MECP 2026

7th Multi-step Enzyme Catalyzed Processes congress

March 29th – April 1st 2026, Milano, Italy

BOOK OF ABSTRACTS

Welcome to Milano!

The biocatalysis community of Milano and Pavia is delighted to offer you the chance to spend a few days of stimulating lectures, engaging discussions and fruitful networking in the field of enzyme-mediated synthesis, together with the opportunity of a pleasant stay in the lively city of Milano while enjoying the charming atmosphere of early Italian spring, alongside art, culture, food and fashion.
During this meeting, the latest advances in the following key areas will be presented:

- multi-enzymatic and chemo-enzymatic cascades for sustainable industrial processes;
- bioprocess engineering to design and use living cells for the synthesis of valuable chemical products;
- enzyme engineering, discovery, and design, including methods based on computational tools and/or machine learning strategies;
- flow biocatalysis, from the recent achievements for enzyme immobilisation, to new compartmentalisation strategies and to integration of process analytical technologies;
- …and even more, according to the breadth of your ongoing research.

Welcome to Milano and to MECP 2026!

POLITECNICO MILANO 1863

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Acknowledgements

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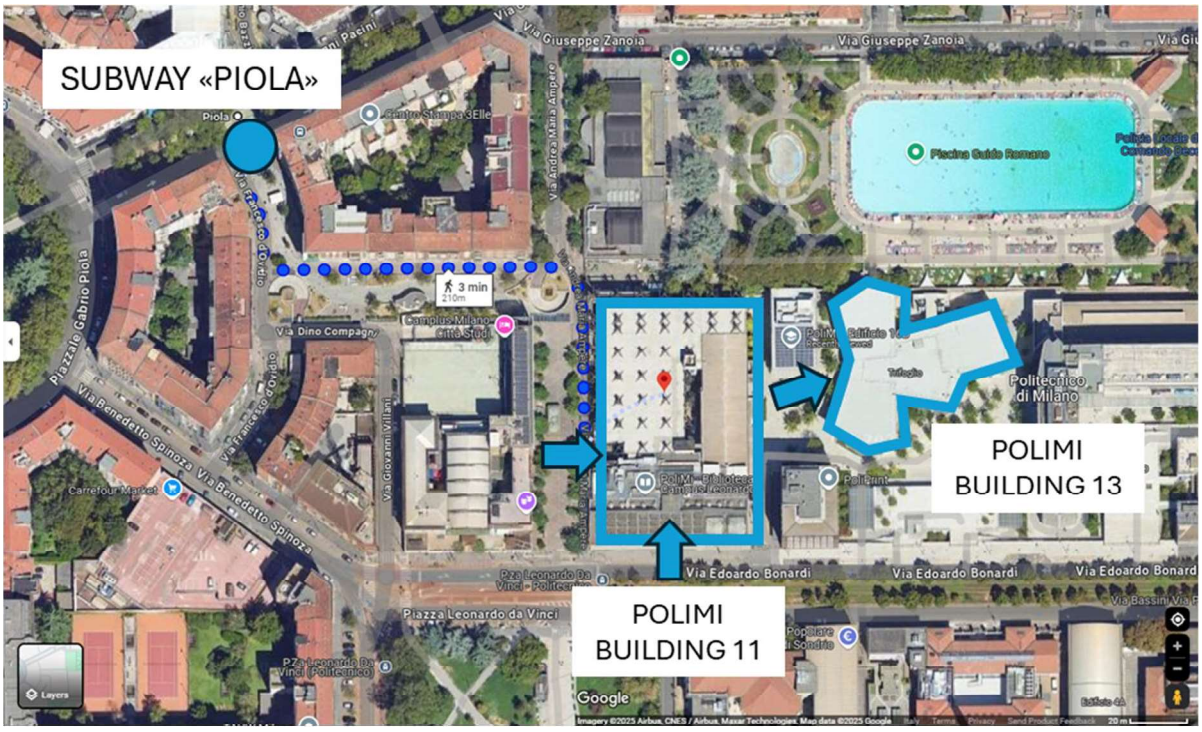
Venue

MECP 2026 will take place in the campus buildings of **Politecnico di Milano**. Lectures will be held in the Rogers Lecture Hall (building 11), with access either from Via Bonardi 11 or from Via Ampere 3. Poster sessions and coffee/lunch breaks will be in the “trifoglio” hall (building 13).

Google Maps link:
https://maps.app.goo.gl/cUyp1qCyM7cfWNUL7



Read more about the Architecture Campus here:
https://lightaz.com/led-project-details/the-politecnico-di-milano%E2%80%99s-architecture-campus?p=3542

Social dinner

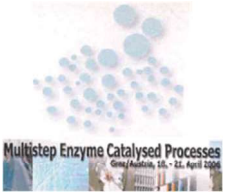
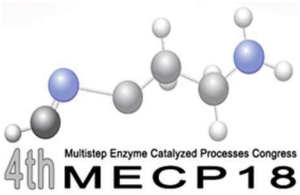
The social dinner will take place at the picturesque restaurant **I 12 GATTI** (the 12 cats), located in the heart of the city, very close to Piazza Duomo and Piazza della Scala. The entrance is tucked away inside the famous Galleria Vittorio Emanuele II, on the corner with Piazza della Scala, shared with the Leonardo Museum. Follow the corridor, take the elevator to the 6th floor and enjoy the breathtaking views.

Google Maps link:
https://maps.app.goo.gl/CcpjP3r4RPfekue18

Brief story of this establishment

Once upon a time, there was an old woman who lived in one of the attics of the Galleria Vittorio Emanuele II. The old woman was known, because through the dormers she fed the pigeons and her pair of black cats who roamed free on the rooftops. The day when the old woman, too lonely and too old to continue to live up there, left the attic, her cats remained. Too wild and too wary to be captured, contemptuous of danger and curious enough to venture more than once through the warehouses and shops of the Galleria, the cats thrived. The cats of the old woman have multiplied and today, on the rooftops of the Galleria, live a colony of ***twelve cats****, all black. With great enthusiasm we, along with the City Council of Milan and the association of animal protection, have made a commitment to contribute to their care, leaving them the space and the tranquillity they need, because a roof without cats is anonymous. By eating here, you are contributing to their care and especially their freedom.*

20 years of MECP

2006		**1st edition** Graz (Austria)
2012		**2nd edition** Graz (Austria)
2014		**3rd edition** Madrid (Spain)
2018		**4th edition** Trondheim (Norway)
2021		**5th edition** Aachen (Germany) held online
2024		**6th edition** Vienna (Austria)
2026		**7th edition** Milano (Italy)

Program

SUNDAY 29/03/2026

14.00-16.00	REGISTRATION
	SESSION 1 – Chair: Elisabetta Brenna
16.00-16.15	WELCOME ADDRESS AND INTRODUCTION
16.15-17.00	**OPENING KEYNOTE LECTURE** *Process solutions for multi-step biocatalytic cascades* **John Woodley**
17.00-19.00	WELCOME RECEPTION

MONDAY 30/03/2026

	SESSION 2 – Chair: Sergio Riva
9.00-9.50	**INVITED LECTURE 1** *Intensification of chemo-enzymatic processes towards industrial volumetric productivities* **Selin Kara**
9.50-10.10	**ORAL PRESENTATION 1** *Multi step cascades catalyzed by a single enzyme* **Wolfgang Kroutil**
10.10-10.30	**ORAL PRESENTATION 2** *Production of biobased monomers for polyesters and polyamides using (chemo)enzymatic cascades* **Volker Sieber**
10.30-11.00	COFFEE BREAK
	SESSION 3 – Chair: Daniela Ubiali
11.00-11.20	**ORAL PRESENTATION 3** *Immobilization for cascade biocatalysis* **James Weltz**
11.20-11.40	**ORAL PRESENTATION 4** *100 years of enzyme immobilization, What's next?* **Rob Schoevaart**

11.40–12.00	**ORAL PRESENTATION 5** *Engineering alcohol dehydrogenase for enhanced cofactor recycling* **James L. Galman**
12.00–12.20	**ORAL PRESENTATION 6** *Enzymatic synthesis of human milk fat substitute and other high value triglycerides by immobilized lipases: role of enzyme carriers and regulatory implications* **Alessandra Basso**
12.30–13.30	LUNCH BREAK
13.30–15.00	POSTER SESSION
	SESSION 4 – Chair: Davide Tessaro "Circular Bioengineering" Cluster of Excellence session
15.00–15.50	**INVITED LECTURE 2** *Multi-enzyme cascades for the asymmetric synthesis of cyclic amines* **Joerg Schrittwieser**
15.50–16.10	**ORAL PRESENTATION 7** *Biocatalytic four-step cascade turning phenols into tyramines* **Isabella E. E. Kroschel**
16.10–16.40	COFFEE BREAK
16.40–17.00	**ORAL PRESENTATION 8** *Sustainable phosphate-catalyzed synthesis of non-symmetric pyrazines in water: mechanistic insights, biocatalytic applications and industrial potential* **Jorge González-Rodríguez**
17.00–17.20	**ORAL PRESENTATION 9** *Valorisation of sinapine-rich side streams: an enzyme cascade for the synthesis of biobased hydroxystyrenes* **Daniel Kracher**

TUESDAY 31/03/2026

	SESSION 5 – Chair: Francesco Molinari
9.00-9.50	**INVITED LECTURE 3** *Biocatalytic cascades in continuous flow* **Sebastian Cosgrove**
9.50-10.10	**ORAL PRESENTATION 10** *Flowing green: intensified multi-step biocatalysis enabled by enzyme immobilization and advanced reactor technology* **Martina L. Contente**
10.10-10.30	**ORAL PRESENTATION 11** *Reductive amination-based multi-enzyme approach for the synthesis of D-phenylalanines* **Krisztina Boros**
10.30-11.00	COFFEE BREAK
	SESSION 6 – Chair: Daniela Monti
11.00-11.20	**ORAL PRESENTATION 12** *ATP-dependent multi-enzymatic cascade for sustainable amide bond formation* **Žiga Gerdina**
11.20-11.40	**ORAL PRESENTATION 13** *Design and development of photobiocatalytic methods for the stereoselective synthesis of chiral alcohols* **Laura Rodríguez-Fernández**
11.40-12.00	**ORAL PRESENTATION 14** *The enzymatic chameleon: rethinking alcohol dehydrogenases for the synthesis of amides and thioesters* **Matteo Damian**
12.00-12.20	**ORAL PRESENTATION 15** *From C1 molecules to ethyl formate: a sustainable concatenated catalytic process* **Nina Klos**

	SESSION 7 – Chair: Francesco Gatti
12.20-12.50	**FLASH PRESENTATIONS (5 min)** *Engineered phototrophic mixed-species biofilms for continuous production of polycaprolactone precursors from cyclohexanol in multi-stage drip-flow reactors* **Mahir Bozan** *Continuous δ-viniferin synthesis in a microreactor using magnetite-immobilized combined cross-linked enzyme nanoaggregates* **Marko Božinović** *Photobiocatalytic deracemization of 3-hydroxypropano-nitriles: advances in asymmetric synthesis* **Sara Filgueira** *Selective oxyfunctionalization of aromatic hydrocarbons by recombinant CYP153A – Acetobacter malorum* **Sara Foiadelli** *Engineered enzymatic cascades: overcoming challenges in P450 monooxygenase biocatalysis* **Carolin Mügge** *From a transcriptomic approach towards a multi-step biodegradation process of polyethylene by Rhodococcus opacus R7 laccase and oxygenase enzymes* **Jessica Zampolli**
13.00-14.00	LUNCH BREAK
14.00-15.00	POSTER SESSION
15.00-19.00	FREE AFTERNOON
19.00-22.00	SOCIAL DINNER – **Ristorante pizzeria "I 12 GATTI"**

WEDNESDAY 01/04/2026

	SESSION 8 – Chair: Marina Lotti
9.00-9.20	**ORAL PRESENTATION 16** *Scale-up of multi-step fungal glycosylation of chlorinated chalcones and integrated by-product valorization* **Agnieszka Krawczyk-Łebek**
9.20-9.40	**ORAL PRESENTATION 17** *Developing fluorescence-based coupled enzyme cascade to screen NAD(P)H-dependent oxidoreductases* **Trisha Ghosh**
9.40-10.00	**ORAL PRESENTATION 18** *Heterotrophic and phototrophic multistep biocatalysis for the production of nylon building blocks from renewables* **Lyn K. Möhrlin**
10.00-10.30	COFFEE BREAK
	SESSION 9 – Chair: Fabio Parmeggiani
10.30-10.50	**ORAL PRESENTATION 19** *ThDP-dependent enzyme for multi-step biocatalytic synthesis* **Francesco Presini**
10.50-11.10	**ORAL PRESENTATION 20** *CalA engineering: selectivity improvement, mechanistic insights, and unexpected applications* **Daniela Quaglia**
11.10-12.00	**CLOSING KEYNOTE LECTURE** *Multi-step enzymatic processes: selecting, coupling and completing multiple reaction steps* **Roland Wohlgemuth**
12.00-12.20	CLOSING REMARKS AND **CONFERENCE PHOTO**
12.30-13.30	LUNCH BREAK

List of posters

1* *Engineered phototrophic mixed-species biofilms for continuous production of polycaprolactone precursors from cyclohexanol in multi-stage drip-flow reactors*
Mahir Bozan

2* *Continuous δ-viniferin synthesis in a microreactor using magnetite-immobilized combined cross-linked enzyme nanoaggregates*
Marko Božinović

3* *Photobiocatalytic deracemization of 3-hydroxypropanonitriles: advances in asymmetric synthesis*
Sara Filgueira

4* *Selective oxyfunctionalization of aromatic hydrocarbons by recombinant CYP153A – Acetobacter malorum*
Sara Foiadelli

5* *Engineered enzymatic cascades: overcoming challenges in P450 monooxygenase biocatalysis*
Carolin Mügge

6* *From a transcriptomic approach towards a multi-step biodegradation process of polyethylene by Rhodococcus opacus R7 laccase and oxygenase enzymes*
Jessica Zampolli

7 *Lipase-catalyzed production of esters of hydroxycinnamic acids with antihypertensive and antimicrobial potential*
Abirami Baskaran

8 *One-pot enzymatic cascades for the conversion of biomass-derived phenolic acids into chiral aromatic building blocks*
Martina Bigliardi

9 *Immobilization of aldehyde reductase integrated in multi-enzymatic cascade for the production of bioplastic precursors from agricultural fatty acids*
Devesh Mohne

10 *Multi-enzymatic cascade for the synthesis of D-phenylalanine derivatives*
András-Ernő Iszlai

11 *Chemo-enzymatic platforms for the synthesis of L-(hetero)arylalanines*
Celeste Nobbio

12 *Discovery and characterization of a novel aromatic prenyltransferase from Aspergillus melleus (AmaPT)*
Matteo Corti

13 *Leveraging biodiversity for the biocatalytic production of esters via hemiacetal oxidation*
Sofia Ceccarossi

14 *Robust industrial biocatalysts with peroxygenase, phenol-oxidase and furfuryl-oxidase activities from bacterial and fungal hosts*
Letizia Rossato

15 *Two-cell biosynthesis of (–)-deoxypodophyllotoxin from ferulic acid in Escherichia coli*
Jonas Barsig

16 *Merging photo- and biocatalysis for sustainable synthesis of benzonitriles*
Luca Nespoli

17 *Chemo-enzymatic cascade for the synthesis of (R)-citronellyl nitrile*
Federico Acciaretti

18 *Oxidase-driven chemo-enzymatic cascade toward rare sugars*
Elena Karnisova Potocka

19 *FLEXIZYME: a flexible enzymatic platform for sustainable bio-based fatty amine production*
Ognjen Pećanac

20 *Characterisation of lytic polysaccharide monooxygenases: activity and interaction assays*
Leonor Vieira Carneiro

21 *Chemo-enzymatic flow synthesis of chiral piperidine scaffolds*
Sara Vicinanza

22 *One-pot multicatalytic cascades to chiral β-hydroxy esters combining organocatalysis and biocatalysis for the preparation of valuable chiral building blocks*
Marta Menéndez-González

23 *Enzymes in action: converting lignocellulosic phenolics into functional aromatics*
Gloria Zucchi

24 *Green extraction and biotransformation of bioactive compounds from oil industry by-products using NADES*
Aleksandra Grudniewska

25 *Three-step one-pot chemoenzymatic cascade for the stereoselective synthesis of secondary allylic alcohols*
Pablo López-Fernández

26 *REUSE: Enzymatic CO_2 capture in a rotating packed bed and electrocatalytic CO_2 reduction to useful products*
Sara Barricella - Milica Milić

27 *Enzymatic modification of acyl chains in natural phospholipids*
Witold Gładkowski

28 *One-pot cascade preparation of dicarboxylic acids using engineered Acetobacter malorum*
Stefano Giuliani

29 *NADPH-dependent aldehyde synthesis from carboxylic acid catalysed by CAR enzyme*
Ana-Katarina Marić

30 *Rational design of a biocatalytic cascade to simplify vanillin biosynthesis from eugenol*
Christian Ascaso-Alegre

31 *Harnessing photosynthetic ATP for whole-cell biocatalysis in the cyanobacterium Synechocystis*
Giovanni Loprete

32 *From primary alcohols to nitriles: a one-pot chemoenzymatic cascade strategy*
Enol De Prado Fernández

33 *Optimizing transaminase catalysis for eco-friendly pyrazine production*
Carlotta Chiesa

MECP 2026

ABSTRACTS

Process Solutions for Multi-step Biocatalytic Cascades

John M Woodley
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Biosynthetic systems using microbial cell factories, where many natural metabolic pathways are present, have long been proposed as an alternative to conventional methods of organic synthesis [1,2]. Driven by metabolic engineering, many examples of product biosynthesis have been demonstrated in the laboratory, and some also at larger scale. However, with only a few exceptions, scaling such fermentations comes with a number of inherent limitations, including the need for complex downstream processing. Hence, inspired by Nature, but now driven by chemists, multi-step biocatalytic cascades have been developed, the primary theme of this conference series. Here too, enormous progress has been made in the laboratory in the last few decades [3], and today several excellent examples run effectively in industry. Nevertheless for wider implementation at a larger scale, process solutions are still required [4]. Interestingly, such solutions are quite different to those required for cell factories, and in this lecture, I will outline the logic for the further development of biocatalytic cascades. I will also describe some of the process solutions, including considerations for biocatalyst format, substrate feeding, media selection, product recovery, and reactor design. Topics requiring further research will also be highlighted [5-7].

