MŰEGYETEM 1782

1st George Olah Conference

Innovative research at the Faculty of Chemical Technology and Biotechnology, BME

23 September 2019

BME VEGYÉSZMÉRNÖKI ÉS BIOMÉRNÖKI KAR
VJA
Varga József Alapítvány
1873

1st George Olah Conference

Program

EVENT DATE: 23th SEPTEMBER 2019

EVENT VENUE: BME CH BUILDING, SZENT GELLÉRT SQUARE 4., CHC14

7^{45}-8^{25} Registration

8^{25} Opening ceremony

ORAL PRESENTATIONS

Chairman: Prof. Dr. László Poppe

8^{30}-9^{00} **Prof. Dr. Romas J. Kazlauskas** – Changing enzyme function: esterases to hydroxynitrile lyases – Invited lecturer from University of Minnesota

9^{00}-9^{30} **Dr. Kinga Nyíri** – Structural background of pathogenicity island regulation in *Staphylococcus aureus* **–** George Olah Prize winner in 2018

Chairman: Prof. Dr. György Keglevich

9^{30}-9^{55} **Dr. József Kupai** – Asymmetric synthesis with cinchona-based cyclodextrin organocatalysts in a synthesis separation integrated continuous flow reactor – Invited lecturer from Department of Organic Chemistry and Technology

9^{55}-10^{10} **Gergő Dargó** – In vitro, tissue-specific permeability models in lead optimization

10^{10}-10^{25} **Zsófia Molnár** – Immobilized whole-cell transaminase biocatalysts for continuous-flow kinetic resolution of amines

10^{25}-10^{40} **Zsófia Bata** – Structural dynamics of the catalytic inner lid loop of MIO enzymes

10^{40}-11^{00} Coffee Break

Chairman: Prof. Dr. Beáta G. Vértessy

11^{00}-11^{25} **Dr. Áron Németh** – Ten years in development of lactic acid fermentation technology – Invited lecturer from Department of Applied Biotechnology and Food Science

11^{25}-11^{50} **Dr. Benjámin Gyarmati** – Poly(amino acid) derivatives for gellable formulations – Invited lecturer from Department of Physical Chemistry and Materials Science

11^{50}-12^{05} **Martiz Chalen Jose Alejandro** – Synthesys of ceramic materials for Preparation of nanocomposites

12^{05}-12^{20} **Rawan Abukharian** – Interaction of MLL4 with long non-coding RNAs

12^{20}-12^{35} **Borbála Tegze** – Photoinduced processes of dyes in mesoporous titania sol-gel coatings

12^{35}-13^{40} Lunch Break – Poster Session (CH 201)

Chairman: Prof. Dr. László Nyulászi

13^{40}-14^{10} **Dr. Balázs Volk** – Cooperation between the Faculty of Chemical Techology and Biotechnology of BME and Egis Pharmaceuticals Plc – Invited lecturer from Egis Zrt.

14^{10}-14^{35} **Dr. Julianna Oláh** – Accurate modelling of biochemical systems: How far can we go? – Invited lecturer from Department of Inorganic and Analytical Chemistry

14^{35}-14^{50} **Réka Mokrai** – Investigation of heteroelement containing conjugate systems

14^{50}-15^{05} **Flóra Horváth** – Nucleating agents with dual nucleating ability in isotactic polypropylene

15^{05}-15^{30} Coffee Break

Chairman: Prof. Dr. Krisztina László

15^{30}-15^{55} **Dr. Edit Székely** – Innovative applications of pressurized carbon dioxide – Invited lecturer form Department of Chemical and Environmental Process Engineering

15^{55}-16^{10} **Ayshan Khalafli** – Process Design and Automation in brownfield projects in example of Methanol Production Company with Agile approach

16^{10}-16^{25} **Zsolt Benedek** – Quantum chemistry aided design of biomimetic complexes for atmospheric pressure ammonia synthesis

16^{25} Closing ceremony

POSTERS

1. **Ahmed Mohamed Abdelhamied Rozza:** Mapping pathways of diatomic ligands migration into H-NOX domains as a model of sGC activation
2. **Amer Aljamal:** Effect of Phosphorus Flame Retardants on the Properties of Bio-based Epoxy Resins
3. **Aysel Mammadova:** The effect of cationic side groups on the aqueous stability of thiolated polyaspartamides
4. **Balázs Decsi:** Application of a biomimetic oxidation system in homogeneous and heterogeneous, continuous flow conditions
5. **Bence Varga:** Resolution of secondary phosphine oxides and H-phosphinates as P-stereogenic intermediates
6. **Dániel Vadas:** Physical and Chemical Foaming of Flame Retarded Poly(lactic acid)
7. **Edina Jaksics:** Rheological behavior of wheat lines with altered amylose content
8. **Emese Farkas:** Continuous flow chemoenzymatic cascade reactions aiming diastereopure amine synthesis
9. **Emese Pregi:** Effect of fiber content on the properties of hybrid PP composites containing lignin and flax
10. **Erzsébet Madaras:** Investigation of Ligand Exit Paths in the MIO Enzyme Family with the Help of Random Acceleration Molecular Dynamics (RAMD)
11. **Eszter Supala:** High-throughput electrosynthesis and read-out of protein MIPs with microelectrospotting-coupled fluorescence imaging
12. **Éva Pusztai:** Process capability indices when the usual assumptions fail: a tolerance interval approach
13. **Gergely Koppány:** Development and in vitro testing of covalent inhibitors targeting oncogenic KRAS mutants
14. **Gergely Rácz:** Knock-out of dUTPase in mice leads to early embryonic lethality and is not rescued by concurrent knock-outs of both UNG and SMUG
15. **Gergő Dargó:** In vitro, non-cellular permeability assay to predict corneal absorption of APIs using the PAMPA model
16. **József Kozma:** Comparison of Conducting Polymers and High Capacitance Materials for the Fabrication of Solid-Contact Ion-Selective Electrodes
17. **Kata Decsov:** Development of bioepoxy resin microencapsulated ammonium-polyphosphate for flame retardancy of polylactic acid
18. **Katharina Preißinger:** Dissection of *Plasmodium falciparum* developmental stages with multiple imaging methods

19. **Kinga Juhász:** Heterogeneous catalytic 1,4-addition reaction in the presence of supported metal catalysts
20. **Krisztina Lévay:** Selective heterogeneous catalytic hydrogenation of nitriles to primary amines over palladium
21. **László Ferenc Simon:** Sequence independent quantification of the surface density of DNA probes on DNA microarrays by SPRi
22. **Lu Cui:** Physical Ageing of Poly(Lactic acid): Factors and Consequences for Practice
23. **Marietta Szentmiklóssy:** Improvement of bioactive component composition in wheat with breeding
24. **Marwa Ahmed:** Optimization of the polymerization conditions of thermoresponsive poly(NIPAm-co-AAc-co-TBAm) microgels for the molecular imprinting of lysozyme
25. **Miklós Bosits:** Development of a lab-scale continuous crystallization process with turbidity-based safety and control method
26. **Muriel Józó:** Degradation of PLA fibers containing an enzyme
27. **Nikolett Nagy:** Tissue- and development-specific expression pattern of dUTPase isoforms
28. **Péter Kisszékelyi:** Cinchona-decorated cyclodextrin - a recyclable organocatalyst
29. **Zsófia Bognár:** Enzymatic methods for microRNA detection with surface plasmon resonance imaging

Abstract

George Olah Prize winner in 2018

STRUCTURAL BACKGROUND OF PATHOGENICITY ISLAND REGULATION IN *STAPHYLOCOCCUS AUREUS*

Kinga Nyíri[*,1,2] and Beáta G. Vértessy[1,2]

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[2] *Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest 1117, Hungary 2*
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Staphylococcus aureus pathogenicity islands (SaPIs) spread virulence within bacteria by hijacking the capsids of specific so-called helper phages, while also interfering with phage propagation. The identification of the helper phages by SaPIs happens *via* direct protein-protein interaction of SaPI master life cycle regulator protein, Stl and a specific phage protein. Interestingly the same SaPI regulator Stl protein can target structurally unrelated proteins with identical function[1]. In case of SaPIbov1-Stl it has been shown that it binds to the structurally highly different all-α-helical dimeric and β-pleated trimeric dUTPases[2,3], and the interaction leads to inhibiton of enzymatic function in both cases. We set out to investigate how this Stl protein can recognize this two distinct types of dUTPases.

Based on a range of biochemical and biophysical methods, we created a structural model of a trimeric dUTPase–Stl complex[4] and by using hydrogen deuterium exchange mass spectrometry (HDX-MS) we also identified protein regions directly involved in the complex formation of Stl with both types of dUTPases[5].

Trimeric dUTPases form DUT_3Stl_2 and DUT_3Stl_3 complexes with Stl, while in the case of dimeric enzyme the complex is a DUT-Stl heterodimer[3,4,5]. Based on our results Stl binds directly to the active site of trimeric dUTPases and it acts as a competitive inhibitor of these enzymes[3]. Direct evidence from native mass spectrometry shows that the mechanism of inhibition of dimeric dUTPases by Stl is different from that of trimeric dUTPases. In case of dimeric dUTPases reduction of the enzyme activity results from disruption of the active site architecture, which resides at the dimer interface of the protein[5].

Our HDX-MS results suggested highly different interaction surface of Stl with the dimeric and trimeric dUTPases[5]. We found that the 98Y–113Y segment of Stl protein has a major contribution in the interaction with trimeric dUTPases, while peptides from the 60 residue-long region situated at the very C-terminal part of the Stl sequence play a key role in the binding of dimeric dUTPases. Similar results were reached by crystallization of the complexes reinforcing the conclusions of both studies[6]. Our results open a way towards design of species specific dUTPase inhibitors. What has driven this SaPI to evolve to target key enzymes of uracil metabolism is still an open question.

[1] Bowring, J. et al. *Elife* **2017**, *6*, 1–23.
[2] Hill, R.L.L. et al *J. Mol. Biol.* **2016**, *428*, 142–152.
[3] Szabó, J.E. et al *Nucleic Acids Res.* **2014**, *42*, 11912–20.
[4] Nyíri, K. et al *Sci. Rep.* **2018**, *8*, 4326.
[5] Nyiri, K. et al *Biomolecules* **2019**, *9*, 488.
[6] Rafael Ciges-Tomas et al *Nat. Commun.* **2019**, *10*, 3676.

Abstracts

Oral presentations of students from the George Olah Doctoral School

IN VITRO, TISSUE-SPECIFIC PERMEABILITY MODELS IN LEAD OPTIMIZATION

Gergő Dargó*; Anna Vincze and György T. Balogh

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The permeability across biological membranes and tissue barriers has major influence on oral absorption and bioavailability of drug molecules. To predict permeability of drugs during different stages of the drug discovery several models exist, with differing complexity, throughput, accuracy and costs. In vitro non-cellular models provide a high-throughput, robust and cost-effective tool for permeability prediction in the early stages of drug discovery. Since its development in 1998[1], the parallel artificial membrane permeability assay (PAMPA) has been widely used for measurement of permeability in the gastrointestinal tract. Over the last two decades, by modification and improvement of the original PAMPA model, novel tissue-specific methods have also been developed to predict the blood-brain barrier (BBB)[2], buccal[3], skin[4] penetration of drugs.

Our research aimed to investigate permeability efficiency of several urea and amide type fragments based on experimental lipophilicity and permeability values using the PAMPA-BBB method[2]. We also developed an in vitro non-cell-based, high-throughput screening model for the prediction of corneal permeability as a novel alternative to the expensive, and time-consuming ex vivo and in vitro cell-based models[5].

[1] Kansy, M.; Senner, F.; Gubernator, K, *J. Med. Chem.*, **1998**, 41, 1007–1010.
[2] Müller, J.; Esső, K.; Dargó, G.; Könczöl, Á.; Balogh, Gy. T. *Eur. J. Pharm. Sci.*, **2015.** 79, 53–60.
[3] In vitro dissolution-permeation evaluation of an electrospun cyclodextrin-based formulation of aripiprazole using µFlux. *Int. J. Pharmaceutics*, **2015**, 491, 180-189.
[4] Sinkó, B.; Garrigues, T. M.; Balogh, Gy. T.; Nagy, Zs. K.; Tsinman, O.; Avdeef, A.; Takács-Novák, K. *Eur. J. Pharm. Sci.*, **2012**, 45 (5), 698–707.
[5] Dargó, G., Vincze, A., Müller, J., Kiss, H. J., Nagy, Z. Zs., Balogh, Gy. T., *Eur.J. Pharm. Sci.*, **2019**, 128, 232-239.

IMMOBILIZED WHOLE-CELL TRANSAMINASE BIOCATALYSTS FOR CONTINUOUS-FLOW KINETIC RESOLUTION OF AMINES

Zsófia Molnár[1*]; Emese Farkas[1] and László Poppe[1,2,3]

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Enantiopure amines are essential chiral building blocks for the synthesis of a wide variety of active pharmaceutical ingredients. Chemical synthesis of these compounds usually employs transition metal catalysts of relatively high toxicity, and may require harsh reaction conditions. In recent years, there is a growing interest in transaminases (TAs), which offer a sustainable alternative to these synthetic chemical processes. For the sustainable, industrial production of enantiopure amines the intensification possibilities offered by immobilized TA biocatalysts and by the continuous-mode operations are needed.

This study aimed for the preparation of robust, stereoselective TA biocatalysts capable of operating under batch and continuous-flow conditions to produce enantiopure amines. *E. coli* cells containing overexpressed transaminases of various selectivities and hollow silica microspheres as supporting agent were immobilized by an improved sol-gel process. The immobilized cells proved to be easy-to store, cheap and durable biocatalysts, and were applied successfully in the continuous kinetic resolution of racemic amines. By using the most suitable immobilized ω-transaminase biocatalysts, this study describes the first transaminase-based approach for the production of both pure enantiomers of 1-(3,4-dimethoxyphenyl)ethan-1-amine.

