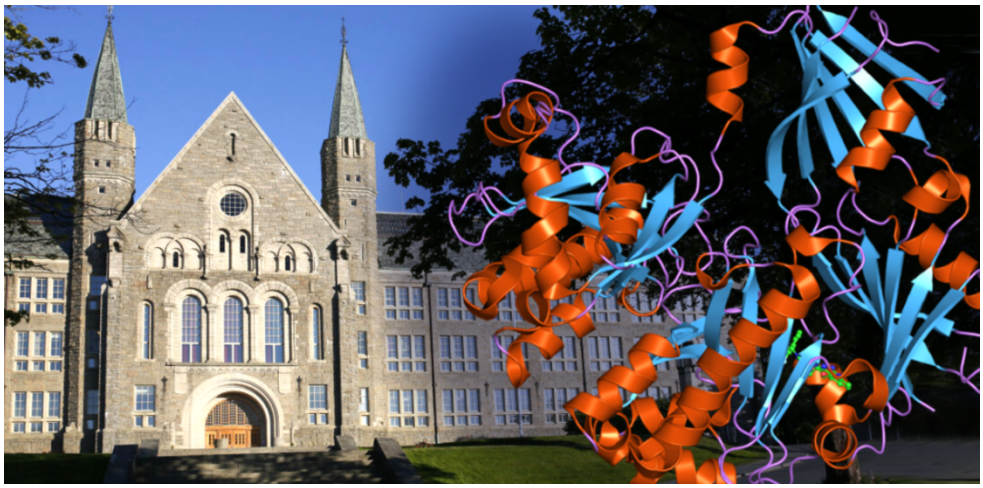
7th International Conference on Biocatalysis in Non-Conventional Media

NTNU, Trondheim, Norway, May 26th-29th, 2024



SPECIAL HONORARY LECTURE

DISTINGUISHED PROFESSOR
ROGER SHELDON

University of the Witwatersrand, Johannesburg, South Africa,
Technical University of Delft, Delft, The Netherlands

Reflections on Five Decades of Biocatalysis in Non-Conventional Media

The Research Council of Norway

https://www.ntnu.edu/bncm2024

Distinguished guest

It gives me great pleasure to welcome you all to the 7th International Conference on Biocatalysis in Non-Conventional Media organised at Department of chemistry, Norwegian University of Science and Technology in Trondheim 26th to 29th of May 2024. In particular, we are honoured to welcome Distinguished Professor Roger Sheldon (South Africa and The Netherlands), Professors Selin Kara (Germany and Denmark), Polona Žnidaršič-Plazl (Slovenia), Dörte Rother (Germany), Pedro Lozano (Spain), Frank Hollmann (The Netherlands) and Distinguished Scientist Fabrice Gallou, Novartis Pharma, Switzerland.

The opening lecture of the conference will be presented by Professor Selin Kara, who is also the project manager of the EU funded DECADES project. The conference has a session dedicated to the DECADES project, where both experts in the field and PhD students and early career scientists will present their work.

This conference series was initiated in 1986 and organised by Professors Hans Tramper and Urs von Stockar at University of Wageningen, The Netherlands, both in 1986 and in 1992. In 2005, the conference was organised by Prof. Rein Uljin, University of Manchester, UK. In 2008, Professor Vytas Svedas, Lomonosow State University in Moscow, Russia, chaired the conference. In 2017, the fifth edition of this series was organised by Professors Jan von Langermann, Selin Kara and Udo Kragl at University of Rostock, Germany. The hybrid edition of the sixth BNCM conference was held in Milano in May 2022 and chaired by Professors Sergio Riva, Francesco Molinari, Daniela Ubiali and Francesco Secundo.

BNCM 2024 gathers nearly 70 delegates from both academia and industry from 15 countries presenting and discussing research progress on the topics of novel non-conventional reaction media for biocatalysis, water- and solvent-free reaction systems, non-conventional media in flow biocatalysis, biphasic and multi-phase biocatalytic systems, BNCM: from labscale to industrial scale, kinetics and thermodynamics of enzyme-catalyzed reactions in non-conventional media, non-conventional media for whole-cell versus cell-free biocatalytic systems, sustainable biocatalytic reaction engineering in non-conventional media.

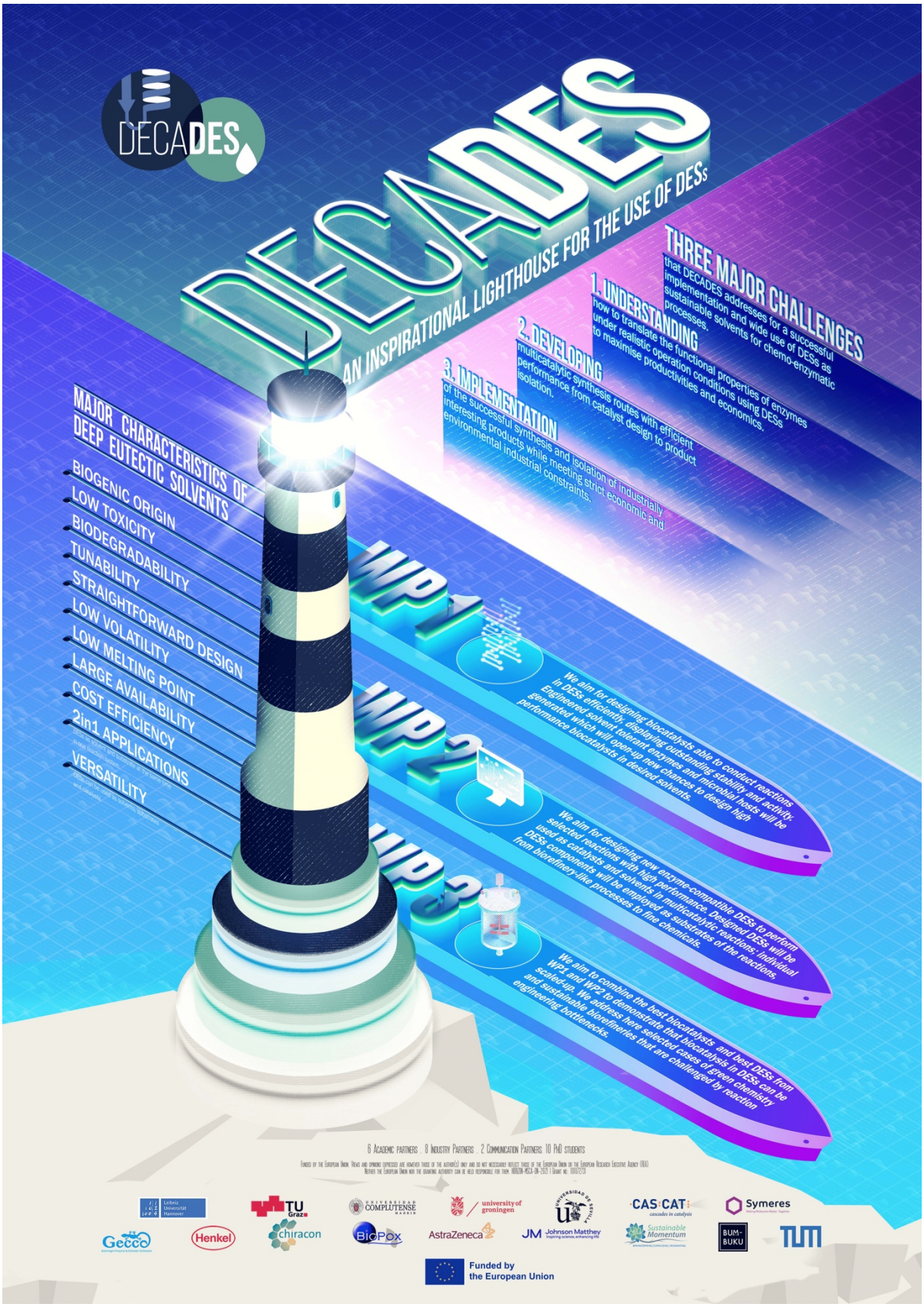
We are grateful for the support from The Research Council of Norway, DECADES EU project, Syncozymes Co., Ltd, (Shanghai, China), Amano Enzyme (UK), MDPI Catalysts (Switzerland), Dipl. Ing HOUM, AS (Oslo Norway), Trondheim Kommune, and Department of Chemistry, NTNU. Please visit the stands belonging to Amano Enzyme and Dipl. Ing. Houm- and pay serious attention to their products and services. You might even be able to secure a special deal on enzymes and equipment for your research!

I hereby also express my thanks to the Scientific committee of the conference, and to the Organizing committee. Special thanks to MSc Petter Daleng, who has been assisting me daily the last six months with all the "paper-work", and kept the conference webpage up to date. I also hope that you will experience a nice and "historic" time visiting Sverresborg Folk Museum May 28th -followed by the conference dinner at Borgstua.

I wish you all an inspiring conference with fruitful discussions in Trondheim, and I quote a special message to you all from our friend, Distinguished Professor, Nobel Laureate Frances Arnold, who was sorry to have to decline my invitation (due to her heavy work for President Biden!):

"I wish you all the best for a marvelous conference! France".

On behalf of the BNCM 2024 Scientific committe
Elisabeth E. Jacobsen
Chair BNCM 2024


DECADES
DECADES
AN INSPIRATIONAL LIGHTHOUSE FOR THE USE OF DESs
THREE MAJOR CHALLENGES
that DECADES addresses for a successful implementation and wide use of DESs as sustainable solvents for chemo-enzymatic processes.
1. UNDERSTANDING
how to translate the functional properties of enzymes under realistic operation conditions using DESs to maximise productivities and economics.
2. DEVELOPING
multicatalytic synthesis routes with efficient performance from catalyst design to product isolation.
3. IMPLEMENTATION
of the successful synthesis and isolation of industrially interesting products while meeting strict economic and environmental industrial constraints.
MAJOR CHARACTERISTICS OF DEEP EUTECTIC SOLVENTS
BIOGENIC ORIGIN
LOW TOXICITY
BIODEGRADABILITY
TUNABILITY
STRAIGHTFORWARD DESIGN
LOW VOLATILITY
LOW MELTING POINT
LARGE AVAILABILITY
COST EFFICIENCY
2in1 APPLICATIONS
VERSATILITY
WP1
We aim for designing biocatalysts able to conduct reactions in DESs efficiently, displaying outstanding stability and activity. Engineered solvent tolerant enzymes and microbial hosts will be generated which will open up new chances to design high performance biocatalysts in desired solvents.
WP2
We aim for designing new enzyme-compatible DESs to perform user selected reactions with high performance. Designer DESs will be selected as catalysts and solvents in multicatalytic reactions. Individual DESs components will be employed as substrates of the reactions from biorefinery-like processes to fine chemicals.
WP3
We aim to combine the best biocatalysts and best DESs from WP1 and WP2 to demonstrate that biocatalysis in DESs can be scaled-up. We address here selected cases of green chemistry and sustainable biorefineries that are challenged by reaction engineering bottlenecks.
6 Academic partners . 8 Industry Partners . 2 Communication Partners. 10 PhD students
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bncm 2024

Program

ESAB European Society of Applied Biocatalysis

7th International Conference on Biocatalysis in Non-Conventional Media
Trondheim, Norway, May 26th-29th, 2024

Sunday May 26, 2024

13:00-15:50	**Registration**	EL5 area
Session Chair: Bård Helge Hoff, NTNU, Norway		
16:00-16:20	**Opening of the Conference** Words of welcome: **Kent Ranum**, Mayor of Trondheim **Øyvind Weiby Gregersen**, Dean of Faculty of Natural Sciences, NTNU **Elisabeth Jacobsen**, Chair of BNCM 2024, NTNU	EL5
16:20-17:20	DECADES Special Opening Lecture **Selin Kara, Germany and Denmark** Non-Conventional Media for Enzymatic Decarboxylations: Transitioning From Lab To Industrial Scales.	EL5
17:20 -17:30	Short break	EL5 area
17:30 -18:30	Opening lecture **Frank Hollmann, The Netherlands** To Be or Not to be Green With Biocatalysis in Neoteric Solvents	EL5
19:00 -22:00	**Wine and Tapas Reception in EL5 and poster area**	EL5

Scientific committee:
Elisabeth Jacobsen NTNU, Trondheim, Norway. Treasurer of ESAB
Roland Wohlgemuth University of Lodz, Lodz, Poland. President of ESAB
Jennifer Littlechild University of Exeter, Exeter, United Kingdom. Vice President of ESAB
Sergio Riva Institute of Chemical Sciences and Technologies, SCITEC-CNR, Milano, Italy
Antonio Ballesteros CSIC, University of Madrid, Spain.
Jan von Langermann Otto-von-Guericke-University, Magdeburg, Germany
Vytas Svedas Lomonosov Moscow State University, Moscow, Russia

Organising committee:
Elisabeth Jacobsen Department of Chemistry, NTNU, Norway
Bård Helge Hoff Department of Chemistry, NTNU, Norway
Susanne H. Troøyen Department of Biotechnology and Food Science, NTNU, Norway
Mughilan Selvarajah Department of Physics, NTNU, Norway
Petter Daleng Department of Chemistry, NTNU, Norway
Marcin Krzysztof Makosa-Szczygiel Department of Chemistry, NTNU, Norway
Lucas Bocquin University of Bielefeld, Bielefeld, Germany
Kristofer Gunnar Paso Department of Chemical Engineering, NTNU, Norway

68 delegates (see alphabetical name list) from 15 countries:
Austria, Croatia, Denmark, Finland, France, Germany, Hungary, Italy, The Netherlands, Norway, Poland, Slovenia, Spain, Switzerland, United Kingdom.

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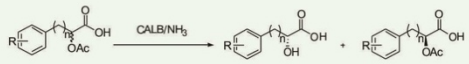
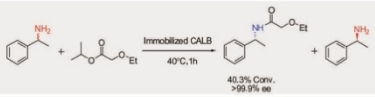
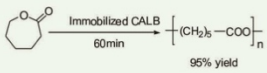
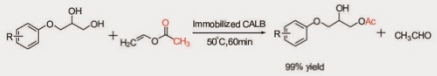
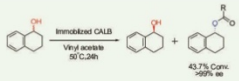
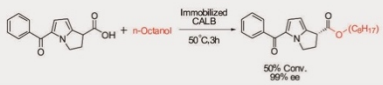
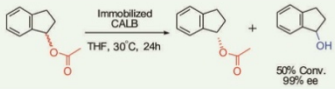
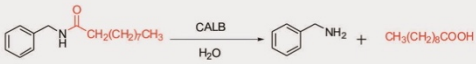
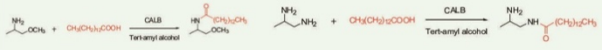
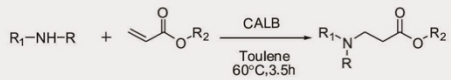
Product Code (Immobilized CALB): SZ-PLE-100(CALB)-IMMO.

Application Examples

Example 1(Aminolysis)(1) :	
Example 2(Aminolysis) (2) :	
Example 3(Ring opening polyester synthesis) (3) :	
Example 4(Transesterification, regioselective of hydroxyl group) (4):	
Example 5(Transesterification, kinetic resolution of racemic alcohols)(5):	
Example 6(Esterification, kinetic resolution of carboxylic acid) (6) :	
Example 7(Esterolysis, kinetic resolution) (7) :	
Example 8(Hydrolysis of amides) (8) :	
Example 9(Acylation of amines) (9) :	
Example 10(Aza-Michael addition reaction) (10) :	

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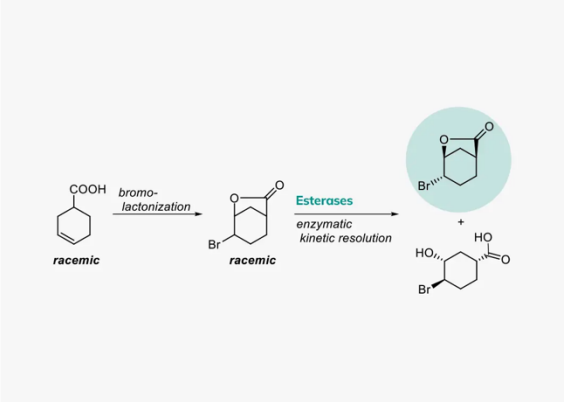
E-mail: sales@syncozymes.com services@syncozymes.com

Monday May 27, 2024

Session chair: Roland Wohlgemuth, University of Lodz, Lodz, Poland		
09:00 - 09:45	DECADES plenary lecture **Pablo Domínguez de María, Spain** There Is No "Away": The CO_2 Invoice When using Non-Conventional Media or Water in Biocatalysis	EL5
09:45 -10:10	DECADES lecture **Francesco Napoletano, Germany and Johannes Zechner, The Netherlands** Design of Catalytic Processes with Deep-Eutectic-Solvents	EL5
10:10 - 10:35	DECADES lecture **Ningning Zhang, Germany** Holistic Understanding of Alcohol Dehydrogenase Catalysis in Deep Eutectic Solvents	
10:35- 11:15	**Coffee break - with posters**	EL5 area
11:15 - 12:00	DECADES plenary lecture **Robert Kourist, Austria** Enzymatic Decarboxylation in Deep Eutectic Solvents: From Stability Engineering to Application in Continuous Flow	EL5
12:00- 12:20	DECADES lecture **Jan Philipp Bittner, Germany** Solvent Effects on Alcohol Dehydrogenase: Insights from Molecular Dynamics Simulations	EL5
12:20 -12:40	DECADES lecture **Chiara Falcini, Spain** Synthesis and Reactivity of β-Ketosulfides in Deep Eutectic Solvents	EL5
12:40 -13:00	DECADES LECTURE **Daniel Alonzo Durante Salmeron, Spain** Promiscuous Activity of Hydrolases on Chitosan in Water/Des Media	EL5
13:00-14:15	**Lunch**	Kjelhuset Cantina
Session chair: Susanne Hansen Troøyen, NTNU, Norway		
14:15- 15:00	**Fabrice Gallou, Switzerland** (online digital) Sustainability as a Driver for Innovation	EL5
15:00- 15:30	**Fredrik Bjørnes, Norway** Chemo-Enzymatic Synthesis of Enantiopure Vasodilator (–)-Nebivolol	EL5
15:30- 16:00	**Marina Cvjetko Bubalo, Hungary** Enzymes in Deep Eutectic Solvents: Balancing Enzyme Activity and Stability	EL5
16:00- 17:30	**Poster session with snacks and something to drink**	EL5 area

Amano Enzyme Europe Limited is a subsidiary of Amano Enzyme Inc. which has been a major producer of enzymes in Japan. We are located in the United Kingdom and supply customers across Europe with Amano's speciality enzymes for organic synthesis, digestive aids, anti-inflammatory, diagnostics, food processing and other industrial use. Our strength is mainly hydrolases, but we have been extending our collection of biotransformation enzymes to other classes such as ketoreductases, transaminases, etc. over recent years, many of those are indeed unique to Amano Enzyme.

Examples of chemical synthesis alternatives Edoxaban intermediates



Source: Michida M. et al., Org. Process Res. Dev., 23, 524 (2019)

Edoxaban, an oral anticoagulant, is conventionally produced by chemical methods, but it has recently been reported that a portion of the intermediate synthesis can be replaced by enzymatic methods, thereby reducing organic solvents, raw material costs and the number of filtrations without compromising yield.

Environmental burden	Reduction Effect
Organic solvents used	-90%
Raw material cost	-50%
Filtration times	7→3

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Additionally, we have recently revamped our website with a new look, please visit

www.amano-enzyme.com/en.

Tuesday May 28, 2024

Session chair: Sergio Riva, SCITEC-CNR, Milano, Italy		
09:15-10:00	**Polona Žnidaršič-Plazl, Slovenia** Non-Conventional Media in Flow Biocatalysis	EL5
10:00- 10:30	**Erika Ferrandi, Italy** Two-Step Biocatalytic Synthesis of (1*S*)-Nor(Pseudo)Ephedrine using Immobilized Enzymes	EL5
10:30- 11:30	**Coffee break - with posters**	EL5 area
11:30- 12:00	**Ricardo Semproli, Italy** From Dairy Waste to Bio-Based Surfactants: Enzymatic Synthesis of *n*-Butyl 6-*O*-Acyl-Galactosides	EL5
12:00- 12:30	**Juliet Joanna Victoria, Denmark** Challenges in Analysis for Biocatalysis in Non Conventional Media	EL5
12:30- 13:00	**Panel discussion** ESAB activities	EL5
13:00- 14:15	**Lunch**	Kjelhuset Cantina
Session chair: Lucas Bocquin, Bielefeld University, Bielefeld, Germany		
14:15- 15:00	**Roger Sheldon, South Africa and The Netherlands (online digital)** Reflections on Five Decades of Biocatalysis in Non-Conventional Media	EL5
15:00- 15:30	**Bård Helge Hoff, Norway** Pyrrolopyrimidines and Purines as CSF1R Inhibitors	EL5
17:00- 22:00	**Sverresborg Folk Museum**-Tour 17.30-18.30. **NB:** Meet at the Museum 17.10, see Bus table from Lerkendal line 11 or 13. **Conference Dinner Borgstua** at 19.00, same place.	Sverresborg Folk Museum

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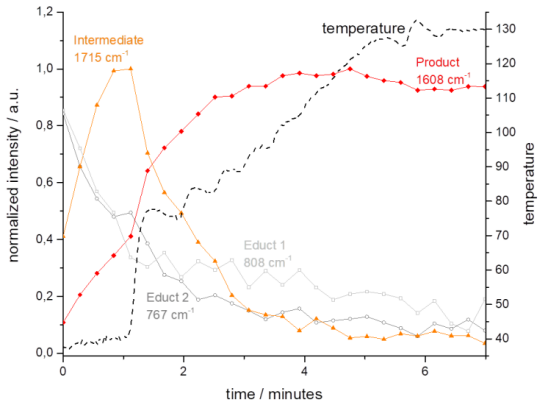
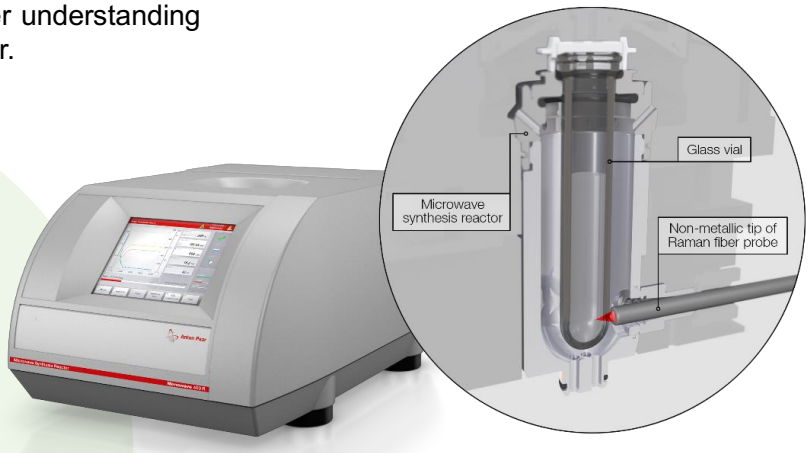


Convinced yet?

