synmikro

SCIENTIFIC REPORT 2010–2014

LOEWE CENTER FOR SYNTHETIC MICROBIOLOGY

LOEWE
Exzellente Forschung für Hessens Zukunft

Philipps Universität Marburg

MPI

MAX-PLANCK-GESELLSCHAFT

Preface

Research on microorganisms has been a long-standing focus of the Philipps-Universität and has made Marburg one of the best places in Germany to study microbiology. The fruitful collaborations between groups working at the university and the local Max Planck Institute for Terrestrial Microbiology have heightened the national and international visibility of Marburg and have fortified its reputation for excellence. These concerted activities provided the appropriate framework for the foundation of the Center for Synthetic Microbiology (SYNMIKRO) within the LOEWE excellence program of the state of Hessen in a joint effort between Philipps-Universität and Max Planck Society in the year 2010. We are grateful for the continued support of the state of Hessen without which the center could not have been established. On the occasion of the fifth anniversary since its inception, this brochure surveys the many activities of SYNMIKRO. As the president of the Philipps-Universität Marburg, I am very proud of what has been achieved in this time.

Scientific progress requires creative minds, excellent working conditions and a stimulating environment. All these elements come together in an ideal manner in SYNMIKRO. Research at the center is carried out by internationally highly regarded and recognized scientists. The center provides access to a state-of-the-art infrastructure for modern molecular biology. The large number of cooperations across the traditional disciplines from life sciences and natural sciences all the way to the humanities shows that Marburg provides an atmosphere that is conducive for interdisciplinary and trans-disciplinary research. In the near future, key groups in synthetic microbiology from both the Philipps-Universität and the Max Planck Institute will be housed in a large, well-equipped new research building in the center of the science campus Lahnberge. This new building will foster even closer scientific interactions and the cost-effective sharing of equipment.

The collaboration between groups from the Philipps-Universität with departments and research groups from the Max Planck Institute for Terrestrial Microbiology laid the foundation for further projects like the international Max Planck Research School and DFG-funded coordinated programs, including the microbiology-oriented Collaborative Research Center 987. Regionally, SYNMIKRO interacts with groups at the Justus-Liebig-Universität Giessen and the Technische Hochschule Mittelhessen and thereby strengthens the research alliance among the universities. SYNMIKRO is thus also a prime example of fruitful collaborations between institutions.

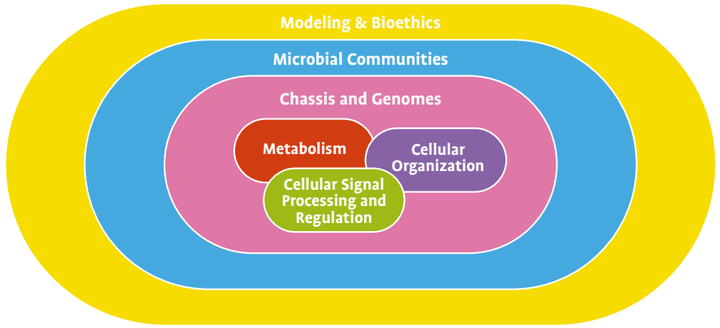
The manifold research activities and the broad range of microorganisms studied under the umbrella of SYNMIKRO extend to dedicated teaching activities that provide superb training for the next generations of microbiologists. Excellence in teaching in the field of microbiology is a nationally and internationally recognized hallmark of the Philipps-Universität.

Along with the many scientists working in SYNMIKRO, I feel that it is both necessary and worthwhile to summarize for a broader audience what has been accomplished so far and to look forward to future activities of the center. I thank the group leaders, scientists, and the large number of students for their hard work and their concentrated efforts to make SYNMIKRO a success. They have all contributed to making this brochure an interesting and stimulating read that I hope you will enjoy.

Prof. Katharina Krause
President
Philipps-Universität Marburg

Synthetic Microbiology in Marburg

Microorganisms are omnipresent in the biosphere. They occupy even the most exotic ecological niches and have developed an impressive variety of physiological and sensory functions that allow them to respond to and cope with environmental challenges. Some of these traits already provide the basis for large-scale production of chemicals and pharmaceuticals, for biofuels, food-processing, biosensing or bioremediation. Nevertheless, the vast natural toolbox developed by microorganisms over billions of years of evolution undoubtedly contains many undiscovered processes that wait to be explored and harnessed. The field of synthetic microbiology is at the center of this enterprise. Much of the fascination with this emergent field derives from the ability to freely combine myriads of genes, cellular processes, and biochemical functions in very much the same way as engineers combine different parts to form larger functional units and products.



Topics integrated within SYNMIKRO.

However, the challenges encountered by synthetic biology in its early years revealed that many processes within cells are still poorly understood and that not all synthetic pathways sketched on the drawing board actually work in cells. A better quantitative, dynamic, and theoretical understanding of how organization and interaction of cellular components generates emergent properties is required before we can use natural systems as prototypes for the construction of predictable, streamlined and robust systems with novel properties. Vice versa, the process of building synthetic systems or of modifying natural systems facilitates understanding, as the performance of these constructs helps to understand their function. All of this is made possible by advances and new technologies in several disciplines, in particular in cell biology, genetics, molecular biology, structural biology, biochemistry, and computational biology.

In 2010, the Philipps-Universität Marburg and the Max Planck Institute for Terrestrial Microbiology joined forces to bring synthetic microbiology to Marburg. They established SYNMIKRO, the Center for Synthetic Microbiology, within the LOEWE excellence program of the state of Hessen. Research at the center follows the two-pronged approach – building to understand and understanding to build – to gain insights into the basic principles of microbial life and to provide the fundamental knowledge and tools needed to tap in novel ways the potential of microorganisms as cell factories or sensor/reporter systems. Since its foundation, the center has grown to become a major, internationally visible research institution that today represents one of the hot spots of research in quantitative and synthetic microbiology in Europe.

SYNMIKRO is an interdisciplinary center that consists of members from seven faculties of the university and the four departments of the Max Planck Institute. Drawing on Marburg's highly-rated expertise in microbiology, it integrates almost 30 laboratory groups at the university and the Max Planck Institute to cover all aspects of microbial life from the molecular level to whole cells and communities. In addition, theory- and ethics-focused groups provide testable mathematical models, systematic overviews and guidance. With its inter- and trans-disciplinary expertise, SYNMIKRO offers a unique opportunity to develop synthetic microbiology, from the identification and characterization of modules in natural systems to the development of synthetic modules and their integration into natural host organisms or synthetic chassis-cells.

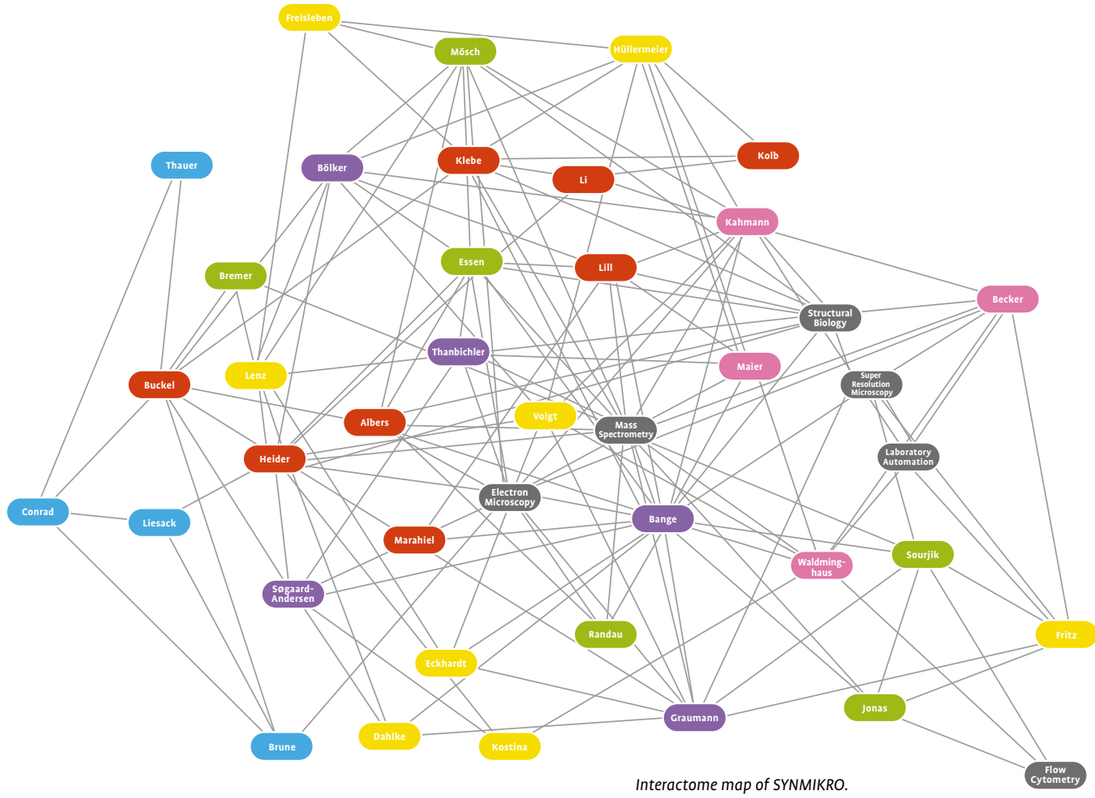
For optimal support of the scientific projects, SYNMIKRO has invested in state-of-the-art infrastructural units for laboratory automation, flow cytometry, protein spectroscopy, super resolution microscopy, electron microscopy, structural biology and mass spectrometry. These infrastructural units provide critical support for the projects pursued by SYNMIKRO researchers and their collaboration partners, and are engaged in the development of enabling technologies in key areas in synthetic microbiology.

The impact of SYNMIKRO goes well beyond the dedicated research projects. With the thematic focus in synthetic microbiology within the M.Sc. program *Molecular and Cellular Biology* and with our successful iGEM team, we recruit graduate and undergraduate students to this fascinating new field early in their career. Furthermore, a structured doctoral program, summer schools, workshops and seminars aim at scientists in academia and industry. In addition, we are committed to promoting the public and scientific discourse on pioneering developments. The public is regularly invited to learn about synthetic microbiology in open lectures and plenary discussions, and a fruitful dialogue between basic natural sciences and the humanities was initiated.

With this brochure, we introduce the members of SYNMIKRO and their projects, explain the work of the infrastructural units and describe key activities of the center in the years 2010-2014. We hope that you can see from this report that SYNMIKRO provides a vibrant environment for top-level education and first-class science, and that it's worth to keep an eye on SYNMIKRO and its diverse activities.

Bruno Eckhardt
Director

Anke Becker
Vice Director



Interactome map of SYNMIKRO.

Scientific Advisory Board

SYNMIKRO seeks feedback from independent experts: Its Scientific Advisory Board of internationally renowned scientists convenes every two years and advises the center regarding research planning and organization. We thank the members of the Scientific Advisory Board for this valuable contribution.

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PROF. DR. JULIA VORHOLT
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Content

1.1 RESEARCH

Cellular Signal Processing
and Regulation

PROF. DR. ERHARD BREMER

Osmotic Stress Responses of Microorganisms

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SYNMIKRO member since 2010

SHORT CV

1995– Professor of Molecular Microbiology, Philipps-Universität Marburg
2013 Visiting Professor, Princeton University, USA
1993–1995 Adjunct Professor, Philipps-Universität Marburg
1992–1995 Independent Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
1984–1992 Staff Scientist and Assistant Professor, University of Konstanz
1989 Habilitation, University of Konstanz
1982–1984 Postdoctoral Fellow, National Cancer Institute, Frederick, MD, USA
1982 Ph.D. in biology, Universität Tübingen
1980 Diploma in biology, Universität Tübingen

AWARDS & HONORS

2011 Elected as Member of the *European Academy of Microbiology* (EAM)
1991 Young Investigator Award from the *Deutsche Gesellschaft für Hygiene und Mikrobiologie* (DGHM)
1982–1984 Postdoctoral fellowships from the German Research Foundation (DFG) and the German Academic Exchange Service (DAAD)

Osmoprotectants in bacterial stress responses and cell integrity

Water is the foundation of life. It is essential for vital processes like the replication and transcription of the genetic material and biochemical reactions, but at the same time, these processes need protection from adverse environmental influences. The development of semi-permeable lipid membranes that let the water pass, but severely restrict the crossing of ions and most organic molecules therefore was a key step in the evolution of proto-cells. Since the osmotic potential of the cytoplasm is considerably higher than that of the surrounding, water will flow along this osmotic gradient into the cell and thereby generate an intracellular hydrostatic pressure, the turgor. As the cytoplasmic membrane is a fragile structure that is unable by itself to restrain turgor, microorganisms eventually developed firm, yet elastic cell walls (Fig. 1). Turgor is considered as the driving force for expansion of the cell wall sacculus during growth and is therefore considered an essential attribute of most cells.

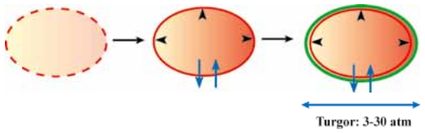

Figure 1. *From a proto-cell to a cell-walled microorganism and the concomitant development of turgor.*

In their natural ecosystems, free-living microorganisms will experience fluctuations in the osmolarity that will either result in water influx (at low osmolarity) or water efflux (at high osmolarity). To timely respond to these environmental challenges, highly integrated cellular stress responses are engaged. Understanding these responses and the underlying signal transduction mechanisms is the core of our research efforts. Besides, as adequate turgor regulation is an essential function for most microorganisms, all efforts to synthetically construct proto-cells, or to design microbial cells with minimalized genomes ("chassis") must take care to implement systems for osmoregulation. Therefore, our group also embarked on the development of synthetic osmoprotection gene clusters and of synthetic derivatives of natural osmoprotectants with novel properties.

Cellular responses to high osmolarity

When challenged by high osmolarity surroundings, bacteria engage in carefully timed stress responses to curb the outflow of water. This cellular adjustment entails the accumulation of organic osmolytes, so-called compatible solutes; these can be amassed to exceedingly high concentrations and in a fashion that is sensitively linked to the degree of the osmotic stress experienced by the cell. In *Bacillus subtilis*, the model organism for Gram-positive bacteria that we study, important compatible solutes are glutamate, proline, and glycine betaine. Other *Bacilli* additionally accumulate ectoine and its derivative 5-hydroxyectoine. We have elucidated the synthesis pathways for these stress protectants and study the biochemical and structural properties of key enzymes; e.g., that of the ectoine hydroxylase (Fig. 2) (Höppner *et al*, 2014). Compatible solutes can also be accumulated through import (Broy *et al*, 2015), and our genetic and structural analyses of the involved transport systems in *B. subtilis* have broken new ground for their understanding in other microorganisms and their functional annotation in genome sequencing projects. In Fig. 3, the crystal structure of the extracellular solute receptor protein (OpuBC) for choline, the biosynthetic precursor of the potent osmostress protectant glycine betaine, is shown (Pittelkow *et al*, 2011).

Compatible solutes as effective temperature stress protectants

In addition to providing osmostress protection, our work shows that compatible solutes also serve as effective temperature stress protectants for cells threatened by death both at the upper and at the lower boundaries of the temperature spectrum that *B. subtilis* cells can tolerate (Hoffmann & Bremer, 2011; Broy *et al*, 2015). This occurs with a degree of effectiveness that is unmatched by other microbial temperature stress defense systems; e.g., the heat- and cold-shock proteins and the general stress system of *B. subtilis*.

Harnessing compatible solute systems for synthetic microbiology

Compatible solutes are not only excellent osmolytes and temperature stress protectants, but also serve as stabilizers of macromolecules, ensure the functioning of biosynthetic processes and can even preserve the integrity of entire cells. The function-preserving properties of compatible solutes have aroused considerable interest as they could be exploited for biotechnological purposes, cosmetics, and medical applications. The compatible solutes ectoine and 5-hydroxyectoine are currently produced on an industrial scale, and products containing them (in particular those used in skin care) have reached consumer markets. Production of these compounds relies on so-called bacterial milking, a procedure that involves the high-density fermentation of a highly salt resistant bacterium (*Halomonas elongata*) followed by a severe osmotic down-shock, which releases the ectoines via mechanosensitive channels from the producer

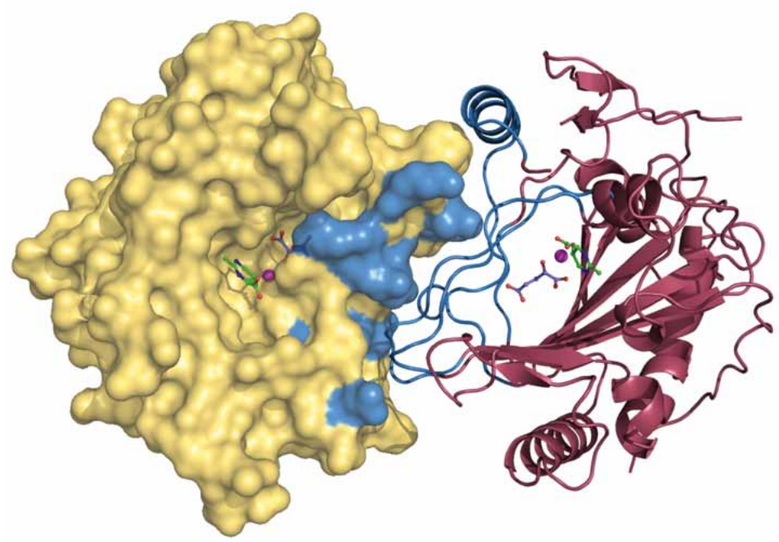

Figure 2. *Crystal structure of a dimer of the ectoine hydroxylase (EctD) in complex with its co-substrate 2-oxoglutarate, the catalytically important iron atom and the reaction product 5-hydroxyectoine.*

cells. We are studying the molecular biology and biochemistry of the biosynthetic routes for ectoine and hydroxyectoine production. In collaboration with the group of Christoph Wittmann (TU Braunschweig), we successfully transplanted a synthetically engineered gene cluster for ectoine production into the industrial workhorse *Corynebacterium glutamicum* (Becker *et al*, 2013). In addition to this proof of principle, we are now exploring the possibility of exploiting the ectoine hydroxylase (EctD) for the *in vivo* and *in vitro* synthesis of ectoine derivatives that do not exist naturally but which might possess interesting novel properties. Structural analysis of the EctD enzymes (Fig. 2) (Höppner *et al*, 2014) helps us to design appropriate substrates. Likewise, we found that synthetic derivatives of naturally occurring compatible solutes (e.g., dimethylsulfoniopropionate; DMSP) can be smuggled into osmotically stressed *B. subtilis* cells via a type of transport system (OpuC) that is widely distributed in nature (Broy *et al*, 2015), opening up the way for novel applications of these remarkable compounds.

Figure 3. *Crystal-formation of the purified OpuBC ligand binding protein (left) and its three-dimensional structure in complex with the ligand choline (right).*

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Hoffmann T, Bremer E (2011). Protection of *Bacillus subtilis* against cold stress via compatible-solute acquisition. *J Bacteriol* 193, 1552-62.

Pittelkow M, Tschapek B, Smits SH, Schmitt L, Bremer E (2011). The crystal structure of the substrate-binding protein OpuBC from *Bacillus subtilis* in complex with choline. *J Mol Biol* 411, 53-67.

Becker J, Schäfer R, Kohlstedt M, Harder BJ, Borchert NS, Stöveken N, Bremer E, Wittmann C (2013). Systems metabolic engineering of *Corynebacterium glutamicum* for production of the chemical chaperon ectoine. *Microb Cell Fact* 12:110.

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Broy S, Chen C, Hoffmann T, Brock NL, Nau-Wagner G, Jebbar M, Smits SH, Dickschat JS, Bremer E (2015). Abiotic stress protection by ecologically abundant dimethylsulfoniopropionate and its natural and synthetic derivatives: insights from *Bacillus subtilis*. *Env Microbiol*, doi: 10.1111/1462-2920.12698

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SHORT CV

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1996–2001 Research Group Leader, Max Planck Institute for Biochemistry, Munich
1995–1996 Postdoctoral Fellow, Laboratory of Molecular Biology, MRC, Cambridge, UK
1995 Ph.D. in biochemistry, Max Planck Institute for Biophysics, Frankfurt
1991 Diploma in biochemistry, ETH Zürich, Switzerland

AWARDS & HONORS

2012 Lee-Wee-Nam Visiting Professor, NTU, Singapore
1996 TMR Marie-Curie Fellowship
1991 Silver medal of the ETH Zürich

Structural biology of photoreception & yeast cell walls

Photoreceptors allow all forms of life to monitor their environmental status in terms of light quality & quantity, and thus the time of day, or their location in soil, sediment, water column and hosts. We study the mechanisms of photoreceptors from different microorganisms by combining structural biology with biochemical and biophysical techniques. When understanding how photon absorption triggers conformational changes and affects downstream signaling we can engineer these photoreceptors for optogenetic applications, e.g. the light-induced degradation of proteins (Fig. 1) (Renicke *et al*, 2013) or the biosynthesis of second messengers.

The ultrastructure of fungal cell walls is a second focus of our work. Yeasts are unicellular fungi, which can switch to a wide range of multicellular phenotypes such as biofilms, filaments, flocs and flors. Given their lack of motility, these phenotypes enable yeasts to colonize various habitats and to escape for example unfavorable nutrient conditions. A prerequisite for multicellular phenotypes is cell adhesion *via* either cell-cell and/or cell-surface contacts. We analyze the N-terminal A-domains of fungal adhesins in terms of structure and specificity, because these domains determine the physical nature of cell-cell interactions.

From structures to evolution

Photoreceptors of the photolyase-cryptochrome family mediate responses to blue light. Amongst other things, these proteins catalyze light-driven repair of UV-lesions in DNA, and act in blue-light dependent signaling, e.g., for the control of flowering or gene expression. For this they utilize a U-shaped flavin adenine dinucleotide (FAD) chromophore together with a second chromophore, that harvests light like an antenna. Our studies on class II photolyases, mostly found in animals and plants, and the recently discovered cryptochromes from proteo- and cyanobacterial species (CryPro) showed that the intramolecular transfer of electrons to this cofactor takes different routes than previously known (Fig. 2). Likewise, the architectural features relevant for DNA recognition of class I and II photolyases reflect different topological restraints imposed by pro- and eukaryotic DNA packaging. In class II photolyases the antenna cofactor, 8-hydroxydeazaflavin, is utilized throughout the green lineage, but not in higher plants, which have developed a hitherto unknown pigment for this function.

The structure of *Rhodobacter sphaeroides* cryptochrome B, a regulator of the photosynthetic apparatus genes, revealed not only the photolyase-like fold and a third type of an electron-transfer pathway to FAD, but also two cofactors only found in the CryPro subfamily: 6,7-dimethyl-8-ribityl-lumazine in its antenna-binding domain, and a $[4Fe\text{-}4S]^{2+}$ cluster within the catalytic domain (Geisselbrecht *et al*, 2012). Especially the latter resembles the large primase subunit PriL, a key component of eukaryotic and archaeal DNA replication. This finding implies that primases are evolutionary closely related to the CryPro subfamily of cryptochromes.

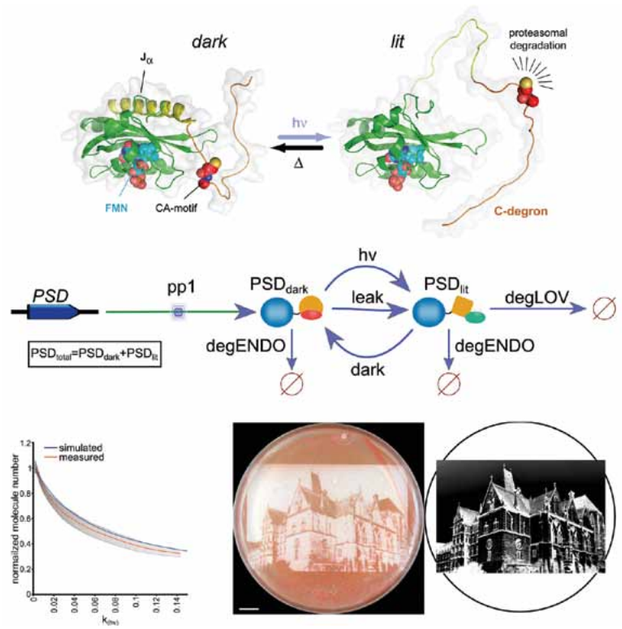

Figure 1. *The photon-sensitive degron developed in collaboration with the group of Christoph Taxis, Philipps-Universität Marburg, allows light-dependent degradation of fused protein partners. Top, structural and biophysical analyses found that upon illumination the end (yellow) of a light-sensitive domain (LOV, green) gets loosened and hence exposes a degron motif for proteasomal degradation. Middle & bottom, quantitative modeling allows to predict its sensitivity to light and can be manifold applied, for example to get yeast cells acting as a photographic medium.*

Fungal adhesins with different mechanisms

A hallmark of fungi is their thick cell wall that provides not only a strong physical barrier against host defense, but also the basis for cell-cell communication. Accordingly, a concise understanding of yeast adhesion and its different modes may pave the way for controlling the adhesive properties of yeast cells, e.g., by the development of anti-adhesive antimycotics.

In bakers yeast, flocculins (Flo), a family of adhesins, mediate aggregation of yeast cells into protective flocs by self-recognition. Although flocculation plays an eminent role in biotechnology and food industry (e.g., for beer & wine), the mode of flocculin-based surface recognition and the nature of cognate ligands remained elusive. By analyzing crystal structures of the adhesion domain of flocculin 5 complexed to its cognate ligands, we found a PA14-like lectin fold that utilizes a unique D*cis*D calcium-binding motif for carbohydrate binding (Veelders *et al*, 2010). The structure allowed to engineer yeast strains, whose flocculation is controlled by glucose and are hence applicable in biotechnology and the brewing industry.

The epithelial adhesins (Epa) from the human pathogen *Candida glabrata* are not only responsible for host-cell adhesion, but highly related to flocculin domains. Therefore, we studied structures of the different Epa domains complexed to different carbohydrate ligands (Fig. 3). In doing so, we showed how the mucins of human hosts are specifically discriminated by various Epa subtypes (Maestre-Reyna *et al*, 2012), and that specificity is mainly governed by variation of two inner loops, CBL1 and CBL2.

Not all fungal adhesins utilize a lectin-based mode of interaction, though. Flocculin 11, which confers the formation of different multicellular structures such as biofilms, filaments or flors in bakers yeast, is one example: The Flo11A domain structure reveals a β-sandwich of the fibronectin type III type covered by a unique hydrophobic apical region. We show that homophilic Flo11-Flo11 and heterophilic Flo11-plastic interactions just depend on the Flo11A domain. EM 3D-reconstructions of yeast cell-cell contact sites indicate that Flo11 acts as an unusual membrane-tethered hydrophobin that mediates long-range cell-cell interactions.

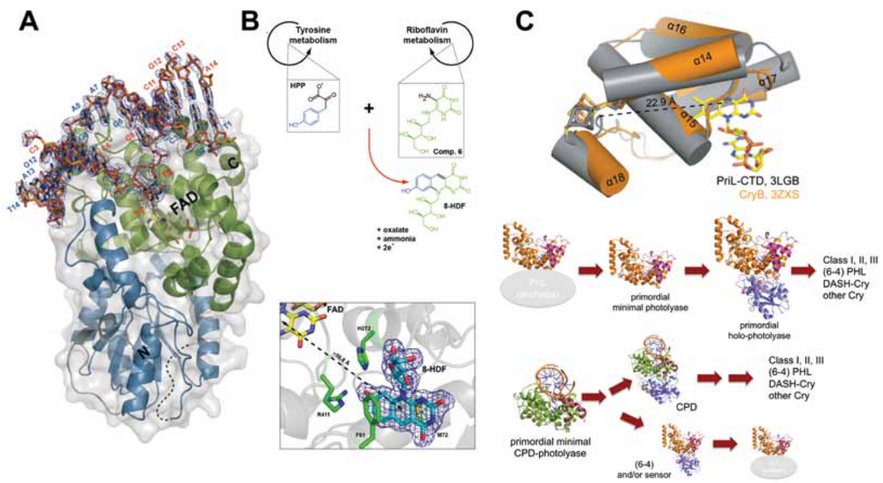

Figure 2. *(A) Structure of the class II photolyase from Methanosarcina mazei in complex with UV-damaged DNA (Kiontke et al, 2011). This enzyme is highly related to plant photolyases and has apparently been acquired by this archaeon via horizontal gene transfer. (B) The biosynthesis route for its antenna pigment (8-HDF) was artificially introduced into Escherichia coli and allowed to characterize the recombinant photolyase in complex to 8-HDF. (C) The catalytic domain of photolyases and cryptochromes structurally resembles the large subunit of eukaryotic/archaeal primases (PriL), an enzyme class essential for DNA-replication. This raises the question of who was first: photolyases or primases?*

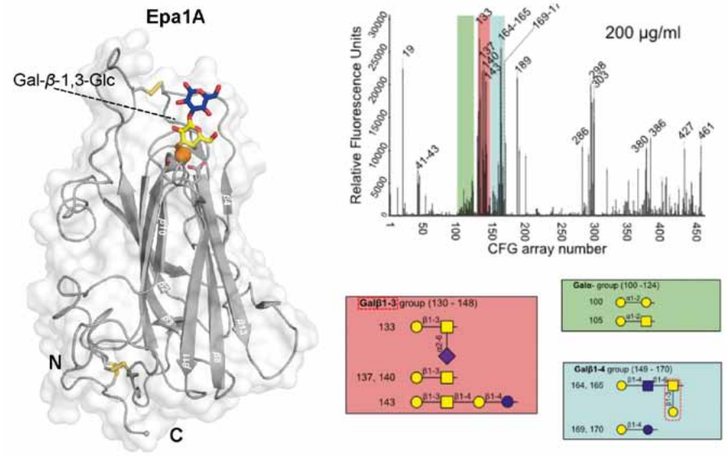

Figure 3. *Structural analysis of the adhesion domain of Epa1 from Candida glabrata allowed to map its carbohydrate binding site, whereas specificity profiling by glycan chips allowed to identify human mucins as predominant interaction partners.*

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Geisselbrecht Y, Frühwirth S, Schröder C, Pierik AJ, Klug G, Essen LO (2012). CryB from *Rhodobacter sphaeroides*: A unique class of cryptochromes with novel cofactors. *EMBO Rep* 13, 223-29.

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Maestre-Reyna M, Diderrich R, Veelders M, Eulenburg G, Kalugin V, Brückner S, Keller P, Rupp S, Moesch HU, Essen LO (2012). Structural code for adhesin-mediated invasion of host epithelia by *Candida glabrata*. *Proc Natl Acad Sci USA* 109, 16864-9.

Renicke C, Schuster D, Essen LO, Taxis C (2013). A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem Biol* 20, 619-26.

Veelders M, Brückner S, Ott D, Unverzagt C, Mösch HU, Essen LO (2010). Structural basis of the social function of flocculins. *Proc Natl Acad Sci USA* 107, 22511-16.

DR. GEORG FRITZ

Computational Microbiology

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SYNMIKRO member since 2014

SHORT CV

2014– Independent Research Group Leader, Philipps-Universität Marburg
2012–2014 Postdoctoral Fellow, LMU Munich
2006–2012 Ph.D. in theoretical physics, LMU Munich
2007 Visiting Scientist, University of California, San Diego, USA
2006 Diploma in physics, Albert-Ludwigs-Universität Freiburg

AWARDS & HONORS

2008 CeNS Publication Award

Computational approaches to microbial gene regulation

Mathematical modeling and theoretical simulations have become valuable tools in the analysis of biological networks. In our group, we use such computational approaches to elicit how microbes adapt to environmental changes. For instance, we study the response of *Bacillus subtilis* against antimicrobial peptides and scrutinize the adaptation of *Escherichia coli* to fluctuating sugar concentrations. Through quantitative modeling of the *in vivo* adaptation dynamics, our computational approach gives unprecedented insight into the mechanisms of signal transduction and gene regulation. On a more general level, this enables us to link molecular regulation mechanisms to phenotypic behavior and, hence, to provide model-driven hypotheses for the evolutionary consequences associated with different regulation strategies. In addition to these reverse engineering efforts, we apply computational strategies in the forward design of synthetic circuits, where our analyses serve as a theoretical guide for the efficient implementation of synthetic gene expression programs and help to decipher some of the design principles of biological signal processing.

Modeling the anatomy of antimicrobial resistance modules

In natural habitats with limited amounts of nutrients, many bacterial species produce a variety of antimicrobial agents to impede their competitors. Key molecules in this "chemical warfare" are so-called peptide antibiotics that often target important steps in cell wall biosynthesis. Bacitracin, for instance, binds undecaprenyl pyrophosphate (UPP), a lipid carrier of glycan biosynthetic intermediates, and thereby blocks its recycling. Hence, for bacteria to successfully thrive in such environments, it is of vital importance to mount specific stress responses, which allow them to cope with endo- and exogenously produced peptide antibiotics.

The BceRS-BceAB system of *B. subtilis* is one of these widely conserved peptide sensing and detoxification modules, conferring resistance to a broad spectrum of compounds including bacitracin. It consists of the ABC transporter BceAB, which facilitates removal of the antibiotic from its target site by a so far unknown mechanism, and the two-component system BceRS, which in turn induces the expression of BceAB (Fig. 1). While in many two-component systems the histidine kinases perceive their stimuli autonomously, in Bce-like systems the ABC transporter is also strictly required for stimulus perception. Recently, we used a combined experimental and theoretical approach to discriminate between alternative models that might explain this mutual requirement. In this work, we could show that the regulatory dynamics in the Bce system is compatible with a model in which BceS and BceAB form a sensory complex that detects transport activity, *i.e.*, the antibiotic *flux* perceived by individual ABC transporters, rather than the external *concentration* of the antibiotic itself (Fig. 1). Such an unusual mode of stimulus perception might have evolved as a cost-efficient 'produce-to-demand' strategy, which induces gene expression only if the antibiotic flux per transporter exceeds a certain threshold and, hence, if an increased demand for detoxification exists.

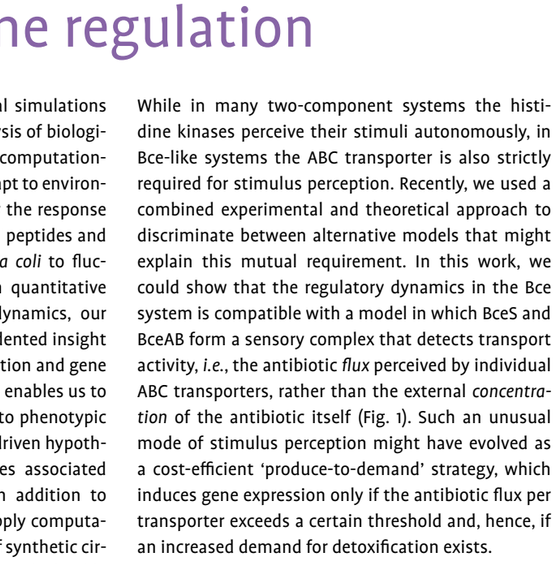

Figure 1. *Need-based activation of antibiotic resistance by a flux-sensing mechanism. If the bacitracin flux per BceAB transporter exceeds a certain threshold (upper panel), signaling via the two-component system BceRS leads to the up-regulation of bceAB expression levels, thereby reducing the bacitracin flux per transporter (lower panel).*

From modules to networks: The cell envelope stress response in *B. subtilis*

In addition to specific resistance modules, *B. subtilis* also mounts more unspecific systems to stabilize and protect its cell envelope against the action of antimicrobial peptides. One example for such 'protection modules' is the *liaIH* operon that is strongly induced by a wide spectrum of peptide antibiotics. We recently showed that LiaI is a small membrane protein that acts as a membrane anchor for the phage-shock protein homologue LiaH (Dominguez-Escobar *et al*, 2014). Upon envelope stress, LiaI and LiaH copy numbers increase drastically and co-localize into almost static foci (Fig. 2). Their highly sub-diffusive motion suggests that heterooligomers of LiaI and LiaH become trapped – potentially at sites of cell wall damage, where they might exert their putative role in maintaining the integrity of the cytoplasmic membrane. Currently, we elucidate how resistance modules and protection modules are coordinated and interconnected within the larger cell envelope stress response network of *B. subtilis*.

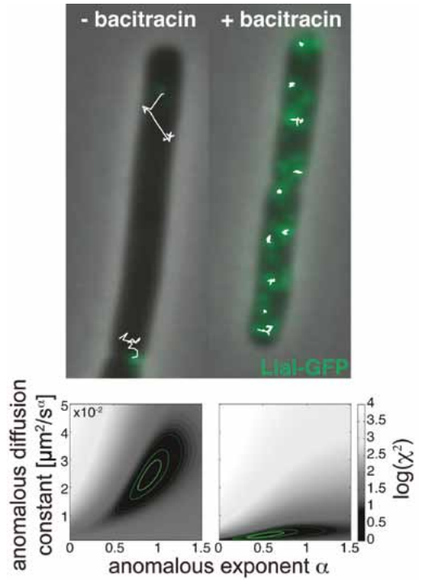

Figure 2. *Diffusional trapping of LiaI-GFP foci under cell wall antibiotic stress. The upper panel shows tracks of individual LiaI-GFP foci under unstressed (left) and stressed (right) conditions. The lower panel shows the results of our computational analysis of these tracks, indicating highly sub-diffusive motion (α<1) of LiaI-GFP under antibiotic stress conditions.*

Forward design and implementation of synthetic genetic circuits

A central goal of synthetic biology is to rationally engineer living cells with new functions. To guide this forward design process theoretically, we perform *in silico* simulations based on the *in vivo* characteristics of biological parts and thereby explore the signal processing potential of novel circuit modules. For instance, it is well-established that bacteria can use their *cis*-regulatory transcription machinery to implement combinatorial logic gates (AND, OR, etc.), allowing them to integrate multiple external stimuli to generate an appropriate transcriptional response. However, less is known about how microbes condition their responses upon signals perceived in the past, that is, how and to what extent they can "memorize" their environment. In a theoretical work, we showed that simple protein-protein interactions and protein-DNA binding are sufficient, in principle, to implement genetic circuits with the capabilities of memory devices, *i.e.*, latches and flip-flops, known from digital electronics (Hillenbrand *et al*, 2013). However, while in digital memory circuits the timing of all operations is rapidly updated by virtue of a synchronizing 'clock', biological memory circuit designs must compensate for their inherently slow and asynchronous dynamics. In synthetic biology, such 'sequential logic' circuits could be used, *e.g.*, to memorize whether bacteria have encountered specific chemicals in the past.

As a first step towards the implementation of novel genetic modules and devices, we established a standardized and well-characterized genetic toolbox comprising a basic set of vectors, promoters, reporters and affinity-tags for the Gram-positive model organism *B. subtilis* (Radeck *et al*, 2013). However - despite all standardization efforts - the success of implementing complex circuits into microbes is challenged by the inherent interconnectivity of virtually all compounds within a living cell. Therefore, one goal of our ongoing work is to better understand the context-dependence of synthetic biology building blocks, and to ultimately engineer "orthogonal", i.e., context independent synthetic circuits.

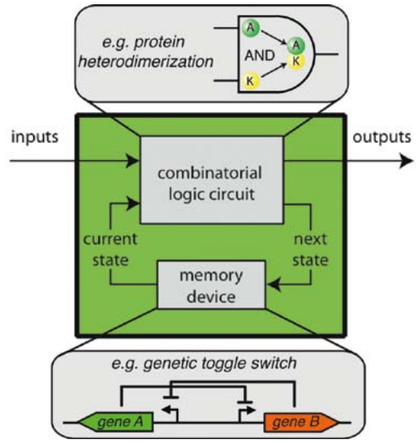

Figure 3. *Sequential logic design using cis-regulatory transcription elements. Bacteria can 'memorize' transient signals by using bistable genetic circuits, such as the genetic toggle switch. If the output of a regulatory circuit depends both on the present inputs as well as on the state of this internal 'memory bit', microbes can condition their responses upon signals perceived in the past.*

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Mechanisms of bacterial cell cycle control

Important cellular processes are generally orchestrated by regulatory circuits, which are composed of numerous genes and proteins. Our group studies the operation principles, organization and evolution of such circuits, focusing on the regulatory mechanisms that govern cell cycle progression in bacteria. In particular, we are interested in understanding how cells adjust their cell division cycle in response to inputs from the environment. As a primary model we use the α-proteobacterium *Caulobacter crescentus*, which divides asymmetrically and whose cell cycle can be easily synchronized. In addition, we also make use of other model organisms such as *Escherichia coli* to learn how the cell cycle circuit of *Caulobacter* relates to that of other bacteria. Based on our insight into the mechanisms governing cellular information processing, we develop novel tools for controlling cellular behaviors in synthetic biology settings.

Modularity of complex regulatory systems

Complex regulatory circuits are often built of simpler parts or modules that carry out specific, but separable functions. Such a modular architecture is thought to enhance evolvability as it allows for the generation of new functions by simply reusing existing modules in different contexts rather than inventing entire new networks from scratch. Our previous work demonstrated that the task of regulating DNA replication during the *C. crescentus* cell cycle is distributed between two genetically separable modules (Fig. 1) (Jonas *et al*, 2011). One of them centers on DnaA, a positive regulator of DNA replication that dictates the periodicity of replication and hence regulates replication in a temporal manner. The second control module centers on the response regulator CtrA that governs the asymmetric replicative fates of the two daughter cells, thereby acting as a spatial control module (Jonas *et al*, 2011; Chen *et al*, 2011). Both DnaA and CtrA activities are controlled by their own regulatory sub-circuits, which allow them to function largely independently of each other. Importantly, the genetic separability of spatial and temporal control modules is reflected in their evolutionary history. DnaA is the central component of an ancient and phylogenetically widespread circuit that governs replication timing in *Caulobacter* and most other bacteria. By contrast, CtrA, which is found only in asymmetrically dividing α-proteobacteria, was recruited later in evolution as an additional control layer to enforce replicative asymmetry of daughter cells. Currently, we investigate the organization and function of stress response circuits and study how they have become interlinked with the core modules driving cell cycle progression during evolution.

Cell cycle control by environmental cues

Research in the past has led to the identification of the key factors required for cell cycle progression and we have by now a relatively detailed understanding of how these factors are wired in higher-order circuits to drive cell cycle progression under optimal conditions. However, it is only poorly understood how cell cycle progression is modulated in response to environmental cues to ensure optimal adaptation and survival under changing conditions (Jonas, 2014). Our recent work revealed that distinct stress conditions cause *C. crescentus* to arrest the cell cycle with a single chromosome in G1-phase, which is mediated by a drop in

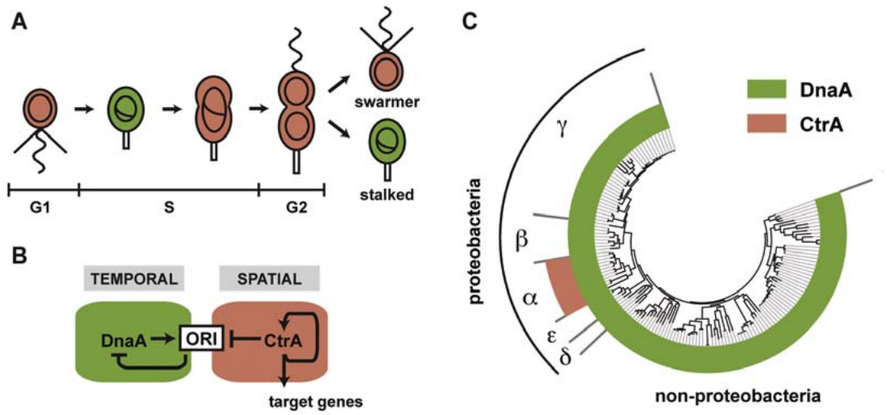

Figure 1. *Modularity of the Caulobacter crescentus cell cycle. (A) Schematic of the asymmetric cell cycle of C. crescentus. (B) DnaA and CtrA compose two control modules that separate the temporal and spatial control of DNA replication. (C) The genetic separability of DnaA and CtrA functions is reflected in phylogeny.*

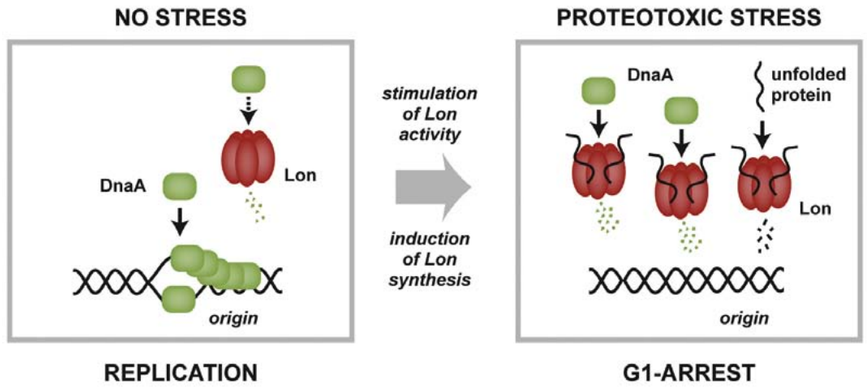

Figure 2. *Stress-mediated control of DNA replication. In optimal conditions the replication initiator DnaA accumulates and initiates DNA replication. Stress-induced protein unfolding causes the upregulation and activation of Lon, which induces DnaA degradation and a block of replication initiation.*

the levels of the conserved replication initiator DnaA (Fig. 2). We could show that a sophisticated network encompassing a chaperone system as well as the AAA+ protease Lon tightly controls degradation of DnaA in response to changes in the global protein folding state (Jonas *et al*, 2013). Under optimal growth conditions, DnaA is relatively stable and accumulates at high abundance to initiate DNA replication with maximal frequency. By contrast, stress conditions causing the unfolding of proteins, for example heat stress or chaperone failure, induce transcription of the *lon* gene and stimulate activity of the Lon protease, which then promotes degradation of DnaA (Jonas *et al*, 2013).

