universität wien

Department of Analytical Chemistry

MPA VIENNA 2025

10th International Conference
Metabolic Pathway Analysis
July 28 - August 1, 2025

Scientific Program

Arcaded Courtyard and BIG lecture hall
Universitätsring 1, 1010 Vienna, Austria

https://mpa2025.univie.ac.at
mpa2025@univie.ac.at

Contents

Monday, July 28, afternoon

Registration

Arcaded Courtyard

15:30

A0 Welcome, Key note

Chair: Jürgen Zanghellini

BIG Lecture room

17:00 **Diethard Mattanovich**
^{13}C tracer analysis for single carbon pathway analysis in yeasts

18:00 **Stefan Schuster**
Happy Birthday, MPA!

P1 Poster Session, Wine & Cheese

Arcaded Courtyard

18:15

Tuesday, July 29, morning

A1 One carbon metabolism

Chair: Jürgen Zanghellini — BIG Lecture room

9:15 **Elad Noor**
Reinventing the CO_2 fixation cycle

10:00 **Matthias Steiger**
In vitro and *in vivo* characterization of a new-to-nature pathway for formaldehyde assimilation in methylotrophic yeast

10:15 **Luz Francy Yañez-Meneses**
Methanol to isopropanol in *Methylorubrum extorquens* AM1

10:30 **Soumen Konar**
Placental proteomics and early metabolic programming: A Constraint-based multi-omics perspective on pediatric obesity

coffee break

Arcaded Courtyard

10:45

B1 One carbon metabolism

Chair: Steffen Waldherr — BIG Lecture room

11:15 **Jasmin Bauer**
Using metabolic modeling to analyze thermodynamic constraints of the acetogen *Acetobacterium woodii*

11:30 **Nico Claassens**
Using synthetic and systems biology to ramp up synthetic C1-metabolism: towards higher yields and faster growth

11:45 **Blanca Araya**
Triacylglycerols accumulation in *Rhodococcus opacus* DSM 43205 from CO_2 and hydrogen and C1-C5 soluble derivatives

12:00 **Michael Baumschabl**
Conversion of CO_2 to organic acids in engineered *Komagataella phaffii*: Integrating synthetic biology and isotopically nonstationary metabolic flux analysis

Lunch break

Arcaded Courtyard

12:15

Tuesday, July 29, 2025, afternoon

C1 Theory and methods in network analysis

Chair: Jürgen Zanghellini — BIG Lecture room

13:15 **Justin Chitpin**
Atomic elementary flux modes explain the steady state flow of molecular constituents in genome-scale metabolic flux networks

13:30 **Emma Crisci**
Constraint-Driven Enumeration of Biologically relevant Elementary Flux Modes in Metabolic Networks Using Hybrid Logic-Linear Programming

13:45 **Paz Torres-Praderio**
Genome assembly and genome-scale metabolic model reconstruction of *Rhodococcus opacus* DSM 43205, an oleaginous and hydrogen-oxidizing bacterium

14:00 **Anne Richelle**
Integrating Data-Driven GeM Reduction and Bayesian Rate Calculation for Enhanced Cell System Modeling

14:15 **Steffen Waldherr**
Validation of genome-scale metabolic network models with proteomics data

coffee break

Arcaded Courtyard

14:30

D1 Applications in Metabolic Engineering

Chair: Marcelo Rivas-Astroza — BIG Lecture room

15:00 **Cong Trinh**
Modular Cell Design with Resource Allocation for Scalable Biomanufacturing: A Multi-Objective Optimization Framework

15:15 **Steffen Klamt**
Enabling New Biochemical Conversions by Combining Fermentative Metabolism with Selected Respiratory Modules

15:30 **Guido Schlögel**
OptFed: Advancing Bioprocess Control with Data-Driven Optimization

15:45 **Rebeca Gonzalez-Cabaleiro**
Glycolysis in anaerobic fermentation: how nature efficiently resolves the electron puzzle

P2 Poster Session

Arcaded Courtyard

16:00

Wednesday, July 30, morning

A2 Cellular economics

Chair: Markus Arthur Köbis — BIG Lecture room

9:15 **Avi Flamholz**
The proteome is a terminal electron acceptor

10:00 **Titania Insani Sugiarto**
Modeling the Effect of Cell Membrane on the Protein Allocation Model

10:15 **Maarten Droste**
Principles governing shifts in metabolic strategies

10:30 **Diana Szeliova**
Why do cells invest in compartmentalization? The thermodynamic benefits of mitochondria

Coffee break

Arcaded Courtyard

10:45

B2 Cellular economics

Chair: Oliver Ebenhöh — BIG Lecture room

11:15 **Constance Le Gac**
Global Enzyme Cost Minimization: A resource allocation framework that integrates kinetic and thermodynamic constraints for genome-scale metabolic networks

11:30 **Kate Meeson**
Can we predict the metabolic objectives of a cell?

11:45 **Maaike Remeijer**
Biodiversity in bioenergetics and precursor usage in microbial growth

12:00 **Lukas Korn**
Optimal resource allocation in defence mixtures: Modelling the effects of substrate competition and competitive inhibition

12:15 **Luis A. Salinas-Te**
From Mixed Acid to Homolactic Fermentation: Understanding ATP Supply and Demand in *Lactococcus cremoris*

Lunch break

Arcaded Courtyard

12:30

Wednesday, July 30, afternoon

C2 AI in MPA

Chair: Steffen Klamt — BIG Lecture room

13:30 **Vassily Hatzimanikatis**

14:15 **Charlotte Merzbacher**
Accurate prediction of gene deletion phenotypes with Flux Cone Learning

14:30 **Mathias Gotsmy**
Integrating metabolic networks into hybrid kinetic bioprocess models

14:45 **Wassili Dimitriew**
Enhancing genome-scale metabolic model reconstruction by utilizing large language models

coffee break

Arcaded Courtyard

15:00

D2 Apps in SysBiol & Methods

Chair: Diana Szeliova — BIG Lecture room

15:30 **Nadine Töpfer**
Putting metabolic networks into context – Understanding leaf functioning through computational modelling

15:45 **Marcelo Rivas-Astroza**
Thermodynamically consistent and phenotype-specific estimation of metabolic fluxes

16:00 **Nathan Fargier**
Unbiased metabolic flux inference through combined thermodynamic and 13C flux analysis

16:15 **Alejandro Leon**
Unraveling Genetic and Environmental Interactions in *Arabidopsis thaliana* Using a Multi-Scale Life Cycle Model

Traditional Wine Tavern

Heuriger 10er Marie

18:00

Please make your way to **10er Marie**, located at: Ottakringer Straße 222/224, 1160 Wien

The venue is easily accessible via **tram line 44**, which departs directly from the University of Vienna. Get off at “Johannes-Krawarik-Gasse”. The 10er Marie is approximately 50 meters from the tram stop.

For those who don’t want to venture there on their own, we will lead a group to the venue:

- **Meeting point**: In front of the main entrance of the University (right ramp when facing the building)
- **Departure**: 17:15 (sharp) by public transport — please bring a valid ticket

Thursday, July 31, morning

A3 Microbial Communities

Chair: Zita Soons — BIG Lecture room

9:15 **Christine Moissl-Eichinger**
Archaeal-bacterial metabolic interactions in the human microbiome

10:00 **Jordi Roma**
Linking Microbiome-Derived Butyrate Production to Host Metabolism via SIRT1 Regulation

10:15 **Tim Hensen**
Metabolic modelling links gut microbiota to metabolic perturbations in Parkinson's disease

10:30 **Ashley Beck**
Maize root exudates stimulate a more diverse microbial community: A theoretical analysis of metabolic interactions within a synthetic root-associated bacterial community

Coffee break

Arcaded Courtyard

10:45

B3 Microbial Communities

Chair: Christoph Flamm — BIG Lecture room

11:15 **Hyun-Seob Song**
Simulating the Emergence of Microbial Cooperation from Individual Growth Maximization via Cybernetic Modeling

11:30 **Stefan Müller**
The geometry of cooperation: decoding microbial interactions

11:45 **Ross Carlson**
Cellular Economics of Exchanged Metabolites Alter Ratios of Microbial Trading Partners in Predictable Manner

Brown bag lunch

Arcaded Courtyard

12:00

Thursday, July 31, afternoon

Social activities

12:45	Climbing Forest Rope Park	12:45	Dancing Ballsaal Wien	13:30	Guided city walk

Climbing at Forest Rope Park

- **Meeting point:** In front of the University (main entrance, left ramp when facing the building)
- **Departure:** 12:45 (sharp) by bus
- **Climbing starts:** 13:30 at the Forest Rope Park
- **Return:** Bus departs at 16:30, arrival at the University around 17:00

Dancing at Baalsaal Wien

- **Meeting point:** In front of the University (main entrance, right ramp when facing the building)
- **Departure:** 12:45 (sharp) by public transport — please bring a valid ticket
- **Dancing starts:** 13:30 at the Ballsaal Wien

Vienna City Tour

- **Meeting point:** In front of the University (left ramp when facing the building)
- **Departure:** 13:30

Gala Dinner

House of Industry

18:00

Please make your way to the **House of Industry**, located at: Schwarzenbergplatz 4, 1030 Wien

The venue is accessible via the **U2 subway line**, which departs directly from the University. Take the train in the direction of *"Karlsplatz"* and get off at the final stop. The **House of Industry** is a 10-minute walk from the station.

For those who would like guidance, we will lead a group to the venue:

- **Meeting point:** In front of the University (main entrance, right ramp when facing the building)
- **Departure:** 17:15 by public transport — please bring a valid ticket

Friday, August 1, morning

A4 Human Metabolism

Chair: BIG Lecture room

9:15 **Camille Siharath**
From dysregulation to compensation, a modelling approach to energy metabolism in neuromuscular diseases.

9:30 **Marian Breuer**
Identifying metabolic subtypes in heart disease via metabolic task analysis

9:45 **Bram Nap**
Modelling human metabolism: the interaction between nutrition, the gut microbiome and host metabolism.

10:00 **Almut Heinken**
Systematic characterization of inborn errors of cobalamin metabolism and their effect on metabolism through genome-scale modeling

10:15 **Zita Soons**
Integrative Multi-Omics Metabolic Modeling Reveals Mechanisms of Drug-Induced Liver Toxicity

10:30 **Thomas Sauter**
Epigenetic control of metabolic identity across cell types

Coffee break

Arcaded Courtyard

10:45

B4 Tools & Theory

Chair: BIG Lecture room

11:10 **Pavlos Stephanos Bekiaris**
COBRA-k: a new modeling framework linking constraint-based and kinetic modeling approaches

11:25 **Katharina Nöh**
Tackling Aleatory and Epistemic Uncertainty using Metabolic Network Ensembles

11:40 **Hugo Dourado**
The emergence of oscillations on reaction networks in growing cells

11:55 **Anika Küken**
Kinetic modules are sources of concentration robustness in biochemical networks

12:10 **Michael Predl**
From Microbes to Interactions with PyCoMo – Tackling the Challenges in Community Metabolic Modelling

Brown bag lunch

Arcaded Courtyard

12:25

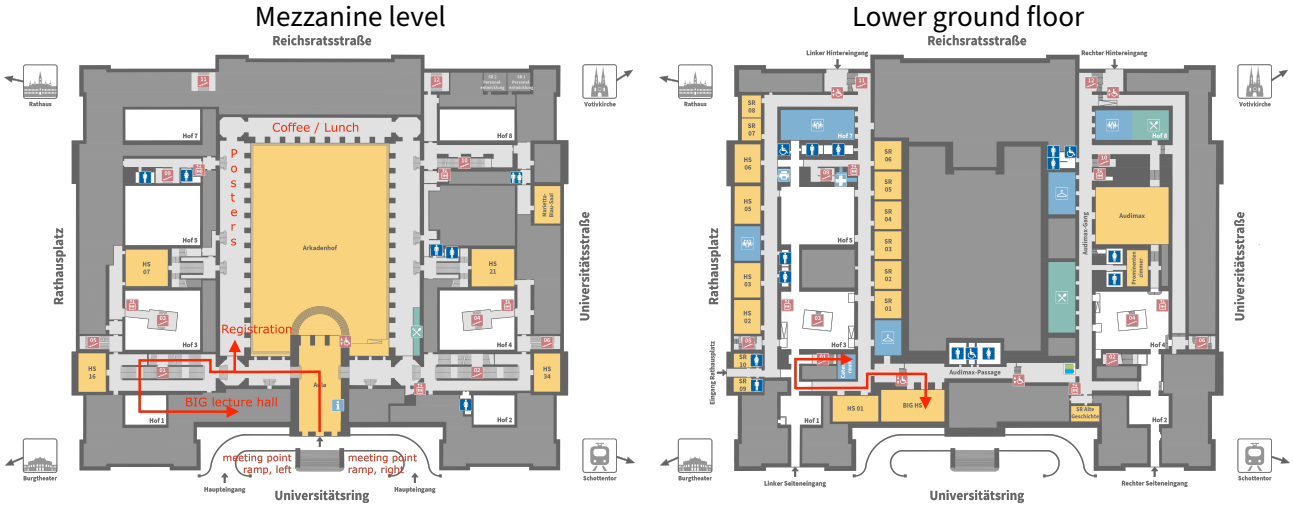
Venue

The conference takes place at the **Main Building** of the **University of Vienna**, located at Universitätsring 1, 1010 Wien

The university is conveniently accessible by public transport. Take the U2 subway line or one of several trams (2, 37, 38, 40, 41, 42, 43, 44) to “Schottentor” and use the exit “Universität”. The main entrance is just a short walk from the stop.

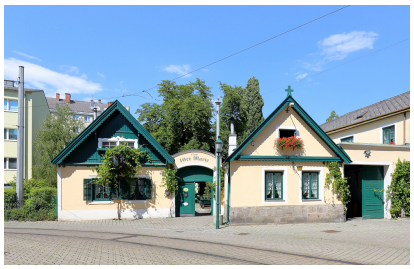
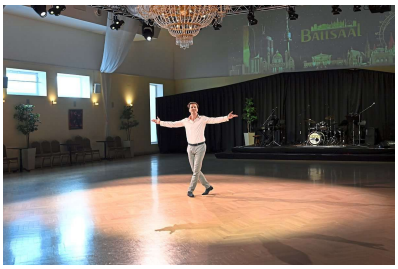
On-site Locations

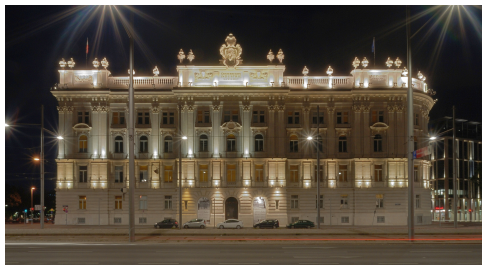
- Registration, Coffee, Lunch, and Poster Sessions will take place in the Arcaded Yard (Arkadenhof), located on the mezzanine level.



- All lectures will be held in the BIG Lecture Room, located in the lower ground floor.

Off-site Locations



- Traditional Wine Tavern: **10er Marie**, Ottakringer Straße 222/224, 1160 Wien
- Dancing Class: **Ballsaal Wien**, Kundmanngasse 30, 1030 Wien
- Gala Dinner: **Haus der Industrie**, Schwarzenbergplatz 4, 1030 Wien

WiFi / Internet Access

Eduroam is available throughout all university buildings for members of participating institutions.

If you do not have access to Eduroam,

- Please create an u:account here: https://account.forms.univie.ac.at/uaccount-anlegen/index.html
- Once you’ve registered your u:account, let us know your `UserID` at the conference check-in so we can activate your WiFi access.

Good to know

Oral Presentations

- Talks are limited to 12 minutes + 3 minutes Q&A
- Please approach IT support during the **break before your session** to upload your presentation to the conference system.

Posters

- Kindly dismount your posters by Thursday at 13:00 at the latest. Unclaimed posters may be removed by the organizing team.

Electronic payment in Austria

While electronic and card payments are widely accepted in Austria, some places may accept cash only. For your convenience, there is an **ATM** located in the University's lower ground level, just to the left of the BIG Lecture Room.

List of Abstracts

AI in MPA

Predicting sample-specific metabolic routes through omics integration

Jelle Bonthuis, *Maastricht University*
Poster, P1-01

1. Introduction
Metabolism can be modelled using genome-scale metabolic models (GEMs), but due to the large size of animal-derived GEMs, their interpretation is often challenging or focussed on a limited set of specific metabolic pathways or metabolites. These problems persist when integrating transcriptomic data to predict sample-specific metabolism or to score overall activity. One approach is to interrogate small metabolic tasks that have predefined inputs and outputs, but which lack fixed reactions to accomplish these tasks [1]. The initial approach that utilized this methodology was to pre-calculate a set of "essential" reactions per such metabolic task and to project sample-specific expression onto these pathways of essential reactions. Using this projected expression, the metabolic activity per task could be quantified and compared among samples. This approach however lacks the ability to utilize gene expression to inform which reactions are likely active for a specific metabolic task.
2. Approach
By converting the regular linear programming approach to solve constraint-based GEMs into a mixed integer linear problem, specifically through introducing a binary variable to denote reaction activity, we created a minimization problem that finds the "route of least resistance" or the shortest weighted path (SWP), where the resistance or weight is inversely proportional to the potential enzymatic activity associated with each reaction. A solution to SWP is then calculated for each metabolic task and sample of interest, and the resulting routes are compared between the samples. Potential enzymatic activity is derived from sample-specific transcriptomics, combined with tissue-specific transcriptome-proteome ratios (how many proteins expected for a normalized quantity of mRNA expression), as well as enzymatic activity per-reaction predicted through machine learning [2]. The resulting sample-specific and task-specific metabolic routes are subsequently analyzed for each sample's ability to accomplish a task (the task specific activity), as well as the specific route/reactions taken to accomplish it. This information is then combined with clustering and phenotypical classification in order to identify potential trends in overall and task-specific metabolism between groups of samples.
3. Results
The described approach allows for identification of sample-specific changes in enzymatic activity and their quantification, while not being limited to fixed pathways as each route is determined by the sample's enzymatic activity for each reaction, nor limited to a fixed set of routes of interest; tissue- or condition-specific tasks can be added or removed with minimal effort, allowing for rapid exploration of research questions and hypotheses. Specific scenarios of interest include the ability to find alternative routes in metabolic disorders where canonical pathways are perturbed such as in DCM [3,4]. Patients might show reduced activity by fixed-route or standard pathway analyses, yet only a subset may exhibit disease-phenotypes. It is possible that the phenotypically healthy group compensates for the decreased activity through other non-canonical routes. Our method can distinguish such cases and predict which reactions/enzymes are involved in these compensatory mechanisms.
4. Discussion
Due to minimizing nature of the SWP method, it can only find a single route for each task-sample pair, and thus is not yet suitable for identification of perturbations in multiple redundant pathways. In future iterations of SWP we aim to integrate any alternative routes in found among samples, to characterize a larger portion of total metabolic activity for a given task.

[1] Richelle, Anne, et al. Model-based assessment of mammalian cell metabolic functionalities using omics data. Cell reports methods 1.3 (2021).
[2] Yu, Han, et al. UniKP: a unified framework for the prediction of enzyme kinetic parameters. Nature communications 14.1 (2023): 8211.
[3] Heather, Lisa C., et al. Redefining diabetic cardiomyopathy: perturbations in substrate metabolism at the heart of its pathology. Diabetes 73.5 (2024): 659-670.
[4] Nihant, Bastien, et al. Metabolic task analysis reveals distinct metabotypes in end-stage dilated cardiomyopathy. bioRxiv (2025): 2025-03.

Enhancing genome-scale metabolic model reconstruction by utilizing large language models

Wassili Dimitriew, *Friedrich Schiller University Jena*
Oral

Advancements in science and engineering have progressively enabled the resolution of increasingly complex biological problems through increasingly sophisticated methodologies. This progression ranges from the development of early mathematical models capable of producing numerical solutions to the advent of genome-scale metabolic models (GEMs) that strive to capture the complete metabolic network of an organism, and further to toolboxes that streamline the GEM reconstruction process. Most recently, large language models (LLMs) have emerged as valuable tools that facilitate the reconstruction of GEMs and address challenges encountered during the process. *Lichtheimia corymbifera*, a pathogenic fungus belonging to the Mucoromycota phylum, is desig-

nated as a high-priority fungal pathogen on the WHO's priority list. Besides causing severe infections in humans, it is also used as a fermentation agent in Southeast Asia without apparent adverse health effects. To date, GEMs of Mucoromycota fungi remain scarce, with only a few published examples, none of which belong to the Lichtheimiaceae family. The procedurally reconstructed GEMs are prone to biologically implausible artifacts, which are often challenging to detect. A notable example includes erroneous energy-generating cycles (EGCs) [1], or more generally, reaction sets that can sustain flux in an isolated system. These sets may involve dozens of reactions and are difficult to identify manually. Existing methods for detecting EGCs rely on energy dissipation reactions involving energy carrier molecules; however, this approach is not universally applicable to all flux-carrying reaction sets. We reconstructed a GEM for *L. corymbifera* by aligning its proteome with those of organisms for which GEMs already exist (template models) [2]. The initial phase involved unifying metabolite identifiers and names using publicly available databases such as KEGG and ChEBI, performed using the "bioservices" Python package. This unification was further refined using LLMs to recognize identical metabolites in cases when their instances in different models lack sufficient identifiers. LLMs were also employed to identify reaction sets capable of carrying flux in the absence of environmental exchange. These sets often comprised reactions that, while functionally identical, met the formal criteria for distinct reactions within the reconstruction framework. Nullspace analysis was also applied to detect such reaction sets, and the outcomes were compared with those obtained by using LLMs. Particular attention was paid to the sets that regenerate energy carrier molecules and to those violating the principle of mass conservation. This LLM-based approach requires no specialized programming skills, thereby lowering the barrier to GEM reconstruction for researchers with diverse scientific backgrounds. In several instances, prompts as concise as a single sentence were sufficient to retrieve valuable insights. The resulting model provides a foundation for investigating complex metabolic processes in Mucoromycota fungi.

[1] Claus Jonathan Fritzemeier et al. Erroneous energy-generating cycles in published genome-scale metabolic networks: Identification and removal. In: PLoS computational biology 13.4 (2017), e1005494.
[2] Hao Wang et al. "RAVEN 2.0: A versatile toolbox for metabolic network reconstruction and a case study on *Streptomyces coelicolor*". In: PLoS computational biology 14.10 (2018), e1006541

Integrating metabolic networks into hybrid kinetic bioprocess models

Mathias Gotsmy, *ETH Zürich*
Oral

Bioprocesses are widely used in the production of pharmaceuticals and chemicals. However, their full potential is rarely harnessed and their optimization remains challenging. A reason for this is the poor understanding of underlying process kinetics which arise from complex biological interactions that are dependent on the environment, organism, and product. Moreover, available data is limited, with often only few bioprocess runs and measurements reported per study. One possible way to overcome these limitations consists of formulating hybrid models that incorporate (bio-)physical knowledge into data-driven process models. Hybrid-model frameworks have been shown to consistently outperform purely data-driven models for bioprocess optimization, yet how to optimally build them (i.e., which type of knowledge should be used and how much) remains unclear. Metabolic networks are built from omics data of microorganisms based on the principles of mass and electron balance. Their constraint-based optimization, i.e., flux-balance analysis, is a powerful tool for the prediction of unobserved metabolic fluxes of cells. Databases of highly curated metabolic networks (e.g., BIGG) provide a source of knowledge that could be incorporated into hybrid bioprocess models for the discovery of kinetic bioprocess models. Although initial studies exist, they focus on the parameterization of an existing model structure rather than model discovery, leaving a largely untapped potential. Here, we introduce a new framework that integrates knowledge of metabolic networks into hybrid kinetic bioprocess models. Designed for compatibility with modern machine learning libraries, the framework supports easy integration with various hybrid modeling architectures. It incorporates optimality constraints from the metabolic network using a flux balance analysis surrogate model, which enforces mass and electron balance and predicts both observed and unobserved nutrient uptake and byproduct formation rates. To demonstrate the effectiveness of our framework, we apply it to a case study focused on protein production in *Escherichia coli*. The kinetic bioprocess model, constrained by the metabolic network, is trained and validated using experimental data. Additionally, we use the model to predict optimal bioprocess conditions and benchmark its performance against other state-of-the-art bioprocess modeling approaches. We believe our framework can enhance the reliability and accuracy of bioprocess models, leading to greater efficiency and reduced costs in development and production. Furthermore, it paves the way for advanced multi-scale bioprocess modeling approaches that can better support data-driven decision-making in the biotechnological industry.

Synthesising unhealthy samples: A modified StyleGAN approach for imbalanced medical datasets

Tim Hulshof, *University of Galway*
Poster, P1-02

Inherited metabolic diseases are rare diseases caused by single gene deficiencies in metabolic pathways. They are often associated with significant changes in the blood or urine concentration of a few metabolites, which also serve as disease biomarkers. Due to the rarity of the individual diseases, obtaining sufficient biochemical data from patients for *in silico* studies is challenging. For instance, this data imbalance in metabolomic datasets hampers the training and validation of robust diagnostic models. Hence, the generation and use of synthetic data could be beneficial. However, many popular oversampling methods used for the generation of synthetic data treat each feature in isolation, undermining the real-life dependencies between metabolites. Here, we develop a

novel approach using an adaptation of StyleGAN, a generative neural network originally built for images but here redesigned for high-dimensional, interdependent metabolomics data. The model is trained only on healthy samples, learning a compact latent "style" space that captures normal metabolic variation. By sampling from regions of that space, applying the "styles" of the scarce unhealthy data, and controlling diversity with a kernel-density bandwidth parameter, we synthesise realistic, privacy-preserving profiles that mirror the statistical properties, and inter-feature correlations, of real patients. Using three metabolomics datasets for the inherited metabolic diseases phenylketonuria, IVA, and classic galactosemia, we demonstrate that modified StyleGAN outperforms CopulaGAN, a widely used model for the generation of tabular synthetic data, while better capturing the natural variability of the data. Taken together, modified StyleGAN learns joint distributions directly, preserving the structure and interactions that drive metabolic dysregulation. This approach translates into more faithful representations and improved predictive robustness in practice, while safeguarding sensitive data and enriching training sets wherever authentic unhealthy examples are scarce.

Unraveling Genetic and Environmental Interactions in *Arabidopsis thaliana* Using a Multi-Scale Life Cycle Model

Alejandro Leon, *University of Cologne*
Oral

Constraint-based metabolic models provide a powerful framework for analyzing biochemical processes and resource allocation in plants. While such models enable large scale simulations of plant metabolism, their connection to development and phenology remains underexplored. In this project, we address these challenges by developing and employing *in silico* analyses of a multi-scale life cycle model to study the adaptive potential and ecological performance of *A. thaliana*. Our framework integrates (1) a germination model that accounts for dormancy levels, soil temperature, and moisture; (2) a phenological model that predicts life cycle duration based on core flowering-regulating genes; and (3) a whole plant model composed of root, leaf and stem encompassing 861 metabolites and 891 biochemical reactions per tissue, which examines key biochemical processes affecting growth in response to resources such as light and nitrogen. By integrating these three models, we develop a comprehensive multi-scale framework that captures key physiological and metabolic processes shaping the life cycle of *A. thaliana*. To complement our computational approach, we incorporate experimental data from the "TRR 341 Plant Ecological Genetics", providing empirical validation and deeper insights into the life cycle strategies of urban *A. thaliana* populations. Our findings demonstrate how plants adapt to varying levels of soil macronutrients and contrasting warm and cold environments. Traits such as the leaf-to-root biomass ratio, germination timing, and growth strategies respond dynamically to these conditions. In warmer climates, plants often complete their life cycle within a single year, adjusting their phenology to avoid excessive heat. In colder environments, some cohorts delay germination for several years to evade harsh winter conditions, resulting in diverse growth trajectories. These results underscore the critical role of environmental factors in shaping plant life history strategies. This research provides mechanistic insights into how genetic variation influences life history strategies, resource utilization, and resilience to environmental challenges. The knowledge gained will advance our understanding of plant adaptation and resilience, with broad applications in agriculture, ecology, genetics, and conservation biology.

Fluxome-wide association study for age using personalized microbiome community models based on general population cohort data

Ameneh Mehrjerd, *University Medicine Greifswald*
Poster, P2-03

Background and objective:
The gut microbiome plays a critical role in host metabolic regulation and aging. Through the gut-brain axis and metabolic signaling, microbiota-derived metabolites can influence systemic inflammation, energy balance, and age-related pathophysiology. While chronological age correlates with shifts in microbial composition, biological age, an integrated measure of functional aging, may be a more responsive marker for identifying modifiable metabolic pathways. Here, we utilized personalized microbiome community modelling for deriving metrics of biological age through machine learning paradigms.
Data and Methods:
We analyzed 1,837 metagenomics shotgun sequencing samples from the SHIP-Trend0 cohort. Microbiome abundances were generated using MetaPhlAn 4.0.5. After filtering sparse and invariant species, 477 microbial features remained for modeling. To get flux net secretion as a readout of functionality, we mapped each microbiome to AGORA2 to construct personalized metabolic models for each individual. The total number of analyzed metabolite net secretions was 281 across all modelled microbiomes. We then predicted biological age via the flux data using multiple machine learning models (TabPFN, CatBoost, Random Forest, XGBoost, LightGBM, MLP, SVR, ElasticNet). Data were stratified by sex (941 females, 899 males) additionally to generate sex-specific biological age scores. Each model was trained with 90/10 splits repeated over 10 iterations. We further optimized performance using RandomizedSearchCV with 5-fold cross-validation. To validate the biological age scores, we conducted linear regressions adjusting for sex and chronological age, linking biological age-scores to 21 clinical and metabolic markers (e.g., triglycerides, Gammaglutamyltransferase (GGT), HDL-cholesterol (HDL), Alanin aminotransferase (ALAT/GPT) (µmol/sl), Hb A1 c (%) (HbA1c), inflammatory markers, and lifestyle behaviors like smoking and alcohol use). Finally, we performed a fluxome-wide association study on chronological age to identify microbiome net secretion fluxes associated with chronological age.
Results:

TabPFN and CatBoost demonstrated the highest predictive power ($R^2 \approx 12.77\%$) towards chronological age, highlighting that microbiome functions carry a substantial age-related component, which is however less pronounced than for other omics layers. Intriguingly, flux-based biological age scores showed negative associations conditional on chronological age with smoking, elastase, GGT, and triglycerides were negatively associated with biological age, while diabetes, fibrinogen, and HbA1c showed positive associations across the full sample set. These results indicate that higher flux-based scores do not necessarily indicate better health in contrast to conventional age-scores, where higher scores are markers of less healthy states. Importantly, clinical associations were far more pronounced in men than in women. We also conducted a Fluxome-Wide Association Study (FWAS) to assess the relationship between fluxes and chronological age. Out of 281 fluxes analyzed, 148 showed significant associations in the overall cohort with age, albeit with small effect sizes. When stratified by sex, 101 fluxes were significantly associated with age in males, and 72 were significant in females. The top-hit functions associated with age were related to bile acid metabolism and carbohydrate fermentation.
Conclusion:
These results show that microbiome functions shift broadly with aging, while showing substantial inter-individual variance and sex-specificity leading to small effect sizes. These insights into microbial mediated metabolic pathways offer promising avenues for personalized interventions aimed at reducing biological age and improving metabolic resilience. However, the biological meaning of the found associations remains to be clarified.

