Final report on Postdoctoral OTKA project PD-131467

Nanostructured catalyst systems for sustainable biotransformations

Enzymes catalyze the production of biologically active molecules under mild conditions and due to their substrate selectivity and high catalytic efficiency enzyme-based biocatalysis has a growing potential in many fields of science and industry. For example, the development of synthetic chemistry, bioanalytical methods and medical treatments are the most common sectors which could be supported by enzymatic processes. However, enzymes could have unique advantages their structures are quite sensitive against environmental conditions, thus their biocatalytic function could be lost. In addition, enzymes in their native forms lead to difficulties in separation and purification of valuable products. Enzyme immobilization can solve these problems due to the appropriate methods enzymes can be stabilized and separated easily. Among several enzyme immobilization strategy, the combination of bio- and nanotechnology can be promising novel research field. The structural and functional nanomaterials can be potential carriers with stabilizing effects for enzyme molecules without limiting their natural functionality.
This research focused on the systematic development of nanostructured enzyme carriers as nonporous networks, surface functionalized nanoparticles, tubes and fibers. To cover wide range of enzymes and demonstrate the general applicability of nanomaterials, wild-type crude enzymes and recombinant proteins were also applied for immobilization studies. The basic aim of this project was the fusion of different, rationally designed nanomaterials and enzyme immobilization technology for stable, efficient and easy-to-handle nanobiocatalyst, which can be efficiently applied for selective biotransformation.

Objectives

Objective 1. Enzyme attaching onto functionalized nanomaterials
By the development of rationally functionalized non-magnetic (SiO_2) and magnetic (Fe_3O_4) nanoparticles, so called smart particles could be achieved, which have ideal surface properties (inert and reactive linkers for enzyme attaching) for the binding of protein molecules. This objective was accomplished in the first two years of the project (*1st year from 01.12.2019 to 30.11.2020. 2nd year from 01.12.2020. to 31.11.21.*).

Novel functionalized SiO_2 and iron-oxide based nanoparticles (SNPs and MNPs respectively) and carbone nanotubes which were able to immobilize enzymes via the mixing of adsorptive and covalent interactions were performed.
SNPs were synthetized at different size form 50 nm–1000 nm from alkoxysilanes applying Stöber method and modified by alkyl-, and aryl-substituted organosilanes (propyl-, vinyl-, octyl- and phenyl-trimethoxysilanes), which can immobilize enzymes by adsorptive interactions. The preliminary experiments showed that the particle size has significant effect on the immobilization efficiency and biocatalytic activity of immobilized *Ca*L B (*Candida antarctica* lipase B) enzyme. Spherical SNPs at 600 nm particle size had been selected for further carrier optimization, when the hydrophobic organosilanes were combined with amino-group containing surface modifier silane agents (3-aminopropyltrimethoxysilanes) to compose binary mixtures. The application of the binary mixture of inert (alky or aryl-substituted organosilanes) and reactive (amino-substituted organosilanes) modifiers so-called

mixed-grafted surface of SNPs could be produced. These mixed-functionalized particles are able to represent adsorptive enzyme immobilization by their inert groups and potential covalent binding place based on the chemical cross-linking of amino-group on the surface and amino acid side chains (mainly Lys, Cys, Ser) of the enzyme via bis-epoxy cross-linking agents. By this method so-called cross-linked enzyme adhered nanoparticles (CLEANs) could be created, which brings internal nanostructure, however, presents micro-sized solid particles. If the structure of CLEANs and simple particle-free CLEAs (cross-linked enzyme aggregates) are compared, it can be observed, that particles can form more porous and definitive structure than CLEAs which has a dried gel structure (**Figure 1**).

