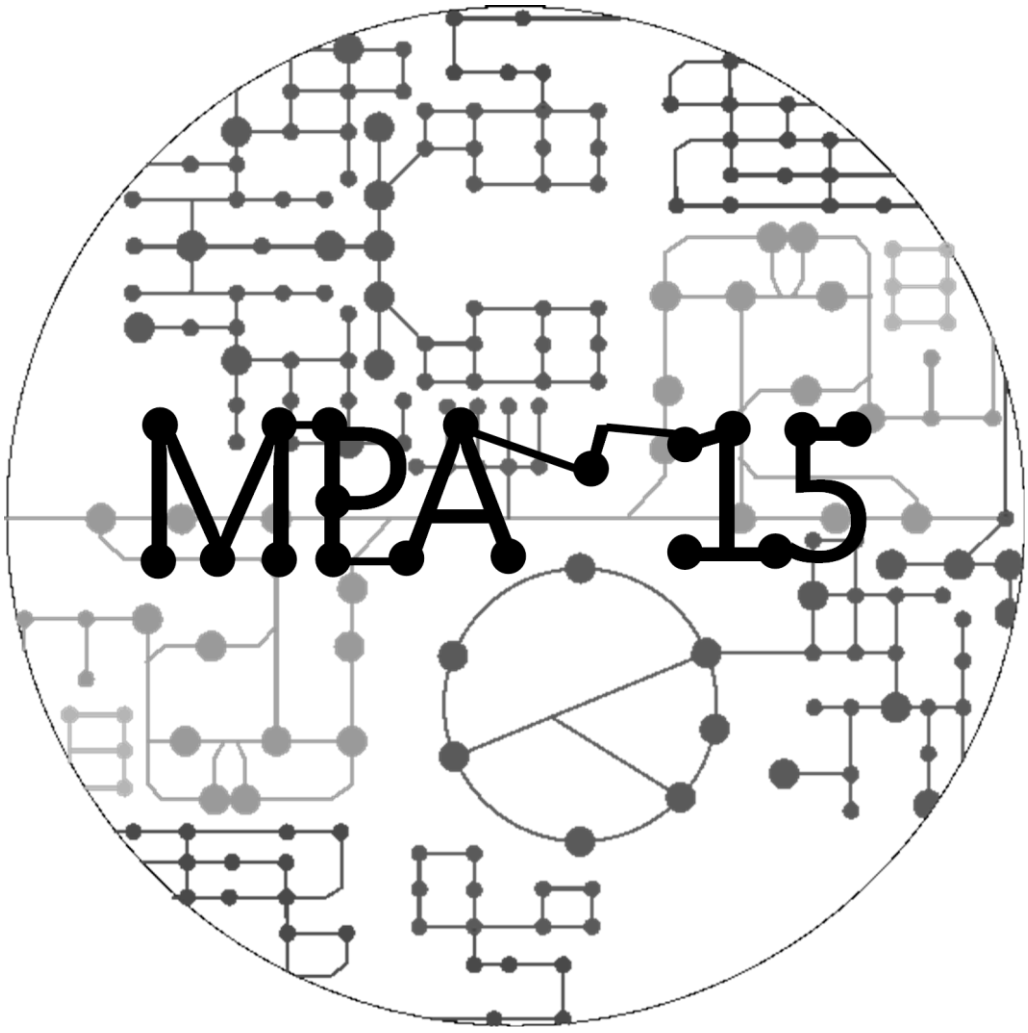
Programme & Abstracts

Metabolic Pathway Analysis 2015

8-12 June

Braga – Portugal



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Sociedade Portuguesa de Bioquímica SPB

BIOCHEMICAL SOCIETY

Contents

Welcome and Introduction

Dear participants at the MPA 2015,

Welcome to Braga!

The MPA 2015 conference is the fifth meeting of that name, previous meetings having been held in 2013 in Oxford, 2011 in Chester, 2009 in Leiden, and in 2005 at the University of Jena.

The meeting has a primary focus on the structural analysis of metabolic networks, and in particular techniques allied to linear algebra, linear programming and computer modelling. We will have more than 90 scientific contributions, including posters and oral presentations, covering novel methods and tools applicable to metabolic pathways, whole organisms or mixed populations. The most relevant applications covered include metabolic engineering, research in health sciences or plants.

We chose to organize this conference in a special location. Bom Jesus is, besides a pleasant natural park, a hilltop with a unique set of architectonic Baroque jewels that will hopefully soon be part of the UNESCO world heritage list. We also hope you enjoy the city center of Braga, which is one of the oldest and most lively towns in Portugal.

In common with previous MPA meetings, one of the major objectives of MPA2015 is to provide time and the adequate environment to allow delegates to participate in informal meetings in addition to the plenary sessions to foster collaborations between participants. We hope that the unique location characteristics, the fact that the meeting format will be a residential meeting and the social events will promote this type of interaction.

We made our best effort to provide you a great experience in both Scientific and Social terms and hope you enjoy our MPA2015!

Isabel Rocha, chair of MPA 2015

Overview

All lectures will take place in the Congress Center *Colunata de Eventos*.

All participants will be hosted at *hotel do Lago* (students), *hotel do Templo* and *hotel do Parque.* All four buildings are at walking distance from each other in Bom Jesus, Braga.

Breakfast will be served at each participant's hotel.

The registration fee includes lunches, dinners and refreshments for the duration of the conference, and the Conference Dinner on June 10. Lunch and refreshments will be served in Colunata de Eventos.

Monday, 8 June 2015	
10:00 -17:00	Check-in
10:00 – 17:00	Workshop – Computational Metabolic Pathway Analysis with OptFlux (registration required)
17:30 – 17:45	Opening Session
17:45 – 19:30	Session 1 - Metabolic Engineering 1
19:30 – 20:30	Welcome Reception
20:30	Dinner
Tuesday, 9 June 2015	
09:00 – 10:20	Session 2 – Uncovering Biological Principles
10:20 – 10:50	Coffee break
10:50 – 12:50	Session 2 – Uncovering Biological Principles (continued)
12:50 – 14:00	Lunch
14:00 – 15:20	Session 3 – Methods and Tools
15:20 – 15:40	Coffee break
15:40 – 17:00	Session 3 – Methods and Tools (continued)
17:00 – 20:00	Poster Session 1 & drinks
20:00	Dinner
Wednesday, 10 June 2015	
08:30 – 10:30	Session 4 – Applications to Photosynthetic Organisms and Microbial Communities
10:30 – 11:00	Coffee break
11:00 – 13:00	Session 4 – Applications to Photosynthetic Organisms and Microbial Communities (continued)
13:00 – 14:00	Lunch
14:00 – 19:00	Free afternoon (Tour and Activities in Braga Historical Center)
19:00	Conference Dinner
Thursday, 11 June 2015	
09:00 – 10:20	Session 5 - Applications in Health
10:20 – 10:50	Coffee break
10:50 – 12:50	Session 5 - Applications in Health (continued)
12:50 – 14:00	Lunch
14:00 – 15:20	Session 6 – Metabolic Engineering 2
15:20 – 15:40	Coffee break
15:40 – 17:00	Session 6 - Metabolic Engineering 2 (continued)
17:00 – 20:00	Poster Session 2 & drinks
20:00	Dinner
Friday, 12 June 2015	
08:30 – 10:30	Session 7 – Omics Data Integration
10:30 – 11:00	Coffee break
11:00 – 12:20	Session 7 – Omics Data Integration (continued)
12:20 – 12:40	Closing Session
12:40	Lunch

Sponsors and Exhibition

The Organizers would like to express their thanks and gratitude to the conference sponsors:

Universidade do Minho

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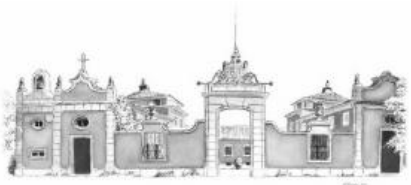

Casa de Sezim

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Organizers

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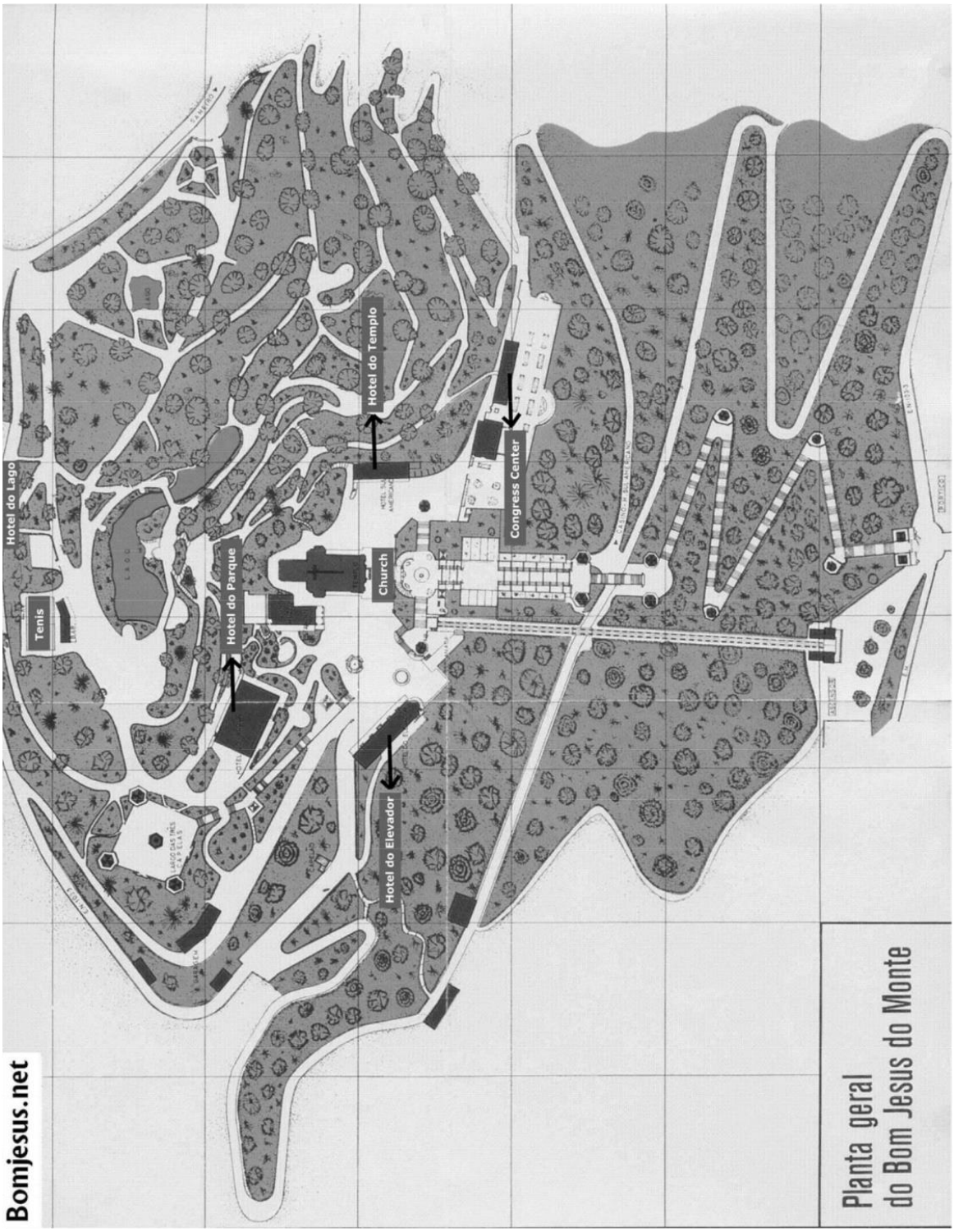
Venue

Venue Address: Bom Jesus do Monte, 4715-056 Braga, Portugal

Registration, all lectures and poster sessions will take place in the Congress Center *Colunata de Eventos*.

All participants will be hosted at *Hotel do Lago* (students), *Hotel do Templo* and *Hotel do Parque*. All four buildings are at walking distance from each other.

Lunches will be served at *Colunata de Eventos.* The welcome reception and dinner on June 8 and dinner on June 9 will be served at Sala Arcada at *Hotel do Elevador*. Dinner on June 11 will be served at *Colunata de Eventos*. The conference dinner on June 10 will take place at *Convento do Carmo*, Travessa do Carmo, Braga (at the city center).



Participant Information

Poster Session

Poster Session 1 – Tuesday June 9
Poster Session 2 – Thursday June 11

The Poster Session will take place in *Colunata de Eventos*.

Posters allocated to Poster Session 1 will be displayed from Monday 8 to Wednesday 10 12h00m and Posters allocated to Poster Session 2 will be displayed from Wednesday 10 13h00m until Friday 12 13h00m. Each poster has been assigned a number, which appears alongside its abstract in this booklet. Presenting authors are requested to stand alongside their posters during their poster session. Velcro will be provided at the registration desk.

Poster Prizes

A poster prize will be given at the Thursday Dinner to the best posters selected by an *ad-hoc* committee nominated by the Scientific Committee.

"Metabolic Pathways Analysis 2015" has been approved for the purposes of Continuing Professional Development (CPD) by the Society of Biology. These points are valid if attendees are registered on the Society of Biology CPD scheme. Approval signifies that the Society of Biology recognizes the "Metabolic Pathways Analysis 2015" event is of merit to the development needs of participants. If you require a CPD certificate for this conference please request one during the post event feedback questionnaire which will be sent to you by email shortly after the event.

Security

Badges must be worn for the duration of the conference, both for security purposes and for entry to the lectures and social events.

Facilities Information

Accommodation

Residential registration includes accommodation in Hotéis do Bom Jesus do Monte, Braga from June 8 to June 12. Students will be staying in *Hotel do Lago* (GPS: 41º33'16.27"N | 8º22'27.79"W). Other participants will be staying in *Hotel do Parque* (GPS: 41º33'21.16"N | 8º22'39.06"W) and *Hotel do Templo* (GPS.: 41º 33' 16" N | 8º 22' 38" W).

Check-in is from 14h30m on the day of arrival and check out is at 12h00m.

Parking

There is free parking near by the hotels.

Internet access

WiFi is available in the hotels and in *Colunata de Eventos*. Login details will be provided to each participant upon check-in for access within each participant's hotel. All participants can access internet at *Colunata de Eventos* (limited to 50 users simultaneously) and *Hotel do Templo* (50 m away from *Colunata*)

Password for *Colunata de Eventos*: colunata.2013

Login details for *Hotel do Templo*: Username: hotel; Password: templo

Further Information

Biochemical Society Transactions

All Speakers have been invited to prepare a manuscript for publication in Biochemical Society Transactions. Single issues of Biochemical Society Transactions are available for purchase (£30). You can order one by emailing conferences@biochemistry.org

Certificates of Attendance

Certificates of Attendance for the meeting will be provided to all participants and are available at the registration desk.

Attendees at the Conference

A delegate list will be sent by email to all attendees after the conference. Please note that this list is intended for use only to promote networking between scientists. You do not have permission to use this list for any other purpose, and any other use may infringe the Data Protection Act 1998. The list contains the names and affiliations of all attendees. Contact details are included only for attendees who gave their permission during the registration process.

Liability

The Organizers will assume no responsibility whatsoever for damage or injury to persons or property during the meeting. Participants are advised to arrange their own personal travel and health insurance.

Tweeting and Blogging

The Biochemical Society encourages the discussion of its conferences via Twitter, Facebook and similar social networks. In order to promote discussion and the exchange of information, delegates who wish to Tweet are asked to use the hashtags:

#MPA_2015
#MPA_15
#MPA15
#MPA2015

Speakers will be made aware of this policy, and have the right to ask delegates not to disseminate their research via the Internet. If a Speaker makes this request, delegates are asked not to discuss the relevant work in this way.

Delegates are respectfully asked to refrain from communicating using mobile devices whilst lectures are in progress.

