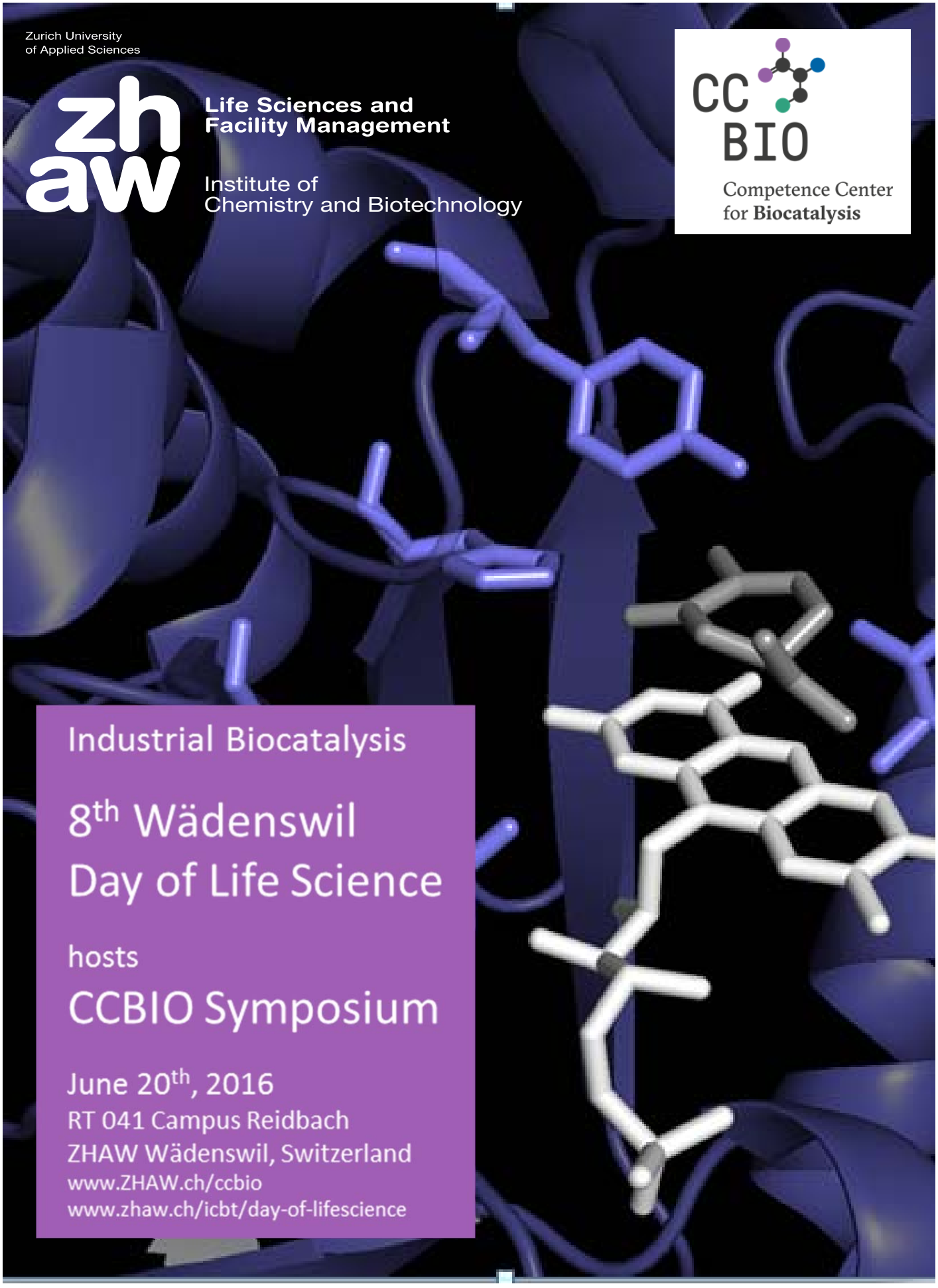
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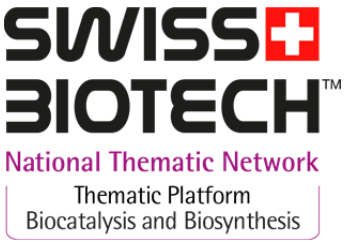
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Program– Monday, June 20th

