Leibniz Bioactive Compounds and Biotechnology

Abstract Book

Leibniz Conference on Bioactive Compounds

September 27. 2021

IPB Leibniz Institute of Plant Biochemistry

It is a great pleasure…

to welcome you to the Leibniz Conference on Bioactive Compounds. The pandemic situation allows us to meet online: scientists from various disciplines present and discuss their latest research related to the topics of drug discovery, (non-)medical applications of bioactive compounds, method development, novel targets and biotechnology.

We hope you enjoy the program and we thank all for their contributions to this conference!

The organizing committee

Prof. Ludger A. Wessjohann (IPB)andDr. Anna Rusznyak (IPB)

And the speakers of the alliance

Prof. Ludger A. Wessjohann (IPB), Dr. Pierre Stallforth (HKI), and Dr. Dirk Janasek (ISAS)

LEIBNIZ RESEARCH ALLIANCE BIOACTIVE COMPOUNDS AND BIOTECHNOLOGY

Involving 16 institutions, the Leibniz Research Alliance Bioactive Compounds and Biotechnology bundles the Leibniz Association's broadly-based research on molecules with biological effects.

Speaker:
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Conference Program

09:00 - 09:15	**Welcome addresses** **Ludger A. Wessjohann** (IPB Bioorganic Chemistry) **Thomas Gutsmann** (Borstel Research Center)
Session Chair	*Ludger A. Wessjohann IPB Halle*
09:15 - 09:45	**Pierre Stallforth** HKI Jena *Bioactive natural products from interacting microorganisms*
09:45 – 10: 00	**Victoria Shpacovitch** ISAS Dortmund *Development of SPR-based immunoassays for measuring anti-CoV-2 antibodies and for detecting interactions between CoV-2 spike protein and virus entry receptor ACE2 at very low concentrations*
10:00– 10:20	**Andreas Brunschweiger** TU Dortmund *DNA-encoded chemical libraries: cheminformatics - reaction development - compound identification*
10:20 – 10:40	Meet the speakers
10:40 - 11:00	**Bernd Rupp** FMP Berlin *Wirkstoffradio and Leibniz News Portal – an update*
11:00 - 11:30	Meet the speaker
11:30 – 12:30	lunch break
Session Chair	*Dirk Janasek ISAS Dortmund*
12:30 – 12:50	**Frank Broda** IPB Halle *The Cloud Resource & Information Management System (CRIMSy): supporting collaboration and data management in the life sciences – the past, present & future*
12:50 - 13:00	Meet the speaker
13:00 – 13:20	**Yvonne Mast** DSMZ Braunschweig *SARP-driven activation of antibiotic gene clusters*
13:20 – 13:40	**Maik Behrens** Leibniz-LSB@TUM Freising *Substances isolated from bitter mushrooms activate a subset of human bitter taste receptors*
13:40 – 14:00	Meet the speakers
Session Chair	*Pierre Stallforth HKI Jena*
14:00 – 14:30	**Lackner et al.** HKI Jena – Leibniz Drug of the Year 2021 *Metabolomics-driven discovery of mycofactocin, a redox cofactor crucial for ethanol utilization by mycobacteria*
14:30 - 14:45	**Ulschan Bathe** - University of Florida *Producing libraries of diterpenoids by combinatorial biosynthesis in yeast for anticancer compound discovery*
14:45 - 15:00	**Ana Rodriguez Humpierre** IPB Halle *Expanding the scope of Ugi multicomponent bioconjugation to produce pneumococcal multivalent glycoconjugates as vaccine candidates*
15:00 – 15:15	**Vito Valiante** HKI Jena *Biosynthesis of sphingofungins in filamentous fungi*
15:15 - 15:45	Meet the speakers
15:45 - 16:00	Closing remarks and future plans: Leibniz Research Network
16:00 - 17:00	Further discussion (unmoderated)

Bioactive natural products from interacting microorganisms

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The search for new bioactive natural products has prompted scientists to exploit both environmental and organismal diversity. We describe our efforts regarding this endeavor and we provide an evolutionary perspective to natural product diversification as well as to access otherwise hidden natural products. In particular, we will describe a variety of Pseudomonas-derived natural products [1-4] (Fig. 1) that show potent bioactivity and we discuss their biosynthesis,[1,2] evolution,[3] and regulation.[4]

