```
class Data4Omics_Symposium {
      String Date = “1st June, 2023”;
      String Location = “Tulln, Lower Austria”;

      public void Book_Of_Abstracts() {
            this.Gather_Abstracts();
            book = this.Write_Book_Of_Abstracts();
            Copy2Website(
                  “https://metabolomics-ifa.boku.ac.at/
                   Data4Omics_Symposium/BookOfAbstracts.pdf”,
                  book);

            // Have a pleasant symposium!

            return;
      }
}
```

Funding

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Agenda

9.00-10.00h	**Registration and welcome coffee**
10.00-10.10h	**Welcome**
Session 1:	Chairs: Rainer Schuhmacher, Jonathan Samson
10.10-10.50h	**Gerhard Thallinger** (Keynote): *Reliable metabolite identification: From spectrum scoring to decision rules to deep learning*
10.50-11.10h	**Kevin Mildau**: *specXplore: An interactive dashboard for flexible exploration of mass spectral data*
11.10-11.30h	**Markus Riedl**: *Comprehensive meta-analysis of the CHO coding transcriptome*
11.30-11.50h	**Roman Ufimov**: *ParalogWizard: A novel method for detecting and incorporating paralogues in phylogenetic tree inference from target enrichment data*
11.50-13.20h	**Lunch and poster session**
Session 2:	Chairs: Christoph Büschl, Markus Aigensberger
13.20-14.00h	**Thomas Schwarzl** (Keynote): *Bioinformatics binding site detection of enigmatic RNA-binding enzymes*
14.00-14.20h	**Markus Aigensberger**: *Comparison of untargeted metabolomics processing software, data treatment, & statistical analysis methods*
14.20-14.40h	**Jonathan Samson**: *TopN Simulator: A software tool for the optimization of data dependent analysis settings*
14.40-15.00h	**Katharina Munk**: *Holomics: an R Shiny application for the holistic integration and analysis of multiple omics data*
15.00-15.30h	**Coffee break and posters**
Session 3:	Chairs: Günter Brader, Katharina Munk
15.30-16.10h	**Ulrik Sundekilde** (Keynote): *Using multiomics to gain insight into the mother-milk-infant triad*
16.10-16.30h	**Muhammad Ahmad**: *Integrating high-throughput phenotyping with transcriptomics and metabolomics to gain insight into drought stress responses in Norway spruce seedlings*
16.30-16.50h	**Michael Santangeli**: *Deciphering the interplay of plant gene expression, root exudation and soil microbial community in the rhizosphere of field-grown maize across the vegetation period*
16.50-17.10h	***Anela Tosevska**: Cytokine-derived transcriptomic signatures, cellular crosstalk and synovial pathotypes in rheumatoid arthritis*
17.10-17.25h	**Poster award and concluding remarks**
17.30h – open end	**Closing reception with snacks and drinks**

Invited Speakers

Keynote 1:
Reliable metabolite identification: From spectrum scoring to decision rules to deep learning

Dr. Gerhard Thallinger
Principal Investigator at Thallinger Lab TU Graz
Foto (c) Lunghammer-TU Graz

Keynote 2:
Bioinformatics binding site detection of enigmatic RNA-binding enzymes

Dr. Thomas Schwarzl
Research Staff Scientist at EMBL Heidelberg, Hentze group

Keynote 3:
Using multiomics to gain insight into the mother-milk-infant triad

Dr. Ulrik Sundekilde
Researcher at DEPARTMENT OF FOOD SCIENCE, Aarhus University

Content

Talks

Posters

Talk 1:
specXplore: An interactive dashboard for flexible exploration of mass spectral data

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Untargeted metabolomics promises to elucidate the metabolic composition of diverse chemical matrices ranging from human, plant, and fungal tissue samples to environmental probes. However, current workflows are hampered by poor annotation rates of compounds achieved via library matching alone. Here, computational metabolomics tools such as molecular networking play an important role in assisting researchers in prioritization and spectral comparison of measured spectra. In this research, we present *specXplore*, an interactive dashboard providing a flexible means of spectral data exploration complementary to molecular networking.
specXplore makes use of *ms2deepscore* machine learning-based embeddings as the foundation for similarity matrix computation and graph construction. This allows for graphs to more closely correspond to those based on chemical structural fingerprint similarity and, correspondingly, can lead to better analog grouping. Since machine learning-based graphs tend to be denser, alternative visualization approaches to molecular families were implemented to allow for a more efficient and interactive local and global data exploration. Specifically, a t-SNE based embedding of the graph is used for global data representation, while different node-link diagram visualizations are used for detailed inspection of local clusters. *specXplore's* data processing protocols further provide means of including chemical classifications, which in turn provide a straightforward means of finding chemical spaces of interest.
specXplore is a hybrid of 1) a data processing pipeline implemented via a Jupyter Notebook, and 2) an interactive dashboard for data visualization and inspection. Furthermore, it's machine-learning-based similarity scores used as foundation for molecular networking allow for more effective analog finding, while its flexible data visualizations and on the fly setting modifiability make it suitable to explore both sparse and dense similarity graphs.

