MegaSyn Symposium on Megasynthases

29th September to 1st October 2020

Bad Nauheim near Frankfurt, Germany

(Booklet does not contain Conference Programme; will be provided separately)

TABLE OF CONTENTS

Welcome to the MegaSyn Symposium!

It is our great pleasure to welcome you to Bad Nauheim for the MegaSyn Symposium on Megasynthases. Thank you for your attendance in these difficult times!

The idea to gather the experts in the field on natural compound biosynthesis, with a focus on megasynthases, was already born in 2017 with the start of the MegaSyn Research Cluster. We are glad that you followed our invitation, so that we could assemble a program that showcases many of the recent important scientific advances in the field. As the entire Cluster, this Symposium is funded by the LOEWE program (Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz) of the state of Hesse. We are grateful for the support over the last years.

This Symposium intends to highlight the latest, cutting-edge discoveries in the field of polyketide synthases and non-ribosomal peptide synthetases, and will also be a platform for the members of the MegaSyn Research Cluster to present their data in talks and poster presentations. The scientific program will foster discussions, and hopes to inspire participants to initiate collaborations within and across disciplines for the advancement of our field. The poster session is scheduled for Tuesday, but posters will be displayed throughout the whole Symposium, giving multiple opportunities for in-depth discussions.

Finally, a few more sentences about the Corona pandemic, as it has an enormous impact on all aspects of our daily and professional life. We are convinced that holding a conference with the participants in attendance is possible in these difficult times, if compliance with safety regulations is guaranteed. We have assured ourselves in advance that this spacious hotel complex with its responsible staff makes this possible. Despite all of this, there remains always a residual risk of infection, which mainly depends on how we behave as participants in the conference. This is why we appeal to compliance with the safety regulations even in places that may encourage casual interaction, such as a conversation during the coffee break or during a drink at the bar in the evening. We urge you to consistently wear a face mask in closed rooms, i.e. whenever you have not taken a “fixed position” (seated or standing) with sufficient distance. Let's please act responsibly together to make this conference venue a safe environment for everyone.

We’re looking forward to an excellent meeting with great scientists and sharing new and exciting results on natural compound biosynthesis.

Helge and Martin

-Frankfurt, 15th September 2020-

MegaSyn Research Cluster

The MegaSyn Research Cluster started in 2017. It is a Hesse-wide research initiative, funded with about 4.6 M€ by the Hessen State Ministry of Higher Education, Research and the Arts, which focuses on the understanding of megasynthases in structure and function, and controlling megasynthases for directed natural product synthesis. The MegaSyn research program includes 12 groups from 5 different institutions.

The MegaSyn project started with the idea to enlarge the chemical space of polyketides and non-ribosomal peptides by engineering a set of relatively well understood and simple megasynthases (PKSs and NRPSs). The synthetic potential of those megasynthases shall be harnessed in new reaction pathways by employing a broad set of methods from fields of molecular and structural biology, biotechnology and computational modelling. The approach is based on two synergistic developments to which MegaSyn PIs had substantially contributed during the years: the newly established knowledge on the function and manipulation of PKSs and NRPSs, and new and improved methods in structural biology, which were essential to pave the way for handling these complex proteins. As such, the MegaSyn project is complementary to traditional approaches in the field that focus on identifying new compounds from natural sources occurring as secondary metabolites.

Several substantial scientific contributions to the understanding of megasynthases were made during the almost four years of MegaSyn funding of which some are presented during the MegaSyn conference by the PhD students actually collecting those data.

Helge and Martin

-Frankfurt, 15th September 2020-

HOSTS

- Martin Grininger grininger@chemie.uni-frankfurt.de
- Helge Bode h.bode@bio.uni-frankfurt.de

ORGANIZATION

megasyn-symposium@uni-frankfurt.de

- Theodora Ruppenthal office.grininger@chemie.uni-frankfurt.de
- Karthik S. Paithankar paithankar@chemie.uni-frankfurt.de

SPONSORS

LOEWE

Exzellente Forschung für Hessens Zukunft

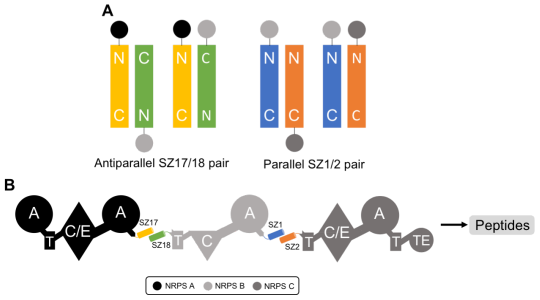
Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz - in short: LOEWE - is the title of the research funding program with which the state of Hesse has been setting scientific policy impulses since 2008 with the aim to sustainably strengthen the Hessian research landscape. In the period from 2008 to 2019, around € 869 million were made available for LOEWE to promote outstanding joint scientific projects (source: https://wissenschaft.hessen.de).

SYNZIPs AS A TOOL FOR THE RAPID CONSTRUCTION OF ENGINEERED NON-RIBOSOMAL PEPTIDE SYTHETASES

Nadya Abbood, Kenan A. J. Bozhueyuek, Jonas Watzel, Helge Bode, Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany

Nadya Abbood
Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
abbood@bio.uni-frankfurt.de

Peptides generated by microbial non-ribosomal peptide synthetases (NRPS) are clinically used as drugs (antibiotic, anti-cancer or immunosuppressive drugs)[1]. Engineering these mega synthases to access new or altered non-ribosomal peptides (NRPs) is of great interest[2]. Even though recent concepts allowed the de novo design of new-to-nature NRPS systems[3], NRPS engineering remains an ongoing task. This presentation introduces you into a method that allows the rapid construction of dozens of engineered NRPS systems by the re-combination of SYNZIP linked NRPS units from different biosynthesis pathways and species. SYNZIPs are heterospecific synthetic alpha helices that have specific affinities to their SYNZIP partner and allow a strong non-covalent interaction[4,5]. Once they are introduces into the C-A linker or other regions of the system as novel NRPS parts, they can connect the split NRPS to a functional enzyme. When re-combining SYNZIP linked NRPS units with each other, the number of engineered systems increases rapidly with the number of building blocks. Therefore, SYNZIPs represent a good tool to do NRPS engineering in a high-throughput manner which simplify the access to new bioactive compounds.



*SYNZIPs as a tool for NRPS engineering. **A** Overview of used antiparallel (left) and parallel (right) SYNZIP pairs. **B** Schematic representation of a SYNZIP linked NRPS assembled from three different synthetases.*

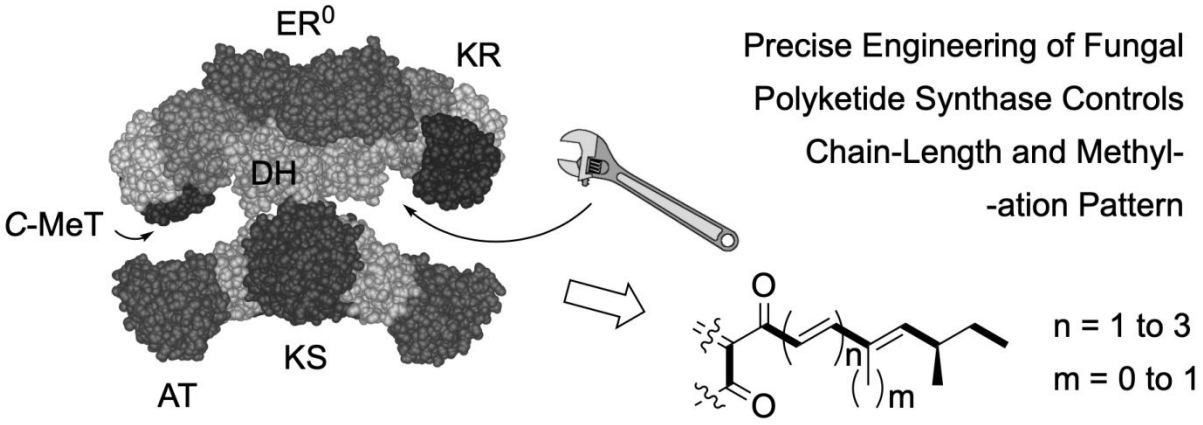
References

(1) Süssmuth, D. R., Mainz, A. (2017). Nonribosomal peptide synthesis- principles and prospects. Angew Chem Int Ed Engl 56, 3770-3821.
(2) Calcott,M.J.,Ackerly,D.F.(2014).Geneticmanipulationofnon-ribosomalpeptidesynthetases to generate novel bioactive peptide products. Biotechnol Lett 36, 2407-2416.
(3) Bozhüyük,K.A.J.,Fleischhacker,F.,Linck,A.,Wesche,F.,Tietze,A.,Niesert,C.,Bode,H.B. (2018). *De novo* design and engineering of non-ribosomal peptide synthetases. Nat Chem 10, 275-281.
(4) Thompson, K. E., Bashor, C. J., Lim, W. A., Keating, A. E. (2012). SYNZIP protein interaction toolbox: *in vitro* and *in vivo* specifications of heterospecific coiled-coil interaction domains. ACS Synth Biol 1, 118-129.
(5) Reinke, A. W., Grant, R. A., Keating, A. E. (2009). A synthetic coiled-coil interactome provides heterospecific modules for molecular engineering. J Am Chem Soc 132, 6025-6031.

INVESTIGATIONS OF PROGRAMMING AND REPROGRAMMING IN FUNGAL HR-PKS

Russel Cox
Institute for Organic Chemistry, BMWZ, Leibniz University, Hannover, Germany
russell.cox@oci.uni-hannover.de

Fungal highly reducing polyketide synthases (hr-PKS) have a domain structure almost identical to the vertebrate Fatty acid synthase (vFAS). However, in contrast to vFAS, hr-PKS are highly programmed, controlling starter unit selection, chain-length, methylation pattern, reduction pattern, and in some cases even *E/Z* geometry. This is remarkable because hr-PKS, like vFAS, are iterative, using the same suite of catalytic domains repeatedly. The Cox group have taken two complementary approaches to understanding and engineering the programming of hr-PKS. First, extensive domain swaps have been made between differently programmed synthases; and secondly isolated WT and mutant domains have been studied *in vitro* to determine their native selectivities. The presentation will focus on: the high levels of similarity between vFAS and hr-PKS; the use of extensive modelling to predict and verify programming elements; and the rational reprogramming of individual catalytic domains.[1,2,3]



References

(1) K. M. Fisch, W. Bakeer, A. A. Yakasai, Z. Song, J. Pedrick, Z. Wasil, A. M. Bailey, C. M. Lazarus, T. J. Simpson, R. J. Cox, *J. Am. Chem. Soc.,* **2011**, *133*, 16635–16641.
(2) X.-L. Yang, S. Friedrich, S. Yin, O. Piech, K. Williams, T. J. Simpson, R. J. Cox, *Chem. Sci.*, **2019**, *10*, 8478–8489.
(3) O. Piech and R. J. Cox, RSC Chemical Biology, **2020**, submitted.

SPECIALIZED METABOLISM AT THE HOST-GAMMAPROTEOBACTERIAL INTERFACE

Jhe-Hao Li,[1,2,7] Joonseok Oh,[1,2,7] Sabine Kienesberger,[3] Nam Yoon Kim,[1,2] David J. Clarke,[4] Ellen L. Zechner,[3,5] and Jason M. Crawford[1,2,6,]*

[1]Department of Chemistry, Yale University, New Haven, CT 06520, USA

[2]Chemical Biology Institute, Yale University, West Haven, CT 06516, USA

[3]Institute of Molecular Biosciences, University of Graz, A-8010 Graz, Austria

[4]School of Microbiology and APC Microbiome Ireland, University College Cork, Cork, Ireland

[5]BioTechMed-Graz, A8010 Graz, Austria

[6]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536, USA

[7]These authors contributed equally: Jhe-Hao Li, Joonseok Oh

Jason Crawford

Department of Chemistry, Yale University, New Haven, USA

Jason.crawford@yale.edu

Gammaproteobacteria produce diverse natural products and are well represented in the human microbiota. In this lecture, we highlight two distinct strategies for the formation and processing of peptidic natural products in select gammaproteobacterial microbiome members that regulate host responses. While the selected peptides are produced through differing mechanisms – nonribosomal peptide synthesis versus ligase-mediated peptide synthesis – both groups of molecules undergo proteolytic transformations that alter their structural and functional properties. We also discuss how these biosynthetic operations are associated with host pathogenesis.

A TRANSESTERIFYING THIOESTERASE EXTENDS G PROTEIN INHIBITOR BIOSYNTHESIS

Cornelia Hermes, René Richarz, Daniel Wirtz, Wiebke Hanke, Stefan Kehraus, Evi Kostenis, Gabriele M. König, Max Crüsemann

Max Crüsemann
Institute of Pharmaceutical Biology, University of Bonn, Germany
cruesemann@uni-bonn.de

The depsipeptide natural product FR900359 (FR) selectively inhibits Gαq proteins and thus signaling of many G protein-coupled receptors. This unique mechanism of action makes FR an indispensable pharmacological tool to study Gαq-related processes, as well as a promising drug candidate.(1)
FR is a complex natural product with seven nonproteinogenic building blocks; it was isolated from the plant *Ardisia crenata*, but is not produced by the latter. Instead, the endosymbiotic bacterium "*Candidatus* Burkholderia crenata" contains the biosynthetic gene cluster of FR (*frsA-frsH*) encoding two nonribosomal peptide synthetase (NRPS) systems.(2)
In this work, the successive assembly of the FR side chain, *N*-propionylhydroxyleucine was achieved *in vitro*, by utilizing the monomodular NRPS FrsA and the non-heme diiron monooxygenase FrsH. The final step of FR biosynthesis is an intermolecular transesterification reaction, conjugating the side chain with a macrocyclic depsipeptide intermediate (FR-Core) that is assembled by the heptamodular NRPS FrsD-G, and was shown to be overproduced by a *ΔfrsA* mutant. We show that the FrsA thioesterase domain catalyzes this unusual reaction, and harnessed the substrate promiscuity of the FrsA domains for the chemoenzymatic production of unnatural FR derivatives with altered side chains.
Our *in vivo* and *in vitro* studies demonstrate that the side chain of FR is crucial for its remarkable Gαq inhibition properties and we furthermore present an evolutionary scenario leading to this important biosynthetic optimization.

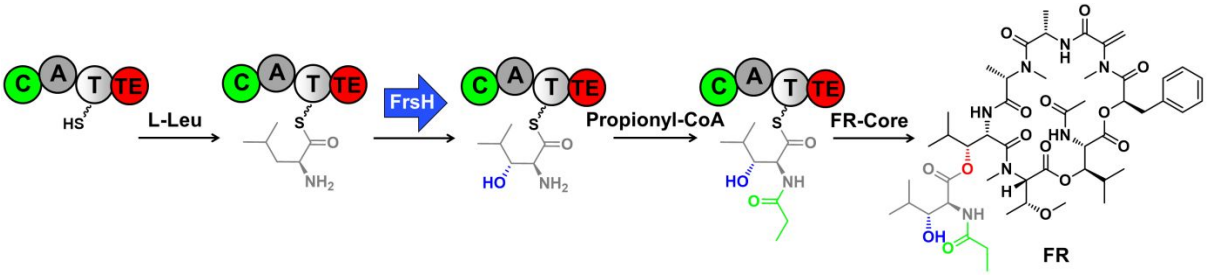


Figure 1: Formation of the FR900359 side chain by the monomodular FrsA and attachment to the heptacyclic FR-Core by an intermolecular transesterification reaction catalysed by FrsA-TE.

References

(1) R. Schrage et al. The experimental power of FR900359 to study Gq-regulated biological processes. *Nat. Comm*. **2015**, 6, 10156.
(2) M. Crüsemann et al. Heterologous Expression, Biosynthetic Studies, and Ecological Function of the Selective Gq-Signaling Inhibitor FR900359. *Angew. Chem. Int. Ed*. **2018**, 57, 836-40.

UNRAVELING EVOLUTIONARY SCENARIOS FOR THE DIVERSIFICATION OF NONRIBOSOMAL PEPTIDE SYTHETASES

Martin Baunach[1] and Elke Dittmann[1]
[1]Department of Microbiology, University of Potsdam, Golm, Germany

Elke Dittmann
Institute of Biochemistry and Biology Department of Microbiology, University of Potsdam, Germany
editt@uni-potsdam.de

Nonribosomal peptides (NRP) are of tremendous significance in microbial ecology as well as for pharmacological applications. Nevertheless, the evolution of the multitude of individual megasynthetases that correlates with the stunning structural diversity of NRPs is poorly understood. Starting with the assembly line catalyzing the synthesis of the cyanobacterial toxin microcystin (1), we have systematically dissected recombination events in bacterial NRP synthetases (NRPS) genes across distant bacterial phyla that have guided structural diversification in a plethora of NRP families. The comparison of a large number of individual recombination events did not only unveil a striking plurality in the nature and origin of the exchange units but allowed deducing overarching principles enabling efficient exchange of adenylation (A) domain substrates whilst keeping the functionality of the dynamic multienzyme complexes. In the majority of cases, recombination events were targeting variable portions of the A_{core} domains, yet domain interfaces and the flexible A_{sub} domain remained untapped. The present-day variety of compounds and the mosaic-like pattern observed in biosynthesis genes as well as in NRPS family distribution likely reflect the ongoing evolution of NRPs as gene collectives in a transforming genetic background shaped by genome streamlining as well as horizontal gene transfer. Our results strongly contradict the assumption that adenylation and condensation (C) domains coevolve and significantly challenge the attributed role of C domains as stringent selectivity filter during NRP synthesis. Moreover, they teach valuable lessons on the choice of natural exchange units in the evolution of NRPS diversity, which may guide future engineering approaches.