[1] C.T. Marshall and J.M. Woodley, *Nature Biotech.,* 1995, **13**, 1072.

[2] J.M. Woodley, *Adv. Appl. Microbiol.*, 2006, **60**, 1.

[3] P.A. Santacoloma, G. Sin, K.V. Gernaey and J.M. Woodley, *Org. Proc. Res. Dev.*, 2011, **15**, 203.

[4] R. Xue and J.M. Woodley, *Bioresource Technol.*, 2012, **115**, 183.

[5] J.M. Woodley, *Appl. Microb. Biotechnol.*, 2019, **103**, 4733.

[6] J.M. Woodley, *ChemSusChem*, 2022, **15**, e202102683.

[7] J.M. Woodley, *ChemCatChem*, 2025, **17**, e202500794.

Multi-Step Enzymatic Processes – Selecting, Coupling and Completing Multiple Reaction Steps

Roland Wohlgemuth

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Key features of living biological cells are characterized by a complex organization in space and time of numerous biocatalytic reaction networks, coded in the cell-specific genome and evolved over more than 3 billion years. Multiple enzymatic reaction steps involved in conversions of simple building blocks to complex molecules, or the breakdown of complex compounds into building blocks, have been translated into viable industrial processes. The metabolic pathways, along which enzymes work together in catalyzing the required reaction steps from the available raw materials, provide thereby valuable information about resource- and energy-efficient transformation routes. The design of multi-step enzymatic processes can benefit from the growing knowledge of enzyme functions and reaction types. Nature provides a blueprint for multi-step enzymatic processes, with the reaction architecture of core and specialized pathways, orphan, cryptic, or silent pathways, enzymatic housekeeping and maintenance systems essential for damage repair, clearance of toxic side products, and waste removal [1-4]. For the selection of suitable routes and enzymes backward analysis of reactions towards desired target compounds is important, in both chemical and biological domains, while forward analysis of reactions is of much interest for starting material oriented diversification [5,6]. From straightforward functional group interconversions to total synthesis of small molecules, raw material aspects, well-established enzymatic reaction platforms and their coupling are highly valuable for the molecular aspects of route design [7,8]. Rapid prototyping, identifying and overcoming bottlenecks are helping in the engineering of resource-efficient processes towards complete conversions and product recovery [9]. Analytical tools and methods with high information content are thereby instrumental. Multi-step enzymatic processes are highly attractive for overcoming challenges and limitations in biological and chemical manufacturing processes [10].

[1] R. Wohlgemuth, *Life*, 2024, **14(3)**, 364.

[2] R. Wohlgemuth, *New Biotechnol*. 2021, **60**, 113-123.

[3] R. Wohlgemuth, *Biotechnol. J.* 2018, **13(6)**, 1700620.

[4] A.R. Alcántara, P. Domínguez de María, J.A Littlechild, M. Schürmann, R.A. Sheldon, R. Wohlgemuth, *ChemSusChem*, **15(9)**, e202102709.

[5] R. Wohlgemuth, *React. Chem. Eng.* 2023, **8(9)**, 2109-2118.

[6] R. Wohlgemuth, *ChemSusChem* 2022, **15(9)**, e202200402

[7] L. Shen, M. Kohlhaas, J. Enoki, R. Meier, B. Schönenberger, R. Wohlgemuth, R. Kourist, F. Niemeyer, D. van Niekerk, C. Bräsen, J. Niemeyer, *Nat. Commun*. 2020, **11**, 1098.

[8] P. Gruber, F. Carvalho, M.P. Marques, B. O'Sullivan, F. Subrizi, D. Dobrijevic, J. Ward, H.C. Hailes, P. Fernandes, R. Wohlgemuth, F. Baganz, *Biotech. Bioeng*. 2018, **115(3)**, 586-596.

[9] R. Wohlgemuth, *React. Chem. Eng*., 2025, **10**, 278-293.

[10] R. Wohlgemuth, *Chimia* 2025, **79**, 352–358.

Multi-Enzyme Cascades for the Asymmetric Synthesis of Cyclic Amines

Joerg H. Schrittwieser,[a] Natália Alvarenga,[a,b] Philipp Petermeier,[a] Alejandro Mata,[a] Christoph Kohlfuerst,[a] Ana Torvisco,[c] Martín López,[a] Tobias Fleiss,[a] Alexander List,[a] André Luiz Meleiro Porto,[b] C. Oliver Kappe,[a] Wolfgang Kroutil[a]

[a] Department of Chemistry, University of Graz (Graz, Austria)

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Nitrogen heterocycles are a frequent structural motif in pharmaceutical drugs and bioactive natural products, in particular alkaloids.[1] Among the saturated N-heterocycles found in these molecules, chiral piperidine and pyrrolidine derivatives are common, yet their asymmetric synthesis remains a challenge. Biocatalysis offers an attractive entry to these important chiral amines, as it enables their preparation from simpler, open-chain precursors through enzyme cascades.[2]

In recent years, our group has developed several multi-enzyme cascades that target chiral piperidines and pyrrolidines. Initially, our efforts have focused on dihydropinidine, an alkaloid found in pine and spruce trees, which we have accessed in one chemo-catalytic and three biocatalytic steps (esterase, transaminase, IRED) from commodity chemicals.[3] Using enzyme-based stereocontrol, either enantiomer of the target compound could be prepared in high yield and optical purity, requiring only a single hydrochloride precipitation as the sole purification step in the entire synthetic sequence.

More recently, we used a diketoester intermediate from the dihydropinidine project as starting material for the preparation of chiral, trisubstituted piperidines through chemo-enzymatic or multi-enzymatic sequences of transamination, cyclisation, and reduction.[4] The target compounds were obtained with excellent enantio- and diastereocontrol, and in one example we observed true stereochemical cooperativity between two of the involved enzymes (transaminase, IRED). In related, as yet unpublished, work, we combined ADHs, transaminases, and IREDs to achieve the redox-neutral transformation of hydroxyketones into chiral pyrrolidines and piperidines.

In my talk, I will discuss the development and optimisation of the above-mentioned cascades and highlight the advantages that the multi-enzyme one-pot approach offers in each of these cases.

[1] E. Vitaku, D. T. Smith, J. T. Njardarson, *J. Med. Chem.*, 2014, **57**, 10257–10274.

[2] S. P. France, S. Hussain, A. M. Hill, L. J. Hepworth, R. M. Howard, K. R. Mulholland, S. L. Flitsch, N. J. Turner, *ACS Catal.*, 2016, **6**, 3753–3759.

[3] N. Alvarenga, S. E. Payer, P. Petermeier, C. Kohlfuerst, A. L. Meleiro Porto, J. H. Schrittwieser, W. Kroutil, *ACS Catal.*, 2020, **10**, 1607–1620.

[4] P. Petermeier, C. Kohlfuerst, A. Torvisco, R. C. Fischer, A. Mata, D. Dallinger, C. O. Kappe, J. H. Schrittwieser, W. Kroutil, *Adv. Synth. Catal.*, 2023, **365**, 2188–2202.

Biocatalytic cascades in continuous flow

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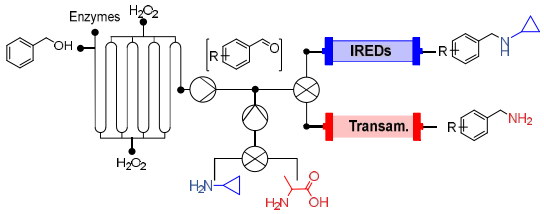
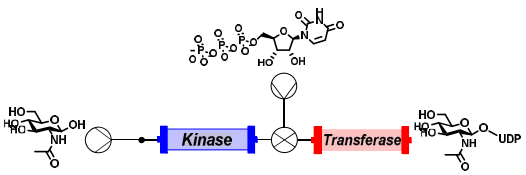
Biocatalytic cascade reactions are cited as being highly efficient when compared to analogous synthetic transformations due to commonality in conditions across all enzymes.[1] Whilst most enzymes do work under ambient, aqueous conditions, there is often more nuance than this due to pH and temperature requirements, and simply that some biocatalysts are not able to operate under identical conditions. Compartmentalisation can allow enzymes to be separated and enable different reaction conditions to be realised, which can be enabled with continuous flow.[2]

This talk will discuss some approaches our group has used to enable multi-step biocatalytic cascade reactions in continuous flow. This includes both immobilised and soluble enzymes, with discussion of case studies based on oxidase enzymes,[3] carbohydrate active enzymes,[4] and nucleoside kinases.[5]



Continuous biocatalytic cascades



Sugar nucleotide synthesis

1 S. P. France, L. J. Hepworth, N. J. Turner and S. L. Flitsch, *ACS Catal.*, 2017, **7**, 710–724.
2 S. C. Cosgrove and A. P. Mattey, *Chem. Eur. J.*, 2022, **28**, e202103607.
3 A. P. Mattey, G. J. Ford, J. Citoler, C. Baldwin, J. R. Marshall, R. B. Palmer, M. Thompson, N. J. Turner, S. C. Cosgrove and S. L. Flitsch, *Angew. Chem. Int. Ed.*, 2021, **60**, 18660–18665.
4 T. L. Roberts, J. P. Dolan, G. J. Miller, M. A. D. Lima and S. C. Cosgrove, *React. Chem. Eng.*, 2025, **10**, 1221–1226.
5 A. Naramittanakul, S. Sari, C. Benckendorff, J. Cheang, Y. Sanghvi, G. Miller and S. Cosgrove, *ChemRxiv*, 2025, preprint, DOI: 10.26434/chemrxiv-2025-snvjk-v2.

Intensification of chemo-enzymatic processes towards industrial volumetric productivities

P. Petermeier, Aarhus University/Denmark, J.P. Bittner, TU Hamburg/Germany,
T. Jonsson, Diduco AB/Sweden, S. Serban, Sunresin/China,
A. Basso, Sunresin/China, P. Domínguez de María, SusMom/Spain,
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The application of nature's catalysts, "enzymes," for chemical synthesis is a crucial emerging field in industrial biotechnology that meets current and future societal needs for sustainable chemical manufacturing. Nature employs an elegant and efficient synthetic strategy: coupling enzymes in multi-step pathways, without intermediate isolation or purification, with precise spatial control of catalysis. Inspired by nature, the design of multi-step biotransformations has been attracting significant attention within the biocatalysis community. The talk will introduce enzymatic decarboxylation reactions (in cascading systems), exploring the use of non-conventional media [1,2], enzyme immobilization, and different operational modes [3] to enhance the volumetric productivity of these biocatalytic applications. [4-6]

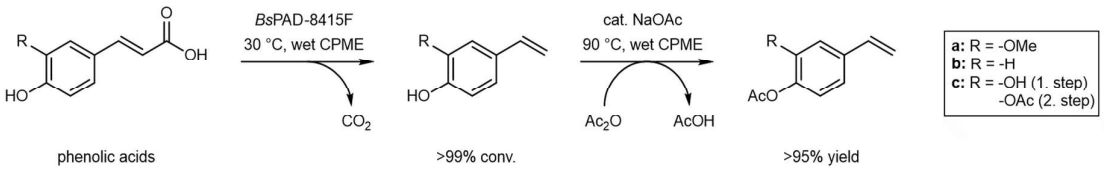

Figure 1: Chemoenzymatic synthesis of acetylated hydroxystyrenes from phenolic acids.

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Multi step cascades catalyzed by a single enzyme

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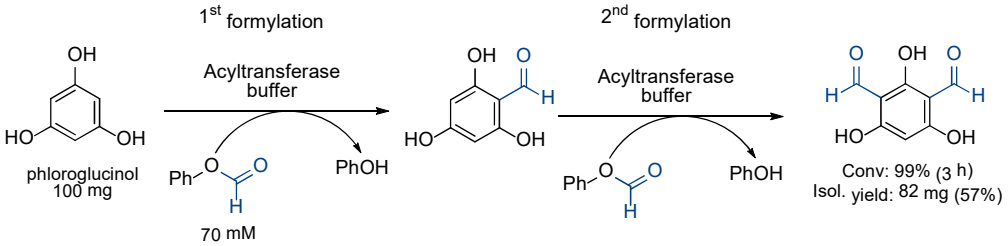
Most artificial cascades are performed by combining at least two enzymes to transform a substrate to the desired product via various intermediates [1]. During some of our recent work we came across some cases where a single enzyme may catalyze several reaction steps.

For instance, the formylation of phloroglucinol using an acyltransferase allowed to obtain selectively the di-formylated product via the mono-fomylated intermediate with 99% conversion [2] (Scheme 1).



Scheme 1. Di-formylation of phloroglucinol catalyzed by a single enzyme.

While in this first example, the enzyme enables the same type of reaction (acyltransfer) twice, we exploited in other examples, that a single enzyme may perform also different reactions such as C-H hydroxylation or alcohol oxidation or aldehyde oxidation (although all best on the same reaction concept). Alcohol/aldehyde oxidation has been used for the oxidation of two functional groups at the same molecule to oxidise HMF to the corresponding di-carboxylic acid [3]. A very interesting case is, when the second step works in a cooperative fashion with the first one to achieve for instance an improvement of the optical purity. For instance, the ee of α-hydroxylation of a fatty acid was improved by further oxidizing in an enantioselective fashion the minor enantiomer formed in the hydroxylation step to enhance the ee from 96 to 99% [4].

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Production of biobased monomers for polyesters and polyamides using (chemo)enzymatic cascades

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Replacing fossil monomers for polyesters and polyamides requires conversion processes that tolerate real biomass feedstocks, couple redox-intensive steps efficiently, and converge chemically diverse, heterogeneous substrates into a small set of value-added building blocks.

Here, a unifying cascade design concept is presented that combines enzyme promiscuity, cell-free pathway engineering, intrinsic cofactor balancing, and targeted chemo-catalytic steps to convert mixed carbohydrate streams and carbohydrate based furanics into polymer-relevant diols, diacids, and amino acids. Across different cascade architectures. It is demonstrated how convergent conversion of heterogeneous sugars, one-pot redox management, and chemoenzymatic integration enable compact processes to access key monomers such as 1,4-butanediol, succinic acid, furandicarboxylic acid and aminomethyl-furancarboxylic acid from renewable feedstocks.

Beyond specific products, the examples illustrate general design principles for multi-enzyme cascades, matching enzyme specificities to real feedstocks, coupling thermodynamically challenging steps, minimizing isolation steps, and embedding sustainability metrics early in process development. This could provide a blueprint for translating biocatalytic cascades into scalable routes for sustainable polyester and polyamide precursors. [1], [2], [3]

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Immobilization for Cascade Biocatalysis

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Multi-enzyme cascades enable intensified chemical synthesis by eliminating isolated intermediates, shifting equilibria through intermediate consumption, recycling cofactors catalytically, and minimizing downstream separations. In practice, however, one-pot cascades are often forced to operate under compromise conditions due to differing enzyme stability and activity optima. Stability and total turnover number (TTN) become particularly critical in these systems, as the use of multiple enzymes increases overall catalyst cost contribution and process sensitivity to enzyme deactivation.

Cascade Biocatalysts offers a solution to these cost and stability challenges through an immobilization platform that stabilizes enzymes via engineered covalent [1] and non-covalent [2-4] enzyme-polymer interactions. Building on prior academic work demonstrating significant increases in enzyme stability and TTN under challenging conditions, this approach enables stabilization strategies using commodity acrylic copolymers grafted onto conventional heterogeneous catalyst supports. These materials provide substantial increases in enzyme longevity under elevated temperatures, organic (co)solvents, and solvent-free conditions.

Individual cascade components may be immobilized on separate polymer-functionalized supports to maximize stability, then combined in batch or packed-bed flow configurations with minimal impact on overall catalytic rates. Alternatively, co-immobilization of compatible enzymes is possible where appropriate. This flexibility permits stabilization of heterogeneous enzyme ensembles while maintaining process design freedom.

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100 years of enzyme immobilization, what's next?

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In 1916 Nelson and Griffin discovered that invertase “exhibited the same activity when absorbed on a solid (charcoal or aluminum hydroxide) at the bottom of the reaction vessel as when uniformly distributed throughout the solution”. This discovery was the first of various enzyme immobilization techniques currently available. Besides adsorption, different covalent methods of enzyme immobilization were developed in the 1950s and 1960s.

To make a 'green process greener' cellulose beads can be used as enzyme carriers to replace non-renewable polluting plastic beads currently used in most industrial biocatalytic processes. Cellulose is a natural biopolymer extracted from wood, a renewable feedstock that can be sourced sustainably. Cellulose is thermally and mechanically stable while at the same time being biodegradable in the presence of microbes (e.g. in wastewater treatment sludge).

In collaboration with Naturbeads and Bath University a new cellulose carrier was developed that can act as a replacement for standard acrylic beads. The cellulose beads have well defined properties such as uniform size, spherical shape and high porosity (>90% pore volume). A series of experiments was run to introduce a variety of functional groups including covalent and ionic binding groups. This functionalization allows for binding of enzymes via different binding modes ensuring high loading, good activity recovery and stable performance.