Currently, we are studying additional mechanisms that transduce environmental information into the cell cycle machinery of *C. crescentus*. For example, we have evidence that in addition to DNA replication, the process of cell division is also subject to environmental control. Our work shows that distinct stress conditions cause a reversible block of cell division. We want to unravel the regulatory mechanisms leading to this stress-mediated cell division block and try to understand how a tight control of cell cycle processes can enhance the fitness of microorganisms in changing environments (Fig. 3).

Tools for the development of synthetic circuits

Understanding the general principles of bacterial decision-making processes will help us to reprogram bacterial behaviors for custom-made applications. Based on our mechanistic insight into regulatory processes, we aim to design and establish tools for manipulating and utilizing bacterial behaviors. For instance, understanding the molecular mechanisms of inducible protein degradation will enable the engineering of synthetic degradation systems, which can eliminate proteins of interest in a highly regulated manner. Therefore, we currently dissect the rules governing substrate selectivity of the protease Lon and investigate how the degradation of specific proteins can be induced in response to certain input signals. In a next step, we aim to reprogram substrate selectivity to trigger the destruction of pre-defined proteins upon protease stimulation. Such controllable degradation systems are critical for the development of synthetic circuits, which so far largely rely on transcriptional control mechanisms.

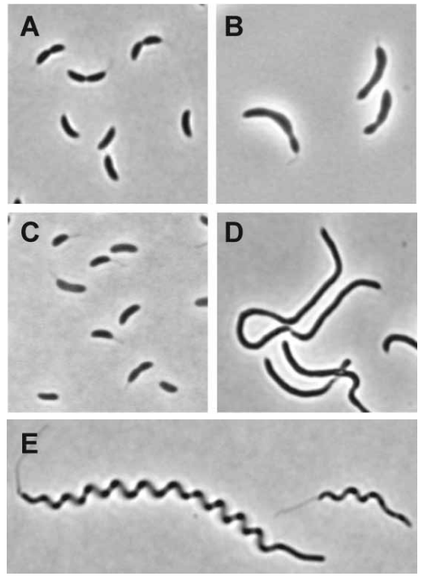

Figure 3. *Morphological responses of wild type C. crescentus to distinct stress conditions. (A) no stress, (B) heat shock, (C) carbon starvation, (D) salt stress, (E) long-term growth in stationary phase.*

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Dynamics of signal transduction and multicellular development in yeast

Biological signaling circuits and networks are often capable of processing several distinct stimuli, and yet faithfully elicit appropriate cellular programs in a signal-specific manner. In many cases, the wiring of the individual signaling components and the according dynamic behavior of signaling pathways are not yet well understood. We are studying the interplay of such pathways in the budding yeast *Saccharomyces cerevisiae*, which can choose between different lifestyles (Brückner & Mösch, 2012) and, depending on the correct environmental stimuli, undergoes for example sexual cell fusion or multicellular development (Fig. 1A). In doing so, we want to get detailed insights into (i) the topology and dynamic behavior of the responsible signal transduction pathways, and into (ii) the precise spatial organization and temporal assembly dynamics of the resulting multicellular growth forms.

Cellular wiring and dynamic behavior of MAPK signaling pathways

Lifestyle adaptation of *S. cerevisiae* is under control of several evolutionary conserved signal transduction pathways including a MAPK cascade. This pathway controls a number of cellular programs, for example multicellular development in response to nutrient availability and cell fusion upon stimulation by sexual pheromone. The MAPK pathway contains several signaling components, which are required for both programs, for instance the central MAPK module and the transcription factor Ste12 (Fig. 1B). It also harbors components that are required for only one of the two programs, for example the TEA family transcription factor Tec1 that controls multicellular development in combination with Ste12. We have previously shown that in response to sexual pheromone, Tec1 is rapidly degraded to ensure efficient execution of the mating program (Heise *et al*, 2010). More recently, we found that Tec1 protein stability is also under control of the nutrient-sensitive TORC1 signaling pathway, which plays a major role in cell growth and development in most eukaryotes (Brückner *et al*, 2011). These findings indicate that the MAPK and TORC1 signaling pathways are linked via Tec1 to coordinate control of cellular development in response to sexual and nutritional stimuli. In our attempt to further elicit the cellular wiring of the MAPK pathway, we lately discovered that Tec1 and Ste12 can associate with the transcriptional co-regulators Msa1 and Msa2, which were previously found to control cell cycle specific transcription (van der Felden *et al*, 2014). These findings add an additional layer of complexity to the cellular wiring of the MAPK cascade and the control mechanisms exerted by this signaling pathway.
In order to study the dynamic behavior of MAPK signaling, we have established a fluorescence microscopy-based system for quantitative measurement of MAPK signaling *in vivo* at single cell level with a focus on

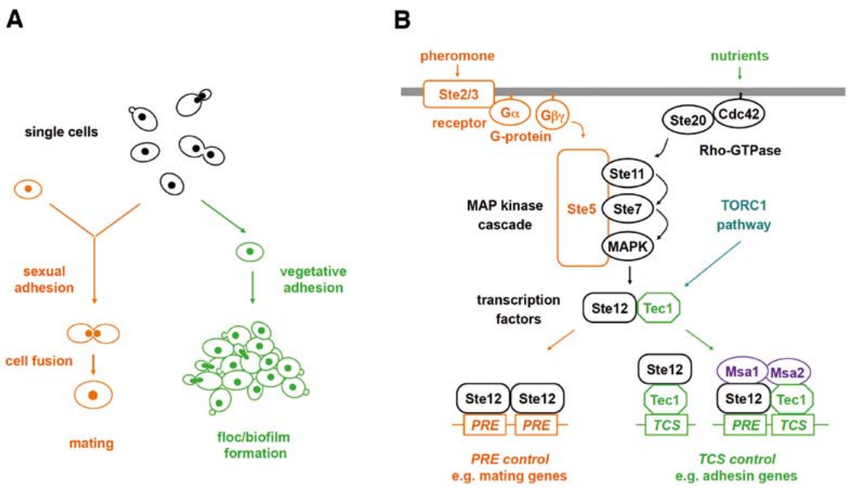

Figure 1. *Control of vegetative and sexual development in Saccharomyces cerevisiae. (A) Vegetatively growing single yeast cells (black) can either (i) adhere to and fuse with sexual partner cells to undergo mating (orange pathway), or they can (ii) express adhesins for aggregation with other vegetative cells to form flocs/biofilms where they then reproduce asexually via budding (green pathway). (B) MAPK signaling cascade for regulation of mating genes in response to pheromone and for control of adhesin gene expression. Components required for mating and adhesin gene expression are shown in black, mating-specific proteins are orange. The position of the mitogen-activated protein kinases MAPKs in the signaling cascade is indicated. Tec1 is shown in green to indicate its specific requirement for vegetative adhesion. Ste12-binding sites (PRE) and Tec1-binding sites (TCS) are shown. The control of Tec1 protein stability by the TORC1 pathway and complex formation between Ste12, Tec1, Msa1 and Msa2 are indicated.*

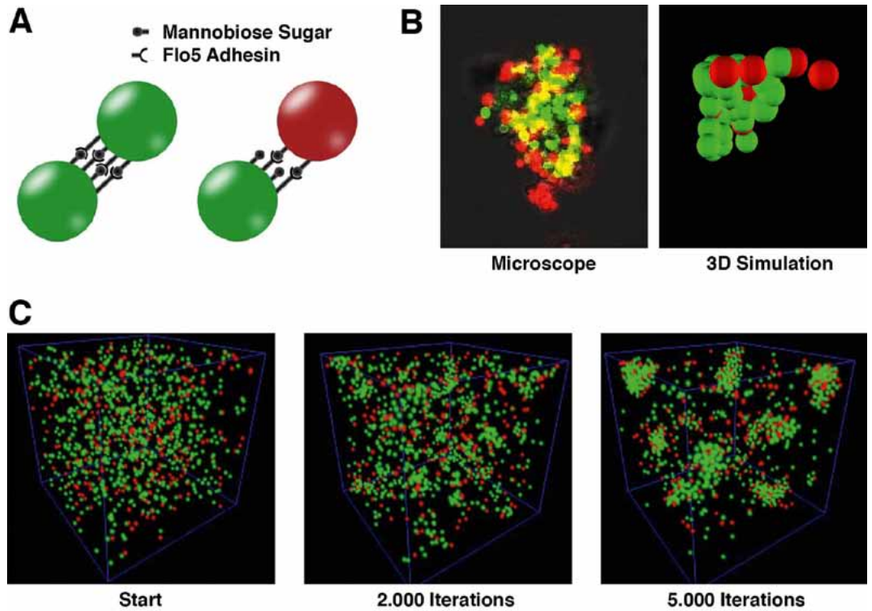

Figure 2. *Spatial analysis and simulation of yeast flocculation. (A) Illustration of cell-cell adhesion between two bearer cells that both present the mannobiose ligands and the Flo5 adhesin (green) or between a bearer cell and a cheater cell (red), which presents the ligand only. (B) Microscopic picture (left) and simulated 3D visualization (right) of a small floc consisting of a mixed population of bearer (green) and cheater (red) cells. (C) 3D real-time visualization of yeast flocculation with OpenCL showing screen shots after 0 (Start), 2.000 and 5.000 iteration steps, respectively.*

Ste12 and Tec1 activity. In collaboration with the group of Peter Lenz, we have also started to develop mathematical models to describe the activity of the transcription factors in response to activation of the upstream MAPKs. Our preliminary data indicate that our system is valid, because we find that the average data obtained by single cell measurements are comparable to previous biochemical measurements at population level. In our future efforts, we want to uncover significant cell-to-cell variation of MAPK activity and to analyze the contribution of different regulatory sub-circuits to the dynamic behavior of the MAPK pathway.

Spatial analysis and computer simulation of yeast flocculation

The multicellular development options of *S. cerevisiae* include the process of flocculation, which is the adhesion-dependent interaction among yeast cells leading to multicellular aggregates, so-called flocs. The ability to form flocs can provide an evolutionary benefit to a yeast population, for instance by protecting individual cells from harmful conditions in the environment. Importantly, flocculation is not only relevant for the study of fundamental biological questions, but also for biotechnological applications, for instance in the brewing and wine industry.
Within SYNMIKRO, we have established a fluorescence microscopy-based system for the precise spatial analysis of single cells within individual flocs (Fig. 2). In collaboration with the groups of Peter Lenz and Bernd Freisleben, we have further developed a detailed mathematical model for yeast flocculation, which allows us to analyze the dependence of this process on various relevant parameters *in silico*. To simulate our model process with acceptable runtimes, we have further developed a graphics processing unit (GPU) implementation that allows us to track the cell movement in a detailed manner by 3D visualization during execution (Leinweber *et al*, 2014). This GPU implementation is up to a factor of 736 faster than a multithreaded C/C++ implementation and allows simulating up to 1.000.000 yeast cells in total. Initial simulations have led to several theoretical predictions that can now be tested by appropriate *in vivo* experiments. Here, we will focus for example on the behavior of mixed populations, where defined fractions of cells do no produce the adhesion proteins required for flocculation. Our preliminary data indicate that such *cheater* cells are not only pushed to the boundary of the flocs, but are also restricted to specific sites within these protective multicellular structures.

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Modulation of DNA sequences via prokaryotic immune systems

Many bacteria and most archaea have simple immune systems, the so-called CRISPR-Cas systems, which provide adaptive immunity against mobile genetic elements like phages and conjugative plasmids. In these systems, specificity is conferred by the CRISPR arrays, repeated DNA sequences that are interspersed with spacer sequences representing fragments of such foreign DNA (Fig. 1). Transcription and processing of a CRISPR array yields small CRISPR RNA (crRNA) molecules that are then incorporated into a Cas ribonucleoprotein complex termed Cascade (type I) or Cas9 (type II). In case of a reinfection, these complexes utilize the base-complementarity between crRNA and foreign DNA to induce site-specific DNA cleavage. Only recently, the design of custom spacer sequences allowed for targeted DNA cleavage during genome engineering, and the type II protein Cas9 is now frequently used to change the sequence of bacterial and eukaryotic genomes *in vivo*. Our work within SYNMIKRO focuses on the processing, assembly and mechanism of three different type I Cascade complexes, and on constructing a synthetic minimal variant as a potential genome editing tool, which would be a smaller alternative to the Cas9 system.

An anti-crRNA for regulation

CRISPR/Cas systems are highly diverse – even though they all contain crRNA and ribonucleoprotein complexes, both their Cas protein composition and the way the CRISPR arrays are processed differ significantly. In our studies, we focus on the three CRISPR/Cas subtypes I-A (in *Thermoproteus tenax*), I-B (in *Methanococcus maripaludis* C5 and *Clostridium thermocellum*), and a minimal I-F variant (in *Shewanella putrefaciens*). To investigate the processing of these subtypes, we utilize a combination of computational, RNA-Seq and biochemistry approaches. E.g., RNA-Seq analyses verified transcription and maturation of crRNAs from all investigated CRISPR loci. Furthermore, the cleavage sites and the relative abundance of individual crRNA molecules were determined (Fig. 2) (Randau, 2012; Su *et al*, 2013). Our differential RNA-Seq approach with selective treatment of crRNAs with T4 polynucleotide kinase verified the presence of 5'-OH termini in crRNAs. Also, we identified the endonucleases responsible for crRNA maturation and reconstituted this reaction *in vitro*. For subtype I-B, we discovered and biochemically characterized a crRNA maturation endonuclease that we termed Cas6b. *M. maripaludis* Cas6b can form dimers in the presence of non-cleavable substrates and harbors two catalytic histidine residues in a general acid/base catalytic triad.

The profile of crRNA abundance for the five CRISPR loci found in *C. thermocellum* revealed several surprises (Zöphel *et al*, 2013): While promoters were already known to exist within a leader region upstream of the CRISPR clusters, we could show that a spacer sequence with a potential promoter element can stimulate internal transcription and dramatically raise the level of downstream crRNAs containing spacers which likely were acquired earlier in the organism's evolution. Moreover, spacer sequences were also shown to promote anti-crRNA transcription which could be used to regulate the abundance of potentially harmful crRNAs. Indeed, we found one striking example of such a problematic self-targeting crRNA that is complementary to a tRNA-Proline of the host *C. thermocellum*. Here, a second crRNA was identified that in turn is complementary to the tRNA-targeting crRNA, and that might act as an RNA sponge to remove the harmful crRNA. The mechanism of antisense RNA-mediated regulation of crRNA abundance will be investigated in the future - understanding the mechanisms that regulate crRNA abundance is pivotal to ensure that synthetic crRNAs are functional in the cell.

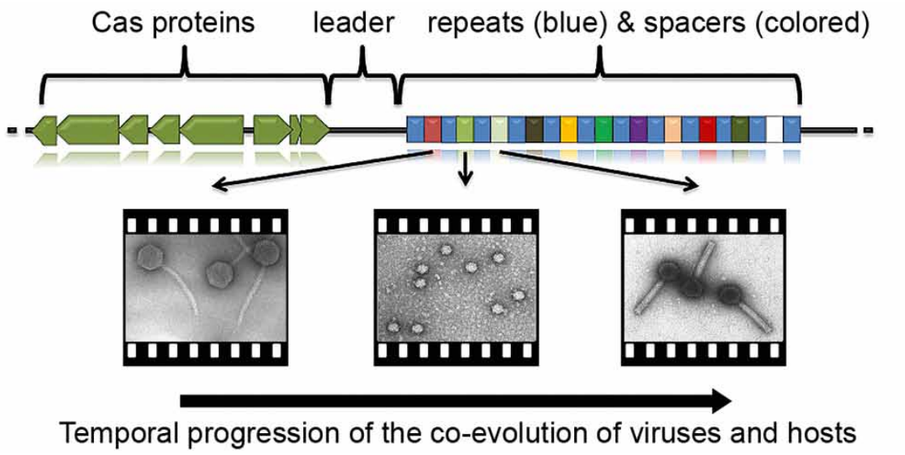

Figure 1. *Composition of CRISPR-Cas systems. A CRISPR-Cas system consists of genes coding for Cas proteins and a CRISPR array with repeat DNA elements and interspersed spacers that can be derived from previously encountered mobile genetic elements.*

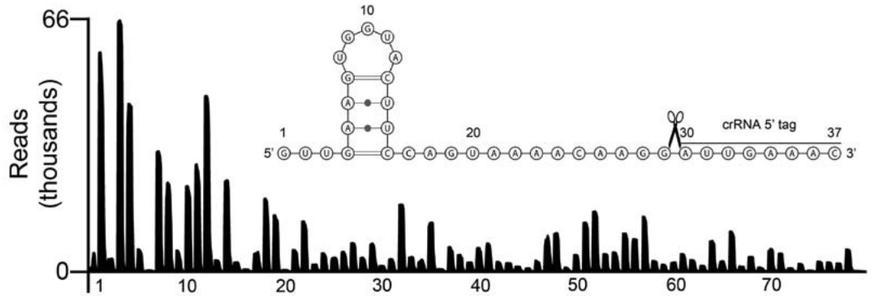

Figure 2. *RNA-Seq profile of a CRISPR cluster. A long C. thermocellum cluster displays abundance variability of individual crRNAs. The Cas6b cleavage site within the repeats is indicated.*

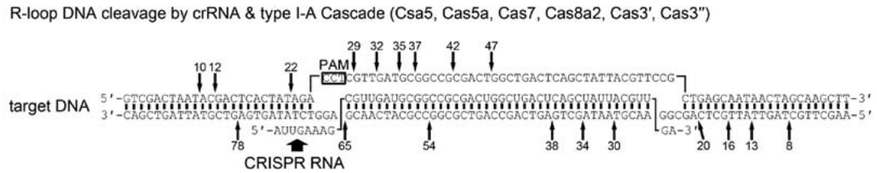

Figure 3. *DNA interference by a type I-A Cascade/crRNA complex. The T. tenax Cascade complex was reconstituted in the presence of crRNA and displayed target DNA cleavage (cleavage sites are indicated) in dependence of the correct protospacer-adjacent motif (PAM) and crRNA complementarity.*

Furthermore, our group aims to establish the functional reconstitution of Cascade effector complexes *in vitro,* and of a minimal Cascade effector complex in the heterologous host *Escherichia coli*. Here, the parallel analysis of three ribonucleoprotein complexes allows for the elucidation of mechanistic differences and similarities and enables us to investigate the function and evolution of individual Cas protein subunits. Until now, we have successfully assembled the first complete type I-A Cascade complex consisting of six Cas protein subunits and synthetic crRNAs (Plagens *et al*, 2014; Daume *et al*, 2014). This ribonucleoprotein complex was shown to be active and to cleave DNA in dependence of crRNA complementarity and a three nucleotide protospacer-adjacent motif (Fig. 3). Furthermore, we could show that the endonuclease responsible for target DNA cleavage, the Cas3 protein, is an integral part of the type I-A Cascade of *T. tenax*, whereas in the type I-E Cascade of, e.g., *E. coli*, Cas3 was shown to be a separate protein only temporarily recruited to the complex. Therefore, we hypothesize that this integral Cas3 is an adaptation to the mostly hot environments of organisms with CRISPR-Cas subtypes I-A. Also, the composition of a type I-B Cascade from *C. thermocellum* was identified, and all five Cas protein subunits can be produced as soluble recombinant proteins. Finally, a minimal type I-F system can be reconstituted from only four Cas protein subunits. Future studies will address how these systems differ in their recognition of the DNA targets using different subtype-specific Cas protein subunits. A minimal Cas3-guided interference complex might represent a viable alternative (with different target DNA specificity and restrictions) to currently popular Cas9-mediated genome editing methods.

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AWARDS & HONORS

2011 ERC Advanced Grant
2007 Chica und Heinz Schaller Research Award
2006 EMBO Young Investigator Award
1990 DAAD Scholarship

Design principles of microbial networks

All complex functions in a cell are executed by groups of interconnected proteins. Understanding the structure and operation of these protein networks is one of the next grand challenges in biology, and it is the first step to the rational design of novel synthetic networks. In our group, we combine tools of quantitative fluorescence microscopy with mathematical modeling in order to characterize the real-time functioning of networks in microorganisms. We are particularly interested in elucidating mechanisms behind evolutionary selected properties that are common to most networks, such as the capability to function robustly in a noisy environment, to detect and integrate multiple extra- and intracellular cues with high sensitivity, and to self-organize into macromolecular complexes within a cell. Ultimately, we would like to understand why the observed network designs were evolutionary selected out of a large number of possibilities, and to use such established principles of "evolutionary design" for synthetic biology applications.

Signal processing and integration by the bacterial chemotaxis network

Bacterial chemotaxis is one of the best-studied model systems for signal transduction. Chemotactic signals – e.g., nutrients like sugars – are perceived and processed by the chemosensory clusters and subsequently transmitted to flagellar motors (Fig. 1A). Over the last years, we used fluorescence resonance energy transfer (FRET) combined with computer modeling and simulations to quantify the function of the chemotaxis pathway in *Escherichia coli*. We applied FRET (Fig. 1B) to map all interactions within the pathway and to study their dynamics upon chemotactic stimulation (Kentner & Sourjik, 2009). These studies revealed how chemotactic stimuli are amplified through allosteric interactions between receptors within the chemosensory clusters. They also showed how multiple signals are integrated within the pathway, including such general physic-chemical stimuli as pH and temperature, and sugar-uptake related stimuli mediated by the cytoplasmic phosphotransferase system (PTS) (Neumann *et al*, 2012). Here, receptor interactions serve an additional function to couple the response and adaptation to different stimuli, which is necessary for optimal navigation in chemoeffector gradients (Krembel *et al*, 2014).
Currently, we investigate the relation between the chemotactic and metabolic preferences of *E. coli*, elucidating mechanisms that enable cells to optimally adjust chemotactic signaling dependent on their metabolic requirements and growth conditions. We also utilize the potential of signal amplification and integration within the chemotaxis pathway to develop novel biosensors that can sensitively detect a variety of chemicals such as xenobiotics, and their combinations.

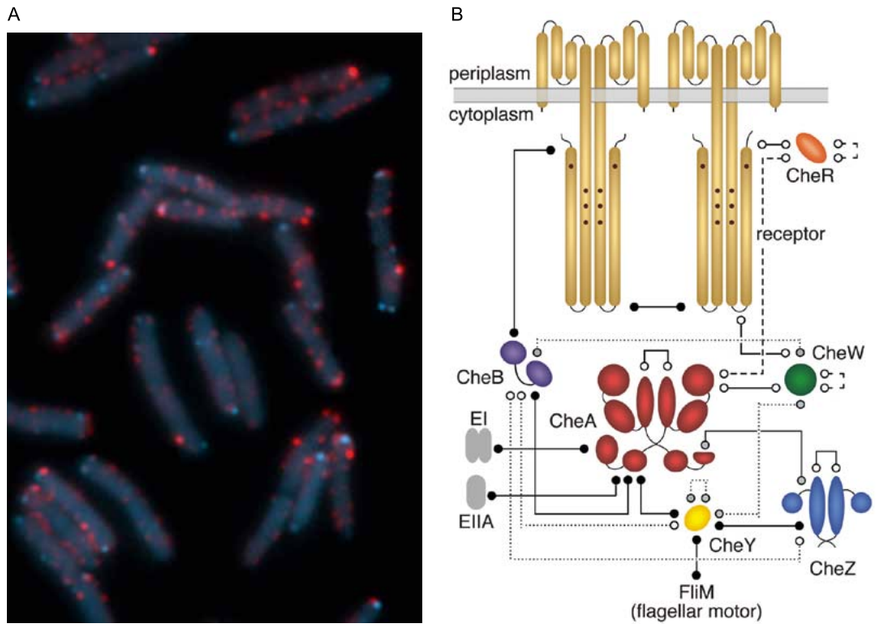

Figure 1. *Spatial organization and interaction map of the chemotaxis network in Escherichia coli cells. (A) Localization of chemosensory clusters, labelled by a CheR-CFP (blue), and of flagellar motors, labelled by FliM-YFP (red). (B) FRET-based interaction map of the E. coli chemotaxis pathway. Positive FRET pairs correspond to direct interactions (solid lines) or proximities (dashed or dotted lines) between chemotaxis proteins, flagellar motor (FliM) and PTS components EI and EIIA^{Glc}. Interactions that are independent of chemotactic stimulation are indicated by open circles, those dependent on chemotactic stimulation by filled circles.*

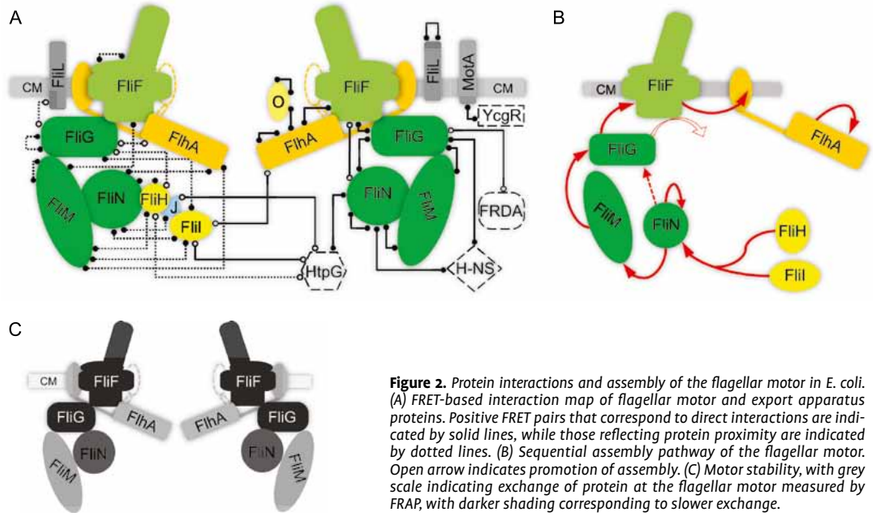

Figure 2. *Protein interactions and assembly of the flagellar motor in E. coli. (A) FRET-based interaction map of flagellar motor and export apparatus proteins. Positive FRET pairs that correspond to direct interactions are indicated by solid lines, while those reflecting protein proximity are indicated by dotted lines. (B) Sequential assembly pathway of the flagellar motor. Open arrow indicates promotion of assembly. (C) Motor stability, with grey scale indicating exchange of protein at the flagellar motor measured by FRAP, with darker shading corresponding to slower exchange.*

Robustness of signaling in chemotaxis

Robustness is believed to be one of the major underlying properties of cellular networks, but its implementation in specific cases remains poorly understood. We showed that signaling in chemotaxis is robust against stochastic variations in protein levels or gene expression noise, and demonstrated that this robustness is an evolved property of the pathway topology, being achieved through the balance of opposing enzymatic activities at the level of the pathway output. More recently, we have analyzed the robustness of the chemotaxis pathway against another common type of perturbation, namely variations in ambient temperature. We demonstrated that such thermal robustness is achieved through several compensatory mechanisms, primarily because similar temperature effects on opposing reactions cancel each other, but also due to the growth-temperature dependent adjustment of the enzyme levels (Oleksiuk *et al*, 2011). Currently, we apply a similar framework to analyze robustness of the mating pathway in the budding yeast *S. cerevisiae*. Finally, we have developed a general mathematical framework for the analysis of network robustness based on topology, which will be useful for the future design of robust synthetic networks.

Assembly and dynamics of macromolecular complexes

Another focus of our work is to understand general principles behind assembly and dynamics of macromolecular complexes in bacteria, using several well-studied protein networks and structures in *E. coli* as model systems. Thus far, we have studied the chemosensory complexes and flagellar motors, as well as the network of multiple chaperone systems that regulates refolding of protein aggregates. In all cases, we used a combination of FRET with imaging to elucidate the order of the structure assembly and the interactions involved in this process (Fig. 2A). Furthermore, we applied fluorescence recovery after photobleaching (FRAP) to explore the dynamics of these assemblies. For all of these structures, we demonstrated that the assembly proceeds through a well-defined sequence of steps, where formation of each subsequent structure stabilizes the entire assembly (Fig. 2B). We believe that such combination of the hierarchical order with gradual stabilization represents a general rule for the assembly of large macromolecular structures.
We further showed that both chemosensory clusters and flagellar motors consist of several subcomplexes with different stability (Fig. 2C), which ensures that the structure can be gradually recycled while retaining its function. Recently, we have further demonstrated that cellular assembly of the motor and of the chemosensory clusters is assisted by a molecular chaperone HtpG, a highly conserved member of the Hsp90 chaperone family that up to now has not been assigned a clear physiological function in bacteria (Press *et al*, 2013). Using FRET, we have also observed physical interactions between the flagellar motor and several other systems, such as membrane-embedded energy complexes or second-messenger binding proteins that directly tune bacterial motility dependent on environmental or growth conditions (Fig. 2A).

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1.2 RESEARCH
Metabolism

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2001–2002 Postdoctoral Fellow, University of Groningen, Netherlands
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AWARDS & HONORS

2013 ERC Starting Grant
2012 Research Award of the Association for General and Applied Microbiology (VAAM)
2008 Max Planck Research Group appointment
2006 VIDI grant by the Dutch Science Organization (NWO)
2003 VENI grant by the Dutch Science Organization (NWO)

Synthetic microbiology in *Archaea*

The central theme of our research is the biology of thermoacidophilic archaea from the genus *Sulfolobus* which grow optimally at 75˚C and a pH of 2-4. Mainly, we use genetic approaches to identify systems in *Sulfolobus* that are involved in the assembly of cell surface appendages like archaella, the "archaeal flagella", and to investigate how these appendages are regulated. Furthermore, we biochemically characterize the subunits and their interplay in the assembly process. As the growth conditions preferred by our model organisms are used for the pretreatment of (ligno)cellulosic waste before fermentation, we also engineer *Sulfolobus acidocaldarius* to streamline the fermentation process.

Sulfolobus strains for biofuel production from (ligno)cellulosic waste

Before (ligno)cellulosic waste can be fermented by engineered yeast strains, an expensive five step process ensures that it has the right composition to sustain yeast growth. One of these is an acid treatment at high temperature. However, *S. acidocaldarius* can grow at the conditions used for this pretreatment, and volatile products like alcohol would directly evaporate at such high temperature, which would circumvent the need to isolate alcohol resistant strains of *S. acidocaldarius*. Therefore, synthetic *Sulfolobus* strains will be constructed which can directly grow on (ligno)cellulosic waste at high temperature and can simultaneously convert it to volatile products. This would be a cost-effective alternative to the hitherto used process. In our lab, different cellulolytic enzymes and sugar transporters are thus tested for their capability to enable *S. acidocaldrius* to efficiently degrade (ligno)cellulosic waste and take up the obtained carbohydrates to channel the energy into the production of second generation biofuels. So far, strains were engineered that contain different cellulases from other *Sulfolobus* strains, which enabled *S. acidocaldarius* to use extracellular cellulose for growth. Moreover, a transcriptional regulator, termed MalR, was identified which is involved in the regulation of the α-amylase and the maltose ABC transporter gene expression (Wagner *et al*, 2013). We will now use the MalR controlled promoters to manipulate the expression of genes important for (ligno)cellulosic waste degradation.

An old structure with a new name: the archaellum

Like almost all other archaea, *Sulfolobus* has one cytoplasmic membrane that is covered by a proteinaceous layer, called the S-layer (Fig. 1). Inserted into the cytoplasmic membrane are the archaea's organs of locomotion: the archaellum and two types of pili. Archaellins, the subunits of the archaellum, have class III signal peptides. The ATPase FlaI and the integral membrane protein FlaJ in turn share high homologies with the respective components from bacterial type IV pili assembly machineries and type II secretion systems, whereas the bacterial flagellum is assembled by a type III secretion system. This and other marked differences led Ken Jarrell and our group to propose the name "archaellum" instead of "archaeal flagellum" (Jarrell & Albers, 2012).
Using thermomicroscopy, we could demonstrate that *S. acidocaldarius* uses its archaellum for swimming with speeds up to 15 µm/s. When tethered to a surface via their archaella, cells rotate, implying that the archaellum is rotating in the cell envelope. This is especially interesting in view of the fact that the archaellum is structurally type IV pilus-like.
To this point we have characterized four of the seven subunits of the *S. acidocaldarius* archaellum biochemically and structurally (Fig. 2). The soluble domain of the monotopic membrane protein FlaX was shown to form very stable ring-like oligomers with a diameter of 30 nm in single particle analysis (Banerjee *et al*, 2013). The structure of FlaI, the only active ATPase in the archaellum operon, showed a hexameric organization with large conformational changes dependent on the nucleotide-bound state. In contrast to other type IV pili assembly ATPases, FlaI is bifunctional: It first serves as an assembly ATPase and, after a so far unknown switch, it is essential for archaellum rotation (Reindl *et al*, 2013).
FlaH is a predicted ATP-binding protein as it has a Walker A motif, but a non-canonical Walker B motif. As we could also show by single particle analysis and biochemical interaction studies that FlaX, FlaH and FlaI interact with each other with high affinity, we propose that these three proteins form the cytoplasmic motor complex of the archaellum (Banerjee *et al*, 2014).
The crystal structure of the monotopic membrane protein FlaF was dominated by β-sheets. As we had so far only identified cytoplasmic components of the archaellum, we hypothesized that FlaF might be involved in anchoring the archaellum in the cell envelope. As almost all extracellular proteins in archaea are N-glycosylated, we tested FlaF for sugar binding and could demonstrate that it binds chitobiose, the central sugar of the N-glycan of *S. acidocaldarius*, with high affinities. Therefore, FlaF might act as the stator of the archaellum.

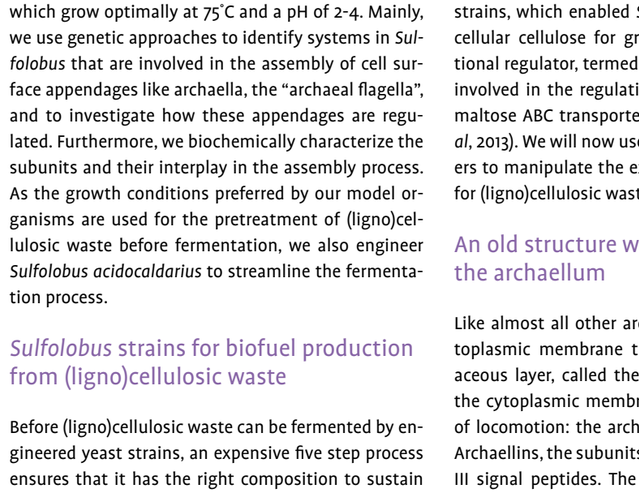

Figure 1. *Model of the cell envelope of S. acidocaldarius. After insertion into the membrane, the archaellins or pilins are processed by PibD, a class III signal peptidase, and assembled into either the archaeal adhesive pili (aap), the UV induced pili (ups) or the archaellum.*

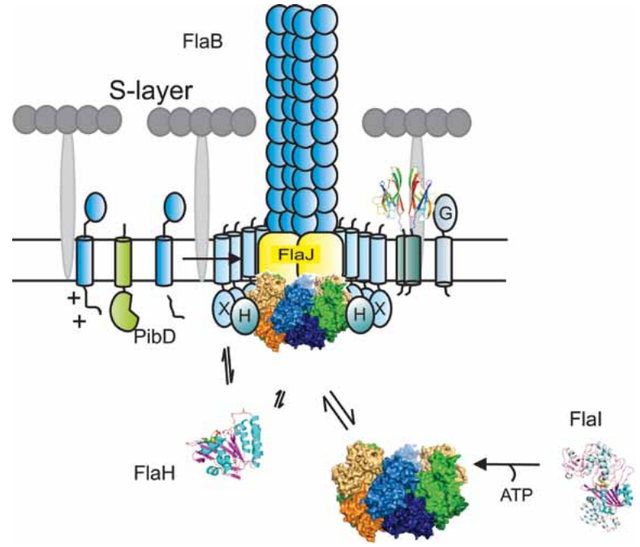

Figure 2. *Current model of the S. acidocaldarius archaellum. FlaI hexamerizes upon ATP binding and interacts with FlaH and FlaX in the motor complex. FlaF is probably acting as a stator as it binds to the N-glycans of the S-layer and thereby anchors the archaellum in the cell envelope.*

Role of UV induced pili in gene transfer

All *Sulfolobales* have a UV inducible pili system that causes the cells to aggregate after UV irradiation – in *S. acidocaldarius*, up to 90% of the cells can be found in aggregates 3 hours after UV stress. Using a conjugation assay, we could demonstrate that the cells in these aggregates exchange DNA. Therefore, we hypothesized that these systems might be part of a community effort to repair DNA damage by homologous recombination. Using specific FISH probes, we demonstrated that the aggregation in mixed cultures containing different *Sulfolobus* species is strain-specific. This fact of course makes sense if UV-induced DNA exchange is indeed used as an alternative DNA repair mechanism.

Biofilm formation by *Sulfolobus* species

Although it is known that archaea are present in virtually all environmental niches and also have been described in natural mats or communities, surprisingly little is known about the molecular details of community formation in archaea. Therefore, we have established *Sulfolobus* species as a model system to identify the molecular drivers of archaeal biofilm formation. An –omics approach indicated Lrs14-like regulators as possible players in this process. *S. acidocaldarius* contains six Lrs14 regulators in its genome, four of which had an effect on biofilm formation. By far the highest influence had AbfR1 that acts as an inhibitor of biofilm formation by inducing archaella biosynthesis and inhibiting EPS production (Orell *et al*, 2013). Deletion of AbfR1 therefore led to a high increase of EPS production and subsequent community formation. Moreover, it was shown that AbfR1 is phosphorylated *in vivo*, and ongoing experiments will clarify whether this phosphorylation is involved in the regulatory mechanism of AbfR1.

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Banerjee A, Ghosh A, Mills DJ, Kahnt J, Vonck J, Albers SV (2013). FlaX, a unique component of the crenarchaeal archaellum, forms oligomeric ring-shaped structures and interacts with the motor ATPase FlaI. *J Biol Chem* 287, 43322-30.

Jarrell KF & Albers SV (2012). The archaellum, an old structure with a new name. *Trends Microbiol* 20, 307-12.

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AWARDS & HONORS

2008–2017 Fellow of the Max Planck Society
2011 and **2008** Visiting Professor at the University of Kyoto, Japan
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Anaerobic biofactories for valuable carboxylic acids

The amino acids occurring in proteins are the essential building blocks of life and are synthesized by almost all organisms. Removal of the amino group from amino acids yields carboxylic acids, which are used as fungicides (propionate) and antioxidants (indolepropionate), or as technically important precursors for the syntheses of polymers and pharmaceuticals (succinate, glutaconate, glutarate, adipate, 3-hydroxypropionate, 4-hydroxybutyrate). To produce carboxylic acids from the renewable resource glucose, the amino acid biosynthesis route has to be diverted in such a way that the 2-oxoacid precursor is not converted to the amino acid, but reduced to the desired carboxylic acid. Thus, the pathway from glucose via oxaloacetate to the amino acid aspartate has been redirected to succinate by the chemical company BASF, already on a 1000 tons scale. However, enzymes that catalyze the reduction of oxaloacetate to succinate are widespread, whereas the production of the other carboxylic acids, which have no essential biological function, requires enzymes that only occur in some strict anaerobic clostridia. The key enzymes of these transformations are the oxygen-sensitive (*R*)-2-hydroxyacyl-CoA dehydratases that need an unpaired electron for catalysis, which is introduced by an ATP-dependent electron transfer from reduced ferredoxin (Buckel *et al*, 2014).

Recombinant production of glutarate and adipate

We engineered *Escherichia coli* into a glutaconate producer by introducing six genes from strict anaerobic glutamate fermenting clostridia, one gene for (*R*)-2-hydroxyglutarate dehydrogenase, two genes for glutaconate CoA-transferase, and three genes for (*R*)-2-hydroxyglutaryl-CoA dehydratase (Fig. 1) (Djurdjevic *et al*, 2011). Using its own enzymes, *E. coli* converts glucose to 2-oxoglutarate, which is then taken over by the recombinant enzymes to be reduced and dehydrated to glutaconate. Alternatively, reduction of the intermediate glutaconyl-CoA would lead to glutaryl-CoA and further to glutarate (Fig. 1). The same enzymes may be applied to convert 2-oxoadipate to adipate, one constituent of Nylon® (Parthasarathy *et al*, 2011). The reductions of glutaconyl-CoA to glutaryl-CoA and of homoglutaconyl-CoA to adipyl-CoA could be catalyzed by glutaryl-CoA dehydrogenase from *Syntrophus aciditrophicus* (Gdh_{Sa}). The reaction requires the bifurcating electron transferring flavoprotein (Etf_{Sa}) to direct the electrons from NADH to Gdh_{Sa}. We have produced recombinant Gdh_{Sa} and currently try to improve the formation Etf_{Sa} in *E. coli*.

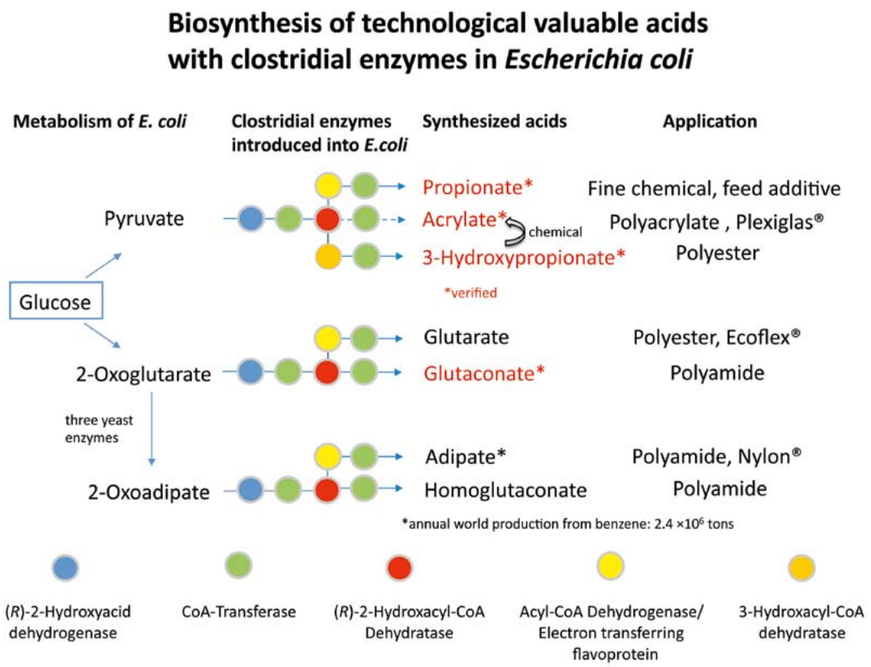

Figure 1. *Biosynthesis of biotechnological valuable acids in recombinant Escherichia coli strains. The figure demonstrates the central role of (R)-2-hydroxyacyl-CoA dehydratases in these conversions.*

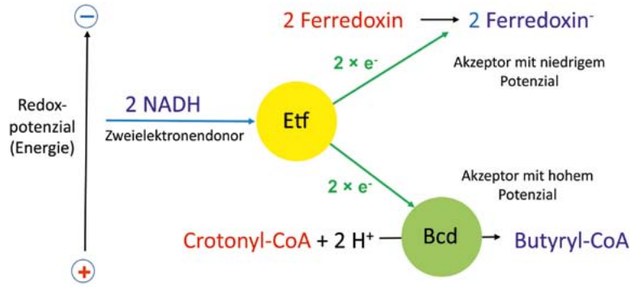

Figure 2. *Schematic presentation of electron bifurcation. Etf, electron transferring flavoprotein; Bcd, butyryl-CoA dehydrogenase. The figure shows how the two electrons from NADH bifurcate, whereby the 'energy rich' reduced ferredoxin is formed.*

Flavin based electron bifurcation

We studied this novel energy conserving process in more detail and characterized the bifurcating Etf_{Af} and butyryl-CoA dehydrogenase (Bcd_{Af}) from *Acidaminococcus fermentans* which couple the exergonic reduction of crotonyl-CoA to butyryl-CoA (equation 1) to the endergonic reduction of ferredoxin (Fd, equation 2) both with NADH (Fig. 2) (Chowdhury *et al*, 2014).

