



Gesellschaft für Biochemie  
und Molekularbiologie e.V.

March 22 - 24, 2018  
Mosbach / Baden, Germany

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# 6<sup>th</sup> Mosbacher Kolloquium

## SYNTHETIC BIOLOGY - FROM UNDERSTANDING TO APPLICATION

# Abstracts



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### *Determination of growth-coupling strategies and their underlying principles*

Presenting author:

**Tobias B. Alter**

Author(s):

Tobias B. Alter, Lars M. Blank, Birgitta E. Ebert

Metabolic coupling of product synthesis and microbial growth is a prominent approach for maximizing production performance. Growth-coupling (GC) also helps stabilizing target production and allows the selection of superior production strains by adaptive laboratory evolution. We have developed the computational tool gcOpt, which identifies knockout strategies leading to the best possible GC by maximizing the minimally guaranteed product yield. gcOpt implicitly favors solutions resulting in strict coupling of product synthesis to growth and metabolic activity while avoiding solutions inferring weak, conditional coupling.

GC intervention strategies identified by gcOpt were examined for GC generating principles under diverse conditions. Curtailing the metabolism to render product formation an essential carbon drain was identified as one major strategy generating strong coupling of metabolic activity and target synthesis. Impeding the balancing of cofactors and protons in the absence of target production was the underlying principle of all other strategies and further increased the GC strength of the aforementioned strategies. Thus, generating a dependency between supply of global metabolic cofactors and product synthesis appears to be advantageous in enforcing strong GC.

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### *Dynamic Blue Light-Inducible T7 RNA Polymerases (Opto-T7RNAPs) for Precise Spatiotemporal Gene Expression Control*

Presenting author:

**Armin Baumschlager**

Author(s):

Armin Baumschlager, Stephanie Aoki, Mustafa Khammash

Light has emerged as a control input for biological systems due to its precise spatiotemporal resolution. We developed a light-inducible transcription system that is independent from cellular regulation through the use of an orthogonal RNA polymerase. Our engineered blue light-responsive T7 RNA polymerases (Opto-T7RNAPs) show properties such as low leakiness of gene expression in the dark state, high expression strength when induced with blue light, and an inducible range of more than 300-fold. Following optimization of the system to reduce expression variability, we created a variant that returns to the inactive dark state within minutes once the blue light is turned off. This allows for precise dynamic control of gene expression. The regulators, which only require blue light from ordinary light-emitting diodes for induction, were developed and tested in the bacterium *Escherichia coli*, which is a crucial cell factory for biotechnology. Opto-T7RNAP, with minor alterations, should be extendable to other bacterial species as well as eukaryotes such as mammalian cells and yeast in which the T7 RNA polymerase and the light-inducible Vivid regulator have been shown to be functional. We anticipate that our approach will expand the applicability of using light as an inducer for gene expression independent from cellular regulation and allow for a more reliable dynamic control of synthetic and natural gene networks.

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### *Synthetic mammalian gene circuits: from fundamentals to applications*

Presenting author:

**Yaakov (Kobi) Benenson**

Author(s):

Yaakov (Kobi) Benenson

Synthetic gene circuits that 'compute' in situ with endogenous molecular inputs have the potential to control and reprogram cell behavior in complex fashion. Focusing on mammalian cells, our group develops biological computing circuits that are tightly integrated with their host cells via endogenous inputs and outputs. In the talk I will describe some of the mammalian circuit design platforms we have established, such as combinatorial logic with microRNA and transcription factor inputs in order to target specific cell states. I will also introduce the concept of dynamic control of circuit genetic encoding and show how this idea can lead to dramatic consequences for circuit operation: from orders-of-magnitude improvement in sensor dynamic range, to the possibility of compressing circuit genetic encoding in a manner similar to 'zipping' a computer file. Lastly, I will describe some of the open questions and challenges that still need to be addressed before such circuits can gain a widespread acceptance and translated into (medical) practice.

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### *Interrogating and tuning stochastic gene expression by optogenetic transcription factor control*

Presenting author:

**Dirk Benzinger**

Author(s):

Dirk Benzinger, Marc Rullan, Gregor Schmidt, Andreas Miliadis-Argeitis, Mustafa Khammash

Gene expression can exhibit substantial cell-to-cell variability, which partly stems from inherent stochasticity of the transcription process. Nevertheless, its precise and dynamic regulation is crucial for most biological processes as well as in biotechnological applications. The complexity of signal transduction and gene regulation hampers our ability to analyze how the dynamic activity of transcription factors (TFs) affects transcription and cellular heterogeneity. We have established a synthetic biology approach that makes use of a fast-acting, light-responsive TF to quantitatively study multiple aspects of transcriptional regulation in *S. cerevisiae*.

We found that pulsatile TF regulation can reduce cell-to-cell variability in protein expression and that expression mean and variability can be independently tuned by adjusting the frequency of input signals. By combining this approach with live-cell quantification of nascent RNAs, we found that transcription occurs in discontinuous bursts whose duration and timing are modulated by TF activity. Probing the system with pulsed TF inputs uncovered that promoter activation is largely memoryless and that bursts are terminated upon TF unbinding. This led us to propose a mechanistic model of transcriptional bursting based on TF binding that quantitatively reproduces our experimental observations.

Our results demonstrate the merit of using easily controllable synthetic systems to gain new insight into fundamental biological processes.

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### *Tetracycline-regulated gene expression in Haloarchaea*

Presenting author:

**Johannes Born**

Author(s):

Johannes Born, Felicitas Pfeifer

Introduction: Tetracycline-regulated systems are based on the high affinities of the repressor protein TetR to the operator tetO and of tetracycline (Tc) to TetR. In absence of Tc, TetR binds to tetO and represses the transcription. Binding of Tc to TetR leads to a conformational change in TetR what prevents DNA-binding and enables the start of transcription. The aim of our study is to apply Tc-regulated gene expression in haloarchaea by fusing the haloarchaeal activator GvpE to TetR.

Methods: A plasmid containing PpA-gfp as reporter was used to test the activation of PpA by GvpE in the haloarchaeon *Haloferax volcanii*. Cells lacking GvpE exhibit the basal PpA activity. The GvpE recognition sequence (UASE) upstream of PpA was replaced by tetO, a second plasmid carried the tetR-gvpE fusion. The binding of TetR-GvpE to tetO should lead to PpA activation by GvpE.

Results: Compared to the basal activity of PpA, GvpE leads to a 13-fold higher activity of PpA. PpA lacking tetO is not activated by TetR-GvpE and possesses the basal PpA activity. The presence of TetR-GvpE results in a significant higher activity of PpA when the UASE is exchanged with tetO.

Conclusion: GFP is useful as reporter to quantify promoter activities in haloarchaea. The fusion protein TetR-GvpE activates PpA, which shows that TetR recognize tetO and bind under high salt conditions (2M KCl). This suggests that Tc-regulated gene expression in haloarchaea might be possible.

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### *Genetically encoded biosensors – valuable tools for white biotechnology*

Presenting author:

**Michael Bott**

Author(s):

Michael Bott

Microorganisms have evolved a large repertoire of sensors that enable them to detect and respond to their environment. Typical representatives are one-component transcriptional regulators or two-component signal transduction systems. In recent years, these sensors were employed as “biobricks” to construct devices that are useful in white biotechnology. Examples will be presented for the application

of transcription factor-based biosensors for FACS-based high-through screening in microbial strain development and for the use of sensory histidine kinases as basis for in vitro biosensors.

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### *Creating Diversity in Prodiginines – Synthetic Biology meets Chemistry*

Presenting author:

**Hannah Braß**

Author(s):

Hannah Braß, Andreas S. Klein, Thomas Classen, Anita Loeschcke, Thomas Drepper, Luca Laraia, Sonja Sievers, Karl-Erich Jaeger, Jörg Pietruszka

Prodiginines are microbial secondary metabolites produced by Gram positive and negative bacteria such as *S. coelicolor* and *S. marcescens* exhibiting pharmacological properties including anticancer activities and antibiotic activities against pathogens such as *S. typhi*, *P. aeruginosa*, and *S. aureus*.<sup>1</sup>

As *S. marcescens* is an opportunistic pathogen accumulating the red tripyrrol prodigiosin in low amounts, the development of alternative strategies for an efficient synthesis independent of the native producer is of great interest.

Therefore, we utilized the transfer and expression (TREX) system that facilitates transfer, chromosomal integration and concerted expression of large gene clusters in bacteria.<sup>2</sup> The prodigiosin gene cluster from *S. marcescens* was integrated into the genome of the GRAS certified bacterium *P. putida* accumulating prodigiosin at substantial levels.

Based on that, a mutasynthesis approach was established to create easy access to new prodigiosin derivatives. Thus, a prodigiosin deficient mutant of the *P. putida* production strain was created by blocking the biosynthesis towards an early key intermediate. By feeding this mutant synthetic precursors, new prodigiosin derivatives were produced, the substrate scope of the mutasynthesis was investigated, and the bioactivity of the new derivatives evaluated.<sup>3</sup>

<sup>1</sup> Hu, D. X., et al., Chem Rev 2016, 116, 7818-7853

<sup>2</sup> Loeschcke, A., et al., ACS Synth Biol 2013, 2, 22-33

<sup>3</sup> Klein, A. S., et al., ACS Synth Biol 2017, 6, 1757-1765

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### *A Bacterial Bandpass Assay for Protein-Protein Interactions*

Presenting author:

**Katherine Brechun**

Author(s):

Katherine Brechun, Katja Arndt, Andrew Woolley

We have developed a genetic circuit in *Escherichia coli* acting as a bandpass filter for protein-protein interactions, which allows one to select for interacting proteins of different strength by changing antibiotic concentrations in the media. This assay uses a genetic circuit that links protein-protein interaction strength to beta-lactamase activity, while simultaneously imposing tuneable positive and negative selection pressure for beta-lactamase activity. Resultingly, selection recovers cells expressing interacting proteins with affinities that fall within the set high- and low-pass thresholds. The activity of this circuit was demonstrated by analyzing a series of coiled-coils with known affinities. The bandpass function enabled pure cultures to be separated from a mixture of cells expressing different coiled-coils, based on the interaction strength of each protein pair. We additionally incorporated an inhibitor targeting the interacting pair into the circuit. We demonstrated the ability to detect inhibitors of various strengths by co-expressing a series of inhibitors targeting CREB and AP-1. This circuit provides improved control in the selection of interacting proteins, and is expected to facilitate the development of tuned protein-protein interactions.

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*Co-factor binding of PAPSS2 APS kinase compensates destabilizing effects of the cellular environment*

Presenting author:

**Oliver Brylski**

Author(s):

Oliver Brylski, Jonathan Wolf Mueller, Simon Ebbinghaus

PAPS synthase (PAPSS) provides the cell with its sole activated sulfate source 3'-phosphoadenosine-5'-phosphosulfate (PAPS). The enzymes bifunctionality is split into well separated domains, ATP sulfurylase and APS kinase. Deficiencies in either of the two domains result in disease states such as malformation in bones and cartilage as well as metabolic diseases. First biophysical studies performed in vitro revealed that PAPSS isoforms 1 and 2 differ in their thermal stability with PAPSS2 being naturally fragile at physiological temperatures. In vitro studies cannot account for the heterogeneity of the cellular environment such as varying metabolite concentrations or its physicochemical properties including excluded-volume effects or quinary interactions. The question remains whether these in vitro findings can be transferred to a complex cellular system.

Using fast fluorescence microscopy in combination with temperature jumps we investigated the in cell stability of PAPSS2 APS kinase inside the cellular environment. We further modified the domain using two classes of mutations, disease relevant and catalysis inhibiting mutations. Thermodynamic profiles allow a classification of the mutants into disease related, destabilized and native like states. Furthermore, co-factor binding counteracts the destabilizing effects of the cellular environment and helps to retain stability at physiological conditions.

### *Measuring beyond the resolution of light with the branched proximity hybridization assay*

Presenting author:

**Marco Cavallari**

Author(s):

Marco Cavallari, Shuangshuang Zheng, Michael Mitterer, Michael Reth, Jianying Yang

The concept of plasma membrane organization has recently undergone a paradigm shift from the continuum to the partitioned fluid model. Accordingly, most surface receptors are non-randomly organized at nanoscale distances. This nanoscale organization of membrane proteins plays a central role in receptor activation and signaling, for both healthy and neoplastic cells.

However, advanced microscopy techniques allowing protein studies at the nanoscale are limited by their low-throughput, time-consuming data collection and complicated analysis. More importantly, no more than two targets can be monitored simultaneously by these methods.

To tackle these challenges, we have developed a new proximity detection method: The branched proximity hybridization assay or bPHA. In this assay, the distance between a given set of targets is measured by the size of their recognition elements (antibodies, antibody fragments or aptamers) and the coupled oligonucleotides. Only if the targets are in close vicinity to each other, a sequential DNA hybridization process amplifies such an individual interaction signal about 400 times. Intra- and extracellular bPHA can be measured by flow cytometry in a multiplexing, high-throughput and quantitative manner.

Deploying bPHA, we reliably detected the nanoscale reorganization of B cell receptor isotypes and the intracellular dynamics of Syk kinase recruitment to the BCR signaling subunit after treatment of B cells with different stimuli.

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### *Reprogramming the Genetic Code*

Presenting author:

**Jason Chin**

Author(s):

Jason Chin

The information for synthesizing the molecules that allow organisms to survive and replicate is encoded in genomic DNA. In the cell, DNA is copied to messenger RNA, and triplet codons (64) in the messenger RNA are decoded - in the process of translation - to synthesize polymers of the natural 20 amino acids. This process (DNA RNA protein) describes the central dogma of molecular biology and is conserved in terrestrial life. We are interested in rewriting the central dogma to create organisms that synthesize proteins containing unnatural amino acids and polymers composed of monomer building blocks beyond the 20 natural amino acids. I will discuss our invention and synthetic evolution of new 'orthogonal' translational components (including ribosomes and aminoacyl-tRNA synthetases) to address the major challenges in re-writing the central dogma of biology. I will discuss the application of the approaches we have developed for incorporating unnatural amino acids into proteins and investigating and

synthetically controlling diverse biological processes, with a particular emphasis on understanding the role of post-translational modifications.

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### *Shaping E. coli cells to study protein patterns and chromosome structure and dynamics*

Presenting author:

**Cees Dekker**

Author(s):

Cees Dekker

We use the tools of nanotechnology (nanofabrication, tweezers, fast AFM, fluorescence...) to explore biology at the single-molecule and single-cell scale. Our research ranges from single-molecule biophysics studies of DNA-protein interactions to DNA translocation through solid-state nanopores to exploring biophysics of bacteria with nanofabricated shapes, see <http://ceesdekkerlab.tudelft.nl>

In this talk, I will present our work where we shape E. coli cells to study protein patterns and chromosome structure and dynamics. We study cell-division proteins and DNA in live E.coli bacteria that are molded into user-defined arbitrary shapes and sizes. Clarifying the effects of cell shape will elucidate the guiding principles for the spatiotemporal organization of the cell-division machinery. We can shape live E. coli bacteria into novel shapes such as rectangles, squares, triangles and circles and study spatiotemporal oscillations of Min proteins – associated with cell division – in such artificial geometries of live E. coli cells [1].

We also exploit cell shaping to enable direct imaging of the circular chromosome in live E. coli bacteria through cell broadening [2]. The chromosome exhibits a torus topology with a 4.2- $\mu\text{m}$  toroidal length and 0.4- $\mu\text{m}$  bundle thickness, which on average shows dense right and left arms that branch from a lower-density origin of replication, and connect at the terminus of replication by an ultrathin flexible string of DNA. At the single-cell level, the DNA density along the torus is strikingly heterogeneous, with blob-like Mbp-size domains that undergo major dynamic rearrangements, splitting and merging at a minute timescale. We show that prominent domain boundaries at the terminus and origin of replication are induced by MatP proteins, while weaker transient domain boundaries are facilitated by the global transcription regulators HU and Fis.

If time allows, I will also present our work on proteins and DNA in nanofabricated chambers. We use a bottom up approach to study the basic divisome components in vitro exploiting the full control provided by nanochambers. This approach can resolve the spatial organization of the fascinating patterns of Min proteins [3] and chromatin that dictate the localization of the division ring.

[1] F. Wu, B.G.C. van Schie, J.E. Keymer, C. Dekker, Nature Nanotechn. 10, 719 (2015)

[2] F. Wu, A.Japaridze, X. Zheng, J.W.J. Kerssemakers, C. Dekker  
<https://www.biorxiv.org/content/early/2018/01/11/246389>

[3] Y. Caspi and C. Dekker, eLife doi:10.7554/eLife.19271 (2016).

### *Mechanisms of selective recruitment of RNA polymerases II and III to snRNA gene promoters*

Presenting author:

**Oleksandr Dergai**

Author(s):

Oleksandr Dergai, Pascal Cousin, Jerome Gouge, Alessandro Vannini, Nouria Hernandez

Core RNA polymerase (Pol) II snRNA promoters and type 3 Pol III promoters contain a proximal sequence element which recruits the factor SNAPc. Type 3 promoters contain moreover a TATA box, which determines Pol III specificity. We examined how TBP, TF2B, and TF2A, required for Pol II transcription, and TBP, BRF2, and BDP1, required for Pol III transcription, assemble to ensure specific Pol recruitment at SNAPc-dependent promoters. We aimed to understand whether it's possible to construct a functional promoter for both Pol II and Pol III. To address these questions, we used minimal sets of transcription factors capable of nucleating specific PICs on Pol II or Pol III SNAPc-dependent promoters and examined the specificity of various individual protein-protein interactions. Our results reveal mutually exclusive protein-protein associations and a key role for TBP in both favoring BRF2 recruitment to TATA-containing promoters and preventing BRF2 recruitment to TATA-less promoters. Our results provide a model for specific Pol recruitment at SNAPc-dependent promoters. By changing position of TATA-box and its sequence it's possible to achieve a PSE-based promoter sequence which can serve as a functional promoter for both Pol II and Pol III.

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### *Combining inteins and optogenetics to control protein activity in living cells*

Presenting author:

**Barbara Di Ventura**

Author(s):

Barbara Di Ventura

Inteins are autocatalytic protein domains that excise themselves out of proteins and in doing so connect the flanking regions with a peptide bond. The splicing reaction requires the presence of specific amino acids that are conserved in all inteins, such as a cysteine or serine at the N-terminus and an asparagine at the C-terminus. Split inteins are constituted by two separate domains that need to associate to reconstitute a functional intein. Therefore, during the process of splicing, split inteins make fusions between two previously independent proteins or peptidic fragments. Inteins are extremely useful tools in synthetic biology, because they can be used to modify proteins of interest inside living cells. Split inteins can be for instance used to reconstitute a functional protein out of two dysfunctional split halves. Being able to control the splicing reaction in space and time would open up new possibilities to perturb cells and study the effects of such perturbations on cellular behavior. Here we report the engineering of a system based on two recently developed optogenetic tools, LEXY and LOVTRAP, to control the splicing reaction with blue light in muscle cells.

### *Biosynthetic access to regioselectively methoxylated flavor compounds*

Presenting author:

**Martin Dippe**

Author(s):

Martin Dippe, Anne-Katrin Bauer, Danilo Meyer, Ludger Wessjohann

Regiospecific hydroxylation and O-methylation are common modifications of secondary metabolites, which are often indispensable for the biological activity of the produced substances. In particular, these reactions are crucial steps in the biosyntheses of plant phenolics, which contain a so-called vanilloid motif (such as the aroma compound vanillin or the bitter-masking flavone homoeriodictyol) but are difficult to achieve using conventional chemosynthetic methods.