Various enzymes were immobilized on functionalized cellulose beads and various applications were developed. Equal to even better performance and recyclability compared to the same amount of enzyme immobilized on a standard epoxide acrylic beads was demonstrated. This proofs that an enzyme immobilized on a cellulose bead can be a biodegradable and renewable alternative for plastic beads while at the same time having good or even better economics.

In collaboration with Technip Energies (former Ecovyst), a leading supplier of innovative silica based catalyst carriers, we investigated various types of silica’s (i.e. different porosities, pore sizes) and derivatizations thereof (e.g. installing ionic or hydrophobic groups) which can be used to immobilize enzymes.

One of the carriers that was used to immobilize lipase B from Candida antarctica (CaLB) actually performed better than formulations based on macroporous polyacrylic and polystyrene resins. In particular the esterification activity measured in propyl laurate units (PLU) was with >12000 PLU / g more active compared to Novozyme 435 (NZ435), the current industry standard with 10000 PLU/g. These results indicate that silica definitely has the potential to be a sustainable alternative to crude oil based resins for the immobilization of lipases without compromising on performance.

Engineering Alcohol Dehydrogenase for enhanced Cofactor recycling

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Biocatalysis is an advancing technology in chemical synthesis, highly regarded for its capability to generate chiral molecules with high selectivity under mild temperature and pressure conditions. It is widely utilised in the production of chiral compounds like alcohols and amines, which are essential to the pharmaceutical and agrochemical sectors.

Enzymes involved in these transformations typically rely on the cofactor NAD(P)H for reduction[1], necessitating its continuous regeneration through the addition of a catalytic system. The conventional glucose dehydrogenase (GDH) system provides several benefits, including its irreversible reaction-driven by the spontaneous hydrolysis of gluconolactone in water-which pushes the equilibrium toward product formation, as well as the high enzyme activity that allows for low enzyme loadings. However, this system also presents drawbacks, such as the requirement for dedicated pH monitoring equipment, limitations in substrate concentration due to glucose's limited solubility, and low atom efficiency, given that glucose serves as the final reductant.

At Johnson Matthey, we have developed a robust ADH enzyme that operates efficiently with isopropanol as the hydride donor, offering a viable alternative to the traditional GDH system. We report an integrated structure-guided mutagenesis and directed evolution approach for optimising the ADH system to the next level. Our findings demonstrate the successful development of self-sufficient systems for keto reduction, along with proof-of-concept for nitro reductions, using an ADH as an auxiliary enzyme in bi-enzymatic fashion.

This study underscores the potential of alternative biocatalytic systems to advance the synthesis of high-value chiral molecules, offering a more sustainable and efficient platform for industrial-scale applications.

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ENZYMATIC SYNTHESIS OF HUMAN MILK FAT SUBSTITUTE AND OTHER HIGH VALUE TRIGLYCERIDES BY IMMOBILIZED LIPASES: ROLE OF ENZYME CARRIERS AND REGULATORY IMPLICATIONS

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The industrial relevance of immobilised enzymes is primarily application driven, in the sense that there must be a differentiating advantage offered by such biocatalyst over soluble enzymes, whole cells or chemical catalysts. Therefore, the use of immobilised enzyme biocatalysts requires a good understanding of both technical and economic factors.

The manufacture of high value lipids as human milk fat substitute (HMFS) requires strict control over the enzyme and the enzyme carriers. In this presentation we review the effect of chemical composition of different enzymme carriers for lipase immobilization and their effect on enzyme activity, performances and also on regulatory aspects. Diffrent polymeric materials can be used as Polyethylene, methacrylate, epoxy carriers and styrenic. Each of them offer different benefits and are differently regulated in the market.

Sunresin has developed specific enzyme carriers suitable for lipase immobilization used in the manufacture of HMFS and other structured lipids. In addition to technical suitability such as high enzyme activity and extensive recycling, the enzyme carrier has to comply with stringent worldwide regulations. HMFS is the only source of food for human babies and infants in their first months of life therefore rigorous regulatory requirements are in place for its manufacture. Here, we review the global regulatory requirements for enzyme carriers and immobilized enzymes, by considering the chemical structure of the carrier and its combined effect with the lipase for an efficient and safe biocatalyst to use for HMFS manufacture.

Biocatalytic four-step cascade turning phenols into tyramines

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Biocatalytic cascades are of increasing interest for the synthesis of complex molecules such as APIs.[1] They are showing higher time- and cost-efficiencies as well as less waste generation than multi-step synthesis approaches, while making it possible to overcome unfavorable equilibria.[2]

Within our recent work we present a four-step cascade from phenol towards N-methyltyramine. This cascade starts with the formation of tyrosine by the tyrosine phenol lyase *Cf*TPL M379V, which is then deaminated by the L-amino acid oxidase AncLAAO. The formed α-keto acid is then decarboxylated by the indolepyruvate decarboxylase ipdc_Ec and the aldehyde aminated by IRED 35. Additionally, a commercial catalase preparation and the phosphite dehydrogenase PtDH Opt13 are added for cofactor recycling and side product removal.
The cascade was first established using the enzymes as CFE-preparations, causing elevated levels of aldehyde degradation and therefore limiting the amount of product formation. In order to avoid this effect, the cascade was then optimized with purified enzymes (except *Cf*TPL M379V). Within this optimization, the stoichiometry of enzyme activity was adjusted aiming for maximum product formation. The best enzyme loading was found to be 500 U/mL *Cf*TPL M379V CFE, 250 U/mL AncLAAO, 375 U/mL ipdc_Ec and 1125 U/mL IRED 35, yielding in 27% product formation in a one-pot-two-step approach.
Additionally, a substrate scope was performed where 11 different alkylamines as well as 17 *ortho*- and *meta*-substituted phenols were tested. For the amine donors, methylamine was the most suitable for the 2-step setup. In terms of the phenols, small substituents such as chloro- fluoro-, methoxy- or ethyl groups were accepted.

In conclusion, we successfully established and optimized a four-step cascade from phenol to N-methyltyramine and investigated its substrate scope in terms of amine donors and substituted phenols.

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Sustainable Phosphate-Catalyzed Synthesis of Non-Symmetric Pyrazines in Water – Mechanistic Insights, Biocatalytic Applications and Industrial Potential

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Keywords: *Biocatalysis, Biocompatibility, Cascade synthesis, Electrophile, Food industry, Pyrazines*

Pyrazines are pivotal flavor compounds with widespread applications in the food, pharmaceutical, and chemical industries. Their natural abundance is low, and traditional synthetic methods often involve hazardous conditions unsuitable for the food sector. In this study, we present a novel biocatalytic methodology for synthesising non-symmetric trisubstituted pyrazines using aminoacetone dimerisation followed by electrophile incorporation under environmentally benign conditions, catalyzed by phosphate anion. The approach includes the employment of an L-threonine dehydrogenase to generate aminoacetone *in situ* from natural L-threonine, integrating biocatalysis with green chemistry principles. Detailed mechanistic investigations, supported by control experiments and DFT calculations, revealed the critical role of phosphate buffering, an E1cB elimination, and a tautomerisation-driven pathway for product formation. The methodology demonstrates broad substrate scope and scalability, yielding pyrazines with diverse structural modifications up to 96% yields. This work establishes a starting point for the industrial production of non-symmetric pyrazines, addressing current regulatory and environmental demands in the flavor and fragrance sector.

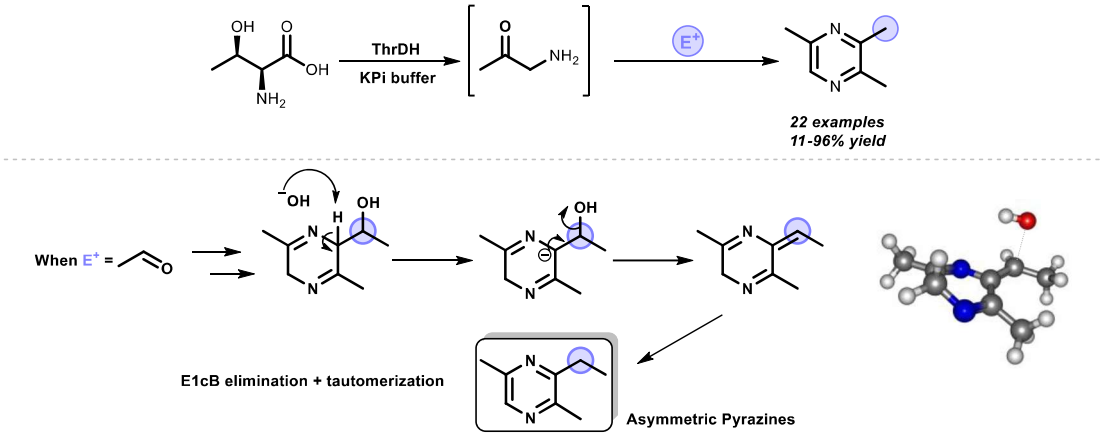

Figure 1. Schematic representation of the synthesis of pyrazines under biocompatible conditions.

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Valorisation of Sinapine-Rich Side Streams: An Enzyme Cascade for the Synthesis of Biobased Hydroxystyrenes

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The transition to a circular bio-economy requires efficient up-cycling of renewable biomass components. Lignocellulose-derived hydroxystyrene monomers, such as 4-vinyl syringol, are valuable precursors for the production of polymers and fragrances. 4-vinyl syringol can be obtained from the enzymatic decarboxylation of sinapic acid[1]. However, this substrate occurs predominantly as a choline ester in plant cell walls, and free sinapic acid has a limited solubility in aqueous solution and is only poorly converted by wild-type decarboxylases due to steric hindrance.

Here, we present an enzymatic cascade that overcomes these limitations by combining *Aspergillus niger* feruloyl esterase (*An*FAE), which efficiently releases sinapic acid,[1] with an engineered phenolic acid decarboxylase (PAD) to produce the corresponding hydroxystyrene. To address the solubility limitation of the hydrophobic sinapic acid, the cascade was established in deep eutectic solvents (DESs). While high DES concentrations impact enzyme activity, we optimised the composition of the DES and the buffer-to-DES ratio, allowing us to maintain high activity of both the esterase and the decarboxylase to obtain high conversion yields

To further improve the performance of the cascade, we engineered a previously reported reconstructed PAD ancestor (N31) with a melting temperature of 78 °C.[2] Rational engineering of the active site resulted in the N31-SSA variant, which exhibits an 11-fold increase in catalytic efficiency for sinapic acid and retains high thermostability ($t_{1/2}$ > 1 day at 50°C).[3]

This work introduces a biocatalytic cascade that combines enzyme engineering with solvent engineering. We show that coupling the release of phenolic acids from renewable feedstocks with their decarboxylation offers a sustainable path for producing high-value biobased monomers.

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Flowing Green: Intensified Multi-Step Biocatalysis Enabled by Enzyme Immobilization and Advanced Reactor Technology

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Biocatalysis is increasingly shaping the future of sustainable chemical manufacturing by enabling selective, low-impact routes to pharmaceuticals, food ingredients, cosmetics, and fine chemicals. [1] In particular, multi-step enzyme-catalyzed processes are emerging as powerful tools to streamline synthesis, reduce intermediate purification, and enhance overall process efficiency. Modern approaches combine enzyme immobilization, reactor technology, and process intensification strategies to integrate sequential biotransformations into scalable and resource-efficient platforms.

Continuous-flow biocatalysis provides an ideal framework for implementing enzymatic cascades, allowing precise control over reaction parameters, improved mass transfer, and seamless coupling of multiple catalytic steps. Immobilized enzymes play a key role in these intensified systems by enabling catalyst reuse, enhancing operational stability, and facilitating modular reactor configurations suitable for sequential or tandem transformations. [2,3] In parallel rotating bed reactors (RBRs), further support multi-step processing by improving mixing and mass transfer, thereby enhancing catalytic performance. [4] These integrated systems have demonstrated strong potential in the multi-step synthesis of pharmaceuticals, key building blocks, aroma compounds as well as the valorization of renewable feedstocks, enabling the efficient conversion of agro-industrial by-products into bioactive molecules with significant environmental and economic value (Fig.1).

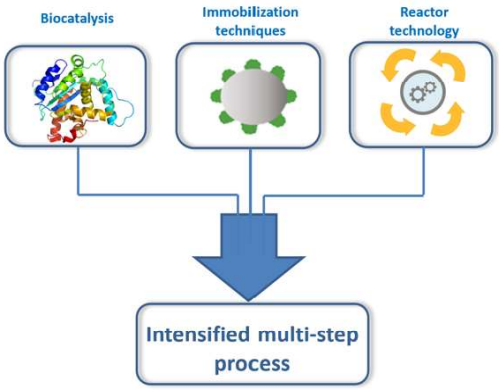

Figure 1: Merging biocatalysis, protein immobilization and reactor technology for intensified multi-step enzymatic processes

Overall, the convergence of enzymatic cascades, immobilization strategies, and innovative reactor technology highlights how biocatalysis is evolving into a robust, scalable, and high-productive technology for sustainable chemical manufacturing.

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Reductive amination-based multi-enzyme approach for the synthesis of D-phenylalanines

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Aromatic D-amino acids (D-AAs) have emerged as valuable building blocks, due to increasing demand for enantiopure chiral starting materials [1]. Biocatalytic approaches provide an environmentally friendly and highly stereoselective alternative to traditional chemical routes. Therefore, we investigated the reductive amination-based synthesis of D-phenylalanine derivatives, using engineered D-amino acid dehydrogenases (DAADHs) [2].

The stereoselective synthesis of D-phenylalanines was first approached through a chemo-enzymatic cascade, integrating a Knoevenagel-Doebner condensation chemical step, followed by sequential biotransformations mediated by phenylalanine ammonia-lyase (PAL), L-amino acid deaminase (LAAD), and DAADH. To improve the efficiency and economic viability of the multi-step cascade, we optimized the immobilization of DAADH by evaluating different solid supports and various binding approaches. Covalent attachment to Purolite® ECR8415F resin and co-entrapment of DAADH with its cofactor regenerating glucose dehydrogenase (GDH) on aldehyde-activated agarose beads [3] proved to be efficient and economically viable approaches and were therefore selected for further refinement.

Following optimization, site-specific immobilization of DAADH was achieved *via* maleimide-thiol coupling. Finally, preparative-scale reactions were performed to assess the catalytic performance, stability, and recyclability of the immobilized biocatalysts.

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ATP-Dependent Multi-Enzymatic Cascade for Sustainable Amide Bond Formation

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Amide bond formation is a key transformation in pharmaceutical synthesis and represents a substantial portion of medicinal chemistry reactions. Conventional coupling methods rely on stoichiometric activating reagents, which reduce atom economy and generate considerable waste [1,2]. ATP-dependent amide bond synthetases (ABSs) provide an alternative approach based on enzymatic carboxylate activation through formation of a reactive acyl–adenylate intermediate [2,3], enabling amine coupling under mild aqueous conditions.

This work describes the development of an ATP-dependent multi-enzyme cascade for amide bond formation using cinnamic acid as a model substrate. The sequence involves ABS-mediated activation followed by amine coupling, combined with pyrophosphate hydrolysis using inorganic pyrophosphatase to support the overall driving force of the reaction [4].

This study identifies the key factors influencing the productivity and stability of a multienzymatic cascade and discusses their implications for process design. In addition to pH, temperature, substrate concentrations, and enzyme ratios, various reaction media—including deep eutectic solvents—were systematically evaluated. Initial results on the immobilization of both enzymes and their integration into a microfluidic reactor are also presented, highlighting the potential for continuous and intensified multi-step biocatalytic processing.

Acknowledgement: The research was funded by the Interreg Central Europe project CE0200857 GreenChemForCE, co-funded by the European Union. TM and PŽP were supported by the Slovenian Research Agency (Grant P2-0191) and EU Horizon Europe project FlowCat (Grant No. 101160108).

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Design and development of photobiocatalytic methods for the stereoselective synthesis of chiral alcohols

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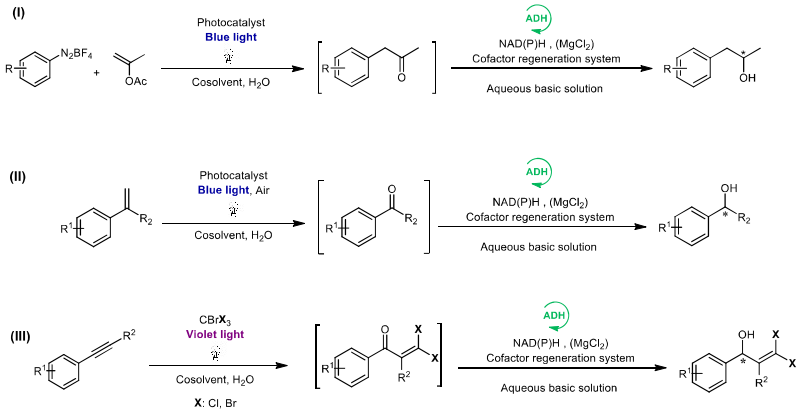
Photochemistry and biocatalysis are complementary disciplines for accessing molecular architectures difficult to obtain by conventional synthetic methods. Their integration enables efficient and sustainable transformations under usually sustainable conditions [1]. This contribution summarizes one-pot photobiocatalytic cascades combining visible-light photocatalysis and enzymatic carbonyl reduction of the so-obtained intermediates using alcohol dehydrogenases (ADHs). Thus, the stereoselective synthesis of optically active secondary alcohols in aqueous medium is successfully achieved starting from different organic molecules (aryl diazonium salts, alkenes or alkynes, Scheme 1).