Keywords: mass spectral molecular networking, exploratory data analysis, computational metabolomics, interactive visualization, untargeted metabolomics

Talk 2:
Comprehensive meta-analysis of the CHO coding transcriptome

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Chinese Hamster Ovary (CHO) cells are amongst the most important cell factories in biotechnology. They are invaluable in the production of biopharmaceutical products and therefore steadily developed to improve yield and product quality through cellular engineering and by optimizing bioprocesses. High-throughput omics technologies have drastically influenced these endeavours by enabling a comprehensive molecular insight at multiple levels, leading to more rational and informed engineering decisions. Oftentimes, such datasets are created to elucidate one specific biological question, and the potential to put these individual datasets into greater context in left unused. With numerous RNA-seq datasets accumulated, we aim to seize the opportunity to conduct a large-scale meta-analysis of the CHO transcriptome to study gene expression across various cell lines and culture conditions. We used publicly available RNA-seq datasets as well as several datasets that were produced in-house. The datasets span a wide variety of different cell lines, culture conditions and growth phases as well as different sequencing approaches and sequencing depths. This unique heterogeneity, however, requires careful consideration of each individual data processing step. Starting from raw reads in FASTQ format, we consistently process all datasets through a reproducible workflow that incorporates state-of-the-art bioinformatic tools. RNA-seq reads were aligned and quantified using the most recent reference genome assembly of the Chinese Hamster. Our approach involves novel batch adjustment techniques and sophisticated normalization methods in order to address the challenges arising from inhomogeneous data. Finally, custom-developed R scripts assess gene expression patterns across several biological conditions of various cell lines.
Our work aims to elucidate the complex circuitry of the CHO coding transcriptome. We conduct a large-scale investigation of the gene expression patterns across various cell lines under different biological conditions. This meta-analysis of RNA-seq data can reveal transcriptomic programs that cohere with industrially relevant phenotypes and/or differences that are specific to culture conditions or recombinant cell lines. On the long term, we aim for extensive insights into the regulatory circuits that ultimately determine gene expression, and in consequence the phenotype of CHO cells. With our approach, we demonstrate the vast potential of publicly available omics datasets for elucidating molecular and phenotypic relations on a large scale. The pipeline is end-to-end reproducible and can be easily adapted for similar intentions.

Keywords: Transcriptomics, RNA-seq, Meta-analysis, Biotechnology, CHO

Talk 3:
ParalogWizard: A novel method for detecting and incorporating paralogues in phylogenetic tree inference from target enrichment data

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Target enrichment data is becoming an increasingly popular tool in the field of phylogenetics. It involves the capture and sequencing of specific genomic regions from multiple individuals or species, providing a cost-effective alternative to whole-genome sequencing. However, analysing target enrichment data presents some challenges, one of which is the presence of paralogous sequences. Paralogs are genes that are related through gene duplication events. When two genes are duplicated, they are initially identical, but over time they can evolve in different ways. This means that the two genes may have different evolutionary histories, which can lead to conflicting signals in phylogenetic analyses. To address this challenge, a novel method for detecting and incorporating paralogous genes into phylogenetic tree inference has been developed. This method involves obtaining both orthologous and paralogous copies of genes and then creating orthologous alignments to build gene trees. This approach has been implemented in ParalogWizard. To test the efficacy of ParalogWizard, it was used to analyse target enrichment data from three different plant groups that have undergone whole-genome duplication events: the subtribe Malinae (family Rosaceae), the genus *Oritrophium* (family Asteraceae), and the genus *Amomum* (family Zingiberaceae). We used Angiosperms353 and Malinae481 probes for the Malinae group, Compositae1061 probes for *Oritrophium*, and Zingiberaceae1180 probes for *Amomum*. The results showed that incorporating paralogous genes into the phylogenetic analysis led to a reduction in gene tree discordance and an increase in species tree support in the Malinae group, but not in the other two groups. This may be due to the proportion of paralogous loci being higher in the Malinae data set. The implementation of ParalogWizard can aid researchers in identifying paralogous genes and integrating them into the phylogenetic analysis.

Keywords: angiosperms; bioinfomatics/phyloinfomatics; paralogy; species tree

Talk 4:
Comparison of untargeted metabolomics processing software, data treatment, and statistical analysis methods

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Untargeted metabolomics describes the analysis of small molecules in biological samples without prior knowledge about their composition. Liquid chromatography coupled to mass spectrometry, one of the most popular techniques for untargeted metabolomics analysis, generates highly complex data. Thus, bioinformatic tools are necessary for data processing. While there is a variety of options for processing raw data, data treatment, and statistical analysis, no general standard exists.
The aim of this project is to compare different platforms for raw data processing, data treatment, and statistical analysis. Bovine saliva extracts were spiked with a mixture of 42 compounds including carboxylic acids, nucleotides, and sugar phosphates at three different concentration levels (300; 100; 30 ng/mL). The spiked and native samples were analysed by anion exchange chromatography coupled to high resolution mass spectrometry (HR-MS) on an Orbitrap instrument. The raw data were processed using Compound Discoverer (CD, Thermo Scientific), XCMS (open source R package), and MS-DIAL (RIKEN). The resulting data in form of tables with feature information (accurate mass and retention time) as well as abundance information (peak areas) were imported to RStudio. Different options for imputing missing peak areas were applied. The data then were either kept as they were, or transformed using log10-, square-root-, or cube-root-transformation. Data were scaled using either auto-, pareto-, level-, range-, or vast-scale. Primary component analysis (PCA) was used to assess the impact of each processing step on the grouping in scores plots. Partially least squares discrimination analysis (PLS-DA), as well as univariate statistics were used to determine significant features between groups. The number of correctly recovered compounds was used to analyse the impact of each processing step.
The imputation technique had no major impact on the results. Transforming the data alters the grouping in PCA but did not notably impact the statistical analysis. Interestingly, the scaling method altered the appearance of the PCA scores plot, however, it had no impact on further statistical analysis. All three platforms produced sound data where the statistical methods were able to recover most of the spiked compounds. MS-DIAL was able to recover all of the spiked compounds. CD has the most sophisticated user interface for interacting with the results. XCMS is the most complicated tool to learn, but has the most versatile applications.

