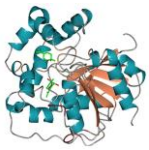

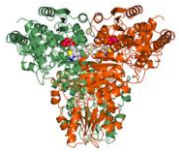

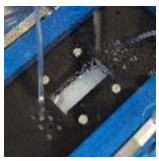

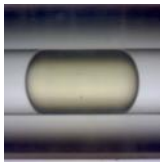

ESAB Webinar

Novel Enzymes

January 24th 2025

09.00-11.00 Greenwich Mean Time (GMT)
10.00-12.00 Central European Time (CET)
10.00-12.00 South Africa Time (SAT)
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 Australian Eastern Daylight Time (AEDT

Chairs: Roland Wohlgemuth (Lodz University of Technology)
Uwe Bornscheuer (University of Greifswald)
Jennifer Littlechild (University of Exeter)
László Poppe (Budapest University of Technology and Economics)

PROGRAMME

10.00 CET **Prof. Dr. Rebecca Buller, Zurich University of Applied Sciences, Waedenswil, Switzerland**

Engineering protein catalysts for synthetic applications

Chemical synthesis of small molecules can be profitably complemented by biocatalysis. This green technology is used in countless applications from bench scale to industrial production and allows practitioners to access complex organic molecules, often with fewer synthetic steps and reduced waste[1]. Yet, before being applied, biocatalysts typically need to be optimized as most wildtype enzymes are just marginally stable in the selected reaction conditions and perform at scales well below what is required to drive an industrial process.

Improved catalysts can be constructed in the laboratory by applying enzyme engineering strategies, among them the directed evolution of proteins. Accelerating implementation, advances in computational protein structure and property prediction are increasingly informing enzyme engineers[2]. By predicting how protein sequence encodes function, researchers aim to leverage the computational models to select a reduced number of optimized sequences for laboratory measurement with the aim to lower costs and shorten timelines of enzyme engineering campaigns[3-5]. In this presentation, an overview of the current status of biocatalysis will be complemented by successful engineering examples from our laboratory, including work carried out on Fe(II)/α-ketoglutarate dioxygenases[6], as well as on additional enzyme families of synthetic interest[7].

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10.30 CET **Prof. Dr. Takahiro Mori, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan**

Structure-function analysis of a novel cysteine dioxygenase in the biosynthesis of sulfonamide antibiotics altemicidin

Altemicidin, isolated from *Streptomyces sioyaensis* SA-1758, is a sulfonamide antibiotic showing promising bioactivity[1,2]. In the biosynthesis of altemicidin, a unique cysteine dioxygenase (CDO) homolog SbzM is involved in the formation of 2-sulfamoylacetic aldehyde (2-SA)[3,4]. While canonical CDOs generally use Fe^{2+} to catalyze oxidation of cysteine to produce cysteine sulfinic acid[5], SbzM catalyzes the conversion of L-cysteine into 2-SA. Although the catalytic function of SbzM has been confirmed, the metal dependency and catalytic mechanism of SbzM remain unclear. In this research, we performed structure-function analysis of SbzM homolog to elucidate the catalytic mechanism for the sulfonamide formation reaction.

In vitro analysis of tag-cleaved AmSbzM (a SbzM homolog from *Actinokineospora mzabensis*) with various divalent metal ions revealed that SbzM is an unprecedented Ni^{2+}-dependent sulfonamide synthase. The X-ray crystal structure of AmSbzM indicated that the enzyme has a conserved β-barrel structure and three metal-binding sites. The structure-based mutagenesis study of the active site residues of AmSbzM showed that all variants exhibited a significant decrease in 2-SA forming activity compared to that of the wild type. Remarkably, a AmSbzM variant generated a new compound as the main product instead of 2-SA. Mechanistic analysis of AmSbzM will also be discussed in the presentation[6].

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11.00 CET Prof. Dr. Frank Hahn, Department of Chemistry, University of Bayreuth, Bayreuth, Germany

New biosynthetic enzymes for chemoenzymatic natural product synthesis and biocatalysis

Natural product biosynthetic pathways are a virtually inexhaustible source of novel biocatalysts, often exhibiting enzymatic activities not found in primary metabolism. Their natural involvement in the assembly of natural product-like molecular architectures makes them particularly suitable for the chemoenzymatic synthesis of complex bioactive molecules.

