The 14th International Conference on Protein Stabilization 2025 ProtStab 2025

September 21st-24th, 2025
Timișoara, ROMANIA

Editura POLITEHNICA

Colecţia “CONFERINŢE”

Book of Abstracts

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EDITORI

FRANCISC PETER ANAMARIA TODEA

DIANA MARIA DREAVĂ

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EDITURA POLITEHNICA
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WELCOME TO PROTSTAB 2025

On behalf of the Scientific and Organizing Committees, we are glad to warmly welcome all participants at the 14th International Conference on Protein Stabilization - ProtStab 2025. The conference is organized by Politehnica University Timişoara in partnership with the European Society of Applied Biocatalysis (ESAB) and hosted by the Conference Center of Politehnica University. For the first time, a scientific conference endorsed by ESAB is held in Romania.

This event will continue the series of meetings initiated in 1992 in Maastricht, The Netherlands, providing an overview of the current achievements and trends in the field of protein stabilization. The scientific program will involve 7 plenary lectures and 5 keynote lectures presented by leading scientists in the field, covering a comprehensive area of protein science, from protein design using bioinformatics and enzyme engineering to enzyme stabilization by immobilization and a wide range of applications in industrial bioprocesses, therapeutics, cosmetics, foods, or nutraceuticals. The main developments will be illustrated by 18 oral communications and 25 poster presentations. ProtStab 2025 will bring together more than 70 participants from universities, research institutes, and industry from 17 countries, allowing them to present their latest results, exchange information, engage in fruitful discussions, and initiate new cooperations.

We are grateful to the management of Politehnica University Timişoara, the board of ESAB, and the members of the Scientific Committee for their reliable assistance throughout the whole period of conference organization. We are also grateful to our sponsors for their generous support.

Timişoara is a vibrant multicultural city with stunning history, also called 'The Little Vienna" of Eastern Europe due to its richness in Baroque and Secessionist architecture. It was in 2023 one of the three Cultural Capitals of Europe, becoming an even more important destination.

We are confident that your participation at the ProtStab 2025 conference in the capital of the historical province of Banat will be an unforgettable event!

Anamaria Todea
Francisc Péter

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PROGRAMME OVERVIEW

SUNDAY, 21st September 2025		MONDAY, 22nd September 2025		TUESDAY, 23rd September 2025		WEDNESDAY, 24th September 2025	
		08.00	Registration				
		09.00	PL 2 L.H. Østergaard	09.00	PL 4 R. Silaghi-Dumitrescu	09.00	PL 6 L. Gardossi
		09.40	KL 1 M.L. Contente	09.40	KL 4 P. Žnidaršič-Plazl	09.40	OP 15 D. Abella López
		10.10	OP 1 C.G. Boeriu	10.10	OP 8 H. Hronská	10.00	OP 16 C. Paul
		10.30	OP 2 F. Papatola	10.30	OP 9 C. Danielli	10.20	Coffee break
		10.50	Coffee break	10.50	Coffee break	11.00	PL 7 D. Bednář
		11.30	KL 2 J.A. Littlechild	11.30	KL 5 B. Binay	11.40	OP 17 F. Pucci
		12.00	OP 3 A. Paloyan	12.00	OP 10 M. Mangiagalli	12.00	OP 18 R.B. Tomoiagă
		12.20	OP 4 A. López-Teijeiro	12.20	OP 11 P. Abellanas-Perez	12.20	Awards and conference closing
		12.40	OP 5 L.C. Bencze	12.40	OP 12 F. Zappaterra		
		13.00	Lunch	13.00	Lunch		
		14.30	PL 3 L.O. Martins	14.30	PL 5 A. Beloqui		
15.00	Registration	15.10	KL 3 A. Vogel	15.10	OP 13 K. Vasić		
		15.40	OP 6 A. Siladi	15.30	OP 14 I. Plazl		
17.00	Conference opening	16.00	OP 7 A. Vișa	15.50	Altium International		
17.20	Musical performance	16.20	Coffee & Poster Talks	16.05	Laboratorium SRL		
17.40	PL 1 M. Moracci			18.00	Conference Dinner		
18.20	ESAB R. Wohlgemuth	18.00	Guided City Tour				
19.00	Welcome Reception						

CONFERENCE PROGRAMME

DAY 1 – SUNDAY September 21st

Location: Conference Center of the Politehnica University Timișoara
Auditorium Hall, Vasile Pârvan 2B Blvd., Timișoara

15.00-17.00	**Registration**
17.00-17.20	**Conference opening** Assoc. Prof. Anamaria Todea (*ProtStab Co-Chair*) Prof. Alina-Gabriel Dumitrel (*Vice-Rector of Politehnica University Timişoara*) Prof. Liviu Cădariu-Brăiloiu *(President of the Senate of Politehnica University Timişoara*) Prof. Liviu Marșavina (*Vice-Rector of Politehnica University Timişoara*) Prof. Roland Wohlgemuth (*ESAB Chair*)
17.20-17.40	**Musical performance**, presented by **Sebastian Covaci** (piano) and **Casiana Cioată** (soprano)
	Chair: Prof. Francisc Péter
17.40-18.20	**Opening Plenary Lecture – Prof. Marco Moracci** **Enzymatic Hydrolysis of PET at High Temperature: Challenges and Opportunities** Marika Gargano[1], Roberta Iacono[1,2], Carmen Ercolano[1], Nicola Curci[3], Donato Giovannelli[1], Domenico Palatucci[1], Wolfgang R. Streit[4], Pablo Pérez-García[4], Beatrice Cobucci-Ponzano[3], Andrea Strazzulli[1,2], Marco Moracci[1] *[1]Department of Biology, University of Naples Federico II, Complesso Universitario di Monte S. Angelo, Naples, Italy* *[2]National Biodiversity Future Center (NBFC), Palermo, Italy* *[3]Institute of Biosciences and BioResources, National Research Council of Italy, Naples, Italy* *[4]Department of Microbiology and Biotechnology, University of Hamburg, Germany*
18.20-18.35	**In memoriam Prof. Antonio Ballesteros** Roland Wohlgemuth *European Society of Applied Biocatalysis*
19.00-22.00	**Welcome Reception** Mihai Eminescu 11 Blvd., including **Traditional Romanian Folk Music Performance**, presented by **Ansamblul Ceatăra Carei**

DAY 2 – MONDAY September 22nd

Location: Conference Center of the Politehnica University Timișoara
K1 Amphitheatre, Vasile Pârvan 2B Blvd., Timișoara

Exhibition Desks of the partner companies will be available during the whole day at the 1st floor lobby.

Posters will be displayed on September 22nd and September 23rd at the Poster Exhibition Space, 2nd floor lobby of the Conference Center.

8.00-9.00	**Registration**
	Chair: Prof. Roland Wohlgemuth
9.00-9.40	**Plenary Lecture 2 – Dr. Lars H. Østergaard** **From Lab to Industry: Enhancing Enzyme Stability for Industrial Use** Lars H. Østergaard *Novonesis, Lyngby, Denmark*
9.40-10.10	**Keynote lecture 1 – Prof. Martina Letizia Contente** **Stabilizing Biocatalysis: Harnessing Protein Immobilization for Scalable and Sustainable Processes** Martina Letizia Contente *Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy*
10.10-10.30	**OP 1** **Natural Deep Eutectic Solvents Enhance the Stability and the Catalytic Performance of Enzymes** Carmen Gabriela Boeriu *Politehnica University Timişoara, Faculty of Chemical Engineering, Biotechnology and Environmental Protection, Timişoara, Romania*
10.30-10.50	**OP 2** **Novel hydrolytic enzymes screening: a design of experiment approach for biocatalyzed polycondensation reactions** Francesco Papatola[1], Filippo Fabbri[2], Virender Kumar[3], Chiara Siracusa[2], Doris Ribitsch[2], Cristiano Varrone[3], Georg M. Guebitz[2,4], Alessandro Pellis[1] *[1]Università di Genova, Dipartimento di Chimica e Chimica Industriale, Genova, Italy* *[2]Institute of Environmental Biotechnology, Department of Agrobiotechnology, IFA-Tulln, BOKU University, Tulln an der Donau, Austria* *[3]Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark* *[4]Austrian Centre of Industrial Biotechnology, Tulln an der Donau, Austria*

10.50-11.30	**Group Photo**, Main entrance of the Conference Center **Coffee break** Conference Center, 1st floor lobby
	Chair: Prof. Marco Moracci
11.30-12.00	**Keynote lecture 2 – Prof. Jennifer A. Littlechild** **Thermophilic Genomes and Metagenomes as a Source of Novel Enzymes for Industrial Biocatalysis** Jennifer A. Littlechild *The Henry Wellcome Building for Biocatalysis, Biosciences, College of Life and Environmental Sciences, University of Exeter, United Kingdom*
12.00-12.20	**OP 3** **Two Glycoside Hydrolases Characterized from Jermuk Hot Spring Metagenomes** Ani Paloyan[1], Anna Krüger[2], Hasmik Grigoryan[1,3], Garabed Antranikian[4] *[1]Scientific and Production Center "Armbiotechnology" National Academy of Science of Armenia, Yerevan, Armenia* *[2]Authority for the Environment, Climate, Energy and Agriculture in Hamburg, Hamburg, Germany* *[3]Research Center of Genetics, Selection and Feeding of Agricultural Animals, Armenian National Agrarian University, Yerevan, Armenia* *[4]Center for Biobased Solutions TUHH, Hamburg, Germany*
12.20-12.40	**OP 4** **A Novel Platform for the Self-Immobilization and Stabilization of Plastic-Degrading Enzymes** Adrián López-Teijeiro[1], Natalia Barreiro-Piñeiro[1], Gemma Eibes González[2], José Manuel Martínez-Costas[1] *[1]CiQUS, Department of Biochemistry and Molecular Biology, Universidade de Santiago de Compostela, A Coruña, Spain* *[2]CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, A Coruña, Spain*
12.40-13.00	**OP 5** **Exploring and Unlocking the Functional Diversity of Aromatic Ammonia-Lyases** László Csaba Bencze, Raluca Bianca Tomoiagă, Krisztina Boros, Levente Csaba Nagy *Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj-Napoca, Romania*
13.00-14.30	**Lunch** Conference Center, 1st floor lobby

	Chair: Dr. Francesco Secundo
14.30-15.10	**Plenary Lecture 3 – Prof. Lígia O. Martins** **Network Dynamics as Fingerprints of Thermostability in an In Silico-Engineered DyP-type Peroxidase** Carolina F. Rodrigues[1], Diogo Silva[1], Constança Lorena[1], Patrícia T. Borges[1], Laura Masgrau[2], Lígia O. Martins[1] *[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal* *[2]Department of Chemistry, Universitat Autònoma de Barcelona, Bellaterra, Spain*
15.10-15.40	**Keynote lecture 3 – Dr. Andreas Vogel** **Stability Matters: Industrial Realities in Enzyme Applications** Andreas Vogel *c-Lecta, Leipzig, Germany*
15.40-16.00	**OP 6** **Challenges to transport liquid dairy protein concentrates** Armenia Siladi, Alfredo Walter *Prolactal GmbH, Hartberg, Austria*
16.00-16.20	**OP 7** **Green-Synthesized Metal–Organic Frameworks for Improved Adsorption and Catalytic Efficiency** Aurelia Vișa[1], Marcela Iosivoni[1], Adriana Popa[1], Nicoleta Pleșu[1], Bianca Mărănescu[2], Lavinia Lupa[3] *[1]Romanian Academy,"Coriolan Drăgulescu" Institute of Chemistry, Timişoara, România* *[2]Department of Biology-Chemistry, Faculty of Chemistry, Biology, Geography, West University, Timişoara, Romania* *[3]Faculty of Chemical Engineering, Biotehnology and Environmental Protection, University Politehnica Timişoara, Romania*
16.20-17.50	**Coffee & Poster Talks** Conference Center, 1st floor lobby & 2nd floor Poster Exhibition Space
18.00-20.00	**Guided City Tour** (walking)

DAY 3 – TUESDAY September 23rd

Location: Conference Center of the Politehnica University Timișoara
K1 Amphitheatre, Vasile Pârvan 2B Blvd., Timișoara

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Posters will be displayed on September 22nd and September 23rd at the Poster Exhibition Space, 2nd floor lobby of the Conference Center.

	Chair: Assoc. Prof. Anamaria Todea
9.00-9.40	**Plenary Lecture 4 – Prof. Radu Silaghi-Dumitrescu** **Blood Proteins and Light: Spectroscopy with a Vampire Touch** Radu Silaghi-Dumitrescu *Department of Chemistry, University Babeș-Bolyai of Cluj-Napoca, Romania*
9.40-10.10	**Keynote lecture 4 – Prof. Polona Žnidaršič-Plazl** **Immobilized Biocatalysts in Miniaturized Flow Systems** Polona Žnidaršič-Plazl *Faculty of Chemistry and Chemical Technology University of Ljubljana, Slovenia*
10.10-10.30	**OP 8** **Enhanced Stability and Functional Versatility of Immobilized Hexosaminidase** Helena Hronská, Mária Bláhová, Vladimír Štefuca, Michal Rosenberg *Institute of Biotechnology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia*
10.30-10.50	**OP 9** **2,5-Furandicarboxaldehyde as a Bio-Based Bifunctionalized Agent for Covalent Enzyme Immobilization** Chiara Danielli[1], Luuk van Langen[2], Lucia Gardossi[1] *[1]Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy* *[2]ViaZym B.V., Delft, The Netherlands*
10.50-11.30	**Coffee break** Conference Center, 1st floor lobby

	Chair: Prof. Lucia Gardossi
11.30-12.00	**Keynote lecture 5 – Prof. Barış Binay** **Immobilisation of Selected Enzymes for More Stability: Case Studies and Applications** Kübra Akbulut[1], Buse Susamaz[1], Mine Nazan Kerimak-Öner[2], N.Ece Varan[3], Deniz Yildirim[3], Barış Binay[1,4] *[1]Department of Bioengineering, Gebze Technical University, Kocaeli, Türkiye* *[2]Kocaeli University, İzmit Vocational School, Department of Medicinal and Aromatic Plants, Kartepe, Kocaeli, Türkiye* *[3]Cukurova University, Faculty of Ceyhan Engineering, Department of Chemical Engineering, Ceyhan, Adana, Türkiye* *[4]BAUZYME Biotechnology Co., Gebze Technical University Technopark, Kocaeli, Türkiye*
12.00-12.20	**OP 10** **Effects of Levoglucosenone-Derived Solvents on the Stability of Immobilized Lipase B from *Candida antarctica*** Marco Mangiagalli[1], Diletta Ami[1], Laura Legnani[1], Alessandro Pellis[2], Marina Lotti[1], Antonino Natalello[1] *[1]University of Milano Bicocca, Department of Biotechnology and Biosciences, Milano, Italy* *[2]University of Genova, Department of Chemistry and Industrial Chemistry, Genova, Italy*
12.20-12.40	**OP 11** **Use of Glutaraldehyde Chemistry to Coimmobilize Enzymes Enabling the Re-Use of the Most Stable Immobilized Enzyme** Pedro Abellanas-Perez[1], Diandra de Andrades[1,2], Diego Carballares[1], Maria de Lourdes T. d M. Polizeli[2], Roberto Fernández-Lafuente[1] *[1]Departamento de Biocatálisis. ICP-CSIC, Campus UAM-CSIC Cantoblanco, Madrid* *[2]Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Brazil*
12.40-13.00	**OP 12** **Upcycled Keratin Scaffolds Confer Process-Ready Robustness on *Pseudomonas stutzeri* Lipase, Enabling Batch-to-Flow Catalysis** Federico Zappaterra, Marco Bottin, Pier Paolo Giovannini, Lindomar Alberto Lerin, Daniele Ragno, Alessandro Massi *Department of Chemical, Pharmaceutical and Environmental Sciences, University of Ferrara, Italy*
13.00-14.30	**Lunch** Conference Center, 1st floor lobby

	Chair: Prof. Bariș Binay
14.30-15.10	**Plenary Lecture 5 – Assoc. Prof. Ana Beloqui** **"Plastified Proteins" to Improve the Stability and Broaden the Use of Proteins in Functional Materials** Ana Beloqui *University of the Basque Country, Donostia-San Sebastian, Spain*
15.10-15.30	**OP 13** **Immobilization of β-Lactamase onto Magnetic Nanoparticles for Antibiotic Degradation Pathway** Maja Leitgeb[1,2], Željko Knez[1,2], Katja Vasić[1,3] *[1]University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory of Separation Processes and Product Design, Maribor, Slovenia* *[2]University of Maribor, Faculty of Medicine, Maribor, Slovenia* *[3]University of Maribor, Faculty of Electrical Engineering and Computer Science, Institute of Electrical Power Engineering, Laboratory for applied electromagnetics, Maribor, Slovenia*
15.30-15.50	**OP 14** **Addressing Sensitivity and Non-Uniqueness Challenges in Determining Enzyme Kinetics Parameters for Biocatalytic Reactions** Mitja Lakner[1], Igor Plazl[2] *[1]Faculty of Civil and Geodetic Engineering, University of Ljubljana, Ljubljana, Slovenia "Independent Scholar"* *[2]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia*
15.50-16.05	**Redefining Protein Stability Testing with Stunner** Elena Apostu *Altium International, Bucharest, Romania*
16.05-16.20	**Advanced analytical technologies for protein characterization** Alex Bercuci *Laboratorium SRL, Bucharest, Romania*
18.00-21.00	**Conference Dinner** at Recaș Wineries

DAY 4 – WEDNESDAY September 24th

Location: Conference Center of the Politehnica University Timișoara
K1 Amphitheatre, Vasile Pârvan 2B Blvd., Timișoara