Accurate prediction of gene deletion phenotypes with Flux Cone Learning

Charlotte Merzbacher, *University of Edinburgh*
Oral and poster, P01-04

Predicting the impact of gene deletions is crucial for biological discovery, biomedicine, and biotechnology. For example, identifying lethal deletions is key for new cancer therapies or antimicrobial treatments that bypass drug resistance. In biotechnology, non-lethal deletions are a powerful strategy to redirect chemical flux toward production of high-value compounds for the food, energy, and pharmaceutical sectors, using genetically engineered cells as alternative to petrochemicals. Owing to the cost and complexity of large-scale deletion screens, there is a growing interest in computational models that can leverage such data for predictive modelling. Here, we present Flux Cone Learning, a best-in-class framework for predicting the impact of metabolic gene deletions on many phenotypes of interest. Flux Cone Learning is based on high-dimensional Monte Carlo sampling of the metabolic space in tandem with supervised learning of fitness scores from deletion screens. We demonstrate unparalleled predictive accuracy for metabolic gene essentiality in organisms of varied complexity (*E. coli*, *S. cerevisiae*, Chinese Hamster Ovary cells), as well as the first predictive model for small molecule production from deletion screening data. Flux Cone Learning is a significant advancement in predictive biology and lays the groundwork for the development of metabolic foundation models across the kingdom of life.

Integrative Analysis of Breast Cancer Metabolism Using Genome-Scale Models and Machine Learning

German Preciat, *University of Guadalajara*
Poster, P2-05

The metabolic heterogeneity of breast cancer poses a major challenge for identifying robust biomarkers and therapeutic targets. To address this, we integrated genome-scale metabolic models (GEMs) with supervised machine learning to characterize disease-specific metabolic phenotypes. We generated 90 individualized metabolic models using clinical and gene expression data from the TCGA-BRCA dataset (66 tumor, 24 normal). Metabolic fluxes were estimated by minimizing a weighted L2 norm guided by gene expression. A Mann-Whitney U test with Benjamini-Hochberg correction was applied to identify significantly different reactions between phenotypes. Five classification algorithms—K-Nearest Neighbors (KNN), Support Vector Machines (SVM), Logistic Regression, Decision Tree, and Naive Bayes—were evaluated using stratified 5-fold cross-validation. KNN and SVM achieved the best results, with accuracies near 0.98 and ROC-AUC scores of 1.00, effectively distinguishing tumor from normal profiles. Key differentiable pathways included extracellular transport (up to 60 reactions), fatty acid oxidation, and nucleotide interconversion. These findings support the utility of combining GEMs with machine learning for phenotype classification and metabolic insight. Nonetheless, further experimental validation and statistical refinement are necessary to enhance interpretability and clinical relevance.

MetNetComp: Database for minimal and maximal gene-deletion strategies for growth-coupled production of genome-scale metabolic networks

Takeyuki Tamura, *Kyoto University*
Poster, P2-06

Growth-coupled production, in which cell growth forces the production of target metabolites, plays an essential role in the production of substances by microorganisms. The strains are first designed using computational simulation and then validated by biological experiments. In the simulations, gene-deletion strategies are often necessary because many metabolites are not produced in the natural state of the microorganisms. However, such information is not available for many metabolites owing to the requirement of heavy computation, especially when many gene deletions are required for genome-scale models. A database for

such information will be helpful. However, developing such a database is not straightforward because heavy computation and the existence of replaceable genes render difficulty in efficient enumeration. In this study, the author developed efficient methods for enumerating minimal and maximal gene-deletion strategies and a web-based database system. MetNetComp provides information on 1) a total of 85,611 gene-deletion strategies excluding apparent duplicate counting for replaceable genes for 1,735 target metabolites, 11 constraint-based models, and 10 species; 2) necessary substrates and products in the process; and 3) reaction rates that can be used for visualization. MetNetComp is helpful for strain design and for new research paradigms using machine learning. MetNetComp database is available on https://metnetcomp.github.io/database1/indexFiles/index.html

Multi-omics driven genome-scale metabolic modeling improves viral vector yield in HEK293

Leopold Zehetner, *University of Vienna*
Poster, P2-07

Human Embryonic Kidney 293 (HEK293) cells are widely utilized for recombinant protein and viral vector production, including adeno-associated virus (AAV) vectors essential for gene therapy. Despite their adaptability and high transfection efficiency, challenges persist in optimizing AAV yields. This study employs a multi-omics approach combined with genome-scale metabolic modeling (GSMM) to investigate metabolic and cellular mechanisms influencing AAV production in two HEK293 strains: a high-producing (HP) and a low-producing (LP) variant. A comprehensive dataset integrating lipidomics, exometabolomics, and transcriptomics under controlled bioprocessing conditions was generated. GSMMs reconstructed for both strains revealed that over 99.8% of cellular resources were allocated to growth rather than AAV production. A key bottleneck was identified in the LP strain, which exhibited pseudohypoxia characterized by upregulated hypoxia-inducible factor 1-alpha (HIF-1α), limiting AAV yield. Inhibition of HIF-1α halted cellular growth and enhanced AAV capsid production by 2.5-fold; however, it simultaneously reduced viral genome synthesis, negatively impacting the full-to-empty particle ratio. This trade-off highlights a critical challenge in optimizing AAV vector manufacturing, as balancing capsid assembly with genome packaging is necessary for maximizing functional viral vector yields. Additionally, metabolic analysis showed distinct energy storage strategies between the strains, with HP favoring triacylglycerides and LP accumulating glycogen, further indicating metabolic constraints in LP. Spent media analysis identified key nutrient limitations, including aspartate and glutamate in LP and serine in HP, providing insights for process optimization. Ultimately, this study demonstrates the utility of GSMMs for metabolic characterization and targeted process improvements in HEK293-based AAV production, offering new strategies for enhancing viral vector manufacturing efficiency.

Microbial Communities

Maize root exudates stimulate a more diverse microbial community: A theoretical analysis of metabolic interactions within a synthetic root-associated bacterial community

Ashley Beck, *Carroll College*
Oral

Microbes play a vital role in plant development, health, and resilience to stress, yet relatively little is known about the specific metabolic mechanisms driving interactions in these host-associated communities. Systems biology models enable a computational approach toward understanding experimentally elusive metabolic interactions, but the large number of species identified in natural communities can still present challenges for analyzing interactions. Synthetic communities provide a tractable alternative to investigate metabolite exchange in a more targeted manner. Here, we use an experimentally isolated, seven-member maize root-associated bacterial community as a platform for investigating metabolic-scale interactions, accounting for the plant host context through medium design. Metabolic models were constructed for each of the seven bacterial species and were curated with phenotype data to more accurately capture substrate uptake potential. A representative suite of maize root exudate compounds was compiled from literature to formulate an environmentally relevant medium for predicting potential interactions. Simulations of individual bacterial models comparing the root exudate medium with a standard laboratory cultivation medium demonstrated a substantial difference in growth rate predictions, reflecting the variability of each species with respect to substrate specificity as well as efficacy in processing substrates for growth. Community model simulations predicted the root exudate medium to support a more diverse community composition compared with the standard laboratory medium, and predicted abundances of community members aligned closely with experimentally measured proportions. Predicted exchange metabolites provide specific hypotheses to guide future experiments. Altogether, our *in silico* findings provide insight into the mechanisms by which root exudates influence and structure microbial interactions.

Modeling the metabolic basis for snow algae microbiome interactions

Ashley Beck, *Carroll College*
Poster, P1-76

Snow algae (psychrophilic single-celled eukaryotes) are keystone members of a diverse microbiome; they coexist with bacteria, fungi, and protozoa to colonize seemingly uninhabitable environments – snow and glacier surfaces typically at high altitude. The pigmentation of snow algae influences the environment through increased light absorbance (and thus decreased light reflectance by the snow surface) and creates a positive feedback loop increasing the melt rate, a phenomenon known as “bioalbedo”. Studies have examined the taxonomic composition of snow algal communities in a variety of blooms across polar and mid-latitude regions, and while symbiotic relationships are likely critical in these communities, relatively little is known about the interaction mechanisms that drive the assembly of these communities and sustain their development over a season. Here, we use environmental samples and sequencing data to identify dominant algae and bacteria in blooms from Montana, USA, and begin reconstructing a simplified community network. We reconstructed the main metabolic pathways in the dominant *Sanguina* snow alga, along with genome-scale models for major bacterial guilds (including the phyla Pseudomonadota, Bacteroidota, and Rhodothermota) in the community. We then used this system to explore bacterial-algae interactions in this unique habitat, investigating potential routes of carbon, nitrogen, sulfur, and vitamin exchange supporting this community via flux balance analysis simulations. Simulation results are interpreted in light of resource partitioning as a guiding ecological hypothesis, as resource scarcity is likely a major driving force for community structure and function in these harsh environments. Ongoing work is collecting additional experimental data to refine the community network, as well as expanding simulation analysis. The presented work provides an exciting first step toward dissecting metabolic interactions in these unique and environmentally important communities, with the long-term goal of linking metabolic effects to climate impacts.

Feeding the future: predicting infant microbiome health through simulating bacterial community dynamics

Coen Berns, *University of Amsterdam*
Poster, P1-09

During the first 1000 days of life, the microbiome undergoes dramatic transitions driven by dietary changes and exposure to external microbes. Breastfed infants, as compared to formula-fed infants, typically host a *Bifidobacteria*-richer microbiome that efficiently metabolizes human milk oligosaccharides (HMOs). This shift in microbial abundance may impact the production of key short-chain fatty acids (SCFAs), including formate, lactate, and butyrate, which are associated with gut health and metabolic development. Understanding how microbial communities influence the production of these beneficial metabolites is essential for designing interventions to promote a healthy state. Since *in vivo* testing remains challenging and *in vitro* experiments are both time-consuming and costly, genome-scale metabolic models (GEMs) offer a powerful tool to prioritize key microbial species and metabolites. However, ensuring the predictive accuracy of these models requires validation against experimental data. To address this issue and refine our GEMs, we use growth and SCFA production data of eight prevalent infant-gut microbes (*B. breve*, *B. fragilis*, *B. bifidum*, *A. caccae*, *B. pseudocatenulatum*, *B. luti*, *C. aerofaciens*, and *B. infantis*) grown in monoculture on YCFA minimal media with a single

HMO. Subsequently, models are integrated into community-scale GEMs and different combinations of input fluxes and bacteria were simulated using dynamic flux balance analysis. By modeling microbial interactions and SCFA fluxes, we aim to identify microbial consortia and feeding interventions, such as specific HMOs, that optimize butyrate (and potentially other beneficial SCFA) production. These results will be validated *in vitro* to assess the predictive accuracy of the GEMs and community simulations. The outcomes could be used to inform microbiome-based interventions, potentially supporting infant metabolic health in the future.

Cellular Economics of Exchanged Metabolites Alter Ratios of Microbial Trading Partners in Predictable Manner

Ross Carlson, *Montana State University*
Oral and poster, P2-10

Most microorganisms exist in interacting consortia, but the principles behind consortia assembly, including trading partner structure, are largely unknown despite being central to understanding, building, and controlling consortia. This study tests a cellular economy-based hypothesis that predicts consortia engaged in obligate metabolite exchange will assemble at ratios where the metabolic burden associated with the exchanges is predictably split. The metabolic burden was quantified using ATP equivalents, the ATP that could be produced if the exchanged metabolites were instead retained and catabolized for cellular energy. The hypothesis was tested using *Escherichia coli* cocultures engineered for obligatory exchange of pyruvate and L-arginine. The burden of metabolite exchanges was manipulated by changing O_2 availability, altering bioavailability of substrate energy, and by deactivating the ATP synthase enzyme, preventing oxidative phosphorylation. The three synthetic cocultures assembled at predicted strain ratios as a function of the perturbations. The strain ratios represented an equivalent metabolic burden between the trading partners even though the exchanged metabolites varied substantially in flux magnitude (300+ fold), molecular size (174 vs. $88\,\mathrm{g\,mol^{-1}}$), enzyme requirements (8 enzymes vs. none), and biological energy density (27.5 vs. 9 ATP molecule-1). The strain ratios could be rationally altered up to 20-fold using O_2 availability and cellular phenotype. The metabolic burden theory was applied to seven additional published cocultures and found to accurately predict trading partner ratios. Quantifying exchanged metabolites on an ATP equivalents basis provides a theory for interpreting natural consortia and a toolbox for controlling bioprocess consortia.

Christine Moissl-Eichinger, *Medical University of Graz*
Oral

Microbial interactions within the human microbiome profoundly influence host physiology, immune regulation, and metabolic health. Among these, the often-overlooked interplay between bacteria and archaea is now recognized as a critical determinant of microbiome functionality, particularly in anaerobic environments such as the gut. This lecture will explore the complex metabolic interdependencies between bacterial and archaeal taxa, highlighting how integrative pathway analysis and genome-scale metabolic modeling are transforming our understanding of these cross-domain dynamics.

Archaea—especially *Methanobrevibacter* species—play a pivotal role at the end of microbial food chains by consuming bacterial fermentation end products such as hydrogen and carbon dioxide. This syntrophic relationship not only enhances bacterial metabolic efficiency but also stabilizes the gut ecosystem. Notably, up to 40% of the Central European population exhibits a high-methanogen phenotype, characterized by a 1,000-fold increase in Methanobrevibacter abundance, detectable via breath methane measurements. This phenotype has been associated with beneficial health outcomes, including lower body mass index and increased longevity, likely due to more efficient fiber degradation.

However, an overabundance of methanogens in specific niches can shift their role from symbionts to contributors to disease. Emerging evidence links local Methanobrevibacter overgrowth to conditions such as periodontitis and potentially colorectal cancer, where enhanced interactions with pathobionts like Fusobacterium nucleatum may drive pathogenesis.

Pan-genome-scale metabolic modeling to understand metabolic functions of plant-associated microbes

Martina Feierabend, *University of Cologne*
Poster, P2-75

In natural environments, plants share their habitat with a myriad of microorganisms which include bacteria, fungi, oomycetes, archaea, and viruses. Plants and their associated microbiota engage in complex interactions in which metabolic dependencies and metabolic cross-feeding are a driving forces. The plant-associated microbiome conveys a fitness advantage to the plant host by promoting plant health, improving nutrient acquisition and resilience against pathogen attacks and abiotic stress conditions. *Sphingomonas* is a highly abundant member of the core microbiota in various plant species, often described as a plant-growth promoting bacterium. Meta-analyses have shown that irrespective of plant species or type of pathogen, *Sphingomonas* is often depleted following biotic stress conditions. We work on identifying metabolic factors causative for this depletion using the combined knowledge about the plant's metabolism, its effects on bacterial metabolism and interaction effects. To this end, we utilize pan-genome-scale metabolic models, which capture the metabolic potential of multiple members within a taxonomic group, while also enabling strain-specific metabolic characterization. By applying pan-genome-scale metabolic models of different *Sphingomonas* strains, we aim to identify metabolic commonalities across different strains, enabling functional predictions about the role of *Sphingomonas* in microbial plant-associated communities under different environmental conditions. 1.) We present a large set of

Sphingomonas metabolic network reconstructions charaterizing the pangenome of *Sphingomonas*. Using these models, we can identify conserved and unique metabolic mechanisms. 2.) We show that the pan-genome-scale metabolic model can be used as a reference model to create high-quality strain-specific models, which can be used to study the diversity of *Sphingomonas*. 3.) We present the metabolic reactome of *Sphingomonas*, which we will further used to study its diversity. This extended computational framework also is an important step toward gaining a mechanistic understanding of plant-microbial interactions, with the goal of applying it to sustainable agricultural practices, such as the targeted manipulation of microbiota to enhance plant yield.

Isolation and Agricultural Potential of Indole-3-Acetic Acid (IAA) Producing Plant Growth-Promoting Rhizobacteria (PGPR)

Reyhan Gül Güven, *Dicle University*
Poster, P1-14

Rhizosphere microorganisms (PGPR) that promote plant growth hold significant potential for achieving sustainability goals in agriculture. In this study, the indole-3-acetic acid (IAA) production capacities of rhizobacteria isolated from Diyarbakır city cotton roots were investigated. Samples were collected, and pure bacterial cultures were obtained using standard microbiological techniques. IAA production was quantified spectrophotometrically by treating culture supernatants grown in L-tryptophan-supplemented media with Salkowski reagent. IAA concentrations were calculated based on a standard curve prepared using standard IAA solutions ranging from 1 to $150\,\mu g\,mL^{-1}$ and results were expressed as micrograms per optical density unit. IAA production levels among the isolates ranged from 12.28 to $28.04\,\mu g\,mL^{-1}$, with the highest concentrations observed in Samples 14, 17, and 18. These results demonstrate the potential of selected PGPR strains to directly promote plant growth through IAA biosynthesis, offering valuable prospects for biofertilizer development and sustainable agricultural practices.

Characterization of Nitrogen-Fixing and Phosphate-Solubilizing Bacteria from Cotton Field Soils

Kemal Güven, *Dicle University*
Poster, P1-12

This study aims to characterize the plant growth-promoting bacteria (PGPR) isolated from the rhizosphere of healthy cotton plants in Diyarbakır, Turkey. Pure bacterial isolates were identified using MALDI-TOF and evaluated for PGPR traits including phosphate solubilization and nitrogen fixation. Nitrogen fixation ability was assessed on nitrogen-free medium, where isolates causing a color change from green to blue were considered positive. Among the isolates, 5 (Bacillus sp.) and 33 (Pseudomonas sp.) demonstrated nitrogen-fixing capacity. Phosphate solubilization was evaluated on NBRIP agar, with isolates 5 (Bacillus sp.), 18 (Pantoea sp.), 33 and 44 (Pseudomonas sp.) exhibiting clear halo formation and solubilization indices ranging from 1.8 to $2.8\,mm$. The morphological, physiological, and biochemical characteristics of these bacteria have been determined. The dual functional traits of isolates 5 and 33 underline their potential as effective biofertilizers to enhance soil fertility and promote sustainable cotton production.

Metabolic modelling links gut microbiota to metabolic perturbations in Parkinson's disease

Tim Hensen, *University of Galway*
Oral

Human gut microbiota can contribute to metabolic disruptions in Parkinson's disease (PD) through metabolic crosstalk with the host. However, mechanistic pathways linking gut microbiota to these metabolic disruptions remain largely unknown. Recent advances in constrained-based metabolic modelling have enabled *in silico* simulations of host-microbiome interactions, enabling the identification of mechanistic links between gut microbial shifts and metabolic perturbations in PD. Using this *in silico* modelling approach, we systematically profiled gut microbial contributions to blood metabolites associated with PD. To this end, we recontextualised a previously described gut metagenomics dataset comprising 435 PD patients and 219 healthy controls by generating and analysing microbiome-personalised whole-body metabolic models. We selected 116 metabolites with replicated metabolomic associations with PD diagnosis and predicted their blood accumulation fluxes *in silico*. These predictions were then mechanistically linked with gut microbial species with altered relative abundances in PD. Our analysis revealed consistently lower gut microbiome contributions to blood levels in PD of L-leucine, leucylleucine, butyrate, nicotinic acid, pantothenate, and myristic acid. Lower relative abundances of *Faecalibacterium prausnitzii* in PD microbiomes were identified as a major contributor to these lower blood metabolic fluxes. Furthermore, the predicted lower gut microbial contributions to L-leucine and leucylleucine were associated with *Methanobacter smithii*, an archaean methanogen and L-leucine consumer enriched in PD microbiomes. Taken together, this study linked gut microbial shifts with metabolic disruptions in PD and identified *F. prausnitzii* and *M. smithii* as potential modulators of blood metabolomic alterations in PD.

System-Level Analysis of Bacterial Poly-ADP-Ribosylation in *Deinococcus radiodurans*

Chun-hua Hsu, *National Taiwan University*
Poster, P1-24

Poly-ADP-ribosylation (PARylation) is a post-translational modification best known in eukaryotic systems for regulating DNA repair, transcription, and stress responses. Our recent studies reveal that *Deinococcus radiodurans*, a radiation-resistant extremophile, possesses an active bacterial PARylation system. We structurally and biochemically characterized DrPARG, an ADP-ribosyl glycohydrolase upregulated following UV-induced DNA damage. The ADP-ribose–bound crystal structure of DrPARG reveals a solvent-accessible 2'-hydroxy group, suggesting endo-glycohydrolase activity. Deletion of DrPARG led to PAR accumulation and impaired DNA damage recovery, confirming its functional relevance. To explore the system-wide role of PARylation, we established a proteomic workflow to define the UV-induced ADP-ribosylome, identifying 111 target proteins. STRING network analysis and GO enrichment revealed that PARylated proteins are functionally clustered into modules related to DNA repair (NER, MMR, DSB repair), redox regulation, central metabolism (TCA cycle, nucleotide biosynthesis), protein translation, and detoxification. These data support the view that bacterial PARylation coordinates key stress-responsive pathways beyond genome maintenance. This study represents the first integration of structural biology, proteomics, and pathway network analysis in the context of bacterial ADP-ribosylation. It highlights PARylation as a regulatory layer that enables prokaryotes to orchestrate adaptive metabolic and genome repair responses under extreme environmental conditions.

Magnitude of Resource Fluctuations Drives the Evolution of Microbial Metabolic Strategies

Sergei Maslov, *University of Illinois Urbana-Champaign*
Poster, P2-53

Microbial populations supplied with mixed carbon sources can evolve as single-resource specialists, sequential (diauxic) utilizers, or simultaneous co-utilizers, yet the environmental cues that favor each strategy are still unclear. We integrate a mechanistic proteome-allocation model with eco-evolutionary serial-dilution simulations of microbial communities. At every growth cycle, the ratio of up to four supplied resources is redrawn from a Dirichlet distribution whose concentration parameter sets fluctuation magnitude. Small mutations in the enzyme fraction allocated to non-preferred resources let lineages move smoothly through strategy space. After more than ten thousand evolutionary steps, the system converges on three mutually exclusive evolutionarily stable states: balanced inputs promote rapid-growing specialists, moderately skewed ratios favor sequential utilizers that minimize switching costs, and highly skewed supplies reward co-utilizers that split enzyme investment evenly. Because variance in resource ratios is the sole control knob, the framework offers a simple quantitative predictor linking environmental fluctuation strength to the long-term diversification of microbial metabolic strategies and provides testable hypotheses for both natural and engineered communities.

The geometry of cooperation: decoding microbial interactions

Stefan Müller, *University of Vienna*
Oral

Understanding microbial communities is crucial for advancing fields like ecology, biotechnology, and human health. Recently, constraint-based metabolic models of individual organisms have been combined in various ways to study microbial consortia. In this work, we present a comprehensive geometric approach to characterize all feasible microbial interactions. First, we project community models onto the relevant variables for interaction, namely exchange fluxes and community compositions. Next, we compute elementary compositions/exchange fluxes, thereby extending the concept of minimal metabolic pathways from single species to entire communities. Every feasible community is a combination of these elementary compositions/exchange fluxes, and, surprisingly, every elementary vector represents a fundamental ecological interaction (such as specialization, commensalism, or mutualism). Hence, our geometric approach allows us to decode the metabolic interactions underlying microbial cooperation. Moreover, it provides a foundation for rational community design. Since it treats exchange fluxes and community compositions equally, we can directly apply existing constraint-based methods and algorithms.

Modelling human metabolism: the interaction between nutrition, the gut microbiome and host metabolism

Bram Nap, *University of Galway*
Oral and poster, P1-15

Human metabolism and human gut microbiome co-metabolism are difficult to investigate *in vivo* due to the complexity of the systems involved. Dietary habits, gut microbiome composition, and human physiology all have high variance between individuals, increasing the complexity. Genome-scale metabolic modelling with human whole-body metabolic models (WBMs) offers a computational approach to address this complex question. However, personalisation and analysis of WBMs require comprehensive knowledge of metabolic modelling and can be daunting for beginners. To reduce the computational expertise required and to standardise the process of working with and analysing WBMs, we present the nutrition toolbox and Persephone: a personalisation and evaluation pipeline for human whole-body metabolic models. Persephone has functionalities to create community microbiome models from FASTQ files or taxonomy-assigned read tables; use physiological and metabolomic data to create personalised WBMs; combine community microbiome models with WBMs or personalised WBMs to create community microbiome (personalised) WBMs; optimise WBMs according to user-defined metabolic reactions; perform statistical analyses to describe the

data; and visualise significant results in various figures. The nutrition toolbox is designed to convert diets, defined by food items, to *in silico* diets. It does so by mapping the food items, defined by keywords and macronutrient composition, to public databases that have metabolite content measured for food items. In this way, the nutrition toolbox can map the dietary food items to a database alternative and create an *in silico* diet that is defined by measured metabolite content. This subsequently can be set as constraints on the WBMs, and personalised nutrition can be modelled. Persephone and the nutrition toolbox are designed for use by anyone with basic research-computing experience and access to modest computer hardware. Applications include the investigation of human-microbiome co-metabolism and the evaluation of the effects of various physiological parameters, such as age and weight, on human metabolism. Alternatively, the effects of dietary interventions on human metabolism and changes to diet to achieve desired changes in human metabolism can be modelled.

Linking Microbiome-Derived Butyrate Production to Host Metabolism via SIRT1 Regulation

Jordi Roma, *Université de Lorraine*
Oral and poster, P2-16

Genome-scale metabolic models (GEMs) offer a powerful framework to study cellular and host-level metabolism through Constraint-Based Reconstruction and Analysis (COBRA) methods. When integrated with transcriptional regulatory networks (TRNs), these models, enable simulation of context-specific metabolic states under regulatory control. However, capturing how microbiome-derived metabolites impact host regulatory systems remains an open challenge. In early life, the gut microbiome plays a crucial role in shaping host development. Notably, butyrate, a short-chain fatty acid produced by gut microbes, has emerged as a key metabolite with potential epigenetic and metabolic effects on the host. One of its known targets is SIRT1, a NAD^+-dependent deacetylase that serves as a nutrient-sensing regulator involved in metabolic homeostasis, inflammation, and epigenetic regulation. Butyrate has been shown to inhibit SIRT1 activity, potentially altering host gene expression and metabolism. To mechanistically model the interaction between butyrate and host metabolism, we used regulatory flux balance analysis (srFBA) in the MEWpy framework. Wet-lab experiments were performed in Caco-2 cells treated with varying concentrations of butyrate, and SIRT1 protein levels were quantified via western blot. This produced a regression model linking extracellular butyrate concentration to SIRT1 expression. Metagenomic data from the gut microbiomes of 20 infants (sampled at five days, one month, six months, and one year) and 13 mothers had previously been mapped onto the AGORA2 resource of 7,302 microbial reconstructions [1]. Microbiome-derived butyrate production rates had been predicted from personalized community models based on the infant metagenomic data. These predicted fluxes were used to estimate SIRT1 expression levels for each host sample via the regression model. Host metabolism was simulated using the Recon3D GEM, extended with a SIRT1-centered TRN. We modified the srFBA class to support continuous-valued regulatory states, allowing simulation of partial inhibition of metabolic genes, reflecting a more nuanced representation of transcriptional regulation. Simulations were performed across all infant and maternal samples. Flux distributions were analyzed using hierarchical clustering and principal component analysis (PCA). Clear temporal trends were observed, with distinct metabolic profiles emerging at each time point. Differences between infants born vaginally versus via cesarean section were also evident. Specific metabolic subsystems, particularly fatty acid oxidation and nucleotide metabolism, showed strong sensitivity to SIRT1-driven regulatory modulation as influenced by microbial butyrate fluxes. These differences suggest that early-life microbial composition and metabolism may influence host metabolic programming via regulatory pathways. Our integrative framework provides a mechanistic approach to study host-microbiome interactions by linking microbial metabolite production to host gene regulation and metabolism. By incorporating continuous regulatory states, we improve the biological realism of TRN-GEM integration and capture graded regulatory effects. This study highlights the importance of microbial butyrate as a modulator of host metabolic regulation through SIRT1, with potential implications for early-life development and disease susceptibility. The model is currently being expanded to include additional microbial metabolites and transcriptional regulators, aiming to map broader regulatory-metabolic interactions across developmental stages.

[1] Heinken, A., Hertel, J., Acharya, G. et al. Genome-scale metabolic reconstruction of 7,302 human microorganisms for personalized medicine. *Nat Biotechnol* **41**:1320 (2023). doi:10.1038/s41587-022-01628-0

Using metabolic modelling to understand trophic dependencies in the *Arabidopsis* leaf and root microbiota

Frowin Reichhardt, *University of Cologne*
Poster, P2-17

Longer extreme weather and growing population increase the need for efficient agriculture and stress-resistant crops. The reliance on agrochemicals to enhance crop yields has contributed to the deterioration of soil quality, further complicating efforts to ensure global food security. Studies in plant-microbe interactions have revealed the prospects of employing artificial microbial communities to aid in crop management and increase crop productivity as a sustainable alternative to the usage of agrochemicals. However, understanding microbial community structure means investigating a highly complex system of metabolic exchanges between the host and its associated microbes interconnected with competitions and cross-feeding in the microbial community itself. How the presence of certain microbes enables the presence of others and how these various members integrate into an interdependent food web has hardly been investigated. To address this knowledge gap, we simulate growth succession and hierarchical trophic exchanges in the phyllo- and rhizosphere communities of *Arabidopsis thaliana* by making use of genome-scale metabolic

models generated from microbial genome sequences found in the At-Sphere database. This database, contains *Arabidopsis* leaf- and root-derived microbiota culture collections representing the majority of bacterial species that are reproducibly detectable by culture-independent community profiling and hosts hundreds of microbial genome sequences. We generated draft versions of genome-scale metabolic models for all 197 root-associated microbes and all 224 leaf-associated microbes using the automated reconstruction tool CarveMe and subsequently manually curated all of them to match community standards. To reflect the indirect effect of the plant on the native community, a constraint-based modelling algorithm, the microbial community succession module, was employed. This modelling framework enables the iterative simulation of microbial community members growth in a metabolomics-based *Arabidopsis* root- or leaf-like environment. Exchange metabolites produced by microbes in one iteration are used to update the list of available compounds in the environment used for the next iteration, thus allowing us to pinpoint key metabolites for microbial growth in each iteration and the respective microbes secreting them. Our preliminary results show that only 22% of root-associated microbes are capable of initial growth in the defined plant-mimicking medium without any prior microbial secretions, while this number increases to 80% when secretions from these initial colonizers are included. A similar trend was observed in the leaf-associated community, where 40% of microbes grew initially, expanding to 80% with the help of metabolic secretions from the first cohort. These results highlight the strong trophic interdependencies within both the rhizosphere and phyllosphere microbiota and demonstrate the potential of our iterative modeling approach to identify keystone species and metabolic facilitators. By revealing the layered structure of microbial community assembly and metabolic complementarity, this method offers a valuable tool for the rational design of synthetic communities tailored to support plant health and resilience.

