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Hot from the press!

Biocatalysis – Key Technology to Meet Global Challenges
CCBIO Symposium at the 8th Waedenswil Day of Life Science

Elsbeth Heinzelmann, science and technology journalist

Abstract: In a world of dwindling fossil-based energy, global air pollution and warming, biocatalysis may be a perfect problem-solver. It has the potential to procure sustainable raw materials and energy from biomass, and enables chiral and highly functionalized compounds to be produced ecologically for the chemical and pharmaceutical industry. At ZHAW Waedenswil on June 20, 2016, the Competence Center for Biocatalysis (CCBIO) gave European experts the opportunity to present the latest findings from science, research and practice in the future-oriented field of biocatalysis.

Keywords: Biocatalytic and biosynthetic processes · Biocatalytic toolbox of enzyme libraries · Chemo-enzymatic cascade reactions · Customized biocatalysts · Engineered enzymes for new biosynthetic applications · Novel asset molecules

Biocatalysis exploits the catalytic power of enzymes to perform highly chemo-, regio- and stereoselective organic transformations. Enzymes are 'useful little helpers', evolved over millions of years, and have become efficient catalysts for chemists. As technologies in molecular and synthetic biology develop, chemists can now specifically custom-fit the active sites of many enzymes to their synthetic requirements.

Building Better Enzymes

Enzyme design – even today a conceptual and technical challenge – is one priority of a leader in the field of chemical biology, Professor ***Donald Hilvert*** at the Laboratory of Organic Chemistry, ETH Zurich. While decades of mechanistic and structural studies have yielded understanding and applications relating to enzyme action, far less is known about structure–function relationships in these macromolecules. For the most part, reproducing efficiencies achieved by natural enzymes remains elusive.

Researchers around the world are racing to engineer enzymes for novel applications, including those not known in nature. Approaches range from repurposing existing active sites to generating antibodies with tailored catalytic properties. "Computational design enhanced by directed evolution is a particularly promising pathway," states Professor Hilvert, whose research interests include enzymology and enzyme engineering, as well as molecular evolution and chemical biology. Computational enzyme design provides made-to-order catalysts for a variety of reactions lacking biological counterparts, including simple proton transfer reactions, multi-step retro aldol transformations, Diels-Alder cycloadditions, and several metal-dependent processes. Although the initial activities of these artificial enzymes are typically low, they can be vastly increased by directed evolution. In favourable cases, activities approaching those of natural enzymes have been achieved. "Analysis of sometimes serendipitous evolutionary trajectories provides valuable feedback for design as well as a window into natural protein evolution," Hilvert explains. "Progress in computational design and evolutionary optimization of artificial enzymes is providing clear advancement toward broad therapeutic and industrial enzymatic applications."

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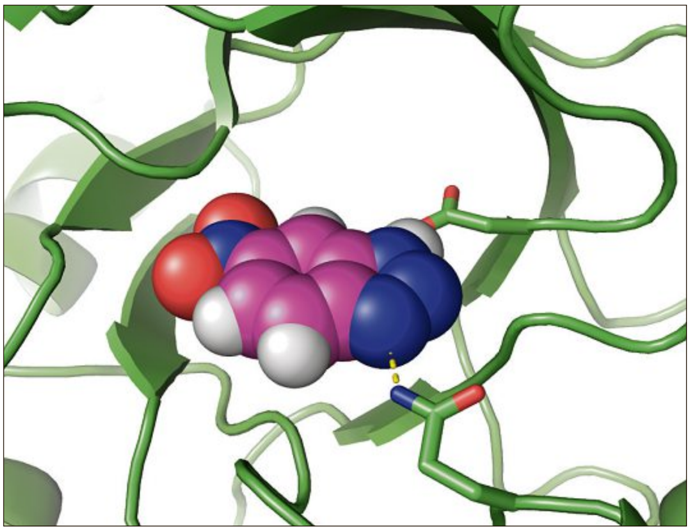
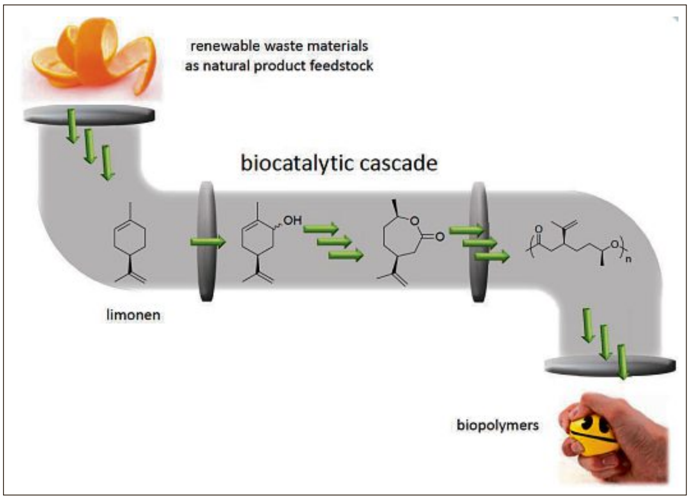


