Annual Congress Biotechnologie 2020+

Preliminary program | October 4th, 2018 at Fraunhofer-Forum Berlin

»Biological Transformation: Cutting-Edge Technologies in Biomanufacturing«

09:00	**Registration**	
09:30	***Welcome addresses***	**Dr. Patrick Dieckhoff** Fraunhofer-Gesellschaft
		Andrea Noske Bundesministerium für Bildung und Forschung, BMBF
	Keynote presentation	**Prof. Dr. Christine Lang** Bioökonomierat
10:15	**Coffee Break** *(15 min)*	
10:30	***Session 1 - Lighthouse Projects***	*Dr. Patrick Dieckhoff*
	»MaxSynBio - Avenues towards creating cells from the bottom up«	**Dr. Seraphine Wegner** Max Planck Society
	»Micro-scale production of industrially relevant molecules«	**Dr. Vito Valiante** Leibniz Association
	»Structure-based design of versatile biosensors for small molecules«	**Prof. Dr. Michael Bott** Helmholtz Association
	»Molecules off the conveyor belt«	**Prof. Dr. Frank Bier** Fraunhofer-Gesellschaft
11:30	**Break** *(5 min)*	
11:35	***Panel discussion*** *(in german)*	
12:30	**Lunch** *(45 min)*	
13:15	***Session 2 - Automated and Molecular Systems in Biotechnology***	*Dr. Stefan Kubick*
	»Modular molecular systems in biotechnology«	**Dr. Stefan Schiller** Albert-Ludwigs-University of Freiburg
	»How the automated lab can contribute to design the digital cell and new bioprocesses«	**Prof. Dr. Peter Neubauer** Technische Universität Berlin
	»KnowVolution: Redesigning proteins for innovations in catalysis and material science«	**Prof. Dr. Ulrich Schwaneberg** RWTH Aachen
14:00	**Postersession & Coffee** *(30 min)*	
14:30	***Session 3 - Synthetic Biology***	*Dr. Stefan Kubick*
	»Bionics beyond the Macroscopic World: from Biosynthesis to Bioactivity«	**Prof. Dr. Michael Müller** Albert-Ludwigs-University of Freiburg
	»Smart Membrane Compartments«	**Prof. Dr. Reinhard Lipowsky** Max Planck Institute of Colloids and Interfaces
	»Expanding the Synthetic Scope towards Industrial Chemicals through Combination of Chemocatalysis and Biotechnology«	**Prof. Dr. Harald Gröger** University of Bielefeld
	»Bioswitches, robotics and biodigitalization for synthetic biology«	**Prof. Dr. An-Ping Zeng** Hamburg University of Technology
15:45	**Postersession & Coffee** *(30 min)*	
16:15	***Session 4 - Glyco Biotechnology***	*Prof. Dr. Frank Bier*
	»N-glycans, a diagnostic tool for ovarian cancer«	**Prof. Dr. Véronique Blanchard** Charité - Virchow Klinikum
	»The Golgi Glycan Factory (GGF) - Optimized enzyme cascades for glycoconjugate synthesis«	**Prof. Dr. Lothar Elling** RWTH Aachen
	»Taylored Systems for High Performance Glycoanalysis in Golgi Glycan Factory 2.0«	**Dr. Erdmann Rapp** Max Planck Institute for Dynamics of Complex Technical Systems Magdeburg
	»Cell-free Synthesis and Functional Analysis of Membrane Proteins and Glycoproteins«	**Dr. Stefan Kubick** Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses
17:15	**Final Statements**	
17:30	**End**	

MaxSynBio - AVENUES TOWARDS CREATING CELLS FROM THE BOTTOM UP

Dr. Seraphine Wegner
Max Planck Institute for Polymer Research

The MaxSynBio consortium investigates living systems from a fundamental perspective and relies solely on the bottom-up approach to Synthetic Biology. In MaxSynBio, essential processes of life are reconstituted into minimal synthetic systems staring from molecular building blocks. This bottom-up approach to synthetic biology focuses on the detailed analysis and understanding of essential functions of life with the ultimate goal to construct a basic living unit form non-living components. In this approach, life processes are assembled from well understood parts that can be assembled into modules and modules can be integrated into systems. The fundamental comprehensions gained from the activities in MaxSynBio can eventually be utilized for establishing a new generation of biotechnological processes, which would be based on synthetic cell constructs that replace natural cells currently used in conventional biotechnology.