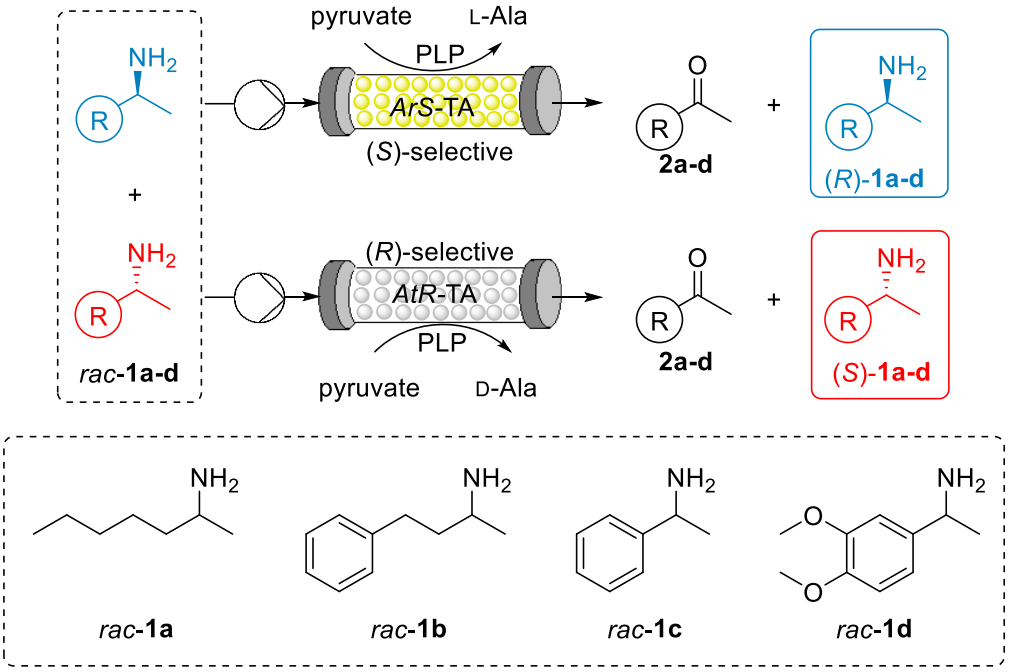

Figure 1. Scheme of the continuous-flow kinetic resolution of amines.

STRUCTURAL DYNAMICS OF THE CATALYTIC INNER-LOOP OF MIO ENZYMES

Zsófia Bata[1,2*]; Erzsébet Madaras[1]; Bence Molnár[1,2]; Andrea Varga[3]; Ibolya Leveles[2,4]; Renzhe Qian[5]; Friedrich Hammershmidt[5]; Csaba Paizs[3]; Beáta G. Vértessy[2,4]; László Poppe[1,3]

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[2] *Institute of Enzymology, HAS-Research Center of Natural Sciences.*
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[4] *Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics.*
[5] *Institute of Organic Chemistry, University of Vienna.*
* *Corresponding author: bata.zsofia@mail.bme.hu*

Aromatic amino acid ammonia-lyases and 2,3-aminomutases contain the post-translationally formed prosthetic 3,5-dihydro-4-methylidene-5H-imidazol-5-one (MIO) group [1]. The so-called MIO-enzymes may be used for the stereoselective synthesis of enantiopure α- or β-amino acids, making these chemical processes more environmentally friendly and more affordable. Although, a number of structures are available in the PDB for MIO enzymes, the catalytically important inner-loop [1] is either missing or is in a catalytically inactive conformation eukaryotic phenylalanine ammonia-lyase (PAL) structures. Lack of protein structure in a catalytically competent conformation impeded understanding the PAL mechanism and the key reasons for the enantioselectivity of the enzyme.

Our recently determined crystal structure complexed with a covalently bound 2-methylidene substituted phosphonic acid inhibitor [2] at 1.9 Å resolution comprising the catalytically essential Tyr-loop in a catalytically competent conformation (PDB: 6F6T) allowed a detailed structural and mechanistic analysis of parsley PAL (*Pc*PAL). The high resolution *Pc*PAL structure compared to the previously determined structure of *Taxus* phenylalanine PAM (*Tc*PAM) enabled straightforward molecular dynamics studies of the tetrameric proteins. Results suggest that small protein motions are sufficient for ligand access/exit and that multiple possible ligand access/exit paths exit in PAL and PAM. Furthermore, these access/exit paths seem to be conserved through the whole MIO enzyme family, except in prokaryotic (*S*)-PAMs. Single residue mutations to the prokaryotic (*S*)-PAM counterparts aimed to close the ligand access paths and create the amino mutase activity.

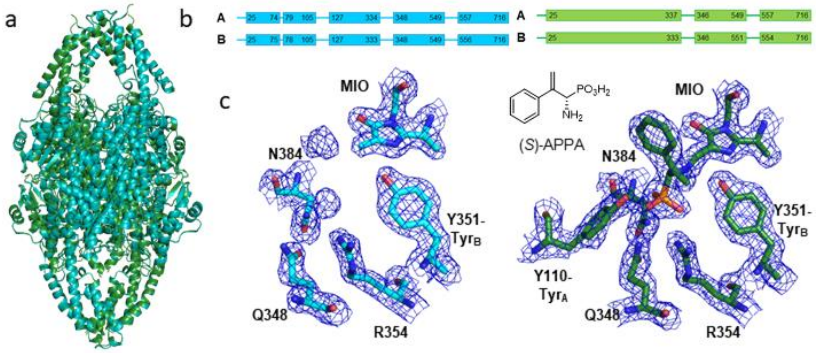

Figure 1. Global features of the PcPAL structures. ***a*** *Overlay of the cartoon representation of the two PcPAL structures (6H2O cyan, 6F6T green).* ***b.*** *Schematic representation of the residues modeled in the structures.* ***c.*** *Active site electron densities for the catalytic residues and the carboxylate binding residues.*

[1] Poppe, L., Rétey, J. *Angewandte Chem.* **2005**, 24:3668-3688.
[2] Bata, Z.; Qian, R.; Roller, A.; Horak, J.; Bencze, L. C.; Paizs, C.; Hammerschmidt, F.; Vértessy, B. G.; Poppe, L. *Adv. Synth. Catal.* **2017**, *359*, 2109–2120.

SYNTHESYS OF CERAMIC MATERIALS FOR PREPARATION OF NANOCOMPOSITES

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Ceramic composite materials (CMC), are tailored to possess superior properties (mechanical, thermal, corrosion/oxidation resistance), due to effect of a reinforcement which can enhance the positive properties of ceramics matrix.[1] Currently, ceramic materials which serve as precursors for composites, are prepared by sophisticated routes such as electrospinning, hydrothermal, sol-gel and plasma synthesis, with the aim to obtain materials at a less-explored scale (submicron and nanoscale) then are fervently used at industrial scale (micron size particles) at this time. ZrC has been considered to be a promising candidate as a matrix material, because of characteristics such as high melting temperature (3500°C), hardness (28.7 GPa), low density and thermal conductivity.[2] Reinforcements can improve the properties of ZrC ceramics like microstructural properties, or oxidation and thermal shock resistance, by avoiding the diffusion of oxygen and forming oxide film in the surface correspondingly.

Mo-based ceramics materials (MoO_3, MoC) are well known by their uses in a wide range of fields like catalysis, refractory, protective and reinforcement material. Reinforcing phases for CMCs can be discontinuous phases (particles, whiskers, or short fibers) and continuous fibers. Almost all of Mo-based ceramic fibers are mainly obtained by sol-gel synthesis. However, these methods have the disadvantage of producing fibers with a relatively low length/diameter (L/D) relationship. Otherwise, electrospinning (ELS) is a versatile process for the production of fibers, which produce the highest L/D relationship, however this is a poorly explored field for Mo-based ceramic fibers.[3]

In the current work, we present the preparation of ZrC nanopowder and Mo-based ceramic fibers for its further use as matrixes and reinforcement in the preparation of nanocomposites. Two different methods are used for ZrC nanopowders synthesis like sol-gel and plasma reactions. Both cases use Zirconium propoxide as Zr source. The main differences in the products lie in the control of the particle sizes, cost and morphology. On the other hand, Mo-based ceramics fibers are obtained by ELS (Figure 1), starting from a pH-stable hybrid solution (polymeric base). Later, the obtained fibers are calcined in a range of temperature between 500°C and 850°C, producing different fiber morphologies depending on many parameters along the process.

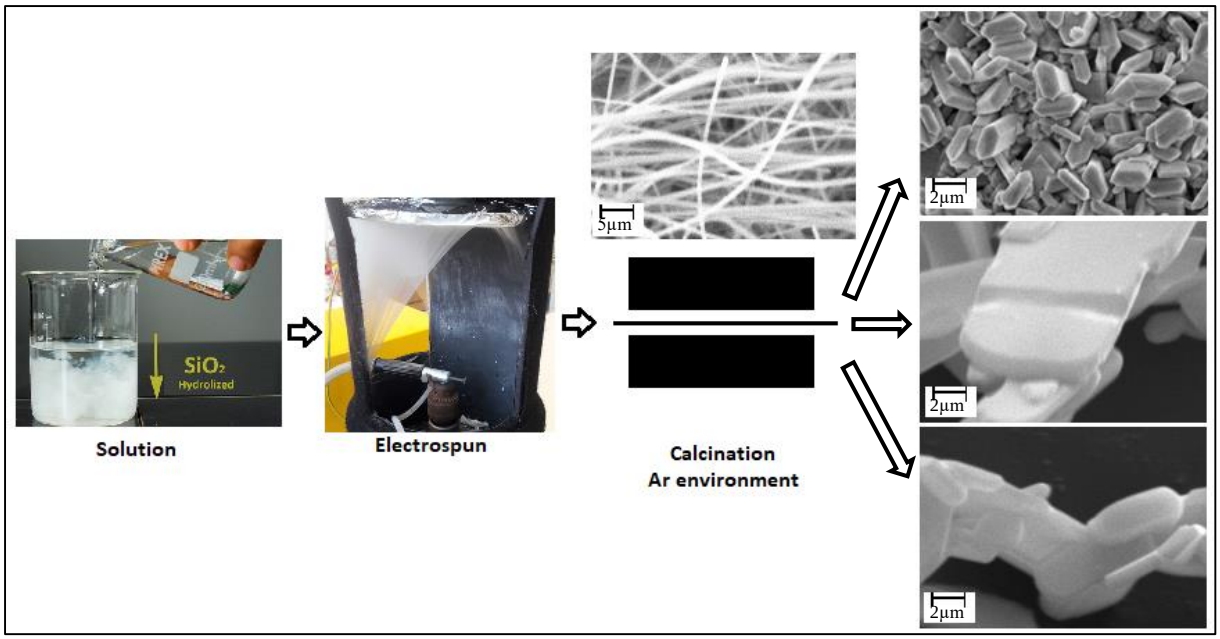

Figure 1. Stages of the Mo-based ceramics fiber formation process.

Finally, the morphologies of ZrC and Mo-based fibers are characterized by scanning and transition electron microscopy (SEM and TEM), while the phase compositions by X-ray diffraction (XRD). Surface chemical compositions are determined by X-ray photoelectron spectroscopy (XPS).

XRD analysis of the sol-gel and plasma synthesized product powders clearly confirmed the formation of ZrC with high conversion rate (95% for sol-gel and 98% for plasma synthesis). According to TEM micrographs, the plasma synthesized ZrC powders had a particle size less then 100 nm. SEM and particle size analyzer reveal that sol-gel produced particles exhibit agglomeration.

SEM characterization of the fibers, before of calcination, exhibit a L/D relationship bigger than 100, with a nominal diameter around 400nm. Final morphology depends of the thermal treatment. But the composition depends exclusively of the calcination environment. At atmospheric condition high presence of MoO_3 was obtained with a low yield due to its sublimation at 750°C. Under Ar environment was obtained MoO_3 together with MoC, with a higher yield because of the conversion is carried out at lower temperatures (590°C) than sublimation.

[1] Zapata-Solvas E., Jayaseelan D.D., Brown P., Lee W.E., J. Eur. Ceram. Soc. 33, (2013) pp 1373-1386
[2] Nisar A., Ariharan S., Balani K., Directions 15 (1) (2015) 55-65.
[3]Hamid E., Rajan J., Seeram R., Materials, 10 (2017). 1238.

INTERACTION OF MLL4 WITH LONG NON-CODING RNAs

Rawan Abukhairan[1*]; Beáta Szabó[1], Nikoletta Murvai[1], Bálint zeder[1], András Micsonai[2], József Kardos[2], Ágnes Tantos[1]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

[2] ELTE NAP, Neuroimmunology Research Group Department of Biochemistry, Eötvös Lorand University, Budapest, Hungary

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Long non-coding RNAs (lncRNAs) are transcribed molecules longer than 200 nucleotides that are not translated to proteins. The heterogeneity of lncRNAs resonates in the diversity of their functions as they can interact with DNA, proteins and other RNAs to participate in processes from transcription, intracellular trafficking to chromatin remodeling.

Histone lysine methyltransferases (HKMTs) induce histone modifications via catalyzing mono-, di-, and tri-methylation of lysine residues[1]. Mechanisms by which histone methylation is regulated remain elusive. One proposed pathway is HKMT-lncRNA interaction: lncRNAs might serve as scaffolds bridging HKMTs to transcription factors and recruit them to the promoter region of the target gene.

In a recent publication[2], we have provided evidences for a so far unrecognized interaction between MLL4 and different lncRNAs (HOTAIR, Meg3 and hNEAT1). MLL4 (Mixed Lineage Leukemia 4) is the methyltransferase subunit of COMPASS-like complexes. It catalyzes mono-methylation of H3K4 and acts as a tumor suppressor. The aim of this study was to prove that not only one COMPASS component, WDR5, is able to bind lncRNAs, but also MLL4. The tested MLL4 region is between 3500 and 3630 of the wild type protein (MLL4-130). This region harbors several cancer-related point mutations. It also has a poly glutamine stretch (polyQ) directly preceded by a predicted RNA-binding region. During the RNA binding experiments phase separation-like behavior of the protein upon binding to the lncRNA $HOTAIR_{440}$ was observed. As MLL4 is the only H3K4 methylase that contains polyQ stretches, phase separation might be a regulatory step specific for this protein. Since it has been suggested that polyQ regions are involved in phase separation of RNA binding proteins[3], we hypothetised that the polyQ region of MLL4-130 might be involved in this phenomenon.