For more information and application examples, contact Hanne Svergja:

Hanne Svergja
mail: hs@houm.no
phone: 97477442



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Mail: post@houm.no

Wednesday May 29, 2024

Session chair: Jennifer Littlechild, University of Exeter, Exeter, United Kingdom		
09:00-10:00	**Dörte Rother, Germany** The Effect of Organic Solvents, Ionic Liquids and Micro-Aqueous Reaction Systems on Biotransformations: Three Case Studies	EL5
10:00 -10:30	**Morten Andre Gundersen, Norway** Lipase Catalyzed Synthesis of Enantiopure Precursors and Derivatives for β-Blockers Practolol, Pindolol and Carteolol	EL5
10:30 -11:00	**Stefania Gianoglu, Switzerland** Biocatalytic Decarboxylation of Amino Acids in Deep Eutectic Solvent using Immobilized Decarboxylases	EL5
11:00 -11:45	**Coffee break - with posters**	EL5 area
11:45 -12:30	**Jan Deska, Finland** Amphiphile-Stabilized Emulsions and Ionic Liquids as Media in Haloperoxidase Biocatalysis	EL5
12:30 -13:00	**Ricardo Moro, Spain** Enzymatic Depolymerization of Polyurethanes through Alcolysis	EL5
13:00 -14:15	**Lunch**	EL5 area
Session chair: Jan von Langermann, Otto-von-Guericke-University in Magdeburg, Germany.		
14:15 -15:00	**Pedro Lozano, Spain** When Enzymes met Ionic Liquids: A Journey to ”Dream” Chemistry	EL5
15:00 -15:15	**Elisabeth Jacobsen, Norway** Concluding remarks -End of conference	EL5
16:00 -18:00	**ESAB General Assembly** Meeting, Room E2-127, Department of chemistry	E2-127

Scientific Topics

- Novel non-conventional reaction media for biocatalysis
  - Water- and solvent-free reaction systems
  - Non-conventional media in Flow Biocatalysis
  - Biphasic and Multi-phase biocatalytic systems
  - BNCM: from labscale to industrial scale
- Kinetics and Thermodynamics of Enzyme-catalyzed Reactions in Non-conventional Media
  - Non-conventional Media for Whole-cell versus Cell-free Biocatalytic Systems
  - Sustainable Biocatalytic Reaction Engineering in Non-conventional Media

Please submit your work to the BNCM 2024 Special Issue in Catalysts by October 31, 2024

catalysts

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Biocatalysis in Non-conventional Media 2024

Guest Editors:

Dr. Elisabeth Egholm Jacobsen
Department of Chemistry, Norwegian University of Science and Technology, Trondheim, Norway

Prof. Dr. Jennifer Littlechild
The Henry Wellcome Building for Biocatalysis, Biosciences, Biocatalysis Centre, University of Exeter, Exeter, UK

Prof. Dr. Roland Wohlgemuth
MITR, Institute of Applied Radiation Chemistry, Faculty of Chemistry, Lodz University of Technology, Lodz, Poland

Message from the Guest Editors

Dear Colleagues,

The present Special Issue will feature the works presented at BNCM 2024 (https://www.ntnu.edu/bncm2024, organised in Trondheim, Norway, 26–29 May) as well as additional contributions in the field of biocatalysis, ranging from synthesis and characterizations to catalytic applications. In detail, this Special Issue will cover the following topics: (i) novel nonconventional reaction media for biocatalysis; (ii) water- and solvent-free reaction systems; (iii) non-conventional media in flow biocatalysis; (iv) biphasic and multi-phase biocatalytic systems; (v) BNCM, from labscale to industrial scale; (vi) kinetics and thermodynamics of enzyme-catalyzed reactions in non-conventional media; (vii) non-conventional media for whole-cell versus cell-free biocatalytic systems; and (viii) sustainable biocatalytic reaction engineering in non-conventional media.

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Deadline for manuscript submissions:
31 October 2024

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Special Issue

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LECTURE ABSTRACTS

OPENING LECTURE # 1
NON-CONVENTIONAL MEDIA FOR ENZYMATIC DECARBOXYLATIONS: TRANSITIONING FROM LAB TO INDUSTRIAL SCALES

Philipp Petermeier[1], Jan Philipp Bittner[2], Tobias Jonsson[3], Pablo Domínguez de María[4], Emil Byström[5], and Selin Kara[1,6*]

[1]*Aarhus University, Aarhus, Denmark*
[2]*Hamburg University of Technology, Hamburg, Germany*
[3]*Diduco AB, Umeå, Sweden*
[4]*Sustainable Momentum S. L, Canary Islands, Spain*
[5]*SpinChem AB, Umeå, Sweden*
[6]*Leibniz University Hannover, Hannover, Germany*
** Corresponding author: selin.kara@bce.au.dk*

The application of nature's catalysts, "enzymes," for the synthesis of chemicals is a crucial emerging field of industrial biotechnology to meet the current and future needs of our society for sustainable manufacturing of chemicals. Nature uses an elegant and efficient synthetic strategy: Coupling enzymes in multi-step pathways without intermediate isolation and purification steps 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] for enhancing the volumetric productivity of these biocatalytic applications. [4,5]

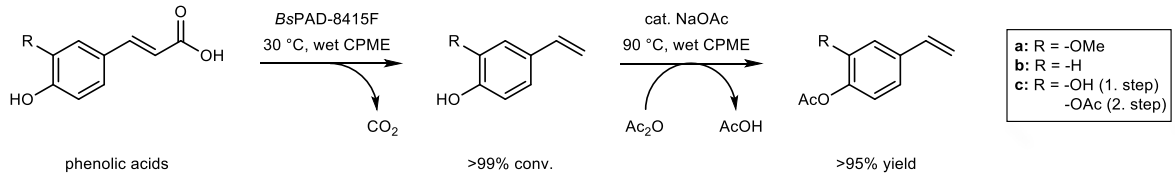

Figure 1: Chemoenzymatic synthesis of acetylated hydroxystyrenes from phenolic acids.

[1] Domínguez de María, P.; Kara, S; Gallau, F., Biocatalysis in Water or in Non-Conventional Media? Adding the CO_2 Production for the Debate, *Molecules* **2023**, *28*(18), 6452.
[2] Zhang, N.; Domínguez de María, P.; Kara, S., Biocatalysis for the Synthesis of Active Pharmaceutical Ingredients in Deep Eutectic Solvents: State-of-the-Art and Prospects, *Catalysts* **2024**, *14*(1), 84.
[3] Vernet, G.; Hobisch, M.; Kara, S., Process intensification in oxidative biocatalysis, *Current Opinion in Green and Sustainable Chemistry* **2022**, 38, 100692.
[4] Petermeier, P.; Bittner, J. P.; Müller, S.; Byström, E.; Kara, S., Design of a green chemoenzymatic cascade for scalable synthesis of bio-based styrene alternatives. *Green Chemistry* **2022**, *24*(18), 6889-6899.
[5] Petermeier, P.; Bittner, J. P.; Jonsson, T.; Domínguez de María, P.; Byström, E.; Kara, S., Integrated Preservation of Water Activity as Key to Intensified Chemoenzymatic Synthesis of Bio-Based Styrene Derivatives. *Communications Chemistry* **2024**, accepted.

OPENING LECTURE # 2
TO BE OR NOT TO BE GREEN WITH BIOCATALYSIS IN NEOTERIC SOLVENTS

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We all view our work within the context of a more sustainable future and aim to contribute to this with our research. However, sometimes our enthusiasm leads us to overestimate the impact of our work, especially when it comes to sustainability. Biocatalysis, for example, is reputed to be particularly sustainable due to its operation in water and under mild reaction conditions. But is this sufficient to label biocatalysis as 'green'?

The biocatalysis community is also notably enthusiastic about considering novel solvents as sustainable. However, similarly, are very high boiling points and (apparent) non-toxicity and (apparent) renewable origin sufficient?

Figure 1: Whether biocatalysis or novel solvents shine, whether greener they are, remains undecided, fine.

In this contribution, I intend to provoke. I will present theses that challenge our common perceptions and discuss them. This piece is expressly designed to serve as a stumbling block to initiate interesting debates during the conference.

DECADES PLENARY LECTURE # 1
THERE IS NO "AWAY": THE CO_2 INVOICE WHEN USING NON-CONVENTIONAL MEDIA OR WATER IN BIOCATALYSIS

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Biocatalysis has emerged as a sustainable alternative for many synthetic processes.[1] To sustain the greenness of enzymatic procedures the simple qualitative use of the Green Chemistry Principles is oftentimes employed. This has created the misleading perception that biocatalysis is green *per se*, regardless of other important aspects of the reaction (e.g. substrate loadings, the downstream unit, etc.). To validate the greenness of the reactions, the need of incorporating quantitative green chemistry metrics appears mandatory.[2]
This is particularly relevant when the reaction media is considered. Biocatalytic processes can be performed in water or in non-conventional media. Once the synthesis is terminated, two main waste streams are generated: i) a wastewater effluent if reactions are performed in water as reaction system; ii) and an organic fraction, coming from enzymatic reactions in non-aqueous media, and from the downstream processing. The fate of the wastewater treatment can be the Wastewater Treatment Plant (WWTP), whereas incineration is commonly applied for the organic fractions and water-based recalcitrant effluents. Both approaches generate CO_2, what can be used as the common metric to compare (bio)catalytic reactions (kg CO_2 · kg product^{-1}).[3,4]

This presentation will discuss how biocatalysis can become a green and efficient alternative for chemical processes when environmental metrics – in particular, the CO_2 production –, are measured in relation to the fates of the wastewater and the organic fraction. Several scenarios will be presented, emphasizing the need of setting recycling loops and intensified processes to reach decent sustainable metrics. In particular, a forward-discussion on the fate of Deep Eutectic Solvents (DES) and their environmental impact will be provided.[5]

References.

[1] Alcántara, A.R.; Domínguez de María, P.; Littlechild, J.; Schürmann, M.; Sheldon, R.; Wohlgemuth, R. Biocatalysis as Key to Sustainable Industrial Chemistry. *ChemSusChem* **2022**, *15*, e202102709.
[2] Domínguez de María, P. Biocatalysis, sustainability and industrial applications: Show me the metrics. *Curr. Op. Green Sust. Chem*., **2021**, *31*, 100514.
[3] Onken, U.; Koettgen, A.; Scheidat, H.; Schueepp, P; Gallou, F. Environmental metrics to drive a cultural change: Our Green Eco-Label. *Chimia* **2019**, 73, 730.
[4] Biocatalysis in water or in non-conventional media? Adding the CO_2 production for the debate. *Molecules* **2023**, *28*, 6452.
[5] Domínguez de María, P.; Kara, S. On the fate of Deep Eutectic Solvents after their use as reaction media: The CO_2 production during downstream and ultimate disposal. *RSC Sustainability* **2024**, *2*, 608-615.

DECADES PLENARY LECTURE # 2
ENZYMATIC DECARBOXYLATION IN DEEP EUTECTIC SOLVENTS: FROM STABILITY ENGINEERING TO APPLICATION IN CONTINUOUS FLOW

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Enzymatic decarboxylation of bio-based hydroxycinnamic acids gives access to phenolic styrenes for adhesive production. Phenolic acid decarboxylases are proficient enzymes that have been applied in aqueous systems, organic solvents, biphasic systems, and deep eutectic solvents, which makes stability a key feature. Stabilization of the enzyme would increase the total turnover number and thus reduce energy consumption and waste accumulation associated with biocatalyst production. In this study, we used ancestral sequence reconstruction to generate thermostable decarboxylases. Investigation of a set of 16 ancestors resulted in the identification of a variant with an unfolding temperature of 78.1 °C and a half-life time of 45 hours at 60 °C. Crystal structures were determined for three selected ancestors. Structural attributes were calculated to fit different regression models for predicting the thermal stability of variants that have not yet been experimentally explored. As bacterial PADs do not convert sinapic acid, simultaneous saturation mutagenesis was used for an expansion of the substrate scope of the ancestor of the highest stability.

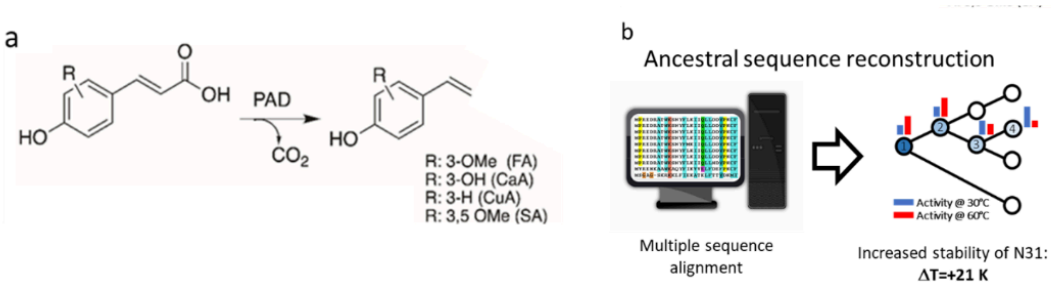

Figure 1: Ancestral sequence reconstruction for the improvement of the stability of phenolic acid decarboxylase.

A further stabilization of the enzyme was achieved by the application of mixtures of natural deep eutectic solvents and buffer. This approach substantially improves productivity, rendering our approach a straightforward option for enhancing the industrial application of the process..

[1] K. Myrtollari, E. Calderini, D. Kracher, S. Galušić, A. Slavica, A. Taden, D. Mokos, A. Schrüfer, G. Wirnsberger, K. Gruber, B. Daniel, R. Kourist (2024), Stability increase of phenolic acid decarboxylase by a combination of protein and solvent engineering unlocks applications at elevated temperatures, *ACS Sust. Chem. Eng.*, *accepted manuscript*

PLENARY LECTURE # 3
SUSTAINABILITY AS A DRIVER FOR INNOVATION

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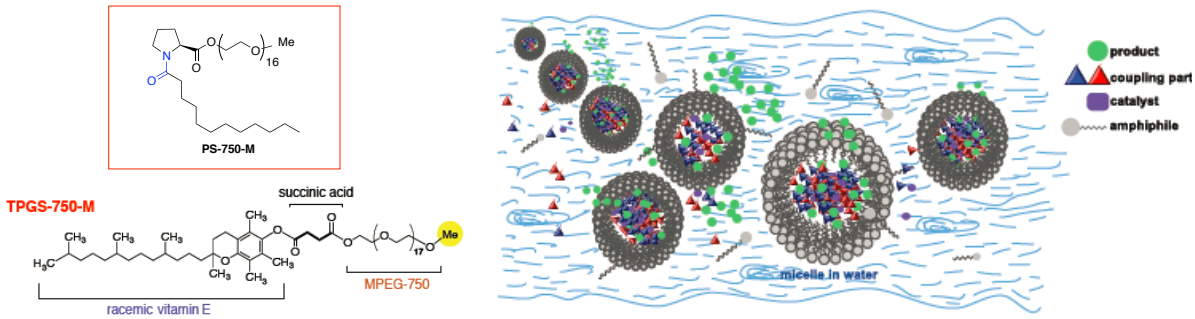
During our evaluation of the potential of surfactant technology, we have identified a variety of straightforward and highly advantageous transformations and applied them successfully on-scale on various chemo and biocatalytic transformations.[1] Implementation of the technology typically results into significant benefits across our entire portfolio, not just from an environmental standpoint but also from an economic and productivity perspective. To name a few: reduction of organic solvent consumption, water use and cycle time, milder reaction conditions, improved yields and selectivities,[2] much greener footprint,[3] which all contribute to improved process performance and lower manufacturing costs.



Modern non-ionic surfactants for micellar catalysis in water.

These surfactant mediated reactions can be up-scaled in the already existing multi-purpose facilities of pharmaceutical or chemical organizations, using a catalytic amount of a combination of a non-ionic designer surfactant (e.g. TPGS-750-M, PS-750-M) in water, and a well-chosen organic co-solvent instead of traditional and undesirable organic solvents. We now start gaining insight onto the physical phenomena involved and the role of the various components of the reactions and utilize this know-how to design even better catalytic systems.[4]

[1] ACS *Sustain. Chem. Eng.* **2022**, 10, 5148. *Chem. Catal.* **2023**, 3, 100485. *Chimia* **2020**, 74, 538. *Chem. Rev.* **2023**, 123, 5263.
[2] *Green Chem.* **2016**, 18, 14. *Curr. Opin. Green Sust. Chem*. **2017**, 7, 13.
[3] *Chimia* **2019**, 73, 9, A681. *Org. Process Res. Dev.* **2021**, 25, 900.
4 *Curr. Opin. Colloids and Interface Sci*. **2021**, 56, 101506. *J. Colloid Interface Sci.* **2022**, 628, 819.

PLENARY LECTURE # 4
NON-CONVENTIONAL MEDIA IN FLOW BIOCATALYSIS

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Continuous-flow processing and miniaturization have recently changed the paradigm of biocatalytic process design and significantly contribute to process intensification.[1] Since many organic substrates or reaction products are only sparingly soluble in water, the use of non-conventional media is receiving considerable attention, especially with the possibility for *in situ* product removal in two-liquid phase systems.[2] Current trends to use solvents that combine high solubilization capacity and cost-effectiveness with a low environmental footprint, such as deep eutectic solvents and protic ionic liquids that can even improve biocatalyst selectivity, might gain momentum by using high-throughput microflow system for their selection.[3]

In this presentation, the application of both free enzymes in cosolvent or two-liquid phase systems, as well as immobilized biocatalysts in non-conventional media within microfluidic devices will be highlighted. Examples include lipase-catalyzed esterifications and transesterifications in selected ionic liquids with immobilized enzyme, or in aqueous/organic solvent and ionic liquid/organic solvent systems with dissolved enzyme[4-7]. Moreover, amine transaminase-catalyzed transamination using selected deep eutectic solvent in a magnetic-field assisted microreactor will be presented, and the effect of various cosolvent and two-liquid phase systems on laccase-catalyzed tyrosol acetate oxidation in a microflow system will be discussed. Finally, the application of deep eutectic solvent for tuning the characteristics of a copolymeric hydrogel used for biocatalyst immobilization will be presented.[8]

[1] Žnidaršič-Plazl, P. Biocatalytic process intensification via efficient biocatalyst immobilization, miniaturization, and process integration. *Curr. Opin. Green Sustain. Chem*., **2021,** 32, 100546.
[2] Žnidaršič-Plazl, P. Enzymatic microreactors utilizing non-aqueous media. *Chimica Oggi – Chem. Today*, **2014**, 32: 54-61
[3] Žnidaršič-Plazl, P. Biotransformations in microflow systems: Bridging the gap between academia and industry. *J Flow Chem.,* **2017**, 7 (3-4): 111-117
[4] Pohar, A; Plazl, I; Žnidaršič-Plazl, P. Lipase-catalyzed synthesis of isoamyl acetate in an ionic liquid/n-heptane two-phase system at the microreactor scale. *Lab Chip*, **2009**, 9: 3385-3390.
[5] Pohar, A; Žnidaršič-Plazl, P; Plazl, I. Integrated system of a microbioreactor and a miniaturized continuous separator for enzyme catalyzed reactions. *Chem. Eng. J.* **2012**, 189– 190: 376– 382.
[6] Cvjetko, M; Vorkapić-Furač, J; Žnidaršič-Plazl, P. Isoamyl acetate synthesis in imidazolium-based ionic liquids using packed bed enzyme microreactor. *Process Biochem.,* **2012**, 47: 1344–1350
[7] Novak, U; Žnidaršič-Plazl, P. Integrated lipase-catalyzed isoamyl acetate synthesis in a miniaturized system with enzyme and ionic liquid recycle. *Green Proc Synth*., **2013**, 2: 561-568
[8] Menegatti, T; Kopač, T; Žnidaršič-Plazl, P. Tuning mechanical characteristics and permeability of alginate hydrogel by polyvinyl alcohol and deep eutectic solvent addition. *Bioengineering*, **2024**, 11 (4), 371

HONORARY PLENARY LECTURE # 5
REFLECTIONS ON FIVE DECADES OF BIOCATALYSIS IN NON-CONVENTIONAL MEDIA

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A personal reflection on the development of biocatalysis in non-conventional media over 5 decades is presented. In the 1970s and 1980s there was a clear distinction between processes for bulk chemicals manufacture on the one hand and fine chemicals and pharma on the other hand, and never the twain shall meet. Bulk chemicals production involved the use of heterogeneous catalysts and hydrocarbons as substrates, mainly in the gas phase. In contrast, fine chemicals and pharmaceuticals were produced using stoichiometric quantities of reagents dissolved in organic solvents, i.e. in the liquid phase.