The first approach discloses a two-step photobiocatalytic sequence for the stereoselective synthesis of 1-arylpropan-2-ols from aryl diazonium salts combining a light-driven Meerwein arylation with an ADH-catalyzed reduction. Extending this, the second strategy increases the scope of photobiocatalytic cascades by combining the photo-oxidative cleavage of alkenes into ketones with a carbonyl bioreduction step, enabling access to a range of secondary alcohols with stereoselectivity. Finally, a one-pot sequential process is presented for the synthesis of optically active *γ*,*γ*-dihalo-*β*-enols from alkynes, in which visible-light activation generates *gem*-dihaloenone intermediates that are subsequently bioreduced with efficiency [4].



Scheme 1. Three different photobiocatalytic cascades for the synthesis of optically active alcohols.

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The Enzymatic Chameleon: Rethinking Alcohol Dehydrogenases for the Synthesis of Amides and Thioesters

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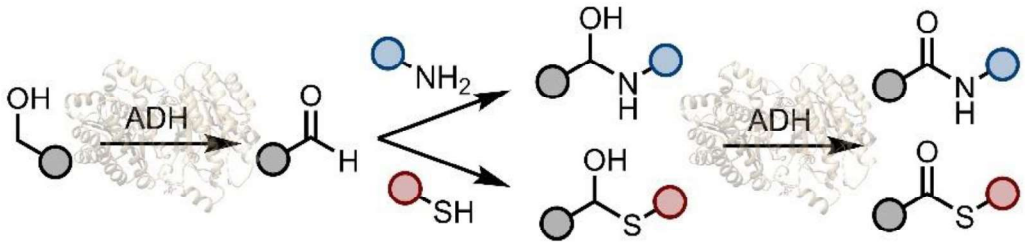
Alcohol Dehydrogenases (ADHs) have long been recognized for a single, yet powerful, skill: the highly enantioselective reduction of ketones.[1-4] However, scattered reports of their catalytic promiscuity hinted at a broader reactivity, including the formation of carboxylic acids,[5-8] lactones,[9] and lactams,[10] an area that has remained largely underexplored.

Building on these observations, we re-examined ADHs as versatile oxidative biocatalysts. We demonstrated that, under carefully tuned reaction conditions, ADHs can promote oxidative coupling reactions, enabling the efficient synthesis of high-value amides and thioesters directly from simple alcohols and amines or thiols.[11] Furthermore, enzyme engineering significantly broadened the substrate scope of this methodology, opening new opportunities for new synthetic routes.



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From C1 molecules to ethyl formate:
A sustainable concatenated catalytic process

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For a circular and sustainable bioeconomy, fossil-based feedstocks that cause global warming and climate change need to be replaced with sustainable ones. We follow an approach in which CO_2 is first converted into reactive C1 building blocks or small molecules, such as formic acid. These C1 intermediates can be further processed by biological, electrochemical, or chemical techniques into a wide range of products, including fine and bulk chemicals or biohybrid fuels. [1] By concatenating chemical catalysts with biocatalysts and microbes in one process, best features of each catalyst can be combined to more sustainable, and efficient processes with reduced required downstream processing steps. [1,2]

The process we based our work on is a combined microbial and chemo-catalytic one-pot process for the production of formic acid and bioethanol including a CO_2 integration step and using renewable resources [3]. Now, we extend the process to produce ethyl formate (see Figure 1) by addition of an enzymatic step.

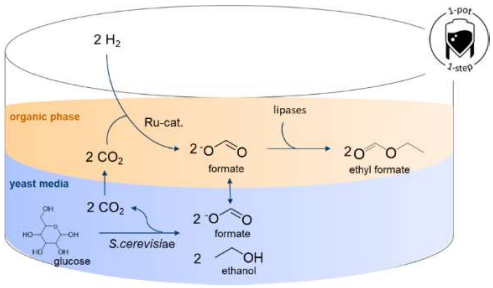

Figure 1: One-pot, one-step process with three different catalyst types.

Determining the operating window is crucial for the process development of such a complex multiphase reaction system. With *Candida antarctica* lipase B (CAL-B) we identified a suitable enzyme to catalyze the esterification. The synthesis should be performed under organic reaction conditions, as ethyl formate hydrolyzes under aqueous conditions. Other parameters as the choice of the organic solvent, the catalyst compatibility, and the ratio between the phases in a two-phasic system are key parameters. By identifying these parameters and limitations, the best process mode was determined. The concept developed can be applied to other formate esters, enabling sustainable production with CO_2 integration.

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Scale-up of Multi-step Fungal Glycosylation of Chlorinated Chalcones and Integrated By-product Valorization

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The enzymatic functionalization of flavonoids is a key strategy for enhancing their bioavailability and biological activity. This study explores the multi-step biotransformation of chlorinated 2'-hydroxychalcones into their 4''-*O*-methylglucopyranosyl-dihydrochalcone derivatives with antimicrobial activity. The process, which combines a double-bond reduction with a regioselective methylglycosylation cascade, was performed using the whole-cell biocatalysts – entomopathogenic filamentous fungi strains *Isaria fumosorosea* KCH J2 and *Beauveria bassiana* KCH J1.5 [1,2].

The study focused on optimizing the bioprocess parameters to facilitate the transition from analytical flasks to a multi-liter orbital bioreactor. A comparative analysis of carbon sources revealed that glucose supports superior biomass growth and conversion efficiency compared to glycerol. To improve the process's energy profile, the cultivation temperature was optimized to 24°C, which allowed for stable production without loss in yield. Utilizing a fed-batch strategy, the bioreactor cultures achieved effective substrate loading of the chlorinated aglycones. Following the biotransformation, the products were recovered via liquid-liquid extraction and purified using flash chromatography and crystallization. This downstream processing workflow consistently yielded glycosylated derivatives with high purity, suitable for advanced bioactivity screening.

In alignment with circular economy principles, the research also addressed the valorization of post-reaction by-products. The fungal biomass, characterized by a rich profile of specialized enzymes and bioactive peptides, was evaluated for its suitability as a functional feed additive component. Simultaneously, the post-culture filtrate was investigated as a prospective source of hydrolytic enzymes for the treatment of fruit seed coats to release bioactive compounds for cosmetic applications. This integrated approach demonstrates a scalable and sustainable model for the production of halogenated flavonoid glycosides.

Acknowledgement: This research was funded by the National Centre for Research and Development (NCBR), Poland, under the LIDER XIV programme, grant No. LIDER14/0106/2023.

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Developing fluorescence-based coupled enzyme cascade to screen NAD(P)H-dependent oxidoreductases

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Enzymes play a pivotal role in green chemistry as tools for biocatalysis, enabling a wide range of chemical transformations that may not be accessible through conventional synthetic approaches. Oxidoreductases are particularly valuable due to their ability to catalyze key electron transfer (redox) reactions, including asymmetric hydrogenation, oxygenation, hydroxylation, epoxidation, and Baeyer–Villiger oxidation[1,2]. Many of these enzymes depend on NAD(P)H as an electron-donating co-substrate or $NAD(P)^+$ as an electron acceptor. Protein engineering strategies, such as directed evolution and rational design, rely on screening mutant libraries to enhance enzyme activity, specificity, and efficiency. Selection techniques and screening methods, including coupled enzyme assays and multi-enzyme pathways, are commonly used to evaluate functional variants and facilitate rapid assessment of enzyme performance.

The ability to sensitively detect oxidoreductase activity is critical for identifying useful biocatalysts from nature and for engineering improved variants, particularly when native reaction rates are low. Traditionally, enzyme activity is monitored by measuring changes in absorbance at 340 nm as NAD(P)H is converted to $NAD(P)^+$ (or vice versa). However, the limited sensitivity of absorbance-based assays presents challenges in detecting low levels of enzyme activity, thereby constraining high-throughput screening efforts[3,4].

Herein, we report the development of a fluorescence-based coupled enzyme cascade system for detecting NAD(P)H-dependent oxidoreductase activity with orders of magnitude greater sensitivity than conventional absorbance-based methods. By recycling NAD(P)H from $NAD(P)^+$, the coupled enzyme cascade triggers cleavage of a fluorogenically labeled probe, generating a strong fluorescent signal. This approach enables sensitive, continuous, and high-throughput detection of low levels of NAD(P)H/$NAD(P)^+$-dependent oxidoreductase activity, providing an effective platform for screening and engineering redox enzymes in protein engineering and biocatalysis applications.

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Heterotrophic and phototrophic multistep biocatalysis for the production of nylon building blocks from renewables

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Multistep whole-cell biocatalysis is a versatile approach to facilitate the sustainable synthesis of value-added compounds, such as polyamide (PA) building blocks, from renewables. In contrast to the current fossil-based PA production, which relies on energy- and waste-intensive multi stage processes, whole-cell multistep biocatalysis benefits from highly specific enzymatic reactions under mild conditions, resulting in less by-product formation [1]. Moreover, orthologous pathways (*in-vivo* cascades) allow multiple reactions in a single process step, avoiding intermediary purification steps [2-3]. Nylon 6 (PA6), a major industrial polyamide, is derived from 6-aminohexanoic acid (6-AHA), a compound of dual relevance as both a polymer building block and a pharmaceutical agent for the treatment of aneurysmal subarachnoid haemorrhages.

Our research aims to engineer biocatalysts capable of efficiently producing 6-AHA from renewable hexanoic acid (esters) through an enzymatic cascade assembled from genes of diverse microbial origin. The goal is to engineer *in-vivo* cascades in heterotrophic *Escherichia coli* JM101 and phototrophic *Synechocystis* sp. PCC 6803, the latter for light-driven 6-AHA synthesis, and to maximize, scale, and compare production efficiencies. The substrates hexanoic acid or its methyl ester (HAME) can be derived from waste biomass via an anaerobic digestion process developed at the UFZ.

To quantitatively analyse, detect bottlenecks in, and optimize the transgenic cascade, the enzymes were tested individually and in combinations. Based on monooxygenase, alcohol dehydrogenase, and transaminase catalysis, promising terminal HAME functionalization activities have been achieved. Further, intracellular recycling and supply of alanine as amino donor is targeted involving an alanine dehydrogenase.

The resulting reaction cascade is to be implemented in a suitable bio-process concept mitigating substrate toxicity likely caused by its accumulation in cell membranes [3]. In this respect, various reactor strategies will be presented and discussed, including substrate feeding controlled via off-gas MS.

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ThDP-dependent enzyme for multi-step biocatalytic synthesis

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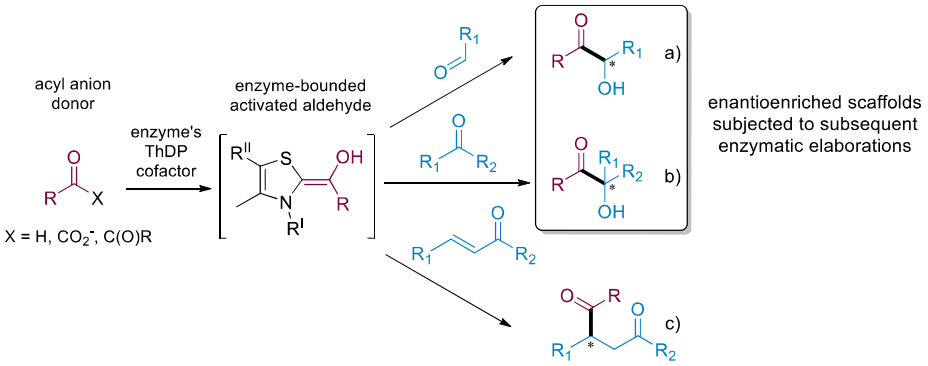
ThDP-dependent enzymes represent powerful biocatalytic tools for the asymmetric synthesis of multifunctional chiral building blocks, which can serve as useful intermediates for subsequent enzymatic transformations.[1] The reactivity of ThDP-dependent enzymes is related to 1,2-addition, including aldehyde–aldehyde and aldehyde–ketone benzoin condensation (*a* and *b* in **Scheme 1**) [2], and to 1,4-addition such as the Stetter reaction (*c* in **Scheme 1**).[3]



Scheme 1. Schematic representation of ThDP-dependent enzymes reactivity

In recent years, the ThDP-dependent enzyme acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR), has been employed by our research group for the synthesis of enantioenriched secondary [4,5] and tertiary α-hydroxyketones [6]. These compounds were subsequently utilized as intermediates in further enzymatic transformations, including reactions catalyzed by NAD(P)H-dependent reductases, lipases and transaminases, enabling the development of multi-step biocatalytic syntheses.

The proposed contribution will present recent examples of multi-step enzymatic synthesis in which the ThDP-dependent enzyme plays a pivotal role.

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CalA engineering: selectivity improvement, mechanistic insights, and unexpected applications.

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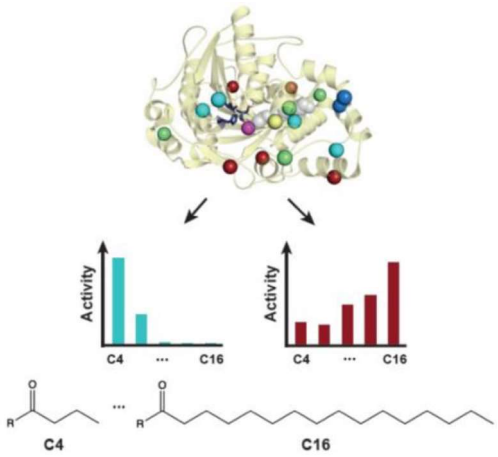
Our laboratory extensively studied the biocatalyst *Candida antarctica* lipase A (CalA) and its potential as a selective catalyst for the hydrolysis of fatty acid esters of different chain lengths.[1–3] CalA was engineered for this purpose, and, while this enabled the desired selectivity, it also revealed a deeper understanding of its mode of action. As previously predicted, CalA's mechanism involves a tunnel region for substrate recognition; however, we unexpectedly found that residues in distal regions are also key for modulating substrate selectivity, and uncovered otherwise hard-to-predict epistatic effects of the introduced mutations.[3]



A deeper understanding of the enzyme led us to hypothesize that CalA might accept bulky substrates in addition to its natural triglycerides. Very recently, we discovered that some previously engineered CalA variants can degrade Impranil DNL, an emulsifiable industrial polyurethane used in our laboratory for the discovery of plastic-degrading enzymes. Pre-existing libraries of thousands of CalA variants were tested for specificity towards this substrate.[2] Several variants were identified that catalyze its hydrolysis. This is a remarkable result that warrants further investigation, which we are currently undertaking. Not only will this help in our quest to identify an effective enzyme for degrading plastic, one of the main scopes of our laboratory, but the data obtained will also improve our understanding of the mechanism of action of CalA, a valuable addition to our growing puzzle.

Here, we present this journey of discovery, what we learnt so far, and our latest results on this versatile and polyvalent biocatalyst.

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Engineered Phototrophic Mixed-Species Biofilms for Continuous Production of Polycaprolactone Precursors from Cyclohexanol in Multi-Stage Drip-Flow Reactors

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Sustainable biotechnological production routes for polymer precursors are essential to diminish reliance on petrochemical processes. In the current study, we present a biofilm-based photobiocatalytic reactor system enabling the conversion of cyclohexanol (CHOL) to ε-caprolactone (ε-CPL) and 6-hydroxyhexanoic acid (6-HHA), both key intermediates in polycaprolactone (PCL) synthesis. The system involves a mixed-species biofilm consortium composed of *Pseudomonas taiwanensis* VLB120 and *Synechocystis* sp. PCC 6803 cultivated in continuous, light-driven, multi-stage drip-flow biofilm reactors (DFR).

Differential species colocalization along the reactor axis was observed evidently, with heterotrophic partner predominating in the upper substrate-loading stage and phototrophic involvement intensifying toward the middle and bottom stages. This axial metabolic zonation was supported by scanning electron microscopy and stage-resolved light–dark shift experiments. Dark operation resulted in a stage-dependent decrease in total product formation of approximately 32% in the upper stage, 57% in the middle stage, and 84% in the bottom stage, indicating differential species localization and increasing reliance on phototrophic metabolism toward the lower reactor zones.

Optimal substrate loading (5–6 mM CHOL) resulted in a maximum product formation rate of 37.9 $g \cdot m^{-2} \cdot day^{-1}$, corresponding to substantially higher effective substrate availability compared to suspended-culture systems (typically 0.3–1mM). Product selectivity was pH-dependent: near-neutral pH (7.5–7.9) supported balanced formation of ε-CPL and 6-HHA, whereas alkaline conditions (pH 8.5–10) promoted lactone hydrolysis, increasing 6-HHA formation.

Overall, these findings demonstrate the technical feasibility of light-driven, biofilm-based whole-cell redox biocatalysis for sustainable PCL precursor production from cyclohexanol.

Continuous δ-viniferin synthesis in a microreactor using magnetite-immobilized combined cross-linked enzyme nanoaggregates

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δ-Viniferin, a resveratrol dimer, exhibits diverse biological activities and shows potential for the prevention and treatment of cancer, cardiovascular disorders, and neurodegenerative diseases [1]. Despite its promising bioactivity, its high market price significantly limits further research and broader application. *δ*-viniferin was successfully synthesized from resveratrol and hydrogen peroxide using horseradish peroxidase (HRP) [2]. However, excess hydrogen peroxide can inactivate HRP by forming inactive enzyme complexes [3]. To mitigate this effect, glucose oxidase (GOx) can be introduced in the enzyme cascade system for *in situ* hydrogen peroxide generation from glucose. This approach enables controlled production and immediate consumption of this substrate by HRP, thereby reducing enzyme deactivation [4].

The aim of this study was to develop a cost-effective and sustainable process for *δ*-viniferin synthesis using HRP–GOx enzymatic cascade reaction in a continuously operated microreactor system. To improve enzyme stability and reusability, both enzymes were co-immobilized as cross-linked enzyme nanoaggregates (combiCLEAs) within a microfluidic system, following a modified method recently developed by our group [5].