In the presented work we report on the rational-re-design of an O-methyltransferase to generate either catalytically improved enzymes or variants of opposite regiospecificity. The tailored enzymes proved to be valuable tools for the production of flavonoids with specific O-methylation patterns as they functionalize catecholic (1,2-dihydroxyphenyl) compounds to the corresponding 3- or 4-O-methylated derivatives. In combination with a cytochrome enzyme which performs the required hydroxylation step, the engineered O-methyltransferase was applied in life whole-cell biocatalysts for the production of the highly prized homoeriodictyol from the waste stream material naringenin.

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### *streamMD: Novel computational methods for synthetic biology.*

Presenting author:

**Maximilian Dombrowsky**

Author(s):

Maximilian Dombrowsky

Synthetic biology requires advanced computational methods in particular, the rational design of novel functionalities of molecules. The combination of Molecular Dynamics (MD) simulations with results from Next Generation Sequencing can provide rich insight into the molecular systems of interest.

However, a pressing need arises to analyse these data efficiently and in semantic detail. We present streamMD, an easy to use package for the widespread statistical system R. streamMD enables the straightforward import and analysis of MD simulation data, as well as lightweight storage formats. Most importantly, it employs advanced graph-based applications to semantically analyze MD trajectories to functionally-mechanically annotate molecular phenotypes suggested by NGS experiments. This enables various molecular design goals; among them riboswitch design.

*streamMD: Novel computational methods for synthetic biology.*

Presenting author:

**Maximilian Dombrowsky**

Author(s):

Maximilian Dombrowsky, Sven Jager, Benjamin Schiller, Benjamin Mayer, Sebastian Stammmler, Kay Hamacher

Synthetic biology requires advanced computational methods in particular, the rational design of novel functionalities of molecules. The combination of Molecular Dynamics (MD) simulations with results from Next Generation Sequencing can provide rich insight into the molecular systems of interest.

However, a pressing need arises to analyse these data efficiently and in semantic detail. We present streamMD, an easy to use package for the widespread statistical system R. streamMD enables the straightforward import and analysis of MD simulation data, as well as lightweight storage formats. Most importantly, it employs advanced graph-based applications to semantically analyze MD trajectories to functionally-mechanically annotate molecular phenotypes suggested by NGS experiments. This enables various molecular design goals; among them riboswitch design.

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### *TOWARDS ENGINEERING OF MAGNETIC NANOSTRUCTURES IN BACTERIA BY SYNTHETIC BIOLOGY*

Presenting author:

**Marina Dziuba**

Author(s):

Marina Dziuba, Dirk Schüler

Magnetosomes (MS) are intracellular organelles of magnetotactic bacteria (MTB), representing membrane-coated magnetic crystals aligned in chains to serve as sensor for magnetic navigation. MS formation is tightly controlled by >30 genes that endows them with unprecedented physical properties, making MS highly attractive for many biotechnological applications. However, difficult manipulation of MTB has stimulated attempts for engineering of the MS in more amenable organisms.

Recently, our lab has for the first time reconstituted the MS formation in the bacterium *Rhodospirillum rubrum* by stepwise transfer of the entire set of MS genes from the MTB *Magnetospirillum gryphiswaldense* (MSR). This prompted us to take a synthetic biology approach to test a broader variety of hosts potentially suitable for MS biosynthesis. To facilitate genetic manipulations we have constructed a compact, single transposable vector harboring all major MS operons from MSR. One-step transfer of the vector fully restored MS formation in non-magnetic mutants of MSR as well as endowed *R. rubrum* with MS chains. Likewise, the transfer of the vector duplicated all MS genes in the MSR  $\Delta$ recA that significantly enhanced MS production (>110 MS per cell vs. ~25 in WT).

Currently we are exploring the transfer of the vector and its derivatives into various hosts. The long-term goal is to create a genetic toolset for the rational design and enhanced production of tailored magnetic nanostructures in different organisms.

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### *Genomic Correction of Mutations in Contractile Proteins for Precision Medicine*

Presenting author:

**Antje Ebert**

Author(s):

Antje Ebert

Site-specific genetic engineering presents a novel platform in synthetic biology for assessing molecular function of cells. We employ a top-down approach combining rational genome modification with metabolomics in higher eukaryotic cells to elucidate complex molecular signaling networks and their relevance for cardiovascular disease. Here, we have studied mutations in contractile proteins, e.g. troponin T (TnT)-R173W, which result in reduced force generation and contractility of cardiomyocytes. Moreover, mutations such as TnT-R173W lead to abnormal signaling in a variety of molecular pathways such as mitochondrial fatty acid conversion. Together, these consequences lead to severe disease, such as dilated cardiomyopathy and heart failure. Here, we designed and characterized isogenic control cells in which mutations in contractile proteins such as TnT-R173W were corrected or introduced via CRISPR/Cas9-mediated site-specific gene editing. Using these engineered isogenic controls, we performed untargeted metabolomics in contractile cells to assess signaling networks altered in presence of disease-causing mutations in contractile proteins, such as mitochondrial fatty acid conversion. Goal of the study is a systems level understanding of molecular dysregulation in pathways relevant to cardiovascular disease, which may apply also to other diseases. Increasing our respective knowledge may contribute to application of synthetic biology methods for developing precision medicine.

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### *Cyclic triterpenoid production with tailored *Saccharomyces cerevisiae**

Presenting author:

**Birgitta E. Ebert**

Author(s):

Birgitta E. Ebert, Eik Czarnotta, Kerstin Walter, Anna Lewandowski, Christoph Knuf, Hao Guo, Simo A. Jacobsen, Thomas Polakowski, Jochen Förster, Jérôme Maury, Lars M. Blank

Triterpenoids are secondary metabolites derived from squalene and consist of six isoprene units (C<sub>30</sub>). Many of them or their synthetic derivatives are being investigated as medicinal products for various diseases. The cyclic triterpenoid betulinic acid is of special interest for the pharma- and nutraceutical industry as it has antiretroviral, antimalarial, and anti-inflammatory properties and potential as anticancer

agent. Despite the obvious industrial potential, the application is often hindered by low abundance in natural plant sources. This poses challenges for biosustainable production of such compounds due to wasteful and costly product purification.

We present a novel bioprocess for betulinic acid production with tailored *Saccharomyces cerevisiae* strains, optimized on different scales, including:

pathway engineering: optimal gene combination/dosage

compartment engineering: increased reaction space for betulinic acid synthesis

strain engineering: implementation of push, pull, and block strategies.

The fermentation process was developed in parallel. Production performance was boosted by optimization of medium composition, cultivation conditions, carbon source and mode of fermentation operation in lab-scale reactors. Product purification was achieved by a one-step extraction with acetone.

The final process was evaluated for economic and ecologic efficiency and rated to be competitive with existing plant extraction procedures and to have potential for further improvement.

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### *Optimized expression cassettes for efficient enzyme synthesis*

Presenting author:

**Birgitta E. Ebert**

Author(s):

Dario S. Neves, Birgitta E. Ebert, Lars M. Blank

Engineering of productive cell factories requires high abundance of pathway enzymes. These levels are achieved by strong promoters characterized by an increased affinity towards sigma factors and RNA polymerases and thus increased mRNA synthesis rates. This can lead to RNA polymerase hijacking and drain of nucleotides and consequently to a shortage of gene expression resources for cellular purposes.

High enzyme abundance can also be achieved without massive allocation of resources by increasing the mRNA half-life. This approach allows the use of weaker promoters and still maintains high mRNA levels and translation rates.

To test this option, we designed an optimized gene expression cassette, harboring a ribozyme, a bicistronic design and an RNase III site<sup>1</sup> and evaluated it in *Pseudomonas* sp. VLB120, an uprising cell factory. We used 5 promoters (3 constitutive, 2 inducible) controlling expression of 2 fluorescent reporter genes (GFP and mCherry) and a 2-step acetoin pathway. In all experiments, the optimized cassette achieved higher production levels: a maximal 10-fold expression increase in fluorescence compared with the traditional and a 2.5-fold increased acetoin titer. A correlation between higher protein synthesis and mRNA stabilization is currently investigated.

This new expression cassette complements the synthetic biology toolbox with a device for high enzyme abundance at reduced metabolic burden in *Pseudomonads* and related organisms.[1 DOI: 10.1016/j.cbpa.2013.10.003

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### *CETCH me if you can: Bringing inorganic carbon into life with synthetic CO<sub>2</sub>-fixation*

Presenting author:

**Tobias Erb**

Author(s):

Tobias Erb

Carbon dioxide (CO<sub>2</sub>) is a potent greenhouse gas that is a critical factor in global warming. At the same time atmospheric CO<sub>2</sub> is a cheap and ubiquitous carbon source. Yet, synthetic chemistry lacks suitable catalysts to functionalize atmospheric CO<sub>2</sub>, emphasizing the need to understand and exploit the CO<sub>2</sub> mechanisms offered by Nature.

In my talk I will discuss the evolution and limitation of naturally existing CO<sub>2</sub> fixing enzymes and pathways, present strategies for the engineering and design of artificial CO<sub>2</sub> fixation reactions and pathways (Peter et al. 2015), and outline how these artificial pathways can be realized and further optimized to create synthetic CO<sub>2</sub> fixation modules (Schwander et al. 2016).

An example for such a synthetic CO<sub>2</sub> fixation module is the CETCH cycle (Schwander et al. 2016). The CETCH cycle is an in vitro-reaction network of 17 enzymes that was established with enzymes originating from nine different organisms of all three domains of life and optimized in several rounds by enzyme engineering and metabolic proofreading. In its version 5.4, the CETCH cycle converts CO<sub>2</sub> into organic molecules at a rate of 5 nanomoles of CO<sub>2</sub> per minute per milligram of protein. This is slightly faster than the Calvin cycle under comparable conditions and notably at 20% less energy per CO<sub>2</sub> fixed.

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Peter D, Schada von Borzyskowski L, Kiefer P, Christen P, Vorholt JA, Erb, TJ (2015) Screening and engineering the synthetic potential of carboxylating reductases from central metabolism and polyketide biosynthesis. *Angew Chem* 45:13457-61.

Schwander T, Schada von Borzyskowski L, Burgener S, Cortina NS, Erb TJ (2016) A synthetic pathway for the fixation of carbon dioxide in vitro. *Science* 354:900-4.

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### *Engineering of fluorescence complementation assay vectors and customized cell lines*

Presenting author:

**Raluca Fleischer**

Author(s):

Raluca Fleischer, Gary Lewin

As shown in knock-out mouse models, Stoml3 has been implicated in the generation and maintenance of neuropathic pain. Stoml3 is believed to fulfill its cellular and pathological functions as a homo-dimer has been identified as a possible drug target for the development of new classes of analgesics. Fluorescence complementation assays (FCAs) offer a possibility to probe and monitor the interaction between two proteins in an in-vivo setting, as opposed to in-vitro methods, i.e. affinity purification and co-immunoprecipitation. An FCA with Stoml3 can be used to screen commercially available small molecule libraries for candidates that impair homo-dimerization of Stoml3. In my PhD project, I set out to design and build user-convenient cloning vectors (Golden Gate cloning of gene of interest) harboring the tripartite GFP FCA for constitutive and inducible mammalian expression. Furthermore, I attempted to engineer customized Tet-ON cell lines harboring entry points for Crispr/Cas9- and PhiC31-mediated down-stream engineering of the Rosa26 locus. For the construction of FCA-vectors and targeting vectors I employed a new cloning technology, namely ligase chain reaction (LCR), which can enable scarless, multi-part assembly of recombinant DNA.

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### *A framework for high-resolution characterization of synthetic biological parts*

Presenting author:

**Timothy Frei**

Author(s):

Timothy Frei

When designing and building genetic circuits one regularly has to deal with partially or completely uncharacterized components. Therefore, it has become common practice to characterize the individual parts prior to the implementation of the full circuit. In mammalian cells, this is usually achieved by transfecting varying ratios of the components and recording their dose-response. However, the number of transfections that can be performed at a time limits the achievable throughput and resolution.

Here, we introduce a novel framework for characterizing dose-response curves based on single-well transient transfections and quantification by flow cytometry. The proposed method exploits the ability of the transfection process to generate a large variability in the number of plasmid copies internalized by the individual cells. Particularly, by performing separated transfections we are able to reduce the correlation between the copy numbers of the co-transfected plasmids. The lower correlation allows for the exploration of regions in the co-expression space that are otherwise inaccessible. By combining flow cytometry with a suitable data analysis pipeline it is possible to extract the dose-response curves from measurements of a single transfected well. The proposed framework allows for higher throughput, higher resolution and is less error-prone.

We believe that our new framework will reduce the time and resources used to predictably construct genetic circuits in mammalian cells.

### *Engineering orthogonal synthetic timer circuits in bacteria*

Presenting author:

**Georg Fritz**

Author(s):

Georg Fritz

The rational design of synthetic circuits is often restricted by cross-reactions between circuit components and physiological processes within the heterologous host. In our work we seek to overcome these restrictions by using extracytoplasmic function  $\sigma$  factors (ECFs), which represent ideal orthogonal regulators because of their high promoter specificity. After evaluating several heterologous ECF switches in *E. coli* and *B. subtilis*, computational modelling allows us to predict cascades with multiple ECFs. These "autonomous timer circuits" activate a series of target genes with defined time delays, which we find in excellent agreement with experimental data. Our results not only serve as a proof of concept for the application of ECFs as organism-independent building blocks in synthetic biology, but could also be used in biotechnological applications, e.g. to introduce a timing hierarchy in the expression of biosynthetic pathway components.

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### *Synthetic gene switches*

Presenting author:

**Martin Fussenegger**

Author(s):

Martin Fussenegger

Abstract not submitted

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### *Study of FtsZ in cell-like microenvironments*

Presenting author:

**Daniela Garcia-Soriano**

Author(s):

Daniela Garcia-Soriano, Ana Raso, Rudy Mendez, Diego Ramirez, Lei Kai, Michael Heymann, Petra Schwillie

The tubulin homolog FtsZ along with other division proteins forms a ring at mid-cell that upon constriction initiates cell division in bacteria. It attaches to the inner membrane by the peripheral membrane anchor FtsA and the integral membrane protein ZipA. To simplify the system and bypass

these natural anchors, a membrane-targeted FtsZ (FtsZ-YFP-MTS) with an additional amphipatic helix (MTS) can be employed to investigate ring formation on supported lipid bilayers (SLBs) in vitro. A previous study on SLBs suggested that i) FtsZ self-organizes into dynamic rings due to a specific interaction with FtsA, and ii) FtsZ-YFP-MTS and FtsZ-ZipA form static bundles of filaments (no-circles). We have demonstrate that FtsZ-YFP-MTS also assembles into circular dynamic patterns, providing evidence that FtsA interaction is not required and the observed self-organization is an intrinsic property of FtsZ when bound to membranes. Now, our aim is to study FtsZ-YFP-mts in cell-like microenvironments. We will employ high-throughput liposome generation to encapsulate the tubuline homolog using either purified protein or in-vitro expression reactions. Later, microfluidics devices will be employed to deform the liposome into bacteria like shape.

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### *Flavin-dependent Halogenases for the Enzymatic Halogenation in Organic Synthesis*

Presenting author:

**Jan Gebauer**

Author(s):

Jan Gebauer, Alexander V. Fejzagic, Thomas Classen

Halogenating enzymes have achieved much interest for biological and pharmaceutical applications. Today it is known, that more than 4,000 halogenated natural products exist.<sup>[1]</sup> It has been shown that these moieties enhance biological activity e.g. by preventing rapid metabolization. Besides these effects, halogen atoms are also well known for the use cross-coupling reactions and thereby derivatization and synthesis of complex structures.<sup>[2]</sup> The focus will be the cloning and heterologous expression of Flavin-dependent halogenases from *Pseudomonas protegens* PF-5 biosynthetic pathways for pyrrolnitrin<sup>[3]</sup> and pyoluteorin<sup>[4]</sup> in *Escherichia coli*. After characterization, including kinetics and substrate scope, it is the aim to produce a variety of halogenated pyrroles enzymatically. One crucial step is the chemical synthesis of reference substances for precise analysis.<sup>[5]</sup> The advantage is the utilization of non-toxic and cheap salts like NaCl.

[1] G. W. Gribble, *J. Chem. Educ.* 2004, 81, 1441.

[2] J. Latham, et al., *Chem. Rev.* 2017.

[3] S. Kirner, et al., *J. Bacteriol.* 1998, 180, 1939-1943.

[4] P. C. Dorrestein, et al., *Proc. Natl. Acad. Sci. U. S. A.* 2005, 102, 13843-13848.

[5] M. D. Morrison, et al., *Org. Lett.* 2009, 11, 1051-1054.

This research is part of the CombiCom project which is embedded in the activities of the Bioeconomy Science Center, funded by the Ministry of Culture and Science within the framework of the NRW Strategieprojekt BioSC (No. 313/323-400-00213).

### *Optimization and Application of a light-switchable Cas9 variant*

Presenting author:

**Renate Gelfert**

Author(s):

Renate Gelfert

The RNA-guided DNA-endonuclease Cas9 (CRISPR associated protein 9) paves the way for genome editing, analysis and controlling in a precise and easy manner. A short RNA directs the Cas9 protein to a specific, complementary DNA sequence followed by a PAM sequence and contributes to DNA-cleavage. Using the catalytically inactive form of Cas9, called dCas9, no cleavage occur but the protein binds to DNA and blocks gene expression. Despite the high precision, undesired effects exist which can be reduced by turning the Cas9 protein on and off via an external trigger, e.g. chemical or, like in this case, a physical stimulus. We have engineered a novel light-inducible Cas9 variant that combines the Cas9 from *Streptococcus pyogenes* and the light-oxygen-voltage domain 2 of *Avena sativa* phototropin 1 (AsLOV2). The AsLOV2 is a light-sensitive photoreceptor and a widely used optogenetic tool for the control of cellular processes via blue light in a non-invasively, spatio-temporal and reversible manner. Preliminary data show that this engineered protein variant binds and cleaves specific DNA target sequences upon blue-light exposure in vivo and in vitro. To generate and select improved variants, mutations are inserted and cell-based assays, biophysical protein characterization and functional tests are performed. Additionally, the optimized constructs are applied in *Danio rerio* and *Drosophila melanogaster*.

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### *Semi-rational protein engineering to improve the activity of surface displayed exocellulase CelK*

Presenting author:

**Katrin Gesing**

Author(s):

Katrin Gesing, Florian Lenz, Iason E.P. Tozakidis, Joachim Jose

We recently developed a whole-cell catalytic approach for the saccharification of lignocellulosic biomass employing the soil bacterium *Pseudomonas putida* with surface displayed cellulases. In this work we focused on the improvement of exocellulase CelK from *Ruminiclostridium thermocellum*, which is the current bottleneck of the hydrolysis process. Therefore a semi-rational approach was chosen by using site saturation mutagenesis (SSM) on six positions (Y543, S547, A475, R683, T797, W791) that were selected based on a low conservation and their location at the entrance of the substrate binding pocket. The variants of CelK were expressed as autotransporter fusion proteins on the surface of *Pseudomonas putida* KT2440. A 96-well plate activity assay was developed to test the variants against the synthetic substrate 4-nitrophenyl- $\beta$ -D-cellobioside (pNPC) and the more natural substrate carboxymethylcellulose (CMC). Overall, the mutation at two of the six positions led to an increase of the activity with pNPC. The mutation of T797A led to a 1.7 fold increase of the activity to  $5.49 \text{ mU} \cdot \text{ml}^{-1} \cdot \text{OD}^{-1}$ , whereas variant A475W even showed an activity increase of 2.9 fold to  $9.35 \text{ mU} \cdot \text{ml}^{-1} \cdot \text{OD}^{-1}$  in comparison to the not mutated CelK ( $3.24 \text{ mU} \cdot \text{ml}^{-1} \cdot \text{OD}^{-1}$ ).