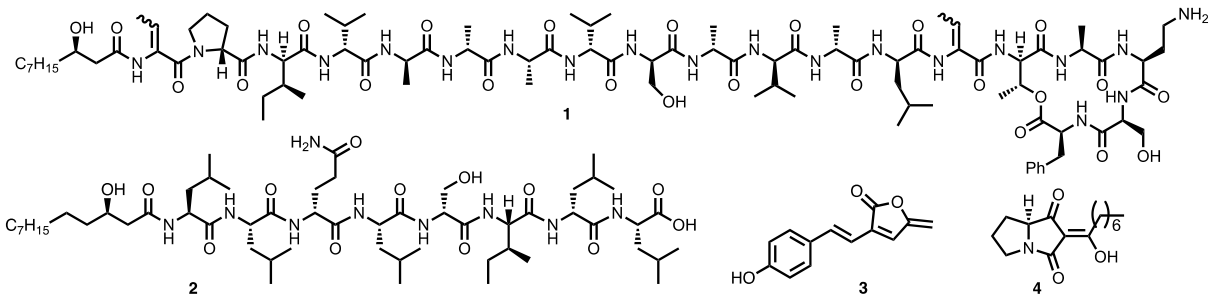

Fig. 1. Jessenipeptin A (1), virginiafacin A (2), styrolide A (3), and pyreudione A (4).

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Development of SPR-based immunoassays for measuring anti-CoV-2 antibodies and for detecting interactions between CoV-2 spike protein and virus entry receptor ACE2 at very low concentrations

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In the series of published works (Shpacovitch, 2015) (Shpacovitch, 2017) (Yayla, 2019), the power of surface-plasmon resonance (SPR)-based sensor in the detection of biological and non-biological nano-particles was successfully proved. Plasmon-assisted microscopy of nano-objects (PAMONO) technique was harnessed for a swift analysis of physical characteristics of viruses, virus-like particles and extracellular vesicles (EVs): for their sizing and quantification. Further, it was planned in the current project to validate the ability of the PAMONO-sensor to study protein-protein interactions (CoV2 S-protein/human ACE2 and CoV2 S-protein/anti-CoV2 antibodies) at very low concentrations. In order to attain this goal, it was necessary to validate analytical accuracy of the reference method - nano-particle tracking analysis (NTA) and to develop protein-nanoparticle conjugation protocols for the PAMONO-sensor. Thus, conducted introductory research activities served not only as a basis for the development of SPR-based immunoassays, but also revealed inherent features of a commonly used nano-particle analytical method-NTA analysis (Usfoor, 2020).

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DNA-encoded chemical libraries: cheminformatics - reaction development - compound identification

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DNA-encoded libraries of chemically synthesized compounds (DELs) are a widely used small molecule screening technology [1]. DELs are efficiently synthesized by encoded combinatorial chemistry that encompasses alternated enzymatic DNA tagging and preparative organic synthesis steps. In contrast to discrete screening libraries, encoded compound formats enable target-based screening for identification of bioactive compounds by selection of compound pools and subsequent barcode sequencing. Successful compound selection depends on both chemical space coverage and functionality of the genetic tag. The prime challenge in the field is the current lack of organic preparative methods for encoded compound synthesis. These need to tolerate water and keep the genetic information intact. Reactions mediated by low pH, oxidants, many metal ions and harsh reaction conditions harm DNA. We exploit heterogeneous reaction systems such as solid phase- and polymer micelle-based approaches to open access to a large scope of reactions on DNA-encoded starting materials [2-8]. A cheminformatics workflow that enables filtering chemistry databases for relevant reaction conditions and clustering of reactions has been developed to design DNA-encoded libraries. Screening of a proof-of-concept DNA-encoded library and analysis of DNA barcode sequencing data with an in-house-programmed algorithm led to identification of an inhibitor of the TEAD4-YAP protein-protein interaction.

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Wirkstoffradio and Leibniz News Portal (leibniz.fy) – an update

Silke Oßwald[1], Gisela Olias[2], Florian Köhler[3] and BerndRupp[1*]

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In the course of the digitization of scientific work, it has become necessary to equip all areas(research, science, administration and PR) with the necessary digital tools. Since neithersuitable tools nor a definitive data format are currently defined for publications from the area ofpress and public relations, this project first developed a suitable data structure. Then, this datastructure was equipped with the necessary "Graphical User Interface" (GUI) to reflect thecorresponding editorial workflow.