Keywords: metabolomics, workflows, comparison, data treatment, data processing

Talk 5:
TopN Simulator: A software tool for the optimization of data dependent analysis settings

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Untargeted metabolomics aims to detect and identify as many metabolites present in a sample as possible, and liquid chromatography paired with high-resolution mass spectrometry (LC-HRMS/MS) is often used towards this goal. This approach separates ions by time through the chromatography phase, and by mass-to-charge ratio (m/z) through the MS phase. For compound annotation and identification, ions must be fragmented, and the fragmentation patterns can be compared to known compounds. In an unknown sample, however, selection of precursor ions for this fragmentation step must be performed globally. The most common method to this end is data-dependant acquisition (DDA). With this approach, precursor ions are selected and fragmented individually “on the fly” based on their intensity, allowing a direct link between fragment spectrum and precursor ion. DDA uses a variety of parameters (loop count, dynamic exclusion window, isotopologue deconvolution, etc), and a typical run can take 20 minutes or more. As there are so many possible combinations for the TopN parameters, optimizing them for each sample is currently very tedious, and would take a lot of measurement time. In order to streamline this process, we have developed DDASim, a software tool that simulates precursor ion selection in silico using a previously-generated full scan of the sample. Depending on the sample and parameters, simulations can take less than a minute, saving time, and also provide the user with plots of precursor ion intensity, and if a peak table is provided as a ground truth, receiver operator curves can be generated, so the user can determine which settings are best suited for DDA measurements.

Keywords: Metabolomics, Untargeted, DDA, TopN

Talk 6:
Holomics: an R Shiny application for the holistic integration and analysis of multiple omics data

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Background: Due to rapid technological development, a vast amount of data is currently being generated from different omics domains. However, analyses are often still performed separately. This opens the door for multi-omics analyses, as these integrate multiple omics datasets to understand biological processes in a holistic way. In many cases, in-depth bioinformatics knowledge is required to use the available tools. To make multi-omics analyses accessible to a broader scientific community, we have developed Holomics.
Results: Holomics is an R Shiny application built on the R package mixOmics, allowing both single- and multi-omics analyses. Holomics provides an easy-to-follow graphical user interface, guiding through the general workflow, ranging from single-omics analyses of the uploaded omics datasets to performing feature selection of these datasets and pairwise and multi-omics analyses. The results of each analysis are presented in a variety of graphs, from simple sample and variable graphs to more complicated graphs, such as the circos plot, which highlights correlations between omics datasets.
Conclusion: As an R Shiny application, Holomics provides a user-friendly and easy way to analyse omics datasets, from single-omics analyses to integrative multi-omics analyses.

Keywords: Multi-omics; Data analysis; R Shiny application

Talk 7:
Integrating high-throughput phenotyping with transcriptomics and metabolomics to gain insight into drought stress responses in Norway spruce seedlings

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Drought stress is a critical factor limiting the current and future distribution of Norway spruce (*Picea abies* (L.) H. Karst.). It is therefore important to investigate which spruce populations are better adapted to drought and the molecular basis of this adaptation. Provenance trials, which have traditionally been used to determine whether populations differ in adaptive variation, are very limited, firstly because of the limited number of sites and seed sources included, and secondly, because they are inherently very labour-intensive. Here, for the first time, we established a high-throughput plant phenotyping (HTPP) method to assess the adaptive potential of Norway spruce at the seedling stage, the most critical stage in the tree's life. We applied the HTPP method to two climatically contrasting populations (provenances) of Norway spruce by exposing them to a 21-day drought stress period under strictly controlled environmental conditions. We further linked the phenomic data with needle transcriptomic and metabolomic profiles to gain deeper insight into the mechanistic basis of the observed phenotypes. Our multivariate analysis shows that more than 50 phenotypic traits derived from the multi-sensor phenomics platform can discriminate between early and late drought stress responses in Norway spruce seedlings and distinguish between populations. In agreement with the phenomics, we obtained similar patterns, with metabolic profile modulations being more pronounced towards the end of the drought stress period. Furthermore, each population showed distinct metabolic signatures under well-watered and drought-stressed conditions, further corroborating our phenomics data. Finally, the mRNA-seq data revealed more than 4000 differentially expressed genes in drought-stressed seedlings as compared to well-watered plants. The majority of the differentially expressed genes appeared to be shared between the two populations. However, a small proportion of differentially expressed genes were provenance-specific, which is consistent with the differences observed between the two populations in the phenomics and metabolomics datasets. Overall, our work has laid the groundwork for assessing the drought sensitivity of natural populations in a high-throughput manner and for elucidating the molecular mechanisms underlying the responses of Norway spruce seedlings to drought stress.