The graphic illustrates the active site of the highly active computationally designed and experimentally optimized enzyme reported in R. Blomberg, H. Kries, D. M. Pinkas, P. R. E. Mittl, M. G. Grütter, H. K. Privett, S. L. Mayo, D. Hilvert, *Nature* **2013**, *503*, 418–421). (Source Donald Hilvert)

Chemo-Enzymatic Cascade Reactions

Professor ***Marko D. Mihovilovic***, Head of the Institute of Applied Synthetic Chemistry and Chair for Bioorganic Synthetic Chemistry at TU Wien, Vienna, studies chemo-enzymatic cascade reactions, one of the mainstays of biocatalysis. "Enzyme-mediated reactions are a highly environmentally benign class of transformations, which – in many cases – cannot be carried out enantioselectively using conventional synthetic approaches", says the organic chemist. "The combination of such stereospecific biotransformations with catalysed chemical conversions is particularly appealing for compiling reaction sequences." However, reaction conditions for biocatalysis and homogeneous or heterogeneous chemical catalysis are often different, consequently requiring certain adaptations in process design. He has produced various case studies regarding chemo-enzymatic cascade reactions. In one of these an artificial cascade composed of an alcohol dehydrogenase, an enoate reductase and a Baeyer–Villiger monooxygenase were investigated *in vitro* to gain deeper mechanistic insights and understanding concerning the benefits and drawbacks of this multi-step biocatalysis. Several substrates composed of different structural motifs were examined and provided access to functionalized chiral compounds in high yields (up to >99%) and optical purities (up to >99%). Hence, the applicability of the presented enzymatic cascade was exploited for the synthesis of biorenewable polyesters.



Exploitation of orange peel as a renewable waste material for the isolation of limonene as the starting material for a mixed whole-cell redox biocatalysis cascade en route to polymerizable products. (Source TU Wien)

A major advantage of enzymes as catalytic entities is their largely identical parameter set for reaction conditions. This allows various types of biocatalysts to be combined in a novel fashion, previously unprecedented in nature and resembling the concept of reaction design exploited in organic synthesis. The specialist in practice-oriented synthetic chemistry concludes: "Combining various types of reductases with (mono)oxygenases opens up an interesting new biocatalytic cascade process capable of accessing a variety of novel compounds of particular interest to fragrance chemistry."

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How to Eliminate the Bottleneck

The group headed by Professor ***Bernhard Hauer*** is convinced: catalysis that utilizes both microorganisms and isolated enzymes has a considerable influence on the synthesis of novel attractive products by developing greener manufacturing processes. But for many important reactions catalyzed in chemical laboratories the corresponding enzymes are missing, constituting a restriction in biocatalysis. "Although nature provides highly developed appropriate mechanisms for catalyzing various non-natural chemical transformations, their potential is often overlooked. This also applies to Brønsted acid catalysis, a powerful method for promoting a myriad of synthetically important reactions", reports Bernhard Hauer, Head of the Institute of Technical Biochemistry at the University of Stuttgart. The scientist, who was awarded the BASF Innovation Prize for developing biotechnological production methods for commercial use, has gained experience with the catalytic diversity of squalene-hopene cyclases (SHCs). In nature, these enzymes convert squalene to pentacyclic products in a process initiated by a unique protonation mechanism that creates a highly acidic aspartic acid in the enzymes' active site. "We have recently revealed that SHCs are enzymatic Brønsted acids that can be harnessed for stereoselective synthesis. This is illustrated by the enzymatic activation of different functional groups (alkenes, epoxides and carbonyls) that facilitate the highly stereoselective C–C bond formation in the synthesis of various cyclohexanoids. Mutants with increased activity for these non-natural chemical transformations were created by active site reshaping, thereby releasing SHCs from its polycyclization chemistry." The result was different selective variants that 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", describes Bernhard Hauer. 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 the systematic investigation of nature's catalytic mechanisms to yield unique catalysts."