HRP and GOx were first precipitated with acetone and subsequently cross-linked with glutaraldehyde using an innovative microfluidic approach, yielding uniform nanoscale combiCLEAs that fully retained the enzymatic activity of both enzymes. The resulting combiCLEAs were then immobilized onto magnetite nanoparticles to ensure efficient retention and handling within a custom-designed, 3D-printed microreactor. Shelf-life studies were performed on the free enzymes, combiCLEA particles, and magnetite-supported combiCLEAs, together with an evaluation of the operational stability under continuous-flow conditions in the microreactor.

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Photobiocatalytic Deracemization of 3-Hydroxypropanonitriles: Advances in Asymmetric Synthesis

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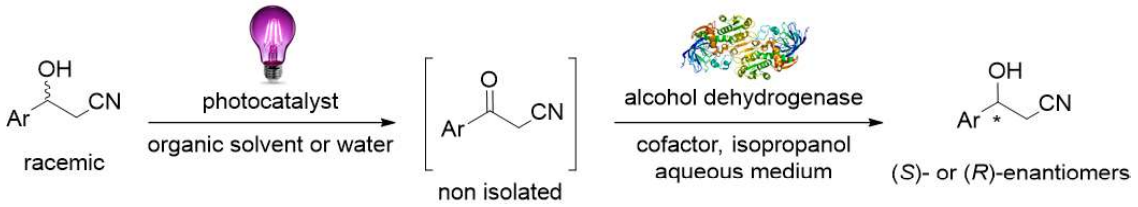
Enantiopure 3-hydroxypropanenitriles are valuable intermediates in organic synthesis, serving as chiral building blocks for the production of biologically active compounds such as Fluoxetine derivatives. In 1994, Lilly Research Laboratories proposed an asymmetric synthesis starting from a racemic mixture of 3-hydroxypropanenitriles prepared by a direct 1,2-addition of acetonitrile to benzaldehyde. However, the overall yield was compromised by a kinetic resolution step that discards half of the starting material.[1] In contrast, a deracemization strategy would provide a full-conversion. This process couples an initial oxidation of the racemic alcohol to the corresponding 3-oxopropanenitrile with a subsequent stereoselective bioreduction.[2,3] In this context, methodologies that employ catalysts, green oxidants, and mild conditions are highly desirable for large-scale applications. Thus, photochemistry emerged as a promising tool for achieving redox transformations. However, current reports on the photobiocatalytic deracemization of secondary alcohols still face challenges in efficiently oxidizing 3-hydroxypropanenitriles.[4,5]

In this study, we propose a one-pot two-step photobiocatalytic methodology for the efficient linear deracemization of 3-hydroxypropanenitriles (Figure 1). This strategy involves an initial selective photooxidation step of the alcohol functionality under violet light irradiation generating the corresponding 3-oxopropanenitrile, followed by a stereoselective bioreduction catalyzed by an alcohol dehydrogenase overexpressed in *Escherichia coli*. Our approach achieves significant progress in the synthesis of enantiopure 3-hydroxypropanenitriles, overcoming the challenging oxidation step and enabling new synthetic pathways for the efficient production of these valuable intermediates with high applicability in the pharmaceutical industry.



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SELECTIVE OXYFUNCTIONALIZATION OF AROMATIC HYDROCARBONS BY RECOMBINANT CYP153A-*ACETOBACTER MALORUM*

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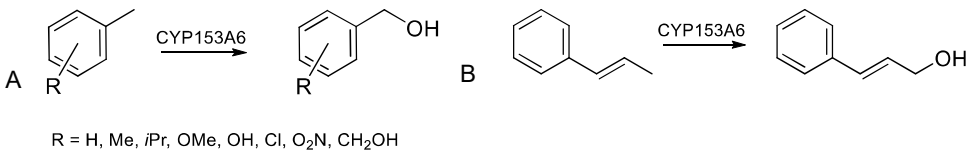
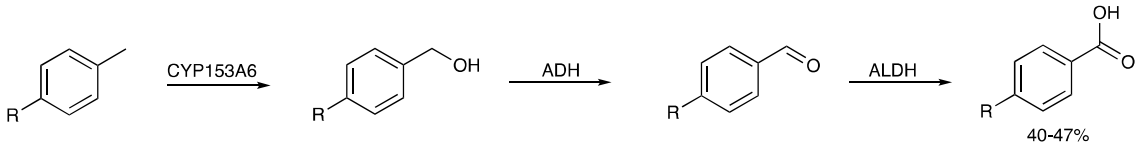
Biocatalytic oxyfunctionalization of aliphatic and aromatic hydrocarbons is crucial for selectively converting inert compounds into reactive, valuable derivatives. CYP153A6, a cytochrome P450 monooxygenase from *Mycobacterium sp*., catalyses the highly selective terminal hydroxylation of linear and alicyclic alkanes [1].
In this work, the CYP153A6 enzyme was expressed in *Escherichia coli*, and tested as crude extract on a panel of aromatic hydrocarbons (Fig.1).



R = H, Me, *i*Pr, OMe, OH, Cl, O_2N, CH_2OH

***Figure 1**: Hydroxylation of A) toluene derivatives and B) Trans ß methylstyrene catalyzed by CYP153A6*

The enzyme selectively hydroxylated the substrates at the benzylic position. In the case of 4-xylene, only one of the two benzylic methyl groups was hydroxylated, demonstrating the high selectivity of the biocatalyst. Among the tested compounds, 4-methylanisole was the most reactive substrate (specific activity of 1.75 U mg^{-1}), whereas 4-cresol, 4-methylbenzyl alcohol, and 4-nitrotoluene showed no detectable activity.



R = Me, *i*Pr

***Figure 2**: Sequential oxidation of toluene derivatives using CYP153A6-Acetobacter malorum*

Next, we engineered *Acetobacter malorum* DSM 112354, an acetic acid bacterium (AAB) isolated in our laboratory [2], to express CYP153A6 using the plasmid pSEVA331Bb encoding for the CYP450 monooxygenase, plus a ferredoxin and a ferredoxin reductase to ensure efficient electron transfer. AAB efficiently oxidize primary alcohols into carboxylic acids, taking advantage of the several native membrane-bound alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH) [3].
By leveraging this intrinsic oxidative capability, engineered CYP153A6-*A. malorum* enabled the direct one-pot conversion of the aromatic hydrocarbons to their carboxylic acid derivatives (substrate concentrations: 5-35 mM) (Fig. 2).

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Engineered Enzymatic Cascades: Overcoming Challenges in P450 Monooxygenase Biocatalysis

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This contribution presents our recent advances in engineered enzymatic cascades, focusing on cytochrome P450 (CYP) monooxygenase fusion constructs to tackle the limitations of these powerful biocatalysts. Cytochrome P450s, renowned for their versatility, face hurdles such as cofactor dependency, electron transfer partner requirements, and stability issues that hinder their industrial scalability. We combine whole-cell biotransformation with fusion constructs that integrate CYP enzymes, electron transfer partners, and an esterification module to streamline product extraction.

We constructed and optimized fusion constructs using diverse class I bacterial CYP enzymes and ferredoxin reductase-ferredoxin electron transport systems. Our enzymatic cascades demonstrated efficient conversion of aliphatic compounds, showcasing the potential of whole-cell biotransformation.[1] Through reaction optimization, we enhanced product yield, minimized side product formation, and controlled substrate toxicity. Resting cell assays with various carbon sources maintained cell activity without promoting growth.[2]

Moreover, we identified optimal ferredoxin reductase-ferredoxin couples as adaptable electron transport systems, driving CYP-catalyzed monooxygenation reactions with improved efficiency.[3] Our approach offers a sustainable solution for producing valuable chemicals, with significant implications for developing efficient and selective hydroxylation processes for various industries.

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From a transcriptomic approach towards a multi-step biodegradation process of polyethylene by *Rhodococcus opacus* R7 laccase and oxygenase enzymes

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Polyethylene (PE), the most widely produced synthetic polymer, significantly contributes to plastic pollution due to its chemical inertness and persistence, ultimately leading to the accumulation of microplastics in the environment. Although several microorganisms have been reported to transform PE, the molecular mechanisms and the enzymatic cascade underlying this process remain insufficiently defined.

This study presents an integrated transcriptomic and enzymatic exploration of PE biodegradation by *Rhodococcus opacus* R7 to delineate a multi-step oxidative process.

R. *opacus* R7 grows on untreated PE as the sole carbon and energy source, and exhibits extracellular laccase activity during the first 14 days of incubation.
RNA-seq approach revealed a coordinated activation of multiple oxidoreductases, including three laccase-like multicopper oxidases (LMCOs), an alkane monooxygenase (*alkB*), and cytochrome P450 hydroxylases [1]. Moreover, gene expression monitored up to 28 days highlighted that PE triggered a temporally regulated transcriptional response. Results showed early activation of *alkB* and *P450II* as well as *LMCO1*, *LMCO2*, *LMCO3*, and *LMCO5* (at 3 days), followed by delayed induction of specific *LMCOs* such as *LMCO6* (at 14 days).

Among these oxidases, LMCO2 and LMCO3 were cloned, expressed, and purified as recombinant enzymes. Biochemical characterization, FTIR spectroscopy, and GC-MS analyses demonstrated their oxidative activity on untreated PE within 24 hours. Structural modeling and density functional theory calculations suggested their peculiar properties supporting the catalytic potential toward PE activation [2].

Specifically, the redox potential of LMCO2 was experimentally examined, classifying LMCO2 as a low-potential laccase. The hydrophobic T1-site and a Met-loop of LMCO2 appear to stabilize PE binding near the copper center. This suggests that substrate positioning and binding mode, rather than redox potential alone, are critical for PE oxidation, along with PE flexibility, allowing it to fit into the enzyme's binding pocket [3].

Other LMCO systems predicted as laccase-like enzymes are under characterization, since their transcriptional response to PE varies according to the PE concentration and mode of administration.

Our findings support a multi-step, enzyme-catalyzed degradation model in which extracellular laccases initiate polymer oxidation, followed by intracellular oxygenase-mediated processing of oxidized fragments, providing a mechanistic framework for sustainable PE bioconversion.

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Lipase-Catalyzed Production of Esters of Hydroxycinnamic Acids with Antihypertensive and Antimicrobial Potential

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Hydroxycinnamic acids (HCAs), including ferulic, *p*-coumaric, caffeic, and sinapic acids, are naturally occurring phenylpropanoids with antioxidant, antimicrobial, and health-promoting properties [1], but their high polarity limits application in lipophilic systems. Esterification with hydrophobic alcohols is an effective strategy to enhance lipophilicity while maintaining or improving biological activity.

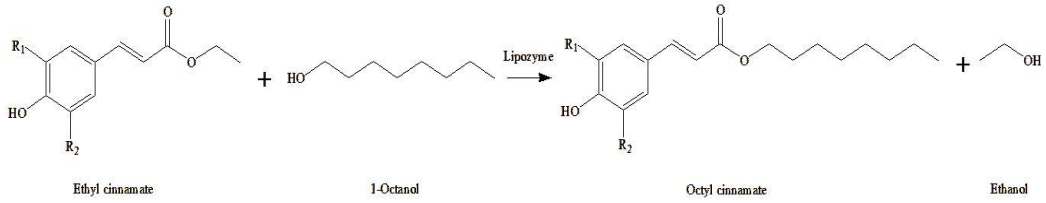

Figure 1. Lipase-mediated transesterification of ethyl esters with 1-octanol: $R_1 = R_2 = H$ (*p*-coumaric acid), $R_1 = OH$ and $R_2 = H$ (caffeic acid), $R_1 = OCH_3$ and $R_2 = H$ (ferulic acid), $R_1 = R_2 = OCH_3$ (sinapic acid).

Selected alkyl esters of HCAs were synthesized via lipase-catalyzed transesterification of ethyl esters with octanol and 2-phenylethanol, using immobilized lipase (Lipozyme®435) in diisopropyl ether (Figure 1). Reactions were performed in batch with conventional heating, batch under microwave irradiation, and in a continuous flow packed-bed reactor. Batch reactions achieved conversions up to 70% within 6 h, while microwave heating provided comparable results. Continuous flow experiments showed steady-state conversions of 45-67% and stable operation for at least 22 h, with reduced enzyme loading achieved using inert glass beads.

The synthesized esters showed enhanced biological activity; octyl *p*-coumarate and octyl and ethyl ferulate exhibited enhanced angiotensin-converting enzyme (ACE) inhibitory activity compared to the parent acids. Increased lipophilicity also enhanced antimicrobial activity against human pathogenic strains *Staphylococcus aureus* ATCC6538, *Escherichia coli* ATCC11229, and *Candida albicans* ATCC10231, highlighting their potential for food, cosmetic, and pharmaceutical applications.

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One-Pot Enzymatic Cascades for the Conversion of Biomass-Derived Phenolic Acids into Chiral Aromatic Building Blocks

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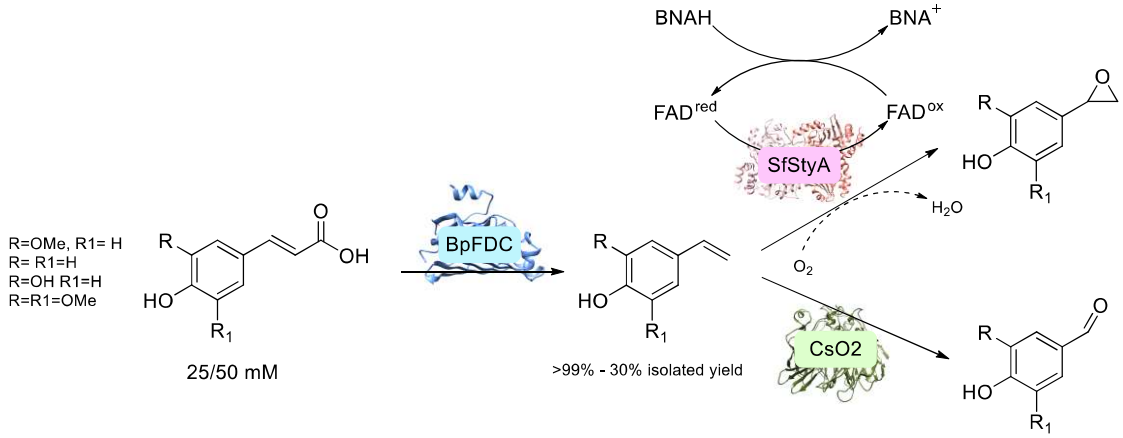
Phenolic acid decarboxylases (FDC/PAD, EC 4.1.1) have attracted growing interest for the valorisation of phenolic acids from renewable resources such as lignocellulosic biomass.[1] These enzymes enable the cofactor-independent conversion of phenolic acids into 4-vinylphenol (4VP) derivatives, which are valuable flavour, fragrance, and industrial intermediates.[1] However, the high reactivity and propensity of 4VPs toward spontaneous polymerisation represent a major bottleneck, requiring rapid and controlled downstream conversion.



Enzymatic cascade reactions offer a sustainable solution by enabling the *in situ* transformation of unstable intermediates into value-added products. The robust phenolic acid decarboxylase from *Bacillus pumilus* ATCC 14884 (BpFDC) efficiently converts hydroxycinnamic acids to 4VPs (>99–30% conversion in 2 h at 30 °C) and can be coupled with complementary downstream enzymes. For example, the coenzyme-independent dioxygenase CsO2 (EC 1.13.11) from *Caulobacter segnis* ATCC 21756 enables aldehyde formation, providing access to compounds such as vanillin.[1]
Moreover, we report for the first time a one-pot, two-step enzymatic cascade for the synthesis of *para*-hydroxystyrene oxide derivatives. Following quantitative decarboxylation by BpFDC, asymmetric epoxidation of the resulting 4VPs is catalysed in less then 2 h by the styrene monooxygenase SfStyA from *Sphingopyxis fribergensis* Kp5.2, involving a new non-enzymatic approach for the reduction of FAD with 1-benzyl-1,4-dihydronicotinamide (BNAH). SfStyA can afford valuable chiral epoxides and when combined with a further one-pot chemical step enables access to optically active molecules *via* regioselective ring-opening reactions.[2]

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Immobilization of aldehyde reductase integrated in multi-enzymatic cascade for the production of bioplastic precursors from agricultural fatty acids

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The annual global plastic production has crossed 400 million metric tons, significantly contributing to environmental pollution and the depletion of fossil resources. In response, this work offers a significant advancement in circular bio-economy and sustainability by enabling the efficient, enzyme-driven transformation of bio-based fatty acids into high-value bioplastic precursors. By immobilizing aldehyde reductase on a metal organic framework (MOF) addresses key industrial challenges such as enzyme degradation, high purification costs, and limited recyclability. The enzymatic cascade efficiently converts ω-hydroxy fatty acids derived from agricultural waste into ω-amino fatty acids, which are crucial for producing sustainable polyamides used in bioplastic production.