Keywords: Norway spruce; Phenotyping; Drought stress; Metabolomics; Transcriptomics

Talk 8:
Deciphering the interplay of plant gene expression, root exudation and soil microbial community in the rhizosphere of field-grown maize across the vegetation period

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Plant root exudates play a key role in fuelling rhizosphere processes and their feedback loops as they mediate the cross-talk between plants, the microbiome, and soil and in turn also affect plant growth and development. Multi-omics data integration can be used to unravel the dynamic interplay of the different factors driving rhizosphere processes. However, the successful application of such an integrated approach depends on the appropriate statistical model used to analyze the different rhizosphere-related datasets. In the present study, we investigated the spatiotemporal dynamics of the rhizosphere environment in field conditions by multi-omics analyses (non-targeted metabolomics, microbiome, and transcriptomic) of two maize genotypes, grown in two different substrates, across three different developmental stages. By conducting a joint sampling campaign and applying a unified, machine learning technique (random forest), we identified maize genes, root exudate metabolites and members of the rhizosphere prokaryote microbiome that explain the differences by maize development and soil substrate. Our results revealed that plant growth stage was the strongest driver affecting root gene expression and root exudation, with the rhizosphere prokaryote microbiome also being equally affected by growth stage and substrate. Our field joint-omics approach supports the view that much of the differences in maize rhizosphere microbiome are caused by the differential production of secondary metabolites, and that part of the changes reflected in root exudation is also present in the gene expression dataset.

Keywords: non-targeted metabolomics, machine learning, field experiment, multi-omics

Talk 9:
Cytokine-derived transcriptomic signatures, cellular crosstalk and synovial pathotypes in rheumatoid arthritis

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There has been a growing interest in investigating the structural reorganization of the synovium in rheumatoid arthritis (RA) with a specific focus on the expansion of fibroblast-like synoviocytes (FLS) and the influx of immune cells. In this study, we employed multi-source transcriptomic and phenotypic data integration to explore the interactions between activated FLS and immune cells, which play a crucial role in synovial tissue remodeling and contribute to distinct synovial pathotypes observed in RA.
Cytokine-treated FLS and co-cultured T cells were subjected to flow cytometry and RNA-Seq to characterize their phenotypes and transcriptomic profiles, respectively. These datasets were then integrated with publicly available bulk and single cell RNA-Seq data obtained from RA patients with different histological synovial pathotypes. To further elucidate the cell-cell interactions, we performed computational prediction analyses and knock-down experiments targeting adhesion molecules in FLS. These experiments aimed to identify the critical adhesion molecules that mediate the physical interactions between FLS and immune cells.

By combining the results of phenotypic profiling, RNA-Seq analysis, computational predictions, and knock-down experiments, we gained valuable insights into the intricate network of cell-cell interactions contributing to synovial tissue remodeling in RA. The integrated analysis allowed us to uncover key molecular signatures associated with FLS and immune cell interactions. The integration of publicly available transcriptomic data from RA patients with different synovial pathotypes allowed us to validate and refine our findings in an *in vivo* context.
Overall, this study highlights the power of integrating multi-source transcriptomics in unraveling the complex interactions between activated FLS and immune cells, shedding light on the underlying mechanisms driving synovial tissue remodeling in rheumatoid arthritis.

Keywords: Transcriptomic Signatures, Rheumatoid Arthritis, Cell Interaction, Cytokines

Poster 1:
Metabolomics of the skin surface

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Metabolomics of the skin surface can provide valuable information about the metabolic activity of skin cells and microorganisms living on the skin and the effects of environmental factors. The applications for skin surface metabolomics range from skin health/disease diagnostics and understanding skin aging to skin microbiome research. Overall, metabolomics of the skin surface has the potential to provide valuable insights into skin health and disease and can even lead to the development of new skin products and diagnostic tools. The sampling is non-invasive and very simple.
Presented are data from the initial – proof of concept – experiment. Samples were collected from 4 persons over the course of three days. Samples were taken by swabbing a small area (5×5 cm) of hand or forehead. The swabs can be conveniently stored at -20 °C until the analysis. For analysis, the metabolites were extracted by water containing 0.15 % (v/v) formic acid in a sonication bath. The extracts were analyzed by LC-HRMS. Obtained data were processed by Compound Discoverer Software (3.3 SP2 2023, Thermo Fisher).
Using principal component analysis, it was possible to separate samples from different individuals into clusters. Also, samples contaminated by hand lotion did not cluster with other samples. This protocol will be extended to include also more hydrophobic compounds, which are not soluble in water.

Keywords: metabolomics, LC-MS, skin surface, sampling, sample preparation

Poster 2:
Untargeted Metabolomics: How to Tackle the Challenge

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Untargeted metabolomics approaches using Liquid chromatography high-resolution mass spectrometry (LC-HRMS) is a powerful technique for the comprehensive analysis of metabolites in biological systems. However, the enormous amount of data generated by LC-HRMS instruments is a major challenge for untargeted metabolomics approaches. The data generated includes not only the desired signals from the biochemical constituents but also background signals, electronic noise, or contaminants, making it difficult to accurately identify metabolites of interest. Furthermore, when biological compounds are detected, the majority remains unknown.
Stable isotopic labelling has emerged as a valuable tool to help address this challenge. By labelling metabolites with stable isotopes, it is possible to filter biological signals from other signals based on successful recognition of labelling-specific isotope patterns in the generated LC-HRMS/MS data. In this work, we applied a tracer labelling approach to study the response of plants to pathogen attack. It allowed us to gain untargeted insights into the phenylalanine-derived submetabolome of wheat plants upon pathogen attack and enabled us to efficiently filter out noise- and contaminant-derived signals from the LC-HRMS data, resulting in improved detection, annotation and identification of a large number of phenylalanine-derived metabolites. By comparing the metabolite profiles of treated and untreated plants, we found different classes of Phe-derived metabolites to be involved in the plant defence response to pathogen attack. Several metabolites extracted from wheat ear material were related to their function as metabolic precursors for cell wall reinforcement. In a follow up study, a protocol for cell wall hydrolysis was developed and used to assign Phe-derived constituents that were released from the cell wall. Interestingly a number of them were significantly differing in abundance upon pathogen treatment. This confirms cell wall reinforcement via Phe-derived polyphenols to be a defence- and resistance mechanism of wheat plants upon attack by the plant pathogenic fungus F. graminearum.
This work illustrates major challenges of LC-HRMS based untargeted metabolomics and exemplifies the great potential of stable isotope-assisted techniques to improve existing untargeted metabolomics approaches. The presented methods are well suited to obtain untargeted, yet reliable and comprehensive, guided insights into the metabolome of plants and other biological systems.