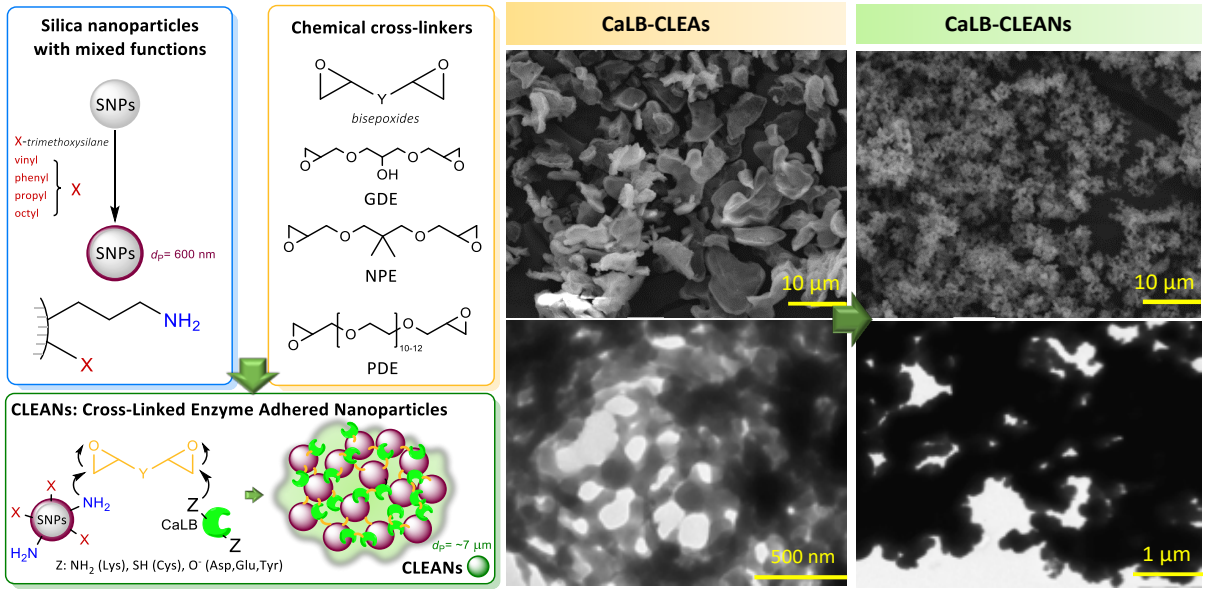

Figure 1. Production of cross-linked enzyme adhered nanoparticles (CLEANs) applying functinalized SiO_2 nanoparticles with binary mixture of organosilanes (vinyl-, phenyl-, propyl- and octyl-trimethoxysilane as inert and 3-aminipropyl-trimethoxysilanes as reactive linkers) (SNPs) and bisepoxy cross-linkers (GDE: glyceroldiglycidyl ether, NPE: neopentylglycol diglycidyl ether and PDE: polyethyleneglycol diglyicidyl ether). Morphological comparison of simple CLEAs (Cross-linked enzyme aggregates, without nanoparticles) and CLEANs by electron microscopic (SEM and TEM) images.

We proofed that the fine-tuning of molar ratio of inert and reactive groups can significantly affect the activity of the immobilized enzyme. Detailed morphological investigation was also carried out, in which electron microscopy (by SEM and TEM), porosity determination (by BET) and particle size analysis (based on Mie-scattering) were used for the characterization of the initial carriers and the immobilized biocatalysts as well. Results clearly showed that there no significant difference between the morphological and physicochemical properties of immobilized CLEANs biocatalyst, thus the binding linkers of the surface and the length of the cross-linkers affect the enzymatic activity. For demonstration of effectiveness of this novel immobilization method and carrier system, lipase B from *Candida antarctica* (*Ca*L B) was immobilized and our biocatalyst was successfully applied at the first time in the continuous-flow solvent-free synthesis of fragrance esters (from cinnamyl alcohol and geraniol). [P1]

Based on the results with SNPs, functionalized magnetic nanoparticles (MNPs) had been also performed for adsorptive and covalent immobilization of enzymes. The systematic investigation of hydrophobic surface modifiers (series of alkyl-and aryls substituted organosilanes with increasing chain-length from methyl- to decyl-substituents) it could be seen that an optimum level can be found in the lipophilicity (log*D*) of the silane modifiers

(phenyl-trimethoxysilane was the most efficient for lipase adsorption). However, enzyme adsorption is an easy and rapid way to immobilize enzyme, the leaching may cause difficulties. Thus, similarly to the study with SNPs, MNPs were functionalized with the binary mixture of phenyl- and 3-glycidoxypropyl-trimethoxysilane, where the epoxy-silane could directly form covalent binding with proteins. Results showed that higher biocatalytic activity could be achieved with the dual functionalized MNPs (where the phenyl and epoxy ratio was 1:4) than simple mono-functionalized MNPs. The developed biocatalyst was applied for kinetic resolution of racemic secondary alcohols (phenyl-1-ethanol, phenyl-1-propanol, and 2-octanol), and magnetic nano-biocatalyst provided better activity, enantioselectivity and reusability to compare with the commercially available covalently attached *Ca*L B (ChiralVision T2-150^{TM}). Now to continue this study, MNP-*Ca*L B biocatalysts are going to apply for the enzymatic degradation of polyesters, which mean a promising alternative for the elimination of plastic waste. *Ca*L B attached to MNPs could have better access to the polymer macromolecule than the commonly applied porous beads (for example Novozym435^{TM}), in which *Ca*L B immobilized inside the deep pores, thus more effective and easy-to-separate process could be achieved. [P2]