References

(1) Meyer et al. Cell Chem Biol. 23: 462-471

SUBSTRATE SELECTIVITY OF PHOSLACTOMYCIN POLYKETIDE SNTHASE AND FUNCTION OF THE ASSOCIATED TYPE II THIOESTERASE

Kyra Geyer, Steffen Hartmann, Tobias J. Erb
Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch-Str. 10, 35043, Marburg (Germany)

Kyra Geyer
Max Planck Institute for Terrestrial, Microbiology, Karl-von-Frisch-Str. 10, 35043 Marburg, Germany
kyra.geyer@mpi-marburg.mpg.de

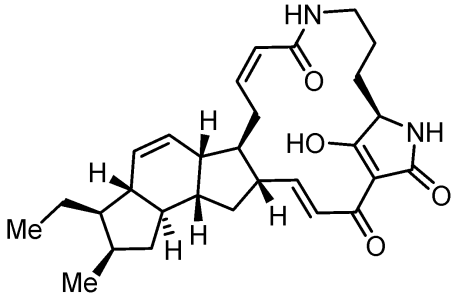
Polyketide synthases (PKS) use simple extender units to synthesize complex natural products. A fundamental question is how different extender units are site-specifically incorporated into the growing polyketide. Here we established phoslactomycin (Pn) PKS that incorporates malonyl- and ethylmalonyl CoA as an *in vitro* model to study substrate specificity. We combined up to six Pn PKS modules with different termination sites for the controlled release of tetra-, penta- and hexaketides and challenged these systems with up to seven different extender units in competitive assays to test for specificity of Pn modules. While malonyl-CoA modules of Pn PKS exclusively accept their natural substrate, the ethylmalonyl-CoA module PnC tolerates different *α*-substituted derivatives, but discriminates against malonyl-CoA. We show that the ratio of extender transacylation to hydrolysis controls incorporation in PnC, explaining site-specific selectivity and promiscuity in the natural context of Pn PKS. Furthermore, we show a beneficial effect of PnG, the type II thioesterase present in the phoslactomycin gene cluster, on the *in vitro* production of polyketides. Biochemical characterization with acyl-ACP substrates show a clear preference for decarboxylated residues. This data, combined with observations made in the production assays support the low specificity model of type II thioesterases and open the path to increasing efficiency of *in vitro* polyketide synthase systems

APPLICATION OF MULTIMODULAR AND TAILORING ENZYMES IN COMPLEX MELCULE (BIO-)SYNTHESIS

Chair of Technical Biochemistry, Technical University of Dresden, Germany.

Tobias A. M. Gulder
Chair of Technical Biochemistry, Technical University of Dresden, Germany.
tobias.gulder@tu-dresden.de

The biosynthesis of complex specialized metabolites is often orchestrated by multimodular polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS), or fusions of these machineries. Modern bioinformatic and analytical tools as well as engineering approaches are key to further expand the known PKS/NRPS structural space by enabling discovery of novel molecules from Nature and by facilitating the artificial design of new structures.
A still largely untapped application of PKS/NRPS enzymes is their use in the synthesis of complex molecules *in vitro*, thereby harnessing their full biocatalytic potential. Within this talk, studies on the unusual bacterial iPKS/NRPS machinery encoding polycyclic tetramic acid containing macrolactams (PoTeMs), such as ikarugamycin, will be presented – from functional studies to applications *in vivo* and *in vitro*. In addition, biocatalytic strategies for late-stage structural diversification of PKS and NRPS products will be discussed, showcasing the tremendous potential of biosynthetic tailoring enzymes in the total syntheses and targeted functionalization of natural and structurally modified microbial metabolites.



Representative structure of a PoTeM: ikarugamycin.

References

(1) Antosch, F. Schaefers, T. A. M. Gulder; *Angew. Chem. Int. Ed.* **2014**, *53*, 3011.
(2) Greunke, J. Antosch, T. A. M. Gulder; *Chem. Commun.* **2015**, *51*, 5334.
(3) C. Greunke, A. Glöckle, J. Antosch, T. A. M. Gulder; *Angew. Chem. Int. Ed.* **2017**, *56*, 4351.

IN VITRO BIOCHEMISTRY AND BIOCATALYSIS OF PKS DOMAINS AND MODULES

Lisa Wagner, Tim Hollmann, Marius Schröder, Johannes Wunderlich, Frank Hahn

Frank Hahn
Department of Chemistry, Faculty of Biology, Chemistry and Earth Sciences, University Bayreuth, Bayreuth, Germany
frank.hahn@uni-bayreuth.de

The action of polyketide synthases (PKSs) is enabled by the fascinating interplay of their individual components, which in turn is controlled by a combination of protein-protein and protein-substrate interactions.[1] The substrate-dependent selectivity of the individual domains is an important factor in complex PKS systems, which must be taken into account for their engineering.[2] We use a combination of synthetic chemistry, in vitro biochemistry and structural biology to investigate such substrate- dependent effects. This provides crucial insights into the catalytic performance of standard and non- canonically acting domains.
A deeper understanding of the structures and mechanisms of PKS megasynthases enables their targeted improvement and biotechnological exploitation.[1] Besides the engineering of entire PKS in vivo, the in vitro biocatalytic use of PKS constituents has potential to provide access to compounds with technological and pharmaceutical value. Their nature as large multi-domain enzymes however makes PKS rather “exotics” in the field of enzymatic biocatalysis. This underlines the importance of studies in which substrate specificity and product selectivity of PKS biocatalysts are comprehensively investigated using structurally diverse substrate surrogates.
In the talk I will present examples of detailed studies on PKS domains and modules together with the application of some of these enzymes in natural product synthesis.[3-8]

References

(1) M. Klaus, M. Grininger, *Nat. Prod. Rep.* **2018**, *35*,1070.
(2) K. J. Weissman, *Beilstein J. Org. Chem.* **2017**, *13,* 348.
(3) S. Friedrich, F. Hahn, *Tetrahedron* **2015**, *10*, 1473.
(4) T. Hollmann, G. Berkhan, L. M. C. Wagner, K. H. Sung, S. Kolb, H. Geise, F. Hahn, *ACS Catal.* **2020**, *10*, 4973.
(5) J. Wunderlich, T. Roß, M. Schröder, *Org. Lett.* **2020**, *22*, 13, 4955.
(6) K. H. Sung, T. Hollmann, G. Berkhan, L. M. C. Wagner, W. Blankenfeldt, F. Hahn, *Angew. Chem. Int. Ed.* **2018**, *57*, 343.
(7) G. Berkhan, C. Merten, C. Holec, F. Hahn, *Angew. Chem. Int. Ed.* **2016**, *55*, 13589.
(8) G. Berkhan, F. Hahn, *Angew. Chem. Int. Ed.* **2014**, *53*, 14240.

ECO-NOMICS: DECIPHERING THE BIOSYNTHESIS, EVOLUTION AND CHEMICAL ECOLOGY OF TRANS-ACYLTRANSFERASE POLYKETIDE SYNTHASE-DERIVED POLYKETIDES

Eric Jan Nikolaus Helfrich
Harvard Medical School, Departement Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Bldg C-643, B C M P, 240 Longwood Ave, Boston MA 02115, USA
eric_helfrich@hms.harvard.edu

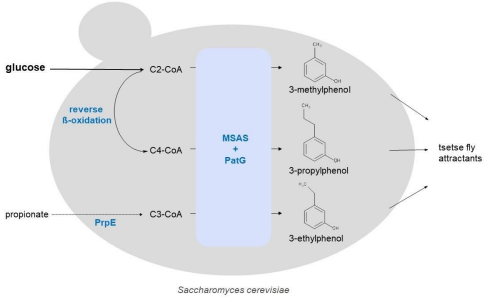
Trans-acyltransferase polyketide synthases (*trans*-AT PKSs) are giant multi-domain enzyme complexes that are responsible for the biosynthesis of structurally diverse polyketides, many of which are of high pharmaceutical value. In comparison to the well studied textbook modular polyketide synthases (*cis*-AT PKSs) the biosynthesis of *trans*-AT PKS-derived polyketides is only poorly understood. Bioinformatic studies on *trans*-AT PKSs revealed a high correspondence between the phylogenetic relationships of ketosynthase domains and the biosynthetic intermediates they recognize. We have exploited this simple correlation for the development of two bioinformatic platforms for the accurate structural prediction of *trans*-AT PKS-derived polyketides (TransATor) in genome mining studies and to explore the evolution of the combinatorial biosynthetic diversity encountered in *trans*-AT PKSs (TransPACT). These studies resulted in the genome mining-guided identification of novel *trans*-AT PKS scaffolds and the recognition that evolution through the exchange, excision or acquisition of conserved sequential arrangements of PKS modules is a widespread phenomenon in *trans*-AT PKS systems. Insights gained into the natural combinatorial diversity of *trans*-AT PKS systems do not only provide evidence for the origin of chemical novelty in *trans*-AT PKS systems but will also pave the way towards evolutionary-inspired engineering of non-natural hybrid PKSs. Moreover, the *trans*-AT PKS correlation rule can be applied to identify the uncultured producers and biosynthetic gene clusters of pharmaceutically important *trans*-AT PKS-derived polyketides in complex metagenomes. Deciphering the biosynthetic blueprints of the PKSs associated with these drug candidates is the first step towards the sustainable biotechnological production of pharmaceutically-important *trans*-AT PKS-derived polyketides from microbial dark matter.

POLYKETIDE SYNTHASE AND DECARBOXYLASE MEDIATED PRODUCTION OF 3-ALKYLPHENOLS IN YEAST ENGINEERED FOR INCREASED PRECURSOR SUPPLY

Julia Hitschler[1], Martin Grininger, Eckhard Boles[1].
[1]*Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany.*
[2]*Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany*

Julia Hitschler
Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
j.hitschler@bio.uni-frankfurt.de

Using polyketide synthase expression in yeast and re-direction of priming unit synthesis, we present a biotechnological approach for inexpensive, simple production of various 3-alkylphenols in microbial fermentations from sugars. 3-alkylphenols (3-methyl, 3-ethyl- and 3-propylphenol), normally occuring in cattle urine, can act as attractants in tsetse fly traps to prevent transmission of the diseases human sleeping sickness and African animal trypanosomiasis (AAT) in sub-saharan Africa.
We have developed a yeast strain with a *de novo* 3-methylphenol (3-MP) production pathway. In this recombinant yeast, heterologous phosphopantetheinyltransferase-activated 6-methylsalicylic acid synthase (MSAS) utilizes acetyl-CoA as priming unit to synthesize 6-methylsalicylic acid (6-MSA) that is further converted by 6-MSA decarboxylase to 3-MP. Through exploitation of the substrate promiscuity of MSAS for the alternative priming units propionyl-CoA and butyryl-CoA as well as the decarboxylase, we produced 3-EP and 3-PP in yeast fermentations.
3-EP formation by activated MSAS and 6-MSA decarboxylase was facilitated by propionate feeding, since yeast can convert extracellularly provided propionate into propionyl-CoA via endogenous enzymes. Nevertheless, additional expression of a heterologous propionyl-CoA synthase gene improved 3-EP production up to 12.5 mg/L. An engineered reverse ß-oxidation pathway provided butyryl-CoA as priming unit and led to a maximum titer of up to 2.6 mg/L 3-PP. As our yeasts reached similarly high concentrations of 3-alkylphenols as those occurring in cattle urine, they might promise an inexpensive production of attractants in rural African communities. Further applications include utilization as preservatives and for the production of cleaning agents or flavors.
This project was supported by the MegaSyn research programme.



Production of 3-alkylphenols in Saccharomyces cerevisiae.

References

(1) Hitschler, J. & Boles, E. *De novo* production of aromatic *m*-cresol in *Saccharomyces cerevisiae* mediated by heterologous polyketide synthases combined with a 6-methylsalicylic acid decarboxylase. Metab. Eng. Commun. 9, e00093 (2019). https://doi.org/10.1016/j.mec.2019.e00093
(2) Hitschler, J., Grininger, M. & Boles, E. Substrate promiscuity of polyketide synthase enables production of tsetse fly attractants 3-ethylphenol and 3-propylphenol by engineering precursor supply in yeast. *Sci. Rep.* 10, 9962 (2020). https://doi.org/10.1038/s41598-020-66997-5

DIRECTED BIOSYNTHESIS OF FLUORINATED POLYKETIDES

Mirko Joppe[1] Alexander Rittner[1], Lara Maria Mayer[1], Jennifer J. Schmidt[2], Dietmar Herzberg[1], Elia Heid[1], David H. Sherman[2] and Martin Grininger[1]

[1]Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany
[2]U-M Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, 48109-2216 Michigan, United states of America

Mirko Joppe
Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany
mirko.joppe@stud.uni-frankfurt.de

Polyketides are a diverse class of natural products with biological and pharmaceutical relevance, and polyketide derivatization has an enormous potential to develop new pharmaceuticals. In particular, fluorination has been used for lead structure optimization in medicinal chemistry.
In nature, polyketides are assembled by polyketide synthases (PKSs) from simple substrates. In this study, we derivatize polyketides in site-specific manner by exchanging the gatekeeping acyltransferase of PKSs with the highly promiscuous malonyl-acetyl transferase (MAT) of the metazoan type I fatty acid synthase (FAS). [1] We use module 6 of the 6-deoxyerythronolide B synthase (DEBS) as a well described model PKS to showcase the versatility of this approach. We demonstrate that the MAT communicates with the acyl carrier protein of DEBS module 6 and that the DEBS/FAS chimeric protein accepts fluoromalonyl-CoA as a substrate. Using this approach, we are able to produce new-to-nature derivatives of 10-deoxymethynolide and narbonolide, particularly interesting as new antibiotic scaffolds (see figure). The same approach can now be used for the site-specific fluorination of other polyketides.

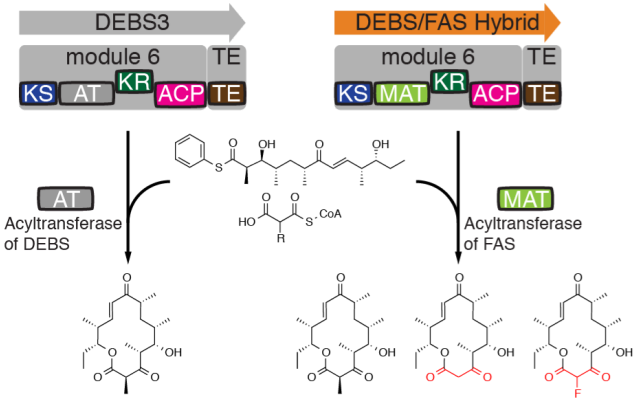


Illustration of the approach, exemplified on the sixth module of DEBS

References

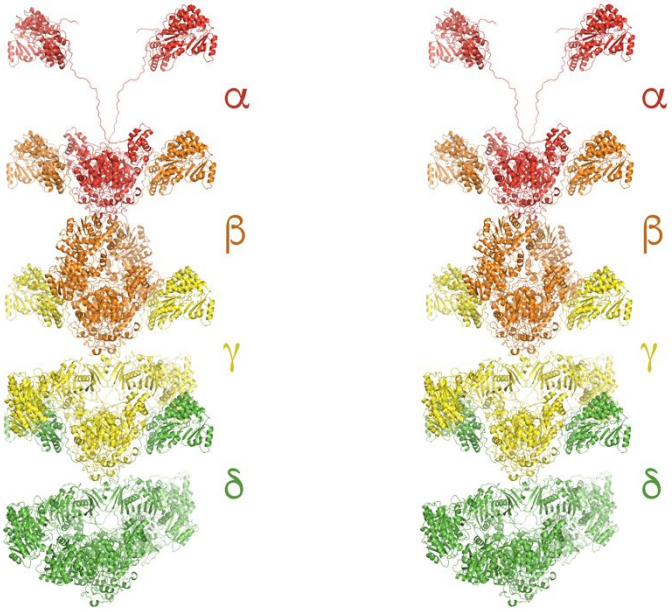
(1) Rittner, A., Paithankar, K. S., Huu, K. V. & Grininger, M. Characterization of the Polyspecific Transferase of Murine Type I Fatty Acid Synthase (FAS) and Implications for Polyketide Synthase (PKS) Engineering. *ACS Chemical Biology* **13**, 723–732 (2018).

ENGINEERING MODULAR PLYKETIDE SYNTHASES USING THE UPDATED DEFINITION OF THE MODULE

Takeshi Miyazawa, Melissa Hirsch, Katherine A. Ray, Zhicheng Zhang, and Adrian T. Keatinge-Clay
Depts. of Molecular Biosciences & Chemistry, University of Texas at Austin, Austin, TX, USA 78712

Adrian Keatinge-Clay
Depts. of Molecular Biosciences & Chemistry, University of Texas at Austin, Austin, TX, USA 78712
adriankc@utexas.edu

Engineers define a module as a set of independent units that can be used to construct more complex structures. The polyketide assembly lines that biosynthesize medicines such as the antibiotic erythromycin and the anticancer agent epothilone are modular. To optimally engineer them to produce new molecules and medicines, their modules must be properly defined. This seminar structurally and functionally describes the redefinition of the polyketide synthase module after 30 years and how our lab is using the updated definition to construct and harness active, hybrid assembly lines.[1-4]



Stereodiagram of how updated modules with downstream KS might appear.
✔ *modules*: *no processing enzymes* 🚲⏭ *KR* ■: *KR & DH* ●: *KR, DH, & ER*

References

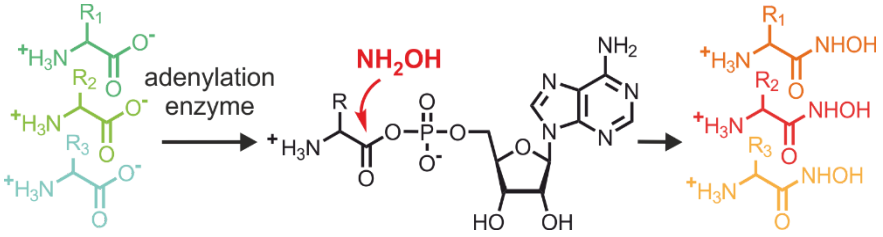
(1) Miyazawa, T., Hirsch, M., Zhang, Z., Keatinge-Clay, A.T. (2020). An in vitro platform for engineering and harnessing modular polyketide synthases. *Nat. Commun.* **11**, 80.
(2) Vander Wood, D.A. & Keatinge-Clay, A.T. (2018). The modules of *trans*-acyltransferase assembly lines redefined with a central acyl carrier protein. *Proteins* **86**, 664-75.
(3) Keatinge-Clay, A.T. (2017). The uncommon enzymology of *cis*-acyltransferase assembly lines. *Chem. Rev.* **117**, 5334-66.
(4) Keatinge-Clay, A.T. (2017). Polyketide synthase modules redefined. *Angew. Chem. Int. Ed. Engl.* **56**, 4658-60.

