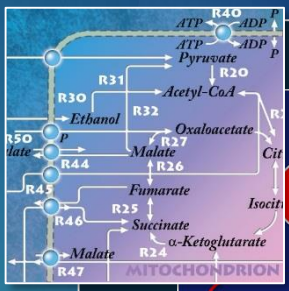
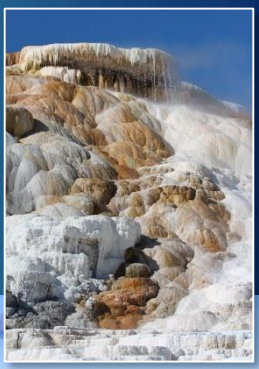


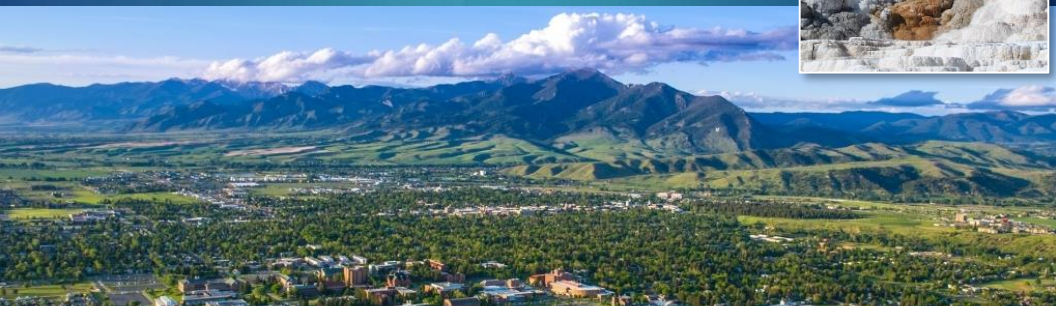
Metabolic Pathway Analysis 2017

Bozeman, Montana USA
24-28 July 2017

In silico & in vitro METABOLISM CONFERENCE: From pathways to cells to communities and tissues

www.chbe.montana.edu/biochemenglab/MPA2017.html





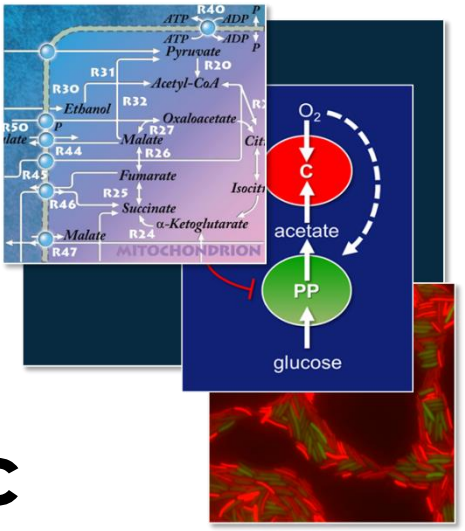
CENTER FOR BIOFILM ENGINEERING

NSF

THERMAL BIOLOGY INSTITUTE

BIOCHEMICAL SOCIETY

processes



Metabolic Pathway Analysis 2017

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Content:

WELCOME TO BOZEMAN!

Metabolic Pathway Analysis 2017 is the sixth conference in a distinguished series of scientific meetings held in **Braga 2015**, **Oxford 2013**, **Chester 2011**, **Leiden 2009** and **Jena 2005**. The MPA 2017 Scientific and Planning Committee is pleased to add **Bozeman 2017** to this prestigious list.

Bozeman, elevation 4,820 feet (1470 m), is located in **Gallatin County**, **Montana** and enjoys on average 300 days of sunshine per year. Bozeman is surrounded by four mountain ranges: the Bridger, Gallatin, Spanish Peaks, and Tobacco Root Ranges and has four alpine ski resorts within 40 miles. Gallatin County has an area of 2,632 mi² (6800 km^2) and a population of approximately 104,000 residents. Gallatin County was the 24th fastest growing US county based on population in 2015 (from 3,235 total US counties) and was ranked in the top 2% of all US counties for general well-being (University of Pennsylvania Well-Being Project).

Bozeman is home of **Montana State University**, a land grant university.

ENROLLMENT
16,440
MONTANA'S LARGEST UNIVERSITY
FALL 2016

FALL 2016 FRESHMEN:
Average HS GPA
3.48
Average ACT score
25.4
Average SAT score
1230

FALL 2016 FRESHMEN:
Montana residents
50%
Nonresident students
50%

TOP 10 STUDENT HOME STATES

Montana	10,122	Idaho	349
Washington	1,158	Oregon	348
California	981	Alaska	270
Colorado	882	Illinois	226
Minnesota	447	Wyoming	193

INTERNATIONAL STUDENTS
721 (FROM 76 COUNTRIES)

ESTABLISHED
1893
BOZEMAN, MT
MONTANA'S LAND-GRANT UNIVERSITY

MSU BOBCATS
NCAA DIV 1
ATHLETICS
BIG SKY CONFERENCE

Estimated Cost of Attendance (per year)

2017 / 2018	Resident	Nonresident
Tuition/Fees	$7,080	$24,070
Room/Board	$9,300	$9,300
Books/Supplies	$1,350	$1,350
Total Estimated Cost	$17,730	$34,720

Scientific and Planning Committee:

Ross P. Carlson, chair, Montana State University, Bozeman, USA
Oliver Ebenhoeh, Heinrich Heine University, Düsseldorf, Germany
David Fell, Oxford Brookes University, United Kingdom
Mark Poolman, Oxford Brookes University, United Kingdom
Herbert Sauro, University of Washington, Seattle, USA
Stefan Schuster, Friedrich Schiller University, Jena, Germany
Hyun-Seob Song, Pacific Northwest National Laboratory, USA
Cong Trinh, University of Tennessee, Knoxville, USA

Sponsors:

The MPA 2017 Scientific and Planning Committee is grateful for the generous support from:

National Science Foundation, CBET

MSU Office of the VPR

MSU Thermal Biology Institute

MSU Center for Biofilm Engineering

***Processes*, MDPI publishing**

Biochemical Society

Conference Accommodations:

MPA 2017 attendees will be staying in Langford Hall dormitory on the Montana State University campus. Please see map.

Meal Services:

All meal services, except for the conference banquet, will be held in the Miller Dining Hall. Please see map. Your name tag is required for meal service.

The **conference banquet** will be held Wednesday 26 July at the **Baxter Hotel** in downtown Bozeman. Please take the free Bozeman Streamline Bus Service from the MSU Student Union to the downtown location or alternatively, attendees are welcome to walk the ~ 1 mile from campus to downtown.

Oral and Poster Presentation Location:

All oral and poster presentations will be held in, or immediately adjacent to **101 Gaines Hall**. Please see map.

Museum of the Rockies: **(https://museumoftherockies.org/)**

Attendees visiting the Museum of the Rockies will find it located a couple blocks south east of campus. Follow 7th Ave south to Kagy St.; the museum will be diagonally across the intersection. There are a replica Lewis and Clark ship, teepee and animal statues visible. Your name tag is required for paid admittance.

Madison River Tubing: **(http://madisonrivertubing.com/)**

Tubing attendees will be picked up in front of Langford Hall. Please meet at 12:15 pm. Please have your waiver filled out when you are picked up. Please see guidelines listed on page 11.

Internet Access:

Please use the MSU-Guest or eduroam connections available throughout campus.

Potentially Useful Contact Information:

Langford Hall front desk, 406-994-3291

Montana State University Ask Us Desk, 406-994-INFO

MSU Police, 406-994-2121 (Emergency: 911)

MSU Museum of the Rockies, 406-994-2251

Madison River Tubing, 406-209-8384

Yellowstone International Airport, 406-388-8321

Greater Valley Taxi, 406-587-6303

Local Public Transportation Information:

Streamline Bus Service, free service throughout Bozeman, many routes starting at the MSU Student Union. For schedules, please see: www.streamlinebus.com

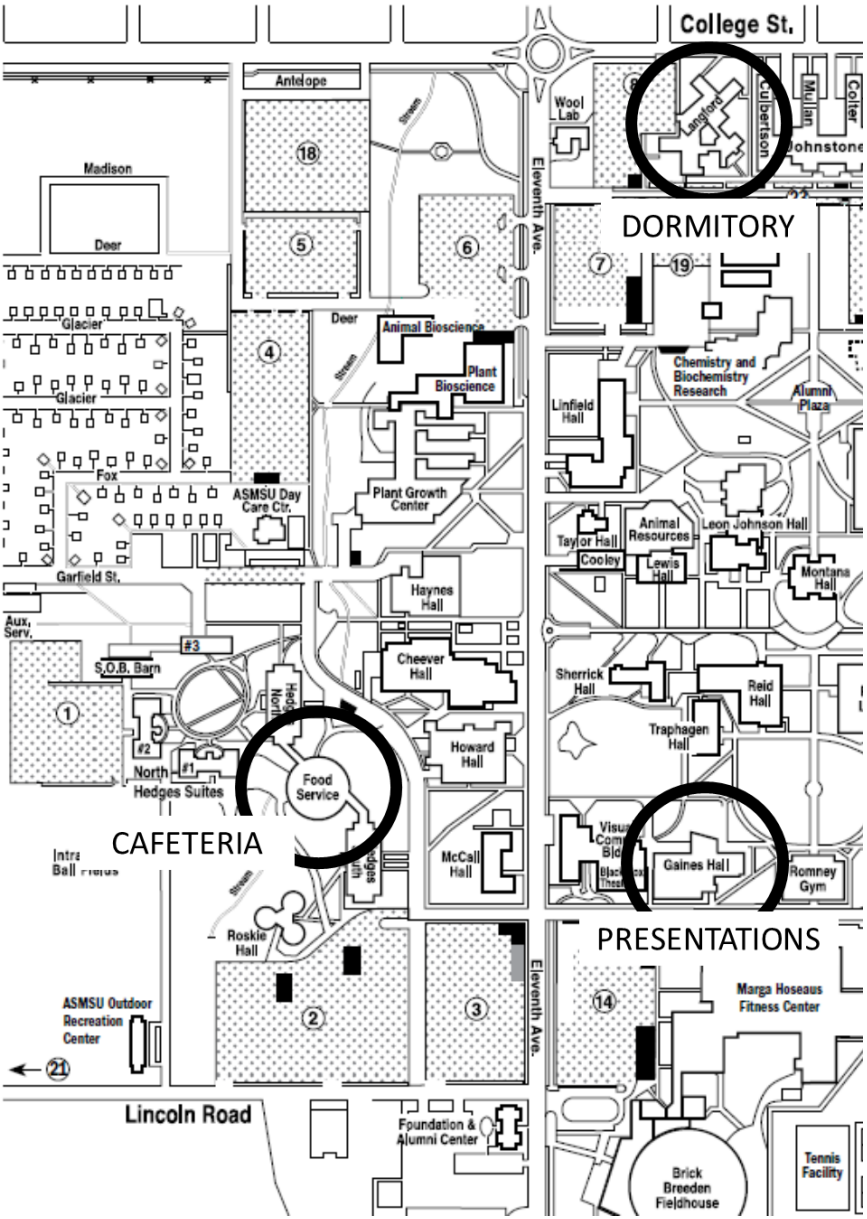
Groceries and Convenience Items:

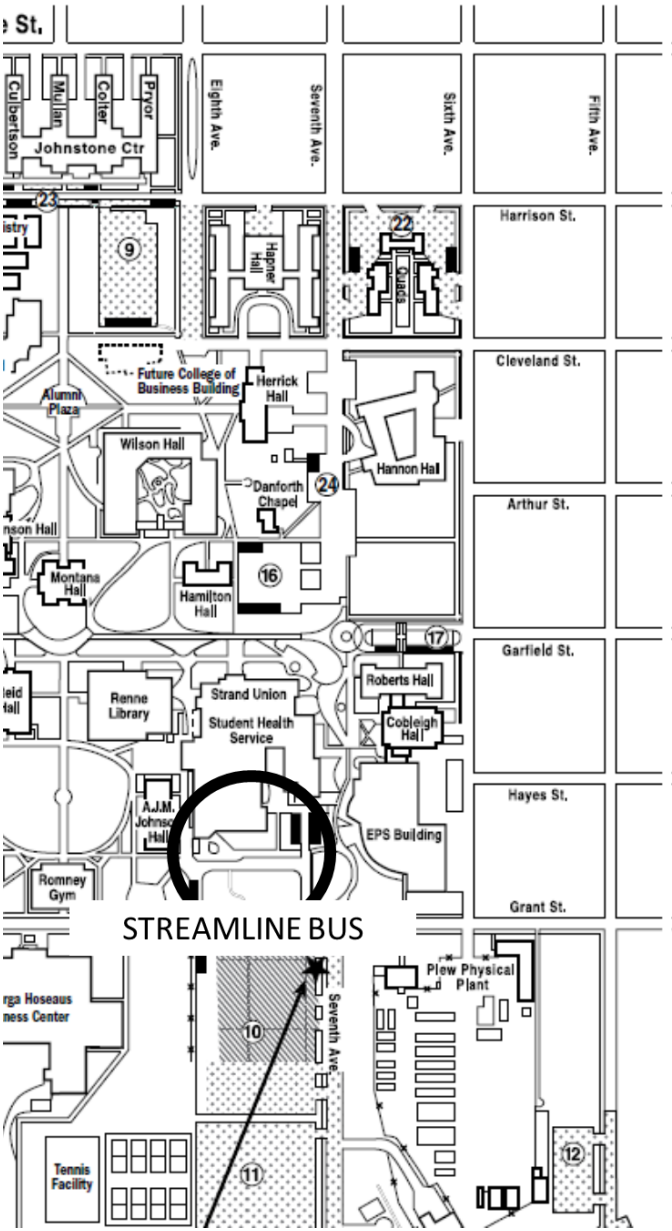
Joe's Parkway (joesparkway.com) is located just north of Langford Hall at 903 W College Street and carries a nice selection of food, alcohol, and toiletries.

Town and Country Foods is a full-sized grocery store located on the southwest side of campus at 1611 S 11th Ave next to Bridger Brewing.

ATM/Cash Machine:

The Strand Student Union Building has four ATMs on the basement level next to the student book store and houses First Interstate Bank, which is a full-service bank, 406-586-0757.


College St.
Antelope
Madison
Deer
Glacier
Glacier
Fox
Garfield St.
Aux. Serv.
S.O.B. Barn
#3
#2
#1
North Hedges Suites
Food Service
CAFETERIA
Intra Ball Fields
Stream
Roskie Hall
ASMSU Outdoor Recreation Center
Lincoln Road
Foundation & Alumni Center
Eleventh Ave.
Wool Lab
Langford
Gulbertson
Mulan
Colter
Johnstone
DORMITORY
Deer
Animal Bioscience
Plant Bioscience
ASMSU Day Care Ctr.
Plant Growth Center
Haynes Hall
Cheever Hall
Howard Hall
McCall Hall
Chemistry and Biochemistry Research
Alumni Plaza
Linfield Hall
Taylor Hall
Animal Resources
Leon Johnson Hall
Cooley
Lewis Hall
Montana Hall
Sherrick Hall
Reid Hall
Traphagen Hall
Gaines Hall
Romney Gym
PRESENTATIONS
Marga Hoseaus Fitness Center
Brick Breeden Fieldhouse
Tennis Facility



http://www.montana.edu/campus-map/

Processes (MDPI publishing) has agreed to host a **special issue focused on methods, protocols, standards and opinions** related to most aspects of metabolic modeling including experimental data generation. Dr. Ross Carlson (rossc@montana.edu) is the special editor for the issue and would be happy to discuss all potential manuscripts with MPA 2017 attendees.

Yellowstone National Park and Madison River Floating Guidelines and Considerations:

Bozeman and Yellowstone National Park are dry, high elevation locations (4,800-7,000 feet/ 1470-2135 m). Temperatures heat and cool quickly; daytime highs in July often reach 95˚F/35˚C (or higher) while the night lows in July can drop to 55˚F/10˚C (or lower). **Please wear comfortable clothes and bring a light coat/shirt for the evening. Please bring a water bottle to stay hydrated.** The UV index is very high due to elevation, low humidity and sunny (usually) skies. **Please bring sun screen, sun glasses and a hat.**

We will walk around some geothermal features in Yellowstone National Park using a system of boardwalks. The longest, optional, boardwalk (Norris Geyser Basin) will be approximately 1-2 miles (1.6-3 km), **please wear comfortable shoes** in addition to comfortable clothes, hat, sun glasses and sun screen. The geothermal features can be very hot, boiling, with extreme pH values (either acidic or basic). **Please use caution around the geothermal features.**

We will hopefully see some wildlife. **Do not approach the wildlife; they are wild animals and can injure or kill you if they interpret your actions as threatening.** Observe wildlife (and some tourists) from a safe distance, 25+ m for herbivores including bison, elk, and deer and 100+ m for carnivores including bears and wolves. For more information: https://www.nps.gov/yell/index.htm. We do not want any MPA 2017 participants to end up in a viral YouTube video!