### *Improvement of a toolkit for characterization of non-canonical amino acid incorporation systems*

Presenting author:

**Robert T. Giessmann**

Author(s):

Robert T. Giessmann, M. Nicolas Cruz-Bournazou, Matthias Gimpel, Peter Neubauer

Recently, a toolkit for characterization of non-canonical amino acid incorporation systems was published.<sup>1</sup> Reproducing the reported findings in our lab, we measured incorporation efficiency and fidelity for a model system. However, when aiming to transfer the described method to bioprocess conditions typical in our lab (i.e. liter-sized stirred tank reactors instead of microtiter plates), we obtained less clear results.

To clarify the differences between our approach and the reported conditions, we investigated important process variables, mainly dissolved oxygen tension (DOT), at both conditions.

Here, we report on the effects of scale-up, respectively scale-down, on growth patterns of the microorganism and on efficiency of non-canonical amino acid incorporation. We found that the published system requires special attention to yield results applicable to both microtiter plates and shake flasks.

Integrating the individual results into one model, we were able to predict the system's behavior under conditions relevant for bioprocess engineering (i.e. short mixing times, high oxygen transfer). Additionally, this kinetic model allowed us to estimate the parameters of the non-canonical amino acid incorporation system in a more robust way than with traditional data handling.

References:

1. "Rapid and Inexpensive Evaluation of Nonstandard Amino Acid Incorporation in Escherichia coli." J. W. Monk, et al. ACS Synthetic Biology 2017 6 (1), 45-54. DOI: 10.1021/acssynbio.6b00192

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### *Bacteriochlorophyll bound to water-soluble chlorophyll protein: a potential photosensitizer for photodynamic therapy*

Presenting author:

**Philipp Girr**

Author(s):

Philipp Girr, Jessica Kilper, Mara Werwie, Harald Paulsen

Photodynamic therapy (PDT) makes use of photosensitizers that upon illumination produce reactive oxygen species like singlet oxygen in order to induce cell death in tumor cells or pathogenic microorganisms. Special requirements for a photosensitizer are a high chemical and physical stability

and the ability to absorb light between 700 and 800 nm, where light penetration into tissue is maximal. As a potential photosensitizer for PDT, we investigate tetrameric type-II water-soluble chlorophyll proteins (WSCPs). These extremely stable plant proteins (so far only found in Brassicaceae) bind one chlorophyll (Chl) per monomer and are known to produce relatively high amounts of singlet oxygen upon illumination. Interestingly, the photostability of non-photosynthetic WSCP is comparable to that of photosynthetic Chl proteins although WSCP, unlike the latter, does not contain any carotenoids to which prevent the formation of singlet oxygen. In this study, we found recombinant WSCP to be able to reconstitute with bacteriochlorophyll (BChl), the photosynthetic pigments of purple bacteria with a strong light absorption at 770 – 780 nm. BChl –WSCP complexes show a similar heat stability, singlet oxygen formation and photoprotection as the Chl complexes. Overall, the high stability and the long-wavelength absorption makes BChl-containing WSCP an interesting candidate for usage in PDT.

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### *Design and synthesis of a minimal bacterial genome*

Presenting author:

**John Glass**

Author(s):

John Glass

Abstract not submitted

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### *Rebuilding pattern formation in a simplified Min system*

Presenting author:

**Philipp Glock**

Author(s):

Philipp Glock, Petra Schwille

The Min system of *Escherichia coli* plays a major role in positioning the division septum to midcell. Our lab and others have shown that two proteins, MinD and MinE, can self-organize into planar surface waves when reconstituted on model membranes in vitro [1,2]. Here, we investigate how one can rebuild pattern formation, starting from a minimal MinE-derived peptide that binds to MinD and enhances its ATPase activity. Intriguingly, we found two ways to do so, using properties innate to the wildtype MinE. First, and contrary to published reports [3], by endowing the minimal peptide with a means of membrane binding, which leads to the formation of patterns several orders of magnitude larger than those formed with wild type MinE. Second, by synthetically dimerizing the minimal peptide. Both approaches lead to chaotic patterns, in stark contrast to the mostly regular, single-wavelength patterns observed with full-length MinE. With the integration of mathematical modelling, our approaches should lead to a better understanding of the principal features of the native Min system. It should further serve useful as a minimized and simplified version of a biological reaction-diffusion system for synthetic biology and nonlinear dynamics research.

### *Engineering fatty acid synthases (FAS) for custom compound synthesis*

Presenting author:

**Martin Grininger**

Author(s):

Martin Grininger

Multienzyme type I fatty acid synthases (FAS) provide compartmentalized reaction space for C-C bond formation. Natively used for the biosynthesis of C16 and C18 fatty acids, recent studies demonstrate the application of FAS for the production of other and more complex compounds, among them short fatty acids (1,2), methylketones and a lactone. The production of the lactone was achieved by the coupling of two FAS modules in a way that module 2 starts synthesis only when loaded with the product of module 1. Key to the successful design of FAS is the well-established understanding of these proteins, and the combined in vitro protein analysis and in silico description of the relevant enzymatic functions performed during this work.

The approach of rewriting fatty acid synthesis also revealed the challenges of in vitro characterization and computational modeling of MDa-sized multienzymes, as well as the limitations and the perspectives of multienzyme engineering. As such, it has model character for the rational design of the evolutionally related, chemically highly versatile polyketide synthases (PKS).

(1) Gajewski, et al. Engineering fatty acid synthases for directed polyketide production. Nat Chem Biol. 13, 363-365, (2017).

(2) Gajewski et al. Engineering fungal de novo fatty acid synthesis for short chain fatty acid production. Nat Commun. 8:14650 (2017).

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### *Targeting the bottleneck – new methods to develop biosensors*

Presenting author:

**Florian Groher**

Author(s):

Florian Groher, Adrien Boussebayle, Cristina Bofill-Bosch, Beatrix Suess

Short oligonucleotides that bind to a specific target molecule with high affinity and specificity can easily be obtained from an in vitro selection process called SELEX. Albeit the invention of this methodology was back in the 1990s, the successful enrichment of aptamer libraries for small molecules stays still challenging, thus creating a bottleneck for the development of synthetic riboswitches, biosensors and 'point-of-care' applications.

Here we present a new method for aptamer selection where there is no need for immobilization of the target molecule leaving all side groups of the small molecule accessible for RNA binding. Additionally, this also eliminates the laborious need for chemical immobilization onto a matrix. Utilization of this new

protocol not only yields aptamers in a shorter timescale than before, but also facilitates subsequent screening steps identifying synthetic riboswitches or intracellular biosensors.

This new approach, together with other improvements in e.g. single cell analysis, will not only lead to more aptamers that can be used as affinity reagents, but also more biosensors that can be used by biotechnology industry for e.g. strain optimization. This will have a sustainable impact on both (circular) bioeconomy and health care.

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### *Temperature-dependent dynamic control of the TCA cycle for increased volumetric productivity of itaconic acid production by Escherichia coli*

Presenting author:

**Björn-Johannes Harder**

Author(s):

Björn-Johannes Harder, Katja Bettenbrock, Steffen Klamt

We recently engineered E.coli (strain ita23) for growth-coupled synthesis of itaconic acid with high yield. Here we aimed to improve the productivity by applying a two-stage process strategy with decoupled production of biomass and itaconic acid.

Based on the design of the E. coli strain ita23, we constructed a strain ita32 (MG1655  $\Delta aceA \Delta pta \Delta pykF \Delta pykA$  pCadCs), which, in contrast to ita23, has an active tricarboxylic acid (TCA) cycle. This enables the strain to grow with a fast growth rate of  $0.52 \text{ h}^{-1}$  at  $37^\circ\text{C}$  with glucose as the only carbon source, thus representing an ideal phenotype for the first stage, the growth phase. To down-regulate the TCA cycle and thus to switch from growth to itaconic acid production in the second stage, we replaced the promoter of the isocitrate dehydrogenase by the Lambda promoter (pR). The expression of this promoter was controlled by the temperature-sensitive repressor CI857 which is active at lower temperatures ( $30^\circ\text{C}$ ). The respective strain ita36A grew with a fast growth rate at  $37^\circ\text{C}$  and switched to production of itaconic acid at  $28^\circ\text{C}$ . To study the impact of the process strategy on productivity we performed one-stage and two-stage bioreactor cultivations. The two-stage process enabled fast formation of biomass resulting in improved peak productivity of  $0.86 \text{ g/L/h}$  (+48%) and volumetric productivity of  $0.39 \text{ g/L/h}$  (+22%) after 120 h in comparison to the one-stage process.

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### *Switching de novo coiled coils at membranes*

Presenting author:

**Leon Harrington**

Author(s):

Leon Harrington, Jordan Fletcher, Derek Woolfson, Petra Schwillie

Protein-based molecular switches are a common feature of natural signaling pathways in cells. In particular, the reversible switching of proteins between cytosolic and membrane-bound states is a critical mechanistic motif underpinning many dynamic processes such as polarization and cell division. We have developed a system of de novo coiled coil peptides whose association and dissociation can be reversibly mediated by phosphorylation and dephosphorylation reactions. The switching kinetics are tunable at the amino acid level as well as through experimental conditions such as temperature and concentration. By coupling the peptides to appropriate membrane anchors, reversible membrane localization can be readily achieved. Such switches could be used as building blocks for the construction of synthetic circuits or dynamic systems. Further, they could be more broadly applied for the reversible switching of protein oligomeric states, as reporters of enzymatic activity, or as interfaces into existing signaling pathways.

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### *In vitro characterization of molecular processes underlying MinD membrane interaction*

Presenting author:

**Tamara Heermann**

Author(s):

Tamara Heermann, Simon Kretschmer, Petra Schwille

As components of a central system for the spatiotemporal organization of E.coli, the cell division regulators MinD, MinE and MinC are crucial for the localization of the division machinery to mid-cell. To achieve this function, MinD and MinE undergo coordinated pole-to-pole oscillations. This gives rise to a concentration gradient of MinC, which inhibits polymerization of the early divisome protein FtsZ at the poles but not at mid-cell. A central step in the molecular mechanism of the MinD/MinE-oscillation is the ATP dependent dimerization of MinD. Upon dimerization, the C-terminal amphipathic helices of MinD-dimers interact cooperatively with the membrane. Membrane-bound MinD dimers then recruit MinE, which stimulates ATP hydrolysis and acts as an antagonist to MinD accumulation on the bacterial membrane. To obtain a comprehensive understanding of the processes involved in the assembly of MinD at the membrane, we will focus on systematically analyzing binding kinetics of WT MinD and mutant proteins with engineered membrane-target sequences. For this purpose, we will utilize quartz crystal microbalance with dissipation monitoring (QCM-D) and isothermal titration calorimetry (ITC) to characterize MTS residues that specifically regulate the binding affinity of MinD to native-like lipid membrane interfaces. Ultimately, this project should result in a better understanding of the molecular-scale processes enabling pattern-forming protein systems to self-organize.

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### *Multicomponent Optogenetics ⇔ Sensing is not Understanding*

Presenting author:

**Peter Hegemann**

Author(s):

Peter Hegemann

Activation or inactivation of a living cell, tissue or animal, either naturally or artificially, initiates small or larger long-term changes within the living system (The biological uncertainty principle). In most cases the researcher intends to learn about the living system without being aware about its own – in many cases -destructive action. Illumination of a cell is probably one the least destructive action one may undertake. We study natural sensory photoreceptors mostly from green algae with respect to the original function within the algal context but also modify these photoreceptors and employ them in host cells to manipulate host processes ideally non-invasively. For a long time the membrane voltage has been the main host parameter and we engineered - supported by spectroscopic and structural information - light-gated channels and light-driven pumps in many direction with respect to color sensing, ion selectivity and kinetics, and converted ion pumps into ion channels or vice versa to understand the principle differences. More recently we focused on second messengers as cAMP and cGMP by employment of fungal photo-activated cyclases. The combination with cyclic nucleotide gated (cNG) channels generated multi-component optogenetic systems with large amplifications providing ultra high sensitivity in host cells. We keep in mind Max Plancks concept: "Understanding precedes application" and we begin to learn that "manipulation always causes distraction from natural behavior". Moreover, large signals do not mean natural responses, it may be just the opposite.

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### *(Opto)genetic control of microbial cell factories for an efficient production of valuable secondary metabolites*

Presenting author:

**Fabiene Hilgers**

Author(s):

Fabiene Hilgers, Fabian Hogenkamp, Dennis Binder, Anita Loeschcke, Jörg Pietruszka, Karl-Erich Jaeger, Thomas Drepper

Secondary metabolites are a valuable source of compounds with pharmaceutical or biotechnological potential. However, the efficient biosynthesis in a heterologous host is often hampered by inefficient metabolic fluxes or accumulation of unwanted or toxic intermediates. Such unwanted effects can be abolished by applying modular systems allowing for a strict control over recombinant biosynthetic cascades. This project therefore aims to achieve a precise and straightforward control over complex gene cluster-encoded pathways by the application of light-triggered optogenetic switches such as photocaged compounds.

As a proof of concept, photocaged arabinose was applied as a novel light-responsive inducer for heterologous gene expression in *E. coli*. Exposure of caged arabinose to UV-A light resulted in a rapid and gradual induction of protein production. The biotechnological applicability of this phototrigger was demonstrated by successful expression of the violacein pathway genes from *Chromobacterium violaceum*.

Next, the photoswitch principle will be transferred to the alternative production hosts *Rhodobacter capsulatus* and *Pseudomonas putida*, in order to optimize the synthesis of novel sesquiterpenes as well as prodiginines. First expression studies with  $\beta$ -caryophyllene synthases were performed in *R. capsulatus*, resulting in a substantial production of the heterologous sesquiterpene with final yields of > 1g/L.

### *Structural basis of human mitochondrial transcription*

Presenting author:

**Hauke Hillen**

Author(s):

Hauke Hillen

The mitochondrial genome is transcribed by a dedicated mitochondrial RNA polymerase (mtRNAP), which also generates the RNA primers required for DNA replication. Unlike the distantly related bacteriophage RNA polymerases, mtRNAP requires auxiliary protein factors for each step of the transcription cycle. However, the molecular mechanisms underlying mitochondrial transcription are poorly understood. While the structures of the mitochondrial polymerase and of some mitochondrial transcription factors have been reported, structural data on the interplay between these factors and the polymerase in functional complexes has been lacking. We have determined the structure of the human mitochondrial transcription initiation complex, which reveals how the initiation factors TFAM and TFB2M facilitate promoter binding and DNA opening, respectively. Furthermore, we have solved the structure of the mitochondrial transcription elongation factor TEFM and of an anti-termination complex consisting of TEFM bound to the transcribing polymerase. These structures illustrate how TEFM interacts with both the nucleic acid and the polymerase in the elongation complex to facilitate processive transcription and drive gene expression over primer formation for DNA replication. Together, these results elucidate the mechanistic basis of transcription initiation and processive elongation in human mitochondria and provide the framework for studying the regulation of mitochondrial gene transcription.

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### *Engineering the Substrate Scope of a Fe(II)-dependent Halogenase*

Presenting author:

**Sabrina Höbenreich**

Author(s):

Sabrina Höbenreich

Asymmetric halogenation is an important reaction for late-stage functionalisation of drug-like molecules. Performing chlorinations under mild conditions using sodium chloride as the chlorine source has great potential for sustainable catalysis. The discovery of non-heme iron (NHI) and 2-oxoglutarate dependent halogenases, acting directly on a small organic molecule and not on acyl-carrier bound substrates,[1,2] has eliminated a major drawback of known NHI-halogenases. Hence, these enzymes represent attractive starting points for developing biocatalytic routes for selective, aliphatic chlorination of C(sp<sup>3</sup>)-H bonds, a paramount challenge in organic synthesis. After solving the crystal structure of WelO15 from *Westiella intricata*, we used enzyme engineering to redesign the active site. New variants are able to chlorinate novel, non-native substrates, thereby presenting a first step towards biocatalytic mild, asymmetric chlorination of C(sp<sup>3</sup>)-H bonds.

[1] M. L. Hillwig, X. Liu, Nat. Chem. Biol. 2014, 10, 921–923. [2] M. L. Micallef, D. Sharma, B. M. Bunn, L. Gerwick, R. Viswanathan, M. C. Moffitt, BMC Microbiology 2014, 14, 213.

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### *Multiple genetic circuits in the yeast *S. cerevisiae* for tight regulation of gene expression*

Presenting author:

**Anja Hofmann**

Author(s):

Anja Hofmann, Johannes Falk, Andreas Christmann, Harald Kolmar

In recent years, the development of regulated systems allowing robust and precise on and off switching of one or more genes of interest, followed by expression or repression has become more and more attractive for research and industrial purpose [1]. Especially the high homologous recombination efficiency renders the yeast *S. cerevisiae* an important model organism for synthetic biology. However, many regulated systems published to date influence the viability of the host cell, show high basal expression or enable only the overexpression of the target gene without the possibility of fine regulation. Herein we describe various genetic switches based on a CRISPR/dCas9 regulated system [2]. Exemplarily, a switch inducible by carbon source change was implemented and combined with tight regulation of gene expression by a heterologous transcription factor,  $\beta$ -estradiol [3]. We achieved a switch active only in the presence of both inducers, which does not influence viability, does not show basal expression and enables the regulated expression of a target gene.

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### *Investigating mechanosignaling by utilizing intra- and extracellular optogenetic switches*

Presenting author:

**Maximilian Hörner**

Author(s):

Maximilian Hörner, Claire Chatelle, Wignand W.D. Mühlhäuser, Katrin Raute, Barbara Hummel, Josef Madl, Wolfgang W.A. Schamel, Winfried Römer, Matias D. Zurbriggen, Christian Friedrich, Susana Minguet, Ritwick Sawarkar, Gerald Radziwill, Wilfried Weber

The mechanical properties of the cellular microenvironment play an important role in various biological processes such as development, cell differentiation and tumor progression. Therefore a deeper understanding of the underlying signaling processes remains crucial. In order to allow the comprehensive investigation of mechanosignaling, we applied optogenetic switches within and outside of mammalian cells.

On the one hand, we engineered the activation of focal adhesion kinase (FAK) blue light-responsive by fusing FAK to the photoreceptor cryptochrome 2 from *Arabidopsis thaliana*. This so-termed optoFAK could be repeatedly activated and deactivated uncoupled from physiological stimuli within minutes by blue light illumination or incubation in the dark, respectively.

On the other hand, we coupled the cyanobacterial phytochrome Cph1 to branched polyethylene glycol. Thereby we synthesized a hydrogel the mechanical properties of which could be adjusted in a wavelength-specific, dose-dependent, spatially controlled and fully reversible manner within seconds. Incorporation of an RGD cell adhesion motif into the hydrogel allowed us to use the material as synthetic extracellular matrix for the analysis of mechanosignaling and cell migration.

We suggest that especially the combination of intra- and extracellular molecular switches will allow valuable insight into mammalian signaling processes.

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### *Investigations into the Folding Mechanism and Conformational Dynamics of a Chimeric Protein*

Presenting author:

**Abhishek Anan Jalan**

Author(s):

Abhishek Anan Jalan, Birte Höcker

(ba)<sub>8</sub>-barrel is an ancient protein fold believed to have evolved by tandem fusion of ancestral (ba)<sub>4</sub> half-barrels. Fusion of half-barrels of histidine biosynthetic enzyme HisF resulted in a topologically similar (ba)<sub>8</sub>-barrel, plausibly recreating an evolutionary event [Höcker, B. Curr Opin Struc Biol 201427, 56]. In addition, the folding mechanism of the barrel was conserved vis-à-vis HisF [Carstensen, L. et al J Am Chem Soc 2012 134, 12786]. These results suggested homologous recombination as a feasible route for (ba)<sub>8</sub>-barrel evolution. The (ba)<sub>4</sub> half-barrel is also topologically similar to a fragment of the (ba)<sub>5</sub> flavodoxin-like proteins. Fusion of fragments from HisF and chemotactic protein CheY results in a chimeric (ba)<sub>8</sub>-barrel, CheYHisF [Eisenbeis, S. et al J Am Chem Soc 2012 134, 4019]. This suggests non-homologous recombination as an alternative route for the (ba)<sub>8</sub>-barrel evolution. In order to investigate this evolutionary hypothesis, we studied the folding mechanism of CheYHisF and whether it is dictated by either fragment or some cooperative combination of both fragments. Here we present preliminary results of investigations into the folding mechanism of the CheYHisF chimera using equilibrium and kinetic (un)folding and NMR-based study of the residual structure and conformational dynamics of unfolded, intermediate and folded states of CheYHisF.