Genome-scale metabolic reconstruction of the plant growth-promoting bacterium *Paraburkholderia dioscoreae*

Pablo Rolle, *University of Vienna*
Oral and poster, P1-18

Paraburkholderia dioscoreae MSB3T is a newly discovered species with potential agricultural impact, isolated from leaf acumens of the 'air potato yam' *Dioscorea bulbifera*. *P. dioscoreae*'s genome encodes a relevant combination of features mediating beneficial plant-associated lifestyle and it exhibits significant growth promotion when applied to agriculturally important plants such as tomato. Here we constructed iPR1691, the first genome-scale metabolic model for *P. dioscoreae* MSB3, which includes 1687 reactions, 1487 metabolites, and 1691 genes. From an available annotation of MSB3's genome, a draft model was constructed using an automated approach followed by automatic gap-filling on KBASE, and was manually curated using constraint-based modeling. *P. dioscoreae* MSB3 can grow on 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor in plants, as a carbon and nitrogen source. As ethylene is a potent growth regulator, associated with fruit ripening, growth arrest, and senescence, ACC consumption has been shown to be a key mechanism in growth promoting bacteria. Based on the reactions and genes present in the model, and on various pathways available on MetaCyc, a pathway for ACC usage in *P. dioscoreae* was constructed. Flux Balance Analysis (FBA) was successfully performed to test the growth of *P. dioscoreae* on different substrates like glucose, succinate, and ACC, and diverse conditions of carbon, oxygen, and nitrogen availability were simulated. The model was evaluated through Metabolic Model test (MEMOTE), getting a score of 89%. For experimental validation, FBA predictions were compared against proteomic data, where for 55% of the active reactions the corresponding enzyme was detected in the proteome. Metabolic models are great tools to gain deeper knowledge and understanding on the metabolism of different organisms. This model provides a representation of the growth of MSB3 on ACC which later could be used to join this model to one of a plant and thus simulate the plant-bacteria interactions. Such a model will allow to further study the plant growth-promoting effects of *P. dioscoreae* MSB3.

Genome-Scale Metabolic Modeling of the OMM19.1 Synthetic Gut Community: Integrating BIOLOG Anaerobic Assays and Model Refinement

Adrian Rendon Schatanek, *Joint Research Center for Computational Biomedicine*
Poster, P2-19

Advances in metagenomic and multi-omics technologies have revealed a strong link between gut microbiota composition and host health, diet, environment, lifestyle and disease. However, the specific metabolic interactions among microbial community members and how these interactions contribute to resilience and compositional stability—remain poorly understood. Gaining mechanistic insight is essential to move from correlative studies to predictive, personalized microbiome-based interventions. To bridge this gap, the Oligo-Mouse-Microbiota (OMM19.1), a synthetic consortium of 19 bacterial strains representing major gut phyla, provides a controlled model for studying microbial function. This consortium, combined with genome-scale metabolic models (GSMMs), represents a powerful framework for predicting compound production by individual species and for determining how production rates are influenced by environmental conditions, nutrient availability, and interspecies interactions. While Weiss et al. have profiled a subset of these strains in rich media, such conditions do not fully reflect the fermentative, substrate-limited environment of the distal gut. We therefore aimed to reconstruct GSMMs for each strain, and integrate them with *in vitro* substrate utilization data generated under anaerobic conditions using the BIOLOG AN system. Our goal was to identify key metabolic traits and interspecies compatibilities relevant to the gut environment. However, the use of BIOLOG AN plates presented several challenges due to substantial variability across samples, data types, and plate triplicates. In this study, we critically evaluate both the opportunities and limitations of BIOLOG AN plates for metabolic phenotyping under anaerobic conditions. We screened 95 carbon sources across all OMM19.1 strains using BIOLOG AN plate triplicates, obtaining high-dimensional absorbance data and readouts

across multiple wavelengths and timepoints. To address the high variability across replicates and conditions, we developed a pipeline that classified each data point based on dataset-specific thresholds. This approach assigned categorical labels (positive, boundary, indifferent) to over 47,000 data points and produced average utilization scores per substrate per strain. From these data, more than 160 substrates were identified as potential metabolic inputs for OMM19.1 strains. Selected substrates were validated through growth assays in a defined minimal medium adapted from Kwoji et al., supplemented with specific carbon sources. Notably, while classic sugars like D-glucose and D-fructose showed expected consumption patterns in many strains, results for amino acids and complex carbohydrates varied widely, highlighting limitations in reproducibility of BIOLOG plates under anaerobic conditions. To enable study of the metabolic flux of each compound we aimed to integrate the *in vitro* assays into GSMMs. For this, we generated draft GSMMs for each strain using gapseq, followed by flux balance analysis and flux variability analysis under defined media conditions. The reconstructed GSMMs contained between 1203-2644 reactions and 1110-2125 metabolites. Each substrate was tested *in silico* for its potential to support ATP maintenance and biomass production. Where no growth was predicted, we examined media completeness and identified key cofactors and metabolites that were iteratively added to model constraints. In total, 42 substrates (13 metabolite classes) were identified as essential across models for accurate simulations. Model predictions were often inconsistent with *in vitro* results, revealing knowledge gaps in pathway annotation. For instance, four GSMMs failed to metabolize D-glucose *in silico* despite strong experimental evidence. Iterative refinement included curation steps based on experimental feedback, literature, and databases, such as the incorporation of palatinose metabolism in *Lactobacillus murinus* and adjustments to sugar pathways in *Mucispirillum schaedleri*. These refinements improved alignment between *in silico* and *in vitro* data. Our study illustrates the strengths and limitations of BIOLOG AN assays in anaerobic settings, emphasizing the importance of iterative model refinement based on integrated experimental and computational data. The resulting GSMMs form a robust foundation for future community-level simulations and metabolic interaction studies. Ultimately, this work contributes to the development of reproducible, predictive models of microbial function in the gut ecosystem.

Analysing plant-microbe interactions via community modelling of metabolic interactions in a seven-member maize SynCom

Lisa Schönherr, *University of Cologne*
Poster, P2-20

Plant microbiomes are complex networks of microorganisms that can be beneficial or commensal. In the rhizosphere, these sophisticated microbial communities can support plant health by improving nutrient uptake, enhancing immune responses, and boosting stress resistance, thus enabling plants to better adapt to challenging environmental conditions. These interactions are mainly driven by metabolic processes such as competition for nutrients, which leads to niche differentiation, as well as through metabolic exchanges and cross-feeding. However, how these factors influence plant-microbe and microbe-microbe interactions remains an open question. Given the complexities of natural systems, simplified synthetic communities offer an attractive platform to dissect these interactions and address them in both experimental and computational contexts. In particular, a seven-member bacterial synthetic community (SynCom) derived from maize roots encapsulates key features of native root microbiomes. This SynCom exhibits metabolic interdependencies, including vitamin auxotrophies, which in the context of niche differentiation can facilitate microbial diversity. Additionally, it features a keystone species whose removal disrupts community assembly and function. These properties make the SynCom an ideal system for exploring microbiome stability and metabolic interaction networks. In this work, we reconstructed and curated genome-scale metabolic models for all seven SynCom members using publicly available genomes and the reconstruction tool CarveMe. Each model comprises approximately 1600 metabolites and 2500 reactions, with manual curation focusing on vitamin biosynthesis and auxotrophic traits to accurately capture metabolic interactions. We then used these models to simulate the key *in vitro* and *in vivo* experiments described in the original study, i.e. testing whether our computational reconstructions can mirror the complex metabolic behaviour that drives niche differentiation and cross-feeding. Our ongoing analyses will explore the metabolic features underpinning community stability—by investigating the impact of keystone species removal—and assess the interdependencies that support microbial diversity. Ultimately, we anticipate that this work will demonstrate the predictive power of metabolic modelling as an effective tool for understanding plant-microbe interactions, while laying the groundwork for designing stable, beneficial plant-associated microbial communities that enhance plant health under challenging environmental conditions.

Applications in Syst & Synt Biology / Biol & Metab Engineering / Sustainable Bioengineering

Regulation of the Carbon Flux in *Synechocystis* Using the PGAM-PirC Switch

Nathalie Becker, *University of Tübingen*
Poster, P1-21

For a sustainable bioeconomy, CO_2 neutrality is pivotal. Cyanobacteria are autotrophic organisms, which perform oxidative photosynthesis. Their ability to oxidize water and use the electrons for reducing carbon dioxide (CO_2) to form organic matter by using solar energy makes them interesting organisms for biotechnological applications. *Synechocystis* sp. PCC 6803 is a unicellular, non-diazotrophic cyanobacterium. Relatively fast growth rates among cyanobacteria, good genetic accessibility and the natural excretion of various metabolites under specific conditions make *Synechocystis* sp. PCC 6803 an interesting candidate for biotechnological applications. Therefore, *Synechocystis* is studied concerning the metabolic flux to further broaden the bioengineering platform. The carbon metabolism of *Synechocystis* can be divided in upper and lower glycolysis. In upper glycolysis, CO_2 is fixated and next to other pathways 1,5-ribulose is regenerated for further CO_2 fixation. Also, under nitrogen starvation, glycogen is accumulating as a carbon storage polymer. On the other hand, in lower glycolysis, the carbon flow is directed towards anabolic pathways, such as the production of amino acids, fatty acids, biopolymers, such as polyhydroxybutyrate (PHB) and more. An important control point of the carbon flux is the 2,3-bisphosphoglycerate-independent phosphoglycerate-mutase (PGAM), which converts the first CO_2 fixation product 3-phosphoglycerate to 2-phosphoglycerate. This reaction directs carbon flow from upper to lower glycolysis. Under nitrogen starvation, *Synechocystis* adapts with a reversible process called chlorosis, where the photosynthesis apparatus is degraded, and the overall metabolism is downregulated. Under these conditions, the PII protein, the sensor protein for the carbon/nitrogen status, releases the small protein PirC. Consequently, PirC binds to PGAM and thereby inhibits its activity. As a result, glycogen is formed as carbon storage as well as PHB is accumulating for yet unknown reason [1]. The aim is to use this PGAM-PirC key hub to direct the metabolic flux towards lower glycolysis and the production of PHB and other feedstock chemicals. Therefore, a strain constitutively overexpressing PGAM was constructed by using constitutive synthetic promoters. Levels of PGAM and glycogen, PHB production and metabolite excretion were analyzed. In addition, several deletion mutants were tested, e.g., a ΔphaEC mutant, which cannot produce PHB. Furthermore, it has been shown that a PGAM variant lacking the C-terminus is less inhibited by PirC [2]. This variant will also be tested in different strain backgrounds. Our results revealed that PGAM overproduction led to lower glycogen levels, whereas PHB amounts were higher under nitrogen depletion compared to the wild type. This indicates the redirection of the carbon flow towards lower glycolysis. Deletion of either pirC or phaEC resulted in increased excretion of the metabolites pyruvate, succinate and 2-oxoglutarate to the medium. The overexpression of PGAM in the ΔphaEC mutant increased the excretion even more, while higher level of PGAM in the ΔpirC mutant resulted in a growth impaired strain. The double mutant lacking pirC and phaEC showed the highest excretion of the tested metabolites. Our work shows the important role the PGAM-PirC switch is playing in the metabolism. By tuning this regulation further, a platform will be established to redirect carbon flow for enhanced valuable chemical production.

[1] Orthwein et al., *Proc Natl Acad Sci U S A* **118**:e2019988118 (2021). doi:10.1073/pnas.2019988118.
[2] Orthwein et al., *mBio* **16**:e0337824 (2025). doi:10.1128/mbio.03378-24.

Identifying metabolic subtypes in heart disease via metabolic task analysis

Marian Breuer, *Maastricht University*
Oral

The combination of genome-scale metabolic models (GEMs) with transcriptomic data allows to delineate the role of individual metabolic functions in health and disease. In particular, the framework of metabolic task analysis allows to study the role of specific metabolic functions ("tasks") with a higher resolution than general metabolic subsystems; without however requiring the construction of full context-specific models, which from transcriptomic data alone is challenging. Here, we apply a metabolic task analysis pipeline to dilated cardiomyopathy (DCM), a heart condition characterized by metabolic shifts that are heterogeneous between patients. In particular, we combine publicly available transcriptomic data from end-stage DCM patients and controls with an extended set of metabolic tasks combined and curated from published task lists; and analyze these tasks and data using the latest human GEM, Human1. By clustering patients on their metabolic task scores, we identify two metabolic subtypes of DCM that not only display distinct metabolic alterations; but also show immune-related differences on the whole-transcriptome level, suggesting an interplay between inflammation, immune response and metabolism in these DCM subtypes. Thus, we demonstrate how GEMs and patient transcriptomic data can directly be combined to identify metabolic subtypes in disease [1].

[1] Nihant et al., *bioRxiv* (2025). doi:10.1101/2025.03.28.645901.

SYNONIM: Synthetic community design based on functional representation

Benjamin Coltman, *CeMESS, University of Vienna*
Poster, P1-22

Environmental microbial communities are inherently complex, making it difficult to experimentally assess the roles of individual members, their interactions, and their responses to perturbations. Synthetic microbial communities (SynComs) simplify this complexity by capturing key metabolic traits while minimizing confounding diversity. However, designing SynComs that accurately represent natural communities remains a challenge. SYNONIM (SYNthetic cOmmunity desigN via approxIMation) addresses this issue using a variety of complementary strategies. It employs a function-first, bottom-up approach that integrates different optimization techniques to design SynComs that best approximate a reference functional profile derived from metagenomic data. This allows for the creation of SynComs that not only reflect essential metabolic functions but also account for ecological principles such as functional redundancy, complementarity, and community size control. Building on existing strategies for SynCom design, SYNONIM offers flexibility by allowing users to tailor constraints to specific needs—including the ability to focus on the coverage of key functions or controlling the diversity of the community. These capabilities enable the generation of reductionist, yet representative, SynComs that retain critical functions and dynamics, facilitating hypothesis-driven experiments that isolate the contributions of individual traits. As a proof of concept, SYNONIM has been used to design SynComs with varied denitrification pathway configurations. Ongoing experiments are assessing how these differences affect soil nitrous oxide emissions. Ultimately, SYNONIM's versatile approach paves the way for more predictable, engineered ecosystems and provides valuable insights into microbial ecology.

Modeling Metabolic Heterogeneity and Crosstalk in the Tumor Microenvironment with scFASTCORMICS: A Single-Cell Framework for Context-Specific Network Reconstruction in Colorectal Cancer

Evelyn Gonzalez, *University of Luxembourg*
Poster, P1-23

Metabolic heterogeneity within the tumor microenvironment (TME) plays a pivotal role in immune evasion and therapy resistance, yet it remains poorly characterized at single-cell resolution. Advances in single-cell RNA sequencing (scRNA-seq) have enabled the identification of diverse cellular populations within tumors, providing an unprecedented opportunity to explore cell-type-specific metabolic programs. However, the translation of scRNA-seq data into mechanistic, genome-scale metabolic models remains a major challenge. This is due to the inherent sparsity and noise of single-cell data, as well as the computational demands of reconstructing biologically accurate, context-specific metabolic networks at scale. Existing methods often fail to capture intercellular metabolic crosstalk or are not robust enough to handle complex, large-scale datasets. Here we show the ongoing development and application of an enhanced version of scFASTCORMICS, a scalable metabolic network reconstruction framework tailored for single-cell data. The algorithm leverages qualitative gene expression and aggregates scRNA-seq data into cell-type-level pseudobulk profiles, allowing for more robust inference while preserving cell identity. This approach reduces data sparsity and noise while simplifying computational requirements, enabling the generation of context-specific genome-scale models for distinct TME populations. The pipeline uses discretized gene activity (on/off states) to construct cell-type-specific metabolic subnetworks and connects them via a union compartment that simulates intercellular metabolite exchange. To enhance model fidelity, we compare discretization thresholds derived from both bulk and single-cell distributions and use bulk RNA-seq data to guide parameter selection. The final metabolic models balance compactness, completeness, and specificity, supported by Pareto-informed optimization strategies. We benchmarked the new scFASTCORMICS implementation against alternative reconstruction methods, including ftINIT and METAFlux, using colorectal cancer (CRC) scRNA-seq datasets. Validation is performed using orthogonal datasets, including tumor-normal LC-MS metabolomics (Jain et al., 2024), single-cell multi-omics data from comprehensive CRC TME characterization (Wu et al., 2023), and RNA-based flux prediction tools like Fluxestimator (Zhang et al., 2023). The models successfully recapitulate known metabolic signatures and reveal distinct immunosuppressive metabolic programs across four TME subtypes. Current applications focus on identifying key metabolites involved in cancer–immune crosstalk and evaluating the metabolic plasticity of immune and stromal cells within the CRC TME. We are particularly interested in testing how targeted nutrient interventions, guided by predicted metabolic vulnerabilities, could shift the TME from tumor-promoting to a tumor-restrictive state. In future work, we aim to extend these simulations to experimentally defined immunosuppressive programs (e.g., PD-L1, CD47, Tregs) using public single-cell atlases. These developments contribute to a generalizable pipeline for single-cell metabolic modeling that bridges transcriptomic data and mechanistic network inference. By addressing key computational and biological challenges, this work enables a deeper investigation of metabolism-driven immune suppression. It opens new avenues for rational, subtype-specific therapeutic strategies across heterogeneous tumor ecosystems.

Glycolysis in anaerobic fermentation: how nature efficiently resolves the electron puzzle.

Rebeca Gonzalez-Cabaleiro, *Delft University of Technology*
Oral

Rationalisation of catabolic pathways can explain their evolutionary selection while simultaneously identifying new opportunities for biotechnology. In anaerobic fermentations, where no external electron acceptor is available, microbial communities must self-balance redox reactions by generating both oxidised and reduced products from a single carbon substrate that simultaneously serves as electron donor and acceptor. These constraints lead to short metabolic pathways and low biomass yields, often requiring cooperative degradation by a microbiome. Specifically, in anaerobic fermentations, there is potential for highly sustainable production of valuable chemicals using low-cost natural communities. In absence of any external electron acceptor, anaerobic fermentors are obligated to self-balance the redox reactions yielding carbon products more reduced and more oxidised than the substrate that acts as a carbon source and both electron donor and acceptor. Furthermore, metabolic pathways are short, needing a

community to completely degrade a carbon substrate. This, subjected to the fact that the efficiency of biomass production is low in fermentative communities, has given us the opportunity to design bioprocesses that inhibit the downstream activity of populations degrading products of interest. However, the industrial exploitation of these metabolic activities remains hindered by our inability to fully predict and explain the overall stoichiometry of these microbiomes activities and its possible change with environmental conditions. Fermentative bacteria possess abundant enzymatic complexes such as HydABC, Rnf, and Hyd, which facilitate flexible electron transfer among electron carriers in the steps post-glycolysis in any catabolic pathway. These systems theoretically enable a wide range of stoichiometries between acetate and butyrate production. From a biotechnological perspective, a more selective product profile—e.g., exclusive butyrate production—would be highly desirable. However, despite efforts in both pure and mixed cultures, fermentation invariably yields mixed carboxylates, and steering the process towards a single product remains elusive without extensive strain engineering. For instance, the fermentation of 1.5 moles of glucose consistently yields 1 mole of acetate and 1 mole of butyrate across various conditions. A pathway typically catalysed by Clostridium pasteurianum (1) and frequently observed as dominant in mixed-culture fermentations (2). This conversion yields 3.33 mol of ATP per mol of glucose, which appears suboptimal when compared to alternative stoichiometries that could, in theory, yield higher ATP by favouring acetate production. To investigate the potential underlying constraints, that limit the observation of other theoretically possible stoichiometries in the fermentation of glucose, we employed a well-known and defined thermodynamic model (3). We have extended it to incorporate constraints specifically associated with fermentative metabolisms: redox potential constraints of electron carriers, ATP synthesis energy thresholds, and limited concentrations of conserved moieties. The model evaluates the feasible distributions of driving forces across different stoichiometries following glycolysis, considering observed stoichiometries but also, hypothetical ones. Our results demonstrate that the observed equimolar acetate:butyrate stoichiometry is not only thermodynamically viable but also optimally balances driving forces across metabolic steps while maintaining high ATP yields. Alternative stoichiometries, though energetically feasible, suffer from inefficient energy distribution and high dissipation at key catabolic steps. This high energy dissipation does not imply a higher efficacy catalysing the process (driving forces above 20kJ/mol) (3), however, these stoichiometries are doomed to be 'rigid': they have reduced capacity to endure environmental changes, as less energy is available for distribution among the catabolic steps of the pathway. After this first analysis on driving forces distribution, we have developed a deeper analysis focusing on redox potential compatibility among electron carriers involved in the catabolic steps of these pathways. This has revealed that only certain stoichiometries enable effective electron exchange via HydABC-, Rnf-, or Hyd- mediated reactions, meanwhile others lead to inefficient pathways with excessive energy dissipation and limited adaptability to environmental changes. Overall, this work provides a mechanistic rationale for the dominance of specific fermentation patterns in nature and highlights the importance of efficiency in energy distribution as selective pressure in systems in which energy is strongly limiting microbial growth like fermentations, and offers new insights into the evolutionary constraints shaping anaerobic catabolic activities, drawing the limits of maximum achievable technological efficiencies when working with non-engineered bacterial strains.

(1) Buckel et al., *Biochim Biophys Acta* **1827**:94 (2013). doi:10.1016/j.bbabio.2012.07.002.
(2) Rombouts et al., *Biotechnol Bioeng* **117**:1281 (2020). doi:10.1002/bit.27301.
(3) Noor et al., *PLoS Comput Biol* **10**:e1003483 (2014). doi:10.1371/journal.pcbi.1003483.

Cloning, Purification and Possible use of a Bacterial Nitroreductase as Biosensor

Kemal Güven, *Dicle University*
Poster, P1-13

Nitroreductases are enzymes that can reduce nitroaromatic compounds to nitrite, amino group or hydroxylamine groups. Nitroaromatic compounds are synthetic industrial chemicals used as explosives, dyestuffs, herbicides, insecticides and solvents. Nitroaromatic compounds generally cause environmental pollution, which causes toxic and mutagenic effects on humans, fish, algae and microorganisms, as they can remain undisturbed in nature for a long time. While nitroreductases are mostly found in bacteria, they are also known to be found in eukaryotes and archaea. Bacterial nitroreductases have the potential to be used in many different biotechnological fields due to their ability to metabolize nitrogenous compounds that are toxic, mutagenic or carcinogenic. The most important of these is the use of nitroreductases in the treatment of GDEPT (gene-directed enzyme prodrug therapy) cancer. In addition, the potential for use in the bioremediation and detection of nitro compounds and as a biosensor has also been the subject of research. There are not enough studies on nitroreductases belonging to Bacillus species. In this context, the potential of the purified nitroreductase (KG5-NTR) as a biosensor in biotechnological applications has been investigated by cloning in *E. coli* and purification of the enzyme. Purification was performed by applying supernatant obtained from the recombinant cell to High Performance Ni-NTA (Sigma) affinity column and obtaining fractions with 100-500 mM imidazole solutions and applying it to SDS-PAGE. Then, purified nitroreductase was used for the biosensor experiment. For the biosensor production, $25\,\mu L$ ($21\,\mu g$) enzyme solution, $25\,\mu L$ 2% gelatin solution and $1\,\mu L$ 2% glutaraldehyde were applied to the surface of a Glassy Carbon Electrode (GCE) and kept overnight at $4\,^{\circ}C$. The biosensor prepared for electrochemical characterization was immersed in a 5 ML cell containing 50 mM Tris-HCl (pH 8.0) buffer and 50 µM nitrofurazone. The working electrode is polarized in the potential range of -0.1V to +0.9V against the Ag/AgCl reference electrode. The potential corresponding to the maximum current was determined using differential pulse voltammetry (DPV) technique. In control experiments, only the electrode signal was measured and minimal current was obtained at $\pm 0.0004\,\mu A$ levels. In the presence of nitrofurazone, significantly increased current values were recorded. In particular, a significant decrease in current intensity was observed in the potential December of 0.3–0.5 V and the highest negative current value was recorded as $-174.88\,\mu A$ at 0.4 V. This result reveals that nitrofurazone is converted into electroactive products through the enzyme KG5-NTR nitroreductase, and the reduction reaction occurs most intensively in this potential December. The KG5-NTR

biosensor developed in this study showed high sensitivity by giving the maximum current response in the first minute after the addition of the substrate (nitrofurazone). The strong reduction signal observed in 1. minute showed that the enzyme-substrate interaction is fast and efficient. In 2. and 5. minutes the decrease and change of direction of the current values in minutes suggest that the enzymatic activity decreases with time and the ambient conditions affect the reaction kinetics. These findings reveal the potential of the biosensor for detecting nitroaromatic compounds to make precise measurements in a short time.

Analysing mitochondrial metabolism across tissues and time with mitoMammal and FBAcomparer

Bianca Habermann, *Cnrs, Ibdm Umr7288*
Poster, P1-70

We want to understand how mitochondrial metabolism adapts to the cellular environment of its host. We employ constraint-based modelling using Flux Balance Analysis (FBA) and using our recently published mitoMammal mitochondrial metabolic network, in combination with OMICS data to investigate how mitochondrial metabolism changes across tissues and across developmental time. In order to compare flux rates across experiments, we have developed a set of statistical and network tools, packaged in a user-friendly Jupyter notebook, the FBAcomparer.I will present data from different tissues and developmental states and demonstrate how FBAcomparer can be used to compare results from metabolic modelling.

Validation of Forbidden Fruits: an algorithm to predict growth-coupled production strategies in *Synechocystis*

Loles Hoogerland, *University of Amsterdam*
Oral and poster, P1-25

Cyanobacterial CO_2 conversion holds great promise for sustainability, although challenges remain. One of them is the instability of production – when production imposes a fitness burden on the cell, revertants that eliminate this burden via spontaneous mutations tend to take over the population, often by impairing the overall productivity. A strategy to overcome this instability is growth-coupled production. This strategy can be achieved by engineering the native metabolism to be dependent on a non-native pathway. By knocking out reactions involved in the production of an essential compound, the mutant is not able to survive, or its growth is severely hampered. A non-native pathway can resolve this auxotrophy while simultaneously producing a target compound. We developed an algorithm – Forbidden Fruits – to find viable strategies for producing non-native compounds in a growth-coupled manner. Through Forbidden Fruits, we found a strategy to produce L-lactate in the cyanobacterium *Synechocystis* sp. PCC6803. By knocking out the enzymes responsible for the production of oxaloacetate (PepC and Mdh), *Synechocystis* becomes dependent on an alternative route to produce oxaloacetate. The non-native reaction catalysed by malate-lactate transhydrogenase (MLTH) can convert malate and pyruvate into L-lactate and oxaloacetate. [1] This results in a mutant that is dependent on L-lactate production for oxaloacetate supply. The production of L-lactate via L-LDH has been found to be unstable after 25 generations [2]. The construction of the growth-coupled mutant is ongoing in the lab. Expression of MLTH hampers the growth rate of the organism by 10%. This indicates that the sole production of L-lactate via MLTH is not beneficial for the cells. We hypothesize that creating the growth-coupled dependency on L-lactate production will result in a stable phenotype. Using a tightly controlled turbidostat, the growth-coupled production mutants will be cultivated for many generations with a small propagation bottleneck (5%). Growth rate will serve as selection pressure. This way, we will be testing the growth-coupled L-lactate production stability under stringent conditions.

Single-cell resolved metabolic modelling of the human kidney

Chenglong Huang, *University Medicine Greifswald*
Poster, P1-26

The kidney is a metabolically complex organ composed of highly specialized cell types, each contributing distinctively to its physiological functions. Conventional metabolic models, which treat the kidney as a homogeneous tissue, overlook this critical cellular heterogeneity. To bridge this gap, we developed a single-cell-resolution metabolic network model of the human kidney by integrating single-cell RNA-seq data from the Kidney Precision Medicine Project (KPMP) with the human genome-scale metabolic reconstruction RECON3D. Leveraging the FastCore algorithm, we generated cell-type-specific metabolic subnetworks for 13 distinct kidney cell types under both healthy and chronic kidney disease (CKD) conditions, optimizing reaction inclusion thresholds based on gene expression profiles. Using constraint-based modeling approaches, including flux balance analysis (FBA), flux variability analysis (FVA), Monte Carlo sampling, we systematically characterized metabolic capabilities across different cell types. Our results revealed pronounced functional specialization, as highlighted for example by the differential capacities of cell types to produce nitric oxide, an important signaling molecule in vasodilatation and inflammation. Moreover, putative disease-specific processes were identified. Healthy proximal tubule cells exhibited strong fluxes through oxidative phosphorylation to meet the high energy demands of reabsorption, whereas CKD-affected proximal tubular cells shifted toward anaerobic glycolysis as the primary ATP-generating pathway. Comparative modeling between healthy and diseased states uncovered key metabolic perturbations in CKD, including dysregulated ATP production and altered transport fluxes in tubular cells. This study presents the first systematic exploration of single-cell-resolved metabolic models of the human kidney, providing novel insights into cell-type-specific metabolic

adaptations and their pathological dysregulation in disease. Our framework enables precise mechanistic exploration of kidney pathophysiology and lays the foundation for future applications in biomarker discovery and targeted therapeutic interventions.

Metabolic Modelling of Earthworms

Axel von Kamp, *Max Planck Institute For Dynamics Of Complex Technical Systems*
Poster, P2-27

Until Charles Darwin published his book on earthworms in 1881 their role in the soil was largely unclear. By now, the importance of earthworms for soil fertility has been recognized and they are widely used in ecotoxicological tests, for instance in relation to the effects of microplastics. However, for these purposes mainly their phenomenology (e.g. amount of earthworms in the soil, reproduction rate) is being evaluated. The investigation of earthworm molecular biology is comparatively underdeveloped with the main interest lying in their immune system and regenerative capabilities. For metabolic modelling of prokaryotes, it is common practice to create genome-scale metabolic models from the genome sequence followed by some manual curation and a variety of tools are available to support this process. For eukaryotes, the situation is more complicated for several reasons: Firstly, gene calling is more difficult because of the intron/exon structure of genes and the possibility of splice variants. Also, eukaryotic cells have multiple compartments and prediction of the cellular location of a protein from the genome sequence alone is still error-prone. To make matters worse, metabolite exchange between compartments can be indirect and is often not well-known. Finally, there can be cell-type specific metabolic differences which are also hard to predict from the genome sequence. This makes it difficult to create metabolic models for eukaryotes from the genome sequence alone. Here, we show how a metabolic model of the compost worm *Lumbricus rubellus* can be constructed from the annotated genome with the help of BioCyc and KEGG. For assigning reactions to compartments, we take a *Caenorhabditis elegans* metabolic model as template whose molecular biology as a model organism is well studied. With this strategy a working draft metabolic model can be constructed that is able to produce all common biomass components. This shows that despite the complications of metabolic modelling in eukaryotes it is still possible to create a meaningful draft model of earthworm metabolism. The metabolic model and BioCyc Pathway Genome Database created in this study are publicly available. We also envision that with scRNA data it will in the future be possible to specialize this model into cell-type specific variants to better understand the earthworm organism as a whole.

Enabling New Biochemical Conversions by Combining Fermentative Metabolism with Selected Respiratory Modules

Steffen Klamt, *MPI Magdeburg*
Oral and poster, P2-28

Many industrial bioproduction processes rely on anaerobic microbial fermentations, where the need for cellular redox balance naturally couples growth of the microbes with high-yield synthesis of fermentation products. However, redox balance requirements under anaerobic conditions also limits the range of fermentable substrate-product combinations. Here, I will present recent work on the targeted design of an *E. coli* platform strain, which facilitates the implementation of specific respiro-fermentative pathways for the production of selected chemicals [1]. In this strain, the transfer of electrons to the ubiquinone pool, and thus respiration, is completely blocked. However, reintroducing selected respiratory modules enables the use of oxygen as a terminal electron acceptor, which helps rebalance otherwise unbalanced substrate-product conversions. The potential of this new platform strain is demonstrated by enabling growth-coupled synthesis of lactate and isobutanol from glycerol - conversions, that would be unbalanced under anaerobic conditions or uncoupled under aerobic conditions in the wild-type strain. We believe that the developed concept of controlled respiro-fermentation is a highly promising approach to enable new-to-nature biochemical conversions by overcoming the limitations imposed by redox balancing.