Three mutants of MLL4-130 were designed with the aim to study the effect of polyQ region on protein structure, RNA binding and phase separation. The mutant proteins were cloned and overexpresed in E. coli, then purified using affinity chromatography. Microscale Thermophoresis (MST) and Electromobility Shift Assay (EMSA) were used to study the protein-RNA interactions. Results show that the polyQ region does not dramatically affect the secondary structure of the protein but it might play a role in the protein specificity toward lncRNAs and might induce RNA-mediated phase separation.

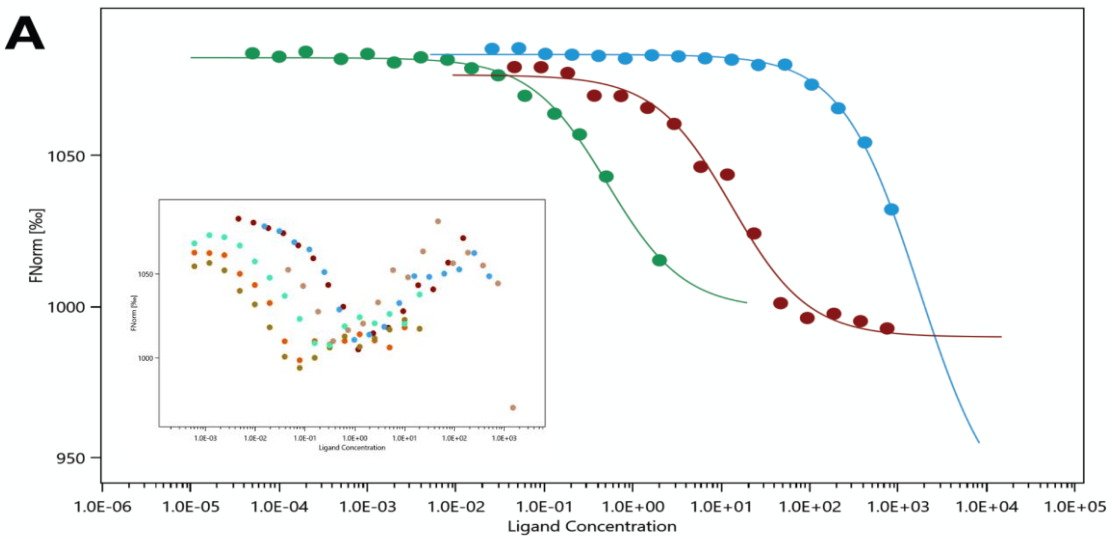

Figure 1. RNA(HOTAIR440) binding detected by MST.MST binding curves of MLL4$_{3500-3630}$ (green) MLL4$_{4210–4280}$ (red) and thymosin beta 4 (blue). In case of MLL4130, saturation of the reaction could not be reached due to marked aggregation above 1:20 RNA:protein ratio (Inset). This abnormal behaviour is RNA-specific.

[1] Herz, H.Met al. Genes Dev. (2012) 26, 2604–2620.
[2] Szabó, B. et al. Int. J. Mol. Sci. (2018) 19.
[3] Langdon, E. M. et al. Science (2018) 360, 922–927.

PHOTOINDUCED PROCESSES OF DYES IN MESOPOROUS TITANIA SOL-GEL COATINGS

Borbála Tegze[1]*, Emőke Albert[1], Péter Basa[2], György Sáfrán[3] and Zoltán Hórvölgyi[1]*

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[2] *Semilab Semiconductor Physics Laboratory Co. Ltd., Budapest, Hungary*
[3] *Centre for Energy Research, Institute for Technical Physics and Materials Science, Budapest, Hungary*
* *Corresponding author: boritegze@mail.bme.hu, zhorvolgyi@mail.bme.hu*

TiO_2 is a semiconductor widely used for its photovoltaic and photocatalytic properties in energy conversion and environmental protection. Dyes are often used as model molecules for studying the properties of photoactive materials. Studying dye photodegradation in porous thin films at the air-solid interface can be a simple model system relevant for practical applications (*e.g.* self-cleaning coatings, dye-sensitized solar cells). In such systems it's also important to consider and investigate the dye adsorption and association processes.

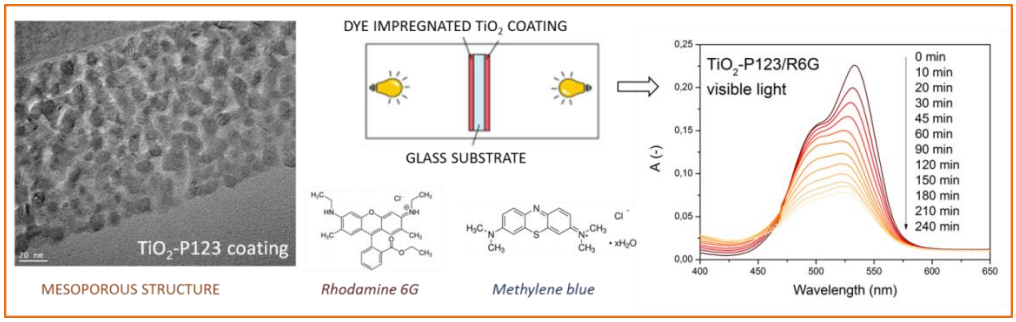

Figure 1. Dye adsorption in the coatings, followed by photodegradation measurements under UV or visible light

In our study mesoporous titania coatings on solid substrates were prepared by sol-gel method. The pore structure of the coatings was formed by different template molecules [1]. Optical properties, porosity, pore structure, crystallinity and morphology of the coatings were investigated by UV-Vis spectroscopy, ellipsometric-porosimetry and HR-TEM methods. Two different cationic dyes (rhodamine 6G and methylene blue) were adsorbed in the pores from aqueous solutions, and their degradation was studied under UV and visible light (Figure 1). Dye photodegradation kinetics was compared for the two dyes and the different pore structures. It was found, that dye molecules adsorbed in the pores as monomer and in their associated forms, and the association processes depended on the dye structure and the pore sizes. Furthermore, photodegradation rates and kinetics were also influenced by the dye association processes. Associated forms of the investigated dyes showed higher photostability compared to the monomers [2].

[1] E. Albert, P. A. Albouy; A. Ayral; P. Basa; G. Csík; N. Nagy; S. Roualdès; V. Rouessac; G. Sáfrán; A. Suhajda; Z. Zolnai; Z. Hórvölgyi; RSC Adv., 2015, 5, 59070–59081.
[2] B. Tegze; E. Albert; B. Fodor; G. Sáfrán; Z. Hórvölgyi; Dye. Pigment. 2019, 167, 109–119.

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INVESTIGATION OF HETEROELEMENT CONTAINING CONJUGATE SYSTEMS

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In the past decade, π-conjugated systems based on heteroatoms have been widely studied following the first reports on their successful incorporation in (opto)-electronic devices[1,2]. For example, heteroatom-based heterocycles are interesting building blocks as the reactivity and physicochemical properties of these π-systems highly depend on the heteroatom's substituents as well as the nature of the heterocycle in which it is embedded[3-6]. Theoretical calculations can be used to rationalize structure/property relationship. In this lecture, two main topics will be presented:

■ Synthesis of new phosphorus-containing polycyclic aromatic hydrocarbons (PAHs). The reactivity and the optical properties of these PAHs have been studied. Furthermore, theoretical calculations have also been performed, which may allow us to gain deeper insight into the local aromaticity, the optical properties, as well as the mechanism of specific organic reactions taking place on these PAHs.

■ New 2,5-diphosphanyl substituted siloles have been synthetized and DFT calculations have been carried out to explain how the nature of the P-substituents affects the physicochemical properties.

In the lecture it will be presented how the combination of the sythetic work and computations can help us to get deeper understanding of the optical, electrochemical and reactivity properties of the new molecules.

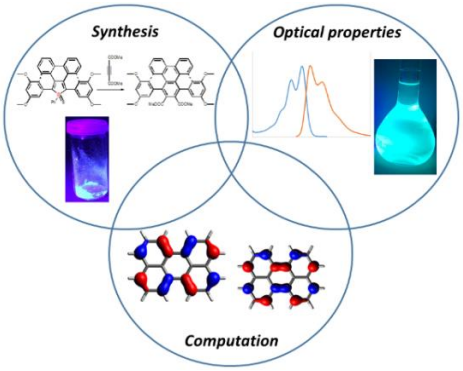

Figure 1.

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NUCLEATING AGENTS WITH DUAL NUCLEATING ABILITY IN ISOTACTIC POLYPROPYLENE

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Isotactic polypropelene (iPP) is a semi-crystalline polymer used in various application fields. The properties of iPP depend mainly on its crystalline structure, which can be manipulated by the application of nucleating agents. Since iPP is a polymorphic polymer, these additives can enhance the formation of either one polymorphic modification of iPP or both (α- and β-iPP) of them. A well-known and commercially available example for dual nucleating agents is N,N'-dicyclohexyl-2,6-naphthalenedicarboxamide (sold under the trade name of NJ Star NU 100), which is an efficient additive in the preparation of iPP samples rich in the β-modification. However, it was shown that it is not selective for the β-iPP, since also the α-iPP forms in its presence [1].

The aim of this work is to describe two other examples for dual nucleating agents of iPP, namely N,N'-dicyclohexyl-terephthalamide [2] and dibenzoyl-terephthalic acid dihydrazide, which were synthesized in our laboratory. In our research we used simultaneous thermal analysis to determine the melting temperature and the decomposition temperature of the compounds, since these characteristics are crucial from the viewpoint of applicability of a material as a nucleating agent. Then we introduced the additives in iPP in a wide concentration range, and investigated the thermal properties of the as prepared samples, by using differential scanning calorimetry. We proved that these compounds possess a dual nucleating ability in iPP, since they enhance the formation of both of the two polymorphic modifications already mentioned. Polarized light microscopy was also used to follow the crystallization process and to observe the presence of the iPP modifications.

Since the crystallization conditions (thermal conditions, presence of additives, etc.) influence the crystalline structure and consequently also the properties of the polymer, targeted manipulation of the structure by adding nucleating agents creates an opportunity to tune the properties of the polymer so that they fit our standards.

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PROCESS DESIGN AND AUTOMATION IN BROWNFIELD PROJECTS IN EXAMPLE OF METHANOL PRODUCTION COMPANY WITH AGILE APPROACH

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Optimized, risk free and accelerated capital projects with maximized return on investment from both brownfield and greenfield projects are always in a focus to be aimed at oil refining, chemical and petrochemical companies. Sooner facility startup, faster production target reach and sustainable operations with peak performance seem possible and can be achieved by combination of the best process design and technology with advanced automaton as per plant owners and operators. [1]

However traditional approaches are often semi-empirical and depend primarily on the ingenuity and experience of engineers. This methods makes hard to define the process in early stages of the project with highest freedom in design and implementation of automation both before and after startup of the facility. [1,2]

Experience has shown that re-use of P&ID's often leads to over-instrumentation and to the exclusion of the best modern technology. [3]

In example of automation, agile approach provide solution how to avoid misinterpretation between engineering, procurement and construction contractor and automation supplier, missing out of the best practice applications due to restrictions and comes with 60-70% of complete engineering of automation for each process before project starts. [1,2]

When it comes to the implementation of transformation in brownfield projects, the relatively simple projects can be more complex, more expensive and delayed. Challenges in this regard can be categorized as documentation, equipment condition, utility system and infrastructure capacity, codes & standards, execution sequence/timing, control of the work site and temporary facilities to support production. [4]

One of the brownfield projects in Azerbaijan was Azerbaijan Methanol Company which acquired the rights to the Inside Battery Limits (ISBL) portion of a decommissioned methanol plant formerly located in Clear Lake, Texas and shipped it to the Baku. During its original use, the Celanese methanol plant shared its Outside Battery Limits (OSBL) and utilities with other facilities and, as such, they could not be relocated along with the ISBL equipment, accordingly OSBL and utilities are designed and constructed newly. [5]

In this paper comparison of the greenfield and brownfield projects in above challenge categories and possible risks and their mitigations will be discussed and Azerbaijan Methanol Company design and construction project will be reviewed over traditional and agile approach.

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QUANTUM CHEMISTRY AIDED DESIGN OF BIOMIMETIC COMPLEXES FOR ATMOSPHERIC PRESSURE AMMONIA SYNTHESIS

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Synthetic iron nitrogenases (Figure 1, left) are promising novel catalysts for ammonia synthesis[1]; these bioinspired single-site metal complexes catalyse thereduction of N_2 (N_2RR; Figure 1a) under atmospheric pressure, which is a significant advantage compared to the traditional Haber-Bosch process. However, this catalytic system has two major drawbacks which hinders any practical application. Firstly, the catalysts are not selective as they also facilitate the undesired hydrogen evolution reaction (HER; Scheme 1b). Secondly, the catalyst complex deactivates quickly due to the formation of inert hydride side products (Figure 2c).

In order to create an industrially applicable molecular catalyst (which is the long term goal of ongoing researches), the lifetime and N_2RR vs HER selectivity needs to be dramatically increased. This can only be achieved by a rational, theory (QM) based re-design of the ligand scaffold.

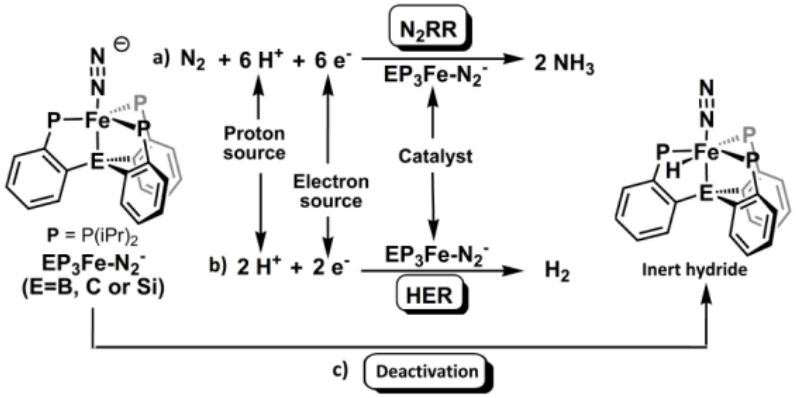

Figure 1. Structure and reactivity of biomimetic ammonia synthesis catalysts.