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### *Spatiotemporal regulation toolbox for cell free synthetic biology*

Presenting author:

**Haiyang Jia**

Author(s):

Haiyang Jia, Tobias Härtel, Michael Heymann, Lei Kai, Petra Schwillle

To investigate fundamental principles of self-organization in natural cells, we seek to encapsulate cell-free transcription/translation into membrane vesicles to ultimately progress toward a synthetic minimal "cell". Due to their complexity and often non-linear nature, prototyping regulatory circuits can be

challenging. To address this problem, we developed a feedback regulation toolbox to enable spatiotemporal regulating protocell processes from transcription/translation to protein-protein interaction. The toolbox includes two kinds of sensors, temperature sensors and light sensors. As the temperature sensors, the synthetic temperature sensitive RNA non-coding sequences (RNA thermometers) provide the transcription and translation level regulation, which enables the protocell behaves in a logic way. For the light sensors, we engineered the PhyB-PIF system as optical tools to spatiotemporally print target proteins on the model membranes. The range of applications extends from controlling protein molecular interaction, caging protein and driving pattern formation onto model membrane, and up to mediating the membrane-membrane adhesion. The tunable temperature sensitive genetic circuits and the reversible light-gated protein-protein interaction will enable a variety of biotechnology applications such as protocell engineering, biomedical research, tissue engineering and industry.

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### *Binding of Pheophytin in Water-Soluble Chlorophyll-Protein Increases Photo-Stability without Decrease of Singlet Oxygen Production*

Presenting author:

**Fabian Jung**

Author(s):

Fabian Jung, Bastian Barthelmess, Anne-Christin Pohland, Harald Paulsen

The Water-Soluble Chlorophyll-binding Protein (WSCP) is quite an atypical representative of the group of chlorophyll (Chl) or Chl derivative-binding proteins of higher plants. Its tetrameric conformation shows extraordinary stability, but knowledge about the physiological function of WSCP is still limited. Although there are no covalent bonds between WSCP and the four bound Chl molecules, the Chl ligation is extremely strong, even under extreme conditions. Additionally, the WSCP-incorporated Chls are partially protected against photooxidation although WSCP contains no carotenoids to prevent the accumulation of singlet oxygen ( $^1\text{O}_2$ ), known to protect Chls in the photosynthesis apparatus of plants. This protection is remarkable, as, the formation of  $^1\text{O}_2$  upon illumination is as high as that of free Chl.

Recently, we found that recombinantly expressed apoprotein of WSCP is also able to bind pheophytin (Pheo) to form tetrameric complexes with similar biochemical properties and heat stability as those with Chl. Consequently, the central Mg, absent in Pheo, does not seem to be essential for Chl binding to the protein. Interestingly, the photostability of Pheo-WSCP is significantly higher than that of Chl-WSCP despite a comparable  $^1\text{O}_2$  formation rate. Thus, Pheo-WSCP is a long-lasting producer of  $^1\text{O}_2$  under illumination in the visible range. Such a system might be an ideal antimicrobial agent and candidate for applications in the context of wastewater-treatment.

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### *Synthetic epigenetics - from engineering of the chromatin state to cellular differentiation*

Presenting author:

**Tomasz Jurkowski**

Author(s):

Peter Stepper, Phil Oberacker, Tomasz Jurkowski

All the cells in a human body stem from a single fertilized egg and contain the same DNA sequence. It is fascinating that each cell type can express different sets of genes and perform different functions in the body. The fact that these different cells contain the same DNA but show very distinct phenotypes indicates that regulation of genetic information is key to understanding cell identity and, therefore, human development and health. Epigenetic modifications enforce a transcriptionally repressed or active state and thus explain the cell's transcriptional profile and consequently its differentiation state over the lifetime of an organism. On the other hand, the epigenetic enzymatic machinery responds to various parameters providing transcriptional plasticity in response to environmental stimuli. This apparent paradox that epigenetic states are stable yet dynamic prompted us to investigate how stability or dynamics of epigenetic states are achieved. We have developed a highly multiplexable EPIC'RISPR epigenetic editing toolbox, which we employ to investigate the molecular mechanisms underlying the maintenance of epigenetic states, with the goal of identifying the molecular machinery that allows maintaining or stably switching of the epigenetic status. Furthermore, using our tools we are able to control the differentiation state of mammalian cells as exemplified by epigenetically programmed lineage conversion of pancreatic alpha to insulin-producing beta cells.

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### *S-adenosyl-L-homocysteine hydrolase and synthetic nicotinamide cofactor biomimetics*

Presenting author:

**Lyn Lisette Kailing**

Author(s):

Lyn Lisette Kailing, Daniela Bertinetti, Caroline Emilie Paul, Tomasz Manszewski, Mariusz Jaskolski, Friedrich Wilhelm Herberg, Ioannis V. Pavlidis

S-adenosyl-L-homocysteine hydrolases (SAHases) catalyse the reversible hydrolysis of SAH to adenosine and L-homocysteine. In many organisms, they are important regulators for methylation processes, as their substrate SAH is a potent inhibitor of S-adenosyl-L-methionine (SAM) dependent methyltransferases. Hyper- or hypoactive SAHases fail to maintain an appropriate cellular SAM:SAH ratio and therefore cause severe health issues. Both directions of the reaction, SAH hydrolysis and synthesis, rely on an NAD<sup>+</sup>-dependent oxidation to activate the substrate for the subsequent reaction mechanism. Interestingly, the regeneration of the initial oxidised form of the nicotinamide cofactor is part of the catalytic cycle itself, i.e., no external regeneration system is needed.

In the present in vitro study, we tested a set of synthetic nicotinamide cofactor biomimetics (NCBs) with regard to their ability to reconstitute or modulate activity of an SAHase from the root-nodulating bacterium *Bradyrhizobium elkanii* in the absence or presence of the natural cofactor NAD<sup>+</sup>, respectively. Since one of the NCBs was identified as an inhibitor, a more comprehensive characterisation of this inhibition was performed.

### *Using light to dissect and direct cellular transport systems*

Presenting author:

**Lukas Kapitein**

Author(s):

Lukas Kapitein

Motor proteins that move over cytoskeletal fibers create long-range order within cells. For example, highly polarized and spatially extended cells, such as neurons, depend on the proper positioning of organelles by a large variety of cytoskeletal motor proteins. To explore how different motor proteins contribute to neuronal transport and to study the site-specific roles of different organelles, we have established optical control of intracellular transport by using light-sensitive heterodimerization to recruit specific cytoskeletal motor proteins (kinesin, dynein or myosin) to selected cargoes. In addition, to unravel how the specialized organization of the neuronal cytoskeleton guides different motor proteins to either axons or dendrites, we have developed novel approaches for optical nanoscopy. One of these, called motor-PAINT, uses nanometric tracking of motor proteins to super-resolve cytoskeletal fibers and determine their polarity. This has revealed a key architectural principle of the neuronal microtubule cytoskeleton that explains how different motor proteins can selectively transport cargoes to either axons or dendrites.

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### *Engineered Polyketide Synthases for Production of Commodity and Specialty Chemicals*

Presenting author:

**Jay Keasling**

Author(s):

Jay Keasling

Engineered modular polyketide synthases (PKSs) have the potential to be an extraordinarily effective retrosynthesis platform. Native PKSs assemble and tailor simple, readily available cellular acyl-CoAs into large, complex, chiral molecules. By successfully rearranging existing polyketide modules and domains, one could exquisitely control chemical structure from DNA sequence alone. As an example of the diverse biosynthetic potential of PKSs, we have concluded that approximately 20 of the roughly 150 commodity chemicals tracked by the petrochemical market information provider ICIS could be produced by mixing and matching naturally occurring PKS domains. To form these chemicals, engineered PKSs would load acyl-CoAs, perform a programmed number of extension reactions, and then release products using previously published mechanisms. However, this potential has only just begun to be realized as the compounds that have been made using engineered PKSs represent a small fraction of the potentially accessible chemical space. In my talk, I will highlight work from our laboratory where we have engineered polyketide synthases to produce a variety of commodity and specialty chemicals.

### *Towards the PDB for enzyme function data – STRENDA DB*

Presenting author:

**Carsten Kettner**

Author(s):

Carsten Kettner

The STRENDA Commission (STAndards for Reporting ENzymology Data, [www.beilstein-strenda.org](http://www.beilstein-strenda.org)) made up of experts from the enzyme chemistry community and supported by the Beilstein-Institut, has developed the STRENDA Guidelines in tight consultation with the community. The aim is to improve the quality of enzyme function data in the literature. Today, more than 55 biochemical journals already recommend authors to refer to these guidelines when reporting enzyme kinetics data.

To enable scientists to easily prepare data for manuscripts, the STRENDA Commission has developed a web-based portal for the direct electronic submission of data by the authors prior to publication. This portal called STRENDA DB provides an assessment tool with which authors, journals' editors and reviewers can check whether the reporting of experimental data is compliant with the STRENDA guidelines and thus matches the instructions for authors from the journals. The data entered are stored in STRENDA DB and will be made publically accessible after they have been published in a journal. In addition, with the establishment of STRENDA DB we propose the change of the current publication workflow: from manuscripts to databases to publication rather than from manuscript to publication to databases.

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### *Theory and design of Cybergenetic Systems*

Presenting author:

**Mustafa Khammash**

Author(s):

Mustafa Khammash

Among the possible applications enabled by synthetic biology is the design and engineering of feedback control systems that act at the molecular scale in real-time to steer the dynamic behavior of living cells. Here I will present our theoretical framework for the design and synthesis of such control systems, and will discuss the main challenges in their practical implementation. I will then present the first designer gene network that attains integral feedback in a living cell and demonstrate its tunability and disturbance rejection properties. A growth control application shows the inherent capacity of this integral feedback control system to deliver robustness, and highlights its potential use as a universal controller for regulation of biological variables in arbitrary networks. Finally, I will discuss the potential impact of biomolecular control systems in industrial biotechnology and medical therapy.

### *Characterization of ubiquitin acetylation*

Presenting author:

**Simon Kienle**

Author(s):

Simon Kienle, Martin Scheffner

Ubiquitination is a diverse post-translational modification (PTM) involved in a variety of cellular pathways. Ubiquitin (Ub) is covalently attached to substrates via a cascade of three enzymes. This cascade involves Ub activating enzymes (E1s), Ub conjugating enzymes (E2s) and Ub ligases (E3s). The various functions of the ubiquitin system are in part achieved by different types of ubiquitination including mono- and poly -ubiquitination. Recently, by the discovery that ubiquitin itself is subjected to PTMs such as phosphorylation and acetylation another layer was added to the complexity of ubiquitin signalling. In contrast to Ub phosphorylation, Ub acetylation and its biological functions are only poorly understood. For Ub acetylation neither acetyltransferases nor deacetylases are known.

To investigate Ub acetylation and its influence on the ubiquitination cascade, site-specifically acetylated ubiquitin was generated via the stop-codon suppression method.

In contrast to the above approach, most studies investigating lysine acetylation use respective lysine to glutamine mutants to examine the effects of acetylation. Here, we compared these apparent "lysine acetylation mimics" to their actual acetylated counterparts on a functional level. Furthermore, the effect of Ub acetylation on the efficiency of the ubiquitination -cascade was examined, since an inhibitory effect of Ub acetylation on ubiquitin chain elongation has been recently reported.

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### *OptoBase: A platform for molecular optogenetics*

Presenting author:

**Katja Kolar**

Author(s):

Katja Kolar, Hendrik Stork, Christian Knobloch, Matej Žnidarič, Wilfried Weber

Optogenetics is a scientific field, where natural light-responsive proteins are engineered into genetically encoded protein switches, which can be utilized to control cellular signaling and behavior in an unprecedented space- and time-resolved manner. Thanks to these powerful characteristics, optogenetic tools, such as the ones for light-controlled protein localization, light-controlled gene expression and light-controlled enzyme activity, enable us to systematically dissect or direct selected biological processes, and thereby gain a deeper understanding of the cellular inner workings. Moreover, they hold a potential for further development into biomedical and other applications.

To provide a means for thoroughly keeping track of the diverse and fast growing pool of optogenetic tools and applications on the one hand, and a comprehensive source of essential information for the researchers who are new to the field on the other, we have established OptoBase, an online optogenetics

platform. The core of OptoBase is a curated database of hand-tagged (categorized) optogenetic publications, which allows one to search for papers involving a specific trait, such as a particular optogenetic switch or a host organism. In addition, there are an exhaustive portfolio of available optogenetic switches, as well as a collection of convenient web tools, for example, an illumination units converter, and a tool to find the most suitable switch candidates for one's upcoming optogenetics project.

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### *Extending a de novo designed TIM-barrel*

Presenting author:

**Sina Kordes**

Author(s):

Sina Kordes, Birte Höcker

The de novo design of functional enzymes is a challenging task and few successful approaches have been reported. Recently, in collaboration with the Baker and Fernandez-Velasco groups we designed a TIM-barrel from scratch and verified the designed fold by X-ray crystallography. This new inert protein with its regular structure and minimalist loops provides a robust platform for engineering of new catalytic activities. In natural occurring TIM-barrels, the functionality is located in the loop regions at the top of the barrel, whereby the bottom regions contribute mainly to the stability. To extend the scaffold and generate binding pockets for further enzyme design we are inserting structured elements into the top loops to increase the surface of the protein. In a first approach, a short antiparallel coiled coil of 20 amino acids was designed using the Rosetta program and introduced into the de novo TIM-barrel. In a second approach, we designed an antiparallel helical bundle on top of the TIM-barrel through the introduction of two coiled coil elements into opposing loops, whose folding will be driven by interactions with the TIM-barrel as well as with each other. For this purpose, a backbone for the helical bundle was calculated using CCCP and an optimal sequence was designed using Rosetta. Using Remodel this is inserted into the TIM-barrel. First constructs are currently being tested in the lab. Here we describe the computational modelling approach.

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### *Activation of translation elongation factor EF-P of Escherichia coli by the non-cognate rhamnosyltransferase EarP*

Presenting author:

**Ralph Krafczyk**

Author(s):

Ralph Krafczyk, Wolfram Volkwein, Pravin Kumar Ankush Jagtap, Zhenghuan Guo, Jakub Macošek, Kirsten Jung, Janosch Hennig, Jürgen Lassak

Translation elongation factor P (EF-P) alleviates ribosomal arrest at polyproline motifs. For effective ribosome rescue, *E. coli* as well as 25 % of sequenced bacteria modify the invariant lysine 34 of EF-P (EF-PLys) by  $\beta$ -lysinylation via the lysyl-EF-P-synthetase EpmA. Conversely, a phylogenetically distinct group of EF-Ps (EF-PRha) is rhamnosylated at a conserved arginine 32 by the recently identified EF-P arginine rhamnosyltransferase EarP. Using the bacterial adenylate cyclase-based two hybrid system in a luminescence based reporter strain we found that EarP of *Pseudomonas putida* interacts with EF-PLys from *E. coli*, suggesting putative cross-reactivity. When we replaced lysine 34 of EF-PLys with arginine, we observed rhamnosylation of the resulting variant by EarP. An additional substitution of proline 32 to serine was sufficient to enable functionality of this EF-PLys variant after rhamnosylation at arginine 34. These results demonstrate that the protein target of the glycosyltransferase EarP can be altered and is not strictly sequence dependent. Apart from this, our structural and biochemical analysis of EarP suggests that the glycosyltransferase exhibits natural substrate promiscuity towards the nucleotide sugar donor, possibly accepting not only TDP- $\beta$ -L-rhamnose but also alternative donors. Taken together, these findings lay the basis for engineering EarP into a glycosynthase for targeted glycosylation of various proteins.

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### *A pipeline for biosynthetic design of antibiotic peptides*

Presenting author:

**Hajo Kries**

Author(s):

Farzaneh Pourmasoumi, Lisa Mahler, Martin Roth, Hajo Kries

Nonribosomal peptide synthetases (NRPSs) that protect microorganisms against environmental threats by producing siderophores or antibiotics, for instance, have a modular molecular structure inviting biosynthetic engineering. We have explored several strategies for the redesign of NRPS specificity. Notable examples are the incorporation of a clickable amino acid through targeted binding pocket mutagenesis [1] or specificity transfer through swapping of small protein fragments [2]. Incorporation of clickable amino acids has further enabled a strategy for high-throughput sorting of mutagenized NRPSs leading to a remarkable switch in substrate specificity from alpha- to beta-phenylalanine [3]. Here, we propose an approach towards directed evolution of NRPSs based on antibiotic activity of the peptide products. Bacteria producing antibiotic peptides are encapsulated in microdroplets together with sensitive reporter strains allowing for fluorescence activated sorting. Screening for non-ribosomal peptides with antibiotic activity in a directed evolution setup could potentially yield new weapons for the fight against increasing antibiotic resistance.

[1] Kries, H.; Wachtel, R.; Pabst, A.; Wanner, B.; Niquille, D.; Hilvert, D. *Angew. Chem. Int. Ed. Engl.* 2014, 53 (38), 10105.

[2] Kries, H.; Niquille, D. L.; Hilvert, D. *Chem. Biol.* 2015, 22 (5), 640.

[3] Niquille, D. L.; Hansen, D. A.; Mori, T.; Fercher, D.; Kries, H.; Hilvert, D. *Nat. Chem.* 2017, AOP.

### *Engineered production of short chain acyl-coenzyme A esters in *S. cerevisiae**

Presenting author:

**Nicolas Krink**

Author(s):

Nicolas Krink

Nicolas Krink-Koutsoubelis, Anne C. Loechner, Anna Lechner, Hannes Link, Tobias J. Erb, Victor Sourjik and Jay D. Keasling

Short chain acyl-coenzyme A esters serve as intermediate compounds in fatty acid biosynthesis, and the production of polyketides, biopolymers and other value-added chemicals. *S. cerevisiae* is a model organism that has been utilized for the biosynthesis of such biologically and economically valuable compounds. However, its limited repertoire of short chain acyl-CoAs effectively prevents its application as a production host for a plethora of natural products. Therefore, we introduced biosynthetic metabolic pathways to five different acyl-CoA esters into *S. cerevisiae*. Our engineered strains provide the following acyl-CoAs: propionyl-CoA, methylmalonyl-CoA, n-butyryl-CoA, isovaleryl-CoA and n-hexanoyl-CoA. We established a yeast-specific metabolite extraction protocol to determine the intracellular acyl-CoA concentrations in the engineered strains. Propionyl-CoA was produced at 4-9  $\mu\text{M}$ ; methylmalonyl-CoA at 0.5  $\mu\text{M}$ ; and isovaleryl-CoA, n-butyryl-CoA and n-hexanoyl-CoA at 6  $\mu\text{M}$  each. The acyl-CoAs produced in this study are common building blocks of secondary metabolites and will enable the engineered production of a variety of natural products in *S. cerevisiae*. By providing this toolbox of acyl-CoA producing strains, we have laid the foundation to explore *S. cerevisiae* as a heterologous production host for novel secondary metabolites.