[1] Schulz-Mirbach et al., *Nat Commun* **15**:6725 (2024). doi:10.1038/s41467-024-51029-x

Development of the MPA-Pathway-Tool towards protein-constrained analysis on the web

Emanuel Lange, *Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.*
Poster, P2-29

Microbiomes play key roles in environmental processes, biotechnology, and human health, largely through their metabolism. Metabolic fluxes can be studied using constraint-based models, which can estimate reaction rates under steady-state assumptions. These models can be enhanced with constraints based on enzyme concentrations to improve predictive accuracy. We demonstrate this by applying an established method for integrating proteomic data into an enzyme-constrained Escherichia coli model using a programmatic approach. To make these methods accessible to non-programmers, we implemented them in the MPA-Pathway-Tool, a user-friendly web application for pathway mapping currently under development. Using Python, MATLAB, and the COBRApy and autoPACMEN packages, we integrated enzyme kinetics parameters and quantitative proteomics measurements into the model via the sMOMENT method. The refined model achieved better flux predictions (RMSE reduced from 3.76 to 2.33) and lower total flux variability (smallest total variability of 17.50% of the original model). The MPA-Pathway-Tool was extended to support flux simulations with sMOMENT, SBML model exchange, and network-based visualization of results. The workflow was significantly accelerated

from about three days to one. We demonstrate that model predictions can be effectively improved by incorporating quantitative proteomic data. The implemented methods have been integrated into the MPA-Pathway-Tool, enabling user-friendly and platform-independent usage. The application is actively being developed, with planned extensions to support protein-constrained modeling of human metabolism.

FedBatchDesigner: A user-friendly dashboard for modeling and optimizing growth-arrested fed-batch processes

Julian Libiseller-Egger, *University of Vienna*
Poster, P2-30

Optimizing fed-batch fermentation strategies is key to maximizing bioprocess efficiency. While mathematical modeling can aid process design, its complexity often limits accessibility for experimental scientists. We present FedBatchDesigner, a user-friendly web tool for optimizing fed-batch processes with a growth-arrested production stage. With minimal input requirements, FedBatchDesigner enables rapid exploration of a process's Titer–Rate–Yield landscape for both constant and exponential feeding strategies. Interactive visualizations allow users to assess trade-offs between productivity and titer, supporting rational decision-making without the need for extensive modeling expertise. We demonstrate FedBatchDesigner's utility via two case studies in *E. coli*: synthesis of (i) L-valine with a microaerobic production stage and (ii) mevalonic acid under sulfur starvation. In both cases, FedBatchDesigner successfully identifies optimized feeding strategies that improve average volumetric productivity.

Metabolic modeling of two-organism consortia located on fibrillar membranes for the production of chemicals using sunlight and carbon dioxide

Liva Kristiana Lukasa, *University of Latvia*
Poster, P2-31

The global chemical systems are primarily linear, fossil-dependent and emissions-intensive. Finding sustainable solutions to produce chemicals using a circular (bio)economy is ambitious but necessary for a sustainable future. Objectives: The main objective of the LivMat project is to capture and utilize natural resources (e.g., natural fibers) and waste resources (e.g., CO_2) for developing catalytic living materials (cat-LMs) that are robust, energy-efficient, and scalable for chemical production. Potential applications: LivMat develops a synthetic microbial consortium reliant on natural and waste resources and constructs cat-LMs-based bioreactor prototypes for continuous monomer production. Impact and potential benefits: The commercialisation of the LivMat concept will capture CO_2 and support the EU's GHG emission reduction targets. Developing bioreactor prototypes for continuous chemical production supports the European Green Deal and the Circular Economy Action Plan. The metabolic engineering of two consortia-building microorganism interactions uses a constraint-based stoichiometric modelling approach. The COBRA Toolbox and the GLPK package were applied in MATLAB 2017b to run the metabolic modelling. The genome-scale metabolic models for *Synechocystis* sp. PCC6803 and *Pseudomonas taiwanensis* VLB120 were combined to develop a two-cell metabolic model with shared substrates under mixotrophic conditions. The term 'shared' means that a specific maximum rate for a substrate was considered in the two-cell model, and the two models of the microbes compete for that substrate. Maximization of the growth rate for both cells was considered as the objective function. For simulation of mixotrophic conditions, the uptake of glucose, bicarbonate, and photons was determined. Transport reactions were added to the two-cell model for the exchange of malate, fumarate, acetate, and oxygen between the two cells. The two-cell model was used to simulate growth under anaerobic conditions. The results indicate that the growth of *Pseudomonas taiwanensis* depends on the transport of oxygen, which is produced under oxygenic photosynthesis by *Synechocystis*. So, *Synechocystis* is the dominant microbe in the consortia, consuming most of the glucose in the medium, and acetate and fumarate produced by *Synechocystis* are transported into *Pseudomonas taiwanensis*. The two-cell model was also used to evaluate the effect of glucose and photon uptake rates on the growth rates of the cells. The results show that the ratio of *Pseudomonas taiwanensis* per *Synechocystis* is higher under lower rates of glucose and photons. The constructed two-cell metabolic model is now available for the evaluation of the production of various bioproducts under different growth conditions.
This research is funded by the Latvian State Budget (Latvian Council of Science) in the frame of M-Era.Net project "Productive catalytic living materials: combining 3D biobased fibrillar membranes with synthetic microbial consortia to produce chemicals" (LivMat), grant number ES RTD/2024/27.

Beyond carbon concentration: A model to understand what defines a heat-resistant plant.

Jérémie Muller-Prokob, *Heinrich-heine Universität*
Poster, P2-32

C4 photosynthesis exhibits good efficiency and resource management under environmental conditions that are becoming increasingly widespread due to climate change (e.g. high temperatures and low water availability). Under these conditions, traditional crop yields decrease. Thus, being able to understand how C4 plants function and evolve, with the goal of engineering a C4 crop capable of withstanding extreme environmental conditions remains a major challenge. Multiple research efforts aim at engineering a C4 crop from a C3 crop. We propose that a new perspective on studying C4 plants is needed to gain a deeper and more comprehensive understanding of the subject. Current models and experimental designs focus either on the biochemical aspects, hydrological

aspect or thermal aspect of the C4 syndrome. Our goal is to develop a holistic model that combines the C4 biochemical pathways model with mechanistic representations of the plant's environment, anatomy, and overall structure. This integrated model will enable us to produce explainable predictions of the fitness and adaptive traits of a plant situated in its environment. By generating explainable predictions, we aim to identify the critical environmental conditions and plant metabolic phenotype and anatomy that favor the use of the C4 pathway. Additionally, by using these predictions, we aim at a producing a more precise description of the selection pressures that played a role in C4 evolution. Using an early implementation of our modeling framework, we examine how air temperature, irradiance, and CO_2 concentration influence the optimal plant phenotype. We first validate the model by benchmarking its outputs against existing experimental data. We then extend our analysis to environmental conditions that are difficult to test experimentally, demonstrating the model's potential to expand the current understanding of what defines a C4 plant.

Fine-tuning a synthetic Entner-Doudoroff pathway in *E. coli*

Henrique Oliveira, *Wageningen University*
Poster, P2-33

The Embden-Meyerhof-Parnas (EMP) pathway is the main glycolytic route in *E. coli* when glucose is the sole carbon source, generating 2 ATP and 2 NADH molecules per glucose oxidized to pyruvate. An alternative route for glucose, for which the genes are also present in *E. coli*, is the Entner-Doudoroff (ED) pathway, which generates 1 ATP, 1 NADH and 1 NADPH. The ED pathway is both attractive for the formation of NADPH and could potentially run with lower protein costs. Even though *E. coli* possesses genes encoding both pathways, on glucose it is programmed to use the EMP pathway only. The ED pathway is only switched on during growth on gluconate as a carbon source. The *E. coli* ED-specific genes edd and *eda* are strictly regulated through the repressive transcriptional regulator GntR, which is inactivated in the presence of gluconate. Therefore, even when blocking the upper part of the EMP, *E. coli* is not able to grow fast on glucose via the ED pathway. We hypothesize that if we use orthogonal enzymes without native control from the natural ED-organism *Zymomonas mobilis*, and use synthetic control elements (promoters and ribosome binding sites), we could fully activate the ED pathway with glucose. However, a key challenge when introducing synthetic control elements for a synthetic pathway is to optimize the enzyme levels for efficient, balanced expression. Hence, we used 4 different orthogonal inducible promoters to allow fine control of the 4 ED-enzymes, and tested a broad range of inducer concentrations to find a well-balanced expression profile. Our preliminary results indicate that we were able to enable fast growth of *E. coli* on glucose via the ED-pathway using specific combinations of inducers for the 4 tested enzymes. Being able to optimize the expression of and the flux through a synthetic pathway of interest would be beneficial for a wide range of applications in synthetic biology and biotechnology.

Dynamic Metabolic Remodeling in *Candida albicans*: Linking Transcriptomics to Pathway Fluxes

Shrutakirti Saha, *Julius-maximilians-universität Würzburg*
Poster, P1-34

To model the metabolic adaptation and gene regulation in *Candida albicans* (wild type and *sky2*Δ gene knock-out) under glucose, succinic acid and malic acid growth conditions, we created an integrative model of the Tricarboxylic acid (TCA) cycle, arginine biosynthesis and the glyoxylate shunt using the software YANAsquare. Our metabolic network consists of 23 enzymatic reactions and 37 metabolites, including the glyoxylate shunt with two reactions, one with ICL1 and the other with MLS1, and the arginine biosynthesis is considered as one reaction to see how it responds along with TCA cycle. 18 functional elementary modes were obtained, and the fluxes were found, revealing that *Candida albicans* bypasses the TCA cycle to glyoxylate shunt and arginine biosynthesis under malic acid growth conditions in *sky2*Δ. We used a flow optimization approach based on convergence to predict relative enzyme activity from transcriptome data. We found that *sky2* knockout strains exhibited a significant change in metabolic routing, with increased flow through the glyoxylate shunt, especially in the presence of malic and succinic acid. By avoiding the TCA cycle's decarboxylation stages, this rerouting most likely represents an adaptive approach to save carbon skeletons. Furthermore, the mutant strains showed a markedly elevated flux in the Arg_Biosyn reaction, a simplified depiction of the arginine biosynthesis route connecting 2-oxoglutarate to fumarate, underscoring its compensatory function in nitrogen metabolism under regulatory disruption. We performed a transcription factor interaction analysis to clarify the regulatory underpinnings of these metabolic changes, discovering important regulators including MCM1, CPH1, and MIG1. A coordinated transcriptional regulation of metabolic flexibility was suggested by the substrate-specific expression patterns of these transcription factors and their substantial correlation with flux variations in their downstream targets. For example, in *sky2*Δ strains exposed to succinic acid and malic acid, increased expression of ICL1 and MLS1 correlated with decreased MIG1 repression and increased MTLA1 expression. As a crucial link between Sky2- and JEN2-associated modules, CEF1 may be able to integrate metabolic and regulatory responses, as it was upregulated in both malic acid and succinic acid growth conditions, suggesting a way for transcriptomic-metabolic adaptations. The phylogenetic analysis of the interactors and the transcription factors revealed that the genes were also evolutionarily conserved in other *Candida*, *Aspergillus*, and *Lichtheimia* species, supporting the robustness of the regulatory-metabolic structure. Together, our findings reveal a multi-layered adaptive strategy in C. albicans that integrates transcriptional control with metabolic network plasticity. This systems-level insight provides a valuable framework for understanding fungal survival in nutrient-variable environments and may identify novel antifungal targets.

Epigenetic control of metabolic identity across cell types

Thomas Sauter, *University of Luxembourg*
Oral

Constraint-based network modelling is a powerful tool for analysing cellular metabolism at genomic scale. Here, we conducted an integrative analysis of metabolic networks reconstructed from RNA-seq data with paired epigenomic data from the EpiATLAS resource of the International Human Epigenome Consortium (IHEC). Applying a state-of-the-art contextualisation algorithm, we reconstructed metabolic networks across 1,555 samples corresponding to 58 tissues and cell types. Analysis of these networks revealed the distribution of metabolic functionalities across human cell types and provides a compendium of human metabolic activity. This integrative approach allowed us to define, across tissues and cell types, i) reactions that fulfil the basic metabolic processes (core metabolism), and ii) cell type-specific functions (unique metabolism), that shape the metabolic identity of a cell or a tissue. Integration with EpiATLAS-derived cell type-specific gene-level chromatin states and enhancer-gene interactions identified enhancers, transcription factors, and key nodes controlling core and unique metabolism. Transport and first reactions of pathways were enriched for high expression, active chromatin state, and Polycomb-mediated repression in cell types where pathways are inactive, suggesting that key nodes are targets of repression. This integrative analysis forms the basis for identifying regulation points that control metabolic identity in human cells.

OptFed: Advancing Bioprocess Control with Data-Driven Optimization

Guido Schlögel, *University of Vienna*
Oral and poster, P1-35

Constraint-based models have become indispensable tools in systems biology and metabolic engineering, enabling large-scale analysis of metabolic networks and guiding strain and process design through approaches like flux balance analysis (FBA). Their ability to leverage stoichiometric information without requiring detailed kinetic parameters makes them especially powerful for exploring cellular capabilities under steady-state conditions. However, applying these models to dynamic bioprocesses, such as fed-batch cultivations, can be challenging, as real-world systems often involve transient behaviors, regulatory effects, and nutrient shifts that extend beyond steady-state assumptions. Notably, production and biomass growth are often uncoupled in flux space, as in recombinant protein production, where product synthesis competes with cellular protein synthesis for shared precursors and energy. To address these limitations, we developed OptFed, a model-based optimization framework that builds upon the structure and insights of metabolic networks, while extending them through kinetic (ODE-based) modeling. This hybrid approach enables a more comprehensive and dynamic representation of cellular behavior under fed-batch conditions, where nutrient availability, growth, and productivity evolve over time. OptFed also adapts commonly used heuristic models (e.g., Monod kinetics) to better describe real-world process behavior. OptFed integrates:- Metabolic networks to identify theoretical optima for growth and production,- Mass balances to construct ODE models, using yields derived from the metabolic model,- Heuristic models to describe substrate utilization, growth, production, and maintenance requirements,- Bayesian state estimation to incorporate off-line and on-line measurements, improving estimates of growth and exchange rates,- Nonlinear optimization to fit models to experimental data,- Cross-validation and statistical testing to reduce model complexity and prevent overfitting,- Optimal control theory to identify optimal process trajectories without relying on predefined feeding strategies. We applied OptFed to a fed-batch process producing recombinant protein L. Compared to traditional optimization based on Response Surface Methodology (RSM), OptFed achieved a 19% yield improvement using the same factorial DoE dataset. To enhance real-time adaptability, we integrated Bayesian state estimation techniques, enabling dynamic model refinement and control adaptation in response to biological variability, an inherent feature of bioprocesses. In a case study under phosphate limitation, we used OptFed to predict the optimal process endpoint by incorporating off-gas data into the model. Our results highlight how constraint-based and kinetic modeling can complement one another to address the complexity of real-world bioprocesses. By embedding metabolic network insights within a dynamic, data-driven optimization framework, OptFed provides a powerful tool for advancing robust and efficient biotechnological production.

Comparative Metabolic Pathway Analysis in CHO Cells Using Genome-Scale Models Under Varying Temperature Conditions

Veronika Schäpertöns, *University of Salzburg*
Poster, P2-36

Chinese Hamster Ovary (CHO) cells are a cornerstone of therapeutic protein production, yet their metabolic behaviour remains complex and context dependent. Genome-scale metabolic models (GEMs) offer a valuable framework to simulate and interpret this complexity, especially when combined with time-resolved experimental data. In this study, we perform a comparative analysis of two CHO GEMs to evaluate their performance for predicting biomass formation and monoclonal antibody (mAb) production across dynamic culture conditions. Our work focuses on NISTCHO, a publicly available cell line developed by the National Institute of Standards and Technology (NIST). This cell line expresses cNISTmAb, a model monoclonal antibody used in biomanufacturing research as a reference for development. We investigated two temperature conditions—constant ($37\,^{\circ}\mathrm{C}$) and mild hypothermia ($32\,^{\circ}\mathrm{C}$)—which are known to influence CHO cell productivity and metabolic efficiency. For each condition, experimental data were collected in between feedings across five temporal windows, spanning both the exponential and the production phase. These data include measurements of extracellular exchange rates, biomass growth, and cNISTmAb production. The data were incorporated into both models using constraint-based modelling to generate flux distributions (fluxomes) that are condition- and time-specific. By comparing the predicted metabolic fluxes, biomass formation rates, and cNISTmAb production levels across models and con-

ditions, we were able to identify both consistent trends and model-dependent discrepancies. Notably, both models predicted the general decline in biomass-associated fluxes under hypothermic conditions, but they diverged in the prediction of certain amino acid utilization pathways and antibody secretion-associated fluxes. These differences point to underlying variations in model assumptions, such as biomass composition, network structure, and reaction reversibility constraints. At $32\,^{\circ}\mathrm{C}$, the fluxomic analysis revealed temperature-dependent rerouting of carbon and energy metabolism, with a shift toward reduced central carbon fluxes and altered nucleotide biosynthesis. This corresponds with known physiological responses such as extended cell longevity and modified protein glycosylation patterns in mild hypothermia. Additionally, the use of time-windowed data allowed us to capture dynamic shifts in metabolism, such as transitions from growth-dominated to production-dominated phases. This comparative modelling approach emphasizes the value of using multiple models to bracket predictions and explore uncertainty in constraint-based simulations. It also highlights the need for careful curation and context-specific parameterization of GEMs for CHO cells. The results from this study can inform model refinement, guide targeted metabolic engineering strategies and improve process development for biopharmaceutical production.

From dysregulation to compensation, a modelling approach to energy metabolism in neuromuscular diseases.

Camille Siharath, *Université Claude Bernard Lyon 1*
Oral and poster, P2-37

Energy metabolism, both systemic and muscular, is a complex process and plays a crucial role in the proper functioning of the neuromuscular system, as it ensures the renewal of the energy required for muscle contraction and the maintenance of cellular activity. Neuromuscular diseases are a group of heterogeneous disorders affecting one of the components of the motor unit, i.e. motor neurons located in the spinal cord, its extension (axon) running in the peripheral nerve and all the muscle fibers it innervates, resulting in impaired muscle function, affecting the metabolism of muscle cell responsible of contractions, atrophy and possibly death. However, by impairing neuromuscular function, this group of pathologies is systematically characterised by primary or secondary metabolic defects, which contribute to disease progression and deterioration in patients' quality of life. Depending on the pathology, but also on inter-individual specificities, metabolic alterations change and remain poorly understood and poorly known, which limits the development of therapeutic approaches. It therefore seems essential to better understand and anticipate the adaptations and compensations of muscle energy metabolism in these diseases, in order to guide preclinical research and develop new, individualised therapeutic approaches. Nevertheless, when the effects on the energy metabolism are not well understood, traditional experimental approaches might be difficult to consider because metabolism constitutes a vast network of metabolites interconnected by a large number of cross-linked enzymatic activities that might need to be tracked. Thus, this necessitates the utilisation of metabolic modelling to focus the study on specific pathways, anticipate and facilitate the understanding of the changes it can undergo. Various approaches to modeling energy metabolism co-exist, including constraint-based approaches, which can be used on models such as the MitoCore model, which can be run in Flux Balance Analysis (FBA). This method can be used to determine which pathways optimise an objective function, such as biomass or, in the case of this study, ATP production. Therefore, this research aims to implement a constraint-based computational model and to account for the alterations in energy metabolism induced by different pathological situations such as neuromuscular diseases and anticipate the development of therapeutic approaches, using calpainopathy as a case study. To achieve this, we first developed a healthy skeletal muscle cell model, ready to adapt to different energy demands, simulating different intensities of physical exercise, using well-known carbon source proportions. Indeed, depending on the intensity, duration, and type of exercise, both the amount and rate of energy consumption changes. As fatty acids, through the β-oxidation, represent the most efficient cellular energy source, their use is constrained by production rates. As a result, increased energy demand leads cells to resort to alternative sources, such as more powerful sources like glucose. Then to simulate the disease state and build a pathological model, experimental data, in the form of enzyme activities or differential gene expression, were then integrated to our healthy muscle cell model as boundary constraints. With the idea of studying the potential of physical exercise as a complementary approach to improve the clinical care of patients with this type of disease, previous research made the hypothesis that an exercise protocol, tailored to each patient's metabolic state, could lead to improvements in motor function and be considered as a clinical care strategy. So as maintaining physical activity could improve adaptation capacity and slow muscle weakness, our model suggests compensatory mechanisms through anaerobic glycolysis during moderate exercise, combined with additional inputs such as amino acids to meet higher energy demands. However, excessive glycolysis may indicate a need to enhance mitochondrial respiration, preventing excess lactate production, common in several diseases. Our disease-like model then suggests moderate exercise supplemented with increased carbohydrate source or transporter activity as potential therapeutic strategies.

Enhanced flux predictions in plants using machine-learning-derived Kcats

Edward Smith, *University of Oxford*
Poster, P2-38

Rational metabolic engineering requires knowledge of the underlying metabolic network and its limitations. Protein allocation constraints can limit the productivity of organisms under certain conditions. Here we integrate machine-learning-derived enzyme turnover numbers (Kcat) as constraints in stoichiometric models of *Arabidopsis thaliana*, *Camelina sativa*, and *Nicotiana tabacum* leaves. Model predictions were validated against 13C-metabolic flux analysis measurements of intracellular fluxes. Using *in silico*

metabolic control analysis, and quantitative proteomic data, we identified enzyme overexpression targets to enhance plant productivity. This pipeline is generalisable to other plant species or tissues, providing a systematic approach for identifying and prioritising metabolic engineering targets.

Investigating cold-induced metabolic reprogramming through machine learning-driven analysis of flux predictions from a time-resolved model of rice metabolism

Fatemeh Soltani, *University of Cologne*
Poster, P2-39

Rice (*Oryza sativa* L.), a staple food crop worldwide, faces significant productivity and geographical distribution constraints due to its sensitivity to cold stress. Thus, understanding cold-induced metabolic reprogramming as a pivotal component of rice's adaptive responses can provide valuable insights for breeders and farmers. Owing to their ability to capture metabolic interactions and temporal dynamics of metabolism, constraint-based models are valuable tools to study cold-induced metabolic reprogramming. In this study, we used the E-Flux algorithm to constrain a series of time-point-specific models which were then coupled to construct a time-resolved model representing dynamics of rice cold stress responses. Moreover, we used pareto frontier analysis to understand the trade-off between growth and cold-adaptive related metabolic processes. This led to a large number of flux data and prompted us to develop a machine learning framework to identify reactions and pathways with most significant changes during the acclimation process. Our model predicted a reduced growth rate when proline accumulation, as an osmoprotectant induced by cold stress, was prioritized. Comparing the flux solutions in two scenarios, highlighted that photosynthates diversion toward increased proline synthesis is the key driver of. Moreover, our results pointed to the importance of down-regulation in photosynthetic light reactions to adjust the imbalance between production and consumption of excitation energy. This imbalance results from cold-induced suppression of the Calvin cycle, which consumes the ATP and NADPH produced by the light reactions. This study provides a network-level overview of rice metabolic reprogramming in response to cold stress, identifying key pathways involved in cold-adaptive responses and can guide efforts aimed at enhancing cold tolerance in rice.

Integrative Multi-Omics Metabolic Modeling Reveals Mechanisms of Drug-Induced Liver Toxicity

Zita Soons, *University Hospital RWTH Aachen*
Oral

Introduction
Adverse drug events remain a significant challenge in both drug development and clinical practice. Conventional pre-clinical assays often rely on static, non-physiological *in vitro* exposure conditions that poorly replicate dynamic in vivo drug profiles. This disconnect hampers accurate prediction of human toxicity. To address this, we employed primary human liver spheroids exposed to physiologically relevant drug concentration-time profiles derived from physiologically based pharmacokinetic simulations, enabling the study of hepatotoxicity under clinically meaningful conditions [1].
Methods
We investigated ten well-characterized hepatotoxic compounds using exposure regimens that mimic repeated administration at therapeutic levels as well as toxic levels equivalent to IC20 values after 14 days of incubation. Multi-omics analyses were conducted over a 14-day period, including time-resolved transcriptomics (RNA-seq), proteomics (mass spectrometry), targeted amino acid quantification, and assessments of spheroid viability and morphology. Transcriptomic and proteomics data for different drug doses and exposure times have been made publicly available [2]. To interpret the molecular changes induced by drug exposure, we reconstructed context-specific genome-scale metabolic models based on the consensus Human1 model [3] using the iMAT algorithm [4], enhanced with probabilistic flux analysis [5]. These models integrated transcriptomic, proteomic, and metabolomic data to characterize cellular state transitions under both therapeutic and toxic exposures.
Results
Transcriptomic and proteomic results demonstrate that the cumulative exposure over time—rather than the nominal therapeutic or toxic dose—was the primary driver of molecular responses. This was particularly evident in the timing of apoptotic signaling, which varied across drugs but correlated with integrated exposure rather than absolute dose. Functional enrichment analyses highlighted consistent alterations in central carbon and nitrogen metabolism. Measurements of metabolite usage showed that toxic exposure led to increased catabolism of amino acids. Metabolic modeling further revealed drug-specific disruptions, including altered bile acid and steroid metabolism, and significant shifts in histidine turnover. In our presentation, a detailed case study of isoniazid will be presented to illustrate the integration process and biological insights derived from multi-omics constraints. We also evaluated the impact of incorporating individual (transcriptomics, proteomics, and metabolomics) versus combined omics datasets into metabolic flux analysis. Notably, inclusion of quantitative metabolite exchange rates significantly refined the model solution space and improved the resolution of metabolic pathway alterations. In summary, this study illustrates a generic framework, which bridges pharmacokinetics and metabolic flux analysis, for further investigations to elucidate the impact of drug concentrations-time profiles on organ- specific cellular biochemistry and mechanisms of drug-induced liver toxicity.
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Integrating a reactive species reactions module with a plant metabolic model to study (a)biotic stress responses

Subasree Sridhar, *University of Cologne*
Poster, P2-40

Plant genome-scale metabolic models are useful for studying plant metabolic systems and their interactions with the biotic and abiotic environment. Reactive Species (RS) are generated via a range of metabolic pathways of which photosynthesis is the major contributor. They are an important part of the plant's immune system in response to abiotic stress conditions and pathogen attacks. However, a quantitative understanding of the effect of RS production on the activity of metabolic reactions is missing. Here, we have reconstructed a RS module for plants which is composed of 303 reactions and 517 metabolites across nine cellular organelles. The module is divided into the following subsystems: reactions of different categories of RS reactions such as reactive oxygen species, reactive nitrogen species, reactive sulphur species, ascorbate and glutathione based antioxidant reactions and other enzymatic reactions involved in RS metabolism, reactions of DNA damage, protein oxidation/nitration etc. This RS model can be integrated with any plant metabolic model. As a case study we have integrated the RS module with a well-curated model of plants' primary metabolism. We used this model to perform a Pareto analysis of the tradeoff between biomass growth and the production of different reactive species like hydrogen peroxide, nitric oxide, superoxide, etc in the RS integrated-plant metabolic model under autotrophic condition and find pathways of primary metabolism to be affected by this shift in cellular objective. We expect our reactive species integrated model to advance our understanding of metabolic flux modes in non-optimal conditions and thus to be useful in identifying metabolic targets for tolerance against biotic and abiotic stresses.

Transcriptomics-based metabolism analysis of macrophages and foam cells mitochondria affected by atherosclerosis

Egils Stalidzans, *Riga Stradins University*
Poster, P2-41

Atherosclerotic cardiovascular disease, including coronary heart disease and stroke, represents 85% of all deaths from cardiovascular disease (CVD), and is the leading cause of death and a major contributor to disability worldwide (World Heart Report 2023). In particular, Central and Eastern Europe, including the Baltic countries, is the region with one of the highest CVD burdens in the world (Bugiardini, 2023; Cenko et al., 2023). In the early stages of CVD, key events occur including the inflammatory activation of both circulating immune cells and resident arterial wall cells, endothelial dysfunction, changes in the blood lipid profile, and oxidative stress. Mitochondria are highly specialized organelles in eukaryotic cells, which are important centres of energy metabolism and signal transduction. In addition to energy production, mitochondria are involved in a number of vital cellular processes: calcium homeostasis, signaling, which is partially performed through generation of reactive oxygen species (ROS), and initiation of apoptosis. Imbalances in mitochondria homeostasis, including fission, fusion, oxidative stress, calcium overload, mitophagy, and biogenesis, may trigger to further cellular dysfunction, uncontrolled ROS production followed by damage of cells and their surroundings, and initiation of the inflammatory response (Morató & Sandi, 2020; Zeng et al., 2022). Two types of macrophages have been identified: pro-inflammatory M1 and anti-inflammatory M2 (De Gaetano, et al., 2016). Their interaction influences the dynamics of the atherosclerotic plaque. In our study we concentrate on the role of mitochondria in the inflammation process analysing transcriptomics data of macrophages and foam cells (Hagg et al., 2008). The MitoCore model (Smith et al., 2017) has been adapted for the needs of CVD-related modeling of mitochondrial metabolism. From the original MitoCore model (445 metabolites, 563 reactions and 378 genes), four genome-scale metabolic models have been created using the Fastcore algorithm (Vlasis et al., 2014): 1) macrophage without atherosclerosis (341 metabolites, 366 reactions and 321 genes), 2) macrophage with atherosclerosis (341 metabolites, 365 reactions and 318 genes), 3) foam cells without atherosclerosis (339 metabolites, 363 reactions and 312 genes) and 4) foam cells with atherosclerosis (340 metabolites, 359 reactions and 316 genes). The models for macrophages with atherosclerosis and Foam cells without atherosclerosis had the highest and lowest number of core reactions, respectively. Models have been analysed for differences in their performance with different objective functions (ATP, H2O2, NO, putrescine, lactate production and others). From the simulation results authors have tried to identify M1 and M2 macrophage features in the analysed samples. The simulation results show that the difference in the expression level of genes in the oxidative phosphorylation pathway plays a key role in the different phenotypes of the four cells. It also indicates that atherosclerosis results in the reduction of the capacity of macrophages for pro-inflammatory responses (M1 state), while atherosclerosis improves the anti-inflammatory capacity (M2 state) of both cell types. This seems logical, considering atherosclerosis is a disease that causes inflammation and more inflammation is not needed for this disease that causes inflammation. RSU internal and RSU with LSPA external consolidation No. 5.2.1.1.i.0/2/24/I/CFLA/005 is financed by the investment of the European Union's Recovery and Resilience Mechanism and the state budget.

