FINAL REPORT ON OTKA PROJECT NN 103242
„STRUCTURE AND MECHANISM OF NOVEL MIO-CONTAINING BIOCATALYSTS"
Principal investigator: Dr. László Poppe

Enzyme-catalyzed reactions can be widely used for synthetic goals, e.g. for preparation of enantiopure compounds. Phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL) and histidine ammonia-lyase (HAL) use the 5-methylene-4*H*-imidazol-4-one (MIO) – forming from Ala-Ser-Gly, or in some instance from Thr-Ser-Gly triade – as prosthetic group for catalyzing the elimination of ammonia from the corresponding amino acids. The phenylalanine and tyrosine 2,3-aminomutases (PAM and TAM), catalyzing the isomerization of the corresponding α-amino acids to their β-isomers, belong also to the family of MIO-enzymes.

Four objectives related to MIO-enzymes and their utilization for preparation of both L- and D-enantiomers of novel unnatural α- and β-amino acid in enantiopure form were selected as subjects of the project. All objectives were performed in co-operation with the research group of Prof. Csaba Paizs (Cluj, Romania).

Our results achieved in the fields in frame of the planned objectives (1-4) witin the research period of the project (2012-05-01 – 2015-10-31) are listed below.

A general review on the activity of our research group containing details on our recent results on MIO-enzymes has been published in *Periodica Polytechnica Chemical Engineering.*[1]

Objective 1)
Production, isolation and purification of various wt- and mutant PAL, PAM and other MIO-enzymes

a) A number of known bacterial PAL / PAM genes were synthesized or cloned. Expression vectors were constructed containing synthetic / cloned genes of various PALs [*Petroselinum crispum* PAL with two mutated cysteines (*Pc*PAL);[2] *Rhodosporidium toruloides* PAL (*Rt*PAL), *Anabaena variablis* PAL (*Av*PAL); *Photorhabdus luminescens* PAL (*Pl*PAL)]. In a similar way, bacterial expression vectors were obtained with genes of various PAMs [*Taxus canadensis* PAM (*Tc*PAM); *Pantoea agglomerans* PAM (*Pa*PAM);[3] *Streptomyces maritimus* PAM (*Sm*PAM)]. The non-expressing gene for *Tc*PAM was redesigned and optimized sequences for genes of further heat stable PALs were designed (e.g. for PAL of *Bambusa oldhamii*) for future gene synthesis.

b) A novel PAL from a thermophilic bacterium *Rubrobacter xylanophilus* (*Rx*PAL) was identified.[4] The sequence of *Rx*PAL from the thermophilic and radiotolerant bacterium having a growth optimum of 65 °C was identified by screening the genomes of bacteria against the known sequence of *Photorhabdus luminescens* PAL. After bioinformatics-based identification, the gene encoding the *Rx*PAL protein with an *N*-terminal His_6-tag was synthetized, cloned and overexpressed in *E. coli* TOP 10 and the produced recombinant *Rx*PAL was purified by Ni-NTA. *Rx*PAL proved quite alkalophylic exhibiting with L-phenylalanine at various pH values a local activity maximum at pH 8.5 and a global activity maximum at pH 11.5. Circular dichroism (CD) studies showed that *Rx*PAL structure was well preserved up to pH 11.0.

c) The sequence of a novel bacterial phenylalanine 2,3-aminomutase from *Stackebrantia nassauensis* (*Sn*PAM) was identified by screening the genomes of bacteria against a characteristic portion of the known sequence of *Taxus canadensis* PAM [an (*R*)-isomer-forming phenylalanine 2,3-aminomutase]. After its bioinformatics-based identification, the gene

encoding the *Sn*PAM protein with an *N*-terminal His_6-tag was cloned out from native DNA of *Stackebrantia nassauensis* and overexpressed in *E. coli*. The produced recombinant *Sn*PAM was purified by Ni-NTA. Activity assays with α- and β-phenylalanine and DL-propargylglycine indicated that all three compounds were accepted as substrate of *Sn*PAM.
A high impact publications on this essentially novel PAM referring to the financial support from OTKA NN 1032424 project is planned in the next year.