	Chair: Prof. Polona Žnidaršič-Plazl
9.00-9.40	**Plenary Lecture 6 – Prof. Lucia Gardossi** **What Biocatalysts for a New Generation of Bio-based Chemicals?** Lucia Gardossi[1], Anamaria Todea[2], Monia Renzi[3], Emanuele Carosati[1] *[1]Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy* *[2]Politehnica University Timisoara, Faculty of Industrial Chemistry, Biotechnology and Environmental Protection, Timisoara, Romania* *[3]Department of Life Sciences, University of Trieste, Italy*
9.40-10.00	**OP 15** **Versatile Nanoencapsulation Platform for the Stabilization and Oral Delivery of Proteins** Daniel Abella López, Adrián López Teijeiro, Natalia Barreiro Piñeiro, José Manuel Martínez Costas *CiQUS - Singular Center for Research in Biological Chemistry and Molecular Materials, University of Santiago de Compostela, Spain*
10.00-10.20	**OP 16** **Enhancing Lipase Stability Through Immobilization in Sol-Gel Systems** Cristina Paul, Corina Vasilescu, Ioana-Cristina Benea, Anamaria Todea, Francisc Péter *Green Chemistry and Biocatalysis Group, Faculty of Chemical Engineering, Biotechnology and Environmental Protection, Politehnica University Timișoara, Romania*
10.20-11.00	**Coffee break** Conference Center, 1st floor lobby
	Chair: Prof. Lígia O. Martins
11.00-11.40	**Plenary Lecture 7 – Assoc. Prof. David Bednář** **FireProt Suite: Advancing Protein Stability Engineering through Integrated Computational Approaches** David Bednář[1,2], Milos Musil[1,2,3], Simeon Borko[1,2], Pavel Kohout[1,2], Joan Planas-Iglesias[1,2], Jan Dvorsky[1,2], David Lacko[1,2], Monika Rosinska[1,2,3], Jan Velecky[1,2], Petr Kabourek[1,2], Rayyan Tariq Khan[1,2], Matej Berezny[1,3], Martin Stepanek[1,3], Andrej Ježík[1,3], Jana Horáčková[1,2], Stanislav Mazurenko[1,2], Jiri Damborsky[1,2] *[1]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic* *[2]International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic* *[3]Department of Information Systems, Faculty of Information Technology, Brno University of Technology, Brno, Czech Republic*

11.40-12.00	**OP 17** **Leveraging Artificial Intelligence and Structural Data to Design Proteins with Improved Solubility** Fabrizio Pucci[1,2], Simone Attanasio[1,2], Marianne Rooman[1,2] *[1]Computational Biology and Bioinformatics, Université Libre de Bruxelles, Brussels, Belgium* *[2]Interuniversity Institute of Bioinformatics in Brussels, Bruxelles, Belgium*
12.00-12.20	**OP 18** **High-Throughput Assays Development for Screening Saturation Mutagenesis-Derived Phenylalanine Ammonia-Lyase Libraries** Raluca Bianca Tomoiagă, László Csaba Bencze *Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj-Napoca, Romania*
12.20-12.45	**Awards and conference closing** ***Best Young Scientist Oral Presentation Award*** *(under the age of 40), sponsored by the BioTech Journal* ***Best Poster Award****, sponsored by the Romanian Chemical Society* ***Poster Awards*** *(3) granted by the European Society of Applied Biocatalysis (ESAB) as free registrations to the 3rd ESAB Digital Conference* *https://esabweb.org/2025_congress.html* ***Closing remarks***

CONFERENCE LOCATION

ProtStab 2025 will take place in Timișoara, Romania, in the Conference Center of the Politehnica University Timișoara.

Conference Center of the Politehnica University Timișoara
Auditorium hall and K1 Amphitheatre, Vasile Pârvan 2B Blvd., Timișoara
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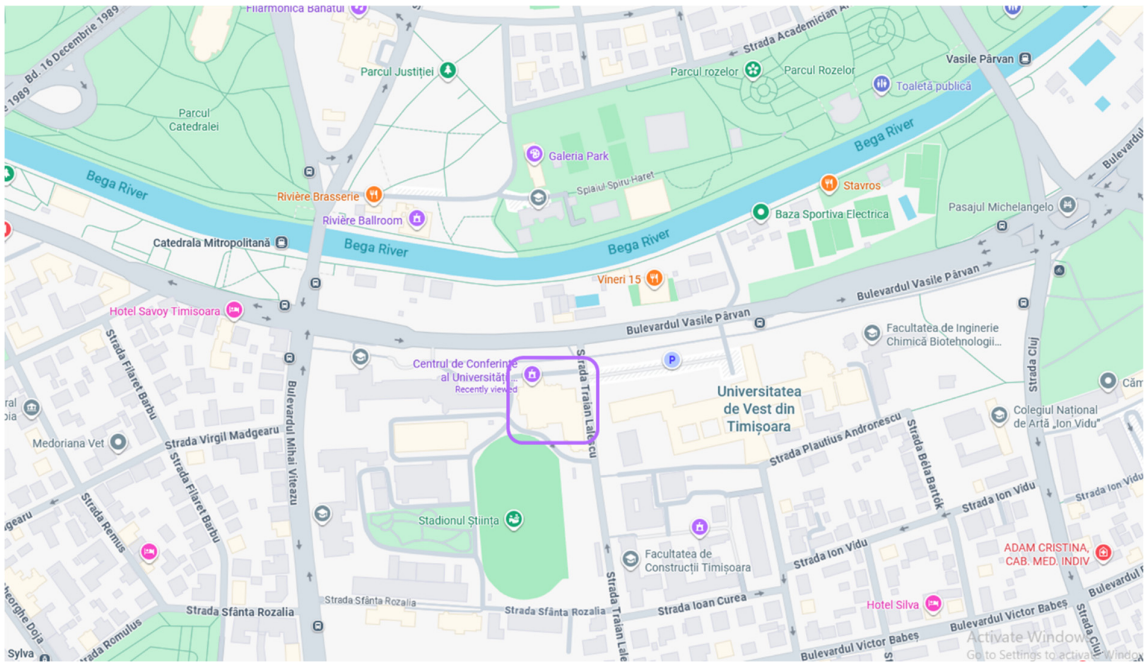

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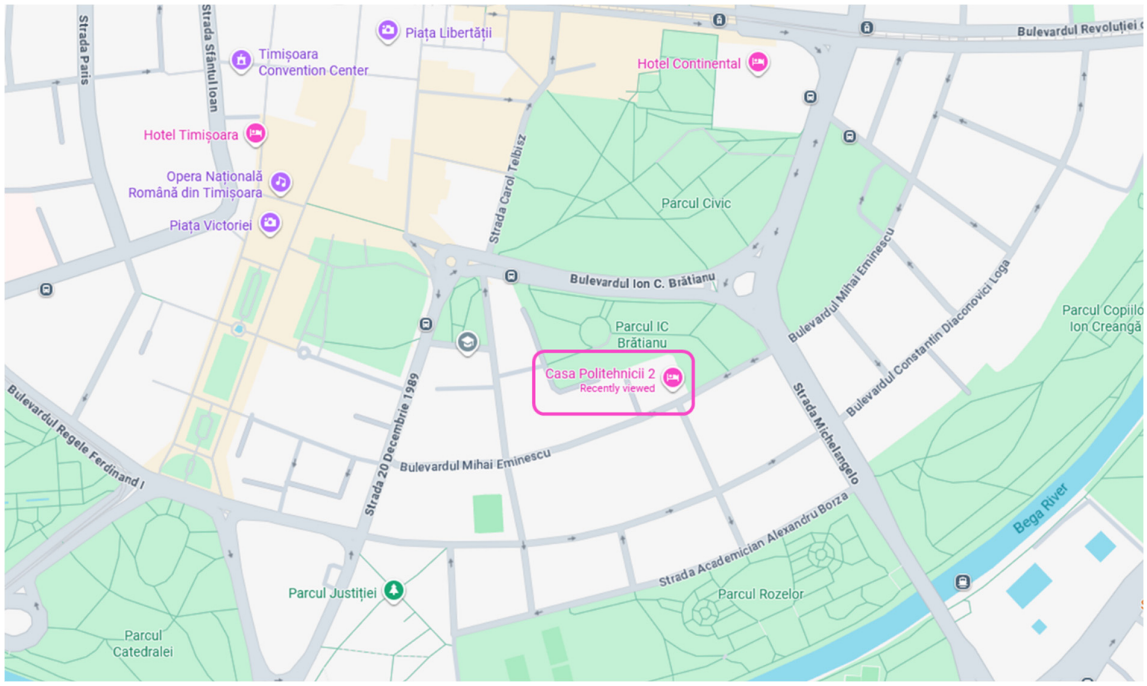

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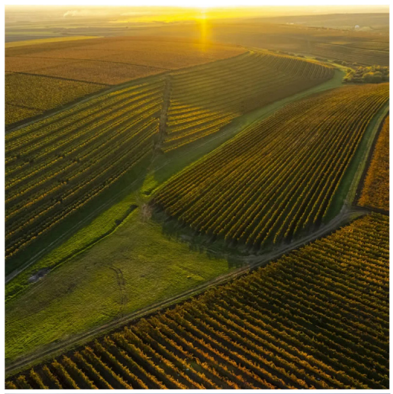
CRAMELE RECAȘ



INVITED SPEAKERS

PL1 - Prof. Marco Moracci

Marco Moracci made all studies in Naples working on enzymes from extremophiles and as post-doc in 1991 at the Chemical Laboratory of the Cambridge University (UK) with prof. Alan Fersht. He worked for more than 20 years (1994-2016) in the National Research Council of Italy in Naples as Research Director. From 2016 he's Full Professor in Biochemistry in the Department of Biology of the University of Naples 'Federico II'. Marco's research interests are: (i) Discovery, characterization and engineering enzymes, with particular focus on extremophiles; (ii) Discovery of drugs for CAZymes involved in genetic metabolic diseases.

PL2 - Dr. Lars Henrik Østergaard

Lars Østergaard earned his degree in Chemistry-Biotechnology from Aarhus University in 1994. He then completed his PhD at the University of Edinburgh, where he specialized in protein engineering with focus on flavocytochrome b2. Following a postdoctoral position at Cambridge University, where he worked on the Erythromycin producing polyketide synthase, he joined Novo Nordisk's enzyme division (later Novozymes/Novonesis) in 2000. His primary work area has been protein engineering to optimize enzymes, which have found their way into multiple commercial products, including household detergents. His industry experience has also enabled him to engage in academic collaborations and contribute to numerous publications.

PL3 - Prof. Lígia O. Martins

Lígia O. Martins is an experienced Enzymologist and the Head of the Microbial and Enzyme Technology Lab at ITQB a research Instituto of Universidade Nova de Lisboa. Her research focuses on designing enzymatic systems for novel functions with applications in Industrial Biotechnology. She combines experimental and computational tools to discover and engineer enzymes with enhanced performance and robustness by integrating molecular biology, biochemistry, and structural biology. She aims to develop sustainable enzymatic processes that valorize renewable feedstock, creating products with applications in medicine, food, and nutraceuticals.

PL4 - Prof. Radu Silaghi-Dumitrescu

Radu Silaghi-Dumitrescu received his PhD degrees from the University of Georgia in Athens, GA, U.S.A. (2004, experimental chemistry) and from the Babeș-Bolyai University (UBB) in Romania (2005, computational chemistry). After a postdoctoral stage at the University of Essex (Colchester, England), he returned to UBB in 2007, where he teaches biochemistry, computational chemistry and related subjects, while also serving as president of the Scientific Council (2012-), director of the Center for University Strategies (2020-) and Coordinator of the Office for Open Science (2024-). His research explores the mechanisms of small molecule activation by metals in biology and medicine (especially involving redox chemistry, unusual metal oxidation/coordination states, and free radicals) in contexts such as enzymes of the nitrogen cycle, blood substitutes, anticancer drugs, oxidative and nitrosative stress in various ranges of, or plant extracts – but also interdisciplinary applications of exact science tools in scientometrics or in cultural studies.

PL5 - Assoc. Prof. Ana Beloqui

Ana Beloqui is a multidisciplinary researcher whose work spans chemistry, enzymology, nanotechnology, and applied polymer chemistry. She leads the PolyZymes Lab at POLYMAT Institute (Spain), where she has pioneered a research line focused on combining proteins and polymers to create advanced functional materials. These hybrids are meticulously designed to enhance the stability of functional proteins and expand their use in applications such as sensing, therapeutics, and bioremediation. Her contributions to the field have been recognized with the "Best Young Group Leader" award by the Group of Chemical Biology of the Spanish Royal Chemical Society.

PL6 - Prof. Lucia Gardossi

Lucia Gardossi received her PhD in Medicinal Chemistry at the University of Trieste and spent two years as an associate researcher at MIT (Cambridge - USA) in the laboratory of prof. Alexander Klibanov. Her research integrates experimental and computational approaches for the development of sustainable enzymatic processes applicable to industry. In 2007 she was co-founder and scientific director of the university spin-off SPRIN SpA. Since 2017 she has been a member of the board of the Italian Technology Cluster for Green Chemistry and Bioeconomy, and she coordinates its Scientific and Technical Committee. She is active in various working groups at European and Italian level which aim to promote the bioeconomy and sustainable chemistry. She was vice chair of the Advisory Group SC2 of the European Commission for the Horizon 2020 program and is currently a member of the Italian National Coordination Group for the Bioeconomy of the Presidency of the Council of Ministers.

PL7 - Assoc. Prof. David Bednář

David Bednář is Associate Professor at the Loschmidt Laboratories, Faculty of Science, Masaryk University, where he also earned his PhD in 2017. He broadened his expertise through research internships at Rutgers University (USA), the University of North Carolina (USA), and Adam Mickiewicz University (Poland). David Bednar has authored over 80 publications and holds several international patents in protein stabilization. His research focuses on applying molecular modelling and bioinformatics to enzymology and medicinal topics. This knowledge is further translated into user-friendly web servers and databases for protein analysis and design, serving over 100,000 users annually.

PLENARY LECTURES

PL 1

ENZYMATIC HYDROLYSIS OF PET AT HIGH TEMPERATURE: CHALLENGES AND OPPORTUNITIES

Marika Gargano[1], Roberta Iacono[1,2], Carmen Ercolano[1], Nicola Curci[3], Donato Giovannelli[1], Domenico Palatucci[1], Wolfgang R. Streit[4], Pablo Pérez-García[4], Beatrice Cobucci-Ponzano[3], Andrea Strazzulli[1,2], Marco Moracci[1,2]*

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Since the discovery of plastics, waste accumulation is one of the most overwhelming environmental issues of this century [1]. Particularly, single-use products made from polyethylene terephthalate (PET), accounting for about 6.2% of global plastic production [2], have heavily contributed to this problem. The discovery in 2016 of enzymes capable of hydrolysing PET (PETases) has sparked research into more environmentally friendly methods for managing PET waste [3].

This study describes how the use of novel PETases from metagenomes obtained from geothermal samples offers the opportunity to develop a sustainable one-step process for the depolymerization of PET waste at mid-high temperatures (60°C) within 4 days, exploiting the biodiversity of extreme environments.

The genes encoding for putative PETases have been identified and produced in recombinant way in active form. The characterization of the PETases demonstrated its activity on high-purity substrates such as bis(2-hydroxyethyl)-TPA (BHET) and nanoPET, as well as semi-crystalline and non-treated PET waste from food packaging materials like food trays.

The depolymerization process was set up and optimized on PET waste by incubating it with different concentrations of enzymes, by monitoring its degradation over time, in terms of macro and microscopic modification of the solid substrate and by analysing products formation through HPLC [4].

These findings suggest that thermophilic PETases could serve for PET recycling, offering a sustainable approach to PET waste management. The study advances the understanding of PETases and opens new possibilities for more efficient and environmentally friendly recycling processes.

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IN MEMORIAM ANTONIO BALLESTEROS

Andrés R. Alcántara[1], Francisco J. Plou[2], Miguel Alcalde[2], Roland Wohlgemuth[3,4]*

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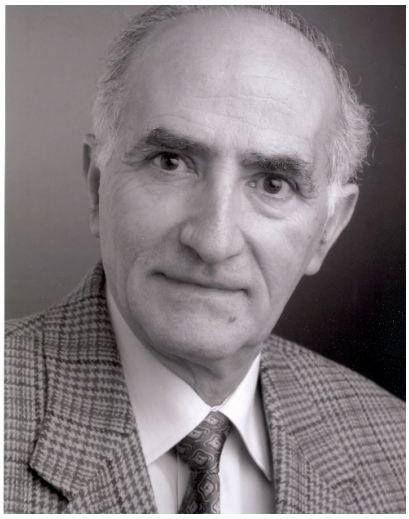

This presentation will pay tribute to the memory of Prof. Antonio Olmo Ballesteros, who recently passed away. Antonio Ballesteros was a great scientific personality in the field of biocatalysis, earning recognition at national and international level. He graduated in Chemical Sciences (with Extraordinary Prize and top of his class) from the University of Seville in 1962 and years later, in 1972, he also earned a degree in Pharmacy from the Complutense University of Madrid, where he obtained in 1966 a Ph.D. in Chemical Sciences. In the 1970s and 1980s, Prof. Ballesteros conducted research during 5 years at institutions and universities in the United States, the United Kingdom, Japan, Germany, and France. He spent two years at the National Institutes of Health, working alongside Prof. Christian Anfinsen, Nobel Prize in Chemistry in 1972 [1].

Prof. Antonio Ballesteros had remarkable contributions in the development of biocatalysis as a distinct scientific field, being the first director of the Applied Biocatalysis Group at the Institute of Catalysis and Petrochemistry at the Spanish Council for Scientific Research, member of the Working Party of Applied Biocatalysis since its beginning, and President (1996-2000) of the Applied Biocatalysis Section of the European Federation of Biotechnology, which in 2020 has become the European Society of Applied Biocatalysis (ESAB). From 2011 until 2024 he was Editor-in-Chief of the journal Biocatalysis and Biotransformation.

Prof. Ballesteros organized in 1998 the international Symposium “Stability and Stabilization of Biocatalysts” (Córdoba, Spain), the precursor of the conference series on Protein Stability, and was standing member of the Scientific Committees of these events. He published more than 250 journal articles with high impact and is considered one of the pioneers of biocatalysis in Europe.