The characterisation of these enzymes usually requires detailed studies involving the tailored chemical synthesis of complex, putative biosynthetic intermediates. A number of such studies on heterocyclases[1,2], Rieske oxygenases[3] and methyltransferases[4], which we discovered in the biosynthetic pathways of polyketides, will be presented. In addition to the elucidation of their biosynthetic role and a biochemical characterisation, some cases of their use in chemoenzymatic synthesis are discussed[5–7].

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11.30 CET Prof. Dr. Gideon Grogan, Department of Chemistry, University of York, Heslington, UK

Synthesis of Pharmaceutical Amides Using Amide Bond Synthetases

The formation of the amide bond is one of the most significant in pharmaceutical synthesis, accounting for as many as 20% of reactions performed in industrial medicinal chemistry. Although straightforward, routinely used methods can suffer from poor atom economy and toxic or hazardous reagents and methodology. Given these limitations, there has been an increasing focus on amide bond formation using enzymes. In this talk we will look at the various enzymatic methods for enzyme formation and focus on our own work, which exploits ATP-dependent ligases for the synthesis of pharmaceutical amides from carboxylic acids and amines in aqueous media.

12.00 CET Dr. Zsófia Bata[1], Dániel J. Incze[1,2], Nikolett Emődi[1,3], Kinga Nyíri[3], László Poppe[2,4]
[1] Dr. Bata Ltd., Research and Development Laboratory, Ócsa, Hungary; [2] Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary; [3] Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary
[4] Biocatalysis and Biotransformation Research Center, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University of Cluj-Napoca, Cluj-Napoca, Romania

Enzymatic methods for the reduction of mycotoxin contamination

Mycotoxins are secondary metabolites produced during the growth, storage, and transportation of crops contaminated by fungi which are toxic to humans and animals. Physical, chemical, and biological methods may be used to prevent the formation and to mitigate the mycotoxin problems. Enzymatic methods offer a selective detoxification and are environmentally friendly, however their practical application pose several challenges. This lecture describes the practical aspects of the discovery, and product development of two mycotoxin degrading enzymatic feed additives.

Fumonisins are sphingolipid-like mycotoxins that cause serious concerns when they contaminate food and feed products. Fumonisin B_1 esterase (FE, EC 3.1.1.87) cleaves the two tricarballylic acid (TCA) moieties of fumonisin B_1 (FB_1) accounting for 70% of fumonisin contamination; thereby representing a biotechnological tool for detoxification of FB_1. We discovered, that FE cleaves the TCA ester at C6 in the first step of FB_1 hydrolysis and performed the kinetic characterization for two FEs. The low K_M values (4.76–44.3 μM) are comparable to concentrations of environmental contaminations, and the high catalytic efficiencies are promising for practical applications. The X-ray structure of FE2 enabled the understanding of FB_1 hydrolysis at the molecular level. One of the FEs (FE2) showed a high potential to eliminate FB_1 at the beginning of the corn refining process, during the soaking step. Computations showed that FE2 can likely detoxify all fumonisins, not just FB_1, indicating its potential applicability in food and feed products. FumErase, based on FE2 have been registered as a feed additive in the European Union and in Brazil.

Zearalenone (ZEN) is a secondary fungal metabolite that causes hormonal disruption in livestock and humans due to its estrogen-like structure. α/β hydrolase lactone hydrolases have attracted considerable attention as they have been shown to degrade ZEN to a non-toxic form by hydrolyzing the lactone ring in the molecule. This research focused on finding new zearalenone degrading enzymes with K_M values comparable to the environmental contamination levels, and high kcat values to enable the very effective degradation of ZEN. Interestingly, changing the production system of the known ZHD101 enzyme resulted in a significantly higher k_{cat} value and reduced its K_M compared to previous literature results. Several formulations were tested to improve the storage and applicability of the enzyme in the food and feed industry.