In this computational study, we compare the thermodynamics and kinetics of conceivable "on-path" (N_2RR) and "off-path" (deactivation, HER) elementary steps by means of DFT-D calculations. Based on the obtained Gibbs free energy profiles,[2,3] along with the discussion of the available experimental data, we determine which of the mechanisms can be competent under the reported catalytic conditions. In this way, we point out which elementary reactions should be influenced by ligand modifications to alter catalyst activity and selectivity in a favorable way.

We believe our research is a good example for how theoretical modeling broadens the horizons of a novel field of chemical research.

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Abstracts

Poster presentations of students from the George Olah Doctoral School

MAPPING PATHWAYS OF DIATOMIC LIGANDS MIGRATION INTO H-NOX DOMAINS AS A MODEL OF SGC ACTIVATION

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Diatomic gas molecules are the ligands of numerous proteins, enabling them to perform their functions. Especially nitric oxide (NO), O2 and CO contribute significantly to gas sensing in living organisms by binding to heme proteins. Heterodimeric heme protein αβ soluble guanylyl cyclase (sGC) is the unique receptor for nitric oxide (NO) in man which regulates various physiological processes through the NO (H-NOX domain)/cGMP (cyclase domain) signaling pathway. Dysfunctioning of this signaling network has been linked to cardiovascular, neurodegenerative and inflammatory diseases making sGC an intereting target for the pharmaceutical industry (with only a single drug presently on market which still need more advanced development for its performance). Therefore, deep mining in the knowledge of sGC would be critical for designing a potent drug.

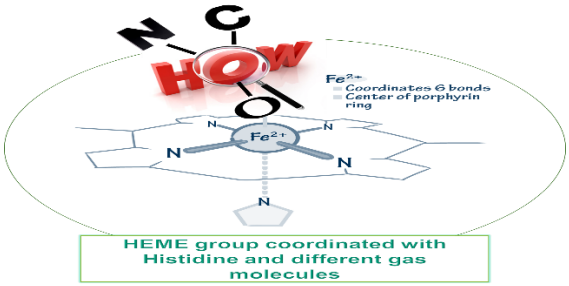

Figure 1.Gas ligands bound to Ferrous heme of H-NOX domain.

In the present work, we tackle the gas-binding mechanism to H-NOX domain which leads to sGC activation. Two starting structures of homologous H-NOX proteins were selected, from the facultative anaerobe *Nostoc* genus and obligate anaerobe *Thermoanaerobacter tengcongensis*, as there is lack of the crystal structure of sGC. We studied the diffusion of NO, O2 and CO using 600 ns long molecular dynamic simulations on these two proteins following a protocol previously published in our preceding work.1 Cluster analysis of obtained trajectories in conjunction with visual inspection allowed us to explore the most likely pathways of gas migration throughout the protein body to distal heme pocket. Quantitative assessment of ligand diffusion through the trajectories enabled us to obtain rate constants for migration, which indicate that ligand diffusion in these proteins is similarly fast as in myoglobin for which experimental data also exists. Finally, we describe a few factors contributing to the differential activity of the diatomic ligands on the studied proteins.

Acknowledgements: The financial support of a Stipendium Hungaricum Fellowship, the Egyptian Government, NKFIH Grants 115503 and 116305, and of the Protein Science and its Applications National Programme (HunProtEx, 2018-1.2.1-NKP-2018-00005) is thankfully acknowledged. MD simulations were carried out at the facilities of the Hungarian NIIF Institute.

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EFFECT OF PHOSPHORUS FLAME RETARDANTS ON THE PROPERTIES OF BIO-BASED EPOXY RESINS

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Fire risk is not a new problem but, as new polymeric materials are developed and used, the potential for fire risk may increase. Thermosetting polymers offer advantages over metals such as lower weight, and no corrosion and are widely used within the engineering fields because of their versatility in tailoring their ultimate properties and performances in terms of strength, durability, thermal and chemical resistances as provided by the highly cross-linked structure [1]. Therefore, the aim of this study was to find the best available flame retardant (FR) for a bio-based epoxy resin (Sorbitol Polyglycidyl Ether (SPE), an aliphatic polyfunctional epoxy resin), by comparing different additive type flame retardants at the same levels of phosphorus content.

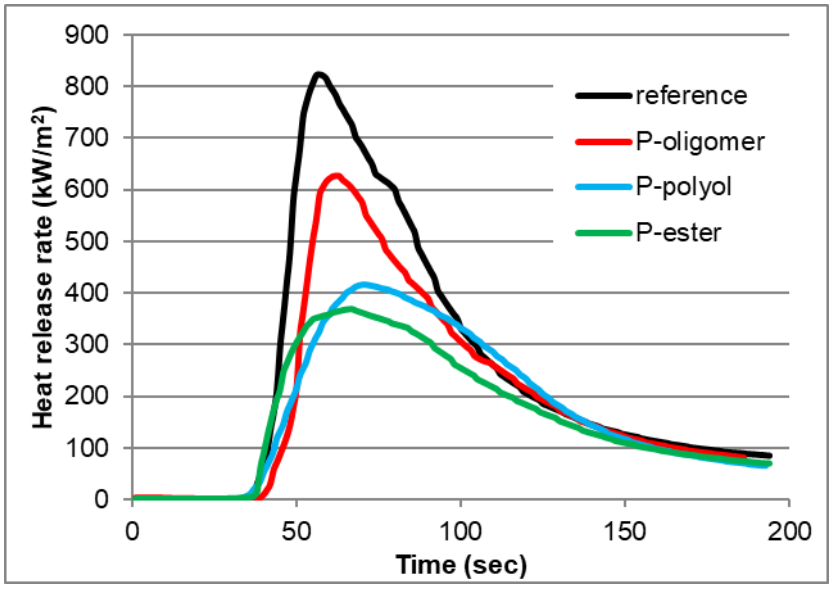

Figure 1. Heat release rate of the different FR formulations at 3% P-content

The different type of flame retardants act very differently: while the P-oligomer ensures high charring, it could not decrease the heat release; while the use of a P-ester-type FR resulted in more than 50% reduced peak of heat release rate.

The use of flame retardants decreased the flammability of GE60 and GE61 epoxy systems by increasing LOI value and significantly decreasing the heat release. The self-extinguishing UL-94 rate can be achieved with certain composition accompanied very good mechanical properties and processability.

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THE EFFECT OF CATIONIC SIDE GROUPS ON THE AQUEOUS STABILITY OF THIOLATED POLYASPARTAMIDES

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The aim of modern drug delivery systems is to overcome both physicochemical and biological barriers for releasing drugs in a desired period of time, dosage and place, and easy removal of empty devices at the end.[1,2] Synthetic poly(aspartic acid) and its derivatives due to their biocompatibility and biodegradability, desirable mechanical properties, tunable sizes from nanometers to micrometers, a large surface area for multivalent bioconjugation, and an interior network for the incorporation of biomolecules gain huge attention in this field.[3]

In this work we synthesized polyaspartamides with cationic side groups by adding *N*,*N*-dimethylethylenediamine (DME) and 3-(dimethylamino)-propylamine (DMP) in various amounts onto cysteamine-modified polysuccinimide. Aqueous stability of the polymers was characterized by the dialysis recovery after storage either in physiological (pH = 7.7) or endosomal (pH = 5.5) pH. The recovery (the amount of remaining polymer after dialysis) was higher at pH = 5.5 compared to pH = 7.7 and the degree of recovery decreased with increasing amount of DME in polymer as shown in Figure 1. Polymers synthesized with high DME content (40% and above) are sensitive to hydrolytic degradation with recovery below 20%, whereas using DMP instead of DME resulted in considerable stability in aqueous solutions at both pH values.

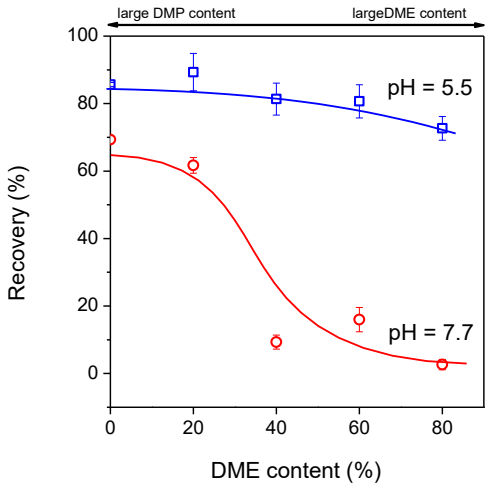

Figure 1. Recovery of polymers in pH=7.7 and pH=5.5. Results are mean of three measuremts.

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APPLICATION OF A BIOMIMETIC OXIDATION SYSTEM IN HOMOGENEOUS AND HETEROGENEOUS, CONTINUOUS FLOW CONDITIONS

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[2] *Biocatalysis and Biotransformation Research Centre, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University of Cluj-Napoca, Arany János Str. 11, RO-400028 Cluj-Napoca, Romania*
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In the early stages of preclinical research of drug candidate molecules metabolism research has an important role. During metabolism research the main goal is to characterize the metabolites of the mother components, and to isolate and identify them. These experiments are carried out traditionally by *in vivo*, on living organizations or by *in vitro* cell based methods. However, this approach presents many challenges from an analytical point of view and ethical issues are raised because animal studies are performed. Biomimetic oxidation can be an alternative solution for metabolic tests. Synthetic metalloporphyrins are feasible catalysts to mimic the liver-based metabolism of xenobiotics in presence of an oxygen donor. Their application is based on the structural similarity to the active site of the cytochrome P450 isoenzyme family.

My work can be divided into two parts. In the first half of my research I developed a synthetic metalloporphyrin based medium throughput screening system, which can produce metabolites in one step from the mother component in homogeneous conditions. The drawback of this method is the rapid degradation of the porphyrin therefore it has short lifetime. It can be extended by immobilization of the catalyst on solid support. In the second half of my research I immobilized meso-tetra(parasulphonatophenyl)ironporphine on surface modified (with aminopropyl groups) silica. The immobilized supported catalyst then was filled in packed bed reactor and used in a continuous flow system to catalyze biomimetic oxidation. Furthermore, I examined the effect of the length of the linker between the catalyst and the supporter surface on the biomimetic reaction. [1]

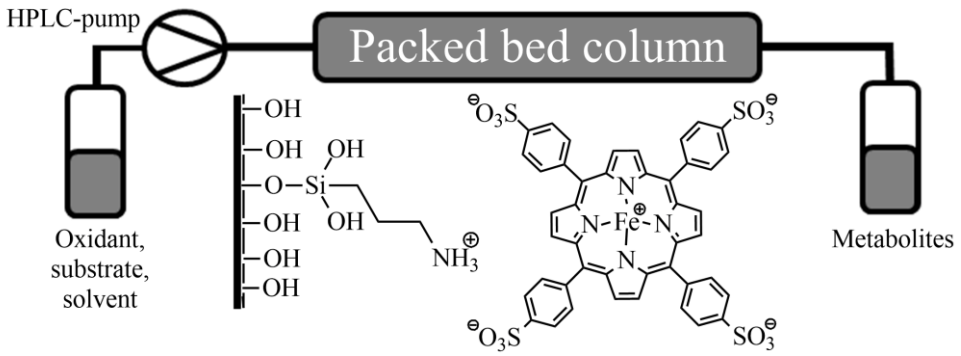

Figure 1. The scheme of continuous flow biomimetic oxidative system.

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RESOLUTION OF SECONDARY PHOSPHINE OXIDES AND *H*-PHOSPHINATES AS *P*-STEREOGENIC INTERMEDIATES

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H-Phosphinates and secondary phosphine oxides are of particular importance in the field of organophosphorus compounds. They might be regarded as intermediates with a stable *P*-stereogenic center which can be substituted in a stereospecific manner to give optically active tertiary phosphine oxides or boranes.[1] Furthermore, these P(O)H compounds are preligands, because the trivalent tautomeric form dominates in the presence of transition metals. Thus, the metal complexes of the corresponding phosphinic or phosphonic acids can be obtained and used as catalysts.[2]

In our research, the resolution methods previously developed for tertiary phosphine oxides were extended to secondary phosphine oxides and *H*-phosphinates.[3] The diaryl- and aryl-alkyl secondary phosphine oxides (**1**), as well as adamantyl *H*-phosphinates bearing aryl or alkyl-groups (**2**) comprised the substrate scope of this study. First, the corresponding *P*-stereogenic P(O)H compounds were prepared in racemic form. The resolution of the secondary phosphine oxides (**1**) and *H*-phosphinates (**2**) were then elaborated with TADDOL-derivatives (**3** and **4**) allowing the preparation of the corresponding pure enantiomers. The main parameters influencing the efficiency of the resolution procedures were optimized. Moreover, a correllation was found between the efficiency of the resolution and molecular sturcture of the racemic compounds.

