







### ESAB Webinar

## Multistep Enzyme Catalyzed Processes

February 23 <sup>rd</sup> 2024	06.00-08.00 Brasília Time (BRT)
	09.00-11.00 Greenwich Mean Time (GMT)
	10.00-12.00 Central European Time (CET)
	11.00-13.00 Eastern European Time (EET)
	14.30-16.30 India Standard Time (IST)
	16.00-18.00 Indochina Time (ICT)
	17.00-19.00 China Standard Time (CST)
	18.00-20.00 Japan Standard Time (JST)
	20.00-22.00 Australia Eastern Daylight Time (AEDT)

Chairs: Florian Rudroff (Vienna University of Technology) Roland Wohlgemuth (Lodz University of Technology)

#### PROGRAMME

**10.00 CET** Prof. Dr. Jun Ogawa, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Multistep enzyme catalyzed processes development through microbial metabolism analysis – application to chemical synthesis and environmental control

Information obtained through detail analysis of microbial metabolism leads to finding of unexpected new reactions and enzymes useful for bioprocess design. Recent accumulation of omics data facilitated to identify the novel enzyme genes and to expand the enzyme library. Here, examples of multistep enzyme catalyzed process development to chemical synthesis and environmental control by applying unique reactions found in the microbial metabolism of amino acids, fatty acids, nucleosides, and inorganic nitrogen metabolism are described.

1) Amino acid metabolism: The expanded library of amino acid-hydroxylating dioxygenase made possible the production of various chiral hydroxy amino acids and chiral amino acid sulfoxides by the cascade reactions coupled with the dioxygenases and the enzymes generating the related derivatives.

2) Fatty acid metabolism: Novel polyunsaturated fatty acids saturation metabolism was found in gut microorganisms such as lactic acid bacteria. The metabolism involved four enzymes of hydratase, dehydrogenase, isomerase, and reductase. The combination of these enzymes enabled to produce various hydroxy, oxo, and enone fatty acids with unique physiological activity useful for health.

3) Nucleoside metabolisms: The reversible reactions involved in nucleoside degradation metabolism were applied to produce deoxyribonucleosides coupled with energy generation system.

4) Nitrifying bacteria play an important role in generating nitrate for crop cultivations. A controllable model consortium for ammonification and nitrification using a co-culture of only three strains selected through metagenomic analysis was established. Denitrification with a bio-degradative polymer as an electron doner was developed by coupling of biopolymer degrader and denitrifying bacteria and applied together with nitrifying consortia to recirculating aquaculture system.

#### PROGRAMME

#### **10.30 CET** Prof. Dr. Colin Scott, CSIRO Environment, Black Mountain Science and Innovation Park, Canberra, and Queensland University of Technology, Brisbane, Australia

#### **Building continuous flow biocatalytic cascades**

There is an increasing focus on discovering methods to lower the expenses of manufacturing chemicals produced biocatalysis, including advanced pharmaceutical intermediates and pharmaceuticals. A promising solution to achieve this goal is continuous-flow biocatalysis, which intensifies the process. Nevertheless, certain enzyme classes depend on diffusible cofactors, such as the nicotinamide cofactors, which pose a substantial technical and economic challenge. In this presentation, I will present our interdisciplinary strategy for overcoming this obstacle by using chemical modification of cofactors to enable cofactor recycling and retention as part of a modular multienzyme cascade. I will also touch on our recent efforts to reduce the cost of producing new cascades by investigating facile and scalable cofactor modification chemistry.

# 11.00 CET Prof. Dr. Laurence Hecquet, Université Clermont Auvergne - Institut de Chimie de Clermont-Ferrand, Aubière, France

#### One Pot Enzymatic Procedures for the Synthesis of α-Hydroxyketones

Conventional syntheses in organic chemistry often use hazardous substances, consume large amounts of energy and generate toxic waste. An alternative is the use of biocatalytic ways performed in one pot by using simultaneously two or more enzymes in cascade avoiding the isolation of intermediates, thus saving time, energy, and limiting waste. The enzymatic approach is particularly useful for obtaining  $\alpha$ -hydroxyketones moieties found in highly diverse classes of natural products that are of great importance due to their bioactivity, and their function as chiral building blocks for the synthesis of pharmaceuticals and agrochemicals. The chemical synthesis of stereoisomerically pure  $\alpha$ -hydroxyketones is difficult and typically requires uneconomical steps for protective group manipulations. Carboligases such as thiamine dependent enzymes are particularly suitable and efficient to obtain  $\alpha$ -hydroxyketones. Among them, the thermostable Transketolase (TK) from *Geobacillus stearothermophilus*, shows great advantages for obtaining a wide range of  $\alpha$ -hydroxyketones according an irreversible reaction from an  $\alpha$ -ketoacid and an aldehyde rendering the reaction irreversible by the release of carbon dioxide. The presentation will focus on the development of different strategies involving TK only (wild type or variants) or coupled with other enzymes (aldolase, transaminase or aminoacid oxidase) in reaction cascades (sequential or simultaneous) for the *in situ* generation of the TK substrates.