(1) Crotonyl-CoA + NADH + H^+ = Butyryl-CoA + NAD^+; $\Delta G' = -52$ kJ mol^{-1}.
(2) 2 Fd + NADH = 2 Fd^- + NAD^+ + H^+; $\Delta G' = +42$ kJ mol^{-1}

Etf_{Af} contains one flavin-adenin-dinucleotide (FAD) in subunit α (α-FAD) and a second FAD in subunit β (β-FAD). NADH reduces β-FAD to β-$FADH^-$ which is considered as the bifurcating cofactor. One electron goes to α-FAD and yields a stable anionic semiquinone, α-$FAD^{\bullet -}$, which donates this electron further to δ-FAD of Bcd_{Af}. The remaining non-stabilized neutral semiquinone, β-$FADH^{\bullet}$, immediately reduces ferredoxin. Repetition of this process affords a second reduced ferredoxin and δ-$FADH^-$ of Bcd_{Af} that converts crotonyl-CoA to butyryl-CoA. The reduced ferredoxin is used for many reductive processes including the production of molecular hydrogen or the formation of an electrochemical Na^+ gradient mediated by a membrane bound ferredoxin-NAD^+ reductase, whereby NADH is regenerated. If glutaryl-CoA dehydrogenase together with Etf_{Sa} works like Etf_{Af} + Bcd_{Af} and both enzymes are introduced in the glutaconate producing *E. coli* strain, then the conversion of glucose to glutarate might follow the very simple equation: Glucose = $Glutarate^{2-}$ + 2 H^+ + CO_2 + 2 H_2.

Enzyme redesign guided by cancer-derived mutations

For the synthesis of adipate, one essential enzyme has to be constructed by mutagenesis. It should catalyze the specific NAD(P)H-dependent reduction of 2-oxoadipate to (*R*)-2-hydroxyadipate without action on 2-oxoglutarate. It was reported earlier that the blood of patients suffering from various types of cancer contains high levels of (*R*)-2-hydroxyglutarate, which most likely inhibits its demethylation of the DNA. In these patients a mutation has converted the isoenzyme 1 of isocitrate dehydrogenase to a (*R*)-2-hydroxyglutarate dehydrogenase. Hence this mutant enzyme catalyzed the reduction of 2-oxoglutarate with NADPH rather than the oxidation of (2*R*,3*S*)-isocitrate with $NADP^+$. We speculated therefore that a similar mutation could convert homoisocitrate dehydrogenase to the desired highly specific (*R*)-2-hydroxyadipate dehydrogenase (2-oxoadipate reductase) (Parthasarathy *et al*, 2011). Based on our idea, this experiment was indeed successfully performed by Reitman *et al*. in 2012. The *Süddeutsche Zeitung* reported this result, and a Californian journalist asked me for an interview.

Propionate, 3-hydroxypropionate (3HP) and indolepropionate

The introduction of (*R*)-lactyl-CoA dehydratase from *Clostridium propionicum* converted *E. coli* either to a propionate or to a 3-hydroxypropionate (3HP) producer (Fig. 1). 3HP is a technically very important acid because it can be polymerized or easily dehydrated to the very useful compound acrylate. The microflora of the human intestine reduces tryptophan to indole-3-propionate, which is excreted into the blood. The propionate side chain allows indolepropionate to cross the blood-brain barrier and the indole moiety protects the brain from reactive oxygen species (ROS), a causative agent of Alzheimer disease. Recently we have shown that *Clostridium sporogenes*, an inhabitant of the intestine, converts tryptophan to indolepyruvate which is reduced and dehydrated further to indolepropionate in an identical manner as pyruvate to propionate (Fig. 1).

4-Hydroxybutyrate

4-Hydroxybutyryl-CoA dehydratase is a wide spread radical enzyme that catalyzes the reversible dehydration of 4-hydroxybutyryl-CoA to crotonyl-CoA. Since crotonyl-CoA is easily derived from acetyl-CoA, the now recombinant available enzyme from *Clostridium aminobutyricum* opens a way to synthesize 4-hydroxybutyryl-CoA from acetate or glucose (Zhang *et al*, 2015). 4-Hydroxybutyryl-CoA can be polymerized to poly-4-hydroxybutyrate or reduced to 1,4-butanediol that is currently produced from oil on a million tons scale.

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Chowdhury NP, Mowafy AM, Demmer JK, Upadhyay V, Koelzer S, Jayamani E, Kahnt J, Hornung M, Demmer U, Ermler U, Buckel W (2014). Studies on the mechanism of electron bifurcation catalyzed by electron transferring flavoprotein (Etf) and butyryl-CoA dehydrogenase (Bcd) of *Acidaminococcus fermentans*. *J Biol Chem* 289, 5145-57.

Zhang J, Friedrich P, Pierik AJ, Martins BM, Buckel W (2015). Substrate induced radical formation in 4-hydroxybutyryl-CoA dehydratase from *Clostridium aminobutyricum*. *Appl Environ Microbiol* 81, 1071-84.

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1985 Ph.D. in computer science, Technische Universität Darmstadt
1981–1982 Research Scientist, IBM Research and Development, Böblingen
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AWARDS & HONORS

2007–2014 Best Paper Awards (IAS'07;CCGRID'08;AINA'09;CEC'11; WETICE'14)
2008 IBM Real-time Innovation Award
2007 IBM UIMA Award
2005 IBM Eclipse Innovation Award
1986 IEEE Award for Outstanding Achievement

In silico methods for modeling, simulation and data analysis

Mathematical modeling, computer-based simulation and data analysis are useful for studying intra- and intercellular processes of microorganisms in order to introduce novel synthetic variants. On the downside, these methods often require high computational runtimes and storage capacities. Our group develops efficient algorithms to significantly speed up such computations and reduce the complexity of handling "big data" in a biological context (Dalman *et al*, 2013; Haunschild *et al*, 2005). In collaboration with other SYNMIKRO research groups, we work on (a) parallel software tools for large-scale structural analyses of protein binding sites to predict protein function and perform similarity retrieval, and (b) parallel simulation tools for studying the spatial organization and temporal assembly dynamics of multicellular growth forms.

Figure 1. *GPU-based Cloud computing for structural analysis of protein binding sites: sequential C++ program, parallel OpenCL program, single GPU execution, multi GPU-based Amazon Cloud execution.*

Efficient structural analysis of protein binding sites

A major goal in synthetic biology is the manipulation of the genetic setup of living cells in order to alter existing and introduce novel biochemical pathways. A prerequisite is a deep understanding of the biochemical function of the proteins of interest. Since assessing protein function experimentally is time-consuming and sometimes infeasible, protein function prediction is a central task in computational biology. Typically, the function of a protein is inferred from similar proteins with known functions, most prominently by sequence comparison, owing to the observation that

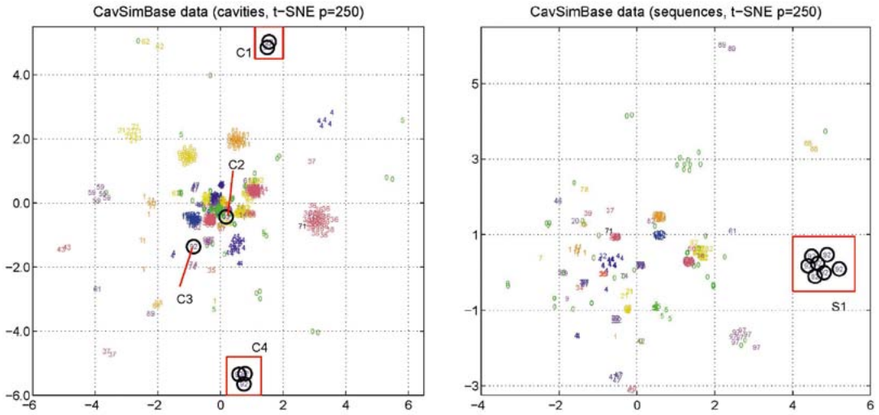

Figure 2. *Top: Embeddings of serine protease data sets based on structural information of binding site geometries (left) and sequence alignments (right) for t-SNE. Bottom: Protein structure images related to two cavity-based t-SNE locations C2:2F6I (left) and C4:1TYF(right) taken from the protein data bank (http://www.rcsb.org/pdb/).*

proteins with an amino acid sequence similarity larger than 40% tend to have similar functions. Yet, below this threshold, results of sequence comparisons often become uncertain.
In this case, a structural comparison can provide further insights, especially when focusing on functionally important regions, such as protein binding sites. Several structural comparison algorithms are known, but they have much longer runtimes than their sequence-based counterparts, severely limiting their use in large-scale comparisons. One option to overcome this obstacle is to pre-compute pairwise similarities of protein binding sites in an all-against-all comparison, and to subsequently make them accessible to data analysis methods. Pairwise similarities can also be used to establish a neighborhood structure to perform similarity retrieval more efficiently. Unfortunately, an all-against-all comparison of the roughly 250.000 binding sites contained in CavBase, a database of protein-ligand data maintained by the Cambridge Crystallographic Data Center, would require 297 years of computation time on a single CPU core of a contemporary computer. Selecting a biologically meaningful subset of about 144.000 binding sites would still require about 100 years of computation time.
By using a novel comparison algorithm, accelerating the algorithm on graphical processing units (GPU) and parallelizing the computations in a GPU-based Cloud infrastructure of a set of Amazon EC2 Cluster GPU Instances (Fig. 1) (Leinweber *et al*, 2012), 24 days instead of 100 years were required. This large-scale study, performed in collaboration with the groups of Gerhard Klebe and Eyke Hüllermeier, resulted in a similarity database called CavSimBase (Fig. 2). Analyzing the similarity relation in its entirety requires methods for dealing with "big data". This is part of our current work, and we invite the community to participate in this endeavor. CavSimBase can be accessed at: http://pc12872.mathematik.uni-marburg.de/.

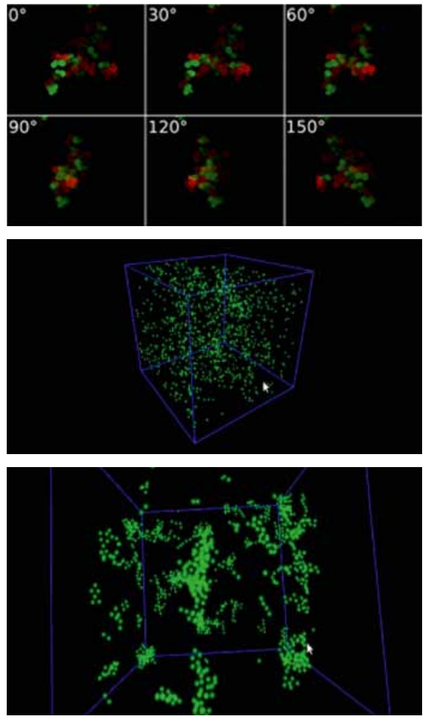

Figure 3. *Top: Microscopic image of a small floc consisting of a mixed population of bearer (green) and cheater (red) cells. Middle and Bottom: 3D realtime visualization of yeast flocculation showing screen-shots after the start, and after a few iteration steps, respectively.*

Fast computational modeling of yeast cell flocculation

Cells of the baker's yeast *Saccharomyces cerevisiae* interact with each other to form multicellular aggregates, called flocs. This reversible asexual interaction is calcium-dependent and provides an evolutionary benefit for a yeast population, e.g., as a protection against environmental stress. Flocculation also is a fast, cost-effective and environment-friendly way to remove yeast cells at the end of fermentation processes in the production of beer, wine, ethanol, biodiesel or pharmaceutical compounds. In addition, flocculent yeast strains that effectively bind calcium ions have been used in bioremediation to remove other divalent ions, such as heavy metals, from contaminated sites.
Unfortunately, experimental optimization and control of flocculation is time-consuming and cost-intensive. Therefore, an understanding of the dynamics and structure of flocculation offers great benefits for several industrial applications. A novel model describing yeast flocculation depending on the cell surface adhesin protein Flo5 has been proposed by the SYNMIKRO research groups of Peter Lenz and Hans-Ulrich Mösch. This kind of flocculation is mediated by the heterophilic interaction between the Flo5 protein of one yeast cell and mannobiose sugars in the cell wall of other yeast cells. The model can be used to perform simulation studies *in silico* and recommend biotechnological adjustments for optimizing the production strains *in vivo*.
To significantly reduce the runtimes of simulations, a GPU implementation of the model has been developed in our work (Leinweber *et al*, 2014). Experimental results indicate that the GPU implementation is up to 736 times faster than a multithreaded C/C++ implementation on a multi-core workstation for simulations of up to 20.000 yeast cells. Moreover, it requires only up to 225 milliseconds to simulate up to 1.000.000 yeast cells. Finally, the quality of the simulation is improved, since the side effects of simulations with limited cell numbers can be reduced, and second-nearest neighbor interaction can be modeled to increase the simulated level of detail (Fig. 3).
Our current work focuses on improving the model and finding optimal parameters to reproduce the natural behavior of yeast cells during flocculation. The algorithms for parameter optimization are designed to run in parallel on a large computing cluster of GPUs.

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Synthetic pathways for production of aromatic compounds

Microorganisms harbor a multitude of biochemical pathways which are involved either in the biosynthesis or in the degradation of chemical compounds. The amount of naturally occurring chemicals produced or degraded by bacteria and archaea exceeds anything eukaryotic organisms are capable of. In addition to this impressive metabolic variety, it is observed that microbes can even adapt to the degradation of most xenobiotics introduced into the environment. Our group studies the biochemistry behind the bacterial degradation of recalcitrant substrates like aromatics or hydrocarbons under anaerobic conditions. Whereas the presence of oxygen allows relatively easy degradation of these compounds even by eukaryotic organisms, their degradation under anaerobic conditions is limited to bacteria and archaea. Since all known aerobic degradation pathways of these compounds include oxygen-dependent enzymes, anaerobic degraders have developed completely new and surprising solutions to overcome difficult reactions and have evolved novel pathways connecting these unusual enzymic reactions. We are making use of the enzymes of these newly identified pathways for the rational design of completely new artificial biosynthetic pathways for interesting bioproducts, using methods of synthetic biology. Our main resource organisms are denitrifying bacteria of the genera *Thauera* and *Aromatoleum*, $Fe^{(III)}$-reducing *Geobacter* species or sulfate-reducing bacteria capable of degrading aromatic or aliphatic hydrocarbons as sole substrates.

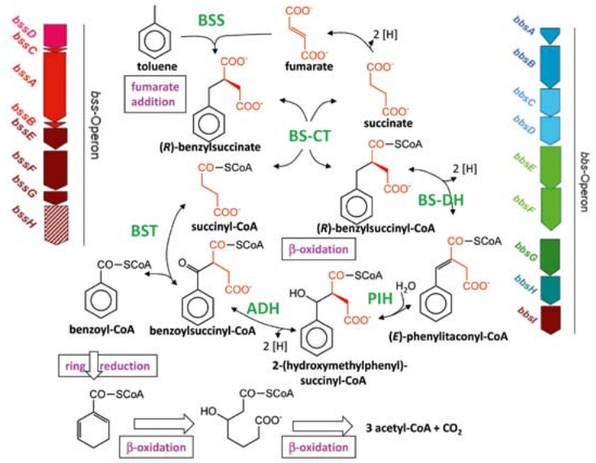

Figure 1. *Pathway of the anaerobic toluene metabolism. The first reaction is catalyzed by benzylsuccinate synthase (BSS), followed by five enzymes of a b-oxidation module for benzylsuccinate to benzoyl-CoA (bbs operon): BS-CT= benzylsuccinate CoA-transferase, BS-DH= benzylsuccinyl-CoA dehydrogenase, PIH=phenylitaconyl-CoA hydratase, ADH=alcohol dehydrogenase, BST=benzoylsuccinyl-CoA thiolase. The successive metabolic modules for further degradation of benzoyl-CoA are also indicated.*

Modularity of biochemical pathways

All biochemical pathways are designed in a modular fashion and lead through a series of common intermediates, which are interconverted by various cascades involving successive enzyme reactions. The enzymes involved in such modules are very often encoded in common operons in bacteria, which eases their coordinate regulation. As an example, the anaerobic degradation of toluene is initiated by an unusual reaction adding the methyl group to a fumarate cosubstrate to yield the first intermediate (*R*)-benzylsuccinate. This reaction is catalyzed by the extremely oxygen-labile glycyl-radical enzyme benzylsuccinate synthase (BSS), which represents the first module of the pathway and is encoded in a common operon with an activating enzyme (BssD) necessary to create the active radical-containing form of BSS (Fig. 1). The next module of this anaerobic toluene metabolism is represented by five successive enzymes involved in activation of benzylsuccinate and its subsequent degradation to benzoyl-CoA and succinyl-CoA by a specialized β-oxidation pathway (Fig. 1). Benzoyl-CoA is the common intermediate of anaerobic degradation of any aromatics and is also generated from many other substrates. For example, the metabolic module leading from benzoate to benzoyl-CoA consists of a benzoate transport system and a CoA-ligase or CoA-transferase as activating enzyme (Fig. 2). The further degradation of benzoyl-CoA involves benzoyl-CoA reductase, another highly unusual enzyme reducing the aromatic ring, and several additional β-oxidation modules involved in modifying and cleaving the dearomatised ring and further degrading the open-chain intermediates to acetyl-CoA and CO_2 (Fig. 1).

Re-design of the metabolic routes of benzylsuccinate

We were attracted by the observation that the unusual metabolite benzylsuccinate occurs in the pathway exclusively as the (*R*)-enantiomer. Like any derivative of the important platform chemical succinate, benzylsuccinate promises a variety of potential uses, e.g., for the synthesis of polymers or as building block for further chemical synthesis. It can also be synthesized chemically, but is rather costly and usually only obtained as racemic mixture of both enantiomers. As seen in Fig. 1, there are two principal ways of designing a biochemical pathway towards the synthesis of benzylsuccinate, using the enzymes of anaerobic toluene metabolism: it can either be approached from toluene and fumarate via the BSS reaction, or from benzoate and succinate, using the enzymes of the β-oxidation module in reverse. Because of the extreme lability of BSS and our previous observations that all enzymatic reactions of benzylsuccinate degradation are principally reversible *in vitro*, we tried to convert the degradation pathway of benzylsucciante into a new backwards-moving synthetic pathway (Fig. 2).

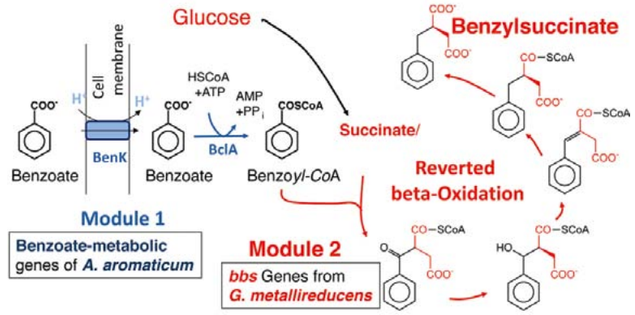

Figure 2. *Synthetic pathway for benzylsuccinate production in E. coli. The modules for benzoyl-CoA production and its conversion to benzylsuccinate are shown, assuming intrinsic succinate generation via fermentative growth conditions.*

Because the intended new synthetic pathway starts with benzoyl-CoA rather than benzoate, we constructed it in a modular fashion containing the genes for benzoate uptake and activation as module I and the genes coding for the benzylsuccinate-degrading enzymes from *Geobacter metallireducens* as a second module on two compatible inducible plasmids that were introduced into *Escherichia coli* as heterologous host (Fig. 2). The transformed cells were grown under either aerobic or anaerobic conditions with either benzoate and succinate as precursor compounds, or benzoate only, as *E. coli* produces succinate in its mixed-acid fermentation when grown anaerobically. Under aerobic conditions, very little benzylsuccinate was identified in the supernatant of induced cultures that were supplemented with benzoate and succinate as precursor molecules, whereas an up to 1000-fold increased benzylsuccinate production was observed under anaerobic growth conditions. Therefore, it appears possible to further optimize this type of synthetic pathways to redirect the metabolism of *E. coli* to produce suitable amounts of "alien" metabolites which may be as diverse as the different existing degradation pathways in nature.

Further developments of synthetic pathways

In our ongoing project, we are modifying further metabolic pathway modules in different directions. The already established modules for synthesizing benzoyl-CoA from externally supplied benzoate, e.g., may be generally useful for the synthesis of many different complex aromatic compounds via benzoyl-CoA as building brick. To prove this point, we are trying to combine this with biphenyl synthase (BIS), a different benzoyl-CoA-metabolizing enzyme from rowan berry trees. This enzyme belongs to the chalcone synthase family and condenses a benzoyl-CoA starter molecule with three malonyl-CoA units to a polyketide, which is then converted to the secondary metabolite 3,5-dihydroxybiphenyl (Fig. 3).

In another extension project, we intend to use enzymes of the anaerobic degradation pathway of indoleacetate to design a similar synthetic pathway leading to phenylsuccinate instead of benzylsuccinate (Fig. 3). This would show the general applicability of our strategy to use degradation pathways based on β-oxidation in reverse for the design of novel synthetic routes.

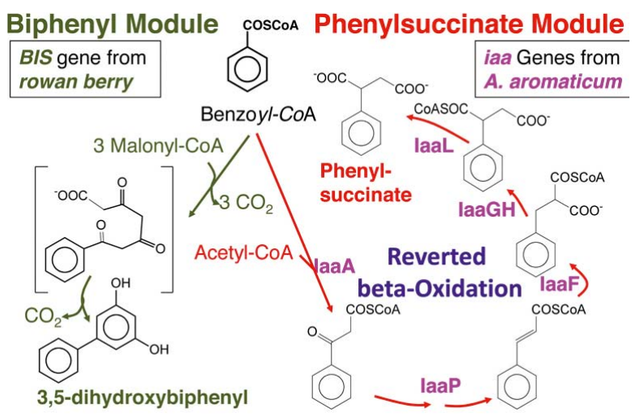

Figure 3. *Additional planned synthetic pathways for aromatic compound synthesis from benzoate. IaaL=thiolase, IaaP=hydratase/dehydrogenase, IaaF=acyl-CoA dehydrogenase, IaaGH=B12-dependent mutase, IaaL=CoA-transferase, all involved in anaerobic indoleacetate degradation; BIS=biphenyl synthase.*

SHORT CV

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1992 Ph.D. in biology, Ludwig-Maximilians-Universität München
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1991 Habilitation, Universität Heidelberg
1983 Research Scientist, University of Bern, Switzerland
1982 Research Scientist, University of Frankfurt/Main
1981 Visiting Scientist, CNRS and Institute Laue-Langevin, Grenoble, France
1982 Ph.D. in physical chemistry, University of Frankfurt/Main
1978 Diploma in chemistry, University of Frankfurt/Main

AWARDS & HONORS

2012 Carl Mannich Medal of the German Pharmaceutical Society
2011 ERC Advanced Grant
2008 Novartis Lecture Prize
1999 Literature price of the German chemical industry association (VCI) for the text book „Wirkstoffdesign“ together with Prof. Dr. H.-J. Böhm and Prof. Dr. H. Kubinyi
1975–1982 Scholar of the „Studienstiftung des deutschen Volkes“

Structural basis of protein-ligand and protein-protein recognition

The research of the Drug Design Group within SYNMIKRO is concerned with the question how small molecule ligands are recognized by macromolecular receptors. Usually, these small molecules are substrates, inhibitors, regulators, or modulators of the function of the target proteins, and the understanding of the recognition processes involved is a prerequisite for the rational design of ligands meant to influence the function of the addressed proteins. In pharmaceutical research, such interactions are used to develop new lead structures for putative drugs. In chemical biology, these techniques aim for the development of chemical probes to interfere in a tailored fashion with the properties of bio-macromolecules. In synthetic microbiology, the targeted interference with protein-ligand recognition is oriented towards a better understanding of metabolic pathways, the minimal requirement of proteins necessary for cellular functions, and the reprogramming of cellular networks. A second focus of our work is the modification of enzyme active sites, which is of relevance, e.g., for the production of pharmacologically interesting compounds by biological system. In order to modify active sites in a way that they can yield other products of relevance for a later pharmaceutical application, a detailed understanding and appropriate modeling techniques to simulate and predict the properties of enzyme variants with altered substrate specificity are required.

In silico simulations of binding pockets

The Drug Design Group tries to assemble a solid knowledge base of how proteins recognize their ligands, and particularly of thermodynamic data correlated with structural information (Klebe, 2015). As experimental techniques, crystallography and microcalorimetry are used along with other biophysical methods such as surface plasmon resonance, thermal stability measurements and micro thermophoresis. The structural information is then linked via computer simulations that try to predict binding properties and dynamics of protein-ligand complexes - the group has developed a number of well-known tools used worldwide to analyze and predict binding characteristics. In particular, methods to analyze and compare proteins and protein binding sites have been released. In close collaboration with the Knowledge Engineering & Bioinformatics Group of Eyke Hüllermeier, our group developed a number of tools to compare protein binding pockets (Fig. 1). Searches across the entire space of protein-exposed binding pockets (> 250.000 entries) can now be accomplished within seconds (Fober *et al*, 2011; Fober *et al*, 2012). This approach allows us to compare proteins with regard to their function, independent of sequence and fold similarity. Such comparisons help to elucidate, e.g., putative cross-reactivities and undesired side effects of existing and putative drug molecules in the field of pharmaceuticals. In the area of synthetic biology, proteins of unknown function can be annotated with respect to binding site similarities of catalytic centers, or proteins can be searched for that possibly catalyze and process similar substrates. Such comparisons can indicate redundancies in the function of proteins, an aspect that could be of interest when genes are knocked out, but their loss-of-function is compensated by the up-regulation of redundant pathways.

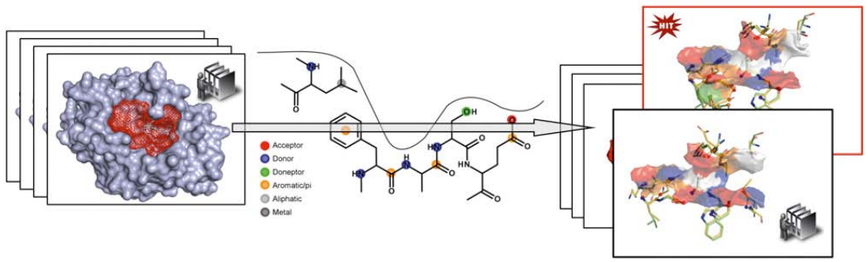

Figure 1. *A database holding more than 250,000 binding pockets has been created. Each pocket is characterized in terms of assigned pseudocenters that describe the exposed physicochemical properties of the binding pocket. The spatial distribution of the pseudocenters is subsequently used to compare individual binding pockets among each other. This approach allows to find functional relationships or putative cross-reactivity between proteins independent of any sequence or fold similarity.*

Fragment-based lead discovery and the influence of water on protein interactions

In order to find new lead structures for the development of a drug, our group is heavily engaged in establishing strategies for fragment-based lead discovery (FBLD), a crystallographic approach starting with small probes (≤ 200 Da, so called ‘fragments’) as initial entry points. Based on the crystal structure of such hits, more potent leads are developed by growing the initial fragment into the binding pocket (Fig. 2). Since in all of these projects the influence and binding of water molecules takes an essential impact, the group embarked onto a detailed study of water molecules and their role in ligand binding. These investigations require crystallographic studies at extremely high resolution with X-rays and with neutrons as well (Fig. 3). Furthermore, molecular dynamics simulations help to understand the influence of water on binding (Biela *et al*, 2013).
An additional focus of the group is put on the understanding of protein-protein interfaces and how they are stabilized energetically and kinetically. Strategies are developed to perturb and destabilize such interfaces by tailored mutations and by interference via small molecule binding. Apparently, also in this case water penetration takes decisive influence on the protein-protein interface formation and stability (Jakobi *et al*, 2014).

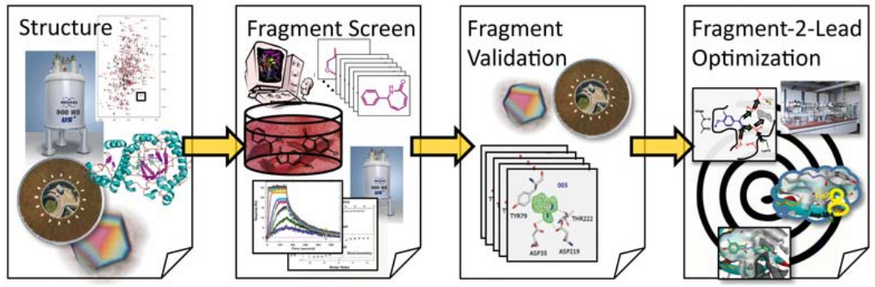

Figure 2. *Fragment-based lead discovery starts with the spatial structure of a protein, determined by X-ray crystallography or NMR spectroscopy. By use of either biophysical methods, computational analysis or crystal structure analysis, the binding of small molecular probes (fragments with ≤200 Da) is characterized. Subsequently, the discovered fragments are optimized by design and synthesis from fragments-to-lead structures.*

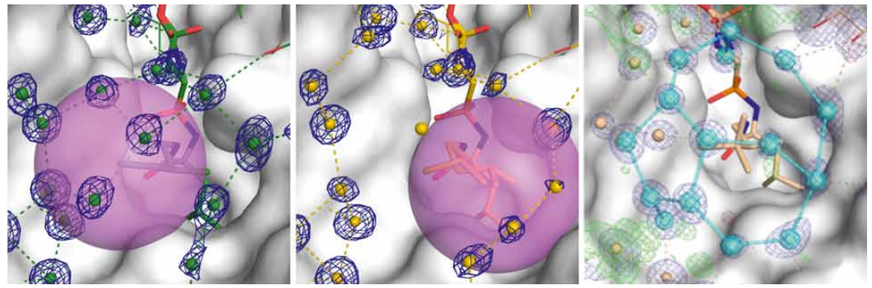

Figure 3. *Protein-ligand complexes are exposed to a surrounding water environment. A layer of surface water molecules is formed which solvates the newly formed complex. By careful design, an optimal network of water molecules can be created that wraps around the exposed ligand functional groups and thus enhances binding affinity of the bound ligand towards its protein.*

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AWARDS & HONORS

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Structure-based prediction of protein ligands and enzyme substrates

Ever since the first X-ray structures of proteins were determined, they have been used to identify and optimize small synthetic molecules that might bind to them. One of the advantages of this approach is that it does not require any known ligands and can thus discover ligands with novel chemistry, or even be applied to orphan targets. In particular, our lab uses docking, a computational technology that screens large libraries of millions of molecules for those that show steric complementarity to the binding site of a target protein. There are two main goals: one, to identify inhibitors or activators of proteins in order to use them to perturb biological systems; two, to identify potential substrates for enzymes of unknown function, assigning reactions to the thousands of proteins that have been crystallized in the various structural genomics initiatives.

Ligands with chemistry previously undescribed for a certain target

The lab is focused on G protein-coupled receptors (GPCRs), a ubiquitous receptor family whose members consist of seven membrane-spanning helices. They sense a broad variety of agents (ranging from peptides down to individual photons) on the outside of a cell and transmit the signal through the membrane. GPCRs are the class of proteins most frequently targeted in pharmaceutical research: over 30% of currently marketed drugs interact with a GPCR.
Through unbiased docking to a number of targets, namely the β_2-adrenergic receptor and the A_1 adenosine receptor, we were able to find novel and potent ligands in high numbers. In the screening campaign against the β_2AR (Kolb *et al*, 2009), six ligands emerged, with the most potent compound binding with a K_i of only 9 nM (Fig. 1). Remarkably, two of the compounds are chemically distant from known ligand chemical space for this receptor, despite the fact that it has been investigated in the pharmaceutical industry for the past 60 years.
Similarly, in the screen against the A_1AR we were able to contribute 25 previously unknown ligands, one fifth with novel chemistry (Fig. 2) (Kolb *et al*, 2012). As no X-ray structure is available for this receptor, we calculated four homology models based on a related receptor. Notably, three of the four models yielded ligands, which can be attributed to the extraordinary conformational flexibility of GPCRs.

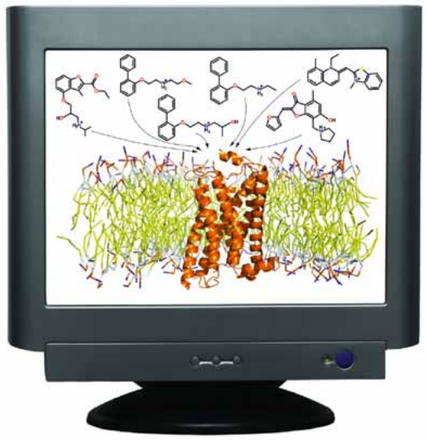

Figure 1. *The six compounds identified by computationally docking one million molecules to the X-ray structure of the β_2AR.*

Ligands with tailored selectivity

The binding profile, i.e. its selectivity, is one of the most important features of a therapeutic molecule. Hence, it is extremely valuable to predict binding patterns already at the screening and design stage. We recently did the proof of concept for two GPCRs from the chemokine subfamily, peptide sensors that are involved in malignancies such as cancer, multiple sclerosis and HIV infections. We extracted both putatively subtype-selective as well as dual binders from our screen. Upon experimental verification, we found that more than 50% of the predictions were correct in each category (Schmidt *et al*, 2014).

Substrate identification for enzymes of unknown function

Through the efforts of structural genomics consortia, thousands of X-ray structures of putative proteins, some of them seemingly enzymes, from various microorganisms are available. Yet, the majority of them have been solved without any potential substrate in the binding site. These enzymes might catalyze useful reactions, however, so annotating them in a high-throughput manner is desirable.
We have focused on enzymes of the amidohydrolase family, which are easily recognized by their characteristic $(\beta/\alpha)_8$-barrel fold. They can catalyze a plethora of different reactions, and the precise subtype cannot directly be deduced from the sequence. *In silico*, the highly labile active complex of putative substrates can be constructed and docked. This is the species that the enzyme is preorganized to stabilize and thus docking screens using such reaction intermediates show a higher hit rate. This way, the substrate specificity and precise reaction mechanism of five amidohydrolases could be elucidated (e.g., Lmo2620, an enzyme that dephosphorylates sugar lactones: Fig. 3 & Xiang *et al*, 2012).

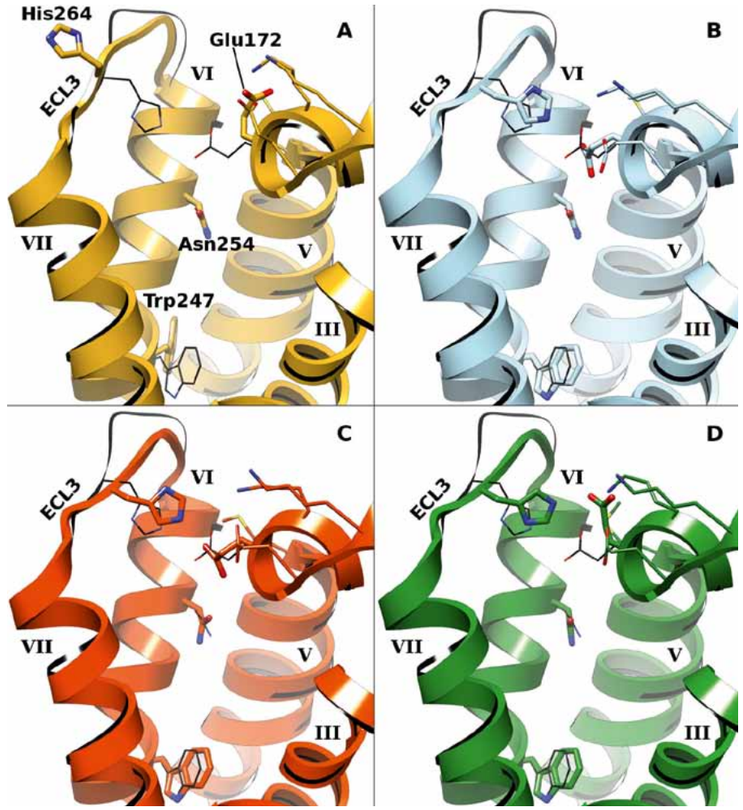

Figure 2. *Four different conformations of the A_1AR used to identify 25 ligands. Each conformation yielded different ligands, except for conformation C, which was unproductive. For comparison the X-ray crystallographic structure of the A_{2A}AR, the template (PDB 3EML), is shown in black. (Copyright PLOS)*

Future directions

Together with the Li lab, we are developing the application of prenyltransferases (PTs) for the modification of small molecule ligands. Initial hits can be chemically modified in a rapid and precise way by PTs, which allows us to explore their structure-activity relationship, i.e. how chemical structure relates to pharmacological potency. PTs are ideal tool enzymes in this respect, because the prenyl-group that they transfer to a small molecule is an apolar moiety that is usually associated with a gain in potency. In a first step, we have cataloged and characterized the substrate and reaction space of PTs. This knowledge is now used to predict additional molecules that might be turned over by a PT. Ultimately, PTs will be used to quickly and efficiently modify small molecules emerging from screens of large libraries against disease-related targets. Thus, the chemical space around a particular hit can be explored in a straightforward manner.

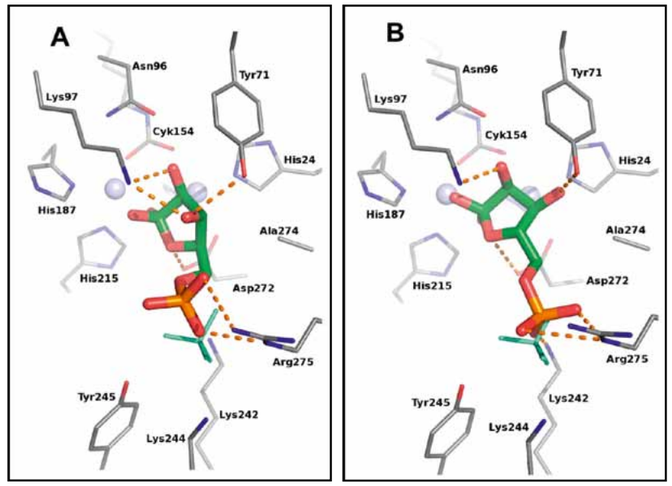

Figure 3. *Docked poses of the substrates with Lmo2620. The position of the phosphate ion from the original structure is colored cyan; the protein and ligands are in grey and green carbons, respectively. (Copyright ACS)*

SELECTED PUBLICATIONS

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2004 Habilitation, University of Tübingen
1995–2004 Assistant Professor of Pharmaceutical Biology, University of Tübingen
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1992 Ph.D. in pharmaceutical biology, University Bonn
1986 M.Sc. in medicinal chemistry, Beijing University, Beijing, China

AWARDS & HONORS

2009 PHOENIX Pharmaceutics Science Awards
2004 PHOENIX Pharmaceutics Science Awards
1997 PHOENIX Pharmaceutics Science Awards

Characterization and application of non-ribosomal peptide synthetases

In the last years, availability of new genome sequences from diverse microorganisms, especially bacteria and fungi, has led to the identification of genes and gene clusters, which can be used for production of novel secondary metabolites by genetic manipulation. These small molecules often show interesting biological and pharmacological activities and therefore are important drug candidates or lead compounds in drug development processes. The scaffold of a large group of biologically active compounds from ascomycetes are often synthesized by modular multifunctional enzymes like non-ribosomal peptide synthetases (NRPS), NRPS-like synthases, polyketide synthases (PKS) or hybrids of PKS and NRPS. The generated fungal peptides or peptide-related products show remarkable diversity both in their structures as well as in their biological activities, and can even be further metabolized by different modification enzymes, *e.g.*, prenyltransferases (PTs) or cytochrome P450 (Li, 2010). Therefore, expressing NRPS, NRPS-like or PKS/NRPS genes in heterologous hosts and thereby generating new "natural" products represent a novel strategy for drug discovery and development.

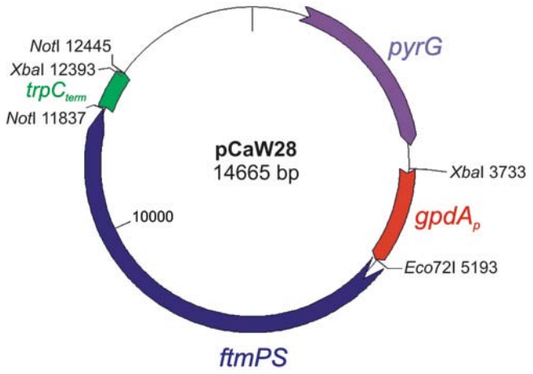

Figure 1. *Map of an exemplary construct for expression of NRPS genes with gpdA promoter, trpC terminator and pyrG selection marker.*

Production and identification of novel secondary metabolites

After successful PCR-amplification of 12 NRPS and NRPS-like genes from six ascomycetes (*Aspergillus nidulans*, *Aspergillus oryzae*, *Aspergillus terreus*, *Chaetomium globosum*, *Neosartorya fischeri* and *Penicillium chrysogenum*), 11 genes were subsequently cloned into the cloning vector pGEM-T Easy to verify the sequence of each gene. Ten of these genes with own or artificial promoter or terminator were finally cloned into the expression vector pJW24 (*pyrG*$^+$) (Fig. 1). Overall, 14 different expression constructs were created, either with own gene promoter or artificial *A. nidulans gpdA* promoter, and with or without *trpC* terminator, and were used for transformation into the fungal strain *A. nidulans* TN02A7 (*pyrG*$^-$, *pyroA*$^-$).

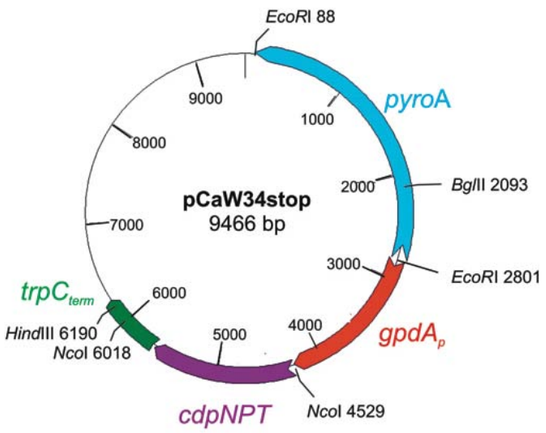

Figure 2. *Map of an exemplary construct for expression of prenyltransferase genes with gpdA promoter, trpC terminator and pyroA selection marker.*

After PEG-mediated protoplast transformation of *A. nidulans* TN02A7 using the expression construct pCaW28 bearing the NRPS gene *NFIA_093690* (*ftmPS*) from *N. fischeri* under the control of *gpdA* promoter and *trpC* terminator, complementation of the uracil auxotrophy was used for selection. Integration of the gene *ftmPS* into the genome of *A. nidulans* was verified for four transformants by PCR (*A. nidulans* CaW02-05). Analysis of secondary metabolites on HPLC showed the presence of one distinct product peak in comparison to its naïve host. Isolation and structure elucidation of the newly accumulated product by NMR and HR-MS analyses revealed unequivocally that the NRPS is responsible for the formation of brevianamide F (*cyclo*-L-Trp-L-Pro). Yields of up to 36.9 mg brevianamide F per liter culture were calculated for the transformants.

For the two orthologous genes *NFIA_074300* from *N. fischeri* and *Pc21g15480* from *P. chrysogenum*, which share a sequence identity of 60 % on the amino acid level, expression constructs with sizes of up to 15 kb under the control of the *gpdA* promoter were created. Both constructs (pKM36 and pKM42) were transformed separately into *A. nidulans* TN02A7, and complementation of the uracil auxotrophy was used for the identification of recombinant transformants. Confirmation of gene integration into the genome of *A. nidulans* TN02A7 by PCR led to identification of three transformants for *Pc21g15480* (*A. nidulans* KM01-03) and 14 transformants for *NFIA_074300* (*A. nidulans* KM45-58), respectively. HPLC analysis of all 17 transformants for production of newly accumulated secondary metabolites showed two additional peaks in each transformant. Isolation on HPLC and structure elucidation by NMR and MS analyses proved that both NRPSs are responsible for the biosynthesis of the cyclic dipeptides *cyclo*-L-Trp-L-His and *cyclo*-L-Trp-L-Pro. Yields of 6 mg l^{-1} *cyclo*-L-Trp-L-His and 4 mg l^{-1} *cyclo*-L-Trp-L-Pro were achieved. The accumulation of such secondary metabolites provides experimental confirmation for our research approach of transforming such large genes into heterologous hosts and thereby creating synthetic organisms with new features.