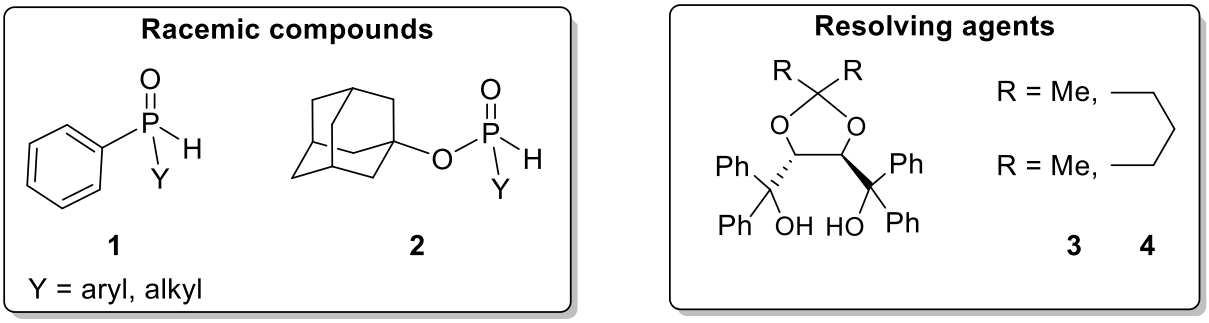

*Figure 1. The racemic secondary phosphine oxdes (**1**), H-phosphinates (**2**) and the resolving agent (**3** and **4**).*

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PHYSICAL AND CHEMICAL FOAMING OF FLAME RETARDED POLY(LACTIC ACID)

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In this work, physical and chemical blowing agents were compared by manufacturing flame retarded poly(lactic acid) (PLA) foams. To obtain dense and uniform cell structures, epoxy-functionalized chain extender (CE) and montmorillonite (MMT) as nucleating agent with flame-retarding effect were applied in the PLA matrix. Besides, ammonium polyphosphate (APP) based intumescent flame retardant (IFR) system was used in order to lower the combustion intensity. The effect of these additives was investigated on the morphology, structure, flammability and mechanical properties of the PLA foams. Foam extrusion was performed on a modular twin-screw extruder, using two different strategies. As physical blowing agent, sc-CO_2 was injected into the extruder using a dual syringe pump.[1] As chemical blowing agent, 30 wt% azodicarbonamide masterbatch was compounded with PLA using the lowest possible temperature profile. At elevated temperatures (180-195°C), during the extrusion foaming process, thermal decomposition of the azodicarbonamide produces N_2, CO and CO_2, thus expanding the PLA matrix. In our experimental work, the produced flame retarded PLA foams were comprehensively characterized by morphological (SEM, density measurement), thermoanalytical (TGA, DSC) mechanical (compression strength) and flammability (UL-94, LOI) testing methods.

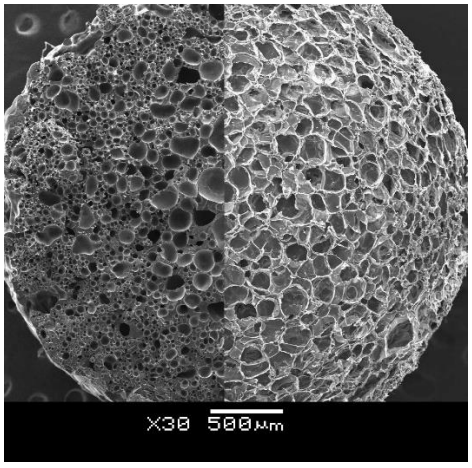

Figure 1. SEM images of PLA foams prepared by chemical foaming(left) and physical foaming (right).

The physical foaming technique proved to be more advantageous than chemical foaming, mostly because of the unique properties of sc-CO_2. The produced PLA foams, due to their beneficial properties, can hopefully gain ground not only in the packaging, automotive, electronics and construction industries, but even in several other sectors.

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RHEOLOGICAL BEHAVIOR OF WHEAT LINES WITH ALTERED AMYLOSE CONTENT

Edina Jaksics[1], Sándor Tömösközi[1*], Renáta Németh[1], Kitti Török[1], Bettina Paszerbovics[1], Blanka Egri[1], László Láng[2], Zoltán Bedő[2], Francesco Sestili[3], Domenico Lafiandra[3], Marianna Rakszegi[2]

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[2]*Agricultural Institute, Center for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary*
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The ratio of amylose to amylopectin is a decisive determinant of the physico-chemical properties of wheat starch, as the susceptibility to enzymatic hydrolysis, the gelling and pasting behaviour or the gel structure and stability. In the case of dough made from wheat flour the composition of starch and its state (hydrolitic degradation, physical brakes in the chains) affect the structure and stability of dough, the process of retrogradation and ultimately the end-use quality of the final product[1,2,3]. The aim of our research was the characterisation of carbohydrate composition, especially the determination of the amylose/amylopectin (AM/AP) ratio and the rheological behaviour of wheat lines with high (Sgp-A1B1D1) and low amylose (Waxy, Wx-A1B1D1) null mutant wheat genotypes, breaded at MTA-MgKI, Martonvásár. The experimental lines were harvested in three years, (2014-2016). Significant variation was identified in the amylose content (1,26-38,3%) and consequently in the AM/AP ratio (0,01-0,6) of the breeding lines. The variability found in the composition of starch resulted significant differences in the mixing characteristics of the dough, mainly in the viscous behavior characterized by Mixolab. We observed that in many cases, alteration in rheological properties can be related to the changes in the starch composition, but not in all instance. It is clear that in parallel with the modification of starch composition, other macromolecules could also be changed. Our further task is the better understandigof the interactions of macromolecules and their effect on the rheological behaviour.

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CONTINUOUS FLOW CHEMOENZYMATIC CASCADE REACTIONS AIMING DIASTEREOPURE AMINE SYNTHESIS

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Transaminases as a pyridoxal-5'-phosphate dependent enzymes are capable of catalyzing kinetic resolution of racemic amines or asymmetric synthesis of enantiopure amines starting from prochiral ketones.[1] Asymmetric synthesis would be more preferred, though, it usually suffers from disfavored reaction equilibrium and requires expensive amino donors. Kinetic resolution is thermodynamically more favored option but one enantiomer of a racemic amine is converted into the corresponding ketone.[2] Thus drawback of KR process is the maximal 50% yield of the desired enantiomeric product. If the formed ketone can be recycled to racemic amine by a proper reductive amination method, the overall efficiency of the KR process may be performed.

Consequently we envisioned a cascade reaction system including immobilized ω-transaminase activity biocatalyst and Pd-based heterogeneous catalyst. Whole-cells of *E. coli* with transaminase activity were immobilized with hollow silica microspheres by sol-gel process. [3] The chemocatalyst was prepared by precipitation of $PdCl_2$ on in-house prepared amino funcionalized silica gel carrier[4] which is suitable for reductive amination of ketones. Fulfilling the requirements of sustainable industry and safety regulations this cascade system was performed in miniaturized packed bed reactor system installed with back pressure regulator. Flow reactor technology consisting of packed-bed enzyme and racemization reactors can significantly improve efficacy of biotransformations.

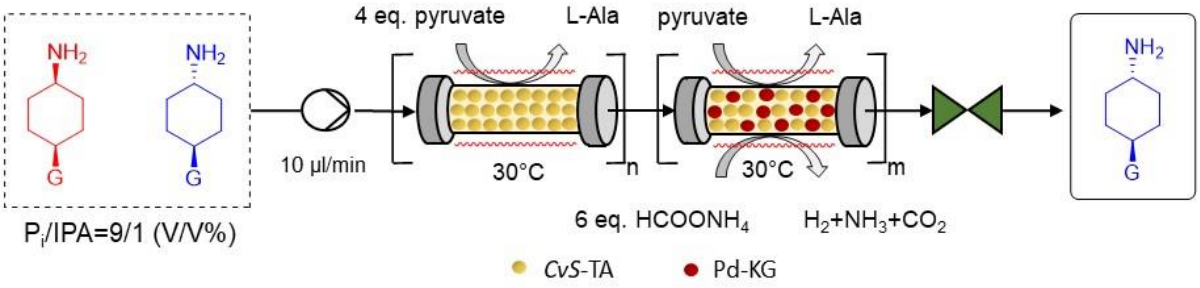

Figure 1. Aliphatic amines tested for reductive aminations and DKR in continuous mode

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EFFECT OF FIBER CONTENT ON THE PROPERTIES OF HYBRID PP COMPOSITES CONTAINING LIGNIN AND FLAX

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Lignin and flax are among the most interesting natural substances which offer great potential as green composites for multifunctional applications. Several attempts have been reported in the literature to use lignin as a low-cost additive for polypropylene (PP). Further results clearly proved that interactions between PP and lignin are weak, resulting in poor properties.[1] Although interfacial adhesion could be improved by the application of a coupling agent, the properties of the blends, especially their deformability, needs further improvement. Therefore, we chose an elastomer modified PP compound as matrix polymer and added flax fibers to the PP/lignin blends to improve their mechanical properties.

PP/lignin/flax fiber composites were prepared in a wide composition range. Strong adhesion was achieved by the addition of MAPP coupling agent. The natural fiber increased both the stiffness and strength (Figure 1.) of the composites. The matrix PP was a reactor blend containing elastomer thus the deformability and the impact resistance of the composites remained at an acceptable level.

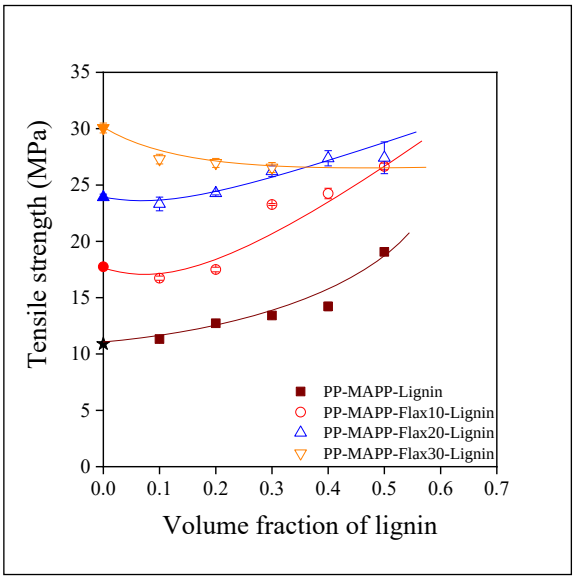

Figure 1. Effect of fiber content on the tensile strength of hybrid composites.

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INVESTIGATION OF LIGAND EXIT PATHS IN THE MIO ENZYME FAMILY WITH THE HELP OF RANDOM ACCELERATION MOLECULAR DYNAMICS (RAMD)

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Aromatic amino acid ammonia-lyases and 2,3-aminomutases contain the post-translationally formed 5-methylene-3,5-dihydro-4H-imidazol-4-one (MIO) electrophilic catalytic group. The aim of this research was to determine the function and mobility of a Tyrosine loop region, which closes the active site region of Phenylalanine ammonia lyases (PAL) and aminomutases (PAM). Furthermore we aimed to investigate its function in the binding and un-biding of the substrate L-phenylalanine and cinnamic acid, the later being a product of biocatalysis.

To achieve our goals we selected and tested several molecular dynamics softwares and force fields. We have determined, that for our purposes the Amber simulation environment and the ff14SB force field are the most suitable. We used these tools to parametrize several non-canonical molecules, namely the MIO, an amino-MIO adduct, the L-phenylalanine, the cinnamic acid and the deprotonated form of tyrosine. We showed through statistical analysis, that the parametrization was successful, the system was stable, and our parameters were in accordance with experimental data.

With the help of homology modelling we were able to complete the crystallographic data of *Tc*PAM (PDB: 2YII) and *Pc*PAL (PDB: 6F6T, unreleased) and build up full protein structures, which we then used for molecular dynamics simulations. We added the ligands through docking and based on the crystal structures. From these starting structures we built up a suitable model system for molecular dynamics, placed in an octahedral water box and with an adjusted ion strength. We minimized and equilibrated the system under 20 ns, then we chose 3 different starting "snapshots" suitable for our RAMD simulations.

Up until this point the general consensus was that there both in the case of PAM and PAL there is only one ligand exit path, which goes from the center of the active site towards the catalytically active Tyr-loop. Through our simulations we have discovered 3 additional paths in which ligands can exit or enter, one of them being as common as the Tyr-loop path itself. Additionally we also question the main theory which says that the loop must open for the ligand to exit. From our 180 simulations not one seems to suggest such flexibility as it was implied up until now. [1,2,3]

Researching the exit paths can open new doors in determining the main differences between PALs and PAMs. The simulation data seems to suggest conserved amino acids, which can influence how a substrate is bound and how much time it can spend in the active site. Currently we are working on mutagenesis experiments to support our simulation data and we are also investigating how these mutations affect the enzyme activity.

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HIGH-THROUGHPUT ELECTROSYNTHESIS AND READ-OUT OF PROTEIN MIPS WITH MICROELECTROSPOTTING-COUPLED FLUORESCENCE IMAGING

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Molecularly imprinted polymers (MIPs) as selective, synthetic receptors can provide cost-effective, robust alternatives to antibodies for protein recognition. During the synthesis of these receptors, functional monomers prearrange around the template molecules via non-covalent or covalent interactions. This intermolecular structure is then fixed by the polymerization of the monomers. The removal of the template leaves behind selective binding sites, which are complementary in shape and functionality to the template, and can be therefore used to selectively detect it.

The main challenge in protein-imprinting is the slow diffusivity of the macromolecules in the polymer matrix resulting in poor template removal. To overcome this problem, surface imprinted nanofilms, comparable in thickness with the template, can be electrosynthesized. For high-throughput optimization of the preparation and characterization of protein MIPs we have introduced a method we called microelectrospotting. [1] Here, we are going to present the coupling of such synthesis, with fluorescence imaging that provides multiplexed, fast and sensitive read-out of the template binding. The preliminary investigations of the MIP-based protein chips were performed using lysozyme as template protein and 3,4-ethylenedioxythiophene as functional monomer.

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PROCESS CAPABILITY INDICES WHEN THE USUAL ASSUMPTIONS FAIL: A TOLERANCE INTERVAL APPROACH

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Statistical indices- like process capability (C_p) or process performance (P_p) index- are widely used in the field of quality management. These clever indicators make illustrative the relationship between the width of the specification interval and the width of the process variability. The latter is characterized by the tolerance interval, which contains major part of the population with high confidence. In the original concept this tolerance interval is calculated using simple models. The ultimate objective of calculating these indices is to give information about the proportion of non-conforming parts in the process (in the population).

However, the use of C_p, P_p is based on certain statistical assumptions. If at least one of them does not fulfill, the calculated value of C_p (P_p) is not able to give information about the process. Our work is dealing with the case that the quality characteristic of interest is a normally distributed random variable, the process is in control, but three sources of variability are present. The models are one-way and two-way nested ANOVA.