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### *A genetically encoded indicator of methionine oxidation*

Presenting author:

**Nikita Kuldyushev**

Author(s):

Nikita Kuldyushev, Roland Schönherr, Stefan Heinemann

Post-translation modifications are essential in the fine-tuning of protein signaling networks. Methionine oxidation, i. e. the formation of methionine sulfoxide, occurs non-enzymatically under oxidizing conditions but also can be catalyzed by recently discovered enzymes. A set of methionine sulfoxide reductases that catalyze the reduction of methionine sulfoxide back to methionine are present in virtually all organisms suggesting that homeostasis of the MetO content in cells is tightly regulated and biologically relevant. Unfortunately, there is lack of convenient instruments to monitor the degree methionine oxidation in living cells and whole organisms.

Here we introduce a genetically encoded fluorescent sensor for methionine oxidation. Variants based on green fluorescent protein (GFP) were designed to provide a ratiometric fluorescence signal reporting on the degree of methionine oxidation. Such fluorescent proteins were produced in a bacterial

expression system and their properties were studied under in vitro conditions in a cuvette. Experimental oxidation with H<sub>2</sub>O<sub>2</sub>, chloramine T, or hypochloric acid resulted in robust changes in the fluorescent ratio F400/F470. Upon expression in mammalian cells (HEK293T), the sensor produced ratiometric fluorescence signals suited for single-cell photometry and life-cell imaging to monitor intracellular methionine oxidation events. This study provides a novel tool for the assessment of long-lasting oxidative protein modification.

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*Synthetic Biology: Lysine-acylation in cellular regulation, ageing and disease.*

Presenting author:

**Michael Lammers**

Author(s):

Michael Lammers, Robert Vogt, Magdalena Kremer, Chuan Qin

Today, more than 15,000 lysine-ac(et)ylation sites have been identified in proteins covering all essential cellular functions in organisms from bacteria to man. Dietary restriction extends lifespan in all model organisms. However, the molecular mechanisms underlying this phenomenon are poorly understood. Due to the tight connection to the cellular energy state and metabolism, post-translational lysine-ac(et)ylation might likely play an important role in the organisms' ageing process. For sirtuins (Sir; silent information regulator), NAD<sup>+</sup>-dependent deacetylases, a protective role against the development of cardiovascular and neurodegenerative diseases as well as cancer has been shown. Despite the huge gain-of-knowledge by mass spectrometry-based approaches, the functional and structural consequences of lysine-acetylation on protein function are only poorly investigated so far. Indeed, for less than 1% of all identified acetylation sites, a thorough functional and structural characterisation has been performed. The genetic-code expansion concept (GCEC) enables to incorporate acetyl-L-lysine co-translationally into proteins using a synthetically evolved, orthogonal acetyl-lysyl-tRNA-synthetase/tRNACUA-pair from *Methanosarcina barkeri*. We use a combined synthetic biological, biophysical and biochemical approach to identify lysine-ac(et)ylation sites with physiological relevance and to judge the consequences of a dysregulation at this site.

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*Birth of a photosynthetic chassis: a MoClo toolbox for synthetic biology in the microalga *Chlamydomonas reinhardtii**

Presenting author:

**Stephane Lemaire**

Author(s):

Pierre Crozet, Francisco Navarro, Felix Willmund, Payam Mehrshahi, Michael Schroda, Alison Smith, Stephane Lemaire

Microalgae have a huge biotechnological potential. These phototrophic microbes are regarded as promising organisms for the development of innovative concepts based on their inherent ability to fix

CO<sub>2</sub>, through a sunlight-driven and sustainable process. The carbon fixed by microalgae can be directed toward production of a wealth of carbon-based biofuels and chemicals. To use microalgae as biotechnological production shuttles and green cell factories, a major challenge is to develop standardized synthetic biology tools to make microalgae easier to engineer. We have developed a Modular Cloning (MoClo) toolkit for the green microalga *Chlamydomonas reinhardtii*. It is based on Golden Gate technology with standard syntax, and is composed of 130 genetic parts, most of which have been functionally validated in diverse strains of *C. reinhardtii*. The kit enables rapid building of engineered cells for both fundamental research and green biotechnology. This work will make *Chlamydomonas* the next chassis for sustainable synthetic biology.

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### *Metabolic engineering for heterologous ethanol production by Pseudomonas putida*

Presenting author:

**Florian Lenz**

Author(s):

Florian Lenz, Svenja Siemer, Iason E. P. Tozakidis, Ruth M. Maas, Joachim Jose

The most cost efficient approaches for the conversion of lignocellulosic biomass to ethanol or other fuels are consolidated bioprocesses, in which the saccharification and fermentation are integrated into one single process. This requires a host organism with good resistance to harsh conditions and metabolic stress. The soil bacterium *Pseudomonas putida* is well suited to meet these requirements. Previously, the bacterium was used as host for surface displayed cellulases to produce glucose from cellulose. The aim of this work was to extend the applicability of the biocatalyst for consolidated bioprocesses by enabling it to produce ethanol. To this end, pyruvate decarboxylase (pdc) and alcohol dehydrogenase B (adhB) from *Zymobacter palmae* and *Zymomonas mobilis* were recombinantly expressed in *P. putida*. Expression of the genes from *Z. palmae* offered a higher ethanol yield (15 mM) than those of *Z. mobilis* (11 mM). These genes were therefore integrated into the chromosome via homologous recombination by simultaneously knocking out lactate dehydrogenase gene (ldhA). Pdc and adhB were set under control of the native promoter of ldhA. A change of the anaerobic metabolite lactate to ethanol was not detectable due to the lack of ethanol in cultivation experiments.

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### *Production of Glycosylated Natural Compounds: Advantages of Nucleotide-sugar Regeneration*

Presenting author:

**Alexander Lepak**

Author(s):

Alexander Lepak, Bernd Nidetzky, Alexander Gutmann

The function of many natural products, such as antibiotics, oligosaccharides, polyphenols and proteins is determined by their glycosylation pattern. Nucleotide diphosphate (NDP) dependent glycosyltransferases (GT) offer selective synthesis as they transfer a sugar moiety from an activated

sugar-nucleotide towards a nucleophilic acceptor molecule. However, their industrial application is limited as the activated nucleotide sugar is the most expensive compound in many GT catalyzed reactions.

To provide the nucleotide sugar we implemented an UDP-glucose in situ regeneration cycle by using sucrose synthase. In this way it is possible to perform the glycosylation in a one-pot reaction by only using the substrate, sucrose and catalytic amounts of UDP. As we show for two test cases this generalized recycling strategy can be used to prepare polyphenol-glucosides in large scale as it can be coupled to a wide range of GTs.[<sup>1</sup>]

Resveratrol 3,5- $\beta$ -d-O-diglucoside showed a 4000-fold increased solubility of about 170 g L<sup>-1</sup> compared to the non-glucosylated molecule while maintaining 90% of its antioxidative power.[<sup>2</sup>]

Further we produced nothofagin which has a C-glycosidic bond instead of the O-glycosidic bond found in most of naturally occurring glycosylation products to enhance the stability of the glucoside against hydrolysis.[<sup>3</sup>]

[1] Gutmann et al., Biotechnol J. 2017, Jul;12(7)

[2] Lepak et al.; ChemBioChem 2015, 16, 1870-1874

[3] Bungaruang et al.; Adv Synth Catal. 2013, 355, 2757-2763

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### *Engineering principles of a targeted intracellular delivery system for protein cargo*

Presenting author:

**Bruce Lichtenstein**

Author(s):

Bruce Lichtenstein, Birte Höcker

Applications of externally introduced proteins in both therapeutics and synthetic biology have been limited by lack of readily accessible protein targeting and delivery carriers. Common approaches like antibodies or receptor ligands only get the protein cargo as far as the cell surface, precluding applications which function in the cytoplasm. These established motifs stand in contrast to the known bacterial AB<sub>5</sub> toxins, which bear structurally and functionally diverse cargo into eukaryotic cells. The carrier B subunits of these proteins are solely responsible for both targeting diverse glycosylated structures on cell surfaces and delivering their cargo to internal cell environments, and are an attractive starting point for creating a platform of protein delivery tools. Using heat labile enterotoxin I (LTI) as a model system for this family of proteins, we identify the sequence and structural parameters necessary to effect co-assembly of the carrier B subunits with non-native protein cargo. As a result, cargo-carrier complexes derived from optimized sequences based upon these principles reveal improved assembly and kinetic stability over the 'wildtype' complexes. These variations in in vitro stability of the complexes in turn correlates with the efficacy of cellular internalization of a fluorescent protein cargo. This improved tool is immediately useful for the delivery of protein cargoes, and on-going efforts seek to extend its scope both in function and cell targeting.

### *Genome-Scale Model Reconstruction of *Ogataea polymorpha**

Presenting author:

**Ulf Liebal**

Author(s):

Ulf Liebal, Constantin Schedel, Aarthi Ravikrishnan, Lisanne Jente, Jörg Mampel, Birgitta Ebert, Lars Blank

*Ogataea polymorpha* is a thermotolerant, methylotrophic yeast with significant industrial applications. It is a promising host to generate platform chemicals from methanol, derived e.g. from carbon capture and utilization streams. Full development of the organism into a production strain requires additional strain design, supported by metabolic modeling on the basis of a genome-scale metabolic model (GSMM). However, to date, no GSMM is available for *O. polymorpha*. To overcome this limitation, we used a published reconstruction of the closely related yeast *Pichia pastoris* as reference and corrected reactions based on KEGG annotations. Additionally, we conducted phenotype microarray experiments to test the suitability of 192 substrates as carbon sources. Over three-quarter of substrate usage was correctly reproduced by the model. However, *O. polymorpha* failed to metabolize eight substrates and gained 38 new substrates compared to the *P. pastoris* reference model. To facilitate strain engineering and identify beneficial mutants, gene-protein-reaction relationships have to be included in the model. This is challenging because the currently available reference genome contains many repeated protein sequences each labelled by different identifiers. The final GSMM of *O. polymorpha* will support the engineering of synthetic metabolic capabilities and enabling the optimization of production processes, thereby supporting a sustainable future methanol economy.

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### *Biological Design Principles: Learning by Building*

Presenting author:

**Wendell Lim**

Author(s):

Wendell Lim

Traditionally, biology has focused on deconstructing and mapping the molecular systems that carry out complex regulatory functions. We still lack, however, a more global understanding of the design principles governing how cells solve problems and make regulatory decisions. To address this problem, we have been complementing deconstructionist approaches with synthetic approaches in which we ask how to build molecular systems that can execute particular regulatory tasks. Are there a limited number of molecular algorithms that evolution can use to solve common physiological tasks? If so, can we learn to recognize them in order to understand the function of complex cellular networks? By systematically rewiring cellular networks, we can test our understanding of cellular logic, as well as engineer cells that execute novel functions. Recent advances have demonstrated that these approaches have the potential to transform medicine by allowing us to engineer smart therapeutic immune cells that can recognize and treat cancer and other diseases with unprecedented precision.

<http://limlab.ucsf.edu/>

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### *Engineering yeast for the production of acylphloroglucinol derivatives*

Presenting author:

**Benye Liu**

Author(s):

Benye Liu, Marco Grull, Lars Fabian Kalz, Till Beuerle, Ludger Beerhues

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are complex natural products possessing an extraordinarily wide range of pharmaceutical activities, e.g. antidepressant, antibacterial, antiviral and antitumor properties. They are mainly found in species of the plant families Hypericaceae and Clusiaceae in low abundance. The complex structure of bicyclo[3.3.1]nonane-2,4,9-trione makes their total chemical synthesis difficult and in general economically impractical. An alternative approach for producing these valuable compounds is to reconstruct their biosynthetic pathways in engineered microbes. The biosynthesis of PPAPs shares the polyketide pathway for the acylphloroglucinol cores and the terpenoid pathway for the C<sub>5</sub> and C<sub>10</sub> side chains. In the present work, enzymes from *Hypericum perforatum* and *Rhodospseudomonas palustris* were expressed in yeast either episomally or by integration into the genome to achieve the biosynthesis of the polyketide cores, 2,4,6-trihydroxybenzophenone and 2,4,6-trihydroxyisobutyrophenone. The production of the two expected products was identified by HPLC and LC-MS in comparison with the authentic reference compounds. When the yeast acetyl-CoA carboxylase 1 (ACC1) double mutant ACC1<sup>ser659ala,ser1157ala</sup> was also co-expressed, the production of the two products increased. This indicates more malonyl-CoAs are available as the ACC1 double mutant bypass the posttranslational downregulation of ACC1 activity by the global regulator Snf1.

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### *Functional protein design from evolutionarily conserved fragments*

Presenting author:

**Francisco Lobos**

Author(s):

Francisco Lobos, Steffen Schmidt, Birte Höcker

Domains are considered structural, functional and evolutionary units of proteins. It is thought that a set of ancestral, sub-domain sized, conserved fragments served as a basis for their emergence and evolution, resulting in the wide range of protein folds and functions present today. Moreover, recombination of such fragments may result in the generation of proteins with novel functions, offering an innovative approach for rational protein design.

To test this hypothesis we have developed a database of evolutionary relationships between protein folds that reports both sequence and structural conservation. A comprehensive analysis of this database

was conducted to find fragments that could be readily combined into proteins with potential new functions.

As a proof of concept, an in silico model of a chimeric protein, TyrA/Fld, was built. It comprises an ATP-binding fragment derived from a Rossmann fold domain recombined with one from an FMN-binding flavodoxin domain. The relative orientation of both ligands in the model suggests that TyrA/Fld could catalyze the formation of FAD. TyrA/Fld is expressed mostly in insoluble form but can be refolded from inclusion bodies. However, its subsequent in vitro characterization deviated from the expected results. Further analysis in the theoretical model showed issues at the interface of both domain fragments. Based on that insight, steps are currently being taken to improve the solubility of the chimera.

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### *Robust Population Control in Synthetic Communities*

Presenting author:

**Anne Loechner**

Author(s):

Anne Loechner

Anne Löchner, Nicolas Krink, Bastian Pook, Victor Sourjik

Using microorganisms for bioremediation and metabolic engineering becomes more and more popular in times of declining amounts of petroleum, increased pollution and awareness of protection of the environment. Even though many organisms could be successfully engineered, a high metabolic burden and a lack of evolutionary stability remain. Therefore the usage of synthetic engineered microbial communities is under investigation for different applications. One problem that remains is the control of population growth. *Escherichia coli* is a model organism for synthetic biology applications and therefore also for bioremediation and metabolic engineering purposes. In order to balance the growth in a mixed synthetic *E. coli* community, we propose a novel quorum stabilizer system. This quorum sensing-based system allows the balance as well as cell density ratio tuning to adapt the community to every specific application. Additionally we explore the implementation of a novel growth inhibition system to stably tune the cell densities in synthetic microbial communities for various applications.

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### *Establishment of optogenetic tools for the control of gene expression and recombination in *Saccharomyces cerevisiae**

Presenting author:

**Fabian Machens**

Author(s):

Lena Hochrein, Fabian Machens, Leslie A. Mitchell, Katrin Messerschmidt, Bernd Mueller-Roeber

Synthetic Biology often calls for specific inducers to control biological processes. As light is an easy to control, cheap and non-toxic alternative to chemical inducers, we developed two red light-regulated tools for yeast: PhiReX and L-SCRaMbLE.

PhiReX allows the programmable and orthogonal control of gene expression based on a light-responsive split transcription factor. The DNA-binding domain is based on TALE proteins, which can be engineered to target any desired DNA sequence. The available PhiReX-systems allow either high level gene expression with moderate background activity, or tight regulation with medium expression levels. To enhance the applicability of PhiReX, we engineered yeast to endogenously synthesize the essential chromophore, which is photolabile and expensive to purchase.

L-SCRaMbLE mediates tight regulation of Cre recombinase, by fusing a split version of the protein to the optical dimerizer, allowing reconstitution of the functional enzyme only upon red light application. L-SCRaMbLE achieved recombination efficiencies of up to 90%, tunable by chromophore concentration and induction time, which can be completely shut down by withdrawal of the chromophore, irrespective of the light condition. Furthermore, L-SCRaMbLE produces a large diversity of recombination events. When applied to Sc2.0 synthetic yeast strains, which will harbor thousands of loxPsym sites across all synthetic chromosomes, L-SCRaMbLE will serve as an excellent tool to control Cre activity.

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### *A universal CRISPR/Cas9 toolkit for multiplexed genome editing and transcriptional reprogramming in *Saccharomyces cerevisiae**

Presenting author:

**Fabian Machens**

Author(s):

Fabian Machens, Bernd Mueller-Roeber

CRISPR/Cas9 is widely used for genome engineering in many different host organisms, including *S. cerevisiae*. The Cas9 nuclease is guided to its target by a short sgRNA. The resulting double strand break can subsequently be repaired by non-homologous end joining or by a template dependent homology directed repair mechanism. In this way, random indels or precise modifications can be achieved. In addition to genome engineering, a nuclease-dead Cas9 (dCas9), fused to a transcriptional activation or repression domain, can be used to modulate gene expression by targeting a promoter.

However, targeting more than three loci for genome editing or transcriptional regulation is difficult, as cloning and expression of many sgRNAs poses several challenges: First, for optimized editing or regulation efficiencies all sgRNAs should be expressed to a similar level. Second, for gene regulation purposes all sgRNAs must be stably maintained, which is challenging due to high recombination frequencies in yeast. Finally, for optimized modulation of gene expression, a screening strategy is needed to find the best combination of sgRNA targets within a promoter.

Here, we set out to develop a comprehensive toolkit allowing simple sgRNA cloning for multiplexed genome editing or stable transcriptional reprogramming with up to eight sgRNAs. We present data on the stability of our sgRNA arrays, efficiency of genome editing, and transcriptional reprogramming using various activation or repression domains.

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### *Stapled Peptides Targeting Protein Kinase A Provide Efficient Inhibition and Cellular Accessibility*

Presenting author:

**Jascha T. Manschwetus**

Author(s):

Jascha T. Manschwetus, N. George Bendzunas, Eileen J. Kennedy, Friedrich W. Herberg

Protein kinases act as dynamic modulators of cellular signaling in eukaryotes. Malfunction of these key enzymes can provoke severe diseases such as cancer or diabetes. Therefore, protein kinases need to be tightly regulated. Numerous synthetic kinase inhibitors are already FDA approved and in clinical use. However, since these compounds target highly conserved ATP-binding pockets, specificity remains a major problem. To overcome these limitations, we designed peptidic inhibitors based on known interaction partners of the cAMP-dependent protein kinase.

Specific protein-protein interactions often rely on  $\alpha$ -helical structures, providing a unique platform for the development of novel inhibitor compounds. Substituting two residues of the  $\alpha$ -helix by non-natural olefinic amino acids allows for introduction of a hydrocarbon staple. Peptides of interest are thus locked in an  $\alpha$ -helical conformation altering both their biochemical and biological properties. Furthermore, proteolytic stability as well as cellular accessibility can be improved while retaining high specificity.

We characterized a set of rationally designed stapled peptides. All compounds were screened for affinity before determining kinetic rate constants and inhibitory potencies of the lead compounds. Finally, cellular uptake of these candidates was monitored using fluorescently labeled variants.

Although further validation is required, these inhibitors are well suited for application in basic research.

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### *Conditional control of fluorescent fusion protein degradation by an auxin-dependent nanobody*

Presenting author:

**Jörg Mansfeld**

Author(s):

Katrin Daniel, Jaroslav Icha, Caren Norden, Jörg Mansfeld

The rapid and reversible degradation of proteins fused to an auxin-inducible degron (AID) is a powerful tool in cell biology. A caveat is the need for genetic engineering, as the AID needs to be inserted into both alleles of each targeted protein. Further, fusion to AID can destabilize the tagged protein. Thus, except for a limited number of cases large-scale application of the AID system in animal cells remains challenging.

In contrast, endogenous proteins tagged with GFP are available in human cells and GFP trap stock collections exist in model systems such as zebrafish and fruit fly. Such GFP-tagged proteins can be efficiently recognized by nanobodies (deGradFP) and targeted for degradation. Compared to the AID system, nanobody-mediated degradation suffers from two disadvantages. First, the onset of degradation cannot be rapidly induced and second, degradation is not reversible as long as the nanobody is present.