In the 1980s it gradually became apparent, to astute observers at least, that environmental concern was encouraging the development of processes that minimize chemical waste, i.e. catalytic processes rather than processes involving archaic stoichiometric reagents.[i] At the same time biocatalytic methods for the synthesis of semi-synthetic penicillins and cephalosporins were being developed, particularly by the companies Gist-Brocades and DSM in the Netherlands. The driving force was the possibility to replace the environmentally unfriendly reagents and solvents used in the classical syntheses, e.g. carbon tetrachloride and dichloromethane.

In the1990s the trend towards the use of catalytic processes in fine chemicals manufacture continued unabated and was reinforced, in 1992, by new legislation introduced by the FDA, that encouraged the marketing of chiral drugs as single enantiomers. This led to widespread interest in catalytic asymmetric synthesis. Initially focused mainly on catalytic asymmetric hydrogenation but it was soon realized that enzymatic methods, aided by developments in protein engineering, had many advantages, in particular the near perfect enantioselectivities and environmentally friendly reaction conditions. This trend, aided by the many developments in molecular biology, continued in the following decades in this century.

No matter which technology is used it generally requires the use of solvent. However, as was already observed in the 1990s: the whole question of solvents in organic synthesis needed to be re-examined. Not only should the solvent be environmentally benign, the overall process should involve its facile removal and recycling in a circular economy. Hence, in the last three decades a wide variety of non-conventional solvents has been studied, both with chemo- and biocatalysts. Major examples are bio-based solvents, ionic liquids, deep eutectic solvents, supercritical carbon dioxide, aqueous micelles and mixtures thereof. Advantages and limitations of the various possibilities are briefly delineated.

. M. A. Murphy, *Substantia*. An International Journal of the History of Chemistry, 2023, 7(2), 41-55, Firenze University Press. www.fupress.com/substantia DOI: 10.36253/Substantia-2140

PLENARY LECTURE # 6
THE EFFECT OF ORGANIC SOLVENTS, IONIC LIQUIDS AND MICRO-AQUEOUS REACTION SYSTEMS ON BIOTRANSFORMATIONS: THREE CASE STUDIES

Dörte Rother[1,2] on behalf of the biocatalysis group at IBG-1 & our cooperation partners

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In nature, enzymes typically function under aqueous conditions. Consequently, aqueous buffers are often used as reaction media in transformations involving enzymes. However, for industrial applications, the poor water solubility of many compounds of industrial relevance means that aqueous systems frequently fail to achieve sufficient substrate and therewith product concentrations. Switching to a non-aqueous solvent system can provide a solution, a practice already common with lipases but more challenging for biocatalysts from other enzyme classes. Recently, several potent green solvents come to market, showing an interesting alternative to classical, environmentally less benign solvents. Some are well suited to biotransformations, when an appropriate formulation is found.

In this presentation, three examples will be focused on. In case studies one and two, the effect of additives on the activity, stereoselectivity, and chemoselectivity of carboligation reactions with thiamine diphosphate-dependent enzymes is demonstrated. Since the chosen reaction is very sensitive to additives in a mono liquid-phasic buffered system, it can be clearly shown how some organic solvents as well as ionic liquids directly interact with areas of the active site, partially competing with the substrates. This affects the selectivity of the enzymes, which can, in the optimal case, lead to increased or inverted selectivity.[1] Thus, solvents can be an interesting parameter for engineering.

As a third example, the use of micro-aqueous reaction systems for single and multi-step enzyme-catalyzed processes will be demonstrated.[2] Due to significantly increased substrate concentrations in these non-buffered systems, high product concentrations and space-time yields can be achieved, while stereoselectivities are maintained. Additionally, the use of an economical catalyst formulation and simplified downstream processing makes its application advantageous.[3] It also offers great potential when biotransformations and chemical transformations are combined in multi-step processes.[4]

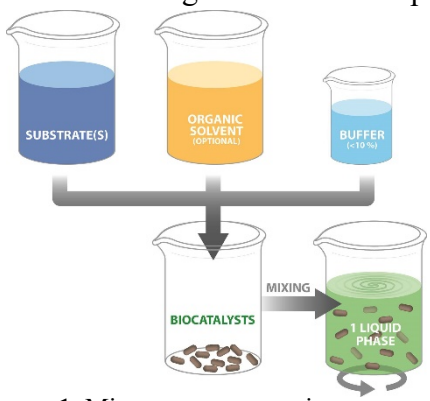

Figure 1: Micro-aqueous reaction system.

[1] Gerhards T, Mackfeld U, Bocola M, von Lieres E, Wiechert W, Pohl M, Rother D. Influence of organic solvents on enzymatic asymmetric carboligations. *Adv. Synth. Catal.* **2012**, 354: 2805-2820.
[2] Van Schie M M C H., Spöring J-D, Bocola M, Domínguez de María P, Rother D. Applied biocatalysis beyond just buffers – from aqueous to unconventional media. Options and guidelines. *Green Chem.* **2021**, 3: 3191-3206.
[3] Oeggl R, Maßmann T, Jupke A, Rother D. Four Atom Efficient Enzyme Cascades for All 4-Methoxyphenyl-1, 2-propanediol Isomers Including Product Crystallization Targeting High Product Concentrations and Excellent E-Factors, *ACS Sus. Chem. Eng.* **2018**, *6*: 11819–26.
[4] Graf von Westarp W, Wiesenthal J, Spöring J D, Mengers H G, Kasterke M, Koß H J, Blank L, Rother D, Klankermayer J, Jupke A. Interdisciplinary development of an overall process concept from glucose to 4, 5-dimethyl-1, 3-dioxolane via 2, 3-butanediol. *Commun. Chem.* **2023**, 6 (1): 253.

PLENARY LECTURE # 7
WHEN ENZYMES MET IONIC LIQUIDS: A JOURNEY TO "DREAM" CHEMISTRY

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Enzymes clearly constitute the most powerful green tools for catalyzing chemical processes, since their activity and selectivity (stereo-, chemo- and regioselectivity) are far ranging. Although enzymes are designed by living systems to work in aqueous solutions, there are numerous potential advantages in employing enzymes in non-aqueous environments (e.g. new chemical transformations, higher solubility of hydrophobic substrates, easy reuse, etc). However, the "*Achilles heel*" of enzymes in non-aqueous system is deactivation, usually related with irreversible structural and conformational changes on its native structure. Water is the key component for all non-conventional media, because of the importance that enzyme–water interactions have in maintaining the active conformation of enzymes.[1]

Since 2000, ionic liquids (ILs) have emerged as exceptionally non-aqueous green reaction media because of their unique solvent properties, headed by their negligible vapour pressure, and their exceptional ability to over-stabilize enzymes, even under extremely harhs conditions (*e.g.* $scCO_2$ at 120 bar and at 150 ºC). ILs are able to preserve the essential hydration layer around enzymes, maintaing all the excellences of its catalytic berhaviour. Another interesting feature for biocatalysis in ILs is the possibility to design two-phase multicatalytic reaction systems (*e.g.* IL-$scCO_2$ biphasic systems), which allow straighforward and clean processes for product recovery and biocatalyst reuse (*e.g.* continuous DKR of sec-alcohols).[2] The discovery of the sponge-like character of hydrophobic ILs (Sponge-Like Ionic Liquids, SILS) was key for designing straighforward and green biocatalytic processes, involving both biotransformation and separation steps for producing nearly pure compounds of high added value (*e.g.* flavour esters, biodiesel, monoglyerides, etc.).[3]

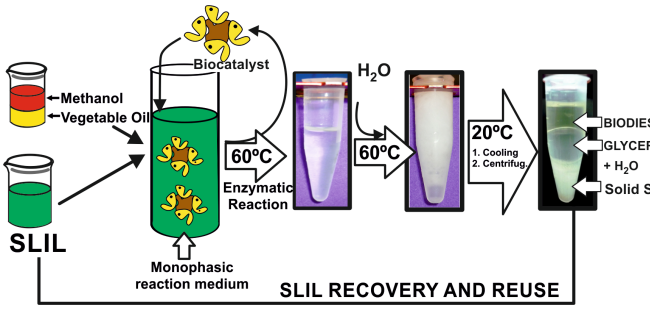

Fig 1. Clean biocatalytic production of biodiesel using SLILs.[3]

Alternatively, biocatalysis in solvent-free systems, based on Deep Eutectic Solvent (DESs) technology, constitutes another sucessfully approach for developing clean and green processes of industrial interest (*e.g.* xylityl laurate, panthenyl monoesters, etc). Multi-enzymatic and/or multi-chemoenzymatic chemical transformations in these non-conventional systems, mimicking the metabolic pathways found in nature, is "dream" chemistry that is getting closer.[4]

Acknowledgements
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[1] *Biocatalysis in Green Solvents*, Ed. P. Lozano, Academic Press-Elsevier London, **2022**.
[2] R. Villa *et al*. Ionic liquids as an enabling tool to integrate reaction and separation processes. *Green Chem*. **2019**, 21, 6527-6544.
[3] P. Lozano *et al*. Sponge-like ionic liquids: a new platform for green biocatalytic chemical processes. *Green Chem*. **2015**, 17, 3706-3717.
[4] P. Lozano *et al*. From green to circular chemistry paved by biocatalysis. *Green Chem*. **2023**, 25, 7041-7057.

DECADES LECTURE # 3
DESIGN OF CATALYTIC PROCESSES WITH DEEP-EUTECTIC-SOLVENTS (DECADES)
DOCTORAL NETWORKS PROJECT

Francesco Napoletano[1] and Johannes Zechner[2]

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[2]*Symeres Netherlands, Groningen, Netherlands*

The choice of solvents – as reaction media and for downstream processing – defines the overall sustainability of chemical transformations. So far, (bio)catalysis has traditionally relied on either aqueous- or classical organic media, or biphasic systems thereof. Selection of solvents has usually implied a case-by-case assessment for every system and step (reaction or purification), leading to several scientific and technological challenges untouched or unsolved. In this respect, Deep Eutectic Solvents (DESs) have been coined as 'the solvents of the 21st century'. In a nutshell, DESs' assets are based on the often biogenic origin of the components, and their properties such as melting points below room temperature, low volatility, high thermal stability, tunable properties depending on their components, biodegradability, large availability at acceptable costs, and straightforward preparation.
Horizon Europe Marie Skłodowska-Curie Actions doctoral networks programme DECADES seeks to become an inspirational lighthouse for the use of DESs as highly advantageous solvents to improve the sustainability of biotechnological processes. With a training focused on scientific excellence, creativity, innovation and entrepreneurship, DECADES aims for a solid employability of its PhD graduates as future technology leaders for a sustainable bioeconomy.

Objective 1: fit biocatalytic activity and stability for DESs in representative reactions in cell-free and cellular systems, all to high-value projects; but, posing different challenges, with a focus on (i) oxidase, (ii) dehydratase, (iii) decarboxylase, (iv) racemase, (v) dehydrogenase, (vi) hydrolase enzymes, and (vii) genome engineering.

Objective 2: gain knowledge on the effects of DESs on enzymes, on bio- and chemo-catalytic systems, and devise strategies for their optimization.

Objective 3: demonstrate the technology with the implementation of production routes of three target products (fine chemicals and bio-based products) and their purification in high yields, including (i) oligosaccharides, (ii) highly functionalized lignin-based phenolics, and (iii) unnatural α-amino acids.

Objective 4: exploit the solvent design and application considering catalyst design, substrate loading, environmental footprint, process design, and downstream processing in a holistic manner.

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them. HORIZON-MSCA-DN-2021 I Grant no.: 101072731.

DECADES LECTURE # 4
HOLISTIC UNDERSTANDING OF ALCOHOL DEHYDROGENASE CATALYSIS IN DEEP EUTECTIC SOLVENTS

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Biocatalysis has been shifting from traditional aqueous to non-aqueous media in the spirit of *Green Chemistry*. The use of oxidoreductases (EC.1) in non-conventional media plays an indispensable role in the synthesis of a variety of value-added chiral compounds.[1a] Deep eutectic solvents (DESs) represent a new class of greener solvents that are highly tunable.[1b] Redox biocatalysis in DESs combines the two dominant assets of selectivity of enzymes and versatility of DESs.[1c, d] The rational design of redox biocatalysis in DESs requires a comprehensive knowledge of the effects of DESs on enzymes. Exemplarily, the impact of DESs on oxidoreductases has been holistically studied by assessing the catalytic performance of alcohol dehydrogenases (ADHs) in DES-water mixtures (e.g., choline chloride-glycerol, ChCl-Gly, 1:2) with the aid of experimental analysis and molecular dynamics (MD) simulations.[2a, b] It was revealed that enzyme activity is positively related to water activity due to solvation changes surrounding enzymes. In addition, the individual DES components showed discrepant effects, e.g., positive (in case of Gly) or negative (in case of ChCl), promoting the generation of an enzyme-compatible eutectic mixture by increasing the Gly fraction (ChCl-Gly, 1:9).[2c] Based on that, the Gly-based DESs with non-ChCl as HBAs (choline acetate and betaine), namely ChAc-Gly (1:2) and Bet-Gly (1:2), are envisioned to be more enzyme-friendly (**Fig. 1**).

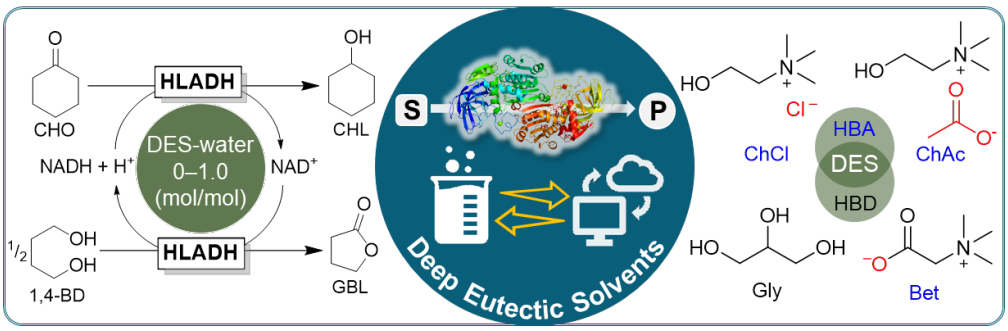

Figure 1: Analysis of horse liver alcohol dehydrogenase in glycerol-based deep eutectic solvents containing various water content (0–100 vol.%) by combining experiments and simulations.

Expectedly, the selected enzyme, horse liver ADH (HLADH), exhibited highly improved stability in both DESs, especially in Bet-Gly. Further studies on different molar ratios and individual components of DESs were conducted to fully elucidate the relationship between the studied DESs and enzymes.

[1] a) M. van Schie, J. D. Sporing, M. Bocola, P. Domínguez de María, D. Rother, *Green Chem.* **2021**, *23*, 3191–3206. b) M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends Biotechnol.* **2019**, *37*, 943–959. c) V. Gotor-Fernández, C. E. Paul, *J. Biotechnol.* **2019**, *293*, 24–35. d) N. Zhang, P. Domínguez de María, S. Kara, *Catalysts* 2024, *14*, 84.
[2] a) L. Huang, J.P. Bittner, P. Domínguez de María, S. Jakobtorweihen, S. Kara, *ChemBioChem* **2018**, *21*, 811–817; b) J. P. Bittner, I. Smirnova, S. Jakobtorweihen, *Molecules* **2024**, *29*, 703. c) J. P. Bittner, N. Zhang, L. Huang, P. Domínguez de María, S. Jakobtorweihen, S. Kara, *Green Chem*. **2022**, *24*, 1120-1131.

DECADES LECTURE # 5
SOLVENT EFFECTS ON ALCOHOL DEHYDROGENASE: INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS

Jan Philipp Bittner[1*]; Ningning Zhang[2]; Pablo Domínguez de María[3]; Irina Smirnova[1]; Selin Kara[2,4] and Sven Jakobtorweihen[1,5]

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Understanding the behavior of alcohol dehydrogenase (ADH) in non-conventional reaction media is crucial for optimizing its catalysis in these solutions. The choice of the solvent plays a pivotal role in enhancing biotransformation efficiency while mitigating issues such as limited reactant solubility and water-induced side effects. Deep eutectic solvents (DESs) are a novel solvent class for biocatalysis that offer a high degree of tunability and have already been applied to various biocatalytic systems.[1] This study explores the impact of various solvents, including DESs, on horse liver alcohol dehydrogenase (HLADH) using molecular dynamics (MD) simulations.[2] The simulations revealed that glycerol has a high affinity for the HLADH surface, which enhances its stability in ChCl-Gly (1:2)-water mixtures. This effect is further improved by adjusting the glycerol molar ratio.[2a] Spatial distribution functions highlight the solvent's impact on enzyme flexibility and structural changes (see **Fig. 1**). Based on these results, we proposed new glycerol-containing DESs with a beneficial effect on HLADH. With the help of MD simulations, we could explain the experimentally observed effects of DES-water mixtures on enzyme activity and stability on an atomistic scale.[2]

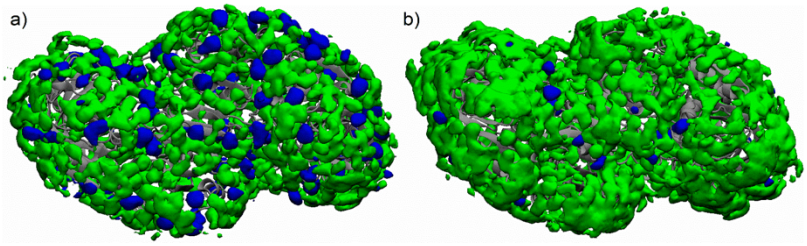

Figure 1: Spatial distribution function of glycerol (green) and choline ions (blue) in the proximity of HLADH (gray) in the MD simulations of (a) ChCl-Gly (1:2) and (b) ChCl-Gly (1:9) in mixtures with 20 vol.% water.

This study further focused on the structural changes of HLADH's active center in diverse reaction environments. The free energy profiles of the substrate molecule (cyclohexanone) along the substrate-binding tunnel to HLADH's active center were quantified using MD simulations. Understanding enzyme behavior at an atomistic level can guide solvent selection for ADH-catalyzed reactions. Thereby, MD simulations provide detailed insights into enzyme-solvent interactions and substrate binding, which can aid in solvent engineering to maintain enzymatic activity under non-conventional reaction conditions.[3]

[1] a) M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends Biotechnol.* **2019**, *37*, 943– 959. b) N. Zhang, P. Domínguez de María, S. Kara, *Catalysts* **2024**, *14*, 84.
[2] a) J. P. Bittner, N. Zhang, L. Huang, P. Domínguez de María, S. Jakobtorweihen, S. Kara, *Green Chem.* **2022**, *24*, 1120–1131. b) L. Huang, J. P. Bittner, P. Domínguez de María, S. Jakobtorweihen, S. Kara, *ChemBioChem* **2020**, *21*, 811-817.
[3] J. P. Bittner, I. Smirnova, S. Jakobtorweihen, *Molecules* **2024**, *29*, 703.

DECADES LECTURE # 6
SYNTHESIS AND REACTIVITY OF β -KETOSULFIDES IN DEEP EUTECTIC SOLVENTS

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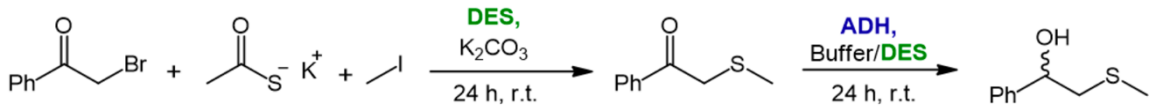
Deep eutectic Solvents (DESs) have appeared in the recent years as an appealingalternative to classical organic solvents, due to their valuable environmental properties [1]. In addition, these compounds, formed by the combination of one hydrogen bond donor with a hydrogen bond acceptor at a defined stoichiometric ratio, present other valuable activities not only as a reaction medium [2]. β-Ketosulfide motif constitutes an interesting organic framework, widely present in natural products and it presents interest in biological and pharmaceutical chemistry [3]. These compounds can be obtained from thiols or other sulfur sources, such as disulfides or silylsulfides employing metals or organocatalysts [4]. Recently, the synthesis of β-ketosulfides has been described employing lipases from from β-alkylsulfide enol esters, but these compounds were obtained using dimethylformamide (DMF) as solvent [5]. The preparation of β-ketosulfides employing DESs will be discussed herein, showing a novel multicomponent one pot reaction under mild conditions employing 2-bromoacetophenone as starting material. The obtained β-ketosulfides have been reduced into optically active β-hydroxyulfides employing alcohol dehydrogenases also in presence of DESs (Scheme 1).



Scheme 1: Multicomponent one pot reaction to prepare β-ketosulfides and bioreduction to optically active β-hydroxyulfides.

[1] Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep Eutectic Solvents (DESs) and their applications. *Chemical Reviews* **2014,** *114*, 11060–11082.
[2] Falcini, C. and de Gonzalo, G., Deep Eutectic Solvents as Catalysts in the Synthesis of Active Pharmaceutical Ingredients and Precursors. *Catalysts* **2024,** *14*, 120.
[3] Perrey, D. A.; Narla, R. K.; Uckun, F. M. Bioorg., Cysteine Chloromethyl and Diazomethyl Ketone Derivatives with Potent Anti-Leukemic Activity. *Bioorganic & Medicinal Chemistry Letters* **2000,** *10*, 547–549.
[4] Vaquer, A. F.; Frongia, A.; Secci, F.; Tuveri, E., Disulfide-based metal-free a-sulfanylation of Ketones. *RSC Advances* **2015,** *5*, 96695–96704.
[5] Heredia A, A.; López-Vidal, M. G.; Kurina-Sanz, M.; Bisogno R, F.; Peñéñory B, A., Thiol-free chemoenzymatic synthesis of β-ketosulfides. *Beilstein Journal of Organic Chemistry* **2019,** *15*, 378–387.