This study demonstrates a sustainable biocatalytic cascade route for producing bioplastic precursors through enzyme immobilization. Aldehyde reductase (AHR) was covalently immobilized onto the metal-organic framework UIO-66-NH_2 via EDC–NHS coupling, resulting in the biocatalyst AHR@UIO-66-NH_2. The immobilized enzyme retained high catalytic efficiency (0.0045 μM min^{-1} μg^{-1}) against model substrate and showed enhanced operational stability, maintaining 80% activity after 30 days and reusability over 21 cycles. When coupled with transaminase from S. Pomeroyi (spTA), the system enabled efficient biotransformation of C8, C10, and C12 hydroxy fatty acids into ω-amino fatty acids with ~90% conversion. These amino fatty acids serve as building blocks for bioplastics. Overall, this work presents an efficient, green strategy for converting biomass-derived fatty acids into bioplastic precursors, highlighting the advantages of enzyme immobilization for scalable biomanufacturing. [1]

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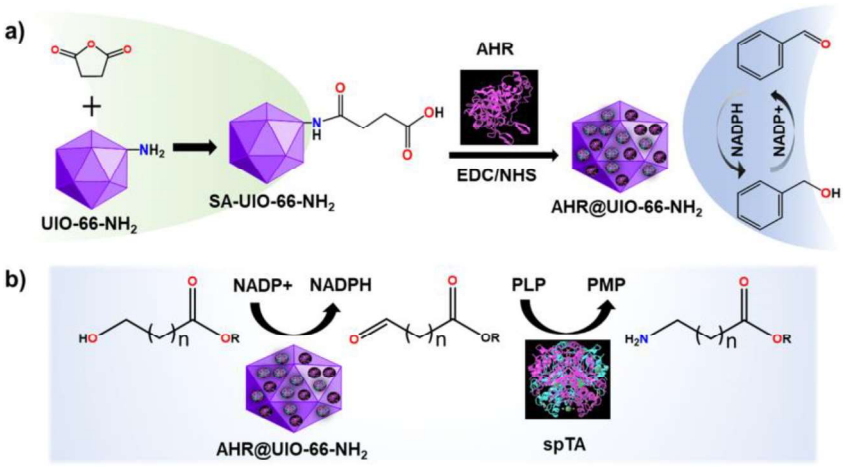

Schematic representation (a) Immobilization of AHR on UIO-66-NH2; (b) synthesis of ω-AFAs from their corresponding ω-HFAs.

Multi-enzymatic cascade for the synthesis of D-phenylalanine derivatives

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Aromatic D-amino acids (D-AAs) have gained increasing attention in both industrial and pharmaceutical fields [1]. Recently, biocatalytic procedures for the synthesis of D-AAs emerged as powerful and environmentally friendly methods. In this study, we investigated a multi-step chemo-enzymatic cascade for the synthesis of D-phenylalanines starting from benzaldehyde analogues using four engineered enzymes.

The cascade was initiated by the chemical conversion of benzaldehydes into *trans*-cinnamic acids via a Knoevenagel–Doebner condensation step. The resulting *trans*-cinnamic acids then underwent a three-step biocatalytic transformation to yield D-phenylalanines. Using whole-cell biocatalysts, recombinant phenylalanine ammonia-lyase from *Arabidopsis thaliana* (*At*PAL) [2] catalyzed the hydroamination of *trans*-cinnamic acids to produce L-phenylalanines, which were subsequently oxidized to their corresponding phenylpyruvic acids by L-amino acid deaminase from *Proteus myxofaciens* (*Pm*LAAD) [3]. In the final step, D-amino acid dehydrogenase from *Ureibacillus thermosphaericus* (*Ut*DAADH) [4] reductively aminated the resulting keto acids, producing enantiopure D-amino acids. Glucose dehydrogenase from *Bacillus subtilis* (*Bs*GDH) was incorporated into the multi-enzymatic system to regenerate the NADPH cofactor required for *Ut*DAADH activity. Several process parameters were systematically optimized, including buffer composition, pH, substrate scope, enzyme loading, and downstream purification steps. The optimization of the chemo-enzymatic cascade enabled the efficient preparative-scale production of four D-phenylalanine derivatives.

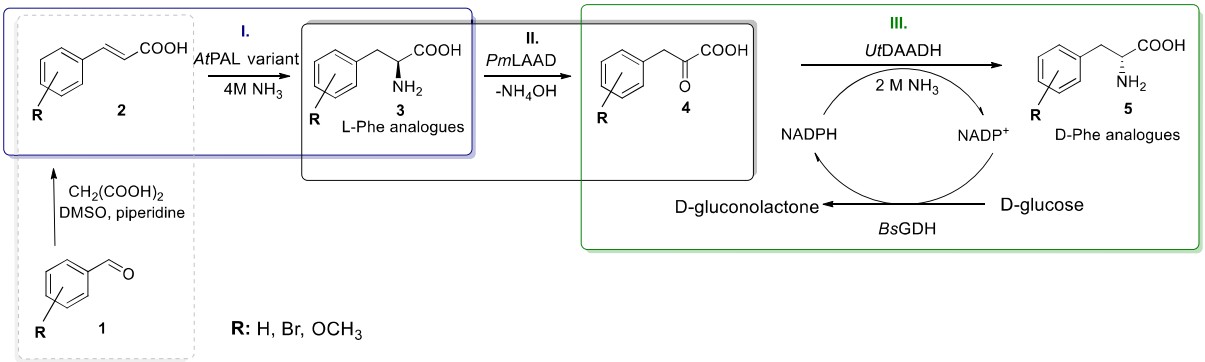

Figure 1. The studied chemo-enzymatic route yielding D-phenylalanine analogues.

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Chemo-enzymatic platforms for the synthesis of L-(hetero)arylalanines

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Non-canonical amino acids are valuable building blocks used for the synthesis of bioactive molecules, as biophysical probes, and for the incorporation into peptides and proteins to introduce new or enhanced functions [1,2]. However, the stereoselective synthesis of structurally diverse amino acids remains a significant challenge using conventional chemical methods.

Biocatalysis offers an attractive alternative due to its high selectivity and mild reaction conditions. In this context, enzymes such as phenylalanine ammonia lyases (PALs) and tryptophan synthases (TrpS) have emerged as powerful tools for the synthesis of non-canonical amino acids [3,4]. By exploiting the substrate promiscuity of these enzymes, a variety of L-heteroarylalanines can be accessed from readily available precursors [Figure 1].

Furthermore, the combination of enzymatic steps with chemical transformations in chemoenzymatic cascades enables the conversion of these heteroaryl amino acids into structurally diverse aliphatic amino acids and peptides. This strategy expands the accessible chemical space of amino acid derivatives and provides a versatile platform for the sustainable synthesis of valuable non-canonical amino acids.

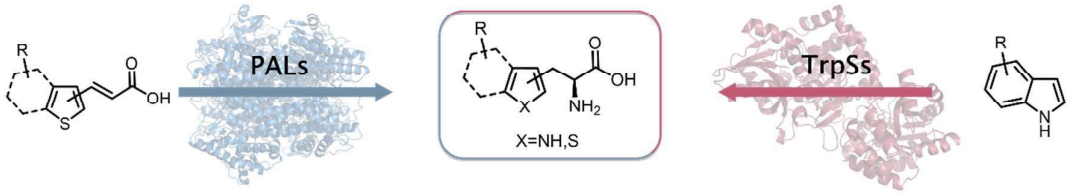

Figure 1. Chemo-enzymatic platform for the synthesis of L-heteroarylalanines.

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Discovery and characterization of a novel aromatic prenyltransferase from *Aspergillus melleus* (*AmaPT*)

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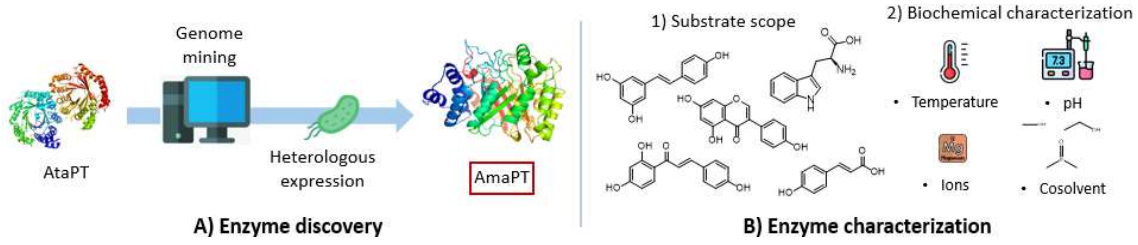
Prenylated polyphenols are a class of natural compounds bearing one or more prenyl $(-C_5H_8)_n$ groups within their structure, and are widely distributed in plants, particularly those used in traditional medicine. Prenylation often enhances biological and beneficial properties of polyphenols with respect to their parent compounds, largely due to the improved lipophilicity. [1] In nature, the biosynthesis of prenylated aromatics is catalyzed by aromatic prenyltransferases (aPTases), enzymes capable of transferring prenyl moieties onto aromatic substrates with high regio- and stereo-selectivity under mild aqueous conditions. [2]

Among these, the aPTase *AtaPT* has shown remarkable capacity of prenylating 46 different aromatic substrates using prenyl donors of varying chain length (C5-C15). Notably, a pronounced preference for geranyl pyrophosphate (GPP, C10) and farnesyl pyrophosphate (FPP, C15) over dimethylallyl pyrophosphate (DMAPP, C5) was observed. [3] Building on these results, we aimed at expanding the substrate scope by identifying a new homologous prenyltransferase derived from the fungus *Aspergillus melleus* (*AmaPT*). Following heterologous expression in *E. coli*, the prenylation activity of *AmaPT* was evaluated on 52 aromatics using DMAPP, GPP and FPP as donors. Also, the optimal reaction parameters have been assessed.

AmaPT showed a clear preference for DMAPP among the tested prenyl donors. Regarding the substrates, the enzyme displayed a notable activity toward chalcones, likely due to a stabilizing π–π interaction of tyrosine 193 present in the active site with the aromatic ring not involved in the prenylation reaction. Furthermore, *AmaPT* exhibited high stability retaining full activity after storage at 4 °C for over two months. Notably, the complementary behaviour of *AtaPT* and *AmaPT*, showing different prenyl donor preferences (GPP/FPP and DMAPP, respectively) as well as regioselective functionalization of the same aromatic scaffold, opens new opportunities for one-pot cascades, enabling synergistic multi-prenylation of the same substrate. Overall, these findings highlight the importance of discovering and characterizing new prenyltransferases to broaden substrate diversity and enable further process intensification in biocatalytic applications.



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Leveraging biodiversity for the biocatalytic production of esters *via* hemiacetal oxidation

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Keywords: biocatalysis, recombinant protein, green solvents, green chemistry

Esters, like methyl formate, ethyl acetate and butyl acetate are widespread commodity chemicals that find application in many industries, like food & beverages, pharma, and textiles. At the current time, they are produced in large quantities exploiting energy intensive chemical processes, starting from fossil feedstocks, with a high environmental impact. Biotechnologies constitute a valid alternative to the well-established chemical methodologies. Enzymatic biocatalysis has severalfold advantages, such as mild reaction conditions, no harsh chemical agents' requirements, and being safer for both humans and the environment. We then selected promising enzymes among alcohol dehydrogenases (ADHs), a class which is still poorly represented in the patent landscape and thus constitute an intriguing category for this research field. Some ADHs have been observed to oxidise hemiacetals, formed by the spontaneous reaction between an alcohol and an aldehyde, to esters, in presence of $NAD(P)^+$ as cofactor. Starting from the commercial Adh1 of *Saccharomyces cerevisiae*, in this work other ADHs from *Homo sapiens*, *Neurospora crassa*, *Clostridium beijerinckii* and *Helicobacter pylori* have been selected, heterologously produced and tested for the synthesis of methyl formate, methyl acetate, ethyl formate and ethyl acetate. Except for ADH from *H. pylori*, all the enzymes displayed the hemiacetal dehydrogenation activity, and showed the capability to regenerate autonomously the cofactors $NAD(P)^+$ allowing the production of high esters titers exceeding stoichiometric limits. This feature, which has never been observed before, is now patent pending. The hemiacetal oxidation route has been explored also for the production of other specialties esters: it has been successfully used for cellulose functionalization with *S. cerevisiae* Adh1 and ADH of *C. beijerinckii*, demonstrating its applicability also to large molecules like polymers in solid state and thus expanding the potential of this technology.

Robust industrial biocatalysts with peroxygenase, phenol-oxidase and furfuryl-oxidase activities from bacterial and fungal hosts

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The implementation of enzyme biocatalysts in the industry requires high-level production, catalytic efficiency and robustness. In the EU-funded project ROBUSTOO, a multidisciplinary consortium aims to address these challenges for three oxidative enzyme families: peroxygenases (UPOs), phenol-oxidase (laccase) and hydroxymethyl furfural oxidase (HMFOs). The objective is to demonstrate their applicability for the synthesis of oxygenated lipophilic compounds and fine chemicals (by UPOs), valorization of industrial lignin into phenolic building blocks and polymers (by laccases), and production of sugar-derived monomers for bio-based plastics (by HMFOs).

To overcome current barriers to industrial deployment, ROBUSTOO integrates computational approaches, synthetic biology, and protein engineering to enhance enzyme robustness, activity, and selectivity under industrial conditions. The project is also scaling up production of optimized recombinant enzymes in tailored microbial hosts and developing efficient, sustainable bioprocesses for commercially relevant bio-based products.

Progress to date includes the development of an advanced computational pipeline for enzyme discovery and design, which have yielded a broad portfolio of recombinant oxidative enzymes, some produced at high titers. Ongoing biochemical characterization and application testing are identifying top candidates and guiding further optimization ahead of pilot-scale demonstrations.
Overall, ROBUSTOO aims to validate the industrial relevance of next-generation oxidative enzymes in resource-efficient, environmentally friendly biocatalytic processes. By enabling the production of bio-based alternatives to fossil-derived chemicals and enhancing the valorization of lignin and biomass streams, the project contributes to EU priorities for a strengthened and innovation-driven bioeconomy.

www.robustoo.eu - Funded by the European Union under Grant Agreement no. 101135119.

Two-cell biosynthesis of (-)-deoxypodophyllotoxin from ferulic acid in *Escherichia coli*

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Lignans are natural products with a wide range of bioactivities and health benefits. For example, plant-derived (-)-podophyllotoxin (PPT) is the direct precursor of the semi-synthetic, clinically relevant anti-cancer drug etoposide. Conventionally, PPT is isolated from endangered plant species such as *Sinopodophyllum hexandrum*. However, due to the long generation cycles, low lignan levels, and laborious downstream processing, this procedure remains inefficient. To establish a more sustainable production alternative, several parts of the natural lignan biosynthetic pathway have been reconstituted in recombinant *E. coli* cells in our group [1,2,3]. These building blocks were now assembled together to establish the biosynthesis of (-)-deoxypodophyllotoxin (DPPT) – the predecessor of PPT – in a two-cell setup starting from ferulic acid (FA). To this end, cell module 1 was optimized towards higher titers for the supply of the expensive lignan (+)-pinoresinol (PIN). Cell module 2 was improved by integrating the eight enzymes catalyzing the conversion of PIN to DPPT into the chromosome of *E. coli*.

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Merging photo- and biocatalysis for sustainable synthesis of benzonitriles

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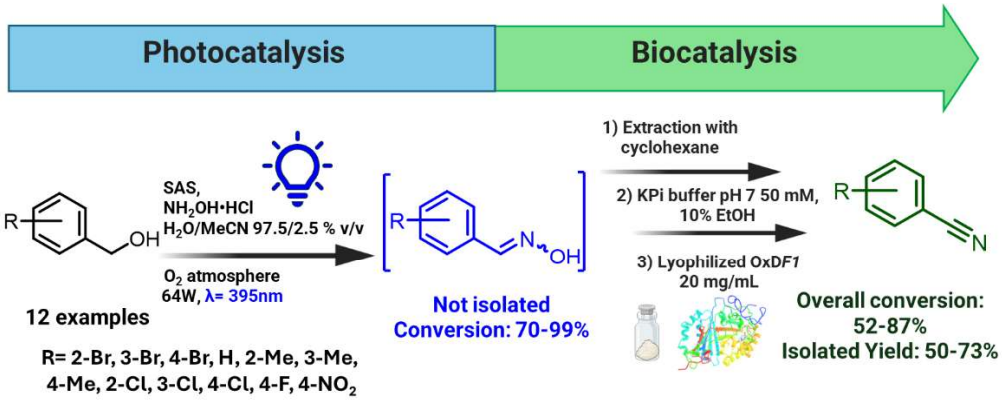
The chemical industry is increasingly seeking efficient and sustainable strategies to replace outdated and not environmentally friendly synthetic processes. Among the most important synthetic targets are nitriles, which are important intermediates for a myriad of molecules and materials. Their wide range of applications, particularly in the polymer industry, has led to their large-scale production as bulk chemicals[1]. However, conventional methods for nitrile synthesis often rely on toxic reagents (especially cyanide salts), high reaction temperatures, and hazardous solvents, which raise serious environmental and safety concerns. Therefore, the development of alternative synthetic approaches based on less toxic starting materials and milder reaction conditions, including biocatalytic methods, is highly attractive from industrial perspectives[2].

Herein, photocatalysis and biocatalysis were merged to produce benzonitriles starting form commercial and unexpensive benzyl alcohols. Sodium anthraquinone 2-sulfonate (SAS) was selected as the most appropriate photocatalyst[3]. In the presence of hydroxylamine hydrochloride, SAS facilitated the rapid, one-pot synthesis of a diverse range of benzaldehyde oximes directly from the corresponding aldehydes, achieving good to excellent conversions. (70-99%, 12 examples, see scheme 1).

This oximation step was followed by an enzymatic dehydration using an aldoxime dehydratase (Oxd*F1* L318F/F306Y) from *Pseudomonas sp*[4] converting the oximes into nitriles. Therefore, a series of benzonitriles were obtained with good to high overall conversion (52-87%) starting directly from benzyl alcohols. The scalability of this telescopic cascade was demonstrated by increasing 10 times the reaction volume (30mL) for the preparation of 4-bromobenzonitrile (200 mg, 78% isolated yield), a relevant building block in organic chemistry for agrochemicals, polymeric materials and pharmaceuticals.