In order to establish the proportion of non-conforming parts the calculation of the ratio of the population beyond the specification limits is needed. According to this, the quantile of the distribution shall be determined that are equal to the specification limits. Thus, the task is to calculate tolerance interval for the $\mathcal{N}(\mu, \sigma_A^2 + \sigma_e^2)$ or $\mathcal{N}(\mu, \sigma_A^2 + \sigma_{B(A)}^2 + \sigma_e^2)$ distribution. In practical cases the variance components are unknown and to be estimated. To estimate the ratio of non-conforming parts two approximate calculating methods which are coherent with the definition of P_p are investigated, as well.

The aim of this work is to compare the results of the two approximate methods with the tolerance interval based (correct) calculation method.

DEVELOPMENT AND IN VITRO TESTING OF COVALENT INHIBITORS TARGETING ONCOGENIC KRAS MUTANTS

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KRAS is a guanine binding signalling protein, which works as a molecular switch in controlling cell growth, differentiation and proliferation. GTP-bound KRAS is in active conformation that can interact with the downstream effectors, like SOS protein, while in the GDP-bound state the signalling decays [1]. Certain mutants of KRAS are locked in the active state causing permanent signalling, which results in uncontrolled cell division and cell growth leading to oncogenic transformation. Mutant KRAS proteins are amongst the most prominent oncogenes and play a significant role in almost 25 percent of all human cancers. Those tumorous malignancies caused by KRAS mutations, such as pancreatic and lung cancer are generally difficult to treat [2].

Despite the oncogenic nature of KRAS is well-known there is no drug against this target, which is efficient and specific enough to be used in therapy so far. However, in the recent years, there were successful attempt to create promising KRAS inhibitors, that bind covalently to the cysteine of the G12C mutant protein [3]. This approach ensures the specificity of the compounds, while provides a strong and irreversible interaction between the inhibitor and the protein. Optimising these inhibitors may lead to a compound, that can be applied in medicine, with some compounds already in phase 2 and 3 trials [4].

In order to test new compounds in vitro we established a test-cascade for testing a large number of samples in short time, to select compounds that are suitable for further examination and development. In the first step we tested the reactivity of the molecules, by measuring the amount of free cysteine in the samples, which refers for the proportion of inhibitor bound KRAS G12C protein. In the second step the thermostability of the protein-inhibitor complexes were examined by differential scanning fluorimetry. Finally, in a functional assay, we measured the rate of nucleotide exchange in the presence of fluorescent nucleotide analogues.

Based on our screenings we were able to identify the molecules MRAS-7 and MRAS-13 which proved to effectively inhibit KRAS activation, thus stop KRAS signal transduction. To help in designing new molecules based on these compounds, we aimed to crystallise the protein-inhibitor complexes for x-ray crystallography. We successfully crystallised GDP-bound KRAS, then used the crystal-soaking approach to get crystals that contain the inhibitors in a complex with the protein.

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KNOCK-OUT OF DUTPASE IN MICE LEADS TO EARLY EMBRYONIC LETHALITY AND IS NOT RESCUED BY CONCURRENT KNOCK-OUTS OF BOTH UNG AND SMUG.

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Sanitization of nucleotide pools is essential for genome maintenance. Deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) is a key enzyme in this pathway since it catalyzes the cleavage of 2'-deoxyuridine 5'-triphosphate (dUTP) into 2'-deoxyuridine 5'-monophosphate (dUMP) and inorganic pyrophosphate. Through its action dUTPase efficiently prevents uracil misincorporation into DNA and at the same time provides dUMP, the substrate for de novo thymidylate biosynthesis. Despite its physiological significance, knock-out models of dUTPase have not yet been investigated in mammals, but only in unicellular organisms, such as bacteria and yeast.

Here we generated CRISPR/Cas9-mediated dUTPase knock-out in mice [1]. We found that heterozygous Dut +/- animals are viable while having decreased dUTPase levels. Importantly, we show that dUTPase is essential for embryonic development since early Dut -/- embryos reach the blastocyst stage, however, they die shortly after implantation. Analysis of pre-implantation embryos indicates perturbed growth of both inner cell mass (ICM) and trophectoderm (TE). We conclude that dUTPase is indispensable for post-implantation development in mice.

We supposed that embryonic lethality might be the consequence of the excessive action of the base excision repair mechanism that removes uracil from the DNA. The elevated uracil level overwhelms the repair process, and consequently, leads to DNA fragmentation and cell death. The two enzymes responsible for the vast majority of the uracil DNA glycosylase activity in mice are UNG and SMUG [2]. To test this hypothesis, we intercrossed mice heterozygous to Dut with Ung -/- Smug -/- mice to determine if the abolished uracil repair can rescue the lack of dUTPase activity. Surprisingly, we found neither live births of Dut -/- offspring, nor homozygous embryos at 10.5 day pc. Our results suggest that the embryonic lethality caused by the lack of dUTPase is not due to excessive uracil repair, rather dUTPase and genomic uracils might be involved in the differentiation process.

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IN VITRO, NON-CELLULAR PERMEABILITY ASSAY TO PREDICT CORNEAL ABSORPTION OF APIS USING THE PAMPA MODEL

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The human eye can be affected by several diseases that might cause visual impairment. For the treatment of the anterior eye segment, the topical, non-invasive routes of drug administration are preferred, using liquid, semisolid and solid ophthalmic drug forms. The absorption of active pharmaceutical ingredients (APIs) may involve the corneal and/or non-corneal routes. In both cases, however, within minutes after application the lachrymal fluid elutes a large portion of the applied drug[1]. Also, topical pharmacodynamic effect can be mainly attributed to API absorbed through the corneal barrier, since the later routes transfer APIs mostly into systemic circulation by the local capillaries[2], resulting in a significantly decreased ocular bioavailability (usually <5-10%)[3]. Thus, prediction of corneal permeability is of utmost importance for the rational selection of APIs for ophthalmic formulations at an early stage of the drug discovery process. To predict corneal absorption several models have been reported: ex vivo assays, using vertebrate animals and in vitro, cellular tissues[4]. However, to our knowledge, no in vitro, non-cellular membrane permeability model has been reported yet, therefore, our research aimed to develop an in vitro non-cell-based, high-throughput screening model to predict corneal permeability as a novel alternative to the expensive, and time-consuming ex vivo and in vitro cell-based models. For this purpose, the parallel artificial membrane permeability assay (PAMPA) was used, studying the effects of composition of the artificial membrane, different buffer solutions and the DMSO cosolvent content in the model. Based on experimental corneal permeability values of 25 APIs, a final model with good predictive ability (R^2= 0.880) was developed and validated.

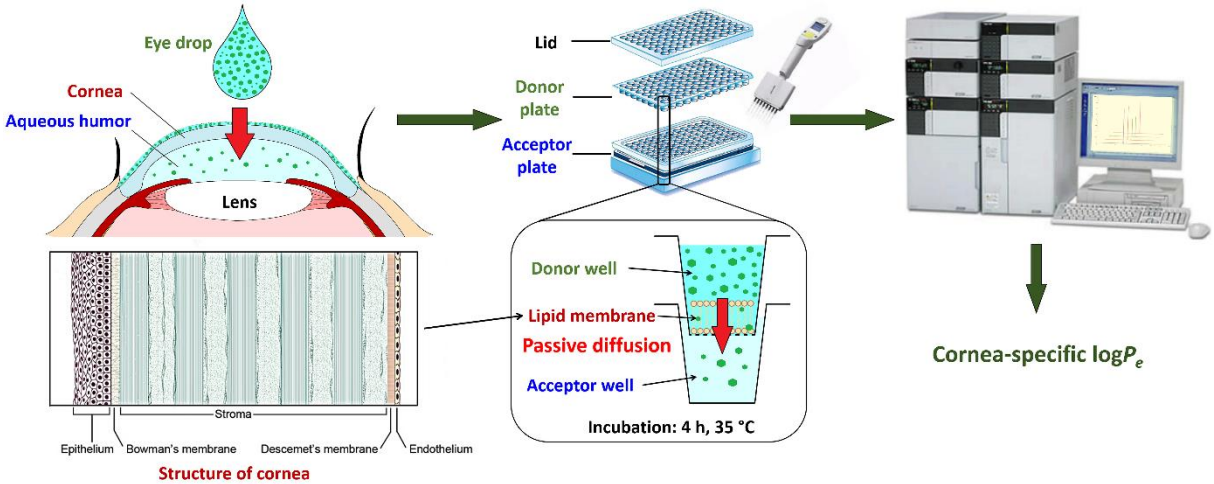

Figure 1. Flowchart of in vitro PAMPA experiments for corneal permeability measurements. (The structure of cornea has been adopted from the NEI Photos and Images Catalog: https://nei.nih.gov/photo).

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COMPARISON OF CONDUCTING POLYMERS AND HIGH CAPACITANCE MATERIALS FOR THE FABRICATION OF SOLID-CONTACT ION-SELECTIVE ELECTRODES

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Solid-contact (SC) electrodes are preferably used to replace conventional ion-selective electrodes with internal filling solution if easy miniaturization or the lack of intense maintenance is desirable (e.g. for wearable sensors)[1]. The reliable fabrication of such electrodes, however, especially in terms of potential stability and reproducibility, is still a challenge. Most typically, electrically conducting polymers (ECPs), nanostructured materials with high electric capacitance or redox active-compounds are utilized as solid-contacts[2].

Here, we present our solutions to better control and understand the E^0 reproducibility during the manufacture of SC electrodes, by using perfluorinated poly(3,4-ethylenedioxythiophene) (PEDOTF) and functionalized carbon materials as solid-contacts, and pre-polarize them before applying the ion-selective membrane (ISM). We found that for PEDOTF film the initial potential stability and reproducibility following the pre-polarization were exceptionally good (±0.5 mV) in the first hour, strengthening the necessity of such a step to successfully control the oxidation state and therefore the potential of the film. Overall, following ISM deposition, the E^0 reproducibility of the potassium-selective electrodes increased to ±3.0 mV, indicating that this is the critical step during the fabrication. The thus manufactured ion-selective electrodes showed excellent potentiometric response with Nernstian slope and LOD ca. 10^{-7} M, while no sensitivity for light, O_2 or CO_2 were observable. Water contact angle (WCA) measurements revealed the high hydrophobicity of the PEDOTF film regardless of its oxidation state (WCA ~ 132°). Aqueous layer test and water uptake measurement with FTIR-ATR confirmed that this hydrophobicity effectively hinders the formation of an aqueous layer[3].

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DEVELOPMENT OF BIOEPOXY RESIN MICROENCAPSULATED AMMONIUM-POLYPHOSPHATE FOR FLAME RETARDANCY OF POLYLACTIC ACID

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During our research, ammonium-polyphosphate (APP), a conventional fire retardant (FR) additive was modified by microencapsulation with special biobased epoxy resin shells. Microencapsulation can provide many advantages, including increase of water resistance which is of key importance in the case of polylactic acid (PLA) products devoted for durable applications. It was found that with the right choice of the encapsulation agent, also the water sensitive components of the intumescent flame retardant system, like the widely used charring agent pentaerythritol (PER), can be substituted. Several combinations of sorbitol and glucose based epoxy components and aliphatic and cycloaliphatic curing agents were investigated to adjust the thermal degradation temperature of the bioresin to that of the APP type acid source, and to obtain the highest char yield at the same time. The most promising bioresins were used to encapsulate APP. The parameters of the encapsulation processes were optimized using experimental design in order to obtain an easy-to-handle combined flame retardant additive, functioning both as acid source and charring agent, with suitable particle size. The flame retardant efficiency of the newly prepared bioresin encapsulated APP was comprehensively investigated, besides evaluating the mechanical properties of the flame retarded PLA samples.

About 30% reduction of the peak of heat release rate (HRR) were measured by cone calorimeter tests and 7% increase of the LOI values were obtained compared to the additive free reference, both with the untreated and the encapsulated APPs. Moreover, the encapsulated APP containing samples reached V-0 rating according to the UL-94 standard, while the neat APP could only improve the PLA's performance from NC to V-2 rating.

Improvement in the mechanical properties were found when bioepoxy resin encapsulated APP was used in the PLA matrix. SEM images taken from the fracture surfaces revealed improved interfacial interaction as a result of the encapsulation of the FR additive.

It was found that bioepoxy resin encapsulated APP can effectively act both as acid source and charring agent and therefore improved flame retardant properties of PLA (such as UL-94 V-0 rating) were achieved at 15 wt% loading. Further investigations are planned to evaluate the water resistance of PLA when flame retarded with the newly developed microencapsulated APP additives.

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DISSECTION OF *PLASMODIUM FALCIPARUM* DEVELOPMENTAL STAGES WITH MULTIPLE IMAGING METHODS

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Every year, more than 200 million people are infected with malaria. Five species of the *Plasmodium* genus cause human malaria infection[1].The protozoon is transmitted into the human body by a mosquito bite, leading to fever, anaemia, splenomegaly and finally death. In the blood stream, malaria parasites invade red blood cells as merozoites, mature to rings and trophozoites, multiply to schizonts and then burst out of the cells, ready to invade further ones. This cycle has been the subject of intense research because it is the cause of clinical symptoms and therefore the major target of antimalarial treatment. The digestion of haemoglobin by all *Plasmodium* species results in the accumulation of a metabolic byproduct, the malaria pigment, and in morphological changes of the red blood cell, which are typically characterized with bright-field microscopy[2,3]. These changes are likely associated with alteration of red-blood-cell topology and mechanics, which are little understood.

To explore correlations of the *Plasmodium*-induced molecular, topographical and mechanical changes, we investigated infected red blood cells with atomic force microscopy (AFM)[4-6], phase contrast[7,8] and total internal reflection fluorescence (TIRF) microscopy[9]. By combining these imaging methods, we could correlate the morphological changes of red blood cells with the Plasmodium falciparum developmental stages. Furthermore, the comparative analysis of the optical and AFM images facilitated a more detailed identification of parasite development stages, compared to bright-field microscopy, without the need of contrast materials.

We are going to extend these studies, carried out on fixed cells, by investigating infected red blood cells with AFM in cold aqueous solutions, slowing down parasite maturation. This protocol may provide conditions close to those realised in the human body to trace morphological and mechanical properties of the infected cells during the maturation of the parasites.