08.30	Registration and coffee	
09.00	**Welcome to the ZHAW, news and outlook** Prof. Dr. Christian Hinderling, Zurich University of Applied Sciences (CH)	
09.15	**Building better enzymes** Prof. Dr. Donald Hilvert, ETH Zurich (CH)	
09.45	**Chemo-enzymatic cascade reactions** Prof. Dr. Marko Mihovilovic, TU Wien (AT)	
10.15	**Integration of an equilibrium-limited cascade reaction and continuous chromatography for production of a rare sugar** Prof. Dr. Sven Panke, ETH Zurich (CH)	
10.45	**Better than naturally designed: Synthetic nicotin- amide cofactors for biocatalysis** Prof. Dr. Frank Hollmann, TU Delft (NL)	
11.15	**Whole cell biocatalysis – status and future prospects** Prof. Dr. Bruno Bühler, Martin-Luther-University Halle-Wittenberg (D)	
11.45	Networking lunch and exhibition	
13.30	**Exploiting squalene hopene cyclases for biocatalysis** Prof. Dr. Bernhard Hauer, University Stuttgart (D)	
14.00	**Unusual terpene cyclization in plants** Dr. Hajo Kries, John Innes Centre, Norwich (UK)	
14.20	**Enzymatic resolution to access agrochemical intermediates** Dr. Régis Mondière, Syngenta (CH)	
14.40	**Biotechnology at Lonza Speciality Ingredients (LSI)** Dr. Marco Mirata, Lonza (CH)	
15.00	Coffee	
15.30	**Competence Center for Biocatalysis (CCBIO)** Dr. Rebecca Buller, Zurich University of Applied Sciences (CH)	
15.50	**Podium Discussion «Industrial Biocatalysis»**	
	Moderation:	Dr. Roland Wohlgemuth, Sigma Aldrich (CH)
	Participants:	Prof. Dr. Donald Hilvert, ETH Zurich (CH) Prof. Dr. Frank Hollmann, TU Delft (NL) Dr. Hans-Peter Meyer, HES-SO Valais-Wallis, Sion (CH) Prof. Dr. Marko Mihovilovic, TU Wien (AT) Dr. Andreas Taglieber, Firmenich SA, Genf, Switzerland
16.50	Closing Remarks	Dr. Hans-Peter Meyer, HES-SO Valais-Wallis, Sion (CH)
17.00	Aperitif & Farewell	

Building better enzymes

DONALD HILVERT

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Enzyme design represents a formidable challenge. Extensive mechanistic and structural studies have provided a solid qualitative understanding of enzyme action. Nevertheless, our knowledge of structure-function relationships in these macromolecules remains incomplete and a quantitative accounting of the incredible efficiency achieved by enzymes still eludes us. Diverse strategies have therefore been explored to engineer enzymes for novel applications, ranging from repurposing existing active sites to generation of antibodies with tailored catalytic properties. Among these approaches, computational design has emerged as particularly promising. Computational enzyme design has afforded made-to-order catalysts for a variety of reactions lacking biological counterparts, including simple proton transfer reactions, multi-step retroaldol transformations, Diels-Alder cycloadditions, and several metal-dependent processes. Although the starting activities of these artificial enzymes are typically low, they can be significantly increased by directed evolution. In favorable cases, activities approaching those of natural enzymes have been achieved. Analysis of the (sometimes surprising) evolutionary trajectories provides valuable feedback for the design process as well as insights into natural protein evolution. Recent progress on the computational design and evolutionary optimization of artificial enzymes will be surveyed in this lecture, highlighting both the opportunities and challenges facing this emerging field.

Chemo-Enzymatic Cascade Reactions

MARKO D. MIHOVILOVIC

Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria

Enzyme mediated reactions (in particular reductions and oxidations) represent a highly environmentally benign class of transformations, which cannot be carried out enantioselectively using conventional synthetic approaches in many cases. The combination of such stereospecific biotransformations with catalyzed chemical conversions is particularly appealing in order to compile reaction sequences. However, reaction conditions for biocatalysis and homogeneous or heterogeneous chemical catalysis are often largely different, consequently requiring certain adaptations in process design.

