EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

EUROCORES Programme
European Collaborative Research

EUROSYNBIO

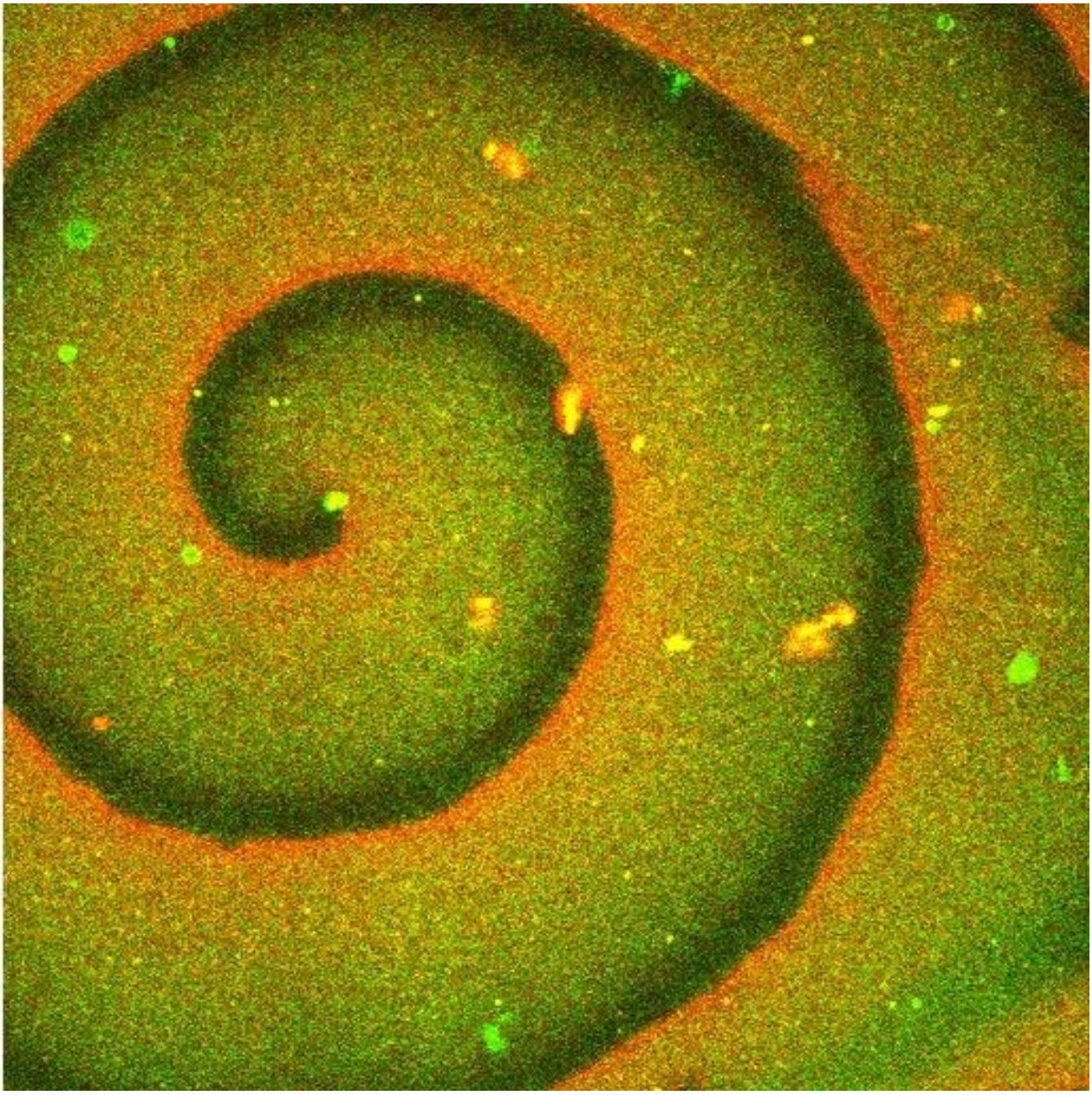
Synthetic Biology: Engineering Complex Biological Systems

First EuroSYNBIO Conference

Cannes-Mandelieu, France

25-27 May 2011

Conference Booklet



EUROPEAN SCIENCE FOUNDATION
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EUROSYNBIO
Synthetic Biology: Engineering Complex Biological Systems

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Programme

Wednesday 25 May 2011 – Poster room (Room "Bart" on first floor of "Le Neptune"-building)

19:00	**Welcome drink & poster session**
20.00	**CONFERENCE DINNER (in "Riviera" restaurant)**

Thursday 26 May 2011 – Plenary room (Room "Surcouf" on first floor of "Le Neptune"-building)

9:00	**Welcome and Introduction** Paul Beckers *(European Science Foundation),* EuroSYNBIO Coordinator / Oscar Kuipers *(University of Groningen),* Project Leader SYNMOD
9:20	**'SynMod: Design and production of novel lantibiotics by synthetic biology** Oscar Kuipers *(University of Groningen),* Project Leader SYNMOD, and Steven Schmitt *(ETH Zurich),* Project member SYNMOD (Panke group)
9:50	**'Lantibiotic precursor and transcriptional element libraries'** Andreas Leiherer *(University of Regensburg),* Project member SYNMOD (Wagner group)
10.20	**COFFEE BREAK (on ground floor, at riverside)**
10.50	**'Towards programing nanocells wih functional units'** Daniel Müller *(ETH Zürich),* Project Leader NANOCELL
11:10	**'Multifunctional block copolymers vesicles for protein reconstitution'** Mihaela Delcea *(University of Basel),* Project member NANOCELL (W. Meier group)
11:30	**'Orthogonalization of in vitro reaction networks by proteomic switches: Transposon-guided engineering of an off-switchable chaperonin'** Sonja Billerbeck *(ETH Zürich),* Project member NANOCELL (Panke group)
11:50	**'Engineering rotor sizes in the ATP synthase nanomotor'** Thomas Meier *(Max-Planck-Institute for Biophysics, Frankfurt am Main),* Principal Investigator NANOCELL
12:10	**'Cellular reconstitution: Rebuilding biological systems from the bottom up'** Daniel Fletcher *(University of California, Berkeley),* Invited speaker
13:00	**LUNCH (in "Riviera" restaurant) and POSTER SESSION (in Poster room)**