Modeling the Effect of Cell Membrane on the Protein Allocation Model

Titania Insani Sugiarto, *RWTH Aachen*
Oral and poster, P1-42

In recent decades, computational approaches have become fundamental tools in biological research, enabling the development of genome-scale models (GEMs) that mimic cellular behavior. However, traditional GEMs often lack key layers of biological complexity, limiting their ability to predict phenomena such as overflow metabolism (e.g., the Crabtree effect in yeast or the Warburg effect in cancer cells) and associated byproduct secretion. To address this, a well established Protein Allocation Models (PAMs), implemented in Python as PAModelpy, introduced a constraint on total cellular protein concentration, dividing proteins into metabolically active, translational, and housekeeping sectors. While PAMs successfully captured overflow metabolism, limitations remained, particularly in predicting maximum growth rates and byproduct secretion, such as acetate excretion. In this study, we introduce a membrane constraint into PAM, resulting in the membrane-constrained Protein Allocation Model (mcPAM). This added constraint explicitly accounts for membrane protein allocation and available membrane space. mcPAM improved predictions of maximum growth rate and acetate secretion in Escherichia coli (*E. coli*) compared to PAM, aligning more closely with experimental phenotypes. Furthermore, a systematic framework was applied to identify the most sensitive enzyme concentrations (sEnz) in PAM, revealing that the membrane constraint emerges as the limiting factor precisely at the onset of growth rate plateaus. Additionally, during protein overproduction scenarios, mcPAM provided more accurate predictions for mutant strains than PAM. Overall, mcPAM enhances the predictive power of protein allocation models and offers a promising framework for studying membrane-limited phenotypes across different organisms with minimal adaptations

Modular Cell Design with Resource Allocation for Scalable Biomanufacturing: A Multi-Objective Optimization Framework

Cong Trinh, *University of Tennessee Knoxville*
Oral

Diversity in cellular metabolism offers vast potential for producing various chemicals. However, conventional metabolic engineering relies on multiple design-build-test-learn (DBTL) cycles, making it costly and labor-intensive to develop optimal strains capable of exploring broad chemical spaces while achieving high product titers, rates, and yields. To address this challenge, the modular cell design (ModCell) concept enables systematic strain engineering with fewer DBTL cycles, using a multi-objective optimization framework based on the mass balance principle, Pareto front optimization theory, and omics data. This approach leverages the inherent modularity of biological systems by organizing metabolic pathways into tractable, interchangeable modules that interface with a modular chassis cell for efficient biosynthesis of a broad library of endogenous and heterologous products. To implement this strategy, we developed ModCell 3.0, a software package driven by evolutionary algorithms and multi-objective optimization. The integration of omics data in ModCell 3.0 enables the construction and analysis of enzyme-constrained genome-scale models that enhance predictive accuracy by incorporating resource allocation (e.g., proteome usage) and metabolic cost (e.g., ATP demand) across different production phenotypes. Unlike traditional single-product strain designs, ModCell 3.0 systematically identifies genetic modifications to design modular cells capable of interfacing with diverse production modules. These designs balance modularity, performance, and robustness for efficient production of target chemicals by systematically analyzing resource reallocation, metabolic burden, and intrinsic metabolic interfaces between modular cells and associated production modules. We envision that ModCell 3.0 will advance the fundamental study of modularity, robustness, and performance in natural and synthetic biological systems, and drive scalable, efficient biomanufacturing.

Putting metabolic networks into context - Understanding leaf functioning through computational modelling

Nadine Töpfer, *University of Cologne*
Oral

Plants use light energy from the sun and CO_2 to build sugars in the process of photosynthesis. Leaves are the main site of photosynthesis in plants. Beyond C3 photosynthesis, which is the most abundant type, different adaptations to hot and dry climates have evolved which rely on temporal or spatial separation of biochemical processes, known as CAM and C4 photosynthesis. This indicates that photosynthetic efficiency is determined not only by plant biochemistry but also constrained by environmental, physical, and anatomical factors. A quantitative understanding of how these factors affect leaf productivity is crucial for ensuring food security in current and future climates. While the interrelationship between form and function in biological systems is well established, the mutual influence between leaf metabolism and anatomy remains underexplored, representing a significant gap in our understanding of plant productivity. We address the question how different types of photosynthesis are enabled or constrained by leaf anatomy and environment. To this end, we use large-scale metabolic models to represent different cell types and tissues in the leaf context, informed by anatomical and physical constraints. To study the effect of temperature and relative humidity on plant metabolism with respect to water conservation, we coupled a time-resolved diel model of leaf metabolism to an environment-dependent gas-exchange model. This combined approach allowed us to study the emergence of CAM as a trade-off between leaf productivity and water saving. We demonstrate that vacuolar storage capacity in the leaf is a major determinant of the extent of CAM. Furthermore, we explore metabolic flux modes and cellular energetics across C3 photosynthesis, intermediate types, and C4 photosynthesis by examining the effects of mesophyll and bundle sheath tissue volumes, bundle sheath cell wall suberization, varying light intensities, and photorespiratory conditions on leaf productivity. Our results not only corroborate our understanding of C3 and C4 leaf characteristics but also provide mechanistic insights and quantitative measures of leaf optimality. Our findings about the interplay between leaf metabolism, anatomy, and environment suggest engineering targets for improved plant productivity,

heat tolerance, and drought resistance.

Validation of genome-scale metabolic network models with proteomics data

Steffen Waldherr, *University of Vienna*
Oral

Genome-scale metabolic networks (GSMNs) are being used widely as a computational model for cellular metabolism in a wide range of organisms from all domains of life. While automated reconstruction pipelines generate model drafts directly from a genome sequence, there are often network gaps or mismatched functional annotations which require a careful manual curation of the model, relying on diverse sources of empirical knowledge and heuristics. Therefore, network reconstruction needs to be done in an iterative cycle between validation of model predictions against experimental data and refinement of the reconstructed network. Classically, GSMNs are validated against experimental data of two types: growth or metabolic activity on different substrates, and lethality of individual gene knockouts. For the substrate-based validation, for example 96-well plates with a range of substrate conditions can be used, mostly different carbon substrates, but also different nitrogen, phosphorus, and sulphur compounds. Metabolic activity (respiration) is determined by a redox dye. With the model, the predicted metabolic activity can be obtained for each substrate by varying the model constraints accordingly, and the results can be matched against the experimental results. Gene knockouts can be tested by creating a knockout mutant library, and comparing model predictions with each gene removed to the phenotype of the corresponding mutant. However, both validation methods require that the considered organism can be cultured in the lab. The construction of a knockout library also requires genetic tools being available for the organism. On the other hand, a validation with different substrates is mostly relevant for heterotrophic organisms, but will be less meaningful for autotrophic organisms which anyway operate on a narrow set of substrates. While the rapid advance and cost reduction of genome sequencing has led to an exponential increase in the number of whole-genome sequences over the past decade, opening a potential for more wide-spread metabolic network reconstructions, the available validation methods become limiting for a large group of newly sequenced organisms. We will need more diverse approaches for experimental validation that also apply to non-model organisms, to organisms that are found in the environment instead of cultured for biotechnical processes, and to organisms operating in narrow ecological niches. A possible additional method of validating GSMN model predictions that has not been used much until now is with data from a proteomics analysis. Proteomics in that context has the advantage that it does not need genetic tools for the considered organism, that it can be done with environmental samples, and that it resolves to the individual reaction level instead of a mere input-output perspective. On the other hand, proteomics is limited by frequently not obtaining complete coverage of an organism's proteome, and by challenges in identifying and distinguishing enzymes with a similar amino acid sequence. In this contribution, I report experiences and conclusions from validating two GSMN reconstructions against proteomics data: one for an ammonium oxidizing archaeon, and another one from a bacterium associated to plant leaves. We found that generally proteomics is a promising way to validate metabolic network models, but needs to take misidentification issues into account. Also, expression of enzymes that is suboptimal in terms of standard flux balance analysis may amplify the number of false negatives in the predicted active reactions. In any case, consideration of proteomics data during the network reconstruction is also a good avenue towards the construction of more elaborate model types that explicitly include enzyme levels, for example resource allocation models, especially if an absolute quantification of the proteome is available.

DBgDel: Database-Enhanced Gene Deletion Framework for Growth-Coupled Production in Genome-Scale Metabolic Models

Ziwei Yang, *Kyoto University*
Poster, P2-43

When simulating metabolite productions with genome-scale constraint-based metabolic models, gene deletion strategies are necessary to achieve growth-coupled production, which means cell growth and target metabolite production occur simultaneously. Since obtaining gene deletion strategies for large genome-scale models suffers from significant computational time, it is necessary to develop methods to mitigate this computational burden. In this study, we introduce a novel framework for computing gene deletion strategies. The proposed framework first mines related databases to extract prior information about gene deletions for growth-coupled production. It then integrates the extracted information with downstream algorithms to narrow down the algorithmic search space, resulting in highly efficient calculations on genome-scale models. Computational experiment results demonstrated that our framework can compute stoichiometrically feasible gene deletion strategies for numerous target metabolites, showcasing a noteworthy improvement in computational efficiency. Specifically, our framework achieves an average 6.1-fold acceleration in computational speed compared to existing methods while maintaining a respectable success rate.

Engineering Cyanobacteria-Rich Microbiomes: Metabolic Interactions Driving Targeted PHB Accumulation in a Multi-Species System

Arianna Zini, *University of Tübingen*
Poster, P2-44

Cyanobacteria represent a powerful platform for sustainable bioproduction, combining photosynthetic efficiency with metabolic

plasticity. In this work, we explore the frontiers of microbial consortia design by integrating metabolic pathway engineering with community-level analysis. Our model system is based on *Synechocystis* sp. PCC 6803 PPT1 (PHB Producer Tübingen 1), a cyanobacterium previously optimized to produce polyhydroxybutyrate (PHB) up to 60% of its cell dry weight under complete photoautotrophic conditions (1). While such high PHB titers are promising, robustness and system stability remain bottlenecks for large-scale application. To address challenges in robustness and scalability, we moved beyond monoculture approaches and developed a synthetic microbiome enriched from a natural microbial community selected for its native ability to produce PHB (2). In the novel microbiome, PPT1 acts as a metabolically dominant “leading species” orchestrating community dynamics. In this work, we observe that the metabolic output of the engineered cyanobacterium PPT1 - particularly the secretion of overflow metabolites like acetate - modulates the recruitment and behaviour of accompanying heterotrophic species, including purple bacteria. Remarkably, these supporting community members initiate PHB biosynthesis only in response to the specific metabolic cues emitted by the engineered cyanobacterium, revealing a tightly coupled interspecies metabolic flow. Through metabolite profiling, we identified acetate and pyruvate as key carbon exchange molecules that direct the structure and function of the synthetic microbiome. Our data demonstrate that different cyanobacterial strains exert species-specific control over microbiome composition and function through unique metabolite fingerprints. This not only influences community composition but also the functional output - particularly PHB accumulation - in the other members of the microbial consortium. Using metabolic analysis combined with community profiling and experimental validation, we present a framework for predicting and directing carbon fluxes across species boundaries. Our approach highlights a new level of metabolic pathway analysis that extends beyond single-species optimization, incorporating interspecies metabolite exchange and division of labor as strategic tools for bioproduction. This work proposes a paradigm shift in metabolic engineering - from single-species optimization to multi-species design - offering new perspectives for future biomanufacturing, where robustness, scalability, and sustainability are achieved through community-level interactions.

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One-carbon Metabolism

Triacylglycerols accumulation in *Rhodococcus opacus* DSM 43205 from CO_2 and hydrogen and C1-C5 soluble derivatives

Blanca Araya, *Universidad de los Andes*
Oral and poster, P2-45

Triacylglycerols (TAGs), three C10-C20+ fatty acids esterified with a glycerol molecule, found use in the chemical and biofuels industries. The transition to renewable energy sources has spurred interest in microbial platforms for sustainable production of TAGs, versatile molecules used in biofuels, food additives, and industrial applications. Bacteria from the genus *Rhodococcus* have been previously identified as oleaginous bacteria, accumulating up to 70% of their cellular dry weight as triacylglycerols, with *R. opacus* PD630 being the most studied organism (Holder et al. 2011). More than 50 years ago, the capacity of an *R. opacus* strain (now DSM 43205) of growing in knallgas (a mixture of H_2, CO_2 and O_2) was described. However, it is unclear whether TAGs are accumulated from this mixture. Previous works from our group have found that this strain grows on methanol, acetic, butyric and valeric acids and propanol. All these substrates can be obtained from H_2 and CO_2 using (bio)chemical processes. Therefore, the metabolic capabilities of *R. opacus* DSM 43205 appear uniquely poised to test, in a single organism, the production of triglycerides directly from knallgas as well as from small molecules derived from this gas mixture. In this contribution, we examine the production of TAGs in *Rhodococcus opacus* DSM 43205 through a quantitative lens, focusing on the interplay between the organism's metabolic network and the chemical pathways required to produce various substrates from knallgas. This study provides the first comprehensive characterization of *R. opacus* DSM 43205 TAGs metabolism, quantifying yields, specific productivity, intracellular TAG content, and fatty acid composition when utilizing formic acid, methanol, acetic acid, propanol, butyric acid, and valeric acid as substrates. As a basis for future process engineering studies and biotechnological applications, we estimate the total H_2 consumption and carbon conservation efficiency of TAGs production via direct gas fermentation compared to two-stage processes involving these small molecules. Glucose was included as a control to benchmark performance. METHODSCultures were carried out in mineral medium under nitrogen (ammonium chloride) limitation to promote TAGs accumulation, using glucose, methanol, acetic acid, 1-propanol, butyric acid, valeric acid (all at an initial titer of $5\,\mathrm{g\,L^{-1}}$) and knallgas as the sole carbon and energy sources in batch cultures. TAGs were determined as methyl esters of their corresponding fatty acids using GC-FID and compared to analytical standards. Dissolved substrates titers were determined using an HPLC-UV-Vis/RID equipment coupled with a Rezex ROA-Organic acid (L22 packing, 8% cross-linked hydrogen column). CO_2, H_2 and O_2 gas molar concentrations were measured in a gas chromatograph coupled with a thermal conductivity detector (GC-TCD, Shimadzu 2014, Japan). Permanent gases were separated in a Carboxen-1000 column (4.6 m x 3.17 mm x 300 µm, Agilent). A reduced central carbon metabolism model for TAG accumulation in *R. opacus* was built based on its annotated genome (RefSeq assembly GCF_001646735). The results obtained from the FBA were utilized to calculate the theoretical mass yield ($\mathrm{g\,g^{-1}}$) of TAGs in the different substrates. Furthermore, the detected products (TAGs and exopolysaccharides (EPS)) were used to construct yield spaces by maximizing the triglyceride synthesis reactions (one specific reaction for each substrate to account for TAGs composition) while varying the lower bound of EPS synthesis (modeled as glycogen synthesis for the sake of simplicity), maintaining the substrate uptake flux at the experimentally determined values and the non-growth associated maintenance energy lower and upper bounds at the previously specified values.
RESULTS AND DISCUSSION
TAGs were accumulated using each substrate, except for formic acid. The highest TAG content of $0.580(95)\,\mathrm{g\,gCDW^{-1}}$ was achieved using acetic acid, with 95.7% of the substrate consumed after 43.4 hours. This TAG content was significantly different ($p = 0.05$) from the values obtained for butyric, methanol and knallgas as substrates, but not from those obtained with glucose, valeric acid or 1-propanol. Glucose, the most rapidly consumed carbon source, reached a TAG accumulation of $0.503(5)\,\mathrm{g\,gCDW^{-1}}$ within 21.8 hours, which was significantly different from the values obtained for methanol and knallgas ($p = 0.05$). In contrast, methanol required the longest cultivation time, achieving complete consumption after 67 hours and a TAG accumulation of $0.305(14)\,\mathrm{g\,gCDW^{-1}}$, significantly different from all other substrates except knallgas. For 1-propanol, butyric acid, and valeric acid, TAG accumulation levels were similar, with no statistically significant differences, with substrate consumption exceeding 90% after approximately 45 hours. The initial biomass for the knallgas culture was deliberately reduced to mitigate the impact of low H_2 and O_2 mass transfer rates on the TAG accumulation rate. Finally, the TAG accumulation on knallgas was found to be $0.325(47)\,\mathrm{g\,gCDW^{-1}}$, comparable to the accumulation on methanol (with no statistically significant difference compared to methanol and butyric acid). Interestingly, not only was the total intracellular TAG content substrate-dependent, but also the distribution of the accumulated fatty acids. Substrates with odd carbons, such as 1-propanol or valeric acid, produced a reduced total TAG content compared to cultures with glucose or acetic acid, but with a higher odd-fatty acid content, especially C15 and C17. A similar shift in the odd fatty acids content was previously reported for *R. opacus* PD630 grown in 1-propanol (Zhang et al., 2019). Moreover, as the chain length of the odd fatty acid substrate increased, the length of the most represented odd fatty acid also increased (from C15 with propionic acid to C17 with valeric acid). These results can be interpreted using the biochemical insights gained from the annotated genome. Results indicate that medium chain fatty acids are produced using FASI, and further elongation and modification to long chain branched lipids occurs via FASII. If the initiation step of FASI can accept not only acetyl-CoA and malonyl-CoA as initiators, but also propanoyl-CoA, butyryl-CoA and valeryl-CoA, this could partly explain the distribution odd and even fatty acids. Considering the hydrogen requirements to produce each substrate used in the fermentation, the highest hydrogen demand was estimated for methanol-derived TAG production, requiring 2.74 kg of H_2 per kg of TAG, along with 18.61 kg of CO_2. Although not significantly different, the second highest hydrogen demand was calculated for direct TAG production from knallgas, with 2.56 kg of H_2. Conversely, the use of knallgas required the least amount of carbon dioxide with 5.95 kg CO_2 per kg TAG. Among organic acids, TAGs production required 5.62 kg of acetic per kg of TAG, translating to 0.95 kg of H_2 and 8.64 kg of CO_2 per kg TAG. Butyric acid (1.85 kg H_2, 11.38 kg CO_2) and va-

leric acid-derived TAG production (1.20 kg H_2, 7.37 kg CO_2) followed similar trends, with valeric acid showing a slightly lower CO_2 requirement compared to acetic acid but higher hydrogen needs. 1-Propanol exhibited a relatively low H_2 requirement (1.33 kg per kg TAG) and a moderate CO_2 demand (7.78 kg per kg TAG). If the theoretical yields for TAGs production, based on the metabolic model, are used instead of the experimental ones, production from acetic acid remains as the chemical synthesis pathway with the lowest H_2 requirement (0.88 kg), closely followed by knallgas with 0.95 kg. On the other hand, methanol (1.13 kg, assimilated using the CBB cycle) and butyric acid (1.21 kg) showed the highest H_2 requirements per kg of TAGs. Notably, if methanol assimilation via the ribulose monophosphate (RuMP) pathway were implemented achieving its theoretical yield shown, the calculated H_2 requirement drops to 0.58 kg, the lowest amongst all the chemical and biochemical pathways explored.
CONCLUSIONS
This study provides the first comprehensive assessment of TAGs production in *Rhodococcus opacus* DSM 43205 using knallgas (CO_2, H_2 and O_2) and organic molecules that can be chemically produced from this gas mixture. TAGs accumulation was demonstrated for knallgas, methanol, acetic, butyric and valeric acid, as well as 1-propanol, but not for formic acid. Odd-carbon substrates (1-propanol and valeric acid, but not methanol or CO_2) increase odd-chain fatty acids to over 80% by propionyl-CoA incorporation. A metabolic model of TAG accumulation allowed the calculation of maximum theoretical TAG yields for each substrate, showing that while the experimental TAG yield on acetic acid reached 96% of its maximum theoretical value, knallgas achieved only 54.3% of the theoretical yield. Process-level analysis, including substrate synthesis requirements, showed that producing 1 kg of TAG from acetic acid required 0.95 kg H_2 and 8.64 kg CO_2, compared to 2.56 kg H_2 and 5.95 kg CO_2 for direct hydrogen fermentation. Metabolic modeling further predicted that implementing the *Bifidobacterium* shunt to bypass pyruvate decarboxylation could increase TAG yield from knallgas to $0.936\,\mathrm{kg\,kg^{-1}}$. These findings position *R. opacus* DSM 43205 as a promising microbial chassis for sustainable TAG production and highlight key metabolic targets for pathway optimization and industrial implementation.
ACKNOWLEDGMENTS
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Conversion of CO_2 to organic acids in engineered *Komagataella phaffii*: Integrating synthetic biology and isotopically nonstationary metabolic flux analysis

Özge Ata, *BOKU University*
Oral

One of the biggest challenges humankind is facing these days is the climate crisis. One clear cause is the increasing atmospheric CO_2 level due to societal activities. Anthropogenic CO_2 emissions exceed the capturing capacity of plants and microorganisms, leading to an imbalance in the global carbon cycle. To move toward a more sustainable future, this balance must be restored. To address this problem, we aim to enable microbial assimilation of CO_2 as a carbon sink by converting it into value-added, bio-based polymers. We have used a synthetic autotrophic yeast, *Komagataella phaffii* (*Pichia pastoris*) which can grow solely on CO_2 for biomass formation while using methanol to harvest energy. We further engineered this strain to use as a platform to produce value-added organic molecules. By balancing the synthetic itaconic acid pathway with the Calvin–Benson–Bassham (CBB) cycle and process design, we achieved a final titer of approximately $12\,\mathrm{g\,L^{-1}}$ itaconic acid in lab-scale bioreactors. To confirm the incorporation of the captured CO_2 into itaconic acid, we performed isotopic non-stationary ^{13}C-MFA (INST-MFA) which allowed us to predict dynamic labeling patterns and quantify the labeling degree of the products. To demonstrate broader applicability, we applied the same approach to the lactic acid production. Our results demonstrate that synthetic autotrophic *K. phaffii* can serve as a robust platform for the sustainable microbial production of value-added chemicals from CO_2 and show that INST-MFA is a powerful tool for analyzing C1-based metabolic pathways.

Using metabolic modeling to analyze thermodynamic constraints of the acetogen *Acetobacterium woodii*

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Oral

Acetogenic bacteria are able to convert CO_2 and H_2 to acetate (and other products) in a process called acetogenesis which is coupled to the net formation of ATP. This conversion is facilitated by the Wood-Ljungdahl pathway (WLP). To enable the operation and thermodynamic feasibility of the WLP, the energy and redox metabolism of acetogens exhibits several special features, for example, the use of ferredoxin as third central redox cofactor in addition to NADH and NADPH. The metabolic capability of acetogens to convert CO_2 and other C1 sources to organic compounds has recently gained much interest as a basis for sustainable biotechnological production processes using substrates that are not in competition with the food and feed industry. Metabolic modeling will be a key tool to gain a better understanding of the complex metabolism of acetogens and to identify key targets for their rational metabolic engineering. One important representative and model organism of acetogens is *Acetobacterium woodii*. A simple model of the central metabolism of *A. woodii* has previously been presented and used [1]. However, this model was not fully charge- and mass-balanced and anabolic routes were included only in a very condensed manner. In this work, we present a larger metabolic model of

A. woodii (520 reactions, 490 metabolites) that is charge- and mass-balanced and integrates anabolic pathways as well as a biomass synthesis reaction that better reflects the biomass composition of *A. woodii*. Since our focus was on thermodynamic analyses, we used the eQuilibrator API [2] to augment the model with standard Gibbs free energy changes. Using available experimental data, we validated the model with respect to stoichiometric and thermodynamic feasibility of observed phenotypes. After performing those tests, we analyzed it with the TCOSA framework [3], a recently introduced approach to study the effect of (hypothetical) redox cofactor swaps on biomass and ATP yields and on the maximally achievable thermodynamic driving force in the network (measured as max-min driving force (MDF) [4]). As one major result, we found that suitable cofactor swaps could, in principle, increase the maximal ATP and biomass yield. Furthermore, our analysis revealed why *A. woodii* requires ferredoxin, in addition to NADH and NADPH, as a third major redox cofactor pool and that the use of NADH and NADPH alone would result in thermodynamic infeasibility or at least very low driving forces for ATP synthesis and growth. In particular, we found that the carbon monoxide dehydrogenase reaction would become the major thermodynamic bottleneck in the absence of ferredoxin as additional low potential redox partner. Finally, we also analyzed beneficial co-factor switches in the central metabolism of *A. woodii* that would either increase the yield and/or the thermodynamic driving force of metabolic conversions of C1-substrates to economically relevant metabolic products such as organic acids, alcohols, diols, etc. These results can serve as a guide for rational metabolic engineering to create bespoke acetogenic bioproduction hosts.

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Using synthetic and systems biology to ramp up synthetic C1-metabolism: towards higher yields and faster growth

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Oral

Microbial bioproduction is a sustainable alternative to traditional manufacturing but often depends on sugar-based feedstocks, linking it to agriculture and limiting its efficiency. Reduced C1-molecules like methanol and formate, which can be produced from CO_2 and renewable electricity, offer a promising alternative. Yet, most organisms cannot use these efficiently. One of the most promising synthetic pathways, the reductive glycine pathway, has been implemented in various hosts — but growth rates and yields remain suboptimal. In our recent work, we used genome integration, lab evolution, and rational engineering to improve formate-based growth and yield in *Cupriavidus necator* and *E. coli*. Quantitative proteomics provides insights revealed how proteome allocation may influence the strains and together with the modelling of fluxes can guide further improvements. Notably, we exceeded the best natural formate yields reported for the Calvin Cycle in *C. necator*. These advances bring us closer to using synthetic C1-metabolism for efficient, scalable bioproduction.

Closing the doors of the peroxisome: towards synthetic methylotrophy

Xavier Farge, *BOKU*
Poster, P1-46

Methanol emerges as a cost-effective and sustainable substrate for microbial bioproduction. In methylotrophic yeasts, methanol metabolism is localized within the peroxisome, a spatial arrangement suspected to confer functional advantages. Understanding this organization is key for advancing synthetic microbial methylotrophy. We aim to relocalize the entire methanol utilization pathway in the cytosol of *Komagataella phaffii*, thereby bypassing the natively optimal peroxisomal conditions. Our initial strategy involves removing peroxisomal targeting sequences from relevant enzymes. Recognizing that this may potentially affect enzymatic activities, we are also testing other strategies, such as completely blocking peroxisomal protein translocation. To enhance this cytosolic methanol metabolism and mitigate oxidative stress, we are replacing the native alcohol oxidases with alcohol dehydrogenases. Engineered strains will be mainly characterized via a multi-omics approach to evaluate metabolic flux, pathway integrity, and biomass yield. This work contributes to the development of next-generation microbial platforms for C1 compound assimilation, supporting sustainable routes to bioproduction.

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Systematic characterization of inborn errors of cobalamin metabolism and their effect on metabolism through genome-scale modeling

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Oral and poster, P1-47

Inborn errors of metabolism were traditionally viewed through the one gene-one disease paradigm and patients were thought to show little inter-individual variation. In recent years, it has become recognized that like in common diseases, patients with rare in-

herited diseases can vary in presented phenotypes and are influenced by environmental factors including diet and the microbiome. The contextualization of multi-omics data (e.g., transcriptomics) through personalized constraint-based modeling could improve our understanding of the altered metabolic phenotypes in patients with rare inherited diseases. Cobalamin (vitamin B_{12}) is the cofactor for two human enzymes, methionine synthase and methylmalonyl-CoA mutase. More than 20 proteins are involved in the absorption, transport, conversion, and delivery of vitamin B_{12}. Due to its direct connection to the folate and methionine cycles, the vitamin B_{12} pathway plays a key role in one-carbon metabolism. Inborn errors of cobalamin metabolism (IECM) are a group of rare inherited diseases affecting the absorption, transport, conversion, or utilization of cobalamin. Patients present with a wide spectrum of metabolic, neurological, hematological, and ophthalmological symptoms. Available treatments include cobalamin injections, dietary restrictions, and antibiotics but are challenging to manage. Here, we used constraint-based modeling to systematically elucidate the altered metabolic pathways in IECM patients. First, we expanded the human genome-scale reconstruction Recon3D with a complete vitamin B_{12} pathway. Using flux variability analysis, we then predicted the outcomes of 11 known IECMs by simulating a complete deletion of the corresponding gene. Next, we leveraged RNA sequencing data from fibroblasts from a cohort of 143 patients with methylmalonic aciduria (MMA) due to a complete or partial defect in the methylmalonyl-CoA mutase (MMUT) gene, 59 patients with other types of MMA, and 19 healthy controls. Personalized models were generated by integrating the RNA sequencing data into a tissue-specific genome-scale reconstruction of the human fibroblast. Metabolic fluxes were predicted across all pathways for each sample through flux variability analysis. Statistical analyses were performed between patients and controls, and between patients presenting with symptoms and those unaffected. Compared with the wild type, 81 reactions were reduced in flux by at least 5% in at least one of the 11 simulated IECMs. Metabolic fluxes in vitamin B_{12} metabolism, methionine and cysteine metabolism, and glycine and serine metabolism were impaired, demonstrating a wide impact on one-carbon metabolism. Unexpectedly, heme biosynthesis was also reduced in flux in the majority of IECMs. Similarities between flux profiles in individual IECMs also partially reflected a recent meta-analysis where IECM patients had been classified based on symptom reports. In personalized simulations, 53 and 397 reaction fluxes differed significantly between patients with complete and partial MMUT deficiency and controls, respectively. Between patients with other types of MMA and healthy controls, 90 reaction fluxes were significantly different. Besides impaired flux in vitamin B_{12} metabolism, MMUT patients showed reduced succinate production, which decreased fluxes into the citric acid cycle and heme biosynthesis pathway. Both MMUT and other MMA patients had reduced flux through enzymes in folate metabolism, suggesting potentially impaired DNA methylation. A variety of reaction fluxes were also altered in patients presenting with symptoms compared with unaffected patients. For instance, 1,277 reaction fluxes across a wide variety of pathways were reduced in flux specifically in MMUT patients with failure to thrive. Overall, our predictions show that a variety of pathways are affected by disturbances in one-carbon metabolism as caused by inherited defects of the pathway. Taken together, we have systematically characterized the effects of inherited gene defects in vitamin B_{12} metabolism on the one-carbon metabolism and beyond. In future efforts, a patient's nutritional data (e.g., vitamin status), transcriptome, proteome, metabolome, gut microbiome, and/or physiological parameters could be simultaneously integrated into genome-scale reconstructions to further define the individual variety in IECMs and other diseases affecting the one-carbon metabolism. Further applications could include the prediction of therapeutic interventions or diagnostic biomarkers for these difficult to treat diseases.

Placental proteomics and early metabolic programming: A constraint-based multi-omics perspective on pediatric obesity

Soumen Konar, *Faculty of Science, Technology and Medicine, University of Luxembourg*
Oral and poster, P1-48

The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that environmental exposures and nutritional conditions during critical periods of early development — particularly *in utero* — can have lasting effects on an individual's risk for chronic diseases later in life. Central to this framework is the role of the placenta as a key interface mediating maternal-fetal exchanges and potentially encoding long-term metabolic programming. Pediatric obesity has become a major global health challenge, affecting both developed and developing countries. In developed nations, the prevalence of childhood obesity is driven by sedentary lifestyles, high-calorie diets, and socio-economic factors, resulting in long-term health consequences such as type 2 diabetes and cardiovascular diseases. In developing countries, this issue is exacerbated by rapid urbanization, shifting dietary patterns, and the coexistence of undernutrition and obesity, often referred to as the "double burden" of malnutrition. The EDEN cohort is a prospective mother-child cohort consisting of deep phenotyping and socioeconomic data as well as epigenomic, metabolomic, and proteomic data from placenta, cord blood, and from children at follow-up time points. In a previous study, we already identified multiple important loci associated with metabolic syndrome outcomes in children at five years of age in an epigenome-wide association study. In this study, we performed proteomic and metabolomic analyses in placenta from 40 participants in the EDEN cohort. Children were stratified into high and low quartiles of body fat percentage (%FM) at age five, disaggregated by sex. Distinct clustering patterns emerged between high and low %FM groups, as well as between males and females, underscoring the presence of sex-specific molecular signatures. To explore the metabolic underpinnings, we employed constraint-based metabolic modeling using the rFASTCORMICS pipeline, a member of the FASTCORE algorithm family. The resulting placental metabolic models revealed key regulatory pathways potentially shaped by intrauterine epigenetic modifications. To our knowledge, this study represents one of the first applications of constraint-based metabolic modeling integrating multi-omic placental data within the DOHaD framework. Our findings underscore the value of placental proteomic profiling in identifying early-life biomarkers of pediatric obesity and provide novel mechanistic insights into fetal metabolic programming and future disease susceptibility.