Within this presentation, several case studies for such reaction cascades will be presented and discussed. Examples will involve (i) hydrogenation via heterogeneous catalysis employing continuous flow chemistry in combination with enzyme mediated oxygenations [1]; (ii) orthogonal C-C bond formation via cross coupling with concomitant redox biocatalysis; (iii) sequential biocatalytic redox cascade reactions [2,3].

A major advantage of enzymes as catalytic entities is their largely identical parameter-set for reaction conditions. This allows for the combination of various types of biocatalysts in a novel fashion previously unprecedented in Nature and resembling the concept of reaction design exploited in organic synthesis [4]. Combination of various types of reductases with (mono)oxygenases opens an interesting new biocatalytic cascade process capable to accessing a variety of novel compounds of particular interest in fragrance chemistry.

[1] M. J. Fink, M. Schoen, F. Rudroff, M. Schnuerch, M. D. Mihovilovic, *ChemCatChem* **2013**, *5*, 724-727.

[2] Oberleitner, N.; Peters, C.; Muschiol, J.; Kadow, M.; Saß, S.; Bayer, T.; Schaaf, P.; Iqbal, N.; Rudroff, F.; Mihovilovic, M.D.; Bornscheuer, U.T. *ChemCatChem* **2013**, *5*, 3524-3528.

[3] Oberleitner, N.; Peters, C.; Rudroff, F.; Bornscheuer, U.T.; Mihovilovic, M.D. *J. Biotechnol.* **2014**, *192*, 393-399.

[4] Muschiol, J.; Peters, C.; Oberleitner, N.; Mihovilovic, M.D.; Bornscheuer, U.T.; Rudroff, F. *Chem. Commun.*, **2015**, *51*, 5798-5811.

Integration of an Equilibrium-limited Cascade Reaction and Continuous Chromatography for Production of a Rare Sugar

SVEN PANKE

Bioprocess Laboratory D-BSSE, ETH Zürich, Basel, Switzerland.

A broad variety of rare sugars can be obtained from cheap abundant sugars such as fructose if epimerases and isomerases are applied for biochemical conversion. The scope of accessible sugars increases if several of such catalysts are used in sequence. The corresponding reactions are, however, limited by the position of the equilibrium, which makes their use unattractive. Continuous chromatography, implemented as a simulated moving bed (SMB), is a well-established method to resolve compound mixtures, with prominent applications in the field of sugar isomer and racemate separations. However, it remains unclear whether it is possible to couple such a method with complex cascade reactions. We applied SMB technology to the efficient production of the rare sugar D-psicose from the discaccharide sucrose by a three-step cascade reaction employing a hydrolase, an isomerase and an epimerase. The process produced psicose of high purity (>99%), high yield (89% on the monosaccharides of sucrose) and, after directed evolution, with high enzyme efficiency (300 g of psicose per g of enzyme). We also evaluated the economics of the process.

Better than naturally designed: synthetic nicotinamide cofactors for biocatalysis

C. E. PAUL AND F. HOLLMANN

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Most biocatalytic reactions involving oxidoreductases are linked to the nicotinamide cofactors as source or sink of redox equivalents. The complex structure of the natural cofactors has triggered the search for simple synthetic but functional analogues (mNADH) as early as the 1950s.[1] While previous research efforts have focused on the use of such analogues to promote alcohol dehydrogenase-catalyzed reactions we have centered our attention to broadening the scope of oxidoreductases.

In contrast to previous reports[2] we found that mNADH are not accepted by all oxidoreductases such as alcohol dehydrogenases (ADHs). This apparent discrepancy will be resolved based on current (unpublished) experimental results. At first sight this represent a disappointing finding but also opens up possibilities for biorthogonal syntheses (*vide infra*).

Unlike alcohol ADHs, enoate reductases (ERs) readily accept a broad range of mNADHs.[3] Their non-acceptance by endogeneous ADHs enables highly chemoselective C=C-bond reductions of conjugated ketones and aldehydes, which using the natural cofactors are only attainable using highly purified ERs. More recent investigations revealed that some mNADHs can actually enable ‘faster than natural’ ER reactions.[4]

Another possible application of mNADHs lies with monooxygenases such as styrene monooxygenases,[5] halogenases (unpublished) and some hydroxylases (unpublished). The structural and mechanistic requirements for efficient usage of mNADH by these enzymes will be discussed.