The immobilization of a novel recombinant aspartate ammonia-lyase (AAL) from *Pseudomonas fluorescens* (*Pf*AAL), which catalyzes non-oxidative ammonia elimination of L-aspartate on functionalized MNPs was also investigated. The AAL coding gene from (*Pf*AAL) R124 was expressed in competent *E. coli*, then the purified *Pf*AAL was directly attached on MNPs via covalent bonds. In this study a two-step procedure was applied for the functionalization of MNP, firstly, primary amine groups were created, then bifunctional epoxy agent was applied to ensure covalent binding place for enzyme molecules. The AAL-MNPs catalysts were well-applicable for traditional enzyme kinetic investigations, which could open new possibilities for optimization studies about covalent immobilization. In addition, results showed that the glycerol addition significantly enhanced the storability of PfAAL-MNP, providing a biocatalyst could be used in the long term. [P3] The immobilized AAL biocatalyst showed good stability and well applicability in different type of magnetic reactor systems as well. The specific enzyme activity of AAL immobilized on epoxy-MNPs in traditional shake vial method was compared to two in-house-made devices designed for magnetic agitation. The first device agitated the magnetic biocatalyst by moving two permanent magnets at two opposite sides of a vial in x-axis direction (being perpendicular to the y-axis of the vial); the second device unsettled the MNP biocatalyst by rotating the two permanent magnets around the y-axis of the vial. In a traditional shake vial, the substrate and biocatalyst move in the same direction with the same moving pattern. In case of magnetic agitation, the MNPs responded differently to the external magnetic field of two permanent magnets. In the axial agitation mode, MNPs formed a moving coherent fluid cloud inside the vial, whereas in the rotating agitation mode, they formed a ring. Especially, the rotating agitation of the MNPs enabling the well mixing of the reaction mixture, leading to enhanced effective enzymatic activity. [P4]

Bis-epoxy agents as covalent binding linkers was also introduced for the modification of single-walled carbon nanotubes (CNTs). A three-step surface modification process was developed in collaboration of Applied Biocatalysis Research Center of Babes-Bolyai University. At first, CNTs with activated carboxylic groups were reacted bisamine compounds with different length, then a bisepoxy agent was applied for modification, similarly than in case of ALL-MNPs biocatalyst. CaLB-CNTs nanobiocatalyst showed high activity in kinetic resolution of racemic

secondary alcohols (1-phenyl ethanol and its *p*-brom, *p*-chlor, *p*-methyl, *p*- and *o*-nitro derivatives) in continuous-flow mode. The effect of various process parameters such as temperature, flow rate and substrate concentration and solvents on the biocatalytic activity was systematically investigated. The long-term activity and selectivity of the biocatalyst were preserved, enabling hundred-gram scale resolution of model substrate rac-1-phenylethan-1-ol. [P5]

Objective 2: Enzyme entrapment into nanostructured materials

In the 2nd and 3rd year (part I: *7 months from 01.12.2021 to 01.07.2022 and part II: 5 months from 01.02.2023. to 05.31.2023) because of maternity leave*) electrospun nanofibers offer unique possibility for the rapid and stable entrapment of biomolecules. In this project water soluble and resistant polymer nanofibers were developed which were successfully applied for the immobilization of crude and recombinant enzymes. Results demonstrated that enzyme loaded nanofibers could be efficient biocatalyst for selective synthesis of biologically active compound and potential drug formula for enzyme-based therapies.

In the project an emulsion electrospinning technique had developed for enzyme immobilization. The *water in oil* emulsion formation during nanofiber production allows the application of polymers with high water and solvent resistance (such as poly lactic acid, which is dissolved in the organic phase) but the introduction sensitive enzymes could be also manageable due to protective effect of isolated aqueous phase. A recombinant phenylalanine ammonia lyase (PAL) with a sensitive heteromeric structure had been successfully encapsulated in polylactic acid (PLA) nanofibers applying two-phase precursors (buffered aqueous phase containing enzyme and organic phases containing PLA). We found that the selection of emulsifier based on their hydrophilic lipophilic balance (HLB) values significantly affect the fiber fabrication (fiber productivity), the fiber morphology (average fiber diameter and distribution) and especially the enzyme activity. Our study showed that HLB has an optimal level, and they can affect directly the enzymatic action and the internal structural of the polymeric nanofibers as well. [P6]

Poly(vinyl alcohol) (PVA) nanofibers were also produced based on electrospinning technique for the entrapment and solid formulation of selected lipases. The aim of these immobilization was to create enzyme-based formulas which could be applied for enzyme replacement therapies (ERT). There are several diseases caused by lysosomal storage disorders or metabolic malfunctions which could be treated by ERT. However, lots of lipase-based solid formulas are available for the treatment of gastrointestinal (GI) problems, they follow the traditional tablet or capsule formulas which have limitations. Nanofibrous lipases could bring new possibilities in ERT, since they can ensure fine-tunable, rapid and sensitive way of enzyme immobilization. For immobilization experiments lipase form *Burkholderia cepacia* and *Aspergillus oryzae* were selected, because of their high activity in the digestion of nutritive fatty acid esters. Lipases were templated with different cyclodextrins (CDs) since they are known to enhance the activity of enzymes in a complex process due to their specific binding. We found that by selecting the appropriate CD:lipase ratio, the activity of the investigated enzyme could be multiplied. Based on Raman-mapping it can be stated that cyclodextrins can support the homogeneous dispersion of lipases inside the PVA nanofibers as well. Entrapment of lipases in PVA nanofibers led to a significant increase in activity compared to the native enzyme or some commercially available formulas. [P7] This research had been extended to lipase from *Porcine pancreas* (*Pp*L), which is a widely used enzyme in medical treatments related to ERT. In case of *Pp*L entrapment, a comprehensive investigation about the

formulation of applying electrospun polymeric nanofibers was performed. Polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), which has good water solubility and water-resistant polylactic acid (PLA) were selected as nanofibrous matrices. In case of PLA, the entrapment in to PLA nanofibers and adsorption on to PLA nanofibers were compared to investigate the effect of enzyme localization. The effect of the type, molecular wight and concentration of polymers were systematically investigated in the immobilization of PpL then PpL loaded nanofibers were tested in ester hydrolysis under standard and GI simulated conditions (**Figure 2**).