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PL 2

FROM LAB TO INDUSTRY: ENHANCING ENZYME STABILITY FOR INDUSTRIAL USE

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With a growing population and with resources under massive pressure, we need solutions for a healthier planet. Biosolution can make a significant difference by using the power of biology to solve problems across many industries and businesses. In our fields, factories and homes, biosolutions are transforming production and consumption. They unlock more sustainable protein sources and help farmers and food producers get more from less. At the same time, they reduce the use of fossil-based resources, chemicals, energy and water. The solutions of the future therefore already exist today enabling us to produce, consume and live with respect for both climate and nature.

For more than a century, Novonesis has been utilizing, engineering and improving the power of biology to solve problems across many industries and businesses. Working with microbiology, we use both the microorganism itself, be that bacteria, yeast or pro- or postbiotics, but we also use the valuable byproducts they produce during fermentation, such as human milk oligosaccharides (HMOs), proteins or enzymes. The enzymes are biocatalysts that are used in many applications, where they each make very specific changes to substances. They can therefore be used to improve a wide range of consumer products and industrial processes. However, the natural enzyme itself will often require modifications to enable its successful application in industry.

Stabilization is very often a crucial target for modification of an enzyme. Enzymes are often challenged by the new environment they need to operate in, e.g. low/high temperature or pH, high salinity and anionic surfactants. Furthermore, enzymes are required to retain good activity during their entire lifetime, which may span years and cover a range of different environments. The first challenge for an enzyme is the production itself, which can be seriously impacted by poor stability. The enzyme product will then undergo various steps before reaching the business customer, where it will experience yet another change in environment presented by an industrial application or a consumer product.

The protein engineering is one of the most valuable tools to target and improve enzyme stability across its entire lifespan. The modifications are introduced by mutagenesis and beneficial substitutions are identified by screening under relevant stress conditions for the desired product. The beneficial substitutions will then be combined, and the resulting enzyme variants will undergo increasingly harsh screening conditions until the set stability target has been reached. The iterative process used in the product development can be described by the design-build-test-learn cycle. The exact methods involved in the individual steps of the cycle are under continuously optimization to incorporate latest developments in automation, molecular biology and AI tools. A real-world example from development of an industrial enzyme will be presented to provide insight into the complex process of enzyme production and to illustrate current state-of-the-art within industrial biotechnology.

PL 3

NETWORK DYNAMICS AS FINGERPRINTS OF THERMOSTABILITY IN AN IN SILICO-ENGINEERED DYP-TYPE PEROXIDASE

Carolina F. Rodrigues[1], Diogo Silva[1], Constança Lorena[1], Patrícia T. Borges[1], Laura Masgrau[2], Lígia O. Martins[1]*

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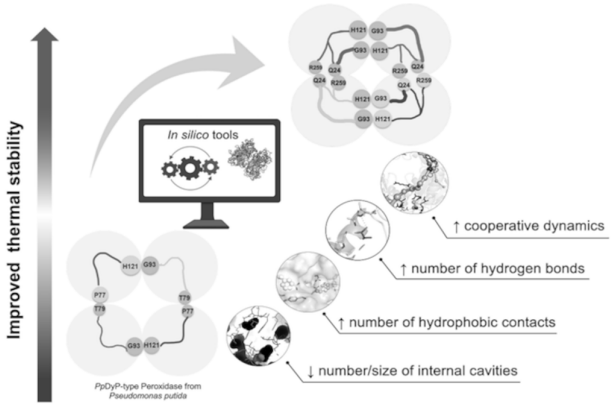
Stabilizing industrial enzymes is essential for advancing environmentally responsible bioprocesses, yet the structural basis of thermostability remains incompletely understood. Here, we engineered thermostable variants of a tetrameric dye-decolorizing peroxidase (DyP) using two independent open-source design algorithms, yielding two variant enzymes with significantly improved thermal performance and prolonged activity at elevated temperatures. Subsequent recombination strategies minimize the mutational burden while maintaining or enhancing stability.



Structural and dynamic analyses of the thermostable variants revealed convergent features, including increased compactness, rigidity, and an enriched network of hydrogen bonds and hydrophobic interactions. Despite differing mutation profiles, stabilizing substitutions clustered in similar structural regions. Notably, the integration of dynamic modeling with protein correlation network analysis uncovered a previously unrecognized stabilization mechanism: highly connected structural networks characterized by denser and more persistent intra- and inter-monomer interactions, greater internal cohesion, and enhanced cooperative dynamics. Tetramers exhibit long-range communication pathways and redundant routes, supporting coordinated motions that can hinder local unfolding and tetramer dissociation. These findings identify dynamic interaction networks as hypothetical critical determinants of protein stability and offer a previously unexplored framework for rational enzyme design.

PL 4

BLOOD PROTEINS AND LIGHT: SPECTROSCOPY WITH A VAMPIRE TOUCH

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This presentation will cover the author's experience with the analysis and modulation of the stability of proteins from (and some related thereto) blood. The two most abundant proteins will be the main (but not only) topic – hemoglobin and albumin [1-4].

Hemoglobin (Hb) has for decades been explored for chemical and genetic modifications that would stabilize it while also curtailing its toxic side-reactions, so that it can be used in cell-free solutions for transfusions in cases of severe blood loss/shortage. Perplexingly, considering the breadth of our knowledge of Hb, such products, dubbed variously as "artificial oxygen carriers", "blood substitutes", "hemoglobin-based oxygen carriers (HBOC)" or variations thereof, are yet to successfully pass clinical trials despite significant efforts to do so. Our own recent contributions to the topic entail exploration of redox reactivity and stability with methods ranging from chemical modification and analysis to spectroscopy (fluorescence, UV-vis, NMR, vibrational, EPR), kinetics and computational/molecular modelling.

Serum albumin's relative abundance in the blood as well as a biochemical reagent in vitro have contributed to a wealth of data and standardized tests where albumin (most often the more accessible bovine form, BSA) is employed as a model protein. The implication is generally that albumin's abundance and its lack of cofactors must make it a simple and predictable protein. We illustrate here some striking illustrations to the contrary in the context of examining albumin's potential as component in blood substitutes but also Hb's and albumin's interactions with therapeutic agents and especially anticancer drugs and drug candidates. We go beyond the propensity of latter classes of compounds to bind to proteins generally (hence also to blood proteins) and also explore the proteins as victims – and not simply carriers or blockers – of the therapeutical agents otherwise targeted towards entirely different parts of the organisms.

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PL 5

"PLASTIFIED PROTEINS" TO IMPROVE THE STABILITY AND BROADEN THE USE OF PROTEINS IN FUNCTIONAL MATERIALS

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The use of artificial materials to stabilize functional proteins, such as enzymes, has been demonstrated to be successful, particularly with the formation of heterogeneous biocatalysts. Herein, I propose an alternative to the use of (in)organic supports that enables the generation of robust and stable biocatalysts. During my presentation, I will demonstrate how the combination of enzymes with polymeric networks, which act as flexible and porous scaffolds, can enhance the stability and robustness of the biomolecules in conditions that would otherwise cause denaturation. These biohybrids open new avenues of applications in chemical synthesis, the alimentary industry, and medicine, particularly in the development of sensors.

I will show the strategies that are being developed in our lab to (1) keep the stability during the chemical modification of enzymes and to (2) generate stable functional protein-polymer biohybrids. I will focus the presentation on the fabrication of "plastified enzymes", biohybrids assembled throughout a molecular dressing strategy in which individual enzymes are wrapped within polymeric networks. This tailored entrapment confers stability to the enzyme when working at high temperatures and in the presence of organic solvents [1].

From the examples developed in the lab, I will delve into the use of a molecular dressing strategy for fabricating heterogeneous biocatalytic reactors [2,3], for enzyme-mediated electrochemical and optical sensors [4], for NADH-cofactor recycling,[5] and for bioremediation applications [6].

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PL 6

WHAT BIOCATALYSTS FOR A NEW GENERATION OF BIO-BASED CHEMICALS?

Lucia Gardossi[1]*, Anamaria Todea[2], Monia Renzi[3], Emanuele Carosati[1]

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Using building blocks obtained from the fermentation or chemical processing of biomass is key to transitioning to non-fossil chemical feedstock. In this respect, enzymes' remarkable selectivity offers a unique opportunities to produce new, complex, bio-based chemicals and polymers. However, the use of biocatalyst and bio-based feedstocks does not necessarily indicate low environmental impact. New, integrated research strategies are needed to develop realistic innovations and solutions able to fulfil the demand for 'safe and sustainable by design' chemicals and polymers, as set out in the European Commission's strategy [1]. In recent years, the University of Trieste and the Polytechnic University of Timișoara have conducted interdisciplinary research that integrate biocatalysis, ecotoxicology, marine biology and computational chemistry, seeking virtuous routes for the rational design of sustainable processes and products. For example, automatic computational workflows integrating molecular dynamics simulations and docking were employed to quickly select enzymes that are efficient at catalysing the hydrolysis and/or synthesis of bio-based polyesters. Bio-based monomers and corresponding enzymatically synthetized oligoesters were tested for biodegradability and ecotoxicity. Furthermore, computational analysis of the structural features of enzymatically synthesised oligoesters enabled the construction of a preliminary structure–biodegradability correlation model. We have also recently developed a rational approach to designing and synthesising functionalised bio-based molecules for use in the pharmaceutical, cosmetic and biomedical sectors. The workflow consists of: i) virtual screening of candidate oligoesters using software that can predict physical and chemical properties (e.g. hydrophilicity, flexibility and permeability. ii) Solvent-free enzymatic synthesis of the selected bio-based oligoesters containing reactive monomers, exploitable for the anchoring of biomolecules; iii) Study of the marine biodegradability and ecotoxicity of the oligoesters. Notably, solvent-free enzymatic polycondensation is scalable, also using enzymes immobilized on lignocellulosic carriers obtained from agricultural residues, which are widely available in Italy at negligible cost. This responds to environmental, social and economic sustainability criteria. Overall, the results of our research suggest that biocatalysts can be fruitfully used in combination with bio-based monomers and building blocks for delivering a new generation of chemicals and polymeric products safe and sustainable by design.

Acknowledgments
University of Trieste is grateful for financial support from ICSC – Centro Nazionale di Ricerca in High Performance Computing, Big Data and Quantum Computing, funded by European Union – NextGenerationEU - PNRR, Missione 4 Componente 2 Investimento 1.4 Grant number CN00000013.

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PL 7

FireProt SUITE: ADVANCING PROTEIN STABILITY ENGINEERING THROUGH INTEGRATED COMPUTATIONAL APPROACHES

David Bednar[1,2,*], Milos Musil[1,2,3], Simeon Borko[1,2], Pavel Kohout[1,2], Joan Planas-Iglesias[1,2], Jan Dvorsky[1,2], David Lacko[1,2], Monika Rosinska[1,2,3], Jan Velecky[1,2], Petr Kabourek[1,2], Rayyan Tariq Khan[1,2], Matej Berezny[1,3], Martin Stepanek[1,3], Andrej Ježík[1,3], Jana Horáčková[1,2], Stanislav Mazurenko[1,2], Jiri Damborsky[1,2]

[1]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Brno 611 37, Czech Republic
[2]International Clinical Research Centre, St. Anne's University Hospital, Brno 656 91, Czech Republic
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Protein stability is a critical characteristic for biotechnology and medicine, yet designing stable proteins remains a challenge. We present a comprehensive suite of computational tools: FireProt^{ASR}, FireProt, FireProt^{DB}, and BenchStab, that integrate evolution-based, energy-based, and machine learning-based approaches in user-friendly interfaces to address this bottleneck in protein engineering. Our evolution-based strategy leverages Ancestral Sequence Reconstruction (ASR) to infer robust, stable protein variants from evolutionary history. We introduce FireProt^{ASR}, a fully automated web server that streamlines the entire ASR pipeline, from homologous sequence searching and multiple sequence alignment to phylogenetic tree construction and ancestral sequence reconstruction, including ancestral gaps. FireProt^{ASR} 2.0 newly introduces a successor sequence predictor, which suggests likely future mutations based on site-specific evolutionary trends, and a generative model based on variational autoencoders, which captures global evolutionary constraints in a low-dimensional latent space.

Energy-based computational design platform, FireProt, offers advanced strategies for stabilizing proteins. FireProt 2.1 introduces an algorithm for constructing multiple-point designs, minimizing antagonistic effects. It further expands capabilities with user-defined mutation sets, saturation mutagenesis, and acceleration thanks to machine learning-based filters for fast evaluation. To overcome the data limitations often faced by machine learning predictors, we developed FireProt^{DB}, a curated database of experimental thermostability data. FireProt^{DB} 2.0 incorporates recently published high-throughput datasets, manually extracted literature data, and in-house experimental results, providing a high-quality resource for training and validating predictive models. Moreover, BenchStab is introduced as a console tool and Python package for the execution and comparative evaluation of 18 different stability predictors, revealing current limitations in the domain.

These tools, together with many others for protein analysis and design, are accessible on our Protein Engineering Portal at https://loschmidt.chemi.muni.cz/portal/.

Acknowledgments
This project was supported by the European Union's Horizon Europe Framework Programme under the grant agreement No. 101136607 (CLARA).

KEYNOTE LECTURES

KL 1

STABILIZING BIOCATALYSIS: HARNESSING PROTEIN IMMOBILIZATION FOR SCALABLE AND SUSTAINABLE PROCESSES

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Biocatalysis is a key technology for the sustainable production of high-value compounds, spanning pharmaceuticals, food, cosmetics, and fine chemicals [1]. To improve efficiency and scalability, process intensification strategies increasingly integrate enzyme immobilization with advanced reactor technologies. In this context, flow biocatalysis has emerged as a powerful approach, enhancing reaction productivity while reducing waste and energy consumption [2]. Enzyme immobilization plays a pivotal role, not only enabling catalyst recovery and reuse, but also providing increased structural and operational stability, crucial for maintaining enzymatic activity under process-relevant conditions. Immobilization can protect enzymes from denaturation due to shear forces, pH fluctuations, and elevated temperatures, extending their functional lifespan and reducing overall biocatalyst costs [3]. These advancements have enabled the continuous synthesis of bioactive molecules, including neurotransmitters, aroma compounds, and pharmaceutical intermediates [4].

In parallel, rotating bed reactors (RBRs) offer an effective platform for immobilized enzymes, ensuring high mass transfer rates and improved catalyst performance. Their application in transesterification and aminolysis reactions has demonstrated significant advantages such as the conversion of triacylglycerols from oleaginous yeasts grown on agro-industrial byproducts into high-value fatty acid amides with potential neuroprotective properties. Another example is the synthesis of perillyl alcohol as an aroma compound from its natural aldehyde precursor [5].

By integrating stabilized, immobilized biocatalysts into both continuous flow systems and RBRs, biocatalysis can reach new heights in efficiency, robustness, and scalability—paving the way toward greener, economically viable chemical manufacturing.

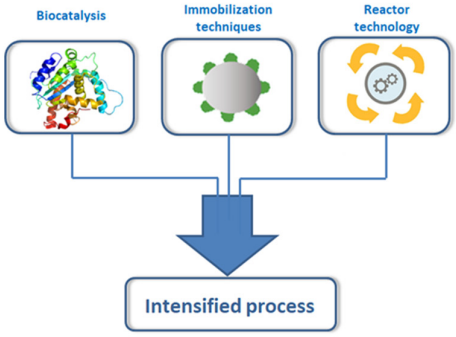

Figure 1. Merging biocatalysis, protein immobilization and reactor technology for intensified enzymatic processes

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KL 2

THERMOPHILIC GENOMES AND METAGENOMES AS A SOURCE OF NOVEL ENZYMES FOR INDUSTRIAL BIOCATALYSIS

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Enzymes identified in thermophilic genomes and metagenomes are providing new novel biocatalysts for Industrial Biocatalysis. These enzymes have evolved under different evolutionary pressures and often have new catalytic properties and stereoselectivity when compared to previously identified proteins. They are finding new applications industrially and are important to enhance the enzyme 'tool box' that is available for new sustainable processes to produce important new pharmaceuticals and healthcare products. These enzymes are not only naturally evolved for stability to temperature but are also stable to solvents and the addition of other formulation additives. They can also have novel protein folds which have not been identified in other know protein structures.

This lecture will illustrate a selection of important enzyme examples including carboxyl esterases, epoxide hydrolases, transketolases, transaminases and phospholipases [1-6].

These enzymes can be further optimised for specific processes by both rational and directed evolution using 'state of the art' microfluidics.

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KL 3

STABILITY MATTERS: INDUSTRIAL REALITIES IN ENZYME APPLICATIONS

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For enzymes to be viable in industrial applications, stability is often as critical as catalytic performance [1]. While a minority of processes require thermostable enzymes due to high operating temperatures, most industrial enzymatic reactions occur under ambient conditions. Nevertheless, enzymes are expected to perform reliably over extended periods in complex and often harsh environments.

Enzyme stability directly impacts process efficiency, consistency, and cost-effectiveness. Stable enzymes reduce dosing requirements, support longer reaction times, and improve overall process robustness. Furthermore, stability during manufacturing and storage is essential for commercial viability.

In this presentation, I will outline several representative industrial applications with defined stability–activity requirements and discuss how enzyme engineering enables us to tailor enzymes to meet these demands. Enzyme engineering — encompassing semi-rational approaches and bioinformatics-guided design — is a key part of our workflow to enhance enzyme properties and bring robust biocatalysts from the lab to the production floor.

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KL 4

IMMOBILIZED BIOCATALYSTS IN MINIATURIZED FLOW SYSTEMS

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The efficient immobilization of enzymes and whole cells in miniaturized flow devices presents significant opportunities for enhancing biocatalyst stability and intensifying biocatalytic processes. The integration of continuous-flow microreactors further accelerates bioprocess development, enabling rapid optimization and scale-down studies [1].

This presentation highlights several successful examples of continuous biocatalytic systems employing diverse immobilization strategies. Case studies include amine transaminase-catalyzed synthesis of various amines performed in aqueous media [2-4], as well as with the addition of selected deep eutectic solvent systems. Furthermore, the microfluidic generation of cross-linked enzyme aggregates (CLEAs) and their subsequent integration into a microbioreactor are demonstrated as a robust approach to enzyme immobilization [5]. Finally, the model-based design of a yeast cell-catalyzed reaction within a hydrogel-coated microflow reactor illustrates how mathematical modeling can support process design and optimization [6].