Keywords: LC-HRMS, stable isotope, wheat, plant defence, cell wall

Poster 3:
Untargeted metabolomics approach enabling the separate investigation of the attack of *Fusarium graminearum* during Fusarium Head Blight on wheat - plant microbe interaction

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Metabolomics studies of plant-microbe interactions are largely hampered by the intimate physical association and potential commonality of metabolites of the respective microorganism and its host. Here, we present a novel liquid chromatography-high resolution mass spectrometry (LC-HRMS) approach for the untargeted screening of microbe-derived metabolites in colonized plant tissue. To this end, a reference list of metabolic features is generated with the software MetExtractII [1] from mixtures of globally 13C labelled and native culture samples of the plant pathogenic fungus *Fusarium graminearum* (Fg) as well as mixtures of 13C labelled and unlabelled wheat plant samples. Next, LC-HRMS data of the biological plant-microbe experiment under investigation are matched against the reference features with the tool MetMatch [2] and filtered with the aim to assign all microbe-derived metabolites. To exemplify the developed workflow, flowering wheat ears were treated with spores of Fg in a time series experiment. Among the 1485 detected low molecular weight biochemical constituents, 100 of these were suggested as Fg-derived metabolites in plant-fungus interaction based on the applied matching- and filtering criteria.
With the aim of confirming the annotation of these assumed metabolites, a more detailed classification process was established. First, the number of potential metabolites was further narrowed down by literature research, keeping only unique metabolite entries, which were then verified with the help of LC-HRMS/MS data of the biological plant-microbe experiment. As indicators of quality, the mass accuracy of the precursor ions (m/z values) from the LC-HRMS measurements, the respective peak shapes, as well as retention time shifts and signal intensities within certain thresholds were taken in consideration. Finally, fragment ions of metabolite entries were compared with standards and reference data from databases, such as MassBank [3] among others. Through this process it was possible to confirm the detection of known Fg metabolites like trichothecenes, culmorins and others in the plant-fungus interaction.

Keywords: Fusarium Head Blight; fungal attack, untargeted metabolomics, time series

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Poster 4:
Efficient Enumeration of Elementary Conversion Modes in Large Metabolic Models

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Metabolic modeling is a powerful tool for studying the intricate web of biochemical reactions that enable living cells to function. However, when it comes to large metabolic models, the sheer number of possible steady-state flux distributions becomes overwhelming, making analysis difficult. One solution is to use elementary conversion modes (ECMs), which capture all the possible ways that input molecules can be transformed into output molecules, without concerning oneself with the details of individual metabolic reactions.
In this study, we present an improved method for computing ECMs that overcomes the challenges of previous approaches, which were limited by high memory requirements and lack of parallelization. Specifically, we integrated the mplrs parallel vertex enumeration method into the ecmtool software package and further optimized the method to make it faster and more efficient for computing ECMs in large metabolic models. This improvement enables efficient computation of ECMs using standard and high-performance computing environments.
To illustrate the effectiveness of our method, we applied it to compute all feasible ECMs in the complete metabolic model of the synthetic minimal cell JCVI-syn3.0, which, despite its simplicity, yields over 4 billion ECMs. Our findings demonstrate that our approach significantly reduces computational time and memory requirements, making exploring the vast space of possible flux distributions in larger metabolic models feasible. This scaling step paves the way to studying emerging properties of microbial metabolic interactions in (small) communities in an unbiased manner, which has important implications for understanding and engineering microbial ecosystems.
In conclusion, our improved method for computing ECMs represents a significant advance in unbiasedly characterizing cellular metabolism, offering researchers a powerful tool for investigating complex metabolic networks in health and disease and for studying microbial communities.

Keywords: Vertex enumeration; High-performance computing; Metabolic interactions

Poster 5:
Improving cell factories with the combination of kinetic and proteomics data in GEMs

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Genome-scale metabolic models (GEMs) are computational representations of an organism's metabolism that utilize gene-protein-reaction associations and experimental data to establish mass-balanced relationships between metabolites. GEMs are primarily used to analyze metabolic fluxes, with Flux Balance Analysis (FBA) being the most commonly used method. As high-throughput technologies have provided an abundance of omics data, GEMs have been enriched with valuable information to enhance their quality and applications, leading to a deeper understanding of metabolism in various organisms. The practical applications of GEMs include drug targeting, predicting enzyme functions, understanding human diseases, modeling interactions between multiple cells or organisms, and strain development for bio-based chemical and material production.
This study focuses on *Bacillus subtilis*, a bacterium with the potential to produce several metabolites, including scyllo-inositol, which is a potential treatment for Alzheimer's disease. The ec_iYO844 model of *B. subtilis* has recently been improved with kinetic-proteomic constraints to provide better flux predictions. However, previous genetic modifications for scyllo-inositol production were based solely on myo-inositol catabolism and were conducted in a trial-and-error manner. Therefore, the objective of this study is to utilize the GECKO method to implement kinetic and proteomic data on the enzymes involved in scyllo-inositol production and provide more realistic flux predictions.
Through the implementation of kinetic and proteomic data, the study successfully constrained the fluxes for scyllo-inositol production reaction, resulting in improved flux predictions. This approach enables more accurate and efficient engineering of *B. subtilis* for the production of scyllo-inositol. The study highlights the significance of integrating kinetic and proteomic information into GEMs to improve their accuracy and applicability in biotechnology and medicine. Furthermore, the continuous refinement of GEMs through the incorporation of omics data provides a robust tool for understanding and manipulating metabolism in a variety of organisms.