MICRO-SCALE PRODUCTION OF INDUSTRIALLY RELEVANT MOLECULES

Dr. Vito Valiante
Leibniz Association

In vitro production of industrially relevant compounds is often limited by the cost of precursors. This is particularly true for all those compounds that use CoA-ligated molecules as biobricks. We recombinantly expressed and purified a malonyl-CoA-synthetase (MatB) and a malonyl-CoA decarboxylase (MatA) isolated from *Rhizobium leguminosarum*. MatB produced high levels of malonyl-CoA out of free CoA and malonate, while acetyl-CoA was obtained by the decarboxylation of malonyl-CoA *via* MatA activity. Adding a third heterologously expressed enzyme, the citrate synthase CitZ isolated from *Bacillus subtilis*, we were able to synthetize citric acid *in vitro* by using only malonate, ATP, free CoA, and oxaloacetate in the reaction mix. Additionally, the whole cycle was functional by using minimal amount of free CoA, the most expensive substrate among the used ones, which was continuously recycled after citrate formation.

All enzymes were shown to be very stable and still active after immobilization on magnetic beads. Using magnetic force, the immobilized enzymes were employed to develop a microfluidic system for the cell-free production of citrate. Last, the efficiency of the bead-bound enzymes within microfluidic systems was studied with both temporal and spatial resolution combining CARS and fluorescence microscope, while CE-UV/MS was employed to unravel the reactions and activities of free and immobilized enzymes using an *in situ* approach.

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STRUCTURE-BASED DESIGN OF VERSATILE BIOSENSORS FOR SMALL MOLECULES

Prof. Dr. Michael Bott
Helmholtz Association

The development of biosensors for in vitro quantification of small molecules such as metabolites or man-made chemicals is still a major challenge. We show that engineered variants of the sensory PAS domain of the histidine kinase CitA of the thermophilic bacterium Geobacillus thermoleovorans represent promising alternatives to established biorecognition elements. By combining binding site grafting and rational design we constructed protein variants binding L-malate, ethylmalonate or the aromatic compound phthalate instead of the native ligand citrate. Due to more favorable entropy contributions, the wild-type protein and its engineered variants exhibited increased (nano- to micromolar) affinities and improved enantioselectivity compared to CitA homologs of mesophilic organisms. Ligand binding was directly converted into an optical signal which was preserved after immobilization of the protein. A fluorescently labeled variant was used to quantify ethylmalonate, an urinary biomarker for ethylmalonic encephalopathy, in synthetic urine, thereby demonstrating the applicability of the sensor in complex samples.

Michael Bott[1]

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MODULAR MOLECULAR SYSTEMS IN BIOTECHNOLOGY:
Topological tecton libraries enabling de novo compartments and biocatalytic systems for the production of ultrapotent biodrugs and mechanical metamaterials for regenerative medicine

Dr. Stefan Schiller
Albert-Ludwigs-University of Freiburg

Modular molecular systems allowing to expand cell functions in a bioorthogonal fashion for *in vivo* and *in vitro* uses in biotechnology are a major focus our research. The systems developed comprise *de novo* organelles and their genetically encoded bioorthogonal covalent modification, new enzyme functions, architecture design, expansion of the genetic code and protocell models. Especially the latter ones may have several interesting implications in redesigning new cell chassis, creating concise reactions spaces with several subspaces and defining new targeting and delivery systems. In order to access such complex systems with high precision we construct topological protein tectons acting as dynamic structural building blocks in most of these systems. The same tectons are currently investigated towards their potential to guide cell function extracellularly and provide responsive bioaccessible material systems constituting mechanical metamaterials with interesting functions *in vitro* and *in vivo*.

HOW THE AUTOMATED LAB CAN CONTRIBUTE TO DESIGN THE DIGITAL CELL AND NEW BIOPROCESSES

Prof. Dr. Peter Neubauer
Technische Universität Berlin

Currently the use of computer based mathematical cell models is limited by the identification of the specific cellular parameters under the distinct environmental conditions. Small changes, e.g. of the genetic system or of environmental conditions can change most of the describing parameters. Therefore, advances in digitalization and automation in biotechnology are necessary that allow the fast identification of cell phenotypes under defined conditions.

Laboratory robot stations combined with analytical instruments and a supervisory intelligent software can help to design, perform and evaluate experiments to support the digital description of the cell. We perform these fully automated experiments fully operated by adaptive design algorithms. An experimental cycle includes the i) design of optimal dynamic experiments, ii) use of the generated data while the experiment is running, iii) online storage and handling of all the data that is being generated, iv) re-fitting the model parameters to the data (learning from the experiment), and v) re-designing the optimal experimental strategy[1]. The integration of all units in a model-based framework is the key to create an intelligent laboratory for rapid characterization of biosystems at industrially relevant conditions[2].