d) The sequence of a novel bacterial phenylalanine 2,3-aminomutase from *Pseudomonas fluorescens* (*Pf*PAM) was identified by screening the genomes of bacteria against the known sequence of *Pantoea agglomerans* PAM [an (*S*)-isomer-forming phenylalanine 2,3-aminomutase]. After bioinformatics-based identification, the primers for cloning out the *Pf*PAM protein with an *N*-terminal His_6-tag from native DNA of *Pseudomonas fluorescens* were designed. Intestingly, besides the bioinformatics-based identification of phenylalanine 2,3-aminomutase (*Pf*PAM) in *Pseudomonas fluorescens* a histidine ammonia-lyase (*Pf*HAL) and a phenylalanine ammonia-lyase (*Pf*PAL) were also identified in this bacterium (cloning of which are also planned).
Activity assays with wild type whole cells of *Pseudomonas fluorescens* with β-phenylalanine and indicated the presence of *Pf*PAM activity.
Because this is the only organism so far which produces three different kinds of MIO-enzymes, a high impact publication referring to the financial support from OTKA NN 1032424 project is foreseen in the next year.

e) Various *E. coli* hosts were tested for recombinant PAL / PAM production. In cooperation with Institute of Enzymology, HAS, with the group of Prof. Csaba Paizs at UBB and with Fermentia Ltd, working expression systems were developed for a number of MIO-enzymes such as PALs of *Petroselinum crispum*,[2] *Rhodosporidium toruloides*, *Anabaena variablis*, *Rubrobacter xylanophilus* (*Pc*PAL, *Rt*PAL, *Av*PAL, *Rx*PAL) and PAMs of *Pantoea agglomerans*[3] and *Stackebrantia nassauensis* (*Pa*PAM and *Sn*PAM). Pilot plant scale production of *E. coli* cells expressing *Pc*PAL, *Rx*PAL, *Rt*PAL, *Pa*PAM and *Sn*PAM were performed in 10 L industrial fermenters.

f) Isolation and purification of the His-tagged *Pc*PAL[2], *Rt*PAL, *Rx*PAL[4], *Av*PAL, *Pa*PAM[3] and *Sn*PAM were already performed. The thermal and operational stability of MIO-enzymes[5] such as His-tagged *Pc*PAL, *Rt*PAL, *Rx*PAL and *Pa*PAM were investigated by kinetics at various temperatures and pH, by thermofluor, CD and gel filtration methods.
A publication on the novel His-tag based purification methods and stability issues related to a Pal and a PAM referring to the financial support from OTKA NN 1032424 project is foreseen in the next year.

Objective 2)
Immobilization of PAL and PAM, and whole cells hosting PAL/PAM production and modifications of MIO-enzymes

a) Various enzyme immobilization methods[6,7] were tested for the isolated *Pc*PAL, *Rt*PAL, *Rx*PAL, *Pa*PAM and *Sn*PAM. The novel immobilization methods performed so far included novel types of cross-linked enzyme aggregates (CLEAs)[8]; adsorption and covalent binding onto various solid supports, including magnetic nanoparticles[9,10,11] and carbon nanotubes[12]; and entrapment in sol-gel matrices[9] or electrospun nanofibers[6]. A combined His-tag affinity binding/covalent attachment immobilization was developed on modified industrial enzyme carriers allowing a one-step purification/immobilization directly from fermentation supernatants of the His-tagged recombinant proteins.

Besides the already published results (points c-e), several further publications on the various unpublished immobilization results with the PALs and PAMs referring to the financial supports from OTKA NN 1032424 project are planned in the next year.

b) The enzyme activity of the immobilized PALs were tested in the elimination and also in the reverse addition reactions by various methods including UV, TLC and HPLC. The covalent immobilization of the PALs and *Pa*PAM resulted in preparations which were active in both the elimination and addition type reactions.