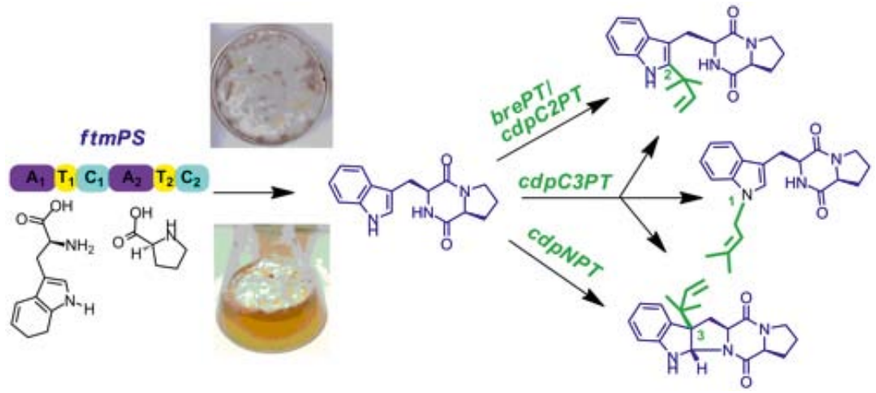

Figure 3. *Production of prenylated derivatives by coexpression of NRPS and prenyltransferase genes in Aspergillus nidulans.*

Targeted production of secondary metabolites

The first project focusing on the targeted gene coexpression was carried out with the NRPS gene *ftmPS* mentioned above and four prenyltransferase genes of the DMATS superfamily, which were known to accept *cyclo*-L-Trp-L-Pro as substrate, but with different features. For this purpose, coding sequences of the prenyltransferase genes *brePT* (Yin *et al*, 2013) from *A. versicolor*, *cdpC2PT* (Mundt & Li, 2013) and *cdpC3PT* (Yin *et al*, 2010) from *N. fischeri* as well as *CdpNPT* (Yin *et al*, 2007) from *A. fumigatus* were cloned separately into a second expression construct (*gpdA*$^+$, *trpC*$^+$, *pyro*A4$^+$) and transformed into the NRPS expression mutant *A. nidulans* CaW03 carrying *ftmPS* (Fig. 2). After complementation of the additional pyridoxine auxotrophy, confirmation of the ectopic integration of prenyltransferase genes into the genome was carried out by PCR for overall 30 transformants. *N*1-regularly, *C*2- and *C*3-reversely prenylated derivatives were isolated from fungal cultures and identified by HPLC, NMR and MS analyses (Fig. 3). Yields between and 5 and 30 mg l^{-1} were calculated for these substances. Our results demonstrate that different prenylated cyclic dipeptides can be produced by synthetic microbiology with scaffold-forming and modification enzymes.

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1981 Diploma in chemistry, Ludwig-Maximilians-Universität München

AWARDS & HONORS

2014 Albrecht Kossel Prize of the German Chemical Society (GDCh), and Luigi Sacconi Medal of the Società Chimica Italiana (SCI)
2010 Feldberg Foundation Prize
2009–2014 Fellow of the Max Planck Society
2007 Elected Member of the German National Academy of Sciences Leopoldina
2003 Leibniz Prize of the German Research Foundation (DFG)

Mechanisms of iron-sulfur protein biogenesis in eukaryotes

Iron-sulfur (Fe/S) clusters are evolutionary ancient, inorganic cofactors of proteins with functions in catalysis, electron transfer and regulation. Fe/S proteins in turn participate in central cellular processes such as DNA synthesis and repair, chromosome segregation, protein synthesis, and respiration. Their biogenesis is thus essential for cell viability, and impairment of Fe/S protein biogenesis causes genome instability, a hallmark of cancer. Our group is interested in identifying and characterizing the molecular machinery catalyzing the synthesis of Fe/S clusters and their insertion into apoproteins in eukaryotes. To date, some 30 biogenesis proteins are known, and they are conserved from yeast to man (Lill, 2009; Netz *et al*, 2014). While we have a good cell biological picture of Fe/S protein biogenesis, the molecular mechanisms underlying the individual reactions are only now being unraveled using biochemical, biophysical, bioinorganic and ultrastructural methods. Knowledge of these reactions will help in synthetic biology to construct artificial pathways for the synthesis of products such as isobutanol or isoprene derivatives, which in turn serve as versatile starting materials for pharmaceuticals, cosmetics and fine chemicals. Our SYNMIKRO project is dedicated to the better understanding of the various pathways of Fe/S protein biogenesis in yeast to render such proteins available as building blocks for synthetic microbiology.

Generation of cellular Fe/S proteins in eukaryotes is accomplished by three conserved biosynthesis machineries in the mitochondria and the cytosol. Biogenesis is initiated by the mitochondrial iron-sulfur cluster (ISC) assembly machinery which was inherited from bacteria during evolution (Fig. 1): Seventeen known ISC proteins first assemble a Fe/S cluster on a scaffold protein and then move the cluster to transfer proteins, from where the cluster is finally inserted into apoproteins by dedicated ISC targeting factors. Cytosolic and nuclear Fe/S protein assembly also depends on this machinery, yet additionally requires the mitochondrial ISC export apparatus and the cytosolic iron-sulfur protein assembly (CIA) machinery. The ABC transporter Atm1 of the export system transports a still unknown sulfur-containing compound (X-S) to the CIA machinery, which then assembles a [4Fe-4S] cluster on a scaffold complex. Specific CIA targeting proteins finally transfer the cluster to recipient apoproteins.

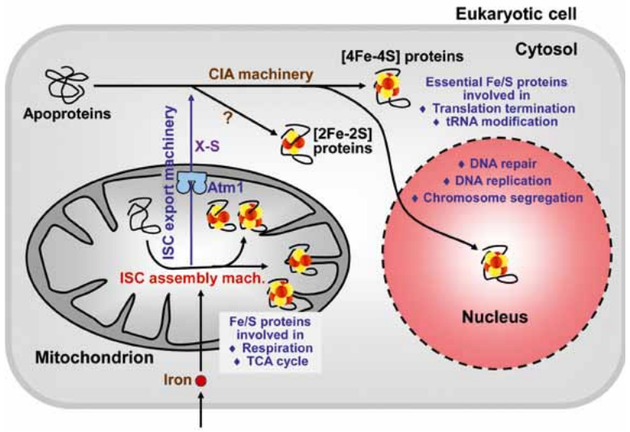

Figure 1. *An overview of some functions and the biogenesis of eukaryotic Fe/S proteins. Generation of all cellular Fe/S proteins depends on the mitochondrial ISC assembly machinery consisting of 17 proteins. The mitochondrial ISC export machinery and the CIA machinery are specifically involved in the maturation of cytosolic and nuclear Fe/S proteins. Red circles: iron ions, yellow circles: sulfide; X-S, an unknown sulfur-containing compound, which is exported from mitochondria by the ABC transporter Atm1 to support Fe/S-protein biogenesis in the cytosol.*

The role of ferredoxin in the reduction of sulfur

In vivo studies have suggested a role of the ferredoxin Yah1 in mitochondrial Fe/S protein biogenesis, but its precise role remained unclear. We biochemically reconstituted the synthesis of a [2Fe-2S] on the Isu1 scaffold and thus showed that Yah1 and ferredoxin reductase are essential for this process (Webert *et al*, 2014). Yah1 in its reduced form specifically interacts with the scaffold protein Isu1 to deliver its electron, thereby possibly reducing the persulfide (-SSH) intermediate of Isu1 to the sulfide needed for Fe/S cluster formation (Fig. 2A). The interaction surface for Isu1 on Yah1 was revealed by NMR spectroscopy and indicates that Isu1 binds close to the [2Fe-2S] of Yah1 (Fig. 2B). The ongoing resolution of the structure of the entire complex will be helpful for understanding the mode of electron transfer. Notably, an electron transfer chain was described in the cytosol for Fe/S protein biogenesis, but it is unclear why reduction is needed (Netz *et al*, 2010).

The 3D structure of Atm1 suggests glutathione as part of the substrate

The central component of the ISC export pathway is the mitochondrial inner membrane ABC transporter Atm1 (Fig. 1). Its human orthologue ABCB7 is mutated in X-linked sideroblastic anemia and cerebellar ataxia (XLSA/A). We recently succeeded to purify and crystallize yeast Atm1 (Srinivasan *et al*, 2014). The 3D x-ray structure revealed a novel feature of ABC transporters (Fig. 3): The two fully resolved C-terminal α-helices were shown to bind to each other, thereby protecting the protein from degradation. Another important feature of Atm1 is its substrate binding cavity which is composed of mainly positively charged residues, suggesting a net negative molecule is bound and exported. In one of our crystal structures we found a bound glutathione in this area (Fig. 3). Since glutathione was earlier shown to be essential for the export process, we suspect that the compound may be part of the exported substrate. The locations of the four residues mutated in XLSA/A suggest an impaired function in substrate binding or membrane transport in disease cells.

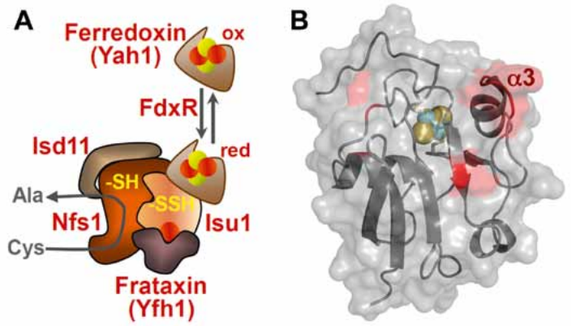

Figure 2. *A mitochondrial electron transfer chain is needed for de novo generation of Fe/S clusters. (A) A persulfide (-SSH) intermediate is generated by the mitochondrial cysteine desulfurase Nfs1-Isd11 on the scaffold protein Isu1. Reduction of the persulfide involves the ferredoxin reductase FdxR and the [2Fe-2S] ferredoxin Yah1, which get their reducing equivalents from NADPH. Yah1 binds to Isu1 preferentially in its reduced form. Frataxin Yfh1 is important for stimulating sulfur transfer. (B) The 3D structure of Yah1 and the interaction surface for Isu1 (pink) as revealed by HSQC NMR experiments.*

A distinct pathway for maturation of cytosolic [2Fe-2S] proteins

A novel mechanistic observation was made in our studies of the construction of metabolic pathways for the engineered synthesis of Isobutanol as part of our collaboration with Gevo Co, Englewood, USA. This process makes use of a bacterial [2Fe-2S] protein in the yeast cytosol, the dihydroacid dehydratase IlvD. We found that the maturation of IlvD to an active enzyme occurs independently of the known components of the CIA machinery, discriminating this pathway from the biogenesis of CIA-dependent [4Fe-4S] proteins. However, maturation still requires the mitochondrial ISC assembly machinery and Atm1. We then realized that all tested cytosolic [2Fe-2S] proteins follow this pathway (Fig. 1). With this knowledge, we currently explore which additional cytosolic biogenesis proteins are needed for the maturation of [2Fe-2S] proteins in the cytosol. This example shows that application-oriented experimental approaches can lead to new discoveries in basic science, too.

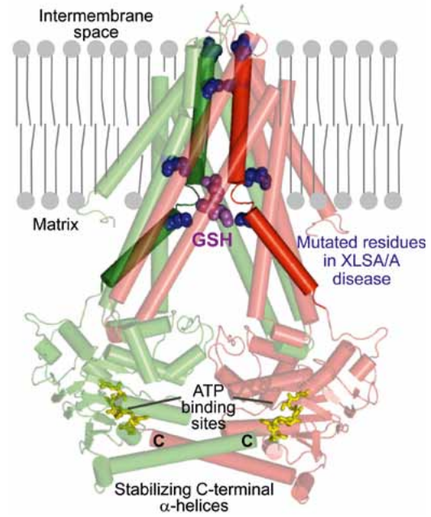

Figure 3. *Crystal structure of the ABC transporter Atm1 of the mitochondrial inner membrane. Atm1 is a homodimer (red and green subunits). The binding site for glutathione (GSH) is within the putative substrate binding cavity. The yellow regions indicate amino acid residues that are involved in ATP binding. Residues mutated in the human Atm1 orthologue ABCB7 in the iron storage disease XLSA/A are shown in blue.*

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1977 Ph.D in microbial biochemistry, Max Planck Institute for experimental medicine/Georg-August University Göttingen
1973 Diploma in chemistry, Georg-August University Göttingen

AWARDS & HONORS

2014 David Gottlieb Memorial Lecture, University of Illinois, USA
2012 Honorary Professorship from Wuhan University, China
2009 Elected Member of the Royal Society of Chemistry, UK
2008 Max Bergmann Medal in gold for work on Non-ribosomal Peptide Synthesis
2004 Elected Member of the German Academy of Sciences Leopoldina

Biosynthesis and engineering of bioactive cyclic peptides

Peptide natural products play an important role in modern medicine for the treatment of many life-threatening diseases. A large fraction of these natural products are either ribosomally synthesized and post-translationally modified peptides (RiPP), or assembled independent of the ribosome on large multi-modular enzymes called nonribosomal peptide synthetases (NRPS). In our group, we investigate the structure, function, biosynthesis and engineering of ribosomally and nonribosomally assembled cyclic peptides that show complex structures and diverse bioactivities.

Characterization of novel lasso peptides

Lasso peptides are a group of natural products that share a unique structural motif: All known lasso peptides consist of an N-terminal macrolactam ring, which is 7 to 9 amino acids in size and is formed between the N-terminus of a Gly, Ser, Ala or Cys residue at position 1 and the side chain of a Glu or Asp residue at position 7, 8 or 9 (Fig. 1). Their distinguishing feature is the threading of the C-terminal tail of the peptide through the macrolactam ring, yielding a structure reminiscent of a lariat knot. This entropically unfavorable confirmation is sustained by the placement of sterically demanding residues (plug amino acids) above and below the ring, entrapping the tail inside and preventing its unthreading.

lasso peptide microcin J25, yielding a potent αvβ3 integrin receptor antagonist (Hegemann *et al*, 2014).
In general, lasso peptide precursors are matured by two enzymes: enzyme B, a cysteine protease homolog, and enzyme C, an adenylate forming enzyme homolog. Interestingly, the B enzyme shares little similarity to enzymes outside of lasso peptide biosynthetic gene clusters. This inspired us to perform a B protein-centric genome mining approach through which we were able to identify 102 putative lasso peptide biosynthetic gene clusters from a total of 87 proteobacterial strains. Ten of these clusters were then heterologously expressed in *Escherichia coli* BL21(DE3), and all twelve corresponding lasso peptides were detected and characterized by high-resolution Fourier transform mass spectrometry (Hegemann *et al*, 2013).

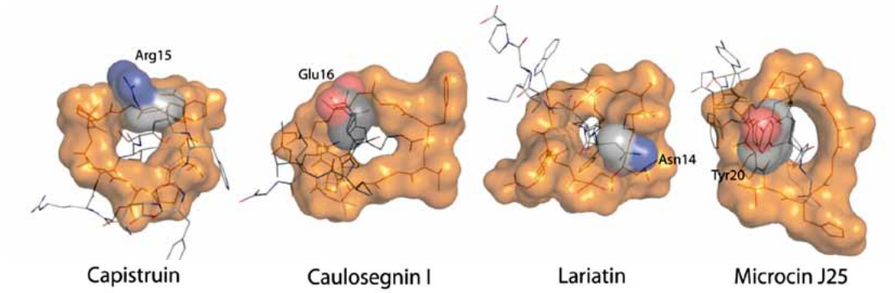

Figure 1. *NMR structures of lasso peptides showing the interactions between the C-terminal tail and the macrolactam ring of capistruin, caulosegnin I, lariatin and microcin J25. The surface of the rings is colored in orange, the surface of the side chains of the plug amino acids positioned below the ring is colored by elements. Steric hindrance caused by the depicted side chains prevents the unthreading of the lasso peptides. All lasso peptides are shown from below the ring. (From Hegemann et al, 2013)*

The majority of known lasso peptides possess various biological properties, ranging from antibacterial and inhibitory to receptor antagonistic activities (Hegemann *et al*, 2013). As lasso peptides are of ribosomal origin and as the enzymatic maturation machinery was shown to have a rather relaxed specificity towards substitutions of most of the residues in the lasso peptide scaffold, they are also promising candidates for epitope grafting approaches. This was demonstrated only recently through the incorporation of the bioactive RGD peptide epitope in the loop region of the

Biosynthesis of modified cyclic dipeptides

In recent years, it has become apparent that aminoacyl-tRNAs are not only crucial components involved in protein biosynthesis, but are also used as substrates and amino acid donors in a variety of other important cellular processes ranging from bacterial cell wall biosynthesis and lipid modification to protein turnover and secondary metabolite assembly. Therefore, we investigated tRNA-dependent biosynthetic pathways that generate different bioactive modified cyclic dipeptides (CDPs). The enzymes responsible for the initial biosynthesis of a CDP-scaffold are referred to as cyclodipeptide synthases (CDPSs) and use loaded tRNAs as their substrates (Fig. 2).
First, we described the identification of the first nocazine biosynthetic gene cluster in *Nocardiopsis dassonvillei* and the elucidation of the biosynthetic pathway leading to nocazine E and XR334 - nocazines are a newly defined family of antibacterial and cytotoxic CDPs produced by different actinobacterial species. We showed that CDP-formation is carried out by a new member of the CDPS family showing a formerly unknown product profile, while tailoring of the CDP scaffold is achieved through the combined actions of a CDP oxidase (CDO) and two distinct SAM-dependent *O*-/*N*-methyltransferases (Giessen *et al*, 2013a).
In a second study, we performed a bioinformatic analysis of a CDPS-containing gene cluster from *Actinosynnema mirum*. We established the biosynthetic pathway leading to two methylated ditryptophan CDPs (cWW) through *in vivo* and *in vitro* analyses. This CDPS was the first member of this enzyme family able to form cWW and the first prokaryotic CDPS whose main product constituents differ from the four amino acids (Phe, Leu, Tyr and Met) usually found in these systems. We also characterized a SAM-dependent *N*-methyltransferase that carries out two successive methylations at the diketopiperazine ring nitrogens of cWW and showed that this enzyme is also able to methylate four other phenylalanine-containing CDPs (Giessen *et al*, 2013b).

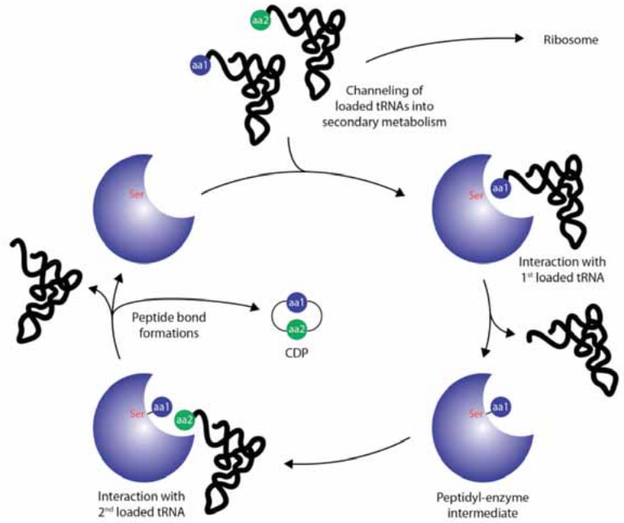

Figure 2. *Overview of the general action of CDPSs and their connection to the primary metabolism. CDPSs hijack aminoacyl-tRNAs and employ them in the formation of CDPs, thus diverting the flow of loaded tRNAs away from the ribosomal machinery. (From Giessen & Marahiel, 2014)*

A synthetic tRNA-aminoacylation catalyst

The incorporation of non-proteinogenic amino acids – i.e., amino acids not naturally used by the translational machinery of any organism – represents a major challenge for the creation of functionalized peptides and proteins. In collaboration with the group of Gert Bange, we recently devised a fusion protein-based design for synthetic tRNA-aminoacylation catalysts by combining NRPS adenylation domains (A-domains, which select and activate the monomers in NRPS pathways) with a small eukaryotic tRNA-binding domain (like Arc1p-C from yeast) (Fig. 3). E.g., we fused the A-domain PheA with the Arc1p-C domain using flexible linkers and thus were able to achieve tRNA-aminoacylation with both proteinogenic (L-Phe) and non-proteinogenic (D-Phe) amino acids (Giessen *et al*, 2015).

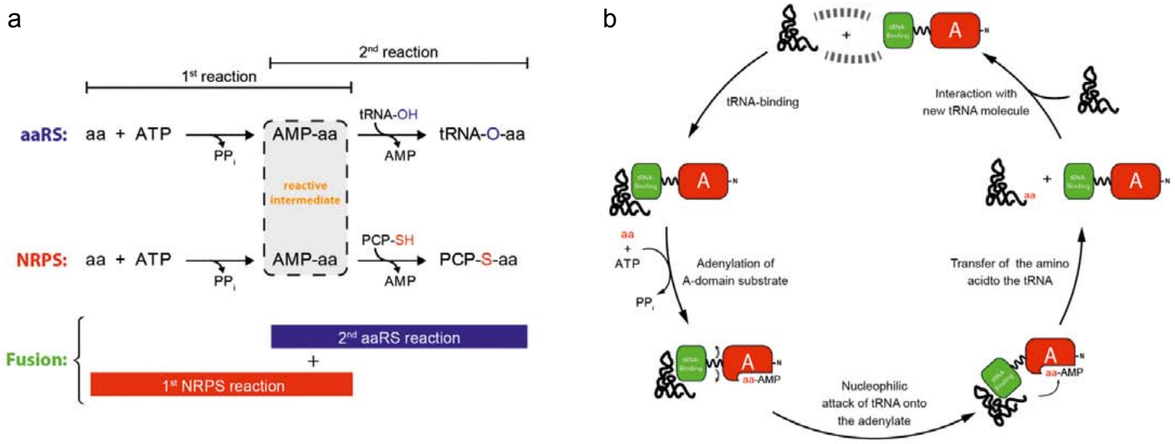

Figure 3. *Design strategy for the construction of synthetic adenylation domain-based fusion proteins for tRNA-aminoacylation based on structural insights and molecular models. (a) Comparative overview of the two reactions catalyzed by aaRSs and NRPS A-domains, respectively. In both cases the first catalyzed reaction consists of the ATP-dependent adenylation of a substrate monomer, generating a high-energy adenylate intermediate (aa-AMP). In the second reaction a nucleophilic attack, either by the 3'-adenosine of the respective tRNA (aaRS) or the 4'-PPant-thiol group of a downstream PCP (NRPS), takes place. Our approach aims to assemble a fusion protein that is able to combine the first reaction shared by aaRSs and NRPS A-domains with the transfer of an activated intermediate to a tRNA-acceptor. (b) Examplary catalytic cycle of A-domain-based tRNA-aminoacylation. Note that the shown order of tRNA-binding and adenylate formation only represents one of several possibilities. (From Giessen et al, 2015)*

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Giessen TW, Altegoer F, Nebel AJ, Steinbach RM, Bange G, Marahiel MA (2015). A synthetic adenylation domain-based tRNA-aminoacylation catalyst. *Angew Chem*, doi: 10.1002/anie.201410047

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2007–2012 Postdoctoral Fellow and Project Leader, University of Heidelberg
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2002 Diploma in biochemistry, Martin-Luther-Universität Halle

AWARDS & HONORS

2013 iGEM gold medal in the European jamboree & admission to finals at MIT in Boston (as advisor of the team)
2012 Peter & Traudl Engelhorn Fellow
2012 F1000 Prime for Kressler & Bange *et al*, Science

Biogenesis and design of bacterial nanomachines

Nanomachines are macromolecular complexes that play important roles in all processes of life, ranging from protein synthesis to motility. A common feature of these nanomachines is their complex subunit architecture in a specific three-dimensional structure. Therefore, their biosynthesis must be highly regulated in time and space to ensure correctness and reproducibility. Our group aims at understanding this spatio-temporal regulation underlying the assembly of biological nanomachines, e.g., the bacterial flagellum, at the molecular level. Furthermore, we want to use the acquired molecular understanding for the rational design of several synthetic tools, for instance for the localization of protein complexes within the cell envelope or for the secretion of target molecules into the medium.

Many bacteria move by rotating rigid, helical organelles, the flagella (Altegoer *et al*, 2014). These flagella represent some of the tiniest complex motors in the biosphere and enable bacteria both to move through liquids (swimming) and through highly viscous environments or on surfaces (swarming). In addition, they act as virulence factors in many pathogenic species, as motility is often required to reach the site of infection. Last but not least, flagella have only recently been recognized to contribute to biofilm formation. Therefore, bacterial flagella seem to fulfill many more functions beyond their role as organelles of locomotion.

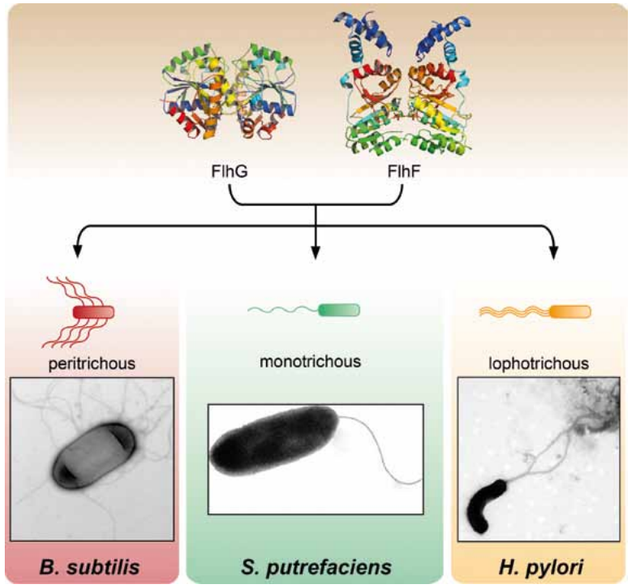

Figure 1. *The two nucleotide-binding proteins FlhG and FlhF control the correct establishment of the flagellation pattern in B. subtilis, S. putrefaciens and H. pylori as well as in many other species.*

Modules for self-organization of regular patterns

Already early in microbial research, it was noticed that bacterial species differ in number as well as localization of their flagella (named 'flagellation pattern' hereafter) (Fig. 1). Despite the enormous amount of bacterial species, there are only a handful of different flagellation patterns known. Establishing the correct flagellation pattern is a prerequisite for efficient motility and for the pathogenicity of many disease-causing flagellated bacteria. In many species (except, e.g., *E. coli*), this pattern is ensured by two nucleotide-binding proteins named FlhF and FlhG (Bange *et al*, 2013; Bange *et al*, 2011; Bange *et al*, 2007). We want to further this molecular understanding of flagellation pattern control and, in doing so, also tackle one of the most challenging questions in today's biology: How do living organisms reproducibly self-organize regular patterns? The 'molecular answer' includes the potential for synthetic modules that specifically localize biosynthetic protein complexes within bacteria or artificial cells.

A high-throughput secretion machine

Despite these different flagellation patterns, the architecture of the bacterial flagellum is conserved and can be divided into three main parts: the transenvelope-spanning basal body with the motor subunits and the extracellular hook and filament structures (Fig. 2). To establish these extracellular structures, a flagellum-specific type III secretion system (fT3SS) consisting of an ~ 9-protein core machinery exports flagella building blocks to the growing tip of the flagellum with both high speed and stringent substrate discrimination. However, an in-depth molecular understanding of how the export apparatus recognizes its substrates within the myriad of cytoplasmic proteins and ensures the correct chronological sequence of export is crucially missing. Previously, we could show that the transmembrane protein FlhA serves as the adaptor for the coordinated delivery of different flagella building blocks to the fT3SS (Bange *et al*, 2010). Now we want to find out how the fT3SS can select the correct export cargos at the right time, and to obtain an in-depth mechanistic and structural view of this process. In the future, we aim at building a minimal T3SS, which could then be used for the controlled secretion of any user-defined target protein from minimal cells.

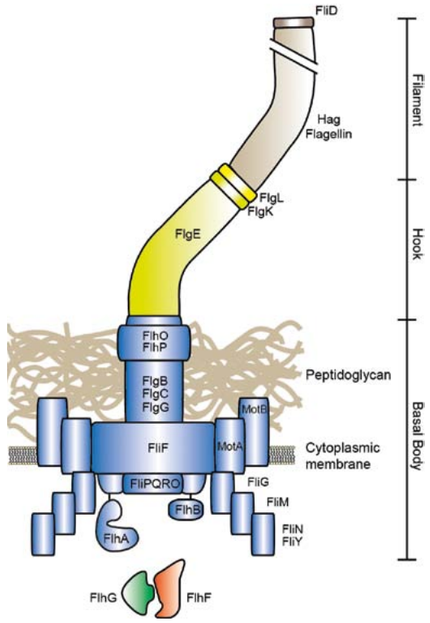

Figure 2. *Architecture of the bacterial flagellum in Gram positive species. The basal-body is composed of integral membrane components including the cytosolic C-ring (blue). The extracellular hook (yellow) and the filament structures (brown) extend from the peptidoglycan forming the outer part of the flagellum.*

The flagellar filament as a hub for synthetic applications

The extracellular filament of the flagellum is a long tubular polymer composed of over 20'000 copies of a single protein named flagellin (Fig. 3). Flagellin consists of a highly conserved part that is essential for its assembly into the flagellar filament, and a variable region (VR) that shapes the surface of the filament and differs significantly between the various bacterial species (Altegoer *et al*, 2014). E.g., the VR of *E. coli* flagellin comprises two domains of approximately 25 kDa, while the VR of *B. subtilis* is virtually non-existent. Based on our crystal structures of different flagellins and the molecular models of the respective filaments, we are currently engineering filaments with a variety of artificial peptides and protein domains within the VR of their flagellin molecules. The so-created synthetic filaments will be useful for the display of peptides (e.g., for vaccine production), scavengers (e.g., for recovery of noble earths), the design of nano-wires and the design and arrangement of microbial communities.

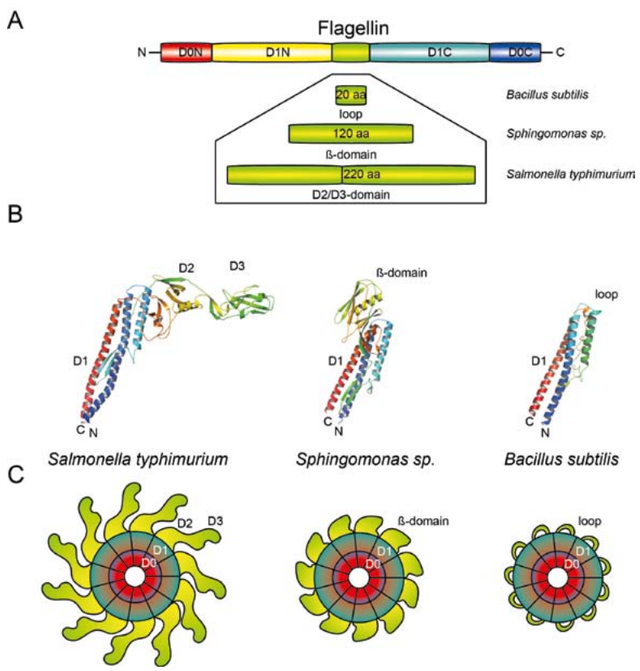

Figure 3. *(A) Domain architecture of Flagellin. The variable region (green) differs significantly between the various bacterial species. (B) Crystal structures of Salmonella typhimurium, Sphingomonas sp. and Bacillus subtilis Flagellin. The disordered D0 domains are missing due to flexibility. D1 domains are conserved among these species. However, the variable region consists of 220 aa forming two distinguishable domains D2 and D3 in S. typhimurium, whereas in Sphingomonas it is reduced to a ß-domain of 120 aa and in B. subtilis only a small loop is formed. (C) Cross sections of flagellar filaments of different species. The variable region is exposed on the surface of the filament, thereby generating distinct properties.*

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Synthetic cell compartments based on peroxisomes

Within each cell, different metabolic pathways compete for substrates and enzymes. Therefore, nature uses cellular compartmentalization as a strategy to prevent unwanted cross-talk in metabolism. Our research group aims at the construction of synthetic cell compartments that can be used to channel metabolic reactions for novel products. Fungal peroxisomes are perfectly suited for this purpose since they are not essential. Thus, it is possible to redesign the peroxisomal proteome and to engineer only selected enzymes into peroxisomes that cooperate in defined and novel pathways. We used synthetic retargeting of enzymes involved in glycolipid biosynthesis to alter the chemical composition of a fungal glycolipid, which serves as a potent biosurfactant. Relocation of the respective enzymes from peroxisomes into the cytosol resulted in incorporation of hydroxylated fatty acids into the glycolipid, which are not accessible within the peroxisomes. This demonstrates that intracellular localization of metabolic enzymes determines the product spectrum of metabolic reactions. We are currently constructing cells where the hydroxylating enzyme is retargeted to peroxisomes. We will use such synthetic compartments with tailor-made proteomes based on peroxisomes to rechannel further metabolic pathway for the production of new metabolites that do not exist in nature.

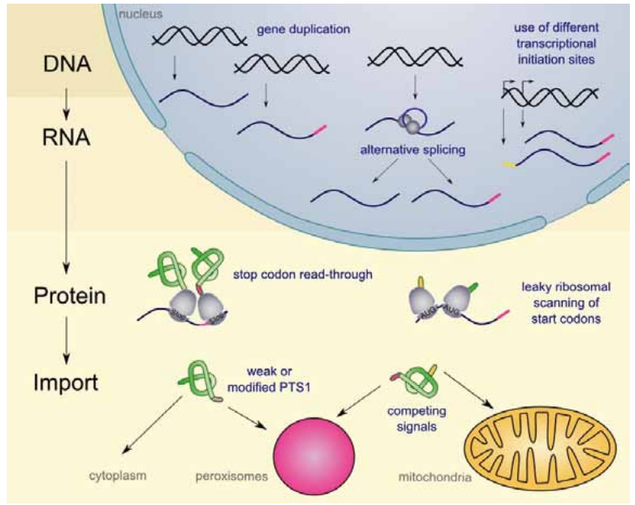

Figure 1. *Dual targeting of intracellular proteins can occur via different molecular mechanisms.*

Dual targeting of intracellular proteins

Eukaryotic cells are organized into defined compartments that differ in their chemical composition and environment. Among these are the nucleus harboring the genetic information, and the mitochondria that provide energy for cellular metabolism. Beside these organelles, all eukaryotic cells also contain peroxisomes that are involved in fatty acid degradation and peroxide detoxification. Remarkably, peroxisomal matrix proteins are transported into these compartments after translation and in a fully folded and co-factor-bound state. Correct targeting of peroxisomal proteins depends on short amino acid sequence motifs that act as intracellular zip codes.
Many important cellular proteins are found not only in a single compartment, but are dually targeted to different organelles (for review, see Ast, Stiebler & Freitag, 2014). We have recently discovered that glycolytic enzymes contain cryptic peroxisomal targeting signals that are post-transcriptionally unveiled by differential splicing or ribosomal read-through (Freitag *et al*, 2012). This unexpected mechanism is evolutionary conserved in fungi and plays an important role in their metabolism. Mutants that are unable to target these enzymes to both compartments displayed a growth defect and were affected in virulence.
Characterization of the sequence elements required for dual targeting via ribosomal read-through revealed that a short stop codon context is sufficient to trigger efficient read-through at this termination codon. Remarkably, this element is conserved from fungi to men and apparently serves as a basal read-through mechanism in a wide variety of species.

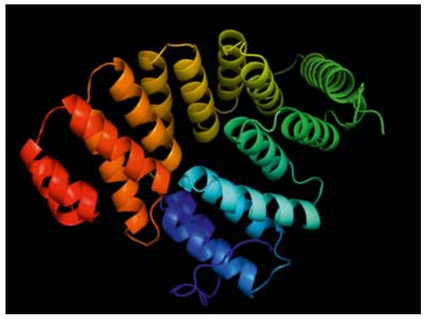

Figure 2. *Predicted molecular structure of the recognition domain of Pex5b, one of the peroxisomal import receptors from Ustilago maydis.*

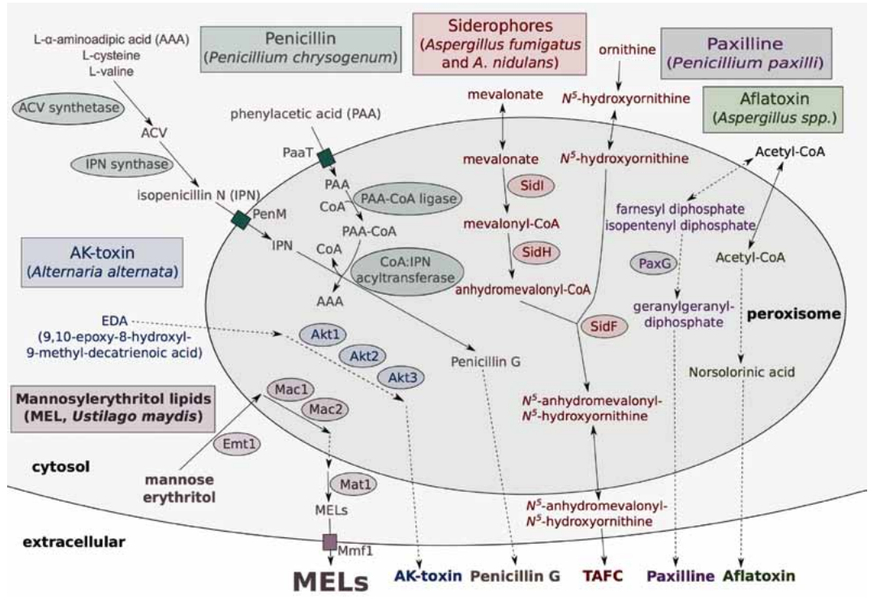

Figure 3. *Diverse secondary metabolic pathways are located in peroxisomes.*

We were able to demonstrate that even in human cells this read-through element is used to target metabolic enzymes such as malate dehydrogenase and lactate dehydrogenase to both the peroxisomes and the cytosol (Stiebler *et al*, 2014). Both enzymes depend on NAD/NADH for their activity and are involved in redox homeostasis via shuttling reduced and oxidized intermediates across the peroxisomal membrane.
To study dual targeting of peroxisomal proteins, we are currently characterizing the peroxisomal import system both in the phytopathogenic fungus *Ustilago maydis* and in the well-studied model organism *Saccharomyces cerevisiae* (baker's yeast). In contrast to yeast, the basidiomycetous fungus *U. maydis* contains two receptors for import of peroxisomal type 1 proteins. Both contain a C-terminal domain, which consists of a number of tetratrico repeats (Fig. 2). They form a binding pocket for the short C-terminal peroxisomal targeting signal typical for most peroxisomal matrix proteins. We could show that both receptors recognize a largely overlapping but not identical set of targeting signals. Currently, we are constructing chimeric receptors to alter the peroxisomal proteome.

Engineering metabolic pathways by retargeting of biosynthetic enzymes

It has become apparent that in fungi many metabolites, in particular those of secondary metabolism, are generated, at least partially, in peroxisomes (Fig. 3). Among these are antibiotics like penicillin produced by *Penicillium chrysogenum*, or toxins like aflatoxin secreted by diverse *Aspergillus* species. Therefore, this organelle constitutes an interesting compartment for the production of bioactive substances (Stehlik *et al*, 2014).
In *Ustilago maydis*, biosynthesis of mannositol erythritol lipid (MEL), an extracellular glycolipid, which serves as an effective biosurfactant, occurs partially in peroxisomes. Two acyl transferases (Mac1 and Mac2) involved in acylation of the sugar moiety of MEL carry peroxisomal targeting signals. Tagging with green fluorescent protein (GFP) demonstrates peroxisomal localization of these key enzymes. Interestingly, glycolipid production is not affected, if peroxisomes are deleted by mutagenesis. This indicates that biosynthesis can also occur in the cytosol. However, retargeting of these enzymes to the cytosol results in the production of a different set of glycolipids. This can be explained by the fact that in the cytosol both enzymes gain access to hydroxylated fatty acids (Freitag *et al*, 2014). These are derived from a competing glycolipid biosynthetic pathway. We are currently trying to further enlarge the potential spectrum of engineered glycolipids by introducing additional enzymatic activities into peroxisomes.

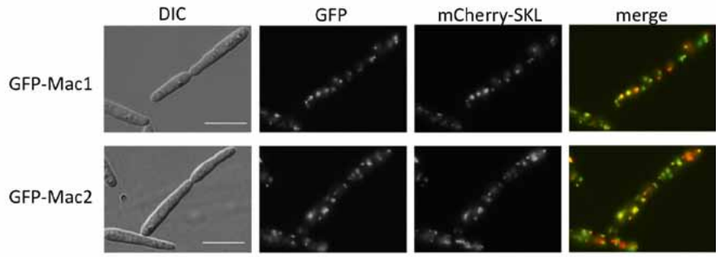

Figure 4. *Peroxisomal localization of MEL acyltransferases Mac1 and Mac2 in U. maydis.*

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Feitag J, Ast J, Bölker M (2012). Cryptic peroxisomal targeting via alternative splicing and stop codon readthrough in fungi. *Nature* 485, 522-5.

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Mathematical modeling of microbiological systems

Within SYNMIKRO, the research group Numerical Analysis is focusing on the mathematical modeling of diverse microbiological systems such as diffusion phenomena in living cells or the regulatory processes underlying cell polarity and flagella localization. In order to gain a deeper understanding of these complex systems, we are developing and studying simplified, but nevertheless appropriate mathematical models. The aim is to make reliable predictions based on these models that help in the design of future wet lab experiments: In an iterative process, the mathematical models will be validated by experiments, and the findings from the mathematical analysis of the models will lead to optimized designs of experiments. Besides the quantitative and qualitative analysis of the resulting mathematical models, we are working on suitable discretization schemes for the numerical simulation and visualization of the solutions.

Mathematical modeling of nonstandard diffusions

One central problem in cell biology is the understanding of diffusion phenomena. In classical diffusion models, which are based on variants of Brownian motions, the square of the distance covered by a particle is proportional to the time t. However, in many applications in cell biology, e.g., in the context of mass transport through membranes or through heterogeneous media inside the cell, one is faced with abnormal diffusion processes. In these cases, the square of the distance is proportional to t^α with $\alpha \neq 1$ ($\alpha > 1$ superdiffusion, $\alpha < 1$ subdiffusion). Transient subdiffusion phenomena can, e.g., be observed in the context of protein translocations on nucleic acids. Recent experiments in the context of single-molecule spectroscopy performed in the group of Peter Graumann for instance strongly indicate the occurrence of subdiffusion phenomena in living cells. By a statistical data analysis, we could confirm this conjecture in many cases. The mathematical modeling of anomalous diffusion phenomena leads to so-called nonstandard fractional diffusion equations, which are highly nonlocal. Usually, the exact solutions to these equations cannot be computed explicitly, so that efficient numerical schemes for their constructive approximation up to a predefined tolerance are needed. Due to the nonlocality, the numerical treatment of nonstandard diffusion equations is quite challenging. We are in particular interested in (adaptive) numerical schemes based on wavelets (Chegini *et al*, 2013), as wavelets are particularly tuned to these kinds of equations, and the research group Numerical Analysis has a long standing expertise in this field (Dahlke *et al*, 2007).

Dynamics of regulatory networks for cell polarity

Spatial-temporal oscillations of proteins in bacterial cells play an important role in many fundamental biological processes. We study these processes in the context of cell polarity in *Myxococcus xanthus* in close collaboration with the group of Lotte Søgaard-Andersen. The motility of *M. xanthus* is due to two motility systems: an A-motility system, and a type-IV pili system. The alignment of both motility systems on the cell surface depends on the correct localization of regulatory proteins at the cell poles which set up a polarity axis. In response to signaling by the Frz chemosensory system, these regulatory proteins are released from the poles and then rebind at the opposite poles. Thus, over time, the regulatory proteins oscillate between the poles. The aim of our project is to derive mathematical models that describe this behavior. Until now, we have studied mathematical models involving two and more regulatory proteins, with and without stochastic influences (Fig.1 & 2) (Rashkov *et al*, 2012; Rashkov *et al*, 2013; Rashkov *et al*, 2014). The models are based on so-called reaction diffusion equations. We are working on the mathematical analysis of these models (stability analysis etc.) as well as on the numerical simulation of the corresponding solutions. It turns out that the models are quite robust, and that a lot of different oscillation scenarios can be reproduced.