We demonstrate that this approach is especially useful for the description and characterization of genetic variants and recombinant expression systems with two case studies.

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KNOWVOLUTION: REDESIGNING PROTEINS FOR INNOVATIONS IN CATALYSIS AND MATERIAL SCIENCE

Prof. Dr. Ulrich Schwaneberg
RWTH Aachen

Keywords: Directed Evolution, Diversity generation, High-throughput screening, adhesion promoter, membranes, microgels.

Protein engineering by directed evolution has matured in academia and industry to a routinely applied algorithm to tailor protein properties [1] to match demands in synthesis and material science. In order to efficiently explore its potential, one has to balance time requirements for a directed evolution campaign, the number of generated enzyme variants, and limitations in state of the art screening technologies. The KnowVolution (Knowledge gaining direct evolution) [2] approach represents such an integrated directed evolution 2.0 strategy, which identifies with limited screening efforts in four phases significantly improved protein variants and ensures a molecular understanding of improved/tailored protein properties. [3]

Protein engineering enables to integrate efficiently biological molecules in materials and to generate protein-polymer hybrid materials and catalysts with exciting novel functionalities and high innovation potentials. In presentation success stories on integrated functionalities in protein-polymer hybrid materials will be presented in respect to hybrid catalysts [3], polypropylene/polystyrene, metal stents and plant leaf functionalizations [4], protein membranes [5], and enzyme loaded microgels [6].

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BIONICS BEYOND THE MACROSCOPIC WORLD: FROM BIOSYNTHESIS TO BIOACTIVITY

Prof. Dr. Michael Müller
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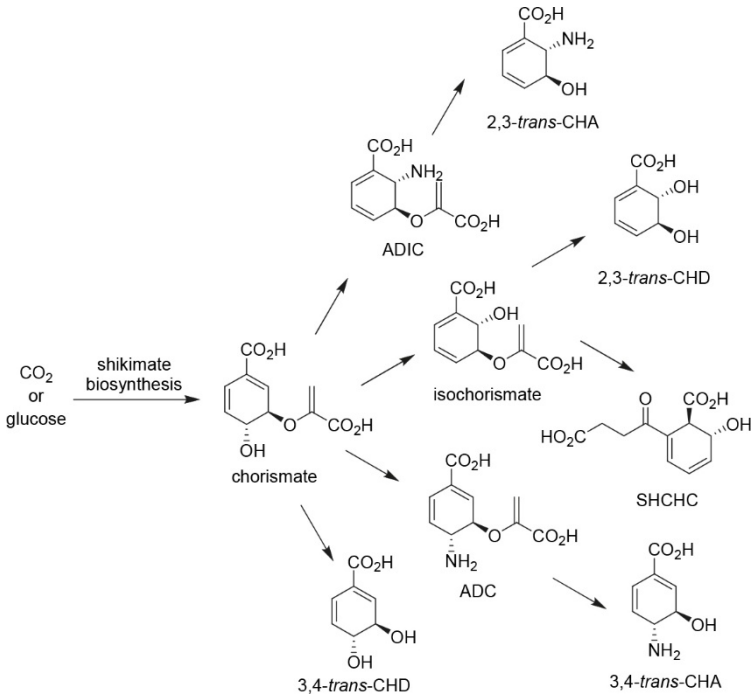
The concept of bionics, which can be described as »the study of mechanical systems that function like living organisms or parts of living organisms«, or according to another source, »application of biological methods and systems found in nature to the study and design of engineering systems and modern technology«, cannot easily be transferred to the molecular level. Reasons for that are, among others, complexity of biological systems, high interdependency of its parts, regulation networks such as allosteric control or inhibition, diversity and promiscuity of functional systems, etc. Nevertheless, biological concepts can be identified, described, and explored towards application in biotechnology and beyond that. We use biosynthetic analysis and biocatalysis as starting points for the identification of ›biomimetic‹ concepts such as diversity-oriented synthesis.[1] Moreover, extension of known biosynthetic traits can result in novel ›unnatural‹ pathways, products, and functions.