NRPS DESIGN GUIDED BY ADENYLATION PROMISCUITY

Aleksa Stanišić, Annika Hüsken, Hajo Kries

Hajo Kries
Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Insitute, Jena, Germany
hajo.kries@leibniz-hki

Adenylation domains control the specificity of nonribosomal peptide synthetases (NRPSs), an important group of enzymes synthesizing numerous bioactive natural products. Despite great efforts invested in adenylation domain engineering in the past, progress has been restrained by the lack of suitable assays for the screening and characterization of mutants (1). We have developed a hydroxamate assay (HAMA) that detects multiple quenched products in a single reaction under substrate competition as in the cellular environment (2). Our assay takes advantage of hydroxylamine to quench activated carboxylates to form hydroxamic acids which are sensitively and specifically detected by UPLC-MS/MS in a multiplexed fashion. HAMA provides a fast and reliable method for simultaneously recording adenylation promiscuity with dozens of substrates. Taking account of promiscuity during directed evolution of adenylation domains opens up new avenues for engineering NRPS specificity.



References

(1) Stanišić, A.; Kries, H. Adenylation Domains in Nonribosomal Peptide Engineering. *ChemBioChem* **2019**, *20*, 1347–1356.
(2) Stanišić, A.; Hüsken, A.; Kries, H. HAMA: A Multiplexed LC-MS/MS Assay for Specificity Profiling of Adenylate-Forming Enzymes. *Chem. Sci.* **2019**, *10*, 10395–10399.

COMPUTATIONAL APPROACHES TO EXPLORE AND ENGINEER MAGSYNTHASE FUNCTIONAL DIVERSITY

Marnix H. Medema
Bioinformatics Group, Wageningen University, Wageningen, The Netherlands
marnix.medema@wur.nl

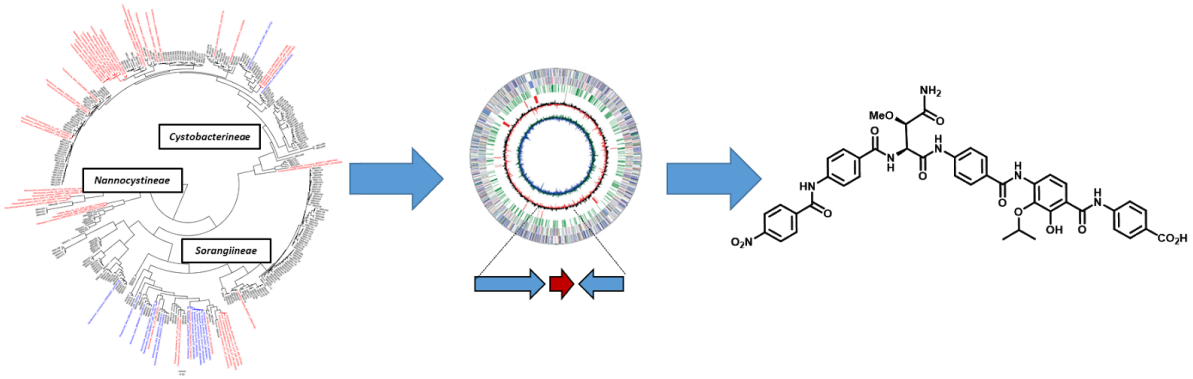
Microorganisms produce a wealth of specialized metabolites, which are of great importance from both ecological and clinical perspectives. Due to the accelerated accumulation of omics data, computational methods have become more and more important to identify these molecules and to assess their biological activities. Here, I will highlight the work performed in my research group on charting diversity of megasynthase-encoding gene clusters and predicting megasynthase substrate specificities and tailoring reactions. Also, I will describe our efforts to engineer a computer-aided design framework for the redesign and de novo construction of megasynthases that can produce custom metabolites as desired by the user.

MYXOBACTERIAL MEGASYNTHASES: A UNDEREXPLOITED SOURCE OF NOVEL ANTIBACTERIAL NATURAL PRODUCTS

Jennifer Herrmann, Ronald Garcia, Daniel Krug, Nestor Zaburannyi, Sebastian Groß, and Rolf Müller
Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS)

Rolf Müller
Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS), Saarbrücken, Deutschland
rolf.mueller@helmholtz-hips.de

The global rise of antimicrobial resistance, mainly due to the mis- and overuse of antibiotics, is one of the most pressing issues of our and future generations. To counteract this development, novel resistance-breaking antibiotics are urgently needed.[1] In this presentation, I will focus on bioactive natural products from soil-dwelling myxobacteria. Amongst the well-established bacterial producers, myxobacteria have a great track record for the discovery of entirely new natural product scaffolds exhibiting promising bioactivities. Comparisons of myxobacterial metabolite profiles with the number of underlying biosynthetic gene clusters encoded in their very large genomes suggest that the biosynthetic potential of myxobacteria is a long way from being exhausted. Many myxobacterial natural products originate from large multifunctional enzyme complexes (megasynthases), such as polyketide synthases (PKS) or non-ribosomal peptide synthases (NRPS).
Our isolation efforts of myxobacteria have shown that the majority of their biodiversity was previously uncultured. In the presentation one example of a new myxobacterial family brought into culture and its biosynthetic potential will be shown. As an example for an important myxobacterial megasynthase, I will present the family of cystobactamids, which is currently under pre-clinical development at the HIPS.[2,3] Cystobactamids are a NRPS-derived compound class with broad antibacterial activity, including gram-negative and other multidrug-resistant pathogens. I will show how we discovered the cystobactamids and elucidated their biosynthesis. Further, I will give insight on our efforts to improve their molecular properties for medical use via heterologous expression and engineering of the underlying megasynthase.



Discovery of Cystobactamids from previously uncultured myxobacteria.

References

(1) Interagency Coordination Group on AMR. Report to the Secretary General of the UN. 2019.
(2) Baumann et al. 2014, Angew. Chem. Int. Ed. Engl. 53 (52), pp. 14605–14609
(3) Hüttel et al. 2017, Angew. Chem. Int. Ed. Engl. 56 (41), pp. 12760–12764

DE NOVO CONSTRUCTION OF MULTIFUNCTIONAL ENZYME ASSEMBLIES

Mislav Oreb
Institute of Molecular Biosciences, Goethe University, Frankfurt, Germany
m.oreb@bio.uni-frankfurt.de

Multifunctional enzymes have evolved to facilitate processing of intermediates of complex reaction cascades in an assembly line-like manner, thereby preventing their diffusion into the bulk cellular fluid. There are obvious advantages of “substrate channeling” between active sites, including the protection of intermediates from competing pathways and shielding cellular environment from their potential toxic or inhibitory effects. Such underlying principles offer attractive possibilities for synthetic biology approaches. Therefore, strategies for the construction of artificial multienzyme assemblies are recently being developed. As one example, I will show how the metabolic flux of pyruvate, a central metabolic intermediate, can be modulated through artificial enzyme complexes. Moreover, I will present evidence that recruiting a xylose isomerase to a xylose transporter via artificial scaffold proteins can direct the utilization of xylose in yeast cells.

TYPE II PKS SYSTEMS: BIOSYNTHESIS OF ANATHRAQUINONES AND ARYL POLYENES

Maximilian Schmalhofer[1], Alois Bräuer[1], Gina Grammbitter[2], Helge Bode[2] and Michael Groll[1]
[1]Lehrstuhl für Biochemie, Technische Universität München
[2]Molekulare Biotechnologie, Goethe-Universität Frankfurt

Maximilian Schmalhofer
Lehrstuhl für Biochemie, Technische Universität München
m.schmalhofer@tum.de

Type II polyketide synthases (PKSs) are multi-enzyme complexes that produce secondary metabolites of medical relevance. Predominantly, the chemical backbones of such polyketides are produced by minimal PKS systems that consist of a malonyl transacylase, an acyl carrier protein and an α/β heterodimeric ketosynthase. A second class of bacterial polyketides are the aryl polyenes (APE). These pigments are synthesized by an unusual type II PKS, that lacks the minimal PKS system.
Here, we present X-ray structures of all ternary complexes that constitute the minimal PKS system for anthraquinone biosynthesis in *Photorhabdus luminescens*[1]. In addition, we provide the enzyme structures involved in the APE Biosynthesis of *Xenorhabdus doucetiae* including two heterooctameric and two heterodimeric complexes[2].

References

(1) Bräuer, A. et al. Structural snapshots of the minimal PKS system responsible for octaketide biosynthesis. Nat. Chem. (2020).
(2) Grammbitter, G. L. C. et al. An uncommon type II PKS catalyzes biosynthesis of aryl polyene pigments. *J. Am. Chem. Soc.* (2019).

COMPLEX POLYKETIDE BIOSYNTHESIS IN SACOGLOSSAN MOLLUSKS AND OTHER ANIMALS

Eric W. Schmidt
Department of Medicinal Chemistry, The University of Utah, Salt Lake City, USA
ews1@utah.edu

Abstract: Many polyketides have been isolated from animals. Relatively simple polyketides were attributed to animal metabolism, while more complex polyketides were unattributed or were linked to biosynthesis in symbiotic bacteria. Recent evidence reveals that many complex polyketides are synthesized by animals, where the enzymes involved are anciently animal in origin rather than resulting from horizontal gene transfer. These enzymes and their products will be described.

DECONSTRUCTING PYRICHALASIN H BIOSYNTHESIS TO RECONSTRUCT NON-NATURAL ANALOGUES

Elizabeth Skellam
Institute for Organic Chemistry, Leibniz University, Hannover, Germany
elizabeth.skallam@oci.uni-hannover.de

"Cytochalasans are PKS-NRPS derived natural products found exclusively in fungi with a diverse range of structures and corresponding biological activities. These molecules are most well known for interacting with the actin cytoskeleton in eukaryotic cells and are used to study cellular function. We have fully elucidated the biosynthetic pathway towards pyrichalasin H, a phytotoxin with a rare methoxyphenyl group, in the blast fungus Magnaporthe grisea. Using a combination of gene knock outs, precursor directed biosynthesis, and combinatorial biosynthesis we were able to generate a library of novel cytochalasans with potential application as alternative cell imaging tools."

NONRIBOSOMAL PEPTIDES IN MICROBIAL PREDATOR-PREY INTERACTIONS

Martin Klapper, Kevin Schlabach, Sebastian Götze, Pierre Stallforth

Pierre Stallforth
Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany
pierre.stallforth@leibniz-hki.de

The search for new bioactive natural products has prompted scientists to exploit environmental niches in which the production of these compounds is ecologically motivated. Microbial predator–prey interactions are particularly rich sources of natural products. We describe one such interaction in which bacterivorous amoebae and their prokaryotic prey meet. Amoebae are voracious and ubiquitous predators to bacteria that cause constant depletion of huge bacterial reservoirs. This puts both organisms under strong evolutionary selection pressure: the bacteria have evolved mechanisms to prevent grazing and the amoebae must counteract or surmount these mechanisms in order to survive. Here, we describe a variety of nonribosomal peptides that show amoebicidal activity [1-3] (**Fig. 1**), along with investigations into their biosynthesis, evolution,[4] and regulation.[5]

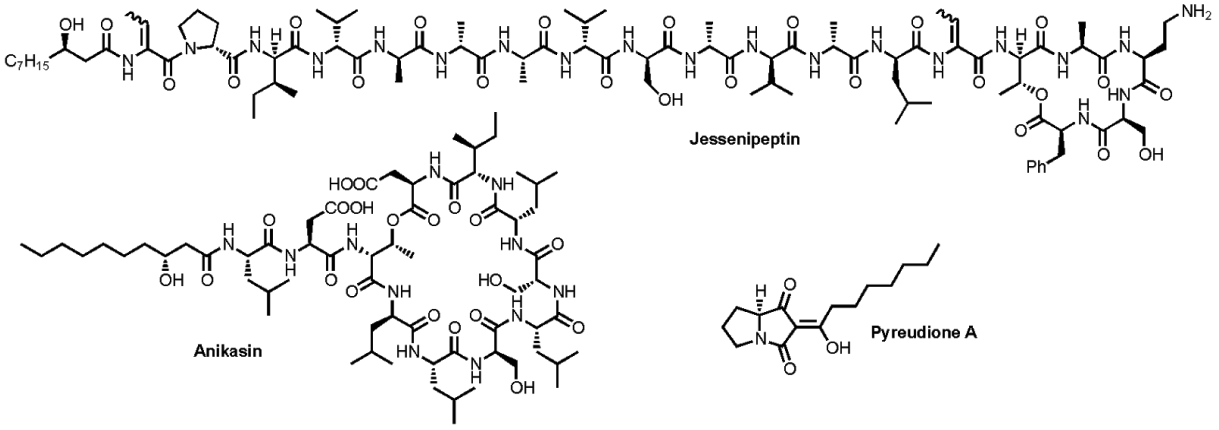


Figure 1. Structures of anikasin,[1] pyreudione A,[2] and jessenipeptin.[3]

References

(1) Götze, S.; Herbst-Irmer, R.; Klapper, M.; Görls, H.; Schneider, K. R. A.; Barnett, R.; Burks, T.; Neu, U.; Stallforth, P. *ACS Chem. Biol.* **2017**, 12, 2498.
(2) Klapper, M.; Götze, S.; Barnett, R.; Willing, K.; Stallforth, P. *Angew. Chem. Int. Ed.* **2016**, 55, 8944.
(3) Arp, J.; Götze, S.; Mukherji, R.; Mattern, D. J.; García-Altares, M.; Klapper, M.; Brock, D. A.; Brakhage, A. A.; Strassmann, J. E.; Queller, D. C.; Bardl, B.; Willing, K.; Peschel, G.; Stallforth, P. *Proc. Natl. Acad. Sci. USA.* **2018**, *115*, 3758.
(4) Götze S.; Arp, J.; Zhang, S.; Kries, H.; Klapper, M.; García-Altares, M.; Willing, K.; Günther, M.; Stallforth, P. *Chem. Sci.* **2019** *10,* 10979.
(5) Mukherji, R.; Zhang, S.; Chowdhury, S.; Stallforth, P. "*Angew. Chem. Int. Ed.* **2020**, *59*, 6192.

STRUCTURE/FUNCTION RELATIONSHIP IN MODULAR PKSs AND THEIR APPLICATION TO GENETIC ENGINEERING

Kira J. Weissman
Université de Lorraine, Molecular and Structural Enzymology Group, Lorraine, France
kira.weissman@univ-lorraine.fr

Despite decades of effort, attempts to manipulate modular PKSs using synthetic biology remain hampered by limited structure-function relationship information for these megaenzymes. This talk will showcase our recent work on several aspects of modular PKS architecture and function, and how the resulting information can be leveraged to generate defined polyketide derivatives.

References

(1) Davison J, Dorival J, Rabeharindranto H, Mazon H, Chagot B*, Gruez A* & Weissman KJ* (2014) Insights into the function of *trans*-AT polyketide synthases from the SAXS structure of a complete module. *Chem. Sci.* **5**, 3081–3095.
(2) Dorival J, Annaval T, Risser F, Collin S, Roblin P, Jacob C, Gruez A*, Chagot B* & Weissman KJ* (2016) Characterization of intersubunit communication in the virginiamycin *trans*-acyl transferase polyketide synthase. *J. Am. Chem. Soc.* **138**, 4155–4167.
(3) Risser F, Collin S, Dos Santos-Morais R, Gruez A*, Chagot B* & Weissman KJ* (2020) Towards improved understanding of intersubunit interactions in modular polyketide biosynthesis: docking in the enacyloxin IIa polyketide synthase. *J. Struct. Biol.* In press. doi: 10.1016/j.jsb.2020.107581.
(4) Massicard J-M, Soligot C, Weissman KJ* & Jacob C* (2020) Manipulating polyketide stereochemistry by exchange of polyketide synthase modules. *Chem. Commun.* Provisionally accepted.

NEW TOOLS FOR DISCOVERY OF NOVEL NATURAL PRODUCTS SYNTHESIZED BY MEGASYNTHASES

Huimin Zhao
University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
zhao5@illinois.edu

Natural products are a rich source of new antimicrobial, antifungal, and anticancer compounds. However, the rate of discovering novel natural products has decreased dramatically over the past decades. In this talk, I will introduce a wide variety of new tools and strategies that my laboratory has developed for discovery of novel natural products with a particular emphasis on those synthesized by megasynthases such as polyketide synthases and non-ribosomal peptide synthases. Particularly, I will highlight the discovery of iterative type I polyketide synthases hidden in Streptomyces species and the development of a rapid and highly efficient direct cloning method.

EVOLUTION AND RESURRECTION OF ANCIENT MEGASYNTHASES

Martina Adamek[1,2], Frauke Adam[1,2], Athina Gavridilliou[1,2], Dumitrita Iftime[1], Evi Stegmann[1], Max Cryle[3,4], Nadine Ziemert[1,2]

[1]Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Microbiology/Biotechnology, University of Tübingen, Tübingen, Germany.

[2]German Centre for Infection Research (DZIF), Partner Site Tübingen, Tübingen, Germany.

[3]Department of Biochemistry and Molecular Biology, The Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, 3800, Australia

[4]EMBL Australia, Monash University, Clayton, VIC, 3800, Australia

Nadine Ziemert

Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Microbiology/Biotechnology, University of Tübingen, Tübingen, Germany.