Scheme 1. Workflow of the cascade reaction to synthesize benzonitriles

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Chemo-enzymatic cascade for the synthesis of (*R*)-citronellyl nitrile

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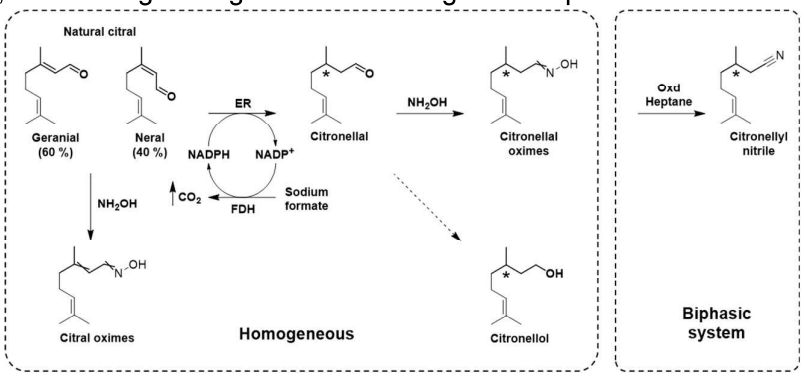
In recent years, the major flavour & fragrance (F&F) companies are focusing more on the sustainability of their products and manufacturing processes. Biocatalysis could play a crucial role in this transition as enzymes are inherently renewables and are usually used in aqueous environment under mild conditions, facilitating the design of multistep cascades.

(*R*)-Citronellyl nitrile (Levocitrile, Takasago) is a commercial chiral fragrance with a cleaner and stronger lemon scent compared to the commonly known racemic mixture, and remarkable stability towards inorganic acid cleaner. Its industrial production relies on a five-step chemical synthesis starting from myrcene, using transition-metal catalysts that could raise environmental concerns [1].

Herein, we propose a more sustainable chemo-enzymatic cascade for the synthesis of Levocitrile starting from natural citral (Scheme 1), widely available in common essential oils and made of 60 % (v/v) geranial ((*E*)-citral) and 40 % (v/v) neral ((*Z*)-citral). The first and crucial step is the stereoselective hydrogenation of citral into (*R*)-citronellal mediated by an ene-reductase (ER), and a modified formate dehydrogenase (FDH) as the cofactor recycling system. The selected ER (KmOYE from *Kluyveromyces marxianus*) was modified by rational design to improve its stereoselectivity and reaction rate, achieving a 7.3 mM yield of (*R*)-citronellal with a 96 % ee starting from 10 mM citral [2]. Moreover, it was produced in *E. coli* RARE strain to minimize the over-reduction to citronellol mediated by endogenous alcohol dehydrogenases (ADHs) [3]. The second step is the formation of (*R*)-citronellal aldoximes achieved by direct addition of hydroxylamine to the same reaction vessel. The third and final step is a dehydration with an aldoxime dehydratases (OxDBr1 from *Bradyrhizobium* sp. LTSPM299) to obtain the final nitrile, performed in a biphasic system after extracting the oximes with heptane, to avoid traces of unreacted hydroxylamine that inhibit the enzymatic activity [4]. By performing the whole cascade, we achieved a 5.8 mM yield of Levocitrile starting from 10 mM citral, maintaining the high ee value throughout the process.



Scheme 1. Schematic representation of the chemo-enzymatic cascade.

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Oxidase-Driven Chemo-Enzymatic Cascade toward Rare Sugars

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Rare sugars such as D-allose and D-gulose are attracting increasing attention as next-generation functional sweeteners with documented physiological benefits, yet their limited natural availability and multistep chemical syntheses hinder broader application. Here we report a chemo-enzymatic strategy for rare sugar synthesis based on an bacterial glycoside-3-oxidase engineered in our laboratory[1](PsG3Ox variant 16F10) that enables selective C3 oxidation of aromatic glycosides, followed by stereoselective chemical reduction and deprotection to access the target rare sugars.

Aromatic β-glycosides were prepared through glycosidase-mediated transglycosylation, or using Mitsunobu benzoylation, both in a one-step protecting-group-free process. The presence of a benzyl or benzoyl aglycone proved essential for directing the oxidase toward selective C3 oxidation. Using purified enzyme as well as whole-cell biocatalysts, complete conversion of benzyl-β-D-glucopyranoside and benzyl-β-D-galactopyranoside was achieved, yielding 150–250 mg of product within 24 h. Implementation of whole-cell catalysis significantly simplified the process while maintaining high selectivity and eliminating the need for enzyme purification.

The oxidized intermediates were subsequently subjected to stereoselective LS-Selectride reduction and deprotection to afford D-allose and D-gulose in yields 95 % for D-allose and 79 % for D-gulose. This modular sequence combines biocatalytic selectivity with chemical stereoselectivity, forming an efficient route to produce rare sugars.

This work demonstrates how enzyme engineering, substrate design and whole-cell process implementation can be integrated into a practical multi-step cascade for sustainable rare sugar synthesis, aligning with current advances in bioprocess engineering and chemo-enzymatic methodologies.

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FLEXIZYME: A Flexible Enzymatic Platform for Sustainable Bio-based Fatty Amine Production

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FLEXIZYME is an EU-funded research project aimed at developing a flexible enzymatic production platform for the sustainable synthesis of fatty amines from industrial side streams, including fat- and protein-rich residues. By integrating biocatalysis with digital tools such as bioinformatics and machine learning, the project seeks to provide scalable alternatives to conventional petrochemical routes, thereby supporting Europe's transition toward a circular bioeconomy. Addressing the entire value chain, from feedstock valorisation to downstream processing, FLEXIZYME targets key industrial bottlenecks to enable robust, cost-efficient production with high productivity and reduced environmental impact [1].

Within the multi-step enzymatic conversion of fatty acids to amines via aldehyde intermediates, GECCO Biotech focuses on the initial reduction step by applying its enzyme discovery and engineering pipelines to carboxylic acid reductases (CARs). Key challenges related to enzyme stability and cofactor regeneration (ATP and NADPH) are addressed to ensure compatibility with continuous, multi-enzyme cascade processes.

Carboxylic acid reductases (CARs) available at GECCO were evaluated for activity toward long-chain fatty acids relevant to the FLEXIZYME process. Lead enzymes were selected based on kinetic performance and are being advanced as starting points for iterative engineering. Initial conversions under optimized CAR conditions demonstrate the feasibility of a sustainable, multi-step enzymatic route to bio-based fatty amines.

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CHARACTERISATION OF LYTIC POLYSACCHARIDE MONOOXYGENASES: ACTIVITY AND INTERACTION ASSAYS

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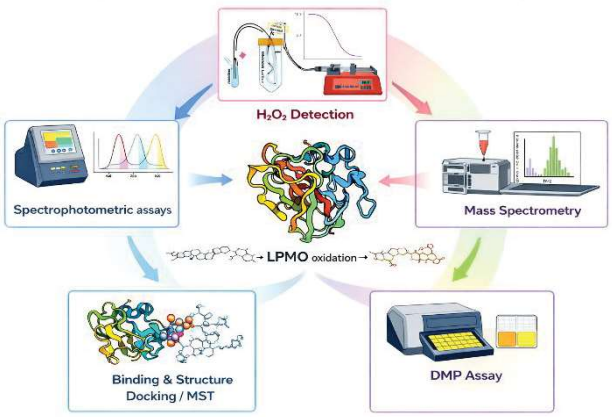
The efficient microbial conversion of recalcitrant biomass, such as cellulose and chitin, relies on the synergy between lytic polysaccharide monooxygenases (LPMOs) and hydrolases. LPMOs perform a critical first step using a copper-dependent oxidative cleavage that creates new access points for hydrolases which enhances enzymatic biomass degradation [1]. However, characterising LPMO activity is challenging due to complex catalytic mechanisms, the involvement of reactive oxygen species, particularly hydrogen peroxide, and the reliance on specialised, time-consuming analytical techniques [2]. To overcome these limitations, we employ complementary and accessible approaches, including substrate-independent spectrophotometric assays [3] and hydrogen peroxide-sensing systems [4], [5] to monitor their activity and determine kinetic parameters. In addition, structural insights and enzyme-substrate interactions are investigated using molecular modelling and microscale thermophoresis (MST) [6]. This combination of activity assays and interaction studies provides a practical and efficient toolkit to investigate LPMO function while reducing dependence on labour-intensive analytical platforms, supporting their application in biotechnology and catalytic bioprocesses.



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Chemo-enzymatic flow synthesis of chiral piperidine scaffolds

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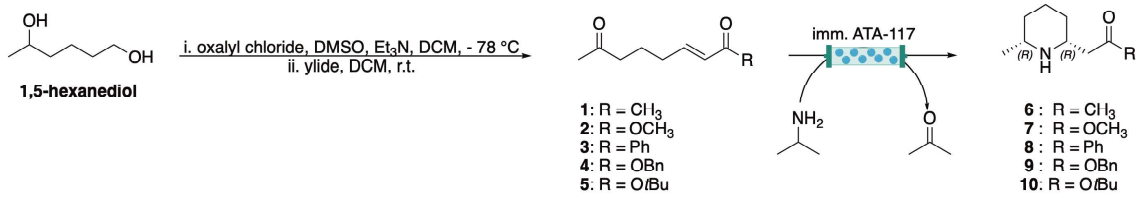
Chiral amines are important building blocks in 40–45% of small molecule pharmaceuticals, as well as in numerous industrially important fine chemicals and agrochemicals[1]. Furthermore, environmental regulations and the increasing demand for enantiopure compounds as high-value products for different sectors require the integration of traditional chemical synthetic methods with greener (bio)catalytic approaches[2]. Since nitrogen-containing heterocycles represent a privileged structure in many APIs[3], in this work we focused our attention on the asymmetric synthesis of enantiopure piperidines as high-value scaffolds for the preparation of different alkaloids[4]. A pyridoxal 5'-phosphate (PLP)-dependent transaminase (ATA-117) was selected and immobilized to perform a stereoselective transamination, followed by a spontaneous intramolecular aza-Michael reaction (IMAMR, Scheme 1), to synthesize natural (-)-pinidinone (**6**)[5]. First, two chemical steps were performed in batch to prepare the desired substrate **1**, namely an oxidation reaction followed by a Wittig olefination using commercially available ylides. Then, after expressing and purifying the enzyme, various trials were conducted to immobilize the (*R*)-selective biocatalyst. Eupergit®C was chosen as the carrier for the covalent immobilization of ATA-117 to enhance its operational stability and reusability. Afterwards, the reaction was optimized in a continuous flow system evaluating substrate concentration, isopropylamine equivalents, reaction temperature, residence time, type and amount of cosolvent. The protocol was extended to different substrates to isolate various 2,6-disubstituted chiral piperidines (compounds **7-10**).



Scheme 1: Chemo-enzymatic asymmetric flow synthesis of 2,6-disubstituted piperidines.

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One-Pot Multicatalytic Cascades to Chiral β-Hydroxy Esters Combining Organocatalysis and Biocatalysis for the Preparation of Valuable Chiral Building Blocks

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The implementation of catalytic and atom-economy processes represents a key aspect in the design of new sustainable chemical products. We have recently demonstrated that L-cysteine (L-Cys) is an efficient and cost-effective catalyst for the hydration of activated alkynes.[1,2] Using sub-stoichiometric loadings, a broad range of β-ketosulfones, amides, and esters was obtained from the corresponding activated alkynes under aqueous conditions in a highly effective and regioselective manner.[3] At a cost of less than €0.5 per gram of catalyst, this approach constitutes a highly economical and environmentally friendly alternative to existing strategies.

Moreover, the mild and biocompatible reaction conditions enable seamless integration with enzymatic processes, providing access to essential chiral synthons and further extending the applicability of this methodology within organic synthesis.

In this contribution, we report the synthesis of chiral β-hydroxy esters from a variety of aryl- and alkyl-substituted propiolic esters through a one-pot combination of organocatalytic hydration and stereoselective carbonyl reduction using ketoreductases (Figure 1). Remarkably, the approach appears to be very general and efficient, giving access to a series of hydroxy esters in good conversions and stereoselectivities.[4,5] The resulting products were subsequently employed as building blocks for the synthesis of chiral compounds of interest in pharmaceutical and chemical applications, underscoring their value as versatile intermediates in sustainable synthetic routes.

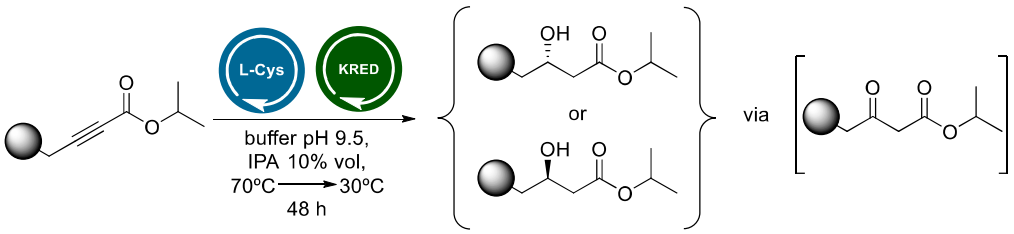

Figure 1: Synthesis of chiral β-hydroxy esters via one-pot sequential cascade.

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Enzymes in Action: Converting Lignocellulosic Phenolics into Functional Aromatics

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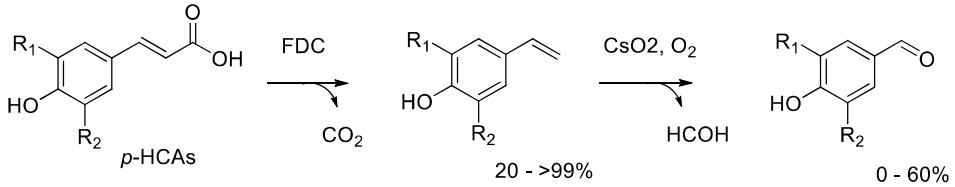
p-Hydroxycinnamic acids (*p*-HCAs) are phenolics embedded in plant cell walls, where they act as linkers between lignin, hemicellulose and cellulose. Abundant in lignocellulose-rich agricultural by-products, they represent attractive renewable feedstocks for the production of value-added chemicals [1].

In this study, a two-step biocatalytic cascade with free enzymes was developed for the valorization of *p*-HCAs into high-value aromatics (Fig.1). In the first step, a ferulic acid decarboxylase (FDC) from *Bacillus pumilis* converted *p*-HCAs into the corresponding 4-vinylphenols, styrene derivatives with relevance as monomers for bio-based polymer production and antioxidant, anti-inflammatory and antimicrobial agents [2]. In the second step, the cofactor-independent dioxygenase CsO2 from *Caulobacter segnis* enabled the oxidative cleavage of the vinyl side chain, yielding *p*-hydroxybenzaldehydes, compounds of interest for pharma/food, and cosmetic applications [3].



R_1 = OCH_3, R_2 = H; R_1 = R_2 = H; R_1 = OH, R_2 = H; R_1 = R_2 = OCH_3

Figure 1. Enzymatic cascade for the valorization of *p*-HCAs into valuable aromatics

To enhance sustainability and catalyst recovery, enzyme immobilization on ferromagnetic nanoparticles (MNPs) [4] was investigated. Immobilized FDC (25 mg/g) retained catalytic activity over five consecutive cycles in 100 mM potassium phosphate buffer pH 8.0, whereas CsO2 displayed significant activity loss upon immobilization. Based on these results, the substrate scope of FDC was explored across different *p*-HCAs (yield ranging from 20 to >99%), and the decarboxylation of ferulic acid (25 mM) to 4-vinylguaiacol was selected as a model reaction to test the performance of immobilized FDC in different green solvents (2-MeTHF, eucalyptol and anisole) with conversions in 24 h quantified by UPLC analysis (0-86%).

Overall, this work highlights the potential of combining biocatalytic cascades and selective enzyme immobilization strategies for the sustainable valorization of lignocellulosic phenolics.

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Green extraction and biotransformation of bioactive compounds from oil industry by-products using NADES

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The oilseed processing industry generates large amounts of by-products that constitute an abundant yet underexploited source of phenolic compounds with high biological potential [1]. In this study, an integrated and sustainable strategy was developed for the extraction and biotransformation of polyphenols from oil industry residues. Environmentally friendly Natural Deep Eutectic Solvents (NADESs) were employed as green extraction media [2,3]. The obtained crude phenolic extracts were subsequently subjected to enzymatic biotransformation aimed at the hydrolysis of sinapic acid derivatives, particularly sinapine, into free sinapic acid. In contrast to conventional biotransformation processes requiring strictly controlled reaction conditions, this approach evaluated the feasibility of applying enzymes directly in crude NADES-based extracts, serving as a complex reaction matrix.

Furthermore, the antioxidant activity of the extracts before and after enzymatic hydrolysis was determined using the ORAC assay. Since sinapic acid exhibits higher free-radical-scavenging activity than its ester derivative sinapine, the conversion of sinapine into sinapic acid was expected to enhance the antioxidant potential of the extracts [4]. As a result of enzymatic treatment a slight improvement in antioxidant activity was observed.

Overall, the proposed approach demonstrates the effectiveness of combining green extraction technologies with targeted enzymatic biotransformation in NADES matrices to enhance the Oxygen Radical Absorbance Capacity of obteined extracts.

This material has been supported by the Polish National Agency for Academic Exchange under Grant No. BNI/PST/2023/1/00046/U/00001.