Useful contacts

Venue

Accommodation:	Tel: +351 253 603 470 (HOTEL)

Local Organizers

mpa@ceb.uminho.pt

Biological Engineering Department, University of Minho	Tel: +351 253 604400 / +351 933267687

Local Taxi Services

Braga Táxis Service:	Tel +351 253 253 253
TAXI24 – Táxis de Braga	Tel +351 919 808 808
Moura & Taveira	Tel +351 253 263 655

Scientific Programme

Monday, June 8 2015

10:00 – 17:00		Workshop - Computational Metabolic Pathway Analysis with OptFlux (registration required)
17:30 – 17:45		Opening session
Session 1 – Metabolic Engineering 1 – Mark Poolman		
17:45 – 18:45	**KN_01**	**Keynote Talk – Engineering metabolic pathways** Friedrich Srienc (University of Minnesota, USA)
18:45 – 19:30	**IS_02**	**Multi-scale modeling of chemical product choices for cell factory development** Markus Herrgård (Technical University of Denmark, Denmark)
19:30		Welcome reception
20:30		Dinner

Tuesday, 9 June 2015

Session 2 – Uncovering Biological Principles – David Fell		
09:00 – 09:40	**IS_03**	**Coping with noisy metabolism in the bacterial microenvironment** Nathan Lewis (University of California San Diego, USA)
09:40 – 10:20	**SS_04**	**Transcriptional vs post-transcriptional regulation of the central carbon metabolism of *E. coli*** Daniel Machado (University of Minho, Portugal)
10:20 – 10:50		Coffee break
10:50 – 11:30	**SS_05**	**Network-level architecture and the evolutionary potential of underground metabolism** Ferenc Pal (Biological Research Centre of the Hungarian Academy of Sciences, Hungary)
11:30 – 12:10	**IS_06**	**Investigating the fitness effects of aerobic fermentation in yeast** Thomas Pfeiffer (Massey University, New Zealand)
12:10 – 12:50	**SS_07**	**Dynamics of NAD-metabolism - everything but constant** Ines Heiland (University of Tromsø, Norway)
12:50 – 14:00		Lunch

Session 3 – Methods and Tools – Stefan Schuster		
14:00 – 14:40	**IS_08**	**Alternate integer linear programming for computing elementary modes from genome-scale metabolic networks** Hyun-Seob Song (Pacific Northwest National Laboratory, USA)
14:40 – 15:20	**SS_09**	**Optimal resource allocation in metabolic networks** Stefan Mueller (Radon Institute for Computational and Applied Mathematics (RICAM), Austria
15:20 – 15:40		Coffee break
15:40 – 16:20	**SS_10**	**Hierarchical decomposition of metabolic networks using k-modules** Arne Reimers (Centrum Wiskunde & Informatica, Netherlands)
16:20 – 17:00	**SS_11**	**Reconciling gene expression data with regulatory network models** José Faria (Argonne National Laboratory, USA)
17:00 – 20:00		Poster Session 1 & drinks
20:00		Dinner

Wednesday, 10 June 2015

Session 4 – Applications to Photosynthetic organisms and Microbial Communities – Eugénio Ferreira		
08:30 – 09:10	**SS_12**	**Investigating lipid production in a genome scale model of *Phaeodactylum tricornutum*** Dipali Singh (Oxford Brookes University, United Kingdom)
09:10 – 09:50	**SS_13**	**Modelling evolutionary reversibility of a metabolic pathway: C4 photosynthesis** David Heckmann (Heinrich-Heine University Düsseldorf, Germany)
09:50 – 10:30	**SS_14**	**A reductionist approach to model self-regulating systems in plants** Anna Matuszyńska (Heinrich-Heine University Düsseldorf, Germany)
10:30 – 11:00		Coffee break
11:00 – 11:40	**IS_15**	**Spatio-temporal models of metabolism in microbial communities** Daniel Segrè (Boston University, USA)
11:40 – 12:20	**SS_16**	**Stoichiometric analysis of primary autotrophy and biomass turnover in a thermoacidophilic iron oxidizing archaeal community** Kristopher Hunt (Montana State University, USA)
12:20 – 13:00	**IS_17**	**Spatiotemporal metabolic modeling of microbial biofilm consortia** Michael Henson (University of Massachusetts, USA)
13:00		Lunch

	Free afternoon (tour and activities in Braga historical center)	
19:00	Conference dinner	

Thursday, 11 June 2015

Session 5 –Applications in Health – Miguel Rocha		
09:00 – 09:40	**IS_18**	**A minimal model for explaining the Warburg effect** Stefan Schuster (University of Jena, Germany)
09:40 – 10:20	**IS_19**	**Reconstruction of *Caenorhabditis elegans* metabolism - a new resource to support healthy aging in nematodes** Christoph Kaleta (Christian-Albrechts-Universität zu Kiel, Germany)
10:20 – 10:50		Coffee break
10:50 – 11:30	**IS_20**	**Metabolic reprogramming in the glioblastoma cell: a systems approach** Anu Raghunathan (National Chemical Laboratory, India)
11:30 – 12:10	**SS_21**	**Analysing the contribution of *Plasmodium falciparum* to whole body glucose metabolism in malaria patients** Jacky Snoep (Stellenbosch University, South Africa)
12:10 – 12:50	**IS_22**	**Exploring *Trypanosoma brucei* energy metabolism using modelling and metabolomics** Fiona Achcar (University of Glasgow, United Kingdom)
12:50 – 14:00		Lunch
Session 6 – Metabolic Engineering 2 – Oliver Ebenhoeh		
14:00 – 14:40	**IS_23**	**Strain design for improved productivity, yield and robustness** Radhakrishnan Mahadevan (University of Toronto, Canada)
14:40 – 15:20	**SS_24**	**Enforced ATP futile cycling increases specific productivity and yield of anaerobic lactate production in *Escherichia coli*** Oliver Haedicke (Max-Planck-Institute, Germany)
15:20 – 15:40		Coffee break
15:40 – 16:20	**IS_25**	**MODCELL- rational design of modular cells for combinatorial biosynthesis of novel molecules** Cong Trinh (University of Tennessee, USA)
16:20 – 17:00	**SS_26**	**Analysing the feasibility of growth-coupled product synthesis in microbial strains using the concept of elementary flux vectors** Steffen Klamt (Max Planck Institute for Dynamics of Complex Technical Systems, Germany)

17:00 – 20:00		Poster Session 2 & drinks
20:00		Dinner

Friday, 12 June 2015

Session 7 – Omics Data Integration – Ross Carlson		
08:30 – 09:10	**IS_27**	**Mapping the fitness landscape of enzyme expression uncovers the cause of antagonism and sign epistasis between adaptive mutations** Christopher Marx (University of Idaho, USA)
09:10 – 09:50	**SS_28**	**Which elementary flux modes are biologically relevant?** Juergen Zanghellini (Austrian Centre of Industrial Biotechnology, Austria)
09:50 – 10:30	**IS_29**	**Pathway level culture media engineering** Rui Oliveira (Faculty of Science and Technology, University Nova de Lisboa, Portugal)
10:30 – 11:00		Coffee break
11:00 – 11:40	**SS_30**	***In vivo* catalytic rates estimated from proteomics match *in vitro* measurements** Dan Davidi (Weizmann institute of science, Israel)
11:40 – 12:20	**SS_31**	**Testing of network completion algorithms and mutant growth-rate predictions using genome-scale datasets** Igor Libourel (University of Minnesota, USA)
12:20 – 12:40		Closing session
12:40		Lunch

Posters

MPA_01	**Integrated contextualisation and analysis of metabolic networks** T. Pfau, M.P. Pacheco, M. Galhardo, J. Lin and T. Sauter
MPA_02	**Shifts in the bacterial metatranscriptome accompanying draught in Namibian agricultural soils** S. Huang, K. Huber, B. Bunk and J. Overmann
MPA_03	**FECorr: An algorithm to improve FBA predictions using transcriptomic data** A.M.A. Desouki, G. Gelius-Dietrich and M.J. Lercher
MPA_04	**Impact of intermediate toxicity on the regulation of metabolic pathways** J. Ewald, M. Koetzing, M. Bartl and C. Kaleta
MPA_05	**Dynamic constraint-based modeling of phototrophic metabolism** A.-M. Reimers, M. Rügen, A. Bockmayr and R. Steuer
MPA_06	**Genome-scale and Flux modeling in the Lemnaceae (*Spirodela polyrhiza*) isoprenoid pathway for predictive metabolic engineering** N. Toepfer, U. Heinig and A. Aharoni
MPA_07	**The steady-state assumption for oscillating and growing systems** A.-M. Reimers and A.C. Reimers
MPA_08	**Biofuel production with cyanobacteria: new strain design strategies revealed by computational modeling** P. Erdrich, H. Knoop, R. Steuer and S. Klamt
MPA_09	**Evaluation of methods for the reconstruction of specific models from omics data** S. Correia and M. Rocha
MPA_10	**Investigation of physiological impacts of knockout mutants using a genome scale model of Arabidopsis** K. Adhikari, M. Zanella, D.A. Fell, S.C. Zeeman and M. Poolman
MPA_11	**Hub Reactions in storage of selected compounds in heterotrophic plant cell network** V.T.N. Vu, M. Beurton-Aimar and S. Colombie
MPA_12	**Drug target identification in a *Salmonella typhimurium* metabolic model** H. Hartman, D.A. Fell and M. Poolman
MPA_13	**A comparison between Flux Balance Analysis and cellular constrained models of simplified metabolic networks** H. Dourado and M.J. Lercher
MPA_14	**Flux variability analysis to understand *Arabidopsis* response to sulfur limitation** A. Calderwood, S. Kopriva and R.J. Morris

MPA_15	**Interpreting systematic properties of the tomato photorespiratory metabolism by using a genome-scale metabolic model** H. Yuan, M. Poolman, D. Fell, G. Zhou, P. Hilbers and N. van Riel
MPA_16	**Ancestral metabolic networks and phenotypic evolution in *E. coli*** T. Pang and M.J. Lercher
MPA_17	**Reconstruction and validation of *i*TR383, a genome-scale metabolic model for *Helicobacter pylori* 26695** T.F. Resende, D.M. Correia, S. Santos and I. Rocha
MPA_18	**The severity of enzyme mutations strongly influences the number of affected metabolic pathways** D. Alzoubi and M.J. Lercher
MPA_19	**Dynamic modelling of cell metabolic behaviour: A work in progress** M. Jolicoeur
MPA_20	**A Model for the expression dynamics of the nicotinic acid degradation pathway in *Pseudomonas putida* KT2440** N. Mesfin, A. Rocco and J. Jimenez
MPA_21	**Imputing enzyme kinetic constants** M.J. Lercher and A.M.A. Desouki
MPA_22	**Dynamic metabolic flux analysis of hybridoma cells cultivated in perfusion mode** S.F. de Sousa, G. Bastin and A.V. Wouwer
MPA_23	**Reconstruction of a genome-scale metabolic model for *Actinobacillus succinogenes*** S. Carneiro, J. Miguel, R. Carreira, P. Vilaça and I. Rocha
MPA_24	**Metabolic modeling of microalgae growth and lipids production during day/night cycles and nitrogen starvation** C. Baroukh, R. Muñoz-Tamayo, J. Steyer and O. Bernard
MPA_25	**Visualizing omics data in the OptFlux workbench** P. Maia, P. Vilaça, I. Rocha and M. Rocha
MPA_26	**Mathematical models of glucosinolate metabolism in plants** S. Sharma and O. Ebenhoeh
MPA_27	**Evaluation of carbon sources for recombinant enzymes production in *E. coli* – an *in silico* analysis of the host metabolism** S. Freitas
MPA_28	***In silico* analysis of retinoid metabolism** J.R. Chase
MPA_29	**The evolutionary footprint in metabolic genes of *Arabidopsis thaliana*** A.A. Mannan, O. Popa and O. Ebenhoeh

MPA_30	**Flux balance analysis of integrated host-virus metabolic models** S. Aller
MPA_31	**An adaptive scenario for the origins of complex innovations** C.J. Fritzemeier, B. Szappanos, B. Csörgő, V. Lázár, G. Fekete, X. Lu, R. Notebaart, B. Papp, C. Pál and M.J. Lercher
MPA_32	**TDPS - Turnover dependent phenotypic simulation: a quantitative constraint-based simulation method that accommodates all main strain design strategies** R. Pereira, P. Vilaça, J. Nielsen and I. Rocha
MPA_33	**The effect of light on the evolution of C4 plants** E. Sundermann, D. Heckmann and M.J. Lercher
MPA_34	**Context-specific metabolic model extraction based on regularized least squares optimization** S. Robaina and Z. Nikoloski
MPA_35	**Analysis of pathways involved in glycerol fermentation by two novel anaerobic bacteria** A. Stams
MPA_36	***Escherichia coli* redox metabolism for the production of polyhydroxybutyrate using different substrates** M.I.V. Alvarez, V. Lobbia, J.J. Heijnen, K.O. Gamez and S.A. Wahl
MPA_37	**Markov-Chain Monte-Carlo sampling of metabolite concentrations to identify thermodynamically feasible reaction directionalities for flux balance analysis** U. Wittelsbürger, K. Schrankel and M.J. Lercher
MPA_38	**Enhancing the production of mannosylglycerate in *S. cerevisiae* through *in silico* driven metabolic engineering** C. Faria, N. Borges, H. Santos and I. Rocha
MPA_39	**Metabolic analysis of EBPR phosphate/glycogen accumulating organisms** L.G. da Silva, K.M. Akkermans, M. van Loosdrecht and S. Wahl
MPA_40	**SAT-based Metabolic Pathways Analysis without compilation** S. Peres, M. Morterol, P. Dague and L. Simon
MPA_41	**Exploring the consequences of species heterogeneity in ^{13}C-Flux Analysis: a case study** S. Azzouzi, S. Niedenführ, W. Wiechert and K. Nöh
MPA_42	**Analysis of 140 published GSMs and identification of the most common representation problems** P. Vilaça, J. Cardoso, I. Rocha and M. Rocha
MPA_43	**Serine and glutamine metabolism in cancer cells** A. Zhukova, A.-K. Bouzier-Sore, E. Obre, R. Rossignol and J. Mazat
MPA_44	**Including cofactor concentrations into dynamic Flux Balance Analysis** A. Succurro, D. Segrè and O. Ebenhöh

MPA_45	**Stochastic modelling of fatty acid synthesis** E. Radmaneshfar
MPA_46	**Integrated analysis of metabolomics and transcriptomics data in tobacco cultivars grown in various regions of China** L. Jin, J.H. Snyder, F. Li, N. Zhai, R. Wang, Q. Chen, X. Chen, P. Liu, Q. Zheng and H. Zhou
MPA_47	***GlobalFit*: Automatically refining metabolic network models by simultaneously matching sets of experimental growth and non-growth data** D. Hartleb
MPA_48	**Modeling nutrient assimilation in a species of *Chloroidium* isolated from the United Arab Emirates** D.R. Nelson, M. Arnoux, A. Chaiboonchoe, A. Jaiswal, B. Khraiwesh and K. Salehi-Ashtiani
MPA_49	**Computer simulation of mitochondrial metabolism in cardiomyocytes during hypoxia** F. Eyassu, A.C. Smith and A.J. Robinson
MPA_50	**Phylogenomic signature fluidity in metabolic network of a key species with plant and animal affinities** A. Chaiboonchoe, L. Ghamsari, B.S. Dohai, P. Ng, A. Jaiswal, K. Jijakli, J. Koussa, D.R. Nelson, H. Cai, X. Yang, R. Chang, B. Khraiwesh, J. Papin, H. Yu, B. Santhanam and K. Salehi-Ashtiani
MPA_51	**Integration of biomass functions of genome-scale metabolic models with experimental data reveals universally essential cofactors in prokaryotes** J.C. Xavier, K. Patil and I. Rocha
MPA_52	**VIRTUAL MITOCHONDRION :a modular and multi level whole-mitochondrion model** J.-P. Mazat, C. Nazaret, S. Ransac and M. Heiske
MPA_53	**Systems level metabolic pathway analysis for understanding antibiotic resistance in *Chromobacterium violaceum*** D. Banerjee, A. Raghunathan, N. Bhattacharya and V. Panchagnula
MPA_54	***merlin* latest developments for pathways analysis** O. Dias, M. Rocha, E.C. Ferreira and I. Rocha
MPA_55	**Metabolic flux prediction in cancer cells with altered substrate uptake** J. Schwartz, M. Barber and Z. Soons
MPA_56	**Elementary flux mode analysis of irradiance-induced stress acclimation strategies in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1** A.E. Beck, H.C. Bernstein and R.P. Carlson
MPA_57	**Uncovering the metabolic capacities of *H. pylori* 26695 using ^{13}C labeling experiments** D.M.M. Correia, R. Carreira, N.F. Azevedo and I. Rocha
MPA_58	**Compensatory mechanisms in mitochondrial diseases revealed by computer modelling** L.P. Zielinski, A.C. Smith and A.J. Robinson

Speaker Abstracts

KN_01

Engineering metabolic pathways

John Barrett, Arkady Khodursky and Friedrich Srienc
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The economic production of chemicals with microorganisms requires the design of reaction sequences that carry out the conversion of the available feedstock into the desired product at the highest possible yield and at the fastest possible rate while maintaining a robust operation that is stable over time. Metabolic pathway analysis offers an invaluable tool for the rational design of pathways since it reveals the complete property space of a metabolic network. Highest yielding pathways can be identified and realized with genetic techniques that eliminate undesired reactions. Furthermore, in combination with thermodynamic analysis and metabolic control theory reaction steps can be rationally identified whose accelerations lead to increased production rates. The advances of this analysis approach, open problems and future possibilities will be discussed.

IS_02

Multi-scale modeling of chemical product choices for cell factory development

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In recent years, bio-based chemicals have gained traction as a sustainable alternative to petrochemicals. However, despite rapid advances in metabolic engineering and synthetic biology, there remain significant economic and environmental challenges. In order to maximize the impact of research investment in a new bio-based chemical industry, there is a need for assessing the technological, economic, and environmental potentials of combinations of biomass feedstocks, biochemical products, bioprocess technologies, and metabolic engineering approaches in the early phase of development of cell factories. To address this need we are developing a comprehensive multi-scale framework for modeling sustainable chemical production. This framework integrates metabolic modeling, pathway finding, bioreactor design, upstream/downstream process modeling, modeling of competing industries and economic/environmental impact assessment. This framework has been demonstrated in case study where the production of two major polymer precursors from two biomass feedstocks through proposed biosynthetic pathways in two host organisms is assessed. In order to further strengthen our ability to select economically and environmentally sustainable chemicals for bio-based production we are also reconstructing a comprehensive model of the petrochemical industry covering the production routes for all major commodity chemicals. The entire framework is built so that it is compatible with constraint-based modeling methods and tools allowing the use of a large number of standard COBRA tools in technoeconomic and environmental assessment. The overall framework allows 1) predicting future trends in bio-chemical demands as a function of feedstock prices and 2) using economy-scale assessment to guide specific strain design decisions in metabolic engineering.