c) Glycerol diglycidyl ether (GDE) proved to be a convenient and inexpensive bis-epoxide cross-linker as demonstrated by the preparation of cross-linked enzyme aggregates (CLEAs) from lipases [*Pseudomonas fluorescens* (AK), *Burkholderia cepacia* (PS) and lipase B from *Candida antarctica* (CaL B)] and further from phenylalanine ammonia-lyase from *Petroselinum crispum* (*Pc*PAL).[8] The novel CLEAs showed improved properties as compared to their glutaraldehyde (GA) cross-linked counterparts. Ultrasonication studies indicated GDE cross-linked CLEAs *Pc*PAL as mechanically more stable than the GA-based forms. GDE-based PAL-bovine serum albumin co-CLEAs could be recycled at least three times when used for the stereoselective ammonia addition in 6M ammonia onto (*E*)-3-(thiophen-2-yl)acrylic acid whereas recycling of conventional GA-based PAL CLEAs from this medium failed.

d) Carboxylated single-walled carbon nanotubes ($SwCNT_{COOH}$) were used as support for covalent immobilization of phenylalanine ammonia-lyase from parsley (*Pc*PAL) by two different methods.[12] The $SwCNT_{COOH}$-PAL^{I} biocatalyst was obtained by direct attachment of *Pc*PAL onto $SwCNT_{COOH}$ after EDC activation. The $SwCNT_{COOH}$-PAL^{II} was abtained by reacting $SwCNT_{COOH}$ after EDC activation with excess 1,3-propanediamine followed by functionalization with glycerol diglycidyl ether and immobilization of *Pc*PAL onto the epoxy functions at the end of the resulting linker.

e) Novel, magnetic nanoparticle bound form of *Pc*PAL was obtained by using epoxy-functionalized magnetic nanoparticles of various particle diameters (250 nm and 600 nm)[13] to use the MNP-PcPAL biocatalysts in magnetic chamber microreactors.[9,10,11]

Co-immobilization of *Pa*PAM and *Pc*PAL on modified industrial enzyme carriers or on magnetic nanoparticles allowed a novel type of kinetic resolution of beta-amino acids based on enantiomer selective isomerization the L-α-amino acids (by *Pa*PAM) which were then digested to (*E*)-arylacrylates (by *Pc*PAL).
A publication on the use of co-immobilized PAL and PAM referring to the financial supports from OTKA NN 1032424 project is planned in the next year.

Objective 3)
Development of biocatalytic procedures mediated by PAL / PAM / TAM and synthesis of inhibitors and substrate analogues

a) A novel method was developed for yeast mediated stereoselective synthesis of the enantiomers of β-phenylalanine as standards for characterization of the products of the various PAMs.[14]

b) A methodology making enantiopure forms of bulky 3-(5-phenylfuran-2-yl)propanoic acids more accessible for synthetic chemists which were too bulky for the previously described PAL-mediated stereoselective ammonia addition to (5-phenylfuran-2-yl)acrylates. An efficient sequential multi-enzyme process was applied for the preparation of enantiopure 5-phenylfuran-2-ylalanines, starting from racemic 2-acetamido-3-(5-phenylfuran-2-yl)propanoic acids. The first step, the CaL-B-mediated dynamic kinetic resolution of the racemic oxazolones provided with 100% theoretical yield the *N*- and *C*-protected L-amino acids (81-92% ee). The protective groups were removed in excellent yields by a second (PLE mediated hydrolysis of the ester) and

a third (Acylase I catalyzed stereoselective hydrolysis of the amide) enzymatic step increasing the enantiomeric excess of the target compounds over 99 %.[15]

c) Novel aromatic and heteroaromatic unnatural α- and β-amino acids were synthesized for applications in reactions of PALs and PAMs[16,17] and the stereoselectivity of the PAL reaction was investigated under non-optimal conditions.[18]

d) The variously immobilized forms of PALs were successfully applied in different miniaturized continuous-flow reactor systems such as IMAc-support bound PAL or SwCNT-PAL packed-bed column minireactors[19,20,21,22] or MNP-PAL filled magnetic chamber microchip reactor.[9,10,11,13,21,23, 24]