15:00	**'Expanding the central dogma'** Philipp Holliger *(MRC Laboratory of Molecular Biology, Cambridge),* Principal Investigator SYNAPTA
15:30	**'Target identification of cell-specific aptamers'** Jan Vinkenborg *(University of Bonn),* Project member SYNAPTA (Famulok group)
16:00	**'Spatial cues for the self-organization of cell division proteins'** Petra Schwille *(Technical University Dresden),* Project Leader SynDiv
16:20	**'Bacterial cell division studied in synthetic cell shapes'** Cees Dekker *(Delft University of Technology),* Principal Investigator SynDiv
16:40	'**Dissection and rebuilding of the Escherichia coli divisome'** David Sherratt *(University of Oxford),* Principal Investigator SynDiv
17:00	**COFFEE BREAK (on ground floor, at riverside)**
17:30	**'Understanding methylotrophy in gram-positive bacilli as a basis for creating synthetic methylotrophs'** Trygve Brautaset *(SINTEF Trondheim),* Project Leader SynMet
18:00	**'From natural to synthetic methylotrophs: Understanding the parts and how they could come together'** Julia Vorholt *(ETH Zürich),* Principal Investigator SynMet
18:30	**'Synthetic biology approaches to Corynebacterium glutamicum: access to new carbon sources and new products'** Volker Wendisch *(Bielefeld University),* Principal Investigator SynMet
19:00	**Closure of day**
20.00	**CONFERENCE DINNER (in "Riviera" restaurant)**

Friday 27 May 2011 – Plenary room (Room "Surcouf" on first floor of "Le Neptune"-building)

9:00	**World Café** Markus Schmidt / Wolfgang Kerbe *(Organisation for International Dialogue and Conflict Management (IDIALOG), Vienna),* Principal Investigator / Project member SYNMOD
11.00	**COFFEE BREAK (on ground floor, at riverside)**
11.30	**World Café continued: Final discussions and collection of results** Markus Schmidt / Wolfgang Kerbe *(IDIALOG Vienna),* SYNMOD
13:00	**Closure of conference** Paul Beckers *(European Science Foundation),* EuroSYNBIO Coordinator / Oscar Kuipers *(University of Groningen),* Project Leader SYNMOD
13:30	**LUNCH (in "Riviera" restaurant)**
14:30 – 16:00	***Scientific Committee meeting*** *(Project Leaders only) – in Plenary room*

Sonja Billerbeck

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Collaborative Research Project (CRP):
NANOCELL

Session: NANOCELL

Orthogonalization of in vitro reaction networks by proteomic switches: Transposon-guided engineering of an off-switchable chaperonin

Sonja Billerbeck, Sven Panke
ETH Zurich

The conditional inactivation of proteins is an important tool for investigating cellular mechanisms as well as for synthetic biology and biotechnology [1-3]. We explore off-switchable protein variants as tool for the topological insulation (orthogonalization) of reaction networks from metabolism as we use these reaction networks for the production of rare fine chemicals in cell free systems[4].

A major problem of recruiting multi-enzyme systems from complex cell free extract is the presence of enzymes which are not part of the desired reaction pathway but sequester starting material, important intermediates or cofactors and which are essential to cell growth and whose genes can thus not be knocked-out. Our goal is to implement an orthogonal reaction network by comprehensive, proteome-wide off-switching of interfering enzyme activities at the in vitro stage.

One potentially proteome-wide strategy for off-switching is to equip proteins with protease recognition sites that are acted upon by a protease that is orthogonal to Escherichia coli´s proteome. Currently, we are testing this strategy with a number of enzyme activities that interfere with our multi-enzymatic production of the fine chemical dihydroxyacetone-phosphate. One major obstacle is the essential and complex folding machine GroEL, which continuously removes ATP from cell free extracts.. An off-switchable variant of GroEL can be engineered by random transposon-guided insertion of cleavage sites into the coding region, followed by selection for functional variants and screening for susceptibility to in vivo and in vitro hydrolysis and complete inactivation by the protease.

By such a random transposon-based insertion and selection approach we could identify a fully off-switchable GroEL variant that is functional in vivo and in vitro. Further, the identified permissive site accepts different amino acid sequences, like a FlAsH binding motive [5], enabling for the engineering of GroEL variants for a variety of applications.

1. Ehrmann, M., et al., TnTIN and TnTAP: Mini-transposons for site-specific proteolysis in vivo. Proceedings of the National Academy of Sciences of the United States of America, 1997. 94(24): p. 13111-13115.

2. Elowitz, M.B. and S. Leibler, A synthetic oscillatory network of transcriptional regulators. Nature, 2000. 403(6767): p. 335-8.

3. Fung, E., et al., A synthetic gene-metabolic oscillator. Nature, 2005. 435(7038): p. 118-22.

4. Bujara, M., et al., Exploiting cell-free systems: Implementation and debugging of a system of biotransformations. Biotechnol Bioeng, 2010. 106(3): p. 376-89.

5. Adams, S.R., et al., New biarsenical ligands and tetracysteine motifs for protein labeling in vitro and in vivo: synthesis and biological applications. Journal of the American Chemical Society, 2002. 124(21): p. 6063-76.

Trygve Brautaset

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Collaborative Research Project (CRP):
SynMet - A synthetic Biology Approach for Engineering of Bacterial Methylotrophy

Session: SynMet

Understanding methylotrophy in gram-positive bacilli as a basis for creating synthetic methylotrophs

Trygve Brautaset
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The thermotolerant bacterium Bacillus methanolicus is a restricted methylotroph that beside of methanol can utilize few alternative multicarbon sources for growth and energy. B. methanolicus methylotrophy is linked to a 19 kb plasmid denoted pBM19 and methylotrophic growth involves the concerted reqruitment of pBM19 and chromosomal genes. We have recently genome sequenced two wild-type B. methanolicus strains representing the first thermotolerant bacilli genomes, and these DNA sequences provide new insight into the genetic and regulatory basis for methylotrophy in thermotolerant bacilli. This information is important basis for the rational modular transfer of methylotrophy to other bacteria by using synthetic biology.