German Centre for Infection Research (DZIF), Partner Site Tübingen, Tübingen, Germany

nadine.ziemert@uni-tuebingen.de

Glycopeptides, such as the last-resort antibiotics vancomycin and teicoplanin, comprise a natural product family with remarkable structural diversity. They typically consist of a glycosylated heptapeptide backbone, which is crosslinked at the aromatic side chains. Each glycopeptide can differ in its glycosylation, methylation, sulfonation, and chlorination pattern, as well as the exact amino acid composition. In general, glycopeptides inhibit cell wall biosynthesis by binding lipid II, however, exact mechanisms and activities differ.

In order to understand the evolutionary mechanisms and structure-function relationships of the different glycopeptide family members, we analyzed the complex evolution of this compound family in bacteria. We used these analyses to reveal the ancestral sequence of the glycopeptide biosynthesis gene cluster enabling us to resurrect the ancient proteins in the laboratory for further investigations.

In this talk we will highlight the diverse evolutionary mechanisms that shape the diversity of glycopeptides and present lessons we can learn for synthetic biology efforts.

Abstracts of posters next page

STRUCTURAL AND FUNCTIONAL ANALYSIS OF TERMIATION DOMAINS IN NONRIBOSOMAL PEPTIDE SYNTHETASES

Maximilian Biermeier, Lars-Oliver Essen
Philipps Universität Marburg, Faculty of Chemistry

Maximilian Biermeier
Philipps University of Marburg, Faculty of Chemistry, Hans-Meerwein-Strasse 4, 35032 Marburg, Germany
biermeie@staff.uni-marburg.de

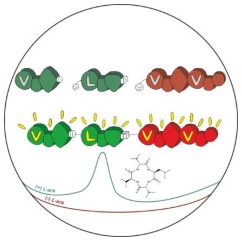
NRPS in general are known to produce a broad range of natural secondary products, finding pharmaceutical applications as antibiotics, immunosuppressants, or cytostatics. Even though being so much in the focus of research, much of the structural dependency of functions within the enzymatic machinery remain unknown.
Previous studies of Bode *et al.* on *Xenorhabdus nematophila* ATCC 19061 have indicated the presence of a tandem thioesterase (Te-didomain) within a xenoamicin biosynthetic gene cluster. We focus on the structural and functional investigation of unusual termination domains from NRPS assembly lines, where bioinformatics have unveiled a larger spread among further microorganisms than anticipated before.
Here we present first structural information and supplementary *in vitro* characterization of native tandem thioesterases (Te). X-ray structural analyses of these TE domains, with the C-terminal Te domain at 1.9 Å and the didomain at 3.4 Å resolution, respectively, reveal the typical compact α,β-hydrolase fold. Our Te-didomain structure shows a domain-domain interface, where a short and dynamic linker region mostly mediates the domain interactions. This linker has a PxxDPR motif that is well conserved among members of the *Xenorhabdus* subfamily of Te-didomains. Based on ESI-MS analysis functionality of Te-didomains towards common serine protease inhibitor was investigated, which showed independent reactivity of active-site serines within the two catalytic sites. The combined information of structure and function highlights the similarity of tandem systems towards classical termination modules and rise further questions regarding their structure dependent activity.

DIVIDE AND CONQUER: A MOLECULAR TOOL KIT TO REPROGRAM THE BIOSYNTHESIS OF NON RIBOSMAL PEPTIDES

Kenan A. J. Bozhüyük[1], Jonas Watzel[1], Nadya Abbood[1], Leonard Präve[1], Helge B. Bode[1,2,3].
1 Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 560438, Frankfurt am Main, Germany.
2 Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, 760438, Frankfurt am Main, Germany.
3 Max-Planck-Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, 35043, Marburg, Germany.

Kenan A. J. Bozhüyük
Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
kenan.bozhueyuek@bio.uni-frankfurt.de

Numerous important therapeutic agents, including widely-used antibiotics, anti-cancer drugs, immunosuppressants, agrochemicals and other valuable compounds, are produced by microorganisms (1, 2). Many of these are biosynthesised by modular enzymatic assembly line polyketide synthases, non-ribosomal peptide synthetases, and hybrids thereof (3). To alter the backbone structure of these valuable but difficult to modify compounds, the respective enzymatic machineries can be engineered to create even more valuable molecules with improved properties and/or to bypass resistance mechanisms (4). In the past, many attempts to achieve assembly line pathway engineering failed or led to enzymes with compromised activity. Thus, engineering of these often giant biosynthetic machineries to produce novel non-ribosomal peptides (NRPs) at high titre is an ongoing challenge. Here we describe a strategy to functionally combine NRPS fragments of Gram-negative and -positive origin, synthesising novel peptides at titres up to 290 mg l^{-1}. Extending from the recently introduced definition of eXchange Units (5), we inserted synthetic zippers (SZs) to split single protein NRPSs into up to three independently expressed and translated polypeptide chains. These synthetic type of NRPS (type S) enables easier access to engineering, overcomes cloning limitations, and provides a simple and rapid approach to building peptide libraries via the combination of different NRPS subunits.



Divide and Conquer: Re-engineering of non-ribosomal peptide synthetases (NRPSs) is challenging due to their protein size up to a few megadalton. The artificial splitting of NRPSs to produce building blocks with moderate size is achieved by the introduction of synthetic zippers, which can be recombined in a plug and play manner to form an in trans regulated synthetic type of NRPSs to produce even peptide libraries.

References

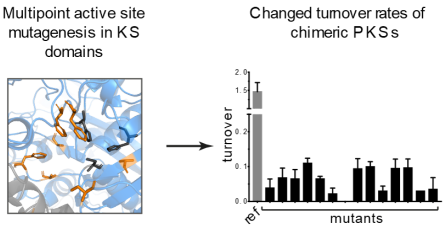
(1) van Belkum, *et al.*, *Nat Rev Microbiol* **2019**, *17*, 51-62
(2) M.G. Moloney, *Trends Pharmacol Sci* **2016**, 32, 689-701
(3) R. D. Süssmuth, A. Mainz, *Angew. Chem., Int. Ed.* **2017**, *56*, 3770–3821.
(4) K. A. J. Bozhüyük, J. Micklefield, B. Wilkinson, *Curr. Opin. Microbiol.* **2019**, *51*, 88–96.
(5) K. A. J. Bozhüyük, F. Fleischhacker, A. Linck, F. Wesche, A. Tietze, C.-P. Niesert, H. B. Bode, *Nat. Chem.* **2018**, *10*, 275–281.

THE KETOSYNTHASE DOMAIN CONSTRAINS THE DESIGN OF POLYKETIDE SYNTHASES

Maja Klaus[a], Lynn Buyachuihan[a], Martin Grininger[a]
[a] *Institute of Organic Chemistry and Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Germany*

Lynn Buyachuihan
Institute of Organic Chemistry and Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Germany
buyachuihan@chemie.uni-frankfurt.de

Modular polyketide synthases (PKSs) produce complex, bioactive secondary metabolites in assembly line-like multistep reactions.[1] Longstanding efforts to produce novel, biologically active compounds by recombining intact modules to new modular PKSs have mostly resulted in poorly active chimeras and decreased product yields.[2–4] Recent findings demonstrate that the low efficiencies of modular chimeric PKSs also result from rate limitations in the transfer of the growing polyketide chain across the non-cognate module:module interface and further processing of the non-native polyketide substrate by the ketosynthase (KS) domain.[5,6] We aimed at disclosing and understanding the low efficiency of chimeric modular PKSs and at establishing guidelines for modular PKSs engineering. Therefore, we used a bimodular PKS testbed and systematically varied substrate specificity, substrate identity, and domain:domain interfaces of the KS involved reactions. We could observe that KS domains employed in our chimeric bimodular PKSs are bottlenecks with regards to both substrate specificity as well as interaction with the acyl carrier protein (ACP). Overall, our systematic study can explain in quantitative terms why early oversimplified engineering strategies based on the plain shuffling of modules mostly failed and why more recent approaches show improved success rates. We moreover identified two mutations of the KS domain that significantly increased turnover rates in chimeric systems and interpreted this finding in mechanistic detail.[7]



Multipoint mutagenesis within the KS binding site of the acceptor module alters turnover rates of chimeric assembly lines.[7]

References

(1) Staunton, J. & Weissman, K. J. Polyketide biosynthesis: A millennium review. *Nat. Prod. Rep.* **18**, 380–416 (2001).
(2) Menzella, H. G. *et al.* Combinatorial polyketide biosynthesis by de novo design and rearrangement of modular polyketide synthase genes. *Nat. Biotechnol.* **23**, 1171–1176 (2005).
(3) Menzella, H. G., Carney, J. R. & Santi, D. V. Rational Design and Assembly of Synthetic Trimodular Polyketide Synthases. *Chem. Biol.* **14**, 143–151 (2007).
(4) Klaus, M. *et al.* Protein-Protein Interactions, Not Substrate Recognition, Dominate the Turnover of Chimeric Assembly Line Polyketide Synthases. *J. Biol. Chem.* **291**, 16404–16415 (2016).
(5) Khosla, C., Tang, Y., Chen, A. Y., Schnarr, N. A. & Cane, D. E. Structure and Mechanism of the 6-Deoxyerythronolide B Synthase. *Annu. Rev. Biochem.* **76**, 195–221 (2007).
(6) Wu, J., Kinoshita, K., Khosla, C. & Cane, D. E. Biochemical analysis of the substrate specificity of the β-ketoacyl-acyl carrier protein synthase domain of module 2 of the erythromycin polyketide synthase. *Biochemistry* **43**, 16301–16310 (2004).

Klaus, M., Buyachuihan, L., Grininger, M. The Ketosynthase Domain Constrains the Design of Polyketide Synthases. *ACS Chem. Biol.* 2020 Aug 12 doi:10.1021/acschembio.0c00405. Online ahead of print. PMID: 32786257.

BIOSYNTHESIS OF OXIGENATED BRASILANE TERPENE GLYCOSIDES INVOLVES A PROMISCUOUS N-ACETYLGLUCOSAMINE TRANSFERASE

Jin Feng,[a] Frank Surup, [b] Maurice Hauser,[a] Anna Miller,[a] Jan-Peer Wennrich,[b] Marc Stadler,[b] Russell J. Cox,[a] and Eric Kuhnert [a]

a. Institute for Organic Chemistry and Centre for Biomolecular Drug Research (BMWZ), Leibniz University Hannover, Schneiderberg 38, Hannover 30167, Germany.

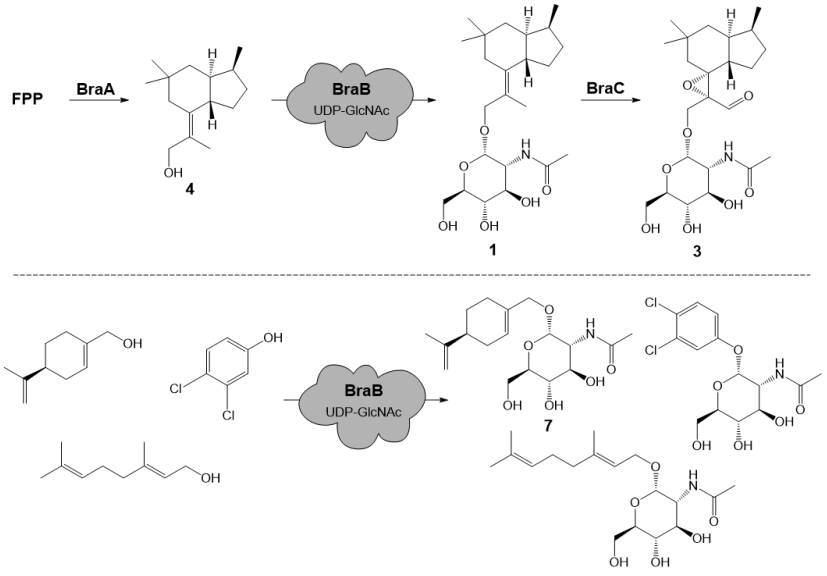
b. Department Microbial Drugs, Helmholtz Centre for Infection Research (HZI), Inhoffenstraße 7, 38124 Braunschweig, Germany.

Jing Feng

Institute for Organic Chemistry and Centre for Biomolecular Drug Research (BMWZ), Leibniz University Hannover, Schneiderberg 38, Hannover 30167, Germany

jin.feng@oci.uni-hannover.de

Investigation of the metabolome of the ascomycete *Annulohypoxylon truncatum* led to the identification of novel oxygenated brasilane glycosides.[1] The respective biosynthetic gene cluster (*bra*) was identified by genome mining of the genome sequenced producer organism. *bra* contains five genes (*braA* - *braE*) with three of them encoding for key and modifying enzymes (BraA - terpene cyclase, BraC - cytochrome P450 monooxygenase, BraB - glycosyltransferase). The function of the cluster and the order of the biosynthetic steps was verified by heterologous expression experiments in *Aspergillus oryzae* NSAR1. *In-vitro* studies of BraB revealed it to be a very rare fungal UDP-GlcNAc dependent *N*-acetylglucosamine transferase. BraB also accepted UDP-glucose as donor and was shown to convert various primary and secondary alcohols into their respective glycoside.



Brasilane biosynthetic pathway and substrate promiscuity of BraB in the presence of UDP-GlcNAc based on experimental evidence.

References

(1) D.-B. Hu, S. Zhang, J.-B. He, Z.-J. Dong, Z.-H. Li, T. Feng and J.-K. Liu, *Fitoterapia*, 2015, 104, 50–54.

ELUCIDATION OF EARLY STEPS IN PELORUSIDE BIOSYNTHESIS

A.E. Fraley, C. L. Dieterich, M. Rust, R. A. Meoded, F. Hemmerling, J. Piel
Eidgenössische Technische Hochschule Zürich, Switzerland

Amy E. Fraley
Eidgenössische Technische Hochschule Zürich, Switzerland
afraley@biol.ethz.ch

The sponge *Mycale hentscheli* is famous for its three distinct anticancer polyketides, among which the tubulin-inhibiting pelorusides exhibit particularly high promise for anticancer drug development, provided that a means of production can be identified. Microbiome sequencing revealed that *trans*-acyl transferase polyketide synthases (*trans*-AT PKSs) are involved in the biosynthesis of these molecules. These multimodular megaenzymes generate the majority of biosynthetically assigned polyketides from sponge symbionts, but remain poorly understood from a mechanistic standpoint as they contain a staggering array of novel biosynthetic components. This work started with the assignment of the *pel* locus to peloruside production based on the knowledge that ketosynthase (KS) sequences can be used to predict partial intermediate structures. However, due to the highly aberrant architecture of modules 2 and 3, the original prediction substantially differed for the exocyclic moiety of the polyketide. These unusual modules feature a series of non-elongating KSs (KS^0), a condensation (C) domain that normally occurs in nonribosomal peptide synthetases (NRPSs) to generate amide bonds, and two internal thioesterase (TE) domains, which are usually positioned at the termini of PKSs and NRPSs to catalyze thioester hydrolysis or macrocyclization. We have biochemically characterized the internal thioesterase from the peloruside biosynthetic pathway and discovered its unusual ability to perform an *O*-acetylation reaction. For comparison, we have characterized a homologous enzyme from the oocydin biosynthetic pathway, providing unique insight for how this novel domain can act in early- or late-stage polyketide biosynthesis.

UNRAVELING A UNIVERSAL DARK MATTER IN PROKARYOTES: THE CHEMICAL STRUCTURES OF ARYLPOLYENE LIPIDS

Gina L. C. Grammbitter[1], Yi-Ming Shi[1], Yan-Ni Shi[1], Sahithya P. B. Vemulapalli [2], Christian Griesinger[2], Christian Richter[3], Anja Schüffler[4] Wolfgang Schuck[4], Matthias Witt[5], Helge B. Bode[1/6].

[1] Institute of Molecular Biological Science, Johann Wolfgang Goethe University, 60438 Frankfurt, Germany.

[2] Max Planck Institute for Biophysical Chemistry, NMR-based Structural Biology, 37077 Göttingen, Germany.

[3] Institute for Organic Chemistry and Chemical Biology, Center for Biomolecular Magnetic Resonance, Johann Wolfgang Goethe University, 60439 Frankfurt am Main, Germany.

[4] University of Kaiserslautern, Paul-Ehrlich-Str. 23, 67663 Kaiserslautern, Germany. [5] Bruker Daltonik GmbH, Fahrenheitstrasse 4, 28359 Bremen, Germany.
[6]Max-Planck-Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, 35043 Marburg, Germany.

Gina Grammbitter
Institute of Molecular Biological Science, Johann Wolfgang Goethe University, 60438 Frankfurt, Germany
grammbitter@bio.uni-frankfurt.de

The aryl polyene (APE) biosynthetic gene cluster (BGC) is one of the most widespread specialized metabolite-associated BGC found in Gram-negative bacteria.[1] In general, APE pigments are a special class of bacterial polyketides that provide anti-oxidative abilities to the producing organism.[2] Only recently, we were able to unravel the unusual biosynthesis of the linear APE compounds, produced by a type II PKS system.[3] The biosynthesis of the APE compounds takes place in a fatty acid-like biosynthetic order with the formation of multiple enzyme complexes. However, the so far described biosynthesis stops with an ACP-bound APE, while the following cleavage mechanism remained obscure. Here, we describe the structure elucidation of the full-length APE-containing lipids that additionally harbor a unique polyunsaturated fatty acid. We were able to isolate several APE lipids from the γ-proteobacterium *Xenorhabdus doucetiae* and thoroughly structure elucidated them by HPLC/MS and NMR techniques. From the structure and detailed mutant analyses, we are also able to propose the biosynthesis of these widespread natural products.