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KL 5

IMMOBILISATION OF SELECTED ENZYMES FOR MORE STABILITY: CASE STUDIES AND APPLICATIONS

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Enzyme immobilization is the confinement of enzymes in inert supports to improve their stability and allow multiple uses. Recently, enzymes trapped in zeolitic imidazole frameworks (ZIF-8) or bounded on various carbon nanotubes (CNTs) or silica supports can maintain their catalytic activity even under harsh conditions. Building on this idea, a thermostable PETase from *Kibdelosporangium aridum* (*Ka*PETase) was first characterized, encapsulated in ZIF-8, and then crosslinked with glutaraldehyde. Analysis confirmed that the framework remained intact after enzyme loading, and kinetic tests showed a roughly 2.7 and 1.7-fold increase in catalytic efficiency, and approximately 3- and 4-fold increase in thermal stability, respectively. Furthermore, *Ka*PETase has also recently studied with functionalized silica and CNTs as an immobilization support. Functionalized silica with amino, aldehyde, and glutaraldehyde groups makes the *Ka*PETase active at a rate of 88, 89, 91%, respectively, after incubation at 75 °C for 24 h. Similarly, CNTs utilization for *Ka*PETase immobilization resulted in enhanced thermal stability with different functional groups, including amino, glutaraldehyde, and sulfone.

In the second study, a calcium-independent α-amylase from *Priestia megaterium* (*Pm*Amy) was successfully synthesized and characterized. The free enzyme was most active at pH 7.0 and 40 °C but lost activity above 50 °C. By immobilizing *Pm*Amy on genipin-coated CNTs and on silica, its recovered activity was increased to 164 % and 134 % of the free enzyme, respectively, and extended its half-life at 40 °C from 15 hours to over 82 hours. After ten reuse cycles, both immobilized forms retained more than 85 % of their initial activity. These results show that choosing the right support material can greatly improve enzyme performance, stability, and reusability for industrial processes.

These material-specific approaches improve enzyme performance, stability, and recyclability, providing clear model for creating robust biocatalysts in a variety of industrial processes and promoting more sustainable enzymatic technologies.

Acknowledgement
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ORAL PRESENTATIONS

OP 1

NATURAL DEEP EUTECTIC SOLVENTS ENHANCE THE STABILITY AND THE CATALYTIC PERFORMANCE OF ENZYMES

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Natural deep eutectic solvents (NADES) emerged recently as a novel class of green solvents and a prospective solution for most problems related to the reaction media in biocatalytic conversions, being nontoxic, biodegradable, and biocompatible. In this study, we discuss an efficient route for the enzymatic esterification of carbohydrate polyols using reactive NADES (R-NADES) functioning both as a solvent and as a reagent pool [1]. We also discuss the remarkable effect of R-NADESs on the stability, selectivity and catalytic efficiency of the enzyme [1].

Binary hydrophilic R-NADESs consisting of choline chloride (ChCl) as hydrogen bond acceptor (HBA) and sugar alcohols (D-sorbitol, xylitol, D-arabitol) as hydrogen bond donors (HBD), were prepared and characterized. R-NADESs were stable fluids with low viscosities between 40-80°C, that is the optimal temperature range for lipase-catalyzed reactions. The polyol-based R-NADESs were compatible reaction media for several native and immobilized lipases. Lipase B from *Candida antarctica* immobilized on acrylic resins (LAR) showed significant esterification activity and high thermal stability in tested R-NADESs, maintaining up to 98% of activity upon incubation at 70°C for 72 hours, that matches an activity decrease of 0.028% per hour. Besides, LAR effectively catalyzed the synthesis of lauryl esters of carbohydrate polyols in the binary ChCl/polyol R-NADESs tested, with high substrate conversion, high selectivity and high product yield. Structural analysis of isolated products demonstrated that the reaction products are solely the diesters of the polyols, i.e. 1,5-dilauryl-D-arabitol, 1,5-dilauryl-xylitol and 1,6-dilauryl-D-sorbitol. A combination of docking and molecular dynamics simulations allowed to rationalize the structural stability of the lipase B from *Candida antarctica* (CalB) active site in R-NADES, as well as the selectivity of the catalyzed esterification. The biocompatibility of hydrophilic NADESs and thermal stability of enzymes are positively associated with the number of hydroxyl groups and the length of the carbon chains.

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OP 2

NOVEL HYDROLYTIC ENZYMES SCREENING: A DESIGN OF EXPERIMENT APPROACH FOR BIOCATALYZED POLYCONDENSATION REACTIONS

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The present work focuses on the expression and characterization of novel thermophilic enzymes belonging to the α,β-hydrolases superfamily (LCC*, leaf-branch compost cutinase*; LCC^{ICCG}, *leaf-branch compost cutinase variant*; ThB from *Thermoanaerobacterales bacterium*) to find possible alternatives to the already known biocatalysts (e.g., *Candida antarctica* lipase B, CaLB; *Humicola insolens cutinase*, HiC; *Thermobifida cellulosilytica cutinase 1*,Thc_Cut1) in biocatalyzed synthetic reactions [1,2,3]. After expression and purification, these enzymes were immobilized onto polypropylene beads and used to perform a design of experiments (DoE) assisted study to investigate their synthetic potential to produce short flavour esters. The software MODDE® Pro 13 (Sartorius) was used to develop a 3-levels full-factorial design to analyze the thermostability and selectivity of the immobilized enzymes towards alcohols and acids with different chain lengths in short-esters synthesis reactions. MODDE® optimized the coefficients involved in the DoE (temperature, alcohol length, acid length, and reaction time) to obtain a visual model that defined the optimal synthetic profile of the biocatalysts. The response measured for each experiment was the conversion rate of the acid as determined by GC-FID analysis. The temperature optima of LCC, LCC^{ICCG} and ThB were 60°C, 55°C, and 80°C respectively corresponding to the maximum percentage of conversion for long-chain alcohols and acids as substrates. Polymerization of dimethyl adipate (DMA) and 1,8-octanediol (ODO) as building blocks was carried out to confirm the applicability of the obtained model for the synthesis of larger macromolecules via polycondensation reactions. Conversion of monomers, studied by Nuclear Magnetic Resonance analysis (NMR), was >83% for all enzymes while the number average molecular weights (M_n) of the polyesters, analyzed by gel permeation chromatography (GPC), were between 3300 and 3600 Da for LCC and its variant LCC^{ICCG} while resulted to reach up to 4200 Da for ThB.

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OP 3

TWO GLYCOSIDE HYDROLASES CHARACTERIZED FROM JERMUK HOT SPRING METAGENOMES

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The use of macroalgal biomass as a renewable energy source has recently attracted considerable interest due to its abundance and the absence of lignin, which simplifies its bioconversion. In particular, valorizing macroalgae currently considered waste could offer substantial environmental and economic benefits [1]. However, a major limitation in developing a macroalgae-based bioeconomy lies in the lack of suitable microorganisms and enzymes capable of efficiently degrading algal polysaccharides or modifying their structure to enhance their functional properties. While several specific enzymes have been identified in recent years, no commercial enzymatic systems are currently available for the effective biodegradation of marine biomass [2].

In this context, the metagenome of the Jermuk hot springs in Armenia was explored to identify novel glycoside hydrolases (GHs), particularly β-1,3-glucanases (EC 3.2.1.39), also referred to as laminarinases. Computational analysis revealed 60 candidate open reading frames (ORFs), which were further evaluated. Based on sequence identity, similarity to known laminarinases, and domain architecture, 10 ORFs were selected for cloning. All candidates possessed GH16 domains characteristic of laminarinases, except for ORF8, which contained a GH5 domain and a CBM4_9 module typically associated with cellulases.

ORF1 encodes a protein of 287 amino acids with a calculated molecular mass of 34.9 kDa, classified within the GH16 family. BLAST analysis showed 69.4% sequence identity with a protein from the *Fidelibacteriota* phylum. ORF8 encodes a 574-amino acid protein with a molecular mass of 65.3 kDa, belonging to the GH5 family, and shares 67.1% identity with a glycoside hydrolase from a *Prolixibacteraceae* bacterium. Both enzymes were successfully expressed, purified to homogeneity, and characterized.

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OP 4

A NOVEL PLATFORM FOR THE SELF-IMMOBILIZATION AND STABILIZATION OF PLASTIC-DEGRADING ENZYMES

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The accumulation of plastic waste in the environment has become one of the biggest challenges facing the world in the 21st century, with severe consequences not only for wildlife but also for human. Polyethylene terephthalate (PET) is the most abundant polyester plastic in the world, with an annual production of 70 million tons. However, less than 20% of PET is recycled, with most of the volume being released into landfills and oceans [1]. In response, enzyme-based biodegradation has recently emerged as an eco-friendly and cost-effective strategy for managing PET waste. In this work, we propose the use of a proper technology, the IC-Tagging system, as a novel platform for the immobilization and stabilization of PET-degrading enzymes in order to overcome some of their constraints, such as efficiency, reusability or thermal stability. Using bacteria as expression system, IC-Tagging allows us to load any enzyme of interest into protein nanospheres, maintaining its correct folding and catalytic activity. Our results demonstrate the capability of this method for the immobilization of active LCC^{ICCG}, the most promising enzyme for large-scale PET degradation. Immobilized enzyme exhibits high stability to temperature and can be reuse up to 10 cycles with a retained activity of over 70%. Furthermore, our stabilized enzyme has proved to nearly fully depolymerized untreated post-consumer plastic from diverse sources as well as different coloured PET at small scale and at a broad range of temperatures (70ºC, 60ºC and 50ºC in 72 hours, 96 hours and 14 days, respectively) when a reuse strategy is performed (Figure 1). These findings, combined with an easy purification process and stability during long-term storage of immobilized enzymes, lay the foundations for the use of IC-Tagging for recycling or upcycling PET residues to value-added products, contributing towards a circular plastic economy.

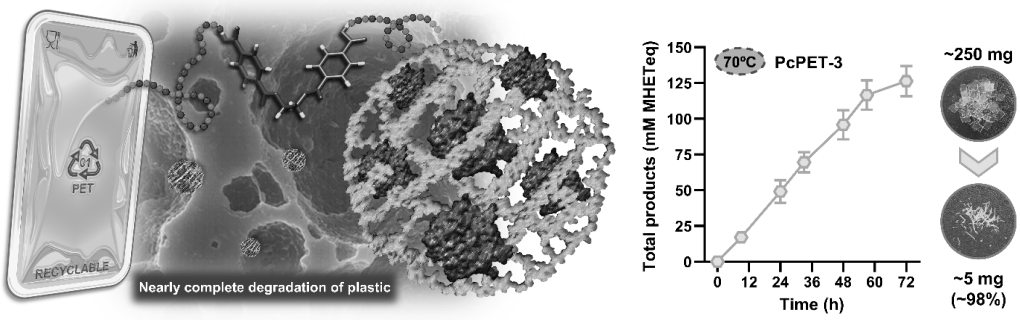

Figure 1. Nearly complete depolymerization of post-consumer PET by immobilized enzyme

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OP 5

EXPLORING AND UNLOCKING THE FUNCTIONAL DIVERSITY OF AROMATIC AMMONIA-LYASES

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Aromatic ammonia-lyases (AALs) have gained increasing attention as versatile biocatalysts for the synthesis of valuable unnatural amino acids [1]. During our efforts to develop a general rational design-based engineering approach of phenylalanine ammonia-lyases (PALs) [2] several improved PALs of high activity for highly valuable substrates have been developed [3]. Further we combined the rational design approach with an extensive saturation mutagenesis strategy, both procedures unveiling variants of improved activity towards the targeted 3,4-dimethoxy-phenylalanine [4]. These efforts combined with the attempt to transfer the improved catalytic activities between PALs of different origin, highlighted the key selectivity modulator residues and also their fluctuation patterns. Using data mining as alternative strategy for identifying novel PALs, with activity toward challenging substrates, we explored several bacterial aromatic ammonia-lyases [5] with unique selectivity modulator residues and substrate specificity distinct from currently explored AALs. The genomic context of the novel bacterial AALs and the phylogenetic analysis of the evolutionary relationships of the AALs with reported HAL, PAL or TAL activities, suggests that the explored bacterial AALs represent a potentially distinct subclass, with the natural reaction and substrate scope modified through evolutionary processes. While our results reveal and highlight several selectivity modulator residues of AALs', the interconversion of the different type of AALs remains challenging, with recently obtained structural data also facilitating loop- and catalytic cavity engineering.

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OP 6

CHALLENGES TO TRANSPORT LIQUID DAIRY PROTEIN CONCENTRATES

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Liquid dairy protein concentrates are being used in the manufacturing of finished products instead of their spray-dried form. The liquids are obtained from skim milk, via membrane filtration as microfiltration or ultrafiltration. The final products are generally protein-enriched fresh products like yoghurts and drinks.

Transport of these liquids is a challenge: from a microscopic point of view, the increase of viscosity is explained by a higher dry matter (compared to skim milk). On top of that, higher protein content leads to more interactions between casein micelles, with the risk of gel formation. [1]

Literature known to us presents results in which the impact of storage is not explored. Viscosity reduction could be achieved with Ca-binding salts, but the effect of storage times longer than 2 days was not reported. [2] The European Food Regulation is very strict on the use of additives and allows their use only in specific products. [3]

Our approach is to collect data during longer storage time (7 to 10 days). The goal is to understand what modifications appear and define good practice.

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OP 7

GREEN-SYNTHESIZED METAL–ORGANIC FRAMEWORKS FOR IMPROVED ADSORPTION AND CATALYTIC EFFICIENCY

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Metal-organic frameworks (MOFs) have emerged as a multifunctional class of porous materials characterized by tunable structures, large surface areas, and diverse functionalities, which make these widely investigated compounds extremely attractive for sustainable applications. Recently, attention has turned to developing environmentally friendly synthetic approaches for MOFs, with the aim of reducing energy consumption, solvent waste, and environmental impact [1]. Several two-component metal-VP or three-component metal-HEDP-Im (metal = Co, Ni, Zn, Cu; VP = vinylphosphonic acid, HEDP = 1-hydroxyethylidene-1,1-diphosphonic acid; Im = imidazole) MOFs, synthesized by green chemistry synthetic approaches such as microwave-assisted synthesis, ultrasound-assisted hydrothermal processes, and solvent-free mechanochemical techniques, offer efficient and environmentally friendly alternatives to conventional solvothermal methods. This work highlights the dual benefits of integrating MOFs and green chemistry: creating advanced hybrid/bio-hybrid materials while reducing the environmental footprint of both material synthesis and catalytic processes [2]. The resulting MOFs have demonstrated optimal results as green adsorbents for water pollutants, as heterogeneous catalysts for organic transformations, and as electrocatalysts for energy conversion processes [3,4]. In addition, biocompatible MOFs have shown promise as supports for biocatalysts, enabling mild and selective reactions in pharmaceutical and biochemical applications [2]. Recently, it was first demonstrated that proteins like lysozyme and carbonic anhydrase B enter MIL-101 pores only when unfolded. Using this approach, mixtures of native and denatured proteins were purified, achieving over 99% native purity, opening new perspectives for MOF applications [5].

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OP 8

ENHANCED STABILITY AND FUNCTIONAL VERSATILITY OF IMMOBILIZED HEXOSAMINIDASE

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β-N-Acetylhexosaminidases (β-NAGA) are glycosidases with growing importance in applied biocatalysis, particularly for the synthesis and hydrolysis of biologically relevant glycosides. Among them, fungal β-NAGA enzymes, such as those from the genus *Penicillium*, offer an excellent source of hexosaminidase activity thanks to their broad substrate range and high catalytic performance.

In our recent work, we focused on the application of immobilized β-NAGA in chemoenzymatic processes aimed at the selective hydrolysis of β-anomers of aromatic-substituted GalNAc derivatives, enabling the preparative-scale isolation of the corresponding α-anomers. These compounds are widely used as chromogenic and fluorogenic substrates in diagnostic and analytical applications.

enhance operational stability and reusability, β-NAGA was immobilized using different techniques, including covalent binding into porous methacrylate carriers and entrapment in lens-shaped polyvinyl alcohol hydrogel particles. The immobilized enzyme systems demonstrated excellent performance in repeated-batch operations, with high hydrolytic yields (>99%) and long-term storage stability (up to 18 months at 4 °C with >90% retained activity).

Furthermore, we developed a simple and effective method for studying reversible inhibition of immobilized β-NAGA in a continuous-flow packed-bed reactor system. This approach was successfully applied to assess the inhibitory effects of two well-known Hex inhibitors, PUGNAc and NAG-thiazoline, with PUGNAc demonstrating strong inhibition characterized by a Ki value of 0.908 μM. These results provide a solid basis for rapid and efficient inhibitor screening and underscore the potential of immobilized Hex in pharmaceutical biocatalysis. Additionally, our findings highlight the advantages of enzyme immobilization in enhancing stability, reusability, and mechanistic characterization.

This work was supported by a grant APVV_22-0161.

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OP 9

2,5-FURANDICARBOXALDEHYDE AS A BIO-BASED BIFUNCTIONALIZED AGENT FOR COVALENT ENZYME IMMOBILIZATION

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Covalent immobilization of enzymes is often performed by binding the protein to a solid carrier through residues on the protein surface. One of the most commonly used bifunctional reagents for this aim is glutaraldehyde, which has several drawbacks due to its widely documented toxicity [1], and environmental hazard [2]. Moreover, its reactivity in aqueous environment is very complex [3], and it is not clear which form reacts with enzyme and carrier in the immobilization process.

We have therefore investigated 2,5-diformylfuran (DFF), a bio-based product derived from the dehydration of sugars, as a bifunctional crosslinker for the covalent immobilization of glucoamylase from *A. niger* on an amino-functionalized carrier. Immobilization experiments and systematic comparison with glutaraldehyde at four different dialdehyde concentrations (200, 20, 2 and 0.2 $\mu mol/g_{carrier}$) showed that the two molecules lead to comparable enzymatic activities. Even at the lowest tested crosslinker concentration, the DFF-immobilized enzyme displayed comparable activity to the glutaraldehyde-immobilized one and did not leach from the carrier despite the low pH (4.5) of the activity assay. NMR studies on DFF in aqueous solution confirmed that it reacts covalently with primary amino groups via imine bond formation only; the formed imine bond is stable also at acidic pH, most likely due to the resonance stabilization of the furan aromatic ring on the conjugated imine bond. Continuous flow experiments, conducted over the course of 13 consecutive days in a viscous reaction medium, confirmed the stability of the DFF-immobilized enzymes at acidic pH.

