UNIVERSITÄT BIELEFELD

CeBiTec Center for Biotechnology

CeBiTec – Quarterly

Spring 2021

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- EU-Project on heterogeneous biocatalysis with CeBiTec-participation started
- Janina Bahnemann appointed as substitute professor for cell culture technology
- Combined CRISPRi and crystal structure analysis to characterize methylotrophy
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Luisa Blöbaum awarded prize of the DECHEMA-Future Forum

For her master thesis conducted at Bielefeld University, Luisa Blöbaum from the Grünberger lab received the prize of the Future Forum, which is annually organized by the DECHEMA-specialist community biotechnology and awarded to interdisciplinary master projects. In her thesis, she investigated the impact of pH changes on *Corynebacterium glutamicum* within microfluidic single-cell systems. The prize is endowed with 3,000 € and half of the prize money is provided by the company Sartorius Stedim Biotech.

L. Blöbaum could obtain novel insights into the growth dynamics and heterogeneity of bacterial populations with great relevance for bioprocess development by using newly developed microfluidic single cell-scale down-reactors to study pH effects.

C. glutamicum is an important organism applied in various biotechnological production processes. Cultivation of this organism in industrial bioreactors is frequently accompanied by the formation of gradients, which influence the organism`s growth, but cannot be analysed during process operation. Averaged information on the behaviour of entire populations can be generated by conventional scale-down approaches, but these approaches

fail to provide insights into single cell responses, which can only be examined by applying microfluidic single-cell cultivation devices. Within her investigations, *C. glutamicum* turned out to be quite resistant towards pH changes and stress responses were disconnected from interval durations, although increasing overproportionately with growing stress intensity.

The award ceremony was part of the spring convention of DECHEMA biotechnologists. Luisa Blöbaum, born in 1995, studied Molecular Biotechnology at Bielefeld University and conducted her master thesis in the CeBiTec group of Prof. Dr. Alexander Grünberger, where she continues her investigations as part of a Ph.D. project.

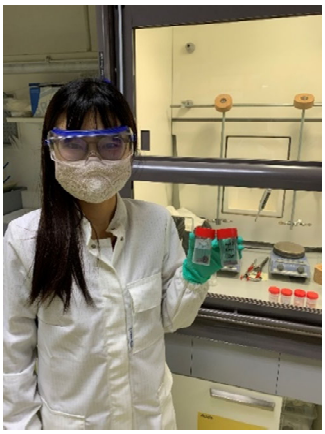
(L. Wobbe)

EU-Project on heterogeneous biocatalysis with CeBiTec-participation started

Within the EU-program "Innovative Training Networks (ITN) – European Industrial Doctorates (EID)" as a part of the H2020 Marie Skłodowska-Curie Actions (MSCA), recently the network project "INTERfaces - Heterogeneous biocatalysis reaction cascades training network" started (grant agreement no. 860414).

The focus of the interdisciplinary project INTERfaces (http://www.h2020interfaces.eu/) is on multi-step synthetic processes using biocatalysts in immobilized form and combines material science, protein and reaction engineering as well as organic synthesis with enzymes. Among the target products are bio-based bulk and fine chemicals of industrial interest. A further goal of INTERfaces is the scale-up of commercially relevant processes together with the industrial partners.

In total, 13 non-academic partners ranging from high-tech SMEs to large producing companies in combination with 9 academic institutions including Bielefeld University are participating in this project INTERfaces and serve as host institutions for 14 Ph.D. candidates. All Ph.D. students will conduct their doctoral research work in both, an academic as well as industrial research environment.



One of the Ph.D. students, Ms. Seika Ishii, will be jointly supervised by scientists from Bielefeld University (being represented in INTERfaces by CeBiTec and the chair of Industrial Organic Chemistry and Biotechnology as its member) and the Spanish start-up AENEAM (http://www.aeneam.com/) founded and owned by Dr. Jonas Gurauskis as a leading expert in the field of ceramic material-based separation technologies. AENEAM`s competencies center on the production of advanced ceramic material membranes with highly efficient flow properties, which can be applied, for example, in heterogeneous catalysis. While so far the AENEAM technology has been utilized successfully for chemocatalytic high temperature applications such as gas separation membranes or solid oxide fuel cells / electrolysers, the goal of the joint collaboration project between AENEAM and Bielefeld

University is to develop immobilized enzymes by means of such ceramic materials and to apply the resulting heterogenized enzymes in biotransformations. Advantages provided by such an approach are the simple recovery of these type of biocatalysts from the reaction mixture and, dependent on their stability, the re-use in further reaction cycles, thus also improving the overall economy of such biocatalytic processes. A further project goal is the integration of these ceramic-based immobilized enzymes in chemoenzymatic cascade processes.

https://twitter.com/INTERfaces2020

Innovative Training Network – European Industrial Doctorates

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860414.