#### 11.30 CET Prof. Dr. Katrin Rosenthal, School of Science, Constructor University, Bremen, Germany

#### Enhancing Efficiency in Multi-Enzyme Cascades through Data-Driven Optimization Strategies

The combination of several enzymes in multi-step reaction cascades enables the synthesis of complex molecules with often shorter reaction pathways compared to chemical routes, thus supporting the development of ecologically sustainable processes. Multi-enzyme reactions have even more advantages such as the shift of the equilibrium towards the product side, no intermediate isolation, and the synthesis of complex molecules in one reaction pot. Recently, several cascades have been developed that led to efficient synthetic pathways that could be of great relevance for the synthesis of anti-viral and anti-cancer compounds.

However, more complex cascades are often limited in their yield and titers, so that a huge optimization effort has to be made in order to find reaction conditions that allow economically viable performance to be obtained. The optimization of an enzyme cascade means that cooperative effects between several design parameters must be taken into account, which involves a high experimental effort. One way to overcome the challenge of optimizing such multi-parameter systems is to combine *in silico* models with experimental design, model calibration and validation. We have therefore investigated computational methods to increase the efficiency of this optimization process.

#### ABOUT THE SPEAKERS

**Jun Ogawa** is a professor of the Laboratory of Fermentation Physiology and Applied Microbiology in Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University. He studied applied microbiology and completed his doctorate in 1995 at Kyoto University and became an assistant professor at the same university. He was a visiting researcher at French National Institute for Agricultural Research (INRA) (2006-2007) and has been appointed as a full professor of the current position in 2009. He has published over 270 papers in applied microbiology such as bioprocess development, microbial metabolism analysis, and microbial enzyme engineering, microbial



community analysis and functional development such as gut microbiota and rhizosphere microorganisms, etc. He was awarded "Oleoscience Award" by the Japan Oil Chemists' Society (2015 and 2020), "Society Award of Japanese Association for Food Immunology" (2018), "Ching Hou Biotechnology Award" (2020) and "Fellow" (2021) by American Oil Chemists' Society, and "Chevreul Medal" by the French association for the study of lipids (2021).

**Colin Scott** is a microbial biochemist and synthetic biologist who works for the Commonwealth Scientific and Industrial Research Organisation (CSIRO), the government research organization in Australia. He completed his BSc in genetics in 1996 at Aberystwyth in Wales and his PhD in 2000 at Sheffield University's Krebs Institute in England.

In 2004, Scott joined CSIRO for a project focused on engineering enzymes to remove environmental pesticide contaminants, which won the DuPont Innovation Award in 2006. He has held several senior leadership positions at CSIRO, including director of the Synthetic Biology Future Science Platform, director of the Advanced Engineering Biology Future Science Platform, and director of the CSIRO BioFoundry. Currently, he is an Adjunct Research Fellow at Sydney University and Murdoch University, and an Adjunct Professor at Queensland University of Technology.



Scott's research interests lie in discovering and engineering microbes and their enzymes for use in industrial and environmental applications. He has been granted over a dozen patents and led projects that resulted in commercial outcomes for companies such as RisingStar Biotechnologies in China, the compostable plastics company Enzide in Australia, and The Coca-Cola Company in the US.

#### ABOUT THE SPEAKERS





**Katrin Rosenthal** is Assistant Professor of Biotechnology with focus on Bioprocess Engineering at Constructor University in Bremen, Germany.

She earned her PhD (2016) from the TU Dortmund University at the Chair of Chemical Biotechnology specializing on the development of single-cell analysis and the development of a microfluidic system for the cultivation and analysis of isolated bacterial cells. From 2016, she worked as a PostDoc in the field of biocatalysis and bioprocess development at the Chair of Bioprocess Engineering at TU Dortmund University. From 2018 to 2022, she has been a group leader focusing on biotransformations and microbioreaction engineering. Since 2023, she is professor at Constructor University and is focusing with her group on biocatalyst screening, cell-free protein synthesis, biotransformations and enzymatically catalyzed reaction cascades, as well as on the development of microbioreactors for biocatalyst characterization and reaction optimization. She tries to combine computational tools with wet-lab experiments in all areas in order to improve the performance of biocatalytic reactions as efficiently as possible.