Here, we combine the best of auxin and nanobody-based technologies and present a customized nanobody (mAID-vhhGFP4) to efficiently target GFP-tagged proteins for degradation in an inducible and conditional manner. Using a panel of ectopic and endogenous GFP-tagged proteins, we show reversible degradation by mAID-vhhGFP4 at different subcellular localizations and for subunits of multi protein complexes in human cells. Further, we establish mAID-vhhGFP4-dependent degradation in zebrafish showing that the AID system can be applied to this vertebrate model organism.

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### *ECFs as orthogonal regulators in *Sinorhizobium meliloti**

Presenting author:

**Doreen Meier**

Author(s):

Doreen Meier, Désirée Körner, Yannick End, Javier Serrania, Stefano Vecchione, Georg Fritz, Anke Becker

The construction of complex synthetic gene circuits in prokaryotes has been hampered by the availability of regulatory parts that can be used in parallel without interference. In frame of a comprehensive pilot study, it was shown that heterologous ECF  $\sigma$ -factors specifically activate their cognate promoters in the  $\gamma$ -proteobacterium *Escherichia coli* (1). Computational phylogenetic analysis identified 43 major ECF groups (2). Thus, they provide an ideal source of orthogonal regulators.

We showed that heterologous ECF/promoter pairs are functional in the  $\alpha$ -proteobacterium *Sinorhizobium meliloti* and characterized a core set of regulatory switches. Varying the copy number of the *ecf* gene and the target promoter affected the overall activity and the dynamic range of the regulatory switches. Changes in the promoter environment slightly improved their activity.

Moreover, we mapped the crosstalk between endogenous and heterologous ECFs of one phylogenetic group in terms of target promoter activation. The analysis revealed a rather high specificity of ECFs for a subset of promoters. Similar observations were made for the respective anti- $\sigma$  which specifically downregulated their cognate ECF.

Our results provide further perspectives for the design of more complex synthetic gene circuits in which ECFs, target promoters and cognate anti- $\sigma$  function as orthogonal regulatory elements.

1. V. Rhodius et al (2013). *Mol Syst Biol* 9: 702

2. A. Staron et al (2009). *Mol Microbiol* 74(3): 557-581

*Construction of a chip-based sensor for glycosylphosphatidylinositol-anchored proteins in complex with phospholipids in extracellular fluids and their potential in differentiating metabolic states*

Presenting author:

**Günter Müller**

Author(s):

Günter Müller, Andreas Herling, Kerstin Stemmer, Matthias Tschöp

The amphiphilic nature glycosylphosphatidylinositol-anchored proteins (GPI-AP) makes them putative candidates for the spontaneous and/or controlled release from the extracellular face of plasma membranes, in particular of those cell types, which are exposed toward mechanical forces and "membrane-active" extrinsic factors. To study this possibility, a chip-based sensor was developed for complete GPI-AP, which may form together with (phospho)lipids so far unknown (non-vesicular) labile extracellular complexes (GLEC). The sensor relies on the induction of phase shifts and amplitude reductions in surface acoustic waves propagating over the chip surface upon specific capturing of the GPI-AP and subsequent interaction of the phospholipid-binding protein annexin-V and renders GLEC isolation unnecessary. GLEC were found to be released from isolated rat adipocyte plasma membranes immobilized on the chip surface, depending on the flow rate and composition of the buffer stream passing along. GLEC were also detected in serum of normal, diabetic and obese rats, enabling the differentiation according to genotype or body weight. Taken together, the ability of the presented SAW chip-based sensing of labile GLEC to delineate correlations with (patho)physiological, in particular metabolic states without being aware of the underlying molecular structures or mechanisms may be regarded as considerable advantage for biomarker research.

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*Directed evolution of lysine deacetylases*

Presenting author:

**Heinz Neumann**

Author(s):

Martin Spinck, Heinz Neumann

Lysine Deacetylases (KDACs) are a prominent class of enzymes featuring roles in almost all physiological processes and many diseases including cancer and aging. These enzymes reverse various types of lysine acylations thereby controlling, e.g., enzyme activities, protein localization and chromatin structure. Here, we report the directed evolution of KDACs towards particular acyl substrates and bioorthogonal lysine modifications using a bacterial selection system. The new enzymes can be used for partial complementation of KDAC deletion strains to reveal the physiological role of particular lysine acylations. Bioorthogonal "eraser" enzymes facilitate the activation of pro-peptides or pro-enzymes by removing protection groups installed on lysine residues. These bioorthogonal "eraser" enzymes may therefore find applications in prodrug strategies of cancer therapy.

### *Comprehensive analysis and cross-species comparison of synthetic promoters*

Presenting author:

**Salome C. Nies**

Author(s):

Salome C. Nies, Dário Neves, Birgitta E. Ebert, Lars M. Blank

Coordinated expression of metabolic pathway enzymes is crucial for efficient bioprocesses, which optimally exploit cellular resources, avoid pathway bottlenecks and the consequent accumulation of intermediates. Fine-tuning of enzyme activities requires controlling gene expression and translation level by using regulatory parts, e.g., promoters and ribosomal binding sites, of defined strength. Synthetic Biologists have developed methods and tools to generate well-characterized libraries of these regulatory parts, but the work done so far mainly focused on *E. coli* and it is unknown how the characterized parts perform in other microorganisms. Here, we created a new library of synthetic promoters and characterized their performance in both *P. taiwanensis* VLB120, a non-model organism with high potential as microbial cell factory, and the industrial workhorse and model organism *E. coli*, using *msfGFP* as a reporter. To avoid differences in promoter strength related to plasmid copy number variation or growth, all promoters were singly genomically integrated and specific GFP expression monitored over time by online biomass and fluorescence measurements. Despite the relatedness of the two organisms and the conservatism of RNA polymerase and  $\sigma^{75}$  promoter interaction, many promoter variants exerted different strengths in the two strains. The characterized synthetic promoters enable fine-tuned gene expression and easy pathway exchange.

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### *Harnessing the chemistry of plant metabolism for synthetic biology*

Presenting author:

**Sarah O'Connor**

Author(s):

Sarah O'Connor

Plants, which make thousands of complex natural products, are outstanding chemists. Through the concerted action of enzymes that are assembled into metabolic pathways, nature creates chemical complexity from simple starting materials. I will highlight some of the unusual enzymatic transformations that plants use to make complex, bioactive natural products, and will also discuss methods by which these pathways can be harnessed for metabolic engineering. The focus is on the biosynthesis of the alkaloids derived from iridoids, known as the monoterpene indole alkaloids. The discovery, functional characterization and mechanistic study of enzymes involved in the biosynthesis of these important compounds in several medicinal plant species will be discussed.

*PRSeq (Promoter RNA Sequencing): Massive and quantitative method for promoter analysis in vitro*

Presenting author:  
**Shoji Ohuchi**

Author(s):  
Shoji Ohuchi

Comprehensive analysis of promoter variants is important for engineering promoters with desired strength and specificity. Here, we report an in vitro promoter analysis method that can achieve both massiveness and quantitiveness. In the method, a template DNA pool carrying a partially randomized promoter and a copy of the respective promoter on its transcribed region is constructed via enzymatic reactions. After in vitro transcription of the DNA pool with an RNA polymerase of interest, the sequences of the resulting transcripts will be analyzed. Since the promoter strength linearly correlates to the copy number of transcript, the strength of each promoter sequence can be evaluated. A model experiment of T7 promoter variants demonstrated the quantitiveness of the method, and the method was applied for the analysis of the promoter for cyanophage Syn5 RNA polymerase. This method would be valuable for the extensive analyses of promoters including those for growing numbers of extracellular function (ECF) sigma factors.

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*Towards easily designable biochemical systems*

Presenting author:  
**Sven Panke**

Author(s):  
Sven Panke

Abstract not submitted

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*Exploring the toolbox of synthetic biology for HIV vaccine design*

Presenting author:  
**David Peterhoff**

Author(s):  
David Peterhoff, Alexandra Hauser, Patrick Neckermann, Winfried Weissenhorn, Rogier Sanders, Ralf Wagner

Vaccination can protect against disease caused by infectious pathogens. Modern vaccine design involves interdisciplinary research of biochemistry, materials science, immunology and experimental medicine. It explores (i) identification and generation of immunogens, (ii) efficient production systems, (iii) optimal delivery systems and (iv) models for qualification in terms of effectiveness, potency and safety.

Investigating a human immunodeficiency virus (HIV) vaccine, we focus on providing stabilized variants of HIV's trimeric envelope protein (Env) which represents the sole viral antigen presented to the human immune system during early infection. Antibodies against Env can protect against infection by virus-neutralization. To provide stable Env immunogens we use structural signatures of stability to identify intrinsically stable HIV-1 clade C isolates and subjoin additional stabilizing features to produce hyperstable Env variants with optimal antigenic properties and expression yields. After pre-screening using mammalian cell display, down-selected variants were characterized biophysically and biochemically as soluble trimers. The resulting variants are substructures of engineering on optimizing the engagement of the B-cell receptor of broad neutralizing antibody precursors. To test immunogenic properties and safety, the immunogens are tested in rabbits. Utilizing the novel vaccine candidates, bioconjugation techniques to provide multivalent delivery formats are explored.

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### *Adsorption of modified proteins from human plasma – potential effects for inflammation and senescence*

Presenting author:

**Veronika Piskovatska**

Author(s):

Veronika Piskovatska, Alexander Navarrete Santos, Andreas Simm

Advanced glycation end products (AGEs) play important role in senescence and age-related diseases by impairing protein structure and functions. These molecules can interact with receptors to AGEs (RAGE) and activate numerous pro-inflammatory and pro-fibrogenic signaling pathways.

The aim of the work is to remove AGE-modified proteins from human plasma, using polymer adsorbent system, and to identify cellular responses towards the retained proteins.

For this aim, a column material from a medical device, used for continuous hemofiltration in sepsis patient, was eluted with denaturing buffer. Amount of eluted protein was quantified by slot-blot, along with albumin standards to obtain standard curve. Posttranslational protein modifications were identified via immunoblotting.

Carboxymethyllysine, argpyrimidine, methylglyoxal-derived hydroimidazolone-1, pentosidine, and carbonyls were identified in eluted plasma proteins. Human umbilical vein endothelial cells (HUVEC) were stimulated with 10, 50, 100, 500 and 1000 ng of eluted proteins. 24 hours of stimulation with 10 ng elution resulted in increased expression of RAGE, compared to non-stimulated cells.

Adsorbed modified proteins induce RAGE expression in HUVECs and activate downstream targets of the RAGE-signaling. Further identification of cytotoxic effects and influence of the isolated modified proteins on cell cycle is required to better understand the physiological effect of repeated hemofiltration procedures in patients.

### *ROC'n'Ribo: Characterizing a riboswitching expression system by modeling single-cell data*

Presenting author:

**Tim Prangemeier**

Author(s):

Leo Bronstein, Christopher Schneider, Jascha Diemer, Heinz Koepl, Beatrix Sues

The design and implementation of riboswitches presents a unique opportunity to manipulate any reporter device in cis, executing tight temporal and spatial control by low metabolic costs. Here, we use a hierarchical stochastic modeling approach to infer and predict the output profiles of a repressor gate constructed by the neomycin and tetracycline riboswitches. A moment approximation together with full Bayesian inference through parallel tempering techniques is employed. The experimentally observed correlation between GFP and transcription factor (TF) abundance can be explained by cell size, which is accounted for by a heterogeneous variable in the stochastic model. The effect of noise on gate performance was taken into account by employing receiver-operator-characteristics (ROC) analysis, deriving a novel performance indicator for logic gates. The prominent features involved for the validation are speed (time to reach half maximum of the effect) and accuracy (separation of responders and non-responders). Our experimental findings suggest different time scales for RNA binding of neomycin and tetracycline. The hierarchical model uncovers the binding dynamics of ligand and mRNA, allowing us to better dissect the temporal sequence of events. The model predicts that the cell-to-cell variability in the gate response cannot be reduced by reducing the variability in the involved TF abundance. We validate this prediction by integrating the transcription factor into the host genome.

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### *Inducible Transcription dynamic for single cell studies on cellular heterogeneity*

Presenting author:

**Tim Prangemeier**

Author(s):

Jascha Diemer, Leo Bronstein, Heinz Koepl

In the past decade, tools and techniques were developed to follow transcription in time and space. Only microscopy provides temporal and spatial information at once and can link other optical features to the transcription dynamic of a single cell. We focus in this study on image based techniques to elaborate transcription in real time and expands the existing methods to estimate the biophysics behind transcription by a mathematical model. Our genetic construct consists of 14 repeats of the PP7 stem loop upstream of the gal10 gene and coexpression of an inducible transcription factor. Cells are incubated and observed in a microfluidic chip designed for single cell studies. Upon induction with beta-estradiol, transcription is initialized and transcription sites become visible through the accumulation of PP7-GFP fusion protein onto the synthesized mRNA. Image analysis of the 4D data give insights in the dynamics of transcription. We used a rigorous Bayesian approach to jointly analyze data from multiple cells in order to calibrate and compare different models for transcription elongation. Initiation, elongation and termination rates and the number of polymerases were estimated. We investigated the evidence for stalling of polymerases due to the simultaneous recruitment of several RNAPs. Our inference framework

is easily extended to related situations and thus demonstrates a general procedure for analyzing time-lapse microscopy data in a principled way.

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### *Engineering RNA-based logic gates from Cell-Free Transcription-Translation (TX-TL) to living cells*

Presenting author:  
**Tim Prangemeier**

Author(s):  
Francois-Xavier Lehr, Marc Vogel, Maleen Hanst, Ulrich Göringer, Beatrix Süß, Heinz Koepl

RNA regulators have recently gained an increasing attention due to the emergence of new powerful RNA design principles. Similar protein-like dynamic ranges and fast signal propagation make them an appealing alternative building or rewiring tool in the world of the synthetic genetic circuits. While having an increasing number of isolated RNA devices reports, a bottleneck remains when it comes to prototype and characterize RNA circuits composed of several transcriptional parts. Here, we build a library of logic gates based on AND computation out of two recently published RNA regulators: The Small Transcriptional Activators RNAs (STARs) and the riboregulators RNAs (Toehold switches). We use Escherichia Coli cell-free transcription-translation (TX-TL) reactions and ordinary differential equations based modeling to design and prototype up a dozen combinations of RNA-based AND gates. We show that our model captures accurately the diverse fold ranges of our different gates taking into account simple crosstalk and compositionality parameters. Finally, we successfully transfer and test those gates in vivo and make conclusions about the transportability of novel RNA logic circuits from a TX-TL environment to living cells.

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### *Design of optimal inducer profiles for the inference of transcriptional circuits on the single-cell level*

Presenting author:  
**Tim Prangemeier**

Author(s):  
Tim Prangemeier, Christoph Zechner, Sung Sik Lee, Matthias Peter, Heinz Koepl

Synthetic biology requires quantitatively predictive models for the design of standardized synthetic parts and circuits. Such models are calibrated on experimental data, whereby the kinetic parameters are computationally inferred with a certain degree of confidence. The information gained from experimental datasets is not only limited by the measurement uncertainty, but also by the specific design of the experiment. Designs differ depending on the manner in which data is to be analysed; direct human interpretation requires different designs than statistical analysis. Rich circuit dynamics in response to complex stimuli, while difficult to interpret directly, often contain more information than responses to standard induction profiles. The recent development of synthetic protein translocation reporters, with

time resolution below one minute, enables experimental access to such rich circuit dynamics. Exploiting this, we present an optimal experimental design strategy to maximize the information gain from a sequence of experiments for the inference of circuit parameters and for hypothesis testing. In a case study, we microfluidically generated optimal inducer profiles for a HOG1 inducible circuit in yeast and measured each individual cell's response. Preliminary results indicate increased information gain, quantified in terms of the Kullback-Leibler divergence, on the order of 40%.

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*The second life of Sleeping Beauty: Mechanism and design of a synthetic DNA transposon for genome engineering*

Presenting author:

**Irma Querques**

Author(s):

Irma Querques, Cecilia Zuliani, Franka Voigt, Orsolya Barabas

Transposons are bits of DNA moving within and between genomes across the tree of life. The synthetic Sleeping Beauty (SB) transposon system is extraordinarily efficient at inserting DNA in vertebrate genomes. This property makes SB the only non-viral integrating vector applied in gene therapy clinical trials to genetically modify patient-derived T cells for cancer treatment. Despite these clinical applications, the molecular principles governing SB's activity and performance remain elusive, greatly hampering its further development by rational design.

Here, we dissect SB's working mechanism. Using biochemical, biophysical and structural techniques together with fluorescence cell imaging, we show that the assembly of the SB machinery follows a distinct pathway from other related transposons. We further demonstrate that the unusual assembly mode of SB largely contributes to its exceptional efficiency and that it can be chemically modulated to control insertion rates in living cells. Building on these mechanistic insights and employing a structure-based protein design approach, we then developed a new generation of SB system that allows safer and more controlled genome modification of several cell types (including stem cells and human T cells), as compared to the state-of-art technology.

Our work sheds first light on the molecular principles of the SB system, providing a unique resource for the rational design of SB-based genome engineering tools for research and medicine.

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*Rebuilding of a mammalian signaling pathway*

Presenting author:

**Michael Reth**

Author(s):

Michael Reth, Christa Kalmbach-Zuern, Kathrin Klaesener, Jianying Yang

One of the goals of synthetic biology is the rebuilding of a biological system, to better understand the working mechanism of each system component. We are studying how B lymphocytes sense foreign antigens and are activated via their B cell antigen receptor (BCR) for antibody production. Many BCR signaling components have been studied by loss-of-function approaches. To gain a better insight into the mechanistic aspect of BCR signaling we have chosen a gain-of-function approach, namely the rebuilding of the BCR and its signaling components in the evolutionary distant environment of a *Drosophila* S2 Schneider. We found that Schneider cells can be efficiently co-transfected with more than 12 different inducible expression plasmids. This allowed us to express the BCR on S2 cells and test its interaction with diverse B cell signaling components.

In our rebuilding project we studied how the signaling subunits of the BCR namely the Iga/Igb (CD79a/79b) heterodimer, interact with protein tyrosine kinases such as the Src family kinase Lyn and the spleen tyrosine kinase (Syk). We found that Syk can phosphorylate and bind to the BCR and thus amplify BCR signaling by a positive feedback. Furthermore, we discovered that Syk changes the conformation of the BCR by a novel inside-out signaling mechanism. During my talk I will explain how a rebuilding approach can be combined with live cell imaging and nanoscale proximity techniques to gain profound insight into the BCR signaling process.

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### *BLADE, a new light-inducible bacterial transcription factor*

Presenting author:  
**Edoardo Romano**

Author(s):  
Edoardo Romano, Barbara Di Ventura

Engineering methods to spatiotemporally control gene expression is one of the most important goals of synthetic biology. The field of optogenetics offers the solution to this need, because light is a spatially and temporally definable external trigger.

The aim of this project is the engineering of a fast responding light-inducible gene expression system based on a single protein. We have engineered BLADE (Blue Light-mediated AraC DimErization), a novel light-inducible transcription factor (TF) made of the light-responsive VVD photoreceptor from *N. crassa* fused to the DNA binding domain of the *E. coli* transcriptional regulator AraC. Light-mediated dimerization of VVD leads to AraC dimerization, which in turn allows binding to the pBAD promoter and induction of mCherry transcription.

BLADE performance is assessed by flow cytometry measuring the expression level of the mCherry reporter. Following rational design and random mutagenesis we found a variant for which the mCherry levels were almost 12-fold higher in cells exposed to 4 hours of blue light compared to control cells kept in the dark.

Importantly, the expression levels obtained by the light-inducible TF are almost as high as those obtained by the full-length AraC, suggesting that the engineered protein functions similarly to the wild type.