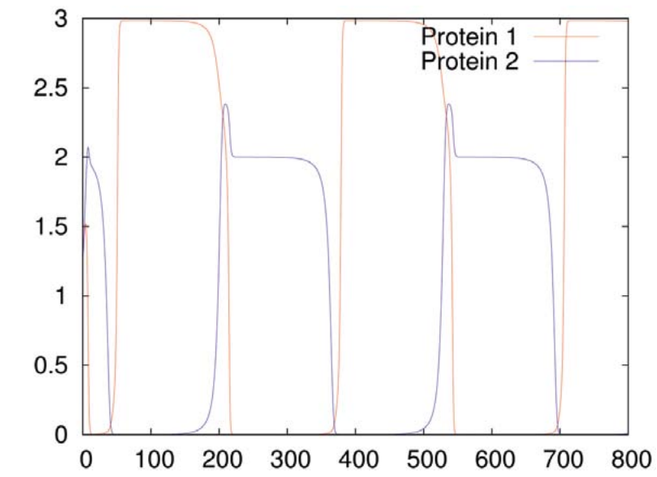

Figure 1. *Visualization of the oscillations of two proteins in a Myxococcus xanthus cell. Both proteins stay at opposite poles of the cell for a relatively long time, followed by a very fast switch, as observed in practice.*

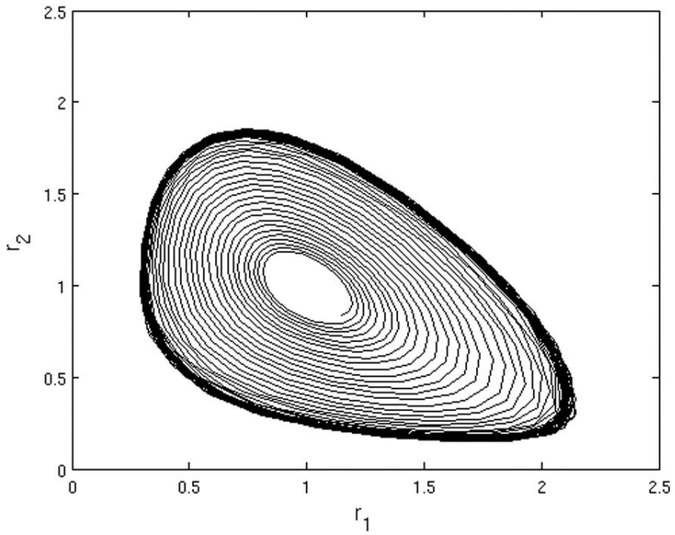

Figure 2. *Oszillations of a protein at one pole in a cell. We observe that stable protein oscillations without external trigger are possible.*

Localization of flagella in bacteria

Only recently, our group embarked on the mathematical modeling of the mechanisms underlying bacterial flagella localization. It has long been known that the localization of flagella as well as their number differs significantly for the various different types of bacteria. The formation of a flagellum is initiated by the localization of specific proteins at the cell membrane. Our aim is to derive new reaction-diffusion equations that describe these protein localizations and to determine the underlying parameters. Within this project, we collaborate with the research group of Gert Bange.

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1995–1996 Assistant Professor, RWTH Aachen
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Diffusive processes in and around bacterial cells

The correct functioning of a cell requires that all its molecular constituents are available at the right time and at the right location. However, molecular diffusion acts against any localization efforts of the cell: collisions with other molecules in the cytosol gradually reduce gradients until the concentration is uniform. The effects of diffusion are reduced for larger assemblies, and they also depend on the degree of molecular crowding or geometrical structuring of space, e.g., by organelles or cytoskeletal elements. Beyond the interior of the cell, diffusion is also a significant factor in the cell's environment, where it impacts nutrient distribution and uptake, and thereby the cell's chemotaxis.

The point of reference for diffusive processes is "normal diffusion", with a Gaussian concentration profile and a displacement Δx^2 that grows linearly with time, $\Delta x^2 \approx t$. Interactions with the structural elements or the motion of cells can cause significant deviations, resulting in either faster or slower spreading. Studying these deviations provides valuable information about the underlying mechanisms and the effects on their function. We are interested in normal and non-normal diffusion in two particular settings: the motion of proteins in membranes, in a collaboration with the group of Peter Graumann, and the stirring of surrounding fluids by swimming microorganisms, together with the groups of Gert Bange and Kai Thormann (JLU Gießen).

Protein dynamics in the cytoplasmic membrane

The cellular membrane contains many proteins that are responsible for important cellular functions. Some handle the exchange of signals and nutrients between the cell and its environment, others act as assembly platforms. In many cases, the details of their function are unknown. Since the membrane provides a fluid environment, the location of the proteins is not fixed, and their diffusive motion complicates their study. High-resolution STED microscopy allows to visualize and track fluorescently labeled proteins and to trace their movements, in order to learn about their dynamics and the interactions and colocalizations with other proteins in the membrane.

Using live cell high-resolution STED microscopy, the motion of YFP-tagged flotillins, membrane proteins that coassemble into discrete microdomains, can be observed in *Bacillus subtilis* membranes (Fig. 1). For the proper interpretation of the observed motions one has to take into account that they are projections of the actual motions into the 2D focal plane, but calculations allow to undo these projections. They further show that the diffusion of flotillins in the membrane is isotropic, i.e. with no directional preference. With the imaging procedures established we can now study other proteins as well, and can characterize their interactions and their spatio-temporal dynamics within the membrane.

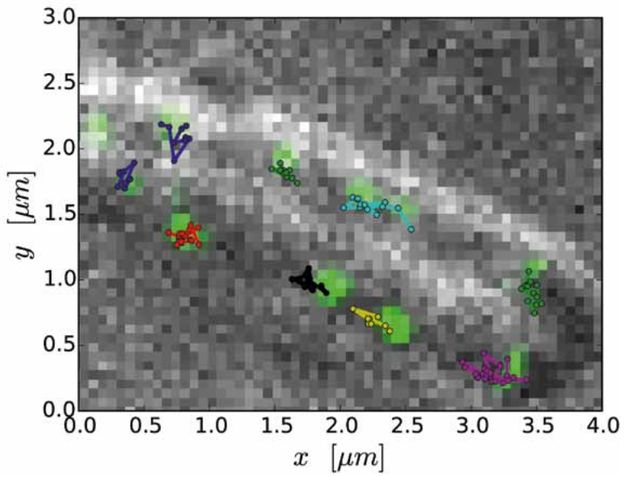

Figure 1. *Bright field image of a single Bacillus subtilis cell, with the initial location of fluorescent proteins overlayed in green and colored lines indicating movements of the respective proteins.*

Stirring and dispersion in bacterial solutions

The motion of microorganisms introduces flows in their environment that affect the dispersal of nutrients and other solutes. The flows can be visualized and measured by the tracking of passively advected particles, which in case of *Escherichia coli* and the marine alga *Chlamydomonas reinhardtii* has revealed that the spreading process does not follow the laws of normal diffusion: the probability density functions are not Gaussian, and the spreading law can change from a normal diffusive law to a super-diffusive law. In order to further explore the origins and implications of this process, we study dispersion in suspensions of *Shewanella*. Genetic engineering allows for the modification of the swimming properties of *Shewanella* by changing the number of flagella, their biochemical composition and the operation of the chemotactic apparatus. This enables us to change the swimming speed and the directionality of the motion, and to explore their impact on particle dispersion in the solvent.

With high frequency cameras and microscopes of sufficient resolution it is possible to track the motion of particles and cells in 2D and 3D. The trajectories of the cells show straight segments during the run phase and sharp directional changes during the tumble phase. The particles in the solvent are affected both by the velocity fields surrounding the swimming cells and the Brownian forces from the solvent. They follow rather ragged trajectories, with sometimes fairly elongated excursions (Fig. 2). When the positions of the particles after some time are collected in a probability density function (pdf), one notices strong deviations from the normal Gaussian distribution: long excursion are much more frequent, and their probability is much better described by an exponential than a Gaussian distribution (Fig. 3). In Ref. (Eckhardt & Zammert, 2012), we have related this behavior to another general class of diffusion processes, so-called continuous time random walks, where long excursions and a persistence of motion are allowed. The fit to the data is encouraging. In ongoing efforts, we now want to explore the effects of changes in the flagellar structure, number, and activity on the efficiency of swimming in the environment.

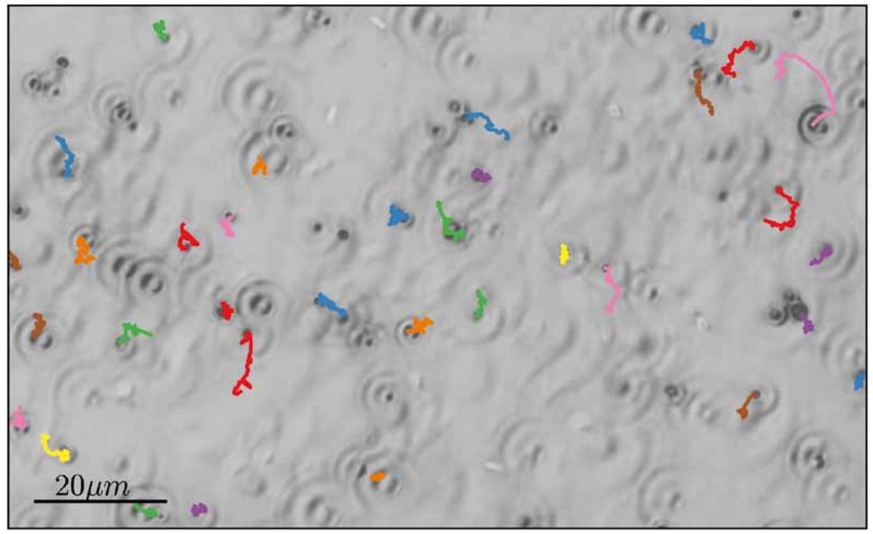

Figure 2. *Traces of small passive particles moved around by swimming Shewanella. Different colors mark different particles.*

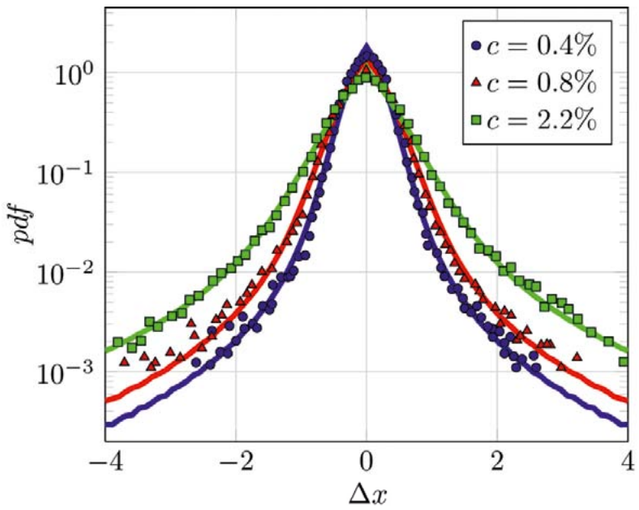

Figure 3. *Probability density functions (pdf) for the displacement of passive particles in a bacterial bath. Experimental data of (Leptos et al, PRL 103 (2009) 198103) for different concentrations together with the fit to a continuous time random walk (Eckhardt & Zammert, 2012).*

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2013–2014 Visiting Professor, University of California, San Diego, USA
2012– Professor of Biochemistry, Philipps-Universität Marburg
2004–2012 Professor of Microbiology, Albert-Ludwigs-Universität Freiburg
2000–2004 Independent Reseach Group Leader, Philipps-Universität Marburg
2003 Habilitation, Philipps-Universität Marburg
1997–2000 Postdoctoral Fellow, Harvard University, USA
1997 Ph.D. in biology/biochemistry, Philipps-Universität Marburg
1994 Diploma in biology, Philipps-Universität Marburg

AWARDS & HONORS

2008 Research Award of the Association for General and Applied Microbiology (VAAM)
2004 Heisenberg Fellow
2000–2004 Emmy Noether Independent Junior Research Group Leader

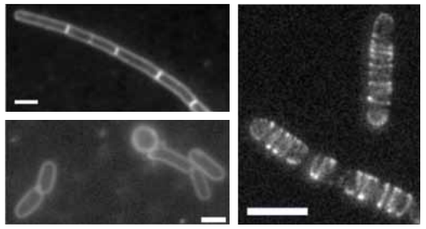
Bacterial cells in 3D

Despite their apparent simplicity, bacteria have a sophisticated cell structure and shape. E.g., they can be round, rod-shaped, bent, or helical. Our group is investigating two bacterial species, the Gram-positive soil-dwelling bacterium *Bacillus subtilis*, and – in a subgroup headed by Dr. Barbara Waidner – the Gram-negative human pathogen *Helicobacter pylori*. As *B. subtilis* is rod-shaped, while *H. pylori* is helical, we analyse the differences and similarities of both organisms in generating their cell shape. Furthermore, we are interested in the general question how bacterial cells are organized in 3D. For instance, not only proteins, but also genes have a defined position within the cell caused by a specific folding of the bacterial chromosome. The unravelling of the molecular network underlying this 3D organization is one of the most fascinating questions of today's cell biology.

Maintenance of cell shape

In the development of cell morphology, filament-forming proteins play a key role. E.g., in many rod-shaped bacteria like *Bacillus subtilis*, the actin-like protein MreB mediates the elongated form with its two defined cell poles and the consequential axes (Graumann, 2007). As such, MreB is essential for viability, and *mreB* depleted *B. subtilis* cells become spherical, enlarged and finally lysed (Fig. 1A). Gram-positive bacteria often have multiple *mreB* homologs, and *B. subtilis* has three: MreB, Mbl and MreBH. However, MreB is not needed for cell shape maintenance and viability in the helical Gram-negative human pathogen *Helicobacter pylori* (Waidner *et al*, 2009; Specht *et al*, 2011). In this organism, four filament-forming coiled coil-rich proteins (Ccrps) influence cell curvature (Fig. 1C). Purified MreB and Ccrps form extended filamentous structures *in vitro* that can be imaged by fluorescence microscopy (Fig. 1B) or by electron microscopy (Fig. 1D). MreB polymers contain ATP or GTP, are dynamically self-remodelling structures, and MreB of *B. subtilis* forms joint filamentous structures with its two paralogs (Fig. 1B). Ccrps do not need a nucleotide cofactor for filament formation and form very stable polymers (Fig. 1D).

Bacillus
A) MreB *in vivo*



B) MreB and homologs *in vitro*

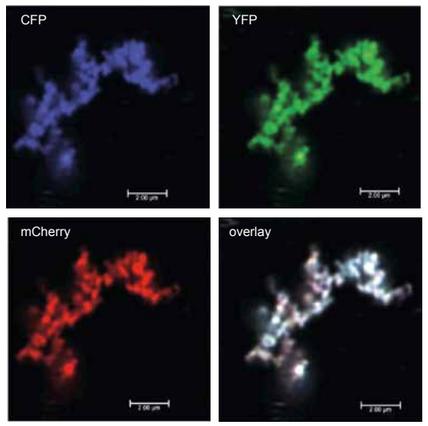
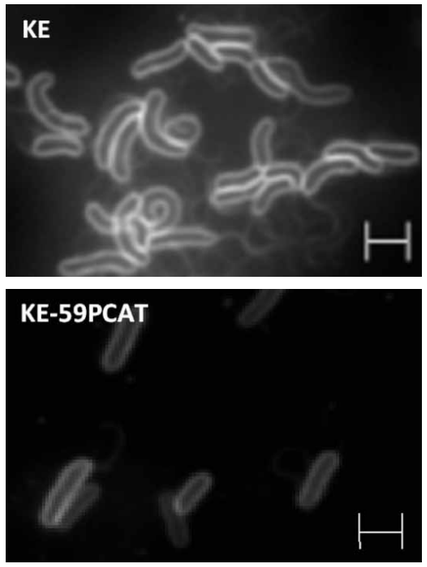
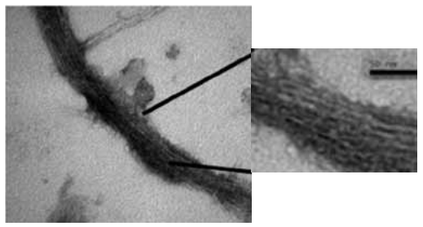


Figure 1. *(A) Fluorescence microscopy images of B. subtilis PY79 membranes demonstrating the effect of mreB depletion (left, top and bottom), and STED super resolution fluorescence microscopy image of YFP-MreB in B. subtilis cells (right). (B) B. subtilis MreB and its two homologs Mbl and MreBH jointly form filamentous structures on a flat membrane as shown via fluorescence microscopy images (using CFP, YFP and mCherry protein fusions, respectively) and the overlay of these.*

Helicobacter
C) Ccrp59 *in vivo*



D) Ccrp59 *in vitro*



(C) Effect of ccrp59 deletion (KE-59PCAT) on H. pylori wt (KE) cells. (D) Electron microscopy of Ccrp59 forming bundles of parallel filaments in vitro.
White bars 2 µm, black bar 50 nm.

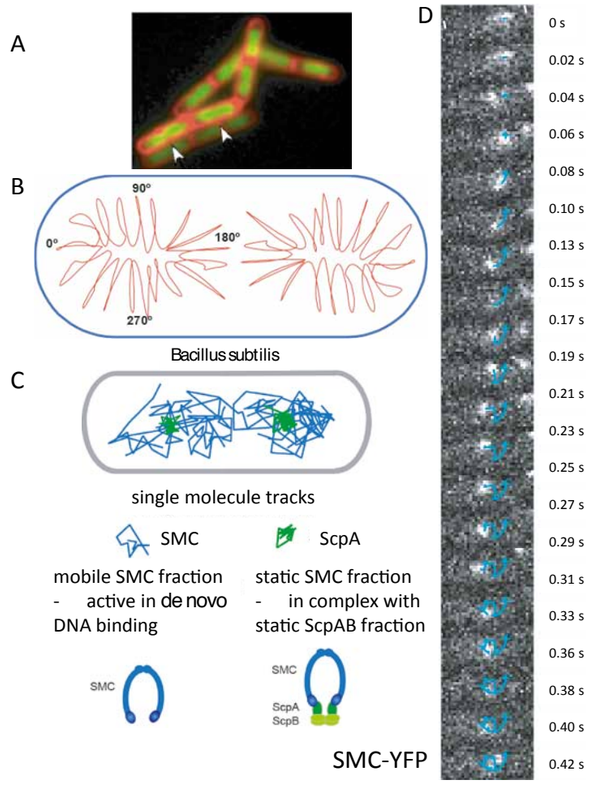


Figure 2. *(A) B. subtilis cells, DNA stained green, membranes stained red. White triangles indicate cells whose sister chromosomes are almost entirely separated into opposite cell halves. (B) Cartoon of a bacterial cell (cell membrane in blue), in which the chromosome (red) is folded in an ordered manner; two chromosomes are shown, after cell division in the middle of the cell, each daughter cell has an origin (0°) at one cell pole, a terminus (180°) at the other pole, and the chromosome arms in between (90° and 270° are indicated). (C) Cartoon of a B. subtilis cell in which ScpAB-bound SMC (green tracks) are static within two condensation centres, while free SMC (blue tracks) moves all over the genome. (D) Movement of a single SMC-YFP molecule in a single Bacillus cell, tracked every 20 ms; tracks are indicated by blue line.*

Separation of sister chromosomes

Bacterial chromosomes are organized into "nucleoids" (green in Fig. 2A), and have a relatively fixed arrangement (Fig. 2B). This specific folding results in defined 3D positions of genes within the cell, and this specific order is even kept during chromosome replication, segregation and cell division. How is the arrangement of the chromosome kept in such a "perfect" order?
One pivotal protein family for this process are SMC (structural maintenance of chromosomes) proteins. These highly conserved proteins are essential for chromosome organisation and segregation in bacteria, and for mitosis and meiosis in eukaryotes. SMC proteins are very large (135-150 kDa) proteins and form flexible dimers with two long arms and head domains at both ends enabling the dimerization in an ATP-dependent manner (Fig. 2C, bottom). This way they can form rings around DNA and hold individual DNA strands together. In *B. subtilis*, the SMC homodimer associates with two regulatory proteins known as ScpA and ScpB. However, how they compact entire chromosomes is still unclear for all kinds of cells. Recently, we have tracked individual SMC molecules in living cells using "slim-field" fluorescence microscopy (Fig. 2D, the "track" of the molecule is shown in blue). Our findings reveal two distinct modes of interaction of SMC with the chromosome: ScpAB-bound SMC stays stationary in co-called condensation centres, while free SMC runs all over the chromosome (Fig. 2C) (Kleine Borgmann *et al*, 2013). Thus, whereas SMC alone dynamically interacts with many sites on the chromosome, it forms static assemblies together with ScpAB. How the two fractions of SMC contribute to the compaction of the whole chromosome, and how the condensation centres are formed and turned over, is subject of our ongoing studies.

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Complex Systems

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2002–2006 Junior Professor of Theoretical Physics, Philipps-Universität Marburg
1999–2001 Postdoctoral Fellow, Harvard University and Stanford University, USA, and Institut Curie, Paris, France
1998 Ph.D. in theoretical physics, Max Planck Institute for Colloids and Interfaces, and University of Potsdam
1995 Diploma in physics, Universities of Regensburg, Hamburg, and Heidelberg

AWARDS & HONORS

2013 Paula und Richard von Hertwig Award (together with collaborators)
1999 Otto Hahn Medal of the Max Planck Society

Physics in synthetic biology

A major insight of the post-genomic era is that the complexity of biological organisms arises not only from the large number of their constituents, but also from the interactions between these components. Consequently, the analysis of living matter has changed dramatically in the last decade. Instead of individual genes and proteins, now modules and networks are in the focus of many studies. In this context, theoretical descriptions become more and more important. Long-term goal of such approaches is to obtain a quantitative understanding of complete organisms. However, even for the best characterized bacteria many constituents and their interactions are still unknown. Therefore, smaller subsystems have to be investigated and mathematically described. In our group, we do this using different physics-based approaches that range from molecular models to effective macroscopic descriptions of biological systems.

Bacterial pattern formation

Periodic stripe patterns are ubiquitous in living organisms. In many cases, however, the underlying developmental processes are complex and difficult to disentangle. In a novel synthetic biology approach in collaboration with groups from San Diego and Hong Kong, we have implemented a genetic circuit coupling cell density and motility into the bacterium *Escherichia coli* (Fig. 1). These cells excrete a small signaling molecule acyl-homoserine lactone (AHL), such that at low AHL levels, cells are motile, while at high AHL levels, cells tumble incessantly and become immotile. On agar plates these engineered bacteria form periodic stripes of high and low cell densities sequentially and autonomously. To theoretically study the origin and mechanism of this process, we developed a kinetic model that includes growth and density-suppressed motility of the cells. In this model, we analyzed the onset of pattern formation by calculating the front profile of a region of cells that spread into an initially cell-free region. From this exact solution, we obtained an analytic approach to determine the phase boundary between the stripe and the no-stripe phases. From this solution, in turn, we were able to make various predictions how the patterns could be tuned experimentally (Liu *et al*, 2011).

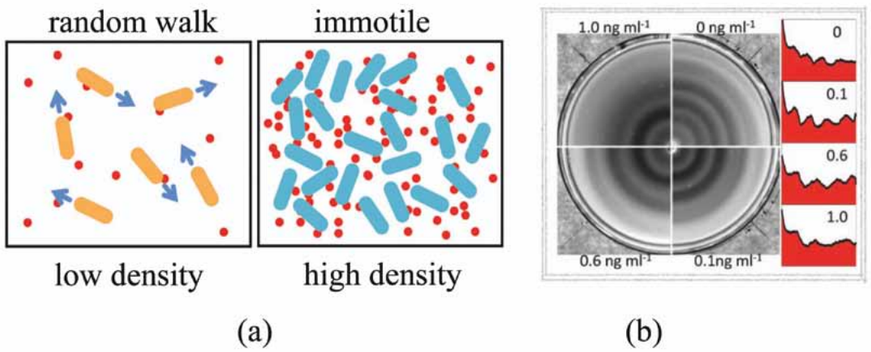


Figure 1. *(a) The engineered bacterium cells execute random walks at low densities, but become immotile at high densities. (b) This coupling between density and motility leads to the formation of sequential patterns with tunable number of rings.*

Regulation of bacterial metabolism

In all domains of life signaling pathways link perception of environmental or intracellular cues to specific adjustments in cellular function. In this project, we have reanalyzed one of the best-studied systems of microbiology, the cyclic AMP (cAMP) dependent pathway in *E. coli* that mediates carbon catabolite repression (CCR) (Fig. 2). In doing so, we could demonstrate that cAMP has a much broader physiological role than merely being the signaling molecule of carbon status that mediates CCR. Rather, the cAMP signaling pathway coordinates the allocation of proteomic resources in response to the global metabolic needs of exponentially growing bacterial cells. More precisely, we revealed surprising relations linking the expressions of catabolic and biosynthetic genes: the expression of catabolic genes increased linearly with decreasing growth rates upon limitation of carbon influx, but decreased linearly with decreasing growth rate upon limitation of nitrogen or sulfur influx. In contrast, the expression of biosynthetic genes exhibited the opposite linear growth rate dependence as the catabolic genes. This striking linear response in gene expression to nutrient limitation can be explained quantitatively by a coarse-grained mathematical model based on two powerful constraints governing cellular metabolism: the balance of metabolic fluxes and the partitioning of the ribosomal load. Key feature of the model is the prediction that carbon precursors, whose intracellular level reflects the difference between the catabolic and biosynthetic fluxes, implement an integral feedback scheme by regulating the level of cAMP which in turn controls the expressions of catabolic enzymes through the regulator Crp (You *et al*, 2013).

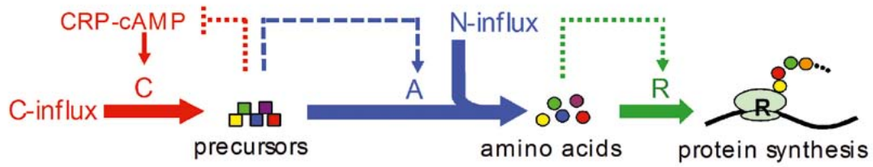
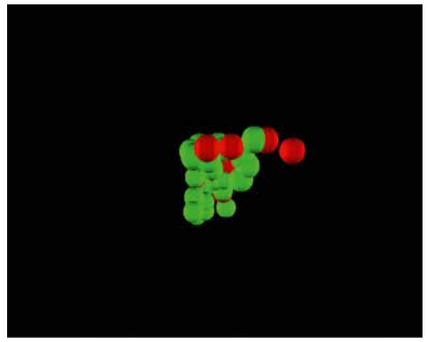


Figure 2. *In our coarse-grained mathematical model, carbon and nitrogen influxes combine to synthesize amino acids which are in turn assembled into proteins by the ribosomes (R). The external carbon source is converted to the pool of carbon precursors that sense the difference between the nutrient fluxes. The carbon and nitrogen fluxes are coordinated by an integral feedback system regulating the catabolic enzymes (C) and anabolic enzymes (A) by the carbon precursors. Cyclic AMP is the messenger regulating the carbon-branch.*

Spatial analysis and modeling of yeast flocculation

Multicellular development of *Saccharomyces cerevisiae* includes the process of flocculation, which is the calcium-dependent interaction among vegetative cells leading to multicellular aggregates, so-called flocs. It has been shown that the ability to form flocs can provide an evolutionary benefit for a yeast population, for instance by protecting individual cells from environmental stress such as ethanol, fungicides or other harmful conditions. In collaboration with Hans-Ulrich Mösch and Bernd Freisleben, two other members of SYNMIKRO, we set up a fluorescence microscopy-based system for the precise spatial analysis of single cells within individual flocs. To interpret the experimental findings, we developed a detailed mathematical model for yeast flocculation, which allows us to analyze the dependence of this process on various relevant parameters, such as, e.g., cell density or strength of the bonds between cells. To simulate our model process with acceptable runtimes, we developed a graphics processing unit (GPU) implementation that allows us to track the cell movement in a detailed manner by a 3D visualization during execution (Leinweber *et al*, 2014). This GPU implementation is up to 736 times faster than a multithreaded C/C++, enabling us to simulate up to 1.000.000 yeast cells in total. From these simulations, we have obtained several predictions that we are now testing experimentally. In our current efforts, we also focus on the behavior of mixed populations, where some cells do not express the relevant adhesion proteins required for flocculation. Our preliminary data indicate that such *cheater* cells are not only pushed to the boundary of the flocs, but also restricted to specific sites within the protective flocs.



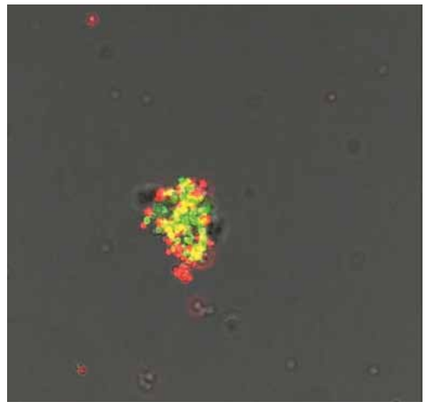


Figure 3. *Comparison between theoretically calculated (top) and experimentally observed (bottom) flocs formed by S. cerevisiae cells. In both cases, cheater cells (i.e., cells that do not express the relevant adhesion proteins required for flocculation) are shown in red.*

SELECTED PUBLICATIONS

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Lenz P & Swain PS (2006). An entropic mechanism to generate highly cooperative and specific binding from protein phosphorylations. *Current Biol* 16, 2150-5.

PROF. DR.
LOTTE SØGAARD-ANDERSEN

Bacterial Development and Differentiation

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SHORT CV

2008– Professor of Microbiology, Philipps-Universität Marburg
2004– Director and Head of the Department of Ecophysiology, Max Planck Institute for Terrestrial Microbiology, Marburg
2002–2004 Professor of Molecular Microbiology, University of Southern Denmark, Denmark
1996–2002 Associate Professor, University of Odense, Denmark
1992–1996 Assistant Professor, University of Odense, Denmark
1994–1995 Visiting Scientist, Stanford University, USA
1991 Ph.D. in molecular biology, University of Odense, Denmark
1990 Visiting Scientist, Institute Pasteur, Paris, France
1988 M.D., University of Odense, Denmark
1984 M.Sc. thesis in molecular biology, University of Odense, Denmark

Synthetic microbiology & modularity of cell polarity systems

All living cells are polarized and contain proteins that localize asymmetrically to specific subcellular regions. In bacteria as well as in eukaryotic cells, many of these proteins are important for fundamental cellular processes such as cell division, growth, motility and differentiation. Therefore, we have as a working hypothesis that streamlined natural cells as well as synthetic cells depend on some level of subcellular organization for optimal function. The overall goal of our work in synthetic microbiology is to generate a module for regulating dynamic cell polarity in streamlined natural cells as well as in synthetic cells.

Dynamic regulation of type IV pili polarity

For most dynamically localized proteins, the localization pattern changes in a cell cycle-dependent manner. An exception to this general rule are the proteins of the type IV pili-dependent motility system in the rod-shaped cells of *Myxococcus xanthus* that localize dynamically to the cell poles in a cell cycle-independent manner. In the case of this motility system, the machinery assembles at the leading pole of a moving cell, and during a reversal it disassembles at the old leading pole and reassembles at the new leading pole. We have shown that 8 of the 10 proteins required for type IV pili function localize to both cell poles and remain stationary during reversals (Fig. 1) (Friedrich *et al*, 2014). Conversely, the PilB and PilT ATPases that energize extension and retraction of type IV pili, respectively, localize to opposite poles with PilB predominantly at the leading and PilT predominantly at the lagging pole, and these proteins switch poles during reversals. In other words, type IV pili pole-to-pole switchings depend on the dynamic localization of PilB and PilT.

Our goal is to identify and characterize the components of the regulatory system that underlies the dynamic polarity of motility proteins in *M. xanthus*. On the basis of this system, we aim to define a minimal module for regulation of dynamic cell polarity in bacteria and to establish this module in other microorganisms as well as in synthetic cells. As part of this research, we are addressing the more fundamental question whether cell polarity systems are indeed modular. In other words, can these systems be transferred between organisms and still function? Or are they so tightly integrated with host cell physiology that function is restricted to the original host?

The polarity module

We recently showed that the regulatory system controlling the polarity of motility proteins in *M. xanthus* is built around the MglA, MglB and RomR proteins, which interact to define the leading/lagging polarity axis of the cell (Fig. 2). MglA is a small Ras-like GTPase that functions as a nucleotide-dependent molecular switch to regulate motility in *M. xanthus* (Leonardy *et al*, 2010; Miertzschke *et al*, 2011; Keilberg *et al*, 2012; Bulyha *et al*, 2013). The MglB protein functions as a GTPase activating protein (GAP) and converts active MglA-GTP to the inactive MglA-GDP. In a moving cell, MglA-GTP localizes to the leading cell pole together with RomR, while MglB localizes to the lagging cell pole, also together with RomR. Between reversals, MglA-GTP sets up the correct polarity of the dynamical motility proteins PilB and PilT by an unknown mechanism. In response to signaling activity of the Frz chemosensory system, MglA, MglB and RomR are released from the poles and then relocate to the respective opposite poles. In total, this results in an inversion of the leading/lagging polarity axis and the relocation of dynamic motility proteins.

In our ongoing research, we focus on defining the parts of this cell polarity system and on elucidating how the various proteins interact to understand how they can localize correctly to the cell poles and how they switch poles in a coordinated manner. In parallel, we are attempting to establish the system in heterologous hosts.

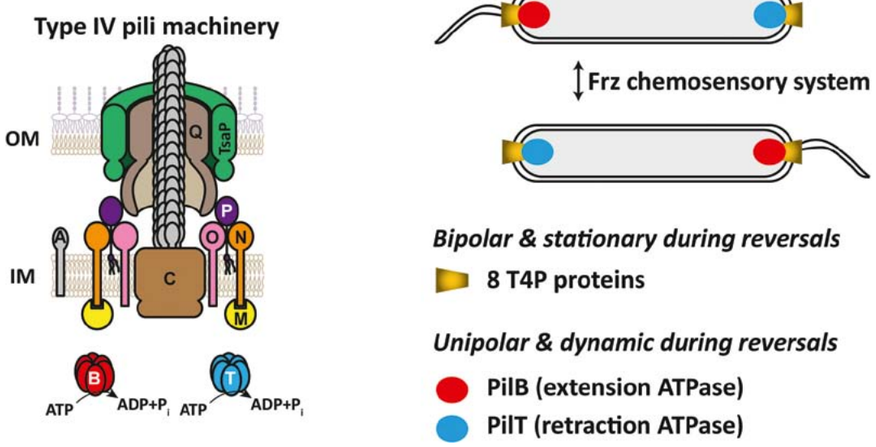

Figure 1. *Type IV pili pole-to-pole switching depends on the dynamic polar localization of PilB (red, at leading pole) and PilT (blue, at lagging pole).*

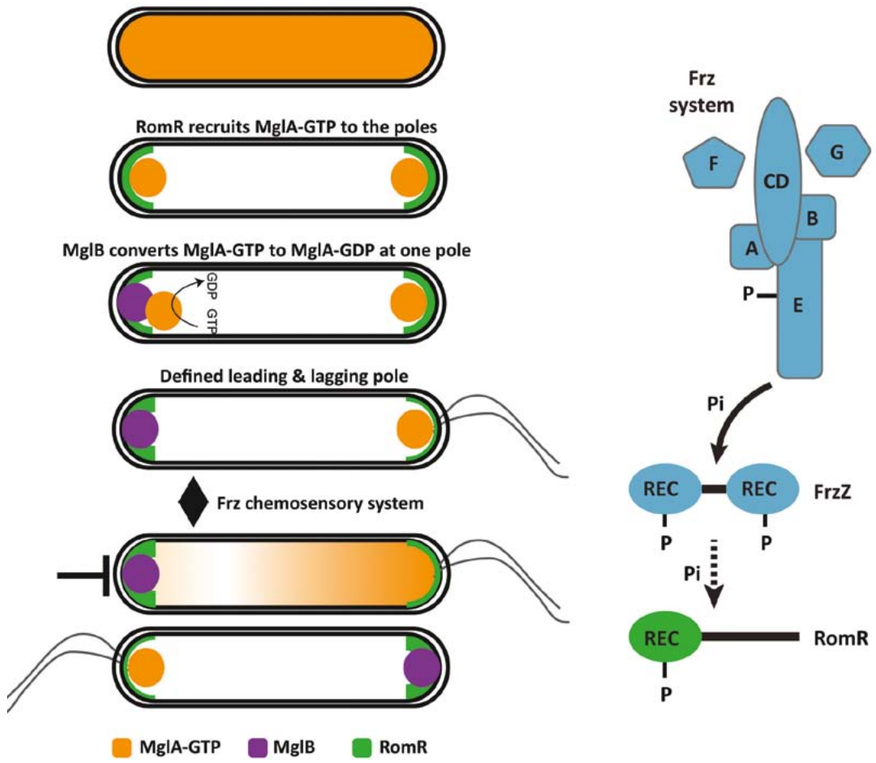

Figure 2. *Left panel, MglA, MglB and RomR establish the leading/lagging cell polarity axis. Right panel, the Frz chemosensory system signals to the RomR response regulator for reversals.*

AWARDS & HONORS

2014 Elected Fellow of the American Academy of Microbiology
2008 Elected Member of the German Academy of Sciences Leopoldina
1999 Research grant from the Danish Research Councils via the Female Researchers in Joint Action program (FREJA-program)
1995 Odense Kommune's Research Award to the Advancement of Science
1992 The Danish Academy of Natural Sciences award for best Ph.D. thesis 1991/1992

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Leonardy S, Miertzschke M, Bulyha I, Sperling E, Wittinghofer A, Søgaard-Andersen L (2010). Regulation of dynamic polarity switching in bacteria by a Ras-like G-protein and its cognate GAP. *EMBO J* 29, 2276–89.

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2007–2014 Max Planck Research Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
2002–2006 Postdoctoral Fellow, Stanford University, USA
2002 Ph.D. in microbiology/biochemistry, Ludwig-Maximilians-Universität München
1998 Diploma in biology, Ludwig-Maximilians-Universität München

AWARDS & HONORS

2011 Research Award of the Association for General and Applied Microbiology (VAAM)
2007 Max Planck Research Group appointment
2003–2005 EMBO long-term postdoctoral fellowship

Mechanisms of spatial organization in bacteria

Life depends on the proper arrangement of macromolecules in time and space to coordinate the complex and highly dynamic processes that underlie the function of biological cells. Unlike eukaryotes, bacteria usually do not make use of membrane-bounded organelles to segregate cellular components into distinct functional units. However, despite the apparent continuity of their cytoplasmic space, they have evolved a variety of mechanisms to recruit proteins and DNA to specific subcellular locations, thereby generating pseudo-compartments with distinct biological functions. Our research focuses on the mechanisms that provide the spatial information required to position cellular components, with an emphasis on essential cellular processes such as cell division, chromosome segregation, and morphogenesis. In particular, we aim at deconstructing the complex machineries involved in these processes, and at characterizing individual functional modules using synthetic biological and reverse-engineering approaches. Such in-depth knowledge of the organizing principles at work in native systems will be critical for engineering machineries that control the function and propagation of synthetic cellular units in the future.

Regulation of division site placement

In most bacteria, cell division is mediated by a multiprotein complex called the divisome, which assembles on a ring-like polymeric structure formed by the bacterial tubulin homolog FtsZ. We have previously identified a novel regulatory mechanism that controls the positioning of the divisome in the model bacterium *Caulobacter crescentus*. It is founded on the ATPase MipZ, which dynamically interacts with pole-associated complexes of the chromosome segregation protein ParB (Fig. 1). As a consequence, MipZ forms a gradient within the cytoplasm, with its concentration being highest at the tips of the cell and lowest at the cell center. MipZ acts as a direct inhibitor of FtsZ polymerization, thus limiting formation of the division apparatus to the midcell region.

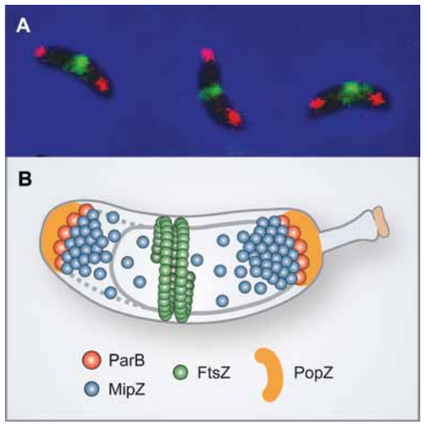

Figure 1. *Positioning of FtsZ by the MipZ•ParB•origin complex. (A) In vivo localization of ParB (red) and FtsZ (green) in C. crescentus cells. (B) Schematic representation of the components involved in the MipZ system.*

Steady-state protein gradients are a well-known regulatory strategy in eukaryotic cells, usually established by localized protein synthesis followed by diffusion and protein degradation. However, such diffusional gradients are extremely unstable at the much smaller scale of prokaryotic cells, suggesting that formation of the MipZ gradient is based on a different principle. Indeed, we have recently shown that MipZ serves as a molecular switch that, similar to small GTPases, uses nucleotide binding and hydrolysis to alternate between two different functional states with distinct interaction networks and diffusion rates. This behavior drives a unique localization cycle, with MipZ molecules oscillating back and forth between the polar ParB complexes and pole-distal regions of the nucleoid. Our results for the first time shed light on the basis of steady-state gradient formation in bacteria and might provide a general mechanistic framework for other gradient-forming systems in both prokaryotic and eukaryotic cells (Kiekebusch & Thanbichler, 2014). They thus form the basis for designing regulatory modules for the positioning of protein complexes in synthetic contexts.

Bactofilins: a new cytoskeletal scaffold

In addition to the cytokinetic FtsZ ring, there are several other cytoskeletal elements with a role in the spatiotemporal organization of bacterial cells. Our group has recently identified a novel class of cytoskeletal proteins named bactofilins. We demonstrated that, in *C. crescentus*, two bactofilin paralogues (BacAB) assemble into membrane-associated polymeric sheets that specifically localize to the old cell pole during defined stages of the cell cycle (Kühn *et al*, 2010). These structures mediate the polar localization of a cell wall biosynthetic enzyme involved in polar morphogenesis (Fig. 2). Bactofilins polymerize spontaneously and in the absence of nucleotide cofactors, forming long, biochemically inert filaments. This behavior is reminiscent of intermediate filaments, even though there is no evolutionary or structural relationship between these two groups of proteins.
Interestingly, bactofilins are almost universally conserved among bacteria. To clarify the spectrum of functions they can perform, we have set out to study the role of bactofilins in other bacterial species.

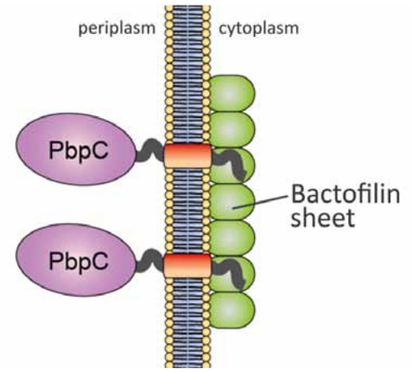

Figure 2. *Model for the recruitment of the cell wall synthase PbpC to membrane-associated bactofilin polymers in C. crescentus.*

In particular, we focus on the delta-proteobacterium *Myxococcus xanthus*, an organism containing four bactofilin homologs. In collaboration with the group of Lotte Søgaard-Andersen, we have shown that three of these proteins co-polymerize into extended bipolar filaments. These structures, on the one hand, mediate the subpolar localization of a small GTPase (MglA) regulating the dynamics of proteins involved in cell motility. On the other hand, they contribute to proper chromosome segregation by controlling the subcellular localization of the chromosome segregation machinery (unpublished). Collectively, these findings suggest that bactofilins serve as multifunctional molecular scaffolds that ensure the proper arrangement of proteins within bacterial cells. It will be interesting to harness these structures for the development of synthetic molecular landmarks that enable the specific targeting of cellular components in artificial or heterologous cellular systems.

Bacterial protein diffusion barriers

Whereas eukaryotic cells contain a variety of membrane-bounded subcellular compartments, bacteria have long been thought to lack mechanisms to segregate their cytoplasmic space. However, we have recently discovered a protein-based diffusion barrier in *C. crescentus* that subdivides cells into physiologically distinct compartments (Schlimpert et *al*, 2012).
The *C. crescentus* stalk is an extension of the cell body that largely consists of cell envelope surrounding a thin thread of cytoplasm. It is interrupted at regular intervals by so-called crossbands, disc-shaped electron-dense structures whose nature and function had remained unknown. We found that these crossbands are polymeric complexes composed of at least four different proteins (StpABCD), which extend from the cytoplasmic core of the stalk to its outer membrane layer (Fig. 3). Blocking the diffusion of both membrane and soluble proteins in the cell envelope, they physiologically separate the stalk from other parts of the cell and thus ensure that newly synthesized envelope proteins are retained in the cell body. As a consequence, cells are able to adapt faster to environmental changes that require the *de novo* synthesis of proteins. Moreover, they need to produce less protein to achieve optimal working levels, thereby gaining a fitness advantage in the oligotrophic environment that *C. crescentus* inhabits. In the future, this machinery could be adapted for generating artificial cellular compartments that may facilitate the storage of toxic biosynthetic products.