The Madison River tubing trip requires a swimsuit or clothing that can get wet; we will be floating in the river. The Madison River is also a high elevation, dry, high UV index location. **Please bring drinking water, sunscreen, sunglasses, and a hat.** We will provide a cooler with ice cold adult only beverages for those who are interested.

SUNDAY JULY 23

1:00pm-5:00pm		Registration Desk Open- Langford Hall
6:00pm-8:00pm		Dinner- Miller Dining Hall

MONDAY JULY 24

7:00am		Breakfast Opens- Miller Dining Hall
8:15am-6:00pm		Thermal Biology Institute Field Trip and Workshop, Yellowstone National Park
6:20pm-8:00pm		Dinner- Miller Dining Hall

TUESDAY JULY 25

7:00am		Breakfast Opens- Miller Dining Hall
All Presentations in 101 Gaines Hall		
Analysis and engineering of metabolic solution space		
8:50am-9:00am		Opening Remarks
9:00am-9:40am	T-1	Exploring the combinatorial space of complete pathways to chemicals- Costas Maranas
9:40am-10:05am	T-2	Design of multi-biosynthetic paths- Elmar Heinzle
10:05am-10:30am	T-3	MODCELL: A multiobjective strain design platform for modular cell engineering- Cong Trinh
10:30am-11:00am		Break
11am-11:40am	T-4	Managing uncertainty in metabolic network structure- Jason Papin
11:40am-12:05pm	T-5	Mentos: a thermodynamics approach for estimating metabolites and fluxes- Jeremy Zucker
12:05pm-12:30pm	T-6	Computing EFMs consistent with equilibrium constants- Sabine Peres
12:30pm-1:35pm		Lunch- Miller Dining Hall
Resource allocation and metabolism		
1:35pm-2:15pm	T-7	Systems analysis of intracellular pH vulnerabilities for cancer therapy- Eytan Ruppin
2:15pm-2:40pm	T-8	The hidden costs of enzymatic catalysis- Elad Noor
2:40pm-3:10pm		Break
3:10pm-3:35pm	T-9	Evolution explains the universality and simplicity of microbial metabolism- Daan de Groot
3:35pm-4:00pm	T-10	Multi-constraint approach for the design of lean-proteome strains- Egils Stalidzans
4:00pm-4:25pm	T-11	Enzymes and substrates are balanced at minimal combined mass concentration-

TUESDAY JULY 25, continued

		Martin Lercher
4:25pm-4:40pm		Break
4:40pm-5:10pm		Panel 1
5:10pm-5:20pm		Rapid Fire Poster Presenters
5:20pm-6:20pm		Posters 1
6:20pm-8:00pm		Dinner- Miller Dining Hall

WEDNESDAY JULY 26

7:00am		Breakfast Opens- Miller Dining Hall
All Presentations in 101 Gaines Hall		
Resource allocation and metabolism		
8:40am-8:45am		Opening Remarks
8:45am-9:25am	T-12	Spatiotemporal dynamic of gut microbiota from *in vitro* and *in silico* models- Terry Hwa
9:25am-9:50am	T-13	How a few tolerant individuals can save a population under stress- Christopher Marx
9:50am-10:15am	T-14	Connecting flux balance at the environmental and organismal levels- Isaac Klapper
10:15am-10:45am		Break
10:45am-11:25am	T-15	Exploring the metabolic potential of human gut microbiota- Ines Thiele
11:25am-11:50am	T-16	*In silico* and *in vitro* analysis of resource allocation in biofilm consortia- Ross Carlson
11:50am-12:00pm		Activities Discussion
12:00pm-6:00pm		Lunch and Activities
6:00pm-9:00pm		Banquet- Baxter Hotel Ball Room, Downtown Bozeman

THURSDAY JULY 27

7:00am		Breakfast Opens- Miller Dining Hall
All Presentations in 101 Gaines Hall		
Fundamentals of metabolic structure		
8:55am-9:00am		Opening Remarks
9:00am-9:40am	T-17	Elementary flux vectors: Closing the gap between elementary flux modes and flux balance analysis- Steffen Klamt
9:40am-10:05am	T-18	Extremum principles in metabolic networks- John Barrett
10:05am-10:30am	T-19	Identifying optimal metabolic nodes using minimal cut sets- Naveen Venayak
10:30am-11:00am		Break
Applied metabolic systems analysis		
11:00am-11:40am	T-20	Model-guided engineering of microbial biocatalysts- Jennifer Reed
11:40am-12:05pm	T-21	Optimizing the production of bulk chemical from carbon monoxide using a genome scale model of *Clostridium autoethanogenum*- Rupert Norman
12:05pm-12:30pm	T-22	Metabolic modeling in food biotechnology- Ahmad Zeidan
12:30pm-1:40pm		Lunch- Miller Dining Hall

THURSDAY JULY 27, continued

Intersection of photosynthesis and central metabolism

1:40pm-2:05pm	T-23	Explaining the asymmetric label incorporation during photosynthesis- Oliver Ebenhoeh
2:05pm-2:30pm	T-24	Elementary modes analysis of photorespiration- David Fell
2:30pm-2:55pm	T-25	Flux analysis of the plant MEP pathway- Johann Rohwer
2:55pm-3:25pm		Break

Dynamic flux analysis

3:25pm-3:50pm	T-26	Dynamic modeling and flux analysis- Mario Jolicoeur
3:50pm-4:15pm	T-27	Towards modeling dynamic regulation in ecosystems- Antonella Succurro
4:15pm-4:30pm		Break
4:30pm-5:00pm		Panel 2
5:00pm-5:15pm		Rapid Fire Poster Presenters
5:15pm-6:20pm		Posters 2
6:20pm-8:00pm		Dinner- Miller Dining Hall

FRIDAY JULY 28

7:00am		Breakfast Opens- Miller Dining Hall

All Presentations in 101 Gaines Hall

Ecology, metabolism and resource storage

8:55am-9:00am		Opening remarks
9:00am-9:40am	T-28	Progressing towards a deep integration of chemistry and biology to discover new protein functions, pathways, and ecological principles- Chris Henry
9:40pm-10:00am	T-29	Growth or storage? Exploring metabolic decisionmaking under feast famine conditions using dynamic 13C flux analysis- Leonor Guedes da Silva
10:00am-10:30am	T-30	Modeling cyanobacterial growth- Ralf Steuer
10:30am-11:00am		Break
11:00am-11:25am	T-31	Dynamic metabolic flux analysis of oil biosynthesis in *Camelina sativa* seeds- Teresa Clark
11:25am-11:50am	T-32	Modeling the phosphorus pools of *Chlorella vulgaris*- Dipali Singh

Methods: Advances, theory and demonstrations

11:50am-12:30pm	M-1	The COBRA Toolbox 3.0 and beyond- Ronan Fleming
12:30pm-1:40pm		Lunch- Miller Dining Hall
1:40pm-2:20pm	M-2	Improving automated model reconstruction- Jose Faria
2:20pm-3:00pm	M-3	Unification of genome scale models- Filipe Liu
3:00pm-3:15pm		Break
3:15pm-3:55pm	M-4	Memote- A testing suite for constraints-based metabolic models- Christian Lieven
3:55pm-4:35pm	M-5	MFAPipe: Open source software for parallel labeling, steady state metabolic flux analysis- Mark Borkum

Oral Presentation Abstracts (in order of presentation)

T-1

Title: Exploring the combinatorial space of complete pathways to chemicals

Author: Costas D. Maranas

Primary affiliation: Penn State, University Park, PA, USA

Abstract: Computational pathway design tools provide a systematic way to traverse production routes to high-value chemicals. Important considerations such as reaction rules, network size, the complexity of pathway topology, co-substrate and co-product choices, mass-conservation, cofactor balance, thermodynamic feasibility, chassis selection, yield, and cost are not generally placed within the same decision framework and are largely dealt with in a posteriori fashion. We recently developed two computational tools: (i) optStoic/minFlux and (ii) rePrime/novoStoic. The optStoic/minFlux is a two-stage MILP-based computational procedure wherein the first step, optStoic, explores the maximum extent of converting carbon substrate(s) to desired product(s) through a non-intuitive combination of co-reactants and co-products while maintaining overall thermodynamic feasibility and mass balances [1]. In the subsequent step, the minRxn/minFlux algorithm identifies the minimal network of reactions to perform the overall conversion. This formalism was applied to identify alternate non-oxidative glycolysis and methane fixation pathways [1]. We recently updated the optStoic/minflux procedure to eliminate the occurrence of thermodynamically infeasible subnetworks in the identified pathways. The updated algorithm was employed to prospect for over 37,000 pathways that are capable of converting glucose to pyruvate while generating pre-determined numbers of ATP. These pathways were filtered based on their thermodynamic feasibility under physiological metabolites concentration bounds and ranked based on the minimal protein cost required to operate them. Pareto analysis of these glycolytic pathways revealed interesting tradeoffs between pathway energy efficiency and protein cost. In particular, the canonical ED and EMP pathways were found to be optimized through natural evolution for energy efficiency. Improved synthetic pathways with lower protein cost were also identified. To further expand our pathway designing capabilities beyond the known repertoire of enzymatic reactions, we incorporate hypothetical reactions predicted using reaction rules. Reaction rules expand our solution space and allow us to explore enzymatic capabilities which are yet to be identified (i.e., due to substrate promiscuity) or those that could be designed through protein engineering. The computational procedure rePrime/novoStoic we present here designs routes that extend beyond the currently known bioconversion space while simultaneously considering all aforementioned design criteria. First, we track and codify as rules, all reaction centers using a novel prime factorization based encoding technique (rePrime). The biochemical tenets, encoded within (reAxiom) reaction rules guide the pathway-designing algorithm (novoStoic) to devise mass balanced bio-conversion strategies. rePrime is a recursive procedure and novoStoic is posed as linear mixed-integer optimization formulation. We demonstrate the use of novoStoic in pathway elucidation towards first predicting intermediates of ill-defined degradation pathways for polycylic aromatic hydrocarbons (PAHs), and by designing novel synthetic routes for small molecules such as 1,4 butanediol and non-natural products such as phenylephrine, naproxen, epinine, and N-methyl-aspartate from aromatic precursors.

1. Chowdhury, A. and C.D. Maranas, *Designing overall stoichiometric conversions and intervening metabolic reactions.* Sci Rep, 2015. **5**: p. 16009.

T-2

Title: Design of Multi-Biosynthetic Paths

Authors: Elmar Heinzle, Lisa Katharina Blass, Christian Weyler

Primary affiliation(s): Saarland University, Germany

Abstract: Complex smaller molecules are of major interest as pharmaceutically active compounds or precursors. Their synthesis is mostly observed in organisms whose physiology is largely unknown and that are usually difficult to cultivate. Modern genetic engineering methods are increasingly powerful in transferring biosynthesis gene clusters into robust, well-known heterologous hosts as *E. coli* or yeast strains. We investigate production using permeabilized cells or designed enzyme cocktails to improve selectivity, yield and titer for one single molecular variant of e.g. polyketides or non-ribosomally synthesized peptides.

We developed a method that using available whole-genome information and builds on existing knowledge about metabolic networks. In a first step the metabolic network of a super organism is created using all data available in KEGG about metabolic reactions. The 7405 metabolites are separated in different groups, e.g. potential start metabolites and cofactors. The network data are combined with thermodynamic data about equilibria using eQuilibrator. We modified a method earlier presented by Pey et al. (2011) by introducing path-finding, stoichiometric and other constraints to generate a set of pathway candidates using MILP. These are ranked using a set of criteria, e.g. length of pathway, identified starting molecules, thermodynamic feasibility, number of heterologous enzymes required, number of cofactors requiring regeneration. The ranked list provides overall balances, the thermodynamic profile of the biosynthesis path, lists of potential side reactions. The pathways are visualized using Cytoscape. This output provides a most useful basis for a following expert assessment.

Concluding, we will present some examples, where we successfully applied this method.

T-3

Title: MODCELL: A Multiobjective Strain Design Platform for Modular Cell Engineering

Authors: Cong Trinh, Sergio Garcia

Primary affiliation(s): University of Tennessee, Knoxville

Abstract: Metabolic engineering has enabled the use of microbial cell factories for industrial production of biochemicals. However, developing an optimal strain for synthesis of one product with the conventional strategy is laborious and expensive. To accelerate and reduce the cost of strain engineering, we develop the modular cell (MODCELL) design principle by exploiting the modular organization of metabolic networks and combinatorial possibilities of metabolic modules that enable the synthesis of a large space of biochemicals. Using the multiobjective optimization methods, we develop novel algorithms to implement the MODCELL design for genome-scale metabolic networks. We demonstrate MODCELL to design the biomass-degrading *Clostridium thermocellum* modular cell for combinatorial synthesis of biochemicals, e.g., alcohols and bioesters from lignocellulosic biomass. We envision the MODCELL will provide a useful platform for modular cell engineering.

T-4

Title: Managing uncertainty in metabolic network structures

Author: Jason Papin

Primary affiliation(s): University of Virginia

Abstract: A major barrier preventing more widespread use of genome-scale metabolic network reconstructions (GENREs), particularly to study non-model organisms, is the extensive time required to produce a high-quality GENRE. Many automated approaches have been developed which reduce this time requirement, but automatically-reconstructed draft GENREs still require curation before useful predictions can be made. We present a novel approach to the analysis of GENREs which improves the predictive capabilities of draft GENREs by representing many alternative network structures, all equally consistent with available data, and generating predictions from this ensemble. This ensemble approach is compatible with many reconstruction methods. We refer to this new approach as Ensemble Flux Balance Analysis (EnsembleFBA). We validate EnsembleFBA by predicting growth and gene essentiality in the model organism *Pseudomonas aeruginosa*. We demonstrate how EnsembleFBA can be included in a systems biology workflow by predicting essential genes in six *Streptococcus* species and mapping the essential genes to small molecule ligands from DrugBank. We found that some metabolic subsystems contributed disproportionately to the set of predicted essential reactions in a way that was unique to each *Streptococcus* species, leading to species-specific outcomes from small molecule interactions. Through our analyses of *P. aeruginosa* and six *Streptococci*, we show that ensembles increase the quality of predictions without drastically increasing reconstruction time, thus making GENRE approaches more practical for applications which require predictions for many non-model organisms.

T-5

Title: MENTOS: a thermodynamic approach for estimating metabolites and fluxes

Authors: Jeremy Zucker, Neeraj Kumar, Bill Cannon

Primary affiliation(s): Pacific Northwest National Laboratory

Abstract: Constraint-based methods such as flux balance analysis (FBA) have been successfully used to predict the steady-state metabolic fluxes that maximize the growth rate, but FBA provides no information about the metabolite concentrations. Genome-scale thermodynamic-based methods have been developed that constrain the reaction direction of metabolic fluxes using measured metabolite concentrations. We presen a new thermodynamic optimization method that can be used to predict both the metabolite concentrations and the fluxes that extract ener from the environment as quickly and efficiently as possible, given the constraints of the network and a suitable set of boundary conditions. The method can also be used to estimate the concentrations of unmeasured internal metabolites, given a set of measured internal metabolites.

T-6

Title: Computing EFMs consistent with equilibrium constants

Authors: Sabine Peres, P. Dague, M. Jolicoeur, S. Schuster

Primary affiliation(s): University Paris-Sud, INRA, University of Jena

Abstract: The notion of elementary flux mode (EFM) is a key concept in the analysis of metabolic networks from a pathway-oriented perspective. The set of EFMs represents the potential pathways in a metabolic network but the biologically feasible pathways are limited by various biological constraints: thermodynamic constraints, kinetics and regulations. We present a method for computing thermodynamically feasible elementary flux modes (tEFMs) using equilibrium constants without need of internal metabolite concentrations. The method is compared with the method based on a binary distinction between reversible and irreversible reactions. When all reactions are reversible, adding the constraints based on equilibrium constants reduces the number of elementary flux modes (EFMs) by a factor of two. Declaring in advance some reactions as irreversible, based on reliable biochemical expertise, can in general reduce the number of EFMs by a greater factor. But, even in this case, computing tEFMs can rule out some EFMs which are biochemically irrelevant. We applied our method to a published model described with binary distinction: the central carbon metabolism of Chinese hamster ovary cells. The suppression of the EFMs that are not consistent with the equilibrium constants appears to be biologically relevant.