IS_03

Coping with noisy metabolism in the bacterial microenvironment

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The cellular microenvironment is dynamic, which fluctuations in nutritional resources and cellular protein composition. Eukaryotes cope with this noise through enzyme regulation, such as enzyme post-translational modification (PTMs). However, for decades it has been asserted that few prokaryotic enzymes are regulated by PTMs. Recent proteomic studies challenge this assumption, having discovered many PTMs on prokaryotic metabolic enzymes. To elucidate the biochemical functions of these PTMs and their influence on *E. coli* physiology, we developed an approach that integrates proteomic data, metabolic network analysis, protein structure analysis, and targeted genome engineering. Using this, we demonstrate that many PTMs aid in rapidly regulating metabolism to cope with fluctuations in the nutritional microenvironment of the cell. Specifically, we use a novel metabolic pathway modeling method, called Regulated Metabolic Branch Analysis (RuMBA), to identify enzymes that should require metabolic regulation in response to noise from fluctuating metabolite concentrations. We show that PTMs are particularly enriched among these enzymes and complement known allosteric regulatory mechanisms. Furthermore, regulated PTM sites are highly conserved and located near enzyme active sites. We further elucidate detailed mechanisms by which these PTMs regulate flux by integrating RuMBA with enzyme assays, protein structure analyses, and screens of PTM mutants generated by multiplexed automated genome editing technologies. Through this we show that PTMs are employed far more than previously anticipated to regulate prokaryotic metabolism in response to intrinsic and extrinsic metabolic noise.

SS_04

Transcriptional vs post-transcriptional regulation of the central carbon metabolism of *E. coli*

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Transcriptomics data are currently one of the most available types of large-scale biological data. A large number of methods have been developed to improve constraint-based simulations using these data. We recently performed a systematic comparison of these methods and observed that, at least for central carbon metabolism, there is no significant improvement in the prediction of flux distributions when gene expression data is used. These results are consistent with recent studies, in different organisms, showing that central carbon metabolism is predominantly regulated at post-transcriptional levels. Central carbon metabolism provides the precursors for the production of multiple compounds used in industrial biotechnology. Hence, it is the main target for intervention in most rational strain design strategies. However, its complexity is still not completely understood. In this work, we analyze the role of allosteric regulation, one of the main mechanisms of post-transcriptional regulation, for the control of central carbon metabolism. We extend a model of central carbon metabolism of *E. coli* with allosteric interactions, revealing a hidden topology in metabolic networks. We use this model to integrate a multi-*omic* dataset containing transcript, protein, flux and metabolite levels to further dissect the contribution of different types of regulation for metabolic flux control in these central pathways. Situations of predominant allosteric control could be identified, highlighting the importance of this kind of regulation in central carbon metabolism.

SS_05

Network-level architecture and the evolutionary potential of underground metabolism

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A central unresolved issue of metabolic network evolution is to understand how these networks can be extended and rewired to produce novel adaptive phenotypes. It is widely stated that weak catalytic side activities of enzymes can provide raw material for the evolution of novel functions. Such physiologically irrelevant 'underground' reactions appear to be frequent, however, it remains unknown to what extent this raw material could generate evolutionary novelties in the context of the entire metabolic network.

Here, we computationally reconstructed the first underground metabolic network of *E. coli* by compiling the known underground reactions into a genome-scale metabolic network. We revealed that most underground reactions are not isolated and nearly half of them are completely connected into the metabolic network. By employing a novel elementary flux mode sampling algorithm we show that many of the underground reactions can form novel pathways producing key biomass precursors. Under standard environmental conditions, typically, these pathways have similar properties to the native ones in terms of length and chemical yield. On the other hand, we estimate that under specific environments at least ∼20% of the connected underground reactions confer a fitness advantage when their activity is increased.

Computational predictions of novel phenotypes showed significant agreement with the *in vivo* evolutionary potential characterized by our genome-wide gene overexpression screen. These findings demonstrate for the first time that the genetic basis of evolutionary adaptations via underground metabolism can be predicted.

IS_06

Investigating the fitness effects of aerobic fermentation in yeast

Thomas Pfeiffer

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To produce ATP from sugars, yeasts can use two different pathways, fermentation and respiration. Respiration provides a high ATP yield (about 18 ATP per glucose), but requires oxygen. Fermentation to ethanol, in contrast, provides a much low ATP yield (2 ATP per glucose) but allows producing ATP in absence of oxygen. Many yeast species, including *Saccharomyces cerevisiae*, however, use the fermentation pathway in the presence of oxygen, when glucose levels are sufficiently high. In my presentation I discuss theoretical explanations regarding the evolutionary costs and benefits of aerobic fermentation, and experimental approaches to disentangle and test them.

SS_07

Dynamics of NAD-metabolism - everything but constant

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NAD as well as its phosphorylated form NADP are best known as electron carriers and cosubstrates of various redox reactions. As such they participate in approximately one quarter of all reactions listed in the reaction database KEGG. In metabolic pathway analysis, mass conservation is usually assumed between $NAD(P)^+$ and the corresponding reduced form NAD(P)H. Changes in the redox state might be considered, but concentration changes of the NAD-moiety are usually neglected. However, a growing number of NAD-consuming reactions have been identified, showing that this assumption does not hold in general. NAD-consuming reactions are common characteristics of NAD^+-dependent signaling pathways, and include mono- and poly-ADP-ribosylation of proteins, NAD^+-dependent deacetylation, and the formation of messenger molecules such as cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate. NAD-consuming reactions are thus involved in major signalling and gene regulation pathways such as DNA-repair or regulation of enzymes in central metabolism. All known NAD^+-dependent signaling processes include the release of nicotinamide (Nam). Thus cellular NAD-pools need to be constantly replenished, mostly by recycling Nam to NAD. This process is, among others, regulated by the circadian clock, causing complex dynamic changes in NAD-concentration. We have in recent years intensively analysed NAD-biosynthetic pathways in different organisms. To gain insights into the characteristics and the physiological relevance of different pathway topologies, we used a wide range of computational methods including phylogenetic approaches and structural network analysis as well as dynamic modelling.

IS_08

Alternate integer linear programming for computing elementary modes from genome-scale metabolic networks

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Enumeration of the full set of elementary modes from genome-scale metabolic networks poses a severe computational challenge. An interest in many cases is, however, often limited to a subset of elementary modes active under a given condition. While sequential pathway identification through iterative optimization is useful for that purpose, typical mixed integer linear programming (MILP)-based formulation becomes ineffective in generating accurate solutions as iteration builds up. To alleviate this drawback, we propose a novel optimization framework for faster and more numerically stable computation. The new method dissembles MILP into integer programming (IP) and linear programming (LP) and seeks a solution through their tandem implementation. At every step, the IP module identifies a set of reactions to be deleted to define a subnetwork, for which an LP problem is subsequently solved to extract an elementary mode. The key element of our approach is to generate a series of subnetworks (by IP) that guarantees all ensuing LP solutions (i.e., elementary modes) are distinct. This strategic division of labor through alternate use of IP and LP (thus, termed AILP) showed significant computational improvement over MILP. Importantly, at no additional cost, AILP also identifies reaction cut sets, the deletion of which disables the network to perform a defined function. I will discuss the usefulness of the proposed algorithm in the context of flux estimation from gene/protein expression profiles.

SS_09

Optimal resource allocation in metabolic networks

Stefan Mueller
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The promise of metabolic engineering has been in part driven by the availability of a formal framework for reasoning about metabolic fluxes. The most prominent such framework is Flux Balance Analysis (FBA). The main objective of FBA is to optimize yield, not rates. When a cell optimizes rates of specific metabolic reactions, this becomes a constrained resource allocation problem, since total enzyme is limited. Understanding the optimization of rates rather than yield helps in making sense of important adaptive scenarios in which metabolic networks do not behave as predicted by FBA.
In our analysis, we go beyond linear methods (such as FBA) based on stoichiometric information. In fact, we explicitly consider kinetic information and arrive at a nonlinear optimization problem with a surprising result: We prove that, for arbitrary kinetics, solutions that optimize rates are elementary flux modes. This is surprising precisely because such flux modes only depend on stoichiometry and yet they show up as optimal states for arbitrary enzyme kinetics, including arbitrary allosteric regulation. Our theoretical result predicts discontinuous metabolic switches and explains the occurrence of low-yield pathways as observed in the Crabtree and Warburg effects.
In our proof, we use the theory of oriented matroids which can be seen as a high-level abstraction of linear programming. Using oriented matroids, many results in stoichiometric network analysis can be viewed in a uniform framework and proved by elementary arguments.

SS_10

Hierarchical decomposition of metabolic networks using k-modules

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The optimal solutions obtained by flux balance analysis (FBA) are typically not unique. Flux modules have recently been shown to be a very useful tool to simplify and decompose the space of FBA-optimal solutions. Since yield-maximization is typically not the primary objective encountered *in vivo*, we are also interested in understanding the space of sub-optimal solutions. Unfortunately, the flux modules are too restrictive and not suited for this task.
I present a generalization, called k-module, that compensates the limited applicability of flux modules to the space of sub-optimal solutions. Intuitively, a k-module is a subnetwork with low connectivity to the rest of the network. Recursive application of k-modules yields a hierarchic decomposition of the metabolic network, which is also known as a branch-decomposition in matroid-theory. In particular, decompositions computed by existing methods like the nullspace-based approach introduced by Poolman and coworkers can be interpreted as branch-decompositions.
With k-modules we can now compare alternative decompositions of metabolic networks to the classical subsystems of glycolysis, TCA-cycle, etc. They can be used to speed up algorithmic problems (theoretically shown for EFM enumeration) and have the potential to present computational solutions in a more intuitive way independently from the classical subsystems.

SS_11

Reconciling gene expression data with regulatory network models

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The reconstruction of genome-scale metabolic models from genome annotations has become a routine practice in Systems Biology research. The potential of metabolic models for predictive biology is widely accepted by the scientific community, but these same models still lack the capability to account for the effect of gene regulation on metabolic activity. Our focus organism, *Bacillus subtilis* is most commonly found in soil, being subject to a wide variety of external environmental conditions. This reinforces the importance of the regulatory mechanisms that allow the bacteria to survive and adapt to such conditions.

We introduce a manually curated regulatory network for *Bacillus subtilis*, tapping into the notable resources for *B. subtilis* regulation. We propose the concept of Atomic Regulon, as a set of genes that share the same "ON" and "OFF" gene expression profile across multiple samples of experimental data. Atomic regulon inference uses prior knowledge from curated SEED subsystems, in addition to expression data to infer regulatory interactions. We show how atomic regulons for *B. subtilis* are able to capture many sets of genes corresponding to regulated operons in our manually curated network. Additionally, we demonstrate how atomic regulons can be used to help expand/ validate the knowledge of the regulatory networks and gain insights into novel biology.

SS_12

Investigating lipid production in a genome scale model of *Phaeodactylum tricornutum*

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Diatoms contribute up to 40% of marine primary production. They can store carbon in the form of lipid and this fact raises new possibilities to increase algal oil production. However, a better understanding of diatom metabolism is required to optimise the quality and quantity of lipid in order to make them an economical source of biofuel. Among diatoms, *Phaeodactylum tricornutum* is studied widely due to the availability of its genome sequence, comparatively small genome size, short generation time and ease of genetic manipulation.

To this end a compartmentalised genome scale model (GSM) of *P.tricornutum* has been constructed. It is capable of producing all major biomass components in phototrophic and mixotrophic conditions. The model is analysed using linear programming, over a range of light intensities to identify potential metabolic responses.

At low light intensity, the precursor for lipid synthesis, acetyl-CoA is produced via the phosphoketolase pathway, which is uncommon in eukaryotes. This potential role of the phosphoketoalse pathway might explain its relevance in *P.tricornutum.*

At high light intensity, photorespiration is active and glycollate is recycled through a novel pathway. Recycling of glycollate along with uptake of HCO3 leads to increase in lipid production at high light condition. It can also be anticipated that in *P.tricornutum,* increase in lipid production at high light intensity might serve as a mode of energy dissipation.

Funding: European Unions Seventh Framework Programme, grant agreement PITN-GA-2012-316427
http://www.accliphot.eu/

SS_13

Modelling evolutionary reversibility of a metabolic pathway: C_4 photosynthesis

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Can environmental conditions drive the reversion of a previously evolved complex trait? Evolutionary biologists have hypothesized that an evolutionary 'ratchet' often prevents such reversions. We address this question for the complex trait C_4 photosynthesis. This add-on to the ancestral C_3 photosynthesis involves the interplay of leaf anatomy, differential gene expression, and specialized biochemistry. Despite its complexity, C_4 photosynthesis has more than 60 independent evolutionary origins. Recent research in our group has shown that patterns in C4 evolution can be predicted through metabolic modelling of a single environment (Heckmann et al. Cell 2013, Mallmann and Heckmann et al. eLife 2014).

Here, we present a kinetic model of C_3-C_4 intermediate photosynthesis that allows us to study reversibility in a variety of environments relevant to C_4 evolution. This model accounts for empirical evolutionary constraints and is parameterized using *in vivo* temperature and light responses of C_3 and C_4 species. Temperature responses of diffusional processes are described through *in vitro* estimates.

We apply this model to predict the fitness landscapes on which C_3-C_4 evolution takes place. We show how the shape of the fitness landscape shifts with environmental conditions. Further, these landscapes allow us to infer condition-dependent reversion probabilities of the C_4 trait, which are used to interpret the phylogenetic distribution of C_3 and C_4 species.

SS_14

A reductionist approach to model self-regulating systems in plants

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One goal of theoretical biology is to discover organisational principles governing the design of biological systems. For this, often small-scale models are more suitable than large and overly detailed models. Small, kinetic models can facilitate in-depth investigation of individual biological components and serve as a valuable source of information in fundamental research on molecular mechanisms. Here, we present a dynamic model of the photosynthetic chain built on a system of ODEs to study the response of a photosynthetic cell to natural light fluctuations.

In natural conditions, plants are exposed to rapid changes in their environment. In order to dynamically react to external stimuli, they developed intrinsic self-regulatory mechanisms to maintain the redox balance and protect them against photodamage.

To study those mechanisms we built a mathematical model of the photosynthetic electron transport chain that describes the dynamics of its components. We simplified all significant light processes within the thylakoid membrane to only 16 reactions and described them with 10 differential equations.

Our model is able to reproduce a large number of experimental results obtained through spectroscopic measurements. Moreover, we provide a theoretical framework to test existing hypotheses on short-term 'light memory', that plants 'remember' previous exposure to light. We present an explanation how self-regulation is obtained under different light conditions and how it is lost in several mutants, supporting the theory that two components involved in photoprotection act cooperatively.

IS_15

Spatio-temporal models of metabolism in microbial communities

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Metabolism, in addition to being the "engine" of every living cell, plays a major role in the cell-cell and cell-environment relations that shape the dynamics and evolution of microbial communities, e.g. by mediating competition and cross-feeding interactions between different species. Despite the increasing availability of metagenomic sequencing data for numerous microbial ecosystems, fundamental aspects of these communities, such as the unculturability of many isolates, and the conditions necessary for taxonomic or functional stability, are still poorly understood. Our lab develops mechanistic computational approaches for studying the interactions between different organisms based on the knowledge of their entire metabolic networks. In particular, we have recently built a new open source platform for the Computation of Microbial Ecosystems in Time and Space (COMETS), which combines metabolic models with diffusion equations to simulate the 3D spatio-temporal dynamics of metabolism in microbial communities. COMETS has been experimentally tested on small artificial communities, and is in principle scalable to hundreds of species in complex environments. I will discuss recent developments and challenges towards the implementation of models for complex microbiomes.

SS_16

Stoichiometric analysis of primary autotrophy and biomass turnover in a thermoacidophilic iron oxidizing archaeal community

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Microbial communities are responsible for the majority of global nutrient cycling, making them prime targets for controlling greenhouse gas production and eutrophication. However, the complexity of most naturally occurring microbial communities limits their tractability due to the large number of species and interactions. Extreme temperature and pH environments, like those found in Yellowstone National Park geothermal springs, typically reduce community species diversity; these relatively simple communities represent ideal model systems for studying primary and secondary nutrient fluxes through multiple trophic levels. An aerobic, thermoacidiphilic archaeal biofilm community, which grows at 60-70°C and pH 2.7-3.8, was modeled using metagenomics data, direct *in situ* measurements and novel stoichiometric modeling approaches. The most abundant autotroph in the system, *Metallosphaera yellowstonensis* MK1, was modeled as an obligate aerobe which oxidizes iron(II) and various reduced sulfur species while respiring on limiting oxygen; MK1 primary productivity was modeled to constrain the potential community compositions and fluxes. The most abundant heterotroph in this system, *Geoarchaeota* archaeon OSPB-1, modeled recycling of nutrients acquired by MKI via primary producer biomass degradation. This study represents the first stoichiometric analysis of nutrient / biomass recycling in a natural microbial community. Characterization of this geothermal system illustrates constraints of electron donors and acceptors on community energetics and nutrient recycling.

IS_17

Spatiotemporal metabolic modeling of microbial biofilm consortia

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Microbial systems in which the extracellular environment varies both spatially and temporally are very common in nature and in engineering applications. While the use of genome-scale metabolic reconstructions for steady-state flux balance analysis (FBA) and dynamic FBA are common, the development of spatiotemporal metabolic models has received little attention. We present a general methodology for spatiotemporal metabolic modeling based on combining genome-scale reconstructions with fundamental transport equations that govern the relevant convective and/or diffusional processes in time and spatially varying environments. Our solution procedure involves spatial discretization of the partial differential equation model followed by numerical integration of the resulting system of ordinary differential equations with embedded linear programs using DFAlab, a MATLAB code for dynamic FBA simulations. We demonstrate our methodology by formulating and solving a spatiotemporal metabolic model for a two species chronic wound biofilm system comprised of *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The biofilm model is used to explore factors that impact the structure and robustness of the two species system. The model is capable of predicting species partitioning where *S. aureus* dominates the hypoxic region while *P. aeruginosa* is only competitive in the aerobic region. Metabolite cross-feeding enhances species partitioning, especially the competitiveness of *P. aeruginosa* in the aerobic region. Based on these encouraging results, we believe that spatiotemporal metabolic modeling represents a powerful tool for understanding biofilm consortia involved in medical, environmental and engineered systems.