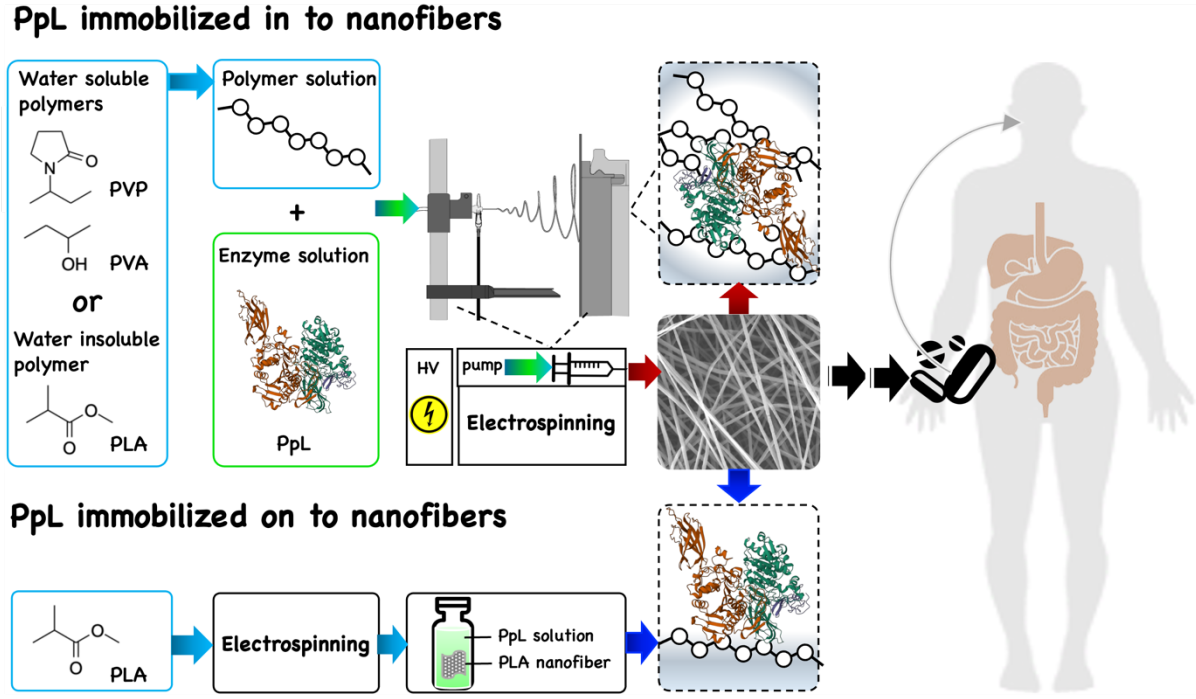

Figure 2. Strategies for the immobilization of the digestive enzyme PpL (lipase from *Porcine pancreas*) into and onto electrospun nanofibrous matrices by the application of water-soluble polymer (PVP: polyvinylpyrrolidone or PVA: polyvinyl alcohol) or water-insoluble polymer (PLA: polylactic acid).

We have found that the feasibility of electrospinning and the structure of the nanofibers produced is largely determined by the viscosity of the precursor used, which is dependent on the quality and concentration of the polymer. Our experiments show that the presence of *Pp*L in any of the precursors reduces the viscosity of the solutions as well as the average diameter of the nanofibers that can be formed from them. This phenomenon is presumably due to conformational changes resulting from interactions between *Pp*L and the matrix polymers, but to be certain this would need to be confirmed by computational chemistry. Raman mapping was used to verify that *Pp*L was successfully immobilized in all the prepared nanofibers and that the distribution of the *Pp*L in the fibers was homogeneous. Karl-Fischer titration has showed that the protein immobilization process has no effect on the residual water content of the fibers. PALS measurements showed that the decreased free volumes of nanofibers could prove the embedding of protein molecules in polymeric nanofibers. The quality of the polymer matrix used had a decisive effect on the extent of enzyme activity achieved. In the case of water-soluble fiber formulations, the use of PVP matrix allowed higher enzyme activity to be achieved than PVA matrix. This is most likely due to differences in the strength of the interactions between the polymer and *Pp*L. The highest enzyme activity could be achieved by using the PLA matrix. Based on a comparison with commercial formulations, we can conclude that *Pp*L formulations immobilized using nanofibers showed comparable

activity with commercial *Pp*L formulas, however *Pp*L entrapped into PLA nanofibers showed higher activity than any of the commercial *Pp*Ls. Based on the experiences presented in this research, we can state that it is worthwhile to continue with the research of nanofibrous formulation of therapeutic enzymes. [P8]