Keywords: genome-scale metabolic models, scyllo-inositol, Alzheimer, GECKO method, FBA

Poster 6:
Antarctic cold-adapted bacteria enhance plant tolerance to cold stress and induce metabolic reprogramming in tomato

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Plant-associated microorganisms can protect plants against cold stress, promoting physiological responses of acclimation to low temperatures in crops. Bacteria associated with Antarctic plants are an understudied source of beneficial microorganisms and the use of these bacteria may represent a sustainable strategy for the protection of crops (e.g. tomato) against cold stress. However, scarce information is available on the molecular mechanisms underlying this process. This work aims at understanding the physiological mechanisms activated by psychrotolerant bacteria on tomato plants and to identify plant metabolites involved in the mitigation of cold stress. Two psychrotolerant bacteria isolated from the Antarctic plant *Colobanthus quitensis* (*Hafnia* sp., *Pseudomonas* sp.) and a well-studied endophyte (*Paraburkholderia phytofirmans* PsJN) were selected for their ability to promote tomato shoot growth at low temperatures. In particular, *Hafnia* sp. produced indolacetic acid in presence of tryptophan at 25°C and at 4°C. To characterize the ability of these bacterial isolates to affect plant metabolism under cold stress, surface-disinfected tomato seeds were inoculated, while mock-inoculated seeds were used as control. Four-week-old plants were exposed to 4°C for 7 days in the dark and incubated at 25°C for zero (control), two and four days to allow recovery from the stress treatment. Phenylalanine-deriving metabolites were analyzed with high performance liquid chromatography coupled with high resolution mass spectrometry (HPLC- HRMS). Bacterium-inoculated plants showed lower concentrations of polyphenolic compounds compared to mock-inoculated plants. Interestingly, bacterium-inoculated plants accumulated higher content of putative phenylalanine-containing dipeptides compared to mock-inoculated plants, suggesting that these compounds may play a role in cold tolerance. The study suggests that Antarctic bacterial isolates were able to modulate phenylalanine metabolism in cold-stressed tomato plants. A deeper knowledge of the transcriptional and metabolic mechanisms activated by beneficial bacteria in tomato plants will open the possibility to develop new sustainable strategies to protect the plants against cold stress.

Keywords: psychrotolerant bacteria, metabolomics, cold stress

Poster 7:
GrUpSe- An Interactive Tool for Evaluating Metabolite Exchange Rates in Growing Cells

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Determining cellular growth rates, and uptake and secretion rates of extracellular metabolites is crucial in systems biology, in particular for constraint-based metabolic modelling methods, such as Flux Balance Analysis (FBA). Widely used in systems biology, FBA is often deployed in metabolic engineering. FBA's predictive power, however, depends on the quality of the experimentally-determined cellular growth and extracellular metabolite exchange rates.
We developed GrUpSe to facilitate intuitive data analysis for growth rates and metabolic exchange rates. A publicly-available, interactive web-based application, GrUpSe allows for the analysis of user-uploaded time-course data from batch cultivation.
GrUpSe can determine the growth rates of cells by fitting the apropriate function to the data using the non-linear least-squares method. The generated growth rates are then automatically incorporated for the fitting of the extracellular metabolites. It can fit simple models using predefined functions (e.g exponential function) and more complex models using splines. The tool has many interactive features, for example allowing users to determine the section of data selected for fitting, whether or not to exclude outliers, or to use a different function for the fitting of glutamine and ammonium to account for degradation. The application also allows users to export graphs and fitting parameters for use in publications or to be integrated into models like FBA.
The user-friendly application could be useful to a wider range of researchers, by allowing for use by researchers not experienced in data science, who are nonetheless interested in FBA or the calculation of exchange rates. GrUpSe is a versatile tool with a wide range of possible applications.

Keywords: Cellular growth rates, Extracellular metabolite exchange rates, Flux Balance Analysis (FBA), Curve fitting

Poster 8:
Computing all feasible compositions of microbial communities via elementary conversion modes

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Communities of microbial organisms and their metabolic capabilities are vital for human health, agriculture, and ecology. However, studying naturally occurring microbial communities is challenging due to the complex interactions among the species involved. Genome-scale metabolic modeling is a promising approach for their study, but applying it to communities requires more attention than for single organisms.
Therefore, we developed an automated workflow that integrates multiple genome-scale metabolic models into a community and computes the community's elementary conversion modes, which describe the full range of feasible metabolic behaviors in the community.
To demonstrate the feasibility of our approach, we applied it to a biogas-producing microbial community and characterized the full range of feasible metabolic behaviors. Our results show that the sum of pairwise interactions between the members of the community can explain 96% of the feasible community behavior.
Our approach provides a powerful tool for understanding the metabolic potential of microbial communities, which is essential for various applications in health, agriculture, and ecology.