DECADES LECTURE # 7
PROMISCUOUS ACTIVITY OF HYDROLASES ON CHITOSAN IN WATER/DES MEDIA

D. Alonzo Durante-Salmerón[1*]; I. Fraile-Gutiérrez[1,2]; J. M. Silvestre[2]; K. Margariti[2,3]; I. Rodríguez-Veiga [1,4]; I. Aranaz[1,2] and Andrés R. Alcántara[2]*

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The enzymatic hydrolysis of chitosan to produce chitooligosacharides (COSs), which displays a plethora of biological activities [1], is mediated in nature by chitosanases [2]. These enzymes, although very specific, are very expensive, so that the employ of other cheaper "promiscuous" hydrolases [3] would be highly beneficial from an economic point of view, allowing the generation of a library of tailor-made COSs with different technological/biological activities [4]. In this presentation, the use of different commercial hydrolases for hydrolysis of chitosan is presented. After a previous characterization of the commercial enzymes, their performance for the modifications of different chitosans was tested, both in aqueous media and in DESs (either reline (basic) and betaine/lactic acid (acidic).

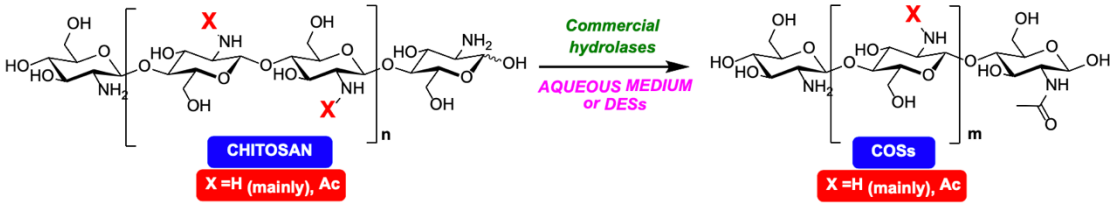

Figure 1: Hydrolase-catalysed depolymerization of chitosan in water/DESs media.

According to the initial results obtained in aqueous solutions, these enzymes were capable to hydrolyse chitosan from different origins, with good recovery yields (up to 94%), and leading to different fractions possessing different Mw and acetylation degree; this fact could suggest that the enzymes can be also acting on the -NH-$COCH_3$ groups (Figure 1, X= Ac). Regarding DESs, enzyme activity is limited by the chitosan solubility in reline/water. Initial results are pointing towards a positive solubilization of starting material in betaine/lactic acid DESs, depending on the polymer properties. Activities are being tested in this DES for this non-previously reported enzymatic-based chitosan depolymerization.

[1] Jagdale, S.; Agarwal, B.; Dixit, A.; Gaware, S. Chitosan as excellent bio-macromolecule with myriad of anti-activities in biomedical applications – A review. *Int J Biol Macromol*. **2024**; 257; 128697
[2] Sinha, S.; Chand, S.; Tripathi, P. Recent Progress in Chitosanase Production of Monomer-Free Chitooligosaccharides: Bioprocess Strategies and Future Applications. *Appl Biochem Biotechnol.* **2016**; 180:883-99.
[3] Poshina, D.N.; Raik, S.V.; Poshin, A.N.; Skorik, Y.A. Accessibility of chitin and chitosan in enzymatic hydrolysis: A review. *Polym Degrad Stabil*. **2018**; 127-129.
[4] Aranaz I.; Castro, C.; Heras, A.; Acosta, N. On the ability of low molecular weight chitosan enzymatically depolymerized to produce and stabilize silver nanoparticles. *Biomimetics*. **2018**; 3 (3), 21.

LECTURE # 1
CHEMO-ENZYMATIC SYNTHESIS OF ENANTIOPURE VASODILATOR (–)-NEBIVOLOL

Fredrik Bjørnes, Sara Aasen[1*], Aleksander Damlien Eide[1], Petter Daleng[1], and Elisabeth Egholm Jacobsen[1]

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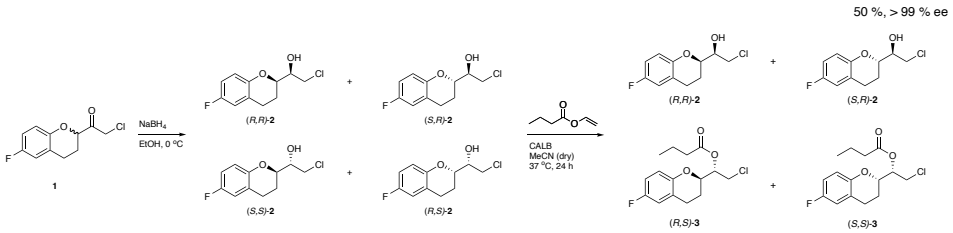
The American Heart Association reported in March 2019 their update on heart diseases and stroke statistics. The report states that the prevalence of high blood pressure in the United States between 2013-2016 was 46% of the total population at ages 20 years and older. It was the cause of death for 82,735 Americans in 2016 and costed the American society approximately $ 55.9 billion in the period 2014-2015 [1]. A class of drugs that have been used both in cardiovascular and non-cardiovascular treatment are the β-adrenergic blocking agents (betablockers). This class of drugs were introduced into clinical medicine in the early 1960's, and since then, the findings of these drugs have led to a discovery of the importance of the sympathetic nervous system in the pathophysiology of a wide variety of cardiovascular and non-cardiovascular disorders [2]. We have for some time developed efficient chemo-enzymatic protocols for synthesis of several betablockers [3a-e]. Here, we present our chemo-enzymatic protocol for intermediates (Scheme 1) of the enantiopure vasodilator (–)-Nebivolol (not actually a beta-blocker), and for the final drug (Fig. 1). Due to the four stereogenic centra in the molecule there are 16 possible isomers when no catalyst is used in the synthesis. However, due to symmetry, there are 10 possible isomers. We have achieved to produce the (*R,S,S,S*)-nebivolol or (–)-nebivolol.



Scheme 1: Synthesis of halohydrines *(R,R)*-**2** and *(S,R)*-**2** was obtained by kinetic resolution with CALB. Separation of *(R,R)*-**2** and *(S,R)*-**2** from esters *(R,S)*-**3** and *(S,S)*-**3** is easily performed by column chromatograpy.

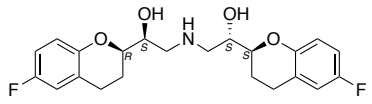


Figure 1. (–)-Nebivolol, (*R,S,S,S*)-nebivolol.

[1]. E. J. Benjamin, *et al. Circulation* **2019**, *139* (10), e56-e528.
[2]. J. W. Black and B. N. C. Prichard. *Br. Med. Bull.* **1973**, *29*, (2), 163
[3]. a) M. A. Gundersen *et al., Catalysts,* **2021,** *11,* 503**.** b) S. H. Troøyen, *et al. Catalysts* **2022**, *12*, 980. c) S. H. Troøyen and E. E. Jacobsen. *Catalysts,* **2022**, *12*, 1645. d) L. H. Y. Bocquin and E. E. Jacobsen. *Catalysts* 2023, *13*, 54. e) P. L. Bøckmann and E. E. Jacobsen. *Topics in Catalysis*, **2024**, 1-9.

LECTURE # 2
ENZYMES IN DEEP EUTECTIC SOLVENTS: BALANCING ENZYME ACTIVITY AND STABILITY

Marina Cvjetko Bubalo*; Marijan Logarušić, Mia Radović, Anja Damjanović, Ivana Radojčić Redovniković

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Deep Eutectic Solvents (DES), neoteric systems that emulate the natural environment for various biomacromolecules, are under extensive study as non-toxic and highly versatile solvents applicable across diverse fields, including synthesis, (bio)catalysis and biomedicine.[1] Originally, the term DES was coined to describe a physical mixture of two or more components that solidifies at a single temperature lower than the crystallization point of any individual component. Over time, these solvents/systems have come to encompass mixtures comprising two or more components that exhibit characteristics similar to a eutectic system, allowing them to maintain a liquid state at a specific desired temperature.[2]

The synergistic combination of DES and biotechnological methods, employing enzymes as catalysts, aligns naturally with the efficient and sustainable production of various organic compounds. Biocatalysis, as a biotechnological approach, enables the catalysis of otherwise challenging transformations with high regio-, chemo-, and enantioselectivity under mild and cost-effective conditions, while DES serve as a robust green medium for modulating and directing reaction pathways to attain the desired product.[3]

Here, we present our extensive experience in the field of biocatalysis in DES, emphasizing the interplay between enzymatic activity and stability in these solvents, and how this interplay influences the fate of DES ability to enhance biocatalytic processes. Specifically, we will focus on our recent research on behavior of oxidoreductive (various dehydrogenases) and hydrolytic enzymes (lipase and lysozyme) in DES and developing Quantitative Structure-Activity Relationship (QSAR) models to predict enzyme's behavior in DES.

[1] Hansen, B.B.; Spittle, S.; Chen, B.; Poe, D.; Zhang, Y.; Klein, J.M. et al, Deep Eutectic Solvents: A Review of Fundamentals and Applications. *Chemical Reviews* **2021**, *10*, 1232-1285.
[2] Abranches, D.O. and Coutinho J.A.P., Everything You Wanted to Know about Deep Eutectic Solvents but Were Afraid to Be Told. Annual Review of Chemical and Biomolecular Engineering **2023**, *14*, 141-163.
[3] Panić, M.; Cvjetko Bubalo, M.; Radojčić Redovniković, I., Designing a Biocatalytic Process Involving Deep Eutectic Solvents. Journal of chemical technology and biotechnology **2021**, *96*, 14-30.

LECTURE # 3
TWO-STEP BIOCATALYTIC SYNTHESIS OF (1*S*)-NOR(PSEUDO)EPHEDRINE USING IMMOBILIZED ENZYMES

Stefania Patti[1]; Ilaria Magrini Alunno[1,2]; Riccardo Semproli[2]; Davide Tessaro[3]; Daniela Monti[1]; Sergio Riva[1]; Daniela Ubiali[2] and Erica Elisa Ferrandi[1*]

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[2] *University of Pavia, via Taramelli 12, Pavia, Italy*
[3] *Politecnico di Milano, P.za L. Da Vinci 32, Milan, Italy*
* *Corresponding author: erica.ferrandi@scitec.cnr.it*

Nor(pseudo)ephedrines (N(P)Es) are vicinal amino alcohols with sympathomimetic activity which have a plethora of applications, especially in the pharmaceutical field. Isolation of N(P)Es in high yield and optical purity from natural sources is not viable on a large scale. Similarly, chemical asymmetric syntheses of NEs and NPEs involve tedious multi-step procedures, thus making it cumbersome to achieve high yield and optical purity simultaneously.[1]

The synthesis of 1(*S*)-N(P)Es (**Figure 1**) and their analogues by a two-step biocatalytic cascade has been recently proposed by some of the Authors. This cascade consists of an acyloin condensation catalysed by the (*S*)-selective acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) from *Bacillus licheniformis*, followed by the transamination mediated by an amine transaminase ((*S*)- or (*R*)-ATA).[2] The use of free enzymes, however, presents several limitations in terms of enzyme reuse and downstream process, especially in the perspective of a large-scale application.

In this study, we successfully immobilized both Ao:DCPIP OR and ATAs and used them in the biosynthetic cascade to N(P)Es. Immobilization yield, activity recovery, and stability of the immobilized enzymes both during the reaction (enzyme recycling) and under storage (shelf-life) were assessed. The two-step biotransformation for the synthesis of (1*S*,2*S*)-NPE and (1*S*,2*R*)-NE (**Figure 1**) using immobilized enzymes was performed on a semi-preparative scale with good to excellent yield and optical purity.[3]

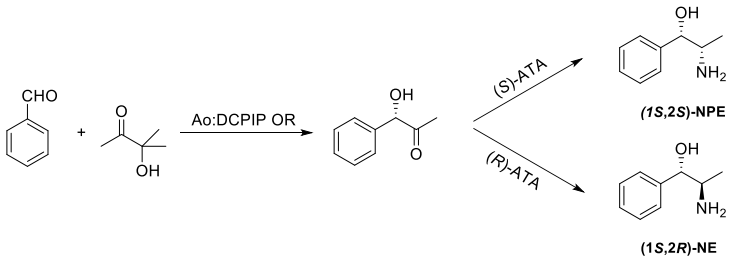

Figure 1: Biocatalysed stereoselective synthesis of (1*S*)-nor(pseudo)ephedrine.

[1] Sehl, T.; Maugeri, Z.; Rother, D., Multi-step synthesis strategies towards 1,2-amino alcohols with special emphasis on phenylpropanolamines. *Journal of Molecular Catalysis B: Enzymatic* **2015**, *114*, 65-71.
[2] Fracchiolla, N.; Patti, S.; Sangalli, F.; Monti, D.; Presini, F.; Giovannini, P. P.; Parmeggiani, F.; Brenna, E.; Tessaro, D.; Ferrandi, E. E., Insight into the stereoselective synthesis of (1*S*)-nor(pseudo)ephedrine analogues by a two-steps biocatalytic process. *ChemCatChem* **2024**, *16*, e202301199.
[3] Patti, S.; Magrini Alunno, I.; Semproli, R.; Tessaro, D.; Monti, D.; Riva, S.; Ubiali, D.; Ferrandi, E. E. Two-step biocatalytic synthesis of (1*S*)-nor(pseudo)ephedrine using immobilized enzymes. Manuscript in preparation.

LECTURE # 4
FROM DAIRY WASTE TO BIO-BASED SURFACTANTS: ENZYMATIC SYNTHESIS OF *n*-BUTYL 6-*O*-ACYL-GALACTOSIDES

Semproli R.[1*]; Robescu M.S.[1]; Sangiorgio S.[2]; Donzella S.[3]; Pargoletti E.[2]; Rabuffetti M.[2]; Bavaro T.[1]; Cappelletti G.[2]; Compagno C.[3]; Molinari F.[3]; Speranza G.[2] and Ubiali D.[1]

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Cheese whey permeate (CWP), a lactose-rich aqueous byproduct of dairy industry, can be exploited as feedstock for the synthesis of fine and commodity chemicals. In the BioSurf project, an integrated bioprocess was set-up for the synthesis of Sugar Fatty Acid Esters (SFAE), valuable non-ionic surfactants.[1] CWP was used both for enzymatic biotransformations and for growing oleaginous yeasts to produce the galactose-based "head" and the lipid "tail" of SFAE, respectively.

Upon the proof-of-concept with commercial lactose,[1] CWP was used to synthesize 1-butyl β-D-galactopyranoside (**1**) through a transglycosylation reaction catalyzed by the immobilized β-galactosidase from *Aspergillus oryzae* in a ternary system (buffer/1-BuOH/acetone) at 30 °C (yield: 45%). At the same time, microbial lipids were produced by growing oleaginous yeasts on CWP (cell lipid content: 45%).[2] Microbial lipids were submitted to acid-catalyzed extraction and *in situ* transesterification giving fatty acid ethyl esters (FAEE, **2a-e**, yield: 80%). 1-Butyl β-D-galactopyranoside (**1**) and FAEE (**2a-e**) were reacted by a *solvent-free* transesterification catalyzed by the immobilized lipase from *Thermomyces lanuginosus* affording a mixture of *n*-butyl 6-*O*-acyl-galactosides (**3a-e**, yield: 40%). The products, characterized by NMR, will be tested for their surfactant properties.

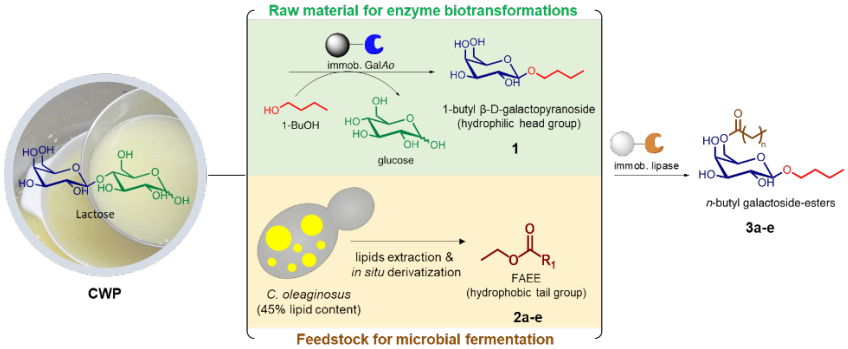

Figure 1: Integrated bioprocess for the upcycling of CWP into bio-based surfactants

[1] Semproli, R.; Robescu, M.S.; Sangiorgio, S.; Pargoletti, E.; Bavaro, T.; Rabuffetti, M.; Cappelletti, G.; Speranza, G.; Ubiali, D., From lactose to alkyl galactoside fatty acid esters as non-ionic biosurfactants: A two-step enzymatic approach to cheese whey valorization. *ChemPlusChem* **2023**, *88*, e202200331

[2] Donzella, S.; Fumagalli, A.; Arioli, S.; Pellegrino, L.; D'Incecco, P.; Molinari, F.; Speranza, G.; Ubiali, D; Robescu, M.S.; Compagno, C., Recycling food waste and saving water: Optimization of the fermentation processes from cheese whey permeate to yeast oil. *Fermentation* **2022**, *8,* 341

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LECTURE # 5
CHALLENGES IN ANALYSIS FOR BIOCATALYSIS IN NON CONVENTIONAL MEDIA

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Biocatalysis in nonconventional media offers higher solubility of many industrially important molecules and therefore has garnered a lot of interest[1]. The higher solubility of the substrates and/or products enables high productivity and also eases the downstream processing, thereby helping the process achieve the tight industrial economic targets[2]. Lipases, peroxygenases and oxidoreductases have been used with non conventional media successfully[3]. The selection of solvents is based on the solubility of the substrates/products and the activity/stablity of the enzymes. In many cases, the difference between the solubility of the substrate and the product necessitates the use of biphasic aqueous-solvent systems which complicates the analysis of the reaction system. This makes establishing mass balances or monitoring reaction progress quite difficult. These challenges present themselvesitself during sampling (representation/location), evaporative losses during the reaction, losses during extraction etc. Addressing these challenges is of utmost importance to enable best use of these solvent systems in biocatalysis.

[1] Mortem, M. C. H. van Schie; Jan-Dirk, Spöring; Marco, Bocola; Pablo, Domínguez de María; and Dorte. Rother., Applied biocatalysis beyond just buffers – from aqueous to unconventional media. Options and guidelines," *Green Chemistry* ***2021***, 23, 3191–3206.

[2] John. M. Woodley, Accelerating the implementation of biocatalysis in industry, *Applied Microbiology and Biotechnology*, **2019**, 103, 4733-4739.

[3] Pablo D. de María and F. Hollmann, On the (Un)greenness of biocatalysis: Some challenging figures and some promising options, *Frontiers in Microbiology*, **2015,** 6.

LECTURE # 6
PYRROLOPYRIMIDINES AND PURINES AS CSF1R INHIBITORS

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The colony-stimulating factor 1 receptor (CSF1R) is a tyrosine kinase expressed among others on monocytes, macrophages, microglial cells, and bone resorbing osteoclasts. Activation of CSF1R and downstream signalling, is necessary for normal function of these cell types. However, in some diseases, overexpression of CSF-1 and/or elevated activity of CSF1R cause a misbalance of the immune cell phenotypes. Thus, CSF1R inhibitors might be relevant in cancers, CNS and bone diseases. We have investigated pyrrolopurimidines and purines as CSF1R inhibitors [1-4]. An X-ray co-crystal structure of one of the front runner inhibitors allowed for rational design and indentification of a high number of compounds being more active than the reference drug PLX3397 in enzymatic studies. Hurdles for progressiong these inhibitors into lead compounds will be discussed, alongside their kinase activation state preferences.

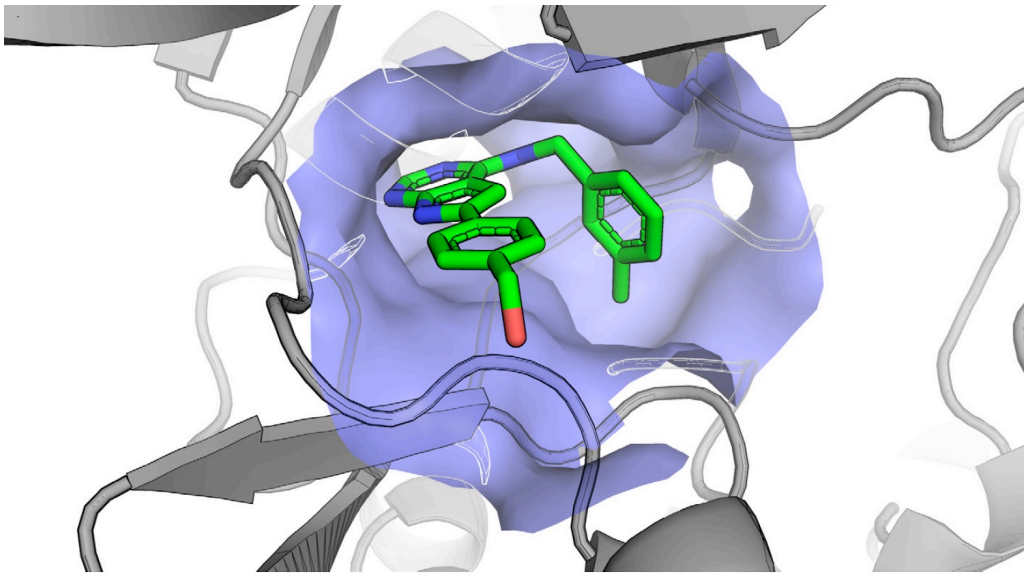

Figure 1: Front view of CSF1R inhibitor co-crystallesed with the CSF1R kinase domain.