Enzymes also can be used as therapeutic agents in basic skin care and medical treatment related to excessive sebum production, acne, and inflammation. The traditional formulations available for skin treatment, such as creams, ointments or gels, are widely applied, however, the good drug penetration properties, stability, or patient adherence commonly mean challenges. Nanoformulated drugs offer the possibility of combining enzymes and small molecule drugs as well, making them a new alternative in this field. In this study polymeric nanofibrous matrices made of polyvinylpyrrolidone (PVP) and polylactic acid (PLA) were developed for the encapsulation of lipases from *Candida rugosa* and *Rizomucor miehei* and antibiotic compound nadifloxacin. The effect of the type of polymers and lipases were investigated, and the nanofiber formation process was optimized to provide a promising alternative in topical treatment. Our experiments have shown that entrapment by electrospinning induced two orders of magnitude increase in the specific enzyme activity of lipases. Permeability on heat-separated epidermis investigations indicated that all lipase-loaded nanofibrous masks were capable of delivering nadifloxacin to the human epidermis, confirming the viability of electrospinning as a formulation method for topical skin medications. [P9]

Poly(vinyl alcohol) is a widely used polymer for electrospinning, PVA nanofibers is a known enzyme carrier as our research and other examples in the literature shows. However comprehensive and detailed evaluation of the use and characterization of PVA is still a missing area. Thus, a study on the effect of PVA's physico-chemical properties and the electrospinning conditions was performed to explore the basic connections between the parameters of PVA precursors, electrospinning process and the effectivity of enzyme immobilization. Thus, the most commonly used, commercially available PVA with various molecular weights (M_W) and degrees of hydrolysis (DoH) were selected to form nanofibrous polymer matrices (in case of DoH = 88% M_W = 24, 31, 67, 130 and 205 kDa, in case of DoH = 98% M_W = 27, 47, 61, 125 and 195 kDa). The effect of fiber formation conditions (precursor composition, voltage, collector-emitter distance, feed rate) on the morphology of the produced nanofiber systems, the rheological properties of the precursor solutions on of the model enzyme (*Burkholderia capacia* lipase, *Bc*L) was comprehensively investigated. Lipase entrapped in PVA nanofibers were examined by DSC measurements as well. Results showed that the molecular wight and degree of hydrolysis of PVA has significant effect on the enzymatic functions. *Bc*L entrapped in the almost fully hydrolyzed (DoH = 98%), medium sized PVA (MW=61 kDa) showed the best catalytic activity. As an additional conclusion it could be observed that PVAs with 98% DoH provided much better enzymatic activity for the immobilized *Bc*L in all cases than PVAs with 88% DoH. For better understanding the results docking simulations were also performed to explore the molecular background of this phenomenon with computational methods. We conducted molecular docking simulations with constraints that allowed us to interpret the results as an estimation how PVA polymer chains may interact with *Bc*L. As for the receptors, an apo and an inhibitor complexed structure of *Bc*L were used (PDB IDs 2LIP and 1YS1). During the docking calculations, a relatively large grid was applied to investigate whether PVA interacts with the surface of the enzyme or rather with the active site pocket. The docked

ligands were PVA oligomers consisting of 12, 15 and 19 monomer units as ligands. Different DoHs (88% and 98%) were investigated, acetyl groups were added to the ligands accordingly. In all the conducted simulations, the docked PVA ligands were positioned in the active center, indicating that PVA is able to interact with the active center forming residues and behave as a bioimprinting agent. This methodology allowed us to keep only polymer chain mimicking positions for further investigation. Examples for the two cases can be seen on **Figure 3**. In the cases of the smaller ligands (12 and 15 monomers), few docked positions could be interpreted as polymer mimicking results owing to their lesser size. However, the oligomer ligands consisting of 19 monomers were suitable for further evaluation. Naturally, larger oligomers would be even better for our purpose, but ligand size was limited in our current computational setup. The affinity results of the conducted dockings with the 19 monomer long PVA ligands for both receptors showed that PVA with both DoH 88% and 98% are able to interact with the active side residues of *Bc*L. Although in the case of the fully open *Bc*L structure, PVA with 88% DoH appears to have higher affinity, but for the ligand-complexed structure of *Bc*L, which corresponds better to the active state of the enzyme, the affinity results with the two types of ligands are alike. Our findings provide new information about the effect of PVA for the entrapped enzyme giving a comprehensive overlook about polymeric nanofiber-based enzyme immobilization. [P10]