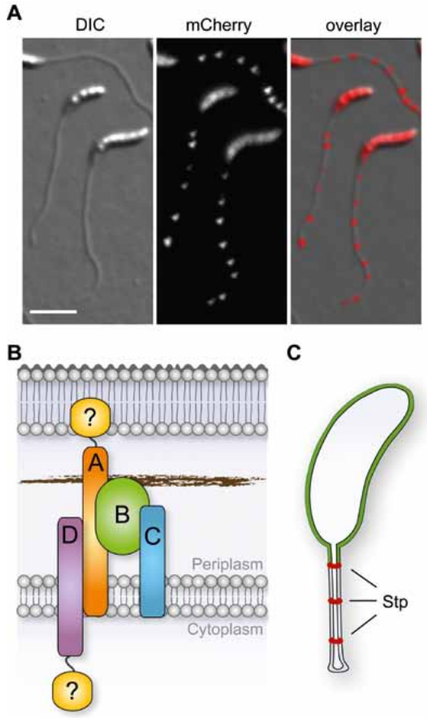

Figure 3. *The StpABCD diffusion barrier complex. (A) Localization of fluorescently tagged Stp complexes in live C. crescentus cells. (B) Model of the Stp complex. (C) Model showing how diffusion barriers (red) retain newly synthesized proteins (green) in the cell body, thereby reducing the effective cell size.*

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1.4 RESEARCH

Chassis and Genomes

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1995–2000 Research Group Leader, Bielefeld University
1994 Ph.D. in microbial genetics, Bielefeld University
1991 Diploma in biology, Bielefeld University

AWARDS & HONORS

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1998–1999 Lise Meitner Fellow, Ministry for Science and Research, NRW
1992–1994 Ph.D. scholarship, German National Merit Foundation
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Engineering of alpha-rhizobial genomes

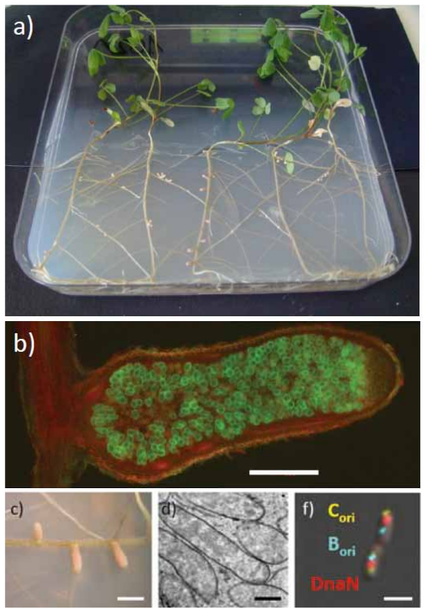
The size of bacterial genomes is highly variable, ranging from less than 0.15 mega base pairs (Mb) in obligate symbionts to more than 10 Mb in free-living soil-dwelling bacteria. Studying naturally occurring large bacterial genomes – which are usually found in bacteria subjected to frequently changing, highly diverse environmental conditions - reveals strategies of genome expansion and provides insights into the upper limit of genome size, as well as into concepts of regulation and genome maintenance. A prominent example of bacteria with large genomes is the group of α-rhizobial species that are able to form a root nodule symbiosis with leguminous host plants. The nodules are invaded by these α-proteobacteria which in the endosymbiotic bacteroid state fix molecular nitrogen to the benefit of the plant. Consistent with changing lifestyles between free-living and endosymbiotic states, multipartite large genomes are prevalent in α-rhizobia. These genomes often consist of one chromosome and two to six large plasmids. The *Sinorhizobium meliloti* genome is composed of a chromosome (3.65 Mb), and the megaplasmids pSymA (1.35 Mb) and pSymB (1.68 Mb) (Fig. 1). We use synthetic biology approaches to investigate the molecular requirements for the different lifestyles and explore the potential of α-rhizobial genomes for synthetic biology applications.

Regulatory circuits

Quorum sensing (QS) is a widespread mechanism of cell-cell signaling that allows bacteria to coordinate their gene expression in response to local population density. Communication occurs via small hormone-like molecules called QS signals. The most common QS signals in Gram-negative bacteria are *N*-acyl-homoserine lactones (AHLs), which are composed of a fatty acid chain of 4 to 18 carbons attached to a homoserine lactone ring (Fig. 2). QS systems provide parts and serve as blueprints for building intercellular communication modules to control and coordinate cellular behavior in a population.

We have investigated the function of a simple QS system in *S. meliloti*. This system consists of only three protein components implementing positive and negative feedback regulation to balance its activity (Fig. 2): AHL signal molecules with acyl chains of 14 or 16 carbons (long-chain AHLS) are produced by the synthase SinI. *sinI* expression is controlled by an interplay of two transcriptional regulators, SinR and the AHL receptor ExpR. Furthermore, ExpR has a broad regulatory role, including repression of motility genes and activation of genes responsible for biosynthesis of secreted sugar polymers (exopolysaccharides) (Charoenpanich *et al*, 2013; Charoenpanich *et al*, 2014).

A widespread mechanism of AHL perception involves binding of the signal molecules by cytosolic transcriptional regulators such as ExpR, which requires uptake of external AHLs. However, the outer membrane is supposed to be an efficient barrier for diffusion of long-chain AHLs. We found that sensing of these AHLs is facilitated by the outer membrane protein $FadL_{Sm}$, a homolog of the *E. coli* $FadL_{Ec}$ long-chain fatty acid



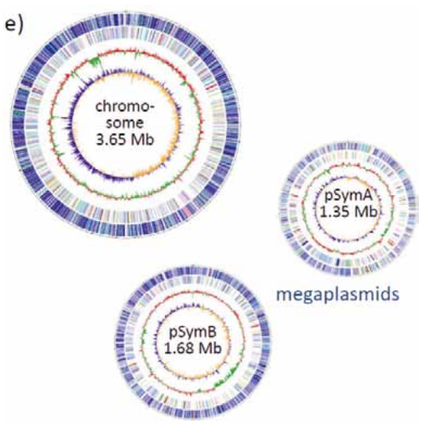


Figure 1. *(a) Medicago sativa plants nodulated by S. meliloti. (b) Root nodule section showing plant cells infected by S. meliloti labeled with the green fluorescent protein. Size bar, 500 µm. (c) Macroscopic view of root nodules. Size bar, 2 mm. (d) Electron micrograph of bacteroids. Size bar, 2 µm. (e) S. meliloti genome architecture. (f) Subcellular localization of the replication origins of the S. meliloti chromosome, pSymB megaplasmid and the DNA polymerase III component DnaN. Size bar 1 µm.*

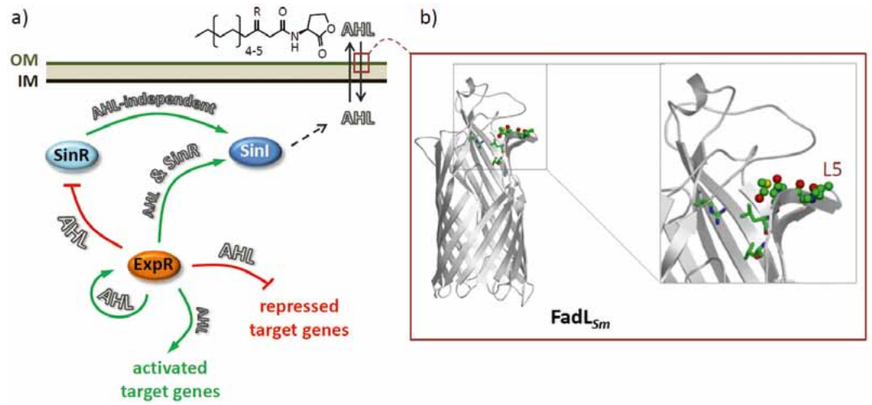


Figure 2. *(a) Scheme of the S. meliloti Sin quorum sensing regulatory system. Expression of the AHL synthase gene sinI requires the transcription activator SinR and is strongly enhanced by the regulator ExpR in the presence of AHLs, resulting in positive feedback. ExpR represses transcription of the sinI activating gene sinR at high AHL concentrations, leading to negative feedback regulation of sinI. Furthermore, ExpR controls expression of a multitude of target genes. (b) 3D structure model of the outer membrane protein $FadL_{Sm}$ facilitating uptake of long-chain AHLs. The extracellular loop L5 conferring specificity to these signal molecules is labeled. OM, outer membrane; IM, inner membrane.*

transporter. By building hybrid FadL proteins we demonstrated that the extracellular loop 5 (L5) of α-rhizobial FadL proteins contains determinants of specificity to long-chain AHLs (Krol & Becker, 2014) (Fig. 2). These experiments delivered a toolbox of L5 sequences that can be used to modify the specificity of native FadL proteins to mediate uptake of long-chain fatty acids and/or long-chain AHLs, which is a requirement for the implantation of long-chain AHL-based QS circuits into a heterologous host.

Population heterogeneity

The ability of clonal cell populations to split into subtypes displaying different phenotypes is a widespread phenomenon among prokaryotes that has recently intrigued the scientific community and has wide-ranging implications both for microbial ecology and biotechnology. Such phenotypic heterogeneity is the basis for strategies like bet hedging and division of labor, prominent examples being the occurrence of persister cells and cell differentiation in biofilms. Mechanisms frequently involve variation in gene expression, caused by fluctuations either in intrinsic or in environmental factors.

We have developed a versatile fluorescent reporter cassette that allows for simultaneous monitoring of activities of three promoters in single bacterial cells. This construct was applied to observe promoter activities related to QS and exopolysaccharide biosynthesis at single cell level in colonies of *S. meliloti* growing in a microfluidics system. QS signals and exopolysaccharides are secreted products that serve as common goods, and monitoring the expression of their biosynthetic genes therefore allows us to study bet hedging strategies and division of labor. In collaboration with Prof. Peter Pfaffelhuber (University of Freiburg), we developed a novel mathematical strategy for classification of the gene expression status in heterogeneous bacterial populations based on the time course of signal intensities of individual cells. Heterogeneous expression of exopolysaccharide biosynthesis and quorum sensing genes in *S. meliloti* populations was observed (Fig. 3). With respect to exopolysaccharide biosynthesis, cells in the population were classified into non-contributors, weak contributors, and strong contributors (Schlüter *et al*, 2015), showing that only one third of the population strongly contributed to the production of exopolysaccharide.

Genome engineering

To facilitate genome engineering in α-rhizobia, novel methods and tools were developed. These include single copy plasmids and controllable gene expression systems. Genome modifications were simplified by development of a cloning-free PCR-based method for gene disruption, deletion and replacement, and insertion of heterologous DNA.

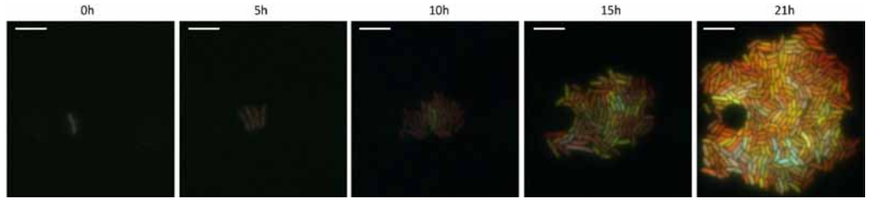


Figure 3. *Time lapse of a S. meliloti colony grown in a microfluidics chamber. Cells carry fluorescence reporter gene fusions to specific promoters of exopolysaccharide biosynthesis and quorum sensing genes. Images represent an overlay of three fluorescent markers. Size bar, 5 µm.*

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1980–1982 Research Associate, Max Planck Institute for Biochemistry, Martinsried
1974–1980 Postdoctoral Fellow and Junior Staff Member, Cold Spring Harbor Laboratory, USA
1974 Ph.D. in genetics, Free University, Berlin
1972 Diploma in microbiology, Georg-August-Universität Göttingen

Adaptation of a pathogen to the plant host

In our SYNMIKRO project, we aim to elucidate how secreted proteins can cross membranes: The biotrophic plant pathogenic fungus *Ustilago maydis* (Fig. 1) secretes a cocktail of about 300 novel protein effectors that modulate host defense responses and reprogram the host metabolism in favor of the pathogen (Djamei & Kahmann, 2012; Lo Presti *et al*, 2015). After secretion, effectors can either remain and function in the interaction zone that is established between fungal hyphae and host plasma membrane (apoplastic effectors), or they can be translocated to the host cell (translocated effectors). Neither the features/motifs required for this translocation nor the uptake mechanism are currently known. We expect that elucidating these requirements and mechanism will provide an important tool for the manipulation of protocells in synthetic microbiology.

Translocated effectors

We have identified the first translocated effector of *U. maydis* by electron microscopy after immunogold labeling (Djamei *et al*, 2011). This effector, a virulence determinant, is an active chorismate mutase (Cmu1) that modulates the metabolism of maize by lowering the level of salicylic acid in infected tissue (Fig. 2).

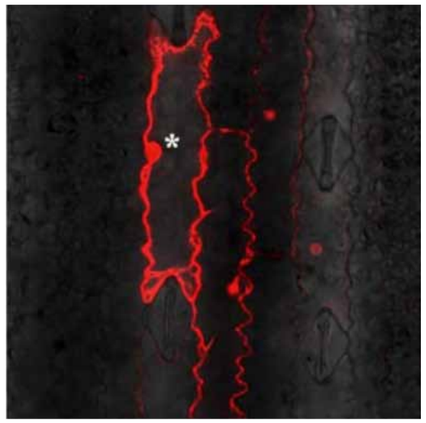

Figure 2. *When transiently expressed in maize cells, a Cmu1-mCherry fusion protein spreads from the transformed cell (*) to adjacent non-transformed cells to prepare these for infection by lowering salicylic acid levels in infected tissue.*

A

B

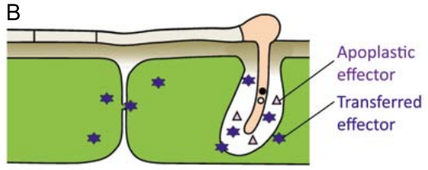

Figure 1. *(A) Corn smut caused by an U. maydis infection. The kernels of the infected maize cob are much-enlarged and form distorted tumors. (B) An U. maydis hypha (pink) infecting maize (green). The hypha secretes effector proteins that either stay in the interaction zone between pathogen and host cell (pink triangles) or are translocated into the host cell (blue stars).*

Cmu1 is initially secreted to the interaction zone between fungal hyphae and host plasma membrane and is then taken up by plant cells. Inside plant cells Cmu1 resides in the cytosol and can spread to neighboring cells through plasmodesmata to prepare them for the upcoming infection. Cmu1 is then speculated to increase channeling of chorismate from the chloroplasts to the cytosol together with the cytoplasmic maize chorismate mutase, by converting chorismate to prephenate, which might return to the plastids to serve the biosynthesis of aromatic amino acids. This would lower chorismate levels in chloroplasts und thus decrease the amounts of precursor that could go into salicylic acid biosynthesis. The plant hormone salicylic acid can induce cell death associated immune responses which would be detrimental for a biotrophic fungus like *U. maydis* that relies on maintaining its host cells alive. Thus, by translocating Cmu1 to plant cells, *U. maydis* is reprogramming these cells metabolically for its own needs.

We have also shown that a catalytically inactive version of Cmu1 ($Cmu1_{KR}$) causes a dominant negative phenotype when overexpressed by *U. maydis*, a phenotype which we now use as an indicator for uptake. In collaboration with the group of Gert Bange, the structure of Cmu1 was recently solved and now serves as a blueprint for defining and mapping the uptake motif, and for site-directed mutagenesis (Bange *et al*, unpublished).

In addition to Cmu1, we have identified a second translocated effector, Tin2. This effector induces anthocyanin biosynthesis and also promotes virulence (Fig. 3). In this case, we have demonstrated a function of Tin2 inside plant cells indirectly, by transiently expressing a

Infection: wild type *tin2* mutant

Figure 3. *Maize plants infected with U. maydis wildtype and tin2 mutant, respectively. Whereas the wildtype induces tumors and anthocyanin production (red patches), leaves infected with the tin2 mutant develop smaller tumors and remain green.*

Tin2 version lacking the signal peptide in maize cells using the red color of anthocyanin as readout. Tin2 interacts with and stabilizes the maize protein kinase ZmTTK1 by masking a ubiquitin–proteasome degradation motif in ZmTTK1. Active ZmTTK1 controls the activation of genes in the anthocyanin biosynthesis pathway and is presumed to lower the metabolite flow into the lignin biosynthesis pathway, which otherwise would have negative consequences for fungal spread and nutrition (Brefort *et al*, 2014; Tanaka *et al*, 2014).

Establishment of an assay for effector translocation

We are unable to detect a conserved sequence motif in the Cmu1 and Tin2 effectors, suggesting that such a motif may not be apparent in the primary amino acid sequence of these effectors. Therefore, we are currently unable to predict which of the 300 novel secreted effectors are translocated. To end this ambiguity, we have recently established an assay that allows to discriminate apoplastic and cytoplasmic effectors. This assay is based on the ability of the biotin ligase BirA to biotinylate a 15 amino acid long sequence tag, the Avitag (Fig. 4). To conduct the assay, effectors are fused with the Avitag and expressed in *U. maydis*. Such strains are then used to infect transgenic maize plants expressing BirA in the cytosol. Biotinylated effectors are enriched by streptavidin affinity purification and visualized by western blot. Biotinylation is observed only if the respective effector is translocated, while apoplastic effectors do not get in contact with BirA. This assay does not only visualize translocation, but also allows to determine the uptake efficiency and will in future permit to screen all 300 effectors for uptake by maize plants (Lo Presti & Kahmann, unpublished).

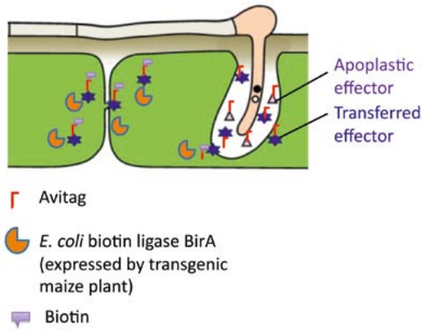

Figure 4. *Model illustrating our translocation assay. Transgenic maize cells express the E. coli biotin ligase BirA (orange). U. maydis effector proteins are fused with the Avitag (red) and, if translocated to the host cells, biotinylated (purple).*

AWARDS & HONORS

2011 TUM Distinguished Affiliated Professorship, Technical University Munich
2011 Honorary doctorate of the Hebrew University, Jerusalem
2008 Elected Member of the German National Academy of Sciences Leopoldina
1998 Medal of Merit (am Bande) of the Federal Republic of Germany
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SELECTED PUBLICATIONS

Djamei A, Schipper K, Rabe F, Ghosh A, Vincon V, Kahnt J, Osorio S, Tohge T, Fernie AR, Feussner I, Feussner K, Meinicke P, Stierhoff Y, Schwarz H, Macek B, Mann M, Kahmann R (2011). Metabolic priming by a secreted fungal effector. *Nature* 478, 395-8.

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Tanaka S, Brefort T, Neidig N, Djamei A, Kahnt J, Vermerris W, Koenig S, Feussner K, Feussner I, Kahmann R (2014). A secreted *Ustilago maydis* effector promotes virulence by targeting anthocyanin biosynthesis in maize. *eLife* 3, e01355.

Brefort T, Tanaka S, Neidig N, Doehlemann G, Vincon V, Kahmann R (2014). Characterization of the largest effector gene cluster of *Ustilago maydis*. *PLoS Pathog* 10, e1003866.

Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, Reissmann S, Kahmann R (2015). Fungal effectors and plant susceptibility. *Annu Rev Plant Biol*, in press.

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1986 Diploma in biology, University of Freiburg

AWARDS & HONORS

1995 Heisenberg Fellow

Solar-powered microfactories for fibers and therapeutics

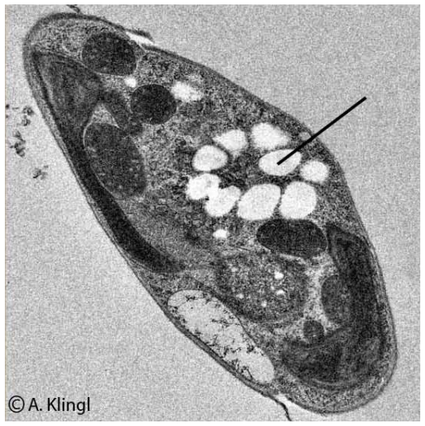

Figure 1. *Bioplastic production in the diatom P. tricornutum. (A) Algal cell culture. (B) Light microscopic picture of P. tricornutum cells. (C) Electron microscopic picture of a cell producing the bioplastic PHB. The bioplastic accumulates in granula-like structures throughout the cytosol (marked with an arrow).*

In the last years, microalgae attracted much attention as resources of valuable natural products, such as lipids for biodiesel production. Furthermore, microalgae are expected to have an enormous potential as biotechnological expression platforms for the production of important products such as fibers or therapeutics. In order to use diatoms as a new expression system for recombinant proteins, our group established these unicellular organisms as solar-powered, CO_2-neutral microfactories in a SYNMIKRO project (Bozarth *et al*, 2009). Accepting this challenge, the diatom expressed portfolio includes production of a bioplastic and spider silk as examples for fibers as well as human monoclonal antibodies and a vaccine.

Bioplastic

If possible, petroleum-based production of synthetic materials should be reversed into production platforms using regenerative resources as the basic materials. Synthesizing and using bioplastics might be attractive aims to reduce the need of oil products and minimizing non-degradable waste. A naturally occurring type of a bioplastic is PHB (poly-3-hydroxybutyrate). Starting with acetyl-CoA, only three enzymes are necessary to synthesize PHB. First attempts to change diatoms into PHB-producing organisms led to a strain synthesizing PHB to up to 11% of its dry weight (Fig. 1) (Hempel *et al*, 2011a). Very promising is that the mentioned production rate can be obtained in 7 days and that the production strain was genetically stable for years.

Spider silk

Spider silk has enormous physical capacities and is very interesting for diverse technical applications. In order to test if this amino acid-based fiber can be produced in diatoms, we expressed spider silk monomers. Up till now, spider silk molecules of more than 150 kDa can be produced at different cellular localizations, highlighting diatoms as an efficient expression platform for huge proteins in biochemically isolated compartments.

Monoclonal antibodies/vaccines

For analytic but also therapeutic applications, monoclonal antibodies are powerful tools. Although their expression in mammalian cell lines is generally established, production of antibodies in other expression platforms is expected to dramatically decrease the production cost. In addition, the synthesis of antibodies in microalgae excludes potential and harmful human pathogenic contaminations. Testing diatoms for such an aim led to the efficient expression of a human monoclonal antibody directed against a Hepatitis B virus protein within the secretory system of the diatom (9% of total soluble protein) (Fig. 2). As shown, the antibody can be purified from the cells and is functionally active (Hempel *et al*, 2011b). Although the expression rate in the diatom is smaller than in comparable mammalian expression systems, the antibody production in diatoms is, additionally to the advantages mentioned, cheap and quick. However, to further decrease production costs and to facilitate the purification process, a protocol was developed to produce the antibody in a way that it is secreted functionally active into the medium by the diatom (Hempel and Maier, 2012). In order to scale up the production, an antibody-secreting strain was successfully grown in flat panel reactors (together with the Fraunhofer IGB Stuttgart). Diatoms can be modified to produce antigens interesting for vaccination as well. This was exemplarily shown by the expression of the target of the monoclonal, anti-Hepatitis B virus protein antibody. Thus for the virus example, an antibody as well as the respective antigen can be produced efficiently by the diatom.

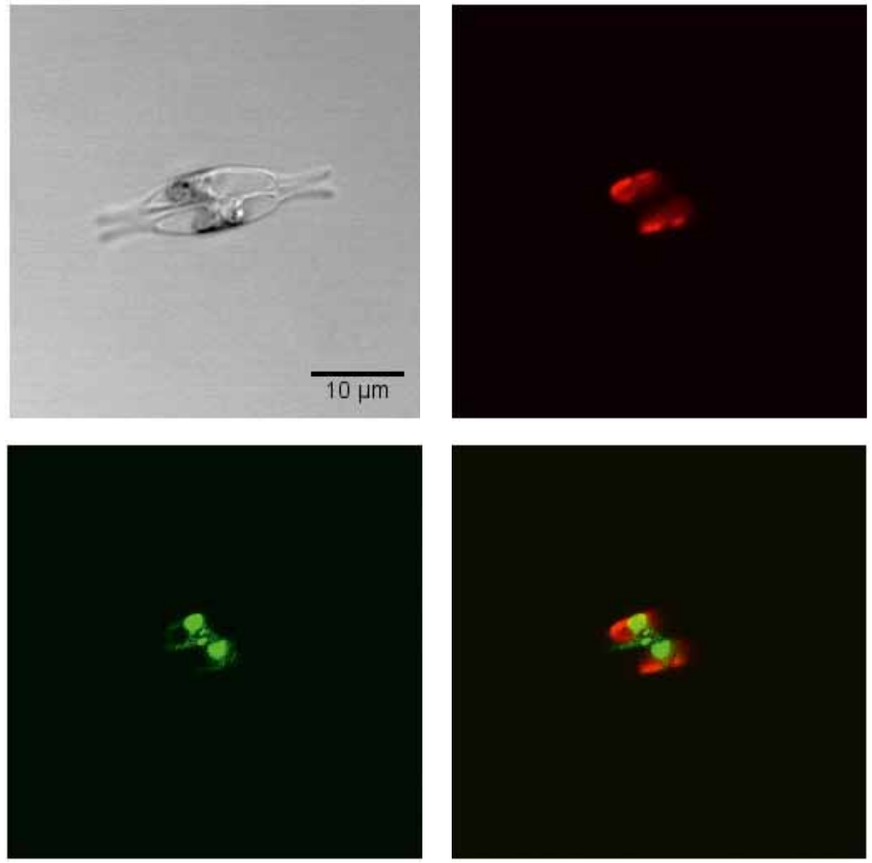

Figure 2. *Expression of the Hepatitis B virus surface antigen in the diatom Phaeodactylum tricornutum. The viral protein is tagged to the green fluorescent protein GFP, the plastid autofluorescence is depicted in red.*

Of course there are several other products, which might be useful to be expressed in microlagae. However, increasing the quality of the expressed products and designing of useful molecules not known from natural systems might be the major new challenges. In this respect, the posttranslational protein-modifying systems of microalgae, such as glycosylations (Peschke *et al*, 2013), have to be humanized for using synthesized molecules therapeutically.

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AWARDS & HONORS

2008 Ruth Massenberg Prize of Ruhr-University Bochum for outstanding Ph.D. thesis
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From natural to synthetic chromosomes and back

We are entering an era in life science in which synthesis of chromosome-sized molecules becomes technically feasible. This leads to the question if we actually understand construction rules of natural chromosomes completely. That is not the case, but such a thorough understanding would be essential to design and build synthetic chromosomes for organisms with new capabilities, e.g., for biotechnological purposes. Therefore, our group studies bacterial chromosome biology using a variety of methods with emphasis on synthetic biology approaches.

Our philosophy:
1. You have to understand chromosome biology to build synthetic chromosomes.
2. You have to build synthetic chromosomes to understand chromosome biology.

The synthetic secondary chromosome synVicII

Many studies in the field of synthetic genomics focus on the question which and how many genes are needed for a minimal genome. However, chromosomes are much more than arrays of genes. They need to organize the genetic material and guarantee its proper transfer to daughter cells – features that are summarized by the term "chromosome maintenance". In order to learn more about the biology of bacterial chromosome maintenance, we establish a synthetic secondary chromosome in *Escherichia coli* as experimental system.
Chromosome maintenance mechanisms usually consist of a DNA binding protein and the respective DNA sequence motif (Messerschmidt & Waldminghaus, 2015), and the distribution of such DNA motifs on natural chromosomes is often crucial for the function of the system. On a synthetic secondary chromosome, in contrast, the motif distribution can be assigned according to any design. By construction and characterization of such synthetic chromosome variants, we want to understand more about the role of DNA motif distribution in chromosome biology. The template for our synthetic secondary chromosome is *Vibrio cholerae*, a bacterium that carries a natural secondary chromosome. Based on the replication origin of this secondary chromosome, we have constructed a 10kb "mini-chromosome" called synVicII (Fig. 1+2) (Messerschmidt *et al*, 2015). This synthetic chromosome replicates in *E. coli* and represents the chassis for more synthetic secondary chromosomes to come. Initial characterization suggests that the replication timing of synVicII in the heterologous host *E. coli* is actually similar to that of the secondary chromosome in *V. cholerae*, indicating a certain independence of chromosome maintenance systems of the biological background. One object of our future work is the optimization of synVicII regarding its stability within the cell. A second goal is to use synVicII as a backbone for building larger chromosomes with different arrangements of chromosome maintenance motifs. A subsequent characterization is expected to provide new insights into chromosome maintenance.

Replication of the two *Vibrio cholerae* chromosomes

While the genome of eukaryotic organisms is divided onto multiple chromosomes, bacteria usually have only one chromosome. An interesting exception to this rule is *Vibrio cholerae*, the causative agent of the cholera disease. This bacterium carries a secondary chromosome (about 1 Mbps) in addition to its primary chromosome (3 Mbps). Over the last years, this two-chromosome system came into focus of scientists for three reasons: First, targeting the unique replication of the secondary chromosome could open up possible routes to therapeutics development specific for *Vibrio* species. Second, the additional *Vibrio*-chromosome can be used as a template for synthetic secondary chromosomes in heterologous hosts (see above). Third, the two chromosomes in *V. cholerae* present the simplest possible system to study general questions of multi-chromosome systems. For all these aspects, a deep understanding of the *V. cholerae* chromosome biology is essential. E.g., we and others have found that the secondary chromosome of *V. cholerae* starts to replicate later than the primary chromosome (Stokke *et al*, 2011), leading to a synchronized termination of the replication of both chromosomes. Now we study the mechanistic basis for this phenomenon using a wide range of molecular biology and bioinformatics approaches.

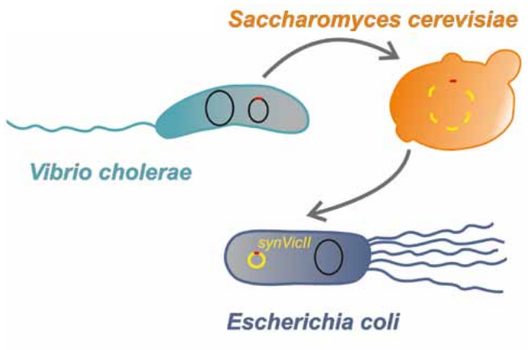

Figure 1. *Construction of the synthetic secondary chromosome synVicII (yellow) in Escherichia coli based on the replication origin of Vibrio cholerae Chromosom II (red). The replication origin of the secondary Vibrio cholerae chromosome including neighboring genes encoding the initiator protein and two segregation proteins was amplified by PCR. The DNA fragment together with other overlapping fragments of different origin was transformed in the yeast Saccharomyces cerevisiae. Homologous recombination in yeast led to assembly of the overlapping fragments into a circular replicon. Successful transformation to Escherichia coli showed the general functionality of the synthetic secondary chromosome synVicII. Figure from Messerschmidt and Waldminghaus, BIOSpektrum 2015 with permission from Springer.*

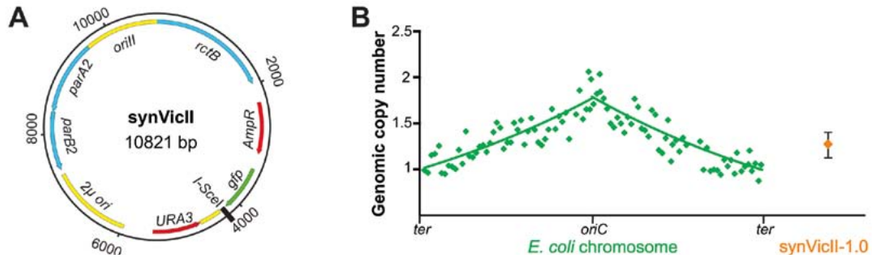

Figure 2. *The synthetic secondary chromosome synVicII in E. coli based on the replication origin of the natural secondary chromosome of V. cholerae. (A) Replicon map with relevant features. (B) Copy number analysis of synVicII in relation to the E. coli primary chromosome using comparative genomic hybridization with a custom microarray.*

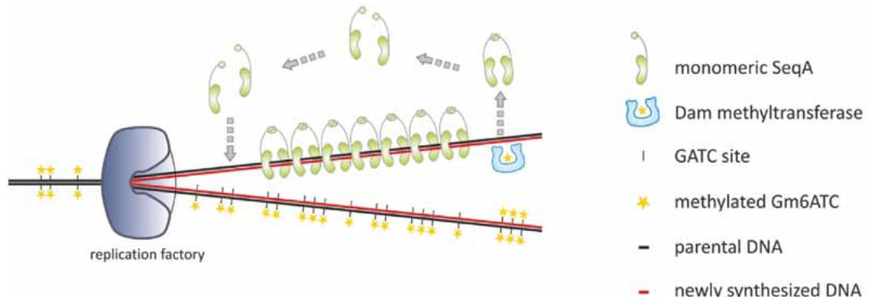

Figure 3. *Model of the SeqA treadmilling. The protein SeqA leaves the DNA at the trailing end of a multimer and rebinds at the leading end near the replication machinery.*

The role of SeqA in *E. coli* DNA replication and chromosome segregation

One important player in chromosome maintenance is SeqA, a fascinating protein with the unique ability to bind hemi-methylated GATC sites in a highly specific fashion (Waldminghaus and Skarstadt, 2009). Such hemi-methylation is characteristic for newly replicated DNA: While the old DNA strand is methylated, the new strand will be un-methylated until the Dam methyltransferase adds the methyl-group at the N6 position of the adenine of the GATC sequence. Binding of SeqA proteins to such hemi-methylated GATCs at the replication origin hinders immediate re-initiation of DNA replication, an effect that is critical to guarantee proper timing of replication rounds. In addition, SeqA follows the newly replicated DNA behind the replication fork, potentially in a treadmilling-like fashion, with a SeqA structure growing at the leading end and shrinking in the trailing end (Fig. 3) (Waldminghaus *et al*, 2012). The movement of SeqA behind the replication fork must be based on the combination of DNA polymerase progression, re-methylation of the DNA by the Dam methyltransferase, and SeqA binding and leaving the DNA. We want to uncover the causality and the details of this molecular treadmilling mechanism using a combination of molecular biology, simulation and bioinformatics.

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1.5 RESEARCH
Microbial Communities

Drivers of microbial community structure in insect guts

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1991–1993 Visiting Research Associate, Michigan State University, USA
1990–1991 Postdoctoral Fellow, Eberhard-Karls-Universität Tübingen
1990 Ph.D. in microbiology, Universität Tübingen
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AWARDS & HONORS

2001 Research Award of the German Society for Hygiene and Microbiology (DGHM)
1991–1993 Feodor Lynen Fellowship of the Alexander von Humboldt Foundation

Termite guts are tiny bioreactors that convert wood and other lignocellulosic matter to microbial fermentation products, which then fuel the metabolism of the host. Our research group studies the role of the gut microbiota in symbiotic digestion, focusing on the structure and function of the gut ecosystem, the biology of the prokaryotic and eukaryotic symbionts and their interactions, and the evolution of the intestinal microbial community. Our studies with germ-free cockroaches are a novel approach to understand fundamental mechanisms governing the assembly of microbial communities and to assess the effects of gut environment and organismic interactions on the activities of individual microbial populations in the digestive process.

The termite gut microbiota reflects host evolution

While the general role of the termite gut microbiota in lignocellulose digestion is slowly emerging, the factors shaping the structure of the intestinal community and the functions of many members are still mostly in the dark. When termites evolved from cockroaches more than 150 million years ago, the acquisition of cellulolytic flagellates as gut symbionts created new habitats for symbiotic bacteria, which now colonize the surface and cytoplasm of these protists in all lower termites. These flagellates were lost again during the evolution of higher termites, whereby wood particles became available for bacterial colonization, providing niches for fiber-digesting species (Brune, 2014).
In the first comprehensive study of a cockroach gut microbiota, we detected numerous bacterial lineages that so far had been found exclusively in termite guts, suggesting a common evolutionary origin of the gut microbiota of termites and cockroaches (Schauer *et al*, 2012). We tested this hypothesis by deep sequencing of the 16S rRNA genes of the bacterial communities in the hindgut of 34 termite and cockroach species, representing the major host groups (Dietrich *et al*, 2014). Comparative analysis documented strong changes in the patterns of bacterial community structure that coincided with major events in termite evolution (Fig. 1).
Although certain bacterial lineages are represented among all termites of the same feeding guild, implicating environmental factors as strong determinants of community structure, the individual taxa typically cluster according to their respective host groups. Evidence of true cospeciation, however, is scarce and rarely spans the entire host range. Rather, the coevolution of termite gut microbiota and host seems to be based on both the selective forces of microhabitat and ecological niche and the faithful transmission of symbionts across host generations, which is facilitated by the social lifestyle of termites (Mikaelyan *et al*, in preparation).

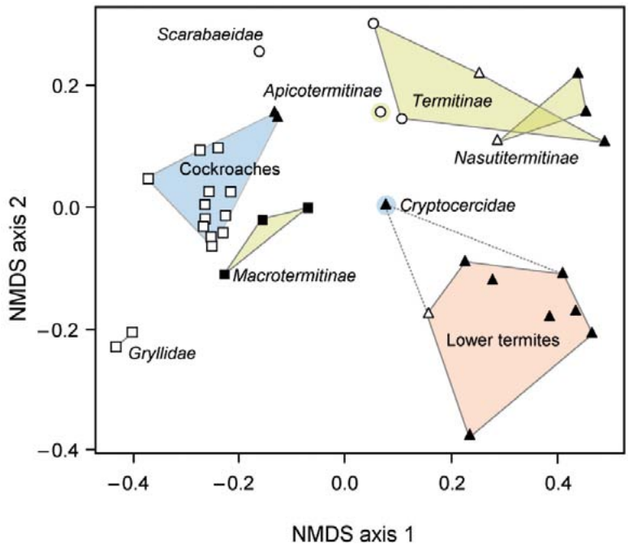

Figure 1. *Phylogenetic patterns in the bacterial gut microbiota of termites and cockroaches. Community similarities are visualized by non-metric multidimensional scaling (NMDS). Colors indicate clusters of samples from the same major host lineages; symbols indicate different feeding groups (Dietrich et al, 2014).*

Germ-free cockroaches as a model for community assembly

A fundamental question in microbial ecology — not only of intestinal ecosystems — concerns the mechanisms of community assembly. In contrast to the distinct patterns observed in termites, the microbial communities in cockroach guts are quite similar, less affected by diet, and highly variable among individuals of the same species (Schauer *et al*, 2014; Bauer *et al*, 2015). Many of the bacterial lineages common to both omnivorous and wood-feeding cockroaches are encountered also in the guts of mammals, indicating their general adaptation to the intestinal environment.

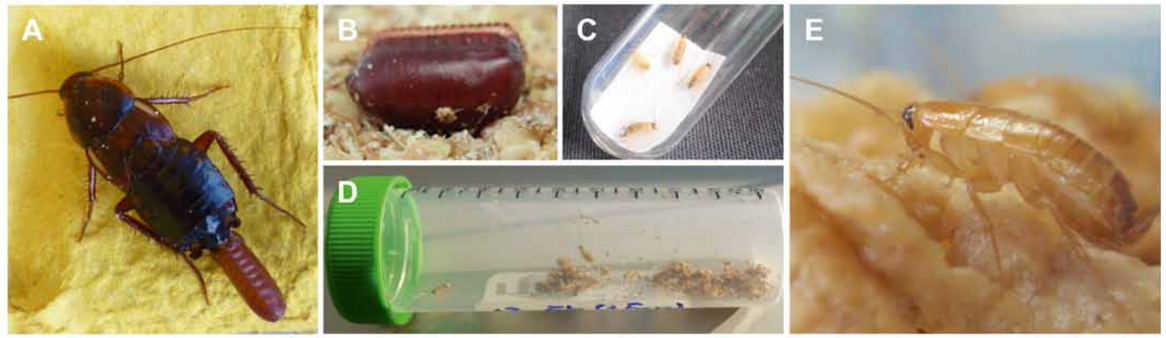

Figure 2. *The cockroach Shelfordella lateralis (A) as model for gnotobiotic studies. Germ-free juveniles are generated by surface sterilization of oothecae (B). After inoculation (C), juveniles are raised under axenic conditions (D). Panel (E) shows a germ-free juvenile two months after hatching.*

Elucidating the fundamental mechanisms of community assembly and succession in insect guts requires an experimental approach. Since termites are obligately dependent on their intestinal symbionts, we established the cockroach *Shelfordella lateralis* as a germ-free model to investigate the drivers of community structure and for gnotobiotic studies – i.e., studies of hosts associated only with certain known microorganisms (Fig. 2). Germ-free juveniles of these cockroaches develop poorly but can be rescued by infection with their natural gut microbiota (Tegtmeier *et al*, in preparation).
Controlled inoculation of germ-free cockroaches with termite microbiota confirmed that the gut microbial community is indeed shaped by the host environment: Irrespective of the source of the foreign inoculum, the bacteria colonizing the germ-free gut came from lineages that are abundant also in conventional cockroaches. However, the foreign strains were rapidly replaced by autochthonous microbiota upon exposure to conventional cockroaches, indicating that host specificity is caused by differences in competitiveness of the bacteria in their particular habitat (Thompson *et al*, in preparation).

Synthetic microbial communities

The complexity of the gut microecosystem with its metabolic networks and the multitude of possible interactions among its biotic and abiotic components make it challenging to identify fundamental mechanisms using conventional insects and their microbiota. Therefore, we chose the germ-free cockroach model also to study metabolic activities of synthetic microbial communities under *in situ* conditions, introducing representative members of different microbial guilds that were isolated from insect guts.
We successfully inoculated germ-free cockroaches with various strains of primary fermenters from the normal gut microbiota of *S. lateralis* and compared their metabolic profiles in pure culture with their activities in the gut environment. To our surprise, both strict and facultative anaerobes successfully colonized the gut in high density if present alone. However, when co-inoculated, the facultative anaerobe outcompeted the strict anaerobe, probably owing to a higher growth yield under the microoxic conditions at the hindgut wall (Tegtmeier *et al*, in preparation).
Controlled inoculation with bacterial strains of natural and foreign microbiota will also allow us to study potential host–microbe interactions, including a differential immune response of the host to colonization by gut symbionts or potential pathogens and, reciprocally, adaptations of the gut microbiota to the host's immune defense.

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AWARDS & HONORS

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2003 Francis Clark Distinguished Lectureship, Soil Science Society of America
1997 Division N Lecture, American Society for Microbiology

Studying methane production with synthetic microbial communities

Wetlands are an important source of the greenhouse gas methane, but the mechanisms of formation and in particular the role of the microbial species involved are still not very well understood. For a long time, the main approach for improving our knowledge has been the rather descriptive analysis of structure and function of the microbial communities in these wetlands. Recently, however, we have started to manipulate these systems in a synthetic approach in order to confirm, extend or refute the theories that have emerged based on analytical studies. As model systems for this manipulation, we have chosen rice roots and bromeliad tanks.

Figure 3. *Tank bromeliad with leaf rosettes forming a water-filled tank. (Photo: Guntars Martinson)*

Methanogenic archaea on rice roots

Rice cultivation is of central importance for feeding the human population, especially in Asia. Unfortunately, flooded rice fields are also an important source for the greenhouse gas methane, which is mainly produced on the roots of rice plants by the anaerobic degradation of photosynthesized organic matter (Fig. 1). Empirical evidence suggests, though, that the extent of methane formation may be dependent on the extent and speciation of root colonization by methanogenic archaea (Conrad *et al*, 2008). In our SYNMIKRO project, we are testing this hypothesis by manipulating the colonization of the rice roots in rice microcosms. The plants are then pulse labeled with $^{13}CO_2$, and the amount of methane emitted by the microcosms is measured (Fig. 2). Furthermore, the microbial colonization of the rice roots is assessed using molecular techniques that target both the composition of microbial communities and the abundance of different microbial species in these communities. Our studies demonstrated close ties between plant physiology, soil properties and microbial turnover of carbon (Pump & Conrad, 2014). They further showed that the rate of methane formation was indeed related to the abundance of methanogenic archaea on the roots (Pump *et al*, 2014). We are presently testing whether the species composition of this methanogenic community is also of importance for CH_4 emission.

Figure 1. *Rice field in the Philippines with flux chambers for measuring CH_4 emission. (Photo: Ralf Conrad)*

In addition to methanogens, rice roots are also a suitable habitat for other archaea, for example those that oxidize ammonia and thus control the cycling of nitrogen (Ke *et al*, 2013). Altogether, the microbial colonization of roots plays a major role for the function of the rice field ecosystem. We are presently studying to which extent this colonization is controlled by the plant and/or by the soil microbiota.

Microbial communities in tank bromeliads

Tank bromeliads are common epiphytes in neotropical forests and early successional vegetation. Their leave rosettes form a tank, in which not only rainwater, but also all kinds of detritus accumulate (Fig. 3). These small wetland systems constitute another significant source in the methane budget of the atmosphere (Martinson *et al*, 2010). The bromeliad plants provide themselves with water and nutrients from the tank, which actually contains a diverse community of methanogenic archaea, analogously to rice roots, but of different composition and structure. The manipulation of the content of the bromeliad tanks can easily be done. Therefore, we decided to establish tank bromeliads as a second model system for studying the interaction between plant and microbial community. For example, we recently studied the effect of desiccation on the tank microbial community by manipulating the water content in the tanks (Brandt *et al*, 2015).