[1] Bjørnstad, F.; Havik, S.; Aarhus, T. I.; Mahdi, I.; Unger, A.; Habenberger, P.; Degenhart, C.; Eickhoff, J.; Klebl, B. M.; Sundby, E.; Hoff, B. H., Pyrrolopyrimidine based CSF1R inhibitors: attempted departure from fatland. *European Journal of Medicinal Chemistry* **2024**, 265, 116053.
[2] Aarhus, T. I.; Teksum, V.; Unger, A.; Habenberger, P.; Wolf, A.; Eickhoff, J.; Klebl, B.; Wolowczyk, C.; Bjørkøy, G.; Sundby, E.; Hoff, B. H., Negishi Cross-Coupling in the preparation of benzyl substituted pyrrolo[2,3-d]pyrimidine based CSF1R inhibitors. *European Journal of Organic Chemistry*. **2023** *26*, e202300052,
[3] Aarhus, T. I.; Eickhoff, J.; Klebl, B.; Unger, A.; Boros, J.; Choidas, A.; Zischinsky, M.-L.; Wolowczyk, C.; Bjørkøy, G.; Sundby, E.; Hoff, B. H., A highly selective purine-based inhibitor of CSF1R potently inhibits osteoclast differentiation. *European Journal of Medicinal Chemistry* **2023**, *255*, 115344.
[4] Aarhus, T. I.; Bjørnstad, F.; Wolowczyk, C.; Larsen, K. U.; Rognstad, L.; Leithaug, T.; Unger, A.; Habenberger, P.; Wolf, A.; Bjørkøy, G.; Pridans, C.; Eickhoff, J.; Klebl, B.; Hoff, B. H.; Sundby, E., Synthesis and development of highly selective pyrrolo[2,3-d]pyrimidine CSF1R inhibitors targeting the autoinhibited form. *Journal of medicinal Chemistry* **2023**, *66*, 6959–6980.

LECTURE # 7
LIPASE CATALYZED SYNTHESIS OF ENANTIOPURE PRECURSORS AND DERIVATIVES FOR β-BLOCKERS PRACTOLOL, PINDOLOL AND CARTEOLOL

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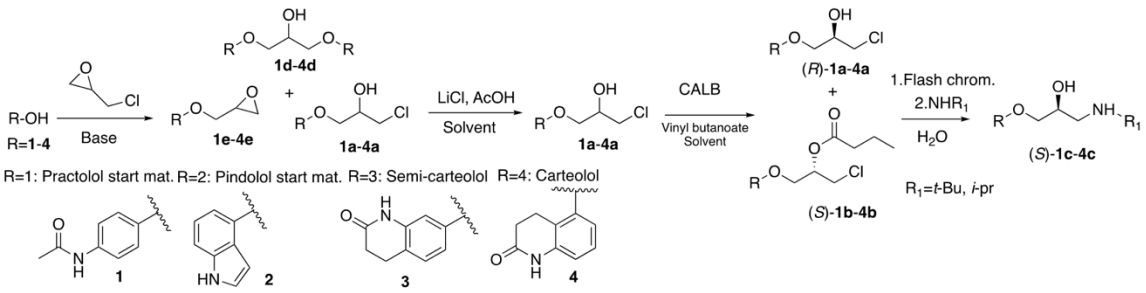
High blood pressure (hypertension) and heart failure are severe global health problems. In Norway, approximately 30% of all deaths are caused by cardiovascular diseases [1]. Approximately 26 million people worldwide live with heart failure, and approximately seven million deaths are caused by hypertension annually [2]. Heart failure cannot be cured, but may be treated by medications in order to increase quality of life for the patients. Both heritage (genetic) and lifestyle may lead to increased risk of cardiovascular diseases. Risk factors for cardiovascular diseases, such as reduced activity, eating more sugar and salt, increased stress, obesity and overweight, may be reasons for the increasing health problems worldwide [3].

Chlorohydrins as building blocks for several β-blockers have been synthesized in high enantiomeric purity by chemo-enzymatic methods. The yield of the chlorohydrins increased by the use of catalytic amount of base. The reason for this was found to be the reduced formation of the dimeric by-products compared to the use of higher concentration of the base. An overall reduction of reagents and reaction time was also obtained compared to our previously reported data of similar compounds.



Scheme 1. Building blocks (*R*)-**1a-4a** synthesized in 92–97% *ee* for use in synthesis of the *S*-enantiomers of the β-blockers practolol, pindolol and derivatives of carteolol ((*S*)-**1c-4c**).

[1]. Causes of Death, Norway. 2019. Available online: http://statistikkbank.fhi.no/dar/
[2]. Ponikowski, P.; Anker, S.D.; AlHabib, K.F.; Cowie, M.R.; Force, T.L.; Hu, S.; Jaarsma, T.; Krum, H.; Rastogi, V.; Rohde, L.E.; et al. Heart failure: Preventing disease and death worldwide. *ESCHeart Fail.* **2014**, *1*, 4-25.
[3]. Klouman, M.; Åsberg, A.; Wideröe, T.-E. The blood pressure level in a Norwegian population-The significance of inheritance and lifestyle. *Tidsskr. Nor. Laegeforen.* **2011**, *131*, 1185-1189.

LECTURE # 8
BIOCATALYTIC DECARBOXYLATION OF AMINO ACIDS IN DEEP EUTECTIC SOLVENT USING IMMOBILIZED DECARBOXYLASES

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Decarboxylases play a crucial role in catalyzing the removal of carboxyl groups from amino acids, offering a sustainable and selective approach for the sustainable production of primary amines. In this study, we explore the application of L-valine decarboxylase from *Streptomyces viridifaciens* [1] and L-tyrosine decarboxylase from *Lactobacillus brevis* [2] in the biocatalytic decarboxylation of amino acids. We use a deep eutectic solvent as the reaction medium, introducing a challenging yet advantageous condition for catalysis, particularly beneficial for downstream processes.

The biocatalysts are immobilized to enhance stability and facilitate their reuse in subsequent reactions. The immobilization strategy consider the physical entrapment of whole cells in alginate matrix, as the most cost-effective options for the primary amines production. The immobilization strategy ensures prolonged enzymatic activity and simplifies the separation of the biocatalyst from the reaction mixture. Our methodology aligns with the principles of green chemistry, promoting efficiency while minimizing environmental impact.

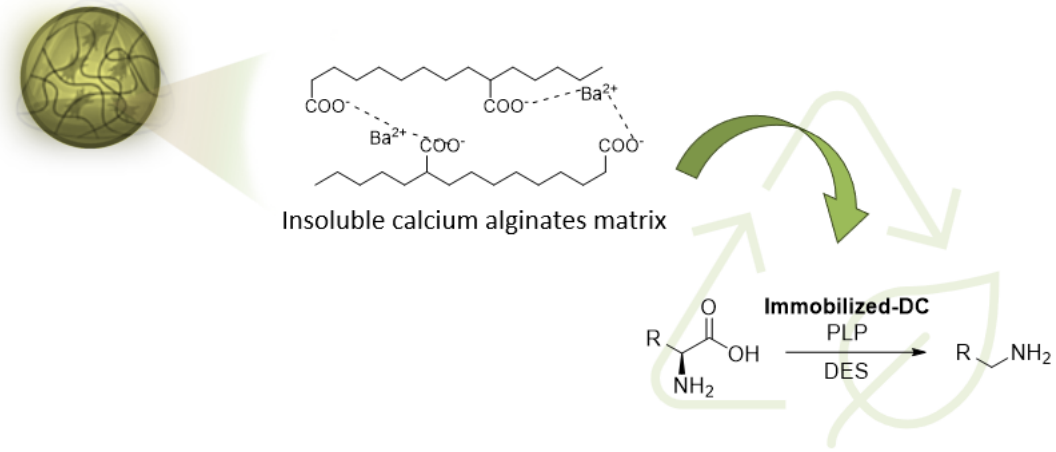

Figure 1: Biocatalytic decarboxylation of natural amino acids in deep eutectic solvent *via* alginate beads.

[1] Kim, D.I., Chae, T.U., Kim, H.U. et al. Microbial production of multiple short-chain primary amines via retrobiosynthesis. Nat Commun 12, 173 (2021). doi.org/10.1038/s41467-020-20423-6.
[2] Zhang, Kai, and Ye Ni. "Tyrosine decarboxylase from Lactobacillus brevis: soluble expression and characterization." Protein expression and purification 94 (2014): 33-39. doi.org/10.1016/j.pep.2013.10.018.

LECTURE # 9
AMPHIPHILE-STABILIZED EMULSIONS AND IONIC LIQUIDS AS MEDIA IN HALOPEROXIDASE BIOCATALYSIS

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Chloroperoxidase from *Caldariomyces fumago* represents an exquisite biocatalyst with a broad range of applications and reactivity modes. In addition to the native halogenation properties,[1] *Cf*CPO also exhibits peroxygenase activity and thus can serve as oxygenating catalyst in a number of synthetic transformations.[2]

As part of our ongoing campaign to identify biological mediators with abilities to address synthetically important reactions beyond the biosynthetic repertoire,[3] an enzymatic halocyclization was developed that allows for the conversion of allenic alcohols and carboxylates to brominated O-heterocycles. Interestingly, the use of micellar reaction media, either stabilized by short non-ionic PEG amphiphiles or by cetyl trimethylammonium bromide proved to be of critical importance to achieve high yields in the enzymatic halogenations.[4] The multiphasic reaction media furthermore enabled direct catalytic cascades where the enzymatically generated vinyl bromides can be cross-coupled by means of palladium catalysts in Suzuki- and Sonogashira-type C-C bond-forming reactions.[5] Beyond its synthetic potential, *Cf*CPO was also investigated as biocatalyst for the decontamination of chemical warfare agents. Here, sulfur mustard-type β-chlorosulfides are readily absorbed by choline acetate that effectively trap the volatile toxins in the non-volatile ionic liquid, where *Cf*CPO (and other biocatalysts) rapidy convert the mustard simulants to less hazardous metabolites.

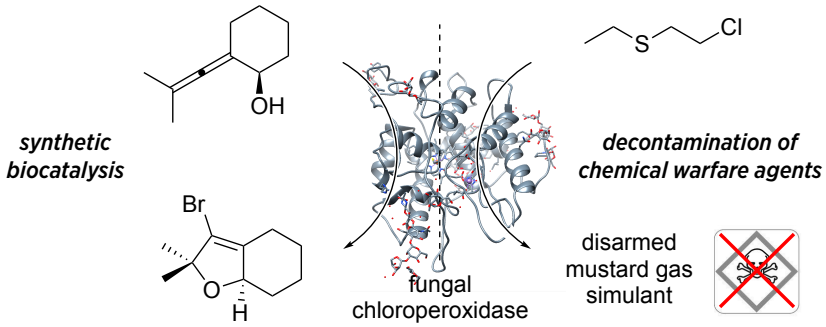

Figure 1: Fungal chloroperoxidase catalyzes bromocyclizations in micellar reaction systems (left) as well as oxidative degradation of chemical warfare agents in choline acetate.

[1] (a) Vázquez-Duhalt, Ayala, Márquez-Rocha, *Phytochem.* **2001**, *58*, 929-933; (b) Getrey, Krieg, Hollmann, Schrader, Holtmann, *Green Chem.* **2014**, *16*, 1104-1108.
[2] (a) Thiel, Doknić, Deska, *Nature Commun.* **2014**, *5*, 5278; (b) Bormann, Gomez Baraibar, Ni, Holtmann, Hollmann, *Catal. Sci. Technol.* **2015**, *5*, 2038-2052.
[3] (a) Kiefer, Liu, Gummerer, Jäger, Deska, Angew. Chem. Int. Ed. 2023, 62, e202301178; (b) Jäger, Haase, Koschorreck, Urlacher, Deska, *Angew. Chem. Int. Ed.* **2023**, *62*, e202213671; (c) Liu, Rolfes, Björklund, Deska, *ACS Catal.*, **2023**, *13*, 7256; (d) Liu, Merten, Deska, *Angew. Chem. Int. Ed.* **2018**, *57*, 12151.
[4] (a) Naapuri, Rolfes, Keil, Sapu, Deska, *Green Chem.* **2017**, *19*, 447; (b) Naapuri, Wagner, Hollmann, Deska, *Chem. Open* **2022**, *11*, e202100236
[5] Naapuri, Åberg, Palomo, Deska, *ChemCatChem* **2020**, *13*, 763-769.

LECTURE # 10
ENZYMATIC DEPOLYMERIZATION OF POLYURETHANES THROUGH ALCOLYSIS

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[2] *CIC biomaGUNE, San Sebastián, Spain*
[3] *CIC biomaGUNE, San Sebastián, Spain*
* *Corresponding author: rmoro@cicbiomagune.es*

Polyurethanes represent a crucial category of polymers with extensive applications across various industries. Unlike more commonly recycled polymers such as PET, polyurethane recycling presents greater challenges due to its unique chemical characteristics and molecular structure.[1] Hydrolysis is the widest approach for chemical depolymerization of PUs, however, this process is high-energy demanding, cannot recover the isocyanates and the quality of the recovered polyols is low. [2] Methanolysis has been proposed as an approach instead of hydrolysis of PUs.[3] Methanolysis allows obtaining the corresponding polyol and diurethane compounds which can be directly recycled into virgin PUs, increasing the sustainability and the efficiecy of the plastic degradation.[3]

Here we aim the **enzymatic methanolysis of PUs** in neat methanol, which has never been projected, to perform a new sustainable and bioorganic process in the plastic depolymerization area. To do so, we have screened **6 lipases**, **3 proteases**, **2 Cutinases** commercial preparations in the methanolsysis of ***p*-nitrophenylbenzyl carbamate** as model substrate in solvent free conditions. **ROL** (*Rhizopus oryzae lipase*) and **TLL** (*Thermomyces lanuginosus lipase*) are the most efficient enzymes studied for this kind of reaction. Additionally, we evaluate the reaction perfomance with different alkyl chain alcohols (figure 1). In these conditions, **ethanol** offered the best results, for which was selected for further experiments. After **24 hours** of reaction, we have achieved the totally convension of *p*-nitrophenol carbamate into corresponding ethylbenzyl carbamate and *p*-nitrophenol.

The best enzymes under the optimal conditions were confirmed in the alcoholysis of dicarbamates and low-molecular weight polyurethanes.

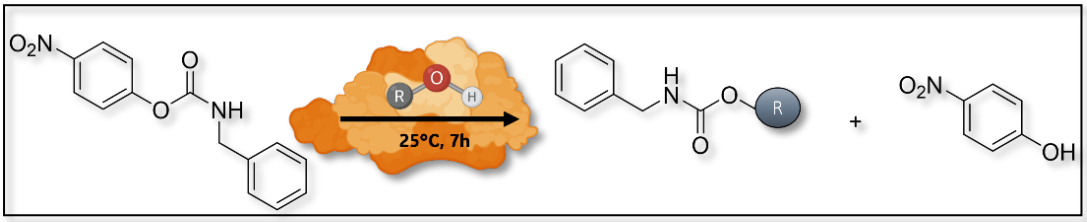

Figure 1: Alcoholysis reaction with lipases.

[1]. H. Sardon, A. P Dove, Plastics recycling with a difference, *Science* **2018**, 360(6387), 380-381.
[2]. A. Kemona, M. Piotrowska, Polyurethane Recycling and Disposal: Methods and Prospects, *Polymers* **2020**, *12*(8), 1752.
[3]. N. Asahi, K. Sakai, N. Kumagai, T. Nakanishi, K. Hata, S. Katoh, T. Moriyoshi, Methanolysis investigation of commercially available polyurethane foam, *Polym. Degrad. Stab* **2004**, 86 (1), 147-151

POSTER ABSTRACTS

POSTER # 1
CHEMO-ENZYMATIC SYNTHESIS OF ENANTIOPURE VASODILATOR (–)-NEBIVOLOL

Sara Aasen[1*], Aleksander Damlien Eide[1], Petter Daleng[1], Katja Liv Benestad[1] and Elisabeth Egholm Jacobsen[1]

[1:]*Department of chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway*
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The American Heart Association reported in March 2019 their update on heart diseases and stroke statistics. The report states that the prevalence of high blood pressure in the United States between 2013-2016 was 46% of the total population at ages 20 years and older. It was the cause of death for 82,735 Americans in 2016 and costed the American society approximately $ 55.9 billion in the period 2014-2015 [1].

A class of drugs that have been used both in cardiovascular and non-cardiovascular treatment are the β-adrenergic blocking agents (betablockers). This class of drugs were introduced into clinical medicine in the early 1960's, and since then, the findings of these drugs have led to a discovery of the importance of the sympathetic nervous system in the pathophysiology of a wide variety of cardiovascular and non-cardiovascular disorders [2]. We have for some time developed efficient chemo-enzymatic protocols for synthesis of several betablockers [3a-e]. Here, we present our protocol for the enantiopure vasodilator (–)-Nebivolol, fig. 1. Due to the four stereogenic centra in the molecule there are 16 possible isomers when no catalysts is used in the synthesis. However, due to symmetry, there are 10 possible isomers. We have achieved to produce the (*R,S,S,S*)-nebivolol or (–)-nebivolol.

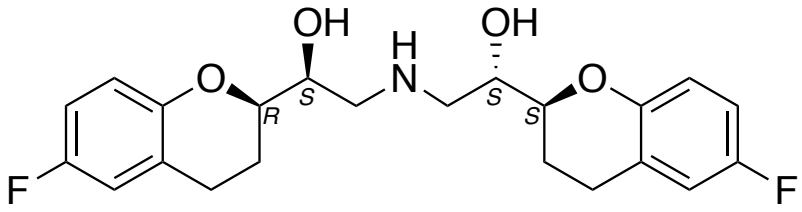

Figure 1. (–)-Nebivolol, (*R,S,S,S*)-nebivolol.

[1]. E. J. Benjamin, *et al. Circulation* **2019**, *139* (10), e56-e528.
[2]. J. W. Black and B. N. C. Prichard. *Br. Med. Bull.* **1973**, *29*, (2), 163
[3]. a) M. A. Gundersen *et al., Catalysts,* **2021,** *11,* 503**.** b) S. H. Troøyen, *et al. Catalysts* **2022**, *12*, 980. c) S. H. Troøyen and E. E. Jacobsen. *Catalysts,* **2022**, *12*, 1645. d) L. H. Y. Bocquin and E. E. Jacobsen. *Catalysts* 2023, *13*, 54. e) P. L. Bøckmann and E. E. Jacobsen. *Topics in Catalysis*, **2024**, 1-9.

POSTER # 2
FATTY ACID PHOTODECARBOXYLASE FOR DROP IN BIOFUELS SYNTHESIS IN DEEP EUTECTIC SOLVENTS

Aikaterini Margariti[1*]; Ningning Zhang[1], Robert Kourist[2], Amin Bornadel[3] and Selin Kara[1, 4]

[1]*Institute of Technical Chemistry, Leibniz University Hannover, Hannover, Germany*
[2]*Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria*
[3]*Johnson Matthey, Cambridge, United Kingdom*
[4] *Department of Biological and Chemical Engineering, Aarhus University, Aarhus, Denmark*
**Corresponding author: katerina.margariti@iftc.uni-hannover.de*

Climate change represents a serious and pressing challenge for our planet. The main cause is the rising levels of greenhouse gases in the atmosphere, such as CO_2. Efforts to decrease CO_2 emissions have escalated, focusing on swiftly transitioning from fossil fuels to low-carbon and carbon-neutral technologies, with biofuels being one proposed solution[1]. "Drop-in biofuels" are biofuels that can be used as a direct substitute for traditional fossil fuels. Enzymes can be employed as a method for synthesizing biofuels[2]. Fatty acid photodecarboxylase (FAP, E.C. 4.1.1.106) is a photoenzyme discovered in 2017[3]. In the presence of blue light, FAP can generate drop-in biofuels from fatty acids (**Figure 1**). The advantages of FAP include the lack of oxygen requirement, there is no overall change in the oxidation state of the reaction and it simply functions by using light. However, addressing the insolubility of FAP's substrates presents a challenge, as most fatty acids do not dissolve well in aqueous solutions. Therefore, alternative, non-conventional media are essential for effective FAP reactions. Biocatalysis is having now transitioning from traditional reaction media to greener solvents. An alternative solution is the deep eutectic solvents (DESs)[4]. A variety of biotransformations have been established with various enzymes in DESs and DES-water mixtures for the synthesis of high-valued chemicals[5]. Several FAP variants were assessed for their activity in DESs with varying water content.

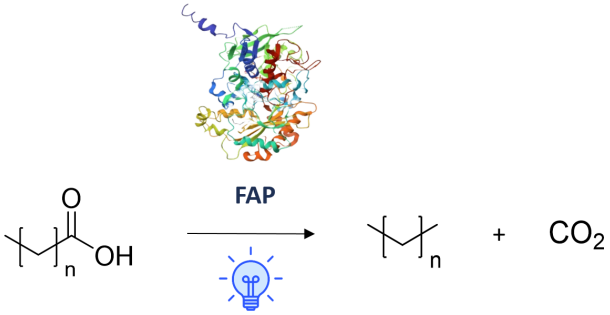

***Figure 1**: Fatty acid photodecarboxylase (FAP)-catalysed synthesis of blended biofuels in presence of blue light (PDB entry: 5NCC). The drop in biofuels that can be synthesized are: Gasoline (C4–C12), Jet fuel (C8–C16), and Diesel (C9–C23) (Image obtained by flaticon.com/free-icons)*

1. Rodionova, M. V., Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., & Allakhverdiev, S. I., *Int. J. Hydrogen Energy*, **2017**, 42(12), 8450-8461.
2. Santner, P., Chanquia, S. N., Petrovai, N., Benfeldt, F. V., Kara, S., & Eser, B. E. *EFB Bioeconomy Journal,* **2023**, 3, 100049.
3. Sorigué, D., Légeret, B., Cuiné, S., Blangy, S., Moulin, S., Billon, E., Beisson, F. *Science,* **2017**, 357 (6354), 903-907.
4. Pätzold, M., Siebenhaller, S., Kara, S., Liese, A., Syldatk, C., & Holtmann, D., *Trends in biotechnology,* **2019,** 37(9), 943-959.
5. Zhang, N., Domínguez de María, P., & Kara, S. *Catalysts*, **2024**, 14(1), 84.