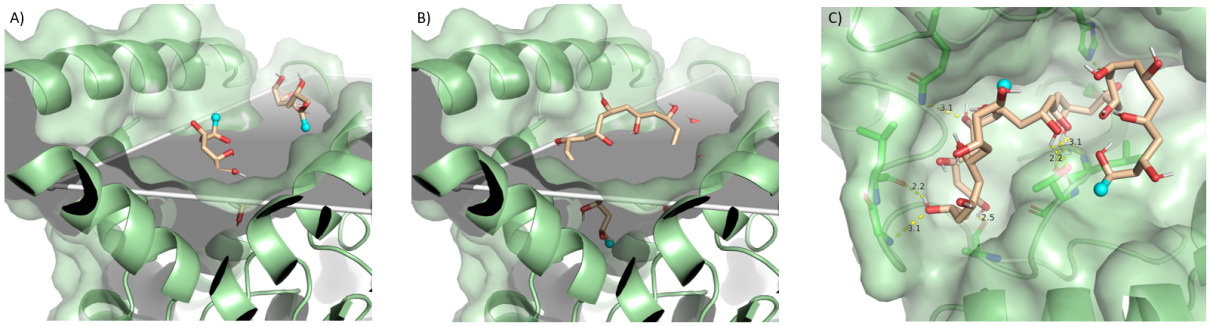

Figure 3. Visualization of docking results on the example of 19 monomer long PVA chain ligand (pale pink, ending C atoms are marked with cyan spheres) and *Bc*L receptor (PDB ID: 2LIP, green). Docking simulation and visualization were carried out with AutoDock Vina program via DockingPie Plugin in PyMol. A) Docked ligand with a position that mimics the possible interactions of a polymer chain. The ends of the ligand (marked with cyan spheres) are above the plane that covers the active center. This conformation can mimic the behavior of a polymer chain. B) Ending atoms of the docked ligand are deeply in the active site pocket, thus it does not represent a part of a longer polymer chain. C) Polar interactions between the docked ligand and the receptor.

Objective 3: Immobilization of biocatalyts with combined nanostructured systems

In the 2nd and 3rd year, we developed novel immobilization methods which produce nanocomposite martials to combine advantageous properties of nanoparticles and nanofibers. Magnetic nanoparticles (MNPs) or SiO_2 nanoparticles (SNPs) with advanced surface properties and polymeric nanofibers as stabilizing, packaging systems were simultaneously applied for the immobilization of enzymes (recombinant transaminase) and enzyme-mimicking agents (CYP450 mimicking metalloporphyrin).

In our study, a novel approach for the purification and immobilization of a recombinant His-tagged transaminase from *Chromobacterium violaceum* (*Cv*TA) has been developed. Simultaneous isolation and immobilization of *Cv*TA was achieved by binding the enzyme on SNPs functionalized with metal affinity linkers (based on IMAC technology) and by entrapping in poly(lactic acid) (PLA) nanofibers (**Figure 4**). Our results showed that the linker length and the nature of the metal ion of the SNPs has significant effect on the enzyme binding efficiency

and biocatalytic activity of *Cv*TA-SNPs. PLA nanofibers produced by electrospinning technique enabled rapid stabilizing embedding of *Cv*TA-SNPs and ensured enhanced stability, activity, and better handling. We showed that this novel approach about in situ isolation and immobilization of a recombinant enzyme could reduce the time required for enzyme isolation, purification and immobilization by more than fourfold compared to traditional stepwise techniques. [P11]