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Three-Step One-Pot Chemoenzymatic Cascade for the Stereoselective Synthesis of Secondary Allylic Alcohols

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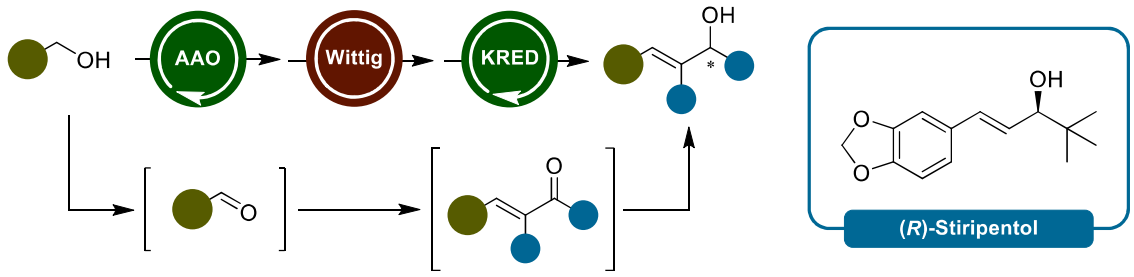
Aryl-alcohol oxidases (AAOs) are flavin-dependent enzymes that catalyze the selective oxidation of alcohols to the corresponding aldehydes under mild and environmentally friendly conditions. These biocatalysts have attracted increasing attention as sustainable alternatives to traditional oxidation methods, particularly in the synthesis of fine chemicals and pharmaceuticals [1]. In previous studies, we described the discovery and characterization of a novel AAO from *Streptomyces hiroshimensis* (*Sh*AAO) and demonstrated its utility as catalyst for the scalable synthesis of aldehydes and enantioenriched intermediates via chemoenzymatic cascades [2,3].

Herein, we report the development of a three-step one-pot chemoenzymatic cascade for the stereoselective synthesis of secondary allylic alcohols. This strategy takes advantage of the compatibility of the Wittig reaction with aqueous media and its integration with biocatalytic systems [4]. Overall, it comprises the *Sh*AAO-catalyzed oxidation of primary alcohols, *in situ* Wittig olefination with stabilized ylides, and a final stereoselective bioreduction of the resulting α,β-unsaturated ketones. The scope of the chemoenzymatic cascade was evaluated using a broad range of aromatic primary alcohols, as well as different ylides, demonstrating high versatility and efficiency. Furthermore, the synthetic potential of this methodology was highlighted through the stereoselective synthesis of the antiepileptic drug (*R*)-Stiripentol.



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REUSE: Enzymatic CO_2 capture in a rotating packed bed and electrocatalytic CO_2 reduction to useful products

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Carbon capture and utilization (CCU) has emerged as a promising strategy for mitigating the ever-increasing carbon dioxide (CO_2) emissions and enabling their conversion into valueable chemicals. However, while we can find examples of chemical carbon capture developed to a commercial scale, it still suffers from high energy demands, solvent degradation, and overall lack of sustainability [1]. Enzymatic carbon capture poses a compelling green alternative, using the highly efficient enzyme carbonic anhydrase to accelerate CO_2 absorption under mild reaction conditions [2].

The EU-funded project REUSE [3] presents an innovative approach to CCU, by combining biocatalysis and electrochemistry to address the key limitations of currently available technologies. In this system, carbon dioxide is absorbed from biogenic flue gas in a rotating packed bed (RPB) reactor, utilizing a combination of immobilized carbonic anhydrase and advanced solvents. The captured CO_2 is subsequently fed into a CO_2 reduction (CO_2R) electrochemical cell, where it is converted into valuable products such as carbon monoxide and formic acid. This integrated process intensifies mass transfer, improves capture efficiency, and provides a direct pathway from dilute CO_2 streams to chemical products. The project also aims at integrating the novel CCU technology in a pilot plant with continuous operation.

REUSE highlights the central role enzymes will play in the development of next-generation carbon capture technologies, and the immense potential of integrating immobilized enzymes in multistep processes. Moreover, it supports the strategic positioning of Novonesis in acceleration, commercialization, and real-world deployment of carbon capture and utilization technologies.

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Enzymatic modification of acyl chains in natural phospholipids

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The fatty acid composition of phospholipids (PL) is crucial for their physical and biological properties. Structured phospholipids (SPL) with defined fatty acids at specific positions on the glycerol backbone can be produced via chemical or enzymatic reactions. In our work to date, we have used various enzymatic methods—such as interesterification and acidolysis with immobilized lipases—to selectively enrich phospholipids isolated from egg yolk with unsaturated n-3 and n-6 fatty acids, as well as conjugated linoleic acids (1-3).

Phospholipids also exhibit characteristic surface-active properties, enabling their use in the food, feed, cosmetics, and pharmaceutical industries (4). Their physicochemical properties can be modified through enzymatically catalyzed changes in the fatty acid composition, thereby expanding their potential applications as emulsifiers.

One of the tasks in the INBIOWAL project is to use enzymatic processes for the biovalorization of lipids isolated from byproducts and waste generated during vegetable oil refining in the oil industry. The project involves research on producing new functional phospholipids containing hydroxy- and oxo-acid residues. These residues are obtained through chemoenzymatic processes from natural fatty acids—such as oleic and linoleic acids—found in those waste products (5). Due to the greater polarity of these modified acyl residues compared to the originals, the resulting phospholipids exhibit different physicochemical properties, including a lower critical micelle concentration (CMC) and greater ability to reduce surface tension. These traits enhance their functional properties, which are valuable for food technology applications (6).

In the model studies, the substrate was commercially available soy lecithin, which consisted almost entirely of phosphatidylcholine (PC). Initial experiments focused on the enzymatic acidolysis of PC with (*R*)-10-hydroxystearic and 10-oxostearic acid, catalyzed by lipase B from *Candida antarctica*.

Acknowledgements:

This research was financed by the Polish National Agency For Academic Exchange (NAWA), project BNI/PST/2023/1/00046.

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One-pot cascade preparation of dicarboxylic acids using engineered *Acetobacter malorum*

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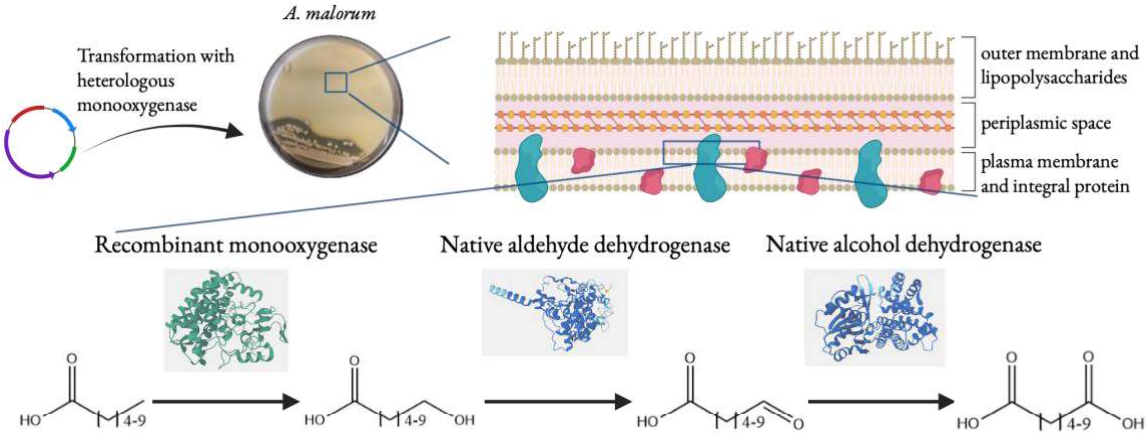
This study addresses the growing demand for sustainable chemical production by exploiting acetic acid bacteria (AAB) as a biocatalytic platform for the development of building blocks for sustainable biopolymers. AAB are Gram-negative, strictly aerobic bacteria with a remarkable capacity to oxidize a broad range of sugars, alcohols and polyols [1]. This metabolic versatility is driven by membrane-bound and periplasmic dehydrogenases, which ensure high stereo- and regioselectivity in oxidation reactions.
In this study, A*cetobacter malorum* DSM 112354 [2] demonstrated high conversion rates (ranging from 42 to 97%) in the oxidation of ω-hydroxycarboxylic acids to their corresponding C7–C12 dicarboxylic acids. Similar results were obtained using *A. malorum* cells immobilized within barium alginate beads.
A triple mutant of cytochrome P450 CYP153A$_{M.aq}$ from *Marinobacter aquaeolei* (M.aqRLT) capable of catalyzing the selective hydroxylation of the terminal methyl group of different fatty acids [3] has been introduced into *A. malorum* to expand its biocatalytic potential. This strategy enables a multi-step, one-pot oxidation cascade that converts fatty carboxylic acids into the corresponding dicarboxylic acids. These compounds are useful building blocks for the synthesis of bio-based polymers, addressing the growing demand for sustainable plastic materials.



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NADPH-Dependent Aldehyde Synthesis from Carboxylic Acid Catalysed by CAR Enzyme

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Aldehydes are essential building blocks for the flavour and fragrance industry. Enzyme-mediated aldehyde synthesis from carboxylic acids circumvents challenges presented by chemical aldehyde production routes [1]. Carboxylic acid reductases (CARs) are enzymes that exemplify principles of biocatalysis in the reaction of carboxylic acid reduction to the corresponding aldehyde. CARs are enzymes found in plants, fungi and bacteria and can act on a broad scope of carboxylic acids [2,3]. However, with synthesis routes relying on carboxylic acid reduction, the risk of overreduction becomes apparent [1]. In addition, the success of the aldehyde synthesis relies on the NADPH cofactor, as CAR activity depends on this costly nicotinamide cofactor. An efficient NADPH regeneration system is crucial for improving aldehyde yield and lowering the production costs by minimising cofactor consumption [4].

Developing an applicable CAR-driven process (Figure 1) requires determining kinetic parameters from experimental data using enzyme activity assays and mathematical modelling. Work to date has focused on optimisation of reaction conditions for the targeted aldehyde synthesis, illustrating the importance of enzyme engineering and mathematical modelling.

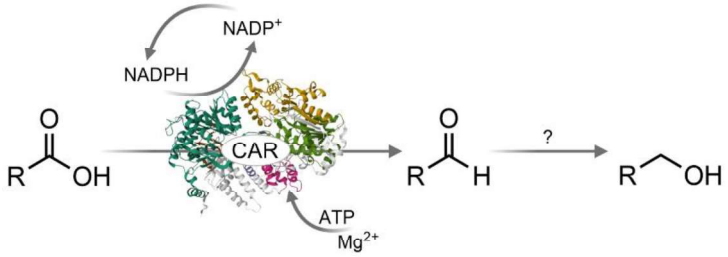

Figure 1 Reaction scheme of enzymatic aldehyde synthesis mediated by CAR

Acknowledgements: This work has been supported by European Union's Horizon Europe Programme under the grant agreement No 101135542 (CirculH2).

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RATIONAL DESIGN OF A BIOCATALYTIC CASCADE TO SIMPLIFY VANILLIN BIOSYNTHESIS FROM EUGENOL

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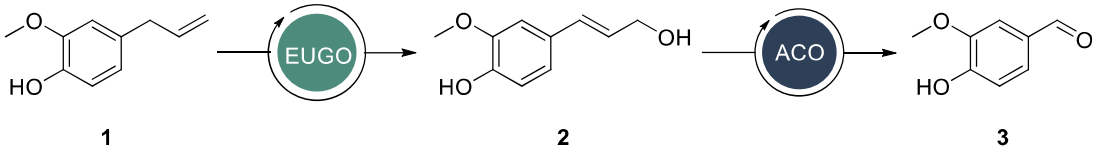
Vanillin is one of the most widely used flavour compounds, traditionally produced either by extraction from vanilla pods or by petrochemical synthesis. Eugenol, a phenylpropanoid that can be readily extracted from clove oil,[1] represents an attractive renewable precursor for the biotechnological production of natural vanillin.

We developed a two-step biocatalytic cascade in which eugenol was first transformed by shifting its double bond into conjugation with the aromatic ring through oxidation to coniferyl alcohol, catalyzed by the eugenol oxidase from *Rhodococcus jostii* (*Rj*EUGO).[2] In a subsequent oxidative step, the C=C bond of coniferyl alcohol was cleaved, yielding vanillin and glycolaldehyde as a side product. To achieve this transformation, a set of putative and previously uncharacterized alkene cleavage oxygenases (ACOs) from the carotenoid dioxygenase family was evaluated for activity toward coniferyl alcohol. The enzyme from a *Sphingomonadales* bacterium (*Sb*ACO) showed the highest conversion efficiency. The resulting enzymatic cascade successfully enabled vanillin production from both pure eugenol and crude clove oil, with similar conversion levels. Compared to currently reported metabolic pathways, which require five steps to convert eugenol into vanillin,[3] this biocatalytic cascade significantly reduces pathway complexity while enabling the production of natural vanillin from naturally derived eugenol.[4]



1 **2** **3**

Scheme 1: Enzymatic synthesis of vanillin from eugenol.

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Harnessing Photosynthetic ATP for Whole-Cell Biocatalysis in the Cyanobacterium *Synechocystis*

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Abstract:

Photosynthetic organisms convert sunlight into ATP and NADPH, the energy sources that sustain their metabolism. Using these molecules to power biocatalytic reactions offers a sustainable alternative to traditional chemical processes. Enzymes belonging to the classes of Baeyer-Villiger monooxygenases, ene-reductases, imine reductases, alkane monooxygenases, cytochrome P450 monooxygenases and alcohol dehydrogenases have already been employed in light-fueled whole-cell biotransformations exploiting photosynthetic NADPH.

We have recently demonstrated that also ATP generated through photosynthesis can directly drive a biocatalytic transformation in living cyanobacterial cells.

This result was achieved by expressing in the cyanobacterium Synechocystis sp. PCC 6803 an ATP- dependent enzyme, the γ-glutamyl-methylamide synthetase from Methylovorus mays No. 9 (MmGMAS). The expressed enzyme was able to drive, in the transgenic strain, the light-driven biosynthesis of L-theanine. Consumption of ATP by the recombinant MmGMAS was even beneficial under strong illumination, protecting the photosynthetic electron transport from photo-damage.

These findings demonstrate the possibility of using photosynthetic microorganisms like Synechocystis as a potential platform for sunlight driven biotransformations with wide potential biocatalytic applications. In this perspective, we further present the tridimensional structure of MmGMAS, which explains its promiscuous in vivo activity and provides the basis for its rational evolution.

From Primary Alcohols to Nitriles: A One-Pot Chemoenzymatic Cascade Strategy

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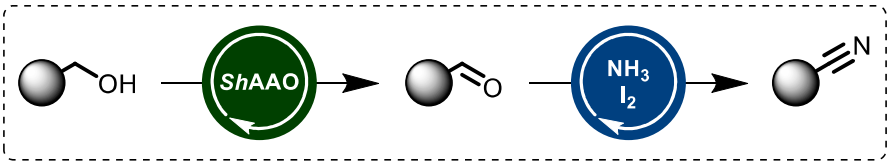
The nitrile functional group is a highly versatile motif in synthetic chemistry, serving both as a key intermediate for the construction of diverse heterocycles and functional groups—including amines, amides, carboxylic acids, and esters—and as a valuable structural element in its own right. Nitriles are widely encountered in fine chemicals, pharmaceuticals, agrochemicals, and fragrances.[1] Consequently, the development of efficient and sustainable methodologies for their synthesis remains an important objective in modern synthetic chemistry. Aldehydes have long been recognized as convenient precursors for nitrile formation, and numerous strategies have been reported for their conversion.[1,2,3] However, aldehydes are often toxic, unstable, and prone to degradation, which can limit their practical utility. In this context, the use of more stable surrogates such as primary alcohols—capable of generating aldehydes *in situ* through (enzymatic) selective oxidation—offers an attractive alternative.[4]

Aryl-alcohol oxidases (AAOs) are FAD-dependent enzymes capable of oxidizing a broad range of primary alcohols, including aliphatic, benzylic, and allylic substrates, to the corresponding aldehydes under mild conditions. Herein, we report a straightforward chemoenzymatic approach for the synthesis of nitriles from primary alcohols (Scheme 1). This strategy combines the biocatalytic oxidation of alcohols using a newly identified bacterial AAO from *Streptomyces hiroshimensis*[5] with a subsequent oxidative conversion mediated by an iodine–ammonia system in aqueous media. The latter promotes imine formation and oxidation, ultimately affording the corresponding nitriles under mild, scalable and operationally simple conditions.



Scheme 1. Graphical abstract of the primary alcohol-nitrile conversion.

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Optimizing Transaminase Catalysis for Eco-Friendly Pyrazine Production

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Pyrazines are volatile, nitrogen-containing heterocyclic compounds renowned for their potent flavor and aroma profiles, making them indispensable in the food, beverage, and fragrance industries[1,2]. Beyond their sensory appeal, these molecules also exhibit bioactive properties, with applications in pharmaceuticals and agrochemicals[3,4]. This project aims to explore and evaluate an alternative, sustainable route for synthesising this class of flavors compared to traditional chemical methods.

Biocatalysis offers a promising strategy by utilizing enzymes or microorganisms as catalysts to drive chemical reactions under milder conditions while avoiding toxic reagents and hazardous processes. In this study, a two-step enzymatic synthesis is implemented and analyzed: amination with a specific amino donor, mediated by a transaminase (fig.1 (I)) , followed by oxidative dimerization (fig.1 (II)).

The ultimate goal is to identify and optimize, using computational methods, a transaminase (ATA) with a broad substrate spectrum, enabling the synthesis of various alkyl pyrazines using a single enzyme. Reaction conditions are then optimized to maximize product formation, yield, and process efficiency.

Preliminary data indicate successful pyrazine production with acceptable yields and promising product purity, marking an important step toward a more sustainable approach to flavor synthesis in the food industry.

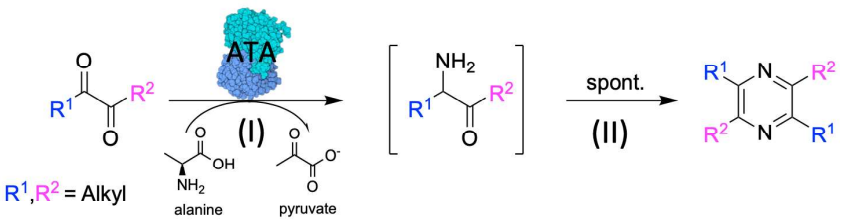

Figure 1 : Transaminases-mediated synthesis of tetra-substituted alkyl pyrazines.

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