References

(1) P. Cimermancic, M. H. Medema, J. Claesen, K. Kurita, L. C. Wieland Brown, K. Mavrommatis, A. Pati, P. A. Godfrey, M. Koehrsen, J. Clardy et al., *Cell* **2014**, *158*, 412.
(2) T. A. Schöner, S. Gassel, A. Osawa, N. J. Tobias, Y. Okuno, Y. Sakakibara, K. Shindo, G. Sandmann, H. B. Bode, *ChemBiochem* **2016**, *17*, 247.
(3) Grammbitter, Gina L. C., M. Schmalhofer, K. Karimi, Y.-M. Shi, T. A. Schöner, N. J. Tobias, N. Morgner, M. Groll, H. B. Bode, *J. Am. Chem. Soc.* **2019**, *141*, 16615.

BIOSYNTHETIC GENE CLUSTERS OF ACETOBACTERACEAE, PHYLOGENETICALLY-DERIVED OR ACQUIRED THROUGH HORIZONTAL GENE TRANSFER?

Juan Guzman,[1] Andreas Vilcinskas[1,2]*
*[1] Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany. *juan.guzman@ime.fraunhofer.de*
[2] Institute for Insect Biotechnology, Justus-Liebig University of Giessen, Giessen, Germany.

Juan Guzmann
Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany
juan.guzman@ime.fraunhofer.de

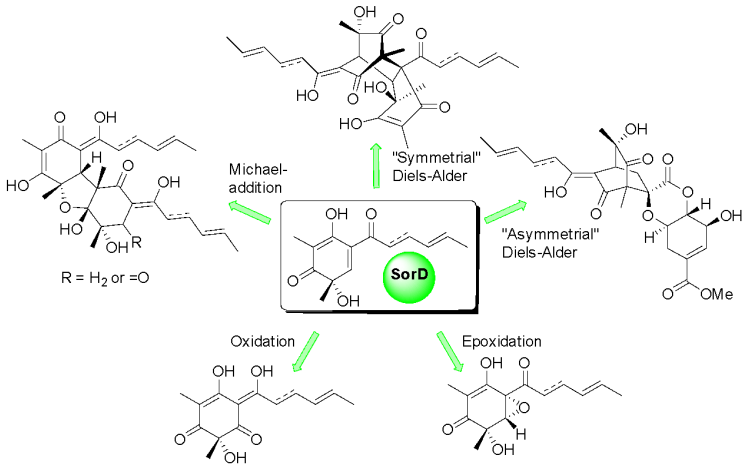
The family *Acetobacteraceae* currently comprises 44 validly published genera, which are phylogenetically grouped into two distinct clades. The acetic acid bacteria group are more homogeneous in their phenotypes and share the ability of oxidize periplasmically a number of substrates, while the second group is more heterogeneous and includes a number of acidophilic, neutrophilic and phototrophic bacteria. The biosynthesis of specialized metabolites has not been explored in the family *Acetobacteraceae*, and preliminary studies indicate that some members encode biosynthetic proteins catalysing the assembly of so far unknown natural products. This study involves the analysis of biosynthetic gene clusters predicted by Antismash v5.0 of more than 150 genomes of the family *Acetobacteraceae*. Phylogenetic signals were also extracted from concatenated house-keeping protein sequences using the same genome dataset. The relation between the presence of certain biosynthetic clusters and the phylogenetic relations was studied in order to have an idea whether the biosynthetic clusters were assembled in the evolution of the family *Acetobacteraceae* or were imported by horizontal gene transfer from other bacterial taxa.

UNVEILING THE BIOSYNTHESIS OF DIMERIC SORBICILLINOIDS: DISCOVERY OF AN UNPRECEDENTED FLAVIN-DEPENDENT MONOOXYGENASE

Lukas Kahlert, Russell J. Cox, Elizabeth J. Skellam
Leibniz Universität Hannover, Centre of Biomolecular Drug Research (BMWZ), Institute of Organic Chemistry, Schneiderberg 38, 30167 Hannover

Lukas Kahlert
Leibniz Universität Hannover, Centre of Biomolecular Drug Research (BMWZ), Institute of Organic Chemistry, Schneiderberg 38, 30167 Hannover
lukas.kahlert@oci.uni-hannover.de

Sorbicillinoids are a structurally diverse class of fungal polyketides, many of which exhibit promising biological activities.[1]The eponymous molecule sorbicillin **1**, which is the product of two consecutively acting PKS, was first isolated in 1948[2] and up to date over 100 sorbicillinoids have been described. The highly reactive intermediate sorbicillinol **2** has been shown to dimerize with itself or other suitable compounds to form various dimeric sorbicillinoids.[3,4] While this dimerization reaction can be induced by organic solvents or during workup steps *in vitro*[5,6] we could demonstrate for the first time that dimerization *in vivo* is catalyzed by a highly versatile flavin-dependent monooxygenase SorD that is peerless in its function.[4] Although sharing little sequence identity, both *Trichoderma reesei* QM6a and *Penicillium chrysogenum* harbour such unprecedented enzyme that does not only fascilitate Diels-Alder and Michael-like dimerization, but also an independent (ep)oxidation of **2**.[4,7] Initial mutation studies showed that covalent binding of the flavin cofactor is essential for enzyme activity.[7]



Versatile reactions catalysed by the flavin-dependent monooxygenase SorD

References

(1) J. Meng, X. Wang, D. Xu, X. Fu, X. Zhang, D. Lai, L. Zhou, G. Zhang, *Molecules* **2016**, *21*, 715
(2) D. J. Cram, M. Tishler, *J. Am. Chem. Soc.* **1948**, *70*, 4238–4243
(3) N. Abe, T. Arakawa, K. Yamamoto, A. Hirota, *Biosci. Biotechnol. Biochem.* **2002**, *66*, 2090–2099
(4) L. Kahlert, E. F. Bassiony, R. J. Cox, E. J. Skellam, *Angew. Chemie - Int. Ed.* **2020**, *59*, 5816–5822.
(5) A. Sib, T. A. M. Gulder, *Angew. Chemie - Int. Ed.* **2017**, *56*, 12888–12891
(6) D. Barnes-Seeman, E. J. Corey, *Org. Lett.* **1999**, *1*, 1503–1504
(7) L. Kahlert, R. J. Cox, E. J. Skellam, *Chem. Commun.* **2020**, accepted

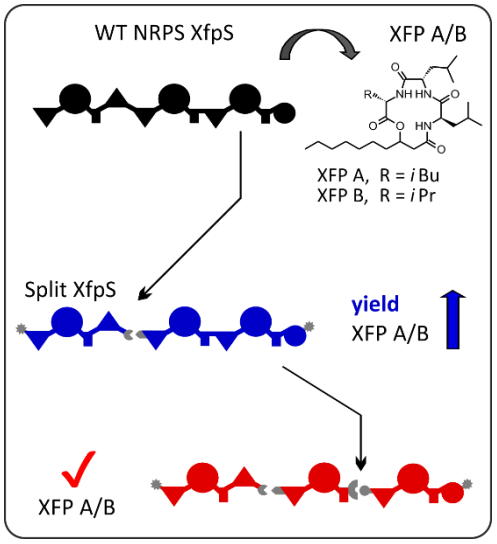
ARTIFICIAL SPLITTING OF A NON-RIBOSOMAL PEPTIDE SYNTHETASE BY INSERTING NATURAL DOCKING DOMAINS

Carsten Kegler[1] and Helge B. Bode[1, 2].

[1]Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
[2]Max-Planck-Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, 35043, Marburg, Germany

Carsten Kegler
Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
kegler@bio.uni-frankfurt.de

The interaction in multi-subunit non-ribosomal peptide synthetase (NRPS) is mediated by docking domains ensuring the correct subunit-to-subunit interaction leading to the desired peptide product. In this work natural docking domains were introduced into the one polypeptide-three module xefoampeptide synthetase (XfpS) creating two to three artificial NRPS XfpS subunits. The enzymatic performance of the split biosynthesis was measured by absolute quantification of the products by use of HPLC-ESI-MS. The connecting role of the docking domains was then probed by deleting integral parts of them. The peptide production data was compared to soluble protein amounts of the NRPS using SDS-PAGE. Reduced peptide synthesis was not a result of reduced soluble NRPS protein concentration but a consequence of the deletion of vital docking domain parts. Splitting the xefoampeptide biosynthesis polypeptide by introduction of docking domains was feasible and resulted in higher amounts of peptide product in one of the two tested module split cases compared to the full-length wild type enzyme.
In a separate NRPS engineering approach we exchanged condensation-starter domains in an NRPS cluster, thereby producing lipopeptides with a variety of fatty acids.



Schematic view of docking domain insertion and biosynthetic production success.

References

(1) C. Kegler and Helge B. Bode Angew. Chem. Int. Ed. 2020, 59, 13463-13467

MULTISPECIFIC AND PROMISCUOUS MONOMODULAR NONRIBOSOMAL PEPTIDE SYNTHETASES

Martin Klapper,[a] Kevin Schlabach,[a] Daniel Braga,[b] Gerald Lackner,[b] Manuel Einsiedler,[c] Katharina Lamm,[c] Tobias A.M. Gulder,[c] Mai Tran,[d] Ute A. Hellmich,[d] Pierre Stallforth[a]

Martin Klapper
Leibniz Institute for Natural Product Research and Infection Biology e.V. - Hans Knöll Insitute (HKI), Department of Paleobiotechnology, Beutenbergstraße 11a, 07745 Jena, Germany
martin.klapper@leibniz-hki.de

Pseudomonads are ubiquitous inhabitants of forest soil, where they face strong predatory selection pressure. As a consequence, some of these prokaryotes display potent toxicity against amoebal predators. Whole genome sequencing of such a toxic strain allowed the identification of a defense-related biosynthetic gene encoding a monomodular nonribosomal peptide synthetase (NRPS). The latter catalyzes the formation of the highly amoebicidal pyreudiones, which was verified by heterologous expression and *in vitro* analysis. Generation of a gene deletion mutant unable to produce these toxins showed that these amoebicides are sufficient and necessary to prevent amoebal predation.[1] The biosynthesis of the pyreudiones was studied in detail, in particular regarding the adenylation (A) and starter condensation ($C_{starter}$) domain, which display a striking plasticity. The broad substrate scope of the A domain was determined *via* an ATP/[^{32}P]pyrophosphate exchange assay and allowed to anticipate novel pyreudione derivatives, which could subsequently be isolated from the producing organism.[2] Heterologous expression of similar monomodular NRPS from other bacteria revealed their promiscuity regarding the $C_{starter}$ domain specificity. Current studies focus on domain swapping and mutagenesis experiments, as well as structural aspects, in order to investigate factors for $C_{starter}$ domain specificity.

References

(1) M. Klapper, S. Götze, R. Barnett, K. Willing, P. Stallforth, *Angew. Chem. Int. Ed.* **2016**, *55*, 8944–8947.
(2) M. Klapper, D. Braga, G. Lackner, R. Herbst, P. Stallforth, *Cell Chem. Biol.* **2018**, *25*, 659–665.

IMPROVING THE PRODUCTION OF NON-ROBSOMAL PEPTIDES BY OPTIMIZING THE INTERFACE BETWEEN THE CONDENSATION AND ADENYLATION DIDOMAIN – FROM A STABLE PLATFORM TO A MULTI-FUNCTIONAL WORKBENCH

Janik Kranz[1], Kenan A. J. Bozhüyük[1] & Helge B. Bode[1,2]
[1]Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
[2]Max-Planck-Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, Marburg, Germany.

Janik Kranz
Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
kranz@bio.uni-frankfurt.de

Besides the canonical ribosomal protein biosynthesis, there are many important natural products produced by non-ribosomal peptide synthetases (NRPSs) including the antibiotic vancomycin, the immunosuppressive cyclosporin A or the cytostatic echinomycin.[1] They are not only restricted to the 20 proteinogenic amino acids, like the analogical ribosomal peptides but can incorporate nonproteinogenic amino acids, fatty acids, β-amino acids, or α-hydroxy acids as building blocks.[2] Furthermore, they can form cyclic peptides or depsipeptides that additionally can be modified after peptide formation via glycosylation and other modifications, leading to a remarkable structural and functional diversity.[3]
Due to the modular nature of NRPSs[4], several laboratories have tried to reprogram these systems. However, except for the A-T-C swapping strategy, denoted as the exchange unit (XU)[5] concept, it has been difficult to develop clearly defined, reproducible and validated guidelines for the engineering of NRPSs. Further development of this concept resulted in the exchange unit condensation domain (XUC) concept, which enables the efficient production of peptides and overcomes the limitations of the XU concept.[6]
Here we will describe how to further affect the NRPS production rate and product spectra by editing the interface of the condensation-adenylation didomain. By comparing the unknown C-A structure of our bicornitin/GameXPeptide homology model with the three known stages of the catalytic cycle - the adenylate forming conformation (AB3403)[7], thioester forming conformation (EntF)[8], and open conformation (SrfA-C)[8] _ an interface forming region was defined. Therein, the modification in a specific position was identified to increase the production rate by up to 140 %.

References

(1) Felnagle, E. A. *et al.* Mol Pharm., Bd. 5(2), pp. 191-211, **2008**.
(2) Walsh, C. T. Acc Chem Res. Bd. 41(1), pp. 4-10, **2008**.
(3) Sieber, S. A. & Marahiel M.A. Chem Rev. 105(2), pp. 715-738, **2005**.
(4) Süssmuth, R. D. & Mainz, A. Angew Chem Int Ed Engl. 56(14), pp. 3770-3821, **2017**.
(5) Bozhüyük, K. A. J. *et al.* Nat Chem. 10(3), 275-281, **2018**.
(6) Bozhüyük, K. A. J. *et al.* Nat Chem., **2019**.
(7) Drake, E. *et al.* Nature, **2016**.
(8) Tanovic, A. *et al.* Science, **2008**.

A TINY MEGASYNTHASE IS INVOLVED IN THE GENERATION OF POLYCHLORINATED BUILDING BLOCKS FOR AMBIGOL BIOSYNTHESIS

I Dewa Made Kresna,[a] Luis Linares Otoya,[a] Elke R. Duell,[b] Ute Mettal,[a] Gabriele M. König,[c] Tobias A. M. Gulder[b,d] and Till F. Schäberle[a,e]

[a] Institute for Insect Biotechnology, Justus-Liebig-University of Giessen, 35392 Giessen, Germany

[b] Biosystems Chemistry, Department of Chemistry and Center for Integrated Protein Science Munich (CIPSM), Technical University of Munich, 85748 Garching, Germany.

[c] Institute for Pharmaceutical Biology, University of Bonn, 53115 Bonn, Germany

[d] Technical Biochemistry (WE), Technical University of Dresden, 01062 Dresden, Germany

[e] Department of Bioresources of the Fraunhofer Institute for Molecular Biology and Applied Ecology, 35394 Giessen, Germany

I Dewa Made Kresna

Institute for Insect Biotechnology, Justus-Liebig-University of Giessen, 35392 Giessen, Germany

idewa92@gmail.com

The cyanobacterium *Fischerella ambigua* 108b is the natural producer of polychlorinated aromatic compounds, the ambigols A-C[1][2]. The biosynthetic gene cluster (BGC) of these highly halogenated triphenyls has been recently identified by heterologous expression[3]. It consists of 10 genes named ab1-10. Two of the encoded enzymes, i.e. Ab2 and Ab3, were characterized *in vitro* and *in vivo* as cytochrome P450 enzymes. This revealed Ab2 to selectively form an *O*,ortho-coupled biaryl ether, whereas Ab3 produced an *ortho*,*ortho*-linked, *C*–*C*-coupled biaryl product[3]. Here, the biosynthetic steps towards the substrates of the P450 enzymes were investigated by *in vitro* assays. Ab7, an isoenzyme of a 3-deoxy-7-phosphoheptulonate (DAHP) synthase is involved in chorismate biosynthesis. The acyl-CoA synthetase Ab6 is necessary to activate hydroxybenzoic acid (4HBA), which is subsequently tethered to the non-ribosomal peptide synthetase system consisting of Ab8 and Ab9. The peptidyl carrier protein domain bound substrate is chlorinated (or brominated) by Ab10 in *meta* position, before 3-Cl-4HBA is released by the thioesterase domain of Ab9. The released product is then expected to be the dedicated substrate of the halogenase Ab1 to produce the monomeric ambigol building block 2,4-dichlorophenol.

References

(1) B. S. Falch, G. M. König, A. D. Wright, O. Sticher, *J. Org. Chem.*, 1993, **58**, 6570-6575.
(2) D. Wright, O. Papendorf, G. M. König, *J. Nat. Prod.*, 2005, **68**, 459-461.
(3) E. R. Duell, T. M. Milzarek, M. E. Omari, L. J. Linares-Otoya, T. F. Schäberle, G. M. König, T. A. M. Gulder, *submitted*

RECONSTRUCTING BACTERIAL BYRROLIZIDINE ALKALOID BIOSYNTHESIS

Katharina Lamm, Manuel Einsiedler, Tobias A.M. Gulder
Technical Biochemistry, TU Dresden, Bergstraße 66, 01069 Dresden, Saxony, Germany

Katharina Lamm
Technical Biochemistry, TU Dresden, Bergstraße 66, 01069 Dresden, Saxony, Germany
katharina.lamm@tu-dresden.de

Bacterial pyrrolizidine alkaloids (PAs) are an as yet underrepresented class of natural products with promising anticancer activity. To date, most of the few identified bacterial PA analogs are derived from Streptomycetes, including mitomycin C[1], a broadly applied antitumoral drug and the dibohemamines which exhibit potency versus lung and liver cancer[2]. Bioinformatic analyses of published bacterial genomes, however, suggest a broad variety of novel PAs awaiting discovery. Interestingly, only two enzymes are needed to build the pyrrolizidine core structure. The initial step in biosynthesis is catalyzed by a dimodular non-ribosomal peptide synthetase (NRPS) to form a heterocyclic intermediate which is then rearranged by a Baeyer-Villiger-type monooxygenase to form the bicyclic pyrrolizidine ring system[3]. Bacterial PA-producing enzymes are encoded within biosynthetic gene clusters (BGCs) of varying complexity, making them interesting targets for discovering novel bioactives. It is the aim to reconstruct this biosynthetic pathway both *in vivo* and *in vitro* via heterologous expression of selected bacterial BGCs in different host systems to gain insights into the mechanism of assembly and furthermore identify yet uncharacterized PA derivatives.