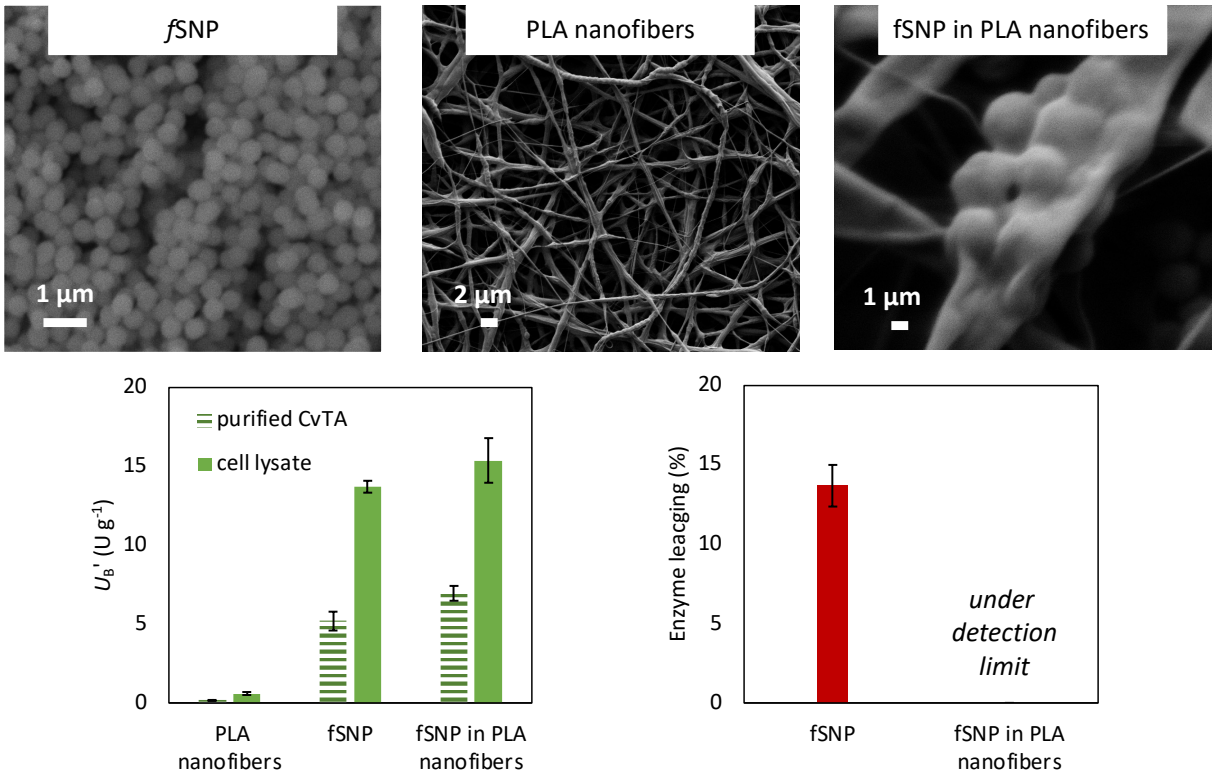

Figure 4. Morphological investigation of CvTA immobilizen on *f*SNP, in PLA nanofibers and purified *Cv*TA immobilized on *f*SNP and entrapped in PLA nanofibers using SEM and the effect of the applied nanocarrier system (*f*SNP, PLA nanofibers and fSNP in PLA nanofibers: *f*SNP loaded PLA nanofibers) on the biocatalytic activity (U_B') of purified *Cv*TA and cell lysate.

In vitro investigations related to enzyme catalyzed biotansformations is a key issue in drug discovery. Among them, metabolic transformations catalyzed by cytochrome P450 (CyP450) enzyme cascade of liver is one of the most important task. Mimicking of certain enzymatic functions has growing importance in many fields since the proper biomimetic agent can replace the cell or enzyme-based test bypassing technological and economical difficulties. Synthetic metalloporphyrins show structural similarity with the heme type prosthetic group of CyP450 as primary hepatic enzyme in oxidative drug biotransformation. Concerning technological and economical aspects, the poor stability and limited solubility of metalloporphyrin, their immobilization onto or into solid carriers can be promising approach to perform sustainable enzyme-mimetic (biomimetic) catalyst. Our work focused on the development a novel immobilized metalloporphyrin (FeTPPS: 5,10,15,20-tetrakis (4-sulfonatophenyl) porphyrin iron III chloride) nanocomposite system and its potential use as biomimetic catalysts. The developed two-step immobilization procedure (similarly to the technique reported with *Cv*TA enzyme) involving the ionic binding of iron porphyrin onto functionalized magnetic nanoparticles (MNP) then embedding the MNP-porphyrin into PLA nanofibers by electrospinning technique. Due to the synergistic morphological and chemo-structural advantages of binding onto nanoparticles and embedding in polymeric matrices the

biomimetic efficiency of metalloporphyrin can be remarkably enhanced, while substrate conversion (amlodipine **1**, a calcium channel blocker medication used against high blood pressure and coronary artery disease as model substrate was selected) value was remarkably larger than which could be achieved with non-immobilized FeTPPS or FeTPPS simply attached to MNPs **(Figure 5**). The structural analysis of test reactions showed that biomimetic system produced the same major metabolite (dehydroamlodipine **2**) as the in vivo system. [P12]

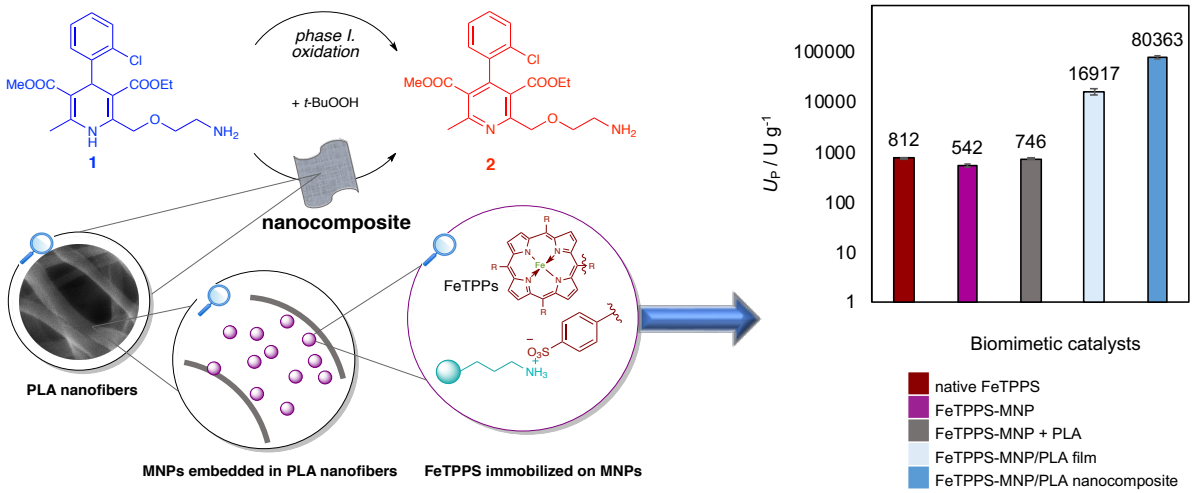

Figure5. Scheme of biomimetic oxidation of amlodipine (**1**) and its human major metabolite dehydroamlodipine (**2**) catalyzed by a nanocomposite catalyst consisting of immobilized FeTPPS on MNPs embedded within PLA nanofibers and specific biomimetic activity (U_P) of FeTPPS in different catalysts tested by the biomimetic oxidation of amlodipine (**1**).