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Collaborative Research Project (CRP):
SynDiv - Synthetic Biology of the Bacterial Cell Division

Session: SynDiv

Bacterial cell division studied in synthetic cell shapes

Cees Dekker
Delft University of Technology

The cell division mechanism in typical rod-shaped bacteria such as Escherichia coli shows a remarkable plasticity in being able to adapt to variety of irregular cell shapes. Here, we investigate the roles of the Min system and nucleoid occlusion mechanism in this adaption. For this study we use squeezed E. coli in shallow nanofabricated channels which despite their highly irregular shapes and large sizes are able to symmetrically partition into two daughters. Imaging of labeled MinD proteins in irregular cell morphologies shows that Min system does not generally generate a stationary pattern of nodes for localization of cell division proteins, i.e. the divisome. In contrast,

	time-lapse imaging of FtsZ proteins and nucleoid show strong anticorrelation between these two molecular systems irrespective of cell shape. Moreover, we estimate that nucleoid occlusion mechanism provides more than one order of magnitude narrower spatial signal for localization of divisome than the Min system. We also find that in addition to nucleoid occlusion mechanism membrane curvature is instrumental in fine scale positioning of the divisome. Progressive FtsZ arcs form only in those positions on the circumference of the squeezed E. coli where their line curvature is maximized.

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Collaborative Research Project (CRP):
NANOCELL

Session: NANOCELL

Multifunctional block copolymers vesicles for protein reconstitution

Mihaela Delcea, Wolfgang Meier
University of Basel

Polymersomes assembled from amphiphilic block copolymers have proven to be useful tools as drug delivery systems [1, 2] nanoreactors and sensors [3-8]. The attachment of targeting ligands or enzymes to the polymersomes, as well as the immobilization of polymer vesicles on surfaces, is of crucial importance in most of the previously mentioned applications.

Our group has developed membrane-forming amphiphilic block copolymers with unprecedented mechanical and chemical stability that allow a functional reconstitution of membrane proteins.

The state of the art on how to functionalize block copolymer vesicle surfaces and their applications in biomedicine, surface- and nanoscience are highlighted. Four different concepts of membrane modification are demonstrated: i) the conjugation of ligands to preformed vesicles, ii) the formation of membranes from end-functionalized block copolymers, iii) the formation of membranes from bio-hybrid block copolymers consisting of synthetic polymers and biologically relevant sugars, peptides, proteins and oligonucleotides, iv) membrane proteins reconstituted in block copolymer membranes that can act as specific recognition and attachment sites.

References:

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2. Broz, P.; Benito, S.M.; Saw, C.; Burger, P.; Heider, H.; Pfisterer, M.; Marsch, S.; Meier, W.; Hunziker, P., J. Control. Release 2005, 102, 475-488.

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Daniel Fletcher

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Collaborative Research Project (CRP):
not applicable

Invited Lecture

Cellular reconstitution: Rebuilding biological systems from the bottom up

Daniel A. Fletcher
University of California, Berkeley

Understanding the molecular basis of cellular behavior is a central goal in biology and a critical guide for medical research. Increasing knowledge of the essential proteins in a complex process such as crawling motility raises the tantalizing question: Do we know enough to build it? In vitro reconstitution provides an import tool for identifying the roles of individual molecules, but defining components is not enough. Progress towards reconstitution of micron-scale cellular structures and processes has been limited by the challenges of generating in vitro reconstitutions that capture the spatial organization, physical constraints, and dynamics of living cells. This talk will describe on-going efforts to create functional reconstitutions of cytoskeletal and membrane processes involved in cellular protrusions and membrane transport. The lessons of what works – and what doesn't – are helping to guide efforts to build biological systems from molecular parts.

Philipp Holliger

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Session: SYNAPTA

Expanding the central dogma

Philipp Holliger
MRC Laboratory of Molecular Biology

Synthetic biology seeks to probe fundamental aspects of biological form and function by construction (i.e. resynthesis) rather than deconstruction (analysis). Synthesis thus complements reductionist

Collaborative Research Project (CRP): SYNAPTA - Encoded synthesis, replication and evolution of unnatural nucleic acid therapeutics	and analytic studies of life, and allows novel approaches towards fundamental biological questions. We propose to exploit the synthesis paradigm to explore the chemical etiology of the genetic apparatus shared by all life on earth. Specifically we ask why information storage and propagation in biological systems is based on just two types of nucleic acids, DNA and RNA. Is the chemistry of life's genetic system based on chance or necessity? Does it reflect a "frozen accident", imposed at the origin of life, or is it functionally superior to simple alternatives. Our work has been focused on expanding the substrate spectrum of polymerases with a view of enabling the synthesis and templated replication of nucleic acid polymers with expanded chemistry. Specifically, we have developed a selection strategies based on self-replication of polymerase genes by their encoded polymerases in a compartmentalized system. Using such strategies, we have isolated a range of polymerases with novel phenotypes, for example, the ability to bypass lesions found in ancient DNA, replicate hydrophobic base analogues or synthesize highly fluorescent DNA. I'll be presenting recent progress on the development and application of 2nd generation selection strategies, which decouple self-replication from selection and allow the evolution of polymerases capable of utilizing exclusively unnatural nucleotide substrates. I'll present progress towards building artificial genetic systems based on alternative genetic materials (XNA) using nucleic acid chemistry and polymerase evolution. Such systems will expand the central dogma and conclusively address questions such as the capacity of nucleic acid polymers other than DNA and RNA for information storage, heredity and evolution.

Oscar Kuipers

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Session: SYNMOD

SynMod: Design and production of novel lantibiotics by synthetic biology

Oscar P. Kuipers[1], Steven Schmitt[2], Manuel Montalban[1], Sven Panke[2]

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Collaborative Research Project (CRP): SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems	Lantibiotics form a group of ribosomally synthesized and posttranslationally modified peptides produced by Gram-positive bacteria. They display strong antimicrobial activity and are promising candidates to be used in clinical treatment of bacterial infections. They all have in common the presence of lanthionine or methyl-lanthionine due to the dehydration of serine or threonine residues and their subsequent reaction with the thiol group of a cysteine.

