5) light source, (6) computer, (7) CNN-based software.

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SYNTHESIS OF SIC NANOCRYSTALS FOR QUANTUM APPLICATIONS

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Silicon carbide (SiC) is a semiconductor recognized for its outstanding optical, thermal, and electrical properties, rendering it highly suitable for high-power, high-temperature electronic applications[1]. When the size of these particles are reduced to the nanoscale, novel properties emerge. Furthermore, by deliberately introducing specific defects into the material's structure, additional unique properties can be attained. For example, defects such as silicon vacancies (V_{Si}) and divacancies ($V_{Si}V_C$) may lead to distinct photoluminescence signals at particular wavelengths, approximately 950 nm and 1100 nm, respectively. The distinctive and recognizable nature of these signals facilitates swift and qualitative defect identification through photoluminescence analysis.

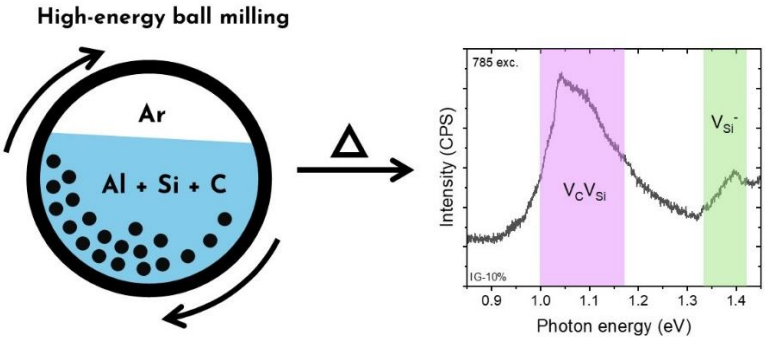

Figure 1. Methodology for reaction and result from the photoluminescence of the defect found in samples.

In this study, an experimental process employing high energy ball milling along with the incorporation of aluminum to synthesize silicon carbide with defects. The resulting samples were characterized using a range of methods, including photoluminescence, electron paramagnetic resonance (EPR), Raman spectroscopy, and X-ray diffraction (XRD). The photoluminescence results indicated the presence of distinct defects within the structure, which were influenced by the amount of aluminum added. In addition to photoluminescence, EPR data indicates the formation of various defects, while XRD analysis reveals changes in polytype formation as the aluminum concentration increases.

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EXPERIMENTAL VALIDATION OF WATER NETWORK PREDICTION TOOLS - STRUCTURE AND THERMODYNAMICS

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Rational drug design focuses on the explanation and prediction of complex formation between therapeutic targets and small molecule ligands. As an often overlooked interacting partner, water molecules play a critical role in the thermodynamics of protein-ligand binding.[1]

We challenged two conceptually distinct modelling methods by predicting and scoring relevant water networks: WaterFLAP, which uses GRID Molecular Interaction Fields to classify the water molecules into structural, displaceable, or bulk characters,[2] and MobyWat, which uses a different principle by applying molecular dynamics in characterizing water molecules.[3] We also investigated the impact of solvent exchange from light (H_2O) to heavy water (D_2O) to provide complete thermodynamic profiling of these ternary systems. Utilizing isothermal titration calorimetry (ITC) experiments and the solvent isotope effects, we gain a deeper understanding of the energetic contributions of various components.[4]

Results show, that water network prediction tools like WaterFLAP and MobyWat can be used to predict and score water molecules within the apo protein binding site and in the protein-ligand complexes in order to: (i) gain information about the thermodynamic stability of the water molecules and their effect on ligand binding, (ii) to interpret ligand affinity variations, and ultimately to guide further ligand optimization efforts. Furthermore, ITC measurements done in both H_2O and D_2O can be used to get an insight into the net effect of the water network on the binding of a ligand.