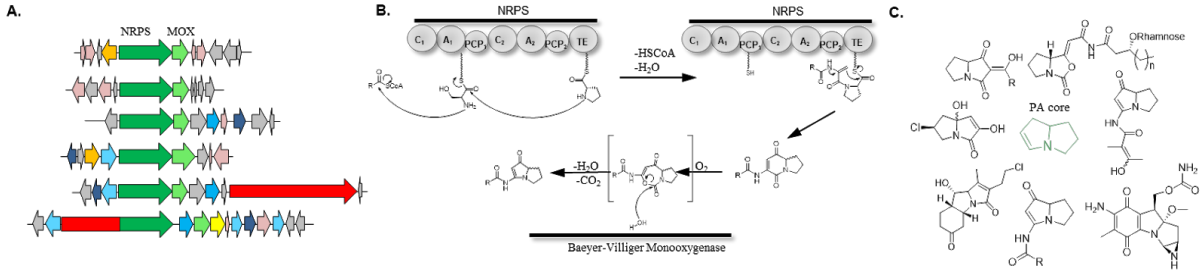


Figure 1. *Principle aspects of bacterial PA biosynthesis. A. Compilation of bacterial BGCs encoding PA biosynthetic genes. B. Mechanism of PA core structure assembly. C. Prominent examples of bacterial PAs.*

References

(1) M. Tomasz, *Chem. Biol.* **1995**, *2*, 575-579.
(2) B. Jiang, W. Zhao, S. Li, H. Liu, L. Yu, Y. Zhang, H. He, L. Wu, *J. Nat. Prod.* **2017**, *80*, 2825-2829.
(3) O. Schimming, V. L. Challinor, N. J. Tobias, H. Adihou, P. Grun, L. Poschel, C. Richter, H. Schwalbe, H. B. Bode, *Angew. Chem. Int. Ed.* **2015**, *54*, 12702-12705

CHARACTERISING ACP TO KS INTERACTIONS IN TYPE I FAS AND PKS SYSTEMS

Felix Lehmann, Christina Heil, Alexander Rittner and Martin Grininger
Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany

Felix Lehmann
Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany
flehmann@stud.uni-frankfurt.de

Fatty acid synthases (FAS) and polyketide synthases (PKS) condense and in part reduce acyl- and malonyl-derivatives to produce fatty acids or complex poleketides. In type I FAS all catalytic domains reside on the same polypeptide whereas in type I PKS the polypeptides can be split into different modules connected by non-covalent interactions. Both systems have in common that the growing substrate is shuttled between the enzymatic domains by the acyl carrier protein (ACP). In order to yield the corresponding product all reactions have to follow a defined choreography. This order seems to be not only regulated by substrate specificity but also domain-domain interactions.[1] Understanding these interactions seems crucial to possibly engineer the product spectrum of FAS and PKS systems. Specifically the recognition of substrate-ACP by the β-ketoacyl synthase (KS) domain is of particular interest, as this interaction determines if the substrate is iteratively elongated by the same module or passed on to the downstream module. We established the side specific introduction of the non-canonical amino acid 4-azido-phenylalanine (AzF) on the ACP-domain of murine FAS constructs.[2] With these functionalized ACP-domains and kinetic assays we are aiming to investigate ACP-KS interactions in type I FAS and PKS constructs.

References

(1) Klaus, M. *et al.* Protein-Protein Interactions, Not Substrate Recognition, Dominate the Turnover of Chimeric Assembly Line Polyketide Synthases. *J. Biol. Chem.* **291**, 16404–16415 (2016).
(2) Heil, C. S., Rittner, A., Goebel, B., Beyer, D. & Grininger, M. Site-Specific Labelling of Multidomain Proteins by Amber Codon Suppression. *Sci. Rep.* **8**, 14864 (2018).

CHARACTERIZATION OF NEU ENZYMOLOGY IN TRANS-ACYLTRANSFERASE POYKETIDE SYNTHASES

Hannah Minas, Franziska Hemmerling, Roy A. Meoded, Reiko Ueoka, Jörn Piel.
Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zürich, Switzerland.

Franziska Hemmerling & Hannah Minas
Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zürich, Switzerland.
minash@ethz.ch, fhemmerl@ethz.ch

Trans-AT polyketide synthases (*trans*-AT PKSs) are enzymatic assembly lines responsible for the biosynthesis of highly complex natural products, which display versatile functionalities in their ecological as well as a pharmacological setting. The modular architecture of these megasynthases relies on a functional repertoire in the form of domains. While many compounds are produced using prototypical module compositions such as sequentially reducing domains, others are the product of unusual additional enzymology. In our quest to expand the toolset for biosynthetic diversification of *trans*-AT PKS products, we combined different genome mining strategies: Analysis of biosynthetic gene cluster (BGC) frequency across all sequenced bacterial genomes revealed potential rich sources of natural products. This is exemplified by *Gynuella sunshinyii*, a talented producer in the previously neglected microbial group of Oceanospirillales, where several cytotoxic compounds were isolated from. Further, we used the unique attribute of ketosynthase domains from *trans*-AT PKSs to phylogenetically group according to the chemical structure of the incoming substrate. The study of unassigned clades was used as a handle to uncover potentially unprecedented enzymatic activities in upstream modules. This lead to the discovery of a FAAL-ligase starter unit and unusual Baeyer-Villiger monooxygenase (BVMO) activity. We have previously characterized *trans*-AT PKS-associated BVMOs for oocydin and lobatamide and showed that they catalyse the regio-specific oxygen insertion into the growing polyketide backbone. Here, we present new module architectures leading to unprecedented enzymology for *trans*-AT PKSs, including different module-integrated oxygenases from different BGCs for the installation of ester and oxime moieties and unique starter modules.

References

(1) Nguyen T. *et al.*, *Nat Biotechnol* 2008, **26**, 225.
(2) Ueoka R. *et al*. *Angew Chem Int Ed Engl*. 2018, **130**, 14727-14731
(3) Meoded R.A. *et al*. *Angew Chem Int Ed Engl*. 2018, **57**, 11644-11648
(4) Ueoka R., Meoded R.A. *et al*. *Angew Chem Int Ed Engl*. 2020, **59**, 7761-7765.

THE DISCRETE ACYLTRANSFERASE KIRCII A POTENTIAL TOOL FOR POLYKETIDE SYNTHASE ENGENEERING

Ewa M. Musiol-Kroll[*1], *Stephanie Grond*[2], *Gavin J. Williams*[3], *Sang Yup Lee*[4], *Tilmann Weber*[4], *and Wolfgang Wohlleben*[1]

[1] *Eberhard Karls Universität Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Mikrobiologie/Biotechnologie, Auf der Morgenstelle 28, 72076 Tübingen, Germany*

[2] *Eberhard Karls Universität Tübingen, Institut für Organische Chemie, Auf der Morgenstelle 18, 72076 Tübingen, Germany*

[3] *North Carolina State University, Department of Chemistry, Raleigh, North Carolina 27695-8204, United States*

[4] *Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kogle Allé 6, 2970 Hørsholm, Denmark*

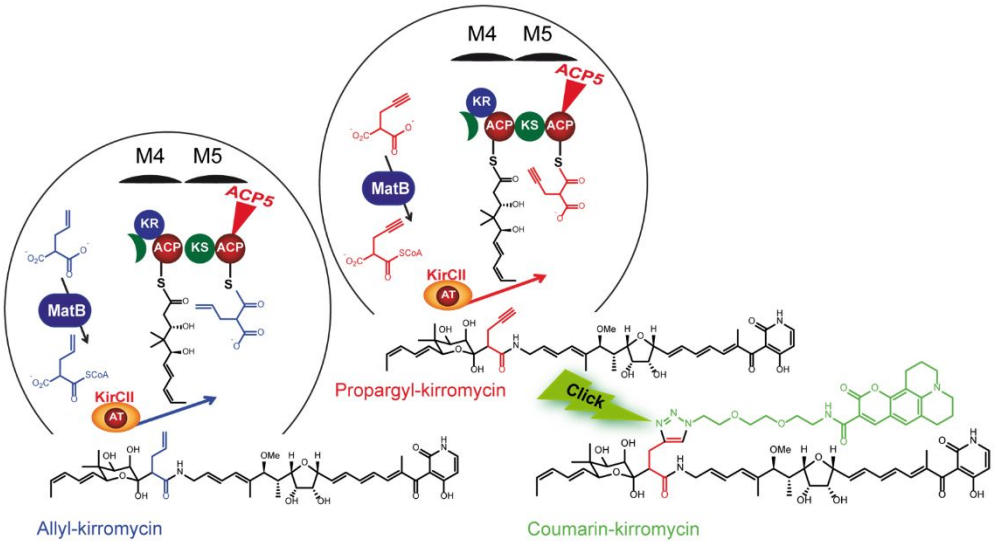
Ewa M. Musiol-Kroll

Eberhard Karls Universität Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Mikrobiologie/Biotechnologie, Auf der Morgenstelle 28, 72076 Tübingen, Germany

ewa.musiol@biotech.uni-tuebingen.de

The biosynthesis of the polyketide antibiotic kirromycin requires the activity of two discrete acyltransferases (ATs), KirCI and KirCII. KirCI is a malonyl-coenzyme A (malonyl-CoA) specific AT and loads malonate onto the assembly line (1). The discrete AT KirCII was confirmed to be specific for ethylmalonyl-Co A and only one acyl carrier protein (KirACP5) (2) in the PKS-assembly line of kirromycin. More recently, *in vitro* studies revealed that KirCII can also use other nonmalonyl-CoA substrates such as allylmalonyl-CoA, propargylmalonyl-CoA, and to a lesser extent azidoethyl- and phenylmalonyl-CoA for loading of KirACP5 (3, 4). The promising spectrum of substrate specificity of KirCII encouraged us to study the flexibility of this enzyme in *in vivo* experiments.

In our “bioderivatization” approach, the malonyl-CoA synthetase T207G/M306I MatB was introduced into the kirromycin producer strain. Non-natural precursors, allyl- and propargylmalonic acid, were fed to the modified strain. The engineered strain activated the substrates to their CoA-forms, which were subsequently utilized by KirCII for the polyketide biosynthesis. This resulted in production of the kirromycin derivatives, allyl- and propargyl-kirromycin (5). The latter was utilized as educt for further derivatization by “click” chemistry, which led to the generation of a fluorescent kirromycin product, coumarin-kirromycin (5). The study demonstrates the promiscuity of the MatB-KirCII/ACP5 system, which can be exploited for the development of molecular tools for generation of polyketide derivatives. The combination of this “bioderivatization” approach and “click” chemistry methods may enable the production of novel molecular probes and analogues of compounds with valuable bioactivities.



References

(1) **Musiol EM**, Greule A, Hartner T, Kulik A, Wohlleben W, Weber T. The AT(2) domain of KirCI loads malonyl extender units to the ACPs of the kirromycin PKS. Chembiochem: a European journal of chemical biology. 2013;14(11):1343-52.

(2) **Musiol EM**, Hartner T, Kulik A, Moldenhauer J, Piel J, Wohlleben W, et al. Supramolecular templating in kirromycin biosynthesis: the acyltransferase KirCII loads ethylmalonyl-CoA extender onto a specific ACP of the trans-AT PKS. Chemistry & biology. 2011;18(4):438-44.

(3) Koryakina I, McArthur J, Randall S, Draelos MM, **Musiol EM**, Muddiman DC, et al. Poly specific trans-acyltransferase machinery revealed via engineered acyl-CoA synthetases. ACS chemical biology. 2013;8(1):200-8.

(4) Ye Z, **Musiol EM**, Weber T, Williams GJ. Reprogramming acyl carrier protein interactions of an Acyl-CoA promiscuous trans-acyltransferase. Chemistry & biology. 2014;21(5):636-46.

(5) **Musiol-Kroll EM**, Zubeil F, Schafhauser T, Hartner T, Kulik A, McArthur J, et al. Polyketide bioderivatization using the promiscuous acyltransferase KirCII. ACS synthetic biology. 2017;6(3):421-7.

NEW SURPRISES FROM AN OLD FELLOW: MEGASYNTHASES ENCODED IN THE MICROBIOME OF THE MARINE SPONGE *MYCALE HENTSCHELI*

Michael Rust[1], Eric J. N. Helfrich[1], Michael F. Freeman[1,2], Pakjira Nanudorn[1], Christopher Field[1], Christian Rückert[3], Tomas Kündig[1], Michael J. Page[4], Victoria L. Webb[5], Jörn Kalinowski[3], Shinichi Sunagawa[1], Jörn Piel[1]

[1] Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zurich, Vladimir-Prelog-Weg 1-5/10, 8093 Zurich, Switzerland.

[2] Department of Biochemistry, Molecular Biology, and Biophysics and BioTechnology Institute, University of Minnesota–Twin Cities, St Paul, Minnesota 55108, USA.

[3] Institute for Genome Research and Systems Biology, Center for Biotechnology, Universität Bielefeld, Universitätsstrasse 25, 33594 Bielefeld, Germany.

[4] National Institute of Water and Atmospheric Research Ltd. (NIWA), P.O. Box 893 Nelson, New Zealand.

[5] National Institute of Water and Atmospheric Research Ltd. (NIWA), P.O. Box 14 901 Kilbirnie, Wellington, New Zealand.

Michael Rust

Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zurich, Vladimir-Prelog-Weg 1-5/10, 8093 Zurich, Switzerland.

rustmi@ethz.ch

Marine sponges are a rich source of bioactive natural products with considerable drug development potential. Many sponges harbor complex microbial communities that account for a significant portion of the host biomass. For an increasing number of cases, it has been shown that the producers of sponge-derived bioactive metabolites are symbiotic bacteria. The New Zealand sponge *Mycale hentscheli* contains three therapeutically relevant polyketides with potent bioactivities in the nanomolar range (1). Here, we present an extreme case of natural product-based mutualism, in which multiple microbiome members contribute to the rich chemistry of their host sponge. We identified biosynthetic gene clusters for the polyketides mycalamide, pateamine and peloruside in three different symbiotic producers and show that all three compounds are produced by *trans*-acyltransferase polyketide synthases. Metagenome mining and binning revealed additional orphan biosynthetic gene clusters distributed among a broad phylogenetic range of bacteria. The data provide a rationale for the chemical variability of *M. hentscheli* (2) and reinforce the concept that uncultivated bacteria harbor diverse producer taxa with as yet unrecognized drug discovery potential.

References

(1) L. J. Habener, J. N. A. Hooper, A. R. Carroll, Chemical and biological aspects of marine sponges from the family Mycalidae. *Planta Med.* 82, 816–831 (2016).

(2) M. Page, L. West, P. Northcote, C. Battershill, M. Kelly, Spatial and temporal variability of cytotoxic metabolites in populations of the New Zealand sponge *Mycale hentscheli*. *J. Chem. Ecol.* 31, 1161–1174 (2005).

THE PAIRED OMICS DATA PLATFORM: STANDARDIZED LINKS BETWEEN GENOMIC AND METABOLOMIC DATA FOR INTERGRATIVE MINING

Michelle A. Schorn[1], Stefan Verhoeven[2], Lars Ridder[2], Florian Huber[2], Pieter C. Dorrestein[3], Marnix H. Medema[4], Justin J.J. van der Hooft[4]

[1]Laboratory of Microbiology, Department of Agricultural and Food Sciences, Wageningen University, Wageningen, the Netherlands, [2]Netherlands eScience Center, Amsterdam, the Netherlands, [3]Collaborative Mass Spectromatry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, USA, [4]Bioinformatics Group, Department of Plant Sciences, Wageningen University, Wageningen, the Netherlands

Michelle A. Schorn

Laboratory of Microbiology, Department of Agricultural and Food Sciences, Wageningen University, Wageningen, the Netherlands, [2]Netherlands eScience Center, Amsterdam, the Netherlands

Michelle.schorn@wur.nl

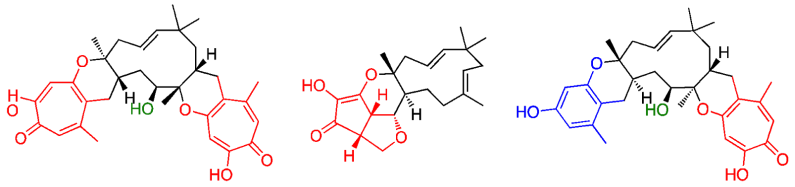
Genomics and metabolomics are widely used to explore microbial and plant biosynthetic diversity. Integrating these types of data from paired samples holds great promise for accelerating natural product discovery by mutually informing each other. However, while many paired (meta)genomes and metabolomes have become publicly available, connections between these datasets remain undocumented, and the same is true for links between biosynthetic gene clusters and mass spectra of their metabolic products. This precludes scientists from exploiting paired data to discover new connections between genes and molecules. Here, we introduce the Paired Omics Data Platform (https://pairedomicsdata.bioinformatics.nl/), a community initiative to systematically document links between metabolome and (meta)genome samples, and between gene clusters and MS/MS spectra. Seeded with >4,500 genome-metabolome links, the platform enables transferring structural knowledge between omics types and more efficiently linking products to producers. Thus, it paves the way towards large-scale computationally-guided natural product analysis.