Reinventing the CO_2 fixation cycle

Elad Noor, *Weizmann Institute Of Science*
Oral

Aside from the usual challenges of metabolic engineering, carbon fixation cycles are notorious for being autocatalytic, sensitive to oxygen, and requiring high CO_2 levels. This is especially the case for the Calvin cycle, which is nevertheless the most common gateway through which CO_2 enters the biosphere. We use a diverse set of computational and experimental tools to design, implement, and analyze the transition of a heterotrophic *E. coli* to a fully autotrophic growth mode by expressing the missing Calvin cycle enzymes and identifying genomic mutations that facilitate the transition. By studying the synthetic autotrophic *E. coli*, we aim to identify and solve the important bottlenecks that limit its growth, and offer it as a flexible and well-understood platform for sustainable bioproduction directly from CO_2.

Metabolic rewiring of *Cupriavidus necator* and *Azohydromonas lata* for autotrophic and heterotrophic production of (R)-3-hydroxybutyrate and pyruvate

Felipe Scott, *Universidad De Los Andes*
Poster, P2-49

Over the long term, biomanufacturing processes can be envisioned as manufacturing of bulk and fine chemicals directly from CO_2, with electrons obtained from renewable energy temporarily stored in the form of H_2, formate and methanol, as opposed to using sucrose or glucose, which offers lower, but often non-negative, CO_2 emissions compared to production from fossil resources[1]. To achieve this long-term goal, microbial strains that can tap on these abundant substrates while producing useful molecules are still to be obtained. In this regard, although impressive, the production of biomolecules from hydrogen, CO_2 and O_2 (knallgas) using chemolithoautotrophs has been hindered by low productivity and titers, especially when these molecules are more reduced than polyhydroxybutyrate (PHB), e.g., alcohols. Two microorganisms stand out as chassis to produce such compounds. *Ralstonia eutropha* (*Cupriavidus necator*) is a natural polyhydroxybutyrate (PHB) accumulating bacteria capable of autotrophic growth in knallgas and heterotrophic growth on sugars and acids. The second microorganism, *Azohydromonas lata* DSM 1123, also accumulates PHB using knallgas, sugars (sucrose, glucose and fructose) and several organic acids. *A. lata* shows the highest volumetric productivity of PHB accumulation up-to-date. Although PHB is an intracellular product, its precursor (R)-3-hydroxybutyryl-CoA can be transformed into (R)-3-hydroxybutyrate (R3HBA), a chiral molecule with several uses in the chemical and pharmaceutical industries. In this work we report on a push and pull engineering strategy to produce extracellular metabolites derived from the key intermediate acetyl-CoA: R3HBA and pyruvate.

Methods

C. necator H16 (DSM428) was used as the parent strain for production in mineral media[2] and *E. coli* DH5α for plasmid replication. Autotrophic cultures were carried out in medium 81 (DSMZ) without nitrogen source and with $300\,\mathrm{mg\,mL^{-1}}$ of kanamycin, under a sterile atmosphere of 10% (v/v) O_2, 10% CO_2 and 80% H_2. Flasks were incubated at $30\,^{\circ}\mathrm{C}$ with shaking at 200 rpm during 96 h. The headspace atmosphere of the flasks was replaced daily, and the gases composition was measured before and after replacement. DNA manipulation techniques, including amplification and restriction cloning, were carried out using standard methods. Gene deletion was done using a homologous recombination-based method with the pT18mobSacB suicide plasmid. Thioesterase overexpression was constructed by inserting the *yciA* gene previously amplified from *C. necator* H16 or *E. coli* DH5α through PCR and flanked with KpnI-HindIII. Then, the gene was digested and ligated into pBBR1MCS-2 as a backbone plasmid previously digested. Fructose, pyruvate, R3HBA and crotonic acid were quantified via HPLC with a UV–Vis instrument (Prominence, Shimadzu, Japan)[3]. Gas composition was measured using a GC-TCD equipped with a Carboxen-1000 column. Batch and chemostat experiments were performed in a 1.5 L total volume, (operated at 1 L and 0.4 L of working volume, respectively. For chemostat experiments, CO_2 concentration in the off-gas was measured using a BlueVary instrument (BlueSens gas sensor GmbH). Maximum metabolic yields of R3HBA and pyruvate on fructose or knallgas were calculated using parsimonious flux balance analysis using the genome scale metabolic model of *C. necator* H16 iCN1361.

Results

Deletion of *phaC* alone or in combination with *hbd* (*C. necator* Δ2 strain) did not reduce the specific growth rate of the mutants relative to the wild type. In contrast, the triple knockout mutant *C. necator* H16 Δ3 (Δ*phaC*1, Δ*hbd*, Δ*yciACn*) exhibited a slight reduction in its specific growth rate from $0.204(11)$ to $0.179(3)\,\mathrm{h^{-1}}$. After 96 hours of growth, none of the mutant strains accumulated PHB, whereas the wild-type strain produced approximately 80% PHB. In *C. necator* H16 Δ*phaC*, R3HBA and pyruvate titers peak at 48 h of culture, gradually decreasing during the remaining 48 hours of cultivation, resulting in a final concentration of $0.054(12)\,\mathrm{g\,L^{-1}}$ and $0.263(59)\,\mathrm{g\,L^{-1}}$, respectively. In strain *C. necator* Δ2, pyruvate titers also peak at 48 h, but in contrast to the *phaC* negative mutant, R3HBA titers continue increasing during the 96 hours of culture, reaching $1.204(135)\,\mathrm{g\,L^{-1}}$. The molar carbon yield of R3HBA on fructose (Y_(3HB/F)^C) for *C. necator* H16 Δ*phaC* was $0.003(2)\,\%$, whereas in *C. necator* Δ2 increases to $8.06(34)\,\%$. Finally, in strain *C. necator* Δ3, the maximum R3HBA titer reduced by two folds compared to strain *C. necator* Δ2, obtaining $0.653(77)\,\mathrm{g\,L^{-1}}$ (Y_(3HB/F)$^C = 4.028(573)\,\%$). Interestingly, the presence or absence of the *yciA* thioesterase gene influenced the consumption of the excreted pyruvate. As expected, no significant production of R3HBA or pyruvate was obtained for *C. necator* H16 culture. Deletion of the (R)-3-hydroxybutyrate dehydrogenase in *C. necator* Δ2 increased the concentration of R3HBA in the broth by reducing its conversion rate towards acetoacetate. Further deletion of the putative thioesterase YciACn (GenBank: CAJ91615.1, gene: h16_A0465), with 84% query cover and 47% identity with YciAEC, not only reduced R3HBA titers, but also increased pyruvate excretion and abolish its uptake from the fermentation broth. These results point to YciACn as a thioesterase

acting on (R)-3-hydroxybutyrate-CoA, whose hydrolytic action produces R3HBA. Since R3HBA is still produced in strain *C. necator* Δ2, other thioesterases may be present in *C. necator* H16 genome. The mutant strain *C. necator* Δ*phaC*-Δ*hbd*, CnΔ2, harboring a copy of *yciaEC* (from *E. coli*) in a pBBR1 plasmid was constructed (along single knockout and triple knockout mutants, complemented with *yciaEC* or *yciaCN*). In shake flasks, this strain achieved the highest R3HBA production of nearly $1.5\,\mathrm{g\,L^{-1}}$ and negligible amounts of pyruvate. Fermentation in batch bioreactor using this strain achieved a R3HBA titer of $2.6\,\mathrm{g\,L^{-1}}$ and negligible amounts of pyruvate. Chemostat cultures of CnΔ2 pLY24 at a dilution rate of $0.1\,\mathrm{h^{-1}}$ achieved $0.46\,\mathrm{g\,L^{-1}}$ of R3HBA, specific R3HBA productivity of 0.08 g R3HBA gcDW^{-1} h^{-1} and a fructose concentration in the outlet stream of $7.95\,\mathrm{g\,L^{-1}}$ (inlet concentration of $0.5\,\mathrm{g\,L^{-1}}$ ammonium sulphate and $10\,\mathrm{g\,L^{-1}}$ fructose). 86% of the carbon in fructose was accounted for, indicating that no other metabolites are produced in important quantities. With an identical inlet stream, but at a dilution rate of $0.015\,\mathrm{h^{-1}}$, the R3HBA titer was $1.26\,\mathrm{g\,L^{-1}}$ with a specific productivity of 0.03 g R3HBA $\mathrm{gCDW^{-1}\,h^{-1}}$. An assay was developed where the R3HBA containing broth is exchanged every 24 hours and replaced by fresh medium containing $10\,\mathrm{g\,L^{-1}}$ fructose and no ammonium sulphate. Under these conditions the titer of R3HBA reached $3.3\,\mathrm{g\,L^{-1}}$, the highest obtained so far, pointing to an inhibitory effect of R3HBA. The production of R3HBA from knallgas using *C. necator* Δ2 plY24 resulted in a titer of $0.661(109)\,\mathrm{g\,L^{-1}}$ after 96 and a carbon mole yield of CO_2 in R3HBA of 67 %. As for *A. lata*, the *phaC* knockout mutant (GenBank: AAC83658.1) was successfully obtained as evidenced by the complete abolishment of PHB production (down to $0.15(1)\,\%$ from 70-80% w/w of PHB in *A. lata* DSM 1123 wild-type). Despite this success, the strain accumulated only $0.28\,\mathrm{g\,L^{-1}}$ R3HBA from $20\,\mathrm{g\,L^{-1}}$ glucose, along with yet unidentified excretion products detected by HPLC (including pyruvate and extracellular (non-bounded to cell) exopolysaccharides Despite these challenges, PHB accumulated in *A. lata* wild type was depolymerized using the native PHB depolymerase at pH 4.0. From either glucose or knallgas, accumulated PHB was depolymerized at close to theoretical yields at low PHB concentrations ($10\,\mathrm{g\,L^{-1}}$), and with a 85% molar yield at high concentrations ($100\,\mathrm{g\,L^{-1}}$, after resuspending pelletized cells).
Conclusion and future work
Our results suggest that it is possible to rewire the metabolism of PHB accumulation to accumulate pyruvate or R3HBA by leveraging on the natural capacity of *A. lata* and *R. eutropha* to funnel CO_2 or glucose/fructose toward these compounds, opening the possibility of autotrophic accumulation of compounds that could serve as standalone products or as intermediates. Further work aims at the production of isopropanol and 1,3 butanediol as more reduced (compared to pyruvate or R3HBA) products using an approach combining division of labor between chemolithoautotrophic production of pyruvate or R3HBA production and final alcohols production. In this regard, a computational framework is being designed for the exploration, design, and optimization of pathway alternatives leading to the production of a given target compound (T) from a set of substrates (in this case, S={O_2,H_2,CO_2}) via an intermediate compound (I, R3BHA). The framework also incorporates pathways growth coupling (for enzyme/operon selection) and uncoupling for production in the bioconversion strain. The challenge lies in finding a minimal set of reactions, including a switch, allowing the coupling of the target compound to growth when the switch is activated, and its uncoupling – to increase product yield – when off.

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In vitro and *in vivo* characterization of a new-to-nature pathway for formaldehyde assimilation in methylotrophic yeast

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Oral

The concept of a methanol economy has gained considerable attention due to its potential as a sustainable alternative to fossil fuels, being (electro-)chemically derivable from CO_2. Formolase (FLS) is the first synthetic enzyme to catalyse the formose reaction, wherein formaldehyde is converted to dihydroxyacetone (DHA). It is thus uniquely suited for the construction of synthetic methanol assimilation cascades, proceeding via methanol oxidation to formaldehyde condensation to DHA, and finally ATP-dependent conversion to dihydroxyacetonephosphate (DHAP). Compared to the native xylulose monophosphate (XuMP) cycle of methylotrophic yeasts, this pathway produces DHAP in fewer catalytic steps, without the need for acceptor recycling and at the cost of less ATP. Here, we implement FLS-based formaldehyde assimilation in Komagataella phaffii, a methylotrophic yeast used on an industrial scale to produce bioproducts, particularly proteins. To this end, an optimized FLS gene with a peroxisomal targeting signal (PTS1) under the control of a methanol-inducible promoter was integrated into the genome of a XuMP-deficient *K. phaffii* strain. Transformants with high copy numbers of the FLS gene $(11(1))$ produced up to $53.65(215)\,\mu\mathrm{M/min}$ DHA in cell-free extract (CFE). In the fed-batch phase of cultivations on methanol feed, the FLS-producing strain showed a biomass yield on methanol of $0.27(9)\,\mathrm{g\,g^{-1}}$ and a biomass formation rate of $0.0113\,\mathrm{g\,h^{-1}}$. This work lays the foundation for the implementation of a more energy-efficient methanol assimilation pathway as the basis for sustainable bioproduction in yeasts.

Genome assembly and genome-scale metabolic model reconstruction of *Rhodococcus opacus* DSM 43205, an oleaginous and hydrogen-oxidizing bacterium.

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Oral and poster, P1-50

Triglycerides (TAGs) are industrially relevant molecules used in biofuels and other applications. They are mainly produced from animal and plant sources, contributing to environmental degradation. Alternatively, TAGs can be produced by microbial fermentation using sugars, although these also have environmental limitations. *Rhodococcus opacus* DSM 43205 is the only known oleaginous bacterium capable of fixing carbon dioxide via the Calvin-Benson-Bassham cycle and oxidising hydrogen using soluble hydrogenases. In addition, it can utilise alternative carbon sources such as methanol and short-chain carboxylic acids, including acetic acid. Given this bacterium's potential for producing TAGs from renewable carbon sources, it is important to understand its oleaginous metabolism and the metabolic functions associated with its versatility. This work presents the first genome assembly and a genome-scale metabolic model (GEM) of *Rhodococcus opacus* DSM 43205. The genome was sequenced using PacBio HiFi technology and assembled with HiFiASM and Flye. Annotation was performed using PGAP and RASTk pipelines, and the PGAP-annotated genome was analysed with Pathway Tools. The assembled genome comprises a multipartite structure with a total length of 9.11 Mbp, consisting of a single circular chromosome and multiple plasmids. The lengths of the main plasmids (pHG201, pHG202, and pHG203) showed high concordance with experimental values reported by Kalkus et al. (1990, doi:10.1099/00221287-136-6-1145), as well as with the length of the restriction fragments of the plasmid pHG201. On plasmid pHG201, the genes responsible for chemolithoautotrophy in hydrogen were identified, encoded by a hydrogenase operon *hoxFUYHWI*, and for carbon dioxide fixation through RuBisCO, encoded by the operon *cbbLSX*. These two operons are located next to each other and are separated by the regulatory gene *cbbR*. The genetic repertoire required for the utilisation of acetate and methanol as carbon sources was identified on the chromosome, along with the complete set of oleaginous-related genes. This set includes the gene involved in short- and medium-chain fatty acid synthesis, genes encoding thioesterases, acyl-CoA ligases, and the four key genes of the Kennedy pathway responsible for triacylglycerols (TAGs) assembly. The draft metabolic model was constructed using Pathway Tools and manually curated using MetaCyc, KEGG, and LipidMaps databases. The biomass equation was refined to sum one gram of biomass, and the growth-associated maintenance (GAM) energy requirement was estimated at 62.6 mmol ATP $\mathrm{gCDW^{-1}\ h^{-1}}$, similar to the values reported in high-quality models of *Escherichia coli* K-12 MG1655 (iML1515 and iAF1260) and *Saccharomyces cerevisiae* S288C (iND750). Additionally, the model was calibrated using experimental data from a simple chemostat with acetic acid as the carbon source under balanced growth conditions. From this experimental data, acetic acid uptake, carbon dioxide production, and TAGs accumulated in the cell were calculated. The experimental graph obtained from the different dilution rates and the specific acetic acid consumption allowed the non-growth-associated coefficient (NGAM) value to be estimated. Furthermore, this plot (experimental dilution rate vs specific uptake of acetic acid) showed a good correlation with the simulated points in the model. The model, hereafter iPT923, was validated using the MEMOTE tool and showed an 87% total score comparable to other reported high-quality models. Furthermore, the model was validated using the observed TAGs yields under heterotrophic conditions using acetic acid and autotrophic conditions with hydrogen reported by Araya et al. (2025, under review, doi:10.2139/ssrn.5181769). Under an uptake of $4.69\,\mathrm{mmol\,gCDW^{-1}\,h^{-1}}$ of acetic acid, the reported observed TAGs yield with acetic acid value was $0.191\,\mathrm{g\,g^{-1}}$, and the calculated value with the model was $0.193\,\mathrm{g\,g^{-1}}$. Complementarily, with an uptake of $2.91\,\mathrm{mmol\,gCDW^{-1}\,h^{-1}}$ of carbon dioxide and $21.89\,\mathrm{mmol\,gCDW^{-1}\,h^{-1}}$ of hydrogen, the observed TAGs yield with hydrogen was $0.54\,\mathrm{g\,g^{-1}}$. With the same carbon dioxide uptake, the modelled yield of TAGs with hydrogen value was calculated at $0.62\,\mathrm{g\,g^{-1}}$ employing an uptake of hydrogen of $21.49\,\mathrm{mmol\,gCDW^{-1}\,h^{-1}}$. Triacylglycerol (TAG) biosynthesis is a highly NADPH-demanding anabolic process, primarily due to the activity of the fatty acid synthesis pathway. For instance, during fatty acid elongation, 14 molecules of NADPH are required for palmitic acid synthesis (C16:0). Therefore, to increase the intracellular NADPH supply while preserving the carbon atoms from acetic acid for TAG biosynthesis, the iPT923 model was used to simulate the co-consumption of acetic acid and formic acid through the activity of a $NADP^+$-dependent formate dehydrogenase (FDH). A formic acid uptake rate of $21\,\mathrm{mmol\,gCDW^{-1}\,h^{-1}}$ was assumed, which is equivalent, in terms of electrons donated, to the hydrogen uptake rate reported in the experimental work of Araya et al., 2025. With the $NADP^+$-dependent formate dehydrogenase (FDH) expression (*in silico*) and the formate uptake rate mentioned above, the calculated TAG yield on acetate increased 2.8 times to $0.54\,\mathrm{g\,g^{-1}}$. Moreover, the carbon yield of TAGs, defined as carbon atoms in TAGs per carbon atom in acetic acid, increased from 0.37 to 1.0. While in the *in silico* wild-type strain, only 32.34% of the acetic acid was directed towards fatty acid synthesis, with the remaining fraction being oxidised through the tricarboxylic acid (TCA) cycle, the expression of FDH enabled the redirection of 95.59% of the acetic acid carbon flux towards fatty acid synthesis. Using the experimental CO_2 uptake rate, the simulation of autotrophic TAG accumulation resulted in a calculated carbon yield of 1.0 mol C in TAGs per mol C in CO_2 with a hydrogen uptake rate of $21.56\,\mathrm{mmol\,gCDW^{-1}\,h^{-1}}$ (98.5% of the experimental value). The CO_2 recycle ratio, defined as the flux through the RuBisCO reaction over the CO_2 uptake rate, resulted in 1.48, slightly higher than the 1.33 theoretical value calculated considering pyruvate decarboxylation. The difference can be explained considering decarboxylation reactions in central metabolic pathways, such as the TCA cycle or anaplerotic reactions operating in a glycolytic direction, and the ferredoxin requirements for TAGs synthesis. Due to this energy burden, a shift to acetate as a carbon source while maintaining hydrogen as an energy source was simulated. The resulting carbon yield reached 1.0 mol C/mol C acetate, and the mass yield was similar to the formate case study. This outcome is expected, as hydrogenase activity provides NADH, which can be converted to NADPH via transhydrogenases. This strategy offers the advantage of relying on the strain's native capabilities without genetic modification, avoiding carbon competition between biomass and product synthesis. Nevertheless, this approach requires experimental validation, as acetate could inhibit hydrogenase activity. In *Cupriavidus necator* H16, hydrogenase expression is known to be highly sensitive to the metabolic state. In diauxic cultures with fructose and glycerol, hydrogenase genes (e.g., *hoxF* and *hoxA*) were strongly upregulated upon the shift to glycerol after fructose depletion, a response linked to cellular energy limitation—suggesting that energy status plays a central role in hydrogenase regulation (Jugder et al., 2015, doi:10.1186/s12934-015-0226-4). This supports the hypothesis that a low-energy substrate such as acetate could be simultaneously used along with hydrogen. It was previously mentioned that *Rhodococcus opacus* DSM 43205 can assimilate methanol, as supported by genome annotation indicating the presence of a nicotinoprotein methanol dehydrogenase (EC 1.1.99.37). To verify the phenotypic expression of this metabolic capability, shake flask cultivations were performed using a mineral medium supplemented with different

concentrations of methanol and conducted in biological triplicate. The highest specific growth rate observed was $0.03\,\mathrm{h}^{-1}$ at 5 g/L methanol. Additionally, in the study conducted by Araya (2025), to which the present author contributed, the accumulation of triacylglycerols (TAGs) under methanol-based growth was demonstrated. However, the experimental yield was low, reaching only 0.087 g TAG per g methanol. Based on the annotated genes, methanol is likely dissimilated to formate and oxidised to CO_2, which is then fixed via the Calvin–Benson–Bassham (CBB) cycle. The central metabolic model constructed in that study predicted a theoretical mass yield of 0.34 g TAG per g methanol if the genes required for the ribulose monophosphate (RuMP) pathway were expressed. This suggests that, although methanol oxidation to CO_2 generates reducing power, it is dissipated into carbon fixation via the CBB cycle, competing with lipogenesis. In contrast, expression of the RuMP pathway would allow direct assimilation of methanol into glycolytic pathways, thereby improving carbon conservation and enhancing TAG yields.
CONCLUSIONS
This work presents the first genome assembly and preliminary genome-scale metabolic model of the chemolithoautotrophic and oleaginous bacterium *Rhodococcus opacus* DSM 43205. The assembled genome showed high agreement with previously reported experimental data. With a MEMOTE score of 87%, the metabolic model accurately predicted TAG yields and identified metabolic strategies—such as alternative assimilation routes and co-substrate use—to enhance reducing power and redirect carbon toward lipogenesis. These findings highlight the potential of *R. opacus* DSM 43205 as a robust microbial chassis for the sustainable production of TAGS from renewable substrates.

Methanol to isopropanol in *Methylorubrum extorquens* AM1

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Oral and poster, P2-51

Greenhouse gases (GHG) are increasing in the atmosphere. There is a growing need to reduce dependence on fossil resources and the chemical products derived from them. These constraints have been the driving force behind the search for sustainable alternatives for the production of substitutes from renewable carbon sources. Consequently, the development of bioproduction processes for producing compounds of industrial interest from renewable carbon sources has become important, including those based on renewable one-carbon (C1) feedstocks ultimately derived from electricity and CO_2, such as methanol, formic acid, CO, CH_4, and hydrogen and carbon dioxide [Park et al., (2024), doi:10.1021/acssynbio.4c00519]. Among these compounds is isopropanol (IPA), a versatile alcohol with various industrial applications, including its use as a solvent, disinfectant, chemical intermediate, and potential sustainable aviation fuel precursor. IPA is traditionally produced via petrochemical processes using propylene or acetone, pathways associated with high greenhouse gas emissions. Biotechnological alternatives for IPA production exist, but most rely on sugars, which have sustainability challenges due to their need for arable land, competition with food supplies and the increase of the concentration of CO_2 in the atmosphere. The bioproduction of IPA from renewable C1 compounds has been poorly explored, with the notable exception of knallgas (CO_2/H_2/O_2) to IPA [Garrigues et al., (2020), doi:10.1016/j.nbt.2019.11.005] using a recombinant *C. necator* strain. Among C1 compounds, methanol derived from CO_2, offers an appealing alternative. Methanol, being a liquid at room temperature, is safer to handle and easier to distribute than gaseous substrates like hydrogen, making it a practical feedstock for microbial fermentation. These factors contribute to the increased viability of methanol in a broad range of research and industrial contexts. The methylotrophic, gram-negative bacterium *Methylorubrum extorquens* AM1 (also known as *Methylobacterium extorquens* AM1) has attracted interest as a potential biotechnology production platform because it is capable of using single-carbon compounds, such as methanol, as the sole source of carbon and energy, and converting it into biofuels [Hu et al., (2014), doi:10.1186/s13068-014-0156-0], polyhydroxybutyrate (PHB), dicarboxylic acids, and alcohols [Ochsner et al., (2015), doi:10.1007/s00253-014-6240-3]. In this study, we report for the first time the production of IPA by *M. extorquens* AM1, highlighting its potential as a versatile chassis for bio-based solvent production. To achieve this objective, we constructed expression plasmids that carried heterologous *adh* and *adc* genes. Each of these genes was driven by a distinct promoter and a ribosome-binding site (RBS). Furthermore, we deleted the native *phaC* gene to prevent PHB accumulation, thus redirecting the carbon flux towards IPA. Initially, expression plasmids compatible with *C. necator* and *M. extorquens* were built and tested in *C. necator* Δ*phaC* and *C. necator* Δ*phaC* Δ*hbd* strains to test IPA production. To conduct the study of IPA production, the *adc** genes from *Clostridium acetobutylicum* (AE001438) and *adh** genes from *Clostridium beijerinckii* (AF157307.2) were codon optimized and ordered from GenScript (USA). Genes were received in a pUC57 plasmid (pUC57::*adc**-*adh**) and were transformed into chemically competent *E. coli* DH5α cells (New England Biolabs, USA) for plasmid replication. Four expression vectors were designed: pFS21, pFS22, pFS23 and pLY25 were constructed. The plasmids pFS21 and pFS22 were assembled using pBBR1MCS-2 as a backbone plasmid. The pFS21 (pBBR1 MCS-2::*adc**-*adh**) plasmid has a constitutive Lac promoter, a non-optimized synthetic ribosome-binding site (RBS) upstream of *adc**, a nucleotide linker (ACAACC) and a RBS (AAAGGAGG) adapted from Grousseau et al., [(2014), doi:10.1007/s00253-014-5591-0], between *adc** and *adh** genes. For pFS22 plasmid, the same genes were inserted but a stronger RBS sequence upstream to *adc** gene was included. pLY25 plasmid was constructed with the same design of pFS22 but using pAWP89 as backbone plasmid and with a Tac promoter. The pFS21 and pFS22 plasmids were assembled through the cut and paste method. The plasmid pFS23 was constructed using a plasmid backbone pTE102 that contains a constitutive mxaF promoter. For the assembly, a fragment of 1846 bp sector of pUC57::*adc**-*adh** that contains the RBS (AAAGGAGG) and the same nucleotide linker mentioned above was included. pLY25 and pFS23 plasmids were assembled by Gibson Cloning. As a first confirmation of IPA production, plasmids were tested in *R. eutropha* Δ*phaC* (a *R. eutropha* strain uncapable to accumulate PHB) and *R. eutropha* Δ*phaC* Δ*hbd* (*R. eutropha* strain uncapable of PHB accumulation and (R)-3-hydroxybutyrate consumption) through electroporation, to test IPA production in a previously untested production strain (*R. eutropha* Δ*phaC* Δ*hbd*) as the strain previously presented by Grousseau et al., [(2014), doi:10.1007/s00253-014-5591-0] carried a knockout (Δ*phaB*) that is lethal in *M. extorquens*. Cultures in shake flasks were carried out in mineral medium

RE1 [Grousseau et al., (2014), doi:10.1007/s00253-014-5591-0] with $20\,\mathrm{g\,L^{-1}}$ of fructose and $1\,\mathrm{g\,L^{-1}}$ of ammonium sulphate. The resulting strains *R. eutropha* Δ*phaC* pFS21, *R. eutropha* Δ*phaC* pFS22, *R. eutropha* Δ*phaC* Δ*hbd* pFS21, *R. eutropha* Δ*phaC* Δ*hbd* pFS22, *R. eutropha* Δ*phaC* pLY25 and *R. eutropha* Δ*phaC* Δ*hbd* pLY25. Results showed that the choice of promoter and RBS configurations significantly affected IPA production, *R. eutropha* Δ*phaC* pFS21, *R. eutropha* Δ*phaC* pFS22 and *R. eutropha* Δ*phaC* pLY25 produced 0.98, 1.64 and 1.83 IPA $\mathrm{g\,L^{-1}}$, whilst *R. eutropha* Δ*phaC* Δ*hbd* pFS21, *R. eutropha* Δ*phaC* Δ*hbd* pFS22 and *R. eutropha* Δ*phaC* Δ*hbd* pLY25 produced 0.85. 1.82 and $1.57\,\mathrm{g\,L^{-1}}$ during 120 h of culture. (R)-3-HBA was detected only in *R. eutropha* Δ*phaC* pFS21 and *R. eutropha* Δ*phaC* Δ*hbd* with $31\,\mathrm{mg\,L^{-1}}$ and $20\,\mathrm{mg\,L^{-1}}$. IPA titers are reported as IPAc, due a corrected concentration considering the evaporation rate of IPA in the culture. Having demonstrated that the plasmids are functional in *R. eutropha* Δ*phaC*, and that the sole deletion of *phaC* is sufficient to funnel acetyl-CoA towards IPA, the plasmids were tested in *M. extorquens* cultured in shake flasks. For this purpose, $0.5\,\mathrm{g\,L^{-1}}$ ammonium sulfate with 0.5% v/v methanol (with methanol refeeding) as a carbon and energy source. *M. extorquens* pFS21 did not produce IPA during the culture, but *M. extorquens* pFS22 and pFS23 were able to produce IPA. *M. extorquens* pFS22 produced IPAc with a titer of $191.5\,\mathrm{mg\,L^{-1}}$ at 240 h of culture and $84.4\,\mathrm{mg\,L^{-1}}$ at 215 h in flask experiments (two biological replicates), with a total methanol consumption of $10.9\,\mathrm{g\,L^{-1}}$ and $9.1\,\mathrm{g\,L^{-1}}$. No acetone production was detected. *M. extorquens* pFS23 exhibited a different trend compared to strain pFS22. IPA was first detected after 24 hours of cultivation and was completely consumed. The depletion of IPA coincided with the exhaustion of methanol in the medium and the presence of the first acetone peak, which reached a concentration of $18.5(25)\,\mathrm{mg\,L^{-1}}$ at 48 hours. Following methanol refeeding at hour 54, IPA production resumed, achieving a maximum IPA concentration of $110.6(25)\,\mathrm{mg\,L^{-1}}$ at 71 h. The strains accumulated XX% of PHB during IPA production. For redirecting the metabolic pathway to IPA instead of PHB, the *phaC* gene (GenBank ACS41044.1) was deleted in *M. extorquens* AM1. Deletion was carried out using a homologous recombination-based method. The suicide plasmid was inserted by electroporation in *M. extorquens* AM1. Then, *M. extorquens* Δ*phaC* was transformed with the plasmids pFS22, pFS23 and pLY25 through electroporation. The cultures were conducted in the same conditions mentioned above and the results showed that over the culture, the IPAc titers and the carbon yields of IPA (carbon mmol per carbon mole of methanol consumed) reached $99.5(5)\,\mathrm{mg\,L^{-1}}$ in M. extorquens Δ*phaC* pFS22 and 36.184 Cmmol $\mathrm{Cmol^{-1}}$; *M. extorquens* Δ*phaC* pFS23 attained $271.2(149)\,\mathrm{mg\,L^{-1}}$ and 100.0 ± 11.2 Cmmol $\mathrm{Cmol^{-1}}$; *M. extorquens* Δ*phaC* pLY25 titer was $341.22(2000)\,\mathrm{mg\,L^{-1}}$ and a carbon yield of 243.5 ± 11.2 Cmmol $\mathrm{Cmol^{-1}}$. Additionally, pyruvate was tested as a carbon source in *M. extorquens* Δ*phaC* pFS23 and produced a maximum concentration of IPAc at 168 h of culture of $304.63(200)\,\mathrm{mg\,L^{-1}}$ with a yield of 97.65 ± 0.64 Cmmol $\mathrm{Cmol^{-1}}$. A fed-batch process with *M. extorquens* Δ*phaC* pFS23 was implemented to potentially improve the production by applying methanol feeding and controlled cultivation conditions, specifically for pH -that decreased due to (R)-3-HBA production and ammonia consumption- and dissolved oxygen. The bioreactor was adapted with 2 diaphragm pumps, a TGS 822 methanol sensor, a DHT22 sensor and a peristaltic pump for methanol dosage. The final IPAc titer, after 90 h of culture, was $420\,\mathrm{mg\,L^{-1}}$ with a total methanol consumption of $10.65\,\mathrm{g\,L^{-1}}$. The highest IPA carbon yield on carbon methanol was attained between 42 and 66 hours, reaching 339.4 Cmmol/mol with a specific IPA productivity of $3.33\,\mathrm{mg\,gCDW^{-1}\,h^{-1}}$ and total biomass of $1.48\,\mathrm{g\,L^{-1}}$. This study is the first to demonstrate IPA production using *M. extorquens* AM1 with methanol as a carbon source. It establishes *M. extorquens* as a viable chassis for renewable IPA synthesis. The promoter and RBS strength critically influenced metabolic flux and product titers, being the best IPA producer strains was *M. extorquens* Δ*phaC* pLY25. Future research directions include addressing IPA toxicity and optimization of the bioreactor configuration for reducing IPA evaporation.