IS_18

A minimal model for explaining the Warburg effect

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For producing ATP, tumour cells mainly rely on glycolysis leading to lactate rather than on respiration. This is known as the Warburg effect (named after German biochemist Otto Warburg) and also applies to striated muscle cells, activated lymphocytes and microglia, endothelial cells and several other cell types. This effect is paradoxical at first sight because the ATP yield of glycolysis is much lower than that of respiration. Although a straightforward explanation is that glycolysis allows a higher ATP production rate, the question arises why the cell does not re-allocate protein to the high-yield pathway of respiration. We tackle this question by a minimal model only including three combined reactions. We consider the case where the cell can allocate protein on several enzymes in a varying distribution and model this by a linear programming problem in which not only the rates but also the maximal velocities are variable. This leads to pure elementary modes, notably pure respiration or pure glycolysis, depending on protein costs. Finally, we propose a way of explaining mixed flux distributions such as in respirofermentation.

IS_19

Reconstruction of *Caenorhabditis elegans* metabolism – a new resource to support healthy aging in nematodes

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While the nematode *Caenorhabditis elegans* represents an important model organism in a broad range of disciplines including developmental biology, aging research and infection research, its metabolic capabilities have not yet been reconstructed on the genome-scale. In my talk I will report on our recent reconstruction of the genome-scale metabolic network of *C. elegans* that will open up *C. elegans* research to a wide array of constraint-based methods. The metabolic network reconstructions further emphasizes the metabolic peculiarities of *C. elegans* that make it distinct from many other animals such as the existence of a glyoxylate bypass. Using halfMADE, a method to map time-course transcriptomic data to metabolic networks, we reconstructed the metabolic state of *C. elegans* during normal aging and under perturbations known to extend life span. Thereby, we were able to show that despite perturbing different parts of metabolism, two life-span extending treatments lead to very similar effects in metabolism on a global scale. This suggests the action of a common mechanism by which the life-span extending effects of the perturbations are mediated.

IS_20

Metabolic reprogramming in the glioblastoma cell: a systems approach

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The complexity of a living system justifies the need for data acquisition at all levels of cell hierarchy from DNA to tissue and organ level delineation. However, just listing candidate genes (From genomic/exome data) or gene expression signatures (from transcriptomic data) are not enough to understand a complex, multi-hit, multifactorial emergent disease like cancer. Glioblastoma, the most severe form of brain cancer is highly complex due to its inherent heterogeneity, and the only drug used to treat it is being rendered less useful due to chemo resistance. To understand the difference between cells of glioblastoma that are resistant or susceptible to temozolomide we have isolated a population of cells from the model cell line U87MG and characterized it extensively using whole exome sequencing, microRNA sequencing, growth-resistance-metabolic profiling and metabolite respiration phenotyping to understand the intrinsic changes in its molecular components and higher order phenotypes. These results will be discussed in the context of a genome-scale flux balance model of human metabolism further constrained by gene expression data. Constraints-based models based on the evolutionary optimality criterion are able to select specific flux patterns that explain the heterogeneity of cells and predict metabolic reprogramming that may be key to investigating resistant mechanisms. This would fill a critical need for predictive models for tumor growth and individualized treatment in personalized medicine.

SS_21

Analysing the contribution of *Plasmodium falciparum* to whole body glucose metabolism in malaria patients

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Malaria, caused by parasitic protozoa from the *Plasmodium* genus, is a dreadful disease from which between 500000 to a million people die yearly, mostly small children in sub-Sahara Africa. Although not generally considered as a metabolic disease, the key-diagnostics for poor chances of survival are hypoglycaemia and lactic acidosis, clearly linked to glucose metabolism. We have developed a modelling framework to analyse the contribution of the parasite to the whole body glucose metabolism in malaria patients. For this we use detailed kinetic models and genome scale structural models at the parasite level together with more coarse grained kinetic and flux based models at the whole body level. The detailed kinetic model is entirely based on experimentally measured parameter values and was validated at the isolated parasite level, at the infected red blood cell level and at the whole body level. The modelling framework makes it possible to analyse drug effects on an individual reaction step in the parasite at the whole body disease state. An inhibitor titration of the glucose transporter is experimentally analysed at the enzyme activity level, at the pathway level in the isolated parasite and at the infected red blood cell level.

IS_22

Exploring *Trypanosoma brucei* energy metabolism using modelling and metabolomics

Fiona Achcar
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Human African Trypanosomiasis is a potentially lethal disease caused by the protozoan parasite *Trypanosoma brucei*. The metabolism of the bloodstream form of the parasite has several unique features that have been investigated, in the search for potential drug targets. Mathematical modelling has been used as a valuable tool to decipher glycolysis, the parasite's only energy source. The earliest models were analysed using a single values for each parameter. We have introduced the notion of uncertainty to the parameter values, and more recently to the topology to the model of glycolysis. This has allowed us to gain a more accurate picture of the model and to highlight parts of the model that do not fit the experimental observations. We then extended this model of glycolysis to include the pentose phosphate pathway, another essential pathway that generates the NADPH used in the cells' protection against oxidative stress, and thus provides a metabolic link to another important drug target in trypanosomes. Mass spectrometry based metabolomics is another valuable tool that enables us to gain a deeper understanding of the parasite's metabolism, either by comparing the metabolic state of cell grown in two conditions, or by using labelled precursors to follow metabolic pathways. Here, we combine modelling and metabolomics to investigate further the energy metabolism and its link with the oxidative stress response in trypanosomes.

IS_23

Strain design for improved productivity, yield and robustness

Radhakrishnan Mahadevan
University of Toronto, Toronto, Canada

Bioprocess development for biofuels and biochemicals typically requires several rounds of metabolic engineering to meet process targets including product yield, titer and productivity, all of which impact the process economics. Similar advances in computational modeling techniques have allowed the development of genome-scale models of metabolism in several organisms. In this talk, the use of such models for metabolic engineering will be presented. In the first part, a rational approach based on bi-level optimization to enhance bioprocess productivity by forcing co-utilization of substrates will be shown. Experimental results from the application of this approach to enforce substrate co-utilization in *Escherichia coli* will be discussed. In addition, we will present a synthetic biology approach for dynamic control of metabolism to improve productivity. In the next part of the talk, a novel nested nonlinear optimization method for metabolic engineering resulting in hundreds of different strain design strategies for biochemicals production will be presented. We will also examine the role of redundant production pathways from a design perspective and present computational results on how these pathways are valuable for robust design.

SS_24

Enforced ATP futile cycling increases specific productivity and yield of anaerobic lactate production in *Escherichia coli*

Oliver Haedicke, Katja Bettenbrock and Steffen Klamt
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The manipulation of co-factor pools such as ATP or NAD(P) H has for long been recognized as key target for metabolic engineering to improve yields and productivities of microorganisms. Several works have already shown that enforcing ATP futile cycling may enhance the synthesis of certain products under aerobic conditions. However, case studies demonstrating that ATP wasting may also have beneficial effects for anaerobic production processes are scarce. Taking lactate as an economically relevant product, we demonstrate that induction of ATP futile cycling in *Escherichia coli* leads to increased yields and specific production rates under anaerobic conditions, even in the case where lactate is already produced with high yields. Specifically, we constructed a lactate producer strain (KBM10111) and implemented an IPTG-inducible overexpression of *ppsA* encoding for PEP synthase which, together with pyruvate kinase, gives rise to an ATP consuming cycle. Under induction of *ppsA*, KBM10111 exhibits a 25% higher specific lactate productivity and an 8% higher lactate yield. Furthermore, the specific substrate uptake rate was increased by 13%. However, trade-offs between specific and volumetric productivities must be considered when ATP wasting strategies are used to shift substrate conversion from biomass to product synthesis. Finally, we discuss potential solutions to design optimal processes. In summary, we conclude that enforced ATP futile cycling has great potential to optimize a variety of production processes and our study demonstrates that this holds true also for anaerobic processes.

IS_25

MODCELL – rational design of modular cells for combinatorial biosynthesis of novel molecules

Cong T Trinh
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Relevant hosts such as *Escherichia coli and Saccharomyces cerevesiae* are commonly engineered and optimized to produce target products through multiple iterative strain optimization cycles. An engineered host that is optimized to produce one target product may not be suitable to function as an optimal host to efficiently produce other target compounds. To address these bottlenecks, we have developed the MODCELL (Modular Cell) tool to design modular cells that can metabolically and genetically couple with a diverse class of exchangeable production modules for combinatorial biosynthesis of novel chemicals in a plug-and-play fashion requiring minimal iterative strain optimization cycles. We will present the design, construction, and validation of an *E. coli* modular cell for a combinatorial biosynthesis of novel bioesters that can be used as fragrances, flavors, solvents, and biodiesels.

SS_26

Analysing the feasibility of growth-coupled product synthesis in microbial strains using the concept of elementary flux vectors

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Growth-coupled product synthesis has become a key principle for metabolic engineering and various constraint-based modeling techniques have been developed to calculate intervention strategies by which a microorganism can only grow if it co-synthesizes a desired by-product. However, growth-coupled synthesis is not feasible for all metabolites. Using geometric techniques we show which structural properties in a network are required such that biomass and product synthesis can be coupled at all. In networks without flux bounds, coupling is feasible if and only if an elementary mode exists that leads to formation of both biomass and product. Setting flux boundaries leads to more complicated inhomogeneous problems. Making use of the concept of *elementary flux vectors*, a generalization of elementary modes, criteria for feasibility of coupling can also be derived for this situation. We applied our criteria to a metabolic model of *E. coli* and determined for each metabolite whether its net production can be coupled with growth and calculated the maximal (guaranteed) coupling yield. The somewhat surprising result is that coupling is indeed possible for all (almost all) carbon metabolites of the central metabolism under aerobic (anaerobic) conditions. Consideration of ATP maintenance requirements may decrease or increase the maximal coupling yields. Overall, our work (i) provides important insights for a central problem of computational strain design and (ii) emphasizes elementary flux vectors as a suitable tool for metabolic pathway analysis in inhomogeneous systems.

IS_27

Mapping the fitness landscape of enzyme expression uncovers the cause of antagonism and sign epistasis between adaptive mutations.

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How do adapting populations navigate the tensions between the costs of gene expression and the benefits of gene products to optimize the levels of many genes at once? Here we combined independently-arising beneficial mutations that altered enzyme levels in the central metabolism of *Methylobacterium extorquens* to uncover the fitness landscape defined by gene expression levels. We found strong antagonism and sign epistasis between these beneficial mutations. Mutations with the largest individual benefit interacted the most antagonistically with other mutations, a trend we also uncovered through analyses of datasets from other model systems. However, these beneficial mutations interacted multiplicatively at the level of enzyme expression. By generating a model that predicts fitness from enzyme levels we could explain the observed sign epistasis as a result of overshooting the optimum defined by a balance between enzyme catalysis benefits and fitness costs. Knowledge of the phenotypic landscape also illuminated that, although the fitness peak was phenotypically far from the ancestral state, it was not genetically distant. Single beneficial mutations jumped straight toward the global optimum rather than being constrained to change the expression phenotypes in a correlated way. Given that adaptation in nature often results from optimizing gene expression, these conclusions can be widely applicable to other organisms and selective conditions. Poor interactions between individually beneficial alleles affecting gene expression may thus compromise the benefit of sex during adaptation and promote genetic differentiation.

SS_28

What elementary flux modes are biologically relevant?

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The calculation of elementary flux modes (EFMs) in metabolic models is known for its combinatorial complexity, which makes their calculation intractable in genome-scale networks. Recently, we introduced thermodynamic EFM analysis (tEFMA), which integrates the metabolome into the EFM analysis. With tEFMA thermodynamically feasible EFMs can be calculated reliably and efficiently even in large-scale networks. Importantly, their number is significantly smaller than the total number of EFMs.

Here we demonstrate the biological relevance of our approach by correctly identifying infeasible pathways in *E. coli* and by unambiguously explaining the experimentally observed behavior of glutamate dehydrogenase under different environmental conditions.

Moreover, we show that only a few out of all thermodynamically feasible EFMs are biologically relevant and can be combined into thermodynamically feasible flux distributions. We identify these largest, thermodynamically consistent sets of EFMs by linear programing. Furthermore, by considering commonly available phenotypic data, we determine that only a handful of these sets can contain a biologically relevant solution. We find that the biologically relevant sets are characterized by their ability to maximize biomass and ATP production, consistent with evolutionary interpretations of cell behavior.

In conclusion, tEFMA avoids the computation of thermodynamically infeasible EFMs and therefore allows for a computationally efficient, unbiased, systems-level analysis of metabolism delivering significant biological insight.

IS_29

Pathway level culture media engineering

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Rational culture media design supported by *in silico* cell models are of paramount importance to decrease the workload of developing novel or custom culture media formulations. In this study we present a method for culture media design using the concept of elementary flux modes. This new method comprises two main stages. In the first stage, a functional enviromics map is built through the joint screening of cell functions and medium factors by the execution of a specific cell culture protocol and exometabolome assays protocol. The functional enviromics map consists of a data array of intensity values of elementary cellular functions against medium factors. In the second stage, optimized cell culture medium formulations are developed that either enhance or repress target elementary cellular functions from columns of the functional enviromics map. The main advantage of this method lies in enabling metabolic engineering through the culture media composition manipulation, wherein an arbitrarily high number of cell functions are optimized through manipulation of medium factors, as opposed to previous methods, which are eminently empirical, are not cell function oriented, and require a much higher number of experiments.

SS_30

In vivo catalytic rates estimated from proteomics match *in vitro* measurements

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The maximal turnover rate of an enzyme, k_{cat}, is a key property constraining protein expression levels and cellular fluxes. Since k_{cat} cannot be measured directly in-vivo, many metabolic models use in-vitro k_{cat} values, measured in conditions that hardly resemble the cytosol. *In-vivo* and *in-vitro* k_{cat} values will deviate because of various factors such as pH, crowding, channelling and regulation. The correspondence between in-vivo and in-vitro k_{cat} values is essential for metabolic flux predictions, yet has never been quantified on a large scale. We present a heuristic approach to estimate *in-vivo* k_{cat} values from catalytic rates of enzymes, derived from recently measured enzyme abundance data and computational flux predictions. The maximal catalytic rate over a large set of cellular conditions is used as a proxy for in-vivo k_{cat} values. We show a strong correlation between *in-vitro* k_{cat} values and our in-vivo maximal catalytic rates, with $r^2=0.6$, $p<10^{-25}$ and a typical error of less then 4-fold. Using a recent decomposition of the Michaelis-Menten framework, we differentiate between thermodynamics, saturation and *vivo-vitro* effects including regulation. This enables investigating the contribution of each aspect to shaping cellular fluxes. The usage of omics data to achieve high throughput kinetic informs our understanding of *in-vivo* enzymatic processes and can serve as input to cellular metabolic models.

SS_31

Testing of network completion algorithms and mutant growth-rate predictions using genome-scale datasets

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Genome-scale metabolic networks promise to facilitate personalized medicine and rational engineering of microbial strains of industrial significance. But, for this promise to become a reality, genome scale-models need to be accurate and have good predictive capability. We investigated the current completeness of draft genome-scale networks and their predictive capabilities.

Bottom-up reconstructed draft networks were unable to utilize a large proportion of included reactions, and were unable to produce a complete set of metabolites required for self-replication. In fact, blast-weighted annotation of the full Rapid Annotation through Subsystems Technology (RAST) biochemistry database (12K reactions) revealed that all tested organisms required the addition of completely unsupported reactions to their networks to produce of a full set of biomass metabolites.

Massive parallel sequencing of a *Shewanella oneidensis* transposon mutant library was used to determine: (1) gene essentiality and (2) mutant growth rates. To call gene essentiality, a probability generating function was developed that accounted for found insertion biases. This approach led to much better agreement with Flux Balance Analysis (FBA) predicted gene essentiality than direct essentiality calls, where any insertion in a gene is interpreted as non-essentiality, and a lack of insertions is interpreted as essentiality. Finally, observed mutant growth rates were used to tests commonly used genome-scale predictive methods.

Poster Abstracts

MPA_01

Integrated contextualisation and analysis of metabolic networks

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Integration of experimental data into metabolic models has become ever more important in the recent years. In particular, the generation of condition and tissue specific networks from generic reconstructions allows to focus the efforts of analysis on the actual target. FASTCORE and its extension, FASTCORMICS, provide efficient ways to incorporate experimental data to obtain consistent models that reflect the current state of the metabolic network. FASTCORE generates consistent models in seconds using a linear problem based on a provided core set. The methodology was extended by FASTCORMICS to allow the use of the solid statistics provided by The Gene Expression Barcode to compute core sets. In addition, low z-scores resulting from the barcode evaluation are used as an indication for absence, further restricting the generated models. FASTCORMICS was able to capture differences in the metabolism of 63 primary human cell types. Another important step in analysis is proper visualisation of the generated metabolic networks and integration of multiomics data. The IDARE Cytoscape app developed in our group along with a web application to tackle this issue. IDARE allows the visualisation of Multiple datasets of omics data on a network structure. It also provides functionalities to divide the network into areas of interest keeping links between the network and thus allows an easy tracking of fluxes.

MPA_02

Shifts in the bacterial metatranscriptome accompanying draught in Namibian agricultural soils

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Even after decades of development, modern agriculture is still highly dependent on water availability. It is known that plant growth-promoting rhizobacteria such as *Paenibacillus polymyxa* can enhance host plant's drought tolerance. The study of the soil microbes can further our understanding in this enhancement mechanism and may one day lead to a less water-dependent farming practice. For these reasons, we took soil samples from Namibia in 2012 and 2013. 2012 received a normal amount of precipitation while 2013 witnessed the worst drought in Namibia for decades. Metatranscriptome analyses were performed on these samples and revealed pronounced differences in bacterial taxonomic and functional compositions among the two conditions. The communities were less diverse in 2013 both in terms of abundances and evennesses. During the drought, Bacteria such as *Paenibacillus* and *Exiguobacteria* were dominating the microbial landscape. On the one hand, samples from 2013 contained fewer nitrogen and phosphorus-related transcripts. In addition, DNA topoisomerases were less frequently detected in 2013. On the other hand, the phosphotransferase system (PTS) was more active in the dry year. Overall, these results suggested that the plants could exert selection on the soil microbes and are more likely to be associated with bacteria that could help the hosts to survive the water shortage. The transcriptome also suggests that the bacterial life strategies shifted from rapid growth to cellular maintenance during the dry year.