e) Results with the *Pc*PAL immobilized on magnetic nanoparticles indicated the usefulness of such biocatalyst in Magne-Chip reactor to screen the natural and five unnatural substrates for degradative kinetic resolution providing the unreacted enantiomer and (*E*)-cinnamate or its analogues. [23,24]

f) By using PAL, immobilized on magnetic nanoparticles and fixed in a microfluidic reactor with an in-line UV detector, we first demonstrated that PAL can catalyze the ammonia elimination from the acyclic propargylglycine (PG) to yield (*E*)-pent-2-ene-4-ynoate indicating new opportunities to extend the MIO-enzyme toolbox towards acyclic substrates.[11]

g) The carbon nanotube-immobilized *Pc*PAL biocatalysts ($SwCNT_{COOH}$-PAL^{I} and $SwCNT_{COOH}$-PAL^{II}) with low diffusional limitation were tested in batch mode kinetic resolution of racemic 2-amino-3-(thiophen-2-yl)propanoic acid [yielding a mixture of the (*R*)-amino acid and (*E*)-3-(thiophen-2-yl)acrylic acid] and in the ammonia addition onto (*E*)-3-(thiophen-2-yl)acrylic acid [yielding enantiopure (*S*)-amino acid].[12] $SwCNT_{COOH}$-PAL^{II} was a stable biocatalyst (>90% of original activity after 6 cycles in the elimination reactions and after 3 cycles in 6 M NH_3 in the ammonia addition reactions). Study of NH_3 addition to (*E*)-3-(thiophen-2-yl)acrylic acid in continuous-flow microreactor filled with $SwCNT_{COOH}$-PAL^{II} (2 M NH_3, pH 10.0, 15 bar) indicated no significant loss of activity over 72 h up to 60 °C. $SwCNT_{COOH}$-PAL^{II} in the continuous-flow system at 30 °C was more productive (r_{flow}=2.39 μmol min^{-1} g^{-1}) than in the batch reaction (r_{batch}= 1.34 μmol min^{-1} g^{-1}).

h) Racemic styrylalanines were synthesized for testing as substrate in the PAL reaction. The fact that these amino acids were accepted as substrate by *Pc*PAL and *Rt*PAL excludes the possibility of the proposed mechanistic alternative via a Friedel-Crafts-like intermediate.[25]

i) The catalytic activity of phenylalanine 2,3-aminomutase from *Pantoea agglomerans* (*Pa*PAM) towards various racemic α- and β-arylalanines was investigated.[26] Experimental results, obtained from ^{1}H NMR spectroscopy and chiral HPLC analysis, indicated that *Pa*PAM favored either the α- or the β-regioisomer depending on the nature and position of the aromatic substituent. Independent from the rate of the reactions the biotransformations proceeded with excellent enantioselectivity. *o*-Substituted α-arylalanines remained unconverted while their β-arylalanine counterparts transformed rapidly. The *m*- and *p*-substituted molecules, in general, were good substrates for *Pa*PAM only as α-isomers but the corresponding β-arylalanines were only poor substrates. Computational results provide strong evidence that the relative energies of the covalent enzyme-substrate complexes were the main factors determining the biocatalytic activities.

Objective 4)
Structural and mechanistic characterization of MIO-enzymes

a) A comparative study was performed using novel homology models and existing experimental structures of various MIO-containing enzymes including TAL, PAL, HAL, TAM and PAM enzymes to identify characteristic differences in PAL structures.[27]

b) The extremely high pH optimum of *Rx*PAL could be rationalized by a three-dimensional homology model indicating possible disulfide bridges, extensive salt-bridge formation and an excess of negative electrostatic potential on the surface.[4] Crystallization trials for X-ray characterization of the novel *Rx*PAL from thermophilic bacteria resulted in protein crystals. To enhance the reflections, synchroton data were collected. Supported by the initial homology model, solution of the x-ray structure is partially ready.
A publication on the structure of *Rx*PALs referring to the financial supports from OTKA NN 1032424 project is planned in the next year.

c) By using three aminophosphonic acids (2 known ones and both enentiomers of a novel one, obtained from Prof. Friedrich Hammerschmidt, TU Wien, Austria) detailed inhibition studies were performed using *Pc*PAL. The strength of inhibition correlated well with the docking studies performed within the active site of *Pc*PAL structure with a corrected Tyr110-loop obtained by partial homology modelling.
When the absolute configuration correlation for the two enantiomers of the novel inhibitor will be ready, a publication on the inhibition data referring to the financial supports from OTKA NN 1032424 project is planned.