T-7

Title: Systems Analysis of Intracellular pH Vulnerabilities for Cancer Therapy

Authors: Erez Persi[1, *, #], Miquel Duran-Frigola[2, *], Mehdi Damaghi[3, *], William R. Roush[4], Patrick Aloy[2, 5], John L. Cleveland[6], Robert J. Gillies[3], Eytan Ruppin[1, #]

Primary affiliation(s):

1 Center for Bioinformatics and Computational Biology, Institute of Advanced Computer Studies, Department of Computer Science, University of Maryland College Park, Maryland 20742, USA
2 Joint IRB-BSC-CRG Program in Computational Biology, Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Catalonia, Spain
3 Department of Cancer Imaging and Metabolism, Moffitt Cancer Center and Research Institute, Tampa, Florida 33612, USA
4 Department of Chemistry, The Scripps Research Institute, 110 Scripps Way, Jupiter, USA
5 Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain
6 Department of Tumor Biology, Moffitt Cancer Center & Research Institute, Tampa, Florida 33612, USA

Abstract: A reverse pH gradient is a hallmark of cancer metabolism, where tumor cells acidify the extracellular milieu yet alkalinize their cytoplasm. While consequences of extracellular acidosis are known, the roles of intracellular alkalization are poorly understood. By reconstructing and integrating enzymatic pH-dependent activity profiles into cell-specific genome-scale metabolic models, we developed a computational methodology that defines how intracellular pH (pH_i) modulates metabolism. The model reveals that alkaline pH_i maximizes proliferation, glycolysis and hypoxia adaptation, whereas acidic pH_i disables metabolic adaptations and compromises tumor cell growth and survival. The modeling also predicted vulnerable metabolic targets, whose experimental knockdown compromised breast cancer cell growth and survival in a selective and pH_i dependent manner. This systems analysis establishes the essential roles of alkaline pH_i in cancer metabolism and provides a framework for exploring pH_i roles across biology.

T-8

Title: The Hidden costs of enzymatic catalysis

Authors: Elad Noor, Meike Wortel, Wolfram Liebermeister

Primary affiliation(s): ETH Zurich

Abstract: The existence of a trade-off between the biomass yield and growth rate of cells has been used to explain aerobic fermentation in cancer cells (Warburg effect), yeast cells (Crabtree effect) and in bacteria such as *E. coli.* This trade-off relies on the assumption that even though fermentation pathways produce 5-10 times less ATP per glucose, respiration requires so much more resources and is therefore inefficient when carbon is not limiting. Is this trade-off a universal constraint imposed by thermodynamics, or a coincidental feature of the specific enzyme kinetic parameters that evolved in these organisms? To answer this question we developed a new method called Flux-balance Enzyme Cost Minimization (fECM) to model the costs of both respiration and fermentation (along with ~1000 other flux combinations called elementary flux modes). We find that the trade-off in *E. coli* is not universal and depends strongly on the availability of oxygen. This framework successfully predicts in vivo enzyme concentrations, and has applications in metabolic engineering where similar candidate pathways can be compared not just by their yields, but also by their costs.

T-9

Title: Evolution explains the universality and simplicity of microbial metabolism

Authors: Daan de Groot, Systems Bioinformatics, Vrije Universiteit, The Netherlands

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Abstract: Many evolutionary distant microbial species show highly similar metabolic behaviour, such as overflow metabolism, diauxic growth and catabolite repression, and mixed substrate usage. Overflow metabolism is also shown by autonomously proliferating higher eukaryotic cells, such as cancer cells (known as the Warburg effect). This raises the question whether and how natural selection drives cells towards those common metabolic strategies. We identify an evolutionary extremum principle that determines metabolic behaviour under growth-promoting conditions: fitness maximisation requires minimisation of metabolic complexity. The principle is a mathematical consequence of maximising metabolic fluxes under (enzymatic) constraints. We prove that the number of active constraints determines the number of active minimal metabolic pathways (Elementary Flux Modes) that carry flux in the optimal solution. These constraints are low in number and universal across species, which explains why different species display common fitness effects of protein expression and similar metabolic behaviour. The theory is used to derive three necessary conditions for a micro-organism to show a mixed strategy. The fundamental principle is presented as a (graphical) mathematical framework that provides a deeper, unifying understanding of (mixed) metabolic strategies. We use it to re-interpret experimental results on overflow metabolism and the co-consumption of substrates, demonstrating that the theory has important operational use in the interpretation and design of experiments that can identify the most likely active constraints.

T-10

Title: Multi-constraint approach for the design of lean-proteome strains

Authors: Egils Stalidzans, I. Morell, A. Seiman, A. Pentjuss, R. Vilu

Primary affiliation(s): Latvia University of Agriculture

Abstract: Modeling aproach is used in metabolic engineering to predict organism behavior after implementation of designed changes. Each model of biological system is a simplification of reality taking into account just part of real life constraints. The available modeling approaches mostly concentrate on metabolism and mass conservation issues leaving out of scope some other cellular processes and parameters that can be matematically calculated or estimated. In case of protein as a target product it becomes important to take into account the potential of engineered organism after removal of some groups of proteins (lean-proteome strains) that are not essential in particular biotechological process.

The proposed SILICON modeling platform integrates constraint based modelling approach for genome scale model and Single Cell Model, where thermodynamic, kinetic constraints, measured input-output fluxes and media composition, proteomics data, and central metabolic network data is taken into account along with cell geometry, cell cycle, necessary trancriptional and translational molecular resources and energetic costs and other parameters. Single Cell Model calculated flux distributions (about five hundred reactions) are integrated in genome scale modeling part and shows possible metabolic flux pattern in a genome scale model. Increased scope of biological and biochemical constraints enables better assessment of design feasibility, limiting factors and other peculiarities of organism behavior under conditions of increased target protein production.

T-11

Title: Enzymes and Substrates Are Balanced at Minimal Combined Mass Concentration

Authors: Martin Lercher, Hugo Dourado, Veronica Maurino

Primary affiliation(s): Heinrich Heine University

Abstract: A fundamental problem in biology is how cells organize their resource investment. Cellular metabolism, for example, typically involves hundreds of enzymes and metabolites, but it is unclear according to which principles their concentrations are set. Reasoning that natural selection will drive cells towards achieving a given physiological state at minimal cost, we derive a general equation that predicts the concentration of a metabolite from the concentration of the most abundant and costly enzyme consuming it. Simulations of cellular growth as well as experimental data demonstrate that costs are approximately proportional to molecular masses. For effectively irreversible reactions, the cell maximizes its metabolic efficiency by investing equally into substrate and unbound enzyme molecules. Without fitting any free parameters, the resulting model predicts in vivo substrate concentrations from enzyme concentrations and substrate affinities with high accuracy across data from *E. coli* and diverse eukaryotes (R^2=0.79, geometric mean fold-error 1.74). The corresponding organizing principle – the minimization of the summed mass concentrations of solutes – may facilitate reducing the complexity of kinetic models and will contribute to the design of more efficient synthetic cellular systems.

T-12

Title: Spatiotemporal dynamics of gut microbiota from *in vitro* and *in silico* models

Author: Terrance Hwa

Primary affiliation(s): University of California, San Diego

Abstract: The human gut harbors a dynamic microbial community whose composition bears great importance for the health of the host. Here, we investigate how colonic physiology impacts bacterial growth behaviors, which ultimately dictate the gut microbiota composition. Combining measurements of bacterial growth physiology with analysis of published data on human physiology into a quantitative modeling framework, we show how hydrodynamic forces in the colon, in concert with other physiological factors, determine the abundances of the major bacterial phyla in the gut. Our model quantitatively explains the observed variation of microbiota composition among healthy adults, and predicts colonic water absorption (manifested as stool consistency) and nutrient intake to be two key factors determining this composition. The model further reveals that both factors, which have been identified in recent correlative studies, exert their effects through the same mechanism: changes in colonic pH that differentially affect the growth of different bacteria. Our findings show that a predictive and mechanistic understanding of microbial ecology in the human gut is possible, and offer the hope for the rational design of intervention strategies to actively control the microbiota.

T-13

Title: How a few tolerant individuals can save a population under stress: a model of growth, death, and phenotype switching during formaldehyde exposure

Authors: Jessica Lee, Siavash Riazi, Christopher H. Remien, Christopher J. Marx

Primary affiliation(s): University of Idaho

Abstract: Apparent lags in population behavior occasionally have been revealed to actually be caused by population heterogenetiy. Rare "persister" cells are one such example, where a small minority of non-growing cells can tolerate lethal doses of antibiotics. These dynamics are well-modeled as two discrete populations that phenotypically switch states and have different growth and death characteristics. We have recently discovered the critical role of phenotypic heterogeneity for survival of *Methylobacterium extorquens* to threshold levels of formaldehyde. Although formaldehyde is an intermediate of methanol metabolism in these organisms, concentrations above 2 mM lead to loss of viability. We have found that there are is a wide, continuous distribution of cellular tolerance to formaldehyde, such that an ever-smaller fraction of cells can survive and grow at ever-higher concentrations. We have developed a novel mathematical model of tolerance using a PDE to capture this continuum of tolerance levels, such that state-switching is best expressed as an advection-diffusion process. Our growth/death/switching model captures the critical features of the system, such as the rate and formaldehyde tolerance of switching, that are nearly impossible to measure experimentally; and to predict the conditions under which a minority of tolerant cells can rescue the whole population through removal of a communal stressor.

T-14

Title: Connecting Flux Balance at the Environmental and Organismal Levels

Author: Isaac Klapper

Primary affiliation(s): Temple University

Abstract: Metabolic pathway analysis, and flux balance in particular, has been extensively studied over the past decades at the organismal level. More recently, attention is also being directed to community level balances and, as a natural extension, to the larger scale environment in which that community functions. Such extensions are warranted as flux at the organismal level is ultimately subject to constraint by flux and transport at the environmental level. We discuss mathematical (space and time multiscale issues) and physical/chemical (transport and energetic issues) aspects that arise in the context of specific examples of phototrophic and medically-related microbial communties. A key issue is how to efficiently connect organismal level processes (e.g. metabolic fluxes) with environmental level processes (e.g. diffusive transport) while respecting differences in spatial and temporal scales.

T-15

Title: Exploring the metabolic potential of human gut microbiota

Author: Ines Thiele

Primary affiliation(s): Luxembourg Centre for Systems Biomedicine, University of Luxembourg, http://thielelab.eu

Abstract: Computational modeling of microbial metabolism has gained increasing attention for phenotypic characterization. Such modeling is achieved by assembling in a bottom-up manner a high-fidelity computational representation of a microbe's metabolic network based on genomic, biochemical, and physiological data. Various computational tools exist to characterize its phenotypic properties (e.g., amino acid or vitamin production capabilities, potential carbon and energy sources). Until recently, only few refined metabolic models of human gut microbes had been published. We have developed a semi-automated pipeline for the assembly of high-fidelity microbial metabolic networks and applied this pipeline to generate a collection of more than 770 gut microbial metabolic networks. We then phenotypically characterized these microbes *in silico* and validated predictions obtained for two microbes *in vitro*. I will show that we can now combine these metabolic networks, e.g., based on metagenomic data, to predict emergent metabolic capabilities of the microbial community and their potential effect on the human host. As such, computational modeling can expand and accelerate the insight gained from metagenomic studies in health and disease.

T-16

Title: *in silico* and *in vitro* analysis of resource allocation in biofilm consortia

Authors: Ross Carlson, Mathew Fields, Tomas Gedeon, Luke Hanley, Michael Henson, Jeffrey Heys

Primary affiliation(s): MSU Bozeman, University of Illinois, Chicago, University of Massachusetts, Amherst

Abstract: Multispecies biofilms are ubiquitous in medical, environmental, and engineered microbial systems. In fact, most naturally occurring microorganisms exist in biofilm consortia. These complicated, self-assembling communities compete for limited resources via interactions between cells and their environment. While foundational to the majority of microbial life, the basic design principles including resource allocation strategies of consortia biofilms are poorly understood. Design principles for community resource allocation have been extracted from three experimental systems including a medical chronic wound consortium, an environmental extremophile consortium and a synthetic, engineered bacterial consortium. The three systems are all being analyzed through a combination of in silico and in vitro studies. Predictive multiscale modeling frameworks including flux balance analysis-based and agent-based models are generating quantitatively accurate predictions of biofilm dynamics, species distributions and responses to perturbations. The in silico research is complemented with in vitro studies including spatially resolved biofilm measurements including dissolved oxygen fluxes and species distributions to quantify the physiologies and resource budgets of consortia members. These three distinct, yet tractable, systems share basic behaviors that can be explained using two powerful ecological theories, namely the resource ratio theory and the maximum power principle. The shared system properties including improved stress tolerance, metabolite cross feeding and enhanced productivity are important design criteria for the rational control of medical, environmental and applied biosystems.

T-17

Title: Elementary Flux Vectors: Closing the Gap between Elementary Flux Modes and Flux Balance Analysis in Metabolic Networks

Authors: Steffen Klamt, Regensburger G, Gerstl MP, Jungreuthmayer C, Schuster S, Mahadevan R, Zanghellini J, Müller S.

Primary affiliation(s): Max Planck Institute, Magdeburg, University of Toronto, Jena University

Abstract: Elementary flux modes (EFMs) provide a valuable approach to explore the space of feasible steady-state flux distributions in metabolic networks. However, EFMs cannot account for inhomogeneous constraints such as known lower or upper flux bounds or allocation constraints frequently used in the context of flux balance analysis. These constraints turn the solution space from a flux cone to a flux polyhedron. In order to generalize EFMs from flux cones to flux polyhedra, the concept of elementary flux vectors (EFVs) was proposed by Urbanczik one decade ago [IET Systems Biology (2007) 1:274-279]. So far it has attracted much less attention than EFMs, possibly because the concept seems, at a first glance, to be more involved. Moreover, apart from some specific uses, the whole spectrum of potential applications of EFVs has not been clearly communicated so far.

We here revisit the concept of EFVs, emphasize the close relationships between EFMs and EFVs, and highlight that almost all applications of EFMs are, in an analogous manner, possible with EFVs in flux polyhedra. In fact, certain properties can only be studied with EFVs. We conclude that EFVs provide a powerful and unifying framework for constraint-based modeling of metabolic networks because they close the gap between EFM analyses (operating on the flux cone) and flux balance analysis and related optimization techniques (usually operating on a flux polyhedron due to inhomogeneous constraints). We also show that EFVs can be calculated by well-established algorithms developed for computation of EFMs, which should boost the applicability of the approach.

Reference: Klamt S, Regensburger G, Gerstl MP, Jungreuthmayer C, Schuster S, Mahadevan R, Zanghellini J, Müller S. (2017) From elementary flux modes to elementary flux vectors: Metabolic pathway analysis with arbitrary linear flux constraints. PLoS Comput Biol 13:e1005409.

T-18

Title: Extremum Principles in Metabolic Network

Authors: John Barrett, Friedrich Srienc

Primary affiliation(s): University of Minnesota, Chem. Eng. / BioTechnology Institute

Abstract: Living cells represent open systems that convert inflowing nutrients via their metabolism into products for excretion. Statements of mass conservation, in the form of balanced stoichiometric reactions, can be used to define metabolic models that describe the fundamental pathways of functioning cells. In addition, the operation of these fundamental pathways is constrained by the principles of thermodynamics. To investigate the relationships between reaction kinetics, material conversions and thermodynamic constraints, we have studied the behavior of a continuous stirred tank reactor as a well-defined representation of an open system. Specifically, we are interested in the operational constraints that result from extremum conditions on thermodynamic quantities such as entropy, Gibbs free energy, and their rates of production. The results of this study provide insight into the operation of metabolic reaction networks and their evolution in growing cell populations.