MPA_03

FECorr: An algorithm to improve FBA predictions using transcriptomic data

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Flux-balance analysis (FBA) is widely used to predict steady-state flux distributions in genome-scale metabolic networks. However, FBA has some limitations, among them the existence of multiple optima and the neglect of genetic regulation. Information about gene regulation is contained in gene expression data, which is widely available and can be used to improve the predictions of FBA. Here we introduce a novel algorithm, FECorr, that combines transcriptomic or proteomic data with constraint-based metabolic modeling to better predict fluxes. FECorr uses multiple transcription datasets and a metabolic network as input. For each catalyzed reaction, FECorr fits a piece-wise linear function to the relationship between flux variability (FVA) ranges and expression levels across different experimental conditions. For each condition, FECorr then identifies the flux distribution that is closest (in terms of Manhattan distance) to the fitted values. When the method was applied to a dataset which was recently used to benchmark different expression-based methods, we found that FECorr provides more accurate predictions than all previously tested expression-based methods, and was also slightly more accurate than the minimization of total flux (parsimonious FBA). We implemented FECorr in sybilEFBA, an extension to the sybil open source library for constraint-based modeling in R (available from http://cran.r-project.org/).

MPA_04

Impact of intermediate toxicity on the regulation of metabolic pathways

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In recent years, the investigation of optimality principles has provided insights in the regulatory strategies controlling metabolism. In our work, we used a dynamic optimization approach to analyze the influence of toxic intermediates on the regulation of metabolic pathways. To accomplish this, we created a model of a linear pathway with additional constraints simulating the toxicity of intermediates. For this model, optimal regulatory programs were determined by dynamic optimization under scenarios of low and high enzyme costs. We observed a sparse regulation, which is characterized by a regulation only of enzymes, if the enzyme costs are low. Further, our results suggests that these key enzymes are dependent on the toxicity of intermediates. The regulation of the first and last enzyme of a linear pathway is changed to a regulation of enzymes producing toxic intermediates. Assuming high enzyme costs, we determined a regulatory strategy involving all enzymes of a linear pathway equally. Due to this, the influence of toxic intermediates on the regulatory program is smaller, since intermediate concentration is lower. The gathered optimality principles were verified by the investigation of metabolite toxicity predicted by QSAR models and linear pathways of several hundred organisms listed in the MetaCyc database. Our results provide new insights in the regulation of metabolic networks and are valuable for metabolic engineering as well as for the identification of targets for antimicrobial interventions.

MPA_05

Dynamic constraint-based modeling of phototrophic metabolism

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Modeling dynamic environmental conditions is crucial for understanding the behavior of photosynthetic organisms, since light availability follows a day-night cycle. In particular, we are interested in a better understanding of the metabolism of cyanobacteria and green algae. Obtaining this understanding is however impossible using static methods, such as flux balance analysis, because the dynamics of the light availability cannot be properly represented. For this purpose, we build up on recent work. The key idea of our method is that metabolism is inherently autocatalytic. More precisely, the metabolic network produces the precursors involved in the formation of enzymes that in turn catalyse metabolic reactions. This way, the metabolism adapts to a dynamic environment by adjusting the enzyme levels via fine tuning the precursor production. Therefore, the method specifically models dynamic enzyme production and then constrains metabolic fluxes according to the enzyme levels. It optimizes a given objective over the whole time period of interest while taking into account variations in the extracellular conditions. We use this method to understand the internal mechanisms of carbon fixation and the biomass dynamics under a day-night cycle. This is of particular interest nowadays, since photosynthetic organisms have enormous potential in the manufacturing of biofuels and industrial chemicals.

MPA_06

Genome-scale and Flux modeling in the Lemnaceae (*Spirodela polyrhiza*) isoprenoid pathways for predictive metabolic engineering

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The aquatic plant Lemna, commonly known as duckweed, is the smallest and morphologically simplest flowering plant. Being a monocot it is related to crop plants such as maize, wheat and rice. Lemna has many applications, including waste water treatment, biofuels and aquaculture, as well as a food source for animals and humans. Here, we present a framework for metabolic flux modeling to study the metabolic network, particularly the isoprenoid pathway in Lemna. It includes two complementary approaches i.e., the generation of a high-quality, genome-scale metabolic model for primary and secondary metabolism as well as the development of a Metabolic (^{13}C) Flux Analysis model for the Lemna cytosolic MEV and chloroplastic MEP isoprenoid pathways. The genome-scale model will be based on the recently published genome of *Spirodela polyrhiza* and a comprehensive 'omics' data set to improve and verify the model. Metabolic Flux Analysis will be performed for different environmental conditions, such as high-light or salt stress. The use of both, Flux Balance Analysis and Metabolic Flux Analysis models will be applied to predict enhancement strategies for the production of high-value isoprenoid derivatives, such as health-promoting carotenoids or isoprenoid-based biofuels. We anticipate that the outcome of this research will be of great value in metabolic engineering of additional crop plants and metabolic pathways generating different high-value compounds.

MPA_07

The steady-state assumption for oscillating and growing systems

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The steady-state assumption is one of the key formalisms that makes an efficient analysis of genome-scale metabolic networks possible. Formally, it is derived from the assumption that the system does not change (physical steady-state). For long time periods this is clearly not the case. An example is the cell-cycle. Thus, we analyze how much effects based on oscillations and metabolite dilution can be neglected. In this talk, we show that the steady-state assumption also applies to oscillating systems. Based on the common reasoning that metabolites must neither accumulate nor degenerate we mathematically derive the steady-state assumption for the average fluxes over long time periods. However, we also show that the average concentrations may not be compatible with the average fluxes. Furthermore, we show that quantitative effects based on dilution of metabolites can typically be neglected, but infinitessimal overproduction of every active metabolite must be possible. We present an efficient extension to steady-state models that incorporates this aspect. In summary, we establish a mathematical foundation for the steady-state assumption over long-term periods that justifies the successful use of the steady-state assumption in many applications. Furthermore, this mathematical foundation also pin-points unintuitive effects in the integration of metabolite concentrations into steady-state models for long time periods. It allows us to ask mathematically: Does the steady-state assumption reduce metabolic capabilities?

MPA_08

Biofuel production with cyanobacteria: new strain design strategies revealed by computational modeling.

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Cyanobacteria are increasingly acknowledged as auspicious cell factories for the production of renewable biofuels and chemical feedstocks. However, most biotechnological applications of these organisms are still characterized by low yields. Increasing the production performance of cyanobacteria remains therefore as a crucial step. Here we present new strain design strategies for biofuel production with cyanobacteria (specifically for ethanol and isobutanol). Based on a stoichiometric network model of *Synechocystis* sp. PCC 6803 we applied CASOP and constrained minimal cut set analysis to identify intervention strategies, first in a medium-scale and then in the full genome-scale metabolic model. As a key result we show that higher-order knockout strategies exist in both models that lead to coupling of growth with high-yield biofuel synthesis under phototrophic conditions. Enumerating all potential knockout strategies (cut sets) reveals a unifying principle behind all identified strain designs, namely to lower the ratio of ATP to NADPH produced by photosynthesis. We show that suitable knockout strategies seek to block cyclic and other alternate electron flows, such that ATP and NADPH are exclusively synthesized via the linear electron flow whose ATP/ NADPH ratio is below that required for biomass synthesis. The products of interest (e.g., ethanol or isobutanol) must then be utilized by the cell as sinks for reduction equivalents in excess. Interestingly, our analyses furthermore suggest that a moderately increased ATP turnover may also lead to increased biofuel yields.

MPA_09

Evaluation of methods for the reconstruction of specific models from omics data

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Recent efforts have allowed the development of genome-scale metabolic models for several organisms, including humans. These models have been used to predict cellular metabolism under the framework of constraint-based modeling. The application in health related research has spanned the issues of drug discovery, biomarker identification and targeting diseases such as cancer or Alzheimer. However, the human organism includes several cell types, each one with a different metabolic profile and functions. So, it is imperative to develop tissue-specific metabolic models. This challenge was addressed by several approaches, namely the Model-Building Algorithm, Metabolic Context specificity Assessed by Deterministic Reaction Evaluation and Task-driven Integrative Network Inference for Tissues methods. All these approaches use a generic model as a template and integrate evidences from omics data, literature and/or network analysis to infer the tissue specific metabolic model. Nevertheless, their results have not yet been adequately and critically evaluated and compared. We analysed the consistency between several omics data sources and reconstructed metabolic models of hepatocytes using different methods and distinct data sources as inputs. The results show that omics data sources have a poor overlapping and, in some cases, are contradictory. Additionally, the hepatocyte metabolic models generated are dependent on the combination of method and omics data source. Finally, we conclude that reliable methods for a priori omics data integration are required to support human cells models reconstruction.

MPA_10

Investigation of physiological impacts of knockout mutants using a genome scale model of Arabidopsis

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Photosynthesis in plants consists of the light reactions and the Calvin-Benson cycle. The activity of some of the Calvin cycle enzymes, sedoheptulose-bisphosphatase (SBPase), fructosebisphosphatase (FBPase), phosphoribulokinase (PRK) and glyceraldehyde-3-phosphate dehydrogenase (GAPdh), are controlled by the thioredoxin system, which activates them in the light and inactivates them in the dark, thus regulating carbon fixation. To investigate their robustness and flexibility, we performed experiments to knockout the genes associated with these enzymes. Although with compromised growth all the mutants, except PRK, were viable. In order to explain how the metabolism can compensate for these knockouts, we repeated the investigation on a genome scale model of Arabidopsis using linear programming and elementary mode analysis. It was found that SBPase and FBPase can play a compensatory role on each other's absence and in conjunction with tranaldolase thus maintaining the flux through the regenerative limb of the Calvin cycle. In case of G3Pdh knockout, its cytosolic isomers were taking over the function of reductive limb thus resulting a feasible solution. The PRK knockout was not feasible. A feasible solution was also possible for a dual knockout of SBPase and FBPase with the help of transaldolase activation, import of glucose-6-phosphate, as opposed to its export in the wild type and the cytosolic isoform of FBPase. Experimental validation for the double knockout is currently in progress.

Funding: AccliPhot (http://www.accliphot.eu/), European Unions Seventh Framework Programme, grant agreement PITN-GA-2012- 316427.

MPA_11

Hub reactions in storage of selected compounds in heterotrophic plant cell network

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Pathway-based analysis of plant metabolism is still interested for example to study the accumulation of interesting metabolites in plant cells. Tools comming from graph theory have been developped, EFMs algorithm (Schuster and Hilgetag 1994) allows to find all minimal and unique feasible pathways and MCSs algorithm (S. Klamt 2005) to set which reactions are able to stop the flux through these pathways. We have combined these two algorithms to study how the essential metabolites can be produced whereas there are not entry of Glucose (carbon source). Our analysis have been performed with a heterotrophic plant cell network - 78 reactions and 55 metabolites (Beurton et al. 2011). We have selected 5 reactions in charge of the production of 5 output metabolites: Starch, Fructose, Glucose, Sucrose, and Glutamate, and from then built 5 matrices of EFMs able to produce them. Analysis of small MCS (size 2, 3 or 4) of these matrices provides a way to find common motifs (sets of reactions) through EFMS matrices. The main result is first a list of 8 reactions which are all mandatory to produce the 5 outputs when glucose uptake is blocked and second a list of 5 alternative reactions which can be viewed as branch points from which it is possible to find similar motifs through the network. In conclusion, we have been able to identify hubs reactions to produce output metabolites of interest.

MPA_12

Drug target identification in a *Salmonella typhimurium* metabolic model

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Salmonella typhimurium (*S. typhimurium*) is a model organism for Gram-negative, intracellular pathogens, for which new antimicrobials are urgently sought. *S.* typhimurium can utilise several nutrients, which, in combination with its robust metabolism, makes identification of metabolic drug targets difficult. Here we identify reaction sets that when removed will impair energy generation. It has previously been shown that the catabolic core (the set of reactions required for generation of energy and precursors for biomass synthesis) can be identified from genome-scale models (GSMs) by simulating changes in ATP demand using linear programming and identifying reactions that co-vary with ATP demand. In previous contributions a single carbon source has been assumed. Here the analysis was repeated assuming the availability of several nutrients, and the superset of responding reactions analysed. We identified all sets of reactions in the core model that were of size less than four and that abolished energy generation when removed from the network. The global catabolic core, i.e. the superset of cores obtained from the different nutrients, contained 170 reactions. Damage analysis indicated that 180 reaction sets, of size two or three, forced an increase in total flux of 25% or more in the GSM when removed. Out of these, 20 reaction sets, primarily involved in pentose phosphate pathway and amino acid biosynthesis, were indicated to be lethal, and were thus identified as potential drug targets.

MPA_13

A comparison between Flux Balance Analysis and cellular constrained models of simplified metabolic networks

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Based on reaction stoichiometries, Flux Balance Analysis (FBA) has been used to simulate genome-scale metabolism. However, FBA fails to predict important metabolic phenomena related to physical constraints other than stoichiometry, e.g., the shift from efficient to inefficient metabolic pathways when nutrients are abundant (overflow metabolism). To go beyond FBA, other important cellular constraints must be considered, such as the limitation in volume for enzymes and metabolites, the limitation in surface area for transporters, and the costs of enzyme production. All these require a more detailed modeling of reaction kinetics; different approximations of kinetics have been used in the literature. We implemented a simple whole-cell model based on previous work, considering concentration-based Michaelis-Menten kinetics. We then compared the whole-cell model simulations to results obtained with the same model using approximations for the reaction kinetics, with results obtained using standard FBA, and with results obtained using FBA with limited volume available for enzymes.

MPA_14

Flux variability analysis to understand *Arabidopsis* response to sulfur limitation.

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Transcriptomics approaches are widely used to study plant responses to various perturbations, however the large data sets generated can be difficult to interpret. Genome scale models of metabolism can be used as a framework to generate further insight into transcriptomic data, and conversely the data can be used to refine the model. Here we use Flux Variability Analysis to compare predictions of plant response to Sulfur starvation to measured changes in transcription, using a genome scale model of the *Arabidopsis* metabolic network, coupled to equations capturing induction of high affinity sulfate transporters, and catabolism of amino acids. We find that the model is largely able to recapture binary genetic responses to S starvation, although not generally the magnitude of the response. Furthermore, through the application of a novel, parsimony based method, we are able to identify regions of the model network requiring further manual curation.

MPA_15

Interpreting systematic properties of the tomato photorespiratory metabolism by using a genome-scale metabolic model

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Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop grown in the world, next to potato. Its growth, yield and fruit quality can be affected by several environmental factors, among which hot and dry conditions are widespread abiotic stresses limiting the growth and yield of crop plants. It is known that the occurrence of photorespiration dramatically increases under hot and dry stressed conditions as the leaf stomata are closed to prevent water loss, resulting in reduced CO_2 available for photosynthesis. Therefore, the regulation of photorespiration has been a target to improve crop production. Thus far, most constraint-based modelling contributions have been in the area of plant growth and primary metabolism with very little work focused on changes in metabolism in response to abiotic stresses such as drought. In this study, we describe the reconstruction of a genome-scale metabolic model representing a developing leaf cell of tomato. To understand the metabolic behaviour of tomato in response to hot, drought stressed conditions, we attempt to capture cellular metabolic characteristics and the interplay of photorespiration with other pathways under stressed conditions. Using flux balance analysis we identify several essential enzymes/reactions for the cell growth across the functional pathways such as the Calvin cycle, and photorespiration, most of which are consistent with published experimental observations. Our predictions also show that plastidic and peroxisomic glycolate transporters play a crucial role in the photorespiratory cycle. The functional roles of the essential enzymes/reactions reported here are to be validated experimentally.

MPA_16

Ancestral metabolic networks and phenotypic evolution in *E. coli*

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How does phenotypic diversification relate to genomic evolution across *E. coli* strains? To answer this question, we took advantage of the availability of well curated genomes and carefully reconstructed metabolic networks for 55 *E. coli* strains. We reconstructed the genomes and metabolic networks of all ancestral strains. For all extant and ancestral strains, we determined nutritional phenotypes, defined as the ability to grow across a set of 654 environments. We applied flux-variability-analysis (FVA) and flux-balance-analysis (FBA) combined with minimization of total flux (MTF) to determine optimal flux distributions in each environment. We defined a reaction as optimal if it has non-zero-flux in the MTF solution, and auxiliary if it can have non-zero-flux according to FVA but is not part of the optimal solution. We then examined how divergence of phenotypic traits (nutritional phenotypes, flux distributions, essentiality, optimality) co-evolves with gene content and amino acid sequence divergence. Furthermore, we studied the association between genes in horizontal gene acquisitions and loss events. We constructed a network with nodes representing genes, connecting gene pairs with statistically significant evolutionary association. The resultant network has a dominant cluster containing 20% of all nodes; the remaining 80% form small isolated clusters. While most edge-connected gene-pairs are close neighbours in the genome and were likely co-transferred, ~20% are separated by large genomic distances and are likely derived from different horizontal gene transfer events.