	These reactions are catalyzed by specific enzymes termed LanB and LanC (type I) or LanM (type II). These enzymes have a relaxed susbtrate specificity and a wide variety of peptides have already been dehydrated and cyclized using them, thus opening the possibility to generate novel peptides with improved biological and physico-chemical properties. We have analyzed the structure, stability, and potency of diverse known lantibiotics in order to select suitable structural modules. These modules will be randomly fitted in a defined architecture, that of nisin, and the nisin induction and modification machinery will be exploited to introduce the desired modifications in Lactococcus lactis. The screening considering biological activity, is expected to render a series of active molecules, which will be submitted to a second engineering step to improve their properties: i.e. improved activity and stability, active against MDR. This will involve the creation of a large library of about 10,000 chimeric lantibiotic sequences to be tested in the first round combining the different selected modules. An innovative culturing system using microcolonies grown in micro-alginate beads will be used for screening employing tools for in situ processing and GFP indicators for activity. Active molecules will be subjected to a second round of selection after random- and directed mutagenesis. We expect not only to obtain novel potent molecules but also to gain insight in the modularity of lantibiotics and thus the structure-activity relationship. Finally, optimized production systems using Staph. carnosus as production host will be developed, also using nanoreactor chemostats for fermentation-parameter optimization. Finally, keen attention will be given to societal impact, safety and communication issues.

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Collaborative Research Project (CRP):
SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems

Session: SYNMOD

Lantibiotic precursor and transcriptional element libraries

Andreas Leiherer and Ralf Wagner
Institute of Medical Microbiology & Hygiene - Molecular Microbiology & Gene Therapy - University of Regensburg

A major prerequisite for the identification of novel active lantibiotics by high throughput screening is the availability of new precursor substrates thereof. For this reason a first-generation DNA library must be synthesized representing all combinations of lantibiotic modules (ring structures and hinge regions). On the basis of known potent lantibiotics more than 30 sub-molecular modules have been selected, which have been pre-determined by evolution for function. These modules comprise sizes between three to twelve amino acids or, alternatively might even be missing. So far, there are no reliable molecular shuffling techniques available to combine such short DNA fragments with sufficient yield to cover the complete combinatorial

	diversity of full-length fragments. Related to this is the absence of conserved positions between the particular building modules, which could act as ligation sites. In the course of this project, we will therefore apply new gene synthesis protocols for the seamless combinatorial synthesis of several libraries for novel lantibiotics. These protocols will also accommodate the need for further modifications of the lantibiotic modules as amino acid substitutions at defined positions or modul-length variations. To support the establishment of new lantibiotoc production pathways, we also set up a collection of mutagenized expression elements, specifically promoters, terminators, and intergenic regions. We therefore employ a primer extension protocol using synthetic oligonucleotides with randomly introduced substitutions. The generation of several libraries of these transcriptional elements and the subsequent selection of a set of 10 candidate sequences from each element pool will be needed for further fine-tuning the lantibiotic modification machinery.

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Collaborative Research Project (CRP):
NANOCELL

Session: NANOCELL

Engineering rotor sizes in the ATP synthase nanomotor

Thomas Meier, Denys Pogoryelov
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F1Fo-ATP synthases are rotary motors, which produce three ATP molecules in their F1 motor complex by every 360°-rotation. A membrane-embedded rotor ring plays a central role in ion translocation during ATP synthesis by proton- or sodium motive force-dependent ATP synthases. In the Na+-binding c11 ring of Ilyobacter tartaricus, the H+-binding c15 ring from Spirulina platensis and the H+-binding c13 ring of Bacillus pseudofirmus OF4, the translocated ions are bound within the grooves of two adjacent c-subunits in coordination networks that include a conserved glutamate. It appears that the structure of these rotor rings represents a finely-tuned adaptation of the enzyme to the environment of the organism. The structures also suggest that the precise coordination chemistry keeps the ion (H+ and Na+) in an ion locked conformation during the passage through the lipid/c-ring interface. The number of ions that are translocated through the Fo motor during this process is determined by the number of n in the cn rotor ring. In the tightly coupled F1Fo ATP synthase, therefore, the turbine stoichiometry n defines the ion-to-ATP ratio (P/O ratio in mitochondria), which represents an important parameter in the energy metabolism of all cells. On the basis of the structures of our c-rings, we started to change the stoichiometry of a given c-ring.

	Recombinant and in vitro expression systems for the biosynthesis of c-rings have been established. By introducing single site mutations into specific residues that are involved in c/c-subunit contact, we are able to change the c-ring stoichiometry. Visual confirmation is provided by structural studies with electron and atomic force microscopy. Our studies pinpoint amino acid residues, which are responsible for the ?-helical contacting in these membrane protein complexes. It appears that the rotor rings quaternary structures are determined by the primary structure of the c-subunits. The results furthermore demonstrate a high grade of flexibility in these bioengineered nanomachines.

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Collaborative Research Project (CRP):
NANOCELL

Session: NANOCELL

Towards programing nanocells wih functional units

Christian Bippes, Mehdi Damaghi, Stefania Mari, Dimitrios Fotiadis, Thomas Meier, Wolfgang Meier
Dept Biosystems Science and Engineering, ETH Zürich; University of Berne; University of Basel; Max-Planck-Institute of Biophysics, Frankfurt

Here we show the first progress of a new concept that allows programing artificial and cellular membranes with single membrane proteins. This step builds a milestone in equipping nanoscopic or macroscopic cells with membrane proteins and thus programing cellular functions.

Markus R. Schmidt

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Collaborative Research Project (CRP):
SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems

Eurosynbio World Café

Markus Schmidt and Wolfgang Kerbe
IDC

The Eurosynbio World Café will be part of the EUROSYNBIO kick-off meeting. It will

- Intensify existing relations within the Eurosynbio Community
- Explore one of the two following questions:

o What are possible societal issues in EUROSYNBIO projects that need closer attention?

o How do we want to communicate our work and insights as synthetic biologists to a broader public?