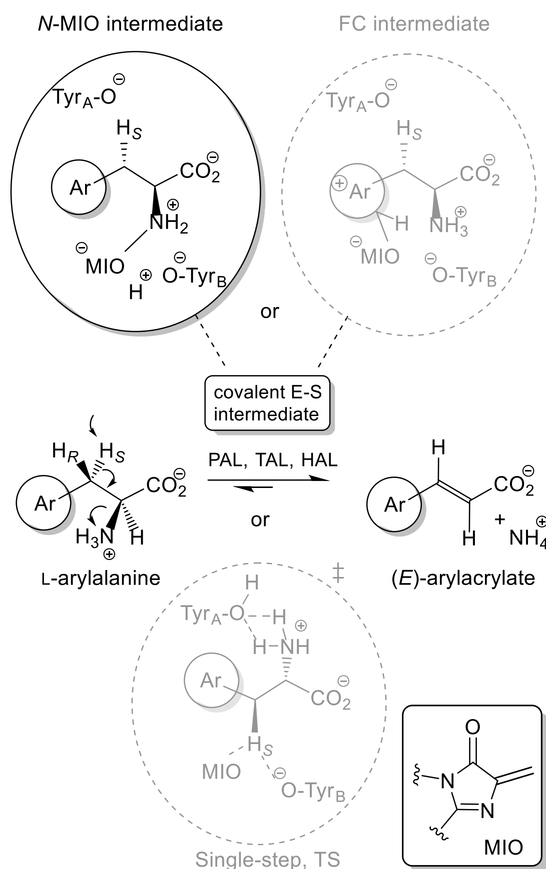
d) The structure of a novel bacterial phenylalanine 2,3-aminomutase from *Stackebrantia nassauensis* (*Sn*PAM) was investigated by homology modeling. This protein is especially interesting because this is the only bacterial MIO-enzyme so far which contains the approximately 120 amino acid long multihelix extension characteristic for the MIO-enzymes of eucariotic origin.
A high impact publications on this essentially novel PAM referring to the financial support from OTKA NN 1032424 project is planned in the next year.

e) Homology modeling was performed on ergothionase *Burkholderia* sp HME13, an enzyme with high sequence similarity to the MIO-enzymes but acting without using MIO to explore its mechanism.[28] It was found that the full catalytic machinery of the homotetrameric ergothionase resembles closely to that of the ammonia-lyases but the MIO prosthetic group which pulls the amino moiety of the regular amino acid during the reaction is replaced by a glutamic acid residue which pulls the permethylated ammonium moiety of ergothionine in the elimination.
A publication on this essentially new mechanism with reference to the financial support from OTKA NN 1032424 project is planned in the next year.

f) Extensive bioinformatics search was performed for novel PAMs of eukaryotic origin because of the of broad substrate acceptance of the eucariotic *Tc*PAM. Our extensive bioinformatics-based search resulted in a partial sequence of postulated PAMs in *Ginco biloba* and a full sequence in *Pseudozyma (Candida) antarctica*.
In the future year attempts will be made to clone the full geneS and express their products from recombinant *E. coli* and characterize the properties of the novel PAMs.

g) Deamination of propargylglycine, being acyclic, cannot involve a Friedel-Crafts-type attack at an aromatic ring. The reversibility of the PAL-reaction, also demonstrated by the ammonia addition to (*E*)-pent-2-ene-4-ynoate yielding enantiopure L-PG, contradicts the proposed highly exothermic single-step mechanism. Computations on the QM/MM models of the *N*-MIO

intermediates from L-PG and L-Phe in PAL, showing similar arrangements within the active site, support a mechanism via the *N*-MIO intermediate (Scheme 1).[11]



Scheme 1. Alternative pathways for the reaction catalyzed by the MIO-containing aromatic ammonia-lyases (PAL, TAL and HAL).