Currently, the implementation of the data structure is running on a suitable server and on thesite (leibniz.fyi) the related documentation of the data structure, the GUI and the related API isbeing created.Extensive testing of the GUI and automated data retrieval via the API is planned for Q4 2021and Q1 2022.In the talk, the goals or benefits of the developed environment and the current state ofdevelopment will be presented.In the second part of the talk, the current status of the "Wirkstoffradio" project will be presented.Here, the current range development will be demonstrated and ongoing or planned projects willbe summarized.

The Cloud Resource & Information Management System (CRIMSy): supporting collaboration and data management in the life sciences – the past, present & future

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Information integration among collaborating research groups is still challenging in many scientific disciplines. Repositories are invoked in late stages of the research data lifecycle, typically for publication and archiving. However, in early stages of research, there is a need for sharing unpublished documents and data among research groups within trustful digital environments, which ideally should support discipline specific data types and processes. Existing solutions often miss such a support and limit the data autonomy of their users.

With strong support by the Leibniz Research Alliance "Bioactive Compounds &Biotechnology" we were able to create the Leibniz Bioactives Cloud (LBAC) as an infrastructure, in which each participating institution maintains its own node with documents provided by its researchers. During the last two years we have extended the LBAC software code base to the Cloud Resource & Information Management System (CRIMSy), which tries to close the previously mentioned gap by providing a distributed data infrastructure for the life sciences. Federated search and document retrieval across the cloud has been designed with semantic integration and chemical understanding in mind and will also allow searches in biological sequence entities using established bioinformatics tools soon. We recently extended the software with a simple electronic lab notebook (ELN) and a chemistry-aware storage and inventory system to aid documentation of laboratory work. Fine grained permissions for user groups as well as individual users control access privileges, thus, each institution retains full control over its data. An institution's node can be member of multiple cloud instances and within each of these instances, trust is established via mutual certificate-based authentication. At the same time, a CRIMSy node is easy to administrate and requires only minor computational resources.

After a short introduction and a report on the current status of LBAC/CRIMSy in the Leibniz Research Alliance, this talk will proceed with an extensive live showcase of CRIMSy's capabilities ranging from LBAC's use cases related to document upload, search and retrieval to the new storage and inventory management and ELN functionalities. Project Website: https://github.com/ipb-halle/CRIMSy

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SARP-driven activation of antibiotic gene clusters

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Actinomycetes, particularly the genus *Streptomyces*, are the most versatile producers of natural compounds. They synthesize about two-thirds of all antibiotics known to date. The antibiotic biosynthesis in these organisms is highly coordinated and subject to diverse environmental and physiological (pre)conditions. Important nodes of regulation are represented by particular pleiotropic, as well as pathway-specific regulators. In my talk, I will give an example how pathway-specific SARP-type regulators can be used in order to exploit the biosynthetic potential of actinomycetes. Pristinamycin is a streptogramin antibiotic produced by *Streptomyces pristinaespiralis*, consisting of two types of chemically unrelated compounds (pristinamycin I and pristinamycin II), which together show synergistic activity. As the pristinamycinsignaling cascade includes all types of important transcriptional regulators, *S. pristinaespiralis* is a suitable model organism to analyze regulatory signaling pathways in antibiotic producers in general. Here, I will illustrate that understanding regulation processes on the one hand is important to optimize antibiotic production processes but also to targetedly activate silent gene cluster expression in order to find new natural compounds.

Substances isolated from bitter mushrooms activate a subset of human bitter taste receptors

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The number of compounds known to taste bitter to humans currently exceeds 1000 and a large fraction of these have been functionally tested in vitro for the activation of individual bitter taste receptors. Among these substances are numerous synthetic molecules, a plethora of plant metabolites, some compounds originate from animal sources or are bacterially-produced, however, not a single substance from bitter mushrooms has been tested so far, even though some of these inedible but non-toxic mushrooms are famous for their bitterness. As a consequence, the chemical space analyzed for bitter substances might be much larger than anticipated bearing the chance to identify new bitter principles, deorphanize additional bitter taste receptors and to discover ultra-potent agonists for research and drug development.