POSTER # 3
CARBOHYDRATE BASED DEEP EUTECTIC SOLVENT SYSTEMS FOR ENZYMATIC CONVERSIONS

Andrea Puglisi[1]; André Pick[2*]

[1,2]*CASCAT GmbH, Straubing, Germany*
** Corresponding author: pick@cascat.de*

Biocatalysis has become an important strategy for converting bio-renewable materials into valuable industrial products, largely as a result of the advances in biotechnology and molecular biology over the past two decades [1]. Although water is the prevalent solvent in many biocatalytic processes, its high polarity is often incompatible with the hydrophobicity of many target substrates, limiting substrate solubility reducing the efficiency of the process [2]. To overcome this challenge, a sustainable non-aqueous media approach to biocatalysis is required [3,4]. In recent years, deep eutectic solvents (DESs) have been raising a lot of attention and have been evaluated as solvents in different biocatalytic reactions [5]. With the aim of defining a biocatalytic system based on an enzyme-catalysed reaction with carbohydrates as substrate, the choice of solvent plays a key role in the efficiency of the overall process; although sugars are soluble in water, unlike most organic solvents [6], the reaction cannot be carried out well in an aqueous environment, as it favours hydrolysis [7]. Among the different applications of DESs for biotransformation, one of the possible approaches is based on the 2-in-1 concept, where one of the components of the DES (HBA or HBD) acts as both reaction medium and substrate [8,6], allowing processes with high substrate conversion, high atomic efficiency, allowing enzymatic reactions under solvent-free conditions, giving the possibility to develop bioprocesses with high substrate loads resulting in high product concentrations.

[1] Sheldon, R. A., & Woodley, J. M. Role of Biocatalysis in Sustainable Chemistry. *Chemical Reviews* ***2018*** *(Vol. 118, Issue 2, pp. 801–838). American Chemical Society.*

[2] Domínguez de María, P. Biocatalysis, sustainability, and industrial applications: Show me the metrics. ***2021*** *In Current Opinion in Green and Sustainable Chemistry (Vol. 31). Elsevier B.V.*

[3] Holtmann, D. and Hollmann, F. Is water the best solvent for biocatalysis? ***2021****, Molecular Catalysis, 517.*

[4] Kumar, A.; Dhar, K.; Kanwar, S. S.; & Arora, P. K. Lipase catalysis in organic solvents: Advantages and applications. *In Biological Procedures Online (Vol. 18, Issue 1)* ***2016****. BioMed Central Ltd*

[5] Nieto, S; Villa, Rocio; Donaire, A and Lozano, P. Chapter 2 – Non conventional biocatalysis: from organic solvents to green solvents. *(Foundations and frontiers in enzymology pp 23-55).* ***2022*** *Biocatalysis in green solvents*

[6] Pöhnlein, M.; Ulrich, J.; Kirschhöfer, F.; Nusser, M., Muhle-Goll, C.; Kannengiesser, B.; Brenner-Weiß, G.; Luy, B.; Liese A., Syldatk, C., and Hausmann, R. Lipase-catalyzed synthesis of glucose-6-O-hexanoate in deep eutectic solvents. *European Journal of Lipid Science and Technology,* ***2015****, 117(2), 161–166.*

[7] Siebenhaller, S., Muhle-Goll, C., Luy, B., Kirschhöfer, F., Brenner-Weiss, G., Hiller, E., Günther, M., Rupp, S., Zibek, S., & Syldatk, C. Sustainable enzymatic synthesis of glycolipids in a deep eutectic solvent system. *Journal of Molecular Catalysis B: Enzymatic,* ***2016*** *133, S281–S287.*

[8] Pätzold, M.; Siebenhaller, S.; Kara, S.; Liese, A.; Syldatk, C.; Holtmann, D. Deep Eutectic Solvents as Efficient Solvents in Biocatalysis. *In Trends in Biotechnology* ***2019*** *(Vol. 37, Issue 9, pp. 943–959).*

POSTER # 4
THE IMPACT OF DIFFERENT SOLVENTS ON LACCASE-MEDIATED TYROSOL ACETATE DIMERIZATION

Borut Šketa[1], Larisa Fabjan[1], Francesca Annunziata[1,2], Janez Košmrlj[1], Lucia Tamborini[2], Andrea Pinto[3], Polona Žnidaršič-Plazl[1]*

[1] *University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia*
[2] *Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milan, Italy*
[3] *Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Celoria, 2, 20133 Milan, Italy*
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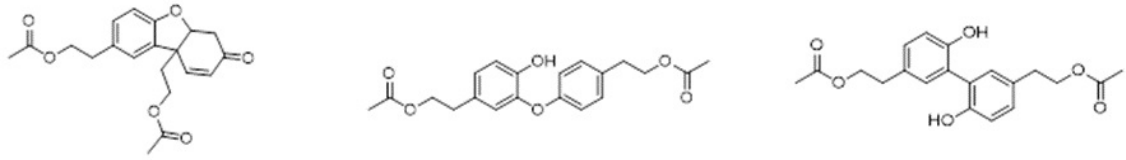
Building upon prior research demonstrating the dimerization of tyrosol acetate to compounds **1** and **2** (Figure 1), catalyzed by *Trametes versicolor* laccase in the presence of oxygen in an ethyl acetate/acetate buffer system[1], we investigated the same reaction within diverse mono and biphasic solvent systems. These systems comprised acetate buffer with acetone, propylene glycol, acetonitrile, and hydrophilic deep eutectic solvent (DES) betaine:propylene glycol, as well as a biphasic solvent comprising buffer and hydrophobic DES menthol:octanoic acid.



1 **2** **3**

Figure 1: Structures of the dimers obtained in various solvent systems: Pummerer ketone (**1**), diphenylether dimer (**2**), and 1,1`-dityrosol-8,8`-diacetate (**3**).

Product profiles from batch reactions varied across solvent systems and were characterised *via* NMR, revealing the presence of three dimers: Pummerer ketone **1**, diphenylether **2**, and 1,1´-dityrosol-8,8´-diacetate **3** (Figure 1), which were previously identified for their bioactive properties [1-3]. In the biphasic system comprising ethyl acetate and acetate buffer (20 mM, pH 3), compound **3** emerged as the main product, while compound **2** was in most cases present in traces. Notably, both the substrate and all resultant products exhibited complete solubility in this solvent system. However, upon transitioning the reaction into a monophasic solvent system with the hydrophylic DES or propylene glycol, a distinctive phenomenon unfolded: while the substrate remained soluble, the products precipitated, forming a white powder. Subsequent NMR analysis of the precipitate generated from the reaction conducted in an 80% buffer and 20% DES betaine:propylene glycol revealed the exclusive presence of compound **1**. The reaction outcomes underscore the potential for directing this biocatalytic reaction towards specific product through the strategic selection of the solvent system.

[1] C. Navarra, C. Goodwin, S. Burton, B. Danieli, and S. Riva, "Laccase-mediated oxidation of phenolic derivatives," *J. Mol. Catal. B Enzym.*, vol. 65, no. 1–4, pp. 52–57, Aug. 2010.
[2] L. Pulvirenti, V. Muccilli, N. Cardullo, C. Spatafora, and C. Tringali, "Chemoenzymatic synthesis and α-glucosidase inhibitory activity of dimeric neolignans inspired by magnolol," *J. Nat. Prod.*, vol. 80, no. 5, pp. 1648–1657, May 2017.
[3] Y.-J. Lee, Y. M. Lee, C.-K. Lee, J. K. Jung, S. B. Han, and J. T. Hong, "Therapeutic applications of compounds in the Magnolia family," *Pharmacol. Ther.*, vol. 130, no. 2, pp. 157–176, May 2011.

POSTER # 5
COMPARATIVE ASSESSMENT OF COMMERCIAL LIPASE ACTIVITY ON AQUEOUS MEDIA AND CHOLINE CHLORIDE/UREA DEEP EUTECTIC SOLVENT

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The use of promiscuous hydrolytic enzymes (different from natural chitinases, chitosanases orhexosaminidases) for processing chitin or chitosan, leading to oligomers with different biological activities, is well-known [1]. Thus, the use of commercial, accessible, and cheap lipases would render a more convenient way to obtain chitooligomers possessing a plethora of biological activities [2]. Conversely, the use of Deep Eutectic Solvents (DESs) for facilitating the solubilization of the starting material would be highly recommended.

In order to stablish a rational comparison of the behaviour of the lipases for the desired modification on chitosan, it is mandatory a previous quantification of the standard activity of these enzymes. For this reason, a comparative study of the hydrolysis of classical chromogenic substrates (*p*-nitrophenyl acetate *p*NPAc and *p*-nitrophenyl butyrate *p*NPBu) catalysed by different commercial lipases in Tris/HCl buffer pH=7 and in reline (choline chloride/urea,1:2) containing 20% water has been carried out.

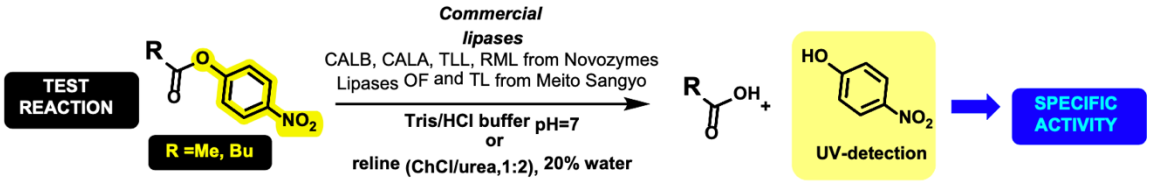

Figure 1: Schematic representation of the assessment of enzymatic activity

As expected, activities in aqueous media are higher (up to 2 orders of magnitude in some cases) using *p*NPBu versus *p*NPAc. Considering the use of DES, results are showing that the behaviour of the lipases is different, as already reported for other lipases [3], as for some enzymes (lipase TL from Meito Sangyo, CALB and lipase TLL from Novozymes), the activity is higher in reline/water (more than 300% for the first one), while for the other lipases tested, the activity notably diminished compared to the aqueous conditions. Therefore, the more active lipases will be the candidates for assessing the promiscuous chitosanolysis both in water and DESs.

[1] Poshina, D.N.; Raik, S.V.; Poshin, A.N.; Skorik, Y.A. Accessibility of chitin and chitosan in enzymatic hydrolysis: A review. *Polym Degrad Stabil*. **2018**; 127-129.
[2] Aranaz, I.; Alcántara, A.R; Civera, M.C; Arias, C,; Elorza, B.; Heras, A; Acosta, N. Chitosan: An Overview of Its Properties and Applications. *Polymers*. **2021**; 13; 3256
[3] Kim, S.H.; Park, S; Yu, H.; Kim, J. H.; Kim, H. J.; Yang, Y. H.; Kim, Y. H.; Kim, K. J.; Kan, E.; Lee, S. H. Effect of deep eutectic solvent mixtures on lipase activity and stability. *J Mol Catal B-Enzym*. **2016**; 128; 65-72.

POSTER # 6
SYNTHESIS OF BRIDGED METAL OXO PHOSPHONATESS AND MO_x FONCTIONALIZED NANOPARTICULES BY NON-HYDROLYTIC SOL-GEL ROUTE.

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In a context of environmental considerations, the decontamination of polluted effluents and the recycling of used materials are of major importance today. New processes are emerging to recycle these materials, but they are often expensive, synthesized in too many steps, or lack selectivity or even stability at low/high pH.

Our study has led to the synthesis of phosphonate-based organic-inorganic hybrid materials [1-2], which are stable and resistant to harsh pH conditions, acting primarily as absorbents and can also be used as supported catalysts.

In this poster, we present our methodology and recent results concerning these original materials (**Fig.1**).

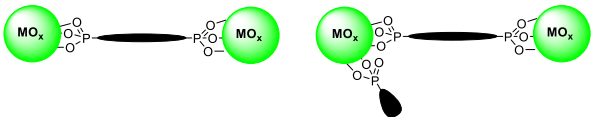

Fig. 1: Schematic representation of metal oxide nanoparticles bridged by bisphosphonate.

These hybrids can be synthesized, from relatively simple and affordable precursors, in 1 or 2 steps, either by functionalizing MOx nanoparticles with phosphonates or by a non-hydrolytic sol-gel (NHSG) route involving the reaction in a non-aqueous medium of precursors with organic oxygen donors instead of water [3] (**Fig.2**).

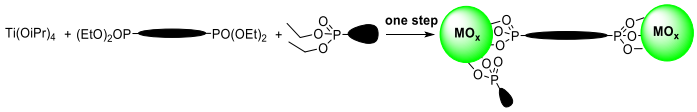

Fig. 2: Route in one step for the synthesis of bridged metal oxo phosphonates.

The number of chelating groups in this type of nanomaterial is relatively high, and they boast interesting textural properties (specific surface area up to 800 $m^2.g^{-1}$, pore volume >1$cm^3.g^{-1}$ and pore diameter between 3 and 20 nm) and remarkable stability in acidic or basic aqueous solutions (pH 1 to 12).

[1] Wang Y. *et al*, Water-stable, nonsiliceous hybrid materials with tunable porosity and functionality: bridged titania-bisphosphonates, *Chem. Mater.*, **2020**, 32 (7), 2910.
[2] Wang Y. *et al*, One-step nonhydrolytic sol–gel synthesis of mesoporous TiO_2 phosphonate hybrid materials, *Beilstein J. of Nanotech.*, **2019**, *10*, 356.
[3] P. H. Mutin *et al*, Nonhydrolytic Processing of Oxide-Based Materials: Simple Routes to Control Homogeneity, Morphology, and Nanostructure*, Chem. Mater.*, **2009**, 21, 582.

POSTER # 7
REDUCTASE-DRIVEN COFACTOR REGENERATION CASCADE IN DEEP EUTECTIC SOLVENTS

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After the pioneering work from Gorke *et al.* (2008)[1], deep eutectic solvents (DESs) have been gaining wide interest as promising non-conventional media for biocatalysis. Due to the tunability and the straightforward preparation, DESs could be potentially applied in all the steps of a biocatalytic process, ranging from upstream to downstream[2]. Furthermore, DESs could be rationally designed as 2-in-1 systems, acting as (co)solvents and substrates at the same time, due to the high variety of compounds that could be used for the generation of the hydrogen bond network[2]. This approach can be used to increase the efficiency and the atom economy of oxidoreductase (EC1) catalysis. The use of oxidoreductases in DESs, such as alcohol dehydrogenases (ADHs) or Baeyer-Villiger monooxygenases (BVMOs), shows promising results for the synthesis of value-added products[3]. To overcome the limitation given by the high cost of the nicotinamide cofactors (NAD(P)H), 2-in-1 DESs could be used for cofactor regeneration cascades[4]. Following this route, a process involving reducing agents as hydrogen bond donors, and Thioredoxin-1/Thioredoxin reductase (Trx-1/TR) from *Thermus thermophilus*, was developed, based on the work from Zhang et al. (2022)[5]. The DES system allows a higher substrate loading than in aqueous medium for the main reaction, and at the same time reduces the Trx-1/TR system allowing the reduction of the $NADP^+$ generated by the ADH or the BVMO (**Fig.1**). The choline chloride-dithiothreitol (ChCl:DTT, 1:2) DES, already generated in a work from Damilano et al. (2022)[6], was prepared and characterized for biotechnological applications. The results from water activity and viscosity analyses, along with the preliminary assessments involving the TR/Trx-1 system, suggest a possible use of this solvent for cofactor regeneration.

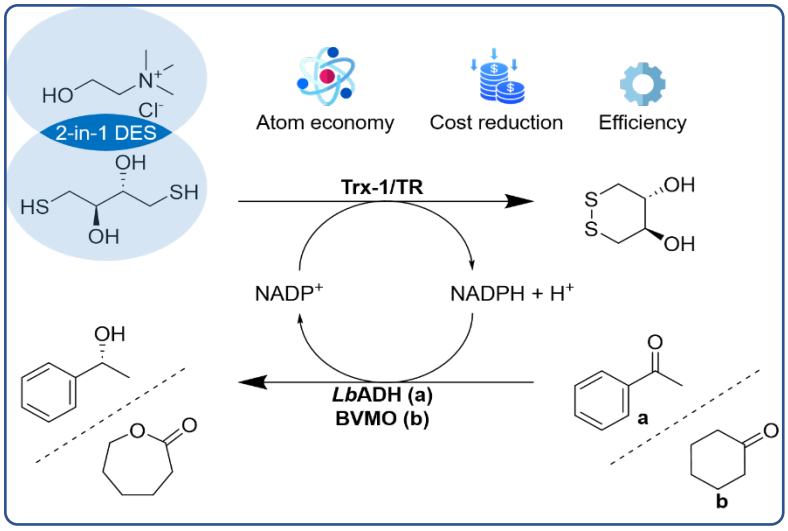

Figure 1. Choline chloride-dithiothreitol DES is used in the cofactor regeneration reaction catalyzed by the Trx-1/TR system, yielding NADPH used by the **a**) *Lactobacillus brevis* alcohol dehydrogenase (*Lb*ADH) for the conversion of acetophenone in (*R*)-phenylethanol or **b**) Baeyer-Villiger Monoxygenase (BVMO) to convert cyclohexanone to ε-caprolactone.

[1] Gorke, J. T., Srienc, F., Kazlauskas, R. J., *Chem. Commun.* **2008**, *10*, 1235-1237.
[2] Pätzold, M., Siebenhaller, S., Kara, S., Liese, A., Syldatk, C., Holtmann, D., *Trends Biotechnol.* **2019,** *37*(9), 943-959.
[3] Gotor-Fernández, V., Paul, C. E., J. *Biotechnol.* **2019,** *293*, 24-35.
[4] Chanquia, S. N., Huang, L., García Liñares, G., Domínguez de María, P., Kara, S., *Catalysts* **2020,** *10*(9), 1013.
[5] Zhang, N., Müller, B., Kirkeby, T. Ø., Kara, S., Loderer, C., *ChemCatChem* **2022**, *14*(7), e202101625.
[6] Damilano, G., Laitinen, A., Willberg-Keyriläinen, P., Lavonen, T., Häkkinen, R., Dehaen, W., Kuutti L., *RSC Adv.* **2022**, *10*(39), 23484-23490.

POSTER # 8
DROPZYMES: PROTEIN CONDENSATES AS NEW UNCONVENTIONAL MEDIA

Greta Bianchi[1*]; Noemi Destefani[1]; Marco Mangiagalli[1] and Stefania Brocca[1]

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In the dynamic field of biocatalysis in non-conventional media, a novel approach emerges from the utilization of proteinaceous biomolecular condensates derived from liquid-liquid phase separation (LLPS). LLPS is a physical process of demixing that often involves macromolecules, namely proteins and nucleic acids. When a critical concentration is reached, phase separation occurs leading to dense, droplet-shaped condensates. Intrinsically disordered proteins (IDPs) are majorly involved in LLPS, due to their low complexity sequences favoring intermolecular interactions. In cellular environment, protein condensation regulates biocatalysis, encapsulating enzymes at high concentrations and including/excluding their substrates. Our investigation focuses on the design of synthetic protein condensates, capable of including enzymes of interest, hereafter referred to as "dropzymes".

We explore the potential of dropzymes as microenvironments where to perform enzymatic reactions, with the aim of shielding enzymes from possible inhibitory effects induced by organic solvents, particularly the well-known competitive inhibition of methanol on the CALB enzyme[1].Within this context, we engineered an IDP tag enriched in charged amino acids[2] to encapsulate CALB *via* a co-condensation strategy. These condensates, being electrostatically-driven, are expected to exhibit stability to organic solvents while remaining sufficiently permeable to substrate molecules.
Notably, our study unveils the robustness of electrostatically-driven condensates in the presence of 20% methanol. Preliminary findings suggest that our dropzymes are permeable to CALB substrate 4-methylumbelliferyl acetate, which is indeed hydrolyzed, underscoring their versatility as biocatalysts. Ongoing investigations will be crucial to assess whether CALB entrapment within condensates is effective in protecting the enzyme against methanol-induced inhibition.

This research not only paves the way for designing robust biocatalytic systems and enzymatic cascades, but also contribute elucidating the benefits conferred by condensates in cellular environments.