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HETEROGENEOUS CATALYTIC 1,4-ADDITION REACTION IN THE PRESENCE OF SUPPORTED METAL CATALYSTS

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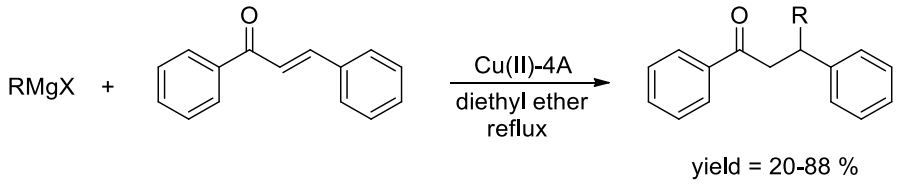
In our research group the applicability of different mineral based supported catalysts in organic reactions has been investigated for years. During this work among others the A^3-coupling reaction[1] and the Chan-Lam-coupling[2] of amines and boronic acids were realized in the presence of a molecular sieve supported copper(II)-catalyst.

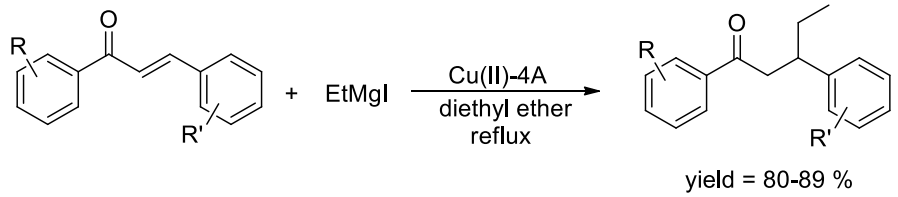
During my research I have investigated the applicability of the heterogeneous molecular sieve supported metal catalysts prepared earlier in our research group. For a model reaction we chose the selective 1,4-addition reaction of various *Grignard*-reagents to chalcone in the presence .[3] molecular sieve supported copper(II) catalyst [Cu(II)-4A]. The reaction conditions were optimized in the reaction of ethylmagnesium-iodide and chalcone in diethyl ether. Under the optimized reaction conditions chalcone was reacted with different *Grignard* compounds (Figure 1).



R = Et, ^{n}Bu, cyklohexyl, Ph, 4-MePh, 3-MePh, 2-MePh, 3-CF_3Ph, 4-MeOPh
X = I, Br

Figure 1. Selective 1,4-addition of different Grignard compounds to chalcone

After the preparation of the corresponding derivatives we wanted to extend the reaction to other chalcone derivatives and examined the effect of different substituents placed on the aromatic rings of the chalcone (Figure 2). Furthermore we investigated the effect of other various supported metal catalysts on the selectivity of the 1,4-addition.[4] During the work the reusability and recyclability of the catalysts were tested as well.



R = H, Me
R' = H, Me, OMe, Cl

Figure 2. Extension of the selective 1,4-addition reaction to substituted chalcones

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SELECTIVE HETEROGENEOUS CATALYTIC HYDROGENATION OF NITRILES TO PRIMARY AMINES OVER PALLADIUM

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Amines constitute important and valuable intermediates in the area of pharmaceutical, plastic and agrochemical industry. There are several methods for the synthesis of amines including the reduction of nitro compounds, and the reductive amination of oxo compounds. However, the heterogeneous catalytic hydrogenation of nitriles is the most widely used process in the pharmaceutical industry.[1]

Although conversion of the nitrile group to a primary amine is relatively easy to accomplish, the selectivity of the reaction may be strongly decreased due to secondary and/or tertiary amines formed in the side reactions (*Figure 1*).[2,3] The amount of secondary and tertiary amines can be effectively minimized by applying excess of ammonia (5–6×), but only in case of Raney® nickel or cobalt catalysts.[4]

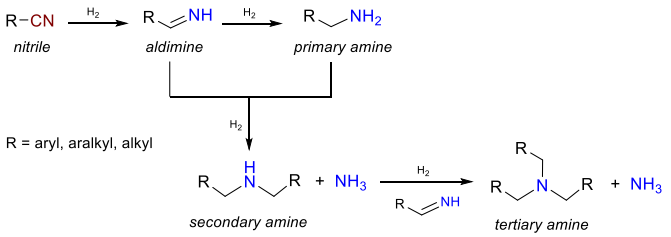

Figure 1. Catalytic hydrogenation of nitriles, reaction mechanism.

Our previously developed process[5] allows the efficient and selective, Pd-catalysed heterogeneous catalytic hydrogenation of nitriles to the corresponding primary amines in liquid phase. Using this method, benzonitrile was hydrogenated to benzylamine under mild reaction conditions (30 °C, 6 bar) with 95% selectivity and 90% isolated yield, but the reduction of benzyl cyanide to 2-phenylethylamine resulted in lower primary amine selectivity (45%).[6] Extending this method to the hydrogenation of 3-phenylpropionitrile, the selectivity to 3-phenylpropylamine and its isolated yield were lesser (26% and 20%, respectively).

To clarify these differences in the selectivities, quantum chemical calculations (DFT) were performed. The computations revealed that the diverse adsorption abilities of the imines could influence the selectivity of these hydrogenations. The calculated adsorption energies (ΔE_{ads}) of these intermediates on Pd(111) showed that their adsorption strength decreases in the following order: benzaldimine > 2-phenylethylimine > 3-phenylpropylimine, *i.e.* there is a much higher possibility for side reactions in case of 3-phenylpropylimine.

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SEQUENCE INDEPENDENT QUANTIFICATION OF THE SURFACE DENSITY OF DNA PROBES ON DNA MICROARRAYS BY SPRi

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In terms of hybridization assays surface plasmon resonance imaging (SPRi) offers high throughput, label-free and real-time monitoring of the binding kinetics. This requires DNA microarrays on bare or modified gold SPRi chips, which are generally premade by an off-line microspotting procedure. Therefore, the surface concentration of the immobilized probes is not known although it is an essential quality control parameter, which in case of self-assembly of thiol-labeled DNAs on gold chips can vary in a very broad range. We show that the small molecular weight ruthenium(III) hexamine complex (RuHex) introduced earlier for electrochemical quantitation of DNA coverage on gold electrodes[1] can be used also in SPRi to assess the surface concentration of DNA probes in DNA microarrays.[2] A single injection of RuHex solution enables the visualization and quantification of all DNA spots on a DNA microarray. This provided an excellent control over the DNA surface concentration, which enabled to study its effect on the hybridization efficiency and selectivity of DNA arrays made by microspotting thiol labelled DNA probes both in prehybridized and single-stranded DNA form as shown through the measurement of hsa-miR-208a-3p microRNA target.

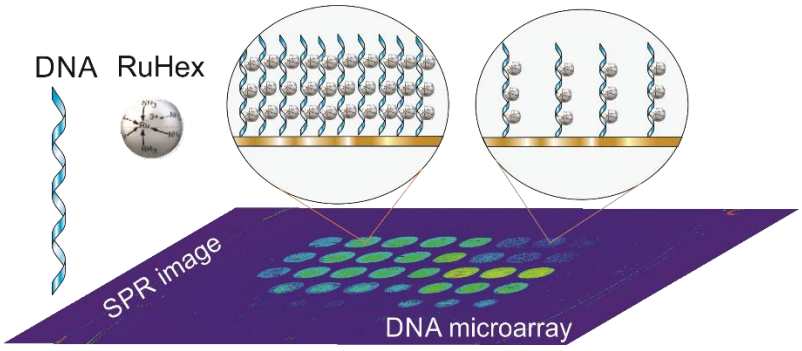

Figure 1.: Schematic illustration of the binding of RuHex to DNA probes on different surface density spots.

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PHYSICAL AGEING OF POLY(LACTIC ACID): FACTORS AND CONSEQUENCES FOR PRACTICE

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Injection molded specimens were prepared from poly(lactic acid) (PLA) and their properties were determined as a function of time to study physical ageing. The mechanical testing of specimens showed that properties change rapidly with time. The stiffness of the specimens increases considerably, while their deformability decreases drastically from 250 % after injection molding to a few percent after less than a day of ageing. Thermal analysis showed that both relaxation enthalpy and the change in the glass transition temperature (T_g) of the polymer increased with time(Figure.1). T_g decreased with increasing ageing time that could not be explained by the generally accepted approach of decreasing free volume[1,2]. The analysis of literature data[3,4] showed that the decrease or increase of T_g depends on the temperature of ageing and on the rate of cooling. T_g decreases at relatively low ageing temperatures, while it increases when ageing temperatures are closer to the T_g of the polymer. Besides the decrease of free volume, the development of internal stresses also plays a role in the determination of the glass transition temperature. Internal stresses result in a decrease of T_g, while decreased free volume leads to an increase. Internal stresses determine deformation and failure mechanism as well; large stresses lead to crazing/cracking and finally to brittle failure, which may hinder the application of PLA in many areas.

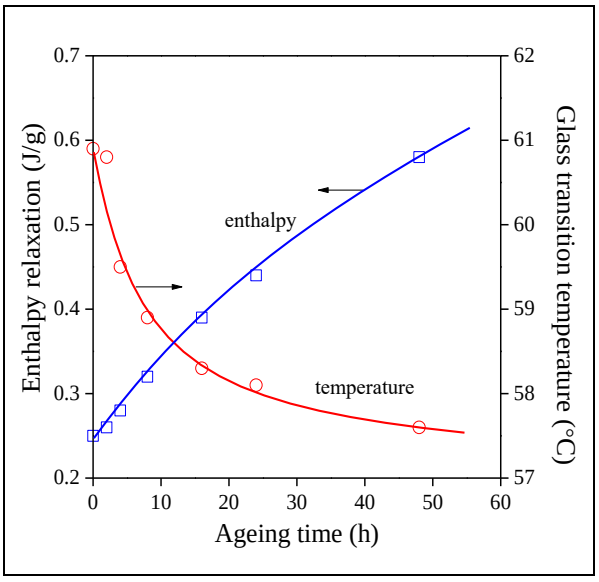

Figure 1. The influence of ageing time on the intensity of enthalpy relaxation and the glass transition temperature of PLA. Symbols: (○) T_g, (□) enthalpy relaxation.

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IMPROVEMENT OF BIOACTIVE COMPONENT COMPOSITION IN WHEAT WITH BREEDING

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The current flour quality characterization methods are primarily based on the qualitative and quantitative analysis of the protein, gluten and starch components. At the same time, the effects of the minor ingredients, such as non-starch, fibre forming polysaccharides, are not or less taken into consideration. As the quality parameters including the bioactive components of the seeds are synthesised in various phases of the grain filling period, next to the genetic background the environmental conditions prevailing during this process is also very decisive in the relocation of the resources in various plant parts. While there is increasing knowledge on the nutritional role of the different bioactive components, only partial information is available on their impact on the technological traits. Additionally, the current flour testing methods are not always suitable for studying the role of minor ingredients, like fibre components.

The main goals of our study were to investigate (a) the GxE variability of the modern wheat varieties and they breeding lines for improving their fibre composition; (b) the rheological character of wheat dough made from selected wheat varieties and lines and (c) the role of AX in the formation of dough mixing properties.

To reach these goals, wheat varieties and breeding lines - produced with targeted breeding – were investigated in different harvest years. After determination of AX properties, samples were narrowed to examine the protein and starch composition and to study the rheological behaviour. After complex evaluation, some lines were selected for model studies.

The arabinoxylan properties of breeding lines show great variability, some of them have higher AX content than their parents. Many breeding lines were promising for different test parameters, but the findings are not yet generalized, further investigations are needed. The effects of genetic variability are obvious, on the other hand, environmental variability has to be taken into account as well. The dough system studies show that the arabinoxylans affect the dough development time, the stability and the degree of softening as well. For deeper investigation the role of AX, model dough systems made from isolated gluten starch and various arabinoxylan addition were also formed and studied.

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OPTIMIZATION OF THE POLYMERIZATION CONDITIONS OF THERMORESPONSIVE POLY(NIPAM-CO-AAC-CO-TBAM) MICROGELS FOR THE MOLECULAR IMPRINTING OF LYSOZYME

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Stimuli-sensitive polymeric microgel/nanogel particles (NPs) exhibiting volume phase transition in response to slight environmental changes, such as temperature, pH, ionic strength, light, etc., have attracted a widespread interest in the past thirty years due to their potential applications in numerous fields.[1] An interesting application originating from Shea's group is the selective and reversible binding and release of certain peptides and proteins by the NPs upon thermal stimulus.[2-3] These NPs have optimized monomer composition; they contain 53 mol% N-isopropylacrylamide (NIPAm), 5 mol% acidic monomer (acrylic acid, AAc), 40 mol% hydrophobic monomer (N-tert-butylacrylamide) and 2 mol% crosslinker (N,N'-methylene-bis-acrylamide). Free-radical emulsion polymerization of these particles is carried out in the presence of sodium dodecyl sulfate (SDS) at 60^0C, whereby narrowly dispersed NPs of approx. 88 nm diameters are formed.

To improve the selectivity of these NPs we were aimed at using molecular imprinting technology i.e. to prepare the particles in the presence of the template protein (lysozyme). For this purpose, however, the polymerization conditions had to be modified, because preliminary investigations showed that the protein was denatured at 60^0C and in the presence of the detergent. Therefore, we opted for surfactant-free polymerization and for the use of a redox-initiator system that allows polymerization at room temperature or at 40^oC (close to body temperature). Two different redox-initiator systems were tested, namely ammonium persulfate (APS)–tetramethylethylenediamine (TEMED) and APS–sodium metabisulfite (SBS). The size, uniformity and shape of the resulting NPs were confirmed by scanning electron microscopy and dynamic laser light scattering (DLS). DLS was also used to investigate the thermoresponsive behaviour of the prepared NPs. HPLC analysis was used to follow the kinetics of polymerization and the rate of incorporation of the different monomers. The molar ratio of the monomers in the final polymer was determined by NMR measurements. Zeta potential measurements confirmed the incorporation of the negatively charged AAc into the NPs. The binding affinity of the different NPs towards lysozyme was also evaluated. Compared to the APS–TEMED redox pair it was found that NPs are colloidally much more stable when the APS–SBS redox pair was used as the initiator. We have found significant differences in their lysozyme binding affinity as well as their thermoresponsive behaviour also.