The overall results of the project (12 published or accepted articles, 1 article prepared for submission and 15 conference presentations) indicated a highly successful and close international co-operation with the Cluj group leaded by Prof. Csaba Paizs.

The facts that our paper recently published in *ChemBioChem* on the mechanism of the PAL reaction aided by the use of the Magne-Chip microreactor has been selected as Front Cover Article for the November 2015 issue of the journal; and that the poster on the biotransformations of styrylalanines by *Pc*PAL has been awarded by the runner-up prize for Catalysis by the poster award board of *International Symposium on Synthesis and Catalysis*, in Evora, Portugal indicate the international recognition of our results.

Publications

1. BOROS, Z.; HORNYÁNSZKY, G.; NAGY, J.; POPPE, L.: From synthetic chemistry and stereoselective biotransformations to enzyme biochemistry – The Bioorganic Chemistry Group at the Budapest University of Technology and Economics. *Per. Polytechn. Chem. Eng.* **2015**, *59(1)*, 59-71.
   [IF 0.296] DOI:10.3311/PPch.7390

2. DIMA, N. A.; FILIP, A.; BENCZE, L. C.; OLÁH, M.; SÁTORHELYI, P.; VÉRTESSY, B. G.; POPPE, L.; PAIZS, C.: Expression and purification of recombinant phenylalanine ammonia-lyase from *Petroselinum crispum*. *Stud. Univ. Babes-Bolyai Chem.* **2016**, (accepted).
   [IF 0.191]

3. Varga, A.; Filip, A.; Bencze, L.C.; Sátorhelyi, P.; Bell, E.; Vértessy, B. G.; Poppe, L.; Paizs, C.: Expression and purification of recombinant phenylalanine 2,3-aminomutase from *Pantoea agglomerans*. *Stud. Univ. Babes-Bolyai Chem.* **2016**, (accepted).
   [IF 0.191]

4. Kovács, K.; Bánóczi, G.; Varga, A.; Szabó, I.; Holczinger, A.; Hornyánszky, G.; Zagyva, I.; Paizs, C.; Vértessy, B. G.; Poppe, L.: Expression and properties of the highly alkalophilic phenylalanine ammonia-lyase of thermophilic *Rubrobacter xylanophilus*. *PLoS ONE* **2014**, *9(1)*, e85943.
   [IF 3.534] DOI:10.1371/journal.pone.0085943

5. Bata, Z.; Kovács, K.; Vértessy, G. B.; Poppe, L.: Aromás aminosav C-N-liáz és 2,3-aminomutáz enzimek vizsgálata. *MKE Vegyészkonferencia.* **2013**. június 26-28, Hajdúszoboszló, Magyarország. (poster)

6. Poppe, L.: Enzyme immobilization – A tool to improve biocatalysts. *COST „Systems Biocatalysis" Training School Conferenc*e, 28 May – 1 July, **2014**, Siena, Italy (lecture)

7. Poppe, L.; Paizs, C.; Vértessy, B. G.; Kovács, K.; Weiser, D.; Varga, A.; Vári, H.; Bánóczi, G.; Bell, E.; Kókai, E.; Boros, Z.: MIO-enzymes – Novel enzymes, immobilization methods and applications. *4th International Conference on Novel Enzymes.*. 14-17 October, **2014**, Ghent, Belgium. (lecture)

8. Weiser, D.; Varga, A.; Kovács, K.; Nagy, F.; Szilágyi, A.; Vértessy, B. G.; Paizs, C.; Poppe, L.: Bisepoxide Cross-Linked Enzyme Aggregates – Novel Immobilized Biocatalysts for Selective Biotransformations. *ChemCatChem* **2014**, *6(4)*, 1463–1469.
   [IF 5.044] DOI:10.1002/cctc.201300806

9. Weiser, D.; Kovács, K.; Ender, F.; Vértessy, B .G.; Poppe, L.: Enzyme coated sol-gel nanoparticles for use in continuous flow microfluidical devices. *XVII International Sol-Gel Conference.* 25-30 August, **2013**, Madrid, Spain. (poster)