[1] Marco Mangiagalli, Henrique Carvalho, Antonino Natalello, Valerio Ferrario, Matteo L. Pennati, Alberto Barbiroli, Marina Lotti, Juergen Pleiss & Stefania Brocca (2020) Diverse effects of aqueous polar co-solvents on *Candida antarctica lipase B*, International Journal of Biological Macromolecules, 1;150:930-940. DOI: 10.1016/j.ijbiomac.2020.02.145

[2] Greta Bianchi, Marco Mangiagalli, Diletta Ami, Junaid Ahmed, Silvia Lombardi, Sonia Longhi, Antonino Natalello, Peter Tompa & Stefania Brocca (2024) Condensation of the N-terminal domain of human topoisomerase 1 is driven by electrostatic interactions and tuned by its charge distribution. International Journal of Biological Macromolecules, 254(Pt 1):127754. DOI: 10.1016/j.ijbiomac.2023.127754

POSTER # 9
DEVELOPMENT OF A CONTINUOUS ENZYMATIC REDUCTION USING DEEP EUTECTIC SOLVENT IN A MICROBIOREACTOR

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Continuous biocatalytic processes in miniaturized reactors facilitate process intensification, enhanced process control, the development of multi-step synthetic pathways with compartmentalized catalysts, and seamless integration with downstream processes, thereby enabling efficient utilization of biocatalysts across various industries.[1] Deep eutectic solvents (DESs) are emerging as promising alternatives to conventional solvents in enzyme-catalyzed processes due to their economic viability and environmentally benign nature.[2]
This research aimed to establish a sustainable alcohol dehydrogenase (ADH)-catalyzed reduction of acetophenone to a chiral pharmaceutical intermediate, (*S*)-1-phenylethanol, along with the regeneration of the NAD^+ cofactor (Figure 1a), utilizing rationally designed DES. Condidering substrate solubility, enzyme stability, and activity, a DES formulation comprising betaine:ethylene glycol with 50% w/w water was identified as the optimal reaction medium. The Michaelis-Menten reaction kinetics was assesed in batch and the reaction was then transferred to a continuous flow system. To enhance the stability of ADH in the flow system, cross-linked enzyme aggregate (CLEA) particles were generated using a microfluidic system.[3] A microbioreactor system between two plates, featuring CLEA-ADH immobilized on the membrane surface (Figures 1b,c) was manufactured utilizing 3D printing A validated 2D model-based design approach, incorporating time-scale analysis (TSA) with characteristic times, was employed to identify the optimal process parameters and operational conditions.[4]

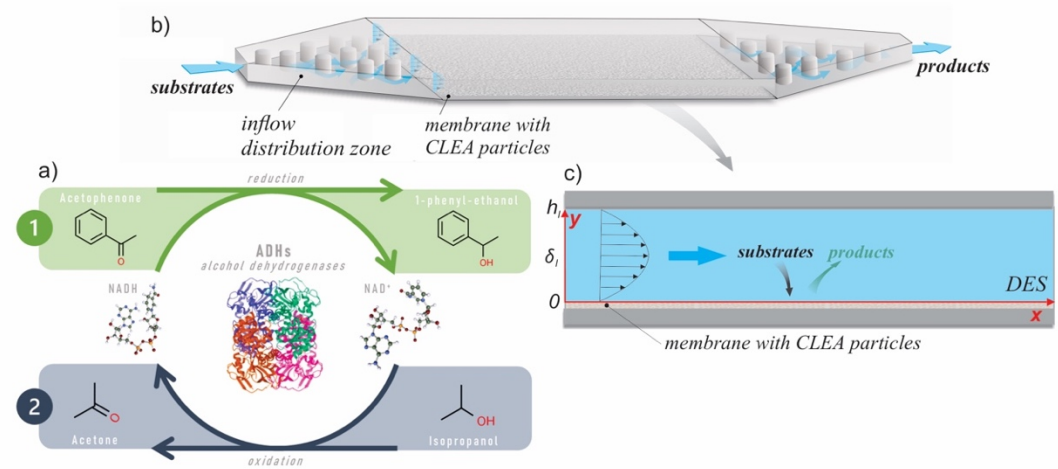

Figure 1: a) Biotransformation reaction scheme. b) scheme of a microbioreactor between two plates with CLEA-ADH immobilized on the membrane surface; c) physical domains od 2D mathematical model.

[1] Žnidaršič-Plazl, P. Biocatalytic process intensification via efficient biocatalyst immobilization, miniaturization, and process integration. *Curr. Opin. Green Sustain. Chem*., 2021, 32, 100546,
[2] Zhang, N.; Domínguez de María, P.;, S., Biocatalysis for the synthesis of active pharmaceutical ingredients in deep eutectic solvents: state-of-the-art and prospects. *Catalysts* **2024**, *14*, 84.
[3] Menegatti, T.; Lavrič, Ž.; Žnidaršič-Plazl, P. Microfluidics-based preparation of cross-linked enzyme aggregates. WO2023175002A1
[4] Menegatti, T.; Plazl, I.; Žnidaršič-Plazl, P. Model-based design of continuous biotransformation in a microscale bioreactor with yeast cells immobilized in a hydrogel film. *Chem. Eng. J.,* **2024,** 483, 149317.

POSTER # 10
MEDIUM ENGINEERING FOR THE ADJUSTMENT OF THE PRODUCT PROFILE OF ENZYMATICALLY HYDROLYZED PET

Tobias Heinks[1], Katrin Hofmann[2], Igor Gamm[3], Simon Last[1], Luise Blach[3], Ren Wei[4], Uwe Bornscheuer[4], Christof Hamel[3], Jan von Langermann[1,*]

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[4] *University of Greifswald, Institute of Biochemistry, Greifswald, Germany*
* *Corresponding author: jan.langermann@ovgu.de*

Polyethylene terephthalate (PET) is one of the most widely used synthetic polymers for several applications, but it is also highly problematic as it does not decompose naturally and thus tends to accumulate as plastic waste in the environment.[1] Chemical depolymerization techniques of PET have been established that lead to terephthalic acid (TPA) and ethylene glycol (EG), which are then used to re-synthesize PET.[2] Since the discovery of PET-hydrolyzing enzymes, more environmentally friendly biocatalytic approaches are targeted to provide an alternative to these conventional processes.[3] In contrast to the present practice of completely hydrolyzing PET to TPA and EG, this study targets the selective depolymerization to MHET that could be used as an alternative monomer for resynthesis (Fig. 1).

A total of 13 selected enzymes were investigated in the first screening round for their ability to hydrolyze the model substrate 3(PET). Based on the results, three enzymes were selected that produced either more BHET, MHET or TPA through medium engineering. These enzymes were analyzed under different solvent-based reaction conditions and the results transferred to the more realistic substrate Nano-PET, highlighting the practicability.

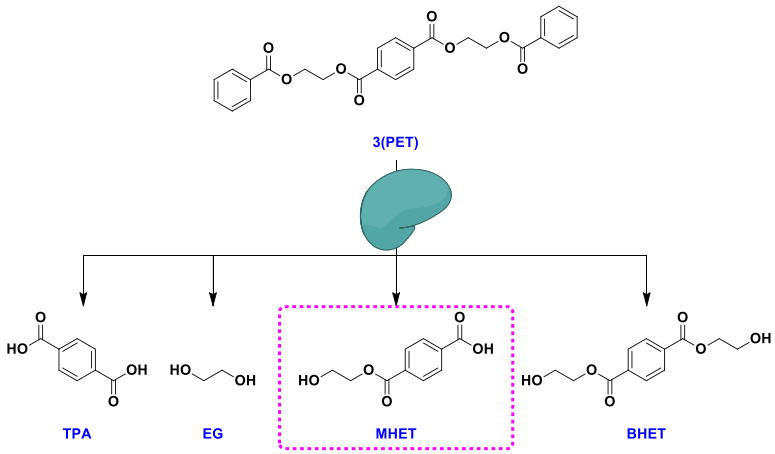

Figure 1: Medium-engineering directs depolymerization of the model substrate 3(PET) to MHET and BHET

[1] Tournier V., Duquesne S., Guillamot F., *et al.* Enzymes' Power for Plastics Degradation. *Chemical Reviews* **2023**, *123*, 5612-5701.
[2] Wei, R., Tiso, T., Bertling, J. *et al.* Possibilities and limitations of biotechnological plastic degradation and recycling. *Nature Catalysis* **2020**, *3*, 867–871.
[3] Arnal G., Anglade J., Gavalda S., *et al.* Assessment of Four Engineered PET Degrading Enzymes Considering Large-Scale Industrial Applications. *ACS Catalysis* **2023**, *13*, 13156-13166.

POSTER # 11
HALOPEROXIDASES IN IONIC LIQUIDS FOR DEGRADATION OF SULFUR MUSTARD

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Chloroperoxidase from a marine fungus *Caldariomyces fumago* (*Cf*CPO) has emerged as a promising biological catalyst for the degradation of the chemical warfare agent (CWA) sulfur mustard (HD) via sulfoxidation into the relatively harmless bis(2-chloroethyl) sulfoxide (HDO) using hydrogen peroxide as a mild oxidant.[1,2] The enzyme belongs in the haloperoxidases, predominantly known as halogenation biocatalysts but exhibiting oxygenative, peroxidase and catalase activities as well. The versatile heme-dependent *Cf*CPO suffers from instability towards the native substrate hydrogen peroxide as the major drawback. Nowadays, more resilient vanadium-dependent haloperoxidases are becoming the preferred choice due to good tolerance towards outside factors, with especially fungal chloroperoxidase from *Curvularia inaequalis* (*Ci*VCPO) featuring in a range of applications.[3]

In the current study, we investigated both *Cf*CPO and *Ci*VCPO for their robustness and applicability in the presence of quaternary ammonium salts, in preparation for introduction of methods incorporating the haloperoxidases in aqueous ionic liquid (IL) mixtures for the decontamination of CWAs such as sulfur mustard. The current focus in the research is the transposition of the findings of these investigations to develop practical applications for sulfoxidations of 2-chloroethyl ethyl sulfide (CEES) and 2-chloroethyl phenyl sulfide (CEPS), the common simulants for the dangerous sulfur mustard. Here are presented the results of biocatalytic sulfur mustard simulant degradations in mainly choline-based aqueous media.

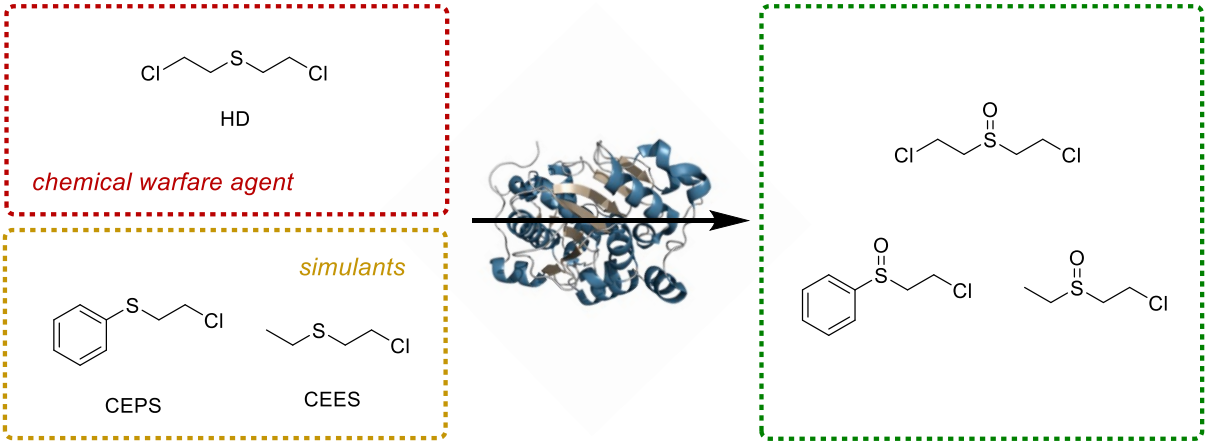

Figure 1: Biocatalytic sulfoxidation of sulfur mustard and simulants.

[1] He, Q.; Song, J.; Li, H.; Zhao, B.; Zhang, Y.; Wang, N.; Liu, B.; Chen, J.; Nie, Z.; Liang, T.; Zhong, W., Chloroperoxidase-catalyzed oxidative degradation of sulfur mustard. *Ecotoxicology and Environmental Safety* **2021**, *225*, 112715.

[2] Popiel, S.; Nawała, J., Detoxification of sulfur mustard by enzyme-catalyzed oxidation using chloroperoxidase. *Enzyme and Microbial Technology* **2013**, *53*, 295-301.

[3] Wever, R.; Renirie, R.; Hollmann. F., Vanadium Chloroperoxidases as Versatile Biocatalysts. In *Vanadium Catalysis*; Sutradhar, M.; da Silva, J. A. L.; Pombeiro, A. J. L., Eds.; The Royal Society of Chemistry, 2021, pp 548-563.

POSTER # 12
PIPETTE SHOW: AN OPEN SOURCE WEB APPLICATION TO SUPPORT PIPETTING INTO MICROPLATES

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Despite increasing automation, manual pipetting remains a daily important task in life science laboratories. However, the creation of an efficient work plan is often time-consuming, and its completion is error-prone. Here, we present Pipette Show[1], a free Vue.js based application that optimizes the generation of an efficient work plan for pipetting into microplates and supports its reliable execution by visual guidance. The basis forms a graphical web interface with a module for building workflows as well as a module displaying the information for each pipetting step by illuminating wells of microplates placed on a tablet.

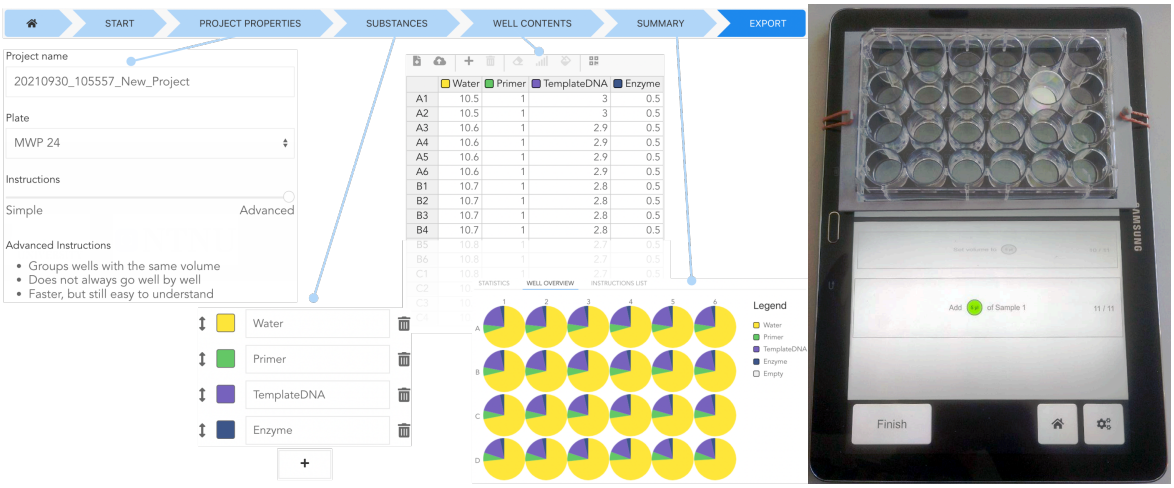

Figure 1: Build (left) and Show (right) modules of pipette show. MTP plates upt to 384-wells can be placed on a tablet with 3d printed holders to light up the well into which to pipette next.

[1] Falk, J., Mendler, M., Kabisch, J., 2022. Pipette Show: An Open Source Web Application to Support Pipetting into Microplates. *ACS Synth. Biol.* 2022, 11, 2, 996-999.

POSTER # 13
EFFECT OF TRIMETHYLAMINE-N-OXIDE (TMAO) AND SORBITOL ON THE CATALYTIC ACTIVITY OF FORMATE DEHYDROGENASE

Julio A. Perez-Erviti[1*]; Yasser Almeida-Hernández[1]; Nicolas Gajardo-Parra[2]; Christoph Held[2]; Roland Winter[3]; Gabriele Sadowski[2] and Elsa Sanchez-Garcia[1]

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Formate dehydrogenase from *Candida boidinii* (*Cb*FDH) is a highly specific NAD^+-dependent oxidoreductase that catalyzes the reversible oxidation of formate to CO_2, coupled to the reduction of NAD^+ to NADH.[1] The main industrial potential of this enzyme derives from its ability to regenerate the expensive NADH with formate as a cost-effective source.[2] However, *Cb*FDH has several drawbacks regarding its moderate chemical and thermal stability, high production cost, and low enzymatic efficiency.[3] The addition of co-solvents such as Trimethylamine N-oxide (TMAO) and sorbitol provides a way of increasing the biocatalytic efficiency of enzymes for industrial applications.[4,5] We carried out extensive molecular dynamics (MD) simulations of *Cb*FDH in water and mixtures of water with TMAO or sorbitol to explore the effects of the co-solvents on the protein structure, solvation shells, and the hydration of the active site. Analysis of the MD simulations indicated that neither co-solvent induced substantial changes in the secondary structure or the protein conformation. However, analysis of the spatial density function and minimum distance distribution function revealed differential distribution of TMAO and sorbitol surrounding the protein. TMAO is excluded from the protein surface and moves to the bulk, whereas sorbitol accumulates closer to the protein. We also found differences in the transit of water molecules through the active site, which could explain the experimentally observed behavior of co-solvents on the catalytical activity of *Cb*FDH.

[1] Tishkov, V. I.; Popov, V. O. Catalytic mechanism and application of formate dehydrogenase. *Biochemistry (Moscow)* **2004**, *69*, 1252–1267.
[2] Tishkov, V. I.; Popov, V. O. Protein engineering of formate dehydrogenase. *Biomolecular Engineering* **2006**, *23*, 89–110.
[3] Schüte, H.; Flossdorf, J.; Sahm, H.; Kula, M. R. Purification and properties of formaldehyde dehydrogenase and formate dehydrogenase from *Candida boidinii*. *European Journal of Biochemistry* **1976**, *62*, 151–160.
[4] Iyer, P. V.; Ananthanarayan, L. Enzyme stability and stabilization—Aqueous and non-aqueous environment. Process Biochemistry 43, 1019–1032
[5] Reichardt, C.; Welton, *T. Solvents and Solvent Effects in Organic Chemistry*. 4th ed. **2011**, Wiley-VCH, Weinheim,

POSTER # 14
ARE OLEATE HYDRATASES STABLE IN DEEP EUTECTIC SOLVENTS?

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Hydroxy fatty acids (HFAs) are derivates of fatty acids (FAs) that hold remarkable industrial value. Due to special properties (high viscosity, reactivity, and degradability) are considered to be promising precursors of pharmaceuticals, lubricants, and a source of bio-based polymers[1,2]. HFAs are primarily obtained by chemical means. While chemical methods lack selectivity, are operated under harsh conditions, and require expensive starting materials, biocatalysis provides the advantages of excellent stereochemistry, mild operating conditions, and high substrate promiscuity. Thus, hydration of unsaturated FAs by oleate hydratases (Ohys) has been widely studied as an alternative approach for HFAs production. A great amount of effort has been put into process optimization together with fine-tuning the performance of Ohys[1,2]. Nevertheless, the hydrophobic nature of FAs still prevents achieving high substrate loadings for industrial purposes. In this context, the addition of co-solvents presents a solution for solubility issues. Deep eutectic solvents (DESs) have been replacing conventional organic solvents for their enhanced physical properties (melting temperatures, viscosity), bio-compatibility, and tunability[3]. Moreover, DESs have been proven to have stabilizing effects on enzymes, improving enantiomeric excess and even increasing the catalytic activity[3]. Recent findings indicate that this is as well the case for Ohys[4]. This work therefore presents a proof of concept study; aiming to explore the hydration of FAs in DESs for the production of value-added chemicals.

Acknowledgements:

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[1] Prem, S. *et al.* Towards an understanding of oleate hydratases and their application in industrial processes. *Microb. Cell Fact.* **2022**, 21.
[2] Zhang, Y., Eser, B. E., Kristensen, P. & Guo, Z. Fatty acid hydratase for value-added biotransformation: A review. *Chin. J. Chem. Eng.* **2020**, 28.
[3] Xu, P., Zheng, G.-W., Zong, M.-H., Li, N. & Lou, W.-Y. Recent progress on deep eutectic solvents in biocatalysis. *Bioresour. Bioprocess.* **2017**, 4.
[4] Zhou, P. *et al.* Effect of Deep Eutectic Mixtures in Hydroxylation of Fatty Acids: A Correlation between Water Activity and Thermostability of FA-HY1. *ACS Sustain. Chem. Eng.* **2024**, 12.

POSTER # 15
EFFICIENT AND SCALABLE ENZYME ENGINEERING BY COMPUTATIONAL DESIGN OF COMBINATORIAL MUTANT LIBRARIES IN AN ITERATIVE MANNER.

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Classical directed evolution is a powerful but often laborious method to improve enzyme properties, as it relies on iterative small steps in sequence space, typically leading to small incremental increases in performance (Fig. 1A).[1] Large steps in sequence space can be achieved by introducing multiple mutations in parallel, potentially enabling larger increases in activity and selectivity (Fig. 1B). However, such combinatorial libraries are huge, leading to massive screening efforts as most of the variants are non-functional.[2] For instance, the simultaneous randomization of an enzyme active site with twelve amino acids generates a mutant library with 4.1×10^{15} variants. Here, we report how *in silico* pre-screening of such large libraries can significantly improve the process of directed enzyme evolution when performed iteratively. In particular, we applied different computational tools[3,4] to identify potentially stable and functional protein sequences and thus condense large combinatorial mutant libraries to an experimentally trackable number of 50 to 4000 variants. This approach was used in an iterative manner to optimize *S*-adenosyl-l-methionine (SAM)-dependent methyltransferases (MTs) for regioselective methylation of *N*-heteroarenes (unpublished data). After three rounds of evolution, dozens of variants with more than 200-fold increase in activity were discovered. As high activities and selectivities could be determined for many substrates, this approach increases not only the speed but also the scale of a directed evolution campaign. The final variants contain up to eleven mutations in the first and second shell and build a small artificial enzyme family with high activity and selectivity on many different substrates.

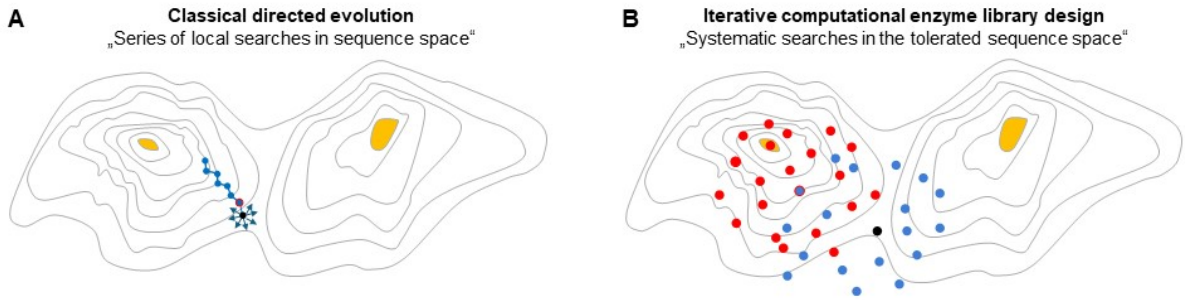

Figure 1: Classical directed evolution and iterative computational enzyme library design. Larger areas of the sequence space can be covered in fewer rounds of evolution using computational tools.