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SMART MEMBRANE COMPARTMENTS

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Giant unilamellar vesicles (GUVs) formed by lipid bilayers represent biocompatible microcompartments that are increasingly used as a versatile research tool in basic membrane science, bioengineering, and synthetic biology. When the vesicle membranes are in their fluid state, they are able to respond to different environments by changing both their morphology and their local molecular composition, in close analogy to cellular membranes. From the theoretical point of view, one key parameter that determines the GUV morphology is the spontaneous membrane curvature, which can vary over several orders of magnitude. [1]

Experimentally, the spontaneous curvature can be varied by exposing the GUVs to solutions of simple sugars with different concentrations. [2] As a result, one observes many »multi-balloon« morphologies, illustrating the morphological complexity of GUVs [3]. Binding His-tagged proteins to anchor-lipids within the GUV membranes, it is even possible to control and measure the density of the membrane-bound molecules. [4] Using the latter approach, the division of GUVs into two identical daughter vesicles has been recently achieved, thereby mimicking cell division in a synthetic manner.

GUV division occurs for relatively small spontaneous curvatures. If these curvatures are large compared to the inverse size of the GUVs, the membranes form many membrane nanotubes. [5,6] These tubulated GUVs exhibit an increased robustness to mechanical perturbations such as osmotic inflation, strong adhesion, and micropipette aspiration. [6] These GUVs represent smart microcompartments that can be used, e.g., as storage and delivery systems for different types of nanoparticles.

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N-GLYCANS, A DIAGNOSTIC TOOL FOR OVARIAN CANCER

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Epithelial ovarian cancer (EOC) is the most frequent cause of death from all gynecological malignancies because of its late diagnosis. As N-glycosylation is modified in the course of ovarian cancer, it is a promising source of tumor biomarkers. In this work, we investigated the glycome of total serum of primary serous ovarian cancer patients, patients suffering from benign ovarian tumors and healthy controls by MALDI-TOF-MS. The areas of the glycan structures that were significantly up- or downregulated were combined as a score named GLYCOV developed in our laboratory. The diagnostic performance of the GLYCOV value was compared with CA125 using Receiver Operating characteristics curves. Sensitivity and specificity were calculated using binary logistic regression. We also investigated the glycome profile in ascitic fluid by MALDI-TOF-MS and in formalin-fixed paraffin-embedded tumor tissues via Glycan Imaging.

GLYCOV was able to diagnose early-stage as well as late-stage serous EOC in serum better than CA125 and even allowed the discrimination between malignant and benign ovarian tumors. Ascites showed qualitatively as well as quantitatively different N-glycosylation pattern compared to healthy serum. Overall, increased antennarity, branching, sialylation and Lewis^X motives were observed in ascites samples. Indeed, different intensities of N-glycans were detected especially for the highly branched N-glycans. In addition, a correlation was established between ascites volume and degree of sialylation. In tumor tissues, first data indicate that specific glycan features can be associated with malignancy.

Conclusion: Our data suggests the power of the glycome to diagnose EOC. In addition, we reported for the first time the N-glycome of ascitic fluid and showed that the glycome modulations detected in EOC serum were also present in ascites. Both serum and ascitic fluid from EOC patients exhibited typical features of inflammatory conditions, when compared with healthy serum.

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THE GOLGI GLYCAN FACTORY (GGF)
OPTIMIZED ENZYME CASCADES FOR GLYCOCONJUGATE SYNTHESIS

Prof. Dr. Lothar Elling
RWTH Aachen

The translation of glycoconjugate multi-enzyme synthesis into larger scale is hampered by multi-parameter optimization of enzyme-modules. In this respect, nucleotide sugars are considered as bottleneck and expensive substrates for glycan synthesis with glycosyltransferases. In our project »The Golgi Glycan Factory 2.0 (GGF 2.0)« we have set up modular multi-enzyme cascades for the synthesis of sixteen different nucleotide sugars starting from monosaccharides and sucrose as substrates. Multiplexed CE (MP-CE) as fast analytical tool was established for optimization of reaction parameters [1]. An excellent space-time-yield of 17 g/L*h was obtained for the synthesis of UDP-α-D-galactose (UDP-Gal) [2]. The repetitive use of enzyme cascades in batch synthesis significantly increased productivity up to a multi-gram product scale [3]. A high mass based total turnover number (TTN_{mass}) of 494 g product/g enzyme and space-time-yield (STY) of 10.7 g/L*h gave 12.8 g UDP-Gal. Synthesis of UDP-*N*-acetylglucosamine (UDP-GlcNAc) in repetitive batch mode resulted in 11.9 g product with a TTN_{mass} of 522 g product/g enzyme and a STY of 9.9 g/L*h. Furthermore, scale-up to a 200 mL repetitive batch was key to the synthesis of 23.3 g UDP-*N*-acetylgalactosamine (UDP-GalNAc) with an exceptional high STY of 19.4 g/L*h. With these basic technologies, nucleotide sugars are readily available for *in vitro* Leloir-glycosyltransferase based glycoconjugate synthesis [4,5].