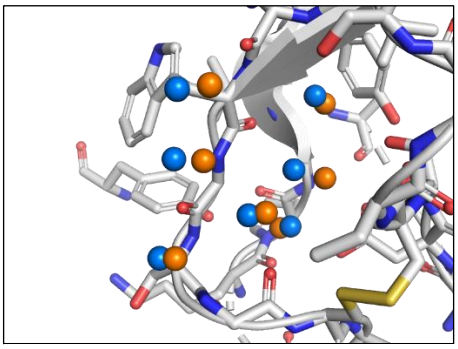

Figure 1. The position of experimentally detected water molecules (blue spheres) in the trypsin binding sites are accurately modeled by WaterFLAP (orange spheres).

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DISCOVERING XYLANOLYTIC ENZYME PRODUCTION OF *SPENCERMARTINSIELLA EUROPAEA* AND *SUGIYAMAELLA NOVAKII*

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Polysaccharide degrading enzymes play a crucial role in many industrial bioprocesses and have a huge potential to be applied in future advanced technologies and biorefineries utilizing lignocellulosic raw materials. Lignocellulose consists of cellulose, hemicellulose and lignin. To degrade lignocellulose, hemicellulase and cellulase are needed. Effective degradation and utilization of cellulose and hemicelluloses is important for the shift towards green bioeconomy, and requires microbes equipped with proper sets of carbohydrate-active enzymes (CAZymes). Knowledge of cellulolytic and xylanolytic enzymes has mainly been generated from bacteria and filamentous fungi, while yeasts have been largely overlooked and may represent an untapped resource in natural CAZymes with industrial relevance.[1] *Spencermartinsiella europaea* (NCAIM Y.01817) and *Sugiyamaella novakii* (NCAIM Y.00987) have been found promising candidates for xylanase production in our previous investigations. Thus, xylanase enzyme production and enyzme activity of *S. europaea* and *Su. novakii* were further investigated in this study.

Fermentations were performed in 100 mL-shake flasks for 72 hours at 30 °C, and 220 rpm. After 3 days of fermentation, xylanase activity was measured from the cell-free supernatant. Xylanase activity measurements were carried out by DNA-method (in 0.05 M acetate buffer and 0.05 M citrate buffer). Experiments were carried out to investigate the pH (pH 2-7) and temperature (25-65 °C) optimum of the produced xylanase enzymes. Side enzyme activities like CMC-ase, β-glucosidase, β-xylosidase and cellobiohydrolase were also measured.

The highest xylanase activity value was 2.3 U/mL and 3.2 U/mL in case of *S. europaea* and *Su. novakii*, respectively. Highest xylanase activity was detected at 55 °C and 35 °C (100% relative activity) in case of *S. europaea* and *Su. novakii*, respectively. In terms of the pH optimum investigation, the maximal activity was obtained at pH 4 (0.05 M) acetate buffer with *S. europaea* and pH 3 (0.05 M) citrate buffer with *Su. novakii*. There was no measurable side activities in fermentation broths of *Su. novakii*. β-xylosidase activity of 0.5 U/mL was obtained from *S. europaea* fermentation's cell-free supernatant.

The wide-type yeasts of *S. europaea* and *Su. novakii* are proved to be promising candidates for xylanase production, thus opening a new chapter in yeast-biotechnology.

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ENANTIOCOMPLEMENTARY BIOREDUCTION OF 1-(ARYLSULFANYL)PROPAN-2-ONES

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In this study, enantiocomlementary bioreduction of 1-(arylsulfanyl)propan-2-ones **2a-e** were investigated using whole cell forms of four wild-type yeast strains[1] (WY1: *P. carsonii*, WY2: *L. elongisporus*, WY4: *C. norvegica* and WY12: *C. parapsilosis*) and two different recombinant alcohol dehydrogenases from *Lactobacillus kefir*[2] (LkADH) and *Rhodococcus aetherivorans*[3] (RaADH) as biocatalysts. The bioreductions proceeded in all cases with excellent enantioselectivity (ee > 99%) and resulted in the formation of enantiopure alcohols (either enantiomer of **1a-e**) with good to excellent conversions. The most efficient enantiocomplementary biocatalysts were the whole-cell forms of WY12 and LkADH producing (*S*)- and (*R*)-enantiomers of the product alcohols **1a-e**, respectively.The bioreductions of substituted 1-(arylsulfanyl)propan-2-ones with these biocatalysts at preparative scale yielded the expected enantiopure forms of the chiral alcohols (*S*)- or (*R*)-**1a-e** in moderate to good yields. Thus, our study offers highly efficient methods to produce sulfur-containing chiral alcohols, (*S*)-**1a-e** with *C. parapsilosis* (WY12) and (*R*)-**1a-e** with *L. kefir* alcohol-dehydrogenase-containing *E. Coli* whole-cells (LkADH) at preparative scale. This study with the aid of LkADH is the first report on preparative scale (*R*)-selective bioreduction from the corresponding ketones leading to enantiopure β-hydroxysulfides.