In conclusion, DFF can be employed as a replacement for glutaraldehyde for enzyme immobilization. This has several advantages: firstly, DFF is a bio-based molecule, unlike fossil-based glutaraldehyde; contributing to lower the environmental impact of the overall immobilization process. Moreover, DFF is a non-volatile solid compound, while glutaraldehyde is a highly volatile liquid that displays high toxicity and respiratory sensitization. The use of DFF allows then to immobilize enzymes minimizing the risks for the operator, and allowing for a fine tuning of the immobilization procedure thanks to the predictable reactivity of DFF in aqueous environment.

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OP 10

EFFECTS OF LEVOGLUCOSENONE-DERIVED SOLVENTS ON THE STABILITY OF IMMOBILIZED LIPASE B FROM *CANDIDA ANTARCTICA*

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Bio-based solvents have received considerable attention as potential substitutes for conventional solvents in polymer biocatalytic syntheses. Cyrene and Cygnet 0.0, high-boiling solvents derived from levoglucosenone that is synthetized from cellulose, are particularly promising candidates. Although both solvents have been successfully used in the synthesis of aliphatic polyesters catalyzed by immobilized *Candida antarctica* lipase B (CALB), their performance differs significantly, with Cygnet 0.0 yielding polymers with higher molecular weights [1]. Considering the underlying mechanisms responsible for this difference, the hypothesis of a structural effect on CALB has been formulated.

In this context, we examined the effects of Cyrene and Cygnet 0.0 on the stability of immobilized CALB by combining functional analyses with Fourier transform infrared micro-spectroscopy (micro-FTIR). This technique was developed to study the structural changes induced by organic solvents in immobilized CALB [2]. Interestingly, both solvents improved the stability of immobilized CALB at the reaction temperature (85 °C) by preserving the enzyme from aggregation. Although Cygnet 0.0 shows slightly better stabilization, our results do not fully explain its better polymerization performance. To further investigate enzyme behavior in the presence of both solvents, molecular dynamics simulations were performed. The computational analysis revealed two distinct interaction mechanisms, with Cygnet 0.0 appearing to stabilize CALB better than Cyrene.

Overall, this work provides new insights into the diverse mechanisms of action of organic bio-based solvents, which are valuable for the design of novel biocatalytic processes and selection of appropriate reaction media for lipase-catalyzed reactions.

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OP 11

USE OF GLUTARALDEHYDE CHEMISTRY TO COIMMOBILIZE ENZYMES ENABLING THE RE-USE OF THE MOST STABLE IMMOBILIZED ENZYME

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Amino-glutaraldehyde supports present three functionalities: cationic, hydrophobic, and covalent. Using preactivated supports (where two molecules of glutaraldehyde per amino group in the support were introduced), later reduced to eliminate the chemical reactivity, the β-galactosidase from *Aspergillus oryzae* was immobilized by physical adsorption but the enzyme could not be later released under conditions compatible with the enzyme's stabilities. However, if the support was modified with only one molecule of glutaraldehyde per amino group, the enzyme could be easily released by just using high ionic strength. This was similar to the situation of immobilizing an enzyme, treating it with glutaraldehyde to get one glutaraldehyde molecule per amino group, and then reduced it, immobilizing a second enzyme via ion exchange [1].

This way, the co-immobilization of two enzymes with very different stabilities was studied using this strategy. Eversa Transform 2.0 was used as a model of a stable enzyme, and β-galactosidase from *Aspergillus oryzae* as an unstable one. First, Eversa Transform 2.0 was immobilized via ionic exchange to MANAE and later was modified with glutaraldehyde to get enzyme-support covalent bonds. The biocatalyst was reduced with sodium borohydride, and the β-galactosidase was coimmobilized with the lipase, in this instance via reversible ionic exchange. This combibiocatalyst was incubated at 55ºC and pH 8, conditions where the β- galactosidase lost its activity while Eversa retained its activity intact. Next, the combibiocatalyst was incubated at high ionic strength to release the inactivated β-galactosidase from the support. After, fresh β-galactosidase was coimmobilized on the biocatalyst and the combi-biocatalyst was inactivated again. This cycle was repeated three times with identical results [2].

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OP 12

UPCYCLED KERATIN SCAFFOLDS CONFER PROCESS-READY ROBUSTNESS ON *PSEUDOMONAS STUTZERI* LIPASE, ENABLING BATCH-TO-FLOW CATALYSIS

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Turning low-value wool waste into high-performance catalysts epitomises the industrial bioeconomy vision. We transformed sulphitolysed keratin into mechanically robust, macroporous sponges (CKS) via hexamethylene-diisocyanate cross-linking and freeze-drying. SEM analysis revealed an interconnected 3-D network (72 µm mean pore size, 68% porosity), with a multimodal porous structure [1]. After hydrolysis of the residual –NCO groups, the resulting sponge (CKS') was employed as support for the immobilisation of *Pseudomonas stutzeri* lipase LC2-8 at pH 7.5. The resulting biocatalyst (PSL-7.5@CKS') attains an enzyme loading of 5.8 mg g^{-1} and a specific activity of 8.54 U g^{-1} (0.5 mM *p*-nitrophenyl butyrate, 25 °C). Compared to the free enzyme, immobilisation significantly improves operational life: PSL-7.5 retains >80% initial activity for >10 consecutive cycles, whereas the free enzyme loses >50% after a single run. n n-hexane, the material enabled the kinetic resolution of *rac*-1-phenylethanol, achieving 52% conversion to the (*R*)-acetate with 96% ee after 5 h, while retaining >90% ee after 17 h. The same pellets catalyse 50% conversion of geraniol to geranyl hexanoate, a fully biobased ester prized as a natural fruity–floral flavour and known sex pheromone for click-beetle pests (*Agriotes* spp.), enabling eco-friendly pest-monitoring traps. For process intensification, PSL-7.5 powder was packed into a 0.7 cm × 4 cm stainless-steel packed column. Continuous flow (5 µL min^{-1}, 60 °C) maintained stable esterification for 45 h without performance loss across successive substrate switches for the production of geranyl hexanoate, isopropyl palmitate, and butyl hydrocinnamate, achieving >80% conversion within 15 h for each of the tested substrates. Throughout operation, back-pressure remained <10 bar, confirming the mechanical integrity of the keratin support. This study shows that waste-derived keratin can be engineered into recyclable, biodegradable supports that stabilise industrial lipases in both batch and flow, unlocking cost-competitive access to high-value esters spanning the flavour, fragrance, and agro-biotech sectors.

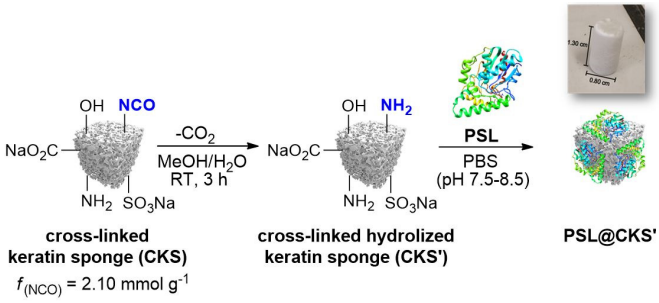

Figure 1. Preparation of PSL@CKS': cross-linked keratin sponge (CKS) to CKS' and used for *Pseudomonas stutzeri* lipase immobilization.

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OP 13

IMMOBILIZATION OF β-LACTAMASE ONTO MAGNETIC NANOPARTICLES FOR ANTIBIOTIC DEGRADATION PATHWAY

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Antibiotics, such as penicillins, are extensively utilized for treatment of bacterial infections for humans and animals and have been spreading in water due to their extremely low metabolic rate. The ever-emerging environmental pollution caused by the abuse of antibiotics and other pollutants has caused a serious threat to the environment and human health, therefore development of effective strategies for degradation, as well as disposal of antibiotic residues in water is urgently needed.

Immobilized β-lactamase onto magnetic nanoparticles (MNPs) was used for the degradation of penicillin (PEN). Thermal stability and reusability of such immobilized β-lactamase was investigated, as well as enzyme kinetics of free β-lactamase and immobilized β-lactamase was determined. Degradation study of PEN was performed with free and immobilized β-lactamase and analyzed using HPLC system. The degradation study of PEN suggested that immobilized β-lactamase degraded PEN more efficiently than free enzyme, since it achieved 98% degradation of PEN after 24 hours, compared to the free enzyme where only 22% degradation was observed. The influence of different PEN concentrations was studied as well, indicating that higher concentrations of PEN slow down the degradation process. PEN with a concentration of 0.05 mg/mL was completely degraded by immobilized β-lactamase after 5 hours, while 45% degradation was achieved after 5 hours by immobilized β-lactamase with PEN concentration of 0.5 mg/mL. Finally, when the concentration of PEN was lowered to 0.01 mg/mL it was found out that after 5 hours 96% was degraded, thereby confirming that the degradation of PEN occured the fastest at its concentration of 0.05 mg/mL.

The results also showed that such immobilized β-lactamase is thermally stable and can be reused for degradation of PEN, therefore providing an efficient tool for successful water treatments in order to ensure environmental safety and have an important practical significance in improving the environment and human health.

Acknowledgements
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OP 14

ADDRESSING SENSITIVITY AND NON-UNIQUENESS CHALLENGES IN DETERMINING ENZYME KINETICS PARAMETERS FOR BIOCATALYTIC REACTIONS

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Accurate determination of enzyme kinetic parameters is critical for model-based design and intensification of biocatalytic processes, particularly in microscale systems. While Michaelis-Menten kinetics provides a foundational framework, its extension to reversible, multi-substrate, and inhibited reactions introduces significant challenges in parameter estimation—most notably, parameter sensitivity and non-uniqueness.

This study systematically investigates these challenges across three case studies of increasing complexity: (i) mono-substrate Michaelis-Menten kinetics, (ii) reversible enzymatic reactions with four parameters, and (iii) a six-parameter ping-pong bi-bi model with inhibition. In the first two cases, we show that vastly different parameter sets can yield nearly indistinguishable model fits to experimental data, exposing the limitations of classical graphical and nonlinear regression methods. In the third case, a theoretical and numerical proof demonstrates the intrinsic non-uniqueness of the parameter estimation problem. Using perturbation analysis and null-space exploration of the Jacobian matrix, we identify families of parameter vectors that produce identical model outputs.

Our findings [1,2] emphasize the need for more robust, structurally-informed modeling approaches in biocatalysis and suggest strategies for overcoming limitations in kinetic model calibration. These insights support the reliable use of enzymatic models in process intensification and design of microreactor systems.

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OP 15

VERSATILE NANOENCAPSULATION PLATFORM FOR THE STABILIZATION AND ORAL DELIVERY OF PROTEINS

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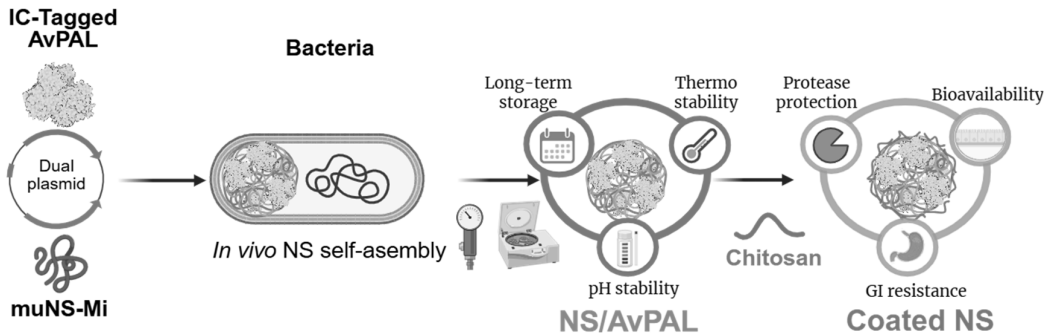
Pharmaceutical research and development is increasingly focusing on biopharmaceuticals, including peptide and protein drugs. Despite their importance, the vast majority are still only available by injection. Permeability barriers and gastrointestinal (GI) instability (pH, digestive enzymes, microbiota, etc.) are major factors influencing the low oral bioavailability of protein drugs.

To address these issues, we propose the use of the IC-Tagging system patented in our laboratory as an advanced single-step, *in cellulo* nanosphere (NS)-encapsulation strategy for protein stabilization and oral delivery. This technology enables the direct production of NS loaded with any protein of interest, maintaining its native functionality, correct folding and quaternary interactions, allowing them to perform complex enzymatic reactions [1]. These NS are easy to purify using straightforward and cost-effective methods. Recently, a highly active version of AvPAL (a candidate enzyme to treat phenylketonuria) was produced to which nanoencapsulation provides formidable thermostability, long-term storage stability, resistance to acidic pH and proteolytic degradation protection. This latter characteristic, essential for oral delivery of polypeptides, is further enhanced by coating with chitosan the NS-encapsulated enzyme (Figure 1). Thus, a similarly nanoencapsulated and chitosan coated luciferase displays sustained enzymatic activity through the entire GI transit when administered orally in mice, indicating the *in vivo* high protective capability of the system while maintaining the availability of the enzyme. Furthermore, a new version of the technology, called MiST-IC tagging, has recently been generated and patented, allowing surface-specific derivatization of the NS by sortase-mediated ligation. This is of great interest for cellular or molecular targeting therapies and for diagnostic applications.



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OP 16

ENHANCING LIPASE STABILITY THROUGH IMMOBILIZATION IN SOL-GEL SYSTEMS

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Lipases (EC 3.1.1.3) are highly versatile enzymes that have found extensive application in biocatalysis. These enzymes are employed in a variety of reactions, including esterification, transesterification, and enantioselective kinetic resolution. However, their industrial applicability is constrained by their low stability in non-aqueous media and the difficulty of reuse. In this regard, entrapment in hybrid sol-gel matrices has been shown to provide a stable and protective environment for lipases, allowing for the tailored design of properties for specific applications through the selection of precursor silanes, additives, and immobilization protocols [1]. An extensive array of organic silane precursors enables a multitude of enzyme-specific methodologies and fine tuning of the desired characteristics. The most prevalent precursors, tetraalkyl orthosilicates (tetraalkoxysilanes) $Si(OR)_4$ (R being methyl or ethyl) in a mixture with trialkoxysilanes ($R'Si(OR)_3$) or dialkoxysilanes ($R'R''Si(OR)_2$) substituted with alkyl or aryl functional groups (R′ and R″), have demonstrated facilitating the modulation of the properties of the xerogel matrix [2].

This work presents the results achieved by the Green Chemistry and Biocatalysis Group from Timisoara in the immobilization of lipases by sol-gel entrapment or by sol-gel entrapment in combination with deposition on a solid support, including functionalized magnetic supports [3-5]. The focal point of this research was the enhancement of the activity, stability, and selectivity of the heterogeneous biocatalyst, with a particular emphasis on its structural and morphological characteristics. The characterization of the immobilized biocatalysts and the correlation of their catalytic efficiency with the morphological and physicochemical properties of the sol-gel matrix were accomplished by scanning electron microscopy (SEM), fluorescence microscopy (FM), FT-IR spectrophotometry, as well as thermogravimetric and differential thermal analysis [2-6].

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OP 17

Leveraging Artificial Intelligence and Structural Data to Design Proteins with Improved Solubility

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Having computational tools to design proteins with improved solubility is of vital importance across many areas of the biotechnology industry. Protein solubility issues are often a bottleneck in various bioprocesses, from antibody engineering to industrial enzyme production, and are also implicated in major diseases such as Alzheimer's. Reliable computational methods can therefore greatly assist in the rational design and optimization of proteins.

In this presentation, I will introduce SOuLMuSiC [1], our computational algorithm developed to predict the effects of mutations on protein solubility. SOuLMuSiC takes as input a set of informative features, including the biophysical properties of the wild-type and mutant residues, energy contributions computed from the three-dimensional structure of the wild-type protein [2], and mutational embeddings extracted from recently introduced protein language models [3]. These embeddings provide a way to capture the evolutionary history and context of each residue in the sequence.

The model combines these features using a simple artificial neural network architecture. It was trained on a high-quality, manually curated dataset of approximately 700 mutations with experimentally measured solubility values collected from the literature. SOuLMuSiC significantly outperforms current state-of-the-art predictors in strict cross-validation, achieving a Spearman correlation coefficient of approximately 0.6 between predicted and experimental solubility values. It also generalizes well to external datasets, including: (1) high-throughput enzyme solubility assay data, where it accurately predicts the impact of single-site mutations on Levoglucosan kinase (LGK); (2) protein aggregation scores for Aβ42 variants; and (3) although primarily designed for single-site mutations, it also performs reasonably well on multiple mutations.

Overall, SOuLMuSiC is a valuable tool for identifying solubility-altering mutations, supporting both the rational design of proteins with improved solubility and the functional interpretation of disease-related genetic variants. The model is freely available to the scientific community through our webserver: http://babylone.ulb.ac.be/SoulMuSiC/.