Other results of the project

Supervising activity related tot he project

In this 3-year long research work 4 PhD students (Balázs Decsi, Gábor Koplányi, Gergő Tóth and Balázs Kenéz) were involved under my supervision and the results of the project provided publications and new scientific thesis for their PhD dissertations.

In addition, 10 BSc or MSc students joined to our research group and prepared their thesis work based on this project (Réka Krammer, Levente Kőnig, Maximilián Kiss, Alexandra Molnár, Réka Farkas, Dalma Lipták, Zsombor Mohácsi, Félix Nagy, Gabriella Sipőcz, Melitta Gedai). Among them 7 students attended on TDK conference, from which 4 student won price (one 1st place: 1, 2nd place: 2 and 3rd place: 1).

This project also ensured a good basis in the application for different grants and fellowships such as New National Excellence Program (ÚNKP supported by National Research, Development and Innovation Office, for 2 student and PhD student), Servier-Beregi Fellowship (supported by Sevier Research Center for 1 PhD student), Richter Talentum Fellowship (supported by Richter Gedeon Talentum Foundation for 1 PhD student). In addition 2 won conference paper won poster award with the project (Koplányi G., Bell E., Molnár Z, Katona G, Neumann P.L., Ender F., Balogh G.T., Žnidaršič-Plazl P., Poppe, Balogh-Weiser D. Functionalized nanoparticles for efficient isolation and immobilization of a recombinant transaminase, CPBCI 2022, Eger Hungary, 6-10 June, 2022, Best poster award of the scienctific committee, Tóth G.D., Molnár A., Kállai N., Lengyel M., Katona G., Zelkó R., Antal I., Balogh Gy.T., Balogh-Weiser D., Nanoszálas enzimkészítmények fejlesztése enzimhelyettesítő terápiához MGYT Konfenferencia 2022, Siófok 2022 október 26-28, Magyarország, Magyar Gyógyszerészeti Társaság szakmai díja.

New collaborations and grants based on the project

Results of the research provided lot of inspiration and idea, which involved new collaborators. From University of Szeged (Dr. Gábor Katona, Dr. Mária Budai-Szűcs and their resach groups from Institute of Pharmaceutical Technology and Regulatory Affairs) and Semmelweis University (Prof. Romána Zelkó, Prof. István Antal and Dr. Nikolett Kállai-Szabó and their research group University Pharmacy Department of Pharmacy Administration and Department of Pharmaceutics). Some experience of the project also supports my ongoing project about nanostructured protein carrier systems, which won an OTKA research Grant (*Development of Lab-on-a-chip platforms for investigation of protein-ligand interaction*, **FK-137582**, supported by National Research, Development and Innovation Office).

New collaborations had been created with University of Ljublaja (Prof. Polona Žnidaršič Plazl and her research group from Faculty of Chemistry and Chemical Technology) and KU Leuven (Prof. Dr. Simon Kuhn and his research group from Department of Chemical Engineering), which won CELSA grant to support our research project (*Self-assembling Multi-Layer Enzyme Network for Flow Biocatalysis*, *supported by Central Europe Leuven Strategic Alliance, CELSA* Grant number: **CELSA-20-243**.)

A Scientific networking grant had been also won based on the project (European network for wider application of electrospun nanofibers ID no. **22310110** supported by Visegrad Fund) with partners from Central Nicolaus Copernicus University in Torun (Prof. Wojciech Kujawski and his research group) and Technical University of Liberec (Dr. Fatma Yalcinkaya and her research group).

Publications related to the project

Based on the research work 9 scientific peer-reviewed paper had been published, 6 of which I am corresponding author.

Total impact factor: 47.892
D1: 1
Q1: 6
Q2: 2

Further 1 paper had been already submitted and 2 papers are under preparation.

[P1] Nagy, Flóra ; Sánta-Bell, Evelin ; Jipa, Monica ; Hornyánszky, Gábor ; Szilágyi, András ; László, Krisztina ; Katona, Gábor ; Paizs, Csaba ; Poppe, László; **Balogh-Weiser, Diána**, Cross-Linked Enzyme-Adhered Nanoparticles (CLEANs) for Continuous-Flow Bioproduction, CHEMSUSCHEM 15 : 2 Paper: e202102284 (2022)

[P2] Tóth, Gergő Dániel ; Koplányi, Gábor ; Decsi, Balázs ; Sánta-Bell, Evelin ; Gyarmati; Benjámin ; Szilágyi, András ; Balogh, György T. ; Poppe, László ; **Balogh-Weiser, Diána**, Engineered magnetic nanoparticles as advanced carrier materials for enzyme catalyst, *scientific paper under prepartion*

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