- The discussion within the setting of the World Café will give all participants the opportunity to talk to people they know or who they have never met before.

	World Café Definition: A World Café is a discussion event for large groups that allows the generation of ideas or to explore a topic intensely. For the whole Café a time of (two to) three hours will be necessary: 15 min: Introduction 3x30 min: Discussions at tables of four people plus 10 min pauses 45min: Final discussion and collection of all results For further references and a detailed description of a World Café visit: www.theworldcafe.com

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Collaborative Research Project (CRP):
SynDiv - Synthetic Biology of the Bacterial Cell Division

Session: SynDiv

Spatial cues for the self-organization of cell division proteins

Jakob Schweizer, Martin Loose, Petra Schwille
TU Dresden, BIOTEC

In the living cell, proteins are able to organize space much larger than their own dimensions. In return, changes of the intracellular space can influence biochemical reactions. However, we still lack knowledge of how intracellular protein distributions respond to the constraints imposed by geometrical boundaries. Using photolithographically structured membranes, we studied the influence of spatial confinement on self-organizing waves of Min proteins, the spatial regulators of bacterial cell division. We found that the emerging pattern can respond to the geometry of the membrane such that, like in vivo, Min protein waves travel along the longest axis of the membrane patch. Our results demonstrate the ability of the Min system to act as geometry sensing mechanism and give insight into the interplay between the geometry of the accessible space and biochemical pattern formation.

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Collaborative Research Project (CRP):

Session: SynDiv

Dissection and rebuilding of the Escherichia coli divisome

Paola Bisicchia and David Sherratt
Biochemistry, University of Oxford

Synthetic Biology is the design and construction of biological systems not found in nature in order to understand normal biological function; it enables the engineering of new functions useful in endeavours to improve the wellbeing of Man and all living organisms. The process of cell division, which underlies the basic life process, and its coordination with chromosome segregation, is not fully understood in any organism, although some success has been made

SynDiv - Synthetic Biology of the Bacterial Cell Division	in reconstituting at least parts of the cell division process in artificial systems. We are dissecting the cell division process in the readily manipulable bacterium, Escherichia coli, by using established genetic tools in combination with pioneering imaging technology, which allows visualization of proteins and of DNA loci in live cells, at the single molecule level. We are also applying new methods of in vivo biochemistry that allow us to activate specific functional proteins and then ablate them over short time-scales. We will apply the resulting information and reagents to the projects of our partners, Petra Schwille and Cees Dekker. To date, we have tagged 9 functional divisome proteins with several fluorescent labels that allow single molecule imaging of two divisome proteins at a time in live E.coli. Time-lapse microscopy will allow us to follow the temporal progession during the assembly of a function divisome, while slimfield and super-resolution microscopy will allow us to analyze both stoichiometry and cellular organization of divisome components. Our initial focus will be on FtsK, which coordinates the late stages of chromosome segregation with cell division, and which assembles onto the replication termination region of the chromosome when there are problems in completion of replication or daughter chromosome unlinking. Key references: Reyes-Lamothe, R., Sherratt, D.J. and Leake, M.C. (2010). Stoichiometry and architecture of active DNA replication machinery within Escherichia coli. Science 328, 498-501 Wang, X., Possoz, C. and Sherratt, D.J. (2005). Dancing around the divisome: asymmetric chromosome segregation in Esherichia coli. Genes.Dev, 19, 2367-22377. Dubarry N & Barre FX (2010) Fully efficient chromosome dimer resolution in Escherichia coli cells lacking the integral membrane domain of FtsK. EMBO J 29, 597-605. Goehring NW, Gonzalez MD, and Beckwith J. (2006) Premature targeting of cell division proteins to midcell reveals hierarchies of protein interactions involved in divisome assembly. Mol Microbiol. 61, 33-45.

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Session: SynMet

From natural to synthetic methylotrophs: Understanding the parts and how they could come together

Julia Vorholt
ETH Zurich

One-carbon (C1) compounds such as methanol are attractive, non-food energy sources for microbial bioprocesses, which can be utilized by methylotrophs, a specialized group of microorganisms. Research

Collaborative Research Project (CRP): SynMet - A synthetic Biology Approach for Engineering of Bacterial Methylotrophy	efforts with different methylotrophic model strains revealed that methylotrophy consists of a set of discrete functional modules that are ultimately linked to central metabolism. The aim of the project is to use a Synthetic Biology approach to create a bacterium that efficiently exploits methanol as a resource. The project encompasses the selection of methylotrophy modules from various natural methylotrophic donor organisms and to develop strategies for their functional assembly to achieve coordinated expression in a biotechnologically relevant bacterial host. Physiological characterization and omics-approaches are used to analyze and evaluate the genetically engineered cells with respect to the acquired methylotrophic properties with the goal to achieve efficient methanol conversion. The generated knowledge will contribute to an increased understanding of bacterial methylotrophy and will facilitate transfer of methylotrophy to biotechnologically relevant bacteria as a new modular platform for methanol-based production of bulk chemicals.

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Session: SYNAPTA

Target identification of cell-specific aptamers

Jan L. Vinkenborg, Thomas Höver, Günter Mayer and Michael Famulok

LIMES Institute, Program Unit Chemical Biology & Medicinal Chemistry, Laboratory of Chemical Biology, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

Collaborative Research Project (CRP):
SYNAPTA - Encoded synthesis, replication and evolution of unnatural nucleic acid therapeutics

The ability to identify a specific cellular phenotype is important for many applications in diagnostics and requires ligands that specifically recognize cell surface markers. Short single strands of oligonucleotides, called aptamers, are very suitable for cellular recognition, as in vitro selection methods can be used to evolve aptamers that bind to their target with high affinity and specificity. To allow identification of the target at the cell surface, the aptamer-protein complex needs to be purified, followed by analysis of the purified protein using mass spectrometry. Crucial in this process is that the aptamer protein complex remains intact during enrichment, which can be achieved by covalently cross-linking the aptamer to its target via a process we coin aptamer-based affinity labeling (ABAL). As a proof-of-principle study for ABAL, we functionalized the aptamer C10.35, which specifically recognizes the Sec7 domain of the exchange factor cytohesin-2, with a tag that contains a phenylazide as photoreactive group and a biotin label to enable visualization of the cross-linked product. Filter retention assays demonstrated that the presence of the tag did not affect the affinity of the aptamer for its target. UV irradiation of samples containing the aptamer and Sec7 followed by analysis using Western blotting revealed clear cross-linking between C10.35 and Sec7 in both a phosphate buffer and in cell lysate. We are currently optimizing the conditions to purify the

	aptamer-protein complex and believe this will pave the way for ABAL to be used in the target identification of cell specific aptamers.