Finally, our first steps towards (enzymatic) *in situ* regeneration systems for mNADH will be discussed.

[1] C. E. Paul et al. *ACS Catal.* **2014**, *4*, 788−797.
[2] H. C. Lo et R. H. Fish, *Angew. Chem. Int. Ed.* **2002**, *41*, 478-481.
[3] C. E. Paul et al. *Org. Lett.* **2013**, *15*, 180-183.
[4] T. Knaus, et al., J. Am. Chem. Soc., 2016, 138, 1033-1039.
[5] C. E. Paulet al. *ACS Catalysis* **2015**, *5*, 2961−2965.

Whole-cell biocatalysis – status and future prospects

BRUNO BÜHLER

Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany
Martin-Luther-University Halle-Wittenberg, Halle, Germany

What are the opportunities of biocatalysis in a future bioeconomy? Production of fuels? Chemicals? Pharmaceuticals? All of them? From what resources and with what technologies? Interesting questions not to be answered simply and maybe not in a comprehensive way, yet. However, whole-cell biocatalysis can be considered a promising tool for a future bioeconomy. The living microbial cell is highly attractive as catalyst unit for energy demanding reactions, since it allows efficient cofactor regeneration, self-renewal, and handling of reactive oxygen species. Thus, whole-cell systems pose big chances as well as challenges in terms of catalyst optimization and process control. Beside technical aspects, a large number of concurrent and highly cross-linked biological processes such as recombinant gene expression, energy metabolism, and toxification have to be considered. With the aim to construct the ideal microbial cell for recombinant oxygenase and multistep catalysis, we follow a systems biotechnology approach considering both biological and technical implications on a systems level. The intracellular coupling of multiple enzymes from diverse origin, i.e., orthogonal pathway engineering, will be presented as a tool to produce high volume products, e.g., polymers, from renewables. Furthermore, prospects for the use of phototrophic microorganisms will be given with the goal to make use of sunlight to drive sustainable production.

Exploiting squalene hopene cyclases for biocatalysis

BERNHARD HAUER

Institute of Technical Biochemistry, Universität Stuttgart, Stuttgart, Germany.

For many important reactions catalyzed in chemical laboratories the corresponding enzymes are missing, constituting a restriction in biocatalysis.[1] Although nature provides highly developed machineries appropriate to catalyze various non-natural chemical transformations, their potential is often overlooked. This also applies to Brønsted acid catalysis, a powerful method to promote a myriad of synthetically important reactions.

Here we report on the catalytic diversity of squalene hopene cyclases (SHCs). In nature, these enzymes convert squalene to pentacyclic products initiated by a unique protonation machinery creating an highly acidic aspartic acid in the enzymes active site.[2-3] We have recently revealed that SHCs are enzymatic Brønsted acids which can be harnessed for stereoselective synthesis. [4] This is illustrated by enzymatic activation of different functional groups (alkenes, epoxides and carbonyls) enabling the highly stereoselective C-C bond formation in syntheses of various cyclohexanoids. Mutants with increased activity for these non-natural chemical transformations were created by active site reshaping, thus, releasing SHCs from its polycyclization chemistry. This yielded different selective variants which catalyze the Prins reaction of citronellal in a stereodivergent manner by binding and activating the substrate under conformational control. Finally, we are evolving SHCs for the selective acidic isomerization of pinene towards various important monoterpenoids. Here active site reshaping is used to guide the rearrangement reactions of the reactive carbocationic intermediates towards a specific product. This work highlights the potential of systematic investigation on nature's catalytic machineries to yield unique catalysts.

1. Nestl BM, Hammer SC, Nebel BA, Hauer B, *Angew. Chem. Int. Ed.* **53**, 3070-3095 (2014).
2. Syrén PO, Hammer SC, Claasen B, Hauer B, *Angew. Chem. Int. Ed.* **53**, 4845-4849 (2014).
3. Hammer SC, Syrén PO, Seitz M, Nestl BM, Hauer B, *Curr. Opin. Chem. Biol.* **17,** 293–300 (2013).
4. Hammer SC, Marjanovic A, Dominicus JM, Nestl BM, Hauer B, *Nat. Chem. Biol.* **11**, 121-126 (2015).