10. Ender, F.; Weiser, D.; Vitéz, A.; Poppe, L.: Enzyme Coated Magnetic Nanoparticles for Use in Continuous Flow Microfluidic Devices. *Lab-on-a-Chip European Congress*, 10-11 March, **2014**, Berlin, Germany. (poster)

11. Weiser, D.; Bencze L. C.; Bánóczi, G.; Ender, F.; Kókai, E.; Szilágyi, A.; Vértessy, B. G.; Farkas, Ö; Paizs, C.; Poppe, L.: Phenylalanine ammonia-lyase catalyzed deamination of an acyclic amino acid - Enzyme mechanistic studies aided by a novel microreactor filled with magnetic nanoparticles. *ChemBioChem* **2015**, *16(16)*, 2283-2288.
    [IF 3.088] DOI:10.1002/cbic.201500444
    (Selected as **Front Cover article** of November 2015 issue of *ChemBioChem*)

12. Bartha-Vári, J. H.; Toşa, M. I.; Irimie, F.-D.; Weiser, D.; Boros, Z.; Vértessy, B. G.; Paizs, C.; Poppe, L.: Immobilization of phenylalanine ammonia-lyase on single-walled carbon nanotubes for stereoselective biotransformations in batch and in continuous-flow modes. *ChemCatChem* **2015**, *7(7)*, 1122-1128.
    [IF 5.044] DOI:10.1002/cctc.201402894

13. Ender, F.; Weiser, D.; Vitéz, A.; Sallai, G.; Németh, M.; Poppe, L.: In-situ measurement of magnetic nanoparticle quantity in a microfluidic device. *Microsyst. Technol.* **2015**, accepted.
    [IF 0.875]

14. Varga, A.; Zaharia, V.; Nógrádi, M.; Poppe, L.: Chemoenzymatic synthesis of both enantiomers of 3-hydroxy- and 3-amino-3-phenylpropanoic acid. *Tetrahedron:Asymmetry* **2013**, *24*, 1389–1394.
    [IF 2.165] DOI:10.1016/j.tetasy.2013.09.007

15. Bencze, L. C.; Komjáti, B.; Pop, L. A.; Paizs, C.; Irimie, F.-D.; Nagy, J.; Poppe, L.; Toşa, M. I.: Synthesis of enantiopure L-(5-phenylfuran-2-yl)alanines by a sequential multienzyme process. *Tetrahedron:Asymmetry* **2015**, *26*, 1095-1101.
    [IF 2.155] DOI:10.1016/j.tetasy.2015.08.004

16. Kókai, E.; Kovács, K.; Weiser, D.; Vértessy, B.G.; Poppe, L.; Nagy, J.: Vizsgálatok nem-természetes alfa- és béta aminosavak előállításához. *MKE Vegyészkonferencia.* **2013**. június 26-28, Hajdúszoboszló, Magyarország. (poster)

17. Filip, A.; Bartha-Vári, J. H.; Bánóczy, G.; Poppe, L.; Paizs, C., Irimie, F. D.: Non-natural aminoacids via the MIO-enzyme toolkit. *29th Annual Symposium of the Protein-Society.* 22-25 July, **2015**, Barcelona, Spain. (poster) Published in *Protein Sci.* **2015**, *24 (SI1, Meeting Abstract: PB-086)*, 54-55.

18. Varga, A.; Nagy, B.; Miklós, M.; Irimie, F. D.; Poppe, L.; C Paizs, C.: Stereoselectivity of PAL under non-optimal conditions. *29th Annual Symposium of the Protein-Society.* 22-25 July, **2015**, Barcelona, Spain. (poster) Published in *Protein Sci.* **2015**, *24 (SI1, Meeting Abstract: PB-086)*, 55-56.