Figure 2. *Device for pulse-labeling of rice microcosms with $^{13}CO_2$ and measuring the flux of CO_2 and CH_4 between the microcosms and the atmosphere. (Photo: Judith Pump)*

SELECTED PUBLICATIONS

Brandt FB, Martinson GO, Pommerenke B, Pump J, Conrad R (2015). Drying effects on archaeal community composition and methanogenesis in bromeliad tanks. *FEMS Microbiol Ecol*, in press.

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Pump J, Pratscher J, Conrad R (2014). Colonization of rice roots with methanogenic archaea controls photosynthesis-derived methane emission. *Environ Microbiol Reports*, doi: 10.1111/1462-2920.12675

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Methanotrophic bacteria and their response to environmental changes

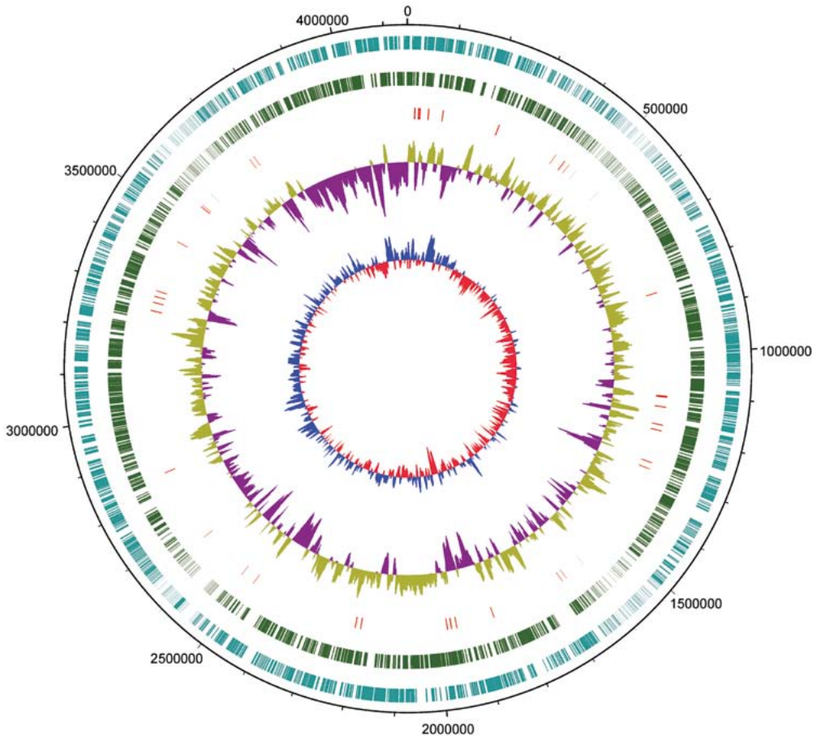

Figure 1. *Whole-genome plot of Methylocystis sp. strain SC2. The circles represent from outside to inside: circle 1, DNA base position (bp), base 1 to 3,773,444 are for the chromosome, followed by plasmids pBSC2-1 and pBSC2-2; circle 2, protein-coding regions transcribed on the plus strand (clockwise); circle 3, protein-coding regions transcribed on the minus strand (anticlockwise); circle 4, tRNA genes; circle 5, G+C content plotted using a 10-kb window (sea-green and magenta indicate values greater than and less than the average G+C content, respectively); circle 6, GC skew ([G+C]/[G-C]) plotted using a 10-kb window (blue indicates values above average and red indicates values below average). The whole-genome plot was generated using DNAPlotter version 1.4 from Artemis 12.0, Sanger Institute.*

Methane-oxidizing bacteria, so-called methanotrophs, are aerobic microorganisms that have the unique ability to grow on methane as their only source of carbon and energy. Their key enzyme, particulate methane monooxygenase (pMMO), converts methane to methanol and thereby attenuates methane emissions from major sources, such as wetlands, rice paddies, and landfills, and constitutes the only biological sink for atmospheric methane in upland soils. Methanotrophs are therefore crucial players in the global cycle of the greenhouse gas methane; nevertheless, their molecular biology is poorly understood. In addition to methane availability, nitrogen source and concentration are major determinants of methanotrophic activity. In particular, ammonia is known to act both as a nutrient for growth and as a competitive inhibitor of pMMO.

Our research aims to improve our understanding of how methanotrophs respond at the molecular and cellular levels to environmental change, using *Methylocystis* sp. strain SC2 as a model system. This strain can adapt to a wide range of methane concentrations due to its ability to produce two pMMO isozymes with different methane oxidation kinetics: The *pmoCAB1* genes encode the well-known type of pMMO (pMMO1) and are expressed only at methane mixing ratios >600 parts per million by volume (ppmv). The *pmoCAB2* genes encode pMMO2 that oxidizes methane at lower mixing ratios, even at the trace atmospheric mixing ratio of 1.8 ppmv (Baani & Liesack, 2008).

A unique denitrification pathway among methanotrophs

To provide a basis for further research into the molecular biology of strain SC2, we sequenced its genome and found it to comprise a 3.77 Mb circular chromosome and two large plasmids of 229.6 kb (pBSC2-1) and 143.5 kb (pBSC2-2) (Fig. 1). The presence of two nearly identical copies of *pmoCAB1* and one copy of *pmoCAB2* was validated, and all other genes required for a methanotrophic lifestyle were identified. The absence of genes encoding the soluble methane monooxygenase was confirmed. Furthermore, a large repertoire of genes involved in nitrogen metabolism was detected (Dam *et al*, 2012a,b).

To verify that strain SC2 has diverse nitrogen metabolism capabilities, we performed several physiological experiments. For example, strain SC2 was found to grow with atmospheric N_2 as the sole nitrogen source, preferably at low oxygen concentrations. Denitrification-mediated accumulation of 0.7 nmol $^{30}N_2$/hr/mg dry weight of cells under anoxic conditions was detected by tracer analysis. N_2 production was related to the activities of plasmid-borne nitric oxide and nitrous oxide reductases. The presence of a complete denitrification pathway in strain SC2, including the plasmid-encoded *nosRZDFYX* operon, is unique among known methanotrophs. The exact ecophysiological role of this pathway still needs to be elucidated (Dam *et al*, 2013).

Differential expression of methane and nitrogen metabolism-related genes

We used Illumina RNA-Seq to identify strain SC2 genes that respond to low (10 mM, AMS) and high (30 mM, NH4) NH_4^+ concentrations in the growth medium, compared to 10 mM NO_3^- (NMS). Strain SC2 cells were incubated under high methane concentrations (20%, v/v) in the different nitrogen treatments (Dam *et al*, 2014).

The majority of the strain SC2 genes showed no significant changes in the expression level. Based on $\log_2$ fold changes of ≥ 2 or ≤ -2, a total of only 198 genes were identified as differentially expressed in NMS/AMS and NMS/NH4 (Fig. 2). A set of 56 genes was differentially expressed in response to both treatments. Their expression levels in AMS and NH4 showed a strong linear correlation. Fifty-two genes were either up- or downregulated in a similar way, while four genes exhibited a reverse expression (Fig. 3). Greatest response to increasing ammonium concentrations showed two genes that encode hypothetical proteins ($\log_2$ fold changes of 6.1 to 9.3).

While the expression of *pmoCAB1* was unaffected, *pmoCAB2* was significantly downregulated ($\log_2$ fold changes of -5.0 to -6.0) in response to increasing ammonium concentrations. Among nitrogen metabolism-related processes, genes encoding hydroxylamine oxidoreductase (*haoAB*) were highly upregulated, while those for assimilatory nitrate/nitrite reduction, high-affinity ammonium uptake, and nitrogen regulatory protein PII were downregulated.

Ammonia competitively inhibits pMMO, which in turn leads to the production of hydroxylamine, a highly toxic molecule that must be transformed or removed quickly to prevent cellular damage. Competitive inhibition and, in consequence, hydroxylamine poisoning would be of ecophysiological relevance particularly in low-methane environments, where only pMMO2, but not pMMO1, is functional. This may be the reason why the expression of *pmoCAB2*, but not that of *pmoCAB1*, is affected by ammonia. The upregulation of *haoAB* expression with increasing ammonium concentration also fits well into such an ecophysiological perspective, assuming that the primary role of HAO is to detoxify hydroxylamine that has been produced by the pMMO isozymes. *Methylocystis* is widely distributed in upland soils. The differential expression of *pmoCAB2* thus provides some explanation as to why ammonium fertilizers have a strong inhibitory effect on atmospheric methane oxidation in such soils.

Methylocystis sp. strain SC2 is one of the very few methanotrophs for which a finished genome sequence exists that enables whole-genome expression studies. While the above study already provided important insights into the response of strain SC2 to different nitrogen sources and concentrations, we want to further improve our understanding of the genome-wide transcriptome response of strain SC2 to environmental change in the future. Another line of research is the development of reverse genetic tools for functional analysis of candidate genes in strain SC2 and other methanotrophic bacteria.

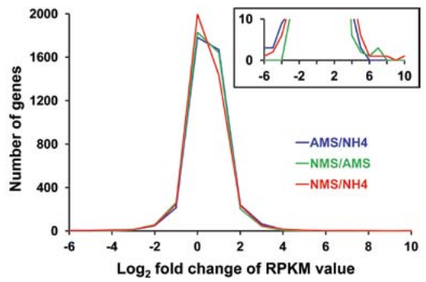

Figure 2. *Differential expression of strain SC2 genes in response to different nitrogen conditions. The histogram indicates the change in expression levels for the complete set of 4,058 genes identified in the genome of strain SC2. High-quality non-rRNA reads were mapped against the concatenated sequence of the chromosome and the two plasmids of strain SC2. RNA-Seq expression data were presented as RPKM (Reads Per Kilobase of CDS model per Million mapped reads) values. Log_2 fold changes of RPKM values were compared for NMS/AMS (green), NMS/NH4 (red), and AMS/NH4 (blue). The inset shows the same graph with the y-axis zoomed in on the range from 0 to 10. Figure was taken from Dam et al, 2014.*

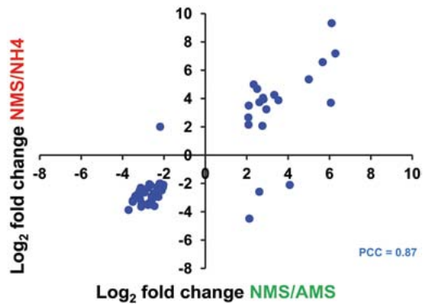

Figure 3. *Correlation between the log_2 fold changes of genes that were differentially expressed in response to both standard (10 mM) and high (30 mM) NH_4^+ concentrations. Comparison was made between NMS/AMS and NMS/NH4. Genes that showed log_2 fold changes of ≥ 2 or ≤ −2 in both treatments were included in the comparison. Figure was taken from Dam et al, 2014.*

SHORT CV

2011– Research Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
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1997–2011 Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
1992–1997 Research Associate, Max Planck Institute for Terrestrial Microbiology, Marburg
1996 Habilitation, Philipps-Universität Marburg
1990 Research Fellow, University of Queensland, Australia
1988 Ph.D. in microbiology, University of Kiel
1984 Diploma in biology, University of Kiel

SELECTED PUBLICATIONS

Baani M, Liesack W (2008). Two isozymes of particulate methane monooxygenase with different methane oxidation kinetics are found in *Methylocystis* sp. strain SC2. *Proc Natl Acad Sci USA* 105, 10203-8.

Dam B, Dam S, Kube M, Reinhardt R, Liesack W (2012a). Complete genome sequence of *Methylocystis* sp. strain SC2, an aerobic methanotroph with high-affinity methane oxidation potential. *J Bacteriol* 21, 6008-9.

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Dam B, Dam S, Blom J, Liesack W (2013). Genome analysis coupled with physiological studies reveals a diverse nitrogen metabolism in *Methylocystis* sp. strain SC2. *PLoS ONE* 8, e74767.

Dam B, Dam S, Kim Y, Liesack W (2014). Ammonium induces differential expression of methane and nitrogen metabolism-related genes in *Methylocystis* sp. strain SC2. *Environ Microbiol* 16, 3115-27.

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Microbiology and Biochemistry

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SYNMIKRO since 2010

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2007– Professor Emeritus, Max Planck Institute for Terrestrial Microbiology, Marburg
1991–2007 Director and Head of the Department of Biochemistry, Max Planck Institute for Terrestrial Microbiology, Marburg
1976–2005 Professor of Microbiology, Philipps-Universität Marburg
1999 Visiting Professor, Stanford University, CA, USA
1991 Visiting Professor, University Frankfurt
1972–1976 Professor of Biochemistry, Ruhr-University Bochum
1972 Postdoctoral Fellow, Case Western Reserve University, Cleveland, Ohio, USA
1971 Habilitation, University of Freiburg
1968 Ph.D. in biochemistry, University of Freiburg
1966 Diploma in biochemistry, University of Tübingen

Construction of a *Methanosarcina* strain that can grow on glucose

In anoxic environments, cellulose, a polymer of glucose, is fermented to CO_2 and methane via a syntrophic association of anaerobic bacteria, protozoa and fungi that ferment cellulose to acetic acid, CO_2 and H_2, and of methanogenic archaea that convert acetic acid, CO_2 and H_2 to methane (see also: Thauer RK, Kaster A, Seedorf H, Buckel W & Hedderich R (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat Rev Microbial* 6, 579-91) (Fig. 1). There is no organism known that can ferment glucose to 3 CO_2 and 3 CH_4 on its own, although the free energy change associated with such an overall fermentation would allow for the largest ATP-gain (mol ATP per mol substrate).

A kinetic theory relating growth rates to the length of metabolic pathways and the number of coupling sites can explain these finding for energy substrate-limited planktonic cells (see also: Pfeiffer T, Schuster S & Bonhoeffer S (2001). Cooperation and Competition in the Evolution of ATP-Producing Pathways. *Science* 292, 504-7). The same theory predicts, however, that methanogens capable of fermenting glucose to CO_2 and methane should exist in biofilms. In collaboration with Prof. Dr. Michael Rother, University Frankfurt, we set out to test this hypothesis. We cloned the genes required for glucose import (glucose transport facilitator *glf* from *Zymomonas mobilis*) and glucose activation to glucose-6-phosphate (glucokinase *glk* from *Escherichia coli*) into *Methanosarcina acetivorans*, a methanogen lacking only these genes for methanogenesis from glucose. Indeed we found that cell suspensions of recombinant *M. acetivorans* are capable of methanogenesis from glucose, albeit presently at only very low specific rates (Fig. 2).

Next, the rate limiting steps in this fermentation will have to be identified and to be overcome. One of the problems already encountered is that, within the recombinant strain, glucose-6-phosphate accumulates to toxic concentrations when glucose is present in the medium. Another consequence of the apparently too rapid glucose phosphorylation is the fact that the ATP concentration, the phosphorylation potential and energy charge drop to values below those required to sustain growth.

In the future, nano-SIMS (secondary ion mass spectrometry) ion imaging will be used to screen biofilms for methanogens that can metabolize glucose or other easy fermentable substrates. Once such methanogens have been identified, efforts will have to be made to isolate and cultivate them. If this is not possible,

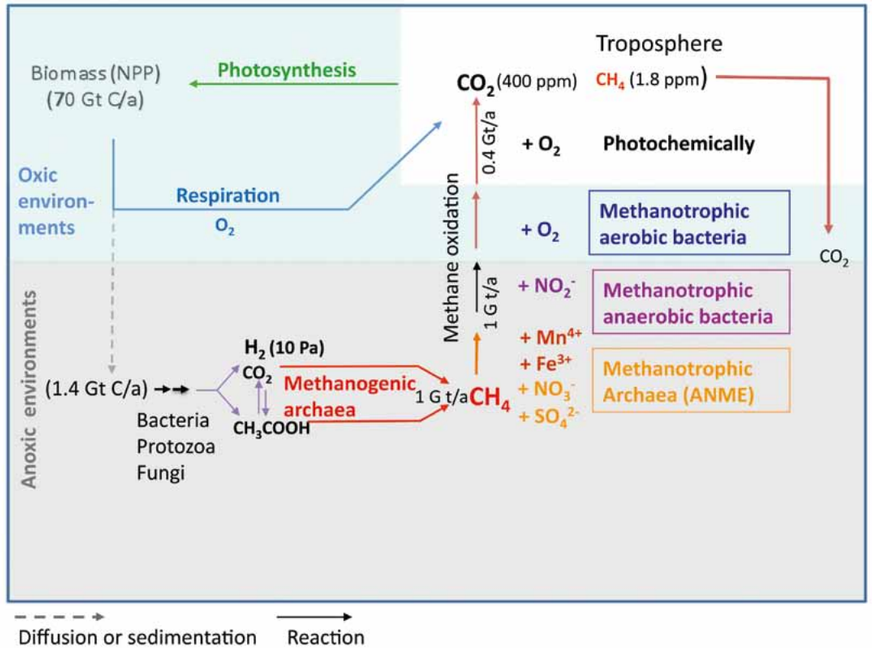

Figure 1. *The global microbial methane cycle. More than 70% of the net primary production (NPP) of plants are lignocelluloses, of which about 2% end up each year in anoxic environments where the cellulose is fermented to CH_4 and CO_2 via a syntrophic association of anaerobic bacteria, protozoa and fungi with methanogenic archaea.*

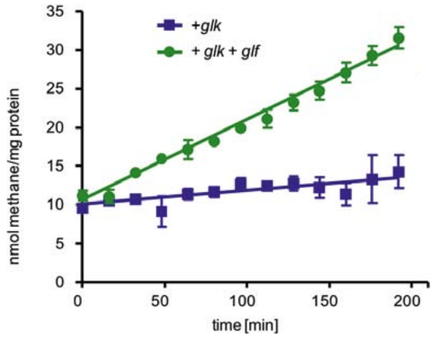

Figure 2. *Methanogenesis from glucose (0.2 mM) in cell suspensions of recombinant Methanosarcina acetivorans with the gene glk for glucokinase from Escherichia coli and the gene glf for the glucose transport facilitator from Zymomonas mobilis.*

which may well be the case, they will have to be characterized on the genome and metabolome level via single cell techniques, some of which are still under development.

From 2013 on, Michael Rother and Christian Sattler, the Ph.D. student involved, have continued the project alone at the Technical University of Dresden, where they moved end of 2011.

AWARDS & HONORS

2013 Medal of Merit of the German Academy of Sciences Leopoldina
2008 Carl Friedrich Gauß Medal of the „Braunschweigische Wissenschaftliche Gesellschaft"
2001 Honorary doctorate of the ETH Zürich, Switzerland
1987 Leibniz Prize of the German Research Foundation (DFG)
1984 Otto Warburg Medal of the „Gesellschaft für Biologische Chemie" (GBM)

SELECTED PUBLICATIONS

Thauer RK, Kaster A, Goenrich M, Schick M, Hiromoto T, Shima S (2010). Hydrogenases from methanogenic archaea: structure and function, nickel regulation, and H_2- storage. *Ann Rev Biochemistry* 79, 507-36.

Scheller S, Goenrich M, Boecher R, Thauer RK, Jaun J (2010). The key nickel enzyme of methanogenesis catalyses anaerobic oxidation of methane. *Nature* 465, 606-9.

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Huang H, Wang S, Moll J, Thauer RK (2012). Electron bifurcation involved in the energy metabolism of the acetogen bacterium *Moorella thermoacetica*. *J Bacteriol* 194, 3689-99.

1.6 RESEARCH
Bioethics

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Bioethics

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SYNMIKRO member since 2011

Ethical assessment of synthetic biology

The Bioethics group deals with the ethical aspects, normative uncertainness and public questions regarding synthetic biology. Based on a consequentialist approach, these ethical conflicts must be confronted with research through differentiated identification, description and classification. Our goal is to develop a model for the ethical assessment of synthetic biology that gives a realistic description of the actual state of research and grades it into different stages from ethically unproblematic via more problematic and riskier procedures up to ideas that are entirely speculative and ethically unjustifiable. This ethical assessment model is developed in close collaboration with all research areas of SYNMIKRO. Through interdisciplinary events and publications, the Bioethics Group also initiates a dialogue with external experts both from life sciences and human sciences, and addresses a wider public.

From describing to classifying: a stage model

With our "stage model for the ethical assessment of synthetic biology", a proposal is made to objectify and de-escalate the debate on ethical risks and changes of synthetic biology (Cf.: Hacker *et al*, 2009). The model includes the methodological steps *describing, assessing*, and *classifying* (Fig. 1), and thereby provides a dynamic and multidimensional criteriology that corresponds to the complexity and distinctiveness of synthetic biological research. In the first step, *levels of descriptions* offer a differentiated *classification of the research field of synthetic biology*, determine *domains of possible actions* and ethically relevant consequences, and present *levels of ethical responsibility*, to which both those responsible for and those affected by the research can be identified and addressed. On the basis of this description, the second step develops criteria for the assessment of current research projects in synthetic biology. These criteria must be realigned with each case and are presented in three dynamic dimensions of assessment: The dimension *identifiability and complexity of the research object*, the dimension *range and reversibility of consequences*, and the dimension *ethical meaning and scientific purpose of research*.
The model concludes the ethical assessment through a third step of classifying the particular research in five *stages of ethical accountability*. The starting point is *stage 1*, the ethically unproblematic and widely accepted case. From this basis, every other stage will be developed, which allows a classification of concrete application cases by their ethical problem sustainability and with regard to the expectable uses and the probable risks. Research classified on *stage 2* is ethically responsible but, due to increasing uncertainty factors, there is an increasing need for ethical reflection. *Stage 3* describes research that is ethical problematic, but can be justified under certain conditions and requirements which must be precisely formulated. *Stage 4* includes applications that are not responsible at the moment. On *stage 5* are extreme positions and absurd scenarios that are obviously not subject of a realistic ethical assessment that remains close to the actual state of research.
In reality, the transitions between the stages become blurred. The demarcation necessary for the assessment between ethically favorable and acceptable research and not anymore justifiable experiments runs between steps 3 and 4. It should be noted, however, that new findings and developments in science can lead to modifications in the criteriological assessment. The ethical evaluation must be aware of these dynamics and transitions. For example, in the research project of synthetic chromosomes of the group of Torsten Waldminghaus, there is a strong intervention and a high grade of complexity at the molecular level, due to its relatively new, constructed character. However, introduced into the cell in addition to the natural chromosome, the synthetic chromosome has only a small influence on the cell's behavior - and no influence on the environment, since the experiments "only" serve fundamental research, and the modified cells are not released. If the cell's own chromosome should eventually be fully replaced, the project must be re-assessed.

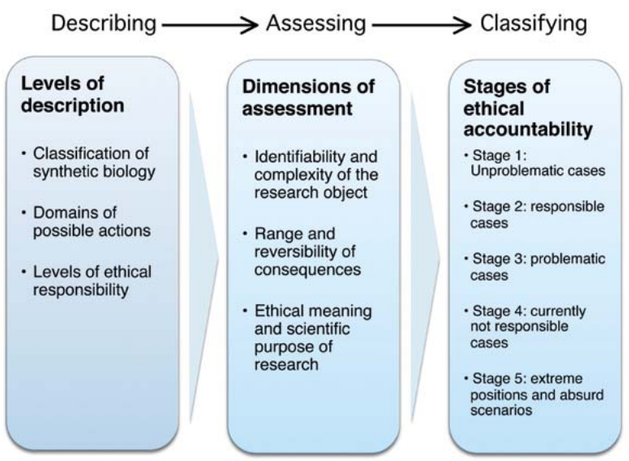

Figure 1. *The methodological steps of the stage model: describing, assessing, and classifiyng.*

SHORT CV

2011– Professor of Social Ethics, Philipps-Universität Marburg
2006–2011 Head of the BMBF Junior Research Group "Religion in bioethischen Diskursen", LMU München
2006 Habilitation, LMU München
1996 Doctoral degree in protestant theology, LMU München
1992 Exam in protestant theology, University of Frankfurt/Main

Public discussions on synthetic biology

In collaboration with SYNMIKRO and the Graduate Center for Life and Natural Sciences of MARA (Marburg University Research Academy), the Bioethics Group organized a series of public events called "Synthetische Biologie im Dialog" in 2013/14 (see also p. 108). The purpose of these events was to illustrate the different approaches, assumptions and beliefs in the current debates about modern life sciences. Based on the central terms and concepts "life", "complexity" (Voigt, 2013) and "natural/synthetic", the different perspectives were reflected and presented in short lectures and subsequent panel discussions with experts of both natural sciences and humanities. Speakers were Prof. Dr. Nedilijko Budisa, Prof. Dr. Dr. hc. mult. Jörg Hacker, Prof. Dr. Gerald Hartung, Prof. Dr. Dr. Kristian Köchy, Prof. Dr. Klaus Mainzer, and Prof. Dr. Sven Panke. Hosts were Prof. Dr. Michael Bölker, Prof. Dr. Bruno Eckhardt, Prof. Dr. Dr. hc. Regine Kahmann, and Prof. Dr. Friedemann Voigt. The publication of the lectures is scheduled for 2015 (Voigt (Ed.), 2015).

Metaphors in the debate on synthetic biology

The ethical debate on synthetic biology is characterized by metaphors such as "engineering life", "living machines", "playing god", etc. These metaphors illustrate how synthetic biology touches culturally and normatively charged distinctions of living and not living matter, and thereby can cause irritation and discomfort. The dissertation project of Daniel Falkner seeks to critically analyze the ethical dimensions of metaphors in debates on modern life sciences and new techno-scientific areas, for which synthetic biology is a prime example.
As exemplified by the metaphor of the "genetic code", this approach leads to a reassessment of synthetic biology as a science between artificial life and living art, and to a new ethical dimension which is in the fundamental relationship of responsibility and trust between science and society (Voigt, 2012). The ethical question then is no longer: „What is life?", but: „How do we want to live together and what role should synthetic biology play in our social life?" (Falkner, 2014). A part of the answer probably lies in the metaphors we use to talk about life and synthetic biology.

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Hacker J, Rendtorff T, Cramer P, Hallek M, Hilpert K, Kupatt C, Lohse M, Müller A, Schroth U, Voigt F, Zichy M. (2009). *Biomedizinische Eingriffe am Menschen. Ein Stufenmodell zur ethischen Bewertung von Gen- und Zelltherapie*, De Gruyter, Berlin.

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Voigt, F (2013). Komplexität. *Zukunft der biomedizinischen Wissenschaften* (ed. by Trutz Rendtorff), 63-5.

Voigt, F (Ed.) (2015). *Synthetische Biologie im Dialog*, Karl Alber (Series: „Lebenswissenschaften im Dialog", edited by Kristian Köchy and Stefan Majetschak), Freiburg i.Br., in press.

Falkner, D (2014). Was ist Leben – eine ethische Frage? *TTN Edition 3/2014*, München (in press).

2 INFRASTRUCTURE

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SHORT CV

2012– Scientific Manager, SYNMIKRO Laboratory Automation Unit
2008–2011 Research Scientist, University of Freiburg
2008 Ph.D. in molecular genetics, Bielefeld University
2005 Diploma in molecular biotechnology, Bielefeld University

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SHORT CV

2014 - Technical Manager, SYNMIKRO Laboratory Automation Unit
2006–2014 Project Manager, Tecan, Switzerland
2004–2006 System and Application Engineer, Tecan, South Africa
1998–2004 Various positions at Tecan, Germany
1997 Diploma in biomedical engineering , University of Applied Sciences Gießen

The Tecan Freedom Evo automation platform with its numerous integrated laboratory devices, such as PCR machine, multimode reader, sealer and centrifuge.

Laboratory Automation Unit

In synthetic biology, high-throughput screens and combinatorial experiments are powerful strategies to support the design–build–test engineering process of gene circuits and biosynthetic pathways. However, manual execution of such experiments is error-prone and often not feasible because of the required throughput. Only recently, advances in computer-aided design and laboratory automation technologies enable such innovative and highly complex experiments: Instead of building and testing of a handful of genetic constructs manually, hundreds to thousands of constructs can now be generated and characterized in an automated way. To tap the potential of these approaches, SYNMIKRO established a Laboratory Automation Unit at its Department of Microbial Comparative Genomics which has been operational since summer 2013. The unit provides access to this technology to the members of the center and is a competent partner both for the design and the execution of automated experiments. In addition, the unit's mission is the development and application of novel experimental methods and processes in the field.

Equipment

The unit operates state-of-the-art laboratory automation platforms. Its current infrastructure is based on one Tecan Genesis and three Tecan Freedom EVO platforms integrating various liquid handling and colony picking options with laboratory devices, such as incubator, shaker, centrifuge, plate sealer, PCR machine, multimode reader, and fluorescence microscope.

Applications

The portfolio of applications comprises

- high-throughput screens as well as combinatorial constructions and performance tests of biological parts and modules including DNA assembly
- automated microscopy of microorganisms including screening of prokaryotic cells for morphology phenotypes and subcellular protein localization

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SHORT CV

2014– Scientific Manager, SYNMIKRO Flow Cytometry and Protein Diffusion & Interactions Unit
2008–2014 Postdoctoral Fellow, Zentrum für Molekulare Biologie, University of Heidelberg
2008 Ph.D. in cellular and molecular biology, The Open University, Milton Keynes, UK and University Vita-Salute San Raffaele, Milan, Italy
2004 Research Associate, University of Milano Bicocca, Italy
2003–2004 Visiting Scientist, ISS inc., Champaign, IL, USA, and Laboratory for Fluorescence Dynamics, UIUC, Urbana, IL, USA
2003 M.Sc. in physics, University of Milan, Italy

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Protein Diffusion & Interactions

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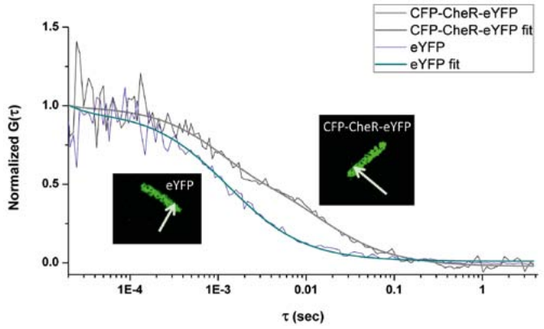
SHORT CV

2014- Postdoctoral Fellow and Technical Manager,
Protein Diffusion & Interactions
2014 Ph.D. in biology/chemistry, University of Konstanz, and Institute of Pharmacology and Structural Biology (IPBS), Toulouse, France
2008 M.Sc. in pharmacological innovation, University of Toulouse, France
2007 Engineering degree in biochemistry, National Institute of Applied Sciences (INSA), Toulouse, France

Flow Cytometry and Protein Diffusion & Interactions Unit

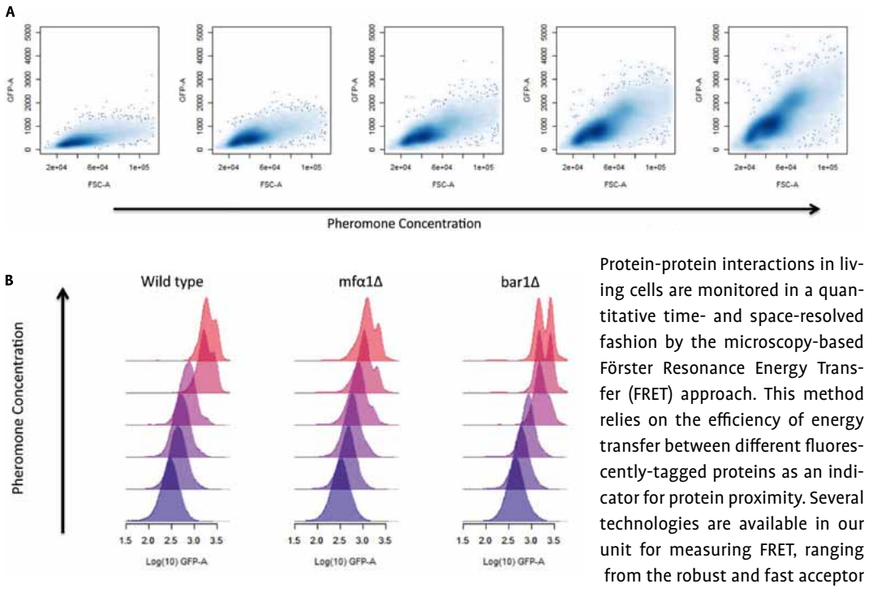
Fluorescently-tagged proteins are ideal reporters for studying the physiological status of living cells. For cells synthetically endowed with new modules or circuits, measuring their properties via relevant fluorescent reporters helps to characterize the effects of these modifications and to optimize the synthetic constructs. Consequently, SYNMIKRO invested in several devices with different key aspects regarding the analysis of fluorescently-tagged molecules for its Flow Cytometry and Protein Diffusion & Interactions Unit launched in October 2014.



Autocorrelation functions of proteins of different molecular weight diffusing in the cytoplasm of Escherichia coli cells obtained from fluorescence correlation spectroscopy data. The arrows in the inset pictures points to the cell volume were the measurements were acquired. In black: normalized autocorrelation function of CFP-CheR-eYFP. In blue: normalized autocorrelation function of eYFP.

Flow cytometry is a laser-based technology that allows the simultaneous measurement of fluorescence in single cells at different wavelengths reporting the cellular concentration of several fluorescently-tagged molecules, and of other physical or biological parameters such as cell size or granularity in a statistically robust fashion. In our instrument, up to thirteen fluorescence channels can be monitored in parallel for every single cell, in thousands of cells per second. Typical applications include single-cell measurements of gene expression, analysis of transcriptional reporters, and cell cycle studies in prokaryotic and eukaryotic microorganisms.

Imaging-based fluorescence experiments, on the other hand, allow us to get more insights into living cells, e.g., by measuring protein properties such as interactions or diffusion. As the design and function of signaling networks depends on protein-protein interactions, which in turn are affected by protein diffusion, resolving such properties in space and time helps to understand signaling up to system-level properties such as signal integration and amplification.



Examples of flow cytometry measurements for Saccharomyces cerevisiae strains containing a GFP reporter stimulated by different concentration of pheromone. (A) Density scatter plots showing GFP intensity versus forward scatter (proportional to cell size) for the wild type. (B) GFP distributions in wild type and two different mutants.

Protein-protein interactions in living cells are monitored in a quantitative time- and space-resolved fashion by the microscopy-based Förster Resonance Energy Transfer (FRET) approach. This method relies on the efficiency of energy transfer between different fluorescently-tagged proteins as an indicator for protein proximity. Several technologies are available in our unit for measuring FRET, ranging from the robust and fast acceptor photobleaching FRET approach to the highly informative fluorescence lifetime imaging approach.

Technical Manager Silvia Gonzales Sierra operating the BD Fortessa Flow Cytometer.

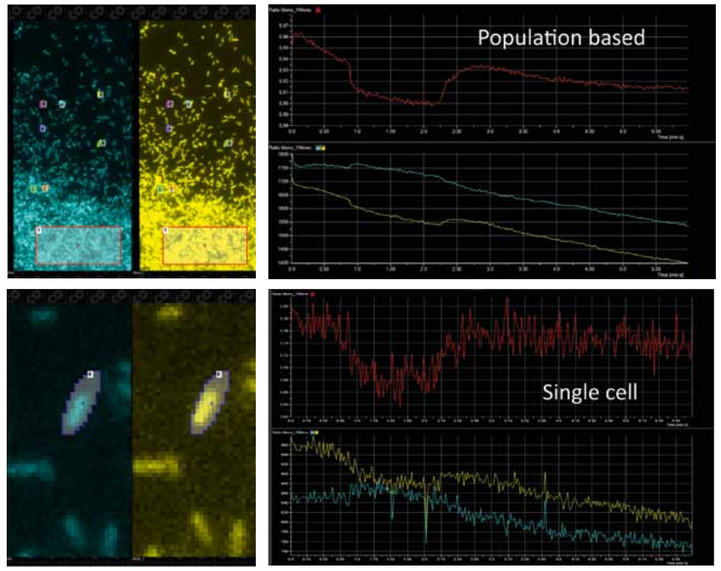
Protein diffusion plays a crucial role in determining what function a protein serves within the cell and how, when and where it may physically interact with other proteins and macromolecules in response to external stimuli. Protein diffusion in living cells is effectively measured by fluorescence correlation spectroscopy (FCS), a fluctuation-based approach that allows to carry out measurements at a physiological protein expression level.

Equipment

The Flow Cytometry and Protein Diffusion & Interactions Unit is part of the Max Planck Department of Systems and Synthetic Microbiology, which is equipped with several fluorescence microscopes dedicated to automatic imaging and FRET applications. The unit helps with the design and execution of protein interactions and protein diffusion experiments. The measurements are run on a Nikon widefield fluorescence microscope customized for automatic acceptor photobleaching and ratiometric FRET measurements, and on a Zeiss LSM880 confocal microscope dedicated to fluorescence correlation spectroscopy and fluorescence lifetime imaging.
The flow cytometry measurements are carried out on a customized BD Fortessa Flow Cytometer with 4 laser lines (445, 488, 514 and 561 nm) and 13 fluorescence channels. Laser lines were selected in order to maximize the fluorescence excitation of fluorescence proteins such as CFP, GFP, YFP and mCherry. The cytometer is endowed with a high throughput sampler that allows to perform automatic measurements of 96-well plates in only 15 minutes.

Applications

- Automatic screening of protein-protein interactions by population-based acceptor photobleaching FRET
- Ratiometric and acceptor photobleaching single-cell FRET
- Fluorescence lifetime imaging microscopy
- Fluorescence correlation and cross-correlation spectroscopy for measurement of protein diffusion and protein-protein interactions
- Confocal imaging
- Measurements of single-cell gene expression in populations, including analysis of gene expression noise
- Measurements of dose-dependent activation of transcriptional reporters in multi-well plates
- Cell cycle studies in prokaryotic and eukaryotic microorganisms



Ratiometric FRET measurements acquired on Escherichia coli cells expressing CheY-eYFP and CheZ-eCFP fluorescent fusions. Plots show data analysis at the population and single cell level reporting chemotaxis response to addition and subsequent removal of Methyl-Aspartate.

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Technical Manager, Flow Cytometry

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SHORT CV

2014- Technical Manager, Flow Cytometry
2010-2014 Technical Manager, Microscopy Laboratory, National Research Center for Human Evolution, Burgos, Spain
2009-2010 Technical Manager, Scanning Electron Microscopy Laboratory, Institute of Nanoscience, University of Zaragoza, Spain
2004-2009 Specialist Technician, Microscopy and FACS Facility, University of León, Spain
2003-2004 M.Sc. in biomedicine, University of León, Spain
2003 Diploma in biology, University of León, Spain

DR. BARBARA WAIDNER

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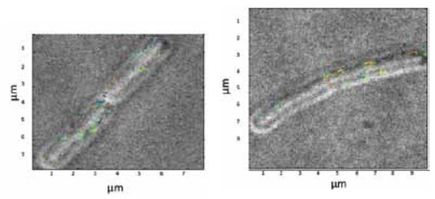
Super Resolution Microscopy Unit

The accurate spatio-temporal visualization of cellular components is of great importance for the understanding of natural cells, which in turn is indispensable for the design and construction of synthetic modules, circuits or entire synthetic cells. Moreover, the ability to visualize synthetic constructs and their effects allows us to test our models and thus brings us closer to a true understanding of the inner workings of biological systems. Modern fluorescence microscopy is the tool that facilitates all of these tasks: It allows the visualization of several types of proteins in parallel, e.g., in the membrane, next to the cell wall and in the cytoplasm. Furthermore, the bacterial cell wall itself, the membrane and the chromosome can also be visualized simultaneously – all of this both in live or in fixed cells, with striking spatial resolution and even in a time-dependent manner.



Dynamics of protein structures. Overlay of a bright field image of a single Bacillus subtilis cell with the first frame of fluorescence (green dots) of a YFP tagged protein and of trajectories, each having a different color, displayed over a time course of 8 seconds.

On these grounds, SYNMIKRO invested in several state-of-the-art fluorescence microscopes for its Super Resolution Microscopy Unit located at the Department of Biochemistry and Cell Biology of Microorganisms. The unit comprises several types of fluorescent microscopes with different features. The most advanced techniques are stimulated emission depletion (STED) and structured illumination (SIM) super resolution microscopy, achieving a resolution of 50 and 125 nm, respectively – several fold lower than the diffraction limit of conventional light microscopy of about 250 nm. Thus, using super resolution microscopy, more details become visible in cells, whose protein molecules are in the range of 5 to 10 nm, and whose protein complexes in the range of 15 to about 100 nm.

Equipment

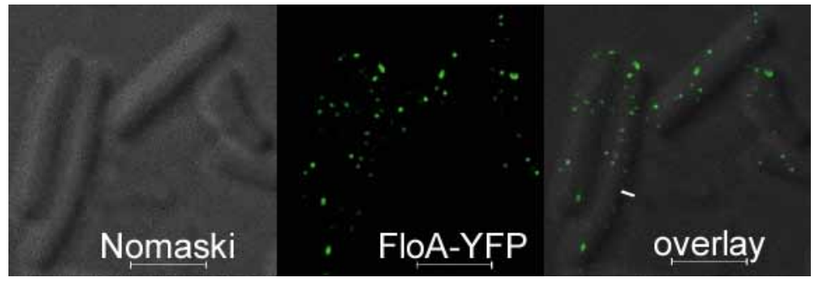
For super resolution microscopy, a STED super resolution scanning confocal microscope (Leica, TCS SP8 STED) and a SIM/PALM (Zeiss, Elyra) microscope are available. The current infrastructure of the unit further comprises several epifluorescence microscopes, some of which are modified for single molecule microscopy (SMM), for total internal reflection fluorescence (TIRF) microscopy and for fluorescence recovery after photobleaching (FRAP).

The stimulated emission depletion super resolution scanning confocal microscope (Leica, TCS SP8 STED).

Applications

The portfolio of applications comprises

- Tracking of single molecules in millisecond time scales (SMM)
- Total internal reflection (TIRF) microscopy
- STED (stimulated emission depletion microscopy)
- Spinning disk confocal microscopy
- SIM (structured illumination microscopy)
- PALM (photoactivation light microscopy)
- FRAP (photo recovery after photobleaching)
- Flow cell set up



Localization of Bacillus subtilis proteins in live cells by STED microscopy. Left panel Nomarski DIC, central panel YFP fluorescence, right panel overlay of Nomarski and fluorescence. Protein structures (FloA-YFP) are on average 75 nm large. White line 2 µm.

SHORT CV

2012– Scientific Manager, SYNMIKRO Super Resolution Microscopy Unit, and head of the *Helicobacter* subgroup at the Department of Biochemistry and Cell Biology of Microorganisms
2011 Interim Professor of Microbiology, University of Freiburg
2007–2010 Independent Junior Research Group Leader funded by the DFG, University Hospital Freiburg
2004–2006 Postdoctoral Fellow, University Hospital Freiburg
2000–2003 Ph.D. in biology/microbiology, University of Karlsruhe
1999 Diploma in biology, University of Karlsruhe

DR. THOMAS HEIMERL

Scientific Manager

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Electron Microscopy Unit

The Electron Microscopy Unit offers researchers in synthetic microbiology the opportunity to literally see the outcome of their work and - so to say - to check the "shape" of created "building blocks" *per se* or in cellular context. Beyond and more profound, electron microscopy can provide essential structural understanding. Thus, it contributes to setting up the foundation the "bricks" are built upon.

Equipment

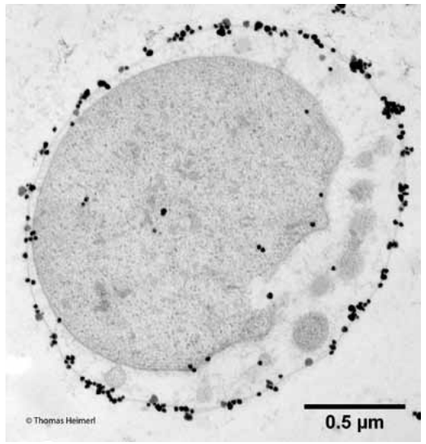
The unit operates a 200kV (JEOL JEM2100) and a 80 kV (Zeiss CEM902) transmission electron microscope. In addition, we can help in advanced structural investigations via focus ion beam scanning electron microscopy (JEOL JIB4610F).