DIVERSITY-ORIENTED COMBINATORIAL BIOSYNTHESIS OF TROPOLONE SESQUITERPENOIDS

Carsten Schotte, Lei Li & Russell J. Cox
Leibniz Universität Hannover, Centre of Biomolecular Drug Research (BMWZ)
Institute of Organic Chemistry, Schneiderberg 38, 30167 Hannover

Carsten Schotte
Leibniz Universität Hannover, Centre of Biomolecular Drug Research (BMWZ) Institute of Organic Chemistry, Schneiderberg 38, 30167 Hannover
carsten.schotte@oci.uni-hannover.de

Tropolone sesquiterpenoids (TS) are an intriguing family of fungal meroterpenoids that are known to be potent antitumor agents (*e.g.* eupenifeldin **1**), psychoactive (*e.g.* xenovulene A **2**) and to induce the production of erythropoietin in human cells (*e.g.* epolone A **3**).[1-3] Biosynthetically, TS natural products are derived from a very unusual *inter*molecular hetero Diels-Alder reaction between tropolones and α-humulene.[4,5] In a systematic synthetic-biology driven approach we engineered a series of unprecedented analogs of **1**-**3** in the fungal host *Aspergillus oryzae* NSAR1: by rational choice of heterologously expressed genes from three different fungal biosynthetic gene cluster (BGC) control over the number of hetero Diels-Alder reactions with humulene, unprecedented ring-contractions and humulene hydroxylation was achieved. The biosynthetic origin of unexpected monobenzopyranyl sesquiterpenoids was further elucidated using isotope-feeding studies to reveal a highly unusual oxidative ring-contraction.



Representative tropolone sesquiterpenoids with key structural features highlighted.

References

(1) T. El-Elimat, H. A. Raja, S. Ayers, S. J. Kurina, J. E. Burdette, Z. Mattes, R. Sabatelle, J. W. Bacon, A. H. Colby, M. W. Grinstaff, et al., *Org. Lett.* **2019**, *6*, 529-534.
(2) P. Thomas, H. Sundaram, B. J. Krishek, P. Chazot, X. Xie, P. Bevan, S. J. Brocchini, C. J. Latham, P. Charlton, M. Moore, et al., *J. Pharmacol. Exp. Ther.* **1997**, *282*, 513-20.
(3) P. Cai, D. Smith, B. Cunningham, S. Brown-Shimer, B. Katz, C. Pearce, D. Venables, D. Houck, *J. Nat. Prod.* **1998**, *61*, 791-795.
(4) R. Schor, C. Schotte, D. Wibberg, J. Kalinowski, R. J. Cox, *Nat. Commun*. **2018**, *9*.
(5) Q. Chen, J. Gao, C. Jamieson, J. Liu, M. Ohashi, J. Bai, D. Yan, B. Liu, Y. Che, Y. Wang, et al., *J. Am. Chem. Soc.* **2019**, *141*, 14052-14056.

GLOBAL ANALYSIS OF BIOSYNTHETIC GENE CLUSTERS REVEALS CONSERVED AND UNIQUE NATURAL PRODUCTS IN ENTOMOPATHOGENIC NEMATODE-SYMBIOTIC BACTERIA

Yi-Ming Shi[1], Merle Hirschmann[1], Yan-Ni Shi[1], Desalegne Abebew[1], Nicholas J. Tobias[1], Timo Obermaier[1], Peter Grün[1], Christian Richter[2], Jan Crames[1], Harald Schwalbe[2], Helge B. Bode[,1,3]*

[1]Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
[2]Institute for Organic Chemistry and Chemical Biology, Center for Biomolecular Magnetic Resonance, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
[3]Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

Yi-Ming Shi
Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
shi@bio.uni-frankfurt.de

Interactions between microbial organisms (e.g. bacteria) and higher eukaryotes are ubiquitous and have essential medical, environmental, and evolutionary significance. Entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* (XP) living in symbiosis with nematodes *Steinernema* and *Heterorhabditis*, respectively, is a promising model to address questions concerning assignments of ecological functions for microbial natural products. The bacteria are able to produce a huge diversity of toxic proteins as well as natural products as signaling molecules and virulence factors to maintain nematode development and protect the insect cadaver from food competitors. However, previous studies either mostly revolved around identification and characterization of BGCs one by one on a single-genome basis, or lacked a comprehensive comparison of intra/interspecies BGCs, which did not reveal to what extent BGCs that might be linked to the special ecological niche are conserved or unique within XP genomes. Here, we enroll newly sequenced genomes and systematically identify and categorize BGCs in 45 XP strains via combining pangenomic and interactive sequence similarity network approaches. Our results revealed that some previously unidentified BGCs are prevalent as highly conserved gene cluster families (GCFs) across XP genomes or as genus-specific GCFs. To rapidly access the unknown prevalent and unique biosynthetic pathways, we then applied a promoter-exchange strategy for BGC homologous overexpression, which led to identification of new natural products featuring unusual architectures.

CELL-FREE SYNTHESIS OF NATURAL COMPOUNDS FROM GENOMIC DNA OF BIOSYNTHETIC GENE CLUSTERS

Ilka Siebels,[a,b,#], Sarah Nowak[c,#], Christina S. Heil[a,b,#], Peter Tufar[a,b], Niña S. Cortina[a,b], Helge B. Bode[b,c,d,] and Martin Grininger[a,b,*]*

[a] *Institute of Organic Chemistry and Chemical Biology, Goethe University Frankfurt, Germany.*

[b] *Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Germany.*

[c] *Fachbereich Biowissenschaften, Molecular Biotechnology, Goethe University Frankfurt, Germany.*

[d] *Senckenberg Gesellschaft für Naturforschung, Frankfurt am Main, Germany*

[#]*These authors contributed equally;*

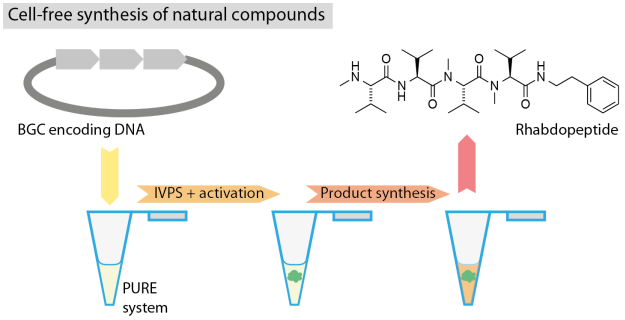
**Correspondence to: h.bode@bio.uni-frankfurt.de, grininger@chemie.uni-frankfurt.de*

Ilka Siebels & Christina Heil

Institute of Organic Chemistry and Chemical Biology, Goethe University Frankfurt, Germany

siebels@chemie.uni-frankfurt.de , christina_heil@urmc.rochester.edu

A variety of chemicals can be produced in a living host cell via optimized and engineered biosynthetic pathways. Despite the successes, pathway engineering remains demanding owing to the lack of specific functions or substrates in the host cell, its sensitivity in vital physiological processes to the heterologous components, or constrained mass transfer across the membrane. In this study, we show that complex multidomain proteins involved in natural compound biosynthesis can be produced from encoding DNA *in vitro* in a minimal complex PURE system to directly run multistep reactions (1). Specifically, we synthesize indigoidine and rhabdopeptides with the *in vitro* produced multidomain non-ribosomal peptide synthetases BpsA and KJ12ABC from the organisms *Streptomyces lavendulae* and *Xenorhabdus* KJ12.1, respectively. The proteins are analyzed in yield produced *in vitro*, post-translational modification and in their ability to synthesize the natural compounds. Recombinantly produced proteins are used for reference. Our study highlights cell-free PURE system as setting for the characterization of biosynthetic gene clusters that can potentially be harnessed for the rapid engineering of biosynthetic pathways.



Workflow of the cell-free synthesis of proteins and in situ product synthesis.

References

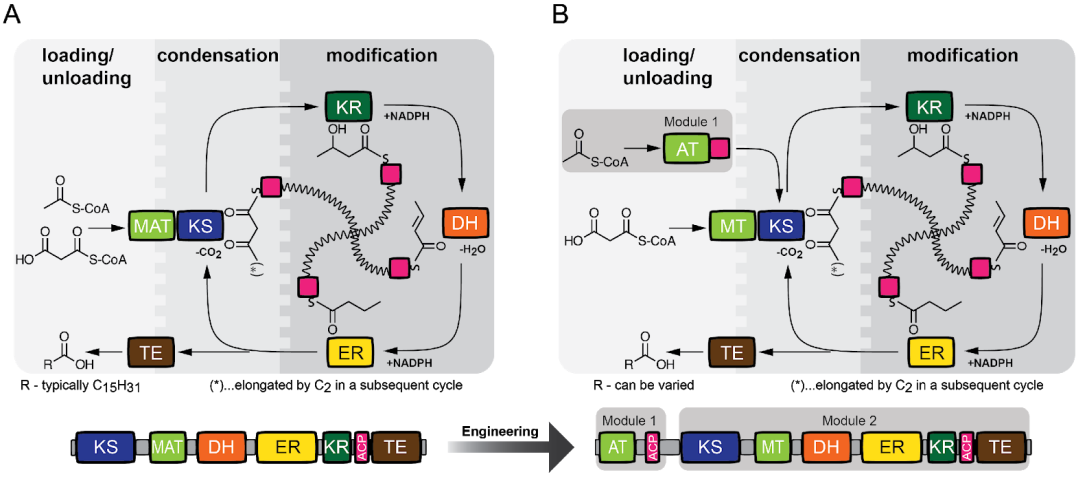
(1) Ilka Siebels, Sarah Nowak, Christina S. Heil, Peter Tufar, Niña S. Cortina, Helge B. Bode, and Martin Grininger 2020 *ACS Synthetic Biology* DOI: 10.1021/acssynbio.0c00186

GENERATION OF AN ARTIFICIAL BIMODULAR MEGASYNTHASE

Franziska Stegemann, Alicia Just, Maren Berlinghof, Amin Fahim, Lynn Buyachuihan, Martin Grininger
Institute of Organic Chemistry and Chemical Biology, Buchmann Insitute for Molecular Life Sciences, Goethe University Frankfurt, Germany

Franziska Stegemann
Institute of Organic Chemistry and Chemical Biology, Buchmann Insitute for Molecular Life Sciences, Goethe University Frankfurt, Germany
f.stegemann@chemie.uni-frankfurt.de

Increasing antimicrobial resistance threatens the effective prevention and treatment of infections. To tackle this threat, the persistent discovery and development of new drugs is essential. Natural products like polyketides are highly valuable sources of pharmaceuticals with i. a. antimicrobial properties. Their synthesis is catalyzed by complex multienzymes – so-called megasynthases.
The megasynthases fatty acid synthases (FASs) and polyketide synthases (PKSs) are using the same set of enzymes and catalyzing the same reactions during biosynthesis. Due to their comparable simplicity, FASs are better characterized than PKSs. [1] In this project, the similarity of both is used in order to design an artificial megasynthase, which can produce new polyketide products. The project aims at proving the concept by generating a bimodular FAS system with dedicated units for priming and elongation.
This project focuses particularly on the acyltransferase domain, which loads the acyl substrates during biosynthesis. Prior to engineering of the FAS, acyltransferases of different PKSs were analyzed. After this characterization, the gained knowledge was used to design an elongation module. Using a simplified model protein, the promiscuous acyltransferase of the mammalian FAS was exchanged with a substrate specific PKS acyltransferase. With this domain swap, a functional hybrid elongation module will be created, which can then be plugged to loading domains for the production of a first set of simple polyketides.



Idea of an artificial bimodular megasynthase for production of new compounds: The natural FAS (A) is modified to create the bimodular system (B). Via domain swapping the promiscuous acyltransferase is exchanged with a substrate specific domain, which solely loads the extension substrate. Additionally, a priming module with an acyltransferase specific for the priming substrate is installed. The natural FAS is engineered to create a bimodular system with separated priming and elongation module.

References

(1) S. Smith and S.-C. Tsai, "The type I fatty acid and polyketide synthases: a tale of two megasynthases," *Nat. Prod. Rep.*, vol. 24, no. 5, pp. 1041–1072, 2007.

THE SPOROTHRIOLIDES. A NEW BIOSYNTHETIC FAMILY OF FUNGAL SECONDARY METABOLITES

Dong-Song Tian,[a] Eric Kuhnert[a] and Russell J. Cox[a]
Centre of Biomolecular Drug Research (BMWZ), Institute for Organic Chemistry, Leibniz University Hannover, Schneiderberg 38, 30167, Hannover, Germany.

Dong-Song Tian
Centre of Biomolecular Drug Research (BMWZ), Institute for Organic Chemistry, Leibniz University Hannover, Schneiderberg 38, 30167, Hannover, Germany.
dong-song.tian@oci.uni-hannover.de

The biosynthetic gene cluster (*spo*) of the antifungal furofurandione sporothriolide **1** was identified from three genome-sequenced producing ascomycetes: *Hypomontagnella monticulosa* MUCL 54604, *H. spongiphila* CLL 205 and *H. submonticulosa* DAOMC 242471. A transformation protocol was established, and genes encoding fatty acid synthase and citrate synthase were simultaneously knocked out which led to loss of sporothriolide production. Heterologous expression of the *spo* genes in *Aspergillus oryzae* then led to the production of intermediates and shunts and delineation of a new fungal biosynthetic pathway originating in fatty acid biosynthesis. Key pathway steps are catalysed by a citrate synthase in a similar fashion as reported for maleidride biosynthesis,[1] an *in vitro* characterized alpha-ketoglutarate dependent dioxygenase and two lactonases, which are proposed to act as heterodimers. A putative hydrolase encoded in the *spo* cluster was revealed by *in vitro* studies to be likely involved in the producer's self-resistance. Additionally, the sporochartines **2**,[2] co-metabolites from the wild-type strains that are thought to be fusion products between **1** and the polyketide synthase derived trienylfuranol A **3**, were shown by *in vitro* experiments to be generated by non-enzymatic Diels-Alder cycloaddition of **1** and **3** during fermentation and extraction process.

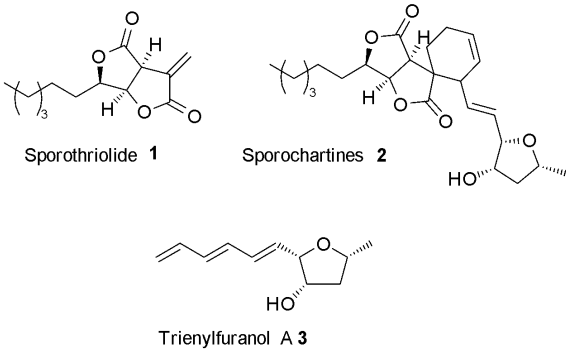


***Figure 1**. Structure of sporothriolide **1**, sporochartines **2** and trienylfuranol A **3**.*

References

(1) K. Williams, A. J. Szwalbe, N. P. Mulholland, J. L. Vincent, A. M. Bailey, C. L. Willis, T. J. Simpson and R. J. Cox, *Angew. Chemie Int. Ed.*, 2016, **55**, 6784–6788.
(2) C. Leman-Loubière, G. Le Goff, C. Debitus and J. Ouazzani, *Front. Mar. Sci.*, 2017, **4**, 399.

T DOMAIN MOONLIGHT AS DOCKING DOMAIN – NMR SOLUTION STRUCTURE OF AN ETENDED DOCKING DOMAIN INTERFACE IN THE PAX PEPTIDE PRODUCING NRPS FROM XENORHABDUS CABANILLASII

Jonas Watzel[1], Elke Duchardt-Ferner[2], Sepas Sarawi[1], Helge B. Bode[1,3], Jens Wöhnert[2]
[1] Molecular Biotechnology, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany.
[2] Institute of Molecular Biosciences and Center for Biomolecular Magnetic Resonance (BMRZ), Goethe University Frankfurt, 60438, Frankfurt am Main, Germany.
[3] Max-Planck-Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, 35043, Marburg, Germany.

Jonas Watzel
Molecular Biotechnology, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany
watzel@bio.uni-frankfurt.de

Nonribosomal peptide synthetases produce a diverse library of natural products, some of them with high relevance to clinical application and agriculture. These commonly multiprotein systems have to maintain a unique protein order, which is achieved via specific protein-protein interactions between terminal docking domains originally termed as “communication mediating domains (1)”, to guarantee a functional NRPS assembly line. In this study we are focusing on the multiprotein NRPS PaxABC producing the peptide-antimicrobial-*Xenorhabdus* (PAX) peptide in different *Xenorhabdus* species. This system contains three *in trans* interacting proteins and provides the first evidence that the “gatekeeper” function identified for N- and C-terminally attached and in part structurally characterized docking domains ($^{N/C}$DDs) (2) is not limited to these domains themselves. Thermodynamic characterization of T-^{C}DD:^{N}DD complexes by ITC determined significant decreased K_D values down to the low nanomolar range if the ^{C}DD preceding T domain was present. Furthermore, we were able to solve the solution structure of a *trans* PaxA T-^{C}DD:PaxB ^{N}DD complex by NMR and confirmed the *in trans* interacting residues by the analysis of PaxA T-^{C}DD and PaxB ^{N}DD mutants. Further functional data of a truncated PAX NRPS assembly line, only composed of the proteins PaxA and PaxB, analysed the effect of these amino acid exchanges *in vivo* by focusing on the absolute peptide production determined by HR-HPLC-MS.

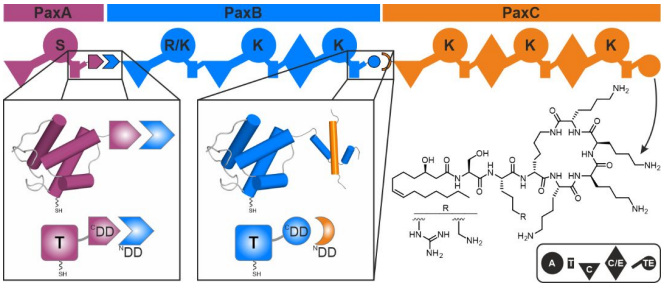


Fig. 1. *Nonribosomal peptide synthetase PaxS. For domain assignment the following symbols are used: A, adenylation domain with centerd amino acid specificity, large circles; T, thiolation domain, rectangle; C, condensation domain, triangle; C/E, dual condensation/epimerization domain, diamond; TE, thioesterase domain, small circle.*

References

(1) M. Hahn, T. Stachelhaus, Selective interaction between nonribosomal peptide synthetases is facilitated by short communication-mediating domains. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15585–15590 (2004).
(2) Watzel, C. Hacker, E. Duchardt-Ferner, H. B. Bode, J. Wöhnert, A new docking domain type in the peptide-antimicrobial-*Xenorhabdus* peptide producing nonribosomal peptide synthetase from *Xenorhabdus bovienii*. *ACS Chem. Biol.* **15**, 982–989 (2020).