We are now testing whether BLADE can be used to regulate the expression of genes whose products control important biological processes such as cell division or chromosome segregation.

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### *Droplet-based screening for artificial metalloenzymes based on the streptavidin-biotin technology*

Presenting author:

**Philipp Rottmann**

Author(s):

Philipp Rottmann, Valentin Senlis, Thomas R. Ward, Sven Panke

Even though nature has created an immense diversity of chemical reaction principles, the toolbox of organic chemistry contains many examples of chemical transformations without any biochemical equivalent. Recruiting such reactions into biochemistry opens exciting novel possibilities in biocatalysis and metabolic engineering. Transition metal carbene complexes are widely used for a versatile set of chemical reactions for the enantioselective production of fine and bulk chemicals. They have been shown to lead to functional hybrid catalysts with promising catalytic activity when integrated into protein environments. Integration of such a transition metal catalyst into a streptavidin scaffold by biotinylation, the resulting artificial enzyme can be evolved for improved specific activity. Here, we implement a fully in vitro droplet-based high throughput screening system of artificial metalloenzymes based on the streptavidin-biotin technology by using sophisticated microfluidic tools. Specifically, we demonstrate the separation and amplification of the streptavidin gene in water-in-oil droplets followed by a compartmentalized cell-free protein synthesis of the individual streptavidin variants, assembly of the artificial metalloenzyme and further screening for improved catalytic activity via fluorescence-activated droplet sorting. This cell-free compartmentalized evolution platform is ideal to expand the biochemical parts used in the field of Synthetic Biology.

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### *A platform for real-time optogenetic regulation and visualization of transcription in single cells*

Presenting author:

**Marc Rullan**

Author(s):

Marc Rullan, Dirk Benzinger, Gregor Schmidt, Andreas Miliadis-Argeitis, Mustafa Khammash

Study of gene expression at the single-cell level has radically changed our knowledge of genetics, exposing the importance of stochasticity for the behavior of cellular systems. Research on cellular noise has relied on observing the process either in response to natural stimuli or to constant gene regulators. However, the ability to probe cells individually and in a controlled fashion offers the promise of new insights. By combining a fast-acting, photo-regulatable transcription factor with nascent RNA quantification in live cells and an experimental setup for precise spatiotemporal delivery of light inputs, we have constructed a platform for the real-time, single-cell interrogation of transcription in *Saccharomyces cerevisiae*. Together with an automated software pipeline for the segmentation, quantification and tracking of single cells, the platform enables the independent and simultaneous real-time tracking and control of a large number of cells. The analysis of the temporal activity of individual

cells showed that transcription occurs in bursts, whose duration and timing are modulated by TF activity. Using our platform, we regulated transcription via light-driven feedback-loops at the single cell level. Feedback markedly reduced cell-to-cell variability, improved control-loop properties, and led to qualitative differences in cellular transcriptional dynamics. Our platform establishes a new, flexible method for studying transcriptional dynamics in single cells.

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### *In situ light control of macromolecular protein assembly in time and space*

Presenting author:

**Maria Florencia Sanchez**

Author(s):

Maria Florencia Sanchez, Ralph Wieneke, Robert Tampé

The organization of molecular triggers at the plasma membrane regulates signaling cascades that involve cell-cell contact. In juxtacrine interactions, the cells come into direct contact and receptor-ligand recognition at the membrane interface elicits the intracellular signaling. In particular, receptor clustering is a key process in the final cell response. Hence, traceless modifications with high spatiotemporal control are required for the on-demand oligomerization of receptors.

We aim to rewire living cells by in situ lateral organization of membrane receptors and thus to exert control over signaling networks by light. We use microscale fabrication techniques to replace one cell with an artificial counterpart to understand the impact of higher order receptor organization in cell response.

Light is a versatile tool as it induces photoreactions with high spatial resolution. We have developed photo-activatable lock and key elements that facilitate non-invasive synthetic protein networks in 2D and 3D. Here, we efficiently immobilized a biotinylated photo-activatable trisNTA on 2D interfaces with various mobility of the anchor units via biotin-streptavidin interaction. This system allows to control the in situ assembly of proteins as well as the mobility, composition and geometry of the generated scaffolds.

Our approach will help to the optical regulation of receptor clustering using non-covalent interactions in real-time and their spatial organization for single-cell analysis.

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### *The mechanisms of gene regulatory networks constrain evolution: A lesson from synthetic stripe-forming circuits*

Presenting author:

**Yolanda Schaerli**

Author(s):

Yolanda Schaeferli, Alba Jiménez, José Duarte, Ljiljana Mihajlovic, Julien Renggli, Mark Isalan, James Sharpe, Andreas Wagner

Phenotypic variation is the raw material of adaptive Darwinian evolution. The phenotypic variation found in organismal development is not truly random – it is biased towards certain phenotypes and against others, but the molecular mechanisms behind such restrictions are still poorly understood. Gene regulatory networks have been proposed as one cause of constrained phenotypic variation. However, most of the evidence for this is theoretical rather than experimental. Here, we study evolutionary biases in two synthetic gene regulatory circuits expressed in *E. coli* that produce a gene expression stripe in response to a morphogen gradient– a pivotal pattern in embryonic development. The two parental circuits produce the same phenotype, but create it through different interactions between the genes, meaning they use different regulatory mechanisms. We show that mutations cause distinct novel phenotypes in the two networks. Because the regulatory mechanisms are well understood, we can use a combination of experimental measurements, mathematical modelling and DNA sequencing to understand why mutations bring forth only some but not other novel gene expression phenotypes. Our results reveal in a tractable experimental system that the regulatory mechanisms of networks restrict the possible phenotypic variation upon mutation. Consequently, seemingly equivalent networks can indeed be distinct in how they constrain the outcome of further evolution.

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### *Optogenetic control of T cell activation and inactivation*

Presenting author:

**Wolfgang Schamel**

Author(s):

Wolfgang Schamel, Sascha Yousefi, Matthias Günther, Julia Chalupsky, Maximilian Hörner, Thomas Höfer, Matias Zurbriggen, Wilfried Weber

T cell activation is a crucial event in immune responses and depends on interactions between T cell receptors (TCRs) and foreign peptides bound to MHC (pMHC) on antigen-presenting cells. The duration of the TCR-pMHC interaction is thought to play a pivotal role in determining the outcome of this stimulation event. It has been proposed that T cells discriminate between self and non-self peptides via the ligand's different binding kinetics. Self-peptides would bind too short to stimulate the TCR, whereas foreign peptides, such as derived from viruses or bacteria, would bind long enough to allow a sequence of biochemical steps to happen leading to T cell activation.

We used optogenetic tools to precisely manipulate TCR-ligand interaction times by light illumination. We fused an Arabidopsis phytochrome B (PhyB)-interacting factor (PIF) to the extracellular part of the TCR. At 660 nm light the PhyB ligand can bind to the PIF-TCR and with 740 nm light the interaction is disrupted. Using the influx of calcium into the cytosol as a read-out for TCR activation, we find that very short pulses of binding do not lead to calcium influx, whereas long durations of the PhyB-PIF-TCR binding event evoke calcium influx. These experiments show that the kinetics of binding indeed can control TCR, and thus T cell, activation.

### *IDENTIFICATION OF MONOTERPENOID RESISTANCE MECHANISMS IN PSEUDOMONAS PUTIDA*

Presenting author:

**Florence M. Schempp**

Author(s):

Florence M. Schempp, Jia Mi, Ferdinand Kirchner, Katharina Hofmann, Hendrik Schewe, Jens Schrader, Markus Buchhaupt

Product toxicity is a common challenge for biotechnological production processes. Therefore, it is essential to enhance the strain's tolerance towards the educts and products and thereby avoid limitations due to inhibitory effects. A high toxicity is known for many monoterpenoids, which are widely used in pharmaceuticals, flavor and fragrance and agriculture.

The bacterium *Pseudomonas putida* shows an inherent extraordinarily high tolerance towards solvents including monoterpenoids. To increase the already high native monoterpenoid resistance of *P. putida* and transfer the underlying resistance mechanisms to suitable host strains for biotechnological production processes, the molecular factors of monoterpenoid tolerance have to be determined. Therefore, an approach, comprising the creation of a mutant library, the selection for monoterpenoid-hyperresistant mutants and further characterization via genome sequencing, deletion and complementation experiments and growth tests, was conducted.

The results show that increased or decreased tolerance for monoterpenoids is mainly related to altered expression levels of efflux pumps. In addition, first evidences for a specificity of the tolerance mechanisms to certain molecular structures were deducible.

The understanding of the underlying factors can help to create suitable monoterpenoid production strains with an improved tolerance towards the bioprocess educts and products, aiding the efficient monoterpenoid production with this organism.

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### *Computer-aided Prediction of DNA Assembly Reactions and Experimental Workflows*

Presenting author:

**Niels Schlichting**

Author(s):

Niels Schlichting, Sven Jager, Felix Reinhardt, Thomas Huxhorn, Michael Schmidt, Heinz Koepl, Kay Hamacher, Johannes Kabisch

Progress in synthetic biology can be facilitated by rapid prototyping approaches to test a wide variety of, e.g., genetic circuits. In the last decades, many methods for assemblies were used to build DNA constructs (e.g., Gibson assembly, Golden Gate assembly, CPEC, BASIC) but with limitations in the usage in robotic platforms. In contrast, the ligase cycling reaction (LCR) fulfills the prerequisites for automated assemblies like the usage of unmodified DNA parts. The assembly order is only determined by single-

stranded oligonucleotides building a bridge (so called bridging oligonucleotide, BO) between adjacent parts. In order to utilize an easy access for scientists to use the LCR for computer-aided DNA assemblies, we developed the web-application CloneFlow. It offers to create LCR workflows based on the input of DNA sequences in a multi-fasta format and experimental parameters. Different output formats are available including csv-files enabling easy down-stream processing e.g. for automation with liquid-handling systems. CloneFlow is modular and includes the design of amplification primers for the DNA parts and the BOs for the assembly. As a unique feature, all oligonucleotides are secondary structure optimized, i. e. our service finds the oligos which have low free energy and thus a small contribution to secondary structures. Our work presents a synergistic collaboration between experimental expertise and computational know-how.

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### *Life from the bottom-up*

Presenting author:

**Petra Schwille**

Author(s):

Petra Schwille

Abstract not submitted

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### *Optogenetic control of inflammasome assembly and cell death*

Presenting author:

**Kateryna Shkarina**

Author(s):

Kateryna Shkarina, Petr Broz

Inflammasomes are multiprotein complexes that are assembled upon the recognition of invading pathogens in cytoplasm. Although inflammasome pathway has been studied for almost two decades, many open questions remain due to pleiotropic effect of pathogens on cell-autonomous immunity and signaling. Optogenetics has recently emerged as a powerful tool to address different aspects of cell physiology. Here, we describe a novel optogenetic approach to precisely control inflammasome assembly and pyroptotic cell death by light-induced oligomerization of inflammasome components. We demonstrate that optogenetic control of inflammasome pathway can be achieved at every signalling level, starting from the cytoplasmic receptors and down to the effector caspases, and that our synthetic optoInflammasome proteins remain fully functional in terms of their protein-protein interactions and downstream signalling response. We show that this system enables unique spatio-temporal control of inflammasome assembly and provides additional insights into molecular interactions governing this process. Moreover, optogenetic induction of cell death enabled us precisely control the timing of caspase activation and gain new insights into the morphological and physiological changes that cells undergo during pyroptosis with unprecedented temporal resolution. Our approach provides researchers

with new tools for studying inflammasome signaling, and can be easily adapted for the study of other cell death pathways.

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### *Cell-free protein synthesis from non-growing, stressed Escherichia coli*

Presenting author:

**Martin Siemann-Herzberg**

Author(s):

Martin Siemann-Herzberg, Jurek Failmezger

Cell-free protein synthesis is a versatile protein production system. Performance of the protein synthesis depends on highly active cytoplasmic extracts. Extracts from *E. coli* are believed to work best; they are routinely obtained from exponential growing cells, aiming to capture the most active translation system. Here, we report an active cell-free protein synthesis system derived from cells harvested at non-growth, stressed conditions. We found a downshift of ribosomes and proteins. However, a characterization revealed that the stoichiometry of ribosomes and key translation factors was conserved, pointing to a fully intact translation system. This was emphasized by synthesis rates, which were comparable to those of systems obtained from fast-growing cells. Our approach is less laborious than traditional extract preparation methods and multiplies the yield of extract per cultivation. This simplified growth protocol has the potential to attract new entrants to cell-free protein synthesis and to broaden the pool of applications. In this respect, a translation system originating from heat stressed, non-growing *E. coli* enabled an extension of endogenous transcription units. This was demonstrated by the sigma factor depending activation of parallel transcription. Our cell-free expression platform adds to the existing versatility of cell-free translation systems and presents a tool for cell-free biology.

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### *Engineering of light-regulated cNMP-specific phosphodiesterases*

Presenting author:

**Robert Stabel**

Author(s):

Robert Stabel, Andreas Möglich

The advantage of optogenetic tools is that they manipulate and control single cells and whole organisms in a non-invasive, reversible, and spatial-temporally precise manner. The light-dependent phosphodiesterase LAPD couples the photosensor module of *D. radiodurans* bacterial phytochrome with the effector module of human phosphodiesterase 2A to degrade the second-messenger cAMP and cGMP in red light-activated manner. LAPD finds application in controlling second-messenger levels in whole cells and we are currently working on further improvements. To this end, we strive to understand the underlying principle design model, as the structural and engineering features of the system have yet to be clarified. To aid in the exploration of these parameters, we established a fluorescence-based assay that can be conducted in *E. coli* crude lysates to accelerate the screening of new light-dependent

constructs. This assay allows medium-throughput screening to identify variants with higher activity, inversed regulation or a better dynamic range. As the enzymatic activity and light-regulation of LAPD strongly depends on the linker length, we are trying to better understand the whole signal transduction process by creating different linker variants. Another field of interest is the creation of new receptor chimeras with altered light regulation and/or altered substrate specificity. Altogether, we have defined simple design rules for the creation of new light-dependent hybrid proteins.

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### *A multiplexed, hydroxamate based UPLC-MS/MS assay for adenylation specificity*

Presenting author:

**Aleksa Stanisic**

Author(s):

Aleksa Stanisic, Hajo Kries

Adenylation domains control the specificity of nonribosomal peptide synthetases (NRPSs), an important group of enzymes involved in the biosynthesis of numerous bioactive natural products. Despite great efforts invested in their engineering, progress has been restrained by the lack of suitable assays for the screening and characterization of mutants. Currently, the pyrophosphate exchange radioassay is the gold standard for characterizing adenylation domains. However, the usage of radioactive isotopes and a tedious experimental procedure make it unsuitable for large scale screening. We propose an assay for adenylation specificity that detects multiple products in a single reaction under substrate competition as found in the cellular environment. Our assay takes advantage of hydroxylamine to quench activated carboxylates to form hydroxamic acids. After a quick derivatization step, these are sensitively and specifically detected by UPLC-MS/MS. Notably, hydroxamate formation is related to the most relevant product of the reaction, the activated amino acid, whereas other adenylation assays detect the reverse reaction or by-products. The hydroxamate assay can potentially provide a fast, technically undemanding and reliable alternative for the determination of adenylation domain specificity in the presence of multiple substrates and thus facilitate NRPS engineering. Applications on other adenylation enzymes, for instance aminoacyl-tRNA synthetases are conceivable.

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### *Harnessing Fungal Nonribosomal Cyclodepsipeptide Synthetases for Mechanistic Insights and Tailored Engineering*

Presenting author:

**Charlotte Steiniger**

Author(s):

Charlotte Steiniger, Sylvester Hoffmann, Andi Mainz, Marcel Kaiser, Kerstin Voigt, Vera Meyer, Roderich D. Süssmuth

Nonribosomal peptide synthetases represent potential platforms for the design and engineering of structurally complex peptides. While previous focus has been centered mainly on bacterial systems,<sup>[1]</sup>

fungal synthetases assembling drugs like the antibacterial cephalosporins or the anthelmintic cyclodepsipeptide (CDP) PF1022 await in-depth exploitation. As various mechanistic features of fungal CDP biosynthesis are only partly understood,<sup>[2]</sup> effective engineering of NRPSs has been severely hampered. By combining protein truncation, in trans expression and combinatorial swapping, we assigned important functional segments of fungal CDP synthetases and assessed their in vivo biosynthetic capabilities.<sup>[3]</sup> Hence, full length artificial assembly lines comprising of up to three different synthetases were generated. Using *Aspergillus niger* as a heterologous host, we obtained new-to-nature octa-enniatin (4 mg/L) and octa-beauvericin (10.8 mg/L) as well as high titers of hexa-bassianolide (1.3 g/L) with an engineered ring size. The hybrid CDPs showed up to 12-fold enhanced antiparasitic activity against *Leishmania donovani* and *Trypanosoma cruzi* compared to the reference drugs miltefosine and benznidazole, respectively. Our findings thus contribute to a rational engineering of iterative nonribosomal assembly lines.

[1] R. D. Süßmuth, A. Mainz, *Angew Chem Int Ed Engl.* 2017, 56(14):3770-3821.

[2] Yu et al., *Nat Commun.* 2017, 8:15349.

[3] Steiniger et al., *Chem. Sci.* 2017, 8:7834-7843.

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### *RNA aptamers as genetic control devices – the potential of riboswitches as synthetic elements for regulating gene expression*

Presenting author:

**Beatrix Suess**

Author(s):

Beatrix Suess

RNA utilizes many different mechanisms to control gene expression. Among the regulatory elements that respond to external stimuli, riboswitches are a prominent and elegant example. They consist solely of RNA and couple binding of a small molecule ligand to the so-called “aptamer domain” with a conformational change in the downstream “expression platform” which then determines system output. The modular organization of riboswitches and the relative ease with which ligand-binding RNA aptamers can be selected in vitro against almost any molecule have led to the rapid and widespread adoption of engineered riboswitches as artificial genetic control devices in biotechnology and synthetic biology over the past decade.

We will highlight proof-of-principle applications to demonstrate the versatility and robustness of engineered riboswitches in regulating gene expression in bacteria and report about new strategies of synthetic riboswitches in eukarya with a special focus on the control of alternative splicing. We will report on strategies and parameters to identify aptamers that can be integrated into synthetic riboswitches, before finishing with a reflection on how to improve the regulatory properties of engineered riboswitches, so that we can not only further expand riboswitch applicability, but also fully exploit their potential as control elements in regulating gene expression.

### *Bottom up approaches to synthetic cellularity*

Presenting author:

**Dora Tang**

Author(s):

Dora Tang

Biology is well equipped in exploiting a large number of out of equilibrium processes to support life. A complete understanding of these mechanisms is still in its infancy due to the complexity and number of the individual components involved in the reactions. However, a bottom up approach allows us to replicate key biological processes using a small number of basic building blocks. Moreover, this methodology has the added advantage that properties and characteristics of the artificial cell can be readily tuned and adapted.

In this talk I will discuss strategies for the design and synthesis of artificial cells based on liquid-liquid phase separation (coacervation) and hydrophobic effects such as lipid vesicles and proteinosomes and describe how these compartments may be used as platforms for implementing dynamic biological behaviours such as intercellular communication.

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### *Optogenetic modules to control protein biosynthesis and proteolysis in budding yeast*

Presenting author:

**Christof Taxis**

Author(s):

Jonathan Trauth, Johannes Scheffer, Sophia Hasenjäger, Lars-Oliver Essen, Christof Taxis

Generation of a sustainable bioeconomy based on renewable resources is a major challenge for the future. An important part of these efforts are metabolic engineering approaches that turn easy-to-handle model organisms into cell factories optimized for the production of valuable compounds. Here, we introduce optogenetic modules that offer novel paths for efficient regulation of enzyme activities in a fast, reliable, and cheap way for optimizing the biosynthesis of valuable chemicals in *Saccharomyces cerevisiae*. Our toolbox of modules comprises blue-light reactive modules for regulation of gene expression and control of protein stability. Our characterization of the different modules demonstrate that differences in their characteristics as well as the facile modulation of inducer strength give numerous possibilities for fine-tuning of enzymatic activities. Overall, we aim to establish our optogenetic modules as platform technology for metabolic engineering approaches using budding yeast.