Therefore, we initiated a collaboration devoted to the identification and purification of bitter principles from mushrooms followed by functional screening of those substances for the activation of human bitter taste receptors. The two mushroom species *Cortinarius infractus* (Bitter webcab) and *Tyromyces stipticus* (Bitter bracket) were subjected to sensory-guided methanol extraction followed by taste-guided isolation of bitter compounds using different chromatography techniques (IPB, Halle). While the structure determinations are in part still ongoing, the highly purified compounds were used for the screening of the 25 human bitter taste receptors (TAS2Rs) by calcium mobilization assays in HEK 293T-Gα16gust44 cells (LSB, Freising).

For all of the six so far isolated bitter compounds responsive TAS2Rs were identified. Whereas one compound, called infractopicrin, activated only a single TAS2R, the TAS2R14, another substance elicited responses from the five receptors, TAS2R1, TAS2R4, TAS2R14, TAS2R41, and TAS2R46. The remaining substances activated subsets of the latter 5 receptors suggesting that this group of TAS2Rs may dominate the perception of mushroom bitter compounds. One substance activated the receptor TAS2R46 at a sub-micromolar concentration matching the, so far, highest detected potency of strychnine at the same receptor. This result indicates that the continuation of such experiments in the future could indeed result in the discovery of ultra-potent bitter compounds from mushrooms.

Since human bitter taste receptors are expressed in numerous extraoral tissues including the airways, the GI tract, brain, and heart, bitter activators do not only represent tastants, but result in physiological TAS2R-mediated responses. Hence, novel and potent bitter activators might represent interesting bioactive compounds useful for medical use as well as important tools for research.

The research was funded in part by a seed-money grant from the Leibniz Research Alliance “Bioactive Compounds and Biotechnology” (to N.A. and M.B., 2019).

Leibniz Drug of the Year 2021

Metabolomics-driven discovery of mycofactocin, a redox cofactor crucial for ethanol utilization by mycobacteria

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Mycofactocin (MFT) is a redox cofactor crucial for ethanol metabolism of mycobacteria including *Mycobacterium tuberculosis*, thus representing a potential drug target. After being postulated only based on a bioinformatics study [1], a preliminary biosynthetic pathway to MFT had been established by *in-vitro* investigations in recent years. However, the chemical structure of natural MFT and key biosynthetic steps remained elusive.
Here, we report the discovery of glycosylated MFT in *Mycolicibacterium smegmatis* by metabolomics and establish a model of its biosynthesis. Structure elucidation using MS/MS, NMR, and enzymatic degradation suggested that MFT is decorated with an oligosaccharide composed of up to nine *β*-1,4-linked glucose residues including 2-*O*-methylglucose [2]. Inactivation of biosynthetic genes demonstrated that the oligoglycosylation is catalyzed by the glycosyltransferase MftF. Furthermore, we demonstrate that glycosylated MFTs can act as a cofactor of a carveol dehydrogenase by activity-based metabolic profiling and show that MFT levels increase during cultivation on ethanol.
These results enable future studies into the physiological roles of MFT in pathogenic mycobacteria as well as its potential exploitation as a drug target.

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Producing libraries of diterpenoids by combinatorial biosynthesis in yeast for anticancer compound discovery

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Natural products and their derivatives constitute a significant part of human pharmacopeia and regularly provide novel drugs. Nonetheless, the potential of natural products is not fully exploited due to low concentration, the difficulty to access specific hosts and to synthesize these complex compounds. In parallel, enormous progress has been made in metabolic engineering of natural products in microorganisms such as *Escherichia coli* or yeast (*Saccharomyces cerevisiae*). For the expression of natural product pathways requiring cytochrome P450 oxygenases (CYPs), e.g. from plants, yeast is a suitable host due the presence of intracellular membrane compartments. For this reason, we chose yeast as a platform for engineering diterpenoid biosynthesis. In previous work, we could show that yeast was a suitable host for the engineering of abietanediterpenoids and could identify novel compounds by combining CYP enzymes from related Lamiaceae species (Scheler et al., 2016; Bathe et al., 2019). In this project, we aim at expanding the range of diterpenoids produced in yeast by expressing all known diterpene synthases and combining them with CYPs known to act on diterpene backbones. Using available genetic information about plant, bacterial and fungal diterpenoid biosynthesis and a synthetic biology approach based on Golden Gate cloning, we generate libraries of genes encoding candidate enzymes involved in those pathways. These include 85 diterpene synthases of class I and class II and 50 CYPs. A high-throughput mass spectrometry screening detects which gene combinations generate new products. After fractionation, the novel metabolites will be tested for activity against cancer cell lines and the structure of compounds with activity will be determined and their mode of action on tumor cells extensively characterized.