Cellular Economics

Spilling the Secrets: Exploring Overflow Metabolism in *Synechocystis*

Janette Alford, *University of Tübingen/IMIT*
Poster, P1-52

Cyanobacteria like *Synechocystis* sp. PCC 6803 are promising platforms for sustainable bioproduction, using light and CO_2 to synthesize valuable compounds like the plastic alternative PHB. However, improving production efficiency and reducing costs requires bioengineering [1]. A deeper understanding of cyanobacterial carbon flux is crucial to optimizing these efforts. In *Synechocystis*, the PirC-regulated PGAM reaction is a central regulatory node in carbon partitioning toward lower glycolysis. Deletion of *pirC* increases flux toward lower glycolysis and elevates intracellular pyruvate and α-ketoglutarate during nitrogen starvation [2]. Under nitrogen limitation, glycogen is a key carbon sink and energy reserve. Deleting *glgC*, which encodes the first step in glycogen biosynthesis, disrupts this sink and induces overflow metabolism, resulting in pyruvate and α-ketoglutarate excretion [3]. Here, we analyze carbon flux in various *Synechocystis* mutants under vegetative and nitrogen-limiting conditions by comparing intra- and extracellular metabolite levels. In doing so, we aim to explore their carbon flow and determine additional deletions that trigger metabolite imbalance, resulting in overflow metabolism. Surprisingly, *pirC* deletion alone did not result in overflow metabolism, despite previous findings of elevated intracellular pyruvate and α-ketoglutarate levels. For a *pirC glgC* double-mutant, overflow metabolism could solely be attributed to the deletion of glgC and the resulting inability to generate glycogen. Similarly, *pgm* deletion, which also impairs glycogen synthesis, produced results akin to the *glgC* knockout. Interestingly, extracellular accumulation patterns did not directly reflect intracellular metabolite accumulation, suggesting selective excretion mechanisms. Our findings suggest that glycogen synthesis buffers excess carbon flow, and its disruption forces rerouting through lower glycolysis, which leads to overproduction and excretion of TCA cycle intermediates. This highlights how metabolic sinks shape carbon allocation and excretion dynamics in cyanobacterial metabolism and offers new insights for engineering cyanobacteria to enhance bioproduction.

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Cell Geometry and Membrane Protein Crowding Constrain Growth Rate, Overflow Metabolism, Respiration, and Maintenance Energy

Ross Carlson, *Montana State University*
Oral and poster, P2-11

A metabolic theory is presented for predicting maximum growth rate, overflow metabolism, respiration efficiency, and maintenance energy flux based on the intersection of cell geometry, membrane protein crowding, and metabolism. The importance of cytosolic macromolecular crowding on phenotype has been established in the literature but the importance of surface area has been largely overlooked due to incomplete knowledge of membrane properties. We demonstrate that the capacity of the membrane to host proteins increases with growth rate, offsetting decreases in surface area-to-volume ratios (SA:V). This increase in membrane protein is hypothesized to be essential to competitive *Escherichia coli* phenotypes. The presented membrane-centric theory uses biophysical properties and metabolic systems analysis to successfully predict the phenotypes of *E. coli* K-12 strains, MG1655 and NCM3722, which are genetically similar but have SA:V ratios that differ up to 30%, maximum growth rates on glucose media that differ by 40%, and overflow phenotypes that start at growth rates that differ by 80%. These analyses did not consider cytosolic macromolecular crowding, highlighting the distinct properties of the presented theory. Cell geometry and membrane protein crowding are significant biophysical constraints on phenotype and provide a theoretical framework for improved understanding and control of cell biology.

Principles governing shifts in metabolic strategies

Maarten Droste, *VU Amsterdam*
Oral

Shifts in metabolic strategies occur throughout the microbial realm. These shifts are typically studied by integrating experiments with genome-scale metabolic models. However, a general understanding of the fundamental principles underlying metabolic shifts is still missing. This has led to recent debates on the cause of overflow metabolism in yeasts and the significance of protein efficiency in this context. In this talk, we provide an extension and generalization of an existing core model for microbial metabolism that displays a metabolic shift as function of the growth rate. The model elucidates two key conditions necessary and sufficient for the emergence of the shift, which can be characterized completely in terms of the growth rate of the organism. To illustrate this, we apply the core model to the yeast *Saccharomyces cerevisiae* and successfully reproduce chemostat data during overflow metabolism. This demonstrates how large genome-scale metabolic models can be reduced to small phenomenological models. Furthermore, the model allows for the exploration of metabolic shifts under different growth conditions and properties of microorganisms.

Global Enzyme Cost Minimization: A resource allocation framework that integrates kinetic and thermodynamic constraints for genome-scale metabolic networks

Constance Le Gac, *ETH Zurich*
Oral and poster, P1-54

Experimental observations show that bacteria may secrete biochemical compounds that they could have metabolized further. This phenomenon is known as metabolic overflow. Metabolic overflows are frequent in certain environments, such as in the human gut, and may involve costly compounds such as amino acids. Because such overflows are not consistent with a maximum yield objective for the bacterium, their biological rationale remains unclear. However, trade-offs in cellular economics can explain metabolic overflows. These trade-offs can be modeled using resource allocation frameworks and have been shown to explain aerobic secretion of acetate by *Escherichia coli* grown in a chemostat. Resource allocation frameworks exist at a variety of scales, from coarse-grained descriptions of enzymatic sectors to enzyme cost minimization models which integrate detailed kinetic and thermodynamic constraints. Nonetheless, the granularity of a given framework usually limits the size of the network it can be applied to. Elad Noor and colleagues developed Enzyme Cost Minimization (ECM) [Noor et al, PLOS Computational Biology, 12, 11 (2016)], which predicts enzyme and metabolite concentrations by minimizing the enzyme cost for a given fixed flux distribution. Predicting an enzyme-cost optimal flux distribution requires enumerating all elementary flux modes (EFMs) and computing the ECM solution for each EFM as in Enzyme-Flux Cost Minimization (EFCM) [Wortel et al, PLOS Computational Biology, 14, 2 (2018)]. As EFM enumeration does not scale well to large metabolic networks, predictions of enzyme-cost optimal flux distributions were effectively limited to core metabolic networks. We present Global Enzyme Cost Minimization (GECM), a biconvex enzyme cost minimization framework that jointly optimizes metabolic fluxes and enzyme concentrations to predict metabolic fluxes, enzyme concentrations, and metabolite concentrations. To account for uncertainty in thermodynamic and kinetic parameter values, GECM samples multiple estimates from a joint parameter distribution. Specifically, we estimate unknown kinetic and thermodynamic parameters as well as their uncertainties using ENKIE [Gollub et al, Bioinformatics, 40,11 (2024)] and eQuilibrator [Beber et al, Nucleic Acids research, 50, D1 (2022)]. The reformulation of the convex fixed-flux ECM problem to a biconvex optimization on both fluxes and concentrations makes GECM scalable to genome-scale metabolic networks, provided that detailed kinetic and thermodynamic constraints are restricted to a subset of reactions. However, this reformulation comes at the cost of losing the global optimum guarantee, which we mitigate by using multi-start optimization. Finding physiologically realistic solutions is made easier by the ability of GECM to integrate both flux constraints and metabolite concentration constraints to further constrain the solution space. We used GECM to study different fermentation strategies in *E. coli*. First, we compared aerobic respiration and aerobic acetate fermentation in a glucose medium. GECM predicts that acetate secretion can be an enzyme cost optimal metabolic strategy, which is consistent with previous studies. Next, we investigated whether an additional reduction in enzyme cost compared to aerobic respiration and aerobic acetate fermentation can explain glycerol secretion by an evolved *E. coli* strain in an aerobic glucose environment. These two examples use condition-specific models whose metabolic strategies are limited to respiration and secretion of acetate and/or glycerol by constraining model exchanges. To evaluate how GECM performs when the model has more degrees of freedom, we studied the minimal cost metabolic strategy of *E. coli* growing in an anaerobic glucose medium. We used minimal pathways analysis to enumerate minimal secretory strategies of a genome-scale metabolic network in this growth condition and constrained exchanges to allow secretion of 44 compounds listed in these enumerated strategies. In this setting, GECM predicts optimal-cost metabolic strategies that are consistent with experimentally observed mixed acid fermentation strategies. We believe that GECM and its applicability to genome-scale metabolic networks will be useful in understanding trade-offs in cellular economics beyond core metabolism. This may lead to a better understanding of metabolic overflows and metabolic crossfeedings that can occur within microbial communities.

Optimal resource allocation in defence mixtures: Modelling the effects of substrate competition and competitive inhibition

Lukas Korn, *Friedrich-Schiller-Universität Jena*
Oral and poster, P2-55

In many interactions between different organisms, the release of defence chemicals (toxins) is a common strategy to defend against pathogens or predators [1], [2]. These toxins are often released in mixtures of chemically similar compounds. For example, tea tree oil of *Melaleuca alternifolia* is a mixture that consists of several terpene, which act as antimicrobials [3]. A drawback of using such mixtures may be that attacking organisms could have evolved a counter defence, for example an enzyme, with broad substrate specificity, which can degrade all compounds of these mixtures. As it is assumed that enzymes have evolved from broad to high substrate specificity, it is likely that this can be observed often in nature [5]. In this theoretical study, it is analyzed whether the effects of substrate competition or competitive inhibition may increase the steady-state concentrations of defence chemicals. For that, a differential equation-based model, that includes terms for substrate competition [5] and competitive inhibition [6], has been developed. This model covers different scenarios of the interactions between toxin mixtures and counter defences. For this model a resource allocation problem has been introduced and solved, with the goal to maximize the sum of steady-state concentrations of all toxins. The analysis revealed that when toxins act solely as competing substrates, the optimal strategy is to invest all resources into a single toxin, that is optimal in respect to maximal reaction velocity, toxicity, and Michaelis-Menten constant. That means in such a case, a mixture of toxins is not optimal. Only when at least some of the toxins act as competitive inhibitors of the counter-defense does it become optimal to allocate resources among two or more toxins. These results may be relevant for calculating the optimal dosage in drug mixtures.

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Can we predict the metabolic objectives of a cell?

Kate Meeson, *The University of Manchester*
Oral and poster, P1-56

At the core of flux balance analysis is an objective function, which should describe both the adaptive evolution and metabolic priorities of a cell. Briefly, the objective function is the reaction(s) towards which all reaction fluxes in the metabolic network are aiming to maximise or minimise. However, the oversimplification of this parameter introduces uncertainty to the analysis of metabolic models. One of the most common objective functions is growth maximisation, but previous work has shown that alternative objective functions could be more representative of the cell's actual physiology, with examples spanning bacterial and mammalian cells. Furthermore, in the instances where an alternative objective function has been considered, the method of choosing the final formulation is inconsistent. Here, we present the development of a Python-based function enabling prediction of the 'optimal' objective function. This method has been tested across multiple systems, namely yeast, bacteria and Chinese Hamster Ovary (CHO) cells to demonstrate its translatability and robustness. The final objective formulation is guided by validation against experimental flux measurements, to prioritise reliability and accuracy. In addition, various multi-objective optimisation approaches have been explored, including lexicographic optimisation and simulated annealing. Using this tool, we have made conclusions on the shifting metabolic priorities of CHO cells over a large-scale culture, which will influence future experiments in the optimisation of bioprocessing.

How Cellular Constraints Shape Metabolic Interactions in Microbial Communities

Benjamin Raach, *ETH Zürich / Eawag*
Poster, P2-57

Microbial communities perform essential metabolic ecosystem functions. The underlying community metabolism is characterized by its collective nature, with metabolic functions distributed among the members of the community. Despite its importance, the underlying drivers of distributed microbial metabolism are not yet understood quantitatively. Proteome allocation has been identified as a crucial factor explaining metabolic strategies in clonal microbial populations. Our study aims to investigate the extent to which this concept can be generalized to microbial communities and explain the distribution of metabolic pathways among different community members. Specifically, we are developing a mathematical model to understand the interplay between metabolic network topologies and physiological constraints, such as an upper bound on intracellular metabolite concentration. Overall, our goal is to contribute to a more mechanistic and quantitative understanding of the drivers of metabolic interactions and how these scale up to determine the dynamics and functions of microbial communities.

Biodiversity in bioenergetics and precursor usage in microbial growth

Maaike Remeijer, *Vrije Universiteit*
Oral

Microbial metabolism supports microbial growth across energetically diverse environments. Microorganisms extract energy from various sources by converting them into metabolites and energy carriers such as ATP and NADPH. These carriers, in turn, power the conversion of carbon sources into biomass and byproducts. Researchers have estimated key bioenergetic parameters such as the biomass yield per unit of ATP (Y_X/ATP). Now, genome-scale metabolic models enable more precise and biochemistry-based calculations of these values. In this study, we developed a systematic approach to dissect metabolism, represented in the form of a genome-scale stoichiometric model, into three core functional subnetworks: energy catabolism, precursor production and biomass production. Using this framework, we simulated the metabolic network of seven different organisms under 50 distinct growth conditions to capture the diversity of microbial metabolism. One striking observation is the wide variability in ATP production during carbon precursor synthesis. Depending on the energy source, this step can contribute anywhere from 0% to 100% of the total ATP demand, an effect observed even within *Escherichia coli* across different growth conditions. This variability stems from the distinct biochemical pathways that are active under each condition. During precursor synthesis, ATP can either be generated or consumed. When ATP is harvested during this step, the biomass yield on the energy source increases. Furthermore, greater energy carrier production at this stage reduces the reliance on catabolic pathways to meet cellular energy demands. These findings have significant implications. In biotechnology, they provide opportunities to enhance product yields by engineering energy efficiency. In microbial ecology, they offer new insight into how microorganisms balance energy use and nutrient exchange within communities.

Simulating the Emergence of Microbial Cooperation from Individual Growth Maximization via Cybernetic Modeling

Hyun-Seob Song, *University of Nebraska-Lincoln*
Oral

A longstanding debate in community ecology contrasts two perspectives: the superorganismal view, which frames ecological systems as integrated entities with cooperative species interactions, and the individualistic view, which attributes community structure and function to individual microbial responses to environmental conditions. This debate poses a dilemma in microbial community modeling, particularly when applying flux balance analysis (FBA) and dynamic FBA, which typically assume the maximization of either individual or community growth. Although community-level optimization can predict cooperative behaviors such as division of labor and cross-feeding, it lacks a strong biological basis, as microbial altruism is difficult to justify. In contrast, models based on individual fitness are more ecologically plausible but often fall short in reproducing the cooperative interactions observed in experiments. We reconcile these two contrasting perspectives by employing the generalized cybernetic control laws formulated by Young and Ramkrishna (Biotechnology Progress, 2007), which describe microbial metabolism as being regulated to maximize return-on-investment over a finite time horizon. Our model captures both cooperative and competitive microbial behaviors without introducing any ad hoc parameters to promote community-level fitness. To demonstrate the model's capabilities, we conducted a case study on a two-species microbial community involved in biopolymer degradation through two sequential stages: (1) the initial breakdown of polymers into oligomers, and (2) the subsequent hydrolysis of oligomers into simpler products such as monomers and dimers. Numerical simulations revealed several emergent ecological dynamics, including the dominance of the primary (early-stage) degrader over the secondary (later-stage) degrader, context-dependent shifts in downstream interactions involving both competition and cross-feeding, and upstream-driven regulation of overall community behavior. These insights highlight complex interaction patterns that conventional models often fail to capture, showcasing the distinctive strength of our approach. The proposed framework serves as a robust and versatile simulation tool for advancing the understanding and design of both natural and engineered microbial ecosystems.

Why do cells invest in compartmentalization? The thermodynamic benefits of mitochondria

Diana Szeliova, *University of Vienna*
Oral and poster, P2-58

Cellular metabolism relies on enzyme-catalyzed reactions to generate energy, synthesize biomass precursors, and process waste—functions that require significant cellular resources. A major evolutionary development is the compartmentalization of metabolic pathways into organelles such as mitochondria, which play key roles in energy production, amino acid metabolism, and redox balance. While prokaryotes perform similar reactions in the cytosol, eukaryotes have retained mitochondria despite their associated costs and continue to import hundreds of nucleus-encoded enzymes. This study investigates whether mitochondrial compartmentalization improves biochemical efficiency by alleviating thermodynamic constraints in metabolic networks. Reaction feasibility depends on thermodynamic driving forces, which are determined by substrate-to-product concentration ratios. In complex metabolic systems, shared metabolites can create conflicting concentration demands, limiting the efficiency of individual reactions. Compartmentalization may resolve these conflicts by allowing distinct metabolite pools in mitochondria and the cytosol. We hypothesize that this spatial separation increases thermodynamic driving forces, reduces enzyme requirements, and enhances cellular fitness—offering a potential explanation for the evolutionary persistence of mitochondria. To test this, we developed OptSDF, a computational framework that maximizes the sum of thermodynamic driving forces across sampled flux distributions. Using genome-scale metabolic models of *Saccharomyces cerevisiae* and *Komagataella phaffii*, we compared native models containing mitochondria to modified versions in which mitochondrial reactions were relocated to the cytosol. Simulations were performed under aerobic and anaerobic conditions and across varying growth rates. Our results show that mitochondria consistently improve thermodynamic efficiency, particularly in central carbon and amino acid metabolism across both organisms and conditions. In some scenarios, relocating mitochondrial reactions to the cytosol rendered them thermodynamically infeasible. Analysis of metabolite concentrations revealed key differences between compartments, particularly in ATP/ADP ratios and several metabolites of central carbon metabolism. These findings support the idea that mitochondria provide an evolutionary advantage by enabling spatial separation of metabolite concentrations, thereby improving reaction thermodynamics. The resulting efficiency gains may free up metabolic resources for growth, stress response or other processes, contributing to selective advantage. This work underscores compartmentalization as a fundamental strategy for overcoming biochemical constraints, with implications for organelle evolution and metabolic engineering.

Understanding the Robustness of *Yarrowia lipolytica* for Upcycling of Depolymerized Plastic Waste

Cong Trinh, *University of Tennessee Knoxville*
Poster, P1-59

The mass production and limited recyclability of petroleum-derived plastics have led to plastic waste polluting our ecosystem for decades. Biological upcycling of plastic waste offers a sustainable solution for promoting decarbonization and reducing environmental pollution. However, direct microbial conversion of plastic waste, particularly recalcitrant polyolefins, is inefficient. Chemical depolymerization of polyolefins yields depolymerized plastic (DP) oil, comprising a complex mixture of saturated, unsaturated,

even, and odd hydrocarbons suitable for biological conversion. Understanding and harnessing the robust metabolic capabilities of microorganisms to upcycle the hydrocarbons in DP oil, both naturally and unnaturally occurring, into high-value chemicals remains limited. Here, we discover that an oleaginous yeast, *Yarrowia lipolytica*, undergoing short-term adaptation to DP oil robustly utilizes a wide range of hydrocarbons for cell growth and production of citric acid and neutral lipids. *Y. lipolytica* demonstrates distinct planktonic and oil-bound phenotypes when grown on hydrocarbons with each exhibiting unique proteomes. Proteome reallocation toward energy and lipid metabolism enables robust growth of *Y. lipolytica* on hydrocarbons, with n-hexadecane as the preferential substrate. We also present ongoing work in modular cell engineering of *Y. lipolytica* for upcycling DP oil into non-native, high-value products.

Theory and methods in network analysis

COBRA-k: a new modeling framework linking constraint-based and kinetic modeling approaches

Pavlos Stephanos Bekiaris, *Max Planck Institute Magdeburg*
Oral and poster, P2-60

Mathematical modeling of metabolic networks has become an essential tool to gain a systems-level understanding of cellular metabolism. Two major modeling approaches frequently used for metabolic networks are constraint-based reconstruction and analysis (COBRA) and kinetic modeling. COBRA centers around the investigation of steady state flux distributions in metabolic networks and is based on linear constraints such as mass balances and linear optimization techniques to explore the solution space. The COBRA framework is computationally very efficient and proved valuable for studying various properties of genome-scale metabolic networks. However, it is limited in its ability to incorporate reaction kinetics hampering quantitative predictions that depend on metabolite concentrations. In contrast, kinetic models are based on differential equations with the metabolite concentrations as state variables, whose dynamics is governed by kinetic rate laws describing the metabolic fluxes as functions of the metabolite (and enzyme) concentrations. While kinetic models are more realistic mechanistic descriptions of metabolic networks and allow for dynamic simulations, they require a large number of kinetic parameters and knowledge of mechanistic details, which are often unknown. In particular, enzyme concentrations (or $v_{\max}$ values) must usually be known when simulating a kinetic model, which limits their ability to explore the space of steady-state flux distributions where the enzyme concentrations can be freely chosen. Here, we introduce COBRA-k (COBRA with kinetic constraints), a new modeling framework that settles between COBRA and kinetic models. In particular, COBRA-k models allow the seamless integration of non-linear constraints from kinetic rate laws with the classical linear COBRA constraints. In comparison to COBRA models, this enables a more precise description of the feasible space of steady-state fluxes as well as associated enzyme and metabolite concentrations. In contrast to kinetic models, COBRA-k models have free enzyme concentrations and can flexibly deal with simplified (or even missing) rate laws and constraints imposed on fluxes and concentrations. Instead of a single deterministic steady state that would result from a simulation in a kinetic model, these degrees of freedom shape the steady-state solution space of the COBRA-k model, which can be explored with optimization techniques. COBRA-k models contain linear and non-linear constraints as well as integer and continuous variables. Their optimization thus results in mixed-integer non-linear programs (MINLP) and are thus much more complex than (mixed-integer) linear programming techniques used in classical COBRA methods. We found that even state-of-the art (global) MINLP solvers fail in finding optimal solutions in larger COBRA-k models. We therefore developed a dedicated iterative algorithm which separates the search for integer variables (representing active and inactive fluxes) and continuous variables (such as fluxes and concentrations). This algorithm turned out to be suitable to tackle large-scale COBRA-k optimization problems. The developed COBRA-k solver and related algorithms and functions have been implemented in the freely available Python package COBRAk. This package also provides support in (semi-) automatic construction of COBRA-k models from constraint-based models, for example, by retrieving relevant kinetic parameters from databases. As a first application of the COBRA-k formalism, we constructed a large-scale COBRA-k model of *E. coli*'s central metabolism based on the model iCH360 [1]. Using maximization of growth under different substrate uptake scenarios, we found that (i) the developed COBRA-k solver converges reasonably fast to a solution that appears to be optimal, and (ii) that the found solutions reproduce well-known major trends of *E. coli*'s metabolism (e.g. regarding metabolic flux distributions, phenomena related to overflow metabolism, metabolite and enzyme abundances), many of which either differ from or cannot be predicted at all with classical COBRA models. We also used the COBRA-k framework to predict other optimal metabolic solutions in the *E. coli* COBRA-k model, e.g. those that could be relevant for metabolic engineering applications to maximize product synthesis. We believe that our developed COBRA-k framework provides a rich and useful methodology for metabolic modeling that naturally extends the classical COBRA approach. COBRA-k requires more knowledge on reaction kinetics, but this data is becoming available in increasing amounts (also based on machine-learning approaches) and COBRA-k is also well-suited to deal with partially missing information. In summary, COBRA-k is the missing piece of puzzle that lies between constraint-based and kinetic modeling approaches and combines the high flexibility of COBRA models with the accuracy of kinetic models.

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Modeling of the metabolism of bacterial predation

Theo Brunet, *Institute of Developmental Biology of Marseilles*
Poster, P1-61

Predation is a fundamental ecological mechanism that plays a critical role in shaping ecosystems by promoting nutrient recycling, structuring communities, and maintaining the delicate balance between predator and prey populations. While macroscopic predation has been extensively studied and is relatively well understood, bacterial predation remains an underexplored and complex phenomenon. Bacteria are remarkable in their diversity of predatory strategies, employing mechanisms that range from the secretion of lytic enzymes and secondary metabolites to direct physical invasion of prey cells (10.1111/1462-2920.13171). In response, prey bacteria have also evolved a variety of sophisticated defense strategies to avoid or resist predation pressures. In this context, the aim of our study is to dissect the metabolic response of prey bacteria during predation. Specifically, we focus on the interaction between *Myxococcus xanthus*, a model predatory bacterium known for its complex social behavior and ability to prey upon a wide range of bacterial and even fungal species, and *Escherichia coli*, a common and well-characterized organism that act as a prey. To-

gether with our collaborators, we established a co-evolution experiment in which populations of *M. xanthus* and *E. coli* were allowed to interact over successive generations. This experimental evolution approach yielded novel strains: an enhanced predatory strain of *M. xanthus* (M1), with increased predatory efficiency, and an evolved *E. coli* strain, referred to as E7, which exhibited resistance to *M. xanthus* WT predation. To elucidate the mechanisms underlying E7's increased resistance, we use a detailed analysis of transcriptomic data generated from both the *E. coli* wild-type (WT) strain and the evolved E7 strain, cultured either in isolation or in co-culture with *M. xanthus*. Transcriptomic profiles provided valuable insights into the global gene expression changes associated with both predatory attack and evolved resistance. To further interpret these changes in a systems biology framework, we integrated the transcriptomic data into the genome-scale metabolic model of *E. coli*, iML1515 (10.1038/nbt.3956). Using the E-flux method (10.1371/journal.pcbi.1000489), we constrained the model based on transcriptomic information, enabling condition-specific simulations of metabolic fluxes via flux balance analysis (10.1038/nbt.1614). This integrative approach allowed us to identify critical metabolic adaptations that may confer a survival advantage to E7 in the face of predation. Preliminary results from the metabolic flux analysis indicate a notable increase in the predicted production of reactive oxygen species (ROS), particularly superoxide anions, in the E7 strain. This finding suggests that oxidative stress responses may play an important role in the defensive strategy of E7 against *M. xanthus*. Complementary experimental assays provided further evidence supporting the involvement of ROS in resistance mechanisms, showing elevated ROS levels in E7 compared to the WT strain upon predatory challenge. Furthermore, a flux sampling approach was employed to comprehensively explore the range of possible metabolic states accessible to the cells under each condition. Flux sampling revealed an intriguing pattern: the metabolic state of E7 in isolation closely resembled that of WT *E. coli* during active predation. This suggests that the E7 strain has adopted a pre-emptive metabolic configuration, primed for defense even in the absence of immediate predatory threat. Such a state likely affords E7 a critical advantage, enabling a rapid and efficient response to the presence of *M. xanthus* without the metabolic delay associated with activating stress responses after the onset of attack. Taken together, these findings demonstrate the power of integrating omics datasets into constraint-based metabolic modeling frameworks to investigate complex biological phenomena. Our study provides valuable insights into how bacterial prey can evolve metabolic strategies to resist predation, highlighting the dynamic interplay between metabolism, stress responses, and ecological interactions. More broadly, this work underscores the potential of systems biology approaches to uncover hidden layers of biological adaptation and evolution at the metabolic level.

Dynamic Modeling of Equilibrium Reactions

Peter Carstensen, *Technical University of Denmark*
Poster, P1-62

Many problems in biotechnology and systems biology can be described by equilibrium reactions. Cellular dynamics and extracellular pH are such examples. Ionization of molecules occurs almost instantaneously, enzymatic reactions in cellular metabolism occur in milliseconds to seconds, cellular growth at minutes to hours, and bioreactor processes extend from hours up to several days. Our modelling approach addresses these multiscale phenomena, by modelling differential equations with an embedded optimization model. The presented methodology models biological species that can adapt to changing environments and have well-established genome-scale models. The model consists of differential equations for substrate, selected enzymes, and biomass concentration. The cellular metabolism is modelled using dynamic flux balance analysis, and is constrained by rates derived from growth media, enzyme concentrations and the stoichiometry of the intracellular metabolism. Extracellular pH is modelled as a separate equilibrium system, affected by the substrates and cellular activity. The model is demonstrated *in silico* with *E. coli* growing on glucose under varying nutrient concentrations and extracellular pH. Results aligns with reported rates from experimental data in literature.

Atomic elementary flux modes explain the steady state flow of molecular constituents in genome-scale metabolic flux networks

Justin Chitpin, *The University of Queensland*
Oral and poster, P1-63

Elementary flux modes (EFMs) are minimal sets of reactions that can sustain steady state flux in a metabolic flux network. First proposed by Schuster and Hilgetag, any set of steady state fluxes in a metabolic network can be explained as a positive, linear combination of their EFM weights. This decomposition, however, is generally not unique, as there are typically exponentially many EFMs. We recently addressed this flux decomposition problem for the special case of unimolecular reaction networks involving single substrates and products. By imposing a Markovian constraint on EFMs, we modelled steady state particle fluxes as a cycle-history Markov chain (CHMC) to uniquely identify EFM weights that reconstruct any network fluxes. Here, we generalize our CHMC analysis to networks containing multispecies reactions. We propose the concept of atomic EFMs (AEFMs) which are steady state pathways that trace the flow of individual atoms through a network. Using a state-of-the-art atom mapping algorithm, we enumerate carbon and nitrogen AEFMs from four genome-scale networks and compute AEFM weights from a fifth HepG2 dataset. We find that enumerating AEFMs is computationally tractable compared to standard "molecular" EFMs, and show how AEFMs characterize nutrient source metabolism through the network. Lastly, our analysis of the HepG2 AEFM weights reveals that the majority of steady state fluxes are explained by a small fraction of AEFMs. Our ACHMC method is a fast and novel tool to quantify metabolic remodelling as technologies to generate large-scale metabolic flux networks advance.