Applications

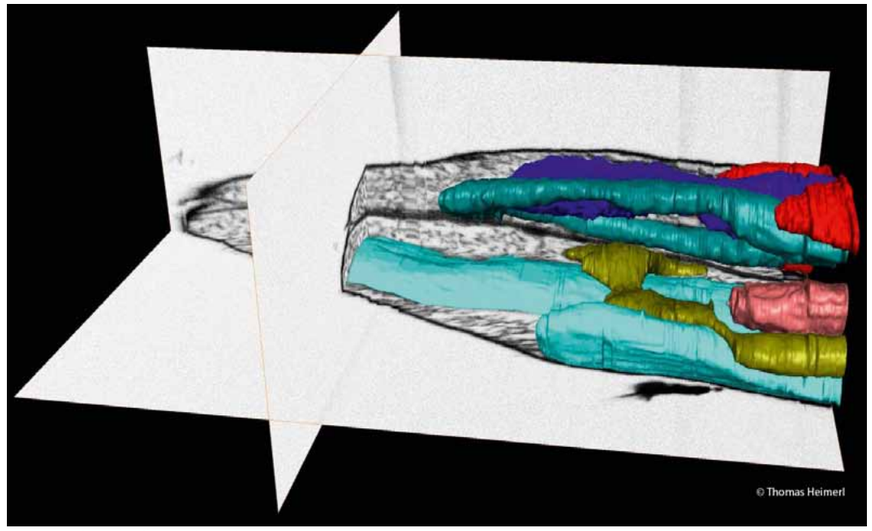
Beside structural investigations of cells and proteins in 2D and 3D, localization of proteins in cells is possible. For this purpose, immunogold labeling represents a powerful technique, especially when limitation in resolution by fluorescent labeling approaches is an issue. To satisfy all these demands, we provide a variety of conventional and advanced methods and techniques:

- Negative staining (e.g., uranylacetate, phosphotungstic acid)
- Carbon coating (Balzers BAE 121)
- Gold coating (Technics Hummer Sputter Coater) Freeze fracturing, freeze etching, platinum/carbon shadowing (Balzers BAF301)
- High pressure freezing (Wohlwend HPF Compact 02)
- Freeze substitution (Leica AFS2)
- Embedding in resins (e.g., Epon, Lowicryl)
- Ultramicrotomy (Leica EM UC7, Reichert Ultracut-E)
- Immunogoldlabeling on ultrathin sections or freeze fracture replica
- 3D analysis by electron tomography of resin embedded samples
- 3D analysis by FIB/SEM tomography
- Processing and visualization of 2D and 3D data (ImageJ, IMOD, AMIRA)

Thus, we can address a broad spectrum of questions in house.



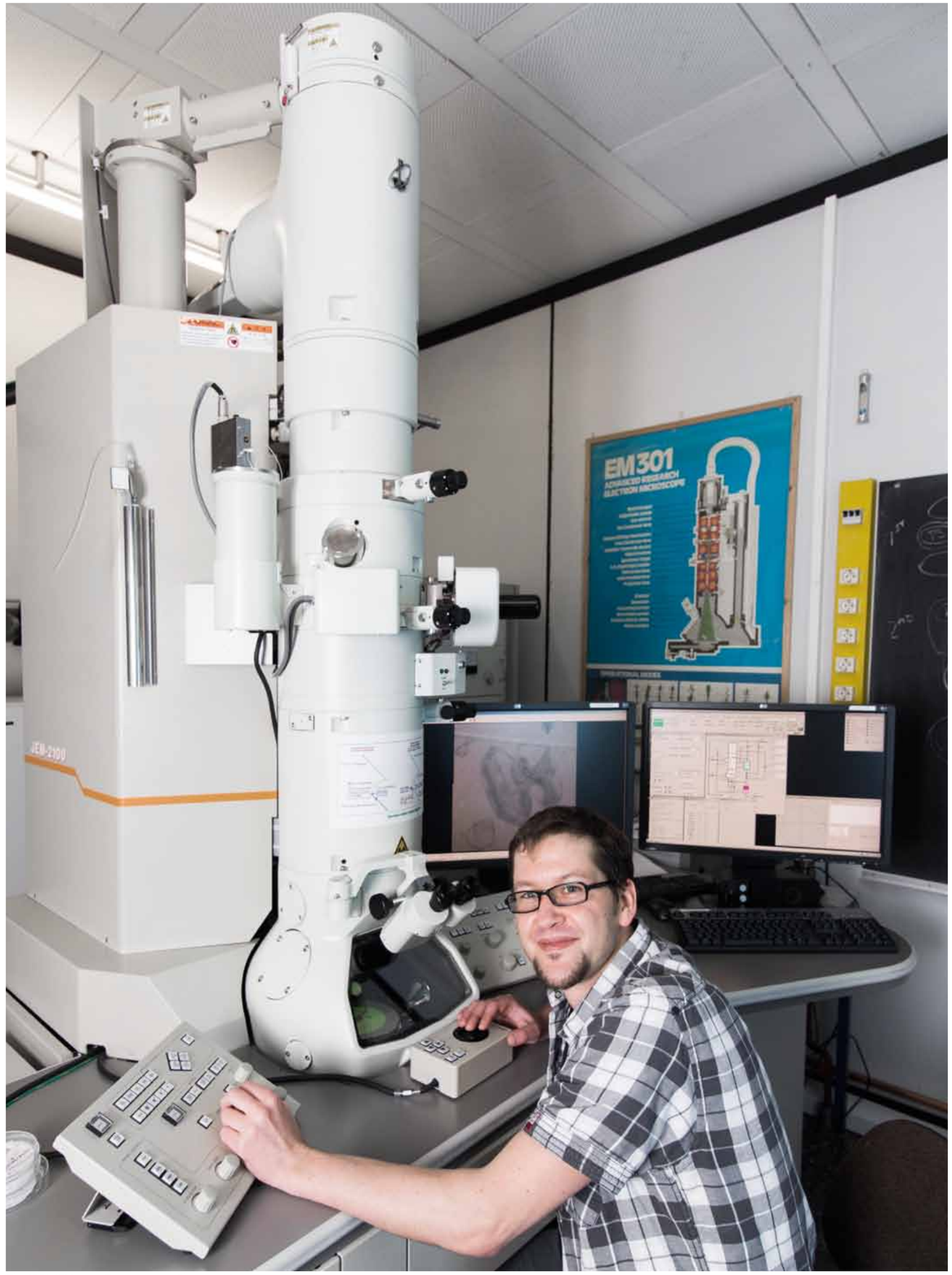
Localization of the A_1A_o ATP synthase of the hyperthermophilic crenarchaeon Ignicoccus hospitalis by immunolabeling on a 50 nm ultrathin section.



3D model of two (half) cells of the diatom Phaeodactylum tricornutum based on a FIB/SEM dataset comprising 971 consecutive images.

SHORT CV

2014– Scientific Manager, SYNMIKRO Electron Microscopy Unit
2014 Ph.D. in microbiology, University of Regensburg
2011 Visiting Scientist, Oak Ridge National Laboratory, Tennessee, USA
2009 Diploma in biology, University of Regensburg



The JEOL JEM-2100 Transmission Electron Microscope.

MarXtal Structural Biology Unit

DR. VASUNDARA SRINIVASAN

Scientific Manager

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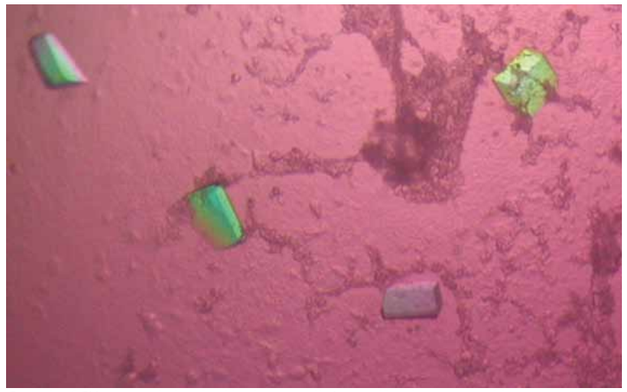
SHORT CV

2014– Scientific Manager, SYNMIKRO Structural Biology Unit
2012–2013 Research Scientist, Philipps-Universität Marburg
2007–2012 Research Group Leader, Free University Brussels & VIB, Brussels, Belgium
2001–2007 Postdoctoral Fellow, Max Planck Institute of Biophysics, Frankfurt
2000 Ph.D. in biochemistry, University of Georgia, USA
1995 M.Sc. in biophysics, University of Madras, India

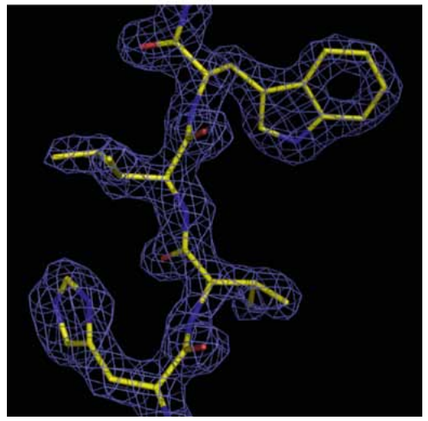
CO-WORKER:

Ralf Pöschke (Technical Assistant)

The determination of the 3D structures of molecules helps in elucidating the molecules' biological functions. This, in turn, is important for the understanding and manipulation of, e.g., disease mechanisms, or the production of biotechnologically relevant metabolites. X-ray crystallography enables such structural insights at atomic resolution, and since there is no size limit for its objects of investigation, small molecules as well as huge macromolecular complexes like ribosomes or whole viruses can be analyzed with this method. The MarXtal Structural Biology Unit localized at the Department of Chemistry, Structural Biochemistry Group, assists its users in every step of the process, from the design of suitable expression constructs for protein production via the optimization of crystals and the collection of data up to the determination and interpretation of the crystal structure.



Crystals of CIA1, a protein involved in the cytosolic Iron-sulfur cluster assembly.

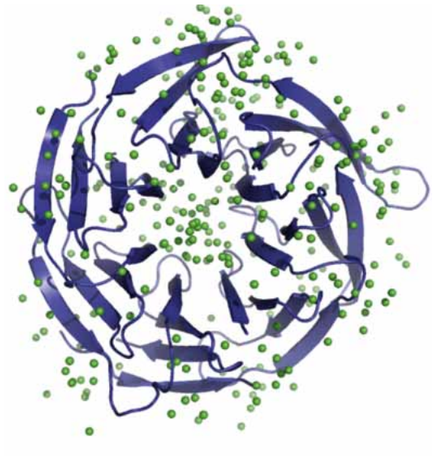


A section of the electron density map of the CIA1 protein at 1.7Å resolution, clearly showing one of the β-sheets and the side chain amino acids.

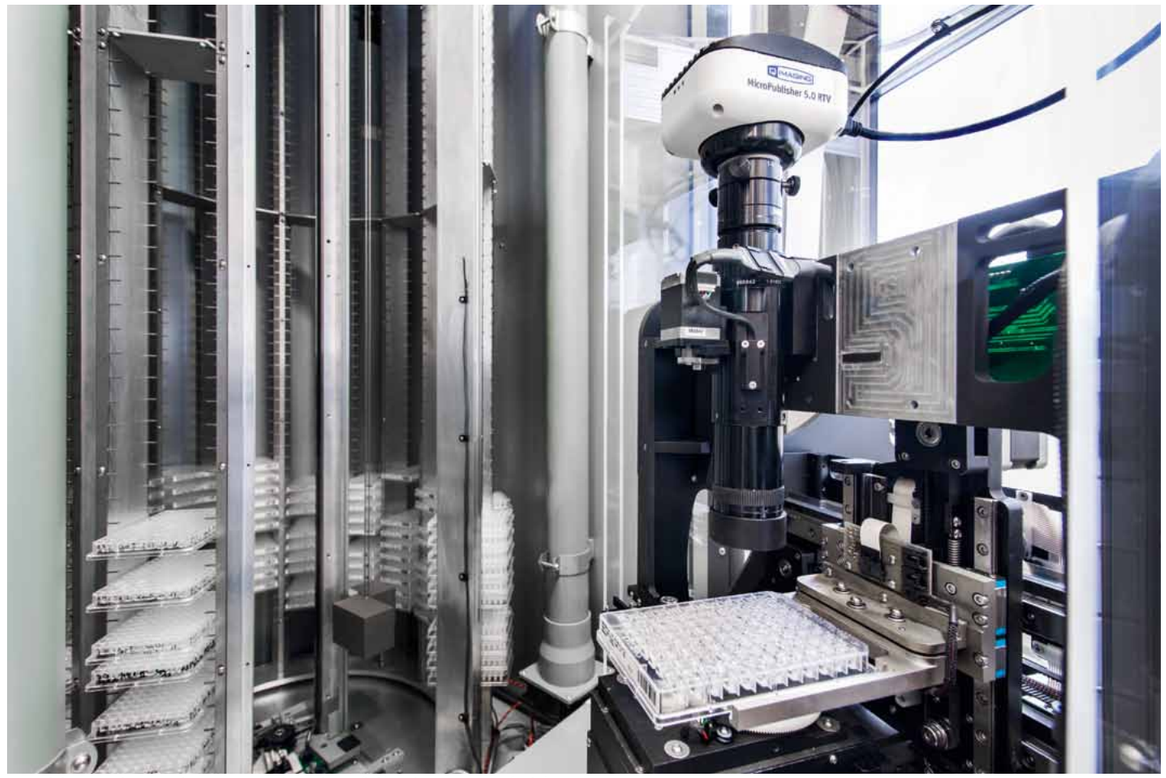
Equipment

To achieve this goal, the facility provides state-of-the-art instrumentation such as a Honeybee 963™ and a Cartesian Microsys ™ SQ 4000 robot for setting up high-throughput crystallization experiments within minutes. Routinely, up to a thousand different crystallization conditions are screened in parallel, with a wide range of buffers and precipitating agents provided both for soluble and for membrane proteins. A liquid-handling system Lissy is available for creating these screening solutions in 96-well deep well blocks. A COY anaerobic glove box is at the user's disposal for proteins/cofactors/ligands that are sensitive to oxygen. Furthermore, light-sensitive proteins can be dispensed in the dark to maintain stability.

Once the drops are set, crystallization experiments can be incubated at 4°C and/or at 18°C and automatically monitored for crystal growth using two Formulatrix Rock Imagers™. These imagers record high-resolution pictures of the drops that the user can access via internet from their own computer. A home rotating anode X-ray generator and high-energy synchrotron beamlines at ESRF in Grenoble, BESSY in Berlin and SLS in Villigen (CH) are then used to check the diffraction properties of the crystals and record images in order to finally solve the atomic resolution crystal structures.



Crystal structure of CIA1 determined to 1.7Å resolution. The structure depicts a seven bladed β-propeller fold with water molecules (green spheres) that can be seen at this high resolution.



One of the Formulatrix Rock Imagers™ with incubator (left) and imaging unit (right).

Applications

- Crystallization trials using 'nanoliter' drops by sitting drop vapour diffusion method
- Automated dispensing of thousands of screening solutions
- Monitoring of crystal growth both at 4°C and at 18°C
- Anaerobic and light-sensitive proteins
- Membrane proteins
- Data collection and structure solution of molecules of any size

Technical Assistant Ralf Pöschke operating the Honeybee 963™.

DR. UWE LINNE

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SHORT CV

2010– Scientific Manager, Core Facility for Mass Spectrometry
2007–2010 Research Scientist, Core Facility for Mass Spectrometry
2001–2007 Research Scientist, Philipps-Universität Marburg
2001 Ph.D. in chemistry, Philipps-Universität Marburg
1998 Diploma in chemistry, Philipps-Universität Marburg

CO-WORKERS:

Jan Bamberger (Chemical Engineer)
Tina Krieg (Laboratory Technician)
Yvonne Ullrich (Laboratory Technician)
Heike Mallinger (Laboratory Technician)
Martina Gerlach (Laboratory Technician)

Core Facility for Mass Spectrometry

The detailed analysis of both small molecules and (bigger) biopolymers is a keystone of today's biology, and mass spectrometry (MS) is the method of choice to address these issues: In Metabolomics, qualitative and quantitative analysis of metabolites by means of HPLC-MS or GC-MS gives insights into the function and catalytic properties of enzymes or even into the metabolic flux within complex systems. In Proteomics, such qualitative or quantitative analysis by means of NanoHPLC-MS/MS identifies proteins within mixtures, quantifies expression levels under different conditions, or gains information on post-translational modifications; the latter, in turn, can be useful to understand enzymatic mechanisms on the molecular level or to get insights into the regulation or modulation of protein function or activity. For structural investigations, HDX MS is a powerful and emerging method that is used to address protein dynamics and to map protein-protein interfaces at the molecular level.

Such an in-depth understanding of natural systems is a prerequisite for the design and construction of synthetic modules or circuits. Moreover, these methods can be used both to track the impact of modifications made to natural systems, and to test the function of actual synthetic systems. Therefore, SYNMIKRO supports the University's Core Facility for Mass Spectrometry – most recently, e.g., the center participated in the purchase of a new mass spectrometer with an HDX-automation platform, which will be used for "high-throughput" analysis. As one of the first institutions worldwide, we want to implement this technology to support crystallization efforts, i.e., to detect "weak spots" of proteins that are highly dynamic and unstructured and thus difficult to crystallize.

Equipment

In the Core Facility for Mass Spectrometry localized at the Department of Chemistry, a number of "state-of-the-art" mass spectrometers are operated:

LTQ-FT Ultra (Thermo Scientific) coupled to an 1100 HPLC (Agilent) or a TLC/MS-Interface (CAMAG)	Ultra-high-resolution mass spectrometer mainly used for the analysis of small molecules
Orbitrap Velos coupled to a nano-RSLC (both Thermo Scientific)	High-resolution mass spectrometer mainly used for all kinds of protein analysis, especially of digests
Synapt with IMS, ETD and HDX automation (Waters)	High-resolution mass spectrometer mainly used for HDX mass spectrometry and mass determination of intact proteins
AccuTOF GCv (JEOL) coupled with a 7890 GC (Agilent)	High-resolution GC-MS system mainly used for small molecule applications
1100 Single Quadrupol HPLC-MS system (Agilent)	Low-resolution HPLC-MS system mainly used for method development
5973N/6890N GC-MS system (Agilent)	Low-resolution GC-MS system mainly used for quantification of small molecules
Element2 ICP-MS system (Thermo Scientific)	High-resolution ICP-MS system used for analysis of trace elements

Adjustment of the nanospray ion source at the Orbitrap Velos Pro mass spectrometer.

Filling liquid nitrogen into the supra-conducting magnet of the LTQ-FT Ultra mass spectrometer. The nano-RSLC seen in the back is coupled to an Orbitrap Velos Pro mass spectrometer.

Applications

The Core Facility for Mass Spectrometry covers almost the whole range of conceivable applications:

- Qualitative or quantitative analysis of small molecules (Metabolomics)
- Qualitative or quantitative analysis of biopolymers, especially of proteins (Proteomics), including protein identification, analysis of post-translational modifications, and mass determination of intact proteins
- HDX mass spectrometry of proteins or protein complexes
- Ion Mobility Separation (IMS) coupled to classical mass spectrometry
- Analysis of trace elements by ICP-MS

3 TEACHING & EVENTS

Building a vibrant community for front line synthetic biology

SYNMIKRO provides training of synthetic biologists at the undergraduate, graduate and postdoctoral level. All our educational programs engage with technology and knowledge at the frontiers of the field.

Mastering synthetic microbiology

Within the Master program *Molecular and Cellular Biology* of the university's Faculty of Biology, SYNMIKRO members offer a specialization in synthetic microbiology. In this specialization, our experts guide hands-on education, combining classic and synthetic biology approaches with modern high-throughput robotics, high-resolution imaging, structural biology and modeling techniques. A major goal is the education of our students towards an in-depth understanding of the function and organization of microbial cells as the basis for successful synthetic approaches.

SYNMIKRO at the iGEM competition

With the support of the center, undergraduate and graduate students of the Philipps-Universität successfully participated in the international Genetically Engineered Machine (iGEM) competition founded by the Massachusetts Institute of Technology (MIT). Every year, student teams from universities all over the world engage in creative scientific projects with an emphasis on synthetic biology. Moreover, the teams are encouraged to present their work to the general public, promoting a dialogue between science and society. After about nine months, they compete with hundreds of other teams at the iGEM jamboree. The challenges associated with this concept lead to an impressive progress in the students' scientific skills, their level of independent thinking and their personal confidence.
In 2013, the Marburg iGEM team used the microalga *Phaeodactylum* to synthesize and secrete an antibody against a Hepatitis B virus protein (PHAECTORY). This approach constitutes an elegant and cost-effective way of producing a high-value molecule important for medical applications, earning the team a gold medal in the European competition and the admission to the world finals at the MIT in Boston. In 2014, our iGEM team engineered synthetic scaffolds and catalysts by creating synthetic units for redirecting functionalities (SURF). Again the team won a gold medal, this time at the Giant Jamboree in Boston. They also started an intense collaboration with the Blindenstudienanstalt (BLISTA) in Marburg and introduced visually impaired people to synthetic biology. Judges at the Giant Jamboree especially honored this approach.

The center's Graduate School

Over 40 doctoral students receive an intense and professional training in the SYNMIKRO Graduate School. The curriculum involves weekly lectures and seminars in synthetic biology offered by external guests or in-house experts and, every other year, a retreat for all students enrolled. In addition, our graduate students participate in at least two workshops to advance their methodological and soft skills. Soft skill training is offered through an intense collaboration of the center with the Marburg University Research Academy (MARA). Moreover, the members of the SYNMIKRO Graduate School profit from activities within the International Max Planck Research School for Environmental, Cellular and Molecular Microbiology (IMPRS-Mic) and the Graduate School of the SFB 987.
Thesis Advisory Committees that consist of three PIs from SYNMIKRO or other research institutions support each graduate student in annual consultations to ensure optimal progress and development. Where relevant for their research projects, our graduate students perform internships in other SYNMIKRO groups or visit labs abroad through the center's short-term fellowships. Moreover, graduate students are obliged to participate in international scientific conferences. Social and scientific meetings further intensify interactions – prominent examples being the "Missions for Synthetic Biology" club where our students and external guests discuss challenges and opportunities of this new emerging scientific discipline, the Postdoc Symposium, or the Twin Talks, pairing up two SYNMIKRO graduate students from different disciplines to present current research topics.

The 2013 iGEM team at the MIT in Boston. (Photo: SYNMIKRO)

Promoting early careers

Furthermore, SYNMIKRO established an easy-to-access exchange program that provides short-term funding to graduate students and postdoctoral researchers to deepen their knowledge and broaden their methodological expertise. The outgoing fellowship allows a three-month stay at another research institution in Germany or abroad. The incoming fellowship enables national or international researchers a three-month visit at SYNMIKRO to profit from the theoretical and methodological expertise available at the center. Consequently, the exchange program has already promoted a significant number of scientific collaborations with other national and international research institutions and represents a spring-board for synthetic microbiologists at an early stage in their career.
In addition to these short-term fellowships, the center also established a long-term fellowship that allows excellent postdoctoral researchers to establish an independent research program and prepare first grant proposals. Since 2013, six long-term fellowships were awarded.

SYNMIKRO students advertising the Master program.

The Schools: interdisciplinary training of early career scientists

SYNMIKRO aims to foster the international and interdisciplinary culture of synthetic biology research at all career levels. To this end, our seasonal schools provide expert training on cutting-edge research and a stimulating environment for graduate students and young postdocs to broaden their knowledge and develop their network of contacts.

SYNMarburg Summer School

The first two international *SYNMarburg Summer Schools* were organized by SYNMIKRO in 2012 and 2013, focusing on „Microbial Cell Biology and Synthetic Signaling Systems“. In 2013, 22 German and international Master and doctoral students as well as young postdoctoral researchers from 11 nations attended this 2-week school to learn about the emerging field of synthetic microbiology. Symposia with lectures by invited experts and laboratory courses covered various aspects of the field, and the participants enjoyed a diverse social program in Frankfurt and Marburg. One evening contained a lecture on ethics in biology, which triggered a lively discussion among the participants. The *SYNMarburg Summer Schools* were kindly supported by the German Academic Exchange Service (DAAD). Building on the great success of these events, early career researchers are invited to the next *SYNMarburg Summer School* „From Microbial Cell Biology to Complex Communities“ taking place from 7th to 18th of September, 2015.

International Autumn School: *Biology featuring Engineering*

SYNMIKRO also joined forces with the Helmholtz Initiative on Synthetic Biology to organize the International Autumn School: *Biology feat. Engineering* in November, 2014. Its realization was a shared effort of the DKFZ Heidelberg, Forschungszentrum Jülich, Karlsruhe Institute of Technology (KIT) and SYNMIKRO, all offering hands-on computational and/or laboratory workshops in Heidelberg, Jülich and Marburg during the first week of the school. The 35 participants from 9 countries could choose among eight courses on topics such as “Synthetic regulatory circuits in microbial and mammalian cells”, “Rewiring cells to explore logic, function and evolution”, or “Single cell analysis in microfluidic chips”.

“Single cell analysis in microfluidic chips” workshop at the Forschungszentrum Jülich, International Autumn School. (Photo: Forschungszentrum Jülich)

“Laboratory Automation” workshop at SYNMIKRO, International Autumn School. (Photo: SYNMIKRO)

This practical part was followed by joint activities in Marburg to foster the exchange of knowledge: Internationally renowned experts in synthetic biology from academia and industry delivered lectures. Moreover, the attendees had the opportunity to present their own research as well as the knowledge and expertise acquired during the workshops to the other participants. This special concept allowed the young researchers to get to know leading institutes in the synthetic biology landscape in Germany and to meet invited national and international speakers. A number of social events supported networking activities.

The International Autumn School: *Biology feat. Engineering* received major financial support from the BMBF and the Helmholtz Initiative; further support came from Carl Roth GmbH, Nikon, Life Technologies, and Hessen Trade & Invest.

“Synthetic regulatory circuits in microbial and mammalian cells” workshop at the DKFZ Heidelberg, International Autumn School. (Photo: DKFZ)

Bringing scientists together to stimulate new ideas and concepts

Prof. Kristala Jones Prather (MIT) presenting her research at the SYNMIKRO Symposium 2014.

Part of SYNMIKRO's self-assigned mission is to foster the exchange between scientists. For this, the center regularly organizes and supports workshops, meetings, and conferences on various topics related to synthetic biology. In 2012, the conference "Mathematical Modeling of Microbiological Systems" brought close to 70 experts from 11 countries to Marburg. In the same year, the third conference on "Molecular Biology of *Archaea*" attracted approximately 150 participants from all over the world to discuss systems biology, biochemistry, structural and cell biology, and the genetics of *Archaea*. In 2014, the first meeting on "Microbial Cell Biology" of the respective section of the German Association for General and Applied Microbiology (VAAM) highlighted new concepts and ideas following recent developments in molecular and imaging techniques. Later that year, almost 90 scientists from 18 countries met in Rauischolzhausen for the EMBO workshop on "Stalked alpha-Proteobacteria and Relatives: from Genes to Structure", at which advances in the field of bacterial cell biology, ranging from genomics and chromosome dynamics over cell cycle regulation, morphogenesis and cell division to modeling and biotechnological applications were presented.

The most prominent outcome of this commitment, though, is the annual SYNMIKRO Symposium, a one-day-conference in spring that brings together scientists from academia and industry. This format, for which the center found a strong partner in Hessen Trade & Invest GmbH (HTAI), was initiated by Erhard Bremer, who together with his co-organizers arranged up-to-date programs with prominent speakers that attracted more and more participants over the years, filling the assembly hall of the scenic Old University in downtown Marburg to the last seat with close to 400 attendees.

This conference was first organized in 2011, then dealing with "Synthetic Microbiology – Perspectives for Biotechnology and Pharmacy", with prominent

A mesmerized audience (from left): Prof. Peter Dürre (Ulm), Prof. Volker Müller (Frankfurt), Prof. Jay Keasling (Berkeley), Prof. Rudolf Thauer (Marburg), Dr. Rainer Waldschmidt (HTAI), and Prof. Erhard Bremer (Marburg).

Stimulating discussions during the lunch break in the courtyard of the Old University in downtown Marburg.

speakers both from academia and industry, e.g., Prof. Bärbel Friedrich (HU Berlin), Prof. Wilfried Weber (U Freiburg), Dr. Holger Zinke (BRAIN AG), Prof. Oskar Zelder (BASF SE), Dr. Markus Schwab (Evolva SA), and Prof. Ralf Wagner (GENE ART AG). In 2012 the symposium addressed "Design of Antibiotics – Innovation Potential of Synthetic Microbiology", with Prof. Jörg Hacker (Leopoldina), Prof. Helge Bode (U Fankfurt), Dr. Claus Lattemann (Sanofi-Aventis GmbH), Prof. Helga Rübsamen-Schaeff (AiCuris GmbH) and Dr. Wolfgang Mutter (Hyglos GmbH) among the speakers. The 2013 conference "From Biological Diversity to Microbial Cell Factories" dealt with the question how modern -omics technologies and synthetic approaches could improve the exploitation of the diverse catalytical properties developed by microorganisms during evolution; speakers included Dr. Guido Meurer (BRAIN AG), Prof. Alfred Pühler (U Bielefeld), Dr. Jan Van den Brulle (MorphoSys AG), and Dr. Esther Gabor (BRAIN AG). The most recent conference – „Microbial Formation of Biofuels and Platform Chemicals" in 2014 – brought experts on metabolic engineering to the podium, including Prof. Jay Keasling (UC Berkeley), Dr. Thomas Haas (Evonik Industries), Prof. Kristala Jones Prather (MIT), Dr. Andrea Herold (BASF SE), and Prof. Ferdi Schüth (MPI Mühlheim).

The 2015 SYNMIKRO Symposium on April 22nd will focus on "Microbial Biosensors and Regulatory Circuits".

Panel discussion on "Complexity" in December, 2013. From left: Prof. Bruno Eckhardt, Prof. Sven Panke, Prof. Friedemann Voigt, and Prof. Klaus Mainzer.

A dialogue between synthetic biology and humanities

Since the early days of the center, SYNMIKRO paid attention to the political and ethical aspects of synthetic biology. In 2010, ethicist Prof. Peter Dabrock, at that time member of the Faculty of Protestant Theology in Marburg and of the center, hosted a panel discussion on the chances and risks of the field. In 2013 and 2014, a whole series of public events was organized in order to promote the mutual understanding of life sciences and humanities in the recurrent debates. At each event, short lectures of prominent experts from both disciplines first demonstrated their different approaches and points of view. According to Prof. Friedemann Voigt, present leader of the SYNMIKRO Bioethics group and initiator of the series, these different perspectives often lead to misunderstanding and even distrust. Therefore, subsequent panel discussions offered an opportunity for the experts to enter into a dialogue, and for the audience to ask questions.

The first event was dedicated to "Life", its definitions, and the question whether or not scientists are allowed to rebuild or even change it; speakers were biologist Prof. Jörg Hacker, President of the German Academy of Natural Sciences Leopoldina, and philosopher Prof. Gerald Hartung, Universität Wuppertal, hosted by Prof. Friedemann Voigt and Prof. Michael Bölker. The second event addressed the "Complexity" of biological systems, with Prof. Sven Panke, biotechnologist from the ETH Zurich, and Prof. Klaus Mainzer, philosopher from the Technical University Munich, as speakers, and Prof. Bruno Eckhardt and Prof. Friedemann Voigt as hosts. The last event dealt with the opposites „Natural/Synthetic" and, e.g., the observation that most advances in synthetic biology are still based on natural molecules or building blocks, whereas a lot of what is perceived as natural in our environment – like the European cultural landscape – already is the result of human intervention; speakers were Prof. Nedilijko Budisa, chemist from the Technical University Berlin, and Prof. Kristian Köchy, philosopher from the University Kassel, hosted by Prof. Regine Kahmann and Prof. Friedemann Voigt.

All events were organized by SYNMIKRO and the Graduate Center for Life and Natural Sciences of the Philipps-Universität Marburg.

Further reading

Voigt, F (Ed.) (2015). Synthetische Biologie im Dialog, Karl Alber (Series: „Lebenswissenschaften im Dialog", edited by Kristian Köchy and Stefan Majetschak), Freiburg i.Br., in press.

Art & Science

The local artist Ingrid Hermentin was repeatedly inspired by SYNMIKRO research. For her exhibition "Transkriptionen_TATA-Box" at the Lutherische Pfarrkirche Marburg in 2013, Hermentin created "synthetic pictures" based on images of the microalga *Phaeodactylum tricornutum* and phylogenetic trees, visualizing her view on "synthetic evolution". For "Transkriptionen_BioBricks", presented 2014 at the Marburger Kunstverein, the artist adapted the iGEM approach by assembling her "synthetic images" (modules) in the given art space (chassis), in order to achieve a change of perspectives.

The exhibitions were each accompanied by public events with talks of SYNMIKRO members on scientific and/or ethical aspects.

Top: The exhibition „Transkriptionen_TATA-Box" 2013 at the Lutherische Pfarrkirche Marburg. Bottom: The exhibition „Transkriptionen_BioBricks" 2014 at the Marburger Kunstverein. (Photos: Bildarchiv Foto Marburg)

Informing and inspiring society

Members of SYNMIKRO and the university's iGEM team regularly show-case interesting features of synthetic biology to the general public, e.g., at the „Hessentag", an annual event of the federal state of Hessen with more than one million visitors, and the annual city festival „3 Tage Marburg". (Photos: SYNMIKRO)

Work hard, party hard

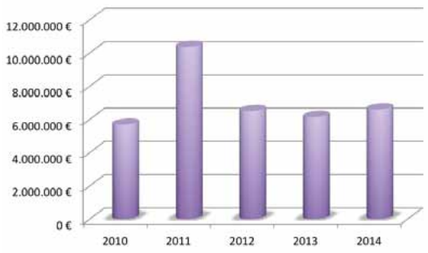
Left: Training for the dragon boat race at the town festival "3 Tage Marburg". Right: Halloween and Christmas Parties. (Photos: SYNMIKRO)

4 FACTS & FIGURES

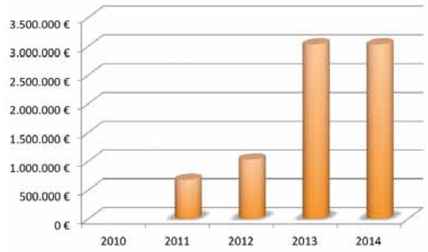
Financial and infrastructural resources

The Mehrzweckgebäude on the science campus Lahnberge, temporary home for six SYNMIKRO groups, the Super Resolution Microscopy Unit, the Laboratory Automation Unit, and the Head Office. (Photo: Reinhold Eckstein)

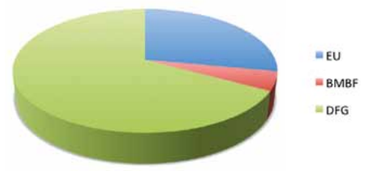
LOEWE funding



External funding



Sources of the external funding



SYNMIKRO was established following the successful application for the excellence program LOEWE of the state of Hessen: For its first three years the center was awarded a total of € 22.4 m, and for the second funding period up to € 21.7 m. This support has been used to build and shape the center via (i) the appointment of two new professors at the university, (ii) the founding of a fourth department at the Max Planck Institute, (iii) the appointment of four temporary groups at the university to attract junior researchers to Marburg, (iv) the provision of seed money to initiate projects that can then be carried on with external funding, and (v) major investments in infrastructure.

External funding

Over the years, these investments in the infrastructure and in the projects paid off and the center attracted additional external funding: By the end of 2014, researchers at the center had been awarded grants totaling more than € 15 m, a major contribution being the Sonderforschungsbereich 987, which alone was granted € 7.058 m over its four years of funding. The largest fraction of grants comes from the German Research Foundation (DFG), followed by the EU via the European Research Council and a EU-Cost action. The aim for the next few years is to expand on funding from the BMBF and industrial collaborations. Among coordinated programs, another Sonderforschungsbereich and a Graduiertenkolleg are planned. Overall, the center is well on its way to establish an annual funding that amounts to at least half of the annual seed funding by the state of Hessen. This, together with the basic support for the Departments at the Max Planck Institute for Terrestrial Microbiology and for the research and infrastructure at the Philipps-Universität Marburg, secures the long-term perspective of the center.

Temporary facilities…

The seed money provided by the LOEWE program enabled us to establish more than 130 positions in research and infrastructure. Some of the new personnel were integrated into the existing groups, but for the largest part new labs and offices had to be provided. The dedicated support of the construction department of the university allowed us to establish temporary, but nevertheless state-of-the-art laboratories and offices within the Mehrzweckgebäude on the science campus Lahnberge. Occupying four levels around stairway C, the groups of Anke Becker, Peter Graumann, Torsten Waldminghaus, Gert Bange, Kristina Jonas and Georg Fritz, and the Head Office of SYNMIKRO make use of about 1100 m² lab space and 450 m² of offices. In addition to special spaces for

The recently completed building for the new Max Planck Department, a junior research group, an independent Max Planck Research Group, and the Flow Cytometry and Protein Diffusion & Interactions Unit.

many microscopes, the building offers a safety-level 2 laboratory and room for a laboratory automation platform. Currently, about 100 people work here.
Space for the fourth Department at the Max Planck Institute was provided in a newly constructed building, located on the Lahnberge between the Mehrzweckgebäude and the Max Planck Institute (and marked “Zentrum für Synthetische Mikrobiologie” on the map on p. 119). Funded through an extra grant by the state of Hessen, the new building was commissioned in 2011 and finished in August 2014. Its 1200 m² lab and office space are occupied by Victor Sourjik and his group, a junior research group and an independent Max Planck Research Group, as well as the new Flow Cytometry and Protein Diffusion & Interactions Unit.

…and a future home

From the very beginning, the aim has been to bring together the key research groups and infrastructural units in one building so as to intensify interactions and, in particular, interdisciplinary collaborations. The decision by the German Council of Science and Humanities (Wissenschaftsrat) in spring 2014 to fund the respective proposal finally paved the way for this SYNMIKRO building. With about 6000 m² lab and office space, it will house the Max Planck Department of Systems and Synthetic Microbiology, five groups from the university’s Faculties of Biology and Chemistry, four junior groups and two groups in biological modeling. Furthermore, most of the center’s infrastructural units, a high-resolution cryo-TEM and a lecture hall will be located in the new building, providing SYNMIKRO with excellent working conditions and the long-sought integrating home. We expect the building to be finished by 2019.

A moment of success. The speaker of the SFB 987 Mohamed Marahiel (right) and the vice speaker Erhard Bremer celebrate the decision of the DFG to establish and fund the Collaborative Research Center Microbial Diversity in Environmental Signal Response. (Photo: SYNMIKRO)

SFB 987: Microbial Diversity in Environmental Signal Response

Logo of the SFB 987.

Microorganisms successfully colonize almost every possible ecological niche, regardless of its conditions. A key factor for this success is their enormous biochemical, physiological, and cellular adaptability: In the course of evolution, microorganisms have developed an array of mechanisms that enables both individual cells and cellular communities to recognize environmental cues with high sensitivity, precision and specificity, to adapt to changing circumstances, and to exploit new opportunities. The scientists that joined forces within the framework of the DFG-funded Collaborative Research Center 987 (SFB 987) focus on these key features of microorganisms and structure their work around the encompassing theme *Microbial Diversity in Environmental Signal Response*.

The title of the SFB 987 heralds one of the strength of microbial research in Marburg: Instead of concentrating on just a few model organisms, the research groups work with a large variety of *Bacteria*, *Archaea*, and *Fungi* and study their properties both under controlled laboratory conditions and in their natural habitats. The central aim is to comprehensively understand specific cellular activities and signaling processes of defined species, the behavior of individual cells and complex microbial communities, the interactions of microorganisms with each other and with their eukaryotic hosts, and the countless contributions that microorganisms make to globally-acting cycles of nutrients and gases operating on our planet. A breadth of approaches and techniques are applied to further these goals.

The SFB 987, which will receive 7 058 000 € from the DFG over the initial four years, began its work in July, 2012. Within this framework, 15 microbiology-focused research teams from the Faculties of Biology, Chemistry and Medicine of the Philipps-Universität and from the Max Planck Institute for Terrestrial Microbiology combine their research activities and expertise. 14 of these groups are also members of SYNMIKRO, and the research activities of the SFB and of SYNMIKRO are thus tightly interwoven. Furthermore, SYNMIKRO attracted strong new research teams to Marburg, providing the members of the SFB 987 with even more interesting partners for future collaborations. On the other hand, a thorough understanding of the genetics, biochemistry, cell biology, and physiology of microorganisms is needed to exploit them for *synthetic microbiological* purposes and to devise cells and processes that do not exist naturally. This expertise is the solid foundation of the activities of the SFB 987.

Erhard Bremer and Mohamed A. Marahiel

Awards & Honors

2014

Roland Lill	Albrecht Kossel Prize, German Chemical Society (GDCh) Luigi Sacconi Medal, Società Chimica Italiana (SCI)
Mohamed A. Marahiel	David Gottlieb Memorial Lecture, University of Illinois, Urbana, IL
Lotte Søgaard-Andersen	Elected Fellow, American Academy of Microbiology

2013

Sonja-Verena Albers	ERC Starting Grant, European Research Council
Peter Lenz	Paula and Richard von Hertwig Award for interdisciplinary cooperation
Rolf Thauer	Medal of Merit, German Academy of Sciences Leopoldina

2012

Sonja-Verena Albers	Research Award, Association for General and Applied Microbiology (VAAM)
Gerhard Klebe	Carl Mannich Medal, German Pharmaceutical Society
Mohamed A. Marahiel	Honorary Professorship, Wuhan University, China

2011

Erhard Bremer	Elected Member, European Academy of Microbiology
Regine Kahmann	Distinguished Affiliated Professorship, TU München Honorary Doctorate, Hebrew University, Jerusalem
Gerhard Klebe	ERC Advanced Grant, European Research Council
Victor Sourjik	ERC Advanced Grant, European Research Council
Martin Thanbichler	Research Award, Association for General and Applied Microbiology (VAAM)

2010

Ralf Conrad	Einstein Distinguished Chair Professorship, Chinese Academy of Sciences
Roland Lill	Feldberg Foundation Prize

Former Members

Ekaterina Kostina	accepted an offer from U Heidelberg (2015)
Sonja-Verena Albers	accepted an offer from U Freiburg (2014)
Andreas Klingl	accepted an offer from LMU Munich (2014)
Eyke Hüllermeier	accepted an offer from U Paderborn (2014)
Alexander Böhm (†)	was a member of Synmikro in 2012
Peter Dabrock	accepted an offer from U Erlangen (2011)

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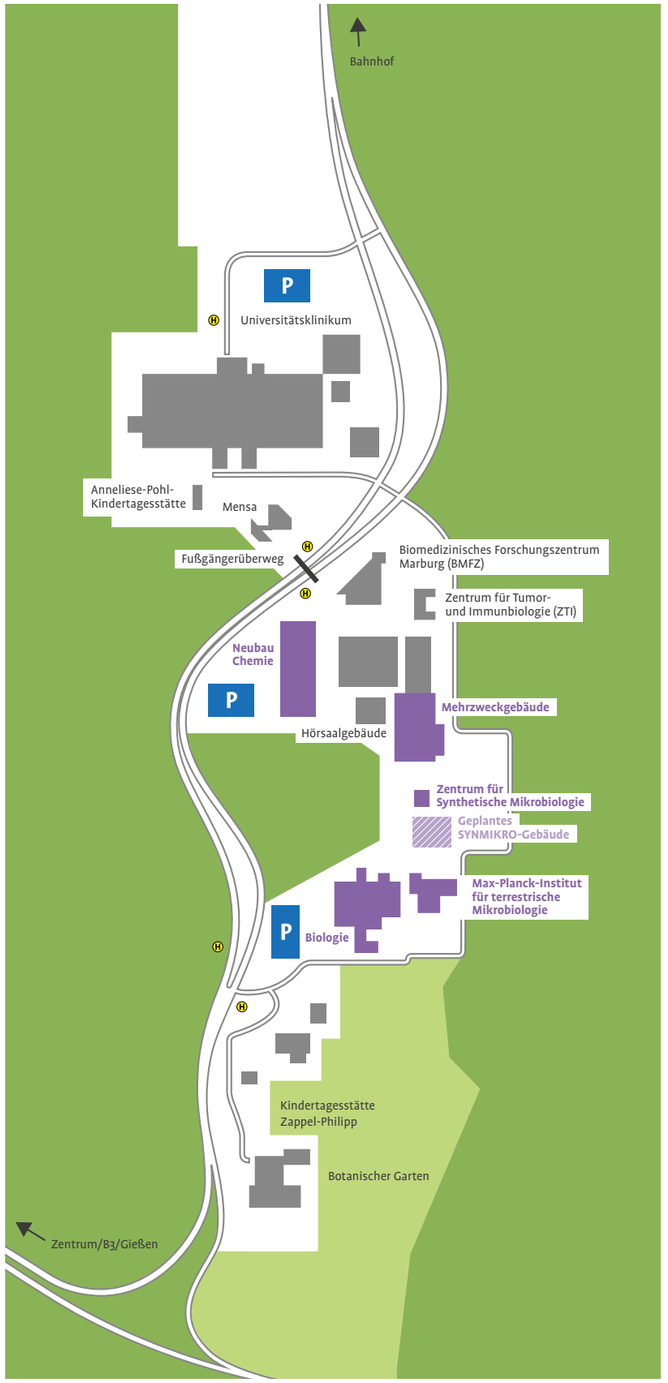
Science Campus Lahnberge



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