T-19

Title: Identifying optimal metabolic nodes and intervention strategies for dynamically controlled microorganisms using two stage minimal cut sets

Authors: Naveen Venayak, Axel von Kamp, Steffen Klamt, Radhakrishnan Mahadevan

Primary affiliation(s): University of Toronto, Max Planck Institute, Magdeburg

Abstract: Microorganisms are capable of producing a large variety of valuable chemicals. Due to stoichiometric constraints, metabolic optimization of these cell factories for chemical production can come at the expense of native functionality, such as cell growth. This trade-off is of immense importance due to its significant impact on overall productivity. To overcome such limitations, there has been recent interest in implementing sensor-actuator circuits to dynamically control metabolism, and gain temporal control of the microbial phenotype. These systems can be used to implement a two-stage fermentation, where the first stage is dedicated to cell growth and the second stage to production. Here, we present the first algorithm to determine optimal interventions to dynamically control metabolism for chemical production, by eliminating unwanted (low yield) phenotypes. This algorithm is based on the minimal cut set (MCS) algorithm and searches for a suitable minimal combination of static and dynamic (valve) interventions. The static interventions allow for a high growth rate (first stage), while additionally switching off the valves in the production stage enables high-yield production. We exploit the computational efficiency of this algorithm to determine metabolic valves which can be applied to a broad spectrum of products, leading to targets for the creation of platform strains. Furthermore, we explore the efficacy of controlling the phenotype based on oxygen availability, which has historically been a preferred method to implement two-stage fermentation. This algorithm presents for the first time a direct and efficient route to determine optimal interventions for dynamically controlled microbes.

T-20

Title: Model-Guided Engineering of Microbial Biocatalysts

Author: Jennifer L. Reed

Primary affiliation(s): University of Wisconsin, Madison

Abstract: Microbes have been engineered to produce a variety of chemicals, including biofuels, commodity chemicals, specialty chemicals, and therapeutics. Chemical production can be enhanced by connecting synthesis pathways to host metabolism, re-wiring regulatory networks, improving precursor production, and optimizing gene expression. A number of computational systems biology approaches have been developed to facilitate metabolic engineering efforts by suggesting which combination of genetic changes would improve chemical production. Network analysis methods can be used to identify paths from inexpensive substrates (e.g., sugars) to high-value chemical products and to identify central metabolic precursors that can be converted into a variety of chemical products. Genome-scale metabolic models can be used to predict how gene deletions, gene additions, and gene expression changes would impact chemical product yields, growth rates, and/or productivities. Additionally, machine learning and active learning algorithms can be used to optimize gene expression constructs to efficiently convert metabolic precursors into desired products. Case studies will be presented that show how computational tools can guide development of strains with enhanced sugar utilization, precursor production, and chemical production. Together this work illustrates how integrating computational and experimental efforts can lead to the rapid development of microbial biocatalysts.

T-21

Title: Optimizing the production of bulk chemicals from carbon monoxide using a genome-scale model of *Clostridium autoethanogenum*

Authors: Rupert Norman, Millat T, Schatschneider S, Henstra AM, Hartman HB, Poolman MG, Fell DA, Winzer K, Minton NP, Hodgman C

Primary affiliation(s): The University of Nottingham, Oxford Brookes University

Abstact: Recent international directives promoting the reduced consumption of fossil fuels have warranted methods for effective carbon recycling. Subsequently, *Clostridium autoethanogenum* has attracted academic and industrial interest due to its ability to convert syngas components (CO, CO_2 & H_2) into valuable platform chemicals, including ethanol and 2,3-butanediol - a jet fuel additive. Developing the metabolic conversions catalysed by *C. autoethanogenum* into an efficient bioprocess requires the accurate prediction of optimal metabolic steady states, which in turn necessitates the construction of a genome-scale model (GSM).

We have successfully constructed a predictive model, suitable for the integration of omics data sets and prediction of gene knock-out targets, consisting of 795 reactions and 786 metabolites. Our model-simulated growth yields agree well with experimentally observed specific growth rates, while elementary modes analysis (EMA) confirms the availability of metabolic routes for acetate, ethanol, lactate and butanediol production. Elevated ethanol production is predicted to result from a reduction in pH levels. Similarly, we found that the switch from acetate to ethanol production occurs with increasing CO uptake rates under non-carbon limited conditions, finally leading to lactate production as a consequence of electron stress. Our results are consistent with trends observed in continuous cultures.

Our interdisciplinary approach for the construction, analysis and application of a genome-scale model provides insight into biological and biochemical principles which govern experimentally observed metabolic behaviour. Our results offer a rationale to aid the optimization of commodity chemicals from waste gases on an industrial scale.

T-22

Title: Metabolic modeling in food biotechnology

Authors: Ahmad Zeidan, Ana Rute Neves

Primary affiliation(s): Chr. Hansen A/S Denmark

Abstract: Genome-scale metabolic network reconstruction and constraint-based modeling (CBM) are increasingly important tools in microbial systems biology. The majority of CBM applications are focused on rationally identifying metabolic engineering strategies for strain improvement. In the food industry, however, strict legislations and the negative perception by consumers of genetically modified foods render the use of recombinant DNA technologies inapplicable.

Here, we illustrate the primary non-GMO-based applications of CBM at Chr. Hansen, a global supplier of food cultures and enzymes. First, the use of strain-specific genome-scale models (GEMs) for unraveling the metabolic potential of industrial strains and optimizing their biomass yield will be presented, using the probiotic bacterium *Bifidobacterium animalis* ssp. lactis BB-12® as an example. An extensively curated and validated GEM for the strain was developed and used to identify its essential nutritional requirements, design a chemically defined medium supporting its growth, and gain new insights into its fructose and sulfur metabolism as well as vitamin and amino acid biosynthesis. Secondly, a framework for CBM-based identification of metabolite analogues for rational strain improvement via non-GMO approaches will be described. The framework provides an alternative approach to strain design by searching for metabolite targets, which when 'knocked-out' in presence of their analogues can result in a desirable phenotype following dominant selection or adaptive laboratory evolution. Finally, the use of CBM for the mechanistic understanding of biological processes in microbial communities in food products will be illustrated. Current limitations and challenges for the full exploitation of CBM in food biotechnology will also be discussed.

T-23

Title: Explaining the asymmetric label incorporation during photosynthesis

Author: Oliver Ebenhöh

Primary affiliation(s): Heinrich Heine University Düsseldorf

Abstract: Sixty years ago in 1957, Martin Gibbs discovered that radioactively labelled carbon dioxide is asymmetrically incorporated into sugars during photosynthesis. This observation, later termed 'Photosynthetic Gibbs Effect', was puzzling and appeared counter-intuitive, because RuBisCO, the enzyme fixing carbon dioxide to a five-carbon sugar,releases two identical three-carbon molecules, from which sugarars e symetrically formed. Many different explanations have been proposed to explain the observed asymmetries, and as usual the simplest were also the most plausible. Already in 1964, James Bassham explained the appearance of asymmetries by different pool sizes of intermediates and argued that other reproducible patterns result from a 'quirk' of carbons by reversible reactions catalysed by transketolase. Despite such plausible qualitative arguments, a quantitative explanation of the observed labelling dynamics has never been given.

Here, we propose a simple model of the Calvin-Benson-Bassham cycle, which is based on thermodynamic considerations of the cycle and focusses on the paths of carbon atoms. We demonstrate that the observed patterns of label incorporation are an emergent property of the cycle's dynamics and do not require any further assumptions beyond the cycle's stoichiometry and thermodynamics. The observed patterns are a result of the particular thermodynamic properties, which clearly separate the enzymatic steps into close-to-equilibrium and far-from-equilibrium reactions.

With our model, we can quantify the effect of single enzymatic steps on the label incorporation and thus we provide the first fully quantitative explanation of the Photosynthetic Gibbs Effect six decades after its discovery.

T-24

Title: Elementary modes analysis of photorespiration

Authors: David Fell, Benazir Huma, Mark G Poolman, Sudip Kun

Primary affiliation(s): Oxford Brookes University

Abstract: Photorespiration is the metabolism associated with recovery of Calvin cycle metabolites after Rubisco catalyses the addition of oxygen to ribulose bisphosphate instead of carbon dioxide. Starting from a genome scale metabolic model of C3 plant metabolism, we extracted a smaller submodel of the reactions associated with photorespiration in the chloroplast, peroxisome and mitochondrion, as well as the transport reactions of the associated metabolites between compartments. This model had 28 elementary modes of photorespiratory metabolism. Amongst these is the classic photorespiratory cycle, but there are also previously undescribed pathways that involve photorespiration coupled with mitochondrial metabolism and ATP production, the glutathione-ascorbate (GSH--ASC) cycle and nitrate reduction to ammonia. Thus these modes demonstrate the underlying basis of the metabolic linkages with photorespiration that have been inferred experimentally, but especially in the case of nitrate assimilation, have not previously been satisfactorily explained. The set of reactions common to all the elementary modes shows good agreement with those of the gene products of mutants that have been reported to have a photorespiratory phenotype. Finally, the set of modes provides a demonstration that photorespiration itself has no intrinsic impact on the assimilation quotient (CO_2 fixed per O_2 released), except in those modes associated with concomitant nitrate reduction, contrary to widespread belief.

T-25

Title: Flux analysis of the plant MEP pathway

Authors: Johann Rohwer, Erica Perreca, Bettina Raguschke, Diego González Cabanelas, Jonathan Gershenzon, Louwrance Wright

Primary affiliation(s): Stellenbosch University & MPI for Chemical Ecology

Abstract: The methylerythritol phosphate (MEP) pathway produces the common precursors for all terpenoids in most bacteria and the chloroplasts of higher plants, playing a central role in the synthesis of plant photosynthetic pigments and defence compounds. We present two studies on the regulation of the MEP pathway. First, we investigated in Arabidopsis whether a futile cycle exists in the transport of the intermediate, methylerythritol cyclodiphosphate (MEcDP), between the chloroplast and cytosol. From isotopic labelling data, plastidic and extraplastidic pool sizes of MEcDP were calculated throughout a 10-h daylight period, which enabled the calculation of an MEcDP export rate. Strikingly, the export flux was orders of magnitude smaller (0.4%) than the main MEP pathway flux, and largely irreversible. Significant futile cycling of MEcDP was thus absent.

In a second study, the effect of drought stress on MEP pathway flux was analysed in spruce. The rate of isoprene emission (the pathway product) was measured directly with mass spectrometry. In parallel, the metabolic pool sizes of three MEP pathway intermediates were measured under the same conditions. MEP pathway flux was calculated from label incorporation into these intermediates from $^{13}CO_2$. While the MEP pathway flux decreased significantly under drought stress, the directly measured rate of isoprene emission was largely unaffected. Under normal conditions, MEP pathway flux was 4–5 times higher than the isoprene emission flux, showing that photosynthetic pigments were the major products. Under drought-stressed conditions, this factor reduced to three-fold, suggesting a change in carbon allocation to pathway products.

T-26

Title: Dynamic modelling and flux analysis

Author: Mario Jolicoeur

Primary affiliation(s): École Polytechnique de Montréal

Abstract: The use of dynamic models to describe a cell behaviour is gaining in interest. Besides its capacity to "animate" a biosystem metabolic network, such models also allow following the time-evolution of metabolic fluxes performing a dynamic flux analysis. Although in silico, such simulation tool enables observations that are tedious or impossible to perform experimentally. The starting cost for developing a model with each flux kinetics described relies on acquiring experimental concentration data of extracellular and intracellular volumes, as well as literature data on kinetic parameters, when available. Expressomic data is obviously a plus. From this knowledge, the process of building the model structure can be initiated. Various optimization algorithms can be applied to estimate parameters value, based on a sensitivity analysis on model behaviour. This process is highly instructive while refining model structure testing hypothesis on the network map and flux regulation mechanisms. Starting with a minimal metabolic network can be a wise idea to accelerate the model development process. Therefore, once anchored on an experimental reality, model simulations, which only require initial conditions and kinetic parameters value, will allow questioning the cell metabolic behaviour and thus visualise flux dynamics with time as well as the specific contribution of each regulation mechanisms. Examples on the use of such dynamic models with bacteria, microalgae, plant and animal cells will be shown, for biomedical and bioprocess applications, under fed-batch and perfusion culture modes. Current limitations and future trends will be discussed.

T-27

Title: Towards modeling dynamic regulations in ecosystems

Authors: Antonella Succurro, Oliver Ebenhoeh, Daniel Segrè

Primary affiliation(s): Cologne University, Heinrich Heine University Düsseldorf, Boston University

Abstract: Ecosystems can be defined as different organisms interacting with each other as well as with the environment. The mechanisms regulating the interplay between each unit are still to be understood, but it is known that metabolic interactions play a central role. To capture the ecosystem dynamics in a quantitative and predictive theory is today a compelling and challenging task.

Constraint based methods like Flux Balance Analysis (FBA) are powerful ways to investigate reaction flux distributions of Metabolic Network Models (MNM) at the steady state. By construction, however, information on metabolite concentrations or reaction kinetics is lost, making it impossible to capture the dynamics of ecosystem interactions. Another aspect lost is the action of cofactors (typically micronutrients, like Iron or vitamins), which are known to be often exchanged for organic carbon sources in mutualistic consortia between eukaryotes and bacteria.

To overcome these limitation we developed an integrated modeling framework coupling dynamic FBA (dFBA) with Ordinary Differential Equations (ODEs) systems in order to interface the metabolic steady state (FBA solutions) with biochemical processes and regulatory mechanisms (pure ODEs). In this way we bridge metabolic adjustements with ecosystem regulation mechanisms and can include the effect of micronutrients availability. We present a case study on a small toy network where we introduce a storage system that allows for the accumulation of metabolites and accounts for regulation of the pool level in a way inspired by control theory and two competing networks are simulate

T-28

Title: Progressing Towards a Deep Integration of Chemistry and Biology to Discover New Protein Functions, Pathways, and Ecological Principles

Authors: Christopher Henry, Jose Faria, James Jeffreyes, Janaka Edirisinghe, Pam Weisenhorn, Nidhi Gupta, Sam Seaver, Andrew Hanson, Keith Tyo, Ronald Taylor, Hyun-Seob Song, Hans Bernstein

Primary affiliation(s): Argonne National Laboratory, Pacific Northwest National Laboratory

Abstract: We are currently exploring how metabolic modeling, cheminformatics, comparative genomics, and omics data integration can be combined to discover new biology in individual microbes and microbial communities. We have applied this approach to discover new protein functions, annotate pathways, and predict interactions between species within a microbiome.

Metabolic models lie at the apex of our approach, serving as the abstraction that is used to integrate all other approaches together. As such, the quality of these models is paramount. Thus we recently invested significant effort improving the quality of our model reconstruction pipeline, ensuring that models represent annotated metabolic functions more comprehensively, and ensuring that the solutions selected by gapfilling algorithms do not result in biologically unreasonable behavior.

Next, we explore how cheminformatics may be merged with metabolic models to discover new protein functions. Specifically, by applying chemical rules to predict potential spontaneous chemistry that may take place inside the cell, we identified regions of metabolism where proteins are required to mitigate the effects of spontaneous metabolite damage. This led to the identification and characterization of new damage control enzymes in the metabolic network, resulting in a more comprehensive understanding of metabolism, including extensive spontaneous reactions that are likely taking place. Our broad application of reactions rules representing both spontaneous and enzymatic chemistry has also led to the identification of numerous unknown peaks in metabolomics datasets.

We then combine cheminformatics with comparative genomics and omics data to identify genes responsible for catalyzing a pathway known to occur in a specific organism. While no single component of this approach is sufficient to completely narrow the list of gene candidates, but applying all of these approaches together, we can arrive at a top set of candidate genes with a high level of confidence.

Finally, we apply all of these approaches to study interactions between multiple species within a microbiome, identifying the metabolites exchanged between species, and filling the gaps required to permit the microbiome system to function in the environment where it is observed to grow. These studies reveal key ecological principles that govern the behavior and structure of the species interactions within these systems.

All of these tools and capabilities have been fully integrated and released within the DOE Systems Biology Knowledge. We will discuss how the tools in KBase may now be applied to perform similar studies on new datasets uploaded by external users. We will also point to KBase Narratives that include all described studies in detail.