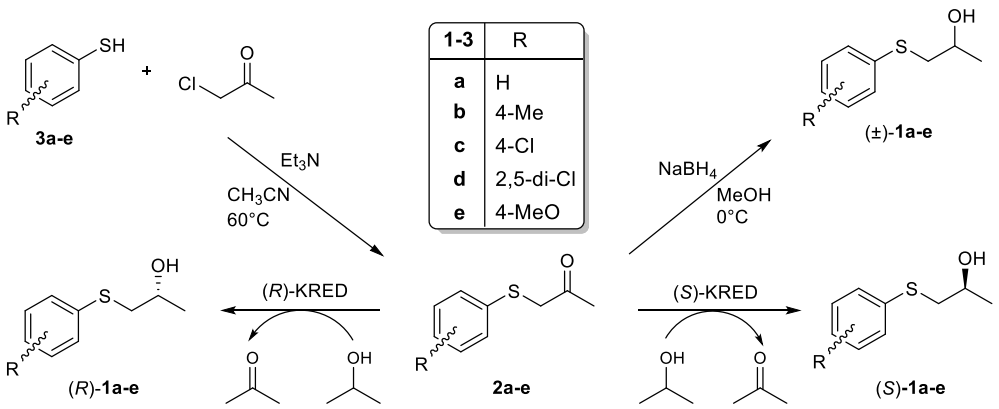

*Figure 1. Synthesis of 1-(arylsulfanyl)propan-2-ones 2a-e and their chemical and bioreduction to racemic and optically active 1-(arylsulfanyl)propan-2-ols [(±)-**1a-e**, (S)-**1a-e**, (R)-**1a-e**]*

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CHANGES IN GENE EXPRESSION AND RNA PROCESSING INDUCED BY THYMIDYLATE SYNTHASE INHIBITORY DRUGS

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Thymidylate synthase (TS) inhibitory chemotherapeutic drugs perturb the cellular dUTP/dTTP ratio by preventing the efficient formation of the DNA precursor dTTP, thus dUTP is used during DNA synthesis, and great amounts of uracil appears in the genome [1]. Uracils are errors in the DNA and can be efficiently processed by the base excision repair (BER) initiated by the uracilDNAglycosylases (UDGs) [2]. The druginduced elevated genomic uracil and dUTP levels lead to hyperactive futile cycles of the BER, which finally leads to thymineless cell death. Interestingly, when the main UDG is inhibited the TS inhibitory drug treatments still lead to cell death, suggesting that these drugs might have some additional mechanisms of action. To better understand the underlying processes during TS inhibitory drug treatments we have analysed the total RNA set of HCT116 cells with different DNA-repair capacities after 48 h treatment with 5-fluoror-2'-deoxyuridine (5FdUR) a covalent inhibitor of TS, or with raltitrexed (RTX) a competitive inhibitor of the same enzyme. We have found many differentially expressed genes in the protein coding group, for example p53 pathway is more affected in case of 5FdUR treatment than during RTX treatment, while in RTX treated cells the upregulation of components involved in RNA processing is stronger than in case of 5FdUR treatment. We have observed enlarged and round nuclear speckles after 48 h drug treatment with immunocytochemistry which appeared more evenly in case of RTX treatment than in case of 5FdUR treatment. Nuclear speckles are phase separated nuclear organelles which contain many proteins involved in RNA transcription, splicing and RNA maturation [3], and it is known that inhibition of RNA polymerase or splicing leads to enlarged and round speckles [4], which eventually suggests altered RNA processing in our case as well, serving additional information of these drugs.