Unusual terpene cyclization in plants

HAJO KRIES

Group of Sarah E. O'Connor, John Innes Centre, Norwich, UK

The enzyme iridoid synthase generates the core of iridoid natural products by cyclizing a monoterpene precursor in a reductive mode fundamentally different from canonical, cationic monoterpene cyclization. In many flowering plants, a plethora of ecologically and pharmacologically important natural products is biosynthesized based on the iridoid scaffold. Recently, the first gene of an iridoid synthase has been described in the medicinal plant Catharanthus roseus and additional homologues have been cloned from olive, snapdragon and catnip. We have solved an inhibitor bound crystal structure of iridoid synthase which sheds light on the conjugate reduction step that apparently triggers cyclization. Currently, we investigate how iridoid synthase controls cyclization and how variations of the iridoid scaffold arise in different plants. Mechanistic characterization of iridoid synthase may pave the way for future biocatalytic applications of this unusual terpene cyclase.

Enzymatic resolutions to access agrochemical intermediates

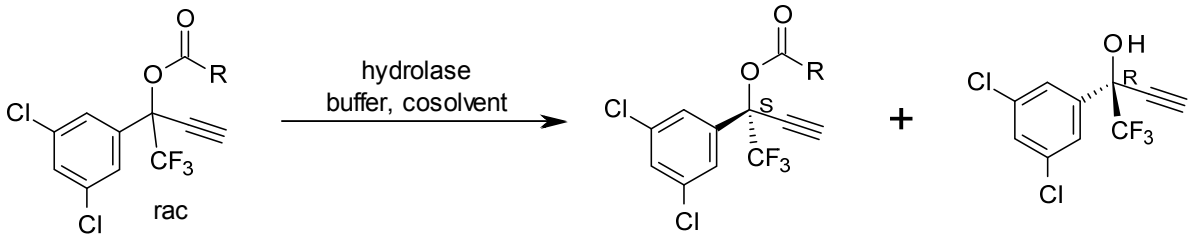
RÉGIS MONDIÈRE,[1] TOMAS SMEJKAL, [1] SEBASTIAN WENDEBORN, [1] JÉRÔME CASSAYRE, [1] MYRIEM EL QACEMI, [1] JULIE TOUEG, [1] NICOLAS POIRIER, [1] BERNHARD BREIT,[2] LISA DIAB. [2]

[1]*Syngenta Crop Protection Münchwilen AG, Stein, Switzerland.*

[2] *Institut für Organische Chemie, Albert-Ludwigs-Universität, Freiburg, Freiburg, Germany.*

The enzymatic resolution of secondary alcohol is nowadays routinely performed to access a large variety of chiral scaffolds.[1] By contrast, the enzymatic resolution of chiral tertiary alcohols has been much less explored, even though one acknowledges that there has been a regain of interest for such topic in the last decade.[2]

In this presentation, we will discuss the enzymatic resolution of esters of the tertiary alcohol depicted below. This will cover the chemical alternatives considered to access this chiral building block, the hydrolase screening to identify the first hits, the optimization of various factors (sense of reaction, choice of cosolvent, concentration, temperature etc.), and the scale up to access multigram amount of the enantioenriched alcohol. Finally, the transformation of this chiral alcohol into novel and useful agrochemical intermediates will be described.



[1] K. Faber, « *Biotransformations in Organic Chemistry, A Textbook* », 6th Edition, Springer Verlag, **2011**.
[2] R. Kourist, P. Dominguez de Maria, U. T. Bornscheuer, *ChemBioChem*, **2008**, *9*, 491 – 498.

Biotechnology at Lonza Speciality Ingredients (LSI)

MARCO MIRATA

Lonza, Visp, Switzerland.

Enzyme design represents a formidable challenge. Extensive mechanistic and Over the past decades, Lonza has accumulated an unparalleled experience in the manufacture of active and functional ingredients for the nutrition, cosmetic, personal care, industrial biotech and pharmaceutical industries by leveraging its expertise in chemistry and biotechnology. Today, the increasing market demand for bio-based products in the Specialty Ingredients Segment (LSI) represents a great opportunity for Lonza to create efficient and sustainable biotechnological processes. Here, recent examples of bioprocesses developed by the LSI R&T PD Group for the Agro Ingredients market and the Consumer Care market using biochemical technologies will be discussed