MPA_17

Reconstruction and validation of *i*TR383, a genome-scale metabolic model for *Helicobacter pylori* 26695

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Helicobacter pylori 26695, a highly pathogenic bacterium, is a human gastric epithelia colonizer, correlated with the development of duodenal and gastric ulcers, and gastric cancer worldwide. Its genome has been previously sequenced and annotated, and two genome-scale metabolic models have been developed; however, since their publication, vast amounts of data and new methodologies have been developed. In order to maintain accurate and relevant the information on this bacterium, and to generate new information and new approaches for its analysis, the assignment of new functions to *H. pylori* 26695's genes was performed and a new genome-scale metabolic model was reconstructed. This work originated the *i*TR383 metabolic model, a compartmentalized model containing 383 genes and composed by 640 different reactions and 412 metabolites.
Gene essentiality analysis and growth simulations were performed using experimental data and nutrient uptake rates to assess the predictive capabilities of the model. Metabolite and flux distribution in the central carbon metabolism pathway using different carbon sources were analysed, as well as pathways for non-essential amino acids biosynthesis, the nitric oxide influx effect, and electron transport and respiratory chain.
This model accurately predicts *H. pylori*'s phenotypic response to different carbon sources and is in agreement with experimental results obtained in minimal and complex media. We believe that this work represents a significant advance in understanding *H. pylori* 26695's metabolism and will provide relevant biological information to the scientific community working on new approaches for enhanced treatments.

MPA_18

The severity of enzyme mutations strongly influences the number of affected metabolic pathways

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We study how the severity of a mutation affects pleiotropy in genome-scale metabolic networks. We measure pleiotropy as the number of biomass components whose maximal production is reduced by the mutation. For each biomass component, we added a new exchange reaction and maximized the corresponding secretion. For each gene, we simulated mutations of different severity by restricting the flux through all reactions catalyzed by the gene to a fixed fraction of the wildtype flux, which we reduced in 10%-steps. We then examined how the maximal production of each biomass component differs between mutant and wildtype. For a genome-scale model of *E. coli* metabolism in minimal medium, we found that mutations for 16% of genes whose knockout resulted in a fitness reduction showed a linear relationship between flux reduction and biomass production. A further 4% showed a steeper decline of biomass production at more severe mutational strengths. The majority of genes show either zero or constant pleiotropy independent of mutation severity. However, about 21% of essential genes show a stepwise increase of pleiotropy with increasing mutation severity. A large fraction of pleiotropy is associated with energy production: when we make ATP freely available, 26% of previously pleiotropic genes no longer affected the production of biomass components, with a further 8% showing reduced pleiotropy.

MPA_19

Dynamic modelling of cell metabolic behaviour: a work in progress

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A kinetic model was first developed to describe plant cells metabolic behaviour, including central carbon and amino acids metabolism, cell respiration and energetics. This model was then successfully used as a culture state estimator for the control of intracellular inorganic cytoplasmic phosphate, and for the identification of medium feeding strategies to maximize the production of a secondary metabolite. The model was then transposed to simulate CHO (Chinese Hamster Ovary) cell culture behaviour, and shown to allow extracting quantitative information such as metabolic flux rates as well as cell energetics, with time, from a limited dataset normally acquired in the industry. This model has also been applied to the analysis of CHO cells clonal variations, while increasing the metabolomic dataset. Used as an *in silico* platform, feedback and feedforward regulatory mechanisms known to occur to either inhibit or activate fluxes of glycolysis were implemented in the model, which has enabled testing various regulatory scenarios for their effects on the cell metabolic response to an hypoxic perturbation. More recently, we have studied the model capacity to describe and define fed-batch strategies, facing serious bottlenecks that were attributed to media composition complexity, with various non-quantified and thus not simulated components, as well as to hard to describe phenomena such as cell viability and cell-cell signalling. Thoughts on the amelioration of the predictive capacity of such dynamic models will be discussed.

MPA_20

A model for the expression dynamics of the nicotinic acid degradation pathway in *Pseudomonas putida* KT2440

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Nicotinic acid (NA), also known as vitamin B3, is found in every living cell as part of pyridine cofactors (NAD and NADP), and is essential in those organisms not able to synthesise it. Bacteria such as *Pseudomonas putida* can also catabolise NA for growth. The process by which bacteria make decisions on how to best utilise valuable resources like NA is tightly regulated. Here we investigate the dynamic response of the *nic* genes responsible for NA degradation by modelling their expression with first order ordinary differential equations (ODEs). The catalytic activities are organized in two pathways: the upper, which takes NA and converts it into the intermediate 6-hydroxynicotinic acid (6HNA), and the lower, which converts 6HNA into 2,5-dihydroxypyridine which is then ultimately metabolised into fumarate and ammonia. Each pathway is regulated by one distinct transcriptional repressor, and these repressors also control each other. We generated a set of seven ODEs describing the system. Simulations confirmed by preliminary empirical data show that the repressors initially keep all activities shut down or at a basal level. In the presence of NA however, we see a transient accumulation of enzymes and metabolites followed by fast decay to steady state. NA metabolism is therefore under strict regulation, with genes only turned on under favorable conditions of NA availability. This is optimal for a system that needs efficient and appropriate responses to changing external environments.

MPA_21

Imputing enzyme kinetic constants

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To realistically model genome-scale metabolism requires knowledge of enzyme kinetic constants. Such data is stored in enzyme databases; however, kinetic parameters are available for at most a few hundred enzymes even for the best studied organisms. To enable realistic genome-scale metabolic modeling with enzyme kinetics, we propose an approach to impute kinetic constants, based on data obtained (i) under different conditions; (ii) for different species; (iii) for different substrates; and (iv) for similar biochemical reactions (based on EC enzyme classifications). Temperature and pH dependence can be modeled by appropriate functions. All other available data will be used to obtain a weighted average, where data from different species is weighted by phylogenetic relatedness (or amino acid sequence similarity), and data from similar biochemical reactions is weighted according to the EC hierarchy. We do not provide an implementation, but present the general approach to solicit collaborations from experts in enzyme kinetics.

MPA_22

Dynamic metabolic flux analysis of hybridoma cells cultivated in perfusion mode

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In this work, metabolic flux analysis is applied to determine the flux distribution within a metabolic network of 72 biochemical reactions representing the metabolism of HB58 hybridoma cells producing antibodies type IgG1, anti-CD54, specific of mouse kappa light chain.

Even though a relatively large set of extracellular measurements are available, including the time evolution of the extracellular concentrations of glucose, glutamine, lactate, alanine, ammonia, 16 amino acids, biomass, IgG and the oxygen uptake rate, mass balancing leads to an underdetermined system of equations and a unique solution cannot be computed. To tackle this problem, a convex analysis approach is used to compute the metabolic fluxes as positive bounded intervals, with the help of the toolbox METATOOL.

The experiments under consideration are performed in a 2-L bioreactor, and involve an initial batch phase followed by a perfusion phase. The application of MFA to the batch phase and the steady state of the perfusion phase is straightforward. More care has however to be exercised to analyze the transient part of the perfusion phase. To this end, the dynamic evolution of the uptake and excretion rates has to be inferred from the experimental data and MFA is applied continuously along time, so as to evaluate the dynamic evolution of the metabolic flux intervals, providing insight into the cell metabolism adaptation when the operating conditions are changed from batch to perfusion.

MPA_23

Reconstruction of a genome-scale metabolic model for *Actinobacillus succinogenes*

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Actinobacillus succinogenes, a gram-negative bacterium, is one the most promising natural producers of succinate. This chemical has been well established as a bio-based chemical platform to produce bulk chemicals (e.g. 1,4-butanediol) and other biomaterials, but the costs associated with the bioproduction of succinate are still discouraging. One of the reasons is that succinate is often produced together with other fermentative products like formate, acetate and ethanol under anaerobic conditions, which reduces the cost-effectiveness of this fermentative bioprocess[1]. Systems biology approaches may be required to provide valuable insights into the metabolism underlying the homofermentative production of succinate and contribute to new developments in the bio-based production of succinate[2]. A genome-scale model of the metabolism of *A. succinogenes,* accounting for 500 genes, 930 reactions, and 690 metabolites, was reconstructed and validated against published experimental data. Flux Balance Analysis and Flux Variability Analysis were used to investigate flux distributions within the metabolic network. A thorough model-driven analysis was performed to explore the metabolism under hetero- and homo-fermentative conditions. The model provided valuable insights into the metabolism of this bacterium and has the potential to predict the phenotypes of perturbed metabolic networks that promote the homo-fermentative production of succinate. Acknowledgements: This work was supported by BRIGIT (FP7 project) and PEM co-funded by the ERDF under the Operational Programme for Competitiveness Factors (COMPETE).

References: 1. Mckinlay, J. B., et al. Appl. Environ. Microbiol. 71, (2005). 2. McKinlay, J. B. et al. BMC Genomics 11, 680 (2010).

MPA_24

Metabolic modeling of microalgae growth and lipids production during day/night cycles and nitrogen starvation

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Microalgae have recently received specific attention in the framework of renewable energies, particularly their ability to produce lipids for biofuels. Despite research efforts in the last decade, production yields are still low and need to be optimized. Metabolic modelling can pave the way to this optimization, by setting up a thorough understanding of carbon storage metabolism in microalgae. However metabolic modeling frameworks rely on the balanced-growth hypothesis and microalgae exhibit an unbalanced growth during day/night cycles.

We developed a new metabolic modeling framework (named DRUM) to represent dynamic unbalanced metabolism. The approach consists in splitting the metabolic network into sub-networks. Then each sub-network is reduced to macroscopic reactions using Elementary Flux Modes analysis, for which simple kinetics are assumed.

The DRUM framework was successfully applied to describe the accumulation of lipids and carbohydrates of the microalgae *Tisochrysis lutea* under day/night cycles and nitrogen starvation, using a simplified metabolic network including 160 reactions. The obtained model contained 10 parameters of freedom and accurately predicted the dynamic of accumulation of lipids and carbohydrates, the total organic carbon and nitrogen content and the chlorophyll content. We showed that a regulation of the metabolism occurs during nitrogen starvation. Several regulation scenarios are however possible, including organic carbon excretion and dissipation of energy (e.g.: non-photochemical quenching).

This work provides new insights into metabolic changes during nitrogen starvation.

MPA_25

Visualizing omics data in the OptFlux workbench

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OptFlux is an open-source and extensible workbench for Metabolic Engineering (ME) tasks. Since 2012, OptFlux adoption has been steadily increasing among users, making it one of the reference go-to platforms among the ME community. The workbench supports common ME tasks such as phenotype prediction for both wild-type and mutant genotypes, metabolic control analysis and pathway analysis as well as strain optimization procedures. Moreover, a visualization plug-in is included, allowing the navigation and edition of biochemical network layouts in a multitude of standard formats. This plug-in also allows the overlap of specific phenotypic conditions in the network layouts, providing an intuitive mechanism to explore and understand the associated flux distributions. Navigation between multiple layouts is also included.
However, for more specialized applications, such as the inclusion of experimental data, this framework was still lagging behind. In this work, the current visualization platform included in OptFlux is extended to support loading generic experimental data sources (e.g. transcript, protein, metabolite and flux measurements) and mapping it to the model information for posterior overlap with the layouts. The visualization features that will represent this data are also fully customizable. The inclusion of multiple conditions or time-dependent measurements is also supported for metabolite-associated data with intuitive bar-plots being displayed for immediate visual comparison. Finally, compound structural information from KEGG is also automatically downloaded and presented.

MPA_26

Mathematical models of glucosinolate metabolism in plants

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Glucosinolates are nitrogen- and sulfur- containing plant secondary metabolites, found principally in the order Brassicales. Glucosinolates are precursors of isothiocyanates, which play an important role in the defense against herbivores. Biosynthesis of glucosinolates takes place in three phases (i) chain elongation of selected precursor amino acids, (ii) formation of core glucosinolate structure, and (iii) secondary modification of the amino-acid side chain. Together with side-chain elongation, secondary modifications result in more than 120 known glucosinolates. A particular difficulty in the analysis of secondary metabolites is the vast diversity of different chemical structures. Considering the types of biochemical transformations involved in secondary metabolite biosynthesis, in principle an infinite number of chemical structures could be produced. Apparently, developing models in which all possible structures are represented as a unique variable are clearly infeasible. To elucidate which factors determine the diversity of secondary metabolites produced, we develop mathematical models simulating the biosynthetic pathways of abundantly found glucosinolates derived from methionine in *Arabidopsis*.

MPA_27

Evaluation of carbon sources for recombinant enzymes production in *E. coli* – an *in silico* analysis of the host metabolism

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Recombinant protein expression has been successful applied to produce low-cost hydrolytic enzymes. The use of high cell density culture (HCDC) is especially interesting to increase the protein production titer based on a good nutrient feeding strategy since it affects the metabolic fluxes, and consequently the maximum cell concentration, specific productivity of rEnzymes and by-products formation. Therefore, a deeper comprehension of the host carbohydrate metabolism in order to improve biomass yield is essential.
This work aimed at evaluating the performance of *E. coli* growing on glucose, glycerol and xylose, as the most relevant carbohydrates in a biorefinery context. *In silico* metabolic flux analysis was used to analyze and better understand the flux distribution under these carbon sources. The model could accurately predict acetate production on glucose and xylose, during the exponential growth phase where a pseudo steady-state condition can be assumed. Acetate production on glycerol-based media was also predicted, despite the fact that experimentally that was not observed. When maximizing biomass production, the specific growth rate was similar, around 1.24 h^{-1}, in these three carbon sources. Growth on glycerol promoted the production of succinic and formic acids, which were observed *in silico* with fluxes of 2.64 and 1.28 mmol/gDW.h, respectively. As a conclusion, xylose appears as a promising alternative carbon source for recombinant hydrolases production in a sugar cane biorefinery stablishment and *in silico* analysis can support optimization studies aiming at HCDC process development.

MPA_28

In silico analysis of retinoid metabolism

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Retinoid metabolism affects a broad range of disease and normal developmental, and cell proliferation states. The main action of retinoids are via retinioc acid (RA) binding to nuclear transcription factors, such as the retinoic acid receptor, in target tissues. The source of retinoid in animals is through dietary sources (β-carotene, retinyl esters), processed in enterocytes, then transported via blood mainly to be in hepatic stellate cells for later mobilization. This complex set of interrelated cell types and reactions can be better understood using a systems-level model than the more typical approach of only a few proteins, such as the modeling in the humancyc.org. We have parsed retinol metabolism from that database into 5 compartments (blood, enterocyte, stellate, hepatocyte, target cells) and updated it to include new human reactions. This stoichiometric model constructed in CellNetAnalyzer was evaluated for elementary flux modes and robustness. The few physiological effects observed in loss-of-function studies in human cells (e.g., stellate PNPLA3) are consistent with the robustness calculations in our model (12% change in blood ROH, loss of 7% of pathways). The availability of a stoichiometric model of retinoid metabolism should serve as a balance of the tendency to oversimplify the role of compartmentation in understanding the basis of disease.

MPA_29

The evolutionary footprint in metabolic genes of *Arabidopsis thaliana*

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The evolution of organisms is driven by random mutations at the level of the genome and constrained by selection pressure acting at the level of the phenotype. While mutations increase allelic diversity, any resulting change to organism 'fitness' invokes selection pressure to act in the direction of that change, subsequently constraining diversification.

Our interest lies in understanding how evolution may be acting within and upstream of genes encoding for metabolic enzymes. Random mutations here would most likely alter enzyme amino acid sequence and its kinetic properties, resulting in attenuated cell fitness. Our working hypothesis is that evolution would act negative selection pressure to purify deleterious forms of the alleles, thereby drastically reducing allelic diversity. Using SNP data from 1001 Genomes project MPICWang2013 we calculate the Shannon Entropy to measure the diversity at every nucleotide base position and average allelic diversity over each gene across the genome, between 343 wild inbred accessions of *Arabidopsis*.

The distribution of average Shannon entropy values of metabolic genes showed significantly lower allelic diversity than non-metabolic genes, with p-value of 1.7×10^{-8}. This conservation was further supported from observations of lower SNP density and a higher proportion of synonymous substitutions in their sequences. Genes of enzymes in central carbon metabolism showed the lowest allelic diversity across the population. We further investigated whether a select few features of the genome-scale metabolic network influenced diversification and evolution of metabolic genes.

MPA_30

Flux balance analysis of integrated host-virus metabolic models

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Flux balance analysis provides an effective approach for analysing the flow of metabolites through a metabolic network and uses linear optimisation for any given objective. For most genome-scale metabolic models this approach can be used to obtain solutions of biological interest such as biomass accumulation. This approach has recently been extended to model host-pathogen metabolic interactions, typically exploring infection of cells by bacterial pathogens. In the case of describing host-virus metabolic interactions, virus models must be directly integrated in the host model, as viruses lack their own metabolism and become entirely reliant on the host's own metabolic network. To model the host-virus system and associated flux distribution within the network, we develop here a multi-objective approach. Host and virus objective functions are weighted against each other and network perturbations identified. Three optimality situations are then created: host optimised; virus optimised; and a range where both the host and pathogen are being optimised. Utilising the constraints based reconstruction and FBA approaches these situations are analysed and the changes in reaction flux, metabolite usage and optima values highlighted. This analysis reveals changes that are conducive to a shift from host to virus optimised system and provides insight into the potential metabolic changes that occur with infection.

MPA_31

An adaptive scenario for the origins of complex innovations

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How innovations originate remains a central challenge in evolutionary biology. Innovation in metabolism allows the utilization of new nutrients, and arises through the integration of new metabolic reactions into the network. In many cases, metabolic innovations depend on the simultaneous acquisition of multiple reactions that provide little or no benefit individually. It has been argued that such complex innovations may arise through the non-adaptive exploration of phenotype space, but it remains unclear if such processes are widespread and fast enough to explain the metabolic diversity observed.

Here, we investigate how complex metabolic evolution can instead arise through purely adaptive processes. We traced *in silico* how bacterial metabolic networks can evolve across hundreds of different nutrient conditions. The analysis revealed that the *Escherichia coli* network can generally utilize novel nutrients through the addition of just one to three metabolic reactions, but the endosymbiont Buchnera has to acquire 80 reactions on average. We also demonstrate that temporally varying nutrient conditions can accelerate the adaptive expansion of metabolic networks: novel environments serve as stepping stones towards the establishment of more complex pathways. Contingent gain of metabolic genes on the bacterial tree of life and results of a short-term laboratory evolutionary study in the same species provided empirical support for the scenario.