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Collaborative Research Project (CRP):
SynMet - A synthetic Biology Approach for Engineering of Bacterial Methylotrophy

Session: SynMet

Synthetic biology aprroaches to Corynebacterium glutamicum: access to new carbon sources and new products

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Amino acid production by Corynebacterium glutamicum amounts to about 2.5 million tons per year and, thus, is a proven large-scale biotechnological process. The traditional product spectrum has recently been widened by metabolic engineering approaches, e.g. for production of 3-aminopropionic acid or ethanol. The generally recognized as safe C. glutamicum has been shown to be robust against a variety of inhibitory compounds and to be able to efficiently co-utilize different carbon source mixtures. The characterization of genetic control mechanisms of carbon metabolism, which are distinct from those of the model bacteria E. coli and B. substilis, enabled strain development for improved carbon substrate utilization. In addition, pathways for access to new carbon sources have been engineered, e.g. for efficient use of glycerol, which arises in large quantities in the biodiesel process as major by-product of plant seed oil transesterification with methanol. Progress and future challenges of synthetic biology approaches to C. Glutamicum as platform for the production of fine chemicals will be discussed.

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Collaborative Research Project (CRP):
NANOCELL

NANOCELL

Reconstitution of ATP-synthase into droplet-on-hydrogel bilayers

Richard Berry and Wei-Meng Ho
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We will present preliminary results in the development of a synthetic lipid bilayer system that will allow high-resolution single-molecule observation of membrane proteins including ATP-synthase.

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Collaborative Research Project (CRP):
SynDiv - Synthetic Biology of the Bacterial Cell Division

SynDiv

Dissection and rebuilding of the Escherichia coli divisome

Paola Bisicchia and David Sherratt
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Synthetic Biology is the design and construction of biological systems not found in nature in order to understand normal biological function; it enables the engineering of new functions useful in endeavours to improve the wellbeing of Man and all living organisms. The process of cell division, which underlies the basic life process, and its coordination with chromosome segregation, is not fully understood in any organism, although some success has been made in reconstituting at least parts of the cell division process in artificial systems. We are dissecting the cell division process in the readily manipulable bacterium, Escherichia coli, by using established genetic tools in combination with pioneering imaging technology, which allows visualization of proteins and of DNA loci in live cells, at the single molecule level. We are also applying new methods of in vivo biochemistry that allow us to activate specific functional proteins and then ablate them over short time-scales. We will apply the resulting information and reagents to the projects of our partners, Petra Schwille and Cees Dekker. To date, we have tagged 10 functional divisome proteins with several fluorescent labels that allow single molecule imaging of two divisome proteins at a time in live E.coli. Time-lapse microscopy will allow us to follow the temporal progession during the assembly of a function divisome, while slimfield and super-resolution microscopy will allow us to analyze both stoichiometry and cellular organization of divisome components. Our initial focus will be on FtsK, which coordinates the late stages of chromosome segregation with cell division, and which assembles onto the replication termination region of the

	chromosome when there are problems in completion of replication or daughter chromosome unlinking. Key references: Reyes-Lamothe, R., Sherratt, D.J. and Leake, M.C. (2010). Stoichiometry and architecture of active DNA replication machinery within Escherichia coli. Science 328, 498-501 Wang, X., Possoz, C. and Sherratt, D.J. (2005). Dancing around the divisome: asymmetric chromosome segregation in Esherichia coli. Genes.Dev, 19, 2367-22377. Dubarry N & Barre FX (2010) Fully efficient chromosome dimer resolution in Escherichia coli cells lacking the integral membrane domain of FtsK. EMBO J 29, 597-605. Goehring NW, Gonzalez MD, and Beckwith J. (2006) Premature targeting of cell division proteins to midcell reveals hierarchies of protein interactions involved in divisome assembly. Mol Microbiol. 61, 33-45.

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Collaborative Research Project (CRP):
SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems

SYNMOD

Development of a Microfluidic Chemostat for Gene Expression Screening

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A prototype microfluidic device (Integrated Microfluidic Chemostat or IMC), with fluid supply and temperature control systems, has been produced. Initial experiments to characterise mixing and test the batch fermentation capabilities of the IMC, using the Gram-positive bacteria, Staphylococcus carnosus, have been performed. An optical system for in situ detection of the induced expression of GFP, by the modified S. carnosus strain, has been developed. Preliminary experiments to measure fluorescence and optical density (OD) variations during microscale batch fermentations were conducted. In situ and real-time monitoring of dissolved oxygen (DO) and pH time profiles has previously been demonstrated (Szita, 2005). Integration of the optical sensors to detect these physicochemical fermentation variables will be straightforward and make use of already incorporated elements of the here presented optical system. Similarly, while the IMC is not currently autoclave sterilised prior to experiments, the material choice will enable this sterilisation method to be utilised in the future.

S. carnosus, possessing a small, but well characterised genome and less diverse metabolism, was chosen as the production chassis to demonstrate the pre-programmed assembly of novel lantibiotics. The IMC is being developed further to incorporate chemostat fermentation and parallelisation for the statistically meaningful analysis of S. carnosus behaviour as a function of growth rate and medium composition.