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DEVELOPMENT OF A LAB-SCALE CONTINUOUS CRYSTALLIZATION PROCESS WITH TURBIDITY-BASED SAFETY AND CONTROL METHOD

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During the past decade, the research and development of continuous processes in pharmaceutical industry have emerged, in parallel with the ongoing evolution of modern PAT (Process Analytical Technology) tools. As a promising alternative to batch-type process, continuous crystallization is a highly investigated topic with several prominent publications [1].

The main objective of our project was to develop a robust continuous crystallization method in laboratory scale that produces a desired polymorph under controlled conditions (*Figure 1*). An MSMPR (Mixed-Suspension-Mixed-Product-Removal) crystallizer was applied to implement the antisolvent crystallization of spironolactone hydrate in acetone-water system.

Our development work focused (1) on the investigation of model ternary system via batch experiments and (2) on the development of an automated, continuous crystallization method to produce drug substance. A novel (3) turbidity-based method was applied for level control to ensure the stability and safety of the process, moreover, (4) stationery state was monitored online by FBRM-probe in a custom-designed measurement cell. Finally, (5) a full factorial DoE (Design of Experiments) was implemented to investigate the effect of process parameters on the main product attribute: the particle size distribution.

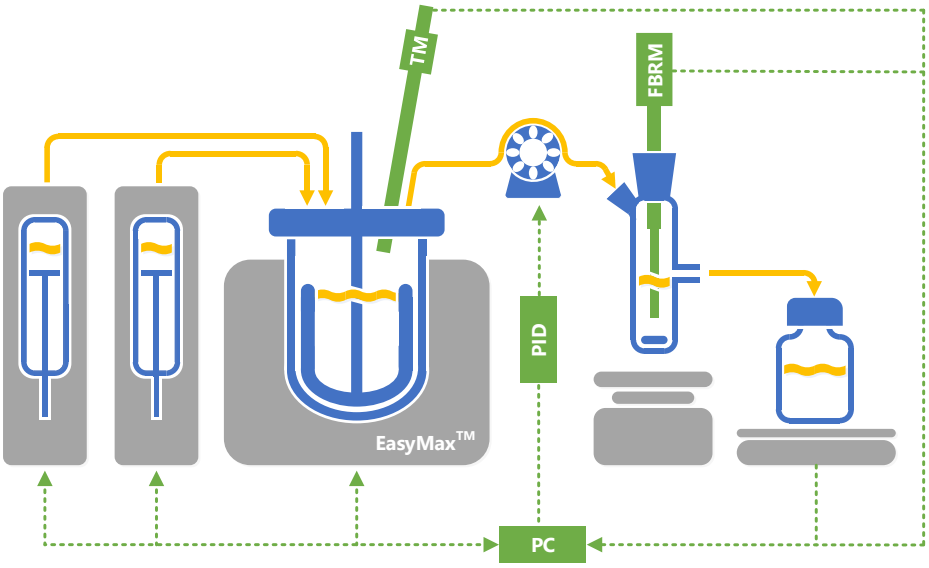

Figure 1. Developed one-stage continuous MSMPR crystallizer.

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DEGRADATION OF PLA FIBERS CONTAINING AN ENZYME

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[2] *Research Institute for Natural Sciences*
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Controlled biodegradation is one of the criterias that a scaffold must accomplish [1]. This can be done with the help of enzymes. In this work proteinase K enzymes were incorporated into PLA fibers via electrospinning technique then their degradation was studied. As reference PLA fibers were degraded by enzymes which were outside of the fibers in the degrading media. Degradation was described by the change in weight, pH and concentration of lactic-acid in the surrounding buffer media.

The degradation was observed to be different on a macroscopic scale. When the enzymes were outside of the fibers we can observe a maximum during degradation, however when the enzymes were inside of the fibers the tendency shows to be constantly increasing (Fig 1a, 1b). The effect was observed at other characteristics as well.

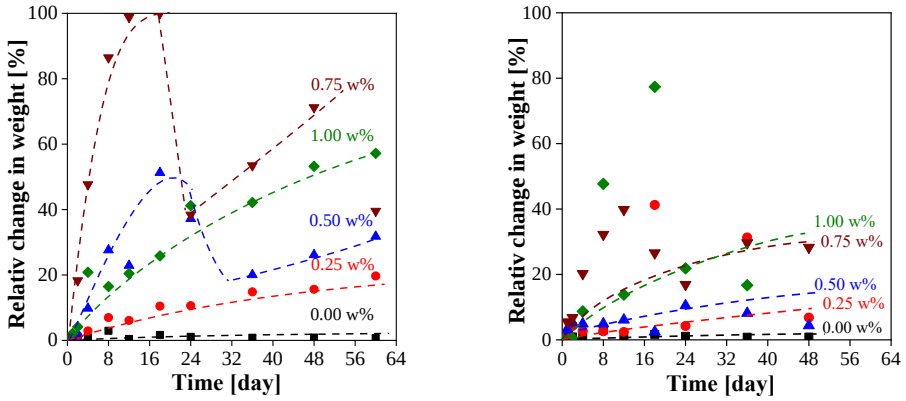

Figure 1: Relativ change in weight during the degradation when the enzymes were in the degrading environment (a) and in the fibers (b)

Although enzymes were succesfully incorporated and they could degrade the fiber from within the exact mechanism needs to be further investigated. The observed effects show differences between the two mechanisms but their exact explanation is still a question of further research.

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TISSUE- AND DEVELOPMENT-SPECIFIC EXPRESSION PATTERN OF DUTPASE ISOFORMS

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The enzyme deoxyuridine 5`-triphosphate nucleotidohydrolase (dUTPase) is a prime example of coupling the nucleotide biosynthesis and genome integrity as it generates dUMP for thymidylate biosynthesis and removes dUTP for synthesis of uracil-free DNA [1]. In eukaryotic organisms, dUTPase is usually present in two isoforms, one of these is nuclear, the other is mitochondrial/cytoplasmic [2]. Because of the significant role of the enzyme, our aim was to describe the tissue- and development-specific expression pattern of the dUTPase isoforms in mice.

We developed a highly reliable and well controlled reverse transcription–quantitative real-time polymerase chain reaction (RT-qPCR) method for the simultaneous quantification of the expression levels of the nuclear and the mitochondrial isoforms of dUTPase. During the optimization procedure, all of the critical design parameters were taken into consideration, in addition the performance of the assay in terms of specificity, efficiency, precision and the linear dynamic range was evaluated as well. Using the developed RT-qPCR method, we carried out a detailed analysis regarding the tissue- and development-specific expression pattern of dUTPase isoforms. To determine the tissue-specific expression pattern of the dUTPase isoforms we analyzed 8 organs (brain, heart, kidney, liver, lung, ovary/testicle, spleen and thymus) of 10-week-old male and female mice and the results were evaluated by statistical analysis of the data. In the development study, 8 organs derived from 2-, 4- and 10-week-old stages for both male and female and 1-year-old stage for male mice were investigated with the optimized and validated RT-qPCR method.

With regard to the tissue-specific expression of dUTPase isoforms in adult mice, we observed that the nuclear isoform is expressed at a high level in thymus, spleen and reproductive organs. Heart, liver, kidney, ovary and brain are organs known to be rich in mitochondria. However, elevated level of expression of the mitochondrial isoform of dUTPase was found only in heart, kidney and ovary. Only a small difference in the expression of the mitochondrial isoform was found in the other organs. Based on our results, it can be stated that the mitochondrial isoform of dUTPase can be regarded as a potential reference gene for studies involving comparisons between different organs in mice [3].

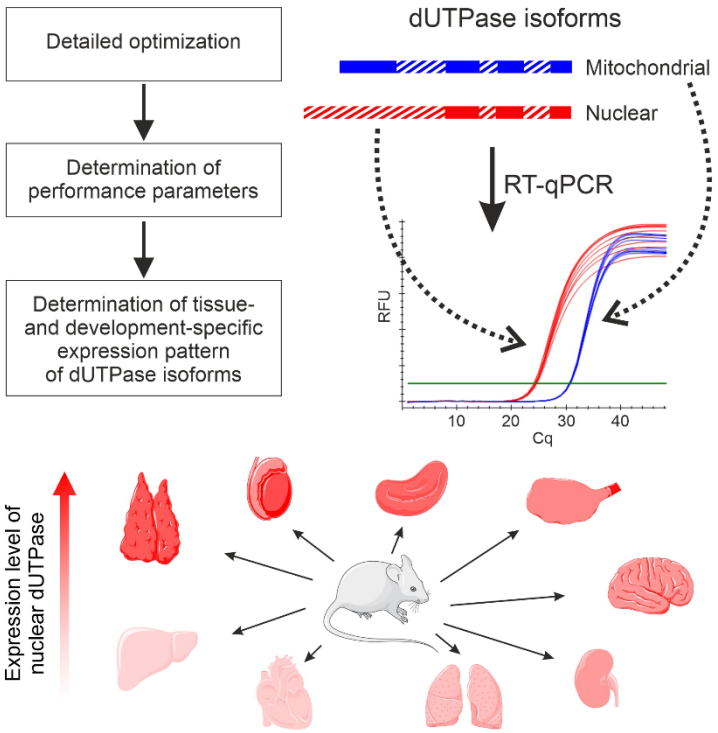

Figure 1. Schematic figure of the method and the tissue-specific expression pattern

With regard to the development-specific expression of dUTPase isoforms, changes in the expression level of the mitochondrial isoform are generally smaller through development as compared to the nuclear isoform, roughly 2-fold differences were the largest observed. In contrast, in case of the nuclear isoform, an approximately 6-fold decrease in the expression level was the largest difference observed in the liver from the 2-week-old to the 10-week-old stage.

It has been previously described, that in human cells the expression level of the mitochondrial isoform of dUTPase is constitutive and independent of cell cycle phase or proliferation status of the cell. In contrast, both the expression level of the nuclear isoform of dUTPase and the mRNA levels are tightly regulated to coincide with nuclear DNA replication [4]. In the adult tissues most cells lose their ability to proliferate actively with the exception of tissues such as thymus, spleen and testicle. The results suggest that the expression level of the nuclear isoform of dUTPase coincide well with DNA replication in proliferating cells through the developmental stages.

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CINCHONA-DECORATED CYCLODEXTRIN - A RECYCLABLE ORGANOCATALYST

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Over the last two decades organocatalytic methodologies have become an attractive tool in asymmetric synthesis. Still, the laborious product purification and recycling of high value organocatalysts call for the design of more flexible and sustainable strategies. The membrane assisted recovery of homogeneous organocatalysts is feasible with low energy consumption, and its scale-up and implementation in continuous and hybrid processing are relatively straightforward.[1] However, the efficiency of separation depends on the molecular weight gap between the catalyst and the other components, and the absolute catalyst retention by the membrane. Thus, size-enlargement of the catalyst is usually required.

This work presents a cyclodextrin-enhanced organocatalytic method for asymmetric synthesis.[2] Cinchona-thiourea and cinchona-squaramide catalysts were covalently anchored to permethyl-β-cyclodextrin and applied in Michael reaction. Both alternative and conventional solvents were screened for the asymmetric addition (up to 99% ee). Continuous organocatalysis was performed in a coiled tube flow reactor coupled with a membrane separation unit (80 g L^{-1} h^{-1}), allowing complete recovery of the catalyst and 50% solvent recycling. Further development of this catalysis-separation methodology could extend the alternatives and facilitate the efficient production of enantiopure chemicals.

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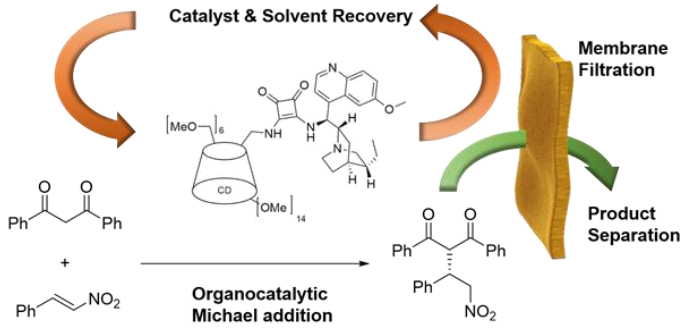

Figure 1. Cinchona-decorated cyclodextrin catalyzed Michael addition in a synthesis-separation integrated flow reactor

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ENZYMATIC METHODS FOR MICRORNA DETECTION WITH SURFACE PLASMON RESONANCE IMAGING

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MicroRNAs are typically 21-25-nucleotide long, natural, non-coding RNAs. [1] It was discovered that miRNAs are playing important role in gene regulation and their level can significantly deviate in various disease states. Since they were found also remarkably stable, the use of miRNAs as tissue specific biomarkers in molecular diagnostics and prognosis generated a considerable interest. However, microRNA concentration in the circulation is generally extremely low, direct methods can hardly guarantee adequate limit of detection. Therefore, their reliable assessment even with highly sensitive detection techniques is required to be complemented by special sample preparation and signal amplification. Here we explored the feasibility of coupling surface plasmon resonance imaging with various enzymatic amplification methods for the detection of hsa-miR-208a microRNA, a potential biomarker of acute myocardial infarction. The selectivity of the microRNA detection was achieved by immobilizing complementary PNA (peptide nucleic acid) probes to a planar gold chip by microspotting. [2] By itself the hybridization largely limits the detection of microRNAs to the nanomolar concentration range. To further improve the limit of detection we explored the use of two enzmyatic amplification methods (Nucleic Acid Sequence Based Amplification — NASBA) and DNA modified nanoparticle-amplified polyadenylation. NASBA was carried out in solution phase prior to detection using a mixture of three enzymes and two primers to exponentially amplify the microRNA sequence. [3] The polyadenylation reaction, however, was conducted in situ as a surface confined enzymatic strand elongation reaction, i.e., a poly(A)-tail is synthesized to the 3' terminus of the microRNA. [4] The signal could be further amplified by coupling the enzymatic elongation with DNA modified gold-nanoparticle hybridization in a subsequent step. With these methods we achieved selective, sensitive and real time detection of microRNA in the diagnostically relevant concentration range.

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