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[4] Lamond AI, Spector DL. Nat Rev Mol Cell Biol. 2003 Aug;4(8):605-12.

Enzymatic interesterification of sunflower oil to biodiesel in a solvent-free process

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In recent years, the production of biodiesel has had a high interest because of its sustainability and environmentally-friendly in comparison with fossil fuel[1,2]. The interesterification reaction for biodiesel production has proved to be the best reaction, especially with enzyme catalysts because it overcomes the problem of glycerol production and inhibition of enzymes[3,4]. Where in this reaction, the enzyme can be used as a catalyst for a longer time without a high reduction of enzyme activity[4]. In this study, biodiesel was produced from sunflower oil by interesterification reaction in a batch process using Novozyme 435 as a catalyst. The reaction conditions used here were temperature (30, 50, and 70 ^{o}C) and methyl acetate to sunflower oil molar ratio (3,9,15, and 30) in 5 h at atmospheric pressure. The better conditions to get a higher yield, which is 63.77 wt%, were 50 ^{o}C, and the molar ratio of methyl acetate to oil was 15 in 5 h. The batch results were used to design a continuous process for biodiesel production and the results showed the expected yield (26.1 wt%) at the best conditions is very close to the measured yield (25.8 wt%) at the same conditions.

Figure 1. Biodiesel life cycle[2]

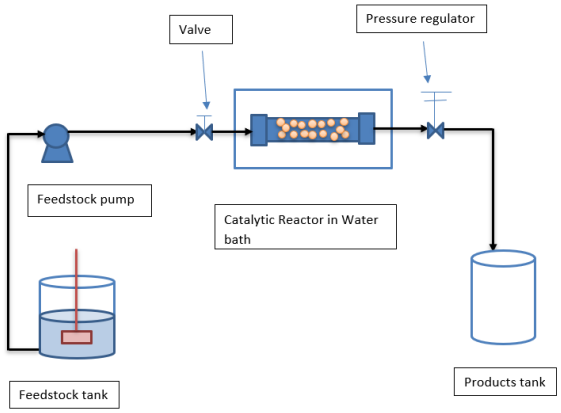

Figure 2. Enzymatic biodiesel continuous process.

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UTILIZATION OF WILD YEASTS IN THE BIOREDUCTION OF BUTAN-2-ONES WITH (PARTIALLY) SATURATED HETEROCYCLIC SIDE CHAINS

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This study explored the bioreduction of butan-2-ones with (partially) saturated heterocyclic side chains in batch mode using wild-type yeast strains and commercially available baker's yeast (*Saccharomyces cerevisiae*). A total of twelve wild yeast strains and baker's yeast were screened for ketoreductase activity on a series of five prochiral ketones. Among the yeast strains tested, *Candida parapsilosis* (WY12) exhibited the most favourable performance, resulting in the corresponding enantiopure alcohols with good to excellent conversions (83-99%) and high selectivities (ee >99%) in the case of several substrates. Other strains, such as *Pichia carsonii* (WY1) and *Lodderomyces elongisporus* (WY2), also showed promising ketoreductase activities, yielding high conversions and selectivities for certain substrates.

