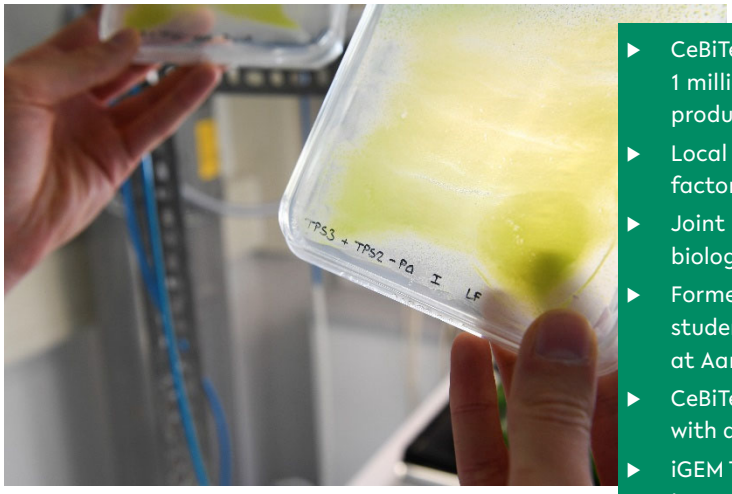
UNIVERSITÄT BIELEFELD

CeBiTec Center for Biotechnology

CeBiTec – Quarterly

Winter 2021/2022



- CeBiTec Young Researcher Dr. Nadja A. Henke receives 1 million Euro funding to develop a bacterial production system for carotenoids
- Local DNA shape is a general principle of transcription factor binding specificity
- Joint DFG tandem project on the chemistry and biology of the plant hormone 12-OPDA approved
- Former CeBiTec employee and Bielefeld University student Dr. Marta Irla appointed Assistant Professor at Aarhus University
- CeBiTec scientists describe a novel SARS-CoV-2 variant with a large deletion
- iGEM Team Bielefeld-CeBiTec 2021 is one of the top10 teams of the annual competition and receives gold

CeBiTec Young Researcher Dr. Nadja A. Henke receives 1 million Euro funding to develop a bacterial production system for carotenoids

The research of CeBiTec member Dr. Nadja A. Henke, who is working in the research group of Prof. Dr. Volker Wendisch (Bielefeld University; Genetics of Prokaryotes), will be funded within the project titled “Validierung einer mikrobiellen Plattformtechnologie zur Produktion natürlicher Karotinoide”, short name KaroTec (03VP09460) and receive 1 million Euro as a BMBF (Federal Ministry of Education and Research)-VIP+-project. Carotenoids are high value-pigments, not only applied in the food and feed industry, but also increasingly demanded as functional ingredients by the cosmetics industry.

The main objective of the 3 years project is to develop a scalable and transferable fermentative production process to sustainably produce natural carotenoids with bacteria.

Figure 1: Dr. Nadja A. Henke (left) and Dr. Florian Meyer from the KaroTec team of the CeBiTec.

Besides creating a high efficiency production strain, a fermentation process will be developed in collaboration with the research group of CeBiTec member Prof. Dr. Alexander Grünberger (Bielefeld University; Multiscale Bioengineering)

and using the technology platform “fermentation”.

The KaroTec team comprises Dr. Nadja Henke (leadership and strain development), Dr. Florian Meyer (process development) and three PhD students.

(P. Peters-Wendisch)

Local DNA shape is a general principle of transcription factor binding specificity

Various *in vitro* and *in vivo* methods have been developed to measure protein-DNA binding. Yet though, it is still challenging to predict those binding events computationally with high confidence. Such predictions for the promoter region of a gene of choice could function as indicators for its expression and would allow improved estimation of gene expression directly from sequences.

Andrea Bräutigam and Janik Sielemann developped a machine learning-based approach to predict protein-DNA binding based on DNA sequence and shape. We trained models by using DAP-seq based, experimentally verified binding sites and incorporated the shape of the DNA[1]. For this, we used 80% of the “ground truth” binding sites for training and hyperparameter tuning and evaluated the resulting models predictions on 20% of the dataset for each transcription factor (TF).

The computational evaluation demonstrated that DNA-shape based predictions improved for each of the 217 transcription factors compared to predictions only made using DNA sequence alone. The CeBiTec compute system provided the computational infrastructure for this endeavor.

Predictions for two transcription factor proteins, At5g11260 (HY5) and At3g10480 (ANAC050), were validated using laboratory-based methods. Competitive electrophoretic mobility shift assay (EMSA), were performed by Donat Wulf using out-of-distribution, novel sequences for which binding or non-binding prediction were generated and validated (Fig. 2). Romy Schmidt provided the necessary details for the design of this experimental procedure.