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OP 18

HIGH-THROUGHPUT ASSAYS DEVELOPMENT FOR SCREENING SATURATION MUTAGENESIS-DERIVED PHENYLALANINE AMMONIA-LYASE LIBRARIES

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Aromatic ammonia-lyases (AALs) of the MIO-enzyme family, particularly phenylalanine ammonia-lyases (PALs), have gained increasing attention as versatile biocatalysts for the synthesis of valuable unnatural amino acids.[1] The development of high-throughput assays that are both easy to implement and efficient, remains essential for efficiently identifying phenylalanine ammonia-lyase variants from large libraries generated through semi-rational design or directed evolution, thereby expanding the versatility of the PAL enzyme toolbox. In this context, a high-throughput solid-phase assay was designed for screening PAL variants in the ammonia addition direction, complemented by a liquid-phase assay targeting the ammonia elimination reaction. The solid-phase assay involved coupling the PAL library with the ancestral L-amino acid oxidase (AncLAAO-N1), leveraging the hydrogen peroxide produced during FAD cofactor regeneration in the LAAO-catalyzed reaction. A peroxidase-coupled colorimetric system detects H_2O_2 via oxidation of a chromogenic substrate, enabling visual identification of active colonies.[2] Additionally, the platform can be adapted to assess reversed enantioselectivity by incorporating D-amino acid oxidase (DAAO), using the same detection principle to screen for D-selective PAL variants.[3] For the liquid-phase assay, crude cellular extracts served as the enzyme source, and the selection of active variants was based on UV-spectrophotometric monitoring of the formation of *trans*-cinnamic acid and its derivatives during the ammonia elimination reaction. This assay enables the screening of PAL libraries for variants with enhanced catalytic activity toward unnatural substrates, as well as for those exhibiting improved thermostability, offering a versatile tool for functional characterization and optimization.

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PS2	**Engineering the Substrate Specificities of D-Hydantoinase and D-Carbamoylase** Hasmik Grigoryan[1,2], Ani Paloyan[1], Lucia Gardossi[3], Anamaria Todea[4] *[1]Scientific and Production Center "Armbiotechnology" National Academy of Science of Armenia, Yerevan, Armenia* *[2]Research Center of Genetics, Selection and Feeding of Agricultural Animals, Armenian National Agrarian University, Yerevan, Armenia* *[3]Università degli Studi di Trieste Department of Chemical and Pharmaceutical Sciences, Trieste, Italy* *[4]Politehnica University Timisoara, Romania, Department of Applied Chemistry and Engineering of Organic and Natural Compounds, Biocatalysis and Green Chemistry Group, Timişoara, Romania*
PS3	**Novel Structural Insights into Phenylalanine Ammonia-Lyases: A Step Toward D-Selective PALs** Krisztina Boros[1], Pauline A. Lang[2], Alina Filip[1], Christopher J. Schofield[2], Jürgen Brem[1], László C. Bencze[1] *[1]Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca, Romania* *[2]Department of Chemistry, Chemistry Research Laboratory, University of Oxford, United Kingdom*
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PS25	**Factors Influencing Protein Opacity in Diabetic Cataract** Mariana Anghel[1], Adina Iuliana Milcu[2,3] *[1]Department of Infectious Diseases, Discipline of Epidemiology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania* *[2]Discipline of Ophthalmology, Department of Surgery I, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania* *[3]Department of Ophthalmology, Municipal Emergency Clinical Hospital, Timișoara, Romania*
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POSTERS

PS 1

COMPUTATIONAL ENGINEERING AND STRUCTURAL CHARACTERIZATION OF THERMOSTABLE PPDYP VARIANTS FOR INDUSTRIAL APPLICATIONS

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Stabilizing enzymes with industrial relevance is pivotal to advancing their implementation in innovative biotechnological processes and for improving predictive tools in protein engineering. In this work, we have used the PROSS [1] and FireProt [2] algorithms to design more stable variants of *Pseudomonas putida* DyP-type peroxidase (*Pp*DyP) and 29 and 21 mutations were inserted in the variants, respectively. The kinetic and biochemical characterization shows that both enzymes exhibit remarkable improvements in their thermal stability. They display an increase of approximately 40 ºC in the optimal temperature relative to the wild-type, and enhanced structural robustness, with apparent melting temperatures of 72°C and 83°C versus 62°C (wild-type), and a remarkable ~400-fold increase in half-life at 60°C. Notably, these variants did not exhibit a stability–activity trade-off, as a 10-fold increase in enzymatic activity was observed. An in-depth analysis of the crystal structures of the variants, supported by molecular dynamics (MDs) simulations, reveals increased protein compactness due to a reduction in the cavities number and size, enhanced backbone rigidity, and the formation of new hydrogen bonds, hydrophobic contacts, and aromatic–aromatic interactions, both within monomeric units and across oligomeric interfaces. Notably, five out of eight mutations predicted by both algorithms appear to contribute directly to key protein stability parameters. Application of MDs simulations and residue interaction network analysis [3] shows that these mutations strengthen enthalpic interactions and promote tighter dynamic coupling, resulting in ring-like communication pathways linking monomers across tetrameric interfaces. These findings suggest that such dynamic connectivity may represent a structural signature of hyperthermostable quaternary proteins.

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PS 2

ENGINEERING THE SUBSTRATE SPECIFICITIES OF D-HYDANTOINASE AND D-CARBAMOYLASE

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The hydantoinase process, a cascade reaction catalyzed by D-hydantoinase and D-carbamoylase enzymes, is one of the methods for the synthesis of D-amino acids [1-2].

The aim of the research was to create an enzymatic cascade containing D-hydantoinase and D-carbamoylase enzymes for the production of D-amino acid derivatives. The cloning of genes for D-specific hydantoinase and carbamoylase enzymes was carried out by using E. coli expression systems. The following substrates were synthesized for the carrying out of biotransformation process: (D,L)-5-(4-hydroxyphenyl)-hydantoin, (D,L)-5-(2-hydroxy-4-bromophenyl)-hydantoin, (D,L)-5-(2-hydroxy-4-chlorophenyl)-hydantoin, (D,L)-5-(2-hydroxy-4-fluorophenyl)-hydantoin, (D,L)-5-(4-methoxyphenyl)-hydantoin, (D,L)-5-(4-methylphenyl)-hydantoin.

Substrate specificity tests showed that (D,L)-5-(4-hydroxyphenyl)-hydantoin and (D,L)-5-(4-methoxyphenyl)-hydantoin was preferred substrats by the enzymes, leading to the synthesis of D-4-hydroxyphenylglycine and D-4- methoxyphenylglycine. The reaction was optimized for temperature, pH, duration, and mixing, followed by purification and identification of the target amino acids.

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PS 3

NOVEL STRUCTURAL INSIGHTS INTO PHENYLALANINE AMMONIA-LYASES: A STEP TOWARD D-SELECTIVE PALS

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In the presence of high ammonia concentrations, phenylalanine ammonia-lyases (PALs) catalyze the synthetically valuable asymmetric hydroamination of cinnamic acid derivatives, yielding optically pure L-amino acids. Considering the broad substrate tolerance of *Petroselinum crispum* PAL (*Pc*PAL) [1-4], as well as the benefits of high atom economy, the absence of cofactor requirements, and the absolute regioselectivity of the PAL-catalyzed ammonia addition, reversing PAL enantioselectivity for the production of D-phenylalanine analogues represents a highly valuable strategy. Despite several engineering efforts [1,5], no D-selective PAL enzymes has been reported to date. All the employed strategies relied on available structural information on arylalanine ammonia-lyases (AALs), which after our exhaustive analysis seems incomplete in key aspects; thus, we aimed to obtain a complete crystal structure of *Pc*PAL complexed with a *trans*-cinnamic acid analogue in the presence of ammonia.

Having at our disposal *Pc*PAL mutants known to exhibit reduced enantiomeric excess in the transformation of substrates bearing electron-withdrawing substituents, we performed the co-crystallization experiments with D-m-CF_3-Phe. The novel ligand-bound structure presented an unreported catalytic site orientation of the corresponding cinnamic acid analogue. While this structure provides valuable insights for further rational design studies, it lacks structural information on the functionally significant loops due to their excessive flexibility. Therefore, in continuation we screened for stabilizing ligands – *via* inhibition kinetics, thermal shift assays, and reverse addition reaction monitored by HPLC – and identified *N*-phenylglycine (NPG) as a strong competitive inhibitor. Co-crystallization of *Pc*PAL I460V variant with NPG yielded the first catalytically competent crystal structure of *Pc*PAL, with well-defined secondary structures of both catalytically essential loops in the active, closed conformation. These two novel structures serve as a solid starting point for the rational design efforts targeting the development of D-selective PALs.

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PS 4

A D-AMINO ACID OXIDASE-BASED SOLID-PHASE ASSAY FOR SCREENING PHENYLALANINE AMMONIA-LYASE LIBRARIES FOR REVERSED STEREOSELECTIVITY

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Phenylalanine ammonia-lyase (PAL) efficiently catalyzes the stereoselective amination of *trans*-cinnamic acid (TCA) to produce optically pure L-phenylalanine (L-Phe). On the other hand, the importance of D-amino acids in the production of pharmaceutical compounds has increased, particularly in the synthesis of antibiotics, antidiabetic drugs, and enzyme inhibitors [1,2]. These aspects have led researchers to develop a PAL enzyme for D-phenylalanine (D-Phe) synthesis using rational and semi-rational design strategies. Despite these efforts, their natural L-selectivity remains the main obstacle. A promising approach which was not applied to PAL yet is directed evolution, a method for generating a mutant library (10^5 - 10^7 versions) by introducing random mutations during genetic amplification. A high-throughput assay for mutants screening is indispensable for this method.

The objective of this research was to develop and optimize a solid-phase high-throughput screening assay based on a coupled enzymatic system involving D-amino acid oxidase (DAAO), designed to efficiently detect active D-selective PAL mutants.

During the optimizations process, the stability of the DAAO was evaluated, considering the composition of the enzymatic cascade and inhibitory effects were checked. As a result of the tests performed, the following protocol was developed: The cells, harboring the plasmids with *pal* library co-transformed with *daao* are transferred on a membrane and after the expression, the cells are pre-treated with horseradish peroxidase (HRP) [3]. The mixture for assay is prepared in 2 M ammonium carbamate: 10 mM TCA, 1.5 mM 4-aminoantipiryne (4-AA), 2 mM vanillic acid (VA) and 50 U/mL HRP.

Acknowledgements
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PS 5

BREAKING THE WALL: TARGETING THE *Mt*DprE1–*Mt*DprE2 COMPLEX IN TUBERCULOSIS

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There is an urgent need for new types of antibiotics, as many bacterial strains have developed multidrug resistance in recent years. This resistance has emerged rapidly, partly because most of the so-called "new" antibiotics are derivatives of older compounds [1]. One promising strategy to overcome this challenge involves targeting protein–protein interactions (PPIs), which are critical for various essential biological processes in bacteria [2].

One such target is the *Mt*DprE1–*Mt*DprE2 complex, which plays a crucial role in the biosynthesis of decaprenylphosphoryl-β-D-arabinose (DPA)—a key building block of the arabinan domain in the *Mycobacterium tuberculosis* (*Mt*) cell wall [3]. This complex consists of two enzymes: decaprenylphosphoryl-β-D-ribose oxidase (DprE1) and decaprenylphosphoryl-2-keto-β-D-erythro-pentose reductase (DprE2), which act sequentially to catalyze the epimerization of decaprenylphosphoryl-D-ribose (DPR) to DPA [4].

In our study, we optimized and screened various expression conditions in case of the co-expressed proteins (varying the growth media, incubation temperature, IPTG concentration) to improve the yield and stability of the *Mt*DprE1–*Mt*DprE2 complex. To further characterize the complex, we applied ion exchange chromatography to separate and validate the individual subunits (DprE1 and DprE2) as well as the assembled complex.

Additionally, using a computationally built-up structure of the *Mt*DprE1–*Mt*DprE2 complex, we investigated the predicted protein–protein interaction interface. Based on this model, we proposed several point mutations aimed at validating the interaction interface and providing insights into the structural basis of complex formation, with future applications in structure-based inhibitor design.

Acknowledgements
This work was supported by the BACPROBIO project, funded by European Union – NextGenerationEU and the Romanian Government, under National Recovery and Resilience Plan for Romania, contract no 760251./28.12.2023, cod PNRR-C9-I8-CF92/31.07.2023, through the Romanian Ministry of Research, Innovation and Digitalization.

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PS 6

STRUCTURE-BASED PHARMACOPHORE MODELING AND PROTEIN COMPLEX MODELING OF DPRE1-DPRE2 FOR ANTI-TUBERCULOSIS DRUG DISCOVERY

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The fight against tuberculosis continues to face critical hurdles, particularly due to the emergence of antibiotic-resistant strains of *Mycobacterium tuberculosis*. A promising strategy to overcome this challenge involves targeting the essential epimerases DprE1 and DprE2, which are crucial for cell wall biosynthesis. In this study, we focused on the stabilization and structural characterization of DprE1 and its interaction with DprE2 to support rational drug design. Using a curated set of high-resolution X-ray crystallographic structures of DprE1, we conducted a comprehensive structural analysis to identify conserved features within the active site. This enabled the development of a robust, structure-based consensus pharmacophore model that captures recurring ligand-protein interaction patterns across diverse chemotypes.

The pharmacophore model was validated through molecular docking (AutoDock Vina with Vina and Vinardo scoring functions), which successfully reproduced known binding poses and informed the selection of optimal protein conformations for virtual screening.

The combined use of pharmacophore filtering and docking enabled more targeted screening of the Enamine database, focusing on novel scaffolds and fragment-derived compounds. Although docking scores alone showed limited correlation with reported IC_{50} and MIC values for over 500 compounds, integration with our pharmacophore model enhanced the relevance of selected hits.

In parallel, we constructed a structural model of the DprE1–DprE2 complex using protein-protein docking and homology modeling approaches, aiming to better understand the interface and allosteric effects that may influence DprE1 function and ligand binding. These models were further examined for possible interactions, using CAVER 3.0 and molecular dynamics simulations.

Ongoing work includes experimental validation and further refinement of the DprE1-DprE2 complex model to explore its implications for protein stabilization and ligand binding dynamics.

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PS 7

A HYPER THERMOPHILIC ENZYME FOR INDUSTRIAL BIOFILM CONTROL

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Biofilms present a significant issue in modern society. They cause issues in several areas including agriculture, medicine, and industry [1]. Biofilms are prolific in the medical industry where it is estimated that 80% of bacterial infections are biofilm related [2]. Many of the current methods of treatment for biofilms are unsustainable due to their use of harsh chemicals and antibiotics. An enzymatic treatment of biofilms is a promising alternative to the current treatment. A number of studies have demonstrated the efficacy of enzymes for treatment of biofilms, but very few have looked at utilising extremophiles for biofilm treatment. There is a huge amount of genomic data freely available for extremophiles enabling ease of mining. Using a variety of microbiological and biochemical techniques, one hyper thermophilic enzyme mined from a thermophilic database (HotZyme [3]) has been characterised and proven to be an effective treatment for biofilm inhibition, as well as a potential anti-virulence treatment.

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PS 8

TAILORED SOL-GEL MATRICES FOR IMPROVED BIOCATALYTIC FUNCTION OF ENTRAPPED HYDROLASES

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The rational design of novel and more efficient biocatalysts has become a central goal in industrial biotechnology over recent decades. Alongside advances in enzyme engineering and de novo design, immobilization techniques have proven essential for enhancing biocatalyst efficiency to meet the demands of large-scale and specialty product manufacturing. Among these methods, sol-gel entrapment has evolved into a versatile approach for immobilizing biomolecules. The ability to finely adjust the physicochemical properties of sol-gel matrices enables the entrapment of diverse enzymes, resulting in robust solid-phase biocatalysts with long-term stability and reusability [1,2].

Typically, ternary silane precursor systems offer enhanced control over matrix characteristics such as hydrophobicity, porosity, and functional group incorporation, tailored according to enzyme properties and intended applications. Additionally, the inclusion of ionic liquids as additives has notably enhanced the catalytic efficiency of lipase-based biocatalysts in our studies [3].

This presentation summarizes the methods developed by our research group to tailor sol-gel entrapment of hydrolases within hybrid organic-inorganic matrices, achieving improved stability while preserving catalytic performance. We highlight the optimization of immobilization parameters for enzymes including *Candida antarctica* lipase B, Subtilisin, Celluclast cellulase, and β-galactosidase, alongside their application in hydrolysis as well as regio- and enantioselective synthesis of industrially relevant bioproducts.

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PS 9

STABILITY ASSESSMENT OF ALDEHYDE OXYDOREDUCTASE FROM AROMATOLEUM AROMATICUM, UNDER VARIABLE THERMIC, SOLVENT AND OXYGEN PARAMETERS

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The Aldehyde Oxidoreductase from *Aromatoleum aromaticum* (AOR*Aa*) is a tungsten-containing enzyme that oxidizes aldehydes to carboxylic acids, but is also capable of catalyzing the reverse reaction, i.e., reduction of non-activated carboxylic acids to the corresponding aldehydes [1]. The enzyme exhibits the highest specific activity at 40°C and is completely inactivated at 50°C [2]. The purified AOR also shows higher resistance to oxygen than archaeal AOR, while being entirely stable in a fresh cell extract [2]. Finally, the enzyme's ability to use benzyl viologen (BV^{2+}) and NAD^+ as electron acceptors or molecular hydrogen as electron donor makes it an interesting catalyst candidate in the green chemical industry.

In this work, we studied the stability of AOR*Aa* in three different contexts: (**1**) the retention of activity as a function of temperature, (**2**) the protective effect of proteins from the fresh cell extract as well as other fractions against inactivation by oxygen, and (**3**) the stability of the enzyme in the presence of organic solvents, especially alcohols. The goal of the study was to optimize conditions that would allow the use of AOR jointly with alcohol dehydrogenase in a cascade, producing benzyl alcohol.

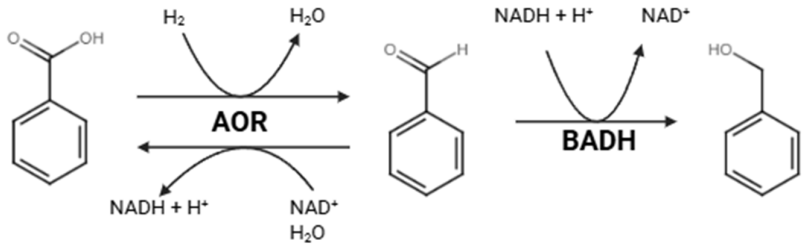

Figure 1. Reactions catalyzed by AOR: acid reduction to aldehyde using molecular H_2, aldehyde oxidation with NAD^+. The cascade uses NADH to reduce aldehyde to alcohol.