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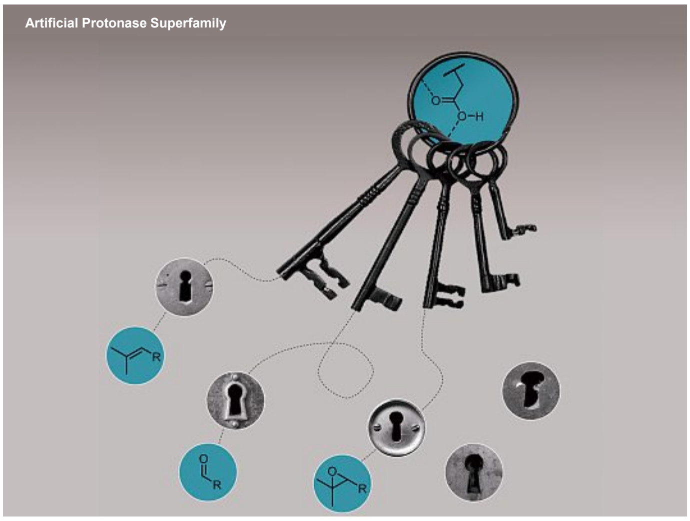
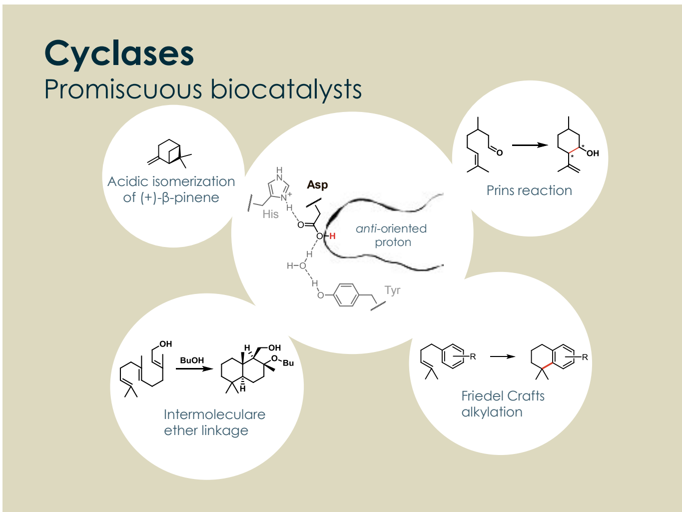
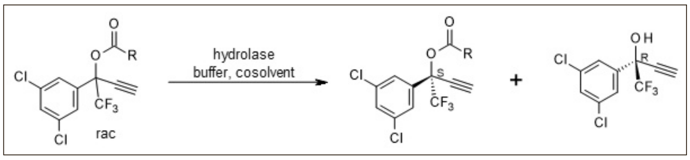




Catalysis that utilizes both microorganisms and isolated enzymes has a considerable influence on the synthesis of novel attractive products by developing greener manufacturing processes. (Source University of Stuttgart)

Enzymatic Resolutions to Access Agrochemical Intermediates

The research centre of Syngenta Crop Protection AG in Stein is one of the company's three most important global sites for research and development. In addition to the discovery and biological characterization of new active substances to combat insects, harmful fungi and nematodes, the scientists focus on plant protection products for the market. Every year they use 10 million test plants and characterize about 24,000 compounds. Researcher Dr. ***Régis Mondière*** conducts studies investigating enzymatic resolutions. "The enzymatic resolution of secondary alcohol is nowadays routinely performed to access a large variety of chiral scaffolds", says the Syngenta scientist. "By contrast, the enzymatic resolution of chiral tertiary alcohols has been much less explored, although it must be acknowledged that there has been a resurgence of interest in this topic over the last decade." With his fellow researchers he has examined the enzymatic resolution of esters of the tertiary alcohol depicted as follows:



The different work stages included the chemical alternatives that are thought to access this chiral building block, the hydrolase screening to identify the first hits, the optimization of various factors such as sense of reaction, choice of cosolvent, concentration, temperature etc., and the scale-up to access multigram amounts of the enantio-enriched alcohol (Scheme 1). And Régis Mondière concludes: "The investigation shows that this is a very useful way to access this chiral alcohol and transform it into novel and useful agrochemical intermediates."