T-29

Title: Grow or store? Exploring metabolic decision making under feast/famine conditions using dynamic ^{13}C flux analysis

Authors: Leonor Guedes da Silva, Koen Verhagen, Andy Wiranata Wijaya, Robbert Kleerebezem, Mark C.M. van Loosdrecht, Aljoscha S. Wahl

Primary affiliation(s): TU Delft

Abstract: Natural habitats of microorganisms are dynamic environments with non-continuous supply of carbon and energy sources, in which intermediate storage of substrates can increase competitiveness. *Plasticicumulans acidivorans* are polyhydroxybutyrate (PHB) accumulating bacteria enriched from activated sludge using carbon feast-famine cycles as selective pressure. Despite growing slowly, *P. acidivorans* outcompetes other bacteria by quickly taking up acetate and storing it intracellularly as PHB to later use it for growth. As soon as acetate is depleted, these bacteria immediately 'switch' their metabolism from PHB production to consumption entailing a very interesting regulatory challenge as parallel activity could lead to significant losses (futile cycling). While the stoichiometry for both feast and famine phases has been extensively described in literature, the switch regulation is not yet fully understood.

To elucidate the responsible regulatory processes, an enrichment of *P. acidivorans* was studied using targeted intracellular metabolite analysis over time, with emphasis on the feast to famine switch. In combination with extracellular rates, the measured intracellular metabolite pools are used to design a labelling experiment to obtain actual intracellular fluxes (dynamic ^{13}C flux analysis). Here the challenge is to create an isotopically non-stationary state (usually mediated by changing the substrate's isotopic composition) to study the metabolic response in the transition from presence-to-absence of substrate.

In this way, we aim to unravel the responsible regulatory mechanism governing the metabolic switch from storage-to-consumption and use this knowledge not only to understand its ecological relevance, but to also propose novel metabolic strategies for microbial cell factory design.

T-30

Title: Modeling cyanobacterial growth

Authors: Ralf Steuer, A-M Reimers, H Knoop, A Bockmayr

Primary affiliation(s): Humboldt-University Berlin

Abstract: Photoautotrophic growth requires a highly coordinated distribution of cellular resources to different intracellular processes, including the de novo synthesis of proteins, ribosomes, lipids, and other cellular components. In our contribution, we present a computational framework to investigate the optimal allocation of cellular resources during diurnal phototrophic growth using a genome-scale metabolic reconstruction of the cyanobacterium *Synechococcus elongatus* PCC 7942. Specifically, we formulate phototrophic growth as an autocatalytic process and solve the resulting time-dependent resource allocation problem using constraint-based analysis. Based on a narrow and well-defined set of parameters, our approach results in the prediction of growth properties over a full diurnal cycle. The computational model allows us to study the optimality of metabolite partitioning during diurnal growth. The cyclic pattern of glycogen accumulation is an emergent property of the model and has timing characteristics that are in excellent agreement with experimental findings. Our approach provides insight into the time-dependent resource allocation problem of phototrophic diurnal growth and may serve as a general framework to assess the optimality of metabolic strategies that evolved in phototrophic organisms under diurnal conditions.

T-31

Title: Dynamic Metabolic Flux Analysis of Oil Biosynthesis in *Camelina sativa* Seeds

Authors: Teresa J Clark, Mike Pollard, and Yair Shachar-Hill

Primary affiliation(s): Michigan State University

Abstract: Seed oil is of great economic importance for food, animal feed, and industrial applications. Triacylglycerol (TAG), the major seed storage lipid, is composed of a glycerol backbone and fatty acid hydrocarbon chains. Fatty acid modification and addition to glycerol can occur through alternative routes within a metabolic reaction network, but the relative importance of fluxes through these alternative routes is unclear. In *Camelina sativa*, a promising oilseed crop, we delineate these fluxes and demonstrate the existence of two distinct metabolically active pools of phosphatidylcholine (PC), which is a key intermediate in oilseed TAG biosynthesis. The first PC pool is used in *de novo* synthesis of diacylglycerol (DAG), the immediate precursor of TAG. This is evidenced by initial DAG products containing higher levels of sn2 labeling compared to sn1 labeling (~9:1). This pool comprises ~60% of total PC. The second PC pool is involved in acyl editing and its formation is catalyzed by the Rod1 enzyme. This is evidenced by ~40% of total PC containing similar labeling in sn1 and sn2 (~1:1). We present a quantitative flux map for TAG biosynthesis, which will facilitate rational engineering of seed oils by providing a means for predicting the effectiveness of altering the expression of genes associated with this pathway.

T-32

Title: Modelling the phosphorus pools of *Chlorella vulgaris*

Authors: Dipali Singh, Ines Hotopp, Oliver Ebenhöh

Primary affiliation(s): Heinrich Heine University

Abstract: In the interdisciplinary collaborative project “AlgalFertilizer” we investigate the capability of *Chlorella vulgaris* to uptake P from waste water and the molecular mechanisms underlying P uptake and storage in algae. For this, we develop mathematical models to understand the uptake of P and the underlying dynamics of the conversion of P pools in algal cells under different environmental conditions.??

The model developed in our study consists of four phosphate pools : the external inorganic phosphate (Pex) pool which provides the phosphate source, and three internal algal phosphate pools. The external phosphate is taken up as free inorganic phosphate (Ortho-P), polyphosphate (Poly-P) is used as short term storage, and organic phosphate (Organic-P) is essential for the algal growth. In addition, the model also takes into account the influence of light on algal growth through an increasing biomass density and the dependence of growth on the availability of growth machinery (proteins). The model is able to simulate the characteristic lag phage at the beginning of the algal growth, the uptake of external phosphorus into an algal cell and the dynamic distribution between the Poly-P and the Ortho-P pools within the cell. It provides an insight on the underlying dynamics of different P pools and supports the hypothesis that the lag phase results from the 'legacy' of the starvation phase, in which many important proteins for reproduction have been degraded, and therefore the growth machinery needs to be re-established. This also lead to quantitative predictions, that can be experimentally tested in growth experiments.?

M-1

Title: The COBRA Toolbox 3.0 and beyond

Authors: Ronan Fleming, The COBRA Toolbox developers' consortium

Primary affiliation(s): University of Luxembourg

Abstract: The COnstraint-Based Reconstruction and Analysis Toolbox (COBRA) is a comprehensive software suite for quantitative prediction of cellular and multicellular biochemical networks with constraint-based modelling. It implements a comprehensive collection of basic and advanced modelling methods, including reconstruction and model generation as well as biased and unbiased model-driven analysis methods. Since its release in 2011, The COBRA Toolbox 2.0 has been widely used for modelling, analysing and predicting a variety of metabolic phenotypes using genome-scale biochemical networks. Here we present the main developments in The COBRA Toolbox 3.0, including expansion of functionality to cover existing and new modelling methods, implementation of support for input and output of new standards for model sharing formats, implementation of support for an extended suite of optimisation solvers, multi-lingual integration with C, FORTRAN, Julia, Perl and Python code for enhanced functionality in the areas of computational efficiency, high performance computing and integration with omics data. This enhanced functionality now enables the modelling of communities of biochemical networks, from microbial communities to multicellular mammalian systems. In particular, the integration with a new set of novel high numerical precision optimisation solvers enables robust and efficient modelling of multi-scale biochemical networks, such as those obtained from integration of metabolism and macromolecular synthesis. With an increasing number of contributions from developers around the world, the code base is evolving at a fast pace. In order to guarantee consistent, stable, and high quality code, a continuous integration approach has been implemented. A semi-automated continuous integration environment ensure that every change in code, as minor as it may be, is fully tested before being released as part of a stable version. Each contribution is verified automatically, and for each code submission, a comprehensive test suite is run in order to detect bugs and integration errors before release. Consequently, the automated testing environment ensures higher quality code and the release of well tested computer code. All documentation and code released as part of the openCOBRA project on https://github.com/opencobra/cobratoolbox and https://github.com/opencobra/cobratoolbox respectively. We conclude with a description of future plans for The COBRA Toolbox.

M-2

Title: Improving automated model reconstruction

Authors: José Faria, Janaka N. Edirisinghe, Filipe Liu, Christopher S. Henry

Primary affiliation(s): Argonne National Laboratory

Abstract: The Department of Energy Systems Biology Knowledgebase (KBase) is a platform designed to solve the grand challenges of Systems Biology. KBase has implemented bioinformatics tools that allow for multiple workflows including genome annotation, comparative genomics, and metabolic modeling. KBase now also includes a comprehensive database of over 80K reference genomes (approximately 5K complete genomes) from NCBI's RefSeq. We have selected a phylogenetically diverse set of approximately 1000 genomes and built draft genome-scale metabolic models constructed using the ModelSEED pipeline. In constructing these models, we were interested in improving the gene functional role – reaction templates and accuracy of biomass compositions used by ModelSEED when producing a draft model. We have curated the existing ModelSEED templates by both fixing and introducing new gene – reaction mappings. Previous ModelSEED biomass compositions often excluded metabolites that are essential for the viability of many organisms, while simultaneously including some non-essential metabolites. These errors in the biomass compositions lead to errors in the growth conditions and gene knockout phenotypes predicted by the models. To correct these problems, we conducted a sensitivity analysis on our full set of models, using a biomass composition that includes all possible essential biomass precursors. We also improved the ModelSEED gap filling database with new rules to restrict the addition of non-biological significant reactions. We then used this data to improve all of our models, validating our improved models with a diverse set of growth and knockout phenotype data. Our improved models are now available for download, viewing, and comparison in the KBase.

M-3

Title: Unification of Genome Scale Models

Authors: Filipe Liu, J. Xavier, F. Ramalho, M. Rocha, I. Rocha

Primary affiliation(s): University of Minho

Abstract: Genome scale metabolic models (GEMs), along with constraint based analysis techniques, are capable of predicting key metabolic characteristics of organisms, revealing their limitations and strengths.

Up until today, hundreds of curated GEMs have been published. These models provide rich curated information for future reconstructions and studies. However, a common drawback of combining several GEMs is the lack of standardization between them. Recently, genome scale model repositories have been developed, however unable to include most of the published GEMs or performing standardization.

An integration pipeline was thus developed to standardize GEMs, and tested against a set of nearly one hundred published models of prokaryotes. These models contain a high diversity of annotation strategies and were built from early 2000 to the present date.

The pipeline follows a cyclic semi-automated integration process. User interaction is necessary only for profiling and report analysis, which was essential to detect faults found in a few GEMs, allowing an easy curation. Identification of the metabolites is fully automated using several methods, such as taking the annotation found within the model, pattern decomposition of the identifiers, name similarity against compound databases, and user provided input (e.g., curation, supplementary materials). The integration scored an average of 70% integration in the model metabolites.

Standardization of drain reactions is essential to enable compatibility between models, and flux balance analysis was used for validation purposes. A total of 75% of the GEMs shown positive growth for default uptake conditions, in 20% no bounded uptake constraints were detected, while the remaining 5% had no growth in all conditions tested.

M-4

Title: Memote - A testing suite for constraints-based metabolic models

Authors: Christian Lieven, Moritz E. Beber, Nikolaus Sonnenschein

Primary affiliation(s): Novo Nordisk Foundation Center for Biosustainability

Abstract: Constraints-based metabolic models have become fundamental and trusted tools in systems biology. Several layers of biological information are combined in a compact format in order to describe a metabolic model. A richly annotated model is required for its various areas of application and represents a veritable knowledge base about an organism's metabolism. However, coherently describing a complex interlinked system such as metabolism is a challenge in and of itself that is only aggravated by the current lack of cohesive, widely-accepted, testable, and modern standards.

Here, we introduce memote (Metabolic Model Tests {https://github.com/biosustain/memote}), a Python package designed to run a given model through a set of hard and soft tests and generate a report that reflects model integrity. Soft tests focus on aspects that do not influence the performance of the model, such as syntactic conventions whereas hard tests determine whether a model is fully functional.

While memote can be run locally as a stand-alone testing suite, it shows its full potential when combined with web-based version controlling (Github) and continuous integration tools (Travis CI). Every tracked edit of a model automatically triggers the memote test suite, and generates a corresponding report that facilitates factual debate of model changes.

Thus, memote not only allows researchers to more quickly iterate through the design-build-test cycle but also provides the scientific community with a measure of quality that is consistent across setups, as well as an opportunity to interact and collaborate by establishing workflows for publicly hosted and version controlled models.

M-5

Title: MFAPipe: Open source software for parallel labeling, steady state metabolic flux analysis.

Author: Mark Borkum

Primary affiliation(s): Pacific Northwest National Laboratory

Abstract: Modern fluxomics studies are enabled by robust, high-performance software packages that empower their users to validate and verify their hypotheses with the support of both experimentally obtained and computationally simulated, multi-instrumental data. Recent advances [1] in the mathematical formulation of stable isotopic labeling (SIL) models have enabled the development of new scientific capabilities; most pertinently, the new ability to fit model parameters to smooth functions of arbitrary isotopic labeling states of heteronuclear moieties; enabling the new ability to fit model parameters to experimentally obtained data from both low- and high-resolution mass spectrometry (MS) experiments, and both low- and high-dimension nuclear magnetic resonance (NMR) experiments.

In this presentation and corresponding workshop, we motivate and discuss the development of MFAPipe; a new, open source software package for flux balance analysis (FBA) and single- and parallel-labeling, steady state metabolic flux analysis (MFA). MFAPipe has no proprietary dependencies and is distributed under an open source software license that permits both academic and non-academic use in both commercial and non-commercial settings.

Workshop participants will be guided, step-by-step, through the MFAPipe installation process, and will be given the opportunity to perform an MFAPipe-supported fluxomics investigation of the citric acid cycle for a model strain of *E. coli* using different combinations of SIL data from MS and NMR instrumentations.

The prerequisites for this workshop are Git (see https://git-scm.org for more information) and The Haskell Tool Stack (see https://www.haskellstack.org for more information). This workshop is tested on macOS and Unix-like operating systems.

References

1. Borkum, Mark I., et al. "Modeling framework for isotopic labeling of heteronuclear moieties." Journal of Cheminformatics 9.1 (2017): 14.

Poster Presentation Abstracts (in no particular order)

Poster 1

Title: Insights Into Acetogen Metabolism Using A Genome-Scale Metabolic Model

Authors: Noah Mesfin, David Fell, Mark Poolman

Primary affiliation(s): Oxford Brookes University

Abstract: Acetogens are microbes which produce acetate as a fermentation by-product of anaerobic fermentation. They are diverse in their phylogeny but have a metabolic feature in common called the Woods-Ljungdahl Pathway (WLP). WLP confers the ability of fixing atmospheric carbon dioxide into central metabolism in a non-photosynthetic route. Electrons for this process are derived from diverse substrates including molecular hydrogen and carbon monoxide. We report the construction of a genome-scale metabolic model of the model acetogen *Acetobacterium woodii* using a recently sequenced and annotated genome of strain DSM1030. An initial draft model was created using the Pathway/Genome Database from BioCyc, and then manually curated using current literature and bioinformatic databases to fill gaps in the metabolic network and produce an analysis ready model. The model consists of 836 metabolites, 909 reactions and 84 transporters and can simulate growth on substrates reported in the literature. Most substrates produce acetate as a by-product of growth but substrates such as 1,2 propandediol result in nonacetogenic growth with propionate and propanol as the fermentation-products.

Using the model, we can calculate theoretical maximum ATP yields of 136 substrate combinations. We are also using the model to elucidate methanol utilisation pathways, explore vitamin B12 biosynthesis, and introduce heterologous reactions for the production of higher added value products.

Poster 2

Title: Comparative evaluation of atom mapping

Authors: German Andres Preciat Gonzalez, L. Assal, Hulda S. Haraldsdóttir, Ronan M.T. Fleming

Primary affiliation(s): University of Luxembourg

Abstract: The reaction mechanism of each chemical reaction in a metabolic network can be represented as a set of atom mappings, each of which relates an atom in a substrate metabolite to an atom of the same element in a product metabolite. Atom mapping data for metabolic reactions open the door to a growing list of applications [wiechert 2001, #haraldsdottir_identification_2016, #pey_refining_2014, #kotera_computational_2004]. Complete manual acquisition of atom mapping data for a large set of chemical reactions is a laborious process. However, until recently many algorithms exist to predict atom mappings. How do their predictions compare to each other and to manually curated atom mappings? For more than five thousand metabolic reactions we compared the atom mappings predicted by six atom mapping algorithms [#first_stereochemically_2012, #chemaxon_standardizer_2015, #rahman_reaction_2016, #kumar_clca:_2014, #latendresse_accurate_2012, #kraut_algorithm_2013].