(H. Gröger)

Janina Bahnemann appointed as substitute professor for cell culture technology

Prof. Thomas Noll started a one-year sabbatical on beginning of April. During his absence, Dr. Janina Bahnemann from Leibniz University Hannover has been appointed as substitute professor and head of cell culture technology group.

Janina Bahnemann is an Emmy Noether fellow and head of the research group "Cell Culture and Microsystems Technology" at the Institute of Technical Chemistry at Leibniz University Hannover since 2017. Using modern, high-resolution 3D printers, her research group in Hannover develops microfluidic prototypes that are used in cell culture technology. Furthermore, the working group focuses on the development of new (bio)sensors for online monitoring of bioprocesses and for point-of-care diagnostics. She received her PhD in 2014 (Institute of Bioprocess and Biosystems Engineering, Technical University Hamburg-Harburg). After one and a half years in industry (PlantTec Medical, Lüneburg), she joined the department of Environmental Sciences at the California Institute of Technology (Caltech, Pasadena, USA - research group of Prof. Michael Hoffmann), where she spent almost two years as a postdoctoral fellow. During her time at Bielefeld University, Prof. Janina Bahnemann intends to combine the expertise in cell culture and bioprocess engineering of the two research groups (in Bielefeld and Hannover) and to initiate innovative joint projects.

(J. Bahnemann & T. Noll)

Combined CRISPRi and crystal structure analysis to characterize methylotrophy

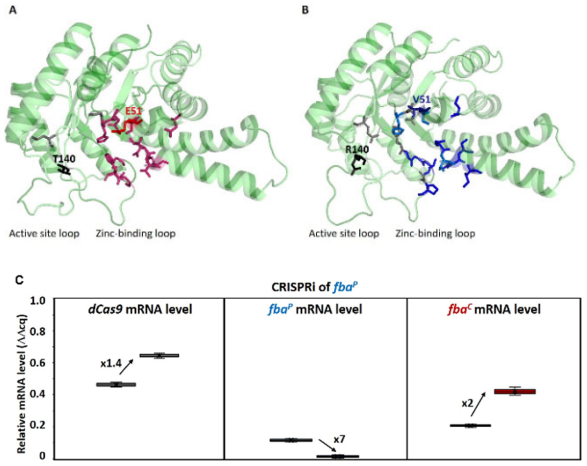
Methylotrophy, the ability to grow and produce with methanol as sole carbon source, of the Gram-positive thermophile *Bacillus methanolicus* depends on the native plasmid pBM19. This facultative ribulose monophosphate (RuMP) cycle methylotroph also grows with mannitol and few other carbon substrates. It possesses two aldolases that function as sedoheptulose bisphosphate aldolases in the RuMP cycle and as

fructose bisphosphate (FBP) aldolase (FBA) enzymes in glycolysis and gluconeogenesis. Both enzymes share 75% identical amino acids, but differ by their kinetic properties. The chromosomally encoded FBA^{C} is the major glycolytic aldolase, while the gene for the major gluconeogenic aldolase FBA^{P} is found on plasmid pBM19 and is induced during methylotrophic growth. Dr. Desirée D. Gütle, Dr. Lin Zhang, Prof. Dr. Oliver Einsle, and Prof. Dr. Jean-Pierre Jacquot from Freiburg and Lorraine universities solved the crystal structures of both enzymes at 2.2 Å and 2.0 Å, respectively. As FBA^{C} and FBA^{P} differ in the FBP binding site (residue 51) and regarding residue 140 for active site zinc atom coordination, these positions were swapped by site-directed mutagenesis.

Dr. Kerstin Schultenkämper and Dr. Marina Gil López (now NTNU, Trondheim) from the Wendisch lab characterized the kinetic properties of the fructose bisphosphate aldolases of *B. methanolicus*. Indeed, the major glycolytic enzyme FBA^{C} could be changed to a gluconeogenic enzyme.

Moreover, the amino acid changes resulted in a complete loss of glycolytic activity of FBA^{P}, turning it into a gluconeogenesis only aldolase. These results confirmed the importance of the structural differences between FBA^{C} and FBA^{P} concerning their distinct enzymatic properties.

In order to investigate the physiological roles of both aldolases, their genes were repressed individually by CRISPR interference (CRISPRi). Dr. Kerstin Schultenkämper and Laura B. Keller revealed a genetic compensation mechanism, i.e, the plasmid gene *fba*^{P} was upregulated when the chromosomal gene *fba*^{C} was repressed and vice versa. Moreover, while CRISPRi targeting *fba*^{P} decreased RNA levels of the downstream transketolase gene *tkt*^{P} of the bicistronic operon, genetic compensation by upregulation of the chromosomal transketolase gene *tkt*^{C} was not observed.