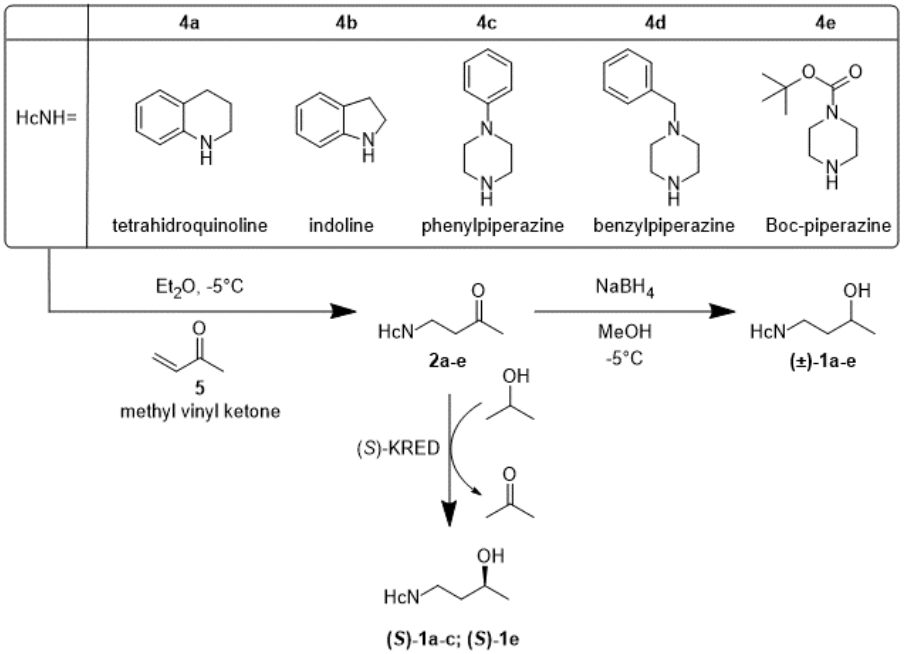

Figure 1. Synthesis of butan-2-ones with (partially) saturated heterocyclic side chains ***2a-e*** *and their chemical and bioreduction to racemic and optically active alcohols (±)-****1a-e*** *and (S)-****1a-c****; (S)-****1e*** *respectively*

Following the initial screening, *C. parapsilosis* cells were immobilized in the form of calcium, zinc, nickel and copper alginate beads. This whole-cell immobilization technique enabled effective recycling, with the biocatalyst retaining considerable activity over multiple cycles, indicating its potential for sustainable biocatalytic applications. Additionally, the study explored the scalability of these bioreductions, with immobilized *C. parapsilosis* delivering promising results. Furthermore, the use of immobilized cells simplified the work-up process and resulted in chiral alcohols with similar conversions and selectivities to those observed in the screening reactions.

EFFECT OF CARRIER MORPHOLOGY ON METAL ION AFFINITY IMMOBILIZATION—A CASE STUDY WITH PHENYLALANINE AMMONIA-LYASE

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In this work, six different substrates were modified and used for the selective immobilization of *Pc*PAL. Three commercially available polymeric supports: Resindion HA 403/S, EA 403/S and ChiralVision T2-150 (HA, EA, CV), two silica-based carriers: MAT 540 and Geduran 60 (MAT, G-Si), and magnetic nanoparticles synthesized by us (MNPs). The CV was modified with ethylenediamine, the G-Si and MNP with aminosilane. Metal ion complexing groups were created from EDTA-dianhydride by reacting it with surface amino groups, then cobalt(II) was complexed on them. The immobilization was performed directly from the crude cell lysate, and all the supports were used at their highest capacity.

The immobilized biocatalysts were tested in the ammonia elimination reaction of L-phenylalanine. The MNP- (21.6 U/g), MAT- (17.7 U/g) and HA-based (16.6 U/g) biocatalysts have sufficiently high activity. The CV has highest capacity but the lowest activity, while the HA produced the best activity among the polymers with the lowest binding enzyme content. It is advantageous if there is sufficient space for conformational mobility, as immobilized enzymes are less crowded and attached to the surface through longer chains.

To compare the efficiency of the different carriers thermal and storage stability tests were performed. The best conversions were earned at 50°C, where a significant activity loss has already occurred, except for the MNP. Long-term stability was tested at -20 °C, 4 °C and room temperature. The highest activity losses were observed in the case of the silica-based supports (MAT and G-Si), while at room temperature the Resindion polymers (EA, HA) and MNP retained 50% of their initial activity by the end of 90 days.

The HA and MNP biocatalysts were used in 5 batch reaction cycles for the resolution of racemic phenylalanine and thienylalanine. Using the HA catalysts, after three reaction cycles, a conversion of 35-40% was consistently achieved for both substrates. In contrast, the conversion of MNP catalysts has decreased cycle-by-cycle. Ammonia addition reactions were carried out in 6 M ammonia solution, which resulted in excellent conversions and enantiomeric excesses (>95%) over 5 repeated cycles. For both types of substrates, the HA biocatalysts preserved higher activity in both reaction directions, which can be explained by the protective effects of the pores, in contrast to the MNP where the enzyme is located on the surface.