As a proof of concept we co-expressed a selected set of diterpene synthases and CYPs in yeast. The candidate genes include ten diterpene synthases from various plant species that cyclized the diterpenoid precursor (+)-copalyl diphosphate to produce in total 16 bi- and tricyclic diterpene skeletons. These served as substrates for four CYPs from conifers, rice, rosemary and sage which have been reported to be involved in specialized diterpene biosynthesis. In a number of combinations with diterpene synthases and CYP from different species, products with oxidations at up to three distinct positions on the diterpene backbone could be observed. This confirms the substrate flexibility of the selected CYPs. Finding out whether the produced compounds are “new-to-nature” or have not been reported yet, will require purification and structure elucidation by NMR. After purification of the products, we will perform activity tests against cancer cell lines.

Expanding the scope of Ugimulticomponent bioconjugation to produce pneumococcal multivalent glycoconjugates as vaccine candidates

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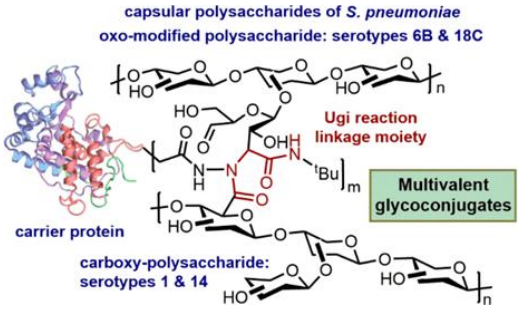

Streptococcus pneumoniae -one of the main causative agents of pneumonia- yearly accounts for more than 500000 deaths in children under 5 years old. Glycoconjugate vaccines confer a long-lasting immune response and efficiently prevent bacterial infections. On the other side, multivalent formulations can provide broad serotype coverage. This work broadens the scope of an efficient multicomponent strategy, leading to multivalent pneumococcal glycoconjugates assembled in a single synthetic operation. The bioconjugation method, based on the Ugi four-component reaction (4CR), enables the one-pot incorporation of two different polysaccharide antigens to a tetanus toxoid carrier, thus representing the fastest approach to achieve multivalency. Glycoconjugateswere characterized using SE-HLPC and NMR. The immunization was carried out in animal models, and the immunogenicity and antigenicity assays were performed by ELISA techniques. The Ugi 4CR proved efficient at the simultaneous conjugation of different oxo-functionalized CPs, such as CPs 6B and 18C, and carboxy-functionalized CPs, such as TEMPO-oxidized CPs 14 and 1 from *S. pneumoniae* to tetanus toxoid. The final carbohydrate/protein ratio and the amount of non-conjugated protein in all conjugates are within the range for commercial antibacterial vaccines recommended by the WHO. The glycoconjugates were able to elicit functional specific antibodies against pneumococcal strains comparable to those shown by mixtures of the two monovalent glycoconjugates.

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Biosynthesis of sphingofungins in filamentous fungi

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Sphingofungins are sphingolipid inhibitors produced by fungi, which specifically inhibit serine palmitoyl transferases, enzymes catalyzing the initial step during sphingolipid biosynthesis. Sphingolipids are integral parts of the eukaryotic cell membrane. Disturbances in sphingolipid biosynthesis have been connected to a wide range of human diseases including obesity, diabetes, and even neurodegenerative disorders including Alzheimer's disease and schizophrenia. It has been suggested that external interventions, via sphingolipid inhibitors, may represent a promising approach for alternative therapies.

We identified and fully characterized the biosynthesis of sphingofungins in the human pathogenic fungus *Aspergillus fumigatus*. In vivo experiments identified novel intermediates, and additionally in vitro analyses revealed that sphingofungin biosynthesis starts with the condensation of a C18 polyketide with the uncommon substrate aminomalonate. Moreover, aside of identifying the role of every biosynthetic gene, we established an enzymatic assay to determine serine palmitoyl transferase inhibition, improving the workflow for the characterization of the related inhibitors.