Crystal structures (pdb entries 7NC7, 7NCC) of aldolases FBA^{C} (A), FBA^{P} (B), and RNA levels of *dCas9*, *fba*^{P} and *fba*^{C} upon CRISPRi targeting of *fba*^{P} (C).

The CRISPRi system was successfully employed to demonstrate the first loss of function analysis of methylotrophic genes. The insight gained by CRISPRi, structural and mutational analysis of the FBA enzymes in methylotrophy of *B. methanolicus* may prove valuable for developing methanol-based fermentation processes within the ERA CoBioTech project C1Pro.

Schultenkämper K, Gütle DD, Lopez MG, Keller LB, Zhang L, Einsle O, Jacquot J-P, Wendisch VF (**2021**) Interrogating the role of the two distinct fructose-bisphosphate

aldolases of *Bacillus methanolicus* by site-directed mutagenesis of key amino acids and gene repression by CRISPRi. *Front. Microbiol.* 12: 669220.
doi: 10.3389/fmicb.2021.669220

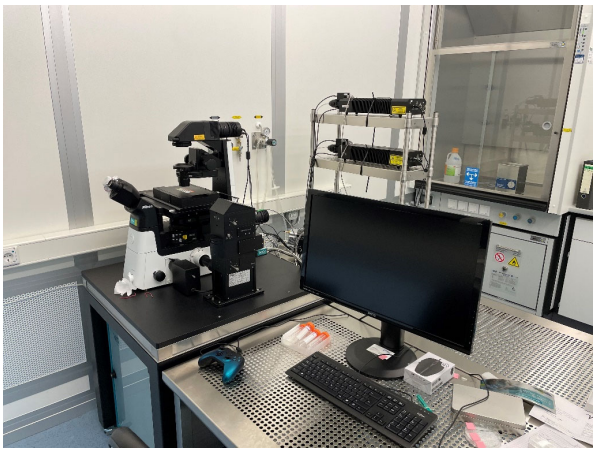
(K. Schultenkämper & V.F. Wendisch)

The state of North Rhine-Westphalia is funding the establishment of a marker-free high-throughput screening platform at the CeBiTec

On the basis of a cross-faculty initiative by colleagues Prof. Dr. Thomas Huser (Physics), Prof. Dr. Karsten Niehaus (Biology), Prof. Dr. Alexander Grünberger (Technical Faculty) and Prof. Dr. Olaf Kruse (CeBiTec Director), the Ministry of Culture and Sciences (MKW) from the state of North Rhine-Westphalia has recently granted 200k€ to set up a marker-free high-throughput screening platform at the CeBiTec.

The systematic screening of single cells is an elementary and essential part of research at the CeBiTec in both, biomedicine and molecular biotechnology. With this approach, changes in the metabolism of cells can be identified, which can be used, among other things, as a basis for therapeutic approaches, the search for new active ingredients or to establish sustainable cell factories in industrial biotechnology. Previous high-throughput screening systems are based on labelled target products, usually by fluorescence, with subsequent identification and sorting using FACS technology. However, this method does not allow the accumulation of secondary metabolites, detected directly in the cells and subsequently sorted.

Raman micro spectroscopy makes it possible to identify and analyze the biochemical composition of dead and living cells with the help of inelastic light scattering. With this principle, light from a laser is scattered at the bonds of biomolecules, with part of the energy being used in the excitation of a molecular bond. Since every molecular oscillation has a well-defined frequency, the biomolecules can be recognized by recording a spectrum of the scattered photons on the basis of values stored in databases.



Raman micro spectroscopy system is set-up and running in the former cleanroom of the technology platform genomic.

The measurement has no influence on the sample and cells are not destroyed. This makes it possible to recognize individual cells such as microalgae or bacteria based on their characteristic spectrum. In this way, individual cells of particular interest can be isolated alive and then replicated, e.g. in order to enrich and cultivate cells in a targeted manner. With this system, an innovative and future-oriented fully

automated marker-free screening platform based on Raman micro spectroscopy at the Center for Biotechnology (CeBiTec) at Bielefeld University will be set up. The platform also offers an ideal basis for further expansion in the future, in order to carry out marker-free cell screens for biotechnology and biomedical drug discovery in high throughput. To increase the throughput of Raman screening, a droplet microfluidics screening platform will be integrated. Such a combined system will significantly improve both, throughput and handling.

(O. Kruse)

Universität Bielefeld
Centrum für Biotechnologie

Dr. Lutz Wobbe
Universitätsstr. 27
33615 Bielefeld
Germany
info@cebitec.uni-bielefeld.de

Concept & Idea:
Dr. Stefan Weidner

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