AQUEOUS MULTICOMPONENT REACTIONS – STEP-BY-STEP TO BIOCATALYSIS

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HNL enzymes are structurally unrelated (non-homologous), isofunctional, plant enzymes found in plant cell walls, which are used by many plants as defence in their defence mechanisms. Their function is to generate α-cyanohydrins by reacting carbonyl-electrophilic centres with the nucleophilic cyanide, but have also been observed to catalyze other nucleophilic reactions. Therefore, they are popular not only for the synthesis of α-cyanohydrins, but also for the formation of β-nitroalcohols in Henry reaction. In such cases, a nitroalkane is coupled as a nucleophile to the electrophilic oxo compound.

In collaboration with the BME Bioorganic Chemistry Research Group, we expressed two types of HNL enzymes, in particular HbHNL and AtHNL that are able to form *S* and *R* cyanohydrins, respectively. Next, aldehydes and ketones were subjected to the Strecker reaction with benzylamine and potassium cyanide in citrate buffer. We have investigated several conditions e.g. temperature of the reaction mixture, reaction time, reagent ratio, pH, buffer concentration and reaction volume, and concluded that the determining factors are substrate and buffer concentration together with the appropriately chosen pH. The optimized reaction was used to prepare twenty 2-(benzylamino)-2-phenyl acetonitriles including biologically important precursors of alpha amino acids (glycine, alanine, methionine, tryptophan etc.). Based on this knowledge, we also extend the reaction to metyl- and ethyl imines that would be small enough to participate in enzymatic reactions as well. Finally, we aimed to perform Strecker and Henry reactions in the presence of the produced HNL enzymes leading to enantiopure cyanohydrins or β-nitroalcohols.

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INSIGHTS INTO ZEARALENONE DEGRADING ENZYMES

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Mycotoxins are secondary metabolites primarily produced by fungi and molds. Grain contamination reduces food and feed quality and causes major losses to the agricultural industry. Mycotoxins produced by *Fusarium sp*., such as zearalenone (ZEN), lead to adverse health issues in humans and livestock upon acute and chronic dietary exposure. Zearalenone's estrogen-like structure disrupts hormone homeostasis, causing reproductive health problems. [1]

Currently, various methods are known for the detoxification of zearalenone. Physical methods, like heating or chemical degradation, are widely used. However, they require harsh conditions, they could damage the nutritional value of the feed and the full decontamination is often incomplete. Immobilizing mycotoxin on adsorbents enables the use of contaminated grain for animal feed. Enzymatic inactivation possesses an alternative solution, by degrading the mycotoxin to its non-toxic form, with excellent repeatability, a simple and environmentally friendly operation, and high safety while keeping the nutritional value of the grain. [2]

Lactone hydrolases attracted significant attention because they can degrade the mycotoxin to a non-toxic form by hydrolyzing the lactone bond in the molecule *(Figure 1.)*. Our research focuses on the improvement of bacterial lactonases isolated from *Rhodococcus erythropolis* and *Streptomyces coelicoflavus*. These enzymes show better substrate affinity and turnover number, than the widely known fungal α/β hydrolases, like ZDH101 from *Clonostachys rosea*. [3] We aim to further improve the stability and activity of these proteins with protein engineering to make them applicable as a feed additive for livestock. Crystallizing these proteins with substrate analogs can reveal their mechanism of action. Additionally, it makes possible the rational design of active site mutations to enhance the enzyme activity towards various mycotoxins.

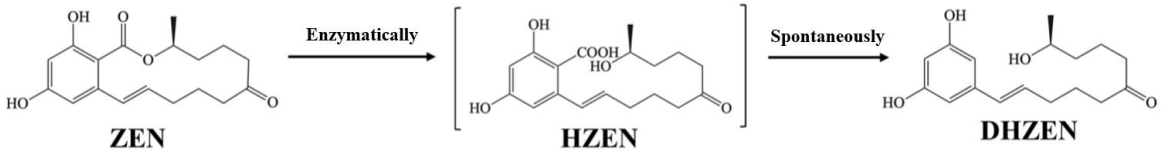

Figure 1. The enzymatic detoxification of Zearalenone.