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PS 10

ENHANCED LONG-TERM STABILITY OF KstD VARIANT VIA IMMOBILIZATION ON FUNCTIONALIZED SILICA MESOPOROUS CELLULAR FOAMS

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Enzyme immobilization is a proven strategy to enhance the stability and reusability of biocatalysts, which is particularly beneficial in industrial and pharmaceutical applications [1]. In this study, we investigated the immobilization of a novel 3-ketosteroid Δ^1-dehydrogenase (KstD), from *Sterolibacterium denitrificans* [2], on mesoporous cellular foams (MCF) functionalized with amino groups (MCF-A) or metal ions (Co^{2+} or Ni^{2+}; MCF-Co, MCF-Ni). Two immobilization methods were employed: conventional covalent binding to glutaraldehyde-activated MCF-A carriers, and specific binding using histidine-tagged enzymes on transition metal-modified silicas (MCF-Co and MCF-Ni). Furthermore, two forms of the enzyme were used for immobilization: purified and crude enzyme preparations. The activity and stability of the resulting heterogeneous biocatalysts were then determined. The studies conducted demonstrated the high activity of catalysts obtained via covalent bonding or bonding via transition metals. Interestingly, similar activity was obtained for heterogeneous biocatalysts based on both purified and unpurified enzymes when using a non-specific (covalent) method of enzyme binding. When these two forms of enzyme were immobilized on transition metal-modified carriers, the biocatalysts obtained using the purified enzyme showed slightly higher activity. An important aspect of immobilization was its effect on the long-term stability of the biocatalysts obtained. After one month of storage at 4°C, the activity of immobilized KstD was significantly higher than that of the free enzyme.

Obtained results suggest that immobilizing KstD on appropriately functionalized MCF effectively anchors the enzyme and significantly extends its shelf life. This immobilization strategy paves the way for future research into KstD mutants or broader substrate profiling, particularly in the field of oxidative transformations of steroid skeletons.

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PS 11

OXYGEN-CARRYING PROTEINS EMPLOYED IN BLOOD SUBSTITUTE CANDIDATES: DIFFERENCES IN INTERACTIONS WITH A MODEL ANTIOXIDANT MOLECULE

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Glutaraldehyde-polymerized hemoglobin (poly-Hb) has long been explored as a key candidate for blood substitute compositions, to be used in transfusions in order to supplement the oxygen-carrying capacity following severe blood loss. Bovine hemoglobin (bHb) has been the standard choice for such efforts, due to its reasonable availability and to its reduced dependence on organic allosteric effectors. We have recently shown that poly-Hb produced from ovine Hb (poly-oHb) outperforms poly-bHb in in vivo tests employing transfusion after up to 30 % blood loss in animals. This improvement was found to correlate with an increased resistance of ovine hemoglobin (oHb) and of poly-oHb towards oxidative and nitrosative stress agents in vitro. The molecular bases for these differences in reactivity offer an interesting challenge, given the high sequence homology between vertebrate hemoglobins. Reported here is an investigation of these molecular bases using different spectroscopic (fluorescence, resonance Raman, NMR, EPR) and computational (molecular docking) methods to assess the interaction with a convenient probe ligand representative of the class of natural antioxidants, caffeic acid. Fluorescence experiments reveal that ovine Hb fluorescence saturates above 25 μ M caffeic acid and indicating full occupancy of fluorescence-responsive binding sites, while resonance Raman and NMR data indicate signals for the heme and indicate the differences between the types of Hbs and the antioxidant binding behavior after polymerization. Computational docking corroborated the spectroscopic data by identifying aromatic residues and distinct affinity patterns for caffeate. The results show that structural differences in oHb may explain a higher redox stability.

PS 12

INTERACTIONS OF SMALL MOLECULES WITH BLOOD PROTEINS

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The two major blood proteins, serum albumin and hemoglobin (Hb), both interact with a variety of small molecules. Albumin's multiple ligand binding pockets are well characterized due to its physiological role in transporting endogenous molecules, and its propensity to also bind exogenous ligands such as therapeutic agents. On the other hand, small molecule binding to Hb has mostly been described in terms of the ability to either coordinate to the iron, or bind to the polypeptide and trigger oxidative changes at the heme active site (such as autooxidation to metHb or the formation of ferryl Hb), or modulate the affinity for molecular oxygen. Recent evidence indicates that beyond Hb's classical ligand sites (the heme iron and the central 2,3-BPG cavity), additional unconventional small-molecule binding sites exist on its surface [1]. These non-canonical sites, though less characterized, may directly modulate Hb's redox reactivity and oxygen affinity [2,3]. Here, we present an integrative approach to identify and characterize such non-canonical binding pockets on Hb, using serum albumin as a reference. We combine molecular docking to predict potential ligand-binding pockets and paramagnetic ^{1}H-NMR spectroscopy to monitor binding induced spectral changes.

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PS 13

UV RADIATION EFFECTS ON PROTEINS: A CASE STUDY ON SERUM ALBUMIN

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This study investigates the effects of UV radiation on serum albumins (BSA, HSA, RSA) and highlights the formation of a unique chromophore. At typical protein assay concentrations, BSA shows fluorescence fluctuations, while at high concentrations and in the presence of PEG, light exposure can induce protein precipitation.

UV radiation (254 nm) generates a well-defined chromophore (Figure 1) at 310–320 nm in albumins [1], dependent on pH (7–9) [2] and independent of salt concentration, buffer type, or the presence of free thiols. The chromophore persists after filtration and proteolytic digestion, indicating it is part of the polypeptide. Its formation involves local covalent modifications of aromatic and sulfur-containing residues, likely including tyrosine and cysteine, without detectable free radical generation.

MALDI-TOF-MS analyses indicate minor mass changes after irradiation, compatible with disulfide rearrangements and local chemical modifications, without major protein degradation. In the presence of aquacobalamin, UV irradiation allows the formation of cobalamin-thiolate complexes, confirming disulfide reduction.

These findings reveal a specific, light-induced modification mechanism in albumins, with implications for photobiology, biophotocatalysis, and protein chromophore design. The results highlight the need to reassess experimental conditions in protein studies and open new avenues for investigating the molecular mechanisms of photoinduced modifications. Furthermore, this work provides insight into protein stability, with potential applications in developing novel methods for biomolecule protection and stabilization.

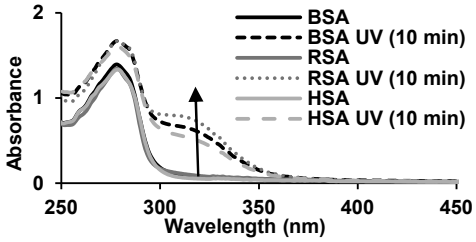

Figure 1. UV-Vis spectra of 30 μM BSA (bovine serum albumin), RSA (rabbit serum albumin), HAS (human serum albumin) before and after irradiation (240 nm) in PBS buffer pH 7.4, at 25°C

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PS 14

SPECTROSCOPIC EVALUATION OF THE INTERACTION OF MYOGLOBIN WITH BIOMEDICAL RELEVANT COMPOUNDS

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This study investigates the redox behavior of myoglobin (Mb) in the presence of several bioactive compounds with biomedical relevance. These include the well-known Pt(II) drug, cisplatin, oxoplatin (a Pt(IV) compound representative for a newer-generation drugs based on platinum), capric acid, fenbufen, plumbagin and fisetin. These molecules were selected based on their structural diversity and documented biological effects, such as anti-inflammatory, chemotherapeutic, or antioxidant properties. The study aimed to evaluate how these compounds affect the structural and redox properties of Mb, particularly in terms of their capacity to bind to the protein and modulate its oxidation state.

Spectroscopic methods (UV-Vis absorption, fluorescence, and NMR) and docking calculations were used to monitor binding and modifications of the redox state of the Mb in reactions such as autoxidation, nitrite-induced autoxidation, and peroxide-induced damage. These experiments are designed to parallel previous findings with similar experiments on hemoglobin with small molecules of biomedical/therapeutic relevance [1–4]. Our results demonstrate a differential response: while some compounds induced minimal or no spectral changes - suggesting limited interaction or redox impact, while others, such as fisetin and plumbagin, displayed notable pro-oxidative effects.

These findings suggest that fisetin and plumbagin may act as pro-oxidants or interact with Mb through mechanisms that alter their redox state, while the other compounds may exhibit stabilizing or potentially antioxidant effects. The observed variability highlights the complex nature of small molecule-protein interactions and underlines the importance of assessing individual compound behavior in biochemical systems.

This work contributes to a better understanding of how diverse small molecules influence the redox chemistry of globins, with implications for pharmacology and redox biology.

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PS 15

HEMOGLOBIN STABILIZATION FOR BLOOD SUBSTITUTES: SYNTHESIS, CHARACTERIZATION, AND LIPID PEROXIDATION STUDY OF AN ULTRAMER AND RELATED POLYMERS

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Most blood substitutes developed at this moment are based on hemoglobin because of its important role in oxygen transport [1]. We have previously reported several protocols for the preparation of reasonably stable poly- and copolyhemoglobins as potential oxygen carriers [2-5]. Here, we report the synthesis and characterization of a hemoglobin ultramer formed via glutaraldehyde cross-linking of rubrerythrin, rubredoxin oxidoreductase (NROR) and serum albumin (Hb-Rbr-NROR-HSA), demonstrated positive results in the relevant evaluation. The findings suggest that the ultramer exibits superior chemical properties compared to simpler forms such as Hb-Rbr, Hb-HSA or poly-Hb.

Additionally, we investigated the oxidative reactivity of the ultramer and a broader range of hemoglobin-based oxygen carriers toward lipids using spectrophotometric assays. It is well known that extracellular native hemoglobin and its copolymers act as active oxidants of unsaturated fatty acids, producing to quantitatively and qualitatively different peroxidation end products depending on the substrate's chemical composition and the nature of reactive species [6,7].

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PS 16

THERMAL STABILIZATION OF LIMONENE-1,2-EPOXIDE HYDROLASE BY COVALENT IMMOBILIZATION ON THE AMINO CARRIER PUROLITE LIFETECH™

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Epoxide hydrolases are enzymes that catalyze the hydrolysis of epoxides by adding a water molecule to form the corresponding vicinal diol. Among them, limonene epoxide hydrolases (LEH) constitute a stand-alone group capable of sequential hydrolysis of both stereoisomers of (+)-limonene-1,2-epoxide [1]. The optically pure product and substrate in question are both valuable chemicals with interesting pharmaceutical properties and applications in organic synthesis [2] and the food industry [3]. LEH from *Rhodococcus erythropolis* exhibits low thermal stability at temperatures above 40°C [4]. Since enhancing thermal stability is one of the most frequently reported improvements resulting from immobilizing epoxide hydrolases [5], the objective of this study was to examine the effect of immobilization on thermal stability of LEH.

In this work thermal stability of free and immobilized LEH from *R. erythropolis* was investigated. LEH was immobilized on Purolite Lifetech™ ECR8309M carrier modified with glutaraldehyde and 1,4-diamonobutane (LEH@Purolite Amino C6-GA-DAB-GA). The temperature optimum for free LEH was determined to be 40°C, while that for immobilized LEH was 60°C. This change was attributed to stabilization due to covalent attachment which prevented aggregation of enzyme molecules. The process of thermal inactivation of biocatalyst was characterized by a simple aggregation mechanism for free LEH, and by the two-step model composed of the first equilibrium and the second irreversible reaction for immobilized LEH. The operational stability of the immobilized biocatalyst was tested in six 30-minute batch reactions at temperatures up to 60°C after which it retained >80 % of its initial activity. Overall, prepared immobilized LEH exhibited enhanced thermal properties, and the results highlight covalent immobilization as a promising method for thermal stabilization of LEH.

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PS 17

METAL-ORGANIC FRAMEWORKS AS A PLATFORM FOR ANTIBIOTIC DEGRADATION USING IMMOBILIZED B-LACTAMASE

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Antibiotics and their uncontrolled abuse of use is posing a serious threat to human health, as well as to the environment. Therefore, an increasing focus on biological methods for antibiotic degradation using enzymes is taking place, since they can degrade antibiotics due to their excellent catalytic properties, eco-friendly nature and high biocompatibility. Metal-organic frameworks (MOFs) are a versatile group of porous materials known for their extensive surface area, adjustable pore size, crystalline structure, and wide range of functionalities suitable as enzymes carriers. Enzyme encapsulated MOFs present a versatile and effective tool for elimination of hazardous pollutants from the environment and utilization for antibiotics degradation in wastewater systems with high efficiency.

ZIF-8 nanoparticles were synthesized. Briefly, zinc acetate (20 mmol/L) in water solution was quickly added to 2-methylimidazole (1.4 mmol/L) in water solution. The mixture was stirred for 12 hours at room temperature and 850 rpm. The synthesized ZIF-8 nanoparticles were collected by centrifugation and washed with deionized water three times. Enzyme encapsulated ZIF-8 (β-lact-ZIF8) were prepared by the same protocol, with 2-methylimidazole mixed with β-lactamase (2,5 mg/mL). The catalytic activity study was performed as follows; penicillin (PEN) solution (with varying concentrations of 0.1 mg/mL, 0.5 mg/mL and 1 mg/mL) was mixed with either free β-lactamase, β-lact-ZIF8 or ZIF-8. Continual agitation was applied using orbital shaker at room temperature. 1 mL of PEN solution was collected during different time periods and monitored by the HPLC method.

The catalytic study and performance of β-lactamase, β-lact-ZIF8 and ZIF-8 for the degradation of PEN was estimated in aqueous solution at room temperature. The results show excellent degradation properties of β-lact-ZIF8, compared to free β-lactamase. 90% of PEN degraded under the catalytic reaction with β-lact-ZIF8 after only 10 min and was fully degraded after 120 min, while free β-lactamase and ZIF-8 degraded only 5% and 66% of PEN after 120 min, respectively with equivalent concentration of the enzyme (2.5 mg/mL). The β-lactamase encapsulated MOFs show excellent properties for the degradation of PEN, which was significantly improved when compared to the use of free enzyme.

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PS 18

MAGNETITE NANOPARTICLE-BASED IMMOBILIZATION OF AMINE TRANSAMINASE FOR SUSTAINED FURFURYLAMINE SYNTHESIS

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Immobilization of enzymes is a widely used strategy in biocatalysis that offers improved enzyme stability, reusability and operational control. It is therefore essential for biotechnological processes in the fields of pharmaceuticals, fine chemicals and biofuels [1]. Among the various immobilization techniques, immobilization on magnetite nanoparticles (MNPs) have shown promise due to their large surface area, biocompatibility and magnetic properties. These properties enable efficient loading of enzymes, improved catalytic activity and easy magnetic separation of reaction mixtures. In addition, the functionalization of MNPs surfaces enables tailored interactions between enzyme and carrier, which further increases the stability of the enzyme and its resistance to denaturation under harsh conditions [2, 3].

Furfurylamine has attracted considerable interest due to its industrial applications, particularly in the manufacture of pharmaceuticals. Recent research has focused on sustainable synthesis methods, particularly those that utilize biocatalytic pathways [4, 5].

In our previous work, we developed an efficient biocatalytic process for the amination of biobased furfural using the amine transaminase *N*-His_6-ATA-wt as biocatalyst and (*S*)-(-)-α-methylbenzylamine as amine donor. To proceed to continuous furfurylamine synthesis, the enzyme was immobilized on synthesized and functionalized MNPs, retaining 94% of its activity. The stability test of the immobilized enzyme showed a total turnover number of $2.04 \cdot 10^7$, highlighting its promising suitability for industrial-scale applications.

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PS 19

ENZYMATIC SYNTHESIS OF AN AROMA ESTER IN A CONTINUOUS SOLVENT-FREE SYSTEM USING SOL-GEL ENTRAPPED LIPASE

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Continuous flow biocatalysis offers numerous advantages for chemical reactions due to enhanced mass transfer and high local catalyst concentration [1]. Enzyme immobilization by sol-gel entrapment results in the formation of stable and robust biocatalysts [2, 3], which can be integrated into continuous reactors. Synergy between these two technologies enables a highly efficient process [1].

The continuous flow esterification of octanoic acid and n-amyl alcohol was carried out in a solvent-free system, using a packed-bed reactor containing 1.5 g immobilized enzyme (20 mg of protein); the main parameters of the ester synthesis were optimized using design of experiments. The biocatalyst used was lipase from *Candida antarctica* B (GenoFocus, South Korea) immobilized by entrapment in sol-gel hybrid matrices obtained with epoxy functionalized silane precursors.

The process optimization was achieved through experimental design, by evaluating the main reaction parameters using the Box-Behnken type (BBD) model between the Response Surface Methodologies (RSM), and statistical modeling and process optimization techniques. The factors studied were the flow rate (ml/min) of the reaction mixture through the reactor, the temperature in the reactor (°C), the substrate (alcohol/acid) molar ratio (alcohol being in excess also acts as a solvent). The response variables were yield in ester (%) and productivity in g ester per hour.

Ester formation was determined by GC-FID analysis of samples collected within a set time frame, following the stabilization of the system. The optimal parameters were determined as the alcohol to acid ratio of 1:1, the flow rate of 0.2 ml/min, and the temperature of 70º C. This study illustrates the applicability of sol-gel entrapped lipases in a packed-bed reactor for continuous aroma ester synthesis in solvent-free conditions.

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PS 20

ENHANCED STABILITY OF LACCASES BY SUBSTRATE-DIRECTED IMMOBILIZATION

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Laccases are multi-copper enzymes found mainly in fungi and higher plants, catalyzing the oxidation of substrates containing a phenolic ring substituted with an electron withdrawing group, but their broad substrate specificity allowed the oxidative transformation of a wide range of compounds and possible applications in various industrial sectors, as well as in environmental protection [1]. The biocatalytic versatility of laccases was extended by the addition of mediators, low molecular weight organic compounds which are in the first reaction step oxidized by the enzyme and subsequently the oxidized form of the mediator enables by either an electron transfer or a radical hydrogen atom transfer route the oxidation of non-phenolic substrates [2]. However, the low stability of laccases in harsh conditions like as extreme pH, high temperature and presence of organic solvents hinders their utilization in industrial processes. Immobilization on a suitable support or by inclusion in a restricted space is a generally accepted approach to increase the stability of enzymes, also allowing the multiple use of the biocatalyst. Immobilized laccases can be versatile biocatalysts for the synthesis of high added-value specialty chemicals, including the oxidation of glycerol to glyceric acid and/or tartronic acid [3, 4].