#### NEXT ESAB EVENTS AND WEBINARS

ESAB aims to promote the development of Applied Biocatalysis and takes initiatives in areas of growing scientific and industrial interest in the field. The full program of the monthly ESAB Webinars and Conferences for the whole year 2024 can be seen on the ESAB website. The Schedule and Topics of the next ESAB Webinars & Conferences in 2024 are:

22 <sup>nd</sup> March	Amine Biocatalysis 5.5
2024	organized by Per Berglund and
14.00-16.00 CET	Dörte Rother

 26<sup>th</sup> April 2024 Joint ESAB-SKB Webinar on
10.00-12.00 CET Biomanufacturing, organized by Roland Wohlgemuth, Jan Lucht and Working Group Bioeconomy

26<sup>th</sup> May 2024 Joint ESAB-SusChem Webinar on 14.00-16.00 CET Sustainable Chemistry organized by Andrés Alcantara, Pablo Dominguez and Working Group Sustainable Chemistry

 28<sup>th</sup> June 2024 Biotransformations in Glycobiology
10.00-12.00 CET organized by Vladimir Kren and Pavla Bojarova

- 6<sup>th</sup> Congress on Multi Step Enzyme Catalyzed Processes, MECP 2024, 14<sup>th</sup>-17<sup>th</sup> April 2024, Vienna, Austria
- 15<sup>th</sup> Carbohydrate Bioengineering Meeting, CBM15, 5<sup>th</sup>-8<sup>th</sup> May 2024, Ghent, Belgium
- 7<sup>th</sup> International Conference on Implementation of Microreactor Technology in Biotechnology, IMTB 2024, 19<sup>th</sup>-22<sup>nd</sup> May 2024, Zadar, Croatia
- 4<sup>th</sup> NextGenBiocat, Next Generation Biocatalysis an International Young Investigator Symposium, May 20<sup>th</sup> and 21<sup>st</sup> 2024, Heraklion, Greece
- 7<sup>th</sup> International Conference on Biocatalysis in Non-Conventional Media, BNCM 2024, 26<sup>th</sup>-29<sup>th</sup> May 2024, Trondheim, Norway
- 20<sup>th</sup> International Conference on Renewable Resources and Biorefineries, RRB 2024, 5<sup>th</sup>-7<sup>th</sup> June 2024, Brussels, Belgium
- 6<sup>th</sup> Symposium on Biotransformations for Pharmaceutical and Cosmetic Industry, BPCI 2024, 17<sup>th</sup>-21<sup>st</sup> June 2024, Kraków, Poland
- 11<sup>th</sup> OXIZYMES Conference, 9<sup>th</sup>-12<sup>th</sup> July 2024, Lublin, Poland
- 11<sup>th</sup> Internat. Congress on Biocatalysis, biocat2024, 25<sup>th</sup>-29<sup>th</sup> August 2024, Hamburg, Germany

#### HOW TO JOIN ESAB

ESAB membership is open worldwide to scientists interested in biocatalysis and its applications. Personal membership is free. You are cordially invited to join ESAB by completing the membership application form online *via* https://esabweb.org/appl.html

Institutional membership is very much welcome and has been established as a new membership category.

Academic, governmental, research and other public Institutions as well as private companies based inside or outside Europe and whose activities are related to the field of applied biocatalysis, are welcome to apply for Institutional Membership. You are cordially invited to join ESAB by completing the membership application online *via* 

#### <u>https://esabweb.org/Membership/Application+f</u> <u>orm+for+institutional+membership.html</u>

ESAB has been founded in 1980 and has the mission of promoting the development of Applied Biocatalysis throughout Europe. The aims of ESAB are to promote initiatives in areas of growing scientific and industrial interest of importance within the field of Applied Biocatalysis.



All talks of the First ESAB E-Congress are now available on the ESAB Science and Technology Platform. After our first digital ESAB Congress last year, we received numerous positive comments from our community and the suggestion to organize a digital congress again this year. The Scientific Committee has therefore decided that a digital ESAB congress will take place again from 25<sup>th</sup>-27<sup>th</sup> November 2024. Further Information will follow soon.

Further information on ESAB activities and initiatives, such as On-site and Digital Conferences, Events, Webinars, the Digital Platform, Jobs and Positions can be found on the ESAB website

#### www.esabweb.org

ESAB - European Society of Applied Biocatalysis (esabweb.org)