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ROLE AND EFFECT OF URACIL METABOLISM ON ZEBRAFISH EMBRYONIC DEVELOPMENT

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Uracil base is a building block of RNA. Its presence in DNA in most cases is mutagenic, which needs to be repaired. Uracil can enter DNA in two ways. On the one hand through the oxidative deamination of cytosine basis, on the other hand DNA polymerases can't distinguish between dUTP and dTTP, so if the dTTP:dUTP ratio is inappropriate in the cells, dUTP will be incorporate into the DNA [1]. dUTPase and Uracil-DNA glycosylase (UNG) are the main repair enzymes which responsible for keeping the genomic uracil level low. dUTPase catalyse the hydrolysis of dUTP to dUMP thus reducing the cellular dUTP:dTTP level, at the same time providing a dUMP substrate for de novo thymidylate biosynthesis [2].

Our experiments investigate the early stages of embryonic development. As a model organism, we used zebrafish, which is nowadays very popular among researchers because it is easy to keep, requires little cost, develops quite rapidly and shows good survivability against various post-fertilization procedures [3]. In previous results we found high uracil-DNA and dUTP levels before the maternal to zygotic transition (MZT). During MZT, the zygotic genome begins transcription, maternal mRNAs are actively cleared, and developmental control is transferred to the nucleus [4]. To find out what is the main reasons for these high levels we measured the mRNA expression levels of genes involved in the uracil metabolism. In addition we have started to measure the dUTPase levels in the embryos of different ages, but these measurements need more optimisation. We have also performed microinjection experiments to figured out what happens when we reduce the dUTP levels, by microinjecting active and inactive form of the dUTPase enzyme into fertilised oocytes. We were also curious to see if there is any pattern in the uracilation of the DNA, to examine that we made U-DNA sequencing using immune precipitation sample preparation.

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CHANGES OF GLUTEN PROTEIN COMPOSITION DURING SOURDOUGH FERMENTATION IN RYE FLOUR

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Celiac disease (CD) is an immune mediated condition triggered by gluten consumption. Yet, the only treatment for CD patients is a strict life-long gluten free diet. For widening the range of safely consumable food for this population, various gluten protein eliminating techniques were examined including traditional technologies like sourdough fermentation. Despite, more studies stated that gluten proteins are partially degrading due to fermentation processes during sourdough making it is still unclear whether the CD toxic epitopes are affected. Most of the available studies examined wheat and barley because of the importance of bread making and beer industry, however, very few studies are available about rye gluten protein degradation during sourdough fermentation.

The aim of our work was to examine the effect of controlled sourdough fermentation on the gluten content with commercially available sourdough starters in rye flour. Various starters were selected including fresh and dried wheat, dried rye, pure lactic acid bacteria (LAB) culture mixture and gluten free (buckwheat and rice based) starters. Gluten content was determined by R5 ELISA according to the Codex Alimentarius recommendations, protein composition was further characterised by size exclusion high performance chromatography (SE-HPLC) and reversed phase high performance chromatography (RP-HPLC)[1].

Despite significant changes in protein size distribution and decrease in high-molecular-weight (HMW)-, ω-, γ-75k- and γ-40k- secalin types during the whole process confirmed by HPLC methods, the R5 ELISA results showed increased gluten content after fermentation. The incomplete degradation of gluten and that the celiac toxic epitopes are supposedly stay intact during sourdough fermentation process may explain our results. As part of the R5 antibody binding (and CD toxic) epitopes are supposed to be unavailable in the native form of the secalins, they might be released and become accessible for the antibodies in the smaller protein fragments.

In comparison of the effect of different starter cultures the results show that the different microbiological composition of the sourdough samples might be the reason of the differences in the protein degradation.

Results highlight the importance of the deeper investigation of the effects of gluten protein degradation on CD toxicity and the standardization of the fermentation process.

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