ABOUT THE SPEAKERS

Rebecca Buller is a biological chemist and Professor for Biotechnological Methods, Systems and Processes at the Zurich University of Applied Sciences. Rebecca Buller studied chemistry at the Westfälische-Wilhelms Universität Münster (D) and the University of California Santa Barbara (US). After completing her PhD with a focus on enzyme engineering at the ETH in Zurich (CH), Rebecca Buller accepted a position as laboratory head at the flavour and fragrance company Firmenich (CH). In 2015 she relocated to the Zurich University of Applied Sciences where she founded the Competence Center for Biocatalysis (CCBIO).
Rebecca serves as an expert on numerous panels, among them the SCNAT Chemistry Platform, the working group Biotransformations of the DECHEMA, and she is founding board member of the Swiss Women in Chemistry. Rebecca and her group seek to expand the biocatalytic toolbox by sourcing and engineering enzymes for synthetic applications using chemical knowledge and bioinformatic tools. Their research program has been recognized with multiple awards, including the 2023 Roche Sustainability Award, the EFMC Prize for a Young Chemical Biologist and the Green & Sustainable Chemistry Award of the Swiss Chemical Society in 2024.

Takahiro Mori is Associate Professor at The University of Tokyo. He received his B.S. from School of Pharmaceutical Sciences, The University of Shizuoka in 2010, and M.S. and Ph.D. from Graduate school of Pharmaceutical Sciences, The University of Tokyo, in 2012 and 2016, respectively. He was appointed Assistant Professor at Graduate school of Pharmaceutical Sciences, The University of Tokyo in 2014. After obtaining his Ph.D. he carried out postdoctoral research in the laboratory of Prof. Donald Hilvert at ETH (2016–2018). Again, he was appointed Assistant Professor at Graduate school of Pharmaceutical Sciences, The University of Tokyo in 2018 and promoted to Associate Professor in 2023. His research interest is the structural analysis and the engineering of natural product biosynthetic enzymes.

ABOUT THE SPEAKERS

Frank Hahn studied Chemistry in Karlsruhe, Bonn and Paris and received his diploma in 2005. After a PhD thesis on polyamine solid phase synthesis and drug delivery with Prof. Ute Schepers und Prof. Stefan Bräse, he moved to a postdoctoral stay with Prof. Peter F. Leadlay at the University of Cambridge (UK) where he worked on the investigation of polyketide biosynthetic pathways. In 2011 he started his independent career as an Emmy Noether Research Group Leader at the Leibniz University of Hanover and moved to his current position as a Professor of Organic Chemistry & Biotechnology at the University of Bayreuth in 2015. His research interests are in the fields of natural products and biocatalysis, with a focus on the discovery of new enzymes in natural product biosynthetic pathways and their establishment for the chemoenzymatic synthesis of bioactive molecules and (hydrogen-powered) biotechnology.

Gideon Grogan is a Professor of Biochemistry at the University of York in the UK. He did his PhD with Stanley Roberts and Andrew Willetts at the University of Exeter, and a postdoc with Nick Turner and Sabine Flitsch at the University of Edinburgh. Since 2000 he has been at the University of York in the Structural Biology Laboratory, where he uses X-ray crystallography to help to understand the structure and mechanism of enzymes, and to inform engineering strategies to make improved biocatalysts.

ABOUT THE SPEAKERS

Zsófia Bata, Scientific Director at Dr. Bata Ltd., specializes in mycotoxin research and biotechnological feed additives. She leads the mycotoxin inactivation research division within Dr. Bata Ltd., that successfully obtained an EU registration for an enzyme-based mycotoxin reducing feed additive in 2024. She obtained her PhD in 2019 in Biotechnology from Budapest University of Technology and Economics under the supervision of Prof. László Poppe. She has extensive international experience. She was a Fulbright Fellow at the University of Minnesota with Prof. Romas J. Kazlauskas and she conducted research at Singapore's Bioinformatics Institute and also graduated in Top Industrial Managers in Europe double degree program from the École Centrale de Nantes in France.
Her work focuses on probiotics, enzymatic engineering, and sustainable animal health. Currently, she is researching new enzyme candidates for mycotoxin reduction in the feed and food industry.

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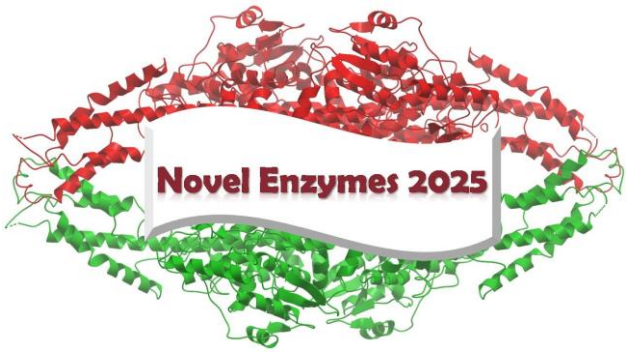

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