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TAYLORED SYSTEMS FOR HIGH PERFORMANCE GLYCOANALYSIS IN »GOLGI GLYCAN FACTORY 2.0«

Dr. Erdmann Rapp
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Owing to rising amount of known health benefits, the attention of science and nutrient industry focuses more and more on the human milk oligosaccharides (HMOs) - an essential part of breast milk research field. Diverse long-chained and more complex HMOs, glint as potential structures for biomedical and nutritional applications. However, they represent only a small part of the human milk composition. Apart from ethical issues, extraction from native samples in suitable amounts is not economical. Chemical synthesis of oligosaccharides is on the other hand extremely challenging, involving many complex synthesis reactions and resulting in relatively low overall yields. Additionally, structural diversity and complexity of HMOs pose a daunting analytical challenge. Therefore, the main goal of the Golgi Glycan Factory 2.0" (GGF 2.0) project is to tackle these challenges, focusing on effective routes for the synthesis of HMOs, software solutions for associated fast reaction screenings as well as analytical methods with high sensitivity, high selectivity and high resolution.

As a part of the BMBF-funded GGF 2.0 project, a modular system for the enzymatic synthesis of HMOs was developed and optimized. The sequential combination of enzyme modules including multi-enzyme cascades for nucleotide sugar synthesis and glycosyltransferase creates an efficient, flexible and economical synthesis route. We introduced high performance multiplexed capillary gel electrophoresis with laser-induced fluorescence detection (xCGE-LIF) technology as a powerful tool for the analysis of even challenging linkage isomers of the Lacto-N-biose-Type (LNTs) and Lacto-N-neo-Type (LNnTs). A further aim was to eliminate data analysis bottleneck when a multiplexed (96) capillary electrophoresis system with UV-detection (xCE-UV) is used for the analysis of nucleotides and nucleotide sugars from enzymatic cascade reactions from microtiter plates simultaneously.

Examples and benefits (incl. the developed software solutions) will be presented for both, the xCGE-LIF based fast monitoring of product formation in complex enzymatic reaction cascades including the verification of desired isomers, and the use of xCE-UV allowing for parallel monitoring of a whole set of enzyme kinetics, thus, for a fast optimization of the reaction parameters. The combination of the multiplexed analytical technologies and dedicated software with a flexible synthesis strategy according to the idea of an artificial Golgi-Glycan-Factory represents a high-potential platform technology to effectively tailor rare but potent HMOs structures and miscellaneous other complex carbohydrates.

CELL-FREE SYNTHESIS AND FUNCTIONAL ANALYSIS OF MEMBRANE PROTEINS AND GLYCOPROTEINS

Dr. Stefan Kubick
Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses IZI-BB

Cell-free protein synthesis (CFPS) is a versatile, flexible, fast and alternative approach for the synthesis of difficult-to-express proteins including glycoproteins, membrane proteins and toxic proteins. CFPS has a high degree of controllability and provides a completely open system allowing direct manipulation of the reaction conditions to optimize protein folding, disulfide bond formation, and incorporation of non-canonical amino acids. One can add detergents, liposomes or make use of the native endogenous microsomes for the solubilization and functional folding of the desired protein. Thus efficient use of CFPS helps in the economic production of functional proteins as active agents as well as targets for drug development.

We present an alternative method for the synthesis of active EPO with an engineered O-glycosylation site by combining cell-free protein synthesis and site-directed incorporation of non-canonical amino acids with subsequent chemoselective modifications. The cell-free synthesis system used in this study is based on lysates derived from cultured *Spodoptera frugiperda* (*Sf*21) cells. Mild lysate preparation ensures the endoplasmatic reticulum (ER) to survive in the form of small vesicles with active translocons. Therefore the system is capable to perform post-translational modifications such as the formation of disulfide bonds, signal peptide cleavage and N-glycosylation. We present the results of the *in vitro* synthesis of glycosylated human erythropoietin (EPO) in a eukaryotic cell-free system.

Based on the versatile properties of cell-free systems, high-throughput production of ion channels, pore-forming proteins and transporters becomes feasible. The subsequent functional investigation of clinically significant proteins paves the way to develop novel functional and pharmacological assays.

Publications:

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Methods Mol Biol. 2018; 1685: 171-186

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Scientific Reports 2018, Volume 8, Article number: 8514

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