[1] Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. Engineering the Third Wave of Biocatalysis. Nature 2012, 485 (7397), 185–194.
[2] Goldsmith, M.; Tawfik, D. S. Enzyme Engineering by Targeted Libraries, 1st ed.; Elsevier Inc., 2013; Vol. 523.
[3] Khersonsky, O. et al. Automated Design of Efficient and Functionally Diverse Enzyme Repertoires. Mol. Cell 2018, 72 (1), 178-186.e5.
[4] Patsch, D.; Eichenberger, M.; Voss, M.; Bornscheuer, U. T.; Buller, R. M. LibGENiE – A Bioinformatic Pipeline for the Design of Information-Enriched Enzyme Libraries. Comput. Struct. Biotechnol. J. 2023, 21 (September), 4488–4496.

POSTER # 16
TOWARDS A GREEN SYNTHESIS OF PRECURSORS TO ORMELOXIFENE: A SELECTIVE ESTROGEN RECEPTOR MODULATOR

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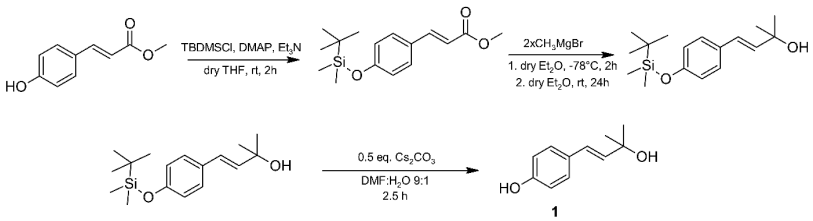
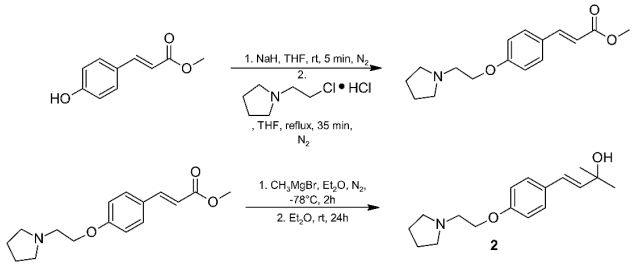
Ormeloxifene is the only female birth control pill that is non-hormonal, which means it exhibits almost none of the side effects commonly associated with other contraceptives [1][2]. As part of a novel pathway to ormeloxifene, two precursors thereof have been synthesized: 4-[(*1E*)-3-hydroxy-3-methylbut-1-en-1-yl]phenol (**1**) (Scheme 1), and (*E*)-2-methyl-4-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)but-3-en-2-ol (**2**) (Scheme 2).



Scheme 1: Synthesis of 4-[(*1E*)-3-hydroxy-3-methylbut-1-en-1-yl]phenol (**1**).



Scheme 2: Synthesis of (*E*)-2-methyl-4-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)but-3-en-2-ol (**2**).

Enzymatic epoxidation of **2** has been attempted using two genetically engineered peroxidases: Eucodis 012 and Eucodis 013. Compound **1** is an aryl allylic alcohol, meaning it has great potential as a future precursor to pharmaceutical compounds. (*E*)-2-Methyl-4-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)but-3-en-2-ol (**2**) is also an aryl allyl alcohol and thereby shares the same potential as compound **1** in addition to being presented as a novel compound.

[1]. Kumar, S.; Rai, R.; Agarwal, G. G.; Dwivedi, V.; Kumar, S.; Das, V. A Randomized, Double-Blind, Placebo-Controlled Trial of Ormeloxifene in Breast Pain and Nodularity. *Natl Med J India* **2013**, *26* (2), 69–74.
[2]. Brahmachari, S.; Bhagat, V.; Patil, P.; Vasuniya, V. Evaluating the Effect of Ormeloxifene on Multiple Fibroadenomas and Mastalgia. *J. Pharm. Bioallied Sci.* **2021**, *13* (Suppl 2), S1386.

POSTER # 17
BIOCATALYZED SYNTHESIS OF SUGAR-BASED POLYESTERS IN A CELLULOSE-DERIVED HIGH BOILING SOLVENT

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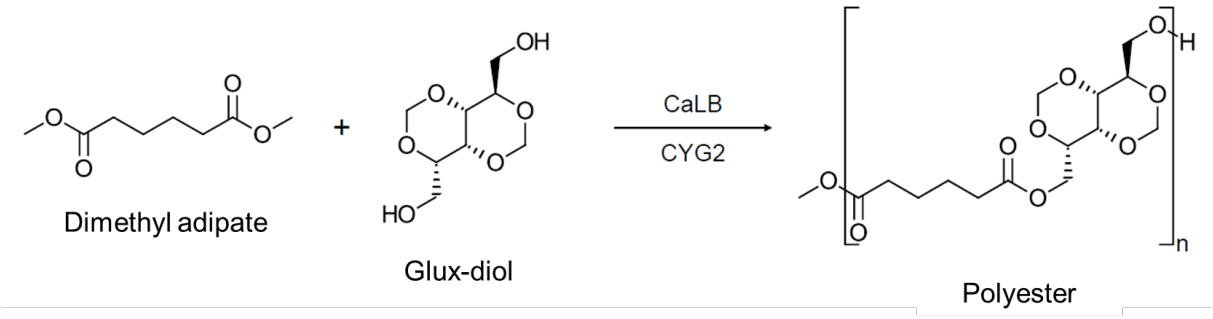
The rational and sustainable use of biomass and the utilization of waste streams as a resource are key concepts in bioeconomy. This study focuses on the enzymatic synthesis of sugar-based polyesters, utilizing glucose derived from the hydrolysis of lactose in cheese whey permeate, a secondary by-product in whey protein production.
Our experimental plan involved the enzymatic oxidation of glucose into D-Glucono-1,5-lactone, subsequently undergoing chemical modification to yield Glux-diol. Due to its rigid scaffold and two terminal primary hydroxyl groups, Glux-diol is a versatile chemical platform that can be used as building block for polymer synthesis. In this work, polyesters were synthesized employing Glux-diol and dimethyl adipate, utilising immobilized *Candida antarctica* lipase B (CALB, Novozym-435) as the catalyst and Cygnet as the solvent. Notably, the employement of Cygnet[1], a bio-based solvent derived from cellulose/levoglucosenone, adds substantial value to our process avoiding the use of the convetional petrol-derived diphenyl ether.
Overall, the chemo-enzymatic synthetic workflow developed in our study yielded a sugar-based oligoester having a number average molecular weight of 2000 Da and a dispersity of 1.52, as determined by gel permeation chromatography analysis. The characterization of this novel sugar-based polyester paves the way for the development of innovative bio-based materials, with new properties and functionalities. This work is expected to meet the increasing demand for sustainable products in the evolving landscape of polymer bioeconomy.



This work has been supported by Fondazione Cariplo, grant n° 2020-0838.

[1] Cicely M. Warne, Sami Fadlallah, Adrian C. Whitwood, James Sherwood, Louis M. M. Mouterde, Florent Allais, Georg M. Guebitz, Con R. McElroy & Alessandro Pellis (2023) Levoglucosenone-derived synthesis of bio-based solvents and polyesters, Green Chemistry Letters and Reviews, 16:1, 2154573, DOI: 10.1080/17518253.2022.2154573

POSTER # 18
RISE YOUR DIOLS WITH MARS

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Stereopure vicinal diols are important building blocks for fine chemicals and pharmaceutical compounds.[1] They can also serve as precursors for biofuels starting from biological resources. Due to the high selectivity of enzymes and their high reactivity under mild conditions, enzymes are potent catalysts for green chiral diol synthesis.[2] This synthesis usually takes place in an aqueous buffer, as most enzymes physiologically operate in an aqueous environment.
Nevertheless, many interesting substrates are hydrophobic, which leads to low substrate loads and therewith product concentration, if a second phase is to be avoided. Biocatalysis in an organic solvent, like cyclopentyl methyl ether (CPME), makes it possible to overcome this problem. To enable biocatalysis under these unconventional reaction conditions, enzymes are formulated as lyophilized whole cells. Due to a certain degree of protection of the entrapped enzymes in the remaining cell envelope high product yields can be achieved even under these harsher reaction conditions. In addition, the micro-aqueous reaction system (MARS) is a convenient environment to combine enzymatic and chemical steps for dioxolane synthesis [3] (Figure 1) in one reaction environment and additionally facilitates product purification.[4]

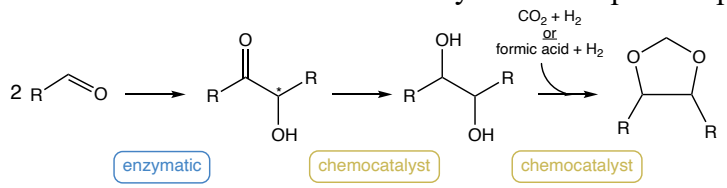

Figure 1: Reaction scheme for the production of cyclic acetal starting from aliphatic aldehyde.

Switching from an aqueous buffer to an organic solvent enabled increased product concentration when starting with more hydrophobic, long-chain substrates (Table 1).

Table 1:Diol concentrations in [mM] in aqueous buffer and organic solvent.

	aqueous buffer	organic solvent
2,3-butanediol	27.18	14.59
3,4-hexanediol	56	63
4,5-octanediol	22.67	34.44

In addition, the concentrations of the intermediate 2-hydroxy ketones, butyroin and valeroin, were also increased from 84 mM and 80 mM to 959 mM and 828 mM, respectively. In addition, the conversion was also increased from 84 % to 95 % for butyroin and from 80 % to 83 % for valeroin. Yet, process optimization to increase the diol concentration is still being investigated.

[1] Zhang, J. *et al.* Characterization of four diol dehydrogenases for enantioselective synthesis of chiral vicinal diols. *Chin J Chem Eng* **47**, 145–154 (2022).
[2] Illanes, A. Enzyme Biocatalysis. (Springer Netherlands, 2008).
[3] Spöring, J. *et al.* Effective Production of Selected Dioxolanes by Sequential Bio- and Chemocatalysis Enabled by Adapted Solvent Switching. *ChemSusChem* (2022) doi:10.1002/cssc.202201981.
[4] van Schie, M. M. C. H., Spöring, J.-D., Bocola, M., Domínguez de María, P. & Rother, D. Applied biocatalysis beyond just buffers – from aqueous to unconventional media. Options and guidelines. *Green Chemistry* **23**, 3191–3206 (2021).

POSTER # 19
ENZYMATIC SORBITYL LAURATE PRODUCTION IN DISSOLVED AND NEAT SYSTEMS UNDER CONVENTIONAL AND MICROWAVE HEATING.

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Glycolipids such as sugar alcohol esters have been demonstrated relevant for numerous applications across various domains of specialty. However, many challenges and limitations remain such as extensive time of production and relatively low productivities amongst others which have to be solved in order to strengthen their biocatalytical production for industrial applications. Therefore, combinations of two heating methods (conventional and microwave) and three solvent systems (organic solvents, DES and neat) were evaluated for the intensification of sorbityl laurate production, as a model biocatalyzed reaction using Novozym 435®. By increasing the reaction temperature from 50°C to 90°C, space time yield and product yield were considerably enhanced for reactions in DES and the organic solvent 2M2B, irrespective of the heating method (conventional or microwave heating). However, positive effects in 2M2B were more pronounced with conventional heating, as 98% of conversion yield was reached within 90 minutes at 90°C equating thus to a nearly four-fold increase in performance yielding 117.96 ± 3.64 g/(L.h) productivity.[1]

With DES, overall yield and space-time yield were lower with both heating methods. However, the effects of microwave heating where more pronounced than in 2M2B. A 7-fold increase in space time yield at 50°C was observed and a 16-fold increase at 90°C when microwave was used instead of conventional heating. Furthermore, microwave irradiation enabled the usage of a neat, solvent free system, representing an initial proof of concept with productivities of up to 13.34 ± 2.34 g/(L.h). Thus, a space-time yield greater than 10 g/(L.h) could be achieved and thus the process can be considered as economically interesting for production of bulk chemicals. However, yields of the solvent-free reaction, as well as the reaction in DES need to be improved even further.

The space-time yield is a widely used metric determining the capital costs and energy requirements to achieve a given productivity. For the production of bulk chemicals the threshold for the space-time yield to achieve a feasible process is 10 g/(L.h). Regarding the results of this study, 10times higher space-time yields than the threshold were achieved for the synthesis of sorbitly laurate in 2M2B. This is complemented by a yield of 98%. This metric yield indicative for the impact of raw material costs has a threshold for bulk chemicals of 95%. The purification costs are normally estimated using the product concentrations. Under the optimized conditions of this study, product precipitation occurs which is of great advantage. In summary, these three process metrics indicate therefore a promising intensification of SL production in 2M2B with respect to feasibility of an industrial process.

[1] Delavault A., Opochenska O., Schönrock S., Hollenbach R., Ochsenreither K., Syldatk S., Intensification of enzymatic sorbityl laurate production in dissolved and neat systems under conventional and microwave heating. *ACS Omega* **2024,** accepted manuscript.

POSTER # 20
ENZYMATIC DEPOLYMERIZATION OF POLYURETHANES THROUGH ALCOLYSIS

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Polyurethanes represent a crucial category of polymers with extensive applications across various industries. Unlike more commonly recycled polymers such as PET, polyurethane recycling presents greater challenges due to its unique chemical characteristics and molecular structure.[1] Hydrolysis is the widest approach for chemical depolymerization of PUs, however, this process is high-energy demanding, cannot recover the isocyanates and the quality of the recovered polyols is low. [2] Methanolysis has been proposed as an approach instead of hydrolysis of PUs.[3] Methanolysis allows obtaining the corresponding polyol and diurethane compounds which can be directly recycled into virgin PUs, increasing the sustainability and the efficiecy of the plastic degradation.[3]

Here we aim the **enzymatic methanolysis of PUs** in neat methanol, which has never been projected, to perform a new sustainable and bioorganic process in the plastic depolymerization area. To do so, we have screened **6 lipases**, **3 proteases**, **2 Cutinases** commercial preparations in the methanolsysis of ***p*-nitrophenylbenzyl carbamate** as model substrate in solvent free conditions. **ROL** (*Rhizopus oryzae lipase*) and **TLL** (*Thermomyces lanuginosus lipase*) are the most efficient enzymes studied for this kind of reaction. Additionally, we evaluate the reaction perfomance with different alkyl chain alcohols (figure 1). In these conditions, **ethanol** offered the best results, for which was selected for further experiments. After **24 hours** of reaction, we have achieved the totally convension of *p*-nitrophenol carbamate into corresponding ethylbenzyl carbamate and *p*-nitrophenol.

The best enzymes under the optimal conditions were confirmed in the alcoholysis of dicarbamates and low-molecular weight polyurethanes.

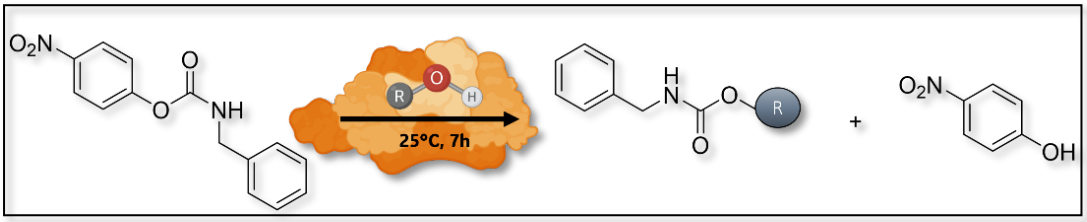

Figure 1: Alcoholysis reaction with lipases.

[1] H. Sardon, A. P Dove, Plastics recycling with a difference, *Science* **2018**, 360(6387), 380-381.
[2] A. Kemona, M. Piotrowska, Polyurethane Recycling and Disposal: Methods and Prospects, *Polymers* **2020**, *12*(8), 1752.
[3] N. Asahi, K. Sakai, N. Kumagai, T. Nakanishi, K. Hata, S. Katoh, T. Moriyoshi, Methanolysis investigation of commercially available polyurethane foam, *Polym. Degrad. Stab* **2004**, 86 (1), 147-151

POSTER # 21
INVESTIGATION OF CO-SOLVENTS FOR THE IN-SITU-CRYSTALLIZATION OF (HOMO-) PHENYLALANINE.

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Transaminases act as catalysts in the production of chiral amines[1]. To improve downstream processes, it is often crucial to purify the product through crystallization [2]. Some products, such as Homophenylalanine, crystallize spontaneously due to their low solubility, while others require modifications in the chemical environment to take advantage of these options. Phenylalanine, which is one chain element shorter than Homophenylalanine, has 50 times higher solubility. The study examined the impact of adding organic solvents to the synthetic solution, which decreases the solubility of Phenylalanine, thereby enabling *in-situ* product crystallization. The effects of this on the biocatalytic process using immobilized enzymes were monitored.

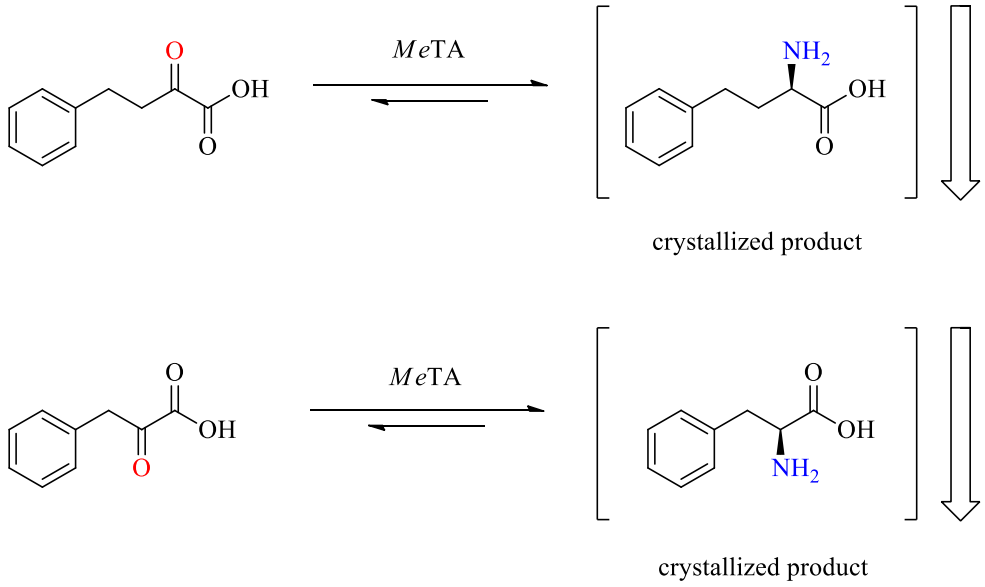

Figure 1: Synthesis of (*S*)-Homophenylalanine and *(S)*-Phenylalanine.

[1] Guo, F.; Berglund, P. Transaminase biocatalysis: optimization and application. *Green Chem.* **2017**, *19*, 333–360.
[2] Hülsewede, D.; Meyer, L.-E.; Langermann, J. von. Application of In Situ Product Crystallization and Related Techniques in Biocatalytic Processes. *Chem. Eur. J.* **2019**, *25*, 4871–4884.
[3] Tiedemann, S.; Neuburger, J. E.; Gazizova, A.; Langermann, J. von. Continuous Preparative Application of Amine Transaminase-Catalyzed Reactions with Integrated Crystallization. *Eur. J. Org. Chem.* **2024.** *manuscript in print*
[4] Tiedemann, S.; Langermann, J. von *manuscript in preparation*

POSTER # 22
EFFECTS OF COSOLVENTS ON PROTEIN DYNAMICS AND BINDING OF PROFLAVINE TO α-CHYMOTRYPSIN

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Biocatalysis with organic solvents is a topic of high relevance for fundamental and applied research.[1,2] In a combined experimental and computational study, we investigated the effect of different organic solvents (TMAO, DMSO, glycine and betaine) in the binding of the proflavine ligand to α-Chymotrypsin. By means of circular dichroism (CD) spectroscopy and extensive molecular dynamics simulations (including τ-Random Acceleration Molecular Dynamics, τ-RAMD[3]) we observed that cosolvents, especially DMSO, act as competitive inhibitors of α-Chymotrypsin. The cosolvent molecules reduce the interactions between proflavine and the catalytic triad of α-Chymotrypsin by modifying the solvent environment around the active site. In addition, the calculated residence times of the ligand in the catalytic site of the enzyme, using τ-RAMD, are in excellent agreement with the experimentally determined binding constants. Altogether, our analysis of the protein structure and dynamics, protein–substrate, protein–solvent, and solvent–solvent interactions, as well as substrate's residence times, provide a molecular rationale for the effect of the cosolvents on the catalytic activity of the enzyme.

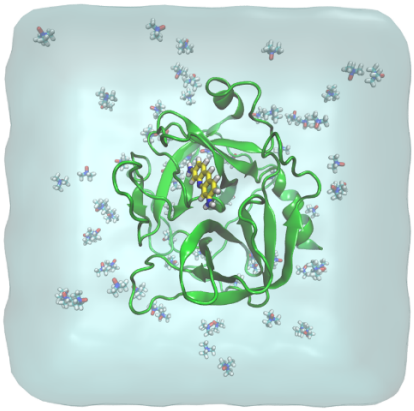

Figure 1: α-Chymotrypsin (green cartoon)/proflavine (yellow sticks) complex in aqueous solution with TMAO at 0.5 M.

[1] Adlercreutz, P. Biocatalysis in non-conventional media. Applied biocatalysis, **2000**;2:295-316.
[2] Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M, & Witholt B. Industrial biocatalysis today and tomorrow. *Nature*. **2001**;409(6817):258-68.
[3] Kokh, D. B., Amaral, M., Bomke, J., Grädler, U., Musil, D., Buchstaller, H. P., ... & Wade, R. C. Estimation of drug-target residence times by τ-random acceleration molecular dynamics simulations. Journal of chemical theory and computation, **2018**;14(7), 3859-3869.

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