We conclude that complex innovations in metabolic networks can evolve through a series of adaptive steps without the need to invoke non-adaptive processes

MPA_32

TDPS - Turnover dependent phenotypic simulation: a quantitative constraint-based simulation method that accommodates all main strain design strategies

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Constraint-based modelling methodologies can expedite the strain engineering process by helping in the search for interesting genetic modification targets. Although the search for gene knock-outs is fairly established with *in silico* methodologies, most computational strain design methods still model gene up/down-regulations by forcing the corresponding flux values to pre-calculated levels without considering the availability of resources.

We have developed a new simulation method, Turnover Dependent Phenotypic Simulation (TDPS), which was designed with the goal of simulating quantitatively the phenotype of strains with diverse genetic modifications in a resource conscious manner. Besides gene deletions and down-regulations, TDPS can also simulate the up-regulation of metabolic reactions as well as the introduction of heterologous genes or the activation of "dormant" reactions. In TDPS the flux values through modified metabolic reactions are modelled by taking into consideration the availability of precursor metabolites in the network, which is accomplished by assuming that the production turnover of a metabolite can be used as an indication of its abundance. The developed method is based on a MILP formulation that manipulates the fractions of metabolite turnovers consumed by the modified reactions. Furthermore, TDPS also integrates a new objective function that promotes network rigidity in order to predict the flux phenotype of modified strains. TDPS was validated using metabolically engineered *S. cerevisiae* strains available in the literature by comparing the simulated and experimental production yields of the target metabolite.

MPA_33

The effect of light on the evolution of C4 plants

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C_4 photosynthesis, an extension of the ancestral C_3 pathway, is more efficient under conditions of high photorespiration, but is energetically more costly. C_4 photosynthesis evolved at least 66 times independently, indicating a low evolutionary barrier for the expression of this trait. It is assumed that the evolution of C_4 is triggered by environmental factors that prompt high photorespiratory rates, such as unlimited access to light and low CO_2 concentrations. To better understand the effects of environmental factors on C_4 evolution, we create an evolutionary mathematical model representing the effect of light on the fitness of C_3-C_4 intermediate species. We solve the non-linear optimization problem for energy partitioning in these plants. C_3 and C_4 photosynthesis are limiting cases of our model, allowing the prediction of evolutionary trajectories that connect these two states. We identify environmental subspaces that are favorable for either C_3 or C_4 photosynthesis. Simulating artificial environments with our model allows the development of optimal evolutionary strategies for experimental evolution, potentially contributing to moving C_3 plants toward C_4 phenotypes.

MPA_34

Context-specific metabolic model extraction based on regularized least squares optimization

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Genome-scale metabolic models have proven highly valuable in investigating cell physiology. Recent advances include the development of methods to extract context-specific models capable of describing metabolism under more specific scenarios (*e.g.,* cell types). However, none of the existing computational approaches allows for a fully automated model extraction and determination of a flux distribution, independent of user-defined parameters. Here we present RegrEx, a fully automated approach that relies solely on context-specific data and ℓ_1-norm regularization to extract a context-specific model and to provide a flux distribution that maximizes its correlation to data. Moreover, the publically available implementation of RegrEx was used to extract 11 context-specific human models using publicly available RNAseq expression profiles. The comparison of the performance of RegrEx and its contending alternatives demonstrates that the proposed method extracts models for which both the structure, *i.e.*, reactions included, and the flux distributions are in concordance with the employed data. Therefore, our study sets the ground for applications of other regularization techniques in large-scale metabolic modeling.

MPA_35

Analysis of pathways involved in glycerol fermentation by two novel anaerobic bacteria

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As just a minority of the microbial world is known, Nature is as an enormous reservoir of microbial biocatalysts with potential use in biotechnology. Research (ERC grant project 323009).was done to show that novel anaerobes with biotechnological potential can be isolated from Nature. We are currently isolating and characterizing novel fermentative anaerobes that produce organic acids and alcohols using glycerol, a cheap side-stream of biodiesel production as feed stock. The metabolic pathways of product formation are studied by employing genomics and proteomics methods.
Ercella succinigenes, a bacterium that forms succinate as main product from glycerol, was isolated from a wastewater treatment plant [1]. The bacterium is phylogenetically related to *Saccharofermentans acetigenes*. Genome-guided physiological studies were done to get insight into the metabolic limitations of the bacterium to improve product formation. Succinate is an important compound in organic synthesis and to produce biodegradable plastics.
A glycerol-fermenting *Trichococcus* strain was isolated by us from methanogenic sludge [2]. It ferments glycerol to 1,3-propanediol (PDO) as main product. PDO is an important organic chemical for synthesis of polyesters and polyurethanes and it can also be used as solvent, antifreeze or protective agent.

[1] van Gelder et al. (2014) *Ercella succinigenes*gen. nov., sp. nov., a new anaerobic succinate-producing bacterium. Int J Syst Evol Microbiol 64: 2449-2454.
[2] van Gelder et al. (2012). 1,3-Propanediol production from glycerol by a newly isolated *Trichococcus* strain. Microb Biotechnol 5: 573-578.

MPA_36

Escherichia coli redox metabolism for the production of polyhydroxybutyrate using different substrates

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The synthesis of industrial compounds through biotechnology has a growing interest and present a promising approach for diverse processes. Especially its applications for the replacement of petrochemical products. Although a significant progress has been achieved in metabolic engineering, the rational design of microbial cell factories is hampered by gaps in knowledge of the metabolic regulation. Particularly limited knowledge of fundamental processes, like a quantitative kinetic description of the growth process. A series of interesting metabolic products require the metabolites acetyl-CoA and NADPH, which are in direct competition with growth. To obtain a significant sink of these metabolites, this study uses as a model pathway Polyhydroxybutyrate (PHB) production, where it will be investigated the metabolite-gene interactions, under different scenarios. The model product PHB allows to manipulate the demands of acetyl-CoA and NADPH drastically. Moreover, the supply of NADPH can be manipulated by introducing alternative pathways, especially a modified Embden-Meyerhof-Parnas (EMP) pathway. Next to genetic modifications, different carbon sources were applied to analyse the metabolic fluxes obtained in each product pathway. In addition, the use of different carbon sources provided an insight about the carbon to electron ratio (e^-/C), growth rate and yield.

MPA_37

Markov-Chain Monte-Carlo sampling of metabolite concentrations to identify thermodynamically feasible reaction directionalities for flux balance analysis

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Flux balance analysis (FBA) is a widely used tool for both the understanding and design of cell metabolism. Without any kinetic data, qualitative as well as quantitative predictions of metabolic flux activity to achieve optimal growth can be made. It is also computationally inexpensive as only linear problems need to be solved. Among its shortcomings, however, is the lack of a rigorous enforcement of the thermodynamical feasibility of reactions; this is due to the fact that FBA's steady-state assumption evades the consideration of metabolite concentrations, which impact changes in Gibbs' free energies. Existing approaches to ensure thermodynamic feasibility are based on computationally expensive mixed-integer linear programming.

Here, we propose a Markov-Chain Monte-Carlo (MCMC) approach to identify metabolite concentrations that ensure thermodynamic feasibility. We sample metabolite concentration vectors, and use these to determine reaction directionalities that violate thermodynamic constraints. We then identify a biomass-producing FBA solution with minimal total flux *F* through these infeasible reactions. Solutions with lower *F* are deemed more likely, and hence the sampled concentrations are expected to converge towards thermodynamically feasible solutions.

We compare our approach to existing ones with regard to solution spaces and runtime and give an overview of potential improvements.

MPA_38

Enhancing the production of mannosylglycerate in *S. cerevisiae* through *in silico* driven metabolic engineering

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Mannosylglycerate (MG) is a compatible solute with major potential applications in the cosmetic industry, as moisturizer and skin protector against UV damage, storage of vaccines and other biomaterials, or protein stabilizer in analytical and clinical kits. Since the production of MG is expensive the development of efficient production systems is mandatory to fully exploit the potential of this solute. *Saccharomyces cerevisiae* was selected to produce MG, which is synthesized by the condensation of GDP-mannose and 3-phosphoglycerate. To better understand the impact of this pathway in yeast metabolism, the two enzymatic reactions were accommodated and evaluated *in silico* using the yeast genome scale metabolic model *i*MM904. Several optimization algorithms were ran to find the sets of genetic modification that lead to maximization of MG production. Results show that the production can be optimized by increasing the flow towards GDP-mannose formation and by introducing a bottleneck in the synthesis of pyruvate. This metabolic engineering strategy that targets the increased supply of biosynthetic precursors was implemented *in vivo*. Results show that MG accumulation increases 1.5-fold by overexpressing the genes involved in formation of GDP-mannose. However, no effect in the production of MG was observed when 3-phosphoglycerate was overproduced. Moreover, higher yields of MG were obtained when the mutants were cultivated in chemostast in comparison with batch mode.

MPA_39

Metabolic analysis of EBPR phosphate/glycogen accumulating organisms

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Enhanced biological phosphorous removal (EBPR) from wastewater is achieved by recycling Phosphate Accumulating Organisms (PAOs) through alternating anaerobic and aerobic conditions. However, industrial EBPR plants are frequently reported as unstable mainly due to PAOs' competitors, Glycogen Accumulating Organisms (GAOs).
The advantage PAOs/GAOs have over regular organisms is that they are capable of taking up substrate anaerobically and storing it for later use (in the form of PHA) at the cost of glycogen and polyPi (PAOs, only). Then, aerobically, their metabolism has to be completely switched around so that the stored substrate can be instantly used to grow and replenish their Gly/PolyPi pools.
With our study we want to answer to how PAOs (and GAOs) switch their metabolism so rapidly and how this is regulated. However, there is a lot of controversy around the metabolism of both organisms (e.g. EMP *vs.* ED, anaerobic TCA cycle (in-)active, PHA dependency on NADH *vs.* NADPH), which can be cleared by metabolomics, an addition and yet unused tool to EBPR modelling.
In order to make full use of our experimental data, model-based design of experiments is fundamental. Here, different network hypotheses are compared so as to design experiments that can discriminate well between stoichiometries. This way, we hope to settle the debate on these organisms' metabolism and use this knowledge to further understand the unique metabolic switch of PAOs and GAOs.

MPA_40

SAT-based Metabolic Pathways Analysis without compilation

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Elementary flux modes (EMs) are commonly accepted tools for metabolic network analysis under steady state conditions. They can be defined as the smallest sub-networks enabling the metabolic system to operate in steady state with all irreversible reactions proceeding in the appropriate direction. However, when networks are complex, the number of EMs quickly leads to a combinatorial explosion, preventing from drawing even simple conclusions from their analysis. Since the concept of EMs analysis was introduced in 1994, there has been an important and ongoing effort to develop more efficient algorithms. However, these methods share a common bottleneck: they enumerate all the EMs which make the computation impossible when the metabolic network is large and only few works try to search only EMs with specific properties. As we will show in this paper, enumerating all the EMs is not necessary in many cases and it is possible to directly query the network instead with an appropriate tool. For ensuring a good query time, we will rely on a state of the art SAT solver, working on a propositional encoding of EMs, and enriched with a simple SMT-like solver ensuring EMs consistency with stoichiometric constraints. We illustrate our new framework by providing experimental evidences of almost immediate answer times on a non trivial metabolic network.

MPA_41

Exploring the consequences of species heterogeneity in ^{13}C-Flux Analysis: a case study

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Metabolic reaction rates (fluxes) provide a detailed explanation for *in vivo* cellular phenotypes. Fluxes, however, cannot be measured directly but have to be inferred from experimental data by means of mathematical modeling. Currently, the most advanced modeling framework is ^{13}C-metabolic flux analysis (^{13}C-MFA). ^{13}C-MFA relies on data from bulk measurements analyzed by metabolomics techniques and therefore reports average cell metabolic activities.
In our contribution we investigate how strong the indirect flux estimation step may depend on the underlying homogeneity assumption. To this end, a sampling-based simulation workflow is implemented that calculates the errors in flux calculation when more than one subpopulation is present in a biological probe. The workflow is able to imitate different levels of variability and different distances to the population mean. In a case study, two species, e.g. a producer and non-producer strain, are mimicked being present in differing ratios. With example network models at hand, ^{13}C-MFA provides well-interpretable outcomes for near-to-linear mappings between measurements and fluxes. To set the findings into a broader picture, we explore the influence statistical identifiability on the results.
Our workflow may help to get a handle on the question whether ^{13}C-MFA can (or should not) be applied when the homogeneity of the biological sample is under question to prevent false predictions of the flux map.

MPA_42

Analysis of 140 published GSMs and identification of the most common representation problems

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The number of publications related to GSMs is increasing exponentially, but as most of these models are scattered across the Internet there is a need to centralize these data in a way that users can easily access and load them into stoichiometric modelling tools. This work presents a web platform to collect scientific work related with the reconstruction of GSMs, providing links to the original publications and the available models (www.optflux.org/models). The platform also indicates which models are compatible with OptFlux, an open-source reference computational platform for the optimization of cellular factories by the application of *in silico* ME methods, designed for non-computational experts by providing a user-friendly interface. The compatible models can be automatically loaded into OptFlux via a repository manager.
This work also presents a thorough analysis on more than 140 published GSMs available in the platform. This analysis highlights some common problems in published models, such as the lack of standards to represent them. The SBML format has been adopted as the main standard by the community, despite some limitations in representing all the information required for modelling purposes. As consequence, this format has been extended ad-hoc by several authors, thus making its automatic interpretation a non-trivial problem.
This analysis provides some insight into the limitations of formats used and the recurrent problems in the representation of GSMs.

MPA_43

Serine and glutamine metabolism in cancer cells

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Glucose and glutamine are the main carbon sources that sustain cell energy and anabolism via glycolysis in cytosol and TCA cycle in mitochondria. Cancer cells undergo reprogramming of their metabolism to adapt to specific cell growth and proliferation, involving in some cases a high consumption of glutamine and synthesis of serine, an amino acid that plays a crucial role in cancer cells anabolism by being involved into phospholipid synthesis, protein synthesis, antioxidant production, epigenetic regulation, etc.
Our ultimate goal is to build a metabolic model of cellular metabolism that would fit the experimental results and help identifying the targets for inhibition of serine biosynthesis in cancer cells in order to alter proliferation.
To identify the pathways that can synthesise serine from glutamine, we combine the results of constraint-based analysis of three metabolic network models: Recon 2, *i*AS253, extended to incorporate serine biosynthesis pathway, and a simplified mitochondrial metabolic network. To identify the pathways of interest, we search for elementary flux modes (EFM) producing serine from glutamine, and apply Flux Balance Analysis to select the optimal combinations of those EFMs and to calculate the involved fluxes. We study the pathways' impact on energy and REDOX homeostasis, as well as oxygen consumption and CO_2 production.
We compare the modelling results to the experimental data obtained on human cancer cells fed with ^{13}C-glutamine and analyzed both by label-free quantitative proteomics, ^{13}C-NMR spectroscopy and bioenergetics.

MPA_44

Including cofactor concentrations into dynamic Flux Balance Analysis

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Constraint based analysis is an efficient way to understand steady state distributions of fluxes without knowing the detailed kinetics of reactions. This makes methods like Flux Balance Analysis (FBA) well suited to investigate complex networks like Genome Scale Models (GSM).
However, the lack of information on metabolite concentrations is a major downside when we want to take into consideration the effect of cofactors. Following the approach of Harcombe *et al*., we develop a dynamic FBA (dFBA) where exchange fluxes are limited by the external metabolite concentrations according to a generic saturation curve. Our dFBA is built as if it were an ordinary differential equation (ODE), therefore we can use commonly available ODE solvers which optimize the computing time.
We then go further by considering internal metabolite concentrations which influence reactions but do not undergo chemical conversion. In this way, we introduce into the dFBA modelling framework the effect of cofactors whose presence directly affects the flux through the reaction they are involved in. Our approach automatically accounts for metabolite dilution.
We test our method on the *i*JO1366 *Escherichia Coli* GSM focusing our attention on the kinetics of the Methionine Synthase reaction which uses Adenosylcobalamin (commonly known as Vitamin B12) as cofactor. Many eukaryotes are Vitamin B12 auxotrophs and our goal is to apply the developed approach to study how different microorganisms establish advantageous mutualistic consortia by exchanging nutrients for cofactors.

MPA_45

Stochastic modelling of fatty acid synthesis

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Essential fatty acids are those fatty acids that they are good for health but cannot be synthesized by mammals (including human). The big source of these fatty acids (i.e. omega-3 and omega-6) are fishes, but overfishing causes a lot of ecological problem. One great source of these essential fatty acids are algae, however producing fatty acids from these micro-organism in industrial scale requires detailed understanding of fatty acid biosynthesis in general. In this work I will present the very first mathematical model which addresses the combinatorial explosion of pathways to synthesis fatty acids which are due to unspecificity of elongase and desaturates enzymes. Our stochastic model will predict the distribution of different fatty acids over time for a known condition.