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Collaborative Research Project (CRP):
SynDiv - Synthetic Biology of the Bacterial Cell Division

SynDiv

Min Oscillations in PDMS based flow channels

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Molecular self-organization is one of the most essential, and most complex characteristics of many biological systems. Well-studied examples are the oscillations of the Min protein system in the bacterium Escherichia coli. These Min proteins oscillate between cell poles of the bacterium, which allows a division site to form at the center. Our group has been able to form planar surface waves of Min proteins on a flat supported lipid membrane in vitro. We extend this work into three dimensions, as well as taking into account curvature of the membrane, leading to a more in vivo like system. We use PDMS based microfluidic flow channels to support the membranes in desired shapes. These membrane coated chambers are used to study the Min oscillations. The PDMS based channels offer a versatile system to vary parameters like curvature, aspect ratios, concentrations and ratios of proteins etc. To study the Min oscillations. These experiments may provide deeper insights into mechanisms and robustness of self-organized biological processes.

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Collaborative Research Project (CRP):
NANOCELL

NANOCELL

Towards asymmetrical membranes for NANOCELLs

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NANOCELLs are artificial vesicular containers designed for specific functionalities. The simplest NANOCELL is binary, i.e. composed of two types of membrane proteins. For example, bacteriorhodopsin (an energy-generating module) establishes a proton gradient across the membrane using light energy, and a proton/solute symporter (translocating module) transports a specific solute in or out of the NANOCELL. Upon assembly of NANOCELLs from detergent-solubilized lipid or copolymer, and membrane proteins, modules are often integrated in both possible orientations leading to a short circuit of the system. Therefore, modules in the unfavorable orientation have to be neutralized. This can be achieved by chemical modification of modules from the outside of the NANOCELL. The position for the directed chemical modification can be selected by mutating a specific single amino acid residue of the protein to a cysteine. Based on existing structural data for certain nanomachines or by random mutagenesis, a position near the active site has to be found to introduce the cysteine residue. Chemical modification of this residue

	will then sterically block substrate binding and thereby protein function. Proteins with favorable orientations do not expose the engineered cysteine to the modifying hydrophilic agent outside of the NANOCELL, thus retaining their functionality. To screen for such mutants the scintillation proximity assay was established in our laboratory. This radioligand based direct binding assay allows high sample throughput using purified, detergent-solubilized protein. To identify mutants that lose their function upon chemical modification, substrate binding before and after modification will be tested. Importantly, this assay will also be crucial for the characterization and screening of engineered transporters with modified substrate specificities. The arginine/agmatine exchanger AdiC and the glucose transporter IICB from the bacterium Escherichia coli were used to establish and validate the scintillation proximity assay.

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Collaborative Research Project (CRP):
SynDiv - Synthetic Biology of the Bacterial Cell Division

SynDiv

Chromosome distribution and membrane curvature localize cell division machinery in Escherichia coli

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We recently discovered that Escherichia coli bacteria that are confined to narrow nanofabricated channels still divide unimpededly, despite the large size and irregular cell shapes that they adopt [1]. Previously, it was shown that normal rod-shaped E. coli bacteria are able to place their cell division plane very accurately in the middle of the mother cell [2]. How are these bacteria able to robustly and accurately localize their cell division proteins? Here, we address this question using two-color fluorescent imaging of bacteria in narrow nanofabricated channels. The non-conventional shape of E. coli in this confinement provides new opportunities to study cell division in bacteria. Using our approach, we are able for the first time to observe the dynamical arrangement of bacterial DNA relative to some of the key cell division proteins. While Min proteins are effective in

	excluding cell division at the poles of rod-shaped bacteria, they do not generate a well-defined pattern for localization of cell division planes in more complicated cell shapes. Instead, we observe that localization of the divisome, i.e., the protein complex pertinent to cell division, correlates strongly with the pattern of DNA arrangement. As the bacterial chromosomes segregate and voids form in its distribution, the early arriving protein of bacterial divisome, FtsZ, localizes in these voids. Not all the voids are selected but only those which are near high circumferential curvature regions of the cell wall in these flattened cells. Our results show that localization of bacterial divisome is to a high degree coordinated with the chromosome distribution. The DNA forms a molecular scaffold which guides this important cellular process. [1] J. Männik, R. Driessen, P. Galajda, J.E. Keymer and C. Dekker, Proc.Natl.Acad.Sci. U.S.A. 106 (2009) 14861. [2] X.C.Yu and W. Margolin, Mol.Microbiol. 32 (1999) 315.

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Collaborative Research Project (CRP):
SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems

SYNMOD

Study of substrate specificity in NisP protease

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Lantibiotics are a group of ribosomally produced peptides that undergo diverse post-translational modifications. Two of these modifications are present in all lantibiotics, the dehydration of serines and threonines and the coupling of cysteins to these dehydrated amino acids to create (methyl-) lanthionine ring(s). These two steps are catalysed either by one enzyme (LanM) in type II lantibiotics or two enzymes (LanB and LanC) in type I lantibiotics. In some lantibiotics, additional enzyme-mediated modifications can occur. When the protein has been modified it is exported and concomitantly cut or transported and cut by a leader peptidase in a second step. Lantibiotics require leader peptide excission to be active.

NisB and NisC enzymes have shown high substrate tolerance thus being able to act on differente peptide sequences attached to their specific leader sequence (nisin leader peptide). On the other hand, the protease NisP is more sensitive to sequence variations. This selectivity sets a hurdle in its use to release nisin leader from the modified peptides. It also makes necessary the use of further purification steps and commercial specific proteases thus increasing the time and cost investment for the production of modified peptides. In this work we report the study of NisP action on a series of lantibiotic sequences in which the cleavage site adjacent aminoacids have been mutated.

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Collaborative Research Project (CRP):
SynMet - A synthetic Biology Approach for Engineering of Bacterial Methylotrophy

SynMet

Establishing methylotrophy in non-methylotrophs

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Methylotrophic bacteria can utilize methanol and other reduced C1 compounds as their sole carbon and energy source. For this purpose they developed a number of specialized enzymes and pathways. Independent of the concrete methylotrophic metabolism, the first steps of carbon conversion are conserved. Methanol is initially oxidized to formaldehyde which is subsequently used to produce energy and biomass. This study aims at inserting genes encoding methylotrophy enzymes into a non-methylotroph to create an artificial methylotroph which can be used for conversion of methanol into value-added products.