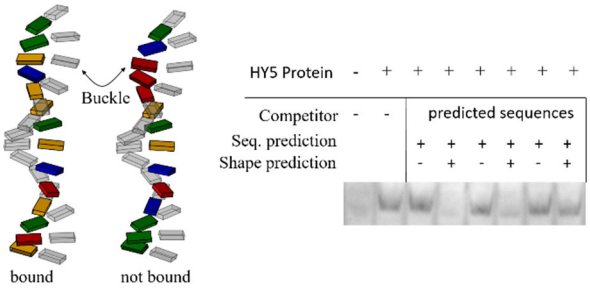


Figure 2: Competition EMSA experiment. All used sequences contain the binding motif. The model assigned different binding affinities, based on the shape features of the sequence. For 5 out of 6 attempts the model predicted binding and non-binding correctly – the sixth attempt showed an incorrect prediction in which completion was predicted but not evident. Note the differences in shape in the bound and non-bound sequences to the left.

In summary, the results show that the binding behavior of TFs depends on the 3D position of bases in its binding site, where different TFs favor different formations even within the same protein family. Experiments were performed using plant transcription factors but the results very likely transfer to animal, human and bacterial DNA binding proteins. Precise understanding of TF binding has the potential to improve the understanding of gene regulatory networks and enable targeted refinements of traits of interest.

Reference

[1]Sielemann, J., Wulf, D., Schmidt, R. *et al.* Local DNA shape is a general principle of transcription factor binding specificity in *Arabidopsis thaliana*. *Nat Commun* **12,** 6549 (2021). https://doi.org/10.1038/s41467-021-268192

(J. Sielemann & A. Bräutigam)

Joint DFG tandem project on the chemistry and biology of the plant hormone 12-OPDA approved

The German Research Foundation (DFG) will fund a joint tandem project of the two CeBiTec research groups headed by Prof. Dr. Karl-Josef Dietz (Biochemistry and Physiology of Plants) and Prof. Dr. Harald Gröger (Industrial Organic Chemistry and Biotechnology) being entitled "Regulation of the synthesis and function of 12-oxophytodienoic acid in plant cellular signal processing" for three years.

The plant hormone 12-oxophytodienoic acid (OPDA; Fig. 3) plays multiple roles in plant signaling and metabolism. Among them, it acts as a precursor for the biosynthesis of jasmonic acid. Jasmonic acid also represents an important industrial chemical in the field of fragrances being produced today, however, by a lengthy chemical synthetic route. Further roles of OPDA are to serve as a binding partner regulating protein functions, covalent modifier of proteins by posttranslational modifications and reactant with other low molecular mass compounds.

This CeBiTec collaboration research project is going to study a fully enzymatic process to OPDA based on nature´s biosynthetic concept. In addition, chemoenzymatic approaches will lead to structurally related novel OPDA analogues that will be utilized to address a set of urgent open questions of plant molecular biology and cell signaling with a particular focus on regulation of the synthetic pathway, the identification of OPDA-binding proteins, OPDAylation and the effect of OPDA and related compounds on plant stress responses.

Figure 3: OPDA as a key plant hormone with multiple roles in plant signaling and metabolism.

The basis of this research project is the long-standing joint research work of the Dietz and Gröger groups in this field of research, which has been ongoing for many years.

(H. Gröger & K.-J. Dietz)

Former CeBiTec employee and Bielefeld University student Dr. Marta Irla appointed Assistant Professor at Aarhus University

Dr. Marta Irla, a former PhD student of the Wendisch Lab who served as student representtative on the Executive Board of CeBiTec, was appointed Assistant Professor at Aarhus University, Denmark, on August 24th 2021.

Figure 4: Dr. Marta Irla

Marta holds a doctoral degree in biology from Bielefeld University, which was awarded in 2016 for her systems biology studies on bacterial methylotrophy and methanol-based bioproduction. She was a PhD student in the CLIB graduate cluster, a joint PhD program of the universities of Bielefeld, Dortmund and Düsseldorf and the Research Center Jülich. From 2017 on, Marta continued her research on the genetics and biotechnology of *Bacillus methanolicus* as postdoctoral researcher at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway. From 2018-2021 the Wendisch Lab and Marta (NTNU, Brautaset Lab) collaborated in the ERA CoBiotech project C1Pro with Marburg University (Germany), the research institutes INSA (France) and SINTEF (Norway) and the companies BASF (Germany) and Acies Bio (Slovenia).

Now, Marta is establishing her research group "Microbial Synthetic Biology" in the Department of Biological and Chemical Engineering of Aarhus University.

Her research focusses on the development of methanol-based bioprocesses for production of value-added compounds.

The former colleagues at CeBiTec, which sees itself as a research incubator and is proud to support the careers of young scientists, wish Marta a successful start at Aarhus University and we are looking forward for fruitful networking in the future.

(V.F. Wendisch)

CeBiTec scientists describe a novel SARS-CoV-2 variant with a large deletion

Scientists from the research groups of Jörn Kalinowski and Alexander Sczyrba have published a study on a SARS-CoV-2 variant with a large deletion in the "Open Reading Frame 8" ORF8 gene.

Genomic surveillance of the SARS-CoV-2 pandemic is widely done and mainly achieved by amplicon sequencing protocols. Overlapping tiled-amplicons are generated to establish contiguous SARS-CoV-2 genome sequences, which enable the precise resolution of infection chains and outbreaks.

The research team investigated a SARS-CoV-2 outbreak in a local hospital and employed nanopore sequencing with a modified sequencing protocol and long amplicons.