IDENTIFICATION OF TWO NOVEL NRPS AND NRPS/PKS HYBRID PRODUCTS IN XENORHABDUS HOMINICKII

Westphalen M.A.[1], *Chekaiban J.*[1], *Shi Y.N.*[1], *Bode H.B.*[1,2,3]
[1]*Institut für Molekulare Biowissenschaften, Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany*
[2]*Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany*
[3]*Max-Planck-Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, Marburg, Germany*

Margaretha Westphalen
Institut für Molekulare Biowissenschaften, Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany
westphalen@bio.uni-frankfurt.de

Entomopathogenic bacteria from the genera *Photorhabdus* and *Xenorhabdus* live in mutualistic symbiosis with their nematode host and together infect and kill insect larvae.[1] The bacteria are known to possess a variety of biosynthetic gene cluster and several of them encode non-ribosomal peptide synthetases (NRPS).[2] NRPS are large multienzyme complexes operating in a modular fashion. One remarkable feature is their ability to produce peptides from unusual building blocks such as non-proteinogenic amino acids via *trans* acting enzymes or the presence of integrated modifying domains working in *cis*.[3]
Here, we investigated two biosynthetic gene clusters in *X. hominickii* by introducing an arabinose-inducible P_{BAD} promoter in front of the first biosynthetic gene respectively, followed by HPLC-MS analysis. Promoter activation in front of gene *xhom03557* lead to the identification of a novel tripeptide harbouring two unusual building blocks: an α-hydroxylated glycine and a β-D-homoserine.[4] The compound was purified by preparative HPLC-MS, followed by structure elucidation via NMR. Additionally, deletion of the monooxygenase domain embedded in the glycine activating adenylation domain allowed production of the non-hydroxylated tripeptide. Secondly, the promoter exchange in front of *xhom04773* lead to activation of an NRPS/PKS hybrid, presumably producing a peptide aldehyde exhibiting a Michael acceptor. Notably is also the presence of two adjacent thiolation domains within the PKS.

References

(1) Goodrich-Blair H, Clarke DJ. Mutualism and pathogenesis in Xenorhabdus and Photorhabdus: two roads to the same destination. *Mol Microbiol*. 2007;64(2):260-268.
(2) Tobias NJ, Wolff H, Djahanschiri B, et al. Natural product diversity associated with the nematode symbionts Photorhabdus and Xenorhabdus. *Nat Microbiol*. 2017;2(12):1676-1685.
(3) Süssmuth RD, Mainz A. Nonribosomal Peptide Synthesis-Principles and Prospects. *Angew Chem Int Ed Engl*. 2017;56(14):3770-3821.
(4) Bode E, Heinrich AK, Hirschmann M, et al. Promoter Activation in Δhfq Mutants as an Efficient Tool for Specialized Metabolite Production Enabling Direct Bioactivity Testing. *Angew Chem Int Ed Engl*. 2019;58(52):18957-18963.

POSTER SESSIONS

1. **STRUCTURAL AND FUNCTIONAL ANALYSIS OF TERMIATION DOMAINS IN NONRIBOSOMAL PEPTIDE SYNTHETASES** * **Maximilian Biermeier** * *Philipps University of Marburg, Faculty of Chemistry, Hans-Meerwein-Strasse 4, 35032 Marburg, Germany*

2. **DIVIDE AND CONQUER: A MOLECULAR TOOL KIT TO REPROGRAM THE BIOSYNTHESIS OF NON RIBOSMAL PEPTIDES** * **Kenan A. J. Bozhüyük** * *Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 560438, Frankfurt am Main, Germany.*

3. **THE KETOSYNTHASE DOMAIN CONSTRAINS THE DESIGN OF POLYKETIDE SYNTHASES** * **Lynn Buyachuihan** * *Institute of Organic Chemistry and Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Germany*

4. **BIOSYNTHESIS OF OXIGENATED BRASILANE TERPENE GLYCOSIDES INVOLVES A PROMISCUOUS N-ACETYLGLUCOSAMINE TRANSFERASE** * **Jing Feng** * *Institute for Organic Chemistry and Centre for Biomolecular Drug Research (BMWZ), Leibniz University Hannover, Schneiderberg 38, Hannover 30167, Germany*

5. **ELUCIDATION OF EARLY STEPS IN PELORUSIDE BIOSYNTHESIS** * **Amy E. Fraley** * *Eidgenössische Technische Hochschule Zuerich, Switzerland*

6. **UNRAVELING A UNIVERSAL DARK MATTER IN PROKARYOTES: THE CHEMICAL STRUCTURES OF ARYLPOLYENE LIPIDS** * **Gina Grammbitter** * *Institute of Molecular Biological Science, Johann Wolfgang Goethe University, 60438 Frankfurt, Germany*

7. **BIOSYNTHETIC GENE CLUSTERS OF ACETOBACTERACEAE, PHYLOGENETICALLY-DERIVED OR ACQUIRED THROUGH HORIZONTAL GENE TRANSFER?** * **Juan Guzmann** * *Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany*

8. **UNVEILING THE BIOSYNTHESIS OF DIMERIC SORBICILLINOIDS: DISCOVERY OF AN UNPRECEDENTED FLAVIN-DEPENDENT MONOOXYGENASE** * **Lukas Kahlert** * *Leibniz University Hannover, Centre of Biomolecular Drug Research (BMWZ), Institute of Organic Chemistry, Schneiderberg 38, 30167 Hannover*

9. **ARTIFICIAL SPLITTING OF A NON-RIBOSOMAL PEPTIDE SYNTHETASE BY INSERTING NATURAL DOCKING DOMAINS** * **Carsten Kegler** * *Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany.*

10. **MULTISPECIFIC AND PROMISCUOUS MONOMODULAR NONRIBOSOMAL PEPTIDE SYNTHETASES** * **Martin Klapper** * *Leibniz Institute for Natural Product Research and Infection Biology e.V. - Hans Knöll Insitute (HKI), Department of Paleobiotechnology, Beutenbergstraße 11a, 07745 Jena, Germany*

11. **IMPROVING THE PRODUCTION OF NON-ROBSOMAL PEPTIDES BY OPTIMIZING THE INTERFACE BETWEEN THE CONDENSATION AND ADENYLATION DIDOMAIN – FROM A STABLE PLATFORM TO A MULTI-FUNCTIONAL WORKBENCH** * **Janik Kranz** * *Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany*

12. **A TINY MEGASYNTHASE IS INVOLVED IN THE GENERATION OF POLYCHLORINATED BUILDING BLOCKS FOR AMBIGOL BIOSYNTHESIS** * **I Dewa Made Kresna** * *Institute for Insect Biotechnology, Justus-Liebig-University of Giessen, 35392 Giessen, Germany*

13. **RECONSTRUCTING BACTERIAL BYRROLIZIDINE ALKALOID BIOSYNTHESIS** * **Katharina Lamm** * *Technical Biochemistry, TU Dresden, Bergstraße 66, 01069 Dresden, Saxony, Germany*

14. **CHARACTERISING ACP TO KS INTERACTIONS IN TYPE I FAS AND PKS SYSTEMS** * **Felix Lehmann** * *Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany*

15. **CHARACTERIZATION OF NEU ENZYMOLOGY IN TRANS-ACYLTRANSFERASE POYKETIDE SYNTHASES** * **Hanna Minas & Franziska Hemmerling** * *Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zuerich, Switzerland.*

16. **NEW SURPRISES FROM AN OLD FELLOW: MEGASYNTHASES ENCODED IN THE MICROBIOME OF THE MARINE SPONGE *MYCALE HENTSCHELI*** * **Michael Rust** * *Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zurich, Vladimir-Prelog-Weg 1-5/10, 8093 Zuerich, Switzerland.*

17. **THE PAIRED OMICS DATA PLATFORM: STANDARDIZED LINKS BETWEEN GENOMIC AND METABOLOMIC DATA FOR INTERGRATIVE MINING** * **Michelle A. Schorn** * *Laboratory of Microbiology, Department of Agricultural and Food Sciences, Wageningen University, Wageningen, the Netherlands, [2]Netherlands eScience Center, Amsterdam, the Netherlands*

18. **DIVERSITY-ORIENTED COMBINATORIAL BIOSYNTHESIS OF TROPOLONE SESQUITERPENOIDS** * **Carsten Schotte** * *Leibniz Universität Hannover, Centre of Biomolecular Drug Research (BMWZ) Institute of Organic Chemistry, Schneiderberg 38, 30167 Hannover*

19. **GLOBAL ANALYSIS OF BIOSYNTHETIC GENE CLUSTERS REVEALS CONSERVED AND UNIQUE NATURAL PRODUCTS IN ENTOMOPATHOGENIC NEMATODE-SYMBIOTIC BACTERIA** * **Yi-Ming Shi** * *Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany*

20. **CELL-FREE SYNTHESIS OF NATURAL COMPOUNDS FROM GENOMIC DNA OF BIOSYNTHETIC GENE CLUSTERS** * **Ilka Siebels & Christina Heil** * *Institute of Organic Chemistry and Chemical Biology, Goethe University Frankfurt, Germany*

21. **GENERATION OF AN ARTIFICIAL BIMODULAR MEGASYNTHASE** * **Franziska Stegemann** * *Institute of Organic Chemistry and Chemical Biology, Buchmann Insitute for Molecular Life Sciences, Goethe University Frankfurt, Germany*

22. **THE SPOROTHRIOLIDES. A NEW BIOSYNTHETIC FAMILY OF FUNGAL SECONDARY METABOLITES** * **Dong-Song Tian** * *Centre of Biomolecular Drug Research (BMWZ), Institute for Organic Chemistry, Leibniz University Hannover, Schneiderberg 38, 30167, Hannover, Germany.*

23. **T DOMAIN MOONLIGHT AS DOCKING DOMAIN – NMR SOLUTION STRUCTURE OF AN ETENDED DOCKING DOMAIN INTERFACE IN THE PAX PEPTIDE PRODUCING NRPS FROM XENORHABDUS CABANILLASII** * **Jonas Watzel** * *Molecular Biotechnology, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany*

24. **IDENTIFICATION OF TWO NOVEL NRPS AND NRPS/PKS HYBRID PRODUCTS IN XENORHABDUS HOMINICKII** * **Margaretha Westphalen** * *Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 560438, Frankfurt am Main, Germany.*

SOCIAL PROGRAMME

Dinner: Hotel Dolce Bad Nauheim

Date: September 30th 2020

Time 6.30 p.m.

Entrance: all

Venue: Elvis-Presley-Platz 1 – 61239 Bad Nauheim

	Name	University	Email
Nadja	**Abbood**	Goethe University Frankfurt	abbood@bio.uni-frankfurt.de
Mohammad	**Alanjary**	Wageningen University	mohammad.alanjary@wur.nl
Friederike	**Biermann**	Friedrich Schiller University Jena	friederike.biermann@uni-jena.de
Maximilian	**Biermeier**	Philipps University Marburg	biermeie@staff.uni-marburg.de
Helge	**Bode**	Goethe University Frankfurt	h.bode@bio.uni-frankfurt.de
Eckhard	**Boles**	Goethe University Frankfurt	e.boles@bio.uni-frankfurt.de
Kenan A. J.	**Bozhüyük**	Goethe University Frankfurt	kenan.bozhueyuek@bio.uni-frankfurt.de
Lynn	**Buyachuihan**	Goethe University Frankfurt	buyachuihan@chemie.uni-frankfurt.de
Russel	**Cox**	Leibniz University Hannover	russell.cox@oci.uni-hannover.de
Max	**Crüsemann**	University of Bonn	mcruesem@uni-bonn.de
Peter	**Czermak**	Justus Liebig University of Giessen	Peter.czermak@kmub.thm.de
Sabrina	**Dirnberger**	University Bayreuth	dirnberger-sabrina@live.de
Elke	**Dittmann**	University of Potsdam	editt@uni-potsdam.de
Jin	**Feng**	Leibniz University Hannover	jin.feng@oci.uni-hannover.de
Amy E.	**Fraley**	ETH Zuerich	afraley@biol.ethz.ch
Achilleas	**Frangakis**	Goethe University Frankfurt	office.frangakis@biophysik.org
Kyra	**Geyer**	Max Planck Institute Marburg	kyra.geyer@mpi-marburg.mpg.de
Gina	**Grammbitter**	Goethe University Frankfurt	grammbitter@bio.uni-frankfurt.de
Martin	**Grininger**	Goethe University Frankfurt	grininger@chemie.uni-frankfurt.de
Michael	**Groll**	Technical University Munich	michael.groll@tum.de
Tobias	**Gulder**	Technical University of Dresden	tobias.gulder@tu-dresden.de
Juan	**Guzman**	Goethe University Frankfurt	Juan.Guzman@ime.fraunhofer.de
Frank	**Hahn**	University Bayreuth	frank.hahn@uni-bayreuth.de
Eric Jan N.	**Helfrich**	Harvard Medical School Boston	Eric_Helfrich@hms.harvard.edu
Franziska	**Hemmerling**	ETH Zuerich	fhemmerl@biol.ethz.ch
Julia	**Hitschler**	Goethe University Frankfurt	J.Hitschler@bio.uni-frankfurt.de
Gerhard	**Hummer**	Max Planck Institute Frankfurt	gerhard.hummer@biophys.mpg.de
Mirko	**Joppe**	Goethe University Frankfurt	joppe@chemie.uni-frankfurt.de

	Name	University	Email
Lukas	**Kahlert**	Leibniz University Hannover	lukas.kahlert@oci.uni-hannover.de
Adrian	**Keatinge-Clay**	University of Texas	adriankc@utexas.edu
Carsten	**Kegler**	Goethe University Frankfurt	kegler@bio.uni-frankfurt.de
Martin	**Klapper**	Hans Knöll Institute Jena	Martin.Klapper@hki-jena.de
Janik	**Kranz**	Goethe University Frankfurt	kranz@bio.uni-frankfurt.de
I Dewa Made	**Kresna**	Justus Liebig University of Giessen	idewa92@gmail.com
Hajo	**Kries**	Hans Knöll Institute Jena	hajo.kries@leibniz-hki.de
Werner	**Kühlbrandt**	Max Planck Institute Frankfurt	werner.kuehlbrandt@biophys.mpg.de
Marcel	**Kulike**	Technical University of Berlin	marcel.kulike@chem.tu-berlin.de
Katharina	**Lamm**	Technical University of Dresden	katharina.lamm@tu-dresden.de
Felix	**Lehmann**	Goethe University Frankfurt	flehmann@stud.uni-frankfurt.de
Shuming	**Li**	Philipps University Marburg	shuming.ki@staff.uni-marburg.de
Marnix	**Medema**	Wageningen University	marnix.medema@wur.nl
Hannah	**Minas**	ETH Zuerich	minash@biol.ethz.ch
Rolf	**Müller**	Helmholtz Centre Saarbrücken	Office.Mueller@helmholtz-hips.de
Ewa Maria	**Musiol-Kroll**	Eberhard Karls University Tuebingen	ewa.musiol@biotech.uni-tuebingen.de
Arne	**Oestreich**	Justus Liebig University of Giessen	Arne.oestreich@lse.thm.de
Mislav	**Oreb**	Goethe University Frankfurt	m.oreb@bio.uni-frankfurt.de
Karhik	**Paithankar**	Goethe University Frankfurt	paithankar@em.uni-frankfurt.de
Silke	**Reiter**	Justus Liebig University of Giessen	silke.reiter@agrar.uni-giessen.de
Theodora	**Ruppenthal**	Goethe University Frankfurt	ruppenthal@chemie.uni-frankfurt.de
Michael	**Rust**	ETH Zuerich	michael.rust@biol.ethz.ch
Till	**Schäberle**	Justus Liebig University of Giessen	Till.F.Schaeberle@agrar.uni-giessen.de
Maximilian	**Schmalhofer**	Technical University Munich	maximilian.schmalhofer@tum.de
Eric W.	**Schmidt**	The University of Utah	ews1@utah.edu
Michelle	**Schorn**	Wageningen University	michelle.schorn@wur.nl
Carsten	**Schotte**	Leibniz University Hannover	carsten.schotte@oci.uni-hannover.de
Yi-Ming	**Shi**	Goethe University Frankfurt	shi@bio.uni-frankfurt.de

	Name	University	Email
Ilka	**Siebels**	Goethe University Frankfurt	siebels@chemie.uni-frankfurt.de
Elizabeth	**Skellam**	Leibniz University Hannover	elizabeth.skellam@oci.uni-hannover.de
Pierre	**Stallforth**	Hans Knöll Institute Jena	pierre.stallforth@leibniz-hki.de
Franziska	**Stegemann**	Goethe University Frankfurt	f.stegemann@chemie.uni-frankfurt.de
Evi	**Stegmann**	Eberhard Karls University Tuebingen	evi.stegmann@biotech.uni-tuebingen.de
Dong-Song	**Tian**	Leibniz University Hannover	dong-song.tian@oci.uni-hannover.de
Jonas	**Watzel**	Goethe University Frankfurt	watzel@bio.uni-frankfurt.de
Kira	**Weissman**	University of Lorraine	kira.weissman@univ-lorraine.fr
Margaretha	**Westphalen**	Goethe University Frankfurt	westphalen@bio.uni-frankfurt.de
Wolfgang	**Wohlleben**	Eberhard Karls University Tuebingen	wolfgang.wohlleben@biotech.uni-tuebingen.de
Nadine	**Ziemert**	Eberhard Karls University Tuebingen	nadine.ziemert@uni-tuebingen.de

GENERAL INFORMATION

- The local currency is €
- Note some small shops may not accepts credit-cards
- If you need any assistance please contact us megasyn-symposium@uni-frankfurt.de