### *Sirtuin transcripts expression in activated T cells from young and old healthy blood donors*

Presenting author:

**Georgiana Toma**

Author(s):

Georgiana Toma, Dagmar Quandt, Barbara Seliger

T cell senescence represents the age-related modifications of T cells. Posttranslational modifications (PTM) of proteins can accumulate within the cells over time thus altering their functionality.

Acetylation is one of the most abundant PTMs and is regulated by 2 types of enzymes: acetyltransferases and deacetylases. Acetyltransferases are called KATs (lysine acetyltransferases) and deacetylases are called HDACs (histone deacetylases) although their substrates vary. The 3rd class of deacetylases is called Sirtuins (SIRT) and SIRT1, 3 and 6 have been linked to ageing.

Our aim is to connect changes in T cell functions to the age-dependent alterations of the T cell acetylome. CD4<sup>+</sup> and CD8<sup>+</sup> T cells were isolated by MACS technology from PBMCs obtained from healthy blood donors of 2 age groups (under 30 and over 60 years old). Constitutive transcript expression of KATs and Sirtuins from primary T cells and the non-T cell fraction were analysed by qPCR. Sirtuin expression was found higher in T cells as compared to non-T cells and decreased in aged CD4<sup>+</sup> T cells compared to young CD4<sup>+</sup> T cells. T cells were also stimulated with aCD3/aCD28 for 24 and 48 hours following Sirtuin analysis. SIRT6 mRNA is highly induced in activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

These results will be linked to proliferation assays and ELISA based analysis of the secretory phenotype of T cells.

Results of our study will give new insights in understanding the involvement of acetylation in T cell senescence.

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### *Synthetic biology strategies for the improvement of biochemical pathways in *Saccharomyces cerevisiae**

Presenting author:

**Joanna Tripp**

Author(s):

Joanna Tripp, Mara Reifenrath, Mislav Oreb, Eckhard Boles

Compartmentalization is a powerful tool to improve the efficiencies of metabolic pathways, minimizing obstacles such as unwanted side reactions, accumulation of toxic intermediates out of the cell, and high diffusion distances. To increase the yield of a heterologous cis, cis-muconic acid (CCM) pathway in yeast, we have developed a novel compartmentalization strategy. The valuable compound CCM is a precursor of the bulk chemical adipic acid, which is used for production of nylon, plastics, lubricants and softeners. In our approach, enzymes of the heterologous CCM pathway are enclosed in endoplasmic reticulum

(ER)-derived synthetic vesicles generated by fusion of these enzymes to plant peptides. Formation of synthetic vesicles was induced in a yeast strain with modifications in enzymes of the shikimic acid pathway (Aro1K<sup>1375</sup>A, Aro3K<sup>222</sup>L, Aro4K<sup>229</sup>L), which leads to an enhanced production of the shikimic acid pathway intermediate 3-dehydroshikimate (3-DHS), the precursor of the heterologous CCM pathway.

Using fluorescence microscopy and cell fractionation techniques, we have proven the formation of synthetic vesicles and the sedimentation of enzymes of the heterologous CCM pathway in membrane fractions. The functionality of the system was demonstrated in fermentation experiments and compared to other compartmentalization strategies.

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### *From sensors to signal quantification to understand self-organization in plants*

Presenting author:

**Teva Vernoux**

Author(s):

Teva Vernoux

Plant aerial organs are produced at the shoot apical meristem, a specialized tissue containing a stem cell niche located at the shoot apex. Organ production follow relatively robust spatio-temporal patterns that control the shoot primary architecture or phyllotaxis. Results from modeling approaches and wet experiments have shown that the dynamics of auxin distribution allows for reiterative organogenesis at the shoot apex, making it a system of choice to understand how morphogenetic signals such as auxin and other plant hormones contributes to patterning dynamics. I will discuss this mechanism as well as recent results from both modeling and imaging approaches using genetically-encoded biosensors that allow understanding the respective contribution of growth and patterning to the dynamics of this unique self-organizing system.

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### *Machine learning with Tetracycline Dimers - A large-scale approach towards the in silico prediction of riboswitch performance*

Presenting author:

**Marc Vogel**

Author(s):

Ann-Chistin Groher, Sven Jager, Marc Vogel, Christopher Schneider, Kay Hamacher, Beatrix Suess

In synthetic biology, the toolbox of regulatory devices has increased immensely in the last couple of years. The devices work very well in the genetic context they were optimized for, but if they are transferred into a new genetic context, this might not be the case. This means that the whole process of optimizing the device has to start all over again if it is transferred into a new genetic context. One method to prevent the optimization step is to use insulators which flank the regulatory device and make it possible to insert it into any genetic context. As we would like to understand the influence of the genetic

context better, we used a different approach. This way we want to be able to predict the switching behavior of a given device in any genetic context it is placed in. To achieve that we started to analyze tc-aptamer dimers with the help of an in silico machine learning approach (i.e. random forest). The random forest was trained to predict the switching behavior of the dimer constructs based on (bio)physical parameters (e.g.  $\Delta G$ , GC content and melting temperature). We predicted more than 300 different constructs and analyzed their switching behavior. This way we were able to significantly increase the switching behavior and the performance of the different dimers.

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### *Controlling Protein Levels in Diverse Gram-negative Bacteria using Acetyl-lysine Dependent Amber Suppression*

Presenting author:

**Wolfram Volkwein**

Author(s):

Wolfram Volkwein, Christopher Maier, Ralph Krafczyk, Kirsten Jung, Jürgen Lassak

In vivo analysis of essential genes often requires tight control of the levels of their protein products. Current methodologies focus mainly on transcriptional or post-transcriptional regulation of mRNAs. We have developed a toolbox, based on amber suppression in the presence of N $\epsilon$ -acetyl lysine (AcK), for translational tuning of protein output. We chose the highly sensitive luminescence system LuxCDABE as a reporter and incorporated a UAG stop codon into the gene for the reductase subunit LuxC to measure the effects of AcK-dependent amber suppression in *Escherichia coli*. We demonstrate that in combination with transcriptional regulation, the system can be used both, for total repression and gradual adjustment of protein production. In parallel, we constructed an *E. coli* strain that carries an isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG)-inducible version of the gene for AcK-tRNA synthetase (AckRS) on the chromosome to lower the level of mischarging with non-cognate amino acids. Furthermore, we assembled a set of vectors that enable the AcK-dependent amber suppression system to control protein output not only in *E. coli*, but also in *Salmonella enterica* and *Vibrio cholerae*.

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### *Synthetic biology-inspired design of a biomaterial-based positive feedback loop*

Presenting author:

**Hanna Wagner**

Author(s):

Hanna Wagner, Raphael Engesser, Kathrin Ermes, Jens Timmer, Wilfried Weber

Molecular switches are at the heart of synthetic biology and serve as basis for building engineered cellular systems performing basic digital or analog functions. In recent years, such circuits have been interconnected to higher order cellular networks paving the way for diverse applications in e.g. the therapeutic, biotechnological and analytical sectors.

Here, we introduce the translation of the underlying design principle and synthetic biology tools to material sciences for the construction of information-processing biomaterial circuits. We demonstrate this concept by developing analytical signal detector devices. To this aim, we functionalized polymer materials with antibiotic-switchable and/or protease-cleavable biological parts to obtain novel types of remote-controlled biomaterials. Following a mathematical model-guided approach, we wired these materials in a positive feedback topology enabling signal amplification and hence sensitive input detection. We applied this system for the detection of antibiotics. The modular combination of biological and polymer parts enables customization of the system's design for detecting other molecules of interest. Based on this flexibility, we propose the generic applicability of the underlying engineering concept for the design of information-processing material systems with broad perspectives in fundamental and applied research.

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### *Synthetic biology: Inspiration for (HIV) vaccine development*

Presenting author:

**Ralf Wagner**

Author(s):

Ralf Wagner, on behalf of EHVA (European HIV Vaccine Alliance)

Recent insight into HIV epidemiology, replication and pathogenesis and a better understanding of our immune system's limitations regarding the control of HIV replication is questing for novel and improved vaccine strategies. Despite the fact that there's no effective HIV vaccine available yet, hypothesis driven research as well as quantitative systems biology approaches inspired the development of a broad range of interdisciplinary technologies now being applied in various fields of vaccine development. These technologies comprise tools for (i) bioinformatic analysis enabling rational T- and B cell immunogen design, (ii) advanced HT DNA- and RNA-design and synthesis to re-program viral transcriptional and translational regulation, (iii) recombination-based protein engineering, selection and manufacturing, (iv) novel delivery systems (nonviral- and viral chassis, protein and (nano)particle based) and modalities (schedule, devices), (v) built-in orthogonal adjuvant strategies as well as (vi) in depth immune monitoring and data de-convolution. Collectively, these tools and devices enable accelerated iterations of vaccine design, construction, testing and data analysis. Using HIV as an example, we will demonstrate how the above tools and devices translate into candidate vaccines with improved immunological properties in preclinical and clinical trials. Furthermore, we will present selected highlights where above strategies were successfully used in other fields beyond HIV.

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### *Learning by Building – Synthetic Neochromosomes in Escherichia coli*

Presenting author:

**Torsten Waldminghaus**

Author(s):

Daniel Schindler, Torsten Waldminghaus

Advances in de novo synthesis of DNA and assembly methodology make construction of synthetic chromosomes a reachable goal. Considering a potential design leads to the question what the essential parts of a chromosome are. Investigations on this question have mainly focused on the minimal set of genes needed to allow cells to live. However, chromosomes are more than arrays of genes. Chromosomes need systems to replicate, segregate and organize the encoded genetic information. To explore such chromosome maintenance systems we have designed and constructed the synthetic neochromosome synVicII. The replication origin stems from the secondary chromosome of the human pathogen *Vibrio cholerae* which carries a natural secondary chromosome. We use synVicII as backbone to construct bigger replicons (~100kbp) with variations in the distribution of DNA motifs relevant for chromosome maintenance. In a pilot project we focus on an epigenetic system that regulates DNA mismatch repair, chromosome segregation and DNA replication in *E. coli*. This system is based on the adenine-methylation of the sequence GATC. A set of three neochromosomes was constructed and used to study the relation between DNA mismatch repair and chromosome segregation factors. Our long term goal is to establish synthetic neochromosomes as experimental system to study chromosome maintenance and to provide chromosome construction rules for biotechnology applications.

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*Engineering optogenetic control of p53 to study the effects of its nuclear accumulation dynamics on cellular outcome independent of stress*

Presenting author:

**Pierre Wehler**

Author(s):

Pierre Wehler, Barbara Di Ventura

p53 is a transcription factor that integrates various stress signals into the most appropriate cellular response by regulating transcription of specific genes.

Under physiological conditions, p53 levels are kept low by a tightly regulated network of proteins, among which is the E3 ubiquitin ligase Mdm2. Upon stress, p53 levels increase and show a pulsatile or continuous response, dependent on the type and severity of the stress. It was proposed that p53 dynamics dictate which downstream gene expression programs are initiated. However, many questions remain open such as whether post-translational modifications play a role alongside p53 dynamics or how many distinct cellular responses can be controlled purely by p53 dynamics.

Here we use optogenetics to take control over p53 nuclear accumulation. Our aim is to generate various dynamical patterns of p53 transcriptional activity by using blue light, in the absence of upstream stress. We use LEXY, a light-inducible nuclear export system developed in our lab, to control the localization of p53, thereby controlling its transcriptional activity. We show that we can repeatedly accumulate the transcriptionally active p53 tagged with LEXY in and out of the nucleus, effectively generating p53 pulses akin to those obtained under certain stress conditions.

Currently, we are studying how p53 dynamics control target gene expression on a single cell level.

### *Rapid cloning, transfer and expression of biosynthetic pathways using the yTREX-toolbox*

Presenting author:

**Robin Weihmann**

Author(s):

Robin Weihmann, Sonja Kubicki, Stephan Thies, Andreas Domröse, Thomas Drepper, Karl-Erich Jaeger, Anita Loeschcke

The efficient production of high-value secondary metabolites often requires the transfer of entire metabolic pathways into amenable expression hosts. Therefore, effective tools for the cloning and heterologous expression of gene clusters in microorganisms are needed.

Here, we developed a novel toolbox based on the transfer and expression system TREX<sup>1</sup> for straightforward expression of gene clusters in bacteria. The system was adapted to allow the cloning via homologous recombination in yeast enabling fast multi-fragment cloning. Effectivity was demonstrated by the rapid cloning and successful implementation of biosynthetic pathways in *Pseudomonas putida*<sup>2</sup>.

Based on that, we developed a toolbox concept consisting of changeable components that allows flexible adaption of expression cassettes to different target pathways, hosts and experiments. Promoters, selection markers, and reporter elements as well as DNA sequences for chromosomal integration can easily be exchanged using designated recombineering sequences. Random integration can be exerted by applying Tn5-transposition to create strong constitutive production strains utilizing host promoters<sup>3</sup>. Aiming for targeted sites, the yTREX vector can be adapted to allow homology-based site-specific chromosomal integration.

In summary, the yTREX-toolbox offers effective strategies for the rapid generation of bacterial strains for the production of valuable compounds.

1 Loeschcke et al. 2013

2 Domröse et al. 2017

3 Domröse et al. 2015

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### *Combining inteins and optogenetics to control protein activity in living cells*

Presenting author:

**Daniel Weis**

Author(s):

Daniel Weis, Barbara Di Ventura

Inteins are autocatalytic protein domains that excise themselves out of proteins and in doing so connect the flanking regions with a peptide bond. The splicing reaction requires the presence of specific amino

acids that are conserved in all inteins, such as a cysteine or serine at the N-terminus and an asparagine at the C-terminus. Split inteins are constituted by two separate domains that need to associate to reconstitute a functional intein. Therefore, during the process of splicing, split inteins make fusions between two previously independent proteins or peptidic fragments. Inteins are extremely useful tools in synthetic biology, because they can be used to modify proteins of interest inside living cells. Split inteins can be for instance used to reconstitute a functional protein out of two dysfunctional split halves. Being able to control the splicing reaction in space and time would open up new possibilities to perturb cells and study the effects of such perturbations on cellular behavior.

Here we report the engineering of a system based on two recently developed optogenetic tools, LEXY and LOVTRAP, to control the splicing reaction with blue light in muscle cells.

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*Investigating the design principles of bacterial gene expression to enable next-generation synthetic biology: Terminator libraries and non-canonical translation initiation devices for genetic engineering in bacteria*

Presenting author:

**Hans-Joachim Wieden**

Author(s):

Hans-Joachim Wieden, Justin R. J. Vigar, Andrew J. Hudson

Genetic circuit design is limited by our current understanding of gene regulatory processes and availability of standardized genetic parts with known properties. Identification of optimal regulatory parts often requires iterative rounds of design and performance testing. We report a simple method for constructing complex intrinsic terminator libraries that can be mined for desired termination efficiencies. These libraries are interrogated in *E. coli* using a dual fluorescence reporter system to evaluate in vivo termination efficiency. Clones are fractionated via FACS and analyzed by Term-seq.

Modulating translation initiation is a universal strategy for controlling gene expression. We characterize a non-canonical translation initiation element lacking a canonical SD, capable of efficient translation initiation in *E. coli*. We use a quantitative, dual-reporter in vivo screening tool, and have mapped the sequence-function relationships using large-scale mutagenesis, next-gen sequencing and SAXS. We have generated a library of synthetic structure-based translation initiation devices able to fine-tune translation initiation over several orders of magnitude.

This work expands the number of available parts for genetic engineering and provides robust combinatorial libraries to select optimal termination and translation initiation elements. We provide critical mechanistic information for the rational design and manipulation of translation initiation and termination in bacteria.

### *Next vivo: Cell-Free Synthetic Biology for the Masses*

Presenting author:

**Hans-Joachim Wieden**

Author(s):

Hans-Joachim Wieden, iGEM team Lethbridge

We aim to develop a standardized, modular, and open-source ex vivo transcription and translation (TX/TL) system available for research and teaching communities worldwide. Ex vivo systems provide several advantages to cell-based platforms, including: simple input and output, non-proliferation, precise control of molecular interactions, and incorporation of unnatural amino acids. Thus, ex vivo systems are highly useful tools in making synthetic biology accessible to novices, providing enthusiasts with inexpensive cell-free synthesis, and empowering experts with modular control over robust expression systems. With our end-users in mind, we have designed, modelled, and built a plasmid-based multi-protein parts collection for the stoichiometric expression of all required biomachinery for coupled TX/TL reactions, as well as a novel purification method that makes these parts readily available from an inexpensive single-step elution. Lastly, we have created a software tool to preempt biosecurity challenges associated with genetic recoding in ex vivo systems.

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### *Optochemical Biology – In-situ Receptor Clustering by Light*

Presenting author:

**Ralph Wieneke**

Author(s):

Ralph Wieneke, Robert Tampé

Cells exhibit biochemical properties that are heavily influenced by the surrounding extracellular matrix (ECM). Especially the confined arrangement of signaling proteins or bioactive molecules within artificial ECMs is key to exert influence on cells. Regarding the study of biological systems, the spatial as well as temporal control by stimuli-responsive frameworks will advance synthetic and chemical biology. A key challenge is the controlled interaction by molecular lock-and-key elements to connect protein activity with macroscopic response.

We present new optochemical tools for the in-situ receptor assembly. We designed photo-activatable lock-and-key elements and scaffolds for the controlled receptor assembly. The high-affinity pair was used for a spatio-temporal protein labeling at defined intracellular localizations. The high-affinity interaction pair enabled fast labeling at subnanomolar concentrations with tunable labeling density, which substantially improved the localization accuracy in live-cell super-resolution microscopy. The two-photon activation in ECMs facilitated the non-invasive 3D protein organization. Gradually increasing protein densities were achieved and protein assembly was followed in real-time after in-situ photo-activation, leading to multi-protein arrays of tunable densities.

Our photo-activatable lock-and-key elements will help to investigate and control receptor clustering in macroscopic 3D cell culture at system-level by optical intervention.

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*Palmitoylation - Emerging roles in TNF-R1 endocytic trafficking and signaling*

Presenting author:

**Philipp Zingler**

Author(s):

Philipp Zingler, Vinzenz Särchen, Timo Glatter, Lotta Caning, Stefan Schütze, Jürgen Fritsch

TNF-R1 as well as other members of the TNF-Receptor superfamily and their ligands have been described to be involved in various physiological processes ranging from regulating immune response to organ formation. On the other hand they have pathological function i.e. in cancer formation, chronic inflammatory and neural diseases. It is known that TNF-R1 can induce diametrically opposed biological effects upon ligand binding: inflammation/proliferation versus cell death. The differences in signal quality depend on the localization of the receptors. Plasma membrane resident receptors activate pro-inflammatory/survival signals, while endocytosed receptors can induce cell death.

We recently showed that TNF-R1 ubiquitination is mandatory for signaling bifurcation and that signaling cascades are regulated by protein complexes, which are assembled in distinct membrane enclosed compartments upon ligand binding. One mechanism to induce or modulate membrane localization of proteins is their post translational modification by palmitoylation. We here present unpublished data showing that TNF-R1 itself is palmitoylated, it induces biological effects which can be modulated by the application of (de-)palmitoylation inhibitors and that it interacts with palmitoyl transferases (PAT) and thioesterases (PTE). Thereby, activation of TNF-R1 induces dynamic changes in the palmitoylation status of various trafficking associated proteins, highlighting their role in endocytic TNF-R1 signaling.

## Abstracts - sorted by presenting author

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