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Collaborative Research Project (CRP):
SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems

SYNMOD

Heterologous expression of pre-galiidermin in Staphylococcus carnosus

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Lantibiotics are ribosomally produced, short peptides with antimicrobial activity, mainly produced by coagulase- negative staphylococcal species. Characterized by the presence of unusual amino acids and lanthionine rings, lantibiotics primary act by inducing pores in the cytoplasmic membrane by binding to the cell wall precursor Lipid II in Gram-positive bacteria.

All lantibiotics are synthesized as inactive precursors and subsequently activated through proteolytic cleavage by specific proteases, generally designated LanP. Gallidermin, produced by Staphylococcus gallinarum, is a typical type A lantibiotic that is considered for the treatment of acne (caused by Propionbacterium acnes) and mastitis (caused by Staphylococcus aureus). Meanwhile the increasing number of multidrug resistant pathogens emphasizes an urgent need for novel antibiotics with new targets and optimized production processes. Therefore, the main objective of this thesis was to establish Staphylococcus carnosus as a new production chassis for improved, heterologous expression of gallidermin.

Expression was accomplished by introduction of relevant biosynthesis genes gdmBCDHTQ into one plasmid, and the structural gene gdmA into another. By deliberately skipping gdmP, S. carnosus was able to

	synthesize an inactive gallidermin precursor peptide which could be activated by selective addition of GdmP to the supernatant. This mechanism is considered to increase yields in future fermentation processes. Successful activation was verified in different bioactivity assays, using S. carnosus TM300 as an indicator strain. Presence and accuracy of the produced peptide were further confirmed by HPLC- and MS- analysis. This two- plasmid expression system can be applied as a useful tool for expression and subsequent identification of a variety of synthetically generated gallidermin derivatives with improved antimicrobial and pharmacokinetic properties.

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Collaborative Research Project (CRP):
SYNMOD - Synthetic biology to obtain novel antibiotics and optimized production systems

SYNMOD

Use of Staphylococcus carnosus as a chassis for the production of lantibiotics

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The chemical synthesis of lantibiotics is too complicated and costly, so biotechnology is the only way to produce derivatives of the interesting lead-structure.

We have first success in establishing a platform for expressing a large number of novel lantibiotics. As a suitable production chassis, S. carnosus TM300 is chosen, due to it's already reduced genome complexity (2.56 Mb). In addition, S. carnosus is a GRAS organism with available genomic data. It can be made a highly efficient production chassis while providing only limited metabolic complexity and a reduced chance for interference. We are establishing an implementation of a context-insensitive and modular lantibiotic production platform in this strain. For this, we are exploiting the relaxed-substrate specificity modification machineries of gallidermin (S. gallinarum, gdmBCD) and nisin (L. lactis, nisBTC), expand the set where required by selected machinery parts from the original hosts that provided the original set of structures. The nisin modification and transport genes are differently structured, than those of the gallidermin production system. They are composed of nisBTC; nisBT are responsible for lantibiotic modification, the transporter, nisT, is part of the operon. The corresponding genes have been de novo synthesized to codon-optimize the genes for use in S. carnosus with optimized ribosomal binding sites for cloning under control of a xylose-inducible promotors. Our aim is to develop and test S. carnosus TM300 as a reduced-genome production chassis for a high variety of lantibiotics.

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Collaborative Research Project (CRP):
SYNAPTA - Encoded synthesis, replication and evolution of unnatural nucleic acid therapeutics

SYNAPTA

Aptamer-based affinity labeling (ABAL): a tool for target identification of cell-specific aptamers

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The ability to identify a specific cellular phenotype is important for many applications in diagnostics and requires ligands that specifically recognize cell surface markers. Short single strands of oligonucleotides, called aptamers, are very suitable for cellular recognition, as in vitro selection methods can be used to evolve aptamers that bind to their target with high affinity and specificity. To allow identification of the target at the cell surface, the aptamer-protein complex needs to be purified, followed by analysis of the purified protein using mass spectrometry. Crucial in this process is that the aptamer protein complex remains intact during enrichment, which can be achieved by covalently cross-linking the aptamer to its target via a process we coin aptamer-based affinity labeling (ABAL). As a proof-of-principle study for ABAL, we functionalized the aptamer C10.35, which specifically recognizes the Sec7 domain of the exchange factor cytohesin-2, with a tag that contains a phenylazide as photoreactive group and a biotin label to enable visualization of the cross-linked product. Filter retention assays demonstrated that the presence of the tag did not affect the affinity of the aptamer for its target. UV irradiation of samples containing the aptamer and Sec7 followed by analysis using Western blotting revealed clear cross-linking between C10.35 and Sec7 in both a phosphate buffer and in cell lysate. We are currently optimizing the conditions to purify the aptamer-protein complex and believe this will pave the way for ABAL to be used in the target identification of cell specific aptamers.

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SynMet

MEP Pathway Modulation for Terpenoid Production in Bacillus subtilis

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Creating novel biosynthetic pathways and modulating the synthesis of important compounds is one of the hallmarks of synthetic biology.

Collaborative Research Project (CRP): SynMet - A synthetic Biology Approach for Engineering of Bacterial Methylotrophy	Understanding the key parameters controlling the flux of chemicals throughout a metabolic pathway is one of the challenges ahead. The well characterized and broadly used production organism Bacillus subtilis forms an ideal background for creating and studying novel synthetic routes. Terpenoids are the most functionally and structurally varied group of natural products from which numerous medicines and relevant fine chemicals are derived. Isoprene is the smallest representative of terpenoids, and also the general precursor of all terpenoids. In comparison to other bacteria Bacillus subtilis emits the volatile compound isoprene in high concentrations and in combination with its well characterized genome it represents an interesting starting point for a terpenoid Cell Factory. In this research, genes involved in the methylerythritol phosphate (MEP) pathway to isoprene in Bacillus subtilis were analyzed. Five genes essential for viability were shown to be essential for the isoprene production. Carotenoid synthetic genes, crtM and crtN, were introduced into Bacillus subtilis to establish carotenoid read out system. As an initial start to set up the system to study the flux of terpenoids, the effect of overexpressing one essential MEP pathway gene, dxs, was investigated and it was shown that the production of the carotenoid compound staphyloxanthin can be increased significantly.

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