Keywords: microbial communities; metabolic capabilities; constraint-based modeling; biogas-producing community

Poster 9:
Comparative evaluation of the secondary metabolome of *Trichoderma atroviride* under three common light conditions

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Many studies try maximizing fungal secondary metabolite production. For some fungi a strong light influence is reported, but this has hardly been systematically studied. Here, we combined an untargeted isotope-assisted liquid chromatography-high resolution mass spectrometry-based metabolomics approach with standardized cultivation of *Trichoderma atroviride* under three defined light regimes (darkness (PD), reduced light (RL) exposure and 12/12 h light/dark cycle (LD)) to determine their effect on secondary metabolite production: Comparative analyses revealed a similar response upon cultivation in PD or RL. LD cultivation had an inhibiting effect on both the number and abundance of metabolites. Spatial distribution under dark and reduced light conditions was analyzed, by cutting the agar into five equally sized zones. This resulted in the detection of more than 500 metabolites. Clustering revealed 85 metabolites, including 6-pentyl-alpha-pyrone, significantly more abundant when fungi were grown in darkness. Overall, PD only modestly raised the metabolite number (less than 2%). The effect on abundance was stronger, with a quarter of metabolites being more abundant when cultivated under RL or PD compared to LD. The remaining metabolites, were not significantly affected by the RL regime. Hence, our study reveals that cultivation of *T. atroviride*. under RL can be used to maximize secondary metabolites.

Keywords: fungal metabolomics, *Trichoderma atroviride*, light response

Poster 10:
Multi-omics analysis to disentangle factors related to storability in sugar beet using the R Shiny app Holomics

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Food production systems need to adapt to climate change but also to the demands of the continuously growing human population. But focusing merely on the production of food is not the only path to attaining food security. Reducing wastage through the extension of storage time and shelf life is critical too. Crop improvement via modern breeding techniques along with efficient agronomic practices, innovations in microbiome applications, and exploiting plant genetic resources is also of utmost importance to meet this storage-related challenges.
Within modern plant breeding techniques, genomics is an important player for trait mapping that has allowed rapid gene identification and characterisation of genetic markers. However, genomics alone is reflecting only one functional layer of an organism. The integration of further layers (transcriptomics, metabolomics, proteomics, microbiomics) –a multi-omics data analysis approach– enables a holistic view into functional processes of the focal organism and can be considered as the basis for a finetuning of the development and characterization of genetic markers that are further applied in selection and breeding programs. The add-on of such a multi-omics approach is the knowledge increase towards additional key factors (e.g., transcripts, metabolites, microbial taxa) linked to (agronomically) important features, enabling an extension of the marker type beyond genetic markers (e.g., SNPs) facilitating a holistic and flexible screening for relevant traits.
To proof the functionality of a multi-omics approach for crop improvement, we used the storability problematic of sugar beet as a case study. During post-harvest storage, sugar beet is prone to storage rot which is leading sucrose loss and ultimately to economic loss. Many factors have been described to influence the storability of sugar beet, from the genotype to environmental impacts, implying that many functional but also external layers are involved. But so far, especially molecular genetic-related factors have been studied predominantly via single-omics. With an integrative bioinformatics analysis of phenomics, transcriptomics, metabolomics and microbiomics data using the R Shiny application Holomics we aimed at linking as many (functional) levels as possible to extract a molecular network of features related to storability.

Keywords: Sugar beet; Storability; Multi-omics; Holomics; R Shiny application

Poster 11:
RNA-seq meta-analysis of lncRNAs in CHO cells

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Long non-coding RNAs (lncRNAs) are a relatively new class of RNAs involved in many regulatory functions, ranging from modulation of gene expression to modification of chromatin states of their target genes in mammalian cell models. The investigation of lncRNA function in Chinese hamster ovary (CHO) cells faces several challenges: lncRNAs are expressed in a lower amount compared to mRNAs, they are not well conserved across species, and they are often located in complex genomic locations. Moreover, for CHO cells in particular, comprehensive studies that could help mapping lncRNAs functions genome-wide are missing and we found that the lncRNA reference annotation is often not reliable. Consequently, our attempts to overexpress promising lncRNA candidates in CHO cells was futile. To overcome these bottlenecks and to provide a comprehensive overview of lncRNA conservation in CHO cells, we collected 15 RNA-seq datasets available from NCBI or from previous in-house studies to initially improve the reference annotation by performing a transcriptome-based assembly. Our RNA-seq samples include different host cell lines, producer andnon-producer cell lines, and cell lines exposed to various culture conditions. Therefore, we next aimed at using the available metadata to perform differential expression analysis and identify lncRNAs classes and candidates that can be used as engineering targets. Principal component analysis (PCA) showed that samples from the same cell line (CHO-S, CHO-K1, or CHO-DXB11), although originating from different studies, cluster together. Generally, most of the annotated lncRNAs are expressed in all cell lines (number) with little difference (up to number) between cell line A and cell line B.
Additionally, when looking at one host cell line (e.g. CHO-K1), the specific culture condition explains most of the variance within a sample set. Next, we evaluated the influence of different parameters (e.g. presence of glutamine in the media, growth rate, productivity, etc.) on the number of expressed lncRNAs and their expression level and assessed the differences between cell lines and samples.
This in-depth differential expression analysis will help identifying lncRNAs that can be considered markers for the cell phenotypes of interest and/or targets of cell engineering to improve cell culture performances.

Keywords: CHO, lncRNAs, RNA-seq, PCA.