They identified a 168-base deletion, which was almost impossible to detect with the classical short amplicon sequencing procedures since it removes two amplicon primer-binding sites, leading to a loss of sequence information in the region of the deletion.

Additionally, the standard data analysis pipelines were not able to correctly identify this large deletion and had to be modified.

The researchers also analyzed public SARS-CoV-2 sequences and sequencing read data from public archives and identified the same deletion in over 100 genomes that belong to different lineages of SARS-CoV-2 stemming from all over Europe, pointing to a mutation hotspot or to positive selection. In almost all cases, the deletion was not represented in the virus genome sequence after consensus building. Additionally, further database searches pointed to other deletions in

the ORF8 coding region that have never been reported by the standard data analysis pipelines. The ORF8 gene product is known to play a role in evasion from the human immune response and a lack of its functionality may result in milder forms of the COVID-19 disease.

Figure 5: Dr. Christiane Scherer (Evangelisches Klinikum Bethel) and CeBiTec members Prof. Dr. Jörn Kalinowski and Prof. Dr. Alexander Sczyrba

Deletions such as this ORF8 deletion and other mutations that may increase infectivity but decrease fatality conceivably play a role in the viruses path towards endemism, as it can be currently observed with the Omicron variant.

Reference:

David Brandt, Marina Simunovic, Tobias Busche, Markus Haak, Peter Belmann, Sebastian Jünemann, Tizian Schulz, Levin Joe Klages, Svenja Vinke, Michael Beckstette, Ehmke Pohl, Christiane Scherer, Alexander Sczyrba, Jörn Kalinowski (2021) Multiple Occurrences of a 168-Nucleotide Deletion in SARS-CoV-2 ORF8, Unnoticed by Standard Amplicon Sequencing and Variant Calling Pipelines. *Viruses* **13(9)**: 1870

https://www.mdpi.com/1999-4915/13/9/1870

(D. Brandt, J. Kalinowski & A. Sczyrba)

iGEM Team Bielefeld-CeBiTec 2021 is one of the top10 teams of the annual competition and receives gold medal.

Figure 6: The 2021 iGEM Bielefeld-CeBiTec team

In October 2021, the iGEM Bielefeld-CeBiTec team was awarded as one of the best teams in the competition and received the gold medal. The team was further nominated for the special prizes “Best new application” and “Best basic part”. The team, comprised of 13 students from Bielefeld University and from different life sciences, participated in the renowned iGEM (international Genetically Engineered Machine) competition, where they designed and implemented their own research project in the field of synthetic biology. Within one year, they implemented an impressing synthetic biology project to tackle the problem of the identification and surveillance of suspected and unknown storage sites of chemical weapons and byproducts, where its degradation poses a threat to the environment. Within the whole project, they stayed in close contact with the *iGEM Biosecurity and Biosafety committee*, as well as the *Organization for the Prohibition of Chemical Weapons* (OPCW)

to prevent any risks when working with less harmful chemical weapon precursors as proof of concept.

The team decided to solve this problem by creating a plant-based biosensor for the detection of chemical weapon degradation products and successfully showed the functionnality of all of its components. The detection is enabled by the specific binding of a receptor, followed by signal transduction and leading to a visual output signal. The team used computational protein design to create receptors which are specific for biosimilars that mimick chemical weapons and proved that the specificity could be increased. They further could show that the signalling cascade is functional and that the use of RUBY, coding for a betalain biosynthesis gene and producing a bright red colour, is favored as visual signal due to lack of requirement of an additional substrate, a good visibility, and a high stability of the pigment.

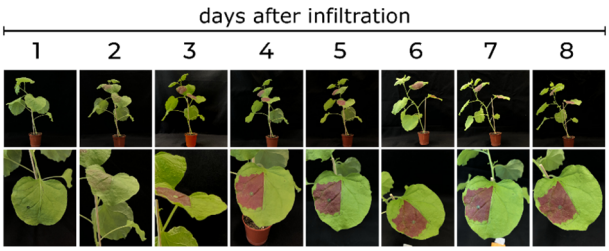


Figure 7: *N. benthamiana* leaves infiltrated with 35S:RUBY, from 1 to 8 days after infiltration with agrobacteria from transient transformation.

The team of 2021 comprised 13 students enrolled in Bachelor and Master programs, including Molecular Biotechnology, Interdisciplinary Biomedicine, Genome Based Systems Biology as well as Molecular Cell Biology. Special thanks go out to the team's advisers Laura Schlüter, Christian Rückert, and Prof. Dr. Jörn Kalinowski, as well as other members of the CeBiTec and Bielefeld University who supported the team at different points during their project. This year's project of our iGEM team Bielefeld-CeBiTec and its success in the iGEM competition underlines the strong position of the CeBiTec in research and education in the Life Sciences.

Teams from the CeBiTec have been successfully participating in the iGEM competition for twelve years and with the 2021 iGEM Bielefeld-CeBiTec team, this era will come to an end. Therefore, we want to thank all the amazing people of different research groups at Bielefeld University who supported our iGEM teams over the last decade.

(L. Schlüter & 2021 iGEM Bielefeld-CeBiTec team)

Impressum

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