We also compared these predictions to those obtained by manual curation of atom mappings for over five hundred reactions distributed amongst all top level enzyme commission number classes. Five of the evaluated algorithms had similarly high prediction accuracy over 91% when compared to manually curated atom mapped reactions. On average, the accuracy of the prediction was highest for reactions catalysed by oxidoreductases and lowest for reactions catalysed by ligases. In addition to prediction accuracy, the algorithms were evaluated on their availability and advanced features such as the ability to identify equivalent atoms and reaction centres, and the option to map hydrogen atoms. In addition to prediction accuracy, we found that availability and advanced features were fundamental to the selection of an atom mapping algorithm.

We selected two different algorithms for the atom mapping of mass balanced Recon 3D reactions due to their high accuracy, ease of availability, and predictions without any unmapped atoms. The Reaction Decoder Tool (RDT) was selected to atom map reaction with implicit hydrogen atoms, while DREAM was chosen to atom map reaction with explicit hydrogen atoms. Using RDT and DREAM atom mapping were obtained for 959 (95%) of the 1,005 biochemical reactions identified so far in mitochondria. For a further 40 (4%) mass imbalanced reactions, CLCA was chosen for atom mapping, leaving a remainder of 6 (0.6%) unmapped reactions with missing chemical structures.

Poster 3

Title: Decomposition of the mitochondrial metabolic network using atom mapped reactions and the left null space of the stoichiometric matrix

Authors: German A. Preciat Gonzalez, Susan Ghaderi, Ronan M.T. Fleming

Primary affiliation(s): University of Luxembourg

Abstract: Genome-scale metabolic network reconstructions have become a relevant tool in modern biology to study the metabolic pathways of biological systems *in silico*. However, a more detailed representation at the underlying level of atom mappings opens the possibility for a broader range of biological, biomedical and biotechnological applications than with stoichiometry alone.

A set of atom mappings represents the mechanism of each chemical reaction in a metabolic network, each of which relates an atom in a substrate metabolite to an atom of the same element in a product metabolite.

From the mitochondrial compartment of latest generic human metabolic reconstruction, Recon 3D, we obtained all the chemical structures of its metabolites (from metabolic databases or manually drawn by consulting the literature). This allowed us to atom map all the internal reactions with the Reaction Decoder Tool algorithm, which was selected after comparing the performance of recently published algorithms. Atom mapped reactions were used to identify a set of conserved moiety vectors that form a sparse non-negative integer basis for the left null space of the stoichiometric matrix and thus, we decomposed the network into a set of graphs, to visualise the path that follows specific sets of moieties facilitating the analysis of this metabolic network using graph theory.

Poster 4

Title: Mathematical model of glucsinolate biosynthesis

Authors: Saraj Sharma, Oliver Ebenhoeh

Primary affiliation(s): Institute for Quantitative and Theoretical Biology, Heinrich Heine University, 40225 Düsseldorf, Germany, Cluster of Excellence on Plant Sciences (CEPLAS)

Abstract: Glucosinolates are sulphur-rich secondary metabolites, found in plants of the *Brassicaceae* family, that upon hydrolysis facilitate defence against plant pathogens. The distinct taste of certain Brassicaceae vegetables (broccoli, cabbage) and condiments (mustard, wasabi) is due to the presence of glucosinolates. For humans, hydrolysis products function as cancer-preventive agents and flavour compounds. To fully exploit the potential of glucosinolates in agriculture and medicine, complete understanding of how plants synthesize glucosinolates is important. A primary difficulty in the analysis of secondary metabolites is the vast diversity of chemical structures. Apparently, developing models in which all possible structures are represented as a single variable is very challenging. Here, we developed a mathematical model of biosynthesis of aliphatic glucosinolates found in Arabidopsis thaliana. Our model exemplifies how biosynthetic rates in the system depend on all other metabolite concentrations, a behaviour originating from broad-range substrate specificity of the metabolic enzymes. Extensive variation is observed in both composition and total accumulation of glucosinolates across different Arabidopsis ecotypes. This could be a result of allelic composition at different biosynthetic loci. We used our model to study the broad-range specificity of the enzymes, associated with one of these loci. Addressing the observed diversity, our model elucidates why and how aliphatic glucosinolates with a particular frequency are produced. Furthermore, by relating the allelic differences to metabolic properties and thus adjusting model parameters, we can reproduce patterns of glucosinolate accumulation from different Arabidopsis ecotypes. Thus, our model provides a framework wherein the link between genotype and phenotype can be investigated.

Poster 5

Title: Engineering and evolving a functional RuMP pathway in place of the serine cycle in *Methylobacterium extorquens* PA1.

Authors: Sergey Stoylar, Dipti D. Nayak, Christopher J. Marx

Primary affiliation(s): University of Idaho

Abstract: The ribulose monophosphate (RuMP) pathway is the most energetically favorable pathway for formaldehyde assimilation, and is markedly more efficient than the serine cycle. We have constructed a strain of *Methylobacterium extorquens* PA1 which lacked hydroxypyruvate reductase from the serine cycle, and instead expressed two genes from *Methylococcus capsulatus* Bath that encode the key enzymes of RuMP pathway. This initial strain, which demonstrated extremely poor growth on methanol, was subsequently evolved in batch culture for 300 generations. Although still well below wild-type levels, growth rate and yield increased dramatically. The genomes of six strains from improved lines were sequenced and the accumulated mutations were analyzed. Based on mutant, gene expression and metabolic analysis and metabolic modeling we have built a conceptual model of improved RuMP-expressing strains, and this has provided the basis for further evolutionary and engineering approaches to improve growth.

Poster 6

Title: Resolving the PFK Paradox in Glycolysis

Author: Herbert Sauro

Primary affiliation(s): University of Washington, Seattle

Abstract: The biochemical networks found in living organisms include a huge variety of control mechanisms at multiple levels of organization. While the mechanistic and molecular details of many of these control mechanisms are understood, their exact role in driving cellular behaviour is not. For example, yeast glycolysis has been studied for almost 80 years but it is only recently that we have come to understand some of the roles of the multitude of feedback and feed-forward controls that exist in this pathway. In this poster, I will apply control theory to resolve one of the paradoxes in metabolic regulation where regulated enzymes such as phosphofructokinase show little control but nevertheless possess important regulatory influence. Control theory will be used to quantify the regulatory importance of PFK and highlight the conceptual difference between control and regulation.

Poster 7

Title: *In silico* and *in vitro* analysis of energy conservation and bifurcating enzymes in *Clostridium thermocellum*

Authors: Zackary Jay, Katherine Chou, Kristopher A. Hunt, PinChing Maness and Ross P. Carlson

Primary affiliation(s): MSU Bozeman, National Renewable Energy Laboratory

Abstract*: Clostridium thermocellum* str. DSM1313 is a fast growing (poly)saccharide fermenter that produces H_2, ethanol, acetate, formate, and lactate as major byproducts, making this organism of interest to consolidated bioprocessing. The metabolic capabilities of this organism have been extensively studied, particularly to understand and optimize the bioconversion of sugars to biomass and/or byproducts. Less is known about the energy conserving pathways, specifically the role enzymatically catalyzed electron bifurcating (BF-) transhydrogenase and BF-hydrogenase reactions play in electron flux and energy generation. The objectives of this study were to, 1) characterize growth, byproduct production, and redox poise of *C. thermocellum* cultured under low or high H_2 partial pressures (pH_2); 2) quantify the thermodynamic limits of reactions catalyzed by enzymes implicated in electron flux; and 3) identify molecular components and design principles which are necessary for enhanced electron-mediated energy conservation. Growth characterization, byproduct production, and redox poise (i.e., [NAD(P)H]/[NAD(P)+]) were determined by culturing *C. thermocellum* on cellobiose and in the presence of either Ar or H_2 headspace (1 bar). Classic thermodynamic modeling of redox reactions associated with electron flow were constrained by *in vivo* measurements to predict catalytic bias and reaction direction under defined pH2 conditions. Stoichiometric metabolic networking, specifically Elementary Flux Mode Analysis (EFMA) and Flux Balance Analysis (FBA), was used to integrate growth data, genomics, and thermodynamic analysis to model energy and byproduct yields under simulated conditions. The results of this study revealed the importance of electron bifurcating reactions in electron-mediated energy conservation of *C. thermocellum* and identified important control points that can be targeted for metabolic engineering and optimization.

Poster 8

Title: EColiCore2: a reference core model

Authors: Oliver Haedicke, Phillip Erdrich, Steffen Klamt

Primary affiliation(s): Max-Planck-Institute Magdeburg

Abstract: Genome-scale metabolic modeling has become an invaluable tool to analyze properties and capabilities of metabolic networks and has been particularly successful for the model organism *Escherichia coli*. The most recent genome-scale reconstruction iJO1366 (Orth et al. (2011)) is widely accepted as the reference *E. coli* network. However, for several applications, smaller metabolic (core) models are needed.

Using the recently introduced NetworkReducer (Erdrich et al. (2015)), we derived a subnetwork, EColiCore2 (ECC2), that preserves predefined phenotypes including optimal growth on different substrates. A major advantage of ECC2 is that it is a strict submodel of its genome-scale parent model by which results from ECC2 can be directly related to iJO1366. All flux distributions in ECC2 are valid solutions in iJO1366 and, likewise, all elementary modes of ECC2 are equally valid in iJO1366.

We also studied the value of the core model for calculating relevant metabolic engineering strategies and demonstrate how reaction knockout sets of ECC2 can be used as a seed and then be extended by further knockouts to a valid strategy for iJO1366. This approach can be used to determine thousands of additional valid knockout strategies for iJO1366 with higher cardinalities which could not be calculated before.

Overall, EColiCore2 is readily usable for e.g. educational purposes due to its appropriate scope, size, and clarity and even holds promise to become a reference model of *E. coli's* central metabolism.

References:

Erdrich, P. et al., 2015, BMC Systems Biology, 9 (48)

Orth, J.D. et al. , 2011, Molecular Systems Biology, 7 (535)

Poster 9

Title: Linking Overflow Metabolism and Growth Cessation in *Clostridium thermocellum*

Authors: Adam Thompson, Cong Trinh

Primary affiliation(s): University of Tennessee, Knoxville

Abstract: As a model thermophilic bacterium for the production of second-generation biofuels, the metabolism of *Clostridium thermocellum* has been widely studied. However, most studies have characterized *C. thermocellum* metabolism for growth at relatively low substrate concentrations. This outlook is not industrially relevant, however, as commercial viability requires substrate loadings of at least 100 g/L cellulosic materials. Recently, a wild-type *C. thermocellum* DSM1313 was cultured on high cellulose loading batch fermentations and reported to produce a wide range of fermentative products not seen at lower substrate concentrations, opening the door for a more in-depth analysis of how this organism will behave in industrially relevant conditions. In this work, we elucidated the interconnectedness of overflow metabolism and growth cessation in *C. thermocellum* during high cellulose loading batch fermentations (100 g/L). Metabolic flux and thermodynamic analyses suggested that hydrogen and formate accumulation perturbed the complex redox metabolism and limited conversion of pyruvate to acetyl-CoA conversion, likely leading to overflow metabolism and growth cessation in *C. thermocellum*. Pyruvate formate lyase (PFL) acts as an important redox valve and its flux is inhibited by formate accumulation. Finally, we demonstrated that manipulation of fermentation conditions to alleviate hydrogen accumulation could dramatically alter the fate of pyruvate, providing valuable insight into process design for enhanced *C. thermocellum* production of chemicals and biofuels.

Poster 10

Title: Reconstruction and simulation of metabolic models of interacting holobionts

Authors: Johannes Zimmermann*, Georgios Marinos*, Wentao Yang+, Nancy Obeng+, Hinrich Schulenburg+, Christoph Kaleta*

Primary affiliation(s): *Institute for Experimental Medicine, Christian-Albrechts-University and University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany, + Zoological Institute, Christian-Albrechts-University Kiel, 24118 Kiel, Germany

Abstract: The holobiont concept was introduced to address the observation that certain organisms have to be studied in their natural communities because they form ecological units [1]. In order to investigate the 'rules of engagement' in a holobiont context, we recently presented a framework called BacArena for modeling metabolic interactions in cellular communities [2]. Here, we extended the approach with respect to the diverse environment in the human gut. Absorption of nutrients by the gastrointestinal tract was considered as well as the nutrient transport by diffusion and peristalsis. The colon two-level mucus layer was represented along niche forming crypts. Colonic host cells were added with their specific activity and reasonable multicellular optimization. Moreover, we modeled microbiome-host interactions in the nematode *C. elegans* which is increasingly being recognized as an ideal model system for microbiome studies [3]. To this end, we reconstructed metabolic models of the native *C. elegans* microbiota and used a combination of modeling as well as RNA-seq data integration approaches to study the interaction between *C. elegans* and its microbiome.

[1] T. Bosch and D. Miller "The Holobiont Imperative", Springer (2016)

[2] E. Bauer*, J. Zimmermann*, F. Baldini, I. Thiele, C. Kaleta "BacArena: Individual-Based Metabolic Modeling of Heterogeneous Microbes in Complex Communities", PLoS Computational Biology (2017), accepted. * Equal contribution.
[3] F. Zhang, M. Berg, K. Dierking, M. Felix, M. Shapira, B. Samuel, H. Schulenburg "*Caenorhabditis elegans* as a Model for Microbiome Research", Frontiers in Microbiology (2017)

Poster 11

Title: Strategy for simultaneous production of butanol and hydrogen as biofuel with a membrane bioreactor system

Author: Zeyi Xiao

Primary affiliation(s): School of Chemical Engineering, Sichuan University

Abstract: Butanol can be prepared by ABE fermentation with clostridium strains under serious anaerobic environment, accompanying gas metabolite hydrogen and carbon dioxide emission. We have been developing a strategy for ABE fermentation with a CCCF system based upon a pervaporation membrane bioreactor. In this system, the fermentor and a pervaporation membrane module were coupled together, and the broth was circulated closely and continuously through the fermentor and the module so that the metabolite acetone, butanol and ethanol could be separated in situ by the membrane. In spacious top of the fermentor, slight pressure was built to ensure effective insulation against air and collect the gaseous products released from the broth and transmit them to the downstream for hydrogen recovery. We have conducted a series of experiments about this technology, and achieved some interesting measurements on microbial growth and culture, fermentable sugar utilization and conversion, product formation and extraction / recovery, as well as other concernings of the process operation. We have thought this strategy as a potential and valuable process technology for production of butanol and hydrogen as biofuel or chemicals.

Poster 12

Title: Computational Network Design and Analysis

Authors: Lisa Katharina Blass, Christian Weyler, Elmar Heinzle

Primary affiliation(s): Saarland University, Saarbrueken

Abstract: With the discovery of new enzymes and enzymatic activities the full exploration of their vast potential for the synthesis of valuable products is becoming increasingly complex. Designing biosynthetic multi-step routes manually using enzymes from more than one organism is very challenging, as the network of potential synthesis pathways quickly grows highly complex with more and more reaction steps.

To more easily harness the full potential of the enzymatic toolbox we developed an in silico toolbox for the directed design of biosynthetic production pathways for multi-enzyme catalysis.

The method combines the reconstruction of a genome-scale pan-organism metabolic network, a path-finding algorithm and the ranking of the pathway candidates for proposing suitable synthesis pathways. The metabolic network is based on data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the thermodynamics calculator eQuilibrator 2.0. We implemented a path-finding algorithm based on a mixed-integer linear program (MILP) which takes into account both topology and stoichiometry of the network to propose synthesis pathways starting from arbitrary metabolites to a target product of interest. The generated pathway candidates are ranked according to different criteria, including pathway length, thermodynamics and other biological properties such as number of heterologous enzymes or cofactor use. For each pathway candidate, a thermodynamic profile, the overall reactant balance and potential side reactions as well as an SBML file for visualization are generated.

The method presented is highly customizable and suitable for in vitro enzyme cascades, cell hydrolysates and permeabilized cells.

Poster 13

Title: Modeling cyanobacterial growth

Authors: Ralf Steuer, A-M Reimers, H Knoop, A Bockmayr

Primary affiliation(s): Humboldt-University, Berlin

Abstract: Photoautotrophic growth requires a highly coordinated distribution of cellular resources to different intracellular processes, including the de novo synthesis of proteins, ribosomes, lipids, and other cellular components. In our contribution, we present a computational framework to investigate the optimal allocation of cellular resources during diurnal phototrophic growth using a genome-scale metabolic reconstruction of the cyanobacterium *Synechococcus elongatus* PCC 7942. Specifically, we formulate phototrophic growth as an autocatalytic process and solve the resulting time-dependent resource allocation problem using constraint-based analysis. Based on a narrow and well-defined set of parameters, our approach results in the prediction of growth properties over a full diurnal cycle. The computational model allows us to study the optimality of metabolite partitioning during diurnal growth. The cyclic pattern of glycogen accumulation is an emergent property of the model and has timing characteristics that are in excellent agreement with experimental findings. Our approach provides insight into the time-dependent resource allocation problem of phototrophic diurnal growth and may serve as a general framework to assess the optimality of metabolic strategies that evolved in phototrophic organisms under diurnal conditions.