Constraint-Driven Enumeration of Biologically relevant Elementary Flux Modes in Metabolic Networks Using Hybrid Logic-Linear Programming

Emma Crisci, *LBBE, Lyon France*
Oral and poster, P2-64

Metabolism represents the entirety of biochemical reactions occurring within an organism to sustain life, including both the degradation processes — catabolism — and the synthesis processes — anabolism — of molecules. Metabolic networks can be represented as oriented hypergraphs, where each reaction connects multiple substrates to products. These networks are encoded by a stoichiometric matrix, quantifying metabolite consumption and production. Under steady-state conditions, the net production of internal metabolites is zero, ensuring balanced consumption and production across the network. Elementary Flux Modes (EFMs) are the fundamental building blocks of metabolic stoichiometric models. EFMs allow the description of the minimal sets of reactions in a metabolic network under steady-state conditions, representing unique and feasible pathways. While EFMs fully characterize the space of all possible steady-state flux distributions, their number can grow combinatorially with network size, rendering exhaustive enumeration computationally infeasible. Furthermore, it is not necessary to calculate all EFMs, as many of them are not biologically relevant. To address this, we propose an original method to enumerate only biologically meaningful EFMs by integrating explicit biological constraints during the enumeration process. Our approach is a combination of logic programming and a linear solver through ClingoLPx, a hybrid solver. Logic programming manages the qualitative structure of EFMs, while linear programming handles the quantitative side by incorporating biological constraints. To encode the targeted search for EFMs and enforce constraint satisfaction, we use Answer Set Programming (ASP), a declarative paradigm well-suited for modeling and solving complex logical problems. This integration enables the cut-off of search paths leading to non-relevant EFMs, drastically reducing computation time and memory usage, while also allowing the simultaneous application of multiple biological constraints. We present a new version of our software, EFM-aspLPx, which allows seamless integration of various biological constraints during EFM enumeration. This redesigned tool achieves a 10-fold speed-up over our previous implementation by optimizing the interaction between the logical and linear components of the solver provided by ClingoLPx. In particular, we incorporate thermodynamic constraints based on reaction Gibbs free energies, which restrict metabolite concentrations to biologically plausible intervals. These constraints are implemented as theory propagators and further reduce the number of EFMs during enumeration. We applied our method to the central carbon metabolism of *E. coli*, showing that incorporating Gibbs energy constraints effectively filters out non-relevant EFMs and significantly improves computational performance. We are currently finalizing an extension of the framework to support additional biological constraints, particularly recent developments in enzyme cost modeling, which limit total enzyme usage within a defined budget.

The emergence of oscillations on reaction networks in growing cells

Hugo Dourado, *Heinrich Heine University*
Oral

The growth of unicellular organisms is known to involve an oscillatory allocation of internal resources even in static growth media providing no external dynamic stimulus. The cause and function of this self-oscillation remains unclear, with different hypotheses including its origin as a byproduct of misguided regulatory processes. Here we present Growth Mechanics (GM) as a general mathematical framework to study the dynamic resource allocation in models of whole self-replicating cells, built exclusively on first principles. This allows us to demonstrate that the observed oscillations may have the function of maximizing the fitness of microbial cells. We derive all our results exclusively from the basic assumptions that the evolution of self-replicating cells is driven by fitness maximization and constrained by mass conservation, nonlinear kinetic rate laws, and constant cell density, resulting in an optimal resource allocation problem that can be understood and solved as a classical physics problem. Our results also indicate how almost linear "growth law" relationships between oscillation frequency, average cell state and growth rate emerge from first principles only. This work contributes to the foundations of theoretical cell biology with general quantitative principles and a bridge to theoretical tools of physics.

Opening the black box: Thermodynamics of Microbial Growth

Oliver Ebenhöh, *HHU Düsseldorf*
Poster, P1-65

Microbial growth is often described as an energy converter, in which the catabolic breakdown of nutrients yields free energy which is partly used to drive the anabolic processes that assemble nutrients into new biomass. The combined effect of anabolism and catabolism can be described by a so-called "macrochemical" equation, which summarizes the chemical conversion of nutrients into catabolic waste products, biomass, and possibly other side products. Because such a description of microbial growth completely ignores the details of intracellular metabolism, it is often termed a "black box" model of microbial metabolism. A diametrically different approach to describe metabolism is by genome-scale metabolic network models. Such models are derived from annotated genome sequences and ideally comprise all known biochemical reactions that can occur inside an organism. We here investigate how these two concepts can be combined to study the thermodynamics of microbial metabolism. We illustrate how experimentally determined macrochemical equations can be systematically separated into a catabolic and an anabolic part. We then characterize these separate chemical equations by their thermodynamic properties and derive the generalized thermodynamic forces and flows that correspond to the affinities of catabolism and anabolism, and the corresponding catabolic and anabolic rates. With these data

we challenge the common view that microbial energy conversion can be described by a linear energy converter model. We systematically analyse chemostat growth data for a variety of microbes under carbon and non-carbon limited conditions to calculate the thermodynamic driving forces. We observe that the catabolic driving force, normalised to carbon mole new biomass formed, is consistently higher for non-carbon limited conditions over carbon limitation. Our observations hold for yeasts as well as bacteria and extend over a variety of limitations (nitrogen, sulfur, potassium, and phosphate). This systematic increase of catabolic driving force per biomass formed under anabolic limitations indicates a novel thermodynamic principle of microbial growth. Understanding this increase systematically will be important for biotechnological applications. Often, anabolic limitations are applied to induce the production of valuable side products, but as a consequence the heat generation drastically increases. A quantitative understanding of the thermodynamic gradients, and thus also heat production, under anabolic limitations will be critical to optimise bioreactor-based biotechnological production processes.

Creating Atom-Resolution Chemical Reaction Networks from Biochemical Models

Casper Asbjørn Eriksen, *University of Southern Denmark*
Poster

Metabolic flux analysis using stable isotope tracing is a key contributor to understanding cell metabolism. However, computational approaches to isotope tracing are difficult at the metabolic scale due to the lack of accurate models containing atom-to-atom mapped reactions. While manually curated datasets of atom-to-atom maps exist, they use different standards and annotation techniques. Furthermore, each source of these maps associates them with reactions from different databases, making direct comparison and usage difficult. We present preliminary work on ChemRecon, a software library for leveraging the combined information of multiple biochemical databases. By constructing and analysing networks based on references and connections within and between databases, ChemRecon is able to make connections and determine consensus associations between different types of database entries. We apply this system to the problem of generating atom-to-atom mapped chemical reaction networks based on metabolic models and the combined information of multiple databases, with the goal of creating a model which is manually curated to the greatest possible extent. When no curated atom-to-atom maps are available, we determine the compatible structures for each compound by a consensus algorithm using the database networks and apply an automatic atom-to-atom mapping tool. Preliminary results on genome-scale *E. coli* models show that ChemRecon is able to associate curated atom-to-atom maps to between 76% and 90% of reactions.

Unbiased metabolic flux inference through combined thermodynamic and 13C flux analysis

Nathan Fargier, *University of Groningen*
Oral

Quantification of cellular metabolic fluxes, for instance with 13C-metabolic flux analysis, is highly relevant for applied and fundamental metabolic research. A still-existing challenge in 13C-flux analysis is that the available experimental data are usually insufficient to resolve metabolic fluxes in metabolic networks without making assumptions about flux directions and reversibility. To infer metabolic fluxes in an unbiased manner, we devised an approach that does not require such assumptions. The developed three-step approach integrates thermodynamics, metabolome and physiological data, involves a comprehensive sampling of the complex thermodynamically-constrained metabolic flux space and scores the obtained posterior flux distributions with 13C labeling data. Applying our approach to budding yeast with its compartmentalized metabolism and parallel metabolic pathways, we could resolve metabolic fluxes in an unbiased manner and found novel flux patterns that had remained unknown until now, probably due to assumptions made in previous 13C flux analysis studies. We expect that our approach will be an important step forward in determining metabolic fluxes with improved accuracy in microorganisms and possibly also in more complex organisms.

Characterising functional redundancy in microbiome communities via relative entropy

Daniel Fässler, *University Medicine Greifswald*
Poster, P1-66

Functional redundancy has been hypothesised to be at the core of the well-evidenced relation between high ecological microbiome diversity and human health. Here, we conceptualise and operationalise functional redundancy on a single-trait level for functionally annotated microbial communities, utilising an information-theoretic approach based on relative entropy that also allows for the quantification of functional interdependency across species. Via constraint-based microbiome community modelling of a public faecal metagenomic dataset, we demonstrate that the strength of the relation between species diversity and functional redundancy is dependent on specific attributes of the function under consideration such as the rarity and the occurring functional interdependencies. Moreover, by integrating faecal metabolome data, we highlight that measures of functional redundancy have correlates in the host's metabolome. We further demonstrate that microbiomes sampled from colorectal cancer patients display higher levels of species-species functional interdependencies than those of healthy controls. By analysing microbiome community models from an inflammatory bowel disease (IBD) study, we show that although species diversity decreased in IBD subjects, functional redundancy increased for certain metabolites, notably hydrogen sulphide. This finding highlights their potential to provide valuable insights beyond species diversity. Here, we formalise the concept of functional redundancy in microbial communities

and demonstrate its usefulness in real microbiome data, providing a foundation for a deeper understanding of how microbiome diversity shapes the functional capacities of a microbiome.

Kinetic modules are sources of concentration robustness in biochemical networks

Anika Küken, *Universität Potsdam*
Oral

Biochemical networks underpin cellular function by transforming metabolites into essential building blocks that support all cellular functions emerging from the coordinated rates of biochemical reactions. Here, we introduce a novel framework for identifying kinetic modules—groups of reactions that maintain fixed rate ratios across various cellular states—by leveraging steady-state reaction rate couplings. Unlike traditional approaches that rely on detailed kinetic parameters (often unavailable at the genome scale), our method extracts kinetic modules directly from the network's structure endowed with mass action kinetics. We then link the kinetic modules of metabolic networks to the robustness of metabolite concentrations in response to perturbations. Analyzing 34 metabolic network models from 26 organisms, we reveal that specific enzymatic mechanisms, such as ordered binding, confer enhanced robustness of metabolite concentrations compared to random binding. These results provide new perspectives on how intrinsic network properties contribute to system robustness—insights that are critical for both systems biology and synthetic biology. Consequently, our findings pave the way for using modules to advance both theoretical understanding and practical applications in synthetic biology and biotechnology.

Mining Metabolic Reaction Modules for Retrobiosynthetic Design and Genome-Scale Model Curation

Konrad Lagoda, *Epfl*
Poster, P2-74

Metabolism is most commonly understood as collections of enzymatic reactions assembled into pathways. While a pathway view aids our comprehension of higher-level phenomena, i.e. cellular metabolism, it is limited in its insight into its own design and origin. Rather than single enzymes, short cascades of sequential enzymatic reactions, referred to as Reaction Modules, are believed to be the composable building blocks of metabolism (1). Such modules, being the very units that nature itself redeploys, may therefore pose as more suitable primitives for retro-biosynthetic pathway design and genome-scale model curation. Previous efforts to access them were limited by their demand for extensive and non-transparent manual curation (2) or methods uninformed by reaction module biology (3). Our approach broadens the scope of enzymatic reactions considered(4), employs a contemporary metabolic network formulation (5), classifies via a manually curated enzymatic mechanism library, and enforces constraints informed by current knowledge of reaction modules. Preliminary results are found to be consistent with results achieved through manual curation. We hope the resulting library will inform studies into metabolisms emergence and guide its rational design.
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Tackling Aleatory and Epistemic Uncertainty using Metabolic Network Ensembles

Katharina Nöh, *Forschungszentrum Jülich GmbH*
Oral

Parameter inference using network models forms the basis for generating quantitative insights into living systems. Here, uncertainty in parameter estimates is typically considered as result of statistical noise in the data (known as aleatoric uncertainty). However, since models are only context-specific approximations of real biological systems, their formulation is also subject to epistemic model uncertainty that arises from incomplete or presumed knowledge about a useful model, which aspects should be included, and at what level of detail. In any realistic case, however, aleatoric and epistemic uncertainty are intertwined and cannot be separated. This leads to the question of whether model formulations can be justified for an inference task on a given data set without risking over- or underfitting, known as the bias-variance tradeoff problem in statistics. Bayesian Model Averaging (BMA) is a statistical framework suitable for inferring parameters and their uncertainty under aleatoric and epistemic uncertainty [1]. BMA is an unsupervised method that considers a set of possible models and their parameter values and rules them in terms of their ability to explain the data [2]. For simple reaction direction variants, we have previously shown that BMA resembles a tempered Ockham's razor, where too flexible models with overly many parameters, but also too simple models are effectively penalized [3]. Here we extend our previous work towards general, not necessarily nested, network model ensembles. Applying BMA in the context of metabolic flux analysis using isotope labeling data, we again find low support for expert models, while robust and unbiased parameter estimates are obtained with models having considerably fewer degrees of freedom. By calculating probabilistic model weights, our approach provides interpretable results and connects structural and practical identifiability analysis based on the more general idea of model ensembles, rather than conditioning the result on a single model.

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[3] Theorell, A., Jadebeck, J. F., Wiechert, W., McFadden, J., & Nöh, K. (2024). Rethinking 13C-metabolic flux analysis - The Bayesian way of flux inference. Metabolic Engineering, 83, 137–149.

From Microbes to Interactions with PyCoMo – Tackling the Challenges in Community Metabolic Modelling

Michael Predl, *University of Vienna*
Oral

Microbial communities play crucial roles in human health, biotechnological applications and natural ecosystems. Interactions between individual members have a profound impact on the formation, stability and functions of the entire community. Yet, their analysis remains challenging due to their inherent complexity and the substantial time and resource requirement of experiments. *In silico* analyses are promising alternatives to gain better understanding of microbial communities and the interactions of their members. In particular, metabolic modeling approaches, which have been highly successful in the analysis of single organisms, can offer novel mechanistic insights. However, taking metabolic models to the level of communities introduces a plethora of new challenges. PyCoMo, a Python package designed to construct compartmentalized, COBRA-compliant metabolic models for multi-species systems presents solutions to the many key challenges in community metabolic modeling. The added layer of community composition transforms the linear single organism model into a bilinear problem, drastically increasing the computational cost for analyses. PyCoMo utilizes a model structure which allows for switching between modes of fixed growth rate and fixed community composition, each yielding a linearized problem for efficient computation. This structure is compliant with the SBML standard and allows for use with standard COBRA methods, predicting interactions between community members. A major pitfall of metabolic models is the presence of thermodynamically infeasible cycles, which can inflate flux values and subsequently lead to incorrect phenotype predictions. This problem is exacerbated in microbial communities, where such cycles can also occur across multiple members. To provide correct solutions, PyCoMo is able to perform loopless flux variability analysis with efficient computation, even in community models with more than tens of thousands of reactions and metabolites. The full capabilities of this approach are exemplified by an analysis of a three-species community for underground biogas production. Based on this journey from genomes to interaction predictions, this talk will cover the most important steps and challenges in constructing metabolic models of microbial communities and the prediction of cross-feeding interactions.

Graph-theoretic interpretation of fundamental subspaces in biochemical networks

Hadjar Rahou, *University of Galway*
Poster, P2-67

A biochemical reaction network is generally represented as a stoichiometric hypergraph, where each node represents a metabolite and each hyperedge represents a reaction. Although this representation presents an accurate algebraic and graphical description of a reaction network, it lacks well-developed graph-theoretic concepts, such as spanning trees and cycles that are central to structural analysis in graph theory. On the other hand, each metabolite can be represented as a graph where each node represents an atom and each edge represents a chemical bond. In this work, we bridge the gap between these two levels of representation by presenting a new method for identifying conserved and reacting moieties. We introduce a moiety-based graph representation and provide an interpretation of the fundamental subspaces of the stoichiometric matrix using graph-theoretic concepts derived from moiety transition graphs. A conserved moiety is a set of atoms whose association is invariant across all reactions in a network. A reacting moiety is a set of bonds that are either broken, formed, or undergo a change in bond order in at least one reaction in the network. Given a directed stoichiometric hypergraph, a molecular graph for each metabolite and an atom mapping for each reaction, we generate a directed atom transition multigraph. We generate a molecular transition graph as the union of the atom transition multigraph and chemical bonds from all molecular species in a biochemical network. Each node of a molecular transition graph represents an atom and each edge represents either a chemical bond within a molecule or an atom transition induced by a chemical reaction. We show that the incidence matrix of a molecular transition graph can be partitioned into a sum of incidence matrices corresponding to its connected components. These components define two types of subgraphs, one noted as conserved molecular transition subgraph and formed entirely by conserved bonds, which do not change across any reaction and one noted as reacting molecular transition subgraph, formed by reacting bonds, which are either broken, formed, or change order in at least one reaction. Conserved moieties are identified from the conserved molecular transition subgraph and reacting moieties are identified from the reacting molecular transition subgraph. This representation allows us to decompose the stoichiometric matrix into biologically meaningful submatrices of the incidence matrices of moiety transition graphs. Most importantly, it enables a direct interpretation of the four fundamental subspaces in terms of the graphical properties of the moiety transition graphs. By linking molecular structure, graph theory, and linear algebra, this framework provides a scalable and interpretable approach to studying fluxes, conservation laws, and network topology in biochemical networks. It offers a novel perspective on how molecular-level transformations give rise to the algebraic structure of metabolic networks, with applications in systems biology, metabolic engineering, and metabolic pathway analysis.

Integrating Data-Driven GeM Reduction and Bayesian Rate Calculation for Enhanced Cell System Modeling

Anne Richelle, *Sartorius Corporate Research*
Oral

Integrating genome-scale metabolic models (GeMs) with accurate metabolic rate calculations is essential for advancing predictive capabilities in cell system modeling. This presentation introduces a novel framework that combines a Bayesian approach to metabolic rate estimation with data-driven GeM reduction, addressing key challenges such as computational complexity and data scarcity. GeMs are vital for simulating cellular metabolism under various conditions; however, their complexity and numerical instability often hinder their use in bioprocess applications. Traditional top-down GeM reduction methods simplify models while preserving key metabolic functions but lack standardized frameworks that ensure biological feasibility and computational stability. Our approach leverages data-driven top-down reduction, where accurate metabolic rate estimates are derived from exometabolomic concentration data (e.g., cell growth, substrate consumption, and metabolite production). Estimating metabolic rates from sparse and noisy data is a significant challenge, which we address using a modular Bayesian non-linear interpolation framework. This framework fits a model to a "pseudo-batch" transformation of measured concentrations, incorporating prior knowledge of cell culture dynamics through specific basis functions. Nested sampling algorithms are used to marginalize the prior, providing credibility bounds for the metabolic rate trajectories and facilitating model comparison. A key advantage of our method is the generation of confidence intervals for metabolic rates, which constrain the model and eliminate the need for arbitrary error coefficients. This improves the reliability of predictions by better accounting for experimental variation. We integrate these credibility bounds into a structured GeM reduction pipeline that uses Mixed-Integer Linear Programming (MILP) to identify and remove unnecessary reactions, ensuring compatibility with experimental data and maintaining model solvability at each step. The proposed framework is demonstrated in a case study of CHO cell culture simulations, where the reduced GeM accurately reflects experimental data. By systematically reducing model complexity and integrating metabolic rate data, our approach enhances the applicability of reduced GeMs for bioprocess modeling, digital twins, and synthetic biology, enabling more reliable predictions and optimization.

From genome to metabolism: Pan genome-scale metabolic model reconstruction of the extremophile genus Sulfolobus

MSc María José Rimón Martínez, *University of Vienna*
Poster, P1-68

The *Sulfolobus* genus includes some of the most widely used thermophilic archaea in biotechnology due to their ability to be manipulated in the laboratory. However, only a few species have been extensively studied and little is known about their shared and unique metabolic traits. In this study, we present the first pan-genome-scale metabolic model (Pan-GEM) for the *Sulfolobus* genus. By reconstructing individual genome-scale metabolic models (GEMs) for multiple species, we seek to explore metabolic diversity, identify central and accessory metabolic functions and investigate gene diversity and evolutionary divergence, among others. This integrative approach provides a comprehensive framework for understanding *Sulfolobus* metabolism and its biotechnological potential.

Thermodynamically consistent and phenotype-specific estimation of metabolic fluxes

Marcelo Rivas-Astroza, *Universidad Tecnológica Metropolitana*
Oral and poster, P1-69

Accurately estimating metabolic fluxes in biological systems is hampered by unrealistic flux patterns that violate thermodynamic laws, alongside the need to reflect specific cellular states. These thermodynamic inconsistencies lead to biologically unrealistic predictions, while phenotype specificity requires integrating data like transcriptomics. We developed teraflux, a method that addresses both challenges by maximizing transcriptomically-weighted entropy within a framework that guarantees thermodynamic consistency and respects known reaction directionality. It unifies principles from Fleming et al. (thermodynamic consistency) and Gonzalez et al. (phenotype-specificity) to compute unique, biologically relevant fluxomes. Teraflux's performance demonstrates significant advantages. Crucially, it ensures thermodynamic consistency, eliminating biologically impossible energy-generating loops. Compared to standard flux sampling on *E. coli* models, teraflux consistently avoids the extreme, biologically unrealistic flux values associated with thermodynamic inconsistencies, instead identifying diverse (high-entropy) states within the biologically relevant, thermodynamically feasible region. This highlights its ability to explore the valid solution space more effectively. Furthermore, teraflux correctly distinguishes between thermodynamically allowed and disallowed cycles. While methods like loopless FVA eliminate all cycles, potentially removing important pathways, teraflux accurately predicts flux through biologically essential, thermodynamically feasible cycles like the *E. coli* glyoxylate shunt, matching experimental data where loopless FVA fails. This ability to preserve necessary metabolic routes while ensuring overall thermodynamic consistency is critical for accurate modeling. When applied to experimental data from *E. coli* grown under various conditions, teraflux achieves high predictive accuracy (Pearson r = 0.89), comparable to other state-of-the-art methods like Pheflux. However, teraflux yields more constrained fluxomes (lower entropy), reflecting the exclusion of thermodynamically impossible states. This increased constraint translates directly to enhanced biological relevance. The biological significance of enforcing thermodynamic consistency is clearly demonstrated under specific conditions, such as *E. coli* growth on pyruvate. Methods lacking this enforcement (like Pheflux) predict biologically implausible

scenarios, including glucose excretion and futile cycling through glycogen metabolism. Teraflux, by maintaining thermodynamic consistency, correctly predicts the absence of these phenomena, aligning with established metabolic principles and experimental observations. It accurately reflects that *E. coli* does not produce glucose from pyruvate under these conditions and only synthesizes glycogen when carbon is abundant relative to other nutrients. Similarly, teraflux prevents unrealistic cycling between acetate and acetyl-CoA. In conclusion, teraflux provides a robust method for obtaining unique, condition-specific metabolic flux estimations that are rigorously thermodynamically consistent. By successfully integrating transcriptomic data with fundamental thermodynamic laws and known biochemical constraints, it avoids biologically unrealistic predictions common in other methods. The results show that teraflux not only achieves high accuracy against experimental data but also generates more biologically meaningful insights by correctly handling metabolic cycles according to thermodynamic principles. This makes teraflux a valuable tool for accurately understanding cellular metabolism, studying metabolic diseases, and guiding metabolic engineering strategies.

From Mixed Acid to Homolactic Fermentation: Understanding ATP Supply and Demand in *Lactococcus cremoris*

Luis A. Salinas-Te, *VU Amsterdam*
Oral

For most organisms, glycolysis serves as the major catabolic pathway for the conversion of sugars into cell biomass precursors, bioproducts, and energy equivalents. Although the stoichiometry of glycolysis is conserved across species, its control and regulation are not, complicating the understanding of the general principles underlying glycolysis operation. In *Lactococcus cremoris*, the control of glycolytic flux and its correlation with bioproduct formation remains a key question. Experimental studies suggest that the control of glycolytic flux is distributed among glycolytic enzymes but can shift to energy demand systems under varying nutrient conditions. High glycolytic flux is associated with lactate production via homolactic fermentation. Conversely, limited glucose or the presence of a "slow" sugar leads to mixed acid fermentation, producing formate, acetate, and ethanol. Since enzyme concentrations hardly vary in the chemostat when the switch occurs, metabolic regulation is believed to underlie the shift. However, a comprehensive and unifying explanation of the switch, and how glycolysis is regulated in this bacterium, remains elusive despite numerous kinetic models in the literature. We revisited these models and designed a kinetic model of glycolysis with a focus on growth-associated ATP supply and demand, phosphate homeostasis, and enzyme kinetics that capture the most important regulatory mechanisms known from the literature. To facilitate the analysis of our glycolysis model, we simplified the model dynamics into two functional blocks: the supply (glycolysis) and the demand (anabolism and maintenance) for ATP. Our model effectively replicates the metabolic shift in *Lactococcus cremoris* and explains various experimental results related to the control and regulation of glycolytic flux and its branches. Our findings indicate that the metabolic shift is primarily driven by allosteric enzyme regulation in the mixed acid branch and influenced by factors such as sugar quality, anabolic and catabolic limitations, and protein overexpression. Furthermore, we have integrated previous suggestions regarding the control of glycolytic flux into a unified model.

GEMCAT: A novel algorithm for predicting metabolite-level changes from gene expression via centrality-based integrative pathway analysis

Suraj Sharma, *University of Bergen*
Poster, P2-73

Understanding how gene expression changes influence cellular metabolism remains a central challenge in systems biology. We introduce GEMCAT (Gene Expression-based Metabolite Centrality Analysis Tool), a novel algorithm that predicts qualitative metabolite changes by integrating transcriptomic or proteomic data with genome-scale metabolic reconstructions. Unlike traditional constraint-based methods, GEMCAT doesn't require artificial constraints such as biomass and energy objectives. It uses a graph-theoretical framework based on directed reaction-metabolite networks, with enzyme abundances inferred from gene-protein-reaction rules. By analysing changes in PageRank centrality, GEMCAT infers directional changes in metabolite centrality, serving as a proxy for concentration changes, without requiring flux optimisations or training data. We demonstrated GEMCAT's predictive capacity across diverse biological systems, including an NAD transporter knockout in HEK293 cells, training-induced adaptations in rat muscle, and proteomic profiles from inflammatory bowel disease patients. GEMCAT accurately predicted metabolite changes (up to 79% accuracy). The method also introduces centrality control coefficients, enabling attribution of metabolite changes to specific genes. GEMCAT is available as an open-source Python package, offering a robust and generalisable framework for integrating omics data into metabolic network analysis. This approach supports biomarker discovery, disease mechanism studies, and systems-level insights into metabolic adaptation.

A disease agnostic pipeline for genome-scale metabolic modeling application in sample-specific drug combination ranking

Rui Tavares, *University of Latvia*
Poster, P2-71

According to the World Health Organisation (WHO), Noncommunicable diseases (NCDs) killed over 43 million people in 2021, around 23 times the population of Latvia indicating acute need for solutions. The current trend in drug development is that it becomes more

expensive and the success rate in new drug development is decreasing. At the same time, there are new opportunities: re-purposing and combination of already registered drugs. The challenge for medical doctors is to predict systemic effects of new therapies. At the same time the growing affordability of genome-scale data (omics) and genome-scale mathematical modelling of metabolic processes in humans allows for an opportunity to utilize mathematical analysis and computational power to assess the effects of drugs and their combinations at tissue level, allowing for a more refined precision medicine approach. Genome-scale modelling with integration of sample-specific omics data enables the modelling of side effects in a given network, such as human metabolism which has more than 10 000 reactions and 10 000 metabolites. The suggestions of mathematical models can serve as a valuable decision support system for medical doctors, especially if a treatment of tissue samples *in vitro* is possible. That is especially true for combination therapies where more than one drug is applied: the re-purposing options and the huge number of possible combinations make computational tools and artificial intelligence irreplaceable. Use of drug databases in combination with artificial intelligence in search for the most promising drug combinations can help deal with the combinatorial explosion problem. There are about 166 million combinations when 1000 drugs are applied in combinations of up to 3. The project aims to develop a disease agnostic genome-scale metabolic modelling computational pipeline for drug combination generation, evaluation and ranking for therapeutic purposes depending on sample-specific omics data, providing a valuable decision support system for medical doctors. Application of the pipeline will result in a complex assessment of metabolic side-effects of drug combination application and ranking of combinations according to multi-component ranking criteria that takes into account parameters like number of drugs in combination, therapeutic effect assessment, side-effect assessment, costs and other relevant aspects. First, a generic model of human metabolism will be obtained and checked/corrected to ensure that the key pathways affecting disease are functioning properly, allowing for the integration of omics data (proteomics/transcriptomics). Following this, a simulation process will be developed in order to identify key aspects of the disease pathway that could be exploited for treatment. Depending on disease, key criteria will be assessed for appealing targets (for instance, in cancer the reduction of cell biomass/proliferation and reduction of ATP production would be useful criteria to evaluate). Assessment/scoring models for existing drugs for the disease of choice will be developed, and hypothetical combinations of drugs will be simulated *in silico*, tested on sample specific models, and appealing combinations will be suggested to medical professionals and compared with existing treatments which will lead to improved precision therapy and personalised treatment. The application of artificial intelligence enables extension of model-based combinatorial space screening efficiency facilitating identification of counter-intuitive and non-traditional approaches or drug re-purposing and combining that can not be screened by a human due to the task complexity and huge number of possible solutions.

Thresholding gene expression data for metabolic modeling: An unresolved challenge

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Abstract
Context-specific genome-scale metabolic models (GEMs) require thresholding gene expression data to define active genes, yet no consensus exists on the optimal thresholding strategy. To enable early-stage method selection, we evaluated four representative thresholding methods (Global, LocalT2, LocalGini, and StanDep) at the preprocessing stage, prior to model construction. Using a normalized housekeeping (HK) recall metric that adjusts for core set size, we benchmarked these methods across four RNA-seq datasets and four broadly defined HK gene lists. Results showed highly similar performance across methods, with no approach consistently outperforming others. Higher thresholds led to improved recall but drastically reduced core size, limiting biological relevance. Our findings suggest that normalized HK recall alone may be insufficient to distinguish between thresholding methods, especially given the ambiguity of HK gene definitions. Nonetheless, pre-model evaluation remains essential, and future work should explore more informative and biologically grounded metrics to guide thresholding decisions.
1. Introduction
Context-specific genome-scale metabolic models (GEMs) integrate omics data, particularly RNA-seq, to represent biological variation across cells, tissues or conditions. Incorporating RNA-seq requires converting continuous expression values into binary gene activity. However, this thresholding step lacks a clear standard. Since thresholding directly impacts model content and predictive capacity, there is a need to systematically evaluate which thresholding method performs best before model-construction stage.
2. Approach
To evaluate thresholding methods independently of downstream modeling, we focused on the preprocessing stage and introduced a normalized housekeeping (HK) recall rate as the primary metric. This metric adjusts HK recall by core set size, enabling fairer comparisons. We tested four representative methods (Global, LocalT2, LocalGini, StanDep1–3) across four RNA-seq datasets and over 200 threshold combinations where applicable. To assess robustness, we repeated evaluations using four broadly defined HK gene lists. This design aimed to reduce bias from varying core sizes and enable systematic comparison.
3. Results
We found that those four methods showed highly similar performance with overlapping HK recall rate across all datasets. No method consistently outperformed the others, and overall trends appeared similar across methods. We also observed that increasing LT and UT thresholds consistently led to higher HK recall, but at the cost of drastically shrinking the core set. This trade-off undermines the biological utility of the core, as extremely small cores are unlikely to be meaningful.
4. Discussion
The normalized HK recall revealed little difference between methods, suggesting either the metric's limited sensitivity or genuine method similarity. Given uncertainties around HK gene definitions, relying on this metric alone is insufficient. However, evaluating thresholding methods prior to model construction remains crucial. Alternative, more informative metrics are needed to guide

method selection.

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