19. POPPE, L.; PILBÁK, S.; FARKAS, Ö.; KOVÁCS, K.; BÁNÓCZI, G.; WEISER, D.; BOROS, Z.; VÉRTESSY, B. G.; PAIZS, C.: MIO-containing ammonia-lyases and 2,3-aminomutases – Mechanism, immobilization and biotransformations in batch and continuous-flow systems. *Biotrans 2013, 11th International Symposium on Biocatalysis*. 21-25 July, **2013**, Manchester, United Kingdom. (lecture)

20. BOROS, Z.; ABAHÁZI, E.; OLÁH, M.; NAGY-GYŐR, L.; FALUS, P.; BÓDAI, V.; SÁTORHELYI, P.; ERDÉLYI, B.; KOVÁCS, K.; WEISER, D.; VÉRTESSY, B. G.; POPPE, L.: Immobilized enzymes for biotransformations in continuous-flow systems. *4th Conferences on Frontiers in Organic Synthesis Technology.* 16-18 October, **2013**, Budapest, Hungary. (poster)

21. POPPE, L.; WEISER, D.; ENDER, F.; BARTHA-VÁRI, H.; PAIZS, C.; VÉRTESSY, B. G.: Nanosupported phenylalanine ammonia-lyase in microreactors. *3rd International Conference Implementation of Microreactor Technology in Biotechnology – IMTB 2015.* 10-13 May, **2015**, Opatija, Croatia. (lecture)

22. BOROS, Z.; WEISER, D.; ENDER, F.; BARTHA-VÁRI, H.; OLÁH, M.; FALUS, P.; PAIZS, C.; VÉRTESSY, B.G.; SERVI, S.; POPPE, L.: Immobilized enzymes in microfluidic systems – phenylalanine ammonia-lyase-, lipase- and protease-catalyzed processes. *Biotrans 2015, 12th International Symposium on Biocatalysis*. 26-30 July, **2015**, Wien, Austria. (poster)

23. WEISER, D.; BENCZE, L. C.; PAIZS, C.; POPPE, L.; ENDER, F.: Magne-Chip: an efficient tool for screening of selective biotransformations. *5th Conferences on Frontiers in Organic Synthesis Technology.* 21-23 October, **2015**, Budapest, Hungary. (poster)

24. ENDER, F.; WEISER, D.; NAGY, B.; BENCZE L. C.; PAIZS, C.; PÁLOVICS, P.; POPPE, L.: Microfluidic multiple cell chip reactor filled with enzyme-coated magnetic nanoparticles – An efficient and flexible novel tool for enzyme catalyzed biotransformations. *J. Flow Chem.* **2015**, accepted.
[IF 1.878]

25. BENCZE, L. C.; FILIP, A.; BÁNÓCZI, G.; TOŞA, M. I. IRIMIE, F. D.; POPPE, L.; PAIZS, C.: Styryl-alanines as novel substrates for phenylalanine ammonia lyase. *International Symposium on Synthesis and Catalysis*, 2-4 September, **2015**. Evora, Portugal.
(poster, awarded with the **runner-up prize for Catalysis**)

26. VARGA, A.; NAGY, B.; BÁNÓCZI, G.; GELLÉRT, Á.; POPPE, L.; PAIZS, C.: Effect of the aromatic substituents in α- and β-arylalanines on biotransformations with phenylalanine 2,3-aminomutase from *Pantoea agglomerans*. *Amino Acids* **2015**, prepared for submittion.

27. BÁNÓCZI, G.; SZABÓ, C.; BATA, Z.; FILIP, A.; HORNYÁNSZKY, G; POPPE, L.: Structural modeling of phenylalanine ammonia-lyases and related MIO-containing enzymes – An insight into thermostability and ionic interactions. *Stud. Univ. Babes-Bolyai Chem.* **2015**, (will appear in the 4th issue in 2015).
[IF 0.191]

28. BÁNÓCZI, G.; BATA, Z.; HORNYÁNSZKY, G.; POPPE, L.; FARKAS, Ö.; HÉBERGER, K.: Enzyme-substrate interactions in *Burkholderia* sp HME13 ergothionase - *Ab initio* prediction of the binding pose and mechanism using bioinformatics. *Schrödinger Hungarian User Group Meeting*, 25 November, **2014**, Budapest, Hungary. (lecture)