Poster 12:
Root exudation patterns and rhizosphere microbiome assembly in contrasting rice genotypes grown on highly phosphate fixing Andosol

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Andosols are soils with positive physical properties for cultivation, such as high water-holding capacity, but with an extraordinarily high phosphate (P) adsorption capacity which leads to high fertilizer consumption in upland rice production areas. The mechanisms favoring rice growth on these acidic, P-fixing soils, as well as the involvement of plant-microbe interactions are poorly understood. To test whether rhizosphere modifications are involved in P mobilization from Andosols, we examined root exudation patterns and rhizosphere microbial community composition of 12 rice genotypes differing in phosphorus efficiency. Photometric assays and metabolomic profiling via LC-TOF-MS were used to characterize root exudates. Amplicon sequencing of the 16S rRNA gene and the ITS1 region was performed to study the root associated microbial community. Carbon exudation rates, especially the exudation rates of phenolic compounds were highest in less efficient genotypes. However, enhanced phenolic compound exudation rates did not translate into distinct microbial community structures. Microbial community composition was significantly affected by the plant genotype. Rice genotypes with the highest P efficiency had fungal community structures with higher relative abundance of Chytridiomycota when compared to the remainder genotypes. Our data suggest that enhanced soil exploration (maintained root growth together with low C exudation rates) is an important strategy of P efficient rice and that root mining for P (enhanced C exudation and modification of the microbiome through exudation of phenolic compounds) is a response of P inefficient genotypes grown on strongly P fixing Andosols. The involvement of rhizosphere microbiota in rice P uptake from highly P fixing soils requires further functional studies.

Keywords: metabolomic profiling, amplicon sequencing, root traits, rice genotypes, low phosphate availability

Poster 13:
Comparison of chromatographic conditions for the targeted tandem mass spectrometric determination of 354 mammalian metabolites

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Metabolomics, the process of measuring a wide range of small molecules in any biological system, has become an increasingly popular "omics" approach in biomedical research and nutritional analysis [1]. Comparatively, the use of modern metabolomics technology in livestock research is still lagging behind [2]. To develop a set of targeted multi-analyte LC-MS/MS methods for metabolite quantification and biomarker discovery, we constructed a metabolite library with 354 mammalian metabolites from 19 compound classes, including sugars, amino acids, carboxylic acids, nucleotides and various lipid classes. We then optimized multiple selected reaction monitoring transitions for each compound on a triple quadrupole mass spectrometer. Subsequently, we compared the retention profiles of our metabolite library across different chromatographic conditions: three reversed-phase (RP) methods (C18, F5, C18 under lipidomics conditions), three hydrophilic interaction liquid chromatography (HILIC) methods (bare silica-, zwitterionic-based HILIC, zwitterionic-based HILIC at pH 9) as well as anion exchange chromatography (IC). Similar as already shown by others [3], our results show that RP and HILIC are complementary to each other. Both IC and RP using apolar solvents increase analyte coverage. Compared to RP, HILIC methods improve the coverage of polar metabolites such as amino acid related compounds or biogenic amines, while carboxylic acids, nucleotides, and sugar phosphates are predominantly targeted by IC or HILIC under basic conditions. This extensive survey of both chromatographic and MS properties provides a diverse and comprehensive dataset, which will facilitate the development of quantitative targeted LC-MS/MS methods for livestock metabolomics [4].

Keywords: metabolomics, mammalian metabolites, targeted LC-MS/MS methods, high-performance liquid chromatography, retention profile

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Poster 14:
Metabolic changes in chemotherapeutically treated Hodgkin Lymphoma cells: complementary analytics by SERS and GC-MS

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Oncometabolites, substances which accumulate in cells due mutations in genes of Krebs cycle enzymes, have been recognized as cancer biomarkers. There is an essential need for simple, user independent hands–on sample preparation, robust measurement and fast data analysis. Raman spectroscopy with its fast, label-free and non-destructive detection of chemical fingerprints provide such a tool and possess the potential to investigate metabolic changes in single cells. Surface-enhanced Raman spectroscopy (SERS) further allows the mapping of metabolic changes at the level of organelles, and the discovery of novel biomarkers, to differentiate between healthy and degenerated cells at the different development stages.
SERS findings regarding metabolic changes in chemotherapeutically treated Hodgkin Lymphoma cells need confirmation by complementary analysis. Untreated control and cells treated with the cytostatic Etoposide were analysed using GC-MS to identify specific metabolites with significantly different concentration between these two classes. Ideally, changes in the cellular Raman spectra could then be matched to these differences. In total, 69 unique compounds were identified, of which 37 were found in at least 50% of samples. Out of these 37 metabolites, 8 are significantly different ($p = 0.05$) in concentration between classes. Three of these significant metabolites – aspartic acid, valine and glutamic acid – are amino acids, which are all more prevalent in Etoposide treated samples. This could potentially correspond to the stronger protein signal found in the Raman spectra of treated cells.
While GC-MS is able to resolve individual compounds, when using Raman spectroscopy, it is often only possible to identify groups of substances. In the case of SERS, affinity towards the substrate also determines which compounds are detected, with heteroatomic molecules often dominating the spectrum. It is therefore no coincidence that the most influential SERS signals belong to proteins and the nucleobase Adenine, both of which contain a large number of nitrogen atoms. On the other hand, Raman spectroscopy can provide a more complete picture of the cellular constituents, as it is not limited to low-molecular-weight metabolites and can detect larger biomolecules as well.
Therefore, Raman spectroscopy/SERS and GC-MS should be considered complementary techniques, rather than being directly compared. Another future goal will be the establishment of metabolite databases for both Raman spectroscopy and GC-MS to make the assignment of metabolites to signals simpler and more robust.

Keywords: Oncometabolites, Cancer treatment, Surface-enhanced Raman spectroscopy, Gas Chromatography - Mass Spectrometry