MPA_46

Integrated analysis of metabolomics and transcriptomics data in tobacco cultivars grown in various regions of China

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We conducted an extensive series of metabolomics analyses of tobacco samples grown in various regions in China. We then used the results of these analyses, in combination with the results of microarray analysis of the same plant materials, to predict the functions of various enzymes in plant metabolism. The plant materials in the study were of several different cultivars, and were grown in different locations around China, including Yunnan, Henan, Hubei, Guizhou, Guangdong, and Hunan. The analytical methods used in the metabolomics part of this project included both targeted and non-targeted methods. The targeted methods included GC-quadropole MS based analyses of free amino acids, organic acids, and phytosterols, as well as GC-QQQ MS analyses of terpenoids, alkaloids, flavonoids, polyphenols, and pigments. The non-targeted MS analyses included LC-qTOF MS analyses of polar extracts as well as non-polar extracts, and CE-MS analysis. The microarray experiments used Affymetrix Gene Chips. The results of the metabolomics and microarrays were then integrated using methods including Pearson correlation analysis and weighted correlation network analysis. These methods of data integration enabled the identification of metabolites, such as arbutin, that had accumulation patterns that closely resembled the expression patterns of both regulatory genes and genes encoding glycolsyltransferase enzymes that may be involved in the biosynthesis of arbutin.

MPA_47

GlobalFit: automatically refining metabolic network models by simultaneously matching sets of experimental growth and non-growth data

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Initial genome-scale reconstructions of metabolic network models are refined through comparisons to experimental gene knock-outs or growth conditions, aiming to minimize erroneous predictions of growth and non-growth. Previous methods considered individual experiments separately. This may result in contradicting network modifications and can prevent the identification of feasible solutions.
Here, we present a novel bi-level optimization method that identifies the minimal set of network changes needed to correctly predict all cases of experimentally observed growth and non-growth across all tested environments or knock-out mutants simultaneously. Network changes include removing, adding, and reversing reactions, as well as adding and removing candidate metabolites from the biomass objective function.
We applied *GlobalFit* to the genome scale metabolic models of *Mycoplasma genitalium iPS189* and *Escherichia coli iAF1260* and could improve the accuracy up to 97.4% and 97.9%, respectively. Although we optimized only for gene knock-out viability predictions, the modified metabolic network of *iAF1260* also predicts more accurately the growth and non-growth of Biolog experiments. We provide a freely available implementation of *GlobalFit* in R, which is integrated with the sybil toolbox for constraint-based analyses.

MPA_48

Modeling nutrient assimilation in a species of *Chloroidium* isolated from the United Arab Emirates

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Chloroidium sp. DN1, salt-tolerant freshwater alga, was sequenced using the Illumina HiSeq 2500 to yield a genome of 52.5 Mb and 10,605 predicted ORFs. We created a draft metabolic network of *Chloroidium sp. DN1* using the software Pathway Tools. The metabolic network is comprised of 1,445 genes in 194 pathways. Full pathways for many major metabolic processes were created automatically and others were completed after manual curation. For example, all 28 reactions that comprised the metabolic pathway from an acetyl group to palmitate were automatically filled in from genomic evidence, but some amino acid metabolism including ornithine and citrulline biosynthetic pathways had to be manually curated to reflect biochemical evidence from Biolog phenotype microarrays. Analysis of reconstructed pathways shows an abundance of sugar and amino acid assimilatory pathways that are further supported by high-throughput phenotype data. Overall 10 carbon compounds and 38 nitrogen compounds were found to promote heterotrophic growth. Resistance to over 470 chemicals/ antibiotics was tested and results were supported by pathways with genomic evidence for responsible enzymes. Models were also created using the software tools in KBase for *Chloroidium sp.* DNA1 and several closely related algae including Chlorella and Coccomyxa species using genomic data from JGI. Comparative analyses were made with these algae as well as more distantly related strains including *Physcomitrella patens* and several crop plant species to reveal phenotypic variation with regards to predicted metabolic flux.

MPA_49

Computer simulation of mitochondrial metabolism in cardiomyocytes during hypoxia

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Ischemia in heart causes a well-characterised metabolic signature that is largely due to changes to mitochondrial metabolism. Some metabolites modify this hypoxic metabolic response, improving cardiac tolerance and increasing ATP levels. To simulate ischemia and quantify the effect on central metabolism of different metabolites and pathways, we used flux balance analysis of a manually-curated computer model of a human cardiomyocyte mitochondrion. The simulations unexpectedly showed activity of an NADH-fumarate reductase system (NFRS), a mechanism for producing ATP in the absence of oxygen, and observed in anaerobic bacteria. The NFRS uses an alternative electron transport chain where complex II uses fumarate as the terminal electron acceptor, making oxygen unnecessary. This allows complex I to continue pumping protons, generating a proton gradient for ATP synthesis and bypassing complexes III and IV. By varying the availability of different metabolites in a succession of simulations, we identified routes that feed into the NFRS, the metabolites involved, and that the end product of central metabolism was succinate. Our collaborators used this to interpret and verify experimental datasets showing when oxygen returned, rapid metabolism of accumulated succinate by complex II was the driver of ischemia-reperfusion injury through reverse electron transfer into complex I producing damaging reactive oxygen species.

MPA_50

Phylogenomic signature fluidity in metabolic network of a key species with plant and animal affinities

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Relationships between shared phylogenetic profiles and gene functions have been previously described. *Chlamydomonas reinhardtii*, a biofuel relevant green alga that has retained key genes with plant, animal, and protist affinities, serves as an ideal model organism to further investigate the interplay between gene function and phylogenetic affinities at multiple organizational levels under different conditions. Here, using detailed topological and functional network analyses, we show that network connectivity has a significant concordance with the co-conservation of genes in the *C. reinhardtii* metabolic network while a distinction between topological and functional relationships is observable. Dynamic and static modes of co-conservation were defined and observed in a subset of gene-pairs across the network topologically. In contrast, genes with predicted synthetic interaction, or genes involved in coupled reactions, show significant enrichment for both shorter and longer phylogenetic distances. Based on our results, we propose that the metabolic network of *C. reinhardtii* is assembled with an architecture to minimize phylogenetic profile distances topologically, while it includes an expansion of such distances for functionally interacting genes. This arrangement may increase the robustness of *C. reinhardtii*'s network in dealing with varied environmental challenges that the species may face.

MPA_51

Integration of biomass functions of genome-scale metabolic models with experimental data reveals universally essential cofactors in prokaryotes

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Knowledge of the core biochemical composition of the cell is critical for genome-scale metabolic modelling. In order to identify the universal core organic cofactors for prokaryotes, we performed a detailed analysis of biomass objective functions (BOFs) of 71 manually curated genome-scale prokaryotic models. These were then compared and integrated with the ModelSEED framework for biomass composition, experimental data on gene essentiality, curated enzyme-cofactor association data and a comprehensive survey of the literature. Surprisingly, no cofactor was present in all the BOFs analysed, including the important redox cofactor nicotinamide adenine dinucleotide (NAD) or its derivatives. Our results indicate not only the redox cofactors but also others such as coenzyme A, flavins and thiamin as universally essential for prokaryotes and therefore as important to include in the BOFs of future genome-scale models of prokaryotic organisms.

MPA_52

Virtual mitochondrion :a modular and multi level whole-mitochondrion model

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Virtual Mitochondrion is a project of a multilevel modelling of mitochondrial bioenergy metabolism. It involves: - A molecular/ atomic level with stochastic modelling (Gillespie) of electrons and protons transfers in respiratory chain complexes and super complexes of respiratory chain. It allowed us to predict a natural bifurcation of electrons in complex III, to clarify the antimycin inhibition constraints and to simulate the ROS production in complex I and III. It also permits to jump to the upper level of enzyme kinetics. - A mitochondrial level with the global modelling of the respiratory chain using simple but thermodynamical correct kinetics equations developed for the respiratory chain complexes (Henri-Michaelis-Menten like equations with the introduction of the proton gradient). The aim is to understand how local changes (pathological mutations for instance, drug effect, competition between respiratory substrates) in respiratory complexes influence the global behaviour of the oxidative phosphorylation. (In collaboration with Edda Klipp, Berlin). - A cell level with the description of simple(s) model(s) of central energy metabolism easy to manipulate and to understand. The aim is to coherently integrate various types of data, metabolomics, fluxomics, transcriptomics and to follow the reroutings of metabolism, their regulations and controlling steps/targets (Metabolic Control Analysis). In this work, our purpose is not only to fit the experimental results but also to evidence inconsistencies that will lead to unveil mechanisms which were not taken into account.

MPA_53

Systems level metabolic pathway analysis for understanding antibiotic resistance in *Chromobacterium violaceum*

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Antibiotic resistance is a serious threat to public health globally. Altered metabolism, in addition to the pathogenicity islands and virulence factors have been implicated in pathogenesis and antibiotic susceptibility. The systems biology paradigm of integrating heterogeneous data-types with computational metabolic models offers a constraints-based framework to understand connections between growth, metabolism and resistance. *Chromobacterium violaceum* (CV) populations resistant to chloramphenicol (chlR) and streptomycin (strepR) have been evolved under controlled laboratory conditions. A Genome scale metabolic model (GSM) of CV was reconstructed including drug metabolic pathways. Constraint-based flux balance analysis was further used to define resistant and susceptible phenotypes of CV and understand metabolic rewiring in the differential phenotypes. The model constrained using physiological, genomic and metabolic profiling (MALDI) data acquired in-house mimicked the multiple pathogen phenotypes *in silico*. Biolog (TM) data was used to validate the model. The differential growth & respiration profiles on exogenous Carbon & Nitrogen sources were predicted with good accuracy. The Antibiotic sensitivity was also calculated as cfu/ml on 30 different carbon and nitrogen sources and TCA cycle intermediates citrate and succinate allow reversal of antibiotic resistance. Flux variability analysis captured the differential metabolic secretome of susceptible and resistant cells and gave insight into the alternate routes available to the pathogen. Metabolic reprogramming in pathogens as a response to antibiotics may allow development of strategies against the emergence of antibiotic resistance.

MPA_54

merlin latest developments for pathways analysis

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merlin is a user-friendly open-source software tool developed for the reconstruction of genome-scale metabolic models. These models are derived from sets of reactions, organised in pathways, which can be used to mimic the behaviour of microorganisms in different genetic and environmental conditions. One of the toughest challenges, when reconstructing models is the identification of gene-protein-reaction associations, a step usually performed by manually searching literature. Thus, a novel approach for automatically predicting, at the genome level, protein subunits using gene association rules retrieved from the KEGG BRITE database was developed and integrated in *merlin*. The presence or absence of the different pathways in the metabolic models may be related with several properties of the microorganism, namely the ability to survive in specific environments. Moreover, the analysis of metabolic pathways is important for finding gaps, which can impair model predictions by blocking the production of a by-product of interest, or a biomass component. Additionally, this analysis may propose more efficient pathways to increase the production of specific metabolites by, for instance, proposing knock-out or knock-in of genes. Therefore, an innovative reactions panel, which organises reactions by pathway allowing the visualisation and analysis of the constructed models' reactions in KEGG pathways was developed and integrated into *merlin*.

MPA_55

Metabolic flux prediction in cancer cells with altered substrate uptake

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Proliferating cells such as cancer cells are known to have an unusual metabolism, which is characterised by an increased rate of glycolysis and a reduced rate of mitochondrial oxidative phosphorylation known as the Warburg effect. Our understanding of this phenomenon is limited but could potentially be used in order to develop new therapies. Computational modelling is important to further our understanding of metabolic flux regulation in these cells by enabling us to formulate and test new hypotheses, and compare predictions to experimental data. Techniques such as Flux Balance Analysis have been used to predict fluxes in various cell types, but remain of limited use to explain the unusual metabolic shifts and altered substrate uptake in human cancer cells. We implemented new flux prediction methods based on elementary modes and structural flux analysis, and tested them against experimentally measured flux data obtained from ^{13}C labelling in a cancer cell line. We assessed the quality of predictions using different objective functions along with different techniques in normalising a metabolic network with more than one substrate input. Results show a positive correlation between predicted and experimental values, with larger discrepancies in some parts of the network, and show that a combination of several objectives is needed to reflect the distribution of fluxes in cancer cells.

MPA_56

Elementary flux mode analysis of irradiance-induced stress acclimation strategies in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1

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Irradiance plays a central role in regulating phototrophic metabolisms, including the metabolism of photoautotrophic cyanobacteria. Oxygenic cyanobacteria are critical primary producers in most aquatic ecosystems and have become industrially relevant as bioprocess hosts for biofuels and secondary metabolite synthesis. Here, the model thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 was studied for metabolic acclimation strategies to irradiance-induced stress using elementary flux mode analysis. Metabolic stress was considered in conjunction with the availability of dissolved inorganic carbon and fixed nitrogen as well as the inhibitory effects of metabolic byproducts. Physiologies and their associated byproduct secretion profiles were analyzed over a gradient of irradiances. Formate was predicted to be the most competitive fixed carbon byproduct under stress conditions, a result interpreted in terms of metabolic pathways. Additionally, this work details the experimental determination of biomass macromolecular composition (carbohydrate, DNA, lipid, protein, RNA) for stoichiometric models, which is an often undervalued activity.

MPA_57

Uncovering the metabolic capacities of *H. pylori* 26695 using ^{13}C labeling experiments

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The determination of nutritional requirements of pathogenic organisms is of great significance for understanding host-pathogen interactions. Despite the knowledge obtained so far concerning amino acid requirements in *H. pylori*, it is still unclear which are the metabolic pathways used for biosynthesis and catabolism. Thus, information on the carbon flow in this organism is required. Glutamate is a very important metabolite in bacterial metabolism that can be used as a carbon and nitrogen source. ^{13}C flux analysis has been largely applied to characterize phenotypes by quantifying *in vivo* the carbon fluxes. One of the most important applications of this approach is the identification of active pathways in less-studied organisms. Thus, in order to clarify the metabolic pathways used by *H. pylori* 26695, ^{13}C labeling experiments with ^{13}C-glutamate were conducted and labeled amino acids in biomass hydrolysates were analyzed by GC-MS. The obtained results confirmed L-glutamate as a potential sole and effective carbon source for *H. pylori*. Overall, all non-essential amino acids, except proline, presented a ^{13}C labeling pattern. We hypothesized that L-proline is produced from L-arginine, while L-alanine is probably produced from pyruvate by alanine dehydrogenase. Additionally, the full usage of complete TCA cycle, under the conditions used, was also demonstrated.

MPA_58

Compensatory mechanisms in mitochondrial diseases revealed by computer modelling

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Dysfunction of the mitochondrial respiratory chain causes a variety of disorders affecting particularly organs with high metabolic demands, such as brain, heart and liver. The causative pathogenic mutations reduce the activity of mitochondrial respiratory complexes, leading to decreased ATP production, and accumulation of lipids, TCA cycle intermediates, lactic acid, and some amino acids. The severity and tissue specificity of the disorders varies between patients by unknown mechanisms, and treatments are limited to compensating for dysfunctional metabolism, and stabilising complexes by dietary supplements of antioxidants, riboflavin and bicarbonate. To better understand how respiratory chain dysfunction impacts metabolism and how metabolites may have compensatory or deleterious effects, we simulated deficiencies of the mitochondrial respiratory complexes using computer models of metabolic networks. Our simulations show complex III and IV deficiencies cause greatest decreases in ATP production. But - depending on which complex is deficient - ATP production can be increased by supplying amino acids, including glutamate, aspartate, arginine and proline. Simulations of complex I disorders show various pathways can compensate the underlying deficiency. Conversely, little compensates for complex II deficiencies. Overall, our simulations are consistent with patient phenotypes, supporting their biological significance and demonstrating how computer modelling can further understanding and treatment of mitochondrial disorders.

MPA_59

Analysis of *Salmonella typhimurium* pathways and metabolic model improvement

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Live attenuated strains of *Salmonella typhimurium* have been extensively investigated as vaccines for several infectious diseases. However, a better knowledge of *S. typhimurium* metabolism is required to develop protocols to improve bioprocesses for the production of biotechnological products in large scale. Currently, genome scale metabolic models are important tools for better understanding the phenotypic behavior of many microorganisms. In this work, a genome-scale metabolic model reconstructed for *S. typhimurium* (STMv1.0 model) was used to determine the *in silico* fluxes distributions of end-products and to compare with *in vivo* data. Experimental data from glucose-limited chemostat at different dilution rates (0.1 to 0.67 h^{-1}) with *S. typhiumurium* LT2 were compared with *in silico* simulations performed with Optflux 3.2.4 software, using the same environmental conditions (glucose and O2 experimental uptake fluxes). *Salmonella* cultures showed deviation of carbon towards acetate formation, starting at dilution rate above 0.1 h^{-1}, with flux of 4.16 mmol acetate/(gDCW . h) at the higher dilution rate. Nevertheless, this model, which was based on *E. coli* model, overestimates the biomass production and, consequently, minimizes the fluxes of acetate. Thus, changes in metabolic model are required to improve its accuracy to predict the fermentation patterns observed experimentally, including changes in model equations related to P/O ratio and ATP yield. ^{13}C Fluxomic analysis are being carried out to better understand the *S. typhimurium* central carbon metabolism and to improve the model predictions.

MPA_60

Elucidate robust redox metabolism of *Clostridium thermocellum*

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Consolidated bioprocessing (CBP) is a potentially feasible route for sustainable production of bio-based fuels that condenses multiple steps of biomass degradation and sugar fermentation into a single step. *Clostridium thermocellum* is an anaerobic, gram positive, thermophilic bacterium that is capable of degrading cellulosic biomass directly into ethanol. Despite its growing popularity, the complete understanding of *C. thermocellum* central metabolism is still lacking with atypical glycolysis, incomplete pentose phosphate pathway and Krebs cycle, and complex redox pathways. In this study, a predictive metabolic model of *C. thermocellum* has been developed for metabolic flux quantification and rational strain design. We experimentally validated the model and investigated the range of phenotypes of *C. thermocellum* in response to significant perturbation of energy and redox pathways. The result revealed a complex, robust redox metabolism of *C. thermocellum*. By incorporating experimental data into the model, we identified redox bottlenecks hindering high-yield ethanol production in *C. thermocellum*. Furthermore, we show the model's rationale for why previous metabolic engineering strategies had low target ethanol yields, and provide different metabolic engineering strategies for reaching the target yield.

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Notes