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Biotechnology at Lonza Specialty Ingredients

The Lonza Group AG is an important global player as a customer-focused and market-driven organization bringing biotech and specialty chemical expertise to its target markets. One of its two segments is Specialty Ingredients, in which Lonza supplies innovative solutions that promote health, wellness, beauty, nutrition, hygiene and materials protection. The segment benefits from market-leadership positions in Personal Care & Preservation, Nutrition and Hygiene as well as in Agro Ingredients, Coatings and Composites and Water Treatment. Over the past decades, Lonza has accumulated an unparalleled experience in the manufacture of active and functional ingredients for nutrition, cosmetics, personal care, industrial biotech and components for the pharmaceutical industry by leveraging its expertise in chemistry and biotechnology. "Today, the increasing market demand for bio-based products in the Specialty Ingredients Segment combined with the significant progress achieved over the past years in the field of biotechnology represent a great opportunity for Lonza to create efficient and sustainable biotechnological processes", explains Dr. ***Marco Mirata***, Project Leader Biotechnology at Lonza RSI. "Our strategic focus for the future is on further developing bioprocesses for the Agro Ingredients market and the Consumer Care market using biological technologies, such as the industrial application of fermentation and biocatalysis for the manufacture of specialty chemicals."

Fermentation laboratory at Lonza Visp. (Source LONZA)

The role of biocatalysis is becoming ever more important as it facilitates the adoption of sustainable production methods, results in far less waste and is resource-saving. "In the ecosystem of Swiss biotechnology, Contract Research Organizations and manufacturers play a key role nowadays: they are the suppliers of synthetics and biologics for globally active companies. Particularly in the manufacture of synthetics, *i.e.* organic compounds, the integration of enzyme- and cell-based production of compounds can be an alternative to, or supplement, traditional chemical processes", explains Professor ***Daniel Gygax***, President of Biotechnet Switzerland. "In his keynote speech at the Swiss Biotech Days 2016 in Basel, Dr. Rudolf Hanko, CEO of Siegfried Pharma, pointed out the need for this option and showed – with the help of examples - how it can be implemented."

June 20, 2016 saw the launch, at ZHAW Waedenswil, of the new Competence Center for Biocatalysis (CCBIO) headed by Dr. Rebecca Buller. (Source ZHAW)

Rebecca Buller sums up positively: "The development, linkage and implementation of biocatalytic-, catalytic- and bioprocess-based methods and processes are our strength. That's why it was important to us to provide a platform for scientific knowledge exchange between creative minds from industry and academia who are active in biocatalysis. For us the symposium was a great opportunity to kick-off the newly founded Competence Center for Biocatalysis (CCBIO) at the ZHAW Waedenswil."

The speakers from all over Europe captivated the audience and answered interesting questions. (Source ZHAW)

Closing the conference, representatives from academia and industry joined the podium discussion on 'Industrial Biocatalysis' and openly exchanged views and ideas for cooperation. (Source ZHAW)

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Contact Point for Creative Minds

Dr. Rebecca Buller of the Competence Center for Biocatalysis CCBIO. (Source ZHAW Waedenswil)

The Competence Center for Biocatalysis (CCBIO) at ZHAW Waedenswil promotes biocatalysis as a complementary method to classic organic synthesis and aims to help bridge the gap between academic laboratories and the production plant. By bundling the relevant research competences, CCBIO strives to develop a comprehensive biocatalytic toolbox consisting of enzyme libraries and methods. CCBIO facilitates the development of biocatalytic and biosynthetic processes for the chemical and pharmaceutical industry.

Head of the CCBIO is Dr. ***Rebecca Buller***. She studied chemistry at the University of California, Santa Barbara, USA, and at the Westfälische Wilhelms Universität Münster, Germany, where she received her degree with honours. For her doctoral work, she moved to ETH Zurich, under the supervision of Professor Donald Hilvert, and was awarded her Ph.D. in 2011. "My work was focused on the directed evolution and rational design of artificial enzymes. The aim was to improve their activity and understand their underlying catalytic principles." Her findings, published in *Nature* in 2013, showed for the first time that computational enzyme design in combination with a directed evolution approach can yield biocatalysts that rival their natural counterparts. Between 2011 and 2015 Rebecca Buller held a position as Laboratory Head at Firmenich SA, Geneva. She worked on the optimization and scale-up of biocatalytic reactions for the production of asset molecules. "During my time at Firmenich, I implemented several biocatalytic processes yielding new or improved commercial products. It was exciting to see how powerful enzymes can be in an industrial context." She was promoted to Senior Scientist in 2014.

Her motivation to return to academia was to help create a sustainable, bio-based economy in a more global context. "In October 2015 I joined ZHAW Waedenswil where I am heading the Center for Biocatalysis and Processtechnology and the national Competence Center for Biocatalysis CCBIO.

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