Poster 14

Title: Metabolite production cost and the evolution of cooperation in microbial communities.

Authors: Diana Schepens, Ashley Beck, Ross Carlson, Jeffrey Heys, Tomas Gedeon

Primary affiliation(s): Montana State University

Abstract: Metabolic cross-feeding between microbes is observed in many microbial communities. It has been experimentally observed that cross-feeding synthetic communities have an increased level of fitness and cell growth as compared to wild type cells.

Our goal is to develop a model to analyze the effects that resource investment into metabolite production has on the evolution of cross-feeding in a microbial community. We first analyze the investment into the substrates and enzymes associated with a metabolic pathway to formulate a function representing the optimal investment cost of producing the metabolite. We then combine this cost function together with traditional mass balance equations to develop a consortia model. With this model, we investigate conditions favorable to the evolution of cooperation in a microbial community.

Poster 15

Title: Stoichiometric network analysis of cyanobacterial acclimation to photosynthesis-associated stresses identifies heterotrophic niches

Authors: Ashley E. Beck, Hans C. Bernstein, and Ross P. Carlson

Primary affiliation(s): Montana State University, Pacific Northwest National Laboratory

Abstract: Metabolic acclimation to photosynthesis-associated stresses was examined in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 using integrated computational and photobioreactor analyses. A genome-enabled metabolic model, complete with measured biomass composition, was analyzed using ecological resource allocation theory to predict and interpret metabolic acclimation to irradiance, O_2, and nutrient stresses. Reduced growth efficiency, shifts in photosystem utilization, changes in photorespiration strategies, and differing byproduct secretion patterns were predicted to occur along culturing stress gradients. These predictions were compared with photobioreactor physiological data and previously published transcriptomic data and found to be highly consistent with observations, providing a systems-based rationale for the culture phenotypes. The analysis also indicated that cyanobacterial stress acclimation strategies created niches for heterotrophic organisms and that heterotrophic activity could enhance cyanobacterial stress tolerance by removing inhibitory metabolic byproducts. This study provides mechanistic insight into stress acclimation strategies in photoautotrophs and establishes a framework for predicting, designing, and engineering both axenic and photoautotrophic-heterotrophic systems as a function of controllable parameters.

Poster 16

Title: Principles for microbial community design: metabolic specialization creates a super-competitor unit

Authors: Ashley E. Beck and Ross P. Carlson

Primary affiliation(s): Montana State University

Abstract: Synthetic cross-feeding consortia were constructed with *Escherichia coli* to examine fundamental principles of organic acid exchange and detoxification in microbial communities. Pairing genetically engineered acetate and lactate producer strains with an organic acid scavenger strain created cross-feeding consortia that were compared with a generalist strain of the same genetic background. Batch systems were cultured using environmentally relevant buffering capacities and analyzed for resource utilization. The initial pH and composition (producer/scavenger ratio) of the systems were varied and shown to influence the overall productivity and degree of acid stress experienced by the consortia. A differential equation-based model was also used to compare and validate the experimental results. Interpreted within the framework of resource ratio theory, the division of labor paradigm leads to a super-competitor unit which more completely utilizes available resources than does the generalist strain. The concepts of microbial interactions and byproduct detoxification lead to important design principles for microbial communities for industrial bioprocesses and managed ecosystems.

Poster 17

Title: Metabolic Flux Analysis of Osteoarthritic Chondrocytes under Dynamic Compression

Authors: Daniel Salinas, Ronald K. June, Brendan M. Mumey

Primary affiliation(s): Montana State University

Abstract: Metabolomics data provides a snapshot of a cell's metabolism via estimates of the concentration of key metabolites. Application of metabolomics analysis over time may be used to estimate the change in abundance induced during the period of time between samples. However, a functional interpretation of the data becomes more difficult as additional metabolites are measured. To interpret the results, metabolic flux analysis (MFA) may be used to infer a set of reaction rates that could have generated the observed changes in abundance, thereby providing a systems approach to integrate the data into a set of pathway activities.

The response of chondrocytes to *in vitro* stimulus designed to mimic the human gait was examined. The hypothesis that dynamic compression induces synthesis of cartilage precursors was tested. Evidence that dynamic compression results in protein synthesis was discovered via metabolic flux analysis of central energy metabolites. ANOVA-simultaneous components analysis (ASCA), an extension of principal components analysis to data exposed to differing levels of treatments, was used to extend the method to analysis of primary chondrocytes.

Finally, research is ongoing into developing an alternative to pathway enrichment analysis from untargeted metabolomics data. Enrichment analysis is a statistical technique whereby a likely set of active pathways can be inferred from a set of metabolites measured using metabolomics, using the principle that those pathways that overlap with the metabolites the most must be active. We are extending the greedy algorithm for the set cover problem to infer pathways from untargeted data.

Poster 18

Title: Strategies for Modeling of Large-Scale Metabolic Models of Microbial Communities

Authors: Sabine Koch, Dirk Benndorf, Fabian Kohrs, Patrick Lahmann, Udo Reichl, Steffen Klamt

Primary affiliation(s): Max Planck Institute, Magdeburg

Abstract: Microbial communities play a major role in ecology, medicine and various industrial processes. A challenge in modeling microbial communities is the large number of organisms involved which results in complex stoichiometric networks. Here, we introduce an approach to handle this complexity. It relies on compartmented models and the concept of balanced growth [1, 2]. First, we construct and validate stoichiometric models of the core metabolism of the organisms. In a next step, we compute bounded elementary flux vectors (EFVs) [3] for each model and reduce them to their overall stoichiometry. Selected EFVs fulfilling a species-level optimality criterion serve as reactions for the community model.

To illustrate our approach, a reduced model was established consisting of nine organisms including *Escherichia coli*, *Clostridium acetobutylicum*, *Acetobacterium woodii*, *Propionibacterium freudenreichii*, *Syntrophobacter fumaroxidans*, *Syntrophomonas wolfei*, *Desulfovibrio vulgaris*, *Methanococcus maripaludis*, and *Methanosarcina barkeri*. These organisms are representatives for typical degradation steps of anaerobic digestion in biogas plants. The model is analyzed with standard methods of constrained-based modeling. It reflects product yields and ratios of a chemostat enrichment culture grown on ethanol. For glucose as a substrate, the expected ratio of approximately 50% methane and 50% CO_2 in the biogas as well as an anti-correlation between acetate and methane yields is obtained. We also show how the reduced model can be used to find intervention strategies for an increase in methane yields.

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Poster 19

Title: Metabolic network analysis of uncultivated oral microbiome organisms

Authors: David Bernstein, Daniel Segré

Primary affiliation(s): Boston University

Abstract: Microbial communities are ubiquitous in nature and influence important processes ranging from global biogeochemical cycles to human health. Genome-scale metabolic networks are powerful tools that can be used to provide mechanistic understanding of the structure and function of these communities. With the advent of methods to automatically reconstruct metabolic networks from sequenced genomes these tools are becoming increasingly widespread. However, additional methods are needed to analyze these networks and extract relevant metabolic information. We have developed a novel algorithm that can be used to analyze genome-scale metabolic networks and provide preliminary insight into an organism's biosynthetic capabilities. Our method uses a probabilistic approach, inspired by percolation theory, to calculate a measure of robustness describing a given networks ability to synthesize a target metabolite or set of metabolites in an environment-independent manner. We used our method to analyze draft metabolic networks for 457 microbial strains from the human oral microbiome, a particularly well-studied microbial community, and focused in particular on uncultivated organisms. Using our method, we have identified interesting metabolic signatures for certain uncultivated organisms and proposed potential exchanged metabolites between the important uncultivated oral strain TM7x and its recently identified partner strain *Actinomyces odontolyticus* XH001. As the number of sequenced microbial organisms continues to increase, our method and other similar methods will be important tools for predicting metabolic functions directly from genomes and will help reveal connections between microbial organisms and their environments.

Poster 20

Title: Towards the identification of pathways for lipids biosynthesis in HCB

Authors: Oscar Dias, Rita Castro, Alcina Pereira, Isabel Rocha

Primary affiliation(s): University of Minho, Portugal

Abstract: Storage compounds, such as lipids, can be used as sources of carbon and energy in animals, plants and microorganisms. Regarding prokaryotes, this approach allows stocking energy for periods in which there is a limited availability of nutrients, thus such mechanism can provide evolutionary advantages for thriving in extreme conditions. Hence, when subjected to stress conditions, like growth-restrictions, excess carbon source or high carbon-nitrogen ratios, almost all prokaryotes are prone to accumulate these compounds.

Hydrocarbonoclastic bacteria (HCB) are a collection of microorganisms that can process hydrocarbons. HCB have the ability to accumulate storage compounds as triacylglycerols (TAGs), wax esters (WEs) and poly-ß-hydroxybutyrates (PHBs), among others. These compounds are essential lipophilic substances, which can be biosynthesized and accumulated in intracellular inclusion bodies or also exported into the extracellular space.

The purpose of this study was to identify the genes involved in the metabolic pathways for the production of TAGs, Wes and PHBs and determining the paths taken by the metabolism of different organisms (*Rhodococcus opacus* PD630, *Rhodococcus opacus* B4, *Acinetobacter baylyi* ADP1, *Alcanivorax borkumensis* SK2 and Pseudomonas putida KT2440) when accumulating these compounds. An existing genome-scale model of A. *baylyi* was updated and used to simulate in silico the production of these lipids using several carbon sources (including glucose, acetate, octane, pimelate and succinate) and throughout a span of nitrogen source concentrations.

The results of this work will allow determining strategies to improve the biotechnological potential of the five bacteria using metabolic engineering and bioinformatics approaches.

Poster 21

Title: Explaining the asymmetric label incorporation during photosynthesis

Author: Oliver Ebenhöh

Primary affiliation(s): Heinrich Heine University, Düsseldorf

Abstract: Sixty years ago in 1957, Martin Gibbs discovered that radioactively labelled carbon dioxide is asymmetrically incorporated into sugars during photosynthesis. This observation, later termed 'Photosynthetic Gibbs Effect', was puzzling and appeared counter-intuitive, because RuBisCO, the enzyme fixing carbon dioxide to a five-carbon sugar,releases two identical three-carbon molecules, from which sugarars e symetrically formed. Many different explanations have been proposed to explain the observed asymmetries, and as usual the simplest were also the most plausible. Already in 1964, James Bassham explained the appearance of asymmetries by different pool sizes of intermediates and argued that other reproducible patterns result from a 'quirk' of carbons by reversible reactions catalysed by transketolase. Despite such plausible qualitative arguments, a quantitative explanation of the observed labelling dynamics has never been given.

Here, we propose a simple model of the Calvin-Benson-Bassham cycle, which is based on thermodynamic considerations of the cycle and focusses on the paths of carbon atoms. We demonstrate that the observed patterns of label incorporation are an emergent property of the cycle's dynamics and do not require any further assumptions beyond the cycle's stoichiometry and thermodynamics. The observed patterns are a result of the particular thermodynamic properties, which clearly separate the enzymatic steps into close-to-equilibrium and far-from-equilibrium reactions.

With our model, we can quantify the effect of single enzymatic steps on the label incorporation and thus we provide the first fully quantitative explanation of the Photosynthetic Gibbs Effect six decades after its discovery.

Poster 22

Title: Automated pathway curation and predicting auxotrophy

Authors: Janaka Edrisinghe, Christopher Henry, José Faria

Primary affiliation(s): Argonne National Laboratory

Abstract: Metabolic models generated by automated reconstruction pipelines are widely used for high-throughput prediction of microbial phenotypes. However, the generation of accurate in-silico phenotype predictions based solely on genomic data continues to be a challenge as metabolic models often require extensive gapfilling in order to produce cell biomass. As a result, the true physiological profile of an organism can be altered by the addition of non-native biochemical pathways or reactions during the gapfilling process. In this study, we constructed draft genome-scale metabolic models for ~1000 diverse set of reference microbial genomes currently available in GenBank, and we decomposed these models into a set of classical biochemical pathways using pathway databases such as KEGG or Metacyc as a reference. We then determine the extent to which each pathway is either consistently present or absent in each region of the phylogenetic tree, and we study the degree of conservation in the specific steps where gaps exist in each pathway across a phylogenetic neighborhood. Based on this analysis, we improved the reliability of our gapfilling algorithms, which in turn, reduce the number of non-native reactions being added and improved the reliability of our models in predicting auxotrophy. This also resulted in improvements to the genome annotations underlying our models. We validated our improved auxotrophy predictions using growth condition data collected for a diverse set of organisms. Our improved gapfilling algorithm is openly available for use within the DOE Knowledgebase platform (https://kbase.us).

Poster 23

Title: Generating tissue-specific metabolic models of *C. elegans*

Authors: Chintan Joshi, Sean Sadykoff, Nathan E. Lewis

Primary affiliation(s): University of California, San Diego

Abstract: Genome-scale metabolic models are often used to understand and predict molecular mechanisms of cellular phenotypes in organisms. However, understanding mechanisms of phenotypes in multicellular eukaryotic organisms requires (1) metabolic reconstructions of single cells belonging to the organism and (2) cellular metabolic interactions amongst single cells of the organism. Over the past decade, various methods have been developed to construct cell-specific and tissue-specific models. However, the choice of method has a significant impact on model content, and therefore, quality. Here, we applied these methods to our reconciled organismal model of *C. elegans*, CeleCon, to generate various tissue-specific models. Further, we also identified possible metabolic roles of various tissues during the growth of the worm from hatchling to adult, thus, highlighting shifts in metabolic flux distribution across various tissues in the worm.

Poster 24

Title: Memote - A testing suite for constraints-based metabolic models

Authors: Christian Lieven, Moritz E. Beber, Nikolaus Sonnenschein

Primary affiliation(s): Novo Nordisk Foundation Center for Biosustainability

Abstract: Constraints-based metabolic models have become fundamental and trusted tools in systems biology. Several layers of biological information are combined in a compact format in order to describe a metabolic model. A richly annotated model is required for its various areas of application and represents a veritable knowledge base about an organism's metabolism. However, coherently describing a complex interlinked system such as metabolism is a challenge in and of itself that is only aggravated by the current lack of cohesive, widely-accepted, testable, and modern standards.

Here, we introduce memote (Metabolic Model Tests {https://github.com/biosustain/memote}), a Python package designed to run a given model through a set of hard and soft tests and generate a report that reflects model integrity. Soft tests focus on aspects that do not influence the performance of the model, such as syntactic conventions whereas hard tests determine whether a model is fully functional.

While memote can be run locally as a stand-alone testing suite, it shows its full potential when combined with web-based version controlling (Github) and continuous integration tools (Travis CI). Every tracked edit of a model automatically triggers the memote test suite, and generates a corresponding report that facilitates factual debate of model changes.

Thus, memote not only allows researchers to more quickly iterate through the design-build-test cycle but also provides the scientific community with a measure of quality that is consistent across setups, as well as an opportunity to interact and collaborate by establishing workflows for publicly hosted and version controlled models.

Poster 25

Title: Reduced Metabolic Models

Authors: Jean-Pierre MAZAT[1,2], Razanne ISSA1,2 and Stéphane RANSAC[1,2]

Primary affiliation(s): 1 IBGC- CNRS UMR 5095, 1 rue Camille Saint Saens, CS 61390, 33077 Bordeaux~Cedex, France 2 Université de Bordeaux France

Abstract: The knowledge of genomes leads to the construction of genome-scale models (GSM) involving all the enzymes possibly encoded in the genome. Due to their big sizes, it is difficult to study these models and the only possible approach is Flux Balance Analysis looking for flux values able, at steady-state, to optimize some objective function. The determination of all their Elementary Flux Modes (EFMs) is impossible and a dynamical study of such big models is difficult.