In this work, firstly the immobilization of the laccase from *Trametes versicolor* was investigated by adsorption and covalent binding on synthetic polymeric supports. Although the highest activities, assessed on 2,6-dimethoxyphenol model substrate, were in the same range for both approaches, the covalent immobilization on epoxy- or amino-activated polymethacrylate resins was considered more suitable to provide higher stability and reusability. Next, the immobilization technique was directed toward improving the enzyme stability and reusability by covalent binding on novel functionalized magnetic supports, compared to the functionalized polymethacrylate supports and targeting the selective conversion of glycerol to glyceric acid. Ni-Zn or Ni-Zn-Co spinel ferrite (MFe_2O_4) microparticles, ranged from 1 to 10 μm, were investigated as carriers. Particularly, the utilization of a Ni-Zn ferrite support functionalized with 3-aminopropyl-trimethoxysilane, via crosslinking by glutaraldehyde and reduction with $NaBH_4$ led to excellent biocatalytic efficiency and stability of the immobilized *Trametes versicolor* laccase. This biocatalyst proved broader pH and higher temperature stability compared to the native enzyme. The operational stability was also significantly improved, the recovered total activity after 5 reaction cycles increasing from about 50% to 80% when the carbon-nitrogen double bonds were reduced to secondary amino bonds. The best immobilized biocatalyst, with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) as mediator, proved high efficiency for the selective oxidation of glycerol to glyceric acid, the conversion exceeding 50%.

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PS 21

CUSTOMIZED IMMOBILIZATION STRATEGY ALLOWS INCREASED OPERATIONAL STABILITY OF β-GALACTOSIDASE AND GLUCOSE OXIDASE FOR ONE-POT PRODUCTION OF GLUCONIC ACID AND GALACTO-OLIGOSACCARIDES

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Recent advances in the field of enzyme-catalyzed cascade reactions offer effective solutions to replacing traditional chemical processes due to their high selectivity and specificity, reduced energy consumption, minimal secondary products, mild reaction conditions, and high yields [1]. However, this approach is limited by the difficulty of maintaining proper operational stability for all the enzymes involved in the process. In this regard, enzyme immobilization has become a key strategy for developing efficient, reusable, and economically viable processes, which contribute to increased productivity and reduced production costs [2].

This research focusses on the customized immobilization of two enzymes, *β*-galactosidase from *Kluyveromyces lactis* and *Aspergillus oryzae*, and glucose oxidase from *Aspergillus niger*, which were further investigated for their combined biocatalytic potential in a novel one-pot biocatalytic cascade system. The aim was to tailor the immobilization of both enzymes toward a synthetic approach with improved operational stability, able to convert lactose into two useful products: gluconic acid, a multifunctional organic acid with applications in food, pharmaceuticals and other industries, and galacto-oligosaccharides, prebiotic compounds with beneficial health effects [3].

The immobilization methodology which led to improved stability of the studied enzymes was the covalent binding onto different supports, novel amino-terminated $Ni_{0.4}Co_{0.2}Zn_{0.4}Fe_2O_4$ and $Ni_{0.5}Zn0.5Fe_2O_4$ magnetic particles for *β*-galactosidase and commercial amino- and epoxy-functionalized methacrylate supports for glucose oxidase, respectively. Among them, higher activity of the immobilized *β*-galactosidase was achieved when stabilized on $Ni_{0.5}Zn0.5Fe_2O_4$ magnetic particles, while glucose oxidase showed a higher affinity for the methacrylate supports with epoxy active groups. The immobilized biocatalysts were thoroughly characterized, revealing enhanced storage, pH, and thermal stability, along with improved operational stability compared to their soluble counterparts. These improvements are relevant for the reusability potential of the biocatalyst system and long-term performance in industrial applications.

The influence of various parameters on the one-pot reaction system was evaluated, including enzyme loading, substrate concentration, and the presence of hydrogen peroxide, a glucose oxidase by-product. Experimental design and response surface methodology were used to determine optimal conditions for the conversion of lactose and product yields. The one-pot system with immobilized enzymes effectively converted lactose to gluconic acid and galacto-oligosaccharides, with the product ratio influenced by factors such as the *β*-galactosidase source, immobilization methods and initial substrate concentration. This work highlights the potential of customized immobilized biocatalysts in a one-pot cascade reaction system, offering a promising route for producing valuable compounds from a readily available and inexpensive resource.

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PS 22

INSTRUMENTAL INVESTIGATIONS REGARDING THE STABILITY OF TWO ENZYMES: PANCREATIN AND PEPSIN

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Pancreatin (PANC) and pepsin (PEP) are two important digestive enzymes commonly used in the pharmaceutical domain to aid in the treatment of digestive disorders. PANC is a mixture of several enzymes - primarily amylase, lipase, and protease derived from the pancreas of pigs or cattle, and is used to support digestion in patients with pancreatic insufficiency, such as those with cystic fibrosis, chronic pancreatitis, or after pancreatic surgery [1]. PEP, on the other hand, is a proteolytic enzyme derived from the stomach lining, often used to assist in protein digestion in patients with impaired gastric secretion. Both enzymes are frequently formulated into oral supplements or tablets to improve nutrient absorption and gastrointestinal function. Their inclusion in pharmaceutical preparations highlights their critical role in managing conditions where natural enzyme production is inadequate [2,3]. PANC and PEP, while effective as digestive aids, exhibit limited thermal stability, which poses challenges in pharmaceutical formulation and storage [3]. These enzymes are proteins, and like most proteins, they are sensitive to high temperatures that can lead to denaturation, rendering them inactive. PEP begins to lose activity significantly above 37°C, and PANC's enzymes (particularly lipase) are highly heat-labile, with substantial loss of function occurring at elevated temperatures. This sensitivity requires that enzyme-containing medications be stored in cool, dry conditions and often manufactured using techniques that minimize heat exposure. Additionally, during processing or encapsulation, care must be taken to avoid thermal degradation, which can compromise the efficacy of the final product.

In this study, we focused on the thermal stability and degradation kinetics of PANC and PEP using isoconversional methods such as Flynn-Wall-Ozawa and Friedman, with the aim of gaining a deeper understanding of how these enzymes degrade under varying thermal conditions. This knowledge is crucial for ensuring their efficacy, shelf-life, and optimal formulation in pharmaceutical products. These model-free kinetic approaches enable the determination of activation energy (Ea) at different stages of degradation without assuming a specific reaction mechanism. By applying the FWO and Friedman methods, we assessed how temperature influences the rate of enzyme degradation and identified the most thermally sensitive phases.

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PS 23

OBESITY-DRIVEN MODULATION OF INFLAMMATORY PROTEIN MARKERS IN CHRONIC VENOUS DISEASE

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Obesity is not only a metabolic disorder but also a chronic pro-inflammatory state that profoundly affects protein stability and turnover. Elevated adiposity disrupts protein homeostasis by increasing oxidative stress, promoting post-translational modifications, and amplifying the production of acute-phase proteins. Inflammatory protein markers such as C-reactive protein (CRP) and fibrinogen, together with the absolute neutrophil count (ANC), which reflects neutrophil activation and the systemic potential for release of inflammatory proteins, are particularly sensitive to obesity-driven dysregulation. These circulating markers illustrate how protein networks become destabilized in obesity and provide an accessible model for investigating the consequences of protein instability in vivo. Chronic venous disease (CVD) offers a clinical framework where obesity-driven protein changes translate into vascular remodeling and tissue damage. We analyzed 619 patients with CVD between 2018–2024. Clinical assessment was combined with measurement of BMI, CRP, fibrinogen, and ANC. Patients were stratified by BMI, and associations between obesity, protein markers, and CVD severity were tested using correlation analysis, logistic regression, and ROC modeling. Exclusion criteria eliminated confounders such as acute infection, autoimmune disease, or malignancy. Obese patients displayed markedly higher CRP (21.1 vs. 5.1 mg/L), fibrinogen (419.5 vs. 319 mg/dL), and ANC (5.24 vs. 3.98 $\times 10^3/\mu L$) compared with normal-weight controls (all $p < 0.001$). BMI correlated strongly with protein markers ($r = 0.61$ for CRP; $r = 0.55$ for fibrinogen; $r = 0.48$ for ANC), indicating obesity-driven destabilization of inflammatory protein regulation. Multivariate regression identified CRP, fibrinogen, and ANC as independent predictors of advanced CVD, while BMI exerted an indirect effect by amplifying these proteins. The integrated model (age + CRP + fibrinogen + ANC) achieved high predictive accuracy (AUC = 0.902). Our findings support obesity as a systemic driver of protein dysregulation, with destabilized inflammatory proteins (CRP, fibrinogen) and neutrophil activity (ANC) contributing to vascular injury. These markers exemplify how protein stability is compromised in obesity and highlight their utility as biomarkers for risk stratification. Chronic venous disease illustrates the clinical consequences of such instability, linking molecular-level changes to structural vascular remodeling.

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IN SILICO EVALUATION OF POLYOL – MUSCARINIC RECEPTOR INTERACTIONS RELEVANT TO XEROSTOMIA

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Xerostomia (dry mouth) is a frequent consequence of salivary gland dysfunction, affecting oral health and quality of life. In menopausal women, declining estrogen levels disrupt salivary gland physiology, reducing saliva flow, altering oral microbiota, and increasing caries risk [1]. Although pharmacological treatments like pilocarpine, an M3 muscarinic receptor agonist, are available to stimulate salivary flow, their use is often limited by systemic side effects such as excessive sweating, gastrointestinal discomfort, and cardiovascular complications [2]. Polyols like xylitol, sorbitol, and erythritol, widely used in sugar-free oral care products, are potential non-pharmacological stimulants of salivary secretion. Their mechanisms, especially under estrogen deficiency, remain unclear. Computational methods can explore their interactions with salivary receptors, offering possible alternatives to pilocarpine for xerostomia management [3].

Stimulation of muscarinic acetylcholine receptors (mAChRs), particularly the M1 and M3 subtypes, play a critical role in regulating saliva secretion. In this comparative study, we assessed the binding potential of four polyols (arabitol, erythritol, threitol, and xylitol) through molecular docking simulations on M1 (PDB ID: 5CXV) receptor models. Among the tested compounds, xylitol exhibited the highest affinity, with docking scores ranging from –4.6 to –4.0 kcal/mol, forming multiple hydrogen bonds and hydrophobic interactions with aminoacid residues as Asn217, Glu1063, Val127. Arabitol and erythritol displayed moderate affinities (–4.1 kcal/mol), interacting with residues such as Asn1067 and Ser126, while threitol showed slightly weaker binding.

These comparative findings highlight xylitol as the most promising candidate for modulating salivary secretion via mAChRs. Further experimental validation is required to confirm its therapeutic potential and support its development as a safe and effective strategy for managing xerostomia.

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FACTORS INFLUENCING PROTEIN OPACITY IN DIABETIC CATARACT

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Protein opacification is the fundamental pathological process underlying cataract formation, occurring when normally transparent crystallins undergo aggregation, denaturation, and abnormal interactions that disrupt lens clarity. Diabetes mellitus (DM) substantially increases cataract risk, primarily through chronic hyperglycemia, which drives excessive production of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals, while simultaneously depleting natural antioxidant defenses (glutathione, ascorbate, antioxidant enzymes). Diabetic cataracts develop earlier and progress faster than age-related cataracts because hyperglycemia chronically activates oxidative and inflammatory pathways at higher intensity. Recent evidence also highlights the role of microRNAs (miRNAs) such as hsa-miR-34a and hsa-miR-146a, which regulate apoptosis and inflammation by binding to the 3′ untranslated regions of target mRNAs, resulting in degradation or translational repression. These miRNAs are expressed in various ocular tissues and fluids (lens epithelial cells, retinal cells, cornea, aqueous humor) and act through molecules including SIRT1, Notch1/2, IRAK1, and TRAF6. Despite evidence of miRNA dysregulation in DM, their profile in diabetic cataract patients—particularly in plasma—remains poorly characterized [1]. This study aimed to evaluate plasma expression levels of hsa-miR-34a and hsa-miR-146a in patients with diabetic cataract compared with non-diabetic cataract controls. We investigated circulating miRNA expression in 79 patients with advanced cataract requiring lens surgery. The case group included patients with previously diagnosed type 2 DM (fasting plasma glucose >125 mg/dL and HbA1c >6.5%). Relative quantification of miRNAs was performed using qRT-PCR, followed by correlation analyses with clinical characteristics. hsa-miR-34a and hsa-miR-146a were significantly upregulated in diabetic cataract patients compared with non-diabetic controls. Overexpression of these miRNAs, likely reflecting the intensification of oxidative stress and inflammation in DM, may enhance apoptosis and crystallin damage in the lens, accelerating opacification and visual decline [2]. Our findings demonstrate that circulating hsa-miR-34a and hsa-miR-146a are significantly increased in diabetic cataract, suggesting their involvement in distinct molecular mechanisms of cataract formation depending on the presence of diabetes. These miRNAs could serve as biomarkers of disease progression and potential therapeutic targets.

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NEW SUPPORT FOR ENZYME IMMOBILIZATION AND STABILIZATION: AMORPHOUS PRECURSOR OF MOF ZIF-8

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MOFs (Metal Organic Frameworks) are nanoporous materials of great structural and compositional versatility, with multiple and varied applications such as gas separation and/or storage, drug delivery, heterogeneous catalysis and enzyme immobilization. These materials, formed by metals and organic linkers, are suitable for *in situ* immobilization of enzymes, where the MOF support is formed in the presence of the enzyme. This is a highly efficient strategy which requires MOFs that can be prepared under sustainable conditions compatible with enzyme activity: in water, at room temperature and at moderate pH [1]. ZIF-8, a zinc imidazolate, is one of the most widely used MOFs for enzyme immobilization but its synthesis at room temperature and in water implies the use of a large excess of linker with respect to the metal source (at least 40:1). We propose the use of a ZIF-8 precursor under sustainable conditions with a stoichiometric 2-methylimidazole/Zn molar ratio of 2, and the use of deprotonating agents such as triethylamine (TEA) [2].

In this work we study the effects on enzyme stability and activity of two different enzymes, alcohol dehydrogenase (ADH) and laccase (Lac) immobilized in a conventional ZIF-8 (Conv) and ZIF-8 Precursor with TEA. 100% immobilization of the enzymes were achieved. Both ADH and laccase immobilized on ZIF-8-TEA precursor gave higher catalytic activity than these immobilized on ZIF-8-Conv, being negligible in the reduction of ethanol to acetaldehyde with ADH. Leaching assays (Fig. 1A), and activity decay with time at 40ºC (Fig. 1B and 1C) of both biocatalysts and free enzymes were evaluated. ADH biocatalyst had the lesser leaching and was the best in terms of thermic stability.

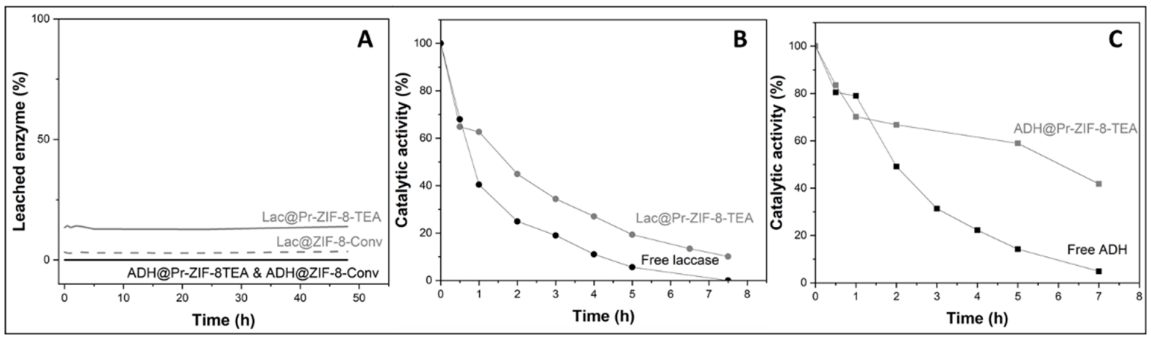

Figure 1. (A)Leaching assay. (B) Lac@Pr-ZIF-8-TEA thermic stability. (C) ADH@Pr-ZIF-8-TEA thermic stability

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INSIGHTS INTO THE MOLECULAR STRUCTURE AND FUNCTION OF POLYESTER SYNTHASE ENZYMES

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Polyhydroxyalkanoate synthases (PhaCs) are essential enzymes for the biosynthesis of biodegradable polyhydroxyalkanoate (PHA) plastics. Structural studies of PhaCs were limited to the catalytic domains, leaving unresolved the role of the N-terminal domain in dimer stabilization. Recently, the complete crystal structure of *Aeromonas caviae* PhaC (class I) has revealed the architecture of the N-terminal helical domain, its stabilizing function in dimer formation, and the existence of a hydrophobic exit tunnel essential for polymer release [1].

However, PhaCs constitute a wide variety of enzymes classified into four classes. Structural studies are limited to class I only, including the full-length PhaCs from *A. caviae*, and the C-terminal domains of *Cupriavidus necator* H8 and *Chromobacterium* sp. USM2 [2]. Our studies extend to all classes. While classes I and II of PhaCs are homooligomeric forms, classes III and IV are heterooligomeric structures and less well known from a structural perspective. Our research focuses on understanding the structures and mechanisms of action of the four classes of PhaCs. The methods used include molecular modeling, X-ray diffraction, molecular dynamics, SAXS, and cross-linking mass spectrometry. We hypothesize that the flexibility of the N-terminal domain in classes I and II is critical for dimeric stabilization and the conformational modulation of the polymer exit tunnel as it forms. The PhaE and PhaR subunits present in classes III and IV are unknown, and in this project, we aim to elucidate these structures to understand their role in the PHA synthesis process.

This integrative approach will not only deepen our mechanistic understanding of PhaCs but also provide a framework for the rational stabilization of industrially relevant enzymes for the development of sustainable bioplastics.

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