For all these reasons, we decided to develop simpler models still representing the main architecture of the whole metabolism but with fewer reactions which are aggregations of the actual reactions with their stoichiometries. Typically, such reduced models involve between 50 to 100 reactions and metabolites to describe the central carbon metabolism. The advantage of such models is to be more easily tractable and more understandable. Furthermore, they can be approached with a greater panel of methods such as analysis of EFMs, FBA and FVA. Their dynamical behavior can be studied with some reasonable hypotheses on their kinetic laws and Metabolic Control Analysis (MCA) is possible leading to the determination of good targets for therapeutic or biotechnology purposes.

With such a simple metabolic model, we have determined the different ways to synthesize serine from glutamine in cancer cells. We were also able to simulate Crabtree and Warburg effects, the metabolic changes accompanying mitochondrial diseases and the interactions between metabolisms in different type of cells.

Poster 26

Title: Culture medium customization by metabolic pathway analysis

Authors: Rui Oliveira, Rui Portela

Primary affiliation(s): University of Minho, Portugal

Abstract: Culture media customization to clone, protein and/or process is still a significant burden in process development in the biopharma sector. Current approaches are based on statistical design of experiments (DoE) guided by experience. Such projects imply a large number of culture experiments for assessing many different combinations of concentrations of a potentially large number of medium components. Mathematical modeling of cellular systems holds the key for rational culture media design, potentially decreasing the experimental burden for culture media customization. However, currently used metabolic modeling methods, such as metabolic flux analysis (MFA) or flux balance analysis (FBA) have produced limited benefits in the context of culture medium optimization. In this study, we investigate cell functional enviromics as a novel technique for systematic culture media optimization. This technique comprises two main stages. In the first stage, a functional enviromics map is built through the joint screening of metabolic pathways and medium factors by the execution of a cell culture/1H-NMR exometabolome assay protocol. The functional enviromics map consists of a data array of intensity values of metabolic pathways activation and/or repression by individual medium factors. In the second stage, optimized cell culture medium formulations are optimized that either enhance or repress target metabolic functions using the information contained in the functional enviromics map. The main advantage of this method lies in enabling metabolic engineering through the culture media composition manipulation. A case study is presented of a Pichia pastoris X33 strain expressing a scFv. It is shown that the optimization of trace elements concentrations linked to critical metabolic pathways, increase the titer of the target scFv by twofold

Poster 27

Title: Theoretical and practical limitations of functionalized hydrocarbon production in a cellulolytic, endophytic fungus

Authors: Kristopher A. Hunt, Natasha D. Mallette, Brent M. Peyton, Ross P. Carlson

Primary affiliation(s): Center for Biofilm Engineering, Montana State University, Bozeman, MT, Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT

Abstract: Functionalized hydrocarbons have a variety of ecological and industrial uses from signaling molecules and antifungal/antibacterial agents to fuels and specialty chemicals. The potential to produce functionalized hydrocarbons by the cellulolytic, endophytic fungus, *Ascocoryne sarcoides*, was quantified using genome-enabled stoichiometric modeling. *In silico* analysis identified available routes to produce these hydrocarbons, which included both anabolic- and catabolic-based strategies, and determined correlations between the type and size of molecules and culturing parameters, such as oxygen and carbon limitation. The analysis quantified the limits of wild-type to produce functionalized hydrocarbons from cellulose-based substrates, as well as identified metabolic engineering targets, including cellobiose phosphorylase (CP) and cytosolic pyruvate dehydrogenase complex (PDH_{cyt}). CP and PDH_{cyt} activity increased the theoretical production limits most substantially under anoxic conditions where less energy was extracted from the substrate. Incorporation of both engineering targets resulted in near complete conversion of substrate electrons in functionalized hydrocarbons. The *in silico* framework was integrated with *in vitro* fungal batch growth experiments to support predictions of electron acceptor limitation and functionalized hydrocarbon production. This is the first reported metabolic reconstruction of an endophytic filamentous fungus and describes pathways for both specific and general production strategies of 161 functionalized hydrocarbons applicable to many eukaryotic hosts.

Poster 28

Title: Multiscale Analysis of Autotroph-Heterotroph Interactions in a High-Temperature Microbial Community

Authors: Kristopher A. Hunt, Ryan deM. Jennings, William P. Inskeep, Ross P. Carlson

Primary affiliations: Thermal Biology Institute, Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717

Abstract: Interactions among microbial community members can lead to emergent properties, such as enhanced productivity, stability, and robustness. Iron-oxide mats in acidic (pH 2 – 4), high-temperature (> 65 ^{o}C) springs of Yellowstone National Park (YNP) contain relatively simple microbial communities and are well-characterized geochemically. Consequently, these communities are excellent model systems for studying the metabolic activity of individual populations and key microbial interactions. The primary goals of the current study were to integrate data collected *in situ* with *in silico* calculations across process-scales encompassing enzymatic activity, cellular metabolism, community interactions, and ecosystem biogeochemistry, and to predict and quantify the functional limits of autotroph-heterotroph interactions. Metagenomic and transcriptomic data were used to reconstruct carbon and energy metabolisms of an important autotroph (*Metallosphaera yellowstonensis*) and heterotroph (*Geoarchaeum* sp. OSPB) from Fe(III)-oxide mat communities. Standard and hybrid elementary flux mode and flux balance analyses of metabolic models predicted cellular- and community-level metabolic acclimations to simulated environmental stresses. *In situ* geochemical analyses, including oxygen depth-profiles, Fe(III)-oxide deposition rates, stable carbon isotopes and mat biomass concentrations, were combined with cellular models to explore autotroph-heterotroph interactions important to community structure-function. Integration of metabolic modeling with *in situ* measurements, including the relative population abundance of autotrophs to heterotrophs, demonstrated that Fe(III)-oxide mat communities maximize total community growth rate, as opposed to the net community growth rate, as predicted from the maximum power principle. Integration of multiscale data with practical ecological theory provides a basis for predicting autotroph-heterotroph interactions and community-level cellular organization.

Poster 29

Title: Linear Programming Model can explain Respiration of Fermentation Products

Authors: Philip Möller, Xiaochen Liu, Daniel Boley, Stefan Schuster

Primary affiliation(s): Friedrich Schiller University Jena, University of Minnesota

Abstract: To produce ATP, tumour cells rely on glycolysis leading to lactate (in addition to respiration) to a higher extent than the corresponding healthy cells. This phenomenon is known as the Warburg effect, named after German biochemist Otto Warburg. A similar effect also occurs in several other cell types such as striated muscle cells, lymphocytes, and microglia, when activated states are compared with the resting state. It seems paradoxical at first sight because the ATP yield of glycolysis is much lower than that of respiration. An obvious explanation would be that glycolysis allows a higher ATP production rate, but the question arises why the organism does not re-allocate protein to the high-yield pathway of respiration. We tackled this question by a minimal model only including three combined reactions. Recently, we have extended the model further by considering the possible uptake and oxidation of fermentation products (e.g. lactate). We consider that the cell can allocate protein on several enzymes in a varying distribution and model this by a linear programming problem. This leads to pure respiration, pure glycolysis, and respirofermentation as a mixed flux distribution, and, as an additional possible solution in the extended model, mixed respiration of glucose and the fermentation product, depending on side conditions and on protein costs. Oxidation of fermentation products is predicted when external glucose (or any equivalent resource) is scarce or its uptake is severely limited.

Poster 30

Title: Trimethylamine-N-oxide Production by Gut Microbiota Populations

Authors: Jesse Peach, Bothner Lab, Stephanie Keene, Miles Lab,

Primary affiliation(s): Montana State University

Abstract: Recently, microbe-host interactions have shown to have a significant impact on health and disease.[1] This trend has highlighted the different metabolite profiles produced by varied gut microbe populations.[2] A metabolite of particular concern, as related to human health, is trimethylamine N-oxide(TMAO).[3] TMAO production by gut microbiota begins with the degradation of precursors, such as choline and carnitine, into the tertiary amine trimethylamine(TMA). While diet certainly has an impact on TMA production levels, gut microbiota population makeup appears to have an important effect on TMA levels as well. TMA is eventually processed in the liver by Flavin Monooxygenase 3(FMO3) into TMAO. Increased TMAO concentrations correlate with cardiovascular diseases(CVD), particularly with atherosclerosis. Although the mechanism is unclear, increased TMAO levels lead to an increase in arterial wall cholesterol deposits and a decrease in arterial wall cholesterol removal.[4]

To elucidate more information between the interaction of gut microbiota population makeup and TMAO production, the gut microbiota response to TMA precursors will be investigated. This will begin by creating a pool of diverse human subjects with known gut microbiota. The subjects will then be given a large quantity of butter to eat and blood samples will be drawn before ingestion and every 2 hours after for 8 hours. These samples will then be analyzed by mass spectrometry[5,6] to determine the relationship between gut microbiota and TMAO production over time with an influx of TMA precursors.

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Poster 31

Title: Computational design of metabolic division of labor for synthetic microbial communities

Authors: Megan Thommes**,** Taiyao Wang, Qi Zhao, Joshua Goldford, Ioannis Ch. Paschalidis, Daniel Segrè

Primary affiliation(s): Boston University

Abstract: The goal of this project is to understand and design metabolic crossfeeding in microbial communities. We have created an algorithm to predict how reactions will be partitioned to different members within a microbial community. Our approach is based on a mixed integer linear programming extension of community flux balance analysis. This method enables the identification of optimal "division of labor" states, in which different organisms can perform different functions to globally maximize a given objective. With the use of our algorithm, we have discovered organisms with metabolism that promotes metabolic crossfeeding by methods other than knocking out reactions to create auxotrophs. We use a version of dynamic flux balance analysis where each metabolic model is optimized separately to predict the growth and to analyze the metabolic exchange of the microorganisms identified by the algorithm.

Poster 32

Title: Metabolic Network Modeling of Interspecies Metabolic Coupling

Authors: Hyun-Seob Song, Hans C. Bernstein, Christopher S. Henry

Primary affiliation(s): Pacific Northwest National Laboratory, Argonne National Laboratory

Abstract: Metabolic network models can serve as a useful tool for a mechanistic understanding of complex interplay between species in microbial communities. In comparison to single species modeling, community metabolic network reconstruction is generally more complex due to the need to account for interspecies interactions. The reliable prediction of interspecies interactions would require the reconstruction of high-quality individual networks, which however becomes challenging for environmental communities whose member species cannot be sufficiently characterized. To address this limitation, we tested a new approach that uses community-level data as a key input for network reconstruction. Incorporation of community data is critical because it provides direct information on interspecies metabolic interactions, which is not necessarily obtainable from axenic cultures. Using a binary photoautotroph-heterotroph consortium, we compared alternative strategies of gapfilling: individual vs. community-level gapfilling. As a result, metabolic networks that were refined using community data provided experimentally validated predictions of how a photoautotrophic cyanobacterium provides organic carbon and nitrogen sources to support the growth of an obligate heterotrophic species. We implemented all processes for network reconstruction and refinement using the DOE Systems Biology Knowledgebase (KBase) platform (www.kbase.us). Reproducible narratives of model building for both single species and community networks are publicly available in KBase (https://narrative.kbase.us/narrative/ws.13807.obj.1).

Poster 33

Title: Extreme pathway analysis of endocrine developmental programming in zebrafish (*Danio rerio*)

Author: David Hala

Primary affiliation(s): Texas A&M, Galveston

Abstract: The interconnected topology of transcriptional regulatory networks (TRNs) lends to mathematical (or *in silico*) representation as a pseudo-stoichiometric matrix. Such a matrix can be 'solved' using the mathematical method of extreme pathway (ExPa) analysis, which identifies uniquely activated genes subject to transcription factor (TF) availabilities. This poster describes construction of *in silico* multi-tissue TRN models of brain, liver and gonad representing reproductive-endocrine developmental programming in zebrafish (*Danio rerio*). Life stages studied spanned from 0.25 hours post fertilization (hpf; zygote) to 90 days post fertilization (dpf; adult life stage). *First*, model calibration simulations showed brain to exhibit lowest proportion of co-regulated genes (19%) relative to liver (23%) and gonad (32%). This 'hierarchy' of co-regulatory capability (brain<liver<gonad) indicated presence of highly gene-specific TRNs in the brain, alluding to its role as 'master controller' of endocrine function. *Second*, TRN models were constrained with varying TF availabilities during zebrafish development. Normalized numbers of genes active during development showed concomitant activations between brain and gonad from 10-12 hpf (embryonic life stage) up to 30-90 dpf (adult life stage). This indicated a putative 'syncing' between the brain and gonad and initiation of an early reproductive-endocrine developmental program. Finally, comparison of *in vivo* active genes with those predicted *in silico* showed relatively good agreement for brain (49%), liver (27%) and gonad (32%). The multi-tissue TRN models presented can help to assess effects of changing environmental and/or genetic constraints on reproductive-endocrine function.

Poster 34

Title: Ten Years of Resource Allocation Analysis using Stoichiometric Models and Pareto Surfaces

Author: Ross P. Carlson

Primary Affiliation(s): Montana State University, Bozeman, MT 59717

Abstract: Resource availability limits growth in most environments driving microbial evolution toward strategies that allocate limiting resources in a manner that favors fitness. Resource-ratio theory was used generate Pareto surfaces to elucidate microbial strategies for extracting and channeling mass and energy from the environment. The theory assumes cellular fitness is maximized by allocating scarce resources in appropriate proportions to multiple stress responses. Presented case studies deconstruct metabolic networks into a complete set of minimal biochemical pathways known as elementary flux modes (EFMs). An economic analysis of the EFMs tabulates enzyme atomic synthesis requirements from amino acid sequences and pathway operating costs from catabolic efficiencies, permitting characterization of inherent tradeoffs between resource investment and phenotype. The theory and predictions[1,2,3] were tested using a compilation of published fluxomic data[4] as well as via an extensive set of nitrogen-, iron- or glucose-limited chemostat experiments[5,6]. The theory accurately predicts metabolic acclimations to nutrient scarcity using only a limited number of assumptions. Major predictions that have been experimentally verified on gradients of nutrient scarcity include 1) use of the catabolic glyoxylate shunt in place of the complete oxidative TCA cycle, 2) use of the Entner-Doudoroff pathway in place of the Embden-Meyerhof-Parnas glycolysis pathway, 3) overflow metabolisms that shift from acetate and formate secretion to lactate secretion, 4) the use of the pyruvate formate lyase enzyme in place of the pyruvate dehydrogenase complex and 5) enhanced function like high enzyme affinity comes at the cost of cellular energetics[4,5,6]. The variety strategies all maximize the functional return on investment of the scares resource at the expense of other resources found in excess.These concepts are relevant at all biological scales, from individual microbes to ecosystems, and appear to play key roles in the composition, organization, and functioning of molecular-level metabolic systems.

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Poster 35

Title: *In silico* approaches to study mass and energy flows in microbial consortia: a syntrophic case study

Authors: Reed Taffs, John E Aston, Kristen Brileya, Zackary Jay, Christian G Klatt, Shawn McGlynn, Natasha Mallette, Scott Montross, Robin Gerlach, William P Inskeep, David M Ward, Ross P Carlson

Primary affiliations: Montana State University

Abstract: Three methods were developed for the application of stoichiometry-based network analysis approaches including elementary mode analysis to the study of mass and energy flows in microbial communities. Each has distinct advantages and disadvantages suitable for analyzing systems with different degrees of complexity and a priori knowledge. These approaches were tested and compared using data from the thermophilic, phototrophic mat communities from Octopus and Mushroom Springs in Yellowstone National Park (USA). The models were based on three distinct microbial guilds: oxygenic phototrophs, filamentous anoxygenic phototrophs, and sulfate-reducing bacteria. Two phases, day and night, were modeled to account for differences in the sources of mass and energy and the routes available for their exchange.
Results: The in silico models were used to explore fundamental questions in ecology including the prediction of and explanation for measured relative abundances of primary producers in the mat, theoretical tradeoffs between overall productivity and the generation of toxic by-products, and the relative robustness of various guild interactions.
Conclusion: The three modeling approaches represent a flexible toolbox for creating cellular metabolic networks to study microbial communities on scales ranging from cells to ecosystems. A comparison of the three methods highlights considerations for selecting the one most appropriate for a given microbial system. For instance, communities represented only by metagenomic data can be modeled using the pooled method which analyzes a community's total metabolic potential without attempting to partition enzymes to different organisms. Systems with extensive a priori information on microbial guilds can be represented using the compartmentalized technique, employing distinct control volumes to separate guild-appropriate enzymes and metabolites. If the complexity of a compartmentalized network creates an unacceptable computational burden, the nested analysis approach permits greater scalability at the cost of more user intervention through multiple rounds of pathway analysis.

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